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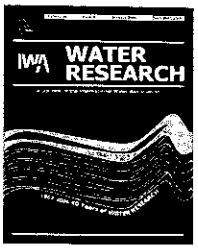

Piggery wastewater treatment using *Alcaligenes faecalis* strain No. 4 with heterotrophic nitrification and aerobic denitrification

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ABSTRACT

Alcaligenes faecalis strain No. 4, which has heterotrophic nitrification and aerobic denitrification abilities, was used to treat actual piggery wastewater containing high-strength ammonium under aerobic conditions. In a continuous experiment using a solids-free wastewater (SFW) mixed with feces, almost all of the 2000 NH_4^+-N mg/L and 12,000 COD mg/L in the wastewater was removed and the ammonium removal rate was approximately 30 mg-N/L/h, which was 5–10 times higher than the rates achieved by other bacteria with the same abilities. The denitrification ratio was more than 65% of removed NH_4^+-N, indicating that strain No. 4 exhibited its heterotrophic nitrification and aerobic denitrification abilities in the piggery wastewater.

1. Introduction

Ammonium treatment is essential for preventing eutrophication. Wastewaters, such as piggery wastewater contain not only high concentrations of nitrogen compounds but also very high concentrations of carbon materials. Among the nitrogenous compounds, the ammonium concentration reaches up to 1000–3000 mg/L, which is 50–100 times higher than in municipal wastewater (Ra et al., 2000; Beline et al., 2001; Su et al., 2001; Cao et al., 2003; Carrera et al., 2003; Uygur and Kargi, 2004). The C/N ratio of the mixture of urine and feces in piggery wastewater is usually in the range of 5–20 (Itokawa et al., 2001; Rostron et al., 2001; Jung et al., 2004; Obaga et al., 2005). However, conventional nitrification using autotrophic bacteria is difficult to apply to such wastewater because autotrophic bacteria are vulnerable to high concentrations of ammonium and organic matters with C/N ratio of 0–2 (Itokawa et al., 2001). Thus, conventional nitrification can be implemented only after pretreating the wastewater to reduce the C/N ratio (Khin and Annachhatre, 2004) or diluting the wastewater (Rostron et al., 2001; Jung et al., 2004). Furthermore, nitrification by autotrophic bacteria requires a long retention time of flowing wastewater in the reactor due to the slow growth rates of these bacteria (Jetten et al., 1997; Muller et al., 2003).

Some bacteria conduct heterotrophic nitrification and aerobic denitrification, and can convert oxidized or nitrified ammonium to denitrified products such as NO, N_2O and N_2 under aerobic conditions (Arts et al., 1995; Dalsgaard et al., 1995; Robertson et al., 1995; Stouthamer et al., 1997; Honda et al., 1998; Nishio et al., 1998; Pai et al., 1999). *Alcaligenes faecalis* is one such bacteria, and research into the possibility of using this bacterium for treating wastewater containing relatively low ammonium loads has progressed (Nishio et al., 1998; Otte et al., 1999; Pai et al., 1999).

Previously, we isolated *A. faecalis* strain No. 4 as an antagonist to plant pathogens (Honda et al., 1998) and confirmed that it has heterotrophic nitrification and aerobic

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denitrification abilities (Wako, 2002; Joo et al., 2005). In the present work to determine whether these abilities of strain No. 4 are exhibited in the treatment of actual piggery wastewater, we applied this strain to batch and continuous cultures using solids-free piggery wastewater (SFW) either alone or supplemented with additional carbon sources. In particular, from the nitrogen balance, we examined the ability of strain no. 4 to remove ammonium from wastewater containing high ammonium concentrations.

2. Materials and methods

2.1. Microorganism

A. faecalis strain No. 4, which was isolated from sewage sludge (Honda et al., 1998) by our laboratory, was used. The cells of the strain No. 4 were stored in a 25% glycerol solution at −80 °C.

2.2. Medium used

The synthetic medium used was prepared by dissolving the following in 1 L of distilled water; 14 g K_2HPO_4, 6 g KH_2PO_4, 51 g trisodium citrate dihydrate, 6 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$ and 2 mL of a trace element solution. The trace element solution contained (g/L): 57.1 g EDTA · 2Na, 3.9 g $ZnSO_4 \cdot 7H_2O$, 7 g $CaCl_2 \cdot 2H_2O$, 5.1 g $MnCl_2 \cdot 4H_2O$, 5.0 g $FeSO_4 \cdot 7H_2O$, 1.1 g $(NH_4)_6MO_7O_{24} \cdot 4H_2O$, 1.6 g $CuSO_4 \cdot 5H_2O$, 1.6 g $CoCl_2 \cdot 6H_2O$ (pH 6.0). The medium was inoculated with 1 mL of a stock solution of strain No. 4 in a 500-mL shaking flask and cultivated at 30 °C at 120 strokes per minute (spm) for 24 h. This culture was used to seed the continuous cultures.

2.3. Piggery wastewater

Piggery wastewater was provided by the Kanagawa Prefectural Livestock Industry Research Institute, Kanagawa, Japan. Solids-free wastewater (referred to as SFW) was obtained by separating the solids from the raw wastewater containing urine, washing water, and feces by centrifugation at 1000 rpm. Table 1 shows the characteristics of the SFW and a sample comprised of SFW supplemented with feces (3:1 on a weight basis) (referred to as MW). Carbon concentration expressed by chemical oxygen demand measured in the presence of chromium (COD_{cr}) in MW was 6.5 times higher than that in SFW.

2.4. Aerated batch experiment of strain No. 4 in SFW and in MW

To determine the basic characteristics of strain No. 4 in piggery wastewater, aerated batch experiments were conducted. In these experiments, 18 mL of a preculture was inoculated into 360 mL of SFW in a 500-mL conical flask and stirred with a magnetic stirrer at 200 rotations per minute (rpm). Air was supplied from a compressor at a flow rate of 0.5 L/min. The experiments were performed by adding sodium citrate to a final concentration of 15 g/L (referred to as C(15)), and sodium citrate and glucose to final concentrations of 10 and 5 g/L, respectively (referred to as C(10)G(5)) to adjust the C/N ratio to around 10. Two-milliliter samples were taken periodically for chemical analysis, measurement of pH, and microbial count. The ammonia stripped from the reactor by aeration was captured in a 35 mM H_2SO_4 solution.

For the MW solution containing SFW supplemented with feces as a carbon source (3:1 on a weight basis), citrate and glucose were not added to the solution. Strain No. 4 was cultivated in the solution undet the same aerated batch culture conditions as described above. For solution MW, the C/N ratio was 7–9 and the pH was around 6.

2.5. Continuous experiments

Continuous treatment of SFW and MW after the inoculation of strain No. 4 was conducted in a reactor at room temperature as shown in Fig. 1. Table 2 lists the experimental conditions. Additional carbon sources were mixed into the SFW solution to raise the C/N ratio.

The culture of strain No. 4 in the synthetic medium was poured into a 2.4-L aeration tank, and the airflow rate was set at 2–2.5 L/min. After 3-day batch cultivation, open continuous experiments were started by supplying SFW.

The continuous operation periods were divided into four parts, denoted a–d, as shown in Table 2. In period a, only SFW was supplied; in period b, 6.25 g/L of citric acid was added to the SFW to adjust the pH to 6; in period c, 27 g/L of organic acids, consisting of 15 g/L of citric acid and 12 g/L of sodium acetate, was added to the SFW. In period d, the mixture of SFW and feces (MW) was supplied. The influent NH_4^+–N concentration was approximately 1000–2000 mg/L, and the hydraulic retention time (HRT) was 34–60 h.

Table 1 – Characteristics of solid-free piggery wastewaters used in this study

	pH	NH_4^+–N (mg/L)	Total N (mg/L)	NO_2–N (mg/L)	NO_3–N (mg/L)
Solid-free wastewater	8.3–8.5	830–1250	1050–1270	<1	<1
Mixed wastewater[a]	5.8–6.1	1850–1960	2050–2350	<1	<1
	OD_{660}	Total solid (mg/L)		COD_{cr}-C (mg/L)	C/N ratio
Solid-free wastewater	4.41	5126–5900		4150–5300	4–5
Mixed wastewater[a]	29	32,500–33,755		13,800–14,650	7–9

[a] Mixed wastewater is a mixture of solid-free wastewater with feces at the ratio 3:1 (on weight basis).

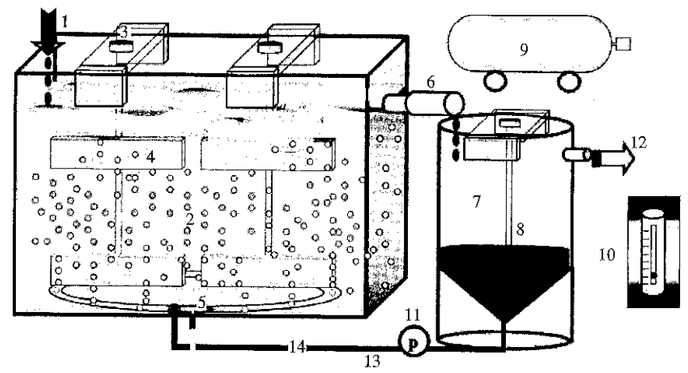

Fig. 1 – Aerobic reactor used for continuous experiments. 1: influent, 2: aeration tank, 3: agitating motor, 4: agitating propeller, 5: air dispersing tube, 6; sludge effluent, 7: clarifier, 8: agitating propeller, 9: air compressor, 10: air flow meter, 11: peristaltic pump, 12: effluent, 13: air, 14: return sludge.

Table 2 – Operation conditions in open continuous system inoculated with strain No. 4 for treatment of solid-free piggery wastewater

Periods (days)	Temperature (°C)	Initial NH_4^+–N (mg/L)	HRT (h)	Air supply (L/min)	Carbon addition	C/N ratio
a 1–10	17–19	1050	48	2.0	—	4
b 11–20	18–21	1050	40	2.5	6.25 g/L of citric acid	6
c 21–52	19–21	1250	34	2.0	27 g/L[a]	15
d 52–80	16–22	1950	50–60	2.0	25% feces[b]	7–8

[a] 15 g/L citric acid and 12 g/L sodium acetate.
[b] 25% feces were added to solid-free wastewater.

2.6. Analytical methods

The NH_4^+ concentration was determined by the indophenol method (Japanese Industrial Standards (JIS) K 0102, 134–145, 1986). NH_2OH, NO_2^-, NO_3^- and citrate concentrations were measured using previously reported methods (Joo et al., 2005). Culture samples were centrifuged at 10,000 rpm and the supernatants were filtered using membrane filters (0.2 μm) and then the filtrates were used for the following analysis.

Total viable cell numbers in SFW or MW expressed as colony-forming units (cfu) were counted on L agar plates to detect common heterotrophic bacteria. The L agar (pH 7.0) medium contained 1% peptone, 0.5% NaCl, 0.5% yeast extract and 1.5% agar. The viable cell numbers of strain no. 4 were also estimated by L agar plating because compared to other bacteria, the colonies of strain No. 4 appeared on the L agar plate much sooner and had a significantly different appearance. Intracellular nitrogen content (mg-N/L) was calculated from the viable cell number and the nitrogen content (%) obtained from the elemental analysis of dry cells. Dry cells were obtained by centrifugation of culture broth at 10,000 rpm, followed by washing with sterilized water and drying at 105 °C for 24 h. The amount of denitrifying products (denitrification ratio) was calculated by subtracting the amount of nitrifying products (NH_2OH–N, NO_2^-–N, NO_3^-–N) and the amount of nitrogen in dried cells from the removed ammonium. The concentration of stripped ammonia was estimated by capturing in 35 mM H_2SO_4 ammonia emitted from a tightly sealed 500-mL conical flask containing 350 mL of culture under aeration conditions with the same air flow rate as was used for the continuous operation. COD_{cr} was determined by a closed reflux titrimetric method using standard ferrous ammonium sulfate (FAS) as the titrant after decomposition in standard potassium dichromate digestion solution and sulfuric acid regent with heating at 150 °C for 2 h.

The ammonium removal rates in batch cultures were calculated from the maximum slope of ammonium removal, and in continuous culture, the rate was obtained from the amount of removed ammonium divided by the HRT. As the concentrations of the denitrification intermediates (NO and NO_2) determined using a NO_x analyzer (AP1 200A, Riken Keiki) were extremely low in our preliminary sterilized continuous cultures, they were not measured in the continuous experiment.

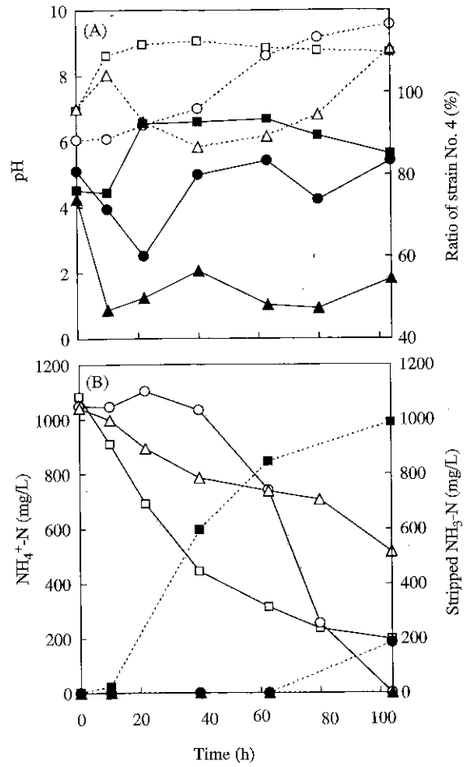

Fig. 2 – The ratio of strain No. 4 cell number to viable cell number and pH (A), and ammonium concentration and stripped ammonia (B) in the batch experiment using strain No. 4. Symbols: A, pH (empty symbols) and ratio of strain No. 4 cell number to viable cell number (filled symbols); B, ammonium concentration (empty symbols) and stripped ammonia (filled symbols). Squares, circles and triangles indicate solids-free wastewater (SFW), the SFW supplemented with 15 g/L sodium citrate (C(15)), and the SFW supplemented with 10 g/L sodium citrate and 5 g/L glucose (C(10)G(5)), respectively.

3. Results

3.1. *Aerated batch experiment of strain No. 4 in SFW*

Fig. 2 shows the results obtained when strain No. 4 was cultivated in SFW, SFW supplemented with 15 g of citrate (C(15)), and SFW supplemented with 10 g of citrate and 5 g of glucose (C(10)G(5)). In SFW, most of the removed ammonium was stripped as ammonia gas, mainly because the scarcity of carbon available to strain No. 4 accelerated the degradation of nitrogenous compounds in SFW to ammonium. This caused a rapid increase in wastewater pH, resulting in enhanced ammonification from ammonium. This pH increase was delayed in the SFW supplemented with C(15) or C(10)G(5), which was reflected by the result that almost no, or a smaller amount (only 10% of initial ammonium), of stripped ammonia than was observed in SFW were detected (Fig. 2B). The ratio of the number of strain No. 4 cells to the total cell number in SFW was estimated as 60–80%. Unlike many bacteria, strain No. 4 utilizes organic acids rather than sugars (Joo et al., 2005). As a result of this characteristic, the percentage of strain No. 4 in C(10)G(5) was lower than in the other two batches owing to increased proliferation of other bacteria by glucose consumption. The results indicate that to achieve more efficient ammonium removal and to avoid the production of stripped ammonia, the addition of carbon and control of pH are important.

The nitrogen balance in the aerated batch experiments after 100 h is shown in Table 3. The ratios of denitrified nitrogen in the experiments using SFW supplemented with C(15) and C(10)G(5) were similar, although the total amount of ammonium removed in the SFW with C(10)G(5) constituted only half that of the initial value. In both cases, the nitrified products were disregarded, because the total amount of such products was less than 0.5% of the removed ammonium (data not shown).

Fig. 3 shows the results obtained when strain No. 4 was cultivated in the MW. Approximately 1700 NH_4^+–N mg/L was removed and the pH increased from 6 to 8, and then 50 NH_3–N mg/L was stripped. The maximum ammonium removal rate in the MW was 27.5 mg-N/L/h, while those in SFW supplemented with C(15) and C(10)G(5) were 29 and 8 mg-N/L/h, respectively. The ratio of the number of strain No.

Table 3 – The nitrogen balance in the aerated batch experiments of strain No. 4 using solid-free piggery wastewater (Unit: mg/L)

Wastewater	Influent NH_4^+-N	Removed NH_4^+-N (%)	Stripped NH_3-N (%)[a]	Assimilated NH_4^+-N (%)[a]	Denitrified NH_4^+-N (%)[a]
Solid-free wastewater	1082	885 (82)	987 (>100)	52 (6)	—
C(15)[b]	1049	1049 (100)	182 (17)	399 (38)	468 (45)
C(10)G(5)[b]	1040	523 (50)	0 (0)	278 (53)	245 (47)

[a] Percentage against the removed ammonium nitrogen.
[b] C(15) and C(10)G(5) are the solid-free wastewaters supplemented with 15 g/L sodium citrate dehydrate and supplemented with a mixture of 10 g/L sodium citrate dehydrate and 5 g/L glucose, respectively.

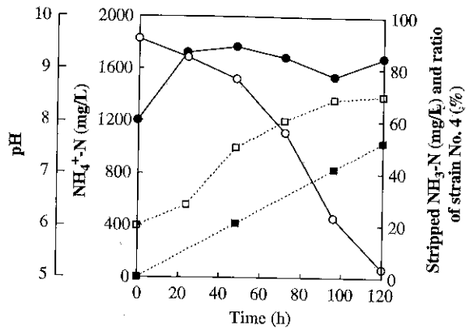

Fig. 3 – The ratio of strain No. 4 cell number to viable cell number, pH, ammonium concentration and stripped ammonia in the batch culture of strain No. 4 using a mixture of solids-free wastewater and feces. Symbols: pH (□), ratio of strain No. 4 (●), ammonium concentration (○) and stripped ammonia (■).

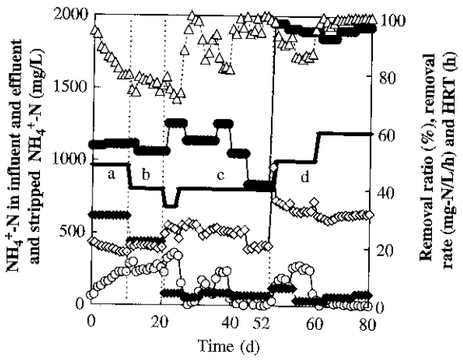

Fig. 4 – Ammonium concentration in influent and effluent, stripped ammonia pH, removal rate and removal ratio of ammonium, and hydraulic retention time (HRT) in the continuous experiment using strain No. 4 to treat solids-free wastewater. Symbols: ammonium in influent (●), ammonium in effluent (○), removal rate of ammonium (◇), removal ratio of ammonium (△), hydraulic retention time (HRT) (solid line) and stripped ammonia (♦). The letters, a–d corresponds to the operation conditions shown in Table 2. On day 52, the sludge was drawn off and the feces were added.

4 cells to the viable cell number was estimated to be approximately 80%. These data indicate that the feces are available as a carbon source for removal of high-strength ammonium by strain No. 4.

3.2. *Continuous experiment using strain No. 4*

Fig. 4 shows the ammonium concentrations in influent and effluent, stripped ammonia, the removal rate and removal ratio of ammonium, and the hydraulic retention time (HRT) during continuous treatment of SFW with strain No. 4. The initial HRT was set at 48 h and the HRTs during the overall period of operation varied in the range of 34–60 h depending on the concentration change of influent ammonium. The ammonium removal ratio for the initial 10 days (period a) decreased, mainly due to a scarcity of available carbon for strain no. 4 in SFW. Then, citric acid was added and the HRT was reduced (period b). Consequently, the ammonium removal ratio was stabilized to about 80%. In period c, the NH_4^+–N concentration in the influent increased by 350 mg/L, and another carbon source (15 g/L citric acid and 12 g/L sodium acetate) was added. As a result, the ammonium removal ratio increased to 100% and the ammonium concentration in the effluent at 40–51 days was reduced to almost 0 mg/L at 40 h HRT. This period was regarded as the steady state in period c. In period d, the influent NH_4^+–N concentration was increased to 2000 mg/L with the addition of feces, and the HRT was increased to 48 h and then to 60 h. Ammonium removal was incomplete at 48 h HRT, but at 60 h HRT, the effluent ammonium concentration was reduced to almost 0 mg/L; thus, the period with a HRT of 60 h was considered to be the steady state in period d. Among the four periods, the ammonium removal rate and ammonium removal ratio in period d were the highest and the most stable. The ratios of stripped ammonia in periods a, b, c and d were 56%, 40%, 4–8% and 2–6%, respectively, indicating that stripped ammonia was significantly lower in periods c and d, when the removal ratio of ammonium was high. These findings thus indicate that stripped ammonia was high in system with lower C/N ratios as shown in batch experiments in Fig. 2.

The pH was in the range of 8.6–9 in periods a and b, and then gradually decreased to 8.5–8.8 in period c. In period d, when MW was supplied, the pH decreased to 7.4–8 (data not shown). The ratio of the number of strain No. 4 cells to the total viable cell number in the culture was estimated to be 75–84% on average.

The ammonium removal rate in periods a and b was approximately 20 mg-N/L/h and in periods c and d they increased to approximately 25 mg-N/L/h and 30–35 mg-N/L/h, respectively (Fig. 4). These removal rates were 2–4 times higher than that achieved in a pilot-scale treatment plant at Kanagawa Livestock Research Institute in which an activated sludge system was used to treat SFW.

Fig. 5 shows the COD_{cr} in influent and effluent, the COD removal ratio, and the HRT in the continuous experiment. During periods a and b, COD_{cr} was completely removed. However, during this 20-day-period, the removal ratio of ammonium was reduced to 80% (Fig. 4). In period c, 30% of the initial value of COD_{cr} remained unused due to the external carbon supplementation, which increased the influent COD_{cr} to 12,000–15,000 mg/L. In period d, when feces were added as a carbon source, the COD_{cr} in the influent was about 12,000 mg/L, and the COD_{cr} at the outlet on days 52–65 was about 700 mg/L at 48 h HRT, and subsequently decreased to almost 0 mg/L at HRT of 60 h. The ammonium concentration in the effuluent was also 0 mg/L. In this period, nitrogen and carbon consumptions were well balanced.

The nitrogen and carbon balances in periods c and d in the continuous experiment are shown in Table 4. For each item,

no significant difference was observed between the two periods. The denitrification ratios in periods c and d were also similar, at 68% and 73%, respectively. These findings imply that for active strain No. 4, feces serve as a carbon source for treating high-strength ammonium. The COD removal ratio, ammonium removal ratio, and denitrification ratio in period d were higher presumably due to the C/N ratio of the MW being appropriate for strain No. 4 activity.

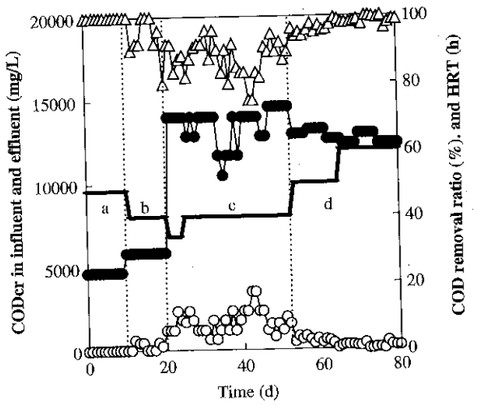

Fig. 5 – COD_{cr} in influent and effluent, removal ratio and hydraulic retention time (HRT) in the continuous experiment using strain No. 4 to treat solids-free wastewater. COD_{cr} is converted to oxygen concentration. Symbols: COD_{cr} in influent (●), COD_{cr} in effluent (○), removal ratio of COD_{cr} (△) and hydraulic retention time (HRT) (solid line). The letters, a–d correspond to the operation conditions shown in Table 2. On day 52, the sludge was drawn off and the feces were added.

4. Discussion

A. faecalis strain No. 4 which has heterotrophic nitrification and aerobic denitrification abilities, was used to treat high-strength ammonium in SFW or MW. In batch culture of SFW, most of the removed ammonium was converted to ammonia gas. This conversion of ammonium to ammonia gas can be attributed to the scarcity of available carbon in SFW for strain No. 4 to utilize abundant nitrogenous compounds, which caused an increase in the pH, and this inturn resulted in chemical conversion of ammonium to ammonia gas. When, however, carbon sources in the form of organic acids or feces were added to the SFW, ammonium removal by assimilation and denitrification by strain No. 4 was observed and conversion of ammonium to ammonia gas was significantly reduced. Consequently, from the nitrogen balance, we estimated that about 45% of the removed ammonium was denitrified (Table 3).

Our previous results indicate that in the aerated batch experiment, approximately half of the ammonium removed by strain No. 4 was denitrified and 90% of the denitrified products was nitrogen gas and 10% was N_2O as determined by the isotope method (Wako, 2002; Joo et al., 2005). Comparison of these data of No. 4 to those of *A. faecalis* TUD (Otte et al., 1999) indicates that strain No. 4 has the advantage to produce much higher N_2 and much lower N_2O.

N_2 gas production rates of *Thiosphaera pantotropha* and *Pseudomonas stutzeri* were calculated to be 0.1 and 2 mg-N/L/h, respectively (Su et al., 2001). From the data for the denitrification of ammonium by strain No. 4 in the batch experiment, we estimated that the N_2 gas production rate was 10 mg/L/h by considering that 90% of the denitrified ammonium was N_2: thus, this indicates that strain No. 4 produces N_2 gas 100 times than *T. pantotropha* and 5 times faster than *P. stutzeri*.

Table 4 – The nitrogen and carbon balances in continuous experiment using strain No. 4 for treatment of the solid-free piggery wastewater. All data was average values in the operation periods

Items	Operation periods used for calculation	
	21–51 days (c)[a]	52–80 days (d)[a]
Load of NH_4^+-N (mg/L/d)	670	837
Influent NH_4^+-N (mg/L)	1084	1901
Effluent NH_4^+-N (mg/L)	116	97
Removed NH_4^+-N (mg/L)	968	1804
Intracellular N[b] (mg/L) (%)[c]	232 (24)	419 (23)
Stripped NH_3-N (mg/L)	74	73
Denitrified N (mg/L) (%)[c]	662 (68)	1312 (73)
Ammonium removal rate (mg-N/L/h)	25	33
Influent COD_{cr}-C (mg/L)	13,491	12,762
Effluent COD_{cr}-C (mg/L)	1679	342
COD removal ratio (%)	87	97
Estimated viable cell number (cfu/ml) of strain No. 4	1.7×10^9	8.8×10^9

[a] Operation time, see Figs. 4 and 5.
[b] Intracellular N is assimilated nitrogen and was calculated by amount of sludge drawn off from reactor and result of elementary analysis of the dry sludge.
[c] Percentage against removed ammonium nitrogen.

The ammonium removal rate obtained in the continuous experiment was about 2–10 times higher than that obtained in the conventional treatment, and about 2–5 times higher than that in the systems using heterotrophic nitrification and aerobic denitrification (Nishio et al., 1998; Gupta and Gupta, 2001; Pollice et al., 2002; Carrera et al., 2003). These data reflect a high ability of ammonia removal of strain No. 4.

We also found that in the batch experiment using a mixture of SFW and feces shown in Fig. 3, the ammonium removal rate was similar to that in the SFW supplemented with 15 g/L sodium citrate in Fig. 2. This indicates that strain No. 4 is active in a mixture of SFW and feces. In the continuous treatment by inoculation of strain No. 4, we observed stable removal of ammonium in both SFW supplemented with external carbon sources and the MW. The number of strain No. 4 colonies was estimated by growth on L agar medium. Although the actual total cell numbers in the SFW and MW systems were not clear, the denitrification ratio and ammonium removal rate in the aerated batch experiment (Table 3 and Fig. 2) and in the continuous experiment (Table 4) were similar to the values in previous experiments in which cells of strain No. 4 were inoculated into sterilized SFM or MW that showed no microbial activities indigenous to these materials (Joo et al., 2005). On the basis of this similarity, we conclude that strain No. 4 is active even in an open system.

In the steady state of the continuous experiments, the denitrification ratio was calculated to be more than 60% and almost no nitrified products were detected. The denitrification ratio was significantly higher in the continuous experiment than in the batch experiment, indicating that strain No. 4 effectively reduces the nitrogen load in continuous flow system in an aqueous environment.

The addition of extra carbon was essential for the efficient removal of ammonium. The criterion for determining the amount of carbon to be added was to maintain a C/N ratio of 7–8. The addition of feces into the SFW provided an appropriate carbon source for strain No. 4 to exhibit its activity both in batch and in continuous cultivation. However, sludge production is inevitable in high-strength wastewater treatment using heterotrophic bacteria (Jetten et al., 1997; Konohana et al., 2000). In the continuous experiment, sludge production corresponding to intracellular N was 23% of the initial ammonium (Table 4). When sludge production was estimated from the influent NH_4^+-N concentration of 2000 mg/L, the dry weight of sludge was approximately 6 g-sludge/L (nitrogen percentage of the dry weight of the produced sludge was about 8%). If the initial total solids (TS) of the mixture, approximately 33,000 mg/L, remained in the sediment in the clarifier, the increase in sludge including the growth of cell mass of strain No. 4 will be 18% of the TS. Thus, composting should be considered as a method for treating this piggery waste sludge that includes less biodegradable organics to minimize the excessive sludge production and to reduce treatment cost of ammonium.

In wastewater treatment using specific microorganisms, the dominance of the inoculated strains is of great importance. Possible means to increase the dominance of specific strains include use of a mixed culture or immobilized cells (Uemoto et al., 2000; Gupta and Gupta, 2001; Seo et al., 2001; Su et al., 2001), or sequencing batch reactors (SBR), which have been used to ensure dominance of specific strains in the conventional treatment of wastewater with high-strength ammonium (Ra et al., 2000; Itokawa et al., 2001; Jung et al., 2004). The removal rates in those reports were 4.8–17 mg-N/L/h. A high removal rate of 64 mg-N/L/h was achieved by supplying diluted and digested piggery wastewater at C/N = 1 (Obaga et al., 2005). In the treatment of piggery wastewater, waste dilution is inevitable because of the high carbon and nitrogen concentrations. However, in the present study, strain No. 4 provided suitable to treat undiluted piggery wastewater with C/N ratios of 8–10, yielding removal rates of 25–35 mg-N/L/h. At present, we are conducting long-term experiments to assess the stability of the treatment efficiency and the survival capacity of strain No. 4.

5. Conclusions

A. faecalis strain No. 4 was used to treat high-strength ammonium in solids-free piggery wastewater (SFW) in both batch and continuous systems.

(1) Strain No. 4 removed ammonium from SFW supplemented with feces at rates of 27.5 and 33 mg-N/L/day in batch and continuous culture experiments. These values are significantly higher than those achieved using other bacteria with similar abilities.
(2) In continuous culture, under well-balanced conditions, the removal ratios of ammonium and COD were almost 100%. These high removal ratios were obtained by controlling the C/N ratio and pH.
(3) Although the exact numbers of cells in the wastewater were not determined, the values of the estimated number of strain No. 4 cells and the denitrification ratio, and the fact that no nitrified products were detected suggested that strain No. 4 exhibited its abilities of heterotrophic nitrification and aerobic denitrification in the piggery wastewater.

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