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ELIMINATION OF *MICROCYSTIS AERUGINOSA* KÜTZ. BY MODIFIED WALNUT AND PEANUT SHELLS WITH PHOSPHORIC ACID

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Abstract

The phosphoric acid modified walnut and peanut shell were prepared and applied for the removal of the blue-green alga (*Microcystis aeruginosa* Kütz.). Orthogonal experiment was used to optimize the modified condition. For the efficient removal, 0.05 g of the modified walnut and peanut shell was added into 50 ml of algal culture and kept standing for 4 hrs. This condition was found best and under which the removal rate of algae can be reached > 90%. At the same time, a comparison between unmodified walnut and peanut shell and modified walnut and peanut shell on the removal of *M. aeruginosa* cells showed that, unmodified walnut and peanut shell have a removal rate of 31.51 and 29.90%, respectively. On the other hand, the modified walnut and peanut shell showed a removal rate of 96.55 and 93.75%, respectively. Walnut and peanut shells modified with phosphoric acid enhanced the removal efficiency of *M. aeruginosa* cells. So, the modified walnut and peanut shell were considered as a potential algaecide with high efficiency of obnoxious algae removal from the environment safely.

Introduction

Algae are ubiquitous in rivers, lakes, dams and reservoirs (Aktas *et al*. 2012). Very rapid growth of algae can therefore pose a serious threat to the water supply industry, commercial fisheries and aquaculture, human health and coastal aesthetics (Beaulieu *et al*. 2005, Jiang and Kim 2008). In particular, cyanobacteria may produce toxic secondary metabolites, the cyanotoxins. The production of cyanotoxins has been the object of scientific attention and concern because of associated public health and environmental hazards, including economical losses and environmental impairment (Beaulieu *et al*. 2005).

To minimize the threat, some studies on removal of cyanobacteria were conducted (Jiang *et al*. 2010, Pei *et al*. 2016, Gao *et al*. 2017). It has been concluded that chemical coagulation-flocculation is the most economical step in the water treatment process for the removal of algae (Chen *et al*. 2009, Wu *et al*. 2011). For example, cations, namely aluminum ions, ferric ions and synthetic polymers such as polyacrylamide, were shown to flocculate algal cells and form flocs (Dong *et al*. 2014). However, chemical reagents may not be environmental friendly or cost effective when used in large quantities in natural waters (Zou *et al*. 2006).

In recent years, extensive attention has been given to research and application of natural algicide, because they are natural low-cost products and characterized by their environmentally friendly behavior.

Under the above background, the modified walnut and peanut shell have been prepared using phosphoric acid as modification reagents to investigate its applicability to remove the cells of *Microcystis aeruginosa* Kütz. (Microcystaceae).

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Materials and Methods

Microcystis aeruginosa which was a common bloom forming species in eutrophic surface water was selected for this study. Culture of *M. aeruginosa* was received from the Institute of Hydrobiology, Chinese Academy of Sciences and was cultured in sterilized BG11 medium (pH 7.4) at 25^{o}C with light intensity of 2500 lux (12 : 12 hrs light: dark cycle). The growth medium of all cultures was BG11 (Rippka *et al.* 1979).

Walnut (fruit of *Juglans regia* L., Juglandaceae) and peanut (fruit of *Arachis hypogaea* L., Fabaceae) shells were initially washed free of debris with tap water and then with deionized water. The materials thus prepared were then dried on trays in an oven at 80^{o}C for 6 hrs. After drying, the walnut and peanut shells were weighed and powdered to obtain 80-mesh particles.

An appropriate amount of the powdered sample was soaked with deionized water for 48 hrs at room temperature and then filtered with qualitative filter paper (10 - 15 µm pore diameter, Newstar, 103 type) to remove the suspended substances. The substances on the filter were dried at 95°C in oven, then stored in dryers until being used for modification process.

Since the effects of phosphoric acid (PA) volume, reaction time and concentration of PA were high on modified walnut and peanut shell, those were standardized for the experiment. The three levels per factor were used with PA volume 35, 50 and 65 ml, the reaction time 0.5, 1.5 and 2.5 hrs and the concentration of PA 0.5, 1.0 and 1.5 mol/l, respectively (Table 1). Nine reacting conditions were designed according to the orthogonal test as L_9 (3^4) (Tables 2-3).

Table 1. Factors and levels of orthogonal test.

Levels	Factors		
	(A) Volume of PA (ml)	(B) Concentration of PA (mol/l)	(C) Reaction time (hr)
1	35	0.5	0.5
2	50	1.0	1.5
3	65	1.5	2.5

In 35 - 65 ml of 0.5 - 1.5 mol/l phosphoric acid solution, 5 g of the preprocessed walnut and peanut shell were dispersed. The mixtures were stirred vigorously for a certain time. The liquid portion of mixtures was removed by centrifugation. Then the modified walnut and peanut shell were washed at pH > 5 with distilled water, followed by drying at 50°C in an oven.

The capacity of the modified walnut and peanut shell to remove harmful algal blooms (HABs) was tested using *M. aeruginosa*. The modified walnut and peanut shell were added into 50 ml of algal culture in a 100 ml beaker and kept standing for 4 hrs. In control groups, the modified walnut and peanut were not added. At the end of the settling period, a sample was collected 2 cm below the surface for analysis. Each experiment was done in triplicate.

M. aeruginosa cells were harvested by centrifugation (at 12000 rpm) and then suspended in 0.5% NaCl solution in order to keep the cells alive. The initial cell concentration for all flocculation experiments carried out was controlled at optical density of 0.100 - 0.150 at the wavelength of 680 nm ($OD_{680\ nm}$) (Clesceri *et al.* 1999). Concentration of chlorophyll *a* (chl *a*) calibrated against the direct microscopic cell counts (Clesceri *et al.* 1999) was used to monitor the concentration change of *M. aeruginosa* cells during the flocculation experiment. Samples of *M. aeruginosa* cells filtrated onto 0.45 µm glass fiber filters (Whatman Maidstone, UK) were completely dissolved in 5 ml of 90% acetone solution and then measured for optical density at the wavelength of 665 nm ($A_{665\ nm}$). The chlorophyll *a* concentration was calculated as chl *a* mg/l = 13.4 × $A_{665\ nm}$ (Clesceri *et al.* 1999).

The clearance of algae (r%) of every sample based on the chl *a* concentration was determined after the 4 hrs exposure by the following formula:

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

where T_1 and T_2 were the chl *a* concentrations of the algae and the control after the flocculation, respectively.

All experiments were performed in triplicate and the data were reported as the mean (± Sd). Statistical analyses were performed using SPSS software. A probability level of 0.05 was used to establish the significance ($p < 0.05$).

Results and Discussion

The orthogonal experimental protocol and the results are presented in Tables 2 and 3, respectively. The results showed that the removal rate of algae can be up to > 48% of all modified walnut and peanut shells. In order to find the optimum combination, the analysis of range was done by calculating the difference between the maximum and the minimum of the averages for each factor (Ren 2003). For the modified walnut shell, the order of factors was as follows: reaction time > concentration of PA > volume of PA. The optimum combination was $A_2B_3C_3$, that is to say,

Table 2. Analysis on results of orthogonal test of modified walnut shell with phosphoric acid.

Processing number	A	B	C	D (null line)	Removal efficiency of algae (%)
1	35	0.5	0.5	1	84.93
2	35	1	1.5	2	48.97
3	35	1.5	2.5	3	90.60
4	50	0.5	1.5	3	61.50
5	50	1	2.5	1	91.64
6	50	1.5	0.5	2	80.90
7	65	0.5	2.5	2	87.62
8	65	1	0.5	3	58.67
9	65	1.5	1.5	1	72.69
K_1	224.50	234.05	224.50	249.26	
K_2	234.04	199.28	183.16	217.49	
K_3	218.98	244.19	269.86	210.77	
$\overline{K_1}$	74.83	78.02	74.83	83.09	
$\overline{K_2}$	78.01	66.43	61.05	72.50	
$\overline{K_3}$	72.99	81.40	89.95	70.26	
R	5.02	14.97	28.90	12.83	

adding 1.5 mol/l phosphoric acid solution 50 ml into 5.0 g walnut shell at room temperature with stirring for 2.5 hrs. For the modified peanut shell, the order of factors is as follows: concentration of PA > reaction time > volume of PA. The optimum combination was $A_3B_3C_2$, that is to say, adding 1.5 mol/l phosphoric acid solution 65 ml into 5.0 g peanut shell at room temperature with stirring for 1.5 hrs.

Table 3. Analysis on results of orthogonal test of modified peanut shell with phosphoric acid.

Processing number	A	B	C	D (null line)	Removal efficiency of algae (%)
1	35	0.5	0.5	1	61.77
2	35	1	1.5	2	72.55
3	35	1.5	2.5	3	74.04
4	50	0.5	1.5	3	72.10
5	50	1	2.5	1	64.19
6	50	1.5	0.5	2	85.38
7	65	0.5	2.5	2	77.62
8	65	1	0.5	3	61.35
9	65	1.5	1.5	1	88.81
K_1	208.36	211.49	208.5	214.77	
K_2	221.67	198.09	233.46	235.55	
K_3	227.78	248.23	215.85	207.49	
$\overline{K_1}$	69.45	70.50	69.50	71.59	
$\overline{K_2}$	73.89	66.03	77.82	78.52	
$\overline{K_3}$	75.93	82.74	71.95	69.16	
R	6.48	16.71	8.32	9.36	

Figs 1 and 2 showed that after modification with phosphoric acid, walnut and peanut shell, the algae removal efficiency increased up to the two adsorbents dose of 0.05 g. At this dose, the algae removal was 96.55 and 93.75%, respectively. Further increase in the adsorbent's doses reduced the removal of algae. Therefore, the most economic and effective algal removal conditions are as follows: 0.05 g modified walnut and peanut shell for 50 ml *M. aeruginosa* culture.

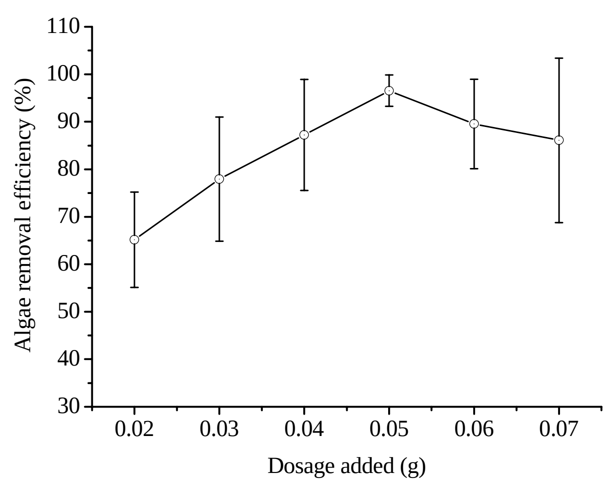

Fig. 1. Effects of the addition of modified walnut shell with phosphoric acid on algae removal.

A comparison between unmodified walnut and peanut shell and modified walnut and peanut shell on the removal of *M. aeruginosa* cells is presented in Fig. 3. There were lower efficiency in removal of *M. aeruginosa* with the unmodified walnut and peanut shell, 31.51 and 29.90% of algae were removed in 4 hrs. In contrast, modified walnut and peanut shell displayed the higher efficiency, with a removal rate of up to 96.55 and 93.75% in 4 hrs, respectively. An Independent-Samples T Test was conducted to compare the removal efficiency between unmodified with modified walnut and peanut shells. The statistical study revealed that walnut and peanut shells showed significant differences at unmodified with modified ($p < 0.01$). The results manifested that the modification by phosphoric acid can enhance the removal efficiency of walnut and peanut shell for *M. aeruginosa* cells.

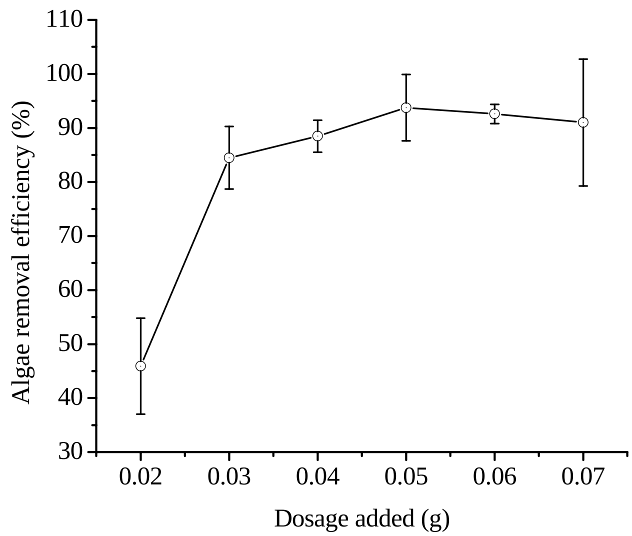

Fig. 2. Effects of the addition of modified peanut shell with phosphoric acid on algae removal.

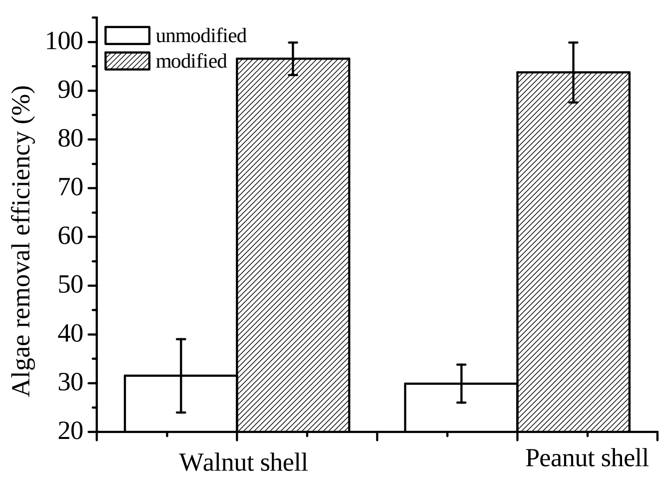

Fig. 3. Effects of unmodified and modified walnut and peanut shell with phosphoric acid on algae removal.

Liu *et al.* (1994) and Jia (2012) determined the chemical composition of walnut and peanut shells and found the main components as cellulose and lignin (Table 4). The modified cellulose and lignin have been found effective as excellent metal and basic dye sorbents (Karsheva *et al.* 2000, O'Connell *et al.* 2008, Gurgel and Gil 2009, Zhang *et al.* 2010, Uraki *et al.* 2006, Gong *et al.* 2008). Therefore, in the present investigation, the use of modified walnut and peanut shells as algaecides might be due to their high contents of cellulose and lignin.

To increase the adsorption performance modified fiber adsorbent was prepared by esterification (Zhou *et al.* 2004), halogenation (Tian *et al.* 2011), oxidation (Mansour *et al.* 2011) and/or etherification (Li *et al.* 2012). However, in the current research, walnut and peanut shells were modified with phosphoric acid and used for the removal of *M. aeruginosa*. With this, the removal efficiency was improved dramatically compared to the removal efficiency of unmodified walnut and peanut shell.

Table 4. Chemical composition analysis of walnut and peanut shell.

	Moisture (%)	Ash (%)	Acid-insoluble lignin (%)	Cellulose (%)	References
Walnut shell	5.16	1.63	53.78	23.42	Jia 2012
Peanut shell	12.27	3.30	33.55	66.36	Liu 1994

In the present investigation, the best experimental conditions for the orthogonal methods were: adding 50 ml (1.5 mol/l) phosphoric acid solution into 5.0 g walnut shell at room temperature with stirring for 2.5 hrs and adding 65 ml (1.5 mol/l) phosphoric acid solution into 5.0 g peanut shell at room temperature with stirring for 1.5 hrs.

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