



(51) International Patent Classification:

C07K 14/405 (2006.01) **C07K 1/00** (2006.01)
C07K 14/795 (2006.01)

(21) International Application Number:

PCT/IB2017/054608

(22) International Filing Date:

28 July 2017 (28.07.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

201621028171 18 August 2016 (18.08.2016) IN

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(54) Title: A PROCESS FOR THE EXTRACTION OF PHYCOCYANIN

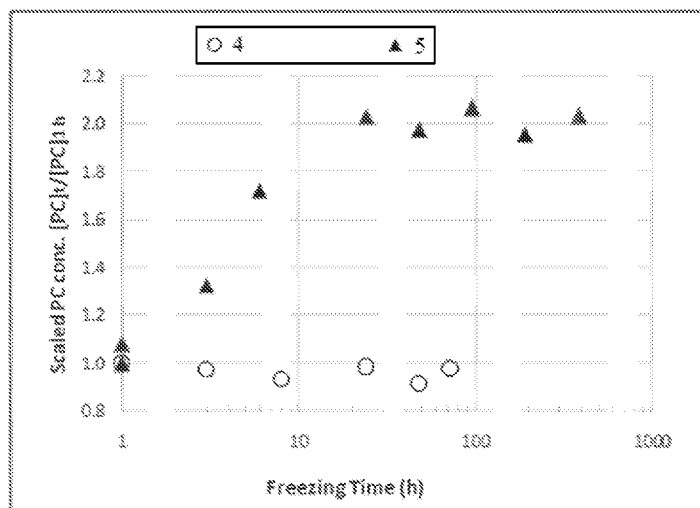


Figure 3

(57) Abstract: The present disclosure relates to the field of extraction of phycocyanin from an algal biomass. The process of the present disclosure relates to a simple yet efficient and rapid method to extract phycocyanin from the algal biomass which is easy to scale up. In the process of the present disclosure, an aqueous slurry is prepared of the algal biomass by adding a predetermined amount of a fluid medium by weight. The aqueous slurry is frozen/chilled and then optionally thawed to obtain a thawed biomass. The liquid from the thawed/chilled biomass can be separated to obtain a supernatant containing phycocyanin and a residue.



(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to the identity of the inventor (Rule 4.17(i))*
- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*

A PROCESS FOR THE EXTRACTION OF PHYCOCYANIN

FIELD

The present disclosure relates to the field of extraction of phycocyanin from an algal biomass.

Tris Buffer: Tris buffer, also known as Tris, or tris(hydroxymethyl)aminomethane, or THAM, is an organic compound with the formula $(\text{HOCH}_2)_3\text{CNH}_2$, which is used as a buffer, especially in biochemistry and molecular biology.

MES Buffer: MES is the common name for the compound 2-(N-morpholino)ethanesulfonic acid, and is used as a buffering agent in biology and biochemistry.

Phosphate buffer: Phosphate buffer is a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, in some formulations, potassium chloride and potassium dihydrogen phosphate solution. It is commonly used in biological research. The osmolarity and ion concentrations of phosphate buffer match those of the human body (isotonic).

BACKGROUND

Algae forms the life-supporting foundation of the natural food chain by providing essential vitamins, minerals, proteins, and nutrients required to support life. The health benefits of some algae have long been appreciated when used as a dietary supplement for promoting and sustaining human health.

Phycocyanin is a pigment-protein complex of the light-harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin. It is an accessory pigment to chlorophyll. All phycobiliproteins are water-soluble, and hence cannot exist within the cell membrane, unlike carotenoids that can exist within the membrane. Instead, phycobiliproteins aggregate to form clusters that adhere to the membrane called phycobilisomes. Phycocyanin comprises a protein and a chromophore, furthermore it exhibits a strong red fluorescence. Phycocyanin has wide range of applications such as, fluorescent markers, antioxidants, immuno-modulant, neuroprotective and hepatoprotective, natural pigments in food and cosmetics and the like.

Conventional methods for isolating phycocyanin from algae/cyanobacteria involve using various cell disruption techniques like Freeze-Thaw, Sonication, Homogenization, etc., thereby releasing cytoplasmic contents, to produce a disrupted cell suspension; separating

solid and liquid phases of the disrupted cell suspension; contacting the liquid phase of the disrupted cell suspension with a non-ionic polyaromatic macroreticular adsorbent resin; collecting the liquid phase from the resin to produce a phycocyanin extract; and optionally dehydrating the phycocyanin extract.

- 5 Some processes involve separation and purification of phycocyanin including precipitation, centrifugation, dialysis and chromatography process. These methods are difficult to carry out and are expensive.

Freeze-Thawing technique is recognized as a superior and versatile technique for cell disruption & downstream processing of algal biomass. Repeated freezing & thawing method
10 is more effective in extracting phycobiliprotein from various cyanobacteria than the cell disruptive methods. The advantage of this technique is that it is mild and non-denaturing. Ice crystals formed during freezing, break the cell wall and the cell membrane and release the phycobiliprotein into the extracting medium. However freezing at extremely low temperatures and thawing at high temperature in freeze-thaw cycle is energy intensive and
15 difficult to scale-up.

Though various processes for the extraction of phycocyanin are known, the extraction rate is not high, resulting in an increased production costs. Also the processes are tedious, time consuming and energy intensive.

It is therefore, desired to provide a simple, economic and rapid phycocyanin extraction
20 process which can be easily scaled up and can be an alternative to the known process and which can also overcome the drawbacks associated with the known process such as complex process steps, time consuming procedure and high process cost.

OBJECTS

Some of the objects of the present disclosure, which at least one embodiment herein satisfies,
25 are as follows:

It is an object of the present disclosure to ameliorate one or more problems of the prior art or to at least provide a useful alternative.

An object of the present disclosure is to provide a process for the extraction of phycocyanin.

Another object of the present disclosure is to provide a simple, economic and rapid phycocyanin extraction process.

Other objects and advantages of the present disclosure will be more apparent from the following description, which is not intended to limit the scope of the present disclosure.

5 SUMMARY

The present disclosure envisages a process for the extraction of phycocyanin from an algal biomass. Initially, the algal biomass is identified and harvested which is followed by preparing an aqueous slurry of the algal biomass by adding a predetermined amount of a fluid medium. The aqueous slurry is then transferred into a container, followed by freezing/chilling
10 at a temperature in the range of -10 to 10 °C for a period of 5 minutes to 400 hours to obtain a frozen/chilled biomass. The frozen biomass can be thawed by keeping the container containing the frozen biomass at a temperature in the range of 20 to 40 °C for 5 minutes to 3 hours to obtain a thawed biomass. The liquid from the thawed/chilled biomass can be separated to obtain a supernatant containing phycocyanin and a residue. In one embodiment,
15 aqueous slurry is concentrated before the freezing or chilling step.

The algal biomass can be at least one selected from the group consisting of *Rhodophyta*, *Cyanobacteria*, *Spirulina*, *Geitlerinema*, *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria*, *Spirogyra* and *Phormidium*. In one embodiment, the algal biomass is a wet algal biomass, and can optionally be washed at least once with water before preparing the aqueous slurry.
20 Typically, the fluid medium can be water and may include at least one buffer, selected from the group consisting of Phosphate buffer, Tris buffer, and MES buffer or any other buffer. In an embodiment of the present disclosure, the buffer is selected from the group consisting of biologically similar buffers, preferably buffers having a neutral pH. The freezing step can be repeated at least once.

25 BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWING

The present disclosure will now be described with the help of the accompanying drawing, in which:

Figure 1 illustrates the impact of freezing time on a single and multiple freeze thaw cycles: wherein 1 denotes single freeze thaw (washed) and 2 denotes multiple freeze thaw (washed).

Figure 2 illustrates the impact of freezing time on dry powder based process; wherein 3 denotes single freeze thaw (not washed).

Figure 3 illustrates the normalized phycocyanin concentration as a function of freezing time; wherein 4 denotes dried biomass and 5 denotes biomass which is not dried.

5 DETAILED DESCRIPTION

Phycocyanin is a water soluble protein and is one of the main pigments of cyanobacteria. It has a blue colour and a red fluorescence. It has a maximum absorption at 620 nm and an emission radiation at 635 nm. This quality makes it a natural fluorescent product which is a favoured label in biomedical diagnostics. At the physiological level, phycocyanin is an
10 important antioxidant particularly in protecting plasma proteins against oxidative modifications. Phycocyanin is a molecule of great interest, because of its beneficial properties for human and animal health. Phycocyanin is consumed particularly for its antioxidant properties and also for its ability to promote the production of stem cells.

Conventional methods for isolating phycocyanin from algae/cyanobacteria involve using
15 various cell disruption techniques like Freeze-Thaw, Sonication, Homogenization, etc., thereby releasing cytoplasmic contents, to produce a disrupted cell suspension; separating solid and liquid phases of the disrupted cell suspension; contacting the liquid phase of the disrupted cell suspension with a non-ionic polyaromatic macroreticular adsorbent resin; collecting the liquid phase from the resin to produce a phycocyanin extract; and optionally
20 dehydrating the phycocyanin extract.

Some processes involve separation and purification of phycocyanin including precipitation, centrifugation, dialysis and chromatography process. These methods are difficult to carry out and are expensive.

Freeze-Thawing technique is recognized as a superior and versatile technique for cell
25 disruption and downstream processing of algal biomass. Repeated freezing and thawing method is more effective in extracting phycobiliprotein from various cyanobacteria than the cell disruptive methods. The advantage of this technique is that it is mild and non-denaturing. Ice crystals formed during freezing, break the cell wall and the cell membrane and release the phycobiliprotein into the extracting medium. However, freezing at extremely low
30 temperatures and thawing at high temperature in freeze-thaw cycle is energy intensive and difficult to scale-up.

Though various processes for the extraction of phycocyanin are known, the extraction rate is not high, resulting in increased production costs. Also, the processes are tedious, time consuming and energy intensive.

5 It is therefore, desired to provide a simple, economic and rapid phycocyanin extraction process which can be easily scaled up and can be an alternative to the known processes and which can also overcome the drawbacks associated with the known processes such as complex process steps, time consuming procedure and high process cost.

10 The present disclosure envisages a process for the extraction of phycocyanin from an algal biomass. Initially, the algal biomass is identified and harvested which is followed by preparing an aqueous slurry of the algal biomass by adding a predetermined amount of a fluid medium. Typically, the fluid medium can be 3 to 8 times the weight of the algal biomass.

In one embodiment, the aqueous slurry is concentrated before the freezing or chilling step. Typically, the aqueous slurry is concentrated by centrifugation at 8000 – 12000 rpm for 10 – 20 minutes at a temperature in the range of 2 – 8 °C to obtain a first mixture.

15 The aqueous slurry is then transferred into a container, followed by freezing or chilling at a temperature in the range of -10 to 10 °C for a period of 5 minute to 400 hours to obtain a frozen/chilled biomass. The frozen biomass can be thawed by keeping the container containing the frozen biomass at a temperature in the range of 20 to 40 °C for 5 minutes to 3 hours to obtain a thawed biomass. The liquid from the thawed/chilled biomass is separated to
20 obtain a supernatant containing phycocyanin and a residue. In an embodiment of the present disclosure, the separation can be carried out by centrifugation at 8000 – 12000 rpm for 10 – 20 minutes at a temperature in the range of 2 – 8 °C

In accordance with the embodiments of the present disclosure, the algal biomass can be at least one selected from the group consisting of *Rhodophyta*, *Cyanobacteria*, *Spirulina*,
25 *Geitlerinema*, *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria*, *Spirogyra* and *Phormidium*. Typically, the algal biomass is a wet algal biomass. Optionally, the algal biomass can be washed at least once with water before preparing the aqueous slurry.

In an embodiment of the present disclosure, *Geitlerinema* is obtained from Gagva, Jamnagar, Gujarat, India.

The fluid medium can be water and may include at least one buffer, selected from the group consisting of Phosphate buffer, Tris buffer, MES buffer [2-(N-morpholino)ethanesulfonic acid] or any other buffer.

5 In another embodiment of the present disclosure, the freezing and the thawing steps can be repeated at least once.

In another embodiment of the present disclosure, where freezing is not done below 0 °C, the thawing steps are bypassed.

In yet another embodiment of the present disclosure, the process for extracting phycocyanin from an algal mass comprises at least one step of centrifugation to obtain phycocyanin.

10 In an embodiment of the present disclosure, envisaged is a process for rapidly extracting the phycocyanin from dry algal biomass or algal powder. Initially an aqueous slurry of the algal biomass is prepared by adding a fluid medium. Typically, the algal biomass is dry algal biomass or algal powder. This aqueous slurry is frozen or chilled at a temperature in the range of -10 to 10 °C for a time period of 5 minutes to 5 hours to obtain a frozen/chilled
15 biomass. The frozen biomass is thawed at a temperature in the range of 20 to 40 °C for 10 – 20 minutes to obtain a thawed biomass. The liquid is separated from the thawed/chilled biomass to obtain a supernatant containing phycocyanin and a residue. In an embodiment of the present disclosure, the separation can be carried by centrifugation at 8000 – 12000 rpm for 10 – 20 minutes at a temperature in the range of 2 – 8 °C.

20 The present disclosure is further described in light of the following experiments which are set forth for illustration purpose only and not to be construed for limiting the scope of the disclosure. The following experiments can be scaled up to industrial/commercial scale and the results obtained can be extrapolated to industrial scale.

Experiment 1: Phycocyanin extraction kinetics (from wet algal biomass)

25 *Geitlerinema* culture from an algal pond was harvested by scooping (40 micron mesh) to produce a harvested biomass containing about 92 % moisture. The scooped biomass was mixed with four times fresh wash water (by weight) and then centrifuged in Clara 20 centrifuge at 9000 rpm to obtain a mixture with 91% moisture. This mixture was then divided in equal parts of about 150 g and transferred in separate reaction vessels. These vessels were
30 then kept in the freezer at -10 °C for varying duration for both single and multiple freeze

thaw cycle studies. The freezing duration and cycle details for different samples are given in Table 1.

Samples 1-9 were treated with single freezing for a time duration of 1 h to 16 days followed by thawing in ambient air for 10-20 minute post freezing based on freezing duration. The thawing time of 10 minute for 1-6 hour freezing duration, 15 minute for 1 to 2 day freezing and 20 minute for 4-16 day freezing duration was maintained. Sample 9 is the duplicate of sample 1.

Samples 10-16 were treated in multiple freeze thaw cycle to study the impact of multiple freeze-thaw on phycocyanin extraction kinetics. For sample 11, 1+2+3 h means that the sample is frozen multiple (three) times, i.e., first time for 1 h, 2nd time for 2 h and 3rd time for 3 h leading to total freezing time of 6 h. After each freezing, the sample was thawed for 10-20 minutes based on the freezing time. It can be noted that sample 11 (multiple) and sample 3 (single) have identical total freezing time. Similarly sample 12 (multiple) & sample 4 (single) have identical total freezing time, and so on for other cases.

Post thawing, samples (thawed biomass) were centrifuged using REMI PR-24 lab centrifuge at 10000 rpm for 15 minutes for separating crude phycocyanin supernatant from the thawed biomass. The phycocyanin (PC) concentration in crude was estimated based on Equation 1.

Equation 1:

$$\text{PC concentration [PC], mg/ml} = (\text{A}_{620} \cdot \text{D.F.} - 0.7 \text{A}_{655} \cdot \text{D.F.}) / 7.38 \quad (1)$$

where A_{620} & A_{655} represent absorbance at 620 & 655 nm and D.F. represents dilution factor.

Figure 1 shows phycocyanin concentration profile, wherein 1 denotes single freeze thaw (washed) and 2 denotes multiple freeze thaw (washed). PC concentration was found to be a strong function of freezing time. It is observed that for the extraction process (where the biomass was not dried), PC concentration increases with time gradually and reaches a plateau at about 24 h, beyond which freezing time has no impact. It is also observed that there was no significant difference between the multiple freeze-thaw over the single freeze thaw. Thus there is no significant merit of conducting multiple freeze thaw over single freeze thaw for these species. Table 2 summarises the different cycles for wet algal biomass and the PC concentration obtained for each cycle.

Table 1: Cycle details for different samples in freezing

Freezing Time (Single & Multiple)			
Sample	Single	Sample	Multiple (except 1h)
1	1 h	9	1 h
2	3 h	10	1 + 2 h
3	6 h	11	1 + 2 + 3 h
4	1 day	12	1 + 2 + 3 + 6 + 12 h
5	2 days	13	(1 + 2 + 3 + 6 + 12) h + 1 day
6	4 days	14	(1 + 2 + 3 + 6 + 12) h + 1 + 2 days
7	8 days	15	(1 + 2 + 3 + 6 + 12) h + 1 + 2 + 4 days
8	16 days	16	(1 + 2 + 3 + 6 + 12) h + 1 + 2 + 4 + 8 days

Table 2: Cycle details for different samples and PC concentration obtained (wet algal biomass)

Single cycle trial using <i>Geitlerinema</i> with washed & wet biomass				
Trial	Freezing Time & Temperature	Thawing Time (min) & temperature	PC conc (mg/ml)	Scaled Conc. [PC]/[PC] _{1hr}
1	1 h, -10 °C	10, room temperature	3.5	1.08
2	3 h, -10 °C	10, room temperature	4.3	1.33
3	6 h, -10 °C	10, room temperature	5.58	1.72
4	1 day, -10 °C	15, room temperature	6.58	2.03
5	2 day, -10 °C	15, room temperature	6.4	1.98
6	4 day, -10 °C	20, room temperature	6.7	2.07
7	8 day, -10 °C	20, room temperature	6.34	1.96
8	16 day, -10 °C	20, room temperature	6.59	2.03
9 (repeat of trial 1)	1 h, -10 °C	10, room temperature	3.24	1.00
Multiple freeze-thaw cycle trial using <i>Geitlerinema</i> with washed & wet biomass				
Trial	Total Freezing Time & Temperature	Thawing Time between cycles, min & temperature	PC conc (mg/ml)	Scaled Conc. [PC]/[PC] _{1hr}
10	1+2h, -10 °C	10-20, room temperature	4.49	1.39
11	1+2+3h, -10 °C	10-20, room temperature	6.09	1.88
12	1+2+3+6+12 h, -10 °C	10-20, room temperature	6.75	2.08
13	(1+2+3+6+12)h+1day, -10 °C	10-20, room temperature	6.60	2.04
14	(1+2+3+6+12)h+1+2 day, -10 °C	10-20, room temperature	6.97	2.15
15	(1+2+3+6+12)h+1+2	10-20, room	6.69	2.06

	+4 day, -10 °C	temperature		
16	(1+2+3+6+12)h+1+2 +4 +8 day, -10 °C	10-20, room temperature	6.84	2.11

Experiment 2: Phycocyanin extraction kinetics (from dry algal biomass)

Geitlerinema culture from an algal pond was harvested by scooping (40 micron mesh) and was sun dried without any washing of the harvested algae. The sun dried *Geitlerinema* biomass had a moisture content of about 10 %.

25 g of the dried *Geitlerinema* biomass was mixed with 100 ml of distilled water in a vessel for 5 minutes using a magnetic stirrer. Six such vessels were prepared and kept in a freezer at -5 °C for varying durations (1h, 3 h, 8 h, 1 day, 2 days and 3 days). After different durations, the vessels were thawed at 27 °C to obtain a thawed biomass, followed by centrifuging at 10000 rpm for 15 minutes for separating the phycocyanin supernatant from the residue. PC concentration profile is depicted in Figure 2, wherein 3 denotes single freeze thaw (not washed). It is observed that phycocyanin concentration is relatively insensitive to freezing time (in contrast to the profile in Figure 1) and even 1 h is sufficient for reaching equilibrium concentration for phycocyanin.

The process of the present disclosure thus demonstrates a rapid PC extraction process (equilibration time not exceeding 1 hour) under less severe cold processing condition (not below -10 °C), which is simple to scale-up. Table 3 summarises the different cycles for washed and unwashed dry algal biomass. It is observed from Table 3 that for dry algal biomass based extraction (both washed & unwashed), phycocyanin concentration was relatively insensitive to extraction time unlike biomass that was never dried. In dry algal biomass based extraction, even 1 h was sufficient for extraction and beyond 1 h, concentration does not change significantly unlike extraction from biomass that was never dried. Also, it was observed that washing helps to achieve higher PC concentration in extraction.

Table 3: Cycle details for different samples (washed and unwashed dry algal biomass)

Trial with washed <i>Geitlerinema</i> dry biomass				
Trial	Total Freezing Time & Temperature	Thawing Time between cycles, min & temperature	PC conc (mg/ml)	Scaled Conc. $[PC]_t/[PC]_{1h}$
17	1 h, -10 °C	10, room temperature	6.2	1.00
18	1+1 h, -10 °C	10, room temperature	7.24	1.17
19	1+1+1 h, -10 °C	10, room temperature	6.73	1.09
20	1+1+1+1 h, -10 °C	10, room temperature	7.18	1.16
Trial with un-washed <i>Geitlerinema</i> dry biomass				
Trial	Freezing time & Temperature	Thawing time, h & temperature, C	PC conc (mg/ml)	Scaled Conc. $[PC]_t/[PC]_{1h}$
21	1 h, -5 °C	0.5, room temperature	2.97	1.00
22	3 h, -5 °C	2, room temperature	2.89	0.97
23	8 h, -5 °C	3, room temperature	2.78	0.94
24	24 h, -5 °C	3, room temperature	2.92	0.99
25	48 h, -5 °C	3, room temperature	2.72	0.92
26	72 h, -5 °C	3, room temperature	2.90	0.98

Comparison of extraction kinetics (dry and wet algal biomass)

Comparison of extraction kinetics between the dry algal biomass based process and the wet algal biomass based process was carried out. For this purpose, PC concentration at any time t,

5 $[PC]_t$ was divided with PC concentration at 1 h, $[PC]_{1h}$ to get the normalized PC concentration, $[PC_n]$ which is given by equation 2,

$$[PC_n] = \frac{[PC]_t}{[PC]_{1h}} \quad (2)$$

Normalized phycocyanin concentration as a function of time was plotted in Figure 3, wherein 4 denotes dried biomass and 5 denotes biomass which is not dried. From this plot, it is
 10 observed that normalized PC concentration does not change much with time for the dry algal biomass based process above 1 h. It is observed that at 24 h normalized concentration for the

dry algal biomass based process is about 1, indicating no significant change in phycocyanin concentration over time. However, normalized PC concentration changes substantially for the wet algal biomass based process. For the process involving wet algal biomass, normalized concentration at 24 h was about 2. There was not much change in the PC concentration in case of the dried algal biomass, while in case of the wet algal biomass, the plateau region was observed to reach at about 24 hour of freezing.

Experiment 3: PC extraction from dry algal biomass powder without thawing

Trials were done where freezing was not done below 0 °C and no thawing was carried out. The exclusion of sub-zero freezing and the thawing step makes the process simpler from the scale-up & process development perspective. Also, it needs to be understood that getting rid of the sub-zero freezing and thawing process helps in reducing the operating and capital cost of the process.

40 g of the dry algal powder [4.2 % moisture & 10.8% ash (moisture free basis)] was mixed with 160 ml of water in a reaction vessel to form a mixture. This mixture was then chilled at 4 °C for 30 minutes. The mixture was then centrifuged, without any thawing step, at 10000 rpm for 15 minutes with REMI-PR24 to obtain supernatant containing phycocyanin (crude PC). PC concentration in supernatant was estimated to be 6.54 - 7.26 mg/ml in two trials under identical process conditions. Table 4 summarises the trial result.

Table 4: Trial with dry algal powder

Trial dry algal powder			
Trial	Freezing time & Temperature	Thawing time, h & temperature, C	PC conc. (mg/ml)
27	0.5 h, 4 °C	No thawing	7.26
28	0.5 h, 4 °C	No thawing	6.54

It is observed that thawing process can be eliminated from dry powder based extraction where freezing was not done below 0 °C. It is also observed that rapid phycocyanin extraction can be achieved in dry powder based extraction at 4 °C, in 30 minutes unlike extraction from biomass that was never dried.

TECHNICAL ADVANCEMENTS

The present disclosure described herein above has several technical advantages including, but not limited to, the realization of:

- 5 – a simple yet efficient & rapid method to extract phycocyanin from an algal biomass and that is easy to scale up; and
- a chemical/enzyme-free extraction of phycocyanin.

The embodiments as described herein above, and various features and advantageous details thereof are explained with reference to the non-limiting embodiments in the description. Descriptions of well-known aspects, components and molecular biology techniques are
10 omitted so as to not unnecessarily obscure the embodiments herein.

The foregoing description of specific embodiments so fully reveal the general nature of the embodiments herein, that others can, by applying current knowledge, readily modify and/or adapt for various applications of such specific embodiments without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended
15 to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. Therefore, while the embodiments herein have been described in terms of preferred embodiments, those skilled in the art will recognize that the embodiments herein can be practiced with modification within the spirit and scope of
20 the embodiments as described herein. Further, it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the disclosure and not as a limitation.

Having described and illustrated the principles of the present disclosure with reference to the described embodiments, it will be recognized that the described embodiments can be
25 modified in arrangement and detail without departing from the scope of such principles.

While considerable emphasis has been placed herein on the particular features of this disclosure, it will be appreciated that various modifications can be made, and that many changes can be made in the preferred embodiment without departing from the principles of the disclosure. These and other modifications in the nature of the disclosure or the preferred
30 embodiments will be apparent to those skilled in the art from the disclosure herein, whereby

it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the disclosure and not as a limitation.

CLAIMS:

1. A process for the extraction of phycocyanin from an algal biomass; said process comprising the following steps:
 - a) preparing an aqueous slurry of said algal biomass by adding a fluid medium;
 - 5 b) freezing or chilling said aqueous slurry at a temperature in the range of -10 to 10 °C for a time period of 5 minutes to 400 hours to obtain a frozen/chilled biomass;
 - c) thawing said frozen biomass at a temperature in the range of 20 to 40 °C for 5 minutes to 3 hours to obtain a thawed biomass; and
 - 10 d) separating the liquid from said thawed/chilled biomass to obtain a supernatant containing phycocyanin and a residue.
2. The process as claimed in claim 1, wherein said algal biomass is at least one selected from the group consisting of *Rhodophyta*, *Cyanobacteria*, *Spirulina*, *Geitlerinema*, *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria*, *Spirogyra* and *Phormidium*.
- 15 3. The process as claimed in claim 1, wherein said algal biomass is a wet algal biomass.
4. The process as claimed in claim 1, wherein said aqueous slurry obtained in step (a) is concentrated before said freezing or chilling step.
5. The process as claimed in claim 1 further comprises a pre-step of washing said algal biomass at least once with water before preparing said aqueous slurry.
- 20 6. The process as claimed in claim 1, wherein said fluid medium is 3 – 8 times by weight of said algal biomass, and is at least one selected from the group consisting of water and/or buffer.
7. The process as claimed in claim 6, wherein said buffer is at least one selected from the group consisting of Phosphate buffer, Tris buffer, and MES buffer .
- 25 8. The process as claimed in claim 1, wherein said steps (b) and (c) are repeated at least once.

9. The process for extracting the phycocyanin from algal biomass as claimed in claim 1; said process comprising the following steps:

- a) preparing an aqueous slurry of said algal biomass by adding a fluid medium;
- b) freezing or chilling said aqueous slurry at a temperature in the range of -10 to 10 °C for a time period of 5 minutes to 5 hours to obtain a frozen/chilled biomass;
- c) thawing said frozen biomass at a temperature in the range of 20 to 40 °C for 10 – 20 minutes to obtain a thawed biomass; and
- d) separating the liquid from said thawed/chilled biomass to obtain a supernatant containing phycocyanin and a residue,

wherein said algal biomass is dry algal biomass or algal powder.

10. The process as claimed in claim 1 or claim 9, wherein said separation is carried out by centrifugation at 8000 – 12000 rpm for 10 – 20 minutes at a temperature in the range of 2 – 8 °C.

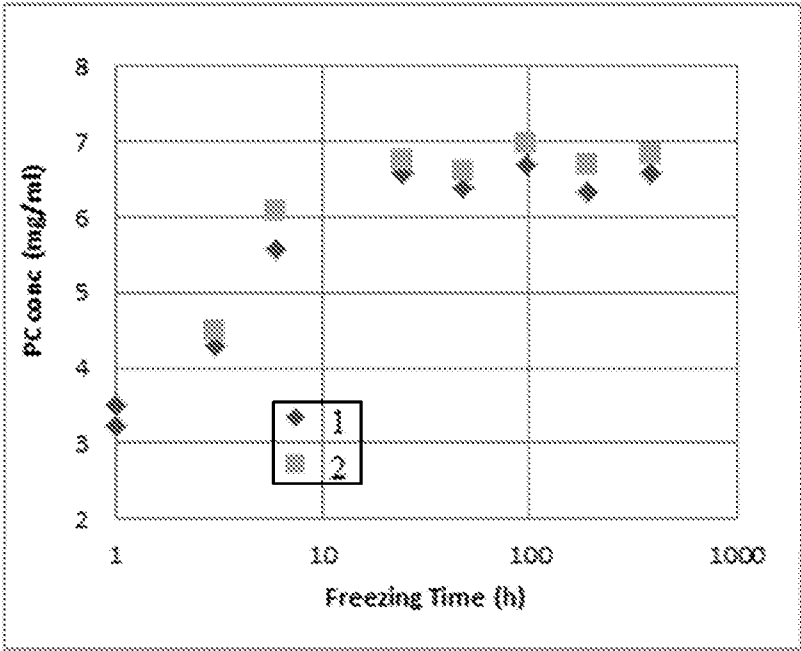


Figure 1

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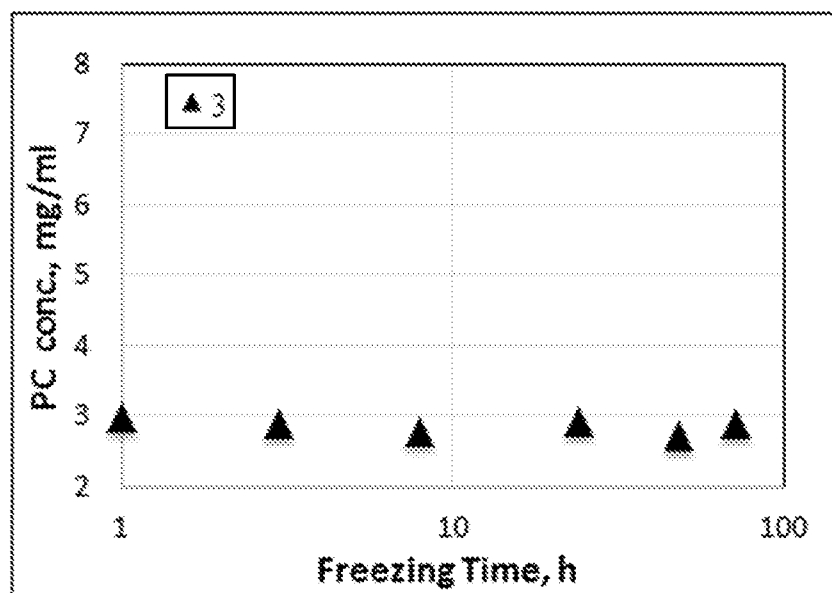


Figure 2

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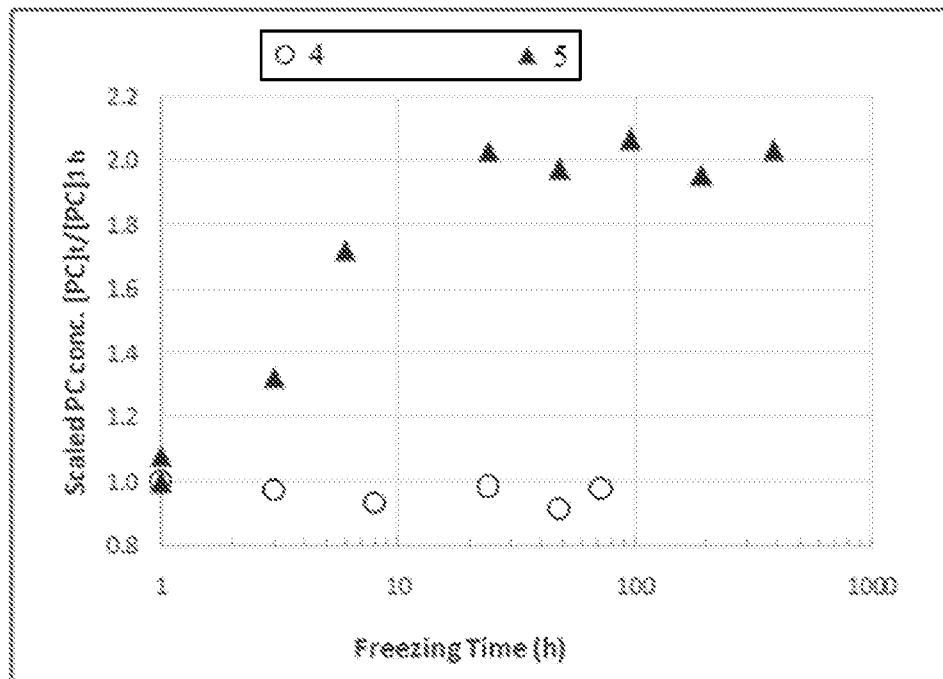


Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/054608

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/405, C07K14/795, C07K1/00 Version=2017.01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: Patseer, IPO Internal Database

Keywords: phycocyanin, Geitlerinema, Isolate, extract

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IN-DEL-2014-03981A (INDIAN COUNCIL OF AGRICULTURAL RESEARCH) 01 Jul 2016 (01-06-2016). *Refer Claims 1, 9; Example 1-2*	1-8
X	KENEKAR A.A et al, February 2014. Effect of Varying Physicochemical Parameters on the Productivity and Phycobiliprotein Content of Indigenous Isolate Geitlerinema sulphureum. BIOTECHNOLOGY, Vol:12, Pages:146-154. *Refer Pages 146, 147 and 153*	1-4, 8-10
A	CN105820236A (DONGTAI CBN BIO ENGINEERING CO LTD) 03 Aug 2016 (03-08-2016). *Refer abstract, Para 0004, 0007, 0026, 0028, 0029, 0034 and 0043*	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

23-10-2017

Date of mailing of the international search report

23-10-2017

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