Biotechnology and Bioprocess Engineering 2006, 11: 538-543 ©KSBB

Nitrate Removal in a Packed Bed Reactor Using Volatile Fatty Acids from Anaerobic Acidogenesis of Food Wastes

Seong Jin Lim[1,2], Yeong Hee Ahn[3], Eun Young Kim[1], and Ho Nam Chang[1]*

[1] Department of Chemical and Biomolecular Engineering, KAIST, Daejeon 305-701, Korea
[2] LG Environmental Strategy Institute, Seoul 120-749, Korea
[3] Department of Environmental Engineering, Dong-A University, Busan 604-714, Korea

Abstract A packed bed reactor (PBR) was fed with nitrate containing synthetic wastewater or effluent from a sequencing batch reactor used for nitrification. The C source introduced into the PBR consisted of volatile fatty acids (VFAs) produced from anaerobic acidogenesis of food wastes. When nitrate loading rates ranged from 0.50 to 1.01 kg N/m^3·d, the PBR exhibited 100~98.8% NO_3^--N removal efficiencies and nitrite concentrations in the effluent ranged from 0 to 0.6 NO_2^--N mg/L. When the PBR was further investigated to determine nitrate removal activity along the bed height using a nitrate loading rate less than 1.01 kg N/m^3·d, 100% nitrate removal efficiency was observed. Approximately 83.2% nitrate removal efficiency was observed in the lower 50% of the packed-bed height. When reactor performance at a C/N ratio of 4 and a C/N ratio of 5 was compared, the PBR showed better removal efficiency (96.5%) of nitrate and less nitrite concentration in the effluent at the C/N ratio of 5. VFAs were found to be a good alternative to methanol as a carbon source for denitrification of a municipal wastewater containing 40 mg-N/L.

Keywords: denitrification, food wastes, nitrate, packed-bed reactor, volatile fatty acids

Direct discharge of wastewater containing nitrogen and phosphorus nutrients into rivers can cause environmental problems such as algal blooms as well as public health concerns [1,2]. Therefore, various biological methods have been developed and employed to remove the nutrients from wastewater [3-9].

Successive nitrification and denitrification is one of the most effective biological processes to remove nitrogenous chemicals from wastewaters. Each of these reactions is performed by two different functional groups of microorganisms [3]. The nitrification process converts ammonium ions to nitrite or nitrate ions under aerobic conditions, whereas the denitrification process reduces nitrite or nitrate ions to nitrogen gas under anaerobic conditions. Generally, wastewater treatment plants employ a heterotrophic denitrification process that uses organic compounds (electron and carbon sources) obtained from external supplementation or the wastewater itself. Phosphorus-removing microorganisms take up phosphorus under aerobic conditions and phosphorus can then be released from the microorganisms under anaerobic conditions when a suitable carbon source, such as acetate or propionate, is available [1].

To facilitate microbial activity with nitrogen and phosphorus nutrients under anaerobic conditions, treatment of wastewaters containing these nutrients requires separation of specific microbial populations into different reactors. One of the most convenient ways to keep specific biomass in different columns has been a biofilm process. Packed-bed reactors (PBR) employing biofilm cells are used most widely for nitrate removal because they can accommodate the appropriate hydraulic retention time required for the biological reaction, and the open water surface in the reactor can be designed to minimize transfer of oxygen by surface aeration [4,7,8].

Since the organic carbon present in wastewater is often limited, a large amount of an external carbon is required for complete removal of nitrate from wastewaters with a high nitrogen concentration. Methanol is commercially used as an electron donor, but the cost may become problematic.

Volatile fatty acids (VFAs) may be an attractive alternative to methanol as an external carbon source for heterotrophic denitrification [10]. VFAs can be produced on-site via anaerobic acidogenesis of organic wastes such as food wastes [11-13]. Representative domestic waste waters generally contain a BOD of 190 and 40 mg/L total nitrogen (organic 15 mg/L, free ammonia and negligible nitrite and nitrate) and are treated using an activated sludge process to yield a final nitrogen concentration of less than 10 mg/L without difficulty [14]. However, typical domestic waste water in Korea contains a BOD of approximately 100 mg/L and a total nitrogen concentration of approximately 35~40 mg/L, which when treated

***Corresponding author**
Tel: +82-42-869-3912 Fax: +82-42-869-3910
e-mail: hnchang@kaist.ac.kr

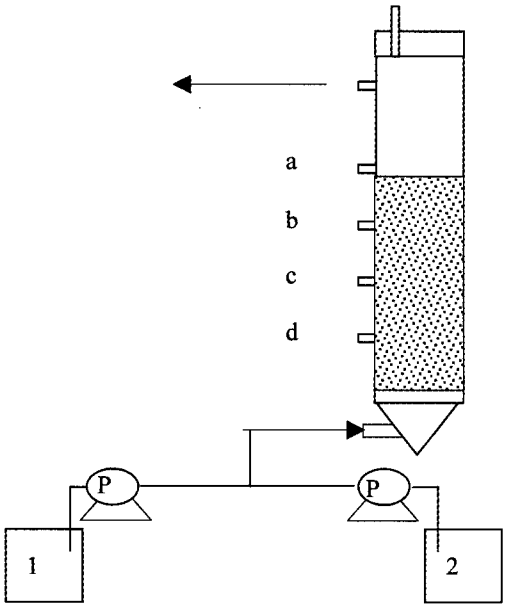

Fig. 1. Schematic diagram of the PBR. 1, Synthetic wastewater containing nitrate or effluent of SBR; 2, C source (acetate or VFA solution); P, pump; a~d, sampling ports.

results in a final nitrogen concentration less than 20 mg/L due to an insufficient amount of external carbon when compared to those of advanced countries such as the U.S.A. Therefore, the Ministry of Environment, Korea set the effluent standard for nitrogen concentration in effluent at less than 20 mg/L in 2008 [15]. However, to avoid eutrophication in lakes and red tides in seas it is necessary to reduce the nitrogen concentration of effluent to less than 10 mg/L.

In this study we will introduce a packed bed reactor (PBR) system for denitrification using VFAs (C source) produced from anaerobic acidogenesis of food wastes [16,17]. Because this technology is designed to reduce the effluent nitrogen concentration rather than treating an incoming waste water (typically 40 mg/L-N), we intend to study effluent from the sequencing batch reactor (SBR) or influent water containing lower levels of NO_2^- or NO_3^- such as 20~30 mg/L to yield final N-concentrations less than 10 mg/L. The performance of the PBR was investigated using different nitrate loading rates and C:N ratios. Nitrate removal efficiency along the reactor bed height was monitored as well.

Fig. 1 shows a schematic diagram of the PBR used in this study. The reactor consisted of a cylindrical acrylic column (4 cm i.d.) 80 cm in length, in which a ceramic medium layer 60 cm in length was installed with the medium size of 4~7 mm. The PBR was maintained at 23.1°C by a heating coil. Inoculum sludge was obtained from a SBR operated for BNR. The PBR was fed with a synthetic wastewater (Table 1) in the upflow mode. The nitrate load was increased stepwise from 0.5 to 1.51 (kg $N/m^3 \cdot d$) by changing the feed flow rate or influent NO_3^--N concentration. Influent flow rates ranged from 10.1 to 16.3 L/d while influent NO_3^--N concentration ranged

Table 1. Composition of the synthetic wastewater used for denitrification in the PBR

Component	Concentration (mg/L)
KNO_3	50~150 as NO_3^--N
$CH_3COONa \cdot 3H_2O$	4 COD per N
KH_2PO_4	20
$MgSO_4 \cdot 7H_2O$	10
$FeCl_3 \cdot 6H_2O$	3
$CaCl_2$	2
$MnSO_4 \cdot H_2O$	5

from 50 to 150 mg/L (Table 2). The synthetic wastewater and carbon source were stored separately and then mixed before they were introduced into the PBR as shown in Fig. 1. The PBR was operated until it reached steady state. Data obtained once steady state was attained are shown in this study.

The SBR was used for phosphate removal and nitrification while the PBR was used for denitrification. The SBR was fed with a synthetic wastewater (Table 3). The effluent from the SBR was supplemented with the VFA solution obtained from anaerobic acidogenesis of food wastes, then introduced into the PBR [16]. The composition of the VFA solution is shown in Table 4. The influent flow rate of the PBR was 16.3 L/d.

The SBR system consisted of a reactor, a controller (for time, solenoid valves, and pH), pumps, ion selective electrodes (DO, pH, and ORP), and tanks as described elsewhere [13]. The reactor was made of an acrylic cylinder (vol. 3 L). Solenoid valves adjusted the gas flow for each cycle according to a time sequence. During reactor operation, the volume of sludge and treated water was kept at 0.5 and 2.5 L, respectively. Solid retention time was 20 days. The SBR was operated at 26°C in a sequence of anaerobic-aerobic phases. The operational cycle for the SBR included 5 min of fill, a 1.5 h anaerobic period with mixing, a 2.5 h aeration period with mixing, 20 min of settling followed by 15 min of decanting, and an 8 min idle period. The oxygen concentration for anaerobic phases was less than 0.2 mg/L, whereas the oxygen concentration for aerobic phases was maintained at greater than 4 mg/L.

For the analysis of NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+, and COD, the samples were immediately filtered through a glass fiber filter (Whatman GF/C). NO_2^-, NO_3^-, NH_4^+, and PO_4^{3-} concentrations and COD were determined using a Hach DR/2010 spectrophotometer system (Hach, Loveland, CO., USA) preprogrammed with calibration data to perform a range of assays using Hach reagents. MLSS (mixed liquor suspended solids) were measured according to the Standard Methods [18]. The pH, ORP, and dissolved oxygen (DO) were measured using ion-selective electrodes (ORION).

The PBR was tested to determine the effect the nitrate loading rate had on nitrate removal efficiency and nitrite accumulation. As the nitrate loading rate was increased

Table 2. Scheme of PBR operation

Influent NO_3^--N conc. (mg/L)	50	50	50	75	75	75	100	100	125	150
Feed flow rate (L/d)	10.1	13.2	16.3	10.1	13.2	16.3	10.1	13.2	10.1	10.1
Nitrate loading rate (kg N/m^3·d)	0.50	0.66	0.82	0.76	0.99	1.22	1.01	1.32	1.26	1.51

Table 3. Composition of synthetic wastewater used for the SBR

Component	Concentration (mg/L)
SCOD*	180
$C_6H_{12}O_6$	40
NH_4^+-N	42.5
PO_4^{3-}-P	5.93
$MgSO_4 \cdot 7H_2O$	50
$FeCl_3 \cdot 6H_2O$	3.75
$CaCl_2$	2.5
$MnSO_4 \cdot H_2O$	5
$NaHCO_3$	102.5

*Carbon source was the VFA solution (Table 4) produced from anaerobic acidogenesis of food wastes.

Table 4. Composition of the VFA solution produced from anaerobic acidogenesis of food wastes

SCOD (mg/L)	39,500 (100%)
Acetate (%, SCOD)	12,900 (32.7%)
Propionate (%, SCOD)	7,580 (23.0%)
Butyrate (%, SCOD)	9,640 (24.4%)
Valerate (%, SCOD)	3,160 (8.0%)
Unidentified (%, SCOD)	4,700 (11.9%)
NH_4^+-N (mg/L)	43.0
PO_4^{3-}-P (mg/L)	72.0

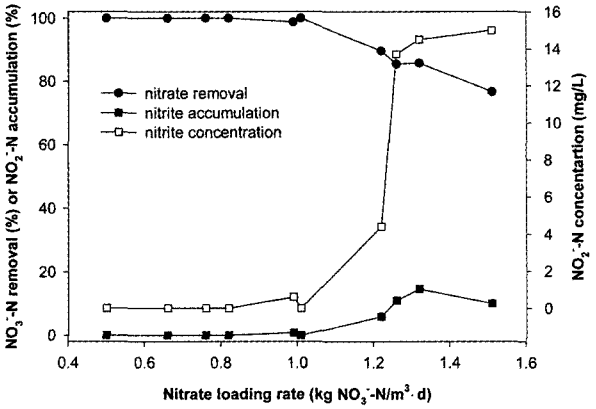

Fig. 2. Effect of nitrate loading rate on nitrate removal and nitrite accumulation in the PBR. Nitrite concentration is measured in the effluent of the PBR.

stepwise from 0.50 to 1.51 kg N/m^3·d, the PBR showed 76.7~100% NO_3^--N removal efficiencies (Fig. 2). When the nitrate loading rate ranged from 0.50 to 1.01 kg N/m^3·d, NO_3^--N removal was in the range of 100~98.8% and the nitrite concentration of the effluent ranged from 0 to 0.6 mg/L NO_2^--N. However, when the nitrate load was greater than 1.01 kg N/m^3·d, NO_3^--N removal was less than 90% and the nitrite concentration of the effluent rapidly increased until the nitrate load was 1.26 kg N/m^3·d, NO_3^--N, then leveled at approximately 14.5 mg/L afterwards. An increased nitrate loading rate has been reported to increase the nitrite concentration of effluent [19]. Additionally, a cumulated nitrite has been shown to inhibit denitrification [20,21].

The PBR was investigated to determine the distribution of nitrate, nitrite and SCOD concentrations along the height of the PBR bed when the influent (100 mg NO_3^--N/L) was supplied at a flow rate of 10.1 L/d. The PBR showed 100 and 94.7% nitrate and SCOD removal efficiencies, respectively, under these conditions (Fig. 3). Most microorganisms in the reactor bed were present in the form of a biofilm. The removal efficiencies of nitrate and SCOD found in the bed suggest that biofilm cells play a major role in the nitrate and SCOD removal activity in the reactor. Most nitrate and SCOD removals were found in the lower part of the reactor bed (Fig. 3). The highest nitrate removal rate was observed a in the bottom 0~15 cm layer of the bed. 83.2% nitrate removal efficiency was observed in the liquid samples obtained from a port located 30 cm (lower 50% of packed-bed height) from the bottom of the reactor.

The nitrate removal result along the bed height agreed with previous reports on nitrate removal efficiency at different bed heights of a PBR [4,21,22]. The observation of more nitrate removal in the lower part of the reactor bed suggests more biomass concentration and/or more microbial activity in that part of the PBR. More biomass concentration was observed in the lower part of a sulfur-packed reactor used for nitrate removal [4] and the biomass distribution also indicated that its nitrate removal activity was found mainly in the lower part. Low concentrations (3~6 mg NO_2^--N/L) of nitrite were detected in the liquid samples obtained from ports located at 15~45 cm bed heights in the PBR but nitrite was not detected in the effluent (Fig. 3).

The C/N ratio for heterotrophic denitrification varies because the C/N ratio required for denitrifying bacteria to completely reduce nitrate to nitrogen gas depends on the nature of the carbon source and the species of bacteria [2]. van Rijn *et al.* [23] supplied acetate at a COD/N ratio of 5.8 for the growth of Pseudomonas stutzeri. Chudoba *et al.* [7] reported that a maximum denitrification capacity (at least 80% denitrification efficiency) was

Table 5. Performance of the BNR system operated in series with a SBR and PBR

Constituent (mg/L)	SBR		PBR (COD/N = 4)		PBR (COD/N = 5)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
SCOD*	180	5	120	18	150	17
NH_4^+-N	42.5	0	0.12	0	0.15	0
NO_3^--N	0	28.6	28.6	3.2	28.6	1.0
NO_2^--N	0	0	0	4.7	0	0.5
PO_4^{3-}-P	5.93	0.12	0.33	0.20	0.38	0.20

*VFA solution in the Table 4.

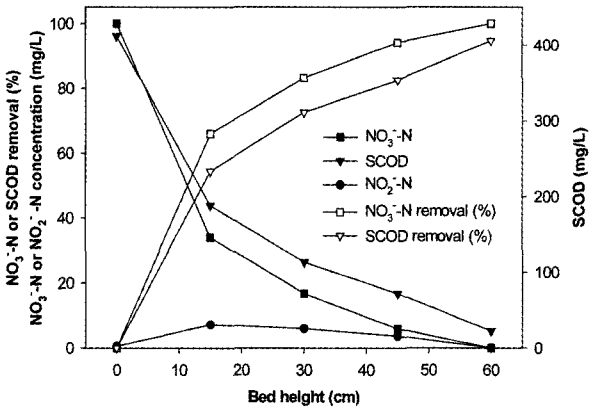

Fig. 3. Concentrations of nitrate, nitrite and SCOD along the PBR bed height. Feed flow and nitrate loading rates were 10.1 L/d and 1.01 kg N/m^3·d, respectively. Influent is indicated as 0-cm bed height.

achieved at a soluble COD/NOx-N ratio of at least 7 during operation of a fixed-film mobile bed bioreactor. In this study, SCOD/N ratios for denitrification ranged from 3.9 to 4.2.

The reported volumetric denitrification rates vary from 2.88 to 100 mg N/L·h [6,9,24]. Conventional BNR process showed 2.88~14.40 mg N/L·h denitrification rates [25]. The volumetric denitrification rate of the PBR observed in this study was 45.8 mg N/L·h. Due to the high denitrifying performance of the PBR, the reactor can offer a highly compact system for denitrification, as much as 2.5 times smaller than that of the anoxic zone of a conventional denitrifying facility employing an activated sludge process.

Table 5 shows the result of the BNR system operated in series with a SBR and PBR. In the SBR system, phosphorous was removed efficiently and nitrification was completed with no accumulation of nitrite. The phosphorous concentration in the effluent of the SBR was consistently less than 0.15 mg/L. Nitrate in the effluent of the SBR was treated by the PBR.

Employing an appropriate C/N ratio is an important factor for heterotrophic denitrification. The PBR was investigated to determine the effect of two different SCOD:N ratios (4 and 5) on nitrate removal. The VFA solution produced by anaerobic acidogenesis of food wastes was used as a carbon source for the PBR. The effluent of the SBR was mixed with the VFA solution and introduced into the PBR for denitrification. When the SCOD:N ratio was 4, NO_3^--N and NO_2^--N concentrations in the PBR effluent were 3.2 mg/L (88.8% removal efficiency) and 4.7 mg/L, respectively (Table 5). When the SCOD:N ratio was increased to 5, the PBR showed a better nitrate removal efficiency (96.5%) and lower nitrite concentration (0.5 mg/L) in the effluent. The results obtained in this study suggest that the PBR is useful to remove not only nitrate but also the VFA solution produced by anaerobic acidogenesis.

Addition of the VFA solution to the SBR effluent slightly increased concentrations of ammonium and phosphate since the VFA solution contains these nutrients as well. However, the increased concentrations did not affect overall nitrogen and phosphorus removal efficiencies of the PBR. This suggests that the nutrients were assimilated by the microorganisms in the PBR and/or that the microbial activity was not inhibited by the concentrations of the nutrient under the PBR conditions.

Recent reviews include cellulosic or chitinoic degradation of biomass for effective utilization by several anaerobic bacteria [26]. Leon and Kumar reviewed biological upgrading of heavy crude oil by enzyme biocatalysts [27]. Tran *et al.* evaluated the ANAMMOX process by granular sludge selected from a digestion reactor in a lab-scale UASB reactor system for 11 months [28]. If the field tests of this work are successful then the problem of denitrification using an additional carbon source such as methanol will be much lower. The authors of this paper are currently studying ways to utilize 4 million tons of food waste (800,000 dry tons/year) for purposes other than soil conditioner or animal feed which have already shown to be of little value. It takes much less time to convert food waste into organic acids than into biogas. Currently, the biggest obstacle is efficient transport of food waste to municipal waste water plants from scattered point sources. Using these methods would allow the required effluent nitrate concentration in Korea to be maintained at 20 mg/L without requiring an additional C source.

A packed-bed reactor (PBR) for denitrification was developed for use in nitrogen removal from municipal waste water effluent. Volatile fatty acids from foodwastes were found to be useful in decreasing the effluent nitrogen concentration to less than 10 mg-N/L. As the nitrate loading rate was increased, the effluent nitrite concentration also increased showing the saturation of removal

capacity when greater than 1.01 kg N/m^3 was loaded. This study shows that VFAs are a good alternative carbon source to methanol for the denitrification process. Using a nitrogen concentration of 100~50 mg-N/L, the PBR showed high removal efficiency in various conditions. The results obtained in this study also suggest that the PBR is useful to remove not only nitrate but also VFAs produced by anaerobic acidogenesis of food wastes.

Acknowledgement This study was supported financially by a Project NM07240 Grant from the Ministry of Commerce, Industry and by Energy, Korea.

NOMENCLATURE

BNR	Biological nutrient removal
COD	Chemical oxygen demand (mg dm^{-3})
DO	Dissolved oxygen
ORP	Oxidation-reduction potential
PBR	Packed-bed reactor
SBR	Sequencing batch reactor
VFA	Volatile fatty acids

REFERENCES

[1] De-Bashan, L. E. and Y. Bashan (2004) Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003). *Water Res.* 38: 4222-4246.
[2] Payne, W. J. (1973) Reduction of nitrogenous oxides by microorganisms. *Bacteriol. Rev.* 37: 409-452.
[3] Khin, T. and A. P. Annachhatre (2004) Novel microbial nitrogen removal processes. *Biotechnol. Adv.* 22: 519-532.
[4] Ahn, Y., W. Park, R. Tatavarty, and I. S. Kim (2004) Comparative analysis of vertical heterogeneity of microbial community in sulfur-packed reactor used for autotrophic nitrate removal. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 39: 1805-1818.
[5] Chang, H. N., R. K. Moon, B. G. Park, S.-J. Lim, D.W. Choi, W. G. Lee, S. L. Song, and Y. H. Ahn (2000) Simulation of sequential batch reactor (SBR) operation for simultaneous removal of nitrogen and phosphorus. *Bioprocess Eng.* 23: 513-521.
[6] Burdick, C. R., D. R. Refling, and H. D. Stensel (1982) Advanced biological treatment to achieve nutrient removal. *J. Water Pollut. Control Fed.* 54: 1078-1086.
[7] Chudoba, P., M. Pannier, A. Truc, and R. Pujol (1998) A new fixed-film mobile bed bioreactor for denitrification of wastewaters. *Water Sci. Technol.* 38: 233-240.
[8] Lemmer, H., A. Zaglauer, and G. Metzner (1997) Denitrification in a methanol-fed fixed-bed reactor. Part 1: physico-chemical and biological characterization. *Water Res.* 31: 1897-1902.
[9] Odegaard, H., B. Rusten, and T. Westrum (1994) A new moving bed biofilm reactor – applications and results. *Water Sci. Technol.* 29: 157-165.
[10] Fass, S., V. Ganaye, V. Urbain, J. Manem, and J. C. Block (1994) Volatile fatty acids as organic carbon sources in denitrification. *Environ. Technol.* 15: 459-467.
[11] Lim, S.-J., D. W. Choi, W. G. Lee, S. Kwon, and H. N. Chang (2000) Volatile fatty acids (VFA) production from food wastes and its application to biological nutrient removal. *Bioprocess Eng.* 22: 543-545.
[12] Choi, D. W., W. G. Lee, S. J. Lim, B. J. Kim, and H. N. Chang (2003) Simulation on long-term operation of an anaerobic bioreactor for Korean food wastes. *Biotechnol. Bioprocess Eng.* 8: 23-31.
[13] Lim, S.-J., R. K. Moon, W. G. Lee, S. Kwon, B. G. Park, and H. N. Chang (2000) Operation and modeling of bench-scale SBR for simultaneous removal of nitrogen and phosphorus using real wastewater. *Biotechnol. Bioprocess Eng.* 5: 441-448.
[14] Tchobanoglous, G., F. L. Burton, and H. D. Stensel (2002) *Wastewater Engineering: Treatment and Reuse.* 4th ed., pp. 186. Metcalf & Eddy, Inc., McGrawhill, USA.
[15] Ministry of Environment. http://me.go.kr.
[16] Chang, H. N., S. J. Lim, W. G. Lee, and D. J. Kim (2005) Method for manufacturing anaerobic fermentation broth from organic wastes for using in nutrient removal. *Korea Patent* 0471963.
[17] Chang, H. N., S. J. Lim, W. G. Lee, and D. J. Kim (2005) Method for biological nutrient removal in effluent from waste water treatment plant using anaerobic fermentation broth from organic wastes and high density microbial cell reactor. *Korea Patent* 0470956.
[18] American Public Health Association (1995) Standard methods for examination of water and wastewater. 19th ed., American Public Health Association, Washington, DC, USA.
[19] Huang, J.-S., J.-J. Her, and C.-G. Jih (1998) Kinetics of denitritification and denitratification in anoxic filters. *Biotechnol. Bioeng.* 59: 52-61.
[20] Almeida, J. S., S. M. Julio, M. A. M. Reis, and M. J. T. Carrondo (1995) Nitrite inhibition of denitrification by *Pseudomonas fluorescens*. *Biotechnol. Bioeng.* 46: 194-201.
[21] Glass, C., J. Silverstein, and J. Oh (1997) Inhibition of denitrification in activated sludge by nitrite. *Water Environ. Res.* 69: 1086-1093.
[22] Jang, A., Y. Ahn, and I. S. Kim (2003) Monitoring the impact of dissolved oxygen and nitrite on anoxic biofilm in continuous denitrification process. *Environ. Monit. Assess.* 87: 133-144.
[23] van Rijn, J., Y. Tal, and Y. Barak (1996) Influence of volatile fatty acids on nitrite accumulation by a *Pseudomonas stutzeri* strain isolated from a denitrifying fluidized bed reactor. *Appl. Environ. Microbiol.* 62: 2615-2620.
[24] Kim, I. S., S. E. Oh, M. S. Bum, J. L. Lee, and S. T. Lee (2002) Monitoring the denitrification of wastewater containing high concentrations of nitrate with methanol in a sulfur-packed reactor. *Appl. Microbiol. Biotechnol.* 59: 91-96.
[25] Kim, E.-W. and J.-H. Bae (2000) Alkalinity requirements and the possibility of simultaneous heterotrophic denitrification during sulfur-utilizing autotrophic denitrification. *Water Sci. Technol.* 42: 233-238.
[26] Ohmiya, K., K. Sakka, and T. Kimura (2005) Anaerobic bacterial degradation for the effective utilization of biomass. *Biotechnol. Bioprocess Eng.* 10: 482-493.

[27] Leon, V. and M. Kumar (2005) Biological upgrading of heavy crude oil. *Biotechnol. Bioprocess Eng.* 10: 471-481.
[28] Tran, H.-T., Y.-J. Park, M.-K. Cho, D.-J. Kim, and D.-H. Ahn (2006) Anaerobic ammonium oxidation process in an upflow anaerobic sludge blanket reactor with granular sludge selected from an anaerobic digestor. *Biotechnol. Bioprocess Eng.* 11: 199-204.

[Received July 14, 2006; accepted November 27, 2006]