Nitrogen removal and sludge reduction in a symbiotic activated sludge system between anaerobic archaea and bacteria

H.-B. Jun*, S.-M. Park*, N.-B. Park* and S.-H. Lee**

* Department of Environmental Engineering, Chungbuk National University, San 48 Kaeshindong Heungduk-gu, Cheongju, Korea 361-763 (E-mail: *jhbcbe@chungbuk.ac.kr*; *psm37@hanmail.net*; *pnb502@hanmail.net*)

** Department of Civil and Environmental Engineering, Korea Advanced Institute Science and Technology, 373-1, Guseongdong Yuseong-gu, Daejeon, Korea, 305-701 (E-mail: *sanghyung@kaist.ac.kr*)

Abstract The possible symbiosis between bacteria and anaerobic archaea was investigated in intermittent aeration (I/A) systems. Archaea solution added to I/A reactor might play an important role in biological activities as well as in improvement of mineralization of organic matter. I/A reactor with archaea solution (I/A-arch) could increase both nitrification and denitrification rate and also reduce the sludge yield remarkably. These results indicate the possibility of the symbiotic activated sludge system with anaerobic archaea by controlling the DO level in the aeration tank. In this study, DO was controlled by intermittent aeration schemes and a successful symbiotic activated sludge system was achieved to reach the following conclusions. 1) SOUR of I/A-arch system was 2.9 mg-O_2/g-VSS·min. SOUR and nitrification rate of the sludge from I/A-arch was higher than those from the I/A and A/S reactors. 2) Removal efficiencies of organic matter ($TCOD_{Cr}$) in I/A-arch, I/A and conventional activated sludge (A/S) reactors were 93, 90 and 87%, respectively. 3) Nitrification occurred successfully in each reactor, while denitrification rate was much higher in the I/A-arch reactor. Efficiencies of TN removal in A/I-arch, I/A and A/S reactors were 75, 63 and 33%, respectively. 4) Observed yield coefficients of I/A-arch, I/A and A/S reactors were 0.28, 0.41 and 0.37 g-VSS/g-COD.

Keywords Archaea; nitrogen removal; sludge reduction; symbiosis

Introduction

Activated sludge systems and their modifications are still used widely for removal of readily biodegradable organic matter and/or nutrients such as nitrogen or phosphorus in the area of wastewater treatment. However, sudden inflow of toxic compounds or non-biodegradable organic matter may cause failure of the treatment system because of the low population of bacteria capable of degrading these inhibitory compounds. Currently, one of the well-known strategies for upgrading an old system and enhancing its efficiency is bio-augmentation (Stephenson and Stephenson, 1992; Quan *et al.*, 2002). In activated sludge systems, bio-augmentation using the microorganisms cultured with specific substrates has been demonstrated to enhance degradation and removal of man-made products such as phenols, chlorinated solvents and aromatic hydrocarbon.

For remediation of polluted water systems and removal of specific pollutants, bio-augmentation with specialized microorganisms could be a powerful tool to improve the wastewater treatment plant (Quan *et al.*, 2002). However, bio-augmentation does not always work due to a variety of reasons that the survival and maintenance of the added microorganisms in the system require to acclimate to indigenous microbial communities. The added microorganism may be washed out from the system or grazed by protozoa.

The combination of both nitrification and denitrification in single-sludge–activated-sludge systems without a distinctive separate anoxic zone may be explained by the mechanisms of symbiosis between nitrifiers and denitrifiers. Activated sludge flocs can

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contain both aerobic and anoxic zones depending on the DO concentration. Nitrate produced by nitrification in the aerobic zone can diffuse into the inner anoxic zone along with substrates so that denitrification occurs within the floc depth. Simultaneous nitrification denitrification (SND) can be accomplished at lower rates by controlling DO level below 0.5 mg/L in oxidation ditches (Trivedi and Heinen, 2000).

Several researchers proposed, not established yet, the hypothesis that eukaryotes have been originated from metabolic symbiosis between eubacteria and methanogenic archaea (Laura and Katz, 1998; Lopez and David, 1999). Also, many studies demonstrated that diverse microorganisms belonging to bacteria as well as archaea are capable of respiratory denitrification. Laurent (2002) reported the similarity of genes between denitrifier and archaea to suggest that they probably have a common original ancestor before the split of bacteria and archaea. Ratsak (1996) reported that symbiosis between bacteria and protozoa might reduce biomass production and enhance the effluent quality.

Methanogens, a major group of archaea, are strict anaerobes. They are ecologically important prokaryotes in the biodegradation of organic matter in nature and anaerobic digestion. These microorganisms might be also incubated in activated sludge flocs by controlling DO concentration in the range of zero to 0.5 mg/L. There have been a few attempts to apply symbiosis between bacteria and methanogenic archaea to remove nutrients in a single-sludge activated sludge system, however, the symbiotic environment could reduce sludge production via mineralization of the organic matter into CH_4 and CO_2 by methanogens incubated in the core of activated sludge floc.

In this study, the effects of periodic addition of anaerobic archaea to a conventional activated sludge system were investigated on nitrogen removal and sludge reduction, and several biological activities were also tested in the activated sludge system with or without archaea addition.

Material and methods

Domestic sewage

Domestic sewage used in this study was taken from a local wastewater treatment plant. The collected raw sewage was stored below 4°C to prevent it from changing the composition before feeding. Average total COD_{Cr} of influent sewage was about 330 mg/L, while soluble COD_{Cr} was as low as 78 mg/L. Total and ammonia nitrogen were 44 and 33 mg/L, respectively, which resulted in influent C/N (TCOD/T-N) ratio of about 7.5. The other components of influent sewage are shown in Table 1.

Experimental apparatus and operational conditions

Figure 1 shows three sets of laboratory scale completely mixed reactors used in this study.

Table 1 Characteristics of domestic sewage used in this study

Components	Range, mg/L	Average, mg/L
Total Suspended Solid (TSS)	100–500	176
Total COD_{Cr} ($TCOD_{Cr}$)	160–540	330
Soluble COD_{Cr} ($SCOD_{Cr}$)	45–150	78
Total nitrogen (T-N)	25–79	44
Ammonium nitrogen (NH_4^+-N)	21–40	33
Nitrate (NO_3^--N)	0–3	0.1
Nitrite (NO_2^--N)	0	0
Total phosphorus (T-P)	4–10	6.3
Ortho phosphorus (PO_4^{3-}-P)	2–6	3.7
pH	6.9–7.3	7.1
Alkalinity (as $CaCO_3$)	80–180	140

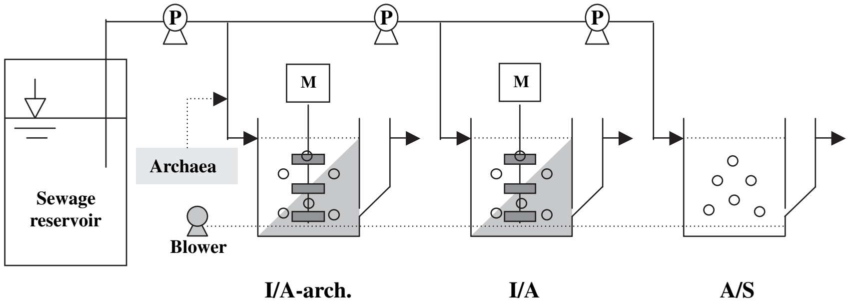


Figure 1 Schematic diagram of I/A-arch, I/A and A/S system used in this study

Table 2 Operational conditions of I/A-arch, I/A and A/S system

Parameters	Intermittent aeration system		Activated sludge system
	I/A-Arch	I/A	A/S
Archaea addition	O	×	×
Flow rate (mL/min)	11.1	11.1	11.1
Working volume (L)	4.0	4.0	4.0
HRT (hr)	6	6	6
SRT (day)	Variable	Variable	Variable
MLSS (mg/L)		3,500	
MLVSS (mg/L)		1,500–2,000	
DO (mg/L) (aerobic condition)	1.0 (at the end of aeration)		1.5–2.0
Archaea solution dosage	4 mL/6 hr	–	–
Temperature (°C)		20 ± 1°C	
Operational mode		Aeration/Mixing: 1 hr/1 hr	Continuous aeration

Working volume of the aeration tank was 4.0 L and a 1.4 L baffled clarifier was attached to separate MLSS from the treated effluent. The first two of the reactors were equipped with both aeration and mixing devices to operate them as intermittent aeration schemes (I/A) and the rest was operated as a conventional activated system (A/S). DO concentration was controlled below 1.0 mg/L after 1 hour aeration for the two I/A reactors, then it was kept below 0.1 mg/L for 1 hour mixing just after aeration stopped. DO concentration in the A/S reactor was about 2.0 mg/L throughout the experimental period. Archaea solution supplied by a local company (Taeheung Co.) was added to an I/A reactor (I/A-arch) once a day in volumetric concentration of 1,000 ppm. The other I/A reactor was operated with the same conditions except addition of archaea solution for comparing the effectiveness of the solution. An A/S was operated to check the baseline performance of the activated sludge system. Hydraulic residence time (HRT) of all reactors was 6 hr, where the treatment capacity was 16 L/day. All reactors were operated at room temperature maintained about 20°C. Sludge yield of each reactor was calculated by monitoring the amount of sludge wasted per day. The amount of waste sludge for each reactor was determined on the basis of MLSS that is adjusted to about 3,500 mg/L. Figure 1 shows the schematic diagram of the I/As and A/S system and operational conditions are summarized in Table 2.

Seed sludge

Seed sludge as indigenous microorganism used in the I/A-arch, I/A and A/S system was taken from a local municipal wastewater treatment plant (city of Cheongju). The raw sludge was sieved and washed with tap water several times to remove inert suspended solids and grits. MLSS in each reactor was about 4,000 mg/L at the start-up of the experiment.

Analysis

Nitrate, nitrite and phosphate were analyzed by an ion chromatograph (Metrohm modular, Switzerland) equipped with A SUPP 4 column (4 mm ID × 250 mm L; Metrohm Ltd., Switzerland). COD_{Cr} was measured by the closed reflux and colorimetric method. Ammonia nitrogen was measured by Nessler method (NH_4^+-N distillation method; HACH Co., USA). The other parameters were analyzed according to *Standard Methods* (APHA, 1995).

Oxygen uptake rate (OUR) was analyzed to compare the autotrophic/heterotrophic activities of sludges in I/A-arch, I/A and A/S reactors. 300 mL BOD bottles equipped with DO meter (YSI 5010, USA) were used for OUR tests with the domestic sewage.

Estimations of population density were measured by heterotrophic plate count (HPC). Pour plate method (*Standard Methods*, 1995) was used to enumerate colonies of anaerobes and aerobes. Cultures of aerobes were incubated and counted on nutrient broth media (Difco Lab.) containing (g/L): peptone 10; NaCl, 5; beef extract, 10; agar 15. Anaerobes were incubated on anaerobic VL (Viande Levure) broth media (Difco Lab.) containing (g/L): tryptose, 10; NaCl, 5; yeast extract, 5; hydrochloride cysteine, 0.6 (Sigma); glucose, 2.5. The final pH of the media was adjusted to 7. The headspace of the anaerobic plate was packed with nitrogen gas and colonies were counted in the anaerobic booth. Aerobes and anaerobes were incubated at 28°C and 45°C for five days, respectively. Population density was expressed in terms of cfu (colony forming unit) per mL.

Results and discussions

Performance of each reactor

Figure 2 shows the performance of TCOD removal throughout the experiment. During the first 30 day operation, settled raw sewage was supplied, after that the raw sewage was supplied directly to the reactors. Before these experiments, I/A-arch and I/A reactors had been continuously operated for another 60 days to reach steady state. The average TCOD removal efficiency of I/A-arch, I/A and A/S reactors were 93%, 90% and 87%, respectively. TCOD removal in I/A-arch was a little higher than other reactors, however, it did not show much difference between the reactors.

Effects of archaea solution on oxygen utilization and nitrification rate

Figure 3 shows the results of oxygen uptake rate (OUR) tests with the activated sludges acclimated to different operating conditions after they reached steady state. The OUR of I/A-arch was higher than other reactors operated without archaea solution. Specific OUR

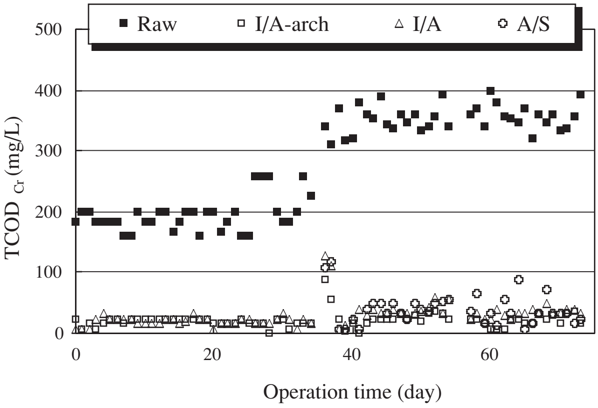


Figure 2 Performance of TCOD removal in each reactor

(SOUR) of I/A-arch was about 2.9 mg-O_2/g-VSS·min, while those of I/A and A/S reactors showed 1.9 and 2.0 mg-O_2/g-VSS·min, respectively. High SOUR means that the sludge incubated with archaea solution has higher activity than other sludges incubated without archaea solution. The higher activity of sludge in I/A-arch reactor would be the result of the symbiotic environment in activated sludge floc.

Table 3 shows the population density of anaerobes and aerobes in each reactor. In the mixed culture of I/A-arch reactor, population density of anaerobes was about 9.6×10^5 cfu (colony forming unit)/mL, while that in the I/A and A/S reactor was 1.9×10^5 and 6.5×10^4 cfu, respectively. From this table, intermittent aeration might provide a good environment for survival and acclimation of anaerobes, such as methanogenic archaea. And addition of anaerobes to the I/A reactor could also increase the population density of anaerobes. High anaerobic population density could enhance heterotrophic activity on the basis of OUR as shown in Figure 3.

On the other hand, population density of aerobes showed a minimum value in the I/A-arch reactor as shown in Table 3. Although the lowest density of aerobes was in the I/A-arch reactor, higher preference for oxygen than the other system implied that anaerobes, such as methanogenic archaea in activated sludge floc could promote the activity of aerobes. From these results, the symbiosis between anaerobic archaea and aerobic bacteria was expected to enhance the activity of the aerobic biocommunity, instead of competition for oxygen or substrate in the activated sludge system. The positive effects of anaerobes on the aerobic activity might possibly be caused by either excretion of certain compounds by anaerobes (methanogenic archaea) stimulating aerobic metabolisms or scavenging non-biodegradable organic compounds such as metabolic byproducts and cell debris by anaerobes. This point of view remains for further research.

Besides OUR, nitrification rate using raw sewage spiked with more ammonia nitrogen was also tested in an aerobic environment. Nitrification rate of the sludge from the I/A-arch reactor was also much higher than that of the sludge from the I/A reactor, and it was even higher than that of the sludge from the A/S reactor as shown in Figure 4. Interesting points

Table 3 Population densities of anaerobes and aerobes in the I/A-arch, I/A and A/S reactors

System	Aerobes (cfu)	Anaerobes (cfu)	Ratio of An./Ae.
Mixed culture of archaea	2.3×10^6	6.2×10^5	2.7×10^{-1}
I/A-arch	9.9×10^6	9.6×10^5	9.7×10^{-2}
I/A	1.5×10^7	1.9×10^5	1.3×10^{-3}
A/S	1.2×10^7	6.5×10^4	5.4×10^{-4}

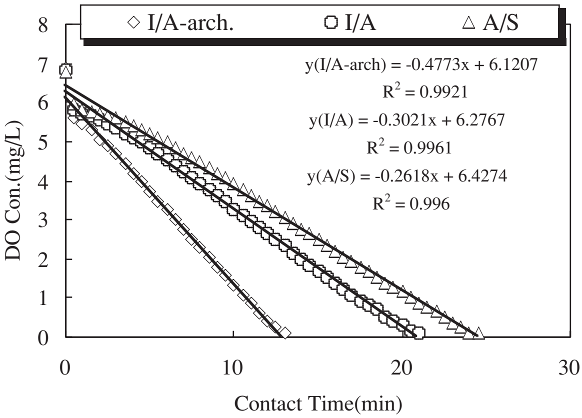


Figure 3 OUR test results with I/A-arch, I/A and A/S sludge

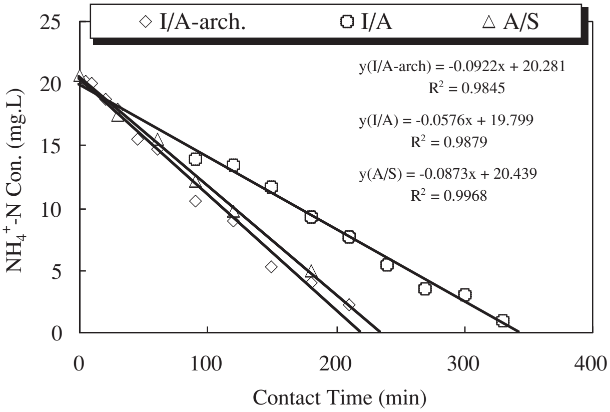


Figure 4 Nitrification rate with I/A-arch, I/A and A/S sludge

of the results are that anaerobes can also enhance the autotrophic activity as well as the heterotrophic one. As the summary of these results, addition of anaerobes (commercial archaea solution) increased the population density of anaerobes in activated sludge floc, which enhanced the biological activity of both heterotrophic and autotrophic bacteria in activated sludge systems.

Effects of archaea solution on the removal of organic matters and nitrogen

Figure 5 shows the archaea solution effects on the removal of organic matters and nitrogen evaluated from the continuous operation of the I/A-arch, I/A and A/S reactor. Removal efficiency of $TCOD_{Cr}$ in each system was 93, 90 and 87%, respectively. TCOD removal in the I/A-arch reactor showed better performance than other reactors and the final effluent $TCOD_{Cr}$ was as low as 21 mg/L.

Figure 6 shows the nitrification efficiency of the 3 reactors. Nitrification occurred successfully in all reactors in a HRT of 6 hr even though the influent ammonia concentration fluctuated widely. Especially, effluent ammonia concentration of the I/A-arch reactor was below 2 mg/L. Addition of anaerobic archaea solution did not inhibit but enhance nitrification efficiency in activated sludge systems. On the contrary to nitrification efficiency, effluent concentration of nitrate showed a different trend in each reactor. Nitrate in effluent from I/A-arch, I/A and A/S reactors was 9, 15 and 24 mg/L, respectively as shown in Figure 7 (a).

In continuous aerobic condition (A/S), nitrate reached as high as 24 mg/L, while in I/A, it was 15 mg/L, which showed the partial nitrate might be denitrified during the anoxic period applied after 1 hr aerobic condition. In the I/A-arch reactor, more nitrates should be

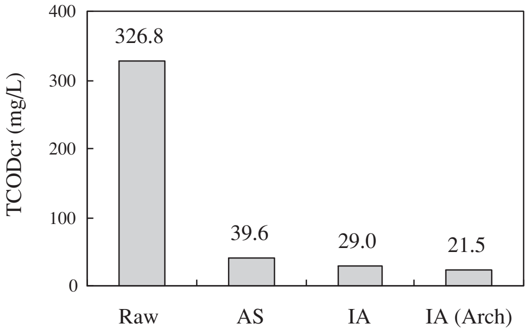


Figure 5 $TCOD_{Cr}$ removal in each system

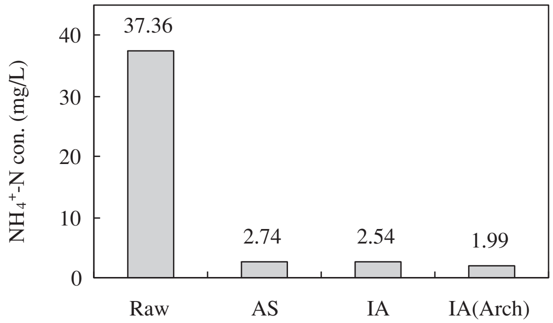
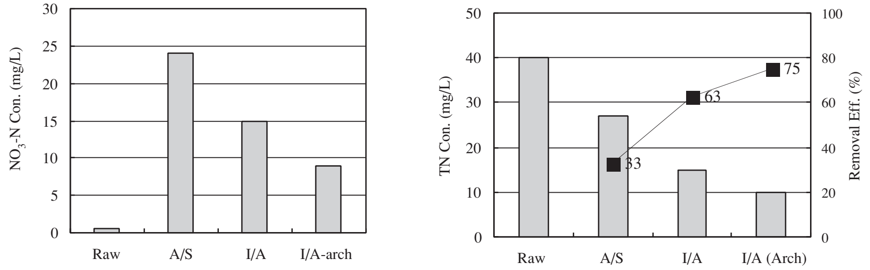


Figure 6 Ammonium removal in each system

denitrified than that in the I/A reactor during the same anoxic period. These results indicate that archaea solution added to activated sludge systems could either increase the denitrification rate or the methanogenic archaea itself reduce nitrate directly in the symbiotic system. Final removal efficiency of total nitrogen was 75%, 63% and 33% in the I/A-arch, I/A and A/S reactors, respectively (Figure 7 b).

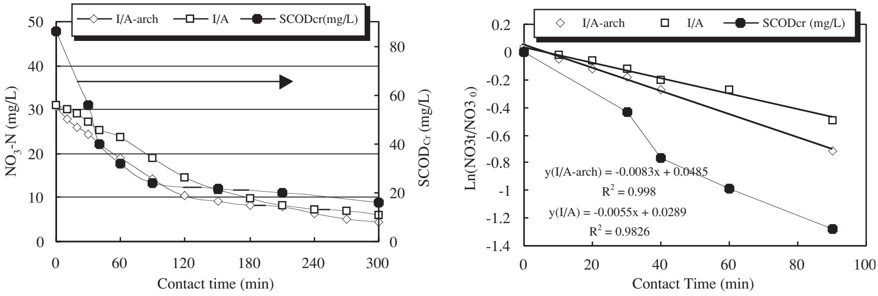
Figure 8 shows the kinetic analysis for denitrification with the raw sewage and sludge from each reactor at steady state. A certain level of nitrate was spiked to the anoxic batch reactors filled partially with raw sewage and settled activated sludge taken from each reactor. Denitrification rate with sludge from I/A-arch reactor was higher than that from I/A. From the results of kinetic analyses, denitrification rate constants in the I/A-arch and I/A system were 0.5 and 0.3 hr^{-1}, respectively as shown in Figure 8 (b).

From the results of both continuous and batch experiments, addition of archaea solution to the I/A activated sludge system could improve the nitrogen removal efficiency. Archaea having the genetic similarity to denitrifiers might play a role in nitrate reduction somewhat



(a) Nitrate (b) TN

Figure 7 Effluent nitrate (a) and TN and its removal efficiencies (b) in I/A-arch, I/A and A/S reactors



(a) Nitrate reduction (b) Kinetics

Figure 8 Results of batch tests for a) denitrification using sewage, and b) denitrification rate in I/A-arch and I/A reactors or promote the activity of denitrifiers. Both archaea inner zone and bacteria outer zone of the suspended microbial floc seemed to form the symbiotic biocommunity that could enhance the denitrification rates at the anoxic stage in the I/A-arch reactor. The I/A activated sludge system operated in this study might provide anaerobic archaea with good conditions for symbiosis with aerobic bacteria.

Effects of archaea solution on biomass yield

To investigate biomass yield of each reactor, observed sludge yield coefficient (Y_{obs}) was evaluated by following simplified equations from net growth rate (r'_g) and substrate uptake rate (r_{su}) with the 30 day-operation results after steady state operation (Metcalf and Eddy, 2003).

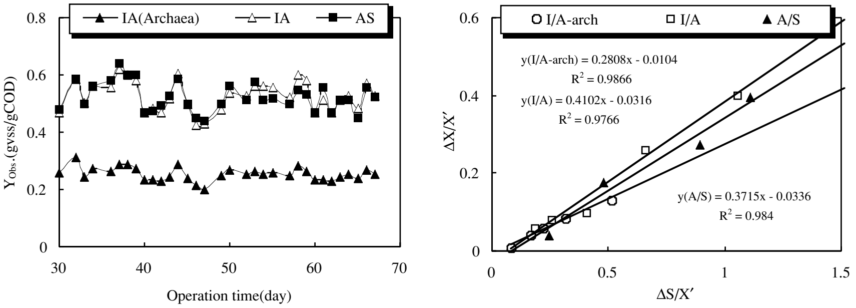
$$Y_{obs} = r'_g/r_{su} = P_x/Q(S_{inf.} - S_{eff.}) \qquad (1)$$

Amount of produced and wasted sludge (P_x) from each reactor was monitored daily, and substrate consumption rate was calculated with the daily operational data of flow rate (Q) and COD (S). Observed yield coefficients of the I/A-arch, I/A and A/S reactors were about 0.26, 0.51 and 0.51 g-VSS/g-COD, respectively (Figure 9 (a)).

Figure 9 (b) shows the batch experiment results with the sludge from each reactor. Five batch reactors for each sludge were filled with same volume of settled sewage and incremental amount of settled sludge, then they were aerated for 24 hours. With the analysis results of both initial and final values of parameters, observed yield coefficient (Y_{obs}) could be evaluated graphically by calculating the amount of increment in biomass and consumed substrate using Eq. (2) as shown in Figure 9 (b).

$$dX/dt = Y_{obs} \cdot dS/dt - k_e X \qquad (2)$$

Y_{obs} of I/A-arch, I/A and A/S reactor were 0.28, 0.41 and 0.37 g-VSS/g-COD_{Cr}, respectively (Figure 9 (b)). With both results of batch and continuous experiments, Y_{obs} of the I/A-arch reactor was much lower than that of the I/A and the A/S reactor. From these results, anaerobic archaea are concerned in sludge yield evidently in activated sludge systems. Sludge reduction in I/A-arch was due to anaerobic conversion of byproducts (some kinds of VFAs) into gases such as methane, hydrogen and carbon dioxide, which prevented yield of activated sludge using these byproducts.



(a) Continuous (b) Batch

Figure 9 Observed yield coefficients (Y_{obs}) of I/A-arch, I/A and A/S reactors in a) continuous and b) batch experiments

Conclusions

The symbiosis between bacteria and anaerobic archaea in intermittent aeration systems could improve nitrogen removal and sludge reduction. Archaea solution added to I/A reactor might play an important role in biological activities as well as in improvement of mineralization of organic matter. The I/A reactor with archaea solution (I/A-arch) could increase both nitrification and denitrification rate and also reduce the sludge yield remarkably. These results indicate the possibility of a symbiotic activated sludge system with anaerobic archaea by controlling DO level in the aeration tank. In this study, DO was controlled by intermittent aeration schemes and a successful symbiotic activated sludge system was achieved to reach the following conclusions.

1. SOUR of I/A-arch system was 2.9 mg-O_2/g-VSS·min. SOUR and nitrification rate of the sludge from I/A-arch was higher than those from the I/A and A/S reactor systems.
2. Removal efficiencies of organic matter ($TCOD_{Cr}$) in the I/A-arch, I/A and A/S reactors were 93, 90 and 87%, respectively.
3. Nitrification occurred successfully in each reactor, while denitrification rate was much higher in the I/A-arch reactor. Efficiencies of TN removal in the I/A-arch, I/A and A/S reactors were 75, 63 and 33%, respectively.
4. Observed yield coefficients of the I/A-arch, I/A and A/S reactors were 0.28, 0.41 and 0.37 g-VSS/g-COD.

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