Biotreatment of Ammonia from Air by an Immobilized *Arthrobacter oxydans* CH8 Biofilter

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A heterotrophic *Arthrobacter oxydans* CH8 that was capable of removing NH_3 from NH_3 containing gas was isolated from livestock farming wastewater. The *A. oxydans* CH8 was immobilized with calcium alginate packed into filter column. Metered NH_3-containing gas was partially humidified and passed through the glass column. Extensive tests including the removal characteristics, the removal efficiencies, and the metabolic products of NH_3 by *A. oxydans* CH8 were conducted. Additionally, the operation criteria for the biofilter was also established. NH_3 removal capacities were elevated by the immobilized-cell (biological conversion) method and the BDST (bed depth service time) method (physical adsorption), respectively. The optimum temperature for removing NH_3 was 30 °C, while the nitrification ability remained 80% at 40 °C. The high efficiency (>97%) in the removal of NH_3 was attained at 36 L/h with pH control and was not decreased because of high NH_3 inlet concentration. In addition, the high maximum removal rate (1.22 g of N/day·(kg of bead)) enhanced the use of the biofilter in industrial-scale NH_3(g) pollution control. The ability to remove NH_3 at high inlet concentration and temperature suggested that the immobilized *A. oxydans* CH8 biofilter has potential in processing NH_3 gas.

Introduction

Ammonia (NH_3) is a colorless air pollutant with a strong and repellent odor. The ammonia emissions range from 10 to 60 ppm in livestock farming in Taiwan (Chung *et al.*, 1996a). A considerable amount of NH_3 is also released by industrial processes, such as petrochemical refining, metal manufacturing, food preparation, paper and pulp manufacturing, and textile industries (Ryer-Power, 1991). Control of NH_3 emissions is essential to mitigate the environmental impact (Prosser, 1989) and to protect public health (Ryer-Power, 1991). The physical and chemical processes that have been used to remove NH_3 from waste gas and wastewater include activated carbon adsorption, wet-scrubber, incineration, and air stripping (Durme *et al.*, 1992; Barth *et al.*, 1984; Mannebeck, 1986). But, the corresponding costs for such technologies and their disposals are economically incompetitive, and secondary-pollutant issues may arise. Recently, the focus has been shifted to investigation of the microbial alternatives to conventional methods (Bohn, 1992). Biofiltration has been proven to be an effective and inexpensive method, especially when applied to dilute, easily biodegradable waste gases under appropriate sets of conditions (Leson and Winer, 1991). However, the biofilter may cause environmental risks because bioaerosols (fungi and bacteria) can be released from the biofilter into the ambient air (Hartikainen *et al.*, 1996). Recently, these disadvantages have been overcome by using an immobilized-cell method.

The immobilized-cell technology has been applied for wastewater treatment because of its many advantages, such as prevention of cell losses, high microbe contents, high tolerance to environmental impact, and high stability during the operation period (Ginkel *et al.*, 1983; Asano *et al.*, 1992). However, little information is available for NH_3(g) removal from air gas by immobilized-cell technology.

In addition to selection of appropriate packing materials, it is also important to use an effective species for optimizing ammonia treatment. Autotrophic bacteria, such as ammonia oxidizers, have been characterized phylogentically Teske *et al.* (1994). The most extensively studied and applied ammonia oxidizer is *Nitrosomonas europaea* (Hunik *et al.*, 1992). Autotrophic nitrifiers have a high nitrification rate, but it would lose superiority in the nitrification processes under certain conditions, such as low oxygen concentration, high NH_3 concentration, and high temperature (Prosser, 1989). Conversely, the heterotrophic nitrifiers, such as *Alcaligenes*, *Pseudomonas*, *Bacillus*, and *Arthrobacter* species, are superior competitors, especially under acid environments (Kuenen and Robertson, 1988). Although the data indicate that the nitrification rate of the heterotrophs is 10^3-10^4 times smaller than that of the autotrophs, the biomass concentrations of heterotrophic nitrifiers are 10^4-10^5 times greater than those of autotrophs which can compensate for the removal capacity (Prosser, 1989). Growth of autotrophic ammonia oxidizers is considered inefficient in comparison to that of heterotrophs due to the small energy gain obtained from the oxidation of ammonia. Hence, the use of heterotrophic ammonia oxidizers for NH_3 containing waste gas was evaluated.

Cells immobilized in calcium alginate beads for H_2S-(g) removal has been regarded as a potential method because of their high removal efficiency, removal capacity (Chung *et al.*, 1996b; Huang *et al.*, 1996), and the economic consideration. In this study, we developed an innovative NH_3 gas treatment method by an immobilized *Arthrobacter oxydans* CH8 biofilter and also conducted a quantitative investigation into the principles and operation of a biofilter system for NH_3 removal.

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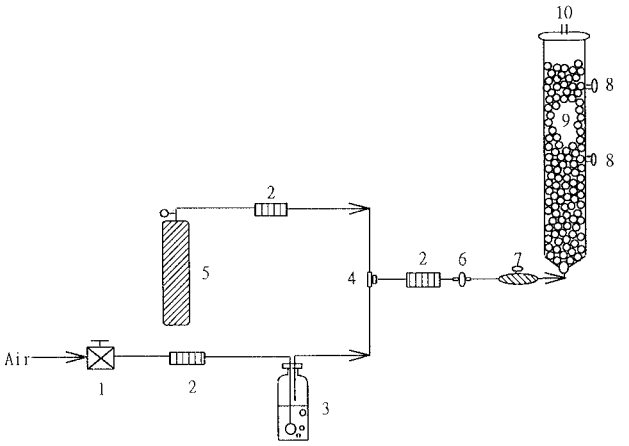

Figure 1. Laboratory-scale experimental biofilter system: 1, air compressor; 2, flow meter; 3, liquid media bottle; 4, three-way valve; 5, H_2S gas cylinder; 6, air filter; 7, inlet chamber; 8, sampling port; 9, glass column; 10, gas outlet.

Materials and Methods

Organism Cultivation and Medium Preparation. The original pure-culture strain of heterotrophic nitrifier was isolated from livestock farming wastewater. During an acclimating period, glucose and ammonium were used as the carbon and nitrogen sources. Stock cultures were grown in a plate count broth at 30 °C. The plate count broth (in grams per liter) contained the following: yeast extract, 5; tryptone, 10; dextrose, 2. The isolated strain was identified as *A. oxydans* CH8 by the Food Industry Research and Development Institute (FIRDI) in Taiwan. We put a basal medium in the humidification bottle in continuous-treatment experiments, and the medium (in grams per liter) contained the following: glucose, 0.2; KH_2PO_4, 2; K_2HPO_4, 2; NH_4Cl, 0.4; $MgCl_2 \cdot 6H_2O$, 0.2; iron(III) citrate, 0.01. The final pH of the culture was adjusted to 7 using 2 N NaOH or HCl.

Immobilized Procedure. *A. oxydans* CH8, grown in 100 mL plate count broth, was harvested by centrifugation (7500*g*, 10 min) and then washed three times with sterile distilled water. The organisms were mixed with a sterile 4% sodium alginate solution, and this cell containing solution was then mixed with a 4% $CaCl_2$ solution. Upon mixing, 3 mm diameter immobilized beads were formed. These beads were then activated by being flushed with sterile buffer solution for 5 h. The initial biomass concentration in the beads was 10^5 cfu/(g of beads) (cfu = colony forming units).

Apparatus and NH_3 Removal for Continuous Operation. Three glass columns (60 mm i.d. × 18 cm working length) connected in series were packed by calcium alginate beads without microbes to investigate the adsorption capacity of NH_3 by alginate beads. NH_3 gas of 5 ppm was supplied to these glass columns at 725 mL/min. The desired concentration of NH_3 at the breakthrough was 2 ppm (i.e., $C_e/C_0 = 0.4$).

A setup of the laboratory-scale experimental biofilter is shown in Figure 1. The description of the laboratory-scale experimental biofilter used was described by Chung *et al.* (1996c). Glass columns (60 mm i.d. × 25 cm of working height) were packed with cell-laden calcium alginate beads supported by a perforated sieve plate to ensure homogeneous distribution of the inlet gas. The packed volume, bead dry weight, and initial cell numbers in each column were 0.7 L, 0.25 kg, and 10^5 cfu/(g of beads), respectively. The column wall contained four sampling ports, drilled 12.5 cm apart, for measuring NH_3 concentration during the experiments. The NH_3(g) was supplied from a gas cylinder, diluted with compressed air, forced through a filter unit (0.45 μm), and then passed through the humidification bottle into the bottom of the biofilter. The immobilized cells were supplied with the air containing basal medium which contained carbon compounds and buffer capability. Relative humidities between 70 and 100% were routinely and continuously achieved during the operation.

In the continuous experiment, NH_3 gas at three concentrations (10, 20, and 60 ppm) was supplied to the biofilter at a flow rate of 36 or 72 L/h. The effects of temperature and flow rate on the removal efficiency and capacity of the biofilter were studied in the ranges of 15−45 °C and 18−210 L/h, respectively. The products of NH_3 oxidation by *A. oxydans* CH8 were also measured during the continuous experiment.

Kinetic Analysis. The NH_3 removal rate in the immobilized-cell biofilter was calculated using the following equation derived from the Michaelis−Menten equation (Hirai *et al.*, 1990):

$$\frac{1}{R} = \frac{K_s}{V_m}\frac{1}{C_{ln}} + \frac{1}{V_m} \tag{1}$$

where R (g of N/day·(kg of beads)) = removal rate, C_{ln} (ppm) = logarithmic mean concentration of NH_3 at the inlet and outlet of the biofilter, V_m (g of N/day·(kg of beads)) = maximum removal rate, and K_s (ppm) = saturation constant. Using the linear relationship between $1/C_{ln}$ and $1/R$, V_m and K_s values were calculated from the slope and intercept, respectively.

Design Criteria of Scale-up Biofilter. The target concentrations of NH_3 at the biofilter outlet were presumed as 0.1 and 1 ppm. The maximum inlet concentrations and critical NH_3 loads needed to satisfy this effluent concentration (0.1 or 1 ppm) were obtained at various SV according to following equation (Tiwaree *et al.*, 1992).

$$SV = \frac{\alpha}{C_0 - C_e} V_m \frac{C_{ln}}{K_s - C_{ln}} \tag{2}$$

where SV = space velocity (d^{-1}) = $F(S_aL)^{-1}$; F = gas flow rate ($m^3\ d^{-1}$); S_a = column cross-section (m^2); L = packing height (m); C_0 = inlet concentration (ppm); C_e = outlet concentration (ppm); α = conversion coefficient (kg of beads/(g of N)). C_e 0.1 or 1 ppm in eq 2, and the maximum C_0 can be estimated at various space velocities. The critical loads (g of N/day·(kg of beads)) of the biofilter can be obtained using eq 3.

$$\text{critical load} = \frac{(SV)C_0}{\alpha} \tag{3}$$

Analytical Methods. The inlet and outlet NH_3 gas concentrations in the biofilter were continuously measured using a single point monitor (MDA Scientific) in the range of 0.1−10 ppm or periodically measured by gas detector tubes (GASTEC, Japan) in the range of 5−100 ppm. A 5 g (wet-weight) amount of cell-laden beads was dissolved in 95 mL of 0.1 M sodium citrate solution, and the nitrogen compounds in the solution were determined. Nitrate and nitrite concentrations in the solution were measured by ion chromatography (Dionex 4500i). Ammonium was determined using an ion-specific electrode. Organic nitrogen was determined by the Kjeldahl method (American Public Health Association, 1992).

Results and Discussion

Effect of Temperature on NH_3(g) Removal Efficiency. The NH_3 removal efficiency at different temperature conditions is shown in Figure 2. Experimental temperatures were controlled in the range of 15−45 °C

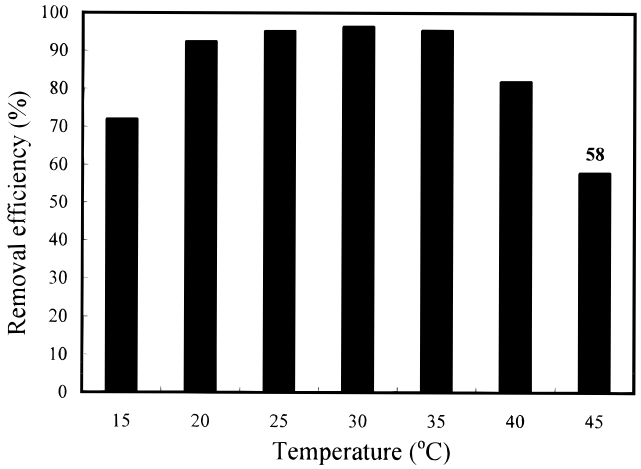

Figure 2. Effect of temperature on NH_3 removal efficiency at 36 L/h. The initial NH_3 concentration was 60 ppm.

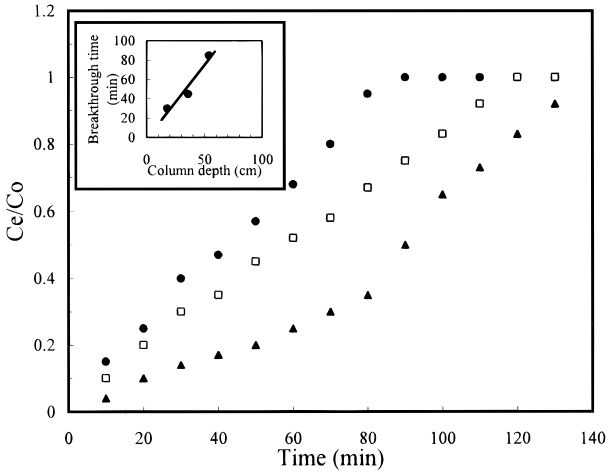

Figure 3. Breakthrough of NH_3 adsorption by the calcium alginate bead column. The inset diagram shows the linear correlation between packing height and breakthrough time according to the calculation of eq 4 by using breakthrough data. Symbols: ●, column I; ○, column II; ▲, column III.

at a flow rate of 36 L/h. The results showed that high NH_3 removal efficiencies (>93%) occurred in the range of 20−35 °C. The maximum removal efficiency of 97% was found at 30 °C. When the operating temperature increased to 45 °C, 58% of the NH_3 removal was achieved. Focht (1977) reported the failure of the autotrophic nitrifiers to grow above 40 °C and was unable to remove NH_3. Therefore, the heterotrophic nitrification by *A. oxydans* CH8 could be of quantitative significance in high-temperature environments.

NH_3 Removal Efficiency in Continuous Operation. The physical adsorption of calcium alginate beads to remove NH_3 is shown in Figure 3. The adsorption process is described by the equation (Chung *et al.*, 1996d):

$$t = \left(\frac{N_0}{C_0 V}\right)X - \frac{1}{kC_0}\ln\left(\frac{C_0}{C_e} - 1\right) \qquad (4)$$

where C_0 and C_e are the inlet concentration and the desired concentration of gas at breakthrough (ppm), respectively; V, t, and X are hydraulic loading (cm/min), service time (min), and bed depth (cm); N_0 and k are adsorptive capacity (ppm) and adsorption rate constant (ppm^{-1} min^{-1}).

The constants of the equation were obtained from the slope and the intercept of Figure 2 by the regression method. The values of the slope and intercept were 1.53 and 1.67. The adsorptive capacity (N_0) and the adsorption rate constant (k) were 195.5 ppm and 0.049 ppm^{-1} min^{-1}, respectively. The NH_3 adsorption capacity by pure calcium alginate beads calculated from this equation can provide a reference value of the removal capacity by cell-laden calcium alginate beads.

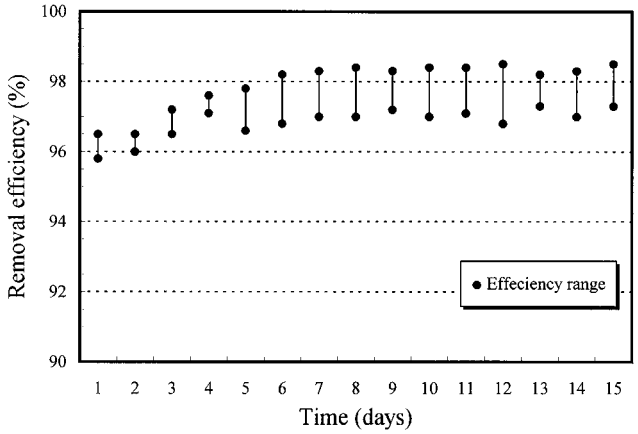

Figure 4. NH_3 removal efficiency of the biofilter at 36 L/h in the continuous operation. The variation ranges were between maximum and minimum removal efficiency for different inlet concentrations.

The effect of fluctuation in the inlet ammonia concentration on NH_3 removal efficiency was examined during continuous operation. The inlet ammonia concentrations were controlled in the range 10−60 ppm. The variation ranges between maximum and minimum removal efficiencies for different inlet concentrations are shown in Figure 4. The acclimation time for the biofilter was unnecessary because the biofilter was an inoculated pure culture; therefore, the biofilter showed excellent removal efficiency (>95%) in 24 h regardless of the inlet concentration. The biofilter reached the steady-state condition after 7 days of operation and achieved 98% removal efficiency. During the operating period, the pH value of the biofilter was decreased from pH 7.5 (the first day) to pH 6.1 (the 15th day), but this decrease in pH did not cause a significant effect on the removal efficiency. Hence, an immobilized heterotrophic nitrifier (*A. oxydans* CH8) has the potential for the acid nitrification due to its high removal efficiency under the acidic conditions. The high biofiltration activity could be maintained for 3 months by exchanging the basal medium periodically. The metabolic products by *A. oxydans* CH8 at different flow rates are presented in Table 1. The ratios of ammonia conversion to nitrite or nitrate were similar and regardless of the flow rates. The production ratios of NO_2^-/NO_3^- were 14.5 (for example: 1.403/0.099) at both 36 and 72 L/h conditions at which the acid products could decrease the pH value of the biofilter. Fortunately, the removal efficiency was still maintained at 95% (Figure 4) under the acidic condition.

Effect of Flow Rate on NH_3 Removal. Gas removal in the biofilter consists of two steps. First, the gas is removed from the gas phase by diffusing into the liquid film and then adsorbed on the solid medium that is metabolized by cell-laden beads. The effect of flow rate on NH_3 removal was studied when 60 ppm NH_3 was fed into the biofilter. The removal efficiencies in the medium zone (12.5 cm) as well as in the end zone (25 cm) of the biofilter were examined and are shown in Figure 5. The removal efficiency decreased with the increasing flow rate. Greater than 94% removal efficiency was achieved when the flow rates were controlled in the range of 18−72 L/h. When the flow rate increased up to 210 L/h, the removal efficiency dramatically decreased by 20%. Only 3% of the total removal was contributed by the 1 s section from the outlet end of the biofilter when the flow rate

Table 1. Nitrogen Mass Balances in the Biofilter Inoculated *A. oxydans* CH8 at 60 ppm NH_3 Supply for 15 Days

flow rate (1/h)	NH_3 removed (g of N/(kg of bead))	NH_4^+ produced (g of N/(kg of bead))	NO_2^- produced (g of N/(kg of bead))	NO_3^- produced (g of N/(kg of bead))	organic N produced (g of N/(kg of bead))
36	2.06	0.025 (1.2%)	1.403 (68.1%)	0.099 (4.8%)	0.523 (25.4%)
72	4.07	0.061 (1.5%)	2.759 (67.8%)	0.183 (4.5%)	1.050 (25.8%)

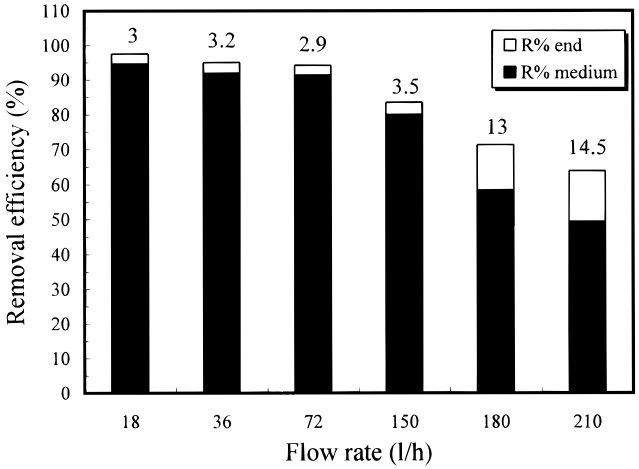

Figure 5. Effect of flow rate on removal efficiency of NH_3 by the biofilter at different heights of the biofilter. The medium and end indicate 12.5 and 25 cm from the bottom of the biofilter. The numbers in the figure refer to the removal efficiencies in the end zone of the biofilter.

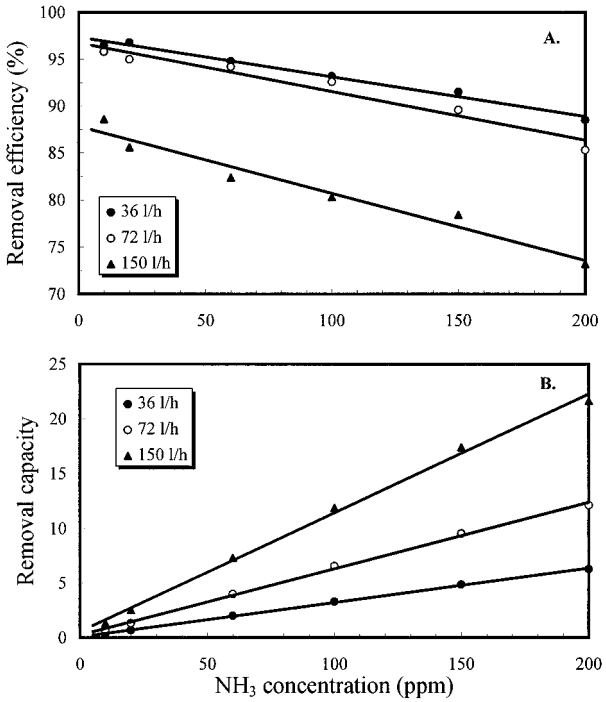

Figure 6. (A) NH_3 removal efficiency vs inlet NH_3 concentration at different flow rates (36, 72, 150 L/h) (B) Removal capacity vs inlet NH_3 concentration at different flow rates (36, 72, 150 L/h).

was lower than 150 L/h. Sublette and Sylvester (1987) indicated that the microorganism could metabolize gas within a short time (several seconds); therefore, the reduction in the removal efficiency at a high flow rate might be caused by the short retention time, and such a short retention would reduce the chance for NH_3 to transfer into the liquid phase.

Effect of Inlet Concentration on NH_3 Removal. The NH_3 removal efficiency as functions of gas flow rates and NH_3 concentrations is shown in Figure 6A. Removal efficiency decreased progressively with increasing gas flow rates and NH_3 concentrations. The NH_3 removal efficiencies showed a little variation at flow rates between

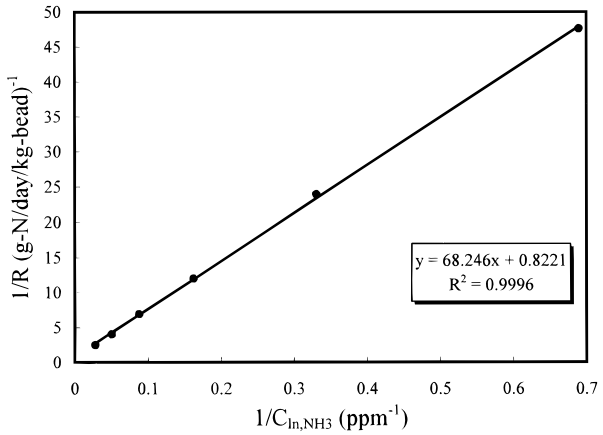

Figure 7. Relationship between NH_3 degradation $1/R$ and $1/C_{ln}$ in the biofilter.

36 and 72 L/h, whereas the significant difference was observed when the flow rate increased to 150 L/h. In contrast to autotrophic *N. europaea* because of the good linear correlation between removal efficiency and inlet concentration, the high inlet concentration of NH_3 did not inhibit *A. oxydans* CH8 removal activity (Hunik *et al.*, 1992). From a practical point of view, it is important for biofilteration that the *A. oxydans* CH8 we obtained are relatively resistant to elevated NH_3 concentrations. The removal capacity vs the inlet NH_3 concentrations at different flow rates (36, 72, and 150 L/h) is shown in Figure 6B. An increase in the flow rate from 36 to 72 L/h yielded a doubled removal capacity, and further increase of the flow rate from 72 to 150 L/h resulted in an advanced improvement of removal capacity. However, the maximum removal capacities were not attained when the inlet concentration was as high as 200 ppm at 36−150 L/h. These results demonstrate that the gas flow rate strongly influences both the removal efficiency (Figure 6A) and the removal capacity (Figure 6B).

Kinetic Analysis. The apparent kinetic parameters of the maximum removal rate and saturation constant to degrade NH_3 under different conditions are calculated by the Lineweaver−Burk method as shown in Figure 7. The kinetic study was performed at 30 ± 1 °C, and the flow rates were controlled below 36 L/h. Greater than 98% removal efficiency was attained at steady state. The regression equation expressed as $y = 68.246x + 0.8221$. The maximum removal rate and the saturation constant for NH_3 by *A. oxydans* CH8 were calculated to be V_m = 1.22 g of N/day·(kg of beads) and K_s = 83.01 ppm (3.3 μM) from the slope and intercept of the regression equation. The saturation constant was higher than the values of 2.0 and 1.4 μM in autotrophic *N. europaea* reported by Bedard and Knowles (1989) and Keener and Arp (1993). If we infer a physical meaning for K_s analogous to enzymatic kinetics, an increase of the K_s value compared with Bedard's and Keener's data indicated the decrease of the enzymatic affinity for NH_3. The lower enzymatic affinity might be because the heterotrophic nitrification was the secondary metabolism and not associated with energy production or growth (Prosser, 1989).

Design Criteria for Scale-up Biofilter. An NH_3 removal rate of 100% can be achieved only when the inlet load is less than the critical load. If this critical load is

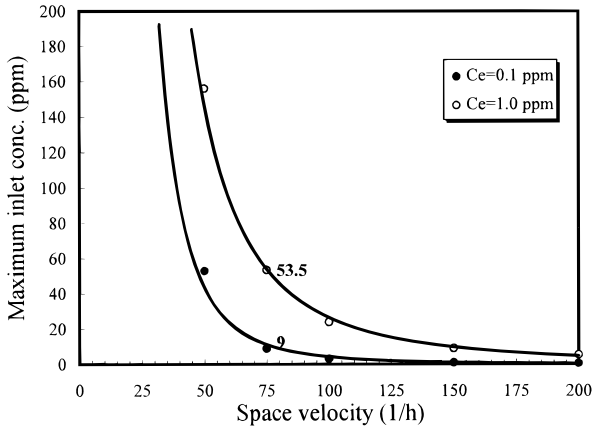

Figure 8. Relationship between the maximum inlet concentration and space velocity for NH_3 removal at 30 °C. The emission standard (C_e) is set at 0.1 or 1 ppm.

exceeded, NH_3 will be present at the outlet of the biofilter. The load is defined as the amount of inlet gas per unit of time and weight of the packing beads (g of N/day·(kg of beads)). Thus, inlet gas concentrations play an important role in the design of a scale-up biofilter if the packing weight and gas flow rate are constant. It suggests that the maximum inlet concentration and the optimum inlet load are two important issues for the design of a biofilter. The relationship between the maximum inlet concentration and space velocity for NH_3 removal is indicated in Figure 8. It suggested that, as lower space velocity was conducted, a higher inlet concentration was allowed to be removed by the biofilter in compliance with the emission standard. The maximum inlet concentrations were 9.0 and 53.5 ppm at a space velocity of 75 h^{-1} when the outlet NH_3 concentrations were limited to 0.1 and 1 ppm. In addition, the maximum inlet loads were calculated to be 0.063 and 0.261 g N/day·(kg of beads), respectively, at the same space velocity and outlet NH_3 limitation.

Conclusions

Our results demonstrate that the immobilized *A. oxydans* CH8 biofilter can remove NH_3(g) from the gas phase with high efficiency. The biofilter achieved 97% removal efficiency at 36 L/h during 15-day operating period with pH control and could be maintained for long periods of time. Additionally, it has the potential for nitrification at higher temperature and NH_3 concentration than autotrophic ammonia oxidizers. The main products of NH_3 oxidation were determined to be nitrite and nitrate, and the conversion ratios were independent of the flow rates. From kinetic analysis, the maximum removal rate and apparent saturation constant were 1.22 g of N/day·(kg of beads) and 83.01 ppm, respectively. When the maximum inlet concentration was 53.5 ppm at 75 h^{-1}, 1 ppm of NH_3 emission standard could be achieved. These results suggested that an immobilized *A. oxydans* CH8 biofilter provided a significant potential in treating NH_3 gas.

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