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Application of Signal Molecule Enhanced ANAMMOX Process in Low Temperature Ammonia Nitrogen Wastewater Treatment

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Abstract: As a new denitrification process, anammox has received extensive attention in the treatment of high ammonia nitrogen wastewater. At low temperature, ANAMMOX bacteria grew slowly and could not stay in the reactor. The reactor performance was poor. As a result, the process is greatly limited in engineering application. Appropriate addition of signal molecules can enhance the activity of ANAMMOX bacteria. The effect of the signal molecule 3-oxo-c8-hsl on the denitrification performance of anammox reactor running continuously at low temperature (15 ℃) was studied experimentally. Two parallel UASB reactors were used, one with 0.67ng/L of 3-oxo-c8-hsl signal molecule (R2) and the other without the addition of signal molecule as blank control (R1). During operation, R2 improved the maximum nitrogen removal rate by 25% compared with R1, and showed better load shock resistance. The batch experiment (SAA) showed that 3-oxo-c8-hsl could significantly improve the activity of anaerobic ammonia-oxidizing bacteria. In addition, 3-oxo-c8-hsl can maintain pH and REDOX potential (ORP) within the appropriate range, providing a stable environment for bacterial growth and reactor operation. The polysaccharides, proteins and total extracellular polymers (EPS) in the reactor increased by 5.4%, 35.2% and 29.7%, respectively, over the original sludge. Particle size and scanning electron microscope (SEM) showed that the sludge particles were compact and particle size was smaller. The addition of signal molecules enhances the reaction performance of ANAMMOX at low temperature, which can promote the application of ANAMMOX process in sewage plants in cold areas.

1. Introduction

Anaerobic ammonia oxidation (anammox) is regarded as a new and promising way of biological denitrification of anaerobic ammonia oxidation under anaerobic conditions with nitrite nitrogen as electron acceptor, ammonia nitrogen as electron donor, complete autotrophic denitrification [1] compared with the traditional nitrification/denitrification, anaerobic ammonia oxidation process can

reduce 63% of the oxygen consumption [2], without additional carbon source, and thus save nearly 90% of the cost [3], in addition also has activated sludge production is low [4], the advantages of low CO2 emission [5] anammox bacteria grow slowly, multiply for a long time, and are extremely demanding on the surrounding environment, which is difficult to retain in the reactor, severely restricting the extensive application of anammox process in engineering. ANAMMOX granular sludge sedimentation performance is good, can be effective for biomass [6], but in high load reactor, easy loss of buoyancy, ANAMMOX sludge particles in the reactor to run unstable [7]. Some scholars conducted research and put forward the corresponding control strategies, such as broken floating sludge particles to add to the reactor [8], or control the right hydraulic shear rate, etc. [9] however, these methods can not fundamentally control condensation of ANAMMOX microbial physiological behavior makes it spontaneously into more close-grained, particle sedimentation performance better.

Phenomenon between bacterial quorum sensing (QS), they through signal points into lines cross flow [10]. Already there are research table Ming ANAMMOX sludge is regulated by the quorum sensing system [11]. ZHAO [12] research shows that under low load, such as zhao inhibit AHLs synthetic signal molecules can cause ANAMMOX EPS content reduce PN/PS particles increases, the disintegration of particle sedimentation performance becomes poor, and led to the sludge activity. These studies indicate that AHLs of ANAMMOX Sludge sedimentation performance and activity have important influence.

Currently, there are few studies on the influence of signal molecules on ANAMMOX sludge without reports on the influence of exogenous signal molecules on the sedimentation performance of ANAMMOX granular sludge, and most studies are conducted at room temperature. Therefore, this paper adopted the externally added signal molecule 3-oxo-c8-hsl with good effect and easy access to conduct long-term test at the reaction temperature of 15 ℃ (80d). A new direction was proposed for the treatment of high ammonia nitrogen wastewater by anaerobic ammonia oxidation at low temperature.

The main purpose of this study was:

(1) Effects of signal molecules on the denitrification performance of anammox process at low temperature Second item.

(2) Effects of signal molecules on the activity of anammox bacteria at low temperature.

(3) Effects of signal molecules on the environmental regulation of anammox bacteria and sludge granulation at low temperature.

2. Material and Methods

2.1. Signal Molecules

ANAMMOX bacteria can release the AHLs signal molecule c8-hsl [13], the addition of such a signal molecule at normal temperature can affect the sludge characteristics through the quorum sensing system of ANAMMOX sludge. Therefore, the AHLs signal molecule c8-hsl was adopted in the experiment to study its influence on the anaerobic ammox reactor at low temperature. The signal molecule 3-oxo-c8-hsl was purchased from sigma-aldrich, USA.

2.2. Microorganism and Culture Medium

The anaerobic ammoxidation sludge used in the laboratory is taken from the reactor operated by continuous culture at low temperature in the laboratory. Table 1 shows its main physical and chemical properties.NLR:2.42 kg/(m^3·d) NRR removal load is 2.06 kg/(m^3·d).

Table 1: Main physical and chemical properties

parameter	SS(mg/L)	VSS(mg/L)	VSS/SS	Particle size(mm)
Sludge particle performance	9291.67	8333.33	0.897	3±1

2.3. Continuous Experiment

Two identical upflow anaerobic sludge beds (UASB) were used in the experiment, with a total effective volume of 7.3L, an effective volume of 5.2L in the sedimentation zone, and a volume of 2.1L in the reaction zone. The inner diameter of the reaction zone is 7 cm, and the outer sleeve is 2 cm thick to realize the water bath cycle and control the temperature of the reaction zone. Inoculate 2 identical UASB reactors (R1 and R2) with the same anaerobic ammoxidation sludge .R1 was not added with signal molecules, and as a blank control, R2 was added with 20ml of 3-oxo-c8-HSL 1.67mg /L. Initial hydraulic residence time (HRT) is R1 and R2 are both 1 day: nitrogen load can be increased by shortening the hydraulic residence time. The whole reactor is surrounded by black cloth to avoid the light, so as to avoid the growth of photosynthetic bacteria.

2.4. Anammox Reactive Batch Test

At the end of continuous operation, microorganisms in the reactor R1 and R2 were taken Batch experiments were conducted to compare the activity of anammox bacteria in detail. In 150 mL serum bottles of 100 mL anaerobic ammox suspension, microbial concentration was 2g VSS/L, NH4Cl and NaNO2 were 100 mg/L and 132 mg/L (according to theoretical stoichiometric ratio), respectively, and the remaining medium was the same as described in section 2.2.Nitrogen was exposed for 1 h to remove dissolved oxygen. The bottle was sealed with a rubber plug and cultured in a shaking table at a constant temperature of 15 °C and 150 r/min under dark conditions.

2.5. Analytical Methods

During continuous operation, samples are collected every 1 d.$NH4^{+}$, $NO2^{-}$, EPS and VSS were determined according to the standard method [14]. PH and ORP A portable analyzer was used for the determination by glass electr [15]. Temperature: Electronic digitai method thermomete; Polysaccharide: Anthrone-Sulfuric Acid method; Protein: Bradford method; The EPS was extracted using the heating method [16]. All chemicals were analytically pure and the result was 2 Secondary measurement average.

2.6. Scanning Electron Microscope (SEM) Observation

The anaerobic ammoxidation sludge in the reactor R1 and R2 on the first and last day of operation was taken and its morphological characteristics were observed by SEM, FEI, USA. The sample was firstly treated with 2.5% (V/V) glutaraldehyde solution with a pH of 7.2 for 2 h. Then the sample was dehydrated by ethanol gradient, with the volume concentration from low to high being 50%, 70%, 80%, 90%, 95% and 100%, respectively. The solution was dehydrated for 10 min at each concentration.

3. Results and discussion

3.1. Continuous Experiments on the Effects of Signal Molecules on the Denitrification Performance of the Reactor at Low Temperature

According to the experimental results Fig. 1. From 1 to 15 days after operation (152mg/L of water in ammonia nitrogen and 200mg /L of nitrite): R1 average removal rate of ammonia nitrogen reached 92.7% and that of nitrite nitrogen reached 95.3%.The average ammonia nitrogen removal rate of R2 reactor was 93.6%, and the average nitrite nitrogen removal rate was 95.4%, slightly better than R1. In the first 15 days, the treatment effect of R1 and R2 reactors was gradually enhanced. Starting from the 16th day, the HRT of R1 and R2 in the two reactors was shortened to 0.7d, and the nitrogen load subsequently increased R1, and $NO2^-$-N accumulation occurred in R2, and $NO2^-$-N/$NH4^+$-N consumption ratio decreased, which significantly reduced the performance of the two reactors. When the high-load operation continued for 50 days, the effect of R1 in the reactor continued to deteriorate, and the average ammonia nitrogen removal rate of R2 at the end of stable operation and high-load operation was 70%, and the average nitrite nitrogen removal rate was 75%. Reset HRT to 1d on day 60. The influented ammonia nitrogen and nitrite nitrogen load of R1 and R2 were reduced to 100 mg/L and 132 mg/L, respectively, and the properties of R1 and R2 were recovered to a certain extent. Finally, the nitrite nitrogen removal rates of R1 and R2 on the 80th day were 89.0% and 92.3%, respectively, and the ammonia nitrogen removal rates were 90.0% and 93.3%, respectively.

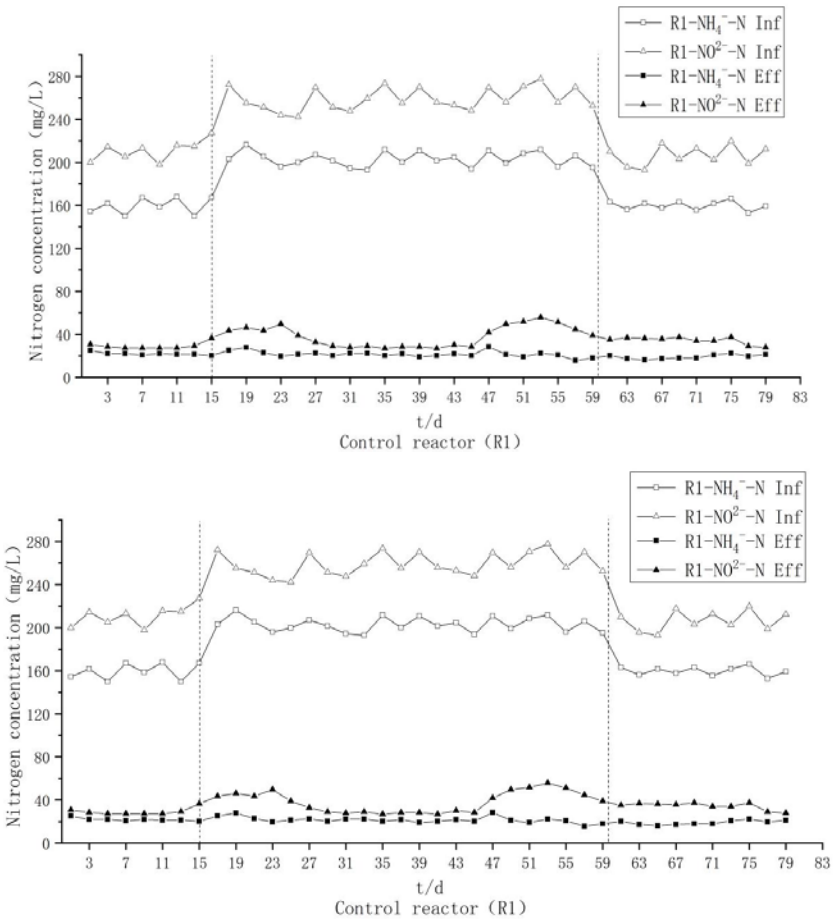

Figure 1: The variations of NH4+ -N and NO2--N concentration in influent and effluent

For each increase in nitrogen load, R2 of the reactor with the addition of signal molecule 3-oxo-c8-hsl showed better impact load resistance, and the concentration of $NH4^+$-N and $NO2^-$-N in the effluent could quickly drop to a low level and become stable after a small increase. The NLR of R1 and R2 reached a maximum of 2.42kg/(m^3 • d), while the NRR of R1 and R2 reached a maximum of 2.15kg/(m^3 • d) and 2.25kg/(m^3 • d).The experimental results showed that the addition of tourmaline could rapidly improve the reactivity of anammox bacteria, improve the denitrification efficiency of the reactor and the impact resistance of the reactor.

3.2. Batch Experiment on the Effect of Signal Molecules on Anaerobic Ammonia Oxidation Activity

After the continuous operation of the reactor for 80 days, the activity of anammox bacteria (SAA) in the two reactors was studied by batch experiment. As shown in Fig. 2. After 24 h of culture, $NH4^+$-N and $NO2^-$-N removal rates of samples from control reactor R1 reached 88.2% and 92.0%, respectively, while $NH4^+$-N and $NO2^-$-N removal rates of samples from addition of signal molecule reactor R2 reached 94%.The $NO2^-$-N consumption of R1 and R2 /$NH4^+$-N consumption mean values are 1.41 and 1.32, respectively. After culture for 3 h, R2 showed obvious SAA growth, and its value was 119.7% of R1.The results showed that the signal molecules improved the denitrification performance of anammox bacteria at low temperature. Reference [17] showed that exogenous addition of c6-hsl could significantly improve the activity of ANAMMOX bacteria under normal temperature and low load (total nitrogen load less than 0.2 kg-N /(m3 • d)). ZHANG [18] showed that the addition of signal molecule c8-hsl in UASB reactor with high load could improve the activity of ANAMMOX granular sludge. This study showed that the addition of signal molecule c6-hsl could also improve the activity of ANAMMOX granular sludge at low temperature.

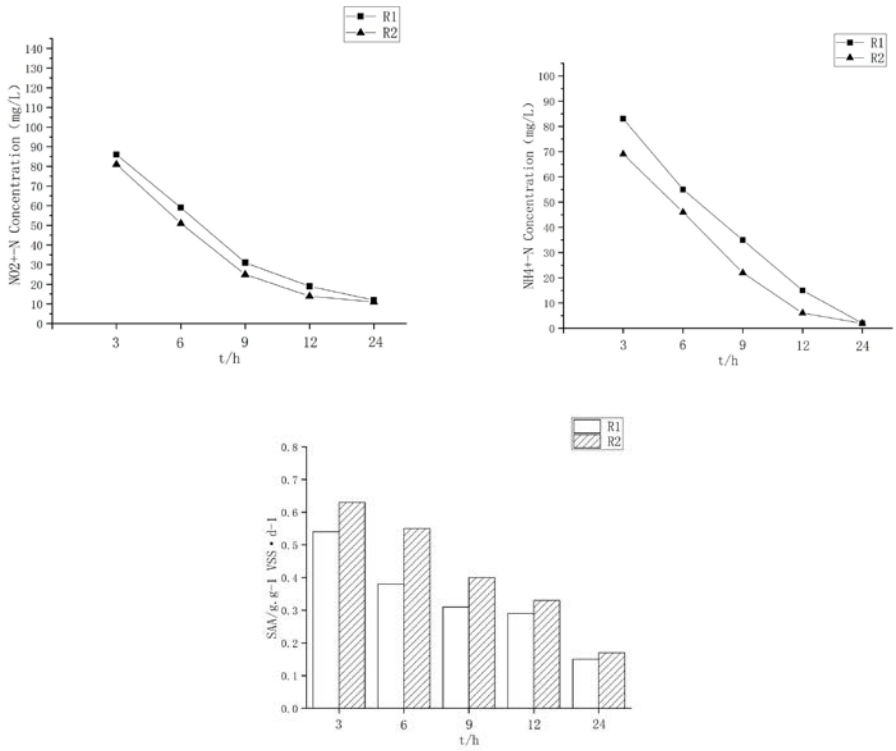

Figure 2: The variations of NH4+-N concentration. NO2--N concentration. Activity of anammox bacteria (SAA) during the cultivation from 3 h to 24h

3.3. Analytical Methods

In Fig. 3, the effluent pH of R2 in the 3-oxo-c8-hsl reactor with the addition of the signal molecule was closer to the optimal pH of 6.7~8.3 for anammox. Under the condition of constant HRT, the increase in the effluent pH could indicate the improvement of anammox reaction performance. In Fig. 4, the ORP of R2 is generally higher than that of R1, which may be caused by the increased activity of anammox bacteria in R2 to generate more NO_2^--N oxidation state.

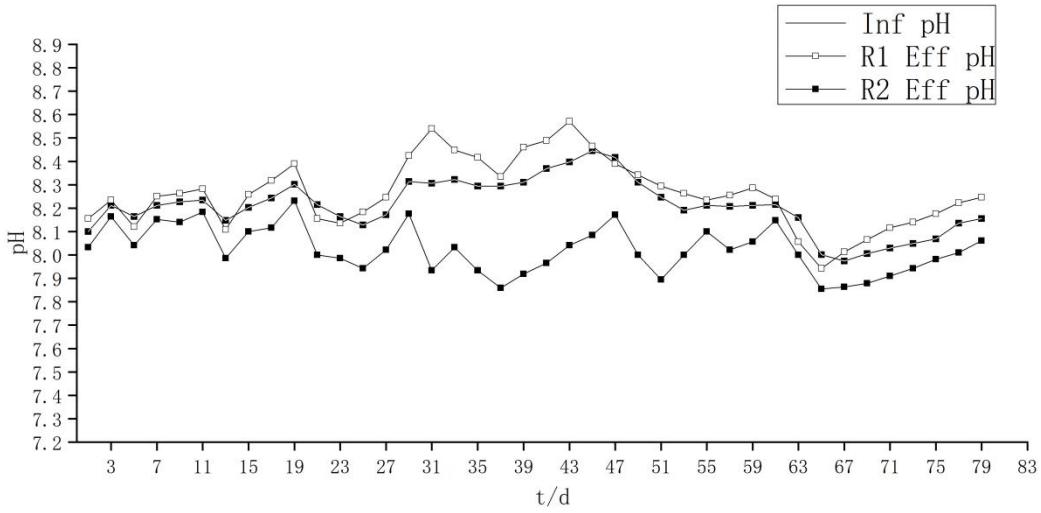

Figure 3: The variations of pH in influent and effluent

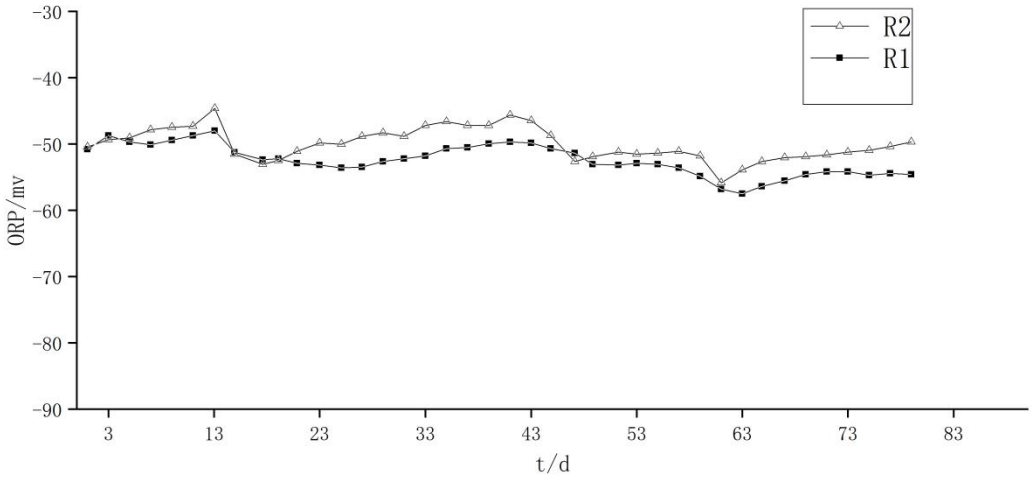

Figure 4: The variations of ORP in R1 and R2

The addition of 3-oxo-c8-hsl can adjust the pH value in the environment, so that the anammox bacteria are in the acid-base balance with the optimal pH condition constant, which is an important condition for maintaining the normal metabolism of anammox bacteria. The appropriate pH range of anammox reaction reported in the literature is 6.7~8.3 [18], and the maximum reaction rate

appears around 8.0.According to the thermodynamic parameters related to anammox reaction, the electric potential of the standard electrode on $N2/NH4^{+}$ and $NO2^{-}/N2$ is 274mV and 976mV respectively (relative to the standard hydrogen electrode), and the standard electromotive force of the whole anammox reaction battery is 702 mV, so anammox reaction needs higher ORP than other anaerobes [19]. Adding signal molecules to the reactor can regulate the pH and ORP in a certain range and provide a stable environment for the growth of bacteria and the operation of the reactor.

3.4. Extracellular Polymer (EPS) and Sludge Granulation

ANAMMOX granular sludge EPS content and composition of stability in the ANAMMOX granules and sedimentation performance plays an important role in [20]. The sludge particles in this experiment reactor R1 R2 when compared with the inoculation sludge EPS content slightly higher (Fig. 5) this is because the high nitrogen load will stimulate the release of a large number of ANAMMOX granular sludge EPS [21]. 40 days reactor in EPS content is 4% lower than the control group R1 R2 MLSS (72 mg/g), which PN content was lower than those of R1 6% (52 mg/gVSS) The content of EPS in R2 of the reactor was 6% lower than that in the control group (77mg/g MLSS). The content of PN was 8% lower than that in R1 (55mg/g MLSS), and the content of PS was 7% higher than that in R1 (22mg/gVSS).

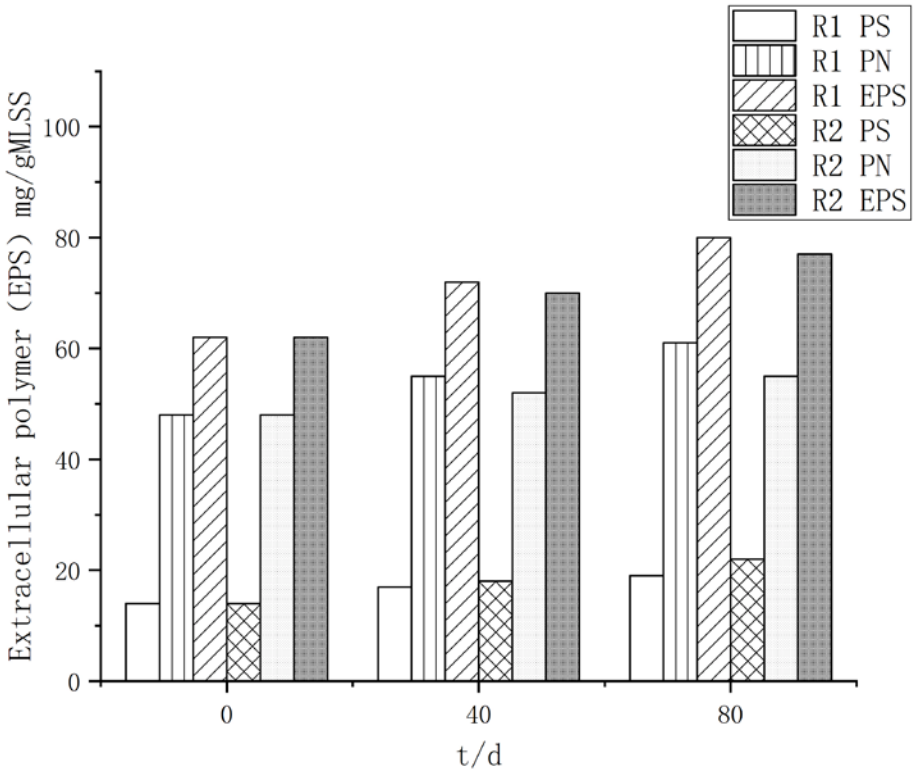

Figure 5: The variations of EPS of anammox bacteria

Yang [22] research points out that excessive EPS can have negative effects on the sludge sedimentation performance, because of excessive EPS bringing water to the sludge produced in the aggregation leads to the low density of the porous flocs. In addition, the proportion of protein and polysaccharide (PN/PS) is usually used to evaluate the strength of the particles and sedimentation

performance.results point such as Zhang [23] PN/PS in the ANAMMOX particle settling properties, shear strength plays an important role in aspects such as higher PN/PS Leads to lower sludge particle shear strength, the fluid viscosity is higher, so the particle sedimentation performance degradation. The sludge particles higher PN/PS means low intensity and poor sedimentation performance, low PN/PS particles is more stable, more tightly, sedimentation performance better. This study than PN/PS values of granular sludge in the R2 R1 decreases, this is the settlement of granular sludge in R2 obviously improve the performance of another.

In Fig. 6, at day 40, the volume-weighted average particle size of R1 in the reactor was 3.5mm and R2 was 3.3mm. At day 80, the average particle size of R1 and R2 increased to 3.7mm and 3.3mm respectively (Fig. 6). Theoretically, low temperature will increase EPS yield (Fig. 5), which will promote sludge granulation, increase sludge particle size and accelerate sludge floating. However at low temperature by adding the R2 signaling molecule in the reactor anaerobic ammonia oxidation bacteria while EPS production but enhance level is lower than the average particle size of anaerobic ammonia oxidation bacteria in the R1 increased levels, visible add signal molecules slow down the speed increase floating sludge particles at low temperatures, the addition of signaling molecules made more stable.

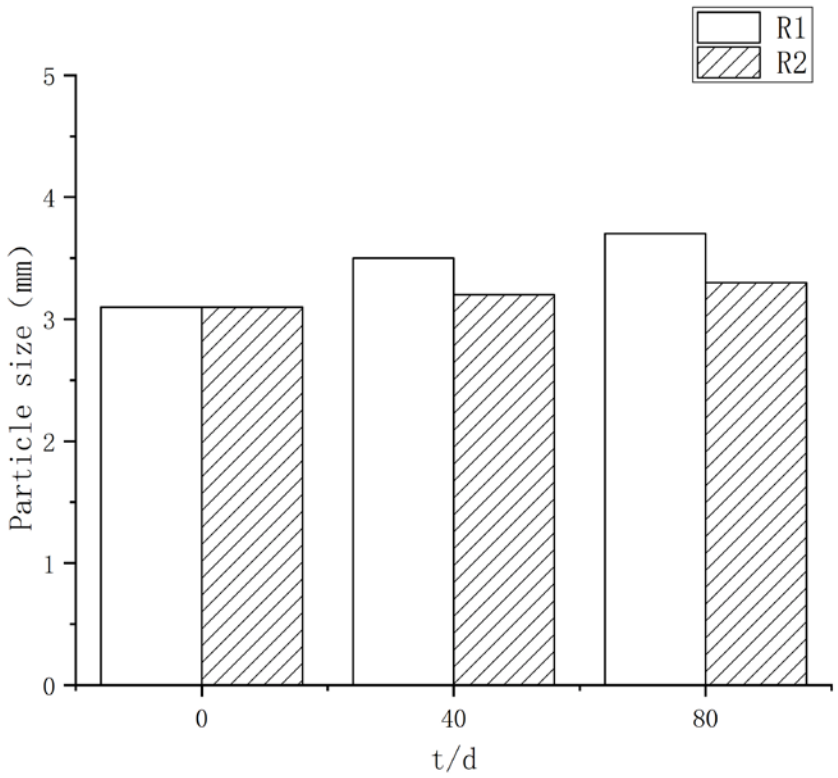

Figure 6: The variations of EPS of anammox bacteria

The growth state of bacteria can be observed by scanning electron microscope (SEM) (Fig. 7). Inoculated anaerobic ammonia oxidation bacteria mainly spherical, running late (80 d), the bacteria in the R1 and R2 are homogeneously spherical, a smooth surface, but didn't add the signal molecular reactor R1 bacteria around the bacteria produce extracellular polymers (EPS) is obvious increase, gather each other between the strains are linked together, so is the sludge particle size of R2 is big. This phenomenon may be caused by the decrease of EPS and PN/PS due to the addition

of signal molecules, so that the sludge has higher strength and better sedimentation performance, which is more conducive to the stable growth of anammox bacteria and improve the reaction activity.

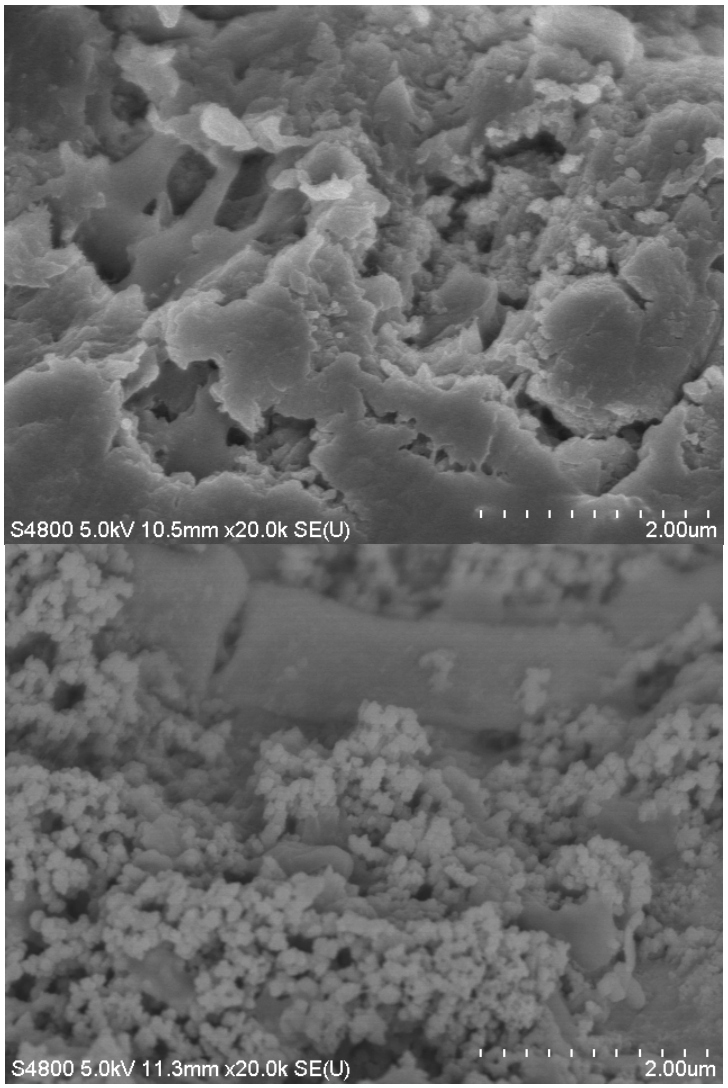

Figure 7: The variations of EPS of anammox bacteria.

4. Conclusions

The addition of the signal molecule 3-oxo-c8-hsl greatly improved the performance of the anammox reactor at low temperature, the impact resistance of the reactor was enhanced, the sludge particles were compacted, and the settling capacity was enhanced. The strengthening method in this experiment has important reference value for the application of ANAMMOX process in cold regions.Specific conclusions are as follows:

(1) During long-term low-temperature operation, add the signal molecule 3-oxo-c8-hsl reactor for nitrogen removal. The performance was improved, the highest NRR was improved by 11.4% compared with the control reactor, and the impact load resistance was better.

(2) At low temperature, the signal molecule 3-oxo-c8-hsl can regulate pH and ORP to make it more suitable for the growth of anammox bacteria and provide a stable environment for the growth of bacteria and the operation of the reactor.

(3) At low temperature, polysaccharide, protein and total EPS of R2 in the reactor with the addition of signal molecule 3-oxo-c8-hsl were increased by 5.4%, 35.2% and 29.7%, respectively, compared with R1, indicating that the signal molecule 3-oxo-c8-hsl promoted cell growth and metabolism.

(4) The batch experiment of anammox activity showed that the addition of signal molecule 3-oxo-c8-hsl at low temperature significantly improved the activity of anammox bacteria, and the maximum SAA was 46.6% higher than the control.

(5) Particle size and SEM test showed that the addition of signal molecule 3-oxo-c8-hsl reduced the particle size of sludge to a certain extent, and the surface tended to be smooth, improving the sedimentation performance.

References

[1] Strous M, Heijnen J J, Kuenen J G, et al. (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Applied Microbiology and Biotechnology, 50, 589-596.

[2] Strous M, Fuerst J A, Kramer E H M, et al. (1999) Missing lithotroph identified as new Planctomycete. Nature, 400, 446-449.

[3] Volcke E I P, van Hulle S W H, Donckels B M R, et al. (2005) Coupling the SHARON process with Anammox: model -based scenario analysis with focus on operating costs. Water Science and Technology, 52(4), 107-115.

[4] Furukawa K, Inatomi Y, Qiao S, et al. (2009) Innovative treatment system for digester liquor using anammox process. Bioresource Technology, 100, 5437-5443.

[5] Shen L D, Hu A H, Jin R C, et al. (2012) Enrichment of anammox bacteria from three sludge sources for the startup of monosodium glutamate industrial wastewater treatment system. Journal of Hazardous Materials, 199/200, 193-199.

[6] Kartal B, Kuenen J G, van Loosdrecht M C. (2010) Sewage treat-ment with anammox. Science, 328(5979), 702 - 703.

[7] Liu S T, Yang F L, Xue Y, et al. (2008) Evaluation of oxygen adaptation and identification of functional bacteria composition for anammox consortium in non - woven biological rotating contactor. Bioresource Technology, 99, 8273-8279.

[8] vander Star W R L, Abma W R, Blommers D, et al. (2007) Startup of reactors for anoxic ammonium oxidation: experiences from the first full -scale anammox reactor in Rotterdam.Water Research, 41(18), 4149-4163.

[9] Tsushima I, Ogasawara Y, Kindaichi T, et al. (2007) Development of high -rate anaerobic ammonium -oxidizing (anammox) biofilm reactor. Water Research, 41, 1623-1634.

[10] Tang C J, Zheng P, Wang C H, et al. (2011) Performance of high-loaded ANAMMOX UASB reactors containing granular sludge. Water Research, 45, 135-144.

[11] Strous M, Kuenen J G, Jetten M S M. (1999) Key physiology of anaerobic ammonium oxidation. Applied Microbiology and Biotechnology, 65, 3248-3250.

[12] Liu S T, Yang F L, Meng F G, et al. (2008) Enhanced anammox consortium activity for nitrogen removal impacts of static magnetic field. Journal of Biotechnology, 138, 96-102.

[13] Liu S T, Yang F L. (2009) The Enhancement by Electrical/Magnetic Field and Multispecies Coupling of Anammox Technology for Autotrophic Nitrogen Removal. School of Environ - mental Science and Technology, Dalian University of Techno- logy, China.

[14] Chen J W, Ji Q X, Zheng P, et al. (2010) Floatation and control of granular sludge in high-rate anammox reactor . Water Research, 44, 3321-3328.

[15] Chen H, Ma C, Yang G F, et al. (2014) Floatation of flocculent and granular sludge in a high-loaded anammox reactor . Bioresource Technology, 169, 409-415.

[16] GAO M J, WANG S Y, PENG Y Z, et al. (2016) Influence of pulsed flow fluctuation on UASB reactor of anaerobic ammonia oxidation. China Environmental Science, 36(8), 2347- 2354.

[17] Lu H F, Zheng P, Ji Q X, et al. (2012) The structure, density and settlability of anammox granular sludge in high-rate reactors. Bioresource Technology, 123, 312-317.

[18] Lu H F, Ji Q X, Ding S, et al. (2013) The morphological and settling properties of ANAMMOX granular sludge in high-rate reactors. Bioresource Technology, 143, 592-597.

[19] APHA, Standard Methods for the Examination of Water andWastewater.

[20] Morgan J.W., Forster C.F., Evison L. (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges, Water Res., 24, 743-750.

[21] Tang X, Liu S T, Zhang Z T, et al. (2015) Identification of the release and effects of AHLs in anammox culture for bacteria communication. Chemical Engineering Journal, 273, 184-191.
[22] ZHANG J, ZHANG L H, JIANG D D, et al. (2018) The effects of signal molecules on the characteristics of ANAMMOX granules in high-loaded UASB reactors. China Environmental Science, 38(02), 532-541.
[23] Jetten M S M, Strous M, van de Pas Schoonen K T, et al. (1999) The anaerobic oxidation of ammonium. FEMS Microbio-logy Reviews, 22, 421-437.