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Response surface methodology as a statistical model for nutrient optimization to enhance biomass and bioactive phycobiliproteins production in cyanobacterium *Nostoc* sp. SW02

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Abstract

Phycobiliproteins (PBPs) are water-soluble pigments involved in the photosynthesis of cyanobacteria. They are valuable natural colorants for biotechnological applications due to their significant biological properties. This research was the first to study the impact of $NaNO_3$, Na_2CO_3, and K_2HPO_4 nutrient variations on the production of biomass and PBPs in the cyanobacterium *Nostoc* sp. SW02. The experiments utilized the response surface methodology with the Box-Behnken design for nutrient optimization. The quadratic equations showed that the models sufficiently aligned with the experimental data and could explain the combined influence of factors with statistical significance. The addition of $NaNO_3$, Na_2CO_3, and K_2HPO_4 at concentrations of 2.0, 0.02, and 0.035 g L^{-1}, respectively, provided the maximum biomass and PBPs production at 0.967 g L^{-1} and 13.89% yield, representing increases of 30.67% and 8.86%, compared to normal culture medium. The validity of the predicted model was confirmed, indicating its potential application in both small and large-scale cultivations. This study developed a model that could be applied for the commercial production of natural cyanobacterial colorants for their potential use in food and cosmetics industries.

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1. Introduction

Cyanobacteria can produce natural bioactive substances including several metabolites that exhibit health-enhancing biological action. Phycobiliproteins (PBPs) are bioactive pigments, a category of colored proteins that participate in the light-harvesting processes of cyanobacteria. PBPs play a crucial role in photosynthesis; they capture light energy and transfer it to chlorophyll, the primary pigments that convert light into chemical energy located on cyanobacterial thylakoid membranes [1]. Cyanobacterial-derived PBPs are water-soluble pigment molecules consisting of proteins and chromophores called phycobilins, which can exhibit intense fluorescence [2]. They are categorized into three major types as phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) according to their phycobilin composition and light absorption properties. Each of these categories has a specific spectrum referred to as deep red (490 - 570 nm), deep blue (610 - 625 nm), and greenish blue (650 - 660 nm), respectively [3 - 5]. PBPs are valuable

natural products that have attracted interest for potential applications across various industries including dietary supplements, cosmetics, and pharmaceuticals because of their brilliant bright colors, fluorescence, and biological antioxidant, anti-inflammatory, and anticancer properties [3, 6 - 10].

Natural colorants and bioactive ingredients are becoming increasingly popular, with a substantial expansion in the global PBPs market. However, the PBPs production differs among cyanobacterial strains and the varieties of PBPs compositions present in cyanobacteria depend on their habitat and the available light conditions. Consequently, efficient large-scale production of biomass and PBPs from cyanobacteria is strongly regulated by species, light quality, and culture conditions [3, 8]. One of the main factors is the optimization of media composition. The primary nutrients required to produce cyanobacterial biomass are carbon and nitrogen [11]. Nitrogen is a critical factor for cyanobacterial cell viability as it regulates the synthesis of PBPs, which serves as the primary nitrogen storage within the cell [12]. Other nutrients such as phosphorus, calcium chloride, and trace elements are often present in low concentrations. These are necessary for maintaining cyanobacterial growth and impact the accumulation of PBPs [13]. Thus, adjusting nutrient concentrations is a key consideration to induce a significant buildup of PBPs.

PBPs are found in most cyanobacteria but their accumulation varies depending on the selected strain. This study enhanced biomass and PBPs production in the cyanobacterium *Nostoc* sp. SW02 through media optimization. *Nostoc* sp. SW02 is a filamentous cyanobacterium that was first isolated from karst caves in Thailand. This strain showed potential in scaling up production of PBPs since it can grow under low light conditions and produce PBP pigments at high yields of up to 12.76% w/w, which is higher than the reported commercial PBP-producing cyanobacterium *Arthrospira platensis* [5]. Moreover, The PBPs produced from *Nostoc* sp. SW02 mainly contain PE and show outstanding antioxidants, antibacterial, and anticancer properties as a bioactive colorant agent [14]. Nevertheless, the low biomass content presents considerable challenges to this strain's commercial-scale manufacturing of PBPs.

Response surface methodology (RSM) is a powerful statistical technique for optimizing processes or products by investigating the combined effects of two or more experimental factors. RSM combines experimental design, statistical analysis, and optimization techniques and requires fewer experiments than conventional methods [15]. This study applied RSM with a Box-Behnken design (BBD) to optimize the concentrations of three crucial nutrient factors, sodium nitrate ($NaNO_3$), sodium carbonate (Na_2CO_3), and dipotassium phosphate (K_2HPO_4) for the efficient large-scale cultivation of the cyanobacterium *Nostoc* sp. SW02. Furthermore, the impact of these nutrients on biomass and PBPs production was also investigated using a response surface and quadratic model. The results demonstrated that adjusting the concentration of critical nutrients can increase the production of biomass and PBPs in cyanobacterium *Nostoc* sp. SW02.

2. Materials and Methods

Cyanobacterial strain and growth conditions

The *Nostoc* sp. SW02 strain used in this research was initially isolated from a karst cave in Eastern Thailand and described as an effective producer of bioactive PBPs [5]. The starter culture was prepared by inoculating cyanobacterial cells in a 2 L Schott Duran glass bottle containing 1.8 L of BG11 medium. The growth condition was adjusted according to the method of Suphan et al. [14]. The culture was maintained at 27 ± 2 °C under continuous aeration with filter-sterilized air and 10 μmol m^{-2} s^{-1} constant illumination from cool white-fluorescent lamps until the cells reached the log phase (OD_{730} ~ 0.4). Cells were then harvested for the experiment.

Experimental design and response surface methodology

Response surface methodology (RSM) with a 3 factorial (3^3) Box-Behnken design (BBD) was used to optimize the concentration of the three nutrients factors (X_1; sodium nitrate

($NaNO_3$), X_2; sodium carbonate (Na_2CO_3), and X_3; dipotassium phosphate (K_2HPO_4)) for biomass and PBPs production by *Nostoc* sp. SW02. The three independent variables were established at three levels (-1, 0, 1), as indicated in Table 1. The BBD generated 17 experimental runs to optimize the nutrient variables, as shown in Table 2. Each experiment was performed in a 2 L Schott Duran glass bottle containing 1.8 L of culture medium (small-scale cultivation), with the initial cell concentration adjusted to an OD_{730} of 0.02. The cells were collected after cultivation under growth condition for 10 days to avoid cell degradation and the release of toxins upon cell lysis under nutrient changes. The biomass production and total PBPs yield (%) were measured as the response values, Y_1 and Y_2, respectively. The experimental data (Table 2) were used to develop an appropriate model with statistical significance using Design Expert 13.0 trial version software (STAT-EASE Inc., Minneapolis, USA). The response values were fitted to a quadratic model as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n-1} \sum_{j>1}^{n} \beta_{ij} x_i x_j + \sum_{i=1}^{n} \beta_{ii} x_i^2 \quad (1)$$

where Y represents the predicted response, x_i and x_j are independent factors, and β_0, β_i, β_{ii}, and β_{ij} signify the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively [16 - 17]. Three-dimensional response (3D) surface plots illustrated the response optimization on the z-axis, with the x- and y-axes representing the two independent variables while keeping the other factors constant at their center point. Analysis of variance (ANOVA) was applied to determine the statistical significance of the model equation and its term. The model's accuracy for the quadratic regression equation was evaluated using the coefficient of determination (R^2). The optimal level of each factor was achieved at the highest possible value in both responses. The control condition was regular BG11 medium with $NaNO_3$, Na_2CO_3, and K_2HPO_4 concentrations of 1.5, 0.02, and 0.04 g L^{-1}, respectively. The three specific nutrients were chosen for investigation due to their critical role in supporting cyanobacterial growth, which relies on carbon and nitrogen supplies, including the regulation of the N:P ratio [11 - 12].

Cyanobacterial biomass determination

After 10 days of cultivation, a 1.2 µm glass microfiber filter was used to filter a 50 mL aliquot of cell culture from the experimental runs. The cell culture was then dried in an oven at 60 °C for 4 h until it reached a consistent weight, and the dry biomass weight was determined.

Phycobiliproteins extraction and determination

An aliquot of cell culture (50 mL) was centrifuged at 2,790×g for 10 min, and the supernatant was discarded. The cell pellet was resuspended in 50 mL of 0.01 M phosphate buffer (pH 7.0) before PBPs extraction using the freeze-thawing method [18]. The freeze-thaw cycle was performed five times by freezing the sample solution at −20 °C for 12 h and thawing at room temperature (27 ± 2 °C), maintained at 4 °C for 12 h, and then centrifuged for 10 min (2,790×g, 4 °C) to obtain the PBPs crude extract. The yield (%) of total PBPs was determined after the concentration of PBPs was examined spectrophotometrically and calculated using the following equations as described by Suphan et al. [14].

$$[PC]\ (mgmL^{-1}) = \frac{(A_{615} - (0.474 \times A_{652}))}{5.34} \quad (2)$$

$$[APC]\ (mg\ mL^{-1}) = \frac{(A_{652} - (0.208 \times A_{615}))}{5.09} \quad (3)$$

$$[PE]\ (mg\ mL^{-1}) = \frac{(A_{562} - (2.41 \times [PC]) - (0.849 \times [APC]))}{9.26} \quad (4)$$

$$[PBPs]\ (mg\ mL^{-1}) = [PC] + [APC] + [PE] \quad (5)$$

$$\%Yield = \frac{[PBPs] \times V}{DW} \times 100 \quad (6)$$

where A_{615}, A_{652}, and A_{562} are the maximum absorbance wavelengths of PC, APC, and PE, respectively, V is the volume of phosphate buffer in mL, and DW is cell dry weight in mg.

Validation of the model under optimum conditions

The predicted model was validated at the optimum concentrations of the three nutrient factors under small and large-scale cultivations. The optimum medium (10 L) was placed in a flat plate photobioreactor for large-scale cultivation. According to Krasaesueb et al. [19], a 0.22 μm cellulose acetate membrane was used to filter the ambient air supplied from the bottom of the reactor and continually purged into the photobioreactor. The experiment was conducted for 10 days by maintaining similar proportions of starting cyanobacterial cells in small-scale cultivation. All experiments were performed as three independent samples (n = 3) and expressed as mean values with standard deviation.

3. Results and Discussion

RSM analysis and model fitting

A Box-Behnken design (BBD) with RSM was applied for the experiment because it is well-suited for investigating interactions between parameters, especially in a three-factor, three-level setting.

Table 1 Range of nutrient factors at three levels used in experimental design.

Factors	Unit	Symbol codes	Coded levels and concentrations		
			-1	0	+1
Sodium nitrate ($NaNO_3$)	g L^{-1}	X_1	1.0	1.5	2.0
Sodium carbonate (Na_2CO_3)	g L^{-1}	X_2	0.01	0.02	0.03
Dipotassium phosphate (K_2HPO_4)	g L^{-1}	X_3	0.03	0.04	0.05

Table 2 Box-Behnken design (BBD) with experimental values of biomass and PBPs production in *Nostoc* sp. SW02.

Runs	Factors			The experimental values	
	X_1 $NaNO_3$ (g L^{-1})	X_2 Na_2CO_3 (g/ L^{-1})	X_3 K_2HPO_4 (g/ L^{-1})	Response: Y_1 Biomass production (g L^{-1})	Response: Y_2 Total PBPs (%yield)
1	0	0	0	0.745 ± 0.012	12.91 ± 0.087
2	0	-1	-1	0.377 ± 0.030	11.60 ± 0.150
3	+1	-1	0	0.426 ± 0.025	10.55 ± 0.120
4	0	0	0	0.815 ± 0.077	12.34 ± 0.215
5	+1	0	+1	0.893 ± 0.013	9.18 ± 0.073
6	0	+1	+1	0.985 ± 0.013	8.04 ± 0.082
7	-1	0	-1	0.877 ± 0.043	7.96 ± 0.197
8	0	+1	-1	1.058 ± 0.021	12.67 ± 0.145
9	0	-1	+1	0.457 ± 0.050	7.38 ± 0.071
10	0	0	0	0.723 ± 0.010	12.34 ± 0.211
11	+1	+1	0	1.069 ± 0.045	12.89 ± 0.334
12	-1	+1	0	1.101 ± 0.063	7.06 ± 0.081
13	+1	0	-1	0.931 ± 0.014	14.47 ± 0.521
14	-1	0	+1	0.868 ± 0.057	10.97 ± 0.324
15	0	0	0	0.725 ± 0.009	12.78 ± 0.101
16	0	0	0	0.710 ± 0.011	13.46 ± 0.509
17	-1	-1	0	0.391 ± 0.006	8.36 ± 0.098

Table 3 The fitted model equations showing coefficients in terms of coded factors.

Responses	Fitted model equations
Biomass production ($g\ L^{-1}$)	$Y_1 = 1.02 - 0.91X_1 + 86.12X_2 - 39.21X_3 - 3.33X_1X_2 - 1.47X_1X_3 - 378.55X_2X_3 + 0.350X_1^2 - 848.91X_2^2 + 605.96X_3^2$
Total PBPs (%yield)	$Y_2 = - 40.73 + 30.12X_1 + 557.33X_2 + 1269.27X_3 + 182X_1X_2 - 415X_1X_3 - 1025X_2X_3 - 4.66X_1^2 - 18867.50X_2^2 - 9567.50X_3^2$

Table 4 Summary of analysis of variance (ANOVA) and statistics of fitted models.

Factors	Responses fitted to the quadratic model			
	Biomass production ($g\ L^{-1}$)		Total PBPs (%yield)	
	***F*-value**	***p*-value**	***F*-value**	***p*-value**
Model	66.21	$<0.0001^{sig}$	8.55	0.0049^{sig}
X_1	9.98	0.0160^{sig}	15.11	0.0060^{sig}
X_2	48.06	0.0002^{sig}	2.78	0.1393
X_3	5.46	0.0521	7.90	0.0261^{sig}
X_1X_2	0.7326	0.4204	3.02	0.1259
X_1X_3	0.1423	0.7172	15.69	0.0055^{sig}
X_2X_3	3.78	0.0930	0.0383	0.8504
X_1^2	21.50	0.0024^{sig}	5.20	0.0566
X_2^2	20.00	0.0029^{sig}	13.66	0.0077^{sig}
X_2^3	10.19	0.0152^{sig}	3.51	0.1031
Lack of Fit	0.7010	0.5989^{ns}	10.50	0.0229^{sig}
R^2	0.9884		0.9166	
Adjusted R^2	0.9735		0.8093	
Predicted R^2	0.9241		-0.1989	
Adequate precision	23.97		10.14	
SD	0.0389		0.1047	
C.V.%	5.03		9.62	

^{sig} means the *p*-value is less than 0.05, indicating the model term is significant at 95%.
SD means standard deviation, C.V. means coefficient of variation.

Seventeen runs of three factors and three levels of BBD were designed by varying the nutrient concentrations of $NaNO_3$ (1.0 - 2.0 g/ L^{-1}), Na_2CO_3 (0.01 - 0.03 g L^{-1}), and K_2HPO_4 (0.03 - 0.05 g L^{-1}). Multiple regression analysis was used to build the statistical model, utilizing the experimental values of the two response variables as biomass production (Y_1) and total PBP yield (%) (Y_2), as shown in Table 2. Results suggested that the second-order fitted model derived by a quadratic equation adequately correlated with the experimental values. The fitted quadratic equations for the correlations between the responses Y_1 and Y_2 and the independent variables $NaNO_3$ (X_1), Na_2CO_3 (X_2), and K_2HPO_4 (X_3) concentrations are shown in Table 3. Analysis of variance (ANOVA) was used to evaluate the statistical significance of the model. The *p*-value for the model terms was < 0.05, indicating that the quadratic model for the two response variables (biomass and total PBPs) was significant at 95% (Table 4).

The empirical models demonstrated a high correlation coefficient (R^2), precisely R^2 = 0.9884 for biomass and R^2 = 0.9166 for total PBPs, indicating that the models accurately depicted the relationship between the experimental values and the predicted theoretical values [20]. In addition, the adjusted R^2 value in the model of biomass was less than 0.2 from the predicted R^2 value, indicating that the model was in reasonable agreement with high accuracy [21]. A negative predicted R^2 in the model of total PBPs suggested that it served as a better predictor of response than the existing model. All the models exhibited adequate precision values exceeding 4.0, signifying the model has a strong signal compared to data noise and were suitable for optimization [22]. A lower coefficient of variation (C.V.%) indicated less variability in the correlation between the predicted and actual values, as shown in Fig. 1A-B. The statistical analyses revealed that the proposed quadratic equations were highly effective in representing the expected responses of the parameter variables.

Influence of nutrient variation on biomass production

The analysis of variance presented in Table 4 indicated that each nutrient factor had a statistically significant influence. The quadratic model revealed that $NaNO_3$ (X_1) and Na_2CO_3 (X_2) significantly influenced biomass production of the cyanobacterium *Nostoc* sp. SW02 (*p*-value < 0.05). The analysis of the correlation between the two factors, illustrated in the 3D response surface graph (Fig. 2A-B), revealed that the factors mutually affected each other. Like other photosynthetic organisms, cyanobacteria require a carbon source to support their growth.

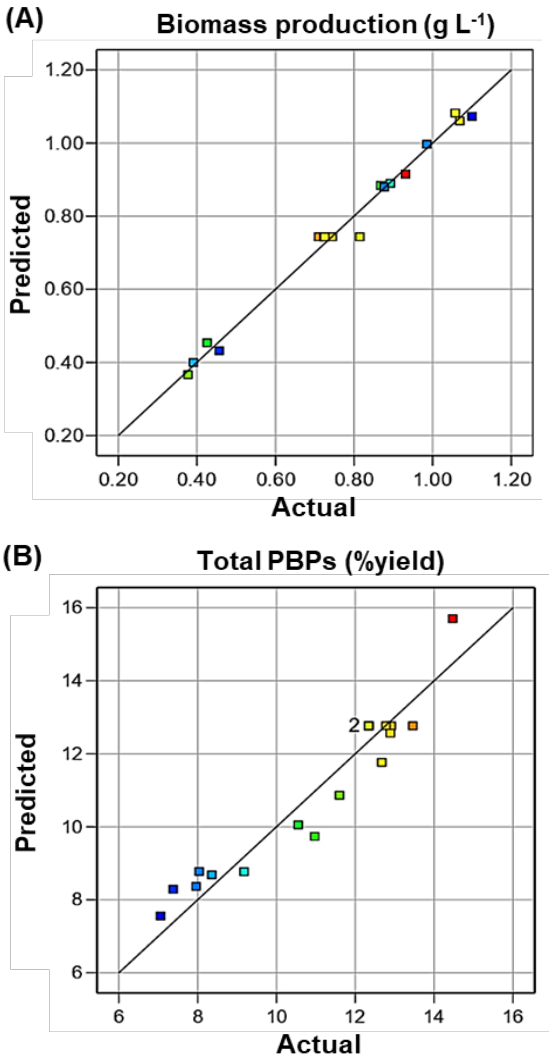

Fig. 1 Parity graphs demonstrate the distribution of actual and predicted values of biomass (A) and total PBPs (B) production in *Nostoc* sp. SW02.

Sodium carbonate provides a source of inorganic carbon, particularly in the form of bicarbonate ions (HCO_3^-), which cyanobacterial cells prefer to utilize the increased bicarbonate ions in the photosynthetic process as an energy source for cellular division and proliferation [23]. Nitrogen is the second most important nutrient for biomass and metabolite production [3]. Previous research demonstrated that cyanobacteria could assimilate many nitrogen sources including ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), urea, and amino acids, with several species also possessing the ability to fix atmospheric N_2 [24]. The combined influence of the two factors (Fig. 2A) showed that at Na_2CO_3 concentrations exceeding 0.025 g L^{-1}, $NaNO_3$ concentration did not influence biomass production by *Nostoc* sp. SW02.

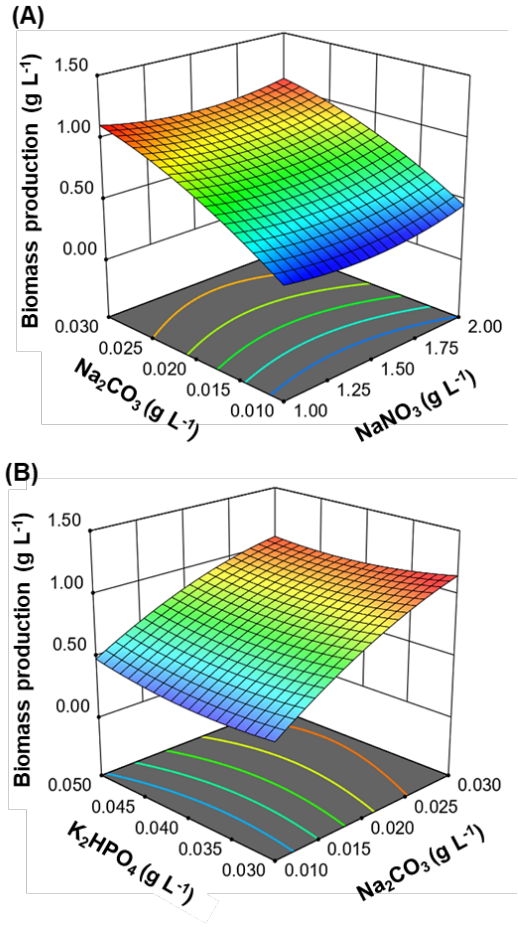

Fig 2. 3D response surface diagram showing the impact of factor variation on biomass production in *Nostoc* sp. SW02 when (A) K_2HPO_4 and (B) $NaNO_3$ were fixed.

This phenomenon was attributed to the production of heterocyst cells that by the cyanobacterium that facilitated nitrogen fixation under nitrogen-deficient conditions. In Fig. 2B, the 3D response surface plot shows the combined effects of Na_2CO_3 and K_2HPO_4 on biomass production of *Nostoc* sp. SW02 when the $NaNO_3$ concentration was fixed. Results also suggested increased cell biomass when Na_2CO_3 exceeded 0.025 g L^{-1}, with no influence shown by K_2HPO_4. Phosphorus involved in the metabolism of nucleic acid synthesis, high-energy substances such as ATP, and the process of producing phospholipids in cells [25]. However, previous studies found no effects on microalgal growth when subjected to phosphorus starvation [26 - 27]. Singh & Kumar [28] discovered that reduced phosphorus levels increased biomass productivity in the cyanobacterium *Leptolyngbya foveolarum*. Phosphorus is strain-specific and higher phosphorus concentrations result in irregular N:P ratios under phosphorus assimilation, significantly impacting biomass yields [29]. Therefore, the relationship between the three nutrient factors indicated that carbon sources had the greatest influence on promoting the biomass of cyanobacterial strain *Nostoc* sp. SW02. Changing the amounts of nitrogen and phosphorus in the normal culture medium did not affect the increase in biomass when a high carbon source was provided. *Nostoc* sp. SW02 produced high biomass in the range ranging from 0.985 to 1.101 g L^{-1} when the cells were cultured by adding 0.03 g L^{-1} Na_2CO_3, as shown in Table 2.

Influence of nutrient variation on phycobiliproteins production

The quadratic model indicated that $NaNO_3$ (X_1) and K_2HPO_4 (X_3) mutually influence the total PBPs yield of *Nostoc* sp. SW02 (p-value < 0.05), as shown in Table 4. The 3D response surface plots showed the interactive effects of nutrient variation on the total PBPs yield of *Nostoc* sp. SW02 is illustrated in Fig 3A-B. The response surface exhibited a curved slope, indicating the presence of an optimal region surrounded by lower PBPs yield. These findings implied that increasing the $NaNO_3$ concentration led to greater PBPs production because nitrogen

promotes protein synthesis in living cells [12, 30]. Nitrogen is crucial for cyanobacterial cell viability as it regulates the synthesis of PBPs, which are the primary nitrogen storage compounds within the cells [31]. The marine cyanobacterium *Oscillatoria willei* produced lower PBPs during nitrogen limitation because of the loss of one major Rubisco isoenzyme [32]. Under nitrogen restriction, *Synechococcus* strain PCC 7942 showed reduced PBPs production and the breakdown of phycobilisomes (PBS) [33]. The cyanobacterial cells selectively degrade their stored PBPs when nitrogen levels are reduced, while the optimal nitrogen source to produce PBPs varies depending on the cyanobacterial species. Many studies on optimizing PBPs production utilizing different nitrogen sources in various cyanobacteria, including nitrogen-fixing strains, found that high concentrations of $NaNO_3$ in a normal BG11 medium promoted PBP production [3]. By contrast, enhanced concentrations of K_2HPO_4 and Na_2CO_3 did not impact PBPs production by *Nostoc* sp. SW02. The sodium carbonate (Na_2CO_3) does not directly induce the accumulation of PBPs in microalgae and cyanobacterial cells because it can enhance growth and photosynthetic activity by providing an inorganic carbon source, leading to increased production of chlorophyll *a* [34]. Phosphorus produces high-energy metabolites in the form of ATP, essential for the metabolic pathways of cyanobacteria [35]. Several cyanobacteria had high amounts of PBPs at the concentration of K_2HPO_4 used in the BG11 medium (0.04 g L^{-1}) [36]. The experimental results indicated that enhancement of biomass and PBPs yield of the cyanobacterium *Nostoc* sp. SW02 required the regulation of carbon and phosphorus sources supplied to the cells. The optimal conditions could help *Nostoc* sp. SW02 achieved a maximum yield of PBPs at 14.47% with $NaNO_3$ at a concentration of 2.0 g L^{-1}, Na_2CO_3 at 0.02 g L^{-1}, and K_2HPO_4 at 0.03 g L^{-1}, respectively (Table 2).

Validation of the model under nutrient optimization

The accuracy of the statistical model and the quadratic equations were validated by experiments, giving high levels of biomass and PBPs yield by the cyanobacterium *Nostoc* sp. SW02. Nutrient optimization was predicted through the fitted quadratic equations in Table 3 with varying concentrations of $NaNO_3$, Na_2CO_3, and K_2HPO_4 as 2.0, 0.02, and 0.035 g L^{-1}, respectively.

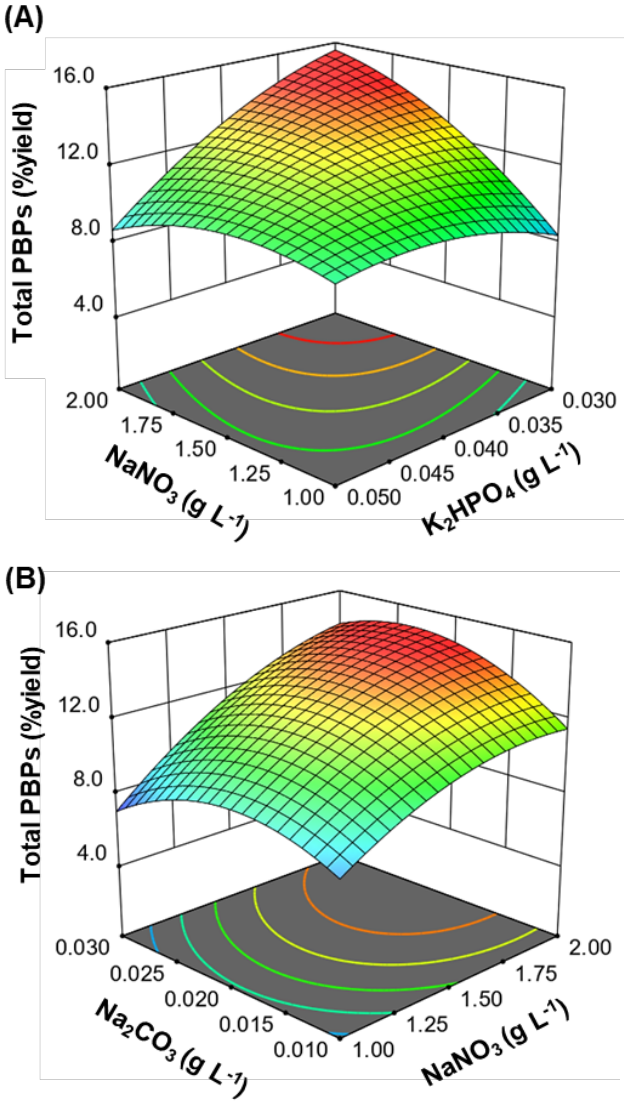

Fig 3. 3D response surface diagram showing the impact of factor variation on total PBPs yield (%) in *Nostoc* sp. SW02 when (A) Na_2CO_3 and (B) K_2HPO_4 were fixed.

The point prediction exhibited a simultaneous increase in biomass and PBPs yield values. Moreover, *Nostoc* sp. SW02 was cultured under nutrient optimization by two experiments such as small-scale cultivation and large-scale

photobioreactor cultivation. The predicted and observed experimental values of the two responses are shown in Table 5. Results confirmed there were no significant differences between the observed values for the small and large-scale production (Table 5). The observed values were close to the predicted values, suggesting that the statistical model of RSM applied to nutrient optimization enhanced growth and production of PBPs in the cyanobacterium *Nostoc* sp. SW02. Therefore, nutrient optimization provided the maximum biomass and PBP production at 0.967 g L^{-1} and 13.89% yield, representing increases of 30.67% and 8.86%, respectively compared to normal BG11 culture conditions. Many studies have concentrated on enhancing the overproduction of a specific metabolite and PBP pigments by one variable at a time, which is a time-consuming process [13]. These findings indicate that the RSM is a robust methodology for identifying critical nutrients for biomass overproduction in *Nostoc* sp. SW02 and can be applied to PBP industrial-scale production.

Table 5 Model validation under nutrient optimization for biomass and PBPs production in *Nostoc* sp. SW02.

Responses under nutrient optimization	**Predicted value**	**Observed values**	
		Small-scale cultivation	**Large-scale Photobioreactor**
Biomass production (g L^{-1})	0.851	0.967 ± 0.077	0.831 ± 0.068^{ns}
Total PBPs (%yield)	13.98	13.89 ± 1.858	13.17 ± 0.548^{ns}

Mean ± S.D. (n = 3). "ns" indicates not significant ($p > 0.05$) in the observed values present between small and large-scale production.

Table 6 Comparison of biomass and PBPs production in different cyanobacteria under nutrient optimization.

Cyanobacterial strain	**$NaNO_3$ (g L^{-1})**	**Na_2CO_3 (g L^{-1})**	**K_2HPO_4 (g L^{-1})**	**Biomass production (g L^{-1})**	**PBPs production**	**Reference**
Nostoc sp. SW02	2.0	0.02	0.035	0.967	13.89% (w/w)	This study
Scytolyngbya sp. LKK05	1.5	0.02	0.04	0.71	13.42% (w/w)	[5]
Nostoc sp.	1.5	0.008	0.04	0.625	13% (w/w)	[37]
Euhalothece sp.	1.67	0.02	0.04	-	4.5% (w/w)	[38]
Synechocystis sp. PCC 7120	1.5	0.02	0.04	-	6.5% (w/w)	[39]
Phormidium ceylanicum	4.5	0.02	0.04	0.97	0.73 g L^{-1}	[13]
Arthrospira platensis	2.25	16.8 g L^{-1} of $NaHCO_3$	0.05	1.0	12.47% (w/w)	[23]

4. Conclusion

Cyanobacterial phycobiliproteins (PBPs) serve as an essential natural colorant in the food and pharmaceutical industries. Statistical modeling of using response surface methodology for nutrient optimization was first reported to promote the production of biomass and PBPs in *Nostoc* sp. SW02. The validity of the predicted model was confirmed, indicating its potential application in both small- and large-scale

cultivations. Under nutrient optimization, the results could increase the productions up to 30.67% and 8.86% for biomass and PBPs compared to normal BG11 medium. Table 6 showed comparison of biomass and PBPs yield of other reported cyanobacteria under nutrient optimization, nitrogen concentrations influence the production of PBPs depending on the cyanobacterial species. Interestingly, the cyanobacterial *Nostoc* sp. SW02 produced higher biomass and PBPs content than the several reference strains. Therefore, these results indicate that the RSM is an effective method for estimating important nutrients needed for high biomass production in *Nostoc* sp. SW02 and can be applied in large-scale PBP production for use in the food and pharmaceutical industries. Future studies should focus on providing further insights into the possible interactions between nutrient availability and abiotic factors such as light and temperature.

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6. References

[1] Bryant, D. A., Guglielmi, G., de Marsac, N. T., Castets, A.M., & Cohen-Bazire, G. (1979). The structure of cyanobacterial phycobilisomes: a model. *Archives of Microbiology*, *123*, 113-127. https:// doi.org/10.1007/ BF00446810.

[2] Hsieh-Lo, M., Castillo, G., Ochoa-Becerra, M. A., & Mojica, L. (2019). Phycocyanin and phycoerythrin: Strategies to improve production yield and chemical stability. *Algal Research*, *42*, 101600. https://doi. org/10.1016/j.algal.2019.101600.

[3] Pagels, F., Guedes, A. C., Amaro, H. M., Kijjoa, A., & Vasconcelos, V. (2019). Phycobiliproteins from cyanobacteria: Chemistry and biotechnological applications. *Biotechnology Advances*, *37*(3), 422-443. https://doi.org/ 10.1016/j.biotechadv.2019.02. 010.

[4] Montero-Lobato, Z., Fuentes, J. L., Garbayo, I., Ascaso, C., Wierzchos, J., Vega, J. M., & Vílchez, C. (2020). Identification, biochemical composition and phycobiliproteins production of *Chroococcidiopsis* sp. from arid environment. *Process Biochemistry*, *97*, 112- 120. https://doi.org/10.1016/j. procbio.2020. 07.005.

[5] Limrujiwat, K., Supan, S., & Khetkorn, W. (2022). Cyanobacterial biodiversity from Thai karstic caves as a potential source for phycobiliprotein production. *Algal Research*, *64*, 102666. https://doi. org/10.1016/j.algal.2022.102666.

[6] Eriksen, N. T. (2008). Production of phycocyanin—a pigment with applications in biology, biotechnology, foods and medicine. *Applied microbiology and biotechnology*, *80*, 1- 14. https://doi.org/10.1007/ s00253-008-1542-y.

[7] Manirafasha, E., Ndikubwimana, T., Zeng, X., Lu, Y., & Jing, K. (2016). Phycobiliprotein: Potential microalgae derived pharmaceutical and biological reagent. *Biochemical Engineering Journal*, *109*, 282-296. https://doi.org/ 10.1016/j.bej.2016.01.025

[8] Ji, L., Qiu, S., Wang, Z., Zhao, C., Tang, B., Gao, Z., & Fan, J. (2023). Phycobiliproteins from algae: Current updates in sustainable production and applications in food and health. *Food Research International*, *167*, 112737.
https://doi.org/10.1016/j.foodres.2023.112737.

[9] Ashaolu, T. J. (2024). The powerful phycobiliproteins-phycocyanin and phycoerythrin: Pleiotropic applications and biofunctional uses. *Algal Research*, *82*, 103636. https://doi.org/10.1016/ j.algal.2024. 103636

[10] Galván-Colorado, C., Chamorro-Cevallos, G. A., Chanona-Pérez, J. J., Zepeda-Vallejo, L. G., Arredondo-Tamayo, B., González-Ussery, S. A., ... & García-Rodríguez, R. V. (2024). Phycobiliprotein from *Arthrospira maxima*: Conversion to nanoparticles by high-energy ball milling, structural characterization, and evaluation of their anti-inflammatory effect. *International Journal of Biological Macromolecules, 275*, 133679. https://doi.org/10.1016 /j.ijbiomac.2024.133679

[11] Quintero-Dallos, V., García-Martínez, J. B., Contreras-Ropero, J. E., Barajas-Solano, A. F., Barajas-Ferrerira, C., Lavecchia, R., & Zuorro, A. (2019). Vinasse as a Sustainable Medium for the Production of *Chlorella vulgaris* UTEX 1803. *Water*, *11*(8), 1526. https://doi.org/10.3390/ w11081526.

[12] Lamela, T., & Márquez-Rocha, F. J. (2000). Phycocyanin production in seawater culture of *Arthrospira maxima*. *Ciencias marinas*, *26*(4), 607-619. https://doi. org/10.7773/cm.v26i4.619.

[13] Singh, N. K., Parmar, A., & Madamwar, D. (2009). Optimization of medium components for increased production of C- phycocyanin from *Phormidium ceylanicum* and its purification by single step process. *Bioresource Technology*, *100*(4) , 1663- 1669. https://doi.org/10. 1016/j.biortech. 2008.09.021.

[14] Suphan, S. , Limrujiwat, K. , Kula, K. , Maneeruttanarungroj, C., Raksajit, W., & Khetkorn, W. (2023). Characterization and exploration of

biological properties of phycobiliproteins purified from Thai karstic cave cyanobacterium *Nostoc* sp. SW02. *Biocatalysis and Agricultural Biotechnology*, *52*, 102826. https://doi. org/10.1016/j.bcab.2023. 102826.

[15] Baş, D., & Boyacı, İ. H. (2007). Modeling and optimization I: Usability of response surface methodology. *Journal of food engineering*, *78*(3), 836- 845. https://doi. org/10.1016/j.jfoodeng. 2005.11.024.

[16] Lu, Z., He, F., Shi, Y., Lu, M., & Yu, L. (2010). Fermentative production of L (+)-lactic acid using hydrolyzed acorn starch, persimmon juice and wheat bran hydrolysate as nutrients. *Bioresource Technology*, *101*(10) , 3642- 3648. https:// doi.org/10.1016/j.biortech. 2009.12.119.

[17] Thakur, A., Panesar, P. S., & Saini, M. S. (2018). Parametric optimization of lactic acid production by immobilized Lactobacillus casei using Box-Behnken Design. *Periodica Polytechnica Chemical Engineering*, *62*(3), 274-285. https://doi.org/10.3311/ PPch.11403.

[18] Lauceri, R., Zittelli, G. C., & Torzillo, G. (2019). A simple method for rapid purification of phycobiliproteins from *Arthrospira platensis* and *Porphyridium cruentum* biomass. *Algal Research*, *44*, 101685. https://doi.org/10.1016/j.algal. 2019. 101685.

[19] Krasaesueb, N., Incharoensakdi, A., & Khetkorn, W. (2019). Utilization of shrimp wastewater for poly-β-hydroxybutyrate production by *Synechocystis* sp. PCC 6803 strain ΔSphU cultivated in photobioreactor. *Biotechnology Reports*, *23*, e00345. https://doi.org/10.1016/j.btre.2019.e00345.

[20] Khuri, A. I., & Mukhopadhyay, S. (2010). Response surface methodology. *Wiley interdisciplinary reviews: Computational statistics*, *2*(2), 128- 149. https://doi. org/10.1002/wics.73.

[21] Myers, R. H. (2002). Response surface methodology (RSM). *Process and Product optimization using designed experiments*, 489-492.

[22] Fazeli Burestan, N., Afkari Sayyah, A. H., & Taghinezhad, E. (2020). Mathematical modeling for the prediction of some quality parameters of white rice based on the strength properties of samples using response surface methodology (RSM). *Food Science & Nutrition*, *8*(8) , 4134- 4144. https://doi.org/10.1002/fsn3.1703.

[23] Castro, G. F. P. d. S. d., Rizzo, R. F., Passos, T. S., Santos, B. N. C. d., Dias, D. d. S., Domingues, J. R., & Araújo, K. G. d. L. (2015). Biomass production by *Arthrospira platensis* under different culture conditions. *Food Science and technology*, *35*, 18-24. https://doi. org/10.1590/1678-457X.6421.

[24] Herrero, A., Muro-Pastor, A. M., & Flores, E. (2001). Nitrogen control in cyanobacteria. *Journal of bacteriology*, *183*(2), 411-425. https://doi.org/10.1128/ jb.183.2.411-425.2001.

[25] Richmond, A. (2004). *Handbook of microalgal culture: biotechnology and applied phycology* (Vol. 577). Wiley Online Library.

[26] Solovchenko, A., Khozin-Goldberg, I., Selyakh, I., Semenova, L., Ismagulova, T., Lukyanov, A., Mamedov, I., Vinogradova, E., Karpova, O., & Konyukhov, I. (2019). Phosphorus starvation and luxury uptake in green microalgae revisited. *Algal Research*, *43*, 101651. https://doi.org/10.1016/j.algal. 2019.101651.

[27] Lavrinovičs, A., Murby, F., Zīverte, E., Mežule, L., & Juhna, T. (2021). Increasing phosphorus uptake efficiency by phosphorus- starved microalgae for municipal wastewater post- treatment. *Microorganisms*, *9*(8) , 1598. https:// doi.org/10.3390/microorganisms9081598.

[28] Singh, P., & Kumar, D. (2021). Biomass and lipid productivities of cyanobacteria- *Leptolyngbya foveolarum* HNBGU001. *BioEnergy Research*, *14*, 278-291. https://doi.org/10.1007/s12155-020-10170-3.

[29] Ernst, A., Deicher, M., Herman, P. M., & Wollenzien, U. I. (2005). Nitrate and phosphate affect cultivability of cyanobacteria from environments with low nutrient levels. *Applied and Environmental Microbiology*, *71*(6) , 3379- 3383. https://doi.org/10.1128/ AEM.71.6.3379-3383.2005.

[30] Humphrey, S. J., James, D. E., & Mann, M. (2015). Protein phosphorylation: a major switch mechanism for metabolic regulation. *Trends in Endocrinology & Metabolism*, *26*(12) , 676- 687. https:// doi.org/10.1016/j.tem.2015.09.013.

[31] Luo, Z., Li, S., Arifeen, M. Z. U., Fu, F. X., Gao, H., Sun, T., ... & Jiang, H. B. (2025). Physiological and molecular evidence for phycobilisome degradation in maintaining carbon and nitrogen balance of cyanobacteria. *Marine Life Science & Technology*, 1-13.https://doi.org /10.1007/s42995-025-00290-0

[32] Saha, S. K., Uma, L., & Subramanian, G. (2003). Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. *FEMS microbiology ecology*, *45*(3), 263-272. https://doi.org/10.1016/S0168-6496(03)00162-4.

[33] Richaud, C., Zabulon, G. r., Joder, A., & Thomas, J.C. (2001). Nitrogen or sulfur starvation differentially affects phycobilisome degradation and expression of the *nblA* gene in *Synechocystis* strain PCC 6803. *Journal of bacteriology*, *183*(10), 2989-2994. https://doi.org/10.1128/jb.183.10.2989-2994.2001.

[34] Zhou, W., Sui, Z., Wang, J., Hu, Y., Kang, K. H., Hong, H. R., ... & Que, Z. (2016). Effects of sodium bicarbonate concentration on growth, photosynthesis, and carbonic anhydrase activity of macroalgae *Gracilariopsis lemanei formis*, *Gracilaria vermiculophylla*, and *Gracilaria chouae* (Gracilariales, Rhodophyta). *Photosynthesis Research*, *128*, 259-270. https://doi.org/10.1007 /s11120-016-0240-3

[35] Geider, R. J., & La Roche, J. (2002). Redfield revisited: variability of C [ratio] N [ratio] P in marine microalgae and its biochemical basis. *European Journal of Phycology*, *37*(1), 1-17. https://doi.org/10.1017/S0967026201003456.

[36] Zuorro, A., Leal-Jerez, A. G., Morales-Rivas, L. K., Mogollón-Londoño, S. O., Sanchez-Galvis, E. M., García-Martínez, J. B., & Barajas-Solano, A. F. (2021). Enhancement of phycobiliprotein accumulation in thermotolerant *Oscillatoria* sp. through media optimization. *ACS Omega*, *6*(16), 10527-10536. https://doi.org/10.1021/acsomega.0c04665.

[37] Johnson, E. M., Kumar, K., & Das, D. (2014). Physicochemical parameters optimization, and purification of phycobiliproteins from the isolated *Nostoc* sp. *Bioresource technology*, *166*, 541-547. https://doi.org/10.1016/j.bior tech.2014.05.097

[38] Mogany, T., Swalaha, F. M., Kumari, S., & Bux, F. (2018). Elucidating the role of nutrients in C-phycocyanin production by the halophilic cyanobacterium *Euhalothece* sp. *Journal of Applied Phycology*, *30*, 2259-2271. https://doi.org/10.1007/s10811-018-1477-3

[39] Xing, W., & Lusan, L. (2013). Effects of the different nitrogen, phosphorus and carbon source on the growth and glycogen reserves in *Synechocystis* and *Anabaena*. *African Journal of Microbiology Research*, *7*(23), 2820-2827.