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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 Fe_3O_4 immobilized in polyvinyl alcohol (PVA)/sodium alginate (SA) as a flocculant (Fe_3O_4/PS) is investigated. Using the Fe_3O_4/PS immobilized beads could reduce the amount of soluble ferrous ions (Fe^{2+}) released from naked Fe_3O_4 in acid treatment, leading to easy recovery. The characterization was performed under different dosages and pH values of Fe_3O_4/PS. The results show that the Fe_3O_4/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. Fe_3O_4/PS can be directly reused without adjusting the pH value. The recycled Fe_3O_4/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 (Fe_3O_4) and iron yttrium oxide ($Y_3Fe_5O_{12}$) 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 (Fe_3O_4) are widely used as a reusable adsorbent/flocculant for wastewater treatment [14,15]. Especially ferrous ions (Fe^{2+}) of Fe_3O_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 Fe^{2+} ions have an impact on cytotoxicity for algae and cell growth inhibition [17,18].

The immobilization of iron oxide (Fe_3O_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 Fe_3O_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 Fe_3O_4/PS particles through a mixed PVA and SA solution embedded with iron oxide (Fe_3O_4), with boric acid and phosphate as a spherical solution. Fe_3O_4/PS has a high mechanical performance and performs well regarding magnetic separation for algae harvesting. Batch experiments on Fe^{2+} ions released from an acidic environment, their dosage, different pH value conditions, and contact time were conducted, and the reusability of Fe_3O_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 ± 0.7 °C under continuous illumination by a fluorescent lamp (26 W, EFHS26L-GE, China Electric, Hsinchu, Taiwan) with a light intensity of 67.5 µmol protons m^{-2} 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 °C for 24 h. The microalgae biomass concentration was estimated using Equation (1):

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

where biomass is in milligrams per liter (mg/L) and OD_f is the supernatant value.

2.2. Fe_3O_4/PS Preparation

Naked iron oxide Fe_3O_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 °C, for 20 min. Then, the naked Fe_3O_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% KH_2PO_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 ± 0.15 mm.

2.3. Characterization Analysis

The soluble iron Fe^{2+} concentration of Fe_3O_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_3O_4/PS and naked Fe_3O_4 was determined by using a digital biological microscope (DMWB1-223, Motic, Xiaman, 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_3O_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 ± 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_3O_4 were 20, 40, 60, 80, 100, and 120 mg/L, respectively. The dosage of Fe_3O_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_3O_4), respectively. Both flocculants, as mixtures, were rotated using stirrers at 300 rpm for 1 min in the algae medium. Afterwards, a 10 × 10 × 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):

$$\text{Harvesting efficiency }(\%) = \frac{(OD_i - OD_f)}{OD_i} \times 100 \tag{2}$$

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_3O_4/PS was harvested, the supernatant was carefully discarded with pipettes, and the Fe_3O_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_3O_4 was mixed in the NaOH solution in vials, and the pH value was adjusted to 10. The mixture of algae and naked Fe_3O_4 was rotationally stirred at 500 rpm for 1 min, after which, the magnet was placed under the vials to collect the naked Fe_3O_4. The adsorbed algal biomass was separated from the detached naked Fe_3O_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_3O_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_3O_4 and Fe_3O_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. Fe_3O_4/PS Characteristics

The soluble iron Fe^{2+} concentration of Fe_3O_4/PS and Fe_3O_4 under different pH values for 30 min is shown in Table 1. The concentration of dissolved ion Fe^{2+} of Fe_3O_4/PS was insignificant, since the pH value changed ($p > 0.05$). However, the soluble iron Fe^{2+} of Fe_3O_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 Fe oxides or oxyhydroxides in solution, as per Equation (3).

$$Fe_3O_4 + 2H^+ \rightarrow Fe_2O_3 + Fe^{2+} + H_2O \tag{3}$$

Table 1. Concentration of soluble iron Fe^{2+} of Fe_3O_4/PS and naked Fe_3O_4 under different pH values.

Condition	Concentration of Soluble Iron Fe^{2+} (mg/L)			
	pH 3	pH 4	pH 5	pH 6
Fe_3O_4/PS [1]	16 ± 0.02 [a]	15.9 ± 0.03 [a]	15.9 ± 0.03 [a]	15.9 ± 0.02 [a]
Naked Fe_3O_4 [2]	2220 ± 31 [d]	1077 ± 67 [c]	132 ± 8 [b]	22 ± 3 [a]

[1] The concentration of Fe_3O_4/PS calculates at 2.4 g/L (*w*/*w*). [2] The concentration of Fe_3O_4 was 120 mg/L. Means ± standard deviations. Different lowercase letters ([a], [b], [c], 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 Fe^{2+} is beneficial to microbial attachment [27], but high concentrations of Fe^{2+} could decrease microbial attachment [28]. Ren et al. [29] reported that Fe_3O_4, as a magnetic material, can successfully develop granules with the addition of Fe^{2+} and Fe_3O_4, which could decrease negative charges on bacterial attachment. Wang et al. [12] also determined a slight iron dissolution of naked Fe_3O_4 at pH 3.01. This result indicated that PVA/SA immobilization could protect Fe_3O_4 and avoid oxidization from the high amount of dissolved oxygen in the algae medium. Moreover, removing soluble iron Fe^{2+} from the algae harvesting process could require additional processes.

Figure 1a–c shows the surface morphology of the prepared Fe_3O_4/PS. Fe_3O_4/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; Figure 1a). Figure 1b shows the individual microsphere of Fe_3O_4/PS exhibiting paramagnetism (Figure 1c,d). Huang et al. [20] reported that the coercive force of the magnetization hysteresis loop of Fe_3O_4/PS was 5 emu/g, indicating that the Fe_3O_4/PS had a magnetic response and separation capability. Those results indicate that Fe_3O_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 *Nannochloropsis oculata* interaction, the zeta potential can determine electrostatic repulsion for microalgal cells and material. Figure 2 shows the zeta potential measurement of *N. oculata* and naked Fe_3O_4 under different pH values. They were found to maintain a predominantly negative surface charge (−3 to −11 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 *N. oculata* SAG 38.85 cultivated in an artificial seawater medium was −7 mV. Boli et al. [31] also determined marine microalga *Nannochloropsis oceanica* CCMP1779 and magnetic microparticles under different pH values, finding different surface charges. Fe_3O_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]. Prochazkova 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].

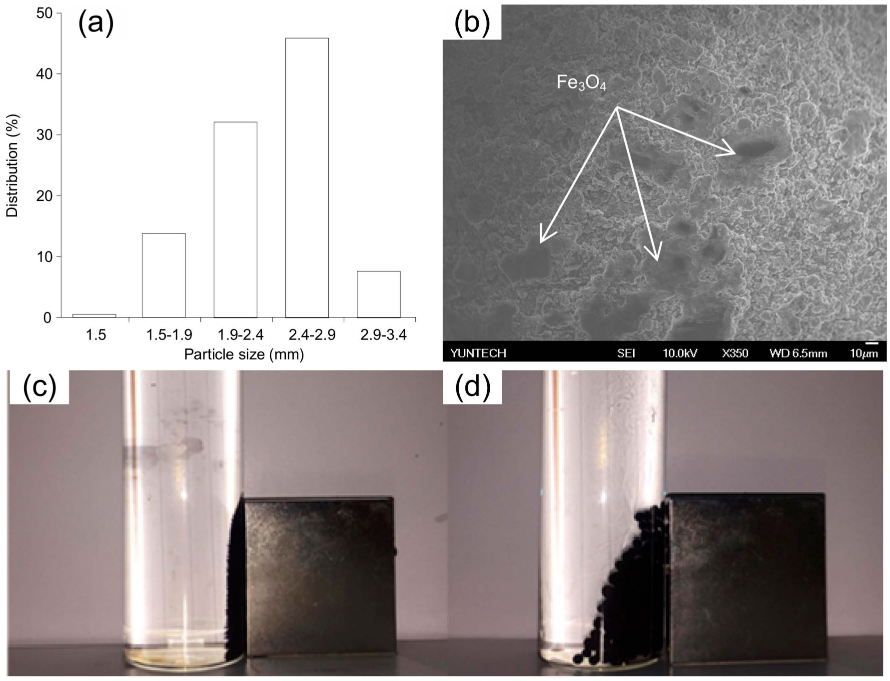

Figure 1. Surface morphology of prepared polyvinyl alcohol (PVA) and sodium alginate (SA)-coated Fe_3O_4 nanoparticles (Fe_3O_4/PS). (**a**) Size distribution (%) of Fe_3O_4/PS; (**b**) Iron oxide immobilized in Fe_3O_4/PS determined by field emission scanning electron microscopy (SEM). (**c**) Magnetic separation of naked Fe_3O_4 and (**d**) Fe_3O_4/PS attracted in water under a 200 mT magnet.

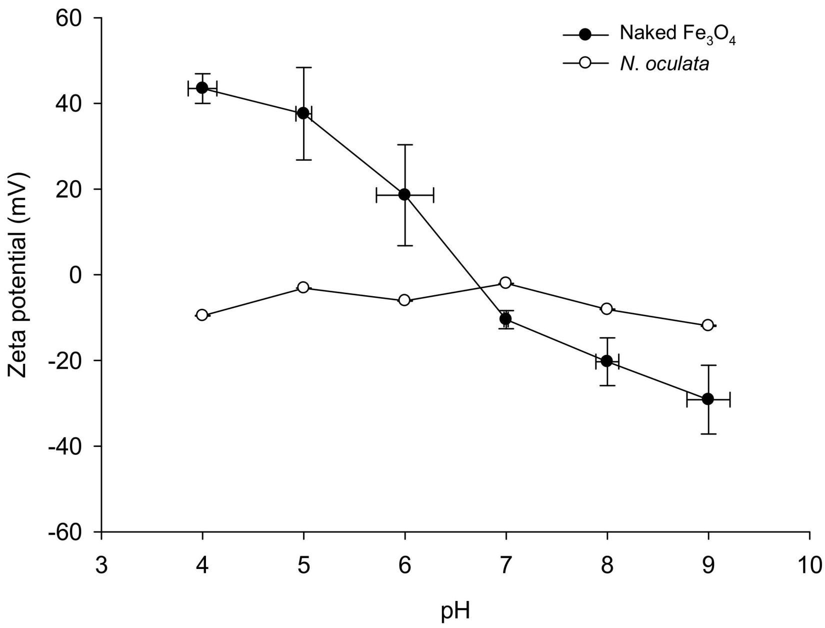

Figure 2. Zeta potentials of naked Fe_3O_4 and *Nannochloropsis oculata* as a function of pH.

3.2. Effects of Dosage Concentration on Magnetic Harvesting

The performance of *Nannochloropsis oculata* harvesting efficiency, using different dosages of Fe_3O_4/PS and naked Fe_3O_4, is shown in Figure 3. Harvesting efficiency reached 99 ± 0.7% and 92 ± 0.6% at the optimal dosage of naked Fe_3O_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_3O_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 Fe_3O_4 particles and also successfully flocculated oleaginous microalgae, reaching 93% efficiency at an optimal dosage of 25 g/L.

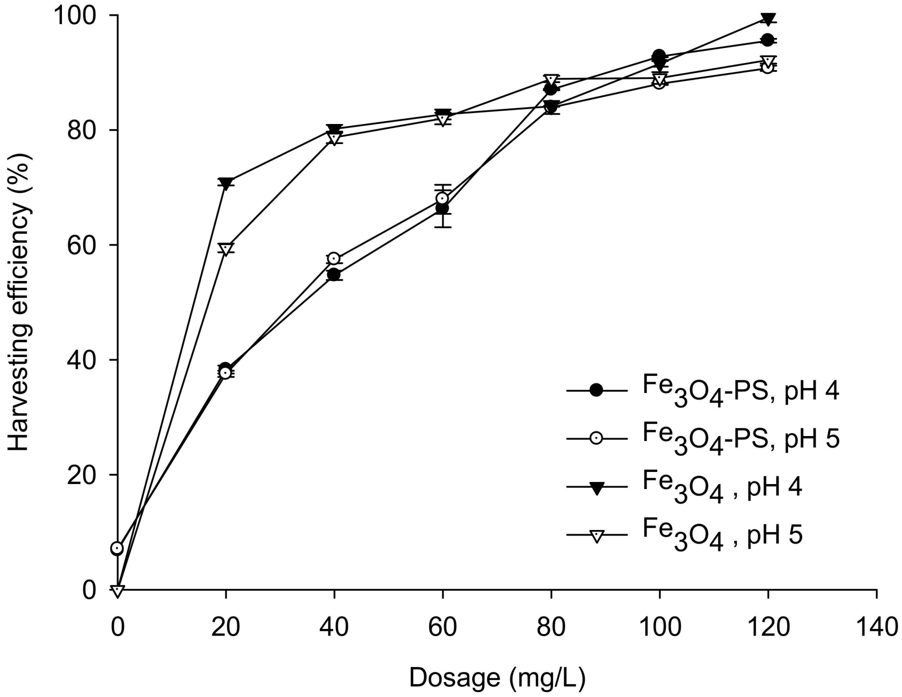

Figure 3. Comparison of different dosages of Fe_3O_4/PS and naked Fe_3O_4 on *Nannochloropsis oculata* harvesting efficiency with an initial concentration of 500 mg/L.

Dosage increments from 40 to 120 g/L of naked Fe_3O_4 reached > 80% harvesting efficiency for *N. oculata*. This is because the Fe^{2+} ions from the Fe_3O_4 detected in this study gradually increased with a decreasing pH value (Table 1). This could be ascribed to Fe^{2+} ions being able to potentially oxidize into Fe^{3+} ions in an acidic environment, thereby acting as flocculants [32]. The harvesting efficiency of Fe_3O_4/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 Fe_3O_4/PS had a positive effect on harvesting *N. oculata*. The coating of a polymer was sufficient to cover Fe_3O_4 surfaces and ensure the colloidal stability of Fe_3O_4/PS over a wide range of pH levels without oxidation [12]. These findings reveal the stability of Fe_3O_4/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 Fe_3O_4/PS under different pH values. The effectiveness of Fe_3O_4/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.

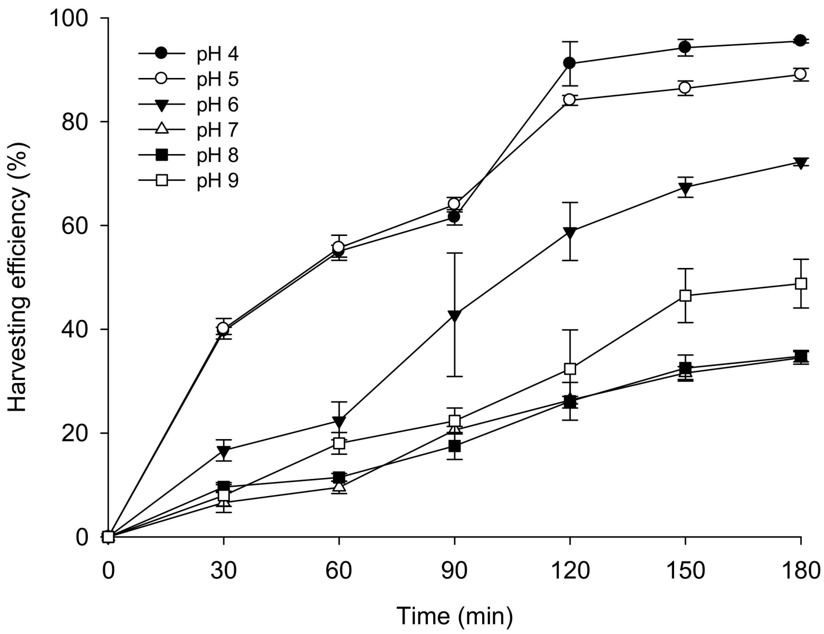

Figure 4. *Nannochloropsis oculata* harvesting efficiency (with initial concentration of 500 mg/L) at different Fe_3O_4/PS pH values. Results are in the form of average ± standard deviation.

Fe_3O_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 microalga *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 reusing the culture medium, 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_3O_4/PS Reusability

Figure 5 shows the reusability of Fe_3O_4/PS. The harvesting efficiency of *Nannochloropsis oculata* was established under conditions of a pH of 5. The separation process of Fe_3O_4/PS from the solution was conducted with an external magnet after reaction for 180 min. Afterwards, Fe_3O_4/PS was washed three times with diluted water and then reused. The method of Fe_3O_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_3O_4/PS, which was reduced to 80% after five recycles. Fe_3O_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_3O_4 (Fe_3O_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.

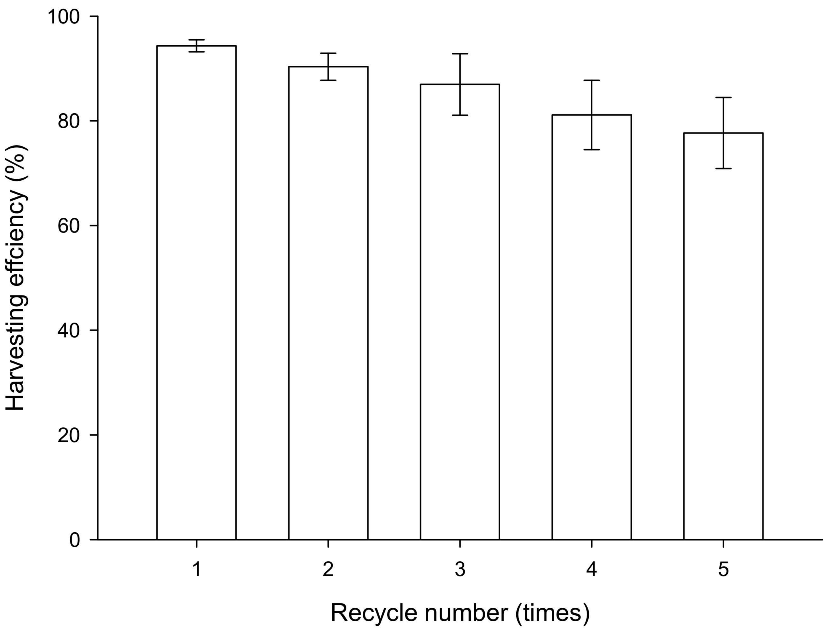

Figure 5. *Nannochloropsis oculata* harvesting efficiency (with an initial concentration of 500 mg/L) at different Fe_3O_4/PS recycles. Results are in the form of average ± standard deviation.

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_3O_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_3O_4/PS can reduce the risk of environmental damage associated with the application of nanomaterials.

4. Conclusions

Nano Fe_3O_4, immobilized in polyvinyl alcohol (PVA)/sodium alginate (SA), was successfully applied as a flocculant for magnetophoretic harvesting in this study. The Fe_3O_4/PS, with a larger size than naked Fe_3O_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_3O_4 is unstable at an acidic pH value, and that microalgae harvesting with Fe_3O_4/PS could be a suitable method to overcome soluble ion Fe^{2+} release. The reusability of Fe_3O_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_3O_4/PS will be conducted in a future study.

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