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Comparative study of protozoan communities in full-scale MWTPs in Beijing related to treatment processes

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ABSTRACT

The potential influence of process principles and system conditions on shaping protozoan community structures in eight full-scale municipal wastewater treatment systems in Beijing, including four process types, i.e., anaerobic/anoxic/aerobic or anoxic/anaerobic/aerobic process (A2O), anoxic/aerobic or anaerobic/aerobic process (AO), oxidation ditch (OD) and sequencing batch reactor (SBR), was evaluated with the aid of cluster analysis and principal components analysis (PCA). The species richness and abundance distribution of protozoa varied significantly with the process types: the A2O ecosystems harbored more diverse protozoan communities with higher relative abundance of crawling and sessile ciliates than the other systems. Cluster analysis revealed that the protozoan community structures were in high coordination with the process types, i.e., different systems with the same process principles exhibited similar community structures. The A2O processes displayed a distinctively higher similarity of protozoan community structures than the AO processes, suggesting that the A2O ecosystems were more stable than those of AO. The PCA analyses demonstrated that swimming and carnivorous ciliates were correlated with poor settleability of sludge, and that amoebae were sensitive to DO level. We therefore concluded that protozoan community structures were primarily shaped by treatment process principles, whilst they were also modified by system conditions in terms of operational properties and water quality.

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1. Introduction

Biological removal of pollutants from sewage is performed by a mixed community of microorganisms in municipal wastewater treatment plants (MWTPs). The success of this biological process is dependant not only on the single role-play of functional bacteria, who are foremost responsible for the biotransformation of waste materials and accordingly has attracted much more special attentions in research fields, but also on the overall “healthy” ecological interactions among

the microscopic residents all together (Curds, 1982; Madoni and Ghetti, 1981; Madoni et al., 1993; Martín-Cereceda et al., 1996). In this artificial ecosystem, protozoa serving as primary consumers of bacteria and fungi as well as food sources for metazoa, construct an essential trophic link in food web and therefore make a numerically and functionally significant contribution (Pernthaler et al., 1997; Martín-Cereceda et al., 2001; Degans et al., 2002; Nicolau et al., 2005). In addition to improving the effluent quality through grazing of dispersed bacteria and suspended particles (Curds and Cockburn, 1970;

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Nomenclature			
Symbols and abbreviations			
A2O	anaerobic/anoxic/aerobic and anoxic/anaerobic/aerobic process	MLSS	mixed liquor suspended solids
AO	anoxic/aerobic or anaerobic/aerobic process	MWTP	municipal wastewater treatment plant
BOD _e	biological oxygen demand of effluent	NH ₃ -Ne	the concentration of ammonia nitrogen in effluent
BOD _i	biological oxygen demand of influent	NH ₃ -Ni	the concentration of ammonia nitrogen in influent
DO	dissolved oxygen	NO ₃ -N	the concentration of nitrates
HRT	hydraulic retention time	OD	oxidation ditch
		SBR	sequencing batch reactor
		SRT	solids residence time
		SVI	sludge volume index

Curds, 1982; Salvadó et al., 1995; Lee et al., 2004), protozoa exert potential influence on the treatment system by regulating the morphological and taxonomic structures of bacterial community through top-down predatory behaviors (Šimek et al., 1997; Jürgens et al., 1999; Hahn and Höfle, 1999, 2001). Giving an insight into protozoan communities is hereby of great significance in expanding the holistic understanding of activated-sludge ecosystem, and thus improving the performance of the existing biological treatment processes.

It is accepted that the protozoan communities will be influenced in two pathways: to be manipulated directly by virtue of their inherent sensitivities to environment, and meanwhile to be modified indirectly in accordance with the potential changes of bacterial community structures through food chain (Curds and Cockburn, 1970; Zarda et al., 1998; Jezbera et al., 2005; Nicolau et al., 2005). For building the species-habitat relationship, most previous studies have been devoted to monitoring protozoan communities for predicting effluent quality and plant performance (Curds and Cockburn, 1970; Poole, 1984; Al-Shahwani and Horan, 1991; Salvadó et al., 1995; Martín-Cereceda et al., 1996). The species of protozoa present in activated sludge have been well inventoried as important bio-indicators since last century (Curds and Cockburn, 1970; Madoni and Ghetti, 1981; Madoni et al., 1993; Nicolau et al., 2001). Recently, with the awareness of their potential indications for operating conditions, some researchers have focused on evaluating the association of protozoan species with operational parameters (Esteban et al., 1991; Salvadó and Gracia, 1993; Salvadó, 1994; Martín-Cereceda et al., 1996; Lee et al., 2004). Although the process principle of a treatment system, such as configuration, flow regime as well as aeration mode, etc., also affects the habitat of its microscopic individuals to some extent, its relationship with protozoan communities has been poorly understood.

In this context, systematic analyses on protozoan communities of 8 biological treatment systems in 5 full-scale MWTPs of Beijing were carried out in support of statistical approaches involving multivariate methods, such as cluster analysis and principal components analysis (PCA). These 8 systems include mainly 4 types of process, anaerobic/anoxic/aerobic or anoxic/anaerobic/aerobic process (A2O), anoxic/aerobic or anaerobic/aerobic process (AO), oxidation ditch (OD) and sequencing batch reactor (SBR).

The objectives of this study were to characterize (1) the distribution patterns of protozoan populations in different sewage treatment systems; in order to provide an elucidation

on (2) the potential impact of process principles on protozoan communities, i.e. whether the structures of protozoan community vary with different treatment processes, and how; and further to determine (3) the potential contributions of system conditions in terms of operational properties and water quality on characterizing community structures of protozoa.

2. Materials and methods

2.1. Description of wastewater treatment systems investigated

Five full-scale MWTPs, including eight different treatment systems, in Beijing, China, were investigated. The characteristics of each plant are outlined in Table 1 and the schematic flow diagram of each system is shown in Fig. 1. Plant G, the largest MWTP in China, comprises three complete treatment systems: one anoxic/anaerobic/aerobic process (G1) and two anoxic/aerobic processes (G2 and G3), each of which receives one-third of inflow. Plant Q consists of two parallel treatment systems of A2O processes, i.e. a modified one (anoxic/anaerobic/aerobic, Q1) and a normal one (anaerobic/anoxic/aerobic, Q2). These two MWTPs serve as representative in-situ models for comparing the effects of process principles and wastewater sources on protozoan communities. Plant B with an anaerobic/aerobic process, Plant J (the Pasveer type of OD) and Plant W (SBR) were selected to confirm the potential contributions of process principles on characterizing community structures of protozoa.

2.2. Sampling protocol

Samples were collected for three times (October 2005, April 2006 and November 2006). Samples of influent and effluent were collected at the same time, and their characteristics were determined according to standard methods for the examination of water and wastewater (APHA, 1995). The water quality of the influent and effluent during the sampling period was estimated on an average of the three samples and is shown in Table 2. Sludge samples for microscopical examination were collected randomly with 1 l plastic bottles from the mixed liquor of the aeration stage immediately prior to solid-liquid separation. For meeting the oxygen demands, it was ensured that only less than two-thirds of the volume of

Table 1 – Description of eight objecting full-scale municipal wastewater treatment systems in relation to four types of process

MWTP	Treatment capacity (m ³ /d)	Process principle	Sample name
Gaobeidian 1	250,000	Anoxic/anaerobic/aerobic	G1-iA2O
Gaobeidian 2	250,000	Anoxic/aerobic	G2-AO
Gaobeidian 3	250,000	Anoxic/aerobic	G3-AO
Qinghe 1	100,000	Anoxic/anaerobic/aerobic	Q1-iA2O
Qinghe 2	100,000	Anaerobic/anoxic/aerobic	Q2-A2O
Beixiaohe	40,000	Anoxic/aerobic	B-AO
Jiuxianqiao	200,000	Oxidation ditch-Pasveer	J-OD
Wujiacun	80,000	Sequencing batch reactor	W-SBR

each bottle was occupied with the mixed liquor, and that the bottles were lidded untightly. The temperature during sampling was approximately 20°C.

2.3. Microscopical examination of protozoa

All grab samples were completed for microscopic analysis within 5 h after collection (Madoni and Ghetti, 1981). After shaking, sub-samples of 25 µl in volume were extracted with an automatic micropipette for microscopic analysis (Martín-Cereceda et al., 1996). Slides were viewed under an Axioskop 2 epifluorescent microscope (Carl Zeiss, Jena, Germany) mounted with a video camera, using bright-field and differential interference contrast. Direct counting procedures were carried out at magnifications of $\times 100$ – $\times 400$, according to the sizes of species, and were expressed in number of individuals per ml of mixed liquor. Identification of protozoa was mainly based on morphology and movement (Shen and Zhang, 1990; Patterson, 1996). Whenever necessary, images were taken and documented with the standard software package provided by Zeiss (Axio Vision 4.1). The results for each sample were determined by averaging the counts of three replicates.

2.4. Statistical analyses

Since this survey was particularly made with interests in process principles of MWTPs, to minimize any possible confusion resulting from the seasonal changes in temperature and operational malfunctions on occasion, estimation was performed here with averaged data of the three samplings.

In order to reveal the existence of potential correlation between treatment systems and distribution of protozoan populations, multivariate exploratory techniques, which are especially suitable for processing large-sized data containing high dimensionalities and correlations, were carried out with the aid of software STATISTICA for Windows Version 6.0. Prior to conducting statistical analysis, normalization of all measured data was employed with this program to stabilize variance.

Cluster analysis, as one type of multivariate statistics, provide a classification algorithm to link together objects sufficiently similar to each other and then amalgamate

successively larger clusters, typically illustrated by the structure of a hierarchical tree with distinct branches (Hansel-Welch et al., 2003; Jiang et al., 2007). Hereby, to characterize the community structures of protozoa with various treatment processes, tree clustering on the amalgamation rule of unweighted pair-group average was conducted. To interpret the relative similarity reflected by linkage distance of neighbors across clusters, correlation coefficients in forms of 1–Pearson r was selected as distance measure in data computing.

On the other hand, to capture the statistical behavior of various treatment system variables, PCA was performed. By way of compiling and classifying these high-dimensional factors simultaneously, PCA was considered appropriate for recovering a vector space of lower dimension and displaying the important information derived from the measured data compacted in a complex data set (Kalin et al., 2001; Yoo et al., 2003; Hansel-Welch et al., 2003; Jiang et al., 2007). Furthermore, to facilitate its visual interpretability, via the correlation matrix of active variables, 2D plots of factor coordinates were produced in this paper.

3. Results

3.1. Species richness and relative abundance of protozoa in relation to various treatment processes

Data presented here were collected from the eight treatment systems. A total of 67 protozoan morphospecies, attributed to 33 genera were identified, including 48 ciliates, 12 amoebae and 7 flagellates. For data analyses, protozoa were further categorized into eight groups in terms of lifestyles, phenotypic traits and other food preference (Shen and Zhang, 1990; Madoni, 1994; Martín-Cereceda et al., 1996; Lee et al., 2004). Their population distributions in abundance categories were screened on analysis of normal distribution and were listed in Table 3. Changes of protozoan community structures in different treatment systems were depicted in Fig. 2.

Protozoan compositions in terms of species richness and relative abundance in the eight systems rendered generally the same characteristics: first, ciliates > amoebae > flagellates; second, ciliates were overwhelmed by sessile and crawling ones, which have been considered as the key functional components and their dominance usually indicated the high

Fig. 1 – Flow charts of eight full-scale municipal wastewater treatment systems investigated in this study.

Table 2 – Physico-chemical and operational characteristics of eight full-scale municipal wastewater treatment systems investigated in this study

Plants	Influent		Aeration tank					Effluent		
	BOD (mg/l)	NH ₃ -N (mg/l)	MLSS (mg/l)	SVI (ml/g)	HRT (h)	SRT (d)	DO (mg/l)	BOD (mg/l)	NH-N ₃ (mg/l)	NO ₃ -N (mg/l)
Gaobeidian 1	204±52	21.1±6.3	1540±273	152±32	7.2	15	1.20±0.1	11.3±4.7	8.0±5.1	13.5±8.3
Gaobeidian 2	215±58	25.8±8.1	2533±523	130±66	7.0	15	2.00±0.3	10.9±5.4	13.3±10.4	12.6±11.1
Gaobeidian 3	194±47	23.1±6.9	2318±477	146±59	7.0	15	1.70±0.3	12.5±3.1	8.5±7.9	9.6±9.1
Qinghe 1	170±37	45.2±8.5	1841±203	71±27	9.6	6	1.20±0.3	11.0±8.6	2.6±2.1	21.4±8.5
Qinghe 2	158±34	40.7±7.5	1805±211	76±22	10.8	6	1.50±0.4	11.2±7.5	2.3±1.9	19.6±7.6
Beixiaohe	211±67	42.1±12.7	1039±263	197±153	8.0	10	1.20±0.1	10.5±8.1	25.7±15.6	6.6±4.1
Jiuxianqiao	223±71	46.9±7.5	4041±473	56±27	15.7	16	2.00±0.3	10.3±7.1	2.8±1.7	5.2±2.7
Wujiacun	151±47	45.0±6.7	4520±513	62±44	4.0	15	1.60±0.2	9.4±4.1	16.0±12.1	13.0±8.1

efficiency of an activated-sludge system (Madoni, 1994). As compared with each other, however, protozoan distribution varied in correspondence with treatment processes. The A2O ecosystems of Plant G1, Q1 and Q2, harbored the most diverse protozoan communities in a range of species numbers from 41 up to 50. Whilst, all AO systems including Plant B, G3 as well as G2 exhibited relatively lower numbers of species (approximately 40), followed by OD (34) and SBR (below 30).

Accompanied by varying characteristics of taxonomic compositions, relative abundance appeared a pronounced distinction with regard to different processes. Along with its preponderance in numerous taxa, the three A2O systems represented the most extraordinary proportions of sessile and crawling ciliates, suggesting that the A2O process might be more stable and efficient in comparison with the other processes studied in this survey (Madoni, 1994). It is noteworthy that although virtually poor species richness was encountered in SBR, the relative abundance of sessile and crawling ciliates in this process reached the level equivalent to A2O. It is interesting that testae and naked amoebae, whose indicative significance in activated sludge ecosystems are still not so clear, were relatively abundant in the OD process. For the AO systems, testae and naked amoebae as well as carnivorous ciliates appeared relatively diverse and abundant. Carnivorous ciliates are believed to function indicatively as deficient sedimentation of the sludge (Martín-Cereceda et al., 1996; Lee et al., 2004). It is noted that Plant B (B-AO) had a markedly higher relative abundance of carnivorous ciliates, which is in correspondence to its relatively bad sludge settleability (average SVI, 197 ml g⁻¹).

3.2. Association of protozoan community structures with process principles

For further comparison of the entire protozoan community structures among different processes, a more systemic algorithm of tree clustering was adopted, depending on both taxonomic and numerical distributions of protozoa in the eight treatment systems, and a horizontal dendrogram

derived from this multivariate analysis was constructed in Fig. 3.

Four major clusters of protozoan communities were in coordination with the four major types of treatment process. The nearest neighbors of Plants Q1, Q2 and G1 with the same process formed the A2O cluster, which declared a much higher similarity than the other clusters. Successively larger clusters aggregated by process OD and then adjacent SBR indicated a relative similarity of these two processes to A2O. Meanwhile, away from them, the furthest grouping of process AO, which demonstrated a marked distinction from the uppers, comprised a sub-group of anoxic/aerobic process in Plants G2 and G3, and an anaerobic/aerobic process in Plant B in addition. The long distance within this AO cluster denoted a relatively low similarity to one another. On all account, the emerging clusters apparently formed in process order: Group A2O-OD-SBR-Group AO, which clearly mirrored a strong ecotype-habitat relationship. That is, ecosystems with respect to the same process principles were colonized by populations of the most similar structures.

However, even in the A2O process group, the three systems of G1-A2O, Q1-A2O and Q2-A2O, still presented some differences from each other in terms of protozoan community structures. Q1-A2O and Q2-A2O are two parallel systems in Plant Q, and their main difference is in the sequence of anaerobic and anoxic units (Fig. 1). The difference between G1-A2O and Q1-A2O is mainly in their wastewater sources and operating conditions. The higher similarity between Q1-A2O and Q2-A2O systems suggested that, besides the process principle, the source of raw wastewater and the operating conditions of a treatment system might be another two important factors determining the similarity of protozoan community structures. It also seemed that the sequence of anaerobic and anoxic units in A2O systems have less significant effect on community structures.

The overall state of a treatment system can be defined mainly by two parts, namely process principles and system conditions in terms of operational properties and water quality. These two definitive features exert their potential influence together on characterizing protozoan communities

Table 3 – Population distributions of protozoa expressed in abundance categories

Functional groups	Species	G1-iA2O	G2-AO	G3-AO	Q1-iA2O	Q2-A2O	B-AO	J-OD	W-SBR
Swimming ciliates	<i>Colpoda</i> sp.	–	+	++	–	–	++	–	–
	<i>Loxophyllum uninucleatum</i>	+	–	–	–	+	–	–	–
	<i>Paramecium caudatum</i>	–	+	+	–	–	+	–	–
Crawling ciliates	<i>Aspidisca costata</i>	++++	–	–	++	+++	–	+++	++
	<i>Aspidisca lynceus</i>	+++	++	++	++	++	++	++	++
	<i>Aspidisca sulcata</i>	+++	++	++	+	+	+++	++	++
	<i>Chilodonella aplanata</i>	+	–	++	+	+	++	++	–
	<i>Chilodonella capucina</i>	–	–	–	–	–	–	++	–
	<i>Chilodonella uncinata</i>	+	++	++	+	+	++	+++	++
	<i>Euplotes affinis</i>	+	++	++	+	+	+	+++	–
	<i>Euplotes muscicola</i>	–	–	–	+	+	–	–	–
	<i>Euplotes patella</i>	+	–	–	–	–	–	–	–
Sessile ciliates	<i>Carchesium polypinum</i>	++	–	–	++	++	–	+++	++
	<i>Epistylis lascastris</i>	+++	+++	+++	++++	+++	+++	+++	+++
	<i>Epistylis plicatilis</i>	++++	+	+++	++++	++++	+++	+++	+++
	<i>Epistylis</i> sp.	++	+	++	+	++	+	+	++
	<i>Epistylis urceolata</i>	+++	+++	++++	+++	+++	+++	+++	+++
	<i>Haplocaulus</i> sp.	++	–	–	–	–	–	+	–
	<i>Opercularia cylindrata</i>	+	+	++	+	++	+	–	+
	<i>Opercularia microdiscum</i>	+	+	+	+	++	+++	–	+
	<i>Opercularia phryganeae</i>	+	+	++	+	++	++	++	+
	<i>Pseudovorticella moninata</i>	–	–	–	–	+	–	–	–
	<i>Vorticella aequilata</i>	+	–	–	++	++	–	++	–
	<i>Vorticella campanula</i>	++	–	–	+	++	–	–	–
	<i>Vorticella convallaria</i>	+++	+	++	++	++	+	+	+++
	<i>Vorticella cupifera</i>	+	–	–	+	+	–	–	–
	<i>Vorticella elongata</i>	+	–	–	+	+	–	–	–
	<i>Vorticella fromenteli</i>	+	–	–	+	++	–	–	–
	<i>Vorticella hamata</i>	++	++	++	++	++	+	–	+
	<i>Vorticella microstoma</i>	+	++	+	+	+	+	++	+
	<i>Vorticella octava</i>	+	+	+	+	++	+++	+	+
	<i>Vorticella picta</i>	++	–	–	+	++	–	–	–
	<i>Vorticella putrina</i>	++	+++	+	+	++	+	–	–
	<i>Vorticella striata</i>	+	+	++	+	+	+	++	++

Carnivorous ciliates	<i>Acineta tuberosa</i>	+	+	+	+	+	+	–	–
	<i>Coleps hirtus</i>	+	+	+	+	+	+	+++	+
	<i>Coleps elongatus</i>	+	+	+	+	+	+	–	–
	<i>Hemiophrys agilis</i>	–	–	+	–	–	++	–	+++
	<i>Hemiophrys pectinata</i>	–	–	–	–	+	–	–	–
	<i>Hemiophrys pleurosigma</i>	–	–	+++	–	–	–	–	–
	<i>Hemiophrys punctata</i>	–	+	+	–	–	+	–	++
	<i>Litonotus carinatus</i>	+++	++	++	+	+	+++	–	–
	<i>Litonotus fasciola</i>	–	–	–	–	+	–	–	–
	<i>Litonotus lamella</i>	+	++	++	–	–	++	–	–
	<i>Litonotus obtusus</i>	–	–	–	+	+	–	–	–
	<i>Podophrya fixa</i>	+	–	–	–	–	–	–	–
	<i>Tokophrya quadripatite</i>	+	–	+	+	+	+	+	+
	<i>Trachelophyllum pusillum</i>	+++	–	++	++	+	+++	–	–
Naked amoebae	<i>Cashia limacoides</i>	–	+	+	–	–	+	–	–
	<i>Hartmanella cantabrigiensis</i>	–	–	+	–	–	–	++	–
	<i>Mayorella cypressa</i>	–	+	+	–	–	+	–	–
	<i>Mayorella hohuensis</i>	+	+	+	+	–	+	++	–
	<i>Saccamoeba gongornia</i>	–	+	+	–	–	+	+	–
	<i>Striamoeba striata</i>	+	+	+	+	+	+	++	–
	<i>Thecamoeba sphaeronucleolus</i>	+	+	+	–	+	+	+	–
Testae amoebae	<i>Arcella gibbosa mitriformis</i>	+	+	–	–	–	–	+	–
	<i>Arcella hemisphaerica</i>	+	++	++	+	++	+	++	+
	<i>Arcella nulgaria</i>	+	++	+	+	++	+	++	+
	<i>Arcella catinus</i>	+	+	+	–	+	+	+	–
	<i>Euglypha tuberculata</i>	–	+	–	–	–	+	+	–
Heterotrophic flagellates	<i>Bodo</i> sp.	+	+	–	+	+	+	+	+
	<i>Peranema</i> sp.	+	–	–	–	–	–	–	+
	<i>Peranema trichophorum</i>	+	+	–	–	–	–	+	+
Autotrophic flagellates	<i>Anisonema acinus</i>	+	–	–	–	+	–	+	–
	<i>Entosiphon sulcatum</i>	+	+	+	+	+	+	–	–
	<i>Oikomonas socialis</i>	+	+	+	–	–	++	–	–
	<i>Polyoecca dumosa</i>	–	–	–	+	+	–	–	–

Absence: (–); scarce: 1–50 ind ml^{–1}(+); moderate: 51–100 ind ml^{–1}(++); abundant: 101–500 ind ml^{–1}(+++); more abundant: > 500 ind ml^{–1}(++++).

Fig. 2 – Species richness and abundance distribution of protozoa in functional groups.

in a treatment system. It is therefore also essential to know how protozoan community structures are affected by system conditions.

3.3. Association of protozoan community structures with operational properties and water quality

To understand the effects of system conditions in terms of operational properties and water quality, two PCA analyses with extracted principal components subjected to Varimax Rotation were designed representatively. Loading biplots of PCA with protozoan communities imposed on were performed to detect how these variables interacted to modify protozoan communities. In these analyses, DO was used both as an operational and water quality variables.

Table 4 showed the PCA of operational properties and protozoan communities. The first two factors with eigenvalues of 2.45 and 1.45, respectively, captured nearly 78% of the accumulated variance and were selected as a pair of effective axes to generate a bidimensional vector space. The PCA biplot

with a correlation circle was illustrated in Fig. 4. Two well-defined groups were acquired on the basis of regions occupied by variables, i.e. the closer the pattern the stronger the interrelation. MLSS, DO and two kinds of amoebae formed one group. The close correlation between MLSS and DO was also reported in previous research (Yoo et al., 2003). Statistically significant variables derived from the PCA analyses were further quantitatively correlated in Pearson coefficients with populations of protozoa in the eight systems, as shown in Table 5. It also revealed that DO was correlated markedly with amoebae (Pearson = 0.86, $P < 0.05$), which indicated that amoebae were especially sensitive to DO level in activated sludge systems. In the other group, the close relationship between SVI and carnivorous and swimming ciliates reiterated that these ciliates might have affected sludge settleability significantly as discussed above. SVI showed a close positive correlation to carnivorous and swimming ciliates with relatively higher values of 0.92 and 0.86, respectively ($P < 0.05$) (see Table 5). Our results are in good agreement with previous studies, in which these functional ciliates such as *Litonous lamella* and

Fig. 3 – Dendrogram of protozoan communities in relation to various treatment processes.

Table 4 – Contributions of operational and protozoan variables derived from PCA with 5 PCs

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigenvalue	2.45	1.45	0.78	0.27	0.05
Cumulative eigenvalue	2.45	3.90	4.68	4.95	5.00
% Total variance	48.99	28.94	15.72	5.35	1.00
Cumulative variance %	48.99	77.93	93.65	99.00	100

Amphileptus sp. were observed in activated sludge with poor settleability (Martín-Cereceda et al., 1996; Lee et al., 2004).

Another PCA analysis on the association between water quality and protozoan communities was interpreted in Table 6. The first two PCs were significant with a cumulative variance of more than 64% and respective eigenvalue of 2.13 and 1.73. The ordination of this bidimensional space was plotted in Fig. 5. NO_3^- -N and sessile ciliates could be grouped together, suggesting that sessile ciliates might be related with nitrifiers to some extent. However, the correlation significance that resulted from Pearson correlation analysis was relatively low (data not shown). This predator–prey interaction in relation to nitrification is continued in our lab. It is interesting that DO, NH_3 -Ne and BODi were clumped together with most protozoan communities into a large species-quality cluster. Similar relationship between DO and amoebae was also observed in Fig. 4, which depicted the inter-relation between operational and protozoan community variables. A positive correlation of 0.73 ($P < 0.05$) between NH_3 -Ne and carnivorous ciliates (see Table 5) corroborated that carnivorous ciliates were related not only with SVI but also with NH_3 -Ne. The reason, however, requires further research.

The above PCA and Pearson analyses results demonstrated that while protozoan community structures were determined by the process principles to a large extent, they were also

modified by system conditions in respect of operational properties and water quality.

4. Discussion

4.1. Changes of community structures with various processes

While the microbial community structures of activated sludge have some general characteristics in common, it is reasonable to propose that pronounced differences do exist in various treatment systems (Madoni and Ghetti, 1981; Forney et al., 2001). The assumption that protozoan ecotype was somewhat associated with process principles, in terms of different configurations or regimes, was confirmed by the above results. The taxonomic composition and population distribution of protozoa appeared to vary greatly with types of treatment processes. The ecologically significant groups of crawling and sessile ciliates were noted as an extraordinary diversity and abundance in the A2O systems.

To date, the underlying reason that treatment processes specifically drive the pattern of protozoan distributions in this way is not well known. Further studies aiming for explaining this are conducted in details by our group.

Necessarily speaking, besides the ciliates, who act certainly as the predominant inhabitants in the activated sludge and thus were studied preferably by researchers, testae amoebae were observed with relatively higher frequencies and sometimes with considerable abundance in this study. Although these amoebae have been assumed to be

important in terms of their equivalent body sizes and appetites for bacterial populations to ciliates, there still remain scarce data in related studies (Shen and Zhang, 1990; Foissner, 1999). Upon that, we pose a challenging notion of more extensive studies emphasized on their ecological significance.

Fig. 4 – PCA analysis of operational characteristics and protozoan populations. Protozoan variables were labeled as follows: carnivorous ciliates, CaC; sessile ciliates, SeC; crawling ciliates, CrC; swimming ciliates, SwC; testae amoebae, TeA; naked amoebae, NaA; heterotrophic flagellates, HeF; autotrophic flagellates, AuF.

Fig. 5 – PCA analysis of water quality and protozoan populations. Protozoan variables were labeled as follows: carnivorous ciliates, CaC; sessile ciliates, SeC; crawling ciliates, CrC; swimming ciliates, SwC; testae amoebae, TeA; naked amoebae, NaA; heterotrophic flagellates, HeF; autotrophic flagellates, AuF.

Table 5 – Correlation coefficients between protozoa and significant treatment variables derived from PCA

	Carnivorous ciliates	Sessile ciliates	Crawling ciliates	Swimming ciliates	Testae amoebae	Naked amoebae	Heterotrophic flagellates	Autotrophic flagellates
SVI	0.92*	−0.21	0.06	0.86*	−0.19	−0.02	0.30	0.59
DO	−0.46	−0.54	−0.03	−0.08	0.86*	0.61	−0.11	−0.62
NH ₃ -Ne	0.73*	−0.53	0.16	0.61	−0.39	−0.20	0.68	0.35
* $p < 0.05$.								

Table 6 – Contributions of water quality and protozoan variables derived from PCA with 6 PCs

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Eigenvalue	2.13	1.73	1.39	0.45	0.26	0.04
Cumulative eigenvalue	2.13	3.86	5.25	5.70	5.96	6.00
% Total variance	35.45	28.90	23.12	7.45	4.31	0.77
Cumulative variance %	35.45	64.35	87.47	94.92	99.23	100

4.2. Community stability in relation to different process principles

From cluster interpretation, the community structures of protozoa emerged clearly in a very high coordination with process principles rather than influent sources or operating conditions. However, the difference between Group A2O and Group AO in terms of the similarity within each group revealed that the A2O process have a much higher stable protozoan communities in comparison with AO.

As shown in Fig. 3, the A2O process marked a distinctively higher similarity of protozoan community structures among different treatment systems. In contrast, AO (anoxic/aerobic) process displayed another sub-cluster with much lower similarity of structures even in the parallel trains of Plant G working under almost identical conditions. Accordingly, a higher ecotype stability of process A2O could be concluded. And as a higher level in food chain, this stable structure of protozoa might be considered informative of the structural stability of bacterial communities and thus functional stability of the overall ecosystem.

To assess the functional aspects of a community, both species richness and community structures should be integrated (Dighton, 1997). The underlying reasons that A2O ecosystem appears to be more stable could be therefore given in such two pathways. The highest species richness as described above was considered as one of the most important properties for A2O to resist disturbance. That is, the higher degree of redundancy in species, the generally higher degree of stability in biological functions (Griffiths et al., 2000, 2001). The relatively higher species richness of the A2O process (see Fig. 2) is related with its more complicated configuration: there exist three different environments, i.e., anaerobic, anoxic and aerobic, in the treatment system. Compared with AO (anoxic/aerobic), the A2O process has an additional anaerobic unit to improve the biodegradability of wastewater by fermentation and to accomplish biological phosphorus removal. There thus occurred a much richer composition of bacteria, especially phosphate accumulation organisms, as well as richer actinomycotic and yeast populations (Liu et al., 2007). As an ecologically trophic link with bacteria, a predatory diverse and complex community of protozoa was born out accordingly. In addition to species richness, characteristics of community structures are also responsible for the high stability of A2O. As mentioned above, A2O was typically colonized by the most predominant populations of attached and crawling ciliates, which preferred to dwell in the flocs and was therefore testified by Madoni (1994) to maintain dynamic stability in an efficient activated-sludge ecosystem. These results were also corroborated by other observations that the attached and crawling ciliates were the most representative groups in a stable aeration tank (Martín-Cereceda et al., 1996; Salvadó et al., 1995).

It is noteworthy that the AO process exhibited fairly less-stable community structures and was thus unsuccessful to maintain treatment performance, which was also reflected in the factual operating practice of Plants G2 and G3. Even then, it is unlikely to conclude that the A2O ecosystem somewhat displays a certain competence for decomposition or degradation with higher efficiency than the others.

5. Conclusions

- I. The protozoan community structures were primarily determined by treatment process principles, whilst they were also modified by system conditions in terms of operational properties and water quality.
- II. The A2O ecosystems exhibited higher similarity and diversity of protozoan community structures with higher relative abundance of crawling and sessile ciliates than the other systems investigated. Cluster analysis revealed that the A2O ecosystems were more stable than those of AO.
- III. PCA demonstrated that: (1) swimming and carnivorous ciliates were correlated with poor settleability of sludge; (2) amoebae were sensitive to DO level; and (3) sessile ciliates were associated with nitrifiers to some extent.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2007.11.020

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