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Performance enhancement of MBR operated with aerobic granules on membrane filterability improvement

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ABSTRACT

Due to the classic pitfalls of activated sludge processes and increasingly stringent water quality requirements, leading to progressively tighter limits on BOD and nutrient discharge, there is a need to remediate and manage our water resources more efficiently and in a more cost-effective and sustainable manner. Although membrane bioreactor (MBR) has been considered as a technology that guarantees a relatively small footprint and high water quality, they are still susceptible to membrane fouling. Membrane fouling in MBRs is mainly caused by the accumulation of microbial substances, such as extracellular polymeric substances and soluble microbial substances on or in the membrane. Here, an aerobic granule is suggested as a solution to reduce membrane fouling; accordingly, a compact MBR with aerobic granules was studied in an attempt to improve the quality of effluent related to activated sludge processes. Even though various granular sizes were formed, the granule sizes were from 0.1 ± 0.15 to 0.5 ± 0.25 mm, rarely exceeding 0.75 mm.

Keywords: Aerobic granules; Extracellular polymeric substance (EPS); Fouling; Membrane bioreactor (MBR); Soluble microbial substances (SMP)

1. Introduction

Globally, since the amount of potable water available for human use is becoming limited, changes in the hydrological resource base have potentially significant effects on environmental quality, economic development and human welfare. As such, water has to be considered as a limited natural resource in the twenty-first century, of which the most obvious symptom is that 1.1 billion people lack access to improved water supply sources [1]. To this end, climate change caused by global warming is one of the pressures facing water resources [2]; it has been observed that some parts of the world are characterized by an arid to semi-arid climate due to a significant reduction in precipitation (e.g. the annual rainfall in Jordan ranges

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from 50 to 600 mm [3]). In addition, population growth is a fundamental barrier to the sustainable use of water resources [4], especially as the world population is forecast to reach 9 billion by 2050 [5]. With such a high population growth rate and intensive anthropogenic activities, a steep increase in water consumption-resulting in a stress on water supplies, and greater amounts of wastewater production—the global demand for potable water is out of balance. For this reason, wastewater should be treated before it is returned to lakes, rivers and estuaries. As such, since biological wastewater treatment is the most promising and versatile approach being studied to meet economic demands and effectively treat biological oxygen demand (BOD) and nutrients (mainly nitrogen and from municipal and phosphorus) industrial discharges; a large amount of research on biological pollutant removal processes has been carried out.

Recent developments in compact bioreactors with very high volumetric conversion capacities have become a focus in the field of biological wastewater treatment. In order to meet economic demands and effectively treat BOD and nutrients from municipal and industrial discharges, the combination of a membrane and bioreactor (membrane bioreactor [MBR]) has gained considerable attention due to the following advantages: complete solids removal, significant physical disinfection capability, superior organic and nutrient removals and a small footprint. In addition, by simply adjusting the programmable logic control settings (i.e. change of cycle times and flow rates) various biological reactions (i.e. aerobic, anaerobic or anoxic conditions) can be switched to encourage the growth of desirable micro-organisms [6]. The resulting microconsortia not only provide for the sequential degradation of xenobiotics due to their co-metabolic activity, but they also have the flexibility to withstand fluctuating loading rates and to increase the volumetric conversion capacity. However, membrane fouling in MBRs, which increases the operational cost, limits their usage.

According to literature, membrane fouling is a ubiquitous phenomenon in MBRs, mainly caused by microbial substances such as extracellular polymeric substances (EPS) and soluble microbial substances (SMP) [7]. Accordingly, considerable efforts have focused on the development of an advanced MBR that can reduce the flux decrease resulting from membrane fouling. Indeed, our early research indicated that biomass in a sequential batch reactor (SBR) produces settling granules, which facilitate a good solid–liquid separation and the accumulation of high amounts of active biomass [8]. Granulation is well documented in anaerobic processes, as in the upflow anaerobic sludge blanket (UASB); however, it has been recently reported that aerobic micro-organisms could also be self-immobilized without a carrier and then form compact granules [9]. Note that the operation conditions for forming aerobic granules are very different from those of anaerobic granules due to the differences in aeration.

Other studies have attempted to develop an algorithm to optimize the cycle length to a short fill period, and thereby create a feast-famine in the formation of aerobic granules in order to minimize effluent organic carbon and nutrient concentration in a SBR. Although information pertaining to the formation of granules under aerobic conditions is still subject to discussion [10-12], it has been suggested that suspended cells or non-settling flocs interact with the negatively charged biopolymers in activated sludge to create the accumulated floc-like sludge; they then sequentially aggregate as various types of granules, according to the physicochemical conditions of the reactor. The use of aerobic granules is a new and very promising approach for overcoming the conventional drawbacks of MBRs because they have the same properties as the biofilm. In addition, MBRs with an aerobic granules process incur lower membrane biofouling and have excellent membrane permeability. As such, it can be expected that MBRs with aerobic granules may be one of the technologies most suitable for simultaneously removing BOD and nutrients in wastewater, while saving space and operating at a higher rate than conventional biofloc methods. In spite of the merits of MBR with aerobic granules, however, only a few studies on the elimination of organic and nitrogenous compounds in wastewater have been reported [13-16].

Therefore, the combination of MBR and SBR with aerobic granules is studied here in attempt to achieve effective, one-step biological pollutant removal from wastewater treatment plants for water reuse and reclamation.

2. Materials and methods

2.1. Aerobic granule sludge

In order to make aerobic granular sludge, seeding sludge was fed with the activated sludge taken from a municipal wastewater treatment plant. The characteristics of the activated sludge were 5,000 MLSS mg/L, pH 7.6 and a 170 mL/g sludge volume index (SVI). In order to choose the most effective metal ion for aerobic granulation, mono and divalent metal ions such as K^+ , Na^+ , Mg^{2+} and Ca^{2+} were added to each chamber of a jar-tester (PHIPPS & BIRD, Richmond, VA 23228) at the beginning of each experiment. Various

Table 1

concentrations (0, 250, 750 and 1,250 mg/L) of each cation were then added to the jar-tester, where the pure seeding sludge was mixed at 60 rpm for 12 h for adjustment; the reactor was thermostatically maintained at 25°C.

2.2. Reactor operation

Fig. 1 shows the laboratory scale MBR–SBR (M-SBR) reactor, with an effective volume of 18 L. The hollow fibre membrane ($0.4 \,\mu$ m pore size; hydrophilic polyethylene) module was potted with epoxy and then submerged in a direction perpendicular to the airflow. The airflow rate was kept at 10 L/min. The electric controls of the time box in each phase are shown in Table 1.

Table 1 states the control settings for each phase in terms of nitrogen removal and reduced membrane fouling; Table 2 shows the experimental sections for the step feed operation as organic loading rate (OLR) and aeration fill and anoxic fill times. Note the following conditions: one cycle was 8 h in this system, the hydraulic retention time was 1 d, the solid retention time was controlled to 30 d and the temperature was maintained at 20°C. Table 3 presents the components of the synthetic wastewater, where glucose was used as the carbon source and the C:N:P ratio was 100:10:1.2.

2.3. Filtration test of M-SBR sludge

For the different sludge conditions in the M-SBR, gravitational filtration tests were conducted; the gravitational filtration apparatus was described by Jang et al. (2006). In the filtration test, membrane

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Time	controls	of M-S	SBR pl	hases

Timer	Phase	Lane 1 (feed)	Lane 2 (air)	Lane 3 (level sensor and suction pump)
T1	Aeration Fill (AF-feed)	ON	ON	OFF
T2	Aeration (AE)	OFF	ON	OFF
T3	Anoxic Fill (AX-feed)	ON	OFF	OFF
T4	Anoxic (AX)	OFF	OFF	OFF
T5	Aeration (AE-drain)	OFF	ON	ON

resistance (R_m), pore blocking resistance (R_{pore}) and cake resistance (R_c) were calculated using Eq. (1):

$$J = \frac{\Delta p}{\eta_0 (R_{\rm m} + R_{\rm pore} + R_{\rm c})} \tag{1}$$

where, $R_{\rm m}$ is the resistance from pure water flux, $R_{\rm t}$ is the resistance from flux after finishing the filtration, $R_{\rm pore}$ is the resistance from pure water flux after cake cleaning and $R_{\rm c} = R_{\rm t} - R_{\rm pore} - R_{\rm m}$.

2.4. EPS and SMP analyses

The EPS concentrations were measured in terms of carbohydrates and proteins using a cation exchange resin (CER; Dowex® Marathon® C, Na⁺ form, Sigma-Aldrich, USA) extraction method. The exchange resin (75 g CER/g volatile suspended solids [VSS])



Fig. 1. Schematic of M-SBR.

Phases of each experimental section						
Phase section	AF-feed	AE	AX-feed	AX	AE- drain	OLR
1	40 min	240 min	0 min	100 min	100 min	Loading 0.25 g COD/L/day
2	40 min	240 min	0 min	100 min	100 min	Loading 0.50 g COD/L/day
3	30 min	240 min	10 min	100 min	100 min	Loading 0.50 g COD/L/day

Table 2 Phases of each experimental sect

Table 3 Compositions of synthetic wastewater

	Chemical	Concentration	Remark
Carbon & nutrient	C ₆ H ₁₂ O ₆ NH ₄ Cl K ₂ HPO ₄	234.4 mg/L 95.5 mg/L 16.8 mg/L	COD 250 mg/L TOC 100 mg/L T-N 25 mg/L T-P 3 mg/L
Buffer Mineral	NaHCO ₃ CaCl ₂ ·2H ₂ O MgSO ₄ ·7H ₂ O MnSO ₄ KCl FeSO ₄ ·7H ₂ O	300 mg/L 3.8 mg/L 50.0 mg/L 1.7 mg/L 7.0 mg/L 2.2 mg/L	

was added to a 200 mL sample and mixed at 600 rpm for 2h at 4°C. The mixture was then centrifuged for 15 min at 12,000g to remove the mixed liquor suspended solids (MLSS). After CER addition, the centrifuged supernatant of the sample represented the sum of the EPS and SMP concentrations. The untreated mixed liquid was then centrifuged for 15 min at 12,000g (VS-21SMT, Vision Scientific, Korea); and the protein and carbohydrate concentrations of the supernatant were determined as representing the soluble fraction of the SMP; the difference between these measurements was the EPS concentration. The carbohydrate and protein concentrations of the supernatant were the measured using the methods established by Dubois et al. [17] and the advanced protein assay reagent (Cytoskeleton, USA), respectively. Note that bovine serum albumin and dextrose were used as the protein and carbohydrate standards, respectively.

2.5. Analysis

The MLSS, mixed liquor volatile suspended solids (MLVSS), and SVI were measured using Standard Methods [18]. In addition, the total organic carbon (TOC) was then analysed using a TOC analyzer (SIEVERS 820, General Electric, CO, USA); the chemical oxygen demand (COD), ammonia and nitrate were analysed by Humas kits (Humas, Korea). And the pH

measurement was obtained using a pH meter (Model 205A, Thermo, USA). The sludge observations were then carried out based on a differential interference contrast image analysis by employing confocal laser scanning microscopy (CLSM) (Carl ZEISS, LSM 5 PASCAL, Germany).

3. Results and discussion

3.1. M-SBR operation

The M-SBR reactor operation results are summarized in Table 4. The total operation period was about 100 d. The increased transmembrane pressure (TMP), ranging between 0.09–0.35 bar, indicated that membrane fouling occurred during the reactor operation. In the table, the MLVSS concentration was stabilized around 2,800 mg/L; and the total nitrogen removal ratios were 23.8 and 73.4% in Phase Sections 2 and 3, respectively. It was assumed that the carbon source of the step feed in Phase Section 3 assisted the denitrification process.

The combination of an MBR and SBR is one promising solution for MBR problems. Here, the SBR for wastewater treatment is a fill-and-draw activated sludge system. In this system, wastewater is added to a single "batch" reactor, treated to remove undesirable components, and is then discharged. It is posited here that an M-SBR could reduce membrane fouling due to the cleaning time of AF-feed and AE. In the M-SBR, the fill, anoxic, aeration and suction steps can be arranged based on the wastewater characteristics,

Table 4 Summaries of reactor operations

Phase section (operation day)	Average MLVSS (mg/L)	Average TOC removal (%)	Average T-N removal (%) (Sample No.)	TMP (bar)
1 (30)	1,158	94.12	_	0.09-0.125
2 (30)	2,808	85.2	23.8 [11]	0.125-0.25
3 (30)	2,795	86.8	73.4 [11]	0.25-0.35

which can maximize the treatment efficiency of nutrient (e.g. nitrogen, phosphorous) removal.

Also, MBRs offer economic advantages, including an extremely compact footprints. In particular, since this apparatus makes it possible to keep the cell concentration in the reactor high, high strength wastewater can be handled. Moreover, since biological nutrient removal and sludge settling take place in a timed sequence in a single reactor [19], there is no subsequent need to expand existing wastewater treatment plants, which commonly have extreme space constraints.

The application of MBRs is dramatically increasing in operations worldwide, especially in industrial, municipal, domestic, building wastewater and landfill leachate treatments [20]. Due to the continuous reduction in membrane costs, increase in membrane performance and increase in process efficiency, the application of MBR has progressively been extended to recycling and reuse, as well.

3.2. M-SBR filtration tests

Short-term filtration tests (120 min) were conducted with new membranes to determine the characteristics of sludge in the M-SBR. As shown in Table 5, the total resistance of the membrane in Phase Section 1 was lower than that of Phase Sections 2 and 3, as predicted from the lower MLVSS concentrations in Phase Section 1. However, even though the EPS, SMP and SVI concentrations were different in Phase Sections 2 and 3, their total resistances were similar.

Generally, membrane fouling results from the interaction between the membrane material and the components of the activated sludge liquor, which include biological flocs formed by a large range of living micro-organisms along with soluble and colloidal compounds. However, frequent membrane cleanings (inside and outside) and replacements that induce high operating costs are considered as the primary disadvantages of MBRs (including S-MBR) compared to the use of other biological processes.

3.3. Fouling factors of MBR processes

Fouling factors related to MBRs are very complex, with their relationships being extremely hard to determine, due to fact that all factors are linked to and depend on the characteristics of the micro-organic communities within the bioreactor. Kim et al. (2009) simplified the complex matrix of components—factors affecting biofouling—by dividing them into two categories: suspended solids and soluble materials in the mixed liquor. Fig. 2 shows the biological fouling components, key variables and fouling parameters for various factors in an MBR. They reported that the rheological and physiological characteristics of suspended solids (sludge or biological floc) influence the filterability of sludge and the formation of a cake layer on the membrane surface.

The MLSS concentration, however, is not always proportional to membrane fouling. Some researchers have suggested that a higher sludge concentration resulted in less fouling, implying that membrane fouling is related not only to the sludge quantity, but also to its characteristics [21,22]. For example, the overgrowth of filamentous bacteria is not always associated with increasing membrane fouling; soluble materials are also able to block membrane pores and solutes accumulated at the membrane surface then affect the concentration gradient of solutes between the membrane surface and the bulk solution. This gradient results in a diffusive flow of solutes or particles from the membrane surface back into the bulk solution. In this case, SMPs are the main soluble materials in the biological treatment process.

3.4. Aerobic granules

Granules facilitate good solid–liquid separation and contain multiple active layers of high amounts of biomass [8,23–31]. Since a granule can be defined as a collection of self-immobilized cells, some authors have regarded the granule as a suspended spherical biofilm, such as microbial cells, degradable particles and EPS [32]. According to literature, biofilm processes play a major role in many water reclamation and reuse technologies [33] due to their potential merits,

Table 5Results of M-SBR filtration tests

Phase section	<i>R</i> _t (1/m)	<i>R</i> _m (%)	R _c (%)	R _p (%)	EPS Mg/g MLVSS	SMP Mg/g MLVSS	SVI
1	8.8×10^{11}	16.6	60.3	23.1	23.6	63.5	120
2	$2.6 imes 10^{12}$	7.1	80.1	12.8	42.4	3.1	45.8
3	2.4×10^{12}	5.8	80.9	13.3	80.0	17.6	105.4



Fig. 2. Factors influencing membrane fouling in MBR.

such as higher concentrations of relevant organisms, very long-biomass residence times, small reactor space, ease of operation (automation), no clarification, no need to return sludge to the biological reactor, less sensitivity to adverse environmental conditions such as pH, temperature and concentration of toxic substances, potential for co-metabolism to mineralize certain xenobiotic contaminants, denitrification occurring inside the biofilm even under aerobic wastewater conditions and lower amount of waste sludge production. Thus, a submerged membrane system with aerobic granules can be applied to the biodegradation of many types of organic pollutants.

It has also been previously reported that granulation is initiated by bacterial adsorption and adhesion to inert matters, to inorganic precipitates [34] and/or to each other through physicochemical interactions and syntrophic associations [35]. Among other substances, an aqueous matrix of EPS can change the surface negative charge of bacteria, and thereby bridge two neighbouring bacterial cells to each other as well as other inert particulate matters, and settle out as a stable granule [36]. These initial granules then grow into compact mature granules, if favourable conditions pertaining to bacteria are maintained [37]. Moreover, it was found that extracellular polymers prefer to bind divalent ions (such as Ca^{2+} , Mg^{2+} and Fe^{2+}) when they are available, due to the formation of more stable complexes [34,38]. In particular, calcium is considered a constituent of extracellular polysaccharides and/or proteins because of its ability to bridge electronegative carboxyl and phosphate groups associated with bacterial surfaces [39]. In addition, as microbes aggregate, the presence of calcium enhances opportunities for cross-feeding, co-metabolism and interspecies hydrogen and proton transfers—which may further stimulate growth of microcolonies [40]. Thus, calcium may act not only to facilitate cell–cell bridging but also to indirectly promote the growth of aggregates [34].

The granulation properties of the biomass sludge influenced by the addition of each different cation were then characterized via the SVI. After 5 d of operation using a jar-tester for granule formation, the granulation trends for each sample were examined by SVI changes measured in a laboratory. With the passage of time, it was found that the SVI value did not decrease as much as that for the initial seeding sludge (about 190 mL/g), indicating that the raw seeding sludge could be granulated with no chemical additives. However, when the SVI was measured after adding various cations (K⁺, Na⁺, Mg²⁺ and Ca²⁺), the change in SVI significantly differed in each case. Table 6 presents the results of SVI of granular sludge

Table 6

Final SVI of granular sludge influenced by various cations (unit: mL/g)

Amount of cation (mg/L)	Monova cation	alent	Divalent cation	
	Na^+	K^+	Mg ²⁺	Ca ²⁺
0 (S-1)	185	181	174	173
250 (S-2)	184	181	165	157
750 (S-3)	177	181	155	129
1,250 (S-4)	178	174	149	139

influenced by four different cations measured after 5d of mixing. The amount of each cation added was as follows: 0 (S-1), 250 (S-2), 750 (S-3), and 1,250 (S-4) mg/L were added as chloride salts into the raw activated sludge samples in four jar-tester beakers. There were no significant changes of SVI between the measurements and final at 5d of mixing in the granular sludge when monovalent cations were added; in contrast, the SVI value of the granular sludge supplied with divalent cations conspicuously changed after 5d of experimentation. As a possible explanation for these results, Higgins and Novak (1997) and Novak et al. (1998) found that increased monovalent cation concentrations resulted in floc deterioration and decreased sludge performance (poor sludge settling and dewatering) through possible ion exchange mechanisms. On the other hand, sludge settling and floc strength were improved when divalent cation concentrations were increased; Mg²⁺ and Ca²⁺ exhibited similar chemical properties that change in a regular way because they are in the same group of the periodic table. However, if the ionization energies of Mg²⁺ (1,450.7 kJ/mol) and Ca²⁺ (1,145.4 kJ/mol) are compared, the ionization energy of Ca²⁺ is lower than that of Mg^{2+} , meaning that Ca^{2+} can be the more chemically active metal ion and can also be more easily negatively charged than the Mg²⁺ ion. Therefore, in this experiment the sample mixed with calcium ions was shown to have a lower SVI value than for the magnesium ions, indicating that calcium ions positively influence granule formation.

Kim and Jang (2006) reported that a low calcium concentration showed an 11 times higher steady state fouling rate than the optimum calcium concentration in their laboratory scale MBR operation. They assumed that the cake layer resistance was reduced due to a decrease in filamentous bacteria, in addition to the better flocculation caused by calcium bridges and the increased hydrophobicity of EPS under optimum calcium operation conditions.

Most studies on MBRs with aerobic granules have researched the removal efficiency rather than the properties and structure of the granules in the reactor [36,41]. However, physical characteristics of the granules play an important role in the performance of the MBR processes. Thus, granules need to be characterized and their activities need to be estimated using sensitive, accurate and representative methods that are quick and easy to use. The results from successful monitoring of granules may then be used to improve the process efficiency in MBR systems with aerobic granules. As shown in Fig. 3, the size and detailed microstructures of granules were examined using CLSM. It was observed that the granules had an



Fig. 3. Image of an artificially cultured granule after *in situ* double hybridization with FITC-labeled probe EUB338 (green) and Cy3-labeled probe Nsm156 (red).

irregular size and compact bacterial structure, in which cells were tightly linked, and rod-shaped species were found to be dominant. This distribution of micro-organisms was similar to the results of Tay et al. (2001), which showed cells tightly linked together with rod-like species predominant in the outer surface. With the help of the microscale bar in the eyepiece of the microscope and CLSM image analysis software, the granule diameters were determined. Even though various granular sizes were formed, the granule sizes were from 0.1 ± 0.15 to 0.5 ± 0.25 mm, rarely exceeding 0.75 mm.

4. Conclusions

Membrane biofouling in MBR processes has been attributed to physicochemical interactions between the biofluid (soluble materials suspended solids) and the membrane. Membrane fouling is currently the most serious problem affecting membrane separation performance, leading to permeate flux decline, requiring frequent membrane cleaning and replacement, which then increases operating costs. Different cleaning scenarios require different tools to ensure the quickest and most cost-effective cleaning. One of the best ways to clean a membrane is to backwash it, meaning reversing the flow at which liquid passes back through the membrane, though this method is relatively expensive.

The use of aerobic granules is a new and very promising approach to overcome the negative aspects of conventional MBRs because they have the same properties as the biofilm mentioned above. In particular, the EPS content plays a critical role in building and maintaining the structural integrity of aerobic granules through the cohesion and adhesion of microbial cells, which significantly affects their settleability efficiency. Here, it has been posited that large amounts of aerobic granular sludge can reduce membrane fouling. Combined with MBR systems, in particular, aerobic granules could offer several operational and economic advantages over typical conventional activated sludge processes, including an extremely compact footprint, simplified operation and consistently higher quality effluent. However, there are still many considerations to be overcome prior to their widespread application, including a complete analysis of substrate compositions, investigation of substrate limitations, measurement of granule diameters, comparison of turnover rates with an active biomass and testing under different operation strategies to determine optimal MBR operation conditions. In the long term, with more research, this method may be a benchmark for future biological wastewater treatment technologies.

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