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Improved phenol degradation in high-phenol-fed MBR by membrane-driven containment of non-settling biodegradation microbes

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ABSTRACT

Phenol is one of major pollutants in industrial wastewater with high remediation priority. In this study, the role of membrane in phenol degradation in high-phenol-fed MBR (membrane bioreactor) was explored. Phenol elimination in high-phenol-fed MBRs resulted in complete mineralization and the high-phenol-fed MBR exhibited greater biomass-specific phenol removal rates (0.4–1.5 mg phenol/(mgVSS·d) than the low-phenol-fed MBR. In the high-phenol-fed MBR, filamentous non-settling microbes were more abundant than in the low-phenol-fed MBR. In the following batch experiment, high-acclimated and non-settling microbes were separately collected from the high-phenol-acclimated bioreactor, and their specific phenol degradation was determined at 5.1 mg phenol/(mgVSS·d). The greater specific phenol degradation rate of the non-settling microbes than the observed phenol elimination rate in the high-phenol-fed MBR indicates that the high-phenol-acclimated and non-settling microbes had greater degradation activity than the rest of sludge microbes in the bioreactor. According to these findings, the role of membrane in the high-phenol-fed MBR was identified as the containment of non-settling and biodegradative microbes in bioreactor, and in turn, the membrane-driven increase of non-settling phenol degrading microbes enhanced phenol elimination in the high-phenol-fed MBR.

Keywords: Membrane bioreactor (MBR); Phenol microbial degradation; Filamentous non-settling microbes

1. Introduction

Phenol and its related compounds are known to be major pollutants in wastewater from industrial plants, such as oil refineries, petrochemical plants, coking plants, and phenol resin industry plants [1,2]. Phenol has been ranked 182 in the 2007 the Comprehensive Environmental Response, Compensation, and Liability Act (CER-CLA) Priority List of Hazardous Substances. The United States EPA lifetime health advisory level for phenol in water is 2 mg/L [3].

Physical and chemical processes are relatively simple and highly efficient approach toward the treatment of phenol-containing wastewater. Representative physical and chemical treatment processes may be ultrasonic process and advanced oxidation processes (AOP) [4,5], but they do not seem to be economically feasible when applied to a large scale of industrial wastewater treatment [6]. An economically feasible treatment option for large scale wastewater treatment is biological approach. However, when high concentration of phenol in wastewater was treated by conventional biological processes such as aerobic activated sludge techniques, proliferation

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of filamentous and non-flocculating populations tended to reduce the treatment efficiency and prevent normal operation of the conventional biological reactors [7,8]. The active biomass concentration was kept insufficient to treat wastewater, as microorganisms are washed out and lost together with the filamentous and non-flocculating populations generated by the phenol-containing wastewater. There have been attempts to apply the microorganisms adjusted to phenol wastewater in granular type to SBR (sequencing batch reactor) [9], or to isolate and cultivate a single strain microorganism that is capable of degrading phenol and apply it to aerobic systems such as RBC [10] or biofilter [11] in order to induce efficient biological degradation. However, even though the SBR with granular microorganisms and the RBC to which microorganisms were attached and grown are applied to the treatment of high concentration phenol-containing wastewater, microorganisms were continuously lost and the treatment efficiency was lowered, due to the filamentous fungi or the non-settled populations that cannot form microbial flock easily. An alternative approach is to keep the floating non-settling populations in a bioreactor using a forced membrane filtration process, i.e., MBR (membrane bioreactor).

Despite the potential application of MBR in treating highly phenol-loaded wastewater, the role of membrane has yet to be confirmed [12]. Especially, whether the degradation rate of non-settling microbes is greater than that for free swimming microbes is not clear. In addition, when phenol exposure level is higher than 1,000 mg/l, kinetic information on phenol microbial degradation is very limited. In this study, the role of membrane in phenol degradation in high-phenol-fed MBR was explored. For this, phenol elimination was characterized and compared in low- and high-phenol-fed MBRs. In addition, high-acclimated and non-settling microbes were separately collected from the high-phenol-acclimated bioreactor, and their degradation kinetics was examined in independent batch experiments.

2. Materials and methods

2.1. Membrane bioreactor operation

In this study, to conduct the phenol eliminating experiments for phenol-containing wastewater of high and low concentration in parallel, two aerobic bioreactors in which identical type of hollow fiber membrane is combined were operated in a continuous manner. Each of the reactors was made of Pyrex with 2.2L volume and operated in 1.8 L of the effective volume. The hydrodynamic residence time was 8 h with 3.75 ml/min of inflow rate. The temperature was maintained at 25°C in a bath. The membranes submerged in the reactors were of PVDF hollow fiber membranes with 0.4 μm of pore size and 14 cm^2 of active membrane area. At the bottom of the reactor, a glass air spray was installed so that the sterilized air that passed through 0.2 μm filter could be supplied in flow rate of 2–3 LPM and maintain sufficiently aerobic condition. The material for all the reactors and their parts as well as the pipes except the dosing pump head pipe was Teflon or glass. A pH and ORP meter (Lab-215, Sechang Inc.) and a dissolved oxygen (DO) probe (YSI Inc.) were also installed in the MBRs for the pH, ORP and DO in reactors. The inflow of the reactors was synthetic wastewater of which composition was as follows [13]: 2.13 g Na_2HPO_4, 2.04 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.067 g $CaCl_2{\cdot}2H_2O$, 0.248 g $MgCl_2{\cdot}6H_2O$, 0.5 mg $FeSO_4{\cdot}7H_2O$, 0.4 mg $ZnSO_4{\cdot}7H_2O$, 0.002 mg MnCl2·4H2O, 0.05 mg $CoCl_2{\cdot}6H_2O$, 0.01 mg $NiCl_2{\cdot}6H_2O$, 0.015 mg H_3BO_3, 0.25 mg EDTA [per litter]. For the experiment, Reactor A (low phenol-fed MBR) to which 100 mg/L of low phenol concentration wastewater was flowed and Reactor B (high phenol-fed MBR) to which 1,000 mg/L of high phenol concentration wastewater was flowed were simultaneously operated. The recycle sludge from wastewater treatment plant was seeded to each reactor (Fig. 1). To monitor the phenol-eliminating efficiency in each reactor, 5 ml of the effluent from Reactor A and Reactor B was sampled with vials of which top was coated with Teflon and 50 mL of sample was also taken for TOC analysis and stored in Teflon-treated glass bottles.

2.2. Chemical analysis

The samples for phenol concentration analysis was filtered again with a 0.2 μm filter and analyzed with high-performance liquid chromatography (HPLC). The analysis was performed with a C18 (Waters) column with 254 nm UV detector. The mobile phase, methanol: water = 40:60, was with the fed in 1 mL/min flow rate. Total organic carbon (TOC) was analyzed with TOC analyzer (Shimadzu). For biomass quantification, volatile suspended solids (VSS) was measured.

2.3. Biokinetic parameters determination

The kinetic test of phenol biodegradation was performed in a separate batch reactor to determine kinetic parameters, which were cell growth yield (*Y*), maximum specific growth rate (μm) and maximum specific utilization rate (*k*) [14]. The batch test was carried out to verify the phenol-eliminating function in non-settled fungal populations that are usually generated much in high (~1,000 mg phenol/L) phenol-fed MBR and to obtain the biokinetic parameters. The isolated microorganism was cultured with phenol as the only carbon source to investigate the microbial growth curve. 0.2 L of the culture medium with 1,000 ± 100 mg/L of phenol concentration was fed into 0.5 L volumetric flask and non-settled and fungal populations was seeded with the concentration ≥0.02 abs (OD at 600 nm). The cultivation was carried out at 25°C, stirring at 125 rpm. To monitor the phenol elimination depending on the microbial growth, the microbial concentration and the phenol concentration were

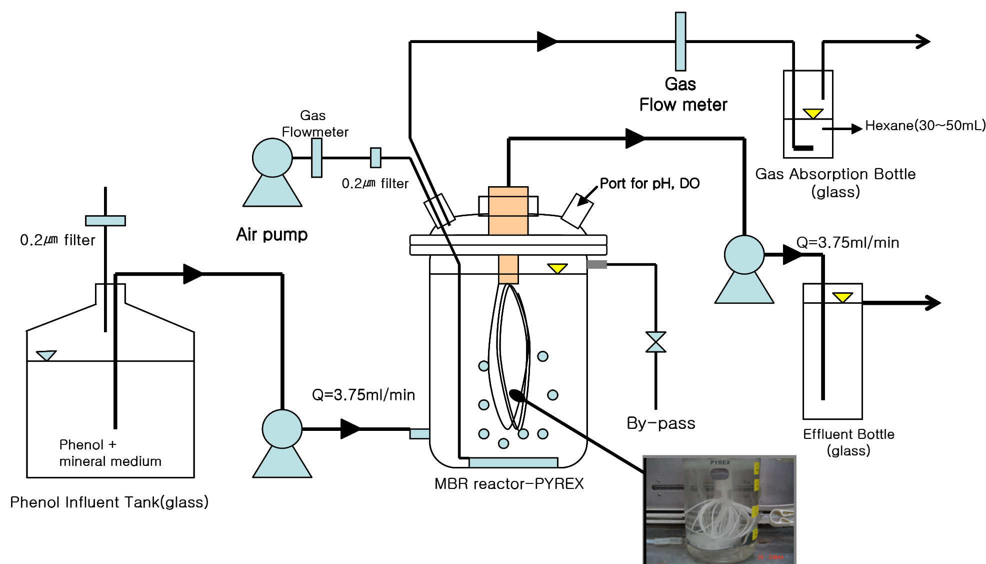

Fig. 1. Diagram of the experimental apparatus.

measured at different times. For this, the cell density was determined by measurements of the optical density at wavelength of 600 nm using a semi-micro cuvette. Cell densities were also derived from protein determinations. A modified Lowry protein test was used for the bioreactor samples. For the calculation of the cell dry weight (cdw) from the measured protein content it was assumed that 50% of the cell mass was protein [15].

3. Results and discussion

3.1. Laboratory membrane bioreactor operation

The low phenol concentration (100 mg/L) wastewater and the high phenol concentration (1,000 mg/L) wastewater were continuously flowed in activated sludge inoculated (2,500 mg VSS/L) Reactor A and Reactor B, respectively. Under these conditions, pH in each reactor was monitored (Fig. 2). According to Marrot (2006), pH is a marker of phenol degradation and plays an important role in biological treatment [16]. Stable biological treatment of phenol can be performed in the range of pH 6.5–7.5. When the initial phenol concentration was high, the microbial growth was inhibited and the pH variation range increased. While the pH values were not widely fluctuated in the case of Reactor A, the pH values for Reactor B were more widely fluctuated. These results indicate that, although the neutral ranges of pH showed stable biological treatment of phenol in the low and high-loaded reactors, the high-phenol loaded reactor showed less stable performance than the low-loaded reactor.

When the lower phenol concentration wastewater (100 mg/L) was fed into Reactor A, the effluent phenol concentration was about 10 mg/L, and then no phenol was detected in the effluent (Fig. 3). The instantaneous

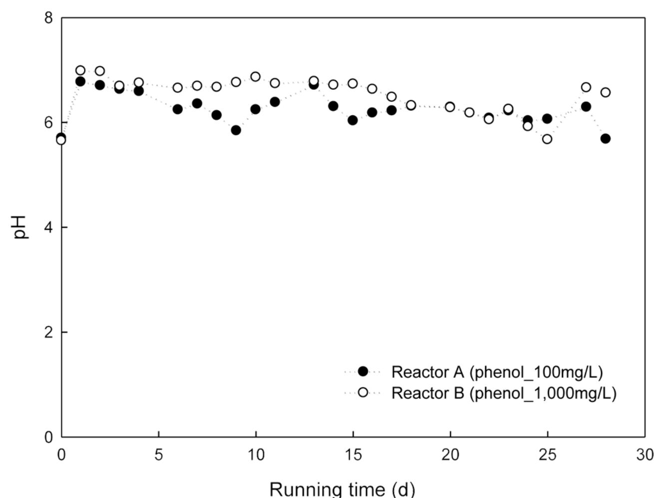

Fig. 2. pH in low and high phenol-fed MBRs.

removal of phenol suggests no lag of phenol degradation by the microbes in Reactor A. However, the removed phenol may have not been completely mineralized into CO_2 since the TOC remained detected although complete phenol was detected.

In the case of Reactor B which high phenol concentration wastewater (1,000 mg/L) was fed into, the introduced phenol was flowed through without any removal at all for the first three days, and then the reactor exhibited complete removal of phenol (Fig. 4). This suggests that an extended lag phase was required prior to the phenol removal in the reactor. The observed lag phase or adaptation phase may be due to any results from phenol toxicity [17,18]. According to the TOC data, no phenol degradation intermediates remained when the complete

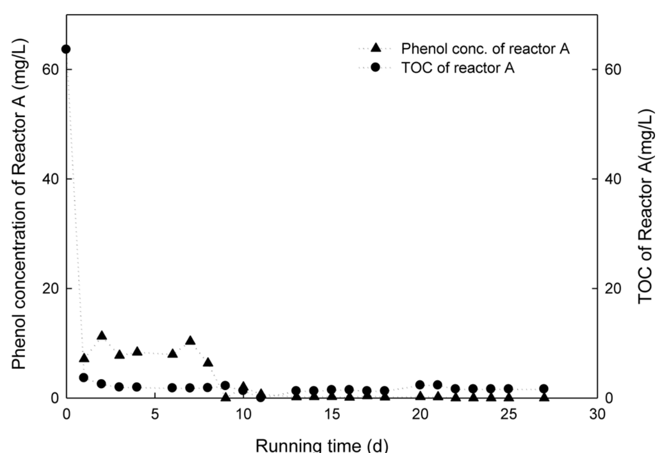

Fig. 3. Effluent phenol and TOC concentrations in low phenol-fed MBR (Reactor A).

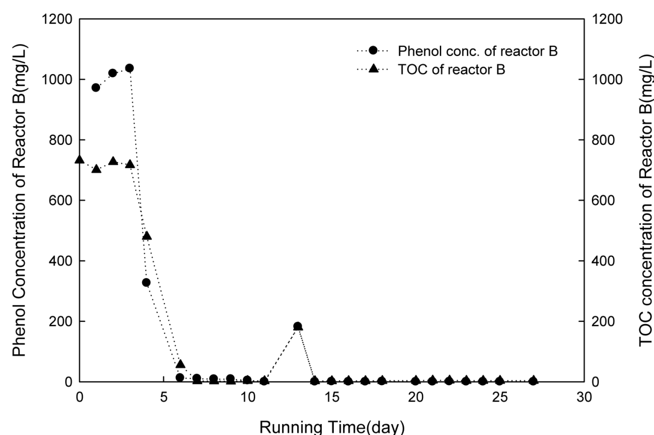

Fig. 4. Effluent phenol and TOC concentrations in high phenol-fed MBR (Reactor B).

phenol removal was detected. This indicates complete mineralization of phenol into CO_2

On the 13th day, an abrupt increase in effluent phenol concentration was observed in Reactor B (Fig. 4). Since abrupt microbial growth in Reactor B caused membrane blockage, a portion of the sludge was removed out of the reactor on the 13th day of operation (resulting into microbial concentration of 7,000 ± 100 mg VSS/L). During the sludge control, the effluent phenol concentration temporarily increased (Fig. 4). However, complete phenol and TOC removals were recovered within 24 h). This suggests that the high-phenol adapted microbes remained phenol degradation capability even after the perturbation due to the withdrawn sludge microbes.

Phenol elimination rate per biomass was calculated using the data from Fig. 3 and Fig. 4. The calculated values were compared between Reactor A and Reactor B (Fig.5). Reactor A showed a fairly constant value of phenol elimination rate (0.22 ± 0.04 mg phenol/mg VSS/d) during the period of operation. On the other hand, the results for Reactor B showed a widely fluctuated trend between 0.4–1.5 mg phenol/(mgVSS·d) during the acclimation period. After this acclimation period (after 15th day), phenol elimination rate per biomass became stable at 0.78 ± 0.05 mg phenol/(mgVSS·d). These results showed that the high-phenol fed bioreactor (Reactor B) exhibited greater phenol degradation activity than that for the low-phenol fed bioreactor (Reactor A).

3.2. *Kinetic characterization of microbial growth on phenol*

When monitoring morphology of microbial consortia in the reactor B, filamentous non-settling microbes increased between 5–10 days, and both filamentous non-settling and free-swimming microbes were present after 15th day. This led us to further investigate the

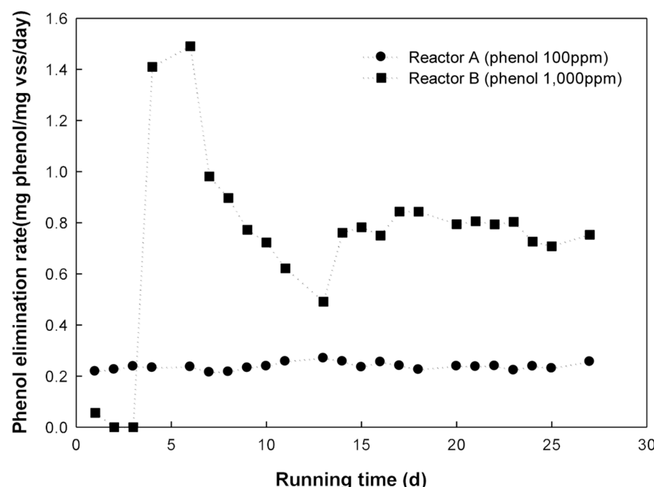

Fig. 5. Phenol elimination rates of low and high phenol-fed MBR.

biokinetics of phenol degradation by filamentous non-settling microbes. Phenol and biomass concentrations were monitored in batch experiments with the non-settled filamentous populations separated from the mixed sludge of Reactor B (Fig. 6). During the first 20 h, there was a lag phase. After this lag phase, phenol degradation and microbial growth occurred. Using the post-acclimation data, maximum specific growth rate (μ_m) was estimated using Monod model [Eq. (1)}.

$$\mu_m = \ln\left(X_t / X_0\right) / t \tag{1}$$

Here X_t indicates biomass when time is t; X_0 indicates biomass when time is zero.

The value of μ_m for non-settled filamentous populations was estimated at 0.1388 h^{-1} (Fig. 7). Because the

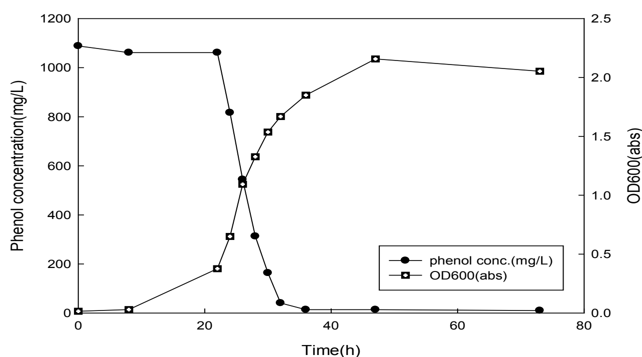

Fig. 6. Cell growth of non-settled filamentous populations and phenol degradation curve.

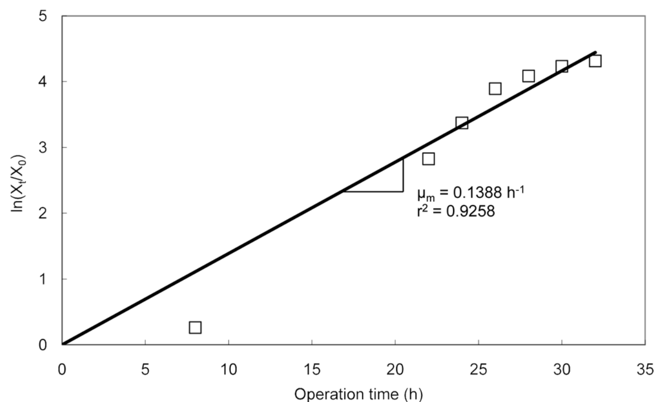

Fig. 7. Determination of μm using ln(Xt/Xo) vs. time plot.

applied phenol concentration was high, our approach is feasible for estimating maximum specific growth rate but insufficiently feasible for half-saturation constant, K_M [14].

To compare with the observed phenol elimination rate in the continuously flow Reactor B (Fig. 5), maximum specific phenol degradation rate (*k*) was calculated from the measured μ_m value. For this, cellular growth yield (*Y*) is required. The ratio of grown biomass (ΔX) to utilized phenol (ΔS), which is the definition of cellular growth yield, was independently determined at 1.2976 mg-protein/mg-phenol. Using the *Y* value and Eq. (2), the maximum specific phenol degradation rate was estimated at 2.568 mg phenol/(g protein·d)

$$k = \mu_m / Y \qquad (2)$$

By assuming that 50% of VSS is protein [14], the estimated k value can be converted into 5.136 mg phenol/(gVSS·d) The specific phenol degradation rate for non-settling microbes is 4–13 times greater than the observed values in Reactor B. This indicates that the non-settling microbes exhibited much higher degradation activity than the rest of sludge microbes in the bioreactor. If the membrane of Reactor B did not keep the non-settling microbes in the bioreactor, most of high-degradative non-settling microbes would have been washed out from the bioreactor and, in turn, phenol removal by the reactor would have been much less efficient. Thus, these findings revealed the significant role of membrane in improving phenol elimination in the MBR by capturing non-settling microbes.

The specific phenol degradation rate of the non-settling microbes (5.136 mg phenol/mgVSS·d) was within the range of phenol degradation rate values from previous other studies for bacteria and/or fungi [19,20,22–28], but much smaller than the value for yeast [21]. In the previous other studies, however, the measured degradation rate values were from microbes that were exposed to phenol concentration lower than 500 mg/l.

4. Conclusions

In this study, the role of membrane in phenol degradation in high-phenol-fed MBR was explored. For this, phenol degradation was characterized in low- and high-phenol-fed MBRs, and their phenol elimination rates were compared. In addition, high-acclimated and non-settling microbes were separated from the high-acclimated bioreactor, and their phenol degradation kinetics was determined in independent batch experiments. It is first to report the specific phenol degradation rate of the extremely high (1,000 mg/L)-phenol adapted and non-settling microbes. The specific phenol degradation rate of the non-settling microbes was greater than the observed phenol elimination rate in the high-phenol-degrading MBR. This indicates that the high-phenol-acclimated and non-settling microbes had greater degradation activity than the rest of sludge microbes in the bioreactor. These findings suggest that the role of membrane in high-phenol-fed MBR was the containment of non-settling microbes with sufficient phenol-degradative activity in bioreactor, and in turn, that the increase of non-settling phenol degrading microbes by membrane enhanced phenol elimination in the high-phenol-fed MBR.

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