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PREPARATION OF ALCOHOL/ZNSO$_4$-MODIFIED SHRIMP SHELL ADSORBENT AND ITS CYANOBACTERIAL REMOVAL EFFICIENCY

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MICROCYSTIS AERUGINOSA
MICROCYSTIN
SODIUM HYDROXIDE
MODIFIED SHRIMP SHELL
ADSORBENT

ABSTRACT. – Water pollution caused by toxic cyanobacteria is a worldwide problem that increases with eutrophication. A shrimp shell adsorbent modified by alcohol/ZnSO$_4$ was employed herein to remove *Microcystis aeruginosa*. The modification effects of the volume ratios of the alcohol/ZnSO$_4$ solutions, modification reaction temperature, and modification reaction time on the shrimp shells were studied using single factor experiments. The optimum modification parameters were also obtained. Under optimal conditions, the removal efficiency of the alcohol/ZnSO$_4$-modified shrimp shell adsorbent on the *M. aeruginosa* cells was 78.38 % when the added concentration of the modified shrimp shell adsorbent was 14 g/L. The present study provides a promising technique of removing toxic cyanobacterial blooms.

INTRODUCTION

Microcystis aeruginosa, a worldwide species of cyanobacteria, is frequently related to toxic water blooms (Tonietto *et al*. 2012). These blooms and their metabolites can produce several kinds of toxins, as well as unpleasant tastes and odors, which, along with water quality problems, are considered severe (Jiang *et al*. 2010). Specifically, hepatoxic microcystins are members of a remarkable family of more than 90 cyclic peptides that inhibit protein phosphatase (PP1 and PP2A) (Welker & von Döhren 2006), cause increases in protein phosphorylation, and have been linked to tumorigenic effects (Carmichael 1997, Ding *et al*. 1999) promoting liver cell necrosis (Takenaka & Otsu 2000). Therefore, the control and elimination of cyanobacterial blooms have become significant goals in the restoration and protection of lake ecosystems (Wang & Zhang 2017).

To date, many cyanobacterial removal techniques have been conducted by applying physical, chemical, and biological methods, such as coagulation, allelopathy, adsorption, and application of algicides (Qian *et al*. 2010, Pei *et al*. 2016, Wang *et al*. 2016, Gao *et al*. 2017a, b). As a contingency measure for algae removal, adsorption is one of the most common methods to treat water blooms. For example, the composite material of magnetite/hydrotalcite has been added to an *M. aeruginosa* culture and let stand for 60 min. Most of the algal cells settled to the bottom, and the removal rate reached 98 % (Jiang 2016). At present, modified clays are most widely used for algae removal. These clays with various reagents all exhibited higher removal efficiencies on algae than did natural clay (Kim *et al*. 2016, Liu *et al*. 2016). However, the dose of clay must be controlled to reduce its impact on aquatic life (Zhang *et al*. 2019). Walnut and peanut shells modified by phosphoric or citric acid also have a better effect on algae removal (Wang *et al*. 2016, Wang *et al*. 2018). Compared with clay, these biomaterials are easy to degrade and have little impact on the environment. In fact, Pan *et al*. (2006) thought that algicides produced from natural materials have become a popular research topic because of their distinct advantages, such as their natural origin and safe and nontoxic nature, abundant raw material, and low cost.

Nearly 45 %-48 % of the weight of the shrimp raw material is discarded as waste from seafood industries, and this is now a growing problem with a significant environmental impact (Sachindra *et al*. 2005, Ambigaipalan & Shahidi 2017). On a dry weight basis, shrimp shell offals also contain 17.8 % chitin (Synowiecki & Al-Khateeb 2000), which is used as a flocculant (Naghizadeh *et al*. 2017). Accordingly, the application of natural materials as adsorbents may be a more feasible and sustainable alternative considering the abovementioned issues.

The present study aims to determine a preparation method of an alcohol/ZnSO$_4$-modified shrimp shell adsorbent and investigate the removal efficiency of cyanobacteria using this adsorbent.

MATERIALS AND METHODS

Algal culture: Axenic, unicellular *M. aeruginosa* was obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences. The algae were

cultured in sterilized BG11 medium (pH 7.4) at 25 °C at a light intensity of 2500 lux with a 12:12 h light:dark cycle. The algae were cultured for 4 days to the exponential phase at a density of 10^6 cells/mL and were then used to assay the adsorptive property of the alcohol/$ZnSO_4$-modified shrimp shell adsorbent. The growth medium for all the cultures was BG11 (Rippka *et al*. 1979).

Preparation of the modified shrimp shell: The shrimp shell material was obtained from the supermarket of Pingdingshan in Henan Province, China. It was washed free of debris with tap water and later by deionized water, then dried on trays in an oven at 60 °C for 4 h. It was smashed and sieved using an 80-mesh screen after drying.

The specific preparation process of the alcohol/$ZnSO_4$-modified shrimp shells was referenced from the modification methods of a peanut shell (Lu *et al*. 2012), which is described herein. First, a 0.1 mol/L zinc sulfate solution was prepared. Alcohol was added to the zinc sulfate solution, and 6 g of the pretreated shrimp shell was then slowly added to 300 mL of the alcohol/$ZnSO_4$ solution. The mixed suspension was slowly stirred with a magnetic stirrer. In the end, the suspension was filtered with a qualitative filter paper (10-15 μm). The modified shrimp shell was washed four to five times with distilled water, then dried to constant weight in an oven at 50 °C. The dried, modified shrimp shell was the final adsorbent.

Removal of M. aeruginosa: The capacity of the alcohol/$ZnSO_4$-modified shrimp shell to remove harmful algal bloom was tested using *M. aeruginosa*. The alcohol/$ZnSO_4$-modified shrimp shell was added to 50 mL of algae culture in a 100 mL beaker and let stand for 30 h. The alcohol/$ZnSO_4$-modified shrimp shell was not added in the control groups. A sample was collected 2 cm below the surface for analysis at the end of the settling period.

Analysis methods for the chlorophyll-a concentration: The chlorophyll-a concentration was measured as an indicator of the change in the *M. aeruginosa* cell concentration during the flocculation experiment. The chlorophyll-a concentration was determined using standard methods (Chinese EPA 2012).

The clearance of algae after a 30 h exposure (r, %) in every sample was based on the chlorophyll-a concentration as determined by the following formula:

$$r = \frac{T_2 - T_1}{T_2} \times 100 \qquad (1)$$

where T_1 and T_2 are the chlorophyll-a concentration after adsorption and control, respectively.

RESULTS AND DISCUSSION

Effect of the volume ratio of the alcohol/ $ZnSO_4$ solution

The total volume of the alcohol/$ZnSO_4$ solution was 300 mL with alcohol:zinc sulfate solution ratios of 1:1, 1:3, 1:5, 1:7, and 1:9. The corresponding removal efficiencies of the *M. aeruginosa* cells were 55.26 %, 75.86 %, 67.24 %, 58.62 %, and 40.52 % (Fig. 1). The results showed that the volume ratio of the ethanol/$ZnSO_4$ solution could affect the removal efficiency of the alcohol/$ZnSO_4$-modified shrimp shell adsorbent on the *M. aeruginosa* cells. Therefore, the present study suggests that the optimum volume ratio of the alcohol/zinc solution must be 1:3.

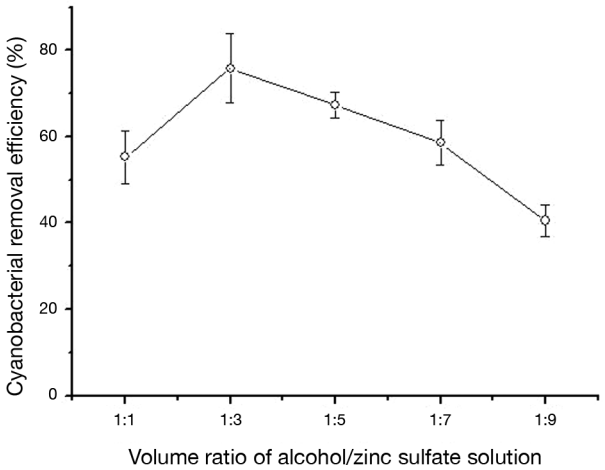

Fig. 1. – Effect of the volume ratio of the alcohol/zinc solution on the efficiency of the *M. aeruginosa* cell removal.

Effect of the reaction temperature modification

Figure 2 shows the removal efficiency trends of the modified shrimp shell adsorbent on the *M. aeruginosa* cells. The removal efficiency first increased with the increasing modification reaction temperature, then slowly decreased as the temperature further increased. The *M. aeruginosa* removal using the modified shrimp shell adsorbent was significantly influenced by the modifica-

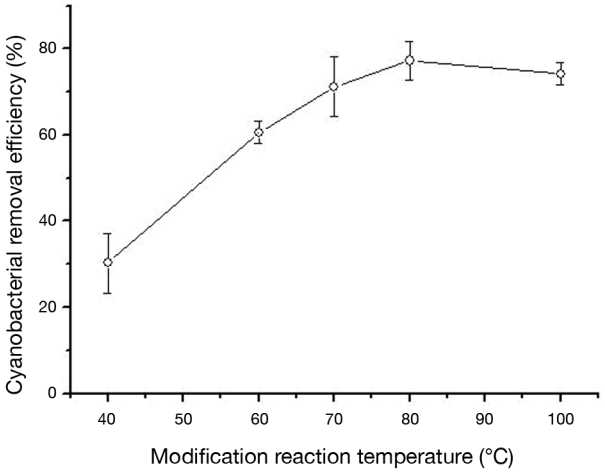

Fig. 2. – Effect of the modified reaction temperature on the *M. aeruginosa* cell removal.

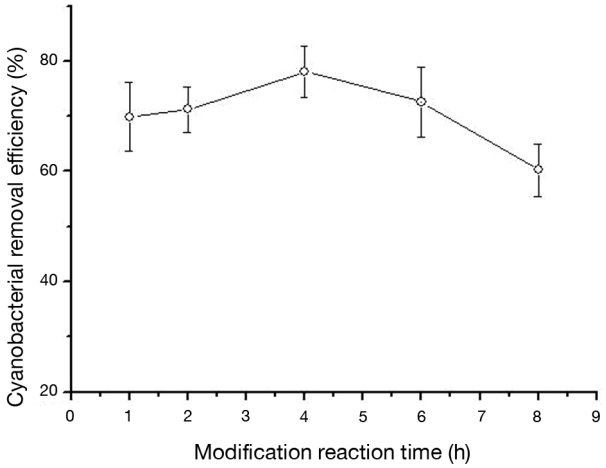

Fig. 3. – Effect of the modified reaction time on the *M. aeruginosa* cell removal.

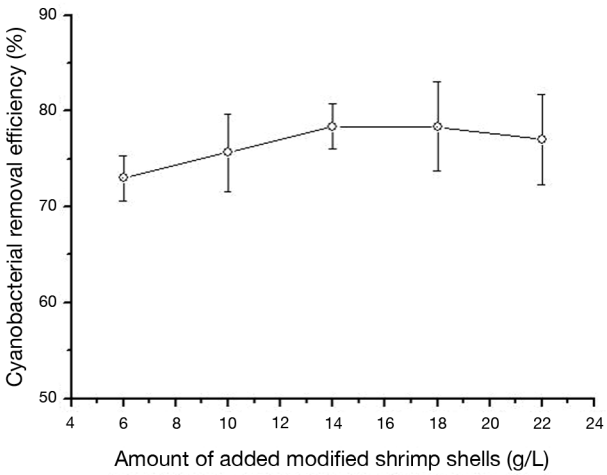

Fig. 4. – Effect of the amount of the modified shrimp shells on the *M. aeruginosa* cell removal.

tion reaction temperature. For example, the removal efficiencies doubled when the temperature increased from 40 °C to 60 °C. Therefore, a modification reaction temperature of 80 °C was chosen for the subsequent experiments.

Effect of the reaction time modification

Figure 3 shows the effects of increasing the modification reaction time on the *M. aeruginosa* cell removal using the modified shrimp shell adsorbent. The removal efficiency of the *M. aeruginosa* cells increased from 69.86 % to 78.08 % as the modified reaction time increased from 1 to 4 h, then gradually decreased with the increasing modification reaction time. The optimized modified reaction time of 4 h was used for the subsequent experiments based on these results.

Effect of the amount of the modified shrimp shell

The data in Fig 4 show that at dosages of 14 g/L and below, the *M. aeruginosa* removal efficiency generally increased with an increase in the dosage of the modified shrimp shell. Further increases in the removal efficiency were observed as the dosage increased. A slight decrease at a dosage of 22 g/L was then seen after a plateau.

In our previous study, walnut and peanut shell adsorbents were modified by citric acid, and these modified walnut and peanut shells exhibited an excellent algae removal performance (Wang *et al*. 2016). The modification by citric acid can enhance the removal efficiencies of the walnut and peanut shells with regard to the *M. aeruginosa* cells. In the present work, the shrimp shell was modified with an alcohol/$ZnSO_4$ solution. As an adsorbent, the shrimp shells are less expensive and could increase the benefit of shrimp products.

Chitin is well known to be recognized as an excellent flocculant or adsorbent for metal ion removal (Hoshi *et al*. 1997, Ahmed *et al*. 2014). Shrimp shell is the main raw material of chitin, which is considered to be a biodegradable, biocompatible, and environmentally friendly material (Ahmed *et al*. 2014). Therefore, low-cost, fresh shrimp shells can be an alternative chitin source. Alcohol/$ZnSO_4$ was used herein as a modifier to improve the algal removal efficiency of the shrimp shells. The shrimp shells contained 34.2 % protein (Lee *et al*. 2018), and ethanol was employed to remove these organic substances in the adsorbent, making some active sites fully exposed and increasing the adsorbent's specific surface area. In addition, the structure of the modified adsorbent became rough, loose, and porous, showing fish-like scales, which was more conducive to the adsorbate adsorption process (Lu *et al*. 2012). In conclusion, the shrimp shell, which is the by-product of a living aquatic resource and employed herein to prepare an adsorbent for the cyanobacterial bloom removal, demonstrated a good potential prospect with economic benefits.

In Jiang's (2016) study, the adsorption algae of the composite material were washed with anhydrous ethanol, dried after being put it in a muffle furnace, calcined for 6 h under 450 °C, and be re-used as an adsorbent. The removal rate of the composite material on the algae slowly decreased with the increase of the cycle times, but still reached more than 85 % after three cycles, indicating that the composite material had a good recycling performance (Jiang 2016).

CONCLUSIONS

This study demonstrated that modification is an efficient method for enhancing the removal efficiency of shrimp shells as an adsorbent for the *M. aeruginosa* cells. The influence of the factors associated with the modification reaction, such as the volume ratio of the alcohol/$ZnSO_4$ solution, modification reaction temperature, and

modification reaction time, were also investigated and identified.

The preparation process of the alcohol/$ZnSO_4$-modified shrimp shell is as follows: 1) 75 mL alcohol was added to 225 mL zinc sulfate solution (0.1 mol/L); 2) 6 g pretreated shrimp shell was slowly added to the alcohol/$ZnSO_4$ solution, and the mixed suspension was slowly stirred with a magnetic stirrer; and 3) the suspension was filtered with a qualitative filter paper (10-15 μm), and the modified shrimp shell was washed four to five times with distilled water before drying to a constant weight in an oven at 50 °C. The dried alcohol/$ZnSO_4$-modified shrimp shell was the final adsorbent. The removal efficiency of the alcohol/$ZnSO_4$-modified shrimp shell adsorbent of the *M. aeruginosa* cells was 78.38% under optimal conditions. Overall, the alcohol/$ZnSO_4$-modified shrimp shell adsorbent may be an interesting and green method for removing *M. aeruginosa* cells.

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