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Development of an Energy-Efficient Rapid Microalgal Cell-Harvesting Method Using Synthesized Magnetic Nanocomposites

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Abstract: Due to high consumption and non-renewable nature of fossil fuels, rapid development of potential renewable energies such as biofuel derived from microalgae is necessary for achieving the goals of sustainable growth and carbon neutrality. However, the high energy consumption required for microalgal biomass harvesting is regarded as a major obstacle for large-scale microalgal biofuel production. In the present study, the marine green microalgae *Tetraselmis* sp. was used to investigate a rapid and energy-efficient biomass collection method among different methods such as gravity sedimentation, auto-flocculation (at target pH), flocculation by polymers followed by magnetic separation, and centrifugation. The results showed that sufficient high cell densities of microalgae were obtained under the optimized growth conditions after 21 days of cultivation, and the microalgae could be easily flocculated and collected by magnetic separation using synthesized magnetic nanocomposites. The results also showed that among the different methods, magnetic separation was more efficient for biomass harvesting because of its simple and fast processing steps as well as low energy consumption. However, further investigation on different target microalgal species and their cultivation conditions, such as salinity and medium pH, will be required before application for large-scale biofuel production in the future.

Keywords: microalgal biofuel production; magnetic nanoparticles; auto-flocculation; biofuel downstream processes; magnetic separation; effective biomass harvesting

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1. Introduction

Increasing global energy consumption for urban development has generated a vast amount of carbon dioxide emissions over the decades, resulting in unusual climate changes [1]. Renewable energies such as solar energy, wind energy, and biofuel have been developed and adopted for applications to achieve sustainable growth and development in the future. Recently, microalgae-based biofuel has been proposed as a promising option for generating clean energy [1,2]. The advantages of using microalgae for biofuel production include its simple cultivation conditions and faster growth rate versus other plant species. Compared to other crop species, the higher CO_2-capturing ability of microalgae can remove more CO_2 from the environment. The microalgal lipid production yield is the highest for which more microalgae-based biofuel can be produced [3–5]. In addition, microalgal biofuel contains a lower content of nitrogen and sulfur than that of traditional fossil fuels; thus, both nitrogen oxide and sulfur oxide emissions can be significantly reduced [5,6]. Moreover, microalgae have been applied for wastewater treatment to remove nutrients [7,8], and they can be cultivated to produce food supplements with high commercial value [9,10]. However, their diluted cell densities (4–10 g/L) and small cell size (2–20 μm) properties have resulted in low efficiency of their biomass separation [11].

Additionally, their negative cell surface charge and high neutral buoyancy due to their high lipid content significantly increase the difficulty of microalgal biomass collection using conventional methods such as gravity sedimentation (>3 h), disc-stack centrifugation (25–30 min), and cross-flow membrane filtration. As a result, slow processing, high energy consumption, and frequent maintenance of essential collection equipment such as centrifuges and filters have been reported [12,13]. Furthermore, the cells of certain marine microalgal species such as *Dunaliella salina* possess a very high lipid content, which has been reported to have high sensitivity against both centrifugal and compression forces. As a result, cell rupture and the release of significant amounts of cell components to the spent medium have been observed after centrifugation, and so extraction or post-concentration processes would be required. Eventually, the biofuel production yield is significantly affected [14].

In general, 20–30% of the overall energy consumption has been reported among different microalgal cell harvesting methods [11,12]. In order to increase the possibility of large-scale microalgal biofuel production, this high energy consumption for their biomass harvesting should be significantly reduced [15,16]. In fact, the microalgal cell surface is negatively charged due to the presence of carboxyl and hydroxyl groups. An alternative approach has been proposed for their biomass collection. For example, charge neutralization is conducted by adding chemical flocculants. The aggregated microalgal cell clusters can be easily collected by gravity [16,17]. Past studies have indicated that inexpensive and common flocculants such as cationic polymers (e.g., polyethylenimine (PEI), chitosan, poly(diallyldimethylammonium chloride)) can be used to achieve a higher separation efficiency for microalgal cells. Previous results showed that cationic polyelectrolytes were very effective at low dosages applied for flocculation (i.e., 5–20 mg/L), so that the treatment cost and energy consumption required per unit mass of microalgal cell collection could be reduced [18–20]. However, chemical flocculants mixed with the biomass collected would significantly affect the lipid extraction and reduce the production yield of biofuel [15,20]. In addition, in a case in which significant amounts of residual chemical flocculants could not be recovered, this resulted in serious environmental contamination after their disposal. It also increased both reagent and wastewater treatment costs [20]. Therefore, an effective recovery method of chemical flocculants should be developed for their reuse. An example is the immobilization of the flocculant followed by a magnetic separation process [21,22]. In addition, the spent culture medium can be easily reused for next batch of cultivation after rapid biomass separation, and thus the overall energy and water footprint can be significantly reduced [23]. In this study, a marine green microalgal species, *Tetraselmis* sp., was used as the target species to investigate the microalgal biomass harvesting efficiency and time required for different commonly used or newly proposed collection methods, including sedimentation, auto-flocuulation, centrifugation, and flocculation by magnetic nanocompsites.

2. Materials and Methods

2.1. Cultivation of Microalgae Species

Deionized water (dI H_2O) was used in the following experiments (Milli-Q water system, Millipore, MA, USA). Stock culture of marine green microalgae *Tetraselmis* sp. was obtained from the commercial company TCK Aquarium Company Ltd. (Ping Tung, Taiwan). The cultivation was conducted in a 250 mL flask with 100 mL modified BG-11 medium in the presence of artificial sea salt (Instant Ocean®). Two different concentrations of artificial sea salts (10‰ vs. 33‰) were prepared to investigate the effect of salinity towards the cell growth and cell harvesting process. Both 10‰ and 33‰ artificial sea salt contained 1200 mg/L magnesium, 350 mg/L potassium, and 400 mg/L calcium ions. The medium was autoclaved (Hirayama HVE-50, Japan) for 15 min at 121 °C, and 20 mM filter-sterilized phosphate was added into the medium after autoclaving to prevent precipitation. The pH of the medium was adjusted to 7.8–8.0 using 1 M HCl and 1 M NaOH. The light intensity of the incubating shaker (Yihder Technology LM-2530RD, Taiwan) was fixed at a light irra-

diance of 4000 lux, and the light–dark cycle was programmed at 14:10 (light: dark). Further, the temperature and shaking speed were set at 23 °C and 150 rpm, respectively. Gaseous exchange from surroundings was allowed by using sealing plastic film (0.22 µm filter) to prevent contamination. Cell densities were monitored every 2 or 3 days by direct cell count using a haemocytometer, and the measurement of the absorbance signal (A600nm) of the cell culture was conducted using spectrophotometer (Shimadzu UV1800, Japan) [5]. Due to the limited linearity of the signal (A600nm), samples exceeding the limit (i.e., >0.8) were diluted with blank medium. The undiluted cell densities can be calculated by multiplying the dilution factor.

2.2. Synthesis of Bare Iron Oxide and Citrate-Coated Iron Oxide

An amount of 10.8 g of $FeCl_3.6H_2O$ and 4.0 g $FeCl_2.4H_2O$ were dissolved in 25 mL 0.8 M HCl and later added with dI H_2O into 50 mL. Bare iron oxide (bare Fe_3O_4) was prepared by dropwise addition (6 mL/min) of the above iron solution into a 1 L three-neck flask containing 500 mL 1 M NH_3 solution (alkaline co-precipitation), and the flask was purged with N_2 gas to prevent oxidation and mechanically stirred (DLAB Scientific Inc, OS40-PRO, CA, USA) at 400 rpm under room temperature. The mixture was stirred for 30 min, and the bare iron oxide synthesized was collected by magnetic separation (200 mT permanent magnet) and washed with 500 mL dI H_2O 4 times. The bare iron oxide solution was kept in 125 mL dI H_2O (36–39 g/L bare Fe_3O_4). An amount of 1.828 g of citric acid monohydrate and 2.145 g trisodium citrate dihydrate were dissolved in the above solution of bare iron oxide. The solution mixture was transferred into a 1 L three-neck flask, purging with N_2 and 400 rpm mechanical stirring in an 80 °C oil bath. The citrate-coating process on bare iron oxide was maintained for 1.5 h, and then it was cooled down to room temperature. A total of 500 mL acetone was added to the cooled mixture to facilitate the magnetic separation of the citrate-coated iron oxide. The citrate-coated iron oxide was collected by magnetic separation and washed with 150 mL of 95% ethanol four times and then further washed with dI H_2O four times to remove the ethanol. The citrate-coated iron oxide was dispersed in 120 mL dI H_2O (named citrate-80C-Fe_3O_4) [24].

2.3. Synthesis of Amine-Functionalized Iron Oxide

A total of 500 mL of solution containing 0.5 wt% of the above citrate-coated iron oxide was prepared. The pH of the solution was adjusted to 11.5 by 1 M KOH. Then, 1.5 mL TEOS (~0.4 g SiO_2, 6.7 mmol Si) was added into the solution, and the reaction was maintained for 3 h at room temperature with 1200 rpm mechanical stirring. The solution was heated to 60 °C, and 8 mmol of 3-aminopropyltrimethoxysilane (97%) (1.44 mL) was added into the mixture and further reacted for 2 h. The mixture was cooled down to room temperature with an ice bath. The functionalized iron oxide was collected by magnetic separation and washed with 200 mL dI H_2O four times. The product was redispersed in 50 mL dI H_2O (named NH_2-Fe_3O_4) [25,26].

2.4. Synthesis of (Polydiallyldimethylammonium Chloride) PDDA-Coated Iron Oxide

The above bare iron oxide solution obtained (mentioned in 2.2) was pre-concentrated with a 200 mT permanent magnet to 80 g/L concentration, then 15 mL of the bare iron oxide solution (~80 g/L) was transferred into 50 mL plastic tube. An amount of 1.83 g of citric acid monohydrate and 2.15 g trisodium citrate dihydrate were added into the plastic tube. The coating process was conducted by shaking at 150 rpm for 20 h at room temperature (RT), then the citrate-coated RT iron oxide was collected with a permanent magnet, and the supernatant was discarded. The mixture was repeatedly washed with 30 mL dI H_2O 3 times to remove the unbound citrate. The citrate-coated RT iron oxide (~40 g/L iron oxide) was transferred into a 50 mL plastic tube in the presence of NaCl (0.5 M), and 15 mL of the final volume of a PDDA (100–200 K, 20 wt%) (20 g/L) mixture was kept by adding dI H_2O. The mixture was shaken at 150 rpm for 1 h at room temperature, after which the polymer-coated iron oxide was collected with a permanent magnet, and

the supernatant was discarded. The mixture was repeatedly washed with 30 mL dI H_2O 3 times to remove the unbound reagents. The final product was redispersed in 15 mL dI H_2O (named PDDA-Fe_3O_4) [27–29].

2.5. Characterization of Synthesized Magnetic Nanoparticles

The magnetic hysteresis loops analysis of synthesized magnetic nanoparticles was conducted using a vibrating sample magnetometer (VSM) (Lake Shore Cryotronics, Inc., VSM7307, OH, USA) at room temperature. Zeta potential analysis of different nanoparticles at a selected pH (i.e., 7.5–8.0) was conducted with a Zetasizer (Malvern Panalytical Ltd., Nano ZS90, UK). Transmission electron microscopy (TEM) images of synthesized magnetic nanoparticles were performed with a JEOL JEM 2010F electron microscopy (JEOL USA, Inc., MA, USA).

2.6. Microalgal Cell Harvesting Process

A total of 5 mL cell culture of *Tetraselmis* sp. with the highest cell densities (after 21 days of cultivation) were transferred into different plastic tubes (15 mL). Their initial cell densities before the harvesting step were first measured at A600nm. Different amounts of magnetic flocculants (in terms of Fe_3O_4 mass) such as bare iron oxide (8 mg of Fe_3O_4), citrate-coated iron oxide (16 mg of citrate-80C-Fe_3O_4), NH_2-coated iron oxide (16 mg of NH_2-Fe_3O_4), and PDDA-coated iron oxide (8 mg of PDDA-Fe_3O_4) were added into different tubes to flocculate the microalgal cells. The initial pH of sample mixtures after the addition of magnetic nanoparticles was about 8.7–9.0. The tubes were shaken at 150 rpm for 2 min and then underwent magnetic separation using a permanent magnet (200 mT) placed next to the plastic tubes for 30–120 s. The final cell densities of the supernatant (0.5 to 1 mL upper portions of the mixture) were measured at A600nm to determine the cell harvesting efficiency (Equation (1)).

$$\text{Cell harvesting efficiency } (\%) = \frac{\text{Initial A600nm} - \text{Final A600nm}}{\text{Initial A600nm}} \times 100\% \quad (1)$$

A control experiment was conducted by directly settling 5 mL cell culture (no magnetic flocculant) for 30 min and 18 h to investigate the cell harvesting efficiency by gravity sedimentation. Similarly, auto-flocculation (no magnetic flocculant) was conducted by adjusting the 5 mL cell culture into different pH conditions, i.e., 3, 5, 7, 10, and 11.5, by 1 M HCl or 1 M NaOH. The tubes were subjected to direct settling for 30 min and 18 h. Furthermore, the effect of salinity (10‰ vs. 33‰) regarding cell sedimentation and auto-flocculation was also investigated. Finally, 5 mL cell culture was directly centrifuged (Hermle Z206A, Germany) at 6000 rpm for 3 min to compare the results with different cell collection methods.

3. Results

3.1. Magnetic Nanoparticle Properties

Figure 1 shows the TEM image and particle size distribution of the synthesized nanoparticles. The diameter of bare Fe_3O_4, NH_2-Fe_3O_4, and citrate-80C-Fe_3O_4 nanoparticles was observed to be 12.95 ± 3.10 nm, 12.28 ± 3.03 nm, and 12.09 ± 3.08 nm, respectively. The nanoparticles were observed to be spherical to slightly irregular shapes without a significant change after their surface coating (the results of PDDA-coated iron oxide are not shown in the present study). Figure 2 shows the saturation magnetization of the above three synthesized nanoparticles (the results of PDDA-coated iron oxide are not shown in the present study). The initial saturation magnetization of bare iron oxide was 86.3 emu/g. Upon coating with non-magnetic citrate or amine groups, it dropped to 73 emu/g. However, a high superparamagnetic property was still observed from those surface-coated nanoparticles, so that rapid magnetic separation could be achieved after they were flocculated with the microalgal cells.

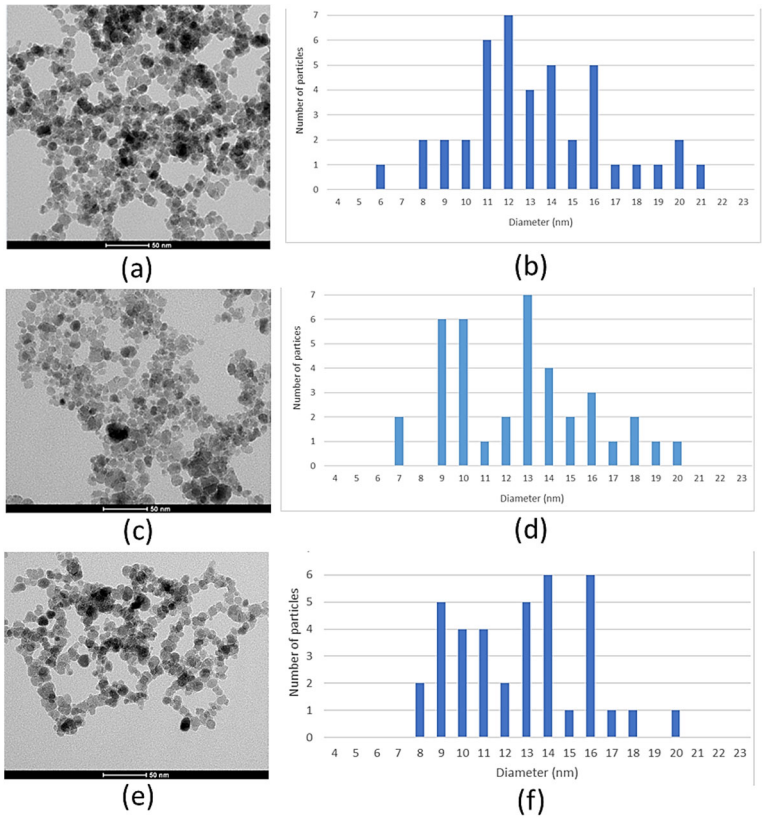


Figure 1. (**a**) TEM image of bare Fe_3O_4, (**b**) particle size distribution of bare Fe_3O_4, (**c**) TEM image of NH_2-Fe_3O_4, (**d**) particle size distribution of NH_2-Fe_3O_4, (**e**) TEM image of citrate-80C-Fe_3O_4, and (**f**) particle size distribution of citrate-80C-Fe_3O_4.

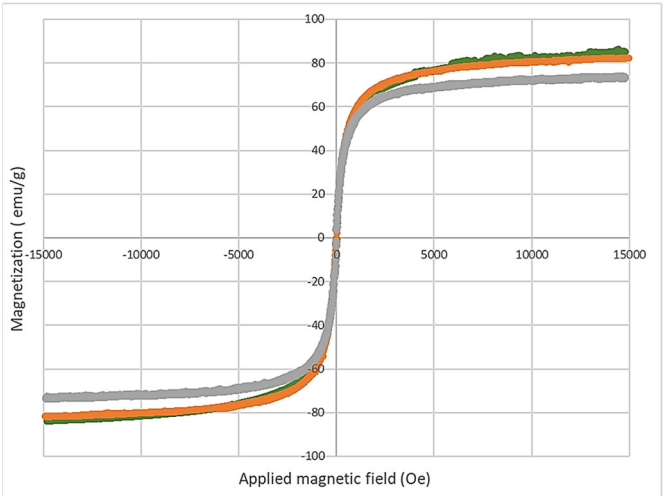


Figure 2. Magnetization loop of bare Fe_3O_4 (green), citrate-80C-Fe_3O_4 (orange), and NH_2-Fe_3O_4 (grey).

3.2. Microalgal Cell Collection by Sedimentation and Auto-Flocculation

After 21 days of cultivation, the highest cell densities of the marine green microalgae *Tetraselmis* sp. were shown and estimated in ~1.3×10^7 cells/mL. In addition, the pH of the cell culture medium was changed from 7.8 to 10.1–10.2. Table 1 shows the cell harvesting efficiency of the microalgae after 30 min gravity sedimentation (direct settling without adding flocculant); ~37% of cells can be collected in both 10‰ and 33‰ salinity conditions. Prolonged sedimentation (i.e., 18 h) further increased the efficiencies to ~94% and ~82% in 10‰ and 33‰ salinities, respectively. Although no significant effect of medium salts

was shown in the gravity sedimentation, and the treatment cost would be relatively low, it required a very long processing time (i.e., 18 h) and an additional large treatment tank, which would be a barrier to scale up the process for industrial application.

Table 1. Cell harvesting efficiency under different salinity conditions using sedimentation.

Salinity	Settling Time	Initial A600nm	Final A600nm	Cell Harvesting Efficiency (%)
10‰ (pH 10.13)	0.5 h	3.888	2.451	36.96%
33‰ (pH10.21)		3.835	2.389	37.71%
10‰ (pH 10.13)	18 h	3.888	0.236	93.93%
33‰ (pH 10.21)		3.835	0.687	82.09%

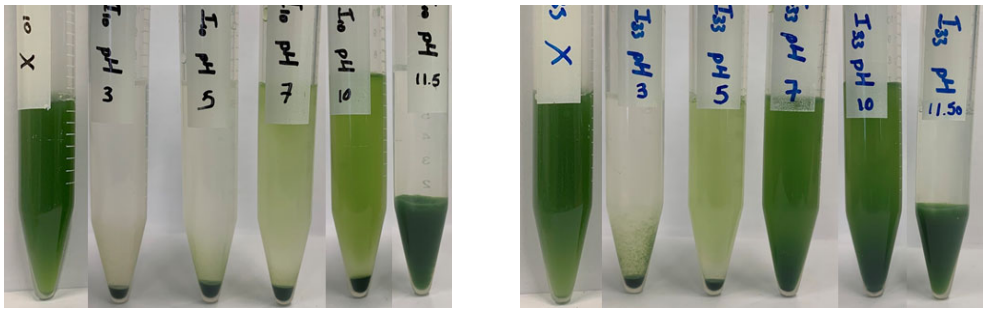
Auto-flocculation of microalgal cells was conducted by adjusting the samples' culture into different pH conditions followed by the sedimentation process. Table 2 shows the effects of salinity and different pH conditions on the cell settling process after 30 min and 18 h of auto-flocculation, respectively. A higher cell harvesting efficiency (i.e., ~40–65%) can be achieved at a relatively low pH (i.e., pH 3–5) and low salinity (i.e., 10‰) conditions within a short period of the sedimentation process (i.e., 30 min).

Table 2. Cell harvesting efficiency at different pH conditions used for auto-flocculation.

Salinity	pH Adjusted	Settling Time	Initial A600nm	Final A600nm	Cell Harvesting Efficiency (%)
10‰	3	0.5 h	3.917	1.374	64.92%
33‰	3		3.767	2.600	30.98%
10‰	5		4.002	2.377	40.60%
33‰	5		4.022	2.581	35.83%
10‰	7		3.996	2.468	38.24%
33‰	7		4.014	2.666	33.58%
10‰	10		4.016	2.525	37.13%
33‰	10		4.008	2.716	32.23%
10‰	11.5		4.241	3.540	16.53%
33‰	11.5		4.036	3.391	15.98%
10‰	3	18 h	3.917	0.015	99.61%
33‰	3		3.767	0.126	96.66%
10‰	5		4.002	0.096	97.60%
33‰	5		4.022	0.159	96.05%
10‰	7		3.996	0.141	96.47%
33‰	7		4.014	0.949	76.36%
10‰	10		4.016	0.505	87.43%
33‰	10		4.008	0.877	78.12%
10‰	11.5		4.241	0.003	99.93%
33‰	11.5		4.036	0.009	99.78%

On the other hand, in the presence of high salt content (i.e., 33‰), when the cell culture was kept in neutral to alkaline pH conditions (i.e., pH 11.5), a relatively low cell harvesting efficiency was observed due to the medium salt interference (i.e., ~16–37%). Compared to sedimentation alone (Table 1), the results indicated that a slightly higher

harvesting efficiency can be achieved (i.e., >76–99%) using auto-flocculation. However, a long processing time was still required (i.e., 18 h). Figure 3 shows the sample tubes after 18 h auto-flocculation, followed by sedimentation at different pH and salinity conditions.



(**a**) (**b**)

Figure 3. Comparison of the effects of different pH and salinity conditions on 18 h auto-flocculation and sedimentation process for *Tetraselmis* sp.: (**a**) 10‰ salinity medium, (**b**) 33‰ salinity medium (tubes from left to right: initial 5 mL cell culture at start, pH 3, pH 5, pH 7, pH 10, pH 11.5).

3.3. Microalgal Cell Collection by Flocculation and Magnetic Separation of Nanoparticles

Four different synthesized magnetic nanoparticles, i.e., bare Fe_3O_4, NH_2-Fe_3O_4, citrate-80C-Fe_3O_4, and PDDA-coated Fe_3O_4, were tested and compared for the microalgal cell flocculation and magnetic separation processes. Table 3 summarizes the results of flocculation and magnetic separation after 2 min gentle shaking at an initial pH 8.7–9.0 under different salinity conditions (i.e., 10‰ and 33‰).

Table 3. Flocculation and magnetic separation of microalgal cells under different salinities.

Magnetic Nanoparticles	Salinity	Magnetic Nanoparticles Added (mL)	Magnetic Separation Time (s)	Initial A600nm	Final A600nm	Cell Harvesting Efficiency (%)
Control (5 mL cells culture alone)	10‰	/	/	4.089	4.389	/
Control (5 mL cells culture alone)	33‰	/	/	3.881	3.881	/
8 mg Bare Fe_3O_4	10‰	0.106	45	4.089	3.066	25.02
8 mg Bare Fe_3O_4	33‰	0.106	45	3.881	3.182	18.01
16 mg NH_2-Fe_3O_4	10‰	0.205	45	4.089	2.395	41.43
16 mg NH_2-Fe_3O_4	33‰	0.205	45	3.881	2.630	32.23
16 mg citrate-80C-Fe_3O_4	10‰	0.125	45	4.089	1.632	60.09
16 mg citrate-80C-Fe_3O_4	33‰	0.125	45	3.881	1.854	52.23
8 mg PDDA-Fe_3O_4	10‰	0.127	45	4.089	0.136	96.67
8 mg PDDA-Fe_3O_4	33‰	0.127	45	3.881	1.797	53.70

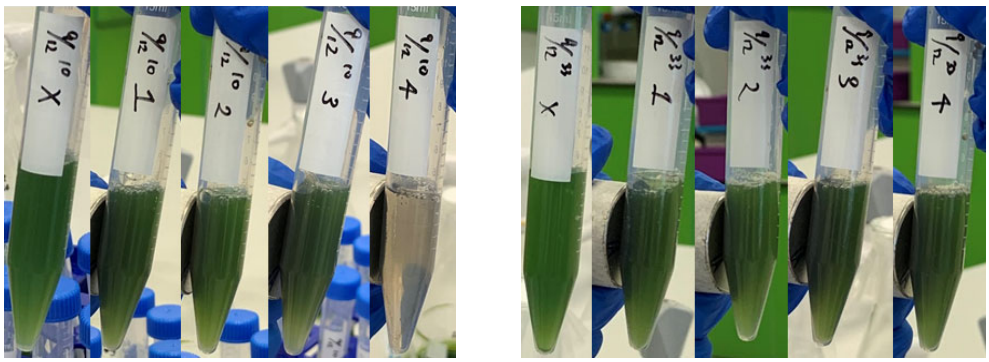
The results showed that in the presence of 10‰ salinity, PDDA-Fe_3O_4 achieved 96.67% cell harvesting efficiency after 45 s of magnetic separation. Citrate-80C-Fe_3O_4 and NH_2-Fe_3O_4 both showed a lower cell harvesting efficiency (i.e., 41–60%), and bare iron oxide showed a very poor result (i.e., 25%). A higher salinity (i.e., 33‰) caused a significant drop in performance in PDDA-Fe_3O_4 (i.e., 53.7%), and the three other types of nanoparticles were slightly affected. Table 4 shows the preliminary trials of the zeta potential of different magnetic nanoparticles conducted at pH ~7.5–8.1 without adding the medium salts. The results indicated that both bare Fe_3O_4 and citrate-80C-Fe_3O_4 were negatively charged (−19.3 to −21.5 mV), whereas both NH_2-Fe_3O_4 and PDDA-Fe_3O_4 were positively

charged (21.3 to 36.8 mV). Therefore, a higher cell flocculation performance can be expected in both NH_2-Fe_3O_4 and PDDA-Fe_3O_4, as the microalgal cell surface was predominantly negatively charged [17].

Table 4. Zeta potential of different magnetic nanoparticles.

Type of Magnetic Nanoparticles	Zeta Potential (mV)	pH
Bare Fe_3O_4	−19.3	8.03
NH_2-Fe_3O_4	21.3	7.90
citrate-80C-Fe_3O_4	−21.5	8.11
PDDA-Fe_3O_4	36.9	7.45

Figure 4 demonstrates a simple setup using a permanent magnet (200 mT) to provide an external magnetic field for conducting the magnetic separation process. By comparing to auto-flocculation and sedimentation, effective microalgal cell flocculation (2 min) and rapid magnetic separation (45 s) can be achieved within several minutes using PDDA-coated Fe_3O_4. As a result, the processing time, treatment tank volume, and energy requirement can be significantly reduced. On the other hand, direct centrifugation of cell culture samples (5 mL) at 6000 rpm was conducted for comparison. Results showed that almost 99.9% of the cell harvesting efficiency was easily achieved after 3 min of processing without showing negative impacts due to pH effect or salinity interference. However, this is highly energy consuming and may not be practical for handling a large volume of samples [11,12].



(**a**) (**b**)

Figure 4. Comparison of different synthesized magnetic nanoparticles and salinity effects on magnetic separation (45 s) for *Tetraselmis* sp.: (**a**) 10‰ salinity medium, (**b**) 33‰ salinity medium (tubes from left to right: control (5 mL cell culture at start), bare Fe_3O_4, NH_2-Fe_3O_4, citrate-80C-Fe_3O_4, PDDA-Fe_3O_4).

4. Discussion

The optimal conditions required for microalgal cultivation should first be investigated before the real application. This is because some parameters, such as pH and salinity, may significantly affect the microalgal growth and the downstream cell harvesting efficiency of biofuel production. For example, when using a marine microalgal species, a high salinity level may be required for their optimal growth, and the freshwater footprint for cell cultivation can be significantly reduced or may even be unnecessary. On the other hand, the adverse effects of high salt content must be addressed, because using the flocculation process may greatly inhibit the cell harvesting, and thus a low salinity medium would be selected. In this study, the effect of 10‰ and 33‰ salinities were investigated for the selected marine green microalgae *Tetraselmis* sp. A past study has shown that this microalgae has a high lipid content and fast growth rate; it is regarded as a potential species that can be used for biofuel production [30]. In addition, this species can also tolerate a wide range of salinities, so a more flexible design could be used for their cultivation [30]. The results showed that *Tetraselmis* sp. can grow well in both salinity levels for 21 days of

cultivation; thus, it provides an opportunity for saving the consumption of freshwater by the direct usage of filtered marine seawater for large-scale application. On the other hand, 10‰ salinity of seawater (diluted by freshwater) would be proposed for *Tetraselmis* sp. when using flocculation and magnetic separation for its cell harvesting, as this can reduce the negative impacts of such high salt content towards the processes. Moreover, the overall freshwater consumption in 10‰ salinity medium can be reduced, because the spent culture medium can be easily collected and reused after rapid magnetic separation.

Without using a buffer system, the pH value of the culture medium was increased to ~10.2 due to the uptake of CO_2 by microalgae for growth [31]. At a pH higher than 10, the essential elements such as calcium ions were precipitated into calcium phosphates or calcium carbonates, whereas magnesium ions were precipitated into magnesium hydroxide [32]. Past studies have indicated that the presence of a high content of calcium, magnesium, and phosphate ions induced flocculation at pH 11. Due to more positively charged precipitates being formed, electric neutralization was further facilitated. As a result, a higher cell flocculation efficiency could be achieved [16,32]. On the other hand, auto-flocculation of microalgae can also be induced by lowering the culture medium to a pH below 4 [33]. The carboxylic acid groups on the microalgal cell surface were protonated at acidic conditions, and then the surface charge of the microalgal cell became more neutral to enhance the cell flocculation process. Similar results were also observed in the present study. For example, 65% cell harvesting efficiency can be achieved at pH 3 and increased to 99.6% after 18 h of prolonged auto-flocculation.

Four different magnetic nanoparticles were synthesized to investigate their performances on cell flocculation and magnetic separation for *Tetraselmis* sp. All three magnetic nanoparticles were observed to have small particle size (<15 nm) and bore a high superparamagnetic property (86.3 to 73 emu/g) (data for PDDA-coated iron oxide is not shown in the present study). Such a small nanoparticle size and high superparamagnetic nature can facilitate both the microalgal cell flocculation and magnetic separation processes. PDDA-coated iron oxide was easily collected by a short period of magnetic separation, as shown in Figure 4a. In addition, it also showed the highest cell flocculation efficiency among the different magnetic nanoparticles because of its higher positively charged density (+36.9 mV at pH 7.45). In general, the negatively charged microalgal cells were stable in diluted medium [16,17]; the added positively charged flocculants such as PDDA-coated iron oxide can neutralize and destabilize the cell surface charge to allow for cell flocculation [17,20].

On the other hand, NH_2-Fe_3O_4 (zeta potential +21.3 mV at pH 7.90) showed a lower efficiency than PDDA-coated Fe_3O_4, which may be due to its lower positive surface charge density, resulting in less amounts of cell surface neutralization that hinder the cell flocculation. Citrate-80C-Fe_3O_4 (zeta potential −21.5 mV at pH 8.11) showed the usual high cell harvesting efficiency (i.e., 60%) versus NH_2-Fe_3O_4, and both citrate-coated nanoparticles and microalgal cells were observed to be negatively charged at pH8.7–9. A past study has also reported that the performance of cell flocculation may not be solely explained by charge neutralization theory, particularly in the presence of medium interferences [34]. Thus, further study would be required to investigate the details of the interactions. All newly synthesized magnetic flocculants cannot perform well in 33‰ salinity. Such a high salinity significantly increased the shielding of the electrostatic interaction between the magnetic flocculants and the microalgal cells, and thus it suppressed the charge neutralization as well as the flocculation process [17,34]. A larger amount of magnetic flocculants or a higher ratio of PDDA polymer coating on bare iron oxide would be suggested to improve the flocculation performance under such high salinity conditions. However, the processing time required for both flocculation (i.e., >30 min) and magnetic separation (i.e., 5–10 min) may be longer in order to achieve a higher cell harvesting efficiency (i.e., >95%). For *Tetraselmis* sp., the results showed that a flexible range of salinity, such as <10‰, would be suitable for both the microalgae's growth and cell harvesting using the magnetic flocculation method.

A practical harvesting method for microalgal cells should be designed for large-scale biofuel production. By comparing the results of the different methods for flocculation-magnetic separation, this newly proposed method can fulfill most of the requirements [35]. For example, the nanoparticle flocculation process can be easily conducted in a batch-wise operation mode using a small treatment reactor, and the magnetic separation can be designed using an online small magnetic separator for handling a large volume of flocculation mixture [36]. The cost for a large sedimentation tank can be saved. In addition, the electricity consumption for a large-scale centrifugation or filtration unit can also be prevented. In addition, the benefits of using this new method include simple design, rapid processing, and a low possibility of microalgal cell damage or contamination during the short processing period [20,33]. Further, the spent culture medium and magnetic flocculant can be easily collected for further reuse [21–23]. This can reduce the daily operational costs and prevent the discharge of reagents to the environment. Table 5 summarizes the advantages and disadvantages of different cell harvesting methods [20,35]. In the coming future, large-scale microalgal cultivation and an effective cell collection system would be required for more applications, such as carbon capture and utilization (CCU), biofuel production, and nutrient removal in tertiary wastewater treatment design [37–39]. This study can provide a new and alternative approach for conducting effective microalgal biomass collection.

Table 5. Comparison of different microalgal cell harvesting methods.

Cell Collection Methods	pH Effect	Salinity Effect	Processing Time	Cell Harvesting Efficiency	Energy Consumption
Sedimentation	No	No	Long	Low to moderate	Low
Auto-flocculation	Yes	Yes	Long	Moderate to high	Low
Centrifugation	No	No	Very short	Very high	High
Flocculation-magnetic separation	Yes	Yes	Very short	Very high	Low

5. Conclusions

Cationic polymer-coated magnetic nanoparticles and PDDA-iron oxide were successfully synthesized, and high performances of cell flocculation and magnetic separation were shown for the marine green microalgal species *Tetraselmis* sp. Salinity had a major effect in this flocculation-magnetic separation process. The optimal dosage between the magnetic flocculant to the microalgal cells and the amount of polymer coating on iron oxide, as well as different microalgal species, should be investigated. Gravity sedimentation and auto-flocculation processes were also compared, and the results showed that only an 82–94% cell harvesting efficiency can be achieved, and a longer processing time was also required compared to the flocculation-magnetic separation process.

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