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From 3G biofuels to high-value-added bioproducts

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Abstract
The paper focused on the co-production of high-value-added product thermostable C–phycocyanin (C–PC) and biomass, further utilized in pyrolysis. The photobiosynthesis of C–PC was carried out by the thermophilic cyanobacteria *Synechococcus* PCC6715 cultivated in helical and flat panel photobioreactors (PBR). Despite the application of different inorganic carbon sources, both PBRs were characterized by the same growth efficiency and similar C–PC concentration in biomass. To release the intracellular C–PC, the biomass was concentrated and disintegrated with the freeze-thaw method. The crude C–PC was then further purified with foam fractionation (FF), aqueous two-phase extraction (ATPE), membrane techniques (UF) and fast protein liquid chromatography (FPLC). Although each method can be used separately, a three-stage purification system (FF, FPLC and UF) was proposed from a practical and economic point of view. The purity ratio of the final C–PC was about 3.9, which allows it to be classified as a reactive grade. To improve the profitability of 3G biorefinery, the solid biomass residue was used as a substrate to pyrolysis process, which led to production of additional chemicals in the form of oils, gas (containing e.g. H_2) and biochar.

Keywords
microalgae, biorefinery, biofuels, value-added products, phycocyanin

1. INTRODUCTION

One of the main goals of biorefineries is to contribute to a more sustainable industry by using biomass instead of fossil fuels and by reducing greenhouse gas emissions and other pollutants. Biorefineries can be classified according to four main raw materials. The first generation of biorefinery using food crops as feeds is in an obvious conflict between energy and food. Therefore, there is no chance of further development of 1G biorefineries. The second-generation biorefineries (2G) using lignocellulosic biomass (forest and agricultural residues, energy crops, etc.) are still in intensive development in Europe. There are approx. 300 commercial and demonstration biorefineries in the EU. The third-generation (3G) biorefineries aim to utilize microbial cell factories to convert renewable energies and atmospheric CO_2 into biofuels and chemicals in a carbon-neutral manner. 3G biorefineries transform renewable energy and CO_2 into algae biomass that can be used to produce a wide variety of food, feed, pharmaceuticals, chemicals, and biofuels. This is the transition from lignocellulosic raw materials (2G biorefinery) to the use of CO_2 for the production of biofuels and bio-chemicals (3G biorefinery). The fourth generation (4G) biorefinery uses genetically modified plants and microorganisms with high CO_2 absorption capacity to produce biofuels and biochemicals (Cavelius et al., 2023; Sriariyanun et al., 2024).

It is estimated that downstream processes make up from 50% to 90% of total production costs (Pais et al., 2016). The final products from algae result from a series of costly processes such as disintegration and separation (extraction, purification of the main target component). Economic challenges still limit the large-scale exploitation of microalgae-based biorefineries, particularly for the cost-intensive cultivation step and low biomass yield. According to Rafa et al. (2021) analysis, priority should be given to combining microalgae and aquaculture farming in wastewater and coproducing high-value multi-products to achieve a cost-effective 3G biorefinery. It is worth emphasizing that during the last conferences of the European-algae-industry-summit (Annual European Algae Industry Summit), the topic of 3G biofuels was practically paid no attention, while both scientists and industry focused on the production of value-added products such as pharmaceuticals, cosmetics and others, and their economically viable applications.

One of the leading examples of value-added bioproducts is phycocyanin (C–PC), with therapeutic features including antioxidant, anti-inflammatory and anti-cancer properties (price € 106/mg) (Jiang et al., 2017). Phycocyanin (C–PC) occurs as the major phycobiliprotein in many cyanobacteria and as a secondary phycobiliprotein in some red algae. The pigment has a single visible absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~ 650 nm. C–PC is a soluble phycobiliprotein synthesized in cyanobacteria and is considered a high-value product due to its brilliant blue color and fluorescent properties. The factor limiting the wider use of C–PC (from mesophilic strains Spirulina, *Arthrospira platensis*) is the narrow range of its thermal and pH stability. C–PC from *A. platensis* is denatured

at $> 45\,^{\circ}C$ and outside the pH range of 4–7, which causes loss of blue color, fluorescence and antioxidant properties and limits its use in the food, cosmetic and textile industries. Moreover, the procedures typical for Spirulina were ineffective (low C–PC recovery) and required many purification steps (Manirafasha et al., 2016).

The thermostable phycocyanin from thermophilic organisms would have an advantage over currently used pigments and could have expanded their utilization in the food industry. The *Synechococcus* 6715 strain is an extremely promising, and rarely investigated, source of thermostable pigments. As was demonstrated in our previous paper (Liang et al., 2018), C–PC extracted from *Synechococcus* 6715 showed good long-term stability characteristics when compared with Spirulina protein and is one of the most stable proteins of this type reported to date. Analysis of the molecular model of the C–PC and crystal structures of other phycocyanins reveals an interesting pattern of amino acid substitutions that are present in thermophilic proteins that could have a significant impact on their much higher thermostability (Liang et al., 2018).

Moreover, in this paper attention is paid to the maximum utilization of biomass from the photobiosynthesis of a high-value, highest-purity product (C–PC) in combination with further processing of biomass residues through pyrolysis. The scheme of raw material circulation and product utilization, shown in Fig. 1, is in line with the principles of circular economy.

As shown in Fig. 1, the microalgae biomass obtained by photobiosynthesis after centrifugation is subjected to disintegration by the method of freezing and thawing and extraction of crude phycocyanin with PBS buffer. The obtained crude extract is then subjected to various purification methods tested to examine and compare the efficiency and selectivity of the downstream processing methods. The solid residue after phycocyanin removal from microalgae biomass can be used for biogas production or subjected to thermochemical processes such as pyrolysis. Integration of biological and thermochemical platforms for biorefinery is a common practice and could significantly lead to various outcomes which guide towards the circular economy (Velvizhi et al., 2022). The remaining liquid fraction can be returned to the photobiosynthesis process after supplementing an alternative carbon source e.g. by CO_2 from flue gases or $NaHCO_3$.

It is assumed that the optimization of thermostable C–PC biosynthesis as well as the development of a sequence of efficient downstream processing (DSP) operations, including new methods, not used so far for C–PC purification (Foam Fractionation – FF, Aqueous Two-Phase Systems – ATPS, Membrane Filtration – UF, Fast Protein Liquid Chromatography – FPLC), will allow for the elaboration of an effective method for production of C–PC of required purity.

2. MATERIALS AND METHODS

2.1. Photobiosynthesis

The thermophilic strain *Synechococcus sp.* PCC6715, from the Collection of Cyanobacteria Institute Pasteur (Paris, France), was cultivated at $45\,^{\circ}C$ in a non-sterile BG11 medium prepared according to UTEX guidelines in two photobioreactors (PBR) presented in Figure 2: a laboratory helical-tube photobioreactor Biostat PBR-2S (Sartorius) with a working volume of 2.7 L and in a flat panel photobioreactor Labfors 5 Lux LED (Infors HT) with 1.9 L flatbed culture vessel with an efficient airlift mixing. The construction, gas and liquid flow patterns inside the vessels and therefore hydrodynamics and mass transfer conditions in both bioreactors are entirely different. The helical bioreactor consists of two volumes connected in-series: one is the aerated glass container ("tank"), where the ideal mixing conditions of the liquid may be assumed and where all sensors (e.g. temperature sensor, pO_2, pH and others) are placed, and the other – a helical glass tube where the flow of the liquid is forced by a peristaltic pump and plug

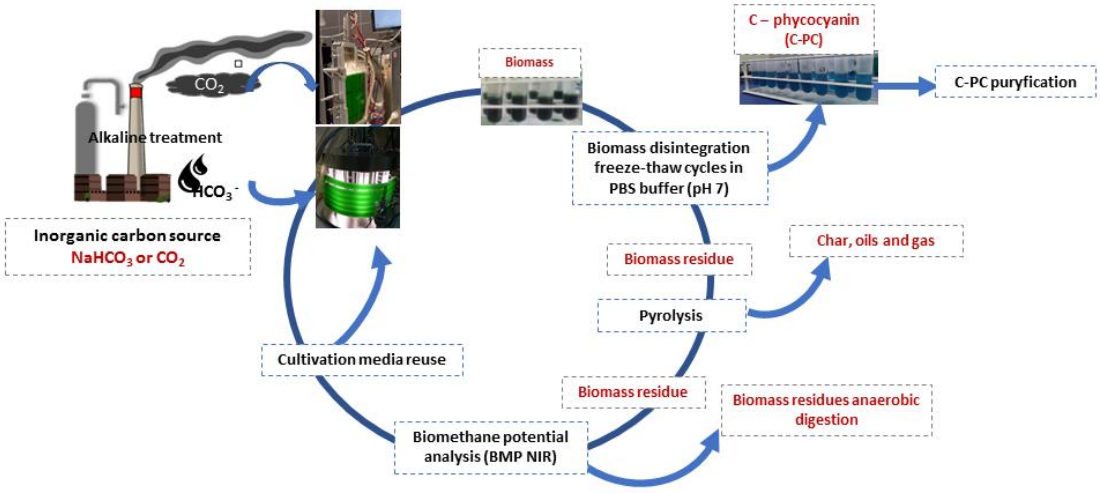

Figure 1. The idea of circular economy approach to the production of thermostable C–PC.

flow may be assumed. The flat panel bioreactor consists of only one flat vessel containing all the nutrient liquid which is aerated by means of a perforated-pipe aerator and then mixed according to the bubble column principle. The light path in this PBR, i.e. the gap between two vertical glass walls of the flat vessel, is 0.02 m.

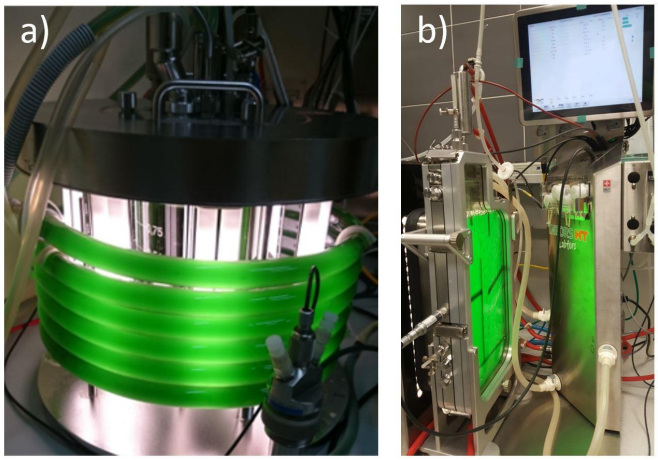

Figure 2. Photobioreactors applied for the cultivation of *Synechococcus* sp. PCC6715; a) Biostat PBR-2S (Sartorius); b) Labfors 5 Lux LED (Infors HT).

Hydrodynamics and mass transfer characteristics of the Biostat PBR-2S were thoroughly investigated and described elsewhere (Gluszcz et al., 2018). The investigated hydrodynamics parameters were: mean liquid circulation rate, liquid velocity/residence time in the tubular part of the apparatus and mixing time, measured in the wide range of rotary speed of the circulation pump. The influence of the aeration intensity on these parameters was also checked. Next, the volumetric oxygen and carbon dioxide transfer coefficients in the liquid phase and their dependency on the liquid circulation velocity and gas inflow rate were determined, using the dynamic (gassing-in) method (Gluszcz et al., 2018).

The hydrodynamics and gas transfer investigations in the flat panel PBR were not so extensive. The most important volumetric gas transfer coefficient in the liquid phase, $k_L a$, and its dependency on the liquid aeration rate was determined, as in the helical bioreactor, using the gassing-in method. Experiments were performed in tap water and then in the real three-phase cultivation broth at the end of thermophilic cyanobacteria cultivations.

The light intensity for Biostat PBR-2S, provided by the unit consisting of 8 fluorescent lamps (Osram Dulux L, Osram GmbH) of cool light was 60 µmol/(m·s). In the case of Labfors PBR light with an intensity of 50 µmol/(m·s) was delivered by the panel of white LED lighting. Photobiosynthesis experiments in both PBRs were carried out using a photoperiod 16 h day/8 h night.

In the course of photobiosynthesis experiments the volumetric liquid flow rate ensuring turbulent flow inside the helical tube of PBR-2S was $4.6 \cdot 10^{-2}$ L/s (Gluszcz et al., 2018), while the volumetric gas flow rate in the flat panel bioreactor ensuring an efficient airlift mixing was $5 \cdot 10^{-2}$ L/s.

In the bioreactors, two different sources of inorganic carbon were used: in the case of Biostat PBR-2S liquid 0.1 M $NaHCO_3$ solution (10% v/v) and gaseous CO_2 7.5 mL/min incorporated into the inlet air stream for Labfors.

The biomass concentration was assessed spectrophotometrically (Agilent BioTek Epoch microplate spectrophotometer) at 680 nm. Volatile solids (VS) were determined by the weight method according to PN-EN 12879:2004. After 6 days of cultivation, the biomass sample for C–PC extraction was harvested by centrifugation, washed with distilled water and again centrifuged, suspended in a minimal amount of distilled water and stored in freezing conditions (–20 °C) before further proceedings.

2.2. Recovery of crude C–PC extract

The defrosted thickened biomass was suspended in PBS buffer and disintegrated by 6 cycles of alternating freeze-thaw (the single-cycle 12 h freezing –20 °C and thawing at room temperature with manual mixing). The supernatant was collected after 10 min centrifugation at $5,000\times$ g. Further, the biomass was resuspended in PBS buffer, mixed thoughtfully and left at the temperature of 4 °C for 24 h with repeated manual mixing. Then biomass was separated by centrifugation, the supernatant was collected, combined with the supernatant from the first series and subjected to spectrophotometric analysis (scanning 200–800 nm Agilent BioTek Epoch microplate spectrophotometer). The extraction was carried out for 4 repetitions, and an error of measurement is presented as a standard deviation. Biomass debris was washed out with distilled water and frozen for further processing. Based on the spectrophotometric analysis the following parameters were calculated: C–PC concentration in PBS according to (Bennett and Bogobad, 1973) C–PC purity (Liu et al., 2005) and C–PC concentration in biomass as yield, respectively Equations (1), (2) and (3).

$$C_{C--PC} = \frac{A_{615} - 0.474 \cdot A_{652}}{5.34} \quad \left(\frac{\text{mg}}{\text{mL}}\right) \tag{1}$$

$$P_{C--PC} = \frac{A_{616}}{A_{280}} \tag{2}$$

$$Y_{C--PC} = \frac{C_{C--PC} \cdot V_{PBS}}{VS \cdot V_B} \quad \left(\frac{\text{mg}_{C--PC}}{\text{g}_{VS}}\right) \tag{3}$$

where: C_{C--PC} – concentration of C–PC, mg/mL, A – absorbance in a given wavelength, [–], V_B – volume of culture medium, mL, V_{PBS} – volume of PBS buffer, mL, V_S – volatile solids, g_{VS}/mL, Y_{C--PC} – C–PC concentration in biomass as yield, mg/g_{VS}.

2.3. C–PC purification methods

An application and comparison of four selected methods for thermostable C–phycocyanin concentration and purification were investigated. Chromatographic separation (FPLC) was run in ÄKTA pure 25 system (GE Healthcare, USA) equipped with an anion exchange Resource Q column (1 mL). Proteins were eluted with a linear gradient of 0–0.2 M NaCl in 10 mM Na acetate buffer (pH 5.0) at a flow rate of 4 mL/min. The ultrafiltration/diafiltration (UF) process was performed with the use of the Sartoflow Smart (Sartorius Stedim Biotech GmbH, Germany) system or the cassette filter connected to the peristaltic diafiltration pump (Millipore, USA). In the experiment, the sample with a C–PC was equilibrated with PBS buffer and transferred from a vessel to the Sartocon Slice 50 Hydrosart membrane with a cut-off point of 5 and 10 kDa. The initial pressure of 2 bar, separating the permeate from the retentate, was applied. The permeate was collected and the retentate was recirculated back to the feed tank for further concentration. The foam fractionation (FF), which is a bubble separation technique, allows for the separation of amphiphilic molecules, such as proteins, from their aqueous solutions. It is carried out not only under mild conditions for biological molecules, but also suitable for diluted solutions. FF was run in a glass column of 0.62 m in length and inner diameter of 0.03 m, a foam collector, a Büchner flask connected to a pump producing low under-pressure, and a compressed air distributor. The column was equipped with a porous glass disperser at the bottom, as was described by Blatkiewicz et al. (2017) After fixing the airflow, 100 mL of the crude extract was poured into the column and the foaming started. Each experiment was conducted until the foam was no longer able to reach the top of the column before collapsing. The process was run in different pH and temperature values and with the addition of surfactants. An aqueous two-phase system (ATPS) consists of two immiscible phases, made by aqueous solutions of specific compounds, such as polymer and salt. It is an attractive method of biomolecule concentration, due to its mild conditions, relatively low cost, and scale-up potential. The systems of phosphate salt and polyethylene glycols (PEGs) with different MW and different concentrations of solutions were investigated. The extraction experiments were performed in a specially designed extraction vessel presented in Blatkiewicz et al. (2018) consisting of a flask and a burette. During experiments the components: water solutions of salt, PEG and crude extract of phycobiliproteins were transferred into the flask and were stirred for an hour at 300 rpm in a thermostated incubator at a temperature set to 25 °C to achieve phase equilibrium between the phases. After mixing, the vessels were carefully flipped upside-down, to introduce the mixture into the burette part and stored in this position for twenty-four hours to separate the phases. Finally, the phases were separated into different vessels and weighed. ATPE was conducted in the batch mode, by simply mixing the components and separating the phases.

To determine and compare the efficiency of purification processes, three main parameters were calculated, as earlier presented in Antecka et al. (2022). The partitioning coefficient (K), which informs about the ratio of C–PC concentrations between the phases in the given process; the recovery yield (R), which informs about the recovery of the C–PC in the given process and was calculated as the ratio of C–PC content after the specific purification process to initial C–PC content in the sample; and the purification factor (PF), which informs about the increase of C–PC purity as a result of the given purification process.

2.4. Pyrolysis of microalgae in thermobalance coupled with a mass spectrometer

The studies of biomass before C–PC extraction and biomass debris after extraction were conducted in a thermobalance (Mettler–Toledo TGA/SDTA851 LF) coupled with a mass spectrometer (MS) (Balzers Thermostar, QMS 200) by a fused silica capillary (0.22 mm i.d.) heated at 200 °C. The end of the capillary was placed very close to the crucible to avoid secondary reactions. The mass spectrometer was equipped with an ionization chamber (70 eV) and the secondary electron multiplier as a detector (1000 keV). Dry feedstock samples (about 20 mg) were heated from 30 to 900 °C under an inert gas flow (argon). Three heating rates of 5, 10, and 20 °C/min were applied. The argon flow through the furnace was equal to 100 mL/min. The small mass of the sample, the low heating rate of the furnace, and the high flow of argon through the furnace allowed for reducing the occurrence of secondary vapor-solid reactions at higher temperatures and decreased the influence of heat and mass transfer on the pyrolysis process. For each sample, TG analysis was carried out in triplicate and the standard deviation never exceeded 3%. Kinetic computations were conducted using Kinetics software (Netzsch). All analytical procedures were performed following Standard Methods. Prior to the pyrolysis experiment, the biomass debris was freeze-dried (Alpha 1-4 LSC, Christ).

3. RESULTS AND DISCUSSION

3.1. Mass transfer in photobioreactors

The differences between liquid flow pattern and mass transfer conditions in the Biostat and Labfors bioreactors arise from the different construction and operation of these PBRs, mentioned in Section 2.1. One of the main parameters, influencing the Biostat PBR-2S performance is velocity of the liquid circulating through the bioreactor, forced by a pump, which is absent in the Labfors. That is why the obtained values of volumetric mass transfer coefficient, $k_L a$, (of the order of 0.03 1/s) in both bioreactors (Figures 3a and 3b) are concurrent only in the absence of liquid flow in the helicoidal bioreactor. In this case the liquid in both bioreactors

(i.e. strictly speaking in the glass tank of the Biostat and in the main vessel of the Labfors) is mixed only by the inlet gas stream and then a similar dependency of the k_La on the gas flow rate is observed. When the liquid flow is forced in the helicoidal bioreactor, where there is no contact of the medium with the gas bubbles and hence no gas-liquid mass transfer inside the helicoidal tube, the k_La values calculated for the whole bioreactor significantly decrease. Therefore, in this bioreactor it is definitely worth using sodium bicarbonate as a carbon source instead of gaseous CO_2. However, in the case of a flat plate bioreactor, the use of $NaHCO_3$ does not fulfil its role, because it is necessary to use gas anyway in order to maintain the airlift mixing system.

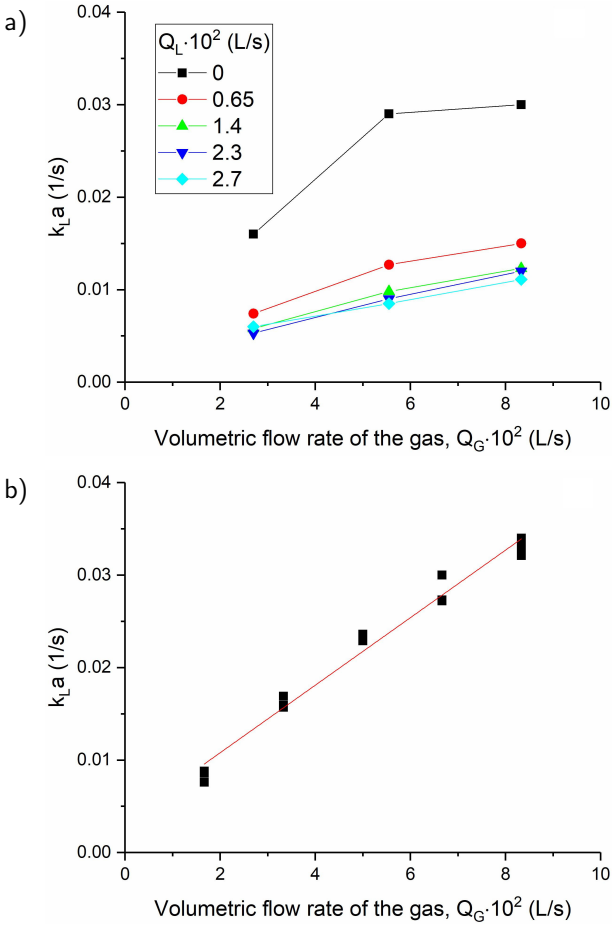

Figure 3. Volumetric mass transfer coefficients in a) Biostat PBR-2S (Sartorius) and b) Labfors 5 Lux LED (Infors HT).

3.2. Biomass growth and crude extract production

Kinetics of *Synechococcus* sp. PCC6715 growth in both photobioreactors is presented in Figure 4. *Synechococcus* sp. PCC6715 was able to grow on gaseous CO_2 and bicarbonate. The lag phase was not distinct, however, characterized by a similar growth for both forms of inorganic carbon used. In the next stage of growth, the higher affinity of the strain towards carbonate than for gaseous CO_2 can be observed, resulting in the same biomass concentration achieved on the 5th and 6th day of cultivation. In the case of Biostat and bicarbonate, after the six-day, the biomass decline starts without a stationary phase, whereas in the case of gaseous CO_2 and Labfors the growth ceases, but cells remain metabolically active. The inorganic carbon source did not influence C–PC concentration in biomass as well as its purity as shown in Table 1.

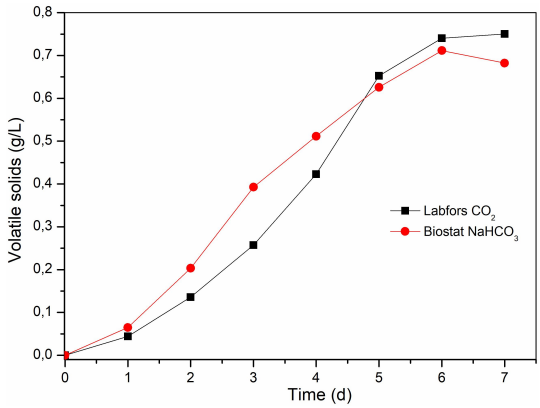

Figure 4. Kinetics growth of *Synechococcus* sp. PCC6715 in both photobioreactors Biostat PBR-2S (Sartorius) and Labfors 5 Lux LED (Infors HT).

Table 1. The comparison of final concentration of C–PC and its purity in both photobioreactors.

	Y_{C--PC} (mg/g$_{VS}$)	P_{C--PC} (–)
Labfors CO_2	258.88 ± 12.80	1.67 ± 0.08
Biostat $NaHCO_3$	257.71 ± 12.60	1.57 ± 0.08

3.3. Crude extract purification

Three methods: foam fractionation, aqueous two-phase extraction and ultrafiltration, have been previously studied and the best results were presented (Antecka et al., 2022). However, further research concerning different process conditions allowed for new achievements and estimation of averaged values of parameters. Also, another method, chromatography, has been added and thoroughly investigated. Table 2 presents

Table 2. The comparison of four tested methods for separation and purification of C–PC.

Purification method	R (%)	K (–)	PF (–)
FF Flow rate 2.4 L/h	52.90 ± 3.47	40.56 ± 13.30	1.32 ± 0.11
ATPE PEG 6000-phosphate salt	80.96 ± 2.01	75.18 ± 16.71	1.51 ± 0.07
UF Hydrosart 10 kDa	76.50 ± 7.56	–	1.44 ± 0.05
FPLC Resource Q column	78.77 ± 0.03	–	3.29 ± 0.05

the selected averaged results, regarding the recovery yield (R), partitioning coefficient (K) and purity factor (PF) as the most important parameters for subsequent processes. Each of the methods can be used separately, both in a batch and continuous mode.

The FF process, as was earlier demonstrated (Antecka et al., 2022), did not require pH adjustment, or the addition of surfactants. Also, a temperature other than room temperature was not valuable and did not improve the results. The studied conditions of the process resulted in a recovery yield of about 53%, higher than the previously mentioned partitioning coefficient of 40.56 and a purification factor of up to 1.32. In the ATPE process, regardless of the system used, C–PC was concentrated in a PEG phase. The most effective ATPS consisted of polyethylene glycol 6000 and phosphate salt with a recovery yield of almost 81%, partitioning coefficient of about 75 and purification factor of 1.51. Ultrafiltration gave a recovery yield of 76.5% and a purification factor of 1.44. Compared to that, the ion exchange chromatography resulted in a recovery yield of about 79% and the highest purification factor of 3.29. During elution, fractions containing C–PC were found in two major peaks (Figure 5) and it was possible to separate allophycocyanin in the separate fraction. The purity ratio of the final bioproduct was about 3.9, which allows it to be classified as a reactive grade (Wu et al., 2016).

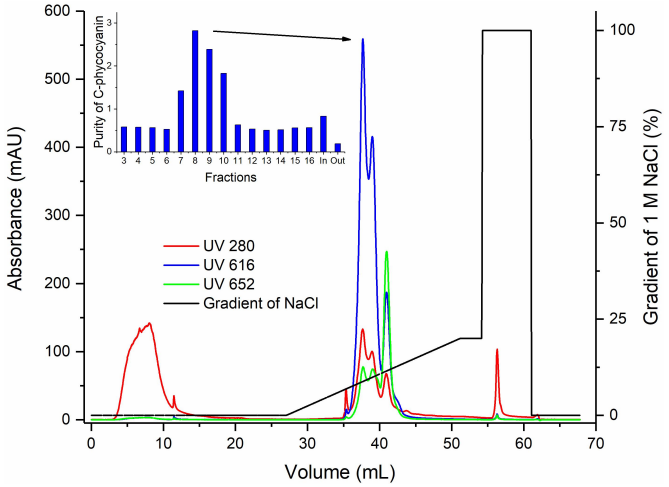

Figure 5. Purification of C–phycocyanin with anion exchange chromatography on a Resource Q column.

The results confirmed that the classical methods, such as ultrafiltration and chromatography, are the most effective, but they are also very expensive and time-consuming. Therefore, the presented results indicate the possibility of their at least partial replacement or combination with new approach methods, foam fractionation or aqueous two-phase extraction. From a practical and economic point of view, a three-stage purification system has been proposed and is planned to be investigated (Figure 6).

FF will be used as the first stage, as it is suitable for diluted solutions, then chromatography, as the most effective purification method, followed by UF for further concentration and diafiltration. ATPE, which separately resulted in quite effective purification as well as concentration, seems to be slightly difficult to combine with other methods because of residual PEG, which is hard to remove by all three investigated methods. Therefore, it is proposed as a good alternative to the above-mentioned three-stage process.

3.4. Biomass debris valorization

The proximate and ultimate analysis of cyanobacteria biomass before and after extraction is shown in Table 3. Biomass before extraction is characterized by higher volatile and ash content and lower fixed carbon than after extraction.

Table 3. Proximate and ultimate analysis of algae before and after extraction.

	Before extraction	After extraction
	Proximate analysis	
Moisture [wt.%]	4.55 ± 0.07	3.38 ± 0.05
Volatiles [wt.%]	71.53 ± 0.93	69.85 ± 0.89
Fixed carbon [wt.%]	16.94 ± 0.24	21.14 ± 0.33
Ash [wt.%]	6.98 ± 0.08	5.33 ± 0.05
	Ultimate analysis	
N [wt.%]	10.62 ± 0.02	9.18 ± 0.01
C [wt.%]	47.74 ± 0.06	48.46 ± 0.03
H [wt.%]	6.90 ± 0.02	6.91 ± 0.02
S [wt.%]	0	0
O [wt.%]*	27.77	30.11

*Calculated by a difference

Figure 6. A three-stage method to achieve a C–PC of a high degree of purity.

DTG curves received from a thermobalance indicated three phase decomposition of biomass (Figure 7a). The second phase which occurred in the range of 200–600 °C was related to the main pyrolysis process. In this phase carbohydrates (hemicellulose, cellulose, lignin), proteins and lipids are decomposed (Ślęzak et al., 2022). Extraction of microalgae caused slight changes in DTG curves in 2nd phase (Figure 7a). The activation energy of the pyrolysis process was determined using the Friedman method described in an earlier study (Matusiak et al., 2020). Biomass after extraction needed less energy to carry out the pyrolysis process than biomass before extraction (Figure 7b). Lower activation energy could be a result of cell wall disruption during the extraction process. Biomass after extraction was characterised by a higher yield of char and tar production during the pyrolysis process than biomass without extraction (Figure 7c). During the biomass pyrolysis the yield of water production was about 19 wt.%. Pyrolytic oil from a slow pyrolysis process contained much more water than from fast pyrolysis and can decant the aqueous phase from tar (Bridgwater, 2012). The applied pyrolytic gas analysis technique (MS) allowed only for methane accurate measurement. The concentration of higher hydrocarbons was estimated to be below 7% as described in Almeida et al. (2022). When analysing the effect of biomass extraction on the pyrolytic gas composition at 600 °C, no significant changes were noted (Figure 7d). The gas produced during algal pyrolysis contained four dominant components H_2, CH_4, CO and CO_2. The pyrolytic gas from the algae before and after extraction contained a high concentration of hydrogen (19.8 and 22.9%, respectively).

4. CONCLUSIONS

Synechococcus sp. PCC6715 can use various forms of inorganic carbon and transform them into high-value-added product thermostable C–PC. Helical tube and flat panel photobioreactors were similarly effective in the microalgae growth and C–PC productivity. Alternative methods to traditional DSP such as FF and ATPE have been tested, with ATPE having higher recovery yield and partitioning coefficient. A three-stage purification system (FF, FPLC and UF) is recommended. The purity ratio of the final bioproduct was about 3.9, which allows it to be classified as a reactive grade. The pyrolysis of microalgae debris after C–PC extraction leads to bioproducts: oils, gas, biochar. The results obtained on the biosynthesis and DSP of C–PC may improve process profitability and support 3G biorefinery development.

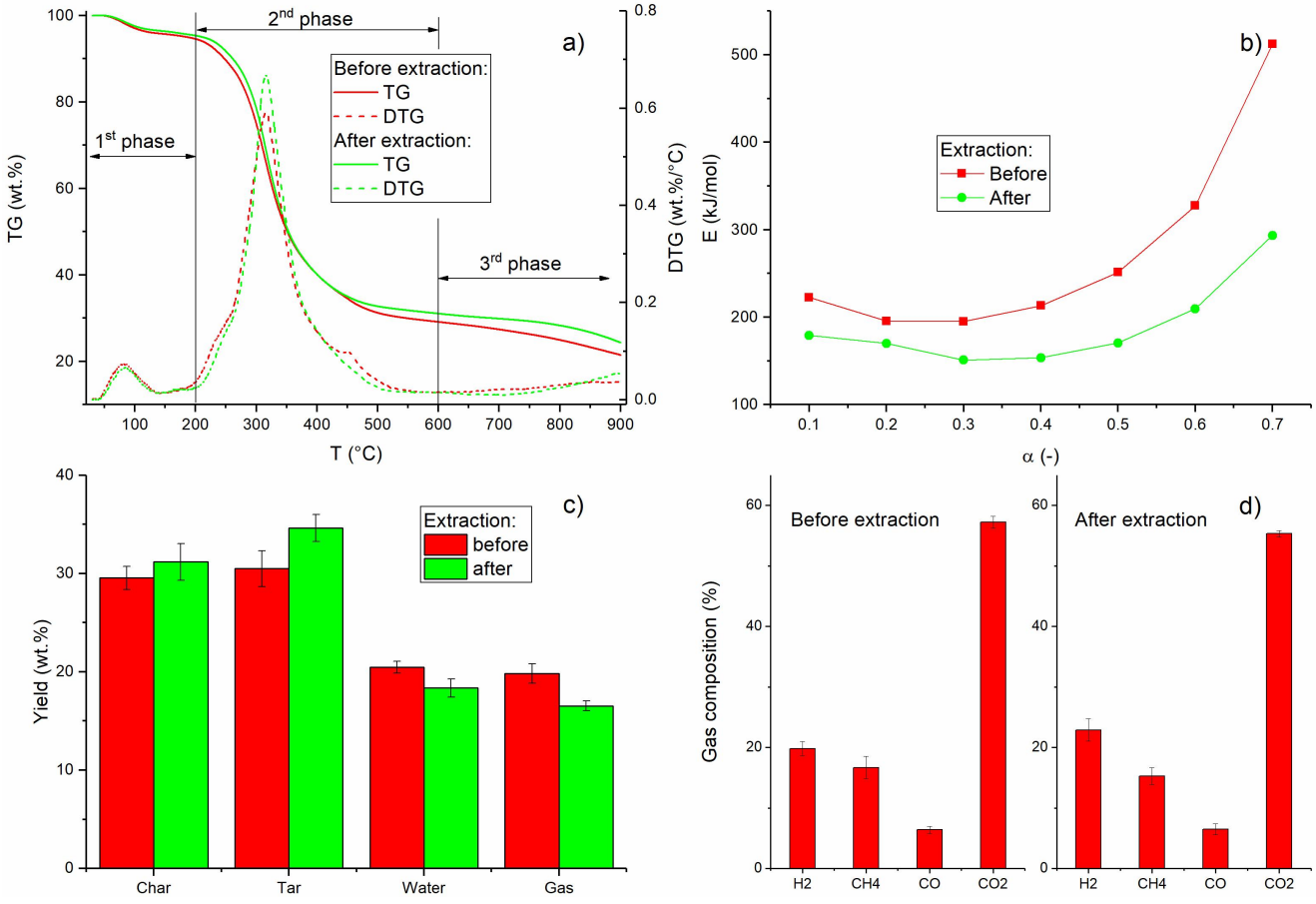

Figure 7. Pyrolysis of algae before and after extraction: a) DTG curves at furnace heating rate of 10 K/min, b) activation energy, c) yield of pyrolytic products at 600 °C, d) composition of pyrolytic gas at 600 °C.

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SYMBOLS

A	absorbance, –
C_{C--PC}	concentration of C–PC, mg/mL
DTG	derivative thermogravimetry, wt.%/°C
E	energy of activation, kJ/mol
K	partitioning coefficient, –
$k_L a$	volumetric mass transfer, 1/s
P_{C--PC}	C–PC purity, –
PF	purification factor, –
Q_G	volumetric flow rate of gas, L/s
Q_L	volumetric flow rate of liquid, L/s
R	recovery yield in purification, %
T	temperature, °C
TG	thermogravimetry, wt.%
V_B	volume of culture medium, mL
V_{PBS}	volume of PBS buffer, mL
VS	volatile solids, g_{VS}/mL
Y_{C--PC}	C–PC concentration in biomass as yield, mg/g_{VS}

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