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Functional graphene-based magnetic nanocomposites as magnetic flocculant for efficient harvesting of oleaginous microalgae

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ABSTRACT

Magnetic graphene oxide (GO) nanocomposite was assigned to decorate with cationic polymer PDDA (diallyldimethylammonium chloride) to a sponge like nanocomposite GO-Fe_3O_4/PDDA, and employed in magnetic harvesting of oleaginous *Chlorella* sp. HQ. The results showed that huge surface area, light weight and stable dispersion of GO make contribution to the high harvesting efficiency of GO-Fe_3O_4 (80.14%), compared to Fe_3O_4 NPs (62.65%) with 70 mg of nanomaterials / L of algal cells dose. In addition, graphene base provides more active sites to further decorate with PDDA, which imparts a positive zeta potential charge (21.94 mV) to nanoflocculants, thereby achieving 95.35% harvesting efficiency within 5 min. The adsorption followed Langmuir isotherm and pseudo-second-order kinetic model, with the maximum adsorption capacity of 14.06 g dry-biomass / g. Moreover, the harvesting efficiency decreased dramatically when pH increased for Fe_3O_4 NPs, while GO-$Fe_3O_4$and GO-Fe_3O_4/PDDA could maintain in a wide pH range. Besides,GO-Fe_3O_4/PDDA can be effectively reactivated (88.68% harvesting efficiency after 5 times reactivation) with 70 mg L^{-1} dose (add 30 mg L^{-1} after every time). It can be expected that this novel graphene-based magnetic nanocomposite has great potential to be a promising magnetic flocculant applied to efficient harvesting of oleaginous microalgae.

1. Introduction

Microalgae are considered as a promising renewable source for biodiesel production, as the result of their ability to grow rapidly and accumulate large amount of lipid [1], alongside the application in environmental bio-mediation processes, such as wastewater treatment by using and removing nitrogen and phosphorous from waste water [2]. While the industrial-scale microalgae-based biodiesel production was limited in the light of high operational cost, especially in harvesting step, accounting for at least 20%–30% of the total production cost [3]. In order to achieve the biodiesel production from microalgae, it is urgently required to exploit an efficient harvesting method to obtain a meaningful decrease in operation cost.

Although several conventional approaches such as filtration [4], centrifugation [5] and electrolysis [6,7] has been applied to microalgae harvesting, their energy-intensiveness make them inapplicable [8]. Flocculation is currently the most commonly used method which can significantly reduce the costs and energy demand [9]. While the time-consuming process of flocculant sedimentation makes lower harvesting efficiency [10]. Harvesting algae by magnetic separation was thought of as a promising approach because of the rapid, easy and efficient capture of algal cells from liquid solution applied by an external magnetic field. Numerous research efforts have been developed on microalgal harvesting utilizing magnetic nanomaterials recently for high efficiency and economic, especially the use of Fe_3O_4 nanoparticles which are in good biocompatibility. However, naked magnetic nanoparticles are often insoluble in water and the harvesting effectiveness could be suppressed due to agglomeration [11]. Tremendous studies which focus on the modification of Fe_3O_4 nanoparticles have been reported for application of microalgal harvesting. Zhao et al. studied the utilization of Fe_3O_4NPs coated with polyaluminium chloride and polyacrylamide for magnetic flocculation of *Chlorella vulgaris*, and demonstrated that the optimal dosing strategy was adding the composite PACl/Fe_3O_4 first and then PAM, and the main harvesting mechanism was charge neutralization and sweeping action [12]. Lee et al. reported the magnetic harvesting for *Chlorella* sp. using Fe_3O_4 magnetic nanoparticles, and the materials could be recycled for 10 times with 99% harvesting efficiency [13]. Hu et al. fabricated Fe_3O_4-PEI nanocomposites with large amount of $-NH_2$ groups and applied to efficient magnetic harvesting of *C. ellipsoidea* [14]. Lin et al. reported magnetic nanoparticles applied in *Microcystis aeruginosa* harvesting and also studied the reactivation property of nanomaterials [15]. The influences of surface coating, UV irradiation and magnetic field on the algal harvesting using magnetite nanoparticles were investigated by Ge et al. [11], and in another research, the recovery property of magnetic SA-Fe_3O_4-ZnO nanocomposites from algal

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cells was demonstrated based on hydrophobicity shift and UV irradiation [16]. In our recent report, a novel magnetic nanomaterial Fe_3O_4@PAMAM was designed by coating Fe_3O_4 nanoparticles with amino-riched polyamidoamine dendrimer, and applied in magnetic harvesting of *Chlorella* sp. HQ; the harvesting efficiency reached over 95% within 2 min at the dosage of 80 mg L^{-1}, and the nanomaterials could be regenerated and reused effectively [17].

Owing to the excellent property including huge surface area, easy to synthesis, good chemical stability and low cost, graphene material has been widely studied and applied in multi-disciplined field. Graphene oxide is rich in oxygen containing functional groups on its surface, which enable it to be used as a super adsorbent [18]. In earlier researches, several magnetic graphene materials have been synthesized and shown good adsorption performance for heavy metal ions [19] and organic pollutants [20]. However, the application of magnetic graphene in the separation of microalgae from liquid solution is still scarce. Understanding and studying the characteristic, electrical property, surface functional groups of algal cells essentially determines the design of algal harvesting materials [21]. The major functional groups on the surface of microalgae are carboxyl, phosphate and amine or hydroxyl groups, and algal cells possess negative zeta potential in culture medium [22]. This may explain the limited use of magnetic graphene for separating microalgae as they carry the same kind of charge. Therefore, modification of magnetic graphene is of vital importance to improve its adsorption property.

Herein, we firstly fabricated a functional magnetic graphene material for the harvesting of microalga *Chlorella* sp. HQ (a potential oil-producing algal species with high lipid content demonstrated in our previous study [23]), which was synthesized by depositing magnetic (Fe_3O_4) NPs onto graphene oxide sheet, and was further modified with water-soluble cationic polymer (PDDA) by electrostatic self-assembly and hydrogen bonding. The key operating parameters including dosage and pH value were investigated, and adsorption isotherm as well as adsorption kinetic models were also used to fit and study harvesting process. Furthermore, a relatively simple reactivation method was investigated to recycle materials and we estimated the operation costs roughly. The aim of this research is to present a strategy for a simple and facile method utilized in efficient microalgae harvesting process by adsorption bridging action, flocculation as well as rapid magnetic separation.

2. Material and methods

2.1. Microalgal cultivation

The microalgae *Chlorella* sp. HQ (Collection No. GCMCC7601 in the China General Microbiological Culture Center) used in this study was isolated in our previous study [23]. In this work, *Chlorella* HQ was cultivated at initial algal density of 2×10^5 cells mL^{-1} in 500 mL conical flasks containing 300 mL axenic culture medium, and the flasks were put in an artificial climate chamber (HPG-280H, HDL, China) at 25 °C with a light density of 60 μmol photons m^{-2} s^{-1} and light/dark cycle of 14/10 [23]. The culture media used to cultivate the algae in this study was SE medium, with the compositions of (per liter) 250 mg $NaNO_3$, 75 mg $K_2HPO_4 \cdot 3H_2O$, 75 mg $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2 \cdot 2H_2O$, 175 mg KH_2PO_4, 25 mg NaCl, 5 mg $FeCl_3 \cdot 6H_2O$, 0.81 mg $FeCl_3$, 10 mg Na_2EDTA, 2.86 mg H_3BO_3, 1.81 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnSO_4 \cdot H_2O$, 0.079 mg $CuSO_4 \cdot 5H_2O$, and 0.039 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ [23]. The experiments in this study used *Chlorella* HQ samples on Day 20 in the cultivation medium of pH 7.0 to achieve the dry cell weight (DCW) of 0.2 g L^{-1}.

2.2. Preparation of magnetic graphene oxide composites and surface modification

Magnetic NPs was prepared by modified chemical coprecipitation method [24]. Briefly, 0.99 g of $FeCl_2 \cdot 4H_2O$ (>99%) and 2.70 g of $FeCl_3 \cdot 6H_2O$ (>98%) (Molar ratio 1:2) were dissolved in 50 mL of deionized water, and the suspension was then heated to 353 K under nitrogen atmosphere. Then 10 mL of NH_4OH (25%) was added drop-wise to reach pH of 10 to 11 and the mixture was stirred continuously for another 30 min. The color of the solution turned from brown to black color, indicating the formation of Fe_3O_4. The prepared products were magnetically separated and washed with water several times.

Graphene oxide (GO) were obtained from graphite by modified Hummers method [25]. One gram of graphite powder (>99%) was added to 23 mL of concentrated H_2SO_4 and the mixture was stirring for 12 to 24 h. $NaNO_3$ (0.5 g) was then added and was stirring for 3 h. $KMnO_4$ (3 g) was then added slowly with stirring and cooling to keep the temperature of the reaction mixture below 283 K. The temperature of the reaction mixture was increased and maintained at 308 K for 30 min, and then maintained at 311 K for 2 h. Then 3 mL of deionized water was added slowly to this mixture, and another 3 mL was added after 5 min, and another 40 mL was added after 5 min. After 15 min, 140 mL of deionized water was added followed by 10 mL of 30% H_2O_2 solution. The mixture was settled for >4 h to get solid product. Then the products were washed repeatedly with 5% HCl solution, until the sulfate ions were removed, and then washed with distilled water until free of chloride ions. The residue was freeze dried for 24 h.

The nanocomposites of GO-Fe_3O_4 (GF) were synthesized by a simple and facile approach under room temperature [26]. The GO (80 mg) was added in deionized water (80 mL) and sonicated for 1 h to disperse uniformly. For another solution, Fe_3O_4 NPs (80 mg) was added to 1 M HNO_3 under sonication treatment to increase the concentration of H^+ ion to make Fe_3O_4 NPs protonated for 30 min, and then separated by magnet. Then Fe_3O_4 NPs after acid treat were added to 60 mL of GO solution and stirred continuously for 3 h. GO-Fe_3O_4 nanocomposites were removed by using magnet, and washed with deionized water several times and then freeze dried. The modification of PDDA was subsequently completed. GO-Fe_3O_4 powder (60 mg) in 60 mL deionized water was sonicated for 10 min to form a homogeneous suspension, and 0.5 mL of 20 wt% PDDA solution was mixed with the GO-Fe_3O_4 suspension under vigorous stirring for 7 h at 318 K. The products obtained were isolated, and the residual PDDA was removed by washing and centrifugation at 10,000 rpm for 5 min, followed by consecutive cycles several times.

2.3. Microalgal harvesting test

When the microalgae reached their maximal biomass in broth, different given amount of magnetic nanomaterials (10–450 mg L^{-1}) were added to 10 mL algal suspension and mixed by shaking in the speed of 250 rpm for 20 min at 25 °C. The nanomaterial coated microalgal cells were concentrated and separated from the suspension medium by a permanent magnet outside the vessel. After the magnetic separation, the density of algal cells in the supernatant was measured. The optical density of algal cell suspension before and after harvesting was conducted at 690 nm with a UV spectrophotometer to determine the harvesting efficiency of microalgal cells. The lowest dosage of nanomaterials required for achieving 95% of harvesting efficiency for *Chlorella* sp. HQ was defined as the optimal dosage of this material in this work. Furthermore, the pH effect of the microalgae medium on harvesting was also investigated. The pH value was adjusted in the range of 4 to 12 using either 0.1 M HCl or 0.1 M NaOH. The concentration of algal suspension could be calculated based on the linear correlation between optical density at 690 nm (OD_{690}) and dry cell weight (DCW) which was developed gravimetrically [27] (Fig. S1).

The harvesting efficiency and recovery capacity (g-DCW/g-NMs) were calculated by Eqs. (1) and (2) respectively [11].

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

$$\text{recovery capacity } (g\ g^{-1}) = (C_0 - C_t)V/m \quad (2)$$