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Utilization of Organic Acid Solution Prepared for High-Strength Ammonium Treatment Using *Alcaligenes faecalis* No.4

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Abstract

For efficient treatment of ammonium utilizing *Alcaligenes faecalis* No.4 (No.4) which is capable of heterotrophic nitrification and aerobic denitrification, organic acid solution as a main carbon source for No.4 was prepared by anaerobic degradation of organic matter and sugar. The prepared solution contained total organic carbon (TOC) of 20,049 mg/L and this value was similar to the value of the carbon content of eight organic acids 20,754 mg-C/L, and thus TOC was used as an index to determine the optimal carbon amount to be added to the wastewater. The solution was used to two high-strength ammonium wastewaters, leachate and anaerobic digestion wastewaters, to supplement the carbon, which were deficient in carbon relative to the high ammonium concentration. Complete removal of ammonium of 600-700 mg-NH_4-N/L in the two wastewaters was attained by No.4 based on the ratio of carbon of TOC/ammonium -N 10. During the treatment, the changes in carbon content of eight organic acids were well-correlated with the changes in TOC. The ammonium removal rates of these experiments were approximately 100 times higher than that of conventional ammonium treatment system.

Keywords: Heterotrophic nitrification; Aerobic denitrification; *Alcaligenes faecalis*; Organic acid solution

Introduction

Many bacteria are known to be capable of heterotrophic nitrification and aerobic denitrification [1-9]. The use of these bacteria is more advantageous than the conventional nitrogen removal process of an aerobic nitrification and an anaerobic denitrification because ammonium removal is achieved in one reactor using one type of bacteria under aerobic condition. The ammonium removal rates are higher than those in the conventional ammonium removal process and the short hydraulic retention time is possible. However, the experiments using these bacteria were conducted under low-strength ammonium conditions.

In a previous study [10], we showed that *Alcaligenes faecalis* No.4 (No.4) has the ability to carry out the following heterotrophic nitrification and aerobic denitrification, $NH_4^+ \rightarrow NH_2OH \rightarrow N_2O \rightarrow N_2$. Approximately 40% of ammonium was converted into N_2 gas and 60% in cell mass. Only a small percentage of NO_2^- and NO_3^- was produced from ammonium. No.4 removed more than 90% of high-strength ammonium and chemical oxygen demand (COD) from crude piggery wastewater without dilution of the wastewater [11]. No.4 also exhibited an ammonium removal rate of 3 kg-NH_4-N/m^3/day in the treatment of anaerobically digested sludge from a municipal wastewater plant [12]. Wastewater from a chemical company that contained a high concentration of ammonium (5,000 mg-NH_4-N/L) and a small amount of BOD was treated using No.4 and the average ammonium removal rate was 1.1 kg-NH_4-N/m^3/day [13]. These removal rates were several hundred-fold higher than that in the conventional treatment method.

As No.4 utilizes primarily organic acids as a carbon source and no sugar is available, a chemical agent of citrate was used as a carbon source in those previous experiments. In the previous paper [10], we demonstrated the optimal ratio of organic carbon to ammonium-nitrogen for No.4 was 10, indicating that at this ratio, nitrogen and carbon sources were simultaneously consumed. In practical treatment, inexpensive production and supply of organic acids is a key for the materialization of No.4 in ammonium treatment. In this study, we conducted anaerobic fermentation using leachate as seed supplemented with sugar to obtain high organic acid solution. Then, he prepared mixture of organic acid solution was supplemented with two high-ammonium and low-carbon wastewaters by balancing C/N ratio around to 10 and the effectiveness of the prepared solution was confirmed.

Materials and Methods

Strain

The detailed characteristics of No.4 are described in a previous paper [10]. Cultured cells of No.4 were mixed with a 50% glycerol solution in vials and stored at -84℃. For each pre-culture, one vial was used as the No.4 inoculum.

Synthetic medium

A synthetic medium used was described in detail in the previous papers where citrate was used as a carbon source [12,13]. However, in this study, 12.5 g of sodium lactate was used as a carbon source because the lactate concentration in the prepared organic acid solution was the highest among eight organic acids and the degree of consumption was higher than other organic acids.

Wastewater

The leachate wastewater from a landfill area in B city where the city garbage was land filled was used for ammonium treatment. The total organic carbon (TOC) and ammonium concentration were 4,310 mg/L and 880 mg NH_4-N/L, respectively. The anaerobically digested sludge from a municipal waste treatment plant in Y city which contained approximately 900 mg NH_4-N/L and almost no TOC was also used for

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ammonium treatment. The low TOC was primarily because most of the organic acids produced in the anaerobic digester were converted to methane during digestion.

Preparation of the organic acid solution

Forty milliliter leachate wastewater, and 20 g of glucose were mixed in a 1-liter plastic container and statically incubated at 30°C for two weeks. The TOC and concentrations of eight organic acids in the prepared solution were determined at KURITASU Analyzing Co., Ltd., (Tukuba, Japan).

Reactor

A small-scale jar fermenter (total volume of 1 liter, working volume of 300 mL; BMJ-01PI, Able Corp., Tokyo, Japan) was used. The dissolved oxygen (DO) concentrations and pH values were monitored using a DO sensor (SDOC-12F, Able Corp., Tokyo, Japan) and a pH sensor (Easyferm Plus 225, Hamilton Bonaduz AG, Bonaduz, Switzerland) inserted into the fermenter. The temperature was maintained at 30°C. The agitation speed was fixed at 600 rpm and air was supplied at a constant rate of 30 mL/min.

Experimental procedure in the jar fermenter

The No.4 cells were pre-cultivated in 100 mL of the synthetic medium in a 500-mL shaking flask at 30°C with a shaking speed of 100 strokes per min (spm) for two days, and the culture was used as an inoculum.

The following four experiments were conducted using a jar fermenter:

Experiment 1: Ammonium treatment in the synthetic medium using No.4 and organic acid solution

To verify the use of organic acid solution, 125 mL of the synthetic medium devoid of lactate, 30 mL of No.4 culture and 145 mL of organic acid solution were mixed and the ammonium treatment was conducted. The initial NH_4-N concentration was set at 1,200 mg/L using $(NH_4)_2SO_4$. The volume of organic acid solution was determined so as to be C/N ratio 10.

Experiment 2: Ammonium treatment of leachate wastewater only is using No.4 culture

265 mL of leachate wastewater and 35 mL of No.4 culture were mixed and the ammonium treatment was conducted.

Experiment 3: Ammonium treatment of the leachate wastewater using No.4 culture and organic acid solution

230 mL of leachate wastewater, 30 mL of No.4 culture and 40 mL of organic acid solution were mixed and the ammonium treatment was conducted.

Experiment 4: Ammonium treatment of anaerobically digested sludge using No.4 culture and organic acid solution

For a sample containing high NH_4-N concentration and the least amount of carbon, anaerobically digested sludge wastewater was used. 180 mL of the wastewater, 30 mL of No.4 culture and 90 mL of organic acid solution were mixed and the ammonium treatment was carried out. This volume of organic acid solution was also determined that C/N ratio was 10.

Analytical method

The ammonium concentration was determined using an ammonium sensor (SNH-10, Able Corp., Tokyo, Japan). The nitrite and nitrate concentrations were intermittently determined by the method described in the previous paper [13]. The ammonium exhausted from the reactor by aeration was trapped in the 0.1 N H_2SO_4 solutions and the accumulated ammonium was determined. To determine the number of No.4 cells, the sampled culture was diluted and plated on synthetic agar plates containing the synthetic medium and 1.5% agar, and the plates were then incubated at 30°C for two days. Because it was previously confirmed that No.4 grew on plates significantly faster than other cells indigenous to the leachate sample or anaerobically digested sludge and that No.4 exhibited characteristic morphological features, the colonies that appeared on the plates after two days were counted as No.4 cells, and the cell concentration was expressed as cells/mL. The initial and final values of TOC and the concentrations of eight organic acids in the initial and final samples were determined at KURITASU Analyzing Co., Ltd., (Tukuba, Japan).

Results and Discussion

Prepared highly concentrated organic acid solution

After a 2 week anaerobic incubation of the leachate wastewater, the resulting solution contained 20,049 mg/L of TOC and 52,103 mg/L of total organic acid content of eight types, oxalate, citrate, lactate, formate, acetate, propionate, *iso*-butyrate and *n*-butyrate. The estimated carbon content from the organic acid data was 20,754 mg/L, as shown in Table 1. As the carbon contents in the TOC and organic acid solution were almost similar, TOC was used as an indicator to adjust to the necessary carbon content required to treat ammonium completely by balancing C/N ratio 10.The content of NH_4-N in the prepared organic acid solution was detected to be 600 mg/L and thus the NH_4-N added to the treatment sample was considered to determine the volume of organic acid solution.

Ammonium treatment in the synthetic medium using No.4 and organic acid solution

The result is shown in Figure 1. As 1,200 mg-NH_4-N/L was the initial concentration, for the complete consumption of ammonium, the carbon content should be 10 times the NH_4-N. Therefore, 145 mL of organic acid solution was added to the synthetic medium and the initial TOC was 11,300 mg/L. The ammonium was completely treated and the abrupt increase in DO reflected the exhaustion of ammonium and stop of cell synthesis. The final NH_4-N was 0 and the final TOC was 300 mg/L,

	Content(mg/L)	Carbon content(mg/L)
Oxalate	250	64
Citrate	250	94
Lactate	41202	16481
Formate	479	125
Acetate	4750	1900
Propionate	2402	613
iso-Butyrate	250	102
n-Butyrate	2520	1375
Total	52103	20754

Table 1: Organic acid distribution and carbon content in the prepared organic acid solution.

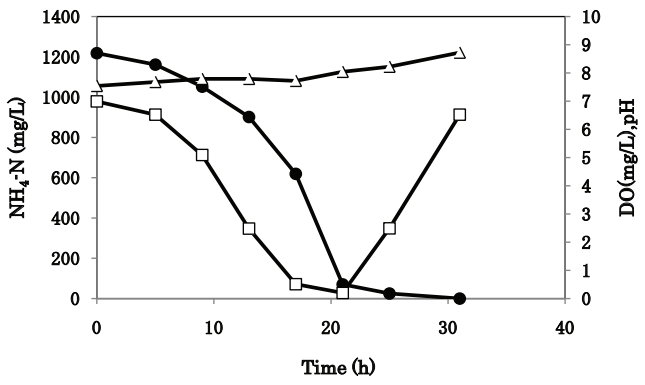

Figure 1: Ammonium removal when the synthetic medium and organic acid solution were mixed. Symbols: NH_4-N(●), dissolved oxygen concentration (DO) (□), and pH(△).

indicating that 97% of TOC was utilized. The changes in carbon content of the 8 organic acids at initial and final samples were shown in Table 2. The initial and final carbon contents were 10,460 mg-C/L and 387 mg-C/L, respectively. These values were almost similar to those measured as TOC. During the treatment, almost no nitrite or nitrate was detected and the evaporated ammonium from the fermenter was less than 0.5% of the initial ammonium concentration. The initial cell number, 7×10^8 cells/mL increased to 4×10^{10} cells /mL at the final stage of the treatment.

Ammonium treatment of leachate wastewater using No.4 culture only

The leachate sample, which contained 4,310 mg/L TOC and NH_4-N 880 mg/L, was mixed with No.4 culture and the ammonium treatment was conducted. From TOC and NH_4-N data, incomplete treatment of ammonia was expected. The result is shown in Figure 2. The initial NH_4-N concentration of 780 mg/L decreased to approximately 240 mg/L of NH_4-N, which remained untreated due to lack of organic acids in the leachate wastewater.

Ammonium treatment of the leachate wastewater using No.4 culture and organic acid solution

As shown in Figure 2, approximately 240 mg/L NH_4-N remained untreated. For complete ammonium treatment, 40 mL of organic acid solution was added to the mixture and the ammonium treatment was conducted. The result is shown in Figure 3. The initial TOC was 7,017 mg/L and the initial NH_4-N concentration was 659 mg/L. The initial value of 8 kinds of organic acids was 17,750 mg/L in which the estimated carbon content was 6,500 mg/L (Table 3). The final value of TOC was 900 mg/L and the final carbon value of 8 kinds of organic acids was 827 mg/L as shown in Table 3. Complete ammonium removal was observed and thus the effectiveness of the use of organic acid solution and the use of TOC as an index to determine C/N ratio was confirmed.

	Initial carbon content(mg/L)	Final carbon content(mg/L)
Oxalate	64	13.3
Citrate	94	9.4
Lactate	8400	296
Formate	65	6.5
Acetate	880	10
Propionate	255	28
iso-Butyrate	102	10.2
n-Butyrate	600	13.6
Total	10460	387

Table 2: Change in the initial and final carbon contents of organic acids in Figure 1.

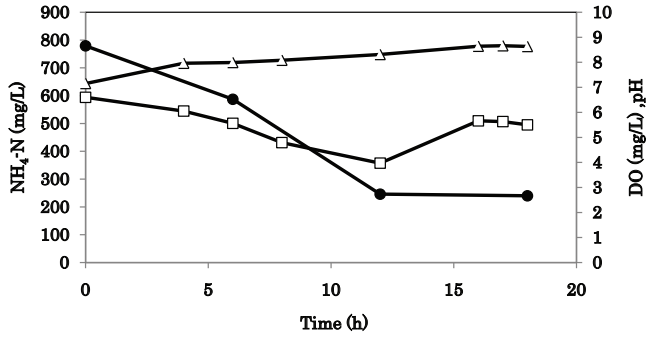

Figure 2: Ammonium removal when leachate wastewater was treated with No.4 culture: Symbols: NH_4-N(●), dissolved oxygen concentration(DO) (□), and pH(△).

Ammonium treatment of anaerobically digested sludge using No.4 culture and organic acid solution

In the previous paper [12] the high-strength ammonium from anaerobically digested sludge, the product of an anaerobic reactor in which methane was produced from excess municipal sewage sludge in Y city, was removed using No.4 with addition of citrate. In this study, a similar sample that contained 900 mg NH_4-N/L and 20 mg/L of organic acids was obtained from Y city plant. The low organic acid content indicated that the available carbon for No.4 is scarce, and supplementation of the organic acid solution is essential for complete removal of ammonium. For the initial 180 mL sludge sample, 90 mL of organic acid solution and 30 mL of No.4 culture were mixed and the ammonium removal was monitored, as shown in Figure 4. The initial NH_4-N concentration 635 mg/L was completely removed. The initial TOC and total carbon of organic acid content were 7,030 mg/L and 7,314 mg/L (Table 4), respectively and the final carbon content of organic acid content was 348 mg/L as shown in Table 4. In this case, consumed TOC/consumed NH_4-N was approximately 9.5. The initial cell number, 3.8×10^8 cells/mL increased to 1.4×10^{10} cells /mL at the final stage of the treatment. In the previous study [12] where citrate was a carbon source, the ammonium removal rate was 3 kg-NH_4-N/m^3/day, but in this study the removal rate decreased to 0.8 mainly because lactate was a main carbon source. This indicates that quality of organic acids gives influence on the activity of No.4.

The ammonium removal rates in 4 experiments are shown in Table 5. The rates varied depending on the kinds of wastewaters. However, these rates were approximately 100-fold higher than those in conventional ammonium treatment system [14,15]. As a source of organic acids to No.4 which is essential for the utilization of No.4 in ammonium removal, unused organic acids can be obtained from different areas such as citrus fruits and the dairy industry. Mixed organic acid production also can be easily conducted in anaerobic fermentation when excess organic matter

	Initial carbon content (mg/L)	Final carbon content (mg/L)
Oxalate	64	64
Citrate	94	94
Lactate	3600	100
Formate	65	65
Acetate	1680	100
Propionate	842	156
iso-Butyrate	102	102
n-Butyrate	136	136
Total	6500	817

Table 3: Change in the initial and final carbon contents of organic acids in Figure 3.

	Initial carbon content (mg/L)	Final carbon content (mg/L)
Oxalate	6.4	6.4
Citrate	9.4	9.4
Lactate	6000	100
Formate	22	6.5
Acetate	720	138
Propionate	181	64
iso-Butyrate	10.2	10.2
n-Butyrate	365	13.6
Total	7314	348

Table 4: Change in the initial and final carbon contents of organic acids in Figure 4.

	Exp.1	Exp.2	Exp.3	Exp.4
Ammonium removal rate (kg-N/m^3/day)	**1.2**	**0.6**	**1.1**	**0.8**

Table 5: Ammonium removal rates in four experiments

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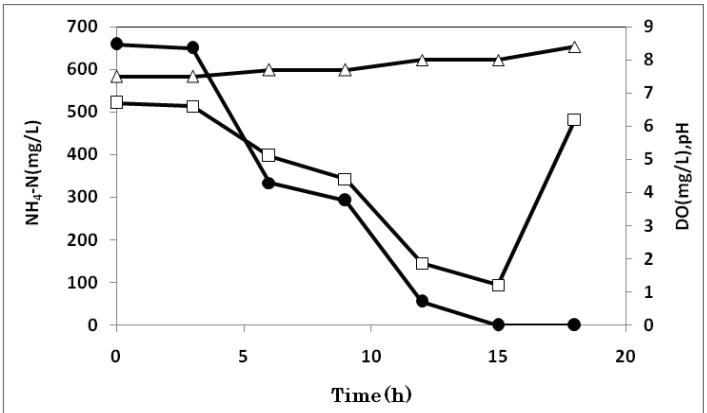

Figure 3: Ammonium removal when leachate wastewater was treated with No.4 culture and organic acid solution. Symbols: NH_4-N(●), dissolved oxygen concentration (DO) (□), and pH(△).

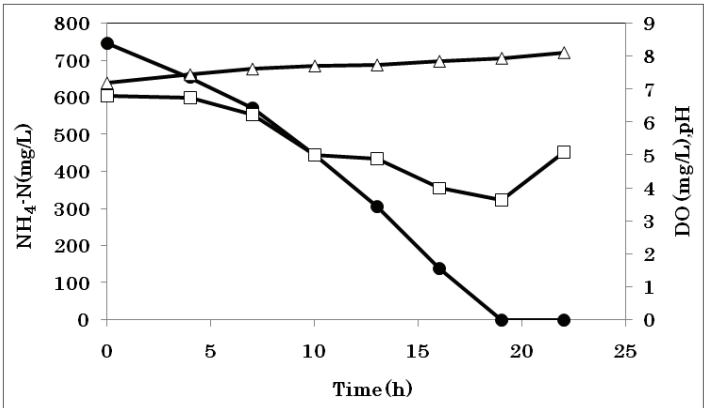

Figure 4: Ammonium removal when anaerobically digested sludge was treated with No.4 culture and organic acid solution. Symbols: NH_4-N(●), dissolved oxygen concentration (DO) (□), and pH(△).

is available. As an index of evaluation of treat ability of the mixture of organic acids, TOC is simple and useful because most organic acids are available for No.4.

As we showed in previous papers [12,13], No.4 is useful for ammonium removal under high saline condition [10,11] and the cells of No.4 have ability to suppress the plant pathogens [16] and to reduce methane production from rumen [17]. This suggests that the reutilization of the excess cells of No.4 after treatment is possible and this reduces the problem of handling of the excess sludge produced after wastewater treatment.

Conclusions

As *Alcaligenes faecalis* No.4 primarily utilizes organic acids as a carbon source, a mixture of highly concentrated organic acid solution was prepared using anaerobic fermentation for practical supplementation of carbon to treat high-strength ammonium by No.4. As TOC and carbon content of eight organic acids produced in the prepared mixture were almost similar and the change in TOC and the change in carbon content of eight organic acids were well-correlated, the volume of organic acid solution which should be added to wastewaters was able to determine by using C/N ratio. By the C/N ratio adjustment, No.4 effectively removed 600-700 mg NH_4-N/L from the two wastewaters within 24 h

By the treatment of the two different wastewaters which exhibited high ammonium concentration, the adjustment of the supply volume of organic acid solution resulted in complete ammonium removal and significant decrease in TOC. As the ammonium removal rates were significantly higher than those in conventional nitrogen removal systems, a compact reactor system will be possible to treat high-strength ammonium, in which ammonium can be converted to N_2 gas and the cell mass of No.4 via heterotrophic nitrification and aerobic denitrification.

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