Influence of operating pH on biodegradation performance and fouling propensity in membrane bioreactors for landfill leachate treatment

Samunya Sanguanpak^a, Chart Chiemchaisri^{a*}, Wilai Chiemchaisri^a and Kazuo Yamamoto^b

^a Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand (E-mail: samunys@mtec.or.th; fengccc@ku.ac.th; fengwlc@ku.ac.th)

^b Environmental Science Center, University of Tokyo, Tokyo 113, Japan
(E-mail: yamamoto@esc.u-tokyo.ac.jp)

*Corresponding author, Tel. 662 797 0999 ext. 1010, Fax. 662-579-0730

Abstract

This research has investigated the effects of varying mixed liquor pH (i.e. 5.5, 6.5, 7.5, 8.5) on the landfill leachate biodegradability and fouling behavior in two submerged membrane bioreactors (MBR) for a 150-day period. In the experiments, variations in organic removal and dissolved organic matters (DOM) transformation, microbial community and mixed liquor properties as well as the fouling behavior were monitored and systematically analyzed. The findings indicated that the organic removal in both MBRs was insignificantly different for the entire experimental pH range and that the pH levels influenced the DOM transformation. In the analysis of microbial community, PCR-DGGE revealed considerable structural changes, pointing to an effect of pH on the dynamic shift in bacterial species. In addition, clone library analysis showed that *Proteobacteria* was the dominant phylum in the MBRs and that the dominant bacterial community belonged to the classes *Betaproteobacteria* and *Gammaproteobacteria*, both of which were more abundant at pH 8.5 than at pH 5.5. The experiment also showed that the mixed liquor properties were affected by the pH variations. The extracellular polymeric substance (EPS) formation in the mixed liquor at pH 5.5 significantly contributed to membrane fouling while hindering the inorganic and humic substances fouling. Nevertheless, the organic fouling under the low pH condition could be effectively removed by membrane chemical cleaning. It is expected that the experimental results could be applied to enhance the biodegradability and mitigate the fouling in the MBR process.

Keywords: Landfill leachate; MBR; Biodegradability; Membrane fouling; Mixed liquor pH

1. Introduction

Landfill leachate is the putrid liquid that drains or leaches from a landfill. It varies considerably in composition but typically contains large amounts of organic matters, ammonia and other inorganic compounds (Zhao et al., 2013). The biodegradability of leachate is inversely correlated to the landfill age. In other words, as the landfill ages, the organic fractions are replaced with non-biodegradable compounds. Previous research showed that dissolved organic matters (DOM) accounted for the largest proportion of organic substances in leachate. DOMs are a highly heterogeneous matrix comprising a variety of

molecular weights (MW) of humic substances, proteins, carbohydrates and hydrophilic acids (He et al., 2006; Huo et al., 2009). Therefore, the removal of organic compounds from landfill leachate through effective treatment technologies is thus necessary prior to discharge into the receiving environment. The membrane bioreactor (MBR) technology is a recent innovation suitable for landfill leachate treatment due to its complete solids removal ability, high pollutants removal efficiency, and lower sludge production vis-à-vis the conventional activated sludge. In addition, since the MBR technology is operable under the long sludge retention time and high biomass concentration condition, the biotransformation of organic pollutants could be enhanced through specialized and slow-growing bacteria (Ahmed and Lan, 2012). In our previous study (Chiemchaisri et al., 2011; Sanguanpak et al., 2013), a pilot-scale two-stage MBR system (i.e. an anoxic tank followed by an aerobic reactor) was applied to landfill leachate treatment in Thailand for a period of over two years. The results showed that biodegradable organic substances could be efficiently removed by biological activity in the MBR process, while partial removal of non-biodegradable organic constituents, e.g. humic substances, was achieved through adsorption and filtration mechanisms. Furthermore, membrane fouling attributable to organic substances and inorganic elements (i.e. calcium and magnesium) was observed in the MBR operation.

Despite extensive research studies on MBR, additional efforts should be expended to improve the performance of the MBR technology. Of particular challenge is the need to normalize the treatment efficiencies along with the mitigation of membrane fouling. According to Ahmed and Lan (2012), the performance of MBRs in wastewater treatment was subject to the operating conditions and environmental factors, e.g. pH level. pH is a major factor that significantly influences the membrane characteristics (Nanda et al., 2010), mixed liquor properties (Gao et al., 2010), and microbial activity (Wu et al., 2010). Moreover, pH variations were reported to influence the cell morphology, flocculation and adhesion phenomena (Baily and Ollis, 1986). With the increasing popularity of MBR in treating wastewater of various types, interest in research studies on the effects of pH levels on the treatment performance and membrane fouling has also increased. Wu et al. (2010) found that a pH range of 6.0-9.0 insignificantly affected the microbial activity and that the activity declined when the mixed liquor pH decreased toward 4.0. Gao et al. (2010) reported that an alkaline surge (pH 9-10) adversely affected the COD removal and membrane permeability. Sweity et al. (2011) noted that a decrease in pH from 8.3 to 6.3 altered the EPS formation and swelling characteristics, thereby an increase in filtration permeability. Zhang et al. (2014) found that, with increase in mixed liquor pH, the resistance of cake layer significantly increased. On the other hand, a decrease in pH contributed to an increase in the adhesion of sludge flocs on the membrane surface. However, previous studies (e.g. Wu et al., 2010; Sweity et al., 2011; Zhang et al., 2014) have focused on the effects of mixed liquor pH in the MBR process only for a very short term period of one day or less. Thus, it is likely that, due to those short-term experimental conditions, the existing researches could offer a mere fragmented picture of the complex biological treatment mechanism and membrane fouling of the MBR process. Nevertheless, this research has extended the study period to 150 days. The longer study period has enabled a more thoroughly investigation into the treatment performances of MBR with regard to DOM transformation changes in microbial population

and sludge properties as well as the correlation of the properties of sludge to membrane fouling.

This paper aims to investigate the effects of mixed liquor pH on the biodegradability and fouling behavior of the MBRs treating landfill leachate. In this research, two identical lab-scale MBRs were utilized, one of which was for the experiments under the mixed liquor pH 5.5 and 6.5 conditions, while the other was for those under the pH 7.5 and 8.5 conditions. The entire experimental period was 150 days. The biological treatment performances with respect to organic removal and DOM transformation as well as the changes in microbial population under the acidic and alkaline conditions were identified. In addition, changes in sludge properties as a function of pH and their correlation to membrane fouling were systematically analyzed. It is expected that the study findings would help identify an optimal mixed liquid pH level that induces normalized biological treatment performances and minimal membrane fouling in the MBR process.

2. Materials and Methods

2.1 Laboratory-scale MBR setup and operation

This research utilized two identical 12-liter laboratory-scale MBRs individually outfitted with a submerged hollow-fiber microfiltration membrane module (Mitsubishi Rayon, Japan), as shown in Fig.1. The membranes were of polyvinylidene fluoride (PVDF) materials, with a membrane surface area of 0.07 m² per module and a nominal pore size of 0.4 μm. To maintain the dissolved oxygen (DO) concentration within a range of 3-4 mg L⁻¹, two sets of air pumps were employed to supply air through diffusers at the base of MBR tanks at an air flow rate of 5 L min⁻¹. Due to low variations in DO concentrations controlled in this study, the air flow rate was kept constant throughout the entire operations. A hydraulic retention time (HRT) of 1.5 days was selected, which corresponded to an approximate 4.5 L m⁻² h⁻¹ permeate flux. To maintain a constant volume in both MBRs, the influent pumps were regulated with level sensors. Permeate was intermittently withdrawn using a peristaltic pump with an operating mode of 10 min suction and 2 min relaxation. The feed leachate was prepared by diluting fresh leachate with tap water in a ratio of 1:10 (v v⁻¹), resulting in an organic loading of approximately 3.7±0.5 kg BOD m⁻³ d⁻¹ and 5.4±0.9 kg COD m⁻³ d⁻¹. Both MBR tanks were seeded with activated sludge from an integrated MBR-RO pilot plant in Thailand. The initial mixed liquor suspended solids (MLSS) concentration was 7 g L⁻¹ and was subsequently maintained within a range of 10-15 g L⁻¹ throughout the MBR process without sludge wastage operation except for sampling purposes.

Insert Figure 1

The initial ambient of mixed liquor pH in both experimental MBR tanks (i.e. MBRs 1 and 2) was 7.9±0.1. The experiments were divided into two successive phases of 75 days each. In MBR 1, the mixed liquor pH was first reduced from the ambient pH (i.e. 7.9±0.1) to pH 6.5±0.3 during the first phase (days 1 to 75) and subsequently lowered to pH 5.5±0.3 during

the second phase (days 76 to 150). On the other hand, in MBR 2 the pH was first raised to pH 8.5±0.2 and then adjusted to pH 7.5±0.1 during the same periods. The pH of the MBRs were manually adjusted to the targeted pH levels using analytical-grade HCl (1 M) and NaOH (1 M) solutions, and the measurements of mixed liquor pH were taken every hour using a digital pH meter. In addition, the permeate flux was monitored volumetrically and the trans-membrane pressure (TMP) was measured with a pressure gauge on a daily basis. The operation of the treatment system was continuous and under ambient conditions (27-30°C). Membrane cleanings were individually performed at the end of each experimental run (i.e. 75 days for each pH operation), by which the membrane modules were removed and rinsed with tap water to wash off the foulants on the membrane surface. Furthermore, chemical cleaning was undertaken by soaking the membranes in a 0.1 percent sodium hypochlorite (pH ~12) for 60 min, followed by 0.1 percent hydrochloric acid (pH ~2) for another 60 min.

2.2 Analytical methods

2.2.1 Water sample analysis

In leachate analysis, the pH levels were measured with a pH meter. In addition, suspended solids (SS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined in accordance with standard methods (APHA, 2005) without pre-treatment. The samples were diluted and filtered with a GF/C filter prior to determination of dissolved organic matters (DOM). DOC was measured using the Shimadzu TOC-5000. UV₂₅₄ was determined by a spectrophotometer (Thermo Scientific™ Evolution 60S) and the results were employed to calculate specific UV absorbance (SUVA₂₅₄), i.e. scaling UV₂₅₄ by DOC. Fluorescence excitation-emission matrix (EEM) spectroscopy was determined on a Jasco FP-8200 spectrofluorometer using a 1 cm quartz cell. All the samples were diluted to the DOC concentration of <10mg L⁻¹ prior to analysis. The EEM spectra were collected at the excitation (Ex) wavelengths between 200-500nm and emission (Em) wavelengths between 250-600nm with 5nm increments. The spectra were scanned with a 5nm slit bandwidth at a scan rate of 2000nm min⁻¹. The spectrum of de-ionized (DI) water was recorded as blank and the equipment was auto-zeroed before analysis. The measurement was carried out in a temperature-controlled room at 25°C.

2.2.2 Bacterial community analysis

The PCR-DGGE technique was employed to investigate the effects of mixed liquor pH at 5.5 and 8.5 on the bacterial community and the foulant layer in the MBR process. At the end of each pH operation, the samples of mixed liquor sludge and foulant layer were collected and subjected to DNA extraction using the PrepEase® Tissue and Cell DNA Spin kit (Invitrogen, USA). Universal bacteria primers of 338GC-F (5'-ACTCCTACGGGAGGCAGC-3') and 518R (5'-ATTA-CCGCGGCTGCTGG-3') were then used to identify the bacterial community. DGGE analyses of 16s DNA amplified PCR products were performed using DGGE-2000 system apparatus (CBS Scientific Company, Del Mar, CA). The procedures for PCR amplification and DGGE analyses of the PCR products were detailed in Boonnorat et al.

(2014). The DNA sequencing was manually performed using the BLAST software on the NCBI website.

In the analysis of microbial community structures, the Shannon diversity index (H) is used to compare the microbial species diversity at pH 5.5 and at 8.5. The index can be expressed as (Shannon, 1948):

$$H = - \sum P_i \ln P_i \quad (i = 1, 2 \dots S) \quad \text{Eq. 1}$$

Margalef's richness index (D) is used to determine the number of microbial species in the MBR system and is written as (Gao et al., 2014)

$$D = (S-1) / \ln N \quad \text{Eq. 2}$$

where P_i is the relative abundance of the species, S is the number of microbial species, and N is the total number of species.

2.2.3 Determination of mixed liquor properties

The microbial floc size was measured by a Malvern Mastersizer 2000 particle size analyzer with a detection range of 0.02-2000 μm . The microbial flocs were morphologically characterized by a microscope (BHM, Olympus). The supernatant samples were separated from solid fraction by gravitational sedimentation of the mixed liquor for 30 min, and surface charges of the samples were determined as zeta-potential in 0.01 N NaCl at pH 7.0 on an electrophoresis measurement apparatus (Zetasizer nano ZS, Malvern Instrument) with a disposable zeta cell. In each run, 10 scans per sample were performed. The unsettled suspended solids (SS) in the supernatant samples were measured and represented the flocculation performance of the mixed liquor.

Soluble microbial products (SMP) and extracellular polymeric substances (EPS) were extracted from the mixed liquor using heat treatment (Le-Clech et al., 2006). The mixed liquor was centrifuged for 30 min at 6000 rpm before the supernatant was collected and filtered through a 0.45 μm membrane. The filtered supernatant samples represented the concentration of SMP. The remaining sludge pellet was washed and re-suspended in saline water (0.9 percent NaCl). The sludge mixture was then diluted to its original volume. The NaCl solution for dilution was pre-heated to 100°C for 1 h prior to re-centrifugation under the same condition. The supernatant was collected and treated as EPS of the sludge. The extracted solutions of SMP and EPS were analyzed for total carbohydrates and proteins. Proteins were colorimetrically determined according to the folin method with bovine serum albumin (BSA) as standard, while carbohydrates were determined by the phenol-sulfuric acid method with glucose as standard.

2.2.4 Membrane fouling determination

The permeate flux and membrane resistance were analyzed using Darcy's law (Eq. 3 and Eq.4):

$$J = dV/dtA = TMP/\eta\Sigma R_t \quad Eq. 3$$

$$R_t = R_m + R_c + R_p + R_{ir} \quad Eq. 4$$

where J is the permeate flux ($\text{m}^{-1} \text{h}^{-1}$), V is the collected volume, A is the membrane surface area, t is time. TMP is the trans-membrane pressure (Pa), η is the dynamic viscosity (Pa.s), and R_t is the total membrane resistance (m^{-1}), which is a function of time and operating conditions which include the initial membrane resistance (R_m , m^{-1}) and resistance due to fouling. Fouling resistance can be classified into three categories: (a) removable fouling resistance (R_c , m^{-1}), that which can be removed by physical cleaning; (b) irremovable fouling resistance (R_p , m^{-1}), that which can be removed by chemical cleaning; and (c) irreversible fouling resistance (R_{ir} , m^{-1}), that which cannot be removed by any methods including chemical cleaning (Meng et al., 2009). The resistances were evaluated as follows: R_m was determined by measuring the flux of pure water from new membrane modules; R_t was determined by measuring the flux of sludge wastewater at the end of experiments; and R_c , R_p , and R_{ir} were obtained after the physical cleaning and chemical cleaning, respectively.

To investigate the relationship between mixed liquor pH and foulant properties, the fouled membranes were removed from the bioreactors at the end of operation. Two liters of DI water was used to desorb foulants from the membrane surface. The desorbed foulant properties, including SS, DOC, proteins, carbohydrates, inorganic contents (Ca and Mg) and the FEEM properties of DOM in the membrane foulants, were subsequently determined in accordance with the analytical methods described in sub-section 2.2.1.

2.2.5 Statistical analysis

SPSS statistical analysis was performed to identify the correlation between mixed liquor properties and the TMP increasing rate at different pH values. Pearson's product momentum correlation coefficient (r_p) was used for verification of the accuracy of linear correlation between two factors. The relationship can be ignored for $-0.4 < r_p < 0.4$; otherwise, their correlation is considered statistically significant at 95 percent confidence interval.

3. Results and discussion

3.1 Effects of mixed liquor pH on the removal and DOM transformation in the MBRs

Both experimental MBRs were operated for a total period of 150 days with an average organic loading of $3.7 \pm 0.5 \text{ kg BOD m}^{-3} \text{ d}^{-1}$ and $5.4 \pm 0.9 \text{ kg COD m}^{-3} \text{ d}^{-1}$. During the initial period, the biomass concentration in the aerated MBR tanks steadily rose from 7 g L^{-1} to almost 12 g L^{-1} . After the steady condition, the organic removal efficiencies, i.e. COD, BOD

and DOC, were relatively constant with an overall average of roughly 97 percent. MLSS levels were maintained between 10 and 15g L⁻¹ and the mixed liquor pH values were further adjusted to the targeted values. The influent and effluent characteristics of the leachate under different pH conditions are presented in Table 1. At pH 6.5, 7.5 and 8.5, the average removal efficiencies were approximately 99 percent for COD, 99 percent for BOD and 97 percent for DOC. However, as the mixed liquor pH decreased to 5.5, the removal efficiencies slightly worsened. Similar to Tadkaew et al. (2011), the optimum pH for biological performance of MBR was near neutral (pH 6-7). A slight decrease in organic matters removal performance for the mixed liquor pH of 5.5 could be attributed to bacterial population changes in the MBRs. Under the mild acidic condition (pH 5.5), some bacterial species in the bioreactors could become inactive (Baldwin and Campbell, 2001), which would in turn influence the substrate utilization and degradation mechanism, thereby leading to a decrease in treatment performance. Wu et al. (2010) also found that when the mixed liquor was acidified to a pH of 4, the microbial activity, as indicated by specific oxygen uptake rate (SOUR), was significantly reduced.

Specific UV absorbance at 254nm (SUVA₂₅₄) is a parameter that indicates the presence of aromatic carbon contents and treatability of DOM. According to Comstock et al. (2010), an increase in SUVA₂₅₄ during biological treatment generally indicated the presence of higher aromatic organic carbon content and transformation of DOM through microbial activities. In Table 1, the influent leachate has a low SUVA₂₅₄ of 0.35 L mg⁻¹m⁻¹, suggesting an abundance of non-aromatic compounds. After the treatment, SUVA₂₅₄ of effluent showed a significant increase to 1.70-2.96 L mg⁻¹m⁻¹, suggesting the biodegradation of non-aromatic in the MBRs. In addition, SUVA₂₅₄ in the effluent samples at pH 5.5 was lower than that of the other pH levels. This finding suggested that the removal and transformation of DOM became poorer as the mixed liquor pH decreased toward 5.5.

Insert Table 1

Fig. 2 illustrates the fluorescence properties of DOM during the MBR treatment under different pH conditions of mixed liquor. In this research, the analysis of four principal fluorescence peaks was based on the fluorescence EEMs of DOM in landfill leachate in Lu et al. (2009) and Comstock et al. (2010). In Fig.2, peaks A and B, where Ex/Em respectively are 230-240/330-340 and 270-290/300-340, represent protein-like peaks, in which the fluorescence is respectively linked to aromatic protein-like substances and tryptophan protein-like substances. Peak C, where Ex/Em=315-355/395-425, indicates fulvic-acid-like substances, while peak D, where Ex/Em=245-260/435-480, designates humic-like substances. In the MBR process, under the mild acidic condition of pH 5.5, the fluorescence peaks of protein-like substances (peaks A, B) in fresh leachate partially disappeared and were almost completely removed with increase in pH to 6.5 or higher. On the other hand, relative to that of the influent, the fluorescence peaks of humic-like substances (peaks C, D) shifted to the longer wavelengths and became more intense with increase in pH. The extension of fluorescence wavelengths implied an increase in aromatic poly-condensation and a higher degree of humification (Kang et al., 2002; Huo et al., 2009), suggesting the higher

humification degree in the MBR process, particularly under alkaline conditions of mixed liquor. These findings were in good agreement with Amonette et al. (2004), who reported a higher degree of humification in soils under alkaline conditions through the enhancement of condensation step in the humification process. The remaining humic-like substances at peaks C and D in the effluent indicated the presence of non-removable biodegradable organic substances. In addition, a slightly longer wavelength of protein-like substances (peaks A, B) in the mixed liquor relative to that of the effluent indicated the removal of protein-like substances during membrane filtration, which would subsequently deposit on the membrane surface as foulants. The differences in the EEM fluorescence spectra results showed the effects of varying mixed liquor pH conditions on the DOM transformation of leachate in the MBRs and the influence of pH on the system performance with regard to DOM removal.

Insert Figure 2

3.2 Effect of mixed liquor pH on microbial communities

The microbial communities in the MBR treatment system are subject to multiple influencing factors, e.g. the influent wastewater characteristics (Mnif et al., 2012), operation condition (Molina-Muñoz et al., 2009). In this research, the effects of mixed liquor pH on microbial communities were investigated using the PCR-DGGE technique. Fig.3 illustrates the dissimilarity in the diversity of microbial communities in the two MBR tanks under two different mixed liquor pH conditions (i.e. 5.5 and 8.5), indicating a significant role of pH in the composition of microbial communities in the MBR treatment system. In fact, the variations in the bacterial communities had no influence on the bioreactor performances with respect to BOD, COD and DOC removals but exerted some influence on the transformation of DOM. Molina-Muñoz et al. (2009) reported that the microbial community formed in the MBRs under different sets of operation conditions and environment parameters varied significantly with regard to microbial species, and that the resultant sludge possessed dissimilar rates of mineralization; however, these differences had no impact on the quality of the permeate. In addition, the adaptability of bacterial communities to environmental changes was crucial to a stable MBR performance.

The samples were found to make up of two phyla: *Proteobacteria* (84 percent), which was the most dominant and consistent with Hu et al. (2012) and *Firmicutes* (16 percent). The finding suggested that *Proteobacteria* were largely responsible for the removal of various organic substances in the MBRs. For the pH 5.5 samples, *Proteobacteria* accounted for 75 percent, while the figure was 86 percent in the pH 8.5 samples. In the former, *Proteobacteria* were mainly composed of *Betaproteobacteria* (33 percent), followed by *Gammaproteobacteria* (25 percent), *Alphaproteobacteria* (8 percent) and *Deltaproteobacteria* (8 percent). In the latter (pH 8.5), *Betaproteobacteria*, *Gammaproteobacteria* and *Alphaproteobacteria* respectively accounted for 43 percent, 36 percent and 7 percent of *Proteobacteria*. In addition, the dominant members in *Betaproteobacteria* belonged to the order *Burkholderiales*. Consistent with Molina-Muñoz et al. (2009), *Betaproteobacteria*, *Gammaproteobacteria* and *Alphaproteobacteria* were the

dominant bacterial classes in the MBR wastewater treatment system. Ittisupornrat et al. (2014) found that bacterial communities belonging to the phyla *Proteobacteria* and the class *Gammaproteobacteria* were dominant in the MBR operation, and most of them were responsible for protein degradation. Thus, an abundance of *Gammaproteobacteria* in the pH 8.5 sludge samples likely contributed to a high removal rate of protein-like substances during the DOM transformation in the MBRs.

By comparison, the abundance of bacterial species in the MBR at pH 5.5 ($D = 3.8$) was slightly less than at pH 8.5 ($D = 4.4$). In addition, in the pH5.5 samples, the bacteria strains of *Hipaea maritima* DSM 10411 (Band 1), *Thermoanaerobacter siderophilus* SR4 (Band 6), *Methylobacillus flagellatus* KT (Band 7), *Candidatus Accumolibacter phosphatis* (Band 9), and *Methylovorus glucosetrophus* SIP3-4 (Band 16) were detected, whereas the DGGE profile displayed different bacterial populations for the pH 8.5 samples. In the higher pH condition, the analysis results revealed the bacteria strains of *Ralstonia pickettii* 12J (Band 3), *Ralstonia eutropha* JMP134 (Band 8), *Frateuria aurantia* DSM 6220 (Band 11), *Nitrosomonas* sp. Is79A3 (Band 12), *Thioalkalivibrio sulfidophilus* HL-EbGr7 (Band 13), *Methylibium petroleiphilum* PM1 (Band 14), and *Burkholderia* sp. CCGE1002 (Band 18). Interestingly, *R. picketti*, *R. eutropha*, *M. petroleiphilum* and *Burkholderia* sp. are capable of degrading a wide variety of aromatic compounds (e.g. aromatic hydrocarbon, chloroaromatic compounds, and phenol). Thus, the increase in aromatic-compounds humic-like substances during the transformation of DOM, particularly under the alkaline condition, probably contributed to the enrichment of such bacteria species.

To quantify the structural diversity of the microbial community in the MBRs, the Shannon diversity index (H) was calculated according to the number and relative abundance of terminal restriction fragments amplified directly from community DNA. According to Miura et al. (2007), the biodiversity is positively related to system stability, e.g. high resistance to disturbance and rapid recovery rate after disturbance. In this research, the Shannon diversity indices at pH 5.5 and 8.5 were respectively 2.43 and 2.58. In comparison with a study by Miura et al. (2007), the Shannon diversity indices of this research were considerably higher than that in the MBRs for municipal wastewater treatment (i.e. 1.3-1.6). The disparity could be attributed to the more complex composition of landfill leachate and long sludge age condition of this research study. It could be concluded that the dissimilarities in the proportions of *Proteobacteria* and the bacterial species in the mixed liquor pH of 5.5 and 8.5 may influence the biodegradability based on the transformation of DOM during landfill leachate treatment by the MBR system of this study.

Insert Figure 3

Insert Figure 4

3.3 Effects of mixed liquor pH on MBR fouling and mixed liquor properties

Fig.5 presents the TMP and permeate flux profiles relative to time (days) under four experimental mixed liquor pH conditions (i.e. 5.5, 6.5, 7.5, and 8.5). It is observed that, under all four pH conditions, the TMP pressure minimally rose during the first 50 days, followed by rapid elevation. According to Meng et al. (2009), the fouling mechanisms in the constant flux mode involved three fouling stages of: an initial short-term rapid rise in TMP, steady fouling, and TMP jump. The sudden jump of TMP was found to be closely related to the sudden increase in the EPS concentration at the bottom of cake layer. Moreover, the likelihood of TMP jump depended on the operating conditions and the incidence was more frequently observed in small-scale experiments. In this research, no obvious fouling was observed during the first 50 days under all four pH conditions. Afterward until termination, the TMP pressure drastically rose at the average rates ($dTMP/dt$) of 0.91, 0.33, 0.31 and 0.64 kPa day⁻¹ for the mixed liquor pH 5.5, 6.5, 7.5 and 8.5, respectively. The membrane fouling was most severe at pH 5.5, followed by pH 8.5, 6.5 and 7.5. Zhang et al. (2014) reported that a decline in pH induced stronger adherence of sludge flocs on the membrane surface and reduced the repulsive energy barrier between sludge and membrane, a phenomenon which facilitates the attachment of foulants. Nonetheless, since this research applied the identical feeding and operating conditions to all four pH conditions, the dissimilarity in fouling characteristics could thus be attributed to changes in the sludge properties induced by variations in the mixed liquor pH. Therefore, the effect of pH levels on mixed liquor properties and the correlation of sludge characteristics to the membrane fouling are next investigated.

Insert Figure 5

The mixed liquor characteristics that contribute to membrane fouling, including the particle size of sludge flocs, unsettled particle in supernatant, zeta potential, carbohydrate and protein contents of SMP and EPS under the four pH conditions, were examined and tabulated in Table 2. As presented in Table 2, the mean floc sizes remained relatively constant between 54-58 μ m for the entire experimental pH range, while the absolute values of zeta potential decreased with pH. The low absolute value of zeta potential at low pH possibly enhanced the adherence capacity of sludge flocs onto the membrane surface, thereby resulting in more severe membrane fouling (Zhang et al., 2014). The unsettled SS in supernatant could be another important factor contributing to membrane fouling (Meng et al., 2006) as these solids are mostly made up of colloid and small particles which can easily penetrate into the membrane pores. The highest concentration of solids in the suspension was at pH 5.5, demonstrating the weak structure of sludge flocs under the acidic condition and thereby more dispersed form of particles. The abundance of unsettled dispersed particles as pH decreased toward 5.5 was associated with the resultant increasing rate of TMP.

SMP and EPS are two other important factors that contribute to membrane fouling in the MBR process (Le-Clech et al., 2006). Table 2 presents the effects of mixed liquor pH on the SMP and EPS composition. Proteins were found dominant in both SMP and EPS for all the entire experimental pH range. Huang et al. (2012) indicated that proteins of different molecular sizes played an important role in the foulant formation and that small MW proteins were responsible for the fouling of large-pore membrane. The highest quantities of proteins

and carbohydrates in SMP occurred under the pH 8.5 and 5.5 conditions, respectively, while the highest concentrations of both proteins and carbohydrates in the extracted EPS were found at pH 5.5. SMP and EPS are typically originated from microbial activity during unstable conditions (Wu et al., 2010) and the pH adjustment in the MBRs of this current research to pH 5.5 apparently contributed to the production of SMP and EPS. Gao et al. (2010) reported that the sludge properties with regard to the contents of proteins and carbohydrates in EPS could be affected by pH shocks. In addition, changes in the sludge properties as a function of pH were confirmed by microscopic observation. The sludge flocs at pH 5.5 exhibit a loose structure vis-à-vis those at the other experimental pH levels. This implied that the higher EPS production under the pH 5.5 condition could cause the outer EPS layer to be loosely bound to the cells. This weak linkage could easily be dissociated under turbulent fluid in the MBR process, which would result in significant cell erosion and subsequently increase in the unsettled particles in the mixed liquor supernatant. The loosely bound EPS weakened the floc structure, resulting in poor bio-flocculation (Li and Yang, 2007).

To determine the main contributing factors to membrane fouling, the correlations between the mixed liquor characteristics and TMP increasing rate at each of the experimental pH levels were analyzed. As presented in Table 2, the unsettled SS ($r_p = 0.810$) and EPS protein ($r_p = 0.862$) were strongly correlated to the TMP increasing rate, while EPS carbohydrate ($r_p = 0.611$), SMP protein ($r_p = 0.63$) and SMP carbohydrate ($r_p = 0.769$) were moderately related to the fouling rate. In addition, the low correlations of the TMP increasing rate to the mean particle size of sludge flocs ($r_p = -0.110$); and to the zeta potential of supernatants ($r_p = 0.218$) were observed.

Insert Table 2

3.4 Effect of mixed liquor pH on foulant characteristics

Table 3 presents the effects of mixed liquor pH on the foulant characteristics in the experimental MBRs. For the entire experimental pH range (pH 5.5–8.5), suspended solids (SS) were the major component of membrane fouling, accounting for 58-68 percent by weight, followed by Ca and Mg (inorganic elements) (16-33 percent) and soluble organic substances (7-15 percent). The proportions of soluble organic substances with respect to DOC, protein and carbohydrate were most abundant in the foulant at pH 5.5, while the SS and inorganic elements were highest in the foulant at pH 8.5. It is of paramount importance to note that the fouling behavior is largely subject to the sludge characteristics. The differences in the membrane foulant composition corresponded to the variations in the mixed liquor characteristics in response to changes in its pH. Thus, the ubiquity of loosely bound EPS at the mixed liquor pH of 5.5 could be attributed to the higher deposition of soluble organic substances on the membrane surface. Furthermore, the higher SS and inorganic contents fractions in the membrane foulant at pH 8.5 could be a result of the chemical precipitation on the membrane surface.

Insert Table 3

To identify the organic composition of membrane foulants, the EEM fluorescent spectra for the four experimental pH levels were analyzed and depicted in Fig.6. For the membrane foulants at pH 5.5, two peaks, one of which represented aromatic protein-like substances (peak A) and the other tryptophan protein-like substances (peak B), could be identified. With increase in the pH level, two additional peaks (peaks C and D), representing humic-like substances, were identified. Moreover, the fluorescence peaks of humic-like substances (peaks C, D) were red-shifted (5-50nm) along the excitation and emission axes, while the fluorescence peak of protein-like substances (peak A) was blue-shifted (5-15nm) along the excitation axis. A red shift was related to an increase in aromatic poly-condensation and a higher degree of humification, while a blue shift was associated with a decomposition of condensed aromatic moieties and the break-up of large molecules into smaller fragments. This observation indicated that the MBR operation at the varying mixed liquor pH levels yielded different organic structures and components in the membrane foulants. Under the mild acidic conditions (i.e. pH 5.5 and 6.5), the membrane foulants were mainly composed of protein-like substances with different molecular structures. However, they were dominated by humic-like substances when the pH level rose above 7.5.

Insert Figure 6

To determine the main contributors to the membrane fouling under the varying experimental pH levels, the components of hydraulic resistance (i.e. R_t , R_c , R_p , R_{ir}) were measured using a resistance-in-series model. In Table 4, the removable fouling resistance (R_c) is the largest contributor (80-90 percent) to the total resistance (R_t), followed by the irremovable fouling resistance (R_p , 9-18 percent) and irreversible fouling resistance (R_{ir} , 0.5-1.5 percent). The findings indicated that R_c caused by the cake layer formed on the membrane surface was mainly responsible for the TMP rise. At pH 5.5, R_t was the highest as a result of R_c , while R_p was highest at pH 8.5. Removable fouling fractions became dominant on the membrane surface at pH 5.5, while the proportion of irremovable foulants increased with increase in pH from 5.5 to 8.5. This phenomenon can be ascribed to the characteristics of sludge suspension and membrane foulants in the MBRs. At pH 5.5, the mixed liquor had the highest amount of EPS, which generated a highly hydrated gel matrix covering on the membrane surface and increased the removable fouling fractions. Cosenza et al. (2013) found a positive relationship between the bound EPS and the specific cake layer resistance. On the other hand, as the mixed liquor pH was increased from 5.5 to 8.5, the chemical precipitation of Ca and Mg was induced. These precipitates could intensify the accumulation on the membrane or penetrate into the membrane pores, thereby resulting in an increase in the irremovable foulants due to the cohesive properties (Meng et al., 2009).

Insert Table 4

4. Conclusions

This research study examined the effects of varying mixed liquor pH levels (i.e. 5.5, 6.5, 7.5 and 8.5) on the biodegradability and fouling behavior in the MBRs for landfill leachate treatment. The experimental results showed that the organic removability under the pH 5.5 and 8.5 conditions was insignificantly different. Nonetheless, a significant increase in inorganic removal was observed with the increase in the mixed liquor pH. In addition, the differences in EEM spectra during the MRB treatment revealed dissimilarity in the transformation of DOM components under the various mixed liquor pH levels. The experiments also showed that variations in the mixed liquor pH between 5.5 and 8.5 had an impact on the microbial community. Moreover, dissimilar proportions of *Proteobacteria* and microbial community diversity between the mixed liquor pH of 5.5 and 8.5 influenced the biodegradability in the MBR tanks, particularly the transformation of DOM. The membrane fouling was found to be most severe at pH 5.5, followed by at pH 8.5, while the fouling at pH 6.5 and 7.5 were quite similar and less severe than that at pH 5.5 and 8.5. The sludge properties were significantly affected in response to changes in the mixed liquor pH. In addition, EPS proteins and the unsettled SS appeared to be positively correlated to the membrane fouling rate. The foulant layer on the membrane surface at pH 5.5 was composed of protein-like substances, whereas humic-like substances and inorganic precipitation were prevalent in the foulant layer at pH 8.5. Chemical cleaning of membranes revealed that the proportion of irremovable foulants increased with increase in the mixed liquor pH.

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Figure 1

Figure 1 Schematic of the experimental MBR system

Table 1

Table 1 Treatment performance of the MBRs at four mixed liquor pH conditions					
Parameter	Influent	Effluent	Effluent	Effluent	Effluent
		at pH= 5.5	at pH= 6.5	at pH= 7.5	at pH= 8.5
pH	5.47±1.0	5.66±0.30	6.61±0.23	7.96±0.10	8.79±0.06
COD ((mg L ⁻¹)	5445±900	-	-	-	-
		104±40	41±2	59 ±11	51±23
BOD (mg L ⁻¹)	3659±476	(98.10%)	(99.25%)	(98.91%)	(99.06%)
		53±8	18±9	23±5	20±10
DOC (mg L ⁻¹)	1154 ±205	(98.55%)	(99.51%)	(99.37%)	(99.48%)
		48±2	26±10	25±2	30±2
UV ₂₅₄ (cm ⁻¹)	4.11±0.09	(95.84%)	(97.75%)	(98.18%)	(97.14%)
		0.79 ±0.10	0.74±0.12	0.74 ±0.05	0.85 ±0.05
SUVA ₂₅₄ (Lmg ⁻¹ m ⁻¹)	0.35±0.06	(80.78%)	(82.00%)	(82.00%)	(79.32%)
		1.70 ±0.04	2.88 ±0.03	2.96±0.01	2.84 ±0.02
		-	-	-	-

Figure 2

Figure 2 Fluorescence EEM spectra of leachate during the MBR operation as a function of mixed liquor pH

Figure 3

Figure 3 The DGGE profiles of PCR amplification of the pH 5.5 and 8.5 MBR sludge samples.

Figure 4

Figure 4 The proportions of bacterial community in the pH 5.5 and 8.5 sludge samples

Figure 5

Figure 5 Changes in trans-membrane pressure (TMP), permeate flux, and specific permeate flux (Flux/TMP) with time (days) under the four experimental pH conditions for an entire period of 150 days. The initial pH were 6.5 in MBR1 and 8.5 in MBR2 during the first phase (days 1-75) and subsequently adjusted to 5.5 and 7.5, respectively, at the start of the second phase (day 76-150).

Table 2**Table 2** The effects of pH on the characteristics of MBR mixed liquor and their correlation to the TMP increasing rate

Floc characteristics	pH=5.5	pH=6.5	pH=7.5	pH=8.5	TMP increasing rate
					r_p
Mean floc size (μm)	55 $\pm$ 2.3	58 $\pm$ 2.1	54 $\pm$ 2.1	55 $\pm$ 2	-0.110
Zeta potential (mV)	-13 $\pm$ 1.8	-14 $\pm$ 1.5	-18 $\pm$ 1.9	-21 $\pm$ 2.0	0.218
Unsettled SS(mg L^{-1})	80 $\pm$ 30	18 $\pm$ 3	20 $\pm$ 2	41 $\pm$ 5	0.810 ^a
SMP- Protein (mg L^{-1})	119.1 $\pm$ 10	99.6 $\pm$ 10	98.8 $\pm$ 4	132.2 $\pm$ 12	0.630 ^a
SMP-Carbohydrate (mg L^{-1})	44.6 $\pm$ 4	24.3 $\pm$ 6	27.0 $\pm$ 5	27.7 $\pm$ 3	0.769 ^a
EPS-Protein (mg gSS^{-1})	68.3 $\pm$ 5	50.4 $\pm$ 3	51.7 $\pm$ 3	53.9 $\pm$ 3	0.862 ^a
EPS-Carbohydrate (mg gSS^{-1})	29.4 $\pm$ 1.2	20.8 $\pm$ 1.2	22.8 $\pm$ 1.1	18.4 $\pm$ 1.1	0.611 ^a

^a Significant at 95percent confidence level.

Note: The mixed liquor samples were collected after 60 days of each pH operation to determine the effect of pH on MBR mixed liquor characteristics and to investigate their correlation with the TMP increasing rate ($dTMP/dt = (TMP_{end} - TMP_{initial}) / TMP \text{ rising time}$). Based on the results after the 60-day treatment, the changes in sludge characteristics could be attributed to the pH variations.

Table 3**Table 3** The characteristics of desorbed membrane foulants as a function of mixed liquor pH

Parameters	pH=5.5	pH=6.5	pH=7.5	pH=8.5
SS (g/m ²)	11.286	6.571	7.429	18.429
DOC (g/m ²)	2.463	0.947	0.917	2.183
Protein (g/m ²)	0.996	0.417	0.368	0.628
Carbohydrate (g/m ²)	0.243	0.077	0.083	0.225
Mg ⁺² (g/m ²)	0.786	0.834	1.500	1.937
Ca ⁺² (g/m ²)	2.000	2.880	2.629	6.320

Figure 6

Figure 6 Fluorescence EEMs of the desorbed membrane foulants as a function of mixed liquor pH.

Table 4**Table 4** Resistances through the membrane fouling layer as a function of mixed liquor pH

	Fouled membrane ($\times 10^{11} \text{ m}^{-1}$)	After water cleaning ($\times 10^{11} \text{ m}^{-1}$)	After chem. cleaning ($\times 10^{11} \text{ m}^{-1}$)	percent R_c (Removable)	percent R_p (Irremovable)	percent R_{ir} (Irreversible)
pH = 5.5	32.60	4.57	1.77	90.44	9.03	0.53
pH = 6.5	12.00	2.72	1.74	89.11	9.58	1.31
pH = 7.5	13.50	3.65	1.78	82.82	15.66	1.52
pH = 8.5	21.40	5.42	1.78	80.69	18.40	0.91

Note: Removable fouling resistance (R_c) refers to the foulants that can be removed by physical cleaning; irremovable fouling resistance (R_p) refers to the foulants that can be removed by chemical cleaning; and irreversible fouling resistance (R_{ir}) refers to the foulants which cannot be removed by any method including chemical cleaning.