Water Science and Technology **Vol 49 No 2 pp 255–261** © IWA Publishing 2004

Microbial community structure of membrane fouling film in an intermittently and continuously aerated submerged membrane bioreactor treating domestic wastewater

B.-R. Lim*, K.-H. Ahn*, P. Songprasert, J.W. Cho* and S.H. Lee***

* Future Technology Research Division, Korean Institute of Science and Technology, P.O.Box 131, Cheongryang, Seoul, 130-650 Korea
(E-mail: *limbr@kist.re.kr*; *khahn@kist.re.kr*; *cogitol@kist.re.kr*; *seocklee@kist.re.kr*)

** School of Environment, Resources and Development, Asian Institute of Technology, Bangkok, Thailand
(E-mail: *piyapornna@hotmail.com*)

Abstract There was an observable difference in microbial community structure between suspended microorganisms and membrane biofouling film in intermittently and continuously aerated SMBRs. The dominant quinone type of membrane biofouling film in an intermittently aerated SMBR was ubiquinone (UQs)-8, -10 followed by menaquinone (MKs)-8(H_4) and -8(H_2). But that of the continuously aerated SMBR was UQs-10, -8 followed by MKs-6 and -8(H_4). The experimental results also showed that the conditions of an intermittently aerated SMBR may contribute to biofouling by *Pseudomonas, Moraxella, Vibrio* (quinone type UQ-8), *Staphylococcus warneri* (quinone type MK-7), *Micrococcus* sp. (quinone type MK-8(H_2)) and *Nocardia* sp. (quinone type MK-8(H_4)), but biofouling in a continuously aerated SMBR may be due to *Paracoccus* sp. (quinone type: UQ-10) and *Flavobacterium* species (quinone type: MK-6). The microbial diversities in the intermittently aerated SMBR were 10.9 and 9.4 for biofouling film and suspended microorganisms, respectively. For the continuously aerated SMBR, the results were 10.4 and 10.5 for biofouling film and suspended microorganisms, respectively.

Keywords Biofilm; biofouling; microbial community structure; microbial diversity; quinone profile; submerged membrane bioreactor

Introduction

One of the most widely used wastewater treatment processes is the conventional activated sludge process. It is a cost effective treatment method under optimal conditions, but bulking can be a chronic problem in some plants. The membrane bioreactor (MBR) process eliminates the weakest link in the activated sludge process and makes effluent quality independent of the settling characteristics of the biomass. The 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 (Nah *et al*., 2000; Hasar *et al*., 2001; Chang and Simon, 2002; Zoh and Stenstrom, 2002). 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. This increases costs because of the need to clean or replace clogged membranes. Fouling is the result of complex phenomena but is essentially caused by the extracellular polymers produced during lysis of bacteria (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; Mukai *et al*., 2000; Huang *et al*., 2001). However, there is little information available on the relationship between membrane fouling and accumulation of bacterial cells and/or community structure of microorganisms attached to the membrane surface.

In this study, respiratory quinone profiles were applied as a tool for identifying the

microbial population of the biofouling film formed on 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 (Hess *et al*., 1979; Collins and Jones, 1981). 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 (Hedrick and White, 1986; Hiraishi, 1988; Fujie *et al*., 1994; Hu *et al*., 1997; Lim *et al*., 2001).

The objective of this study is to investigate the microbial community structure of the biofouling film formed on hollow-fiber membrane surfaces in the intermittently and continuously aerated submerged membrane bioreactors treating domestic wastewater using the respiratory quinone profile. In addition, the major differences in quinone composition between the suspended microorganisms and biofouling film in two SMBRS were investigated.

Methods

Apparatus and operating conditions investigated in this study

The experimental system consists of an activated sludge bioreactor in which a membrane module is submerged. The lab-scale intermittently aerated submerged membrane bioreactor (SMBR1) was a rectangular tank of 90 mm × 300 mm × 450 mm, having an effective volume of 8.1 L. Aeration was conducted intermittently (60 minutes off, 90 minutes on) in cyclic anoxic and oxic conditions in the reactor to promote nitrification-denitrification. The seed sludge and domestic wastewater were obtained from the influent of a wastewater treatment plant in Kuri, Korea where a conventional activated sludge process has been adopted. A bench-scale continuously aerated SMBR (SMBR2) system (reactor volume; 40 L), located in a municipal wastewater treatment plant in Kwangjoo, Korea, was constructed to investigate the microbial community structure of the biofouling film formed on hollow-fiber membrane surfaces.

The type of membrane used in this study was a hollow-fiber membrane module made of polyethylene (Mitsubishi Rayon Co., Japan) with a pore size of 0.4 μm and an effective filtration area of 0.2 m^2/module. The influent was taken from the feed tank to the bioreactor by the use of a peristaltic pump. Membrane-filtered effluent was extracted by a suction pump connected to the membrane module, which was operated with a mode of 8 min on and 2 min off. The operation of the pumps and valves within a cycle was automatically controlled with time control system (PLC). Flux through the membrane in both SMBRs was set at around 0.24 m/day. Air feed rate was passed through the modules at 10 L/min. Hydraulic retention times (HRTs) of the feed water in the intermittently and continuously aerated SMBRs were around 8.4 hr and 4.4 hr, respectively. During the whole period of the study, no sludge was removed from the plant intentionally except for sampling. No backwash of the membrane was carried out during this period.

Analytical methods

Water qualities. The COD concentrations in the influent and effluent of the SMBR were measured using the *Standard Methods* (APHA, 1992). COD_{Cr} (COD, hereafter) was determined by a HACH (Loveland, Colorado) DR/3000 direct reading spectrophotometer using a HACH COD reactor. The concentration of MLSS in bioreactor was determined according to *Standard Methods*. The concentrations of ammonium, nitrate and nitrite were measured

by autoanalyzer (BRAN+LUEBBE, A United Dominion Co.) and by ion chromatography (DX-120, Dionex), respectively. Total nitrogen was determined by a UV/VIS spectrophotometer (DU 520, Beckman).

Microbial quinone. Microbial quinones in suspended microorganisms and biofilm were analyzed using previously described methods (Hu *et al*., 1999a, 2001). Quinones were initially 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 paper, the quinones are 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.

Results and discussion

Pollutant removal performance

The average water qualities of the treated wastewater of the intermittently (SMBR1) and continuously (SMBR2) aerated SMBRs are summarized in Table 1. The effluent TCOD concentration of both SMBRs was less than 12.8 mg/L regardless of the changes in influent. NH_4^+-N in both SMBRs was completely converted to NO_3^--N. No NO_2^--N was detected in the effluent. The average removal efficiencies of COD and T-N in SMBR1 during the course of this study were greater than 96% and 70%, respectively. The COD and T-N removal efficiencies of SMBR2 were average 90% and less than 50%, respectively. The removal efficiency of suspended solid (SS) was observed as high as 100%, which demonstrated a better separation effect of the hollow-fiber membrane module in the submerged membrane bioreactor than that of the settling tank in the conventional activated sludge process. MLSS concentration of 4,800–5,800 mg/L in both SMBRs was achieved over the experiment period and this guaranteed the efficient removal of pollutants.

Suction pressure and filtration stability of membrane

Figure 1 shows the filtration stability of the lab-scale intermittently aerated SMBR in terms of suction pressure and flux with operating time. The upward tendency of suction pressure became serious over a short period at day 20 from the start, which implied rapid acceleration of membrane fouling. The formation of a thick gel layer was observed on the membrane surface. After the washed membrane by brush was reinstalled in the reactor, the operation restarted. The suction pressure was restored almost to its initial zero value and maintained for about 3 days, but then started to increase again. It is not clear if this second increase of suction pressure was due to the hard deposition and internal pore plugging by

Table 1 Characteristics of the influent and treated wastewater of each SMBR

Items	Intermittent aeration (SMBR1)		Continuous aeration (SMBR2)	
	Influent	Effluent	Influent	Effluent
$TCOD_{Cr}$ (mg/L)	78.5–283	3.0–32	50.0–313	8.0–24
T-N (mg/L)	15.7–33.9	7.2–15.9	8.7–45.6	3.7–34.9
NH_4^+-N (mg/L)	10.3–18.9	ND	8.0–26.6	0–4.7
NO_3^--N (mg/L)	0–0.03	4.5–15.8	0–18.84	8.4–40.6
SS (mg/L)	23–174	ND*	83–473	ND
Turbidity (NTU)	84	0.02–0.43	–**	0.1–0.26

*; Not detected, **; Cannot be detected

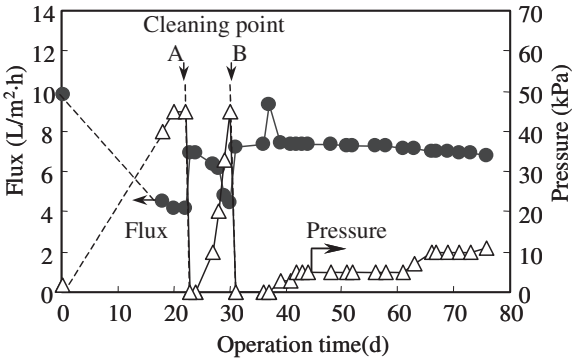

Figure 1 Variations of flux and transmembrane pressure in the intermittently aerated SMBR during the operation time

particles that were not easily cleaned by physical washing. When the suction pressure reached about 40 kPa on day 30, the chemical cleaning was done after washing as before. Cleaning of the membrane was conducted by submerging the membrane into 300 mg/L NaOCl solution. The restored zero value of suction pressure was subsequently maintained for a longer time compared with physical cleaning.

Microbial community structure

Quinone compositions. The analytical results of 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. Three types of ubiquinone (UQs-8, -9, and -10) were found in all the samples. The suspended microorganisms and biofilm on membrane contained 11–14 types of menaquinones. 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-9(H_2), however, was found only in the raw conventional activated sludge and suspended microorganisms in both SMBRs. Conversely, MKs-9 and -11, however, were observed only in the suspended microorganisms and biofilm of SMBR1 and SMBR2. As shown in Table 2, the dominant quinone types of biofilm on the membrane surface in SMBR1 were UQs-8, -10, followed by MKs-8(H_4), -8(H_2) and -7. But those of biofilm on membrane in SMBR2 were UQ-8, MK-10(H_4), followed by MKs-6, -8(H_2) and -7. The dominant quinone types of suspended microorganisms in SMBR1 were UQs-8, -10, followed by MKs-8 and -6, but those of suspended microorganisms in SMBR2 were UQs-8, -10 followed by MKs-6, -10(H_4) and -7.

The molar ratios of ubiquinone to menaquinone (UQ/MK) for the suspended microorganisms and biofilm on the membrane surface are also illustrated in Table 2. The values of UQ/MK ratio for the suspended microorganisms ranged from to 0.81–1.4, which are higher than that for biofouling film on the membrane surface (0.42–0.92). This suggests that the ratio of aerobic bacteria in suspended bacteria might be higher than that in the biofouling film on the membrane.

The major differences in quinone composition between the suspended microorganisms and biofilm on the membrane surface in SMBR1 and SMBR2 are shown in Figure 2. The microbial community structure of biofilm on the membrane surface differed from that of the suspended microorganisms in both SMBRs. The molar fractions of MKs-7, -8, -8(H_2), -8(H_4) and -10(H_4) of biofilm on membrane in SMBR1 were higher than those of suspended microorganisms. On the other hand, the molar fractions of MKs-6, -7, -8, -8(H_2),

-9(H_2), -9(H_4) and -10(H_4) of the biofilm in SMBR2 were higher than those of suspended microorganisms. The bacteria containing MK-7 and MK-6 in biofilm on the membrane surface may represent *Bacillus* sp., *Staphylococcus warneri* and *Flavobacterium* (Ridgway *et al*., 1983; Flemming and Schaule, 1988; Gryta, 2002), and those containing MK-8(H_2) may represent *Arthrobacter* sp., *Corynebacterium* and *Brevibacterium* sp. Menaquinone-8(H_4) was reported to represent *Nocardia* sp. The above-mentioned bacteria may be contributing to microbiological fouling in SMBR1 and SMBR2 tested in this study.

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

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

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

Table 2 Composition (molar fraction) of quinones in the intermittently (SMBR1) and continuously (SMBR2) aerated SMBRs in this study

Quinone type	Activated sludge		SMBR1		SMBR2	
	Kuri plant	Kwangjoo plant	Biofilm	Suspended	Biofilm	Suspended
Ubiquinones						
UQ-8	0.332	0.415	0.281	0.352	0.171	0.281
UQ-9	0.051	0.059	0.060	0.059	0.042	0.046
UQ-10	0.199	0.18	0.138	0.161	0.084	0.12
Menaquinones						
MK-6	0.043	0.034	0.059	0.080	0.144	0.115
MK-7	0.068	0.058	0.072	0.047	0.085	0.078
MK-8	0.033	0.024	0.039	0.135	0.071	0.061
MK-9	–	–	0.014	0.004	0.001	0.001
MK-10	0.003	0.005	0.003	0.003	–	0.024
MK-11	–	–	0.002	0.003	–	0.001
MK-12	–	0.003	–	0.003	–	–
MK-6(H2)	–	–	–	–	0.002	–
MK-7(H2)	0.009	0.008	0.016	0.045	0.027	0.015
MK-8(H2)	0.060	0.045	0.074	0.004	0.086	0.062
MK-9(H2)	0.006	0.016	–	0.001	–	0.016
MK-10(H2)	0.001	–	–	–	0.007	0.01
MK-8(H4)	0.111	0.081	0.133	0.039	0.050	0.065
MK-9(H4)	0.037	0.024	0.048	0.062	0.069	–
MK-10(H4)	0.047	0.047	0.059	–	0.152	0.106
UQ/MK	1.39	1.89	0.92	1.40	0.42	0.81

–; Not detected

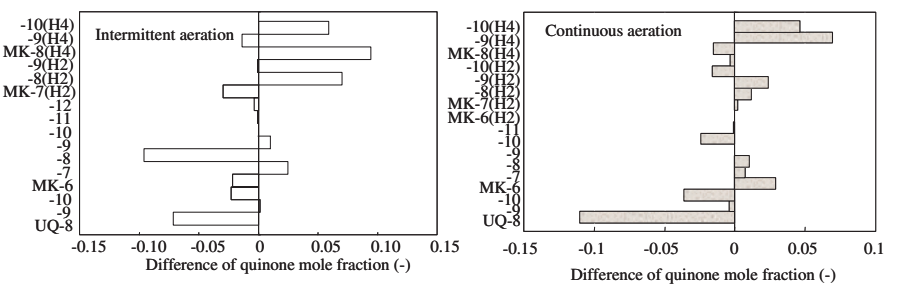

Figure 2 The differences in quinone composition between the suspended microorganisms and biofilm in the intermittently and continuously aerated SMBRs (Biofilm-Suspended)

Table 3 Microbial diversity and equability in the suspended microorganisms and biofilm on membrane tested in this study

	Activated sludge (Pilot Plant)		Intermittent aeration (SMBR1)		Continuous aeration (SMBR2)	
	Kuri	Kwangjoo	Biofilm	Suspended	Biofilm	Suspended
DQ(–)	9.9	9.6	10.9	9.4	10.4	11.2
EQ(–)	0.71	0.68	0.78	0.64	0.69	0.75

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.

The microbial diversity calculated from the composition of all quinones (including ubiquinones and menaquinones), DQ_q, which reflects the diversities of the heterotrophic bacteria, for the suspended microorganisms in the intermittently and continuously aerated SMBRs and conventional activated sludge were 9.4–11.2 and 9.6–9.9, respectively. The DQ_q for the biofilm on the membrane surface was 10.4–10.9, which was similar to that for the suspended microorganisms.

The microbial equabilities (*EQ*, defined as $EQ = DQ/n$, Hu *et al*., 1999b) for suspended and attached microorganisms 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 SMBR were less than in conventional activated sludge, which was in turn less than biofilm. The microbial equitability for Biofilm in SMBR1 was 0.78, which was larger than that for the suspended microorganisms (0.64).

Summary and conclusions

The differences in microbial community structure between suspended microorganisms and biofilm on the membrane surface in the intermittently and continuously aerated SMBRs treating domestic wastewater were evaluated by analyzing microbial quinone composition. The average removal efficiencies of COD and T-N in SMBR1 during the course of this study were greater than 96 % and 70 %, respectively. The COD and T-N removal efficiencies of SMBR2 were average 90% and less than 50%, respectively. There was an observable difference in microbial community structure between biofilm and suspended microorganisms in the SMBR1 and SMBR2. The dominant quinone types of biofilm on the membrane in both SMBRs were UQs-8, -10, followed by MKs-8(H_4), -8(H_2), -6 and -7, but those of suspended microorganisms were (UQs)-8, -10 followed by MKs-8, -9(H_4) and -6. The change in quinone profiles of biofilm on the membrane surface suggested that *Pseudomonas vesicularis*, *Staphylococcus warneri, Arthrobacter* sp., *Corynebacterium* sp. and *Nocardia* sp. contributed to microbiological fouling in the SMBR process. The microbial diversity of microorganisms attached to the membrane surface was calculated based on the composition of all quinones, and was similar to that of suspended microorganisms.

Acknowledgement

This study was supported by grant No. (R03-2001-000-00036-0) from the Basic Research Program of the Korean Science and Engineering Foundation.

References

APHA (1992). *Standard Methods for the Examination of Water and Wastewaters*, 17th edn. American Public Health Association, Washington, D.C.

Chang, I.S. and Simon, J.J. (2002). Air sparging of submerged MBR for municipal wastewater treatment. *Process Biochemistry*, **37**, 915–920.

Collins, M.D. and Jones, D. (1981). Distribution of isoprenoid quinones structural types in bacteria and their taxonomic implications. *Microbiol. Rev.*, **45**(2), 316–354.

Defrance, L., Jaffrin, M., Gupta, B., Paullier, P. and Geaugey, V. (2000). Contribution of various constituents of activated sludge to membrane bioreactor fouling. *Bioresource Tech.*, **73**, 105–112.

Flemming, H.C. and Schaule, G. (1988). Biofouling on membranes – a microbiological approach. *Desalination*, **70**, 95–119.

Fujie, K., Hu, H.-Y., Tanaka, H. and Urano, K. (1994). Ecological studies of aerobic submerged biofilter on the basis of respiratory quinone profiles. *Wat. Sci. Tech.*, **29**(7), 373–376.

Gryta, M. (2002). The assessment of microorganism growth in the membrane distillation system. *Desalination*, **142**, 79–88.

Hasar, H., Kinaci, C., Unlu, A. and Ipek, U. (2001). Role of intermittent aeration in domestic wastewater treatment by submerged membrane activated sludge system. *Desalination*, **142**, 287–293.

Hedrick, D.B. and White, D.C. (1986). Microbial respiratory quinones in the environment. I. A sensitive liquid chromatographic method. *J. Microbiol. Methods*, **5**, 243–254.

Hess, A., Hollander, R. and Mannheim, W. (1979). Lipoquinones of some spore-forming rods, lactic-acid bacteria and actinomycetes. *J. Gen. Microbiol.*, **115**, 247–252.

Hiraishi, A. (1988). Respiratory quinone profiles as tools for identifying different bacterial population in activated sludge. *J. Gen. Microbiol.*, **34**, 39–56.

Hodgson, P.H. and Fane, A.G. (1992). Crossflow microfiltration of biomass with inorganic membranes: the influence of membrane surface and fluid dynamics. *Key Eng. Mater.*, **61–62**, 167–174.

Huang, X., Liu, R. and Qian, Y. (2001). Behaviour of soluble microbial products in a membrane bioreactor. *Process Biochemistry*, **36**, 401–406.

Hu, H.-Y., Fujie, K., Tanaka, H., Makabe, T. and Urano, K. (1997). Respiratory quinone profile as a tool for refractory chemical biodegradation study. *Wat. Sci. Tech.*, **35**(8), 103–110.

Hu, H.-Y., Fujie, K. and Urano, K. (1999a). Development of a novel solid phase extraction method for the analysis of bacterial quinones in activated sludge with a higher reliability. *J. Biosci. Bioeng.*, **87**(3), 378–382.

Hu, H.-Y., Fujie, K., Nakagome, H., Urano, K. and Katayama, A. (1999b). Quantitative analyses of the change in microbial diversity in a bioreactor for wastewater treatment based on respiratory quinones. *Wat. Res.*, **33**(15), 3263–3270.

Hu, H.-Y., Lim, B.-R., Goto, N. and Fujie, K. (2001). Analytical precision and repeatability of respiratory quinones for quantitative study of microbial community structure in environmental samples. *J. Microbiol. Methods*, **47**, 17–24.

Kunihiro, D., Hu, H.-Y., Lim, B.-R., Goto, N. and Fujie, K. (2002). Comparative study on the community structure of suspended and sessile microorganisms in rivers based on quinone profile. *J. Gen. Appl. Microbiol.*, **48**, 35–41.

Lim, B.-R., Huang, X., Hu, H.-Y., Goto, N. and Fujie, K. (2001). Effects of temperature on biodegradation characteristics of organic pollutants and microbial community in a solid phase aerobic bioreactor treating high strength organic wastewater. *Wat. Sci. Tech.*, **43**(1), 131–138.

Mukai, T., Takimoto, K., Kohno, T. and Okada, M. (2000). Ultrafiltration behaviour of extracellular and metabolic products in activated sludge system with UF separation process. *Wat. Res.*, **34**, 902–908.

Nah, Y.M., Ahn, K.-H. and Yeom, I.T. (2000). Nitrogen removal in household wastewater treatment using an intermittently aerated membrane bioreactor. *Environ. Technol.*, **21**, 107–114.

Ridgway, H.F., Kelly, A., Justice, C. and Olson, B.H. (1983). Microbial fouling of reverse osmosis membranes used in advanced wastewater technology: chemical, bacteriological and ultrastructural analyses. *Appl. Environ. Microbiol.*, **45**, 1066–1084.

Zoh, K.-D. and Stenstrom, M.K. (2002). Application of a membrane bioreactor for treating explosives process wastewater. *Wat. Res.*, **36**, 1018–1024.