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Enhancing the harvesting efficiency of *Tetraselmis* sp. biomass from aquaculture wastewater via electrocoagulation technology

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Abstract. Harnessing nutrients in wastewater to cultivate microalgal biomass offers significant industrial and environmental benefits. However, the tiny size of marine microalgae *Tetraselmis* sp. makes traditional filtration methods ineffective, leading to high harvesting costs and energy consumption. This study deals with electrocoagulation and flocculation in recovering *Tetraselmis* sp. biomass from aquaculture wastewater, both individually and in combination, regarding their effectiveness. The results show that with an algal density of $1.16 \pm 0.28 \times 10^6$ cells/mL in shrimp farming wastewater (salinity 25.0 ± 1.8‰, pH 8.3), combining flocculation (2.5 mg chitosan/L, pH adjusted to 8.2) with electrochemical treatment (TiO_2 and RuO_2-IrO_2-TiO_2 electrodes, 12 V, 3.5 A) achieved a recovery efficiency of 92% after 21 min. This method also minimized cell damage, yielding biomass with 8.5% protein, 2.4% lipid, and 23.9% carbohydrate. However, when applied individually, electrochemical treatment attained a recovery efficiency of only 37%, and flocculation reached an efficiency of 22%. These findings highlight the significant potential of electrocoagulation for harvesting *Tetraselmis* sp. biomass from aquaculture wastewater.

Keywords: marine microalgae, *Tetraselmis* sp., chitosan, electrocoagulation, biomass harvesting

1 Introduction

Microalgae hold significant potential in various fields ranging from wastewater treatment and CO_2 absorption to renewable energy production. They are capable of producing biofuels such as biodiesel, biomethane, bioethanol, biohydrogen, and biobutanol, which can replace fossil fuels and meet up to 25% of global energy demand [1].

Additionally, algal biomass is widely utilized as aquaculture feed, livestock feed, and the production of dietary supplements, cosmetics, and pharmaceuticals.

However, the commercial production of microalgal biomass faces numerous challenges, particularly in the harvesting process, which accounts for 20–30% of the total biomass production cost [1, 2]. The small size (5~50 μm), low biomass concentration (0.5~5 g/L), and negatively charged surface (–7.5~–40 mV) of microalgae make the harvesting process complex [1–3]. As a result, developing efficient and economical harvesting technologies is essential for scaling up microalgal biomass production to meet industrial demands [1].

Tetraselmis (Chlorodendrophyceae, Chlorophyta) is a marine microalga with a size of 5–20 μm that contains a high level of lipids and has potential for biofuel production [4, 5]. However, its small size makes it difficult to separate from the cultivation environment with traditional filtration methods. Therefore, searching for an efficient method to harvest *Tetraselmis* sp. without

affecting its nutritional quality is a crucial step for the industrial production of this microalga.

Physical, chemical, biological, and electrochemical methods [1–3, 6–17] have been used to improve efficiency and reduce harvesting costs. Among these, flocculation-settling combined with centrifugation is commonly used [1]. However, a high concentration of chemical coagulants (such as aluminum sulfate and ferric chloride) can negatively affect cell quality and the environment [1, 3, 6]. The electrochemical method stands out thanks to its advantages, such as minimal impact on the quality of bioactive compounds, low operational costs, high efficiency, and low toxicity [1, 3, 7, 8]. This method utilizes metal ions from electrode oxidation and small bubbles to coagulate the microalgae [7], neutralizing the negative charge on the cell surface and leading to cell aggregation and floatation [8]. Electrochemical methods have a lower harvesting cost (0.35 USD/m^3) than chemical coagulation (0.47 USD/m^3) and centrifugation (0.53 USD/m^3) [9]. The drawbacks of electrochemical methods include a short electrode life and a risk of biomass contamination with metal oxides [7].

An ideal algae harvesting process should ensure high efficiency with reasonable costs, avoid contaminating the biomass, not create secondary pollutants, and allow the reuse of the culture solution [1, 2]. The choice of a harvesting method depends on the final product and specific characteristics of the microalgae, such as acceptable moisture levels, salt concentration, and cell damage [1].

Chitosan, a natural biopolymer with cationic amine groups, is commonly used as an environmentally friendly flocculant in microalgae harvesting [10]. Since colloidal particles (polymers) in nature often carry a negative charge (including microalgae), electrostatic interactions between cationic and anionic polymers lead to the formation of flocs [10]. However, because chitosan has a low surface charge density, it needs to be used in large quantities or combined with other strong flocculants to achieve effective coagulation. Moreover, the chemical processes involved in these combined methods can be challenging to control [10]. For example, polyaluminum chloride is frequently added to enhance chitosan's flocculation efficiency by improving charge neutralization. While alkaline conditions can increase this effectiveness, they are difficult to control and pose environmental risks [10]. Thus, a simpler and more sustainable approach is needed to enhance chitosan's performance in microalgae harvesting. This paper evaluates the efficiency of harvesting Tetraselmis sp. biomass from aquaculture wastewater using electrocoagulation with chitosan and electrolysis.

In this study, electrolysis using inert electrodes consisting of a titanium dioxide (TiO_2) cathode and a ruthenium oxide-iridium oxide-titanium dioxide (RuO_2-IrO_2-TiO_2) anode without chemical additives, easily controlled by an electric switch, helpd to safely and efficiently harvest microalgal biomass as a feed supplement. These electrodes were chosen for their high stability and corrosion resistance, preventing chemical contamination and ensuring clean biomass production [18]. Although more expensive than other alternatives like stainless steel or iron, these elctrodes have better durability and long-term performance, making them ideal for sustainable operations. The coagulation with chitosan and electrolysis was tested both individually and in combination to evaluate their feasibility in harvesting microalgae from brackish water under different conditions of chitosan dosage, pH, and electrolysis time.

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2 Methods

2.1 Cultivation of marine microalgae in aquaculture wastewater

The marine microalgae *Tetraselmis* sp. were cultivated in the wastewater collected from shrimp farming in Vinh Thanh commune, Phu Vang district, Thua Thien Hue province. According to previous experiments, *Tetraselmis* sp. did not grow well in untreated aquaculture wastewater because of the competition from bacteria and unwanted algae. To address this, we pre-treated the wastewater by filtering through sand to remove suspended solids (SS) and disinfected using UV radiation (417 mJ/cm^2) in combination with ozone (100%) and liquid-thin films (65 L/min) for 30 min to eliminate microorganisms [19]. The wastewater collected in May 2024 had the following characteristics: salinity 25.0 ± 1.8‰, pH 8.2 ± 0.4, alkalinity 86.4 mg $CaCO_3$/L, total dissolved iron (Fe) 0.15 mg/L, total nitrogen concentration 10.8 ± 0.8 mg/L, and total phosphorus 0.7 ± 0.4 mg/L. *Tetraselmis* sp. inoculum was added at a ratio of 10% (V_inoculum/V_wastewater), with continuous aeration (9.4 L/min), illumination with white LED lights at an intensity of 101 µmol/m^2/s, and a light/dark cycle of 18 hours light/6 hours dark, maintaining a temperature of 29–32 °C. After 10 days, the algae grew from light green to dark green, reaching a cell density of 1.1–1.2 × 10^6 cells/mL, equivalent to 0.56 g/L (dry weight), and were used for biomass harvesting experiments. *Tetraselmis* sp. cells are green, oval, slightly elongated, and measure 20.2 ± 0.2 × 15.8 ± 0.6 µm (measured under an Olympus BX51 microscope, Japan).

2.2 Cell density determination

Cell density was determined by using the cell counting method with a Sedgewick Rafter counting chamber (Germany) (1 mm^2, 1000 grid squares, corresponding to a volume of 1 mL) and a cover glass on an Olympus CX33 optical microscope (Japan). The cells were counted under optical magnification at 10× and 20× [20].

2.3 Determination of dry biomass weight

The dry biomass weight of *Tetraselmis* sp. was determined by using a standardized method, following the SMEWW-2540-TSS-D protocol [21]. The procedure involved filtering the algal suspension through pre-weighed, standard-grade filter paper (GF/F Whatman, UK) with a pore size of 0.45 µm. The filtered samples were then dried at 103–105 °C in a drying oven until a constant weight was achieved. The increase in weight, as compared with the initial weight of the filter paper, was attributed to the dry algal biomass (expressed in g/L). This method ensured accurate quantification of the algal biomass, which is crucial for evaluating the efficiency of the harvesting techniques employed.

2.4 Biomass harvesting via gravity sedimentation

The harvesting of *Tetraselmis* sp. biomass was conducted by using the gravity sedimentation method within a 20-litre acrylic column ($D \times H$ = 192 × 692 mm) (Fig. 1). Eighteen litres of microalgal suspension were thoroughly mixed and then allowed to settle undisturbed for 12 hours. Water samples were collected at specific time intervals of 0, 2, 4, 6, 8, 10, and 12 hours. For each time point, a composite sample was prepared by combining three subsamples collected at varying depths (150, 250, and 350 mm). After a 12-hour sedimentation period, the supernatant was carefully decanted, and the settled biomass was filtered through a 20-µm mesh cloth filter. The experiment was performed in triplicate to ensure reproducibility.

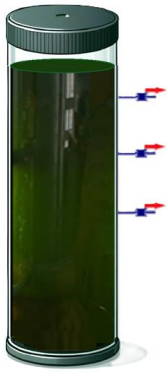

Fig. 1. Sedimentation experiment setup

2.5 Biomass harvesting via electrocoagulation

The electrocoagulation process was conducted in 500 mL beakers with a titanium dioxide (TiO_2) cathode and a ruthenium oxide-iridium oxide-titanium dioxide (RuO_2-IrO_2-TiO_2) anode (Huetronics, Vietnam) (Fig. 2). These electrodes, each with a surface area of 7 cm^2, were positioned 4 cm apart to ensure efficient ion migration and interaction. The electrodes were connected to a 12-volt direct current (DC) power supply. The setup included an adapter (LE350-24 Jetek, Huetronics) to convert alternating current (AC) to DC, along with a voltage regulator (3590S-2-104L, Bourns®, Mexico) for precise voltage control. The current flowing through the algae suspension was monitored with an ammeter when the DC current facilitated the release of cations from the anode and hydroxyl ions from the cathode. These ions disrupted the stability of the negatively charged microalgal cells, effectively neutralizing their charge and promoting coagulation. This coagulation resulted in the aggregation of the microalgae, allowing for efficient biomass recovery.

The electrocoagulation efficiency was assessed under gentle stirring conditions to enhance ion distribution and minimize shear stress on the algal cells [12]. A magnetic stirrer (MSH-20A, Korea) was used, with an electrode influence area of 56 cm^2 and a current density of 0.1 A/cm^2. The electrolysis duration was varied across multiple time points (5, 10, 15, 20, 25, 30, 60, 120, 180, 240, and 300 seconds) to determine the optimal conditions for biomass recovery. After each electrolysis period, the solution was stirred for an additional 30 seconds to promote uniform settling, then allowed to rest for 10 min. A 5 mL water sample was subsequently collected from a depth of 5 cm below the surface to measure the cell density. This procedure was repeated three times to ensure the reliability and reproducibility of the results.

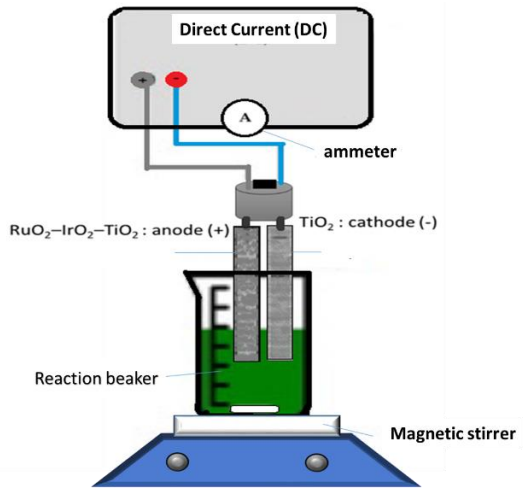

Fig. 2. The electrocoagulation experiment setup

2.6 Biomass harvesting via flocculation with chitosan and sedimentation

A chitosan flocculant solution was prepared by dissolving 10.0 g of chitosan $(C_6H_{11}NO_4)_n$ (China) in 1.0 L of 4% acetic acid (Merck), yielding a final concentration of 10.0 g/L with a pH of 2.9. The solution was stored at ambient temperature and utilized within the same day to ensure optimal flocculation performance.

The flocculation experiments were conducted by using a Jartest apparatus (JLT4 Velp Scientifica, Italy) with four stirring units, under controlled conditions at 24–26 °C (Fig. 3). Each 500 mL beaker contained 200 mL of microalgal suspension, to which chitosan was added at

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concentrations ranging from 2.5 to 875 mg/L. Rapid stirring at 100 rpm for 1 min was initially applied to ensure even dispersion of the flocculant. This was followed by slow stirring at 30 rpm for 14 min to facilitate floc formation. The suspension was then allowed to settle undisturbed for 20 min, after which a 5 mL sample was drawn at a depth of 5 cm below the surface to assess cell density. Each experiment was performed in triplicate to ensure reproducibility.

The pH of the suspension was adjusted with 0.10 N HCl and 1.0 N NaOH solutions (Merck), and monitored with a HANNA HI8424 pH meter, as the efficacy of chitosan flocculation is pH-dependent. Following the determination of the optimal chitosan dose, the biomass was harvested by allowing the suspension to settle for 35 min. The supernatant was carefully removed by using a flexible plastic tube and bulb, and further clarified by passing the remaining solution through an Imhoff funnel (750 mL) for an additional 10 min. The concentrated biomass was then filtered through a 20-µm mesh cloth filter to obtain the final product.

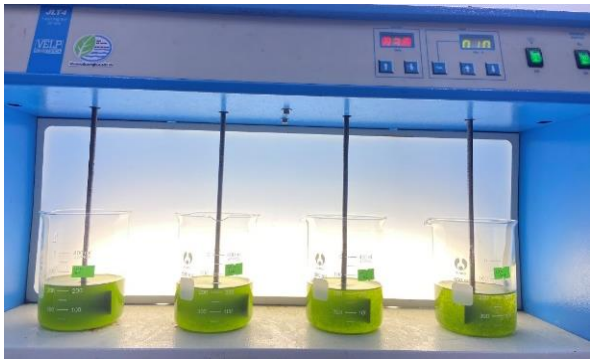

Fig. 3. Chitosan flocculation setup using Jartest apparatus

2.7 Biomass harvesting via electrocoagulation flocculation

The experimental setup for electrocoagulation-flocculation is described in Fig. 2, with the same electrochemical apparatus detailed in Section 2.5. Each 500 mL beaker contained 200 mL of microalgal suspension. Chitosan was added to the suspension, and the pH was adjusted to 8.2 with 0.10 N HCl and 1.0 N NaOH solutions. The mixture was first stirred rapidly at 100 rpm for 1 min to ensure the even distribution of chitosan. This was followed by slow stirring at 30 rpm for 10 min to facilitate the formation of flocs. In the last minute of flocculation, the electrocoagulation device was activated, applying a current to enhance floc formation and promote the aggregation of microalgae.

After the flocculation-electrocoagulation process, the suspension was allowed to settle for 10 min. A 5 mL sample was then carefully drawn at a hight of 5 cm above the bottom of the beaker to measure the cell density. The efficiency of biomass recovery was evaluated across varied chitosan concentrations (2.5 to 10 mg/L) and electrolysis time (10 to 60 seconds). Each experiment was conducted in triplicate.

2.8 Nutritional analysis of algae

The nutritional composition of the harvested *Tetraselmis* sp. biomass was analyzed to determine moisture content, protein, lipid, and carbohydrate levels. The analyses were conducted according to the standardized procedures outlined in methods KT2.QT.CH-059, KT2.QT.CH-057 (2022), KT2.QT.CH-058 (2022), and KT2.QT.CH-054 (2022) provided by the Quality Assurance and Testing Centre 2 (QUATEST 2), Vietnam.

2.9 Data analysis methods

Experimental data were collected, processed, and presented by using the Microsoft Excel software. The efficiency of algae biomass harvesting was calculated according to formula (1), described by Harith et al. [13].

$$\text{Efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where c_i is the initial cell density (cells/mL); c_f is the cell density in the solution after sedimentation.

3 Results and discussion

3.1 Algal biomass harvesting via gravity sedimentation and filtration

The initial density of *Tetraselmis* sp. was determined at 1.19 ± 0.21 × 10^6 cells/mL, setting a baseline for evaluating the effectiveness of the gravity sedimentation method. Over time, the sedimentation efficiency improved significantly, beginning at 40% after 2 hours, increasing to 61% after 6 hours, and ultimately reaching 80.8% after 12 hours. This progressive increase in efficiency reflects the natural settling behaviour of the algal cells as they gradually aggregate and settle to the bottom of the column.

The subsequent filtration of the sedimented biomass proceeded at a rate of approximately 4 min/L, culminating in an overall harvesting efficiency of 83%. However, the small cell size of *Tetraselmis* sp. posed inherent challenges during filtration. These challenges include the potential for filter cloth clogging, which not only extends the filtration time but also risks reducing the efficiency of biomass recovery if the filtration process was disturbed by excessive agitation. Such agitation can cause algal cells to bypass the filter pores, leading to lower recovery rates. While this method demonstrates a high recovery efficiency (>80%), it is notably time-intensive and requires substantial space, which may limit its scalability and practicality in industrial applications. These considerations are crucial when evaluating the method's feasibility for large-scale biomass production, where efficiency, time, and space are critical factors.

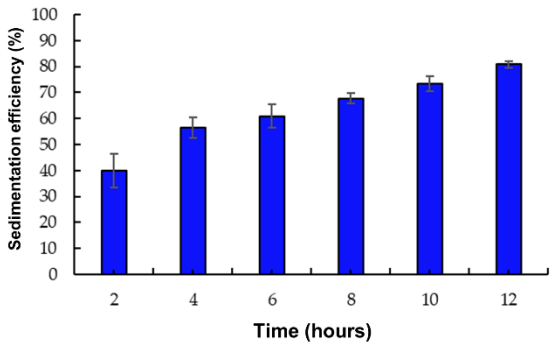

Fig. 4. Gravity sedimentation efficiency of *Tetraselmis* sp. over time

3.2 Biomass recovery via electrocoagulation

Mechanical stirring during electrocoagulation, as noted by Lee et al., enhances the mobility of charged particles, facilitating floc formation and reducing electrolysis time [12]. In our study, gentle magnetic stirring was employed to optimize the recovery of *Tetraselmis* sp. biomass.

Starting with a density of 1.19 ± 0.21 × 10^6 cells/mL, salinity of 25‰, and pH of 8.3, the electrocoagulation process induced rapid changes (Fig. 5 and Fig. 6). Within the first 5 to 25 seconds, white bubbles formed, a chlorine-like odour was detected, and the suspension color shifted from dark green to pale (Fig. 5). This was similar to the electrolysis of NaCl in high-salinity brackish water, producing NaClO, which may have contributed to cell membrane disruption and subsequent degradation. Microscopic observations at 40× magnification showed that the initially motile, green *Tetraselmis* sp. cells progressively lost mobility and chlorophyll pigmentation (Fig.s 5b–f).

The recovery efficiency increased from 16.4% after 5 seconds to 58.3% after 300 seconds (Fig. 6a). After 60 seconds, the biomass aggregated at the bottom of the beaker, yielding a dry weight of 0.049 g/L, significantly lower than the initial 0.56 g/L (Fig. 6b). Prolonged electrolysis resulted in salt crystal formation, indicating cell

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dehydration (Fig. 6c). While effective in destabilizing and aggregating cells, electrocoagulation may not be suitable for nutritional biomass recovery because of cell damage, but it shows promise for environmental applications, such as controlling harmful algal blooms.

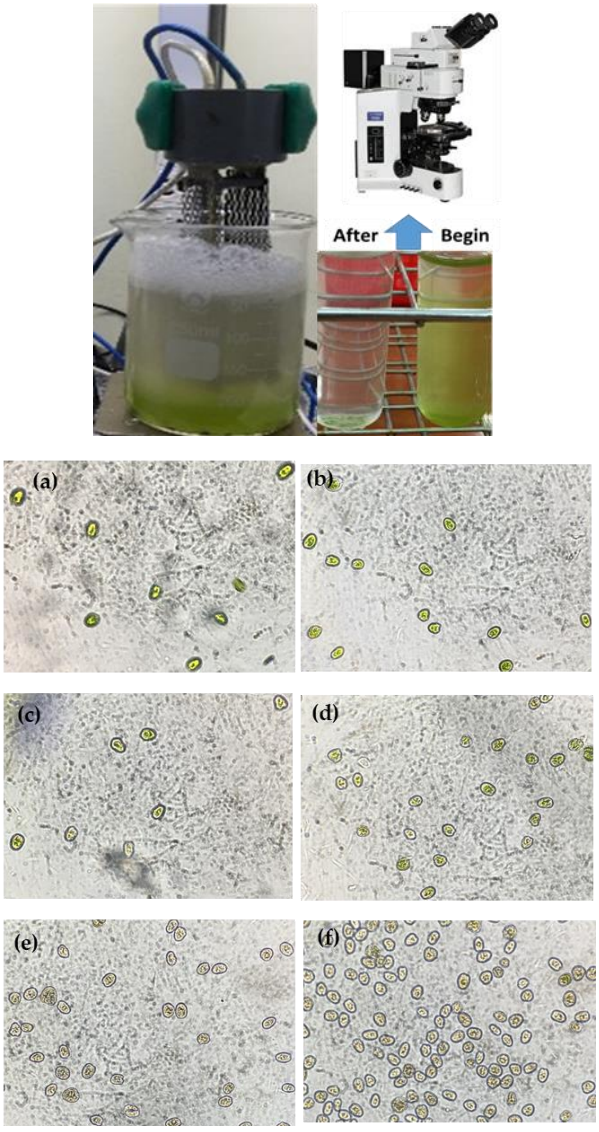

Fig. 5. Effects of electrocoagulation on *Tetraselmis* sp.: Microscopic images at 40× magnification showing cells at (a) initial; (b) 5 seconds; (c) 10 seconds; (d) 15 seconds; (e) 20 seconds; (f) 25 seconds

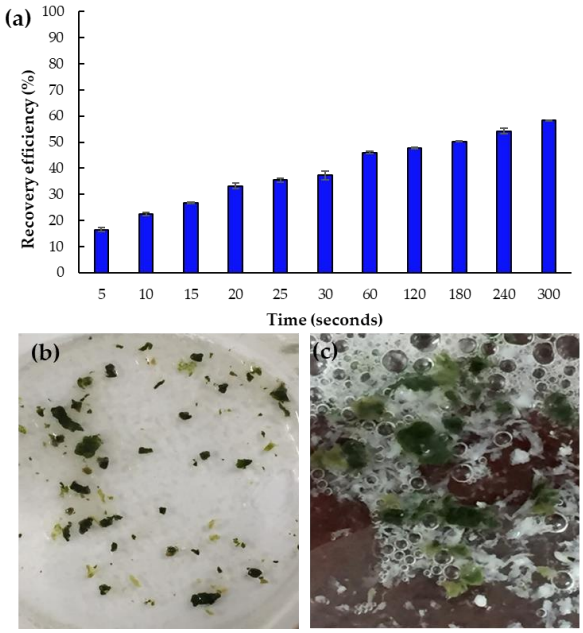

Fig. 6. Biomass recovery of *Tetraselmis* sp. by using electrocoagulation combined with magnetic stirring: (a) Biomass recovery efficiency; (b) Biomass precipitation after 60 seconds of electrocoagulation; (c) Biomass precipitation after 300 seconds of electrocoagulation

3.3 Biomass harvesting via flocculation with chitosan

The flocculation experiments were conducted at 25 °C with an initial *Tetraselmis* sp. density of 1.31 ± 0.14 × 10^6 cells/mL and an initial pH of 8.3. The recovery efficiency was found to be strongly dependent on both chitosan concentration and pH. As the chitosan dosage increased from 2.5 to 875 mg/L, the pH of the suspension decreased correspondingly from 7.4 to 2.7, which adversely affected the flocculation process. Without pH adjustment, the recovery efficiency remained suboptimal, and water separation was challenging. For instance, at a chitosan concentration of 625 mg/L, the pH dropped to 2.9, causing over-protonation of chitosan, which hindered its interaction with negatively charged cell surfaces, resulting in smaller, less stable flocs and a recovery efficiency of only 42.5% after 35 min of sedimentation. In contrast, adjusting the pH to a neutral level of 7.0 resulted in the formation of larger, more robust flocs,

significantly enhancing recovery efficiency. Under these conditions, the efficiency improved markedly from 20 to 82% after 35 min (Fig. 7a), and further increased to 40−100% after 10 hours of sedimentation (Fig. 7b). This underscores the pivotal role of pH in the flocculation process.

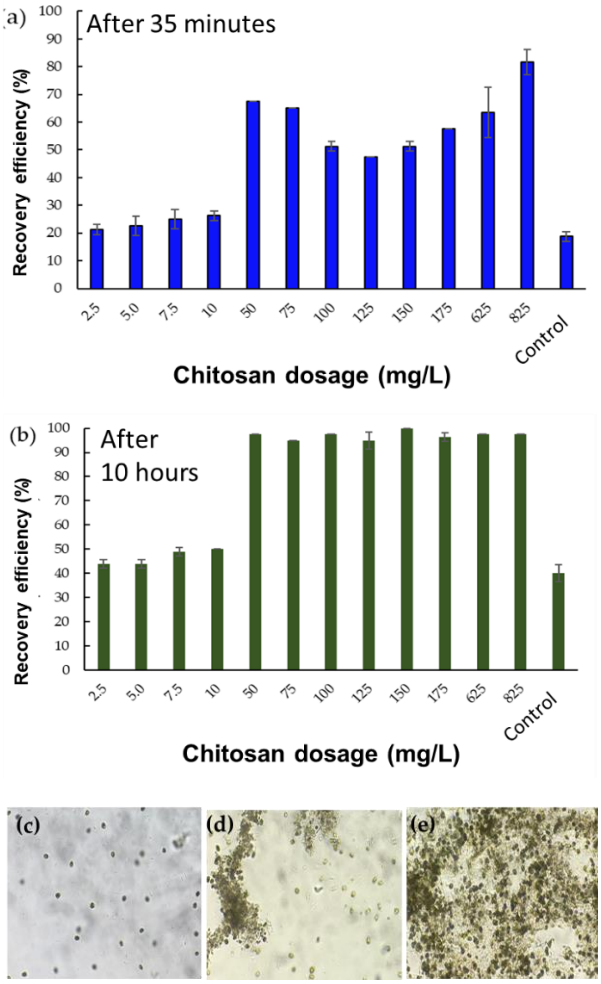

Fig. 7. Effects of chitosan dosages (2.5–875 mg/L) at pH 7.0 on the recovery efficiency of *Tetraselmis* sp. after (a) 35 min and (b) 10 hours. Microscopic images (10× magnification) of cells in (c) control, (d) 625 mg/L chitosan at pH 2.9, and (e) 625 mg/L chitosan at pH 7.0 after 35 min

Further analysis revealed that recovery efficiency peaked at higher pH levels when chitosan concentration was optimized to fully neutralize the negatively charged cell surfaces, thereby enhancing flocculation efficiency. This indicates that the optimal chitosan concentration depends on the cell density in each sample, promoting more effective aggregation and separation. At a chitosan dosage of 50 mg/L, the recovery efficiency increased from 38.2% at pH 5.9 to a peak of 92.0% at pH 8.1. In contrast, the control experiments conducted without chitosan at pH 8.3 showed a significantly lower recovery efficiency of only 21.8% (Fig. 8). This comparison highlights the synergistic effect of combining optimal chitosan concentration with pH adjustment, which together facilitates more effective biomass aggregation and recovery. Lubián's study also demonstrated that when the pH was adjusted to 8 with chitosan dosages ranging from 40 to 80 mg/L, the efficiency of marine microalgae biomass recovery improved, exceeding 75% [16].

These findings emphasize the necessity of carefully controlling both chitosan dosage and pH to achieve a high-efficiency biomass recovery. Such optimization is particularly critical for scaling up the flocculation-based harvesting processes in industrial applications, where maximizing yield while minimizing resource input is essential.

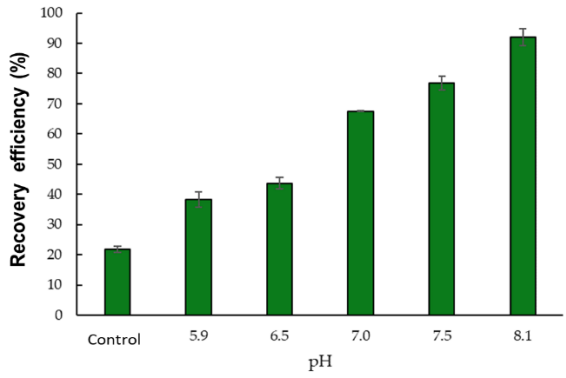

Fig. 8. Effect of pH (ranging from 6 to 8) and 50 mg/L chitosan dosage on the recovery efficiency of *Tetraselmis* sp. after 35 min. The control sample is algae without added chitosan

The effect of pH (6, 7, and 8) on flocculation efficiency at different chitosan dosages (75, 100, and 150 mg/L) revealed that flocculation

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efficiency increased as pH rose from 6 to 8 (Table 1). The microscopic observations showed that *Tetraselmis* sp. cells formed the largest clusters at a chitosan dosage of 150 mg/L at pH 8.2 (Fig. 9). At this optimal dosage and pH (150 mg/L chitosan at pH 8.2), the highest recovery efficiency was achieved, reaching 98.6 ± 1.2% after 35 min. In comparison with the study by Kwon et al. on *Tetraselmis* sp. KCTC12236BP (algal density of 3 g/L), where the recovery efficiency reached only 85.6% with a dosage of 1.2 g/L $Al_2(SO_4)_3$ after 30 min [17]. In this study, we used chitosan—a biological polymer—ensures safer biomass recovery.

Table 1. Recovery efficiency of *Tetraselmis* sp. at different chitosan dosages and pH levels

Chitosan dosage (mg/L)	Recovery efficiency (%)		
	pH 6	pH 7	pH 8
50	46.2 ± 8.7	67.5 ± 0.1	92.0 ± 2.7
75	27.5 ± 3.5	65.0 ± 0.1	90.1 ± 0.2
100	27.5 ± 3.5	51.3 ± 1.8	87.6 ± 3.7
150	28.8 ± 3.6	52.6 ± 1.8	98.6 ± 1.2

After flocculation (150 mg/L chitosan, pH 8.2, 35 min), the supernatant was decanted, and the biomass was filtered and rinsed through a 20 μm mesh cloth filter. The resulting algae biomass was dark green, with the characteristic odour of green algae, containing 85.2% moisture and yielding 5.2 g of wet algae per litre. The nutritional composition (% by dry weight) was as follows: 15.6% protein, 6.4% lipid, and 44.2% carbohydrate.

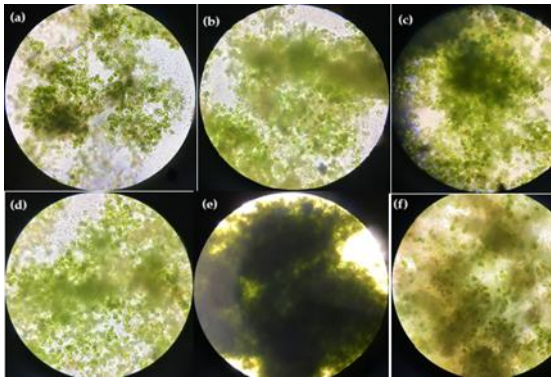

Fig. 9. Microscopic images (40× magnification) of *Tetraselmis* sp. biomass after flocculation with varied chitosan dosages: (a) 50 mg/L; (b) 75 mg/L; (c) 100 mg/L; (d) 125 mg/L; (e) 150 mg/L; (f) 175 mg/L at pH 8 after 35 min

3.4 Algal biomass harvesting by electrocoagulation

In this study, we systematically evaluated the effectiveness of several biomass harvesting techniques, and the combined electrocoagulation and chitosan flocculation method proved to be the most advantageous, offering superior recovery efficiency over other methods. The experiments were conducted at varied chitosan dosages (2.5 to 10 mg/L) at pH 8.2, with electrocoagulation using inert electrodes at a current density of 0.1 A/cm² for 30 seconds at 30 °C. After chitosan was added and pH adjusted, the mixture was rapidly stirred for 1 min and then slowly stirred for 10 min to promote floc formation. The 10 mg/L chitosan dosage produced larger flocs compared with the 2.5 mg/L dosage (Fig.s 10c and 10g). Electrocoagulation, applied during the last 30 seconds of floc formation, generated small bubbles that floated the algal flocs to the surface (Fig.s 10d and 10h). After 10 min of settling, the biomass floated to the surface (Fig.s 10e and 10i), with cells remaining green but losing motility (Fig.s 10f and 10j). The combined method achieved a recovery efficiency of 91.9–94.3% with an initial algal density of 1.16 ± 0.28 × 10^6 cells/mL in a medium with 25‰ salinity (Fig. 10a).

The effect of electrolysis time was further investigated at intervals from 10 to 60 seconds with 2.5 mg/L chitosan at pH 8.2. The flotation

process became pronounced after 30 seconds of electrocoagulation, with the recovery efficiency increasing from 48.0 to 95.2% as the electrolysis time was extended from 10 to 60 seconds (Fig. 10b). However, extending the electrolysis time beyond 40 seconds resulted in cell damage. This was attributed to chlorophyll degradation, cell membrane disruption, and oxidative stress. Therefore, the optimal conditions were identified as a chitosan dosage of 2.5 mg/L, pH 8.2, rapid stirring for 1 min, slow stirring for 10 min, followed by 30 seconds of electrocoagulation, and 10 min of settling. Under these conditions, the biomass recovery efficiency reached 91.9 ± 0.8%. The harvested biomass, after filtration through a 20 µm mesh, was dark green, with the characteristic odour of green algae, containing 80.2% moisture and yielding 5.1 g of wet algae per litre. The nutritional content was 8.5% protein, 2.4% lipid, and 23.9% carbohydrate, according to the dry weight.

Compared with a study by Lee et al., which used electrocoagulation and gravity sedimentation to recover *Tetraselmis* sp. in F/2 medium with 149.5 × 10^4 cells/mL at pH 8.4, 10 V, 9.9 A, and a recovery time of 45.5 min, achieving 91% efficiency [12], this study achieved a similar efficiency (92%) in a shorter time (21 min) and with a lower current intensity (3.5 A) when recovering biomass from shrimp farming wastewater.

In this study, we introduced a combined electrocoagulation and flocculation method that significantly improved microalgae biomass recovery. The approach delivered a high efficiency (>90%), preserved biomass nutritional quality, and reduced energy consumption and processing time. Scalable for industrial applications, particularly in sustainable biofuel and bioproduct production, this method utilized chitosan, a natural biopolymer, as an eco-friendly flocculant [10], offering a greener alternative to chemical coagulants. The optimized parameters paved the way for more efficient, cost-effective, and sustainable large-scale microalgal production, with potential applications in bioenergy, wastewater treatment, and beyond.

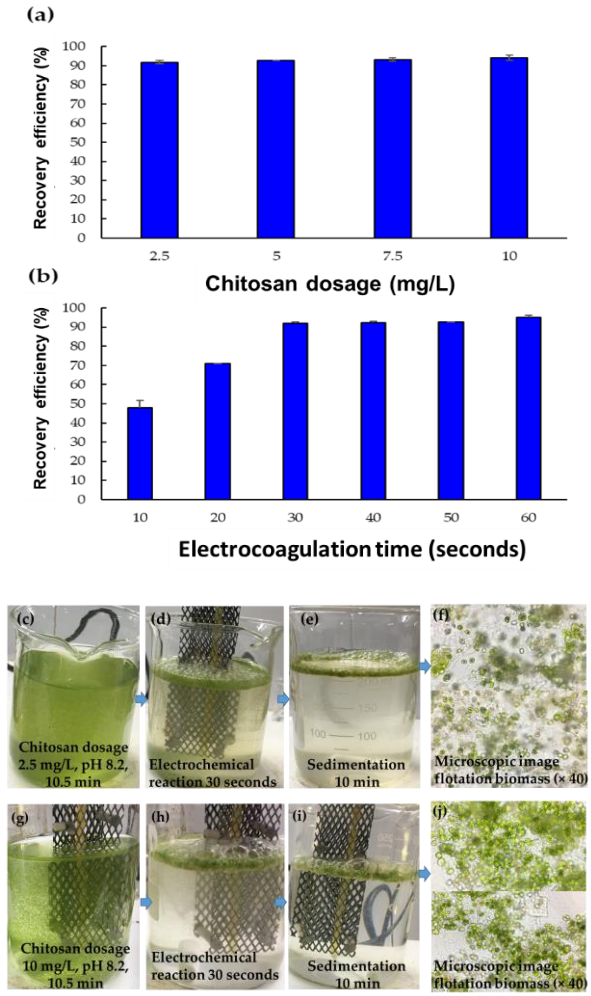

Fig. 10. Biomass recovery efficiency by using electrocoagulation with (a) Chitosan dosages from 2.5 to 10 mg/L and 30 seconds of electrocoagulation; (b) 2.5 mg/L chitosan dosage and electrocoagulation time from 10 to 60 seconds; (c–e) Electrocoagulation with 2.5 mg/L chitosan; (g–i) Electrocoagulation process with 10 mg/L chitosan. Microscopic images (40× magnification) of *Tetraselmis* sp. biomass after electrocoagulation with chitosan dosages of (f) 2.5 mg/L and (j) 10 mg/L at pH 8.2 after 21 min

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4 Conclusion

We successfully develop a highly efficient, safe, and environmentally sustainable method for recovering *Tetraselmis* sp. biomass from shrimp farming wastewater. By integrating electrocoagulation with a minimal chitosan dosage—approximately 1.7% of what is required in traditional flocculation—the method achieves an impressive biomass recovery efficiency of 91.9%. This process involves 2.5 mg/L of chitosan at pH 8.2, with an optimized sequence of rapid stirring (100 rpm for 1 min), slow stirring (30 rpm for 10 min), 30 seconds of electrocoagulation with simultaneous stirring, followed by a 10-min settling period.

Compared with conventional methods, this approach stands out for its practicality and efficiency. Gravity sedimentation, while achieving over 80% efficiency, is hindered by the extensive time and space required, limiting its industrial viability. Standalone electrocoagulation, though simple, yields only 37.2% efficiency and leads to chlorophyll degradation, rendering it unsuitable for nutritional purposes. Although traditional flocculation achieves high recovery rates (>98%), it requires significantly higher chitosan dosages (150 mg/L) and a lower pH, making it less favorable from both environmental and economic perspectives.

The developed electrocoagulation-flocculation method thus represents a significant advancement, offering a balanced solution that maximizes recovery efficiency while minimizing environmental impact. Its potential for scalability and application in industrial contexts, particularly in sustainable aquaculture and biofuel production, positions it as a feasible and innovative approach for large-scale microalgal biomass harvesting.

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Conflict of Interest

The authors declare no conflicts of interest related to the publication of this article.

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