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Magnetic-Nanoflocculant-Assisted Water−Nonpolar Solvent Interface Sieve for Microalgae Harvesting

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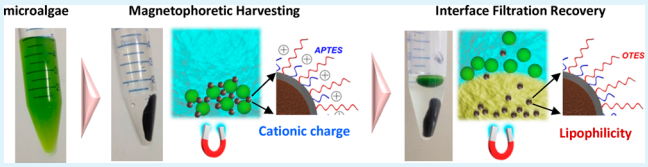
Supporting Information

ABSTRACT: Exploitation of magnetic flocculants is regarded as a very promising energy-saving approach to microalgae harvesting. However, its practical applicability remains limited, mainly because of the problem of the postharvest separation of magnetic flocculants from microalgal flocs, which is crucial both for magnetic-flocculant recycling and high-purity microalgal biomasses, but which is also a very challenging and energy-consuming step. In the present study, we designed magnetic nanoflocculants dually functionalizable by two different organosilane compounds, (3-aminopropyl)triethoxysilane (APTES) and octyltriethoxysilane (OTES), which flocculate negatively charged microalgae and are readily detachable at the water-nonpolar organic solvent (NOS) interface only by application of an external magnetic field. APTES functionalization imparts a positive zeta potential charge (29.6 mV) to magnetic nanoflocculants, thereby enabling microalgae flocculation with 98.5% harvesting efficiency (with a dosage of 1.6 g of dMNF/g of cells). OTES functionalization imparts lipophilicity to magnetic nanoflocculants to make them compatible with NOS, thus effecting efficient separation of magnetic flocculants passing through the water-NOS interface sieve from hydrophilic microalgae. Our new energy-saving approach to microalgae harvesting concentrates microalgal cultures (~1.5 g/L) up to 60 g/L, which can be directly connected to the following process of NOS-assisted wet lipid extraction or biodiesel production, and therefore provides, by simplifying multiple downstream processes, a great potential cost reduction in microalgae-based biorefinement.



KEYWORDS: *magnetic materials, functionalization, microalgae, harvesting, recovery, interfacial tension*

1. INTRODUCTION

Soaring oil prices and increasing greenhouse gas emissions obligates consideration of suitable alternatives that can be efficiently incorporated into existing fossil-fuel-based infrastructures. In this view, biomass-based biofuel production through biological conversion of CO_2 has received wide attention. Among the various feedstocks, microalgae, the third-generation biomass constituent, are looked upon as one of the most promising sources not only of biomass[1,2] but also of valuable biochemicals[3,4] for biodiesel-production applications. Microalgal biomass offer several advantages over conventional oil crops, which include nonedibility, high productivity per area, and high lipid content. Unfortunately, microalgae-based biodiesel production remains expensive because of factors associated with several steps in the downstream process.[5] A breakthrough is urgently required, especially in the harvesting process, which accounts for 20−30% of the total biodiesel production cost, compared with other steps such as oil extraction and conversion, which have been well established with first and second generation biomasses.[6] The challenges of microalgae harvesting originate in their low cell concentration (<2 g/L), small size (on the order of a few micrometers), and stable dispersion. Although application of any of several conventional methods including centrifugation,[6] filtration,[7] and electrolysis[8,9] has been suggested, their energy-intensiveness renders them problematic.

Flocculation is an alternative, nonconventional, *minimal-energy-use* technology that has been considered for application to microalgae harvesting.[10,11] Magnetic-particle-based flocculation recently has been offered as a means of overcoming conventional chemical-based flocculation's critical drawbacks, which include slow processing and contamination.[12−15] Microalgae harvesting by magnetic-particle flocculants is characterized by a three-step process including flocculation, magnetic separation, and recovery of magnetic particles. First, nanometer-to-micrometer-sized magnetic particles decorated with

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positively charged surface components generate flocculation by electrostatic interaction with negatively charged microalgae in the culture. Second, unlike gravity sedimentation for microalgae separation in conventional flocculation, the flocs are separated from the culture within a few minutes by application of an external magnetic field, thereby preventing retention of flocculant residues in the culture. More importantly, no additional energy input, other than potential energy in the form of electrostatic energy and the magnetic field of a permanent magnet, is required. Lee et al. reported better-than-99% microalgae harvesting using chitosan-Fe_3O_4 composite; their microalgae, moreover, could be recultured without adverse effect on cell growth in the used medium postharvesting.[14] However, one key challenge to be overcome in the final step, in order to successfully apply the magnetic-particle flocculation process to microalgal biofuel production, is to recover the magnetic particles after separation of the microalgae-magnetic-particle flocs from the culture. This is important, not only for magnetic particles recycling but also for obtainment of a high purity of biomass for the subsequent oil extraction and conversion processes.

Notwithstanding the importance of that final step, little attention has been paid to it in the magnetic-particle flocculation literature. Seo et al. reported on the use of amine-functionalized magnetic nano/microparticles for microalgae harvesting.[13] They showed that magnetic-particle flocculants can be recovered from microalgae only by mechanical shaking with the aid of pH adjustment, and that recovery efficiency is size-dependent, meaning that larger particles are more suitable. They obtained a recovery efficiency of up to 85% with 1.2 μm particles for a final total suspended solids (TSS) concentration of about 20 g/L. Nonetheless, an energy-efficient method for recovery of magnetic-particle flocculants, one that will minimize the total energy consumption through the three steps, is required. Furthermore, reducing the dosage of flocculants by decreasing the size of magnetic particles and increasing the concentration of microalgae will lower the cost incurred in the harvesting step.

Herein, we report a novel strategy for energy-efficient magnetic-nanoflocculant-based microalgae harvesting that utilizes a water-nonpolar organic solvent (NOS) interface for selective filtration of magnetic nanoparticles for recovery. We take advantage of the hydrophilicity of the surfaces of microalgae trapped in the water phase while magnetic nanoparticles traverse the water-NOS interface, to be collected in the NOS phase by means of a magnetic field applied from the NOS side. For effective and efficient harvesting of microalgae and recovery of magnetic-particle flocculants, we synthesized dual-functionalized Fe_3O_4 magnetic-nanoparticle flocculants (dMNF) coated with two functionally different organosilane molecules, (3-aminopropyl)triethoxysilane (APTES) and octyltriethoxysilane (OTES). APTES plays the role of flocculating negatively charged microalgae by electrostatic interaction, which originates from the amine (NH_2) group by protonation (NH_3^+). On the other hand, hydrophobic OTES molecules provide magnetic nanoparticles the compatibility with the NOS phase, thereby ensuring their recovery in that phase.

2. EXPERIMENTAL SECTION

2.1. Preparation of Silica-Coated Fe_3O_4 Nanoparticles and Organosilane Functionalization. First, the Fe_3O_4 nanoparticles were prepared by a modified coprecipitation method reported previously.[16,17] Briefly, 26 mmol of iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, > 98%, Sigma-Aldrich) and 13 mmol of iron(II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, > 99%, Sigma-Aldrich) were dissolved in 125 mL of distilled water. After thorough mixing, the solution was heated to 85 °C under a nitrogen environment for 30 min. Then, 8.4 mL of ammonium hydroxide solution (NH_4OH, 25% NH_3 in H_2O, Sigma-Aldrich) was slowly added to the mixture, which was maintained for 30 min. After the mixture was cooled to room temperature, Fe_3O_4 nanoparticles were washed with distilled water and ethanol by magnetic decantation. Subsequently, SiO_2 was coated onto the Fe_3O_4 nanoparticles (Fe_3O_4@SiO_2) by hydrolysis of tetraethyl orthosilicate (TEOS, $Si(OC_2H_5)_4$, > 98%, Sigma-Aldrich). For this, Fe_3O_4 nanoparticles were added to a mixture of 200 mL of ethanol, 30 mL of distilled water, 3 mL of ammonium hydroxide solution and 60 mmol of TEOS. The resulting mixture was shaken for 12 h at room temperature. After washing with ethanol, organosilane functionalization of Fe_3O_4@SiO_2 nanoparticles finally was performed by co-condensation with APTES ($C_9H_{23}NO_3Si$, > 98%, Sigma-Aldrich) and OTES ($C_{14}H_{32}O_3Si$, > 96%, Sigma-Aldrich). The molar ratio of both APTES and OTES was set as APTES/(APTES+OTES) = 0.35. In detail, APTES and OTES were added to the Fe_3O_4@SiO_2 suspension in ethanol. The total concentration of both APTES and OTES was 35 mmol. Following ultrasonication treatment, the mixture was shaken for 12 h and washed with ethanol, thus enabling obtainment of dual-functionalized Fe_3O_4 magnetic-nanoparticle flocculants (Fe_3O_4@SiO_2-OTES/APTES or dMNF).

2.2. Strain and Cell Culture. *Chlorella* sp. KR-1 is a newly isolated oleaginous microalga that can accumulate significant amounts of lipid (0.35−0.41 g-FAME or fatty acid methyl ester/g cell) under nitrogen-deficient growth conditions.[18] Briefly, using a previously reported method,[14] the microalgae were cultivated for 7 days in 7 L Pyrex bubble-column photobioreactors (6 L working volume) supplied with 10% (v/v) CO_2 in air at a rate of 0.75 L/min using a modified N8 medium. The light intensity, temperature and pH were maintained during cultivation at, respectively, about 80 μmol photons/m^2·s (using 12 fluorescent lamps), 28−31 °C, and approximately 6.5.

2.3. Harvesting. Harvesting of microalgae (around 1.6 g/L in dry cell weight) using the as-prepared dMNF was performed by the following previously reported method.[14] First, the dMNF were added to freshly sampled 5 mL of microalgae culture, which mixture was vortexed for 30 s. It is in this step that electrostatic-interaction-induced flocculation of microalgae and dMNF takes place. Separation of the microalgae-dMNF flocs was completed within 2 min by application of an external magnetic field using a permanent NdFeB magnet block (length: 20 mm, width: 9 mm, height: 4 mm) of 3400 G surface magnetic field strength measured by a Gauss meter (TM-701, KANETEC Co., Japan). After measuring the concentration of the supernatant solution, the harvesting efficiency was calculated by the equation[14]

$$\text{harvesting efficiency (\%)} = \left(\frac{OD_i - OD_f}{OD_i}\right) \times 100 \quad (1)$$

where the concentration of microalgae was evaluated by optical density (OD at 660 nm) measurement using a UV−vis spectrophotometer (Optizen 2120 UV, Mecasys Co., Korea), and where OD_i and OD_f indicate the OD of culture before and after magnetophoretic separation, respectively. Microalgae-dMNF slurry was obtained after discarding of the clear supernatant liquid. All of the experiments were carried out in duplicate.

2.4. Recovery. After magnetophoretic harvesting of microalgae with the 1.6 g dMNF/g cell dosage, the microalgae were detached from the microalgae-dMNF flocs by addition of NOS, dichloromethane (DCM, density 1.33 g/cm^3, Sigma-Aldrich, USA), hexane (0.655 g/cm^3, Junsei, Japan) or dodecane (0.75 g/cm^3, Sigma-Aldrich, USA) to the harvested microalgae-dMNF slurry, by which the water-based slurry was separated from the NOS phase. Before adding 2 mL of NOS, the pH of the separated microalgae-dMNF slurry was adjusted to around 12 by adding 100 μL of 0.1 N NaOH solution, thus imparting a negative surface charge to the dMNF. The zeta potentials

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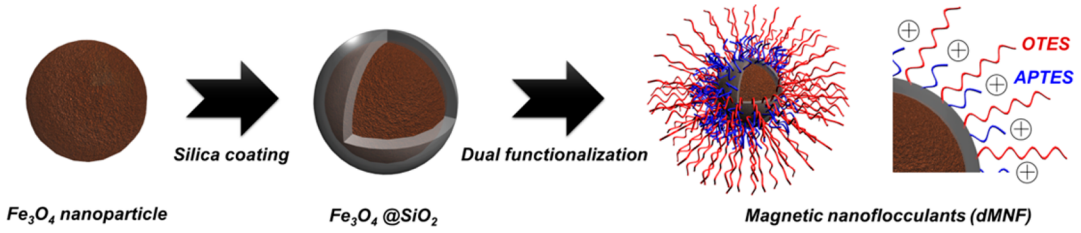
Scheme 1. Schematic of dMNF Preparation by Silica Coating of Fe_3O_4 Nanoparticle Followed by Dual Functionalization with OTES and APTES



of Fe_3O_4@SiO_2−OTES and Fe_3O_4@SiO_2−OTES/APTES were measured to −33.4 and −40.7 mV at pH 12, respectively. The slurry was drawn toward the NOS phase by the external magnetic field, thereby enabling collection of microalgae and dMNF in the water and NOS phases, respectively. The on−off pulse of the magnetic field was applied to the microalgae-dMNF flocs by sliding a falcon-tube-contained sample along a magnet rod (500 mm length, 22 mm diameter, JAMAGNET, Korea) with 16 segmented areas of magnetic field (the maximum surface magnetic-field strength, 9200 G), as shown in Movie S1. This procedure was consistently repeated 10 times for each of the samples. The recovery efficiency, as indicated by the percentage of microalgae detached from the microalgae-dMNF flocs, was calculated by the equation

$$\text{recovery efficiency}\ (\%) = \frac{OD_r}{OD_i - OD_f} \times 100 \quad (2)$$

where OD_i and OD_f indicate the OD of the culture before and after magnetophoretic separation in the harvesting step, and OD_r represents the OD of the supernatant after detachment of microalgae from the microalgae-dMNF flocs and volume adjustment to the initial state. All of the experiments were carried out in duplicate.

2.5. Analytical Methods: HR-TEM, XPS, Zeta Potential, Contact Angle, Optical Microscope, Confocal Microscope Observation at Water−Oil Interface. High-resolution transmission electron microscopy (HR-TEM) images and energy-dispersive spectrometer (EDS) mapping of Fe_3O_4@SiO_2 were obtained by FE-TEM (Tecnai TF30 ST, 300 kV, FEI Company). The zeta potential of the dMNF was measured using a Zetasizer (ZS90, Malvern, UK). The data were recorded as the average values of three measurements.

The surface-elemental compositions of the Fe_3O_4 nanoparticles before and after SiO_2 coating and organosilane functionalization were obtained by X-ray photoelectron spectroscopy (XPS, Thermo VG Scientific, Sigma Probe) equipped with microfocused monochromatic Al Kα X-ray sources. These sources utilized pass energies of 100 eV for low-resolution survey-scan spectra and 50 eV for high-resolution elemental spectra. The binding energies of all of the XPS spectra were calibrated by setting the C 1s peak maximum to 285 eV. All XPS peak-fitting was performed by a combination of Gaussian (70%) and Lorentzian (30%) functions with Shirley-typed background correction. The surface-elemental composition data were acquired by Thermo Advantage 4.75 software installed in the XPS system.

For contact-angle measurement, a few drops of ethanol dispersion containing each sample were positioned on slides, under which a magnet was utilized to densely concentrate the magnetic powders. The contact angle of the superhydrophobic sample (Fe_3O_4@SiO_2-OTES) was obtained using a contact angle analyzer (Phoenix300, SEO), whereas the Fe_3O_4@SiO_2-OTES/APTES could not be measured, due to soaking of drops into the samples. In the contact angle measurements, the drop volumes ranged between 7 and 9 μL.

A square-type glass capillary (Biosilicate square tubing, Harvard Apparatus; outer diameter = 1.5 mm, inner diameter = 1.05 mm, length = 150 mm) was used in order to microscopically observe the separation of microalgae and dMNF at the water-NOS interface. Here, cyclomethicone oil was used in place of NOS. The glass capillary was sequentially filled with an aqueous solution of algae-magnetic-nanoparticle aggregates and oil by capillary force. The oil−water interface was observed under laser-scanning confocal microscopy (LSM 5 PASCAL, Zeiss) while a magnetic field was applied from the oil side.

3. RESULTS AND DISCUSSION

3.1. Dual-Functionalized Magnetic-Nanoparticle Flocculants (dMNF). As illustrated in Scheme 1, the dMNF were designed to have a magnetite-silica (Fe_3O_4@SiO_2) core−shell structure functionalized with two organosilane molecules. Successful synthesis of the core−shell-structure dMNF and its functionalization were confirmed by high-resolution transmission electron microscopy (HR-TEM; Figure 1), X-ray

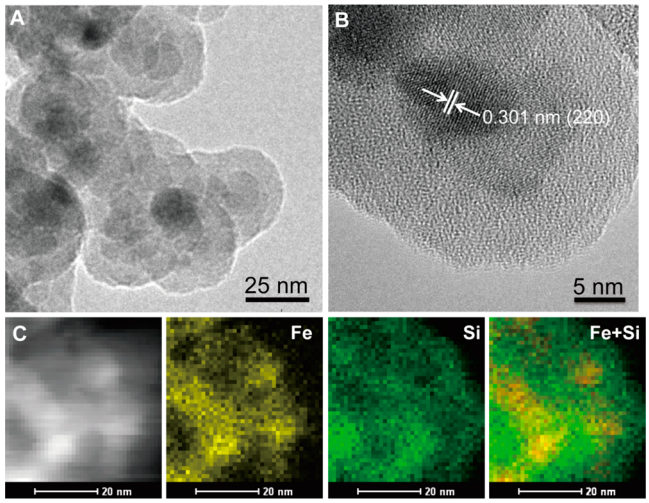


Figure 1. (A, B) HR-TEM and (C) EDS mapping images of dMNF.

diffraction (XRD; Figure S1) and X-ray photoelectron spectroscopy (XPS; Figure 2). First, TEM showed a 10−20 nm Fe_3O_4 core (Figure 1A). Considering that the critical size for superparamagnetism is under 20 nm,[19] use of synthesized magnetic nanoparticles as core dMNF material is desirable in order to minimize aggregation of magnetic-particle flocculants. The microalgae harvesting capacity per unit gram of magnetic-particle flocculants can be maximized when interparticle aggregation is minimized.[20] The position and relative intensity of the XRD peaks of the synthesized Fe_3O_4 matched the JCPDS card (No. 65−3107) of Fe_3O_4 (Figure S1). The lattice spacing of the core crystal, as shown in the HR-TEM image, was 0.301 nm, which was assigned to the (220) plane of Fe_3O_4, magnetite (Figure 1B). However, phase identification of Fe_3O_4 only by diffraction-based analysis is insufficient, since the crystal structures of Fe_3O_4 and γ-Fe_2O_3 (maghemite) are very similar, as are, correspondingly, the two materials' lattice spacing and XRD patterns.[21] XPS analysis provides more information, based on which Fe_3O_4 can be clearly differentiated from γ-Fe_2O_3.[22,23] XPS spectrum of the γ-Fe_2O_3 shows a satellite peak at around

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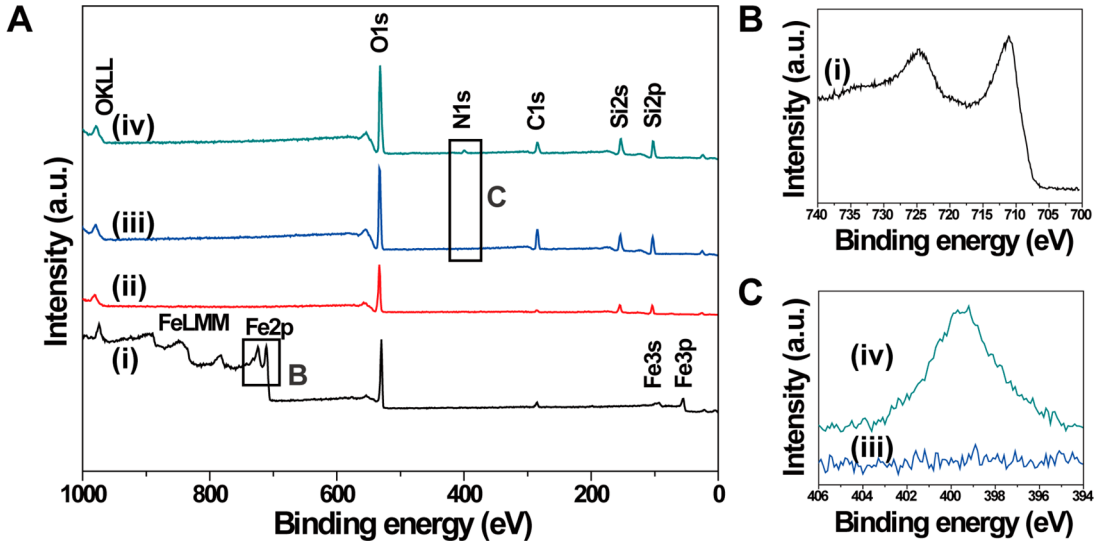

Figure 2. (A) XPS survey-scan spectra of (i) Fe_3O_4, (ii) Fe_3O_4@SiO_2, (iii) Fe_3O_4@SiO_2-OTES, and (iv) Fe_3O_4@SiO_2-OTES/APTES (dMNF). (B) Fe 2p spectrum and (C) N 1s core spectra of i and iii–iv, respectively.

718 eV in addition to two peaks at 711 and 724 eV, which were commonly assigned to the Fe 2p peaks from Fe_3O_4 and γ-Fe_2O_3. As shown in Figure 2A and B, the absence of the satellite peak proved that we obtained the pure magnetite phase. This is important in that magnetite has a higher magnetic moment than maghemite, and that therefore, magnetophoretic separation will be more efficient with magnetic flocculants of higher magnetic moment.

A silica intermediate layer was introduced to provide compatibility for the functionalization of organosilane molecules. The diffraction pattern after SiO_2 coating of the Fe_3O_4 core did not show any changes except for the broadened peak at around 23°, which originated from the amorphous phase of SiO_2 (Figure S1). HR-TEM showed that the amorphous layer was coated onto the Fe_3O_4 core at a thickness of about 10 nm (Figure 1A and B). Energy-dispersive spectrometer (EDS) mapping confirmed the core–shell structures by delineating the distribution of Fe elements surrounded by Si (Figure 1C). Furthermore, XPS confirmed the homogeneity of the SiO_2 coating on the Fe_3O_4. For all of the survey-scan spectra of Fe_3O_4@SiO_2, the Fe peaks were undetectable; only Si 2s and Si 2p were detected, as shown in Figure 2A (ii–iv). Considering the 10 nm information depth and approximately 400 μm^2 beam focus of XPS, these results clearly demonstrated that SiO_2 was homogeneously coated onto the Fe_3O_4 nanoparticles at a thickness of over 10 nm, which could be observed only on the microscopic scale by HR-TEM.

As the final dMNF preparation step, organosilane molecules were functionalized on Fe_3O_4@SiO_2 core–shell structures. In order to obtain high harvest and recovery efficiencies, this step needs to provide two different functionalities to dMNF: one is a positive surface charge for efficient flocculation of negatively charged microalgae by electrostatic neutralization; the other is lipophilicity, which will enhance dMNF compatibility with the NOS phase so as to facilitate detachment of dMNF from hydrophilic microalgae at the water-NOS interface. When Fe_3O_4@SiO_2 was functionalized only with the alkyl-ending group, the OTES molecule, Fe_3O_4@SiO_2-OTES, exhibited high lipophilicity and dispersed only in the oil phase (inset of Figure S3A). The contact angle measurement, 160°, demonstrated the superhydrophobicity of Fe_3O_4@SiO_2-OTES (Figure S3A). Whereas lipophilicity of magnetic-nanoparticle flocculants is desirable for recovery from microalgae after harvesting using the water-NOS interface, dewetting of flocculants in the water phase does not allow any chance of contact between flocculants and microalgae. After functionalization of Fe_3O_4@SiO_2 by APTES with the amine-ending groups, the magnetic particles lost lipophilicity and became wet from water droplets (Figure S3B). Most of the Fe_3O_4@SiO_2-OTES/APTES (or dMNF) stayed at the water–oil interface, which fact shows the Janus-like property of dMNF, possessing as it does both hydrophilicity and lipophilicity (inset of Figure S3B). The nitrogen peak (N 1s) at 399.4 ± 0.1 eV, revealed in the XPS analysis and corresponding to free amine, confirmed that the APTES molecules had successfully coated onto the particles (Figure 2A–C).[24] The dMNF contained 3.28 at. % of nitrogen and 19.55 at. % of carbon, while Fe_3O_4@SiO_2-OTES contained 28.55 at. % of carbon without nitrogen. The NH_2-ending groups of APTES generated a positive dMNF surface charge by protonation of NH_2 to NH_3^+. The zeta potential value of dMNF was 29.6 mV at pH 7, while that of Fe_3O_4@SiO_2-OTES was −22.8 mV (Figure 3). The surface charge of dMNF was

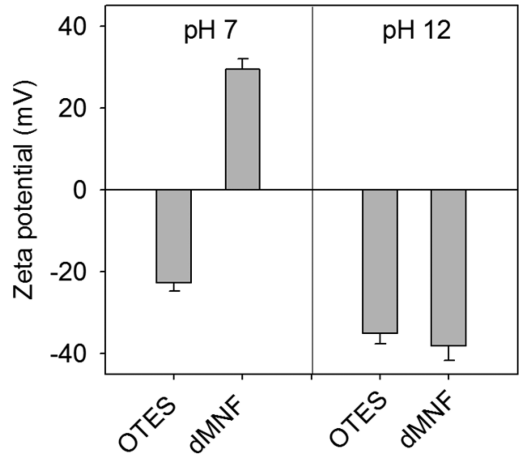

Figure 3. Zeta potentials of Fe_3O_4@SiO_2–OTES and dMNF at pH 7 and 12.

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sufficiently high to generate flocculation of the microalgae (zeta potential of *Chlorella* sp. KR-1 ∼−18 mV) by electrostatic interaction, without changing the pH of the culture.[13] The positive surface charge of dMNF was switched to negative by increasing pH, which is desirable for effective detachment of dMNF from microalgae in the recovery step (Figure 3). Treating microalgae at high pH (especially 12) can induce hydrolysis of cell wall, which is beneficial for the subsequent step of lipid extraction. In principle, microalgae could be recovered under mild condition of pH, for example pH 8, which is just above isoelectric point of magnetic particles, as reported by Lee et al.[20]

3.2. Harvesting Efficiency. As shown in Figure 4A−C, when dMNF was mixed with green microalgal culture, flocculation took place spontaneously, and subsequent magnetic separation by application of an external magnetic field yielded a clear supernatant within 2 min. The optical-microscopic observation of the flocs showed that the brownish dMNF were attached to the microalgal cells, the existence of which was clearly observed under red autofluorescence (Figure 4D−E). On the other hand, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2-OTES only hardly adhered to the microalgal cells (data not shown). Similarly, Lim JitKang et al. reported that flocculation of *Chlorella* sp. was achieved only after positive-charged functionalization by cationic polyelectrolytes on iron oxide nanoparticles. Employing an extended Derjaguin−Landau−Verwey−Overbeek (XDLVO) analysis, they determined that the electrostatic interaction plays the major role in particle attachment to microalgal cells in fresh water, especially with *Chlorella* sp. as a model system, while the van der Waals and Lewis acid−based interactions are negligible.[25]

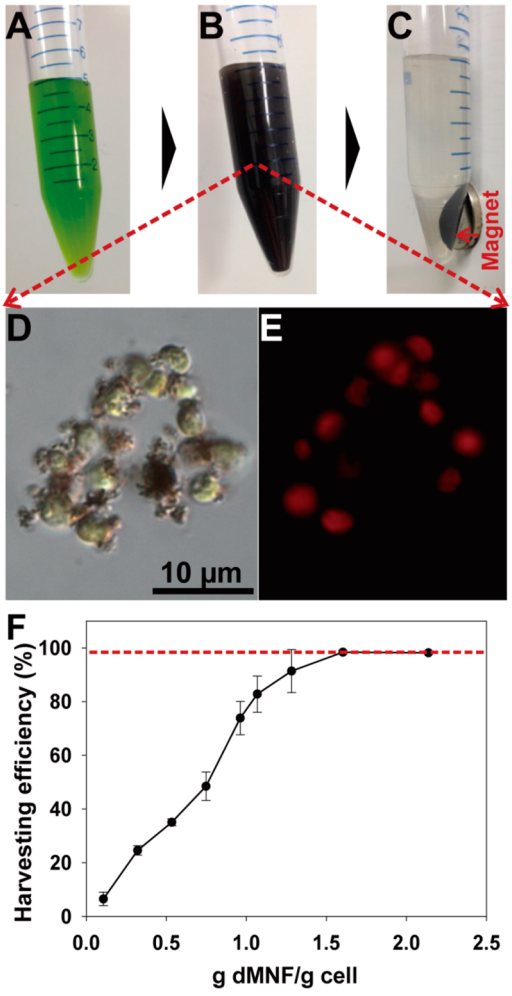

Figure 4. Magnetophoretic harvesting of microalgae by dMNF: (A) microalgal culture (pH 6.5), (B) flocculation of microalgae by dMNF (pH 6.8), (C) magnetophoretic separation of microalgae-dMNF flocs, (D) optical-microscopic image of microalgae-dMNF flocs from panel B showing microalgae and dMNF (brownish particles), (E) red autofluorescent microscopic image of panel D, and (F) plot of harvesting efficiency of microalgae as a function of dMNF dosage.

Figure 4F plots an OD-measurement-based harvesting efficiency curve according to the dMNF dosage per gram of microalgae in dry cell weight (DCW). The curve shows that the harvesting efficiency increased with the dosage, finally attaining 98.5% with 1.6 g of dMNF/g cell. The dMNF dosage for the following experiment was fixed, based on the curve, to 1.6 g of dMNF/g cell. Compared with the relevant previous magnetophoretic harvesting work with the same *Chlorella* sp. KR-1 microalgal strain, the dMNF dosage used in this work to harvest unit grams of DCW was lower than those of the other types of flocculants such as bare Fe_3O_4 (2.5−50 g/g-cell), aminoclay-nZVI (13.3 g/g-cell) and APTES-$BaFe_{12}O_{19}$ (>2.3 g/g-cell) composites.[13,20,26] It should be noted that the dosage varies largely from 0.015 to 12.8 g/g-cell depending on the species of microalgae and magnetic flocculants.[27] For practical application of magnetophoretic separation of microalgae, the adsorption capacity of the dMNF should be further improved and several issues such as increasing surface charge or minimizing aggregation of flocculants could be taken into account. Interestingly, harvesting efficiency increased with increasing cell concentration of microalgae under the same dosage of dMNF per gram microalgal cell (g dMNF/g cell): 56.7% at 0.85 g/L, 98.5% at 1.60 g/L, and 99.9 at 3.30 g/L. For the reason for this phenomenon, we speculate that the higher populations might have higher chances of contact and interaction between microalgae and dMNF.

3.3. Recovery Efficiency. Detaching microalgae after their magnetophoretic separation from magnetic-particle flocculants has been a challenging task. It was reported that simple switching of the electrostatic interaction between the two to electrostatic repulsion was not sufficient to generate their spontaneous separation.[20] Rather, for separation, additional energy such as shaking or sonication needs to be applied under an external magnetic field. In the present work, microalgae could be successfully recovered from microalgae-dMNF flocs at the water-NOS interface only by the aid of potential energy originating from interface tension and the external magnetic field. When DCM was added to the microalgae-dMNF flocs after discarding the clear supernatant in the harvesting step (Figure 4C), phase separation was observed as shown in Figure 5A−B, owing to the microalgae-dMNF flocs' overall hydrophilicity. The flocs were floating on hydrophobic DCM, the density (1.33 g/cm^3) of which is higher than that of water (Figure 5B), whereas in hexane (0.655 g/cm^3) and dodecane (0.75 g/cm^3), the flocs sank (Figure S2). When the external magnetic field was applied from the direction of the NOS, the dMNF were drawn to and trapped in the DCM phase while the microalgae remained in the water phase. By repeating this, microalgae could be separated from the dMNF, as shown in Movie S1, and the culture was concentrated up to 61 g/L. Figure 5E plots the recovery efficiencies for the different types of NOS. We obtained between 68 and 83% recovery efficiencies

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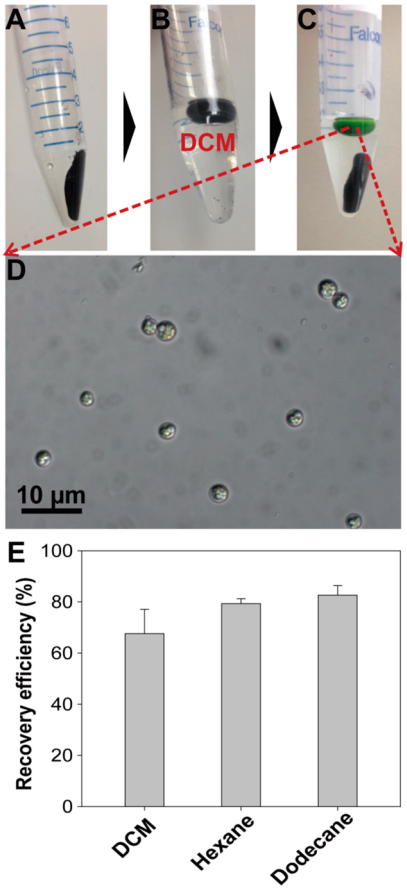

Figure 5. Recovery of microalgae from microalgae-dMNF flocs: (A) separated microalgae-dMNF flocs after discarding of supernatant, (B) addition of NOS (DCM), (C) magnetophoretically separated microalgae using water−DCM interface, (D) optical-microscopic image of separated microalgae, and (E) recovery efficiencies of microalgae (or dMNF) with different NOS.

using DCM, hexane and dodecane. It is believed that recovery efficiencies are strongly related to the interfacial tension between the two liquids, because at higher interfacial tensions, microalgae are more effectively trapped at the interface. The measured interfacial tensions between water and the DCM, hexane and dodecane were 28.31, 51.0, and 52.8 dyn/cm, respectively.[28] Correspondingly, the recovery efficiencies at the water-hexane (79%) and water-dodecane (83%) interfaces were higher than at the water-DCM interface (68%). On the basis of the recovery efficiency, dodecane is the most promising solvent among three solvents tested. Especially, dodecane solvent is known as a biocompatible solvent which was successfully used for the selective extraction of high-value astaxanthin pigment from *Haematococcus* cell while keeping its viability up to 98%.[29] However, hexane in the mixture form with methanol has been preferred for the extraction of lipid (significantly cheaper than astaxanthin) than dodecane considering overall extraction cost. For the regeneration of dMNF, the remaining microalgae should be further detached from dMNF and additional physical treatment such as ultrasonic cleaning can be applied. However, a complete separation of attached algal cells might be difficult only by such physical separation methods considering the recent report by Toh et al., who showed that the some magnetic nanoparticles were internalized into *Chlorella sp.* cells during the magnetophoretic separation based on TEM observation.[30] Nonetheless, the biodiesel quality produced from *Chlorella* biomass was not affected by the internalization of magnetic nanoflocculants.

In principle, this strategy for separation of microalgae from dMNF was conceived by taking advantage of the hydrophilic nature of the microalgal surface. Physicochemical studies show that microalgae have hydrophilic surfaces with 30−40° contact angles.[31] The hydrophilic nature of the cell surface originates from the hydrophilic end of the phospholipid layer of the outer cell membrane.[25,32] It is reasonable to infer that dMNF-tagged microalgae will tend to stay in the water phase while dMNF are detached from them and selectively transferred to the NOS phase under a magnetic field, as illustrated in Figure 6A. Here

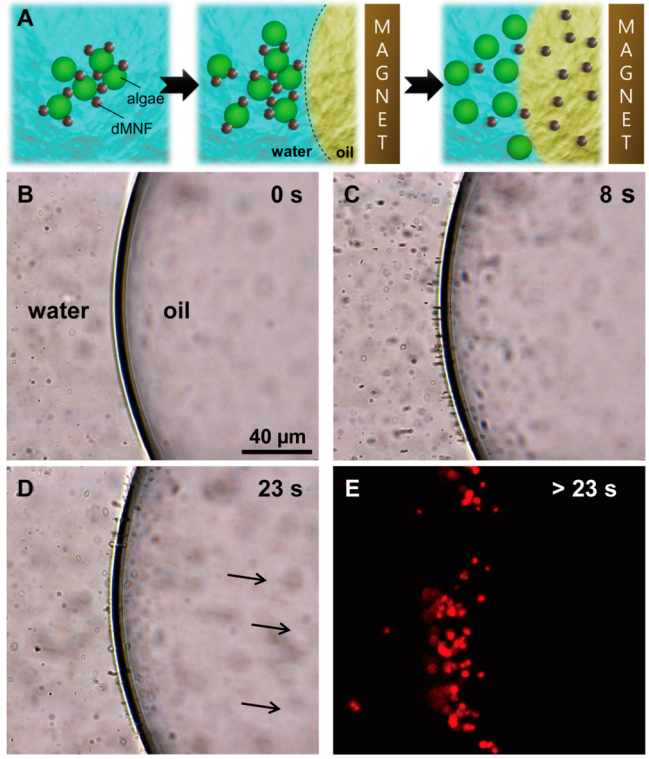

Figure 6. (A) Schematics and (B−E) microscopic observation of magnetophoretic separation of dMNF from microalgae-dMNF flocs at water−oil interface. The arrows in panel D indicate the direction of the movement of the separated dMNF toward the permanent magnet. The red autofluorescence in panel E indicates the trapped microalgae at the water−oil interface.

the water-NOS interface plays the role of a sieve for selective filtration of dMNF, but, crucially, not by size difference but by hydrophilicity difference. For efficient filtration, the water-NOS interface tension must be overcome by a magnetic field strong enough to drag the dMNF away. For this, other than applying such a strong magnetic field, we partially endowed the dMNF with lipophilicity by OTES functionalization, thus enhancing the compatibility of dMNF with the NOS phase. Microscopic observation of the water−oil interface clearly indicated successful filtration (Figures 6B-E). When the magnetic field was applied from the oil phase, the flocs in the water phase moved toward the interface (Figure 6C). And while the movement of dMNF was observed even after the interface and in the oil phase (Figure 6D, indicated with arrows), microalgae were blocked at the interface. The autofluorescence originated from the chlorophyll of the microalgae that were observed only

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in the water phase, and most of them were concentrated along the interface (Figure 6E).

The energy-intensive primary harvesting step forming 2−7% TSS is followed by a secondary, dewatering step to obtain 15−25% TSS, which is followed in turn by extraction and drying steps.[33] It is noteworthy that in this present work, the microalgal culture could be concentrated up to ∼60 g/L (6% TSS) simply by utilization of potential energy such as from electrostatic energy, the magnetic field of a permanent magnet, and by the interfacial tension of liquids. When the same process was applied for 0.5 L scale, we observed the efficient harvesting and could concentrate microalgal cells by about 15 folds (Figure S4), which requires further optimization for higher yield. Furthermore, wet lipid extraction or even biodiesel production can be carried out directly from concentrated microalgae in the presence of hexane, as demonstrated by Cao et al. or Chen et al., respectively,[34,35] which, by simplifying multiple downstream processes, would provide a great potential cost reduction in microalgae-based biorefinement.

4. CONCLUSIONS

We fabricated dual-functionalized magnetic nanoflocculants (dMNF) of core−shell structure with an Fe_3O_4 core of organosilane-functionalized silica shell for efficient magnetophoretic harvesting of microalgae. Two types of organosilane compounds, (3-aminopropyl)triethoxysilane (APTES) and octyltriethoxysilane (OTES), were introduced to exhibit cationic charge and enhance lipophilicity, respectively. A small dosage of dMNF induced rapid *Chlorella* sp. KR-1 flocculation of almost 100% efficiency. Considering that the microalgal surface is hydrophilic, a lipophilic level of dMNF is controlled by tuning the APTES/OTES ratio, which lipophilicity impelled the selective separation of magnetic nanoflocculants from microalgal flocs at the interface of the water and nonpolar organic solvents (NOS). This was clearly confirmed by microscopic observation. The recovery efficiencies of the magnetic nanoflocculants corresponded, from highest to lowest, to the magnitudes of interfacial tension between the water and NOS: dodecane, hexane, and dichloromethane. We believe that this recovery strategy utilizing the controlled lipophilicity of magnetic particles represents an important new approach to the resolution of a particularly thorny problem in the field of microalgae harvesting. As such, it paves the way to the full realization of microalgal-based bioenergy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b04098.

X-ray diffraction patterns of Fe_3O_4 and Fe_3O_4@SiO_2, recovery of microalgae from microalgae-dMNF flocs using hexane and dodecane, contact angle measurements of Fe_3O_4@SiO_2-OTES and Fe_3O_4@SiO_2-OTES/APTES (dMNF), samples dispersed in water−oil phase-separated solution, and scaled-up microalgae harvesting using dMNF and dodecane (PDF)

Recovery of dMNF from microalgae-dMNF flocs using DCM (AVI)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

NOS = nonpolar organic solvent
TSS = total suspended solids
dMNF = dual-functionalized Fe_3O_4 magnetic-nanoparticle flocculants
DCW = dry cell weight

■ REFERENCES

(1) Wijffels, R. H.; Barbosa, M. J. An Outlook on Microalgal Biofuels. *Science* **2010**, *329*, 796−799.

(2) Georgianna, D. R.; Mayfield, S. P. Exploiting Diversity and Synthetic Biology for the Production of Algal Biofuels. *Nature* **2012**, *488*, 329−335.

(3) Foley, P. M.; Beach, E. S.; Zimmerman, J. B. Algae as a Source of Renewable Chemicals: Opportunities and Challenges. *Green Chem.* **2011**, *13*, 1399−1405.

(4) Teixeira, R. E. Energy-efficient Extraction of Fuel and Chemical Feedstocks from Algae. *Green Chem.* **2012**, *14*, 419−427.

(5) Lam, M. K.; Lee, K. T. Microalgae Biofuels: A Critical Review of Issues, Problems and the Way Forward. *Biotechnol. Adv.* **2012**, *30*, 673−690.

(6) Milledge, J.; Heaven, S. A Review of the Harvesting of Microalgae for Biofuel Production. *Rev. Environ. Sci. Bio/Technol.* **2013**, *12*, 165−178.

(7) Zhang, X. Z.; Hu, Q.; Sommerfeld, M.; Puruhito, E.; Chen, Y. S. Harvesting Algal Biomass for Biofuels using Ultrafiltration Membranes. *Bioresour. Technol.* **2010**, *101*, 5297−5304.

(8) Gao, S. S.; Yang, J. X.; Tian, J. Y.; Ma, F.; Tu, G.; Du, M. A. Electro-coagulation-flotation Process for Algae Removal. *J. Hazard. Mater.* **2010**, *177*, 336−343.

(9) Kim, J.; Ryu, B. G.; Kim, K.; Kim, B. K.; Han, J. I.; Yang, J. W. Continuous Microalgae Recovery using Electrolysis: Effect of Different Electrode Pairs and Timing of Polarity Exchange. *Bioresour. Technol.* **2012**, *123*, 164−170.

(10) Vandamme, D.; Foubert, I.; Muylaert, K. Flocculation as a Low-cost Method for Harvesting Microalgae for Bulk Biomass Production. *Trends Biotechnol.* **2013**, *31*, 233−239.

(11) Farooq, W.; Lee, Y.-C.; Han, J.-I.; Darpito, C. H.; Choi, M.; Yang, J.-W. Efficient Bicroalgae Harvesting by Organo-building Blocks of Nanoclays. *Green Chem.* **2013**, *15*, 749−755.

(12) Xu, L.; Guo, C.; Wang, F.; Zheng, S.; Liu, C. Z. A Simple and Rapid Harvesting Method for Microalgae by in situ Magnetic Separation. *Bioresour. Technol.* **2011**, *102*, 10047−10051.

DOI: 10.1021/acsami.5b04098
ACS Appl. Mater. Interfaces 2015, 7, 18336−18343

(13) Seo, J. Y.; Lee, K.; Lee, S. Y.; Jeon, S. G.; Na, J.-G.; Oh, Y.-K.; Park, S. B. Effect of Barium Ferrite Particle Size on Detachment Efficiency in Magnetophoretic Harvesting of Oleaginous *Chlorella* sp. *Bioresour. Technol.* **2014**, *152*, 562−566.
(14) Lee, K.; Lee, S. Y.; Na, J.-G.; Jeon, S. G.; Praveenkumar, R.; Kim, D.-M.; Chang, W.-S.; Oh, Y.-K. Magnetophoretic Harvesting of Oleaginous *Chlorella* sp. by using Biocompatible Chitosan/Magnetic Nanoparticle Composites. *Bioresour. Technol.* **2013**, *149*, 575−578.
(15) Cerff, M.; Morweiser, M.; Dillschneider, R.; Michel, A.; Menzel, K.; Posten, C. Harvesting Fresh Water and Marine Algae by Magnetic Separation: Screening of Separation Parameters and High Gradient Magnetic Filtration. *Bioresour. Technol.* **2012**, *118*, 289−295.
(16) Liu, X.; Ma, Z.; Xing, J.; Liu, H. Preparation and Characterization of Amino−silane Modified Superparamagnetic Silica Nanospheres. *J. Magn. Magn. Mater.* **2004**, *270*, 1−6.
(17) Melnyk, I. V.; Zub, Y. L. Preparation and Characterization of Magnetic Nanoparticles with Bifunctional Surface Layer ≡Si-$(CH_2)_3NH_2$/≡$SiCH_3$ (or ≡SiC_3H_7−n). *Microporous Mesoporous Mater.* **2012**, *154*, 196−199.
(18) Na, J.-G.; Lee, H. S.; Oh, Y.-K.; Park, J.-Y.; Ko, C. H.; Lee, S.-H.; Yi, K. B.; Chung, S. H.; Jeon, S. G. Rapid Estimation of Triacylglycerol Content of *Chlorella* sp. by Thermogravimetric Analysis. *Biotechnol. Lett.* **2011**, *33*, 957−960.
(19) Cornell, R. M.; Schwertmann, U. *The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses*. John Wiley & Sons: Weinheim, Germany, 2006.
(20) Lee, K.; Lee, S. Y.; Praveenkumar, R.; Kim, B.; Seo, J. Y.; Jeon, S. G.; Na, J.-G.; Park, J.-Y.; Kim, D.-M.; Oh, Y.-K. Repeated Use of Stable Magnetic Flocculant for Efficient Harvest of Oleaginous *Chlorella* sp. *Bioresour. Technol.* **2014**, *167*, 284−290.
(21) Darbandi, M.; Stromberg, F.; Landers, J.; Reckers, N.; Sanyal, B.; Keune, W.; Wende, H. Nanoscale Size Effect on Surface Spin Canting in Iron Oxide Nanoparticles Synthesized by the Microemulsion Method. *J. Phys. D: Appl. Phys.* **2012**, *45*, 195001.
(22) Fujii, T.; De Groot, F.; Sawatzky, G.; Voogt, F.; Hibma, T.; Okada, K. In situ XPS Analysis of Various Iron Oxide Films Grown by NO_2-assisted Molecular-beam Epitaxy. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1999**, *59*, 3195.
(23) Zeng, Y.; Hao, R.; Xing, B.; Hou, Y.; Xu, Z. One-pot Synthesis of Fe_3O_4 Nanoprisms with Controlled Electrochemical Properties. *Chem. Commun.* **2010**, *46*, 3920−3922.
(24) An, Y.; Chen, M.; Xue, Q.; Liu, W. Preparation and Self-assembly of Carboxylic Acid-Functionalized Silica. *J. Colloid Interface Sci.* **2007**, *311*, 507−513.
(25) Toh, P. Y.; Ng, B. W.; Ahmad, A. L.; Chieh, D. C. J.; Lim, J. The Role of Particle-to-Cell Interactions in Dictating Nanoparticle Aided Magnetophoretic Separation of Microalgal Cells. *Nanoscale* **2014**, *6*, 12838−12848.
(26) Lee, Y.-C.; Lee, K.; Hwang, Y.; Andersen, H. R.; Kim, B.; Lee, S. Y.; Choi, M.-H.; Park, J.-Y.; Han, Y.-K.; Oh, Y.-K. Aminoclay-templated Nanoscale Zero-Valent Iron (nZVI) Synthesis for Efficient Harvesting of Oleaginous Microalga, Chlorella sp. KR-1. *RSC Adv.* **2014**, *4*, 4122−4127.
(27) Wang, S.-K.; Stiles, A. R.; Guo, C.; Liu, C.-Z. Harvesting Microalgae by Magnetic Separation: A Review. *Algal Res.* **2015**, *9*, 178−185.
(28) Demond, A. H.; Lindner, A. S. Estimation of Interfacial Tension between Organic Liquids and Water. *Environ. Sci. Technol.* **1993**, *27*, 2318−2331.
(29) Kang, C. D.; Sim, S. J. Selective Extraction of Free Astaxanthin from Haematococcus Culture using a Tandem Organic Solvent System. *Biotechnol. Prog.* **2007**, *23*, 866−871.
(30) Toh, P. Y.; Ng, B. W.; Chong, C. H.; Ahmad, A. L.; Yang, J.-W.; Lim, J. Magnetophoretic Separation of Microalgae: the Role of Nanoparticles and Polymer Binder in Harvesting Biofuel. *RSC Adv.* **2014**, *4*, 4114−4121.
(31) Ozkan, A.; Berberoglu, H. Physico-chemical Surface Properties of Microalgae. *Colloids Surf., B* **2013**, *112*, 287−293.
(32) van Oss, C. J. Hydrophobicity of Biosurfaces — Origin, Quantitative Determination and Interaction Energies. *Colloids Surf., B* **1995**, *5*, 91−110.
(33) Uduman, N.; Qi, Y.; Danquah, M. K.; Forde, G. M.; Hoadley, A. Dewatering of Microalgal Cultures: a Major Bottleneck to Algae-based Fuels. *J. Renewable Sustainable Energy* **2010**, *2*, 012701.
(34) Chen, M.; Liu, T.; Chen, X.; Chen, L.; Zhang, W.; Wang, J.; Gao, L.; Chen, Y.; Peng, X. Subcritical Co-solvents Extraction of Lipid from Wet Microalgae Pastes of *Nannochloropsis* sp. *Eur. J. Lipid Sci. Technol.* **2012**, *114*, 205−212.
(35) Cao, H.; Zhang, Z.; Wu, X.; Miao, X. Direct Biodiesel Production from Wet Microalgae Biomass of *Chlorella pyrenoidosa* through in situ Transesterification. *BioMed Res. Int.* **2013**, *2013*, 930686.

DOI: 10.1021/acsami.5b04098
ACS Appl. Mater. Interfaces 2015, 7, 18336−18343