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Studies on Bioflocculation efficiency of Moringa leaf and Soybean seed protein

Pratibha Kushwaha
University of Allahabad
Sushma Kumari
University of Allahabad
K. Suresh Kumar
ksuresh2779@allduniv.ac.in

University of Allahabad

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Abstract

Algae are a group of diverse photosynthetic organisms, that have a wide range of applications. Bioflocculation has emerged as a new eco-friendly approach for harvesting algae. The composition of algae influences the bioflocculation process; therefore, in this study, we evaluated the protein content of marine microalgae *Synechococcus* sp., *Dunaliella* sp., and *Chlorella* sp. (50±0.2%, 36.2±0.4%, and 40.16±2.22% respectively); the protein content of *Moringa*leaf and soybean (16 and 55 %) were also evaluated. The genus *Moringa*(also called the miracle tree), has coagulation/flocculation abilities and is used for wastewater treatment, pharmacy, food, and feed. A study on the efficiency of *Moringa* to flocculate the mentioned under varying operating parameters (pH and dose), revealed that in the case of *Dunaliella*sp. maximum bioflocculation efficiency (91.3%) was achieved with Moringa leaf protein followed by soybean protein (85.3%). Apart from being simple, non–perilous, and effective, this bioflocculation method involves no algal pre-treatment costs; moreover, it can be scaled up for large-scale harvesting of microalgae.

Introduction

Algae comprise a diverse assemblage of photosynthetic organisms, encompassing microscopic forms as well as large seaweeds that can attain several meters in size. Microalgae are widely distributed across aquatic habitats and occur in marine, freshwater and brackish environments including oceans, rivers, lakes and ponds [4]. Microalgae play an important role in aquatic ecosystems by contributing to primary production and supporting aquatic food webs. Through photosynthesis, they also contribute to oxygen production and carbon fixation. Beyond their ecological importance, their lipid, protein, carbohydrate and other bioactive components make them attractive feedstocks for biofuel production and a range of value - added products [26, 1].

The recovery of microalgal biomass remains a major challenge in the development of algal biofuel processes. Microalgal cultures generally contain relatively dilute biomass, while the cells are small and often possess a negatively charged surface, which keeps them dispersed in the culture medium and makes their separation more difficult. Consequently, harvesting and subsequent concentration of the biomass can contribute substantially to the overall production cost, although the actual cost varies with the cultivation system and harvesting technology employed [43]. The cost of biomass recovery can be substantial in algal biomass production. Depending on the cultivation system, algal species, and downstream processing requirements, harvesting may account for a considerable proportion of the overall production cost, with some studies reporting contributions of up to 50–60% of the total process cost [20]. Apart from biofuel, the nutritional value of algae has made them suitable for use as nutritional supplements in human and animal feed. Siddik et al. [31] reported that algae are rich in protein, carbohydrates, vitamins, antioxidants, and trace elements along with several bioactive compounds such as astaxanthin, lutein, beta-carotene, vitamin E, chlorophyll, phycobiliproteins, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, γ-linolenic acid, oleochemical fatty acids, and Beta-1,3-glucan.

Algal biomass can be harvested using various techniques, including mechanical methods (filtration, sedimentation, centrifugation, flotation and ultrafiltration), electrical approaches (electrophoresis), and chemical or biological flocculation using inorganic, organic and biodegradable agents. Despite their widespread use, physical techniques such as centrifugation, tangential filtration, magnetic flocculation and electrocoagulation along with chemical approaches involving coagulants and biopolymers have several limitations. These include high energy consumption, shear-induced cell damage, membrane fouling, slow processing, limited scalability, chemical contamination, reduced culture medium recyclability, and high operational costs [30]. The bioflocculation offers a potential eco-friendly approach to microalgal harvesting, in which interactions between the flocculant and algal cells promote aggregation through ionic bridging. This method is particularly relevant to biomass recovery for food and feed application [44].

The microbial flocculation facilitated by bacteria, fungi and other microalgae, offers a potential strategy for integrating microalgal biomass recovery with wastewater treatment for biofuel production [36]. Achieving efficient microalgal harvesting requires optimization of the flocculant dosage to reduce the electrostatic repulsion arising from the negatively charged cell surfaces and facilitate cell aggregation [2].

A range of natural and modified materials has been investigated for algal biomass harvesting, including mung bean protein extract and *Moringa oleifera* seed extract, *Cassia angustifolia* derived materials, vegetable tannins, Nirmali (*Strychnos potatorum*) seeds, common bean (*Phaseolus vulgaris*, sugar and red maize, chitosan, and modified soil [9]. Several bioflocculants including chitosan, poly-γ-glutamic acid, cationic starch, guar gum, and mung bean protein extract have been reported to facilitate the flocculation of diverse microalgal species such as *Thalassiosira pseudonana, Nanochloropsis oculate, Chlorella vulgaris, Phaeodactylum tricornutum, Scendesmus dimorphus, Chlamydomonas* sp., *Chlorella* sp., and *Botryococcus* sp [12]. This study aims to develop an efficient and convenient method for harvesting microalgae using varying concentrations of *Moringa* leaf protein and soybean seed protein as bioflocculants under different pH levels. This could probably reduce the cost of microalgae biomass production in various industries.

Material and methods

Culturing of microalgae

Inoculum of *Synechococcus* sp. *Chlorella* sp. and *Dunaliella* sp. were procured from National Facility for Marine Cyanobacteria (NFMC) and Bioinformatics Centre Bharathidasan University, Tiruchirappalli, Tamil Nadu, India (10.68°N, 78.74°E). The marine microalgae were axenically cultured in a laboratory growth chamber maintained at 24 ± 0.1°C temp, and a 16:8 light-dark cycle with white fluorescent lights and a photon flux of ~60 µmol photon/m^2 /s using ASNIII media. Temperature, pH, and optical density (OD) were measured daily.

Growth of Microalgae

The growth of *Synechococcus* sp., *Dunaliella* sp., and *Chlorella* sp., was monitored over a 15-days cultivation period by measuring cell concentration. The initial cell densities were approximately 1×10^7, 2×10^6, and 3×10^6 cells/mL, respectively. The growth exhibited an initial lag phase, followed by an exponential phase characterized by an increase in cell density, during which the maximum cell concentration was attained.

Extraction of Moringa leaf protein

The *Moringa* leaves were collected directly from the Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, Uttar Pradesh, India (25° 27’ 42.2352” N,81°5’ 7.278” E).

Protein was extracted from the fresh *Moringa* leaves following the method described by Niu et al. [23]. 500 mg of fresh leaves tissue was thoroughly homogenized using a pre-chilled mortar and pestle in 0.5mL of lysis buffer containing trichloroacetic acid (TCA). The homogenate was transferred to a centrifuge tube and centrifuged at 10,000 rpm for 5 min at 4℃. The resulting supernatant was discarded, and the protein containing pellet was stored at -20℃ for 1 h. Chilled acetone was then gradually added to the pellet, followed by thorough mixing. The suspension was centrifuged at 10,000 rpm for 5 min at 4℃, after which the supernatant was discarded. The pellet was subsequently washed three times with acetone at room temperature to remove residual pigments. The resulting pellet was reconstituted in rehydration buffer and vortexed thoroughly to obtain a homogenous suspension. The suspension was then centrifuged at 10,000 rpm for 5 min, and the resulting supernatant containing the total crude protein extract was collected. The protein content of the *Moringa* leaf extract was determined according to method described by Sarkar et al. [30].

Isolation of protein from soybean seed

Soybean seeds used in this study were obtained from local commercial sources in Prayagraj, Uttar Pradesh, India. Soybean seeds were sorted to remove foreign materials and damaged seeds, followed by dehulling and grinding into a fine flour. The flour was defatted by soaking in hexane and subsequently air-dried for 1h. Protein was then isolated using an acid-base extraction procedure following Verfaillie et al. [35]. The defatted flour was suspended in distilled water, and the pH was adjusted to 9.0 using 1N NaOH. The suspension was centrifuged at 1000 rpm for 20 min and the protein-containing extract was collected. Protein precipitation was achieved by adjusting the extract to pH 4.0 with 1N HCl. The resulting precipitate was collected, washed with distilled water, and neutralized to pH 7.0 using 1N NaOH. The resulting protein slurry was dried in an oven at 55°C, followed by grinding and sieving. The dried soybean protein was subsequently used for further experiments. Protein content was determined according to the method of Tian et al. [33].

Flocculation experiment

A modified jar test method described by Rodrigues et al. [29] was used to study the effects of flocculant concentration, settling time, and pH on the flocculation efficiency of the protein flocculants. Briefly, 1 L of microalgal suspension was placed in a 2-L jar and stirred at 500 rpm. The required amount of protein flocculant was added gradually during stirring. After 5 min, stirring was stopped and the suspension was allowed to settle. The settling time was 15 min for the *Moringa* protein and 120 min for the soybean protein. After settling the supernatant was collected at a depth of 3.0 cm from the surface of algal suspension. Its absorbance was measured at 540 nm using a UV-visible spectrophotometer to determine the flocculation efficiency.

The original algal medium's absorption was measured before adding the flocculant. With the help of either 1.0 N HCl or 1.0 N NaOH, the pH was changed in order to assess its impact on flocculation. Flocculation efficiency was calculated from the following eq-

Flocculation efficiency (%) = (Ci - Cs)/Ci × 100

Where *Ci* is the initial cell concentration and *Cs* is the cell concentration in supernatant.

Result and discussion

The crude protein content of *Moringa oleifera* leaves was found to be 8.63± 0.561%, which was comparable to the values reported by Jamil et al. [10], who recorded crude protein contents of 8.75%, 8.88%, and 9.84% in leaves collected from different locations in Punjab, Pakistan. The protein content of the *Moringa* leaf was higher than other leafy vegetables like Amaranthus leaf (3.5%) and Spinach (3.2%) [39]. The protein content of soybean seeds obtained in our study (33.38± 0.55%) was comparable to the value reported by Oli et al. [25], who reported content of 37.85 ± 0.33% in black cultivated soybean (*Glycine max*) from Uttarakhand, India.

The protein content of *Moringa* leaf varied from 5-16%, while protein content of the soybean seed varied from 35% to 55%; this could be because various environmental and genetic factors [5]. Overall *Moringa* leaf protein showed higher harvesting efficiency for microalgae than soybean seed protein under the condition tested in the present study. This higher efficiency may be related to the difference in protein content and protein characteristics of the two plant sources. *Moringa* derived protein have previously been reported to promote microalgal aggregation through interactions with the negatively charged algal cell surface [42,11]. Therefore, the greater flocculation efficiency observed with M*oringa* leaf protein in the present study may be attributed, at least in part, to differences in the protein composition and surface interactions of the two flocculants. Table 2 shows the flocculation efficiency of current study and reported microalgae species.

Effect of pH on flocculation efficiency

Malik et al. [21] point out that pH does not only influence the cell surface charge of microalgal cells but also influences the flocculants involved; therefore, pH is an important parameter to be considered. *Moringa* leaf protein (150 ml/L) was used as a flocculant to harvest *Chlorella* sp., *Dunaliella* sp. and *Synechococcus* sp. After 15 min settling time at pH 2, the flocculation efficiency of *Moringa* leaf protein (1, 1 and 1.5 mg/mL respectively) for *Chlorella*, *Dunaliella*, and *Synechococcus* was 81.790±2.82% Figure 4(a), 91.3043±3.286% Figure 5(a) and 77.8246±1.805 % Figure 6(a) respectively. Ineffective flocculation was observed by increasing the pH from 3-5. At acidic pH protonation of negatively charged groups present on the algal cell surface reduces the overall surface charge thereby decreasing the electrostatic repulsion between cells and promoting their aggregation. In contrast an increase in pH favours deprotonation of carboxyl groups, resulting in more negative surface charges. The increase negative charge can strengthen electrostatic repulsion and consequently reduce cell aggregation and flocculation.

Just as in case of *Moringa* leaf protein, pH 2 and 30 mg/15ml soybean protein was optimal for bioflocculation of *Chlorella*, *Dunaliella* and *Synechococcus* sp. wherein 78.70±3.33% Figure. 4(b), 85.3±3.18% Figure 5(b) and 75.62±2.46% Figure 6(b) bioflocculation efficiency were recorded). The flocculation efficiency for *Synechococcus* obtained in our study was similar to the report by Oliveira et al. [27]. Purwono et al. [28] tested the flocculation efficiency of *Dunaliella* sp. by the electrocoagulation method and obtained 91% efficiency.

Similar to the present study, pH-dependent changes in microalgal harvesting efficiency have been reported in previous studies. Kuzhiumparambil et al. [13] demonstrated that pH-induced flocculation influenced the harvesting efficiency of *Chlorella vulgaris*, highlighting the importance of culture pH during microalgal biomass recovery. According to Maji et al. [20], for *Chlorella vulgaris* pH of 3.5 and 9.5 resulted in highest flocculation, whereas pH of 4.0 and 9.0 provided maximum flocculation in *Scendesmus obliquus*. Further, they observed cells of *Chlorococcum* sp flocculated at higher levels at pH 3.5 and 9.0. At low pH values, the flocculation efficiency was higher even at higher biomass concentration.

Effect of flocculant dose on flocculation efficiency *Moringa* leaf protein

A number of studies were conducted to identify the exact dose influence on flocculation efficiency. The dose concentration is a crucial factor in the flocculation process. The flocculant dose influenced bioflocculation of microalgae.

Maghfiroh et al. [19] studied harvesting efficiency of *Chlorella* sp. biomass using bioflocculant *Moringa* seeds; they observed dose, and pH-dependent changes in flocculation efficiency. According to them the optimum pH for harvesting *Chlorella* sp. using *Moringa* seeds bioflocculant was at pH 11 with the bioflocculant concentration of 0.15 g/L. At highly alkaline pH, ionization of functional groups in the protein can alter its surface charge and interaction with algal cells. Under these conditions, changes in the charge state of amino and carboxyl groups may reduce electrostatic repulsion and facilitate interaction between the protein and algal cells, resulting in the formation of larger aggregates or flocs. In

our study, 1mg/ mL concentration of *Moringa* leaf protein gave maximum flocculation in case of *Chlorella* and *Dunaliella*, however, 1.5mg/mL was appropriate dose in case of *Synechococcus* at pH 2. According to Maghfiroh et al. [19], the effect of dose of *Moringa* seeds powder bioflocculant showed decreasing of biomass concentration even though the decrease was gradually. They suggest that one of the mechanisms of flocculation is the charge neutralization; further, the surface of the cells will result in the reduction of electrostatic repulsion force between the cell particles so that the cells will be coagulated until finally be flocculated.

A fixed algae concentration was used with different doses of Soybean protein extract from 0.5 g/L to 2.5 g/L at pH 2. In the case of *Chlorella* and *Dunaliella* sp. 2.0 g/L Soybean protein showed (78.7037±3.338% Figure 4(b) and 85.30±3.187% Figure 5(b). respectively, 90 min), while 2.5g/L of the same was suitable for *Synechococcus* showed (75.622±2.466% Figure 6(b) bioflocculation efficiency. These results are higher than the report of Ma et al. [16], who reported 40-50% flocculation efficiency of marine algae using the chemical coagulant $Al_2(SO4)_3$ (114.4 mg/L). Xu et al. [37] reported that the use of seed-based proteins as coagulants for negatively charged contaminants due to the presence of the abundance of -NH_2 groups.

Effects of algal cell size on flocculation efficiency

The bioflocculation efficiency of any bioflocculant would vary with the algal species.

As seen in Table 1, Despite having a larger cell surface area than the other two algal species, *Dunaliella* required a relatively low coagulant dose for effective charge neutralization. These differences may be attributed to species-specific surface properties and interaction between the algal cells and the coagulant [3, 14].For *Dunaliella*, the maximum removal efficiency was achieved at coagulant doses of 2.0g/L using soybean protein and 1mg/mL *Moringa* leaf protein at pH 2.0 (with a flocculation efficiency of 85.30% and 91.30% respectively).

The lower removal efficiency of *Synechococcus* sp. may be associated with its small cell size and low biomass density, which can limit the formation and sedimentation of larger flocs. The harvesting mechanism may involve charge neutralization, while additional mechanisms such as sweep flocculation and adsorption/bridging can contribute depending on the flocculant and operating conditions [6, 42, 8].

Effect of salinity on flocculation efficiency

Salinity can alter the surface and electrokinetic properties of microalgal cells, thereby influencing flocculation efficiency. At elevated salinity, microalgae experience osmotic stress and undergo physiological and metabolic adaptations. In some species, salt stress promoted lipid accumulation; however, excessive salinity may inhibit cell growth and biomass productivity. Therefore, the effect of salinity on dry biomass and flocculation efficiency is species- and concentration-dependent [40, 15].

The high ionic strength of marine environments can reduce the effectiveness of conventional cationic polyelectrolytes for microalgal flocculation. This effect has been attributed to ionic shielding of charged groups and changes in polymer confirmation, including polymer coiling, which can reduce the effective dimension of the polymer and hinder adsorption-bridging between algal cells [36]. However, the effect of salinity is dependent on polymer characteristics, and salt-tolerant flocculants can remain effective under marine conditions . Bioflocculation using plant-derived proteins offers an alternative approach because it avoids the addition of conventional synthetic chemical flocculants. In the present study, *Dunaliella,* achieved flocculation efficiencies of 91.30% and 85.30% with 1 mg/ml *Moringa* leaf protein and 30mg/15mL soybean seed protein, respectively. These doses were approximately 6-8 fold higher than those required for the other algal species tested, suggesting that the higher flocculant requirement may be associated with the marine nature and surface characteristics of *Dunaliella*.

The present study demonstrated that both *Moringa* leaf protein and soybean seed protein were capable of harvesting *Dunaliella*, *Chlorella* and *Synechococcus*. However, their performance was not uniform across the tested algal species. The highest flocculation efficiency was obtained with *Dunaliella* indicating that the interaction between algal cell and a bioflocculant is strongly dependent on the characteristics of the target cell. Differences in cell size, surface charge, surface functional groups, and extracellular organic material can alter the extent to which a flocculant attaches to algal cells and promote their aggregation. Malik et al. [20] also reported that flocculation behaviour can differ not only between algal species but also among strains of the same species under different cultivation conditions. The cell size may be one of the factors contributing to these differences. Smaller microalgal cells generally possess a greater surface area to biomass ratio, consequently, a larger amount of flocculant may be required to achieve effective aggregation of an equivalent biomass compared with larger cells. Nevertheless, cell size alone cannot explain the observed variation in harvesting efficiency because algal surface characteristics and biochemical composition also determine flocculant – cell interactions. The composition of the growth medium, extracellular polymeric substances such as polysaccharides and proteins, biomass concentration, nutrient availability, pH, and physiological state or growth phase may therefore modify the flocculation response [25]. The physical conditions during flocculation are equally important. Appropriate mixing and shear can increase cell-flocculant collision and facilitate floc formation, whereas excessive shear may disrupt the aggregates that have already formed. Thus, the shear conditions need to be controlled to obtain sufficiently large and stable flocs [6]. Eichhorn et al. [7] reported high flocculation activity up to 93% for *Moringa* seed derived material, although the resulting flocs were relatively susceptible to disruption under low shear. In contrast, cactus-derived flocculants produced more stable aggregates but showed a lower maximum flocculation activity (54%). The combination of the two materials improved the balance between harvesting efficiency and floc stability, achieving approximately 76% flocculation. These observations highlight that flocculation efficiency alone may not adequately describe the practical performance of a bioflocculant, the mechanical stability of the resulting flocs is also important for subsequent harvesting and downstream processing. The increasing interest in bio-based flocculants has encouraged the evaluation of a variety of naturally derived materials for microalgal harvesting. Yang et al. [39] experimentally compared several natural polymeric flocculants,

including chitosan, Tanfloc, cationic starch and *Moringa oleifera*, for harvesting *Chlorella vulgaris* and *Scendesmus obliquus*. Their results demonstrated that the harvesting performance depended on the type and dosage of the flocculant, culture pH, cell density and algal organic matter, with *Moringa oleifera* promoting flocculation primarily through bridging interactions. Konucu et al. [11] further demonstrated the potential of *Moringa oleifera* seed powder for harvesting a mixed culture of *Chlorella vulgaris* and *Desmodesmus* sp., achieving 75% harvesting efficiency with the seed powder and up to 91% following water extraction. More recently a plant-based flocculant derived from pomegranate seed waste achieved upto 85% harvesting efficiency, highlighting the potential of plant-derived materials as alternatives for microalgal biomass recovery. Collectively, these studies support the continued development of plant-derived and other bio-based flocculant for efficient microalgal harvesting. Other bio-based flocculants, particularly chitosan have also demonstrated considerable potential for microalgal harvesting. In an experimental study, chitosan achieved a flocculation efficiency of 89% for *Chlorella vulgaris*, which increased to 98% when combined with walnut protein extract [37]. The experimental studies have demonstrated that inorganic coagulants can achieve high harvesting efficiencies, in *Chlorella vulgaris.* Aluminium sulfate achieved efficiencies of 89.8-96.4%, while iron- and aluminium -based coagulants showed efficiencies of 93.5-98.8% under optimized conditions [17]. However, the addition of inorganic coagulants may introduce metal residues into the harvested biomass, potentially affecting its composition and subsequent downstream processing. In comparison, the results obtained in the present study demonstrate the potential of plant-derived proteins as bio-based alternatives for microalgal harvesting. The relatively high efficiency obtained with *Moringa* leaf protein and soybean seed protein, particularly for *Dunaliella* suggests that these readily available plant-derived material can serve as a promising flocculants. Their practical suitability, however should be evaluated not only on the basis of flocculation efficiency but also in terms of dosage requirement, floc stability, biomass quality, cost and the compatibility of the harvested biomass with subsequent downstream applications.

Conclusion

In the present work, *Moringa* leaf Protein and soybean seed protein were tested for the harvesting of three microalgal species, namely *Synechococcus, Dunaliella* and *Chlorella*. Both plant proteins showed flocculation activity, although the extent of biomass recovery differed among the algal species and between the two flocculants. For *Dunaliella* the harvesting efficiency reached 91.30% with *Moringa* leaf protein and 85.30% with soybean seed protein. The difference in harvesting performance may be related to the distinct cell surface characteristics and physicochemical properties of the algal species. Overall, the results show that plant-derived proteins can serve as potential bioflocculants for microalgal biomass harvesting. Further work on floc stability, optimization of flocculant dosage, biomass quality and process economics would help in evaluating their application at a larger scale.

Declarations

Ethics declaration

The *Moringa* leaves used in this study were obtained from cultivated sources and were not collected from protected or natural habitats. Their procurement and use complied with applicable local and national guidelines.

Soybean seeds used in this study were obtained from local commercial sources in Prayagraj, Uttar Pradesh, India. The procurement and use of the Soybean seeds complied with applicable local and national guidelines. No specific collection permits or licences were required for the materials obtained through routine commercial procurement.

Consent to participate

Not applicable. This study did not involve human participants.

Consent to publish

Not applicable. This manuscript does not contain any individual person's data or identifiable information requiring consent for publication.

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Author Contribution

Author Contributions:Pratibha: Conceptualization, investigation, data analysis, and manuscript preparation.Sushma Kumari: Experimental work, data collection, and review of the manuscript.K. Suresh Kumar: Supervision, methodology, manuscript review, and corresponding authorship.

Data Availability

This is appropriate for your manuscript because the study on bioflocculation efficiency of Moringa leaf and soybean seed proteins involves experimental data.

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Tables

Table 1 shows the cell diameter, surface area and cell surface area of the three algae tested.

Species	Cell diameter	Surface area	Cell surface area	Stationary phase	Reference
Dunaliella	8 μm	$4\pi r^2$ Where r=4.00	201.06	2.0×10^6	[44]
Chlorella	4 μm	$4\pi r^2$ Where r=2.00	50.24	3.0×10^6	[14]
Synechococcus	2 μm	$4\pi r^2$ Where r=1	12.56	1.0×10^7	[14]

Table 2 shows the flocculation efficiency of current study and reported microalgae species.

Plant product	Microalage	FE (%)	Reference
Moringa leaf protein	*Chlorella* sp.	81.7	This study
Soybean protein	*Chlorella sp.*	78.7	
Moringa leaf protein	*Dunaliella* sp.	91.3	
Soybean protein	*Dunaliella sp.*	85.3	
Moringa leaf protein	*Synechococcus* sp.	77.8	
Soybean protein	*Synechococcus sp.*	75.6	
Guar gum	*Chlamydomonas* sp.	92.7	[6]
Inulin	*Botryococcus* sp.	88.6	[8]
Mung bean protein extract	*Nannochloropsis* sp.	>90	[45]
Moringa seed flour	*Chlorella vulgaris*	89	[46]
Poly-γ-glutamic acid	*Chlorella vulgaris*, *Nannochloropsis oculata*, and *Phaeodactylum tricornutum*	>90	[47]
Neem leaves	*Chlorella* sp. *Scendesmus* sp. *Synechocystis* sp. *Spirulina* sp.	52.3	[48]
Banana peels	*Synechocystis* sp. *Spirulina* sp. *Scendesmus* sp.	48.5	[48]
Moringa seed protein	*Chlorella* sp. *Scendesmus* sp.	75	[11]
Cactus pod	*Chlorella* sp. *Scendesmus* sp. *Spirulina* sp.	60.1	[48]
Pomegranate peels	*Chlorella* sp. *Scendesmus* sp.	50	[48]
Moringa oleifera De-oiled seed cake	*Scendesmus obliquus*	80.33	[49]

Figures

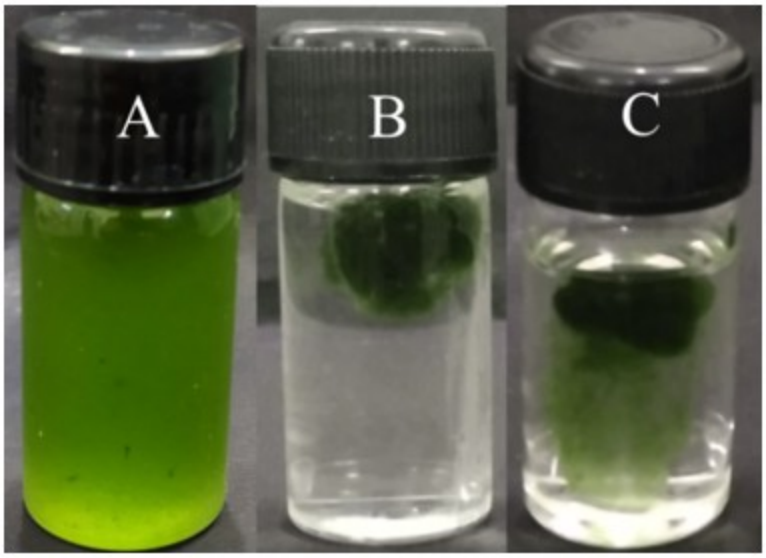


Figure 1

Bioflocculation efficiency of *Chlorella*sp. (a) Control (b) *Moringa* leaf protein (c) soybean protein at pH 2.0

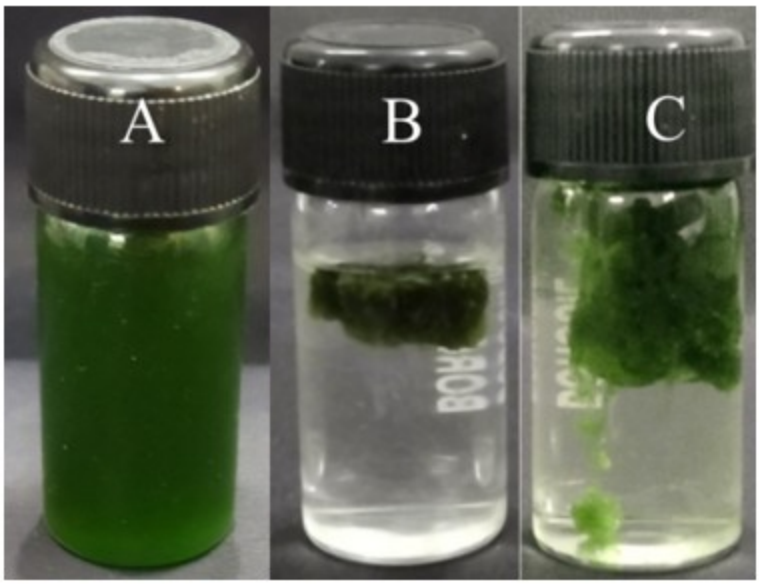


Figure 2

Bioflocculation efficiency of *Dunaliella* sp. (a) Control (b) *Moringa* leaf protein (c) soybean protein at pH 2.0

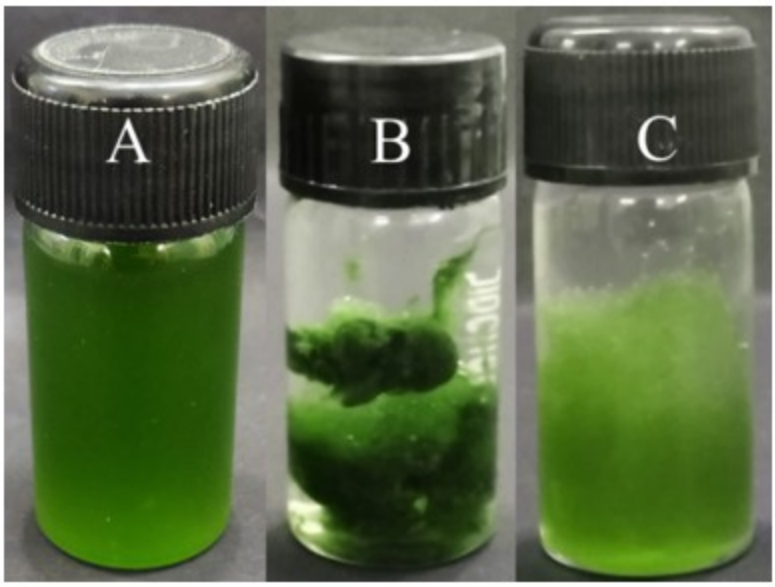


Figure 3

Bioflocculation efficiency of *Synechococcus* sp. (a) Control (b) *Moringa* leaf protein

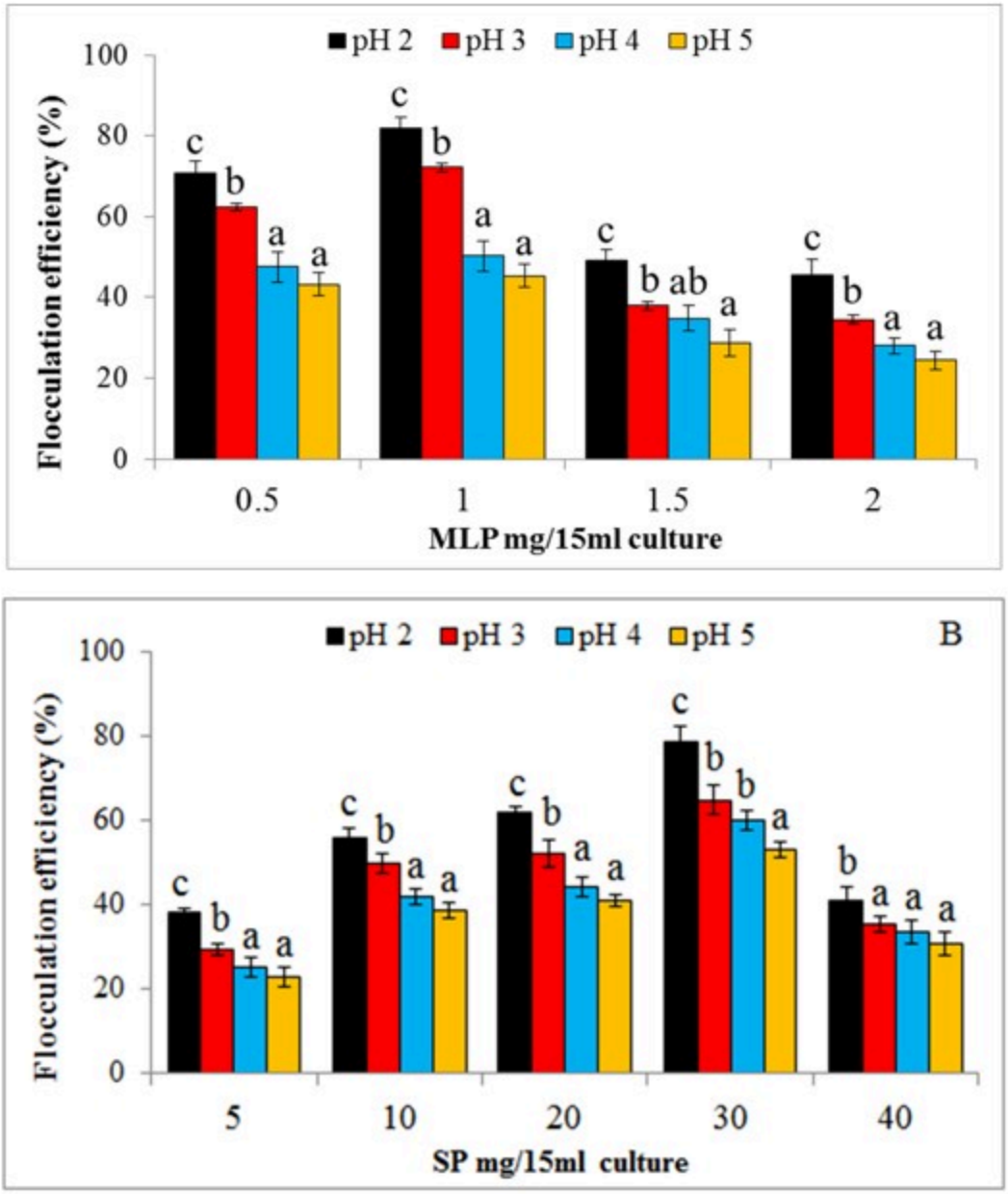


Figure 4

Effect of MLP (a) and SP (b) dose and pH on the bioflocculation efficiency of *Chlorella* sp.

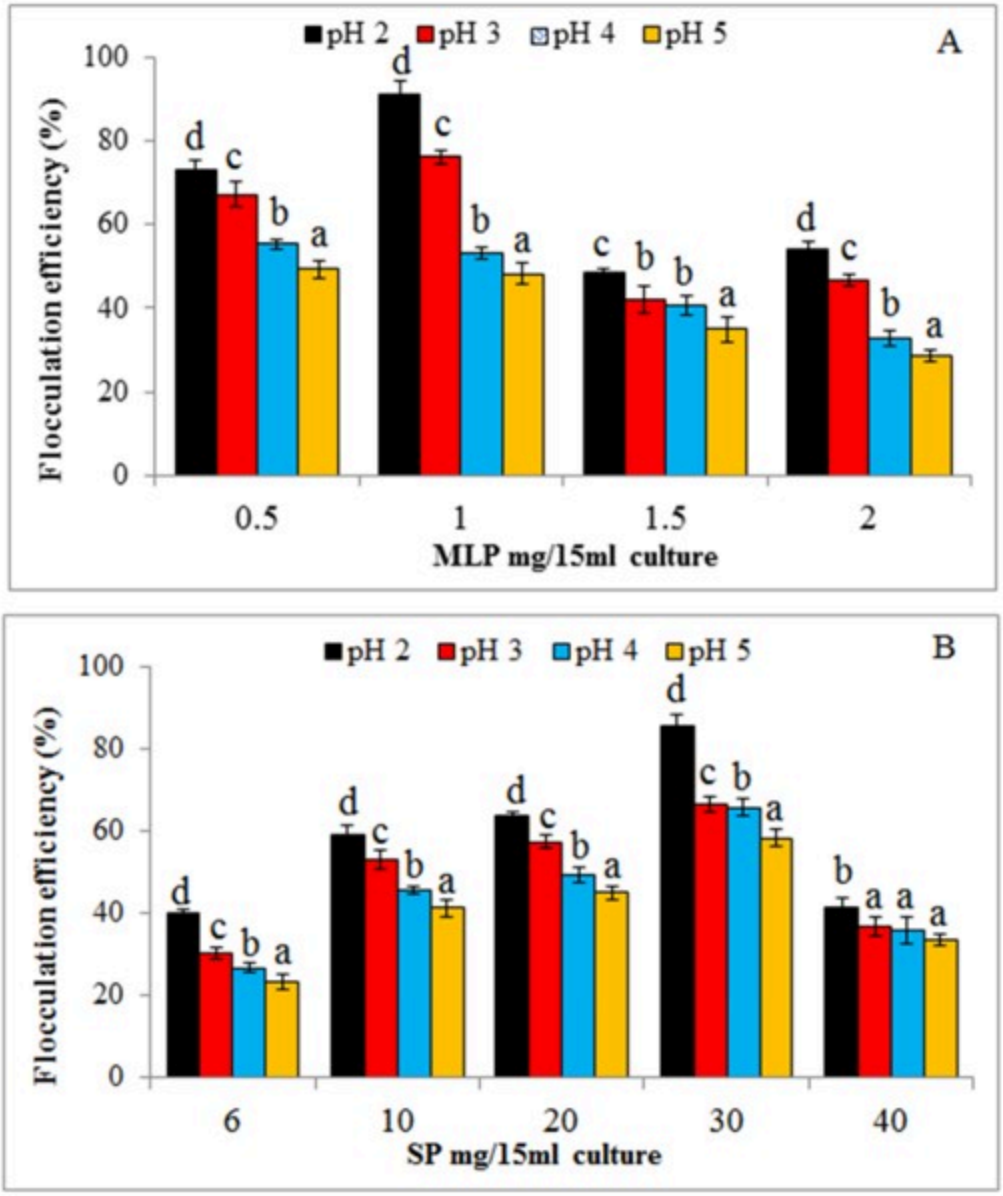


Figure 5

Effect of MLP (a) and SP (b) dose and pH on the bioflocculation efficiency of *Dunaliella* sp.

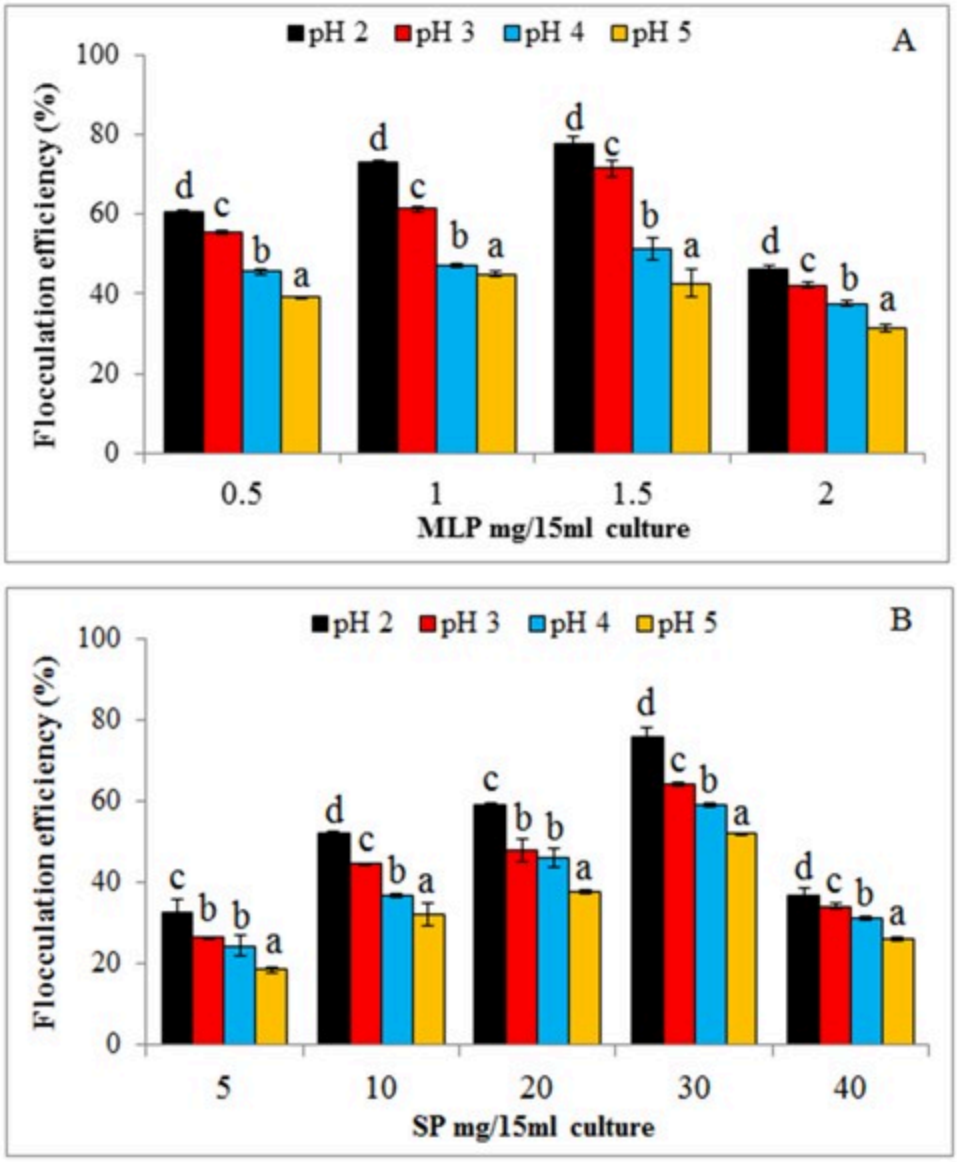


Figure 6

Effect of (MLP (a) and SP (b) dose and pH on the bioflocculation efficiency of *Synechococcus* sp.

Supplementary Files

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