J. Gen. Appl. Microbiol., **49**, 135–139 (2003)

Short Communication

Application of quinone profiling method to primary evaluation of the impact of domestic effluent on the microbial population in a stream

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(Received December 12, 2002; Accepted February 10, 2003)

Key Words——attached microorganism; domestic wastewater; microbial community structure; microbial diversity; planktonic microorganism; quinone profile; stream

A huge amount of domestic wastewater is discharged in modern human daily life. In the cities and towns of developed countries, the domestic wastewater is discharged to natural bodies of water such as rivers and lakes after appropriate treatment. However, in most developing countries and some remote villages in developed countries, the domestic wastewater is usually discharged into bodies of water without sufficient treatment. The discharge of untreated or insufficient treated domestic wastewater into a stream will cause water pollution and destroy the ecosystem of the stream (Baker and Farr, 1977; Fukushima and Fukushima, 1997). Microorganisms in rivers (including planktonic and attached microorganisms) play key roles in degrading the pollutant and thereby prevent the river ecosystem from being destroyed (Coleman et al., 1974; House and Denison, 1998; Nuttall, 1982a). Many studies have been carried out on the role of microorganisms in river ecosystems and the relationship between water quality and planktonic microorganisms or attached microorganisms (Geesey et al., 1978; Kunihiro et al., 2002; Nuttall, 1982b). Nuttall (1982a, b) has studied the effect of environmental factors on the planktonic microorganisms in a river. Some researchers have suggested that attached microorganisms are greatly affected by the environments they inhabit (Geesey et al., 1978; Lai and Chen, 1997). However, the information on the influence of effluent discharge on the microbial community structure and microbial diversity in rivers is still limited because we have few reliable tools for characterization of microbial community structure. For this reason, sensitive tools for microbial analysis to detect the influence of discharged water are needed.

In recent years, analytical techniques for microbial community structure based on specific biomarkers (Morgan and Winstanley, 1997) in microorganisms such as rDNA (Amann et al., 1995), phospholipid fatty acids and microbial quinones (Hedrick and White, 1986; Hiraishi, 1988; Hu et al., 1993) have been developed. Molecular techniques using PCR based on rDNA such as denaturing gradient gel electrophoresis (DGGE) and restriction fragment length polymorphism

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(RFLP) have not yielded information on microbial biomass because the copy number of rDNA in each bacteria species is different (Farrelly et al., 1995). The fluorescent in situ hybridization (FISH) technique as a molecular analytical technique based on the enumeration of bacteria in various environments has been developed (Amann et al., 1995). However, the FISH technique for analysis of a microbial community requires much time and a skilled operator to get reliable results. On the other hand, techniques of quantitative chemical analysis such as phospholipid fatty acid (PLFA)-profiling and microbial quinone-profiling have a high correlation with the biomass. The profile of PLFA does not represent individual taxonomic groups (Katayama and Fujie, 2000). Microbial quinone, which is one of the components of the electron transport chain in microbial cells, can be divided in two groups: respiratory quinone and photosynthetic quinone. Respiratory quinones (including ubiquinone and menaquinone) exist in the bacteria gaining energy by the way of respiration. Almost every aerobic gram-negative bacterium examined has ubiquinones; anaerobic gram-positive bacteria have menaquinones, and some facultative gram-negative bacteria have demethylmenaquinones as well as ubiquinones and/or menaquinones (Collins and Jones, 1981). In general, ubiquinones are used for aerobic or nitrate respiration and menaquinones for anaerobic or aerobic respiration (Jones, 1988). Photosynthetic quinones (including plastoquinone and vitamin K1) are in photosynthetic microorganisms such as micro-algae and cyanobacteria (Hiraishi, 1999; Hiraishi et al., 1999; Jones, 1988). So the quinone profile, which is usually represented as the molar ratio of each quinone species, should be specific to a microbial community structure. The technique of using quinone profiles has been considered a simple and useful tool for analysis of microbial population dynamics in mixed cultures. The aim of this study was to evaluate the effect of domestic wastewater on microbial community structure in a stream by using the technique of quinone profiles.

The Gonmo River, a small stream flowing into the Umeda River at Toyohashi City, Aichi Prefecture, Japan, was selected as the sampling points of this study (Fig. 1). The stream water and small stones on the streambed (water depth: 30–50 cm) were collected from 3 sampling points at 12 a.m. October 6, 1999. At that time, the stream flows of the upper, inflow and lower point were 8.4, 2.6 and 9.6 m^3/min, respectively. Water depth at sampling points was approximately 30 cm. Planktonic microorganisms in the stream were collected from 10 L of the stream water by filtration with 0.3 μm glass filters (GF-75, ADVANTEC, Tokyo, Japan). Attached microorganisms were collected by brushing the biofilm attached on the surface of more than 10 stones (diameter: 5–10 cm) taken from the sampling points and then suspended in distilled water. These samples were used to measure quinone, dissolved organic carbon (DOC), total organic carbon (TOC) and chlorophyll-*a* (Chl-*a*).

The physical/chemical properties such as temperature, pH, electric conductivity (EC, EC-meter: Cyber Scan Con-100, Iuchi, Osaka, Japan), dissolved oxygen (DO, DO-meter: DO-14P, Toa Electronics Ltd., Kobe, Japan), total nitrogen (T-N) and total phosphate (T-P) in the stream water were analyzed according to the standard methods (JIS, 1997). Concentrations of DOC and TOC were determined by TOC analyzer (TOC-500, Shimadzu, Kyoto, Japan) with and without the micro-filtration using a membrane filter (0.45 μm-pore-size), respectively. Microbial biomass in the stream water and biofilm were measured as particulate organic carbon (POC), which was calculated from the difference between TOC and DOC of the same sample. Chl-*a* was measured by the spectrophotometric method (Standard Methods for JIS, 1997).

Microbial quinones in planktonic and attached microorganisms were analyzed using a method describe in our previous papers (Hu et al., 1999b, 2001; Kunihiro et al., 2002). Quinones were firstly extracted from the microorganisms using a mixture of chloroform-methanol (2 : 1, v/v) and then re-extracted into hexane. Menaquinones and ubiquinone contained in the crude extract were separated and purified using Sep-Pak® Plus Silica. The species and the concentration of quinones were determined using a HPLC equipped with an ODS column (Zorbax-ODS, 4.6 (I.D.)× 250 mm, Shimadzu-Dupont, Kyoto, Japan) and a pho-

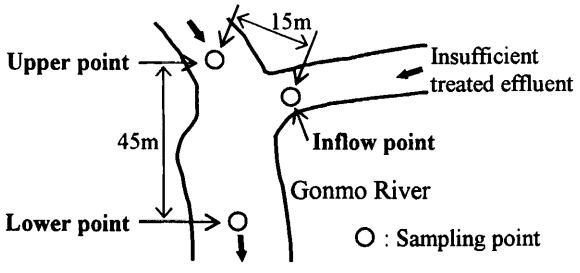

Fig. 1. Sampling points in this study.

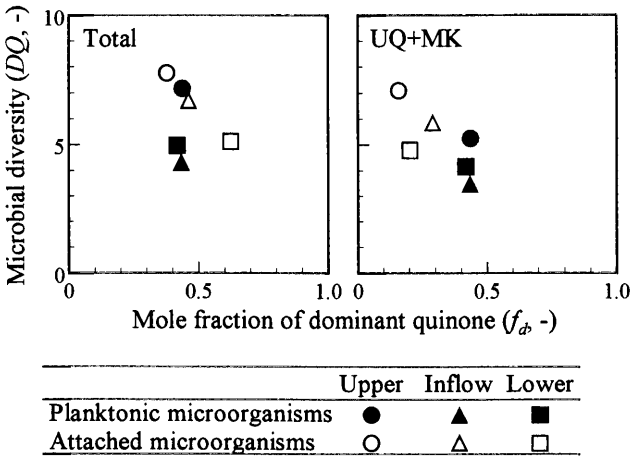

Fig. 2. Effect of the effluent discharge on microbial diversities of planktonic and attached microorganisms.
f_d shows the mole fraction of dominant quinone in a sample.

todiode array detector (SPD-M10A, Shimadzu).

In this paper, the quinones are named as follows: the abbreviation of the type of quinone (ubiquinone: UQ, menaquinone: MK, plastoquinone: PQ), 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(H_2) represents a menaquinone with 9 isoprenoid units and one of the 9 units is hydrogenated with 2 hydrogen atoms. In addition, vitamin K1, which has a similar molecular structure to menaquinone, is abbreviated as VK1.

The microbial diversities (*DQ*) for planktonic and attached microorganisms calculated from the quinone composition using Equation (1) (Hu et al., 1999a) are shown in Fig. 2 versus the molar fraction of dominant species of quinone in each sample (f_d).

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

Where, f_k is the molar fraction of quinone species k and n is the number of quinone species with a mole fraction of no less than 0.001.

Physical and chemical properties of the stream waters and biofilms are shown in Table 1. POC, DOC, BOD, T-N and T-P of the Gonmo River were increased by receiving the discharge water. Chl-*a* in water downstream was lower than that upstream. Similarly, Chl-*a* in the biofilm downstream was also lower than that upstream. Possible reasons for the observation are the interception of solar light by suspended materials contained in the effluent and high concentration of pollutants downstream.

Table 1. Physical and chemical properties of stream waters and biofilms in the samples.

Items	Units	Sampling points		
		Upper	Inflow	Lower
Stream water				
Temperature	(°C)	25.3	24.7	26.2
Flow speed	(m/s)	0.2	0.4	0.1
pH	(—)	7.07	6.72	6.91
EC	(mS)	0.39	0.43	0.43
DO	(mg/L)	6.8	5.0	5.7
POC	(mg/L)	ND[a]	65.9	22.3
DOC	(mg/L)	5.5	31.2	9.4
BOD	(mg/L)	5.6	119.9	31.1
T-N	(mg/L)	9.1	17.5	10.6
T-P	(mg/L)	0.2	1.0	0.3
Chl-*a*	(μg/L)	1.6	1.0	0.7
Biofilm				
POC	(mg/cm^2)	0.4	0.3	0.2
Chl-*a*	($\mu g/cm^2$)	4.1	4.3	3.3

[a] ND: not detected.

The analytical results of microbial quinones in planktonic and attached microorganisms are shown in Table 2. UQ, MK and VK1 were observed in both planktonic and attached microorganisms for all the samples. Ten species of quinones were observed in planktonic microorganisms upstream, but only 7 species downstream. The total concentration of quinones, which reflects the concentration of planktonic microorganisms in the stream, varied markedly from 0.3 to 3.3 nmol/L. Compared to the upstream, quinone concentrations of the planktonic microorganisms downstream were slightly higher. The attached microorganisms had 15 species of quinone upstream, but only 11 species downstream. The quinone contents for attached microorganisms were in the range of 7.6×10^{-2} to 10.7×10^{-2} $nmol/cm^2$. Unlike the planktonic microorganisms, the quinone contents of attached microorganisms downstream were lower than those upstream.

UQ, MK, and photosynthetic quinones are the electron transport chain used for aerobic/nitrate, anaerobic/aerobic and photosynthesis, respectively (Collins and Jones, 1981). So, the relative content of each group of quinones may reflect the energy metabolic characteristics of an ecosystem (Iwasaki and Hiraishi,

Table 2. Quinone concentrations of planktonic and attached microorganisms in this study.

	Planktonic microorganisms			Attached microorganisms		
	Upper	Inflow	Lower	Upper	Inflow	Lower
Quinone species	Quinone concentrations (nmol/L)			Quinone contents (10^{-2} nmol/cm^2)		
UQ-8	0.136	0.261	1.359	1.563	2.664	1.527
UQ-9	0.017	0.241	0.583	1.437	0.663	0.398
UQ-10	0.025	—[a]	0.122	0.448	0.777	0.373
MK-6	0.014	0.011	0.930	0.041	—	0.027
MK-7	0.017	—	—	0.186	0.147	0.121
MK-8	0.004	0.024	0.041	0.195	0.185	0.078
MK-9	—	—	—	0.016	0.022	—
MK-10	—	—	—	0.036	0.027	—
MK-8(H_2)	—	—	—	0.006	0.023	—
MK-9(H_2)	—	—	—	0.006	0.049	0.002
MK-8(H_4)	0.003	0.011	0.008	0.065	0.051	0.009
MK-9(H_4)	—	—	—	0.084	0.014	—
MK-9(H_8)	0.004	—	—	0.070	—	0.003
VK1	0.032	0.055	0.217	2.431	0.333	0.317
PQ-9	0.067	—	—	4.074	4.227	4.735
Total	**0.320**	**0.602**	**3.260**	**10.656**	**9.182**	**7.591**
Mole ratios						
UQ/MK	4.56	10.76	2.11	5.22	7.91	9.55
(UQ+MK)/(PQ-9+VK1)	0.15	0.08	0.44	0.08	0.06	0.04

[a] —: not detected.

1998). The values of UQ/MK and (UQ+MK)/(PQ-9+VK1) for planktonic and attached microorganisms calculated from the data are also shown in Table 2. The values of UQ/MK for planktonic microorganisms decreased from 4.56 upstream to 2.11 downstream. This suggests that the ratio of aerobic bacteria in planktonic microorganisms upstream might be higher than that downstream because of the effluent discharge. The values of UQ/MK for attached microorganisms increased from 5.22 upstream to 9.55 downstream. This suggests that the ratio of anaerobic bacteria in attached microorganisms upstream might be higher than that downstream. Furthermore, the values of (UQ+MK)/(PQ-9+VK1) for planktonic microorganisms increased from 0.15 upstream to 0.44 downstream. The ratio of heterotrophic bacteria in planktonic microorganisms downstream was higher than that upstream. Unlike the planktonic microorganisms, the values of (UQ+MK)/(PQ-9+VK1) for attached microorganisms upstream was lower than that downstream.

The microbial diversity calculated from the composition of all quinones (including ubiquinones, menaquinones, and photosynthetic quinones), DQ_q, for the planktonic microorganisms upstream and downstream were 7.1 and 4.9, respectively. Compared to the upstream, the microbial diversity of the planktonic microorganisms downstream was relatively low. The microbial diversities of the attached microorganisms upstream and downstream were 7.7 and 5.1, respectively. Like the planktonic microorganisms, the microbial diversity of attached microorganisms at downstream was also lower than that upstream. In addition, the diversity calculated from the composition of respiratory quinones (including ubiquinones and menaquinones), DQ_{uq+mk}, which reflects the diversity of heterotrophic bacteria in the planktonic and attached microorganisms showed a similar trend as DQ_q.

In summary, our experimental results clearly demonstrated that the discharge of domestic wastewater lower the microbial diversity of planktonic and attached microorganisms in the receiving stream.

Acknowledgments

This study was partly funded by a Grant-in-Aid for Scientific

Research (B) (Project No. 04832020) from the Ministry of Education, Culture, Sports, Science and Technology in Japan, Major State Basic Research Development Program of China (Project No.: G1999045711) and Tsinghua University, Beijing, China.

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