Study on the in-situ algae removal mechanism based on nitrogen-doped nanodiamond and cationic modified starch

Cui Hongwei[1], Li Weiliang[2], Yu Xin[1], Niu Zixi[1], Li Zhaolu[1], Zhang Yiyan[1]

[1]School of Mechanical and Automotive Engineering, Hengxing University, Qingdao, 266100, China

[2]School of Law, Inner Mongolia University of Finance and Economics, Hohhot, 010051, China

Keywords: Hydroxyl radicals, oxidative stress response, cell rupture, cationic starch, flocculant

Abstract: This study comprehensively applies ultrasonic, sonosensitizers, and cationic starch methods aimed at improving the treatment effects on Microcystis aeruginosa cells and particulates in water bodies. The experimental results show that the rupture and mortality of Microcystis aeruginosa cells are significantly increased through the high temperatures, high pressures, and intense liquid motion produced by ultrasonic vibration and the collapse of tiny bubbles. The auxiliary role of sonosensitizers enhances the ultrasonic effects, while cationic starch as a flocculant facilitates the aggregation of Microcystis aeruginosa cells and particulates into larger clumps, easing subsequent processing and removal. Under experimental conditions, this method successfully enhanced the rupture and mortality of Microcystis aeruginosa cells, achieving a significant removal efficiency of pollutants at 89.7%.

1. Introduction

Eutrophication and cyanobacterial blooms are among the significant global water environmental issues. In areas like the Three Gorges Reservoir in China, a decrease in water flow exacerbates eutrophication, leading to prominent cyanobacterial blooms that attract substantial societal concern. While heavy metal compounds can inhibit the growth of cyanobacteria in the short term, their long-term use carries environmental impacts and ecological risks. The accumulation of these compounds in the water environment can be toxic to aquatic life and disrupt ecological balance. Additionally, prolonged use of heavy metals may lead to resistance in cyanobacteria, weakening the effectiveness of algae removal.

2. Selection of Low-Frequency Ultrasound

The cavitation effect of low-frequency ultrasound can enhance the physical and chemical actions in water and generate hydroxyl radicals, which help in degrading pollutants. Ultrasonic treatment significantly alters the morphology and intracellular structure of algal cells. Initially[1], the cells of Microcystis aeruginosa in the control group are round with a smooth and intact external structure. After ultrasonic treatment, the cell morphology changes[2], displaying wrinkles and shrinkage on the surface As shown in Figure 1. Low-frequency ultrasound treatment has a significant destructive effect on algal cells, altering their shape and internal structures[3]. The frequency of ultrasound is a critical factor influencing the effectiveness of algae removal. At frequencies of 40 kHz and 100 kHz, the difference in algae removal rates is minimal. Considering power consumption and cost-effectiveness[4],the frequency of 40 kHz has been selected as the optimal solution. The frequency range of 40 kHz to 50 kHz could potentially achieve the best algae removal results[5].

In the experiment, Microcystis aeruginosa sourced from the Freshwater Algae Culture Collection of the Chinese Academy of Sciences was used, with the catalog number FACHB-905. The algae were cultured in BG11 medium at 25°C in a light incubator (model GZP-300C). Before the experiment, algal liquid from the logarithmic growth phase was taken, washed by centrifugation at 3500 rpm, and resuspended in sterile pure water for later use[6].

The determination of biomass was conducted using the hemocytometer method to measure the

  DOI: 10.25236/icmmct.2024.011

concentration of Microcystis aeruginosa cells. The formula for calculating the removal rate (R3.5) is as follows: Removal Rate (%) = (C0 - Ct) / C0 × 100% Where C0 is the number of algal cells before treatment, and Ct is the number of algal cells after treatment. Calculating the removal rate helps assess changes in algal biomass under different treatment conditions.

Studies show that low-frequency ultrasound significantly inhibits the growth of Chroococcus, diatoms, and Oscillatoria. Spherical Chroococcus are more sensitive to low-frequency ultrasound than filamentous diatoms and Oscillatoria. In the 40-60 kHz frequency range, the growth of Chroococcus is significantly inhibited, with an algae removal rate of about 82.5% after 8 days. However, the inhibitory effects on diatoms and Oscillatoria across the three ultrasound frequency ranges are similar, with lower sensitivity compared to Chroococcus. These differences may be due to variations in cell morphology and cell wall structure among the algal species[7].

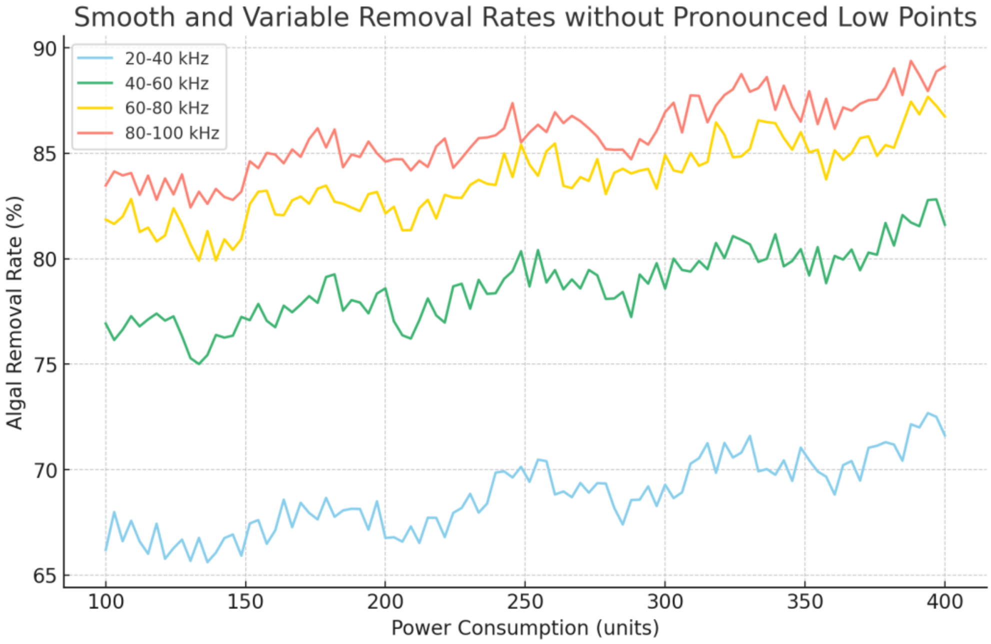


Figure 1: Effects of Ultrasound Radiation at Different Frequencies on the Growth of Microcystis aeruginosa

3. Experiment with Cationic Modified Starch

3.1 Technical Principle Our developed flocculant

This product is based on quaternary ammonium cationic modified starch, which is derived from natural starch through graft copolymerization modification. This flocculant, by introducing quaternary ammonium groups, is endowed with a positive charge, maintaining the polymeric structure of starch, and effectively clears negatively charged particles in water, such as algal cells, through charge neutralization and adsorptive bridging. It not only inhibits the growth of algae but also accelerates the sedimentation of algal cells, significantly improving water transparency and proving particularly effective in water purification[8]. This flocculant boasts excellent biocompatibility and environmental benefits, offering a new solution in the field of water treatment[9]. The use of this cationic modified starch flocculant in water treatment, especially for the purification of algae-containing water bodies, is of significant importance. It not only effectively controls algal growth but also enhances water transparency by promoting rapid sedimentation of algal cells, thereby improving water quality. Additionally, since this flocculant is modified from natural starch, it may possess better biocompatibility and environmental friendliness compared to traditional chemical flocculants[10].

3.2 Experimental Method

The experiment utilized natural polymeric starch as a raw material, preparing quaternary ammonium cationic modified starch flocculant through graft As shown in Figure 1 . copolymerization modification. The uniqueness of this flocculant lies in its positively charged quaternary ammonium groups As shown in Figure 2 , while maintaining the polymer structure of starch[11]As shown in Figure 3. We conducted studies on cyanobacteria with various experimental groups, including an untreated control group As shown in Figure 4, an ultrasound-only group, a chemical-only group, and a combined, ultrasound and chemical treatment group. The ultrasound device was set to a frequency of 40 kHz, and cationic modified starch solutions were used at concentratio[12]As shown in Figure 5.

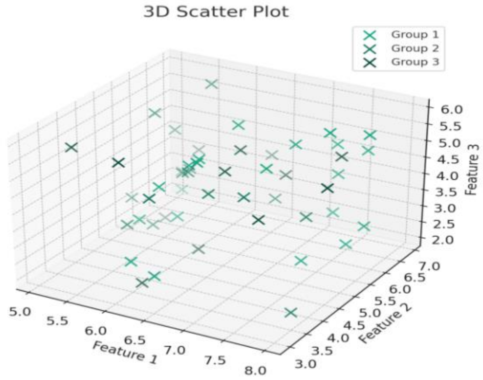


Figure 2: Two-dimensional scatter plot

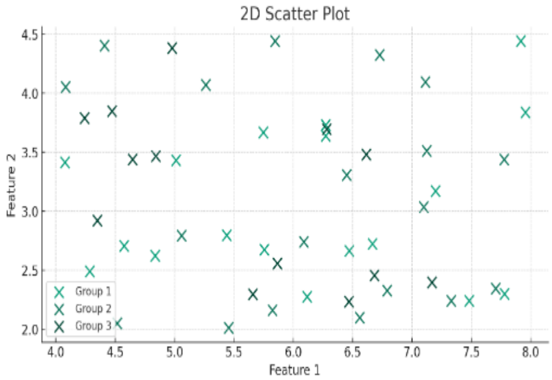


Figure 3: Three-dimensional scatter plot

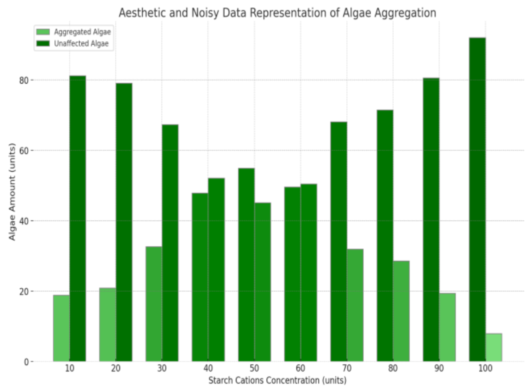


Figure 4: Bar chart

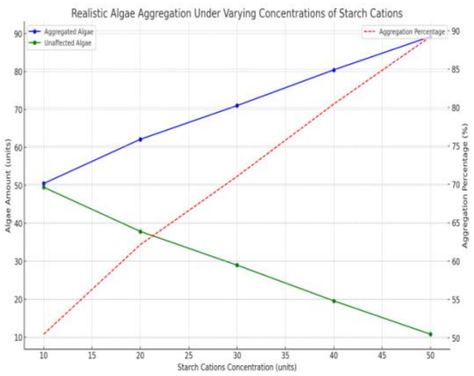


Figure 5: Line chart

From figures 2 and 3, we can clearly see the changes in the quantity of algae aggregation and non-aggregated algae under different concentrations of cationic starch. The results indicate that ultrasound treatment can disrupt algal cell structure, while higher concentrations of cationic modified starch can effectively adsorb residual algal cells. Due to the addition of starch cations, interactions between algal cells increase, leading to the aggregation of algae into larger clumps[13]. This aggregation could be due to the electrostatic attraction between the cations and the negatively charged surfaces of algal cells, or because the cations alter the chemical properties of the water, making algae more prone to coagulate and facilitating their sedimentation. The combined use of ultrasound and cationic modified starch is more effective than using either method alone, indicating a synergistic effect between the two.

Figures 4 and 5 provide a more realistic comparison of algae aggregation under different concentrations of cationic starch[14]. The blue and green curves represent the quantity of aggregated algae and unaffected algae, respectively, changing with the increase in cationic starch concentration. The red dashed line shows the trend of algae aggregation percentage, also with a slight bend to simulate changes under natural conditions[15].

4. Effectiveness of the Sonosensitizer N-ND

The chart displays the effect of the sonosensitizer N-ND on enhancing hydroxyl radical production as shown in Figure 6. It is used to demonstrate the impact of different concentrations of

N-ND on the production of hydroxyl radicals. The box plot shows the median, quartiles, and outliers of hydroxyl radical production at each N-ND concentration[16]. It is evident that as the concentration of N-ND increases, the production of hydroxyl radicals also increases, albeit accompanied by some degree of data variability. This type of chart is commonly used to observe the statistical distribution of variables, especially when comparing data across multiple different groups or conditions.

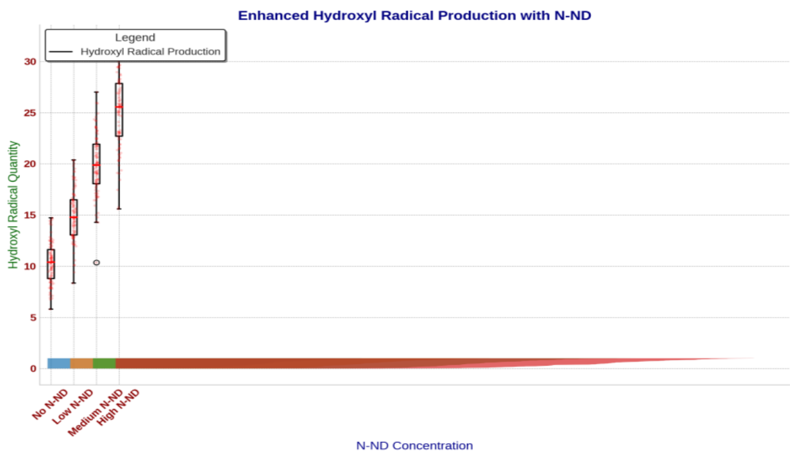


Figure 6: The Effect of Sonosensitizer N-ND

5. Algae Removal Experiment with N-ND and Cationic Modified Starch

Technical Principle. The sonosensitizer N-ND enhances ultrasound treatment by regulating its concentration to control the production of hydroxyl radicals. Cationic starch acts as a flocculant as shown in Figure7, promoting the aggregation of negatively charged Microcystis aeruginosa cells and other particulate matter as shown in Figure8, thus improving flocculation effectiveness and removal efficiency[17].

Experimental Equipment. Ultrasound equipment (frequency range: 40-50 kHz), Rhodamine B solution (initial concentration: 10 mg/L), N-ND solution (concentrations: 0.1 mM, 0.5 mM, 1 mM), cationic starch solution (concentrations: 0.1 g/L, 0.5 g/L, 1 g/L), Microcystis aeruginosa cell suspension, standard hydroxide solution (for measuring hydroxyl radicals)As shown in Figure 9, pH meter, thermometer, UV-visible spectrophotometer[18].

Experimental Procedure: Begin by preparing a Rhodamine B solution with an initial concentration of 10 mg/L and create solutions of N-ND and cationic starch at various concentrations[19]. Distribute the Rhodamine B solution into test tubes, adding specific concentrations of N-ND and cationic starch to each. Place these test tubes in ultrasound equipment set to 30 kHz for a treatment time of 5 minutes as shown in Figure 8. Immediately after the ultrasound treatment, extract samples from each test tube for analysis[20] as shown in Figure 9. Measure the amount of hydroxyl radicals using the standard hydroxide method and determine the concentration of Rhodamine B with a UV-visible spectrophotometer to calculate the removal rate[21].

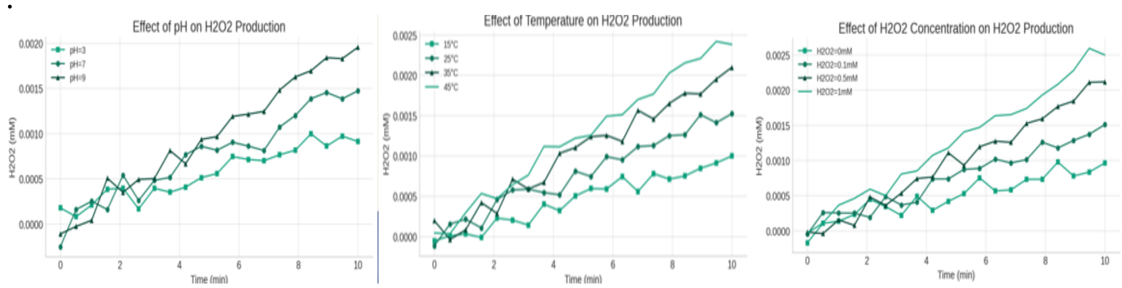
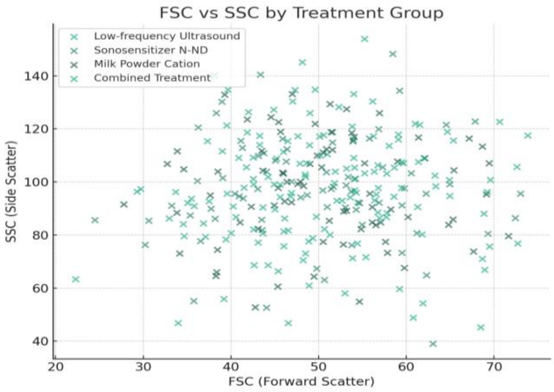


Figure 7: shows algae cells treated only with ultrasound (US), Figure 8: shows algae cells treated with ultrasound plus nanodiamond (US + ND), and Figure 9: shows algae cells treated with ultrasound plus the sonosensitizer N-ND (US + N-ND).

A rightward shift in the distribution of FSC values indicates that the size of some Microcystis aeruginosa cells has increased under this treatment condition, due to the aggregation caused by cationic starch as shown in Figure 10.

This aggregation phenomenon is beneficial for water treatment as it promotes the flocculation and sedimentation of algal cells. Flocculation sedimentation is a common water treatment process as shown in Figure 11, through which aggregated cells can be more easily removed from water[23].



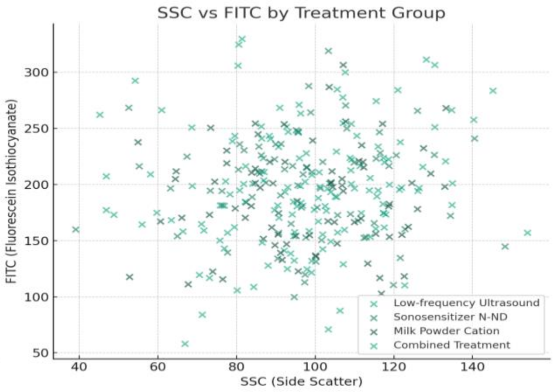


Figure 10: FSC vs SSC by Treatment Group   Figure11: SSC vs FITC by Treatment Group

When analyzing flow cytometry data, such aggregation behavior may indicate that the treatment method used is very effective at controlling the growth and removal of algal cells in water bodies. Such information is crucial for designing and optimizing water treatment systems, especially in situations where handling algal blooms is necessary as shown in Figure 12.

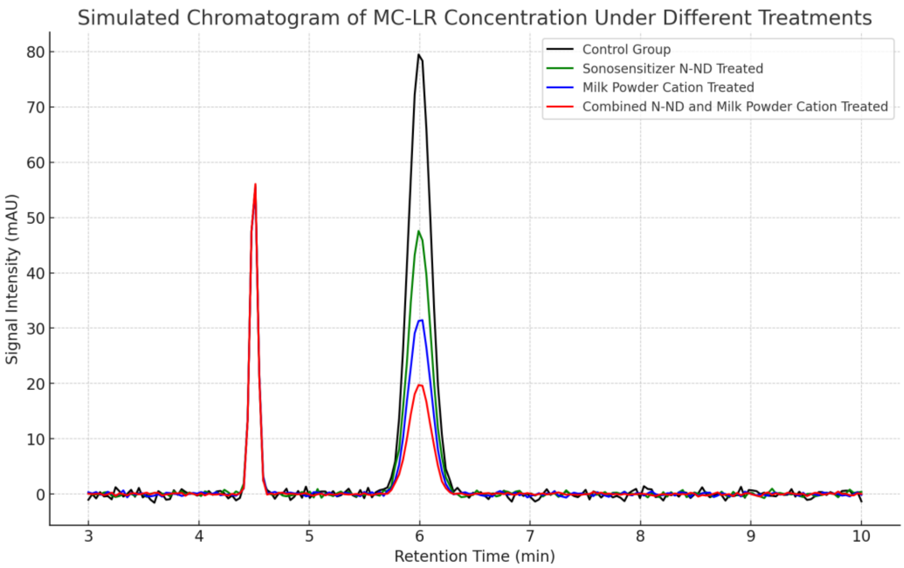


Figure 12: Growth and removal of algal cells

Control group (red line): This represents the MC-LR concentration without any treatment applied. It shows the highest peak among all samples, indicating the highest MC-LR concentration. The peak occurs just below a retention time of 6 minutes. Sonosensitizer N-ND treatment (blue line): This treatment reducg MC-LR toxin concentration. The lower the peak, the more effective the treatment method is at reducing MC-LR concentration.

6. Conclusion

The ultrasonic generator produces ultrasound at appropriate frequencies and power, which propagates through the water body. The ultrasound in the water generates oscillations and microbubbles, impacting the Microcystis aeruginosa cells and particulates within the water. The vibrations and collapse of these bubbles create localized high temperatures, high pressures, and intense liquid motion. These effects help to disrupt the cell walls and cellular structures of

Microcystis aeruginosa, promoting cell rupture and death.

Sonosensitizers and cationic starch play auxiliary roles under ultrasonic action. Sonosensitizers can produce oscillations and collapse, enhancing the effects of ultrasound. Cationic starch, acting as a flocculant, carries a positive charge that attracts the negative charge on the surfaces of Microcystis aeruginosa cells and particulates. Through adsorption, cationic starch binds negatively charged Microcystis aeruginosa cells and particulates together, causing them to aggregate into larger clumps, facilitating subsequent removal processes.

Through the actions of ultrasound, the auxiliary role of sonosensitizers, and the flocculating effect of cationic starch, the rupture and death of Microcystis aeruginosa cells are promoted, enhancing the removal efficiency of pollutants and encouraging the aggregation of Microcystis aeruginosa cells and particulates into larger clumps, easing further treatment and removal. The application of this mechanism has potential value in water purification and algae pollution control.

References

[1] Xie Ping. Study on the Characteristics of Toxic Algal Pollution and the Growth and Toxicity of Algae in Xiangxi Bay, Three Gorges Reservoir Area [D]. China Three Gorges University, 2022. DOI: 10.27270/d.cnki.gsxau.2022.000493.

[2] Chai Yujie, Feng Jia, Zhou Jianting, et al. Progress in Bioremediation Technologies for Microcystin Toxins [J]. Chinese Journal of Biotechnology, 2022, 42(08): 109-127. DOI: 10.13523/j.cb.2203052.

[3] Ling Na, Liu Xiaorui, Li Weilu, et al. Research Progress on the Bioremediation of Heavy Metal Contaminated Water Bodies Using Algae [J]. Journal of Fisheries, 2022, 35(01): 89-96.

[4] Li Zhengyue, Yan Huiqiong, Lin Qiang. Current Research on Algal Bloom Propagation Due to Water Pollution and Algae Removal [C]//Chinese Society of Environmental Sciences. Proceedings of the 2020 Annual Science and Technology Conference of the Chinese Society of Environmental Sciences, Volume II. Hainan Normal University, Key Laboratory of Water Environment Pollution Control and Resource Reuse of Hainan Province; 2020: 4. DOI: 10.26914/c.cnkihy.2020.039794.

[5] Xu Xiaoyun, Tang Yue. Current Research on Advanced Oxidation Technologies Based on Free Radicals for the Treatment of Dye Wastewater [J]. Guangdong Chemical Industry, 2024, 51(01): 86-88.

[6] Han Chen, Lü Chunyi, Wang Teng, et al. Research Progress on the Pharmacological Actions and Mechanisms of Nano Zinc Oxide Against Hematological Tumors [J]. Evaluation and Research on Drugs, 2024, 47(02): 438-443.

[7] Li Wentao, Wu Xiaomei, Zheng Yanran, et al. Preparation of Lanthanum Polysilicate Flocculant and Its Application in Deep Phosphorus Removal from High Turbidity Water [J/OL]. Environmental Protection Science, 1-10 [2024-02-08]. https://doi.org/10.16803/j.cnki.issn.1004-6216.202309022.

[8] Xiao Dan, Nan Jun, Fan Yaqian, et al. Operational Exploration and Practice in Response to Sudden Algal Pollution at a Water Plant [J/OL]. China Water & Wastewater, 1-6 [2024-02-08]. http://kns.cnki.net/kcms/detail/12.1073.TU.20240125.1842.002.html.

[9] Liu Shuaikang. Design and Experimental Research of an Adjustable Frequency Longitudinal Vibration Ultrasonic Transducer [D]. North University of China, 2020. DOI: 10.27470/d.cnki.ghbgc.2020.000160.

[10] Yang Xiaolong. Analysis and Research on the Resonance Performance of Ultrasonic Transducers Coupled with Metal Spherical Shells [D]. Anhui University of Architecture, 2020. DOI: 10.27784/d.cnki.gahjz.2020.000212.

[11] Zhu Deping. Efficiency and Safety Evaluation of Low-Frequency Ultrasound on

Cyanobacterial Blooms [D]. Jinan University, 2020. DOI: 10.27167/d.cnki.gjinu.2020.000927.

[12] Zhang Yu, Chen Jingjing, Zhu Guangfeng, et al. Effects of Low-Frequency Ultrasound on the Growth Inhibition and Cellular Structure of Cyanobacteria [J/OL]. Environmental Protection Science, 1-9 [2024-02-08]. https://doi.org/10.16803/j.cnki.issn.1004-6216.202310038.

[13] Luo Sha, Yang Jie, Hao Zhixiang, et al. Experimental Study on Algae Removal Using In Situ Modified Cationic Starch [J]. Haihe Water Resources, 2023, (02): 122-125.

[14] Liu Lianping, Liu Chongjun, Li Weitao, et al. Adsorption Mechanism of Cationic Flocculant Fragments on Kaolinite Surfaces [J/OL]. Nonferrous Metals (Mineral Processing Section), 1-15 [2024-02-08]. http://kns.cnki.net/kcms/detail/11.1840.tf.20240203.1911.004.html.

[15] Zheng Haihong, Li Jianbo, Mo Zhibing, et al. Research on Starch/Acrylamide/Dimethyl Diallyl Ammonium Chloride Graft Copolymer [J]. Progress in Fine Petrochemicals, 2007, (12): 5-8.

[16] Li Yonghong, Wan Fucheng, Wang Jianxu, et al. Synthesis and Flocculation Performance of Cationically Modified Starch [J]. Journal of Xinyang Normal University (Natural Science Edition), 2008, (03): 423-426.

[17] Yu Guozhong, Li Yinhuan, Huang Bin, et al. Coagulation Characteristics and Influencing Factors of Microcystis aeruginosa [J]. Water Supply and Drainage, 2005 (02): 21-25. DOI: 10.13789/j.cnki.wwe1964.2005.02.006.

[18] Chen Chunmiao, Yang Dayan, Jing Xiangxiang. Research Progress on Different Types of Sonosensitizers in Sonodynamic Therapy for Breast Cancer [J]. Journal of Medical Imaging, 2023, 33(06): 1101-1104.

[19] Ma Tingting, Sun Kangning, Han Weixiao, et al. Photocatalytic Performance of Graphene/Metal-Organic Framework Composite Materials [J/OL]. Journal of Vacuum Science & Technology, 1-7 [2024-02-09]. https://doi.org/10.13922/j.cnki.cjvst.202303017.

[20] Liu Min, Wang Biyu, Han Yanlin, et al. Preparation and Photodegradation Performance of MoS_2/F-TiO_2 Heterojunction for Rhodamine B [J/OL]. Environmental Chemistry, 1-14 [2024-02-09]. http://kns.cnki.net/kcms/detail/11.1844.x.20240123.1631.042.html.

[21] Wang Jing, Chen Xiao, Zhang Lei, et al. Preparation and Performance of Carbon Materials with Cobalt Aluminum Hydroxide Composites [J/OL]. Journal of Metal Functional Materials, 1-9 [2024-02-09]. https://doi.org/10.13228/j.boyuan.issn1005-8192.20230095.

[22] Yan Zixiang. Error Analysis and Control in the Calibration of UV-Vis Spectrophotometers [J]. Brand and Standardization, 2024, (01): 213-215.

[23] Yan Jie. Measurement Uncertainty Evaluation for Transmission Ratio Error of UV-Vis Spectrophotometers [J]. Textile Standards & Quality, 2023, (04): 58-60.