Influence of Sulphate Nutrition on Growth Performance and Antioxidant Enzymes Activities of *Spirulina platensis*

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Abstract

The growth of *Spirulina platensis* is dependent on culture conditions. This study has established adequate conditions for the quality and quantity production of *S. platensis*. The effect of sulphate salts nutrition on growth performance and biochemical status of *S. platensis* was assessed in vitro. Prior to culture, the Paracas strain of *S. platensis* from SAGRIC pond was analysed in different magnesium sulphate (MgSO₄; 0.08, 0.16, 0.32, 0.64 and 1.28 g/L), potassium sulphate (K₂SO₄; 0.08, 0.16, 0.32, 0.64 and 1.28 g/L) and MgSO₄/K₂SO₄ (0.16/0.00, 0.08/0.08, 0.04/0.12, 0.02/0.14 and 0.01/0.15 g/L) concentrations. Culture media pH, total dissolved solids (TDS) and conductivity rate were monitored. Microscopic analysis revealed sulphate salt concentrations influenced the number of whorls and filaments of *S. platensis*. K₂SO₄ (1.28 g/L) produced the highest number of whorls and filaments. Moreover, pH level fluctuated by sulphate treatments. K₂SO₄ (1.28 g/L) had a pH level of 8.77±0.01 (day 5 of culture incubation). TDS and conductivity rate, protein and cysteine contents increased with culture age and K₂SO₄ concentration in a culture medium. Conversely, negative correlations between protein and cysteine contents were observed, and sugar content decreased. Sulphate salt type and concentrations affected polyphenol oxidase (PPO) and peroxidase (POD) activities. MgSO₄/K₂SO₄ (0.02/0.14 g/L) displayed the best PPO and POD activities. Both enzymes appeared to be negatively correlated to the decreasing sugar content. These results indicate growth performances and biochemical status of *S. platensis* are significantly improved with the adequate supplementation of sulphate salts (MgSO₄ and K₂SO₄) in culture media.

Keywords: *Spirulina platensis*, sulphate salts, growth, antioxidant activity

1. Introduction

*Spirulina platensis* is a multicellular, filamentous and microscopic photosynthetic cyanobacterium commonly found in the brackish lakes of Central Africa and Mexico. *S. platensis* has been consumed for centuries by the Aztecs and bordering populations on Lake Chad (Shigekatsu et al., 2019). This microalga is characterized by a high content of protein (including enzymes such as polyphenol oxidase and peroxidase) and high amounts of essential fatty acids, essential amino acids, minerals, vitamins (especially B12), polysaccharides and antioxidant pigments (chlorophyll, carotenoids, phycobiliprotein, phycocyanin and carotenoids) (Budiyono et al., 2014; Ben Amor et al., 2017; Jung et al., 2019; Fatemeh & Choopani, 2020).

This microalga being studied, not only for its nutritional properties but also for its reported therapeutic properties related to its hypolipidemic effect (Al-Saman et al., 2020), protective effect against diabetes and obesity (Azabji-Kenfack et al., 2011; Gómez-Téllez et al., 2020), inhibitory effect on anemia and cancer (Abdel-Daim et al., 2013; Barakat et al., 2015), stimulatory effect on the immunological system (Ngo-Matip et al., 2015; Ama Moor et al., 2020), nephrotoxicity effect on pharmaceuticals and toxic metals and protective effect against harmful radiation (Mohan et al., 2006; Priyanka Yadav et al., 2019). Because of its multiple properties, the production of *S. platensis* has gained worldwide attention for use in human food supplements, animal feed and pharmaceuticals industries.
In Cameroon, culture of spirulina remains rudimentary, not controlled by producers and its biochemical status uncertain. Biomass, specific growth and biochemical composition of spirulina depend on many factors which include farming practices, environmental parameters and culture medium composition (Madkour et al., 2012). However, composition of culture medium is a major factor which influences growth rate, biomass production and biochemical status of this cyanobacterium. Hence, it is possible to improve the growth performance, biochemical status and antioxidant activity of *S. platensis* while acting on composition of culture medium in order to fulfil pharmaceutical and nutritional requirements.

Therefore, the present study was undertaken to study the incidence of exogenous sulphate salts (*K*₂SO₄ and *Mg*SO₄) supplementation on growth performance and biochemical profile (including antioxidant enzymes activities) of *S. platensis* cultured in vitro. Green algae growth is negatively influenced by harmful reactive oxygen species (ROS) from diver’s physiological metabolic processes. Polyphenol oxidase and peroxidase are important antioxidant enzymes in stress control of the cell (Yakelin et al., 2001; Mostafa Mahmoud et al., 2016).

2. Material and Methods

2.1 Microorganism Strain

The cyanobacterium *S. platensis* strain «Paracas» used in the present study was obtained from the freshwater culture pond of SAGRIC Common Initiative Group (CIG) farm, Douala-Cameroon. The strain was grown and maintained in 500 mL sterilized Erlenmeyer flasks containing 100 mL Jourdan’s medium (Table 1) (Jourdan, 2013) at pH 9 in an illuminated growth room at 28±0.5 °C under 12/12 hours photoperiod and daily manually shake (thrice).

2.2 Culture Media and Experimental Design

Jourdan’s medium (Jourdan, 2013) was used as the reference medium. Sulphate salts (*Mg*SO₄ and *K*₂SO₄) were brought in variable concentrations in Jourdan’s medium. *Mg*SO₄ was varied in absence of *K*₂SO₄. Conversely, *K*₂SO₄ was varied in absence of *Mg*SO₄. Also, the ratios *Mg*SO₄/*K*₂SO₄ varied with fixed content of SO₄²⁻ (Table 1). The algae *S. platensis* cells were inoculated at a concentration of 15% (V inoculation/V media) in 1000 mL erlenmeyer flasks. The pH of all culture media was adjusted to 9 before sterilization, cool and addition of *S. platensis* cells (15% v/v). Cultures were incubated at 12/12 hours (light-darkness) photoperiod under temperature 28±0.5 °C for 5 days. Cultures were manually shook (for 3 min) thrice daily. Samples were collected every day for assessment of the cyanobacteria growth as well as estimation of biochemical status and antioxidant enzymes activities. All experiments were carried out in triplicate.

| Constituents                   | Composition (g/L) |
|-------------------------------|-------------------|
| Urea ((NH₂)₂CO)               | 0.05              |
| Di-ammonium phosphate ((NH₄)₂HPO₄) | 0.12             |
| Potassium nitrate (KNO₃)      | 2                 |
| Magnesium sulphate (MgSO₄)    | 0.16              |
| Calcium chloride (CaCl₂)       | 0.02              |
| Ferrous sulphate (FeSO₄)       | 0.02              |
| Sodium chloride (NaCl)         | 5                 |
| Sodium bicarbonate (NaHCO₃)   | 8                 |

2.3 Monitoring of Physico-Chemical Parameters of Culture Media

Physico-chemical parameters (temperature, pH, conductivity, and total dissolved solids (TDS) of media were recorded daily using of multi-parameters (HI 98130, HANNA Instruments, Rhodes Island, USA).

2.4 Assessment of *S. platensis* Growth Parameters

*S. platensis* cell populations (number of filaments and whorls) were evaluated using light and fluorescence microscope (Cysscope® HP, Sysmex-Partec, Japan) by direct microscopic counting method described by Usharani et al. (2012). Biomass concentration (g/L) was determined every day by measuring the optical density at 560 nm. A standard concentration was used to determine the biomass of individual samples (culture media and daily monitoring) based on optical density and use the coefficient of correlation (C = 0.782 X, where X is the biomass concentration (g /L) according to Tsarahevitra et al. (2003). The calculated biomass was used to obtain maximum specific growth rate (μₘ) and productivity (P) from the following equation of (Madkour et al., 2012):

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\[ \mu_m = \ln(X_1/X_2)/t_2 - t_1 \]  

where, \( \mu_m \) = specific growth rate (div/day); \( X_1 \) = biomass concentration at time \( t_1 \); \( X_2 \) = biomass concentration at the time \( t_2 \).

Productivity (\( P \)) was estimated as follow:

\[ P = (X_m - X_i)/t_m \]

where, \( P \) = productivity (mg /L/day); \( X_i \) = initial biomass density (g/L); \( X_m \) = biomass density at time \( m \) (g/L); \( t_m \) = time interval (day) between \( X_i \) and \( X_m \).

2.5 Chemical and Biochemical Analysis

2.5.1 Chemical Composition Analysis of \( S. platensis \) Strain Used

The \( S. platensis \) sample from SAGRIC pond was aseptically filtered and dried during 48 hours at 50 °C in a sterilizer (Binder, Germany). Subsequently, the sample was analyzed to find out its chemical composition. Total protein was determined by the conventional Micro-Kjeldahl method (AOAC, 1995). Lipids were extracted using a Soxhlet apparatus and analyzed according to the AOAC (1990) method. Total ash and fibers were determined by the standards method of AOAC (1990) and Wolff (1968). The determination of minerals (Ca, Mg, K, Na, Fe and P) was carried out using atomic absorption spectrophotometer after extraction in a mixture of nitric-hydrochloric acid (75v/ 25v).

2.5.2 Biochemical Analysis of \( S. platensis \) in Experimental Design

1) Reducing Sugars and Cysteine Extraction and Analysis

Reducing sugars and cysteine were extracted in 80% ethanol. One mL of homogenized algal suspension was added in 5 mL of 80% ethanol in the mortar and then centrifuged (3000 g, 10 min). The supernatant was collected and used for reducing sugars and cysteine contents quantification. Reducing sugars were assayed by mixing 0.1 mL of reducing sugars crude extract with 1.5 mL of water and 0.5 mL of Müller reagent \([1\% \text{ (w/v)} \text{ DNS (3,5-dinitro salicylic acid)}, 1.6\% \text{ NaOH (w/v)} \text{ and 30\% (w/v) sodium-potassium tartrate}]\). The mixture was homogenized and incubated at 100 °C for 10 min in the water bath to allow colour development. Optical density was measured at 575 nm using glucose as standard.

Cysteine content was estimated by the method described by Gaitonde (1967). Cysteine crude extract (0.15 mL) was added to 0.35 mL of acidic ninhydrin reagent \([1, 3\% \text{ (w/v)} \text{ Ninhydrine in 1:4 HCl:CH}_3\text{COOH conc}]\). The mixture was homogenized and heated at 100 °C for 10 min followed cooling in ice. A volume of 1 mL ethanol 95° was added and the optical density read 560 nm against a blank where cysteine crude extract was replaced equal volumeof ethanol 80°.

2) Antioxidant Enzymes and Proteins Extraction

Polyphenol oxidase and peroxidase were extracted by homogenizing 1g of fresh \( S. platensis \) sample in a mortar containing 10 mL potassium phosphate buffer (50 mM, pH 6.0). The homogenate was subsequently centrifuged (6000g, 30 min at 4°C) and the supernatant was collected. The pellet was re-suspended in the same buffer centrifuged under the same conditions as previously. The second supernatant was added to the first to obtain polyphenol oxidase and peroxidase preparation extract which was used for the analysis of proteins contents, polyphenol oxidase and peroxidase activities.

3) Proteins Quantification

The protein content was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a blank.

4) Polyphenol Oxidase and Peroxidase Activities

a) Polyphenol Oxidase Activity

Polyphenol oxidase (PPO) activity was determined by measuring the increase in absorbance at 330 nm using the method of Van Kammen and Broumer (1964). The reaction mixture incubated at 25 °C was made of: 2.7 mL of phosphate buffer \((1/15 \text{ M}, \text{ pH 6.1})\) and 0.3 mL catechol (10 mM). The reaction was initiated by adding 40 μL of enzymatic extract. The enzyme activity was monitored through change of optical density at 330 nm after 30 s. PPO activity was expressed in unit per μg of proteins content.

b) Peroxidase Activity

Peroxidase (POD) activity was determined using the Thorpe and Gaspar method (1978). Guaiacol transformation was followed at 420 nm. A volume (5 mL) of the reaction mixture \((1 \text{ V of 0.2% H}_2\text{O}_2; 2 \text{ V of 1% guaiacol; 5 V})\)
of 1/15 M phosphate buffer pH 6) was added to 10 μL of enzymatic extract. The enzyme activity was evaluated by monitoring optical density change at 420 nm. Peroxidase activity was expressed in unit per µg of proteins contents.

2.6 Data Analysis

The data obtained were represented as the mean±standard deviation (SD) of three independent experiments. All of the statistical analyses were conducted using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) with Student-Newman-Keuls tests was used to compare differences between treatment means when significant F values were observed at p < 0.05.

3. Results

3.1 Physico-Chemical Parameters of Culture Media

In this study, variation of physico-chemical parameters of culture media was dependent on different sulphate salts (MgSO₄, K₂SO₄ and combination of MgSO₄/K₂SO₄) concentrations. It was noticed that S. platensis was grown at 27.7±0.03 °C.

The present study revealed pH fluctuation along the culture process. Before autoclaving, pH of all culture media was adjusted to 9.0. From this pH value, all the treatments displayed a decrease in pH. The difference between the initial pH (9) and daily pH (for each culture media) displayed a characteristic pattern similar to all regulatory factors (MgSO₄, K₂SO₄ and MgSO₄/K₂SO₄ ratio) (Figures 1a, 1b, and 1c).

![Figure 1](https://example.com/figure1.png)

Figure 1. pH fluctuation in culture media using separately MgSO₄ (a), K₂SO₄ (b) and MgSO₄/K₂SO₄ ratio (c) as regulatory factors. Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

Conductivity displayed time-increase pattern for all regulatory factors (MgSO₄, K₂SO₄ and MgSO₄/K₂SO₄ ratio). However, the increase rates varied from one sulphate salt concentration to another and from one regulatory factor (MgSO₄, K₂SO₄ and MgSO₄/K₂SO₄ ratio) to another. Hence, the conductivity rate showed peaks with MgSO₄ (0.16 g/L) and MgSO₄/K₂SO₄ (0.02/0.14). Though, conductivity rate appeared to increase with K₂SO₄ content in culture media (Figures 2 and 3).

![Figure 2](https://example.com/figure2.png)

Figure 2. Variation of conductivity (as function of time) in culture media using separately MgSO₄ (a), K₂SO₄ (b) and MgSO₄/K₂SO₄ ratio (c) as regulatory factors. Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)
Figure 3. Variation of conductivity rates versus concentrations of regulatory factors (MgSO₄, K₂SO₄ and MgSO₄/K₂SO₄ ratios). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

Total dissolved solids (TDS) versus time (days) showed an increase pattern as observed with conductivity. TDS rate exhibited a pattern similar to conductivity rate (Figures 4 and 5).

Figure 4. Variation of TDS (as function of time) in culture media using separately MgSO₄ (a), K₂SO₄ (b) and MgSO₄/K₂SO₄ ratio (c) as regulatory factors. Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

Figure 5. Variation of TDS rates versus concentrations of regulatory factors (MgSO₄, K₂SO₄ and MgSO₄/K₂SO₄ ratios). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

3.2 Microscopic Identification

*S. platensis* was identified based on microscopic characteristics like a dark blue-green filament with solitary coiled or spiral shape filaments and typical arrangement of multicellular cylindrical trichomes in an open helix usually of relatively large diameter, sometimes attenuated at the ends and with evident cross-walls floating freely in the medium. The filaments were made up of many cells with clear and visible whorls. The number of whorls varied between 4 and 22, with average of 6 whorls per filament (Figure 6).
3.3 Growth Performance

In the present study, *S. platensis* was successfully cultured in five concentrations of MgSO₄ (0.08; 0.16; 0.32; 0.64 and 1.28 g/L), K₂SO₄ (0.08; 0.16; 0.32; 0.64 and 1.28 g/L) and MgSO₄/K₂SO₄ ratios (0.16/0.00, 0.08/0.08, 0.04/0.12, 0.02/0.14 and 0.01/0.15 g/L).

Growth of spirulina as number of filaments (Nₓ), number of whorls (Nₚ), cell productivity (Pₓ) and maximum specific growth rate (µₚ) showed a sulphate salt concentration dependent response presented in Table 2. Number of filaments, number of whorls, cell productivity and maximum specific growth rate in 0.16 g/L MgSO₄ (17569±1070 Nₓ/mL, 6±0 Nₚ/mL, 0.22±0.007 mg/L/day, 0.82±0.07 div/day respectively), 1.28 g/L K₂SO₄ (17737±251 Nₓ/mL, 6±0 Nₚ/mL, 0.24±0.008 mg/L/day, 0.94±0.08 div/day respectively) and 0.02/0.14 g/L of MgSO₄/K₂SO₄ (17972±637 Nₓ/mL, 6±0 Nₚ/mL, 0.26±0.009 mg/L/day, 0.99±0.05 div/day respectively) were significantly higher than those obtained in media supplemented with other concentrations of MgSO₄, K₂SO₄ and combination of MgSO₄/K₂SO₄. A higher concentration of MgSO₄ (1.28 g/L) and low concentration of K₂SO₄ (0.16 g/L) could not support the growth of *S. platensis* and resulted in a significantly low number of filaments, cell productivity and maximum specific growth rate. However, with the decrease of the concentrations ratio of MgSO₄/K₂SO₄ significant increase of number of filaments, cell productivity and maximum specific growth rate were observed. Therefore, in media supplemented with different concentrations of MgSO₄, K₂SO₄ and the MgSO₄/K₂SO₄ combination, the best growth performance was recorded on the medium supplemented with MgSO₄/K₂SO₄ (0.02/0.14 g/L) (Table 2).

Table 2. Number of filaments (Nₓ), number of whorls (Nₚ), biomass (X), cell productivity (Pₓ) and maximum specific growth rate (µₚ) of *S. platensis* (Paracas) of culture media on day 5 with different sulphate salts concentrations.

| Media       | Treatment (g/L) | Nₓ (Nₓ/mL) | Nₚ (Nₚ/mL) | X (g)   | Pₓ (mg/L/day) | µₚ (div/day) |
|-------------|----------------|------------|------------|---------|---------------|--------------|
| MgSO₄       | 0.08           | 14992±793a | 5±0        | 1.01±0.03a | 0.19±0.005b  | 0.76±0.05b   |
|             | 0.16           | 17569±1070b| 6±0        | 1.06±0.03b | 0.22±0.007a  | 0.82±0.07a   |
|             | 0.32           | 14790±938b | 6±0        | 0.98±0.02ab| 0.17±0.01b   | 0.73±0.06b   |
|             | 0.64           | 12537±793b | 6±0        | 0.92±0.01b | 0.15±0.011bc | 0.69±0.09bc  |
|             | 1.28           | 9428±901d  | 6±0        | 0.86±0.02  | 0.13±0.010   | 0.67±0.06c   |
| K₂SO₄       | 0.08           | 7899±295c  | 5±0        | 0.80±0.03c | 0.15±0.010c  | 0.55±0.07c   |
|             | 0.16           | 10799±292d | 5±0        | 1.01±0.02b | 0.17±0.010   | 0.78±0.09b   |
|             | 0.32           | 12148±202a | 5±0        | 1.03±0.02b | 0.20±0.011b  | 0.81±0.07b   |
|             | 0.64           | 14138±248b | 6±0        | 1.06±0.02b | 0.21±0.012b  | 0.90±0.06a   |
|             | 1.28           | 17737±251  | 6±0        | 1.13±0.01  | 0.24±0.008b  | 0.94±0.08a   |
| MgSO₄ + K₂SO₄| 0.16 + 0.00    | 9633±513c  | 5±0        | 1.02±0.01c | 0.19±0.006c  | 0.80±0.06c   |
|             | 0.08 + 0.08    | 14172±439b | 5±0        | 1.07±0.01c | 0.21±0.010b  | 0.91±0.07b   |
|             | 0.04 + 0.12    | 14870±401b | 5±0        | 1.11±0.02ab| 0.24±0.006c  | 0.93±0.07ab  |
|             | 0.02 + 0.14    | 17972±637a | 6±0         | 1.24±0.03a | 0.26±0.009ab | 0.99±0.05a   |
|             | 0.01 + 0.15    | 13241±1637 | 5±0         | 1.04±0.02b | 0.20±0.004b  | 0.88±0.06b   |

*Note.* Data are presented as mean±standard deviation (SD). Values in the same column with the same superscript letters (a > b > c > d > e) are not statistically significant at P-value 0.05.
Biomass appeared to increase with culture age. The increase rate was affected by sulphate salt type and concentration. The use of MgSO4 as regulator factor exhibited a peak at 0.16 g/L. With K2SO4, rate of biomass accumulation increased with K2SO4 concentration in culture medium. The ratio MgSO4/K2SO4 displayed a peak at 0.02/0.14 g/L (Figures 7 and 8).

Figure 7. Variation of biomass (as function of time) in culture media using separately MgSO4 (a), K2SO4 (b) and MgSO4/K2SO4 ratio (c) as regulatory factors. Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

Figure 8. Variation of biomass rates versus concentrations of regulatory factors (MgSO4, K2SO4 and MgSO4/K2SO4 ratios). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

3.4 Chemical and Biochemical

3.4.1 Chemical Composition of S. platensis Strain Used

The chemical profile of S. platensis strain obtained from the SAGRIC Common Initiative Group (CIG) pond was determined. Results obtained in Table 3 revealed the presence of high level of protein content (63.9%), ash (12.4%), calcium (987.4 mg), magnesium (398.7 mg), potassium (1444.0 mg), iron (59.0 mg) and phosphorous (831.0 mg). Whereas lipids content (2.5%), fibers (6.8%) and sodium (8.3 mg) were found to be lower.

Table 3. Chemical profile of S. platensis strain grown in SAGRIC Common Initiative Group (CIG) pond, Douala-Cameroon

| Components      | Contents |
|-----------------|----------|
| Proteins (%)    | 63.90    |
| Lipids (%)      | 2.50     |
| Fiber (%)       | 6.80     |
| Ash (%)         | 12.40    |
| Potassium (mg/100 g) | 1444.00 |
| Calcium (mg/100 g)  | 987.40   |
| Phosphorous (mg/100 g) | 831.00   |
| Magnesium (mg/100 g) | 398.70   |
| Iron (mg/100 g)  | 59.00    |
| Sodium          | 8.30     |
3.4.2 Proteins, Cysteine and Reducing Sugars Contents in *S. platensis* Cultured in Media Supplemented With Sulphate Salts

Proteins, cysteine and reducing sugars contents in *S. platensis* cultured in media supplemented with five concentrations of MgSO₄ (0.08; 0.16; 0.32; 0.64 and 1.28 g/L), K₂SO₄ (0.08; 0.16; 0.32; 0.64 and 1.28 g/L) and the MgSO₄/K₂SO₄ combination (0.16/0.00, 0.08/0.08, 0.04/0.12, 0.02/0.14 and 0.01/0.15 g/L) were monitored and in media supplemented with different concentrations of MgSO₄, K₂SO₄ and the MgSO₄/K₂SO₄ combination, the best proteins, cysteine and reducing sugars contents were recorded on the medium supplemented with MgSO₄/K₂SO₄ (0.02/0.14 g/L) (Table 4).

Table 4. Protein, cysteine and reducing sugars contents of *S. platensis* (Paracas) at 5 days of growth in media with different MgSO₄, K₂SO₄ and combination of MgSO₄ + K₂SO₄ concentrations

| Media   | Treatment (g/L) | Protein (mg/L) | Cysteine (mg/L) | Reducing sugars (mg/L) |
|---------|----------------|----------------|----------------|------------------------|
| MgSO₄   | 0.08           | 837.09±21.34   | 82.61±5.12     | 35.22±0.03             |
|         | 0.16           | 933.02±29.91   | 100.01±4.03    | 28.63±0.03             |
|         | 0.32           | 733.70±20.85   | 104.35±4.50    | 26.73±0.02             |
|         | 0.64           | 696.35±33.01   | 104.44±4.58    | 26.07±0.01             |
|         | 1.28           | 652.33±37.74   | 117.40±4.46    | 25.60±0.02             |
| K₂SO₄   | 0.08           | 748.37±25.59   | 69.57±4.59     | 36.51±0.81             |
|         | 0.16           | 800.40±35.03   | 73.91±4.80     | 32.62±0.31             |
|         | 0.32           | 896.45±35.86   | 78.26±4.50     | 30.24±1.17             |
|         | 0.64           | 933.80±36.11   | 95.65±4.43     | 28.82±0.61             |
|         | 1.28           | 986.19±38.68   | 113.05±3.68    | 26.28±0.72             |
| MgSO₄ + K₂SO₄ | 0.16 + 0.00 | 763.04±38.11  | 100.07±3.83    | 27.87±0.87             |
|         | 0.08 + 0.08    | 859.76±19.63   | 112.06±3.33    | 32.30±1.18             |
|         | 0.04 + 0.12    | 913.13±25.56   | 116.35±3.83    | 38.16±0.92             |
|         | 0.02 + 0.14    | 1067.20±26.58  | 122.35±5.42    | 34.29±0.84             |
|         | 0.01 + 0.15    | 807.74±34.89   | 99.05±5.42     | 27.94±0.53             |

*Note.* Data are presented as mean±standard deviation (SD). Values in the same column with the same superscript letters (a > b > c > d) are not statistically significant at P-value 0.05.

Protein contents of *S. platensis* biomass increased with culture age independently of sulphate salt type and concentration. However, the increase rate of protein contents appeared to be affected by sulphate salt (content and type) in culture medium. Hence, the use of MgSO₄ as regulatory factor exhibited the lowest protein contents increase rate between 0.16 and 0.64 g/L MgSO₄. When K₂SO₄ was as regulatory factor, a peak of protein contents increase rate was obtained with 0.16 g/L protein contents increase rate. A peak of protein contents increase rate was also observed with MgSO₄/K₂SO₄ combination (0.02/0.14) (Figures 9 and 10).

Figure 9. Proteins contents (as function of time) of *S. platensis* cultured in media supplemented with MgSO₄ (a), K₂SO₄ (b) and MgSO₄/K₂SO₄ ratio (c). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)
As with protein content, an accumulation (with time) of cysteine contents in *S. platensis* was observed in all culture media. Though, the accumulation rate was varied with sulphate salt type and concentration. Peak of cysteine accumulation rate appeared at 0.16 MgSO₄ and MgSO₄/K₂SO₄ (0.02/0.14). These peaks seemed to match with peaks of protein contents increase rate (Figures 11 and 12).

Reducing sugars contents increased with culture ages for all culture media. But, when MgSO₄ or K₂SO₄ were used separately as regulatory factors, the increase rate of reducing sugars contents displayed an opposite pattern compared to cysteine contents rate pattern (Figures 13 and 14).
3.5 Antioxidant Enzymes Activities in *S. platensis* Cultured in Media Supplemented With Sulphate Salts

The activities of antioxidant enzymes (PPO and POD) increased with cultures age in all sulphate salt types and concentrations. But, the increase rate of polyphenol oxidase activity decreases when the concentration of MgSO₄ increases. Reversely, increase rate of polyphenol oxidase activity increases with K₂SO₄ concentration in culture media. The use of MgSO₄/K₂SO₄ as regulatory factor displayed a peak of increase rate of polyphenol oxidase activity at 0.02/0.14 (MgSO₄/K₂SO₄). This peak matches with proteins and cysteine contents rates for the same regulatory factor (MgSO₄/K₂SO₄) (Figures 15 and 16).

Figure 13. Reducing sugars contents (as function of time) of *S. platensis* cultured in media supplemented with MgSO₄ (a), K₂SO₄ (b) and MgSO₄/K₂SO₄ ratio (c). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

Figure 14. Variation of reducing sugars contents rates versus concentrations of regulatory factors (MgSO₄, K₂SO₄ and MgSO₄/K₂SO₄ ratios). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)

Figure 15. Polyphenol oxidase activity (as function of time) of *S. platensis* cultured in media supplemented with MgSO₄ (a), K₂SO₄ (b) and MgSO₄/K₂SO₄ ratio (c). Values are expressed in term of: Mean±SD (n = 3 × 3 = 9)
Variation in peroxidase activity was observed with time and sulphate salt types and concentrations. The increase activity rate of peroxidase exhibited peaks with MgSO\(_4\) (0.16 g/L), K\(_2\)SO\(_4\) (0.16 g/L) and MgSO\(_4\)/K\(_2\)SO\(_4\) (0.02/0.14) (Figures 17 and 18).

Correlation analysis between variables was conducted. When K\(_2\)SO\(_4\) was used as regulatory factor, negative and significant correlations were observed between K\(_2\)SO\(_4\) and reducing sugars; indicating that, K\(_2\)SO\(_4\) supply in culture media lead to the use of reducing sugars. Also, negative and highly significant correlations were obtained between reducing sugars contents and biomass or cysteine contents; showing that, K\(_2\)SO\(_4\) supply promote the use of reducing sugars for the synthesis of cysteine which increases biomass. Polyphenol oxidase activity appeared to be also negatively correlated to reducing sugars. In summary, correlations found between variables indicate dependant link between K\(_2\)SO\(_4\) supply and biochemical status and biomass of spirulina (Table 5). Similar correlations (but not always significant) were also found when using MgSO\(_4\) or MgSO\(_4\)/K\(_2\)SO\(_4\) as regulatory factors.
Table 5. Pearson correlation between K₂SO₄, Conductivity, TDS, number of filament, Density, Biomass, Proteins, Cysteine, Reducing sugars, Polyphenol oxidase and Peroxidase.

|                  | K₂SO₄ Corr | Conductivity Corr | TDS Corr | No. of filament Corr | Density Corr | Biomass Corr | Proteins Corr | Cysteine Corr | Reducing sugars Corr | Polyphenol oxidase Corr | Peroxidase Corr |
|------------------|------------|-------------------|--------|---------------------|--------------|--------------|---------------|---------------|----------------------|------------------------|-----------------|
| K₂SO₄           | Corr 1     |                   |        |                     |              |              |               |               |                      |                       |                 |
| Conductivity     | Corr 0.979 * | Corr 0.961 *       | Sig. 0.004 | Corr 0.965 * | Corr 0.978 * | Sig. 0.009 | Corr 0.995 * | Sig. 0.000 | Corr 0.999 * | Corr 0.997 * | Corr 0.998 * | Sig. 0.001 |
| TDS              | Corr 0.961 * | Corr 0.995 *       | Sig. 0.004 | Corr 0.965 * | Corr 0.978 * | Sig. 0.009 | Corr 0.995 * | Sig. 0.000 | Corr 0.999 * | Corr 0.997 * | Corr 0.998 * | Sig. 0.001 |
| No. of filament  | Corr 0.970 | Corr 0.979 *       | Sig. 0.006 | Corr 0.990 * | Corr 0.996 * | Sig. 0.000 | Corr 0.999 * | Sig. 0.001 | Corr 0.999 * | Corr 0.997 * | Corr 0.998 * | Sig. 0.001 |
| Density          | Corr 0.805 | Corr 0.889 *       | Sig. 0.100 | Corr 0.812 | Corr 0.840 | Corr 0.933 | Corr 0.551 | Corr 0.839 | Corr 0.826 | Corr 0.812 | Corr 0.818 | Corr 0.866 | Corr 0.513 | Corr 0.855 |
| Biomass          | Corr 0.019 | Corr 0.066         | Sig. 0.095 | Corr 0.095 | Corr 0.098 | Corr 0.377 | Corr 0.335 | Corr 0.019 | Corr 0.066 | Corr 0.095 | Corr 0.098 | Corr 0.377 | Corr 0.335 | Corr 0.019 |
| Proteins         | Corr 0.176 | Corr 0.262         | Sig. 0.122 | Corr 0.555 | Corr 0.723 | Corr 0.795 | Corr 0.461 | Corr 0.778 | Corr 0.670 | Corr 0.557 | Corr 0.723 | Corr 0.795 | Corr 0.461 | Corr 0.778 |
| Cysteine         | Corr 0.937 | Corr 0.954 *       | Sig. 0.030 | Corr 0.965 | Corr 0.964 | Corr 0.911 | Corr 0.721 | Corr 0.778 | Corr 0.723 | Corr 0.795 | Corr 0.461 | Corr 0.778 | Corr 0.461 | Corr 0.778 |
| Reducing sugars  | Corr 0.995 * | Corr 0.914 *       | Sig. 0.012 | Corr 0.879 | Corr 0.912 | Corr 0.940 | Corr 0.785 | Corr 0.902 | Corr 0.668 | Corr 0.898 * | Corr 0.995 * | Corr 0.914 * | Sig. 0.012 |
| Polyphenol oxidase | Corr 0.778 | Corr 0.670         | Sig. 0.030 | Corr 0.555 | Corr 0.723 | Corr 0.795 | Corr 0.461 | Corr 0.778 | Corr 0.670 | Corr 0.557 | Corr 0.723 | Corr 0.795 | Corr 0.461 | Corr 0.778 |
| Peroxidase       | Corr 0.937 | Corr 0.954 *       | Sig. 0.030 | Corr 0.879 | Corr 0.912 | Corr 0.940 | Corr 0.785 | Corr 0.902 | Corr 0.668 | Corr 0.898 * | Corr 0.995 * | Corr 0.914 * | Sig. 0.012 |

Note. ** Significant of correlation at 0.01 probability level, * Significant of correlation at 0.05 probability level.

4. Discussion
This study highlights that sulphate nutrition has an important influence on the growth performance and biochemical status including antioxidant enzymes activity of the cyanobacterium *Spirulina platensis*.

In the mass culture of microalgae, the medium quality is one of the key factors controlling growth, productivity and biochemical status (Madkour et al., 2012). The algae *S. platensis* has been studied with the basic aim of screening for magnesium sulphate (MgSO₄) and potassium sulphate (K₂SO₄) concentrations. The cyanobacterium *S. platensis* was cultured at pH 9 and temperature of 28 °C. However, we noticed progressive increase of conductivity and total dissolved solids (TDS) in media with different concentration of MgSO₄, K₂SO₄ and the MgSO₄/K₂SO₄ combination. This increase of conductivity and total dissolved solids (TDS) could be explained by the presence of electrically charged atoms which increase with the evaporation of water in media and to the change of the other variables of the culture media due to uptake of nutrients brought by the different concentration of sulphate salt (Anna, 2018).

The morphological feature of *S. platensis* identified like a blue-green filamentous cyanobacterium was reported by Luo and Jiang (2015). Microscopic analysis revealed that the number of whorls and filaments are influenced by sulphate salts concentration. Growth of *S. platensis* as number of filaments, biomass, cell productivity and maximum specific growth rate in 0.16 g/L MgSO₄, 1.28 g/L K₂SO₄ and 0.02/0.14 g/L MgSO₄/K₂SO₄ combination was significantly higher than those obtained in media supplemented with others concentrations of MgSO₄, K₂SO₄ and combination of MgSO₄/K₂SO₄ (Table 2). These results could be explained by the fact that the higher concentration of MgSO₄ (0.32, 0.64 and 1.28 g/L) have negative effect in media involving the reduction of photosynthetic activity of *S. platensis* (Nyabuto et al., 2015). According to Ndjouondo et al. (2017), 0.1 and 0.2 g/L of magnesium sulphate are used as optimum concentration for growth of *S. platensis* and growth delay was observed at concentration higher than 0.2 g/L and the low biomass yield at the highest concentration could be attributed to substrate toxicity (Wakte et al., 2011). The lower number of filaments, biomass, cell productivity and maximum specific growth rate at lower concentration of K₂SO₄ (0.16 g/L) are in agreement with those reported by Wagih El-Shouny et al. (2015) which showed that the reduction of sulphur in the culture medium involved non significant reduction in the growth of the biomass and productivity of *S. platensis*. Moreover low yield of growth recorded in media in with low potassium sulphate concentrations could be
because sulphur deficiency could cause a reduction in the cell multiplication while influencing on metabolism of carbon in photosynthetic activity as reported by Carfagna et al. (2015) to *Chlorella sorokiniana*. Therefore, in media supplemented with different concentrations of sulphate salts, the best number of filaments, biomass, cell productivity and maximum specific growth rate were recorded on medium supplemented with MgSO$_4$/K$_2$SO$_4$ (0.02/0.14 g/L). This could be due to the presence of mineral nutrients (K and Mg) brought by the MgSO$_4$/K$_2$SO$_4$ combination that might play a critical role in the metabolic activities, as essential components of enzymes and other cellular components (Kaushik et al., 2006) and the presence in the medium of ions Mg$^{2+}$ and K$^+$ which could play a significant role in the mechanism of photosynthesis.

Culture medium composition has been reported as one of the most important factors with determining role in biochemical status of microalgae (Çelekli et al., 2016). The biochemical profile of *S. platensis* strain in pond of SAGRIC Common Initiative Group (CIG) farm, Douala (Cameroon) has shown highest content of protein (63.9%) compared to the 37.5% of protein harvested by Ama Moor et al. (2016) in Nomayos-Cameroon, 58.6% and 50.2% of protein by Ngakou et al. (2012) but lower than 69.2% of protein obtained by Mbaïguinam et al. (2006). This could be attributed to the availability of essential elements (N and P) in Jourdan's medium as well as the tendency of algae for bioaccumulation and incorporation of these elements into their macromolecules. Furthermore this analysis revealed that total ash and some minerals (Ca, Mg, K, Fe and P) were much higher than those reported by Ngakou et al. (2012) and Ama Moor et al. (2016). These differences could be explained by either the influence of culture media, the difference in climate, or caused by the differentiated cellular metabolism in as much as these elements are actively involved in the metabolism of *S. platensis*. Reversely, lipids, fibers and sodium contents appeared to be lower. This could be due particularly for lipids content to a variation of the extraction method or the type of solvent used (Ama Moor et al., 2016). Thus the strain of *S. platensis* used in this experiment contains macronutrients and essential micronutrients absorbed from its growth medium become chelated with amino acids and are therefore more easily assimilated by the body and is considered as an excellent food supplement, nephrotoxicity effect of pharmaceuticals and toxic metals, immunological properties and acts as a potent antioxidant.

Considering, magnesium sulphate (MgSO$_4$), the concentration 0.16 g/L gave higher content of protein. Protein contents were increased with K$_2$SO$_4$ content in culture media (the highest protein contents were obtained with 1.28 g/L). The combination of both salt generated the highest protein content with 0.02/0.14 g/L MgSO$_4$/K$_2$SO$_4$. These results highlight the benefit effect of sulphate salts nutrition on proteins accumulation in *S. platensis* biomass. However, this benefit effect depends on sulphate salt type and Mg$^{2+}$/K$^+$ ratio. *S. platensis* seems to not tolerate high concentration of Mg$^{2+}$. This might indicate the toxicity of magnesium sulphate at highest concentration in *S. platensis* biomass (Wakte et al., 2011). Reversely, high K$^+$ promotes protein accumulation in *S. platensis* biomass.

Cysteine content analysis in *S. platensis* biomass (relation sulphate nutrition) revealed the influence of sulphate salt type and Mg$^{2+}$/K$^+$ ratio on content of this sulphurous amino acid (as with protein content). The accumulation of cysteine with increasing content of K$_2$SO$_4$ might indicate that, the availability of sulphate promote the assimilation of sulphur for the synthesis of cysteine (a sulphur-containing amino acid). However, this promoting effect appeared to be stimulate by high content of K$^+$ in culture media; but altered by high content of Mg$^{2+}$ (above 0.16 g/L) in culture media. The increase in SO$_4^{2-}$ concentration caused an increase in cysteine due to essential role of sulphur in synthesis of amino acids like cysteine which make up proteins and enters in the composition of chlorophyll and has a direct implication in the enzymatic catalysis (Schwenk, 2012).

Reducing sugars contents in *S. platensis* biomass displayed a reverse pattern compared to protein and cysteine contents. This might reveal that, the sulphate supply in culture media leads the use of reducing sugars for synthesis of cysteine which is utilized for protein building. Negative and significant correlation was found between cysteine contents, protein contents and reducing sugars.

These changes in biochemical composition could be correlated with the essential role played by the ions K$^+$ and Mg$^{2+}$ in the assimilation of sulphur and the growth of *S. platensis* (Dea Prianka et al., 2019).

Algae are negatively affected by harmful reactive oxygen species (ROS) produced by photosynthetic electron transport, photorespiration, respiration and other metabolic processes which may cause the deterioration of cell metabolism and damage cellular components (Foyer et al., 2011; Mostafa Mahmoud et al., 2016). To alleviate the harmful effects of ROS, *S. platensis* have developed several mechanisms such as antioxidants enzymes in which the polyphenol oxidase (PPO) and peroxidase (POD) play a significant role. The enhanced activity of PPO and POD in *S. platensis* biomass in lower MgSO$_4$ and higher K$_2$SO$_4$ concentrations observed in the present study may suggest a cooperative role of these antioxidants enzymes in protection of *S. platensis* cells against ROS.
Highest PPO and POD activities matched with highest protein, cysteine contents and biomass production. These set of results might reveal that an adequate sulphate supply leads to optimal biomass production, protein and cysteine accumulation under appropriate PPO and POD activities which preserved spirulina cells against ROS (Mostafa Mahmoud et al., 2016; Panahi et al., 2019).

5. Conclusion

Medium composition is one of the key factors that control S. platensis growth, biochemical status and antioxidant activity. From the present study, it could be concluded that high yield of biomass (number of filaments, biomass concentration, cell productivity and specific growth rate), biochemical status (protein, cysteine and reducing sugars) and antioxidant enzymes activities (PPO and POD) were sulphate salt type and concentration dependent. S. platensis cultured in medium supplemented with both MgSO₄ and K₂SO₄ was characterized by highest growth performance, biochemical status (protein, cysteine and reducing sugars) and antioxidant activities (PPO and POD). These sets of results draw attention to the importance of selecting the source and concentration of sulphate salts for S. platensis culture. Sulphate nutrition appeared to be useful to improve growth performance and biochemical status (nutritional value and antioxidant activity) of this cyanobacterium which is important in further explored for their use for medicinal products and additives in pharmaceutical, food, cosmetic or other industrial applications.

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References

Abdel-Daim, M. M., Abuzead, S. M. M., Halawa, S. M., & Partha, M. (2013). Protective Role of Spirulina platensis against Acute Deltamethrin-Induced Toxicity in Rats. Public Library of Science, 8, 1371-1371. https://doi.org/10.1371/journal.pone.0072991

Al-Saman, M. A., Doleib, N. M., Ibrahim, M. R., Nasr, M. Y., Tayel, A. A., & Hamouda, R. A. (2020). In vitro and in vivo hypolipidemic properties of the aqueous extract of Spirulina platensis, cultivated in colored flasks under artificial illumination. PeerJ, 8, e10366 https://doi.org/10.7717/peerj.10366

Ama Moor, V. J., Pieme, C. A., Nkeck J. R., Nya Biapa, C. P., Ikomey, M. G., Kouanfack, C., … Ngogang, J. (2020). Spirulina platensis enhances immune status, inflammatory and oxidative markers of HIV patients on antiretroviral therapy in Cameroon. Research Square, 1602-6494. https://doi.org/10.21203/rs.2.22360/v1

Ama Moor, V. J., Pieme, C. A., Nya Biapa, C. P., Ngo-Matip, M. E., Moukette, B. M., Tankeu, F. N., … Ngogang, J. (2015). Chemical composition of Spirulina platensis of nomayos- Yaoundé (Cameroon). Annals. Food Science and Technology, 17, 524-528.

Anna, F. R. (2018). Correlation between conductivity and total dissolved solid in various type of water: A review. IOP Conference Series: Earth and Environmental Science, 118, 012019. https://doi.org/10.1088/1755-1315/118/1/012019

AOAC (Association of Official Analytical Chemists). (1990). Official Methods of Analysis (15th ed.). Association of Official Analytical Chemists, Arlington, USA.

AOAC (Association of Official Analytical Chemists). (1995). Official Methods of Analysis (16th ed.). Association of Official Analytical Chemists, Arlington, USA.

Azabji-Kenfack, M., Loni, G. E., Sobngwi, E., Onana, E. A., Edie, D. S., & Von Der Weid, D. (2011). The Effect of Spirulina platensis versus Soybean on Insulin Resistance in HIV-Infected Patients: A Randomized Pilot Study. Nutrients, 3, 712-724. https://doi.org/10.3390/nu3070712

Barakat, W., Shima, M., & Mahmoud, A. (2015). Spirulina platensis Lacks Antitumor Effect against Solid Ehrlich Carcinoma in Female Mice. Advances in Pharmacological Sciences, 2015, Article ID 132873. https://doi.org/10.1155/2015/132873

Ben Amor, F., Barkallah, M., Elleuch, F., Karkouch, N., Dammak, M., Baréa, B., … Fendri, I. (2017). Cyanobacteria as source of marine bioactive compounds: Molecular specific detection based on D9 desaturase gene. International Journal of Biological Macromoles, 105, 1440-1445. https://doi.org/10.1016/j.ijbiomac.2017.07.139

Bradford, M. (1976). A rapid and sensitive method for the quantitative of microgram quantities of protein utilizing the principle of protein-Dye binding. Analytical Biochemistry, 72, 248-254. https://doi.org/
Budiyono, Syaichurrozi, I., Sumardiono, S., & Budi Sasongko, S. (2014). Production of *Spirulina platensis* biomass using digested vinasse as cultivation medium. *Trends in Applied Sciences Research, 9*, 93-102. https://doi.org/10.3923/tasr.2014.93.102

Carfagna, S., Salbitani, G., Bottone, C., De Marco, A., & Vona, V. (2015). Cross-effects of nitrogen and sulphur starvation in *Chlorella sorokiniana* 211/8K. *Natural Resources, 6*, 221-229. https://doi.org/10.4236/nr.2015.64020

Çelekli, A., Topyürek, A., Markou G., & Bozkurt, H. (2016). A Multivariate approach to evaluate biomass production, biochemical composition and stress compounds of *Spirulina platensis* cultivated in wastewater. *Applied Biochemistry and Biotechnology, 2*, 2122-2128.

Dea Prianka, A. I., & Taufik, T. (2019). Biomass and Phycocyanin Production from *Spirulina platensis* cultivated in Anaerobically Digested Dairy Manure Waste (ADDMW) with Sodium Bicarbonate Addition. Joint Symposium Plant Sciences and Product, Sith Itb & UKM Malaysia.

Fatemeh, M., & Choopani, A. (2020). Spirulina, food of past, present and future. *Health Biotechnology and Biopharma, 3*(4), 1-20.

Foyer, H., & Shigeoka, S. (2011). Understanding oxidative stress and antioxidant functions to enhance photosynthesis1. *Plant Physiology, 155*, 93-100. https://doi.org/10.1104/pp.110.166181

Gaitonde, M. (1967). A Spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochemistry Journal, 104*, 627-633. https://doi.org/10.1042/bj1040627

Gómez-Téllez, A., Sierra-Puente, D., Muñoz-Gómez, R., Ibarra-Pitts, A., Guevara-Cruz, M., Hernández-Ortega, M., & Gutiérrez-Salmeán, G. (2020). Effects of a Low-Dose Spirulina/Turmeric Supplement on Cardiometabolic and Antioxidant Serum Markers of Patients with Abdominal Obesity. *Frontiers in Nutrition, 7*, 65. https://doi.org/10.3389/fnut.2020.00065

Jourdan, J. P. (2013). Cultivez votre Spiruline. Manuel de culture artisanale pour la production de spiruline. *Antenna Technologies* (Consulté le 24/08/2018, p. 143). Retrieved from http://www.spirulinasource.com/microjourdan.html

Jung, F., Kruger-Gengeb, A., Waldeck, P., & Kupper, J.-H. (2019). *Spirulina platensis*, a super food? *Journal of Cellular Biotechnology, 5*, 43-54. https://doi.org/10.3233/JCB-189012

Kaushik, R., Prasanna, R., & Joshi, C. (2006). Utilization of anaerobically digested distillery effluent for the production of *Spirulina platensis* (ARM 730). *Journal of Scientific and Industrial Research, 65*, 521-525.

Leustek, T. (2002). Sulphate Metabolism. *The Arabidopsis Book 1: e0017* (p. 17). American Society of Plant Biologists. https://doi.org/10.1199/tab.0017

Liu, G., & Wang, J. (2012). Effects of nano-copper (ii) oxide and nano-magnesium oxide particles on activated sludge. *Water Environment Research, 84*, 569-576. https://doi.org/10.1017/106143012X13373575830593

Luo, J., & Jiang, L. (2015). Production of aquatic feed grade algal powder from turtle breeding wastewater using a locally isolated *Spirulina* sp. JXSC-S1. *African Journal of Microbiology Research, 9*, 2404-2409. https://doi.org/10.5897/AJMR2015.7602

Madkour, F., Abd El-Wahab, K., & Hodam, S. (2012). Production and nutritive value of *Spirulina platensis* in reduced cost media. *Egyptian Journal of Aquaculture Research, 38*, 51-57. https://doi.org/10.1016/j.ejar.2012.09.003

Mbaïguinam, M., Tarkodjiel, M., & Maoura, N. (2006). Culture and comparison study of the chemical composition of blue algae of Kanem-Lake (*Spirulina platensis*). *Applied Sciences and Health, 1*, 10-21.

Mohan, I. K., Khan, M., Shobha, J. C., Naidu, M. U., Prayag, A., Kuppusamy, P., & Kutala, V. K. (2006). Protection against cisplatin-induced nephotoxicity by *Spirulina* in rats. *Cancer Chemotherapy and Pharmacology, 58*, 802-808. https://doi.org/10.1007/s00280-006-0231-8

Mostafa, M. S., Yassin E. M., & Michele, P. N. (2016). Role of pH on antioxidants production by *Spirulina* (*Arthrospira*) *platensis*. *Brazilian Journal of Microbiology, 47*, 298-304. https://doi.org/10.1016/j.bjm.2016.01.003

Ndjouondo, G. P., Dibong, S. D., Wamba, F. O., & Taffouo. V. D. (2017). Growth, Productivity and Some
Physico-chemical Factors of *Spirulina platensis* Cultivation as Influenced by Nutrients Change. *International Journal of Botany, 13*, 67-74. https://doi.org/10.3923/ijb.2017.67.74

Ngakou, A., Wague, R., Mbaïlao, M., & Namba, F. (2012). Changes in the physico-chemical properties of *Spirulina platensis* from three production sites in Chad. *Journal of Animal and Plant Sciences, 3*, 1811-1822.

Ngo-Matip, M. E., Pieme, C. A., Azabji-Kenfack, M., Moukette, B. M., Korosky, E., Stefanini, P., … Mbofung, C. (2015). Impact of daily supplementation of *Spirulina platensis* on the immune system of naïve HIV-1 patients in Cameroon: A 12-months single blind, randomized, multicenter trial. *Nutrition Journal, 4*, 15-58. https://doi.org/10.1186/s12937-015-0058-4

Nyabuto, D., Cao, K., Mariga, A., Kibue, G., He, M., & Wang, C. (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. https://doi.org/10.5897/AJAR2015.10210

Panahi, Y., Khosroshahi, A. Y., Sahebkar, Y., & Heidari, H. R. (2019). Impact of Cultivation Condition and Media Content on *Chlorella vulgaris* Composition. *Advanced Pharmaceutical Bulletin*, 182-194. https://doi.org/10.15171/apb.2019.022

Priyanka, Y., Jyoti, S., & Vishal, M. (2020). Biosorption-Cum-Bioaccumulation of Heavy Metals from Industrial Effluent by Brown Algae: Deep Insight. *Microbial Genomics in Sustainable Agroecosystems*, 978-981-13.

Schwenk, J. R. (2012). Effects of Magnesium Sulfate, Digestate and Other Inorganic Nutrients on the Phototrophic Growth of the Green Microalga Scenedesmus Dimorphus. *ETD Archive* (p. 353).

Shigekatsu, S., Haruyo, Y., & Masanobu, K. (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

Soundarapandian, P., & Vasanthi, B. (2008). Effects of chemical parameters on *Spirulina platensis* biomass production: Optimized method for phycocyanin extraction. *International Journal of Zoology Research, 4*, 1-11. https://doi.org/10.3923/ijzr.2008.1.11

Thorpe, T. A., & Gaspar, T. (1978). Changes in isoperoxydase during shoot formation in tobacco callus. *In Vitro, 14*, 552-559. https://doi.org/10.1007/BF02616094

Tsarahevitra, J., Charpy, L., & Vicente, N. (2003). *Culture de Spiruline en eau de mer à Toliara (Madagascar)*. Colloque d’Ecologie Microbienne, Carry Le Rouet.

Usharani, G., Saranraj, P., & Kanchana, D. (2012). *In vitro* cultivation of *Spirulina platensis* using rice mill effluent. *International Journal of Pharmaceutical and Biological Archives, 3*, 1518-1523.

Van Kammen, A., & Broumer, D. (1964). Increase of polyphenol oxidase activity by the local virus infection in uninoculated parts of leaves. *Virology, 22*, 9-14. https://doi.org/10.1016/0042-6822(64)90042-X

Wagih, E.-S.,Sharaf, M., Abd, A. E.-F., & Abo-Eleneen, M. (2015). Production enhancement of some valuable compounds of *Arthrospira platensis*. *Journal of Basic and Environmental Sciences, 2*, 74-83.

Wakte, P., Mohite, Y., & Bhusare, D. (2011). Influence of metal ions on growth and c-phycocyanin production in *Arthrospira (Spirulina) platensis*. *Recent Research Science and Technology, 3*, 104-108.

Wolff, J. P. (1968). Analytical Manual of fatty acids. *Azoulay* (p. 519). Paris, France.

Yakelin, R., Pérez, E., Solórzano, E., Meneses, A., & Fernández, F. (2001). Peroxidase and polyphenol oxidase activities in tomato roots inoculated with *Glomus clarum* or *Glomus fasciculatum*. *Cultivos Tropicales* (pp. 11-16).

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