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Microalgae harvesting by buoy-bead flotation process using Bioflocculant as alternative to chemical Flocculant

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ABSTRACT

To evaluate the use of bioflocculants as potential alternatives to chemical flocculants in improving the harvesting efficiency of buoy-bead flotation (BBF) process, harvesting experiments for *Scenedesmus obliquus* (*S. obliquus*) and *Chlorella vulgaris* (*C. vulgaris*) were first carried out by the BBF method combined with pre-flocculation in a bench scale flotation column. Chitosan and ferric chloride were used as pre-flocculants in this study. Measurements of zeta potential and algal cell surface architecture were performed to reveal the mechanisms of action of ferric chloride and chitosan as pre-flocculants. The results showed that the BBF method with chitosan pre-flocculation provided harvesting efficiencies as high as 83.77% and 92.47% for *S. obliquus* and *C. vulgaris*, respectively. The harvesting efficiency was found to be related directly to the characteristics of microalgae-microsphere aggregates formed. The addition of a small number of flocculants could significantly change the size and density of aggregates and increase the rising velocity of aggregates. The characteristics of aggregates were dependent on the effects of different pre-flocculants. The effect of chitosan was stronger than that of ferric chloride in improving the rising velocity. The faster the rising velocity, the more the microalgae enriched on the suspension surface. Therefore, the harvesting efficiency could be improved by enhancing the rising velocity. These results suggest that bioflocculants like chitosan are good substitutes for chemical flocculants to improve the harvesting efficiency in BBF process.

1. Introduction

Microalgae are considered as an important future raw material for health foods, animal feeds, nutraceuticals, pharmaceuticals, biofuels, and cosmeceuticals, due to their high contents of carotenoids, lipids, proteins, and polysaccharides [1,2]. However, there are still several issues regarding the commercial and sustainable production of microalgae, such as sustainable nutrient sources in the process of microalgae culture, harvesting microalgae in a cost-effective manner and developing an efficient method to dry microalgae biomass [3]. Among them, a key problem is how to harvest microalgae from water in an economical and ecofriendly manner, since this step accounts for 20–30% of the total cost of biomass production [4]. The current common harvesting methods include centrifugation [5], gravity sedimentation [6], flocculation [7], filtration, dissolved air flotation [8], and integrated techniques [9,10]. As the size of microalgae is under 30 μm and they grow in very dilute cultures with densities close to that of water, harvesting microalgae from the culture medium is not easy and the majority of the above harvesting methods are of either low efficiency or high cost.

Recently, a buoy-bead flotation (BBF) method has been demonstrated to be effective for harvesting microalgae [11]. In this method, microspheres with low density are used for air flotation instead of bubbles. These microspheres act as carriers to transport suspended microalgae to the liquid surface where microalgae are removed. It is reported that the BBF process consists of three main steps: collision, attachment, and detachment of algae and microspheres [12]. As essential reagents, flocculants are generally added to the BBF system to promote the attachment of microalgae onto microspheres, and then to enhance the microalgae harvesting efficiency [13,14].

Multivalent chemical flocculants like ferric sulfate, ferric chloride, aluminum sulfate and aluminum chloride are generally used to facilitate the flotation separation process [15] because they form flocs easily due to the neutralization effect [16]. For example, Jarvis et al. [11] used ferric sulfate as a pre-flocculant to harvest algae. However, the usage of chemical flocculants might be harmful for the aquatic environment and downstream products due to their high toxicity and non-biodegradability. Therefore, it is of practical interest to select

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environment-friendly flocculants as potential alternatives to chemical flocculants for harvesting microalgae [14].

Bioflocculants have similar physio-chemical properties as chemical flocculants, but with the additional advantages of low toxicity, biodegradability and production from renewable resources. Thus, the usage of bioflocculants in harvesting microalgae has been attracting a great deal of attention [17]. Chitosan ($C_{56}H_{103}N_9O_{39}$), a linear polysaccharide, is generated by the deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. Chitosan is the second most abundant biopolymer in the world [18]. The molecular weight of commercially produced chitosan is generally between 3800 and 20,000 Da. Chitosan can easily break into small fragments in weakly acidic solutions. Therefore, it is considered as a bioflocculant in flocculation methods for wastewater treatment and microalgae harvesting [19,20]. However, chitosan is reported to have a lower efficiency than ferric chloride in the flocculation harvesting method. Moreover, there are no studies on harvesting microalgae with chitosan as a pre-flocculant in the BBF system. Therefore, it is necessary to further improve the performance of bioflocculants for microalgae harvesting, and to investigate the effectiveness of chitosan in the BBF method.

The objective of the present study is to examine the usage of bioflocculant (chitosan) as a potential alternative to chemical flocculants (ferric chloride) to achieve high harvesting efficiency in the buoy-bead flotation process. For this purpose, microalgae harvesting experiments were conducted via the BBF method with pre-flocculation in a laboratory scale flotation column. The effects of the two types of flocculants on the formation of "microalgae-microsphere" aggregates and harvesting efficiency of microalgae were examined based on the measurement results of zeta potential and algal cell surface architecture. Mean rising velocity of microalgae-microsphere aggregates was then calculated by a Stokes' model. Finally, the harvesting performance of microalgae in BBF system was analyzed by explaining the pre-flocculation mechanism of chitosan and ferric chloride.

2. Materials and methods

2.1. Microalgae culture

The microalgae species, *Chlorella vulgaris* (*C. vulgaris*) and *Scenedesmus obliquus* (*S. obliquus*), were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB-collection, China). *C. vulgaris* was cultured using BG-11 medium (constituents: 1.500 g/L $NaNO_3$, 0.040 g/L K_2HPO_4, 0.075 g/L $MgSO_4{\cdot}7H_2O$, 0.036 g/L $CaCl_2{\cdot}2H_2O$, 0.006 g/L Citric acid, 0.006 g/L Ferric ammonium citrate, 0.001 g/L EDTA-2Na, 0.020 g/L Na_2CO_3, and 1 mL/L A5 trace metal solution). Before transferring to a photobioreactor (PBR, Shanghai Guangyu Biological Technology Company, China) as the inoculum, *C. vulgaris* was grown in a 1 L conical flask at 25 ± 3 °C for 10 days under light intensities between 3000 and 3500 Lux, using a light: dark cycle of 12:12 h. Air was continuously supplied at a flow rate of 15 $L{\cdot}h^{-1}$. The pH was maintained at 7–7.5 by automatically supplying 1 $mol{\cdot}L^{-1}$ NaOH and HCl with a peristaltic pump. *S. obliquus* was cultured in Selenite Enrichment (SE) medium (constituents: 0.250 g/L $NaNO_3$, 0.075 g/L $K_2HPO_4{\cdot}3H_2O$, 0.075 g/L $MgSO_4{\cdot}7H_2O$, 0.025 g/L $CaCl_2{\cdot}2H_2O$, 0.175 g/L KH_2PO_4, 0.025 g/L NaCl, 40 mL/L Soilextract, 0.005 g/L $FeCl_3{\cdot}6H_2O$, 1 mL/L Fe-EDTA, 1 mL/L A5 trace metal solution). *S. obliquus* was grown in a 1 L conical flask for 12 d and then inoculated into a PBR. *S. obliquus* was cultured in the PBR at 28 ± 3 °C under a light source at the intensity of 6800 Lux (12 $h{\cdot}d^{-1}$), with the air flow rate adjusted at 18 $L{\cdot}h^{-1}$ and the pH at 7–7.5. Microalgae *C. vulgaris and S. obliquus* entered the stationary growth phase (less than 5% increases in cell numbers per day) after 13 days and 15 days of cultivation, respectively. After reaching the stationary growth phase, the microalgae were washed twice with distilled water and then used to prepare microalgae suspension samples for the following measurements and experiments.

2.2. Pre-flocculation process

In these experiments, *C. vulgaris* cells were harvested every 3 days between days 13 and 19, and *S. obliquus* cells were harvested at 2 day intervals between days 15 and 21. As they were both at the stationary growth phase, the change in biomass concentrations of *C. vulgaris* and *S. obliquus* suspensions can be ignored and their concentrations were $7.60 \pm 1.8 \times 10^6$ $cells{\cdot}mL^{-1}$ and $9.40 \pm 0.9 \times 10^6$ $cells{\cdot}mL^{-1}$, respectively. In separate experiments, the ranges of both ferric chloride and chitosan concentrations selected for harvesting test were 0.00–0.20 g/L. Harvesting experiments were carried out in 2 L flotation column containing 2 L of microalgae culture. The suspension was rapidly mixed for 30 s at 250 rpm followed by a standing period for 15 min [21]. An aliquot of the supernatant was taken 2 cm from the surface of the liquid and its absorbance was measured at 540 nm in a 10-mm path length plastic cuvette using a DU720 spectrophotometer (Shimadzu, Japan). Microalgae harvesting efficiency was measured using Eq. (1). Zeta potential was analyzed by a zeta meter (DelsaTM Nano Beckman Coulter, USA). Scanning electron microscopy (SEM) images were taken with a Hitachi S-4800 microscope (Japan).

$$\text{Harvesting efficiency}(\%) = 100 \times \left(1 - \frac{c_0}{c_1}\right) \quad (1)$$

where C_0 is the concentration of microalgae still in the supernatant, and C_1 is the concentration of microalgae before the addition of flocculants.

Furthermore, the dissolved organic materials (DOM) of samples were determined by ultraviolet (UV) spectrophotometry. The microalgae suspension was treated with flocculants, and then it was filtered with a 0.45 μm cellulose acetate membrane filter. The ultraviolet absorbance (UVA) at 254 nm by the filtered microalgae suspension was used for determining the concentration of DOM in solution. All experiments were performed in triplicate. All results shown in the following graphs and table were the mean values of $n = 3$ and the bars represent standard deviations from the mean.

2.3. Buoy-bead harvesting experiments

In this study, hollow microspheres (Shanghai, Mingbo New Material Technology Co., Ltd., China) composed of a thin wall sodium borosilicate glass and with a low density were employed to support flotation. According to our previous screening tests, the best removal efficiency could be obtained when microspheres had a diameter of 55 μm and a density of 0.40 $g{\cdot}cm^{-3}$. In this case, the rising velocity of the microspheres was moderate, which could be helpful for flotation.

Bench scale tests of both BBF systems were performed under verified optimum conditions, in which pH was 7.5 and microsphere dosing was 0.55 g/L. Fig. 1 shows the process of BBF. The main body of the flotation equipment was a self-made flotation column with a working volume of 2 L. Chitosan and ferric chloride of aforementioned concentrations were used as flocculants. For better solubility, 100 mg of chitosan was added into 10 mL of 1% HCl solution and mixed with a stirrer at 100 rpm for 30 min [22]. The microspheres were dosed into the water before adding the flocculant with stirring at 200 rpm for 30 s, to ensure that the microspheres were dispersed in the column. Subsequently, the stirring was stopped and most of the microalgae-microsphere aggregates that floated to the top of the column after two minutes were collected.

2.4. Characteristics of aggregates

2.4.1. Aggregate dimension measurement

In the microscope method of size analysis, direct measurements are made on enlarged images of the aggregates. In the simplest technique,