Determine the Best Ultrasonic Parameters of Enhanced Anammox by Response Surface Method

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Abstract: Based on the single factor experiment, the effects of ultrasonic intensity and ultrasonic time on the efficiency of anammox denitrification were studied by response surface method. The results show that 0.3 (w/cm^2) has the best effect in the single factor test of ultrasonic intensity. In the single-factor ultrasonic time test, with the increase of ultrasonic time, SAA first rises and then falls, and different ultrasonic intensity corresponds to different ultrasonic time Response surface method was used to establish a model to predict the interaction between ultrasonic intensity and ultrasonic time on the effectiveness of anaerobic nitriding. When the temperature was 15℃ and the ultrasonic intensity was 0.35 (w/cm^2), the ultrasonic time was 4 (min), SAA was the highest. Anaerobic nitrous oxide sludge has the best activity. The comparison between the predicted value of SAA model and the actual test value at low temperature shows that the deviation is small, indicating that the model is reasonable and effective.

Keywords: response surface methodology; anammox; low temperature; ultrasonic

1. Introduction

Anammox technology has been widely concerned by scholars at home and abroad since its discovery. Anammoxidation is based on NH_4^+ under anaerobic conditions .For electron donor, NO_2^- is an electron acceptor. It is a biological process of converting ammonia nitrogen and nitrous nitrogen into stone. Compared with the traditional biological nitrogen removal technology, it has the advantages of no organic carbon source, low energy consumption and low sludge yield. However, anammox is sensitive to temperature [1], and its optimal growth temperature is 30 ~ 35℃, so low temperature conditions severely limit the use of anammox process. At the same time, the reaction rate of anammox bacteria was not the same under different pH environments. A large number of single-factor tests have proved that both ultrasonic intensity and ultrasonic time can affect the anammox reaction, but there are few studies on the combined effect of ultrasonic intensity and ultrasonic time on the anammox process. Therefore, this paper intends to use the response surface method to explore the combined effect of ultrasonic intensity and ultrasonic time.

The response surface method [2-4] is an optimization method, which takes the response of the system as a function of one or more factors, USES graphic technology to display the functional relationship, and selects the optimal conditions in the test design or predicts the test results through intuitive observation. In environmental engineering, there are many examples of using the response surface method. For example, Zhang Qian et al. [5] used the response surface method to study the optimal operating parameters of denitrifying biofilters. Wang Yong et al. [6] used the response surface method to optimize the conditions of homogeneous Fenton depth treatment process.

In this experiment, specific anammox activity (NRR) was taken as the objective index. Firstly, the influence of ultrasonic intensity and ultrasonic time on anammox reaction was analyzed by a single factor through static test. hen, response surface method was used to study the influence of ultrasonic intensity and ultrasonic time on the denitrification efficiency of anAMmox process. Based on the results, a model was established to seek for the regulation means to improve the denitrification efficiency of anammox process at low temperature.

2. Materials and Methods

2.1. Test Equipment and Operating Conditions

The ananmox reaction device adopts 150 mL conical flask. In the test process, the temperature and pH were adjusted, and the DO in the water was blown off by high-purity nitrogen to eliminate the influence of DO on the anaerobic ammoxic bacteria, and the exterior was treated by avoiding light.

2.2. Test Sludge

The test sludge was taken from the laboratory UASB reactor, which was anaerobic ammonia-oxidized granular sludge for long-term cultivation under the environment of 15℃ and pH value of 7.6.

2.3. Test Water

The test waste water is artificial prepared waste water, and its main components are shown in Table 1.

Table 1. Main Components of Simulated Wastewater in Anammox Reactor

Composition	Centration/(mg • L^{-1})	Composition	Centration/(mg • L^{-1})
$NaHCO_3$	1000	EDTA	6.25
KH_2PO_4	27.2	NH_4Cl	70
$CaCl_2$	300	$NaNO_2$	90

2.4. Ultrasonic Strength Single Factor Test

The sludge concentration (MLVSS) was 1.667 g/L from the anaerobic ammonia oxidation reactor. The sludge was inoculated into 5 150 mL conical bottles, the sludge inoculation amount was 20 mL, 80 mL simulated wastewater was poured, and high purity nitrogen was filled for 5 min to remove DO in the water. The conical flask was placed in an oscillating incubator in dark for 8 h. The temperature was set at 15℃, the speed was 140 r/min, and the ultrasonic time was 3min.Water samples were taken for determination at 4h of culture. The strain test period was 1d, culture was resumed for 1d, ultrasonic intensity was changed, other conditions were unchanged, and the above test was conducted. Three groups of parallel experiments were set for each condition, and the results were averaged.

2.5. Ultrasonic Time Single Factor Test

The experimental inoculation sludge and the first part of the sludge from the same source, inoculation sludge and wastewater dosage is the same as the first part of the sludge, before inoculation by clean water wash. The experimental sludge was inoculated into 5 150 mL conical bottles, filled with high-purity nitrogen for 5 min, and then placed in a vibrating incubator to be cultured in darkness for 8 h. The rotating speed was 140 r/min. The ultrasonic intensity was kept unchanged at 3w/cm^2, and the ultrasonic time was changed. After 4h of reaction, the reaction was measured. Three groups of parallel tests were set for each condition, and the results were averaged.

2.6. Central Composite Design

The test adopts the response from the surface method of 2 factors 3 horizontal center composite design. The results were shown in Table 2. In this test, the ultrasonic intensity and ultrasonic time of two factors in the single-factor test were taken as independent variables, respectively represented by X1 and X2, and the independent variables were coded according to the principle of the central composite test [7].

Table 2. Design Code Levels for Response Surface Methodology Test

The independent variables	Code	The coding level		
		-1	0	1
The intensity of ultrasonic	X1	0.1	0.3	0.5
The time of ultrasonic	X2	1	4	6

3. Results

3.1. Effect of Ultrasonic Intensity on Anaerobic Ammoxidation Bacteria

The "Figure 1" shows the experimental results of batch experiments with different ultrasonic intensities. All the data were measured at 40h. Because the original nitrate nitrogen concentration table data in and out of the water were too numerous, the calculated parameter total nitrogen SAA was used to represent the activity of the anaerobic ammonia-oxidizing bacteria in the whole reactor. It can be seen from the figure that the sludge activity after ultrasonic treatment with 0.1w/cm^2, 0.2w/cm^2, 0.3w/cm^2, 0.4w/cm^2, 0.5w/cm^2 and 0.6/cm^2 was higher than that of the untreated group and the activity increased significantly. The activity of the anaerobic ammox bacteria in the treatment group after 0.3w/cm^2 ultrasound increased by 37% from 0.52g $^{-1}$ VSS • d^{-1} to 0.71g^{-1}VSS • d^{-1}. In the range of 0.1~0.3w/cm^2, the activity of anammoxic bacteria tended to increase with the increase of ultrasonic intensity. When the ultrasonic intensity was higher than 0.3w/cm^2, the activity of anammox was improved compared with that of the control group without ultrasonic treatment, but the activity of anammox denitrification did not continue to increase, but showed a downward trend. That is to say, within a certain range of ultrasonic intensity, the amplitude of activity improvement of anammoxic bacteria is positively correlated with the ultrasonic intensity. When the ultrasonic intensity exceeds a certain threshold, such a trend does not exist.

Cavitation effect may be the reason why the reaction results appear in this state. When the ultrasonic intensity is low, a stable cavitation effect occurs, which promotes mass transfer and liquid-mass mixing, forming a positive effect on the improvement of the activity of anAMmoxic bacteria. Excessive ultrasonic intensity may cause irreversible damage to microbial cells [8], so that the enhancement effect of activity of anaerobic ammox bacteria is not obvious or even decreased.

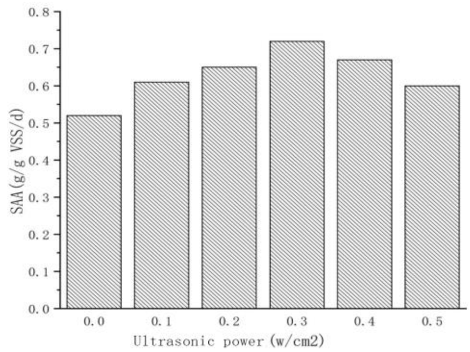

Figure 1. Diagram of SAA changes with ultrasonic intensity

3.2. Effect of Ultrasonic Time on Anaerobic Ammoxidation Bacteria

As shown in the experimental data “Figure 2”, the effects of different radiation time on the activity of anAMmox were investigated under the effects of optimal ultrasonic intensity and ultrasonic frequency. It was found that when the radiation time was 4 min, the activity of anammox was 132% higher than that of the control group. When the radiation time exceeded 4 minutes, the reactivity of anammox bacteria began to decrease instead of increasing. When the radiation time was 7 minutes, the anAMmox activity SAA was lower than the blank experimental value without ultrasound. This phenomenon indicated that the prolonged ultrasonic radiation time would not improve the denitrification activity of the anaerobic ammox bacteria as expected, but would produce negative effects, which would reduce the anaerobic ammox activity in the reactor. This indicated that the ultrasonic irradiation time would not improve the denitrification activity of the anaerobic ammox bacteria, but would decrease the activity of the anaerobic ammox bacteria. Liu et al. used ultrasonic radiation on the callus of aloe vera and found that the cell wall and cell membrane of the cells might be damaged due to the excessive energy of the applied ultrasound.: It is also believed in experiments that, in certain ultrasonic radiation intensity and ultrasonic radiation time, low-intensity ultrasound will stimulate the secretion of intracellular metabolites, and long-term ultrasound radiation will have a negative effect on microorganisms. The results of this experiment are generally consistent with this view.

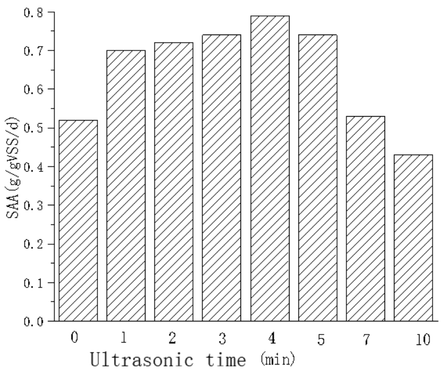

Figure 2. SAA varies with ultrasonic time

3.3. Response Surface Method

According to the experimental data, a functional relation expression is designed. The results were shown in Table 3. In the later stage, whether the data fits the equation is judged through analysis. If the model is not fitted, the equation is replaced to express the influence of ultrasonic intensity and ultrasonic time value on the interaction of SAA.

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii} X_i^2 + \sum_i \sum_j \beta_{ij} X_i X_j \cdot \quad (1)$$

Table 3. Experimental design and results

Number	intensity/(w/cm2)	Time	SAA/(g/gVSS/d)
1	0.30(0)	3.50(0)	0.72
2	0.58(1.41)	3.50(0)	0.49
3	0.30(1)	3.04(-1)	0.69
4	0.50(-1.41)	6.00(0)	0.51
5	0.30(1)	4.00(1)	0.75
6	0.50(0)	1(0)	0.53
7	0.30(0)	7.04(0)	0.49
8	0.30(0)	0.1(1.41)	0.64
9	0.10(0)	6.00(0)	0.51
10	0.30(0)	3.04(1.41)	0.70
11	0.02(-1)	3.50(1)	0.52
12	0.30(0)	3.50(0)	0.68
13	0.10(-1)	1.00(-1)	0.53

According to the experimental design, the ultrasonic time and ultrasonic intensity were compared with the combined effect of the anaerobic ammox activity SAA, and the software was used for statistical analysis and mathematical model was established. The optimal fitting quadratic polynomial equation was established by regression fitting the experimental data by polynomial regression analysis method. The model equation is as follows.

$$Y = -2.034 + 0.02831X_1 + 0.588X_2 - 0.0003X_1^2 . \\ - 0.04667X_2^2 - 0.001846X_1X_2 \quad (2)$$

According to the application of active model equations obtained by fitting analysis of variance, known, P = 0.000 < 0.05, shows that the model is significant in the fitting analysis, the model of the adjusted determination coefficient R2 is 0.9912, conditions that affect the test results of ultrasound intensity, ultrasonic time two factors about 99.12% distribution in the two factors, only 0.88% can't from the fitting model to explain, so that the test model fitting in good condition prediction can be used for the result of the experiment.

The variance of each coefficient of the regression equation shows that the first term, the second term and the interaction term of the equation have significant influence, and the influence of each specific experimental factor on the response value is not a simple linear relationship. X_1 and X_2 have a very significant influence (P<0.05). The influence of quadratic term X_{22} was extremely significant (P<0.05), while that of X_{12} was significant (P<0.05). The influence of interaction term is very significant. The influence of missing fitting term was not significant (P > 0.05). Judging by the value of F, the larger the value of F is, the stronger the correlation of this test factor is. The correlation comparison results show that the influence of ultrasonic frequency is greater than that of ultrasonic time. The whole analysis shows that within the range of two factors, the model obtained can reflect the relationship between the parameters, and the model can be used to analyze and predict the ultrasonic parameters of anaerobic ammoxidation bacteria, and find the best ultrasonic parameters.

According to the previous section of the model equations, application software, draw the response value of SAA and intensity of ultrasonic, ultrasonic time response surface and contour curve, the contour curve and response chart can show that the effect of single factor, but also can reflect the interaction of size, when the steep curve flat, surface, the influence of the factors effect, best level range near the surface vertex. The effects of ultrasonic intensity and ultrasonic time on response value SAA and isoline are shown in the figure.

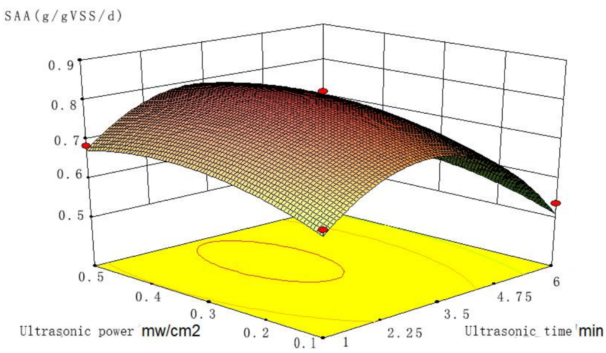

Figure 3. SAA varies with ultrasonic time

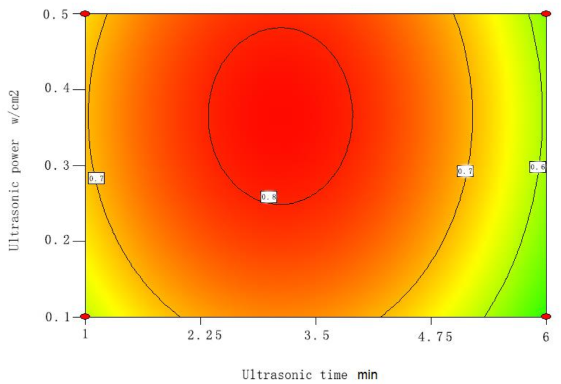

Figure 4. SAA varies with ultrasonic time

It can be seen from “Figure 3” that in the test, there is an optimal range of ultrasonic waves that play an obvious role in the strengthening of anammox. “Figure 4” However, once the selected ultrasonic parameters are beyond this range, the enhancement effect will become worse. In other words, one of the two factors, such as ultrasonic intensity and ultrasonic time, is beyond the optimal range, while the other parameter is within the optimal range, which also fails to achieve good ultrasonic optimization effect.

In order to verify the rationality and effectiveness of the model, the optimal experimental conditions were used to verify it. The results were shown in Table IV. The results showed that the predicted values were close to the experimental values, indicating that the model was reasonable and effective.

Table 4. Test Optimal Condition Design

	intensity/(w/cm2)	Time	SAA/(g/gVSS/d)
predict	0.35	4	77.078
experiment	0.35	4	78

4. Conclusion

In the range of 0.1~0.3w/cm^2, the activity of anAMmoxic bacteria tended to increase with the increase of ultrasonic intensity. When the ultrasonic intensity was higher than 0.3w/cm^2, the activity of anAMmox was improved compared with that of the control group without ultrasonic treatment, but the activity of anAMmox denitrification did not continue to increase, but showed a downward trend. That is to say, within a certain range of ultrasonic intensity, the amplitude of activity improvement of anAMmoxic bacteria is positively correlated with the ultrasonic intensity. When the ultrasonic intensity exceeds a certain threshold, such a trend does not exist.

It was found that the treatment effect was best when 20 kHz low-intensity ultrasound was used and the ultrasonic time was 4 min. It was found that for the intensification of anaerobic ammoxic bacteria, the ultrasonic intensity used in the intensification was not the bigger the better, and the excessive ultrasonic intensity would have the side effect chance to slow down the reaction speed. Similarly, for the ultrasonic processing time, the enhanced ultrasound time is not the better, too long ultrasound time will have side effects to slow down the reaction speed.

Response surface method is used to design the ultrasonic power, ultrasonic time and anaerobic ammonia oxidation bacteria (SAA) of the relationship between regression model, it has been verified by predicting results compared with the actual result, the model is reasonable, can well explain two variables of ultrasonic power, ultrasonic treatment time, and the sludge than the relationship of anaerobic ammonia oxidation activity.

The optimal ultrasonic parameters of strengthening and strengthening ammox at low temperature (15℃) were investigated. The frequency of 20kHz, power of 0.35w/cm^2 and time of 4 min significantly improved the effectiveness of anammox. The results of this experiment can be used as a reference for the optimal ultrasonic parameters of strengthening the activity of anammox at low temperature (15℃) with low intensity ultrasonic. As this test is a static batch test, whether this conclusion is applicable to dynamic test needs to be proved in the follow-up study. At the same time, it was found in subsequent experiments that the most suitable ultrasonic parameters would change at different growth stages of anammox. Therefore, compared with the optimal ultrasonic parameters measured in this part of the experiment, this method of using response surface method to calculate the optimal ultrasonic parameters of low-intensity ultrasonic intensification of anaerobic ammonia-oxidizing bacteria has a stronger reference significance.

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