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ARTICLE

Integrated aquaculture wastewater treatment and biodiesel production using mixed algal consortia

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ABSTRACT

This study investigates the feasibility of integrating the process of waste treatment into aquaculture facilities, and biodiesel production can be achieved using multiple algae species. A mixed consortium consisting of *Anabaena* sp., *Chlorella* sp., together with *Oscillatoria* sp., *Oedogonium* sp., and *Scenedesmus* sp., was grown in untreated aquaculture wastewater for 15 days alongside F/2 synthetic medium cultivation. The combined system outperformed by effectively eliminating total nitrogen by 90.5% and removing total phosphorus along with orthophosphate at 100% and achieving 91.1% and 76.8% removal of nitrate and nitrite, respectively. Biomass production reached 7.3 g/L during wastewater-based cultivation, which doubled the results obtained from the F/2 control. The wastewater-grown cells accumulated lipids corresponding to a weight fraction of 43.9%, which generated 3.20 g/L lipid production. After the transesterification process, biodiesel yield matched the lipid yield (3.20 g/L), which produced an 81% greater output than in synthetic media. Laboratory tests on fatty acid methyl ester (FAME) composition showed high levels of palmitic acid (C16:0) and oleic acid (C18:1) that met all international biodiesel specifications according to ASTM D6751 and EN 14214. The research verifies how mixed microalgal cultures promote simultaneous water purification with sustainable biodiesel outcomes. A unified method publishes bioeconomy circularity through the conversion of aquaculture wastewater into renewable energy materials while it improves both aquatic farming sustainability together with operational energy efficiency.

1. Introduction

The Aquatic ecosystems depend on microalgae as essential primary producers that serve as foundation feeders for food chains while performing vital functions in global biochemical processes (Tsai et al., 2023). The taxonomy of photosynthetic microorganisms exists

between singular cell life forms and basic complex organisms which inhabit ocean water as well as freshwater systems and ground-based territory as well as harsh ecological settings (Bhat et al., 2014). Microalgae display remarkable photosynthetic efficiency together with physiological flexibility, which allows them to extract CO₂ and nutrients from their environment to produce organic matter using solar radiation. The combination of oxygen production and biomass

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accumulation creates valuable opportunities for climate mitigation strategies through their substantial contribution to atmospheric CO₂ reduction (Ramaraj et al., 2022). Microalgae have become important subjects of attention in environmental biotechnology research due to their multiple values in ecosystems. Their fast multiplication rates, along with strong oil-producing abilities and their successful growth in wastewater areas, make them optimal agents for environmental cleanup and renewable energy development (Krishnamoorthy et al., 2021).

Microalgae cultivation exhibits superior advantages since they avoid ending up in disputes with food crops or land allocation for agriculture, and continue growing throughout the entire year within isolated cultivation areas (Bhat et al., 2023a,b). The massive global growth of aquaculture for protein production resulted in environmental challenges due to wastewater that contains high nutrients. The utilization of recirculating aquaculture systems (RAS) results in highly enriched wastewater that contains nitrogen and phosphorus unless proper treatment occurs, because improperly treated water causes eutrophication, which then produces algal blooms that ultimately degrade aquatic ecosystems. The wastewater that contains high nutrients serves as a valuable resource because microalgal cultivation can transform these waste disposal matters into sustainable resource recovery initiatives (Pimpimol et al., 2020). Third-generation biofuel feedstocks are classified because they produce energy-dense lipids when exposed to nutrient restrictions, thus making them suitable for biodiesel manufacturing.

The combination of wastewater purification from aquaculture operations with microalgal farms to create biodiesel represents a promising sustainable solution that handles environmental and energy systems effectively. The sustainable principles of the circular bioeconomy apply through this approach by reusing nutrients with simultaneous emission reduction and high-value biofuel generation (Tongsiri et al., 2023). Studies proved that *Chlorella*, *Scenedesmus*, and *Nannochloropsis* can operate as single-species monocultures that remove aquaculture waste while making biodiesel-quality lipids. The functional properties of monocultures tend to decrease when wastewater exposure conditions change, and these cultures become vulnerable to environmental temperature shifts. Mixed algal consortia utilize synergy effects between different species to boost system stability as well as both nutrient consumption capability and lipid yield (Wicker et al., 2023). The metabolic capabilities of such systems expand because consortia that contain a wide array of functionally and taxonomically diverse microorganisms provide environmental stress protection. A mixture of nitrogen-fixing cyanobacteria, *Anabaena*, together with fast-growing chlorophytes *Chlorella* and *Scenedesmus*, and filamentous algae *Oedogonium* and *Oscillatoria* optimizes the process of nutrient removal and lipid production.

The production of biodiesel from microalgae provides an environmentally sustainable answer to lower dependency on fossil fuels and enables worldwide carbon neutrality (Ramaraj et al., 2016). Among all biofuel feedstock options, which include plants, algae, microbes, crop residues, and manure, microalgae emerge as the top choice due to their exceptional lipid content and quick growth rates and their capability to cultivate in non-farmland situations and salty or wastewater environments. Microalgal biodiesel production efficiency and its quality rely mainly on the fatty acid profile of the lipids. The plastids function as the site of fatty acid synthesis that uses acetyl-CoA carboxylase (ACCase) to convert acetyl-CoA into malonyl-CoA, which then extends through acyl carrier protein (ACP). Biodiesel

conversion becomes optimal when cells produce energy-rich TAGs through the Kennedy and monoacylglycerol biochemical pathways (Shahid et al., 2020). Scientists investigate methods to enhance the productivity of oil production during microalgal growth, together with methods for extracting microalgal products. Current processes to transform lipids into biodiesel function successfully, but researchers need to find better ways to increase lipid production and alteration of fatty acids. When microalgae encounter stressful environments, they mostly produce lipids of the neutral variety, which contain desirable saturated and monounsaturated acids suitable for biodiesel production (Unpaprom et al., 2015). Microalgal biodiesel functions as a promising diesel fuel substitute because it has renewable properties, along with biodegradability and high cetane number and better combustion efficiency, and low sulfur content.

The present study investigates the feasibility of using a mixed microalgal consortium to simultaneously treat aquaculture wastewater and produce biodiesel precursors. The research combines a mixed microalgal consortium for the dual purpose of wastewater treatment from aquaculture while generating biofuel precursors. Bioreactors designed for cultivation received either untreated sea farming wastewater or synthetic F/2 medium as a reference solution. Scientists monitored essential performance parameters such as population growth patterns, together with the removal effectiveness of nutrients (total nitrogen, total phosphorus, nitrate, nitrite, and orthophosphate) and lipid generation, as well as fatty acid methyl ester (FAME) composition during 15 days. The biodiesel production process started with acid-catalyzed transesterification of extracted lipids from harvested biomass to evaluate the biofuel system completely (Waidee et al., 2018; Whangchai et al., 2021). The unified strategy enables both the sustainable expansion of aquaculture operations and renewable energy installations through an environmentally friendly solution. The research outcomes address the disconnect between organizing wastewater and biofuel manufacturing through evidence showing algal consortia work effectively under authentic wastewater situations. Through this study, scientists can utilize gained insights to create better algal biorefineries, which will help adapt to climate change and develop sustainable energy systems in environmental biotechnology.

2. Materials and Methods

2.1 Algal culture and experimental setup

A mix of *Anabaena* sp., *Chlorella* sp., *Oscillatoria* sp., *Oedogonium* sp., and *Scenedesmus* sp. algae grew together in controlled laboratory conditions. The laboratory cultivation of *Anabaena* sp. with *Chlorella* sp. and *Oscillatoria* sp., along with *Oedogonium* sp. and *Scenedesmus* sp. was conducted in controlled conditions. The cultures started their growth cycle in F/2 medium (Guillard & Ryther, 1962) until they reached exponential growth. Next, the culture moved to 14-L PBR units, which received 10.8 L of F/2 synthetic medium or untreated aquaculture wastewater as the control. Laboratory experiments utilized an inoculum density, controlling the initial organism density at 1.3 g/L.

The reactors were continuously aerated with air enriched to 2% CO₂ to enhance photosynthetic efficiency and prevent carbon limitation (Pirt, 1986). White LED illumination was provided at an intensity of 8.4 W/m², and the light regimen followed a 16:8 hour light-dark cycle, consistent with optimal photoperiods for microalgal growth (Richmond, 2004). Cultures were maintained at ambient laboratory

temperature (approximately 25°C), and water losses from evaporation were replenished with distilled water to maintain volume consistency.

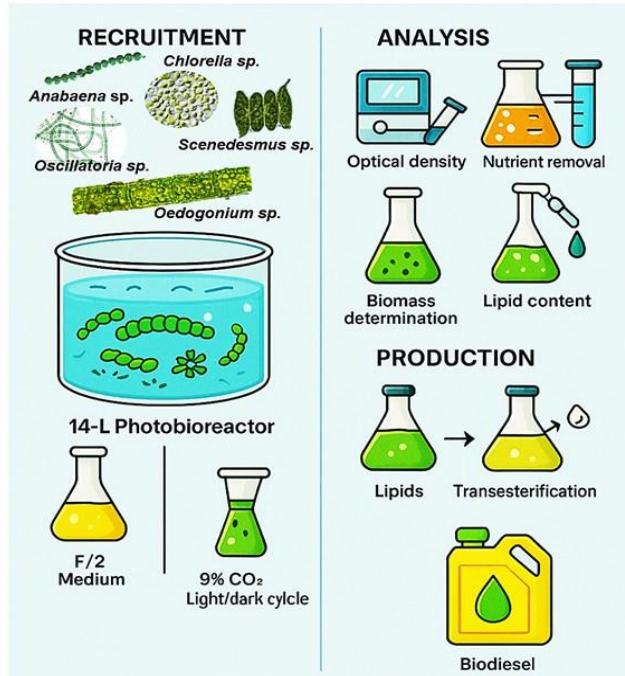


Figure 1. Methodology flowchart illustrating microalgae-based biodiesel production using aquaculture wastewater

2.2 Source and characterization of wastewater

Wastewater from aquaculture operations is collected from the recirculating aquaculture system (RAS) at Maejo University. A mechanical filter system removed large debris from wastewater before its use as an experiment while maintaining the original microbial components and nutrients present. The initial levels of total nitrogen and total phosphorus in the sample exceeded 34.33 ± 0.30 mg/L, while total phosphorus at 3.60 ± 0.01 mg/L. The analysis also evaluated nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and orthophosphate (PO_4^{3-}) using APHA (2017) standard methods.

2.3 Analytical methods

The measurement of algal biomass occurred using spectrophotometry alongside weight measurements. Daily measurements of optical density (OD) at 680 nm wavelength occurred with the UV-Vis spectrophotometer (UV-1800, Shimadzu). Known culture volumes underwent filtration before the retained biomass was dried at 105°C until it reached a constant weight as per Lee et al. (2010). Previous research methods described by APHA (2017) measured the nutrient concentrations present in culture supernatants spectrophotometrically. Analyses of total nitrogen and total phosphorus involved persulfate digestion before colorimetric measurement detection took place. The measurement of nitrate-N and nitrite-N, and ammonium-N followed diazotization methods and indophenol blue analysis techniques, respectively. The measurement of orthophosphate involved the molybdate-ascorbic acid technique (Murphy & Riley 1962). The total lipid measurement involved Soxhlet

extraction with hexane according to Bligh and Dyer (1959), following method modifications for dried algal samples. The percentage of dry biomass was used to determine the weight of lipids through gravimetric analysis. FAME analysis required a lipids' transformation using methanolic sulfuric acid into FAMEs, which were examined by GC-FID according to AOAC official method 996.06 (AOAC, 2000).

2.4 Biodiesel production procedure

The extraction of lipids allowed the production of biodiesel through a conventional acid-catalyzed transesterification reaction method. The preparation included 1.0 grams of algal lipid along with 20 mL of methanol solution and 1% v/v sulfuric acid (H_2SO_4) adding as catalyst. The reaction operation ran at 60°C temperature while operating at constant stirring speed of 600 rpm throughout the 4-hour time period inside a sealed glass reactor. The mixture required temperature reduction to room temperature before it went into the separation funnel.

The collected biodiesel was the upper layer before washing it several times with warm distilled water to remove catalyst and methanol content, while anhydrous sodium sulfate was utilized for drying this product. The researchers determined biodiesel conversion by measuring weight before storing the processed substance at 4°C in amber glass vials for analysis. The biodiesel quality determination included analysis of yield and FAME composition results obtained through the GC-FID method.

2.5 Statistical analysis

The experimental procedures were performed in triplicate for each setup. The researchers presented results as mean values plus or minus standard deviation (SD). The statistical analysis based on one-way analysis of variance (ANOVA), together with Tukey's Honest Significant Difference (HSD) post hoc test, evaluated treatment significance. The researchers evaluated differences with significance set at $p < 0.001$ according to Zar (2010). A comparison of results took place through IBM SPSS Statistics version 25.

3. Results and Discussion

3.1 Removal of nitrogen and phosphorus from fish pond wastewater

The mixed microalgae culture demonstrated remarkable capability for nutrient removal over a ten-day growth cycle in raw aquaculture wastewater. A complete breakdown of nutrient measurements shows in Figure 2 how the mixed algal consortium demonstrated high efficiency in removing total nitrogen (TN), total phosphorus (TP), nitrate (N-NO_3^-) and nitrite (N-NO_2^-), and orthophosphate (PO_4^{3-}) throughout the cultivation period. Biomass of algae consumed 90.5% of total nitrogen while assimilating this nutrient into new growth. All total phosphorus concentrations vanished from the test sample, indicating their transformation into polyphosphate stored inside algal cells and biomolecular structure compounds (Saengsawang et al., 2020). The measured substances nitrate and nitrite were reduced by 91.1% and 76.8%, respectively, which indicated that nitrate-assimilating taxa Chlorella and Anabaena performed biomass uptake

and denitrification-nitrification route transformation. The laboratory data showed complete orthophosphate removal (100% removal), which indicates conditions favorable to lipid production in microalgae according to Chisti (2007).

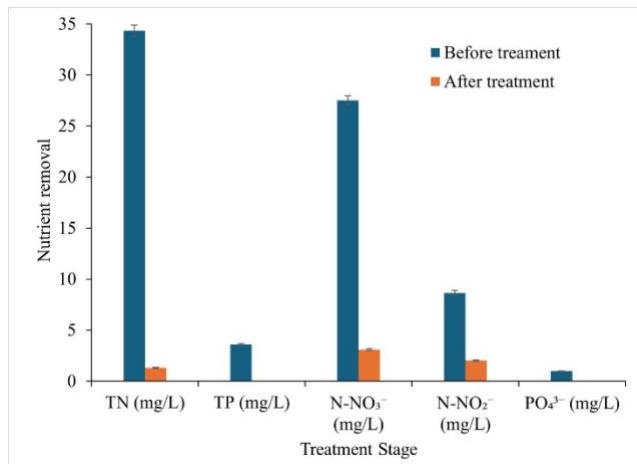


Figure 2. Nutrient concentration in fishpond wastewater before and after algal cultivation

Numerous synchronously working mechanisms produce the observed nutrient removal results. The respiratory functions of different algae species inside the consortium produced a combined effect for efficient dual-nutrient acquisition. Each species focuses on extracting particular phosphorus or nitrogen compounds from the water. The cell wall polysaccharides of the consortium adsorbed the surface of nutrients, which resulted in both phosphate and ammonium ion removal. Laboratory observations verify that polyphosphate granule accumulation inside cells serves as an established survival mechanism of aquatic creatures when confronted with phosphorus scarcity (Powell et al., 2009). The efficient nutrient removal by this system simultaneously protects the environment from aquaculture wastewater while establishing opportunities for resource reuse. Research documents show that combining various microalgae species into cultures creates improved wastewater tolerance alongside enhanced operational stability (Zhou et al., 2022). The remediation functions and material production capabilities of this system demonstrate its potential for adoption in sustainable aquaculture systems and circular bioeconomic models.

3.2 Microalgal growth in fish pond wastewater

This study evaluated the growth pattern of microalgal biomass during fifteen days when cultivated through WW aquaculture wastewater together with synthetic F/2 medium. Biomass levels rose significantly during the research period, as shown in Figure 3, especially among the wastewater cultivation systems. A 3.05 g/L/day productivity rate was developed during the first 8 days when biomass concentration in wastewater cultivation rose from 1.3 g/L to 7.3 g/L. The biomass concentration under synthetic F/2 medium reached only 3.6 g/L, yet the actual performance exceeded it by reaching 7.3 g/L. The biomass levels decreased gradually after Day 8 because high-density conditions led to nutrient exhaustion and increased competition, together with self-shading effects during the experimental period. Aquaculture wastewater proves to be an effective growth medium for microalgae because it provides cost-efficient nutrients

without requiring synthetic supplements. During the exponential phase of the experiment, the biomass increased rapidly because the wastewater effectively utilized its concentrated bioavailable nitrogen and phosphorus content. According to Rawat et al. (2011), aquaculture effluents function well as large-scale algal cultivation media.

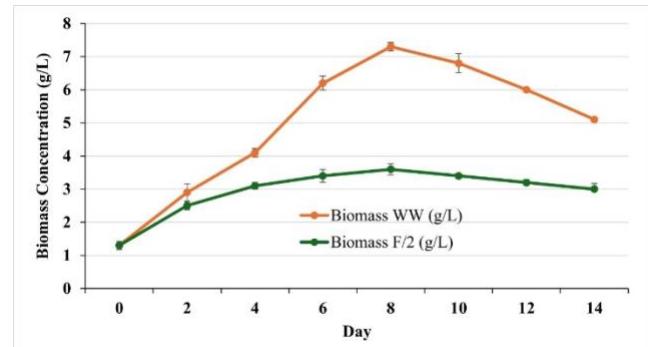


Figure 3. Biomass concentration during cultivation

The experiment used the OD680 measurement technique in addition to biomass gravimetry to track changes in population growth without relying on invasive methods. Free total chlorophyll content correlated strongly with the concentration of microalgae dry biomass through a positive relationship with a value of $r = 0.81$, which proved the precision of spectrophotometric methods for determining biomass amounts. The laboratory results showed that OD values maintained a steady upward trend, which matched the photosynthetic pigment accumulation and metabolic activity as shown by green color intensification. Multiple essential components played a role in the outstanding expansion that occurred within the wastewater system. Both nitrogen and phosphorus components in aquaculture effluents work together to enhance the efficient consumption of nutrients by microorganisms when undertaking biosynthetic mechanisms.

The native microbial community cells in the wastewater contributed to higher algal growth because they could metabolically cooperate by releasing growth-promoting substances or dissolving necessary nutrients. The ability of the mixed algal culture to adapt allowed it to successfully colonize diverse nutrient substrates that frequently appear in actual wastewater systems. The research reveals that aquaculture wastewater contains vast bioresource potential after combining it with resilient mixed-species algal cultures. The improved biomass productivity results achieved in WW systems prove that integrating microalgae into aquaculture operations can be both sustainable and effective for nutrient recovery and renewable biomass production. The combined strategy helps treat aquaculture waste streams and helps establish circular bioeconomy approaches that achieve better environmental and economic performance.

3.3 Lipid content

Microalgal biomass viability for biofuel feedstock depends directly on its capability to produce lipids. The research evaluated how different nutrient supplies affected lipid production by measuring microalgae lipid quantities during regular timepoints when cultivating them through WW aquaculture wastewater and synthetic F/2 medium. The data regarding lipid accumulation appears in Figure 4. The lipid content from both cultivation conditions demonstrated steady growth until reaching its peak at 43.9% for wastewater-based biomass and

49.2% for F/2-based biomass during an 8-day cultivation period. The observed lipid accumulation pattern matches the lipids' behavior under insufficient nutrient situations that mainly affect nitrogen and phosphorus levels. The presence of specific environmental factors prompts microalgae to shift metabolic activities, which encourages them to create neutral lipids called TAGs primarily for biodiesel precursor production (Chisti, 2007; Hu et al., 2008).

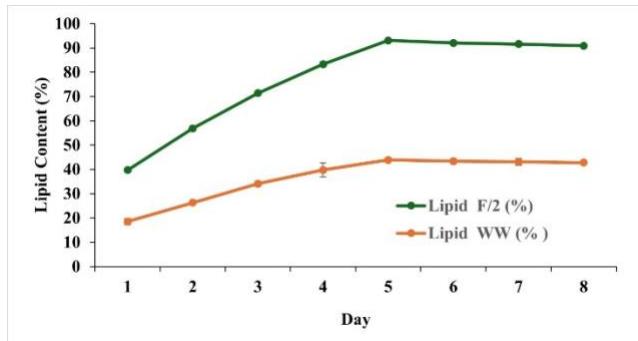


Figure 4. Lipid content during cultivation

The form of lipid production proved higher per biomass unit in F/2 medium yet the wastewater system delivered greater total biomass output for superior volumetric lipid output. The use of real wastewater demonstrates its double advantage because it performs environmental remediation while simultaneously generating high-value lipid products. The tested mixed algal culture successfully evolved to process complex marine waste products from aquaculture facilities which allowed it to grow with significant lipid production accumulations. Scientific studies have thoroughly documented how cells accumulate lipids when they experience nutrient deprivation. When cells experience either nitrogen stress or phosphorus stress they decrease their protein levels together with chlorophyll production resulting in increased carbon available for lipid accumulation inside the cell. During stationary-phase cell cultures grow more slowly and cells transition their metabolic priorities toward storage activities instead of cell division. Observations show that the measured lipid content increase during Days 4 to 8 matches the availability of nutrients.

The observed lipid accumulation might benefit from collaborative effects that occur between the microorganisms grown in mixed-species cultures. The exchange of nutrients and metabolites between different species creates subsequent microorganisms to cultivate lipid-producing environments. Environmental stress within co-cultured algal consortia leads to better lipid productivity according to González-González et al. (2022). The biodiesel production optimal lipid range between 20–50% corresponds to the lipid contents obtained in this research. Mata et al. (2010) together with recent research confirms that nutrient-deprived conditions lead to similar lipid accumulation in *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Nannochloropsis* sp. The effectiveness of using aquaculture wastewater to enhance lipid accumulation remains validated when production rate remains unaffected by this process. The research findings demonstrate that wastewater-treated algal systems function as both environmental contaminants remediaters and bioenergy systems. When using these production conditions scientists can create biofuel feedstock from lipid-rich biomass.

3.4. Fatty acid profile

Biodiesel efficiency, performance, and marketability derived from microalgal sources largely depend on the fatty acid content profiles of microalgal lipids. Biodiesel properties that include cetane number and oxidative stability and cold flow characteristics, and viscosity receive specific impacts from each fatty acid methyl ester (FAME). Research into FAME composition allows experts to determine how suitable microalgal lipids function for biofuel uses. The research examined microalgal lipids extracted from biomass cultures using both WW aquaculture wastewater and synthetic F/2 medium. The researchers extracted samples at Day 8 when lipids reached their maximum accumulation point to perform analysis, showing data in Table 1.

Table 1. Fatty acid profile of algal lipids

Fatty Acid	WW Lipid (%)	F/2 Lipid (%)
C14:0 (Myristic)	2.4	2.8
C16:0 (Palmitic)	28.7	26.5
C16:1 (Palmitoleic)	6.2	7.1
C18:0 (Stearic)	5.1	4.9
C18:1 (Oleic)	33.4	36.2
C18:2 (Linoleic)	16.5	15.4
C18:3 (Linolenic)	7.7	7.1

Both cultivation conditions yielded lipid fractions containing saturated and monounsaturated fatty acids primarily consisting of palmitic acid (C16:0) and oleic acid (C18:1) that formed the main components of FAME pool. Wastewater cultivation yielded microalgae with a minor increased amount of palmitic acid that leads to better cetane numbers and oxidation resistance in biodiesel production. The microalgae cultivated in F/2 medium contained a different proportion of oleic acid which improves fuel flow behavior and ignition performance. The fuel stability standards set by EN 14214 allowed linolenic acid measurements up to 8% while the detected linoleic (C18:2) and linolenic acid (C18:3) polyunsaturated fat content remained at moderate levels below these thresholds. The lipid sources display a favorable fatty acid composition which makes them appropriate for biodiesel production regardless of climatic conditions. The biodiesel precursors receive support for oxidative stability and maintain optimum viscosity from stearic acid (C18:0) and palmitoleic acid (C16:1). The experimental data reveals that growing algae with aquaculture wastewater produces biodiesel precursors with superior qualities than what synthetic media can achieve.

The experimental data matches past research related to lipid production in microalgae under stressful environments. Scientists continue to support the established knowledge that FAME composition shifts favorably together with enhanced lipids production when microorganisms face nutrient deficiencies particularly nitrogen and phosphorus starvation. *Scenedesmus* sp. under nutrient stress produced elevated levels of palmitic acid and oleic acid which improved its potential as a biodiesel source according to Li et al. (2021). The research of Rajesh et al. (2022) showed that lipids in wastewater-grown *Chlorella vulgaris* contained abundant MUFA and SFAs that met EN 14214 specifications. Researchers at Nguyen et al. (2023) discovered that FAME composition and efficiency levels were better in mixed algal consortia when grown in aquaculture effluents compared to synthetic media monoculture cultivation. Modern research confirms the effective operation of the mixed algal culture used in this investigation while establishing aquaculture wastewater as a sound

choice for economical biodiesel production.

The recent developments in microalgal biofuel investigations confirm that limiting nutrients function as major stress factors which powerfully affect both lipid creation and fatty acid composition patterns. Research results from this study demonstrate identical trends established through previous studies by Hu et al. (2008) and Gouveia et al. (2009) through additional evidence provided by recent investigations. The researchers at Wang et al. (2021) determined that *Chlorella sorokiniana* produced superior palmitic acid and oleic acid content because of nitrogen-deficient irrigation which enhanced biodiesel quality levels. *Scenedesmus obliquus* underwent changes in metabolic flux direction toward monounsaturated fatty acid synthesis when deprived of phosphorus according to Kumari et al. (2022). These modified fatty acids improved biofuel properties. The combined cultivation of *Nannochloropsis* with bacterial consortia in aquaculture wastewater according to Zhang et al. (2023) produced enhanced levels of lipids and uniform FAME compositions. Weathered wastewater systems prove efficient in generating appropriate biodiesel profiles through their ability to enhance microalgal consortia metabolic adaptability under stress. The biological findings from this research fully support previous published results which show nutrient-deprived environments to be solid bases for sustainable biofuel manufacturing.

3.5. Biodiesel production

A biodiesel production process was applied to Day 8 microalgal biomass lipids to examine the usable potential of algal biomass as biodiesel feedstock. The experiment was conducted using two growth conditions, namely aquaculture wastewater (WW) and synthetic F/2 medium, where Table 2 reveals the biomass yield, lipid content and resulting biodiesel yield statistics. The biomass production from wastewater algae cultivation reached 7.3 g/L while F/2 medium algae yielded 3.6 g/L despite the minor lipid content difference (49.2% compared to 43.9%). The wastewater culture produced 81% more biodiesel yield than F/2 liquid media because it generated 3.20 g/L lipids, while F/2 media produced only 1.77 g/L lipids. Biodiesel production systems need cost-effective, high-throughput design because of these findings about optimally improving biomass and lipid productivity (Jain et al., 2023). Through wastewater-fed biofuel production systems, both wastewater treatment efforts and clean energy attainment enhance each other as they achieve superior volumetric energy outputs. Table 2 showcases the data about biomass accumulation along with lipid content percentages and biodiesel yields obtained from each cultivator medium.

Table 2. Lipid and biodiesel yields

Condition	Biomass (g/L)	Lipid Content (%)	Biodiesel Yield (g/L)
Wastewater (WW)	7.3	43.9	3.20
F/2 Medium	3.6	49.2	1.77

The fuel quality evaluation of biodiesel from microalgal lipids concentrated on FAME composition assessment according to Section 3.4. The palmitic acid (C16:0) concentration in both conditions increases the cetane number and strengthens storage fuel stability. The high concentration of oleic acid (C18:1) enhances combustion ability as well as cold flow performance. The biodiesel exhibited linolenic acid (C18:3) content below the threshold values set by EN 14214 standards which ensures the protection against oxidative degradation

and polymerization. High oxidative stability and low temperature operability together with efficient energy release are possible features of biodiesel derived from these specific compositional elements. The characteristics that microalgal biodiesel exhibits enable its appropriate use across a wide range of geographic environments including locations experiencing temperature extremes. Microalgal biodiesel stands viable as a renewable fuel because its observed quality parameters match both ASTM D6751 and EN 14214 guidelines.

The combination of microalgal farming with aquaculture wastewater treatment provides multiple environmentally friendly benefits apart from high-quality fuel production. The utilization of nutrient-rich effluents as growth media in this system promotes resource reuse and cuts down requirements for synthetic nutrients and environmental wastewater releases. The photosynthetic abilities of algae fix carbon dioxide, which works as a greenhouse gas (GHG) mitigation approach that subtracts fossil carbon releases from the atmosphere. Appropriate scaling of this approach has the potential to transform aquaculture into an improved energy-efficient operation, which creates a self-contained system while reducing environmental footprints and generating extra value from biofuel manufacturing. This research upholds and develops findings that already exist regarding the subject. Researchers using wastewater media with various microalgal species found that biodiesel production yielded between 2.1 and 3.5 g/L according to Gouveia et al. (2009) and Rawat et al. (2011). The experimental yield of 3.20 g/L turned out to be competitive and demonstrates that mixed algal cultures effectively operate in nutrient-stressful conditions. The findings strengthen the argument for implementing microalgal biofuel production within wastewater valorization systems and aquaculture facilities to create a sustainable bioeconomy structure.

4. Conclusion

This study highlights the effectiveness of integrating aquaculture wastewater treatment with biodiesel production using a mixed microalgal consortium. The selected algal species exhibited strong adaptability to untreated aquaculture effluents, supporting both environmental remediation and renewable energy generation. The microalgal consortium effectively assimilated nutrients, demonstrating significant potential for reducing nitrogen and phosphorus loads from wastewater while simultaneously producing lipid-rich biomass suitable for biofuel synthesis. The dual functionality of this approach—treating wastewater and generating valuable bioenergy feedstocks—aligns well with the principles of circular bioeconomy. The fatty acid profiles of the lipids extracted from the cultivated biomass were favorable for biodiesel applications, showing compatibility with recognized fuel quality standards. This confirms the potential of microalgae not only as an environmental tool but also as a reliable and sustainable source of biodiesel. By utilizing aquaculture effluents as a nutrient medium, the system minimizes the need for synthetic inputs, lowers operational costs, and promotes resource recovery. Moreover, it supports carbon capture through photosynthesis, contributing to climate change mitigation efforts. The study reinforces the value of mixed algal consortia in improving process resilience and performance under real wastewater conditions. Therefore, this integrated approach presents a promising pathway for enhancing the sustainability of aquaculture operations while contributing to renewable energy goals. It offers a scalable, eco-efficient solution with strong implications for waste valorization, energy security, and environmental management.

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Declaration of competing interest

The authors declare that they have no known financial conflicts of interest or personal relationships that could have influenced the work reported in this paper.

Reference

Bhat, O., Unpaprom, Y., & Ramaraj, R. (2023a). Effect of photoperiod and white LED on biomass growth and protein production by Spirulina. *Molecular Biotechnology*, 1-9. <https://doi.org/10.1007/s12033-023-00787-y>

Bhat, O., Unpaprom, Y., & Ramaraj, R. (2023b). Spirulina cultivation under different light-emitting diodes for boosting biomass and protein production. *Molecular Biotechnology*, 1-9. <https://doi.org/10.1007/s12033-023-00842-8>

Bhat, O., Unpaprom, Y., & Ramaraj, R. (2024). Exploring the impact of light-emitting diodes and pH on microalgae Spirulina biomass production optimization using a central composite design. *Biomass Conversion and Biorefinery*, 14(8), 9839-9847.

Bhuyar, P., Shen, M. Y., Trejo, M., Unpaprom, Y., & Ramaraj, R. (2022). Improvement of fermentable sugar for enhanced bioethanol production from *Amorphophallus* spp. tuber obtained from northern Thailand. *Environment, Development and Sustainability*, 24, 8351-8362.

Capilla, M., San-Valero, P., Izquierdo, M., Penya-roja, J. M., & Gabaldón, C. (2021). The combined effect on initial glucose concentration and pH control strategies for acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* DSM 792. *Biochemical Engineering Journal*, 167, 107910.

da Silva, D. B., Fernandes, B. S., & da Silva, A. J. (2022). Butanol production by *Clostridium acetobutylicum* DSMZ 792 from cassava starch. *Environmental Sustainability*, 5(1), 91-102.

Eloka-Eboka, A. C., & Maroa, S. (2023). Biobutanol fermentation research and development: feedstock, process and biofuel production. In *Advances and developments in biobutanol production* (pp. 79-103). Woodhead Publishing.

Guillard, R. R. L. and R.L. Ryther (1962). Studies of marine planktonic diatoms. I *Cyclotella nanahustedt* and *Detonula confervalea* (cleve) gran. *Canadian Journal of Microbiology*. 8, 229-239.

Hargono, H., Jos, B., Kumoro, A. C., & Haryani, K. (2019). Low Temperature Enzymatic Hydrolysis (LTEH) and Fermentation for Bioethanol Generation from Suweg (*Amorphophallus campanulatus* B) Starch. In *Journal of Physics: Conference Series* (Vol. 1295, No. 1, p. 012035). IOP Publishing.

He, Y., Cassarini, C., & Lens, P. N. (2021). Bioethanol production from H₂/CO₂ by solventogenesis using anaerobic granular sludge: effect of process parameters. *Frontiers in Microbiology*, 12, 647370.

Jain, A., Bora, B. J., Kumar, R., Saleel, C. A., Sharma, P., Ramaraj, R., & Balakrishnan, D. (2023). Estimation of the potential of Nahar biodiesel run diesel engine at varying fuel injection pressures and engine loads through exergy approach. *Alexandria Engineering Journal*, 84, 262-274.

Krishnamoorthy, N., Unpaprom, Y., Ramaraj, R., Maniam, G. P., Govindan, N., Arunachalam, T., & Paramasivan, B. (2021). Recent advances and future prospects of electrochemical processes for microalgae harvesting. *Journal of Environmental Chemical Engineering*, 9(5), 105875.

Kuila, A., & Mukhopadhyay, M. (Eds.). (2023). *Production of Biobutanol from Biomass*. John Wiley & Sons.

Lee, H. L., Ashoor, S., Yao, Z., & Jang, Y. S. (2024). Characterization of acidogenic phase metabolism in *Clostridium acetobutylicum* ATCC 824 (pCD07239) under different culture conditions. *Applied Biological Chemistry*, 67(1), 80.

Li, S., Huang, L., Ke, C., Pang, Z., & Liu, L. (2020). Pathway dissection, regulation, engineering and application: lessons learned from biobutanol production by solventogenic clostridia. *Biotechnology for biofuels*, 13, 1-25.

Liu, H., Zhang, J., Yuan, J., Jiang, X., Jiang, L., Zhao, G., ... & Liu, B. (2019). Omics-based analyses revealed metabolic responses of *Clostridium acetobutylicum* to lignocellulose-derived inhibitors furfural, formic acid and phenol stress for butanol fermentation. *Biotechnology for biofuels*, 12, 1-20.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, 31(3), 426-428.

Naleli, K. (2016). Process modelling in production of biobutanol from lignocellulosic biomass via ABE fermentation (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Nandhini, R., Rameshwar, S. S., Sivaprakash, B., Rajamohan, N., & Monisha, R. S. (2023). Carbon neutrality in biobutanol production through microbial fermentation technique from lignocellulosic materials-A biorefinery approach. *Journal of Cleaner Production*, 413, 137470.

Pimpimol, T., Tongmee, B., Lomlai, P., Prasongpol, P., Whangchai, N., Unpaprom, Y., & Ramaraj, R. (2020). Spirogyra cultured in fishpond wastewater for biomass generation. *Maejo International Journal of Energy and Environmental Communication*, 2(3), 58-65.

Ramaraj, R., Kawaree, R., & Unpaprom, Y. (2016). Direct transesterification of microalga *Botryococcus braunii* biomass for biodiesel production. *Emergent Life Sciences Research*, 2, 1-7.

Ramaraj, R., Junluthin, P., Dussadee, N., & Unpaprom, Y. (2022). Potential evaluation of biogas production through the exploitation of naturally growing freshwater macroalgae *Spirogyra varians*. *Environment, Development and Sustainability*, 1-12.

Ray, S., Mukherjee, A., & Ghosh, U. (2024). Advancements in microbial fermentation of agro and food processing wastes for generation of biofuel. *Solid - Gaseous Biofuels Production*, 245-285.

Richmond, A. (Ed.). (2004). *Handbook of microalgal culture: biotechnology and applied phycology* (Vol. 577). Oxford: Blackwell Science.

Saengsawang, B., Bhuyar, P., Manmai, N., Ponnusamy, V. K.,

Ramaraj, R., & Unpaprom, Y. (2020). The optimization of oil extraction from macroalgae, *Rhizoclonium* sp. by chemical methods for efficient conversion into biodiesel. *Fuel*, 274, 117841.

Shahid, A., Rehman, A. U., Usman, M., Ashraf, M. U. F., Javed, M. R., Khan, A. Z., ... & Mehmood, M. A. (2020). Engineering the metabolic pathways of lipid biosynthesis to develop robust microalgal strains for biodiesel production. *Biotechnology and Applied Biochemistry*, 67(1), 41-51.

Shao, M., & Chen, H. (2015). Feasibility of acetone–butanol–ethanol (ABE) fermentation from *Amorphophallus* konjac waste by *Clostridium acetobutylicum* ATCC 824. *Process Biochemistry*, 50(8), 1301-1307.

Tongsiri, S., Bhat, O. A., Ramaraj, R., & Pimpimol, T. (2023). Harnessing aquaculture wastewater with *Chlorella protothecoides* for biodiesel and bioethanol production. *Maejo International Journal of Energy and Environmental Communication*, 5(2), 21-28.

Tsai, D. D., Ramaraj, R., Unpaprom, Y., Bhuyar, P., Ramaraj, R., & Chen, P. H. (2023). Autotrophic production of bio-functional proteins from freshwater microalgae using natural water medium for an economical and ecofriendly approach. *Maejo International Journal of Energy and Environmental Communication*, 5(2), 29-34.

Unpaprom, Y., Tipnee, S., & Ramaraj, R. (2015). Biodiesel from green alga *Scenedesmus acuminatus*. *International Journal of Sustainable and Green Energy*, 4(1), 1-6.

Waidee, T., Unpaprom, Y., & Ramaraj, R. (2018, December). Evaluation of Biodiesel Production from Pre-Heated Castor Oil (*Ricinus communis*). In International Conference on Renewable Energy (MEICRE 2018) (Vol. 14, p. 15).

Whangchai, K., Souvannasouk, V., Bhuyar, P., Ramaraj, R., & Unpaprom, Y. (2021). Biomass generation and biodiesel production from macroalgae grown in the irrigation canal wastewater. *Water Science and Technology*, 84(10-11), 2695-2702.

Wicker, R. J., Kwon, E., Khan, E., Kumar, V., & Bhatnagar, A. (2023). The potential of mixed-species biofilms to address remaining challenges for economically-feasible microalgal biorefineries: a review. *Chemical Engineering Journal*, 451, 138481.