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Comparison Performances of Membrane Bioreactor and Conventional Activated Sludge Processes on Sludge Reduction Induced by *Oligochaete*

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Pilot-scale experiments were carried out to compare sludge reduction induced by *Oligochaete* in a submerged membrane bioreactor (MBR) and a conventional activated sludge (CAS) reactor for 345 d. Worm growth in the CAS reactor was much better than in the MBR. The average worm density of the aeration tank in the CAS reactor was 71 total worms/mg of volatile suspended solids (VSS), much higher than that in the MBR (10 total worms/mg of VSS). Worms did not naturally produce in the MBR, and the dominant worm type in the MBR depended on sludge inoculation from the CAS reactor. Only two types of worms were found in the MBR, *Aeolosoma hemprichicii* and *Nais elinguis*. Worm presence and disappearance in the MBR alternated. Worms in the CAS reactor occurred nearly throughout the operating period and were continuously maintained at over 30 total worms/mg of VSS in the aeration tank for 172 d. Three types of worm were found in the CAS reactor, *A. hemprichicii*, *Pristina aequiseta*, and *N. elinguis*, but *P. aequiseta* was present only occasionally. The alternating dominance of worm types in both reactors changed between *Aeolosoma* and *Nais*, and the time of *Aeolosoma* dominance was longer than that of *Nais* dominance. Worm growth in the MBR contributed to neither sludge reduction nor improvement of sludge settling characteristics because of low density. But worm presence and bloom in the CAS reactor greatly decreased sludge yield and improved sludge settling characteristics at high density. Both the average sludge yield (0.17 kg of suspended solids (SS)/kg of chemical oxygen demand removed ($COD_{removed}$)) and sludge volume index (60 mL/g) in the CAS reactor were much lower than those in the MBR (0.40 kg of SS/kg of $COD_{removed}$ and 133 mL/g). *Nais* had more potential for sludge reduction than *Aeolosoma*. Worm growth had little impact on effluent quality in the MBR but affected effluent quality very much in the CAS reactor.

Introduction

The activated sludge process is the most widely used biological wastewater treatment for both domestic and industrial plants. One of the drawbacks of the conventional activated sludge (CAS) processes is high sludge production. Currently, sewage sludge treatment and disposal represents a rising challenge for wastewater treatment plants (WWTPs) because of economic, environmental, and regulation factors, and its costs accounts for about half, even up to 60%, of the total operation costs of the WWTPs (*1*, *2*). There is therefore considerable impetus to develop strategies for reducing excess sludge production in the biological wastewater treatment processes.

One way to reduce sludge production is to exploit the organisms in the activated sludge process that predate on the bacteria. During energy transfer from low to high trophic levels, energy is lost because of inefficient biomass conversion. Under optimal conditions, the total loss of energy will be maximal and the total biomass production will be minimal (*3*). It is well-known that the presence of protozoa and metazoa in aerobic wastewater treatment processes plays an important role in keeping the treated wastewater clear by consuming dispersed bacteria. Recently, many researchers have focused on sludge reduction induced by grazing on bacteria (*3*−*18*).

Worms are the largest organisms observed during the microscopic investigation of activated sludge (*19*) and may have more potential for sludge reduction in practical application than protozoa because of their bigger sizes. The main types of worms present in activated sludge system and trickling filters are *Naididae*, *Aeolosomatidae*, and *Tubificidae*. *Naididae* and *Aeolosomatidae* are free-swimming worms. *Tubificidae* are sessile worms that do not normally occur in activated sludge suspensions but are sometimes present in sludge "bankets" on the basin bottom (*13*). Under normal conditions, *Naididae* and *Aeolosomatidae* propagate by division into an anterior and a somewhat smaller posterior part. The reproduction of *Tubificidae* takes place sexually in most cases (*4*, *12*).

A dynamic energy budget (DEB) model was extended to describe the growth of *Nais elinguis*, and a numerical method was developed to predict *N. elinguis* asexual reproduction (*4*). The behavior of *Oligochaete* worms in an activated sludge plant in The Netherlands was investigated for 1.5 yr (*6*, *7*). Different worms were found (*N. elinguis*, *Pristina* sp., and *Aeolosoma hemprichicii*) but *N. elinguis* was predominant. Sludge reduction occurred at over 20−30 worms/mL of sludge mixture. Because of the uncontrollability of *Aeolosomatidae* and *Naididae*, Rensink and Rulkens (*11*) and Rensink et al. (*12*) used *Tubificidae* in a trickling filter and an activated sludge system for sludge reduction. Sludge reduction of 18−67% was achieved in the trickling filter, and the sludge yield in the activated sludge system with worms was 0.15 g of mixed liquor suspended solids (MLSS)/g of chemical oxygen demand removed ($COD_{removed}$) as compared to 0.4 g of MLSS/g of $COD_{removed}$ without worms. The performances of worms on sludge reduction in different biological wastewater treatment processes were compared (*13*). The sludge yield in the oxidation ditch with worms was 0.17 g of MLSS/g of $COD_{removed}$ as compared to 0.22 g of MLSS/g of $COD_{removed}$ without worms. The sludge reduction of 10−50% in the lava

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TABLE 1. Reactor Configurations, Operation Conditions, and Fed Municipal Wastewater Characteristics in MBR and CAS Processes

	MBR	CAS
Configurations		
vol of presettling tank (L)	230	230
vol of aeration tank[a] (L)	1000 (700)	1000 (640)
type of aeration tank	plug flow	plug flow
vol of separation tank (L)	300	160 (clarifier)
membrane module	flat plate, polyethylene, pore size 0.4 μm, membrane area $10 \times 0.8 = 8$ m^2	
Operation Conditions		
	average ± SD	average ± SD
T (°C)	20.4 ± 2.25	21.4 ± 2.75
pH	7.0 ± 0.26	6.7 ± 0.32
DO (mg/L)	4.10 ± 3.01	3.40 ± 2.86
HRT[b] (h)	8.47 ± 0.95	6.69 ± 1.00
SRT (d)	33.32 ± 12.78	38.31 ± 11.85
F/M (kg of COD/kg of VSS·d)	0.13 ± 0.08	0.26 ± 0.17
recycle ratio	0.60 ± 0.31	0.92 ± 0.36
TSS (g/L)		
aeration tank	4.84 ± 2.14	6.07 ± 1.05
returned sludge	12.79 ± 4.19	12.17 ± 2.87
VSS (g/L)		
aeration tank	3.97 ± 1.78	4.98 ± 0.86
returned sludge	10.44 ± 3.49	9.96 ± 2.36
ratio of VSS/TSS		
aeration tank	0.82 ± 0.016	0.82 ± 0.017
returned sludge	0.81 ± 0.016	0.82 ± 0.018
Fed Municipal Wastewater in Both Reactors		
total COD (mg/L)	average: ± SD: 341.25 ± 162.35	range: 88.40−1096.00
soluble COD (mg/L)	111.84 ± 39.17	23.90−197.00
SS (mg/L)	155 ± 128	36−878
NH_4^+-N (mg/L)	36.64 ± 12.40	4.08−58.20
NO_3^--N (mg/L)	0.10 ± 0.17	0.00−1.11
NO_2^--N (mg/L)	0.31 ± 0.89	0.00−4.46
PO_4^{3-}-P (mg/L)	3.74 ± 2.19	0.41−9.90

[a] The value in parentheses is the value of working volume in aeration tank. [b] HRT in MBR and CAS is calculated on the basis of aeration tank.

medium of the trickling filter was achieved with worms as compared to 10−15% without worms. With the plastic medium of the trickling filter, the sludge reduction with worms was 10−45% as compared to 10% without worms. However, no attachment of worms occurred on the carrier of the biorotor. The worm growth in submerged MBRs was studied to increase worm density with membrane cutoff (*16, 17*). Their results showed that occurrences of *N. elinguis*, *Pristina* sp., and *A. hemprichicii* were observed, but *A. hemprichicii* was predominant. The sludge yields in a suction submerged MBR varied from 0 to 0.12 kg of suspended solids (SS)/kg of $COD_{removed}$ at over 100 worms/mL of mixed liquor, and those in a gravitational submerged MBR were 0.1−0.32 kg of SS/kg of $COD_{removed}$ with 1008−3800 worms/mL of mixed liquor. However, the same phenomenon was observed in their experiments that unstable worm growth occurred, and worms suddenly disappeared in MBRs after some time.

A definite connection between worm presence and process conditions in aerobic wastewater treatment processes is still missing. Although the presence of worms may lead to a substantial sludge reduction, the practical application is still uncontrollable in the activated sludge system because of unstable worm growth. High worm density once occurred in MBRs, but it is necessary to further explore stable worm growth and investigate membrane impacts on worm growth in the long term. Another challenge of sludge reduction induced by *Oligochaete* is how to maintain stable worm growth as long as possible. Therefore, the objective of this paper was to compare performances of a submerged MBR and a CAS reactor on sludge reduction induced by *Oligochaete* for an extended period in order to know how to control stable worm growth for longer time. The impact of worm growth on COD and nutrient removals was discussed. Meanwhile, operation parameters of affecting worm growth in both reactors were investigated.

Materials and Methods

Pilot Plant Configurations. Two different reactors were used in this study (Table 1). Both reactors consisted of presettling, aeration, and separation tanks. The plug-flow aeration tank was comprised of four aerated compartments. A submerged membrane module (Kubota Company, Japan) was used in the MBR as a solid and liquid separation instead of a clarifier in the CAS reactor. The permeate from the MBR was continuously obtained by the gravity. The membrane was chemically cleaned (0.5% NaOCl) at about 40 °C once every 4 months for controlling membrane fouling.

Wastewater. The fed wastewater (Table 1) was municipal wastewater from a neighboring community of TNO Environment, Energy and Process Innovation in Apeldoorn, The Netherlands. The raw wastewater was screened (0.1 mm) before entering presettling tanks.

Calculations of Sludge Loading Rate and Sludge Yield. The sludge loading rates in the MBR and the CAS reactor were calculated as following:

$$(F/M)_{MBR} = QS/(V_aX_a + V_sX_r) \quad (1)$$

$$(F/M)_{CAS} = QS/V_aX_a \quad (2)$$

where $(F/M)_{MBR}$ and $(F/M)_{CAS}$ are the sludge loading rates of

TABLE 2. Characteristics of Sludge Yield, SVI, Effluent Quality, and Nutrient Removal with Dominant Worm Growth in MBR and CAS Processes

time	dominant worm	Y (kg of SS/kg of $COD_{removed}$)	SVI (mL/g)	COD removal (%)	COD effluent (mg/L)	SS removal (%)	SS effluent (mg/L)	total inorganic N[a] (mg/L) influent	total inorganic N[a] (mg/L) effluent	PO_4^{3-}-P (mg/L) influent	PO_4^{3-}-P (mg/L) effluent
MBR											
days 57−152, 175−228	*Aeolosoma*	0.48 ± 0.19	137± 52	95 ± 2	17 ± 4	nd	nd	39 ± 12	37 ± 9	4.50 ± 1.98	3.83 ± 2.08
days 231−301	*Nais*	0.30 ± 0.16	131± 40	95±2	18 ± 4	nd	nd	40 ± 11	28 ± 9	3.60 ± 2.13	3.77 ± 1.84
CAS											
days 21−189, 245−270, 319−336	*Aeolosoma*	0.19 ± 0.26	60 ± 21	86 ± 8	43 ± 16	71 ± 24	31 ± 19	38 ± 12	33 ± 11	3.66 ± 2.05	3.27 ± 2.09
days 193−242, 273−315	*Nais*	0.13 ± 0.17	51 ± 12	83 ± 7	53 ± 15	71 ± 19	33 ± 15	36 ± 11	33 ± 12	4.09 ± 2.56	5.22 ± 2.71

[a] Total inorganic N = NH_4^+-N + NO_3^--N + NO_2^--N.

the MBR and the CAS reactor (kg of COD/kg of VSS·d); Q is the flow of influent (L/h); S is the COD of the influent (mg/L); V_a and V_s are the volumes of aeration and separation tanks (L); X_a and X_r are the volatile suspended solids (VSS) concentrations in aeration tank and returned sludge (g/L).

The sludge yield in a given time was calculated as

$$\Delta TSS = TSS_{n+i} - TSS_n + TSS_w = 10^{-3}[(V_aX_a + V_sX_r)_{n+i} - (V_aX_a + V_sX_r)_n + iQ_wX_r] \quad (3)$$

$$\Delta COD_{removed} = (COD_{removed})_{n+i} - (COD_{removed})_n = 10^{-6} \times 24i\sum_{n}^{n+i} Q_{in}(\sum_{n}^{n+i} COD_{in} - \sum_{n}^{n+i} COD_{out}) \quad (4)$$

$$Y = \frac{\Delta TSS}{\Delta COD_{removed}} \quad (5)$$

where Y is the sludge yield during i days (kg of SS/kg of $COD_{removed}$); ΔTSS is the amount of sludge (total suspended solids) produced during i days (kg of SS); $\Delta COD_{removed}$ is the amount of COD removed during i days (kg of $COD_{removed}$); TSS_n, TSS_{n+i}, and TSS_w are the amount of sludge on day n and $n+i$, the amount of sludge wasted during i days; V_a and V_s are the volumes of aeration and separation tanks (L); X_a and X_r are TSS concentrations in aeration tank and returned sludge (g/L); COD_{in} and COD_{out} are the COD concentrations of influent and effluent (mg/L); Q_{in} is the influent flow (L/h); Q_w is the flow of wasting sludge, (L/d); 24 is the time transfer factor; 10^{-3} and 10^{-6} are the mass transfer factors.

Operation Conditions. Both reactors had been running for some time before this study began. Parts of returned sludge in both reactors were therefore wasted once a week in the beginning of this study in order to make them in the steady state. Both of pilot systems were fed with the presettled municipal wastewater. The influent pumps (Watson-Marlow 601S, 701S, and 701S/R, U.K.) were controlled by level controllers. Parts of sludge in the membrane separation tank of the MBR and the clarifier of the CAS reactor were returned to the aeration tank by recycle pumps (Watson-Marlow 603S and 604S, U.K.), respectively. The dissolved oxygen (DO) concentrations (WTW TriOxmatic 160R, Germany) in the first compartment of the aeration tank in both reactors were daily monitored as well as pH (PHM2, Germany) and temperature.

The worm growth depends on temperature (*6*, *7*, *13*, *17*). The temperature in the aeration tank of both reactors was hence set at 20 °C (Table 1). The pH was stable, and the average DO concentrations in both reactors were kept at or over 2.0 mg/L. Both the aeration tank and the membrane separation tank in the MBR are included in calculating F/M because they are aerobic. Therefore, the average F/M in the MBR was half of that in the CAS reactor. Except some days (days 70−91, 95−116, and 119−152 for the MBR; days 117−154 for the CAS reactor), parts of returned sludge in both reactors were wasted once a week. The sludge age was determined and adjusted through wasting different amounts of returned sludge on the basis of mass balance in the whole system.

The average TSS concentrations in aeration and separation tanks of the MBR were 4.84 and 12.79 g/L, respectively, and those of the CAS reactor were 6.07 and 12.17 g/L, respectively. The average ratio of VSS and TSS in both MBR and CAS processes was stable at 0.82 (Table 1), which showed that little inert solids accumulated in biomass.

Worm Incubation. The tested sludge was from aeration tanks of both reactors. Different sludge concentrations were made through thickening activated sludge samples. Worm incubation of each sludge concentration was done by triplicate samples. A total of 100 mL of sludge sample was put in an Erlenmeyer bottle of 500 mL. The bottles were

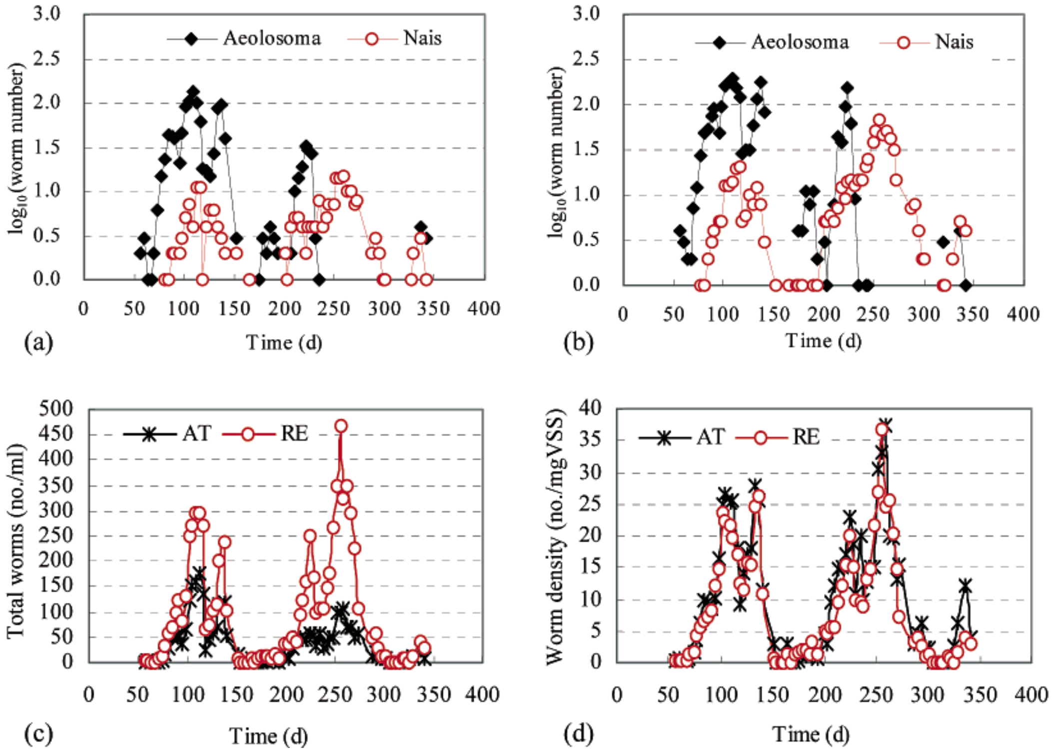

FIGURE 1. Profiles of worm growth in the MBR: (a) aeration tank; (b) returned sludge; (c) total number of worms; (d) ratio of total worms and VSS (AT, aeration tank; RE, returned sludge).

closed by cotton plugs and placed on the orbital shaker (100 rpm) in a 20 °C room for 2 or 3 weeks. Worms were counted every 2 or 3 d.

Analysis. Samples were taken from both reactors twice a week. The COD and NH_4^+-N concentration in feed and effluent were determined with standard vial tests of Dr. Lange: LCK 314 (15−150 mg/L of COD), LCK 114 (150−1000 mg/L of COD), and LCK 303 (2-47 mg/L of NH_4^+-N). The soluble COD was measured with the filtrate samples through a 0.22-μm membrane. The COD value was the average of duplicate samples. The concentrations of total suspended solids (TSS), ash, and volatile suspended solids (VSS) were determined at 104 (4 h) and 600 °C (2 h), respectively. NO_2^--N, NO_3^--N, and PO_4^{3-}-P were measured by a DX-120 ion chromatograph (Dionex Corp., USA), and each of their values were the average of duplicate samples. Worms in both reactors were counted at 1 mL of sludge sample by a stereomicroscope (Wild M7, Switzerland). The values of worms were the average of triplicate samples. Total worms were calculated as *A. hemprichicii* according to 1 *Nais* = 7 *Aeolosoma* = 2.5 *Pristina* (*17*). Photomicrographs of activated sludge were taken by a Sony camera (Sony 3CCD, Japan) connected to a microscope (Leitz Aristoplan, Germany). Statistical analysis, including Pearson and Spearman's rank correlations, was carried out with a soft of SPSS 11.0 produced by SPSS Inc.

Results and Discussion

Worm Growth. No worms were found in the MBR during the first 53 d, and then a given volume of returned sludge containing worms from the CAS reactor was first inoculated in the MBR on day 56. Since day 56, *Aeolosoma* was dominant during day 57−152. For the purpose of promoting worm growth by increasing sludge concentration, no returned sludge was wasted in days 70−91, 95−116, and 119−152, respectively. However, worm density did not reach the level as high as expected. Worms suddenly disappeared on day 154. Since then, worm inoculation was repeated three times on days 172, 193 and 315, respectively. Notably, worms did not naturally produce in the MBR, and the dominant type of worms in the MBR depended on the sludge inoculated from the CAS reactor. Two worm types alternatively dominated during days 175−341, first *Aeolosoma* (days 175−228) and then *Nais* (days 231−301) (Figure 1). And *Nais* became the only worm found during days 249−301. Worm presence and disappearance alternated in the MBR (Figure 1), which was the same as previous observations (*16*, *17*). The average MBR worm density in the aeration tank was only 10 total worms/mg of VSS, and the maximum was just 37 total worms/mg of VSS on the 259th day. The maximums of *Aeolosoma* and *Nais* in aeration tank were 21 no./mg of VSS on day 133 and 5 no./mg of VSS on day 259, respectively. The sizes of *Aeolosoma* and *Nais* were among 0.5−2 and 1−6 mm, respectively.

Worm growth in the CAS reactor was much better than in the MBR as shown in Figure 2. Except for the first 3 weeks, worms occurred throughout the operating period and were continuously maintained at over 30 total worms/mg of VSS in the aeration tank for 172 d (days 70−242). The average CAS worm density in the aeration tank was 71 total worms/mg of VSS, which was higher than that in the MBR. Three types of worms (*A. hemprichicii*, *P. aequiseta*, and *N. elinguis*) were found. But *P. aequiseta* was present only occasionally. The number of *Aeolosoma* was more than that of *Nais* during days 21−189, 245−270, and 319−336, respectively. *Nais* was dominant during days 193−242 and 273−315, respectively. The alternating worm dominance changed between *Aeolosoma* and *Nais* (Figure 2). The maximal worm density in the aeration tank was 302 total worms/mg of VSS on day 84, and those of *Aeolosoma* and *Nais* in the aeration tank were 141 no./mg of VSS on day 109 and 25 no./mg of VSS on day 84, respectively. Because of low sludge yield (0.10 kg of SS/kg of $COD_{removed}$) caused by high worm density during days 21−116, no sludge wasting during days 117−154 was carried out to investigate how long it was possible to keep no sludge discharging at high worm density. However, the aftermath

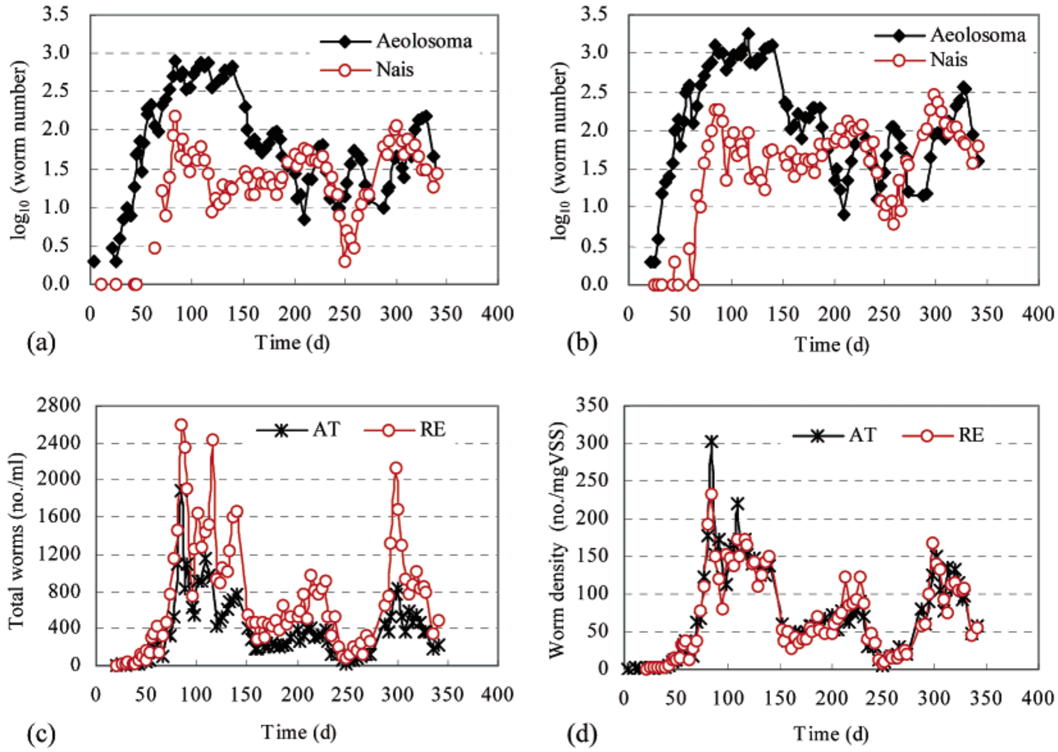

FIGURE 2. Profiles of worm growth in the CAS reactor: (a) aeration tank; (b) returned sludge; (c) total number of worms; (d) ratio of total worms and VSS (AT, aeration tank; RE, returned sludge).

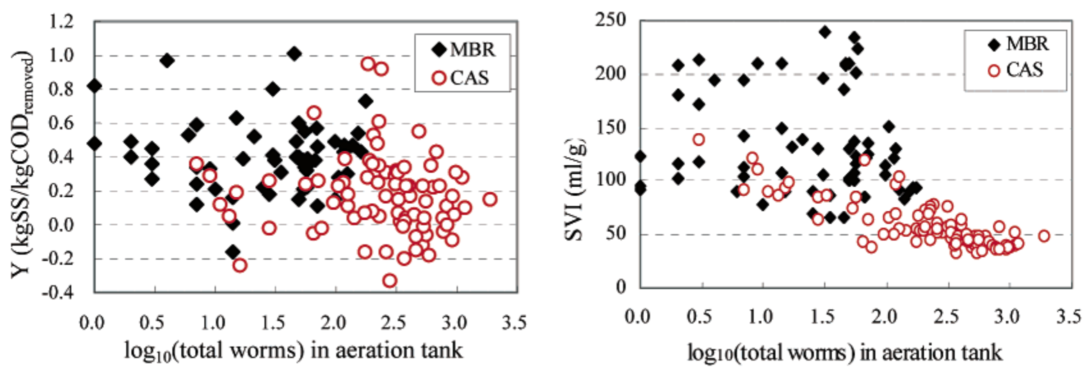

FIGURE 3. Relationships of sludge yield, sludge volume index (SVI), and worm density in MBR and CAS processes.

TABLE 3. Impacts of Different Worm Densities (Aeration Tank) on Sludge Yield, SVI, and Effluent Quality in CAS Process

worm density (total worms/mg of VSS)	*Y* (kg of SS/kg of $COD_{removed}$)	SVI (mL/g)	COD effluent (mg/L)	COD removal (%)	SS effluent (mg/L)	SS removal (%)	PO_4^{3-}-P influent (mg/L)	PO_4^{3-}-P effluent (mg/L)
<50	0.22 ± 0.25	77 ± 27	39 ± 16	87 ± 7	23 ± 16	79 ± 21	3.34 ± 2.16	3.04 ± 2.31
50−100	0.21 ± 0.24	56 ± 12	44 ± 17	84 ± 8	35 ± 16	69 ± 22	4.51 ± 2.66	5.16 ± 2.79
100−150	0.10 ± 0.22	41 ± 5	50 ± 15	81 ± 10	30 ± 13	70 ± 24	3.33 ± 1.64	3.40 ± 1.77
>150	0.14 ± 0.13	44 ± 7	59 ± 10	83 ± 7	51 ± 18	55 ± 24	3.99 ± 1.10	2.96 ± 0.75

of long-term no sludge wasting was that worm density sharply decreased from 152 total worms/mg of VSS on day 119 to 48 total worms/mg of VSS in aeration tank on day 154. *Aeolosoma* became smaller and weaker than that during days 21−116. The sizes of *Aeolosoma* and *Nais* were among 0.5−2 and 2−8 mm, respectively, and they were strong and active in their bloom state.

In prior studies, *Aeolosoma* (*16*, *17*) or *Nais* (*6*, *7*) were the only dominant type of worms in their observations. But both *Aeolosoma* and *Nais* alternatively became dominant in this study. Zhang (*16*) thought the size of *Aeolosoma* was related to its population (i.e., the higher the density of *Aeolosoma* was and the smaller the size of *Aeolosoma* was). It was found in this study that the sizes of worms changed little at different worm density when worm growth was in the same state. Worms were bigger in their growing and bloom state than in their decreasing state, no matter what the worm density was. It therefore indicated that the sizes of worms were principally determined by the state of worm growth.

Sludge Yield and Sludge Volume Index (SVI). Both the average sludge yield and SVI in the CAS reactor were much lower than those in the MBR (Figure 3). The average sludge

TABLE 4. Correlations among Worm Number (Aeration Tank), Sludge Yield, and SVI in MBR and CAS Processes[a]

	Aeolosoma		*Nais*		*Y*		SVI	
	MBR	CAS	MBR	CAS	MBR	CAS	MBR	CAS
Pearson Correlations								
total worms	0.812**	0.819**	0.778**	0.784**	0.090	−0.218	−0.165	−0.581**
Aeolosoma			0.266*	0.285**	0.193	−0.058	−0.195	−0.444**
Nais					−0.067	−0.263*	−0.061	−0.478**
Y							−0.018	0.452**
SVI								
Spearman's Rank Correlations								
total worms	0.620**	0.688**	0.915**	0.809**	0.049	−0.313*	−0.091	−0.761**
Aeolosoma			0.332**	0.235*	0.338**	−0.089	−0.123	−0.590**
Nais					−0.080	−0.324**	−0.029	−0.542**
Y							−0.039	0.508**
SVI								

[a] Key: **, correlation is significant at the 0.01 level (2-tailed); *, correlation is significant at the 0.05 level (2-tailed).

TABLE 5. Results of COD and Nutrients Removals in MBR and CAS Processes

	MBR		CAS	
	average ± SD	range	average ± SD	range
Total COD				
effluent (mg/L)	17.45 ± 3.83	9.32−30.78	44.49 ± 16.43	18.63−100.55
removal (%)	93.96 ± 2.91	79.85−98.18	84.45 ± 8.22	62.37−96.51
Soluble COD				
effluent (mg/L)	20.60 ± 3.41	16.61−31.19	43.37 ± 14.65	25.52−73.58
removal (%)	78.88 ± 7.01	66.09−87.04	60.52 ± 7.78	41.31−75.61
NH_4^+-N				
effluent (mg/L)	0.16 ± 0.67	0−4.98	0.19 ± 0.66	0.01−5.55
removal (%)	99.56 ± 1.49	87.73−100	99.48 ± 1.47	88.65−99.98
NO_3^--N (mg/L)				
influent	0.10 ± 0.17	0.00−1.11	0.10 ± 0.17	0.00−1.11
effluent	32.86 ± 10.02	4.09−51.72	32.83 ± 11.32	9.33−59.63
NO_2^--N (mg/L)				
influent	0.31 ± 0.89	0.00−4.46	0.31 ± 0.89	0.00−4.46
effluent	0.03 ± 0.17	0−1.13	0.03 ± 0.16	0−1.33
Total Inorganic N[a] (mg/L)				
influent	38.19 ± 11.66	8.81−58.20	38.19 ± 11.66	8.81−58.20
effluent	33.07 ± 9.92	9.11−55.70	33.03 ± 11.27	9.76−59.73
PO_4^{3-}-P (mg/L)				
influent	3.74 ± 2.19	0.41−9.90	3.74 ± 2.19	0.41−9.90
effluent	3.43 ± 2.05	0.49−8.30	3.80 ± 2.45	0.32−10.19

[a] Total inorganic N = NH_4^+-N + NO_3^--N + NO_2^--N.

yield and SVI in the MBR were 0.40 kg of SS/kg of $COD_{removed}$ and 133 mL/g, respectively; but those in the CAS reactor were 0.17 kg of SS/kg of $COD_{removed}$ and 60 mL/g, respectively. In addition, the average sludge yield in the CAS reactor in this study was much lower than that (0.7−1.0 kg of MLSS/kg of BOD_5) in CAS processes without worm (*20*).

Nais had more potential for sludge reduction and improvement of sludge settling characteristics than *Aeolosoma*. Results clearly showed that both the average sludge yield and the SVI during periods of dominant *Nais* were lower than those during periods of dominant *Aeolosoma* (Table 2). Worm growth in the MBR contributed to neither sludge reduction nor improvement of sludge settling characteristics because of low density. On the contrary, worm presence and bloom in the CAS reactor greatly decreased sludge yield and improved sludge settling characteristics at high density. Sometimes worm bloom in the CAS reactor resulted in zero or even negative sludge production (Figure 3). The average sludge yield and SVI at over 100 total worms/mg of VSS were nearly half of those at less than 50 total worms/mg of VSS in the CAS reactor (Table 3). Pearson test results showed that the numbers of total worms, *Aeolosoma* and *Nais*, in the MBR process had no linear correlations with sludge yield and SVI, but Spearman's rank test showed the number of *Aeolosoma* significantly correlated with the sludge yield (Table 4). Different from the MBR, both Pearson and Spearman's rank tests showed that all the numbers of total worms (*Aeolosoma* and *Nais*) in the CAS reactor had significant correlation with SVI. But the number of *Aeolosoma* in the CAS reactor had little correlation with sludge yield (Table 4). The statistical analysis also showed that *Nais* had more impact on sludge yield than *Aeolosoma* in the CAS reactor. The possible explanation is that *Nais* is bigger than *Aeolosoma* and so can consume more sludge than *Aeolosoma*. Notably, the average sludge yield during days 274−329 in the CAS reactor was as low as 0.03 kg of SS/kg of $COD_{removed}$ because water flea bloom occurred, and they also consume sludge and compete with worms. Pearson test clearly showed that water flea growth had significant linear correlation with sludge yield (i.e., their Pearson correlation was −0.730 at the 0.01 level (2-tailed)). Water fleas increased quickly, from 4 no./mL on day 273 to 141 no./mL on day 312. However, water flea bloom brought the worsened quality of effluent (i.e., more turbid and higher COD of effluent) although it

TABLE 6. Correlations among Worm Number (Aeration Tank) and Effluent Quality in MBR Process[a]

	Aeolosoma	*Nais*	COD	COD removal	NH_4^+-N	NO_3^--N	PO_4^{3-}-P
			Pearson Correlations				
total worms	0.812 **	0.778**	−0.024	0.177	0.057	0.286**	0.027
Aeolosoma		0.266*	−0.153	0.156	0.122	0.399**	−0.106
Nais			0.125	0.124	−0.037	0.037	0.161
COD				−0.156	0.187	0.060	−0.016
COD removal					−0.025	0.237*	0.101
NH_4^+-N						−0.113	−0.163
NO_3^--N							0.219*
PO_4^{3-}-P							
			Spearman's Rank Correlations				
total worms	0.620**	0.915**	−0.036	0.162	0.041	0.273*	0.250*
Aeolosoma		0.332**	−0.131	0.130	0.021	0.414**	0.117
Nais			0.035	0.095	0.078	0.159	0.254*
COD				−0.178	0.270*	0.109	−0.015
COD removal					0.110	0.193	0.031
NH_4^+-N						0.074	−0.121
NO_3^--N							0.261*
PO_4^{3-}-P							

[a] Key: **, correlation is significant at the 0.01 level (2-tailed); *, correlation is significant at the 0.05 level (2-tailed).

TABLE 7. Correlations among Worm Number (Aeration Tank) and Effluent Quality in CAS Process[a]

	Aeolosoma	*Nais*	COD	COD removal	SS	SS removal	NH_4^+-N	NO_3^--N	PO_4^{3-}-P
				Pearson Correlations					
total worms	0.819**	0.784**	0.372*	−0.161	0.395**	−0.269*	−0.061	0.406**	−0.020
Aeolosoma		0.285**	−0.245**	−0.119	0.340**	−0.223	−0.006	0.437**	−0.201
Nais			0.362**	−0.167	0.280**	−0.220	0.063	0.203	0.191
COD				−0.521**	0.345**	−0.369**	0.084	0.428**	0.094
COD removal					−0.234*	0.525**	−0.026	−0.204	−0.027
SS						−0.681**	0.189	0.331**	−0.032
SS removal							0.019	−0.243*	−0.048
NH_4^+-N								−0.081	−0.107
NO_3^--N									0.290**
PO_4^{3-}-P									
				Spearman's Rank Correlations					
total worms	0.688**	0.809**	0.413**	−0.230*	0.441**	−0.316**	0.160	0.451**	0.178
Aeolosoma		0.235*	0.125	−0.039	0.286*	−0.187	0.194	0.328**	−0.018
Nais			0.435**	−0.291**	0.414**	−0.290*	0.102	0.295**	0.347**
COD				−0.527**	0.332**	−0.320**	0.258*	0.364**	0.195
COD removal					−0.223	0.443**	−0.181	−0.189	−0.177
SS						−0.759**	0.125	0.313**	0.030
SS removal							−0.118	−0.285*	−0.070
NH_4^+-N								0.239*	0.142
NO_3^--N									0.366**
PO_4^{3-}-P									

[a] Key: **, correlation is significant at the 0.01 level (2-tailed); *, correlation is significant at the 0.05 level (2-tailed).

reduced sludge production. At present, little is known about how water fleas occur and bloom in the CAS reactor.

COD Removal. It was obvious that MBR had much higher COD removal efficiency (94%) than CAS (85%) and that the quality of effluent in the MBR was excellent: low COD (<20 mg/L), no SS, and clear (Table 5). Notably, the soluble COD of effluent in the MBR was similar to the total COD of effluent. The reason is that the MBR essentially filtered all solids because the difference between total and soluble COD is simply due to particulate organic matter. Statistical analysis clearly showed that worm growth had impact on neither COD removal nor the effluent quality in the MBR (Table 6), and the effluent quality in the MBR was nearly the same during different dominant worm type (Table 2). Contrary to the MBR, worm growth significantly affected the effluent quality, the average COD, and SS removals in the CAS reactor (Table 7), and the average COD removal and the effluent quality during dominant periods of *Nais* were lower and worse than those during dominant periods of *Aeolosoma* (Table 2). The higher the worm density was, the worse the effluent quality was (Table 3).

The effluent in the CAS reactor became turbid as worm bloom occurred, which was the same as other observations (*6*, *13*). The possible explanation is that parts of sludge flocs disintegrate into smaller flocs or dispersed solids due to predation by worms and then cause smaller flocs or dispersed solids increase in effluent. In addition, the negative impact of water fleas on COD removal and the effluent quality should not be neglected. Both COD removal and the effluent quality were down as water fleas bloomed (i.e., heavy foam and turbid effluent, 74% of COD removal, and 59 mg/L of effluent COD on day 312).

Nutrient Removal. Both reactors had excellent performances on nitrification, regardless of variations in input wastewater and worm growth. Except occasional results over 1 mg/L, NH_4^+-N of effluent in both reactors was stable and very low (<0.50 mg/L) (Table 5) and had little correlations with worm growth (Tables 6 and 7). The average NH_4^+-N removals in both reactors were very high, over 99%, whichever *Aeolosoma* or *Nais* was predominant (Tables 3 and 5). The NO_3^--N concentration in the fed wastewater was very low, less than 0.5 mg/L, but that in the effluent was high, over 30

TABLE 8. Correlations of Operation Parameters and Worm Number (Aeration Tank) in MBR Process[a]

	Aeolosoma	*Nais*	TSS	HRT	SRT	*F*/*M*	recycle ratio	temperature	pH	DO
Pearson Correlations										
total worms	0.812**	0.778**	0.128	0.209*	0.393	−0.155	0.041	0.139	−0.169	−0.112
Aeolosoma		0.266*	0.250*	0.412**	0.217	−0.206	0.334**	0.287**	0.053	0.052
Nais			−0.058	−0.100	0.363	−0.033	−0.294**	−0.078	−0.336**	−0.242*
TSS				0.279**	0.685**	−0.409**	0.525**	0.082	−0.429**	−0.044
HRT					0.420*	−0.380**	0.807**	0.382**	0.294**	0.359**
SRT						−0.398	0.162	0.139	−0.156	0.019
F/*M*							−0.284**	−0.036	−0.001	−0.219*
recycle ratio								0.492**	0.180	0.296**
Temperature									0.130	0.181
pH										0.510**
DO										
Spearman's Rank Correlations										
total worms	0.620**	0.915**	0.113	0.124	0.433*	−0.053	−0.057	0.118	−0.186	−0.162
Aeolosoma		0.332**	0.189	0.451**	−0.101	−0.165	0.399**	0.491**	0.219*	0.120
Nais			0.054	−0.069	0.503*	−0.011	−0.267*	−0.030	−0.340**	−0.246*
TSS				0.407**	0.721**	−0.484**	0.555**	0.217*	−0.333**	0.046
HRT					0.131	−0.445**	0.839**	0.534**	0.212*	0.354**
SRT						−0.440*	0.082	−0.027	−0.225	0.058
F/M							−0.356**	−0.073	0.106	−0.192
recycle ratio								0.594**	0.157	0.313**
temperature									0.107	0.229*
pH										0.524**
DO										

[a] Key: **, correlation is significant at the 0.01 level (2-tailed); *, correlation is significant at the 0.05 level (2-tailed).

TABLE 9. Correlations of Operation Parameters and Worm Number (Aeration Tank) in CAS Process[a]

	Aeolosoma	*Nais*	TSS	HRT	SRT	*F*/*M*	recycle ratio	temperature	pH	DO
Pearson Correlations										
total worms	0.819**	0.784**	0.246*	0.347**	0.111	−0.201	−0.234*	0.480**	−0.212*	0.038
Aeolosoma		0.285**	0.194	0.253*	0.049	−0.121	−0.123	0.551**	−0.455**	−0.145
Nais			0.212*	0.306**	0.137	−0.222*	−0.226*	0.193	0.052	0.263*
TSS				−0.018	0.356**	−0.165	0.076	0.087	−0.340**	−0.256*
HRT					0.134	−0.518**	0.329**	0.266*	0.121	0.098
SRT						−0.117	−0.066	0.013	0.080	−0.064
F/*M*							−0.111	−0.188	0.210*	−0.172
recycle ratio								−0.200	0.081	−0.022
temperature									−0.421**	−0.178
pH										0.101
DO										
Spearman's Rank Correlations										
total worms	0.688**	0.809**	0.088	0.409**	0.187	−0.185	−0.220*	0.536**	−0.127	0.088
Aeolosoma		0.235*	−0.004	0.100	0.192	−0.002	−0.199	0.572**	−0.465**	−0.185
Nais			0.064	0.367**	0.051	−0.248*	−0.231*	0.336**	0.062	0.292**
TSS				−0.056	0.426**	−0.077	0.143	0.105	−0.321**	−0.259*
HRT					0.071	−0.493**	0.382**	0.251*	0.195	0.095
SRT						−0.012	0.068	−0.197	0.285	0.005
F/*M*							−0.100	−0.166	0.076	−0.256*
recycle ratio								0.013	0.074	−0.181
temperature									−0.428**	−0.197
pH										0.182
DO										

[a] Key: **, correlation is significant at the 0.01 level (2-tailed); *, correlation is significant at the 0.05 level (2-tailed).

mg/L (Table 5). There was few or nearly zero NO_2^--N in effluent, which means no NO_2^--N accumulation happened. These results indicated that ammonium nitrogen was efficiently nitrified into NO_3^--N and confirmed that worm growth does not disturb nitrification process (*6*, *7*, *12*, *13*, *16*). However, it should be noted that NO_3^--N concentration in effluent significantly correlated with worm growth in both reactors (Tables 6 and 7). The total inorganic nitrogen concentrations in both influent and effluent changed little (Table 5), which clearly showed that worm growth did not cause the total inorganic nitrogen increase in effluent.

Different from the MBR, the average PO_4^{3-}-P concentration in effluent in the CAS reactor was a little higher than that in influent (Table 5). It was interestingly found in both reactors that worm growth did not cause PO_4^{3-}-P increase in effluent during *Aeolosoma* dominance, but PO_4^{3-}-P increase in effluent occurred as *Nais* was dominant (Table 2). Spearman's rank test also showed that *Nais* growth had an impact on PO_4^{3-}-P concentration in effluent. However, such impact of *Nais* growth on PO_4^{3-}-P increase in effluent was not heavy.

Operation Parameters Affecting Worm Growth. The impacts of eight operation parameters (TSS, HRT, SRT, F/M, recycle ratio, temperature, pH, and DO) on worm growth in both reactors were investigated. Only sludge loading rate (F/M) had no impact on worm growth in the MBR (Table 8),

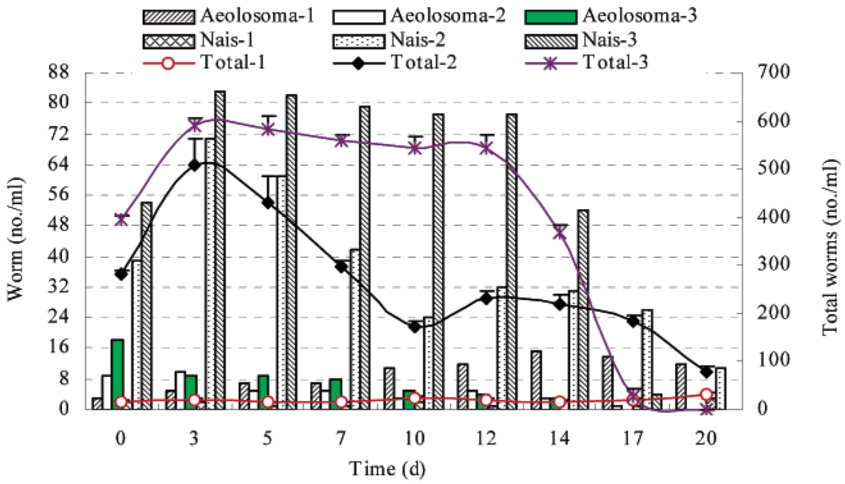

FIGURE 4. Profiles of worm growth in the first worm incubation experiment: (1) MBR, TSS = 3.55 g/L; (2) CAS, TSS = 6.15 g/L; (3) CAS, TSS = 12.50 g/L.

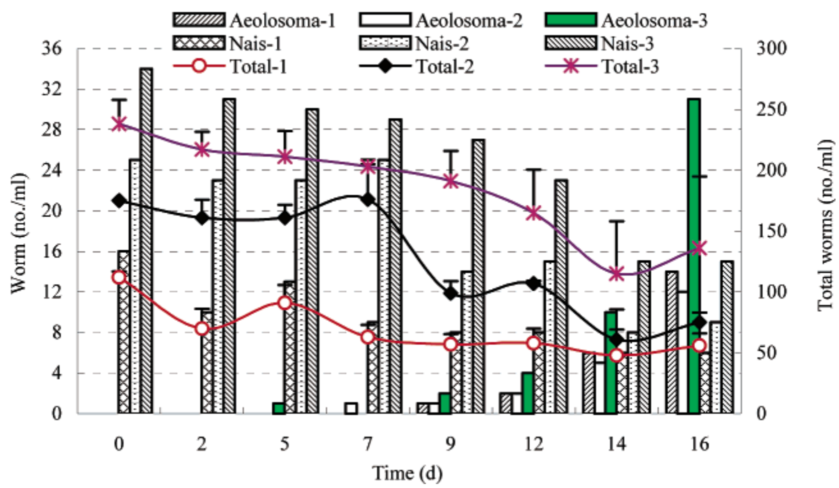

FIGURE 5. Profiles of worm growth in the second worm incubation experiment: (1) MBR, TSS = 3.77 g/L; (2) MBR, TSS = 6.54 g/L; (3) MBR, TSS = 9.40 g/L.

and sludge age (SRT) was the only parameter that did not affect worm growth in the CAS reactor (Table 9). Statistical analysis showed that five parameters (HRT, recycle ratio, temperature, TSS, and pH) significantly affected *Aeolosoma* growth in the MBR; and factors of affecting *Nais* growth in the MBR were pH, recycle ratio, DO, and SRT (Table 8). The results of statistical analysis indicated that *Aeolosoma* growth in the CAS reactor was influenced very much by temperature, pH, and HRT; and five factors (HRT, DO, recycle ratio, F/M, TSS, and temperature) had much impact on *Nais* growth in the CAS reactor (Table 9). Hence, it is possible to maintain stable growth of different worm types through controlling different operation parameters for a long time.

Microbial Community. *Microscopic Investigation.* The activated sludge in the MBR had high viscosity, small and fine sludge flocs, and poor sludge settling and dewatering characteristics, even as worm presence. As opposite to this, the activated sludge in the CAS reactor consisted of firm and compact flocs with large size and had very good settling and dewatering characteristics as worm occurrence and bloom. The color of activated sludge in the MBR was light yellow even as worms presented and that in the CAS reactor gradually became deep, from light yellow to black/brown with worm occurrence and bloom. The floc size (about 30−40 μm) of activated sludge in the MBR experienced little change, but that in the CAS reactor changed too much with worm occurrence and bloom. Grazing of sludge flocs is limited by worm physical properties (size of the mouth and pharynx), and the floc size of activated sludge in the CAS reactor became big and huge (even about 1000 μm) when the dominance of worms transferred from *Aeolosoma* to *Nais*.

In this case, the activated sludge in the CAS reactor was alike a "zoo", containing worms, nematodes, daphnias, tardigrades, and rotifers. But protozoa and metazoa, not including worms, were absent in the activated sludge of the MBR except those occasionally found. This phenomenon was the same as previous observations in different MBRs (submerged and side stream) (*21*−*26*). However, little is known why protozoa and metazoa were absent in MBR processes.

Worm Incubation. Worm incubation experiments were carried out to investigate the impacts of activated sludge on worm growth in both reactors. The sludge samples used in the first experiment were taken from both reactors on day 204. Results of the first one showed that worm growth in activated sludge from the CAS reactor was better than that from the MBR in the first 3 d (Figure 4). To further investigate the impact of activated sludge from MBR on worm growth, a second test was done at different sludge concentrations (Figure 5). The sludge samples used in the second test were taken from the MBR on day 258. The amounts of total worms after several days decreased, but *Aeolosoma* occurred and increased along with time. This phenomenon of *Aeolosoma* increasing with time was the same as that in the first experiment (Figures 4 and 5). These results indicated that the activated sludge from the CAS reactor was more beneficial for worm growth than that from the MBR. The activated sludge in the MBR was a principal biotic factor of determining worm growth.

Operational Problems. The principal operational problem in the MBR was heavy foam that occurred throughout the operation period, which was not caused by *Nocardia* but perhaps resulted from cell lysis due to shear occurring on the surface of membrane. Sometimes large amounts of straight filaments were found and then caused very high SVI in the MBR (i.e., 202−240 mL/g during days 203−224). However, such poor sludge settling characteristics did not influence MBR operation and effluent quality because of membrane filtration. No rising sludge occurred in MBR.

The most common operational problems encountered in the operation of activated sludge processes were bulking sludge, rising sludge, and foam (*27*). In this study, all of these three problems occurred in the CAS reactor. Rising sludge caused by denitrification in the clarifier was often observed during days 41−68. It was controlled through increasing the returned sludge or wasting parts of returned sludge or their combinations. Bulking sludge suddenly happened during days 264−265. Microscopic investigation showed large amounts of straight filaments found in activated sludge. Such large amounts of straight filament presence were possibly caused by high sludge loading rate (0.47 kg of COD/kg of VSS·d) on day 263. Through decreasing aeration and wasting parts of returned sludge, bulking sludge was controlled. Due to no influent, the CAS reactor was only operated with continuously returning sludge during days 210−211. Such operation conditions perhaps resulted in cell lysis and subsequently heavy foam and turbid effluent during days 214−229. Foam sometimes happened again during days 256−259 and days 298−312. Microscopic examination showed that many water fleas were found in activated sludge. The combination of decreasing aeration and increasing sludge age was more effective on controlling it than only increasing sludge wasting. However, operational problems in the CAS reactor influenced COD removal and effluent quality very much. In addition, the pH rise was observed during the time of foam occurred in both reactors (i.e., 6.74−6.99 in the MBR and 6.51−6.97 in the CAS reactor). A possible explanation of this phenomenon was the protein released into mixed liquor due to cell lysis.

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