Characterization of Microbial Community in Biological Wastewater Treatment System Using Respiratory Quinone Profiles

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Abstract

The dynamics of microbial community structure of the various domestic wastewater treatment processes were examined using a novel approach of quinone profiles. The compositions of microbial quinone of 5 sites for plant and lab-scale activated sludge were analyzed. More than 14 kinds of quinones were observed in the activated sludges tested in this study. The microbial community structure of the plant activated sludge processes a little differed from that of the lab-scale submerged MBR systems. The dominant quinones were UQ-8, UQ-10 followed MK-8(H_4), MK-7 and MK-6.The molar ratio of ubiquinones to menaquinones (UQ/MK) changed from 0.81 to 1.9, indicating that aerobic bacteria dominated the microbial community of the activated sludge examined. The microbial diversity of the activated sludges calculated from the all quinone compositions was 9.5-11.9 and the microbial equability of the activated sludges was 0.64-0.79.

Keywords: Activated sludge; microbial community structure; microbial diversity; quinone profile

Introduction

The biological wastewater treatment process is a complex microbial ecosystem composed of many kinds of bacteria, protozoa and metazoa. Analyses of the population dynamics of such ecosystem are of great importance in selecting the optimal operating conditions to give a good effluent water quality from the treatment process. However, only limited information is available on the microbial community activities and interactions associated with these processes. It is very difficult, however, to clarify the microbial community change in the treatment process by conventional techniques using selective medium and so on.

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 (Hiraishi 1988; Fujie *et al.*, 1998; Lim *et al.*, 2001; Kunihiro *et al.*, 2002). Microbial respiratory quinones are components of 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. 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 a microbial community of a mixed culture of microbes could be quantified using the quinone profiles. The objective of this study is to clarify the microbial community structure in various biological treatment processes treating domestic wastewater. Microbial quinone profile was used as a new tool for the study on microbial community structure.

Methods

Source of activated sludges investigated in this study

Plant activated sludge was taken from the domestic wastewater treatment plants located in Guri (activated sludge process, AS1), Gwangju (activated sludge process, AS2) and Gwangdongri (intermittently decanted extended aeration process, KIDA), Gyeonggi, Korea. The laboratory activated sludges used in our study were obtained from the intermittently aerated (SMBR1) and continuously aerated submerged membrane bioreactor (SMBR2) systems.

Analytical methods

Microbial quinones in activated sludge 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.) x 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. For example, UQ-10 represents a ubiquinone with 10 isoprenoid units, and MK-9(H2) represents a menaquinone with 9 isoprenoid units and one of the 9 units is hydrogenated with 2 hydrogen atoms.

Results and discussion

The analytical results of quinone profiles in the activated sludges examined in this study are shown in Table 1. Three types of ubiquinone (UQs-8, -9, and -10) were found in all the samples. The activated sludge in plant activated sludges and lab-scale submerged MBR systems contained 14-15 types of menaquinone. The dominant type of ubiquinone was UQ-8 followed by UQ-10 and UQ-9 in all the activated sludges examined. The dominant type of menaquinone in plant activated sludge was MK-8(H_4) followed by MK-7 and MK-8(H_2). But that of the SMBR systems was MK-6 followed by MKs -8, -7 and -10(H_4).

Table 1. Composition (mole fraction) of the quinones in the activated sludges

	Plant activated sludge			Laboratory activated sludge	
Quinone type	AS1	AS2	KIDEA	SMBR1	SMBR2
Ubiquinones					
UQ-8	0.332	0.415	0.300	0.352	0.281
UQ-9	0.051	0.059	0.054	0.059	0.046
UQ-10	0.199	0.180	0.118	0.161	0.120
Menaquinones					
MK-6	0.043	0.034	0.071	0.080	0.115
MK-7	0.068	0.058	0.087	0.047	0.078
MK-8	0.033	0.024	0.084	0.135	0.061
MK-9	-	-	0.006	0.004	0.001
MK-10	0.003	0.005	0.027	0.003	0.024
MK-11	-	-	0.018	0.003	0.001
MK-12	-	0.003	-	0.003	-
MK-7(H2)	0.009	0.008	-	0.045	0.015
MK-8(H2)	0.060	0.045	0.029	0.004	0.062
MK-9(H2)	0.006	0.016	0.015	0.001	0.016
MK-10(H2)	0.001	-	-	-	0.010
MK-8(H4)	0.111	0.081	0.104	0.039	0.065
MK-9(H4)	0.037	0.024	0.040	0.062	-
MK-10(H4)	0.047	0.047	0.083	-	0.106
UQ/MK	1.39	1.89	0.89	1.40	0.81

The values of UQ/MK ratio in plant activated sludge and lab-scale submerged MBR systems varied from 0.81 to 1.89. It is believed that ubiquinones and menaquinones are specific indicators of aerobic bacteria and anaerobic bacteria, because ubiquinones and menaquinones are involved during aerobic and anaerobic respiration, respectively. Therefore, a UQ/MK value of >1 would suggest that aerobic bacteria were dominant in the activated sludges examined in this study.

The microbial diversities (*DQ*) for suspended and attached microorganisms calculated from the quinone composition using Equation ($DQ= \sum[(f_k)^{1/2}]^2$) (Hu *et al.*, 1999b) are shown in Table 2. Where, f_k is the mole fraction of quinone species k and n is the number of quinone species with the mole fraction higher than or equal to 0.001.

The microbial diversity calculated from the composition of all quinones (including ubiquinones and menaquinones), DQ_q, which reflects the diversity of heterotrophic bacteria, for the plant activated sludges changed from 9.6-11.9. The average of DQ_q for lab-scale SMBR systems was 9.5-11.2, which was similar to that for the plant activated sludges.

In this study, we stressed dissimilarity rather than similarity, and thus defined the dissimilarity index (D) as follows (Hiraishi *et al.*, 1991): $D\ (i, j) = 1/2\sum | f_{kj}-f_{kj} |$ Where, f_{kj} and f_{kj} are the percentages of the k quinone component for the i and j samples, respectively. The dissimilarity values for the 5 sludge samples were obtained from total quinone profiles (Table 3). The dissimilarity level at which the plant activated sludges and lab-scale submerged MBR systems could be separated form each other, it is suggested that a D level between 10-20% and 15-30% can be used as a criterion for classifying bacterial communities of wastewater sludges.

Table 2. Microbial diversity and equability

	Plant activated sludge			Laboratory activated sludge	
	AS1	AS2	KIDEA	SMBR1	SMBR2
DQuq (-)	1.6	1.7	1.3	1.5	1.2
DQmk(-)	3.6	3.2	5.4	3.4	5.1
DQq(-)	9.9	9.6	11.9	9.5	11.2
EQ(-)	0.71	0.68	0.79	0.64	0.75

Table 3. Dissimilarity level

	Plant activated sludge			Laboratory activated sludge	
	AS1	AS2	KIDEA	SMBR1	SMBR2
AS1		0.107	0.178	0.240	0.218
AS2			0.229	0.240	0.251
KIDA				0.250	0.146
SMBR1					0.302

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