Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Article
Volume 11, Issue 2, 2021, 8895 - 8903
https://doi.org/10.33263/BRIAC112.88958903

Cultivation of *Spirulina platensis* Having Humic Acid as Substrate for Soap Production

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Received: 12.07.2020; Revised: 24.08.2020; Accepted: 26.08.2020; Published: 30.08.2020

Abstract: *Spirulina* is a pure and natural whole food, containing no synthetic ingredients or preservatives, consumed as Single Cell Proteins (SCP) by human beings and animals. In this study, *Spirulina* was cultivated with 0.9 % Humic acid (HA) along with the OFERR medium for the enhanced production of *Spirulina platensis*. The obtained *Spirulina platensis* was used for manufacturing natural soap with various components such as Olive oil, *Aloe vera*, and NaOH by employing RSM (Response surface methodology) –CCD (Central composite design). The soap produced having the optimal condition was found to have TFM (Total fatty matter) - 80%, and alkalinity - 9.0. The ideal composition for the improvement of soap was *S. platensis* - 3.75 g, Olive oil - 37.5 mL, *Aloe vera* gel – 15 g, and NaOH – 2 g with *S. platensis* being the key component.

Keywords: *Spirulina plantensis*; Humic acid; Olive oil; Aloe Alkalinity; soap production.

1. Introduction

Cyanobacteria, also known as blue, green algae are aquatic, photosynthetic small, and usually unicellular bacteria that often grow in colonies large enough to see [1]. Besides being the oldest known fossils, they were crucial in shaping the course of evolution and ecological change. They are important providers of nitrogen by fixing the atmospheric nitrogen. A few examples are *Spirulina*, *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii*, *Anabaena circinalis*, *Cyanophora paradoxa*, *Nostoc commune*, etc. *Spirulina* is a biomass of cyanobacteria (blue-green algae) that can be consumed by humans and animals [2]. It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries and has been commercialized in various countries as they are also used for a therapeutic purpose [3]. This is also a source of natural pigments [4].

Spirulina spp is loaded with many nutrients that include proteins, carbohydrates, and vitamins, minerals, carotene, and super antioxidants apart from trace elements. The protein content is as high as 60-70% of its dry weight 5g with a concentration of vitamins and lipid in the amount of 4-7%. Owing to the use of *Spirulina* spp as feed, methods to grow them on a large scale with different kinds of supplements is always an interesting area of research. Generally, mass cultivation methods like open ponds, photobioreactors, immobilized culture systems, and photoactivity bioreactor methods are used [5, 6]. The cost and composition of cultivation media, along with the growth rate of the algae, are the challenging factors for commercially viable production. Different media have been tried for the cultivation of *Spirulina* spp, such as Zarrouk's media, Rao's media, CFTIR media, OFERR media, and Bangladesh medium [7].

Humic substances are nutrients which support to grow plants, make the soil fertile and productive, increases water holding capacity, and seed germination. Humic acid also reduces the other fertilizer requirements, increases aeration of the soil, increases the protein and mineral contents of most crops [8]. Apart from these uses, The present study focused on enhancing the production of *S. platensis* using humic acid as a substrate, and an attempt was given here to make use of *S. platensis* in the production of soaps to enrich in antioxidants and an anti-allergic compound, which is good for human skin. Where the production of this soap using *S. platensis* was done by RSM, where RSM is commonly used for the optimization studies and activity-based studies [9-12].

2. Materials and Methods

2.1. Sample collection.

The algal culture was obtained from OFERR - Spirulina Nallayan Research Centre, Chennai, Tamilnadu, The strain was maintained in OFERR medium.

2.2. Effect of humic acid in S. platensis.

1000 mL of OFERR medium with different concentrations of Humic Acid (0.1% to 1%) was prepared. The medium was adjusted to pH 9 and inoculated with 2 mL of mother culture and was incubated for 14 days under 1500 lux. After the incubation period, the biomass was filtered using a muslin cloth and sundried till the moisture gets removed.

2.3. S. platensis cultivation.

5 × 3 ×1(length×breadth×height) meter tank was prepared with polythene sheet, and 100 L of the medium was added along with 0.9% of Humic Acid in the tank and inoculated with 1 L of mother culture. After 14 days, the biomass was harvested and dried.

2.4. Optimization of soap production using response surface methodology.

The quadratic equation model for predicting the optimal point was expressed according to Eq. 1, which was obtained from ANOVA suggested by Design expert V.7.0.0.

$$Y = b_0 + b_1A + b_2B + b_3C + b_4D + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_{44}D^2 + b_{12}AB + b_{13}AC + b_{14}AD + b_{23}BC + b_{24}BD + b_{34}CD \qquad \text{Eq. 1}$$

The experiment was designed by the design expert 8th trial version. The optimization process was carried out by RSM using *S. platensis*, Olive oil, *Aloe vera*, and NaOH. 32 trials were attempted. CCD was employed to analyze the optimal conditions for soap production from *S. platensis*. The optimization experiment was carried out in a 250 mL flask by four chosen independent process variables: *S. platensis* (X_1, grams), olive oil (X_2, mL), NaOH (X_3, grams) and *Aloe vera* (X_4, grams). The quantity of soap was used as the dependent output variable. A second-order polynomial quadratic equation was used to predict the optimum value and subsequently to elucidate the interaction between the variables. Where Y_i is the predicted response, A, B, C, D, are independent variables, b_0, is the offset term, b_1, b_2, b_3, b_4 are coefficient of linear effects, b_{11}, b_{22}, b_{33}, b_{44} are coefficient of squared effects and b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34} are coefficient of interaction terms. The regression equation contains four linear terms (A, B, C, D), four square terms (A^2, B^2, C^2, D^2) and six cross-interactions (AB, AC, A D, BC, BD, CD) terms plus 1 block term.

2.5. Production of soap.

NaOH was dissolved in distilled water along with *S. platensis*, *Aloe vera*, then it was mixed together with Olive oil and heated to room temperature again. The mixture was carefully poured into NaOH solution and blended until to "trace" form and allowed to mold. The procedure was modified from the previous study of Arasaretnam and Venujah [13]. According to the RSM design concentrations, dried *S. platensis*, Olive oil, and *Aloe vera* were taken. *S. platensis* powder was mixed with lye, olive oil, and *Aloe vera* in a batch reactor. The mixture was poured into the beaker and allowed to settle for 24 h. The solution in the beaker was centrifuged to separate soap as a product and glycerol as residue. The soapy material was dried in the required shape.

2.6. Evaluation of alkalinity and total fatty matter (TFM).

Alkalinity and TFM were done as described earlier by [13, 14].

2.7. Statistical methods.

The analysis was performed by the statistical tool – Design expert and IBM SPSS 23.

3. Results and Discussion

3.1. S. platensis cultivation using humic acid.

Experiments were carried out to evaluate the effect of humic acid on the growth of *S. platensis* based on the biomass obtained, and the results are provided in figure 1.

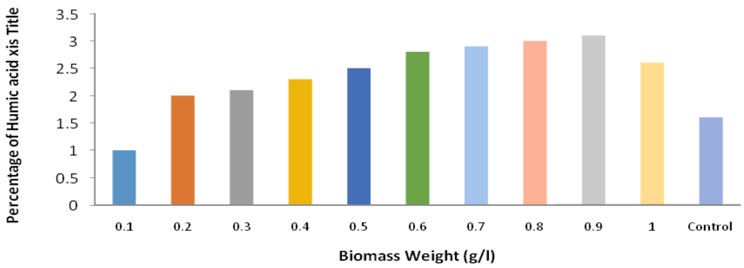

Figure 1. Effect of Humic acid on the growth of *S. platensis.*

S. platensis is an important SCP when consumed has several benefits, such as antioxidant, anti-obesity, and anti-carcinogen. Among various concentrations of humic acid, 0.9% has given maximum biomass (Fig 1) when compared with other trials. Another study also reported a maximum growth rate of 0.2% of Humic acid in the Zarrouk medium. Reports suggest that formulated humic acid with seaweed liquid fertilizer (0.5 : 8.5%) enhanced the growth of *Abelmoschus esculentus* [15-17].

3.2. Optimization of key components for soap production.

The components significant for the production of *S. platensis* were assessed using Response surface methodology (RSM), a statistical approach, and the results are provided in Table 1. The objective of RSM is to optimize the output variable, which is influenced by input variables [18]. In this study, four variables were selected, namely *S. platensis*, Olive oil, *Aloe vera*, and NaOH (Table 1). *Spirulina* contains rich gamma-linolenic acid 4.07-22.51%, and Olive oil contains rich of Triacylglycerols (98 -99%). TGA is a group of glycerol esters with different fatty acids [19, 20]. Among them, monounsaturated oleic acid is the predominant fatty acid. It contains phenolic and polyphenolic compounds that have significant antioxidant and anticancer activity [21]. Also, olive oil is the best soap, as it is alkaline [13]. *Aloe vera* has been used for medicinal, and skin care properties and the role of NaOH is for saponification [22,23]. The Central Composite Design has given 30 trials, and the response was soap on a weight basis. Among the trials, maximum soap production was obtained in 30th trial. The results obtained in the present investigation showed R^2 value to be 99.5 %, which is in accordance with the report by Okolie *et al*. [23]. He reported an R^2 value of 100 % when the variables for soap production were optimized by RSM. When *S. platensis* was added as a component along with olive oil, *Aloe vera*, and NaOH, there was significant p-value, thereby suggesting that RSM was suitable to identify the important components. Another study carried out by Salguero *et al.* revealed that maximum soap yield was obtained at trial $A_2B_2C_1$ where the composition was (Endothermic + 15% NaOH + 12 h)[24]. The TFM value was 63.18 to 63.54 %, and the pH ranged from 9 to 11. Similarly, Pathirana *et al*. [25] reported that soap production was optimized by the Taguchi method with four variables, namely coconut oil, palm oil, caustic soda, and temperature. They obtained maximum yield at $a^1b^1c^1d^1$ (Coconut oil, NaOH 18%, and Temperature 80ºC) with TFM to found be (76.66 %).

Table 1. Experimental design matrix using RSM with CCD and responses for soap production.

Std	Run	A: *S. platensis* g	B: Olive oil mL	C: NaOH g	D: *Aloe vera* g	Soap (g)	
						Actual	Predicted
1	1	2.5	25	1	10	20.7	20.6
2	13	5	25	1	10	21	21.1
3	20	2.5	50	1	10	22.1	22.1
4	3	5	50	1	10	20.8	20.6
5	16	2.5	25	3	10	20.6	20.6
6	9	5	25	3	10	20.1	20.1
7	30	2.5	50	3	10	22.1	22.0
8	7	5	50	3	10	19.4	19.5
9	6	2.5	25	1	20	18.7	18.7
10	21	5	25	1	20	20.2	20.3
11	2	2.5	50	1	20	19.9	19.9
12	23	5	50	1	20	19.5	19.5
13	19	2.5	25	3	20	19.5	19.7
14	26	5	25	3	20	20.2	20.2
15	27	2.5	50	3	20	20.8	20.8
16	5	5	50	3	20	19.2	19.3
17	22	1.25	37.5	2	15	21.6	21.6
18	8	6.25	37.5	2	15	20.8	20.7

Std	Run	A: *S. platensis* g	B: Olive oil mL	C: NaOH g	D: *Aloe vera* g	Soap (g)	
						Actual	Predicted
19	12	3.75	12.5	2	15	18.2	18.1
20	11	3.75	62.5	2	15	18.7	18.7
21	14	3.75	37.5	0	15	20.3	20.4
22	24	3.75	37.5	4	15	20.4	20.2
23	29	3.75	37.5	2	5	22.4	22.5
24	28	3.75	37.5	2	25	20.6	20.4
25	4	3.75	37.5	2	15	23.4	23.6
26	10	3.75	37.5	2	15	23.1	23.6
27	15	3.75	37.5	2	15	23.8	23.6
28	18	3.75	37.5	2	15	23.4	23.6
29	17	3.75	37.5	2	15	23.6	23.6
30	25	3.75	37.5	2	15	24.2	23.6

Our results are promising when compared to these findings and hence can be upscaled in terms of production. Usharani *et al*. [26] mentioned that *Spirulina plantensis* has potential antimicrobial activity, and Sedef *et al*. [27] reported that *Spirulina plantensis* extracts showed no genotoxicity and was also incorporated in skin cream. Additionally, it was found to have anticancer activity against the L929 HS2 cell line, which was determined by an *in vitro* scratch assay. It is an added advantage that *S. platensis* is contained soap.

3.3. Fit summary for model fitting and ANOVA.

ANOVA as the analysis is performed to identify the significance of the experiment and the compounds used in the experiment (Table 2).

Table 2. Analysis of variance (ANOVA) for the experimental results of the CCD quadratic model for soap production.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Model	77.9345	14	5.56675	87.05604	< 0.0001	Significant
A-Spirulina	1.306667	1	1.306667	20.4344	0.0004	
B-Olive oil	0.601667	1	0.601667	9.409209	0.0078	
C-NaOH	0.026667	1	0.026667	0.417029	0.5282	
D- *Aloe vera*	6.406667	1	6.406667	100.1911	< 0.0001	
AB	4	1	4	62.5543	< 0.0001	
AC	1.1025	1	1.1025	17.24153	0.0009	
AD	1.21	1	1.21	18.92268	0.0006	
BC	0.0225	1	0.0225	0.351868	0.5619	
BD	0.09	1	0.09	1.407472	0.2539	
CD	0.9025	1	0.9025	14.11381	0.0019	
A^2	10.15048	1	10.15048	158.739	< 0.0001	
B^2	46.05762	1	46.05762	720.2755	< 0.0001	
C^2	18.48048	1	18.48048	289.0083	< 0.0001	
D^2	7.801905	1	7.801905	122.0107	< 0.0001	
Residual	0.959167	15	0.063944			
Lack of Fit	0.230833	10	0.023083	0.158467	0.9932	not significant
Pure Error	0.728333	5	0.145667			
Cor Total	78.89367	29				

AB - *S. platensis* Olive oil, AC- *S. platensis* NaOH, AD,- *S. platensis Aloe vera*, BC–Olive oil NaOH, BD–Olive oil *Aloe vera*, CD- NaOH *Aloe vera*, A^2- *S. platensis*, B^2- NaOH,C^2- NaOH, D^2–Aleovera.

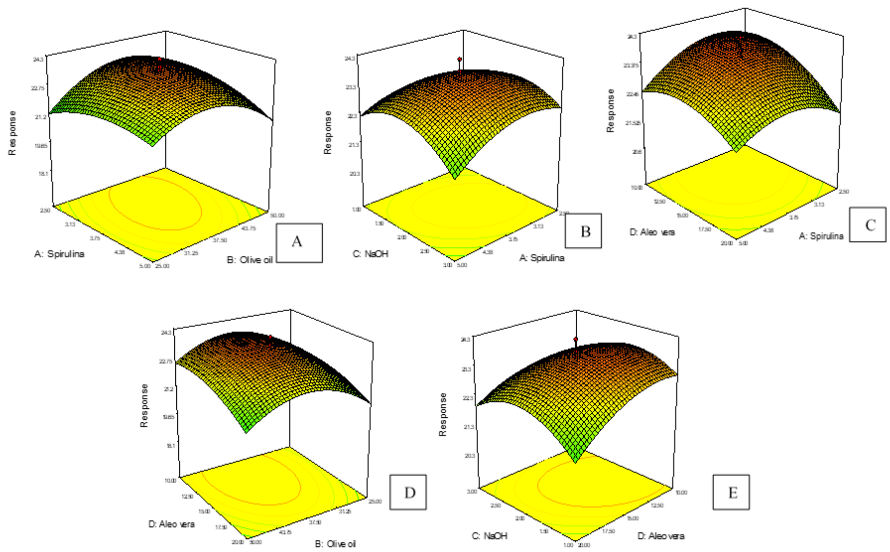

Figure 2. Response Surface Curve (3D plot) of soap production from *S. platensis* mass showing the interaction between a) *S. platensis* and Olive oil b) NaOH and *S. platensis* c) *Aloe vera* and *S. platensis* d) *Aloe vera* and Olive oil e) NaOH and *Aloe vera.*

ANOVA was used to estimate the significance of coefficients, and the p values indicated the significance ($p > 0.005$), which also showed the interaction strength between each independent variable. The model in terms of actual variables of soap weight was regressed by mainly considering the significant terms and was expressed by Eq. 2.

$$SOAP = 23.58-0.233A +0.1583B -0.033X_3-0.517D -0.5AB-0.2625AC +0.275AD -0.037BC-0.075BD + 0.2375CD -0.6083A^2-1.2958B^2- 0.8208C^2-0.53D^2 \quad \text{(Eq. 2)}$$

Table 2 shows the analysis of variance (ANOVA) for the experiment. Multiple regression analysis was carried out considering full quadratic model equation- (2) on the responses to evaluate the adequacy of fit, and results are reported in Table 2. The model is highly significant ($p < 0.001$), and the coefficient determination (R^2-values) was shown as R^2=0.995, indicating 99.5% of the variability in the soap production could be explained the model and only less difference 0.5% total variation were not explained by the model. The adjusted R^2 value is 0.9904, which is indicating 99.04% and Predicted R^2= 0.9763 for the responses as reported in Table 2. Therefore, the models were found to be adequate in representing the response data of the quantity of soap the can be further used for analysis and prediction purposes. Figure 2 demonstrates the interaction between the variables in three-dimensional response surface plots. Fig 2 (a) indicates the combined effect of *S. platensis* and olive oil on soap production increased due to the influence of *S. platensis* and olive oil at NaOH 2 g and *Aloe vera* 15g hence maximum soap production (24.2 g) was obtained. The interaction between the *S. platensis* and olive oil were most effective for soap production. The reason for the increase in soap production observed from the ANOVA table is due to the higher F value of AB, which indicates the great extent of soap production. Fig 2 (b) shows the combined effect of NaOH and *S. platensis* soap yield is obtained lower, may be due to an increase in NaOH. Since the ANOVA table is the lower F value AC and the p-value is relatively not significant (0.0009). Fig 2 (c) *Aloe vera* and *S. platensis* on soap yield are slowly decreased due to the increase of *Aloe vera* and *S. platensis* concentration at NaOH 2g and Olive oil 37.5 mL. *Aloe vera* is a significant compound in this reaction as the ANOVA table F value shows 100.19, and the p-value is less than 0.0001. But combined with *S. platensis* the soap yield is lower. The ANOVA table shows the lower F value of AD. Interaction between the *Aloe vera* and olive oil a part of the experimental study was determining the optimum concentration of *Aloe vera* and olive oil Fig 2 (d). Since the yield was increased to 22.75% but the ANOVA table is the lower value of F of BD. Fig 2 (e) Interaction of NaOH and *Aloe vera* the quantity of soap yield is decreasing. Similarly, the ANOVA table shows the lower F value of CD. This study provides a possibility of using *S. platensis* grown with humic acid for soap production as the materials used in this study are natural and organic and devoid of harsh chemicals.

3.4. Evaluation of alkalinity and total fatty matter (TFM).

Determining alkalinity and Total fatty matter (TFM) are important parameters for evaluating the acceptance level of soap.

Table 3. Results of soap alkalinity and TFM.

Parameters	Range
Alkalinity	9.0
Total fatty mater	80 %

The 30th trial soap production was subjected to estimate for its alkalinity, and TFM was found to be pH - 9.0 and 80 % TFM, respectively (Table 3), and this is considered to be ideal as far as the conditions for bathing soaps are concerned [28-31]. Hence the results suggest that *S. platensis* soap would be suitable for skin usage.

4. Conclusions

This study revealed that *S. platensis* could be produced by the various medium. But OFERR medium, along with humic acids was enhancing maximum growth. Thus, produced *S. platensis* can be used for soap production. Among the optimization trails, maximum soap production was obtained at 30th trial and optimized at the concentration of *S. platensis* - 3.75 g, Olive oil - 37.5 mL, *Aloe vera* gel – 15 g, and NaOH – 2 g along with acceptable parameters like TFM and Alkalinity was observed in the same. This study identifies that the soap from *Spirulina platensis* can be beneficial for human skin nourishment. Generally, *Spirulina* has been used only a single-cell protein (SCP), but this study stated that it could also be used for soap production.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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