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Simultaneous Nitrification and Denitrification with Excess Sludge Reduction in an Attached Growth System Combining Anaerobic Fermentation and Aerobic Swim-Bed Processes

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Abstract

Simultaneous nitrification and denitrification (SND) with excess sludge reduction were evaluated in an attached growth treatment system consisting of a down-flow anaerobic fermentation (AF) reactor and an aerobic swim-bed (SB) reactor operated as an integrated fixed-film activated sludge (IFAS) process. The two reactors were packed with biomass carriers consisting of different configurations of the same acrylic-fiber material: a net-type carrier biofill® (BL) in the AF reactor and a multi-directional thread-type carrier biofringe® (BF) in the SB reactor. The system was operated continuously under various organic loading rates (OLRs) from 1.5 to 4.5 kg COD m^{-3} d^{-1} and nitrogen loading rates (NLRs) from 0.1 to 0.3 kg N m^{-3} d^{-1} using moderately high-strength synthetic wastewater. The AF reactor, located upstream of the SB reactor, provided hydrolysis, fermentation and anaerobic ammonification. During 184 days of operation, stable total nitrogen (TN) removal efficiencies ranging from 85% to 97% were obtained primarily due to SND in the SB reactor when the OLR was higher than 2.7 kg COD m^{-3} d^{-1}. In addition, observed sludge yields for the whole system ranged from 0.13 to 0.17 kg MLSS kg^{-1} $COD_{removed}$; furthermore, mixed liquor suspended solids (MLSS) were maintained at about 10,000 mg l^{-1} in the SB reactor. Exceptionally high COD and TN removal rates of 5.9 kg COD m^{-3} d^{-1} and 0.43 kg N m^{-3} d^{-1}, respectively, were observed in the SB reactor. These results demonstrated that excess sludge reduction and SND could be achieved concurrently by this combined AF-SB system.

Keywords: swim-bed, IFAS, biofringe®, SND, excess sludge reduction

INTRODUCTION

The conventional activated sludge (CAS) process has a long history of being the most widely used biological wastewater treatment method. However, there are still some problems which attract much attention, such as high excess sludge production with sludge yield coefficients typically ranging from 0.3 to 0.5 kg MLVSS kg^{-1} $COD_{removed}$[1]. Costs for disposal of excess sludge range from 25% to 60% of the total operating costs of wastewater treatment plants (WWTPs)[2]. Moreover, costs for disposal of excess sludge continue to increase due to restrictions stemming from environmental legislative constraints[3]. Compared to commonly used methods for disposal of excess sludge such as land application, landfill, incineration, and sea disposal[4], an ideal way to solve this problem

is to reduce sludge production at the source, i.e., at the wastewater treatment, before it becomes a problem[5].

Furthermore, the efficiency of nitrogen removal in CAS processes is another critical issue due to eutrophication in receiving water bodies. Conventional nitrogen removal systems are based on the combination of nitrification and denitrification processes with spatial or temporal separation due to different environmental conditions required for the autotrophic nitrifying and heterotrophic denitrifying bacteria[6]. The obvious drawbacks of these conventional nitrogen removal processes are the relatively large space requirements and relatively complicatedly operational controls. However, recent studies have revealed that nitrification and denitrification processes can occur concurrently in the same reactor[7]. This process has been termed as simultaneous nitrification and denitrification (SND). Such process excludes the need for two separate reactors or intermittent aeration, thus requiring a smaller footprint for the treatment plant and simplifying the treatment system. Other merits of SND process include reduced organic carbon (electron donor source) requirement for denitrification, less sludge yield, reduced alkalinity and less energy consumption[8].

For upgrading activated sludge processes, several types of packing materials for biomass attachment, in both suspended phase and fixed phase, have been developed. A term used to label these processes is integrated fixed-film activated sludge (IFAS)[9,10]. The advantages claimed for the IFAS process over the CAS process are increased treatment capacity due to greater biomass retention in the aeration tank, improved process stability, reduced sludge yield, enhanced sludge settleability and lower operation and maintenance costs[11]. The swim-bed technology using BF carriers[12] is featured in that the "swimming motion" of the carrier results in enhanced mass transfer of nutrients to the biofilm. Yamamoto et al.[13] reported that the MLSS level in an aerobic IFAS swim-bed reactor reached 16,800 mg l^{-1} with a sludge volume index (SVI) less than 50 ml g^{-1} in partial nitritation treatment for anaerobic digester liquor of swine wastewater.

During the past decade, many researchers have investigated the benefits of IFAS for enhancements of COD removal, partial nitritation, nitrification, denitrification and even enhanced biological phosphorus removal (EBPR) in CAS processes, by packing suspended or fixed biomass carriers into separate anoxic and aerobic reactors[10,14-16]. Using ringlace® media, Randall et al.[17] documented that between 30% and 88% of the produced nitrates were denitrified under aerobic conditions. Recently, various biofilm processes, both in suspended and attached phases, have demonstrated the SND ability in a single aerobic reactor by having anoxic zones within the biofilm depth[18-20]. Therefore, it is proposed that an aerobic IFAS process could accomplish steady total nitrogen removal via SND under suitable operational conditions.

In addition, anaerobic hydrolysis and fermentation (also referred to as acidogenesis) are commonly used as pretreatment for treating high-strength organic wastewater[21,22]. Furthermore, anaerobic hydrolysis can be an effective method for improving denitrification efficiency by providing favorable electron donors such as sulfide and acetate[23,24].

In this paper, a combined system, which included an anaerobic fermentation reactor and an aerobic IFAS swim-bed reactor, was constructed for treating moderately high-strength synthetic organic wastewater. The purpose of this study is to evaluate such a system in terms of COD removal, TN removal and excess sludge reduction. The possible mechanisms involved in SND were also discussed.

MATERIALS AND METHODS

Synthetic wastewater The synthetic wastewater used as influent during the main experimental period of this study was prepared by diluting concentrated corn steep liquor (CSL, San-ei Sucrochemical Co., Ltd., Japan) with tap water free of any buffer or nutrient additions. The characteristics of the influent were as follows: COD from 1000 to 2000 mg l^{-1}, TN from 65 to 130 mg l^{-1}, and pH from 3.6 to 4.9 (Table 1). The C:N:P ratio of the influent was about 30:2:1. The CSL

stock was produced by lactic acid fermentation and was thus acidic and high in lactate and volatile fatty acids (VFAs). During the acclimation period, another synthetic wastewater composed of bonito extract and peptone mixture was used.

Experimental setup This system consisted of an AF reactor, a SB reactor and a sludge settling tank, which were all constructed of acrylic resin, with effective volumes of 6, 10, and 2.5 *l*, respectively (Fig. 1a).

Sixty pieces of the BL (biofill®, NET, Japan) (Fig. 1b) carriers were packed into the middle section of the AF reactor with a packing ratio of 50%. A centrifugal pump with flow rate of 20 l min^{-1} was used to provide a down-flow internal circulation with a velocity of 2.5 cm s^{-1} to enhance the substrate-biomass contact in the AF reactor. The AF reactor was operated at constant temperature of 35 ± 1℃ by a thermostat heater. A gas-liquid separating device was provided to collect the evolved gas for

Table 1 Operational conditions over the main experimental period

Parameters	Run 1	Run 2	Run 3	Run 4
Duration (day)	0–22	23–56	57–115	116–184
OLR_{wh} (kg COD m^{-3} d^{-1})	1.5	2.7	3.5	4.5
OLR_{SB} (kg COD m^{-3} d^{-1})	2.4	4.0	5.1	6.1
NLR_{Wh} (kg N m^{-3} d^{-1})	0.1	0.18	0.23	0.30
NLR_{SB} (kg N m^{-3} d^{-1})	0.16	0.28	0.36	0.47
HRT_{wh} (h)	16	10.67	10.67	10.67
HRT_{AF} (h)	6	4	4	4
HRT_{SB} (h)	10	6.67	6.67	6.67
HRT_{ST} (h)	2.5	1.67	1.67	1.67
Temperature in SB (℃)	25 ± 1	26 ± 2	30 ± 2	20–32
Influent COD (mg l^{-1})	1000	1200	1555	2000
Influent TN (mg l^{-1})	65	80	105	130
Influent pH	4.7 ± 0.2	4.3 ± 0.2	4.1 ± 0.2	3.8 ± 0.2

Subscripts: (wh) whole system; (SB) SB reactor; (AF) AF reactor; (ST) settling tank.

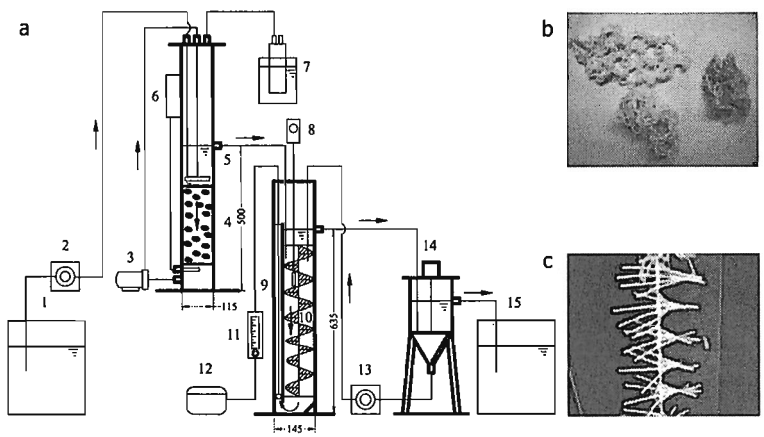


Fig. 1 (a) Schematic of the AF-SB system: (1) influent tank; (2) influent pump; (3) AF recycle pump; (4) BL carriers; (5) AF reactor; (6) thermostat heating device in AF reactor; (7) gas-liquid separator; (8) thermostat heater in SB reactor; (9) SB reactor; (10) BF carrier; (11) air flow meter; (12) air pump; (13) sludge return pump; (14) settling tank; (15) effluent tank; (b) photograph of BL carriers; (c) photograph of BF carrier.

quantification and composition analysis.

The SB reactor had downdraft and updraft sections of 115×115 and 115×30 mm respectively, separated by a vertical baffle. Support filament of the BF carrier (biofringe®, NET, Japan) was 500 mm in length. Fringe yarns of the BF carrier were attached symmetrically, extending equal distances beyond each side of the support filament and twisting to give an even 3-dimensional distribution of the attachment matrix (about 30 circles per meter) (Fig. 1c). Air was introduced near the bottom of the updraft section, with a fixed flow rate of 10 l min^{-1} to aerate the wastewater and induce a down-flow velocity of 20 cm s^{-1} in the downdraft section, leading to a strong "swimming motion" for the carrier filaments. The SB reactor was operated at constant temperature of 25 ± 1℃, except when higher room temperatures occurred in summer.

The surface area of settling tank was 0.017 m^2. Sludge in the settling tank was gently mixed at 2 rpm by a chain and returned to the SB reactor at a 100% recycle rate.

System start-up and sludge acclimation Both AF and SB reactors were initially inoculated with approximately 4,000 mg l^{-1} (as MLSS) of activated sludge from a fill-and-draw batch reactor. During the sludge attachment period without feeding influent, the recycle pump was started for internal recirculation of the AF reactor, and the airflow rate of SB reactor was maintained at 5 l min^{-1} to enhance biofilm attachment under relative weaker velocity condition. After 24 hours of biofilm attachment, with the air flow rate in the SB reactor adjusted to 10 l min^{-1}, the system was operated continuously for about 1 month using synthetic wastewater composed of bonito extract and peptone mixture under low OLRs (0.5 to 1.5 kg COD m^{-3} d^{-1}) for acclimation of the sludge. The system was then shut down to collect all sludge for biomass calculation. Then the sludge obtained from the acclimation was inoculated again (anaerobic and aerobic sludges were used for the AF and SB reactors, respectively) at the start-up for the first stage (Run 1) during the main experimental period. Subsequently, the influent was shifted into CSL.

Experimental procedure and operational conditions During the 184 days of operation, the experiment was divided into four runs according to various OLRs. The operational conditions during the main experimental period are summarized in Table 1. Due to the objective of excess sludge reduction, no sludge was withdrawn intentionally. The initial OLR of each run after start-up was kept at a relatively low value for 3 to 5 days and then increased to the intended OLR. For accurate calculation of sludge yield, all sludge in the system was collected and sampled at the end of each run. Then, the sludge obtained from the previous run was inoculated again in the two reactors with a suitable volume as the seed sludge for the new run.

At the ends of Runs 1, 2 and 4, the MLSS concentration in the SB reactor was determined by sampling the suspended sludge. Then, attached biofilm on the BF carrier was detached completely in the reactor. The MLSS concentration of this mixed sludge was also determined after thorough mixing. The calculated weight difference between the two sludges was considered to be the estimated weight of attached biofilm in the SB reactor. At the end of Run 3, a protocol was adopted for separating the suspended and attached sludge in the SB reactor. After stopping the influent and sludge return from the settling tank, tap water was feed at a flow rate of 30 l d^{-1} directly into the SB reactor with aeration. Almost all suspended sludge was washed out from the SB reactor and only attached biofilm remained in the reactor after 24 h.

Analytical methods Concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD), TN, nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), suspended solids (SS), MLSS, mixed liquor volatile suspended solids (MLVSS), and alkalinity were measured according to standard methods[25] (APHA, 1995). The final effluent samples were filtered (1 μm) before analysis. Ammonium nitrogen (NH_4-N) was measured by the *o*-phenylphenol method[26]. VFAs concentrations were determined by using a CTO-10AS liquid chromatography (Shimadzu, Japan). Gas produced from the AF reactor

was analyzed by using a GC-14B gas chromatograph (Shimadzu, Japan). A digital pH meter (IM-22P, TOA Electronics, Japan) was used to measure pH. Dissolved oxygen (DO) level in the SB reactor was measured using a portable digital DO meter (OM-51, Horiba, Japan). Microorganisms in sludge of the SB reactor were observed by an electron microscope (Nikon Eclipse E600, Japan) with a digital camera (Nikon 4500, Japan). Floc size distribution analysis was conducted using a laser diffraction particle size distribution analyzer (Horiba LA-920, Japan).

Calculation methods In this study, observed sludge yield (Y_{obs}) was calculated according to Eq. (1):

$$Y_{obs} = (W_{end} - W_{start} + \sum QX_e + \sum W_{out})/ \sum Q(S_o - S_e), \quad (1)$$

where W_{end} = total amount of biomass at the end of each run, including all suspended biomass, biofilm biomass in AF and SB reactors and the biomass in the settling tank (mg); W_{start} = total amount of seed biomass at the start-up of each run, (mg); Q = influent flow rate (l d^{-1}); X_e = effluent SS concentration (mg l^{-1}); W_{out} = total amount of sampled sludge for analysis and occasional washout sludge (mg); S_o = influent COD concentration (mg l^{-1}); S_e = effluent COD concentration (mg l^{-1}).

Nitrification efficiency in the SB reactor was calculated according to Eq. (2) :

$$En = 100 \times (TN_{AF} - NH_4\text{-}N_{fi}) / TN_{AF}, \quad (2)$$

where En = nitrification efficiency in the SB reator (%); TN_{AF} = AF effluent TN concentration (mg l^{-1}); $NH_4\text{-}N_{fi}$ = final effluent NH_4-N concentration (mg l^{-1}).

RESULTS AND DISCUSSION

Hydrolysis and fermentation performance in the AF reactor Table 2 shows the changes in VFAs and lactate concentrations during Runs 3 and 4. The average influent total VFAs (as COD) of about 250 and 340 mg l^{-1} increased to 870 and 1,200 mg l^{-1} in the AF effluent in Runs 3 and 4, respectively. The increase of about 250% in total VFAs suggests that a strong acid fermentation reaction occurred in the AF reactor. Meanwhile, most influent lactate was fermented to acetate during AF treatment. At the end of Run 4, the gas production rates ranged from 1.5 to 2 l d^{-1}, and the main components were methane (CH_4) and carbon dioxide (CO_2) at about 50% and 15%, respectively. However, the calculated CH_4 conversion efficiency was only about 0.015 l g^{-1} $COD_{removed}$, which is much lower than a standard value of 0.40 l g^{-1} COD at 35℃[11]. Zhang et al.[27] obtained a gas production level (CH_4 was about 76%) of up to 0.38 l g^{-1} COD in an up-flow anaerobic

Table 2 VFAs and lactate concentrations in Runs 3 and 4

Day	OLR_{wh}[a] (kg COD m^{-3} d^{-1})	Sa.[b]	Acetate (mg l^{-1})	Propionate (mg l^{-1})	Isobutyrate (mg l^{-1})	Butyrate (mg l^{-1})	Isovalerate (mg l^{-1})	Lactate (mg l^{-1})	Total VFAs (mg COD l^{-1})	VFAs & lac[c] (mg COD l^{-1})
		Inf	23	140	0	0	0	374	236	636
80	3.5	AF	299	235	6	78	19	4	866	870
		SB	0	0	15	0	0	0	27	27
		Inf	30	145	0	0	0	383	252	662
102	3.5	AF	326	226	8	79	17	9	881	891
		SB	0	0	0	0	0	1	0	1
		Inf	25	210	0	0	0	488	343	865
132	4.5	AF	389	339	7	62	40	9	1136	1145
		SB	0	0	0	0	0	0	0	0
		Inf	36	203	0	0	0	499	344	878
158	4.5	AF	398	346	7	95	51	10	1238	1248
		SB	0	0	0	0	0	0	0	0

Superscript: (a) OLR for the whole system; (b) sample: Inf: influent; AF: AF effluent; SB: SB effluent; (c) sum of total VFAs and lactate.

sludge bed (UASB) reactor using PVA-gel beads as the biomass carriers for treating high-strength CSL. Compared with these results, the methanogenic activity of the AF reactor was negligible due to different operational conditions, such as hydraulic retention time (HRT), pH, OLR, substrate concentration and biomass carriers. In this study, the HRT of the AF reactor was usually only about 4 h that was not long enough for further VFAs fermentation, leading to accumulation of VFAs and reduction in pH, further inhibiting methanogenic activity.

Influent organic nitrogen, as in protein form, was hydrolyzed to amino acids and then further converted into the ammonium form in the AF reactor. The release of ammonium by anaerobic ammonification in the AF reactor would be preferable so as to allow for sequential aerobic nitrification[28]. This was considered beneficial to SND performance in the SB reactor. Moreover, the increase in alkalinity caused by the release of ammonia replenished the alkalinity consumed during acid fermentation as was evidenced by the elevated reactor pH, which ranged from 5.0 to 5.5 during most of the main experimental period (Fig. 2).

Effluent SS concentrations from the AF reactor fluctuated in the range of 30 to 150 mg l^{-1} and were usually under 100 mg l^{-1} due to the good sludge retaining capacity of the BL carriers. At the end of each run, the attached biofilm on the BL carriers was collected and quantified. The biomass levels (as MLSS) in AF reactor were estimated to be 3,950, 5,500, 8,800, and 11,000 mg l^{-1} in all runs sequentially, while the MLVSS/MLSS ratios of all samples were about 0.93. These high biomass concentrations could be responsible for the stable fermentation performance in the AF reactor.

COD removal performance Fig. 3 shows daily COD removal performance for the whole system. Influent COD was removed efficiently over the whole system with average removal efficiencies of 94% to 99% during steady-state operations. Due to acid fermentation rather than methanogenesis being exhibited in the AF reactor, total COD removal efficiencies for the AF reactor were in the range of only 5% to 20%, with an average of 13.5%. Thus, the actual OLRs for the SB reactor (OLR_{SBS}) were calculated to be 2.4, 4.0, 5.1 and 6.1 kg COD m^{-3} d^{-1} over Runs 1, 2, 3 and 4, respectively.

From Runs 1 to 3, slightly higher system effluent COD concentrations (100 to 120 mg l^{-1}) were observed during the start-up period of each run due to sludge acclimation in the SB reactor. However, after 7 to 10 days of operation, final effluent COD concentrations gradually decreased to 70, 50 and 40 mg l^{-1}, with COD removal efficiencies of 94%, 96% and 97%, respectively. However, just after the start-up period in Run 4, the final effluent

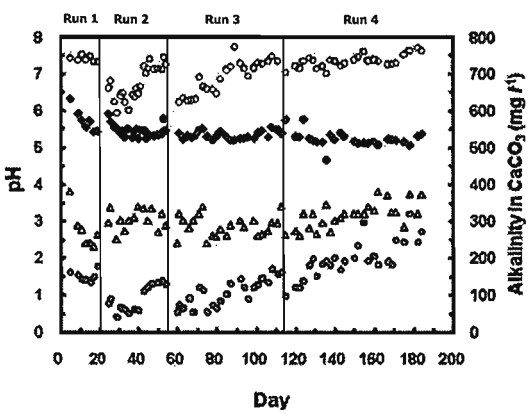


Fig. 2 Profiles of pH and alkalinity in AF and final effluents: (◆) AF effluent pH; (○) final effluent pH; (△) AF effluent alkalinity; (◍) final effluent alkalinity.

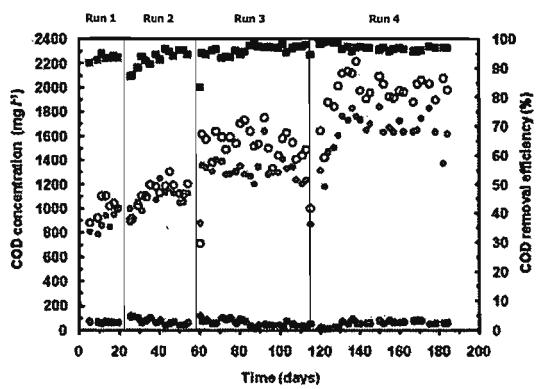


Fig. 3 Changes in COD removal performance: (■) COD removal efficiency; (○) influent COD; (◍) AF effluent COD; (●) final effluent COD.

COD concentration was only 30 mg l^{-1} even though the influent COD was higher than previous periods. This exceptionally high COD removal performance is attributed to the difference in seeding sludge in Run 4. Firstly, the initial amount of sludge for Run 4 was 147 g (dry weight), which was larger than those for other runs. Secondly, the biofilm attached on the BF carrier was separated from suspended sludge in the SB reactor at the end of Run 3, thus all this part of the sludge was inoculated in Run 4, accounting for a ratio of 33% of the total seed biomass. Therefore, the large amount of seed sludge and the high proportion of attached biofilm in the seed sludge enhanced the removal performances during the early period of Run 4. Subsequently, the COD removal efficiencies were greater than 97% with the final effluent COD level below 80 mg l^{-1}.

The SB reactor thus demonstrated high COD removal efficiencies irrespective of OLR, with the highest COD removal rate of 5.9 kg COD m^{-3} d^{-1} occurring at an OLR_{SB} of 6.1 kg COD m^{-3} d^{-1} in Run 4. In a similar study[14] focused on COD removal in a suspended hybrid biological (suspended packing IFAS) reactor treating domestic wastewater, a COD removal efficiency of 83.7% was obtained at an ORL of 3.04 kg COD m^{-3} d^{-1}.

Nitrogen removal performance Nitrogen removal performance is shown in Fig. 4. By calculation[29], it can be inferred that no nitrogen loss due to volatilization of ammonia gas in the AF reactor occurred because of the low pH (5 to 5.5) throughout most of the main experimental period (Fig. 2). TN concentrations in the AF effluent were measured without filtration, so that these values were fluctuated and sometimes higher than the influent TN concentrations. The average TN removal efficiency of the AF reactor was about 3% attributable to bacteria growth requirements. Consequently, the actual NLRs for the SB reactor (NLR_{SB}s) were calculated to be 0.16, 0.28, 0.36 and 0.47 kg N m^{-3} d^{-1} for Runs 1 to 4, respectively.

In Run 1, effluent NH_4-N levels from the AF reactor increased from 50 to 62 mg l^{-1} and effluent NH_4-N levels from the SB reactor were in the range of 10 to 15 mg l^{-1} with about 82% of nitrification efficiency. However, NO_2-N accumulation was observed in the SB reactor with a concentration of 19 mg l^{-1} on day 5 with no NO_3-N detected in the final effluent. Nitrite-oxidizing bacteria (NOB) are more sensitive than ammonia-oxidizing bacteria (AOB) to free ammonia inhibition[29], which could have caused nitrite accumulation during the early period of operation. Free ammonia concentration was estimated to be 0.2 mg l^{-1} on day 5, which would inhibit NOB more than AOB[29]. However, final effluent NO_2-N levels gradually decreased to 1.5 mg l^{-1} by day 17 while NO_3-N levels increased to 14 mg l^{-1}. This result indicated that NOB were gradually enriched. During that period, pH values decreased from 7.6 to 7.3 (Fig. 2), accompanying with a gradual improvement of nitrification. TN removal with an efficiency of 38.5% was observed at the end of Run 1.

Within 5 days after the start-up of Run 2, the TN removal efficiency reached 42.6%.

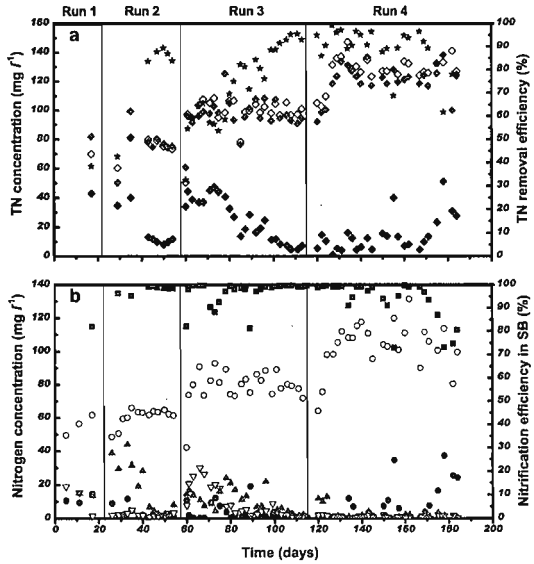


Fig. 4 (a) Changes in TN removal performance: (★) TN removal efficiency; (◇) influent TN concentration; (◈) AF effluent TN concentration; (◆) final effluent TN concentration; (b) profiles of nitrogen concentrations and nitrification efficiency in SB: (■) nitrification efficiency in SB; (○) AF effluent NH_4-N concentration; (●) final effluent NH_4-N concentration; (▽) final effluent NO_2-N concentration; (▲) final effluent NO_3-N concentration.

Then, the efficiencies gradually increased to above 84% from day 43 to the end of this run with final effluent TN levels less than 12 mg l^{-1}. Concurrently, final effluent NH_4-N levels fluctuated around 10 mg l^{-1} during the start-up period, but were always below 3 mg l^{-1} with nearly complete nitrification efficiencies reaching more than 98%. Furthermore, no NO_2-N accumulation in the SB reactor occurred during Run 2. Final effluent NO_3-N levels were about 40 mg l^{-1} during the first 10 days in this run and decreased sharply to below 10 mg l^{-1} afterwards.

In Run 3, influent TN levels were kept low during the start-up period, then increased to about 105 mg l^{-1} on day 60. On day 61, TN removal efficiencies reached 54.6%, while final NH_4-N, NO_3-N and NO_2-N concentrations were 2, 17 and 21 mg l^{-1}, respectively. In addition, NO_2-N accumulation was similar to that observed during the start-up period of Run 1. TN removal efficiencies gradually increased to 82% on day 85. The final effluent NO_2-N levels decreased to 5 mg l^{-1}, while NH_4-N and NO_3-N concentrations oscillated in a range from 1 to 12 mg l^{-1} and 5 to 24 mg l^{-1}, respectively. On day 88, trouble with the air supply resulted in a lack of aeration for a whole night, leading to a sudden increase in NH_4-N to 18.9 mg l^{-1} with a decrease in TN removal efficiency to 71.6%. However, the system recovered quickly and TN removal efficiencies were restored to 84% by day 92, and then reached the highest value of 95% by the end of Run 3.

During the start-up period of Run 4, a TN removal efficiency of 94.9% was obtained in only 5 days of operation. The reasons for this quick response were thought to include the low DO concentration (1.7 to 2 mg l^{-1}) in the SB reactor and the large amount of inoculum applied. From day 125, the influent TN was increased to 130 mg l^{-1}, but TN removal efficiencies were still above 90% and reached 98% on day 127. On days 134 and 155, TN removal efficiencies decreased with final effluent NH_4-N levels increasing to a maximum of 35 mg l^{-1}, though NO_3-N and NO_2-N levels were unchanged (<3 mg l^{-1}). These deteriorations of TN removal were caused by the sludge washout which occurred twice due to deteriorated settling characteristics and pipe clogging. However, these deteriorated TN removal efficiencies were recovered within 2 or 3 days. Owing to temperature decrease (from 26 to 20℃) and sludge washout occurred in the SB reactor, TN removal efficiencies decreased from 90% to 78% from day 175 to the end of this experiment with only leaving NH_4-N in the final effluent. Over the whole period in Run 4 when elevated NH_4-N levels appeared in the SB reactor, final effluent NO_3-N and NO_2-N levels were still maintained below 3 mg l^{-1} (Fig. 4b). These results show that the nitrification process is the rate-limiting step for TN removal in the SB reactor.

Over the course of this study, TN removals mainly occurred in the SB reactor. After the initial phase (Run 1), nitrifying organisms accumulated in the SB reactor and then TN removal efficiencies of 85% to 97% were achieved during steady-state operations in all subsequent runs. During the brief period in Run 4 (days 120 to 171), the highest averaged TN removal rate of 0.43 kg N m^{-3} d^{-1} occurred in the SB reactor.

Sludge characteristics in the SB reactor

Fig. 5 shows the changes in MLSS and SVI in the SB reactor during the main experimental period. MLSS levels gradually increased and reached a maximum of 16,000 mg l^{-1} with a lowest SVI value of about 50 ml g^{-1} by the end of Run 3. This MLSS concentration was several times higher than that in CAS processes and higher than that in most IFAS processes, and comparable to that in some membrane bioreactors (MBRs)[8].

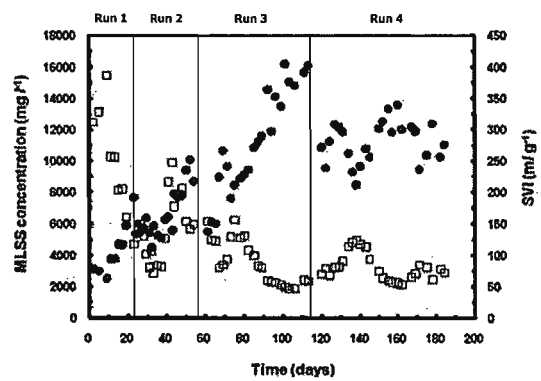
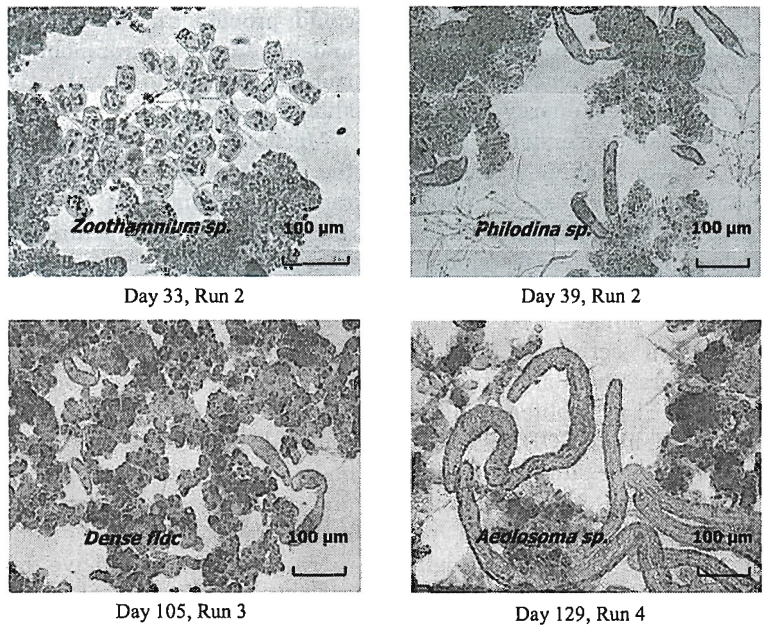


Fig. 5 Profiles of MLSS level and SVI value of sludge in the SB reactor: (●) MLSS; (□) SVI.



Day 33, Run 2 Day 39, Run 2

Day 105, Run 3 Day 129, Run 4

Fig. 6 Microscopic observations of sludge in the SB reactor.

Subsequently, the MLSS oscillated around 11,000 mg l^{-1} during Run 4. Such high MLSS concentrations enabled the SB reactor to remove COD and NH_4-N at extremely high rates.

The low SVI and high MLSS in the SB reactor could be explained by following factors. Firstly, the IFAS process is widely known to enhance sludge settleability[11] as has been verified by Gebara[30]. Secondly, the "swimming motion" of the BF carrier could be inferred to enhance the sludge settleability due to the strong hydraulic shearing force produced by the down-flow water, which caused (1) the biofilm to consolidate or become more densely packed[31], and (2) the detached biofilm and suspended flocs to consistently obtain a uniformed shape[32] as shown in Fig. 6 (day 105). Thirdly, the sludge settleability was improved by enhanced aggregation due to the massive proliferation of rotifiers (*Philodina sp.*)[3] (Fig. 6, days 39 and 105). Lastly, the increment of floc size (Fig. 7) was also in favor of the improvement of sludge settleability.

In addition, the MLVSS/MLSS ratios of aerobic sludge were high (92-94%), which indicates ideal conditions for biological treatment.

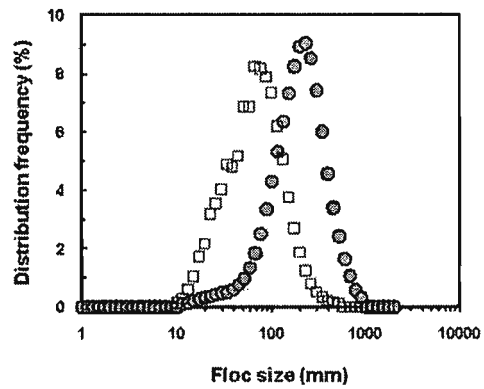


Fig. 7 Floc size distribution of sludge in the SB reactor: (□) Seed sludge, mean size: 76 μm; (●) Run 4, day 149, mean size: 213 μm.

Excess sludge reduction Y_{obs} values in this study were calculated to be 0.16, 0.13, 0.14 and 0.17 kg MLSS kg^{-1} $COD_{removed}$ in the four runs, sequentially. These values were markedly lower than typical values for CAS processes (0.3 to 0.5 kg MLVSS kg^{-1} COD), which is a point of great interest for WWTPs.

One reason for these low observed sludge

yields is regarded to be the microfaunas (protozoa and metazoa) predation on bacteria in the SB reactor. During transfer from a low trophic level to a high level, energy is lost due to inefficient biomass conversion[33]. It is well known that aerobic biofilm processes generally have a more complex microbial ecology than that for activated sludge processes[34]. Therefore, the low excess sludge production for the SB reactor was regarded to be related to the longer food chain compared with that in activated sludge. However, most predators such as protozoa and rotifers were reported to proliferate only in aerobic systems with high COD removal and nitrification efficiencies due to their sensitivity to DO limitation and inhibitory substances such as high concentration of ammonia[11]. Owing to the constantly high COD removals and nitrification efficiencies in the SB reactor, various kinds of protozoa and metazoa appeared in the sludge of the SB reactor. Among these, *Philodina sp.* was predominant in Runs 2 and 3 (6,800 ind. ml^{-1}, on day 113). Especially, *Aeolosoma sp.*, which has more potential for sludge reduction than other predators due to its bigger size[5] was counted in 280 ind. ml^{-1} on day 130 in Run 4. Another reason for the effective reduction of excess sludge in this study is the unique microenvironment of the biofilm attached on the BF carrier. The configuration of the attached biofilm is shown in Fig. 8. The biofilm was very thick, containing black zones in poorly exposed areas due to formation of anaerobic conditions. Thus, the biofilm could provide an integrated aerobic, anoxic and anaerobic environment, allowing various trophic levels to exist, thereby reducing sludge yield[35].

Mechanisms of nitrogen removal via SND in the SB reactor To clarify the nitrogen removal mechanism in this study, the microbial growth was firstly evaluated. The sludge yield and the nitrogen content (about 7%) of sludge were measured, so that the nitrogen removal by cell assimilation was estimated to be about 14% to 18% of the TN removal. Additionally, only 3% of TN was removed in the AF reactor. Therefore, TN removal in our treatment system appeared to be primarily due to simultaneous nitrification and denitrification (SND) occurred in the SB reactor.

Many researchers have reported SND in various kinds of reactors, such as biofilm reactors[18–20] and modified airlift MBRs with continuous aeration rather than intermittent aeration[8, 36, 37] as shown in Table 3. In addition, He et al.[38] investigated the effect of biological factors such as DO, COD/N ratio and pH on SND performance in a membrane bioreactor. Those key factors are discussed below in light of the results observed.

DO level for SND in the SB reactor DO level is regarded to be the most important factor for the occurrence of SND in most studies as shown in Table 3. He et al.[38] also implied that a good SND performance was generally obtained at a DO level of 0.8 mg l^{-1} in a MBR. However, biofilm process could achieve SND at a considerably higher DO

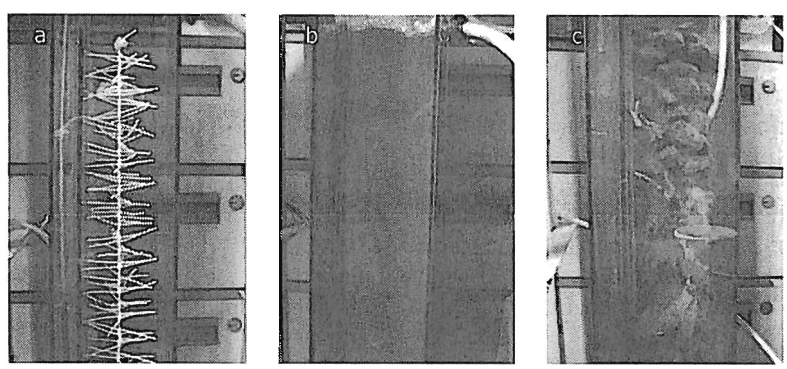


Fig. 8 Photograph of biofilm attached the BF carrier: (a) vacant reactor; (b) running state in Run 3; (c) attached biofilm after suspended sludge removal at the end of Run 3.

Table 3 Comparison of SND performances among different reactor setups

Reactor	Carriers	Influent	COD/N ($g\ g^{-1}$)	TN_{inf}[a] ($mg\ l^{-1}$)	COD removal ($kg\ COD\ m^{-3}\ d^{-1}$)	TN removal ($kg\ N\ m^{-3}\ d^{-1}$)	DO ($mg\ l^{-1}$)	pH	MLSS ($g\ l^{-1}$)	Reference
Moving Bed biofilm	PE balls[b]	Domestic wastewater	9.2	35	0.95	0.13	2	N/M	N/M	19)
Fluidized bed	Rubber[c]	Sanitary wastewater	9	30–45	N/M	0.14	2	7–8	N/M	20)
Immobilized-cell	PVA gel[d]	Beef + peptone	7.5	48	3.37	0.25	2.4–2.7	7.5	5–7[e]	18)
MBR[f]	Fibrous[g]	Glucose + starch + NH_4Cl	N/M	160	N/M	63.6%[h]	0.3[i]	N/M	12.6–13.5	8)
AIC-MBR[j]	None	Sugar + NH_4Cl	9.59	42	0.86	0.073	0.8 (0.4)[k]	7.8	5 ± 0.3	36)
Modified MBR[l]	None	Sucrose + NH_4Cl	9.3	215	1.28	0.13	N/M	7.6–8.5	N/M	37)
Swim-bed	Biofringe	Fermented CSL, VFAs	13–15	80–130	3.9–5.9	0.24–0.43	1.45–3.75	7–7.5	11–16	This study

Superscript: (a) influent TN concentration; (b) polyethylene carriers shaped like small balls with a diameter of 25 mm; (c) fragmentized rubber with a mean diameter of 3mm; (d) phosphorylated polyvinyl alcohol gel beads; (e) VSS (volatile suspended solid) concentration, immobilized on PVA gel beads; (f) internal-loop airlift submerged MBR; (g) fibrous carrier composed of polythene and polyamide; (h) TN removal efficiency; (i) DO level in anoxic zone (47% of the total volume of reactor); (j) airlift internal circulation MBR; (k) 0.8 and 0.4 mg l^{-1} in aerobic and anoxic zones, respectively; (l) airlift internal circulation MBR.
N/M: not mentioned.
The presented results in all literatures and this paper are all the optimal parameters in these studies.

level (1.5 to 3.75 mg l^{-1}, Table 3) due to unique DO gradients in the biofilm.

In this study, for the uniformly distributed aerobic condition in the SB reactor, the average DO concentrations in the bulk liquid were 6.3, 3.75, 2.75 and 1.5 mg l^{-1} during Runs 1, 2, 3 and 4, respectively (Fig. 9). This indicates that the DO levels of the bulk liquid in the SB reactor were much higher than 0.5 mg l^{-1}, which is generally considered to be the limitation for denitrification[19]. However, the thick biofilm attached on the BF carrier (Figs. 1c and 8c) could create a favorable anoxic environment for denitrification.

The DO transfer limitation also might exist in some bigger suspended flocs in the SB reactor. Fig. 7 shows that the mean size of the suspended flocs in Run 4 was 213 μm. Although the average DO concentration in the bulk liquid was around 6.3 mg l^{-1} in Run 1, denitrification occurred with the final effluent NO_3-N below 15 mg l^{-1}. At such a high DO level in the bulk liquid, biofilm could limit DO penetration much more than suspended flocs, so that denitrification might

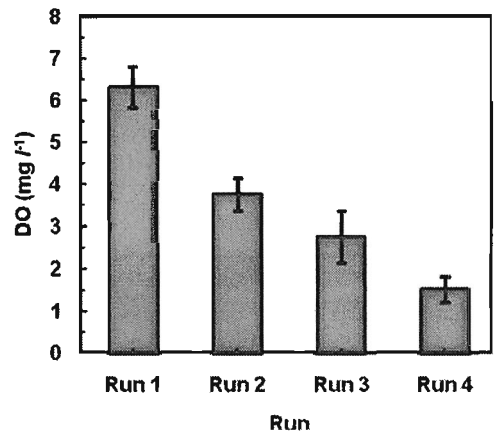


Fig. 9 DO levels in the SB reactor in different runs: bars indicate standard deviation.

occur in the biofilm. Furthermore, TN removal efficiencies deteriorated in Run 4 (days 134 and 155) because of sludge washout and the subsequent sharp decrease in MLSS concentration (Fig. 5). Accompanying the washout of suspended sludge, the TN removal deteriorated owning to the decrease in nitrification capacity rather than

denitrification capacity. Thus, it can be deduced that the suspended sludge was closely engaged in nitrification and denitrification occurred mainly in the biofilm.

pH for SND in the SB reactor The relationship between the TN removal efficiency and the pH is shown in Fig. 10, which indicates clearly that the most suitable pH for SND in this study was 7 to 7.5. This value is a little lower than the reported values shown in Table 3, but very close to the result reported by He et al.[38]. In addition, the alkalinity of final effluent increased due to denitrification (Fig. 2).

COD/N ratio and VFAs for SND in the SB reactor The stoichiometric requirement for conventional denitrification is reported to be 2.86 g COD g^{-1} N[11]. However, previous studies indicated that the suitable COD/N ratio ranged from 7.5 to 9.6 g COD g^{-1} N for good SND performance (Table 3). High COD/N ratio will provide enough electron donors for denitrification, but TN removal will be impaired because the nitrification reaction will be inhibited due to oxygen competition with heterotrophic bacteria[36]. Thus, it would appear that the COD/N ratio (13–15 g COD g^{-1} N) in this study was too high for optimal SND performance; however, this was not the case, apparently due to the good nitrification efficiencies (>98% during steady-state periods) in the SB reactor.

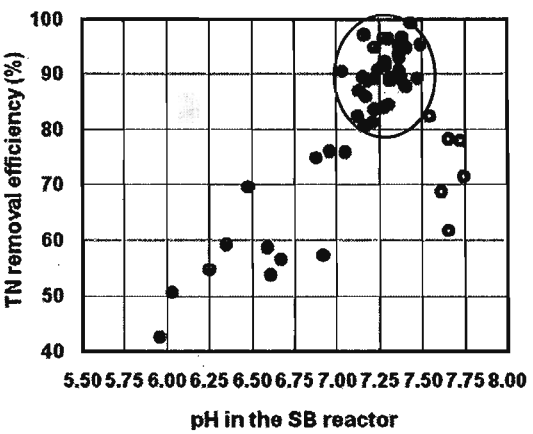


Fig. 10 Relationship between pH in the SB reactor and TN removal: (●) results in normal operation; (○) results in mechanical troubles or sludge washout.

Denitrifying organisms were reported to utilize acetate and propionate as electron donors preferentially[39]. In this study, the main components of the VFAs produced by the AF reactor were acetate and propionate, which were completely consumed in the SB reactor (Table 2). This result could explain high SND performance in the SB reactor.

In addition, Lakshmi et al.[40] reported that the external mass transfer coefficient increased with increase in biofilm thickness, possibly due to the increases in bioparticle diameter and biofilm porosity concurrently. The very thick biofilm attached on the BF carrier could facilitate the mass transfer of substrates. Thus, VFAs produced in the AF reactor and NO_3-N produced in the SB reactor could diffuse into the biofilm and satisfy the growth requirement of denitrifying organisms within it.

CONCLUSION

In this study, an attached growth system was evaluated for carbon and nitrogen removals in treating moderately high-strength synthetic organic wastewater. The results revealed excess sludge reduction of the whole system and SND in the aerobic SB reactor.

(1) The combined system demonstrated 84% to 97% of TN removals mainly via SND under OLRs from 2.7 to 4.5 kg COD m^{-3} d^{-1} and excess sludge productions were from 0.13 to 0.17 kg MLSS kg^{-1} $COD_{removed}$.

(2) The AF reactor contributed hydrolysis and fermentation to the treatment train. VFAs and ammonia produced in the AF reactor were successfully utilized as substrates for the subsequent SND process.

(3) The SB reactor, applied as an IFAS process, demonstrated good treatment performance for COD and TN removals and excess sludge reduction. Maximum COD and TN removal rates of 5.9 kg COD m^{-3} d^{-1} and 0.43 kg N m^{-3} d^{-1}, respectively, were obtained. Moreover, the MLSS concentration in the SB reactor reached a maximum of 16,000 mg l^{-1}.

(4) Nitrification occurred in the SB reactor was mainly carried out by suspended bacteria, while denitrification occurred mainly in the biofilm. The thick biofilm attached on the BF

carrier allowing for good SND performance over a very wide range of bulk DO level (1.5 to 3.75 mg l^{-1}) was considered to be a key factor for SND.

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