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# Systematic analysis of microfauna indicator values for treatment performance in a full-scale municipal wastewater treatment plant

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**Abstract**

The indicator values of microfauna functional groups and species for treatment performance were systematically evaluated based on the continuous monitoring of the entire microfauna communities including both protozoa and metazoa over a period of 14 months, in two parallel full-scale municipal wastewater treatment systems in a plant in Beijing, China. A total of 57 species of ciliates, 14 species (units) of amoebae, 14 species (units) of flagellates and 4 classes of small metazoa were identified, with *Arcella hemisphaerica*, *Vorticella striata*, *Vorticella convallaria*, *Epistylis plicatilis* and small flagellates (e.g. *Bodo* spp.) as the dominant protozoa, and rotifers as the dominant metazoa. The abundance of the sessile ciliates was correlated with the removals of $BOD_5$ (Pearson's $r = 0.410$, $p < 0.05$) and $COD_{Cr}$ ($r = 0.397$, $p < 0.05$) while the testate amoebae was significantly positively related to nitrification ($r = 0.523$, $p < 0.01$). At the same time, some other associations were also identified: the abundances of the large flagellates ($r = 0.447$, $p < 0.01$), the metazoa ($r = 0.718$, $p < 0.01$) and species *Aspidisca sulcata* ($r = 0.337$, $p < 0.05$) were positively related to nitrification; the abundance of *Aspidisca costata* was correlated to the TN (total nitrogen) removal ($r = –0.374$, $p < 0.05$ ); the abundances of the sessile species *Carchesium polypinum* ($r = 0.458$, $p < 0.01$) and *E. plicatilis* ($r = 0.377$, $p < 0.05$) were correlated with the removal of suspended solids.

**Key words**: protozoan; metazoan; activated sludge; treatment performance; bioindicator

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## Introduction

Microfauna play important roles in municipal wastewater treatment systems. They maintain the density of bacteria, contribute to sludge flocculation and to some extent stimulate the bacterial activity in activated sludge systems, being responsible for the improvement of treatment systems (Curds, 1982; Pussard and Rouelle, 1986; Woombs and Laybourn-Parry, 1986; Martín-Cereceda et al., 1996; Ratsak et al., 1996; Pauli et al., 2001).

Microfauna have long been used as a bioindicator to evaluate the performance of biological wastewater treatment systems. In 1930s, Ardern and Lockett reported the association between some protozoan genera and the quality of treated effluents, which might be the first report on this subject (Curds and Cockburn, 1970). Based on the investigation of 56 sewage treatment plants in the UK, Curds and Cockburn (1970) reported that certain protozoan species were more frequently observed over certain effluent BOD (biochemical oxygen demand) ranges, which laid a solid base for relevant studies (Al-shahwani and Horan, 1991). Madoni (1994) synthesized previous findings, and proposed a "sludge biotic index" (SBI) for the evaluation of sewage treatment performance. Except for some special species like *Vorticella microstoma* and *Opercularia* spp., which are often related to bad conditions (e.g., lack of oxygen and occurrence of toxicants), sessile ciliates have been found to be the most important protozoa in activated sludge systems to indicate good treatment performance characterized mainly by high BOD removal, low effluent BOD and good effluent clarity (Madoni, 1994; Martín-Cereceda et al., 1996). At the same time, crawling ciliates have been found to be positively associated with BOD removal (Madoni, 1994), and testate amoebae have been considered as indicators for good nitrification performance (Madoni et al., 1993; Madoni, 1994), while swimming ciliates have exhibited tolerance to toxic and low dissolved oxygen conditions and their dominance has been associated with bad effluent quality (Martín-Cereceda et al., 1996).

To date, most of the relevant studies have mainly concentrated on the indicator values of ciliates (Madoni et

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al., 1993; Martín-Cereceda et al., 1996; Lee et al., 2004), and to a lesser extent, testate amoebae (Madoni, 1994; Chen et al., 2004; Zhou et al., 2006). Although ciliates usually (and sometimes, testate amoebae as well) represent a dominant group of microfauna in activated sludge, there exist other groups including metazoa and flagellates, which should also play roles in the activated sludge processes. Moreover, microfauna compositions may be impacted by operational conditions (Salvadó et al., 1995), temperature (Martín-Cereceda et al., 2001), raw water quality, etc., and thus vary from one plant to another, which may lead to different relationships between the microfauna compositions and treatment performance (Curds and Cockburn, 1970; Salvadó et al., 1995; Chen et al., 2004). In order to better understand the indicator values of microfauna, knowledge of the dynamics of a wide spectrum of microfauna communities covering both protozoa and metazoa over a long period is therefore desirable.

In this study, the entire microfauna communities in two parallel full-scale municipal wastewater treatment systems in a plant located in Beijing, China, were continuously monitored over a period of 14 months. The primary objective of this study was to identify the indicator values of some special microfauna groups/species as well as to confirm the previously reported relationships between some important groups/species and system performance. The finding of this study could be helpful for achieving better operations of sewage treatment plants.

## 1 Materials and methods

### 1.1 Investigated systems

Two parallel full-scale municipal wastewater treatment systems in a plant located in Beijing, China, i.e., System 1 (anaerobic/anoxic/aerobic, $A^2O$) and System 2 (anoxic/anaerobic/aerobic, inverted $A^2O$), with treatment capacity of 200,000 $m^3$/day for each system, were investigated. The sludge recycling ratio was about 100% for both systems. The mixed liquor recirculation in System 1 was about 250%. The influent distributions to the anoxic and anaerobic tanks in System 2 were 30% and 70%, respectively. The flow diagrams of the two systems were outlined by Hu et al. (2012). A profile of the system parameters is shown in **Table 1**.

### 1.2 Sampling

Eighteen mixed liquor samples for microfauna analysis were collected from the end of the aeration tanks in each system from July 2009 to September 2010, with a sampling interval of 2 to 3 weeks for the most part. Samples were collected in a 2-L Plexiglas bucket and kept in suspension with a portable air pump until the completion of analysis. Samples from System 1 were taken 1 day after System 2 to assure that every sample could be analyzed in a timely fashion.

**Table 1** Parameters of Systems 1 and 2 during study period (July 2009 to September 2010)

| Variable | System 1 | System 2 |
|---|---|---|
| Influent $BOD_5$ (mg/L) | 193.2 ± 41.3 | |
| Influent $COD_{Cr}$ (mg/L) | 422.4 ± 88.0 | |
| Influent SS (mg/L) | 216.2 ± 66.3 | |
| Influent TN (mg/L) | 59.5 ± 7.8 | |
| Influent TP (mg/L) | 5.6 ± 1.0 | |
| Effluent $BOD_5$ (mg/L) | 4.9 ± 1.6 | 5.4± 2.2 |
| Effluent $COD_{Cr}$ (mg/L) | 49.0 ± 9.7 | 48.4 ± 9.5 |
| Effluent SS (mg/L) | 12.4 ± 2.3 | 11.9 ± 2.8 |
| Effluent TN (mg/L) | 16.8 ± 4.4 | 24.3 ± 6.6 |
| Effluent TP (mg/L) | 0.4 ± 0.5 | 0.3 ± 0.4 |
| Effluent $NO_3^-$ (mg/L) | 11.4 ± 4.6 | 10.3 ± 6.7 |
| $T$ (°C) | 21.1 ± 4.4 | 21.1 ± 4.4 |
| MLSS (mg/L) | 4093 ± 581 | 3670 ± 579 |
| SRT (day) | 14.2 ± 5.7 | 8.8 ± 3.5 |
| HRT (hr) | 13.6 ± 1.0 | 12.2 ± 1.0 |
| MLVSS/MLSS | 0.68 ± 0.08 | 0.67 ± 0.10 |
| SVI (mL/g) | 114 ± 52 | 141 ± 69 |
| DO (mg/L) | 1.60 ± 0.70 | 1.68 ± 1.30 |

$BOD_5$: five-day biochemical oxygen demand; $COD_{Cr}$: chemical oxygen demand with dichromate; SS: suspended solids; TN: total nitrogen; TP: total phosphorus; $T$: water temperature; MLSS: mixed liquor suspended solids; SRT: solids residence time; HRT: hydraulic retention time; MLVSS: mixed liquor volatile suspended solids; SVI: sludge volume index; DO: dissolved oxygen.

### 1.3 Microscopic analysis

Identification of protozoa was performed *in vivo* according to several keys (Kudo, 1966; Shen et al., 1990; Patterson, 1996; Foissner et al., 1999). Most protozoa were identified to the species level according to morphology and movements. Species not able to be identified to the species level were recorded as units, e.g., *Mayorella* spp. Small metazoa were classified into 4 units: rotifers, nematodes, gastrotrichs and tardigrades. Enumeration of protozoa and small metazoa was performed within 5 hr after sample collections (Madoni and Ghetti, 1981). Protozoa (except solitary small flagellates, e.g., *Bodo* spp.) and small metazoa in three replicates of 25 μL sub-samples were counted (Martín-Cereceda et al., 1996). Solitary small flagellates in five replicates of 0.1 μL sub-samples were counted using a Neubauer counting plate (counting chamber: 1 mm length × 1 mm width × 0.1 mm depth; XB-K-25, Shanghai Qiujing Biochemical Reagent & Instrument Co., Ltd., China) and recorded as one unit, i.e., solitary small flagellates. Species not detected in counting but observed in screening were recorded as 1 individual/mL (1 ind/mL) (Madoni, 1994).

### 1.4 Physico-chemical and operational parameters

Physical and chemical variables of the two systems were determined according to standard methods (APHA, 1995) on a daily basis and provided kindly by Beijing Drainage Group Co., Ltd., China (**Table 1**). Intraday values of these parameters were used in data analysis.