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Biodegradation of monoethanolamine in aerobic and anoxic conditions

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Abatract−Monoethanolamine (MEA) is widely used in many industries and its proper treatment is important for protecting the water environment. As MEA contains an amine group, nitrogen removal by nitrification/denitrification as well as biodegradation of MEA is necessary for wastewater treatment. In this study the effects of adaptation and inhibition of MEA on biological degradation, and the removal of amine were investigated in a laboratory scale sequencing batch reactor (SBR). In addition, the denitrification characteristics of nitrate, and nitrite with MEA as the electron donor, were compared to the other electron donor (acetate). In the aerobic SBR, the removal efficiency of 9,000 mg/L MEA reached 92% at the hydraulic retention time (HRT) of 10.5 days. Ammonium hydrolyzed from the MEA was nitrified after 8 weeks from the start-up showing that adaptation time is needed for nitrification. Non-linear curve fitting of the specific MEA biodegradation gave the maximum specific activity (V_{max}), the half saturation constant (K_s), and the inhibition constant (K_i) of 2.81 g/(g VSS·d), 102.1 mg/L, and 1149.6 mg/L, respectively. Batch denitrification showed that MEA is a competitive electron donor to acetate.

Key words: Denitrification, Monoethanolamine, Nitrification, Sequencing Batch Reactor (SBR), Substrate Inhibition

INTRODUCTION

Monoethanolamine (MEA) is one of the most widely used alkanolamines for removing sour gases (e.g., H_2S and CO_2) from natural gas during refining in the so-called 'sweetening process' [1]. As it can absorb CO_2 from combustion gases, MEA has gained attention for the abatement of greenhouse gases [2,3]. Although MEA is recovered in the processes by stripping and distillation, irreversible degradation may also occur, resulting in products from which the MEA is not easily recovered [4,5]. Purge of contaminated solution and make up with fresh MEA reduces the impurity levels for effective performance.

MEA is often used for alkalinization of water in steam cycles of power plants, including nuclear power plants with pressurized water reactors to control corrosion of metals. The water/steam circulation system of nuclear power plants and thermal power stations uses an ion-exchange resin column to capture MEA, and it is released into the wastewater through a resin regeneration process. The wastewater from the purged solution and the regeneration plant contains high concentration MEA that must be appropriately treated to meet the environmental discharge standard. Wastewater from TFT-LCD (thin film transistor liquid crystal display) and PDP (plasma display panel) manufacturing plants also contains as much as 1,000 mg/L of MEA [6]. The disposal of the MEA containing wastewater is a problem because MEA cannot be easily treated in wastewater treatment systems due its toxic effect and slow biodegradability [7].

MEA is biodegraded by a process that involves the hydrolysis to ammonium and acetaldehyde [8,9]. Activated sludge and pure culture (*Pseudomonas aeruginosa*) have been used and compared for MEA degradation at different MEA concentrations under aerobic conditions [10]. Other bacterial cultures have also been investigated for the degradation of amine compounds such as diethanolamine [11], monoethylamine [12], and MEA [13] in aerobic and anoxic conditions.

For the complete degradation of MEA, ammonium hydrolyzed from MEA needs to be nitrified to nitrite (NO_2^--N)/nitrate (NO_3^--N) and denitrified to nitrogen gas (N_2) by the help of electron donors. MEA can be an electron donor for the denitrification of nitrite/nitrate if MEA is not fully oxidized in the aerobic wastewater treatment. When both ammonium and easily biodegradable organics are present in the wastewater, organic compounds are oxidized first before the ammonium oxidation (nitrification). Therefore, pre-denitrification is preferred to save the cost of the electron donors for denitrification.

The objectives of this study were to investigate the effects of adaptation and inhibition of MEA on biological degradation because it is not well studied in the wastewater treatment with activated sludge. The fate of ammonium from MEA was also investigated. In addition, the denitrification characteristics of nitrate and nitrite with MEA as the electron donor were compared to another electron donor (acetate). For this purpose, a laboratory scale sequential batch reactor (SBR) was used for the aerobic biodegradation of MEA and batch experiments were carried out for the denitrification by MEA and acetate.

MATERIALS AND METHODS

1. Aerobic Biodegradation of MEA in an SBR

A laboratory scale SBR with a working volume of 3 L (height: 55 cm, diameter: 10 cm) was used to investigate the biodegradation characteristics of MEA in aerobic condition. The reactor was operated in a sequencing batch mode for a cycle of 7 days (filling: 1 hr,

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reacting: 164 hr, settling: 1 hr, drawing: 1 hr, and idling times: 1 hr) by decanting and replacing 2 L of the wastewater with fresh wastewater (hydraulic retention time=10.5 days). Air was supplied through a diffuser and the flow rate to the reactor was 0.5 L/min to maintain a dissolved oxygen concentration of about 2 mg/L. The reactor temperature was kept at room temperature (22-26 ºC) and the pH was controlled at 7.5±0.1 by the addition of 0.1 N HCl or 0.1 N NaOH. The MEA wastewater composition was as follows: MEA (1,500-9,000 mg/L), $MgSO_4 \cdot H_2O$ (50 mg/L), KCl (50 mg/L), $NaHPO_4 \cdot 12H_2O$ (200 mg/L), $CaCl_2 \cdot 2H_2O$ (100 mg/L), KH_2PO_4 (200 mg/L), $FeCl_3 \cdot 6H_2O$ (50 mg/L). Activated sludge from a municipal wastewater treatment plant was used for inoculation and the initial mixed liquor volatile suspended solids (MLVSS) was 500 mg/L. The specific MEA degradation rate was calculated from the MEA degradation rate and the MLVSS at the MEA concentration of 1,000 to 6,000 mg/L.

2. Denitrification with MEA as an Electron Donor

For the batch denitrification experiment, an excess amount of carbon source (MEA and acetate) was provided to denitrify 200 mg N/L of nitrite/nitrate under the anoxic condition at the MLVSS concentration of 500 mg/L. MEA, acetate, and MEA+acetate were provided as the carbon source with the concentrations of 2,000, 1,970, and 1,000+985 mg/L, respectively. The initial pH was adjusted to 7.4-7.5 and the flasks were incubated in a shaking incubator (150 rpm, 30 ºC) for 3 days. MEA was provided to find its availability as an electron donor for denitrification. Since acetate is the degradation intermediate of MEA, and it is one of the most common electron donors for denitrification, the mixture of MEA and acetate was supplied to the flask to check the inhibition effect and preference of MEA for denitrification.

3. Analytical Methods

MEA and NH_4^+-N were measured by cation chromatograph (CD-5, Shodex) with YK-421 column (Shodex). Nitrite, nitrate, and acetate were measured by anion chromatograph (DX 500, Dionex) with IonPac AS-11 column (Dionex). Chemical oxygen demand (COD) was measured after filtering the wastewater through GF/C (Whatman) by the closed reflux colorimetric method with $K_2Cr_2O_7$ [14]. Temperature and pH were measured by a pH meter (pH-200L, iSTEK). Dissolved oxygen concentration of the SBR was measured periodically by a DO meter (DO-300L, iSTEK) throughout the experiment. The bacterial cell mass was measured as the MLVSS using the procedure of Standard Methods [14].

RESULTS

1. Aerobic Biodegradation of MEA and Nitrification

Wastewater containing 9,000 mg/L MEA was aerobically treated in the SBR. Fig. 1(a) shows a cycle of SBR after 3 weeks of start-up. As two-thirds of the treated wastewater was decanted and replaced by the fresh wastewater at the end of each SBR cycle, the initial MEA concentration of the SBR was reduced to around 6,000 mg/L. MEA was actively degraded to reach 600 mg/L in 7 days with the removal efficiency of 92% and the degradation rate slowed down after 3 days. COD also showed the same behavior with MEA even though they had higher values than MEA. On the other hand, NH_4^+-N concentration increased sharply from 460 mg/L to 1,025 mg/L for 2 days, and then it reached 1,130 mg/L in 7 days. Nitrification did not occur by observing steady NH_4^+-N concentration and low levels of nitrite and nitrate in the wastewater. Only a small amount of nitrite and nitrate (<20 mg N/L) was found at the end of the SBR cycle.

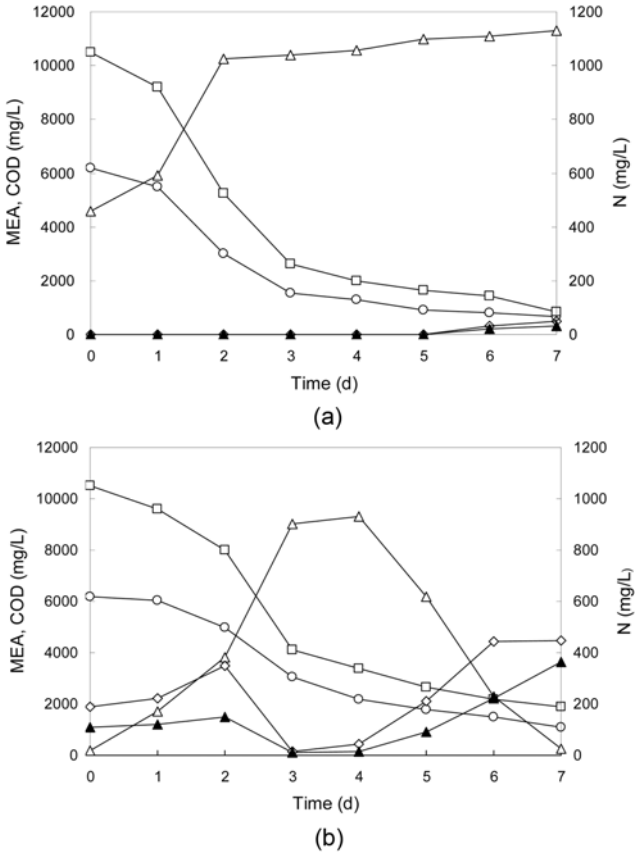

Fig. 1. Aerobic biodegradation of monoethanolamine in a SBR. (a) After 3 weeks, (b) After 8 weeks of the SBR start-up (○: MEA, □: COD, △: NH_4^+-N, ◇: NO_2^--N, ▲: NO_3^--N (mg/L)).

Fig. 1(b) shows the degradation of MEA in the SBR after 8 weeks of start-up. The degradation trends of MEA and COD were similar to the cycle of Fig. 1(a) except for the profiles of nitrogen compounds. As MEA concentration decreased, ammonium concentration increased for the first 4 days and then decreased close to zero at the end of the cycle. Different from Fig. 1(a), nitrification activity was significant at the latter half cycle of the SBR because most of the ammonium was oxidized to nitrite and nitrate. From Fig. 1(a) and (b), the ammonium hydrolyzed from MEA was finally oxidized to nitrite and nitrate by nitrifying bacteria, but it needed time from the start-up to activate or adapt the nitrifying bacteria to MEA.

2. Substrate Inhibition of MEA

For the analysis of the inhibition effect of MEA, the initial MEA concentration in the SBR was gradually increased from 1,000 mg/L to 6,000 mg/L by an increment of 1,000 mg/L. Specific MEA degradation activities of each MEA concentration were calculated from the MEA degradation rate and the MLVSS concentration. Table 1 shows the specific MEA degradation activity at various MEA concentration. Haldane model [15] was used to estimate the kinetic parameters because MEA acts as an inhibiting substrate [16].

Table 1. Specific MEA biodegradation activities measured at various MEA concentrations

MEA (mg/L)	Average MLVSS* (mg/L)	Specific activity (g/g MLVSSd)
1000	265	1.42
2000	250	1.09
3000	390	0.79
4000	595	0.66
5000	755	0.56
6000	490	0.34

*Average MLVSS was calculated from the average value of initial and final MLVSS of each SBR cycle

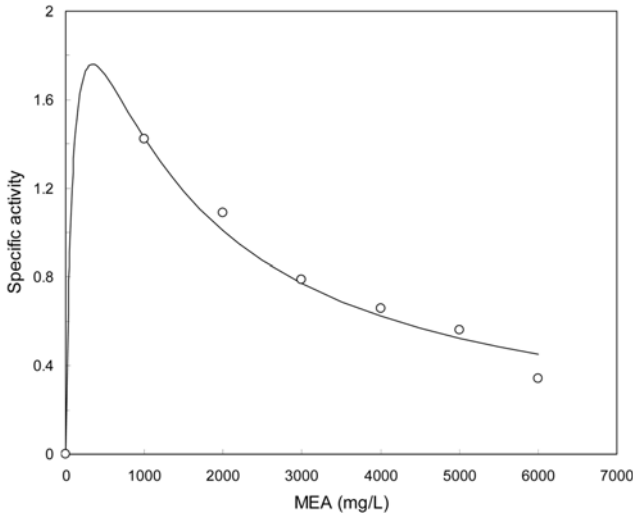

Fig. 2. Curve fitting of the specific monoethanolamine degradation activities at various concentrations. Circle (○) represents experimental data and the solid line represents the values estimated from the Haldane model.

$$v = \frac{V_{max} S}{K_s + S + S^2/K_i} \quad (1)$$

Fig. 2 shows the specific MEA degradation activities at different MEA concentrations and the regression curve. From the non-linear curve fitting of the specific MEA biodegradation, the maximum specific activity (V_{max}), the half saturation constant (K_s), and the inhibition constant (K_i) were 2.81 g/(g VSSd), 102.1 mg/L, and 1,149.6 mg/L, respectively, which were estimated by the Polymath 5.1 software (R^2=0.98). The analysis clearly showed that MEA has an inhibitory effect on its biodegradation at an MEA concentration higher than 1,000 mg/L.

3. Utilization of MEA as an Electron Donor for Denitrification

Ammonium released from the hydrolysis of MEA is oxidized to nitrite/nitrate and denitrified for the complete removal of nitrogen compounds. To determine whether MEA is suitable as the electron donor for denitrification, a batch experiment was carried out with nitrite and nitrate. Before the denitrification with MEA, the sludge from the SBR was incubated for 3 days with acetate as the electron donor to check the denitrifying activity of the sludge. Nitrite and nitrate were completely denitrified with the consumption of acetate (data not shown). From the stoichiometry Eq. (6) of the nitrate reduction in the discussion, 200 mg/L of NO_3^--N requires 745 mg/L of acetate. Therefore, the NO_3^--N (200 mg/L) was completely denitrified as we provided acetate in excess (1,970 mg/L). It proved the presence of denitrifying bacteria and their denitrifying activity in the SBR.

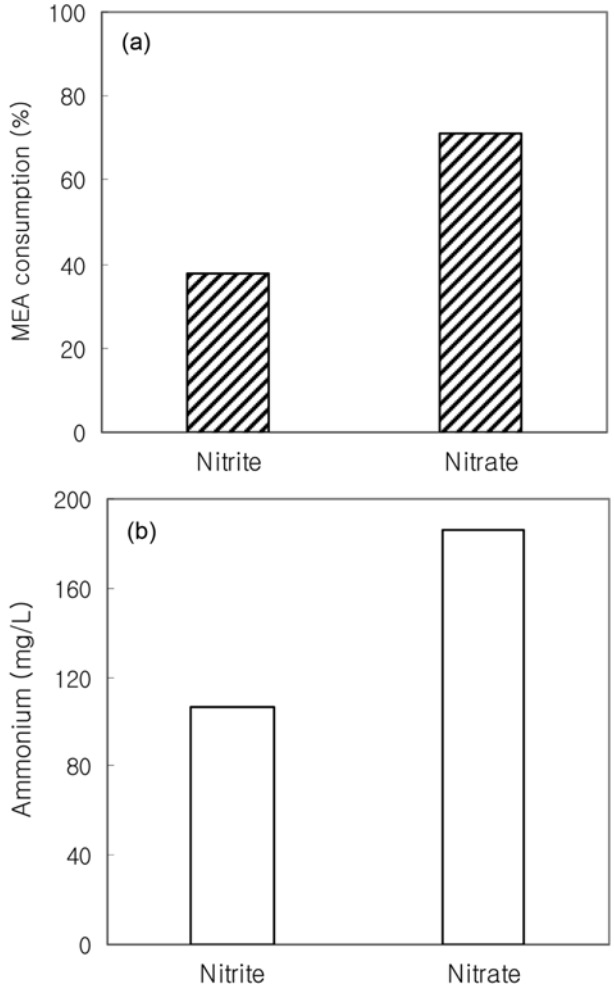

Fig. 3. Denitrification of nitrite (200 mg NO_2^--N/L) and nitrate (200 mg NO_3^--N/L) with 2,000 mg/L MEA. (a) MEA consumed during the denitrification, (b) Ammonium (NH_4-N) produced after the denitrification with MEA.

When 2,000 mg/L of MEA was used for denitrification, most of the nitrite and nitrate were denitrified in 3 days. It seemed that MEA did not inhibit denitrification severely at this concentration. Fig. 3 shows the result of denitrification with MEA. About 71% of the MEA was consumed for the denitrification of nitrate, while nitrite consumed about 38% of MEA for 3 days of incubation (Fig. 3(a)). The total amounts of MEA consumed for nitrite and nitrate reduction were more than the stoichiometry of denitrification with MEA. Some of the MEA were to be used for biosynthesis other than denitrification. In the meantime, ammonium was produced during the denitrification by the degradation of MEA. About 107 and 186 mg/L of NH_4^+-N were produced from the denitrification of 200 mg/L of NO_2^--N and NO_3^--N, respectively (Fig. 3(b)). It is thought that ethanol group of MEA is utilized as the electron donor for the denitrification.

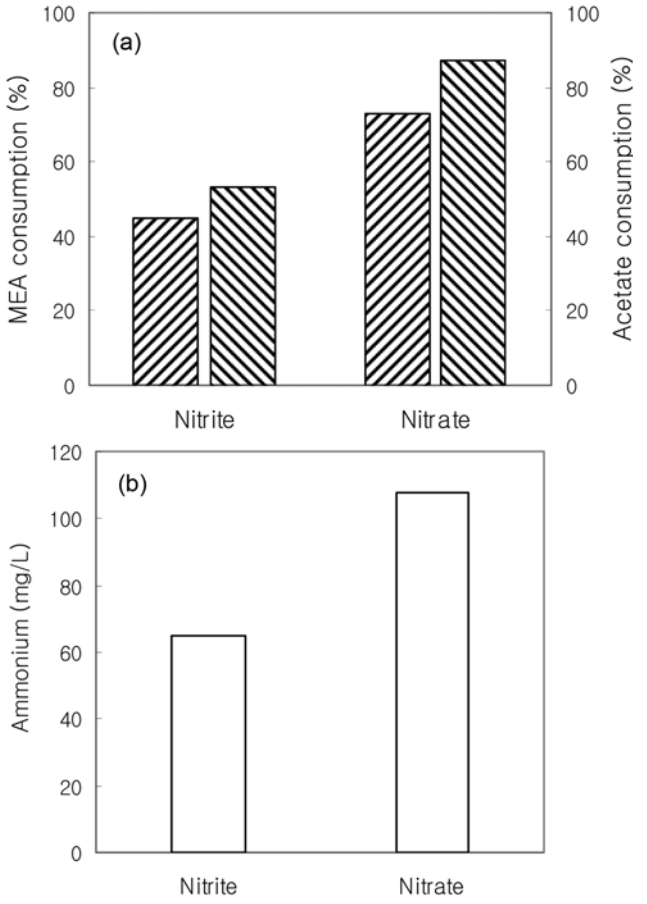

Fig. 4. Denitrification of nitrite (200 mg NO_2^--N/L) and nitrate (200 mg NO_3^--N/L) with acetate (985 mg/L) and MEA (1,000 mg/L) mixture. (a) MEA (▨) and acetate (▧) consumed during the denitrification, (b) Ammonium (NH_4-N) produced after the denitrification with acetate and MEA.

To test the preference of MEA as the electron donor for denitrification, acetate (985 mg/L) and MEA (1,000 mg/L) were provided together and compared for the denitrification of nitrite and nitrate. About 45% of MEA and 53% of acetate were consumed for the denitrification of nitrite, while about 73% of MEA and 87% of acetate was consumed for the nitrate denitrification (Fig. 4(a)). As the amount of electrons needed for the denitrification of nitrite is 40% less than that of nitrate reduction, less MEA and acetate were consumed in case of nitrite reduction than nitrate reduction. As the results of denitrification with MEA, 65 and 108 mg/L of NH_4^+-N were produced after the denitrification of nitrite and nitrate, respectively (Fig. 4 (b)). For the denitrification, more acetate was consumed than MEA, but the activated sludge consumed a significant amount of MEA even though acetate was still available in the reactor. The result proves that MEA is regarded as a competitive electron donor for denitrification. It is also concluded that there is no preference for the electron sources (i.e., acetate versus MEA) for the denitrification.

DISCUSSION

1. Inhibition of MEA on Biodegradation and Nitrification

Alkanolamines are widely used in many industries as acid gas absorbents and corrosion inhibitors, but they also have an antimicrobial effect. Studies of alkanolamines showed their antimicrobial effect to be enhanced at higher pH [17]. The antimicrobial effect of alkanolamines is suggested to be due to their surface-active properties.

The toxicity of MEA, and its effective level in the literature, showed diverse results depending on the test methods and the organisms used in the experiments. From the cell multiplication inhibition test, algae (*Microcystis aeruginosa*) and green algae (*Scenedesmus quadricanda*) were severely inhibited at 1.6 and 0.75 mg/L, respectively, while *Pseudomonas putida* was inhibited at 6,300 mg/L [18]. Bakalova et al. [7] showed that alkaline pH increases inhibitory effect and 200 mM (1,220 mg/L) of MEA at pH 7.2 inhibited 50% of cell growth, while about 30-90% of cell growths were inhibited at pH higher than 9.0 with 20 mM MEA from a bacterial culture.

The antimicrobial effect may be more significant to autotrophic nitrifying bacteria than heterotrophic bacteria [19]. From the study of nitrification inhibition, MEA at 100 mg/L inhibited 16% of its nitrification activity and about 50% of nitrification was inhibited at 12,200 mg/L MEA with *Nitrosomonas* [18]. Based on the experimental data and Haldane model in Fig. 2, about 50% of specific MEA degradation activity was inhibited at 2,300 mg/L MEA with activated sludge. As we used activated sludge instead of pure culture, the inhibition may be different from the above results.

For the acclimation, the sludge was incubated with MEA for 8 weeks in aerobic condition. Half of the MEA was degraded in 2-3 days and the average removal efficiency of MEA in 7 days reached 88%. Mrklas et al. [20] reported that MEA could be degraded in 10 to 20 days depending on the availability of phosphorus. The degradation rate slowed down when the MEA concentration was less than 2,000 mg/L. In the SBR experiments, nitrification occurred after several weeks of adaptation during MEA degradation (Fig. 1(a), 1(b)). During the nitrification, nitrite accumulation was observed (Fig. 1(b)), and Mrklas also observed nitrite accumulation during the aerobic MEA treatment [18]. In general, nitrite is accumulating during the nitrification in oxygen limited condition [19] or inhibiting conditions by toxic compounds such as free ammonia or free nitrous acid [20], which selectively inhibits the nitrite oxidizing bacteria more than the ammonia oxidizing bacteria. After all, most of the ammonium in the SBR was nitrified to nitrite and nitrate (Fig. 1(b)). Interestingly, denitrification occurred between 2 and 3 days as nitrite and nitrate concentration decreased to zero (Fig. 1(b)). The denitrification seemed to be possible by the contribution of MEA as the electron donor.

2. MEA as an Electron Donor in Denitrification

In Fig. 1(b), nitrite and nitrate concentrations decreased in the initial phase of a cycle, which is believed to be removed by denitrification as the MEA wastewater is pumped into the SBR to provide the electron donor. However, it is not clear whether MEA itself could be used as the electron donor for denitrification. Ndegwa et al. [8] found that nitrogen gas evolved during the aerobic phase by simultaneous nitrification and denitrification, and they claimed that ethanol or acetic acid could be the electron donor for denitrification.

MEA is degraded to ammonium and acetaldehyde by ethanolamine deaminase [23]. Stoichiometric equation of the aerobic biodegradation of MEA is as follows:

$$OHCH_2CH_2NH_2+2.5O_2 \rightarrow CO_2+H_2O+NH_4^++HCO_3^- \quad (2)$$

When the ammonium from the MEA is oxidized to nitrate,

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

The overall reaction including biodegradation of MEA and the subsequent nitrification becomes

$$OHCH_2CH_2NH_2 + 4.5O_2 \rightarrow CO_2 + NO_3^- + 2H_2O + 2H^+ + HCO_3^- \quad (4)$$

As one mole of ammonium is produced from one mole of MEA, the stoichiometry yields 1,377 mg/L of ammonium nitrogen (NH_4-N) from 6,000 mg/L MEA. The maximum NH_4-N produced in the aerobic MEA degradation (Fig. 1(a) and (b)) was 1,130 and 930 mg/L, respectively, which was 82% and 68% of the theoretical yields. The other nitrogen of the MEA is assumed to be assimilated to bacterial biomass and converted to N_2 by denitrification. The nitrogen converted to N_2 was more significant in Fig. 1(b) than in Fig. 1(a) where nitrification occurred significantly. Even though oxygen was supplied to the bioreactor, denitrification occurs in many biological wastewater treatment plants by simultaneous nitrification and denitrification (SND), especially where organics are available for denitrification and oxygen is depleted or limited in some parts of the sludge floc during the nitrification [24].

Denitrification of nitrite and nitrate seemed to be possible by the supplement of electrons by MEA itself or the organics (acetate, ethanol, acetaldehyde) produced from the MEA degradation. Ndegwa et al. [8] reported that ammonium, acetate, and N_2 were the dominant by-products in aerobic MEA biodegradation experiment in soil. They suggested that the generation of nitrogen gas was due to the SND because of the existence of anoxic zones resulting from diffusion-limited oxygen transport.

The energy reaction of denitrification with MEA becomes

$$OHCH_2CH_2NH_2 + 2NO_3^- + 2H^+ \rightarrow N_2 + CO_2 + 2H_2O + HCO_3^- + NH_4^+ \quad (5)$$

When acetate is used as the electron donor and nitrate as the electron acceptor and nitrogen source for cell synthesis, the overall denitrification reaction becomes

$$CH_3COO^- + 1.15NO_3^- + 1.15H^+ \rightarrow 0.0976C_5H_7O_2N + 0.5264N_2 + HCO_3^- + 0.5112CO_2 + 1.2336H_2O \quad (6)$$

where the f_s and f_e values are 0.342 and 0.658, respectively, which were obtained by the thermodynamic electron equivalents model [25,26]. The f_s and f_e are the fractions of donor electron converted for cell synthesis and energy generation, respectively.

In the same way the overall denitrification reaction, including energy reaction and cell synthesis, by MEA as the electron donor and nitrate as the electron acceptor becomes

$$OHCH_2CH_2NH_2 + 0.9NO_3^- + 0.1CO_2 + 0.9H^+ \rightarrow 0.275C_5H_7O_2N + 0.45N_2 + 0.725NH_4^+ + 0.725HCO_3^- + 1.175H_2O \quad (7)$$

The above stoichiometric of denitrification equations with acetate and MEA as the electron donors showed that acetate reduces more nitrate than MEA which was shown in Eqs. (6) and (7). The other difference of acetate and MEA in denitrification is that MEA leaves ammonium after denitrification. Overall, 1.9 moles of nitrogen (1 mole from MEA and 0.9 mole from nitrate) leaves 0.725 mole of ammonium nitrogen. From the overall stoichiometry, 1.9 moles of nitrogen leaves only 0.725 mole of nitrogen in water when MEA is used for the denitrification of nitrate. It means that 27.5% of MEA nitrogen goes to biomass and 72.5% goes to nitrogen gas by sequential nitrification and denitrification. Therefore, it can be said that MEA has sufficient electrons for the complete removal of its nitrogen by nitrification and denitrification.

About 38% of MEA was used for the reduction of nitrite, while 71% of MEA was consumed for the reduction of nitrate (Fig. 3) because nitrite needs fewer electrons than nitrate to become N_2. It is also confirmed by the amounts of ammonium produced during the denitrification as nitrite produced less ammonium by consuming less MEA than nitrate.

About 45% of MEA and 53% of acetate were consumed for nitrite reduction when MEA and acetate were supplied together for the denitrification (Fig. 4). In case of nitrate, 73% of MEA and 87% of acetate were utilized. The results are in agreement with the former result with MEA only that nitrate needed more electron donors than nitrite for denitrification. It is also supported by the amounts of produced ammonium during denitrification. When acetate (985 mg/L) and MEA (1,000 mg/L) were provided simultaneously, the amount of ammonium production was different to the case of denitrification with MEA (2,000 mg/L) only. As the total amount of acetate and MEA consumed in Fig. 4 was similar to the amount used in Fig. 3, it can be concluded that MEA is an effective carbon source for denitrification once the microorganisms are adapted to it. Less consumption of MEA for the denitrification of nitrite, and the accumulation of nitrite during nitrification due to the toxic effects of MEA, indicates a possibility of novel process for MEA removal including nitrogen through nitrite pathway.

CONCLUSIONS

In an aerobic SBR, MEA was successfully degraded and the removal efficiency reached 92% with 9,000 mg/L MEA at the HRT of 10.5 days. Ammonium hydrolyzed from the MEA was nitrified in 8 weeks from the start-up, which showed that adaptation time is needed for nitrification. Non-linear curve fitting of the specific MEA biodegradation gave the maximum specific activity (V_{max}), the half saturation constant (K_s), and the inhibition constant (K_i) of 2.81 g/(g VSS·d), 102.1 mg/L, and 1,149.6 mg/L, respectively. It clearly showed that MEA acts as an inhibition substrate. Batch denitrification showed that MEA could work as the electron donor. The activated sludge utilizes MEA under the presence of acetate for denitrification implying that MEA is a competitive electron donor for denitrification. MEA has sufficient electrons for the complete removal of its nitrogen compounds by nitrification and denitrification.

REFERENCES

1. J. R. Gallagher, J. A. Sorenson, S. S. Philbrick, R. Z. Knutson and D. Chollak, *Biol. Treat. Wastewater*, **5**, 269 (1995).
2. B. J. Hwang, S. W. Park, D. W. Park, K. J. Oh and S. S. Kim, *Korean J. Chem. Eng.*, **26**, 775 (2009).
3. S. J. Park, H. Y. Shin, B. M. Min, A. Cho and J. S. Lee, *Korean J. Chem. Eng.*, **26**, 189 (2009).
4. C. J. Kim and G. Sartori, *Int. J. Chem. Kinetics*, **16**, 1257 (1984).
5. O. F. Dawodu and A. Meisen, *Ind. Eng. Chem. Res.*, **33**, 480 (1994).
6. T. K. Chen, C. H. Ni and J. N. Chen, *J. Environ. Sci. Health Part A*, **38**, 2157 (2003).

7. S. Bakalova, V. Mincheva, A. Doycheva, V. Groudeva and R. Dimkov, *Biotechnol. Biotechnol. EQ*, **22**, 716 (2003).
8. A. W. Ndegwa, R. C. K. Wong, A. Chu, L. R. Bentely and S. R. D. Lunn, *J. Environ. Eng. Sci*., **3**, 137 (2004).
9. S. B. Hawthorne, A. Kubatova, J. R. Gallagher, J. A. Sorensen and D. J. Miller, *Environ. Sci. Technol*., **39**, 3639 (2005).
10. J. T. Hyun, I. H. Rhee, S. H. Kwon, D. J. Kim and D. C. Cho, *Kor. J. Biotechnol. Bioeng*., **22**, 157 (2007).
11. J. S. Knapp, N. D. Jenkey and C. C. Townsley, *Biodegradation*, **7**, 183 (1996).
12. B. Lai and W. Shieh, *Water Res*., **30**, 2530 (1996).
13. C. N. Lei, L. M. Whang and H. L. Lin, *Water Sci. Tech*., **58**, 1001 (2008).
14. APHA/AWWA/WEF, *Standard methods for the examination of water and wastewater*, 21st Ed., Washington DC (2005).
15. G. A. Hill and C. W. Robinson, *Biotech. Bioeng*., **17**, 1599 (1975).
16. Water Environment Federation, *Biological and chemical systems for nutrient removal*, WEF, Alexandria (1998).
17. M. Sandin, S. Allenmark and L. Edebo, *FEMS Microbiol. Lett*., **91**, 147 (1992).
18. K. Verscheren, *Handbook of environmental data on organic chemicals*, 4th Ed., Wiley, New York (2001).
19. K. Gernaey, L. Verschuere, L. Luyten and W. Verstraete, *Water Environ. Res*., **69**, 1163 (1997).
20. O. Mrklas, A. Chu, S. Lunn and L. R. Bentley, *Water Air Soil Poll*., **159**, 249 (2004).
21. T. J. Goreau, W. A. Kaplan, S. C. Wofsy, M. B. McElroy, F. W. Valois and S. W. Watson, *Appl. Environ. Microbiol*., **40**, 526 (1980).
22. A. C. Anthonisen, R. C. Loehr, T. B. S. Prakasam and E. G. Srinath, *J. Wat. Poll. Cont. Fed*., **48**, 835 (1976).
23. B. H. Kaplan and E. R. Stadtman, *J. Biol. Chem*., **243**, 1787 (1968).
24. K. Pochana and J. Keller, *Water Sci. Technol*., **39**, 61 (1999).
25. B. E. Rittmann and P. L. McCarty, *Environmental biotechnology: Principles and applications*, McGraw-Hill, New York (2001).
26. P. L. McCarty, *Biotechnol. Bioeng*., **97**, 377 (2007).