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Article

Capacity Enhancement of *Enterobacter aerogenes* for Heterotrophic Nitrification in Integrated Fixed Film Activated Sludge (IFAS) Wastewater Treatment Process

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Abstract. *Enterobacter aerogenes* was reported as a bacterium capable of heterotrophic nitrification. It is typically found that autotrophic nitrification is greater in rate than the heterotrophic nitrification. However, heterotrophs are faster growing and lesser sensitive than the autotrophic nitrification bacteria. In this study, the heterotrophic nitrification of *E. aerogenes* was enhanced with IFAS technology using BioPortz moving media operated at the suspended-growth solids retention time (SRT) of 9 days and temperature of about 28 ^{o}C. The experiments were conducted comparatively in the conventional activated sludge (AS) and IFAS systems containing either mixed culture bacteria or *E. aerogenes* for autotrophic and heterotrophic nitrifications, respectively. The results revealed that *E. aerogenes* could be enhanced with IFAS technology to complete heterotrophic nitrification with the removal efficiency of 100%. There was no significant difference in nitrification between AS and IFAS systems of two microbes if the systems were properly operated. Ammonia stripping was found in the AS systems whenever nitrification was failed and CO_2 was stripped out, resulting in the increase of pH. The clogging of BioPortz media with calcium carbonate precipitates, reducing the IFAS performances, was firstly reported as a result of operating the IFAS systems at the moderate temperature and hardness.

Keywords: Heterotrophic nitrification, *Enterobacter aerogenes*, IFAS, BioPortz, ammonia stripping.

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1. Introduction

Biological ammonium removal process or nitrification in the wastewater treatment system is generally accomplished by autotrophic or heterotrophic nitrification. For autotrophic nitrification, ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) with the nitritation process and nitrite is further converted to nitrate by nitrite-oxidizing bacteria (NOB) with nitratation reaction. Heterotrophic nitrification is not reported to have a significant contribution for nitrification in the wastewater treatment system as compared with the autotrophic nitrification because the heterotrophic nitrification is not conserved the energy for ATP production; therefore, cell yield from the nitrification does not occur [1]. The microbes must oxidize the organics as their energy source for cell growth. Furthermore, the enzymes required for heterotrophic nitrification are probably different from the autotrophic nitrification [2]; however, Robertson and Groffman [3] suggested that the pathway for heterotrophic nitrification is possibly the same as autotrophic nitrification. Ammonia is oxidized to hydroxylamine with ammonia monooxygenes (AMO), which is further oxidized by hydroxylamine oxidase (HAO) to nitrite. Subsequently, nitrite is oxidized by HAO to nitrate [4]. In addition, the heterotrophic nitrification rate is typically lower than the autotrophic nitrification [5-6]. However, bacteria with a capable of heterotrophic nitrification is beneficial to the treatment of wastewater in the competitive systems such as AS process because the competition between heterotrophs and autotrophs for oxygen could be minimized.

E. aerogenes, a facultative heterotrophic bacterium [7] and formerly known as *Aerobacter aerogenes* [8], is capable to perform heterotrophic nitrification under aerobic condition [1, 3]. It was discovered recently that *E. aerogenes* is a bacterium with a capable of degrading acrylamide, which is a carcinogen and hazardous compound [9], at the concentration as high as 5,000 mg AM/L in the culture media [10]. However, Jangkorn et al. [11] reported that *E. aerogenes* in the pilot-scale SBR wastewater treatment system could not degrade acrylamide as much as it was reported due to the inhibition of free ammonia nitrogen (FAN). The FAN was accumulated in the SBR systems because heterotrophic nitrification of *E. aerogenes* was failed as a result of the accumulation of intracellular polyphosphate granules in the cells due to nutritional deficiency and unfavorable environmental conditions [12]. Less energy was available for heterotrophic nitrification when *E. aerogenes* accumulated the polyphosphate granules [12].

To increase the amount of biomass in the wastewater treatment plant (WWTP) without causing overloading problems on the final clarifier, the IFAS technology has been widely used to sustain biomass for enhancement of nitrification at low temperatures [13-14] and to enhance capacity and stability of activated sludge system [15]. The IFAS technology is a hybrid system containing both suspended-growth and attached-growth biomasses. It can reasonably be hypothesized that if *E. aerogenes* could be cultured in the IFAS wastewater treatment system even though the heterotrophic nitrification rate is lower than the autotrophic nitrification, the capacity of WWTP system for heterotrophic nitrification could be enhanced. The AM biodegradation in the IFAS SBR system could be increased.

In this study, the heterotrophic nitrifications of *E. aerogenes* in the SBR wastewater treatment systems operated with the conventional AS and IFAS modes under aerobic condition were comparatively evaluated and the results were compared with the autotrophic nitrifications of mixed culture bacteria in the similar systems.

2. Materials and Methods

2.1. Reactor Setup and Operation

The experiments were conducted in four bench-scale (10 L) Sequencing Batch Reactor (SBR) wastewater treatment systems, named herein as AS-1, IFAS-1, AS-2, and IFAS-2 as illustrated in Fig. 1, in the Environmental Engineering laboratory (Burapha University, Thailand) at the operating liquid temperature of ~ 28 ^{o}C. The AS-1 and AS-2 systems were operated as conventional activated sludge process, which is the suspended-growth system. For comparison, the IFAS-1 and IFAS-2 were the IFAS process containing both suspended-growth and attached-growth systems. Both IFAS systems were integrated with BioPortz moving media (ENTEX Technologies, Inc., USA) at the filling media fraction of 30% (3 L or 510 media). The BioPortz media is made from the high-density polyethylene (HDPE) with the specific surface area of 576 m^2/m^3 [16] and the specific gravity of 0.96, resulting in the specific surface area of 1.73 m^2 in both IFAS systems. Pure culture of *E. aerogenes* was inoculated into both AS-1 and IFAS-1 systems and the mixed culture

of bacteria was seeded into both AS-2 and IFAS-2 systems. *E. aerogenes* was cultured by following the methodologies as described by Jangkorn et al. [11]. Freeze-dried of *E. aerogenes* was resuscitated in the W-minimal medium to obtain *E. aerogenes* colonies for the incubation period of 48 h [17]. Subsequently, *E. aerogenes* colonies were transferred to sterile liquid culture of W-minimal medium for the enrichment, shaking for 24 hours with the mixing speed of 200 rpm at the temperature of 30 °C. The bacterial suspension was adjusted with a 0.5 McFarland standard [18]. Further enrichment of *E. aerogenes* was conducted in a 3-L reactor fed with synthetic wastewater. Finally, *E. aerogenes* was transferred to 10-L AS-1 and IFAS-1 SBR systems. The mixed culture of bacteria taken from a pilot-scale biological nitrogen removal (BNR) wastewater treatment system located in the same laboratory was seeded to the AS-2 and IFAS-2 systems. Each SBR system was operated with two cycles per day consisting of five operating periods of each cycle (12 h), i.e., 15 min filling, 10 h aerobic reacting, 1 h settling, 15 min decanting, and 30 min idling. Three small air fine stone diffusers were installed into each SBR system to provide the dissolved oxygen (DO) at the concentrations of ~ 6.0-7.0 mg O_2/L and to suspend the fixed film media in the IFAS-1 and IFAS-2 systems. Two air pumps with a capacity of 60 L/min each were used to supply oxygen of which each pump provided oxygen for two SBR systems.

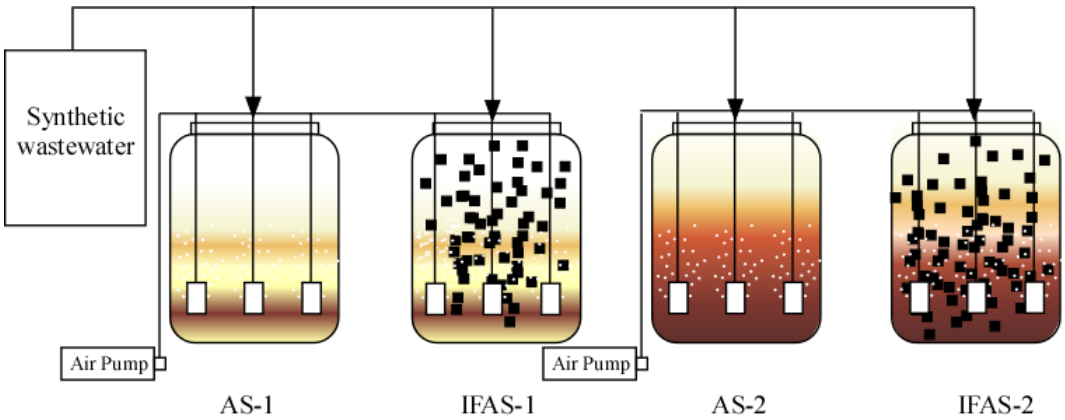

Fig. 1. Four SBR systems containing *E. aerogenes* and mixed culture bacteria operated as AS and IFAS configurations.

All SBR systems were operated at a nominal hydraulic retention time (HRT) of 24 h with the exchange volume ratio of 50%; therefore, the effluent volume of 5.0 L was decanted each cycle. However, the HRT of both IFAS-1 and IFAS-2 systems were reduced to 23 hours at the beginning of experiments as a result of the bulk volume displacement of BioPortz media. The liquid volume displacement of BioPortz media was about 5%; thus, the liquid volume of IFAS systems decreased from 10.0 L to 9.5 L. Furthermore, the suspended-growth biomass was wasted directly from the reactor at the end of reacting period to obtain the operating suspended-growth solids retention time (SRT) of about 9.0 days. The reactor volumes of both IFAS systems were adjusted to take the bulk volume displacement of BioPortz media into account for the SRT calculation. Throughout the studies, all AS and IFAS systems were fed with synthetic wastewater preparing from chemicals as listed in Table 1 dissolved in a 40-L tap water. The wastewater characteristics were as follows: total chemical oxygen demand (TCOD) of about 400 mg COD/L, total kjeldahl nitrogen (TKN) of about 40 mg N/L, pH of 7.2, and total suspended solids (TSS) of 80 mg SS/L.

2.2. Measurement and Analysis

The systems were operated and monitored for about 6 months until the quasi-steady state conditions were achieved. This set of data was referred to as Dataset I. To evaluate the systems at a long operating period under same operating conditions, a set of samples called as Dataset II was collected again after continuously operating the systems for about 10 months. The samples were analyzed for several parameters including mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), TCOD, and Soluble COD (SCOD)(Closed Reflux, Titrimetric Method), TKN (Semi-Micro-Kjeldahl Method), ammonium nitrogen (NH_4^+-N) (Phenate Method), nitrite nitrogen (NO_2^--N) (Colorimetric Method), and nitrate nitrogen (NO_3^--N) (Brucine Method), pH (Cyberscan pH510, Eutech Instruments), and DO (Cyberscan DO110,

Eutech Instruments). These parameters were measured in accordance with Standard Methods for the Examination of Water and Wastewater [19]. For SCOD, ammonium, nitrite and nitrate measurements, the glass membrane filter paper with 0.45- m was used to remove the particulates in samples after centrifuging at 10,000 rpm for 10 min.

Table 1. The compositions of synthetic wastewater in a 40-L tap water.

Chemicals	Chemical Grades and Sources	Amount
Sucrose	Commercial Grade, Wangkanai, Thailand	12.0 g
CH_3COONa	Industrial Grade of 58.8%, Sinoway International, China	24.0 g
K_2HPO_4	Food Grade of 99.2%, Young Jin Chemical, South Korea	2.0 g
KH_2PO_4	ACS Grade, VWR Chemicals, EC	4.0 g
$NaHCO_3$	Food Grade of 99.5%, Tianjin Soda Plant, China	20.0 g
NH_4Cl	Industrial Grade of 99.5%, Tianjin Soda Plant, China	9.0 g
$MgCl_2$	Industrial Grade of 47%, Dead Sea Works, Ltd., Israel	2.8 g
$CaCl_2$	Food Grade of 74.0%, Young Jin Chemical, South Korea	1.6 g

To quantify the amount of attached biomass in the BioPortz media, two BioPortz media were randomly collected from each IFAS system. A high-pressurized water jet made by a syringe connecting with a small pipette tip was used to clean the biomass out from the BioPortz into a beaker. The liquid volume in the beaker was increased to 100 mL with the distilled water. The 5-mL samples were subsequently collected for MLSS and MLVSS analyses. The MLSS and MLVSS concentrations obtained were used to calculate the total attached biomass of the 510-BioPortz media. In addition, equivalent MLSS and MLVSS concentrations of the attached biomass were calculated by dividing the total amount of biomass by the volume of reactor so that both suspended and attached biomasses could be indicated with total MLSS and MLVSS for the comparisons between the conventional AS and IFAS systems.

3. Results and Discussion

3.1. Sludge Productions of *E. aerogenes* and Mixed Culture Bacteria

Four SBR systems were operated under the same experimental conditions until all systems reached the quasi-steady state conditions at which biomass and substrate concentrations were approximately the same over a period of time. At the quasi-steady state conditions of both datasets I and II, samples were collected to determine the amounts of suspended-growth biomass in all AS and IFAS systems and the amounts of attached biomass from the IFAS-1 and IFAS-2 systems. The MLSS and MLVSS concentrations including biofilm density of all SBR systems are listed in Table 2. For dataset I, it appears that MLSS and MLVSS concentrations of the IFAS-1 and IFAS-2 SBR systems containing *E. aerogenes* and mixed culture bacteria, respectively, were lower than the AS-1 and AS-2 systems because the substrates were partially used for the attached growth of microbes in the BioPortz media. As a result of the growth of attached biomass in the BioPortz media, the liquid volume displacement was increased from 5% to 12.8%, resulting in the liquid volume of about 8.3 L and the HRT of about 21 hours in both IFAS systems. If the attached biomass was taken into account for total biomass in the treatment systems, it was found from the total MLVSS in Table 2 that the installation of BioPortz enhanced the amount of biomass in the IFAS systems. In addition, the biofilm density of *E. aerogenes* in the IFAS-1 system was significantly lower than the mixed culture bacteria in the IFAS-2 system because *E. aerogenes* was purely cultured in the system and conducted heterotrophic nitrification; thereby, energy was consumed and cell yield was limited [20]. In contrast, the IFAS-2 contained mixed culture bacteria consisting of heterotrophic and autotrophic bacteria, which could oxidize organics and ammonium as energy sources for the growth; therefore, the growth rates of both bacteria were not limited. Furthermore, the MLVSS/MLSS ratios of *E. aerogenes* from the AS-1 and IFAS-1 systems as shown in Table 2 were relatively high, indicating that the accumulation of intracellular polyphosphate was not accumulated in this study. Jangkorn et al. [11] reported that *E. aerogenes* accumulated intracellular polyphosphate granules when the growth rate of *E. aerogenes* was limited, which could be indicated by low MLVSS/MLSS ratios. For the attached biomass in the BioPortz media, it was found from the dataset I that the MLVSS/MLSS ratios were 0.92 and 0.82 in the IFAS-1 and IFAS-2 systems, respectively, indicating that

both microbes did not accumulate significant amount of inorganic compounds in the cells. It is expected that heterotrophic nitrifications of *E. aerogenes* were substantial in the AS-1 and IFAS-1 systems.

Table 2. MLSS and MLVSS concentrations of *E. aerogenes* and mixed culture bacteria in the AS and IFAS systems.

Dataset	System	MLSS (mg SS/L)	MLVSS (mg VSS/L)	MLVSS /MLSS	Biofilm (g/m^2)	Biofilm MLVSS /MLSS	Total MLVSS (mg VSS/L)
I	AS-1	1033±128	883±92	0.86	-	-	883
	AS-2	1218±60	1010±47	0.83	-	-	1010
	IFAS-1	868±115	773±95	0.90	14.4	0.92	2660
	IFAS-2	780±116	770±116	0.99	23.4	0.82	4825
II	AS-1	913±54	903±54	0.99	-	-	903
	AS-2	855±42	845±42	0.99	-	-	845
	IFAS-1	1233±60	1223±60	0.99	10.9	0..59	3110
	IFAS-2	1668±127	1613±97	0.97	15.0	0.60	4214

Total MLVSS = suspended MLVSS + equivalent MLVSS (biofilm); AS-1 and IFAS-1 contained *E. aerogenes*; AS-2 and IFAS-2 contained mixed culture bacteria.

Subsequently, the quasi-steady state data were collected again after running all systems in parallel for about 10 months after collecting the first dataset. It appears from the experimental data for dataset II in Table 2 that the amounts of suspended-growth biomass in the IFAS-1 and IFAS-2 systems increased significantly as compared with the dataset I. On the other hands, the biofilm densities of IFAS-1 and IFAS-2 in the BioPortz media decreased considerably. It explains from the observations of biomass samples during the MLSS and MLVSS measurements that the biomass contained calcium carbonate scales; thus, the specific surface area inside the BioPortz media for the attachment of microbes must be reduced. The MLVSS/MLSS ratios of attached biomass as listed in Table 2 were 0.59 and 0.60 in the IFAS-1 and IFAS-2 systems, respectively. The total hardness of synthetic wastewater was about 120 mg $CaCO_3$/L. It is generally known that the calcium carbonate solubility product (K_S) decreases with increasing temperature. In addition, the pH values were increased to about 8.5 in all SBR systems due to the stripping of carbon dioxide (CO_2) [21]. It is most likely that hardness precipitated as calcium carbonate in the BioPortz media at the moderate temperature of 28 ^oC and pH of 8.5. As listed in Table 2, the suspended-growth biomass concentrations were much lower in the AS-1 and AS-2 system than in the IFAS-1 and IFAS-2 systems. It can be explained that effective volume of IFAS system was significantly less than the AS system due to the volume replacement of BioPortz media. Grady et al [22] indicated that a small bioreactor volume would contain a higher biomass concentration than the one with larger volume at a fixed SRT, flowrate, and substrate mass removed per unit time.

Finally, it is expected that biomass concentrations were higher than the concentrations listed in Table 2 in all SBR systems at the suspended-growth SRT of 9.0 days. The SRT was carefully controlled for all SBR systems. Biomass were not considerably lost from the effluent of SBR systems. The MLSS concentrations in effluents were about 18.8±4.9, 5.8±4.2, 6.0±4.7, and 6.0±3.6 mg SS/L for dataset I and 5.6±4.8, 13.1±8.1, 1.4±1.4, and 4.4±3.2 mg SS/L for dataset II in the AS-1, AS-2, IFAS-1, and IFAS-2 systems, respectively. The pH was slightly above neutral and the operating temperature was controlled at the moderate temperature of 28 ^oC. It is hypothesized that the synthetic wastewater characteristics in this study limited the growth rates of both microbes, which was probably the alkalinity due to the addition of bicarbonate, or resulted in higher cell maintenances. It was found that the observed yields of microbes were approximately 0.30 and 0.34 g VSS/g COD for dataset I and 0.29 and 0.27 g VSS/g COD for dataset II in the AS-1 and AS-2 systems, respectively. However, the observed yields of biomass in both IFAS systems could not be accurately calculated because the sludge production rate and the COD utilization rate of attached-growth microorganisms on the BioPortz were unknown. It is noted that the observed yield was calculated based on the total suspended-growth biomass production per COD utilization in the AS-1 and AS-2 systems. The suspended-growth biomass produced in the AS-1 and AS-2 systems included both heterotrophs and

autotrophs for the calculations of observed yields because the fraction of heterotrophs and autotrophs was unknown.

3.2. COD Removals of *E. aerogenes* and Mixed Culture Bacteria

Figure 2 illustrates the COD concentrations in the synthetic wastewater at different reacting periods in the AS and IFAS systems containing *E. aerogenes* and mixed culture bacteria during the experimental phases I and II. It is noted that the initial COD concentrations in Fig. 2 were the concentrations after mixing between influent COD and COD in the reactors for a few minutes. The COD removals of all systems were approximately the same between the experimental phase I and II; thus, all data were combined. The COD removal efficiencies of both AS-1 and AS-2 systems were about 78% and both IFAS-1 and IFAS-2 systems were 80%, resulting in the effluent COD concentrations of 90 mg COD/L in the AS-1 and AS-2 systems and about 80 mg COD/L in the IFAS-1 and IFAS-2 systems. It appears that all biodegradable organics in the AS and IFAS systems were removed linearly during the reacting period of 10 hours with approximately the same COD removal rates. The COD removal rates of AS-1, AS-2, IFAS-1, and IFAS-2 systems, which were obtained from the slopes of linear lines in Fig. 2, were 7.4, 7.0, 7.4, and 7.6 mg COD/L-h, respectively. Most organics were removed as the results of the operating suspended-growth SRT of 9 days and moderate temperature of about 28 °C. It is evident that both IFAS-1 and IFAS-2 systems were not superior to the AS-1 and AS-2 systems as a result of additional biomass in the BioPortz media.

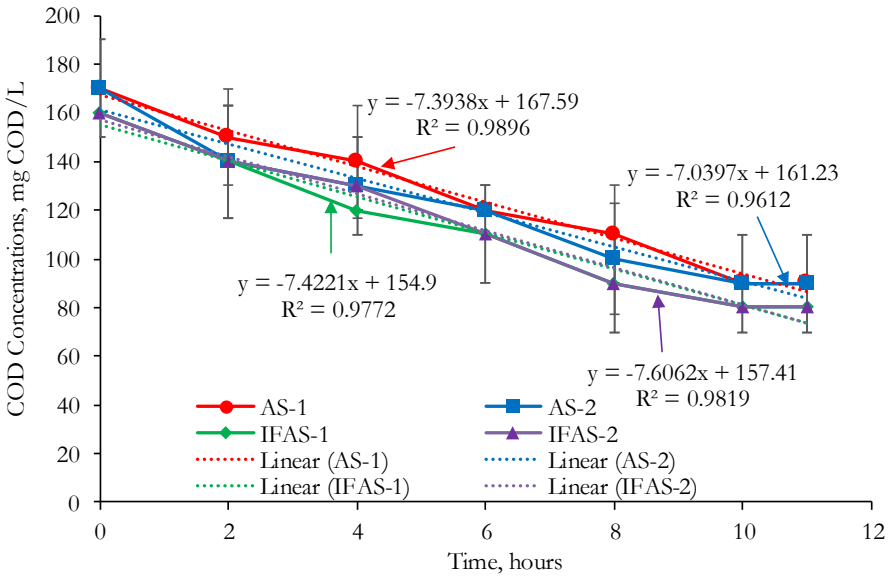

Fig. 2. COD concentration-time profiles of *E. aerogenes* and mixed culture bacteria in the AS and IFAS systems.

3.3. Heterotrophic Nitrification of *E. aerogenes* and Autotrophic Nitrification of Mixed Culture Bacteria

After running several months until the quasi-steady state conditions were achieved in all SBR systems for dataset I, it was found that ammonium removal efficiencies of AS-1 and AS-2 systems were 67.8% and 68.2%, respectively. As illustrated by Fig. 3(a), it appears that the ammonium removals stopped after the reacting period of 6 hours in both AS-1 and AS-2 systems even though the ammonium in the wastewater was not completely exhausted. Figure 4 illustrates the nitrite and nitrate concentrations, resulting from the heterotrophic nitrification of *E. aerogenes* and autotrophic nitrification of mixed culture bacteria in the AS and IFAS systems. As illustrated by Figs. 4(a) and 4(b), the nitrite and nitrate concentrations of both microbes in the AS-1 and AS-2 systems were accumulated minimally in the systems as compared with the ammonium removal efficiencies of about 68%. It was found that the total nitrogen (TN) removal efficiencies of AS-1 and AS-2 systems were 59.3% and 51.2%, respectively. Therefore, it is likely that ammonium nitrogens in

both AS-1 and AS-2 systems were not solely removed by heterotrophic and autotrophic nitrifications, respectively. To verify this assumption, the nitrogen mass balances in the AS-1 and AS-2 systems were calculated and the results are illustrated in Fig. 5. The equations for calculating the nitrogen mass balance in the SBR systems were referred to the methodology of Lee et al. [23] with the nitrogen fraction in the waste sludge of 0.1 g N/g VSS. The total nitrification in the SBR systems was also determined from the mass balance calculations. It was found that the nitrogen mass balances obtained in the AS-1 and AS-2 systems were about 67.3 and 75.6%, respectively. It is evident that certain amounts of nitrogen were lost from the systems. From the calculations, the total ammonium removed from the AS-1 and AS-2 systems were 0.08 g N/cycle, but the oxidized nitrogen (nitrite and nitrate) leaving the AS-1 and AS-2 systems via effluent were only 0.02 and 0.03 g N/cycle or about 25.0 or 37.5%, respectively. It is unlikely to have denitrification in the aerobic zones of both systems in this study because the DO concentrations of about 6-7 mg O_2/L were maintained. Furthermore, *E. aerogenes* has not been reported to denitrify aerobically the oxidized nitrogen. According to the results from the mass balance analysis, it is clear that *E. aerogenes* in the AS-1 system and mixed culture bacteria in the AS-2 system minimally heterotrophically and autotrophically nitrified ammonium nitrogen. It is confirmed that the heterotrophic and autotrophic nitrifications were inhibited. After the investigations, the causes could not be identified. It is presumed that the growths of all bacteria were limited as indicated by low biomass concentrations due to the wastewater characteristics as discussed previously.

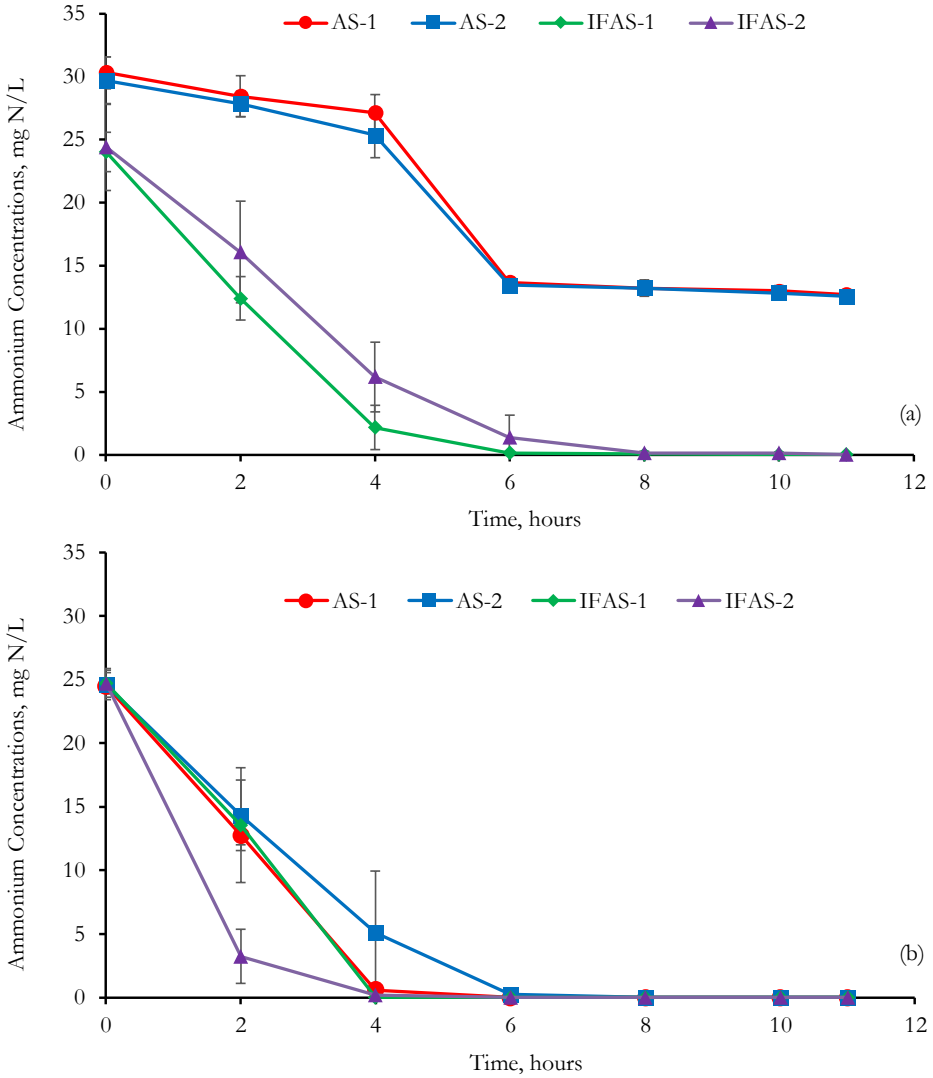

Fig. 3. Profiles of ammonium concentrations versus time of *E. aerogenes* and mixed culture bacteria in the AS and IFAS systems of (a) datasets I and (b) II.

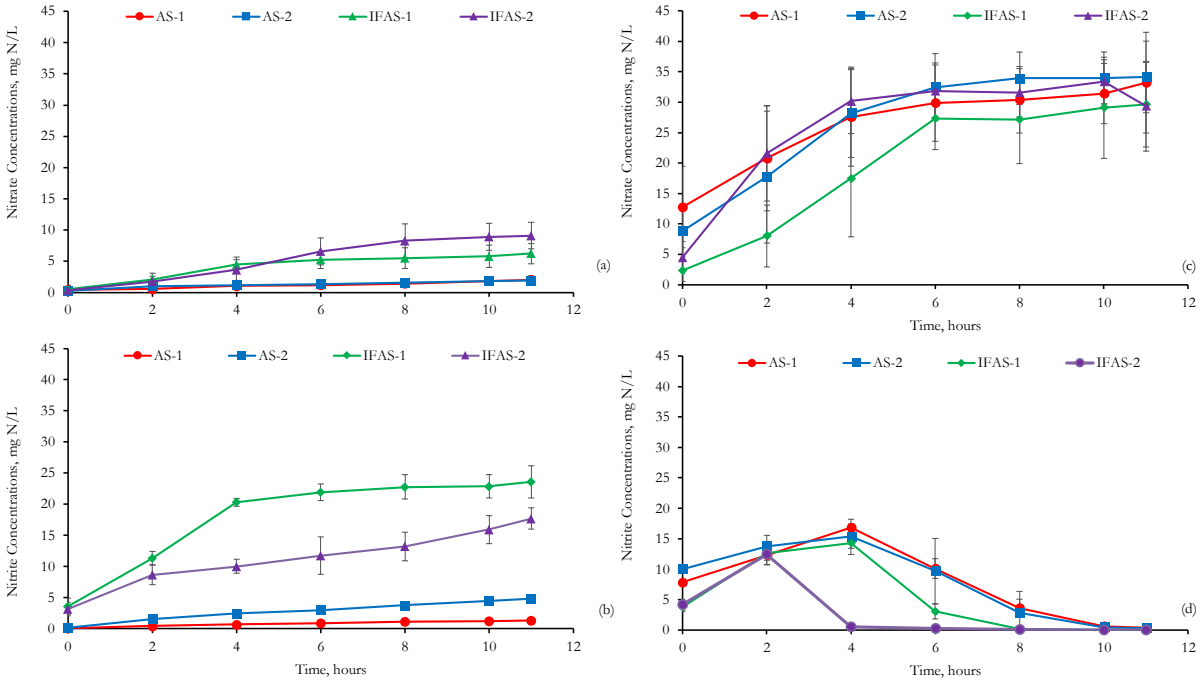

Fig. 4. (a) Nitrate and (b) nitrite concentrations of dataset I and (c) nitrate and (d) nitrite of dataset II of heterotrophic nitrification of *E. aerogenes* and autotrophic nitrification of mixed culture bacteria in the AS and IFAS systems.

Consequently, it was hypothesized that ammonia was removed from both AS-1 and AS-2 systems by using an ammonia-stripping process. According to US.EPA [24], the un-ionized ammonia is about 10.01% of total ammonia species (ammonium and ammonia) at the temperature of 28 °C and pH of 8.20. The pH of wastewater rose from 7.20 to 8.20 due to the failure of nitrification (no acid produced from nitrification) and the stripping of carbon dioxide (CO_2). According to the Henry constants of CO_2 and FAN, CO_2 is stripped faster than the FAN [21]; thus, promoting the raise of pH and resulting in the stripping of free ammonia nitrogen. Therefore, it is reasonable to presume that ammonium nitrogen was stripped out from the solution due to the moderate temperature of about 28 °C, pH of about 8.2, and turbulence of mixing in the systems [21, 25].

In contrast to the AS-1 and AS-2 systems, both IFAS-1 and IFAS-2 systems completely removed ammonium in the wastewater (100% removal efficiencies) during the reacting period of about 6 hours (Fig. 3(a)), suggesting that both heterotrophic and autotrophic nitrifications of *E. aerogenes* and mixed culture bacteria were enhanced in the IFAS-1 and IFAS-2 systems as a result of additional biomass in the BioPortz media. The TN removal efficiencies of IFAS-1 and IFAS-2 systems were 24.6% and 32.3%, respectively, due to the accumulation of oxidized nitrogen. It confirms that *E. aerogenes* was capable of nitrifying ammonium in the biological wastewater treatment systems to nitrite and nitrate nitrogens, respectively [1, 3]. In fact, the heterotrophic nitrification rate as was greater than the autotrophic nitrification, possibly due to the fact that only *E. aerogenes* was grown in the BioPortz media of the IFAS-1 system where as the BioPortz media in the IFAS-2 system contained both heterotrophic and autotrophic bacteria. It is generally known that there is a competition between heterotrophs and autotrophs for oxygen. However, the nitratation process, which converts nitrite to nitrate, of *E. aerogenes* in the IFAS-1 system as illustrated in Figs. 4(a) and 4(b) could not be successfully converted, indicating available energy was likely limited. In addition, it is also possible that oxygen diffusions into the biofilm of IFAS-1 and IFAS-2 systems were limited.

With the assistance of nitrogen mass balance calculations, the total ammonium removals in the IFAS-1 and IFAS-2 systems were 0.16 g N/cycle. It was found that the oxidized nitrogen in the effluent were 0.15 and 0.13 g N/cycle in the IFAS-1 and IFAS-2 systems, respectively. The oxidized nitrogens in the effluents were less than the total ammonium removed from both systems. As indicated by unidentified nitrogen fractions in Fig. 5, it is possible that denitrification in the aerobic zones, resulting from the anoxic zone inside the biofilm, occurred in the IFAS-1 and IFAS-2 systems. However, the denitrification in the aerobic zone of

IFAS-1 was lesser extent than the IFAS-2 as shown in Fig. 5. It is generally known that oxidized nitrogen could be denitrified in the aerobic zone of IFAS systems [13-14] although the DO concentrations were high. The denitrification in the aerobic zone could take place in the BioPortz media containing heterotrophic denitrifiers and oxidized nitrogens. The COD concentrations of IFAS-1 and IFAS-2 systems were lower than the AS-1 and AS-2 systems as shown in Fig. 2, indicating that more COD was utilized due to the denitrification in the aerobic zone. It was of interest to consider the variations in ammonium, nitrite, and nitrate concentrations in the IFAS-1 and IFAS-2 systems as indicated by the standard deviations in Figs. 3(a), 4(a), and 4(b). It is possible that ammonia stripping occurred in the IFAS-1 and IFAS-2 systems, but would be minimal. The pH values of 7.96 and 7.93 in IFAS-1 and IFAS-2 systems were not as high as the AS-1 and AS-2 systems because of acid produced from the nitrification. The amounts of FAN available for ammonia stripping were only 6.01 and 5.64% at these pH values and the temperature of 28 °C [21]. In addition, the mixing was not vigorously found in the IFAS reactors due to the media suspending in the systems. The findings indicated that ammonium stripping minimally involved in the ammonia removal process of both IFAS systems with complete nitrifications.

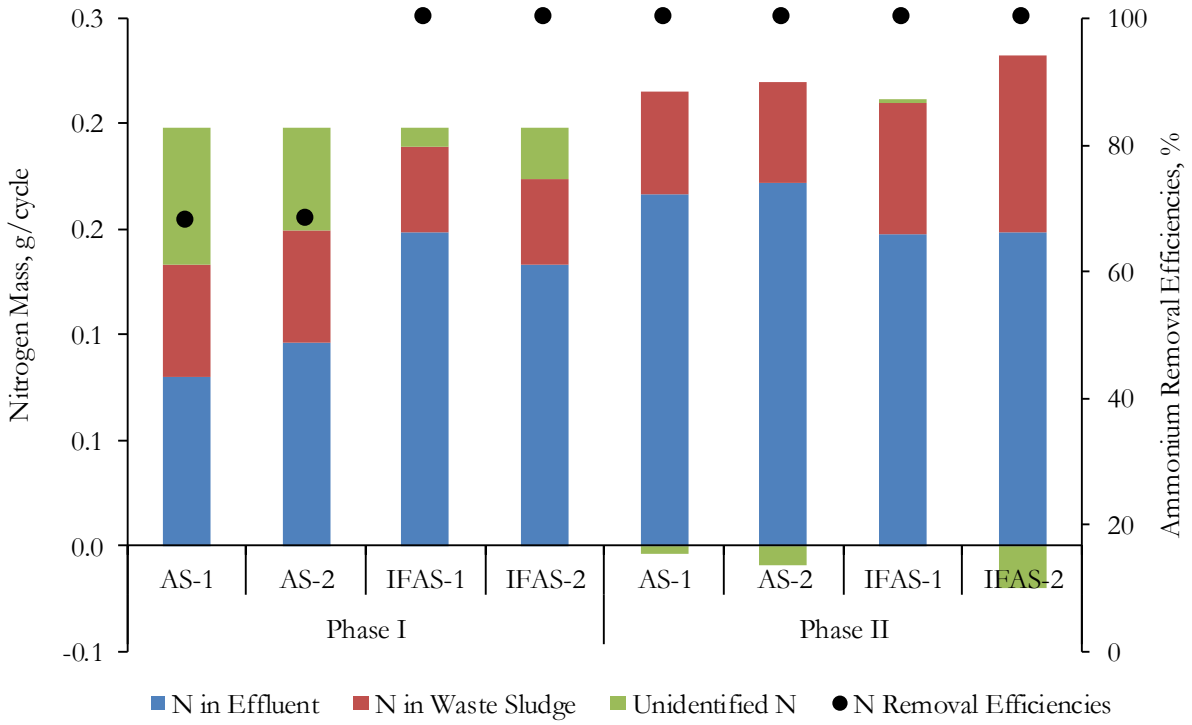

Fig. 5. Nitrogen mass balances and ammonium removal efficiencies of *E. aerogenes* and mixed culture bacteria of datasets I and II in the AS and IFAS systems.

For dataset II, it is interesting to observe that the heterotrophic nitrifications in the AS-1 and AS-2 systems as illustrated in Fig. 3(b) were completed with the ammonium removal efficiencies of about 100% during the reacting periods of 4-6 hours. The TN removal efficiencies of AS-1 and AS-2 systems were 20.4% and 18.5%, respectively. It is suggested that *E. aerogenes* could perform heterotrophic nitrification in equivalent with the autotrophic nitrification of mixed culture bacteria if the system was operated properly and allowed sufficient operating time to achieve the quasi-steady state conditions. From these experimental data, it is possible to postulate that microbial community structure in the AS-1 system might be changed due to the cross-contaminations even though the attempts to avoid the cross-contamination were made in this study by separating all cleaning and operating utilities as well as keeping the systems apart at a certain distance to separate the systems. The microbial structure was not analyzed; however, different in sludge colors were observed between *E. aerogenes* and mixed culture bacteria. *E. aerogenes* was much lighter yellow than the mixed culture bacteria for both datasets. In addition, *E. aerogenes* in the continuous biological wastewater treatment system at the quasi-steady state condition was evaluated so that the possibility to employ *E. aerogenes* in non-axenic conditions of continuous and open biological wastewater treatment system is known.

As illustrated in Figs. 4(c) and 4(d), both nitrite and nitrate nitrogens in both systems increased significantly during the first 4 hours of reacting period, indicating that heterotrophic and autotrophic nitrifications took place in the systems. Subsequently, nitrite in both systems disappeared because ammonium was completely removed during the first 4 hours in both systems and then nitrite was converted to nitrate nitrogen. The variations in ammonium, nitrite and nitrate concentrations were possibly the result of ammonia stripping. The nitrogen mass balances around the AS-1 and AS-2 systems were 101.7 and 104.2%, respectively. The effluent oxidized nitrogen of AS-1 and AS-2 systems were 0.17 g N/cycle, which were approximately equal to the total ammonia removals of 0.164 g N/cycle. The oxidized nitrogen was a little greater than the total ammonia removal because of possible variations in nitrite and nitrate concentrations. It indicates that ammonia stripping was not a significant process when the nitrification was completed even though the pH values were increased to 8.37 and 8.48 in the AS-1 and AS-2 systems, respectively.

During this phase of experiments, it was found that both IFAS-1 and IFAS-2 systems removed ammonium equally as the AS-1 and AS-2 systems due to the relative high SRT and temperature. The TN removal efficiencies of IFAS-1 and IFAS-2 systems were 30.0% and 30.6%, respectively. As a result of limitation of ammonium in the influent, the benefits of IFAS media could not be evaluated during this period. However, it is expected that the benefits of BioPortz media would be minimal in both IFAS systems because both IFAS systems experience the clogging of calcium carbonate precipitates in the BioPortz media. Figures 6(a) and 6(b) reveal the scale deposits in the BioPortz media and in the washed water after cleaning with acid, respectively. It appears that denitrifications in aerobic zone of IFAS-1 and IFAS-2 systems were not observed because of clogging.

(a) (b)

Fig. 6. (a) BioPortz media containing calcium carbonate precipitates after removing biomass from sludge; (b) scale deposits in washed water from BioPortz media after washing with acid.

4. Conclusion

In this study, *E. aerogenes*, which was reported to heterotrophically nitrify the ammonium, was enhanced with IFAS technology to increase its capacity for the heterotrophic nitrification. BioPortz as a moving media was selected to enhance biomass in the IFAS system. The results were compared with autotrophic nitrification of mixed culture bacteria in both conventional activated sludge and IFAS processes. After a long period of system operation, there was no significant difference in the COD removals between IFAS and AS systems containing either mixed culture bacteria or *E. aerogenes* because all systems were operated at the suspended-growth SRT of about 9 days, which was much higher than the minimum suspended-growth SRT for COD removal. In the beginning, both *E. aerogenes* and mixed culture bacteria in the AS systems could not nitrify ammonium at the suspended-growth SRT of about 9 days, whereas the IFAS systems containing both microbes could remove all ammonium in the wastewater, suggesting that heterotrophic nitrification of *E. aerogenes* could be enhanced with IFAS technology. It was found that ammonia stripping occurred in the AS systems when nitrification was failed and CO_2 was simultaneously stripped out, causing the raise of pH. Mixing vigorously with air diffusers could also promote the ammonia stripping. In contrast, BioPortz sustained in the IFAS systems could prevent the ammonia stripping as the results of complete nitrification and reduction of turbulences from mixing. When the AS system containing *E. aerogenes* was operated properly, the heterotrophic nitrification of *E. aerogenes* in the conventional AS system could be completely accomplished and the microbe performed in equivalent with the autotrophic nitrification. In this study, we firstly reported

the clogging problems of BioPortz media in the IFAS system operating at the moderate temperature and hardness due to the precipitation of calcium carbonate.

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