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Significant role of membrane fouling and microbial community on the performance of membrane bioreactor (MBR) system

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A B S T R A C T

The performance of a lab-scale membrane bioreactor (MBR) was evaluated based on its microbial community as well as the mechanism of membrane fouling. The growth of unwanted microorganisms in the system was observed, affecting effluent water quality. The MBR achieved a total-nitrogen removal of 56%. Nitrification and denitrification processes were most likely affected by the presence of *Thiosphaera pantotropha*, a bacterium capable of nitrification and denitrification processes. Phosphorus removal of 51% was attained, which is comparably lower than other existing systems. Furthermore, a chemical oxygen demand removal of 98% indicated the presence of active microbial community. However, this does not guarantee the existence of the target microorganisms. It was found that the increase in transmembrane pressure was caused by sludge-cake resistance rather than membrane-pore fouling. This conclusion was made by analyzing particle-size distribution and quantifying extracellular polymeric substances particularly soluble microbial products. The fouling mechanism was investigated by analyzing the membrane surface image generated by confocal laser scanning microscopy and scanning electron microscopy, and confirmed that MBR sludge were easily removed by physical cleaning. Hindering the growth of undesirable organism and controlling the concentration of soluble microbial product, which causes severe fouling, could lead to an efficient performance of MBR system.

Keywords: Membrane bioreactor; Membrane fouling; Polymeric substances; Nutrient removal; *Thiosphaera*

1. Introduction

The activated sludge process (ASP) is often used for biological treatment of municipal and industrial wastewaters. But due to some disadvantages accompanied by this process such as difficulty in solid–liquid separation, large surface area requirement and high production of excess sludge, membrane bioreactors (MBRs) have gained much attention in the field of wastewater treatment [1,2]. Advantages of MBR process include higher effluent quality, lesser sludge production, higher sludge age, more compact structure

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Table 1
Operating condition of MBR system

Parameter	Target value
Hydraulic retention time (h)	7
Aeration intensity (L/min)	2
Total filtration volume (L/day)	18
Returned sludge (%Inf. flow rate)	
Anoxic reactor 1	100
Anoxic reactor 2	100
Permeate flux (m/d)	0.3
Intermittent suction time: On/Off (min)	9/1

and better performance for nitrification and denitrification [3,4].

Despite the aforementioned advantages of MBR, it is still facing several challenges that restrain some wastewater treatment plants to entertain such new technology. Some of the problems accompanied with the MBR technology include membrane fouling, high membrane cost and pretreatment, greater process complexity, higher aeration requirement, production of less dewaterable sludge and greater sensitivity to shock load [5]. Although MBR has several disadvantages, optimization of the process has proven that MBR is better than the conventional wastewater treatment processes [1,6]. In addition, Judd [7] mentioned various factors that influenced the membrane technology to penetrate the global market. Some of the drivers considered for the implementation of MBR technology include new and more stringent legislation for effluent discharge, local water scarcity, introduction of state incentives, decreasing investment costs, and increasing acceptance of MBR technology. Therefore, a much comprehensive study should be done to find solutions to these problems, especially membrane fouling and effluent quality. In addition, identification of the active microbial community of denitrifying bacteria could provide insights on improving nitrogen removal as well as degradation of other organic pollutants in wastewater by controlling the dominant groups.

This study investigated the role of microbial community of denitrifying bacteria on the effluent quality as well as the effect of membrane fouling on the decrease of filtration flux. Visualization of the fouling mechanism on the membrane surface was also conducted. These factors are considerably essential in the optimization of the performance of an MBR system.

2. Materials and methods

2.1. Experimental set-up

The lab-scale MBR was fabricated and patterned after the design of the pilot plant MBR built in Gapyeong, South Korea. The system is consisted of four reactors. The first two reactors are anoxic with a returned sludge 100% of influent flowrate. The third reactor having an anoxic condition favors the denitrification process. The last reactor is aerated to create an oxic condition for nitrification process. The microbial growth inside the reactors is mainly dependent on the operating condition of the system. MBR operating condition and design is presented in Table 1 and Fig. 1, respectively.

2.2. Membrane and synthetic wastewater

PVDF flat-sheet membrane (TORAY, Japan) with a pore size of 0.08 μm and an effective filtration area of 0.03 m^2 was used in the experiment. Two membrane modules were submerged in the oxic reactor and continuously aerated.

Glucose and bicarbonate were incorporated in the synthetic wastewater to provide organic and inorganic carbon source, respectively, for the target microorganisms to consume. Total nitrogen (TN) concentration of 80 ppm was obtained by using ammonium bicarbonate as N-source, while potassium dihydrogen phosphate

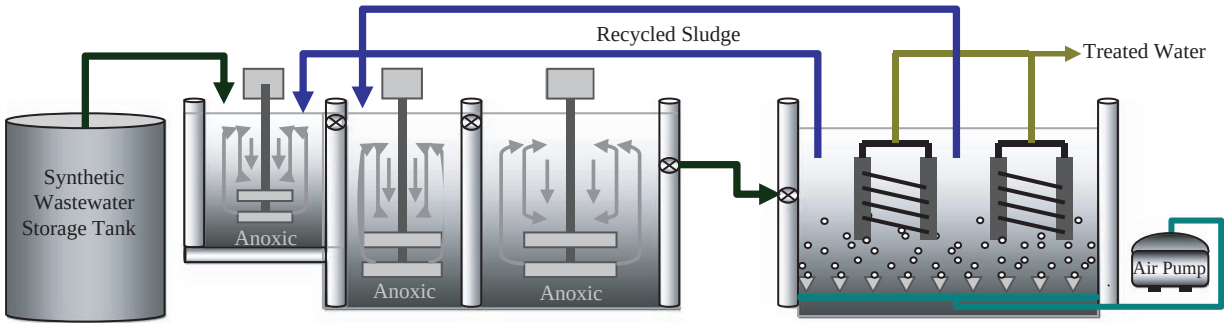

Fig. 1. Schematic of lab-scale MBR System composed of three anoxic reactors (anoxic-1, anoxic-2 and anoxic-3) and one aerobic reactor.

Table 2
Composition of formulated synthetic wastewater

Reagent	Concentration (mg/L)
Glucose	300.00
$NaHCO_3$	300.00
NH_4HCO_3	80.00
KH_2PO_4	5.80
$MgSO_4{\cdot}7H_2O$	50.00
$MnSO_4{\cdot}H_2O$	0.03
$ZnSO_4{\cdot}7H_2O$	0.04
$CaCL_2{\cdot}2H_2O$	10.00
$FeCl_2{\cdot}4H_2O$	0.32
Yeast extract	0.05

was used as P-source to attain a total phosphorus (TP) concentration of 5.8 ppm. Composition of synthetic wastewater is summarized in Table 2.

2.3. Experimental procedure

Two independent studies were conducted to evaluate the effect of microbial community and membrane fouling on MBR performance.

Sludge was first acclimatized for a month inside the membrane bioreactor. Daily monitoring of the effluent quality was done immediately after acclimatization. Sufficient oxygen was supplied in the oxic reactor to maintain microbial activity inside the reactor. Dissolved oxygen (DO) concentration in the anoxic reactors was maintained below 0.5 ppm while in the aerobic reactor, an average concentration of 5 ppm was observed. Temperature of the system was recorded and found to be in the range of 20–23°C. The pH inside the reactor was kept in the range of 7–8 by the addition of sodium bicarbonate. After a month of monitoring, effluent quality was evaluated by analyzing the removal rate of TN, TP and chemical oxygen demand (COD). In connection with this, investigation of the microbial community present in the MBR system was examined to further explain the data gathered during effluent monitoring.

Mechanism of membrane fouling by MBR sludge was investigated by examining the membrane surface using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) analyses. Two- and three-dimensional images were captured by CLSM while visualization of the membrane surface with a magnification of 10 K was done using SEM. Effect of operating flux to the increase of transmembrane pressure (TMP), which was caused by foulants adhered in the membrane during filtration process, was also studied. To further explore the tendency of MBR sludge to cause membrane fouling, particle size distribution and the concentration of extracellular polymeric substances (EPS) of the sludge were also quantified.

2.4. Analytical methods

TN and TP as well as COD of the effluent water were obtained using HACH DR-2800. Protein and carbohydrate contents of EPS and soluble microbial product (SMP) were extracted using modified steaming extraction [8] and method used by Nuengjamnong [9], respectively. Bradford protein assay provided by Bio-Rad Laboratory, Inc. was used in quantifying protein while Anthrone method [10] was employed in measuring carbohydrate concentration.

The total bacterial genomic DNA of activated sludge sample was extracted as protocoled in CoreBio™ Bacterial Genomic DNA extraction kit. Amplification of Cu-containing nitrite reductase genes was made using Eppendorf Thermocycler with the primer pair F1aCu (5′ATC ATG GT(C/G) CTG CCG CG3′) and R3Cu (5′GCC TCG ATC AG(A/G) TTG TGG TT) which corresponds to positions 568–584 and 1021–1040, respectively, in the *nir*K gene of *Alcaligenes faecalis* S-6 (D13155).

Specific resistances of the fouled membrane were determined by conducting dead-end filtration experiment using Amicon Cell (Amicon™) [11,12]. Initial step was to perform a water flux filtration experiment where you can get the value of the membrane resistance. This was followed by filtration of the sludge sample to obtain the total resistance. The fouled membrane was then washed with dI water to remove the foulants adhered in the membrane surface. Finally, the washed membrane was subjected again to water flux filtration where fouling resistance caused by pore blocking can be obtained. Cake resistance was calculated by subtracting the pore and membrane resistances to the total resistance.

Equipment used for SEM and CLSM analyses were Hitachi S-3500 and Olympus OLS 3000, respectively. Particle size distribution (PSD) was analyzed by Particle Size Analyzer - Mastersizer S. The pressure across the membrane was monitored using SMC Digital Pressure Switch with data collection from LabVIEW 8.5 program (National Instrument, Co.).

3. Results and discussion

3.1. Effect of microbial community on effluent quality

One of the essential factors to be considered in attaining high effluent quality is the microbial community living in the MBR system. Primary consideration

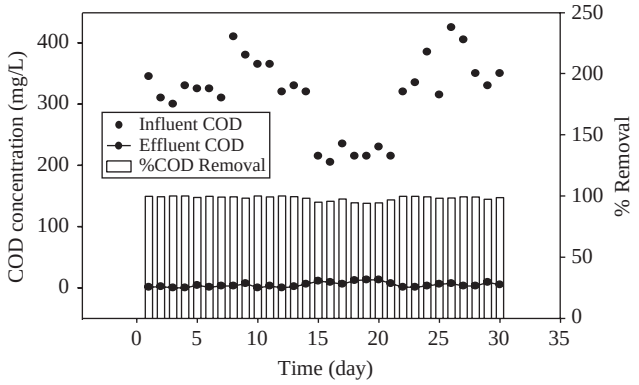

Fig. 2. Reduction in COD concentration (left side) as well as the % COD removal (right side) in the MBR system.

in evaluating a good quality effluent is having low TN and TP content. Thus, growth of the microbes responsible for nitrogen and phoshorus removal should be favored.

Decrease in the concentration of COD, TN and TP is presented in Figs. 2, 3 and 4, respectively. The actual influent concentration was measured from synthetic wastewater fed to the reactor, while the effluent concentration was determined using the permeate obtained during membrane filtration. Values of the influent and effluent concentrations are reflected on the left portion of the graph.

Assessment of an active microbial activity in the MBR system was done by measuring the decrease in COD concentration correlating to the bacterial consumption of biodegradable and nonbiodegrdable organic materials [13]. An influent COD concentration of 300 ppm was fed to the reactor to provide food for the target microorganisms. Based on the results of COD removal, an average percent removal of 98% was attained as shown at the right side of Fig. 2. This indicated that microorganisms utilized the influent COD for their

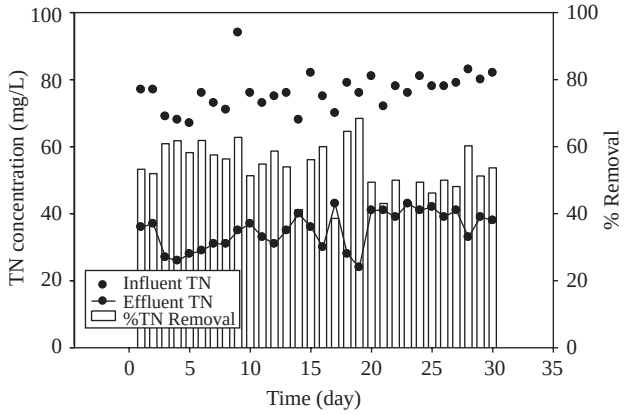

Fig. 3. Reduction in TN concentration (left side) and % TN removal (right side) in the MBR system.

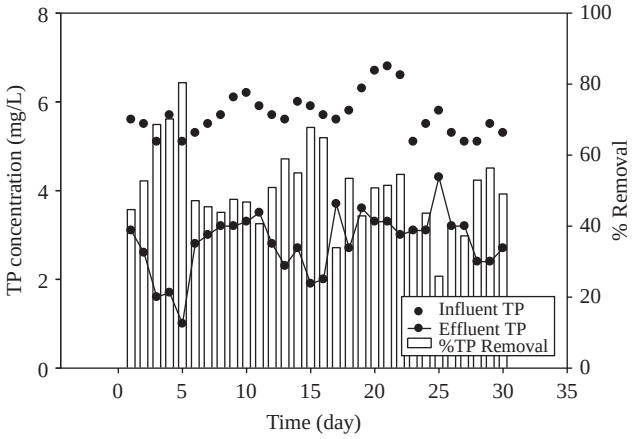

Fig. 4. Reduction in TP concentration (left side) and % TP removal (right side) in the MBR system.

growth. The glucose contained in the influent provides the organic carbon source for heterotrophic bacteria while bicarbonate supplies of inorganic carbon for autotrophic bacteria in the MBR system besides atmospheric CO_2. Sufficient consumption and utilization of these organics by microorganisms inside the reactor resulted to the efficient removal of COD concentration. The decrease in COD concentration from the initial concentration of 300 ppm to the final concentration ranging from 0 to 13 ppm is presented at the left side of Fig. 2.

In the MBR system, the bottom part of the of the aerobic reactor was improperly mixed, in addition to the dead zones located at the sides of the reactor. Some deposition of sludge can be seen in the oxic reactor due to the low aeration rate of 2 L/min. The growth of aerobic nitrifiers may been slowed down resulting to low TN removal in the aerobic reactor.

As seen in Fig. 3, removal rate of TN is fluctuating ranging from 49% to 71%. The influent TN concentration of 80 ppm was reduced to a concentration range of 26–43 ppm. An average percent removal of 56% was obtained, which is lower compared with those existing MBR systems designed for TN removal [14,15]. This might probabaly be due to the incomplete nitrification in the oxic reactor that is likely attributed to the slow growth rate of nitrifiers as affected by the presence of anaerated portion inside the reactor. The number of aerobic nitrifiers cannot handle the large amount of influent ammonia of 80 ppm that should be converted to nitrate, hence, the removal rate for nitrogen was lowered. To address this problem, an efficient air supply, through obtaining the optimum air flowrate, should be provided to ensure an effective circulation of sludge inside the reactor. In doing so, strict aerobic nitrifiers can dominate the oxic reactor resulting to a more efficient nitrification.

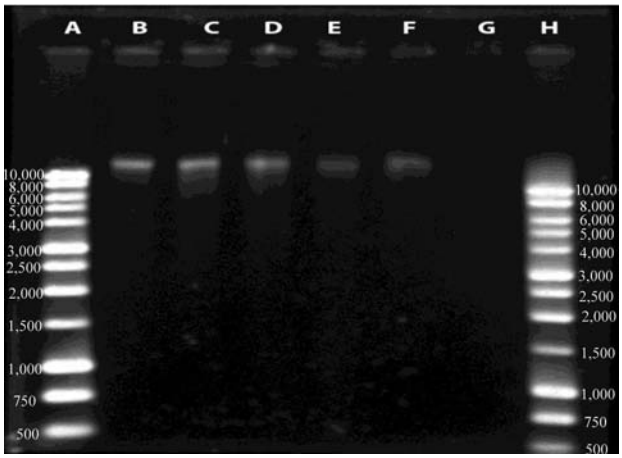

Fig. 5. Electrophoretogram of bacterial community DNA from activated sludge in MBR system. A and H. Promega's 1 kb DNA ladder, B. oxic zone, C. anoxic-1, D. anoxic-2, E. anoxic-3, F. positive control (DNA from unknown bacterial isolate from activated sludge) and G. negative control.

On the average, 51% phosphorus removal was achieved in the experiment as shown in Fig. 4. This reduction in TP concentration is attributed to the possible presence of both ordinary heterotrophic organisms and phosphorus accumulating organisms (PAO). It was reported that the normal value of P incorporated in sludge mass is just approximately 0.02 mg P/mg volatile suspended solids (VSS). However, in the presence of PAO, an additional 0.38 mg P/mg VSS could be removed [11]. The initial TP concentration of 5.8 ppm was decreased to a range of 1.0–4.3 ppm. The removal rate can be further increased by stimulating the growth of PAO, creating a larger portion in the activated sludge. Increasing the amount of PAO relative to the ordinary heterotrophic organisms can increase the capacity for P accumulation; hence, increasing the removal effieciency. Comparing the removal rate obtained from other systems for TP removal [14,15,17], the %TP removal attained was lower. Thus, it was speculated that the decrease in TP concentration was affected by sludge wasting per day rather than the microorganism capability of phosphorus uptake. It was concluded that the number of PAOs in the system was not enough to incorporate the excess phosphorus inside the reactor, thus enhancing the growth of these organisms should be given emphasis. Wentzel [16] mentioned that increasing the influent biodegradable COD for the PAOs to consume will increase the fraction of PAO in the sludge. In addition, it was hypothesized that low TP removal was also affected by PAOs outcompeted by autotrophic nitrifiers, which consumes biodegradable COD.

Selection of the sludge age is one of the most fundamental and important decision in the design of an activated sludge system. Long sludge age of 50 d was chosen due to the fact that long sludge retention time (SRT) can efficiently remove biological N and P as well as COD, and that it also produces very high effluent quality in a very stable condition [18]. One of the advantages of long SRT is the low production of sludge, which in turn decreases the sludge handling cost. But some of its loopholes are having high oxygen demand, large reactor needed, and high capital cost. However, deciding for long sludge age was made due to the aim of improving the efficiency of simultaneous removal of N and P. However, as presented in the results for TN and TP removal, an inefficient reduction of N and P were obtained. Thus, to better understand this phenomenon occurring inside the MBR system, the microbial diversity present in the reactors was explored.

Successful extraction of the total bacterial genomic DNA was confirmed by agarose gel electrophoresis using 0.9% agarose gel, run at 100 V electric current for 15 min. The gel was pre-stained with Lonza® nucleic acid stain to visualize DNA bands. Fig. 5 shows the electrophoretogram of the total bacterial genomic DNA extracted from the different zones of MBR system.

Fig. 6 shows the electrophoretogram of amplified Cu-containing nitrite reductase genes (nir*K*) from the community of activated sludge denitrifying bacteria. The successful amplification of nir*K* genes in the oxic and anoxic zones of the MBR system may have indicated the presence of active community of denitrifying bacteria harboring this functional gene [19]. However, the detection of nir*K* genes in the oxic zone could depict the possible effect of recirculation on the denitrifying

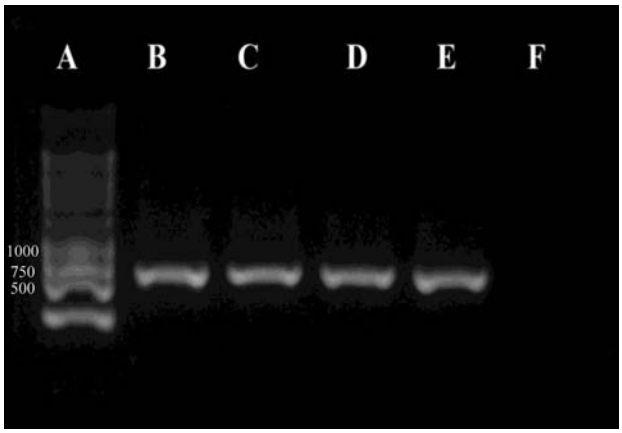

Fig. 6. Electrophoretogram of amplified *nir*K gene from the community of denitrifying bacteria in MBR system. A. Promega's 1 kb DNA ladder, B. oxic zone, C. anoxic-1, D. anoxic-2, E. anoxic-3, and F. negative control. The amplicons were approximately 500bp in size.

community of the MBR system. It is likely that during recirculation denitrifying bacteria are introduced from the anoxic bioreactor towards the oxic bioreactor. Since some anoxic condition may have been occurring in non-aerated zones, these denitrifying bacteria could grow and contribute to the loss of total nitrogen.

The presence of aerobic variant of denitrification also may have indicated the presence of aerobic variant of denitrification. Generally, the expression of nitrite reductase is triggered in the cell by low oxygen tension.

A representative of this type is the genus *Thiosphaera pantotropha* which belongs to the genus *Paracoccus* as a strain of *Paracoccus denitrficans* on the basis of 16S rRNA and high phenotypic similarity. Aerobic denitrification results when activation of the denitrification genes occurs at high oxygen level. Regulatory control usually operates in favor of channeling electrons towards aerobic respiration. However, no fundamental aspect has been uncovered to make aerobic variant a different type of denitrification and it is not a question of oxygen sensitivity of nitrite reductases but rather of regulation at the enzyme and genetic level [20].

During the operation, significantly high mixed liquor suspended solids (MLSS) had been observed. From the results of PCR analysis of nir*K* genes of the community of denitrifying bacteria, it was assumed that the existence of heterotrophic biomass, in which *Thiosphaera sp*. is a good representative, may have been contributing to the observed high MLSS in aerobic bioreactor. Successful isolation of *Thiosphaera sp*. on *Thiosphaera* Agar was made.

3.2. Membrane fouling in MBR system

The main focus of most researchers today is the adverse effect of polymeric substances in the performance efficiency of MBR system [21,22]. EPS are subdivided into bound-EPS and SMP. The soluble EPS in the bulk phase is believed to cause severe fouling during membrane filtration [23]. Thus, quantification of the primary constituent of EPS, namely proteins and carbohydrates, was measured. Based on the results obtained, concentrations of protein and carbohydrates in the bulk phase were 54.1 mg/L IgG standard and 3.2 mg/L glucose standard, respectively. To further investigate the potential of soluble EPS for membrane fouling, comparison with the concentration of bound-EPS was done. It was found that the concentration of protein, with a value of 26.7 mg/L IgG standard, incorporated in the particle sludge was lower than the concentration in SMP. In contrast with the carbohydrate concentration obtained, 5.2 mg/L glucose standard, which was higher than in SMP. However, the total concentration of bound-EPS and SMP is what that matters. Total SMP concentration of 57.4 mg/L was observed to be almost twice as high as the total concentration of bound-EPS with 31.9 mg/L that signifies that the majority of EPS was allocated to the soluble portion, which is a potential source of membrane fouling.

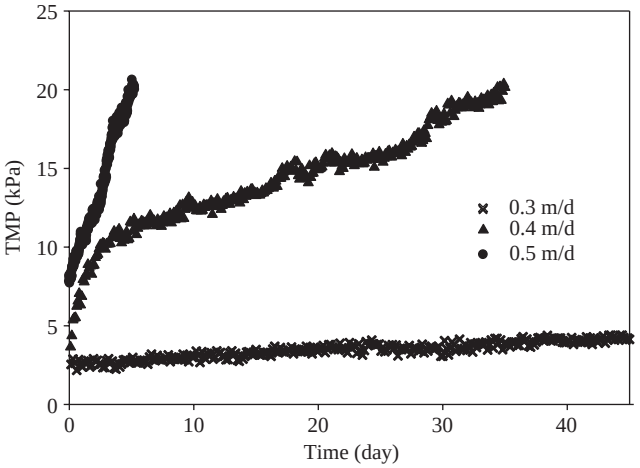

Fig. 7. Variation in TMP at various operating flux.

Another factor that should be considered in minimizing membrane fouling is choosing the suitable operating flux. For the purpose of observing the variation in TMP increase among different filtration flux, the maximum allowable TMP was fixed at 20 kPa. As the flux increases, the length of time to reach the allowable set TMP decreases as shown in Fig. 7. The flux of 0.3 m/d achieved the longest operating time without significant increase in TMP. The suction strength for the other two fluxes, 0.4 and 0.5 m/d, were significantly stronger, thus more particles were adhered in the membrane surface producing thicker film, as compared with the system with the flux of 0.3 m/d. However, operating at lower flux could yield lower filtration volume, which is undesirable for most wastewater treatment plants. But this could be compensated by prolonging the operation time without membrane cleaning, thus reducing the operating cost [24,25].

Consideration in operating at low flux is basically due to the foulants present in the system which considered to the problem in MBR system. These microbial products are produced during cell lysis and bacterial metabolism, and portion of these are distributed in the bulk phase. It was observed that SMP plays a major role in membrane fouling particularly in pore blocking. They are accumulated on the membrane surface and are not easily removed. That these substances are a major membrane foulants reported elsewhere [26,27].

Dead-end filtration experiment was conducted to determine the total resistance during membrane

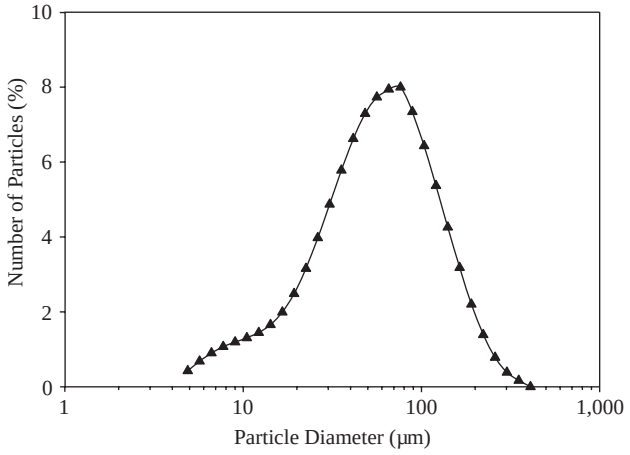

Fig. 8. Specific resistances obtained in dead-end filtration experiment using MBR sludge (Rp – pore fouling resistance; R_c – cake resistance; R_m – intrinsic membrane resistance).

filtration. As illustrated in Fig. 8, the cake resistance was the primary factor that causes membrane fouling, which acquired 94.5% of the total resistance. Pore fouling of 2.4% was incomparable with cake layer suggesting that the foulants were mostly reversible and could be easily removed.

Sludge particles preferred accumulating in the surface of the membrane rather than passing through the membrane pores. This is due to the nature of the MBR sludge that was used which has a greater potential to form thin layer in the membrane surface. The resistance that the pore fouling contributed to the total resistance was due to the undersized polymeric substances that passed through the pores of the membrane. Even though the size of these substances varies, some portions have the capability of pore blocking.

To further investigate the characteristic of MBR sludge, the size distribution of the particles in the reactor was analyzed. PSD experiment predicts the size of the particulates and their distribution in a given sample. According to the results obtained, the mean diameter of the sample sludge was 53.48 μm, which is much bigger than the 0.08 μm pore size of the membrane. In addition, at the lowest size used in the PSD experiment (4.19 μm), only 0.43% passed through. This supports the results obtained in the dead-end filtration experiment where pore fouling having the lowest resistance obtained among others was almost incomparable. To visualize the size distribution of MBR sludge, PSD results was illustrated in Fig. 9. The vertical axis represents the amount of particles present in the sample at specific particle diameter. It can be observed in the graph that a large portion of the particle sludge was allocated in the left side, having smaller particle size. Several studies suggests that further breakdown of these particles occur during MBR operation due to shear force arising from pumping and aeration [1]. Due to this break-up, it generated fine colloids and cells forming a thin layer on the membrane surface. Biological flocs break-up can produce particle size with approximately 2 μm. This phenomenon could not contribute in pore blocking because the resulting break-up size was still not enough to pass through the membrane pores, thus, the membrane fouling was not mainly affected by pore fouling. However, during break-up, EPS present inside the flocs was released to the bulk phase that contributed to 2.4% pore fouling.

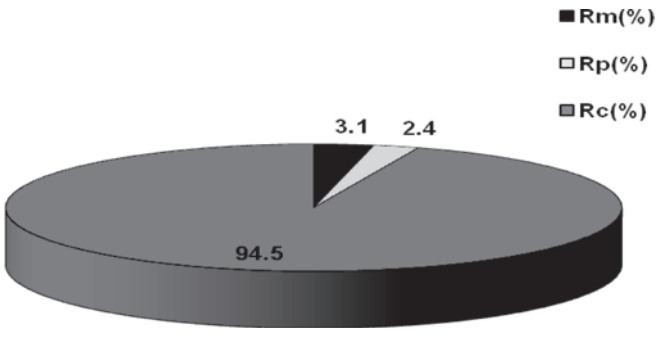

Fig. 9. Particle size distribution of MBR sludge.

In Fig. 10, 2D and 3D images of the membrane surface were presented. The 2D images show that the majority of the particles adhered in the membrane was almost removed after washing. It was further confirmed in the 3D images that the foulants were roughly washed out. This generalization was evidently seen in the smooth surface of the membrane obtained after washing (Fig. 10D).

To further support the generalization made in CLSM analysis, surface of the fouled membrane was examined using SEM analysis. SEM images revealed that the foulants adhered on the membrane surface were almost removed after membrane cleaning (physical washing), which is clearly seen in Fig. 11b. The new membrane was compared to fouled membrane (after washing) to examine the possibility of pore blocking during membrane filtration.

As shown in Fig. 11, the porosity of the fouled membrane was slightly reduced. This can be attributed to the pore blocking obtained in the dead-end filtration experiment. Even though decrease in porosity was observed, the change between new membrane and fouled membrane was minimal and almost incomparable.

4. Conclusions

Microbial community and membrane fouling are two essential parameters to be considered in MBR

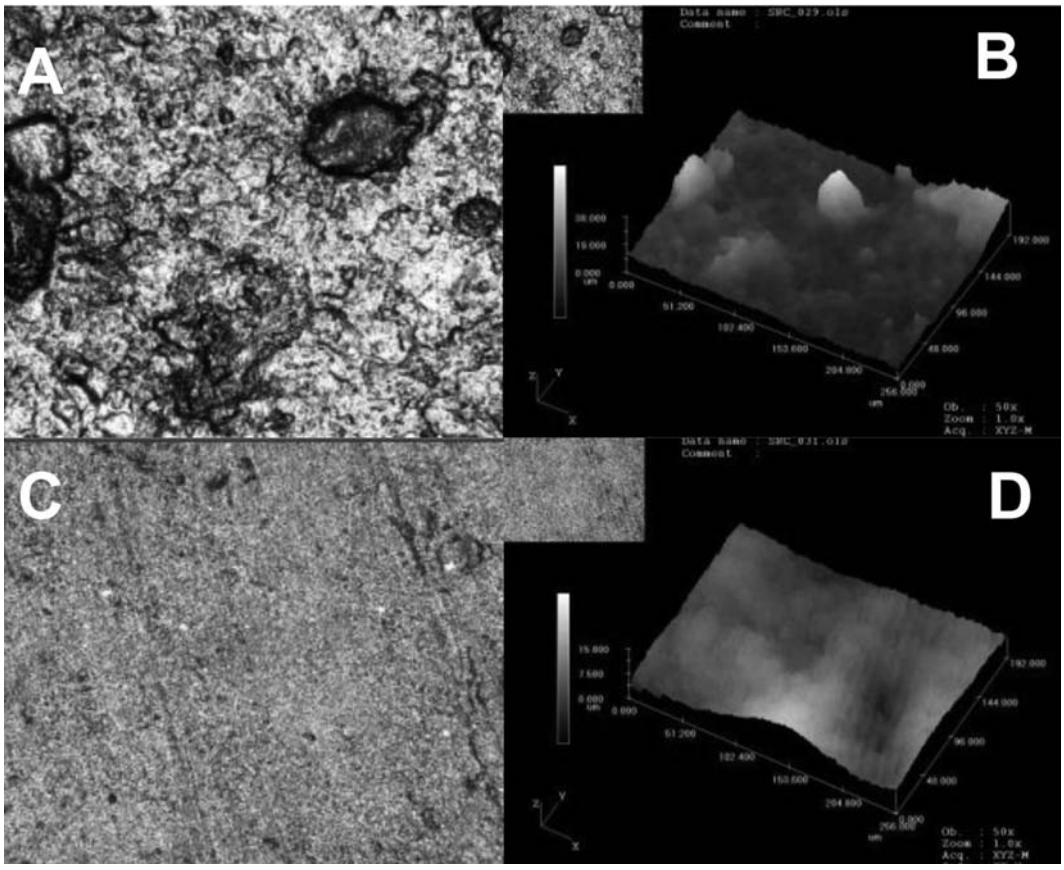

Fig. 10. Two- and three-dimensional CLSM images of the membrane surface: A. fouled membrane (2D), B. fouled membrane (3D), C. washed membrane (2D), D. washed membrane (3D).

system. Presence of the target microbes that are capable of lowering the concentration of TN and TP should be given emphasis to ensure high removal efficiency. Knowing the potential of polymeric substances to membrane fouling could serve as a caution and guide in improving the performance of MBR system.

Thiosphaera, a heterotrophic bacterium, is said to have characteristic of performing both nitrification and denitrification. The bacterium was proven to exist inside the MBR reactor, which probably caused the decrease in TN and TP removal. Even though high percent removal of COD was observed, which is an index of an active microbial community, this does not assure that the target microorganisms were the ones responsible of reducing influent COD concentration. Presence of undesirable bacteria, such as *Thiosphaera*, in the system could hinder the growth of the target microorganisms resulting to inefficient nutrient removal.

Operating at lower filtration flux could minimize the accumulation of EPS, particularly SMP, on the membrane surface that could enhance the membrane filtration efficiency, and consequently prolong the life span of the membrane due to the infrequent membrane cleaning. In addition, the tendency of increasing membrane fouling could be attributed to the amount of EPS present in the bulk phase that could cause severe fouling. However, application of membrane with small pore size could decrease the probability of pore blocking, which is mainly caused by the polymeric substances released during microbial degradation.

Prevention of the growth of the unwanted microorganisms and minimization of membrane fouling could be the factor for the optimization of the MBR design. Awareness of the possible effect of microbial community and membrane fouling on the performance of MBR system should be given importance.

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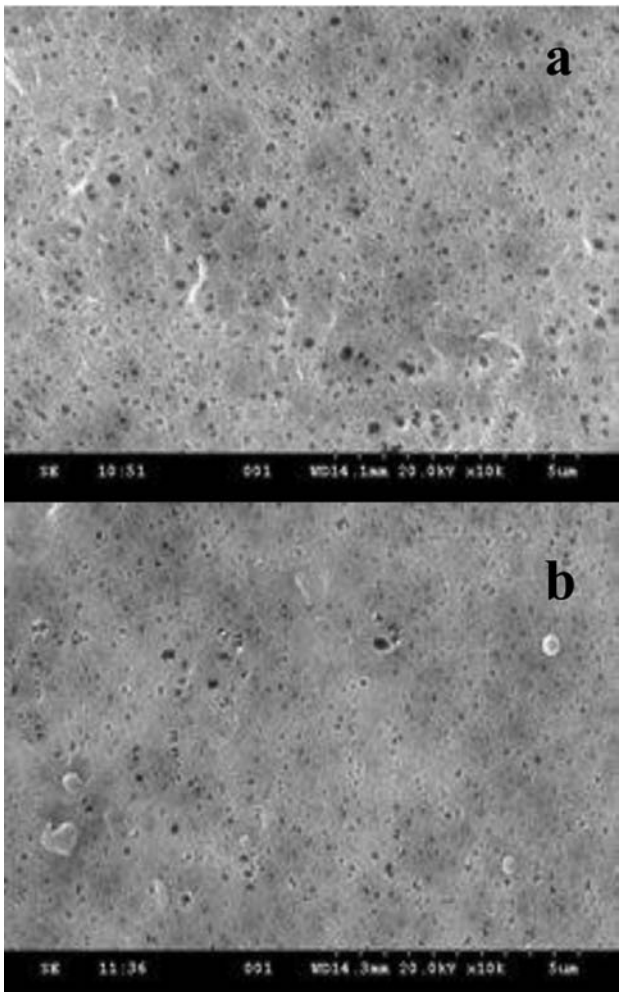

Fig. 11. SEM images of the membrane surface: membrane (2D), B. fouled membrane (3D), C. washed membrane (2D), D. washed (a) new membrane, (b) fouled membrane (after membrane (3D) washing).

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