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Magnetophoretic separation of *Chlorella* sp.: Role of cationic polymer binder

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ABSTRACT

Cationic polyelectrolyte promoted effective attachment of iron oxide nanoparticles (IONPs) onto microalgal cells through electrostatic attraction. Poly(diallyldimethylammonium chloride) (PDDA) and chitosan (ChiL), both are cationic polymer, are feasible to act as binding agent to promote rapid magnetophoretic separation of *Chlorella* sp. through low gradient magnetic separation (LGMS) with field gradient ∇B less than 80 T/m in real time. Cell separation efficiency up to 98% for the case of PDDA and 99% for the case of ChiL can be achieved in 6 min when 3×10^7 cells/mL *Chlorella* sp. are exposed to 300 mg/L surface functionalized-IONPs (SF-IONPs). Different polyelectrolytes do not give significant effect on cell separation efficiency as long as the particle attachment occurred. However, the PDDA is more preferable as the binder for all type of microalgae medium than the chitosan (ChiL) since it is not pH dependent. SF-IONPs coated with PDDA guarantee the cell separation performance for all pH range of cell medium, with $98.21 \pm 0.40\%$ at pH 8.84. On the other hand, the ChiL performance will be affected by the cell medium pH, with only $22.93 \pm 31.03\%$ biomass recovery at pH 9.25.

Keywords: Microalgae; Renewable fuel source; Magnetophoretic separation; Polymer binder; Electrostatic interaction; Sustainable

1. Introduction

The energy crisis has ignited the search of an alternative replacement for fossil fuel which can be more environmental friendly and sustainable. Since the microalgae biomass is capable of producing high oil yield on gallons per acre basis up to two orders of magnitude higher than the other oil producer crops (Johnson, 2009), it makes microalgae an attractive option. The renewable fuel source from microalgae is limitless as its energy source is from the sun itself via photosynthesis (Demirbas, 2010). However, the main restriction in preventing the realization for large-scale third generation microalgal oil production is the high harvesting cost of microalgae biomass. The cost associated to downstream separation processes could reach up to 20–30% of the total production cost of biomass (Grima et al., 2003). On the other hand, magnetophoretic separation of microalgae was introduced since 1970s with the main focus targeted on environmental related problem (Bitton et al., 1975; Yadida et al., 1977). This method is currently revisited and applied on the purpose to harvest the microalgal biomass for biofuel production. It is feasible to achieve cell separation efficiency up to 99% for biofuel production purpose (Lim et al., 2012; Toh et al., 2014). The harvesting cost is estimated at about $180/ton dry biomass through the low gradient magnetic separation (LGMS) (Toh et al., 2012).

Currently there is an active pursue, from research groups all around the globe, on both the fundamental and engineering

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aspects of microalgae separation by magnetophoresis (Xu et al., 2011; Lim et al., 2012; Toh et al., 2012; Cerff et al., 2012; Liu et al., 2009; Hu et al., 2013; Prochazkova et al., 2012; Wang et al., 2014). There are two mechanism utilized to impart the magnetic property on the microalgal cells: electrostatic-mediated-attachment (Xu et al., 2011; Lim et al., 2012; Toh et al., 2012; Liu et al., 2009; Prochazkova et al., 2012) and adsorption-based-attachment (Cerff et al., 2012; Hu et al., 2013). Previous study on the magnetophoretic separation of freshwater *Chlorella* sp. showed that the attachment of magnetic nanoparticles on cell surface do not mediated by the adsorption-based-attachment even though this mechanism has been reported feasible in others study (Toh et al., 2014). The cell separation efficiency can only be achieved at 8% even after extended incubation period up to 2 h (Toh et al., 2014). With the introduction of cationic polymer binder, the cell separation of *Chlorella* sp. is effective (Lim et al., 2012) where it is hypothesized that the attachment is induced by the electrostatic-mediated-attachment. Xu et al. (2011) and Prochazkova et al. (2012), respectively, have further verified this point through a series of pH adjustment by using bare magnetic particles as tagging agent without the use of polyelectrolyte binder. Nevertheless, there is still lacking of experimental data to verify this finding if the cationic polymer binder is employed. Therefore, in this study we demonstrated the feasibility of microalgae separation with the aids of cationic polymer binder to bind together the negatively charged *Chlorella* sp. cells with the anionic iron oxide nanoparticles (IONPs) in culture medium without any pH adjustment. The mechanism associated to this attachment process promoted by the polymer binder is studied in detail. Furthermore, we also identified the extra benefit of polymer binder for the use in this cell separation method.

2. Materials and methods

2.1. Materials

Iron oxide magnetic nanoparticles (IONPs), Fe_3O_4 (98 + % purity, 20–30 nm) was obtained from Nanostructured & Amorphous Materials, Inc. The very low molecular weight poly(diallyldimethylammonium chloride) (PDDA) in water (35 wt% with Molecular weight, M_w < 100,000 g/mol) and the low molecular weight chitosan powder (ChiL) (75–85% deacetylated) were obtained from Sigma–Aldrich, Inc. Deionized water used was obtained by reverse osmosis and further treated by the Milli-Q Plus system (Millipore) to 18 MΩ cm resistivity.

2.2. Cultivation of microalgae

The *Chlorella* sp. strain was obtained from School of Biological Sciences, USM. It was cultivated in 500 mL conical flask that contained 250 mL Bold's Basal Medium (BBM) under continuous illumination (2000 lux) at 25 °C. The medium and flask were autoclaved at temperature 121 °C for 15 min before cell cultivation. Continuous aeration of the culture medium was provided throughout the cultivation period. A cell density of 3×10^7 cells/mL was used for every single test. The cells were determined by using the hemocytometer and the desired cell density were achieved by appropriate dilution of the cell. The pH of cell medium was adjusted by using hydrochloric acid (HCl) and sodium hydroxide (NaOH) and measured by Eutech CyberScan pH 1500.

2.3. Preparation of surface functionalized iron oxide magnetic nanoparticles (SF-IONPs)

In this work, the IONPs used were in spherical shape with dimension of 20–30 nm in diameter. A total amount of 0.02 g of IONPs was dispersed into 20 mL deionized water followed with sonication until a uniform dispersion in concentration of 0.001 g/mL was achieved. For PDDA solution preparation, 786.4 μL of as received polyelectrolyte was added into 18 mL of deionized water (18 mL). The ChiL was prepared by dispersing 30 mg of dry powder into 1% acetic acid solution (15 mL). This dispersion was stirred (500 rpm) for one day to achieve complete dissolution. A total of 3 mL of IONPs dispersion (0.001 g/mL) was added into the polymer solution and then sonicated by low power bath sonicator (40 KHz). This solution was then left on an end-to-end rotating mixer at mixing speed of 40 rpm overnight. Later, a permanent magnet, cylindrical shaped N50-graded neodymium boron ferrite (NdFeB) with surface magnetic field of ~6000 G, was used to collect the SF-IONPs and the remaining supernatant containing excess polymer was discarded. The SF-IONPs collected were further dispersed in 2 mL deionized water. During the microalgae separation experiments, 2 mL of SF-IONPs was added into 8 mL of cell medium sample to achieve particle concentration of 300 mg/L and then followed with simple mixing for 30 s to ensure uniform dispersion. The mixture was further left for another 30 s before the introduction of NdFeB permanent magnet. Particle concentration of 300 mg/L is chosen as it is the optimum concentration for SF-IONPs-attached-cells to achieve complete collection by the permanent magnet in real time (Toh et al., 2012). The electrophoretic mobility and hydrodynamic diameter of samples were measured by Malvern Instruments Nanosizer.

2.4. Magnetophoretic separation of Chlorella sp. by using IONPs or SF-IONPs

The magnetic separation of *Chlorella* sp. cell was carried under LGMS by using an NdFeB permanent magnet with field gradient $\nabla B < 80$ T/m (Toh et al., 2012). The cell separation performance was recorded for 6 min. The absorbance of the sample was measured spectrophotometrically by UVmini-1240 Shimadzu at the wavelength of 660 nm (measured by Agilent Technologies Carry 60 UV-Vis). The cell separation efficiency was determined as

$$\text{Cell separation efficiency (\%)} = \frac{I_0 - I(t)}{I_0 - I_{\text{centrifuged}}} \times 100 \quad (1)$$

where the I_0 represents initial absorbance intensity of microalgae suspension after diluted with 2 mL deionized water, $I(t)$ represents the absorbance intensity of microalgae suspension during magnetophoretic separation at time t, and the $I_{\text{centrifuged}}$ represents the clear centrifuged sample subjected to same dilution factor.

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