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Short Communication

# Magnetophoretic harvesting of oleaginous *Chlorella* sp. by using biocompatible chitosan/magnetic nanoparticle composites

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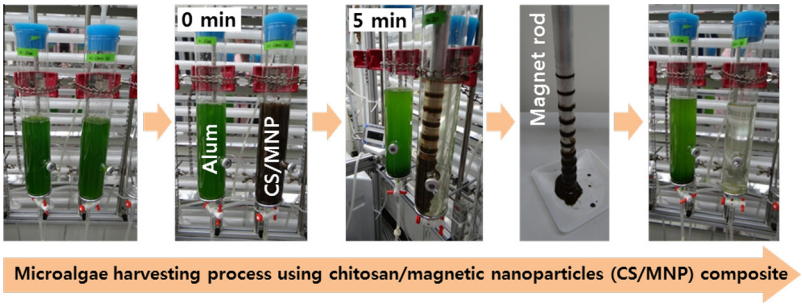
## HIGHLIGHTS

- Biocompatible and rapid magnetophoretic harvesting process of oleaginous microalgae.
- Over 99% harvesting efficiency achieved by using chitosan–$Fe_3O_4$ composites.
- After harvesting, the used medium shows no adverse effect on microalgal growth.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The consumption of energy and resources such as water in the cultivation and harvesting steps should be minimized to reduce the overall cost of biodiesel production from microalgae. Here we present a biocompatible and rapid magnetophoretic harvesting process of oleaginous microalgae by using chitosan–$Fe_3O_4$ nanoparticle composites. Over 99% of microalgae was harvested by using the composites and the external magnetic field without changing the pH of culture medium so that it may be reused for microalgal culture without adverse effect on the cell growth. Depending on the working volume (20–500 mL) and the strength of surface magnetic-field (3400–9200 G), the process of harvesting microalgae took only 2–5 min. The method presented here not only utilizes permanent magnets without additional energy for fast harvesting but also recycles the medium effectively for further cultivation of microalgae, looking ahead to a large scale economic microalgae-based biorefinement.



## 1. Introduction

With increasing petroleum oil price and growing emission of greenhouse gas, biomass-based biofuel production has been receiving much attention, especially since microalgae biomass has many advantages over the grain- and/or tree-based biomass in producing biodiesel (Lam and Lee, 2012; Mata et al., 2010). The microalgae have higher photosynthetic efficiency and high lipid content of 15–77% of cell mass (Chisti, 2007), and they can be cultivated using carbon dioxide directly from power plants. In spite of the many advantages of microalgae-based biorefinery, it involves many steps from cultivation to harvesting, from lipid extraction to oil-to-biodiesel transition, which increase the cost of microalgal biodiesel (Lam and Lee, 2012).

In the various steps in producing microalgal biodiesel, it is necessary to improve the technique of harvesting, which takes up 20–30% of the total cost (Mata et al., 2010). Since microalgae are cultivated in diluted culture medium, normally under 1 g/L, and have a negatively charged surface of a few microns in size, the dispersion of microalgae in the solution is very stable, which

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makes harvesting a task worth challenging. Various harvesting methods have been applied till now. Conventional methods such as centrifugation, filtration and ultrasound have been widely used for microalgal harvesting (Bosma et al., 2003; Zhang et al., 2010). Electrolysis-base technologies have been shown to be powerful methods for harvesting microalgae even under the continuous process (Gao et al., 2010; Kim et al., 2012). However, those methods are not easy to scale-up and also require high energy consumption, which is not desirable for low cost production of microalgal biodiesel. Chemical flocculants such as polyvalent cations and cationic polymers or inorganics have been intensively investigated for large-scale applications (Lee et al., 2013; Sirin et al., 2012). To improve some drawbacks of chemical flocculants such as contaminations of final products and the toxic effects on microalgae (Kim et al., 2013), biocompatible flocculants such as chitosan and starch have been attempted (Beach et al., 2012; Farid et al., 2013). Recently, magnetic microalgal harvesting has been getting much attention. Microalgae are attached with magnetic particles coated with cationic substances by electrostatic interaction and then separated from the culture medium by external magnetic field (Lim et al., 2012; Xu et al., 2011). Lim et al. (2012) who applied the iron oxide magnetic nanoparticles and the cationic polyelectrolyte have reported that the harvesting efficiency of magnetic separation is as high as 99% and the harvesting completed within a few minutes. However, they did not report on the biocompatibility of the flocculent with polyelectrolyte as a functional group and the resulting recycling of medium.

*Chlorella* sp. KR-1 is a newly isolated microalga that accumulates triacylglycerol approximately 37–41% (w/w) of cell mass (Lee et al., 2013; Na et al., 2011). In spite of the high lipid content, the small size of *Chlorella* sp. KR-1, around 3 μm in diameter (Lee et al., 2013), makes the harvesting by conventional methods more challenging. In this study, we synthesized chitosan–$Fe_3O_4$ magnetic nanoparticle (CS/MNP) composites and investigated the composites as biocompatible flocculants for the magnetophoretic harvesting of *Chlorella* sp. KR-1. The efficiency of microalgal harvesting was studied using the dosage and the ratio of chitosan to magnetic nanoparticles of the synthesized composites. Furthermore, the biocompatibility of the composites was tested by reusing both the used culture medium left over from microalgae harvesting and the microalgae-attached composites as inoculums.

## 2. Methods

### 2.1. Synthesis of CS/MNP composites

$Fe_3O_4$ magnetic particles of 10–30 nm in size were synthesized by the previously reported method (Liu et al., 2004). Briefly, two aqueous solutions of $FeCl_2{\cdot}4H_2O$ (⩾99.0%, Sigma–Aldrich, USA) and $FeCl_3{\cdot}4H_2O$ (97%, Sigma–Aldrich, USA) were mixed and heated at 85 °C for 30 min under $N_2$ atmosphere followed by addition of $NH_4OH$. The precipitated nanoparticles were washed several times with ethanol and subsequently with distilled water until pH of suspension reaches to 7. The prepared $Fe_3O_4$ nanoparticles (0.15 g) were dispersed in 12 mL of distilled water with 11 mL of 2 wt% Pluronic F-127 (Sigma, USA) solution using ultrasonication. Chitosan solution was prepared by dissolving 0.2 wt% of chitosan flakes (low molecular weight, Fluka, USA) in 0.5% acetic acid. The $Fe_3O_4$ dispersion and chitosan solutions were mixed for 30 min using a magnetic bar. For cross-linking of chitosan, 140 mL sodium tripolyphosphate solution (0.15 wt%, pH 6; Sigma–Aldrich, USA) was added by a peristaltic pump at 10 mL/min. The solution was aged overnight at 4 °C and then washed with distilled water several times. The total volume of each $Fe_3O_4$-chitosan dispersion solution was set to 50 mL in order to quantify $Fe_3O_4$-chitosan by volume. The final products of CS/MNP composites were named 'CS/MNP α' of which α indicates the calculated weight ratio of CS to MNP (α: 0.13, 0.40 and 0.54).

### 2.2. Cultivation of microalgae

*Chlorella* sp. KR-1, an indigenous freshwater microalga (Na et al., 2011) was used in this study. The microalgae were cultivated in a modified N8 medium in a 7 L Pyrex bubble-column photobioreactor supplied with 10% (v/v) $CO_2$ in air at a rate of 0.75 L/min under illumination of 12 fluorescent lamps (light intensity: 80 μmol photons/$m^2$ s). The medium contained 3 mM $KNO_3$, 5.44 mM $KH_2PO_4$, 1.83 mM $Na_2HPO_4$, 0.20 mM $MgSO_4{\cdot}7H_2O$, 0.12 mM $CaCl_2$, 0.03 mM FeNaEDTA, 0.01 mM $ZnSO_4{\cdot}7H_2O$, 0.07 mM $MnCl_2{\cdot}4H_2O$, 0.07 mM $CuSO_4$ and 0.01 mM $Al_2(SO_4)_3{\cdot}18H_2O$ with pH = 6.5.

For the cultivation of microalgae on agar plate, the agarose gel was prepared by dissolving 1.5 wt% of agar (Bacto™ Agar, BD Bacto™, France) in a modified N8 medium in an autoclave at 121 °C for 15 min. The magnetophoretically harvested microalgae-CS/MNP flocs were plated on the agar plate, which was incubated for 3 days in the growth chamber (temperature, 25 °C; humidity, 60%; light intensity, 15 μmol photons/$m^2$ s).

### 2.3. Harvesting of microalgae with CS/MNP

The harvesting experiment was carried out using microalgae freshly taken from 7 L Pyrex bubble-column after 4 d incubation. The concentration of microalgae was 1.0 g/L (Optical density at 660 nm, $OD_{660nm}$ = 4.5). The harvesting efficiency, in this study, indicates the percentage of microalgae attached on CS/MNP which are separated by the magnetic field subsequently. After injection of the calculated amount of CS/MNP dispersion into 1 mL of microalgal solution, external magnetic field was applied to the mixture. The surface magnetic-field strength of a permanent NdFeB magnet chip (length, 20 mm; width, 9 mm; thickness 4 mm) was measured to be 3400 G by a Gauss meter (TM-701, KANETEC Co., Japan). The supernatant was taken for OD measurement after 3 min of magnetophoretic harvesting, thereby ensuring the measurement of the only concentration of unattached microalgae on CS/MNP. The harvesting efficiency was calculated by following equation (Lee et al., 2013).

$$\text{Harvesting efficiency } [\%] = \left(1 - \frac{OD_f}{OD_i}\right) \times 100$$

where $OD_i$ and $OD_f$ were the initial OD and the OD of the supernatant after magnetophoretic harvesting, respectively.

### 2.4. Reuse of medium solution and microalgae-attached CS/MNP as an inoculum

Microalgae were harvested by using CS/MNP composite and a magnet rod (surface magnetic-field strength, 9200 G; diameter, 22 mm; length, 500 mm) from 0.5 L of microalgal solution (see Graphical Abstract). After adjusting the concentration of nitrate of used medium to 3 mM, new cells were inoculated and cultivated in 1 L bubble columns for 7 d under the same conditions mentioned previously. For comparison, the medium solution was reused for the next cultivation after harvesting microalgae with a widely using flocculant, Alum (Kim et al., 2011). In addition, the magnetophoretically harvested black slurry containing microalgae-attached CS/MNP (the 4th image of Graphical Abstract, Fig. S3a and S4b) was directly used as an inoculum in the new N8 medium to investigate the biocompatibility CS/MNP composite versus microalgae.