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Optimizing Biomass Production of *Spirulina Platensis* Using Urea and Sea Water Combination

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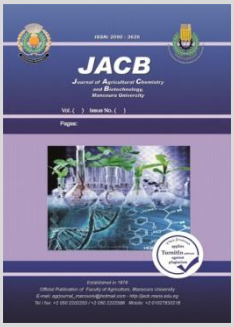

ABSTRACT

The cyanobacterium *Spirulina platensis* is widely recognized as a "food of the future" due to its rich composition of bioactive compounds, including phycobilins, carotenoids, unsaturated fatty acids, and proteins, which contribute to its medicinal and nutritional value. This study aimed to optimize biomass production by evaluating the effect of urea supplementation and seawater incorporation into the growth medium. A modified Zarrouk medium with 0.15 mg/L urea (MZU) was found to enhance *S. platensis* growth more effectively than the standard Zarrouk medium. Furthermore, combining urea with a modified Zarrouk-Provasoli's seawater (PESW) mixture (50:50 v/v) resulted in the highest biomass yield and pigment accumulation, demonstrating the potential of seawater as a partial replacement for conventional freshwater-based media. The use of urea as a nitrogen source proved to be a cost-effective strategy for boosting biomass production while maintaining high levels of valuable bioactive compounds. These findings provide valuable insights for large-scale *S. platensis* cultivation, particularly in regions with limited access to fresh water. The study underscores the potential of urea-seawater combinations in improving *S. platensis* productivity, supporting its application in sustainable food production, nutraceuticals, and biotechnology industries.

Keywords: bio-pigment production, growth rate, *Spirulina*, Zarrouk medium.

INTRODUCTION

Spirulina platensis, a cyanobacterium with unparalleled nutritional and bioactive properties, has emerged as a sustainable superfood and a key player in global food security. It serves as an exceptional source of blue food pigment-phycocyanin, beta-carotenes, and numerous essential nutrients, making it a promising candidate for ideal food production (Oliveira *et al.*, 1999; Barrocal *et al.*, 2010). Historically, Spirulina has been utilized as a nutritional resource in regions such as South America, Asia, and Africa, primarily due to its high protein content (Jung *et al.*, 2019; Bortolini *et al.*, 2022).

Due to its safety and nutritional profile, regulatory agencies like the FDA and ANVISA have approved *Spirulina* biomass for human consumption, further validating its role as a GRAS-certified functional food (Navacchi *et al.*, 2012; El Shafai and Abdallah, 2023). The increasing demand for *Spirulina* stems from its high nutritional value, rich in biologically active compounds, and its potential to be a sustainable, functional food for the future (Sharoba, 2014; Damessa *et al.*, 2021). Classified as generally recognized as safe (GRAS) with no toxic side effects (Matufi *et al.*, 2020), Spirulina has seen a surge in its application in novel, health-focused food formulations (Peshuk and Prykhodko, 2023).

The unique chemical composition of *Spirulina* contributes to its versatility. It contains approximately 60–70% proteins at dry weight, balanced essential fatty acids (notably γ-linolenic acid), vitamins (A, C, and E), antioxidants, minerals (such as Ca, Fe, Mg, P, K, and Zn), and pigments like chlorophyll a, phycocyanin, and carotenes (Bortolini *et al.*, 2022). These attributes have cemented its role in eco-friendly cosmetics (Ragusa *et al.*, 2021), superfoods (Jung *et al.*, 2019), and natural food colorants (Lim *et al.*, 2021). Furthermore, *Spirulina* has demonstrated efficacy in environmental applications, such as treating wastewater by metabolizing nutrients and removing heavy metals in aquaculture (Zhang *et al.*, 2020). Despite its immense potential, one of the primary challenges in cultivating *Spirulina* is the high cost of chemical-based culture media. Media like Zarrouk, Conway, and Kosaric, although effective, contribute significantly to production costs, with chemical media accounting for approximately 35% of total biomass production expenses (Costa *et al.*, 2019; Thevarajah *et al.*, 2022). Researchers have been actively exploring cost-effective alternatives, such as seawater or wastewater enriched with nitrogen and phosphorus, to reduce production costs while maintaining growth and biomass quality (Dineshkumar *et al.*, 2021; Markou *et al.*, 2021).

Nitrogen, an essential nutrient for *Spirulina* cultivation, has been successfully substituted with urea, a more affordable alternative to sodium nitrate (Uddin *et al.*, 2020). Studies reveal that optimizing urea concentration enhances biomass productivity, although excessive urea can adversely affect growth (Mousavi *et al.*, 2022; Ribeiro *et al.*, 2020). Additionally, blending seawater with Zarrouk medium has been proposed to address nutrient limitations and salinity challenges associated with seawater (Bezerra *et al.*, 2020). However, achieving optimal nutrient levels remains a critical area of focus.

This study aims to optimize the cultivation medium for *Spirulina platensis* by incorporating urea as a cost-effective nitrogen source in Zarrouk and seawater-based media to enhance biomass productivity. The research explores the potential of Spirulina-enriched media to support sustainable and economically viable production methods for functional food development. By promoting eco-friendly

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alternatives, this study seeks to reduce reliance on expensive chemical-based media, contributing to more accessible and sustainable Spirulina cultivation practices.

MATERIALS AND METHODS

Algal strain

Inoculants of *Spirulina platensis* used in this study were obtained from the Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig City, Egypt. The strain was cultivated in a 10-liter Erlenmeyer flask containing 5 liters of sterilized Zarrouk medium, prepared according to Stanca (1996).

Preparation of cultivation media

The *Spirulina* growth medium was prepared based on the Zarrouk medium formulation, with its composition (g/L of distilled water) detailed in Table 1. The medium was sterilized at 121 °C for 20 minutes in autoclave. After sterilization and cooling, a *Spirulina platensis* strain was inoculated into a 20-liter transparent glass jar containing 10 liters of the sterilized Zarrouk medium. The prepared medium was adjusted to a pH of 9.5, as recommended for optimal growth. The inoculated jars were maintained at a temperature of 30 °C under continuous illumination provided by cool white fluorescent lamps. Carbon dioxide (CO_2), supplied through aeration with pump-driven air, served as the carbon source for photosynthesis. The cultures were stored in an algal culture chamber as stock cultures. To promote uniform growth, the jars were manually agitated 3 to 4 times per day.

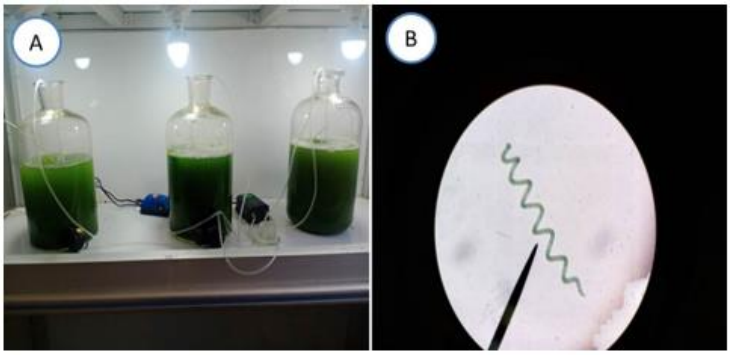

Fig. 1. *Spirulina platensis* stock culture
(A): *S. platensis* in liquid medium; (B): *S. platensis* under light microscope

All procedures involving the transfer of cultures into liquid media were conducted aseptically under a laminar flow hood to ensure sterility. Cells were harvested through filtration using filter paper with an 8 mm pore size and subsequently oven-dried at 75 °C for 4 to 6 hours. After drying, the filter papers containing the Spirulina biomass were allowed to cool before being weighed. The dry weight of the Spirulina biomass was determined by calculating the difference between the initial and final weights. The results were expressed as dry weight in grams per liter (g/L).

Media optimization assay

To optimize the growth of *Spirulina platensis*, a comparative study was conducted using six different culture media: standard Zarrouk medium with sodium nitrate (2.5 g/L), modified Zarrouk medium with potassium nitrate (2.5 g/L), modified Zarrouk medium with urea (0.15 g/L), BG11 medium, Bristol medium, and NPK medium. The compositions of these media are detailed in Tables 1, 2, 3, 4, and 5, respectively. For optimal growth conditions, the pH of each medium was adjusted to 9.5 ± 0.2 using sodium hydroxide (NaOH) and hydrochloric acid (HCl), measured with a portable pH meter. Each medium was tested in triplicate to ensure the reliability and reproducibility of the results.

Incubation conditions

Six 1000 mL conical flasks, each containing 500 mL of one of the prepared media, were uniformly inoculated with 50 mL (10% v/v) of *Spirulina platensis* inoculum obtained from a previously prepared stock subculture. The cultures were maintained at room temperature (25 ± 3 ºC) under continuous illumination provided by three standard cool white LED lamps (9 W), delivering an intensity of 60 µmol photons $m^{-2}s^{-1}$. Aeration was supplied using small air pump devices to ensure adequate oxygenation and mixing. To promote homogeneous distribution of *Spirulina* filaments and ensure uniform light exposure throughout the cultivation system, all culture flasks were manually shaken three times daily.

Measurements of spectrophotometric analysis

At specific time intervals startup (day 0), and days 4, 8, 12, 16, and 20; samples of the medium were aseptically collected for UV-spectrophotometric analysis.

Growth analysis

Growth rates were monitored and determined at the specified time intervals by measuring the optical density at 560 nm, following the method described by Denizot and Lang (1986).

Chlorophyll analysis

The concentrations of photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll, expressed in mg/L) were estimated using 80% acetone, following the method described by Arnon (1956). Spirulina cells (biomass) were harvested and re-suspended in 1 mL of 80% acetone. The suspension was centrifuged, and the resulting supernatant was used for optical absorbance measurements at 663 nm and 645 nm using a UV-VIS spectrophotometer. The content of photosynthetic pigments was calculated using the following equations:

$$\textbf{Chlorophyll a } (\textbf{mg/L}) = (\mathbf{12.7} \times \boldsymbol{A663}) - (\mathbf{2.698} \times \boldsymbol{A645}).$$

$$\textbf{Chlorophyll b } (\textbf{mg/L}) = (\mathbf{12.7} \times \boldsymbol{A645}) - (\mathbf{4.68} \times \boldsymbol{A663}).$$

$$\textbf{Total chlorophyll } (\textbf{mg/L}) = \boldsymbol{chlorophyll\ a} + \boldsymbol{chlorophyll\ b} = (\mathbf{20.2} \times \boldsymbol{A645}) + (\mathbf{8.02} \times \boldsymbol{A663}).$$

Phycobiliproteins analysis

The estimation of biopigments (phycobiliproteins) was conducted following the methods described by Bennett and Bogorad (1973) and Devanathan and Ramanathan (2012). Around 10 mL aliquot from each sample was centrifuged at 4500 rpm for 20 minutes, after which the supernatant was discarded. The remaining filaments were washed with deionized water, re-suspended in 10 mL of phosphate buffer (0.05 M, pH 7.0), and homogenized. The suspension was subjected to repeated freeze-thaw cycles and centrifuged at 4500 rpm for 10 minutes. Phosphate buffer was

used as a blank, and the absorbance of the supernatant was measured at 652 nm, 615 nm, and 562 nm using a UV-VIS spectrophotometer. The concentrations of phycocyanin (PC), allophycocyanin (APC), and total phycobiliproteins were calculated using the following equations:

$$\text{Phycocyanin (PC)} = OD_{615} - 0.474\,(OD_{652}) \div 5.34$$
$$\text{Allophycocyanin (APC)} = OD_{652} - 0.208\,(OD_{615}) \div 5.09$$
$$\text{Phycobiliproteins} = PC + APC$$

Table 1. The nutrient elements of standard Zarrouk with sodium nitrate and modified Zarrouk with potassium nitrate media (Pai *et al.*, 2008).

Ingredients	Standard Zarrouk's media (g/L)	Modified Zarrouk's media (g/L)
NaCl	1.0	1.0
$CaCl_2.2H_2O$	0.04	0.04
KNO_3	-	2.5
$NaNO_3$	2.5	-
$FeSO_4 \cdot 7H_2O$	0.01	0.01
EDTA (Na)	0.08	0.08
K_2SO_4	1.0	1.0
$MgSO_4 \cdot 7H_2O$	0.2	0.2
$NaHCO_3$	16.8	16.8
K_2HPO_4	0.5	0.5
A 5-micronutrient (H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.4H_2O$ Na_2MoO_4, $CuSO_4.5H_2O$)	1 ml	-
PH	9.5	9.5

Table 2. The nutrient elements of modified Zarrouk with urea medium (Fagiri, *et al.*, (2013).

Ingredients	Modified Zarrouk's with urea (g/L)
NaCl	1.0
$CaCl_2.2H_2O$	0.04
Urea	0.15
$FeSO_4 \cdot 7H_2O$	0.01
EDTA (Na)	0.08
K_2SO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.2
$NaHCO_3$	16.8
K_2HPO_4	0.5
pH	9.5

Table 3. The nutrient elements of ingredients of selective (BG11) medium, (Dineshkumar *et al.*, 2016).

Ingredients	BG11medium (g/L)
$NaNO_3$	1.5
$CaCl_2.2H_2O$	0.036
Citric acid	0.006
$FeSO_4 \cdot 7H_2O$	0.006
$MgSO_4 \cdot 7H_2O$	0.075
Na_2CO_3	0.001
K_2HPO_4	0.04
EDTA	0.02
pH	9.5

Table 4. The nutrient elements of selective NPK-based culture medium, (Kumari *et al.*, 2014).

Ingredients	NPK-based culture medium (g/L)
NPK	0.076
NaCl	1.0
$FeSO_4$	0.01
$MgSO_4 \cdot 7H_2O$	0.2
Urea	0.0207
silica	0.0469
EDTA	0.08
$CaCl_2$	0.04
pH	9.5

Table 5. The nutrient elements of selective medium Bristol medium, (Muriel Bristol Roach, 1927).

Ingredients	Bristol medium (g/L)
K_2HPO_4	7.5
$NaNo_3$	25
$MgSO_4 \cdot 7H_2O$	7.5
$CaCl_2$	2.5
KH_2PO_4	17.5
NaCl	2.5
pH	7.0

Figure 2. *Spirulina platensis* growth pattern in six different cultured media.

Sea water media optimization assay

In the natural environment of Qarun Lake (Birket Qarun) in Fayoum City, nutrient availability is primarily regulated by tidal ebb and flow, drainage from irrigated lands, and rainfall that washes soil nutrients into the lake. Under these conditions, algal filaments exhibit rapid growth, reaching high densities and numbers. Eventually, as nutrients are depleted, the algal population declines. The subsequent decomposition of these filaments releases nutrients back into the environment, enabling the initiation of a new seasonal growth cycle, particularly when additional nutrients enter the lake. Building on this natural model, the present study hypothesizes that utilizing seawater-based media for the cultivation of *Spirulina platensis* could significantly reduce the costs associated with chemically formulated media while promoting varied growth and biochemical characteristics. Therefore, this study aims to evaluate the effectiveness of different cyanobacterial culture media prepared with natural seawater in combination with other components.

To ensure consistency and suitability of the Qarun Lake seawater used in the study, its salinity and ion composition were assessed. Qarun Lake water is characterized by a salinity of approximately **(**40 g/L**)** with dominant ions including sodium **(**Na^+**),** chloride **(**Cl^-**),** sulfate **(**SO_4^{2-}**),** calcium (Ca^{2+}**),** and magnesium (Mg^{2+}**),** comparable to brackish or marine environments. The seawater used in the culture media underwent boiling, filtration, and pH

adjustment to remove impurities and maintain reproducibility across experimental replicates.

Furthermore, to verify nutrient availability post-filtration, nitrogen (N) and phosphorus (P) levels were considered. While direct nutrient measurements were not conducted in this study, batch consistency was maintained by using a single pre-filtered seawater source across all replicates. Growth patterns of Spirulina platensis were monitored to confirm uniformity, ensuring that variations in culture performance were due to the tested media formulations rather than fluctuations in nutrient composition. Therefore, this study aims to evaluate the effectiveness of different cyanobacterial culture media prepared with natural seawater in combination with other components, as follows:

1- Qarun Lake seawater as control (100%).
2- Provasoli's enriched sea water (PESW) (100%).
3- PESW medium supplemented with modified Zarrouk with 0.15 g/L urea (MZU), at ratio (50:50 v/v).
4- PESW medium supplemented MZU at ratio (70:30 v/v)
5- PESW medium supplemented MZU (30:70 v/v)

Natural seawater was collected from Qarun Lake in Fayoum City, transported immediately to the laboratory, boiled at 100 ºC, filtered using Whatman filter paper No. 50, and adjusted to a pH of 9.5. All prepared media were sterilized at 121 ºC for 20 minutes and then cooled to ambient temperature. A net volume of 500 mL from each of the five media was transferred into 1-liter flasks and inoculated with 50 mL of *Spirulina platensis* subculture, obtained from the stock culture. The culture flasks were placed on a shelf at 28 ± 2 ºC under continuous illumination provided by white fluorescent lamps and was aerated consistently. Cultivation was conducted for 20 days, with measurements recorded at intervals of 0 (startup), 4, 8, 12, 16, and 20 days. The experiment was performed in triplicate to ensure reliability and reproducibility of the results.

Table 6. The nutrient elements of Provasoli's Enriched Seawater (PESW) medium, Aung, (2020).

Nutrient Elements	Provasoli's Enriched Seawater (PESW) g/L
$NaNO_3$	0.7
NaH_2PO_4. $6H_2O$	0.1
Fe (EDTA: 1:1 molar)	0.05
P II metal	0.05
Tris Buffer	0.001
Vitamin B12	0.00002
Biotin (B6)	0.00001
Thiamine HCl (B1)	0.000001
Fe (EDTA: 1:1 molar)	Amount
Distilled water	500 mL
$Fe(NH_4)_2(SiO_4)2.6H_2O$	0.351 g
Na_2EDTA	0.3 g
P II metal solution	Amount
Distilled water	100 mL
H_3BO_3	114.0 mg
Na_2EDTA	100 mg
$MnSO_4.4H_2O$	16.4 mg
$FeCl_2.6H_2O$	4.9 mg
$ZnSO_4.7H_2O$	2.2 mg
$COSO_4.7H_2O$	0.48 mg

Statistical analysis

Statistical analysis was performed using SPSS software. The analysis of variance (ANOVA) and Duncan's test were used for comparing different means. Statistical significant was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of standardized culture media

Media type and component cost are key challenges for cyanobacterial growth and cultivation of cyanobacteria. Therefore, the biomass yield and constituents including phycocyanin, allophycocyanin, phycobiliproteins, chlorophyll a, b and total chlorophyll of *Spirulina platensis* mainly differed. Generally, there was various growth media implemented to cultivate microgreen algae of *Spirulina platensis*.

However, in our study, six growth media were examined such as standard Zarrouk with 2.5 mg/L sodium nitrate (SZSN), modified Zarrouk with 2.5 mg/L potassium nitrate (MZPN), modified Zarrouk with 0.15 mg/L urea (MZU), BG11, Bristol, and NPK. Our study was primarily aimed to provide a new-modified low cost media with simple components, in place of the conventional media that has been distinguished not only expensive cost ingredients, but also scarce market availability and a use limit unless approved by security agencies.

Optimizing algae reactors and greenhouses by controlled ambient conditions can increase growth rate and productivity than the outdoor environment systems (Ragaza *et al.,* 2020). Table (7) shows growth rate of *Spirulina platensis* as affected by six different growth media. Overall, *Spirulina* growth rate increased as long as days of cultivation extended, regardless the used media, and then reached to its maximum growth rate at 8, 12 and 16 days of cultivation, compared to the startup (zero time) day.

Comparatively, among six different growth media, the modified Zarrouk with 0.15 mg/L urea (MZU) resulted in relative growth rate closer to those obtained by modified zarrouk with 2.5 mg/L potassium nitrate (MZPN) or standard Zarrouk with 2.5 mg/L sodium nitrate (SZSN) media, respectively. However, growth rate of *Spirulina platensis* was fluctuated significant or non-significant differences between previously indicted treatments along different cultivation periods. Thus, the study demonstrated that, the present study proved that the modified Zarrouk with 0.15 mg/L urea (MZU) gave better growth rate relatively to standard Zarrouk with 2.5 mg/L sodium nitrate (SZSN) or modified zarrouk with 2.5 mg/L potassium nitrate (MZPN) media. Similarly, the modified medium was better than Zarrouk 's medium in terms of the performance assessment, productivity, and specific growth rate (Fagiri *et al.,* 2013; Soni *et al.,* 2019). With little urea which is an effective alternative and significantly provides higher cell growth rates when compared to Zarrouk media, and ammonium nitrate media (Fagiri *et al.,* 2013; Volkmann *et al.,* 2008). Other researchers stated cultivation cyanobacteria of *spirulina* with urea as nitrogen source gives better biomass yield. (Quinn *et al.,* 2011; Bernard and Rémond, 2012; Soni *et al.,* 2019).

Our results showed that the highest *Spirulina* growth rate by 71.32% and 79.69% was recorded from the growth media of MZPN and MZU, orderly at 20 day of cultivation, compared to the startup (zero time) day. Urea as a nitrogen source facilitates higher growth rate of *Spirulina platensis*, due to significant influence on the chlorophyll content (Tables; 1 and 3), leading to higher biomass yields as seen (Danesi *et al.,* 2004; Soni *et al.,* 2019). Confirmed results posted by Danesi *et al.,* (2002) and Soni *et al.,* (2019) found the best results for cell growth rate when urea was substituted

as nitrogen source in the modified media. Worthy, nitrogen is an essential component for amino acids and protein production that could enhance growth and cell biomass (Chow, 2012). Also, similar outcomes proved that the best cellular growth was observed with 500 mg/Lof urea at a light intensity of 5600 lux, whereas the highest concentration of chlorophyll in the biomass was observed with 500 mg/L of urea at a light intensity of 1400 Lux (De Oliveira Rangel-Yagui *et al.*, 2004).

Table 7. Effect of different growth media on *Spirulina platensis* growth rate during twenty cultivation days.

Media	*Spirulina* growth rate (%) per day					
	Zero	**04**	**08**	**12**	**16**	**20**
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	0.40^{a}	0.52^{a}	0.66^{ab}	0.84^{b}	1.29^{a}	1.30^{a}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	0.39^{a}	0.51^{ab}	0.69^{a}	1.02^{a}	1.22^{ab}	1.36^{a}
Modified Zarrouk with Urea (0.15 gm/L)	0.27^{b}	0.49^{b}	0.61^{b}	1.00^{a}	1.21^{b}	1.33^{a}
BG 11	0.24^{c}	0.40^{c}	0.45^{d}	0.20^{d}	0.83^{c}	0.48^{c}
Bristol	0.24^{c}	0.38^{d}	0.38^{e}	0.83^{b}	0.30^{d}	0.33^{d}
NPK	0.26^{b}	0.38^{d}	0.58^{c}	0.36^{c}	0.25^{e}	0.56^{b}
SE±	0.028	0.025	0.045	0.128	0.177	0.181

Mean values in columns within the same small superscript letters are non-significantly different ($P \leq 0.05$), SE+: standard error.

Blue pigments (*e.g.*, phycocyanin, allophycocyanin, phycobiliprotein contents), it is a remarkable natural colorants used in food processing, produced from cyanobacteria *Spirulina* as the most cultivated microalga worldwide (Matos *et al.*, 2017; Shao *et al.*, 2019). Such compounds increase their biological function and commercial uses (El-Sheekh *et al.*, 2021). As all organisms, *Spirulina platensis*, are influenced by several factors such as growth medium factors components, and little fluctuations may influence growth and phycocyanin production (Khazi *et al.*, 2021; Jiang *et al.*, 2023). The outcomes in Table 8 show that six different growth media accelerate the accumulation of phycocyanin, allophycocyanin and phycobiliprotein (mg/L) in microgreen algae of *Spirulina platensis* across 20 days of cultivation, compared to the startup (zero time) day. Our results revealed also that *Spirulina platensis* took 12 days for reaching the best accumulation of phycocyanin, allophycocyanin and phycobiliprotein, and still showed a trend toward increase.

Table 8. Effect of different growth media on biopigments phycocyanin content (mg/L) of *Spirulina platensis* during twenty cultivation days.

Media	Phycocyanin content (mg/L) per day					
	Zero	**04**	**08**	**12**	**16**	**20**
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	0.010^{d}	0.013^{e}	0.019^{e}	0.040^{d}	0.093^{c}	0.072^{c}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	0.039^{a}	0.013^{e}	0.037^{c}	0.017^{e}	0.120^{b}	0.128^{b}
Modified Zarrouk with Urea (0.15 gm/L)	0.027^{b}	0.029^{b}	0.084^{a}	0.184^{a}	0.151^{a}	0.142^{a}
BG 11	0.028^{b}	0.046^{a}	0.050^{b}	0.097^{b}	0.070^{d}	0.039^{e}
Bristol	0.022^{c}	0.033^{c}	0.044^{c}	0.079^{c}	0.060^{e}	0.070^{c}
NPK	0.029^{b}	0.026^{d}	0.040^{c}	0.086^{c}	0.053^{f}	0.054^{d}
SE±	0.004	0.005	0.008	0.021	0.014	0.017
Allophycocyanin content (mg/L) per day						
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	0.08^{f}	0.11^{d}	0.12^{e}	0.36^{e}	0.47^{f}	0.63^{c}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	0.14^{d}	0.24^{c}	0.32^{d}	0.37^{e}	0.53^{e}	0.46^{d}
Modified Zarrouk with Urea (0.15 gm/L)	0.29^{a}	0.40^{a}	0.74^{a}	1.71^{a}	1.41^{a}	1.22^{a}
BG 11	0.21^{b}	0.25^{b}	0.42^{b}	0.75^{c}	1.04^{b}	0.20^{e}
Bristol	0.18^{c}	0.24^{c}	0.37^{c}	0.69^{d}	0.62^{d}	0.63^{c}
NPK	0.12^{e}	0.25^{b}	0.34^{cd}	0.85^{b}	0.82^{c}	1.12^{b}
SE±	0.028	0.034	0.075	0.184	0.134	0.146
Phycobiliprotein content (mg/L) per day						
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	0.09^{f}	0.12^{e}	0.13^{e}	0.40^{e}	0.52^{f}	0.70^{c}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	0.13^{e}	0.16^{d}	0.36^{d}	0.41^{e}	0.59^{e}	0.51^{d}
Modified Zarrouk with Urea (0.15 gm/L)	0.28^{a}	0.44^{a}	0.83^{a}	1.89^{a}	1.56^{a}	1.36^{a}
BG 11	0.26^{b}	0.27^{c}	0.47^{b}	0.83^{c}	1.16^{b}	0.24^{e}
Bristol	0.21^{d}	0.32^{b}	0.41^{c}	0.77^{d}	0.69^{d}	0.70^{c}
NPK	0.24^{c}	0.28^{c}	0.38^{cd}	0.95^{b}	0.91^{c}	1.25^{b}
SE±	0.028	0.043	0.085	0.203	0.148	0.161

Mean values in columns within the same small superscript letters are non-significantly different ($P \leq 0.05$), SE+: standard error.

On the other hand, when comparing between six different growth media used in this study, the modified Zarrouk with 0.15 mg/L urea (MZU) promoted the accumulation of phycocyanin, allophycocyanin and phycobiliprotein in the cells of *Spirulina platensis* more than the other media, however still showed maximal increase in particular at 12 or 16 days of cultivation, comparable to startup (zero time) day. In this regard, when urea as a source of nitrogen was added to the modified Zarrouk media promoted feasibly biopegments of *Spirulina Platensis* culture. It's evidenced a strong correlation between biomass and phycocyanin/chlorophyll ratio, rather than phycocyanin, revealed the dependence of *Spirulina Platensis* growth on coordinating regulation of photosynthetic pigments (Zittelli *et al.*, 2022; Jiang *et al.*, 2023). Similar result posted by Jiang *et al.* (2023) found that phycocyanin production reached 70 mg/L/d and 11 mg/L/d from freshwater and seawater medium, respectively.

It is worth to note that, the highest significant mean values of phycocyanin (by 85.33% or 82.12%), allophycocyanin (by 83.04% or 79.43%), and phycobiliprotein (by 85.19% or 82.05%) were attained at 12 or 16 days of cultivation in the modified Zarrouk with 0.15 mg/L urea (MZU), respectively compared to the startup (zero time) day. Manirafasha *et al.*, (2018) and Mohy El-Din (2020) stated that media nutrients content affected the c-phycocyanin content in *Spirulina platensis*,

especially nitrate content which could lead to the reduction or inhibition of c-phycocyanin. Our results (Tables; 2, 3 and 4) show the impact of urea in the modified Zarrouk leading to higher biomass yields as well as biopegments phycocianins contents. In this aspect, Bernard and Rémond (2012) and Salunke *et al.*, (2016) observed higher phycocyanin content associated to the better growth rate in *Spirulina platensis.* The loss of biomass under nitrogen deficient condition also increased with increasing light intensity for four strains of *Botryococcus* spp. (Yeesang and Cheirsilp, 2011).

Our study examines six different growth media on chlorophyll a, b and total chlorophyll concentration (mg/L) of *Spirulina platensis*, as seen in Table 9. Across days of culture, the aforementioned photosynthetic pigments were gradually increased under six different growth media, and reached to the highest concentration after 8th days of cultivation, compared to the startup (zero time) day. Comparatively, between six different growth media, our study explores that the best results of photosynthetic pigments content was recorded when the *Spirulina platensis* inoculated into the modified Zarrouk supplemented with 0.15 mg/L urea (MZU), and then incubated for 12th or 16th days, compared to the other rest growth media. Instantly, the most significant records for chlorophyll a (by 75.41% or 74.04%), chlorophyll b (by 76.82% or 75.26%) and total chlorophyll (by 76.32% or 74.84%) were obtained from the modified Zarrouk with 0.15 mg/L urea (MZU) at 12 or 16 days of culture, respectively, in comparison with the startup (zero time) day of the other growth media. Studies that had been previously conducted showed that nitrogen availability and its concentration affected on the chlorophyll and protein contents of microalgae *Spirulina platensis platensis* (Markou, 2015; El-Sheekh *et al.*, 2021). Consistently, various authors have reported that the photosynthetic pigments namely chlorophyll a, b and total chlorophylls are a key factor of cyanobacteria genera, and increased with the he modified Zarrouk supplemented urea as a nitrogen source (Salunke *et al.*, 2016; Soni *et al.*, 2019; El-Sheekh *et al.*, 2021), showed a trend of compatibility with our results. By adjusting the nutrient media content, *Spirulina platensis* able to regulate their photosynthetic efficiency (Bernard, 2011; Soni *et al.*, 2019).

Table 9. Effect of different growth media on photosynthetic pigments content (mg/L) of *Spirulina platensis* during twenty cultivation days.

Media	Chlorophyll a content (mg/L) per day					
	Zero	**04**	**08**	**12**	**16**	**20**
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	1.67^{d}	2.42^{d}	3.88^{d}	7.76^{c}	10.31^{a}	8.21^{c}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	3.41^{a}	3.83^{c}	0.89^{e}	7.02^{e}	13.18^{b}	6.45^{e}
Modified Zarrouk with Urea (0.15 mg/L)	3.89^{a}	4.38^{b}	8.03^{a}	15.82^{a}	14.99^{a}	13.00^{a}
BG 11	2.89^{c}	4.67^{a}	4.66^{b}	9.40^{b}	9.66^{d}	1.32^{f}
Bristol	2.77^{c}	3.45^{c}	4.17^{c}	7.30^{de}	6.39^{f}	6.90^{d}
NPK	3.10^{b}	3.40^{c}	3.74^{d}	7.65^{cd}	8.87^{e}	10.78^{b}
SE±	0.28	0.30	0.85	1.25	1.15	1.50
Chlorophyll b content (mg/L) per day						
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	1.92^{d}	0.46^{d}	6.94^{d}	12.21^{d}	17.80^{c}	15.23^{c}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	1.16e	6.04^{c}	6.84^{d}	12.21^{d}	22.31^{b}	11.61^{e}
Modified Zarrouk with Urea (0.15 gm/L)	6.80^{a}	8.49^{a}	14.49^{a}	29.34^{a}	27.49^{a}	23.36^{a}
BG 11	4.90^{c}	8.24^{a}	8.39^{b}	17.35^{b}	18.02^{c}	19.85^{b}
Bristol	5.05^{b}	6.19^{b}	7.55^{c}	13.41^{c}	11.62^{e}	12.53^{d}
NPK	5.52^{b}	6.20^{b}	6.66^{d}	14.15^{c}	15.88^{d}	2.41^{f}
SE±	0.82	1.08	1.12	2.46	2.04	2.72
Total chlorophyll content (mg/L) per day						
Standard Zarrouk with Sodium Nitrate (2.5 gm/L)	7.82^{c}	9.46^{c}	10.82^{d}	19.24^{e}	35.49^{b}	23.44^{c}
Modified Zarrouk with Potassium Nitrate (2.5 gm/L)	10.67^{a}	12.61^{b}	13.02^{b}	26.74^{b}	27.67^{c}	18.06^{e}
Modified Zarrouk with Urea (0.15 gm/L)	10.69^{a}	13.16^{a}	22.52^{a}	45.15^{a}	42.48^{a}	36.36^{a}
BG 11	9.64^{b}	9.60^{c}	11.72^{c}	21.80^{c}	28.11^{c}	30.63^{b}
Bristol	3.60^{d}	7.79^{d}	10.39^{d}	20.71^{cd}	24.75^{d}	19.43^{d}
NPK	8.05^{bc}	9.88^{c}	10.62^{d}	19.97^{de}	18.00^{e}	13.73^{f}
SE±	0.99	0.77	1.74	3.70	3.18	3.15

Mean values in columns within the same small superscript letters are non-significantly different ($P \leq 0.05$), SE+: standard error.

Our study was carried out with the primary objective of providing a simple and low coast media, and our results clearly showed that the modified Zarrouk with 0.15 mg/L urea (MZU) is better than standard Zarrouk's medium. In this concern, the addition of urea into the modified Zarrouk culture media has an important role in manifesting the growth rate and biomass production of *Spirulina platensis* during the 20th days of culture period study.

Effect of seawater media optimization

The purpose of this study was to grow *Spirulina platensis* using modified Zarrouk and provasoli´s enriched sea water media as well as sea water. The growth rate (%), phycocyanin, allophycocyanin, phycobiliprotein contents (mg/L), chlorophyll a, b and total chlorophyll contents (mg/L) of *Spirulina platensis* were studied at different alternative growth media namely; sea water (control), provasoli´s enriched sea water (PESW), PESW: modified Zarrouk with urea (0.15 gm/L) 50:50 [PESW: MZU-50:50 (v/v)], PESW: modified Zarrouk with urea (0.15 gm/L) 70:30 [PESW: MZU-70:30 (v/v)], and PESW: modified Zarrouk with urea (0.15 gm/l) 30:70 [PESW: MZU-30:70 (v/v)].

The results listed in Table 10 showed the highest significant growth rate of *Spirulina platensis* attained with modified media of [PESW: MZU-50:50 (v/v)], followed by [PESW: MZU-70:30 (v/v)] and then by [PESW: MZU-30:70 (v/v)], compared to the sea water (control). The maximum growth rate was obtained at 16 and 20 days of cultivation in modified media [PESW: MZU-50:50 (v/v)] by 64.48% and 68.59%, orderly, compared to startup (zero time) day.

These results indicate that Zarrouk medium served as the standard control, while modified organic media were prepared by substituting the nitrogen source (Soni *et al.*, 2019). In this investigation, higher growth rates of *Spirulina* were observed when grown on the media of [PESW: MZU-

50:50 (v/v)], which formulated based on nutrient wise permutations and combinations, in particular components served as nitrogen source (Quinn *et al.*, 2011; James *et al.*, 2013). This means, urea increased growth feasibility and cell concentration. Similarly, earlier authors have been evidenced that the newly modified medium is better than Zarrouk 's medium growth performance assessment (Markou, 2015; Li *et al.*, 2022). Many researchers have reported that thylakoid membranes are damaged when the *Spirulina platensis* grown under sea water conditions. In this sense, they assumed salt stress of sea water decreased oxygen supply that would inhibit PSII electron transport, and diminished amount of the functional PS2 reaction centers (Chentir *et al.*, 2017). All of these can reduce photosynthetic activity of microalgae when cultured under sea water conditions (Zhang *et al.*, 2010).

Table 10. Effect of different alternative growth media on the growth rate (%) *Spirulina platensis* for 20th days cultivation period.

Alternative media	Spirulina growth rate (%) per day					
	Zero	04	08	12	16	20
Sea Water (Control)	0.18^{d}	0.12^{e}	0.08^{e}	0.17^{d}	0.18^{e}	0.13^{e}
Provasoli´s Enriched Sea Water (PESW)	0.21^{c}	0.27^{c}	0.24^{d}	0.20^{d}	0.22^{d}	0.24^{d}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	0.38^{a}	0.49^{a}	0.57^{a}	0.79^{a}	1.07^{a}	1.21^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	0.31^{b}	0.32^{b}	0.40^{b}	0.68^{b}	0.94^{b}	0.91^{b}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	0.30^{b}	0.23^{d}	0.36^{c}	0.49^{c}	0.57^{c}	0.57^{c}
SE ±	0.03	0.05	0.07	0.11	0.16	0.18

Mean values in columns within the same small superscript letters are non-significantly different ($P \leq 0.05$), SE+: standard error.

Generally, phycocyanin, allophycocyanin and phycobiliprotein concentrations (mg/L) of *Spirulina platensis* were increased as long as cultivation days increased (Table 11). Among different growth media, variation in the aforementioned parameters were considerably endorsed with modified media of [PESW: MZU-50:50 (v/v)], followed by followed by [PESW: MZU-70:30 (v/v)] and [PESW: MZU-30:70 (v/v)], in comparison with the other growth media as well as control. Our investigation affiant that phycocyanin is commonly produced by *S. platensis*, however, phycocyanin yield is depending upon the nutritive of the growth medium with the limitation of high cultivation cost and low yield. The maximum concentration of phycocyanin and phycobiliprotein were observed after 12 and 16 days of cultivation by (19.82% and 27.34%), (22.61% and 32.06%), respectively, compared to startup (zero time) day. Whereas, at 8 and 12 days of cultivation displayed the maximal allophycocyanin content by 23.30% and 33.61%, respectively. Most research about optimizing phycocyanin production by *S. platensis* were done in Zarrouk medium of which the chemicals and freshwater account for a non-negligible part of phycocyanin production cost (Xie *et al.*, 2015; Nur *et al.*, 2019; Jiang *et al.*, 2023). However, phycocyanin occurs in thylakoid membranes as a photosynthetic accessory pigment (Dejsungkranont *et al.*, 2017) and its biochemical pathways are closely related to nitrogen and light sources. The phycocyanin content in *Spirulina platensis* is affected by nutrient contents in the culture media, especially nitrogen source, which could lead to the reduction or inhibition of c-phycocyanin, (Manirafasha *et al.*, 2018; Mohy El-Din, 2020). It has been suggested that phycocyanin belongs to a family of phycobiliproteins which have obtained a secondary role as intracellular nitrogen storage compounds that are mobilized for other purposes in times of nitrogen shortage (Eriksen, 2008). This is also supported by several studies, which have demonstrated that nitrogen depletion causes a reduction in protein content, along with an enhancement of energy-rich compounds, such as lipids and carbohydrates (Ho *et al.*, 2012; Procházková *et al.*, 2014).

Table 11. Effect of different alternative growth media on biopigment phycocyanins content (mg/L) of *spirulina platensis* during twenty cultivation days.

Alternative media	Phycocyanin content (mg/L) per day					
	Zero	04	08	12	16	20
Sea Water (Control)	0.008^{e}	0.014^{d}	0.008^{e}	0.013^{e}	0.012^{e}	0.019^{e}
Provasoli´s Enriched Sea Water (PESW)	0.014^{d}	0.034^{c}	0.021^{d}	0.023^{d}	0.022^{d}	0.025^{d}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	0.093^{a}	0.063^{a}	0.051^{a}	0.116^{a}	0.128^{a}	0.064^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	0.070^{b}	0.039^{b}	0.039^{b}	0.083^{b}	0.098^{b}	0.047^{b}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	0.053^{c}	0.034^{c}	0.036^{c}	0.055^{c}	0.085^{c}	0.039^{c}
SE ±	0.01	0.01	0.01	0.02	0.02	0.01
Allophycocyanin content (mg/L) per day						
Sea Water (Control)	0.06^{e}	0.12^{e}	0.12^{e}	0.10^{e}	0.18^{e}	0.06^{e}
Provasoli´s Enriched Sea Water (PESW)	0.12^{d}	0.29^{d}	0.21^{d}	0.20^{d}	0.22^{d}	0.19^{d}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	0.79^{a}	0.54^{a}	1.03^{a}	1.19^{a}	0.54^{a}	0.43^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	0.59^{b}	0.35^{b}	0.75^{b}	0.88^{b}	0.40^{b}	0.34^{b}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	0.44^{c}	0.31^{c}	0.48^{c}	0.51^{c}	0.33^{c}	0.30^{c}
SE ±	0.12	0.06	0.15	0.18	0.06	0.06
Phycobiliproteins content (mg/L) per day						
Sea Water (Control)	0.07^{e}	0.14^{e}	0.07^{e}	0.13^{e}	0.12^{e}	0.20^{e}
Provasoli´s Enriched Sea Water (PESW)	0.13^{d}	0.32^{d}	0.21^{d}	0.24^{d}	0.22^{d}	0.25^{d}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	0.89^{a}	0.60^{a}	0.48^{a}	1.15^{a}	1.31^{a}	0.61^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	0.66^{b}	0.39^{b}	0.38^{b}	0.83^{b}	0.97^{b}	0.45^{b}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	0.49^{c}	0.34^{c}	0.34^{c}	0.54^{c}	0.56^{c}	0.37^{a}
SE ±	0.14	0.07	0.06	0.17	0.20	0.07

Mean values in columns within the same small superscript letters are non-significantly different ($P \leq 0.05$), SE+: standard error.

The effects on chlorophyll a, b and total chlorophyll contents (mg/L) of *Spirulina platensis*, was presented in the Table 12. Regardless different growth media, our results showed that chlorophyll a, b and total chlorophyll contents (mg/L) were increased as cultivation days extended, comparatively to the startup (zero time) day. In the present study, the inoculation of *Spirulina platensis* in the modified media of [PESW: MZU-50:50 (v/v)], correspondingly yielded the highest significant chlorophyll a, b and total chlorophyll contents, followed by [PESW: MZU-70:30 (v/v)]

and then by [PESW: MZU-30:70 (v/v)], compared to the sea water (control) or the other different growth media. In our study, the optimum growth media composition for viable mass cultivation and instantly attained the highest chlorophyll a (by 46.51% or 53.71%), chlorophyll b (by 44.75% or 52.32%) and total chlorophyll (by 46.15% or 53.47%) were obtained at 12 or 16 days of cultivation, orderly, is the modified media of [PESW: MZU-50:50 (v/v)]. Various researchers have reported that the nutritional content of growth media is a key factor influencing growth, and chlorophyll content (Xie *et al.*, 2017; Piazzi *et al.*, 2019; El-Sheekh *et al.*, 2021). The obtained data showed that in the modified media of [PESW: MZU-50:50 (v/v)] medium was the optimum for *Spirulina platensis* with the highest chlorophyll content. Nitrogen source and its concentration had a great effect on the growth of *Spirulina* (Soletto *et al.*, 2005; Çelekli and Yavuzatmaca, 2009). Our results suggest that modified Zarrouk's medium was the most popular medium used for *Spirulina* cultivation (El-Monem *et al.*, 2021); which supplemented with urea, it gave the best chlorophyll a, b and total chlorophyll contents than that was achieved from the others. Accordingly, the concentration of chlorophyll in *S. platensis* biomass increases with an increase of nitrogen concentration in the cultivation medium (Piorreck *et al.*, 1984). The conventional *S. platensis* nitrogen sources are nitrates. However, Stanca and Popovici (1996) demonstrated that the utilization of urea as a nitrogen source in *S. platensis* cultivation leads to an increase in both the total biomass and the biomass chlorophyll content. Urea is easily assimilated by *S. platensis*, probably due to its spontaneous hydrolysis to ammonia under alkaline cultivation (Danesi *et al.*, 2002) and/or urease action (Carvajal *et al.*, 1980). According to Boussiba (1989), when both ammonia and nitrate are present in the medium, ammonia is assimilated preferentially. However, ammonia can be toxic to *S. platensis*, when present at high concentrations.

Table 12. Effect of different alternative growth media on photosynthetic pigments content (mg/L) of *Spirulina platensis* during twenty cultivation days.

Alternative media	Zero	04	08	12	16	20
	Chlorophyll a (mg/L) per day					
Sea Water (Control)	0.75^{d}	1.43^{d}	1.01^{d}	1.37^{d}	1.17^{e}	2.18^{c}
Provasoli´s Enriched Sea Water (PESW)	2.66^{c}	2.88^{c}	2.12^{c}	1.82^{d}	2.64^{d}	2.51^{c}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	5.60^{a}	5.71^{a}	4.88^{a}	10.47^{a}	12.10^{a}	7.77^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	5.36^{a}	3.96^{b}	3.80^{b}	8.13^{b}	9.17^{b}	7.71^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	3.27b	3.40^{b}	4.50^{ab}	4.75^{c}	5.38^{c}	4.35^{b}
SE ±	0.80	0.63	0.66	1.58	1.81	1.09
	Chlorophyll b (mg/L) per day					
Sea Water (Control)	2.52^{d}	1.72^{e}	3.86^{e}	2.40^{e}	2.04^{e}	1.49^{d}
Provasoli´s Enriched Sea Water (PESW)	5.96^{c}	3.80^{d}	4.44^{d}	3.36^{d}	4.72^{d}	2.36^{c}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	10.58^{a}	8.49^{a}	13.00^{a}	19.15^{a}	22.19^{a}	10.35^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	7.09^{b}	6.55^{b}	12.27^{b}	14.76^{b}	16.66^{b}	9.87^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	5.58^{c}	5.82^{c}	7.68^{c}	8.70^{c}	9.85^{c}	8.36^{b}
SE ±	1.16	1.04	1.71	2.89	3.34	1.70
	Total chlorophyll (mg/L) per day					
Sea Water (Control)	2.38^{d}	2.73^{d}	6.04^{d}	3.77^{e}	3.22^{e}	3.96^{e}
Provasoli´s Enriched Sea Water (PESW)	2.67^{d}	5.92^{c}	6.94^{c}	5.18^{d}	7.36^{d}	8.15^{d}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 50:50	13.37^{a}	15.95^{a}	20.71^{a}	29.62^{a}	34.28^{a}	16.29^{a}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 70:30	10.35^{b}	15.24^{a}	20.04^{a}	22.88^{b}	25.83^{b}	11.05^{b}
PESW: Modified Zarrouk with Urea (0.15 gm/L) 30:70	9.21^{c}	12.03^{b}	12.86^{b}	13.46^{c}	15.23^{c}	9.23^{c}
SE ±	1.95	2.33	3.11	4.48	5.15	1.80

Mean values in columns within the same small superscript letters are non-significantly different ($P \leq 0.05$), SE+: standard error.

It should be summarizing that, our results proved that, the best cell growth rate, as well as phycocyanin, allophycocyanin, phycobiliprotein contents (mg/L), and chlorophyll a, b and total chlorophyll contents (mg/L) of *Spirulina platensis* were obtained when the urea (0.15 mg/L) was added to the modified zarrouk plus provasoli´s enriched sea water (PESW) by 50:50 (v/v) during all days of cultivation. Similar results are match to our study (Henrikson, 1989; Danesi *et al.*, 2002; Soni *et al.*, 2019). Thus modified media [PESW: MZU-50:50 (v/v)] can be considered as an alternative economically superior source of nitrogen and can be substituted as a conventional nitrogen source.

CONCLUSION

This study demonstrates that *Spirulina platensis* cultivation can be significantly optimized through cost-effective and sustainable media modifications. The modified Zarrouk medium supplemented with 0.15 g/L urea (MZU) combined with Provasoli's enriched seawater (PESW) in a 50:50 ratio achieved the highest biomass yield (68.59% increase) and pigment production (32.06% phycobiliproteins), outperforming traditional nitrate-based media. Urea proved to be an efficient nitrogen alternative, reducing production costs while maintaining high growth rates, while partial seawater substitution (50%) offered an eco-friendly strategy for freshwater conservation. These findings provide a practical framework for scalable, low-cost *S. platensis* production, particularly in regions facing freshwater scarcity. Future research should focus on large-scale validation of these media formulations and explore integrated biorefinery approaches to maximize the economic and environmental benefits of *Spirulina* biotechnology.

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تحسين إنتاج الكتلة الحيوية لطحلب الأسبيرولينا بلاتنسيس بإستخدام مزيج من اليوريا ومياه البحر

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الملخص

تعرف الطحالب الخضراء المزرقة سبيرولينا بلاتينسيس على نطاق واسع بأنها "غذاء المستقبل" نظرًا لتكوينها الغني بالمركبات النشطة بيولوجيًا، بما في ذلك الفيكوبيليبنات والكاروتينات والأحماض الدهنية غير المشبعة والبروتينات، مما يُسهم في قيمتها الطبية والغذائية. هدفت هذه الدراسة إلى تحسين إنتاج الكتلة الحيوية من خلال تقييم تأثير إضافة اليوريا ودمج مياه البحر في وسط النمو. وُجد أن بيئة زروق المُعدّلة بتركيز 0.15 ملغم/لتر من اليوريا (MZU) يُعزز نمو سبيرولينا بلاتينسيس بشكل أكثر فعالية من بيئة زروق القياسية. علاوة على ذلك، أدى دمج اليوريا مع خليط زروق-بروفاسولي المُعدّل من مياه البحر (50:50) (PESW حجم/حجم) إلى أعلى إنتاجية للكتلة الحيوية وتراكم للصبغات، مما يُظهر إمكانات مياه البحر كبديل جزئي لبيئة الزراعة التقليدية القائمة على المياه العذبة. أثبت إستخدام اليوريا كمصدر للنيتروجين أنه إستراتيجية فعّالة من حيث التكلفة لتعزيز إنتاج الكتلة الحيوية مع الحفاظ على مستويات عالية من المركبات الحيوية النشطة القيّمة. تُقدّم هذه النتائج رؤى قيّمة لتنمية طحالب سبيرولينا بلاتينسيس على نطاق واسع، لا سيما في المناطق ذات الوصول المحدود إلى المياه العذبة. تُبين الدراسة إمكانات مزيج اليوريا ومياه البحر في تحسين إنتاجية طحلب سبيرولينا بلاتينسيس، ودعم تطبيقها في صناعات إنتاج الغذاء المستدام، والمُغذّيات الدوائية، والتكنولوجيا الحيوية.

الكلمات الداله: إنتاج الصبغة الحيوية، معدل النمو، سبيرولينا، وسط زروق.