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ENHANCEMENT OF BIOCHEMICAL COMPOSITION OF MICROALGAL BIOMASS THROUGH FRUIT WASTE NUTRIENT SUPPLEMENTATION FOR BIOFUEL APPLICATIONS

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ABSTRACT

Microalgae represent a promising avenue for production of biodiesel and bioethanol. In this study, microalgae isolated from wastewater were cultured using a combination of Blue Green 11 (BG-11) medium and fruit waste nutrients at different ratios (1:0, 1:3, 1:1, and 3:1) to enhance the carbohydrates, lipids, and protein levels. The microalgae culture was maintained under controlled conditions, including pH of 8, temperature of 25°C, a light intensity of 60 m^{-2} s^{-1} and 16h light/8h dark cycle. Carbohydrates, lipids, and protein levels were estimated over eight days with two-day intervals. There were no significant changes observed in the carbohydrates, lipids, and protein levels at Day 2, and Day 4 across different ratios of BG-11 and fruit waste nutrient, whereas, a significant increase in these levels was observed at Day 8 with 1:1 ratio of BG-11 and fruit waste nutrient. These findings suggest that equal proportions of BG-11 medium and fruit waste nutrients enhance the production of microalgal carbohydrates, lipids, and protein levels. This optimised approach holds promise for the sustainable production of biofuels using microalgae.

Introduction

Microalgae mostly represent a non-consumable biofuel source, capable of exponential growth in various environments, including saline, industrial, agricultural, and municipal wastewater (Ebhodaghe *et al.*, 2022). Moreover, some microalgae species are rich in nutritional compounds and used directly as food or feed. Microalgae are an excellent industrial raw material as they have high photosynthetic efficiency and capacity to accumulate by-products within the cells (Sundaram *et al.*, 2023). Waste oils such as cooking oil waste and palm oil waste have higher oil content; the algal source stands out as particularly simple feedstocks for biodiesel production (Zulqarnain *et al.*, 2021). Algae derived biofuels are devoid of sulfur, making them extremely safe for use (Mat Aron *et al.*, 2020). The microalgae utilisation as a main source of fuel is gaining serious consideration due to the escalating prices of crude oils and mounting concerns about poor air quality, ecological imbalances, health implications, and global warming associated with fossil fuel combustion (Ali *et al.*, 2022; Khoo *et al.*, 2023; Hinduja *et al.*, 2024; Rai *et al.*, 2025).

Interestingly, various studies have demonstrated that microalgae can produce lipids using alternative media, especially wastewater and food waste (Kiran *et al.*, 2014; Barua &

Munir, 2021; Wang *et al.*, 2024). The photoperiod and intensity of light are critical factors that influence the algae biomass production (Parmar *et al.*, 2011; Metsoviti *et al.*, 2020). Based on technique and algal species, a variety of fuels can be produced (Peter *et al.*, 2021). Triglycerides and fatty acids which are obtained from algal biomass can be used as biofuel after transesterification, therefore lessening fossil fuel usages (Pandey *et al.*, 2024). Algae oil could be used to produce biodiesel whereas the obtained residual biomass is rich in sugars, which could be used for bioethanol production (Chew *et al.*, 2021; Wang *et al.*, 2021).

An ideal concentration of nutrients is needed so that the cell can quickly assimilate nutrients such as phosphorus and nitrogen, which are essential for their growth (Chen *et al.*, 2013). The composition of culture medium also plays a key role in amplification of biomass and to increase the algal lipid content. Similarly, factors such as pH, temperature, and lighting must be considered for growth of algae and production of energy yielding bio molecules like lipids and carbohydrates (Perez-Garica *et al.*, 2011; Zhang *et al.*, 2022). High lipid accumulation has been reported in microalgal species under nitrogen deficiency conditions (Udayan *et al.*, 2022). *Euglena* sp. is reported as an important source of lipids (Mahapatra *et al.*, 2013). Hence, the optimisation of medium achieves elevated of value-added products from microalgae (Singh *et al.*, 2025). Using fruit waste to produce biofuel is one of the best options, due to its abundant availability, cost-effectiveness, and environmental benefits (Shilpa *et al.*, 2013). Fruit waste is the cheapest and most readily available feedstock for formation of biofuel (Vishwakarma *et al.*, 2014). Utilisation of fruit waste not simply diminishes the environmental burden of waste accumulation but also minimises reliance on conventional, expensive raw materials (Lee *et al.*, 2024).

The present study was planned to enhance carbohydrates, lipids, and proteins levels with fruit waste nutrients as natural source and Blue Green 11 (BG-11) medium in microalgae isolated from wastewater, while also assessing the importance of natural nutrients and BG-11 medium in development of biomass production.

Materials and Methods

Isolation of Microalgae

Microalgae were isolated from wastewater near Lotus Pond (pH 6.5), Sri Venkateswara University, Tirupati. The wastewater was serially diluted and streaked on agar Blue Green medium (BG-11) (Rippka *et al.*, 1979). The pH of the medium was adjusted with 1N HCl to 7.3. The obtained individual colonies were collected and inoculated in 150 ml of BG-11 at 25°C in an Erlenmeyer flask. A photon flux of approximately 60 $\mu molm^{-2}$ s^{-1} was maintained (light intensity was measured with Lux meter) with 16h:8h light and dark cycles for 12 days on an orbital shaker at 110 rpm. The identification of the algal culture was performed under a light microscope, based on characteristics such as solitary, free swimming, elongated forms, lack of a cell wall, spindle shape, and tapering ends, the algal culture could be identified as Euglenaa genus of microalgae euglenoid (Bhatia *et al.*, 2020).

Preparation of Fruit Waste Nutrients

Fruit waste, consisting of banana, apple, fig, pineapple, and mango in equal proportions of whole fruits was collected from a local fruit market in Tirupati. Tap water was added to the mixed fruit waste, which was then crushed. The yielding slurry was filtered through Whatman No.1 filter paper and centrifuged at 3,000 rpm for 10 minutes to obtain a supernatant. This supernatant was sterilised in an autoclave at 121°C for 15 minutes, yielding a liquid fruit waste nutrient solution that was used as a growth medium for microalgae.

Microalgae Culture Conditions

Isolated microalgae were inoculated into BG-11 medium and fruit waste nutrients at various ratios. The cultures were maintained under the following conditions, as detailed in Table 1.

Table 1: Microalgae culture condition with different ratio of BG-11 medium and fruit waste nutrient

Groups	Ratio	pH	Temperature (°C)	Approximate Light Intensity (μmol m^{-2} s^{-1})	Light and Dark Cycle (Hours)
I	1:0	8	25	60	16:8
II	1:3	8	25	60	16:8
III	1:1	8	25	60	16:8
IV	3:1	8	25	60	16:8

Microalgal Biomass

Every two days up to eight days, the culture medium from different groups were centrifuged for five minutes at 3,000 rpm to obtain microalgal biomass as pellet. Then the microalgal biomass was shade dried and used for biochemical analysis.

Carbohydrate Content

The estimation of carbohydrate content was done by using anthrone-sulfuric acid method (Van Wychen & Laurens, 2020). A sample of 0.2 g shade dried algal biomass and distilled water (40:1) was heated in a water bath for 90 minutes at boiling point. The mixture was then centrifuge to separate the pellet and supernatant. The pellet was treated with 80% ethanol and placed in a water bath (70°C) for 15 minutes, followed by centrifugation (3,500 rpm) for 10 minutes. Perchloric acid (30%) was added to obtained sediment and stirred at 25°C for 20 minutes and centrifuged to achieve total carbohydrate hydrolysis. Sulfuric acid and anthrone were added to 0.3 ml of the supernatant to quantify glucose spectrophotometrically at 620 nm.

Lipid Content

Lipid content was analysed by using Chen *et al.* (2018) method. Briefly, biomass was suspended in 20 μL of 0.05 M phosphate buffer (pH 7.4). Then, methanol (25%) and glass beads (400 μm) were added to the microalgae cells and disrupted by the cell disruptor. An additional amount of methanol was added and saponified to release fatty acids. At room temperature, 600 μl of sample was added to 900 μl of a chloroform/ methanol solvent mixture and vortexed for two minutes. The mixture was centrifuged at 11,000 rpm for two minutes, and organic phase (200 μL) was transferred to two separate tubes; one containing 1M triethanolamine: 1N acetic acid (9:1, v/v) and the other of 1M triethanolamine: 1N acetic acid: Copper (II) nitrate hydrate ($Cu(NO_3)_2 \cdot 3H_2O$). The contents were vigorously mixed and centrifuged at 11,000 rpm for two minutes, and reading was recorded at 260 nm in a UV/visible spectrophotometer for organic phase.

Protein Content

Lowry *et al*. (1951) method was followed to estimate the protein content. Approximately 0.05 g shade dried microalgae were hydrolysed with 1N NaOH at 100°C for one hour and the mixture was centrifuged. The protein content was estimated in obtained supernatant. To the supernatant, TCA was added and centrifuged, and then the pellet was suspended in NaOH (1N), alkaline copper reagent and folin-phenol reagent. Measurement of color was taken by using UV–vis spectrophotometer (Hitachi model U-2000) at 600 nm.

Statistical Analysis

The statistical analysis was performed using SPSS (IBM). Significant differences $p < 0.05$) in different groups of lipid, carbohydrate, and protein were analysed by using one way-ANOVA (Tukey's HSD test) (n = 6).

Results

Different ratios of BG-11 medium and fruit waste nutrients were used to elevate the yield of carbohydrates, lipids, and proteins in microalgae. Carbohydrates are key nutrients for microbes to generate alcohols, serving as an energy source, participating in metabolism of cholesterol and triglyceride, and aiding in fermentation process. The digestive tract breaks down carbohydrates into glucose, which is used for energy. Most carbohydrates were transformed into monosaccharides through dilute acid saccharification, with fermentable sugars later convert into bioethanol by a process of hydrolysis and fermentation. In the present study, different ratios of BG-11 medium and fruit waste nutrients were used to increase carbohydrate levels in microalgae. The 1:1 ratio showed a significant increase in carbohydrates at Day 8, followed by Day 6, while no significant increase was observed at Day 2 and 4 across all ratios (Figure 1).

Biofuels derived from lipids have greater energy density and enhanced compatibility with existing infrastructure, with performance improvement possible by altering the composition of chemical of lipid feedstocks. In the process of biodiesel production, lipids and fatty acids are obtained from dry biomass sourced from natural materials. Microalgae, for instance, are utilised to store lipids which are later converted into fatty acid methyl esters through transesterification, ultimately leading to the creation of biodiesel. In the latest examination, different ratios of BG-11 and fruit waste nutrients were used to enhance biomass lipid content. The 1:1 ratio of BG-11 medium and fruit waste nutrient showed a significant increase in lipids at Day 8 whereas no significant increase in lipid content was observed in remaining ratios at all selected time intervals (Figure 2). In addition to lipid content, A significant increase in microalgae protein consist was noted at Day 8 in the 1:1 ratio of BG-11 medium and fruit waste nutrient, while no significant difference in protein content was observed at Day 2, 4, and 6 in between all ratios (Figure 3).

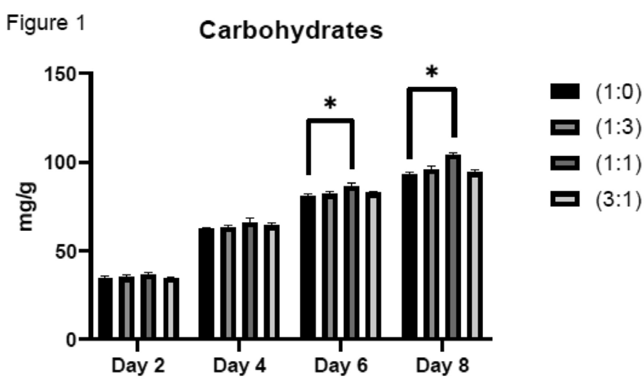

Figure 1: Carbohydrate content in different ratios of BG-11 medium and fruit waste nutrient at Day 2, 4, 6, and 8

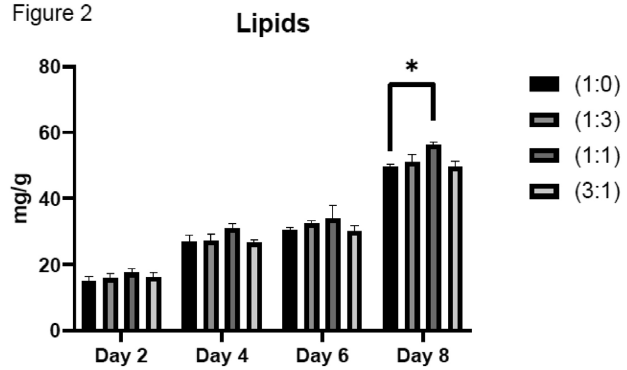

Figure 2: Lipid content in different ratios of BG-11 medium and fruit waste nutrient at Day 2, 4, 6, and 8

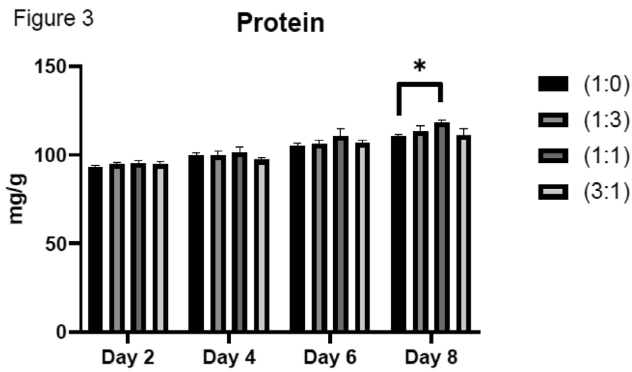

Figure 3: Protein content in different ratios of BG-11 medium and fruit waste nutrient at Day 2, 4, 6, and 8

Discussion

Microalgae biomass can be utilised through various thermo chemical and biochemical conversion pathways to produce fuels such as biodiesel, ethanol, bioelectricity, biomethane, biohydrogen, and biokerosene (Marangon *et al.*, 2023). It has the capability to grow in saline, industrial, agricultural, and municipal wastewater and accumulates the neutral lipids in biomass, which can be converted into biodiesel by a process called transesterification (Rismani-Yazdi *et al.*, 2012; Chen *et al.*, 2018). To optimise microalgae production, an ideal concentration of nutrients is necessary for the rapid assimilation of nitrogen (N) and phosphorus (P), including water (H_2O) and carbon dioxide (CO_2) in culture medium (Chen *et al.*, 2013; Vasistha *et al.*, 2021; Osman *et al.*, 2023). However, elevated levels of lipid content were noticed in *Chlorella pyrenoidosa* under nitrogen, phosphorus, or sulfur deprivation, which also enhanced the quality of the resulting biodiesel (Zhang *et al.*, 2019). The nutrient medium composition plays a crucial role in amplifying biomass and lipid content in algal cells (Zhang *et al.*, 2022).

Photosynthesis is essential for carbohydrate accumulation and algal growth, requiring light, oxygen, carbon dioxide, and water (Meyer, 2015; Cheng *et al.*, 2017). Carbohydrates in microalgae derive from starch in chloroplasts and from polysaccharides/cellulose (Domozych *et al.*, 2012; Moreira *et al.*, 2022). BG-11 medium is well-known for freshwater microalgal biomass and lipid production. Its composition is often optimised with nutrient supplementation to enhance biomass yield based on the cultivation mode (Pandey *et al.*, 2023). In this study, we maintained specific photoperiods, artificial light intensity, pH, and different ratios of BG-11 medium and fruit waste nutrients to improve carbohydrates and lipid contents. Therefore, current findings indicate that a 1:1 ratio of BG-11 medium and fruit waste nutrients yields the highest carbohydrate content and suggests that natural nutrients and BG-11 medium may have synergic effect on carbohydrate production in microalgae. The elevated microalgal growth could be significantly accelerated by BG-11 medium, which is widely recognised for its effectiveness in promoting large-scale biomass production (Chakraborty *et al.*, 2023; Fatima *et al.*, 2025). Due to its nutrient-rich composition, BG-11 medium is commonly utilised in the cultivation of various microalgae species to achieve optimal growth and biomass production (Gao *et al.*, 2024).

Use of microalgae in biodiesel production involves extracting cytosolic lipid bodies, which can be refined into biodiesel (Parapouli *et al.*, 2020). Microalgae strains producing triacylglyceride (TGA) are potential feedstocks for biodiesel and other algal oil-based products (Ghasemi Naghdi *et al.*, 2016). The TGA values are highest at high temperatures when exposed for shorter durations. Effective lipid extraction from microalgae requires proper cell disruption, regardless of whether organic solvents are used (Zhang *et al.*, 2022). Pre-treatments can enhance lipid recovery efficiency by degrading the microalgal cells, enabling easier lipid extraction (Pôjo *et al.*, 2021). Mass transfer limitations, lipid accessibility, insoluble biomass residues, and emulsification influence the lipid recovery from wet algal biomass. Various methods for lipid recovery include microwave treatment, ultrasound, bead milling, osmotic shock, and enzymatic or acid hydrolysis (Rivera *et al.*, 2018).

In this study, spectrophotometric method was used to estimate microalgal lipids. The 1:1 ratio of BG-11 medium and fruit waste nutrients resulted in elevated lipid and protein levels by Day 8, highlighting the positive effect of combining natural and BG-11 medium on lipid and protein production in microalgae. A recent study by Hwang *et al.* (2021) reported that the production of cyanobacteria (*Anabaena cylindrica*) biomass was higher when grown in papaya peel and pineapple peel medium. These findings support the current study, suggesting that combining fruit waste with BG-11 medium can enhance the biomass production significantly.

There remains significant potential for follow-up studies to comprehensively assess, from both techno-economic and environmental perspectives, organic matter application rates, and agitation, when applied at larger scales (Dębowski *et al.*, 2025). While optimised parameters can be established at the laboratory scale, scaling up to industrial or commercial levels may present challenges (Marangon *et al.*, 2023). Productivity outcomes might differ due to variations in system dynamics, necessitating further engineering modifications and operational adjustments to ensure consistent performance and efficiency.

Conclusions

Microalgae are promising candidates for producing biofuels, bioactive medicinal products, and food ingredients. Carbohydrate and lipids derived from microalgae could be used for biodiesel and bioethanol production. Under favourable conditions such as pH, light intensity, photoperiod, and temperature, microalgae can grow exponentially. In the contemporary analysis, we used fruit waste nutrients combined with BG-11 medium to enhance microalgae growth. Equal quantities of natural nutrients and BG-11 medium produced elevated levels of carbohydrates, lipids, and proteins. The proteins produced by microalgae can serve as additional nutrients for living organism, making microalgae a versatile and sustainable resource for multiple applications.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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