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Dual-purpose shrimp-pond effluent treatment and biomass production for biodiesel using *Desmodesmus sp*

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ABSTRACT

Intensive shrimp farming in Vietnam's coastal regions generates significant organic and nutrient pollution. This study optimizes *Desmodesmus sp.* cultivation for treating shrimp-pond wastewater and producing biodiesel in tropical conditions. Microalgae were cultivated in 4 L photobioreactors with 2–10 % (v/v) shrimp-pond sludge, identifying 6 % as the optimal concentration (growth rate: 0.378 day^{-1}). In a 40 m^3 high-rate algal pond (HRAP), *Desmodesmus sp.* achieved 90 % NH_4-N, 76 % PO_4-P, and 85 % COD removal over 4 days. Biomass harvesting via $Ca(OH)_2$ flocculation at pH 10 was cost-effective, yielding biodiesel-grade biomass with 34 % oleic acid (C18:1) and 33 % palmitic acid (C16:0), meeting EU and US standards. This is the first study to demonstrate efficient treatment of high-salinity (33 g L^{-1}) shrimp-pond wastewater in large-scale HRAPs, offering a scalable, low-cost solution for environmental sustainability and economic benefits in aquaculture-intensive regions like Vietnam's Mekong Delta. However, challenges such as zooplankton proliferation and climatic variability highlight the need for robust operational strategies before broader deployment.

1. Introduction

Shrimp aquaculture is a cornerstone of the global seafood industry, contributing over $40 billion annually to the world economy and providing livelihoods for millions, particularly in coastal regions of developing nations like Vietnam (FAO, 2024). In Vietnam, the Mekong Delta alone produces approximately 1.7 million tons of shrimp each year, accounting for 70 % of the country's aquaculture output and making it one of the world's leading shrimp exporters (Vietnam_Ministry_of_Agriculture, 2024). However, this thriving industry comes at a significant environmental cost. Approximately 30 % of Vietnam's coastal areas suffer from pollution due to the discharge of untreated wastewater from shrimp ponds, laden with organic matter, ammonium (NH_4-N), phosphate (PO_4-P), and chemical oxygen demand (COD) (Nguyen et al., 2023). These pollutants contribute to eutrophication, oxygen depletion, and degradation of coastal ecosystems, threatening biodiversity and the sustainability of aquaculture itself.

The environmental challenges posed by shrimp farming are not unique to Vietnam but reflect a global issue in intensive aquaculture. Conventional wastewater treatment methods, such as chemical precipitation or membrane filtration, are often costly, energy-intensive, and generate secondary waste streams, making them impractical for small- and medium-scale shrimp farmers in developing countries (Kashem et al., 2023). Consequently, there is an urgent need for cost-effective, sustainable solutions that can simultaneously address water pollution and create economic value, particularly in regions like Vietnam where shrimp farming is a vital economic driver.

Microalgae-based wastewater treatment has emerged as a promising alternative due to its ability to assimilate nutrients, reduce organic loads, and produce valuable biomass (Chisti, 2007). Microalgae, such as *Chlorella* sp. and *Scenedesmus* sp., have been successfully used to treat municipal and agricultural wastewater, achieving removal efficiencies of up to 90 % for NH_4-N and 80 % for PO_4-P (Guo et al., 2013; Posadas et al., 2015). Moreover, the biomass generated can be harvested for

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applications such as biofuel production, animal feed, or biofertilizers, creating a circular economy model (Toplicean and Datcu, 2024). However, most studies have focused on low-salinity wastewater (e.g., 5 g L^{-1}), which does not reflect the high-salinity conditions (up to 33 g L^{-1}) typical of shrimp-pond wastewater in coastal regions like Ninh Thuan, Vietnam (Shin et al., 2025). Additionally, while laboratory-scale experiments have demonstrated the potential of microalgae, few studies have scaled up to high-rate algal ponds (HRAPs) under

The microalga *Desmodesmus sp.* is particularly promising for treating saline wastewater due to its tolerance to high salinity and robust nutrient uptake capabilities (Kumar et al., 2024). Unlike other species, *Desmodesmus sp.* can thrive in tropical semi-arid climates, such as that of Ninh Thuan, characterized by high temperatures (28–35°C) and intense sunlight (2000–2500 μmol m^{-2} s^{-1}), which enhance algal growth and pollutant removal efficiency. Furthermore, the use of shrimp-pond sludge as a nutrient source offers a novel approach to reduce treatment costs and recycle waste, creating a closed-loop system with minimal environmental footprint. However, no studies have reported the integration of *Desmodesmus sp.* in large-scale HRAPs for treating high-salinity shrimp wastewater, nor have they explored the simultaneous optimization of wastewater treatment and biodiesel-grade biomass production in such conditions.

This study addresses these gaps by investigating the optimal cultivation conditions for *Desmodesmus sp.* to treat shrimp-pond wastewater in a tropical climate, using shrimp-pond sludge as a low-cost nutrient source. Conducted in Ninh Thuan, Vietnam, the research evaluates the performance of *Desmodesmus sp.* in 4 L photobioreactors and a 40 m^3 HRAP, focusing on the removal of NH_4-N, PO_4-P, and COD. Additionally, it assesses the potential of harvested algal biomass for biodiesel production, analyzing its fatty acid composition against EU and US standards. Unlike previous studies limited to low-salinity wastewater or laboratory-scale systems (e.g., (Shin et al., 2025)), this work pioneers the treatment of high-salinity (33 g L^{-1}) shrimp wastewater in large-scale HRAPs, offering a scalable solution for small- and medium-scale shrimp farmers.

2. Materials and methods

2.1. Geographical and climatic characteristics of the study site

The study was conducted in a shrimp farm in Ninh Thuan Province, Vietnam (11°30′33.4"N 109°00′35.6"E) (Fig. 1), situated within one of Vietnam's major fishing regions. The shrimp farm covers an area of 168 ha and typically operates 2–3 production cycles per year. Ninh Thuan's coastal location in the south-central region ensures consistently favorable water quality conditions for shrimp cultivation. As part of Vietnam's semi-arid zone, the area benefits from high solar irradiance suitable for microalgae production. It features a tropical savanna climate (Aw) with an average annual temperature of 26.78°C and moderate rainfall (~93 mm $year^{-1}$), concentrated in 170 rainy days, representing about 47 % of the year.

2.2. Research methodology

The research methodology comprised two main phases. The first focused on evaluating nutrient removal efficiency across different cultivation systems. Microalgae were first grown in a laboratory-scale photobioreactor to determine optimal nutrient conditions by varying sludge concentrations. This was followed by an outdoor pilot-scale experiment (results not presented) and a full scale HRAP system to assess large-scale applicability as described in Section 2.2.1. The second phase addressed harvesting, dewatering, and biomass extraction as described in Section 2.2.2. An overview of the workflow is shown in (Fig. 2).

2.2.1. . Cultivating microalgae strain Desmodesmus sp

2.2.1.1. Incubating microalgae with shrimp-pond sludge in photobioreactor. The microalgae strain was selected based on previous research identifying a local Vietnamese *Desmodesmus sp.* as an adaptive strain for shrimp wastewater in Ninh Thuan province (Luu et al., 2020). The microalgae were grown in a Tris-Acetate-Phosphate (TAP) (Harris, 1989) under controlled conditions of the temperature at 25 ± 1 °C, with continuous illumination at 40 ± 15 W m^{-2} over 24 hours), in various

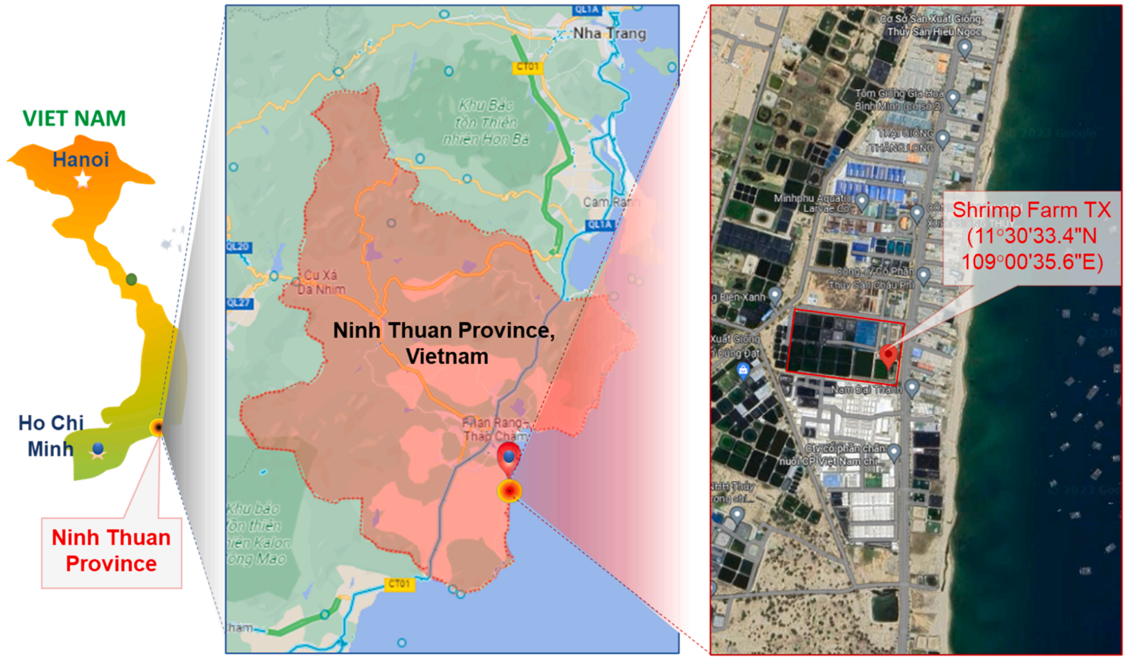

Fig. 1. Location of the study site in Ninh Thuan Province, south-central Vietnam. The map highlights the national context, provincial boundaries, and the shrimp farm TX (11°30′33.4″N, 109°00′35.6″E) where the field experiment was conducted.

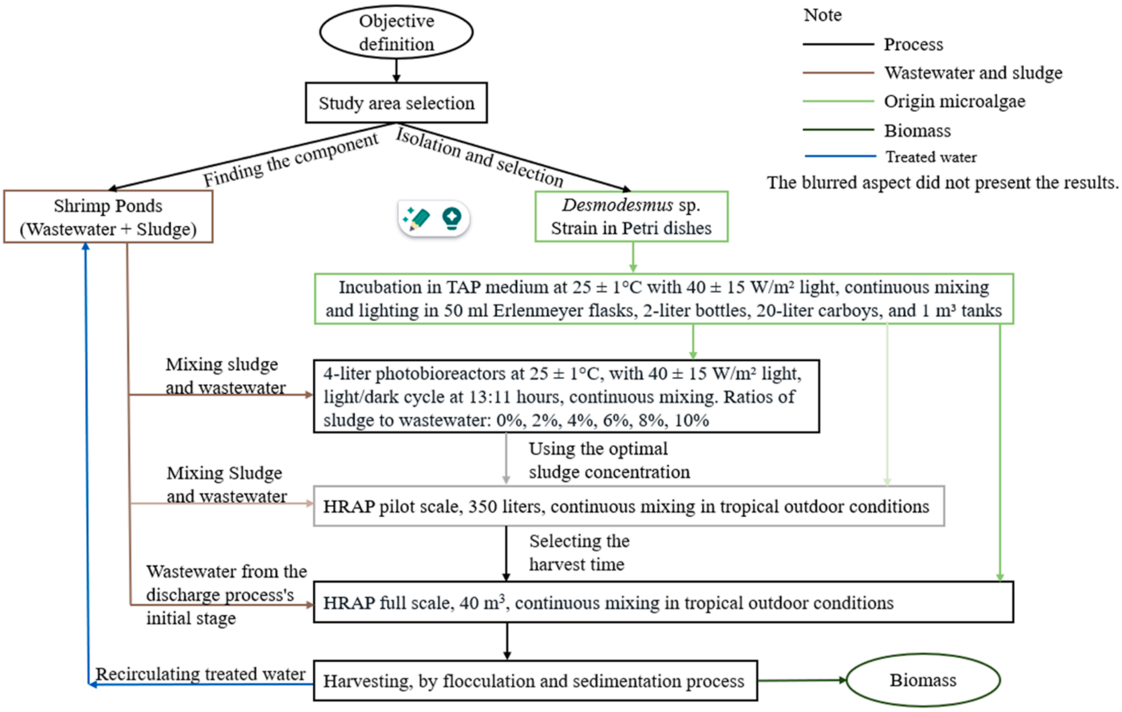

Fig. 2. Conceptual framework illustrating the treatment of shrimp-pond effluent (wastewater and sludge) using *Desmodesmus sp.* and the subsequent biomass production process. The system includes laboratory-scale photobioreactors, pilot-scale, and full-scale HRAPs, with nutrient recovery and treated water recirculation.

containers (10 mL tubes, 250 mL Erlenmeyer flasks, 20 L carboys, and 1.5 m^3 tanks) (Fig S1).

At full scale, *Desmodesmus sp.* was cultivated in 2 m^3 outdoor tanks, sized to supply the initial inoculum required for the HRAPs. Tanks operated at ambient temperature, with sunlight as the primary light source during the day and fluorescent lamps at night. Sterilized seawater with a chlorine concentration of 10 ppm was used, and TAP medium was added to enrich the culture.

To assess the potential of shrimp-pond sludge as a nutrient source for *Desmodesmus sp.*, sludge was incorporated into wastewater at ratios of 0 %, 2 %, 4 %, 6 %, 8 %, and 10 % (Ji et al., 2015). The prepared medium was aerated, allowed to mineralize, and sterilized using a 10 % chlorine solution before the initiation of the cultivation process. The transparent plastic photobioreactors having a volume of 5 L and a working volume of 4 L were used. The cultivation medium was a mixture of sludge and wastewater collected from a two-week-old shrimp pond. Sludge was collected from the shrimp-pond bottom and decanted after settling for 30 minutes. The aeration system consisted of air stones, silicone tubing with an internal diameter of 4 mm, and two air pumps (50 Hz, 200 W) (Fig S2). *Desmodesmus sp.* was inoculated at an initial concentration of 1-2 million cells per ml, and under controlled conditions at 25°C, with fluorescent light (40 ± 15 W m^{-2}) in a 13:11 hours light-dark cycle.

2.2.1.2. Scaling up to full scale HRAP. The full scale HRAP had a total capacity of 130 m^3, with working volumes of 40, 80, and 100 m^3 corresponding to water depths of 0.25, 0.50, and 0.65 m, respectively. The system comprised four zigzag compartments (Fig S3, Fig S4) and was equipped with an airlift aeration system (Royaux, 2018). Operation took place during the dry season (from June to August 2018) under tropical climate conditions, with solar radiation reaching up to 770 W m^{-2}, and an average temperature of 36.6 ± 1.2°C. Mixed sludge/wastewater was pumped directly from the shrimp-pond discharge outlet. Aeration was carried out for 8 hours in the evening using aeration disturbance to inhibit the growth of other algal species. Subsequently, in the early morning, 12 % of the initial microalgae volume was introduced.

2.2.2. *Harvesting biomass using flocculation*

A jar test experiment was carried out to determine optimal coagulating conditions by varying pH and coagulant type. The experiment was performed in TAP medium containing *Desmodesmus sp.* biomass at a TSS concentration of 1 g L^{-1}. The pH range tested was 6 to 10, using NaOH, KOH, and $Ca(OH)_2$ as coagulants.

Based on the laboratory-scale results, an optimal pH was selected for pilot-scale flocculation trials. The pilot-scale tests were conducted in three 1.5 m^3 polyethylene tanks, with continuous monitoring of TSS and settling height over 180 minutes.

At full-scale, biomass was harvested from the microalgae cultivation pond, a 40 m^3 HRAP. The HRAP’s airlift system orifice served as a gravity sedimentation tank for biomass collection. Following flocculation and sedimentation, a portion of the harvested biomass broth was refrigerated, while the remainder was sun-dried. Biomass samples were subsequently sent to an external laboratory for analysis of total lipid and fatty acid content.

2.2.3. *Sampling and analytical procedures*

Sampling and analytical procedures were carried out to assess key parameters related to microalgae cultivation and wastewater treatment. Water and microalgae samples were collected at pre-determined intervals to monitor chemical oxygen demand (COD), ammonium (NH_4-N), dissolved orthophosphate (PO_4-P), total suspended solids (TSS), lipid content, fatty acid methyl ester (FAME), pH, and temperature. Sampling was performed daily between 8:00-9:00 AM, after which samples were centrifuged at 402 rcf for 20 min to separate the water fraction.

The COD was measured using the potassium dichromate method (ISO 6060:1989). NH_4-N concentrations (0.02 to 2.5 mg L^{-1}) were analyzed using the Hatch salicylate method, and dissolved PO_4-P concentrations were determined via spectrophotometry with ascorbic acid reagent (ISO 6878:2004). Total lipid content was quantified using the Folch method (Folch et al., 1957).

FAME analyses were conducted by GC-MS on a Trace GC2000-PolarisQ ion trap mass spectrometer (ThermoScientific, Waltham, MA, USA) coupled with CTC Combi-Pal autosampler (CTC Analytics, Zwingen, Switzerland). The biodiesel properties of *Desmodesmus sp.* biomass were estimated based on the fatty acid methyl ester (FAME) profile (Luu et al., 2020; Shin et al., 2025).

2.2.4. Microalgal growth rate and biomass recovery

The algal growth rate (μ, day^{-1}) was calculated as the slope of the linear regression of the natural logarithm of cell concentration during the exponential phase (Silkina et al., 2025). Cell concentration was determined twice daily by direct cell counting. For each sample, a minimum of five readings were taken, and the mean of the values was used for growth rate calculations (Fig S5).

The biomass recovery efficiency at each biomass concentration value was calculated using the equation:

$$\%R = \left(B_f / B_i\right) * 100$$

Whereas, B_i is the initial dry-biomass equivalent (g) of the known culture volume, and B_f is the filtered dry-biomass.

3. Results and discussion

3.1. The characteristics of wastewater and sludge from shrimp ponds

In a survey of 30 shrimp ponds in the Ninh Thuan region, wastewater discharge was found to range from 2 % to 20 % of the total pond volume (Craeye, 2019). Discharge frequency and volume depended on factors such as shrimp age, feed input, and the amount of uneaten feed remaining in the ponds. Water exchange typically began when shrimp reached a least 12 days of age, coinciding with seawater addition. Based on group data, the production of 1,000 kg of white-leg shrimp (*Litopenaeus vannamei*) in lined ponds on sandy land at a stocking density of 120 individuals m^{-2} required 1,380 kg of feed, resulting in the release of 264 kg of nitrogen, 5 kg of phosphorus, and 12,175 m^3 of wastewater into the coastal environment.

Table 1 presents the analysis of wastewater and sludge samples. The wastewater exhibited relatively low pollutant concentrations, nearing compliance with discharge standards when assessed on a volumetric basis. In contrast, the sludge, accounting for only 2-8 % of the total volume (v/v), contained substantially higher pollutant concentrations and was predominantly in solid form, making it amenable to gravitational settling. These findings highlight sludge management as a critical component in mitigating wastewater pollution from shrimp-pond operations.

Previous studies have demonstrated the suitability of aquaculture wastewater for microalgae cultivation (Christodoulou et al., 2025; Shitu et al., 2024; Simionov et al., 2025). In this study, we investigated the feasibility of enhancing wastewater treatment by incorporating sludge into the process, thereby increasing the treatment system's capacity while supplying essential nutrients for microalgae growth. This approach necessitated a comprehensive evaluation of the adaptive capacity and tolerance of the selected microalgal species within the cultivation medium.

Table 1
Physicochemical characteristics of wastewater and sludge collected from shrimp ponds in this study.

Parameter	Unit	Wastewater	Sludge
pH	-	7.5 ± 0.5	6.6 ± 0.85
Salinity	‰	26.2 ± 7.6	26.2 ± 7.6
Turbidity	NTU	23.3 ± 15.3	2500 ± 700
Dissolved oxygen (DO)	mg•O_2•L^{-1}	3.5 ± 2.2	2.2 ± 1.2
Biochemical Oxygen Demand (BOD_5)	mg•O_2•L^{-1}	50 ± 30	1100 ± 900
Chemical Oxygen Demand (COD) filtered	mg•O_2•L^{-1}	130 ± 100	2250 ± 1750
Chemical Oxygen Demand (COD) non-filtered	mg•O_2•L^{-1}	295 ± 175	5600 ± 1200
Nitrite (NO_2-N)	mg•N•L^{-1}	2.8 ± 4.5	2.5 ± 3.73
Nitrate (NO_3-N)	mg•N•L^{-1}	1.4 ± 2.4	1.05 ± 1.73
Ammonium (NH_4-N)	mg•N•L^{-1}	5.7 ± 4.2	185 ± 148
Total Nitrogen Kjeldahl (TKN)	mg•N•L^{-1}	10.7 ± 9	290 ± 226
Phosphate (PO_4-P)	mg•P•L^{-1}	2.8 ± 3.5	3.25 ± 2.75
Total Phosphorus (TP)	mg•P•L^{-1}	2.5 ± 1.3	3.02 ± 1.2

3.2. Correlation between microalgal growth rate and organic nutrient removal efficiency

Desmodesmus sp. exhibited notable adaptability and growth performance in response to various sludge mixtures (0, 2, 4, 6, 8, and 10 %), as illustrated in Fig. 3. During the lag phase, the adaptation periods extended by 2, 3, 4, and 5 days as the mixed sludge concentrations increased from 0 to 6 %. In the exponential phase, most growth curves were sustained for at least five days, except for 0 % mixed sludge curve (4 days) and the 10 % mixed sludge curve (1 day). By the 11th day, all treatments entered the stationary phase, followed by the death phase. The highest growth rate of *Desmodesmus sp.* (0.387 ± 0.139 day^{-1}) was achieved at 6 % sludge, with growth stabilizing on day 9. The lowest growth rate occurred at a 10 % mixed sludge concentration, where growth slowed markedly by day 7 of the cultivation period.

The results indicate that a mixture of sludge and wastewater from shrimp ponds can support the growth of *Desmodesmus* sp., although nutrient availability remains insufficient for high biomass yields. In the control group (0 % mixed sludge), rapid growth occurred after a brief two-day lag phase, followed by nutrient depletion after day 4 of cultivation. In contrast, adding mixed sludge at concentrations ranging from 2 % to 8 % resulted in elevated NH_4-N levels (from 15 to 42 mg L^{-1}) and increased turbidity (from 71 to 199 NTU). *Desmodesmus sp.* demonstrated improved growth characteristics in response, including an extended exponential phase, with the highest growth rate observed at 6 % mixed sludge.

The addition of mixed sludge also increased turbidity in the cultivation medium, primarily due to organic matter, such as uneaten food, fecal matter, and phytoplankton (Tabrett et al., 2024). High turbidity can inhibit microalgal growth by reducing light penetration and limiting photosynthetic activity. At 10 % mixed sludge concentration, *Desmodesmus sp.* experienced adverse growth conditions, featuring NH_4-N levels of 45.5 mg L^{-1} and turbidity of 232 NTU, resulting in the onset of the death phase within 6 days. This decline was likely driven by the

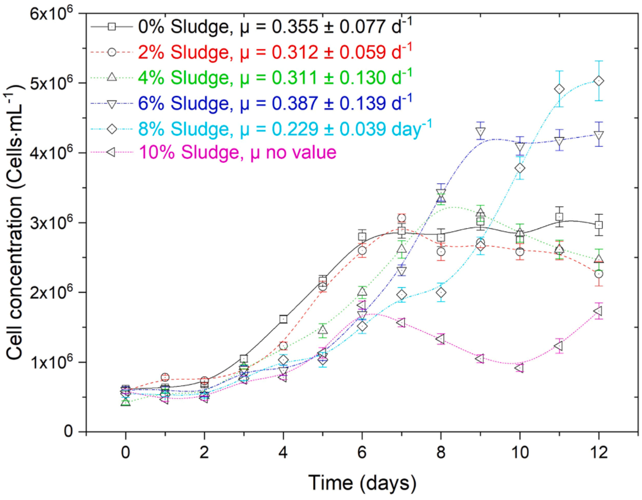

Fig. 3. *Desmodesmus* sp. cultivated in photobioreactors supplemented with shrimp-pond wastewater containing varying sludge concentrations (0–10 %). Cell concentration (cells mL^{-1}) was monitored over a 12-day period, and specific growth rates (μ) were calculated for each treatment.

combined effects of high initial turbidity, self-shading, and reduced light penetration (Shayesteh et al., 2023). While previous studies have shown that turbidity up to 417 NTU did not negatively affect *Chlorella* (Serejo et al., 2021), high NH_4–N concentrations such as those in this study are generally inhibitory to algal growth. However, the exact cause of inhibition remains unclear, as *Desmodesmus sp*. has previously grown well in TAP medium with NH_4-N concentrations as high as 400 mg L^{-1} (Luu et al., 2020).

In this study, *Desmodesmus sp.* demonstrated growth rates ranging from 0.23 to 0.39 day^{-1} in mixed sludge wastewater, exceeding values reported in prior studies conducted under stable temperature conditions with alternative nutrient sources. For comparison, Gang et al. (2015), Pan et al., (2011), and Eze et al., (2018) reported growth rates ($μ_{max}$) of *Desmodesmus sp.* ranging from 0.12 to 0.21 day^{-1} in various nutrient media (Eze et al., 2018; Gang et al., 2015; Pan et al., 2011). Conversely, LongZao et al. (2019) observed growth rates fluctuating from 0.26 to 0.56 day^{-1} in piggery wastewater . Notably, de Mattos and Bastos (2016) achieved growth rate of up to 3.6 day^{-1} when cultivating green *Desmodesmus sp.* under heterotrophic conditions at 25°C (De Mattos and Bastos, 2016; Gaspar et al., 2017). Ferro (2019) reported a growth rate of up to 1.18 day^{-1} for *Desmodesmus sp.* RUC2 strain in outdoor cultivation with municipal wastewater, showcasing the substantial growth potential of *Desmodesmus sp.* in outdoor culture conditions, particularly when utilizing nutrient sources derived from the solid sludge of shrimp ponds (Ferro, 2019).

Table 2 shows that all of mixed sludge wastewater treatments met the regional discharge standards established by QCVN 24: 2009 type B, the national technical regulation of Vietnam on industrial wastewater for nutrient (NH_4-N and PO_4-P) and organic carbon (COD) removal. NH_4-N removal efficiency exceeded 99 % within a 12-day period for mixed sludge concentrations of 4 %, 6 %, and 8 %, corresponding to removal rates ranging from 3.8 to 4.5 mg L^{-1} day^{-1}.

Comparative studies on *Desmodesmus* across different cultivation systems have reported considerable variation in nitrogen removal rates. For instance, Luo et al. (2019) achieved a total nitrogen removal efficiency of approximately 98.3 %, with a removal rate of 20.05 mg L^{-1} day^{-1} using undiluted piggery wastewater over eight days at 35°C (LongZao et al., 2019). Ji et al. (2014) observed complete NH_4-N removal (100 %) from anaerobic digestion wastewater, with a removal rate of 5.3 mg L^{-1} day^{-1} over 14 days at 24°C (Ji et al., 2014). Ammonium, the preferred nitrogen source for algae, is readily assimilated into algal cells, with treatment efficiency depended on hydraulic retention time and influent concentration.

In contrast, PO_4-P and COD removal efficiencies (ranging from 60 % to 88 % and 41 % to 72 %, respectively) were lower than those of NH_4-N. Phosphate reduction was primarily attributed to biomass absorption and phosphate precipitation (De Godos et al., 2009). Additionally, the N:P ratio of the cultivation medium also significantly influenced nutrient removal efficiencies. In this study, N:P ratios spanning from 14 to 27 in mixed sludge wastewater affected NH_4-N and PO_4-P removal efficiencies, both exceeding 99 % and 60 %, respectively. By comparison, (Ji et al., 2014) reported complete removal of both NH_4–N and PO_4–P from anaerobic digestion wastewater with an N:P ratio of 0.83. The observed nutrient uptake ratios did not strictly follow the N:P ratio in the elemental composition of *Desmodesmus* sp., suggesting the species' ability to over-assimilate nitrogen or phosphorus when the medium's N:P ratio deviates from the optimal balance.

3.3. Performance assessment of full scale high-rate algal ponds

Fig. 4 illustrates the effectiveness of *Desmodesmus sp.* over a 4-day cultivation period. Although laboratory trials indicated peak growth at day 6, pilot-scale experiments were hindered by substantial zooplankton grazer proliferation. To address this effect at full scale, a harvesting strategy was implemented at day 4. Under these conditions, *Desmodesmus sp.* demonstrated rapid growth, bypassing the lag phase and entering directly into the exponential growth phase, with a growth rate (μ) of 1.076 ± 2.157 day^{-1}, and reaching the stationary growth phase by day 4.

A comparative overview of cultivation conditions, growth performance, and nutrient removal efficiency between the photobioreactor and full-scale HRAP systems is presented in Table S1. Furthermore, additional data on water parameters, including the daily fluctuations in dissolved oxygen (DO), pH levels, and environmental variables, such as solar irradiation and temperature, are also provided in Fig S6.

The most pronounced reduction was observed for NH_4-N, with a removal efficiency of 89.76 % (±1.4 %). Concentrations decreased from an initial 8.86 mg L^{-1} to 0.9 mg L^{-1}. This high removal rate may be attributed to the strong NH_4-N assimilation capacity of *Desmodesmus sp.* strain, potentially supplemented by ammonia stripping due to the pH elevation. However, at the pH value shown in Fig S6, most nitrogen remained in the NH_4^+ form rather than NH_3, and stripping was estimated at less than 20 % for $pH < 8.5$ at 30 °C (Wu et al., 2012). The NH_4-N removal efficiency in this study aligns well with the average values reported in previous research. For instance, Sutherland et al. (2020) studied HRAPs applied to municipal wastewater treatment with working volumes of 90 m^3 and 2900 m^3 in New Zealand, achieving NH_4-N removal efficiencies of 69 ± 7 % and 90 ± 4 %, respectively (Sutherland et al., 2020). Similarly, Banat et al. (1990) reported 90 % NH_4-N removal rate in two 25 m^3 HRAPs in arid regions for municipal wastewater treatment, utilizing Oscillatoria over a 7-day period (Banat et al., 1990), while Arbib et al. (2013) demonstrated approximately 65 % NH_4-N removal in four 1.25-ha HRAPs treating primary settled wastewater in New Zealand (Arbib et al., 2013). Although NH_4–N is the preferred nitrogen source for algae, elevated concentrations can inhibit *Desmodesmus sp*. growth (Hao et al., 2017).

The removal of phosphate from wastewater followed a distinct sequence of mechanisms. Initially, phosphate was adsorbed onto algal

Table 2
Maximum removal efficiency of NH_4-N, PO_4-P, and COD by *Desmodesmus sp.* cultivated in shrimp-pond wastewater with varying solid sludge concentrations (0–10 %), measured at the end of the exponential growth phase. Values represent means ± standard deviation of triplicate photobioreactors (n = 3).

Sludge Con.	Turbidity	NH_4-N			PO_4-P			COD		
%	(NTU)	Inf. ± STD (mg L^{-1})	Eff ±STD (mg L^{-1})	Effic. ± STD %	Inf. ± STD (mg L^{-1})	Eff. ± STD (mg L^{-1})	Effic.± STD %	Inf. ± STD (mg L^{-1})	Eff. ± STD (mg L^{-1})	Effic. ± STD %
0	2.12	6.85 ± 0.19	0.21 ± 0.06	96.9 ± 0.75	0.26 ± 0.13	0.09 ± 0.01	89.42 ± 2.49	255 ± 35	70 ± 14	73 ± 1.8
2	71.7	15.19 ± 0.09	0.12 ± 0.06	99.2 ± 0.39	0.36 ± 0.04	0.24 ± 0.04	19.8 ± 2.74	345 ± 21	100 ± 21	71 ± 7.9
4	128.58	27.47 ± 1.92	0.08 ± 0.03	99.7 ± 0.1	1.04 ± 0.08	0.49 ± 0.21	53.04 ± 24.07	410 ± 42	150 ± 25	64 ± 9.8
6	174.01	29.49 ± 0.19	0.13 ± 0.01	99.6 ± 0.03	2.16 ± 0.21	1.06 ± 0.4	50.77 ± 15.69	425 ± 64	185 ± 7	56 ± 8.3
8	199.16	42.39 ± 1.97	0.08 ± 0.01	99.8 ± 0.02	2.14 ± 0.47	1.12 ± 0.22	47.58 ± 4.84	450 ± 71	250 ± 57	44 ± 3.9
10	232.13	45.54 ± 0.42	0.05 ± 0.03	99.9 ± 0.07	1.48 ± 0.09	0.67 ± 0.17	54.63 ± 8.97	500 ± 141	290 ± 42	42 ± 8.3

Sludge concentration: Sludge Con.
Influent: Inf.
Effluent: Eff.
Efficiency: Effic.

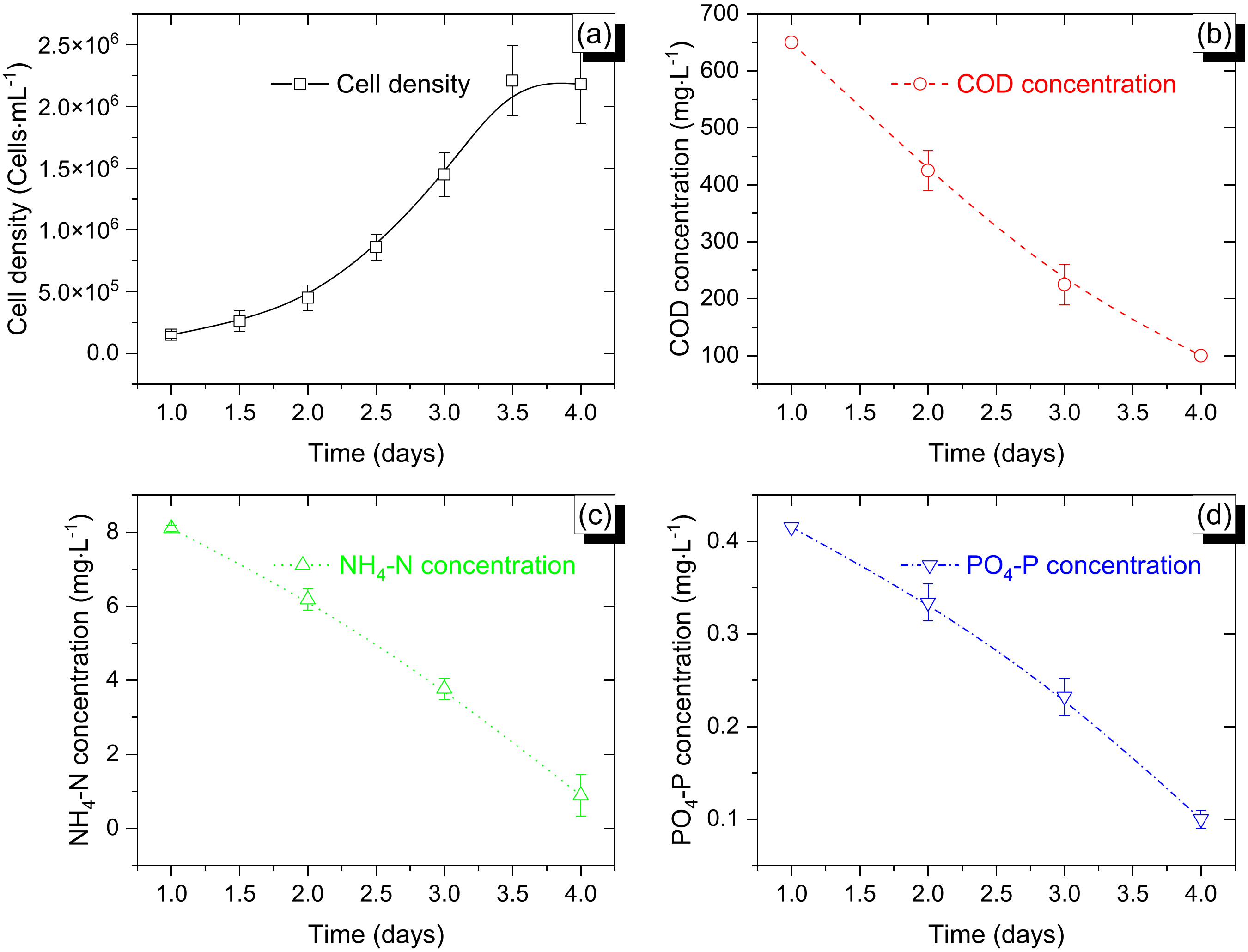

Fig. 4. Changes in control parameters during 4-day outdoor HRAP treatment of shrimp-pond wastewater by *Desmodesmus* sp. (a) Cell density, (b) COD concentration, (c) NH_4-N concentration, and (d) PO_4-P concentration.

cell surfaces, then utilized as a nutrient during microalgal growth, and finally precipitated chemically due to elevated pH (Delgadillo-Mirquez et al., 2016). Under our experimental conditions, phosphate concentrations decreased from 0.42 mg L^{-1} to 0.10 mg L^{-1} in shrimp-pond wastewater through *Desmodesmus* sp. cultivation in a 40 m^3 HRAP over 4 days, resulting in a removal efficiency of 75.94 ± 3.28 %. Similar results have been reported under open pond cultivation conditions. For instance, Posadas et al. (2015) employed an HRAP with a volume of 180 L (1.33 m^2) to treat aquaculture wastewater with an initial phosphate concentration of 14 ± 7 mg L^{-1}, achieving 94 % removal after 10 days (Posadas et al., 2015). In another study, Pagand et al. (2000) treated marine aquaculture effluent in an open pond with a volume of 5900 L (11.8 m^2) and observed a mean phosphate removal efficiency of 56 % within 3.6 days (Pagand et al., 2000). Sutherland et al. (2014) examined the treatment of primary settled domestic wastewater in an HRAP (8000 L, 31.8 m^2) and achieved a phosphate removal efficiency of 58 ± 29 % after 4 days (Sutherland et al., 2014). Phosphorus uptake is influenced by factors including medium composition, environmental conditions, light intensity, and concentration (Choi and Lee, 2012).

The removal efficiency of chemical oxygen demand (COD) reached 85.38 %, even with an initial COD concentration of 650 mg L^{-1}. Some earlier studies reported relatively lower COD removal efficiencies in HRAPs (40 % - 70 %), largely due to the limited biodegradability of the wastewater (Chen et al., 2003; De Godos et al., 2009; Nacir et al., 2010). Nevertheless, several studies have reported high organic carbon removal. For instance, Aguirre et al. (2011) utilized an HRAP with a surface area of 1.54 m^2 to treat piggery wastewater, achieving over 90 % COD removal within 4-8 days (Aguirre et al., 2011). In another study, Buchanan et al. (2018) treated septic tank effluent in an open pond with a volume of 61,440 L and a surface area of 192 m^2, achieving more than 90 % BOD removal in just 4.5 days (Buchanan et al., 2018). Additionally, Muñoz et al. (2004) reported that all biodegradable organic matter was controlled by the oxygen supply and therefore by the algal activity (Muñoz et al., 2004). In our study, temperature was identified as significant factors contributing to high COD removal efficiency at full scale, distinguishing it from earlier findings (Chen et al., 2003).

3.4. Biomass harvesting and lipid composition

Flocculation efficiency was evaluated at different pH levels using NaOH, KOH, and $Ca(OH)_2$, with the highest efficiency observed at pH 10. In the full scale 40 m^3 HRAP system, biomass was harvested after 4 days of exponential growth, yielding 10 kg of dried biomass after 4–5 days of sun drying. The HRAP airlift shaft system effectively facilitated gravity sedimentation, resulting in a high recovery rate. These findings confirm that chemical flocculation at pH 10 is an efficient and scalable approach for harvesting *Desmodesmus sp***.** from shrimp-pond wastewater.

Fig. 5 shows the flocculation efficiency by measuring OD420 nm before and after flocculation and settling process in the Jartest test at pH of 6, 7, 8, 9, 10 of three agents of NaOH, KOH, $Ca(OH)_2$. Flocculation

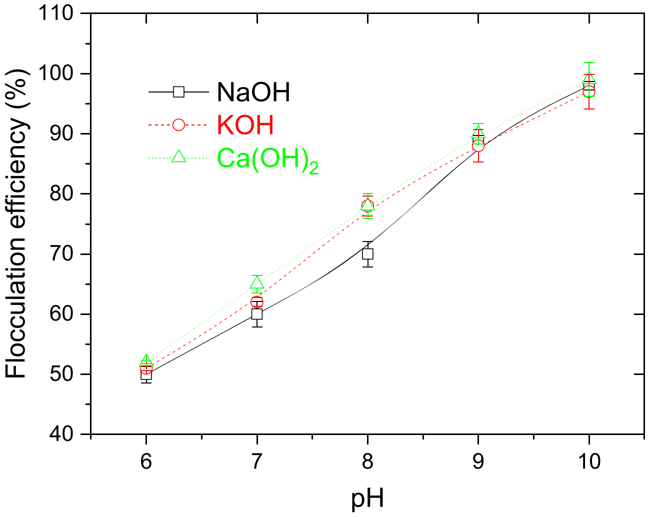

Fig. 5. Flocculation efficiency of *Desmodesmus* sp. using three alkaline agents (NaOH, KOH, and Ca(OH)₂) at pH values ranging from 6 to 10. The microalgae were cultivated in TAP medium with an initial biomass concentration of 1 g·L^{-1} (TSS), and results represent the average of triplicate experiments (n = 3).

efficiency is strongly influenced by pH, as it affects both the surface charge of microalgal cells and the solubility of flocculants. At higher pH, multivalent metal ions (e.g., Ca^{2+} in Ca(OH)₂) undergo hydrolysis, forming precipitates such as Ca(OH)₂ and CaCO₃ that act as bridging agents, physically entrapping microalgal cells into larger flocs and enhancing aggregation (Gregory and Duan, 2001). All three agents had no significant difference in flocculation efficiency at pH of 6,7, 9, and 10, confirming that pH elevation significantly enhances microalgal flocculation by facilitating charge neutralization and precipitate formation, which are critical for optimizing large-scale microalgal harvesting.

In this study, *Desmodesmus sp.* with a biomass concentration of 1 g L^{-1} achieved 99 % harvesting efficiency at pH 10 in TAP medium. This result exceeds 85.33 % efficiency reported by Zhao et al. (2019b) for the same species and pH 10, although differences exist in the cultivating medium (BG-11) and the harvest method (self-flocculation) (Chen et al., 2020b; Zhao et al., 2019b). In a separate study, Chen et al. (2020b) reported > 99 % harvesting efficiency for *Desmodesmus sp.* PW1 in piggery wastewater using the self-flocculation method (Chen et al., 2020b). Various other flocculants have been employed for *Desmodesmus* strains, such as chitosan in Bold's basal medium (efficiency around 99 %) (Oliveira et al., 2019). B. licheniformis broth (efficiency approximately 99 %), ozone-flotation (efficiency from 55.4 % to 83.9 %) (Komolafe et al., 2014), and γ-PGA bio-flocculant (efficiency over 98 %) (Ndikubwimana et al., 2016).

Increasing pH for microalgae harvesting and the potential reuse of flocculated medium has been applied for various microalgae species, including *Tetraselmis suecica, Chaetoceros calcitrans, Chlorella muelleri, Skeletonema sp., Rhodomonas salina, Attheya septentrionalis, Nitzschia closterium, Chlorella muelleri, Thalassiosira pseudonana, Chlorella vulgaris, Scenedesmus sp., and Chlorococcum sp.* (Deepa et al., 2023; Oliveira et al., 2019; Zhao et al., 2019b; Zhu et al., 2024). The pH-based microalgae flocculation process employed in this study proved to be a rapid, cost-effective, and relatively straightforward approach, yielding efficient biomass harvesting. Moreover, it appears to cause less cell damage, allowing biomass to be readily resuspended in seawater, producing material suitable for various applications.

Of the three pH-increasing flocculant agents tested, Ca(OH)$_2$, (or lime), emerged as a cost-effective and locally available option (Table 3), making it a practical choice for scale-up. While elevated pH is often a challenge in wastewater management, it can benefit shrimp farming. In the study area, shrimp farmers store water in reservoirs for 2–3 days to stabilize it, as they mix groundwater with seawater to achieve optimal salinity, with lime primarily used to increase pH (Phụng et al., 2013). Since lime is commonly applied to regulate algal growth, prevent diseases, and promote optimal shrimp growth (Ho et al., 2025), reusing treated wastewater at pH 10 offers two advantages: (i) natural pH neutralization when mixed with lower-pH water, reducing chemical adjustments, and (ii) residual microalgae serving as inoculum to support beneficial algal communities, enhancing shrimp productivity (Oscanoa et al., 2022). Rather than posing an environmental concern, alkaline-treated water can be repurposed to optimize water quality while lowering operational costs and resource use.

Table 3

Estimated cost of chemical flocculants (NaOH, KOH, and Ca(OH)₂) used in the jar test for *Desmodesmus* sp. harvesting. Values are calculated based on base load per gram of biomass and current market prices.

Chemicals	Base (g L^{-1})	Biomass (g L^{-1})	Base load (mg g $^{-1}$ biomass)	Cost ($ Kg $^{-1}$ agent)	Cost ($ ton^{-1} biomass)
NaOH	0.204	1	204	0.31	63.24
KOH	0.324	1	324	0.65	210.6
Ca(OH)$_2$	0.385	1	385	0.1	38.5

The settling height of biomass in the 1.5 m^3 tank was continuously monitored over 180 minute (Fig. 6). Within the first 30 minutes, the treated water occupied more than half the tank height. Fig. 6 also provides insights into biomass quality over time, enabling operators to make informed decisions on when to initiate clean-water drainage and harvest biomass from the tank bottom, as applied in the full scale model.

The dried *Desmodesmus sp.* biomass in this study exhibited lipid and crude protein contents of 17 % and 39 %, respectively (Fig S7). Although lipid contents varied from 15.86 % to 64.13 % in other studies Chen et al. (2020b); Gaspar et al. (2017), our observed lipid content was slightly lower in comparison to studies involving the same algal strain (Chen et al., 2020b; Gaspar et al., 2017). Various factors influence lipid content, including culture conditions, nutrient composition, light exposure, and stress levels across different cultivation scales. For instance, *Desmodesmus sp.* displayed lipid contents of 23.51 % in domestic wastewater (JeongMi et al., 2019), 21.7 % to 27.06 % in a scalable 140 L outdoor photobioreactor (Xia et al., 2014), 22.75 % in mixed wastewater with 5 % CO_2 (Yao et al., 2015) and 24.48 % in vinasse wastewater (Ferreira et al., 2018). Higher lipid yields are achievable under specific conditions, including 52.8 % in tropical climates on a standardized platform (Chen et al., 2013), 58 % after nitrogen starvation, and even 73 % with ultrasound or ozone treatments

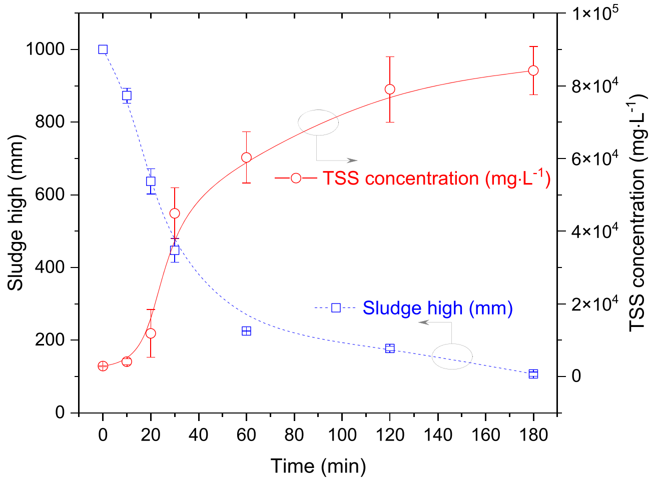

Fig. 6. Sedimentation profile and biomass concentration (TSS) over time during the harvest phase of *Desmodesmus* sp. cultivated in shrimp-pond wastewater. The experiment started with an initial TSS of 1664 mg L^{-1}. Sludge height (mm) and TSS concentration (mg L^{-1}) were measured over 180 minutes.

(González-Balderas et al., 2020). *Desmodesmus* sp. strains are notable for their ease of harvesting and high lipid extraction efficiency, underscoring underscoring their strong potential for sustainable biofuel production.

Table 4 displays the fatty acid (FA) composition, a critical determinant of biodiesel quality. In this study, saturated fatty acids (SFA) accounted for 48 %, while monounsaturated fatty acids (MUFA) represented 59 %. Polyunsaturated fatty acids (PUFA) constituted 35.14 %, with a subfraction of 14.09 %. Mandotra et al. (2014) highlighted that long-chain saturated and monounsaturated FAs enhance oxidative stability and have minimal impact on cold flow properties, making them suitable for biodiesel production (Mandotra et al., 2014). In our study, biomass-derived algal biodiesel contained up to 83.73 % saturated and monounsaturated FAMEs, demonstrating its potential as both a feedstock and a biodiesel source.

Additionally, our results comply with European biodiesel production standard EN 14214, which limits fatty acid methyl esters (FAMEs) with four or more double bonds to 1 % and caps the content of C18:3n3 at 12 %. Notably, our findings align with these criteria, as there were no C18:4 with ≥4 double bonds, and C18:3 was only 1.7 %, well below the 12 % limit. In Table 5, oleic acid (C18:1) dominated algal cells at 34.04 %, followed by Hexadecenoic acid (C16:0) at 32.72 %. For biodiesel production, the quality of *Desmodesmus sp.* biomass can be assessed using six biodiesel properties (Luu et al., 2020): kinematic viscosity (at 40°C, mm^2 s^{-1}), specific gravity (Kg L^{-1}), cloud point (°C), cetane number, iodine value (g I$_2$ 100 g^{-1}), and higher heating value (Mj Kg^{-1}). The results demonstrate that *Desmodesmus sp.* Biomass meets the biodiesel production standards of both Europe (EN 14214) and the United States (ASTM D6751) (Knothe, 2007).

Recent studies show that *Desmodesmus sp.* is a promising microalgae to produce biodiesel (Abbas et al., 2024; El-Sheekh et al., 2023; Javed et al., 2024) and has potential for protein production (Ganguly et al., 2024; Kumar et al., 2024; Oscanoa et al., 2022) across production scales. Moreover, its biomass is a good source of live feed for aquaculture species such as crustaceans, oysters, scallops, clams, mussels, and fish (Graham and Wilcox, 2000; Kumar et al., 2024; Oscanoa et al., 2022). On an industrial scale, *Desmodesmus sp.* Biomass has potential for vegetable oil production. However, biofuel production requires substantial facility investments and is best suited for regional or national applications.

3.5. Research implications, limitations, and mitigation strategies

This study provides practical insights into the use of microalgae-based systems for shrimp aquaculture effluent treatment and biomass valorization. While promising results were obtained at both laboratory and full-scale levels, several limitations and constraints must be acknowledged when considering broader implementation.

Table 4
Fatty acid profile of *Desmodesmus sp.* biomass cultivated in the 40 m^3 HRAP system using shrimp wastewater under tropical climate.

Fatty acid	**Common name**	**Value (%)**
C12:0 Methyl laurate	Lauric	1.97 ± 0.024
C14:0 Methyl myristate	Myristic	2.93 ± 0.054
C16:0 Methyl palmitate	Palmitic	32.72 ± 0.133
C16:1 Methyl palmitoleinate	Palmitoleic	1.09 ± 0.035
C18:0 Methyl stearate	Stearic	9.94 ± 0.025
C18:1 Methyl 10-octadecenoate (CIS)	Oleic	34.04 ± 0.417
C18:2 Methyl linoleate (CIS)	Linoleic	12.86 ± 0.203
C18:3 Methyl linolenate (alpha-ALA)	Linolenic	1.7 ± 1.074
C20:0 Methyl arachisate	Arachidic	1.78 ± 1.037
C22:0 Methyl behenate	Behenic	0.97 ± 0.071
SFA	Saturated fatty acid	48.59 ± 1.54
PUFA	Polyunsaturated fatty acids	14.09 ± 1.94
MUFA	Monounsaturated fatty acid	35.14 ± 0.38

Table 5
Biodiesel properties of *Desmodesmus sp.* biomass.

Biodiesel properties	Value	Standards	
		US (ASTMD 6751-08)	Europe (EN 14214)
Kinematic viscosity (40 ° C mm^2 s^{-1})	4.89 ± 0.167	1.9–6.0	3.5–5.5
Specific gravity (Kg L^{-1})	0.88 ± 0.001	0.85–0.9	-
Cloud point (°C)	13.37 ± 3.52	-	-
Cetane number	59.56 ± 1.76	min 47	min 51
Iodine value (g I$_2$ 100 g^{-1})	49.61 ± 19.59	-	Max 120
Higher heating value (Mj Kg^{-1})	39.41 ± 0.46	-	-

First, the performance of HRAP systems can vary under different climatic conditions. Factors such as light intensity, temperature fluctuations, and precipitation can influence algal productivity and nutrient removal efficiency (Ali et al., 2024). This highlights the importance of conducting long-term and seasonal monitoring before any scaling efforts. Additionally, the full-scale system requires relatively flat land and a reliable source of seawater and sludge, factors that may limit replicability in certain geographic areas (Razaviarani et al., 2023). Another critical challenge observed during the operation is the proliferation of zooplankton, particularly rotifers and ciliates, which can significantly reduce algal biomass yield through grazing pressure (Alam et al., 2024). This biotic limitation, often overlooked in pilot studies, underlines the need for more robust biological control strategies, especially in open or semi-open cultivation systems.

Operational complexity and risk of microbial contamination also remain concerns, particularly in systems exposed to environmental fluctuations. Although the use of locally available flocculants like lime for biomass harvesting appears effective and economically viable, further assessment is needed to evaluate long-term impacts on water chemistry and system stability. Despite these constraints, the dual-function approach explored here, i.e., integrating wastewater treatment with biomass production, represents a step toward more resource-efficient and circular aquaculture practices. Rather than positioning this system as a ready-to-deploy model, we suggest it as a proof-of-concept that may inform decentralized treatment strategies in aquaculture-intensive regions, provided that appropriate technical and ecological adaptations are made. Future research should focus on improving system resilience under variable conditions, developing strategies to control zooplankton proliferation, evaluating life-cycle environmental and economic costs, and assessing integration with other aquaculture and agro-industrial processes.

4. Conclusions

This study provides evidence for the potential integration of *Desmodesmus* sp. in high-rate algal ponds (HRAPs) for the treatment of high-salinity (33 g L^{-1}) shrimp-pond wastewater. The system achieved removal efficiencies of 90 % NH_4–N, 76 % PO_4–P, and 85 % COD, while producing microalgal biomass with a fatty acid profile (34 % oleic acid, 33 % palmitic acid) compatible with current EU and US biodiesel standards.

The use of shrimp-pond sludge as a nutrient source contributes to a low-cost, closed-loop system that simultaneously addresses sludge management and biomass production. Cost-effective biomass harvesting using lime flocculation at pH 10 further supports the potential for upscaling, particularly in small and medium-scale farming contexts.

To our knowledge, this is the first study to assess large-scale HRAP performance under high-salinity aquaculture conditions, offering a proof-of-concept for more sustainable wastewater treatment strategies in the Mekong Delta and similar tropical regions. Further research is needed to address zooplankton control and to evaluate the economic feasibility of broader deployment.

CRediT authorship contribution statement

Thu Thuy Cao: Writing – original draft. **Ngoc Nam Trinh:** Writing – review & editing. **Jean-Luc Vasel:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization. **Claire Remacle:** Writing – review & editing, Validation, Resources, Methodology. **Hung Anh Le:** Project administration. **Gauthier Eppe:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

We, the authors of the manuscript titled "Dual-purpose shrimp-pond effluent treatment and harvesting biomass production by cultivating microalgal Desmodesmus sp.", declare that this research received financial support from ARES (Académie de Recherche et d'Enseignement Supérieur) through a Renewable Project.

We affirm that the research was conducted independently, and the findings presented are solely based on the results obtained. The authors have no other financial, personal, or professional relationships that could be perceived as potential conflicts of interest.

All necessary permissions for the use of data in this study have been obtained, and the work represents original research. Should any potential conflicts arise in the future, the authors will promptly inform the journal.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envc.2025.101273.

Data availability

Data will be made available on request.

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