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Short Communication

Efficient harvesting of marine microalgae *Nannochloropsis maritima* using magnetic nanoparticles

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HIGHLIGHTS

- Magnetic separation method on harvesting tiny marine microalgae was developed.
- Recovery efficiency of *Nannochloropsis maritima* reached above 95% within 4 min.
- Reuse of culture medium from magnetic separation was realized for algal culture.

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ABSTRACT

An efficient magnetic separation technology using Fe_3O_4 nanoparticles was developed for harvesting marine microalgae *Nannochloropsis maritima* from culture broth. Recovery capacity of these nanoparticles was affected by microalgal growth phase and reached the peak value when the microalgal growth reached its maximal biomass after 18 days. The recovery efficiency of microalgal cells from the culture medium reached more than 95% at the particle dosage of 120 mg/L within 4 min. Electrostatic attraction at acidic pH and cell aggregation under neutral and alkaline conditions was beneficial for harvesting the algal cells. Higher operation temperature resulted in higher adsorption capacity of these nanoparticles for microalgawl cells. Reuse of the culture medium obtained from magnetic separation gave similar biomass production in comparison with that from centrifugation separation after 5 recycles. Together with these results provide a great potential in high-efficient and economical harvesting of tiny marine microalgae using magnetic separation technology in practice.

1. Introduction

Marine microalgae used extensively in foods market and pharmaceutical industries have attracted the increasing interest of the researchers around the world (Ríos et al., 2012). Recently, marine microalgae have been regarded as a prospective renewable source for the production of biodiesel, biogas, bioethanol and hydrogen because they possess great advantages including capability to grow in sea water on non-arable land and no competition for resources with conventional agriculture (Sostaric et al., 2012). Among marine microalgae, *Nannochloropsis* sp. was considered as one of the most promising feedstock for biodiesel production due to its high oil content (Bondioli et al., 2012).

Separation of algal cells from a large quantity of their culture medium is a critical challenge. Considerable attentions have been devoted to study of the microalgal harvesting, and various harvesting technologies have been developed such as centrifugation, filtration, flotation, coagulation, flocculation and scraping by using attached culture system (Zheng et al., 2012; Johnson and Wen, 2010). However, these conventional harvesting processes still have some limitations for the purpose of time saving, lower energy consumption and high efficiency because the algal cells are stabilized and suspended in culture broth due to their small size, low concentration and electrical stability (Cerff et al., 2012). Especially, the harvesting process is more difficult for the algal cells of *Nannochloropsis* sp. because the algal cell diameter is relatively smaller (2–4 μm) in comparison with other microalgal cells (Ríos et al., 2012). Therefore, it still remains a challenge to develop an efficient technology to harvest tiny algal cells from extremely dilute solutions.

Magnetic separation has been widely used due to its advantages such as simple operation, fast separation, low running cost and

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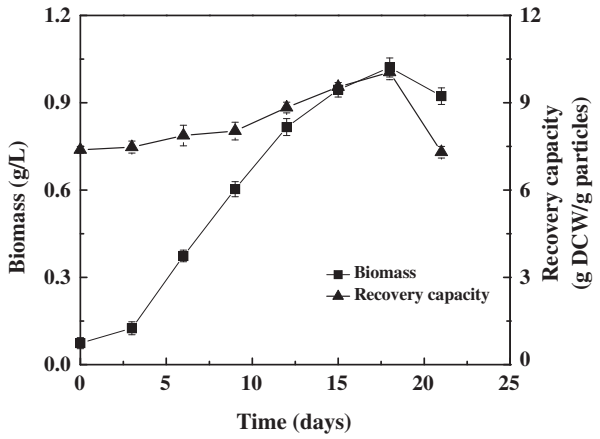

Fig. 1. Time course of *N. maritima* growth and recovery capacity of magnetic nanoparticles at different culture stages.

energy saving (Xu et al., 2011). Magnetic particles coated with silica or cationic polyelectrolyte were utilized for efficiently harvesting of fresh water and marine algae (Cerff et al., 2012; Lim et al., 2012). However, these surface-modified magnetic particles increased the cost of algal harvesting because they were synthesized by complicate steps using functional materials for coating on these naked Fe_3O_4. Naked magnetic nanoparticles (Fe_3O_4) were succeeded in harvesting fresh water microalgae *Chlorella ellipsoidea* and *Botryococcus braunii* (Xu et al., 2011). Here, there is still a great interest to evaluate the harvesting efficiency of *Nannochloropsis* sp. with the naked Fe_3O_4 nanoparticles due to the high salinity environment and much smaller size of the marine microalgae in comparison with those fresh water microalgae. In addition, there is still lack of enough knowledge on some key process parameters for microalgal harvesting technology by magnetic nanoparticles including algal growth phase, harvesting temperature, medium reusability and so on.

The objective of this current study is to investigate the magnetic harvesting of marine microalgae *Nannochloropsis maritima* using low-cost naked Fe_3O_4 nanoparticles. The effect of microalgal culture stages on the recovery capacity of these nanoparticles was studied, and operation parameters including pH, nanoparticle dosage, temperature were carried out. In order to achieve nutrient-saving algal culture process, the reusability of the culture medium after magnetic separation was evaluated for repeatable microalgal growth.

2. Methods

2.1. Synthesis of Fe_3O_4 nanoparticles

Magnetite Fe_3O_4 nanoparticles were prepared by chemical co-precipitation method (Supplementary material-Method 1). The final Fe_3O_4 nanoparticles were dispersed in distilled water for further use. These nanoparticles have an average diameter of about 10 nm.

2.2. Microalgae source and cultivation

The marine microalgae *N. maritima* was purchased from Institute of Hydrobiology, Chinese Academy of Sciences. The initial biomass concentration is 0.074 g DCW/L. Microalgal cells were cultured in 250 mL Erlenmeyer flasks containing 100 mL of the f/2 medium on a rotary shaker at a speed of 100 rpm at 25 ± 1 °C (Supplementary material-Method 2), and illuminated at a light intensity of 35 μmol m^{-2} s^{-1} with a 16 h light/8 h dark cycle. The pH of the final algal culture was around 8.0.

2.3. Magnetic separation procedure

Algal harvesting was conducted in Erlenmeyer flasks by mixing algal suspension with a certain dosage of the magnetic nanoparticles under stirring at 120 rpm for 2 min (Xu et al., 2011). The aggregates resulted from Fe_3O_4 nanoparticles and microalgal cells were separated from the suspension medium by decantation within 2 min when a permanent magnet was placed at the bottom of the flask (Supplementary material-Fig. 1). After the magnetic separation, the cell density in the decanted supernatant was measured. All experiments were carried out at 25 °C if on special notice was mentioned.

To investigate the effect of growth stage on the algal harvesting efficiency, algal suspension was sampled at different growth phases in the course of cultivation. The dosage of the magnetic nanoparticles required for recovering 95% of algae cells was determined, and the recovery capacity of the magnetic nanoparticles was calculated. The biomass concentration for each sample was also measured. To evaluate the influence of pH on the separation of algal cells, the pH value of cell suspension was adjusted to 4–10 using 1 M HCl or 1 M NaOH. The effect of temperature on the algal harvesting was investigated by recovering algal cells with the magnetic nanoparticles at 15, 25 and 35 °C, respectively.

2.4. Analytical method

For dry cell weight (DCW) determination, culture samples at different optical densities centrifuged (10,000 rpm, 5 min) and washed for three times with distilled water in a pre-weighed centrifugal tube were dried at 105 °C for 2 days to a constant weight. A linear correlation between optical density (680 nm) and dry cell weight was obtained, and the biomass concentration can be calculated based on the cell optical density.

To determine the recovery efficiency of microalgal cells (R), the optical density of cell suspension before and after harvesting was measured at 680 nm with a UV spectrophotometer (Unico, UV-2100) and the algal biomass concentration was calculated. Harvesting efficiency (R) and the recovery capacity of the Fe_3O_4 nanoparticles for algal cells (RC, g-DCW/g-particles) were calculated by Eqs. (1) and (2), respectively.

$$R = 100\% - (C_t/C_0) \times 100\% \quad (1)$$

$$RC = \frac{(C_0 - C_t)V}{m} \times 100\% \quad (2)$$

where C_0 is the initial biomass concentration of cell suspension before harvesting (g/L), C_t is the biomass concentration of cell suspension after harvesting (g/L), V is the volume of cell suspension used for harvesting (L) and m is the mass of Fe_3O_4 nanoparticles used for harvesting (g).

Among the mathematical models used in description of adsorption isotherms, Langmuir and Freundlich models are the most popular ones due to their reasonable accuracy and relative simplicity (Xu et al., 2011). To quantitatively evaluate the adsorption ability of Fe_3O_4 nanoparticles for *N. maritima*, the adsorption isotherms were tested at the particle dosage of 120 mg/L with different biomass concentration (0.1–2 g/L) and the equilibrium data were analyzed using Langmuir and Freundlich models (Supplementary material-Method 3).

pH measurement was performed with a digital pH meter. Zeta potential and cells size were measured using a DelsaNano C Particle Size and Zeta Potential Analyzer (Beckman Coulter Inc.). Morphology of both free magnetic nanoparticles and Fe_3O_4-coated algal cells was screened by light microscope (Leica Microsystems CMS GmbH, Germany).