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## Full Paper

# Analysis of microbial community structure in a biofilm on membrane surface in the submerged membrane bioreactor treating domestic wastewater on the basis of respiratory quinone profiles

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**The objective of this study was to investigate the microbial community structure of the biofouling film formed on hollow-fiber membrane surfaces in the submerged membrane bioreactor (SMBR) with a nitrification-denitrification process. In this experiment, aeration was conducted intermittently (60 min off, 90 min on) cyclic anoxic and oxic conditions in the SMBR. The dominant quinone types of biofilm on the membrane surface in an intermittently aerated SMBR were ubiquinone (UQs)-8, -10, followed by menaquinones (MKs)-8($H_4$), -8($H_2$) and -7, but those of suspended microorganisms were UQ-8, UQ-10 followed by MKs-8, -9($H_4$) and -6. The change in quinone profiles of biofilm on the membrane surface suggested that UQ-9, MK-7, MK-8($H_2$) and MK-8($H_4$) contributed to microbiological fouling in the intermittently aerated SMBR treating domestic wastewater. The microbial diversities of suspended microorganisms and biofilm, calculated based on the composition of all quinones, were 9.5 and 10.9, respectively.**

**Key Words**——biofilm; biofouling; microbial community structure; quinone profile; submerged membrane bioreactor

## Introduction

The membrane bioreactor (MBR) process, a technological combination of biological treatment with a membrane separation device, has many advantages due to the efficient interception performance of the membrane (Chang and Simon, 2002; Zoh and Stenstrom, 2002). By combining the submerged membrane bioreactor (SMBR) with a nitrification-denitrification process, nitrogen can be removed from wastewater very effectively because of the maintenance of a high concentration of mixed liquor suspended solids (MLSS) including both nitrification bacteria and denitrification bacteria (Hasar et al., 2001; Nah et al., 2000).

However, the MBR process for domestic wastewater treatment has been limited by problems of membrane fouling during filtration of the activated sludge, which decreases the filtration flux and the treated water output flow. The membrane fouling could originate from adsorption of organic species and adhesion of microbial cells at the membrane surfaces (Hodgson and Fane, 1992). Recent studies have quantified the relative contribution of SS, colloids and dissolved molecules to the resistance to filtration caused by fouling (Defrance et al., 2000; Huang et al., 2001; Mukai et al., 2000). Ridgway et al. (1983) recovered a total of six different generic groups of bacteria and one unidenti-

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fied group of microorganisms from cellulose acetate RO membranes fed with pretreated activated-sludge effluent.

In this study, respiratory quinone profiles were applied as a tool for identifying the microbial population of the biofouling film formed on the membrane surface. Microbial respiratory quinones are components of the bacterial respiratory chain and play an important role in electron transfer during microbial respiration. Quinones exist in almost all bacteria, and in general, one species or genus of bacteria has only one dominant type of quinone (Collins and Jones, 1981; Hess et al., 1979). So the quinone profile, which is usually represented as the mole fraction of each quinone type, should be specific for a microbial community. Changes in the microbial community of a mixed culture of microbes could be quantified using the quinone profiles. In recent years, the technique of using quinone profiles has gained increased recognition as a simple and useful tool for the analysis of microbial population dynamics in mixed cultures (Fujie et al., 1994; Hiraishi et al., 1998; Hiraishi, 1999; Hu et al., 1997; Kunihiro et al., 2002; Lim et al., 2002).

The objective of this study was to investigate the microbial community structure of the biofouling film formed on the membrane surface in SMBR with a nitrification-denitrification process. In addition, the difference in community structure between biofilm and suspended microorganisms was investigated by using the technique of quinone profiles.

## Materials and Methods

*Experimental apparatus and operation.* The lab-scale intermittently aerated SMBR was a rectangular tank of 90 mm×300 mm×450 mm, having an effective volume of 8.1 L. The system consisted of a hollow-fiber membrane module made of polyethylene (Mitsubishi Rayon Eng. Co., Ltd., Japan) with a pore size of 0.4 μm and an effective filtration area of 0.2 $m^2$/module. The influent was fed into the lower part of the bioreactor.

In this experiment, aeration was conducted intermittently (60 min off, 90 min on) under cyclic anoxic and oxic conditions in the bioreactor to promote nitrification-denitrification. Permeate was obtained only during the oxic period. The small propeller was rotated during the anoxic period to prevent settling of the sludge and to detach the accumulated sludge from the membrane surface. Flux through the membrane was set at around 0.24 $m^3/m^2/d$. An air diffuser was set just below the membrane module so that rising air bubbles could provide the membrane surface with enough shear stress, which is effective for removing attached sludge from the membrane. Air feed rate was regulated in the range 10 L/min. Hydraulic retention time (HRT) of the feed water in the bioreactor was around 8.4 h.

The operation of the pumps and valves within a cycle was automatically controlled with time control system (PLC). The seed sludge and wastewater was obtained from a domestic wastewater treatment plant in Guri, Korea, where a conventional activated sludge process has been adopted. During the whole period of the study, no sludge was removed from the plant intentionally except for sampling.

*Analytical methods.* The $COD_{Cr}$ (COD, hereafter) concentrations in the influent and effluent of the intermittently aerated SMBR were determined by a HACH (Loveland, USA) DR/3000 direct reading spectrophotometer using a HACH COD reactor. The concentrations of mixed liquor suspended solid (MLSS) and volatile solids in MLSS (MLVSS) were measured according to the procedures outlined in "Standard Methods" (APHA, 1992). Total-N and T-P were determined by a UV/VIS spectrophotometer (DU 520, Beckman Coulter, Inc., CA, USA). The concentration of ammonium was measured by an auto-analyzer (BRAN+LUEBBE, Germany). Nitrite and nitrate nitrogen were measured by an ion chromatograph (DX-120, Dionex, USA). Turbidity was measured with a HACH 2100AN (Loveland, USA).

*Quantification of microbial quinones.* Microbial quinones in suspended microorganisms and biofilm were analyzed using previously described methods (Hu et al., 1999a, 2001). Quinones were extracted from the centrifuged microbes using a mixture of chloroform-methanol and subsequently extracted into hexane. Menaquinones and ubiquinones contained in the crude extract were separated and purified using Sep-Pak® Plus Silica. The types and concentrations of the quinones were determined using a HPLC equipped with an ODS column (Mightysil RP-18, 4.6 (I.D.)× 250 mm, Kanto Chemical Co., Japan) and a photodiode array detector (SPD-M10A, Shimadzu Co., Japan). In this study, the respiratory quinones were named as follows: the abbreviation of the type of quinone (ubiquinone: UQ, menaquinone: MK), a dash, and the number of isoprene units in its side chain. For

example, MK-9($H_2$) represents a menaquinone with 9 isoprenoid units and one of the 9 units is hydrogenated with 2 hydrogen atoms.

The microbial diversity using quinone composition as an index (*DQ*) is defined with the following equation (Hu et al., 1999b)

$$DQ = \left( \sum_{k=1}^{n} (\sqrt{f_k}) \right)^2 \quad (1)$$

where $f_k$ indicate the molar fraction of quinone species *k* and *n* is the number of quinone species with molar fractions higher than or equal to 0.001.

The microbial equabilities (*EQ*) for suspended microorganisms and biofilm in an intermittently aerated SMBR and conventional activated sludge are defined with the equation $EQ = DQ/n$ (Hu et al., 1999b), where *n* is the number of quinone species in samples.

## Results and Discussion

*Operating conditions and treatment performance*

The variations of transmembrane pressure and flux are shown in Fig. 1. MLSS concentration of 4,800–5,800 mg/L was achieved over the experiment period and this guaranteed the efficient removal of pollutants. Mixed liquor volatile suspended solid (MLVSS) to MLSS ratio was stable throughout the operation period in the bioreactor showing no accumulation of the inorganic material (data not shown). The rapid increase of filtration resistance that was observed during the first few days of operation indicates such a rapid attachment of bacterial cells to the membrane. This might be caused by accumulation of bacteria metabolic substances inside the bioreactor because the operating mode was changed. After the experiment had been conducted for 30 days, the membrane was chemically cleaned to remove fouling (300 mg/L sodium hypochlorite solution for 30 min) and the experiment was continued under sample operation conditions for the long-term experiment. The change in transmembrane pressure after chemical cleaning was very stable.

The characteristics of influent and treated wastewaters are shown in Table 1. The volumetric loading of COD to the treatment process varied from 0.08 to 0.41 (average: 0.23) kg-COD/$m^3 \cdot$d. The COD (87.6–98.1%) component in the wastewater could be removed, with an average of over 92.8%. The removal efficiencies of T-N and T-P were 35–70% and 10–60%, respectively.

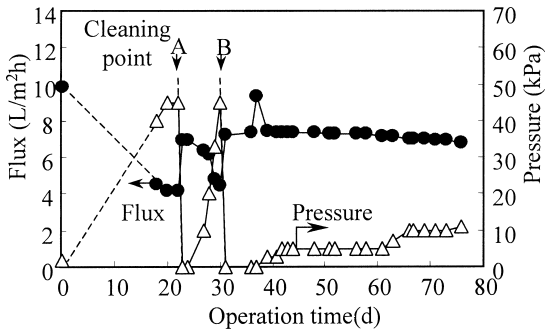


Fig. 1. Variation of transmenbrane pressure and flux with operating time (cleaning A: physical cleaning, cleaning B: NaOCl cleaning).

Table 1. Characteristics of the influent and effluent wastewater.

| Items | Influent | Effluent |
|---|---|---|
| $TCOD_{Cr}$ (mg/L) | 78.5–283 (180) | 3.0–32 (12.8) |
| T-N (mg/L) | 15.7–33.9 (23.9) | 7.2–15.9 (12.1) |
| $NH_4^+$-N (mg/L) | 10.3–18.9 (14.2) | ND |
| $NO_3^-$-N (mg/L) | 0–0.03 | 4.5–15.8 (10.7) |
| T-P (mg/L) | 1.9–4.1 (2.7) | 1.2–2.7 (1.95) |
| SS (mg/L) | 23–174 (84.7) | ND |
| Turbidity (NTU) | 84.0 | 0.02–0.43 (0.12) |

ND: Not detected.

Part of the organic nitrogen and almost all ammonium nitrogen were nitrified to $NO_3^-$-N and denitrification was inhibited. This indicated that T-N removal was affected by various intermittent aeration modes. The influent turbidity removal was 97.0–99.8%.

*Microbial population characteristics*

The quinone compositions are summarized in Table 2. The activated sludge was taken from a full-scale conventional activated sludge process treating the same domestic wastewater. The composition of ubiquinone was much simpler than that of menaquinone. Most of the samples of suspended microorganisms and biofilm contained UQ-8 and UQ-10 as the most abundant quinone type. In addition, considerable levels of MKs-6, -7, -8, -8($H_4$) and -9($H_4$) were detected in all samples. Menaquinone-10($H_2$), however, was found only in the full-scale conventional activated sludge. Conversely, MKs-9 and -11 were observed only in the biofilm and suspended microorganisms of the intermittently aerated SMBR. Furthermore, no large difference in quinone profile between the conventional activated

Table 2. Composition (molar fraction) of quinones in an intermittently aerated SMBR.

| Quinone type | Activated sludge | Submerged MBR | | |
|---|---|---|---|---|
| | | Biofilm | Suspended (oxic) | Suspended (anoxic) |
| Ubiquinones | | | | |
| UQ-8 | 0.332 | 0.281 | 0.296 | 0.409 |
| UQ-9 | 0.051 | 0.060 | 0.052 | 0.065 |
| UQ-10 | 0.199 | 0.138 | 0.153 | 0.170 |
| Menaquinones | | | | |
| MK-6 | 0.043 | 0.059 | 0.089 | 0.071 |
| MK-7 | 0.068 | 0.072 | 0.050 | 0.044 |
| MK-8 | 0.033 | 0.039 | 0.160 | 0.111 |
| MK-9 | — | 0.014 | 0.005 | 0.004 |
| MK-10 | 0.003 | 0.003 | 0.003 | 0.002 |
| MK-11 | — | 0.002 | 0.004 | 0.002 |
| MK-12 | — | — | 0.004 | 0.002 |
| MK-7($H_2$) | 0.009 | 0.016 | 0.054 | 0.037 |
| MK-8($H_2$) | 0.060 | 0.074 | 0.005 | 0.003 |
| MK-9($H_2$) | 0.006 | — | 0.001 | — |
| MK-10($H_2$) | 0.001 | — | — | — |
| MK-8($H_4$) | 0.111 | 0.133 | 0.046 | 0.032 |
| MK-9($H_4$) | 0.037 | 0.048 | 0.077 | 0.047 |
| MK-10($H_4$) | 0.047 | 0.059 | — | — |
| UQ/MK | 1.39 | 0.92 | 1.0 | 1.81 |

—: Not detected.

sludge and the suspended microorganisms in an intermittently aerated SMBR was found. As shown in Table 1, the dominant quinone types of the biofilm on the membrane surface were UQs-8, -10, followed by MKs-8($H_4$), -8($H_2$) and -7. But those of suspended microorganisms at oxic and anoxic conditions were UQs-8, -10, followed by MKs-8, -6 and -9($H_4$). In particular, the molar fractions of UQ-8 and -10 in anoxic condition were much higher than those of suspended microorganisms in oxic condition. This fact suggest that the denitrifying bacteria such as aerobic autotrophic or heterotrophic microorganisms could be switched into anoxic growth when nitrate is used as the electron acceptor. The molar ratios of ubiquinone to menaquinone (UQ/MK) for the suspended microorganisms and biofilm are also illustrated in Table 2. The values of UQ/MK ratio for all the samples varied from 0.92 to 1.81. It is believed that UQs and MKs are specific indicators of aerobic gram-negative bacteria and anaerobic gram-positive bacteria (Collins and Jones, 1981).

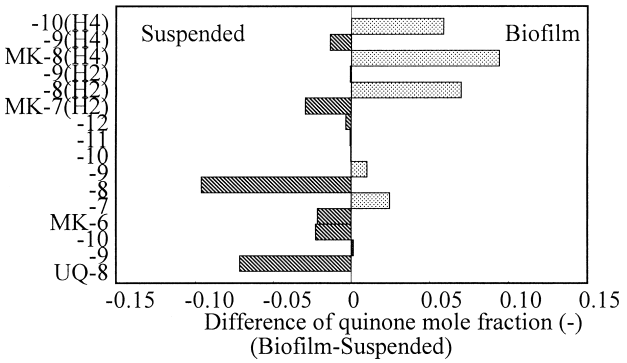


Fig. 2. The differences in quinone composition between the biofilm and suspended microorganisms in the intermittently aerated SMBR (biofilm-suspended).

Therefore, a UQ/MK value of $<1$ would suggest that anaerobic gram-positive bacteria were dominant in the microorganisms attached to the membrane surface examined in this study. The major differences in quinone composition between the suspended microorganisms and biofilm on the membrane surface in an intermittently aerated SMBR are shown in Fig. 2. Zero is a same molar fraction of quinone between biofilm and suspended microorganisms. The microbial community structure of the biofilm differed from that of the suspended microorganisms. The molar fractions of ubiquinone (UQ)-9, menaquinones (MKs)-7, -8($H_2$), -8($H_4$) and -10($H_4$) of the biofilm on membrane surface were higher than those of suspended microorganisms. The bacteria containing above-mentioned quinone species may be contributing to microbiological fouling in an intermittently aerated SMBR tested in this study (Flemming and Schaule, 1988; Ridgway et al., 1983).

In this study, we calculated dissimilarity, and thus defined the dissimilarity index ($D$) as follows (Hiraishi et al., 1998): $D(i,j)=1/2\sum|f_{ik}-f_{jk}|$ where, $f_{ik}$ and $f_{jk}$ are the mole fraction of the $k$ quinone component for the $i$ and $j$ samples, respectively. Clustering based on the total quinone profiles revealed that the suspended microorganisms formed a cluster at a dissimilarity level of less than 10%, whereas the biofilm and suspended microorganisms crossed each other at a dissimilarity level of 25%. This indicates that there are significant differences in the community structure among these operational conditions (e.g., oxygen, temperature).

The microbial diversities ($DQ$) for suspended microorganisms and biofilm on the membrane surface calculated from the quinone composition using Eq. (1) are shown in Table 3.

Table 3. Microbial diversity and equability in the suspended microorganisms and biofilm.

| | Activated sludge | Submerged MBR | | |
|---|---|---|---|---|
| | | Biofilm | Suspended (oxic) | Suspended (anoxic) |
| DQ (−) | 9.9 | 10.9 | 11.7 | 10.7 |
| EQ (−) | 0.71 | 0.78 | 0.69 | 0.67 |

The microbial diversity calculated from the composition of all quinones (including ubiquinones and menaquinones), $DQ_q$ for the suspended microorganisms in an intermittently aerated SMBR and conventional activated sludge were 9.4 and 10.7–11.7, respectively. The $DQ_q$ for the biofilm was 10.9, which was similar to that for the suspended microorganisms.

The microbial equabilities for suspended microorganisms and biofilm in an intermittently aerated SMBR and conventional activated sludge are also shown in Table 3. Note that when the fractional contents of all quinone species in a sample are equal to each other, the microbial equability takes the maximum value of 1. The microbial equability magnitude for total respiratory quinones was as follows: suspended microorganisms in the intermittently aerated SMBR were less than conventional activated sludge, which was in turn less than biofilm on the membrane surface. The microbial equability for biofilm on the membrane surface was 0.78, which was larger than that for the suspended microorganisms (0.67–0.69).

In summary, the difference in microbial community structure between suspended microorganisms and biofilm on the membrane surface in an intermittently aerated SMBR treating the domestic wastewater was evaluated by analyzing microbial quinone composition. The information could be enhanced by numerical analysis of the profiles for attached and suspended microorganisms can be objectified. On the other hand, the microbial diversity of biofilm on the membrane surface was calculated based on the composition of all quinones, and was similar to that of suspended microorganisms.

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