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An in situ method for synthesis of magnetic nanomaterials and efficient harvesting for oleaginous microalgae in algal culture

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ABSTRACT

An in situ approach for magnetic nanomaterial synthesis and harvesting of oleaginous *Chlorella* sp. HQ in an algal culture was explored. Fe-based nanomaterials were synthesized by an in situ reduction reaction using $NaBH_4$ and $FeCl_3{\cdot}6H_2O$ in an algal culture. The various reactant molar ratios and the reaction system of a water solution or an algal culture determined the different formation pathways of magnetic nanomaterials. With the optimal reactant molar ratio of 2:1, this in situ magnetic harvesting method obtained a high harvesting efficiency in a broad pH and temperature range and different algal concentration levels with cost of $505 US per ton of algal biomass harvested. During this in situ process, the algal antioxidant defense system efficiently eliminated the cellular reactive oxygen species caused by nanomaterials via catalase and superoxide dismutase. Fatty acid methyl ester composition analysis indicated that the Fe-based nanomaterials affected the relative contents of several components, but the predominant types were still palmitic acid (C16:0), linoleic acid (C18:2) and γ-linoleic acid (C18:3), which were suitable for biofuel production. Additionally, the recycled cultivation medium maintained normal algal cell growth within 18 days after the biomass had reached the harvesting level. This novel in situ method has promising potential for the harvest of oleaginous microalgae with good biocompatibility.

1. Introduction

Microalgae are regarded as a good fossil-fuel-replacement candidate to conquer the ever-worsening energy crisis due to microalgae's benefits, such as their ability to grow rapidly, high lipid productivity, low-area occupation, potential in bioremediation treatment and viability for diverse valuable by-products [1–3]. Among the overall procedures of microalgae-based biofuel production, harvesting is considered to be one of the key points, which accounts for at least 20%–30% of the total production cost and limits the commercial and industrial-scale production [4]. Efforts devoted to harvesting microalgae using magnetic nanomaterials (NMs) have attracted much attention as a result of the efficient capture of algal cells from an aqueous solution using an external magnetic field. Although magnetic harvesting has come into the spotlight, it still has disadvantages regarding the relatively high fabrication cost of nanomaterials [5], and the toxic effects of nanoparticles on algal cells cannot be ignored.

As one type of producer in an aqueous ecosystem, microalgae play a critical role in maintaining the balance of the whole system. It was previously mentioned that nanoparticles could interact with and be transported through their surrounding environment, thus, affecting the fate of microalgae [6]. Microalgae are the first target of the toxic effects and transmit the influence to other organisms in an ecosystem [7]. Various ions in the microalgae cultivation medium could be adsorbed onto cell surfaces, released to extracellular exudates, or uptaken into the intracellular space [8]. The widely accepted mechanism of toxicity produced by nanoparticles is the internal generation of reactive oxygen species (ROS), which are considered as by-products of normal biological metabolism [9,10] and exist at a proper level in an organism without external stress. When organisms are exposed to external stress and stimuli, excessive ROS will cause a disturbance in the balance of the cells and lead to oxidative stress, cell membrane damage, cell lysis or even death or stimulate nanoparticle accumulation in cells, thus, producing toxic effects [10–12]. Under stressful circumstances, specific enzymes in an antioxidant defense system function as a protection strategy to alleviate oxidative stress [13,14]. Among these enzymes, catalase (CAT) and superoxide dismutase (SOD) are the most important scavengers for eliminating ROS (mainly $OH\cdot$ and $O_2\cdot^-$, respectively) [15].

Cutting down the fabrication cost of nanomaterials and exploiting a

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more environmentally friendly approach are dire requirements for the promotion of the sustainable development of magnetic microalgae harvesting. In this work, we first utilized a cost-effective in situ method for the synthesis of magnetic nanomaterials and the harvesting of microalgae *Chlorella* sp. HQ (an oil-producing algal species with high lipid content, as demonstrated in our previous study [16]) from an algal culture. Parameters, including the reactant molar ratio, algal culture concentration, pH value and temperature, were investigated. Additionally, the effects of magnetic nanomaterials on total lipid and triglyceride (TAG) content were studied. We first analyzed the fatty acid methyl ester (FAME) composition of *Chlorella* sp. HQ with and without nanomaterials. The biocompatibility of the in situ method with the algal cells was determined by evaluating the activities of CAT and SOD and the ROS level. Considering the potential practical application and economical cost, recycling of the cultivation medium after the in situ method was performed was also carried out by utilizing the recycled medium for a 30 day cultivation and observing the cell growth and dry cell weight accumulation. The aim of this work is to present a novel and cost-effective in situ approach for magnetic nanomaterial fabrication and efficient microalgae harvesting and to provide evaluation of the in situ method in terms of biocompatibility and cultivation medium recycling.

2. Material and methods

2.1. Microalgal cultivation

The oleaginous microalgae used in this study were *Chlorella* sp. HQ, which was isolated in our previous study [16] and collected in the China General Microbiological Culture Center (No.GCMCC7601). In this work, *Chlorella* sp. HQ was cultivated using an axenic Selenite Enrichment (SE) culture medium in an artificial climate chamber (HPG-280H, HDL, China) at 25 °C, and the light density was 60 μmol photons $m^{-2} s^{-1}$ with light/dark cycles of 14/10 [16]. The initial concentration of the *Chlorella* sp. HQ solution was 0.2 g L^{-1} (dry cell weight-DCW) after being cultivated for 18 days and was concentrated to 0.5 and 1.0 g L^{-1} before the harvesting process.

2.2. In situ preparation of magnetic nanomaterials in water solution and algal culture

Magnetic nanomaterials with different reactant molar ratios were first prepared in a water solution. Then, $NaBH_4$ (15.12 mg/7.56 mg/3.78 mg/1.89 mg) and $FeCl_3{\cdot}6H_2O$ (27.05 mg) (molar ratio 4:1/2:1/1:1/1:2) were added to deionized water (10 mL) in sequence. The reaction was initiated by shaking the tube lightly, and the magnetic nanomaterials were rapidly fabricated in a few minutes (1–2 min). Then, the magnetic nanomaterials were prepared in situ and applied in an algal culture for microalgae harvesting. The same amounts of $NaBH_4$ and $FeCl_3{\cdot}6H_2O$ (molar ratio 4:1/2:1/1:1/1:2) were sequentially added to the algal suspension (10 mL), fabricating the magnetic nanomaterials while combining with the algal cells in a single step.

2.3. In situ microalgae magnetic harvesting test

The nanomaterial-coated microalgae cells were concentrated and separated from the suspension medium using a permanent magnet outside the culture tube. After the magnetic separation, the density of algal cells in the supernatant was measured. The optical density of the algal cell suspension before and after harvesting was measured with a UV spectrophotometer at 690 nm to determine the harvesting efficiency of microalgae cells.

The effects of the molar ratio, pH value, algal culture concentration and temperature on the in situ magnetic harvesting were investigated. The pH value was adjusted in the range of 4.0 to 12.0 using either HCl (0.1 M) or NaOH (0.1 M). The initial algal cell density in this study was 0.2 g L^{-1} in dry cell weight (DCW) and was concentrated to 0.5 g L^{-1} and 1.0 g L^{-1} before the harvesting process. The temperature of the algal suspension was set in the range of 25–65 °C using a thermostat water bath.

The concentration of the algal suspension could be calculated based on the linear correlation between the optical density at 690 nm (OD_{690}) and the dry cell weight (DCW), which was developed in our previous work [5]. The harvesting efficiency was calculated by Eq. (1) [17].

$$\text{Harvesting efficiency}\ (\%) = (C_0 - C_t)/C_0 \times 100 \quad (1)$$

where C_0 and C_t are the biomass concentration (g L^{-1}) of microalgae before and after magnetic harvesting, respectively [17].

2.4. Biocompatibility evaluation of magnetic nanomaterials

The algal cells with and without nanomaterials were collected for biocompatibility evaluation of the magnetic nanomaterials, including the total lipid and TAG contents, CAT and SOD activities, ROS level, and FAME composition. For total lipid and TAG content determination [16], the algal suspension (40 mL), which had its biomass weighed beforehand, was frozen centrifuged at 14,800 × *g* for 10 min and concentrated to 0.8 mL. Then, methanol (2 mL), chloroform (2 mL) and deionized water (1 mL) were added to the suspension and shaken well. After frozen centrifugation at 1644 × *g* for 10 min, the bottom chloroform layer was transferred to a pre-weighed clean glass tube and evaporated using a nitrogen evaporator until a constant weight was achieved; the difference value between the two measurements was the total lipid yield, and the ratio of the lipid yield to the biomass weight was the lipid content. After the measurement of the total lipid yield, the obtained dried lipids were dissolved in 0.4 mL isopropanol, and the TAG content was determined by an enzymatic colorimetric method using a triglyceride assay kit (Nanjing Jiancheng Bioengineering Institute). CAT and SOD activity measurements and ROS level determination were conducted using a corresponding assay kit (Beyotime Institute of Biotechnology). For the analysis of the algal lipid FAME composition, gas chromatography–mass spectrometry (GC–MS) was performed. The microalgae samples were frozen centrifuged and freeze-dried at −80 °C. Afterward, a solvent mixture of chloroform (1 mL), methanol (0.85 mL) and concentrated H_2SO_4 (0.15 mL) was added to the powdered samples and heated at 100 °C for 2.5 h. After the mixture cooled down, deionized water (0.5 mL) was added to the sample, vortexed for 1 min and centrifuged at 1644 × *g* for 8 min. The bottom organic layer was transferred and dewatered with Na_2SO_4. For biocompatibility evaluation, the same treatments described above were conducted to algal cell-NM flocs, which were formed in the algal solution by the in situ method.

2.5. Recycling of culture medium after magnetic harvesting

To investigate the influence of residual magnetic nanomaterials in the culture medium on the growth of microalgae, the culture medium after magnetic harvesting was collected for the next microalgae cultivation process. Algal cells with the same initial density (5×10^6 cells mL^{-1}) were inoculated in the initial SE culture medium (30 mL) and the recycled SE culture medium (30 mL) and were then cultivated for 30 days. The algal density was determined by counting the number of cells using a hemocytometer every 24 h, and the growth curves were obtained. At the same time, the algal biomass was determined by measuring the optical density at 690 nm (OD_{690}), and the biomass changing curve was drawn.

2.6. Analytical methods

The morphology of the nanomaterials and algal cell-NM flocs were investigated using transmission electron microscopy (TEM), which was carried out on an FEI Tecnai T20 microscope. The samples were