Efficient harvesting of green microalgae cells by magnetic flocculated Fe_3O_4 nanoparticles combined with chitosan

Sifen Liu, Suping Fu, Zhongjie Wen, Xiang Wang, Tianjiu Jiang, Hongye Li*

(*Key Laboratory of Eutrophication and Red Tide Prevention of Guangdong Higher Education Institutes, College of Life Science and Technology, Jinan University, Guangzhou 510632, China*)

Abstract: Microalgae harvesting remains a challenging step in microalgae industrialization, thereby provoking the necessity to explore sustainable and economically feasible approaches. This research investigated the use of magnetic flocculated nanoparticles in the harvesting of the common microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. The results showed that magnetic flocculated nanoparticles efficiently adsorbed negatively charged microalgae cells, and a magnetic field could adsorb the magnetic flocculated nanoparticles, thereby harvesting the microalgae cells. Harvesting efficiency was remarkably increased at the optimum magnetic field strength of 0.5 T with the magnetic flocculated nanoparticles at 0.738 g/L, and microalgae broth at pH 9.0, whereas the recovery rates of both *C. pyrenoidosa* and *S. obliquus* were around 97% and the sedimentation speed of both was above 2.63 cm/min. This study exemplified the magnetic flocculated nanoparticles-based approach to effectively harvest the microalgae cells.

Keywords: magnetic flocculated nanoparticles, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, recovery rate, sedimentation speed

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1 Introduction

The increase in global populations and industrialization has led to higher energy consumption, resulting in an energy crisis on a global scale and climate change[1-2]. The ongoing depletion of finite conventional energy sources is unable to meet the rapidly increasing demand for energy; additionally, the extensive use of fossil fuels is one of the primary causes of global warming[3]. As a result, there is an urgent need to globally explore renewable, sustainable, and eco-friendly biofuels[4]. Among the potential biological feedstocks for biofuel production, microalgae have emerged as promising resources due to their characteristics, including rapid growth rate, feasibility, and not requiring arable land[5]. Furthermore, they are known to synthesize a diverse range of biochemicals with commercial and health implications, including polyunsaturated fatty acids, carotene, triacylglycerides, and polysaccharides[6-8]. Despite these advantageous characteristics, large-scale production of microalgae biochemicals in a commercially viable manner is still in its infancy due to various technological and biological hindrances. However, due to the slow autotrophic growth of microalgae, low biomass and technical limitations of algae harvesting, the development of the microalgae industry is extremely limited. To break through these limitations, it is urgent to increase the biomass of microalgae and to harvest microalgae efficiently. For harvesting microalgae, the existing flocculation techniques include auto-flocculation, bio-flocculation, chemical flocculation, particle-based flocculation, and electrochemical flocculation, each with its own strengths and weaknesses[9]. Among them, microalgae harvesting is regarded as the most challenging, expensive, and energy-intensive process. To overcome the existing bottlenecks, exploring alternative methods is of paramount importance[10-12].

Thanks to the adsorption and degradation capacity of microalgae, microalgae are widely used in purification of domestic wastewater and treatment of industrial effluents. This is of great significance to environmental management, and incidentally can produce active substances to bring some economic benefits[5,13,14]. Of course, in these practical applications, there are still time-consuming and labor-intensive technical problems of microalgae harvesting, with the consequent economic problems. Then we must discover an efficient and simple technology to promote microalgae widely used in these industries. Due to the negative charge on the surface of microalgae and their microscopic nature, the harvesting of these cells is considered cumbersome in the microalgae industry[15]. Traditional techniques such as centrifugation, gravity sedimentation, ultrafiltration, and membrane filtration are used for algae harvesting but have limitations in terms of low efficiency and high cost[10, 12]. Thus, it is crucial to explore strategies that improve the efficiency of harvesting, enabling the large-scale production of algae compounds to be commercially feasible. Magnetic separation was originally applied to decolorization or wastewater treatment in steel factories and power plants, the removal of specific elements in the food industry, the removal of radioactive cesium in the aqueous solution, etc[16,17]. Magnetic separation has gradually gained popularity in the industry for harvesting microalgae cells as it continuously develops. Although the algae cells gathered

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Biographies: Sifen Liu, PhD candidate, research interest: microalgae biological resources and disease treatment, Email: 277939765@qq.com; **Suping Fu**, MS, research interest: algal biotechnology and bioengineering, Email: 1785662189@qq.com; **Zhongjie Wen**, MS, research interest: algal biotechnology and bioengineering, Email: 495294285@qq.com; **Xiang Wang**, PhD, Associate Professor, research interest: microalgae biological resources and environmental applications, Email: xiangwang@jnu.edu.cn; **Tianjiu Jiang**, PhD, Professor, research interest: algal biotechnology, bioengineering, Email: tjiangtj@jnu.edu.cn.

***Corresponding author: Hongye Li**, PhD, Professor, research interest: microalgae biosynthesis and biological resources and environmental applications. College of Life Science and Technology, Jinan University, Guangzhou 510632, China. Tel: +86-20-85220219, Email: thyli@jnu.edu.cn.

magnetically do not meet industrial application requirements, this technique significantly reduces energy consumption and processing costs[18]. Magnetic flocculation, as a cost-effective technique, has broad applications in various industries. This study exemplifies a potential strategy, utilizing positive magnetic flocculated nanoparticles combined with chitosan, for the magnetic adsorption of negatively charged algae cells; thereafter, under the external magnetic field, the magnetic flocculated nanoparticles are gathered together to efficiently harvest *C. pyrenoidosa* and *S. obliquus*.

2 Materials and methods

2.1 Algae strains and cultivation conditions

Chlorella pyrenoidosa and Scenedesmus obliquus were preserved in Jinan University. Algae cells were grown in a modified BG-11 medium in an artificial climate incubator at (25±1)°C with 1500 μmol photons/m^2/s illumination at 12 h : 12 h of time period. The initial pH of the medium was 6.8-7.5. The algae cell density of *C. pyrenoidosa* used for sedimentation was 2×10^9 cells/mL, while the algae cell density of *S. obliquus* was 1×10^9 cells/mL.

2.2 Preparation of magnetic flocculated nanoparticles

First, chitosan-acetic acid solutions with varying concentrations were prepared by dissolving chitosan at concentrations of 3, 4, and 5 g/L in a 1% acetic acid solution, adjusted to a pH of 4.0-6.0. Fe_3O_4 magnetite (0.5 g) was weighed and evenly dispersed in the chitosan-acetic acid solution through 20 min of sonication. Next, 6 mg of a sodium tripolyphosphate solution was added dropwise to the Fe_3O_4-chitosan-acetic acid solution, with volumes of 15, 20, and 25 mL, along with 3 mL of Fe_3O_4-chitosan-acetic acid solution at different concentrations. The solution was mixed at a speed of 300 rpm/min while continuously stirring for 20-30 min[19]. Finally, the prepared magnetic flocculated nanoparticles were separated and dried by using an electromagnet. The final dosage of chitosan and acetic acid in different groups of magnetic flocculated nanoparticles is shown in Table 1.

Table 1 Composition of magnetic flocculated nanoparticles with different combinations

No.	Chitosan/mg	Acetic acid/mL	Sodium tripolyphosphate/mg	Fe_3O_4 nanoparticles/g
1	45	15	6	0.5
2	60	20	6	0.5
3	75	25	6	0.5
4	90	30	6	0.5
5	60	15	6	0.5
6	80	20	6	0.5
7	100	25	6	0.5
8	120	30	6	0.5
9	75	15	6	0.5
10	100	20	6	0.5
11	125	25	6	0.5
12	150	30	6	0.5

2.3 Analytical methods

2.3.1 Definition of recovery rate

The recovery rate is defined as the ratio between the difference between the initial optical density (OD) value of the liquid containing algae after sedimentation and the initial OD value at 540 nm.

$$\mu = \frac{OD_a - OD_b}{OD_a} \times 100\% \quad (1)$$

where, OD_a is the initial OD value of the algae liquid and OD_b is OD value of the liquid after the sedimentation of microalgae.

2.3.2 Definition of compactness

The compactness rate is the ratio of the volume of the algae mud after sedimentation to the volume of the original algae liquid.

$$F = V_0/V_1 \quad (2)$$

where, V_0 is the volume of the algae mud after sedimentation and V_1 is the volume of the original algae liquid.

2.3.3 Definition of sedimentation speed

The sedimentation speed is defined as the rate of change in the optical density (OD) value of the liquid containing algae per unit time during the period when the OD value at a depth of 10 cm below the surface decreases to 50% of its initial value.

$$v = 10/T \quad (3)$$

where, T is the time required for the OD value of 10 cm below the surface to drop to 50% of the original.

2.4 Statistical analysis

All experiments were performed in triplicate, and the results were given as mean ±SD (standard deviation of the mean). The data were analyzed by GraphPad Prism. The differences between the two groups were subjected to a one-way analysis of variance (ANOVA), followed by the Waller-Duncan test at the $p<0.05$ level of confidence. Different lowercase letters on the bars of the columns indicate the significant differences at $p<0.05$.

3 Results and discussion

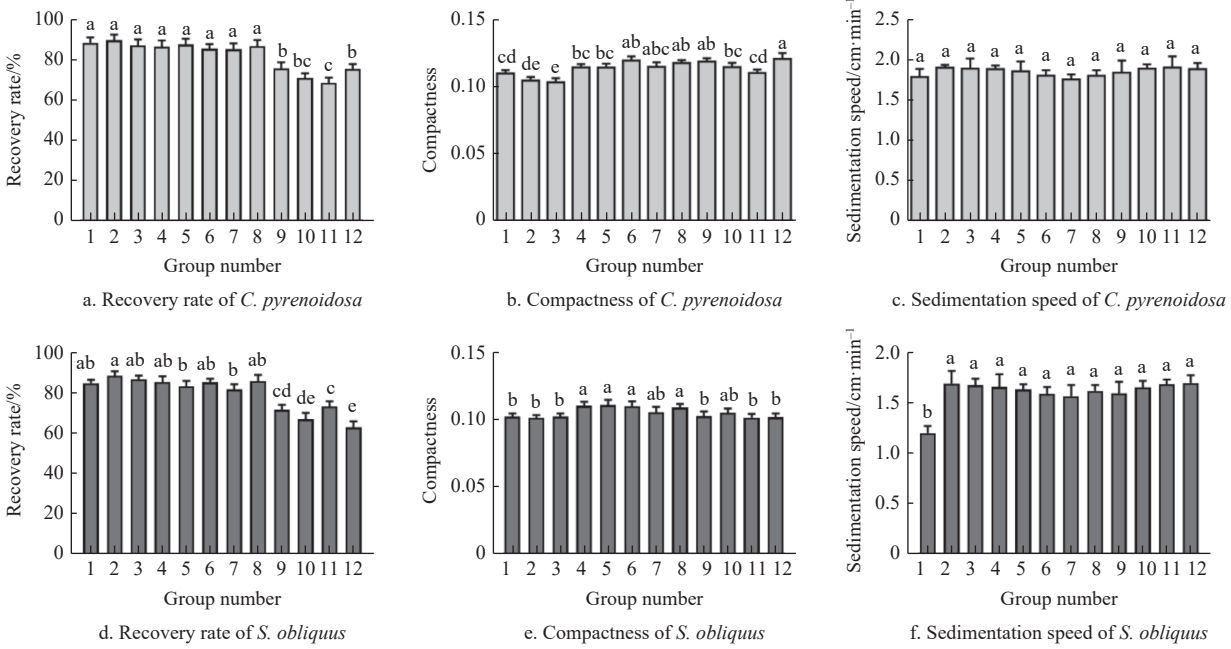
3.1 Effects of different compositions of magnetic flocculated nanoparticles on the harvesting of microalgae

Various particles, such as organic and biological polymers, are added to the algae suspension for harvesting algae cells. Commonly used organic polymers for this purpose include polyacrylamide (PAM), polyethyleneimine (PEI), and poly dimethyl diallyl ammonium chloride (PDDA), while biological polymers such as chitosan, cationic starch, and plant polyphenols are also utilized. Chitosan, a cationic organic polymer formed by the deacetylation of chitin, is considered a sustainable and environmentally friendly material with diverse applications[20]. Moreover, chitosan can harvest microalgae cells by destabilizing the negative charges on their surfaces cells[21]. Furthermore, chitosan has abundant amino groups along its polymer chain backbone, which can offer adsorption sites for microalgae cells[22]. Bare-Fe_3O_4 magnetic particles (bMP) were used as flocculants to efficiently harvest *Chlorella* sp. KR-1, resulting in harvest efficiencies of 94% to 99%[23].

Therefore, 12 groups of different compositions of magnetic flocculated nanoparticles were applied to harvest microalgae cells. Different varying concentrations of chitosan-acetic acid solution were prepared, while the amount of sodium tripolyphosphate solution and magnetite (Fe_3O_4) remained constant (Table 1). The broth of *C. pyrenoidosa* and *S. obliquus* was treated with these 12 groups of magnetic flocculated nanoparticles. The recovery rate, compactness, and sedimentation speed of *C. pyrenoidosa* and *S. obliquus* were determined by adding these 12 groups of magnetic flocculated nanoparticles.

The results indicate that 12 groups of magnetically flocculated nanoparticles exhibited a high recovery rate for both *C. pyrenoidosa* and *S. obliquus* (Figures 1a and 1d). magnetically flocculated nanoparticles No. 1-8 was relatively higher, exceeding 85% for both *C. pyrenoidosa* and *S. obliquus*. However, with the addition of more chitosan, groups No.9-12 showed a lower recovery rate for both *C. pyrenoidosa* and *S. obliquus*. The results are consistent with the report by Ahmad et al.[24], where the harvesting efficiency increased

with increasing concentrations of chitosan at lower levels, but resulted in inefficient harvesting at higher concentrations. In the previous study, chitosan was modified into nano-chitosan through crosslinking with sodium tripolyphosphate, and then used as a flocculant for harvesting the microalga *Nanochloropsis* sp. with an added chitosan dosage of 60 mg/L, nano-chitosan demonstrated significant algae cell harvesting and achieved the most cost-effective process[19]. However, the study by Kurniawati et al.[25] reported different conclusions, as the harvesting efficiency increased with increasing concentrations of chitosan. This variation in results could be attributable to the differences in solvents utilized. Notably, No.2 exhibited the most noticeable effect on microalgae harvesting, with a recovery rate of 89.35% for *C. pyrenoidosa* and 88.57% for *S. obliquus*.



Note: Different lowercase letters on the bars imply statistically significant differences at $p<0.05$ level. Each bar represents mean ± SD (n=3).

Figure 1 Effects of 12 different groups of magnetic flocculated nanoparticles on recovery rate, compactness and sedimentation speed of *C. pyrenoidosa* and *S. obliquus*

Lower compactness signifies an efficient recovery effect. As depicted in Figures 1b and 1e, No.1-8 exhibited lower compactness, with No.3 demonstrating the lowest value. The compactness of both *C. pyrenoidosa* and *S. obliquus* was approximately 0.103, confirming that No.3 possessed the most significant magnetic property.

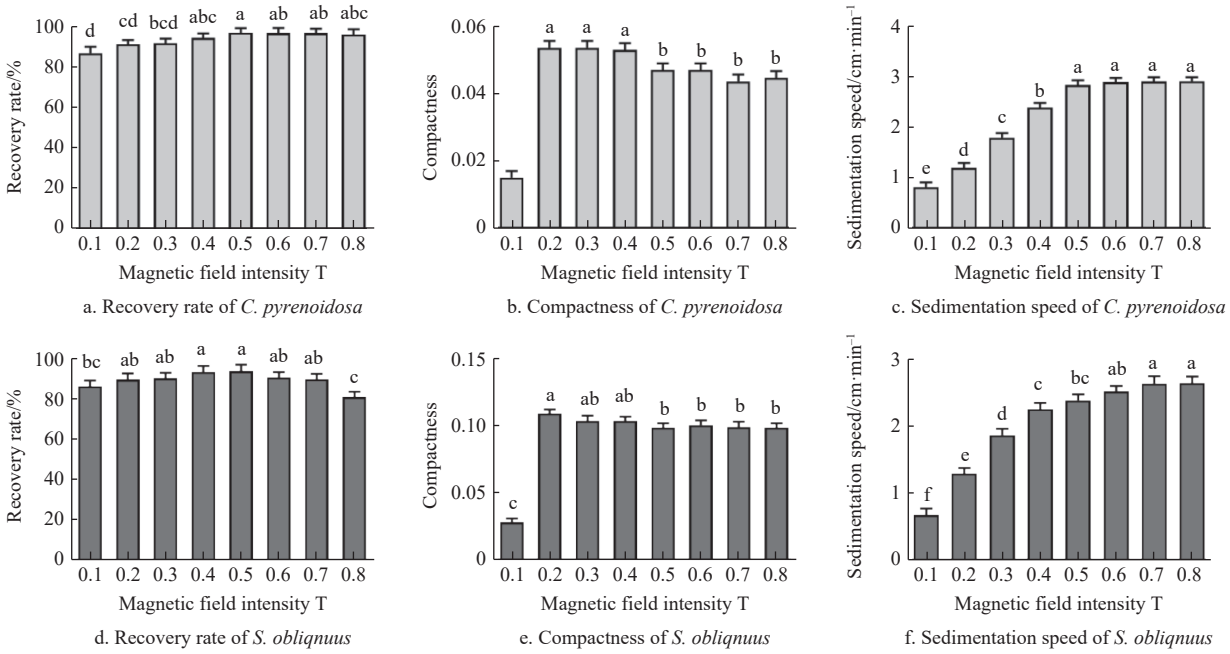
Sedimentation speed is another crucial factor indicating microalgae cell recovery efficiency. The greater the magnetic properties of the magnetic flocculated nanoparticles, the faster and shorter the sedimentation speed of microalgae harvest. When harvesting *C. pyrenoidosa*, all groups of magnetic flocculated nanoparticles did not show significant differences (Figure 1c). Among the magnetic flocculated nanoparticles, No.1 led to a lower sedimentation speed, while the other 11 groups exhibited similar sedimentation speeds in harvesting *S. obliquus* (Figure 1f). The highest sedimentation speeds observed were 1.8 cm/min for *C. pyrenoidosa* and 1.6 cm/min for *S. obliquus*. Considering these three indicators collectively, No.2 of magnetic flocculated nanoparticles was considered the most efficient group for harvesting microalgae.

Flocculation was utilized to aggregate microalgae and subsequently extract valuable biological products without eliminating the flocculants. In this context, the use of various compositions of magnetic flocculated nanoparticles results in notable disparities in both flocculation and extraction outcomes. Daniel et al.[26] observed an 8% increase in the yield of ferritic MNPs doped with Zn and Mg, compared to undoped ferritic MNPs.

3.2 Effect of magnetic field strength on the harvesting of microalgae by magnetic flocculated nanoparticles

After determining the optimal group (No.2) of magnetic flocculated nanoparticles, the impact of magnetic field strength on algae harvesting efficiency was investigated. Magnetic nanoparticles increasingly contribute to the removal of harmful algae in lakes, making them vital in wastewater treatment[9,27]. Hence, this study manipulated the magnetic field intensity and adjusted the current flow to generate different magnetic field strengths, namely 0.1 T, 0.2 T, 0.3 T, 0.4 T, 0.5 T, 0.6 T, and 0.7 T. Surprisingly, there were no significant differences observed in the ability to harvest *C. pyrenoidosa* and *S. obliquus* cells. This lack of distinction may be attributed to the insufficient time for the magnetic flocculated nanoparticles to fully adsorb the microalgae, which had settled at the bottom of the broth due to the magnetic field.

As shown in Figures 2a and 2d, when the magnetic field strength was less than 0.6 T, the recovery rate of *C. pyrenoidosa* and *S. obliquus* gradually increased. However, when the magnetic field strength exceeded or equaled 0.6 T, there was no change observed in the recovery rate of *C. pyrenoidosa*, while the recovery rate of *S. obliquus* slightly declined. This phenomenon can be attributed to the magnetic flocculated nanoparticles being adsorbed to the bottom before fully absorbing the microalgae, leading to an incomplete harvest. Notably, at a magnetic field strength of approximately 0.5 T, the recovery rate for both *C. pyrenoidosa* and *S. obliquus* remained at around 97%.



Note: Different lowercase letters on the bars imply statistically significant differences at $p<0.05$ level. Each bar represents mean ± SD (n=3).

Figure 2 Effects of different magnetic field intensities on recovery rate, compactness and sedimentation speed of *C. pyrenoidosa* and *S. obliqnuus*

Compared to other magnetic field strengths, a magnetic field strength of 0.1 T resulted in the lowest densities for C. pyrenoidosa (0.015) and S. obliquus (0.027). Above 0.1 T, the compactness of both *C. pyrenoidosa* and *S. obliquus* increased significantly, with *C. pyrenoidosa* reaching around 0.05 and *S. obliquus* around 0.105. This increase in compactness may be attributed to the excessive strength of the magnetic force, causing the gap between the magnetic flocculated nanoparticles to become too narrow for complete absorption of the algae cells.

For magnetic field strengths below 0.5 T, the sedimentation speed gradually increased as the magnetic field strength increased. The magnetic field expedited the sinking of flocculants and algae. At a magnetic field strength of 0.5 T, the sedimentation speed of *C. pyrenoidosa* was the fastest at 2.82 cm/min. On the other hand, at a magnetic field strength of 0.7 T, *S. obliquus* exhibited a sedimentation speed of 2.63 cm/min.

Certainly, the effect of static magnetic fields on microalgae should be considered based on the previous report[28]. Exposure to a magnetic field strength of 10 mT was found to increase the biomass of *Chlorella kessleri* and induce physiological changes such as chloroplast enlargement and decreased thylakoid order. Additionally, cultivation disturbance caused by a permanent magnetic field resulted in increased protein content.

3.3 Effect of magnetic flocculated nanoparticle dosage on the harvesting of microalgae

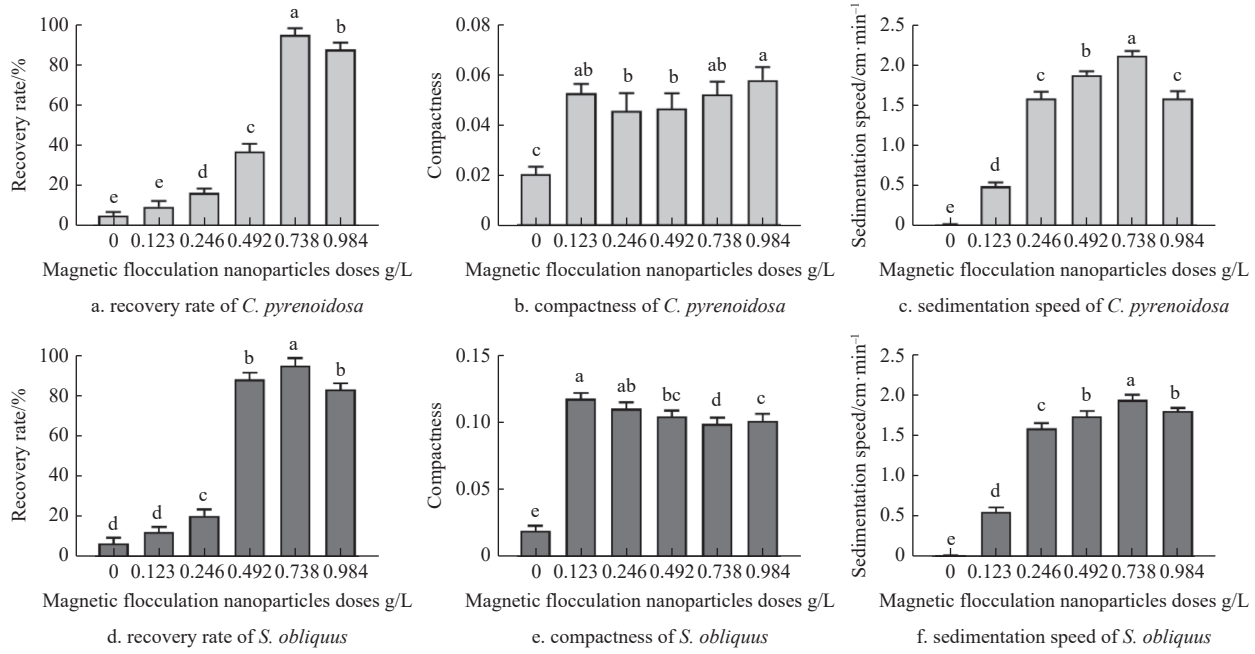
After optimizing the combination of the magnetic flocculated nanoparticles (set No.2) and determining the optimal magnetic field strength (0.5 T), varying doses of magnetic flocculated nanoparticles were added to the microalgae media at concentrations of 0, 0.123, 0.246, 0.492, 0.738, and 0.984 g/L. The microalgae broth was stirred, and electromagnets were used to absorb and harvest the microalgae when floccules formed.

The results revealed significant differences in the recovery rate, compactness, and sedimentation speed of *C. pyrenoidosa* and *S. obliquus* due to the magnetic flocculated nanoparticles ($p<0.05$). It can be seen from Figure 3, that the natural recovery rate, compactness, and sedimentation speed of both *C. pyrenoidosa* and *S. obliquus* were comparatively slow. The recovery rate for both cells remained low at approximately 9.02%. However, as the dose of magnetic flocculated nanoparticles added to the microalgae broth increased up to 0.738 g/L, the recovery rate exhibited an incremental trend. Notably, at a dose of 0.738 g/L, both *C. pyrenoidosa* and *S. obliquus* experienced significantly higher recovery rates, with 95.21% and 94.82%, respectively. Conversely, when 0.984 g/L of nanoparticles was added to the algae broth, the recovery rate declined. This contrasts with the previous report, which found that increasing the dose of magnetic flocculated nanoparticles improved the recovery efficiency for *B. braunii* and *C. ellipsoidea*, demonstrating that higher dosage does not always yield better results[29]. Similarly, among the four different flocculants studied, two achieved the highest recovery efficiency at maximum dose[30].

The observations indicated that all groups had lower compactness, with both *C. pyrenoidosa* and *S. obliquus* exhibiting lower compactness in the absence of magnetic flocculated nanoparticles (Figures 3b and 3e). Specifically, the compactness of *C. pyrenoidosa* was below 0.06, while that of *S. obliquus* was below 0.13. Adding magnetic flocculated nanoparticles to the microalgae broth resulted in decreased compactness. As the nanoparticles adsorbed more microalgae and settled under gravity, the presence of a magnetic repulsive force between the nanoparticles prevented closer clumping of the microalgae.

Figures 3c and 3f showed that the natural sedimentation speed without magnetic flocculated nanoparticles was close to zero. However, upon adding magnetic flocculated nanoparticles, the sedimentation speeds of both *C. pyrenoidosa* and *S. obliquus* increased significantly. Notably, at a concentration of 0.738 g/L, the sedimentation speeds were the highest, with *C. pyrenoidosa* and *S. obliquus* reaching 2.12 cm/min and 1.94 cm/min, respectively. Figure 3 demonstrates that the sedimentation speed was directly proportional to the recovery rate. Considering the recovery rate, compactness, and sedimentation speed, a dose of 0.738 g/L was selected as the optimal amount of added magnetic flocculated

nanoparticles.



Note: Different lowercase letters on the bars imply statistically significant differences at $p<0.05$ level. Each bar represents mean ± SD (n=3).

Figure 3 Effects of different doses of magnetic flocculated nanoparticles on recovery rate, compactness and sedimentation speed of *C. pyrenoidosa* and *S. obliquus*

Previous studies have used different compositions of Fe_3O_4 flocculants to flocculate microalgae, especially in *Chlorella*. The dosage of flocculants varied widely, ranging from 1 to 5000 mg/L, with the majority of studies using around 200 mg/L[31].

3.4 Effect of pH on the harvesting of microalgae with magnetic flocculated nanoparticles

After optimizing the magnetic field intensity (0.5 T) and the optimal addition dose of magnetic flocculated nanoparticles (0.738 g/L) for efficient microalgae harvesting, this study investigated whether the flocculation effect of the magnetic flocculated nanoparticles was influenced by pH. A pH range of 4 to 12 was chosen to examine its effect on magnetic flocculated nanoparticles-mediated microalgae harvesting. The recovery rate, compactness, and sedimentation speed were determined to assess the recovery effect. Previous studies have suggested that an alkaline condition induces microalgae sedimentation without the need for extra flocculants[32-34].

Figure 4 demonstrates that acidic conditions are not conducive to the harvesting of *C. pyrenoidosa* and *S. obliquus* using magnetic flocculated nanoparticles. When the pH was 4.0, the recovery rate of *C. pyrenoidosa* was approximately 75%, while the recovery rate of S. obliquus was about 54%. However, at pH ≥ 6.0, the recovery rate of both *C. pyrenoidosa* and *S. obliquus* was higher, exceeding 90%. This finding contradicts the previous study that pH is insignificant for microalgae harvesting and that recovery rates can surpass 95% in the pH range of 2-12[35]. Nevertheless, another study indicated that harvesting *Chlorella* sp. could be accomplished with pH 7 and pH 12 for detachment[23]. Specifically, at pH 6, the recovery rate of *C. pyrenoidosa* was the highest at 95.53%, whereas at pH 12, the recovery rate of *S. obliquus* reached its peak at 95.76%. Furthermore, a different study found that *B. braunii* showed faster recovery in acidic conditions, while *C. ellipsoidea* had faster recovery in neutral conditions[29].

Figures 4b and 4e illustrated that the compactness of magnetic flocculated nanoparticles harvesting *C. pyrenoidosa* was lowest under neutral conditions, measuring approximately 0.04. The compactness increased in acidic or alkaline conditions, indicating that *C. pyrenoidosa* was not sufficiently compact. In contrast, the compactness of magnetic flocculated nanoparticles harvested from *S. obliquus* remained relatively consistent, ranging between 0.09 and 0.12. Another finding suggested that the compactness of magnetic flocculation is regulated by the rate of protonation and deprotonation at different pH values[36].

Figures 4c and 4f revealed that the trend of sedimentation speed aligned with that of the recovery rate. In acidic conditions, the sedimentation speed was lower, whereas it accelerated under neutral or alkaline conditions.

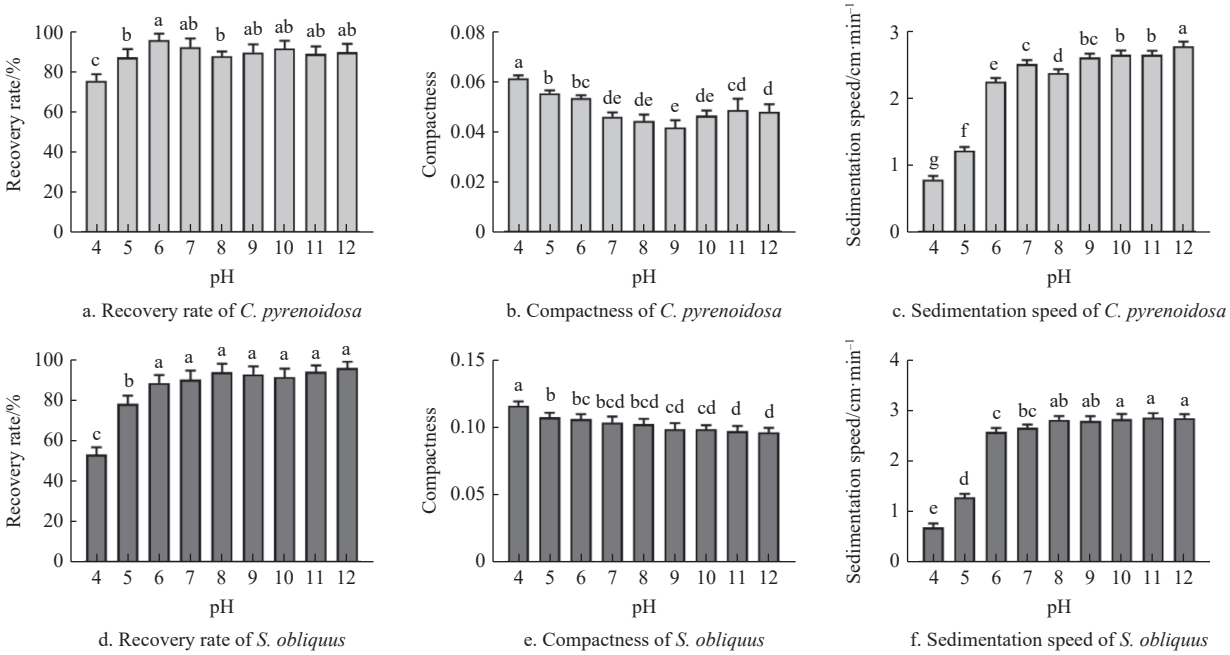
In this study, microalgae exhibited fast sedimentation speed at pH 9.0; *C. pyrenoidosa* had a sedimentation speed of 2.6 cm/min, while *S. obliquus* had a sedimentation speed of 2.8 cm/min. Interestingly, another report concluded that the sedimentation speed of *Chlorella sorokiniana* was faster in acidic conditions when using four different flocculants[30].

Considering the recovery rate, compactness, and sedimentation speed, neutral and alkaline conditions are considered optimal for magnetic flocculated nanoparticles to harvest *C. pyrenoidosa* and *S. obliquus*.

It is worth further investigating the magnetic flocculation of microalgae under alkaline conditions, as certain flocculants can affect the flocculation effect by altering the pH of the medium. Additionally, the change in pH affects the proportion of active substances in microalgae cells, and in the presence of Ca^{2+} and Mg^{2+}, the alkalinity of the medium can result in auto-flocculation[37].

4 Conclusions

In this study, we present the optimal conditions for harvesting microalgae using magnetic flocculated nanoparticles, a critical bottleneck in the commercialization of algae. The best-performing group of magnetic flocculated nanoparticles was No.2, with a magnetic field strength of 0.5 T. The optimal dose of added



Note: Different lowercase letters on the bars imply statistically significant differences at $p<0.05$ level. Each bar represents mean ± SD (n=3).

Figure 4 Effects of pH on recovery rate, compactness and sedimentation speed of *C. pyrenoidosa* and *S. obliquus*

magnetic flocculated nanoparticles was found to be 0.738 g/L, and the most favorable pH of the microalgae broth was determined as 9. Under these conditions, the recovery rate of both *C. pyrenoidosa* and *S. obliquus* exceeded 97%, with a sedimentation speed above 2.63 cm/min for both species. These findings demonstrate that the magnetic flocculated nanoparticles optimized in this study provide an efficient method for microalgae harvesting, thereby offering significant potential for future industrial applications.

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