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Bacterial community dynamics in a full-scale municipal wastewater treatment plant employing conventional activated sludge process

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To elucidate the bacterial community dynamics in a full-scale wastewater treatment plant (WWTP) and the relatedness among bacterial communities in the influent, effluent and sludge, the structure and metabolic ability of the bacterial community throughout a full-scale WWTP employing a conventional activated sludge process was investigated during a period of 10 months. The bacterial community structure was analyzed by terminal-restriction fragment length polymorphism targeting eubacterial 16S rRNA genes, while a Biolog assay was applied to assess the metabolic ability of the activated sludge. Influent bacterial community structure was generally stable. In contrast, the bacterial community structure in the effluent was similar to that in the influent in some cases, while in other cases it was unique and differed greatly from that in the influent and sludge. These results suggest that temporal variations of the effluent bacterial community may be useful to predict the wastewater treatment performance and settleability of activated sludge. The bacterial community structure in the sludge was relatively stable and was rarely impacted by the influent populations. Biolog assay also revealed that activated sludge maintained a remarkably similar metabolic potential of organic compounds over time due to functional redundancy, in which the minor populations played a significant role.

[**Key words:** Activated sludge; Bacterial community; Biolog assay; Full-scale wastewater treatment plant; Terminal-restriction fragment length polymorphism]

Activated sludge treatment has been used worldwide in secondary treatment systems in municipal and industrial wastewater treatment plants (WWTPs). The wastewater purification performance of activated sludge treatment depends heavily on the metabolism and interactions of the microbial community, with bacteria playing a key role in the purification process. Previous efforts have enabled efficient removal not only of suspended solids (SS) and biochemical oxygen demand (BOD), but also of nitrogen, phosphorus and xenobiotic compounds, via the appropriate design and operation of treatment systems. Nevertheless, activated sludge treatment is still subject to crucial problems, such as unsuccessful sludge settlement associated with sludge bulking and foaming caused by non-floc forming or filamentous microorganisms (1–3), as well as failure of wastewater treatment due to the loss or inactivation of key populations (4,5). Accordingly, it is necessary to understand the dynamics of the bacterial community to solve these operational problems and develop promising strategies for improved process performance and high-efficiency operation.

There have been many attempts to evaluate the diversity and variations in the bacterial community during activated sludge treatment; however, previous studies have focused on lab-scale systems. Because the microbiology in full-scale WWTPs is more complex than that in lab-scale systems operated under artificial conditions (6), it is impossible to directly relate the microbial community dynamics observed in lab-scale studies to the operation of full-scale WWTPs. Consequently, more studies in full-scale systems are needed to obtain practically useful knowledge.

To date, relatively few studies have explored the temporal dynamics of the entire activated sludge bacterial community (in aeration tanks) for relatively long periods (more than half a year) in full-scale WWTPs treating municipal wastewater (7–9) or pulp mill effluent (10) to clarify the influence of operational and environmental parameters on the diversity and composition of activated sludge bacterial communities. Moreover, to our knowledge, there have been no in-depth investigations of the long-term dynamic behavior of the bacterial community in activated sludge and the influent and effluent of a full-scale WWTP. The dynamics of the bacterial community in an activated sludge reactor are determined by the sum of the growth and decay of bacterial populations in the bioreactor, the inflow of wastewater-derived populations, and the discharge of unsettled populations in treated water. Therefore, whole-plant monitoring including the inflow and outflow populations is required to fully grasp the dynamics of the activated sludge bacterial community. Furthermore, there have been few attempts to understand structural variations in activated sludge bacterial communities by linking these variations with their metabolic ability, which can have considerable effects on wastewater treatment performance. To our knowledge, Yang et al. (11) conducted the only study that compared the bacterial community in four activated sludge samples collected from geographically

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distinct WWTPs by both phylogenetic and metabolic fingerprinting methods. The results of their study suggested that the activated sludge communities had broad carbon source utilization profiles because of the enrichment of various degraders by long-term acclimation to pollutant-rich wastewater, and that the phylogenetic diversity appeared to be associated with metabolic diversity in the activated sludge microbial community. However, a periodical monitoring study of a WWTP to clarify the dynamic changes of the structure and function of the activated sludge community and their possible links is still needed.

In this study, the bacterial community throughout a full-scale WWTP was monitored over 10 months. The primary objectives of this study were to gain a thorough understanding of the dynamic behavior of bacterial community in a full-scale WWTP in light of the phylogenetic structure and metabolic ability of organic compounds and elucidate possible correlations among the bacterial communities in influent wastewater, effluent water and activated sludge. Bacterial community structure was analyzed by terminal-restriction fragment length polymorphism (T-RFLP) analysis (12) targeting eubacterial 16S rRNA genes. T-RFLP analysis has frequently been used in the long-term monitoring of the bacterial community in full-scale WWTPs (7,8,10). In addition, the carbon source utilization potential of the activated sludge community was evaluated by a Biolog assay (13), which is capable of examining the ability of the community to utilize 95 different carbon sources, to understand the activated sludge bacterial community based on its metabolic properties.

MATERIALS AND METHODS

Wastewater treatment plant and samples A municipal WWTP in Osaka, Japan that employs a conventional activated sludge (CAS) process for secondary treatment was investigated in this study. This WWTP treats 82,400 m^3/d of wastewater (over 90% domestic wastewater and small amounts of industrial discharges) for a population of nearly 110,000 people. The hydraulic retention time (HRT) in the biological treatment tank during the sampling period was 8.6–10.3 h, and the sludge retention time (SRT) was 5.2–7.2 d (Table 1). Samples for T-RFLP analysis and the Biolog assay were collected in July, August and November of 2008 and January and April of 2009. The following four grab samples were collected during each sampling event: influent (Inf), effluent (Eff), and activated sludge (AS) samples from the outlet of the primary settling tank, the final settling tank and the aeration tank, respectively, as well as a return sludge (RS) sample from the return sludge pipe. Hereafter, these samples are referred as the sample type (Inf, Eff, AS or RS)-sampling month (7, 8, 11, 1 or 4). For example, Inf-8 and AS-1 indicate the Inf sample taken in August 2008 and AS sample collected in January 2009, respectively. An AS sample was also collected for Biolog assay in April 2010. All samples were collected in 1-L sterile polyethylene bottles, transported to the laboratory on ice, and subjected to DNA extraction within 3 h and to Biolog assay within 12 h.

Water temperature, pH and dissolved oxygen (DO) concentration at the points at which the Inf, Eff and AS samples were collected were recorded on site using a WQC-22A portable water quality checker (DKK-TOA, Tokyo, Japan). Concentrations of mixed liquor suspended solid (MLSS), SS, dissolved organic carbon (DOC), total nitrogen (T-N), NH_4–N, NO_2–N, NO_3–N, PO_4–P and heterotrophic bacteria were determined in the laboratory. Samples were filtered through a qualitative filter paper (pore size, 5 μm; Advantec, Tokyo, Japan) prior to measurement of the DOC, T-N, NH_4–N, NO_2–N, NO_3–N and PO_4–P. DOC was analyzed using a total organic carbon analyzer (TOC-5000A: Shimadzu, Kyoto, Japan). MLSS/SS, T-N, NH_4–N, NO_2–N, NO_3–N and PO_4–P were analyzed according to the test methods of the Japan Industrial Standards K0102, with minor modifications. Heterotrophic bacteria were enumerated using a CGY agar plate (14).

DNA extraction Approximately 2 ml of sludge samples and 10 ml of wastewater samples were used for DNA extraction. DNA in the samples was extracted using an ISOIL for Beads Beating kit (Nippon Gene, Tokyo, Japan) and subsequently purified with a MagExtractor-PCR&Gel Clean up-kit (Toyobo, Tokyo, Japan) according to the manufacturer's instructions.

T-RFLP analysis The bacterial community structure was analyzed by T-RFLP targeting eubacterial 16S rRNA genes as described in our previous study (15). Briefly, 16S rRNA genes were PCR amplified using the 27F primer with a 5′-end labeled with 6-FAM and the 1392R primer (16). PCR was terminated during the exponential amplification phase to maintain the composition ratios of the 16S rRNA genes from different bacteria in the DNA template. The PCR products were purified with a Montage PCR kit (Millipore, Billerica, MA, USA) and digested with *Hha*I, which is a restriction enzyme that can yield a great number of terminal-restriction fragments (T-RFs) (17). Fluorescently labeled T-RFs were separated and detected using an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA), and their size and abundance were determined with GeneScan ver. 3.7 (Applied Biosystems). Because the PCR products were nearly 1400 bp, GeneScan 2500 ROX Size Standard (Applied Biosystems) was used as the internal standard to measure the size of labeled T-RFs. T-RFs with a peak height of 100 fluorescence units were considered to be positive peaks. The phylogenetic positions representing predominant T-RFs were presumably identified using a database [Microbial Community Analysis (MiCA) III, http://mica.ibest.uidaho.edu] (18).

Biolog assay The potential of AS samples to utilize 95 different carbon sources was evaluated with a Biolog GN2 plate (Biolog, Hayward, CA, USA) (Supplementary data Table S1). Briefly, AS samples were washed twice with phosphate buffer (pH 7.5), homogenized with an ultrasonicator (50 W, 2.5 min), and diluted with saline solution (0.85% (w/v)) to obtain an MLSS concentration of 1 mg/L. Aliquots (150 μl) of the pretreated samples were then added to wells of a Biolog GN2 plate. Next, the plates were statically incubated at 28°C in the dark for 72 h, during which time the absorbance at 595 nm (A_{595}) was measured periodically. All assays were performed in duplicate. The average well color development (AWCD) for all 95 carbon sources (13) was calculated as an index to evaluate the carbon source utilization potential. Wells with an A_{595} of over 0.25 were considered to be positive (utilizable), and the number of utilizable carbon sources was used as the other index.

Numerical analysis Microbial community diversity was evaluated using the Shannon-Weaver index (H'). H'(T-RFLP) (19) and H'(Biolog) (20) were calculated from the T-RFLP and carbon source utilization profiles, respectively, by the following equations:

$$H'(\text{T-RFLP}) = -\Sigma\,(Pi \times \ln Pi) \quad (1)$$

$$H'(\text{Biolog}) = -\Sigma\,(Ri \times \ln Ri) \quad (2)$$

TABLE 1. Operational parameters and wastewater treatment performance in WWTP investigated in this study.[a]

Sampling month	Sample	SRT (day)	HRT (h)	Temp (°C)	pH	Heterotrophic bacteria (CFU/ml)	Concentration (mg/L) of								Removal (%) of			
							DO	MLSS/SS	DOC	T-N	NH_4–N	NO_2–N	NO_3–N	PO_4–P	SS	DOC	T-N	NH_4–N
July 2008	Inf-7	6.7	8.8	26.8	7.6	2.4×10^6	0.06	55.2	54.3	14.7	16.5	0.2	0.2	1.5	95.1	77.7	23.8	89.1
	AS-7			26.9	6.8	9.7×10^6	2.6	1212	15.7	10.3	3.6	0.3	6.6	0.02				
	Eff-7			26.9	6.6	1.7×10^5	0.05	2.7	12.1	11.2	1.8	1.1	4.7	0.04				
August 2008	Inf-8	7.2	8.8	29.9	7.4	1.3×10^7	0.5	35.0	59.2	12.9	12.6	0.03	0.2	3.0	94.9	66.0	20.9	83.3
	AS-8			30.4	6.7	9.8×10^6	3.0	1013	22.1	10.4	3.3	0.2	8.4	0.1				
	Eff-8			30.4	6.6	2.7×10^5	0.5	1.8	20.1	10.2	2.1	0.3	8.3	1.2				
November 2008	Inf-11	5.2	9.2	23.7	7.6	7.1×10^7	0.4	74.5	84.2	29.0	23.0	0.1	0.3	3.0	82.6	69.5	45.2	90.4
	AS-11			25.1	6.9	3.5×10^7	2.5	1332	16.8	12.2	4.9	0.7	6.0	0.05				
	Eff-11			24.7	6.7	4.1×10^4	0.8	13.0	25.7	15.9	2.2	0.1	10.5	0.9				
January 2009	Inf-1	5.5	8.6	19.5	7.6	5.7×10^6	0	74.5	NA	NA	31.8	0.2	0.5	1.3	89.4	NA	NA	87.1
	AS-1			20.7	7.0	6.5×10^7	2.4	1617	13.7	NA	14.4	0.6	0.2	0.01				
	Eff-1			20.3	6.5	1.0×10^5	0.2	7.9	5.8	NA	4.1	4.9	3.4	0.03				
April 2009	Inf-4	6.8	10.3	23.1	7.1	1.6×10^6	0	83.0	56.6	26.3	23.5	0.03	0.1	3.1	100	83.9	32.3	64.3
	AS-4			23.6	7.1	1.4×10^7	3.0	1239	13.1	13.1	8.9	0.3	0.6	0.4				
	Eff-4			23.2	7.0	2.1×10^4	0	ND	9.1	17.8	8.4	1.6	3.9	0.1				

[a] NA, not analyzed; ND, not detected.