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The microfauna communities and operational monitoring of an activated sludge plant in China

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Abstract

The composition of the microfauna community in aeration tanks at the Baoding Sewage Treatment Plant in China was analysed each week from July 2002 to July 2003. The community composition of these microfauna populations was compared with effluent quality data recorded on the same days. A total of 94 species of ciliates, 40 species of amoebae and 13 species of large flagellates were identified in the 50 samples analysed. Numbers of metazoa including rotifers, nematodes, gastrotrichs and oligochaeta were also recorded. Although, *Aspidisca cicada* showed the highest mean abundance and was present in 98% of the samples, factor analysis revealed, among other things, that high *Vorticella convallaria* and *Arcella hemisphaerica* populations correlated with good performance of the treatment plant, while high numbers of *Litonotus obtusus* indicated poor conditions for settlement of sludge. These results show some agreement with an earlier study of sewage plants in Beijing, but analysis of more plants having a diversity of input components working under a range of different operating conditions should be performed to gain a general understanding of the value of indicator species for predicting the efficiency of activated sludge plants in China.

Keywords: Protozoa; Metazoa; Activated sludge plant; Bioindicators; Factor analysis; Water treatment fauna

Introduction

The activated sludge sewage treatment process is based on the formation of suspended bacterial flocs, which, with associated organisms, may be easily separated from the effluent in sedimentation tanks. Microfauna play an important role in this system; they limit the density of bacterial populations by predation and contribute to the flocculation process, being thereby responsible for an improvement in the quality of the effluent.

Microfauna are very sensitive to environmental variations and changes in the microfauna community may affect the whole food web of these artificial ecosystems, thus affecting the biological performance of the wastewater treatment plant; the structure of the microfauna community is therefore an indicator of the operating conditions of the plants (Curds 1975; Madoni 1994, 2002, 2003; Lee et al. 2004; Chen et al. 2004).

A quantitative and qualitative survey of microfauna from Baoding Sewage Treatment Plant in China has been carried out during a period of 1 year from July 2002 to 2003. The aim of this study is to determine the

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relationship between the community composition and density of these microfauna populations and effluent quality in this activated sludge plant, and to compare the results with data from sewage plants in Beijing (Chen et al. 2004) so as to strengthen understanding about which microfauna might provide good indicators of the performance of activated sludge plants in China.

Materials and methods

The Baoding Sewage Treatment Plant

The plant, which is 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 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 two sewage treatment plants in Baoding City. Typical operational characteristics of the Baoding Sewage Treatment Plant are shown in Table 1.

Sampling

Samples were collected from the circulating mixed liquor of the aeration tank in 1 l plastic bottles. Collections were made once a week from July 2002 to 2003.

Enumeration and identification of microfauna

Microscopical examinations were made within 3 h after collection in order to avoid progressive temporal changes in density and in richness. Protozoan and metazoan abundances in the mixed liquor were determined using a sub-sampling technique: the microfauna in three 25 μl replicates taken from the sample with an automatic micropipette were identified and counted.

Table 1. Operational parameters of the Baoding Sewage Treatment Plant

Total capacity of the system (tons/d)	8×10^4
Mean feeding rate (m^3/h)	3833
Residence time in the aeration tank (h)	8.5
Sewage retention time (h)	16.7
Food to microorganism ratio[a] (kgBOD$_5$/(kgMLSS d))	0.15

[a]This relates the biodegradable organic food available to bacteria (represented by the BOD) to the weight of microorganisms (represented by the volatile suspended solids) and gives information on the potential for microorganism growth.

The identification of species was carried out 'in vivo'. Several keys (Kudo 1966; Shen and Zhang 1990; Patterson 1996; Foissner et al. 1999) were employed. All ciliates, most sarcodina and large flagellates were identified to species level. Meanwhile, the numbers of rotifers, nematodes, tardigrades and oligochaetes were each recorded.

Physico-chemical and operational parameters

Physical and chemical variables of the effluent were monitored at two stages in the treatment using standard methods (APHA 1989) as follows: (A) from the end of the aeration tank: mixed liquor suspended solids (MLSS), sludge volumetric index (SVI), temperature (°C), dissolved oxygen (DO); and (B) from the final effluent: biological oxygen demand (BOD$_5$), chemical oxygen demand (COD), total nitrogen (TN), ammoniacal nitrogen (NH_3-N), total phosphorus (TP) and suspended solids concentration (SS). Minimum, maximum and mean values of each parameter are shown in Table 2.

Statistical analysis

Two methods were selected for data analysis and evaluation: correlation coefficients (Pearson) and factor analysis (factor extraction method: principal components, transformation method: Varimax), using programs in the STATISTICA 6.0 package. The abiotic variables selected for the correlation analysis were effluent BOD$_5$, effluent total nitrogen (TN), effluent phosphorus (P), effluent suspended solids (SS) and sludge volume index (SVI). All the results were normalized according to the logarithmic transformation, $x = \mathrm{Ln}(x + 1)$.

Results

A total of 50 samples were analysed from Baoding Sewage Treatment Plant, and in these samples 94 species of ciliates, 40 species of amoebae and 13 species of large flagellates were identified. Numbers of rotifers, nematodes, gastrotrichs and oligochaeta were recorded without further identification.

The frequency of occurrence (the percentage of samples in which a particular species was found) and abundance of key microfauna in activated sludge of the Baoding Sewage Treatment Plant during the sampling period are shown in Table 3. As can be seen in this table, the five species with the highest maximum density were *Litonotus obtusus*, *Aspidisca cicada*, *Carchesium polypinum*, *Epistylis urceolata* and *Vorticella convallaria*, the five species with the highest average density were

Table 2. Physico-chemical and operational parameters in Baoding Sewage Treatment Plant

	Maximum	Minimum	Average	S.D.
Temperature of mixed liquor in aeration tank (°C)	25.7	11.9	18.69	3.98
Effluent BOD_5 (mg/l)	19.7	8.1	15.50	2.74
Effluent COD (mg/l)	119.12	28	56.80	16.37
Dissolved oxygen in aeration tank (mg/l)	6.5	0.15	3.12	1.92
MLSS (mg/l)	4850	1321	2462.5	905.43
SVI (ml/g)	127.6	38.6	76.72	20.37
Effluent TN (mg/l)	31.4	15.5	25.07	4.72
Effluent NH_3-N (mg/l)	24	0.8	11.58	9.50
Effluent TP (mg/l)	3.67	0.33	2.09	0.79
Effluent SS (mg/l)	28	10	15.84	2.77

Table 3. Frequency of occurrence and abundance of key microfauna in activated sludge of the Baoding Sewage Treatment Plant

	Abundance (ind./ml)		Frequency (%)
	Mean	Maximum	
Ciliates			
Vorticella convallaria	302	1980	92
Vorticella octava	329.6	1920	70
Epistylis lacustris	211.6	1240	64
Opercularia coarctata	163.2	840	68
Carchesium polypinum	332.8	2640	62
Aspidisca cicada	830.8	2800	98
Aspidisca lynceus	392	1420	90
Trochilia minuta	234.8	1440	64
Litonotus obtusus	576.8	3360	86
Epistylis urceolata	222.8	2180	30
Amoebae			
Arcella hemisphaerica	211.6	1800	72
Metazoa			
Rotifers	410.8	1500	92

Aspidisca cicada, *Litonotus obtusus*, *Aspidisca lynceus*, *Carchesium polypinum* and *Vorticella octava*, and the five species that occurred most frequently were *Aspidisca cicada*, *Vorticella convallaria*, *Aspidisca lynceus*, *Litonotus obtusus* and *Arcella hemisphaerica*. The maximum density of microfauna was 120 540 ind/ml, and the minimum density was 11 700 ind/ml. According to the frequency and abundance of the microfauna, we selected 11 taxa (*Aspidisca cicada*, *Aspidisca lynceus*, *Trochilia minuta*, *Litonotus obtusus*, *Vorticella convallaria*, *Vorticella octava*, *Epistylis lacustris*, *Opercularia coarctata*, *Carchesium polypinum*, *Arcella hemisphaerica* and Rotifers) for statistical analyses.

Results obtained from the correlation analysis shown in Table 4 help to identify relationships determined in this study between physico-chemical parameters and key taxa of microfauna. *Vorticella convallaria* and *Arcella hemisphaerica* showed the strongest negative correlation with effluent BOD_5, with correlation coefficients of −0.402 and −0.415, respectively. Therefore, these two species are likely to be useful positive bioindicators for the effectiveness of this plant, since their numbers decreased when the process produced poor quality effluent. *Litonotus obtusus* had a significant positive correlation with SVI (correlation coefficient of 0.548), suggesting that this species may be an indicator of bad settlement conditions of sludge. In addition to effluent BOD_5, *Arcella hemisphaerica* also had significant negative correlations with effluent TN, SS and SVI, which means that this species of amoeba may be an indicator of overall good performance of the activated sludge system. *Opercularia coarctata* had significant positive correlations with effluent BOD_5 and SS, indicating that this species of ciliate may be an indicator of lower quality effluents.

Table 5 presents the results of a factor analysis of the population density of these selected microfauna and the physico-chemical parameters. Seven factors were extracted, explaining 78.519% of the total variance. The first principal component (factor 1) accounts for the largest amount of the total variation (16.653%) in the data, and represents the weighed linear combination of the physico-chemical parameters and the populations of microfauna. Effluent TN, SS and BOD_5 correlated positively with each other, while *Arcella hemisphaerica* correlated negatively with them. The second factor, accounting for 13.646% of the total variance, indicated that the density of *Vorticella convallaria* and *Vorticella octava* correlated positively with each other, while *Opercularia coarctata* correlated negatively with them. The third factor explaining 10.990% of the total variance, found that some microfauna: *Aspidisca cicada*, *Trochilia minuta* and *Epistylis lacustris* correlated positively with each other. The fourth factor accounting for 10.636% of the total variance, indicated that the density of *Litonotus obtusus* correlated positively with

Table 4. Correlation analyses between the average population density of selected species of microfauna and some physico-chemical parameters in the Baoding Sewage Treatment Plant

	Effluent BOD_5	Effluent P	Effluent TN	Effluent SS	SVI
Aspidisca cicada	−0.233	0.025	−0.242	−0.123	0.021
Aspidisca lynceus	−0.226	0.078	0.099	−0.047	0.150
Trochilia minuta	−0.199	0.057	0.114	−0.219	0.018
Litonotus obtusus	0.316*	0.203	0.055	0.262	0.548**
Vorticella convallaria	−0.402**	0.176	−0.086	−0.293*	−0.103
Vorticella octava	−0.268	−0.163	0.184	−0.156	0.256
Epistylis lacustris	−0.149	0.189	0.092	−0.199	−0.115
Opercularia coarctata	0.358*	0.205	−0.053	0.296*	−0.193
Carchesium polypinum	−0.041	0.181	−0.199	−0.228	−0.023
Arcella hemisphaerica	−0.415**	0.118	−0.659**	−0.341*	−0.384**
Rotifers	−0.040	−0.007	−0.073	−0.163	−0.122

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

Table 5. Factor analysis of the population density of selected species of microfauna and some physico-chemical parameters in the Baoding Sewage Treatment Plant

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Aspidisca cicada	−0.326	−0.322	0.574	0.246	0.032	0.485	−0.144
Aspidisca lynceus	0.013	0.260	0.058	0.059	0.111	0.871	0.121
Trochilia minuta	0.031	0.188	0.820	−0.004	−0.317	−0.029	0.040
Litonotus obtusus	0.101	−0.285	−0.023	0.827	−0.144	0.008	0.202
Vorticella convallaria	−0.176	0.607	0.200	−0.200	−0.139	0.061	0.400
Vorticella octava	0.051	0.845	−0.057	0.050	0.111	0.230	−0.084
Epistylis lacustris	0.126	0.134	0.609	−0.256	0.449	0.098	0.260
Opercularia coarctata	0.157	−0.649	−0.301	−0.085	0.025	0.096	0.427
Carchesium polypinum	−0.231	0.039	0.066	0.139	0.578	−0.549	0.232
Arcella hemisphaerica	−0.749	−0.043	−0.282	−0.185	0.200	−0.064	0.083
Rotifers	−0.099	0.000	−0.162	−0.121	0.837	0.108	−0.079
Effluent BOD_5	0.714	−0.397	−0.201	0.153	0.045	−0.190	0.066
Effluent P	−0.168	−0.081	0.077	0.135	0.027	0.005	0.862
Effluent TN	0.890	0.187	0.072	0.046	0.025	0.035	−0.187
SVI	0.275	0.281	−0.019	0.845	0.007	0.055	−0.067
Effluent SS	0.666	−0.303	−0.293	0.111	−0.144	0.104	−0.019
Variance explained (%)	16.653	13.646	10.990	10.636	9.177	8.986	8.432
Accumulated variance (%)	16.653	30.299	41.289	51.925	61.102	70.087	78.519

the value of SVI. These four factors interpret approximately 52% of the data on a cumulative basis.

Discussion

This study has provided further evidence that the identification and enumeration of microfauna in the activated sludge mixed liquor can provide rapid information on the environmental conditions and the performance of the plant. Changes in the abundance of bioindicator species of the microfauna community can give advance warning of impending problems in plant performance. Changes in operating conditions of the plant may cause rapid changes in the structure of the communities of microscopic organisms, and it is hoped to work towards an understanding of how to regulate the operating conditions to maintain an ideal balanced community of protozoa and micrometazoa.

Our findings suggest that in this plant one should attempt to minimize populations of *Litonotus obtusus* (associated with high sludge volume index) and *Opercularia coarctata* (associated with high BOD_5 and SS) and to maximise populations of *Arcella hemispherica* (associated with low BOD_5 TN and SS).

In previous work Madoni et al. (1993) found that the shelled amoebae *Arcella* and *Euglypha* were associated with nitrifying conditions, while Curds and Cockburn (1970) and Salvadó et al. (1995), found an association between *Opercularia* species and poor effluent quality, and Klimowicz (1970) and Esteban et al. (1991) found that *Opercularia* spp. were abundant when sludge loadings were high or of poor quality.

In comparison with the study by Chen et al. (2004) of five sewage plants in Beijing, more species were identified in the present work. The microfauna community structure at the Baoding plant was very similar to that in the Gaobeidian activated sludge plants in Beijing. *Vorticella convallaria* and *Arcella hemisphaerica* were abundant in both studies and the latter was associated with good sludge settlement at both plants, while *Epistylis plicatilis* and *Epistylis sulcata,* which were frequent at Beijing plants, were also found in the Baoding plant, although less abundantly. Chen et al. (2004) also found a negative association between the abundance of rotifers and the reduction of BOD_5, COD and the removal of suspended solids at two plants in Beijing, but we did not find evidence of this at Baoding.

We have found that principal component analysis is a useful technique for reducing the number of variables to be taken into account 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 species and physico-chemical parameters are in accordance with the data obtained from correlation analysis.

In order to make generalizations about the relationship between physico-chemical parameters and microfauna community composition in China, analysis of more plants having a diversity of input components, and working under a range of different operating conditions should be performed.

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