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Magnetic–cationic cassava starch composite for harvesting *Chlorella* sp. TISTR8236

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ABSTRACT

Microalgae are cultured for their useful chemicals, but their development is restricted by the costs and difficulties of harvesting them. New composites, magnetic-cationic cassava starch, were successfully developed to facilitate harvesting. Compared to naked magnetic particles, the composites exhibited higher potential for algal cell recovery. In addition, harvest capacity through the composites was positively related to the degree of cationic substitution (DS) in modified cassava starch. The application of 500 mg L^{-1} composites with DS value ≥ 0.76 to recover 1 g L^{-1} *Chlorella* sp. TISTR8236 (1.67 ± 0.01 g-dry cell weight g-composites^{-1}) from medium broth at pH 9.5 was significantly ($p \leq 0.005$) > 98% effective. The separation efficiency of this composite for 1 g L^{-1} algal biomass was evaluated under different composite doses (200 to 800 mg L^{-1}) and pH levels (4 to 10). Ninety-five percent of the algal cell separation efficiency of the magnetic and 0.76 DS cationic starch composite was recorded, when applied to the 300 mg L^{-1} composite to harvest 1 g L^{-1} *Chlorella* sp. TISTR8236 at pH 10 within 2 min. The adsorption isotherm fit the Langmuir model. In addition, desorption of algal cells was conducted by adjustment of the pH of the culture medium. The highest desorption (9.69%) was recorded at pH 12. These composite magnetic-cationic cassava starch particles were shown to be a new adsorption material for algal cell harvesting. This alternative composite for magnetic separation may represent an effective method for saving time and energy in algal industrial processing.

1. Introduction

Microalgae are a valuable natural source of beneficial pigments, protein, lipids and compounds that have wide applications including nutraceuticals, pharmaceuticals, biofuel, food and feed additives [1–3]. However, large-scale production of microalgae requires a high investment cost for cultivation, harvesting, extraction and purification processes. The harvesting cost of microalgae is 20–30% of the total production cost, which is accounted for by the high energy and time consumption in biomass separation because of their small size (3–30 μm diameter), low density (< 1 g L^{-1}) and colloidal stability [1,3–5]. A major contribution to the use of microalgae would be improved harvesting.

Magnetic separation is a promising technique that has been used for biomass collection for both marine and freshwater microalgae [6]. The principle of magnetic separation is the application of magnetic particles to attach to microalgae cells and subsequently separate the biomass from the liquid culture medium via an external magnetic bar [7]. The advantages of magnetic separation are that it is simple, fast, and inexpensive with no energy and chemicals requirements, which makes it an energy efficient and cost effective technique [5,8]. However, simple naked magnetic particles have the problems of particle oxidation and low electrostatic interaction between the negative charge of algal cell surfaces and the neutral charge of the magnetic particle surface, resulting in the requirement of a high dosage of magnetic particles [9]. Coating the particles with a polymer reduces contact with oxygen. In addition, giving the coated polymer a positive charge was shown to enhance the efficiency of algal biomass harvesting [10].

Coating naked magnetite surfaces with polymers to make magnetic composites before the adhesion of algal cell is called the “immobilized-on” strategy, which was shown to achieve higher efficiency than the “attached-to” approach, where cells are coated with a polymer and are subsequently attached by naked magnetics [4,5]. Electrostatic interaction between polymers and algae was a main factor that affected the performance of harvesting efficiency [5]. Synthetic polymers that have been used include poly-(diallyldimethylammonium chloride) [11,12],

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polyethylenimine [9,13], diethylaminoethyl [14] and cationic polyacrylamide [7]. Furthermore, natural polymers have been used for coating with magnetic composites, including: chitosan [3,15], starch [16] and silica [6]. Harvesting of *Chlorella ellipsoidae* cells, a green microalga, by using 20 mg L^{-1} of magnetic nanocomposites coated with polyethylenimine, achieved 97% of harvesting efficiency after 2 min, which was a tenfold lower particle dosage requirement compared to that of naked Fe_3O_4 magnetic nanoparticles of 250 mg L^{-1} [9,13]. The application of silica-coated magnetic particles for *Chlamydomonas reinhardtii, Chlorella vulgaris, Phaeodactylum tricornutum* and *Nannochloropsis salina* separation provided high separation efficiencies > 95% with maximum particle loads for *C. reinhardtii, C. vulgaris*, and *P. tricornutum* of 30, 30 and 77 g g^{-1}, respectively [6]. Moreover, Zhao et al. [5] examined the hybrid strategy by mixing “immobilized-on” and “attached-to” together with the composite (polyaluminum chloride (PACl) + Fe_3O_4 or polyacrylamide (PAM) + Fe_3O_4 and flocculants (PAM or PACl)), respectively. The results showed that 99% of algal biomass could be harvested within 30 s by adding PACl + Fe_3O_4 (0.625 mmol Al L^{-1} + 10 g L^{-1}) and PAM (3 mg L^{-1}).

From the previous studies mentioned above, it is clear that coated magnetic composites resulted in increased separation efficiency. Therefore, the polymer used for coating and the dosage of magnetic composites are the main factors influencing the harvesting efficiency, but different types of polymer coatings on naked particles showed different efficiencies in algal biomass harvesting. Therefore, the objective was to test natural polymers with cationic properties for coating naked Fe_3O_4, that are ecologically sound and, could increase algal biomass harvesting efficiency.

Starch is the second-most abundant natural polymer on earth. Cassava starch has attracted increasing attention as a raw material for various industrial uses because of its low price, renewability and biodegradability. However, the utilization of native starch was reported to be limited by its physicochemical properties, such as water insolubility and its tendency to form unstable pastes and gels [17]. Therefore, the physical and chemical modification of native starch could improve its functionality for other applications. Cationic starches and modified starches, which are widely applied in the paper and textile industry and in waste water treatment, have thermoplasticity and water dissolution properties. In addition, two commercial cationic starches, which are commonly used in waste water treatment and in the paper industry, were reported to be efficient flocculants for freshwater algae, *Parachlorella* and *Scenedesmus*. Another advantage is that cationized polysaccharides are biodegradability, have a relatively low price and are more effective flocculants for highly negatively charged colloidal particles, such as algae [18]. Therefore, this study aimed to synthesize magnetic-cationic cassava starch composite material of different degrees of substitution (DS) with cations into the molecule of starch. In addition, the potential of a novel composite for algal biomass separation was examined by harvesting the microalgae, *Chlorella* sp. TISTR8236. Moreover, the pH is an important factor that affects the protonation/deprotonation of the composite and the microalgal surface and, consequently, affects the algal cell harvesting efficiency [13]. Thus, the present experiments were performed under different pH levels and dosages of new composites. Moreover, the adsorption isotherm between the composites and the algal cell was investigated.

2. Materials and methods

2.1. Synthesis of composites magnetic-cationic cassava starch

The magnetic particles were synthesized by the procedure previously reported by Mikhaylova and her group [19]. Cationic cassava starch was synthesized following the approach of Wang et al. [17], and the ratio of glycidyl trimethyl ammonium chloride:anhydroglucose unit (GTMAC:AGU) was modified to vary its DS value. DS was calculated according to the following Eq. (1) [20].

$$DS = \frac{162N\%}{1400 - 151.5N\%} \quad (1)$$

where N% is nitrogen content determined by a carbon hydrogen nitrogen element analyzer (TruSpec CHN), and 162 and 151.5 are the molecular weights of AGU and GTMAC, respectively.

The synthetic magnetic composite was modified according to Lee et al. [3]. Two grams of modified cationic cassava starch was dissolved in 10 mL of acetic acid at pH 4; next, 1 g of magnetic particles were gradually added into the solution, which was mixed in a sonication bath at 60 Hz for 3 h. For cross-linking between cationic cassava starch and magnetic particles, 15 mL of 0.15% (w/v) sodium tripolyphosphate solution in distillated water was added and incubated overnight. The composites were collected using a small permanent magnet bar. The supernatant was tested by the phenol-sulfuric acid method [21] to examine the cationic starch to ensure that there was sufficient cationic starch for synthesizing it with magnetic material and to confirm that all magnetic particles were formed into the composites. The composite was washed with distilled water several times and redispersed and preserved in distilled water for further use.

2.2. Microalgae source and cultivation

Chlorella sp. TISTR8236, was supplied by the Thailand Institute of Scientific and Technological Research and cultured in BG-11 medium [22] (pH 7.0) in a 500 mL Erlenmeyer flask that was incubated in a shaker at 120 rpm at 25 ± 1 °C and was illuminated continuously 12 h per day at 45 μmol photons m^{-2} s^{-1} for 24 days. The initial *Chlorella* sp. biomass concentration was 0.075 g L^{-1}, and after 24 days of culture, the cell concentration was 1 g L^{-1}, and the culture pH was 9.5.

2.3. Harvesting efficiency of the composites

Algal cells were harvested by centrifugation at 1008 × *g* for 10 min and washed twice with distilled water to remove the secondary metabolites or organic matter, which may affect the electrostatic interaction between algal cells and composites during an experiment. *Chlorella* sp. was resuspended in BG-11 medium with a final concentration of 1 g L^{-1} of cells and was used for investigation of the harvesting efficiency. Naked magnetic or the composite magnetic particles were added to the 10 mL of microalgal suspension, which was then mixed by a vortex mixer for 1 min, and cell separation was performed by placing the vial on a permanent magnetic bar for 2 min. The supernatant was sampled and measured for optical density (OD) using a spectrophotometer at 750 nm to determine the microalgae concentration before harvesting (OD_i) and after harvesting (OD_f).

The optimal magnetic dosage was preliminarily investigated at pH 9.5 by varying the quantity of naked magnetic and composite magnetic agents at 200, 300, 400, 500, 600, 700 and 800 mg L^{-1}. The dosage of adsorption material that provided moderate harvesting efficiency was selected for the following study. The influence of DS values of composites were studied using the composite with different DS values in the range of 0.22 to 0.91. The effect of pH on the algal harvesting efficiency was studied by varying the pH of microalgal suspension in the range of pH 4 to 10, which had been reported as the optimum pH for several microalgal species [8,23]. The optimum dosage and DS values of the adsorption material and optimum pH were chosen and were validated for harvesting efficiency and recovery capacity.

The harvesting efficiency and recovery capacity of the magnetic composites-cationic cassava starch for the *Chlorella* sp. cells (g-DCW g-composites^{-1}) were calculated using Eqs. (2) and (3), respectively.

$$\text{Harvesting efficiency}(\%) = \frac{OD_i - OD_f}{OD_i} \times 100 \quad (2)$$

where OD_i is the initial OD before harvest and OD_f is the OD of the supernatant after harvest.

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