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Removal Behavior of Biological Nitrogen and Phosphorus, and Prediction of Microbial Community Composition with Its Function, in an Anaerobic-Anoxic System from Weak Sewage

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Abstract An easier way of understanding the BNR system was proposed from the study on substrate, nutrient removal tendency, microbial community and its metabolic function by applying the municipal settled sewage. During the anaerobic period, the phosphorus release rate per VFACOD was varied depending on the phosphorus content in the sludge. When the phosphorus content in the sludge was 6% VSS, according to influent VFACOD, the phosphorus release rate and PHA production were 0.35 gPO_4P/gVFACOD and 1.0 gPHA/gVFACOD, respectively. The NO_3N requirement for the phosphorus uptake as an electron acceptor was about 0.5 gNO_3N/gPO_4P_{uptake}, based on the proposed equation with PHA, biomass production, and the concentration of phosphorus release/uptake. Bacterial-community analysis of the sludge, as determined by FISH and 16S rDNA characterization FISH, revealed that the beta-subclass proteobacteria were the most abundant group (27.9% of the proteobacteria-specific probe EUB338), and it was likely that representatives of the beta-subclass played key roles in activated sludge. The next dominant group found was the gamma-proteobacteria (15.4% of probe EUB338). 16S rDNA clone library analysis showed that the members of β- and γ-proteobacteria were also the most abundant groups, and 21.5% (PN2 and PN4) and 15.4% (PN1 and PN5) of total clones were the genera of denitrifying bacteria and PAO, respectively. Prediction of the microbial community composition was made with phosphorus content (Pv, % P/VSS) in wasted sludge and profiles of COD, PHA, PO_4P, and NO_3N in an anaerobic-anoxic SBR unit. Generally, the predicted microbial composition based upon metabolic function, i.e., as measured by stoichiometry, is fairly similar to that measured by the unculturable dependent method. In this study, a proposal was made on the microbial community composition that was more easily approached to analyze the reactor behavior.

Key words: Electron acceptor, microbial community, nutrient removal behavior, PAO, PHA, phosphorus content in wasted sludge

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Performance of a given BNR process is related to the composition of the microbial community and its metabolic functions. Microbial composition depends on many factors, with the types of substrate and the operating conditions being the major factors. Effluent water quality and some stoichiometric parameters, which are varied depending on the types of substrates and operating conditions, were measured in an anaerobic-anoxic SBR unit, treating weak sewage at room temperature. This study focused on the effective BNR system with weak sewage. BNR is a highly complicated system in which the microbial behavior varies with influent characteristics; that is, substrate, nitrogen, and phosphorus. For the evaluation of a BNR system, several biokinetic models can be used, such as ASIM™, BioWin™, and GPS-X™. However, their dynamic simulation requires many stiochiometric coefficients and kinetic parameters. In addition, there are various limitations and assumptions in modeling this dynamic system.

Chang and Ouyang [5] estimated the biomass fraction through experimental result and theoretical calculation. Vollertsen *et al.* [23] compared methods for determining microbiological cell mass and calculating biomass from OUR (oxygen uptake rate) measurements in wastewater. The effect of COD/N ratio on denitrification was also evaluated for the development of a denitrification process [20]. Utilizing the single strain of *Spirulina platensis*, Kim *et al.* [14] tried to remove nitrogen and phosphorus from swine

waste. However, investigations of the biomass distribution of various microorganisms are still scarce and complex. This study mainly focused on attaining a more easily accessible reactor analysis method, and on the use of a low concentration domestic sewage, phosphorus content in the sludge, sludge production, and substrate removal tendency.

The main purpose of this study was to determine the bacterial community structure of activated sludge samples obtained from the reactor by using rRNA target-probes and 16S rDNA characterization. The microbial community composition in this study determined by the unculturable dependent method, RFLP of 16s rDNA clones of unculturable bacteria [9], was compared with the predicted results.

MATERIALS AND METHODS

Operations and Analysis of Reactor

A laboratory SBR unit was operated by feeding settling domestic sewage (as shown in Table 1) at room temperature. The unit contained a total SRT (sludge retention time) of 10 days with 0.25(0.25) h filling time, and 2.25(1.75) h anaerobic, 4(3) h anoxic, 1(0.75) h settling, and 0.5(0.25) h withdrawal times to make a total of 8(6) h (Fig. 1). Approximately 15 to 25 mg/l nitrate was added during the anoxic period, immediately after the anaerobic period. When the effluent quality reached a steady state condition, the batch test was carried out with the extracted sludge in a settling cycle of SBR.

PHA (PHB and PHV) was measured by the method proposed by Smolders *et al.* [21]. The extracted sludge was dried in an oven and analyzed by using GC (Shimazu-14B, Japan) FID with an attached capillary column (Supelcowax™-10; 30 m×0.32 mm×0.25 μm, U.S.A.). Benzoic acid was used as an internal standard. PHA (poly-3-hydroxybutyric acid-co-3 hydroxyvaleric acid; PHV content 12 wt 12%, Sigma Aldrich Chem. Co., U.S.A.) was used as a standard. NO_3N and PO_4P were analyzed by Ion Chromatography (DIONEX model DX-500, U.S.A.). All other analyses were made in accordance with *Standard Methods for the Examination of Water & Wastewater* [2].

Table 1. Characteristics of settled sewage.

Parameters		Concentration, mg/l*	
		Range	Average
pH		7.1–7.5	-
Alkalinity		70–95	-
Suspended solid	Total	74–110	80
	Volatile	45–70	-
COD	Total	150–200	150
	S_S	23–40	30
	S_I	15–25	15
	X_S	75–90	80
	X_I	25–30	25
Nitrogen	TKN	11–25	20
	NH_4N	8–17	-
Phosphorus	Total	4.5–6.6	5.0
	Soluble	3.5–4.5	-

*: except pH.

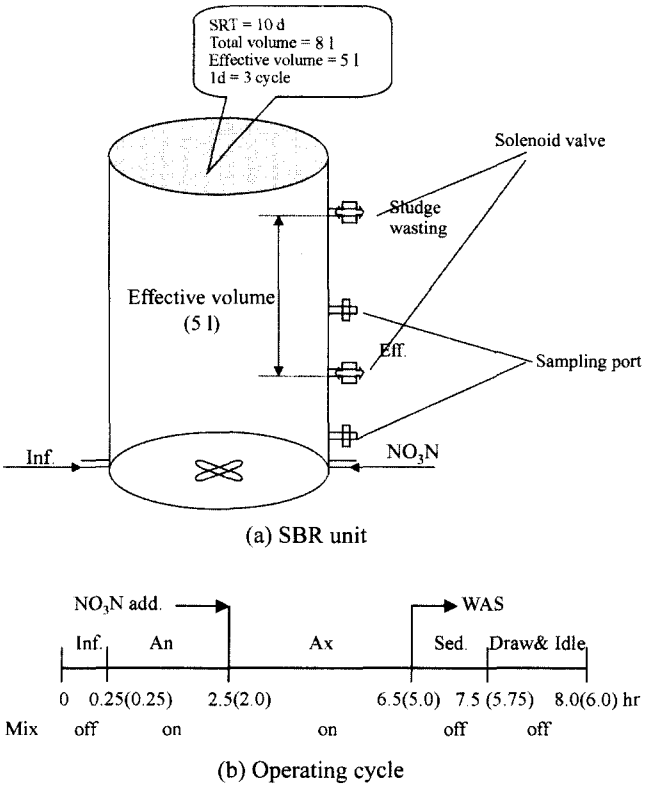

Fig. 1. SBR unit and operating cycle.

Boundary Conditions for the Prediction of Microbial Community Composition

For a prediction of microbial community composition, the calculations included the following boundary conditions:

1) All of PAO, GAO denitrifier, and ordinary heterotrophic organisms are heterotrophic microbes.
2) PHA is stored by PAO and GAO utilizing the influent VFACOD, but phosphorus is released only by PAO during the anaerobic period.
3) PAOs grow on stored PHA and simultaneous uptake of the phosphorus, utilizing nitrate as an electron acceptor in the anoxic period.
4) GAOs grow on stored PHA and they do not take up phosphorus.
5) Denitrifiers grow on the substrate, utilizing nitrate as an electron acceptor during the anoxic period.
6) OHOs grow on the deducted substrate that is utilized by PAO, GAO, and denitrifier.

Microbial Community Analysis

DNA extraction. Genomic DNA of the isolates was purified by the procedure of Hallin and Lindgren [10]. Genomic DNA extractions from the activated sludge were performed using a modified method of Lee *et al.* [15]. The quality of extracted DNA was analyzed by applying the standard agarose electrophoresis. DNA concentrations were measured by absorbance at 260 nm.

16S rDNA amplification. PCR and amplification of 16S rDNA from chromosomal DNA were carried out in a DNA thermal cycle (Model480; Perkin-Elmer, Norwalk, Conn., U.S.A.) with universal bacterial primers, 27F (AGAGTTTGATCMTGCTCAG) and 1492R (GGTTACCTTTGTTACGACTT). After an initial heating to 94°C for 4 min, 30 cycles consisting of 94°C (1 min), 57°C (1 min), and 72°C (2 min), with a final 10 min extension period at 72°C, were performed.

The PCR product was purified with a QIAquick PCR purification kit (Qiagen, Germany), and was cloned into pGEM-T vector (Promega, Madison, WI, U.S.A.) according to the manufacturer's instruction (Promega, Madison, WI, U.S.A.). Clones containing appropriate-sized inserts were identified by agarose gel electrophoresis of PCR products, which were obtained from host lysates by PCR with primers complementary to the vector at sites flanking the insertion site. Unique clones were identified by RFLP analysis of the insert [15]. Seven and five clones representing the dominant restriction fragment groups were selected from a reactor, respectively. These clones were sequenced by PCR cycling sequencing of quick-prepped recombinant plasmids, by using the ABI PRISM BigDye Terminator cycle sequencing kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, U.S.A.).

Oligonucleotide probes. The following oligonucleotide probes were used to evaluate the microbial population in a sludge: EUB338, specific for the eubacteria [17, 18, 22]; ALF1b, BET42a, and GAM42a, specific for the α-, β-, and γ-Proteobacteria, respectively [17, 18, 22]; CF, specific for *Cytophaga-Flavobacterium-Bacteriodes* cluster [17, 18, 22]. Sequences of all probes, their hybridization conditions, and references are given in previous studies [17, 18, 22]. The probes were labeled with tetramethylrhodamine-5-isothiocyanate (TRITC) at the 5' end (Takara Biochemicals Co., Ltd., Japan).

DAPI staining and FISH. DAPI was used to determine the total number of cells in the samples [12]. FISH of whole cells was performed as reported by Kawaharasaki *et al.* [12]. Samples were fixed with para-formaldehyde and immobilized by dry air on glass slides with a heavy teflon coating of 9 mm-diameter wells [22]. These samples were hybridized with probes as described by Amann [1]. BET 42a and GAM 42a were used with competitor oligonucleotides [17]. Hybridization was carried out for 90 min at 46°C in a sealed moisture chamber. Washing was conducted under conditions suitable for each probe [17, 18, 22]. The hybridized and DAPI-stained samples were examined with an epifluorescence microscope (Axioplan; Zeiss, Axiophot 2) with filter sets 01 (for DAPI staining) and 15 [for tetramethylrhodamine-5-isothiocyanate (TRITC) labeled probe]. Means were calculated from 10 to 20 randomly chosen fields on each filter section, corresponding anywhere between 800 and 1,000 DAPI-stained cells. Two replicate samples were counted for each hybridization.

Nucleotide sequence accession number. The 16S rDNA partial sequences obtained in this study are available from the EMBL nucleotide sequence database under accession nos. AF387323 to AF387330.

RESULTS AND DISSCUSSION

The Behavior of SBR

Figure 2 shows COD, PHA, NO_3N, and PO_4P behaviors with time, disregarding the possibility of chemical precipitation or dissolution by adjusting pH at 7.0. The results of SBR are shown in Table 2. Phosphorus release and PHA synthesis rate with influent VFACOD were 0.35 mgPO_4P/mg$VFACOD_{inf.}$ and 1.0 mgPHACOD/mg$VFACOD_{inf.}$ at the anaerobic condition, respectively. Removed PO_4P and NO_3N at the anoxic condition, 1st h SDNR and SPUR, were 3.4 mgNO_3N/gVSS/h and 5.1 mgPO_4P/gVSS/h, respectively. Sludge production and phosphorus content in sludge after the anoxic period were 0.22 gVSS/gCOD and 6 %VSS, respectively.

Phosphorus release rate with applied VFACOD varied depending on the phosphorus content in sludge. The population of PAO can be predicted by using the phosphorus content in sludge. The phosphorus release concentration to influence the VFACOD ratio can also be

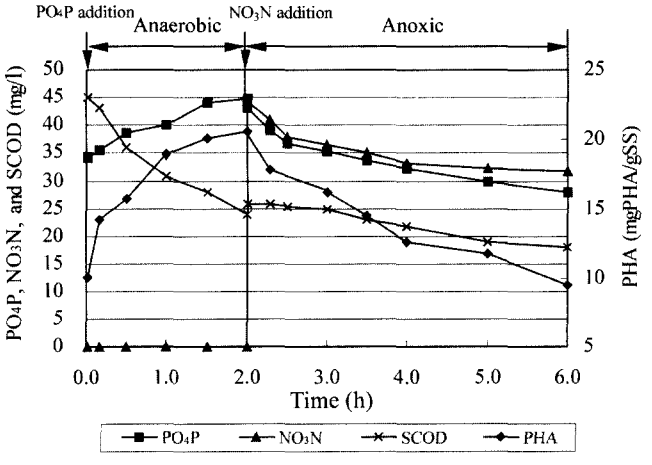

Fig. 2. Phosphorus, nitrate, SCOD, and PHA profiles.

Table 2. Summary of result at An-Ax reactor.

Anaerobic condition:	
Y_{PO4P}	0.35 $mgPO_4P/mgVFACOD_{inf}$
Y_{PHA}	1.0 $mgPHACOD/mgVFACOD_{inf}$
Anoxic condition:	
SDNR[a]	3.4 $mgNO_3N/gVSS/h$
SPUR[a]	5.1 $mgPO_4P/gVSS/h$
Px	6.0% VSS
Sludge production	0.22 $gVSS/gCOD_{removal}$
COD utilized:	
COD requirement for P and N removal by PAO=	3.05 $mgPHACOD_{requirement}/mg(N+P)_{removal}$
COD requirement for N removal by denitrifier =	2.86 $NO_3N/(1-1.134\ Y_{net})$ =3.8 $mgCOD_{requirement}/mgNO_3N_{removal}$

[a]1st h anoxic.

determined by measuring the phosphorus content in sludge, as shown in Fig. 3, which compared our results and results obtained in other researchers.

It is important to estimate the NO_3N requirement for the phosphorus uptake, because only PAO used NO_3N as an electron acceptor in the anoxic condition. Smolders *et al.* [21] reported that the value of NO_3N requirement per PO_4P uptake was 0.36 and 0.64 $gNO_3N_{require}/gPO_4P_{uptake}$ at aerobic and anoxic conditions, respectively, whereas Kerrn-Jespersen *et al.* [13] reported 0.5 $gNO_3N_{requirement}/gPO_4P_{uptake}$. In this study, equation (1) was made by sludge production, using PHA and concentrations of phosphorus release and uptake. The NO_3N requirement for the phosphorus uptakes was about 0.5 $gNO_3N_{require}/gPO_4P_{uptake}$.

$$C_2H_4O_2+0.10\ NH_4^+ +1.09\ PO_4^{3-}+1.19\ NO_3^{2-} \rightarrow 0.12\ C_5H_7O_2N+1.42\ CO_2+1.09\ HPO_3 +4.18\ OH^-+0.7\ N_2+0.25\ H_2O \quad (1)$$

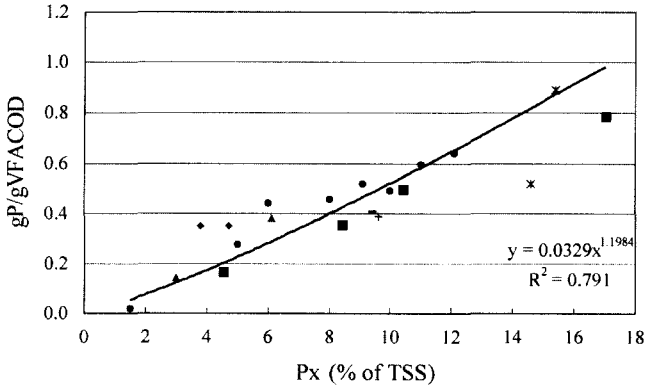

Fig. 3. Px vs $gPO_4P/gVFACOD$.
Ref. Liu *et al.* [16] (●); Mino *et al.* [19] (▲); Copp and Dold [unpublished result] (■); Fukase *et al.* [8] (–); Cech and Hartman [4] (+); Wentzel *et al.* [24] (*); This study (◆).

COD requirement for PAO was compared to that for denitrifier in Table 2. The COD requirement for NO_3N and PO_4P removal by PAO was 3.05 $mgCOD_{requirement}/(N+P)_{removal}$ and COD requirement for NO_3N removal by denitrifier was 3.8 $mgCOD_{requirement}/N_{removal}$, which was calculated by equation (2) in Fig. 4 [7]. The result demonstrated that PAO required 20% less carbon compared to the denitrifier.

Microbial Community Analysis

The total cell numbers were determined by DAPI staining. The total cell numbers in a reactor were 2.48×10^9 cells/ml of activated sludge sample. FISH quantified bacteria with domain specific probes, and showed that up to 76.2% of total DAPI cell counts were hybridized to the proteobacteria-specific probe EUB338. The specific probe EUB338 of the reactor was set to 100%, and the subclass probes (BET, GAM, and CF) indicated the number of proteobacteria (Fig. 4). Among the proteobacteria, the β-proteobacteria members were the most abundant group in the reactor, accounting for as much as 27.9%, and it was likely that representatives of the β-subclass in activated sludge played key roles, such as degradation of organic materials, removal of nutrients, and formation of flocks. The next dominant subclass in the reactor was the gamma group (15.4%). The label "OTHER" refers to the percentage of bacteria detected by probe EUB338 that could not be explained by the sum of the bacteria detected with the four group-specific probes used.

To more fully characterize the bacterial community, 16S rDNA directly extracted from the activated sludge were cloned and analyzed. One-hundred-and-thirty different types

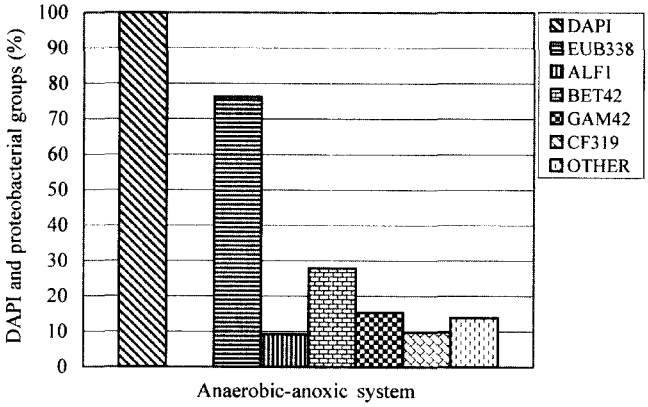

Fig. 4. Comparison of DAPI and proteobacteria community compositions obtained from anaerobic-anoxic system.
EIB338, probe of eubacteria; ALF1, probe of alpha subclass of the class proteobacteria; BET42, probe of beta subclass of the class proteobacteria; GAM42: probe of gamma subclass of the class proteobacteria; CF, probe of *Cytophaga-Flavobacterium-Bacteroid*; OTHER, probe of other bacteria. Assessment of relative abundances (%) of probe-positive cells relative to those visualized by probing with EUB338 or with ALF1, BET42. The specific probe EUB338 of CR-I and CR-II were set to control 100%, and then the relative subclass probe number of proteobacteria was measured.

Table 3. Phyla from the dominant bacteria represented in the SBR clone libraries determined by BLAST comparisons of partial clone insert sequences.

Clones of SBR	16S rRNA gene sequencing			Subclass of proteobacteria
	Closest match	No. of nucleotide compared	% Similarity with closes match	
PN1(11)[a]	*Thauera terpenica* (AJ005817)	457	97%	β
PN2(10)	Denitrifying Fe-oxidizing bacteria strain=BrG2 (U51102)	405	97%	β
PN3(11)	Uncultured CFB-group bacterium 279ew(AJ287669)	291	89%	CF
PN4(18)	*Pseudomonas halodenitrificans* (X90867)	420	94%	γ
PN5(3)	*Arcobacter nirofigilis* (L14627)	293	87%	ε
PN6(9)	*Azoarcus* sp. MXyN1 (X83533)	421	90%	β
PN7(4)	*Arcobacter* sp. D1a1 (AJ271654)	425	93%	ε
PN8(6)	*Arcobacter skirrowii* (L14625)	445	91%	ε

[a]The parenthesis is the number of colonies involved in the same group.

of clones were identified by RFLP. Among them, eight dominant groups (PN1, PN2, PN3, PN4, PN5, PN6, PN7, and PN8) were selected and partially sequenced (Table 3). PN3, PN1, PN2, and PN4 groups were the dominant clones from the reactor. PN4 and PN3 groups were the most closely matched with *Pseudomonas halodenitrificans* (X90867) and the uncultured CFB-group bacterium 279ew (AJ287669), respectively. The PN1 group matched with *Thauera terpenica* (AJ005817) and the PN6 group matched with *Azoarcus* sp. mXyN1(X83533). *Thauera terpenica* and *Azoarcus* sp. belong to the *Rhodocyclus* group within the β-proteobacteria [3, 6], and they are known to be PAOs. The presence of the *Rhodocyclus* group in the EBPR process was first reported by Bond *et al.* [3]. When

Table 4. Computation of microbial composition.

1) PAO population
P/VSS= 6% (measured)
Maximum phosphorus content; 34% (Henze *et al.* [11]), other solids: 2%
PAO fraction: PAO×0.34+(1-PAO)×0.02=0.06
(0.06-0.02)/(0.32)=12.5%

Inf. COD=150 mg/l (VFACOD=30 mgVFACOD/l), Eff. COD=30 mg/l
PHA production (20.5-10) mgPHA/gSS×1.67 mgCOD/mgPHA×1.7 gSS/l=30 mgPHACOD/l
Y_{PHA}=30 gPHACOD/30 gVFACOD=1.0 gPHACOD/gVFACOD
P release Y_{PO4P}=(44.7-34.2) mgP/20 mgVFACOD=0.35 mgP/mgVFACOD
0.35 gP/gCOD×30 mgSCOD/l=10.5 mgP/l
Yobs 0.22 gVSS/gCOD
(Sludge Production) (0.22 gVSS/gCOD)×(150-30) mgCOD/l=26.4 mgVSS/l
VSS prod. by PAO 26.4 mg/l×(0.125)=3.3 mgVSS/l

Stored PHA by PAO (From the equation (1))=
(3.3 mg$C_5H_7O_2N$/l)/(0.12 m-$C_5H_7O_2N$/m-$C_2H_4O_2$×113 gr/64 gr)=15.6 mgPHACOD/l

2) GAO population
Stored PHA by GAO=(30-15.6)=14.4 mgPHACOD/l
GAO fraction=(14.4 mgPHACOD/l×0.22 gVSS/gCOD)/(26.4 mgVSS/l)×100=12.0%

3) Denitrifier population
$COD/NO_3N=2.86/(1-1.134\times Y_{NET})$ [7]-------(2)
COD requirement=2.86/(1-1.134×0.22)=3.8 gCOD/gNO_3N
$NO_3N_{req.}/P_{uptake}$=(1.19 mN/1.09 mP)×(14 gr/31 gr)=0.5 gN/gP (From equation (1))
NO_3N removed by PAO for poly P formation.=0.5 g/g×10.5 mgP/l=5.2 mg/l
COD removed at Ax (except PAO)=(13-5.2)×3.8=29.8 mgCOD/l
Denitrifier fraction=(29.8 mg/l×0.22 gVSS/gCOD)/26.4 mgVSS/l×100=24.8%

4) OHO population
OHO fraction: 100-12.5-12.0-24.8=50.7%

the PCR cloning approach was applied to two EBPR reactors, 15 out of 189 isolated clones phylogenetically belonged to the *Rhodocyclus* group [3]. Recently, FISH showed that the *Rhodocyclus* group dominated in the EBPR reactor. Therefore, 15.4% (PN1 and PN6) of the total clones were known as phosphate accumulating organisms. Among eight dominant groups, the genera of denitrifying bacteria were PN2 and PN4 and they dominated in the reactor (21.5 % of total clones). PN2 closely matched with denitrifying Fe-oxidizing bacteria strain=BrG2 (U51102). Based on these results, it could be inferred that important groups of PAOs and denitrifying bacteria in the reactor were actually bacteria which were closely related to the *Rhodocyclus* group within β-proteobacteria and the denitrifying Fe-oxidizing bacteria strain, along with *Pseudomonas halodenitrificans*, respectively.

Prediction of Microbial Community

PAO population. By using the assumption that the maximum P content of PAO is 34% [11] and other microbial masses including inert particulates have 2% P, the PAO population was predicted from % P of the wasted sludge, as shown in Table 4. This indicates that the PAO population makes up 12.5% of the total population.

During the anaerobic stage, 10.5 mgPHA/gSS PHA equivalent to 30 mg PHACOD/l was produced (Fig. 2). About 10 mg/l of particulate COD was converted to PHA via glycogen, as suggested by the Mino Model [19].

GAO population. PAO and GAO were assumed to store PHA by utilizing the VFACOD during the anaerobic stage, and the stored PHA was consumed in the anoxic stage for phosphorus uptake and denitrification by the PAO. The stored PHA by GAO was calculated to be (30–15.6)= 14.4 mgPHACOD/l. This would indicate that the GAO population makes up 12.0% of the total population.

Denitrifiers and OHO. The requirement for denitrification was computed for operating SRT for 10 days with the measured sludge production rate. The COD requirement/ NO_3N was removed, since the amount of NO_3N removed in the anoxic stage was 13 mg/l, which was computed as 3.8 g/g. The denitrifiers removed 29.3 mg/l COD as shown in Table 4. This would indicate a denitrifier population of 24.8% of the total population. Therefore, the ordinary heterotrophic organism (OHO) was estimated to be 50.7%.

The overall results are summarized in Fig. 5. In this research, the predicted microbial composition based on metabolic function, i.e., as measured by stoichiometry, and compared with the results by the unculturable dependent method, were presented for the first time. The predicted microbial composition results are fairly similar to that measured by the unculturable dependent method.

The % microbial compositions of the predicted and measured were 12.5 and 15.4 for PAO, 24.8 and 21.5 for denitrifier, and 12.0 and 8.5 for GAO and unknown

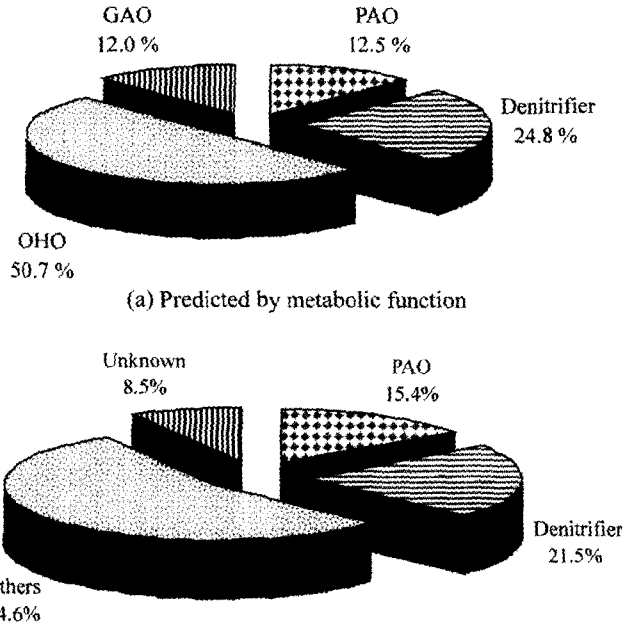

Fig. 5. Microbial community composition.

cultures, respectively. OHO and other microbes were 50.7% and 54.6%, respectively, which were in fact closely matched.

For the model simulated to analyze the reactor behavior, i.e., ASM2d [11], many factors (stoichiometric coefficients and kinetic parameters) should be included, but they were difficult to obtain from the experiment and require more time. In order to get more accurate prediction and analysis of the reactor behavior, the prediction method of microbial community composition needs to be refined. However, these results indicated that the method was an easier approach to analyze the reactor behavior.

Acknowledgments

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NOMENCLATURES

BNR : Biological nutrient removal
COD : Chemical oxygen demand
DAPI : 4, 6-diaidino-2-phenylindole
EBPR : Enhanced biological phosphorus removal
Eff. : Effluent
FISH : Fluorescence in situ hybridization
GAO : Glycogen accumulating organism
Inf. : Influent

NH_4N : Ammonium nitrogen
OHO : Ordinary heterotrophic organism
PAO : Phosphorus accumulating organism
PCR : Polymerase chain reaction
PHA : Poly-hydroxy-alkanonate
PHB : Poly-hydroxy-butyrate
PHV : Poly-hydroxy-valerate
Px : Phosphorus content in wasted sludge
RFLP : Restriction fragment length polymorphism
SBR : Sequencing batch reactor
SDNR: Specific denitrification rate
S_I : Soluble nonbiodegradable COD
SPUR : Specific phosphorus uptake rate
SRT : Sludge retention time
S_S : Soluble readily biodegradable COD
SS : Suspended solid
TKN : Total kjeldahl nitrogen
VFA : Volatile fatty acid
VSS : Volatile suspended solid
WAS : Wasting activated sludge
X_I : Particulate nonbiodegradable COD
X_S : Particulate slowly biodegradable COD
Y_{obs} : Sludge production rate per removed COD
Y_{PHA} : PHA synthesis rate per influent VFACOD
Y_{PO4P} : Phosphorus release rate per influent VFACOD

REFERENCES

1. Amann, R., L. Krumholz, and D. A. Stahl. 1990. Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J. Bacteriol.* **172:** 762–770.
2. APHA, AWWA, WEF. 1995. *Standard Methods for the Examination of Water & Wastewater*, 19th ed., Washington DC, U.S.A.
3. Bond, P. L., R. Erhart, M. Wagner, J. Keller, and L. L. Blackall. 1999. The identification of some of the major groups of bacteria in efficient and nonefficient biological phosphorus removal activated sludge systems. *Appl. Environ. Microbiol.* **65:** 4077–4084.
4. Cech, J. B. and P. Hartman. 1993. Competition between polyphosphate and polysaccharide accumulating bacteria in enhanced biological phosphate removal systems. *Wat. Res.* **27:** 1219–1225.
5. Chang, S. H. and C. F. Ouyang. 2000. The biomass fraction of heterotrophs and phosphate-accumulating organisms in a nitrogen and phosphorus removal system. *Wat. Sci. Tech.* **34:** 2283–2290.
6. Crocetti, G. R., P. Hugenholtz, L. Bond, A. Schuler, J. Keller, D. Jenkins, and L. L. Blackall. 2000. Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Appl. Envir. Microbiol.* **66:** 1175–1182.
7. EPA. 1993. *Manual of Nitrogen Removal*, EPA/695/R-93/10.
8. Fukase, T., M. Shibata, and Y. Miyaji. 1984. The role of an anaerobic stage on biological phosphorus removal. *Wat. Sci. Tech.* **17:** 68–80.
9. Geffrey, S. B. and M. T. James. 1992. Isolation and characterization of a nitrite-reduction gene and its use as a probe for denitrifying bacteria. *Appl. Environ. Microbiol.* **58:** 374–384.
10. Hallin, S. and P.-E. Lindgren. 1999. PCR detection of genes encoding nitrite reductase in denitrifying bacteria. *Appl. Envir. Microbiol.* **65:** 1652–1657.
11. Henze, M., W. Gujer, T. Mino, T. Matsuo, M. C. Wenzel, G. R. Marais, and M. C. M. van Loosdrecht. 1999. Activated sludge model no. 2d ASM 2D. *Wat. Sci. Tech.* **39:** 165–182.
12. Kawaharasaki, M., H. Tanaka, T. Kanagawa, and K. Nakamura. 1999. In situ identification of polyphosphate-accumulating bacteria in activated sludge by dual staining with rRNA-targeted oligonucleotide probes and 4',6-diamidino-2-phenylindol (DAPI) at a polyphosphate-probing concentration. *Wat. Res.* **33:** 257–265.
13. Kerrn-Jespersen, J. P. and M. Henze. 1993. Biological phosphorus uptake under anoxic and aerobic conditions. *Wat. Res.* **27:** 617–624.
14. Kim, M. H., W. T. Chung, M. K. Lee, J. Y. Lee, S. J. Ohh, J. H. Lee, D. H. Park, D. J. Kim, and H. Y. Lee. 2000. Kinetics of removing nitrogenous and phosphorus compounds from swine waste by growth of Microalga, *Spirulina platensis*. *J. Microbiol. Biotechnol.* **10:** 455–461.
15. Lee, S. Y., J. B. Bollinger, D. Bezdicek, and A. Ogram. 1996. Estimation of the Abundance of an unculturable soil bacterial strain by a competitive quantitative PCR method. *Appl. Environ. Microbiol.* **62:** 3787–3793.
16. Liu, W. T., K. Nakamura, T. Matsuo, and T. Mino. 1997. Internal energy base competition between poly phosphate and glycogen accumulating bacteria in biological phosphorus removal reactors effect of P/C feeding ratios. *Wat. Res.* **31:** 1430–1438.
17. Manz, W., M. Amann, W. Ludwig, M. Wagner, and K. H. Schleifer. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: Problems and solutions. *Sys. Appl. Microbiol.* **15:** 593–600.
18. Manz, W., R. Amann, M. Vancanneyt, and K. H. Scheifer. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in natural environment. *Microbiology* **142:** 1097–1106.
19. Mino, T., V. Arun, Y. Tsuzuki, and T. Marais. 1987. Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process. pp. 27–30 *In* R. Ramadori (ed.), Biological Phosphate Removal from Wastewater, Pergamon Press, Oxford, U.K.
20. Park, E. J., J. K. Seo, J. K. Kim, K. H. Suh, and S. K. Kim. 2000. Denitrification characteristics and microorganism composition of acclimated denitrification consortium. *J. Microbiol. Biotechnol.* **10:** 410–414.
21. Smolders, G. I. F., J. van der Meij, M. C. M. van Loosdrecht, and J. J. Heijnen. 1994. Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry

and pH influence. *Biotechnology & Bioengineering* **43:** 461–470.

22. Snaidr, J., R. Amann, I. Huber, W. Ludwig, and K. H. Schleifer. 1997. Phylogenetic analysis and in situ identification of bacteria in activated sludge. *Appl. Environ. Microbiol.* **63:** 2884–2896.
23. Vollertsen, J., A. Jahn, J. L. Nielsen, T. Kvitved-Jacobsen, and P. H. Nielsen. 2001. Comparison of methods for determination of microbial biomass in wastewater. *Wat. Res.* **35:** 1649–1658.
24. Wentzel, M. C., G. A. Ekama, P. L. Dold, R. G. Lowental, and G. V. R. Harais. 1988. Enhanced polyphosphate organism culture in activated sludge systems part I: Enhanced culture development. *Water SA* **14:** 81–92.