Response Surface Design for the Optimization of the Removal of *Chlorella pyrenoidosa* Low Frequency Ultrasonic Irradiation

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Water blooms caused by blue and green cyanobacteria has been shown to be hazardous to both animals and humans. Ultrasonic algae removal technology can be good for controlling algae growth by appropriate power, frequency and irradiation time. First of all, the approximate ranges of the ultrasonic parameters, frequency, power and irradiation time, were estimated with single factor experiments. Then the optimized values of the three parameters were determined by central composite design of response surface design. Results showed that the removal rate of *chlorophyll-a* reached to 64.1 % after the irradiation for 7 min by ultrasonic of 77 kHz and 100 W. Ultrasonic technology can remove *Chlorella pyrenoidosa* in water quickly and effectively, so as to achieve the purpose of water purification.

Key Words: Ultrasonic irradiation, Algae removal, *Chlorella pyrenoidosa*, Response surface design.

INTRODUCTION

With the development of the social industrialization, a large number of pollutants containing nitrogen, phosphorus have been let out into water, greatly accelerating the process of eutrophication of natural waters, resulting in overgrowth of algae in the water and the water bloom outbreaks more and more frequently. Because of the water bloom caused by the blue and green algae, the water became foul and the algal toxins appeared. At present there are many ways to control the algae in water. Such as control the pollution load, add algicide, artificial aeration mixing, clay or activated carbon adsorption and biological methods. For the waterworks, the commonly used unit processes are chemical methods, biological flotation and direct filtration treatment^{1,2}. But due to technical limitations of the various methods, in terms of safety, affordability and efficiency of algae removal does not meet the requirements. The removal of algae by ultrasonic, which is clean and efficient, referred to as environment-friendly technologies^{1,3,4}, has advantages of moderate operation conditions. It can be used alone or in combination with other water treatment technology. Previous studies have shown that the control parameters such as ultrasonic frequency, ultrasonic power and ultrasonic irradiation time have a significant impact on effectiveness of the removal of algae and energy consumption. The selection

of control parameter is critical. At present, the number of species of green algae and blue algae is the most in Phytoplankton community structure in fresh water. Green algae is the dominant algae in some areas^{5,6}. *Chlorella pyrenoidosa* is the typical type of green algae, which is common and easy to leading to algal bloom. Study on removal and inhibition of microcystis cyanobacteria, spirulina and other algae using ultrasonic technique has been more focused^{1,2,4,7,8}, however, research on *Chlorella pyrenoidosa* is still relatively little.

Response surface method for the design is an analysis method of test condition optimization with a combination of mathematics and statistics. It considers both test random error and complex relationship between unknown function fitted with multiple regression equations within the small area, which is a valid method used to optimize production conditions, reduce costs, improve product quality and solve actual problems in the production⁹. In this article, ultrasonic frequency, power and irradiation time are used as control parameters to study the influence of ultrasonic removal of *Chlorella pyrenoidosa* for selecting a suitable scope for further testing; and then central composite design and response surface is used to analyze the best values of control parameters, for providing theoretical basis of the ultrasonic control of *Chlorella pyrenoidosa* blooms in water.

EXPERIMENTAL

Experimental apparatus of ultrasonic: ATANA AT3020 signal generator is used as ultrasonic signal source. Power amplifier is predicted by the Buddha in Nanjing technology industrial production, model HFVA-62. Ultrasonic transducer for reactor is ultrasonic horn launch, developed by the Institute of acoustics, Chinese Academy of Sciences. The rest reactor is self-made, ultrasonic apparatus show as Fig. 1.

Fig. 1. Experimental apparatus of ultrasonic (1. Signal Generator; 2. Power Amplifier; 3. Oscilloscope; 4. Transducer; 5. Hydrophone; 6. Telescopic stand; 7. Cooling; 8. Glass flask; 9. Thermometer)

Determination of experimental parameters: Detection of ultrasonic frequency and power use the Ut2025C oscilloscope produced by unit-trend group limited in Hong Kong and CS-3A hydrophone developed by the Institute of acoustics, Chinese Academy of Sciences. Aqua fluor handheld chlorophyll fluorescence tester was used to detect *Chlorophyll-a*, which was developed by United States Turner Designs, model 8000-010.

Algae species and culture methods: *Chlorella pyrenoidosa* was provided by fresh water algae culture collection of the Institute of Hydrobiology of Chinese Academy of Sciences (FACHB-9). SE medium, medium formulations: 0.25 g/L NaNO_3 , 0.075 g/L $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.075 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.025 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.175 g/L KH_2PO_4 , 0.025 g/L NaCl , 0.005 g/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1 mL/L Fe-EDTA , 1 mL/L A_5 , 40 mL/L soil extract. In which, the solution formula of A_5 is: 2.86 g/L H_3BO_3 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1.81 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 220 mg/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 79 mg/L, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 39 mg/L. The solution formula of Fe-EDTA is Na_2EDTA 10 g/L, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 810 mg/L. The solution formula of soil extract (1 L) is using a beaker or flask to take garden soil 0.5 kg without fertilization, adding distilled water 1000 mL. Sealing the beaker or flask with a breathable stopper, heating in a boiling water bath for 2 h, then cooling it for several hours. Filter under sterile conditions. Then take the supernatant, add sterile distilled water to the total volume of 1000 mL. Soil extract stored in 4 °C for use. Algae-laden liquid initially placed in the 500 mL triangle flask, put in light training incubator at 25 °C, 2000 lux and keep ventilation and appropriate daily mixing. Later, expand in 20 L glass fish tank. Aerate 3 times every day. In this experiment, the algae we choose is in Logarithmic phase, it is that the algae density reached at 10^7 cells/mL.

Experimental methods: 800 mL algae fluid was put respectively into a group of 1 L beakers. Put the ultrasonic transducer 30 mm below the liquid. Irradiate the algae solution using different frequency, power and irradiation time. And then, determine the *chlorophyll-a* concentration in supernatant and calculated the *chlorophyll-a* removal rate, which is used as assessment index. The expressions is shown as eqn. (1).

$$\text{Removal of chlorophyll-a} = \frac{\text{Chla}_0 - \text{Chla}_1}{\text{Chla}_0} \times 100\% \quad (1)$$

where, Chla_0 is the *Chlorophyll-a* concentration of *Chlorella pyrenoidosa* before ultrasonic treatment, $\mu\text{g/L}$, Chla_1 is *Chlorophyll-a* concentration of *Chlorella pyrenoidosa* after ultrasonic treatment, $\mu\text{g/L}$.

RESULTS AND DISCUSSION

Single-factor experiments

Experiment of different ultrasonic frequency: With 80 W ultrasonic, algae liquid was irradiated by frequency 20, 40, 80, 100, 120 and 150 kHz for 5 min for the research on removal effective of different ultrasonic frequency. The results were shown in Fig. 2.

Fig. 2. Effect of ultrasonic frequency on removal of *chlorophyll-a* of *Chlorella pyrenoidosa*

From Fig. 2, it is clear that under the condition with power of 80 W and irradiation time of 5 min, for different frequencies, the content chlorophyll will lower with the increasing of ultrasonic frequency at first. It is that the removal rate increase with the increasing of ultrasonic frequency. When the frequency is 80 kHz, the immediate removal rate will reach to 62.35 %. When the frequency is greater than 80 kHz, the removal rate of chlorophyll will decrease with the increasing of ultrasonic frequency. When frequency is 80 kHz, the immediate removal rate will be the maximum. With the frequency continues increasing; the immediate removal rate will have a trend of decrease. So the removal effect will be the best when the frequency is at 40 kHz-100 kHz. The removal rate will be the maximum when the frequency is about 80 kHz. At this time, the removal rate of *chlorophyll-a* can reach to 62.35 %. Existing researches generally believe that cavitation is the main reason for the removal of algae cells^{2,4}. Biological tissue and tissue fluids contain many tiny air bubbles. Expansion, contraction, vibration, crashes and other cavitation process are generated on micro-bubbles when ultrasonic irradiates,

resulting in the sound hole effect¹⁰. Algal cells at the velocity wave nodes in the standing wave would continue colliding with large particles and accumulate, leading to death or deposition. When the ultrasonic frequency super closes to the resonant frequency of algal cells, the damage of algal cells would be more obvious and the removal of algal cells would be more effective^{10,11}. When the ultrasonic with the frequency of f_0 generates cavitation, harmonics with the frequency of $2f_0$, $3f_0$, etc. will be produced. The higher the ultrasonic frequency is, the closer between harmonic frequencies and resonant frequency of algal cells are. However, the excessive frequency makes cavitation difficult to generate, leading to decrease of cavitation intensity¹⁰. Therefore, the combined effects of these reasons lead to the removal rate of *chlorophyll-a* increasing first and then decreasing (Fig. 2).

Experiment of different ultrasonic frequency: With 80 kHz ultrasonic, algae liquid was irradiated by different power for 5 min for investigating the removal efficiency with ultrasonic power 20, 40, 60, 80, 100, 120 and 140 W. The results are shown in Fig. 3.

Fig. 3. Effect of ultrasonic power on removal of *chlorophyll-a* of *Chlorella pyrenoidosa*

Fig. 3 showed that when the ultrasonic power is at 0-60 W, the greater the power is, the slower the increasing rate of removal rate of algal cells is. When the power is 60 W, there comes a trough. And when the power is at 60-80 W, the removal rate will increase sharply. The removal rate is 62.35 % when the power is 60 W. When the ultrasonic power is at 80-140 W, with the ultrasonic power increasing, the removal rate of *Chlorella pyrenoidosa* increase slowly, almost remains unchanged. The main reason is that, there is driving sound pressure during the process of cavitation bubbles' movement. The higher excitation sound pressure increases, the more obvious the shock effect is³. When ultrasonic frequency and the initial radius are in certain circumstances, the larger excitation sound pressure is, the stronger the degree of bubble collapse is, so is cavitation⁶. Therefore, the higher the ultrasonic power, *chlorophyll-a* removal rate increases gradually when the ultrasonic power rises. At the same time, high sound pressure makes bubbles difficult to collapse⁶, therefore, the rise of *chlorophyll-a* removal rate tend to slow.

Experiment of different ultrasonic irradiation time: With 80 kHz, 200 W ultrasonic, algae liquid was irradiated for different periods of time for the research on removal effective of different ultrasonic irradiation time (1, 5, 10, 20 and 30 min). The results are shown in Fig. 4.

Fig. 4. Effect of ultrasonic irradiation time on removal of *chlorophyll-a* of *Chlorella pyrenoidosa*

Fig. 4 showed that under the condition with frequency of 80 kHz and power of 80 W, the removal rate of *chlorophyll-a* increased with the time in the first 5 min. After 5 min, there's no obviously increase of removal rate. This showed that ultrasonic's damage to algae cells is mainly happened within the first 5 min. Therefore, the effective time range of algae removing can be 3 min to 7 min. The result is similar to the ultrasonic removal of *Microcystis aeruginosa*¹¹, because of the related diameter of *Chlorella pyrenoidosa* and *Microcystis aeruginosa*.

Optimized experiment conditions: According to the removal effect above and the energy consumption, the optimized ultrasonic frequency level range of response surface experiment of removal *Chlorella pyrenoidosa* by ultrasonic irradiation is 40 kHz to 100 kHz. The ultrasonic power range is 60 W - 100 W and the irradiation time range is 3 min to 7 min.

Response surface analysis

Response surface design: Based on the design principles of response surface method, the beaker experiment of *Chlorella pyrenoidosa* removal by ultrasonic irradiation is designed by using the statistical software named Design Expert 7.0 and central composite design model. Factors and levels design is shown in Table-1. Where X1, X2, X3 respectively are ultrasonic frequency (kHz), ultrasonic power (W) and ultrasonic irradiation time (min). Choose Y, the removal rate of *chlorophyll-a* (%), as the assessment indicators. Combined experimental design factors and the results are shown in Table-2.

TABLE-1 FACTORS AND LEVELS OF DESIGN					
Factor	Variable	Units	Level		
Ultrasonic frequency	X ₁	kHz	40.00	70.00	100.00
Ultrasonic power	X ₂	W	60.00	80.00	100.00
Irradiation time	X ₃	min	3.00	5.00	7.00

The results of the model adaptability analysis and multiple model analysis are shown in Table-3. The analysis result of lack of fit is shown in Table-4. And the comprehensive analysis of R² is in Table-5.

These indicate that, the second-order model can adapt the response significantly, while the third-order model is aliasing. As can be seen by the Table-3, the model test results should be the second-order model. In which, Prob > F value is less

than 0.0001. It is to say, comparing with the 2 FI model, there is a comparability not more than 0.01 % for which is more suitable for the forecast of the removal rate of *chlorophyll-a*. Because of the aliasing, the third-order model will not be considered.

TABLE-2
CENTRAL COMPOSITE DESIGN TEST SCHEME AND RESULTS

No.	Factors			Res.	No.	Factors			Res.
	X ₁	X ₂	X ₃	Y		X ₁	X ₂	X ₃	Y
1	40	60	3	26.17	11	70	60	5	48.84
2	100	60	3	33.68	12	70	100	5	63.92
3	40	100	3	48.10	13	70	80	3	49.15
4	100	100	3	43.33	14	70	80	7	61.45
5	40	60	7	28.45	15	70	80	5	60.10
6	100	60	7	40.50	16	70	80	5	58.60
7	40	100	7	46.53	17	70	80	5	59.85
8	100	100	7	64.60	18	70	80	5	56.75
9	40	80	5	49.79	19	70	80	5	60.13
10	100	80	5	51.07	20	70	80	5	59.50

For the lack of fit, in terms of model, the bigger the Prob > F value is, the better. From Table-4 we can see that, the Prob > F value is greater than 0.0001 both in second-order model and third-order model. So, both of these two models can adapt the simulation model. But, because of the aliasing in third-order model, it will not be considered. Therefore, based on the analysis of lack of fit, the second-order model has a better adaptability.

According to the R² comprehensive analysis, the residual sum of squares which predicted by second-order model is the minimum. So it should be selected to model.

Thus, the second-order model is more suitable to be the mathematical model of the removal rate of *chlorophyll-a*.

Therefore, the second-order model is chosen as the response regression model of *chlorophyll-a* to find the optimal response factor levels.

Use ultrasonic frequency (X₁), ultrasonic power (X₂) and irradiation time (X₃) as the independent variables and removal rate of *chlorophyll-a* (Y) as response value to establish the quadratic regression model, showed as eqn. (2):

$$Y = -108.329 + 1.425 X_1 + 1.826 X_2 + 7.213 X_3 + 1.304 \times 10^{-3} X_1 X_2 + 0.057 X_1 X_3 + 0.033 X_2 X_3 - 0.011 X_1^2 - 9.101 \times 10^{-3} X_2^2 - 1.180 X_3^2 \quad (2)$$

Make an analysis of variance for the model, to ensure the model can reflect the real surface and ensure it can reflect and predict the *Chlorella pyrenoidosa* removal effect of the ultrasound accurately. Analysis of variance results are in Table-6. Reliability analysis results of model are in Table-7.

Table-7 shows that R² = 0.9651 and standard deviation = 2.95, indicating that regression equation can simulate the real surface. Adeq accuracy is used to measure the signal to noise ratio of a model. When this ratio is greater than 4, the model can be considered reliable. According to the reliability analysis of the model, the value of Adeq accuracy of this model is 17.696, indicating that the model is reliable and has enough signal to respond the design. It further indicated that, this model can make optimization analysis of the ultrasound parameters when the ultrasound is used to remove *Chlorella pyrenoidosa*.

Response surface analysis of removal rate of *chlorophyll-a*: The effect of ultrasound frequency and ultrasound power to the removal rate of *chlorophyll-a* and the interaction between the two factors are shown in Fig. 5.

Fig. 5 shows that, in the experiment that use ultrasonic to remove *Chlorella pyrenoidosa*, the removal rate of *chlorophyll-a* increased at first, decreased after with the increasing of the

TABLE-3
SEQUENTIAL MODEL SUM OF SQUARES FOR CENTRAL COMPOSITE DESIGN

Source	Sum of Squares	df	Mean Square	F-value	p-Value Prob > F	Notes
Mean vs Total	51056.52	1	51056.52			
Linear vs Mean	1074.73	3	358.24	4.03	0.0259	
2FI vs Linear	112.65	3	37.55	0.37	0.7739	
Quadratic vs 2FI	1221.78	3	407.26	46.80	< 0.0001	Suggested
Cubic vs Quadra	76.02	4	19.00	10.36	0.0073	Aliased
Residual	11.01	6	1.83			
Total	53552.71	20	2677.64			

TABLE-4
LACK OF FIT TESTS FOR CENTRAL COMPOSITE DESIGN

Source	Sum of squares	df	Mean Square	F-value	p-value Prob > F	Notes
Linear	1412.92	11	128.45	75.22	< 0.0001	
2FI	1300.27	8	162.53	95.19	< 0.0001	
Quadratic	78.49	5	15.70	9.19	0.0147	Suggested
Cubic	2.47	1	2.47	1.45	0.2828	Aliased
Pure error	8.54	5	1.71			

TABLE-5
MODEL SUMMARY STATISTICS FOR CENTRAL COMPOSITE DESIGN

Source	Std. dev.	R ²	Adjusted R ²	Predicted R ²	Press	Notes
Linear	9.43	0.4305	0.3238	-0.0625	2652.14	
2FI	10.03	0.4757	0.2337	-3.0521	10114.90	
Quadratic	2.95	0.9651	0.9338	0.4932	1265.11	Suggested
Cubic	1.35	0.9956	0.9860	-0.2211	3048.02	Aliased

TABLE-6
ANALYSIS OF VARIANCE OF THE REGRESSION EQUATION

Source	Sum of squares	df	Mean Square	F- value	p-value Prob > F	Note
Model	2409.16	9	267.68	30.76	<0.0001	Significant
X ₁	116.55	1	116.55	13.39	0.0044	Significant
X ₂	789.25	1	789.25	90.69	<0.0001	Significant
X ₃	168.92	1	168.92	19.41	0.0013	Significant
X ₁ X ₂	4.90	1	4.90	0.56	0.4704	Not significant
X ₁ X ₃	93.71	1	93.71	10.77	0.0083	Significant
X ₂ X ₃	14.05	1	14.05	1.61	0.2327	Not significant
X ₁ ²	252.94	1	252.94	29.06	0.0003	Significant
X ₂ ²	36.45	1	36.45	4.19	0.0679	Not significant
X ₃ ²	61.28	1	61.28	7.04	0.0242	Significant
Residual	87.03	10	8.70			
Lack of Fit	78.49	5	15.70	9.19	0.0147	Significant
Pure error	8.54	5	1.71			
Cor total	2496.19	19				

TABLE-7
MODEL RELIABILITY ANALYSIS

Index	Std. dev.	Mean	C.V. (%)	Press	R ²	Adj. R ²	Pred. R ²	Adeq precision
Results	2.95	50.53	5.84	1265.11	0.9651	0.9338	0.4932	17.696

Fig. 5. Interactive effect of ultrasound frequency and power (a) 3D surface (b) 2D

ultrasonic frequency in the detection area. According to the calculated optimum experimental parameters, the optimum frequency is 77 kHz. That is keep the ultrasonic power and ultrasonic time invariant, when the frequency is 77 kHz, the removal rate of *chlorophyll-a* is the maximum. While in the single-factor factorial experiment, when the frequency is 80

kHz, the rate of *chlorophyll-a* is the maximum. In comparison, there has a small shift in frequency. This may be due to the interaction effect of ultrasonic power. Otherwise, because of the wide interval of the single-factor factorial experimental values, the frequency which corresponding to the maximum value of removal rate may not been taken.

It is also found that, with the ultrasonic power increases, the removal rate of *chlorophyll-a* is also increasing, but the increasing trend has been observed. It can be expected that, when the ultrasonic power increased to a certain extent, a limit value of removal rate, which indicate that the rate cannot increase significantly even the ultrasonic power continued increasing. According to the selected range of ultrasonic power, when the ultrasonic power is the maximum, 100W, the removal rate of *chlorophyll-a* also reached the maximum. When the power increased, the input energy of the algae fluid increased, also the power density of the algae fluid. However, with the input energy of the algae fluid increasing, the cavitation bubbles became difficult to break, which slowed the increasing trend of the removal rate of *chlorophyll-a*. It is identical to the results of single-factor factorial experiment.

The interaction between ultrasound frequency and ultrasound power manifested in that, when the ultrasound frequency is close to the optimal experimental conditions, that is 77 kHz, with the ultrasonic power increasing, the removal rate of *chlorophyll-a* reached to the limit value and this value is closer to the best removal rate. Because of the interaction, there is an optimal area of the removal rate of *chlorophyll-a*, which is the semi-elliptical area shown in the Fig.5, where the frequency is about 50 kHz to 100 kHz and power is about 80 W to 100 W. In this area, the removal rate is more than 60.86 %.

The effect of ultrasound frequency and irradiation time to the removal rate of *chlorophyll-a* and the interaction between the two factors are shown in Fig. 6.

Fig. 6 shows that, in the experiment that use ultrasonic to remove *Chlorella pyrenoidosa*, with the increasing of the ultrasonic frequency in the detection area, the removal rate of *chlorophyll-a* increased at first, then decreased.

Fig. 6. Interactive effect of ultrasound frequency and irradiation time (a) 3D surface (b) 2D

With the irradiation time increase, the removal rate of *chlorophyll-a* also increased, but the increasing trend has been slowing. It is similar to the effect of ultrasonic frequency for the removal rate of *chlorophyll-a*. This may be due to the harmonics, which caused by the cavitation derived the vibration of a single cell. Meanwhile, the collisions between algal cells in the algae fluid is also be exacerbated, which make the algae cell damage and death, finally lead to the decreasing *chlorophyll-a* content. With the ultrasonic irradiation time increase, the number of algae cell decreased. While the algal density decreased, the collision chances of algae cells with each other decreased. This is the reason for the removals rate of *chlorophyll-a* increased slowly.

The interaction between ultrasound frequency and irradiation time manifested in that, the closer the irradiation time is to the optimal experimental conditions (7 min) and the closer the ultrasound frequency is to the optimal experimental conditions (77 kHz), the greater the removal rate is.

Because of the interaction, there is an optimal area of the removal rate, which is the semi-elliptical area shown in the figure. Where the frequency is about 60 kHz-90 kHz and irradiation time is about 4.5 min-7.0 min. In this area, the removal rate is all above 63.12 %.

The effect of ultrasound power and irradiation time to the removal rate of *chlorophyll-a* and the interaction between the two factors are shown in Fig. 7.

Fig. 7. Interactive effect of ultrasound power and irradiation time (a) 3D surface (b) 2D

The interaction between ultrasound power and irradiation time is not very obvious. So, the response surface is close to flat. But, there are still optimal area of the removal rate, which is the semi-elliptical area shown in the figure, where the power is about 210 W to 100 W and irradiation time is about 4.5 min to 7.0 min. In this area, the removal rate is more than 62.25 %.

In summary, this experiment indicated that, there are significant interactions among the three factors of ultrasonic frequency, ultrasonic power and irradiation time. Correspond to the maximum value of the removal rate, both of the ultrasonic frequency and the irradiation time has an optimal value.

Regression equation can be written as eqn. (3):

$$Y = a + X^T b + X^T c X \quad (3)$$

In which:

$$X = [X_1, X_2, X_3]^T \quad (4)$$

Firstly,

$$\frac{\partial Y}{\partial X} = 0 \quad (5)$$

Equal to:

$$\frac{\partial Y}{\partial X} = \left[\frac{\partial Y}{\partial X_1}, \frac{\partial Y}{\partial X_2}, \frac{\partial Y}{\partial X_3} \right] = 0 \quad (6)$$

Among them, the limit condition is $X_2 \leq 100$

After implementing, the eqn. (7) is

$$\begin{bmatrix} 0.022 & 1.304 \times 10^{-3} & -0.057 \\ 1.304 \times 10^{-3} & 18.202 \times 10^{-3} & -0.033 \\ 0.057 & 0.033 & -2.360 \end{bmatrix} \bullet \begin{bmatrix} X_1 \\ X_2 \\ X_3 \end{bmatrix} = \begin{bmatrix} 1.425 \\ 1.826 \\ -7.213 \end{bmatrix} \quad (7)$$

The solution is: $X_1 = 77$, $X_2 = 100$, $X_3 = 7.0$

That is to say, the optimal condition for the *Chlorella pyrenoidosa* removal is ultrasound frequency of 77 kHz, ultrasonic power of 100 W, irradiation time of 7 min.

Verification of the model: In order to verify the prediction results, select the ultrasonic frequency as 77 kHz, ultrasonic power as 100 W, irradiation time as 7 min to be the reaction conditions. Taking three groups and a control group to test, the average of removal rate as 64.4 % was received. Comparing with the result of verification experiments using the same conditions, the error is 2.87 %, between which the predicted values are of good fit. Therefore, the model has some practical value for the accurate analysis and forecasting of ultrasonic parameters of *Chlorella pyrenoidosa* removal.

Conclusion

According to the single factor analysis, algae removal effect as ultrasonic frequency increases first and then decrease, in the optimal values of 80 kHz. Improvement of ultrasonic power can improve the effect of removal. After 5 min's irradiation, increasing irradiation time makes no significant impact.

Using CCD model of response surface method for optimization in ultrasonic frequency, power and irradiation time of the experiment, the quadratic polynomial regression equation established in response to removal rate of *chlorophyll-a* has a high degree of distinctiveness ($P < 0.0001$), $R^2 = 0.9987$ and loss of the proposed items is not significant. Regression equation was well fit with the actual situation. Ultrasonic frequency, ultrasonic power and irradiation time and the

interaction of the three had significant impacts on treatment effect.

When use the ultrasound to remove the *Chlorella pyrenoidosa* in the logarithmic phase, the optimal parameters are ultrasonic frequency of 77 kHz, ultrasonic power of 100 W, irradiation time of 7 min.

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