

# Study on Enhancement of Single Stage Autotrophic Biological Denitrification System by Ultrasonic Wave

Li Zhang, Lei Zhang, Qi Jiang and Diannan Huang\*

*School of municipal and environmental engineering, Shenyang Jianzhu University, Shenyang, Liaoning Province, China*

**Keywords:** Ultrasonic Strengthening, Canon, Extracellular Polymer.

**Abstract:** The application of one-stage autotrophic nitrogen removal is hampered by its overlong start-up and instability under disturbance. A lab-scale batch tests were used to explore the optimal ultrasonic strengthening parameters, which were determined based on changes in the activity of the Canon system and EPS. The optimal ultrasonic intensity was 0.3 W/cm<sup>2</sup>, and the activity of Canon system was 40.68 mg N(gVSS<sup>-1</sup>)d<sup>-1</sup>. The optimal ultrasonic time was 4 min, and the activity of Canon system was 41.02 mg N(gVSS<sup>-1</sup>)d<sup>-1</sup>. Under the best ultrasonic intensity and ultrasonic time, it can be maintained for 6 days.

## 1 INTRODUCTION

The single-stage autotrophic biological nitrogen removal process is one of the new biological nitrogen removal processes promoted in recent years. It has the advantages of low oxygen consumption, no need to add organic carbon source, small reactor footprint, etc. In addition, this process can improve nitrogen removal efficiency, reduce nitrogen removal cost, and develop the theory and engineering application of nitrogen removal (Tang, 2015).

In order to reduce the possibility of instability of nitrogen removal system and stimulate the growth of Anammox bacteria, low-frequency ultrasonic with mild action conditions, wide application range and no secondary pollution was adopted in this study for enhancement (Zhang, 2017). Ultrasonic wave is a mechanical wave with multiple parameters, and the corresponding parameters of different biological nitrogen removal systems are not the same. Therefore, it is of certain significance to explore the influence of ultrasonic parameters on the efficiency of single-stage autotrophic biological nitrogen removal system.

Therefore, in order to increase the nitrogen removal efficiency of the single-stage autotrophic biological nitrogen removal system, low-frequency ultrasonic batch test was used in this part to strengthen the single-stage autotrophic biological nitrogen removal system to study the optimal working parameters. Fixed ultrasonic time, gradient

change of ultrasonic intensity, the effect of ultrasonic intensity on biological denitrification system strengthening efficiency, extracellular polymer and each component were studied.

## 2 TEST MATERIALS AND METHODS

### 2.1 Test Device and Operation

A certain amount of Canon system sludge was inoculated and placed into 100 mL serum bottles, and artificially simulated wastewater was added to the 100 mL scale line. The initial biomass concentration of batch experiments was 1g VSS/L. The ultrasonic generator has a fixed ultrasonic frequency of 25KHz, and the power is between 40 and 200W.

The entire serum vial is covered with a black film. Each serum flask was cultured in a shaker at (30±1) °C. Samples were taken every 12 hours with a long syringe needle and the concentrations of NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N in the water samples were measured. The Canon system activity was calculated based on the change of total nitrogen concentration over time. Take the average in triplicate as the result.

### 2.2 Test Sludge Inoculation

In the ultrasonic enhancement stage, the inoculated sludge was Canon sludge that was started in the

laboratory. When the Canon system was started and stable, the NLR was  $0.84 \text{ kg N}/(\text{m}^3 \cdot \text{d})$ , and the nitrogen removal rate was  $0.68 \text{ kg N}/(\text{m}^3 \cdot \text{d})$ .

### 2.3 Test Water Quality

To prepare the synthetic wastewater, Ammonia nitrogen is added to the mineral medium in the form of  $\text{NH}_4\text{Cl}$ . The composition of the synthetic wastewater was reported in Jin (Jin, 2013) et al.

## 3 RESULTS AND DISCUSSION

### 3.1 Influence of Different Ultrasonic Intensity on the Activity of Canon System

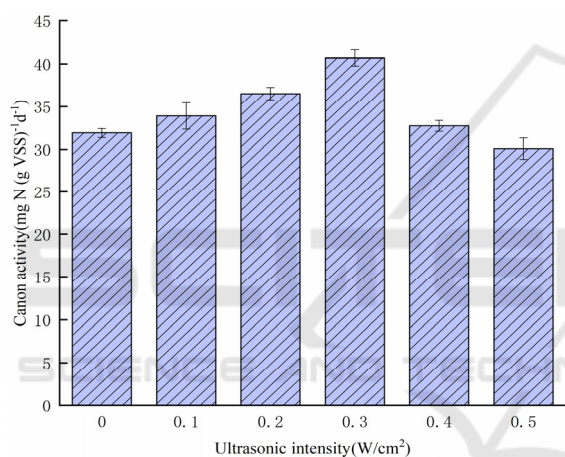


Figure 1: Activity of Canon system under different ultrasonic intensities.

Five different ultrasonic intensity values were selected from  $0.1\text{--}0.5 \text{ W}/\text{cm}^2$ , and  $0.1 \text{ W}/\text{cm}^2$  was increased each time, and the fixed ultrasonic time was 3min. As shown in Figure 1, the activity of the Canon system increased by a gradient when the ultrasonic intensity was in the range of  $0.1\text{--}0.4 \text{ W}/\text{cm}^2$  compared to  $0 \text{ W}/\text{cm}^2$ . The enhancement peak was reached at  $0.3 \text{ W}/\text{cm}^2$ , when the activity of blank group was  $31.91 \text{ mg N (g VSS)}^{-1}\text{d}^{-1}$ , and that of  $0.3 \text{ W}/\text{cm}^2$  group was  $40.68 \text{ mg N (g VSS)}^{-1}\text{d}^{-1}$ , 27.48% higher than that of blank group. Compared with the group without ultrasound enhancement, it increased by 27.48%. The activity of Canon at  $0.4 \text{ W}/\text{cm}^2$  was  $32.74 \text{ mg N (g VSS)}^{-1}\text{d}^{-1}$ , which was lower than that at  $0.3 \text{ W}/\text{cm}^2$ , but still higher than that of the group without ultrasound enhancement. When the ultrasound intensity was  $0.5 \text{ W}/\text{cm}^2$ , the Canon activity

continued to decline, which was 5.73% lower than that of the group without strengthening, indicating that excessive ultrasound intensity would cause damage to microorganisms, so the ultrasonic intensity at this time had certain damage to the treatment effect of the Canon system flora. The exchange rate between matrix and cell was accelerated, and the growth and metabolism of bacteria were promoted.

### 3.2 Influence of Different Ultrasonic Intensity on EPS in Canon System

EPS is a kind of polymer organic matter secreted by microorganisms, which mainly contains proteins, carbohydrates, nucleic acids, humus, etc (Zhou, 2020).

In the sludge system, the main components of EPS are polysaccharides and proteins, so the contents of polysaccharides and proteins are mainly analyzed in the study (Wu, 2013). As shown in Figure 2, compared with the blank group, EPS content in the enhanced group was increased. This indicates that under the strengthening effect of the sludge system, a certain amount of polysaccharides and proteins are released from the microbial biofilm to reduce the damage caused by external changes. When the optimal ultrasound intensity was  $0.3 \text{ W}/\text{cm}^2$ , the polysaccharide and protein contents were  $25.07 \text{ mg/g VSS}$  and  $85.31 \text{ mg/g VSS}$ , respectively, which were 22.71% and 20.94% higher than those of the blank group. When the ultrasonic intensity was  $0.5 \text{ W}/\text{cm}^2$ , the contents of polysaccharide, protein, and total EPS were significantly increased, and the contents were  $32.22 \text{ mg/g VSS}$ ,  $122.65 \text{ mg/g VSS}$ , and  $154.87 \text{ mg/g VSS}$ , respectively, which were 57.71%, 73.87%, and 70.24% higher than those of the blank group. Although ultrasonic enhancement can promote the sludge treatment system and increase the secretion rate of EPS, under the adjacent intensity gradient, according to the normal secretion rate, the increase of EPS content is larger when the ultrasonic intensity is  $0.5 \text{ W}/\text{cm}^2$ . The reason may be that the high-intensity ultrasound causes excessive damage to microorganisms, the cell wall is destroyed, and the permeability of the cell membrane is increased, leading to the release of a large number of polysaccharides and proteins in cells, thus causing a sudden increase in EPS content.

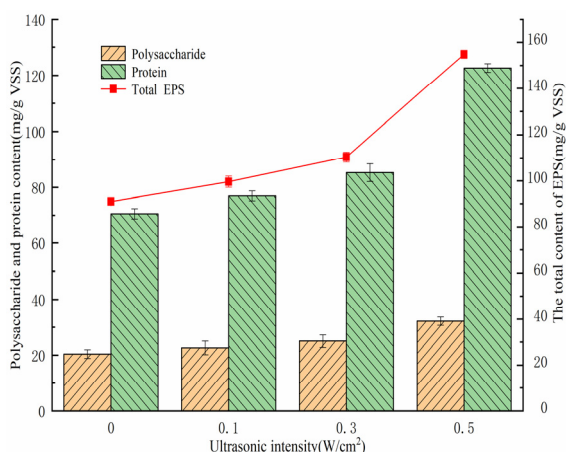


Figure 2: EPS content and composition under different ultrasonic intensities.

### 3.3 Influence of Different Ultrasonic Time on the Activity of Canon System

Ultrasonic intensity is an important parameter in the ultrasonic enhancement treatment of biological systems, and ultrasonic time is also one of the selected parameters. Pulse ultrasonic time refers to the interval between two ultrasonic times, usually several seconds; Continuous ultrasound time refers to the duration of an ultrasound, usually a few minutes to a few hours. In actual bioaugmentation, pulsed ultrasound is rarely used, and most of it is continuous (Yan, 2016).

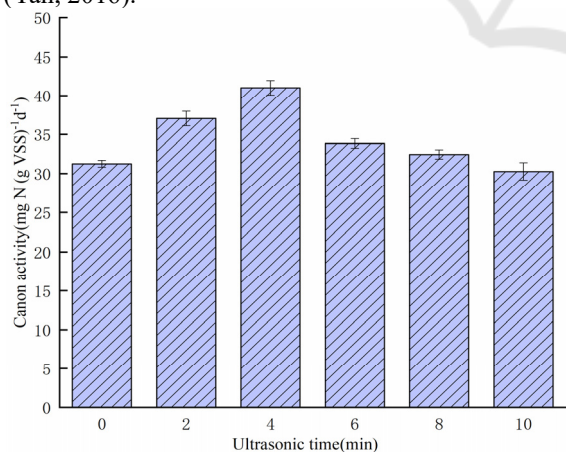


Figure 3: Activity of Canon system after different ultrasonic time.

When investigating the influence of ultrasonic time on the activity of Canon system, the optimal fixed ultrasonic intensity was 0.3W/cm<sup>2</sup>, and the ultrasonic time was set to 0 min, 2 min, 4 min, 6 min,

8 min, and 10 min, respectively. The effect was shown in Figure 3. The maximum activity of Canon system was 41.02 mg N(gVSS<sup>-1</sup>)d<sup>-1</sup> at 4 min of ultrasonic time, which was 31.25% higher than that of the untreated group. After 4 min, the activity of the Canon system did not continue to increase but began to decrease. At 10 min, the activity of the Canon system was 3.16% lower than that of the unenhanced group, indicating that prolonged ultrasound time did not further improve the activity of the Canon system, but caused negative feedback and ultimately decreased activity. Schlafer (Schläfer, 2002) also believed that stimulation of the biological system within a certain range can improve microbial activity, and when the range is exceeded, it will cause inhibition.

### 3.4 Influence of Different Ultrasonic Time on EPS in Canon System

Studies on the effects of ultrasound on other microorganisms show that low-intensity ultrasound can cause micro-damage to cells and destroy the structure of the biological walls and cell membranes, in which microorganisms produce more EPS to protect themselves from adverse environmental conditions. In order to explore the influence of ultrasonic time on the activity of Canon system, the contents of polysaccharide, protein, and total EPS at the fixed ultrasonic intensity of 0.3W/cm<sup>2</sup> and ultrasonic time of 0 min, 2 min, 4 min, and 10 min were selected to study.

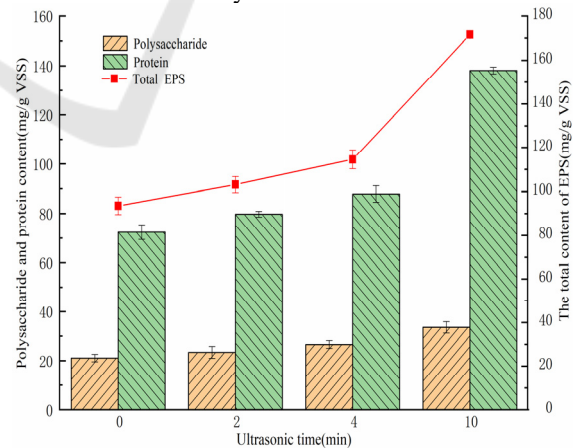


Figure 4: EPS content and composition after different ultrasonic time.

The result is shown in Figure 4. When the short-time ultrasound time was 2 min and 4 min, the total EPS content increased gradually. When the short-time ultrasound time was 4 min, the polysaccharide,



protein, and total EPS contents were 26.64mg/g VSS, 87.91mg/g VSS, 114.55mg/g VSS, respectively. Compared with the group without ultrasound enhancement, it increased by 26.50%, 21.32%, and 22.49%, respectively. When the ultrasound time was 10min, the total EPS showed an obvious increase process, and the contents of polysaccharide, protein, and total EPS were 33.56mg/g VSS, 138.05mg/g VSS and 171.61mg/g VSS, which increased by 59.35%, 90.52%, and 83.50% compared with the group without ultrasound enhancement.

### 3.5 Activity of Canon System under Different Ultrasonic Cycles

Prolonged ultrasound can exceed the tolerance limit of microorganisms and cause irreversible cell damage. In order to maximize the enhancement effect of low intensity ultrasound, the ultrasonic cycle was studied after the optimal ultrasonic intensity and ultrasonic time were discussed. In this part of the study, the ultrasonic intensity was set at 0.3W/cm<sup>2</sup> and the ultrasonic time was set at 2min and 6min respectively. Based on the optimal ultrasonic time of 3min, the maximum number of active maintenance cycles of the Canon system was studied.

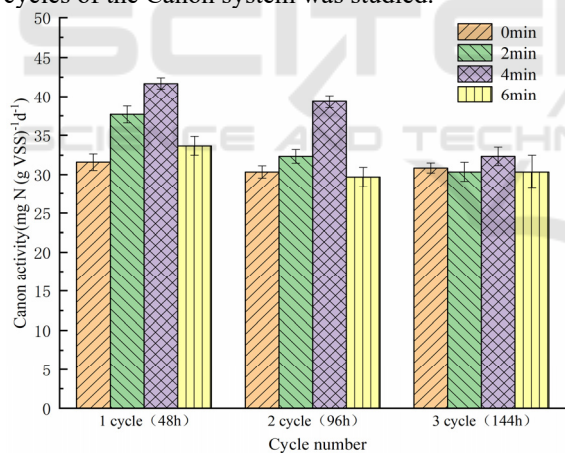


Figure 5: Changes in the activity of the Canon system under the optimal ultrasonic intensity and different ultrasonic time.

Under the conditions of ultrasonic intensity of 0.3W/cm<sup>2</sup> and ultrasonic time of 0min, 2min, 4min and 6min, the activity test results of each Canon system are shown in Figure 5. The test period shall be divided into 48 hours each. In the second cycle, the activity of the test group was close to that of the blank group when the ultrasonic time was 2min and 6min. Therefore, it was proved that the effect maintained for two cycles when the ultrasonic time was 4min and

6min. However, when the ultrasonic time was 4min, the activity of the Canon system was similar to that of the untreated group only in the third cycle. This indicates that when the ultrasonic intensity is 0.3W/cm<sup>2</sup> and the ultrasonic time is 4min, the maximum period can be maintained for 6days, which is one more period than the 2min and 6min test groups, indicating that the Canon system has certain adaptability to ultrasonic radiation and can promote the activity of the Canon system in a relatively short period of time (Liu, 2003).

### 3.6 Influence of Different Ultrasonic Periods on EPS in Canon System

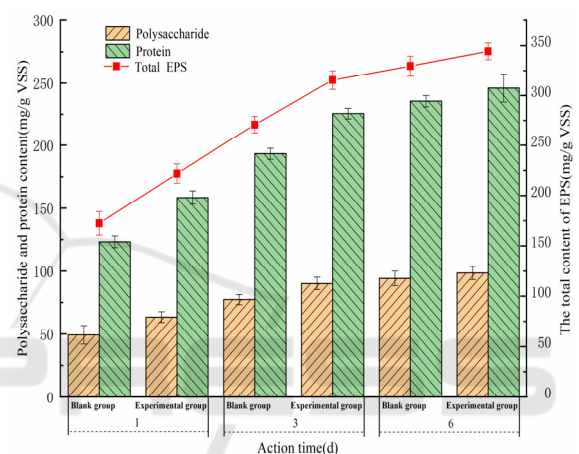


Figure 6: Comparison of EPS content and composition between ultrasound and non-ultrasound treated Canon systems.

Figure 6 shows the comparison of polysaccharide, protein, and total EPS contents between the ultrasound enhanced group and the non-enhanced group after the ultrasonic intensity of 0.3W/cm<sup>2</sup> and ultrasonic time of 4 min. On the first day after ultrasonic enhancement, the contents of polysaccharide, protein and total EPS in the experimental group were 63.39mg/g VSS, 158.48mg/g VSS, and 221.87mg/g VSS, respectively, increased by 28.63%, 32.69%, and 31.53% compared with those in the group without ultrasonic enhancement, respectively. On the third day after the completion of the enhancement, the growth rate of each component decreased, but the contents of polysaccharide, protein, and total EPS in the experimental group were still higher than those in the non-ultrasonic enhancement group. The production rates of polysaccharide, protein, and total EPS were 13.39 mg/g VSS/d, 31.97 mg/g VSS/d and 45.36 mg/g VSS/d, respectively, 1-3 days after ultrasonic

treatment. On the sixth day of ultrasound treatment, the contents of all components in the ultrasound enhanced group and the group without ultrasound were basically the same, but the production rates of polysaccharide, protein, and total EPS decreased to 4.11 mg/g VSS/d, 9.51 mg/g VSS/d and 13.62 mg/g VSS/d during 4-6 days of ultrasound treatment, respectively. The results showed that low intensity ultrasonic stimulation increased the metabolic rate of cells, and more metabolites were excreted out of cells (Pitt, 2003).

## 4 CONCLUSIONS

(1) In the range of 0.1-0.4 W/cm<sup>2</sup>, the activity of the Canon system was higher than that of the blank group, and the maximum value of 40.68 mg N(gVSS<sup>-1</sup>) d<sup>-1</sup> was reached at 0.3W/cm<sup>2</sup>, which was 27.48% higher than that of the blank group. When the ultrasound intensity was 0.5W/cm<sup>2</sup>, the activity was 5.73% lower than that of blank enhancement group.

(2) In the ultrasonic time range of 2-8min, the activity of the Canon system was higher than that of the group without ultrasound enhancement, and the maximum enhancement was 41.02 mg N(gVSS<sup>-1</sup>) d<sup>-1</sup> at the ultrasonic time of 4 min, which was 31.25% higher than that of the group without ultrasound enhancement. The activity of the Canon system was 3.16% lower than that of the untreated group.

(3) when the optimal ultrasonic intensity was 0.3W/cm<sup>2</sup> and the optimal ultrasonic time was 4 min, the maintenance time of ultrasonic enhancement was 6 days, one cycle longer than that of adjacent ultrasonic time of 2 min and 4 min. On day 1 after ultrasonic treatment, the content of polysaccharide, protein, and total EPS in the Canon system was 63.39mg/g VSS, 158.48 mg/g VSS, and 221.87 mg/g VSS, respectively, which increased by 28.63%, 32.69%, and 31.53% compared with the group without ultrasonic enhancement, respectively. With the extension of time, the secretion rate of each component decreased, and the ultrasonic enhanced group was similar to the unenhanced group on day 6.

## ACKNOWLEDGEMENT

This research was financially supported by General project of scientific Research Plan of Education Department of Liaoning Province (LJKZ0601)

## REFERENCES

- Liu Y, Takatsuki H, Yoshikoshi A, et al, (2003). Effects of ultrasound on the growth and vacuolar H<sup>+</sup>-ATPase activity of aloe arborescens callus cells. *Colloids and surfaces B: Biointerfaces*, 32(2): 105-116.
- Pitt W G, Ross S A, (2003). Ultrasound increases the rate of bacterial cell growth. *Biotechnology progress*, 19(3): 1038-1044.
- R.C. Jin, C. Ma, J.J. Yu, (2013). Performance of an Anammox UASB reactor at high load and low ambient temperature. *Chem. Eng. J.* 23217-25.
- Schläfer O, Onyeche T, Bormann H, et al, (2002). Ultrasound stimulation of micro-organisms for enhanced biodegradation. *Ultrasonics*, 40(1-8): 25-29.
- Tang X, (2015). Removal of ammonia nitrogen from wastewater by a single-stage autotrophic denitrification process with low intensity ultrasound. *Dalian University of Technology*.
- Wu Z S, Peng C, Hu X B, et al, (2013). Experimental study on enhanced sludge hydrolysis by SDS and SDBS. *Civil, construction and environmental engineering*, 35(05):25-29+43.
- Yan Y X, Liu H, (2006). Mechanism of low intensity ultrasound enhanced biological treatment of wastewater. *Environmental Science*, 27(4): 647-650.
- Zhang D D, (2017). Study on start-up and operation performance of anaerobic ammonia oxidation enhanced by ultrasonic wave. *Hebei University of Technology*.
- Zhou P, (2020). Effect of low intensity ultrasound on the activity of ANAMMOX at low temperature. *Shenyang Jianzhu University*.