Magnetophoretic Harvesting of *Nannochloropsis oculata* Using Iron Oxide Immobilized Beads

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**Abstract:** In this work, the harvesting of *Nannochloropsis oculata* microalgae through the use of nanosized Fe3O4 immobilized in polyvinyl alcohol (PVA)/sodium alginate (SA) as a flocculant (Fe3O4/PS) is investigated. Using the Fe3O4/PS immobilized beads could reduce the amount of soluble ferrous ions (Fe2+) released from naked Fe3O4 in acid treatment, leading to easy recovery. The characterization was performed under different dosages and pH values of Fe3O4/PS. The results show that the Fe3O4/PS, when applied to the algae culture (500 mg dry cell weight/L), achieves a 96% harvesting efficiency under conditions of a pH of 4 with 200 mT magnetic field intensity. Fe3O4/PS can be directly reused without adjusting the pH value. The recycled Fe3O4/PS shows stability in terms of its surface properties, maintaining more than 80% harvesting efficiency after five recycles. Magnetophoretic harvesting, using immobilized magnetic iron oxide as a particle-based flocculant, is a potential method to reduce challenges related to the cost-effective microalgae-harvesting method.

**Keywords:** magnetophoretic harvesting; *Nannochloropsis oculata*; magnetic field; iron oxide; immobilization; polyvinyl alcohol; alginate

1. Introduction

As the flocculant plays a major role in the flocculation harvesting process, and as microalgae characteristics are small and stable when in a suspension with a negatively charged surface, the availability of low-cost flocculants and process scale-ups should be properly considered [1]. Low-cost options for harvesting microalgae by replacing chemical coagulants can solve both the problems of high costs and contamination associated with harvesting microalgae [2,3]. To date, several studies have successfully applied a flocculant to achieve a recyclable, low-toxicity, and low-cost harvesting process [4,5].

*Nannochloropsis oculata* has been widely developed, not only for the production of therapeutic agents in the pharmaceutical industry [6,7], but also for the production of nutrients [8], food [9], and biofuel. *N. oculata* is also regarded as attractive because it can be grown in brackish water and seawater.

Harvesting techniques for marine species of *N. oculata* are worth exploring, and have been developed due to marine species having a limited dependence of freshwater resources [8]. Recently, algae harvesting using charge neutralization and electrostatic bridging with functional nanoparticles,
based on the magnetophoretic harvesting technique, has been intensively investigated [1]. The characteristics of magnetophoretic harvesting flocculating materials, such as iron oxide ($\text{Fe}_3\text{O}_4$) and iron yttrium oxide ($\text{Y}_3\text{Fe}_5\text{O}_{12}$) are a high harvesting efficiency, ease of operation and recovery, low energy consumption, and magnetization and biocompatible properties [3,10–12]. In addition, the effective recycling of material could reduce the manufacturing costs of particle-based flocculants, which is important in terms of achieving a sustainable and low-cost process [13].

Magnetic nanomaterial iron oxides ($\text{Fe}_3\text{O}_4$) are widely used as a reusable adsorbent/flocculant for wastewater treatment [14,15]. Especially ferrous ions ($\text{Fe}^{2+}$) of $\text{Fe}_3\text{O}_4$, which can be used as Fenton catalysts when under an acidic environment [16]. However, by using magnetic nanomaterials in the microalgae harvesting process, the $\text{Fe}^{2+}$ ions have an impact on cytotoxicity for algae and cell growth inhibition [17,18].

The immobilization of iron oxide ($\text{Fe}_3\text{O}_4$) nanocomposites within polyvinyl alcohol (PVA) and sodium alginate (SA) is a safe process that is widely used in other applications, such as wastewater treatment [19–21], pharmaceuticals [22,23], and immobilized lipase for biodiesel production [24].

To our knowledge, the synthesis of PVA and SA-coated $\text{Fe}_3\text{O}_4$ nanoparticles and the use of them as a magnetic flocculating material for harvesting marine microalga N. oculata has not been reported. Hence, this work aims to synthesize $\text{Fe}_3\text{O}_4/$PS particles through a mixed PVA and SA solution embedded with iron oxide ($\text{Fe}_3\text{O}_4$), with boric acid and phosphate as a spherical solution. $\text{Fe}_3\text{O}_4/$PS has a high mechanical performance and performs well regarding magnetic separation for algae harvesting. Batch experiments on $\text{Fe}^{2+}$ ions released from an acidic environment, their dosage, different pH value conditions, and contact time were conducted, and the reusability of $\text{Fe}_3\text{O}_4/$PS was studied.

2. Materials and Methods

2.1. Algae Culture

The microalga used in this study was Nannochloropsis oculata, which was obtained from the Fisheries Research Institute Biotechnology Research Center (Tungkang, Taiwan). N. oculata was cultured in a 1 L photobioreactor with Walne's medium for 10 days [25]. The cultivated temperature was maintained at $24 \pm 0.7 \, ^\circ\text{C}$ under continuous illumination by a fluorescent lamp (26 W, EFHS26L-GE, China Electric, Hsinchu, Taiwan) with a light intensity of 67.5 $\mu\text{mol protons m}^{-2} \text{s}^{-1}$. Cultures were aerated (EP-12000, Rambo, New Taipei City, Taiwan) at a rate of 0.25 vvm.

Biomass optical density was measured at a wavelength of 680 nm using a UV/VIS spectrophotometer (DR/4000U, Hach, Loveland, CO, USA). Dry biomass was washed with distilled water 3 times and then centrifuged (CN-820, Hsiangtai, New Taipei City, Taiwan) at 2500 rpm for 5 min, then dried in an oven (DO45, Dengyng, New Taipei City, Taiwan) at 105 $^\circ\text{C}$ for 24 h. The microalgae biomass concentration was estimated using Equation (1):

$$\text{Biomass concentration (mg/L)} = 494.59 \times \text{OD}_i - 66.862, \quad R^2 = 0.995 \quad (1)$$

where biomass is in milligrams per liter (mg/L) and $\text{OD}_i$ is the supernatant value.

2.2. $\text{Fe}_3\text{O}_4/$PS Preparation

Naked iron oxide $\text{Fe}_3\text{O}_4$ magnetic nanoparticles were immobilized in PVA and SA according to the method described by Huang et al. [20]. Briefly, 8 g of PVA and 2 g of SA were dissolved into 90 mL of diluted water and then autoclaved (TM-321, Tomin, New Taipei City, Taiwan) at 1.2 kg cm$^{-2}$ (15 psi), at 121 $^\circ\text{C}$, for 20 min. Then, the naked $\text{Fe}_3\text{O}_4$ (4.99 g) was mixed into the PVA/SA solution with a rotation speed of 150 rpm for 1 h until the mixture cooled down to room temperature. The mixture was added dropwise to the treatment mixture (containing 1% boric acid solution and 5% calcium chloride) with a peristaltic pump (RP-2000, Eyela, Tokyo, Japan). The formed beads were then transferred to a 5% $\text{KH}_2\text{PO}_4$ solution for 3 h to reinforce the mechanical strength. The resulting beads were quite uniform in size, with a diameter range of 2.4 $\pm$ 0.15 mm.
2.3. Characterization Analysis

The soluble iron Fe$^{2+}$ concentration of Fe$_3$O$_4$/PS under different pH values was determined by using an inductively coupled plasma optical emission spectrometer (ICP-OES; OPTIMA 5100 DV, Perkin Elmer, Waltham, MA, USA) for 30 min. The morphology of Fe$_3$O$_4$/PS and naked Fe$_3$O$_4$ was determined by using a digital biological microscope (DMWB1-223, Motic, Xiamen, China) and a field emission scanning electron microscope (JSM-6710F, JEOL, Akishima, Japan). The zeta potential and particle size distribution of N. oculata and naked Fe$_3$O$_4$ were established using a zeta/nanoparticle analyzer (NanoPlus-3, Micromeritics, Norcross, GA, USA). All sample zeta potentials were measured 3 times to obtain average values.

2.4. Biomass Harvesting

The method of microalgae harvesting was according to that described by Zhu et al. [13]. The microalgae were cultivated for 10 days to achieve a biomass concentration of $0.5 \pm 0.002$ g/L as a dry weight. The algae solution was adjusted from pH 4 to pH 9 by using 0.1 M HCl and 0.1 M NaOH. The different dosages of naked Fe$_3$O$_4$ were 20, 40, 60, 80, 100, and 120 mg/L, respectively. The dosage of Fe$_3$O$_4$/PS was calculated as 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 g/L (per gram beads contained 50 mg naked Fe$_3$O$_4$), respectively. Both flocculants, as mixtures, were rotated using stirrers at 300 rpm for 1 min in the algae medium. Afterwards, a $10 \times 10 \times 1$ cm external magnet (Magtech Magnetic Products Co., Taipei, Taiwan) with a 200 mT intensity was placed under the vials. Magnetic materials and biomass were passively harvested by the external magnetic field. Harvesting efficiency was determined by the optical density of the supernatant, which was collected from the water surface, and the harvesting efficiency of N. oculata was calculated according to Equation (2):

\[
\text{Harvesting efficiency (\%)} = \left(\frac{\text{OD}_i - \text{OD}_f}{\text{OD}_i}\right) \times 100
\]

where OD$_i$ is the value of the initial culture and OD$_f$ is the value of the supernatant after harvesting.

2.5. Biomass Separation and Reusability

After Fe$_3$O$_4$/PS was harvested, the supernatant was carefully discarded with pipettes, and the Fe$_3$O$_4$/PS was transferred by an external magnet into 100 mL of diluted water and then washed 3 times without ultrasonic treatment. The naked Fe$_3$O$_4$ was mixed in the NaOH solution in vials, and the pH value was adjusted to 10. The mixture of algae and naked Fe$_3$O$_4$ was rotationally stirred at 500 rpm for 1 min, after which, the magnet was placed under the vials to collect the naked Fe$_3$O$_4$. The adsorbed algal biomass was separated from the detached naked Fe$_3$O$_4$. Afterwards, the supernatant of the algae was carefully discarded with the pipettes, then, 100 mL of distilled water was added into the detached naked Fe$_3$O$_4$ particles and treated with ultrasound at 40 kHz for 20 min.

In the reusability test, N. oculata was at an initial concentration of 500 mg/L and a pH of 5 for each cycle, and 120 mg/L and 2.4 g/L of Fe$_3$O$_4$ and Fe$_3$O$_4$/PS were applied, respectively. All separations of the detached measurements were considered as one recycle, with 5 reuse recycles performed in total.

2.6. Statistical Analysis

The results are in 3 replicates and have been calculated as the mean with standard deviation. Statistical analysis was performed using SPSS V20 (IBM, Chicago, IL, USA) analysis of variance (ANOVA), followed by Tukey’s test at a probability level of $p < 0.05$. 
3. Results and Discussion

3.1. \( \text{Fe}_3\text{O}_4/\text{PS} \) Characteristics

The soluble iron \( \text{Fe}^{2+} \) concentration of \( \text{Fe}_3\text{O}_4/\text{PS} \) and \( \text{Fe}_3\text{O}_4 \) under different pH values for 30 min is shown in Table 1. The concentration of dissolved ion \( \text{Fe}^{2+} \) of \( \text{Fe}_3\text{O}_4/\text{PS} \) was insignificant, since the pH value changed \((p > 0.05) \). However, the soluble iron \( \text{Fe}^{2+} \) of \( \text{Fe}_3\text{O}_4 \) was increased when the pH value decreased \((p < 0.05) \). Taylor and Owen [26] discussed that magnetite and hematite can be expressed in incongruent dissolution, which could yield various other less stable \( \text{Fe} \) oxides or oxyhydroxides in solution, as per Equation (3).

\[
\text{Fe}_3\text{O}_4 + 2\text{H}^+ \rightarrow \text{Fe}_2\text{O}_3 + \text{Fe}^{2+} + \text{H}_2\text{O}
\]  

(3)

| Condition         | Concentration of Soluble Iron \( \text{Fe}^{2+} \) (mg/L) | pH 3 | pH 4 | pH 5 | pH 6 |
|-------------------|----------------------------------------------------------|------|------|------|------|
| \( \text{Fe}_3\text{O}_4/\text{PS} \) ¹ | 16 ± 0.02 \(^a\)                                         | 15.9 ± 0.03 \(^a\) | 15.9 ± 0.03 \(^a\) | 15.9 ± 0.02 \(^a\) |
| Naked \( \text{Fe}_3\text{O}_4 \) ² | 2220 ± 31 \(^d\)                                         | 1077 ± 67 \(^c\)   | 132 ± 8 \(^b\)      | 22 ± 3 \(^a\)      |

¹ The concentration of \( \text{Fe}_3\text{O}_4/\text{PS} \) calculates at 2.4 g/L (w/w). ² The concentration of \( \text{Fe}_3\text{O}_4 \) was 120 mg/L. Means ± standard deviations. Different lowercase letters \((a, b, c, \text{ and } d)\) in the same line correspond to significant differences between all assays \((p < 0.05) \) as found by Tukey’s test.

It was reported that \( \text{Fe}^{2+} \) is beneficial to microbial attachment [27], but high concentrations of \( \text{Fe}^{2+} \) could decrease microbial attachment [28]. Ren et al. [29] reported that \( \text{Fe}_3\text{O}_4 \), as a magnetic material, can successfully develop granules with the addition of \( \text{Fe}^{2+} \) and \( \text{Fe}_3\text{O}_4 \), which could decrease negative charges on bacterial attachment. Wang et al. [12] also determined a slight iron dissolution of naked \( \text{Fe}_3\text{O}_4 \) at pH 3.01. This result indicated that PVA/SA immobilization could protect \( \text{Fe}_3\text{O}_4 \) and avoid oxidation from the high amount of dissolved oxygen in the algae medium. Moreover, removing soluble iron \( \text{Fe}^{2+} \) from the algae harvesting process could require additional processes.

Figure 1a–c shows the surface morphology of the prepared \( \text{Fe}_3\text{O}_4/\text{PS} \). \( \text{Fe}_3\text{O}_4/\text{PS} \) was shown to exist in the form of spherical particles with a 2.4–2.9 mm particle size, found via a particle analyzer \((n = 150; \text{Figure 1a}) \). Figure 1b shows the individual microsphere of \( \text{Fe}_3\text{O}_4/\text{PS} \) exhibiting paramagnetism \((\text{Figure 1c,d}) \). Huang et al. [20] reported that the coercive force of the magnetization hysteresis loop of \( \text{Fe}_3\text{O}_4/\text{PS} \) was 5 emu/g, indicating that the \( \text{Fe}_3\text{O}_4/\text{PS} \) had a magnetic response and separation capability. Those results indicate that \( \text{Fe}_3\text{O}_4 \) immobilized by PVA and SA not only reduces the risk of water recycling with toxic materials but also facilitates magnet recovery.

In order to understand the impact of pH on the \( \text{Nannochloropsis oculata} \) interaction, the zeta potential can determine electrostatic repulsion for microalgal cells and material. Figure 2 shows the zeta potential measurement of \( \text{N. oculata} \) and naked \( \text{Fe}_3\text{O}_4 \) under different pH values. They were found to maintain a predominantly negative surface charge \((-3 \text{ to } -11 \text{ mV}) \) over a wide pH range of 2 to 11 pH. This result was supported by Lama et al. [30], who estimated that the zeta potential of \( \text{N. oculata} \) SAG 38.85 cultivated in an artificial seawater medium was -7 mV. Boli et al. [31] also determined marine microalga \( \text{Nannochloropsis oceanica} \) CCMP1779 and magnetic microparticles under different pH values, finding different surface charges. \( \text{Fe}_3\text{O}_4 \) was positively charged under acidic conditions, with an isoelectric point between pH 6 and 7. This behavior is consistent with other studies in the literature [13]. Prochazková et al. [32] also reported that the most pronounced difference between zeta potentials of interacting cells and iron oxide magnetic microparticles occurs at pH 4, resulting in the strongest electrostatic interactions and contributing to the highest efficiencies as compared to other pH values. Moreover, high pH values could result in decreased harvesting efficiency [30].
Figure 1. Surface morphology of prepared polyvinyl alcohol (PVA) and sodium alginate (SA)-coated Fe$_3$O$_4$ nanoparticles (Fe$_3$O$_4$/PS). (a) Size distribution (%) of Fe$_3$O$_4$/PS; (b) Iron oxide immobilized in Fe$_3$O$_4$/PS determined by field emission scanning electron microscopy (SEM). (c) Magnetic separation of naked Fe$_3$O$_4$ and (d) Fe$_3$O$_4$/PS attracted in water under a 200 mT magnet.

Figure 2. Zeta potentials of naked Fe$_3$O$_4$ and Nannochloropsis oculata as a function of pH.

3.2. Effects of Dosage Concentration on Magnetic Harvesting

The performance of Nannochloropsis oculata harvesting efficiency, using different dosages of Fe$_3$O$_4$/PS and naked Fe$_3$O$_4$, is shown in Figure 3. Harvesting efficiency reached 99 ± 0.7% and 92 ± 0.6% at the optimal dosage of naked Fe$_3$O$_4$ 120 mg/L at pH 4 and 5, respectively. It reached 96 ± 0.3% and 90 ± 0.5% at the same dosage after PVA and SA modification at pH 4 and 5, respectively. Specifically, at any Fe$_3$O$_4$/PS dosage, there were insignificant differences ($p > 0.05$) in harvesting efficiency between
the pH values, suggesting a great influence from the acidic environment. A similar effect has been reported in other studies; Wang et al. [12] used polyphenol-coated Fe₃O₄ particles and also successfully flocculated oleaginous microalgae, reaching 93% efficiency at an optimal dosage of 25 g/L.

Figure 3. Comparison of different dosages of Fe₃O₄/PS and naked Fe₃O₄ on Nannochloropsis oculata harvesting efficiency with an initial concentration of 500 mg/L.

Dosage increments from 40 to 120 g/L of naked Fe₃O₄ reached > 80% harvesting efficiency for N. oculata. This is because the Fe²⁺ ions from the Fe₃O₄ detected in this study gradually increased with a decreasing pH value (Table 1). This could be ascribed to Fe²⁺ ions being able to potentially oxidize into Fe³⁺ ions in an acidic environment, thereby acting as floculants [32]. The harvesting efficiency of Fe₃O₄/PS when using a dosage of 80 mg/L showed that it required nearly 2-fold the dose to obtain a comparable level of harvesting efficiency where immobilization was absent.

Earlier studies revealed that, when using PVA for surface immobilization, PVA adsorption increases with polymer molecular weight, which could provide sufficient functional groups and positive active sites for adsorption [33]. Although PVA and SA are promising potential alternative coagulants, they do not achieve efficient microalgal harvesting when directly used in flocculation (Figure 2). The improved activity of Fe₃O₄/PS had a positive effect on harvesting N. oculata. The coating of a polymer was sufficient to cover Fe₃O₄ surfaces and ensure the colloidal stability of Fe₃O₄/PS over a wide range of pH levels without oxidation [12]. These findings reveal the stability of Fe₃O₄/PS to repeated harvesting, supporting its application in marine microalgae harvesting.

3.3. Effects of pH on Magnetic Harvesting

Figure 4 shows the Nannochloropsis oculata harvesting efficiency of Fe₃O₄/PS under different pH values. The effectiveness of Fe₃O₄/PS was tested at several pH values, from pH 4 to 9, using a dosage of 120 mg/L (2.4 g w/w). The highest efficiency was obtained at an acidic pH of 4, with 91 ± 4.2% within the first 120 min, and reached a maximum of 96 ± 0.3% at a sedimentation time of 180 min. Under acidic conditions, a clear supernatant was observed, while poor efficiency was obtained in alkaline conditions. This finding was supported by Fuad et al. [34] who achieved the greatest flocculation efficiency at an acidic pH value using a tannin-based natural biopolymer (AFlok-BP1) to harvest marine Nannochloropsis sp. throughout 2 h of sedimentation.
Figure 4. Nannochloropsis oculata harvesting efficiency (with initial concentration of 500 mg/L) at different Fe$_3$O$_4$/PS pH values. Results are in the form of average ± standard deviation.

Fe$_3$O$_4$/PS occurred at pH 8 and 9, but it was less effective, which affected sample efficiency. The alkaline environment was established by adding NaOH in this study. A previous study reported that using polydiallyldimethylammonium chloride (PDADMAC) for marine microalgae Nannochloropsis salina at an alkaline pH of 10 resulted in a decrease of efficiency, since a high dosage was required because of the adsorption of salt anions on the polymer or algae [35].

Wiśniewska et al. [33] demonstrated that the main reason for polyvinyl alcohol adsorption reduction at pH 9 was because a high pH value causes a significant increase in same charge mutual repulsion, which means that PVA can only undergo adsorption through the formation of hydrogen bonds by hydroxyl groups occurring in a high pH solution.

Despite the benefits of alkaline pH, such as low toxicity and the potential of reuse, the alkaline environment still affects lipid extraction and the fatty acids profile (Fraud et al., 2018). Changing the conditions of the adsorption process impacts the macromolecular compound-binding mechanism. Additionally, the stability mechanism was precisely determined due to the application of the immobilization method. A higher molecular weight is likely to be nonselective, significantly contributing to the adhesion between microalgae. It can be successful for industrial microalgae strains [32], but also in the case of removing harmful microalgae [5,36].

3.4. Fe$_3$O$_4$/PS Reusability

Figure 5 shows the reusability of Fe$_3$O$_4$/PS. The harvesting efficiency of Nannochloropsis oculata was established under conditions of a pH of 5. The separation process of Fe$_3$O$_4$/PS from the solution was conducted with an external magnet after reaction for 180 min. Afterwards, Fe$_3$O$_4$/PS was washed three times with diluted water and then reused. The method of Fe$_3$O$_4$/PS detachment between algae cells and magnetic particles was carried out without adjusting the pH condition. A harvesting efficiency of 96% was achieved with the original Fe$_3$O$_4$/PS, which was reduced to 80% after five recycles. Fe$_3$O$_4$ immobilization within PVA and SA has advantages such as easy separation and no limits in aerobic conditions. Wang et al. [12] indicated that the zeta potential of the natural polymer Fe$_3$O$_4$ (Fe$_3$O$_4$–PP) insignificantly changed after recycling the microalgae harvested five times. This result is supportive of the advantageous properties of an immobilization technique for application in microalgae harvesting.
Figure 5. Nannochloropsis oculata harvesting efficiency (with an initial concentration of 500 mg/L) at different Fe$_3$O$_4$/PS recycles. Results are in the form of average ± standard deviation.

In addition, flocculants cannot hinder downstream bioprocesses, and metallic nanoparticles can negatively impact usability. Zhu et al. [3] suggested that pH adjustment for the separation of naked Fe$_3$O$_4$ has a negative impact, due to the decreased efficiency of microalgal biomass. However, the algae organic matter released by algal cells contain amounts of carbohydrates, nitrogen source compounds, and various organic acids [37,38], which are actively excreted during microalgae growth. Therefore, the effect of a decrease of the efficiency of the harvesting process by organic algae matter on flocculant surface structure should be investigated in future studies [12].

Overall, Fe$_3$O$_4$/PS can reduce the risk of environmental damage associated with the application of nanomaterials.

4. Conclusions

Nano Fe$_3$O$_4$, immobilized in polyvinyl alcohol (PVA)/sodium alginate (SA), was successfully applied as a flocculant for magnetophoretic harvesting in this study. The Fe$_3$O$_4$/PS, with a larger size than naked Fe$_3$O$_4$, can be easily recovered from the microalgae harvesting process without the need to adjust the pH for separation. The concentration of soluble ion Fe$^{2+}$ indicated that naked Fe$_3$O$_4$ is unstable at an acidic pH value, and that microalgae harvesting with Fe$_3$O$_4$/PS could be a suitable method to overcome soluble ion Fe$^{2+}$ release. The reusability of Fe$_3$O$_4$/PS was successfully achieved by recycling during 5 cycles. To make it more attractive and understand the relationship between harvesting efficiency and high concentrations of microalgal cells, an analysis of the outdoor scale performance and harvesting efficiency when using Fe$_3$O$_4$/PS will be conducted in a future study.

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