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Utilization of Banana Peel Extract for Biomass, Lipid and Carbohydrate Production in *Chlorococcum humicola* (Nägeli)

Inaam Sh. Al fiadh, Thaer Mohammed Ibrahim
Department of Biology. College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

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ABSTRACT

The high cost of synthetic culture media remains a major limitation for large-scale microalgal cultivation. Therefore, this study investigated the potential use of banana peel extract (BPE) as a low-cost alternative culture medium for the cultivation of the green microalga *Chlorococcum humicola*. The alga was grown in BPE at concentrations of 10, 25, and 50 mg.L^{-1} and compared with Chu13 medium as a control. Cultures were maintained for 21 days at 25 ± 2 °C under a light intensity of 3000 lux and a photoperiod of 8:16 h (light). Biomass production, total lipids, fatty acid composition, total carbohydrates, and carbohydrate profiles were evaluated. The results showed that the highest biomass production (459 mg.L^{-1}), total carbohydrate content (587.2 mg.g^{-1}), and α-linolenic acid content (6.31 mg.g^{-1}) were obtained at 10 mg.L^{-1} BPE. In contrast, the maximum lipid accumulation (288 mg.g^{-1}) was recorded at 25 mg.L^{-1} BPE. Fatty acid analysis revealed that α-linolenic acid was the predominant fatty acid in algal biomass grown in the alternative medium. Furthermore, BPE supported the production of several carbohydrates, including glucose, fructose, sucrose, galactose, mannose, and xylose. These findings demonstrate that banana peel extract can be utilized as an economical and sustainable alternative culture medium for *Chlorococcum humicola* cultivation, enhancing biomass production and valuable biochemical compounds while contributing to the valorization of agricultural waste.

Corresponding Author:

Inaam Sh. Al fiadh
Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham),
University of Baghdad, Baghdad, Iraq.
Email: inaam.shaker2202m@ihcoedu.uobaghdad.edu.iq

1. INTRODUCTION

Microalgae are unicellular microscopic organisms characterized by a short life cycle and a simple cellular structure, so they grow quickly [1] and they have the ability to tolerate extreme environmental conditions [2]. They are factories of live cell powered by solar light, they are able to exchange solar energy into chemical energy through their ability to capture carbon dioxide to synthesize carbohydrates through process of photosynthesis and stabilize CO2 to reduce greenhouse gases [3, 4] as it has fats, proteins and bioenergy [5, 6]. Microalgae research has been developing generally for more than a period due to its very different submissions in several fields, as it represents a renewable and environmentally friendly raw material for producing a variety of products [7]. So it is utilized in agriculture as fodder and organic fertilizers [8], in the pharmaceutical field as diurnal food, nutritional complements, or medicines to inhibit or treat numerous diseases [9], and in the field of renewable energy used as raw materials to Biofuel, Biohydrogen [10, 11, 12], and produce some types of Solar cells [13, 14]. Meanwhile in the field of cosmetics, it is used as a moisturizing, thickening and antioxidant agent, algal cells produce a variety of biologically active compounds that represented by proteins, fats, carbohydrates, phenols, flavonoids, vitamins, free amino acids, enzymes, growth regulators, pigments, toxins, and antibiotics [4, 5, 13]. Microalgae cells have the potential to produce and extract fats, which can then be utilized and transformed, particularly biodiesel, biofuel [15]. Due to its high content of vitamins and minerals, it is also served as food supplements for human function

[16]. The cultivation, harvesting and processing of algae requires a lot of energy and cost [17, 18, 19]. Current cultivation and processing techniques are not sustainable or cost-effective, particularly for the production of algal biofuel [20, 21].

Researchers are exploring the use of low-nutrient growth media for algal biomass production, as current methods cause environmental imbalances [17, 18]. Algae require light, carbon, growth medium, and nutrients, and their growth rate and concentration are significantly impacted by the medium's production and preparation [14]. However, the high cost of synthetic media makes industrial cultivation of microalgae impractical [5, 22]. Low-cost alternative media, such as nutrient waste from industry and agriculture, have gained interest for large-scale algae cultivation [19, 23, 24].

Bananas are a common herbal plant found in humid tropical and subtropical regions. They belong to the Musaceae family and the genus Musa [25]. Raw bananas are nutrient-dense and easily digestible, and are used in various products like snacks, food coloring, and insecticides [26]. Green bananas, including their pulp and peel, are considered beneficial for human health due to their rich content of resistant starch [27, 28], polyphenols, protein, essential amino acids, polyunsaturated fatty acids, potassium [29], carotenoids, natural antioxidants, and other physiologically active substances [30, 31]. Banana peels are orange-yellow and fragrant when ripe [32]. Bananas are used as food products for both their pulp and peel, providing vitamins and physiologically active substances. Minerals, including macroelements like calcium, magnesium, phosphorus, potassium, sodium, chloride, and sulfur, are essential for the body's overall health and metabolism. Studies show that fresh banana peels contain higher levels of potassium, calcium, and sodium minerals than dried ones [33].

The study aimed to form a culture medium that has a significant impact on both the growth rate and the final concentration of microalgae.

2. METHOD (10 PT)

2.1. Isolates

Pure isolate of *Chlorococcum humicola* was used, according to the following classification Division: Chlorophyta, Class: Chlorophyceae, Order: Chlorococcales, Family: chlorophaeraceae, Genus: *Chlorococcum*, Species: *humicola* and by method of Prescott [34] which isolated by the Dilution method and the Streaking method by Alukilly [35] kept in a sterile incubator.

2.2. Extraction

Banana samples were collected from the local market, the peels were separated, washed with tap water to remove dust, cut into small pieces, and left to dry at 25°C in the laboratory for a week. An electric grinder was used to grind the peels and obtain a powder that was stored in glass bottles, after that, 100 g of banana peel powder was taken and 500 ml of distilled water was added to it, and the mixture was placed on a magnetic stirrer for 24 hours at room temperature, the extract was filtered using sterile gauze and then Whatman No. 0.1 filter papers [36]. Chu13 culture medium that prepared by [37] as a control medium for the growth of the isolated alga.

The culture medium was prepared in the form of stock solution and stored in4C° until use, where specific quantities of it were mixed and the volume was accomplished with distilled water according to the required volume Table 1. Then the culture medium was sterilized by an autoclave at a temperature of 121°C and a pressure of 1.5 pounds/inch for 15 minutes [37].

Table 1. Chemical components of the medium Chu13.

No	Components	Nutrient solution [ml]	Stock solution [L/g]
1	KNO_3	10	40
2	K_2PO_4	10	8
3	$CaCl_2$	10	10.7
4	$MgSO_4.7H_2O$	10	20
5	Citric acid	10	10
6	Ferric citrate	10	2
7	Micro element		
	a. H_3BO_3	1	5.720
	b. $CoCl_2$	1	0.02
	c. $ZnSO_4.7H_2O$	1	0.44
	d. $CuSO_4.5H_2O$	1	0.16
	e. Na_2MoO_4	1	3.62
	f. $MnCl_2$		0.084

2.3. Preparing The Alternative Culture Media Preparing

Three concentrations of the previously prepared extract from banana peels were prepared, three replicates for each concentrations (10, 25, 50) m l.L^{-1} [38].

2.4. Experimental Design

An axenic culture of *Chlorococcum humicola* was used in this study. Three concentrations of banana peel extract (10, 25, and 50 ml.L^{-1}) were prepared, with three replicates for each treatment, in addition to Chu13 medium as a control. Each culture flask contained 1 L of the prepared medium and was inoculated with the algal culture at a ratio of 1:10 (v/v). Cultures were maintained for 21 days at 25 ± 2 °C under a light intensity of 3000 lux and a photoperiod of 8:16 h (light: dark).

2.5. Total Lipids Measurement

The research used a 2:1 methyl alcohol: chloroform mix to estimate total lipids. Dry alga was weighed, added, and stored at 25°C for 18 hours. After shaking, chloroform was added, and distilled water was added. Samples were centrifuged at 2000 RPM for 10 minutes, collected, and concentrated in fat. The sample was then dried at 80°C for 50 minutes and weighed. The total lipids were calculated using an equation [39]. Examinations were conducted at the Ministry of Science and Technology

P=(C x DCW) ÷ T

P= Lipid gm.L^{-1}.

C= lipid content of algal cells (g.$g.^{-1}$).

DCW= Dry weight of cells (g.L^{-1}).

T= Harvest time (in days).

2.6. Fatty Acid Diagnosis

Fast Liquid Chromatographic (FLC) was used to determine the amount of fatty acids. The mixture of fatty acids was separated on a column. FLC (Fast Liquid Chromatography) on Chromatometer Liquid Raf Shimadzu 10AV-LC [40, 41].

2.7. Determine the Total carbohydrates

To calculate carbohydrates, weigh algae and dissolve it in distilled water. Place samples in an ice water bath, add Anhtron, and heat for 10 minutes. Return to ice water, and absorbance measured. Prepare glucose concentrations to create a standard curve, and compare with previous treatments to determine carbohydrates [42].

2.8. Determine types of carbohydrates

Determine types of carbohydrates Fast Liquid Chromatographic (FCL) was used depending on the source [43]. Carbohydrates were calculated as follows: Sample (mg.g^{-1})= Sample area/Standard area × Standard concentration × dilution factor.

2.9. Statistical analysis

Data were analyzed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). All experiments were conducted in triplicate and results are presented as mean ± standard error (SE). Data normality was assessed using the Shapiro–Wilk test and homogeneity of variances was verified before applying one-way analysis of variance (ANOVA). Significant differences among means were determined using the Least Significant Difference (LSD) test at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Results showed that the biomass of alga *Chlorococcum humicola* when grown in different concentrations of aqueous of banana peels extract (BPE). It recorded 459, 416, 225 mg.L^{-1} dry weight at concentrations of 10, 50, 25 ml.L^{-1} respectively and recorded 724 mg.L^{-1} for the Chu13 as control, the highest dry weight value was 459 mg.L^{-1} in 10 ml.L^{-1} of (BPE) (Figure 1).

The enhanced biomass production observed at 10 mg L^{-1} BPE may be attributed to the balanced supply of organic carbon, potassium, calcium, and other nutrients naturally present in banana peel extract. These nutrients support cellular metabolism and photosynthetic activity, thereby promoting algal growth. In contrast, increasing the concentration of BPE beyond the optimum level may have reduced nutrient balance and light penetration, leading to lower biomass accumulation. Similar observations have been reported in studies utilizing fruit-waste-derived media for microalgal cultivation [33].

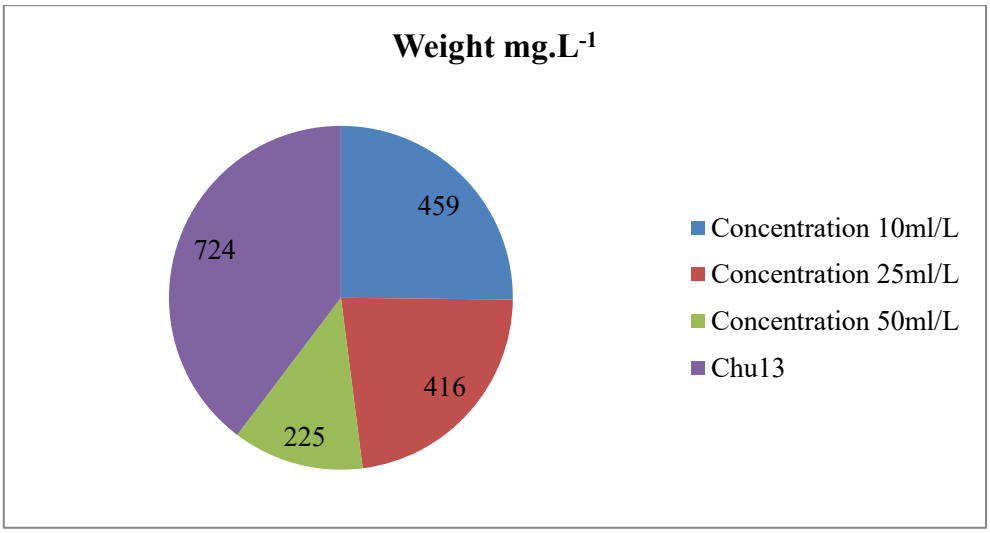

Figure 1. Dry mass of algae at different concentrations of (BPE) medium (mg.g^{-1}).

Algae record (64, 288, 176) mg.g^{-1} total lipids in the three concentrations 10, 50, 25 ml.L^{-1}, respectively, compared to the control medium, which recorded (0.220) mg.g^{-1}. The highest value of total lipids was at a concentration of 25 ml.L^{-1} of the alternative medium and was higher than the value the comparison Chu13 medium (Figure 2). This is consistent with [44] and [45].

The increase in lipid accumulation at 25 mg L^{-1} BPE may be associated with mild nutrient stress conditions that redirect cellular metabolism from growth toward storage compound synthesis. Under such conditions, microalgal cells tend to accumulate neutral lipids as an energy reserve. This metabolic response has been widely reported in microalgae cultivated under nutrient-limited conditions and is considered beneficial for biofuel-related applications [39].

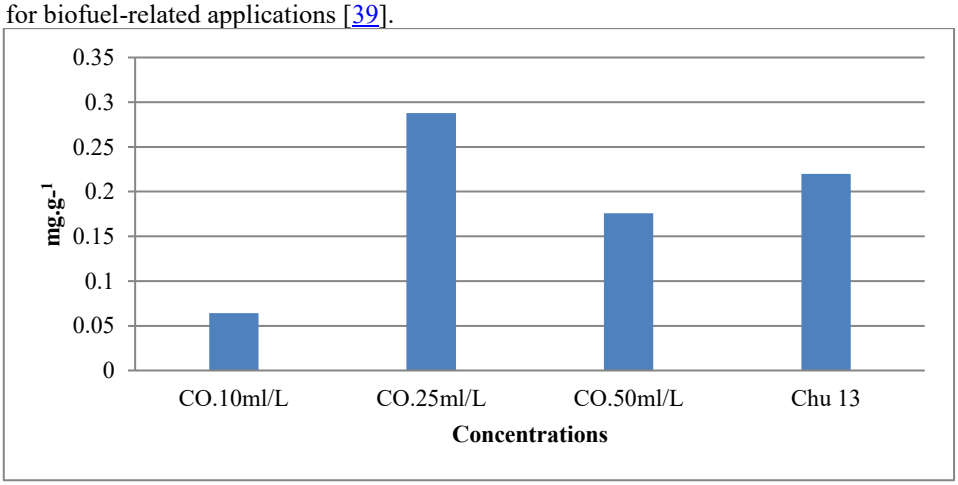

Figure 2. Total lipids of alga *C.humicola* in the banana peel aqueous extract medium (BPE).

The fatty acids of the algae were identified as in Table 2 and Figure 3, where fatty acid was recorded: Palmitic 3.53, 3.22, 4.14 mg.g^{-1} for concentrations 10, 25, and 50 ml.L^{-1} respectively, while Chu13 recorded 1.73. Stearic acid recorded 2.11, 1.63, 2.37 mg.g^{-1} for concentrations 10, 25, and 50 ml.L^{-1} compare with Chu13 who registered 1.01, as well as recorded Oleic acid 4.66, 2.03, 3.36 respectively meanwhile Chu13 registered 3.05 mg.g^{-1} for concentrations 10, 25, and 50 ml.L^{-1} respectively. Linoleic acid recorded 2.25, 3.03, 2.49 mg.g^{-1} for concentrations 10, 25, and 50 ml.L^{-1} respectively, while Chu13 recorded 3.33 mg.g^{-1} on respectively. Finally, acid.α-linolenic recorded 6.31, 2.88, 3.76 for concentrations 10, 25, and 50 ml.L^{-1} compare with Chu13 which registered 4.68 mg.g^{-1}. The highest fatty acid value recorded was for the fatty acid α-linolenic at concentration 10 ml.L^{-1}, it reached 6.31 mg.g^{-1} and the lowest value of total fatty acid fat Stearic acid in concentration Chu13, it was reached 1.01 mg.g^{-1}. This is consistent with [46] and [47].

The predominance of α-linolenic acid in cultures grown on BPE suggests that banana peel extract provides a favorable nutritional environment for the synthesis of polyunsaturated fatty acids. The enhancement of α-linolenic acid is particularly important because of its nutritional and industrial value. Variations in fatty acid composition among treatments may reflect changes in carbon allocation and enzymatic activities involved in lipid biosynthesis [31].

Table 2. Fatty acids of alga *Chlorococcum humicola* in banana peel aqueous extract (mg.g^{-1}).

Fatty acid mg.g^{-1}	Co.10ml.L^{-1} Mean±S.E.	Co.25ml.L^{-1} Mean±S.E.	Co.50ml.L^{-1} Mean±S.E.	Chu13(Control) Mean±S.E.
Palmitic acid	A3.53±0.044^{a}	A3.22±0.049^{b}	A4.14±0.009^{c}	1.73±0.031^{d}
Stearic acid	B2.11±0.032^{a}	B1.63±0.031^{b}	B2.37±0.006^{c}	1.01±0.018^{d}
Oleic acid	C4.66±0.057^{a}	C2.03±0.065^{b}	C3.36±0.009^{c}	3.05±0.040^{d}
Linoleic acid	B2.25±0.054^{a}	D3.03±0.074^{b}	B2.49±0.014^{c}	3.33±0.029^{d}
α-linolenic acid	E6.31±0.088^{a}	E2.88±0.111^{b}	E3.76±0.017^{c}	4.68±0.068^{d}
p-value		0.001*		

Values are presented as mean ± SE (n = 3). Different lowercase letters within the same row indicate significant differences among treatments, while different uppercase letters within the same column indicate significant differences among variables at $p \leq 0.05$ according to the LSD test.

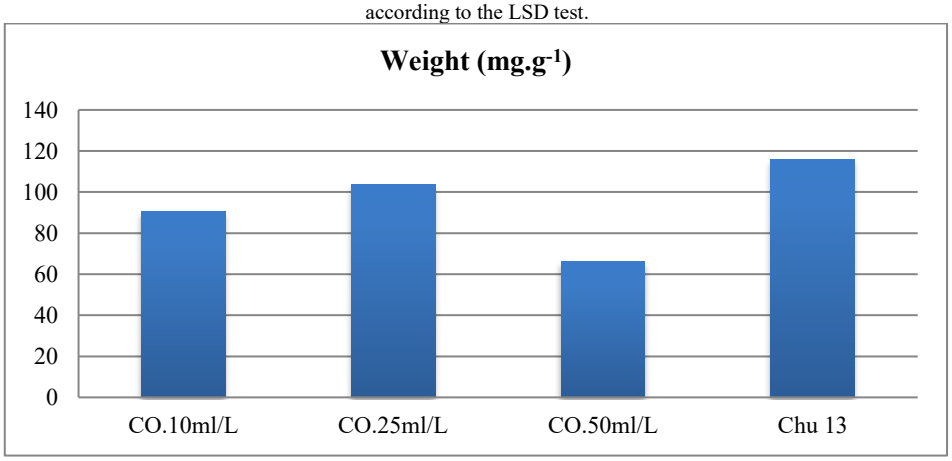

Figure 3. Fatty acid composition of alga *C.humicola* in the banana peel aqueous extract medium (BPE).

The values of sugars extracted from the grown alga were measured in BPE it was as follows: Glucose record 1.62, 1.20, 0.81 mg.L^{-1} for each of the concentrations 10, 25, and 50 ml.L^{-1} and Chu 13 record 2.07 mg.L^{-1}. Fructose record 1.12, 0.93, and 1.01 mg.g^{-1} for the three concentrations respectively and Chu 13 was 0.096, while sucrose recorded 1.71, 2.62, 0.74, 1.42 mg.g^{-1}. Galactose recorded 0.08, 1.62, 0.09, 1.03 mg.L^{-1}, moreover mannose recorded 0.91, 0.98, 1.08, 0.93 mg.L^{-1} and finally xylose recorded 0.11, 1.69, 0.04, 2.7 mg.g^{-1} for the three concentrations and Chu13 respectively. The highest value recorded in xylose was 2.70 mg.m^{-1} in Chu 13 followed by sucrose 2.62 mg.g^{-1} at concentration 25 ml.L^{-1}. The lowest value was for xylose 0.04 mg.g^{-1} at concentration 50 ml.L^{-1} (Table 3).

Table 3. Types of Carbohydrate of alga *Chlorococcum humicola* in banana peel aqueous extract (mg. g^{-1}).

Carbohydrate mg.g^{-1}	10 ml.L^{-1} Mean±S.E.	25 ml.L^{-1} Mean±S.E.	50 ml.L^{-1} Mean±S.E.	Chu13 (Control) Mean±S.E.
1.Glucose	A1.62±0.013^{a}	A1.20±0.014^{b}	A0.80±0.018^{c}	A2.07±0.012^{d}
2.Fructose	B1.12±0.010^{a}	B0.93±0.014^{b}	B1.01±0.016^{c}	B0.96±0.009^{d}
3.sucrose	C1.71±0.006^{a}	C2.62±0.009^{b}	C0.74±0.011^{c}	C1.42±0.006^{d}
4.Galactose	D0.08±0.005^{a}	D1.62±0.005^{b}	D0.09±0.008^{a}	D1.03±0.005^{d}
5.Mannose	E0.91±0.008^{a}	E0.98±0.009^{b}	E1.08±0.010^{c}	E0.93±0.005^{a}
6.Xylose	F0.11±0.003^{a}	F1.69±0.005^{b}	F0.04±0.005^{c}	F2.70±0.003^{d}
p-value		0.001*		

Values are presented as mean ± SE (n = 3). Different lowercase letters within the same row indicate significant differences among treatments, while different uppercase letters within the same column indicate significant differences among variables at $p \leq 0.05$ according to the LSD test.

The effect of the alternative medium on the fatty acid content appeared, as the amount of the five fatty acids recorded for the algae in the alternative medium for the two concentrations of 10 and 25 ml.L^{-1} was higher than the medium Chu13. Potentially increased carbon uptake organic led to increased levels of fatty acids, similar to what was mentioned in [46] and [48] studies. In addition, a study of [49] stated that palmitic acid and 12-Hydroxystearic acid are found in banana peel.

As for carbohydrates, the results recorded for the total carbohydrate for the three concentrations of the BPE medium were less than the value recorded in the control medium Chu13. However the amount of sugars extracted from the algae grown in the three concentrations of the extract BPE were higher than the

Chu13, except Galactose which recorded values lower than the control medium at the concentrations of 10 ml.L^{-1} and 25 ml.L^{-1}., and 1 ml.L^{-1} Xylose in concentration 10 ml.L^{-1}.

The maturation of fruits involved an increase in soluble sugar content and, at the same time, a decrease in starch. The degradation of starch under endogenous enzymes may explain the increase in the soluble sugar content in banana peel extract. This degradation of starch may be attributing to the action of endogenous enzymes, which may explain the increase in the soluble sugar content [50].

Microalgae require minimal nutrients to grow and provide a large biomass. When levels of nutrients are high, but some algae species will go into decline. Due to lack of nutrients in their environment in this experiment *C. humicola* alga was grown. In an environment where the minimum nutrients available from banana peel extract are available, such as potassium and calcium, which play a role in increasing biomass. This is consistent with [51] nitrogen deficiency also leads to low chlorophyll and more carotenoids accumulation of sugars and some oils and more production fats as noted in [44]. As indicated by [52] that extract obtained from fruit waste mostly contains on glucose and fructose, making it a great carbon source for microalgae biomass production.

Previous studies stated that the banana peel is rich in chemical compounds such as: the phenolic compounds [53], gallocatechin. Ripe banana peel also contains other compounds: anthocyanins (delphinidin and cyanidin) [54] and catecholamines [55]. On the other hand, carotenoids have been identified in the banana peel, such as β-carotene, α-carotene, and various xanthophylls as well as sterols and triterpenes, such as β-sitosterol, stigmasterol, campesterol, cycloalkanol, cycloartenol, and 24-methylenecycloartanol [50]

4. CONCLUSION

The present study demonstrated that banana peel extract can serve as an effective and economical alternative culture medium for the cultivation of *Chlorococcum humicola*. Among the tested concentrations, 10 ml L^{-1} supported the highest biomass production (459 mg.L^{-1}), carbohydrate content (587.2 mg.g^{-1}), and α-linolenic acid accumulation (6.31 mg.g^{-1}), whereas the highest lipid content (288 mg.g^{-1}) was obtained at 25 ml.L^{-1}. The results indicate that banana peel extract provides sufficient nutrients and organic carbon sources to support algal growth and biochemical productivity. These findings highlight the potential utilization of agricultural waste as a sustainable and low-cost resource for microalgal cultivation and value-added bioproduct production.

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