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EFFECT OF HRT AND INFLUENT SUBSTRATE CONCENTRATION ON NUTRIENT REMOVAL AND MICROBIAL COMMUNITY DYNAMICS IN AN ANOXIC/ANAEROBIC-AEROBIC MBR

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Abstract: In this study, effects of hydraulic retention time (HRT) and influent COD concentration on biological nutrient removal and changes in microbial community structure in an Membrane bioreactor (MBR) based system were investigated. Three different levels of CODinf and three HRTs (4, 5 & 8 hrs) were applied. Total nitrogen and total phosphorus removal efficiencies were 74% and 89%, respectively when high CODin was supplied to the MBRs. The comparison of microbial community structure between the sludges from the MBR performing poor biological phosphorus removal and from the MBR demonstrating enhanced phosphorus removal pointed out that microorganisms containing ubiquinone UQ-9, and menaquinone MK-7, -8(H2), -9(H4) and -10 (H4) were responsible for improvement in treatment efficiencies. The analysis of microbial community structure indicated that subclass γ- Proteobacteria, species of the genera Rhodoccocus, members of the genus Gordonia and Microlunatus phosphovorus can be considered potential phosphate accumulating organisms in the MBR.

Keywords: *Surface hardness, Membrane bioreactor, Hydraulic retention time, Substrate concentration, Microbial community dynamics, Nitrogen removal, Quinine profile*

1.0 Introduction

In Korea, primary effluent usually contains low concentration of soluble organic carbon (COD approximately 100 mg·L-1) and relatively high concentration of nutrients (4 mg phosphorus L-1 and 25 mg nitrogen L-1)(Choi *et al.*, 1998). The limited availability of organic substrate is a concern in the design and operation of biological nutrient removing (BNR) systems. Enhanced BNR processes largely depends on the composition of the wastewater (Temmink *et al.*, 1996). The availability of organic compounds during the anaerobic phase is important for the storage of polyhydroxybutyrate (PHB) by polyphosphate accumulating organisms (PAOs). During the subsequent aerobic phase, PHB is required for growth of the PAO's and for P-uptake (Wentzel *et al.*, 1991). The process doesn't proceed in cases of availability of limited organic substrate. Therefore, wastewater treatment plants designed for EBPR often encounter problems due to low organic loading even for short periods (Temmink *et al.* 1996). In MBR based denitrification systems, where internal recycling is utilized to create anoxic conditions (Ahn *et al.* 2003), substrate concentration becomes very important for efficient BNR. The organic loading rate can be increased to achieve good BNR by decreasing the hydraulic retention time (HRT), which is possible in MBR based systems due to presence of membranes (Cho *et al.*, 2005). In the nutrient removing MBR based systems, microbial community structure fluctuates with variation of operating conditions (Ahmed *et al.*, 2007). To our knowledge, little information is available regarding dynamics of microbial community in MBRs related to HRT. Therefore a thorough study is required to optimize the operating conditions of MBR based systems for enhanced BNR and to identify microbial community responsible for enhanced BNR in the MBR systems.

The objective of this study was to investigate the effects of different HRTs and influent substrate concentrations on changes in microbial community structure in the MBR utilized for nutrient removal from domestic wastewater. The quinone profile method (Hedrick and White 1986; Hiraishi 1999) was used to investigate the structure of microbial communities, which has been widely used for the analysis of microbial population dynamics in mixed cultures (Lim et al, 2004; Ahmed *et al.*, 2007). The comparison of microbial community structure at different applied conditions was performed to identify potential phosphate accumulating organisms (PAOs).

2.0 Materials and Methods

Two sequencing anoxic/anaerobic-aerobic membrane bioreactor were set up, each composed of a sequencing anoxic/anaerobic reactor (SAAR) and an aerobic reactor (AR). Detailed description can be found elsewhere (Ahmed *et al.*, 2007). The operating conditions adopted in this study are given in Table 1. The operational sludge retention time (SRT) was maintained at 60 days in the MBRs.

Table 1: Operating conditions of lab-scale MBR used in this study.

Operating factors	Phase		
	I	II	III
Influent Flow , L· day^{-1}	30	60	48
Flux, constant flux mode, (L· m^{-2}· hr^{-1})	12.5	25	20
HRT (hours)	8	4	5
Internal Recycling rate	2.5 × of influent flow		

The MBRs were fed with synthetic wastewater with nutrient (Acetate/glucose: 7/3). The current study was composed of three phases. The applied concentrations of chemical oxygen demand (COD), total nitrogen (TN) and total phosphorous (TP) in different phases of the experiment are given in Table 2. In phase-I, low and moderate strength synthetic water (in terms of COD) was introduced to the MBRs, which were operated at longer HRT. In phase-II, the HRT was decreased to 4 hrs aiming to increase the organic loading. In phase III, a high ratio of COD/TP was applied (HRT of 5 hr). The influents were named, according to concentration of COD in influent, as low CODinf: 109~109.8 mg·L-1, moderate CODinf: 216.2~227.5 mg·L-1,and high CODinf: 331 mg·L-1. The influent COD, TN, and TP concentrations (low and moderate CODinf) were similar in phase I & II but levels of COD and TP were increased in phase III.

Table 2: Influent characteristics used in this study.

Parameters, $mg{\cdot}L^{-1}$	Phases				
	I		II		III
	Low	Moderate	Low	moderate	High
COD	109.8 ±7.3	227.5 ±20.4	109 ±7.2	216.2 ±10.7	331.6 ±17.5
TN	22.7 ±1.5	22.73 ±0.4	23.1 ±1.8	22.5 ±1.3	21.8 ±0.5
TP	5.7 ±0.5	5.64 ±0.5	5.9 ±0.3	5.7 ±0.5	3.2 ±0.3
NH_3-N	24.4 ±0.3	22.61 ±1.2	21.1 ±1.1	22. 3 ±2.3	21.3 ±1.9

3.0 Analytical methods

Total nitrogen (TN), total phosphorus (TP) and COD were measured using Hach Digestion Vials (Hach, USA) and a Beckman spectrophotometer (DU 520, Germany). Measurement of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) followed the standard method (APHA 1992). Microbial quinones in suspended microorganisms were analyzed using the previously described method (Hu *et al.*, 1999). Microbes were centrifuged (7,000×g, 4oC for 20 minutes) and quinones were extracted using a mixture of chloroform-methanol (2:1, v/v) and subsequently extracted into hexane. Menaquinones and ubiquinones contained in the crude extract were separated and purified using a Sep-Pak® Plus Silica cartridge. The types and concentrations of the quinones were determined using a high pressure liquid chromatographer equipped with an ODS column (Mightysil RP-18, 4.6 ID x 250 mm, Kanto Chemical, Japan) and a photodiode array detector (SPDM10A, Shimadzu, Japan). Quinones are named as follows: abbreviation of the type of quinone (ubiquinone: UQ, menaquinone: MK), a dash, and the number of isoprene units in its side chain.

4.0 Results and discussions

4.1 Treatment efficiencies at long HRT

The COD removal efficiencies were above 97% during phase I and II when low and moderate CODinf were introduced in the MBRs. The MBR fed with low CODinf (HRT= 8 hrs) did not demonstrate nitrogen removal and just behaved like a nitrification reactor with almost all the nitrogen converted into nitrate (99.9% NH3-N conversion), and no nitrite detected in the effluent. The dominance of nitrification processes was probably due to the low COD/TN ratio (4.8) in the MBR fed with low CODinf as compared to the case of moderate CODinf (COD/TN= 10), and about 1.5 times higher specific TN loading rate in the MBR fed with low CODinf (0.024 kg TN· kg-1 MLVSS·day-1) than that in the MBR fed with moderate CODinf (0.017 kg TN·kg-1 MLVSS·day-1). The TN and TP removal efficiencies were slightly higher in the case when the MBR was supplied with moderate CODinf (Table 3).

4.2 Treatment efficiencies at Short HRT

The treatment efficiencies achieved at reduced HRT (4 hrs) in the MBRs supplied with low CODinf and moderate CODinf are summarized in Table 3. It is evident that the TN and TP removal efficiencies were enhanced at short HRT due to the slight increase in organic loading rates in the MBRs compared with the MBRs operated at long HRT. However, the extent of organic loading rate was not high enough to caused an enhanced biological nitrogen and phosphorus removal (volumetric organic loading rate less than 0.6 kg COD·m-3·day-1). In a previous study, enhanced nutrient removal was observed when volumetric organic loading rate was 3.95 kg COD·m-3·day-1, corresponding to average influent concentration of 304 mg COD·L-1 (Kim *et al.*, 2009). It seems that phosphorus release and uptake became less significant at low COD/TP. Lee *et al.* 1997) found that a higher COD/TP ratio was necessary for luxury uptake of phosphorus. Enhanced biological nutrients removal was observed by Zhang *et al.* (2009) in a MBR based system at COD/TN/TP ratio of 28.5/5.1/1. The phosphorus release during the anaerobic period may have been influenced by nitrate in recycled sludge due to the increased sludge recycling rate at short HRT.

Table 3: Treatment efficiencies achieved in different phases of the experiment.

Parameter	Phases (removal, %)				
	I		II		III
	Low COD_{inf}	Moderate COD_{inf}	Low COD_{inf}	Moderate COD_{inf}	High COD_{inf}
COD	97.5 ±2.7	99.9 ±0.05	99.9 ±0.04	99.9 ±0.04	99.85 ±0.2
TN	0.8 ±6.2	31.7 ±2.3	17.2 ±2.4	57.6 ±3.1	74.16 ±4.9
TP	5.5 ±o.6	13.1 ±0.7	7.0 ±1.3	17.2 ±2.1	89.2 ±4.5
NH_3-N	99.9 ±0.05	99.9 ±0.05	97.5 ±1.29	98.9 ±0.52	99.89 ± 0.1

4.3 COD/TP ratio

In the MBR operated at short HRT (4 hrs), low treatment efficiencies were observed. Therefore, the concentration of influent COD was increased and the concentration of TP was reduced (COD/TP = 103). In addition, the HRT was set at 5 hrs in the phase III. At steady state, the TP and TN removal efficiencies were increased up to 89.2% and 74.16%, respectively (Table 3). It can be concluded that increased organic loading and higher COD/TN and COD/TP ratios positively influence performance of the MBR.

4.4 Specific organic loading

The TN and TP removal efficiencies achieved in the three phases of the experiment are shown as a function of specific loading rate in Figure 1. The increase in TN removal efficiency was linear as the specific organic loading rate increased. However, the TP removal efficiency improved sharply when applied specific loading rate was increased up to 0.3 kg COD kg-1· MLVSS· day-1. This sharp increase in TP removal can be explained by focusing on the fact that the COD/N and COD/TP ratios (15.2 and 103.2 respectively) were comparatively higher than the MBRs fed with low and moderate CODinf (ranges 4.8~10 and 18.7~40.4 respectively).

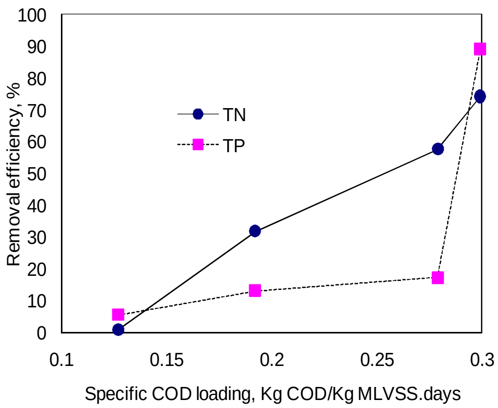

Figure 1: TN and TP removal efficiencies at different specific organic loadings.

4.5 Microbial community structure

Isoprenoid quinones were extracted from the sludge of the MBRs which had reached steady state conditions. The dominant quinone type in the sludges of operated MBRs was UQ-8, usually derived from subclass β-Proteobacteria (Hiraishi *et al.*, 1998). The second most dominant ubiquinone was UQ-9. Differences in quinone compositions of suspended microorganisms between sludges from the MBR fed with high CODinf (HRT=5 hrs) and the MBR fed with low CODinf (HRT=8hrs) are shown in Figure 2. In Figure 2, equal mole fractions of quinones in both conditions have assigned a value of zero whereas positive and negative values indicates increase and decrease in the mole fractions, respectively. It is important to recall that the MBR fed with high CODinf demonstrated high TP removal (89.2%) and the MBR fed with low CODinf (HRT=8 hrs) exhibit poor TP removal. Therefore, comparison of the microbial community structures should be helpful in identification of microorganisms responsible for EBPR. Figure 2 also reveals that the mole fraction of UQ-8 was higher in the MBR fed with low CODinf (HRT 8 hrs) than that in the MBR fed with high CODinf (HRT 5 hrs), whereas the mole fraction of UQ-9 were higher in the MBR fed with high CODinf. A relative increase of mole fraction of UQ-9 was observed when HRT was reduced from 8 hours to 4 hours in both the MBRs (data not shown). As reduction in HRT and increase in influent COD concentration caused improvement in TP removal efficiency along with increase in mole fraction of UQ-9, microorganisms containing UQ-9 can be considered as potential PAOs in the MBR. An increase of UQ-9 along with relatively stable EBPR activity has been reported previously (Lin *et al.*, 2000), therefore, microorganisms containing UQ-9 (subclass γ- Proteobacteria)(Hiraishi *et al.*, 1998) can be considered responsible for improvement in TP removal.

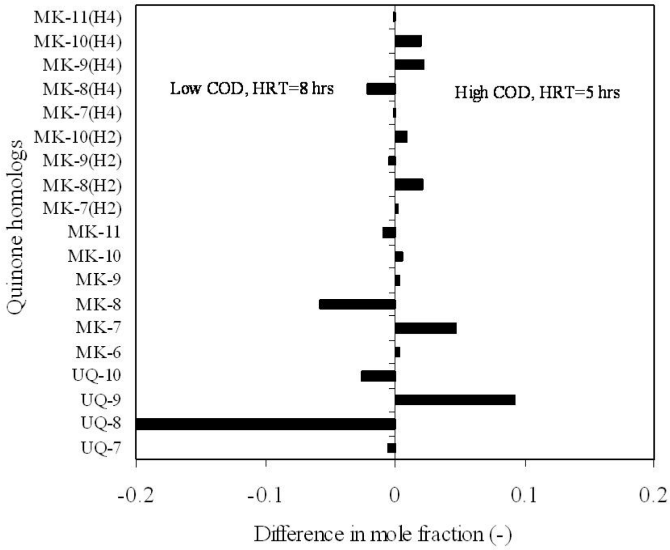

Figure 2: The differences in quinone composition between sludge from MBR fed with low CODinf (HRT= 8 hrs) and from MBR fed with high CODinf (HRT= 5 hrs).

The comparison of menaquinone mole fractions in sludge between the MBR (HRT 8 hrs, low CODinf) and the MBR (HRT 5 hrs, high CODinf) (Figure 2) reveals an increase in MK-6, -7, -9, -10, -8(H2), -10(H2), -9(H4) and 10(H4) in the MBR fed with high CODinf. Microorganisms which contains these quinone homologs can also be considered potential PAOs. MK-7 and MK-6 are reported to be derived from gram-positive bacteria with a low G+C content, δ- and ε-subclass Proteobacteria and members of the Cytophaga-Flavobacterium cluster (Hiraishi 1999; Kurisu *et al.*, 2002). MK-7 to 11 and partially hydrogenated MKs are observed in Bacteroidetes and Actinobacteria (High G+C gram-positive bacteria). Species of the genera Rhodoccocus contain MK-8 (H2) as the major quinone type, and a member of this genus can utilize numerous carbon sources for respiration(Briglia *et al.*, 1996). Member of the genus Gordonia, isolated from an autotrophic denitrification reactor unit contain MK9(H2) as the predominant menaquinone (Kim *et al.*, 2003). Presence of MK-9(H4) and MK-10(H4) among partially hydrogenated MK suggest that actinobacterial PAO may have been contributing to the EBPR process in the MBR fed with high CODinf. Nakamura *et al.* (1995) isolated Microlunatus phosphovorus, polyphosphate-accumulating bacteria from activated sludge. These organisms were gram-positive, coccus-shaped, aerobic chemoorganotrophs and contained MK-9(H4) as a major quinone. An important finding was a low mole fraction of MK-8(H4) in the MBR fed with high CODinf. It is in contrast with reported findings that MK-8(H4) was a dominant homolog from a sequential batch reactor performing efficient EBPR (Onda *et al.*, 2002). In our study, MK-8(H4) was identified in the MBR fed with low CODinf (HRT 8 hrs) which demonstrated poor EBPR activity. It indicates that Microsphaera multipartite (Yoshimi *et al.*, 1996) and Kineosphaera limosa (Liu *et al.*, 2002), a genus within the Dermatophilaceae were populated in the MBR fed with low CODinf. These bacteria contain MK-8(H4) as a major menaquinone but show low EPBR activity.

5.0 Conclusions

Different organic loading rates were applied in an MBR based biological nutrient removal (BNR) system by varying influent concentration and by changing hydraulic retention time. The results demonstrated that increases in organic loading rate had a positive impact on BNR process. The optimum specific organic loading rate was found to be 0.3 kg COD·kg-1 MLVSS·day for the MBR based BNR system used in this study. Microbial community structure was studied using the quinine profile method. Comparison between mole fractions of quinones, extracted from sludges of the MBRs demonstrated enhanced and poor biological phosphorus removal, revealed that microorganisms containing ubiquinone UQ-9, and menaquinone MK-7, -8(H2), -9(H4) and -10 (H4) were responsible for enhanced biological phosphorus removal. The microorganisms belonging to subclass γ- Proteobacteria, species of the genera Rhodoccocus, members of the genus Gordonia and Microlunatus phosphovorus can be considered potential phosphate accumulating organisms in the MBR system used in this study.

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