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Responses of protists with different feeding habits to the changes of activated sludge conditions: A study based on biomass data

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Abstract

Changes of protists, which were categorized into different functional groups primarily according to their feeding habits, in two full-scale municipal wastewater treatment systems experiencing sludge bulking were investigated over a period of 14 months. Protist biomass represented 3.7% to 5.2% of total biomass on average under normal sludge conditions, and the percentage increased significantly ($p < 0.05$) under sludge bulking conditions. The biomass of *Chilodonella* spp., capable of eating filamentous bacteria, tended to decrease in both systems when sludge bulking occurred, showing that the abnormal growth of filamentous bacteria did not lead to a biomass bloom of this group of protists. On the other hand, the bactivorous protists represented more than 96% of total protist biomass, and the biomass of this group, particularly the attached ciliates, increased significantly ($p < 0.05$) when sludge bulking occurred. The significant increase of the attached ciliates may have possibly facilitated the growth of filamentous bacteria through selectively preying on non-filamentous bacteria and further exacerbated sludge bulking. The redundancy analysis and correlation analysis results showed that the biomass changes of the attached ciliates were primarily related to the sludge volume index and to some extent related to five-day biochemical oxygen demand loading and hydraulic retention time.

Key words: protist; biomass; activated sludge; feeding habit; sludge bulking

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Introduction

In wastewater treatment systems, compact activated sludge flocs, which are critical for the effective solid-liquid separation, are generally formed through the cooperation of filamentous and non-filamentous bacteria, with the filamentous ones forming the backbone of flocs. Protists, which can prey on unflocced (dispersed) bacteria and excrete special compounds to promote bacteria flocculation, have been considered to promote the formation of activated sludge flocs (Curds, 1982; Pauli et al., 2001). However, sometimes the balance between filamentous and non-filamentous bacteria is destroyed due to the abnormal growth of some filamentous bacteria, leading to the destruction of floc structures (Jenkins et al., 2003; Martins et al., 2004; Rossetti et al., 2005). This is the major reason for sludge bulking, a phenomenon frequently encountered in biological wastewater treatment systems and one of the biggest challenges for operators. It is easy to speculate that the protist communities will undergo change in order to adapt to the changing sludge conditions, particularly the changing bacterial forms, when sludge bulking occurs.

Due to the differences in feeding habits and phenotypic traits, different types of protists may have different functions in the activated sludge ecosystems and represent different relations with environmental conditions (Pratt and Cairns, 1985; Shen et al., 1990; Madoni, 1994a; Martín-Cereceda et al., 1996). In municipal wastewater treatment systems (MWTSs), while most of the bactivorous protists are generally considered to feed on non-filamentous bacteria, some bactivorous protists, such as *Chilodonella* spp. and *Nassula* spp., are capable of eating filamentous bacteria (Inamori et al., 1991). Therefore it is interesting to know which type of protists will take the opportunity to grow when the abnormal growth of filamentous bacteria occurs, and what this means to sludge bulking.

In this study, changes of protists, which were categorized into different functional groups primarily according to the feeding habits (Pratt and Cairns, 1985; Shen et al., 1990; Madoni, 1994a; Martín-Cereceda et al., 1996), in two full-scale MWTSs experiencing sludge bulking were investigated over a period of 14 months. The main objective of this study was to reveal the responses of protists with different feeding habits to sludge condition changes. Because of the fact that the cell sizes of protists are significantly different between different species, all of the protist numbers were transformed into protist biomass according to previous studies (Finlay, 1982; Gates et al., 1982; Børsheim and Bratbak, 1987; Beare et al., 1992;

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Amblard et al., 1993). This is a new attempt to reveal the responses of protists with different feeding habits to the changes of sludge conditions based on protist biomass data, and the results will contribute to the better understanding of the trophic web in MWTSs and their proper operations.

1 Materials and methods

1.1 Investigated systems

Two parallel full-scale MWTSs with different treatment processes, i.e., System 1 (A^2/O, anaerobic/anoxic/aerobic) and System 2 (inverted A^2/O, anoxic/anaerobic/aerobic), in Beijing, China, were investigated. The flow diagrams of the two systems are shown in Fig. 1. The treatment capacity for each system was 200,000 m^3/day. In the past 3 years, the two systems experienced sludge bulking during winter and spring (mainly between January and April). Some important parameters are shown in Table 1.

1.2 Sampling

Mixed liquor samples for analysis of protists in each system were collected from July 7, 2009 to September 10, 2010, with an interval of 2 or 3 weeks mostly (17 samples for each system in total), from the end of the aeration tanks. A plexiglass bucket with a volume of 2 L was used to hold the mixed liquor samples, which were kept in suspension with an air pump until the analysis was completed. Sample collections in Systems 1 and 2 were approximately synchronous (with 1 day difference to ensure that each sample could be analyzed in time). Physical and chemical parameters were determined on a daily basis according to standard methods (APHA, 1995), and provided kindly by Beijing Drainage Group Co., Ltd., China.

Table 1 Operational parameters of Systems 1 and 2

Variable	System 1	System 2
SVI (mL/g)	115 ± 53	143 ± 71
BOD_5 loading (g/(g MLSS·day))	0.08 ± 0.02	0.11 ± 0.03
HRT (hr)	13.7 ± 1.0	12.1 ± 1.0
SRT (day)	14.0 ± 5.6	8.8 ± 3.6
DO (mg/L)	1.62 ± 0.71	1.66 ± 1.31
T (°C)	20.9 ± 4.4	20.8 ± 4.4
MLVSS/MLSS	0.69 ± 0.08	0.67 ± 0.09

SVI: sludge volume index; BOD_5: five-day biochemical oxygen demand; HRT: hydraulic retention time; SRT: solids residence time; DO: dissolved oxygen; T: water temperature; MLVSS: mixed liquor volatile suspended solids; MLSS: mixed liquor suspended solids.

1.3 Microscopic examination

Protist identification was carried out according to several keys (Kudo, 1966; Shen et al., 1990; Patterson, 1996) with live observations. Images of different individuals of most protist taxa (except some rare species with very scarce quantities) observed were recorded using AxioVision Rel. 4.8 software (Carl Zeiss, Jena, Germany) at ×200 or ×400 magnification. Only individuals with normal cell morphology were recorded. Protists were enumerated according to the method outlined in previous studies (Madoni and Ghetti, 1981; Madoni, 1994a; Martín-Cereceda et al., 1996). For most protist species except single-living small flagellates, three replicates of 25 μL sub-samples were enumerated. For the single-living small flagellates, five replicates of 0.1 μL sub-samples, using a Neubauer counting plate with a counting chamber of 1 mm length×1 mm width×0.1 mm depth, were counted.

1.4 Estimates of protist biomass

For protist species having electronic images, dimensions of different individuals were measured using AxioVision Rel. 4.8. For some rare species without electronic images, dimensions were acquired from literature. Biovolumes were estimated from the formula for the most appropriate standard geometric shape (Gates et al., 1982; Madoni, 1994b). Estimates of ciliate and amoeba biomass were made using a biovolume to dry weight conversion factor of 0.279 pg/μm^3 (Gates et al., 1982). Estimates of flagellate biomass were made by first using a biovolume to carbon biomass conversion factor of 0.1 pg C/μm^3 (Børsheim and Bratbak, 1987) and then converted to dry weight by assuming carbon represents 47.1% of the dry weight (Finlay, 1982; Beare et al., 1992; Amblard et al., 1993).

1.5 Statistical analysis

Sludge conditions were classified into three categories according to the SVI (sludge volume index) values: normal

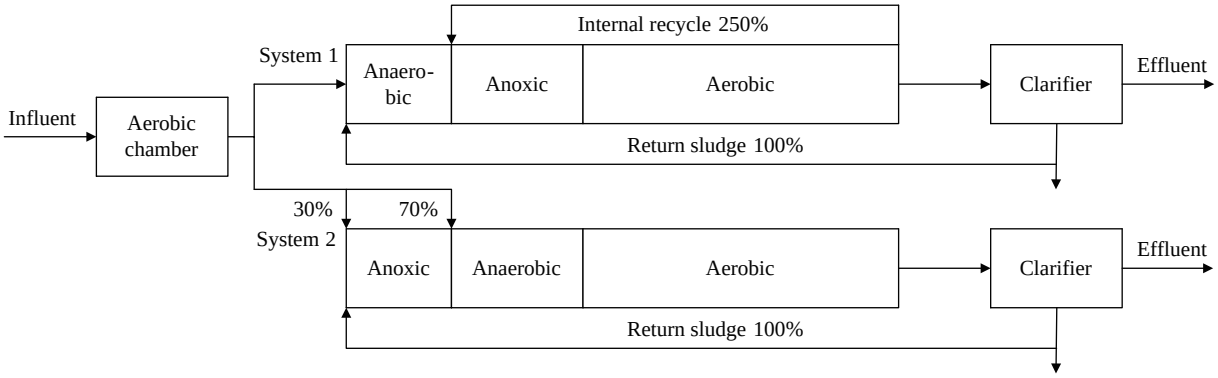

Fig. 1 Flow diagrams of Systems 1 and 2.

state (SVI ⩽ 120 mL/g), transient state (120 mL/g < SVI < 180 mL/g) and sludge bulking state (SVI ⩾ 180 mL/g) (Parker, 1983; Kruit et al., 2002). Sample numbers corresponding to different sludge conditions in System 1 were: 8 (normal), 5 (transient) and 4 (sludge bulking); the numbers in System 2 were 5, 4 and 8, respectively. Protist biomass was expressed as the dry weight per g mixed liquor suspended solids (MLSS). ANOVA (analysis of variance) and calculation of correlation coefficients were conducted using STATISTICA Version 8.0. RDA (redundancy analysis) was carried out using CANOCO version 4.5 (Lepš and Šmilauer, 2003). Data for RDA analysis were logarithm transformed, i.e., $x = \ln(x+1)$.

2 Results and discussion

2.1 Contribution of protist biomass to total biomass

Total biomass was expressed as the mixed liquor volatile suspended solids (MLVSS) per g MLSS (Madoni, 1994b). As shown in Table 2, on average, protist biomass represented 5.2% and 3.7% of total biomass in Systems 1 and 2, respectively, under normal sludge conditions. The proportion of protist biomass increased with the increase of SVI values, and it was 8.0% and 4.4% under transient sludge conditions, and 9.1% and 7.0% under sludge bulking conditions, respectively. Comparing to the values under normal conditions, the proportions under sludge bulking conditions almost doubled, showing that protists were very active during sludge bulking.

The distributions of protist functional groups categorized according to feeding habits are outlined in Table 3. Bactivorous protists represented most of the protist biomass (> 96% on average), which increased further when sludge bulking occurred. Carnivorous protists represented no more than 3.3% on average of the protist biomass among all sludge conditions. The proportions of other functional groups (algivorous, saprotrophic and photoautotrophic protists) in protist biomass were even less (sum ⩽ 0.5% on average) (Table 3). Thus bactivorous protists are the most important protist group in the activat-

Table 2 ANOVA analysis of total biomass and protist biomass under different sludge conditions in Systems 1 and 2

Variable	System 1				System 2			
	Normal	Transient	Sludge bulking	*p* value	Normal	Transient	Sludge bulking	*p* value
Sludge biomass (g/g MLSS)	0.665 ± 0.040	0.757 ± 0.031	0.763 ± 0.015	0.000*	0.650 ± 0.069	0.666 ± 0.038	0.761 ± 0.009	0.000*
Protist biomass (g/g MLSS)	0.035 ± 0.019	0.061 ± 0.023	0.069 ± 0.007	0.015*	0.024 ± 0.008	0.030 ± 0.013	0.053 ± 0.016	0.003*
Protist proportion (%)	5.2 ± 2.6	8.0 ± 2.9	9.1 ± 0.9	0.042*	3.7 ± 1.4	4.4 ± 1.8	7.0 ± 2.1	0.016*

Average SVI in System 1: 88 mL/g (normal), 155 mL/g (transient) and 190 mL/g (sludge bulking); average SVI in System 2: 92 mL/g (normal), 153 mL/g (transient) and 235 mL/g (sludge bulking).
* $p < 0.05$.

Table 3 ANOVA analysis of biomass of protists with different feeding habits under different sludge conditions in Systems 1 and 2

	Biomass (g/g MLSS)			
	Normal	Transient	Sludge bulking	*p* value
System 1				
Bactivorous protists[a]				
Total	0.034 ± 0.019	0.061 ± 0.023	0.069 ± 0.007	0.013*
Chilodonella spp.[b]	0.00014 ± 0.00012	0.00020 ± 0.00025	0.00008 ± 0.00015	0.550
Carnivorous protists	0.00097 ± 0.00090	0.00022 ± 0.00015	0.00047 ± 0.00025	0.145
Others[c]	0.00012 ± 0.00005	0.00009 ± 0.00009	0.00003 ± 0.00004	0.116
System 2				
Total	0.023 ± 0.008	0.029 ± 0.013	0.053 ± 0.016	0.003*
Chilodonella spp.[b]	0.00028 ± 0.00037	0.00031 ± 0.00010	0.00010 ± 0.00013	0.234
Carnivorous protists	0.00086 ± 0.00075	0.00036 ± 0.00016	0.00022 ± 0.00016	0.056
Others[c]	0.00011 ± 0.00008	0.00004 ± 0.00003	0.00004 ± 0.00005	0.083
	Proportion in protists (%)			
	Normal	Transient	Sludge bulking	*p* value
System 1				
Bactivorous protists[a]	96.2 ± 3.7	99.4 ± 0.4	99.3 ± 0.3	–
Carnivorous protists	3.3 ± 3.7	0.4 ± 0.2	0.7 ± 0.3	–
Others[c]	0.5 ± 0.3	0.2 ± 0.2	0.0 ± 0.1	–
System 2				
Bactivorous protists[a]	96.3 ± 1.7	98.6 ± 0.6	99.4 ± 0.4	–
Carnivorous protists	3.3 ± 1.9	1.3 ± 0.6	0.5 ± 0.4	–
Others[c]	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	–

[a] This group consists of *Chilodonella* spp. and other bactivorous protists; [b] protists capable of eating filamentous bacteria; [c] this group consists of algivorous protists, saprotrophic protists and photoautotrophic protists.
* $p < 0.05$.

ed sludge of MWTSs.

2.2 Changes of different types of bactivorous protists

Protists capable of eating filamentous bacteria in wastewater treatment systems have a special organ (cytopharyngeal basket) (Inamori et al., 1991). In this study, only several species of protists belonging to the genus *Chilodonella* were observed with a cytopharyngeal basket. So only this group of *Chilodonella* spp. may have the ability to prey on filamentous bacteria. The biomass of *Chilodonella* spp. under different sludge conditions is shown in Table 3. The biomass proportion of *Chilodonella* spp. in bactivorous protists was no more than 1.5% under normal sludge conditions (data not shown), showing that this group of protists may not be an important one in activated sludge. Our previous study (Hesham et al., 2011) found that *Microthrix parvicella* and *Nostocoida limicola* may be responsible for sludge bulking in the two systems studied. With the occurrence of abnormal growth of filamentous bacteria (under transient and sludge bulking conditions), no significant changes in the biomass of *Chilodonella* spp. was observed and the biomass of this protist group even tended to decrease under sludge bulking condition in both systems, suggesting that the abnormal growth of filamentous bacteria did not lead to the biomass bloom of *Chilodonella* spp. capable of eating filamentous bacteria. It was found that extra motions, e.g., ejecting cell wall after digestion of cells, were needed for protists preying on filamentous bacteria (Inamori et al., 1991), which may lead to the extra consumption of energy. As a group of crawling ciliates, *Chilodonella* spp. may prey on the particles loosely adhering to the activated sludge flocs (Madoni, 1994a; Martín-Cereceda et al., 1996). Thus filamentous bacteria may be not the preferred food source for *Chilodonella* spp. if alternative foods (bacteria loosely attached to the flocs, for example) exists, and these protists may not be able to suppress the abnormal growth of filamentous bacteria when sludge bulking occurs. In contrast, the biomass of other bactivorous protists (non-filamentous bacteria-eating protists) increased significantly ($p < 0.05$) as the sludge condition deteriorated (data not shown).

In order to know which types of non-filamentous bacteria-eating protists responded to sludge bulking the most, these protists were further categorized into 6 functional groups according to their phenotypic traits and other lifestyles (Shen et al., 1990; Madoni, 1994a; Martín-Cereceda et al., 1996), as shown in Table 4. It was found that only the biomass of the attached ciliates increased significantly ($p < 0.05$) in the two systems. The relationship between the biomass of this functional group and SVI for both systems is shown in Fig. 2. A clear increasing trend was observed for the biomass of the attached ciliates in both systems. The attached ciliates, primarily consisting of the peritrich ciliates with developed peristomial ciliatures, are good predators of dispersed bacteria which are distributed in wastewater and are considered as the most important ciliates in activated sludge systems (Martín-Cereceda et al., 1996). During sludge bulking, activated sludge flocs are destroyed due to the abnormal growth of filamentous bacteria, releasing abundant non-filamentous bacteria originally held within the flocs. This could be proved by the existence of good correlation between the suspended solids of effluents and SVI (Pearson's r, 0.508 and 0.768 in Systems 1 and 2, respectively; $p < 0.05$). The release of abundant non-filamentous bacteria during

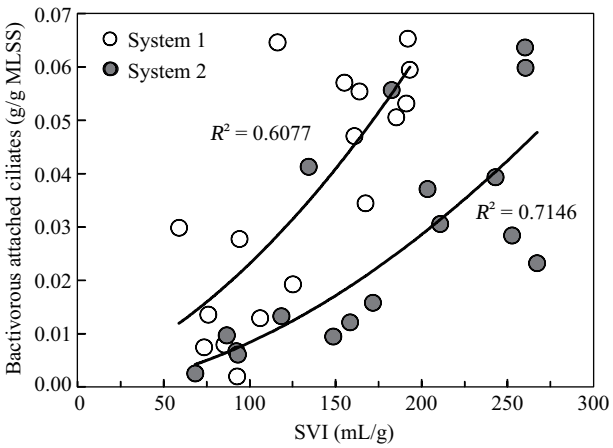

Fig. 2 Relations between biomass of bactivorous attached ciliates and SVI in Systems 1 and 2.

Table 4 ANOVA analysis of biomass of 6 groups of non-filamentous bacteria-eating protists under different sludge conditions in Systems 1 and 2

Functional group[a]	Biomass (g/g MLSS)			
	Normal	Transient	Sludge bulking	p value
System 1				
Attached ciliates	0.021 ± 0.020	0.043 ± 0.016	0.057 ± 0.007	0.009*
Crawling ciliates	0.0010 ± 0.0009	0.0020 ± 0.0026	0.0005 ± 0.0008	0.351
Swimming ciliates	0.00013 ± 0.00012	0.00015 ± 0.00013	0.00016 ± 0.00012	0.898
Testate amoebae	0.011 ± 0.005	0.015 ± 0.006	0.010 ± 0.002	0.316
Naked amoebae	0.00043 ± 0.00049	0.00051 ± 0.00040	0.00005 ± 0.00006	0.227
Flagellates	0.00012 ± 0.00007	0.00021 ± 0.00014	0.00044 ± 0.00024	0.009*
System 2				
Attached ciliates	0.008 ± 0.004	0.020 ± 0.015	0.042 ± 0.015	0.001*
Crawling ciliates	0.0006 ± 0.0007	0.0009 ± 0.0007	0.0025 ± 0.0025	0.173
Swimming ciliates	0.00016 ± 0.00021	0.00005 ± 0.00003	0.00012 ± 0.00012	0.505
Testate amoebae	0.014 ± 0.008	0.008 ± 0.005	0.008 ± 0.002	0.143
Naked amoebae	0.00013 ± 0.00011	0.00019 ± 0.00010	0.00003 ± 0.00003	0.013*
Flagellates	0.00021 ± 0.00012	0.00044 ± 0.00009	0.00032 ± 0.00035	0.429

[a] Protists capable of eating filamentous bacteria were not included.
* $p < 0.05$.

sludge bulking may provide suitable food for the growth of the attached ciliates. The significant increase of the attached ciliates may have possibly facilitated the growth of filamentous bacteria through selectively preying on non-filamentous bacteria. If this speculation is true, the attached ciliates may possibly play a role in escalating the sludge bulking process, which requires further study.

2.3 Relationship between protist biomass and environmental factors

To better understand the environmental factors associated with protist biomass changes in the sludge bulking process, RDA was performed to illustrate the relationship between protist biomass and 6 frequently used environmental variables, as shown in Fig. 3. In System 1, the first two ordination axes explained 32.4% of total variability in the biomass data (four axes: 38.9%) and 81.2% of biomass-environment relations; in System 2, the first two axes explained 37.3% of total variability in the biomass data (four axes: 43.4%) and 85.2% of biomass-environment relations. Pearson correlation coefficients between protist biomass and environmental variables corresponding to RDA are shown in Table 5.

The biomass of the attached ciliates were highly correlated with SVI and water temperature, and correlated to some extent with BOD_5 (five-day biochemical oxygen demand) loading and HRT (hydraulic retention time) (Fig. 3; Table 5). This result further proved that the significant increase of the attached ciliates was highly associated with sludge bulking due to abnormal growth of filamentous bacteria. The increase of BOD_5 loading could lead to the increase of bacterial mass as food to the protists. The implications for HRT, however, were not very clear. On the other hand, SVI was significantly inversely correlated to water temperature in this study (Pearson's $r_{System\ 1} = -0.903$, Pearson's $r_{System\ 2} = -0.897$; $p < 0.05$). The optimum growth temperatures for most of ciliates range from 25 to 30°C (Sudo and Aiba, 1971; Shen et al., 1990). Thus the increase of the attached ciliates at low water temperature may not be explained by the direct effect of temperature. It is known that low temperature is a driving force for sludge bulking (Horan et al., 2004; Martins et al., 2004; Rossetti et al., 2005), which could be used for the interpretation of the correlation between the biomass of the attached ciliates and water temperature. As shown in Fig. 3 and Table 5, the correlations between the biomass of *Chilodonella* spp. and other environmental variables are not very clear. However, at least we can conclude that sludge bulking would not favor the growth of this group of protists.

3 Conclusions

Changes of biomass of protists categorized into different functional groups in two full-scale municipal wastewater treatment systems experiencing sludge bulking were investigated, and the following conclusions were obtained.

(1) Protist biomass represented 5.2% and 3.7% (on average) of total biomass in Systems 1 and 2, respectively, with the dominance of bactivorous protists under normal sludge

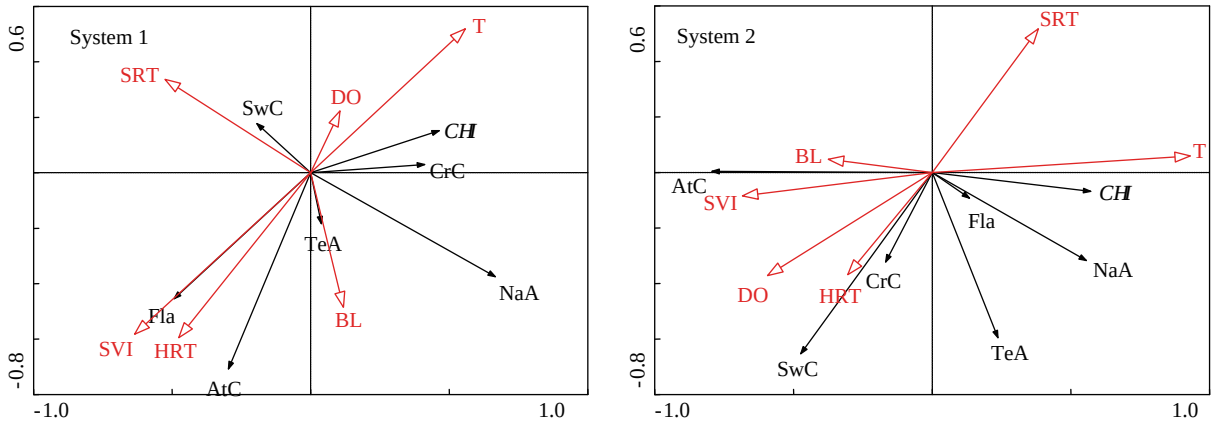

Fig. 3 Bactivorous protist biomass-environmental factors biplot diagrams in Systems 1 and 2. BL: BOD_5 loading; AtC: attached ciliates; CrC: crawling ciliates; SwC: swimming ciliates; TeA: testate amoebae; NaA: naked amoebae; Fla: flagellates; *Chi*: *Chilodonella* spp.

Table 5 Correlation coefficients (r) between biomass of protist functional groups and environmental variables in Systems 1 and 2 (corresponding to RDA)

Functional group	SVI		BOD_5 loading		HRT		SRT		DO		T	
	1	2	1	2	1	2	1	2	1	2	1	2
Attached ciliates[a]	0.66*	0.85*	0.33	0.31	0.76*	0.28	0.11	–0.41	0.04	0.31	–0.80*	–0.89*
Chilodonella spp.[b]	–0.39	–0.28	–0.01	–0.26	–0.31	–0.04	–0.15	0.21	0.01	–0.33	0.35	0.48
Crawling ciliates	–0.26	0.32	–0.00	–0.02	–0.11	0.34	–0.15	–0.13	0.19	0.28	0.12	–0.26
Swimming ciliates	0.28	0.35	0.21	0.15	–0.22	0.39	–0.01	–0.53*	0.06	0.50*	–0.29	–0.46
Testate amoebae	0.10	–0.28	0.02	–0.28	–0.08	0.35	–0.33	0.00	–0.16	0.22	–0.08	0.28
Naked amoebae	–0.19	–0.46	0.29	–0.11	–0.17	–0.29	–0.56*	–0.07	–0.05	–0.24	0.22	0.54*
Flagellates	0.48	0.05	0.16	–0.12	0.33	0.05	0.08	–0.02	–0.63*	–0.14	–0.21	0.05

[a] Mostly changed functional group in sludge bulking process feeding on non-filamentous bacteria; [b] protist group capable of eating filamentous bacteria.
* $p < 0.05$.

conditions, and the proportion increased significantly ($p < 0.05$) under sludge bulking conditions.

(2) With the occurrence of abnormal growth of filamentous bacteria, the biomass of *Chilodonella* spp. capable of eating filamentous bacteria tended to decrease in both systems under sludge bulking conditions, suggesting that the filamentous bacteria may not be the preferred food for this group of protists.

(3) The significant increase of the attached ciliates may have possibly facilitated the growth of filamentous bacteria through selectively preying on non-filamentous bacteria. Thus it is possible that the bactivorous attached ciliates may play a role in escalating the sludge bulking process, which requires further study.

Supporting materials

Supplementary data associated with this article can be found in the online version.

Acknowledgments

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Supporting materials

Table S1 Average cell length and estimated individual biomass of some protist species observed in the two investigated systems

Protist type	Species	Average cell length (μm)	Average estimated individual biomass (pg/individual)
Ciliates	*Acineta foetide*	49.1	2959
	Aspidisca costata	32.3	2051
	Aspidisca sulcata	42.5	4374
	Carchesium polypinum	75.4	21254
	Chaetospira mülleri	95.1	27931
	Chilodonella uncinata	39.6	1875
	Cinetochilum margaritaceum	17.1	237
	Coleps bicuspis	56.3	8904
	Epistylis plicatilis	133.6	48694
	Epistylis urceolata	63.2	18603
	Euplotes eurystomus	109.2	24124
	Hemiophrys fusidens	130.5	8354
	Opercularia coarctata	53.9	7568
	Opercularia phryganeae	114.4	30730
	Plagiocampa mutabilis	42.1	2021
	Prorodon discolor	90.7	46054
	Thuricola folliculata	184.0	56942
	Tokophrya quadripatita	117.1	20745
	Trachelophyllum pusillum	28.0	210
	Vorticella convallaria	82.7	19639
	Vorticella cupifera	38.3	3978
	Vorticella octava	31.6	2488
	Vorticella striata	37.0	3198
Amoebae	*Arcella hemisphaerica*	61.5*	62401
	Euglypha tuberculata	66.8	40942
	Trinema lineare	33.6	4397
Flagellates	*Entosiphon sulcatum*	23.1	2062
	Peranema deflexum	27.5	1814
	Peranema trichophorum	55.5	4836
	Petalomonas mediocanellata	12.9	128
	Petalomonas steinii	23.6	817
	Sphaeroeca volvox	6.4	82

* Average diameter of the shell.

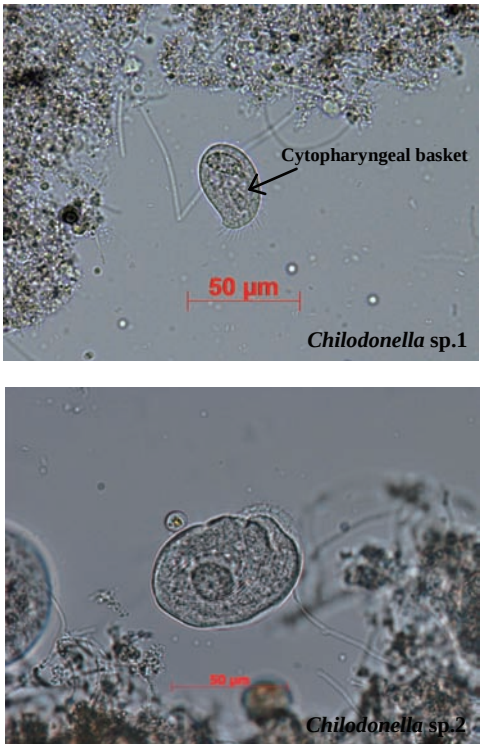

Fig. S1 Two species of the genus *Chilodonella* observed in the two investigated systems.

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