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HONGQIANG WANG[1], LIEYU ZHANG[2]

PREPARATION OF HYDROGEL BEADS BASED ON SODIUM ALGINATE AND AQUEOUS EXTRACT FROM POMEGRANATE PEEL AND ITS CYANOBACTERIA REMOVAL PERFORMANCE

In recent years, harmful cyanobacterial blooms frequently occurred all over the world, causing great damage to ecosystems, fishery resources, and recreational facilities. Therefore, the removal of harmful cyanobacterial blooms is a crucial step for the maintenance of safe water supplies and for the safety of aquatic products. Hydrogel beads based on sodium alginate and an aqueous extract from pomegranate peel were prepared, and used for removing *Microcystin aeruginosa*. The removal efficiencies of hydrogel beads to *M. aeruginosa* and the factors affecting its removal from solutions (the ratio of material to solvent, the dosage of hydrogel beads, and the density of *M. aeruginosa* cells) were investigated. The optimum ratio of material to solvent, the dosage of hydrogel beads and the density of *M. aeruginosa* cells were 0.03 g/cm^3, 100 g/dm^3 and less than 8.68×10^6 cells/cm^3, respectively. When the density of *M. aeruginosa* cells was below 8.68×10^6 cells/cm^3, the removal efficiency of cyanobacteria was above 99.21% at the dose of 100 g/dm^3. The pomegranate peel and sodium alginate, as natural materials, were safe and innocuous, providing simple operation, low cost and high availability. Therefore, the hydrogel beads offer favorable characteristics in *M. aeruginosa* removal.

1. INTRODUCTION

Cyanobacteria are wide-spread organisms that draw attention due to their mass expansion in aquatic reservoirs linked with eutrophication [1]. Their bloom is highly toxic due to microcystins (MCs), their secondary metabolites [2]. Exposure to MCs represents

[1]School of Environmental and Safety Engineering, University of South China, Changsheng West Road, Zhengxiang District, Hengyang City, Hunan Province, 421001, China.

[2]Laboratory of Water Environmental System Engineering, Chinese Research Academy of Environmental Sciences, Dayangfang Beiyuan Road 8, Chaoyang District, Beijing City, 100012, China, corresponding author, e-mail: zhanglieyu@163.com

a health risk to aquatic organisms, wild life, domestic animals, and humans upon drinking or ingesting cyanobacteria in water [3]. The high incidence of primary liver cancer in eastern China is considered to be related to the presence of MCs in drinking water [4]. In 1996, acute exposure to MCs led to the death of 76 patients during renal dialysis treatment in Caruaru, Brazil [5]. Therefore, the removal of harmful cyanobacterial blooms is a crucial step for the maintenance of safe water supplies and for the safety of aquatic products [6].

At present, three types of bloom control employ physical, chemical or biological methods. Although these methods have been proposed to control harmful algal growth, each of these has advantages and disadvantages. To be sure, flocculation is an efficient method for the removal of toxic cyanobacteria and other microalgae [7-9]. Especially for treatment of cyanobacterial blooms, methods using nontoxic flocculants without damaging cell integrity are generally preferred.

Sodium alginate (SA) is a water-soluble salt of alginic acid and naturally occurring non-toxic polysaccharide found in all species of brown algae [10]. Sodium alginate can be used as a curing agent to prepare hydrogel beads. Alginate gelation occurs when divalent cations (usually Ca^{2+}) react with blocks of guluronic acid residues, resulting in formation of a three-dimensional network [11]. This has been used in water treatment [12, 13]. Some studies also showed that hydrogel beads can be used for better regeneration [12] and long-term stability, which avoids introducing new pollution into the water environment [13]. Hence, the hydrogel beads may have a great practical value in water treatment.

Hydrogel beads based on sodium alginate and flocculants were prepared and used for removing harmful cyanobacteria. Hydrogel beads can be recycled after eliminating cyanobacteria, thus reducing the impact on the environment. Therefore, this work aimed to prepare the sodium alginate-aqueous extract from pomegranate peel (SA/AEPP) composite hydrogel beads and to employ them to flocculate and remove *Microcystin aeruginosa* cells. To our knowledge, this is the first report about the flocculation of SA/AEPP composite hydrogel beads on *M. aeruginosa*.

2. MATERIALS AND METHODS

M. aeruginosa culture. *M. aeruginosa*, a common species in eutrophic surface water, was selected for this study. It was obtained from the culture collection of algae at the Institute of Hydrobiology, Chinese Academy of Sciences. The *M. aeruginosa* were cultured in sterilized BG11 medium (pH 7.4) at 25 °C and the light intensity of 2500 lux, 12:12 h light:dark cycle. Cultured *M. aeruginosa* were used for the assay of growth inhibition. The growth medium of all cultures was BG11[14].

Preparation of aqueous extract and composite hydrogel beads. Pomegranate peel was naturally dried on trays away from sunlight at room temperature. The dry peel were weighed and powdered to obtain particles of an 80 mesh size (177 μm mesh size). The pomegranate peel was extracted with proper volumes of ultrapure water at room temperature (25 °C) for 24 h. Various ratios of material to solvent (weight of pomegranate peel to the volume of ultrapure water, g/cm^3) were adopted, which were 0.004, 0.01, 0.03, 0.05, 0.07 and 0.09 g/cm^3, respectively. The extract was filtered through qualitative filter paper (10–15 μm) for removal of residues, and the final volume was readjusted to match the starting volume. The aqueous extract was stored at 4 °C until used for follow-up studies.

25 cm^3 of aqueous extract from pomegranate peel was diluted with 25 cm^3 of deionized water, then 1.0 g of sodium alginate (SA) was dissolved in the solution to produce a viscous solution of the concentration of 2 wt. % SA after mechanical agitation, and then ultrasonic irradiation for 30 min (40 W, 40 kHz, 25 °C) was applied to achieve homogeneous dispersion. The solution was injected through a dropper into a coagulation bath containing a 10 wt. % aqueous solution of $CaCl_2$ and coagulated for 1 h, then the filtered composite hydrogel beads were washed three times with deionized water. Finally, the composite hydrogel beads incubated in deionized water for 2 h and were then dried for 4 h in an oven at 40 °C. The diameter of the resulting composite beads was approximately 2.0–3.0 mm.

Flocculation experiments. The coagulation experiments were performed on cyanobacterial suspensions at room temperature (ca. 25 °C). Composite hydrogel beads were added to a 50 cm^3 of *M. aeruginosa* suspension in a 100 cm^3 beaker and were then settled for 24 h. The hydrogel beads were not added to the control group. At the end of the settling period, a sample was collected 2 cm below the surface for analysis.

A compound microscope (OLYMPUS BH-2) was used to count the number of *M. aeruginosa* cells per unit volume in a hemocytometer. Each sample was counted three times. Cell density was recorded in number of cells/cm^3. *M. aeruginosa* cells were harvested by centrifugation (at 12 000 rpm) and then suspended in 0.5 wt. % NaCl solution to maintain live cells. The initial cell concentration for all flocculation experiments was set to an optical density of 0.100–0.150 at the wavelength of 680 nm ($OD_{680\ nm}$) [15]. The concentration of chlorophyll-a, calibrated against direct microscope cell counts [15], was used to monitor the concentration of *M. aeruginosa* cells during the flocculation experiment. Samples of *M. aeruginosa* cells filtered onto 0.45 mm glass fiber filters were completely dissolved using 5 cm^3 of 90 wt. % acetone solution and then measured for optical density at the wavelength of 665 nm ($A_{665\ nm}$). The chlorophyll-a concentration (T) was calculated according to the relationship: T (mg/dm^3) = 13.4×$A_{665\ nm}$ [15].

The clearance of *M. aeruginosa* (r, %) in every sample based on the chlorophyll-a concentration after flocculation or control was determined after exposure for 12 h. The computational formula is as follows:

$$r = \frac{T_2 - T_1}{T_2} \times 100\% \tag{1}$$

where, T_1 and T_2 are the chlorophyll-a concentrations after flocculation and in a control sample, respectively.

Data analysis. All experiments were performed in triplicate and the data were presented as the mean±standard deviation. The standard deviation was calculated using the STDEV formula in Excel. Statistical analyses were performed using a SPSS software, with significant differences implied at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF THE RATIO OF MATERIAL TO SOLVENT

Removal efficiencies of cyanobacteria in function of the ratios of material to solvent of SA/AEPP composite hydrogel beads are shown in Fig. 1.

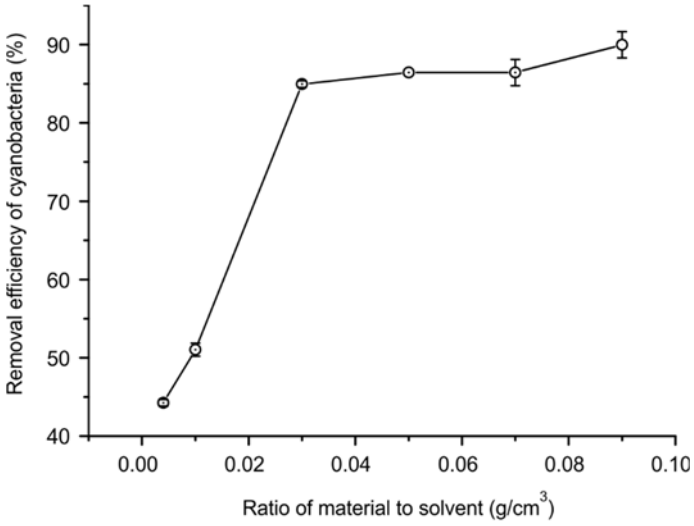

Fig. 1. Removal efficiencies of cyanobacteria in function of the ratios of material to solvent; density of cyanobacteria 8.68×10^6 cells/cm^3, addition dosage of SA/AEPP composite hydrogel beads 100 g/dm^3

It is evident that the ratio of material to solvent played an important role in enhancing cyanobacterial cell removal. The removal efficiency increased from 44.25% to 84.96% upon increasing the ratio of material to solvent from 0.004 to 0.03 g/cm^3. The increase of the ratio of material to solvent resulted in the higher concentration of effective constituent in aqueous extract of pomegranate peel, hence, the removal efficiency was increased. However, for the ratios of material to solvent exceeding 0.03 g/cm^3, the removal efficiency only slightly increased because the extraction yields of effective components were close to saturation. Therefore, the optimum ratio of material to solvent was balanced at 0.03 g/cm^3.

3.2. EFFECT OF THE ADDITION OF SA/AEPP COMPOSITE HYDROGEL BEADS

The effect of SA/AEPP composite hydrogel beads dosage on the *M. aeruginosa* removal is shown in Fig. 2. When the SA/AEPP composite hydrogel beads dosage increased from 70 to 100 g/dm^3, there was an obvious increase in the level of *M. aeruginosa* reduction up to 98.75%, then it slightly decreased. The main reason for the decrease was an overdosing effect of the SA/AEPP composite hydrogel beads.

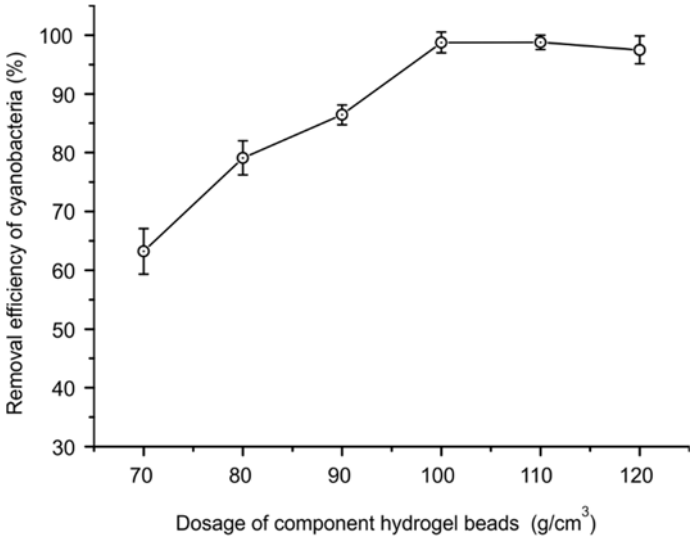

Fig. 2. Effect of the addition of SA/AEPP composite hydrogel beads on cyanobacteria removal; density of cyanobacteria 8.68×10^6 cells/cm^3, ratio of material to solvent 0.03 g/cm^3

3.3. EFFECT OF THE DIFFERENT DENSITIES OF *M. AERUGINOSA* CELLS

The density of *M. aeruginosa* cells presents an obvious influence on the flocculation effect (Fig. 3). The *M. aeruginosa* cell removal was 100% when its density was in the

range of (2.17–4.34)×10^6 cells/cm^3. As the density of *M. aeruginosa* cells increased from 8.68×10^6 to 17.36)×10^6 cells/cm^3, the removal efficiency decreased rapidly from 99.21 to 50.00 %, and decreased slowly upon its further increase. These results suggest that the removal efficiency could be significantly affected by the cell density.

The surface of *M. aeruginosa* cells is negatively charged which can affect the removal efficiency of algae cells by flocculation. Therefore, the removal of *M. aeruginosa* is more difficult than removing suspended solids. In the current work, the results also showed that the removal efficiency of algae cells could be affected significantly by the density of *M. aeruginosa* cells.

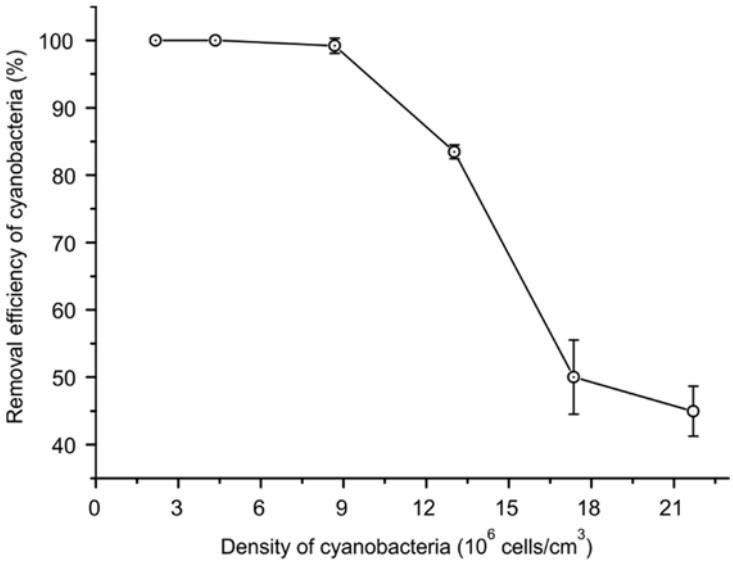

Fig. 3. Effect of SA/AEPP composite hydrogel beads on the removal of cyanobacteria of various densities; dosage of SA/AEPP composite hydrogel beads 100 g/dm^3, ratio of material to solvent 0.03 g/cm^3

Alginate, extracted mainly from brown seaweeds, which are abundant in all the sea areas of the world, is one of the more effective biological adsorbents [16]. Sodium alginate could also be used as a gelling agent due to its ability to form gels with divalent cations, such as calcium, under mild conditions [17]. The removal of methyl orange (MO), uranium ions, Pb(II) ions, and radiostrontium from aqueous solutions using SA gel beads has been reported [18–21]. For example, Ca-alginate beads can be successfully used for uranium recovery from aqueous solutions. The percent adsorption for uranium ions was 91±1 under the optimized experimental conditions [19]. In addition, Meng et al. [22] found that modified alginate flocculant had obvious flocculation for heavy metal ions at varied concentrations. Hu and Ning reported that the

tannin content in the pomegranate peel was around 25–30% of its dry weight [23]. Tannins are a new source for coagulant and flocculant agents [24] that are widely used in wastewater treatment [25–28]. Therefore, pomegranate peel could be used as raw material to extract natural flocculant. In the current work, pomegranate peel was soaked with ultrapure water to extract liquid flocculant. Liquid flocculant immobilized with sodium alginate was prepared to treat cyanobacterial blooms. The immobilized flocculant used is more convenient and easier to transport than liquid flocculant. Hydrogel beads have long-term stability, which can reduce the side effects to the water environment.

4. CONCLUSIONS

- A novel material, the SA/AEPP composite hydrogel beads as an effective flocculant for *M. aeruginosa* removal has been prepared. The flocculant has high removal rates toward cyanobacteria.
- The results demonstrate that the removal efficiency of cyanobacteria is affected by various conditions such as the ratio of material to solvent, the dosage of hydrogel beads and the density of *M. aeruginosa* cells.
- The pomegranate peel and sodium alginate, as natural materials, were safe and innocuous, providing simple operation, low cost and high availability. Thus, SA/AEPP composite hydrogel beads can be utilized as environment friendly flocculant for the removal of cyanobacteria from water body.

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