



Characteristics of microfauna and their relationships with the performance of an activated sludge plant in China

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Abstract

The occurrence and abundance of the microfauna groups were compared with the physico-chemical and operational parameters of the Baoding Lugang Sewage Treatment Plant in China. Attached and crawling ciliates were the dominant groups of ciliates. Crawling ciliates and testate amoebae showed a strong association with effluent BOD₅ (biochemical oxygen demand). Therefore, these two groups are likely to be useful bioindicators since their number decreased as the process produced poor quality effluent. Testate amoebae also had significant negative correlations with effluent TN (total nitrogen), NH₄⁺-N, SS (suspended solids) and SVI (sludge volumetric index), which means that this group of ciliates may be indicators of good performance of the activated sludge system. Carnivorous ciliates and flagellates had significant positive correlations with SVI, suggesting that these two groups may be indicators of bad settlement conditions of sludge. As identification of the microfauna species is difficult and time-consuming, we recommend using microfauna functional groups to evaluate the performance of the activated sludge system.

Key words: microfauna; activated sludge; indicators; sludge biotic index (SBI)

Introduction

Protozoa, especially ciliates, are generally predominant taxa when activated sludge performs adequately. Most of the protozoa found in activated sludge system are ubiquitous appearing all over the world (Madoni *et al.*, 1993; Salvadó *et al.*, 1995). About 230 species of protozoa have been identified in the wastewater treatment plants: 33 flagellates, 25 rhizopodi, 6 actinopodi, and 160 ciliates (Madoni, 1994). The majority of ciliates present in biological water treatment plants feed upon dispersed populations of bacteria. The ciliates in activated sludge can be subdivided into four groups on the basis of behaviour: free-swimmers, crawlers, attached and carnivorous (Madoni, 1994).

Rotifers were shown to have two distinct effects on suspended particulate matter: consumption of dispersed bacteria; improved settling and enhanced aggregation of flocs. Lapinski *et al.* (2003) demonstrated the potential for the use of bdelloid rotifers in an enhanced wastewater treatment process, with reduced biomass production and improved effluent clarity. Chen *et al.* (2004) found that rotifers showed a negative association with the reduction of BOD₅, COD and suspended solids removal at two plants in China. Nematode abundance in activated-sludge systems generally represents less than 1% of the microfauna;

their presence is limited by the short residence time of the biomass in the system (Salvad, 1994). Tardigrades, gastrotrichs and oligochaetes were rarely recorded in daily microscopic examination of activated sludge (Klimowicz, 1970; Madoni *et al.*, 1993).

This sludge biotic index (SBI) is based on the abundance and diversity of the protistan community in the activated sludge of the aeration tank and on the different sensitivity revealed, by some of the microfauna groups, to physical-chemical and operational factors prevailing in the system (Madoni, 1994).

The routine analysis of the microfauna as an indicator of activated-sludge plant performance is becoming more and more common. This analysis gives useful information on the biological activity of the sludge based on the community structure of the microorganisms present. Identification of groups of microfauna according to their behavior and morphology is much easier and applicable. Thus, we recommend an integrated approach to assess the performance of the activated sludge.

1 Materials and methods

The plant which located in Baoding City, Hebei Province, was built in September 1996. Before the plant was built, wastewater from Baoding City was discharged into the Fuhe River upstream of Baiyangdian Lake, the

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largest natural freshwater body in the North China Plain, whose water quality was consequently severely degraded. In recent years, the water quality of the Baiyangdian Lake has improved greatly because most water flowing into it had been treated by sewage treatment plant in Baoding City. The hydraulic retention time (HRT) of the plant is 8.5 h, F/M is 0.15 kg BOD₅/(kg MLSS·d), mixed liquor suspended solids (MLSS) is 3500 mg/L, dissolved oxygen (DO) is above 2.0 mg/L in the aeration tank.

Samples were collected from the mixed liquor at the end of the aeration tank in 11 plastic bottles. Sampling was conducted on a weekly basis from July 2002 to July 2003. In order to avoid random temporal changes in density and in richness, microscopic examination was carried out within 3 h of collection, using both bright field and phase contrast microscopy. Microfauna abundance was determined with a sub-sampling technique: A 25-μl volume of the mixed liquor was taken with a automatic micropipette, and samples were examined in triplicated (Zhou *et al.*, 2006). The identification of species was carried out “*in vivo*”. Several keys (Kudo, 1966; Shen *et al.*, 1990; Patterson, 1996; Foissner *et al.*, 1999) were employed for the identification of microfauna. All ciliates, most sarcodina and flagellates were identified to species level, while, the numbers of rotifers, nematodes, tardigrades and oligochaetes were each recorded.

The following physical and chemical variables of the effluent were analysed according to the Standard Methods for Water and Wastewater Analysis (APHA, 1989): biological oxygen demand (BOD₅), sludge volumetric index (SVI), total nitrogen (TN), ammoniacal nitrogen (NH₄⁺-N), total phosphorous (TP) and suspended solids (SS). Minimum, maximum, mean values and Std. of each parameter are shown in Table 1.

Data handling were performed using the application program STATISTICA for Windows 6.0. Two types of multivariate analysis were employed in this study (Madoni *et al.*, 1993; Martin-Cereceda *et al.*, 1996): partial correlation analysis and factor analysis (method of extraction of factors: varimax rotation). The abiotic variables selected

for the correlation analysis were effluent BOD₅, effluent TN, TP, effluent SS and SVI. All the results were normalized according to the logarithmic transformation, $x = \ln(x+1)$.

The sludge biotic index may range from 0 (indicating the poorest condition) to 10 (indicating the best condition). SBI values are grouped in four classes corresponding to different levels of quality: class I includes SBI values of 10, 9 and 8; class II includes 7 and 6; group III includes 5 and 4; class IV includes the remaining values (Madoni, 1994).

SBI value was calculated to evaluate the performance of the activated sludge system.

2 Results

Number of species, occurrence and abundance of each microfauna groups in the Lugang Sewage Treatment Plant are shown in Table 2. A total of 50 samples were analyzed. Among 94 species of ciliates, 15 species were free-swimming forms, 13 species were crawling forms and 33 species were attached forms. Thirty-three species of carnivorous ciliates were recorded. Forty species of amoebae were found, of which 10 species were testate amoebae and 30 species were naked amoebae. Thirteen species of large flagellates were identified. Metazoa including rotifers, nematodes, gastrotrichs and oligochaeta were also recorded.

As shown in Table 2, the ratio of attached ciliates density to the whole ciliates density was 44.6%, while crawling ciliates 34.6%, carnivorous ciliates 16.8% and free-swimming ciliates 4.0%. So, attached ciliates and crawling ciliates were the dominant groups of ciliates in the Plant.

Annual variations of the density of each microfauna group are shown in Fig.1.

Pearson correlation coefficients among the density of each microfauna groups are shown in Table 3. Free-swimming ciliates had significant positive correlations with carnivorous ciliates, which are shown in Fig.1b.

Table 1 Physico-chemical variables of the effluent in the Baoding Lugang Sewage Treatment Plant

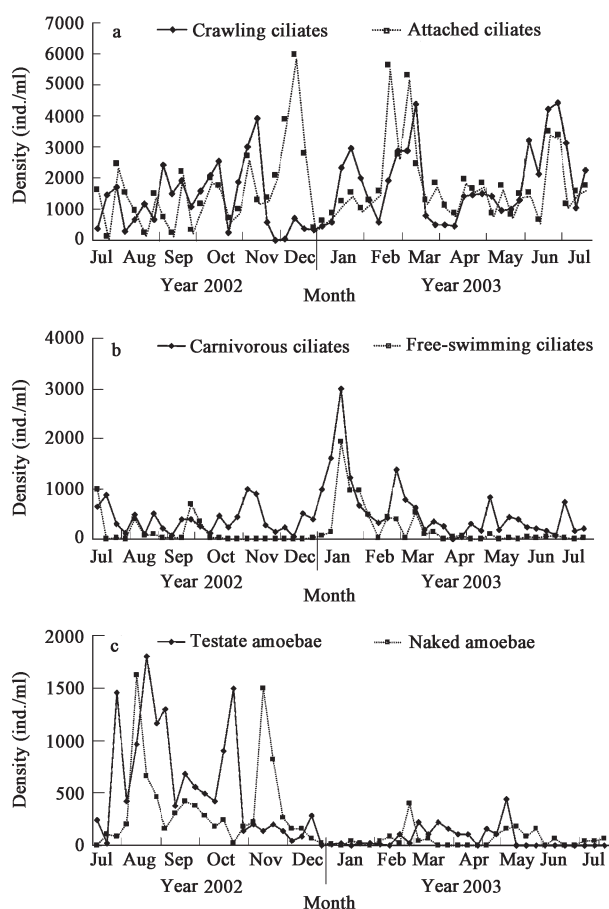
Value	BOD ₅ (mg/L)	SVI (ml/g)	TN (mg/L)	NH ₄ ⁺ -N (mg/L)	TP (mg/L)	SS (mg/L)
Maximum	19.7	127.6	31.4	24	3.67	28
Minimum	8.1	38.6	15.5	0.8	0.33	10
Average	15.50	76.72	25.07	11.58	2.09	15.84
Std.	2.74	20.37	4.72	9.50	0.79	2.77

Table 2 Number of species, number of occurrence and abundance of each microfauna groups in the Lugang Sewage Treatment Plant

	Number of species	Frequency	Abundance (ind./ml)		
			Average	Minimum	Maximum
Free-swimming ciliates	15	65	192.4	0	1940
Carnivorous ciliates	33	205	808	0	3460
Crawling ciliates	13	189	1662.8	240	5860
Attached ciliates	33	413	2148	260	7420
Testate amoebae	10	85	306.4	0	1800
Naked amoebae	30	191	374	20	1620
Large flagellates	13	44	107.6	0	860
Metazoa	4 (groups)	75	439.6	0	1640

Table 3 Correlation analysis among the density of each microfauna groups

	Crawling ciliates	Free-swimming ciliates	Attached ciliates	Carnivorous ciliates	Testate amoebae	Naked amoebae	Metazoa	Flagellates
Crawling ciliates	1	0.206	0.266	0.126	0.057	0.022	-0.029	-0.024
Free-swimming ciliates	0.206	1	-0.080	0.440**	-0.093	-0.065	-0.168	0.257
Attached ciliates	0.266	-0.080	1	0.070	-0.012	0.174	0.177	-0.281*
Carnivorous ciliates	0.126	0.440**	0.070	1	-0.161	-0.044	-0.059	0.114
Testate amoebae	0.057	-0.093	-0.012	-0.161	1	0.157	0.225	-0.267
Naked amoebae	0.022	-0.065	0.174	-0.044	0.157	1	0.390**	-0.194
Metazoa	-0.029	-0.168	0.177	-0.059	0.225	0.390**	1	-0.214
Flagellates	-0.024	0.257	-0.281*	0.114	-0.267	-0.194	-0.214	1

* $P < 0.05$, ** $P < 0.01$.**Fig. 1** Annual variations of the density of crawling ciliates and attached ciliates (a), carnivorous ciliates and free-swimming ciliates (b), and testate amoebae and naked amoebae (c).

Naked amoebae had significant positive correlations with metazoa. Attached ciliates had significant negative correlations with flagellates (Fig. 1a).

Table 4 Correlation analysis between density of each microfauna groups and physico-chemical parameters

	BOD ₅ in effluent	TP in effluent	TN in effluent	NH ₄ ⁺ -N in effluent	SS in effluent	SVI
Crawling ciliates	-0.394**	0.076	-0.207	-0.048	-0.283*	0.032
Free-swimming ciliates	0.078	0.258	-0.247	-0.117	0.073	0.267
Attached ciliates	-0.247	0.322*	0.084	-0.030	-0.200	0.154
Carnivorous ciliates	0.090	0.300*	-0.089	-0.043	0.164	0.368**
Testate amoebae	-0.367**	0.003	-0.587**	-0.542**	-0.281*	-0.522**
Naked amoebae	-0.172	0.039	-0.154	-0.171	-0.247	0.057
Flagellates	0.024	-0.292*	0.178	0.373**	0.213	0.466**
Metazoa	-0.092	0.014	-0.109	-0.226	-0.192	-0.097

* $P < 0.05$, ** $P < 0.01$.

Table 4 presents the correlation analysis results between density of each microfauna groups and physico-chemical parameters. Crawling ciliates and testate amoebae showed a strong association with effluent BOD₅, where the correlation coefficient value was -0.394 and -0.367, respectively. Carnivorous ciliates and flagellates had significant positive correlations with SVI (correlation coefficient value was 0.368 and 0.466, respectively).

Factor analysis was performed and the corresponding results are given in Table 5, showing five factors and explaining the 74.273% of the total variance. The first factor and explains 22.066% of the total variance, implicating effluent TN, NH₄⁺-N and SVI correlated negatively with testate amoebae. The second factor, explaining 14.872% of the total variance, correlated free-swimming ciliates and carnivorous ciliates. The third factor, accounting for 14.095% of the total variance, means that effluent BOD₅ and SS correlated negatively with crawling and attached ciliates. These three factors interpreted approximately 51.033% of the data on a cumulative basis.

According to the calculation method of SBI, the average density of microfauna (except small flagellates) in the Lugang Sewage Treatment Plant was 6038.8 ind./ml, the average species number recorded in each sample was 25.34. Attached ciliates and crawling ciliates were the dominant groups. The average number of small flagellates counted along the Fuchs-Rosenthal chamber diagonal was less than 10. The SBI value was 9, reached the class quality I, which meant a very well colonized and stable sludge, excellent biological activity and very good performance.

3 Discussion

Previous studies focused mainly on the relationships between microfauna species and certain characteristics of

Table 5 Factors derived by varimax rotation (PCA) and relationships between microfauna groups and physico-chemical parameters

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
BOD ₅ in effluent	0.362	0.106	0.774	0.087	-0.146
TP in effluent	-0.218	0.552	-0.005	0.637	0.022
TN in effluent	0.858	-0.286	0.277	0.021	-0.082
NH ₄ ⁺ -N in effluent	0.870	-0.195	0.120	-0.270	-0.187
SS in effluent	0.425	0.093	0.612	-0.060	-0.304
SVI	0.680	0.481	-0.124	-0.255	0.184
Crawling ciliates	0.021	0.151	-0.772	0.089	-0.236
Free-swimming ciliates	-0.088	0.794	-0.027	-0.179	-0.124
Attached ciliates	0.299	0.034	-0.484	0.643	0.215
Carnivorous ciliates	0.109	0.779	0.023	0.077	-0.032
Testate amoebae	-0.740	-0.206	-0.132	0.005	0.076
Naked amoebae	-0.057	0.008	-0.121	-0.006	0.857
Metazoa	-0.155	-0.203	0.166	0.396	0.678
Flagellates	0.300	0.248	-0.043	-0.751	-0.093
Variance explained (%)	22.066	14.872	14.095	12.408	10.832
Accumulated variance (%)	22.066	36.938	51.033	63.441	74.273

activated sludge (Curds and Cockburn, 1970; Klimowicz, 1970; Esteban *et al.*, 1991; Madoni *et al.*, 2000; Lee *et al.*, 2004; Zhou *et al.*, 2006). One of the major drawbacks of using protozoa for wastewater treatment diagnostics is the need for expert on protozoology due to difficulty in species identification. So, using functional groups of microfauna is more applicable. Some relevant researches were done in recent years (Madoni *et al.*, 2000; Chen *et al.*, 2004).

With regard to the relative abundance of the microfauna groups, our results agreed with those obtained by other authors, who also observed that attached and crawling ciliates are the most representative groups in a stable aeration tank (Madoni, 1994; Martin-Cereceda *et al.*, 1996). Attached and crawling ciliates are the best adapted to the activated sludge environment through their ability to associate with the flocs. Attached and crawling ciliates are both associated with the flocs; attached are firmly fixed by a peduncle, while crawling ciliates have cirri for moving on the flocs surface.

Carnivorous ciliates had significant positive correlations with free-swimming ciliates. Maybe the reason is that their relationship is predator and prey.

From correlation analysis, crawling ciliates and testate amoebae showed a strong association with effluent BOD₅. Therefore, these two groups are likely to be useful bioindicators since its numbers decreased as the process produced poor quality effluent. Testate amoebae also had significant negative correlations with effluent TN, NH₄⁺-N, SS and SVI, which means that this group of ciliates may be indicators of good performance of the activated sludge system. Carnivorous ciliates and flagellates had significant positive correlations with SVI, suggesting that these two groups may be indicators of bad settlement conditions of sludge. Martin-Cereceda *et al.* (1996) and Lee *et al.* (2004) also observed that carnivorous protozoa, like *Litonous lamella*, presented high positive correlation with SVI.

Principal component analysis (PCA) is a useful technique for reducing the number of variables in a data set by finding linear combinations of those variables that explain most of the variability. Since some of these variables are highly correlated, there may be one or two linear combinations of the variables that could be formed to explain

variations. The results of principal component analysis performed on microfauna groups and physico-chemical parameters are in accordance with the data obtained from correlation analysis.

The routine analysis of the microfauna as an indicator of activated-sludge plant performance is becoming more and more common. This analysis quickly gives useful information on the biological activity of the sludge based on the community structure of the microorganisms present. However, the identification of the microfauna species is difficult and time-consuming, for their size is so small and their species number is so huge. Only experts with a lot experience can do that. Identification of groups of microfauna according to their behavior and morphology is much easier and applicable. It allows diagnosis of the particular state of functionality of the plant.

Thus, we recommend an integrated approach to assess the performance of the activated sludge—from the species level to the group level, since this can provide a more precise knowledge of the activated sludge system with much easier procedure.

4 Conclusions

In this study, the occurrence and abundance of the microfauna groups were compared with the physico-chemical and operational parameters of the Baoding Lugang Sewage Treatment Plant in China.

Crawling ciliates and testate amoebae are likely to be useful bioindicators since their number decreased as the process produced poor quality effluent. Testate amoebae may be indicators of good performance of the activated sludge system. Carnivorous ciliates and flagellates may be indicators of bad settlement conditions of sludge.

As identification of the microfauna species is difficult and time-consuming, we recommend using microfauna functional groups to evaluate the performance of the activated sludge system.

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