J Ind Microbiol Biotechnol (2012) 39:19–25
DOI 10.1007/s10295-011-0994-8

ORIGINAL PAPER

Structure and dynamics of microbial community in full-scale activated sludge reactors

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Received: 11 October 2010 / Accepted: 14 May 2011 / Published online: 2 June 2011

Abstract Phospholipid fatty acid (PLFA) profiles in four full-scale activated sludge reactors (ASR1 ~ 4) treating municipal wastewater, South Korea, were monitored to evaluate the influence of influent water quality on microbial community structure (MCS) and the effect of the MCS on effluent water quality. In ASR1 ~ 3, PLFA profiles were very similar, regardless of the influent water quality and seasonal differences, and 16:17c/15:0*iso*2OH and 16:0 were dominant. PLFA profiles in ASR4 during summer and autumn were very similar to those in ASR1 ~ 3, but increases in specific fatty acids, 16:1ω5c, 11methyl18:1ω7c and 15:0*iso*3OH, were found in ASR4 during winter and spring, with relatively high total suspended solid (TSS) concentrations in the effluent. 16:1ω5c and 15:0*iso*3OH, possibly related with *Flexibacter* sp., caused a bulking problem in the activated sludge. The community diversity indices such as Shannon diversity and equability decreased in summer but increased in autumn in all the ASRs. Canonical correspondence analysis results suggested that the influent BOD concentration played the most important role in changing MCS, followed by influent TSS concentration. In addition, the TSS and total phosphorus concentrations in the effluent were significantly affected by the change of the MCS.

Keywords Phospholipid fatty acid · Microbial community structure · Full-scale activated sludge reactor · Canonical correspondence analysis

Introduction

Activated sludge technology is one of the most widely used for wastewater treatment. The efficiency of an activated sludge process is heavily dependent upon the performance of the biological stage because this is where the removal of organic pollutants mainly occurs. Complex microbial communities in activated sludge processes are essential for maintaining stable removal efficiency, although conventional activated sludge processes have been successfully operated for treating a broad range of wastewaters. Activated sludge is typically prone to sludge settlement problems, such as bulking and foaming caused by non-floc forming or filamentous microorganisms [5, 8], due to fluctuations in the wastewater and variation in the wastewater composition, as well as toxic chemicals [16]. The key to an efficient biological wastewater treatment relies on understanding the microorganisms involved and how they respond to different operational and environmental conditions [21]. Few studies have been conducted on enhancing the understanding of the microbial community structure (MCS) in activated sludge reactors (ASRs). Eichner et al. [10] reported the effect of the feed strength and operating conditions on the population structure of the biomass. Zhuang et al. [30] and Wilen et al. [26] focused on the bacterial diversity dynamics of aerobic sludge flocs using DNA-based molecular tools, and suggested that flocculation and deflocculation were affected by the microbial composition of the floc structure.

Electronic supplementary material The online version of this article (doi:10.1007/s10295-011-0994-8) contains supplementary material, which is available to authorized users.

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Despite past efforts, knowledge on the microbial communities in full-scale ASRs, as affected by the operational and environmental variations, is still insufficient, as most studies for practical purposes have focused on lab/pilot-scale reactors [11, 15, 17, 21]. In most cases, the microbial composition found in lab/pilot-scale reactors has been inconsistent or misleading, due to the different scales of the technological equipment, which makes it impossible to reproduce process parameters, such as the degree of oxygenation, hydrodynamic conditions in the aeration tank, the recycle ratio, and sludge age [3]. The lab-scale reactors and artificial conditions cause the microbial community to experience a huge transition in adapting to a new environment [3].

Few studies have been aimed at investigating the fate of microbial communities in full-scale wastewater treatment systems during sufficiently long operation periods [1, 24]. However, the main question of the relationships between MCS and the influencing factors remains unanswered; i.e., how the influent water quality affects the MCS.

In this study, the relationship between the MCS and water quality parameters was evaluated by choosing four full-scale wastewater treatment plants, operating conventional activated sludge systems as their main treatment process, with similar operational conditions and types of wastewater, i.e., municipal wastewater.

Materials and methods

Activated sludge sampling

The activated sludge was sampled from the ASRs in four full-scale wastewater treatment plants, located in Seoul (WWTP1, WWTP2, and WWTP4) and one (WWTP3) in Kyonggi-do, South Korea, to evaluate the MCS. The operational conditions of the wastewater treatment plants are shown in Table 1. The treatment capacities of WWTP1, 2, 3, and 4 were 1,710,000, 1,100,000, 1,000,000, and 2,000,000 m^3 day^{-1}, respectively. The mixed liquor suspended solid (MLSS) concentrations of the ASRs were all around 2,000 mg l^{-1}. The food-to-microorganism ratio (F/M) was within the range of 0.15–0.3 mg mg^{-1} d^{-1}. The hydraulic retention time (HRT) and solid retention time (SRT) were 6–10 h and 4.5–10 days, respectively. The biochemical oxygen demand (BOD) and nutrient concentrations in influent and effluent are shown in supplementary data (Figs. 1S and 2S), which were obtained from the following Web sites and personal communications (www.seonam.seoul.kr/; www.tancheon.com/natural/natural_06.ASR; www.nanjihasu.seoul.go.kr/index.html; http://jhasu.seoul.go.kr/). The average of influent BODs in ASR1, ASR2, ASR3, and ASR4 were 116, 146, 121, and 105 ml^{-1}, respectively. The range of the influent concentrations of total nitrogen (TN) and total phosphorus (TP) were 22–38 and 13–24 mg l^{-1}, respectively. The average effluent concentrations of TN in ASR1 to ASR4 corresponded to 63, 54, 49, and 72% influent TN contents, respectively. The BOD:TP and BOD:TN ratios were within normal ranges (BOD:TN = 20–5:1 and BOD:TP = 53–31:1) in all the ASRs [4, 27].

Table 1 Operating parameters of activated sludge reactors

Activated sludge reactors	ASR1	ASR2	ASR3	ASR4
MLSS (mg l^{-1})	2,000	2,200	2,000	1,800
F/M ratio	0.3	0.25	0.2–0.15	0.21
HRT	7	6	9–10	7
SRT	6	4.5	7–10	6.5

F/M ratio food-to-microorganism ratio (mg mg^{-1} d^{-1}), *HRT* Hydraulic retention time (h), *SRT* Solid retention time (day)

Activated sludge samples were taken at different times from the wastewater treatment plants on May 21, 2004 (in spring; ASR1-1, ASR2-1, ASR3-1, and ASR4-1), July 30, 2004 (in summer; ASR1-2, ASR2-2, ASR3-2 and ASR4-2), September 25, 2004 (in autumn; ASR1-3, ASR2-3, ASR3-3, and ASR4-3) and January 6, 2005 (in winter; ASR1-4, ASR2-4, ASR3-4, and ASR4-4). Activated sludge samples were carried in a potable container, with ice packs, and stored at 4°C prior to treatment.

Analyses of the MCS using PLFAs

Phospholipid fatty acids (PLFAs) were extracted from the activated sludges using a modification to the method proposed by Drenovsky et al. [9]. In brief, 1 ml of 3.75 M NaOH solution in a mixture of methanol (MeOH) and H_2O (1:1, v/v) was added to 0.3 g of each of the harvested activated sludges in 50-ml centrifuge bottles (Nalge Company, New York, USA). The samples were vortexed for 1 min and sealed completely using Teflon tape. The samples were then boiled in a 100°C water bath for 25 min. Following this saponification step, the fatty acids (FAs) were converted to the fatty acid methyl ethers (FAMEs) by adding 2 ml of 6.0 HCl:MeOH (1:0.85, v/v) to each sample. The samples were sealed with caps and vortexed for 1 min, placed in an 80°C water bath for 10 min, with 1.25 ml of the hexane:methyl *tert*-butyl ether (MTBE, 1:1, v/v) solution then added to each sample to extract the FAMEs. Following the addition of hexane:MTBE (1:1, v/v) solution, each sample was shaken smoothly for 10 min, with the bottom layer (organic phase) then

removed using a Pasteur pipette. The supernatant was washed with 5 ml of a mild base (0.3 M NaOH) and then gently mixed for 5 min. A few drops of saturated NaCl solution were added to each sample and allowed to stand until separation of the layers. The top layer was transferred with a Pasteur pipette into a gas chromatography (GC) vial and dried and concentrated with N_2 gas. The extracted PLFA samples were analyzed using GC (HP6890, Agilent Technologies Inc., Palo Alto, CA, USA), as previously described [25]. The PLFA peaks were tentatively identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE, USA). A standard sample was used from a calibration standards kit for the identification of the microbial system (Microbial ID, Inc., Newark, DE, USA), as specified by the manufacturer. The PLFAs were designated using the nomenclature described in Frostegård et al. [12].

Diversity and statistical analysis

Based on the relative abundance values of each PLFA (p_i) and total numbers of PLFA (S), the Shannon diversity index (H) for microbial community diversity, and equitability index (J) for microbial community evenness were calculated by using the following equations [19].

$$H = -\sum p_i \log p_i$$
$$J = H/\log S$$

Principal component analysis (PCA) was performed using p_i value to evaluate the similarities of the microbial communities between the sampled activated sludges using SPSS software version 17.0 K (SPSS Inc., Chicago, IL, USA).

The effect of the influent water quality, such as BOD, TP, TN, and total suspended solid (TSS), on the MCS, and the effect of MCS on the effluent water quality were evaluated via a canonical correspondence analysis (CCA) using Canoco version 4.5 software (Biometris, Netherlands), with the ordination plots of species and environmental variables characterized using biplots. A manual forward selection, applying a partial Monte Carlo permutation test (499 permutation), including unrestricted permutation, was used to select key variables that strongly influenced the microbial community composition. The marginal effects of environmental variables were produced via a CCA. The partial Monte Carlo permutation test provided the conditional effect of each variable. The ordination diagram represents not only a pattern of microbial community distribution but also features of the distribution of species coupled with the environmental variables [22].

Chemicals

The following chemicals were used for this study: HCl (hydrochloric acid, Showa Chemical Co., Ltd., Tokyo, Japan), hexane (Merck KGaA, Darmstadt, Germany), MeOH (methanol, Merck KGaA, Darmstadt, Germany), MTBE (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), NaCl (sodium chloride, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and NaOH (sodium hydroxide, Sigma–Aldrich Chemie GmbH, Steinheim, Germany).

Results and discussion

Comparison of PLFA profiles

The MCS were compared between the target ASRs by evaluating the PLFA profiles (Fig. 1). In ASR1, ASR2, and ASR3, 16:1ω7c/15iso2OH and 16:0 were dominant, which are known as possible biomarkers of *Pseudomonas* sp. and is commonly shown as a dominant microorganism in conventional activated sludge systems [7, 18]. PCA also shows that the PLFA profiles were very similar in the ASR1 $\sim$ 3, regardless of seasonal and regional differences. The PLFA profiles as well as dominant PLFAs in the ASR4 during summer and autumn (ASR4-2 and ASR4-3) were similar to those in the ARS1 $\sim$ 3 (Figs. 1 and 2). However, increases in specific fatty acids, 16:1ω5c, 11methyl18:1ω7c and 15:0iso3OH, were found in the ASR4 during winter and spring (ASR4-1 and ASR4-4) (Figs. 1 and 2), with relatively high total suspended solid (TSS) concentrations in the effluent up to 10 mg l^{-1} from 4 mg l^{-1} (data not shown). Kämpfer et al. [13] showed that 16:1ω5c and 15:0iso3OH were possible biomarkers of *Flexibacter* sp., which appears during the bulking problem [6, 14]. Therefore, it was assumed that the increases in these PLFAs as well as TSS concentration were possibly related to the slight bulking problem. However, regardless of any variation in PLFA profiles, stable BOD removal efficiencies were observed in the ASR4 during all season (Figs. 1S and 2S in supplementary data). A possible explanation for stable BOD removal was that the change of dominant species (16:1ω5c and 15:0iso3OH) did not affect the BOD removal as they could utilize the organic compounds present in the municipal wastewater. In many cases, if the composition of the substrate and nutrients, such as the BOD:TN or BOD:TP ratio, is in the normal range, most of the microorganisms present in activated sludge have the ability to utilize the organic compounds present in municipal wastewater. The results showed that similar operating condition led to similar PLFA profiles in different wastewater treatment plants. In addition, the high degree of similarity in PLFA profiles was shown regardless

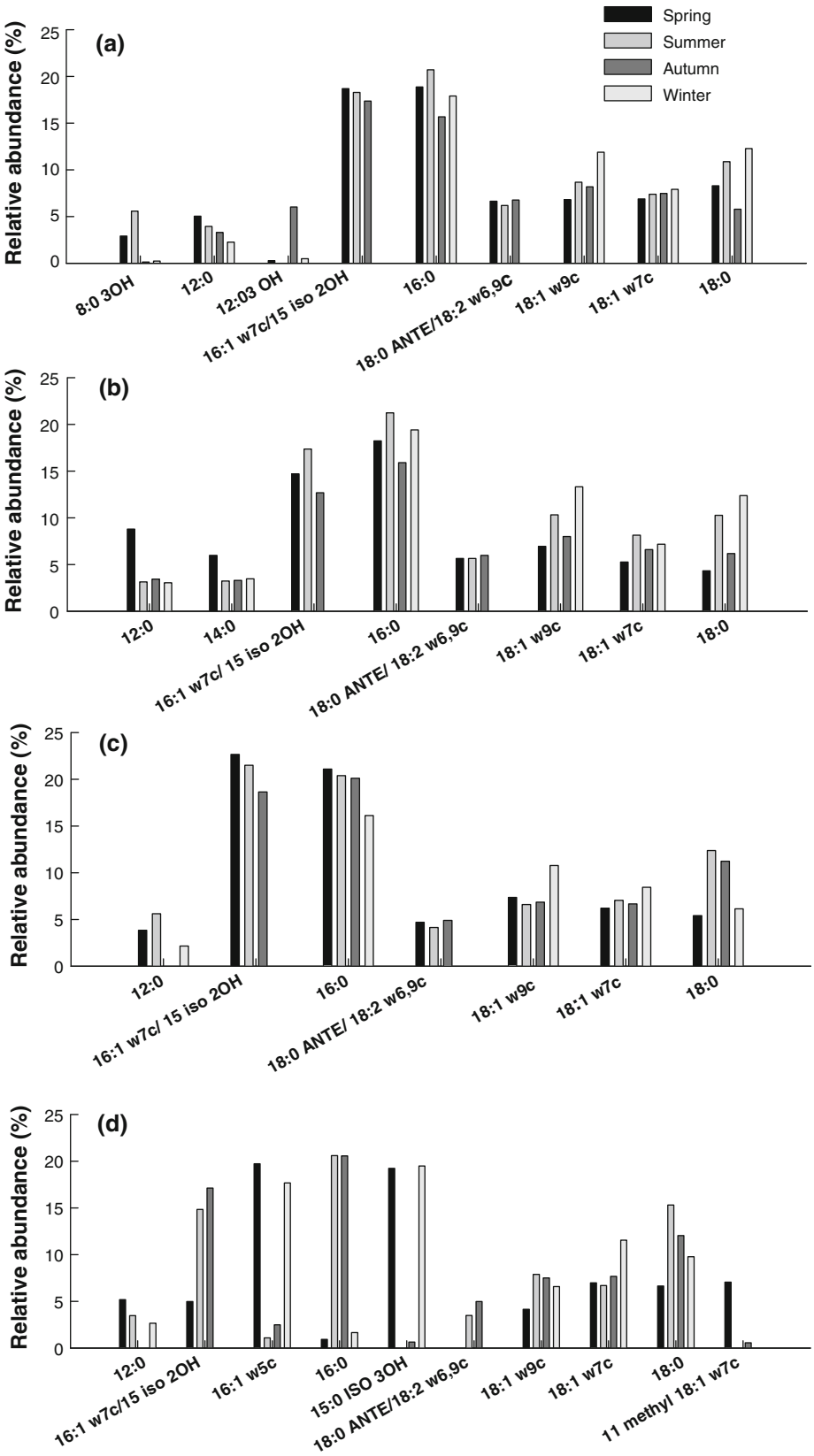

Fig. 1 PLFA profiles in ASR1 (**a**), ASR2 (**b**), ASR3 (**c**), and ASR4 (**d**)

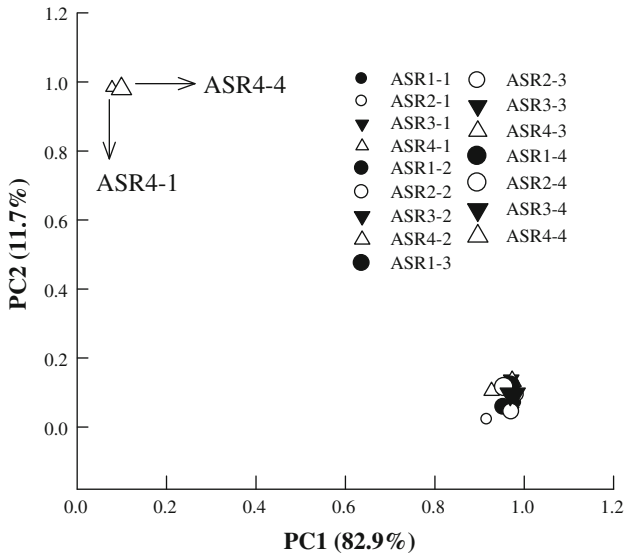

Fig. 2 Principal component analysis (PCA) of the microbial community structures based on the percentage distributions of PLFA derived from four target wastewater treatment plant samples. ASR1-1 (spring in ASR1); ASR1-2 (summer in ASR1); ASR1-3 (autumn in ASR1); ASR1-4 (winter in ASR1); ASR2-1 (spring in ASR2); ASR2-2 (summer in ASR2); ASR2-3 (autumn in ASR2); ASR2-4 (winter in ASR2); ASR3-1 (spring in ASR3); ASR3-2 (summer in ASR3); ASR3-3 (autumn in ASR3); ASR1-4 (winter in ASR3); ASR4-1 (spring in ASR4); ASR4-2 (summer in ASR4); ASR4-3 (autumn in ASR4); ASR4-4 (winter in ASR4)

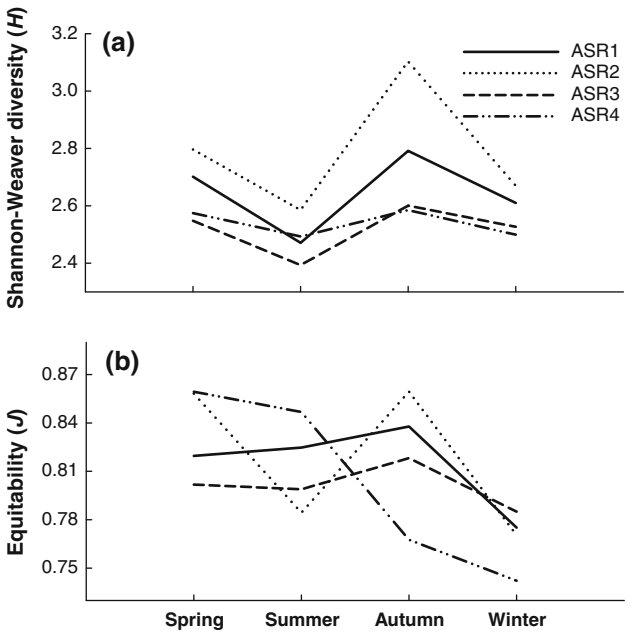

Fig. 3 Comparison of the microbial diversity: **a** diversity (*H*), **b** equitability (*J*), and **c** richness (*S*)

of seasonal variation of nutrient and substrate concentrations. Possibly, stability of wastewater treatment performance was caused by stable MCS (Figs. 1S and 2S in supplementary data).

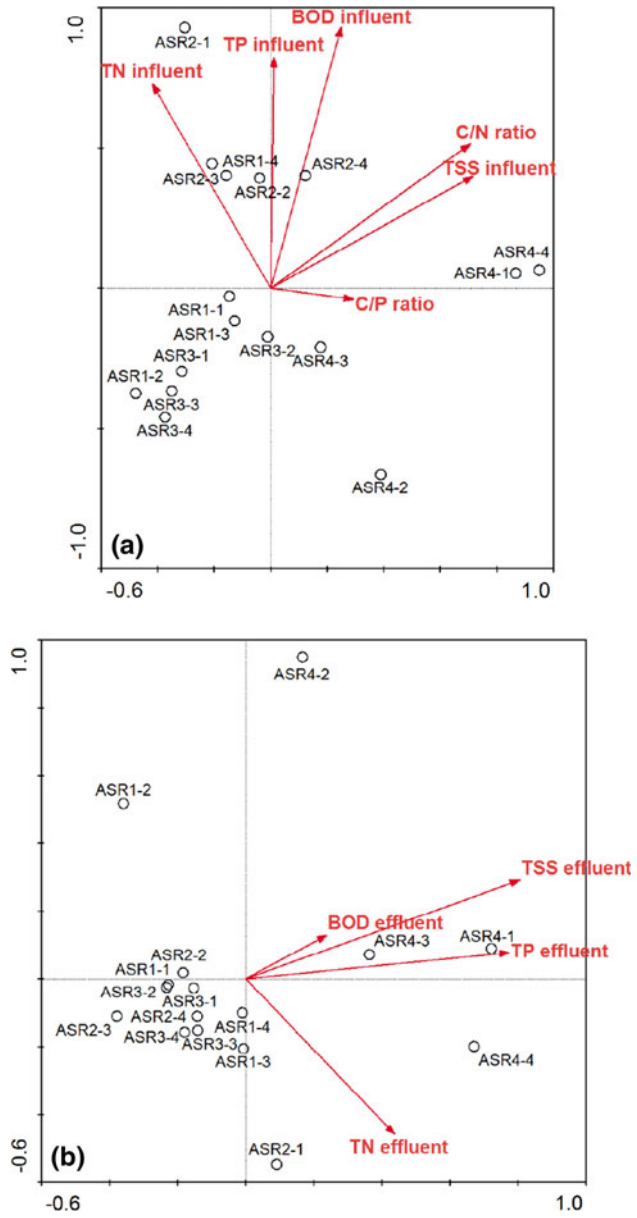

Fig. 4 Correlations between the environmental factors and microbial community structures based on a canonical correspondence analysis (CCA): **a** CCA biplot of influent variables and PLFA profiling data of four ASRs. **b** CCA biplot of effluent variables and PLFA profiling data of four ASRs

Microbial community diversity

Microbial community diversity indices, *H, J*, and *S,* in the ASRs were evaluated using the PLFA profiles (Fig. 3). All the ASRs had high similarities in their *H, J,* and *S* trends; decreases in summer and increases in autumn. This pattern coincided well with the influent BOD profiles (Figs. 1S and 2S in supplementary data), and the most stable BOD removal efficiencies were shown in ASR2, which had the highest BOD loadings and *H* values. In contrast, ASR3 had

Table 2 Marginal and conditional effects of forwardly selected environmental variables produced by canonical correspondence analysis

Community analysis	Water type	Environmental variables	Marginal effects, $\lambda 1$	Conditional effects		
				λA	p	F
Interspecies distances of ASRs	Influent	BOD concentration	0.08	0.12	0.012	3.19
		TSS concentration	0.2	0.2	0.022	4.1
		TN concentration	0.1	0.1	0.062	2.18
		C/N ratio	0.2	0.05	0.424	0.92
		TP concentration	0.05	0.04	0.48	0.9
		C/P ratio	0.06	0.02	0.878	0.52
	Effluent	TSS concentration	0.24	0.24	0.002	5.09
		TP concentration	0.23	0.11	0.032	2.7
		TN concentration	0.11	0.09	0.05	2.61
		BOD concentration	0.06	0.08	0.108	1.78

lowest H values and BOD loadings. Thus, the high influent BOD level decreased the competition between the microorganisms, and it led to high microbial diversity in ASR2. Tan et al. [21] also reported similar observations, and suggested a low substrate concentration leads to high competitive exclusion dominance, which might reduce the diversity.

Relationship between water quality and MCS

The influent water quality will affect the MCS, whereas the effluent water quality is affected by the MCS. Therefore, a CCA analysis was conducted in two dimensions; for the relationship between the influent water quality and the PLFA profiles and between the PLFA profiles and the effluent water quality (Fig. 4; Table 2). Cumulative species-water quality relationships for the axes F1 and F2 of the CCA were 79.9 and 81.9% in influent and effluent water qualities, respectively (data not shown). The species-environment correlations for both axes were >0.84 and >0.93 (data not shown). The influent BOD concentrations displayed a positive correlation with the PLFA profiles (Fig. 4a; Table 2). Another positive correlation was found with the influent TSS concentration (Fig. 4a; Table 2). High BOD loading could influence microbial diversity by reducing competition between the heterotrophic microorganisms [21], which might slightly affect overall MCS. In addition, influent BOD level affects the growth of autotrophic microorganisms by changing the BOD/TN ratio (or COD/TN ratio) [2, 28], which may have arisen as a consequence of the competitive ability of heterotrophic microorganisms being dependent on the quantity of the available carbon substrate. When TSS was fed at a high concentration, the oxygen transfer was inhibited, which lead to competition for oxygen [15], which consequently affected the MCS.

The MCS played the most important role in the variation of the effluent TSS concentration, and the TP concentration might play the second most important role (Fig. 4b; Table 2). The PLFA profiles in ASR4 during spring and winter were clearly separated from the others (Fig. 4b). The effluent TSS concentration is highly sensitive to increased non-floc forming microorganisms in the aeration basin since the filaments and non-floc forming microorganisms are continuously removed via a secondary clarifier as a selector [29]. Tyagi et al. [23] also reported that filamentous microorganisms were relatively high in the effluent from a conventional activated sludge process. Although the influence of the MCS on TP removal remains unclear, TP removal may be affected by specific species, which uptake excess phosphorus, called phosphorus accumulating organisms [20].

Influent water quality affected the MCS and the MCS and had an influence on effluent water quality. In this study, four full-scale activated sludge reactors, which have similar operating conditions and influent quality, were chosen to investigate the dynamic and characteristics of the MCS in activated sludge and the interaction between the MCS and influent and effluent water quality using PLFA profiling. Although seasonal variations in diversity and equality and in influent water quality were observed, the performances and the PLFA profiles of activated sludge processes were stable regardless of the seasonal and regional variation. The results proved that the stable MCS is an important factor for activated sludge performances, and showed that the MCS in the activated sludge was affected by BOD and TSS concentration. This indicates that the change of the MCS was influenced more by substrate concentration than by nutrient concentration. However, the nutrient concentrations such as TP in effluent were affected by MCS. In addition, it is shown that the variation of TSS concentration in effluent was highly attributed to the change of MCS. Conclusively, this research increased understanding of the performance of the activated sludge process by

investigating the structural dynamic of community in activated sludge.

Acknowledgments This study was supported by an RP-Grant 2009 from Ewha Womans University. The authors appreciate the government of Seoul City, South Korea, for supporting the influent and effluent water quality information of WWPTs.

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