

# Influence of culture conditions on the production of extracellular polymeric substances (EPS) by *Arthrospira platensis*

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## Research

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# Abstract

*Arthrospira platensis* is a cyanobacterium that exhibits a large biotechnological interest at food industry because its high protein content, pigments, lipids and carbohydrates. The extracellular polymeric substances (EPS) are co-products of secondary metabolism that present thickening or gelling property. A 3-level factorial design was used to study the combined effect of different nitrate concentrations and photon flux density (PFD) to evaluate the biomass and EPS production by *Arthrospira platensis*. Characterization of the EPS produced the rheological behavior were also evaluated. The best result for biomass production was obtained at condition 6 ( $2 \text{ g.L}^{-1} \text{ NaNO}_3$  and  $600 \text{ }\mu\text{E.m}^{-2}\text{s}^{-1}$ ) leading a biomass concentration of  $1.292 \text{ mg.L}^{-1}$ . Condition 1 ( $0.25 \text{ g.L}^{-1} \text{ NaNO}_3$  and  $200 \text{ }\mu\text{E.m}^{-2}\text{s}^{-1}$ ) produce the major EPS content ( $111 \text{ mg.g}^{-1}$ ) followed by condition 9 ( $2 \text{ g.L}^{-1} \text{ NaNO}_3$  and  $1000 \text{ }\mu\text{E.m}^{-2}\text{s}^{-1}$ ). Rheological studies performed for the product at 5 and  $10 \text{ g.L}^{-1}$  concentrations revealed a dilute solution behavior.

## Introduction

Cyanobacteria consist of a group of prokaryotes, characterized by a variety of forms, staining and habitat. These microorganisms have autotrophic metabolism, can also be grown in heterotrophic conditions, and are found in brackish or marine environments (Baldev et al. 2015). They are responsible for the incorporation of a large part of atmospheric  $\text{CO}_2$  and some of them are  $\text{N}_2$  fixers (Oliver & Atsumi, 2014). Cyanobacteria produces extracellular polymeric substances (EPS), which has a protective function as biofilm formation, mechanical barrier against desiccation (Decho & Gutierrez, 2017), and also absorption of heavy metals (Goo et al. 2013). The composition of EPS depends on the species. Generally, they are formed by various substances, such as proteins, polysaccharides, lipids, humic-like substances, DNA, and liposaccharides, glycoproteins heteropolymers (Can, Gurbuz & Odabas, 2019). EPS may be totally released into the extracellular environment or may be associated with the cell surface, as sheaths, capsules or slime (Pereira et al., 2009).

EPS have attracted industrial interest because of their polyanionic character and renewable source. They can be used as thickeners, emulsifying agents and biosurfactants, with applications in the food and biomedical industry. Anticoagulant (Li et al., 2012), antioxidant (Trabelsi et al., 2016), and anti-inflammatory properties were reported (Xiao et al., 2018).

*Arthrospira platensis*, also called *Spirulina platensis*, is a filamentous blue-green cyanobacteria, belonging to the order *Cyanophyceae*, division *Cyanophyta* (Manirafasha et al., 2018). Its biomass is used in human food because of its high protein content (approximately 70% of dry weight). In addition to proteins, sulfated heteropolysaccharides, frequently formed by neutral (xylose, galactose, glucose, fructose, rhamnose, arabinose and mannose) and uronic acids (galacturonic and glucuronic acids) repeating units, are included in EPS (Trabelsi, M'sakni, Ouada, Bacha, & Roudesli, 2009). It also produces pigments such as carotenoids and phycocyanin, polyunsaturated fatty acids, several vitamins, minerals and other

constituents with antioxidant activity (Gong et al., 2008; Shabana, Gabr, Moussa, El-Shaer, & Ismaiel, 2017).

Although EPSs represent a potential contribution to numerous industrial areas, their cost, low yield and the lack of knowledge about their structure are still disadvantages. An alternative to try to minimize costs and enhance EPS yields could be the optimization of the culture parameters. This is because EPS production by cyanobacteria is influenced by environmental and nutritional conditions, such as temperature (Trabelsi et al., 2009), photonic flux density (Villay et al., 2013) and nitrogen starvation (Arad & Levy, 2010). Furthermore, a specific combination of these parameters could lead to a positive effect on the EPS production. On these issues, only a few studies were reported, with the objective to enhance the EPS yield. For example, the temperature and PFD parameters were optimized for the production of EPSs from *Cyanothece* sp. and *Rhodella violacea* (Ohki et al., 2014; Villay et al., 2013). Regarding to the production of EPSs by *Dunaliella salina* and *Synechocystis* sp., the optimization of NaCl concentration was investigated (Mishra et al., 2011; Ozturk, & Aslim, 2010). Using *A. platensis*, temperature, PFD and concentrations of NaCl and NaNO<sub>3</sub> were optimized by Trabelsi et al. (2009), Chentir et al. (2017) and Dejsungkranont et al. (2016).

On the other hand, the techniques used to extract, purify and characterize the resulting EPS may also be relevant (Dellatre et al., 2016). In general, the culture medium is centrifuged or filtrated to remove cells and then the filtrate is concentrated to reduce the water content. Some authors use a heat bath (Parikh & Madamwar, 2006) or a membrane process, such as tangential ultrafiltration (Han et al., 2014) or microfiltration (Ahmed et al., 2014) to concentrate the medium. However, membrane processing depends on the viscosity of the culture medium, pore size distribution and transmembrane pressure (Li et al., 2011). The frequently used technique to recover EPS consists of alcohol precipitation. Under these conditions, some salts in the culture medium also co-precipitate. Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are also used to extract EPSs, although to a lesser extent (Budarin et al., 2012). It is worth noting that ultrasound waves may also degrade the EPS macromolecules (Du et al., 2011).

No study was found in the literature on the combined effect of nitrate (NaNO<sub>3</sub>) starvation and PFD on the EPS production for the *Arthrospira platensis* culture. Nitrogen starvation is known as a stress condition, which favors cyanobacteria to accumulate some reserve products and to produce EPS (Lupi et al., 1994). PFD is related to the increase of EPS yields (Markou, Angelidaki, & Georgakakis, 2012). However, the knowledge about the events that explain the process is still scarce. Some authors described two events in diatoms: the combined effect of PFD and nitrate starvation decreased the cellular growth and cause an excess of carbon assimilated, which is released as carbohydrates; the electrons accumulated at photosynthetic electron transport chain could induced the oxygen reactive species (ROS) and cause cellular damage – the EPS production acts as a protection barrier against this ROS (Piedras et al. 2010, Miklestad, 1995).

In this work, an experimental design methodology was used to investigate the combined effect of nitrogen concentration and PFD on the biomass concentration and EPS content in cultivation of *Arthrospira platensis*. The resulting products were characterized as for their protein, carbohydrate and metals composition. Further, were characterized by infrared spectroscopy, thermal gravimetric analysis and as for their dynamic rheological properties.

## Materials And Methods

### ***Microorganism***

*Arthrospira platensis* was provided by Elizabeth Aidar microalgae collection from Fluminense Federal University (Niterói, RJ/Brazil). The cellular suspension of microalgae (300 mL), used as inoculum for the cultivations in bottles, was cultivated in Zarrouk's medium, modified by George (1976) in Erlenmeyer flasks. All other reagents were purchased from Sigma Aldrich (São Paulo, SP, Brazil). Distilled and deionized water was used in all cases.

### ***Culture media***

Culture media were formulated in 4L bottles, with 3700 mL of Zarrouk's medium and 300 mL of *A. platensis* inoculum, and incubated in a germination chamber at  $(32 \pm 2)$  °C with constant aeration and lightening from white LED at PFD of  $1000 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Measurements of the photonic flux density (PFD) were carried out with a QSL 2100 dosimeter from Biospherical Instruments Inc. (San Diego, CA, USA). As LED lamps have unequal values of PFD depending on the region of the bottle surface, where light passes through, a complete mapping of PFD values was made along 36 points on the surface.

### ***Experimental design***

Using the Design Expert program, version 10.0.6.0., a 3-level-factorial design ( $3^2$ ) was used to plan culture conditions for the production of *A. platensis* biomass and EPS. A total of twelve runs was planned, nine of which for combinations of three levels and a central point in triplicate (Table 1). The independent variables were  $\text{NaNO}_3$  concentration (0.5, 1.125, and  $2 \text{g} \cdot \text{L}^{-1}$ ) and PFD ( $200, 600$  and  $1000 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ). The two responses evaluated were biomass ( $\text{mg} \cdot \text{L}^{-1}$ ) and EPS yield ( $\text{mg} \cdot \text{g}^{-1}$ ). The effect of each factor was determined by the analysis of variance (ANOVA) for a confidence level of 95%.

### ***Monitoring of growth and determination of the final biomass concentration***

*A. platensis* growth was controlled by measuring daily the optical density (OD) of the cellular suspension at 730 nm for 21 days. The specific growth rate ( $\mu$ ) was determined over the exponential growth phase, and given by Eq. 1

$$\mu = (\ln x_2 - \ln x_1) / (t_2 - t_1) \quad (1)$$

Where  $x_1$  and  $x_2$  are the biomass concentration at exponential phase,  $t_1$  and  $t_2$  are the times corresponding to the beginning and the end of the exponential phase, respectively.

To determine dry biomass weight, aliquots of 20mL were taken from the culture medium at the end of each experiment and filtered through 0.7 -1.2  $\mu\text{m}$  Sartorius glass fiber membranes, previously weighed. After being extensively washed to eliminate excess of salt, the membranes were dried and weighed, giving the dry biomass (g) by difference.

### ***EPS production***

To recover the EPS from the extracellular medium, the procedure reported by Parikh, & Madamwar (2006) was followed. Briefly, an adequate volume of the filtered medium, which still contained biomass, was centrifuged at 20,000 g for 20 min in a Hettich Rotixa centrifuge, model 420R (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The resulting dispersion was heated at 70°C for 12 h to have its volume reduced to  $\frac{1}{4}$ . The EPS product was recovered by adding ice-cooled ethyl alcohol. After 12 h at 4°C, the product was filtered and completely dried in an oven at 60°C. To eliminate residual salt, the samples were resolubilized and then dialyzed against water under constant stirring at room temperature for 24 h. The final product was recovered by adding 95% ethyl alcohol and lyophilized.

### ***Physicochemical characterization of EPS***

The methodology proposed by Dubois et al. (1956) was used to quantify total carbohydrate content for each recovered EPS sample, by adding  $\text{H}_2\text{SO}_4$  concentrated at 80% (m/v) and phenol at 5% (v/v). A standard curve was obtained using D-glucose.

Soluble protein was determined by the Folin's method, using bovine serum albumin as standard (Lowry, Rosebrough, Farr, & Randall, 1951).

Fourier transform infrared spectroscopy (FTIR) analyses were carried out using a Perkin Elmer spectrometer, Frontier model (Waltham, MA, USA) at room temperature, using KBr disks, in the 4000 – 400  $\text{cm}^{-1}$  range, with accumulation of 20 scans and 4  $\text{cm}^{-1}$  resolution.

Thermogravimetric analyses were carried out for the EPS samples under nitrogen atmosphere on a TGA Q-500 equipment from TA Instruments (New Castle, DE, USA). Approximately, 10 mg of sample were heated from 20 to 700°C, at a 10°C/min rate.

The rheological properties of selected EPS samples were investigated at 5g.L<sup>-1</sup> and 10 g.L<sup>-1</sup> at 25°C on an AR G2 controlled stress rheometer (TA Instruments Inc.) with a coaxial cylinder geometry. Initially, a deformation scan was performed by varying the complex modulus ( $G^*$ ) as a function of the oscillatory frequency at 6.28  $\text{rad}\cdot\text{s}^{-1}$ , to determine the region of linear viscoelasticity. After a period of 10 min, an oscillatory frequency scan was performed, from 10<sup>-1</sup> to 7 x10<sup>2</sup> $\text{rad}\cdot\text{s}^{-1}$  (with a deformation value of 10%),

within the region of linear viscoelasticity. Finally, viscosity changes under steady flow regime were investigated as a function of shear rate, from 10 to  $10^2\text{s}^{-1}$ .

### ***Statistical analysis***

All experiments were carried out in triplicate and the results were expressed as mean values  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) was applied to the data by using the PAST 3.20 software, available at <https://folk.uio.no/ohammer/past>. The mean values were compared by the Tukey's test considering a confidence level of 95% level of significance ( $p < 0.05$ ).

## **Results And Discussion**

### ***Evaluation of *Arthrospira platensis* growth under different cultivation conditions***

The growth curves of *A. platensis* cultivated in different culture conditions are presented in Fig. 1. As observed, none of the conditions presented an adaptation phase. The experiment number 6 presented the highest growth rate ( $\mu=0.55$ ), in medium with  $2\text{g.L}^{-1}$  of  $\text{NaNO}_3$  and under  $600\ \mu\text{E.m}^{-2}.\text{s}^{-1}$  of PFD. Condition 2 led to the lowest growth rate, with  $1.125\ \text{g.L}^{-1}$  of  $\text{NaNO}_3$  and PFD of  $200\ \mu\text{E.m}^{-2}.\text{s}^{-1}$ , followed closely by number 1 ( $0.25\ \text{g.L}^{-1}$  of  $\text{NaNO}_3$  and  $200\ \mu\text{E.m}^{-2}.\text{s}^{-1}$  of PFD) and condition 3 ( $2\ \text{g.L}^{-1}$  of  $\text{NaNO}_3$  and  $200\ \mu\text{E.m}^{-2}.\text{s}^{-1}$  of PFD), which correspond to the lowest PFD value and were practically independent on the  $\text{NaNO}_3$  concentration, showing that PFD was the limiting factor in these situations.

The PFD values may affect the growth rate, which increase until reaching a point of light saturation, and, from this point, there may be even a decrease in the rate of growth as a consequence of photo inhibition (Carvalho, Silva, Baptista, & Malcata, 2011). In cultures grown at  $1000\ \mu\text{E.m}^{-2}.\text{s}^{-1}$  and with a concentration of  $2\ \text{g.L}^{-1}$  may have occurred a light saturation, which resulted in a decrease of the cell growth. In cultures using  $600\ \mu\text{E.m}^{-2}.\text{s}^{-1}$ ,  $\text{NaNO}_3$  concentration was probably the limiting factor for the growth.

### ***Final biomass concentration and EPS yield under different growth conditions***

The values of final biomass concentration and EPS yield are given in Table 1. Considering the results, the highest final biomass concentration ( $1.292\ \text{g.L}^{-1}$ ) was observed under conditions with the highest  $\text{NaNO}_3$  concentration ( $2\ \text{g.L}^{-1}$ ). For EPS content, the best condition were the lowest  $\text{NaNO}_3$  ( $0.25\ \text{g.L}^{-1}$ ) and PFD ( $200$

$\mu\text{E.m}^{-2}.\text{s}^{-1}$ ), which generated  $111\ \text{mg.g}^{-1}$  EPS. However, the second highest EPS content ( $100\ \text{mg.g}^{-1}$ ) was obtained under conditions of highest values of  $\text{NaNO}_3$  ( $2\ \text{g.L}^{-1}$ ) and PFD ( $1000\ \mu\text{E.m}^{-2}.\text{s}^{-1}$ ). In other words. EPS content (EPS mass/biomass) attained the maximum values at both extremes of the experimental design.

The results presented in Fig. 2a and 2b showed that, in general, EPS production by *A. platensis* was not directly associated to its growth. As may be observed, the response in which the highest final biomass concentration was obtained differed from that, which generated the highest EPS production. Condition 9 was an exception, since this condition promoted one of the highest EPS contents and at the same time one of the highest final biomass concentration. According to some authors, metabolic stress could affect growth negatively, leading to the production of reserve substances (Santos et al., 2019; Soanen et al., 2016).

Table 2 shows the results of the coefficients and their interactions,  $R^2$ , lack of fit and the p-values for final biomass concentration and EPS concentration by *A. platensis*. A 95% of confidence level was adopted ( $p < 0.05$ ). The model is significant for final biomass concentration with p-value equal to 0.029. PFD and its interactions have significant influence on the model; on the other hand,  $\text{NaNO}_3$  concentration has no influence on biomass concentration. For the EPS production, the model was significant, with p-value equal to 0.0389, and the variables that showed significant influence were PFD and its interactions.

It is noteworthy to observe that lack of fit values for both biomass and EPS production were significant ( $p < 0.05$ ). In this case, the model does not have a good predictive capacity. Despite the lack of adjustment, it is possible to visualize the best working regions.

In Figure 2a, the response surface graphics shows that the conditions, which led to the highest final biomass concentration were  $600 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and  $2 \text{ g}\cdot\text{L}^{-1}$ . A decrease in biomass production was also observed when the values of  $\text{NaNO}_3$  concentration and PFD were smaller,  $0.25 \text{ g}\cdot\text{L}^{-1}$  and  $200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively.

Aikawa et al. (2012) also observed that biomass production was related to PFD values and that *A. platensis* produced the highest biomass concentration ( $1.6 \text{ g}\cdot\text{L}^{-1}$ ) at  $700 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Under the smallest PFD value,  $20 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the highest biomass concentration obtained reached  $0.1 \text{ g}\cdot\text{L}^{-1}$ .

Figure 2b shows the response surface obtained for the EPS content ( $\text{mg}\cdot\text{g}^{-1}$ ). The highest values were observed at the two extremes, 111 and  $100 \text{ mg}\cdot\text{g}^{-1}$ , at conditions 1 and 9, respectively, besides condition 3, which reached an EPS content from  $100 \text{ mg}\cdot\text{g}^{-1}$ .

Chentir et al. (2017) evaluated the maximization of EPS production by *Arthrospira platensis* as a function of variations in NaCl concentration and PFD. Although PFD did not have any positive effect on EPS production, its interaction with NaCl concentration provided a  $0.98 \text{ g}\cdot\text{g}^{-1}$  yield in EPS. On the other hand, Dejsungkranont, Chisti, & Sirisansaneeyakul (2017) studying the effect of PFD on the production of EPS by *Arthrospira platensis* observed that the highest level of PFD of  $203 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  favored EPS production ( $956.4 \pm 37.3 \text{ mg}\cdot\text{L}^{-1}$ ) as well as biomass ( $1.5 \text{ g}\cdot\text{L}^{-1}$ ). Under the smallest value of PFD of  $101 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $0.8 \text{ g}\cdot\text{L}^{-1}$  of biomass and  $637.3 \pm 41.3 \text{ mg}\cdot\text{L}^{-1}$  of EPS were obtained.

Trabelsi, Ouada, & Bacha (2009) carried out a study evaluating the effect of different temperatures and PFD on the final biomass and EPS concentrations by *A. platensis*, and described a possible correlation between the two responses. According to the authors, the production of biomass and EPS are culture dependents, and the increase of EPS production may be associated with the kinetics of growth. To achieve the higher EPS content, it's necessary to optimize the PFD values and the temperature should be maintained between 30-35°C. At its work, *A. platensis* showed the maximum EPS content at the higher PFD value used, 180  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , with  $297.4 \pm 11.1 \text{ mg}\cdot\text{L}^{-1}$ . The results for EPS content founded by Trabelsi, Ouada & Bacha (2009),  $210 \text{ mg}\cdot\text{L}^{-1}$ , was twice higher than our results,  $91 \text{ mg}\cdot\text{L}^{-1}$ .

The results found in this study showed that the choice of the best conditions depended on the response of interest. The results in Figures 2a and 2b showed that the production of EPS by *A. platensis* was not directly associated to growth; nevertheless, it is possible to choose a condition in which EPS as well as biomass could be produced at reasonable values.

Other studies in literature on different microorganisms reported the effect of culture conditions that influenced the EPS content and biomass concentration. For *Cyanothecce* sp. 113,  $\text{NaNO}_3$  concentrations, between 0 and  $200 \text{ mg}\cdot\text{L}^{-1}$ . In this case, the final biomass concentration was observed to increase, reaching  $1.2 \text{ g}\cdot\text{L}^{-1}$  with  $\text{NaNO}_3$  at  $74.3 \text{ mg}\cdot\text{L}^{-1}$  concentration, but decrease at values higher than  $100 \text{ mg}\cdot\text{L}^{-1}$ . However, a decrease in EPS concentration, from  $7 \text{ g}\cdot\text{L}^{-1}$  to  $5 \text{ g}\cdot\text{L}^{-1}$ , was reported elsewhere when  $200 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{NaNO}_3$  concentration was used. In the same work, the PFD effect was evaluated in the 20-100  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  range and the best condition was found to be  $86 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in the range 20-100  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for biomass and EPS production (Chuangdong, Zhenming, & Weidong, 2007).

### **FTIR analyses**

Figure 3 shows the FTIR spectrum for EPS samples from *A. platensis*. The broad bands observed around  $3400 \text{ cm}^{-1}$  is attributed to O-H and N-H stretching. The weak absorptions in the region  $3000 \text{ cm}^{-1}$  to  $2840 \text{ cm}^{-1}$  are associated to C-H asymmetrical and symmetrical stretching modes of methyl and methylene groups. The absorption at  $1650 \text{ cm}^{-1}$  is attributed to C=O stretching of carboxylate and amide groups (amide I band). The high-intensity band with peak at  $1442 \text{ cm}^{-1}$ , was attributed to more complex vibrations, associated to O-H bending (Can, Gurbuz, & Odabaşı, 2019; Trabelsi, M'sakni, Ouada, Bacha, & Roudesli, 2009). The most intense absorption of the spectrum, at  $1046 \text{ cm}^{-1}$ , may be attributed to C-O-C, S-O and P-O-C stretching vibrations. This result evidenced the presence of polysaccharides, proteins/polypeptides, and of sulphate and phosphate groups linked to polymeric substances.

### **Thermal analysis**

Thermal gravimetric analyses is an important technique to measure the thermal stability of EPS (Fig. 4). EPS showed three stages of thermal degradation. In the first stage, up to  $150^\circ\text{C}$ , 10% weight were lost and may be attributed to the loss of water and other volatile substances. In the second stage, the degradation of the polymer chain occurs, between  $225$  and  $350^\circ\text{C}$ , with fifty percent of total mass.

The thermal degradation of an EPS from microalgae and cyanobacteria was recently reported. Two stages were observed for the EPS from *Dunaliella salina*; in the first, 15% weight were lost up to 150°C. In the second, with a temperature of maximum degradation rate of 240°C, approximately 55% weight were lost (Mirsha et al., 2011). On the other hand, for the EPS from *Nostoc carneum*, three stages of thermal degradation were detected. On first stage 15% weight were lost up to 155°C. In the second with temperature of 237°C, 39% weight were lost, attributed when to polysaccharide degradation. The third phase occurred up to 378°C with third two percent of weight loss (Hussein et al., 2015).

### ***Carbohydrate and protein quantification***

The chemical composition of EPS could be depend of environmental conditions and microorganism of interest (Nouha, Kumar, Balasubramanian and Tyagi, 2016). According to Wingender, Neu & Fleming (1999), the majority composition of EPS is carbohydrates and proteins. In *Arthorspira platensis*, the carbohydrate and protein content are estimated in 55% and 13%, respectively, in a photoautotrophic growth at 25 days (Pignolet et al., 2013). Carbohydrate and protein contents determined for EPS are shown in Table 3. The highest contents in carbohydrates,  $39.5 \pm 2.34 \text{ mg.g}^{-1}$  and  $38.73 \pm 2.55 \text{ mg.g}^{-1}$ , were observed under conditions 5 ( $1,125 \text{ gL}^{-1} \text{ NaNO}_3$  and  $600 \mu\text{E.m}^{-2}.\text{s}^{-1}$ ) and 9 ( $2 \text{ gL}^{-1} \text{ NaNO}_3$  and  $1000 \mu\text{E.m}^{-2}.\text{s}^{-1}$ ).

According to Depraetere et al. (2015), when nitrate source is depleted, the protein synthesis is reduced. But ate this work, the highest concentrations in proteins,  $7.05 \pm 0.30$  and  $6.46 \pm 0.20$ , were observed in intermediate values of  $\text{NaNO}_3$ , in the conditions 5 ( $1.125 \text{ gL}^{-1}$  of  $\text{NaNO}_3$  and  $600 \mu\text{E.m}^{-2}.\text{s}^{-1}$ ) and 8 ( $1.125 \text{ gL}^{-1}$  of  $\text{NaNO}_3$  and  $1000 \mu\text{E.m}^{-2}.\text{s}^{-1}$ ), respectively.

### ***Rheological properties***

Aqueous solutions of EPS 01 ( $0.25 \text{ gL}^{-1} \text{ NaNO}_3$  and  $200 \mu\text{E.m}^{-2}.\text{s}^{-1}$ ) and EPS 09 ( $2 \text{ gL}^{-1} \text{ NaNO}_3$  and  $1000 \mu\text{E.m}^{-2}.\text{s}^{-1}$ ) were prepared at  $5 \text{ g.L}^{-1}$  and  $10 \text{ g.L}^{-1}$  and separated and had their rheological properties investigated. The Fig. 5a and 5b show the variation of storage modulus,  $G'$ , and loss modulus,  $G''$ , with the oscillatory frequency. Their rheological behavior is quite similar to that previously observed for other polysaccharides, such as gum algaroba, a galactomannan extracted from *Prosopis juliflora* (Azero, & Andrade, 2006). At very low frequencies, the viscous character predominates. After the crossing of  $G'$  with  $G''$ , the elastic character is predominant. As expected, the value of the frequency at which the crossing occurs is smaller for the higher concentration. The same behavior was observed for the EPS09 supernatant at  $5$  and  $10 \text{ g.L}^{-1}$ .

Our results are different from those reported by Chentir et al. (2017). The EPS solutions of *A. platensis* showed a gel-like behavior, in which the storage modulus values were higher than the loss modulus values at different concentrations, 1%, 2.5% and 5%. Mourhin et al. (1993) found a non-Newtonian character for EPS dispersions of *Spirulina platensis*, which was attributed to its polyanionic nature.

## Conclusions

The present work demonstrated that combined values of NaNO<sub>3</sub> and PFD was capable to enhance the EPS and biomass content in *Arthrospira platensis*. The nitrate starvation and lowest PFD showed the highest EPS production. The EPS production was not growth associated. The FTIR spectra showed that EPS contained carbohydrate and proteins. The rheological studies suggested that EPS exhibited a dilute solution behavior.

## Abbreviations

EPS: Extracellular polymeric substances; NaCl: sodium chloride; NaNO<sub>3</sub>: sodium nitrate; PFD: Photon flux density; ANOVA: analysis of variance; FTIR: Fourier transform infrared spectroscopy; TGA: thermalgravimetric analyses.

## Declarations

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### Authors' contributions

MBFS designed lab work, carried out all the experiments, analyzed data and wrote the manuscript; EA analyzed data; CMLLT analyzed data, managed the team and reviewed the manuscript; CTA analyzed data, managed the team and reviewed the manuscript. Both authors read and approved the final manuscript.

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### Availability of data and materials

Not applicable.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

No potential conflicts of interest were disclosed.

## References

- Adda, M., Merchuk, J. C., Arad, S. M. 1986. Effect of nitrate on growth and production of cell-wall polysaccharide by the unicellular red alga *Porphyridium*. *Biomass* 10 (2), 131-140. <https://doi.org/10.2478/s11535-013-0248-z>
- Ahmed, M., Poortvliet, T. C. W. M., Wijnholds, A., Staland, L. J., Hasnain, S., 2014. Isolation, characterization and localization of extracellular polymeric substances from the cyanobacterium *Arthrospira platensis* strain MMG-9. *European Journal of Phycology* 49 (2), 143-150. <https://doi.org/10.1080/09670262.2014.895048>
- Aikawa, S., Izumi, Y., Matsuda, F., Hasunuma, T., Chang, J. S., & Kondo, A., 2012. Synergistic enhancement of glycogen production in *Arthrospira platensis* by optimization of light intensity and nitrate supply. *Bioresource Technology* 108, 211–215. <https://doi.org/10.1016/j.biortech.2012.01.004>
- Arad, S., Levy-Ontman, O., 2010. Red microalgae cell-wall polysaccharides: biotechnological aspects. *Current Opinion in Biotechnology*. 21, 358–364. <https://doi.org/10.1016/j.copbio.2010.02.008>
- Azero, E. G., Andrade, C. T., 2006. Characterization of *Prosopis juliflora* seed gum and the effect of its addition to k-carrageenan systems. *Journal of the Brazilian Chemical Society* 17 (5), 844-850. <https://doi.org/10.1590/S0103-50532006000500005>.
- Budarin, V., Ross, A.B., Biller, P., Riley, R., Clark, J.H., Jones, J.M., Gilmour, D.J., Zimmerman, W., 2012. Microalgae biorefinery concept based on hydrothermal microwave pyrolysis. *Green Chemistry* 14, 3251–3254. <https://doi.org/10.1039/C2GC36202C>
- Can, H. K., Gurbuz, F., Odabas, M. 2019. Partial characterization of cyanobacterial extracellular polymeric substances for aquatic ecosystems. *Aquatic Ecology* 53, 431-440. <https://doi.org/10.1007/s10452-019-09699-z>
- Carvalho, A. P., Silva, S. O., Baptista, J. M., & Malcata, F. X. 2011. Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Applied Microbiology and Biotechnology* 89 (5), 1275–1288. <https://doi.org/10.1007/s00253-010-3047-8>
- Chentir, I., Hamdi, M., Doumandji, A., HadjSadok, A., Ouada, H. Ben, Nasri, M., & Jridi, M., 2017. Enhancement of extracellular polymeric substances (EPS) production in *Spirulina* (*Arthrospira* sp.) by two-step cultivation process and partial characterization of their polysaccharidic moiety. *International Journal of Biological Macromolecules* 105 (2), 1412-1420. <https://doi.org/10.1016/j.ijbiomac.2017.07.009>

- Chuangdong, S. U., Zhenming, C. H. I., & Weidong, L. U. (2007). Optimization of medium and cultivation conditions for enhanced exopolysaccharide yield by marine *Cyanothece*. *Chinese Journal of Oceanology and Limnology* 25(4), 411–417. <https://doi.org/10.1007/s00343-007-0411-3>
- Cox, W. P., Merz, E. H. 1958. Correlation of dynamic and steady flow viscosities. *Journal of Polymer Science* 28, 619-622. <https://doi.org/10.1002/pol.1958.1202811812>
- Decho, A. W., Gutierrez, T. 2017. Microbial Extracellular Polymeric Substances (EPSs) in Ocean Systems. *Frontiers in Microbiology* 8, 1-28. <https://doi.org/10.3389/fmicb.2017.00922>
- Dejsungkranont, M., Chisti, Y., & Sirisansaneeyakul, S., 2017. Simultaneous production of C-phycoyanin and extracellular polymeric substances by photoautotrophic cultures of *Arthrospira platensis*. *Journal of Chemical Technology and Biotechnology* 92(10), 2709–2718. <https://doi.org/10.1002/jctb.5293>
- Delattre, C., Pierre, G. Laroche, C., Michaud, P., 2016. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotechnology Advances* 34 (7), 1159-1179. <https://doi.org/10.1016/j.biotechadv.2016.08.001>
- Depraetere, O., Deschoenmaeker, F., Badri, H., Monsieurs, P., Foubert, I., Leys, N., Wattiez, R., Muylaert, K., 2015. Trade-Off between growth and carbohydrate accumulation in nutrient-limited *Arthrospira* sp. PCC 8005 studied by integrating transcriptomic and proteomic approaches. *PLOS ONE* 10(7): e0132461. <https://doi.org/10.1371/journal.pone.0132461>
- Du, Z., Li, Y., Wanga, X., Wana, Y., Chen, Q., Wang, C., Lin, X., Liu, Y., Chen, P., Ruan, R., 2011. Microwave-assisted pyrolysis of microalgae for biofuel production. *Bioresource Technology* 102 (7), 4890-4896. <https://doi.org/10.1016/j.biortech.2011.01.055>
- Dubois, M., GillesK. A., Hamilton, J. K., Rebers, P. A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* 28 (3), 350-356. <https://doi.org/10.1021/ac60111a017>
- Ekelhof, A., & Melkonian, M., 2017. Enhanced extracellular polysaccharide production and growth by microalga *Netrium digitus* in a porous substrate bioreactor. *Algal Research* 28, 184–191. DOI: [10.1016/j.algal.2017.11.003](https://doi.org/10.1016/j.algal.2017.11.003)
- Friedman, O., Dubinsky, Z., & Arad, S. M., 1991. Effect of light intensity on growth and polysaccharide production in red and blue-green *Rhodophyta* unicells. *Bioresource Technology* 38 (2), 105–110. [https://doi.org/10.1016/0960-8524\(91\)90139-B](https://doi.org/10.1016/0960-8524(91)90139-B)
- Piedras, F. R., Baisch, P. R. M., Machado, M. I. C. S., Vieira, A. A. H., Giroldo, D. 2010. Carbohydrate release by a subtropical strain of *Spondylosium pygmaeum* (Zygnematophyceae): influence of nitrate availability and culture aging. *Journal of Phycology* 46, 477-483. DOI: [10.1111/j.1529-8817.2010.00823.x](https://doi.org/10.1111/j.1529-8817.2010.00823.x)

- Goo, B. G., Baeka, G., Choi, D. J., Park, Y. I., Synytskyac, A., Bleha, R., Seong, D. H., Lee, C. G., Park, J. K. 2013. Characterization of a renewable extracellular polysaccharide from defatted microalgae *Dunaliella tertiolecta*. *Bioresource Technology* 129, 343-350. <https://doi.org/10.1016/j.biortech.2012.11.077>
- Gong, H., Tang, Y., Wang, J., Wen, X., Zhang, L., & Lu, C., 2008. Characterization of photosystem II in salt-stressed cyanobacterial *Spirulina platensis* cells. *Biochimica et Biophysica Acta* 1777, 488–495. <https://doi.org/10.1016/j.bbabi.2008.03.018>
- Han, P., Suna, Y., Jiaa S., Zhonga, C., Tan, Z., 2014. Effects of light wavelengths on extracellular and capsular polysaccharide production by *Nostoc flagelliforme*. *Carbohydrate Polymers* 105, 145-151. <https://doi.org/10.1016/j.carbpol.2014.01.061>.
- Hussein, M. H., Abou-elwafa, G. S., Shaaban-dessuuki, S. A., & Hassan, N. I., 2015. Characterization and Antioxidant Activity of Exopolysaccharide Secreted by *Nostoc carneum*. *International Journal of Pharmacology* 11 (5), 432-439. Doi: 10.3923/ijp.2015.432.439
- Jesus, C. S., Assis, D. J., Rodriguez, M. B., Filho, J. A. M., Costa, J. A. V., Ferreira, S. E., Druzian, J. I., 2019. Pilot-scale isolation and characterization of extracellular polymeric substances (EPS) from cell-free medium of *Spirulina* sp. LEB-18 cultures under outdoor conditions. *International Journal of Biological Macromolecules* 124, 1106-1114. <https://doi.org/10.1016/j.ijbiomac.2018.12.016>
- Li, H., Mao, W., Hou, Y., Gao, Y., Qi, X., Zhao, C., Chen, Y., Chen, Y., Li, N., Wang, C., 2012. Preparation, structure and anticoagulant activity of a low molecular weight fraction produced by mild acid hydrolysis of sulfated rhamnan from *Monostrom alatissimum*. *Bioresource Technology*, 114 (3), 414–418. <https://doi.org/10.1016/j.biortech.2012.03.025>
- Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 193 (1), 265–75.
- Lupi, F. M., Fernandes, H. M. L., Tomé, M. M., Sá-Correia, I., & Novais, J. M. 1994. Influence of nitrogen source and photoperiod on exopolysaccharide synthesis by the microalga *Botryococcus braunii* UC 58. *Enzyme and Microbial Technology* 16(7), 546–550. [https://doi.org/10.1016/0141-0229\(94\)90116-3](https://doi.org/10.1016/0141-0229(94)90116-3)
- Manirafasha, E., Murwanashyaka, T., Ndikubwimana, T., Ahmed, R. N., Liu, J., Lu, Y., Zeng, X., Ling, X., Jing, K. 2018. Enhancement of cell growth and phycocyanin production in *Arthrospira* (*Spirulina*) *platensis* by metabolic stress and nitrate fed-batch. *Bioresource Technology*. 255, 293-301. <https://doi.org/10.1016/j.biortech.2017.12.068>
- Markou, G., Angelidaki, I., & Georgakakis, D. 2012. Microalgae carbohydrates: An overview of the factors influencing carbohydrates production and of main bioconversion technologies for production of biofuels. *Applied Microbiology and Biotechnology* 96(3), 631–645. <https://doi.org/10.1007/s00253-012-4398-0>

- Mishra, A., Kavita, K., & Jha, B., 2011. Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina*. Carbohydrate Polymers 83(2), 852–857. <https://doi.org/10.1016/j.carbpol.2010.08.067>
- Miklestad, S. M. 1995. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. The Science of the Total Environment 165, 155-164. [https://doi.org/10.1016/0048-9697\(95\)04549-G](https://doi.org/10.1016/0048-9697(95)04549-G)
- Mouhim, F. R., Cornet, J., Fontane, T., Fournet, B., Dubertret, G. 1993. Production, isolation and preliminary characterization of the exopolysaccharide of the cyanobacterium *Spirulina platensis*. Biotechnology Letters 15, 567–572. <https://doi.org/10.1007/BF00138541>
- Nouha, K., Kumar, R. S., Balasubramanian, S., & Tyagi, R. D. (2016). Critical review of EPS production, synthesis and composition for sludge flocculation. Journal of Environmental Sciences (China), 1–21. <https://doi.org/10.1016/j.jes.2017.05.020>
- Ohki, K., Le, N., Yoshikawa, S., Kanesaki, Y., Okajima, M., Kaneko, T., Thi, T. 2014. Exopolysaccharide production by a unicellular freshwater cyanobacterium *Cyanothece* sp. isolated from a rice field in Vietnam. Journal of Applied Phycology 26, 265-272. <https://doi.org/10.1007/s10811-013-0094-4>
- Oliver, J. W. K., Atsumi, S., 2014. Metabolic design for cyanobacterial chemical synthesis. Photosynthesis Research 120 (3), 249–261. <https://doi.org/10.1007/s11120-014-9997-4>.
- Ozturk, S., & Aslim, B. 2010. Modification of exopolysaccharide composition and production by three cyanobacterial isolates under salt stress. Environmental Science and Pollution Research 17 (3), 595–602. <https://doi.org/10.1007/s11356-009-0233-2>
- Parikh, A. Madamwar, D., 2006. Partial characterization of extracellular polysaccharides from cyanobacteria. Bioresource Technology 97 (15), 1822-1827. <https://doi.org/10.1016/j.biortech.2005.09.008>
- Pereira, S., Zille, A., Micheletti, E., Moradas-Ferraira, P., De Philippis, R., Tamagnini, P., 2009. Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiology Reviews 33, 917–941. <https://doi.org/10.1111/j.1574-6976.2009.00183.x>
- Piedras, F. R., Baisch, P. R. M., Machado, M. I. C. S., Vieira, A. A. H., Giroldo, D. 2010. Carbohydrate release by a subtropical strain of *Spondylosium pygmaeum* (Zygnematophyceae): influence of nitrate availability and culture aging. Journal of Phycology 46, 477-483. <https://doi.org/10.1111/j.1529-8817.2010.00823.x>
- Pignolet, O., Jubeau, S., Vacca Garcia, C., Michaud, P., 2013. Highly valuable microalgae: biochemical and topological aspects. Journal of Industrial Microbiology and Biotechnology 40, 781–796. <https://doi.org/10.1007/s10295-013-1281-7>

Piorreck, M., Baasch, K., & Pohl, P., 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry* 23(2), 207–216. [https://doi.org/10.1016/S0031-9422\(00\)80304-0](https://doi.org/10.1016/S0031-9422(00)80304-0)

Santos, R. R., Corrêa, P. S., Dantas, F. M. L., Teixeira, C. M. L. L., 2019. Evaluation of the co-production of total carotenoids, C-phycocyanin and polyhydroxyalkanoates by *Arthrospira platensis*. *Bioresource Technology Reports* 7, 100226. <https://doi.org/10.1016/j.biteb.2019.100226>

Shabana, E. F., Gabr, M. A., Moussa, H. R., El-Shaer, E. A., & Ismaiel, M. M. S., 2017. Biochemical composition and antioxidant activities of *Arthrospira* (Spirulina) *platensis* in response to gamma irradiation. *Food Chemistry* 214, 550– 555. <https://doi.org/10.1016/j.foodchem.2016.07.109>

Soanen, N., Da Silva, E., Gardarin, C., Michaud, P., Laroche, C., 2016. Improvement of exopolysaccharide production by *Porphyridium marinum*. *Bioresource Technology*. 213, 231–238. <https://doi.org/10.1016/j.biortech.2016.02.075>

Trabelsi, L., Chaieb, O., Mnari, A., Abid-Essafi, S., Aleya, L., 2016. Partial characterization and antioxidant and antiproliferative activities of the aqueous extracellular polysaccharides from the thermophilic microalgae *Graesiella* p. *BMC Complementary and Alternative Medicine* 16, 2-10. <https://doi.org/10.1186/s12906-016-1198-6>

Trabelsi, L., M'sakni, N. H., Ouada, H. B., Bacha, H., & Roudesli, S. 2009. Partial characterization of extracellular polysaccharides produced by cyanobacterium *Arthrospira platensis*. *Biotechnology and Bioprocess Engineering* 14, 27-31. <https://doi.org/10.1007/s12257-008-0102-8>.

Trabelsi, L., Ouada, H. Ben, & Bacha, H. 2009. Combined effect of temperature and light intensity on growth and extracellular polymeric substance production by the cyanobacterium *Arthrospira platensis*. *Journal of Applied Phycology* 21, 405–412. <https://doi.org/10.1007/s10811-008-9383-8>

Villay, A., Laroche, C., Roriz, C., El Alaoui, H., Delbac, F., Michaud, P. 2013. Optimisation of culture parameters for exopolysaccharides production by the microalga *Rhodella violácea*. *Bioresource Technology* 146, 732-735. <https://doi.org/10.1016/j.biortech.2013.07.030>

Wingender, J., Neu, R. T., Flemming, H. C., 1999. *Microbial Extracellular Polymeric Substances*. Springer-Verlag Berlin Heidelberg, 1, 1-19.

Xiao, R., Yang, X., Lic, M., Lia, X., Weib, Y., Caod, M., Ragauskasc, A., Thiesg, M., Dingg, J., Zhenga, Y. (2018). Investigation of composition, structure and bioactivity of extracellular polymeric substances from original and stress-induced strains of *Thraustochytrium striatum*. *Carbohydrate Polymers* 195, 515-254. <https://doi.org/10.1016/j.carbpol.2018.04.126>

Zarrouk C (1966) Contribution à l'étuded'unecyanophycée. Influence de diversfacteurs physiques etchimiques sur la croissanacet la photosynthèse de *Spirulina maxima* Geitler. PhD Thesis, University of

## Tables

Table 1: Experimental matrix for EPS production with the corresponding variables and responses

Experiment number	Factors		Response	
	A - NaNO <sub>3</sub> (mg.L <sup>-1</sup> )	B- PFD ( $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Biomass (g.L <sup>-1</sup> )	EPS (mg.g <sup>-1</sup> )
1	0.25	200	0.78	111
2	1.125	200	0.642	70
3	2	200	0.767	100
4	0.25	600	0.868	47
5	1.125	600	1.07	50
6	2	600	1.292	46
7	0.25	1000	0.871	40
8	1.125	1000	0.94	47
9	2	1000	0.913	100
10	1.125	600	1.073	54
11	1.125	600	1.062	51
12	1.125	600	1.087	45

Table 2: R<sup>2</sup>, Lack of fit, p-value, coefficients and effects on quadratic model to responses obtained of biomass and EPS

Source	Biomass yield		EPS	
	Coefficient	p-value	Coefficient	p-value
Model		0.299		0.0389
NaNO <sub>3</sub>	0.06	0.1899	7.76	0.2532
PFD	0.1	0.044	-15.43	0.2532
NaNO <sub>3</sub> x PFD	0.014	0.7805	17.75	0.0503
NaNO <sub>3</sub> x NaNO <sub>3</sub>	0.024	0.6881	12.88	0.2011
PFD x PFD	-0.26	0.0057	24.88	0.0361
R <sup>2</sup>		0.8676		0.8518
Lack of fit		0.0109		0.038

Table 3: Protein and carbohydrate content in dry mass of EPS in different conditions of NaNO<sub>3</sub> concentration and PFD. Equal letters indicate that there was no significant difference

Experiment number	Factors		Response	
	A - NaNO <sub>3</sub> (mg.L <sup>-1</sup> )	B- PFD (μE.m <sup>-2</sup> .s <sup>-1</sup> )	Carbohydrate (mg.g <sup>-1</sup> )	Protein (mg.g <sup>-1</sup> )
1	0.25	200	23 ± 2.27 <sup>a</sup>	4.67 ± 0.28 <sup>a</sup>
2	1.125	200	23.5 ± 1.19 <sup>a</sup>	4.2 ± 1.37 <sup>a,b,c</sup>
3	2	200	24.43 ± 1.40 <sup>a</sup>	5.46 ± 0.40 <sup>a</sup>
4	0.25	600	27.43 ± 0.37 <sup>b</sup>	3.49 ± 0.10 <sup>b</sup>
5	1.125	600	39.5 ± 2.34 <sup>e</sup>	7.05 ± 0.30 <sup>e</sup>
6	2	600	17.53 ± 0.5 <sup>c</sup>	3.14 ± 0.24 <sup>b</sup>
7	0.25	1000	23.67 ± 1.41 <sup>a</sup>	2.56 ± 0.07 <sup>d</sup>
8	1.125	1000	34 ± 1.49 <sup>d</sup>	6.46 ± 0.20 <sup>e</sup>
9	2	1000	38.73 ± 2.55 <sup>e</sup>	4.80 ± 0.38 <sup>a</sup>
10	1.125	600	36.63 ± 0.51 <sup>e</sup>	4.18 ± 0.19 <sup>c</sup>
(Central point)				
11	1.125	600		
(Central point)				
12	1.125	600		
(Central point)				

## Figures

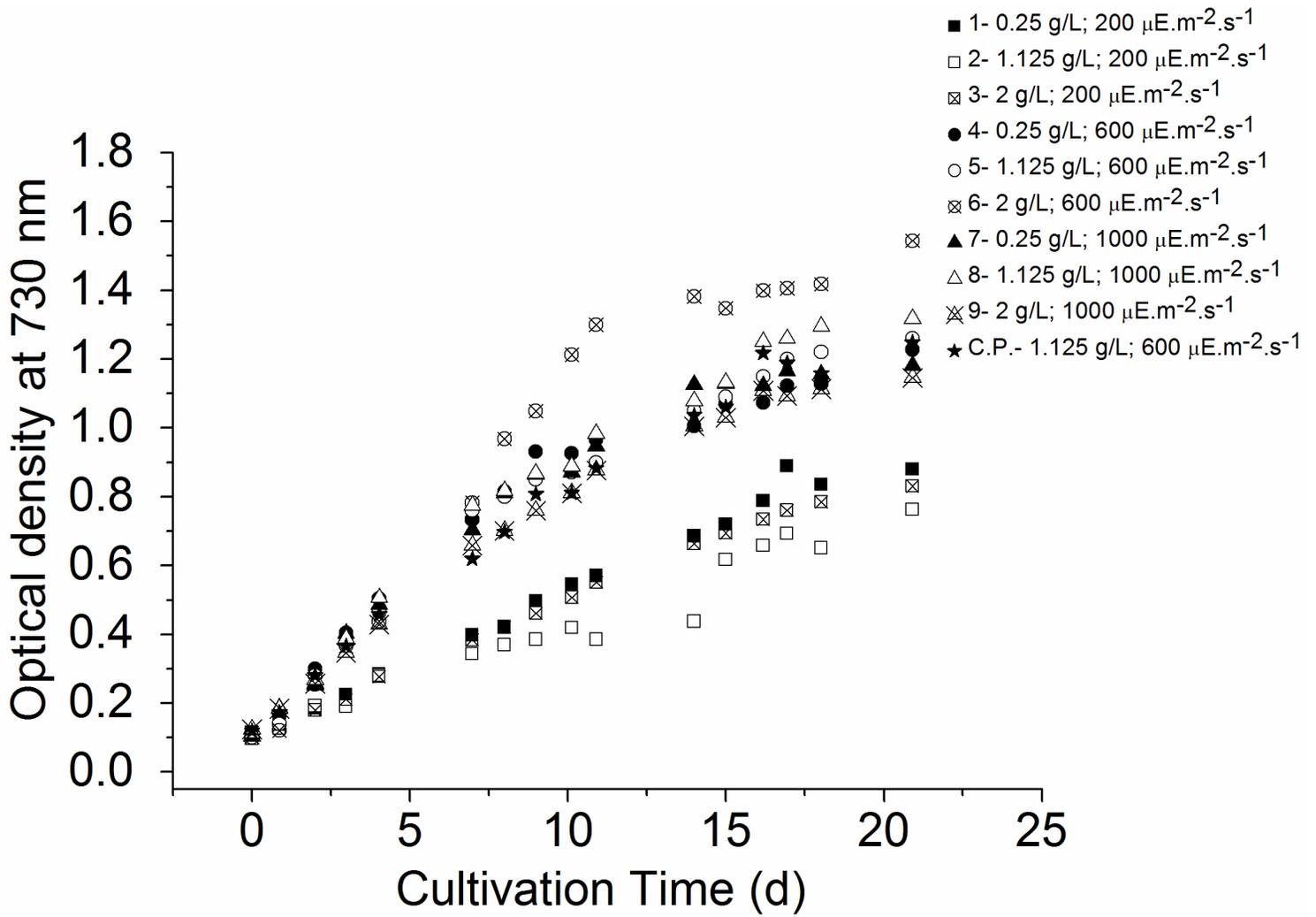
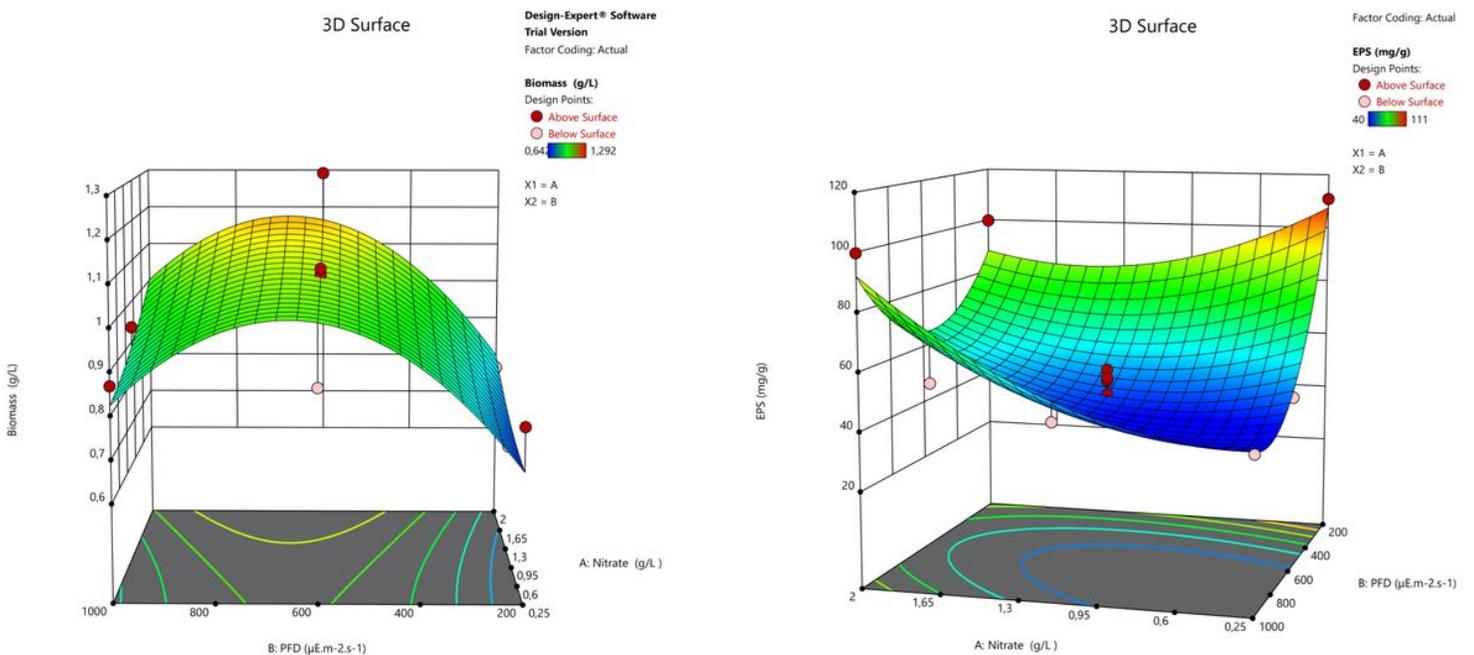


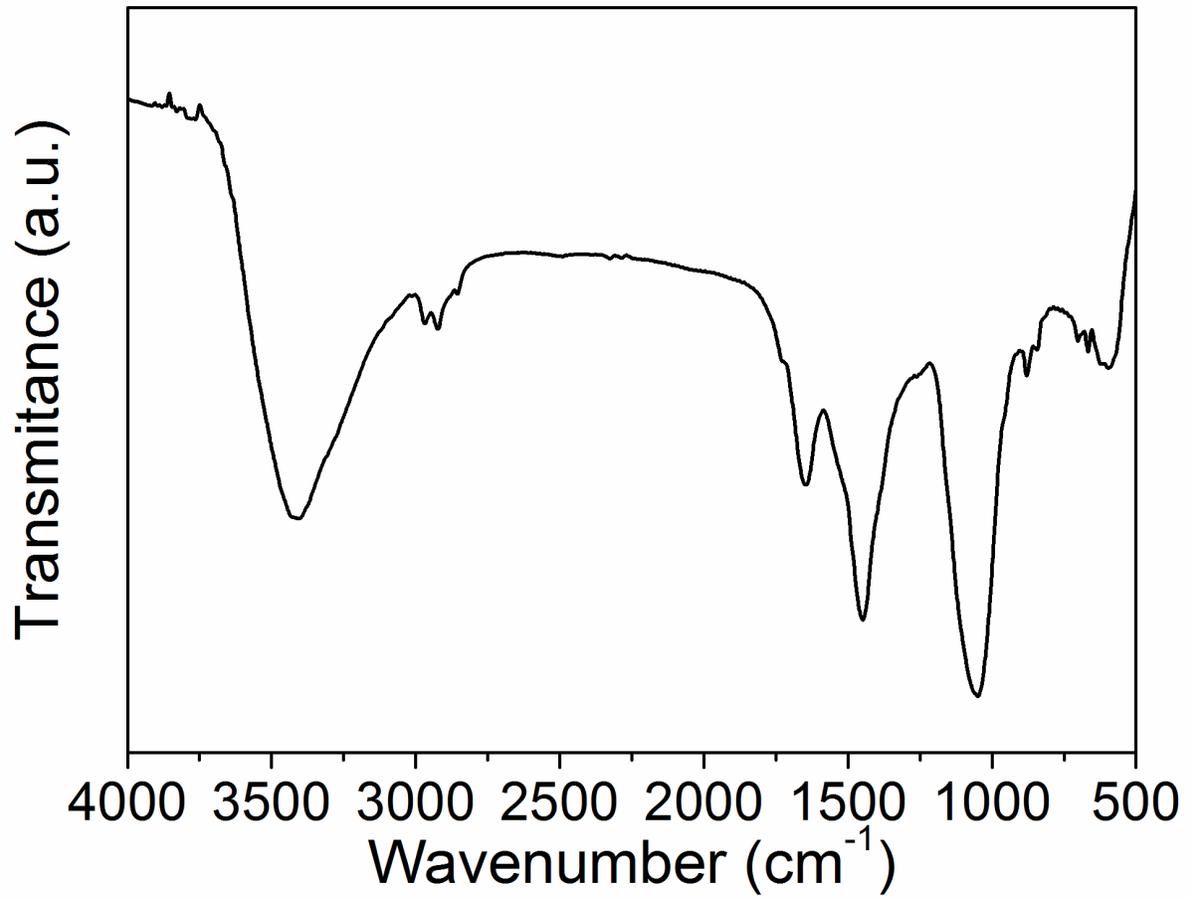
Figure 1

Growth curves of *A. platensis* at different conditions of  $\text{NaNO}_3$  and PFD.



**Figure 2**

Response surface for biomass (a) and EPS production (b).



**Figure 3**

FTIR characteristic of EPS

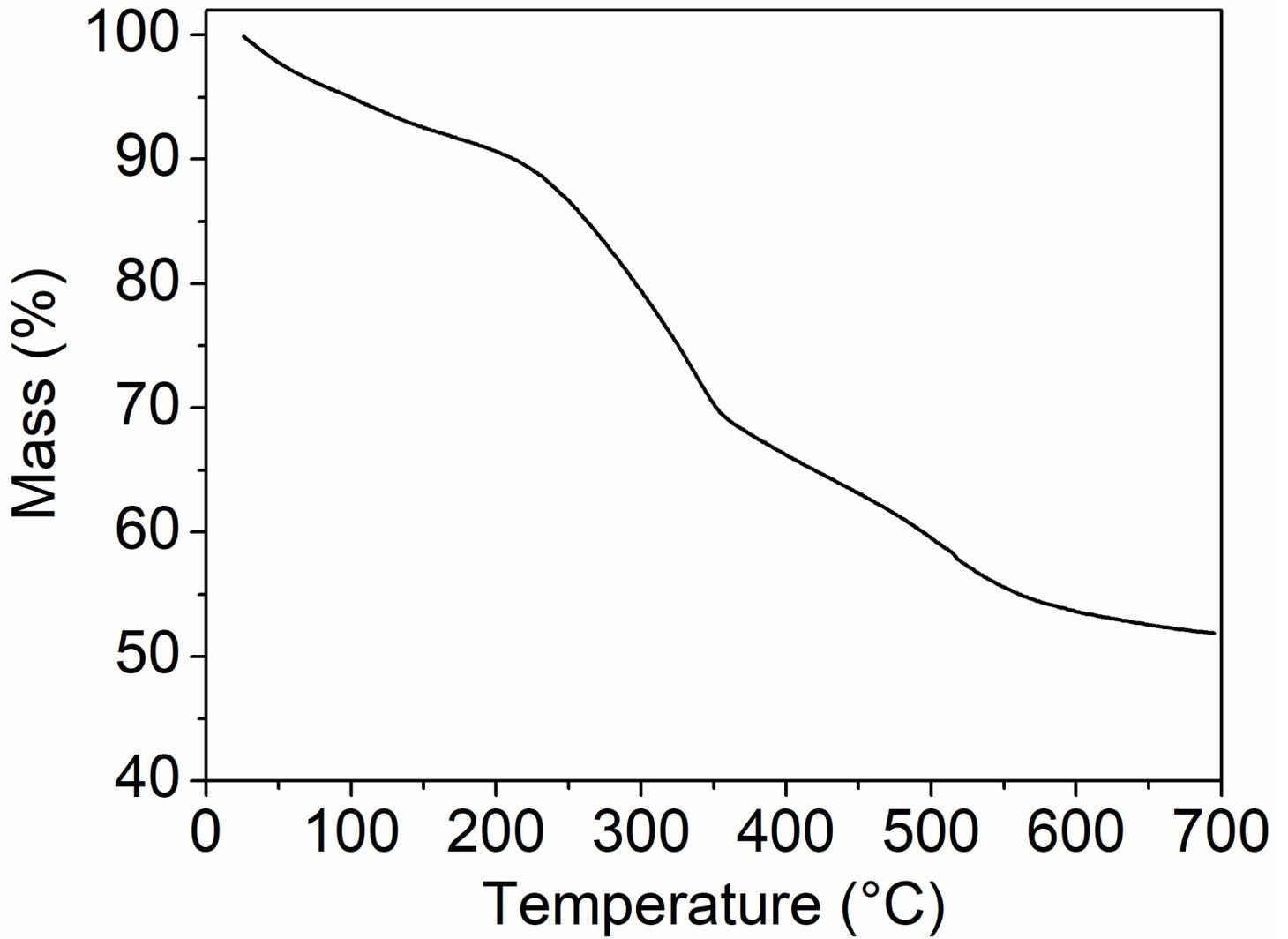


Figure 4

Thermogram of EPS obtained from *A. platensis*

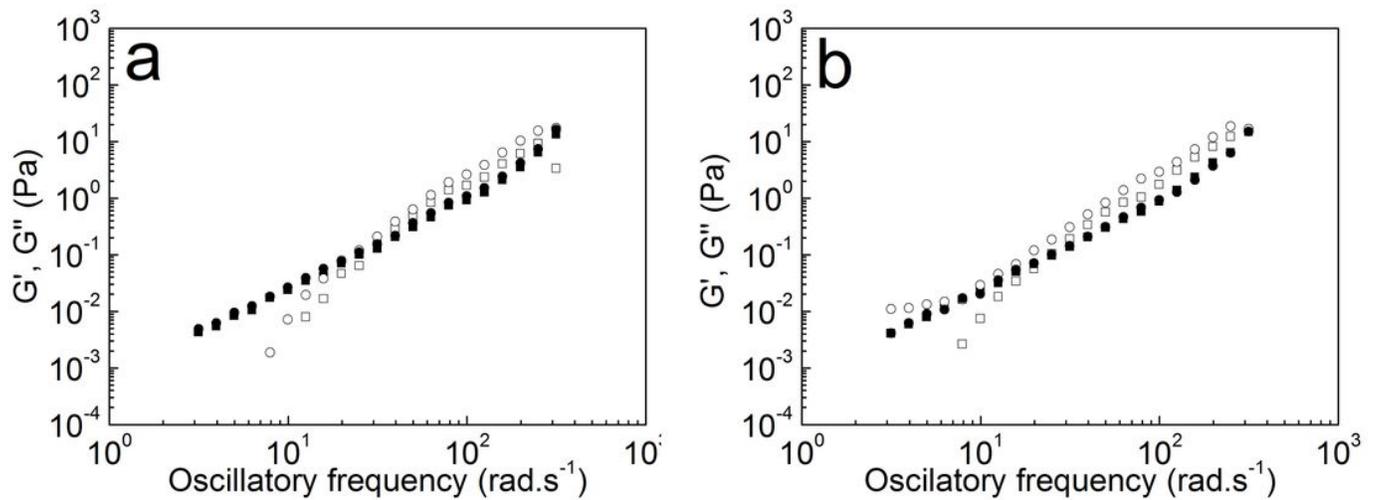


Figure 5

Variation of storage and loss module with oscillatory frequency for the solutions at 5 g.L<sup>-1</sup> (a) and 10 g.L<sup>-1</sup> (b).