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Sedimentation-induced detachment of magnetite nanoparticles from microalgal flocs

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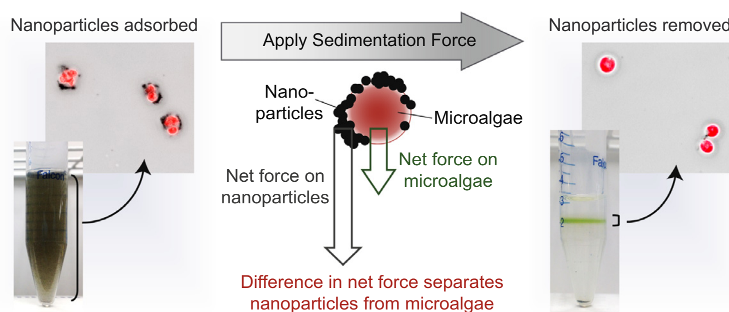
HIGHLIGHTS

- Sedimentation through dense/viscous solution removes nanoparticles from flocs.
- Effectiveness of separation is increased with higher solution density and viscosity.
- Centrifugal and magnetic sedimentation effectively remove nanoparticles from flocs.
- The approach did not show cytotoxicity.
- The mechanism of sedimentation-induced removal is discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study is to develop a simple, one-step approach to separate adsorbed Fe_3O_4 nanoparticles from microalgal flocs for further downstream processing. Using the wild-type strain of fresh-water algae *Chlamydomonas reinhardtii*, effective removal of nanoparticles from microalgal flocs by both centrifugal sedimentation (at 1500 or 2000*g*) and magnetic sedimentation (at 1500 Oe) is demonstrated. At the physiological pH of the solution (*i.e.*, pH 7.0), where the electrostatic force between the nanoparticles and the microalgal cells is strongly attractive, larger separation force was achieved by simply increasing the density and viscosity of the solution to 1.065 g/mL and 1.244 cP, respectively. The method described here offers significant opportunity for purifying microalgal biomass after nanoparticle-flocculation-based harvesting and decreasing the cost of microalgal biotechnology. This may also find avenues in other applications that apply flocculation, such as algal biofilm formation in photobioreactors and wastewater treatment.

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1. Introduction

Microalgae are a diverse group of single-cell, aquatic, photosynthetic organisms (Hallmann, 2007) that have attracted intensive research and development for the production of high-value-added products such as biofuels, feedstocks, drugs and chemicals (Christenson and Sims, 2011; Wijffels et al., 2013). However, the high cost associated with the platforms for production is a significant obstacle for practical commercialization (Chisti, 2013). Harvesting is one of the key elements of microalgal technology that has yet to be optimized for improved economics and efficiency (Georgianna and Mayfield, 2012). Various approaches are used for harvesting microalgae, such as centrifugation, flocculation,

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filtration and flotation (Kim et al., 2013; Lam and Lee, 2012). Of these approaches, flocculation is a unique method that can be combined easily with other approaches to enhance the speed and efficiency of separation (Greenwell et al., 2010; Schlesinger et al., 2012). This is especially appealing for microalgal harvesting, where the low cell concentration (few grams per liter) and the small cell size (typically in the order of a few μm) present a serious challenge for effective separation (Vandamme et al., 2013).

Flocculation is generally achieved by adding flocculants (or coagulants) that disrupt the repulsive force between particles and allow binding to occur to form flocs (Wan et al., 2015). For microalgal cells the repulsive force is electrostatic, due to the surface charge of microalgal cells that are typically negative at physiological conditions (Greenwell et al., 2010). Recently, magnetic nanoparticles (NPs) with positive surface charge have attracted interest as promising flocculants that rapidly adsorb onto the microalgal surfaces, form flocs, and enable rapid, efficient microalgal harvesting through an external magnetic field (Kim et al., 2013; Lee et al., 2015b).

One of the keys to the development of NP-based flocculants is a method for detaching the NPs from the microalgae after harvesting to prevent biomass contamination (Cerff et al., 2012; Wan et al., 2015). However, limited studies have been reported to date on the approaches to remove NPs from microalgal flocs. This includes dissolution of the nanoparticles with acid (Xu et al., 2011), mechanical detachment by ultrasonication or stirring (Ge et al., 2015a; Prochazkova et al., 2013), and alteration of the electrostatic force by changing the solution pH (Seo et al., 2014). An elegant approach was recently reported by Lee et al. and Ge et al. that applies differences in the surface tension of the NPs and microalgae cell surface to adsorb/desorb the NPs from the cell at will (Ge et al., 2015b; Lee et al., 2015a). Although effective, these methods require complex/expensive steps and/or apply toxic chemicals that can increase the economic cost of the process and raise environmental concerns.

In this paper, a simple, one-step procedure for separating NPs from microalgae after flocculation is reported. The concept, which applies a sedimentation force through a high-density solution, is demonstrated using a model system, where *Chlamydomonas reinhardtii* (*C. reinhardtii*) wild-type cells are flocculated with as-prepared Fe_3O_4 NPs. A commercially available, high-density solution (Percoll®) was applied to induce the separation during sedimentation. NP separation was investigated under different solution pH using either centrifugal or magnetic force, and the cell viability after each procedure was assessed with a fluorescence-based assay.

2. Methods

2.1. Synthesis of Fe_3O_4 NPs

Fe_3O_4 NPs were synthesized by a method described in a previous paper (Matsuda et al., 2015). Briefly, 50 mL of aqueous solution containing 0.125 M of spermine, *i.e.*, *N*,*N*′-bis(3-aminopropyl)butane-1,4-diamine (Sigma–Aldrich Japan, Japan), was prepared as base and protective reagent for NP synthesis (Iida et al., 2007). 50 mL of 0.05 M $FeCl_2{\cdot}4H_2O$ (Kanto Chemical Co. Ltd., Japan) aqueous solution was added to the spermine solution and stirred for 4 h at room temperature. The resulting black precipitate of Fe_3O_4 NPs was collected, washed with water and ethanol, and dried before use.

2.2. Microalgae cultivation

CC124 cells, a catalogue for the wild-type strain of *C. reinhardtii* microalgae, were obtained from the Chlamydomonas Resource Center at the University of Minnesota. All chemicals were used as received from Fisher Scientific, unless stated otherwise. CC124 cells were grown in 10 mL of TAP media that was rocked in 50 mL Erlenmeyer flasks under moderate light (Harris, 1989). The cells were collected during exponential growth phase, and when the concentration reached 5.0×10^6 cells/mL. The cell concentration was diluted with TAP media according to the details described in Section 3. Cell counting was performed using a TC20 cell counter (Bio-Rad, USA).

2.3. NP adsorption to CC124 cells

To adsorb the NPs to CC124 cells, 1 mg of NPs and 7.5×10^6 of CC124 cells were mixed in 5 mL of TAP culture media and stirred at 1000 rpm for 15 min. The cells were used immediately after NP adsorption. For all experiments, the pH of the TAP media was adjusted as needed using 0.5 M HCl or 0.5 M KOH.

2.4. Zeta potential measurement

NPs or CC124 cells were dispersed in TAP media for the zeta potential measurement. Approximately 2 mL of the NP or CC124 suspension was assessed with electrophoretic light scattering, using a 90 Plus Zeta Particle Size Analyzer (Brookhaven Instruments Corporation, USA). Zeta potential was calculated using the properties for aqueous solution pre-recorded in the instrument software: *i.e.*, 0.890 cP, 1.330, and 78.54 for the viscosity, reflective index and dielectric constant, respectively.

2.5. Cell sedimentation

Percoll® (GE Healthcare, USA) was diluted to 35 vol% or 50 vol% with TAP media as described in Section 3. From the manufacturer's specification, the as-received Percoll® has a density of 1.130 ± 0.005 g/mL. Assuming the density of TAP media to be about 1 g/mL, the final density of Percoll® after dilution to 35 and 50 vol% is calculated to be 1.046 and 1.065 g/mL, respectively. The viscosity of TAP media and the diluted Percoll® solution was measured using a Cannon–Fenske Routine Viscometer (Cannon instrument company, USA). 1 mL of the TAP media containing 1.5×10^6 of CC124 cells (with or without NP adsorption) was layered carefully onto 1 mL of 35 or 50 vol% Percoll®, as shown in Fig. 1. The layered solution was then either centrifuged at 1500*g* or 2000*g* for 5 min, or placed on a ferrite magnet for 10 min to allow sedimentation. The average magnetic field of the magnet was measured to be 1500 Oe using a 6010 model Gaussmeter (Bell Technologies Inc., USA). After sedimentation, the sample was used immediately for image analysis.

2.6. Cell viability assay

Fluorescein diacetate (FDA) and propidium iodide (PI) were purchased from MP Biomedicals Inc. and Alfa Aesar, respectively. The dye solution, which is 1 μL of 10 mg/mL FDA in dimethyl sulfoxide and 10 μL of 1 mg/mL PI in water, was added into 1 mL of TAP media containing 1.5×10^6 of CC124 cells (with or without NP adsorption), and incubated for 20 min. After incubation, the cells were imaged immediately with a fluorescence microscope to assess their viability (see Section 2.7).

2.7. Image analysis

The samples were imaged with an epifluorescence microscope (Axio Imager M2m Motorized Microscope, Carl Zeiss, Germany). ImageJ software from NIH was used to analyze the obtained images (Rasband, 1997–2014). One hundred particles (either individual

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