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Research Progress on Treatment of Low Ammonia Nitrogen Wastewater by Anaerobic Ammonia Oxidation Process

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Abstract

Anaerobic ammonia oxidation (ANAMMOX) process is a is a novel and cost-effective technology with high nitrogen removal capacity. However, in low ammonia nitrogen wastewater the disadvantages of Anammox bacteria, such as low activity and long doubling time, have become important bottlenecks for its promotion. This paper reviews the influencing factors of anaerobic ammonia oxidation process, such as organic concentration, temperature and pH; this paper also introduced the anaerobic ammonia oxidation process under low ammonia nitrogen. The rapid start-up method of the oxidation process, the choice of sludge source and reactor are profiled; The advantages of anaerobic ammonia oxidation reaction coupling to other processes are discussed. Its autotrophic nitrogen removal system has the advantages of low investment and small footprint; The enhanced techniques for anaerobic ammonia oxidation are enumerated. Finally, it was noted that future research directions should focus on autotrophic nitrogen removal.

Keywords

Anaerobic Ammonia Oxidation; Low Ammonia Nitrogen; Autotrophic Denitrification; Bio-Augmentation.

1. Introduction

Water eutrophication caused by nitrogen has always been an important cause of water pollution in China. At present, the main denitrification process used in urban sewage is nitrification - denitrification process, which requires a large amount of aeration and energy consumption [1]. Therefore, in order to save resources and reduce energy consumption, it is urgent to find a new denitrification process with high efficiency and low energy consumption.

Anammox process is a new biological nitrogen removal phenomenon discovered by Deft University in the Netherlands during the operation of fluidized bed reactor[2]. Anaerobic ammonium oxidation (ANAMMOX) is a biological process in which microorganisms directly use nitrite as electron acceptor to oxidize ammonia into nitrogen under anaerobic or anoxic conditions. This reaction can realize the whole process of autotrophic nitrogen removal without additional organic carbon source. Compared with the traditional nitrification-denitrification process, this process can reduce the aeration amount by 50%, the organic carbon source by 100% and the operation cost by 90%, and the sludge yield is lower[3]. This technology is a new type of high efficiency and energy saving denitrification technology with broad application prospect. It has become a hot spot in the research of new biological denitrification technology for wastewater treatment in recent years, and has been applied to the treatment of high-ammonia-nitrogen wastewater such as sludge water and leachate. However, the content of ammonia nitrogen in urban domestic sewage is low, and the actual water temperature of sewage is low. In the field of low temperature and low ammonia nitrogen municipal sewage treatment, the research is still in laboratory scale.

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In order to provide reference for the treatment of low ammonia-nitrogen wastewater by anaerobic ammonium oxidation (anammox), this paper summarized the influencing factors, rapid start-up methods, coupling processes with other reactions and enhanced anammox reaction technology of the process.

2. Influencing factors of Anammox process

Anammox bacteria culture period is long, the growth conditions are harsh, the reaction process and nitrogen removal effect are easily affected by temperature, organic matter, pH and other factors.

2.1 Organic matter

The presence of organic matter will make other heterotrophic strains compete with AnAOB (Anaerobic Ammonia Oxide Bacteria) for the reaction substrate, resulting in the inhibition of the growth of AnAOB. As shown in Figure 1, glucose [4,5] and other organic matter can promote the anaerobic ammonia oxidation reaction at a lower concentration, and the treatment effect is the best at 56.4mg·L^{-1}, while the reaction is inhibited at a higher concentration.

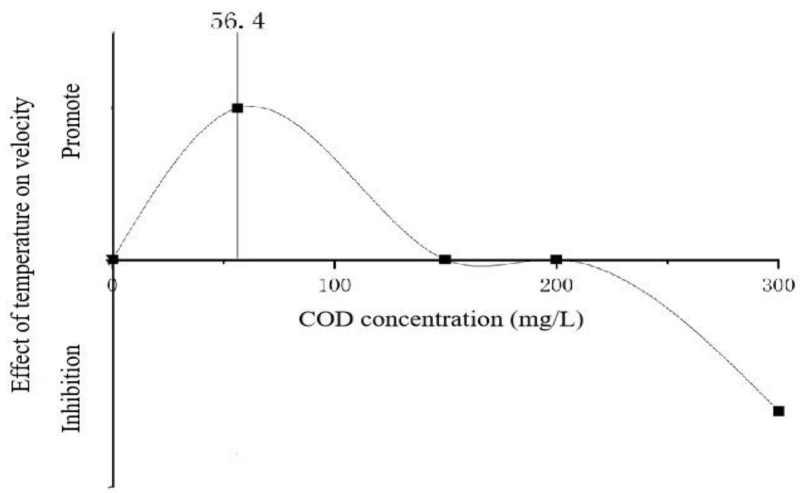

Figure 1. Effect of COD concentration on the reaction

Table 1 lists the influence of common organic matter on the reaction of Anammox. It can be seen that for glucose, sucrose and starch, the effect of maintaining a certain concentration on the reaction is small in the short term, but the reaction will be inhibited if the concentration is maintained at a high level in the long term, and the inhibition on the reaction is reversible.

Table 1. Organic influences on anaerobic ammonia oxidation reactions

Organics	Critical concentration (mg. L^{-1})	Effect on Anammox reaction	References
Sucrose, etc.	80	When organic matter concentration is 80 mg·L^{-1}, the promoting effect of organic carbon source on ANAMMOX is as follows: sucrose > sodium acetate>glucose	[6]
Glucose	150	The COD concentration ≤150mg·L^{-1} can promote the Anammox reaction	[7]
	200	The COD concentration in excess of 200 mg·L^{-1} decreases the activity of ANAMMOX bacteria	
Starch	200	When the concentration of organic matter is 0 ~ 200 mg · L^{-1}, the activity of AnAOB is promoted	[8]
Sodium acetate	40	The COD concentration of 40 mg. L^{-1} promotes the anaerobic ammonium oxidation reaction to the greatest extent	[9]

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2.2 Temperature and pH

Anammox (Anammox) is a functional bacteria (mesophilic bacteria). Anammox can be carried out in the range of 6℃ to 43℃, but when the temperature is lower than 15℃ or more than 40℃, the activity of Anammox will decline sharply[10]. Generally speaking, the optimal growth temperature range is 30 ~ 35℃[11], as shown in Figure 2. Anaerobic ammonia oxidation (SAA) activity is the highest when the temperature is 30 ~ 35℃.

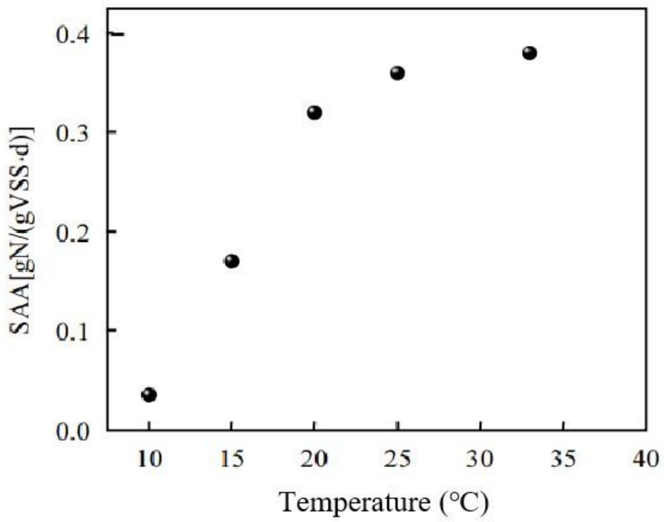

Figure 2. Effect of temperature on anaerobic ammonia oxidation activity

At present, most studies keep the temperature at medium temperature to realize the rapid start of ANAMMOX. However, the actual temperature of domestic wastewater is generally at normal temperature (25℃) or even lower than normal temperature. Studies have shown that the growth rate of AnAOB decreases by 30-40% when the temperature drops by 5℃[12], which is a difficult point in the treatment of municipal wastewater by anaerobic ammonium oxidation process. Especially in Northeast China, the temperature of domestic sewage changes greatly. Therefore, operating the Anammox system at low temperatures requires a sensible startup strategy. First, the required amount of biomass must be produced in separate reactors at near optimal temperatures. Then, biomass is gradually adapted to low temperature in the same reactor, and finally the low-temperature adapted biomass is inoculated into the low-temperature reactor[12]. Through intermittent high-intensity influent and gradual cooling, Bowen Zhang et al.[13] made the ANAMMOX reactor run stably for 28 days at a low temperature (15℃), and the nitrogen load rate reached 1.23-1.34 kg N/m^3/d. The long period and high concentration of ammonia had an inhibitory effect on NOB (nitrite oxidizing bacteria).

Anammox consumes H^+, so the pH increases as the reaction goes on. Too high or too low pH can affect the activity of ANAMMOX bacteria. Van et al.[14] studied the characteristics of Anammox bacteria in a fluidized bed reactor, and found that the suitable pH range of Anammox bacteria was 6.7~8.3, and the optimal pH value was 8.0.

3. Rapid start up of anammox

Anammox bacteria grow slowly, especially in the urban sewage with low ammonia nitrogen at room temperature, which makes it very difficult to start the anaerobic ammonium oxidation process under this condition. The researchers found that the selection of sludge source and reactor is the key to the rapid start-up of the reactor. The selection of both of them should consider how to quickly enrich ANAMMOX bacteria and ensure sufficient biomass to achieve the purpose of rapid start-up.

Anammox bacteria are widely distributed in nature, and the biological density is different in different sludge sources. Therefore, in order to obtain the bacteria with high activity and high density, it is necessary to select the appropriate inoculation sludge. In recent years, researchers have done some

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research on the source of inoculation sludge and achieved some results. Lu Gang et al. [15] inoculated anaerobic granular sludge (R1) and flocculent denitrifying sludge (R2) in a composite anammox reactor, respectively, to investigate the start-up characteristics and granulation degree of anaerobic ammox with different inoculated sludge. The results showed that ANAMMOX was started successfully in R1 and R2 reactors after 45d and 60d, respectively, and the average removal rate of ammonia nitrogen was above 95%. In addition, Anammox red granular sludge with diameter of 0.8 ~ 1.6mm was formed in reactor R1, while the granular sludge in reactor R2 was mainly irregular and flocculent, and the granulation degree was low. This indicates that both anaerobic granular sludge and denitrifying sludge are suitable for inoculation sludge sources. Zhang Zewen et al. [16] inoculated a single type of denitrifying granular sludge and a mixture of denitrifying granular sludge and aerobic nitrifying sludge (volume ratio: 2:1) in two groups of SBR reactor R0 and R1 respectively to start ANAMMOX, and both kinds of reactors successfully started ANAMMOX. However, the results showed that ANAMMOX was successfully started after 64 days of R0, and the total nitrogen removal load was 0.26 kg·(m3·d)$^{-1}$. The total nitrogen removal load of R1 was 0.30 kg·(m3·d)$^{-1}$ in 47 days, which was 17 days shorter than that of R0. This indicates that using mixed sludge as inoculation sludge can accelerate the start-up process of ANAMMOX, and the denitrification performance of the system is more stable after successful start-up. To sum up, the sludge sources that are conducive to the initiation of ANAMMOX reaction are: the mixed sludge of denitrifying sludge and anaerobic sludge.

In recent years, scholars at home and abroad have carried out different transformations on the structure of anaerobic ammonia oxidation reactor, and developed many new reactors. Different reactors have their own advantages and disadvantages. At present, the reactor used to enrich Anammox bacteria is mainly ordered batch bioreactor (SBR), EGSB reactor, membrane bioreactor (MBR), etc. The advantages and disadvantages of the above reactor are compared as shown in Table 2.

Table 2. Comparison of advantages and disadvantages of different reactors

Reactor	Advantage	Disadvantage
SBR reactor	Good biological interception ability, no need for backflow	High requirements for automatic control
EGSB reactor	It can enrich microorganism with high activity and high density	Sludge is easy to drain
MBR reactor	The activity of the bacteria was high and the doubling time was short	Membrane easy to clog, high cost

SBR reactor is the first one to be used to start Anammox reactor. At present, most Anammox processes are SBR reactor. Huang Li et al.[17] used SBR reactor to treat low-matrix simulated wastewater, inoculated anaerobic digested sludge in sewage treatment plant, and successfully started the reactor by gradually shorting hydraulic retention time through 6 stages. The removal rates of ammonia nitrogen and nitrite nitrogen could reach 80.3% and 76.3%, respectively.

In addition to the above two key factors, the influence of temperature, pH, DO and other environmental conditions on the initiation of ANAMMOX reaction should also be considered. Zhang Xiaojing et al.[18] inoculated the ordinary activated sludge in the volcanic rock biofiltration under the condition of 21 ~ 26°C. By controlling the two key influencing factors, low DO value and appropriate pH value, the anaerobic ammox reactor with low ammonia nitrogen could be quickly started and operated stably. Zhang Yonghui et al. [19] adopted low-matrix simulated wastewater to improve the influent nitrogen load by gradually shorting HRT under the condition of (23±0.5) °C. After 83d, the ANAMMOX ASBR reactor was successfully started, and the average removal rates of NH_4^+-N and NO_2^--N were 97.4% and 98.6% respectively.

The above studies show that the rapid start-up of the anaerobic ammonium oxidation reactor with low ammonia nitrogen can be successfully realized by selecting the appropriate reactor configuration,

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inoculating the bottom sludge, and controlling the HRT and DO values and other environmental factors, and high nitrogen removal efficiency can be achieved.

4. Anammox and other coupling processes for the treatment of low ammonia nitrogen wastewater

4.1 Part of the denitrification coupled anaerobic ammonium oxidation process

Despite the high denitrification efficiency of ANAMMOX process, there are still many limitations for a single reaction. In recent years, more studies have been conducted on the coupling of ANAMMOX process with other processes. Some people[20] proposed to completely nitrate ammonia nitrogen in wastewater to nitrate nitrogen, and then reduce nitrate nitrogen to nitrite nitrogen, so as to provide a stable electron donor for ANAMMOX. The process of partial denitrification using heterotrophic denitrification bacteria and then combining with Anammox was developed. The process can realize simultaneous nitrogen and carbon removal, avoid the accumulation of nitrate in the effluent, and provide nitrite for the ANAMMOX reaction through partial denitrification. It has the advantages of simple operation and stable operation.

The realization of partial denitrification coupled Anammox process is affected by many environmental factors, including temperature, pH, DO and so on. The influence of temperature on the process mainly lies in the influence on anaerobic ammonium oxidation bacteria and denitrifying bacteria. Zhao et al.[21] found that in the process of anaerobic ammonium oxidation (ANAMMOX) in the lake, the temperature between 20℃ and 33℃ is more conducive to the coupled reaction of ANAMMOX and denitrification, and the nitrogen removal rate will decrease when the temperature is lower than 20℃ and higher than 33℃. The pH value is an important factor affecting the coupling reaction between ANAMMOX and denitrification. The optimum pH value of anaerobic ammonium oxidation and denitrification coupling process is 7.5. Anammox and denitrifying bacteria are inhibited in aerobic environment. The dissolved oxygen in the coupled reaction of ANAMMOX and denitrification should be controlled below 0.5mg/L[22].

4.2 Partial nitrosation-coupled Anammox process

Nitrite reaction plays an important role in biological nitrogen removal. Nitrite process refers to the conversion of ammonia nitrogen to nitrite nitrogen under the action of nitrite bacteria. This process does not remove nitrogen in the form of gaseous or assimilation into the residual sludge, and other technologies are needed to achieve wastewater denitrification. Therefore, researchers combined it with Anammox (Anammox) process to develop two new nitrogen removal processes. One is a two-stage reaction, represented by Sharon-Anammox; The other is a single stage reaction, represented by Canon. The Sharon-Anammox process first converts about 53% ammonia nitrogen to nitrite nitrogen in the front-end reactor, and then converts nitrite nitrogen and the remaining ammonia nitrogen to nitrogen in the back-end reactor. In the CANON process, nitrite bacteria and anaerobic ammonium oxidation bacteria coexist in the same reactor. Nitrite bacteria located in the outer layer of the biofilm first oxidizes nitrite nitrogen, and then, through mass transfer, the anaerobic ammonium oxidation bacteria located in the inner layer of the biofilm converts nitrite nitrogen and the remaining ammonia nitrogen into nitrogen. Table 3 shows some experimental studies of coupling with Anammox reaction. It can be seen that the coupling process has a good denitrification efficiency and is expected to become the mainstream process of Anammox in the treatment of municipal sewage.

There are limitations to the use of two-stage Sharon-Anammox reaction in municipal sewage. On the one hand, the effluent of nitrosation reaction needs to maintain stable NH_4^+/NO_2^- for the anaerobic ammonium oxidation reaction. On the other hand, the influent ammonia nitrogen concentration of Sharon-Anammox process is generally very high and requires a relatively high temperature condition, which is difficult to maintain for the municipal sewage with low substrate at normal temperature. Under the condition of low ammonia nitrogen, it is difficult to form inhibition of NOB[30], and it is difficult to form stable nitrosation reaction. AnAOB has low activity at low temperature, which leads to a decrease in denitrification efficiency. This problem also exists for Canon process. Table 4 for

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several pilot reactor autotrophic denitrification system (CANON process) operating conditions and denitrification performance, can be seen from the table, in actual operation, the CANON process can realize full autotrophic denitrification, compared with the two levels of response greatly improve sewage treatment, energy consumption and high efficiency of denitrification, especially the SBR denitrification rate reached more than 80%.

Table 3. Partial experimental study of reaction coupling with Anammox

Reaction	Reactor	Volume (L)	Temperature (°C)	DO ($mg\cdot L^{-1}$)	HRT (d)	Nitrogen removal rate (%)	N load ($kg\cdot N\cdot m^{-3}\cdot d^{-1}$)	Refe-rence
SHARON	CSTR	2100	29	2.7	1.05-1.1	85-99	0.56	[23]
SHARON	CSTR	10	35		1		1.2	[24]
Partial nitrification	SBR	20	36	2	1.5		1.5	[25]
Partial nitrification	Biological membrane	10.8	25	5	1	73	1.0	[26]
Partial nitrification	Upflow fixed bed biofilm reactor	11	30	0.8-2.3		62	0.27-1.2	[27]
Partial nitrification	Columns containing PEG carriers	8.0	30	2.5-6.5		77	3.8	[28]
Partial nitrification	MBR	14	35	0.3-0.5	0.67		0.450	[29]

Table 4. Operating conditions and nitrogen removal performance of autotrophic nitrogen removal systems in several pilot reactors

Type of Reactor	Volume (m^3)	Influent type	Temperature (°C)	DO ($mg\cdot L^{-1}$)	Removal rate (%)	Reference
SBR	500	Sludge liquid	25-30	0.3	84	[31]
	400	Sludge liquid	25-30	0.3	90	[32]
UAFB	600	Sludge liquid	30-35	2.0-3.0	75-80	[33]
Moving bed	21	Sludge liquid	23-27	1.2-2.6	62	[34]
RBC	265	Landfill leachate	27-30	0.7-1.0	40-70	[35]
	240	Landfill leachate	10-28	0.8-1.2	30-70	[36]

The above studies show that the partial nitrosation coupled anaerobic ammonium oxidation process is feasible to treat low ammonia nitrogen wastewater, especially the autotrophic nitrogen removal system has outstanding advantages and broad prospects.

5. Strengthening method of anaerobic ammonium oxidation process with low ammonia nitrogen

Anammox (ANAMMOX) is difficult to enrich due to its extremely slow growth and long doubling time. Especially for low ammonia nitrogen wastewater, the application of ANAMMOX process is extremely difficult. To solve this problem, scholars at home and abroad have strengthened the anaerobic ammonium oxidation process from various aspects through many studies, in order to improve the nitrogen removal efficiency. The enhancement of Anammox process is to promote the formation and aggregation of Anammox bacteria and provide a suitable growth environment for them. At present, the common methods for strengthening Anammox process mainly include adding chemical substances and applying physical field, etc.

5.1 Add chemicals

Researchers have found that substances such as MnO_2, graphene oxide (GO), iron ions, and Anammox metabolic intermediates have an effect on the Anammox process, as shown in Table 5. The addition of appropriate amounts can increase the activity of Anammox bacteria.

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Table 5. Effect of adding different chemicals on the anaerobic ammonia oxidation reaction

Added substance	Additive amount	Effect on Anammox reaction	Reference
MnO_2		The denitrification performance of adding MnO_2 was twice that of without adding MnO_2, and the enzyme activity was increased by 78.2%	[37]
GO	0.1g/L	The activity of Anammox was increased by about 10.3%	[38]
Fe^{2+}	0.085mmol/L	Ammonia nitrogen conversion rate is maintained above 90%	[39]
Fe^{3+}	0.08mmol/L	The total nitrogen removal rate reached 85.3% in the activity increasing stage	[40]
K^+	8 mmol/L	The removal rates of NH_4^+-N and NO_2^--N were 89.24% and 84.87%, respectively	[41]
N_2H_4	0.68 mg/L	The total nitrogen removal rate reached about 88.6%	[42]

For urban sewage, methods of adding iron to sewage have been developed for many years. At present, the common method of sludge strengthening is to add a certain amount of ferric salt to the reaction tank to enhance the activity of microorganisms. Generally, ferrous chloride or ferrous sulfate is directly added to the reaction tank to enhance the activity of strains through the action of iron ions, so as to achieve the purpose of improving the treatment effect. But unfavorable long-term cast add these two kinds of ferric salt, the growth that can inhibit microorganism and metabolism.

5.2 Imposed physical field

Extra physical field can affect the structure and metabolism of the organism and the biodegradation rate can be rapidly increased within a certain range of physical field. Based on this characteristic, the influence of ultrasonic wave, electric field and magnetic field on ANAMMOX process was studied. Low intensity ultrasound can speed up the metabolic activities of organisms and improve the activity of enzymes. Duan et al.[43] applied low-intensity ultrasound to Anammox bacteria, and NRR (ammonia nitrogen removal load) increased by 25.5% after 4min of ultrasonic radiation at 25kHz and $0.3W/cm^2$. This effect lasted for 6d, and low-intensity ultrasound may improve the activity of Anammox microorganisms and improve the nitrogen removal efficiency.

In addition to applying ultrasonic wave, electric field can also effectively enhance the nitrogen removal efficiency of Anammox process. Zhang et al.[44] found that the nitrogen removal ability of Anammox was significantly improved when the voltage was $\leq 0.6V$, and the NRR reached $1.21kg/(m^3.d)$, compared with $0.97kg/(m^3.d)$ in the control group. At present, the study of physical field enhancement of Anammox process is not comprehensive enough, and the intensity range of its promotion of activity needs further study.

6. Conclusion and Prospect

For low ammonia nitrogen wastewater, it is difficult for anaerobic ammonium oxidation bacteria to enrich, low temperature and other problems restrict its industrial development. However, in recent years, some breakthroughs have been made in the treatment of low temperature and low ammonia nitrogen wastewater:

(1) The concentration, temperature and pH of organic matter suitable for the growth of ANAMMOX are $56.4mg \cdot L^{-1}$, 30 ~ 35℃ and 6.7~8.3, respectively. The suitable environment can promote the reaction of ANAMMOX.

(2) Under the laboratory scale, an appropriate ANAMMOX reactor is selected, and the mixed sludge of denitrifying sludge and anaerobic sludge is inoculated. By controlling the lower DO value and gradually reducing HRT, the rapid start-up of the ANAMMOX reactor with low ammonia nitrogen can be successfully achieved, and the high nitrogen removal efficiency can be achieved.

(3) Through coupling with other processes, especially for autotrophic nitrogen removal system, the nitrogen removal efficiency can reach 85-99% in the pilot-scale study.

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(4) Adding chemical substances and applying physical field to the reaction can strengthen the ANAMMOX reaction and improve the treatment efficiency of low ammonia nitrogen wastewater.

Among them, autotrophic denitrification system is more feasible than other methods, and has a broad prospect in the treatment of low ammonia nitrogen wastewater. Although there are still some problems in the current research, further research on how to improve the activity of bacteria at low temperature and sustainable research on autotrophic denitrification can be conducted in the future.

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