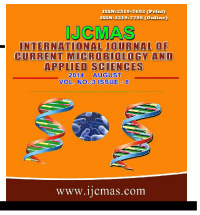
Int.J.Curr.Microbiol.App.Sci (2014) 3(8) 904-909

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 8 (2014) pp. 904-909**
http://www.ijcmas.com



Original Research Article

Comparison of Different Extraction methods for Phycocyanin Extraction and Yield from *Spirulina platensis*

S.Sivasankari*, Naganandhini and David Ravindran

Department of Biology, Gandhigram Rural Institute-Deemed University,
Dindigul, Tamilnadu, India
**Corresponding author*

Keywords

Phycocyanin Extraction, *Spirulina platensis*, blue-green algae

A B S T R A C T

A wide range of phycocyanin extraction methods were studied for the processing of *Spirulina* biomass. Drying methods were resulted in approximately 50% loss of phycocyanin. Therefore fresh wet biomass was suitable for phycocyanin extraction. Among the various extraction methods tried freezing and thawing of cells yielded higher amount of phycocyanin about 0.38 mg/g, homogenization using mortar and pestle yielded 0.06mg/g while organic and inorganic acid treatment yielded up to 0.06 mg/g and 0.28mg/g, sodium phosphate buffer and sonication yielded 0.25mg/g and 0.02mg/g of phycocyanin respectively resulted from 1g of *Spirulina* wet biomass treated with 2ml of volume of solvent which were incubated at 24hr, 48hr and 72 hr to analyse any remarkable yield in the concentration of phycocyanin was examined. The significance of phycocyanin extraction methods under 3 different time interval of incubation were comparatively studied and analysed using two way (ANOVA) which resulted the solvent treated biomass at 24hr, 72hr showed extremely significant and for the variance between 24hr and 48hr showed significant in the yield of phycocyanin at a level of $p < 0.0001$ has the percentage of total variance is 0.95% interaction, 2.49% column factor and 96.17% row factor respectively.

Introduction

Spirulina is a blue-green algae owing to the presence of both chlorophyll (green) and phycocyanin (blue) pigments in its cellular structure . The algae that live in habitats under high solar irradiation have accessory pigments to protect them from radiation damage and oxidation, because of the conjugated double bonds present in the chromophores .This phycocyanin could be extracted from *Spirulina platensis* which has been widely used in commercial applications in the food and cosmetic industry as a natural blue dye, Alfredo walter, *et al.*, (2011). The primary potential of this molecule seems to be their use as a natural dye, but emerging new studies have shown the properties related to health benefits and wide range of pharmaceutical applications. In addition phycobiliproteins are widely used in laboratory tests and

immunological assays , because of their properties such as high fluorescence good storage stability at temperatures between 4 and 10°C ,its isoelectric point close to 4.65 making them easily linkable to antibodies and other proteins by conventional techniques without changing its spectral charecteristics have high molecular absorbance coefficient and emission, oligomeric stability and high photostability.This review describes recent findings about the yield of Phycocyanin, using different extraction methods at various interval of time.

In general the extraction methods are the key factor for the maximum recovery of phycobiliproteins in the natural state from the algae. The phycobili protein extraction involves rupture the cell biomass for the extraction of protein from the cell, Hemlata, *et al.,* (2011). Cyanobacteria are extremely resistant for disruption of their cell walls.Hence the use of variations in the osmotic pressure, abrasive conditions, chemical treatment, freezing and thawing ,sodium phosphate buffer and sonication, Rachen Duangsee, *et al.,* (2009) apart from the usage of these methods mechanical disintegration methods are currently preferred for large scale operations.

The objective of this study is to evaluate the effect of different methods in the yield of phycocyanin. In this work the best method for phycocyanin extraction from *Spirulina platensis* was first investigated subsequently the effects of solvent at three different incubation period. The cell suspension was again examined for any remarkable increase in the yield of phycocyanin.

Materials and Methods

The microalgae Spirulina platensis was studied for the extraction of phycocyanin.The algae was obtained from the laboratory department of biology in Gandhigram Rural Institute Deemed University,Gandhigram -Dindigul district, Tamilnadu, India.The algae was cultivated in an open batch photobioreactor supplemented with zarrouk medium at pH 9.8 illuminated under white lamp and aerated with atmospheric air 8ml/min.

Extraction procedures

The optimized extraction of phycocyanin production from *Spirulina platensis* was studied with 6 extraction methods homogenisation, freezing and thawing, sodium phosphate buffer, organic acid, inorganic acid and sonication. From the open batch photobioreactor *Spirulina* culture was taken for each method and it was centrifuged at 5000 rpm for 20 mins to collect 1 gram of wet biomass.

Homogenisation: This method involves crushing of cells using mortar and pestle in presence of acid washed neutral sand using 50 mm phosphate buffer at pH 6.8. Freezing and thawing: The cell biomass was subjected to repeated freezing and thawing for 24 or 48 hr. The freezing and thawing was repeated twice with 24 hr intervals. Sodium phosphate buffer: The sodium phosphate buffer was prepared at pH 7.0 treated with cell biomass incubated at room temperature.

Inorganic acid extraction: The wet biomass was treated with 12M concentration of hydrochlocric acid in the proportion 1:2 (biomass: acid) and then left for 24 hrs at room temperature. Organic acid extraction: The wet biomass was treated with 1M of acetic acid in the 1:2 proportion and allowed to incubate at room temperature for 24 hrs. Sonication: The biomass was sonicated at 50khz in an ultrasonic bath for 30 mins.

The biomass treated with various solvents in order to disrupt the cell walls and consequently to isolate phycocyanin.The samples were centrifuged at 5000 rpm for 15 mins and the supernatant was examined under the UV-VIS spectrophotometer to verify the phycocyanin extraction yield (equation 1).

Analytical procedures

The evaluation of phycocyanin extraction based on the concentration of phycocyanin was set by equation 1 deduced by Bennett and Bogorad (1973).

$$PC = \frac{A_{620} - 0.474\ A_{652}}{5.34}$$

Where PC: Concentration of phycocyanin in mg/ml ,Abs 620 = Absorbance of the sample at 620 nm and Abs 652 = Absorbance at 652 nm.

The extraction yield was calculated by using the equation 2

$$Yield = \frac{(PC)\ V}{WB}$$

Where yield is the extraction, yield of phycocyanin in terms of mg of phycocyanin /wet biomass (g),V is the solvent volume (ml) and WB is the wet biomass (g).

Statistical Analysis

The results were analysed by two way analysis of variance (ANOVA) followed by Bonferroni post tests statistica. To validate the results reproducibility each assay was done in replicate. All analysis were performed considering a level of 95% of confidence ($p<0.0001$)

Results and Discussion

The *Spirulina* wet biomass undergoes various methods for the extraction of phycocyanin showed the presence of this molecule in the supernatant.Different procedure for phycocyanin extraction was comparatively studied with freshly harvested biomass and the data was presented in the form of mg/g (mg of phycocyanin per g of wet weight of *Spirulina platensis*),termed as extraction yield.

The most important requirement is to obtain the phycobiliprotein from cyanobacterium is optimizing the extraction and yield of phycocyanin at various interval of time 24hr, 48hr and 72 hr incubated for any significant increase in the yield of phycocyanin.The release of phycocyanin is related to the cell rupture but *Spirulina* have resistant multilayered cell walls, making the extraction procedure difficult.

In this study the yield of phycocyanin ranges from 0.02 to 0.76 mg/g for various method of extraction.Homogenisation using pestle and mortar and sonication at 50 khz yielded 0.02-0.06 mg/g where organic acid 12M HCl also resulted 0.06mg/g showed poor yield of phycoyanin , while freezing and thawing at 4°C showed high yield of phycocyanin 0.38mg/g and inorganic acid using acetic acid 1M and sodium phosphate buffer at pH 7.0 exhibited fairly good yield of phycocyanin 0.286mg/g and 0.25 mg/g.The results of phycocyanin concentration (mg/ml) observed under the spectrophotometer were given in the figure1,table1correspondingly. While the yield of phycocyanin (mg/g) at various interval of incubation time is also displayed in the figure 2 ,table 2 respectively.

Figure 1: Phycocyanin extraction from Spirulina platensis wet biomass of 1g undergoes different treatments and incubated at 24hr, 48hr and 72 hr were observed under UV visible spectrophotometer at 620 and 652 nm.1- Homogenization in mortar and pestle at pH 9.5; 2 - Freezing and thawing; 3 - Sodium phosphate buffer at pH 7.0; 4 - Organic acid (12 M HCl at pH 1.20); 5 - Inorganic acid (1m acetic acid at pH 1.20); 6 - Sonication at pH 9.5 calculated by using equation(1).

Figure 2 : The extracted phycocyanin yield for 1g wet biomass was calculated for different treatments 1- Homogenisation; 2- Freezing and thawing; 3- Sodium phosphate buffer; 4-Organic acid; 5-Inorganic acid and 6- Sonication at 24hr,48 hr and 72 hr respectively using equation (2).

Some papers have been reported that the phycocyanin extraction from biomass showed freezing and thawing was considered to be best when compare with other methods studied by the authors, Sarada.R, *et al.,* (1999).This method analysed the significance of solvent treated cell biomass at various interval of time has improved the yield phycocyanin. A comprehensive technique must include quick and efficient disruption treatment for a quantitative extraction and recovery of the released pigment.In the present study the efficient method for phycocyanin extraction from wet biomass was analysed and presented an extraction yield of 64 mg/g at 24hr and 76.0mg/g at 48hr.The concentration of phycocyanin was about 0.32mg/ml at 24hr and 0.38mg/ml at 48 hr.The level of significance at $p<0.0001$results 0.95% of having total variance, Moraes.C.C, *et al.,* (2011), 2.49% of column factor and 96.17% row factor showed extremely significant were analysed by using two way analysis of variance test.

Nomenclature

PC - Phycocyanin concentration
WB - wetbiomass
Abs 620nm - Absorbance at 620
Abs 652nm - Absorbance at 652
V - Solvent volume
Yield - Extraction yield of phycocyanin in mg of phycocyanin /wet biomass

Table.1 The cell biomass was treated with various solvents and incubated at three interval of time to analyse the concentration of phycocyanin using equation.1

Treatment	Phycocyanin concentration from solvent treated biomass at various interval of time								
	24hr mg/ml	%	pH	48hr mg/ml	%	pH	72hr mg/ml	%	Ph
Homogenisation	0.02	2	9.5	0.06	6	9.5	0.06	6	9.5
Freezing and thawing	0.32	32	9.5	0.38	38	9.5	0.38	38	9.5
Sodium phosphate buffer	0.17	17	7.0	0.25	25	7.0	0.25	25	7.0
Organic acid	0.027	2.7	1.2	0.068	6.8	1.2	0.068	6.8	1.2
Inorganic acid	0.262	26.2	1.2	0.286	28.6	1.2	0.286	28.6	1.2
Sonication	0.01	1	9.5	0.04	4	9.5	0.04	4	9.5

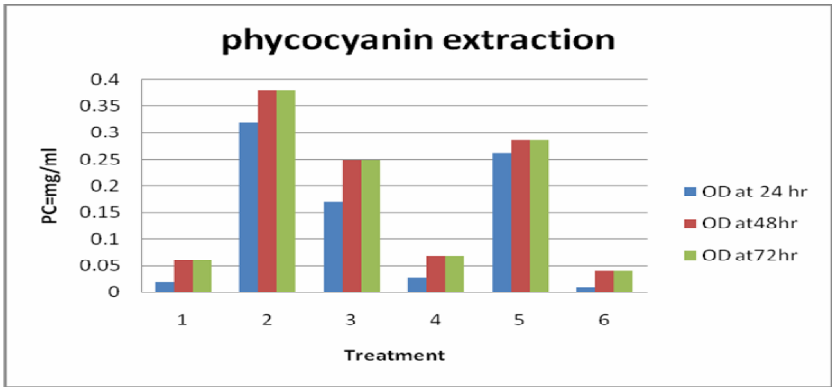
Table.2 Yield of phycocyanin from incubated biomass using equation 2

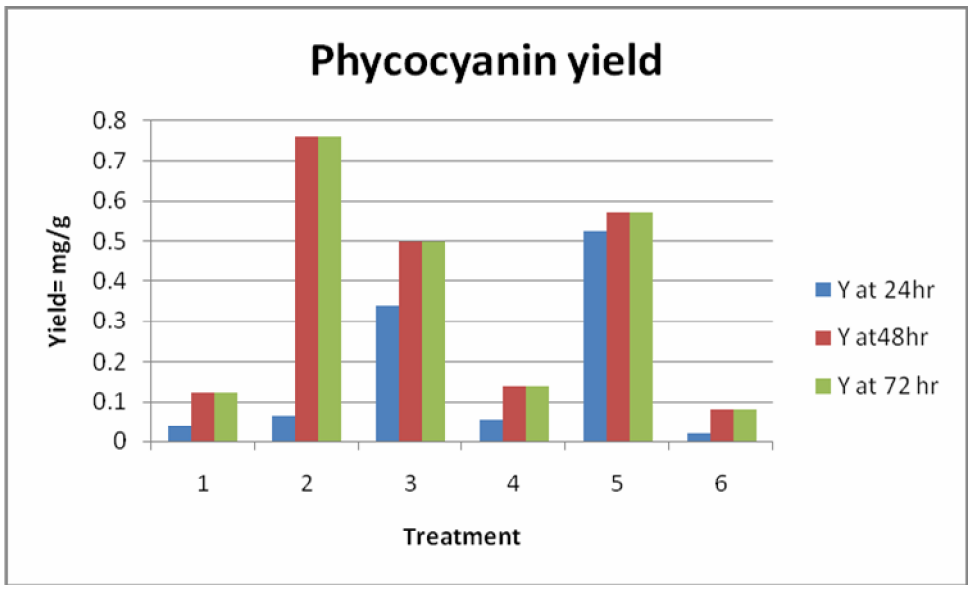
Treatment	Yield of phycocyanin extraction at various interval of time								
	24hr mg/g	%	pH	48hr mg/g	%	pH	72hr mg/g	%	pH
Homogenization	0.04	4	9.5	0.12	12	9.5	0.12	12	9.5
Freezing and thawing	0.064	6.4	9.5	0.76	76	9.5	0.76	76	9.5
Sodium phosphate buffer	0.34	34	7.0	0.50	50	7.0	0.50	50	7.0
Organic acid	0.054	5.4	1.2	0.136	13.6	1.2	0.136	13.6	1.2
Inorganic acid	0.524	52.4	1.2	0.572	57.2	1.2	0.572	57.2	1.2
Sonication	0.02	2	9.5	0.08	8	9.5	0.08	8	9.5

Table.3 Incubation at various interval of time significantly increase the yield of phycocyanin at 24 hr, 48hr and 72 hr

Source of Variation	**% of total variation**	**P value**
Interaction	0.95	< 0.0001
Column Factor	2.49	< 0.0001
Row Factor	96.17	< 0.0001

Source of Variation	**P value summary**	**Significant?**
Interaction	***	Yes
Column Factor	***	Yes
Row Factor	***	Yes





References

Alfredo walter,Julio Cesar De Carvalho,Vanete Thomaz Soccol,Ana Barbara Bisinella De Faria,Fanessa Ghiggi and Carlos Ricardo Soccol , 2011. Study of phycocyanin production from *Spirulina platensis* under different light spectra, *Braz.Arch.Biol.Technol*.,54:675-682.

Bennet, A.and Bogorad, L.1973. Complementary Chromatic adaptation in a filamentous blue green alga, *J.Cell Biol*.,58: 419-435.

Moraes, C.C., Luisa Sala,G.,Cerveira,P and kalil,S.J. 2011. C- Phycocyanin extraction from *Spirulina platensis* wet biomass, *Brazilian journal of chemical engineering*.,28:45-49.

Hemlata ,Gunjan Pandey,Fareha Bano and Tanseem Fatma. 2011. Studies on *Anabaena sp*.NCCU-9 with special reference to phycocyanin, *J.Algal Biomass Utl*.,2:30-51.

Rachen Duangsee,Natapas Phoopat and Suwayd Ningsanond.2009. Phycocyanin extraction from *Spirulina platensis* and extract stability under various pH and temperature,As. *J.Food Ag-Ind*.,2:819-826.

Sarada ,R., Manoj, G.,Pillai ,G and Ravishankar, A.1999. Phycocyanin from *Spirulina sp*: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin, *Process Biochemistry*.,34:795-801.