A Large-Scale Preparation Method of High Purity C-Phycocyanin

Wenjun Song, Cuijuan Zhao, and Suying Wang

***Abstract*—In this paper, a process for large-scale purification of high purity C-phycocyanin (C-PC) has been set up. Spirulina platensis dried powder was incubated with 1 mg/ml lysozyme and then ruptured by using high pressure homogenizer. The crude extract was precipitated by 50% saturation ammonium sulfate, and purified by hydrophobic interaction chromatography using Phenyl Sepharose 6 FF, ion exchange chromatography using DEAE Sepharose FF and gel filtration chromatography using Sephacryl S-100 HR. The final recovery of C-PC was 42.03% with purity ratio (A620/A280) of 5.32.**

***Index Terms*—Spirulina platensis, c-phycocyanin, purification, chromatography.**

I. Introduction

C-phycocyanin (C-PC) is the major component of the phycobiliprotein in Spirulina. The fundamental unit of phycocyanins were α and β subunits. The subunits are associated in an (α β) protomers, which in turn can be associated in trimmers $(\alpha\ \beta)_3$ and hexamers $(\alpha\ \beta)_6$ [1], [2]. The phycocyanins have an apparent molecular mass of 140–210 kDa [3]-[5]. The absorption maxima for C-PC is between 610 and 620 nm, and C-PC usually appear dark cobalt blue [6]. C-PC is not only used as nutrient ingredients and natural dyes for food and cosmetics [7], [8], but also used in diagnostics, biomedical research and therapeutics are possible [9]-[11]. The purity of C-PC is generally evaluated using the absorbance ratio of A620/A280, wherein a purity of 0.7 is considered as food grade, 3.9 as reactive grade and greater than 4.0 as analytical grade [12].Several methods have been developed for the separation and purification of C-PC, such as density gradient centrifugation, ammonium sulfate precipitation, chromatography method and aqueous two phase extraction [13]-[17].

In this study, we designed a method involved ammonium sulfate precipitation, hydrophobic interaction chromatography, ion exchange chromatography and gel filtration chromatography in large scale. The purified C-PC was further characterized and identified by UV–VIS spectra, SDS–PAGE and Native-PAGE.

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II. Materials and Methods

A. Materials and Instruments

Spirulina platensis dried powder was purchased from Yijian Biological Products Co., Ltd (Inner Mongolia, China).

Chromatography equipment was AKTA Purifier (GE Healthcare).

B. C-Phycocyanin Extraction

Spirulina platensis dried powder were suspended at 0.06 g/ml in 20 mM Tris–HCl buffer (contain 10mM EDTA, pH 6.5) then brought to 1 mg/ml lysozyme （Amresco, USA）. The suspension was incubated for 1h at 30 ℃ [18].

The cells were ruptured with high pressure homogenizer D-15M (PhD Technology International LLC) at 10-12,000 p.s.i. and 4-8 ℃. The resultant slurry was centrifuged at 10,000g for 15 min at 4 ℃ to remove the cell debris. The precipitate was discarded and the supernatant crude extract was collected. The pH of crude extract was adjusted to pH 8.1 for the following steps.

C. Study of the Ammonium Sulfate Precipitation

100 ml crude extract was added to eight 250 ml flasks respectively. Different saturation of ammonium sulfate (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%) was gradually added into each crude extract respectively. Resulting solution was kept for 2 h at 4 ℃ and centrifuged at 12,000 g for 30 min. The each supernatant was pooled and subjected to different saturation of ammonium sulfate (10%, 20%, 30%, 40%, 50%, 60%, 70%, 95%) respectively in a manner similar to that of the first ammonium sulfate precipitation operation. Each pellet was resuspended in 50 ml of 20 mM Tris–HCl buffer (pH 8.1) respectively and dialyzed against the same buffer at 4 ℃. The resulting protein solutions were diluted to 100ml and then analyzed by SDS-PAGE, total protein determination and spectroscopic measurement.

D. Phycocyanin Purification

The entire procedure was carried out at 4 ℃.

1) Ammonium sulfate precipitation

Ammonium sulfate was gradually added in 1 L crude extract to achieve 50% saturation with continuous stirring. Resulting solution was kept for 2 h and centrifuged at 12,000 g for 30 min. The pellet was resuspended in 500 ml of 20 mM Tris–HCl buffer (containing 0.86 M ammonium sulfate, pH 8.1) and centrifuged at 12,000 g for 20 min. The supernatant was pooled for the following purification steps.

2) Hydrophobic interaction chromatography

Hydrophobic interaction chromatography was prepared

using 140ml Phenyl Sepharose 6 FF（HS, GE Healthcare, Product code: 17-0973-05) in Column XK 50/20 (GE Healthcare, Product code: 18-1000-71). The column was pre-equilibrated with 20 mM Tris–HCl buffer (containing 0.86 M ammonium sulfate, pH 8.1). Sample was loaded at a flow rate of 100 cm/h. Then the column was washed with 5 bed volumes of the same buffer. The loaded protein was eluted with 10 bed volumes of a lineal gradient from 0.86 to 0 M ammonium sulfate in 20 mM Tris–HCl buffer (pH 8.1) at a flow rate of 120 cm/h. Blue-coloured fractions were pooled and diluted its conductivity below 8 mS/cm by using 20 mM Tris–HCl buffer (pH 8.1) for the following steps.

3) Ion exchange chromatography

Ion exchange chromatography was prepared using 30ml DEAE Sepharose FF (GE Healthcare, Product code: 17-0709-10) in Column XK16/20 (GE Healthcare, Product code: 18-8773-01). The column was pre-equilibrated with 20 mM Tris–HCl buffer (pH 8.1). Sample was loaded at a flow rate of 200 cm/h. Then the column was washed with 10 bed volumes of the same buffer. The column was first eluted with 10 bed volumes of 0.15 M NaCl in 20 mM Tris–HCl (pH 8.1), and the C-PC was then eluted with 0.23 M NaCl in 20 mM Tris–HCl (pH 8.1). Finally, the column was eluted with 1 M NaCl at a flow rate of 400 cm/h.

4) Gel filtration chromatography

Gel filtration chromatography was prepared using 1.8 L Sephacryl S-100 HR (GE Healthcare, Product code: 17-0612-01) in Column XK 50/100 (GE Healthcare, Product code: 18-8753-01). The column was pre-equilibrated with 10mM K-phosphate buffer (containing 0.15 M NaCl, pH 7.4). 80 ml sample was loaded at a flow rate of 12 cm/h, and the column was then washed with same buffer at a flow rate of 25 cm/h. Fractions of every 5 ml were collected when A280 above to 50 mAU.

E. Analysis

1) Spectroscopic measurement

UV–Vis absorbance spectra were recorded on T60 UV-VIS Spectrophotometer (PERSEE, China).The purity of C-PC was evaluated according to the absorbance ratios (A620/A280) [19].The amount of C-PC in the sample was calculated using simultaneous equations of Bennett and Bogorad (1973) as follows [20]:

$$C-PC(mg/ml)=\frac{(OD_{620}-0.7OD_{650})}{7.38} \qquad (1)$$

2) Total protein determination

The total protein contents were determined by the method of Lowry *et al.* (1951) [21],

3) Electrophoretic methods

SDS–PAGE analysis

SDS-PAGE was performed according to Laemmli [22] with stacking gel of 5% and resolving gel of 15% acrylamide concentration. The gel prepared was 1.0 mm thick, containing 0.1% (w/v) SDS. Samples were mixed with equal volume of sample buffer containing 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 0.002% (w/v) bromophenol blue and 60 mM Tris-HCl (pH 6.8), and boiled for 10 min. Electrophoresis was carried out at room temperature and the proteins were visualised with Coomassie brilliant blue (G250) staining. Protein molecular mass standard PR1600-20 (Solarbio, China) having 14.4-97.4 kDa polypeptides were used.

Native gel electrophoresis

Native polyacrylamide gel (1.0 mm thick) consisted of 5% stacking and 10% resolving acrylamide concentration in 25 mM Tris–HCl (pH 8.3) and 250 mM glycine. Samples were mixed with equal volume of sample buffer containing 60 mM Tris–HCl (pH 6.8), 4% (v/v) glycerol and 0.002% (w/v) bromophenol blue. Electrophoresis was carried out at room temperature and the proteins were visualised with Coomassie brilliant blue (G250) staining.

The results were analyzed by Doc-It LS software (UVP, USA).

4) Zinc acetate staining

The resolved native and SDS gels were soaked in 20 mM zinc acetate solution for 5 min at room temperature [23]. The bilin fluorescence was observed under UV light using BioDoc-It Imaging System (UVP, USA)

5) Molecular weight by gel filtration

Gel filtration column Sephadex G-100 (50 ·1.5 cm) (GE Healthcare, Product code: 17-0060-01) was used to determine the molecular weight of native C-PC. The column was pre-equilibrated with 10mM K-phosphate buffer (containing 0.15 M NaCl, pH 7.4). The column was calibrated using ribonuclease (13.7 kDa), egg allumin (45 kDa), bovine serum albumin (67 kDa), and gamma γ-globulin (165 kDa).

III. Results and Discussion

A. Extraction of C-PC

We use high pressure homogenization with lysozyme treatment for the initial protein extraction. The method of high pressure homogenization is perfectly reproducible in industrial scale. It is a continuous mechanical process quick and effective, and no contamination of the product by toxic substances. *Spirulina platensis* dried powder were suspended at 0.06 g/ml in 20 mM Tris–HCl buffer (contain 10mM EDTA, pH 6.5). Before high pressure homogenization, the suspension was incubated with 1 mg/ml lysozyme 1h at 30℃. After lysozyme treatment we could use high pressure homogenization at lower pressure between 10-12,000 p.s.i to break the cells, and the degree of denatured proteins at the end of the process is small. The recovery of C-PC is 20-25 mg/g dried powder, and the purity ratio (A_{620}/A_{280}) could reach 0.8-0.9.

B. Study of the Ammonium Sulfate Precipitation

In this study, we designed eight different concentration gradients of ammonium sulfate to precipitate C-PC from crude extract (Table I). The C-PC was precipitated by two-step ammonium sulfate fractionations. The pellets from the first step precipitation were discarded and the supernatant was precipitated in second step. The pellets

from second step precipitation were dialyzed after resuspending, because high concentration of salt could influence the result of SDS-PAGE. The spectroscopic measurement result showed that no C-PC but 1.54% other proteins were precipitated at 10% saturation (Table I). During the saturation of 10%-50%, the purity ratio (A_{620}/A_{280}) of precipitation is greater then crude extract, and the ratio reaches the highest during the saturation of 20%-30%. During the saturation of 20%-30% and 30%-40%, the recovery ratio of C-PC reach the highest, up to 39.21% and 41.78%, respectively. During the saturation of 50%-95%, the purity ratio (A_{620}/A_{280}) of precipitation is lower then crude extract, and the purity of C-PC was decreased with increasing salt concentration. The result of SDS-PAGE showed that more other proteins were precipitated during the saturation of 50%-95% (Fig. 1). So 50% saturation of ammonium sulfate is suitable for extraction of C-PC.

TABLE I: SPECTROSCOPIC MEASUREMENT RESULT OF THE STUDY OF AMMONIUM SULFATE PRECIPITATION

Ammonium sulfate precipitation	A_{620}/A_{280}	Total proteins (%)	Recovery of C-PC (%)
Crude extract	0.81	100	100
0%-10% saturation	0	1.54	0
10%-20% saturation	1.71	2.81	1.18
20%-30% saturation	1.81	39.21	37.24
30%-40% saturation	1.38	41.78.	40.91
40%-50% saturation	0.98.	17.69	10.13
50%-60% saturation	0.56	12.14	4.16
60%-70% saturation	0.32	7.59	2.64
70%-95% saturation	0.18	10.67	0.89
Total recovery		89.65	96.15

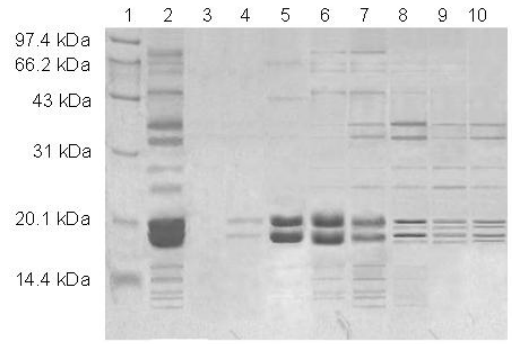

Fig. 1. SDS-PAGE analysis of different concentration gradients of ammonium sulfate to precipitate C-PC. Lane 1, molecular mass standard; lane 2, crude extract; lane 3, 0%-10% saturation; lane 4, 10%-20% saturation; lane 5, 20%-30% saturation; lane 6, 30%-40% saturation; lane 7, 40%-50% saturation; lane 8, 50%-60% saturation; lane 9, 60%-70% saturation; lane 10, 70%-95% saturation.

C. Purification of C-PC

The purification steps are summarized in Table II and the result of SDS-PAGE in Fig. 2. UV–vis absorption overlay spectra of C-PC at each stage of purification was shown in Fig. 3.

According to the result of ammonium sulfate precipitation study, we use one-step precipitation. Ammonium sulfate was gradually added into 1L crude extract to obtain 50% saturation with continuous stirring for 2 h at 4℃. Resulting solution was centrifuged at 12,000 g for 30 min and the pellets were collected. Because the following step was hydrophobic interaction chromatography, the pellets were resuspended in 500 ml 0.86 M ammonium sulfate (20% saturation) in 20 mM Tris–HCl buffer (pH 8.1) and centrifuged at 12,000 g for 20 min to remove insoluble substances. The result of SDS-PAGE showed that some other proteins were removed during this step (Fig. 2).

TABLE II: THE PURITY, YIELD AND THE RECOVERY OF C-PC IN VARIOUS STAGES

Purification step	A_{620}/A_{280}	SDS-PAGE Purity (%)	C-PC content (mg)	Recovery of C-PC (%)
(1)Crude extract	0.89	59.17	1347.84	100
(2)Ammonium sulfate precipitation	2.05	78.72	1152.13	85.48
(3)Phenyl Sepharose 6 FF（HS）	3.06	88.08	864.91	64.17
(4)DEAE Sepharose FF	4.91	96.45	670.42	49.74
(5)Sephacryl S-100 HR	5.32	99.01	566.50	42.03

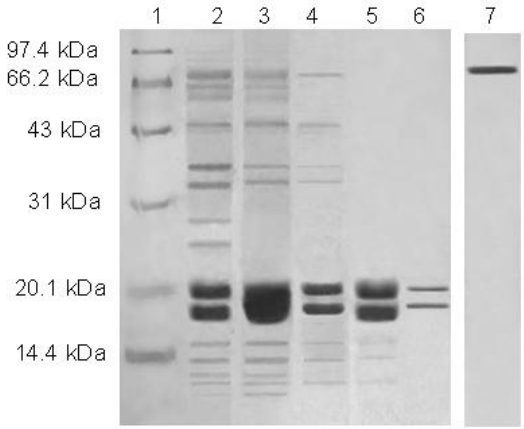

Fig. 2. Lanes 1-6 were 15% SDS-PAGE of C-PC during different steps of purification. Lane 7 was 10% Native-PAGE of purified C-PC. Lane 1, molecular mass standard; lane 2, crude extract; lane 3, Ammonium sulfate precipitation; lane 4, Phenyl Sepharose 6 FF（HS）; lane 5, DEAE Sepharose FF; lane 6, Sephacryl S-100 HR.

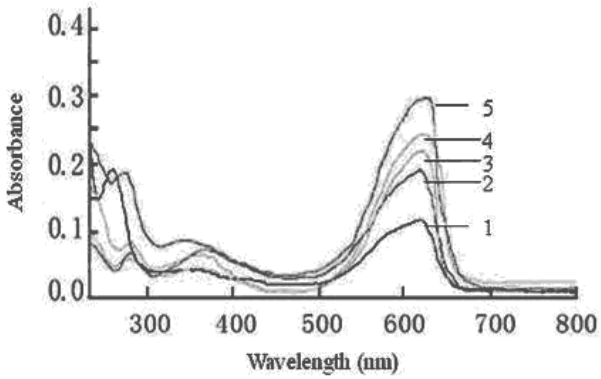

Fig. 3. UV–vis absorption overlay spectra of C-PC at each stage of purification. 1, crude extract; 2, Ammonium sulfate precipitation; 3, Phenyl Sepharose 6 FF（HS）; 4, DEAE Sepharose FF; 5, Sephacryl S-100 HR.

The recovery of C-PC, in this step, was 85.48% with purity ratio (A_{620}/A_{280}) of 2.05 (Table II).

In the step of hydrophobic interaction chromatography, the sample was loaded in 140ml Phenyl Sepharose 6 FF (HS). The loaded protein was eluted with 10 bed volumes of a lineal gradient from 0.86 to 0 M ammonium sulfate in 20 mM Tris–HCl buffer (pH 8.1) at a flow rate of 120 cm/h. The C-PC was eluted at about 0.41 M ammonium sulfate in 20 mM Tris–HCl buffer (pH 8.1), and stop collecting until

A_{280} below 20 mAU (Fig. 4). The recovery of eluted C-PC was 64.17% with purity ratio (A_{620}/A_{280}) of 3.06 (Table II).

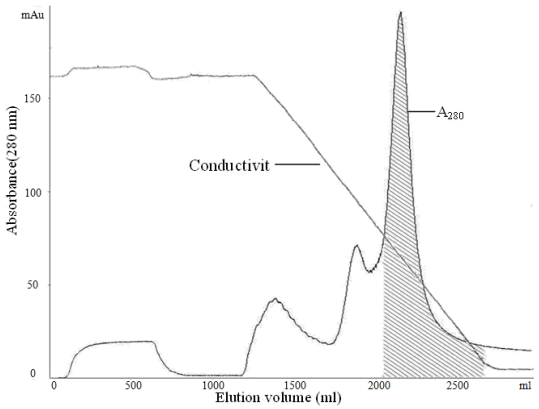

Fig. 4. Phenyl Sepharose 6 FF chromatography of C-PC. The sample was eluted with 10 bed volumes of a lineal gradient from 0.86 to 0 M ammonium sulfate in 20 mM Tris–HCl buffer (pH 8.1) at a flow rate of 120 cm/h.

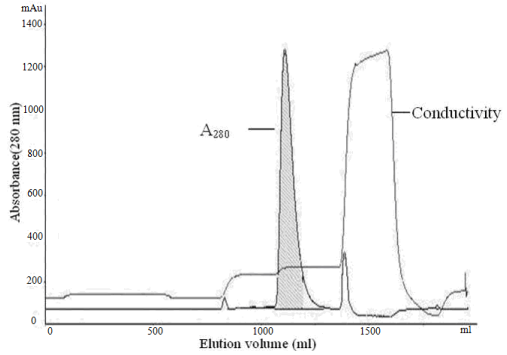

Fig. 5. DEAE Sepharose FF chromatography of C-PC. The column was first eluted with 10 bed volumes of 0.15 M NaCl in 20 mM Tris–HCl (pH 8.1), and the C-PC was then eluted with 0.23 M NaCl in 20 mM Tris–HCl (pH 8.1). Finally, the column was eluted with 1 M NaCl.

The conductivity of C-PC after hydrophobic interaction chromatography was diluted below 8 mS/cm by using 20 mM Tris–HCl buffer (pH 8.1). The sample was loaded in 30ml DEAE Sepharose FF. The column was first eluted with 10 bed volumes of 0.15 M NaCl in 20 mM Tris–HCl (pH 8.1) and a little peak was observed in this point (Fig. 5). The fraction of C-PC was then eluted with 0.23 M NaCl in 20 mM Tris–HCl (pH 8.1). The recovery of eluted C-PC was 49.74% with purity ratio (A_{620}/A_{280}) of 4.91 (Table II). Finally, the column was washed with 1 M NaCl and little other proteins were washed out (Fig. 5).

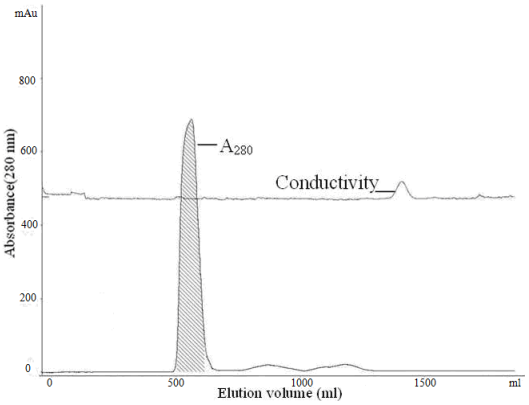

Fig. 6. Sephacryl S-100 HR chromatography of C-PC. 80 ml sample was loaded at a flow rate of 12 cm/h, and the column was then washed with same buffer at a flow rate of 25 cm/h.

After ion exchange chromatography, C-PC was loaded in 1.8 L Sephacryl S-100 HR. During this step, some low molecular weight proteins were removed (Fig. 6). And the buffer of C-PC was exchanged into 10mM K-phosphate buffer (containing 0.15 M NaCl, pH 7.4). The recovery of C-PC was 42.03% with purity ratio (A_{620}/A_{280}) of 5.32 (Table II). From the result of SDS-PAGE and Native-PAGE we can see that the purity of C-PC is up to about 99% (Fig. 2. Line 6, Line7).

D. Determination of Molecular Weight

SDS–PAGE (Fig. 2 Line 6) revealed two subunits of pure C-PC corresponding to α and β subunits of 17 and 21 kDa molecular mass. The molecular weight of the native purified C-PC was determined by gel filtration on a Sephadex G-100 column (50 ·1.5 cm). The molecular weight obtained was 115 kDa (Fig. 7). These date indicated that the aggregation state of the purified C-PC was $(\alpha\beta)_3$. This result is consistent with some reports [24], [25].

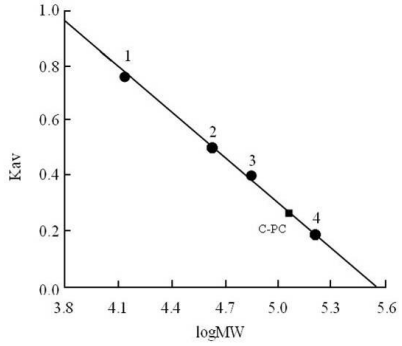

Fig. 7. Determination of the molecular weight of native C-PC. 1, Ribonuclease (13.7 kDa); 2, Egg albumin (45 kDa); 3, Bovine serum albumin (67 kDa); 4, Gamma γ-globulin (165 kDa).

1) Zinc acetate staining

The orange fluorescence of bilins was highly provoked in the presence of zinc ions under UV radiations. The zinc-assisted fluorescence enhancement of SDS–PAGE confirmed both the subunits were bilin-linked polypeptides (Fig. 8 Line 1). And one fluorescence band was observed in native-PAGE (Fig. 8 Line 2).

Fig. 8. Detection of biliproteins of C-PC by zinc-assisted fluorescence enhancement method. Line 1, Purified C-PC was analyzed by SDS-PAGE; Line 2, Purified C-PC was analyzed by Native-PAGE.

IV. Conclusion

In this work we establish a method for the efficient

recovery and purification of C-PC from *Spirulina platensis*. The recovery of C-PC was 42.03% with purity ratio (A_{620}/A_{280}) of 5.32. Moreover, this new method can be used for recovery of C-PC on a large scale. The molecular weight of purified C-PC was 115 kDa, which include two subunits α and β was 17 and 21 kDa molecular mass respectively. These date indicated that the purified C-PC was trimer $(\alpha\beta)_3$.

REFERENCES

[1] P. Freindenreich *et al.*, "Structural studies on phycobiliproteins (II)," *J Biol Chem*, vol. 253, pp. 212-9, 1978.

[2] D. A. Bryant, C. S. Hixson, and A. N. Glazer, "Structural studies on phycobiliproteins (III)," *J Biol Chem*, vol. 253, pp. 220-5, 1978

[3] R. MacColl, "Stability of allophycocyanin's quaternary structure," *Biochem Biophys*, vol. 223, pp. 24-32, 1983.

[4] R. MacColl, D. S. Berns, and N. L. Koven, "Effects of salts on C-phycocyanin," *Biochem Biophys*. vol. 146, pp. 477-482, 1971.

[5] R. MacColl, K. Csatorday, D. S. Berns, and E. Traeger, "The relationship of the quaternary structure of allophycocyanin to its spectrum," *Biochem. Biophys*, vol. 208, pp. 42-48, 1981.

[6] W. A. Sidler, "Phycobilisome and phycobiliprotein structures," in *The Molecular Biology of Cyanobacteria*, D. A. Bryant (Ed.), The Netherlands: Kluwer Academic, Dordrecht, vol. 1, 1994, pp. 140-216.

[7] Z. Cohen, "Products from microalgae," in *Handbook of Microalgal Mass Culture*, A. Richmond (Ed.), CRC Press, Boca Raton, FL, 2004, pp. 421–454.

[8] A. Yoshida, Y. Takagaki, and T. Nishimune, "Enzyme immunoassay for phycocyanin as the main component of Spirulina color in foods," *Biosci. Biotechnol. Biochem*, vol. 60, pp. 57–60, 1996.

[9] J. Zhao, J. Zhu, and L. Jiang, "Study on the energy transfer processes in phycobilisomes from blue-green algae by the use of stochastic simulation approach," *Biochim Biophys Acta*, vol. 1229, pp. 39–48, 1995.

[10] R. Rossano, N. Ungaro, A. D'Ambrosio, G. M. Liuzzi, P. Riccio, "Extracting and purifying R-phycoerythrin from Mediterranean red algae Corallina elongata Ellis & Solander," *Biotechnol*, vol. 101, pp. 289–93, 2003.

[11] R. B. Román, J. M. Alvárez-Pez, F. G. A. Fernández, and E. M. Grima, "Recovery of pure B-phycoerythrin from the microalga Porphyridium cruentum," *Biotechnol*, vol. 93, pp. 73–85, 2002.

[12] G. Patil, S. Chethana, and A. S. Sridevi, "Method to obtain C-phycocyanin of high purity," *Raghavarao Journal of Chromatography A*, vol. 1127, pp. 76–81, 2006.

[13] P. J. Viskari and C. L. Colyer, "Rapid extraction of phycobiliproteins from cultured cyanobacteria samples," *Anal Biochem*, vol. 319, pp. 263–71, 2003.

[14] M. Santiago-Santos, T. Ponce-Noyola, R. Olvera-Ramírez, and J. Ortega-López, "Cañizares-Villanueva RO. Extraction and purification of phycocyanin from Calothrix sp," *Process Biochem*, vol. 39, pp. 2047–52, 2004.

[15] A. Reis, A. Mendes, H. Lobo-Fernandes, J. A. Empis, and J. M. Novais, "Production, extraction and purification of phycobiliproteins from Nostoc sp," *Biores Technol*, vol. 66, pp. 181–187, 1998.

[16] A. V. Galland-Irmouli *et al.*, "One-step purification of R-phycoerythrin from the red macroalga Palmaria palmata using preparative polyacrylamide gel electrophoresis," *J Chromatogr B*, vol. 739, pp. 117–23, 2000.

[17] G. Wang "Isolation and purification of phycoerythrin from red alga Gracilaria verrucosa by expanded-bed-adsorption and ion-exchange chromatography," *Chromatographia*, vol. 56, pp. 509–13, 2002.

[18] E. E. Gardner *et al.*, "Purification and characterization of the c-phycocyanin from Agmenellum quadruplicatum," *Biochimica et Biophysica Acta*, vol. 624, pp. 187-195, 1980.

[19] J. Abalde, L. Betancourt, E. Torres, A. Cid, and C. Barwell, "Purification and characterization of phycocyanin from the marine cyanobacterium Synechococcus sp. IO9201," *Plant Sci*, vol. 136, pp. 109-20, 1998.

[20] A. Bennett and L. Bogorad, "Complementary chromatic adaptation in filamentous blue-green algae," *J. Cell. Biol*, vol. 58, pp. 419-435, 1973.

[21] H. O. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *J.Biol. Chem*, vol. 193, pp. 265-275, 1951.

[22] U. K. Laemmli, "Cleaved of structural proteins during the assembly of head of bacterial phage T4," *Nature*, vol. 277, pp. 680-685, 1970.

[23] T. R. Brekelman and J. C. Lagarias, "Visualization of bilin-linked peptides and proteins in polyacrylamide gels," *Anal Biochem*, vol. 156, pp. 194–201, 1986.

[24] A. Patel, S. Mishra, R. Pawar, and P. K. Ghosh, "Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat," *Protein Express Purify*, vol. 40, pp. 248–255, 2000.

[25] S. Benedetti *et al.*, "Purification and characterization of phycocyanin from the blue-green alga Aphanizomenon flos-aquae," *J. Chromatogr. B*, vol. 833, pp. 12–18, 2006.

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