Aggregation behavior and rheology of culture broths of Rhodosorus marinus

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We report the growth and rheological behavior of culture broths of the red microalga *Rhodosorus marinus*, important as a source of phycobiliproteins. The growing process of the culture broth was controlled with and without a carbon dioxide aeration process. By dynamic light scattering measurements, we investigate the cell-exopolysaccharide (EPS) aggregation at different times of the culture broth and simultaneously, the hydrodynamic radius of the EPS in the supernatant was measured. The results indicate that the cell–EPS aggregation achieves a maximum at the stabilization stage of the culture time and at the end of the growing process, most of the cells remain disaggregated. Measurements of apparent viscosity on both, culture medium and supernatant during the growing process showed a viscoelastic behavior and give a reasonable indication of the cell and EPS maximum growth. The dry cell biomass and EPS production of *Rhodosorus marinus* resulted independent of the carbon dioxide aeration and were 5.7 g/l and 4.1 g/l, respectively. These are important results, compared with the ones obtained with similar microalgae.

Keywords: Microalgae; Rhodosorus marinus; viscosity; light scattering; exopolysachharides.

En este trabajo reportamos el crecimiento y el comportamiento reológico de cultivos de la microalga roja *Rhodosorus marinus*, la cual es una fuente importante de ficobiliproteínas. El proceso de aeración de los cultivos fue controlado en presencia y en ausencia de dióxido de carbono. Por medio de dispersión dinámica de luz investigamos la agregación de células con exopolisacáridos (EPS) producidos por las microalgas a diferentes tiempos del crecimiento de los cultivos. Simultáneamente, determinamos el radio hidrodinámico de los EPS en el sobrenadante de los cultivos. Los resultados indican que la agregación células-EPS logra un máximo en la etapa de estabilización de los cultivos y al final del proceso de crecimiento la mayoría de las células permanecen desagregadas, como consecuencia de reacciones de degradación celular o/y desagregación de EPS. Mediciones de viscosidad de los cultivos de microalgas y los correspondientes sobrendanates durante el proceso de crecimiento, mostraron un comportamiento viscoelástico y dan una indicación razonable del máximo crecimiento de las células y el EPS. Los resultados de la biomasa seca de *Rhodosorus marinus* y la producción de EPS resultaron ser independientes del método de aeración y fueron 5.7 g/l y 4.1 g/l, respectivamente.

Descriptores: Microalgas; Rhodosorus marinus; viscosidad dispersión de luz; exopolisacáridio.

PACS: 36.20.Cw; 47.54.Fj; 83.85.Cg; 87.19.rh

1. Introduction

Many natural biopolymers are widely used in food, cosmetics, pharmaceutical and oil industries as thickening, emulsifying and viscosifying agents [1]. Recently, Huheihel *et al*. [2] have demonstrated that some cell sulfated polysaccharide of red micoralga *Phorpyridium* sp. exhibited impressive antiviral activity against specific viruses. The exopolysaccharides (EPSs) are a special class of biopolymers produced by diverse unicellular microorganisms, such as bacteria, moulds, yeasts and algae [3,4]. The production of EPSs by means of cellular cultures implies the challenge to solve typical viscoelastic problems of complex fluids. It is important to know the rheology of these culture broths in order to improve the design of cell bioreactors [5] with unique characteristics for a better homogenous distribution of air, light, and nutrients that optimize cellular growth [6]. Several studies of the behavior of the culture broths at different times, such as the rheological behavior, EPS production and cell aggregation, have been previously conducted [5,7]. By measuring intrinsic viscosity, some authors [1] have investigated the molecular structure of a EPS sourced from a red microalga. Geresh *et al*. [7] have performed low amplitude oscillatory shear experiments to determine the behavior of EPS aqueous systems. Other important techniques used to investigate both culture broths and EPS are the Static Light Scattering (SLS) and the Dynamic Light Scattering (DLS) [8].

In this work we selected the red microalga *Rhodosorus marinus* as study model, because of its fast and easy growth under laboratory conditions and specifically because of its significant and interesting production of EPS and phycobiliproteins. The aim of our study was to evaluate the potential of growth of this microalga in batch cultures with and without carbon dioxide aeration by performing , dynamic and

static light scattering, capillary and cone and plate viscosimetric measurements. The hydrodynamic radius and the apparent shear viscosity were obtained during the growing process for both, the culture broth and the EPS.

2. Methods

2.1. Cell cultures

Rhodosorus marinus was obtained from UTEX collection (1723) and grown in batch cultures in a Ersdchreiber medium. Cultures were grown at 25°C in a circadian 12-h light (150 μmol/ (m^2 s) and 12-h dark cycle with gentle agitation using continuous bubbling air, provided with a peristaltic pump until 1.5 l level. After this point, cultures were split into two fractions of 18 l each.: one with air containing 5% CO_2 and another one without carbon dioxide. The algae of both cultures were grown under a continuous agitation process provided with a rotating arm (10 rev/min). A continuous aeration process was provided with an air flow rate of 3 cm^3/seg, provided by a mechanical pump and controlled by a flowmeter. Both air and CO_2 were filtered before used.

2.2. Biomass determination

The microalgal cells were counted daily in an optic microscope, using a hemocytometer-type counting chamber, however, due to the cell aggregation, it was impossible to determine the cell concentration. Instead of that, the dry weight was determined according to Bougaran *et al*. [9] by filtering 50 ml of the culture sample onto tarred number 1 Whatman filters, which were dried at 80°C for 12 h, before being used. The filtered sample was then washed three times with 50 mL of a 34 g ammonium formate solution and then dried at 80°C for 24 h before weighing.

2.3. Extraction of EPS

Isolation and extraction of EPS produced by *Rhodosorus marinus* were made according to the method of Geresh *et al.* [7]. Briefly, cells were isolated by centrifugation at 4000 rpm for 15 minutes. Cell pellets were washed repeatedly with distilled water in order to remove EPS from the pellets. EPS were extracted from the cell free supernatant adding a 2% w/w NaCl solution. Polysaccharides were precipitated with two volumes of absolute ethanol at 20°C and left overnight. After this, the precipitate was centrifuged at 4000 rpm for 10 minutes, washed with different ethanol-water solutions of different ratios and left drying.

2.4. Microscopic observations of samples of the culture broth

Microscopic observations of cells at the beginning and the end of the culture broths were carried out in an inverted microscope, model OLYMPUS IX71 with a magnification of 40X.

The static and dynamic light scattering measurements were performed using an ALV -5000 digital correlator system (Langen-GmbH, Germany) fitted with a temperature control set at 25 $\pm$0.1 °C. The scattered light, vertically polarized with a $\lambda_0 = 632$ nm Argon laser (30 mW), was measured at different angles in the range of $40 - 150°$. The reduced elastic scattering $I(q)/KC$, with $K = 4\pi^2 n_0^2 (dn/dc)^2 (I^{90}/R^{90})/\lambda_0^4 N_A$, was measured in scattering angle steps of $10°$, where n_0 is the refractive index of the standard (toluene); I^{90} and $R^{90}$are the intensity and the Rayleigh ratio of the standard at $\theta = 90°$, respectively; the increment of the refractive index (dn/dc) was measured with a refractometer (Mettler-Toledo, model RE4OD). C is the sample concentration in g / cm^3, $I(q)$ is the intensity scattered by the sample and N_A is Avogadro's number. The scattering angle q is given by $q = (4\pi n/\lambda_0)\sin(\theta/2)$ and n is the refractive index of the medium ($n = 1.339$). The data were analyzed using the well established Zimm equation [10].

$$\frac{Kc}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2C \qquad (C \to 0,\ \theta \to 0) \qquad (1)$$

where R_θ is the Rayleigh ratio for the angle θ, A_2 is the second virial coefficient of the molecule, $M$is the weight average molecular mass and $P(\theta)$is the form factor which depends on the shape of molecules and is approached for small q by $1 - (1/3)R_G^2 q^2$ and R_G is the radius of gyration of the molecule. Several form factors were tested for different geometries according to the information given in the software of the instrument (ALV 5000/E/WIN Software). Experiments were performed by triplicate and averaged with a relative error around 5%.

The hydrodynamic radius, R_H, was obtained for diluted samples from dynamic light scattering measurements at an incidence angle of $90°$ through the Stokes–Einstein relation

$$D_0 = \frac{k_B T}{6\pi\eta R_H} \qquad (2)$$

where $k_B$is the Boltzmann constant, $T$is the absolute temperature, η the viscosity of the solvent and D_0 the diffusion coefficient in the limit of low molecular concentration. Triplicate measurements were performed for 60 s each and averaged. Dilutions were performed until the average relative error of the hydrodynamic radius measurements was less than 5%.

EPS were obtained from each culture medium, centrifuged at 4000 rpm for 15 min to eliminate the cells. The polysaccharide concentration of the culture medium was determined indirectly by lyophilizing and weighing the EPS

In order to measure the EPS average molecular weight, we used three EPS solutions (1.4, 0.07, 0.035 mg/ml), prepared by successive dilutions with the original Ersdchreiber culture medium, and filtered with an Acrodisc filter of 0.2 μm. By using the Eq. (1) and varying the scattered angle in the range 40-140° we used the software of the instrument to adjust experimental data to a straight line. The calculations of R_H with the different successive dilutions did not

show appreciable differences (less than 5%), probing the dilute regime of the EPS solutions.

Molecular weight of EPS was also obtained by extrapolation of the straight line of Eq. (1), built with the three different concentrations mentioned and by fixing the scattered angle θ to 90°, as the inverse of the y-intercept. The radius of gyration R_G is obtained from the slope of the straight line of Eq. (1), according to the Zimm method.

2.5. Rheology

Apparent viscosity measurements of the whole cell culture and supernatant at different days were performed by triplicate with a Cannon-Ubbelohde capillary viscometer of 0.53 mm capillary diameter and also with a strain controlled Rheometrics Mod. RFSII Fluids Spectrometer (Piscattaway, N.J., U.S.A.) equipped with a truncated cone and plate geometry (0.0397 radian and 50 mm diameter). Any liquid expulsion of the cone was observed for all the shear rates used. Temperature was controlled with a water bath circulator with a precision $\pm$0.1 C.

We have analyzed the non-Newtonian rheological behavior of both broths with the power law model [11]

$$\tau = K\gamma^n \tag{3}$$

where τ and γ are the shear stress and the shear rate, respectively. K and n are named the consistency index and the power low index, respectively.

Dynamic oscillatory small-deformation rheological measurements of a series of dilutions of the cell biomass were registered from a solution of 3.16×10^{-4} g/ml. This original concentration was obtained by centrifugation of the entire broth as mentioned before and dilutions were done with the same supernatant solvent. Mechanical spectra (within the linear viscoelastic region) were registered in the frequency range of w=0.1 to 100 s-1 (g=10%). The samples were maintained at 25° C. Shear rates were swept from 1 to 100 1/s. Measurements were repeated three times with an average relative error less than 5 %.

3. Results and discussion

3.1. Cell biomass and EPS production

The results of cell biomass and EPS production of *Rhodosorus marinus* at the end of 28 days of cell culture were 5.7 g/l and 4.1 g/l , respectively for the culture broth without using carbon dioxide aeration process (CB). The results of cell biomass and EPS obtained at the day 21 varied less than 5 %. The results for the culture broth with a carbon dioxide aeration (CBCD) were similar with differences also around 5 %. These results are similar to the ones reported by Lapasin *et al*. [12] for the cell culture of *Cyanospira capsulata* (a cyanobacterium which also produces a similar EPS and phycobiliproteins**).** They obtained 4.14 and 3.27 g/l of cell biomass and EPS, respectively. The variation of cell biomass and EPS between the day 21 and the day 28 were also around 5% . Similar results for EPS were reported by Fernandes *et al*. [11]. Fábregas *et al*. [13], using a semicontinuous regime and varying the nutrient concentrations of cultures of the microalga *Porphyridium cruentum*, found a maximum concentration of the sulfated EPS of 90 mg/l. This is a very appreciable difference in comparison with our result.

3.2. Microscopic observations of microalga cells

Microscopic observations of cells show that *Rhodosorus marinus* is a spherical–like unicellular microalga, measuring a diameter between 4 to 5 μm. The cells are red, and as we can see in Fig. 1a, a biofilm is surrounding them, possibly corresponding to EPS. This figure, obtained at the beginning of the culture broth (day 10), shows also some cells connected to one another through a biofilm, possible formed also by EPS excreted by the cells. The microscopic observations were conducted at different days over the development of the culture. You can see that during the first days of culture development, even when the cells concentration is low, the formation of aggregates is initiated. These aggregates are increasing in number and size during the development of the culture, as long as the cellular concentration increases, also the EPS production resulted increased. In Fig. 1b we show an image of some aggregates obtained at the day 23. Some authors have demonstrated that the pattern of polysaccharide production varies with the type of organism and composition of the medium. [14,15]. The aggregation-disaggregation of cells in natural environments is a dynamic process [16] and can be influenced by enzymes [17] which contribute to the disaggregation process. In our case, these enzymes could be similar to the ones found by Simon-Bercovitch *et. al*. [18] obtained from the dynoflagellate *Gymnodinium sp*., which could degrade the EPS of the red microalga *Porphyridium sp*., similar to the one used by us. They observed a total reduction in the viscosity at the end of the culture due to the degradation effect of the mentioned enzyme, also correlated with an increase in reducing sugars.

3.3. Time behavior of cell cultures and EPS

We analyzed the evolution in time of the average hydrodynamic radius of polysaccharides in the supernatant at dif-

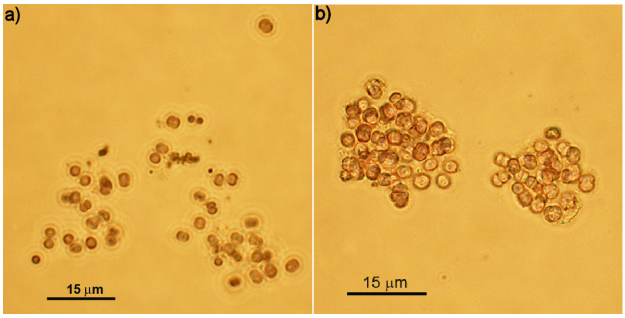

FIGURE 1. Microscopic observations of the culture cells obtained at the day 10 (Fig. 1a) and at the day 23 (Fig. 1b).

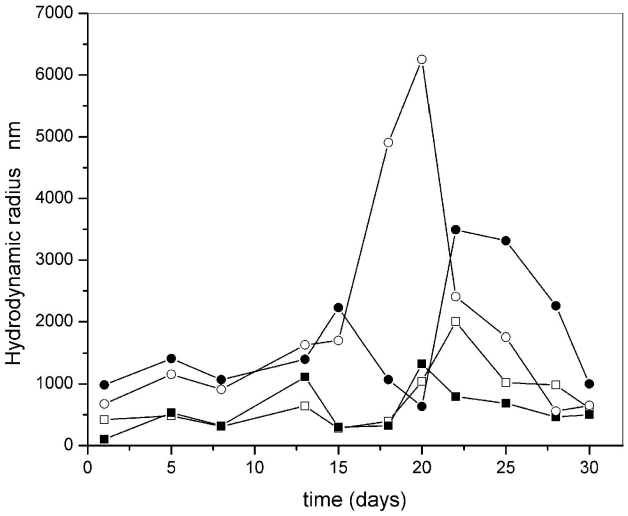

FIGURE 2. Average hydrodynamic radius at different times for the entire culture broth without using carbon dioxide aeration (open circles) and for the culture broth by using carbon dioxide aeration (filled circles). Hydrodynamic radius of polysaccharides in the supernatant from both cultures are also shown. Filled squares for CO_2 aeration and open squares for the culture without CO_2. Measurements were performed at 25 $\pm$1 C.

ferent times by DLS measurements. Simultaneously, the average size of the cell-polysaccharide aggregates were measured from both CB and CBCD cultures. The observation of cell aggregation by using DLS is not new in itself. Sist *et al.* [8] have carried out light scattering and viscosity measurements on the polysaccharide produced by a strain of *Burkholdeira cepacia*, isolated from cystic fibrosis patients. They observed molecular aggregation with DLS and circular dichroism spectra. Recently Bordi *et al.* [19] have demonstrated the usefulness and accuracy of the DLS method to measure the size of human cells. In Fig. 2 it is shown the size behavior of polysaccharides at different times from both CB and CBCD cultures, and as can be observed, the average size does not change significantly during the growing of the cell culture. However, at approximately 20 days after starting the CB culture medium, the average radius of he EPS increased from 350 nm at the day 18 to 2000 nm at the day 22. This behavior was similar for the CBCD culture medium. However, in CBCD culture medium, the EPS aggregation reached an average maximum of 1000 nm at the day 20. After this day, the average hydrodynamic radius of EPS began decreasing until 500 nm approximately for both culture broths. This seems to indicate that an aggregation process can take place due to the concentration increment of EPS.

On the other hand, the average hydrodynamic radius of aggregates of both entire culture media shows a different behavior. At the beginning of the growing process, before the day 15, the average hydrodynamic radius of the aggregates fluctuates approximately between 700 nm and 1700 nm for both culture broths, which means that due to the cell color close to the laser length of the instrument, the light is partially adsorbed by cells and scattered mostly by the EPS. Taking into account that the cell average diameter is 4- 5 μm, we assume that at this stage most of the cells are disaggregated, mostly covered by EPS. As the culture grows, we observe that at approximately 20 days, the average size of the aggregates increases to approximately 12 μm in diameter for the CB culture medium. This probably means that EPS cover the cells and disperse light as if they were 12 μm average diameter objects, similar to the ones observed in Fig. 1b. Beyond this time, the cell-polysaccharide aggregates start disaggregating and become smaller and at the end of the process, the average diameter ends in approximately 2μm, which corresponds to the average size of aggregated EPS and therefore, most of cells remain free of the EPS biofilm, probably because the cell dying process produce enzymes that contribute to the EPS biofilm breaking process [17]. For the case of CBCD culture broth, we found that the maximum average diameter was obtained at the day 22 with an average diameter of 7 μm. After that time, the average diameter of the cell-EPS aggregates decreases to 4.6 μm, which is close to the microalgae cell diameter observed by direct microscopic observations as shown in Fig. 1a. This difference in the cell-EPS aggregates size, compared with the one found in the CB culture medium, could mean that the CO_2 aeration process affects the aggregation in the culture medium, keeping bigger cell-EPS aggregates at the end of the growth process (day 28).

In order to observe the time evolution of the cell–EPS aggregates and the EPS size distribution more clearly, we show in Fig. 3 the hydrodynamic radius distribution of both culture media. In Fig. 3a we show the size distribution of the whole CB culture medium at three different times of culture growth. The y axis represent the relative proportion of dispersed light of molecules and cells of different sizes. At the first day we observe a wide range of sizes varying from 20 nm to 10 μm. However, as observed in Fig. 2, the average radius was 680 nm. This wide size distribution was also found for the CBCD culture medium at the first day of the growing process as can be observed in Fig. 3b. At the day 20 we observe a noticeable difference in the cell- EPS size distribution of both culture broths. For the CB medium, the size ranges from 300 nm to more than 10 μm. On the contrary , for the CBCD culture medium, the hydrodynamic radius varies from 400 nm to 1 μm. This means that at this time the scattered light is mainly obtained from the EPS. Observe in Fig. 2 that at this time for the CBCD culture medium the EPS appears aggregated and probably separated from cells, which seem to be smaller due to the light adsorption from the red laser. At the day 28, the size distribution of both culture media are similar. However, as observed in Fig.2 for the average sizes, the cell-EPS aggregates are larger for CBCD medium than the ones for the CB medium. The results are consistent with the idea that EPS are involved in the formation of cell aggregates. It is known that biofilms in bacteria are synthesized from EPS and other exo-cellular polymers. These biofilms control the state of aggregation of several types of bacteria [20,21]. Burdman *et al.* [22] have found a direct relationship between the

amount of EPS produced by *Azospirillum brasilense* and the state of aggregation of this bacterium.

3.4. Molecular weight of EPS

The molecular weight of the EPS was measured by static light scattering (SLS) with the Zimm method [10]. Shu & Lung [23] have examined the effect of pH on the cell growth and the molecular distribution of exopolysaccharides of *Antrodia camphorata*. They obtained high molecular weight polysaccharides at low pH values (7.9×10^5 Da). In our case, we did not change the pH conditions of the culture medium. pH of both culture mediums were kept at pH 7.0 ± 0.5. The average molecular weight of the EPS was 1.2×10^6Da and resulted independent of the aeration conditions (with or without CO_2 flow). Other authors found variation of the molecular size and viscosity depending on the aeration conditions [24]. The conformation of the EPS was obtained from the ALV instrument software by testing different chain conformations and comparing the results of the radius of gyration given in Eq. (1). The conformation of the EPS resulted similar to a polydisperse coil.

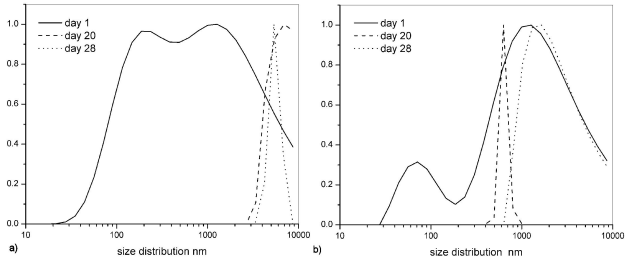

FIGURE 3. Behavior of the hydrodynamic radius distribution at different times for: a) the culture broth without a CO_2 aeration method and b) the culture broth using CO_2.

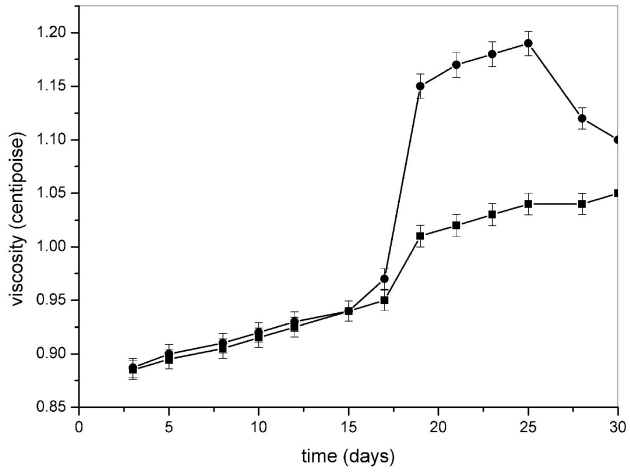

FIGURE 4. Apparent viscosity for the culture medium without CO_2 (circles) and the supernatant (squares) at different times. Viscosity was measured with a Cannon-Ubbelohde capillary viscometer at 25 $\pm$C. Error bars are averaged to 3 %.

3.5. Rheology of culture broths and EPS

Simultaneously with the DLS experiments, we analyzed the cell growth of the CB culture medium and the polysaccharide in the supernatant by measuring the apparent viscosity with a Cannon-Ubbelohde capillary viscometer at different times. Figure 4 shows that at short times and up to day 15 both, culture media and supernatant (*i.e.* EPS) viscosity values are very similar and show a moderate increasing trend with time. Both measurements show differences after approximately 17 days. After this time, the viscosity of the culture medium increases and almost stabilizes at the day 25, near to the time of largest cell-EPS aggregates observed in Fig. 2. In the same period of time, the supernatant also increases its viscosity, indicating an increase of concentration of cells and EPS. Viscosity values of the culture medium remain almost constant until the day 25. After this time the viscosity decreases, probably as a consequence of the cell dying process and the cell-EPS disaggregation which practically ends at the day 30. On the contrary, the viscosity of the EPS supernatant grows slowly after the day 21, indicating a slower EPS production and other cell products.

Viscosity measurements of the culture medium and the supernatant were performed with the Reometrics Spectrometer for different shear rates. We observed a non Newtonian behavior of both, the whole CB and CBCD culture media and their corresponding supernatants containing EPS. Other authors have also found a Non-Newtonian effect after the first days of growth and found a power law equation [1].

Construction of a growth behavior curve of the *Rhodosorus marinus* culture broth was difficult due to the cell aggregation phenomenon, observed even at the first days of the first growing stage. We therefore implemented a complementary viscosimetric method to analyze the growing process using a cone and plate with the Reometrics Spectrometer as mentioned above. Both cultures showed a similar pseudoplastic behavior for different shear rates (not shown). Only at the day 28, where apparently the dying stage starts, the CB and the CBDC media showed the highest viscosity values, probably because the cultures reach the maximum biomolecules concentration or/and the cell-EPS aggregation reaches a maximum, before they start to be digested by enzymes.

In Fig. 5 we summarize the evolution of the viscosity behavior for a fixed shear rate of both media throughout the whole culture time. We have noticed three stages in the whole culture process: induction, exponential growth and cell dying or/and cell disaggregation. It is important to mention that a stationary stage was observed when our culture media were performed at lower (20°C) average room temperature at around days 22-25. Similar stages were identified by Lapasin *et al.* [12] with *Cyanospira capsulata* cultures. We noticed that for both cultures, the three stages started and finished at almost identical time intervals. In the first stage the viscosity does not change appreciably and is interpreted as the time needed by cells to adapt to the new environment [12]

and therefore there is not a noticeable cell growth. We observed at day ten an increase of the size of the aggregated cells by optical microscopy, however, the viscosity only increases up to two times the water viscosity at day 18. This behavior was also noticed by capillary viscometric measurements (Fig. 4). The second stage (exponential) is produced between the day 15 and the day 28. Both cultures reach a maximum of the viscosity values around the day 28. At this stage the viscosity increases more than three (without CO_2) and more than two times (with CO_2) with respect to the water viscosity for a shear rate of 15 1/s. Also, we found that at this stage a greater dependence in viscosity with shear rate is observed (*i.e.* shear thinning). This maximum almost coincides with the one found in Fig. 4, obtained with a capillary viscometer at the day 25. In general, random coil disordered polysaccharides in concentrated solution exhibit a shear thinning behavior [25,26], consistent with our results of the EPS conformation obtained with the static light scattering method used above.

At day 30 the onset of the dead stage is appreciated, where a clear decreasing of viscosity and the number of aggregates is observed for both cultures. The most important difference of both cultures was observed at the maximum viscosity values around day 28.

In order to measure the Non-Newtonian behavior of the cultures, we used the power model given in Eq. (3). This model is equivalent to the one described by the Ostwald-de Waele equation [11] for the apparent shear viscosity and the shear rate. The results of n and K ad are shown in Figs. 6a and 6b. These values were obtained from the experimental data using the least –squares method and are plotted against time. In Fig. 6a we show the change of n with time for both culture mediums. We notice a rather monotonic decreasing of n in both mediums until approximately 20 days. After the day 22 the change of n is more pronounced for the CB medium reaching a value around 0.5 at day 28 in coincidence with the highest viscosity reached, which is a sign of the non-Newtonian behaviour of both media and a structuration of the cell-EPS aggregates. Finally n values almost return to their initial values at the beginning of the growing process, indicating the tendency of the culture medium to return to a more Newtonian behaviour when cells are probably dead or/and cell-EPS are almost disaggregated. This behaviour can be explained assuming that the aggregation cells-EPS builds a gel-like structure until the day 28, observed in Fig. 5 as the largest viscosity value. After that, the liberation of biopolymers in solution can not reach the viscosity values reached by the gel–like structure, also found in the cell biomass rheological behavior observed in Fig. 7. This phenomenon was explained by Simon-Bercovitch *et al*. [18] by the presence of enzymes at the end of the microalgae culture which cause a decreasing viscosity with an increase of reducing sugars.

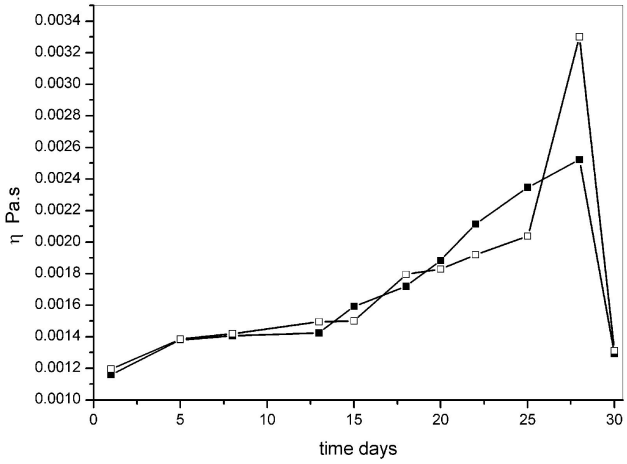

FIGURE 5. Viscosity of both the *Rhodosorus marinus* culture broths (with and without aeration process) at different stages of the growing process at a shear rate of 15 s^{-1} . Measurements were carried on with a RHEOMETRICS Mod. RFSII Fluids Spectrometer at 25° C. Open symbols correspond to culture broth without using CO_2.

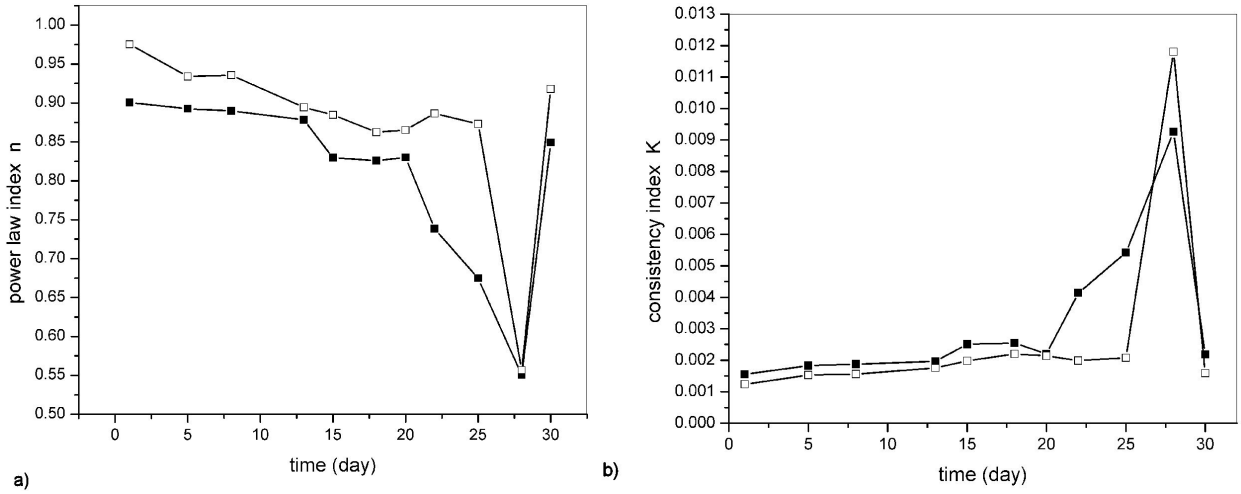

FIGURE 6. Change in the power law index (Fig. 6a) and the consistency index (Fig. 6b) with the progress of both culture broths . Filled symbols correspond to the culture broth aerated with CO_2.

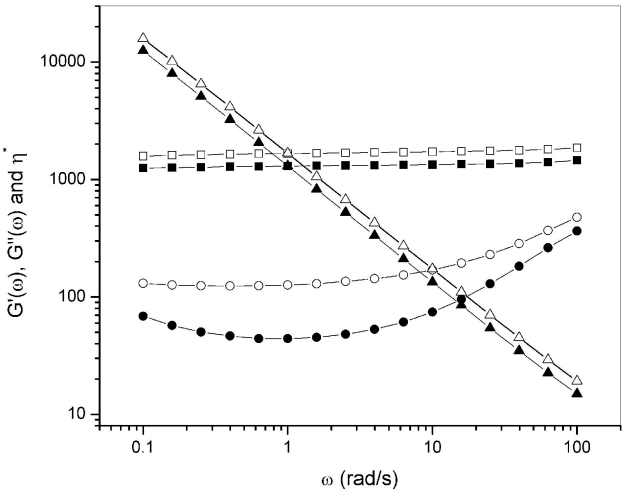

FIGURE 7. Storage, Loss modulus and Complex viscosity as a function of frequency for the cell biomass of both culture broths. Open symbols correspond to the cell biomass of culture broth without using CO_2 and filled symbols to the cell biomass of the culture broth with the CO_2 aeration process. Squares correspond to the storage modulus, circles to the loss modulus and triangles to the complex viscosity.

The behaviour of K is observed in Fig. 6b. For the first 20 days, the behaviour is similar for both culture media. After the day 20, K for the CBCD medium shows a smooth growing until day 28. On the contrary K start rising after day 25 for the CB medium when it increases very fast until day 28. Finally, at day 30 both K values almost return to the initial values. The behaviour of both parameters in time indicate that CB starts its maximum growing later than the CBCD medium, but the final stage of growing coincides very well independently of the aeration process.

3.6. Viscosity of the cell biomass

Measurement of the storage modulus (G', loss modulus (G'') and complex viscosity (η^*) in the range of frequency from 0.1 to 100 rad/s were performed for both culture broths by small-deformation oscillatory rheology. Viscosity of the cell biomass of some dilutions of both culture broths (with and without CO_2) were performed also in the Rheometrics Spectrometer (results not shown). The cell biomass sample was obtained by centrifugation of the culture broth at the end of the process (day 30) and measured in the same day.

In Fig. 7 we show the mechanical spectra. The dependence of the viscoelastic G' and G" moduli and of the complex viscosity, η*, on w exhibited by both types of cell biomass is characteristic of a solid gel-like material. It can be appreciated that the overall magnitude for the culture broth without CO_2 aeration process is slightly greater than that of the aerated one. This is consistent with the viscosity results observed at the time of maximum growth in Fig. 5, probably due to differences in the cell - EPS aggregation process. We can conclude that the presence of CO_2 in the aeration process is not relevant in the the *Rhodosorus marinus* culture broths.

4. Conclusions

We have used three methods in this work to analyze the cell growth and EPS production of the *Rhodosorus marinus* culture broth with and without a CO_2 aeration process. We have performed light scattering experiments on both, culture medium and polysaccharide supernatant suspensions of culture cells of *Rhodosorus marinus*. Capillary and steady-shear viscometric measurements were also carried out on the culture broths at different times.

The experiments of dynamic light scattering indicate that the cells and EPS aggregate at the middle of the culture time and disaggregate at the end after the maximum cell and exopolysaccharide growth are achieved. The average hydrodynamic radius of EPS did not show appreciable changes during the culture growth and showed small changes with the different aeration processes. The influence of the carbon dioxide on the growth of the culture medium was negligible and the exopolysaccharide molecular weight was also independent of the aeration with carbon dioxide. Viscosity measurements of the entire culture and supernatant at different times showed to be sensitive to monitor the continue culture growth process and observed a pronounced non Newtonian behavior at the end of the maximum growth.

The rheological experiments on the culture cells biomass showed a viscoelastic behavior also found by other authors. The origin of this contribution is probably due to the EPS layer attached to the external cellular wall of the microalgae. The flow curves of the cell biomass of the red microalgae have shown an extremely shear thinning response attributed to a shear induced structuration.

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