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Optimization Study of Phycocyanin Ultrasound-Assisted Extraction Process from *Spirulina (Arthospira platensis)* Using Different Solvent

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Abstract

Microalgae *Spirulina (Arthospira platensis)* contains high protein content (55-70%), consisting of approximately 20% phycocyanin compounds as a photosynthetic pigment. Phycocyanin has antioxidant and anti-inflammatory properties. This study aims to obtain the optimum condition for extracting phycocyanin from *Spirulina (Arthospira platensis)* in ultrasound-assisted extraction using three different solvents. A Response Surface Method (RSM) optimization technique is used to obtain the significance of the multivariable effect on the extraction process. Based on the result, it was determined that the sodium buffer phosphate was the most suitable solvent for extraction phycocyanin compared to acetic acid and ethanol. Acetic acid solvent and ethanol solvent failed to provide high yield and purity of phycocyanin as the chlorophyl and its derivative also present on the extract (co-extracted). The optimum conditions of UAE with sodium buffer phosphate were at stirring speed of 1000 rpm, temperature of 40 ^{o}C, and solvent to biomass ratio of 100:1. The results presented that temperature had significant influences on the yield of phycocyanin. The high coefficient of determination (R^2) value obtained in this study (0.9153), which indicate that the model was not only accurate but also adequate and reliable in predicting optimal conditions.

Keywords:Extraction, Phycocyanin, Response Surface Method, Spirulina (Arthospira platensis), Ultrasound.

1. Introduction

The increase in pollution levels exerts adverse effects on various life forms, particularly humans. The increasing use of chemicals and unhealthy lifestyles causes the formation of free radicals in the body and is dangerous for human health. To mitigate and avert the risks of diseases induced by free radicals, the utilization of compounds known as antioxidants is imperative [1]. Specifically, Indonesia relies heavily on imported sources of Vitamin C and Vitamin E to fulfill its antioxidant requirements. According to 2022 data from the Central Bureau of Statistics (BPS), Indonesia is projected to import 5,831,997 kg (USD 33.2 million) of vitamin C and 2,588,553 kg (USD 33.9 million) of vitamin E [2].

With evolving consumer preferences towards natural and healthful products, microalgae present a promising resource of functional, active ingredients with health benefits [3]. Among marine resource-based microalgae, *Spirulina (Arthospira platensis)* emerges as a potential contender for developing active functional ingredients [4]. This blue-green microalgae species thrives in aerial media with salinity levels ranging from 10-40 ‰ [5], displaying optimum growth at pH levels between 8.5 – 10, temperatures of 25-30 ^{o}C, and under light intensities ranging from 1500-3500 lux [6][7].

Nevertheless, using microalgae in the commodity sector, such as energy and agriculture, encounters challenges in attaining economic viability. The pharmaceutical industry presents a realm with the potential to yield high-value products derived from microalgae. In this sector, active components (bioactive compounds) are extracted from microalgae

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for their use as sources of vitamins, antioxidants, antivirals, anti-inflammatories, and immune stimulants. *Spirulina (Arthospira platensis)*, for instance, contains a substantial protein content (55-70%), with approximately 20% constituting phycocyanin compounds [8][13]. These phycocyanin compounds possess antioxidant, anti-inflammatory, hepatoprotective, and anti-radical properties [13][15]. Studies have demonstrated that the antioxidant properties of phycocyanin surpass those of vitamin C by a factor of 20 times higher [11]. Given these attributes, phycocyanin holds the potential to serve as an antioxidant-rich superfood source.

Based on those above, there arises an intriguing proposition to develop an extraction process for phycocyanin from *Spirulina (Arthospira platensis)*. Several variables have been identified as influential factors in the extraction process of active compounds from microalgae, e.g., cell decomposition methods, biomass-to-solvent ratios, and solvent types. While previous research has investigated the single effects of variables such as solvent type and cell disruption method on phycocyanin extraction [13]-[15], a comprehensive study that evaluates the combined impact of multiple variables for process optimization needs to be more present.

This study aims to attain optimal process conditions for phycocyanin extraction, yielding high yields in ultrasonic extraction using different solvents with different operational conditions. This study will optimize temperature, biomass-to-solvent ratio, and time during the extraction process using the ultrasound-assisted extraction (UAE) method. To achieve this, we will use optimization techniques, utilizing the Behnken Box Design Response Surface Method, in conjunction with optimizing stirring speed, biomass-to-solvent ratio, and temperature. Furthermore, we conducted an in-depth analysis of cultivation data for the microalga *Spirulina (Arthospira platensis)*. We studied growth kinetic models, which are crucial in predicting performance and enhancing cultivation conditions.

2. Material and Methods

2.1. Spirulina (Arthospira platensis) Cultivation

The initial phase of this study involved the cultivation of *Spirulina (Arthospira platensis)* in a specific medium. The cultivation process aimed to get the biomass and gather growth data for *Spirulina (Arthospira platensis)* to determine the optimal harvest day. The microalgae cultivation was undergone in open pond units at Nogotirto Algae Park, Sleman, Indonesia, with a scale of 500 L per unit open pond. The chosen medium was saline water supplemented with commercial nutrient fertilizers, including urea, NPK, trisodium phosphate, and soda ash. The cell density was measured daily until the stationary phase was reached. The Logistic, Gompertz, and Richard models were used to forecast the growth kinetics of *Spirulina (Arthospira platensis)*.

The Logistic, Gompertz, and Richard models were chosen for their simplicity and applicability to a wide range of microalgae growth rates, as they are not contingent on substrate type and consumption. The Logistic model, utilizing the maximum growth rate per day as a critical parameter, was employed to project population numbers. The Logistic model was computed by Eq (1), where X, X0, Xmax, and μmax denote cell density, initial cell density, maximum cell density, and maximum specific growth rate, respectively [16]-[17].

$$\frac{dX}{dt}=\mu_{max}\left(1-\frac{X}{X_{max}}\right)X \quad (1)$$

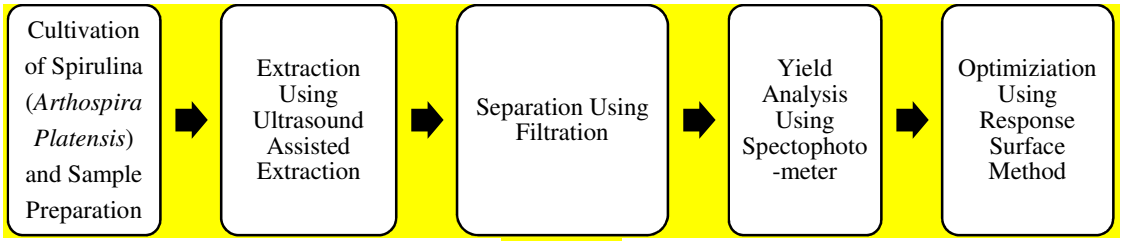
Integration of Eq. (1) resulted in

$$X=\frac{X_0\,exp(\mu_{max}\,t)}{1-\left[\frac{X_0}{X_{max}}(1-exp(\mu_{max}\,t)\right]} \quad (2)$$

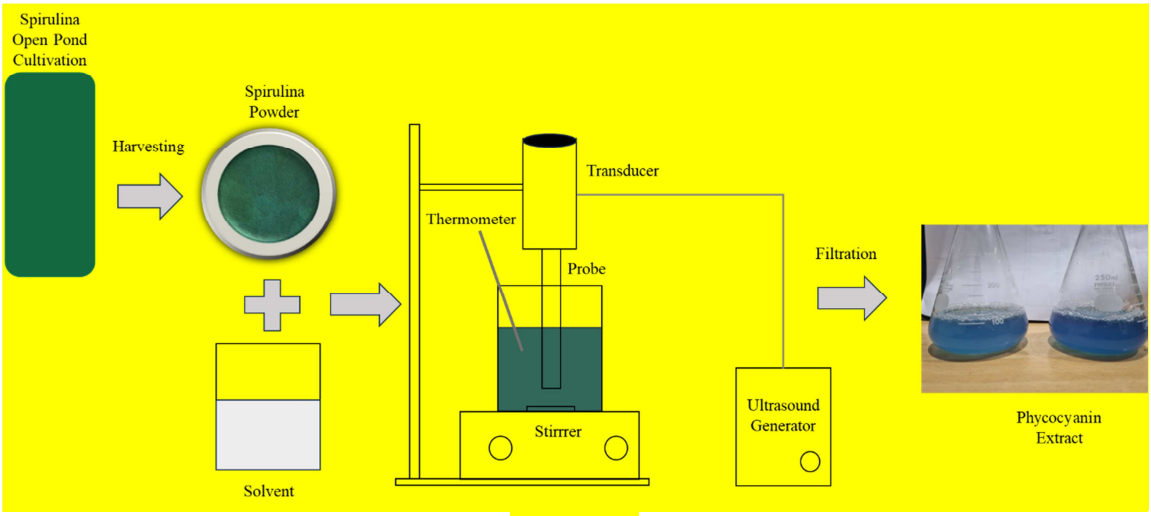
The Gompertz model is also used to determine the cell population of the exponential phase. The parameter is maximum cell production (rm) and lag time (tL).

$$X=Xo+\left[X_{max}.\exp\left[-\exp\left(\left(\frac{r_m.\exp(1)}{X_{max}}\right)(t_L-t)+1\right)\right]\right] \quad (3)$$

Richard's modified kinetic model incorporates parameters such as maximum specific growth rate (μm) and lag time (tl), alongside curve fitting parameter (A) and asymptotic parameters in the stationary growth phase (v) region.



(A) Schematic



(B) Illustration

Figure 1: Method of This Study (A) Schematic (B) Illustration

$$x=A\left(1+v\exp(1+v)\exp\left(\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(tl-t)\right)\right)^{\left(\frac{-1}{v}\right)} \quad (4)$$

The coefficient of determination for the model was carried out using the following formula where SSR is sum square residual and SST is sum square total.

$$R^2 = \left(1-\frac{SSR}{SST}\right) \quad (5)$$

2.2. Phycocyanin Extraction Process

The *Spirulina (Arthospira platensis)* powder used in this study was obtained from the first step cultivation at Nogotirto Algae Park, Sleman. Extraction was done using different solvents: sodium buffer phosphate with pH 7.4, technical-grade ethanol 96%, and technical-grade acetic acid 50 %. The *Spirulina (Arthospira platensis)* microalgae sample was grounded and sieved until a maximum particle size of 80 mesh. The *Spirulina (Arthospira platensis)* biomass and solvent were mixed according to the various combinations of stirring speed, biomass-to-solvent ratios, and temperatures specified in the Response Surface Matrix Design Box Benken in Table 1. An ultrasonic device with a power of 60 Watts and a frequency of 28 kHz was used to generate cavitation bubbles with high shear stress to disrupt the cells. The experiment was conducted in triplicate (3 repetitions).

After extraction, the separation between the phycocyanin extract and the solvent was performed by filtration supernatant, and the remaining solid extract was obtained. The concentration of phycocyanin in the extract was determined by Optical Density (OD) from spectrophotometer analysis referring to the method of [18].

$$PC=\frac{OD_{620}-0{,}474OD_{652}}{5{,}34} \quad (6)$$

Where PC represents the phycocyanin concentration in mg/mL, OD620 is the optical density of the sample at a wavelength of 620 nm, and OD652 is the optical density of the sample at a wavelength of

652 nm. Phycocyanin yield was determined according to the reference provided by [19].

$$\text{Yield} = \frac{\text{PC}\left(\frac{\text{mg}}{\text{mL}}\right) \text{x V(mL)}}{\text{DB (g)}} \text{ x } 100\% \quad (7)$$

Where V is the volume of the solvent, and DB is the dry weight of the *Spirulina (Arthospira platensis)* biomass.

2.3. Optimization Method with Response Surface Method (RSM) Design

The Response Surface Method (RSM) is an advanced Design of Experiment (DOE) technique utilized to comprehend and optimize the impact of variables on processes characterized by optimum points on curved surfaces. This study constructed the experimental setup based on the Box-Behnken design, encompassing 11 factorial experiments and four repeated central point variables. The Box-Behnken design is considered more efficient and more powerful compared to other alternative designs, such as the three-level full factorial design and central composite design. Furthermore, Box-Behnken designs typically involve fewer design points than central composite designs, resulting in lower costs for experimentation while maintaining the same number of factors [20].

The independent variables selected were stirring speed (A), temperature (B), and solvent-to-biomass ratio (C). These variables were configured as follows: stirring speed at 200, 600, and 1000 rpm, the temperature at 30, 40, and 50°C and the biomass to solvent ratio at 1:20, 1:60, and 1:100. The responses assessed for the combinations of these independent variables were phycocyanin yield. To model the yield of phycocyanin about a set of independent variables, a second-order polynomial equation was utilized, as outlined by [21] with modification.

$$\gamma = \beta_o + \beta_1 A + \beta_2 B + + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (8)$$

In this equation, Y represents the response variable, which pertains to the yield of phycocyanin. The constant βi values can be determined using the statistical software Minitab 21. The variable optimization design matrix using the Response Surface Method Box Behnken Design is shown in Table (1) as follows.

Table 1: Design of Optimization Using Response Surface Method Box Behnken

No	Stirring Speed (rpm)	Temperature (°C)	Solvent to Biomass Ratio
1	200	30	60
2	600	30	100
3	600	30	20
4	1000	30	60
5	200	50	20
6	200	50	100
7	600	50	60
8	600	50	60
9	600	50	60
10	1000	50	100
11	1000	50	20
12	200	70	60
13	600	70	20
14	600	70	100
15	1000	70	60

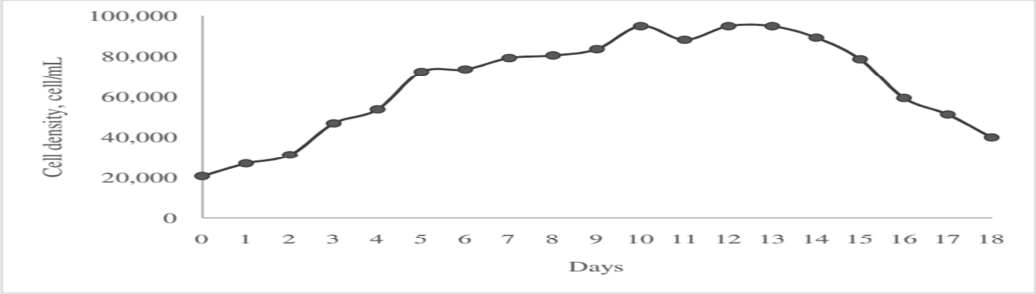


Figure 2: Cell Density Growth Data of *Spirulina (Arthospira platensis)*

3. Result and Discussion

3.1. Spirulina (Arthospira platensis) Cultivation and Growth and Kinetic Modelling

According to Figure 2, the cell growth curve of *Spirulina (Arthospira platensis)* aligns with established microalgae growth theory, encompassing five distinct phases: lag phase, log phase, stationary phase, and death (declining phase) [22]. From day 0 to day 2, the microalgae were in the preparation/adaptation phase (lag phase), characterized by minimal growth, with cell density increasing from 20,625 cells/mL to 31,250 cells/mL. From days 3 to 10, the microalgae enter the exponential phase (log phase), marked by rapid growth, with cell density increasing from 46,875 cells/mL to 95,000 cells/mL. As the highest cell density was reached on the 10th day, it is the optimum time for microalgae harvesting. Following the exponential phase, the microalgae entered the stationary phase, with cell density ranging from 88,333 cells/ml to 95,000 cells/mL from the 10th to the 13th day. On the 13th day, there was a decline in large microalgae, with the density decreasing to 78,750 cells/mL and 40.000 cells/mL on day 16.

To predict performance and optimize cultivation operating conditions, a dynamic approach to microalgae biomass through kinetic modeling, as proposed by [16]-[23], needs to be developed. The number of cells and biomass in samples during the log lag until stationary phases (day 14th) can be approximated using various growth kinetic models. The logistic, Gompertz, and Modified Model were employed for this purpose. The resulting growth curve of *Spirulina (Arthospira platensis)* was then fitted to these kinetic models, with the fitting results depicted in Figure 3.

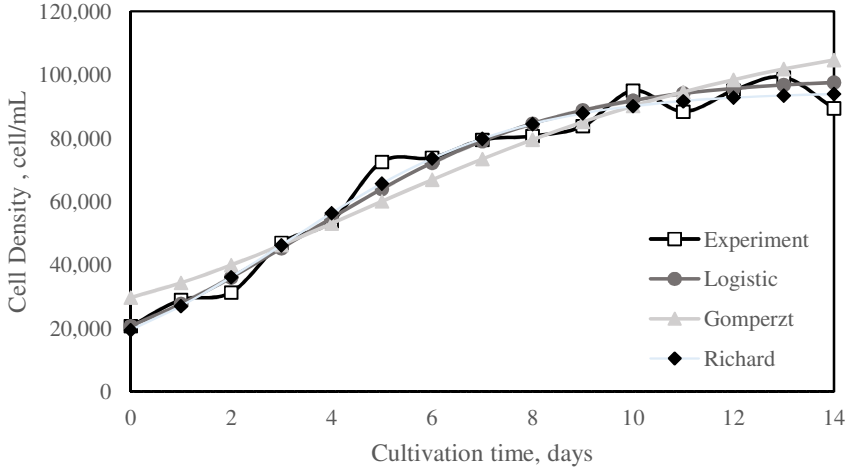


Figure 3: Growth Kinetic Modelling of *Spirulina (Arthospira platensis)* Using Logistic, Gompertz and Modified Richard Method

Table 2: Proximate analysis of Spirulina *(Arthospira platensis)*

Parameter	% Content
Water	5.3%
Ash	6.4%
Lipid	1.5%
Protein	63.8%
Carbohydrate	23.0%
Total	100%

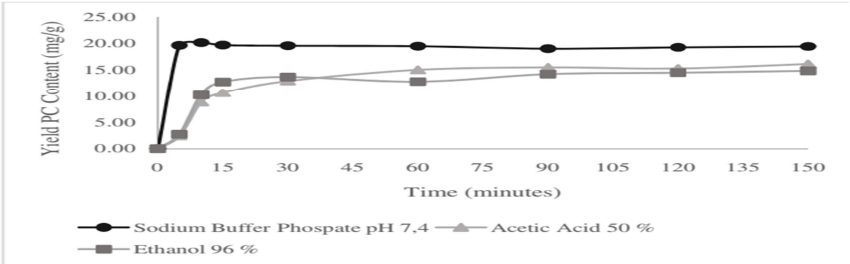


Figure 4: Preliminary Extraction Time Analysis at Stirring Speed 1000 rpm, Temperature 50 ^{o}C, Solvent to Biomass Ratio= 100:1

Among the three non-linear models (Logistic, Gompertz, and Richard), the models were suitable for representing the rapid population growth of organisms like microalgae, owing to their independency on substrate type and consumption. Specifically, based on logistic modelling, the maximum specific growth rate (μmax) of *Spirulina (Arthospira platensis)* was determined to be 0.39/day. In Gompertz modelling, *Spirulina (Arthospira platensis)*'s maximum cell production rate (rm) was estimated at 0.6963 x 10^4cell/mL/day, with a lag time (tL) of 0.65day. The Modified Richard Model yielded a lag time (tl) value of 1.5 days and a specific growth rate (μmax) of 1.0162 x 10^{4s} cells mL/day. Additionally for Richard model, curve fitting parameter (A) and stationary phase asymptotic parameter (v) were 94.76 and 0.97.The R-square error values for the Logistic, Gompertz, and Richard models were 0.9731, 0.9230, and 0.9785, respectively. The Modified Richard Model was identified as the most suitable kinetic model for representing cell density due to the additional parameters it optimizes, namely curve fitting parameter (A) and stationary phase asymptotic parameter (v). Moreover, using an asymptotic curve in the Richard model for approaching the stationary phase leads to more accurate results [24].

3.2. Spirulina Platensis Proximate Analysis

The raw material used in this research was the microalgae *Spirulina (Arthospira platensis)* from Nogotirto Algae Park, Yogyakarta. Table 2 presented proximate analysis of *Spirulina (Arthospira platensis)*. The results show that the protein content reach >60 %. The particle size of the *Spirulina (Arthospira platensis)* was controlled at max 80 mesh (177 micron), to maintain high contact area and increasing damaged cell walls [25].

3.3. Preliminary Extraction Time Analysis

Figure 4 shows preliminary extraction time analysis at a stirring speed of 600 rpm, temperature of 30 ^oC, and to-biomass ratio of 100:1. This stage aims to determine the reference time used for RSM optimization on the variables stirring speed, solvent-to-biomass ratio, and temperature. Based on the results, it was found that in the sodium buffer phosphate solvent pH 7.4, equilibrium was reached after 5 minutes of extraction. Meanwhile, in the solvent 50% acetic acid and 96% ethanol, equilibrium was reached after 150 minutes of extraction. In terms of yield, it was also found that the sodium buffer phosphate solvent was able to provide a yield of around 20 mg/g, while the ethanol solvent gave a maximum yield of 1.6 mg/g, and 50% acetic acid had a maximum yield of 5.5 mg/g. Based on these results, 10 minutes was chosen as the reference time for the optimization stages of stirring speed, solvent-to-biomass ratio, and temperature using the RSM.

3.4. Response Surface Method Plot of Phycocyanin Ultrasound Assisted Extraction Using Different Solvent

The Response Surface Method (RSM) is a mathematical and statistical technique employed for the development, enhancement, and optimization of processes [26]. It is frequently applied to assess the impact of interactions among multiple input variables on the response. The present study studied the three variable interactions of stirring speed, solvent-to-biomass ratio, and temperature vs the response of yield of phycocyanin. The contour plot and surface plot of RSM on different solvents was presented in Figure 5 and Figure 6.

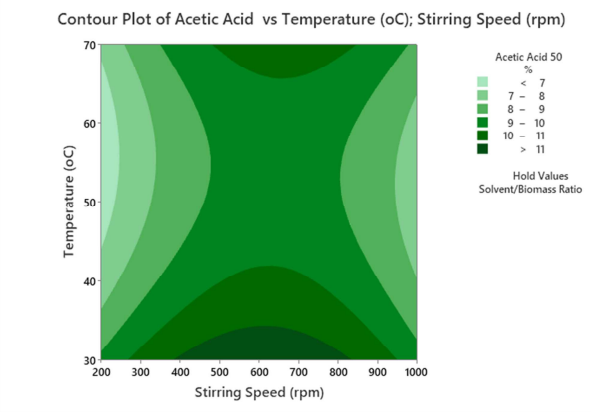
The contour plots of phycocyanin yield as a function of temperature (^oC) and stirring speed (rpm); solvent to biomass ratio and going speed (rpm); solvent to biomass ratio and temperature for acetic acid 50 %, ethanol 96 % and sodium buffer phosphate pH 7.4 was illustrated in Figure 5A, 5B and 5C respectively. It was plotted using a stirring speed range of 200 to 1000 rpm, solvent-to-biomass ratio of 20 to 100, and temperature of 30 to 50^oC.

The results for acetic acid 50 % suggested that the operating conditions (indicated in the dark green region) would be the 400 to 600 rpm stirring speed, higher biomass to solvent ratio (100) and lower temperature of 30 to 35 ^oC (see Figure 5.A1 to 5A3). The surface plot indicates the same result as contour plot (see Figure 6.A1 to 6.A3).

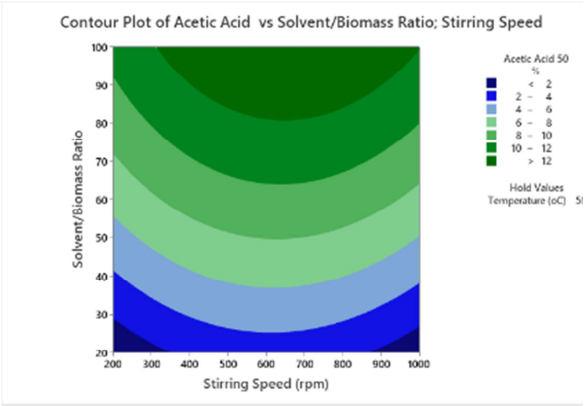
The results for ethanol 96 % suggested that the optimum working conditions (indicated in the dark green region) would be the at higher stirring speed (1000 rpm), higher biomass to solvent ratio (100) and at higher temperature of 70 ^oC (see Figure 5.B1 to 5B3). The surface plot indicates the same result as contour plot (see Figure 6.B1 to 6.B3).

The results for sodium buffer phosphate pH 7.4 suggested that the optimum working conditions (indicated in the dark green region) would be the at both higher and lower stirring speed (200 and 1000 rpm), higher biomass to solvent ratio (100) and at higher temperature of 30 to 40 ^oC (see Figure 5.C1 to 5C3). The surface plot indicates the same result as contour plot (see Figure 6.C1 to 6.C3).

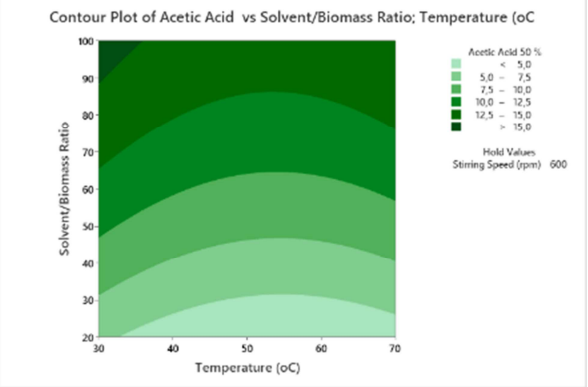
Acetic Acid 50 % (A)



A1

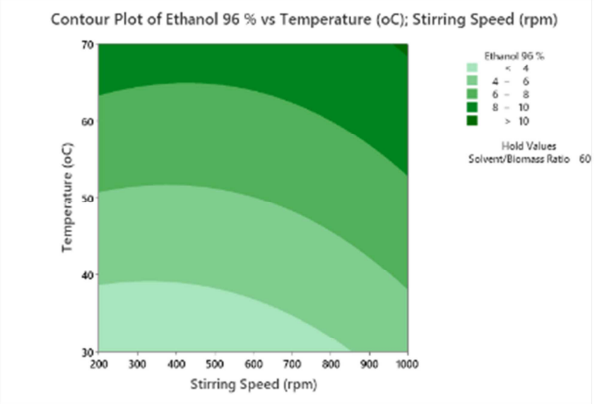


A2

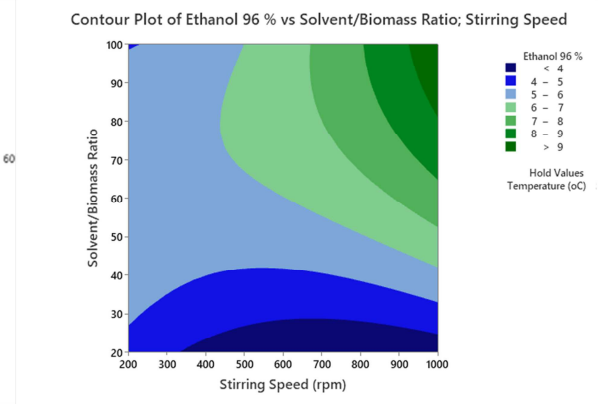


A3

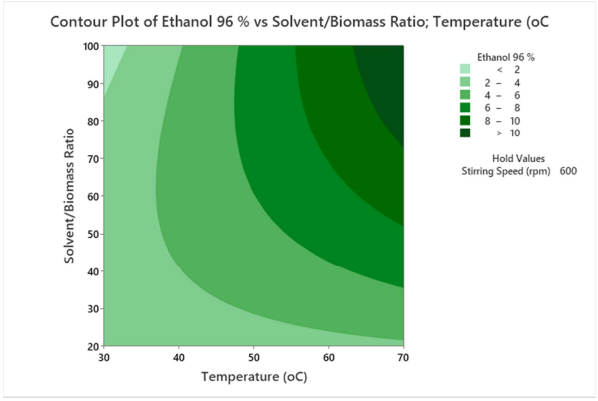
Ethanol 96 % (B)



B1

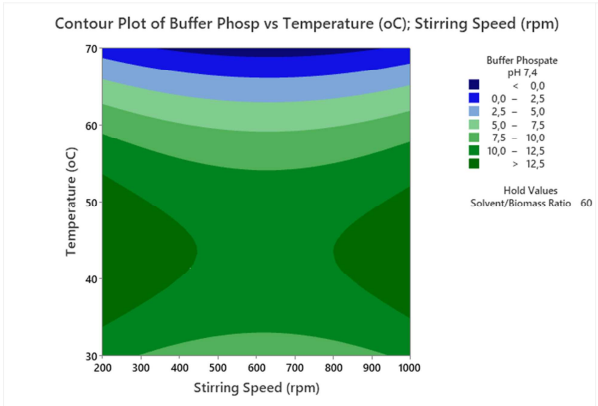


B2

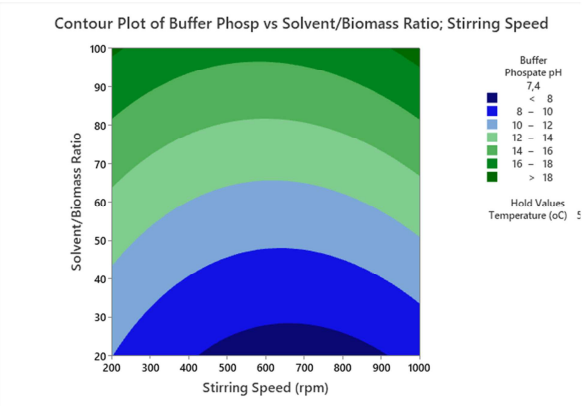


B3

Sodium Buffer Phosphate pH 7.4 (C)



C1



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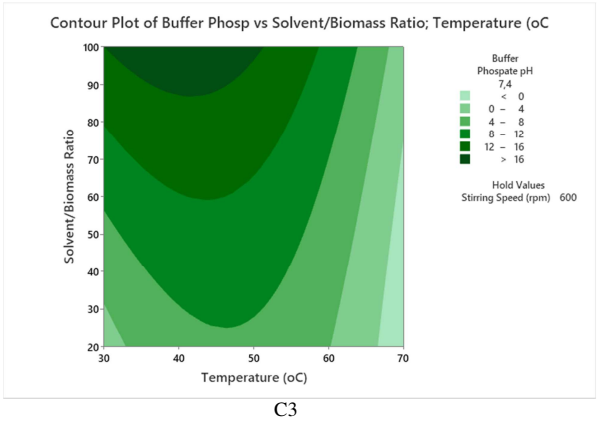
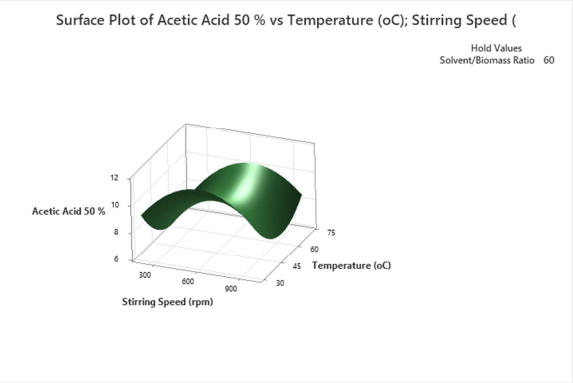


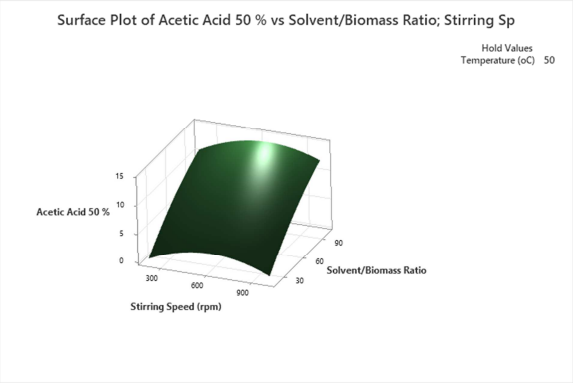
C3

Figure 5: Contour Plot of Response Surface Method Optimization on Different Solvent (A) Aceti Acid 50 %, (B) Ethanol 96 %, and (C) Sodium Buffer Phosphate pH 7.4

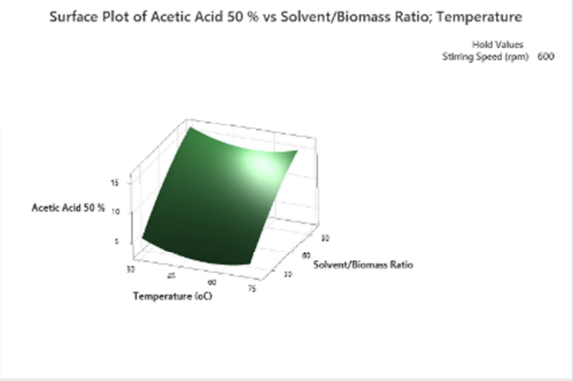
Acetic Acid 50 % (A)



A1



A2



A3

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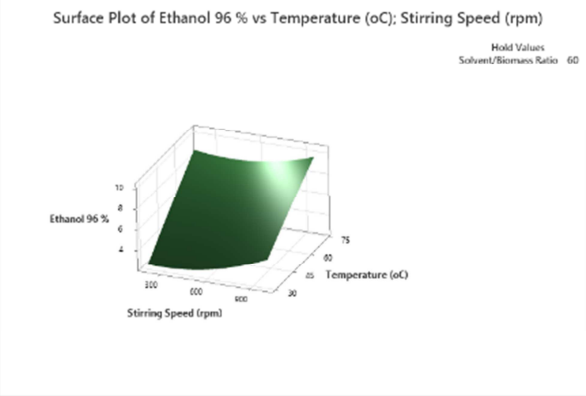
Ethanol 96 % (B)



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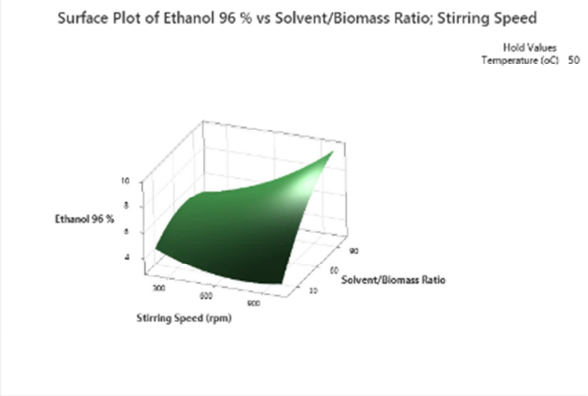

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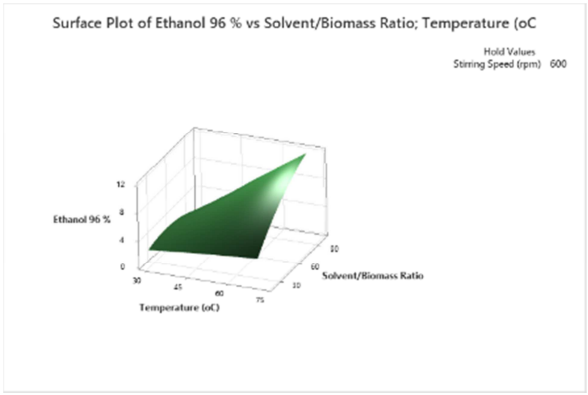
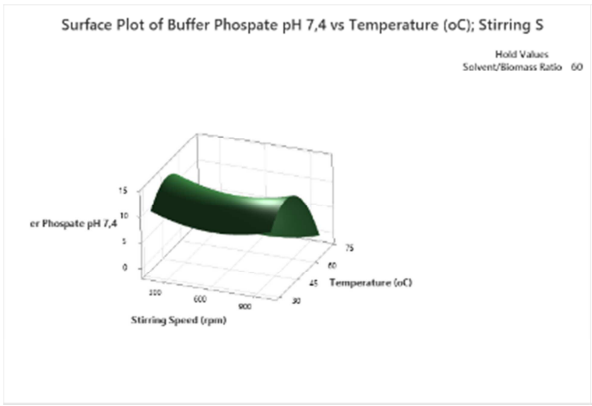
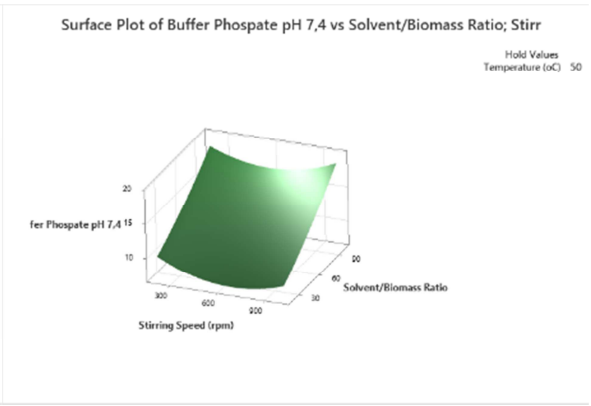


B3

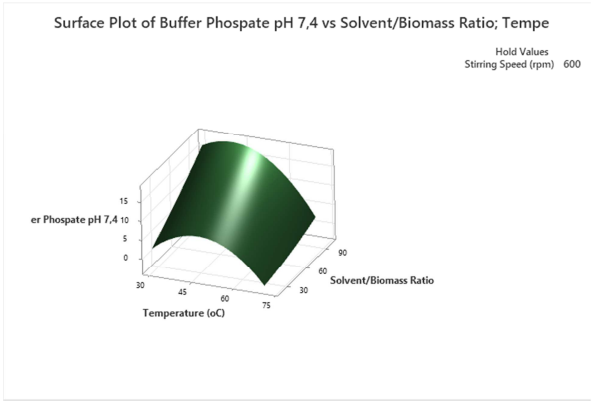
Sodium Buffer Phosphate pH 7.4 (C)



C1



C2

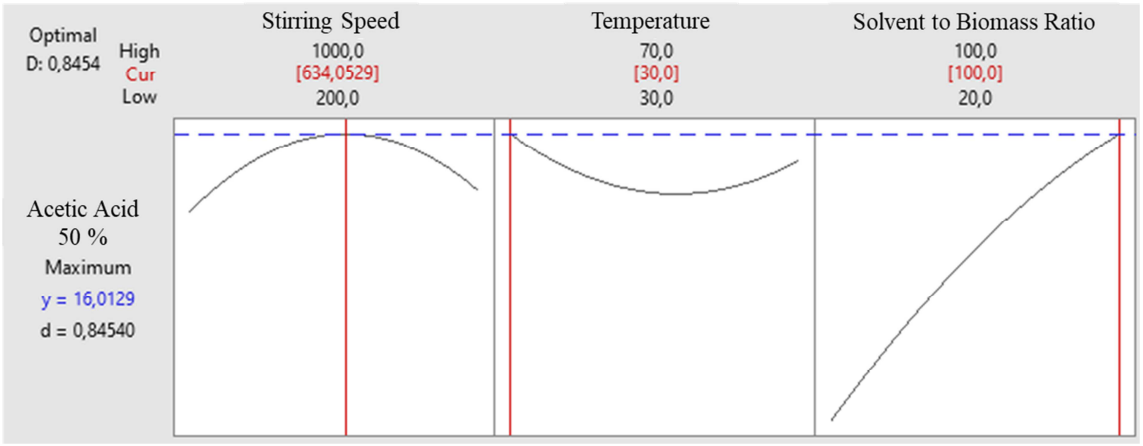


C3

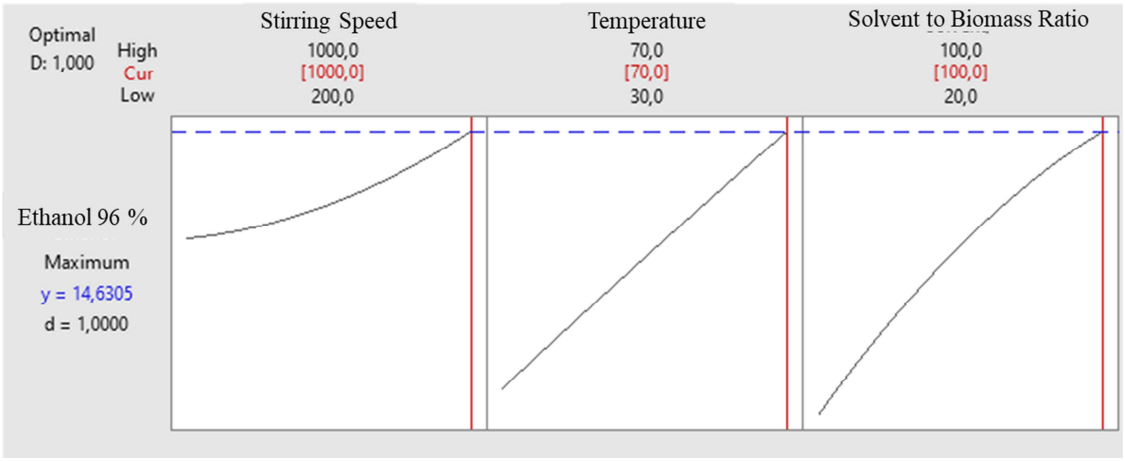
Figure 6: Surface Plot of Response Surface Method Optimization on Different Solvent (A) Aceti Acid 50 %, (B) Ethanol 96 %, and (C) Sodium Buffer Phosphate pH 7.4

3.5. Optimization of Phycocyanin Ultrasound Assisted Extraction Using Different Solvent

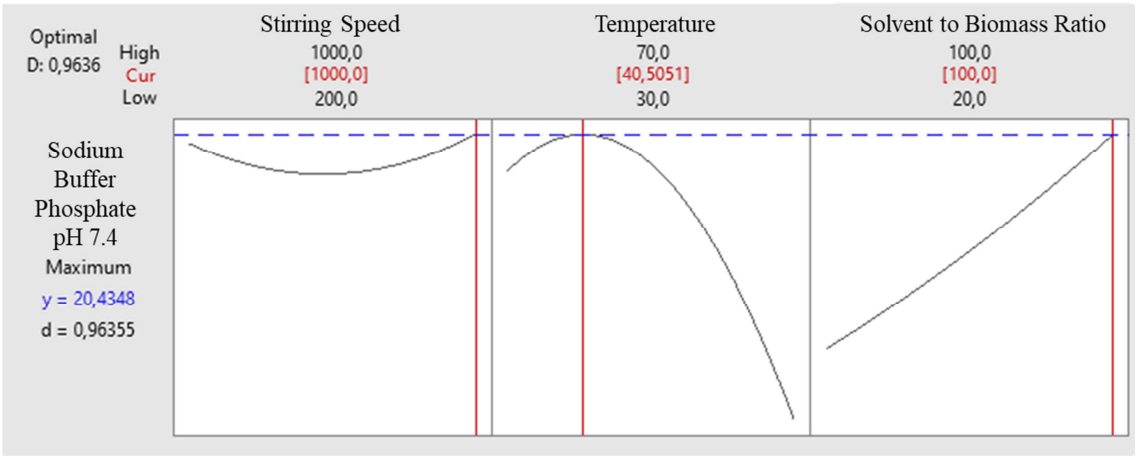
Figure 7 presented the optimum condition based on the RSM result for phycocyanin extraction in different solvents: (A) acetic acid 50 %, (B) ethanol 96 %, and (C) sodium buffer phosphate pH 7.4 For the indicator that optimization desirability (d). The desirability function ranges between 0-1, with the value of 0 indicating that the factors give an undesirable response, in contrast to the value of 1 means the optimal performance (desirable) for the studied factors [27]-[28]. The value of one indicates the ideal case, while one or more response falls outside the desirable limit if the value of d is 0 [29].



Acetic Acid 50 % (A)



Ethanol 96 % (B)



Sodium Buffer Phosphate pH 7.4 (C)

Figure 7: Optimum Condition Based on RSM Result for Phycocyanin Extraction in different Solvent (A) Acetic Acid 50 %, (B) Ethanol 96 %, and (C) Sodium Buffer Phosphate pH 7.4.

In this study, the desirable values of UAE using different solvents acetic acid 50 %, ethanol 96 %, and sodium buffer phosphate pH 7.4 are 0.8455; 1 and 1, respectively. The d value of 1 in ethanol 96 % and sodium buffer phosphate pH 7.4 indicates high suitable performance on RSM optimization, while for acetic acid, a few predictions models fall outside the desirable limit.

Variations in stirring speed in extraction with 50% acetic acid show that the optimum conditions are at a medium speed of 600 rpm (see Figure 7A). Meanwhile, in 96% ethanol (see Figure 7B), the phycocyanin yield increased along with increasing stirring speed. In sodium buffer phosphate pH 7.4 (see Figure 7C), the highest phycocyanin yield can occur at low (200 rpm) or high (1000 rpm) stirring speeds.

The temperature impact was assessed within a temperature range from 30°C to 50°C. The findings revealed that for the acetic acid 50 % and sodium buffer phosphate pH 7.4, a lower temperature is preferred. However, for ethanol, the optimal yield was achieved at a higher temperature of 50°C. As highlighted by [30], for the ethanol solvent, the yield of phycocyanin tends to rise with increasing temperature until it reaches an optimal threshold. Furthermore, it was observed that the yield experienced a decline beyond a temperature of 60°C. This can be attributed to the denaturation of proteins, a phenomenon known to occur once temperatures surpass 55°C [30].

The influence of the solvent-to-biomass ratio was examined within the range of 20:1 to 100:1. As shown in Figure 6 for three different solvents, higher solvent ratios led to an increase in the yield of phycocyanin up to an optimal point.

From Figure 7, it can be shown that the optimum conditions for phycocyanin extraction using acetic acid 50 % solvent was at stirring speed of 634 rpm, temperature of 30 ^{o}C, and solvent to biomass ratio of 100:1. The optimum conditions for phycocyanin extraction using ethanol 96 % solvent was at stirring speed of 1000 rpm, temperature of 70 ^{o}C, and solvent to biomass ratio of 100:1. The optimum conditions for phycocyanin extraction using sodium buffer phosphate pH 7.4 solvent was at stirring speed of 1000 rpm, temperature of 40 ^{o}C, and solvent to biomass ratio of 100:1. To explain the influence of stirring speed, solvent to biomass ratio and temperature to the phycocyanin yield, analysis of variance (ANOVA) will be carried out to determine the significance of this variable.

3.6. Analysis of Variance (ANOVA) of RSM Result

To obtain the best-fitted mathematical model, an analysis of variance (ANOVA) is performed as an indicator [31]. ANOVA for a quadratic model is illustrated in Table 3 to Table 5 below. An indicator of p-value is used to evaluate the variable significance towards phycocyanin yield response. These p-values provide evidence that the parameters predicted by the developed model hold significance [32]. If the p-values are less than 0.05, indicate that model terms were significant. The result shows that the model in UAE using acetic acid 50 % solvent is insignificant (P-value > 0.05). On the other hand, for UAE using ethanol 96 % and sodium buffer phosphate pH 7.4, the P-value is less than 0.05, so the model was significant.

The next stage is to test the significance of the individual linear model terms of variable stirring speed (A), temperature (B), and solvent-to-biomass ratio (C). The result shows that for acetic acid 50 % solvent, only solvent to biomass ratio significantly affects phycocyanin yield (p-value < 0.05). For ethanol, 96 % solvent, temperature, and solvent to biomass ratio have a significant effect on obtained phycocyanin yield. For ethanol solvent, the interaction between temperature and solvent-to-biomass ratio (BC) also indicates a substantial impact. The result for sodium buffer phosphate pH 7.4 solvent also shows that temperature, solvent-to-biomass ratio, and quadratic have a significant effect.

Another indicator for determining the variable significance is using Pareto charts indicating the importance of variables. Pareto charts for standardized effects of phycocyanin yield for different solvent types are shown in Figure 8 to Figure 10. The Pareto chart was plotted to investigate whether the independent variables, their interactions, and their quadratic terms have a positive or negative impact on phycocyanin yield [33].

Table 3: Analysis of Variance of RSM Optimization on Phycocyanin UAE Using Acetic Acid 50 % Solvent

Source	DF	Sum of Square	Mean Square	F-Value	P-Value
Model	9	278.048	30.894	1.93	0.243
Linear	3	238.217	79.406	4.96	0.059
Stirring Speed (rpm) – A	1	1.519	1.519	0.09	0.77
Temperature (^{o}C) B	1	3.661	3.661	0.23	0.653
Solvent/Biomass Ratio – C	1	233.037	233.037	14.56	0.012
Square	3	38.921	12.974	0.81	0.54
Stirring Speed *Stirring Speed (A^2)	1	22.331	22.331	1.39	0.291
Temperature *Temperature (B^2)	1	10.324	10.324	0.64	0.458
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	1	4.108	4.108	0.26	0.634
2-Way Interaction	3	0.91	0.303	0.02	0.996
Stirring Speed*Temperature (AB)	1	0.461	0.461	0.03	0.872
Stirring Speed*Solvent/Biomass Ratio (AC)	1	0.359	0.359	0.02	0.887
Temperature*Solvent/Biomass Ratio (BC)	1	0.089	0.089	0.01	0.944
Error	5	80.051	16.01		
Lack-of-Fit	3	80.048	26.683	21577.44	0
Pure Error	2	0.002	0.001		
Total	14	358.099			

Table 4: Analysis of Variance of RSM Optimization on Phycocyanin UAE Using Ethanol 96 % Solvent

Source	DF	Sum of Square	Mean Square	F-Value	P-Value
Model	9	136.898	15.2109	10.07	0.01
Linear	3	98.408	32.8027	21.71	0.003
Stirring Speed (rpm) – A	1	6.195	6.1952	4.1	0.099
Temperature (^{o}C) B	1	70.145	70.1454	46.43	0.001
Solvent/Biomass Ratio – C	1	22.068	22.0676	14.61	0.012
Square	3	7.105	2.3683	1.57	0.308
Stirring Speed *Stirring Speed (A^2)	1	2.191	2.1911	1.45	0.282
Temperature *Temperature (B^2)	1	0.027	0.027	0.02	0.899
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	1	4.381	4.3806	2.9	0.149
2-Way Interaction	3	31.385	10.4615	6.92	0.031
Stirring Speed*Temperature (AB)	1	0.349	0.3489	0.23	0.651
Stirring Speed*Solvent/Biomass Ratio (AC)	1	9.092	9.0916	6.02	0.058
Temperature*Solvent/Biomass Ratio (BC)	1	21.944	21.944	14.52	0.012
Error	5	7.555	1.5109		
Lack-of-Fit	3	7.372	2.4573	26.92	0.036
Pure Error	2	0.183	0.0913		
Total	14	144.452			

Table 5: Analysis of Variance of RSM Optimization on Phycocyanin UAE Using Sodium Buffer Phosphate pH 7.4

Source	DF	Sum of Square	Mean Square	F-Value	P-Value
Model	9	618.437	68.715	6	0.031
Linear	3	357.913	119.304	10.42	0.014
Stirring Speed (rpm) – A	1	0.507	0.507	0.04	0.842
Temperature (^{o}C) B	1	187.998	187.998	16.41	0.01
Solvent/Biomass Ratio – C	1	169.408	169.408	14.79	0.012
Square	3	239.498	79.833	6.97	0.031
Stirring Speed *Stirring Speed (A^2)	1	15.21	15.21	1.33	0.301
Temperature *Temperature (B^2)	1	210.88	210.88	18.41	0.008
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	1	1.096	1.096	0.1	0.77
2-Way Interaction	3	21.027	7.009	0.61	0.636
Stirring Speed*Temperature (AB)	1	0.026	0.026	0	0.964
Stirring Speed*Solvent/Biomass Ratio (AC)	1	0.898	0.898	0.08	0.791
Temperature*Solvent/Biomass Ratio (BC)	1	20.102	20.102	1.76	0.243
Error	5	57.264	11.453		
Lack-of-Fit	3	57.263	19.088	20562.74	0
Pure Error	2	0.002	0.001		
Total	14	675.702			

For UAE using acetic acid 50 % solvent, aligned with ANOVA result (Table 3), the only significant variable among those examined was the ratio of solvent to biomass. In addition, the remaining variables, as indicated by their bars falling behind the reference line, did not demonstrate their significance. (Figure 8). As established by the p-value in Table 4 for UAE using ethanol 96 % solvent, linear forms of temperature (B) and solvent to biomass ratio (C) together with their interaction (BC) were confirmed to be significant variables. In contrast, the remaining

variable did not demonstrate significance since the bars fell behind the reference line (Figure 9). For UAE using sodium buffer phosphate pH 7.4 solvent, aligned with ANOVA result (Table 5), linear forms of temperature (B) and solvent to biomass ratio (C) together with their interaction (BC) were confirmed to be significant variables. In contrast, the remaining variable did not demonstrate significance since the bars fell behind the reference line (Figure 10).

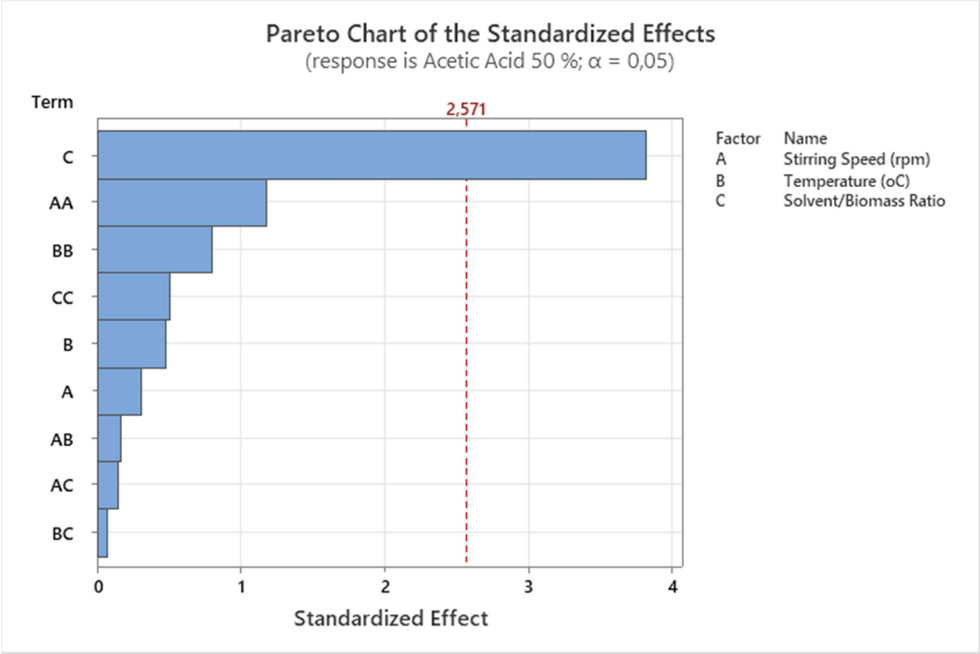


Figure 8: Pareto Charts for Standardised Effects on Phycocyanin Yield on UAE Extraction Using Acetic Acid 50 % Solvent

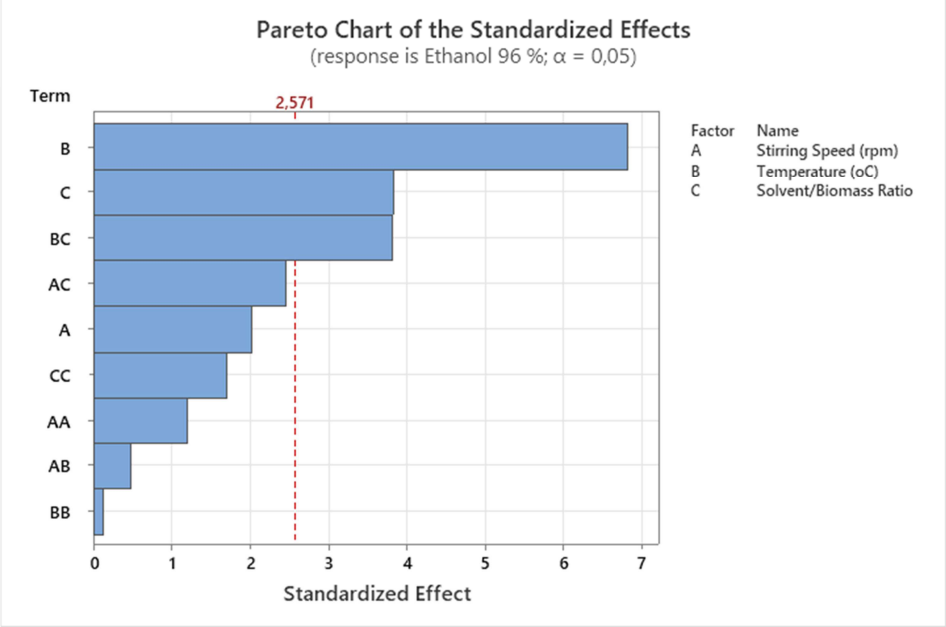


Figure 9: Pareto Charts for Standardised Effects on Phycocyanin Yield on UAE Extraction Ethanol 96 % Solvent

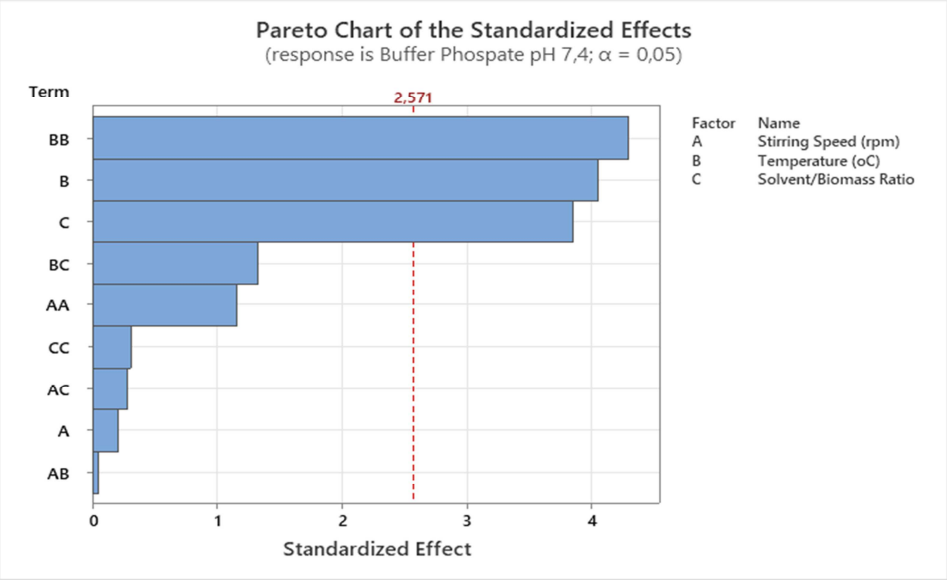


Figure 10: Pareto Charts for Standardised Effects on Phycocyanin Yield on UAE Extraction Ethanol 96 % Solvent

The coefficient of the coded factors for phycocyanin UAE with different solvents is presented in Table 6 to Table 8. This coefficient indicates the expected change in the response for every one-unit alteration in factor value while holding all other factors constant. In an orthogonal design, the intercept represents the overall mean response across all experiments. The coefficients act as corrections around this mean, depending on the factor settings. In instances where the factors are orthogonal, the Variance Inflation Factors (VIFs) are 1; VIFs exceeding 1 indicate the presence of multicollinearity. A higher VIF indicates a more pronounced correlation among factors [34]. Generally, VIFs below ten are considered acceptable, as presented in Table 6 to Table 8 below.

Table 6: Coefficient of Coded Factor of RSM Optimization on Phycocyanin UAE Using Acetic Acid 50 % Solvent

Term	Coefficient Estimate	Standard Error	VIF
Constant	9.45	2.31	
Stirring Speed (rpm) – A	0.44	1.41	1
Temperature (^oC) – B	-0.68	1.41	1
Solvent/Biomass Ratio C	5.4	1.41	1
Stirring Speed *Stirring Speed (A^2)	-2.46	2.08	1.01
Temperature *Temperature (B^2)	1.67	2.08	1.01
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	-1.05	2.08	1.01
Stirring Speed*Temperature (AB)	0.34	2	1
Stirring Speed*Solvent/Biomass Ratio (AC)	0.3	2	1
Temperature*Solvent/Biomass Ratio (BC)	0.15	2	1

Table 7: Coefficient of Coded Factor of RSM Optimization on Phycocyanin UAE Using Ethanol 96 % Solvent

Term	Coefficient Estimate	Standard Error	VIF
Constant	5,978	0,71	
Stirring Speed (rpm) – A	0,88	0,435	1
Temperature (^oC) – B	2,961	0,435	1
Solvent/Biomass Ratio C	1,661	0,435	1
Stirring Speed *Stirring Speed (A^2)	0,77	0,64	1,01
Temperature *Temperature (B^2)	-0,086	0,64	1,01
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	-1,089	0,64	1,01
Stirring Speed*Temperature (AB)	-0,295	0,615	1
Stirring Speed*Solvent/Biomass Ratio (AC)	1,508	0,615	1
Temperature*Solvent/Biomass Ratio (BC)	2,342	0,615	1

Table 8: Coefficient of Coded Factor of RSM Optimization on Phycocyanin UAE Using Buffer Phosphate pH 7.4 Solvent

Term	Coefficient Estimate	Standard Error	VIF
Constant	11,34	1,95	
Stirring Speed (rpm) – A	-0,25	1,2	1
Temperature (^{o}C) – B	-4,85	1,2	1
Solvent/Biomass Ratio C	4,6	1,2	1
Stirring Speed *Stirring Speed (A^2)	2,03	1,76	1,01
Temperature *Temperature (B^2)	-7,56	1,76	1,01
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	0,54	1,76	1,01
Stirring Speed*Temperature (AB)	-0,08	1,69	1
Stirring Speed*Solvent/Biomass Ratio (AC)	0,47	1,69	1
Temperature*Solvent/Biomass Ratio (BC)	-2,24	1,69	1

An analysis of variance was employed to construct the regression model, which took the form of a second-order polynomial. This model was then fine-tuned to align with the parameters of the Response Surface Methodology (RSM) optimization. The coefficient of determination (R^2) was used to express the model's suitability. If the model has an R^2 value in the range of 0.75–1, a good statistical model is attained [31]. The predicted coefficient of the second-order polynomial equation is presented in Table 9. For three different solvents in UAE, an R2 minimum of 0.75 was obtained, which indicates a good correlation between the experimental and estimated values and a good fit of the model. The R2 for the UAE with different solvents, acetic acid 50 %, ethanol 96 %, and sodium buffer phosphate pH 7.4, were 77.65 %, 94.77 %, and 91.53 % respectively.

$$Y=\beta_o+\beta_1A+\beta_2B++\beta_3C+\beta_{12}AB+\beta_{13}AC+\beta_{23}BC+\beta_{11}A^2+\beta_{22}B^2+\beta_{33}C^2$$

Table 9: Predicted Coefficient of Second-Order Polynomial Equation in UAE with Different Solvent

Parameters		Acetic Acid 50 %	Ethanol 96 %	Sodium Buffer Phosphate pH 7.4
R square		77.65 %	94.77 %	91.53 %
Constant	β_o	7.4	4.58	-32.2
Stirring Speed (A)	β_1	0.0163	-0.00739	-0.0171
Temperature (B)	β_2	-0.489	0.016	1.821
Solvent/Biomass Ratio (C)	β_3	0.193	-0.0797	0.197
Stirring Speed*Temperature (AB)	β_{12}	0.000042	-0.0000037	-0.00001
Stirring Speed*Solvent/Biomass Ratio (AC)	β_{13}	0.000019	0.000094	0.00003
Temperature*Solvent/Biomass Ratio (BC)	β_{23}	0.00019	0.002928	-0.0028
Stirring Speed *Stirring Speed (A^2)	β_{11}	-0.000015	0.0000005	0.000013
Temperature *Temperature (B^2)	β_{22}	0.00418	-0.00021	-0.01889
Solvent/Biomass Ratio*Solvent/Biomass Ratio (C^2)	β_{33}	0.00066	-0.000681	0.00034

Table 10: RSM Result on Phycocyanin Yield Using Different Solvent Type

No	Stirring Speed (rpm)	Temperature (^{o}C)	Solvent/Biomass Ratio	Phycocyanin Yield (mg/g)		
				Acetic Acid 50 %	Ethanol 96 %	Buffer Phospate pH 7.4
1	200	30	60	9.50	2.61	11.18
2	600	30	100	**18.74**[a]	**1.30**[b]	12.74
3	600	30	20	1.97	3.52	4.15
4	1000	30	60	9.98	3.67	11.58
5	200	50	20	3.68	3.55	7.85
6	200	50	100	7.61	4.72	**21.21**[a]
7	600	50	60	9.47	6.32	11.36
8	600	50	60	9.48	5.88	11.30
9	600	50	60	9.41	5.74	11.35
10	1000	50	100	8.80	**10.78**[a]	20.91
11	1000	50	20	3.67	3.59	5.67
12	200	70	60	6.68	10.25	0.20
13	600	70	20	**1.11**[b]	3.62	0.39
14	600	70	100	18.47	10.77	**0.01**[b]
15	1000	70	60	8.51	10.13	0.27
	Maximum value			**18.74**	**10.78**	**21.21**
	Minimum value			**1.11**	**1.30**	**0.01**

Note: [a]indicate maximum value of extraction yield, [b]indicate minimum value of extraction yield

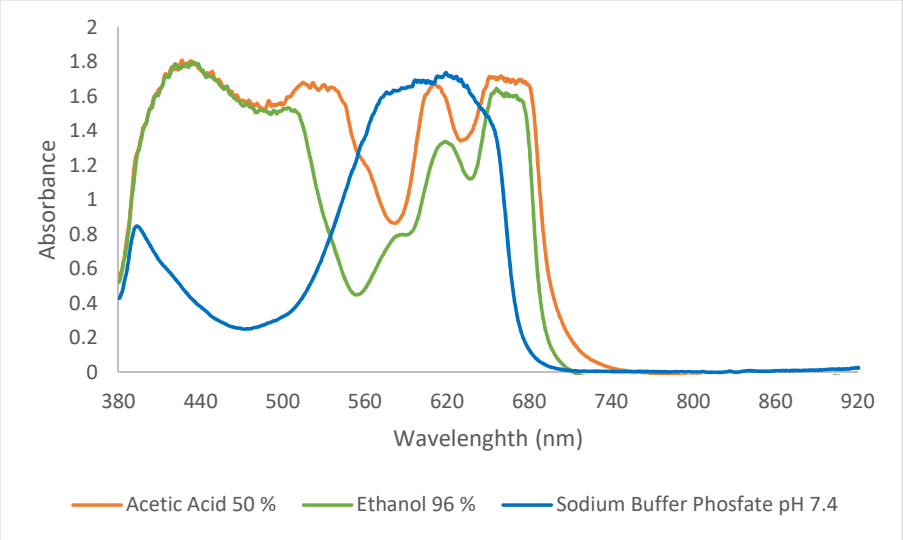
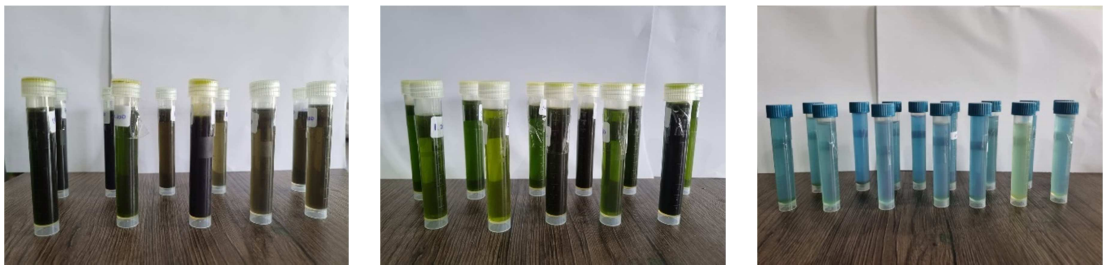


Figure 11: Vis Spectra of the Phycocyanin Extracted by Different Solvent



Acetic Acid 50 % (A) **Ethanol 96 % (B)** **Sodium Buffer Phosphate pH 7.4 (C)**

Figure 12: Image of the Extracted Phycocyanin in Different Solvent Type

3.7. Effect and Selection of Different Solvent on Phycocyanin Yield

Table 10 shows the yield results from UAE with various types of solvents with the RSM Box Behnken experimental matrix. For 50% acetic acid solvent, the yield range for phycocyanin is between 1.11 mg/g to 18.74 mg/g. The maximum value was reached when the stirring speed was 600 rpm, the temperature was 30 C, and the ratio of solvent and biomass was 100:1. In 96% ethanol solvent, the phycocyanin yield obtained was around 1.3 mg/g to 10.78 mg/g with the maximum value reached at a stirring speed of 1000 rpm temperature of 50 ^{o}C and solvent and biomass ratio of 100:1. Phycocyanin yield for sodium buffer phosphate pH 7.4 ranges from 0.01 to 21.21 mg/g with the maximum value reached at a stirring speed of 200 rpm, temperature of 50 ^{o}C and a solvent: biomass ratio of 100:1. Based on the obtained yield results, phosphate buffer is the best solvent to extract phycocyanin from *Spirulina (Arthospria Platensis).*

In addition, from the spectrophotometer absorbance analysis (Figure 1) it was found that for the acetic acid and ethanol solvent, there was another peak at around 430-440 and 680 which indicated the occurrence of chlorophyll co-extraction, while in the sodium phosphate buffer solvent pH 7.4 only a small amount of pheoptyn a was found which was indicated by the presence of a peak in the 400 area. Chlorophylls a and b, pheophytins a and b, and β-carotene can be measured at their appropriate absorption maxima (430, 452, 409, 433, and 452 nm respectively) [35]. Observation with the eye (Figure 12 A and 12 B) also shows a color that tends to be green, which indicates chlorophyll extraction.

4. Conclusion

The production of phycocyanin from microalgae cultivation has been successfully conducted. The highest cell density was reached on the 10th day, indicating the optimum time for microalgae harvesting. From the kinetic modeling, the Modified Richard Model was identified as the most suitable

kinetic model. Phycocyanin ultrasonic-assisted extraction with its optimization has been studied for *Spirulina (Arthospira platensis)*.

The study provided valuable insights into the optimal parameters for phycocyanin extraction using different solvents (50 % acetic acid, 96 % ethanol, and sodium buffer phosphate pH 7.4). Additionally, the validation of the model will also be assessed. Further investigation and application of this model in practical application have the potential to significantly augment the efficiency and productivity of phycocyanin extraction processes, which could lead to advancements in quality control and the ability to scale production at an industrial level.

In conclusion, this study applied response surface methodology to pinpoint the optimal conditions for producing a high purity phycocyanin extract. Through a systematic investigation, it was deduced that the most favorable parameters for this process involved the utilization of sodium buffer phosphate solvent in the optimum conditions at a stirring speed of 1000 rpm, temperature of 40 C, and solvent-to-biomass ratio of 100:1. The results presented that temperature had significant influences on the yield of phycocyanin. Acetic acid and ethanol solvent failed to provide a high yield and purity of phycocyanin as the chlorophyll, and its derivative was also present in the extract (co-extracted).

Furthermore, these conclusions were supported by the high coefficient of determination (R^2) attained in this investigation, registering at 0.9153. This figure provides robust evidence that the model not only demonstrated precision but also proved to be sufficiently reliable in forecasting optimal conditions. The R^2 value signifies the close correlation between the predicted value and the actual experimental findings, underscoring the effectiveness of the experimental design employed in this study.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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7. Acknowledgments

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8. References

[1] Suyono, E. A., Aminin, Pradani, L., Mu'avatun, U., Habiba, R. N., Ramdaniyah, & Rohma, E. F. (2015). Combination of Blue, Red, White, and Ultraviolet Lights for Increasing Carotenoids and Biomass of Microalga Haematococcus pluvialis. Procedia Environmental Sciences, 28, 399–405. https://doi.org/10.1016/j.proenv.2015.07.049

[2] Central Bureau of Statistics (Badan Pusat Statistik Indonesia). (2022). Buletin Statistik Perdagangan Luar Negeri (Impor), Badan Pusat Statistik Indonesia

[3] Guan S. (2016). Extracting phycocyanin from spirulina and hydrothermal Liquefaction of its residues to produce bio-crude oil. Thesis. Ilnois. University of Illinois at Urbana-Champaign

[4] Kore K.J., & Wakte P.S. (2018). Pharmaceuticals bioactive compounds from Spirulina (Arthospira platensis) A review. World Journal of Pharmaceutical Research, 7(10). 1610-1616. https://doi.org/10.20959/wjpr20181-10638

[5] Saif A, & Hafigh A. (2017). Indoor and outdoor culture of Spirulina (Arthrospira platensis) grown in different salinity in Sultanate of Oman. Secheresse. Masters thesis, Universiti Putra Malaysia

[6] Béchet, Q., Shilton, A., & Guieysse, B. (2013). Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation. Biotechnology Advances, 31(8), 1648–1663. https://doi.org/10.1016/j.biotechadv.2013.08.014

[7] Raposo, M. F. D. J., & De Morais, A. M. M. B. (2015). Microalgae for the prevention of cardiovascular disease and stroke. Life Sciences, 125, 32–41. https://doi.org/10.1016/j.lfs.2014.09.018

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

[9] Junique, L., Watier, L., Lejeune, H., Viudes, F., Deblieck, M., & Watier, D. (2021). Determination by response surface methodology of optimal protein and phycocyanin productivity conditions in Arthrospira (Spirulina) platensis under different combinations of photoperiod variation and lighting intensity. Bioresource Technology Reports, 15, 100763. https://doi.org/10.1016/j.biteb.2021.100763

[10] Kissoudi, M., Sarakatsianos, I., & Samanidou, V. (2018). Isolation and purification of food-grade C-phycocyanin from Arthrospira platensis and its determination in confectionery by HPLC with diode array detection. Journal of

Separation Science, 41(4), 975–981. https://doi.org/10.1002/jssc.201701151
[11] Kuddus, M., Singh, P., Thomas, G., & Al-Hazimi, A. (2013). Recent Developments in Production and Biotechnological Applications of C-Phycocyanin. BioMed Research International, 2013, 1–9. https://doi.org/10.1155/2013/742859
[12] İlter, I., Akyıl, S., Demirel, Z., Koç, M., Conk-Dalay, M., & Kaymak-Ertekin, F. (2018). Optimization of phycocyanin extraction from Spirulina platensis using different techniques. Journal of Food Composition and Analysis, 70, 78–88.https://doi.org/10.1016/j.jfca.2018.04.007
[13] Kumar, D., Dhar, D. W., Pabbi, S., Kumar, N., & Walia, S. (2014). Extraction and purification of C-phycocyanin from Spirulina platensis (CCC540). Indian Journal of Plant Physiology, 19(2), 184–188. https://doi.org/10.1007/s40502-014-0094-7
[14] Wu, H.-L., Wang, G.-H., Xiang, W.-Z., Li, T., & He, H. (2016). Stability and Antioxidant Activity of Food-Grade Phycocyanin Isolated from Spirulina platensis. International Journal of Food Properties, 19(10), 2349–2362. https://doi.org/10.1080/10942912.2015.1038564
[15] Nakagawa, K., Ritcharoen, W., Sri-Uam, P., Pavasant, P., & Adachi, S. (2016). Antioxidant properties of convective-air-dried Spirulina maxima: Evaluation of phycocyanin retention by a simple mathematical model of air-drying. Food and Bioproducts Processing, 100, 292–302. https://doi.org/10.1016/j.fbp.2016.07.014
[16] Dewayanto, N., Adhi, K., Negara, N. A. K., Sadewo, B. R., Nisya, A. F., Prakoso, O., Hariyadi, Sigit, U., Suyono, E. A., & Budiman, A. (2023). Study of low cost of microalgae chlorella sp. Harvesting using cationic starch flocculation technique for biodiesel production. IOP Conference Series: Earth and Environmental Science, 1151(1), 012042. https://doi.org/10.1088/1755-1315/1151/1/012042
[17] Phukoetphim, N., Salakkam, A., Laopaiboon, P., & Laopaiboon, L. (2017). Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: Logistic and modified Gompertz models. Journal of Biotechnology, 243, 69–75. https://doi.org/10.1016/j.jbiotec.2016.12.012
[18] Bennett, A., & Bogorad, L. (1973). Complementary Chromatic Adaptation In A Filamentous Blue-Green Alga. The Journal of Cell Biology, 58(2), 419–435. https://doi.org/10.1083/jcb.58.2.419
[19] Silveira, S. T., Burkert, J. F. M., Costa, J. A. V., Burkert, C. A. V., & Kalil, S. J. (2007). Optimization of phycocyanin extraction from Spirulina platensis using factorial design. Bioresource Technology, 98(8), 1629–1634. https://doi.org/10.1016/j.biortech.2006.05.050
[20] Box,G.E.P&Behnken,D.W. (1960). Some new three level designs for the study of quantitative variables, Technometrics, 2, 455–475
[21] Vuong, Q. V., Golding, J. B., Nguyen, M. H., & Roach, P. D. (2012). Production of caffeinated and decaffeinated green tea catechin powders from underutilised old tea leaves. Journal of Food Engineering, 110(1), 1–8. https://doi.org/10.1016/j.jfoodeng.2011.12.026
[22] Steele, D., & Steele, D. J. (2014). Cellular viability and the occurence and significance of chlorophyll allomers during phytoplankton turnover . Cellular viability and the occurrence and significance of chlorophyll allomers during phytoplankton turnover by Faculty of Science and Technology. September 2014
[23] Galvão, R. M., Santana, T. S., Fontes, C. H. O., & Sales, E. A. (2013). Modeling of Biomass Production of Haematococcus pluvialis. Applied Mathematics, 04(08), 50–56. https://doi.org/10.4236/am.2013.48A008
[24] Teleken, J. T., Galvão, A. C., & Robazza, W. D. S. (2018). Use of modified Richards model to predict isothermal and non-isothermal microbial growth. Brazilian Journal of Microbiology, 49(3), 614–620. https://doi.org/10.1016/j.bjm.2018.01.005
[25] Lavilla, I., & Bendicho, C., 2017, "Fundamentals of Ultrasound-Assisted Extraction", Water Extraction of Bioactive Compounds From Plants to Drug Development (pp. 291 316). Elsevier
[26] Carley, K.M., Kamneva, N.Y., Reminga, J., (2004). Response surface methodology. Center for computational analysis of social and organizational systems. Technical Report, CMU-ISRI-04-136
[27] Derringer, G., & Suich, R. (1980). Simultaneous Optimization of Several Response Variables. Journal of Quality Technology, 12(4), 214–219. https://doi.org/10.1080/00224065.1980.11980968
[28] Amdoun, R., Khelifi, L., Khelifi-Slaoui, M., Amroune, S., Asch, M., Assaf-ducrocq, C., & Gontier, E. (2018). The Desirability Optimization Methodology; a Tool to Predict Two Antagonist Responses in Biotechnological Systems: Case of Biomass Growth and Hyoscyamine Content in Elicited Datura starmonium Hairy Roots. Iranian Journal of Biotechnology, 16(1), 11–19. https://doi.org/10.21859/ijb.1339
[29] Cojocaru, C., Khayet, M., Zakrzewska-Trznadel, G., & Jaworska, A. (2009). Modeling and multi-response optimization of pervaporation of organic aqueous solutions using desirability function approach. Journal of Hazardous Materials, 167(1–3), 52–63. https://doi.org/10.1016/j.jhazmat.2008.12.078
[30] Hadiyanto, H., & Suttrisnorhadi, S. (2016). Response Surface Optimization of Ultrasound Assisted Extraction (UAE) of Phycocyanin from Microalgae Spirulina platensis. Emirates Journal of Food and Agriculture, 28(4), 227. https://doi.org/10.9755/ejfa.2015-05-193
[31] Mkruqulwa, U., Okudoh, V., & Oyekola, O. (2020). Optimizing Methane Production from Co-digestion of Cassava Biomass and Winery Solid Waste Using Response Surface Methodology. Waste and Biomass Valorization, 11(9), 4799–4808. https://doi.org/10.1007/s12649-019-00801-y

[32] Zhang, Q.-A., Zhang, Z.-Q., Yue, X.-F., Fan, X.-H., Li, T., & Chen, S.-F. (2009). Response surface optimization of ultrasound-assisted oil extraction from autoclaved almond powder. Food Chemistry, 116(2), 513–518. https://doi.org/10.1016/j.foodchem.2009.02.071
[33] Betiku, E., Akintunde, A. M., & Ojumu, T. V. (2016). Banana peels as a biobase catalyst for fatty acid methyl esters production using Napoleon's plume (Bauhinia monandra) seed oil: A process parameters optimization study. Energy, 103, 797–806. https://doi.org/10.1016/j.energy.2016.02.138
[34] Ngobeni, W. A., & Mulaba-Bafubiandi, A. F. (2023). Using response surface methodology to optimise alkaline gelatinisation of novel depressants. South African Journal of Chemical Engineering, 45, 240–246. https://doi.org/10.1016/j.sajce.2023.06.002
[35] Angeorsa, Franca., Christine Campestre., & Giansante, Lucia. (2006). Olive Oil (Second Edition). AOCS Press, 7, 113-172. https://doi.org/10.1016/B978-1-893997-88-2.50011-0