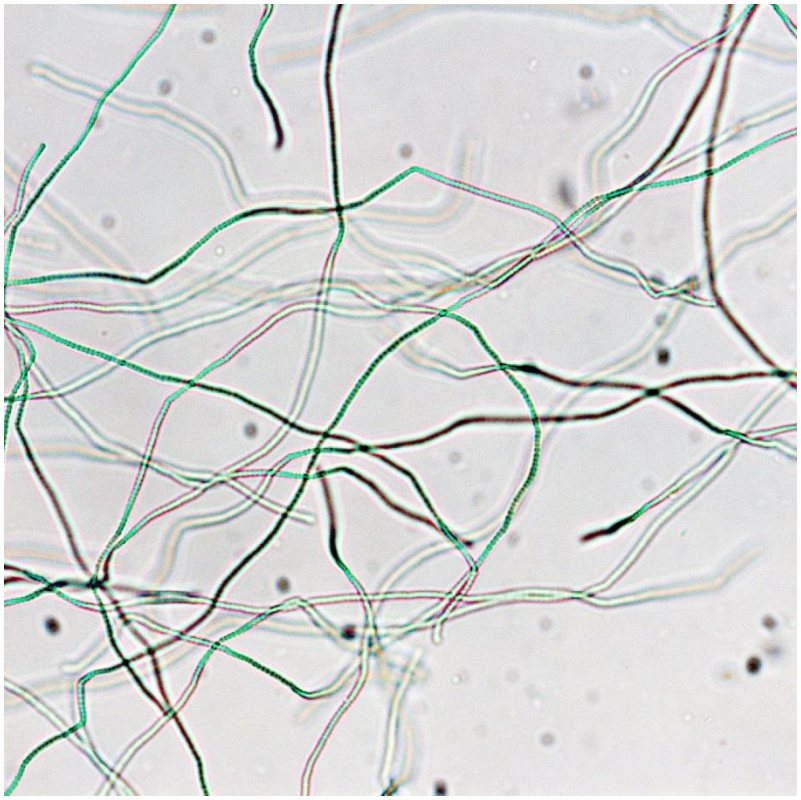
An improved and efficient method for the extraction of phycobiliproteins for optimization of phycocyanin productivity in *Leptolyngbya* sp. QUCCCM 56 under different irradiance and temperature



Student : Filippo Fortunati
Study programme: Master in Suitable Biotechnology
Supervisor(s) Kira Shipper, Maria Barbosa, Peter Westerman

Abstract

One unique feature in cyanobacteria and rhodophytes is the presence of pigments-these water-soluble pigments can absorb light in spectral range poorly absorbed by chlorophyll enhancing the spectral range available for cellular conversion to chemical energy. Efficient detection of these compounds is essential for use in physiological studies. A protocol has been designed to extract phycobiliprotein from *Leptolyngbya* sp. QUCCCM 56, *Arthrospira platensis and Rhodomonas* sp. combining different disruption methods, extraction solutions, biomass amount and incubation times. In all the strains tested the extraction efficiency proved to match what reported in literature while in regards of the purity all the strain achieved higher values (Arthrospira *platensis* 100%, Rhodomonas sp. 288% and *Leptolyngbya* sp. 370%). With the designed protocol it was investigated the effect of light intensity (80–1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and temperature (20–45°C) on phycocyanin productivity in *Leptolyngbya* sp. QUCCCM 56. Maximum phycocyanin productivity of 0.091 $g_{PC} \cdot d^{-1} \cdot l^{-1}$ was measured at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 40°C. The Phycocyanin content decreased at higher light intensities and it increased at higher temperatures. The biomass productivity has one peak at 300 $\mu mol \cdot m^{-2} \cdot s^{-1,}$ while the temperature increases the productivity at higher values.

Contents

1. Introduction

In a world where natural resources are being extracted and consumed at an ever increasing rate, there is a growing need to seek alternatives to provide nutrients, chemicals and energy for mankind in a sustainable way. In recent years, cyanobacteria (blue-green algae) have gained interest thanks to their potential to address this issue. They find applications as food supplements, in the production of fine chemicals, as biofertilizer and in wastewater treatment [1]. In addition, they also produce a wide variety of bioactive molecules with useful therapeutic purpose as strong antiviral, antibacterial, antifungal, antimalarial, antitumoral and anti-inflammatory [2] .

Cyanobacteria are gram-negative oxygenic photosynthetic prokaryotes with a long evolutionary history. They live in diverse and sometimes extreme conditions [3]. Exposure to various environmental stresses like ultraviolet radiation [4] , high temperature, altitude, high/low light irradiation, high pH and high salt concentration have forced cyanobacteria towards gradual adaptation to maintain the cell viability [5] [6] [3]. Especially, an obligate sunlight requirement for photosynthesis have exposed cyanobacteria to the wide range of solar radiation; which in turn, enhanced the synthesis of a number of photon absorbing pigment molecules. In cyanobacteria, the light-harvesting pigments include chlorophyll-a, carotenoids and phycobiliproteins (PBPs)[7]. The latter may comprise up to 60% of their total soluble protein content [8].

The Algal Technologies Program from Qatar University has isolated over 200 microalgae and cyanobacteria from the Qatar Environment. One among these, *Leptolyngbya* sp. QUCCCM 56 displayed a significant phycocyanin (a type of PBP) content. This strain has been isolated from an marine environment with salinities of approximately 40-42 ppt, high solar irradiances (maximum of 22000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and high temperatures (up to 45 ˚C) [9] [10]. With these conditions, this strain could be interesting for a large-scale production, as it does not require fresh water for cultivation and could be robust in terms of coping with high light intensities and temperatures.

1.1 Phycobiliproteins

Phycobiliproteins (PBP) are water-soluble proteins present in cyanobacteria and red algae, which function as light-harvesting proteins that absorb light in regions of the visible spectrum that are absorbed poorly by chlorophylls. Light energy trapped by PBPs is transferred to chlorophyll a with an efficiency approaching 100% during photosynthesis [11] [12].

PBPs are divided into three main classes according to their spectral characteristics: phycoerythrin (PE, λA max = 540-570 nm; λF max = 575-590 nm) with a pink colour pigment, phycocyanin (PC, λA max= 610-620 nm; λF max: 645-653 nm) with a brilliant blue colour pigment and allophycocyanin (APC, λA max = 650-655 nm; λF max = 657-660 nm) with a light blue colour pigment as it can be seen in Figure 1[13] [14].

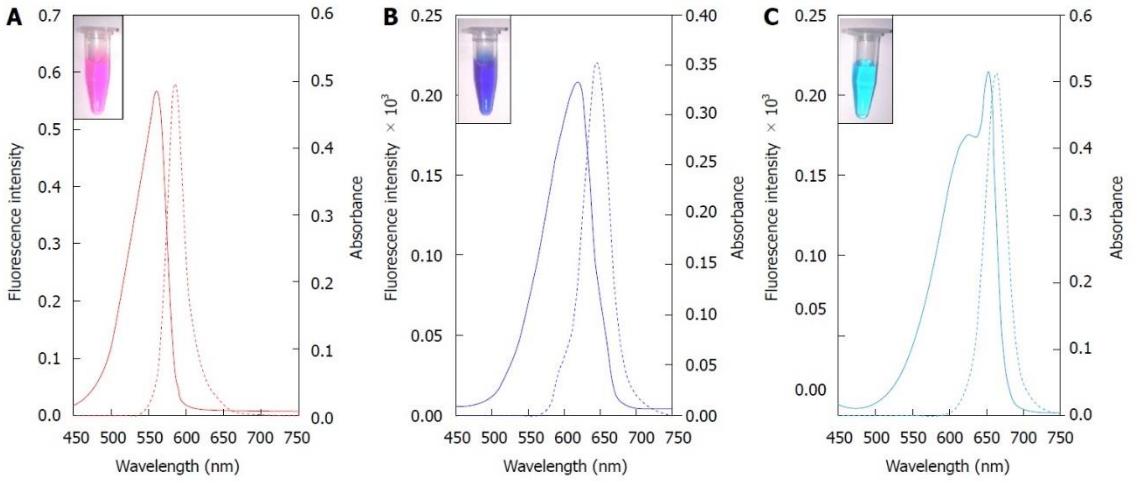


Figure 1: UV-visible absorbance (solid line) and fluorescence emission(dotted line) spectra, and appearance (inset) of A:Phocoerythrin, B: Phycocyanin and C: Allophycocyanin [15].

Phycobiliproteins are assembled into particles named phycobilisomes which are attached in regular arrays to the external surface of the thylakoid membrane. Phycobilisomes consist of allophycocyanin cores surrounded by Phycocyanin and phecoerityrin on the periphery. Phycocyanin is the major constituent while allophycocyanin functions as the bridging pigment between phycobilisomes and the photosynthetic lamella [16]. A schematic representation of these structures is shown in Figure 2.

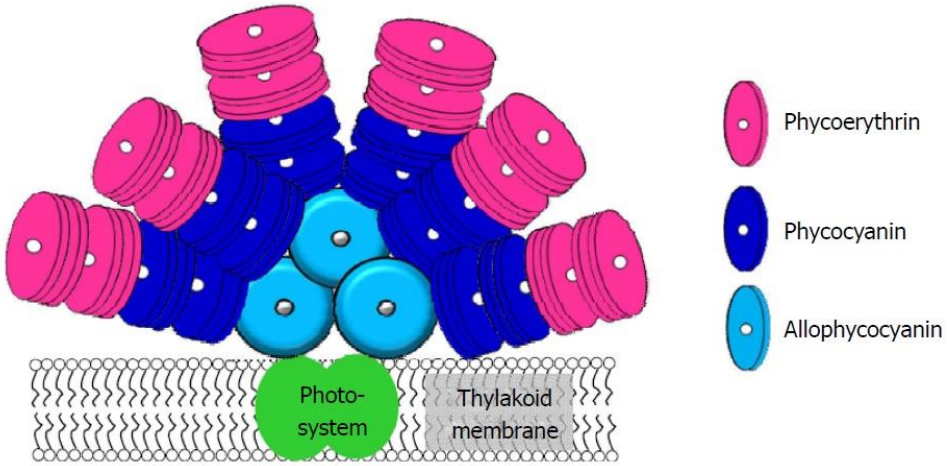


Figure 2: Schematic diagram of the location of phycobilin proteins on the thylakoid membrane [15]

Phycobilliproteins are high valuable products that are gaining importance as natural colorants over synthetic colour, as they are nontoxic and non-carcinogenic . The unique spectral features of PBPs, proteineous nature and, some imperative properties like hepato protective, antioxidants, anti-inflammatory and anti-aging activity of PBPs enable their use in food, cosmetics, pharmaceutical and biomedical industries [17] [18]. Such large range of applications increases the demand of PBPs in commodity market.

1.2 Aim

The aim of this study was to characterize the productivity of phycocyanin in *Leptolyngbya* sp. QUCCCM56 under different cultivation conditions.

The study was divided in two parts, the first consisted in the design of a reliable extraction protocol for PC in *Leptolyngbya* sp. In a second phase the PC productivity in *Leptolyngbya* sp. was investigated in flat panel bioreactors at different temperatures and light intensities using the extraction protocol previously designed.

1.2.1 Aim 1: Extraction protocol

No protocol was found in literature to extract PC in *Leptolyngbya* sp. therefore a new one has been designed. The aim hereof was to fulfil the following features:

- Be a reliable and reproducible method for PC extraction
- Have a limited duration of extraction to allow for fast feedback
- Determinate biomass amounts required for analysis
- Determinate total amount of PC in the cell
- Determinate differences between different strains: Arthrospira platensis, *Leptolyngbya* sp. and Rhodomonas sp.

Reliability and a short duration of the extraction were considered as priorities, given that the protocol's aim is to keep the same extraction efficiency over different cultivation condition with the shortest waiting time possible to be as practical as possible.

PC is generally accounting for approximately the 10-20% of blue-green algae dry weight [19]. Given that the protocol was designed before knowing the actual amount of PC per cell, in *Leptolyngbya* sp. two biomass amounts were tested to ensure reliability of the measurements avoiding to sample big volumes from the bioreactors in the next phase.

The protocol has been designed in order to repeat the extraction until there was no PC left thereby determining the PC content and the extraction efficiency of each method tested.

Even though the protocol was designed for the extraction of PC in *Leptolyngbya* sp., it was also tested if the protocol could be applied to a wider range of strains. Arthrospira platensis is widely known for its production of PC, making it a valid reference for the extraction protocol testing. Furthermore, Rhodomonas sp., red microalgae producing PE, was chosen to test whether there are extraction correlations between different PBPs.

1.2.2 Aim 2: characterization PC productivity in flat panels bioreactors

This characterisation step's aim was to investigate:

- The optimum conditions for the PC productivity in *Leptolyngbya* sp. QUCCCM 56
- The flexibility of the strain in coping with a wide range of light irradiances and temperatures.

In order to find the optimum conditions for the production of PC, temperature and light intensity were chosen as the most influencing features, which are also relevant for the possible outdoor production in Qatar. The major biological role of phycobiliproteins is photosynthetic light harvesting, and their absorption maxima are located at wavelengths where chlorophylls have low extinction coefficients. Therefore, a different phycobiliproteins content on different light intensities can be expected [20]. Temperature was reported to enhance PC productivity at high temperatures [21] [22] . Combination of conditions that could be nicely applied in Qatar.

2. Materials and Methods

2.1 Strain and cultivation conditions

Leptolyngbya sp. QUCCCM 56 was obtained from Qatar University, which isolated the cyanobacteria from the Qatar Environment. Stock cultures were maintained in Zarrouk medium [Appendix 1], in an incubator (Snijders Scientific®; Micro Clima-Series; Economic Lux Chamber) at 30°C under a 12:12 day:night cycle with a light intensity of 80-90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as measured with a LI-250A Light Meter (LI-COR® Biosciences). The culture was agitated using a flask shaker set at 150 rpm (Heidolph Instruments® Rotamax 120).

Rhodomonas sp. is a red marine microalga obtained from Fry marine®(Vlissingen, the Netherlands). Stock cultures were maintained in 20xL1 medium (With the addition of HEPES and NaHCO3 for stable pH in shake flasks cultures pH=7.5) [Appendix 2] in a shaker incubator (INFORS HT®; Multitron Pro) at 25°C, under continuous lighting at an intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 150 RPM, and 2.5% CO2 .

Arthrospira platensis UTEX 1940 was obtained from the UTEX® Culture Collection of Algae (University of Texas, Austin). Stock cultures were maintained in Zarrouk medium in an incubator (Snijders Scientific®; Micro Clima-Series; Economic Lux Chamber) at 25°C, under a 12:12 day:night cycle, with a light intensity of 80-90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as measured with a LI-250A Light Meter (LI-COR® Biosciences). The culture was agitated using a flask shaker set at 150 rpm (Heidolph Instruments® Rotamax 120).

2.2 Extraction Protocol Development

As described in literature, Phycobiliproteins can be extracted using different methods [23] [24] [25] [26]. They all share the same general approach as described in Figure . At the begging the (i) cells are disrupted, followed by (ii) an solubilisation step, for (iii) a certain incubation time. The solid phase is than (iv) separated from the liquid phase, and the (v) PBP concentration in the liquid phase through spectrophotometric analysis.

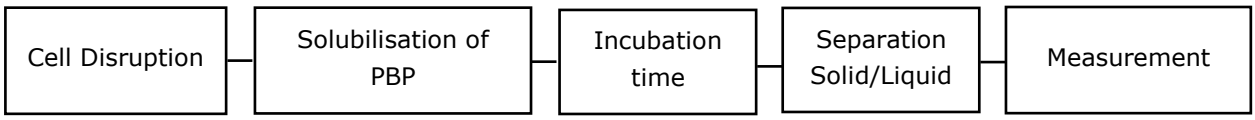


Figure 3: General approach for the Extraction of PBPs

In this study, the same extraction protocol structure was used as described in Figure 3 , but the methods in cell disruption, solubilisation of PBP and incubation time were varied.

Three different cell disruption methods were tested:

(a) Freeze-Thawing, was applied by freezing of the sample at -20°C for 2 hours, followed by thawing and incubation for 24h at 4°C in the dark. It has been reported to be the best technique to extract PC in Arthrospira platensis in regards of extraction yield therefore it was included in the study [23].
(b) Bead beating, a method commonly used for cell disruption for lipid analysis, was achieved with a Bertin® Precellys 24 tissue homogenizer using Lysing Matrix E, 2 mL Tube from mpbio®. The disruption was carried out with three cycles at 2.500 rpm for 25 seconds with a 5 second waiting time between each cycle [27].
(c) Sonication, found by Horvath et al. (2013) and Lawrenz et al, (2010) to be one of the best methods for extraction, was performed on ice, using a VCX 130 Ultrasonic processor (Sonics®) with a continuous pulse at 8W for 5 seconds [28].

To solubilise the PBPs three solutions were tested:

(a) Phosphate buffer 0.1M at pH 6.0 (10.5 $g \cdot L^{-1}$NaH2PO4; 4.4 $g \cdot L^{-1}$; Na2HPO4·12H2O) – commonly used for PBP extraction from Arthrospira platensis with high yields and purities [24].
(b) Calcium Chloride (10 $g \cdot L^{-1}$) was included, for its high purity extractions [26].
(c) MilliQ water was considered due to its low costs and easy availability.

For the sake of simplicity, the collective of these extraction solutions will be referred to as ES (standing for extraction solution).

To assess the efficiency of ES and disruption method, different incubation times were tested, ranging from direct measurement to after 92 hours. After every measurement the supernatant was discarded and the pellet was resuspended in new ES. An overview of all conditions is shown in Figure 4.

After the incubation period, the samples were centrifuged, and the PBPs were measured in the liquid phase with a HACH LANGE® DR 6000 Spectrophotometer using 1-cm polystyrene cuvette (SARSTEDT®). The full protocol is reported in [Appendix 3].

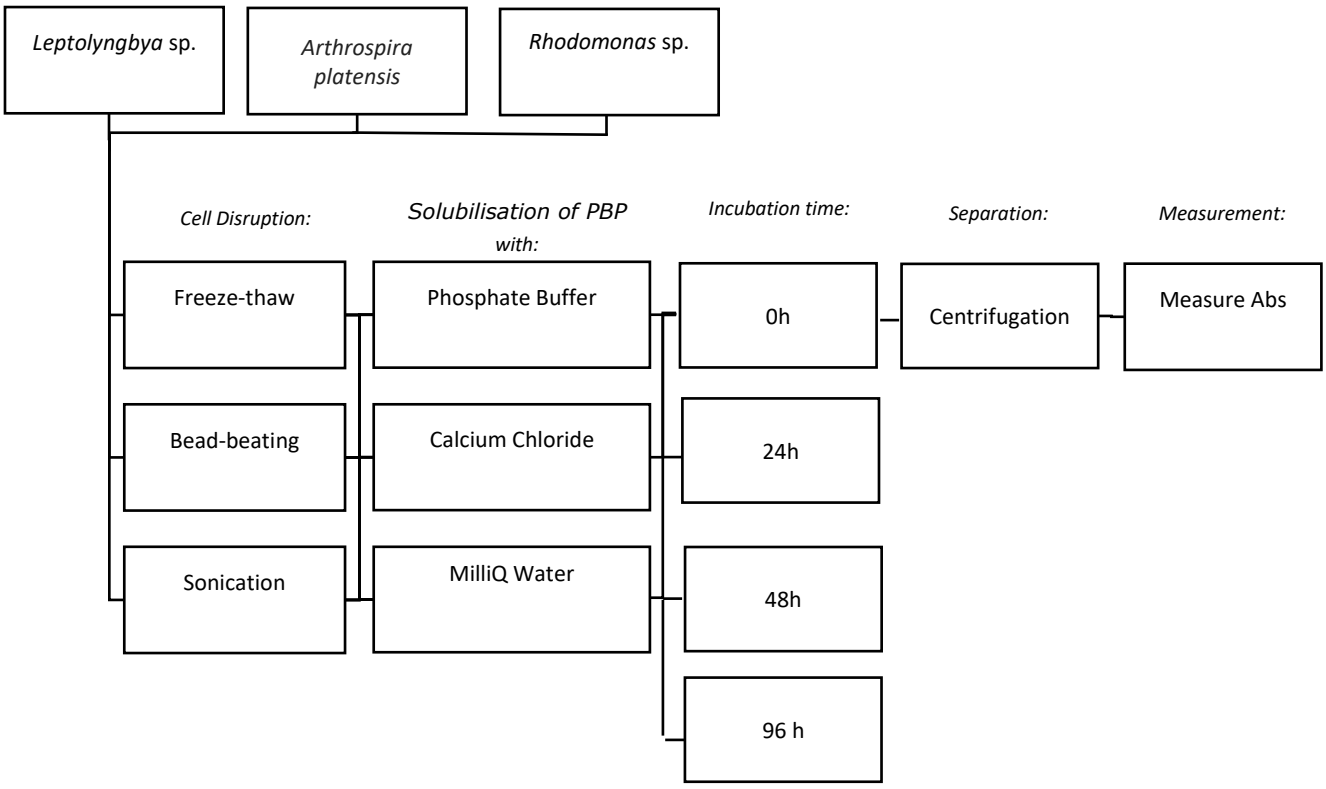


Figure 4: Scheme of the combination tested in tested in the development of the extraction protocol for PBPs.

2.2.1 Spectrophotometric quantification

Absorbance (A) of extracts was measured at 620 and 545 nm for PC and PE respectively, using the appropriate ES as a blank. Absorbance values were scatter-corrected by subtracting the absorbance at 750 nm from the absorbance maximum of either the phycocyanin peak (620 nm) or the phycoerythrin peak (545nm). Phycocyanin and phycoerythrin concentrations (c) (in µg·L−1) were calculated as:

$$c = \frac{A}{\varepsilon d} \cdot MW \cdot \frac{V_{buffer}}{V_{sample}} \cdot 10^6$$

Where MW is Molecular Weight of phycocyanin (264.000 g·mol−1) and for phycoerythrin (241.000 g·mol−1), and ε is the molar extinction coefficients (phycocyanin, 1.9×106 L·mol−1cm−1; phycoerythrin 2.41 × 106 L·mol−1cm−1) and where d is the path length of the cuvette, and Vsample and VES are the volumes of the sample and ES respectively [23].

2.2.2 Extraction purity

The purity of the phycocyanin fraction was measured spectrophotometrically using a HACH LANGE® DR 6000 by the ratio of absorbance at either 620 or 545 nm, divided by absorbance at 280 nm using 1-cm polystyrene cuvette(SARSTEDT®) [29]. This ratio is an indication of the extract purity (EP), with respect to most forms of contaminating proteins, as absorbance at 280 nm reflects the total concentration of proteins in the solution, and the measurements at 620 and 545 nm those for PC and PE specifically [30].

$$EP = \frac{OD_{620\ or\ 545}}{OD_{280}}$$

where EP is the extract purity, OD620 or 545 is the optical density of the sample at 620 nm or 545, and OD280 is the optical density at 280 nm.

2.2.3 Biomass Dry Weight Analysis

In order to determine the amount of phycocyanin and phycoerythrin per biomass dry-weight, the dry weight (DW, $g \cdot L^{-1}$) of the analyses biomass was determined using a gravimetrical analysis reported in the protocol in the [Appendix 4] .

$$DW = \frac{w_2 - w_1}{V_{Sample}}$$

Where w1 (mg) is the weight of the filter dried in the oven at 95 °C for 24 h. W2 (mg) is the weight of the filter after loading the sample and drying it in the oven at 95 °C for 24 h. Vsample is the volume of the sample in L.

2.3 Strain Cultivation in Photobioreactors

Leptolyngbya sp. QUCCCM 56 was cultivated in flat-panel airlift photobioreactors (Algaemist, Technical Development Studio, Wageningen University, the Netherlands, Figure 5), with a working volume of 0.4 L, an optical depth of 14 mm, and an illuminated area of 0.028 m2 (see Fig. 1 for a schematic overview). Light was provided by LED lamps (BXRA W1200, Bridgelux, USA) with a warm-white light spectrum with a 12:12 day:night cycle. The pH was maintained at 9.0 (±0.1) through on-demand addition of CO2 to air, which was continuously supplied at a rate of 200 mL·min−1 (±20), and which also provided agitation for the culture, and prevented oxygen accumulation. The reactors were operated in turbidostat mode to ensure a constant biomass concentration, and therefore a constant biomass DW:light ratio. The reactors were equipped with a planar photodiode (type SLD-70BG2, Silonex, Canada) behind the rear glass panel to measure the transmission of light through the culture. When light transmission decreased below the selected set-point, the Algaemist dilution pump (type 400A1,

Watson Marlow, UK) was automatically engaged, and the culture was diluted with fresh medium. Each reactor was connected to a harvest vessel via an overflow stored in the dark in a fridge at 4°C.

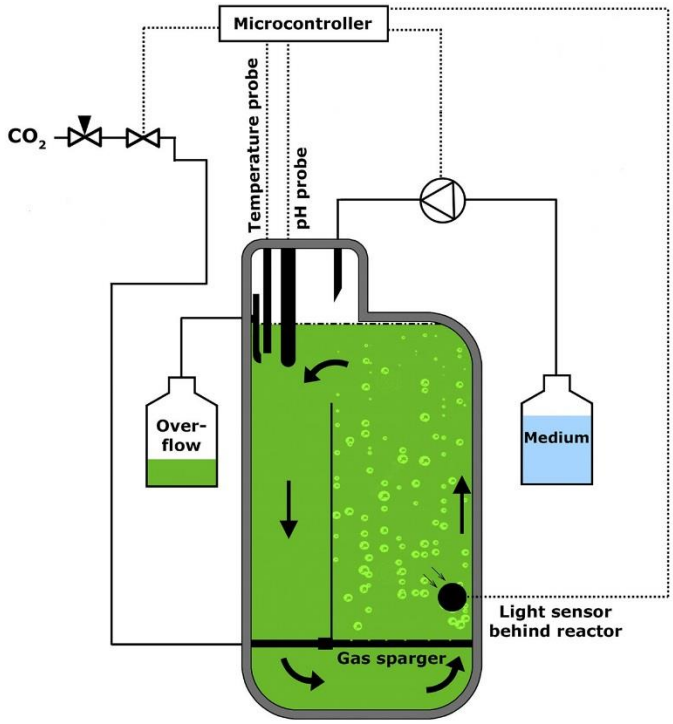


Figure 5: Schematic representation of an AlgaeMist Photobioreactor [31]

2.3.1 Photobioreactor operation

Four reactors were heat-sterilized (30 min at 121 °C), and cultures were first grown in batch mode. After the desired biomass concentration of 1 g·L^{-1} was achieved turbidostat control was initiated. The optical density at 750nm and the harvest volume of all reactors was measured on a daily basis. The biomass concentration was determined from dry weight measurements performed once every 48h in duplicate. A stable steady state was defined as the condition of the reactor where the cell dry weight concentration and the harvest volume were constant after a period of at least three complete dilutions. When the bioreactor was considered in steady state, the PC content was measured for three consecutive days using the PC protocol previously designed.

The equation to calculate biomass productivity was derived from the biomass mass balance, and the definition of biomass productivity r_x (in g·L^{-1}·d^{-1}) as per the following equations:

$$\frac{\mathrm{d}(M_{x,R})}{dt} = \mu \cdot C_{x,R} \cdot V_R - F_{out} \cdot C_{x,out}$$

$$r_x = \mu \cdot C_{x,R}$$

Where ($M_{x,R}$) is the total biomass dry weight amount (g) in the reactor, μ is the growth rate (d^{-1}) , $C_{x,R}$ is the biomass dry weight concentration in the reactor ($g \cdot L^{-1}$), V_R is the reactor volume (L), Fout is the harvest flow collected in the overflow vessel ($L \cdot d^{-1}$), and $C_{x,out}$ is the biomass dry weight concentration of the harvest flow ($g \cdot L^{-1}$). By assuming no biomass accumulation ($\frac{d(M_{x,R})}{dt}$=0), the following equation is obtained:

$$0 = r_x \cdot V_R - F_{out} \cdot C_{x,out} \leftrightarrow r_x = \frac{F_{out} \cdot C_{x,out}}{V_r}$$

Similarly, it can be deduced from the same biomass balance that the specific growth rate μ (d^{-1}) equals the reactor dilution rate D when assuming the reactor volume V_r is constant, and the reactor is ideally mixed

$$C_{x,R} = C_{x,out}, \mu = \frac{F_{out}}{V_R} = D$$

The Phycocyanin content (PC_c in $mg \cdot g^{-1}$) was calculated multiplying phycocyanin concentrations (c) (in $mg \cdot L^{-1}$) by the concentration of the culture c_x ($g \cdot L^{-1}$)

$$PC_c = c \cdot c_x$$

Consequently, from PC_c and r_x was derived the PC productivity (PC_p in $mg \cdot L^{-1} \cdot d^{-1}$) as followed:

$$PC_p = PC_c \cdot r_x$$

2.3.2 Operations light intensity and temperature experiment

Given that PC expected to be expressed more under low light intensities [19], a higher frequency of data points was analysed between 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Subsequently, the light-intensity was increased until reaching 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the closest light intensity to the Qatari environment (maximum of 22000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) that the bioreactor could accommodate [10]. For each light intensity tested the planar photodiode in the rear of the bioreactor had to be adjusted manually to keep the biomass concentration in the reactor constant. The two couples of duplicates were started at the lowest light intensity to then increase the irradiance along the duration of the experiment keeping the bioreactor running. This was done in order to adapt the strain faster to the increase of the light intensity in the shortest time possible.

The light intensity was considered a more relevant feature for the PC characterisation than temperature, therefore it was decided to run the light intensity experiment first [20]. The temperature was maintained at 30 ˚C during the duration of the experiment.

Following the light intensity experiment, the PC productivity was determined under a range of different temperatures. The data points tested were chosen to cover the widest range of temperature highs and lows over the year in Qatar, therefore ranging from 20˚C to 45˚C [9]. The optimal light intensity found for the first experiment was used as light-intensity set-point for the temperature experiment– all other conditions were kept constant over the two experiments.

3 Results

3.1 Extraction protocol

The efficiency of the different extraction methods, with different combinations of: (i) biomass amounts (ii) ESs (iii) cell disruption methods and (iv) incubation times, was determined based on phycobiliprotein extraction yield ($mg_{PC} \cdot g_X^{-1}$) and extract purity (Abs620/Abs280). These results are shown in figures 6 and 7 for *Leptolyngbya* sp. QUCCCM 56, 8 and 9 for *Arthrospira platensis*, and 10 and 11 for Rhodomonas sp.

Leptolyngbya sp.

For the two different biomass amounts tested, higher extraction rates and lower purities were found with 5mg biomass as compared to 10 mg. However, for freeze thawing, the different biomass concentrations resulted in the same extraction yields (per biomass amount). Of the three different disruption methods tested, Sonication had the highest extraction yield in phosphate buffer, however, this method did show the lowest extract purity in all ESs tested. After direct measurement, this method could extract around the 85% of the total PC content. On the other hand, freeze-thawing in phosphate buffer did not extract significant amounts of PC, however, when combined with Calcium Chloride, freeze-thawing was able to extract similar amounts of PC as compared to bead-beating with phosphate buffer (direct measurement). In terms of purity, freeze-thawing, combined with calcium chloride, yielded the highest purity extract, at 6.78, followed closely by bead-beating in phosphate buffer at 6.59. Bead beating with direct measurement yields the same extraction efficiency for the different ESs, however the purity varies significantly. Sonication yielded the highest extraction amounts, without the necessity for additional incubation time – no significant PC extraction was found after 24, 48 or 96 hours of incubation. For bead-beating, up to 71%, 83%, and 87% of total extracted PC was extracted without an additional incubation period in phosphate buffer, calcium chloride, and MilliQ water respectively. Freeze-thawing however, inherently to the procedure, required at least 24 hours incubation time, after which only in Calcium Chloride, significant extraction yields were found. For MilliQ water, after 96 hours still not all the PC was extracted. Overall, bead-beating, in combination with phosphate buffer, reported high extraction yields and extract purity, followed closely by freeze-thawing in Calcium Chloride.

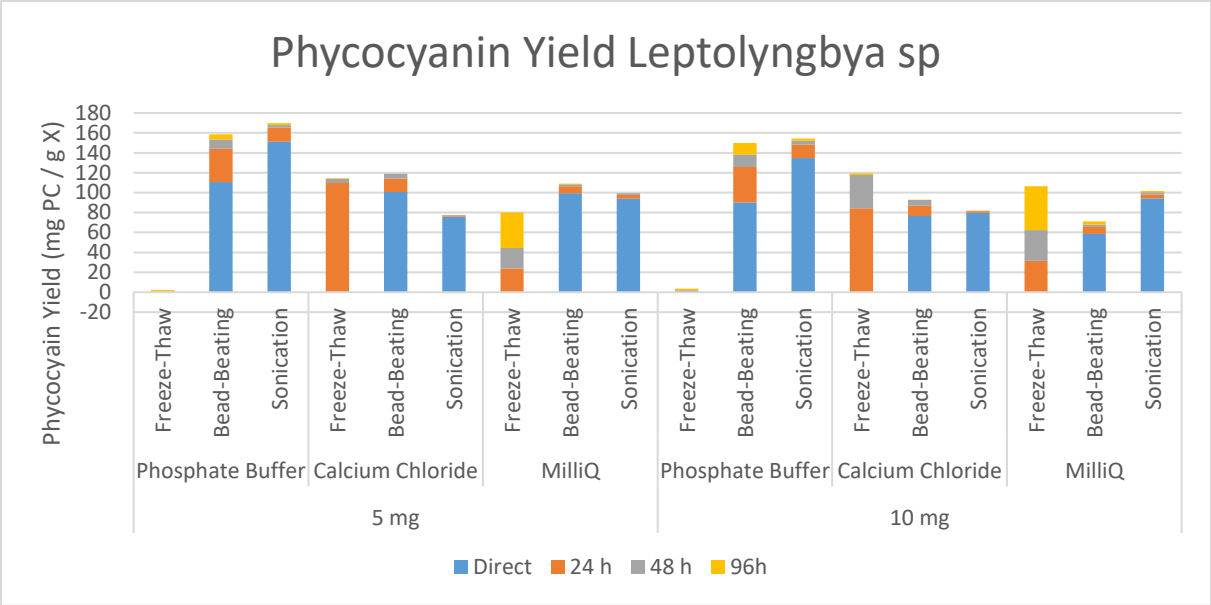


Figure 6: Representation of the extraction yield in Leptolyngbya sp. *for all the combination tested. Therefore, with 2 biomass amounts, 3 disruption methods and 3 ESs tested. Standard deviation was not included, to increase the readability of the graph.*

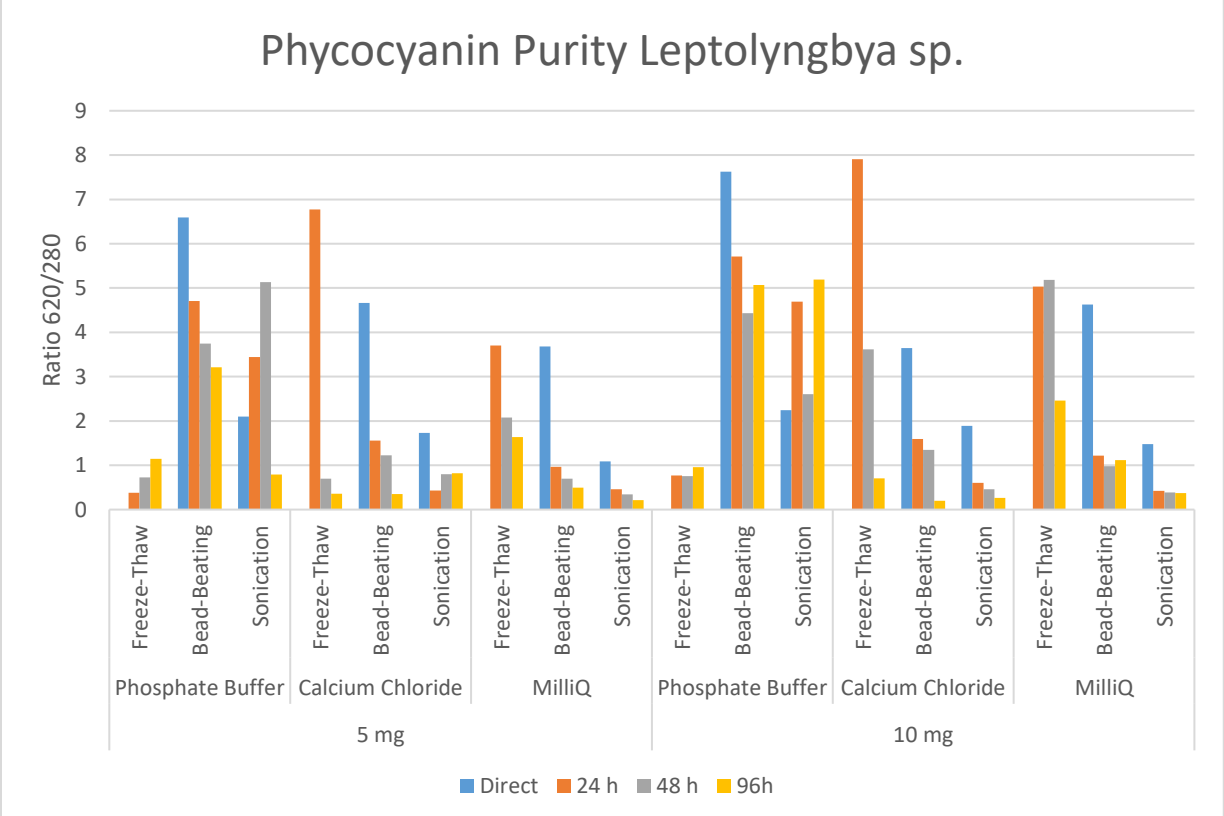


Figure 7: Representation of the purity of the extraction in Leptolyngbya sp. *for all the combination tested. Therefore, with 2 biomass amounts, 3 disruption methods and 3 ESs tested. Standard deviation was not included, to increase the readability of the graph.*

Arthrospira platensis

For the two different biomass amounts tested higher extraction rates and purities were found with 5mg of biomass as compared to 10 mg. Nevertheless, for MilliQ water, the different biomass concentrations resulted in the same extraction yields (per biomass amount). Of the three different disruption methods tested, Freeze thawing showed different extraction yields and purities in each ES. In phosphate buffer, higher extraction yields were found, however with lower purities in comparison to calcium chloride. With direct measurement, bead beating extracts approximately the same amount of PC in phosphate buffer and in calcium chloride, however the purities do differ significantly, ranging from 3.78, 1.23, and 0.41 for phosphate buffer, calcium chloride, and MilliQ respectively. The purity recorded direct measurement of bead beating combined with phosphate buffer was the highest of all tested combinations. Sonication with phosphate buffer, had the highest extraction yield, however the extract purity was very low compared to the other two disruption methods. MilliQ showed the lowest yields and low purities under all conditions tested. In regards to extraction duration, for all 3 methods in phosphate buffer and in Calcium Chloride after 24 hours, at least 90% of the total extracted PC was already recovered. The remaining 10 % was extracted within 48h. Exception was made for freeze thawing in phosphate buffer in 10mg of biomass that extracted approximately 83% or the total PC after 24h. Moreover, bead beating after the direct measurement in Calcium chloride shows higher yields with lower purities than in phosphate buffer.

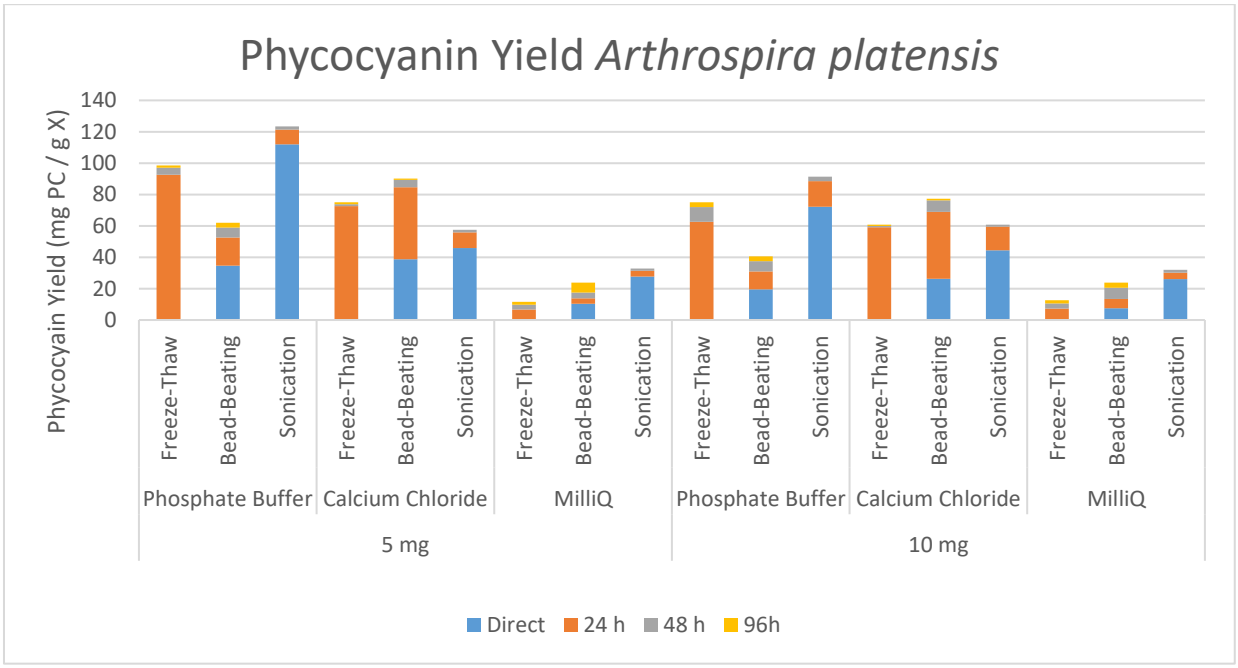


Figure 8: Representation of the extraction yield in Arthrospira platensis for all the combination tested. Therefore, with 2 biomass amounts, 3 disruption methods and 3 ESs tested. Standard deviation was not included, to increase the readability of the graph.

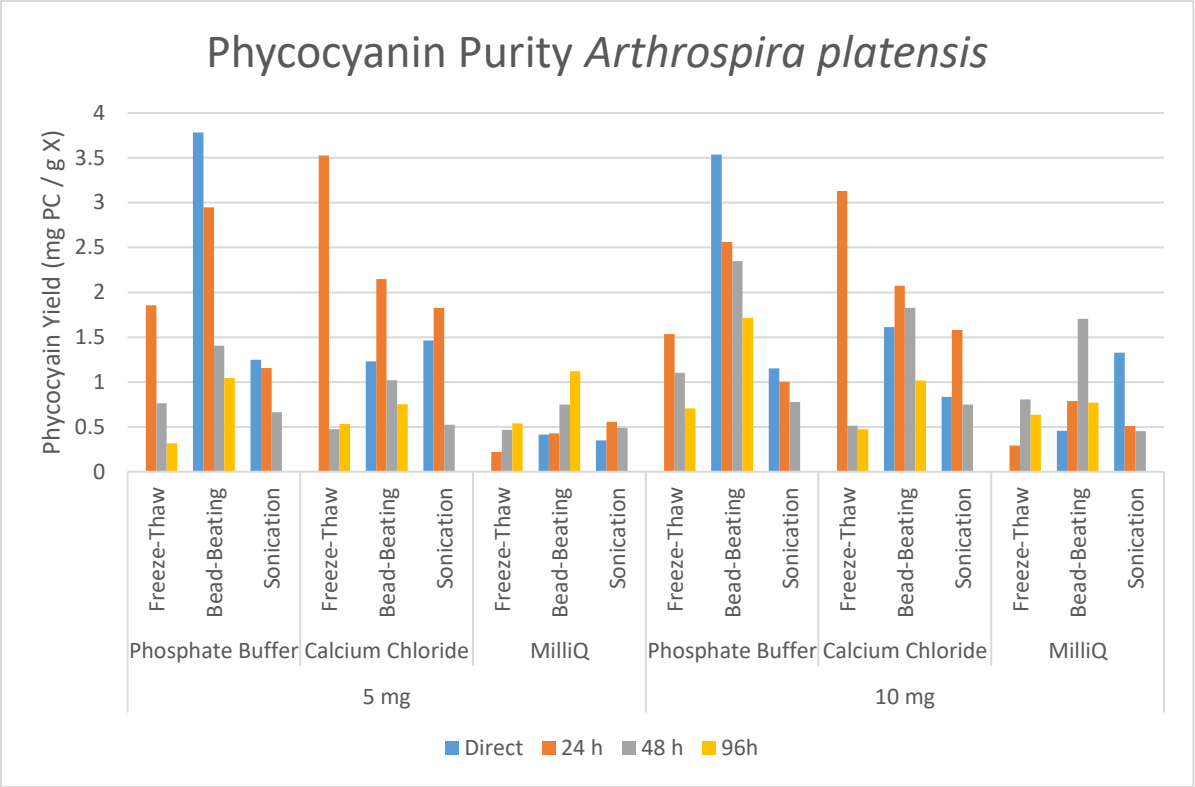


Figure 9: Representation of the purity of the extraction in Arthrospira platensis for all the combination tested. Therefore, with 2 biomass amounts, 3 disruption methods and 3 ESs tested. Standard deviation was not included, to increase the readability of the graph.

Rhodomonas sp.

The PE extraction yield for the two biomass amounts tested was found to be comparable. Of the three-disruption method tested, freeze thawing in calcium chloride showed the lowest PE extraction yield. Disruption of the cells using bead-beating showed similar extraction yields across the different ESs, with the highest purities of all disruption methods tested (direct measurement). Sonication, reported similar or higher extraction yields, however the purities were much lower as compared to bead-beating. In regards to the incubation time, it can be observed that sonication does not require additional incubation times to extract all the PE, while Freeze-Thawing requires up to 48 and 92 hours for 5mg and 10mg of biomass respectively. With Bead beating, direct measurement yields 75 ,82 and 84 % in Phosphate buffer Calcium Chloride and MilliQ water respectively of the total PE extraction, with additional incubation of 24 h it was extract up to 95% in Phosphate buffer while in the other ESs it was reached the 100%.

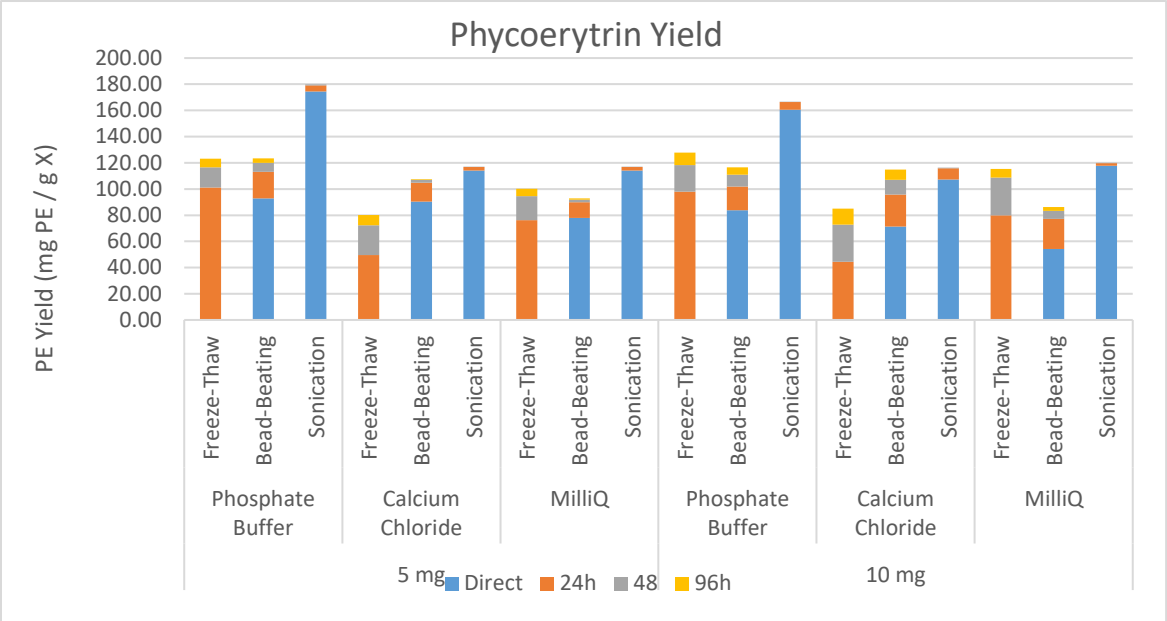


Figure 10: Representation of the extraction yield in Rhodomonas sp. *for all the combination tested. Therefore, with 2 biomass amounts, 3 disruption methods and 3 ESs tested. Standard deviation was not included, to increase the readability of the graph.*

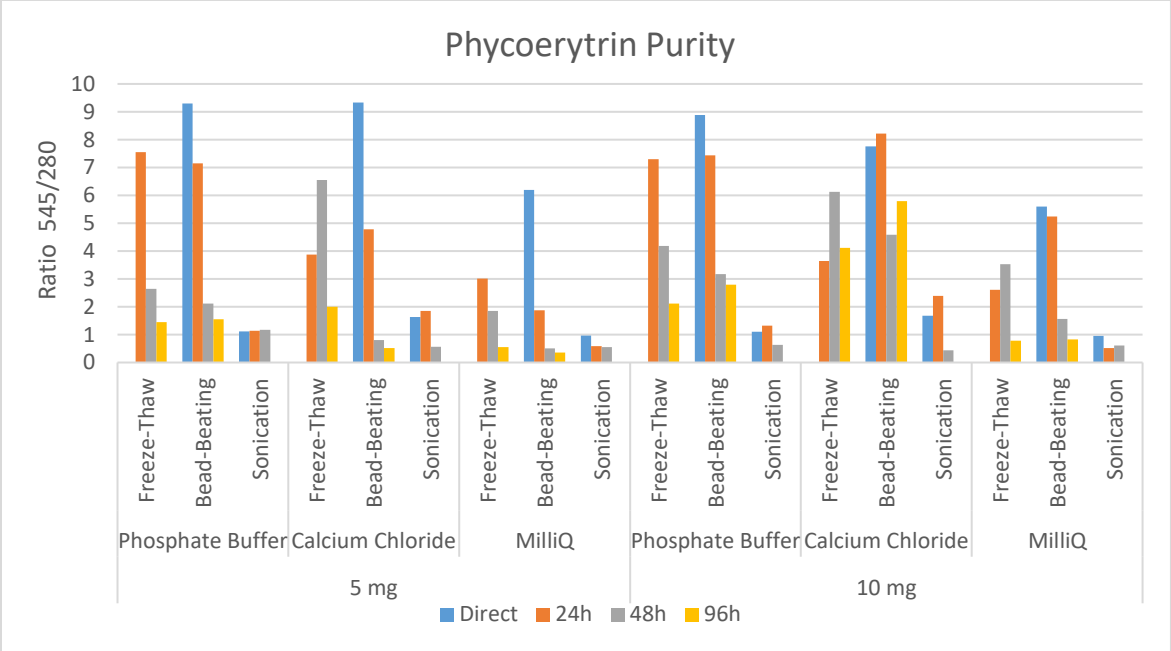


Figure 11: Representation of the purity of the extraction in Rhodomonas sp. *for all the combination tested. Therefore, with 2 biomass amounts, 3 disruption methods and 3 ESs tested. Standard deviation was not included, to increase the readability of the graph.*

3.2 PC productivity of *Leptolyngbya* sp. QUCCCM 56 in terms of light and temperature

Leptolyngbya sp. QUCCCM 56 was cultivated under increasing light intensity and temperature, in Figures 12 and 13 are reported the PC productivity($g \cdot l^{-1} \cdot d^{-1}$) and the Phycocyanin content ($mg_{PC} \cdot g_X^{-1}$). The graphs about biomass productivity are reported in [Appendix 5].

The highest PC productivity was found at 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$, at 38 $mg_{PC} \cdot l^{-1} \cdot d^{-1}$. At increasing light intensities, the PC productivity decreased down to 14 $mg_{PC} \cdot l^{-1} \cdot d^{-1}$at 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the highest PC content

was found at 84 $mg_{PC} \cdot g_X^{-1}$. The PC content decreased significantly for increased light intensities, reducing by 45% at 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and by 75% at 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

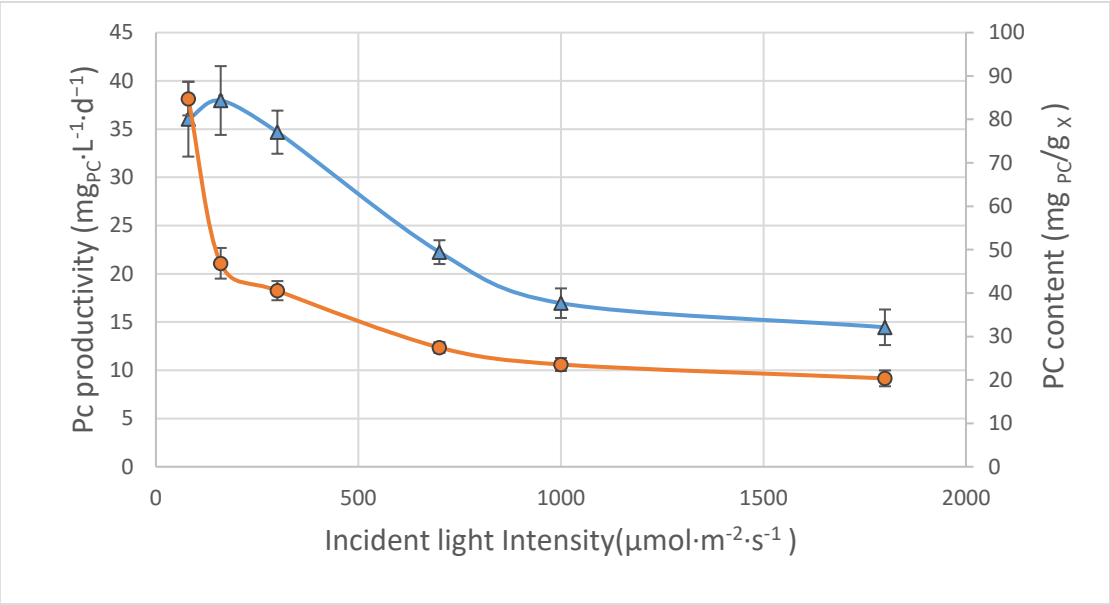


Figure 12: In the graph are reported the PC productivity (Δ dots with blue line) and the PC content (○ dots with orange line) in Leptolyngbya sp. under different incident light intensities.

Regarding temperature, the strain was able to grow in a wide range from 20˚C up to 40˚C. PC productivities were found to increase with increasing temperatures, up to 40˚C (Figure X). At 45˚C, no growth was observed, and the culture died. In terms of PC content, higher temperatures were shown to increase the cells' PC content significantly, from 19.25 $mg_{PC} \cdot g_X^{-1}$up to 75.75 $mg_{PC} \cdot g_X^{-1}$for 20 and 40˚C respectively.

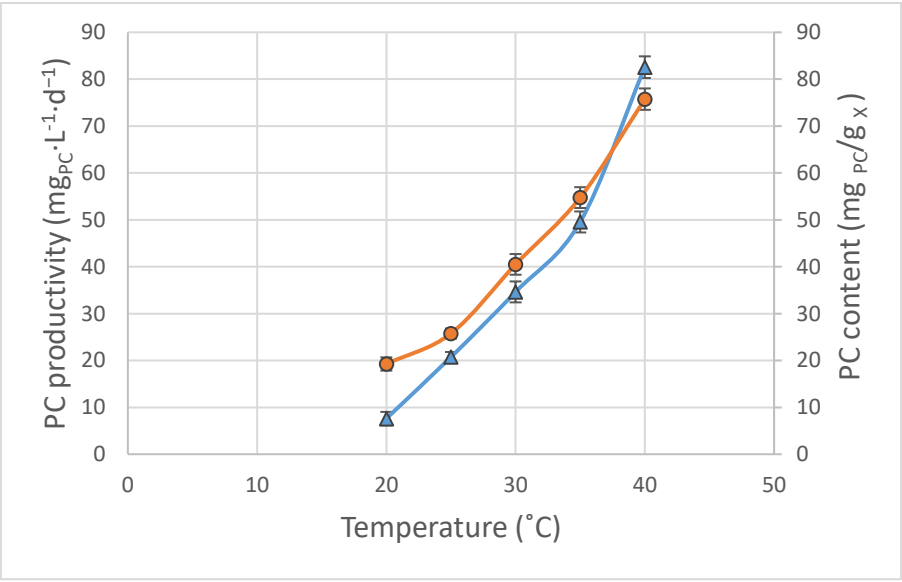


Figure 13: In the graph are reported the PC productivity (Δ dots with blue line) and the PC content (○ dots with orange line) in Leptolyngbya sp. under different Temperatures.

4 Discussion

4.1 Extraction protocol

Disruption method: As to the best of the author's knowledge, no previous studies have been conducted on *Leptolyngbya* sp. in regard to phycocyanin extraction optimization. In many papers it is reported that freezing and thawing in phosphate buffer is considered to be the best for the extraction of *Arthrospira platensis* [32] [24]. On the other hand, the best method for PE extraction reported by Lawrenz et al. (2010), for *Rhodomonas salina*, was freeze thawing or sonication combined with phosphate buffer. Both disruption methods were studied in this work, and it was found that the effectiveness of freeze-thawing is strain dependent, as for *Rhodomonas* sp. and *Arthrospira platensis* it showed significant extraction yields, however for *Leptolyngbya* sp. no PC was extracted using this method. It is hypothesized that this method is unable to disrupt the cells, which was confirmed by microscopic analysis (Figure- 16). On the contrary, in combination with phosphate buffer it achieves the greatest results in *Arthrospira platensis* (98 $mg_{PC} \cdot g_X^{-1}$, with a purity of 1.86 after 24h) in agreement with what reported from Sarada et al, (1998) and Doke et al, (2005) [32] [24]. On the other hand, in *Rhodomonas* sp. this method combined with phosphate better is more efficient than what found in literature as it extracts all the PE (120 $mg_{PE} \cdot g_X^{-1}$) after 48h while in Lawrenz et al. (2010) this technique does not extract higher than 32±5% of the total amount of PE. Therefore, this method can be considered a good cell disruption in *Arthrospira platensis* and in Rhodomonas sp. both. Moreover, it can be said that the extraction yield and the purities are very variable among the ESs tested as the first measurement is taken after 24h and therefore the buffer influences the extraction. Sonication, also found to be an effective disruption method by Lawrenz et al. (2010). From the results obtained it can be stated that it is a very harsh cell disruption method, and is thought to solubilize many other proteins in addition to PBPs. This was confirmed by the absorption scan over different wavelengths for both sonication and bead beating, which is shown in Figure 15. This leads to an overestimation of the yield and to an incorrect purity value. As a matter of fact, Lawrenz et al (2010) did not take into consideration the purity and in that case the yields are the highest.

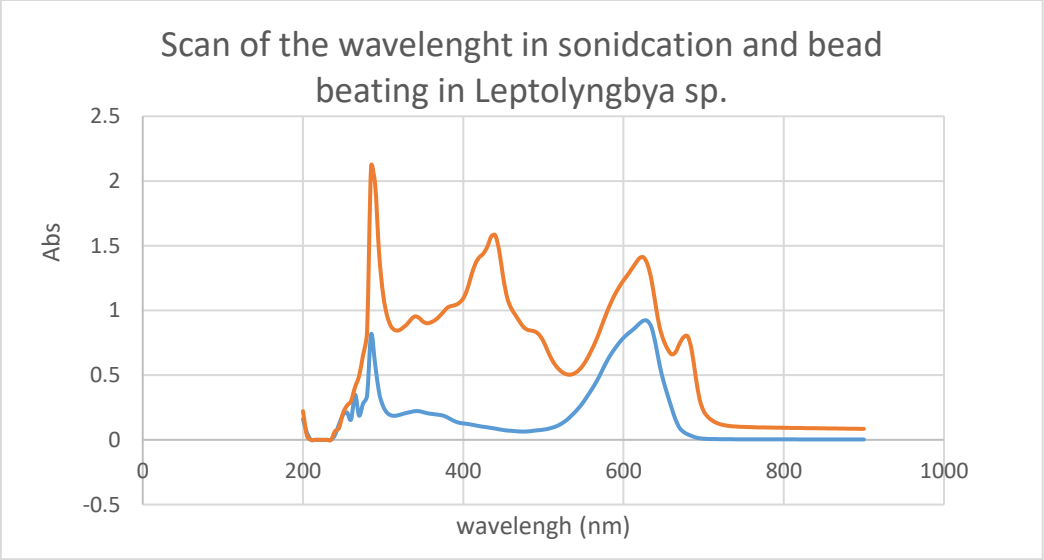


Figure 15: In blue the scan of the wavelength of the supernatant of 5 mg of biomass of Leptolyngbya sp. *subjected to bead beating in phosphate buffer (bottom line). In orange the scan of the wavelength of the supernatant of 5mg of Leptolyngbya* sp. *subjected to sonication in phosphate buffer (top line). The same patter has been noticed in all the strain tested therefore it was reported only one graph.*

No reference was found in literature in which bead-beating was used for cell disruption for protein extraction, however it is a commonly used method for lipid [27] . Nevertheless, in combination with phosphate buffer it proved to be the best in term of having an high extraction yield with high purity in *Rhodomonas* sp. (120 mg of $mg_{pe} \cdot g_x^{-1}$ with 9.25 of purity) and in *Leptolyngbya* sp. (160 $mg_{pc} \cdot g_x^{-1}$ with 6.5 of purity). As it can be seen in Figure 16 *Leptolyngbya* sp. is completely disrupted. Again, the yield is comparable to what found in literature with other disruption methods but the purity is impressive: which is almost 3.5 times more than in *Arthrospira platensis* (1.8 of purity) and 2.9 more times than in red algae *Porphyridium cruentum* (3.2 of purity) compared to *Leptolyngbya* sp. and *Rhodomonas* sp. respectively [33] [24] [25]. Generally, the purities in this method are high, regardless the ES used, meaning that not many other proteins are solubilized. Moreover, the yields of the first measurement are always comparable among different ESs meaning that the method is reliable and consistent. Therefore, it can be considered this disruption method very efficient in extracting PBPs in *Rhodomonas* sp. and in *Leptolyngbya* sp. On the contrary, bead beating in *Arthrospira platensis* does not completely disrupt the cell, Figure 16. As a matter of fact, this strain when resuspended in the ES tends to form slimy clumps that make resistance against this disruption method. Overall it seems that this disruption method is not very severe and this characterize reflects in very high purity values.

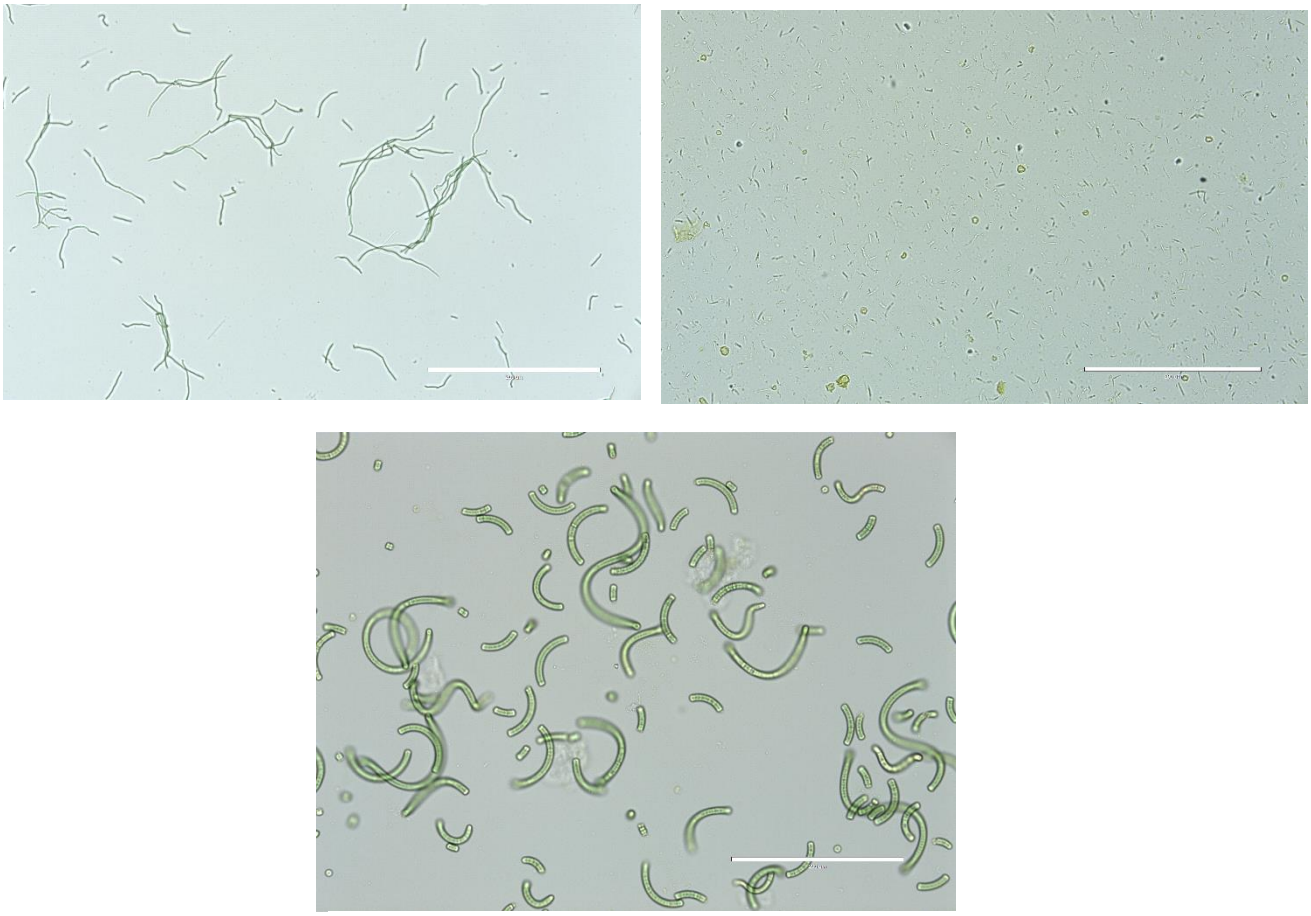


Figure 16: 5mg of biomass of Leptolyngbya sp. after freeze thawing in phosphate buffer (top left). *5mg of biomass of Leptolyngbya* sp. after bead beating in phosphate buffer (top right). 10mg of biomass of Arthrospira platensis after bead beating in phosphate buffer.

ES: During the incubation times phosphate buffer, regardless the disruption method, shows to extract PBPs with the highest yield. As a matter of fact, this ES is widely reported in literature to be the best ES to extract PBPs [23] [24]. Its extraction efficiency thought to be due to pH influences on the charge of the PBPs, thereby enhancing its solubility at charged states while inhibiting the solubility at neutral charged states [34] . Phosphate buffer does maintain the protein away from the isoelectric point, which in *Arthrospira platensis* is found at 5.8 [35]. Unfortunately, the isoelectric point in *Leptolyngbya* sp. and *Rhodomonas* sp. is not known for PC and PE respectively. Nevertheless, from literature it was found that the isoelectric point of PC is ranging between pH 4.74 and 5.8 while for PE is ranging from pH 4.5 to 5.1 [29, 36] [37]. Calcium Chloride and MilliQ water do not have high buffer capacity therefore they are not influencing the pH as the phosphate buffer does. As a matter of fact, after the resuspension of the biomass in the ES, before the disruption method, the pH has been measured and an increase of pH was recorded in Calcium Chloride and in MilliQ water as reported in the Table 3. This feature was noticed especially in the two cyanobacteria as they were grown in a medium with absence of buffer that would have hampered formation of an alkaline culture. Consequently, very high pH can be the cause of hydrolysis explaining therefore the lower extraction yields and purities in Calcium Chloride and MilliQ water compared to phosphate buffer in *Arthrospira platensis* and in *Leptolyngbya* sp. [38]. This hypothesis is confirmed by Patil et al, (2007) where it is also shown that the pH at 6 is having the highest purities as it was the case in the results obtained in this study [39].

Table 1: pH in the three strains before and after the resuspension in the ES.

	ES	pH ES	pH biomass	pH biomass after disruption
Leptolyngbya sp.	Phosphate buffer	6	10.5	6.4
	Cacl2	5.1		7
	MilliQ water	7		10.5
Arthrospira platensis	Phosphate buffer	6	13	6.7
	Cacl2	5.1		8.6
	MilliQ water	7		13
Rhodomonas sp.	Phosphate buffer	6	7.5	6
	Cacl2	5.1		6.5
	MilliQ water	7		7.5

Calcium chloride is having discrete yields and purities when the cell is disrupted but when the disruption method is not efficient and therefore the cell is whole (Freeze thawing in *Leptolyngbya* sp.) or fragmented (bead beading with *Arthrospira platensis*) Calcium chloride is the only ES found able to extract PBPs. It is supposed that the mechanism based on which the extraction occurs is not related to pH as it is in phosphate buffer or in MilliQ water. Given that in literature no information could be found regarding how calcium chloride is extracting PBPs in specific, it generalized that a similar process is occurring as when $CaCl_2$ is used as a treatment for E.coli, in order to facilitate DNA transformation [40]. In this process, $CaCl_2$ increased the permeability of the cells membrane to allow for DNA to pass through. Nevertheless, In *Rhodomonas* sp. resuspended in Calcium Chloride is expected to yield higher extraction yields for the pH effect (6.5 in this case) hypnotized before. The result is supposed to indicate an inhibitor effect that this ES does on the solubilisation of PE. As a matter of fact, it is possible that the positively charged ions of calcium binds with the negatively charged PE (due to pH 6.5) causing precipitation in flocculants as it is described in the egg-box model proposed by Li et al (2006)[41].

Biomass amount and incubation time: The volume of the ES is the same for both the biomass amounts. Given that it directly influences the pH with its concentration (in phosphate buffer more while in calcium chloride and in Milli Q water less), as described before, its effect is lowered when combined with higher biomass amounts increasing, therefore it is noticed a trend in which 10mg always requires more time to extract the same yields. Moreover, with the refreshment of the ES every 24h it has been increased the speed of this process. Therefore it must be taken into account that if it is desired do not change the supernatant every 24h a drop in the yield and purity is expected.

4 .2 PC productivity of *Leptolyngbya* sp. QUCCCM 56 in terms of light and temperature

4..2 1 Light experiment

To obtain high PC productivity, the light intensity should be increased, however low intensity of light was more favourable for PC content. It is known that PC is an antenna pigment used by mainly cyanobacteria and eukaryotic algae to increase efficiency of photosynthesis by collecting light energy at wavelength where chlorophylls poorly absorb, and transferring the energy in high efficiency to chlorophyll a in the thylakoid membranes [42] [43] [44]. The decrease in PC content at higher light intensities is thought to be due a decrease need from the cell to be as efficient in light-absorption, thus it does not waste its energy on producing PC, but it can use it to produce more biomass until photo-inhibition occurred[Appendix 5] [45]. Similarly, Xie et al. (2015) and Chen et al. (2013) reported that higher light intensity results to lower PC productivity in *Arthrospira platensis* [46] [19]. The optimal light intensity for PC productivity study is to be found at 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$, nevertheless, even though 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ reported 10% less PC productivity it was selected to proceed with the temperature experiment as this light intensity would copy better in outdoor systems in Qatar than lower ones.

4.2.3 Temperature experiment

The increased PC productivity found at higher temperatures suggests that the photosynthetic apparatus is more effective at increased temperatures as the Biomass productivity and the PC content were to be found both increasing along the data point tested[Appendix 5]. The productivity is increasing until 40 ˚C, at 45 ˚C the cells experienced death. Per Halldal et al. (1958) reported that the temperature can have different influences over the PC content in combination with different light intensities in Anacystis nidulas drouetand [22]. Therefore, it cannot be stated that the PC productivity would also increase with the same trend with different light intensities in *Leptolyngbya* sp. Nonetheless, Querques et al (2015) reported that higher temperature does not correlate conclusively with high productivity as phycocyanin producing microalgae are known to proliferate in both extreme heat and cold environments. [47]. At 40 ˚C the 5% of the total PC content is already denatured by temperature, at higher temperatures the denaturation is even more drastic therefore the fact that the strain reached 40˚C is very positive [48]. *Leptolyngbya* sp. grew at a very wide range of temperatures 20-40 ˚C proving to be a suitable strain for outdoor production.

5 Conclusions

In *Leptolyngbya* sp. and in *Rhodomonas* sp. the best protocol for the characterization of PC/PE consists in 5 mg of biomass subjected to Bead beating in Phosphate buffer with an incubation time of 24 hours (efficiently of approximately 88% in *Leptolyngbya* sp. and 95% in *Rhodomonas* sp.) .

In *Arthrospira platensis* the best protocol for the characterization of PC consists in 5mg of biomass subjected a freeze thawing in Phosphate buffer with an incubation time of 24h (efficiently of 95% in *Arthrospira platensis*).

Concluding, phosphate buffer is recommended for the extraction of PBPs, as it is recognised the importance of the pH in solubilising the protein. Therefore the findings agree with Lawrenz et al (2010) in this regard. On the other hand, there is not an universal disruption method to break the cell as it is strain dependent. The method must disrupt the cell without exceeding in the harshness in order to have an high purity. In *Leptolyngbya* sp.and in *Rhodomonas* the best is bead beating while in *Arthrospira platensis* is freeze thawing. It is noticed a strong correlation between biomass amount and incubation time, in fact higher biomass amount are requiring more time therefore for all the strains tested is recommended 24h as incubation time with 5mg of biomass.

The quantification of the PC content in the characterisation of PC productivity was achieved with 5mg of biomass with bead beating in phosphate buffer with an incubation time of 24. PC productivity of 91 $mg_{PC} \cdot d^{-1} \cdot l^{-1}$ (added the 12% of PC not extracted after 24h)was obtained when using light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a temperature of 40 ˚C. No other studies are reporting *Leptolyngbya* sp. PC productivity under different light intensities and temperatures, nevertheless, a comparison can be made with *Arthrospira platensis* as it is the most studied in the regards of PC productivity. It is reported inconsistent optimal light intensities for PC productivity from *Arthrospira platensis*. Chen et al. (2013) in his findings reports 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PC productivity of 110 $mg_{PC} \cdot d^{-1} \cdot l^{-1}$) while Xie et al. (2015) reports 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PC productivity of 40 $mg_{PC} \cdot d^{-1} \cdot l^{-1}$). This discrepant might be due to differences in bioreactor configurations and culture strategy. In regards of the temperature instead, Querques et al. (2015) reported that the highest PC productivity (130 $mg_{PC} \cdot d^{-1} \cdot l^{-1}$) is to be found at 30 ˚C in *Arthrospira platensis* [47] .Even though the PC productivity reported in Querques et al. (2015) and Chen et al. (2013) are higher (18% and 30% more respectively) in *Arthrospira platensis*, *Leptolyngbya* sp. proved to be a suitable candidate for outdoor PC productions in Qatar with its good PC productivity at a very wide range of temperatures and light intensities.

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Appendix:

Appendix 1: Zarrouk protocol and recipient.

Zarrouk Medium			
Chemical	**Concentration**		**Added**
Solution A			
NaHCO3	16.8	g/ 500 mL	
K2HPO4	0.5	g/ 500 mL	
Solution B			
NaNO3	2.5	g/ 500 mL	
K2SO4	1	g/ 500 mL	
NaCl	1	g/ 500 mL	
MgSO4*7H2O	0.2	g/ 500 mL	
CaCl2*2H2O	0.04	g/ 500 mL	
FeSO4*7H2O	0.01	g/ 500 mL	
EDTA	0.08	g/ 500 mL	
Micronutrient	1	mL/ 500 mL	
Micronutrient			
H3BO3	2.86	g/L	
MnCl2*4H2O	1.81	g/L	
ZnSO4*4H2O	0.222	g/L	
Na2MoO4	0.0177	g/L	
CuSO4*5H2O	0.079	g/L	
Protocol			
1. Prepare all solutions and autoclave them seperately			
2. After cooling, add solution A and B together aseptically			

Appendix 2: medium recipient L1

	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75.00 g L-1 dH2O	1ml	8.82 x 10-4 M
$NaH_2PO_4 \cdot H_2O$	5.00 g L-1 dH2O	1ml	3.62 x 10-5 M

$Na_2SiO_3 \cdot 9\ H_2O$	30.00 g L-1 dH2O	1ml	1.06 x 10-4 M
trace element solution	see recipe below	1ml	-
vitamin solution	see recipe below	0.5ml	-
Trace element solution			
Na2EDTA · 2H2O	-	4.36 g	1.17 x 10-5 M
FeCl3 · 6H2O	-	3.15 g	1.17 x 10-5 M
MnCl2·4 H2O	178.10 g L-1 dH2O	1ml	9.09 x 10^{-7} M
ZnSO4 · 7H2O	23.00 g L-1 dH2O	1ml	8.00 x 10^{-8} M
CoCl2 · 6H2O	11.90 g L-1 dH2O	1ml	5.00 x 10^{-8} M
CuSO4 · 5H2O	2.50 g L-1 dH2O	1ml	1.00 x 10^{-8} M
Na2MoO4 · 2H2O	19.9 g L-1 dH2O	1ml	8.22 x 10-8 M
H2SeO3	1.29 g L-1 dH2O	1ml	1.00 x 10^{-8} M
NiSO4 · 6H2O	2.63 g L-1 dH2O	1ml	1.00 x 10^{-8} M
Na3VO4	1.84 g L-1 dH2O	1ml	1.00 x 10^{-8} M
K2CrO4	1.94 g L-1 dH2O	1ml	1.00 x 10^{-8} M
Vitamin stock			
thiamine · HCl (vit. B1)	-	200 mg	2.96 x 10-7 M
biotin (vit. H)	0.1g L-1 dH2O	10 mL	2.05 x 10-9 M
cyanocobalamin (vit. B12)	1.0 g $L^{-1}dH_2O$	1 mL	3.69 x 10-10 M

Appendix 3: PBPs extraction protocol

Day 1	
1.1	Determine the biomass concentration of each strain (g DW /L) (triplicate).
1.2	Prepare Phosphate buffer and Calcium Chloride solution.
Day 2	
2.1	Measure filters and determine dry weight – calculate the volume required to have 5 mg and 10 mg of biomass.
2.2	Determine the biomass concentration (g DW /L) (triplicate).
2.3	Prepare 30 tubes with each biomass amount (60 in total).
2.4	Centrifuge the samples for 30 minutes at max speed at 4°C, remove the supernatant.
2.5	For each biomass concentration, place 3 tubes in -80°C for later use (to reconfirm results of best protocol).
2.6	For the 54 other samples, resuspend the pellet (using a vortex) in one of the following ES.

	Phosphate buffer 1.25mL [23] 0.1 M (pH 6.0) 10.5 g/L NaH_2PO_4 4.4 g/L $Na_2HPO_4*12H_2O$ (18 samples)	**Calcium Chloride** 1.25mL [26] 10 g/L (18 samples)	**Milli-Q Water 1.25mL** (18 samples)
2.7	Subject 6 samples per ES (two biomass concentration in triplicates) to each disruption method:		
	Freeze-Thawing [23] Place in -20°C until solid(~2h), remove at place at 4°C until liquid(~24h)	**Bead-Beating [27]** 2500x3 for 25 sec each with a 5 sec wait between each cycle (on ice)	**Sonication [23]** On ice using 5 sec 8W pulses
2.8	Centrifuge the samples at maximum speed for 30 minutes at 4°C.		
2.9	Separate the supernatant from the pellet, and measure the supernatant absorbance at 750,620 and 280 nm for *Leptolyngbya* sp. and *Arthrospira platensis*, and 545 and 280 nm for *Rhodomonas* sp. – make sure to use the adequate blank (Phosphate Buffer / Calcium Chloride / MilliQ water) for each sample		
2.10	Add new extraction ES (phosphate buffer, Calcium Chloride or MilliQ) to the pellet, vortex to resuspend, and incubate at 4°C for 24 h (in the dark).		
Day 3			
3.1	Centrifuge the samples at maximum speed for 30 minutes at 4°C.		
3.2	Separate the supernatant from the pellet, and measure the supernatant absorbance at 750, 620 and 280 nm for *Leptolyngbya* and *Arthrospira platensis*, and 750, 545 and 280 nm for *Rhodomonas* sp.– make sure to use the adequate blank (Phosphate Buffer / Calcium Chloride / MilliQ water) for each sample		
3.3	If the absorbance at 620 is not 0, add new extraction ES (phosphate buffer, Calcium Chloride or MilliQ) to the pellet, vortex to resuspend, and incubate at 4°C for another 24h (in the dark).		
3.4	Measure Filters of 2nd DW analysis.		
Day 4			
4.1	Centrifuge the samples at maximum speed for 30 minutes at 4°C.		
4.2	Separate the supernatant from the pellet, and measure the supernatant absorbance at 750, 620 and 280 nm for *Leptolyngbya* and *Arthrospira platensis*, and 750, 545 and 280 nm for *Rhodomonas* sp.– make sure to use the adequate blank (Phosphate Buffer / Calcium Chloride / MilliQ water) for each sample		
4.3	If the absorbance at 620 or 545 is not 0, add new extraction ES (phosphate buffer, Calcium Chloride or MilliQ) to the pellet, vortex to resuspent, and incubate at 4°C for another 24 h (in the dark)		
Day 5			
5.1	Centrifuge the samples at maximum speed for 30 minutes at 4°C.		
5.2	Separate the supernatant from the pellet, and measure the supernatant absorbance at 620 and 280 nm for *Leptolyngbya* and *Arthrospira platensis*, and 545 and 280 nm for *Rhodomonas* sp.– make sure to use the adequate blank (Phosphate Buffer / Calcium Chloride / MilliQ water) for each sample		

Appendix 4: DW protocol

This Standard Operating Procedure describes how to determine dry weight fresh water microalga species.
Materials
Whatman glass fiber filters, GF/F, Æ 55 mm, nominal pore size 0.7 mm
Equipment
Analytical balance with readability of 0.01 mg and repeatability of ± 0.015 mg Pipette or analytical balance for quantification of culture sample Oven at 95 °C (passive convection)
Procedure

Note: Always handle filters and aluminium trays with tweezers

1. Put filters in an aluminium tray. Put this tray on top of another tray
2. Dry filters in the oven at 95 °C for 24 h.
3. Allow filters to cool down in a desiccator for 2 h or more
4. Make sure the weighing table is clean and weigh dried filters inside only the upper aluminium tray, note values down as (w1).
5. Take 'x' mL (or 'y' g) of culture sample containing between 1 and 10 mg of dry weight. Quantify this amount (x or y) accurately using a calibrated pipette or an analytical balance.
   1st - Dilute the culture sample 5 times with ammonium formate 0.5M (or higher concentration when necessary) in a small flask or tube
   2nd - Pre-wash the filter with 5 ml ammonium formate at low and constant pressure, 20 mm Hg
   3rd - Just before the filter runs dry: add the diluted sample
   4th - Just before the filter runs dry: add 25 ml of ammonium formate (make sure to use more then the total volume of the diluted sample) to rinse the algae off the glass and wash the biofilm on the filter. Perform this step twice and gently release vacuum immediately after filter runs dry.
6. Dry filters in the oven at 95ºC for 24 h
7. Put the filters in the desiccator for 2 h or more
8. Weigh dried filters, again inside the upper aluminium tray, note down values (w2)

Calculations

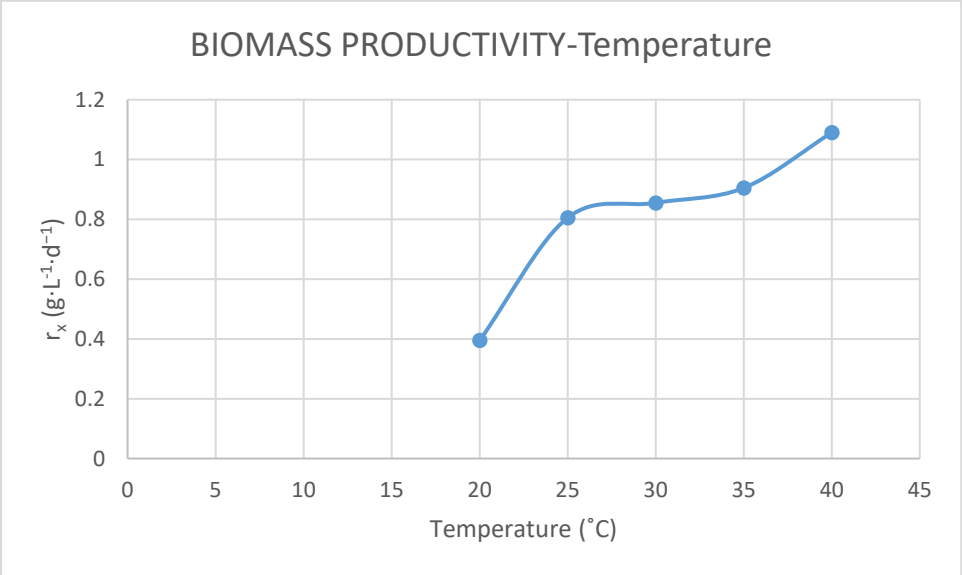
Calculation A: DW (g L^{-1}) = [w2 (g) – w1 (g)] / ['x' (mL) * 0.001]

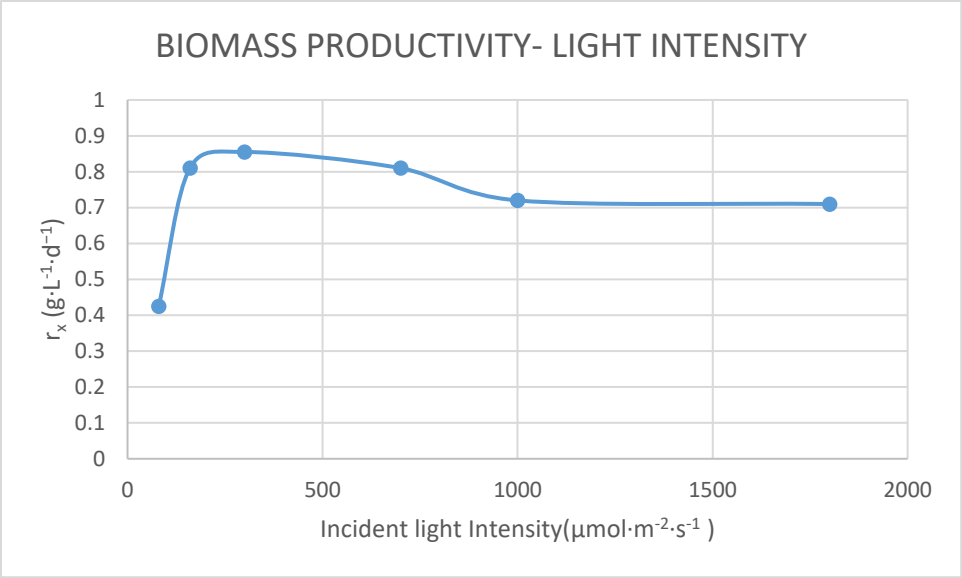
Calculation B: DW (g kg^{-1}) = [w2 (g) – w1 (g)] / ['y' (g) * 0.001]

References

Zhu CJ, Lee YK. 1997. Determination of biomass dry weight of marine microalgae. J Appl Phycol, Vol 9:189-194.

Appendix 5: Biomass productivity at different temperatures and light intensity tested.




BIOMASS PRODUCTIVITY- LIGHT INTENSITY
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
r_x ($g \cdot L^{-1} \cdot d^{-1}$)
Incident light Intensity($\mu mol \cdot m^{-2} \cdot s^{-1}$)