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The Effect of Low-Cost NPK 13-13-21 Fertilizer on the Biomass and Phycobiliproteins Production of *Spirulina platensis*

Magwell Pierre Fils Rodrigue[1, 4], Nedion Nadjimbayel Julien[1], Tavea Marie-Frédéric[1, *], Wamba Fotsop Oscar[2], Tchoffo Djoudjeu Kennedy[1], Maffo Nguena Momo Lucinda[1], Medueghue Fofou Apollin[1], Minyaka Emile[1], Lehman Léopold Gustave[3]

[1]Biochemistry Laboratory, Faculty of Science, University of Douala, Douala, Cameroon

[2]Laboratory of Plant Biology, Faculty of Science, University of Douala, Douala, Cameroon

[3]Laboratory of Animal Biology, Faculty of Science, University of Douala, Douala, Cameroon

[4]Institute of Agricultural Research for Development (IRAD), Nko'olong Kribi Station, Kribi, Cameroon

Email address:

magkdowell@gmail.com (M. P. F. Rodrigue), nedionnadjimbayel39@gmail.com (N. N. Julien), f.tavea@gmail.com (T. Marie-Frédéric), sfotsop@yahoo.fr (W. F. Oscar), kennedytchoffo@gmail.com (T. D. Kennedy), lucindamomomaffo@gmail.com (M. N. M. Lucinda), fappolin@yahoo.fr (M. F. Apollin), minyakae@yahoo.fr (M. Emile), leopoldlehman@gmail.com (L. L. Gustave)

[*]Corresponding author

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Abstract: Cultivating *Spirulina platensis* at a commercial scale depends on the cost and availability of nutrients in the media, as well as the ability to produce byproducts such as phycobiliproteins. The present study assessed the biomass and phycobiliproteins production of *S. platensis* in the low-cost NPK 13-13-21 fertilizer medium. The low-cost NPK 13-13-21 fertilizer medium has formulated using a commercial NPK 13-13-21 fertilizer as a source of the three major nutrients required for *S. platensis* growth and three other ingredients from the modified Jourdan medium (standard). The experiment was conducted over 25 days in concrete tanks under open raceway pond conditions. Standard analytical methods have applied to evaluate the protein and phycobiliproteins production in the *S. platensis* biomass. The low-cost fertilizer medium formulated with 2 g L^{-1} NPK 13-13-21 produced the most biomass as assessed by optical density (0.68 ± 0.03) and biomass dry weight (1.51 ± 0.02 g L^{-1}), as well as higher biomass productivity (0.10 ± 0.004 g L^{-1} d^{-1}) than the standard medium. Likewise, it produced significantly higher ($p \leq 0.05$) amounts of phycobiliproteins (C-phycocyanin 4.29 ± 0.28 mg g^{-1} DW, allophycocyanin 2.40 ± 0.05 mg g^{-1} DW, and phycoerythrin 2.02 ± 0.04 mg g^{-1}). The low-cost fertilizer medium formulated with 2 g L^{-1} NPK 13-13-21 was ideal for optimizing the biomass and phycobiliproteins production compared with the standard medium. These findings suggest that the low-cost NPK 13-13-21 fertilizer medium could be used as an alternative, less expensive medium for maximizing the biomass and producing useful phycobiliproteins in *S. platensis*.

Keywords: *Spirulina platensis*, Biomass Production, Phycobiliproteins Production, Low-Cost NPK 13-13-21 Fertilizer Medium

1. Introduction

Cyanobacteria are microscopic, multicellular, filamentous or colonial photosynthetic organisms that can grow in different habitats under varied environmental conditions [1, 2]. They are considered one of the oldest groups of organisms responsible for the oxygenation of the earth [3]. These organisms can grow efficiently using sunlight, CO_2 and inorganic nutrients [4]. In recent years, cyanobacteria have

been requested in many areas of biotechnological applications such as nutraceutical bioremediation, biofuel, biofertilizers [5, 6], cosmetics, pharmaceutics [7-9], aquaculture [10], and animal and human nutrition [11, 12] because of the biological and commercial value of its products such as polysaccharides, carotenoids and phycobiliproteins (PBPs) [13-15].

Phycobiliproteins are a group of coloured proteins with linear tetrapyrrole prosthetic groups (bilins), which in their functional state, are covalently linked to specific cysteine residues of the proteins [16]. Among the phycobiliproteins, three categories can be distinguished: phycocyanin (C-PC), allophycocyanin (APC) and phycoerythrin (PE). Each of these categories has a specific spectrum called blue (610-620 nm), blue-green (650-655 nm) and pink (540-570 nm) respectively [17]. Due to their nature, unique colour, fluorescence and antioxidant properties, PBPs are used worldwide. The antioxidant properties of PBPs are exploited in a wide range of applications, such as dyes for the food and textile industry (desserts, chewing gums, sorbets and jellies, ice creams and dairy products) and cosmetics (lipsticks, perfumes and eye make-up powders). Similarly, PBPs are involved in the development of drugs with anticarcinogenic, anti-inflammatory, antiangiogenic, neuroprotective and hepatoprotective functions [2, 18, 19]. The cyanobacterium *Spirulina platensis* is among the major commercial producers of phycobiliproteins [17, 20]. The optimization for the enhanced production of biomass and valuable natural products such as PBPs would be vital for the commercial production of biofuels and meet objectives of sustainable development goals adopted by the United Nations in 2015 [2].

PBP production and *S. platensis* growth are highly influenced by factors like environmental conditions, cultivation methodology, harvesting and extraction technology and nutrients availability and high cost [2, 13, 21, 22]. These growth factors are related to the production of various metabolites and the microalgae defence mechanisms. Since commercial-scale *S. platensis* production is carried out in an open raceway, factors like unavailability and high cost of nutrients are major factors as they can account for 25-50% of the total cost of biomass and PBP production [23, 24]. Therefore, the success of commercial-scale production of biomass and PBP depends on the availability and economic development of nutrients. Previously, several studies on cyanobacteria have attempted to find an efficient, less expensive and readily available alternative medium that would help reduce the cost of biomass and PBP production to meet desired objectives. Thus, previous work has focused on the commercial production of *Chlorella vulgaris* and *Spirulina platensis* from commercial fertilizers NPK 16-4-6 [25], NPK 10-26-26 [23], NPK 20-20-20+TE NPK [26, 27], NPK 19-19-19 [28], NPK [24]. These works reported that commercial NPK fertilizers are cost-effective and can be used as a source of nutrients for cultivation and economically viable production of microalgae due to their high availability, solubility, well-defined nutrient composition and similar or better growth than standard Zarrouk and modified Jourdan media [24, 28, 29]. Furthermore, the advantage of using NPK 13-13-21 fertilizer is mainly due to (1) the reduction of ammonia toxicity due to the controlled amount of urea, and (2) economical compared to synthetic nutrients used in standard growing media. (3) Furthermore, it eliminates the need for the addition of N, P and K nutrients separately to the culture medium [23]. So far, the direct use of NPK 13-13-21 fertilizer as a growing medium has not been reported in the literature for *Spirulina platensis* cultivation. Furthermore, given that the purchase price of NPK 13-13-21 fertilizer on the local market is very low at the US 0.77 per 1 kg and its use in the growing medium is limited, it would be important to determine the optimum concentration required for its effective use to optimize the production of biomass and phycobiliproteins from *S. platensis*. Therefore, the objective of this study was to evaluate the effect of low-cost NPK 13-13-21 fertilizer based medium on biomass and phycobiliproteins production of *Spirulina Platensis.*

2. Material and Methods

2.1. Microalgae Strain

The strain of *Spirulina platensis* used in this study was obtained from the stock culture kept at the SAGRIC Common Initiative Group culture pond of Douala, Cameroon. The culture was previously isolated from the algal samples collected from the culture pond. The *Spirulina platensis* strain was maintained in 20 L concrete tanks in Jourdan's modified medium (standard) containing NaCl (5 g L^{-1}), $Na_2CO_3 \cdot 10H_2O$ (3 g L^{-1}), KNO_3 (2 g L^{-1}), $MgSO_4$ (0.16 g L^{-1}), $(NH_4)_2HPO_4$ (0.12 g L^{-1}) of $((NH_2)_2CO$ (0.05 g L^{-1}), $FeSO_4$ (0.02 g L^{-1}), $CaCl_2$ (0.02 g L^{-1}) and $NaHCO_3$ (8 g L^{-1}) at the open raceway conditions. Prior to the onset of this study, the culture was checked under the microscope for contamination detection and was purified by raising the pH and serial dilution techniques to obtain only algal culture.

2.2. Formulation of a Low-Cost Medium with NPK Complex Fertilizer 13-13-21

The low-cost NPK 13-13-21 fertilizer medium was formulated by mixing four ingredients (Table 1). All the ingredients for the low-cost NPK 13-13-21 complex fertilizer are of commercial grade and locally available. The major elements (nitrogen, phosphorus, and potassium) for spirulina growth in the low-cost medium from NPK 13-13-21 fertilizer, a common and well-known fertilizer for growing crops. NPK 13-13-21 is a water-soluble granular complex composed of 13% nitrogen (N) source including 7.8% ammoniacal nitrogen (NH_3-N), 5.2% nitrate nitrogen (NO_3), 13% phosphorus pentaoxide (P_2O_5) and 21% potassium oxide (K_2O), with 3% sulphur (S), 0.02% zinc (Zn) and 0.02% bore (B). The medium was enriched with micronutrients ($MgSO_4$ 0.16; $FeSO_4$ 0.02; $CaCl_2$ 0.02 g/L) and trona (sodium bicarbonate and natron) were added to the culture medium as a carbon source, while sodium chloride

(NaCl) provided an ideal salinity for the medium. NPK 13-13-21 fertilizer is cost-effective and easily accessed in the shops of an authorized distributors of agricultural inputs, whereas 1 kg costs only 462 CFAF (0.77 US) (Table 2).

2.3. Microalgae Inoculation and Cultivation

Table 1 shows the chemicals and composition of the culture media used in the experiment. *Spirulina platensis* was cultivated in an appropriate amount of NPK 13-13-21 fertilizer obtained from an authorized distributor of agricultural inputs from Yara at Sandaga market, Douala-Cameroon. The NPK 13-13-21 fertilizer was weighed at five different concentrations (0, 1, 2, 4 and 6 g L^{-1}) and dissolved in 20 L open concrete tanks (10 L working volume) while Jourdan's modified medium was prepared and considered as standard. Cultures were conducted for 25 days in a greenhouse under daylight exposures. Cultivation trials were carried out in triplicate in 20 L open concrete tanks (10 L working volume) with an initial biomass concentration of 0.4 g L^{-1} and constant aeration provided by a diaphragm pump (flux of 20 $L^{-1}h^{-1}$) (Figure 1).

Figure 1. *Photographic view of the experimental design cultivation using NPK 13-13-21 fertilizer and Jourdan's modified media. (a) Formulation of the low-cost medium with NPK complex fertilizer 13-13-21, (b) Spirulina platensis strain inoculation (c) Spirulina platensis cultivation trials.*

The physicochemical parameters of the media (temperature (°C), hydrogen potential, salinity (mg L^{-1}) and media transparency (cm)) were assessed using a multi-parameter (HI 98130, Hanna Instruments, Rhodes Island, USA).

2.4. Estimation of the Cost of Spirulina platensis Culture Media

The cost estimate of the culture media was made by considering only the concentration and price of each reagent used to make 1 Kg of components. Other expenses such as taxes, electricity consumption and transport costs were not considered. The prices of all reagents and commercial fertilizers used were obtained from https://www.sigmaaldrich.com and an NPK 13-13-21 fertilizer is cost-effective and easily accessed in the shops of authorized distributor of agricultural inputs, whereas 1 kg costs only 462 CFAF (0.77 US) at Sandaga market, Douala-Cameroon (Table 2).

Table 1. *Chemical composition of the low-cost medium formulated with NPK fertilizer 13-13-21 and Jourdan modified medium used in Spirulina platensis biomass and phycobiliproteins production.*

Jourdan modified medium (standard)		**Low-cost medium formulated with NPK fertilizer 13-13-21**		
Components	**Concentration (g/L)**	**Treatments**	**Components**	**Concentration (g/L)**
$(NH_2)_2$ CO	0.05		$NaHCO_3$ *	8
$(NH_4)_2HPO_4$	0.12		Na_2CO_3 *	5
KNO_3	2		NaCl *	5
$MgSO_4$	0.16	NPK-0 (control)		0
$CaCl_2$	0.02	NPK-1		1
$FeSO_4$	0.02	NPK-2	NPK 13-13-21	2
NaCl	5	NPK-4		4
$NaHCO_3$	8	NPK-6		6
Na_2CO_3	4		Micro-nutrients*	1L

* Note: $NaHCO_3$, Na_2CO_3, NaCl and Micro-nutrients (g/l): ($MgSO_4$, 0.16; $FeSO_4$, 0.02; CaCl, 0,02; S, 0.03; B, 0.0002; Zn, 0.0002) were added in all the treatments of the low-cost medium with NPK complex fertilizer 13-13-21

Table 2. *Estimation of the cost of the low-cost medium formulated with NPK fertilizer 13-13-21 and Jourdan modified medium of Spirulina platensis.*

Jourdan modified medium		**Low-cost medium with NPK fertilizer 13-13-21**	
Components	**Price (US / Kg)**	**Components**	**Price (US / Kg)**
$(NH_2)_2$ CO	342.13	$NaHCO_3$	69.08
$(NH_4)_2HPO_4$	130.75	Na_2CO_3	88.91
KNO_3	119.42	NaCl	49.90
$MgSO_4$	233.17	$MgSO_4$	233.17

Jourdan modified medium		**Low-cost medium with NPK fertilizer 13-13-21**	
Components	**Price (US / Kg)**	**Components**	**Price (US / Kg)**
$CaCl_2$	128.57	$CaCl_2$	128.57
$FeSO_4$	82.92	$FeSO_4$	82.92
NaCl	49.90	NPK-13-13-21*	0.77
$NaHCO_3$	69.08		
Na_2CO_3	88.91		
Total Price	1244.85	Total price	653.31

* Note: NPK-13-13-21 with the micronutrients (g/l): (S, 0.03; B, 0.0002; Zn, 0.0002).

2.5. Biomass Production

Biomass production was determined by measuring the dry weight and optical density (OD) at specific time intervals for 25 days. The dry weight was determined every 5 days by filtering a 20 mL culture sample through dried pre-weighed Whatman GF/C filter No. 1 paper. The filtered biomass was washed with distilled water to remove adsorbed salts, oven-dried at 50°C overnight. The filter paper containing dry spirulina was then weighed and the difference in weight between the first (fresh) and last (dry) was the dry weight, which was expressed as weight per volume (g/ ml). The optical density was determined in 5 days intervals at the wavelength of 680 nm with UV/VIS, Biobase – Spectrophotometer. Biomass productivity (P_x, g $L^{-1}d^{-1}$) was calculated from the equation $P_x = (X_i - X_0) / t_i$, where, Xi = biomass density at ti (g L^{-1}) and ti = time interval (d) between X_0 and X_i.

2.6. Protein Extraction and Production

The protein content was assayed according to the method described by Bradford [30] using bovine serum albumin (BSA) as a protein standard. Spirulina sample (5 mg) was homogenized with 2 mL with 50 mM potassium phosphate buffer (pH 6.2) and then centrifuged at 35000 g for 10 min at 4°C. 0.1 mL of supernatant is added to the Bradford reagent (2 mL) and the mixture was incubated thereafter in the dark for 10 min. Then, it was pipetted in spectrophotometer cuvettes and absorbance was measured at 595 nm using a UV/VIS spectrophotometer (Biobase – Spectrophotometer).

The protein productivity (P_{PROT}, mg.L^{-1}.d^{-1}) was calculated based on the biomass dry weight, as described in equations 1, where Xf is the final biomass concentration of a culture (mg L^{-1}), PROT is the protein content (%), and Δt is the cultivation time (d).

$$P_{PROT} \text{ (mg } L^{-1}.d^{-1}) = PROT.X \text{ f } /100.\Delta t \quad (1)$$

2.7. Phycobiliproteins Extraction and Production

The oven-dried 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. 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 (A562), 615nm (A615), and 652nm (A652) against a blank and the concentrations of C-phycocyanin C (C-PC), allophycocyanin (APC), and phycoerythrin (PE) were calculated by using the following equations [31]:

$$\text{C-Phycocyanin (C-PC)} = [A615 \times 0.474(A652)]/5.34 \quad (2)$$

$$\text{Allophycocyanin (APC)} = [A652 \times 0.208(A615)]/5.09 \quad (3)$$

$$\text{Phycoerythrin (PE)} = [A562 \times 2.41(\text{C-PC}) \times 0.849(\text{APC})]/9.62 \quad (4)$$

The phycobiliproteins productivity represents the amount of phycobiliproteins per unit of volume and time in each trial, i.e. considering the content and biomass production during this period.

2.8. Data Analysis

All experiments were performed in triplicate, and data were expressed as the mean ± standard deviation. The statistical analyses were performed by IBM SPSS Statistics 26 Software through analysis of variance (ANOVA). The multiple comparisons of means of each analysis were determined using the Duncan test at the confidence level of 95%. The statistical analysis of differences between Jourdan's modified medium and different treatments was performed with an unpaired T-test. P-values of < 0.05 were considered to be statistically significant.

3. Results and Discussion

3.1. Effect of a Low-Cost NPK Fertilizer Media on S. platensis Biomass Production

Nutrient availability is among the key factors that greatly affect the microalgae growth rate and phycobiliproteins production [32, 33]. However, for large-scale commercial production, excess nutrient supply is an economic challenge [24, 34]. The present study was conducted to investigate the effect of NPK 13-13-21 fertilizer as a low-cost growth medium for biomass and phycobiliproteins production of *Spirulina platensis*.

The results revealed that the growth of *S. platensis* increased with increasing fertilizer content in the culture

medium up to a certain limit and then the growth decreased. The best growth of *S. platensis* was obtained at NPK fertilizer concentration of 2 g L^{-1} with maximum optical density and biomass dry weight (0.68 ± 0.03 g L^{-1} and 1.51 ± 0.02 g L^{-1}) on day 20 of cultivation which were significantly higher ($p \leq 0.05$) compared to Jourdan's standard medium (0.62 ± 0.03 g L^{-1} and 1.38 ± 0.03 g L^{-1}) and other treatments. A progressive decrease in the growth of *S. platensis* was also observed in the control medium (0 g L^{-1} NPK fertilizer) (Figures 2, 3 and Table 3).

The optical density and biomass dry weight values obtained in the present study are higher than those of [23] who reported biomass dry weight values ranging from 0.25 to 1.12 g L^{-1} for *S. platensis* under a medium formulated by varying NPK 10-26-26 fertilizer and sodium bicarbonate. But close to those of [35] with an optical density of 0.519 and 0.849 when cultured *Chlorella sorokiniana* in NPK and BBM media respectively. These results could be explained by the uptake of N, P and K provided by the NPK fertilizer at 2 g L^{-1} compared to the other treatments. Furthermore, in standard culture media, nitrate metabolism occurs through the reduction of nitrate to nitrite and nitrite to ammonium ion with subsequent assimilation of ammonium ions into carbon skeletons resulting in amino acids and proteins in the cell, whereas direct assimilation of ammonium took place in the medium with a controlled amount of NPK fertilizer which improves the efficiency of photosynthesis and thus biomass production in microalgae as reported by [24, 36].

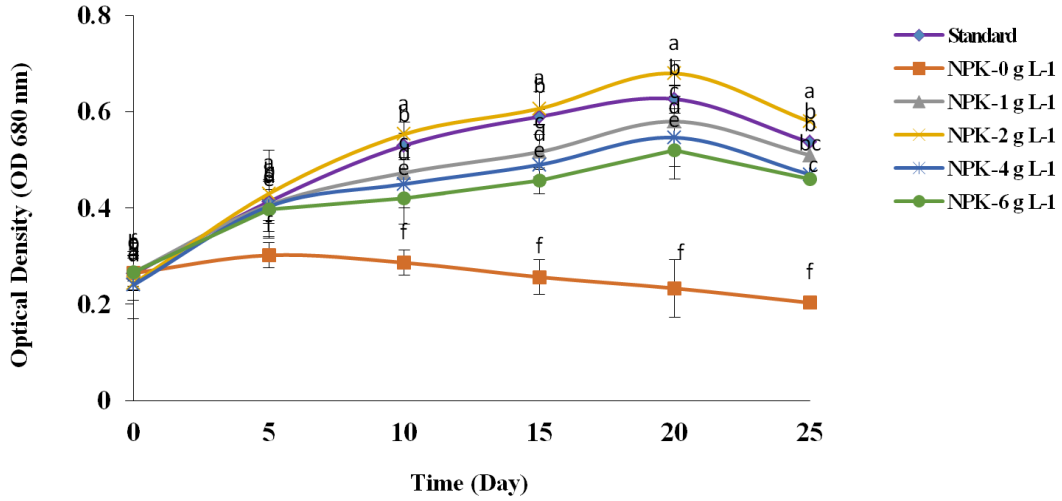

Figure 2. *Variation of optical density in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard). Data are presented as mean ± standard deviation (n = 3). Mean followed by the same superscript letter (a > b > c > d > e > f) indicates that they are not significantly different at (p>0.05) as determined by the Duncan test.*

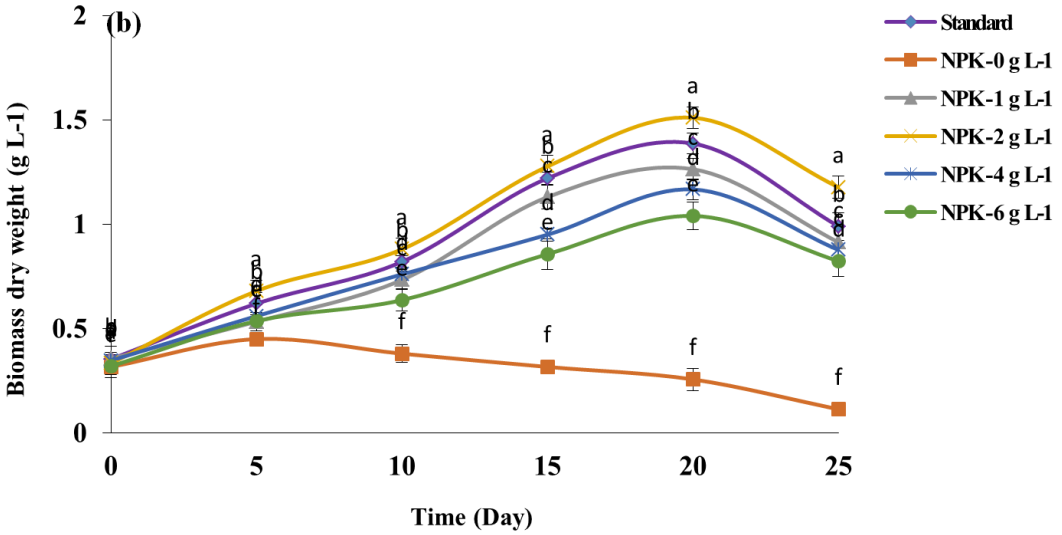

Figure 3. *Variation of biomass dry weight in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard). Data are presented as mean ± standard deviation (n = 3). Mean followed by the same superscript letter (a > b > c > d > e > f) indicates that they are not significantly different at (p>0.05) as determined by the Duncan test.*

Similarly, biomass productivity (Px) increases with culture age and the concentration of NPK fertilizer in the culture medium up to a certain limit and thereafter biomass decreased. It was observed that *S. platensis* did not grow in the culture

medium where NPK 13-13-21 fertilizer concentration was more than 2 g L^{-1}. The highest biomass productivity (0.10 ± 0.004 g L^{-1} d^{-1}) was recorded on the twentieth day of cultivation on the medium formulated with 2 g L^{-1} NPK fertilizer was significantly higher ($p \leq 0.05$) compared to the other treatments and the standard medium (0.09 ± 0.005 g L^{-1} d^{-1}) (Figure 4 and Table 3). These results are higher than the biomass productivity obtained by [24, 32] who reported 0.254 mg mL^{-1} $day^{-1}$and 0.059 mg L^{-1} day^{-1}respectively in the NPK 10-20-20 fertilizer medium of *Arthrospira fusiformis* and the wastewater medium of *Chlorella vulgaris* supplemented with NPK 20-20-10 fertilizer. This difference could be due to the presence of nutrients provided by the NPK complex fertilizer that are favourable to growth in the medium formulated at 2 g L^{-1} rather than high concentrations of NPK 13-13-21 fertilizer [37]. Furthermore, the higher productivity in the medium formulated at 2 g L^{-1} NPK fertilizer 13-13-21 compared to the standard (Jourdan modified medium) could be due to the variation in the percentage of nitrogen present in the NPK fertilizer medium, the difference in the composition of the medium, the concentration and the type of NPK fertilizer used [28].

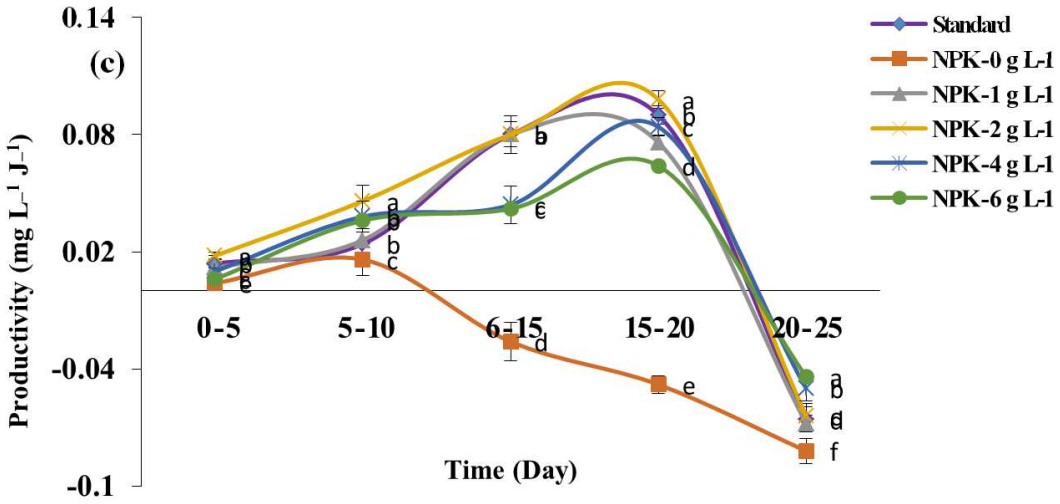

Figure 4. *Variation of biomass productivity in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard). Data are presented as mean ± standard deviation (n = 3). Mean followed by the same superscript letter (a > b > c > d > e > f) indicates that they are not significantly different at (p>0.05) as determined by the Duncan test.*

Table 3. *Biomass production ((Optical density ($OD_{680\ nm}$), biomass dry weight (X) and biomass productivity (P_x) generated by S. platensis after 20 day in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard)).*

Media	Treatments (g L^{-1})	OD (680 nm)	X (g L^{-1})	P_x (g $L^{-1}d^{-1}$)
Jourdan modified medium	Standard	0.32 ± 0.02^{d}	0.62 ± 0.03^{d}	0.04 ± 0.005^{d}
Low-cost NPK fertilizer 13-13-21 medium	NPK-0 (control)	0.32 ± 0.02^{d}	0.62 ± 0.03^{d}	0.04 ± 0.005^{d}
	NPK-1	0.51 ± 0.05^{c}	0.85 ± 0.06^{c}	0.09 ± 0.011^{c}
	NPK-2	0.57 ± 0.06^{c}	1.01 ± 0.08^{b}	0.12 ± 0.015^{b}
	NPK-4	0.65 ± 0.07^{b}	1.07 ± 0.09^{b}	0.13 ± 0.017^{b}
	NPK-6	0.76 ± 0.09^{a}	1.29 ± 0.02^{a}	0.17 ± 0.023^{a}

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 Duncan test.

3.2. Effect of a Low-Cost NPK Fertilizer Medium on S. platensis Phycobiliproteins Productivity

The results revealed that the protein and phycobiliproteins productions of *S. platensis* increased with the concentration of NPK 13-13-21 fertilizer in the culture medium up to 2 g L^{-1} and then the production decreased, indicating that the physiological activity of *S. platensis* was affected by the nutrient concentrations. The concentrations and yield of phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin) extracted from the biomass grown during the experimental optimization period are shown in tables 4 and 5. The concentrations represent the amount of phycobiliproteins per biomass dry weight, while the yield represents the productivity of phycobiliproteins per unit of volume and time in each treatment, considering the content and biomass production during this period. The results show a progressive increase in phycobiliproteins content from the fifth to the twentieth day. Subsequently we noted a decrease in the phycobiliproteins content until the twenty-fifth day of cultivation in the different media formulated with NPK 13-13-21 fertilizer. The maximum content of phycobiliproteins was obtained in the medium formulated with 2 g L^{-1} NPK 13-13-21 fertilizer, while the minimum was observed in the control medium (0 g L^{-1} NPK fertilizer). Phycocyanin was the most abundant phycobiliproteins, followed by allophycocyanin and phycoerythrin (Figure 5a, b, c and table 4).

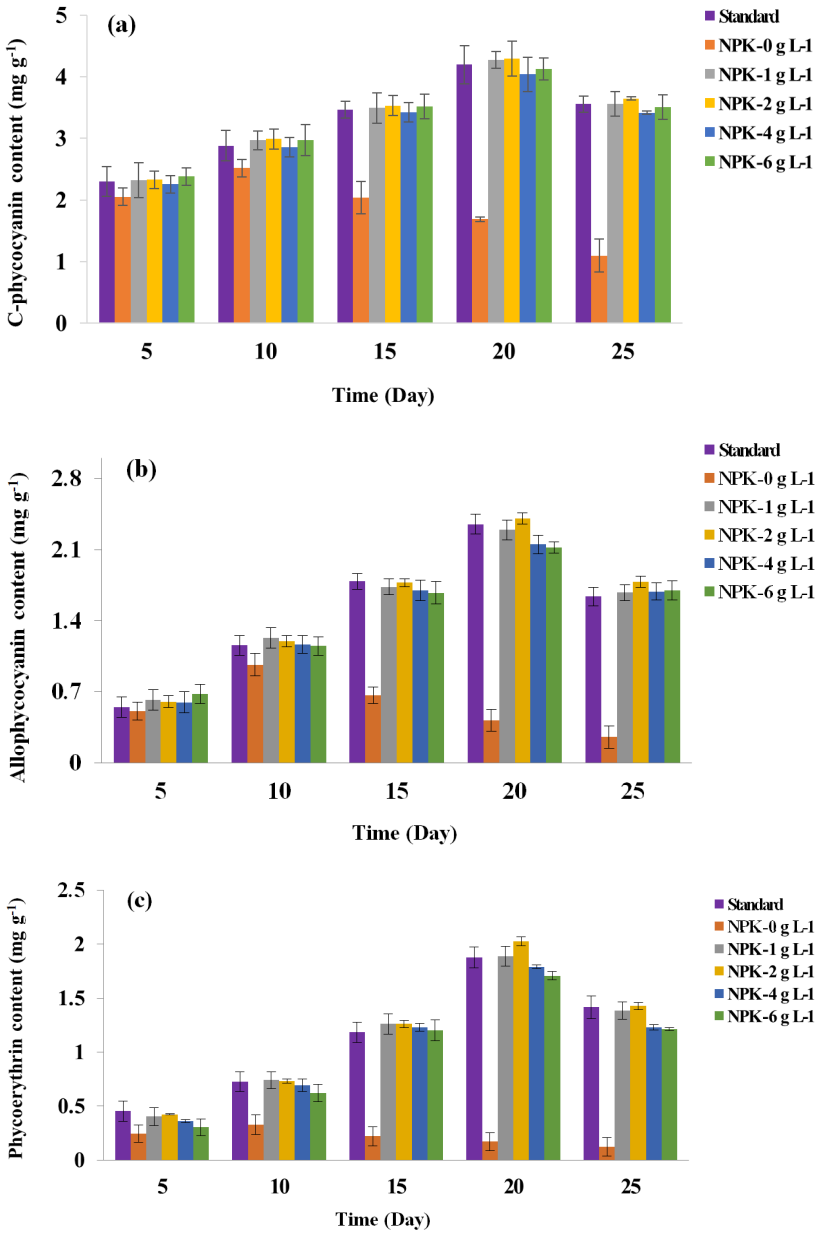

Figure 5. *Variation of phycobiliproteins production in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard). (a) C-Phycocyanin, (b) Allophycocyanin and (c) Phycoerythrin content. Data are presented as mean ± standard deviation (n = 3) as determined by the Duncan test. Bars indicate standard deviation.*

Protein (149.1 ± 4.9 mg g^{-1} DW) and phycobiliproteins (4.29 ± 0.28 mg g^{-1} DW; 2.40 ± 0.05 mg g^{-1} DW; 2.02 ± 0.04 mg g^{-1} DW for C-phycocyanin, allophycocyanin and phycoerythrin respectively) contents were significantly higher ($p \leq 0.05$) in the medium formulated with 2 g L^{-1} NPK 13-13-21 fertilizer compare to the standard and all other media formulated with different concentration of the NPK 13-13-21 fertilizer (Table 4). Similarly, protein productivity (1.12 ± 0.002 mg L^{-1} d^{-1}) and phycobiliproteins productivity (3.24 ± 0.08 × 10^{-2} mg L^{-1} d^{-1}; 1.82 ± 0.02 × 10^{-2} mg L^{-1} d^{-1}; 1.53 ± 0.02 × 10^{-2} mg L^{-1} d^{-1} for C-phycocyanin, allophycocyanin and phycoerythrin respectively) were significantly higher ($p \leq 0.05$) in the medium formulated with 2 g L^{-1} NPK 13-13-21 fertilizer compare to the standard and all other media formulated with different concentration of the NPK 13-13-21 fertilizer (Figures 5a, b, c, tables 4 and 5). These differences could be correlated to the indispensable role of potassium and nitrogen in the activation of enzymes involved in protein synthesis and to changes in metabolic responses following the change in nutrient availability provided by the NPK 13-13-21 fertilizer in the medium [38]. Otherwise, in the form of the nitrogen source as ammonium nitrogen is more easily assimilated than nitrate nitrogen, which requires a reduction process to release the nitrogen. Thus, nitrogen plays a critical role in the overproduction of phycobiliproteins since nitrogen is stored in Phycobiliproteins. Therefore, in case of nitrogen shortage, cells selectively degrade their Phycobiliproteins storage [17, 23].

Table 4. *Proteins and phycobiliproteins contents (C-Phycocyanin, Allophycocyanin and Phycoerythrin content (mg L^{-1})) generated by S. platensis after 20 day in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard).*

Media	Treatments (g L^{-1})	Protein (mg g^{-1} DW)	C-PC (mg g^{-1} DW)	APC (mg g^{-1} DW)	PE (mg g^{-1} DW)
Jourdan modified medium	Standard	139.2 ± 5.3^{b}	2.19 ± 0.30^{a}	2.35 ± 0.06^{a}	1.87 ± 0.005^{b}
	NPK-0 (control)	24.4 ± 2.1^{f}	1.69 ± 0.03^{b}	0.42 ± 0.05^{c}	0.17 ± 0.02^{d}
Low-cost NPK fertilizer 13-13-21 medium	NPK-1	125.5 ± 2.9^{c}	4.27 ± 0.10^{a}	2.29 ± 0.04^{ab}	1.88 ± 0.03^{b}
	NPK-2	149.1 ± 4.9^{a}	4.29 ± 0.28^{a}	2.40 ± 0.05^{a}	2.02 ± 0.04^{a}
	NPK-4	112.9 ± 5.8^{d}	4.04 ± 0.20^{a}	2.15 ± 0.02^{b}	1.79 ± 0.03^{c}
	NPK-6	98.2 ± 5.8^{e}	4.12 ± 0.17^{a}	2.12 ± 0.05^{b}	1.70 ± 0.04^{c}

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 that they are not significantly different at (p>0.05) as determined by the Duncan test.

Table 5. *Proteins and phycobiliproteins productivity (protein productivity (P_{PROT}), C-Phycocyanin productivity ($P_{C\text{-}PC}$), Allophycocyanin productivity (P_{APC}) and Phycoerythrin productivity (P_{PE})) generated by S. platensis after 20 day in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard).*

Media	Treatments (g L^{-1})	P_{PROT} (mg L^{-1} d^{-1})	$P_{C\text{-}PC}$ ($\times 10^{-2}$ mg L^{-1} d^{-1})	P_{APC} ($\times 10^{-2}$ mg L^{-1} d^{-1})	P_{PE} ($\times 10^{-2}$ mg L^{-1} d^{-1})
Jourdan modified medium	Standard	0.96 ± 0.008^{b}	2.91 ± 0.03^{a}	1.63 ± 0.02^{a}	1.30 ± 0.03^{b}
	NPK-0 (control)	0.03 ± 0.001^{f}	0.22 ± 0.03^{b}	0.05 ± 0.02^{c}	0.02 ± 0.03^{d}
Low-cost NPK fertilizer 13-13-21 medium	NPK-1	0.79 ± 0.004^{c}	2.70 ± 0.05^{a}	1.45 ± 0.04^{ab}	1.19 ± 0.04^{b}
	NPK-2	1.12 ± 0.002^{a}	3.24 ± 0.08^{a}	1.82 ± 0.02^{a}	1.53 ± 0.02^{a}
	NPK-4	0.65 ± 0.003^{d}	2.36 ± 0.02^{a}	1.25 ± 0.02^{b}	1.04 ± 0.01^{c}
	NPK-6	0.51 ± 0.005^{e}	2.15 ± 0.05^{a}	1.10 ± 0.02^{b}	0.89 ± 0.01^{c}

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 that they are not significantly different at (p>0.05) as determined by the Duncan test.

3.3. Effect of Physicochemical Parameters of Low-Cost NPK Fertilizer Media on S. platensis Biomass

The experimental results showed that the pH and temperature of the medium ranged between 7.63 and 10.54 and between 29.05°C and 30.37°C respectively for the cultivation period without showing significant differences ($p \leq 0.05$) between the different treatments. This trend of gradual increase in the pH of the medium could be correlated with the regular dissolution of sodium bicarbonate to make hydroxyl ions (OH-) available in the medium as reported by [32]. These temperature and pH values are in agreement with those reported by [33] who indicated that for optimal and flourishing growth, the temperature of *Spirulina platensis* is between 28 and 31°C and the pH value between 8 and 11. Similarly, we observed increasing salinity which is correlated with cultivation time and NPK 13-13-21 complex fertilizer concentration. However, these values were significantly higher ($p \leq 0.05$) on the twentieth day of cultivation in the medium formulated with 2 g L^{-1} NPK 13-13-21 fertilizer compared to the standard and all other media formulated with different concentrations of the NPK 13-13-21 fertilizer (Table 6). This increase in salinity is inversely proportional to the transparency of the culture medium, which decreases progressively with the lowest values (0.51 ± 0.35 cm) recorded on the twentieth day of cultivation in the medium formulated with 2 g L^{-1} NPK 13-13-21 fertilizer compared to the standard medium (1.17 ± 0.074 cm) and all other media formulated with different concentrations of NPK 13-13-21 fertilizer (Table 6). This increase in the salinity could be correlated to the increase in alkalinity and concentration of dissolved ionic salts resulting from the NPK 13-13-21 complex fertilizer formulated media following evaporation of water from the media as reported by [32, 39].

Table 6. *Variation of the physicochemical parameters (temperature (°C), hydrogen potential, salinity (mg L^{-1}) and media transparency (cm)) of medium generated by S. platensis after 20 day in low-cost NPK fertilizer 13-13-21 medium at different concentration levels and Jourdan modified medium (standard).*

Physicochemical parameters	Jourdan modified medium			Low-cost NPK fertilizer 13-13-21 medium concentration (g L^{-1})		
	Standard	NPK-0	NPK-1	NPK-2	NPK-4	NPK-6
Time (day) = 0						
Temperature (°C)	29.34 ± 0.40^{b}	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}
Hydrogen potential (pH)	7.63 ± 0.26^{a}	9.20 ± 0.20^{a}	9.20 ± 0.22^{a}	9.13 ± 024^{a}	9.27 ± 0.23^{a}	9.54 ± 0.50^{a}
Salinity (mg L^{-1})	7.49 ± 0.22^{c}	8.93 ± 0.23^{b}	9.07 ± 0.25^{b}	10.84 ± 0.31^{a}	10.99 ± 0.52^{a}	13.67 ± 0.57^{a}
Transparency (cm)	6.10 ± 1.27^{a}	6.30 ± 1.53^{a}	6.07 ± 0.72^{a}	6.07 ± 0.76^{a}	6.17 ± 0.70^{a}	10.54 ± 0.33^{c}
Time (day) = 20						
Temperature (°C)	30.20 ± 0.34^{a}	30.10 ± 0.14^{a}	30.37 ± 0.22^{a}	30.17 ± 0.19^{a}	30.23 ± 0.31^{a}	30.23 ± 0.31^{a}
Hydrogen potential (pH)	9.06 ± 0.58^{b}	10.40 ± 0.44^{a}	10.47 ± 0.48^{a}	10.50 ± 053^{a}	10.54 ± 0.50^{a}	10.54 ± 0.50^{a}
Salinity (mg L^{-1})	8.76 ± 0.28^{d}	10.15 ± 0.17^{c}	10.50 ± 0.27	12.43 ± 0.37^{b}	13.67 ± 0.57^{a}	13.67 ± 0.57^{a}
Transparency (cm)	1.17 ± 0.074^{a}	4.14 ± 0.026^{b}	0.86 ± 0.003^{b}	0.51 ± 0.035^{c}	0.54 ± 0.033^{c}	0.54 ± 0.033^{c}

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 that they are not significantly different at (p>0.05) as determined by the Duncan test.

4. Conclusion

In summary, the findings suggest that the addition of low-cost NPK 13-13-21 fertilizer up to 2 g L^{-1} stimulated the optical density, biomass dry weight, maximum productivity and phycobiliproteins production of *Spirulina platensis* and thereafter growth decreased. Application of low-cost NPK 13-13-21 fertilizer at 2 g L^{-1} had a significant effect on phycobiliproteins production. It was observed that the increase in C-phycocyanin, allophycocyanin and phycoerythrin was proportional to the increase in biomass of *Spirulina platensis*. These findings indicate a potential use of NPK 13-13-21 fertilizer as a cheap and available alternative medium to the very expensive and less accessible Zarrouk and Jourdan modified medium for optimal production of phycobiliproteins of *Spirulina platensis* biomass for use in the food supplement, cosmetics, textile, pharmaceuticals and biofuel industries.

5. Recommendation

The demand and price of *Spirulina platensis* by-products (polysaccharides, pigments and phycobiliproteins) have increased considerably during the last two decades, although the commercial quantity of its products remains very low. Further research is needed to promote the use of new and cheap sources of nutrients and the development of efficient extraction and purification technologies for these by-products to reduce production costs.

Highlight

NPK 13-13-21 fertilizer formulated at 2 g L^{-1} increases biomass and phycobiliproteins production of *Spirulina platensis.*

NPK 13-13-21 fertilizer could be used as an alternative cheap and available medium for biomass and phycobiliproteins production of *Spirulina platensis.*

Adequate NPK-13-13-21 fertilizer supply in medium leads to optimal biomass and phycobiliproteins production of *Spirulina platensis.*

Author Contributions

Conceptualization: M. P. F. R., N. N. J., W. F. O., M. E., T. M. F. and L. L. G. Methodology: M. P. F. R., N. N. J., W. F. O., T. D. K., M. F. A., M. N. M. L., M. E, T. M. F. and L. L. G. Writing - original draft preparation: M. P. F. R., N. N. J., W. F. O., T. D. K., M. E., T. M. F. Edited, reviewed manuscript and provided with critical input and corrections: M. P. F. R, N. N. J., W. F. O, M. E., T. M. F. and L. L. G. Supervision: W. F. O., M. E., T. M. F. and L. L. G. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any financial relationships that could be construed as a potential conflict of interest.

Ethical Standards

All samples used in this study were microalgae and thus did not involve ethical issues.

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