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Characterization of the polysaccharides chemical diversity of the cyanobacteria

Arthrospira platensis
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Abbreviations

EPS: Exopolysaccharides; HL: High Light condition; HT: High
Temperature condition; LL: Low Light condition; LT: Low Temperature
condition ; -N: Nitrate limitation condition ; +N: Non-limitant Nitrate
condition ; PBR: Photobioreactor; PFD: Photon Flux Density; PS:
Polysaccharides; RSD: Relative Standard Deviation ;

Abstract

Polysaccharides (PS) from photosynthetic microorganisms have gained interest over the last decade, due to very large variety of chemical structures and potential original physico-chemical and/or biological properties. Among them, the cyanobacterium *Arthrospira platensis* is one of the most studied strains. However, depending on studies, diversity of PS (EPS and glycogen) amounts and compositions are proposed, and no clear link could be established to the culture conditions. The aim of this study was to investigate those effects by performing a bibliographic meta-analysis. The incident light was identified as the most influencing parameter and was investigated experimentally in photobioreactors. The produced PS were quantified and analyzed, confirming that glycogen and EPS amounts are strongly affected by this parameter, with EPS/glycogen ratios decreasing from 3.1 at $100 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ to 0.6 at $1200 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$. Moreover, a significant decrease in uronic acids of EPS was observed for high light fluxes.

Keywords: Polysaccharides, Exopolysaccharides, Glycogen, *Arthrospira*, cyanobacteria, culture conditions

1 Introduction

Over the last decade, the use of microalgae for third generation biofuel production and carbon dioxide sequestration has become a challenge worldwide, giving rise to a huge number of studies on these photosynthetic microorganisms (for a recent overview, see [1]). Among them, cyanobacteria are a large and widespread group of photoautotrophic microorganisms that combine the ability to perform oxygenic photosynthesis with typical prokaryotic features [2]. In that phylum, the specie *Arthrospira platensis* is one of the most studied cyanobacteria that is industrially produced mainly for its wealth in proteins and its use as food supplements. Nevertheless, other compounds with potential commercial interest are also accumulated during its growth, such as pigments (phycocyanins) or polysaccharides (PS). Among those high value products, the PS are actually less investigated but could be of first importance since the polyglucans sourced from other biological model (macroalgae or bacteria for example [3, 4]) are exploited by industry as hydrocolloids (gelling, thickening, emulsifying agents) or biological agents (substitutes for glycosaminoglycans, elicitors, antioxidants, etc) [5, 6].

Photosynthetic microorganisms in general and cyanobacteria in particular are able to produce large chemodiversity of PS, playing different roles within the cells. As for higher plants, cellulose, hemicelluloses such as xylan polysaccharides, or pectins, are the main components of cell walls, but a great diversity exists [7]. They also accumulate polysaccharides as storage molecules, especially under non-optimal cultivation conditions, such as starch for eukaryotic microalgae and glycogen for cyanobacteria. But some photosynthetic microorganisms also produce interesting exocellular polysaccharides (EPS), along with growth or under specific conditions that could be, depending on specie, associated with nutrient depletion, high salinity, high light fluxes and so on [8-12]. In those environmental conditions, EPS are supposed to provide protection against

various biotic and abiotic stresses or to facilitate the attachment of the microorganism to a solid substrate [12-15].

These EPS have gained interest due to a very large variety of chemical structures and, as a consequence, a great variety of physico-chemical and potential original biological properties [8]. However, the data available in the literature are somehow confusing because a great heterogeneity exists. Depending on the photosynthetic microorganisms, the polymer can be totally excreted into the surrounding medium or remained more or less tightly bound to the cells. Depending on the studies, the “free” polymers can be found under the denomination of exopolysaccharide (EPS), mucilage, or released exopolysaccharide (RPS). Concerning the bound polymers, they are often called capsular polysaccharides (CPS) or bound polysaccharides (BPS). For the specific case of the cyanobacteria, it is generally admitted that the exocellular PS are mainly constituted of CPS, with only a small amount found as RPS [8, 12]. Nevertheless, this aspect is controversial, as some species does not produce CPS at all, whereas for others, RPS amounts exceeded those of CPS [16]. In the case of CPS, this implies that a chemical or physical treatment is needed to extract them, that may finally induce differences in the quality of the extracted polymer and therefore differences in the results of the compositional analyses, due to methodological biases.

So, when investigating the influence of the culture conditions on the PS abundance or composition (in order to assess for example new insights on the metabolic plasticity of cyanobacteria), it is extremely difficult to compare previous investigations from one study to another since a large heterogeneity of definitions and protocols exists in the literature. Concerning the specific case of *Arthrospira platensis*, no clear consensus arises from the published data about PS (including EPS)

optimized production conditions or characterization. Our statement hypothesis was that the **culture conditions** strongly influence **the** PS accumulation and more particularly **the** EPS composition from *Arthrospira platensis*.

In the present work, it is proposed to bring highlights on that specific topic, and to investigate experimentally the effect of the most influencing **culture parameter** on the PS production by the cyanobacteria *Arthrospira platensis*. **For that purpose, the results coming from a bibliographic meta-analysis will be first interpreted. Specific focus will be on the influence of the culture parameters on the PS amounts, expressed as total sugar, glycogen and EPS content in the biomass.** Data on EPS composition such as uronic acids, sulphate content and monosaccharide composition will be also considered. Once the most influencing parameter will be identified (defined as the one who induces significant variability in PS abundance **and/or** EPS composition), it will be experimentally investigated in photobioreactors and the produced PS analyzed.

2 Material and methods

2.1 Standards and reagents

Solvents and standards were purchased from Sigma-Aldrich (l'Isle d'Abeau, France), Acros Organics (Noisy le Grand, France) or Prolabo (Fontenay sous bois, France).

2.2 *Arthrospira platensis* biomass production

The cyanobacterium *Arthrospira platensis* PCC 8005 (Institut Pasteur, Paris, France) was axenically grown in a modified Zarrouk medium [17].

The cultures (3 independent cultures for each condition) were conducted in the batch mode in a torus-shaped photobioreactor illuminated on one side as described by [18] so that the light transfer attenuation occurred only along the culture depth. Its working volume was 1.3

L with a reactor thickness $L = 0.04$ m. The PBR was exposed to constant illumination with incident Photons Flux Density (PFDs) of 100, 400, 800 or 1200 $\mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ delivered by a panel of light-emitting diodes (LEDs) placed at the surface of the PBR and determined using multi-point measurements with a cosine quantum sensor (LI-190SA, LI-COR®). pH and temperature were monitored and controlled during the experiment (Mettler Toledo® Inpro 3253SG/120/Pt probe). Temperature was set at $35 \pm 1^\circ\text{C}$ and pH was initially set at 9.5 by modifying Zarrouk medium as [19] with 10.5 g L^{-1} NaHCO_3 and 7.6 g L^{-1} Na_2CO_3 and maintained at 9.5 ± 0.1 during the experiment by setting bubbling CO_2 (99.7%, Air liquide®) into the culture at a flow rate of 11 mL min^{-1} . An additional N_2 (99%, Air Liquide®) bubbling was performed continuously at a flow rate of 10 mL min^{-1} . The CO_2 and N_2 gas flow rates were controlled and regulated by mass flow meters (respectively, EL-FLOW F-201CV-020-RAD-33-Z and EL-FLOW F-201C-RAD-33-V, Bronkhorst®). Stirring speed was set to 300 rpm. Each culture was inoculated to reach an initial biomass concentration between 0.02 and 0.15 g L^{-1} in the modified Zarrouk medium.

Growth was followed during cultures using dry weight measurements, by filtering a constant volume V of culture on pre-dried (110°C for 24h) and pre-weighed (Mettler Toledo, AB204) $0.7 \mu\text{m}$ glass fiber filter (VWR or Fisherbrand). Cells were rinsed with de-ionized water before drying at 110°C for 24h. The filter weight difference for a given volume allows estimation of biomass concentrations expressed in g L^{-1} . Dry weight concentration was measured every day for 100 and 400 $\mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ batch cultures and twice a day for 800 and 1200 $\mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ batch cultures.

During growth, nitrate concentration of the culture medium was determined by ionic chromatography from the culture supernatant obtained after centrifugation (12100 g , 30 min, 20°C , Minispin® Eppendorf). NO_3^- anions were analyzed by a Dionex ICS 900 chromatograph (Thermo Scientific) equipped with an AG9-HC

(4x50mm) column and a AS9-HC (4X250mm) pre-column. Nitrate concentration was measured every day for 100 and 400 $\mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ cultures and twice a day for 800 and 1200 $\mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ cultures. The eluate was a sodium carbonate solution (0.77 mM Na_2CO_3 and 0.13 mM NaHCO_3) and was set at a flow rate of 0.8 mL min^{-1} . The eluent for the AMMS@3000 4 mm chemical suppressor is a 25 mM sulfuric acid solution at a flow rate of 1.82 mL min^{-1} .

All analyses during cultures (both dry weight measurements and nitrates quantification) were performed in triplicates.

Total biomass productivity (r_X , expressed in $\text{g}_X \text{L}^{-1} \text{h}^{-1}$ or $\text{kg}_X \text{m}^{-3} \text{s}^{-1}$) was determined for the four investigated operational conditions. It was assessed from linear regression during the observed linear growth phase once the physical limitation by light transfer is achieved. Total biomass productivities were compared to the theoretical value given by the following relationship [20] :

$$r_{X,\text{max}} = \rho_M \times \bar{\phi} \times K \times \frac{2 \times \alpha}{1 + \alpha} \times \frac{1}{L} * \ln\left(1 + \frac{q_0}{K}\right) \quad (1)$$

with :

$$\alpha = \sqrt{\frac{Ea}{Ea + 2 \times b \times Es}} \quad (2)$$

with the maximum energetic yield for photon conversion $\rho_M = 0.80 \pm 0.02$; the mass quantum yield for biomass formation $\bar{\phi} = (1.85 \pm 0.10) \cdot 10^{-9} \text{ kg}_X \mu\text{mol}_{\text{hv}}^{-1}$; the half saturation constant for light harvesting $K = 90 \pm 5 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$; the reactor thickness $L = 0.04 \text{ m}$; the incident photon flux density (PFD) q_0 ($\mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$); the mass absorption coefficient $Ea = 162 \pm 8 \text{ m}^2 \text{ kg}^{-1}$; the mass scattering coefficient $Es = 640 \pm 30 \text{ m}^2 \text{ kg}^{-1}$; the back-scattered fraction for radiation $b = 0.03 \cdot 10^{-3}$ [20].

The volumetric photon absorption rate (r_{hv} , expressed in $\text{mol}_{\text{hv}} \text{m}^{-3} \text{h}^{-1}$) can thus be determined from the measured experimental biomass productivities as follows [20]:

$$r_{hv} = \frac{r_x}{\phi} \quad (3)$$

2.3 EPS purification

Once the culture reached concentrations between 1 and 3 g L^{-1} , cells were harvested during the linear growth phase by centrifugation (5000 g, 30 min, 4°C in a Sorvall® RC-6 Plus Centrifuge) and were washed successively with 0.90% NaCl solution, 0.45% NaCl solution and de-ionized water (milli-Q® Millipore). These successive decreasing NaCl concentrations solutions allowed washing the cells with progressive osmotic modifications, preventing cellular lysis. The supernatants containing RPS (initial and from the washing) were collected and pooled for further purification. CPS extraction from cells was conducted according to a protocol derived from Filali-Mouhim et al., [21]. Briefly, biomass pellet was resuspended in buffer (pH 9.5) to reach a biomass dry weight concentration between 2.4 and 3.0 g L^{-1} . This buffer that limits cell lysis during the subsequent step, consisted in TAPS (0.05 M), EDTA (0.025 M) and NaCl (0.025 M). The resulting suspension was heated at 100°C for 1 hour and a second centrifugation was performed (17000 g, 30 min, 20°C) to recover the cell-free polymer in the supernatant. The cells were re-suspended in the buffer for a second extraction. The resulting supernatants of the two consecutive extractions were precipitated by addition of 10 volumes of 3% cetyltrimethylammonium bromide, which was assumed to allow specific precipitation of acidic polysaccharides. After a new centrifugation step (3600 g, 10 min, 20°C, Micro R22, Hettich), pellet was resuspended in 1.0 M KCl before precipitation with 3 volumes of 96° ethanol (left overnight at -20 °C) and centrifugation (3600 g, 10 min, 20°C, Micro R22, Hettich). These latter solubilization/precipitation steps were repeated 2 times with decreasing KCl concentration solutions (0.75 and 0.3 M) in order to destabilize complex between CTAB and polysaccharide. The last precipitated polysaccharide pellet was then resuspended in pure

water (milli-Q® Millipore). These successive precipitations allowed removing most of the proteins and the other impurities, but the salts were often co-precipitated [22]. In order to achieve greater purity sample, the polysaccharide solution was ultrafiltered. Two different ultrafiltration systems were used, depending on the volumes to treat:

- 1- For large volumes (>200 mL), solutions were diafiltered using vivaflow ultrafiltration system (Sartorius) and a 50kDa NMWCO membrane in order to remove remaining salts. Diafiltration was stopped when conductivity of filtrate was found as lower than $2 \mu\text{S cm}^{-1}$. Polysaccharide solution was then concentrated (volume reduction factor of 5) before freeze-drying, or
- 2- For smaller volumes (<200 mL), solutions were ultrafiltered using Amicon filter cell through a 10 kDa MWCO polyethersulfone membrane (Biomax® Ultrafiltration discs, Millipore) and washed three times with the same volume of the initial sample of de-ionized water (milli-Q® Millipore).

The polysaccharides were further analyzed in solution or after freeze-drying.

2.4 Analytics

All analyses (both biochemical or chromatographical) were performed in triplicate.

2.4.1 Biochemical analyses of biomass and EPS samples

Total sugar content of samples was evaluated by the Dubois method [23] using glucose as standard. Uronic acids and neutral sugar contents of EPS extracts were assayed with *meta*-hydroxyldiphenyl and resorcinol as described by respectively [24] and [25] using glucose and glucuronic acid as standards. Quantification of neutral sugars was

done according to the corrective formula described by [26]. The results were expressed in mg g^{-1} of D-glucose equivalent (GlcEq) for neutral sugars and in mg g^{-1} of D-glucuronic acid equivalent (GlcAEq) for uronic acids. Sulphur content was determined by the turbidimetric method [27] using K_2SO_4 as a standard. Results were expressed in mg g^{-1} of SO_4 equivalent (SO_4Eq). Protein content was evaluated by the Bicinchoninic acid method (Bicinchoninic Acid Protein Assay Kit, BCA1, Sigma Aldrich) using BSA (Bovine Serum Albumin) as a standard. Glycogen assay was performed according to [28]. First, glycogen was extracted and quantified by an enzymatic reaction. One mL of culture sample (at a dry weight concentration between 0.8 and 2.8 g L^{-1}) was centrifuged (12100 g, 30 min, 20°C , Minispin® Eppendorf) and the pellet was suspended in methanol for 45 min at 45°C to extract chlorophylls and carotenoids. After another centrifugation (12100 g, 10 min, 20°C , Minispin® Eppendorf), the supernatant containing chlorophylls and carotenoids was discarded and the pellet containing the glycogen was re-suspended in 200 μL of a 30%_w KOH solution. Hydrolysis was performed at 100°C for 90 min. After cooling down, glycogen was precipitated by the addition of 600 μL of pre-chilled (4°C) absolute ethanol. After an hour at -20°C , samples were centrifuged (12100 g, 5 min, 20°C , Minispin® Eppendorf) and the glycogen was collected in the pellet and washed twice with ethanol using this same procedure. The pellet was finally dried at 60°C for 5-10 min to evaporate the ethanol. The dried pellet was re-suspended in an acetate buffer (100 mM, $\text{pH}=4.5$). One hundred μL of an amyloglucosidase solution (Starch Assay Reagent, S99144, Sigma-Aldrich) were added to 100 μL of the glycogen solution and the glycogen was hydrolyzed into glucose for 15 min at 60°C . Glucose was phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (Glucose Assay Reagent, G3293, Sigma-

Aldrich- 800 μL , 15 min at room temperature). Glucose-6-phosphate was then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH) (Glucose Assay Reagent, G3293, Sigma-Aldrich). During this oxidation, an equimolar amount of NAD was reduced to NADH. The consequent increase in absorbance at 340 nm was directly proportional to the glucose concentration. The total blank has been calculated according to the technical bulletin, taking into account the contribution of the absorbance of the sample, the glucose assay reagent and the starch assay reagent (Starch Assay Kit, Catalog Number SA20).

2.4.2 HPAEC-PAD analysis

Monosaccharide compositions of polysaccharides were evaluated by High Pressure Anion Exchange Chromatography (HPAEC) on an ICS 3000 (Dionex, USA) equipped with pulsed amperometric detection (PAD) and AS 50 autosampler. It was assembled with a guard CarboPac PA1-column (4×50 mm) and analytical CarboPac PA1-column (4×250 mm). Before analysis, polysaccharide samples were hydrolyzed in 2 M HCl for 3 h at 100°C and neutralized with 2M NH_4OH . Samples (1 mg mL^{-1}) were filtered using $0.2 \mu\text{m}$ membrane filter and injection volume was fixed at 25 μL . Before each injection, columns were equilibrated by running during 15 min with 18 mM NaOH. Samples were eluted isocratically with 18 mM NaOH for 25 min, followed by a linear gradient between 0 to 0.5 M sodium acetate in 200 mM NaOH for 20 min to elute acidic monosaccharides. Run was followed by 15 min washing with 200 mM NaOH. The eluent flow rate was kept constant at 1 mL min^{-1} . Columns were thermostated at 25°C . Data were collected and analyzed with Dionex Chromeleon 6.80 software (Sunnyvale, USA). Constituting monosaccharides were first identified regarding elution time as compared to standards (L-Rha, D-Rib, L-Fuc, L-Ara, D-Xyl, D-Man, D-Gal, D-Glc, D-GalN, D-GlcN, D-GalNac, D-GlcNac, D-GlcA, D-GalA), and then confirmed by the internal standard method. Quantification of monosaccharides was

then achieved by injecting several concentrations of standard monosaccharides and plotting response area as function of concentration.

2.5 Bibliographic meta-data organization and treatment

All the total sugar, glycogen and EPS contents of *A. platensis* biomass available in the literature were gathered in the Table A1 (appendix) and depicted in Figure 1. These values were gathered in different categories depending on the growth conditions.

To evaluate the effect of the temperature, the data were classified in two categories. The first category named LT (for low temperature) included data obtained from biomass grown between 20 and 30 °C, while the second category named HT (for high temperature) included data obtained from biomass grown between 32 and 38°C. The temperature was not mentioned for some studies: [29-33].

To evaluate the effect of light intensity, the data were classified in two categories according to the light conditions even though these conditions were not directly comparable because of the different photobioreactor geometries. However, in those particular cases, the categories were formed according to the incident light intensity. The first category named « LL » (for lower light intensities) included data obtained from biomass grown under light intensities between 100 and 200 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$. The second category named HL (for high light intensities) included data obtained from biomass grown between 200 and 360 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$. The light intensity was not mentioned for some studies: [29-32] but were considered to be in the low light category. The light intensity for the data from [34] was estimated to be approximately equal to 250 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ according to the growth curve.

All the cultures in the selected studies were conducted in PBR operated under batch conditions, therefore the limitation was achieved by reducing the initial nitrate concentration in the Zarrouk medium. In the standard Zarrouk medium, the initial nitrate concentration is 1.8 g L⁻¹, this category was named « +N ». In the case of the limitations, the initial nitrate

concentration was between 0.28 and less than 0.6 g L^{-1} . This category was named « -N ». The nitrate concentration or the culture medium was not mentioned for several studies: [29-31].

The category “High Salinity” included results obtained for the biomass grown in media containing from 0.25 to 1 M NaCl added in the Zarrouk medium [35, 36] and for medium consisting of a mix of seawater and deionized water with addition of 2.5 g L^{-1} NaNO_3 , 0.01 g L^{-1} $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.08 g L^{-1} Na_2EDTA [37].

The category “Heterotrophy” included results obtained for the biomass grown in the Zarrouk medium with addition of 0.5, 1.5, and 2.5 g L^{-1} glucose [38]. Other parameters, such as light cycle (continuous or day/night cycle), the age of the culture were not taken into account because there were mentioned in too few studies.

All the monosaccharide composition of *A. platensis* EPS available in the literature were gathered in the Table A2 (appendix) where all the values were expressed in mass percent of the purified PS. Most of the values corresponded to the published values as they were expressed in mass percent fraction. Some EPS compositions were expressed in molar fraction, they were converted in mass percent taking into account the fraction values given in mass percent (for example the sulphated sugars content was expressed in mass fraction in [34] and [30], sulphated and acidic sugars were expressed in mass fraction in [21] so that the sum of sulphated, neutral monosaccharides and acidic monosaccharides was equal to 100%. When the mass fraction was not mentioned, it was supposed to be not measured. For example, the composition given by [29, 35, 39] only took into account the neutral monosaccharides but no sulphated or acidic sugars were mentioned. The composition given by [29-31 34-35, 39-40] only took into account neutral and acidic monosaccharides. Filali Mouhim et al., [21], Lee et al., [31], Shekharam et al., [33] quantified the acidic sugars but

as the amount of each acidic sugar was not mentioned, it was assumed to be half glucuronic acid and half galacturonic acid.

Depending on the culture conditions corresponding to each EPS compositions, several groups were formed. These values were gathered in different categories depending on growth conditions. The definitions of “LT”, “HT”, “LL”, “HL”, “-N” and “+N” definitions are the same as the ones used previously. Additionally, the nature of EPS (RPS or CPS), and the purification mode (P1 and P2), were added.

The EPS compositions published by Depraetere et al. [41], Majdoub et al. [32] and Trabelsi et al. [42] were classified in the « RPS » category because they were harvested in the medium culture and separated from the cells by filtration. The EPS compositions classified in the « CPS » category corresponded to the PS obtained by extraction step involving heating/boiling in water [29-31, 33-35, 39] or in a low ionic strength solution [21]. After extraction the EPS were separated from other molecules by precipitation with CTAB and/or ethanol. The EPS compositions obtained with this type of protocols were named « P1 ». In some studies, the purification steps were more specific as they involved gel separation or ion exchange chromatography and TCA treatment [30-32] leading to highly purified polysaccharide fraction compared to PS obtained by CTAB and/or ethanol precipitation. This second types of purifications were gathered in the category named « P2 ».

2.6 Calculations and graphic plotting

The statistical analysis, data fitting and graphic plots were performed using R 3.2.1 and SigmaPlot 12.5 softwares. Experimental values corresponded to the average values of 3 technical repeats per n=3 biological independent replicates. Standard deviations were calculated for these values. For the meta-bibliographic analysis,

between n=7 and n=50 values were used, depending on the analysed available data.

3 Results and discussion

3.1 Bibliographic meta-analysis of the PS of *Arthrospira platensis*

3.1.1 *Arthrospira platensis* PS content and distribution variability

The first quantitative data published on the EPS of *Arthrospira platensis* dated back to the beginning of the 1990's [19, 21]. Since then, many works have been carried out on this model cyanobacterium, allowing estimating by different methodological approaches, the PS amount synthesized for varied culture conditions. Nevertheless, due to differences in extraction protocols (P1 or P2) as well as in analytical methods, it is extremely difficult to compare previous investigations from one study to another and to clearly demonstrate the influence of the culture conditions on the amount and the composition of the PS. The first part of this study was focused on the meta-analysis of the bibliographic data associated with the global sugar composition found in *Arthrospira platensis* biomass (total sugars, glycogen and EPS). 28 publications were analyzed, with 25 quantification data available for the total sugars, 16 for the glycogen and 50 for the EPS fraction contents. The detailed data and references are listed in the Table A1 (appendix). The compiled data indicated that the polysaccharides (EPS + glycogen) amount was very variable. The distributions summarized in Figure 1 suggest that the median concentrations in EPS were globally low (13 %DW) compared to that in total sugars (35 %DW) but of the same order of magnitude as the storage sugars content (18 %DW). These data have also been analyzed in terms of variability.

However, all information sources taken together, relative standard deviation (RSD ~ [52%-70%], Table 1) **were** quite high, suggesting that a great range of PS content was found according to the tested **culture conditions**. The Figure 1 illustrates also the PS data variability **whenever** the operating conditions were considered (modulation of the temperature, the nitrate content, the light irradiance, the salinity or the use of glucose as carbon source).

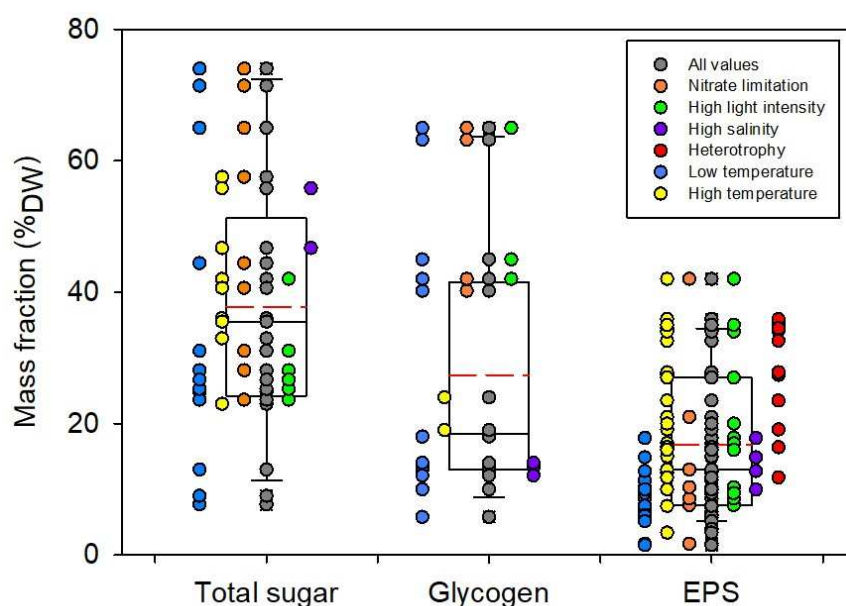


Figure 1. PS variability according to **the** operating conditions such as high temperature, nitrate limitation, high light irradiance, addition of NaCl or the use of glucose as carbon source. **The data** were extracted from 28 publications (each publication **could** contain several values depending on the tested conditions). All conditions **were** grouped together, n=24 for total sugars, n=15 for glycogen, and n=50 for EPS. **The box** plots **included the** medians (black lines) and **the** means (red dashed lines). **The grey points correspond to the whole set of data,**

whenever the operating condition tested. The other colours highlight the data for specific culture conditions.

The data associated with the effect of the operating conditions were also specifically extracted in order to assess the effect of each parameter compared to the reference condition. The median values and RSD were calculated for each operating condition and compared to the median calculated for the whole data set (Table 1).

Table 1. Median values and RSD (%) calculated for each culture parameter. The data were extracted from 28 publications (each publication could contain several values depending on the tested conditions). All conditions were grouped together, n=24 for total sugars, n=15 for glycogen, and n=50 for EPS.

Median mass fraction in % DW [min-max] (RSD %)	Total sugar	Glycogen	EPS
All conditions	35 [7.7-74] (52%)	18 [5.8-65] (70%)	13 [1.5-42] (65%)
High temperature	41 [23-58] (27%)	21 [19-24] (16%)	23 [3.4-42] (45%)
Low temperature	27 [7.7-74] (65%)	16 [5.8-65] (72%)	8.1 [1.5-17.8] (50%)
Nitrate limitation	44 [24-74] (37.5%)	42 [40-65] (26%)	10 [2-42] (89%)
High salinity	51 [47-56] (13%)	13 [12-14] (7%)	14 [10-18] (24%)
Heterotrophy	-	-	28 [12-36] (31%)
High light intensities	27 [23.6-42] (23%)	45 [42-65] (25%)	17 [7-42] (57%)

For the high temperature condition, the results suggested that the PS %DW were greater than the global median composition whatever the fraction was studied. Moreover, the RSD was found lower, suggesting a lower variability in the results for this operating condition. At the opposite, for the low temperature condition, it appeared to decrease each fraction amount, and especially the EPS one (8.1 %DW). Nevertheless, the variability observed remained very high. In fact, the

minimal and maximal values obtained for the total sugars and the glycogen, when all conditions were analysed, corresponded to this low temperature condition. It is thus difficult to conclude on the impact of the temperature on the PS accumulation, as it appeared to be highly variable depending on studies.

Concerning the effect of the nitrate limitation, it appeared to **induce** specifically an increase of the glycogen fraction, counterbalanced by a decrease of the EPS fraction in a proportional manner. Nevertheless, this observation **must** be taken with care. If the increase in glycogen content seemed clear (from 18 %DW all conditions grouped, to 42 %DW in case of nitrate limitation, with RSD reduced to 26 % instead of 70 %), the results of the EPS contents remained very variable (between 2 to 42 %DW depending on studies) with RSD about 89 %. The effect of the salinity did not provoke an increase of those PS, i.e. glycogen or EPS, but **an increase in total sugars was noticed. It has been highlighted by some authors, that a high salinity medium induced accumulation of low molecular weight (LMW) sugars that can act as osmoprotectants for the cells [43]. For the specific case of *Arthrospira*, this has been identified as a nine-carbon sugar named glucosyl-glycerol [44]. It seems then reasonable to propose that the increase in total sugars amount observed could be associated to these LMW sugars (not measured in those literature data).**

Concerning the data extracted for the assessment of the heterotrophy effect, only partial suggestions could be proposed. It seemed to provoke a significant increase of the EPS fraction (28 %DW), but as no information was available concerning the %DW of the glycogen fraction or of the total sugar, it seems difficult to assess if the observed effect could be considered as being specific of the EPS

fraction and to which extend it could be. Complementary investigations are clearly needed to try to elucidate that specific point. Concerning the effect of the light intensity modulation, it appeared very interesting since, when compared to the nitrate limitation, it seemed to increase not only the glycogen fraction (45 %DW), but also the EPS fraction (17 %DW). No counterbalancing effect involving the two fractions could be observed here, as measured for the nitrate effects.

3.1.2 The incident light as the most influencing culture parameter

A closer inspection of the effects of light confirmed its significant influence on the PS abundance and composition, differentiating the EPS fraction from the glycogen fraction. The Figure 2 illustrates the influence of the increasing light intensity on the EPS and the glycogen abundance. These results showed a positive correlation of the glycogen content with the increasing light intensity, reaching 42 %DW for $250 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ [45]. Nevertheless, further increase up to $700 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ did not induce higher accumulation, as the glycogen content was evaluated to be around 45 %DW [46]. For the EPS content, even if a variability was observed for low incident light fluxes ($< 200 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$), values remained below 15 %DW, except in one study. The high value of 34 %DW obtained in [47] at $180 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ could probably be explained by the specific culture conditions. In this study, the culture was conducted at 40°C and the PS were recovered in culture medium. This high temperature could have induced a partial cellular death and lysis,

and no correction of the potential released glycogen has been performed. Thus, an overestimation of the EPS amount could have been encountered. For a greater incident light flux ($> 200 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$), the EPS content progressively increased up to 35 %DW.

Thus, the glycogen content appeared to increase preferentially at lower flux intensities compared to the EPS fraction, for which an almost linear increase was observed. This finding was of first interest since light intensity appeared here to be an important parameter for the modulation of both the glycogen and the EPS amounts.

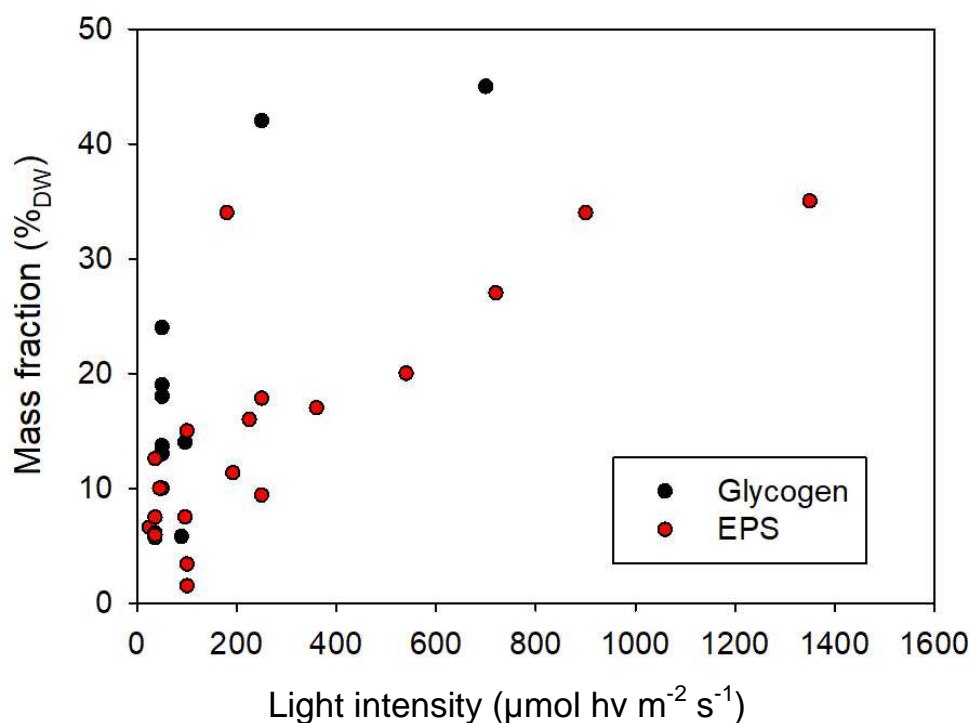


Figure 2. Influence of the incident light intensity on the mass fraction of glycogen and EPS (%). The data were extracted from 19 publications, $n=19$ for the EPS and $n=10$ for the glycogen.

According to the previous data, the PS content seemed to be very variable, depending on the operating conditions. The very specific case

of the light intensity appeared to be interesting to investigate regarding its differential effect on the EPS and the glycogen accumulation. In order to verify if the changes observed on the total concentration of the different fractions did also affect the monosaccharides composition of the EPS, a more specific analysis of the data available in the literature was performed. The Table A2 (appendix) summarized the data obtained after extraction and formatting of the EPS compositional data from the literature (11 studies).

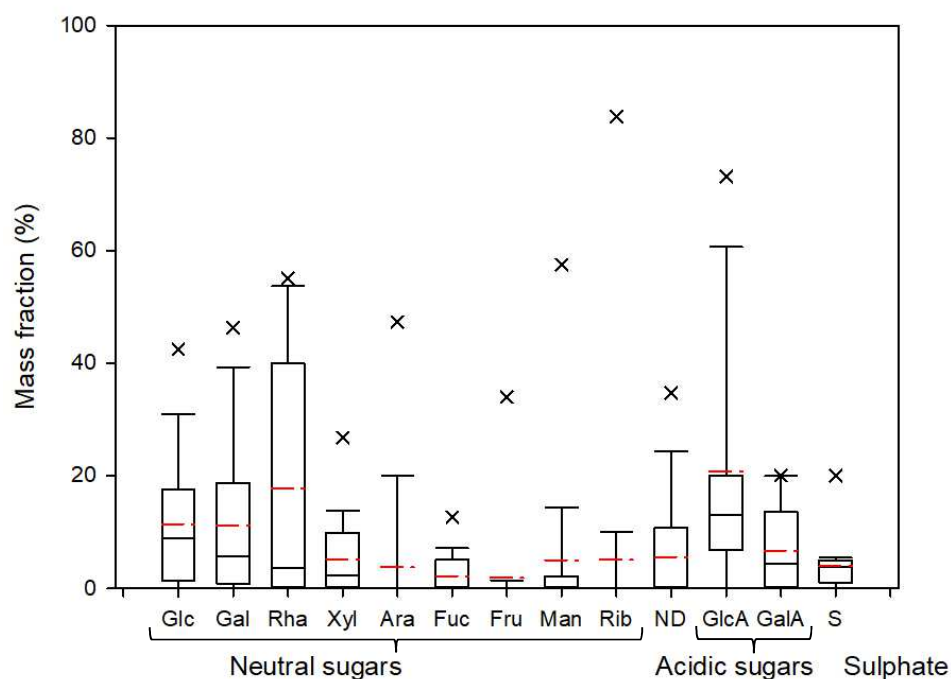


Figure 3. Variability of the monosaccharides composition in the EPS fraction. Nineteen compositions were analysed, coming from 11 publications (each publication can contain several values depending on the culture conditions tested). Glucose (Glc) was found in 17 of the analyzed EPS, galactose (Gal) in 15, rhamnose (Rha) in 14, xylose (Xyl) in 12, arabinose (Ara) in 4, fucose (Fuc) in 6, fructose (Fru) in 2, mannose

(Man) in 9, ribose (Rib) in 3, glucuronic acid (GlcA) in 17, galacturonic acid (GalA) in 11, and sulphate groups (S) in 15. The box plots include the medians (black lines) and the means (red dashed lines). The crosses indicate the highest value encountered for each condition.

When regarding the available EPS compositional data summarized in the Figure 3, a great variability could be observed, with the neutral sugars content ranging from 21 to 100 % and the uronic acids from 0 to 73 %. Depending on the studies, the main monosaccharides found were not identical. As an example, the studies [29-33, 39, 41] have found rhamnose as the main monosaccharide (40 %DW or more), whereas it was not detected or to a very small amount in other studies. Depending on the studies, the main monosaccharides were identified as glucuronic acid and galacturonic acid, glucose, galactose, rhamnose, or xylose, whereas the minor sugars were fucose, arabinose, mannose, fructose, or ribose. This diversity of composition confirmed one of the main characteristics observed in the cyanobacterial EPS, i.e. they often contain at least six monosaccharides. This feature makes them quite different from bacterial EPS that generally account for less than four [12]. These compositions were analyzed in term of variability and whatever the considered methodological approaches (operating or analytical conditions).

Table 2. Median, mean and RSD values calculated for each monosaccharide entering in the EPS composition. Nineteen compositions were analysed, coming from 11 publications (each publication can contain several values depending on the culture conditions tested).

Glucose (Glc) was found in 17 analyzed EPS, galactose (Gal) in 15, rhamnose (Rha) in 14, xylose (Xyl) in 12, arabinose (Ara) in 4, fucose (Fuc) in 6, fructose (Fru) in 2, mannose (Man) in 9, ribose (Rib) in 3, glucuronic acid (GlcA) in 17, galacturonic acid (GalA) in 11, and sulphate groups (S) in 15.

	Glc	Gal	Rha	Xyl	Ara	Fuc	Fru	Man	Rib	ND	GlcA	GalA	S
Median	8.8	5.6	3.6	2.2	0	0	0	0	0	0	13.0	4.3	3.7
Mean	11.4	11.2	17.7	5.1	3.7	2.2	1.9	5.0	5.0	5.5	20.7	6.7	4.0
RSD (%)	102	120	120	135	307	167	418	266	382	180	106	120	110

The obtained results (Table 2) indicated a RSD value close to 140 % for the whole set of measurements made on these sugars. It allowed to determinate the main sugars (galacturonic acid, xylose, rhamnose, galactose, glucuronic acid and glucose) with a variability lower than 140 %, from the minor sugars (fucose, mannose, arabinose, ribose or fructose) for which variabilities may exceed 350 %. This very high variability could be imputed to the culture conditions, the methods of extraction, but also on the analytical method used. In fact, a large diversity of methods can be used for the compositional determination, each of them presenting advantages and drawbacks. In the available studies, many compositional analyses have been performed using gas chromatography (GC). This method has the advantage to be of high resolution and sensitivity [48], but its main drawback consists in needing a mandatory derivatization step of the sugars prior to their analysis. This derivatization step has an impact on the compositional analysis, as all monosaccharides does not present the same reactivity to the treatment, with some sugars that could be underestimated

(especially uronic acids) due to a lower threshold detection [49]. Other methods (HPLC-RID and HPAEC-PAD) present on the contrary the advantage of needing no modification of the samples, but with greater uncertainties regarding the identification when they are not conducted appropriately (shift of retention time for example). Among these 2 liquid chromatographic methods, HPAEC-PAD presents much greater resolution than HPLC, resulting in a better separation of the monosaccharides. Nevertheless, for an optimal identification and quantification, the calibration should be performed for each analysis sequence, and an internal standard should be used to confirm the identification (analysis of sample supplemented with the suspected monosaccharide and comparison with the native sample). Avoiding the systematic usage of an internal standard and a periodic calibration, may therefore provoke strong errors in the identification/quantification. Whatever the method is chosen, the glycosidic linkages should be broken to release the constitutive monosaccharides prior to their analysis. This step is classically performed by acidic hydrolysis, but the choice of the acid, the temperature and the duration have a strong impact on quantification results. A mild hydrolysis condition could lead to an incomplete release of monosaccharides and then to errors in quantification. As an example, when using HPAEC-PAD, disaccharides are often eluted for a retention time close to that of the fructose and can lead to errors in peaks attribution if no internal standards are used. For strong acid hydrolysis conditions, a degradation of the monosaccharides can occur, leading to the underestimation of some monosaccharides [50]. In

the data analyzed for this study, these kinds of errors could have occurred, that could explain a part of the observed variability, more especially for the rare detection of some monosaccharides (such as ribose or fructose which were described only in very few studies). Nevertheless, it has been chosen not to exclude any data and to consider all the available studies.

The comparison of the compositions showed that among the neutral sugars, significant differences appeared between the two types of purifications identified as P1 and P2. The works of Hayashi et al. [30], Lee et al. [31], and Majdoub et al. [32] have enabled to isolate and characterize a polysaccharide fraction employing a protocol of type P2, i.e. steric exclusion or ion exchange chromatography. The EPS isolated was predominantly composed of rhamnose, fructose, xylose and their methylated derivatives, but accounted for only 0.6 % of the biomass after extraction, purification and fractionation. It was therefore not unreasonable to think that the differences observed between P1 and P2 could be explained by the fact that the purified EPS by P2 would not be truly representative of the total EPS, but to a sub-fraction of those EPS.

Indeed, on the figure 4, the newly grouped data, according to the two light intensities ranges (low and high), suggested that an effect could be monitored for those specific lighting conditions. When increasing the light, the total neutral sugars amount in the EPS seemed to be significantly down modulated (from 83.6 %DW to 55.0 %DW, p value = 0.022), whereas the total acidic content seemed to be up modulated (from 13.2 %DW to 40.0 %DW, p value = 0.010), with a sulphate content appearing to be unchanged (3.2 - 4.9 %DW, p value = 0.367). This

observation was really of first importance and was never described before for **such operating condition**. It was then proposed that a modulation of the **composition** of the EPS could be assessed through the simple **change in** the incident light **intensity**. **In** order to confirm this assumption, it was decided to assess quantitatively and precisely the effect of that parameter by conducting experimental cultures at four irradiances (100, 400, 800, and 1200 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$) and to analyze the polysaccharides repartition and fine composition.

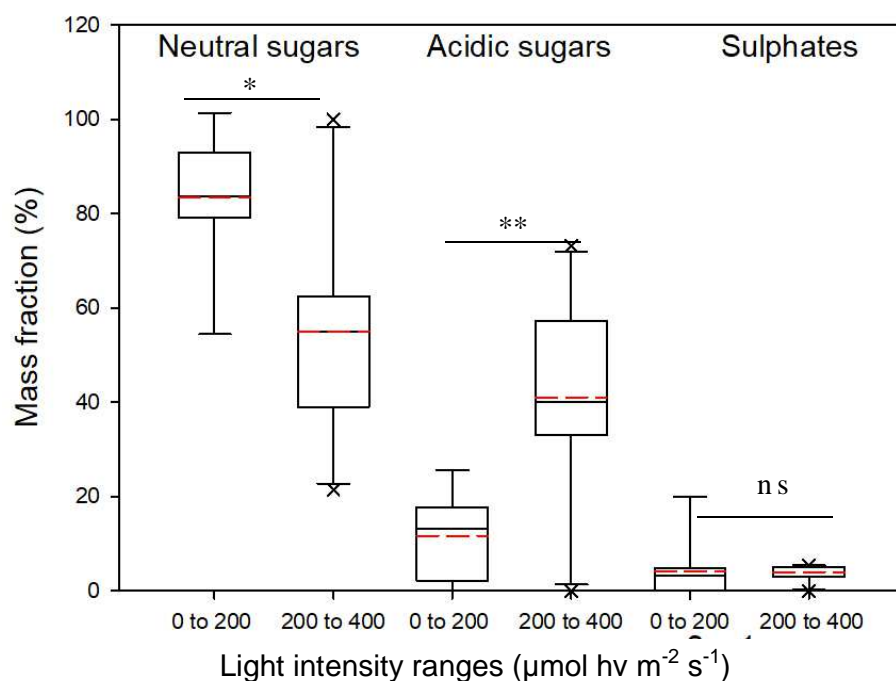


Figure 4. Influence of the light intensity on the EPS composition expressed in neutral, acidic sugars and sulphate content. **The data** were segmented into two groups, **the** high light ($>200 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$) and **the** low light ($<200 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$) **group**. **The data** were extracted from 10 publications (each publication can contain several values depending on the **other culture conditions** tested). For each component (neutral sugars, acidic sugars or sulphates) $n=7$ for low light and $n=10$ for high

light. The box plots include the medians (black lines) and the means (red dashed lines). The significance tests have been performed using the Tukey approach between the considered groups. **: p-value ≤ 0.01 , *: p-value ≤ 0.05 , ns: p-value > 0.05 .

3.2 Experimental validation of the effect of the light on the PS accumulation and composition

In order to verify if the incident light fluxes intensities could be associated to a modulation of the PS abundance and composition, it was decided to conduct comparable cultivations of *Arthrospira platensis* in highly controlled PBR. The incident light flux parameter was modulated in the same ranges as those observed in the meta-bibliographic analysis and extended to extreme values in order to assess the metabolic limits of *Arthrospira platensis* regarding the PS fractions biosynthesis, i.e. the total sugars, the glycogen and the EPS.

3.2.1 Kinetic analysis in PBR

In order to check the robustness of the cultures grown in our PBR cultivation system before analysis, the measured growth rates were first compared to the maximal theoretical values calculated from the Cornet & Dussap formula [20].

The observed biomass productivities as a function of the incident light intensity are reported in the Table 3 and compared to the expected theoretical values. Globally the biomass growth rate increased with the incident PFD, and the experimental vs. theoretically calculated values were in rather good agreement with deviations from the model inferior to 3% for the best results.

Table 3. Biomass productivities (theoretical and experimental) and volumetric photon absorption rate as a function of the incident light fluxes. n=3 independent cultures per condition.

PFD ($\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$)	Biomass theoretical productivity ($\text{g L}^{-1}\text{h}^{-1}$) [20]	Measured biomass productivity r_{XT} ($\text{g L}^{-1}\text{h}^{-1}$)	Photon absorption rate ($\text{mol}_{\text{hv}}\text{m}^{-3}\text{h}^{-1}$)
100	$8.9 \cdot 10^{-3}$	$6.8 \cdot 10^{-3}$	3.7
400	$2.0 \cdot 10^{-2}$	$1.8 \cdot 10^{-2}$	9.7
800	$2.7 \cdot 10^{-2}$	$2.9 \cdot 10^{-2}$	15.5
1200	$3.2 \cdot 10^{-2}$	$3.1 \cdot 10^{-2}$	17.6

These results showed that **the** kinetic behaviour of *A. platensis* was perfectly and rigorously controlled in the PBRs in the light of the previous studies. Moreover, it validated that the data could be considered as being comparable and reproducible to each other since no deviation from the expected behaviour was detected, which was mandatory for the pursuit of the present study. Thus, it allowed sampling the biomass at similar concentrations during their linear growth i.e. for a comparable physiological **state, and** to assess the PS biochemical characterization. For each incident light flux, **the** volumetric photon absorption rates ($\text{mol}_{\text{hv}}\text{m}^{-3}\text{h}^{-1}$) have been calculated and reported in **the** Table 3, showing also an increased value with the PFD. This increase was found to be linear from 100 to 800 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$, while for greater light intensity, an attenuation was observed, with **the** photon absorption rate about 17.6 $\text{mol}_{\text{hv}}\text{m}^{-3}\text{h}^{-1}$ at 1200 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$

while $15.5 \text{ mol}_{\text{hv}} \text{m}^{-3} \text{h}^{-1}$ was reached at $800 \text{ } \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$. The difference between the photon absorption rates at 800 and $1200 \text{ } \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ seemed then quite low and it appeared reasonable to evaluate the light intensity effect within three main PFD ranges, i.e. $< 200 \text{ } \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$, in the range of $200\text{-}400 \text{ } \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$, and $> 400 \text{ } \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$.

3.2.2 Biochemical evolution of the PS fraction for increasing incident light fluxes

As previously discussed, increasing the light seemed to provoke the accumulation of the total PS content, with a modulation of the abundance of the glycogen and the EPS fractions. The global composition of the EPS fraction seemed to be also impacted by this cultivation parameter, with specific modulation of the neutral sugar and acidic sugars. The Figure 5 summarized the results coming from the biochemical analyses of the PS fractions performed on the biomass sampled from the cultivations under increasing light intensities.

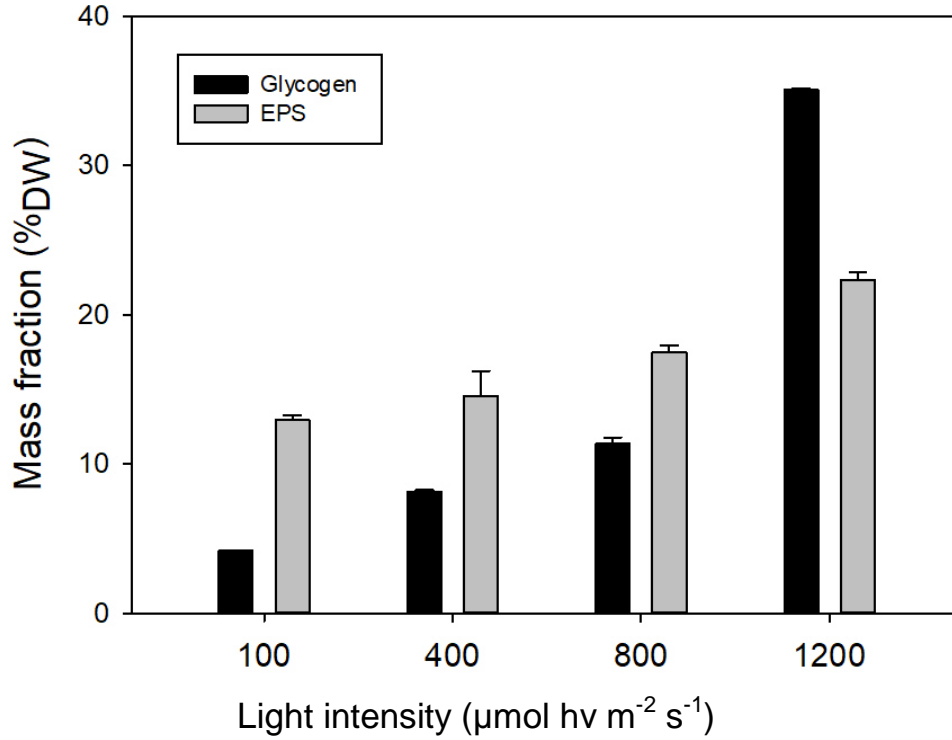


Figure 5. Changes in the glycogen and EPS mass fraction according to the increasing light. Each value corresponds to the average of 3 technical repeats per biological replicates. The number of biological replicates was equal to 3. The error bars correspond to standard deviations.

At a first glance a strong increase of the incident light intensity seemed to induce an exponential accumulation of the glycogen, associated to a linear increase of the EPS fraction. It globally confirmed the tendency observed in the meta-bibliographic analysis (Figure 2), except for the high increase in glycogen content observed for $1200 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$. In the bibliographic analysis, the glycogen amounts appeared to reach a plateau while increasing the light flux up to $700 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$, however no data was available for greater

irradiance. It also suggested that lower light fluxes should be propitious to higher EPS relative accumulation **than extreme fluxes**, where glycogen seemed to be mostly synthesized. To support this hypothesis, EPS/glycogen ratios have been calculated for each incident light flux, giving 3.1 for $100 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$, 1.79 for $400 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$, 1.54 for $800 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$, and 0.64 for $1200 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$. Thus, when increasing the light, the EPS to glycogen ratio seemed to decrease linearly, suggesting a redirection of **the** carbon fluxes towards the accumulation of storage sugars.

The Figure 6 illustrated the **changes in** the repartition of the EPS fractions according to the light, but expressed in term of neutral sugars, uronic acids and sulphate contents. Both bibliographic and experimental results were plotted on that latest figure.

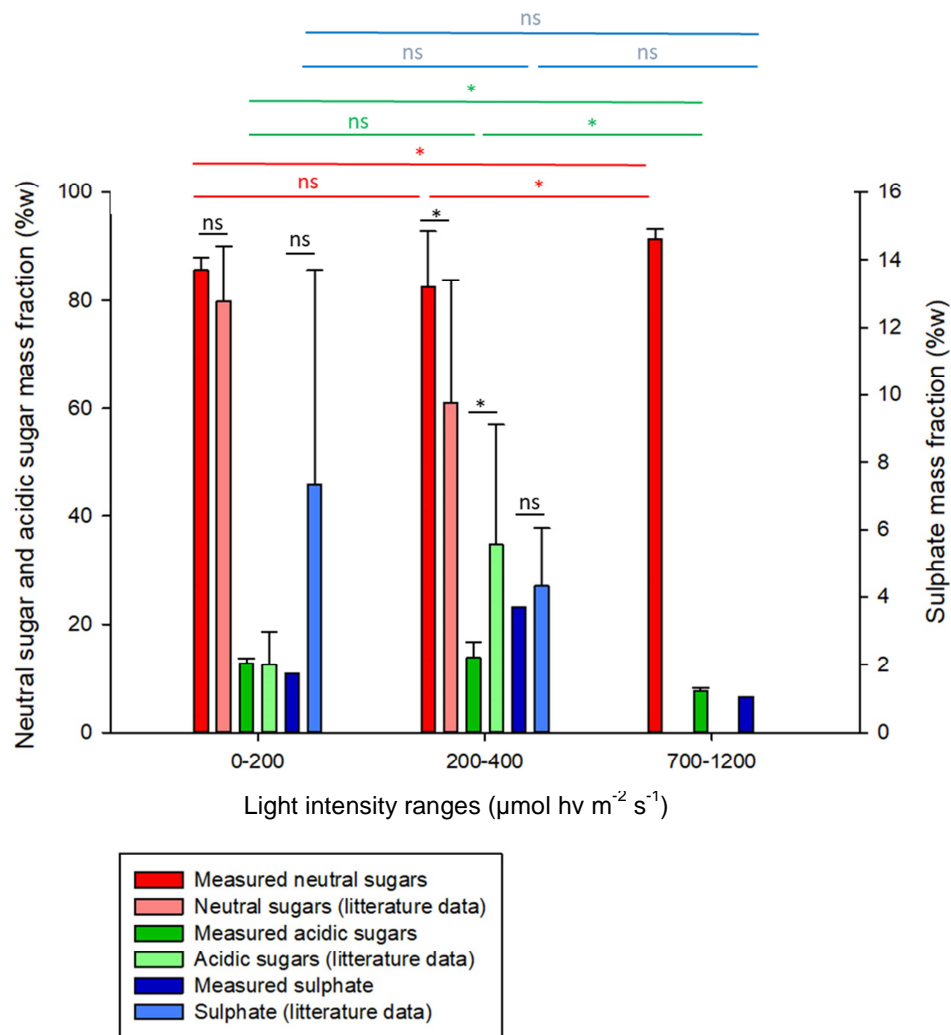


Figure 6. Changes in the glycogen and EPS mass fraction and composition (expressed in neutral, acidic sugars and sulphate content) according to the increasing light for the current experiment and the data extracted from the meta-bibliographic analysis. The bibliographic data were extracted from 10 publications (each publication can contain several values depending on the culture conditions tested). For each component (neutral sugars, acidic sugars or sulphates) $n=7$ for low light and $n=10$ for high light. For the experimental data, each value corresponded to the average of 3 technical repeats per biological replicates. The number of biological replicates was equal

to 3. The error bars correspond to standard deviations. A statistical analysis of the significance has been performed, comparing for each light intensity range, the results from the bibliographic and the experimental studies (black), as well as between the ranges of experimental results for the neutral sugars (red), the uronic acids (green) and the sulphate content (blue). **: p-value ≤ 0.01 , *: p-value ≤ 0.05 , ns: non significant, p-value > 0.05 .

When regarding more deeply the changes in EPS composition according to the increasing light intensity (Figure 6), similarities could be found with the literature data at fluxes lower than $400 \mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ and were confirmed by p-value calculations which were mainly found to be non-significant. The only noticeable difference was a lower acidic sugars content (and then greater neutral sugars amount) in our experimental results as compared to the bibliographic data for the range 200-400 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$. Indeed, when looking at the compositions experimentally obtained for the three light intensities ranges, the acidic sugars content remained quite unchanged for the 2 first ranges (no significant difference, p-value = 0.7), while increasing the light above 400 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ disfavoured the accumulation of the acidic sugars (significant differences, p-values = 0.024) to the neutral ones (significant differences, p-values = 0.024). Our hypothesis is that a metabolic shift can have occurred between 400 and 700 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$, modifying the EPS composition. For the sulphation degree, slight modifications were observed on the average values (increase in sulphate content between the 2 first ranges and then decrease while

increasing the light intensity) but it appeared to be in a restricted manner as differences were found not to be significant.

Sugars analysis indicated moreover that the EPS fine composition was also influenced by the incident light intensity. The monosaccharide composition of the EPS obtained at 100 and 800 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ were reported in the Table 4.

Table 4. Monosaccharide composition (% molar ratio) and fold changes for the EPS obtained at 100 and 800 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$. Each value corresponds to the average of 3 technical repeats per biological replicates. The number of biological replicates was equal to 3.

	Glc	Gal	Rha	Xyl	Ara	Fuc	GlcA	GalA
100 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$	43.3	1.1	14	16.8	0.6	16.5	3.3	2.9
800 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$	77.7	0.1	2.4	12.5	0	5.4	1.1	0
Fold change	+1.8	-11.0	-5.8	-1.3	-	-3.1	-3.0	-

The analysis of the EPS composition produced for 100 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ has shown that the main monosaccharide was the glucose, followed by the xylose, the fucose, the rhamnose and the uronic acids. One important modulation that was observed while increasing the incident light flux up to 800 $\mu\text{mol}_{\text{hv}}\text{m}^{-2}\text{s}^{-1}$ was the strong increase in glucose content (nearly by a factor 2), reaching 77.7 % of the EPS. The other neutral monosaccharides were found to significantly decrease, with fold changes between -1.3 to -11. The fucose was reduced by a factor of 3, while a 6-fold reduction factor was noticed for the rhamnose. For the uronic acids, the increase of the light has induced the disappearance of the galacturonic acid and the decrease in the glucuronic acid content, leading to only 1.1% of the acidic sugars in the EPS instead of 6.2 % at

lower irradiance, confirming the tendency observed by the colorimetric assays. Then, according to these results, and even if the EPS fraction was slightly increased with the incident light flux, it appeared that the composition of the EPS fraction was strongly altered.

The modulation of some monosaccharide that was observed in this study could have significant importance for the potential applications associated to these EPS. The polysaccharides are known to exhibit many biological activities. For instance, glycoaminoglycans constitutes a family of polymers with high sulphate content (except hyaluronan) that have been described as antitumor, antiviral, or antioxidant agents. Many authors have claimed that activity is directly linked to the sulphate content of the polysaccharide, as it may interact with proteins such as receptors or growth factors [51-54]. Over the last decade, the EPS from marine organisms (seaweeds, microalgae and cyanobacteria), that are frequently sulphated, have then gain interest as they may act as mimetics of glycoaminoglycans [5, 55]. However, it is still very difficult to rely a structure and a biological activity. For instance, the level of sulphation appears not to be a sufficient parameter, as shown in 2015 by Roussel *et al.* [56] for antiparasitic activity. Several polysaccharides from algae were tested, demonstrating that the more active one was not the more sulphated, and highlighting a possible implication of the position of the sulphate groups. The same conclusion was obtained by Ropellato *et al.* [57] while testing polysaccharides from the seaweed *Gayralia oxysperma* for antitumor activity. Moreover, and depending on the tested

biological activity, some anionic polysaccharides exhibiting uronic acids in their structure (but no sulphate groups) were shown to be active, hyaluronic acid being one of the most extensively studied example. For instance, Hidari *et al.* [58] have demonstrated that to obtain active fucans against the dengue type 2 virus, uronic acids were needed, as carboxyl-reduction of the glucuronic acids to glucose units abolished the fucans' antiviral properties. Wagner *et al.* [59] proposed immunopotentiating activities of rhamnogalacturonans from *Sabal serrulata* and *Echinacea purpurea*, which resulted in an enhanced phagocytic activity of granulocytes and or macrophages. In this study, polyanionic structures showed an increased activity compared to the neutral polysaccharides but the latter remained active. Then the monosaccharide composition can also have an impact on the efficiency of the measured activity. As an example, some biological activities described for the fucans may be in relation to their high content in the monosaccharide fucose, even if this assumption is still under discussion. Other evidence for the role of the monosaccharide composition is the work of Sheng *et al.* [60] who have tested two fractions of polysaccharides extracted from *Chlorella pyrenoidosa*. Even if, both fractions exhibited a high antitumor activity, one of them was more efficient than the other, differing in its monosaccharide composition exhibiting rhamnose as the main monosaccharide (and galactose for the second one). Hayakawa *et al.* [61] tested sulphated polysaccharides from 23 green algae species for their anticoagulant activities. All samples contained similar amounts of sulphate groups but different main monosaccharides. The arabinose-rich

polysaccharides were found to be more potent thrombin inhibitors than the galactose-rich ones, but the best being the rhamnose-rich polysaccharide for which the inhibition was stronger than that of heparin. However, other parameters should have an implication on the levels of the tested activities (type of activity, differences in acting mechanism, molecular mass and branching pattern of polysaccharides...), explaining the difficulty to establish a clear scheme for the structure-function relationship.

For *Arthrospira platensis* EPS, several biological activities have been demonstrated, such as anticoagulant, antiviral, antithrombogenic or antioxidant [29-30, 32, 55, 62-64]. Nevertheless, all these studies have been performed using the rhamnose-rich EPS, preventing to conclude on the implication of this monosaccharide presence on the biological activities. Regarding the potent implication of the EPS composition on the biological activities, it thus appears that increasing the light flux may have a strong impact on the efficiency of those PS by decreasing their uronic acids, rhamnose and fucose contents.

4 Conclusion

In this work it was possible to obtain, at the best achievable, an overview of *Arthrospira platensis* PS accumulation and composition as a function of the operating conditions. The meta-bibliographic analysis proposed is the first one performed on this kind of data, highlighting the great variability of the results among the authors due to different operating conditions or extraction/analysis methods, but allowing also to define clear tendencies. It appeared that among the operating conditions, the light intensity can strongly modulate the PS

accumulation (EPS and glycogen) and this parameter was studied experimentally to confirm our assumption. The results have shown that the EPS/glycogen ratio was strongly affected (from 3.1 at $100 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$ to 0.6 at $1200 \mu\text{mol}_{\text{hv}} \text{m}^{-2} \text{s}^{-1}$). Moreover, the uronic acids content was decreased for high PFD, and the neutral monosaccharides were modified suggesting potential modulation of the biological activities when obtained under such culture conditions (increase in glucose and decrease in rhamnose and fucose contents).

To our knowledge, this study gives for the first time a clear confirmation of the modulation of an EPS composition and of their potential biological activities as a function of an operating condition, especially as a function of the light. However, it is important noting that this work opens the route to the fine study of the relationship between the illumination conditions and the metabolic response of *Arthrospira platensis*. Indeed the explanation of such a behaviour is much more complex than a straightforward correlation between the light intensity and its polysaccharide composition as already pointed out in this paper. Only absorbed photons leading to charge separation into the photosynthetic electron transport chain have to be taken into account for exploring and discussing changes in the metabolic state. A specific development will be performed in the near future for discussing the results in view of that, focusing also efforts on the elucidation of the carbon fluxes repartition throughout the common glycogen/EPS metabolic biosynthesis pathways.

Conflict of interest

The authors declare that there is no conflict of interest. No conflicts, informed consent, human or animal rights are applicable for this work.

Author's contribution

OG, GC, and GT designed and supervised the research. MP and CL conducted experiments. MP, OG and CL analyzed and interpreted the data and drafted the manuscript. GC and GT critically reviewed the manuscript. All authors read and approved final version of the manuscript.

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Appendix: Table A1 Total sugars, glycogen and EPS contents from bibliographic analysis, as function of the cultivation conditions

References	Total sugar content (% _{DW})	Glycogen content (% _{DW})	EPS content (% _{DW})	Higher temperatures (> 30°C)	Nitrate limitation	Higher light intensities (> 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$)	Addition of NaCl	Glucose as carbon source
[65]	23			x				
	42			x		x		
	36			x				
[66]	44.4				x			
[67]	74				x			
	13							
[41]	24.8							
	7.7							
[40]	74				x	x		
	13							
[34]	31.05				x			
	28.09				x			
	23.62				x			
	25.26				x			
	26.68				x			
[68]	65				x			
	9							
[69]	32.95			x				
	57.53			x	x			

	40.63		x	x		
	35.5		x			
[36]	46.7		x			x
	55.8		x			x
[70]	71.4			x		
		65		x	x	
[46]		45			x	
		18				
[66]		40.2		x		
		63.2		x		
[71]		13.7				
		10				
		13				
[72]		19	x			
		24	x			
		13.3				x
		14				x
[37]		5.8				
		12.1				x
[45]		42			x	
[39]		7.29				
		1.7		x		
[67]		1.5				
		7.6		x	x	
		8.6		x	x	
[34]		10.3		x	x	
		17.83			x	

	9.4			x	
[21]	17	x		x	
[30]	13	x			
[73]	13				
	27				
	6.6				
	7.5	x			
	11.34				
	6.09				
	5.69				
[35]	5.94				
	7.47	x			
	12.57	x			
	5.13				x
	9.94				x
	14.84				x
	17.78				x
	12.77				x
	15	x	x		
[69]	21	x	x		
	13	x	x		
[33]	3.95				
[42]	3.4	x			
	10				
[47]	34	x			
	11.8	x			x
[38]	19.1	x			x
	16.4	x			x

	27.4	x		x
	35	x		x
	35.9	x		x
	23.5	x		x
	34.1	x		x
	27.8	x		x
	32.6	x		x
	34.5	x		x
<hr/>				
	10	x		
	16	x	x	
[74]	20		x	
	27		x	
	34		x	
	35		x	
[19]	42		x	

Appendix: Table A2 Amount and composition data of the EPS after extraction and formatting.

Ara	Fuc	Fru	Glc	Gal	Man	Rha	Xyl	Rib	ND	GlcA	GalA	S	ON	OA	Analytical method	Groups
2.8	6.2		8.8	4.1	-	55.1	10.1	-	-	7.3	5.6	-	86.1	12.6	HPAEC-PAD	[41] RPS, +N, LL, LT
-	-	-	14.5	5.6	2.1	53.7	4.4	10.0	11.1	-	-	-	101.4	0.0	alditol acetate - GC	[29] CPS, P1, +N, LL
0.0	0.0	0.0	42.5	0.0	57.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	TMS derivatives - GC	[39] CPS, P1, +N, LL, LT
47.3	-	-	0.4	0.0	-	2.2	-	0.0	-	47.8	-	2.4	49.8	47.7	HPLC - RID	CPS, P1, -N, HL, LT
0.0	-	-	0.0	0.0	-	0.0	-	83.8	-	13.0	-	3.1	83.8	13.0		CPS, P1, -N, HL, LT
0.0	-	-	1.3	39.3	-	0.0	-	0.0	-	56.2	-	3.3	40.5	56.1		CPS, P1, -N, HL, LT
20.0	-	-	1.3	13.0	-	0.0	-	0.0	-	60.7	-	5.0	34.2	60.7		CPS, -N, HL, LT
0.0	-	-	2.8	18.7	-	0.0	-	0.0	-	73.2	-	5.4	21.4	73.1		CPS, -N, HL, LT
-	5.3	-	16.6	22.4	traces	2.2	8.8	-	?	20.0	20.0	5.0	55.4	39.7	Methanolysis and trimethylsilylation / Alditol acetate - GC	CPS, P1, +N, HL, HT
-	5.1	-	18.7	18.7	traces	2.5	9.9	-	?	20.0	20.0	5.0	54.8	40.1		CPS, P1, +N, HL, HT
-	7.1	-	22.2	10.0	0.0	5.0	10.9	-	?	20.0	20.0	5.0	55.2	39.8		CPS, P1, -N, HL, HT
-	4.9	-	10.8	9.2	2.2	1.5	26.8	-	?	20.0	20.0	5.0	55.2	39.7		CPS, P1, HL, HT
-	-	34.0	4.7	2.4	0.8	40.0	0.5	1.8	-	7.7	4.3	3.7	84.2	12.0	HPLC-RID	[30] CPS, P2, +N, LL

-	-	-	-	-	-	43.5	3.3	-	34.7	6.9	6.9	4.7	81.5	13.7	alditol derivatives + acetylation - GC and GC-MS	[31]	CPS, P2, +N, LL
-	-	1.4	6.8	46.4	9.2	25.5	0.0	-	10.7	-	-	-	100	-	HPLC	[35]	CPS, P1, +N, HL, HT
-	-	-	3.4	4.6	0.7	39.8	4.7	-	1.1	12.1	13.5	20.0	54.4	25.6	Methanolysis and trimethylsilylation - GC	[32]	RPS, P2, LL
1.0	12.6	-	12.7	14.3	0.3	3.6	13.8	-	24.3	13.0	0.9	3.7	82.5	13.8	Methanolysis - GC	[42]	RPS, LL, HT
-	-	-	17.5	0.8	14.3	42.3	1.9	-	6.8	6,6	6,6	3.2	83.6	13.2		[33]	CPS, P1, +N
-	-	-	31.0	2.9	7.9	19.5	2.2	0.0	16.5	9,5	9,5	1.0	80.0	19.0			CPS, P1, +N