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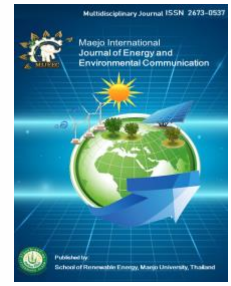

ARTICLE

Harnessing aquaculture wastewater with *Chlorella protothecoides* for biodiesel and bioethanol production

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ABSTRACT

The wastewater treatment potential of the microalga *Chlorella protothecoides* was investigated in the context of fish farming, and it is efficacy was further evaluated through cultivation at different inoculation levels to gauge pollutant removal efficiency. This study examines *C. protothecoides* for growth, nutrient removal, and biofuel potential. Climbing perch culture effluent (CPCE) mediums are employed for algal cultivation, with optical density (OD) measurements capturing growth dynamics that culminate on the 10th day in dense biomass accumulation. Nitrogen and phosphorus, key components of wastewater, exhibited substantial reduction. Ammonia concentrations decreased by 77.88%, nitrite by 93.75%, and nitrate by 95.67%. The most striking reduction was observed in phosphorus levels, with a remarkable 97.87% removal rate. Furthermore, the microalga's pigment composition was explored, showcasing high chlorophyll content, alongside significant carotenoids. High protein content (45.71 g/100 g) offers amino acids for bio-based materials and enzymatic catalysts. Carbohydrates (33.23 g/100 g) represent a valuable energy source for bioethanol production. Lipid content (8.64 g/100 g) suggests biodiesel potential, with unsaturated fatty acids comprising over 82% of the biodiesel content. The study underscores *C. protothecoides*'s potential in growth, nutrient removal, and biofuel production. Therefore, this research contributes valuable insights into sustainable wastewater treatment and bioenergy generation technologies, with empirical data supporting the findings.

1. Introduction

The quest for renewable biofuels has intensified due to multiple factors such as rising greenhouse gas emissions, fluctuating petroleum prices, dwindling fossil fuel reserves, and the urgent need to mitigate climate change (Manmai et al., 2021). Microalgae have come into the spotlight as a promising alternative for sustainable biofuel production. However, challenges remain in developing reliable and economical methods for algal biomass production and harvesting (Behera et al., 2019). Recent research has explored the potential of cultivating microalgae using various wastewater types, including agricultural, industrial, and municipal waste streams (Behera et al., 2021; Palanisamy et al., 2023). This approach has shown that the efficiency of microalgae-based biofuel production largely depends on selecting the right algal strains (Manmai et al., 2022).

Various species, including but not limited to *Chlorella*, *Scenedesmus*, and *Micractinium*, have proven effective in removing nutrients like nitrogen and phosphorus from wastewater (Torres-Tiji et al., 2020). Additionally, the biomass harvested from

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these processes offers potential raw material for producing not only biofuels but also other valuable products such as fertilizers and animal feed. Algae are primary producers in ecosystems, synthesizing their essential nutrients from basic inorganic substances (Pimpimol et al., 2020). These organisms exhibit diverse forms, ranging from single-celled to multicellular structures.

Algae contain essential biomolecules like chlorophyll-a, proteins, carbohydrates, and lipids (Ramaraj et al., 2016a; Tsai et al., 2017). They can grow through photosynthesis (autotrophy), utilizing light to fix carbon dioxide, or by metabolizing organic carbon (heterotrophy). There are over 30,000 identified species of algae, classified into major groups like Cyanobacteria (blue-green algae), Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) based on the Cassidy classification system from 2009 (Tsai et al., 2012; Unpaprom et al., 2015).

Microalgal cultivation primarily occurs through photo-autotrophic methods, using either open systems like ponds or closed systems such as photobioreactors. Since microalgae are photosynthetic, they require well-lit environments for growth. Autotrophic growth relies on light-driven photosynthetic reactions to fix carbon dioxide and generate energy in the form of adenosine triphosphate (ATP). An alternative cultivation method involves heterotrophic growth in dark conditions, replacing the need for photosynthesis with organic carbon sources in the culture medium (Trejo et al., 2020). However, this approach comes with challenges, including high operational costs and potential competition with food and other biofuel resources.

Photosynthesis is a critical process that allows algae to convert light energy into usable biochemical energy. It consists of light-dependent reactions, which capture light energy to form energy-rich molecules like nicotinamide adenine dinucleotide phosphate (NADPH) and ATP, and light-independent reactions that fix carbon dioxide into carbohydrates. Water serves as the electron and proton source, getting split and releasing oxygen in the process. In summary, microalgae hold promise as a source of renewable biofuels, but challenges remain in optimizing cultivation methods and scaling production (Saetang and Tipnee, 2021). The rapid development of land-based aquaculture has led to increasing environmental issues, particularly wastewater treatment.

Traditional methods for treating pollutants like nitrogenous compounds and phosphorus are becoming less eco-friendly and more expensive. Microalgae-based phytoremediation has emerged as a sustainable alternative (Ramaraj et al., 2016b). Microalgae not only assimilate pollutants but also generate biomass that can be used for aquaculture feed and biofuel. However, challenges remain, such as the slow growth rate of microalgae, the uncertainty of how exogenous microalgae interact with real aquaculture wastewater, and the inefficiency of current techniques in rapidly removing pollutants (Tsai et al., 2015). Research suggests that adjusting the initial inoculation dosages of specific microalgal species may speed up the treatment process.

Turning microalgae into biofuel involves two main approaches: biochemical and thermochemical methods. In the biochemical route, key processes are transesterification and fermentation, which yield biodiesel and ethanol, respectively (Zuccaro et al., 2020). Biodiesel is perhaps the most recognized form of biofuel derived from microalgae. During its production through transesterification, glycerol is generated as a byproduct. Additionally, microalgae are a promising source of bioethanol due to their high carbohydrate content and minimal lignin levels. However, the cellulosic material needs to be extracted from the cell walls before the algae can serve as a fermentation substrate (Calijuri et al., 2020). One proposed model integrates the production of biodiesel from microalgae while utilizing the leftover biomass for bioethanol, thereby optimizing resource use. This study aims to identify the most efficient microalgal species and inoculation concentrations for aquaculture wastewater treatment and bioenergy production.

2. Material and methods

2.1 Isolation and identification of microalgal strains

The isolation and identification of microalgae were executed based on methodologies substantiated by previous studies. The microalgae were initially collected from a fish pond at Maejo University using a plankton net featuring a 20-μm mesh size. Following collection, approximately 5 ml of the sample was inoculated into a sterile Bold Basal Medium (BBM) within 20 ml test tubes. These tubes were incubated at a room temperature of 25°C, under a light intensity of 65 $\mu mol^{-1} m^{-2} s^{-1}$ and a 16:8 h photoperiod for a span of 15 days. Post-incubation, individual colonies were carefully isolated and transferred to fresh media for further purification. The purification protocol involved the use of a 250 mL conical flask, and manual shaking of the culture broth was carried out five to six times per day. Stringent microscopic monitoring was conducted to ascertain the purity of the isolated strains.

2.2 Culture media preparation

Isolated and purified strains of microalgae, identified as *Chlorella protothecoides*, were cultured in 250-mL Erlenmeyer flasks containing 125 mL of culture medium formulated with BBM. The medium, designed for the cultivation of microalgae, is formulated from a variety of primary salts and trace elements. The primary stock solutions are prepared by dissolving specific concentrations of various compounds in a liter of deionized water. For instance, sodium nitrate is used at a concentration of 25g/L, while magnesium sulfate heptahydrate and dipotassium phosphate are both included at 7.5 g/L.

Additional elements like sodium chloride, potassium dihydrogen phosphate, and calcium chloride dihydrate are also part of the mix, used at 2.5g, 17.5g, and 2.5 g/L respectively. A separate trace elements mixture enriches the medium with nutrients like zinc sulfate heptahydrate at 8.82 g/L, manganese chloride tetrahydrate at 1.44g, and several others including molybdenum trioxide, copper(II) sulfate pentahydrate, and boric acid. Special additives like ethylenediaminetetraacetic acid, potassium hydroxide, and iron(II) sulfate heptahydrate are used in significant amounts along with a milliliter of sulfuric acid to complete the medium.

2.3 Growth conditions and monitoring

Culture flasks were strategically positioned on a reciprocating shaker set at 120 rpm. The cultures were maintained at a regulated room temperature of 25±1°C for a seven-day incubation period. Illumination was provided through cool white fluorescent lamps, with a predetermined light intensity of 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Algal growth was monitored spectrophotometrically at an optical density of 665 nm using a Spectronic Genesys 20 device from Thermo Fisher Scientific.

2.4 Effluent pre-treatment

The Climbing Perch (*Anabas testudineus*) is a species of freshwater fish commonly found in environments such as small rivers, canals, swamps, and artificial aquaculture settings. Effluent from climbing perch farming was gathered during regular drainage procedures of settling basins at Maejo University's Faculty of Fisheries Technology and Aquatic Resources. This Climbing Perch Culture Effluent (CPCE) served as the growth medium for the isolated *C. protothecoides*. The effluent underwent sedimentation and filtration via a filter cloth, followed by autoclaving at 121°C for 20 minutes. Post-autoclaving, the effluent was stored at 4°C for two days for further sedimentation. The supernatant was then employed for subsequent growth studies.

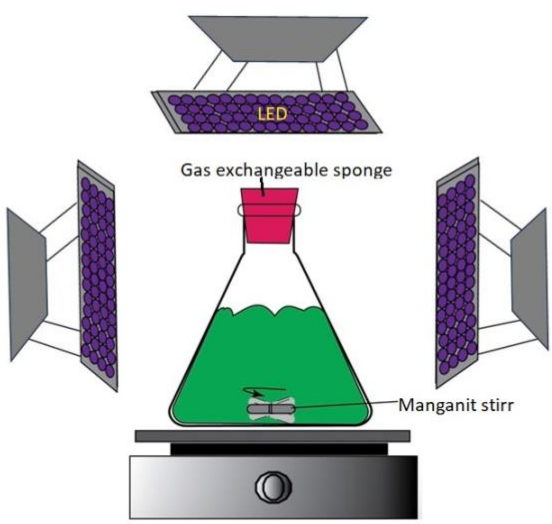

Figure 1. Schematic diagram of photobioreactor

2.5 Experimental framework and setup

The experimental framework for this study was delineated in Figure 1, featuring continuously stirred tank reactors (CSTRs) as the benchmark for reactor design. These reactors were meticulously set up within the premises of the Energy Research Center. All water quality parameters and additional experimental systems of measurement were evaluated at the Fisheries Technology and Aquatic Resources, Program in Biotechnology, Maejo University, Chiang Mai, Thailand. As elucidated in Figure 1, a schematic diagram was utilized to represent the microalgal cultivation system. The reactors were maintained under controlled conditions, specifically a room temperature environment, for a duration of 15 days. LED lamps with an irradiance level of 95 $\mu mol^{-1} m^{-2} s^{-1}$ were employed to provide requisite illumination. Each reactor was subject to a photoperiod of 16:8 h (light:dark). For algal biomass harvesting, ultrasonic devices with the same input power of 3 W and 43 kHz frequencies were used (Honda Electronics Company, Toyohashi, Aichi, Japan).

2.6 Analytical methods

Growth rate: The optical density (OD) was measured to determine the growth rate according to the formula:
Optical Density (OD) = Absorbance at 665 nm

Moisture content: Moisture content was calculated using the formula:

$$W_a=\left(\frac{Gw-Ga}{Ga}\right) x\ 100$$

When W_a represents the initial and final nutrient concentrations in the medium, respectively. Where

W_a represents material moisture content,
G_w is the wet weight of the material, and
G_a is the absolute dry weight.

Removal efficiency: The formula used was:

$$\text{Removal efficieny} = \left(\frac{Co-Gi}{Co}\right) x100$$

For the extraction of pigments such as chlorophyll and carotene, plant material was homogenized and mixed with 80% acetone, followed by centrifugation and absorbance measurement using a spectrophotometer. Similarly, the biochemical assessments for proteins, carbohydrates, and lipids were conducted in accordance with the procedures detailed by Tipnee et al. Protein content was ascertained via the Bradford assay, total carbohydrates were quantified using the Anthrone method, and lipids were extracted and estimated through a chloroform-methanol mixture followed by saponification and transesterification processes. All quantifications were performed by comparing absorbance values against known standards, using equations and calibration curves specified in the original work by Tipnee et al. (2015).

In this study, we followed the biodiesel production methods from Saengsawang et al. (2020). In this study used chemical methods to extract oil from algal biomass and then converted this oil into biodiesel. The procedures for optimizing the extraction and conversion, such as choosing the right catalyst, setting the temperature, and timing the reactions, were all based on Saengsawang et al. (2020)'s guidelines. We used their equations and calibration curves for all our measurements.

In this study, not only did we follow Saengsawang et al.'s 2020 methods for biodiesel production from algal oil, but we also utilized the remaining algal residues for bioethanol production, based on techniques established by Manmai et al. (2020). After the oil extraction process, the algal residues served as the feedstock for bioethanol production. This holistic approach ensured complete utilization of the algal biomass, demonstrating an integrated biorefinery model.

Algal residues were utilized completely for bioethanol production. Incorporating the techniques established by Manmai et al. (2020), the bioethanol production procedure kicks off with the preparation of the feedstock. Following this, both total and reducing sugars are quantified. The feedstock then experiences enzymatic hydrolysis to convert complex sugars into simpler forms. This hydrolyzed material is subsequently blended with a cultured yeast solution and placed in a sealed vessel for the fermentation process. Here, the yeast acts to transform the simplified sugars into ethanol. Once fermentation concludes, the resulting liquid is collected and subjected to analysis to evaluate its ethanol content.

2.7 Statistical Analysis

All the experiments were conducted in triplicate to bolster the reproducibility of the results. Mean values were computed and are represented as mean ± standard deviation (SD). All statistical analyses were executed employing the SPSS statistical software package, with significance levels set at $p<0.05$.

3. Results and discussion

3.1 Microalgal growth dynamics and efficacy of different culture media

Cultivating microalgae emerges as a crucial advancement for successful applications in the biofuel industry, where the significance of growth kinetics becomes paramount in deciphering the complexities of microalgae proliferation (Lee et al., 2015). The microalgal species investigated in this study demonstrated stable and consistent growth, evidenced by the increasing trend of optical density (OD) values shown in Figure 2. During the initial stages of the experiment, the algae exhibited robust growth rates, possibly owing to its inherent ability to thrive in nutrient-rich conditions. This phase of accelerated growth peaked on the 10th day of the experiment, as indicated by the highest recorded OD value. Following this peak, the algal population reached overpopulation status, manifesting as a dense, dark green biomass accumulation.

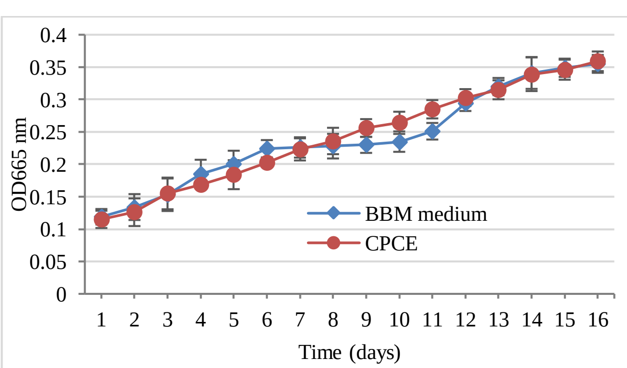

Figure 2. *C. protothecoides* cultivation using a different medium

The study further found that two different culture mediums, BBM and CPCE, produced comparable biomass concentrations, underscoring their shared effectiveness as suitable platforms for microalgal growth. Figure 2 illustrates the impact of wastewater nutrients on the growth curve of *C. protothecoides*. The algae experienced a rapid initial growth spurt, plateauing on the fourth day. Within this period, the algal biomass increased to 1.5 times its initial size, eventually reaching an 8.9-fold increment by the end of the 14-day experimental timeline. Our empirical data support the hypothesis that essential nutrients, such as phosphates and nitrates, are effectively metabolized by the microalgae, contributing significantly to biomass accumulation. This reaffirms the critical roles of nitrogen and phosphorus in facilitating algal growth, thereby lending additional weight to the study's overarching findings.

3.2 Efficacy of algal treatment in nutrient removal from climbing perch culture effluent

Table 1 delineates the characteristics of climbing perch culture effluent (CPCE) in relation to nitrogen and phosphorus content at both the initial and final stages of microalgal treatment. Given that excessive nitrogen and phosphorus in wastewater can lead to eutrophication, it is imperative to treat these elements effectively before discharge into natural water bodies. The nitrogen and phosphorus concentrations in the wastewater sample studied are presented in Table 1. The table underscores the effectiveness of microalgal growth in nutrient reduction from CPCE across multiple parameters throughout the experiment.

Table 1. Nutrition removal from the effluent

Parameter	Initial stage	Final stage	Removal (%)
Ammonia (mg/L)	$0.217\underline{+}0.05^{a}$	$0.048\underline{+}0.10^{c}$	77.88
Nitrite (mg/L)	$0.176\underline{+}0.10$a	$0.11\underline{+}0.02^{a}$	93.75
Nitrate (mg/L)	$1.364\underline{+}0.14^{a}$	$0.059\underline{+}0.17^{b}$	95.67
Phosphorus (mg/L)	$9.992\underline{+}0.11^{b}$	$0.213\underline{+}0.05^{a}$	97.87

Note: Different letters represent statistical difference between treatments and equal letters do not differ statistically, in the same line ($p<0.05$).

For ammonia, the initial concentration was 0.217 mg/L ± 0.05, which was reduced to 0.048 mg/L ± 0.10 at the final stage. This signifies an impressive 77.88% removal efficiency. Similarly, nitrite concentrations displayed a remarkable reduction, starting at 0.176 mg/L ± 0.10 and decreasing to 0.11 mg/L ± 0.02—a 93.75% removal rate. Notably, nitrate concentrations also declined significantly from 1.364 mg/L ± 0.14 to 0.059 mg/L ± 0.17, achieving a 95.67% removal efficiency. Most strikingly, phosphorus levels saw a drastic decline, plummeting from an initial 9.992 mg/L ± 0.11 to 0.213 mg/L ± 0.05, indicating a 97.87% removal rate. The total phosphorus concentration, which initially was 1.1 mg/L, also decreased by 99.1% following the microalgal treatment. To put our findings in context, we compared them to other studies in literature. Gao et al. (2016) reported average reductions of 86.1% for total nitrogen and 82.7% for total phosphorus in aquaculture wastewater using *C. vulgaris*.

Van Den Hende et al. (2011) observed nutrient reductions of 57.9% and 88.6% for nitrogen and phosphorus respectively using a mixed culture of microalgae and bacteria. Furthermore, Singh et al. (2022) confirmed high treatment efficacy in sewage wastewater, reducing nitrogen by 99.19% and phosphorus by 96% with the cultivation of *C. minutissima.* Therefore, our study robustly supports the pivotal role of microalgal growth in achieving highly effective removal of key nutrients such as nitrogen and phosphorus from CPCE. The high removal rates surpass or are competitive with those reported in existing literature, highlighting the potential of this approach for sustainable wastewater treatment.

3.3 Pigment composition and biochemical components of C. protothecoides

C. protothecoides is a green microalga that contains chlorophyll pigments, primarily chlorophyll-a and chlorophyll-b, which are critical for photosynthesis. These pigments capture light energy and convert it into chemical energy. In addition to chlorophylls, Chlorella protothecoides may also contain other accessory pigments like carotenoids (e.g., beta-carotene) that help in light harvesting and protect the cell from oxidative damage. In the study of *C. protothecoides*, the total chlorophyll and carotenoids were found to be 27.55 ± 0.528 mg/g dry weight and 7.68 ± 0.12 mg/g dry weight, respectively (Figure 3).

The high chlorophyll content indicates the algae's strong photosynthetic capabilities, making it suitable for applications that require efficient light utilization (Pirastru et al., 2012). The relatively high carotenoid levels suggest that the algae could be resilient to environmental stresses like high light intensity (Krishnamoorthy et al., 2021). The organism's ability to synthesize additional pigments other than chlorophyll also hints at its adaptability and potential for a range of biotechnological applications, from biofuels to wastewater treatment.

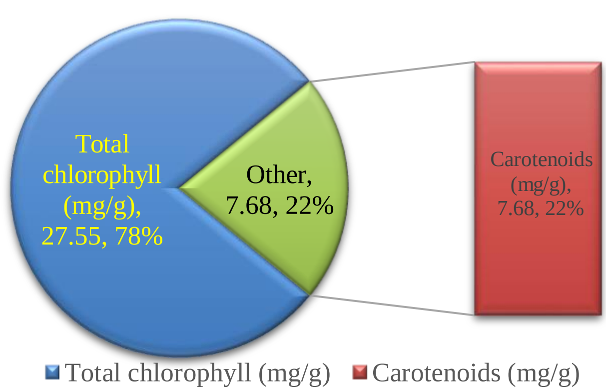

Figure 3. Pigment contents of *C. protothecoides* cultivated using CPCE

The recent analysis of the sample unveils a compelling portfolio of macro- and micronutrients that might have far-reaching implications beyond nutrition, particularly in the field of biofuels (Figure 4). For instance, the sample's high protein content, standing at 45.71 g/100 g, is intriguing. Proteins, crucial in many physiological activities such as enzymatic reactions and immune responses (Skjånes et al., 2021), could be harnessed for their amino acid constituents in the formation of bio-based materials or even enzymatic catalysts for biofuel production processes.

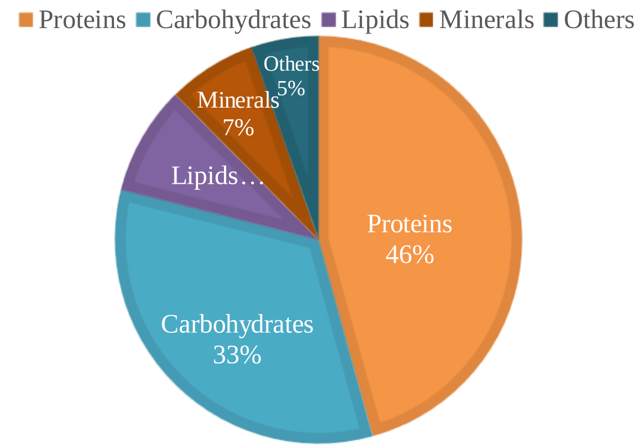

Figure 4. Biochemical components of *C. protothecoides* cultivated using CPCE

The carbohydrate fraction, observed to be 33.23 g/100 g, represents another noteworthy biofuel angle. Carbohydrates are the primary energy currency in biological systems, and their potential for conversion into bioethanol or other biofuels is significant (Williams et al., 2010). The composition of carbohydrates, whether simple sugars or complex starches, determines optimal conversion pathways, guiding biofuel production strategies (Whangchai et al., 2021a). A lipid content of 8.64 g/100 g has biofuel implications, with lipids being well-studied for high-energy biodiesel production via efficient conversion to fatty acid methyl esters (FAMEs).

Lipid quality, categorized by saturated or unsaturated fatty acids, impacts the physicochemical properties of the resulting biodiesel (Abomohra et al., 2020). Furthermore, the mineral content at 7.11 g/100 g and miscellaneous components at 5.31 g/100 g are not to be overlooked. While minerals can act as catalysts or even contaminants in biofuel processes (Do Minh et al., 2020) the "Others" category might include substances like pigments or unique organic compounds that could have novel applications or represent impurities that need to be removed prior to biofuel conversion.

As a result, the sample's abundant nutritional profile provides significant prospects for biofuel research (Trejo et al., 2022). This becomes clear through the unique potential exhibited by every macronutrient class: proteins, carbohydrates, and lipids all offer avenues to contribute to diverse bioenergy conversion route strategies (Whangchai et al., 2021b). The mineral and miscellaneous components add complexity but also potential versatility to the sample. Further studies are warranted to dissect these components at a molecular level, assess their respective bioconversion efficiencies, and explore their interactions within the biofuel production landscape.

3.4 Biodiesel Production from C. protothecoides using climbing perch culture effluent

In the present study, the efficacy of microwave-assisted transesterification for biodiesel production using microalgal oil was thoroughly investigated. Utilizing oil from *C. protothecoides* as the feedstock, along with methanol and potassium hydroxide as catalysts, a detailed exploration of key process parameters was conducted. These parameters included the methanol-to-oil ratio, reaction time, and catalyst-to-oil ratio, each of which was scrutinized for its impact on methyl ester yield (Yaşar and Altun, 2018). The research also evaluated the multipurpose utility of *C. protothecoides*, emphasizing its capacity for concurrent nutrient and CO_2 absorption. This dual-function approach was posited to address existing challenges in biofuel production, including cost-related concerns.

Importantly, the produced biodiesel exhibited a fatty acid composition within the C16–C18 range, confirming its suitability as a high-quality biofuel, evidenced by its high cetane number (Patel et al., 2022). Subsequent analysis revealed that the lipids extracted from *C. protothecoides* were effectively converted into biodiesel via transesterification. The biodiesel predominantly comprised cetane acid methyl ester, linoleic acid methyl ester, and oleic acid methyl ester (data not shown). Unsaturated fatty acid methyl esters constituted over 82% of the total biodiesel content, underscoring the quality and potential utility of the biofuel produced.

3.4 The rise of bioethanol as an eco-friendly and efficient fuel alternative

Bioethanol has gained prominence as a highly favorable fuel alternative, primarily because of its potential for energy security and lower environmental impact compared to fossil fuels (Bhuyar et al., 2021; Vu et al., 2022). Unlike gasoline, bioethanol is an oxygenated fuel, containing 34.7% oxygen, which significantly contributes to its environmentally friendly profile. The oxygen content in bioethanol enhances its combustion efficiency by 15% compared to gasoline. This leads to reduced emissions of nitrogen oxides, a prevalent air pollutant (Khammee et al., 2021). Additionally, when ethanol is mixed with gasoline, the release of other hazardous gases like sulfur oxide and carbon monoxide is remarkably minimized (Sophanodorn et al., 2021).

Such toxic emissions are notorious for causing environmental issues like acid rain and water contamination, posing significant health risks. The production of bioethanol predominantly occurs through fermentation, a metabolic process wherein certain microorganisms convert soluble sugars into alcohol (Vu et al., 2018). In an anaerobic environment, some bacteria and yeast can metabolize simple carbohydrates like monosaccharides and disaccharides to produce ethanol and carbon dioxide (Khammee et al., 2021). Figure 5 reveals the outcomes of yeast fermentation.

When subjected to 1-3% NaOH chemical pretreatment over three days, the total sugar concentrations achieved were 57.163 g/L, 68.325 g/L, and 89.416 g/L, respectively. Post-hydrolysis sugar concentrations ranged from 79.926 g/L to 92.134 g/L over 1 to 3 days. Remarkably, the highest ethanol concentration recorded during a 24-hour fermentation period was 8.86 g/L. In summary, the eco-friendly and efficient nature of bioethanol makes it a compelling alternative to traditional fossil fuels, with tangible benefits in terms of energy security, combustion efficiency, and reduced environmental impact.

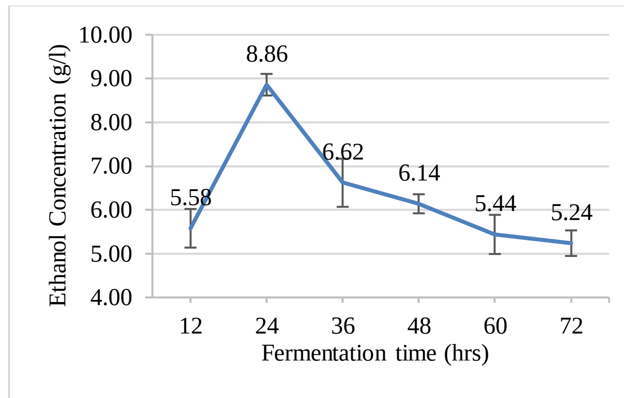

Figure 5. Bioethanol concertation

3.5 Biorefinery perspectives: unlocking the potential of C. protothecoides in tropical aquaculture systems

Microalgal role in nutrient capture and CO_2 sequestration: In tropical land-based aquaculture environments, the microalgae *C. protothecoides* present a compelling case for sustainable biorefinery applications (Ramaraj et al., 2016a; Tsai et al., 2017). These unicellular, photosynthetic microorganisms can capture CO_2 from the atmosphere or industrial emissions, while also effectively assimilating essential nutrients like nitrogen, phosphorus, and carbon from wastewater (Samorì et al., 2013). This dual functionality not only helps in the oxygenation of effluents but also mitigates CO_2 levels, making it a potential tool for environmental restoration.

The bioresource potential: Lipids, protein, and carbohydrates: *C. protothecoides* are rich in lipids and fatty acids, making them an excellent bioresource. The resultant biomass is a complex mixture of lipids, proteins, and carbohydrates, each of which has a distinct bioproduct potential (Yaşar and Altun, 2018). Specifically, lipids can be extracted and converted into biodiesel, proteins can potentially be used for animal feed or enzymatic applications, and carbohydrates can be fermented to produce bioethanol.

Integrated biorefinery approach: From waste to wealth the incorporation of *C. protothecoides* into an integrated biorefinery approach could be a game-changer for both environmental sustainability and economic viability (Kuo et al., 2015). The biomass generated from wastewater treatment can serve multiple purposes. First, it can act as a biofilter, improving the quality of the water released back into natural systems. Second, it can be harvested for lipid extraction, yielding biodiesel as a renewable energy source (Vu et al., 2022). Additionally, residual biomass can be used for biogas production through anaerobic digestion or fermented to yield bioethanol.

Economic implications and revenue streams: Such a circular economy model does not just serve environmental needs; it can also open new revenue streams. By converting waste products into high-value biofuels, the model turns environmental liabilities into economic assets. It also reduces dependence on external raw material sources for biofuel production, making the system inherently more sustainable and cost-effective (Vu et al., 2022).

Global and regional applications: Given the scalability of such systems, there is potential for both localized operations for small-scale aquaculture farms and large-scale industrial applications (Khammee et al., 2021). Tropical regions, which often struggle with water quality and CO_2 emissions, could particularly benefit from this integrated approach. Consequently, the integration of *C. protothecoides* into tropical land-based aquaculture systems offers a comprehensive solution that addresses the challenges of wastewater treatment and CO_2 mitigation while simultaneously providing new avenues for biofuel production. This epitomizes the principle of a biorefinery, converting waste into valuable resources in a sustainable manner.

4. Conclusion

The study reveals the versatile potential of *C. protothecoides* with profound implications. The microalga's consistent growth led to dense biomass accumulation, effectively utilizing climbing perch culture effluent mediums for wastewater treatment. Notably, nutrient removal proficiency was observed, particularly regarding phosphates and nitrates, pivotal for biomass accumulation and the viability of microalgal growth for efficient nutrient removal, promising a sustainable approach to wastewater treatment. The microalga's diverse pigments highlight adaptability and potential for biotechnological applications from biofuels to wastewater treatment. Additionally, the comprehensive nutritional profile uncovers biofuel potential beyond nutrition, with heightened protein, carbohydrate, and lipid levels presenting avenues for innovative biofuel research. Exploring biofuel production, the study showcases efficient microalgal oil-to-biodiesel conversion. Furthermore, bioethanol emerges as a promising sustainable fuel alternative. Thus, the research signifies a notable advancement towards integrated, sustainable solutions encompassing wastewater treatment, climate change mitigation, and renewable energy production.

Conflict of Interest Declaration

The authors assert that there are no conflicts or personal affiliations that might be construed as impacting the outcomes shared in this research.

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