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Heterotrophic nitrification and aerobic denitrification of high-strength ammonium in anaerobically digested sludge by *Alcaligenes faecalis* strain No. 4

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Alcaligenes faecalis **strain No. 4 which is capable of heterogeneous nitrification and aerobic denitrification, was used to remove high-strength ammonium (approximately 1 g NH_4^+-N/l) from digested sludge, the product of an anaerobic digestion reactor, in which methane was produced from excess municipal sewage sludge. Repeated batch operations were conducted at 20°C and 30°C for 550 h, using a jar fermentor. The removal ratios of high-strength ammonium reached 90–100% within 24 h, and the average ammonium removal rate was 2.9 kg-N/m^3/day, more than 200 times higher than that in conventional nitrification–denitrification processes. During these operations, the cell density was maintained at 10^8–10^9 cells of *A. faecalis* strain No. 4/ml. At 3% NaCl in the digested sludge, strain No. 4 exhibited an ammonium removal rate of 3 kg-N/m^3/day.**

[**Key words:** *Alcaligenes faecalis*; Heterotrophic nitrification; Aerobic denitrification; High-strength ammonium; Anaerobic digestion]

Conventional ammonium removal in wastewater treatment plants consists of nitrification by autotrophs under aerobic conditions, followed by denitrification by heterotrophs under anaerobic conditions. The disadvantages of this system are as follows: (i) the rate of nitrification is slow, and (ii) the autotrophs are vulnerable to high loads of ammonium and organic matter. Therefore, treatment of wastewater containing high-strength ammonium by the conventional method requires wastewater dilution and a large-scale treatment plant due to the long hydraulic retention time required for nitrification (1–3).

In a previous paper (4), we showed that *Alcaligenes faecalis* strain No. 4, which has the ability to carry out heterotrophic nitrification and aerobic denitrification, removed significant amounts of high-strength ammonium and COD from crude piggery wastewater. Recently, several bacteria have been reported to carry out heterotrophic nitrification and aerobic denitrification under low-strength ammonium conditions (5–10).

As an alternative to the conventional method, an anammox method has been intensively studied, mainly because this operation does not require the addition of a carbon source (11–15). However, the problems of the extremely slow growth rate of anammox bacteria and the vulnerability of these bacteria to high concentrations of organic carbon and ammonium must be solved to enable the practical development of wastewater treatment system, especially for wastewaters containing high amounts of ammonium and organic matter.

Due to recent trends of limiting fossil energy consumption, sustainable methods of energy production including anaerobic digestion or bioethanol production have been attracting increasing attention. In anaerobic digestion, livestock waste, municipal garbage, and waste from the food industry are used for the digestion, leading to the production of wastewater containing a high concentration of ammonium. Therefore, the development of an effective method of the wastewater treatment is a crucial factor enabling the production of methane.

In this paper, *A. faecalis* strain No. 4 was applied to remove high-strength ammonium from digested sludge generated in a municipal anaerobic digestion plant to assess the possibility of efficient biological treatment of the wastewater and to observe the performance of strain No. 4 under several operational conditions.

MATERIALS AND METHODS

Strain used The detailed characteristics of *A. faecalis* strain No. 4 were described in previous papers (4,16). Cultured cells of strain No. 4 were mixed with a 50% glycerol solution in vials and stored at −84°C. For each pre-culture, one vial was used as the strain No. 4 inoculum.

Medium used The pre-culture of strain No. 4 used a synthetic medium containing 14 K_2HPO_4, 6 KH_2PO_4, 15 trisodium citrate dihydrate, 2 $(NH_4)_2SO_4$, 0.2 $MgSO_4 \cdot 7H_2O$ (all units in g per liter), and 2 ml of trace mineral solution. The trace mineral solution contained the following components (g per liter): 57.1 EDTA (2,2′,2″,2‴-(Ethane-1,2-diyldinitrilo) tetraacetic acid) 2Na, 3.9 $ZnSO_4 \cdot 7H_2O$, 7 $CaCl_2 \cdot 2H_2O$, 5.1 $MnCl_2 \cdot 4H_2O$, 5.0 $FeSO_4 \cdot 7H_2O$, 1.1 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.6 $CuSO_4 \cdot 5H_2O$, and 1.6 $CoCl_2 \cdot 6H_2O$.

The reactor used The reactions were carried out in a small-scale jar fermentor (total volume 1 L, working volume 300 ml) (BMJ-01PI, Able Corp., Tokyo). Dissolved oxygen (DO) concentrations and pH values were monitored with a DO sensor (SDOC-12F, Able Corp., Tokyo) and a pH sensor (Easyferm Plus 225, Hamilton Bonaduz AG, Bonaduz, Switzerland) inserted into the fermentor. The temperature was controlled at 20°C or 30°C. The oxygen transfer coefficients, k_La were varied by changing the agitation speed from 300 to 700 rpm at a constant air supply rate of 300 ml per min.

Experimental material The digested sludge was supplied by Yokohama Municipal Sewage Treatment Center (Yokohama, Japan) where the excess municipal dehydrated activated sludge was digested at 37°C in a 6000 ton-scale anaerobic

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digester. The main characteristics of the digested sludge are as follows: pH 7.3, 24 mg/l volatile fatty acids, 2700 mg/l total nitrogen, 1200 mg/l ammonium-nitrogen, 150 mg/l soluble BOD, 1000 mg/l total BOD, 900 mg/l soluble COD and 20,000 mg/l total COD.

Experimental procedure The strain No. 4 cells were pre-cultivated in 100 ml synthetic medium in a 500 ml shaking flask at 30°C at a shaking speed of 100 strokes per min (spm) for 2 days.

As control experiments, the ammonium removal was measured under the following three experimental conditions using a jar fermentor: (i) 250 ml of digested sludge plus 50 ml of water, (ii) 250 ml of digested sludge plus 50 ml of water and 20 g of trisodium citrate dihydrate, and (iii) 250 ml of digested sludge plus 50 ml of the strain No. 4 culture. The ammonium removal of the three controls was compared with the removal of the sample containing 250 ml of digested sludge, 50 ml of strain No. 4 culture and 20 g of trisodium citrate dihydrate. In each operation, the jar fermentor was operated at 30°C at an airflow rate of 300 ml per min and an agitation speed of 700 rpm.

In repeated batch experiments, 50 ml of the pre-culture of strain No. 4, 250 ml of the digested sludge and 20 g of trisodium citrate dihydrate were mixed in the fermentor, and the treatment of the ammonium was conducted at an air flow rate of 300 ml per min and an agitation speed of 700 rpm. One ml of the culture was sampled periodically, and the concentration of ammonium was determined. After the ammonium concentration was confirmed to be reduced by more than 90% of the initial concentration, 50 ml of the culture was used for the subsequent treatment by adding a fresh 250 ml of digested sludge and 20 g of trisodium citrate dihydrate. The cell numbers of strain No. 4 were determined at the start and at the end of each cycle of batch cultivation.

In the previous paper (16), we demonstrated the optimal C/N ratio for strain No. 4 was 10, indicating that at this ratio, nitrogen and carbon sources were simultaneously consumed. Based on the ratio, 1 g-N and 10 g-C was balanced and thus 10 g-C corresponded to 38 g of trisodium citrate dihydrate. If no other carbon source existed in the sludge, 38 g of trisodium citrate dihydrate should be added. In this experiment, 20 g of trisodium citrate dihydrate was arbitrarily chosen by expecting existence of some carbon sources in the sludge.

Although the anaerobic digestion was conducted at 37°C, the sludge was stored at room temperature in the treatment center. Thus, the temperatures 20°C and 30°C were selected. As the temperature 30°C was the optimal temperature for No. 4 in the removal of ammonium, which was shown in a previous paper (16), the value of removal rate at 30°C will be optimal. The temperature 20°C can be the most probable temperature in practical operation.

Analytical method The ammonium concentration was determined using an ammonium sensor (SNH-10, Able Corp., Tokyo). To determine the number of cells of strain No. 4, the sampled culture was diluted and plated on synthetic agar plates containing the synthetic medium and 1.5% agar, and then the plates were incubated at 30°C for 2 days. As it was previously confirmed that strain No. 4 grew on the plates significantly faster than other cells indigenous to the digested sludge and that strain No. 4 exhibited characteristic morphological features, the colonies that appeared on the plates after 2 days were counted as strain No. 4 cells and the cell concentration was expressed as cells/ml.

RESULTS

Control experiments Fig. 1 shows the change in ammonium concentration under four experimental conditions using the digested sludge. The ammonium removal by the digested sludge containing strain No. 4 cells and citrate as a carbon source was significantly higher than the removal under the other three conditions. The control containing digested sludge and strain No. 4 cells achieved 20–30% ammonium removal in 10 h, presumably because some carbon sources in the digested sludge were consumed by strain No. 4. Therefore, the additional carbon sources were needed for strain No. 4 to remove more than 90% of the ammonium from the sludge.

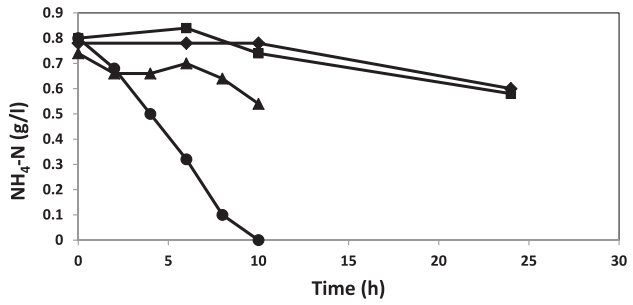

FIG. 1. Ammonium removal from municipal wastewater by *A. faecalis* strain No. 4 under four different experimental conditions using anaerobically digested sludge. Each batch treatment was conducted at 30°C at an agitation speed of 700 rpm in a jar fermentor. The samples consisted of the digested sludge only (diamonds), the digested sludge with trisodium citrate dihydrate (squares), the digested sludge with strain No. 4 cells (triangles) and the digested sludge with both strain No. 4 cells and trisodium citrate dihydrate (circles).

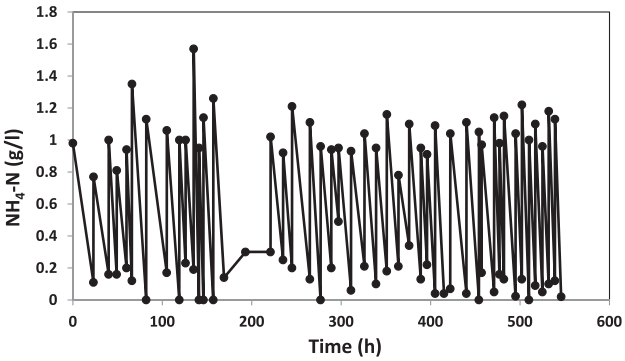

FIG. 2. The ammonium concentration in the digested sludge during repeated batch treatment with *A. faecalis* strain No. 4. These experiments were conducted at 30°C at an agitation speed of 700 rpm in a jar fermentor.

Ammonium removal in the repeated batch experiment Fig. 2 shows the change in ammonium concentration over times in a repeated batch experiment at 30°C, and Fig. 3 shows the change in the number of strain No. 4 cells during the same experiment. Agitation speed was controlled at 700 rpm, resulting in an oxygen transfer coefficient, k_La of 450 h^{-1}, which guaranteed aerobic conditions by maintaining more than 2 mg/l of DO concentration in the reactor. More than 90% of ammonium was removed within 10–20 h, and the number of strain No. 4 cells varied between 10^8 and 10^9 cells/ml. The average ammonium removal rate during the experimental period was 2.9 kg-N/m^3/day. This value is significantly higher than that in conventional nitrification–denitrification processes, and similar to that in an efficient anammox process (11,14). Between 169 and 221 h, the operation was stopped and the jar fermentor was left statically at room temperature. When the operation resumed, ammonium removal was observed without any delay, indicating that interrupted operation causes no adverse effect on the activity of strain No. 4.

Figs. 4 and 5 present the change in ammonium concentration and the change in strain No. 4 cell number, respectively, during operation at 20°C. Although the average ammonium removal rate decreased to 1.5 k-g/m^3/day, half of the rate at 30°C, a relatively high rate of ammonium removal was maintained. The suspension of the operation between 214 and 337 h in Fig. 4 resulted in some delay in the ammonium removal once the operation was re-started. However, the rate of ammonium removal from the second batch

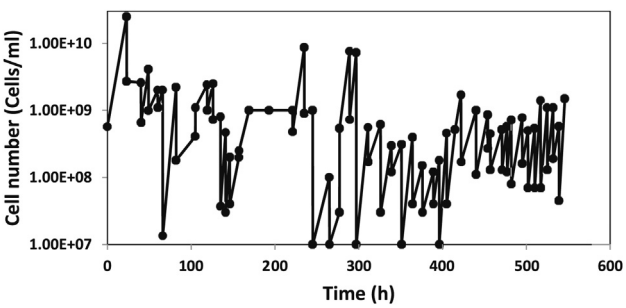

FIG. 3. Change in the number of *A. faecalis* strain No. 4 cells in the same experiment shown in Fig. 2.