International Journal of Sustainable Agricultural Research
2023 Vol. 10, No. 2, pp. 52-63.
ISSN(e): 2312-6477
ISSN(p): 2313-0393
DOI: 10.18488/ijsar.v10i2.3364

Enhanced spirulina platensis growth for photosynthetic pigments production in oil palm empty fruit bunch medium

Simo Claude[1+]
Wamba Fotsop Oscar[1]
Nyabeu Ngnikeu Pascaline Laure[1]
Magwell Pierre Fils Rodrigue[2,4]
Maffo Nguena Momo Lucinda[2]
Tchoffo Djoudjeu Kennedy[2]
Minyaka Emile[2]
Lehman Leopold Gustave[3]

[1]*Laboratory of Plant Biology, Faculty of Science, University of Douala, Cameroon.*
[1]*Email: simoclaude@yahoo.fr*
[1]*Email: sfotsop@yahoo.fr*
[1]*Email: pascalinelaurenyabeu@gmail.com*
[2]*Biochemistry Laboratory, Faculty of Science, University of Douala, Cameroon.*
[2]*Email: lucindamomomaffo@gmail.com*
[2]*Email: kennedytchoffo@gmail.com*
[2]*Email: minyakae@yahoo.fr*
[3]*Laboratory of Animal Biology, Faculty of Science, University of Douala, Cameroon.*
[3]*Email: leopoldlehman@gmail.com*
[4]*Institute of Agricultural Research for Development, Nko'olong Station, Kribi, Cameroon.*
[2+]*Email: magkdowell@gmail.com*


(+ Corresponding author)

Article History
Received: 28 December 2022
Revised: 18 April 2023
Accepted: 3 May 2023
Published: 12 May 2023

Keywords
Enhance
Growth
Nutrients
Oil palm empty fruit bunch
Photosynthetic pigments
Production
Spirulina platensis.

ABSTRACT

S. platensis is a cyanobacterium known for its richness in protein and bioactive compounds, with very captivating therapeutic and nutritional properties. In Cameroon, the medium commonly used for the cultivation of *S. platensis* is Jourdan's medium which is expensive and less available. Thus, it is necessary to find an alternative medium that is more efficient, less expensive and more available. Nutrients present in agro-industrial by-products can be used to enhance the production of biomass and photosynthetic pigments of *S. platensis*. Therefore, this study was conducted to evaluate the growth and Photosynthetic pigments productivity of *S. platensis* on the Oil Palm Empty Fruit Bunch (OPEFB) medium. The culture medium was formulated by distilling different concentrations (0; 2; 4; 6; 8 and 10 g L^{-1}) of ash from previously dried and incinerated OPEFB medium in distilled water. The highest optical density (1.01 ± 0.09), dry biomass (1.35 ± 0.10 g L^{-1}) and productivity (0.032 g $L^{-1}d^{-1}$) were recorded on the medium formulated with 8 g L^{-1} of the OPEFB compared to the standard (Jourdan medium). The same medium showed the highest content of chlorophyll a (4.96 ± 0.31 mg L^{-1}), chlorophyll b (2.21 ± 0.16 mg L^{-1}) and carotenoids (1.43 ± 0.01 mg L^{-1}). However, the protein (156.1 ± 0.70 mg L^{-1}) and sugar (4.50 ± 0.43 mg L^{-1}) contents recorded on the standard medium were significantly high ($P < 0.05$). These results suggest that the OPEFB medium is a potential medium for *S. platensis* culture that can replace the expensive and less accessible Jourdan medium.

Contribution/Originality: The originality of the study lies in the direct use of Oil Palm Empty Fruit Bunch ash-based as an inexpensive alternative medium to the costly and unavailable Jourdan medium to enhance growth performance and the Photosynthetic pigments productivity of *S. platensis*. This study, thus, opens a new avenue for the use of Oil Palm Empty Fruit Bunch as the culture medium for the commercial and economic production of microalgae.

1. INTRODUCTION

Microalgae are widely sought for their potential use as a sustainable source of biofuels and bioactive compounds such as photosynthetic pigments (chlorophylls, carotenoids and phycobiliproteins) (Chakdar & Pabbi, 2012; Saini, Chakdar, Pabbi, & Shukla, 2020). These bioactive compounds have commercial values for pharmaceutical, nutraceutical, food and cosmetic applications (Li et al., 2019; Saini et al., 2020). Among these microalgae, *Spirulina platensis* (*S. platensis*) represents the most abundant photosynthetic, filamentous microalgae common to brackish lakes in Central Africa and Mexico (Suzuki, Yamaguchi, & Kawachi, 2019). It is presented as a green filament wound in more or less tight coils and is characterised by its high digestibility. *S. platensis* is a food source of high nutritional quality due to its high content of protein (50-70% of the dry weight of the alga), fatty acids, essential amino acids, vitamins (provitamins A, β-carotene, vitamin B1) and photosynthetic pigments. Besides this nutritional interest, *S. platensis* has therapeutic properties, particularly in the fight against cellular ageing and cancer, and has been shown to have hepatoprotective and anti-inflammatory activities and to strengthen immunity (Abouzed et al., 2022).

In view of its multiple properties, *S. platensis* is nowadays attracting the attention of the global community. Countries such as China, India and the United States are now the world's largest industrial producers of *S. platensis*, accounting for more than half of global production (Araújo et al., 2021). Cultivation of *S. platensis* to produce photosynthetic pigments is usually carried out in standard Jourdan's medium containing nutrients such as sodium bicarbonate, nitrates, phosphates, sulphates, micronutrients (AlFadhly, Alhelfi, Altemimi, Verma, & Cacciola, 2022). However, the cost and availability of nutrients on the local market are one of the bottlenecks for the sustainable and economic development of *S. platensis*. In Cameroon, the medium commonly used for growing S. platensis is the standard Jourdan medium.

However, this medium is costly, not easily accessible and has too high mineral requirements for large-scale production (Wamba et al., 2021). It would therefore be necessary to look for alternative growing substrates that would reduce the cost of the growing medium for economic production on a commercial scale. Oil Palm Empty Fruit Bunch (OPEFB) is an agro-industrial waste that is still rich in nutrients (nitrogen and phosphorus) essential for the growth of *S. platensis* (Aloo, Makumba, & Mbega, 2020; Windiastuti, Suprihatin, & Hasanudin, 2022). These nutrients present in agro-industrial by-products can be used to enhance biomass and photosynthetic pigment production of S. platensis. Thus, this study was undertaken to improve the growth and productivity of photosynthetic pigments of *S. platensis* on the Oil Palm Empty Fruit Bunch (OPEFB) medium. The novelty of the study lies in the direct use of Oil Palm Empty Fruit Bunch ash-based as an inexpensive alternative medium to the costly and unavailable Jourdan medium to enhance growth performance and the Photosynthetic pigments productivity of *S. platensis*. This study, thus, opens a new avenue for the use of Oil Palm Empty Fruit Bunch as the culture medium for the commercial and economic production of microalgae.

2. MATERIAL AND METHODS

2.1. Oil Palm Empty Fruit Bunch Medium Preparation

Oil palm empty fruit bunch (OPEFB) was collected in a sterile bag from a local palm oil company in Massoumbou, Douala, Cameroon (4° 07' 37" N, 9° 49' 39" E). The OPEFB was washed, shredded into small pieces, and oven dried at 105°C for 24 hours to remove oil and water. The dried OPEFB was then burnt in a muffle furnace at 550°C for 1h to form ash. Different concentrations (2, 4, 6, 8 and 10 g) of the obtained ash were macerated in a 2.5 L capacity glass bottle using 1L of water. The sample was then stirred for 1h using an electric stirrer. The mixture was then filtered through Whatman No.1 filter paper and the filtrate obtained was the aqueous extracts of OPEFB medium. $NaHCO_3$, Na_2CO_3 and NaCl were added to the medium respectively as a carbon source and ideal salinity provider.

2.2. Experimental Setup

Spirulina platensis (*S. platensis*) strain was obtained from the Algal Culture Collection of the SAGRIC (SEMAKO Agriculture) Common Initiative Group (Douala, Cameroon). Growth and maintenance of the culture were done in 20 L concrete tanks in Jourdan's medium (standard) which has a salinity of 12PSU (Practical Salinity Unit), in a greenhouse at 29 °C illuminated by a sunlight source and constant aeration provided by a diaphragm pump Table 1.

The experiment was done using a stepwise approach where *S. platensis* was grown at different concentrations of OPEFB to determine the optimal OPEFB concentration for biomass and pigment production. The algae *S. platensis* was cultured in 15 L working volume in 20 L open concrete tanks placed in a greenhouse at 29 °C illuminated by a sunlight source and constant aeration provided by a diaphragm pump (flux of 20 $L^{-1}h^{-1}$) for 42 days. Ten percent (V inoculation/V media) of inoculum was used for the initial cultivation.

Different concentrations of OPEFB in distilled water were used (0, 0.2, 0.4, 0.6, 0.8 and 1% w/v). The final salinity was set to 11.4 ± 0.5 PSU by using NaCl. The initial pH was set to 9.0 ± 0.4 by using 2 N NaOH. All experiments were carried out in triplicate. The physicochemical parameters of the media (temperature (°C), hydrogen potential, electrical conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}) and salinity (mg L^{-1}) were assessed using a multi-parameter (HI 98130, Hanna Instruments, Rhodes Island, USA).

Table 1. Chemical composition of the oil palm empty fruit bunch (OPEFP) medium and Jourdan medium used in S. platensis biomass production.

Jourdan medium (Standard)		Oil palm empty fruit bunch (OPEFP) medium		
Components	**Concentration (g L^{-1})**	**Treatments**	**Components**	**Concentration (g L^{-1})**
$(NH2)_2CO$	0.05		$NaHCO_3$ *	8
$(NH_4)_2HPO_4$	0.12		Na_2CO_3 *	5
KNO_3	2		NaCl *	5
$MgSO_4$	0.16	OPEFP - 0	OPEFP	0
$CaCl_2$	0.02	OPEFP - 2		2
$FeSO_4$	0.02	OPEFP - 4		4
NaCl	5	OPEFP - 6		6
$NaHCO_3$	8	OPEFP - 8		8
Na_2CO_3	4	OPEFP - 10		10

Note: * NaHCO3, Na2CO3 and NaCl (g L-1) were added in all the treatments of the oil palm empty fruit bunch (OPEFP) medium.

2.3. Analysis

2.3.1. Biomass and Growth Rate

Every 3 days, samples were collected for assessment of the biomass dry weight and the optical density of the cells. The optical density of the cells was measured at 680 nm using a spectrophotometer [(UV/VIS, Biobase - Spectrophotometer, China) (Spectrophotometer brand)].

The biomass dry weight was determined by filtering 20 mL samples through 47 mm GF/C (glass fibre filters brand) glass fibre filters (Whatman, U.K.) (X_1, g). The filters with the cells were washed twice with distilled water, oven-dried at 50 °C for 24 h and weighed (X_2, g). The Biomass dry weight was calculated by Equation 1:

$$\text{Biomass dry weight (X, g } L^{-1}) = (X_1 - X_2) \times 1000/20 \quad (1)$$

The biomass productivity (P_x) and specific growth rate (μ_m) of *S. platensis* were obtained by Equations 2 and 3 respectively:

$$\text{Biomass productivity } (P_x, \text{ g } L^{-1}d^{-1}) = (X_f - X_0) / t \quad (2)$$

$$\text{Specific growth rate } (\mu_m, d^{-1}) = \ln (X_f / X_0) / t \quad (3)$$

Where X_0 and X_f are biomass dry weights at the beginning and the end of the cultivation (t), respectively.

At the end of the exponential growth phase (36 days), the cultures were harvested for proteins, carbohydrates and pigments analysis.

2.3.2. Protein and Carbohydrate

The proteins extracted were quantified through a modified Bradford method (Bradford, 1976). A total amount of sample containing proteins, 0.1 mL, was mixed with 2 mL of Bradford reagent and measured at the wavelength of 595 nm using a UV-Vis spectrophotometer. The readings obtained in absorbance unit were converted into protein concentration using the calibration curve performed while using BSA as standard. Calibration curve of proteins concentration was performed using standards in a range of 0 - 1 mgL^{-1}.

Carbohydrates were measured using the protocol developed by DuBois, Gilles, Hamilton, Rebers, and Smith (1956). Briefly, dry biomass was digested in ethanol 80% (1 mL/2mg DW), then centrifuged at 35000 g for 10 min at 4°C. The supernatant (0.1 mL) was collected and mixed with 2 mL of 96% H_2SO_4 and 500 µL of 90% (w/v) phenol solution at 100 °C for 10 min then cooled. The total carbohydrate in the suspension obtained was determined using colorimetric, by comparing the light absorption of the solution at 490 nm with a calibration curve derived from a glucose standard solution.

2.3.3. Photosynthetic Pigments

2.3.3.1. Chlorophyll a, b and Total Carotenoids

Chlorophyll a, b and total carotenoids were measured using the protocol developed by Lichtenthaler (1987). The microalgae samples (2 mg) were extracted with 1 mL 90% acetone in the dark, for 24 h at 4 °C. Then, the sample was centrifuged for 15 min at 5000 rpm and the supernatant was collected.

Absorbance was measured at 645, 665 and 470 nm for chlorophyll a, b and total carotenoids, respectively. The chlorophyll and total carotenoid content were determined by the extinction coefficient in acetone and calculated by Equations 4, 5 and 6.

$$\text{Chla (mg } L^{-1}) = 12.7 \text{ x } A665 - 2.69 \text{ x } A645 \quad (4)$$

Where chla is chlorophyll a, A665 is the absorbance at A665 nm and A645 is the absorbance at 645 nm.

$$\text{Chlb (mg } L^{-1}) = 22.9 \text{ x } A645 - 4.68 \text{ x } A665 \quad (5)$$

Where chlb is chlorophyll b, A645 is the absorbance at 645 nm and A665 is the absorbance at A665 nm.

$$\text{Total carotenoids (m g } L^{-1}) = ((1000 \text{ x } A470 - 2.86 \text{ x chla} - 129.2 \text{ x chlb})) / 245 \quad (6)$$

Where A470 is the absorbance at 470 nm, chla is chlorophyll a and chlb is chlorophyll b.

2.3.4. Phycobiliproteins

The fresh *S. platensis* biomass was extracted with a phosphate buffer (0.05 M, pH 6.7) ratio 1:3 (w/v), by repeatedly freezing and thawing three times in the dark, for 24 h at 4 °C. Afterwards, the samples were centrifuged for 20 min at 5,000 rpm and the supernatants were analyzed by spectrophotometer (UV/VIS, Biobase – Spectrophotometer). The absorbance was read at 562nm for C-phycocyanin, 615nm for allophycocyanin and 652nm for phycoerythrin. The C-phycocyanin C (C-PC), allophycocyanin (APC), and phycoerythrin (PE) content were calculated by Equations 7-9. (Bennett & Bogorad, 1973):

$$\text{C-Phycocyanin (C-PC)} = [A615 \times 0.474(A652)] / 5.34 \quad (7)$$

$$\text{Allophycocyanin (APC)} = [A652 \times 0.208(A615)] / 5.09 \quad (8)$$

$$\text{Phycoerythrin (PE)} = [A562 \times 2.41(\text{C-PC}) \times 0.849(\text{APC})] / 9.62 \quad (9)$$

2.4. Data Analysis

The data was subjected to one-way ANOVA (with significance $p \leq 0.05$) data analysis and the mean differences were compared using Tukey test. All studies were conducted in triplicate and the values were expressed as mean ± standard error.

The statistical analysis was performed using IBM SPSS statistics software (SPSS version 26.0 for window; IBM Corporation; United States).

3. RESULTS AND DISCUSSION

3.1. Effect of Oil Palm Empty Fruit Bunch Medium on Growth Performance of S. Platensis

This study revealed a change in optical density and dry biomass throughout the cultivation process in all treatments. The optical density and dry biomass values ranged from 0.22 ± 0.02 to 1.01 ± 0.09 and from 0.47 ± 0.02 to 1.355 ± 0.10 g L^{-1}, respectively. The optical density and dry biomass recorded on day 36 on the medium containing 8 g L^{-1} of OPEFB were significantly higher than those of the other treatments and the control Figure 1a and b). This result could be explained by the presence of phosphorus, nitrate and sulphate in the OPEFB, as reported by Marhani, Laban, and Musa (2022) and Udeinya et al. (2021). The medium that received more OPEFB concentration would therefore be more favourable for better growth and consequently high optical density. However, the medium that did not receive OPEFB showed a decrease in optical density and dry biomass. This could be explained by the absence of mineral elements from OPEFB in this medium, which is important for the growth of S. platensis. These results are supported by those obtained by Gnimassoun, Ettien, and Masse (2020) working on the characterisation of the physicochemical properties of compost from a mixture of OPEFB and poultry droppings revealed that OPEFB is very rich in important nutrients for plant growth. On the other hand, the culture medium formulated with 10 g L^{-1} of OPEFB has a low optical density and biomass, which is explained by the fact that at too high concentrations, the medium becomes rather toxic for the crop. This toxicity could be linked to the high pH of the medium. On the other hand, Sharma, Schuhmann, and Schenk (2012) stated that the pH strongly affects biomass production, chemical dissociation and cell physiology.

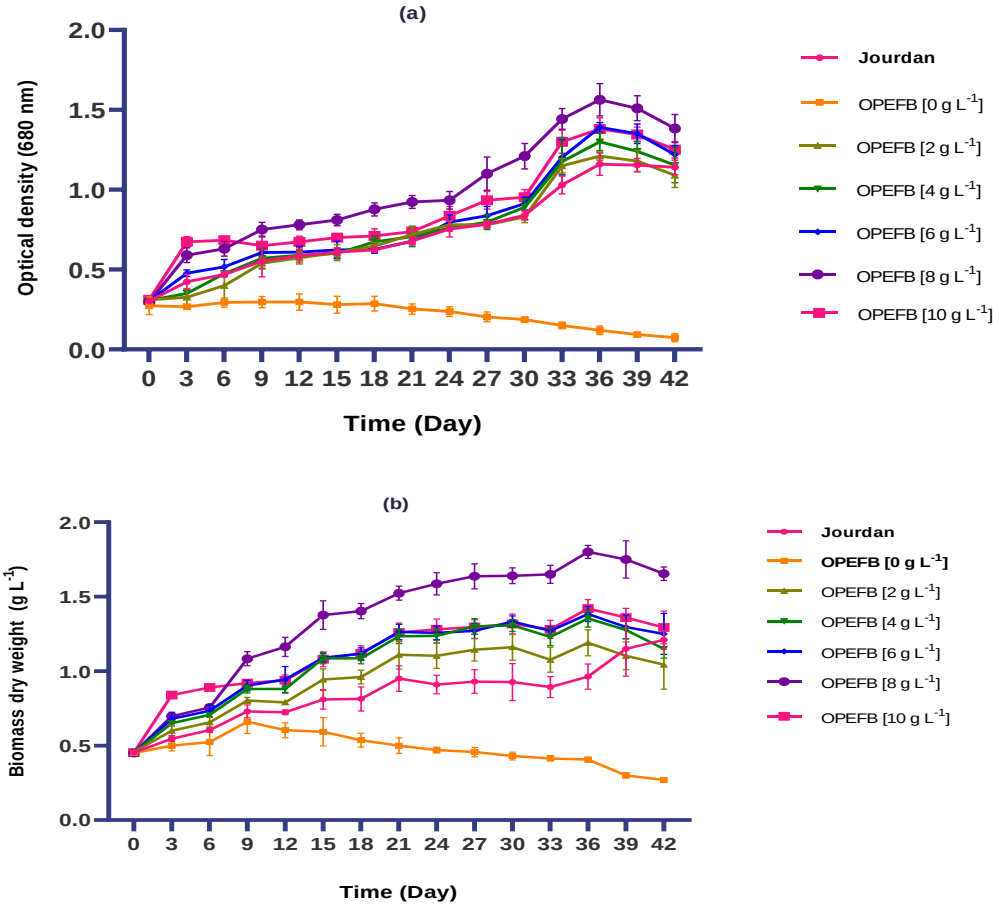

Figure 1. Variation of growth performance of S. platensis in Oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan medium. (a) Optical density and (b) Biomass dry weight. Data are presented as mean ± standard deviation (n = 3) as determined by the Tukey test. Bars indicate standard deviation.

Productivity (Px) and specific growth rate (μm) increased with increasing culture time. The highest values of productivity (0.032 ± 0.005 mg $L^{-1}d^{-1}$) and specific growth rate (0.027 ± 0.003 d^{-1} respectively) were recorded on the 36th day of culture on the medium formulated with 8 g L^{-1} of OPEFB compared to the other trials and the control. Similarly, the standard medium showed significant values of productivity (0.016 ± 0.002 mg $L^{-1}d^{-1}$) and growth rate (0.013 ± 0.003 d^{-1}) of *S. platensis* Table 2. These results could be explained by the addition of nutrients necessary for *S.* platensis growth through the OPEFB, which increases photosynthesis and in parallel the production levels of *S. platensis*. Indeed, for its growth, *S.* platensis needs the essential mineral elements contained in the OPEFB. These results are in agreement with those of Wamba et al. (2021) who demonstrated that *S. platensis* cultivation requires some essential nutrients for its growth.

Table 2. Optical density ($OD_{680\ nm}$), biomass dry weight (X), biomass productivity (P_x) and specific growth rate (μ_m) generated by *S. platensis* after 38 day in Oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan medium).

Media	Treatments (g L^{-1})	OD (680 nm)	X (g L^{-1})	P_x (g $L^{-1}d^{-1}$)	μ_m (d^{-1})
Jourdan medium	Standard	0.74 ± 0.07^{b}	0.84 ± 0.05^{b}	0.016 ± 0.002^{c}	0.013 ± 0.003^{b}
Oil palm empty fruit bunch (OPEFB) medium	Control - 0	0.22 ± 0.02^{d}	0.47 ± 0.02^{f}	-0.009 ± 0.004^{d}	-0.010 ± 0.001^{c}
	OPEFB - 2	0.74 ± 0.08^{bc}	0.94 ± 0.06^{c}	0.021 ± 0.003^{b}	0.020 ± 0.002^{ab}
	OPEFB - 4	0.77 ± 0.08^{a}	1.05 ± 0.07^{a}	0.024 ± 0.004^{b}	0.020 ± 0.001^{ab}
	OPEFB - 6	0.81 ± 0.09^{c}	1.08 ± 0.07^{d}	0.023 ± 0.003^{b}	0.023 ± 0.003^{ab}
	OPEFB - 8	1.01 ± 0.09^{c}	1.35 ± 0.10^{e}	0.032 ± 0.005^{a}	0.027 ± 0.003^{a}
	OPEFB - 10	0.87 ± 0.08^{e}	1.11 ± 0.06^{c}	0.025 ± 0.003^{b}	0.029 ± 0.001^{a}

Note: Data are presented as mean ± standard deviation (n = 3). Mean followed by the same superscript letter (a > b > c > d > e) in the same column indicates that they are not significantly different at ($p>0.05$) as determined by the Tukey test.

3.2. Effect of Oil Palm Empty Fruit Bunch Medium on the Protein and Carbohydrates Content of S. Platensis

S. platensis is one of the most important commercially produced microalgae species because to its nutritional value, especially for its protein content (up to 70%). The protein content of *S. platensis* grown with different concentrations of OPEFB is presented in Figure 2a. This protein content was significantly higher ($P<0.05$) in the medium with 8 g L^{-1} OPEFB (152.6 ± 1.47 mg L^{-1}) than in the other treatments and the control (0 g L^{-1} OPEFB) (71.2 ± 4.42 mg L^{-1}). In the standard medium, we equally recorded high protein content (156.1 ± 7.06 mg L^{-1}). In this study, the results show that the addition of OPEFB significantly ($p < 0.05$) improved the protein content of the crops in all treatments compared to the control. Thus, just as the addition of OPEFB has a positive effect on growth, the mineral elements such as nitrogen, magnesium and potassium present in the OPEFB may be essential co-factors for protein production. In this sense, Asoka, Abu, and Agwa (2021) have shown that an increase in mineral uptake was associated with an increase in protein. These results on protein content are lower than those reported by Nyabuto et al. (2015) with 3.24 mg mL^{-1} on a medium enriched with $NaNO_3$. This difference could be explained by the composition of the culture medium in which the microalgae grow, the size of the inoculum used or the culture conditions. Besides proteins, carbohydrates are also major biomolecular fractions of biomass. The carbohydrates of *S. platensis* are of interest, as we can be use them as a renewable feedstock for various fermentation processes for the production of chemicals and biofuels. Figure 2b below shows the variation in carbohydrate content of *S. platensis* grown with different concentrations of OPEFB. It can be seen from this figure that the highest carbohydrate content (4.50 ± 0.43 mg L^{-1}) was recorded on the Jourdan medium while the lowest carbohydrate content (1.37 ± 0.31 mg L^{-1}) was recorded on the medium that received no OPEFB formulation. Carbohydrates are the main products of photosynthetic metabolism and carbon fixation. They are either stored as reserve materials or transformed into cell wall components (polysaccharides, etc.). Thus, Taufikurahman, Ilhamsyah, Rosanti, and Ardiansyah (2020) reports that carbohydrate accumulation is inversely proportional to protein concentration.

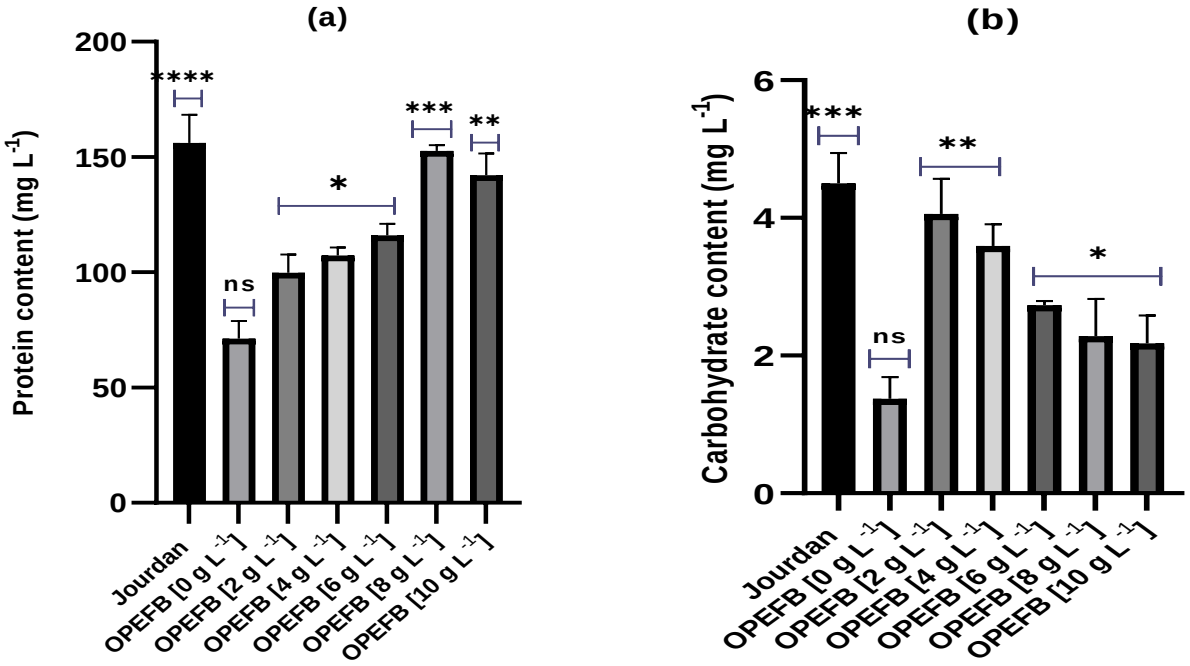

Figure 2. Variation of protein and carbohydrate content of *S. platensis* in Oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan medium. (a) Protein and (b) Carbohydrate content. Data are presented as mean ± standard deviation (n = 3).

Note: Bars indicate standard deviation. Bars followed by the superscript symbol (****>*** > **> * > ns) indicates that they are significantly different at (p>0.05) as determined by the Tukey test.

3.3. Effect of Oil Palm Empty Fruit Bunch Medium on the Chlorophyll and Total Carotenoid Content of S. Platensis

The pigment contents (chlorophylls a, b and total carotenoids) observed in the present study are presented in Table 3 and Figure 3a, b and c.

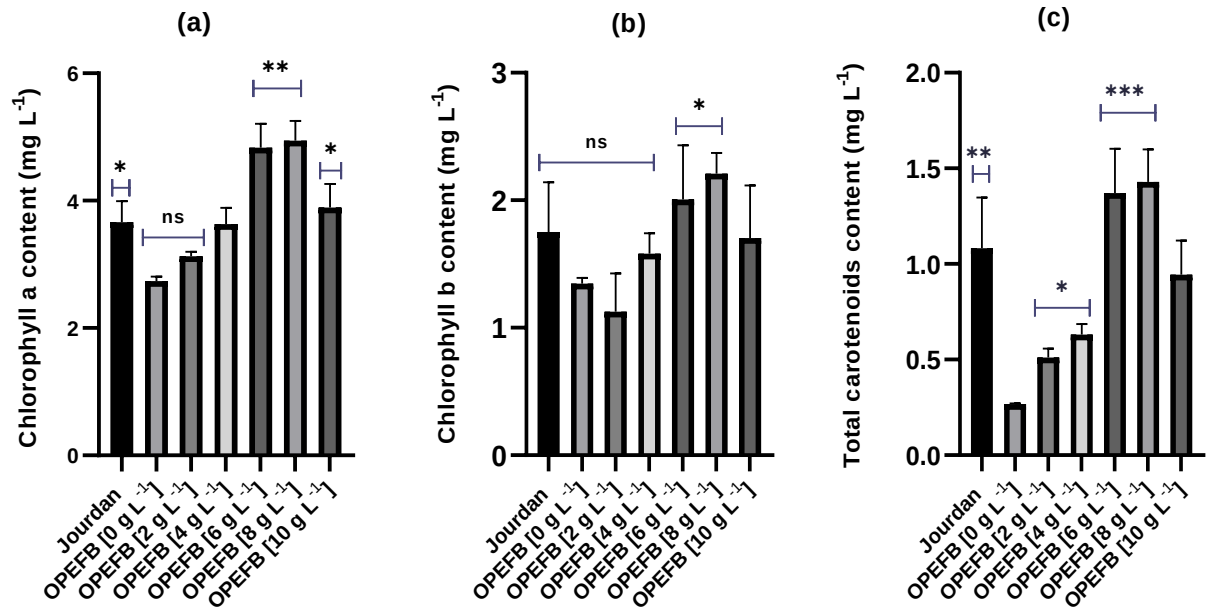

Figure 3. Variation of chlorophylls a, b and total carotenoids content in Oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan modified medium (standard). (a) Chlorophyll a, (b) Chlorophyll b and (c) Total carotenoids content. Data are presented as mean ± standard deviation (n = 3).

Note: Bars indicate standard deviation. Bars followed by the superscript symbol (****>*** > **> * > ns) indicates that they are significantly different at (p>0.05) as determined by the Tukey test.

From this figure, we note an increase in chlorophyll a and b content with the increase of the OPEFB concentration. However, the medium formulated with 8 g L^{-1} OPEFB showed significantly higher content of chlorophyll a (4.96 ±

0.31 mg L^{-1}), chlorophyll b (2.22 ± 0.16 mg L^{-1}) and total carotenoids (1.43 ± 0.01 mg L^{-1}) compared to other's essays. These values of the 8 g L-1 OPEFB medium are higher than those recorded in the standard Jourdan medium with 3.66 ± 0.33 mg L^{-1} for chlorophyll a, 1.75 ± 0.39 mg L^{-1} for chlorophyll b and 1.08 ± 0.26 mg L^{-1} for total carotenoids. Whereas, the lowest values of chlorophyll a (2.74 ± 0.07 mg L^{-1}), chlorophyll b (1.34 ± 0.04 mg L^{-1}) and total carotenoids (0.26 ± 0.03 mg L^{-1}) were recorded on the medium that received no OPEFB input. Indeed, the mineral composition of the OPEFB highlights the presence of mineral elements essential for photosynthesis and consequently for biomass production. Recent studies have shown that the quantity of pigments in microalgae do not only depend on temperature, light and nutrient availability but are also directly influenced by the availability of carbon in the environment. In the same sense, Dolganyuk et al. (2020) in a study, report that changes in inorganic carbon levels in the culture of microalgae can increase the amounts of pigments in the culture of these microorganisms. The results on chlorophyll a and chlorophyll b content differ from those obtained by Osório et al. (2020) who got 3766 µmg $L^{-1}$and 123 µmg L^{-1}, respectively. This difference could be correlated to the species of the microalgae used, the culture conditions, and the composition of the culture medium.

3.4. Effect of Oil Palm Empty Fruit Bunch Medium on the Phycobiliprotein Content of S. Platensis

Phycobiliproteins and in particular phycocyanin are considered a very promising source of natural blue dye, while also having functional properties. In the present study, the phycobiliprotein content showed a similar increasing trend as the chlorophyll content with increasing OPEFB concentration.

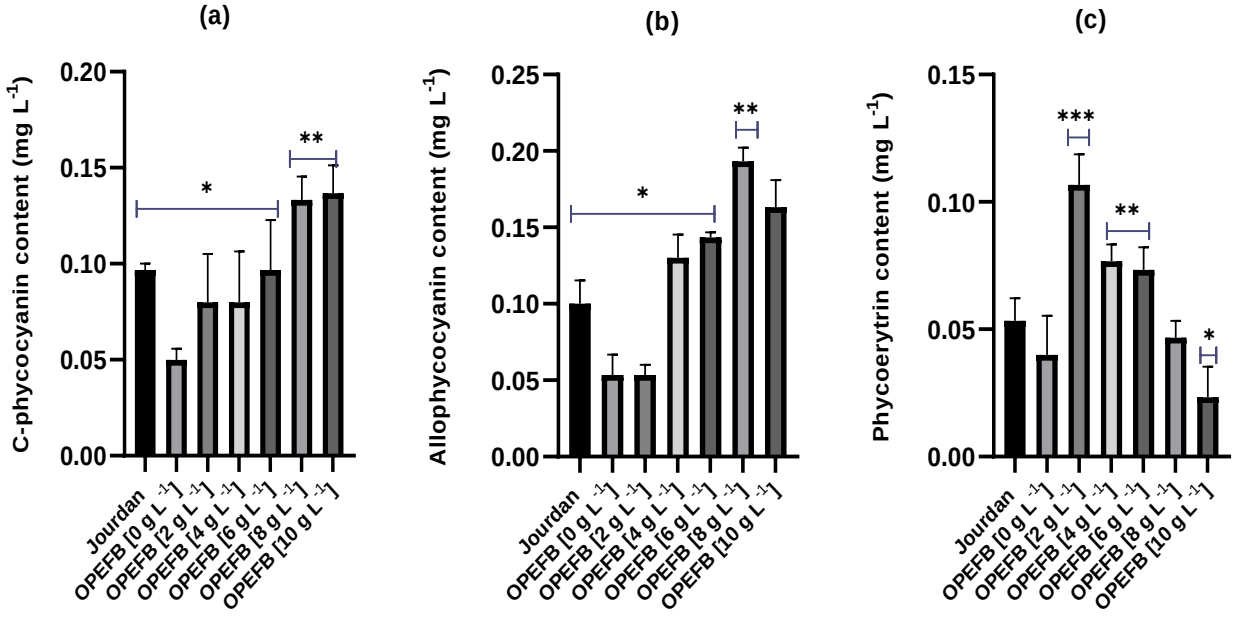

Figure 4. Variation of phycobiliproteins production in Oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan medium (standard). (a) C-Phycocyanin, (b) Allophycocyanin and (c) Phycoerythrin content. Data are presented as mean ± standard deviation (n = 3).

Note: Bars indicate standard deviation. Bars followed by the superscript symbol (****>*** > **> * > ns) indicates that they are significantly different at (p>0.05) as determined by the Tukey test.

Table 3 and Figure 4a, b and c below shows that the Phycocyanin levels got on medium formulated with 8 g L^{-1} (0.14 ± 0.01 mg L^{-1}) and 10 g L^{-1} (0.14 ± 0.03 mg L^{-1}) of the OPEFB were significantly higher than other media. In addition, the medium enriched with 8 g L^{-1} the OPEFB has a significantly high content (0.20 ± 0.003 mg L^{-1}) of allophycocyanin compared to the standard Jourdan medium (0.10 ± 0.01 mg L^{-1}) and the other media. The most notable increase in phycoerythrin (0.11 ± 0.01 mg L^{-1}) was obtained on a medium formulated with 2 g L^{-1} of the OPEFB compared to the contents of the standard Jourdan medium (0.05 ± 0.008 mg L^{-1}) and the other treatments. Indeed, photosynthetic activity is crucial for the synthesis of all pigments. The results obtained on phycocyanin content differ

from those obtained by Chen, Kao, Tsai, Lee, and Chang (2013) and Magwell et al. (2022) who got 0.032, 0.018 and 0.015 mg L^{-1} for C-phycocyanin, allophycocyanin and phycoerythrin respectively in medium formulated with 2 g L^{-1} NPK (fertilizer) 13-13-21 fertilizer. This difference could be associated with the indispensable role of potassium and nitrogen in the medium. Indeed, the activation of enzymes involved in phycobiliprotein synthesis and changes in metabolic responses to changes in the availability of nutrients in the environment influence the production of phycobiliproteins as mentioned by Baslam, Mitsui, Sueyoshi, and Ohyama (2020).

Table 3. Chlorophyll, total carotenoids and phycobiliproteins contents (C-Phycocyanin, Allophycocyanin and Phycoerythrin content (mg L^{-1})) generated by *S. platensis* after 36 day in oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan medium.

Media	Treatments (g L^{-1})	Chl a (mg L^{-1})	Chl b (mg L^{-1})	Cat (mg L^{-1})	C-PC (mg L^{-1})	APC (mg L^{-1})	PE (mg L^{-1})
Jourdan medium	Standard	3.66 ± 0.33^{a}	1.75 ± 0.39^{b}	1.08 ± 0.26^{ab}	0.09 ± 0.003^{ab}	0.10 ± 0.01^{bc}	0.05 ± 0.008^{c}
Oil palm empty fruit bunch (OPEFB) medium	Control - 0	2.74 ± 0.07^{a}	1.34 ± 0.04^{b}	0.26 ± 0.03^{c}	0.05 ± 0.005^{b}	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}
	OPEFB - 2	3.13 ± 0.07^{a}	1.12 ± 0.30^{b}	0.51 ± 0.04^{b}	0.08 ± 0.02^{ab}	0.05 ± 0.01^{c}	0.11 ± 0.01^{a}
	OPEFB - 4	3.63 ± 0.25^{a}	1.58 ± 0.16^{b}	0.63 ± 0.05^{a}	0.08 ± 0.02^{ab}	0.13 ± 0.006^{b}	0.08 ± 0.006^{b}
	OPEFB - 6	4.83 ± 0.38^{a}	2.01 ± 0.42^{a}	1.37 ± 0.02^{a}	0.10 ± 0.02^{a}	0.15 ± 0.01^{b}	0.07 ± 0.008^{b}
	OPEFB - 8	4.96 ± 0.31^{a}	2.22 ± 0.16^{a}	1.43 ± 0.01^{a}	0.14 ± 0.01^{a}	0.20 ± 0.003^{a}	0.05 ± 0.006^{c}
	OPEFB - 10	3.89 ± 0.37^{a}	1.71 ± 0.41^{b}	0.94 ± 0.01^{ab}	0.14 ± 0.01^{a}	0.16 ± 0.008^{b}	0.02 ± 0.01^{c}

Note: Data are presented as mean ± standard deviation (n = 3). Mean followed by the same superscript letter (a > b > c > d > e > f) in the same column indicates tha they are not significantly different at (p>0.05) as determined by the Tukey test.

3.5. Effect of Oil Palm Empty Fruit Bunch on the Physicochemical Parameters of the Medium

The present study revealed a significant increase ($p < 0.05$) in the physicochemical parameters (temperature, hydrogen potential, electrical conductivity, total dissolved solids and salinity) of the *S. platensis* culture medium between the first and 36 days of culture for the different treatments. However, the rates of increase varied tiny between the media with the different concentrations of the OPEFB Table 4.

The highest values of pH (9.42 ± 0.13), electrical conductivity (44.46 ± 2.24 mS cm^{-1}), total dissolved solids (24.53 ± 1.50 mg L^{-1}) and salinity (26.92 ± 1.45m g L^{-1}) were recorded in the media with 8 g L^{-1} of the OPEFB respectively Table 4. This increase can be correlated to the mineral composition of the OPEFB. Indeed, Soni, Sudhakar, and Rana (2019) showed that the ash is basic, which justifies the increase in pH in the media with the addition of the amount of the OPEFB. In addition, the temperature variation is related to environmental conditions. These pH and temperature values agree with those reported by Magwell et al. (2022) who showed that the growth of *S. platensis* in a culture medium is characterised by warm, alkaline water with a pH between 8 and 11.5 and a temperature between 28 and 35 °C. Also, an increase in conductivity, total dissolved solids and salinity could be related to mineral nutrients (Mg, Na, K, Ca, Zn, Cr, Cu) brought by OPEFB ash in the medium as reported by Udoetok (2012) and Asoka et al. (2021).

Table 4. Variation of the physicochemical parameters (temperature (°C), hydrogen potential, electrical conductivity (mS cm^{-1}), total dissolved solid (mg L^{-1}) and salinity (mg L^{-1})) of medium generated by *S. platensis* after 36 day in Oil palm empty fruit bunch (OPEFB) medium at different concentration levels and Jourdan medium.

Physicochemical parameters	Jourdan medium	Oil palm empty fruit bunch (OPEFB) medium concentration (g L^{-1})					
	Standard	Control - 0	OPEFB - 2	OPEFB - 4	OPEFB - 6	OPEFB - 8	OPEFB - 10
Time (day) = 0							
Temperature (°C)	29.36 ± 0.64^{a}	29.37 ± 0.23^{a}	29.05 ± 0.35^{a}	29.21 ± 0.32^{a}	30.23 ± 0.31^{a}	30.13 ± 0.43^{a}	29.36 ± 0.64^{a}
Hydrogen potential (pH)	9.12 ± 0.26^{a}	9.03 ± 0.20^{a}	8.71 ± 0.22^{a}	8.68 ± 0.14^{a}	8.65 ± 0.23^{a}	8.66 ± 0.30^{a}	9.12 ± 0.26^{a}
Electrical conductivity (mS cm^{-1})	13.10 ± 0.50^{a}	12.60 ± 0.43^{a}	12.70 ± 0.40^{a}	13.00 ± 0.13^{a}	12.80 ± 0.25^{a}	13.30 ± 0.60^{a}	13.10 ± 0.50^{a}
Total dissolved solid (mg L^{-1})	22.80 ± 1.27^{a}	22.00 ± 3.84^{a}	22.80 ± 2.94^{a}	22.60 ± 4.42^{a}	23.40 ± 2.85^{a}	23.70 ± 5.62^{a}	22.80 ± 1.27^{a}
Salinity (mg L^{-1})	11.40 ± 0.43^{a}	11.00 ± 0.13^{a}	11.10 ± 0.72^{a}	11.40 ± 0.90^{a}	11.90 ± 0.40^{a}	11.70 ± 0.50^{a}	11.40 ± 0.43^{a}
Time (day) = 36							
Temperature (°C)	28.28 ± 0.36^{a}	28.47 ± 0.38^{a}	28.51 ± 0.38^{a}	28.41 ± 0.35^{a}	28.31 ± 0.34^{a}	28.30± 0.34^{a}	28.32 ± 0.34^{a}
Hydrogen potential (pH)	9.34 ± 0.06^{a}	9.41 ± 0.11^{a}	9.39 ± 0.11^{a}	9.39 ± 0.12^{a}	9.41 ± 0.13^{a}	9.41 ± 0.13^{a}	9.42 ± 0.13^{a}
Electrical conductivity (mS cm^{-1})	44.15 ± 2.33^{a}	43.14 ± 2.46^{a}	43.91 ± 2.36^{a}	43.69 ± 2.30^{a}	44.04 ± 2.34^{a}	44.28 ± 2.21^{a}	44.46 ± 2.24^{a}
Total dissolved solid (mg L^{-1})	24.06 ± 1.58^{a}	23.76 ± 1.69^{a}	24.25 ± 1.63^{a}	24.24 ± 1.63^{a}	24.14 ± 1.59^{a}	24.53 ± 1.50^{a}	24.3 ± 1.55^{a}
Salinity (mg L^{-1})	26.77 ± 1.56^{a}	26.11 ± 1.61^{a}	26.32 ± 1.52^{a}	26.35 ± 1.55^{a}	26.56 ± 1.55^{a}	26.92 ± 1.45^{a}	26.83 ± 1.49^{a}

Note: Data are presented as mean ± standard deviation (n = 3). Mean followed by the same superscript letter (a > b > c > d > e) in the same column indicates that they are not significantly different at (p>0.05) as determined by the Tukey test.

4. CONCLUSION

The composition of the medium is one of the key factors controlling the growth and biochemical status of *S. platensis*. The present study concluded that the growth performance and the photosynthetic pigments productivity of *S. platensis* depended on the concentration of the Oil Palm Empty Fruit Bunch. Spirulina platensis grown in a medium containing 8 g L^{-1} of the Oil Palm Empty Fruit Bunch ash improved the growth performance and Photosynthetic pigments productivity. These results allow us to recommend using a culture medium formulated 8 g L^{-1} of the Oil Palm Empty Fruit Bunch ash as an inexpensive alternative medium for producing *Spirulina platensis* in place of the costly and unavailable Jourdan medium.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study.

REFERENCES

Abouzed, T. K., Althobaiti, F., Omran, A. F., Eldomany, E. B., El-Shazly, S. A., Alharthi, F., . . . Dorghamm, D. A. (2022). The chemoprevention of spirulina platensis and garlic against diethylnitrosamine induced liver cancer in rats via amelioration of inflammatory cytokines expression and oxidative stress. *Toxicology Research*, *11*(1), 22-31. https://doi.org/10.1093/toxres/tfab118

AlFadhly, N. K., Alhelfi, N., Altemimi, A. B., Verma, D. K., & Cacciola, F. (2022). Tendencies affecting the growth and cultivation of genus spirulina: An investigative review on current trends. *Plants*, *11*(22), 3063. https://doi.org/10.3390/plants11223063

Aloo, B. N., Makumba, B. A., & Mbega, E. R. (2020). Plant growth promoting rhizobacterial biofertilizers for sustainable crop production: The past, present, and future. *Preprints*, 1-31. https://doi.org/10.20944/preprints202009.0650.v1

Araújo, R., Vázquez Calderón, F., Sánchez López, J., Azevedo, I. C., Bruhn, A., Fluch, S., . . . Laurans, M. (2021). Current status of the algae production industry in Europe: An emerging sector of the blue bioeconomy. *Frontiers in Marine Science*, *7*, 626389. https://doi.org/10.3389/fmars.2020.626389

Asoka, M. G., Abu, G. O., & Agwa, O. K. (2021). Proximate and physicochemical composition of oil palm empty fruit bunch. *GSC Biological and Pharmaceutical Sciences*, *17*(1), 026-032. https://doi.org/10.30574/gscbps.2021.17.1.0299

Baslam, M., Mitsui, T., Sueyoshi, K., & Ohyama, T. (2020). Recent advances in carbon and nitrogen metabolism in C3 plants. *International Journal of Molecular Sciences*, *22*(1), 318. https://doi.org/10.3390/ijms22010318

Bennett, A., & Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue-green alga. *The Journal of Cell Biology*, *58*(2), 419-435.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chakdar, H., & Pabbi, S. (2012). Extraction and purification of phycoerythrin from Anabaena variabilis (CCC421). *Phykos*, *42*(1), 25-31.

Chen, C.-Y., Kao, P.-C., Tsai, C.-J., Lee, D.-J., & Chang, J.-S. (2013). Engineering strategies for simultaneous enhancement of C-phycocyanin production and CO2 fixation with Spirulina platensis. *Bioresource Technology*, *145*, 307-312. https://doi.org/10.1016/j.biortech.2013.01.054

Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D., . . . Sukhikh, S. (2020). Microalgae: A promising source of valuable bioproducts. *Biomolecules*, *10*(8), 1153-1153. https://doi.org/10.3390/biom10081153

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350-356. https://doi.org/10.1021/ac60111a017

Gnimassoun, E.-G. K., Ettien, J. B. D., & Masse, D. (2020). Characterization of the physico-chemical and chemical properties of a compost resulting from a mixture of palm stalks and poultry droppings in the South-West of Côte d'Ivoire. *International Journal of Biological and Chemical Sciences*, *14*(1), 289-305.

Li, W., Su, H.-N., Pu, Y., Chen, J., Liu, L.-N., Liu, Q., & Qin, S. (2019). Phycobiliproteins: Molecular structure, production, applications, and prospects. *Biotechnology Advances*, *37*(2), 340-353. https://doi.org/10.1016/j.biotechadv.2019.01.008

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol, 148*, 351-382. Academic Press.

Magwell, P. F. R., Nedion, N. J., Tavea, M. F., F.O., W., Tchoffo, D. K., Maffo, N. M. L., . . . Lehman, L. G. (2022). The effect of low-cost NPK 13-13-21 fertilizer on the biomass and phycobiliproteins production of spirulina platensis. *International Journal of Nutrition and Food Sciences, 11*(3), 45-55.

Marhani, S. A., Laban, S., & Musa, Y. (2022). Characterization of biochar empty fruit bunches OPEFB at various temperatures and burning time. *Journal of Experimental Biology and Agricultural Sciences, 10*(3), 599–606. https://doi.org/10.18006/2022.10(3).599.606

Nyabuto, D. K., Kewei, C., Mariga, A. M., Kibue, G. W., Meilin, H. E., & Changhai, W. (2015). Growth performance and biochemical analysis of the genus Spirulina under different physical and chemical environmental factors. *African Journal of Agricultural Research, 10*(36), 3614-3624.

Osório, C., Machado, S., Peixoto, J., Bessada, S., Pimentel, F. B., Alves, R. C., & Oliveira, M. B. P. (2020). Pigments content (chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae. *Separations, 7*(2), 33. https://doi.org/10.3390/separations7020033

Saini, D. K., Chakdar, H., Pabbi, S., & Shukla, P. (2020). Enhancing production of microalgal biopigments through metabolic and genetic engineering. *Critical Reviews in Food Science and Nutrition, 60*(3), 391-405. https://doi.org/10.1080/10408398.2018.1533518

Sharma, K. K., Schuhmann, H., & Schenk, P. M. (2012). High lipid induction in microalgae for biodiesel production. *Energies, 5*(5), 1532-1553. https://doi.org/10.3390/en5051532

Soni, R. A., Sudhakar, K., & Rana, R. (2019). Comparative study on the growth performance of Spirulina platensis on modifying culture media. *Energy Reports, 5*, 327-336. https://doi.org/10.1016/j.egyr.2019.02.009

Suzuki, S., Yamaguchi, H., & Kawachi, M. (2019). The draft genome of a hydrogen-producing cyanobacterium, arthrospira platensis NIES-46. *Journal of Genomics, 7*, 56-59. https://doi.org/10.7150/jgen.38149

Taufikurahman, T., Ilhamsyah, D. P. A., Rosanti, S., & Ardiansyah, M. A. (2020). Preliminary design of phycocyanin production from Spirulina platensis using anaerobically digested dairy manure wastewater. *In IOP Conference Series: Earth and Environmental Science (Vol. 520, No. 1, p. 012007). IOP Publishing.*

Udeinya, I. J., Nubila, N. I., Okoli, U. A., Okafor, T. M., Udeinya, F., Mgbede, S., . . . Shu, N. E. (2021). Oil palm deseeded fruit head ash: Fractionation and characterization. *Advanced Materials Research, 1160*, 75–82. https://doi.org/10.4028/www.scientific.net/amr.1160.75

Udoetok, I. (2012). Characterization of ash made from oil palm empty fruit bunches (OEFB). *International Journal of Environmental Sciences, 3*(1), 518-524.

Wamba, F. O., Magwell, P. F. R., Tchoffo, K. D., Bahoya, A. J., Minyaka, E., Tavea, M. F., . . . Taffouo, V. D. (2021). Physiological and biochemical traits, antioxidant compounds and some physico-chemical factors of Spirulina platensis cultivation as influenced by Moringa oleifera leaves extract culture medium enriched with sodium bicarbonate and kanwa. *African Journal of Biotechnology, 20*(8), 325-334. https://doi.org/10.5897/ajb2021.17358

Windiastuti, E., Suprihatin, B. Y., & Hasanudin, U. (2022). Identification of potential application of oil palm empty fruit bunches (EFB): A review. *IOP Conference Series: Earth and Environmental Science. IOP Publishing, 1063*(1), 012024. https://doi.org/10.1088/1755-1315/1063/1/012024