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Rheological Characterization of *Scenedesmus* Sp. Pastes (Up To100 G/L) Produced In A 200-L Raceway and Harvested By Coagulation-Flocculation-Sedimentation

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Abstract: The aim of this work was to carry out a complete rheological characterization of *Scenedesmus* sp. pastes with or without addition of a flocculant in terms of viscosity and viscoelasticity. Rheological measurements were carried out using a simple viscometer and a rheometer. K and n values from the Power law determined using both the viscometer Brookfiel and the rheometer Physica 1000 systems were quite different. Physica 1000 results are more reliable. In the case of this rheometer, viscosity vs. shear rate lines seem to be divided in three shear rate zones: a) below 0.04 s^{-1}, b) between 0.04 and 6 s^{-1}, and c) up to 6 s^{-1}. For the *Scenedesmus* pastes without chitosan, K values ranged from 0.27 -10.9 $Pa.s^n$, while n values fluctuated between 0.73-0.92 (non-dimensional). R^2 values were quite good for all concentrations (from 0.95-0.98). Viscoelastic behavior of the algal suspensions was observed at any of the assessed concentrations. Viscoelastic properties of microalgae suspensions with chitosan are not very different from those presented by the samples without added chitosan. i.e., the elastic component was greater at low frequencies; whereas to high frequencies, the values of both components are equal.

Keywords: raceway, G'/G'', microalgae, rheological characterization, viscosity

INTRODUCTION

Currently, there is an increasing interest in the cultivation and exploitation of microalgae. The biotechnological applications for microalgae seem to be endless, including biodiesel production, production of pigments, special lipids (omegas), biodiesel, wastewater treatment (tertiary treatment), biomass to be converted in methane or hydrogen, biomass for energy production by pyrolysis, and many more[1]. Microalgae are cultured very often in open systems in autotrophy (i.e., raceways) with microalgal concentrations as high as 1 to 2 g /L. In exceptional cases, closed systems (i.e., air-lift, parallel plate reactor, bubble column) could reach higher biomass concentrations (2-6 g/L). Heterotrophic processes, i.e., where organic substrates are fed to the medium (e.g., saccharose), could reach much higher microalgal concentrations[2], such as 15.5 g/L.

From the point of view of fluid dynamics, all these microalgal suspensions are basically biomass in saline media and depict Newtonian behavior. This is important because the rheology of the media determines mass, heat, and momentum transfer inside the bioreactor. Nevertheless, the next step in microalgae culture is the harvesting processes, which could comprehend operations such as coagulation-flocculation, sedimentation, centrifugation, filtering, etc. After a train of recovering processes, the microalgal concentrations could be enhanced up to 100 g/L (a factor of 6 to 100) and even more.

Now the suspension contains a large amount of microalgal cells and the rheological properties are drastically different from the previous culture. Concentrations up to 40 g/L produce non-Newtonian behavior, although this value is very dependent on the type of microalgae (i.e., size, hydrodynamic radius, form, cilia, etc.), even if microalgae were just sediment or a coagulant-flocculant were added for harvesting or cells were alive or dead[3-5].

Concentrated suspensions are subjected to further processing, such as drying and pumping through pipes, and subjecting to heat; thus, a simultaneous mass and heat transfer process is developed. The rheological behavior of the produced pastes has a profound impact on pumping, mixing, and heat transfer. Because of this, it is very important to characterize rheologically these pastes in terms of viscosity and even viscoelasticity.

Few works have described the rheological characteristics of microalgal suspensions or aggregates. For example Basaca-Loya *et al.*[3] studied the aggregation behavior and rheology of culture broths of *Rhodosorus marinus* microalgae. They observed that viscosity of microalgal aggregates increased as a function of culture time up to 3.2 mPa.s. Regarding the Power law parameters, K and n, they found that the consistency index K was approximately the same during the first 25 hours of culture (2 mPa.s), and suddenly a big jump was observed at 28 hours (12 mPa.s). On the other hand, the flow index n had a value of 0.90 for the first 25 hours and decreased suddenly up to 0.55 at 28 hours of culture. Dos Santos *et al*. [6] studied the rheological behavior of cultures of *Chlorella sp*. and *Scenedesmus sp*. in concentrations from 0g/L to 0.788g/L and 0g/L to 1.352g/L respectively. They observed that the Power Law model adjusted well to the data of shear stress as a function of strain rate. In all concentrations the cultures showed non-Newtonian behavior. At this diluted solutions it was observed to *Scenedesmus sp.* little effect of biomass concentration on the apparent viscosity.

Wileman *et al*.[7] studied the rheological properties of algal slurries to minimize harvesting energy requirements in biofuel production. These slurries had concentrations between 0.5 and 80 g/L of *Nannochloris* sp, *Chlorella vulgaris,* and *Phaeodactylum tricornutum.* They found that under 20 g/L, microalgal suspensions are Newtonian, but over 60 g/L, the suspensions of *Neochloris* and *Chlorella* become non-Newtonian and undergo shear thinning. In the case of *Phaeodactylum,* suspensions were Newtonian up to 60 g/L. Only at concentrations of 80 g/L did *Phaeodactylum* suspensions become non-Newtonian.

Adesanaya *et al.*[4] carried out an excellent characterization of algal suspensions. They studied *Scenedesmus obliquus* suspensions of 70 to 150 g/L. They employed two different rheometers, a piezo-axial vibrator (PAV) and an advanced rheometric expansion system (ARES). They carried out oscillatory measurements to determine the complex viscosity, μ*; storage modulus, G'; and loss modulus, G'', for *Scenedesmus* suspensions. They concluded that the microalgal suspensions showed progressive increases in complex viscosity as a function of biomass concentration. In addition, the value of G' was negligible below 50 g/L concentration and at higher microalgal concentrations there was a progressive increase in G'. The presence of elasticity was suspected to be due to algal cell intraparticle interactions and algal motility.

The aim of this work was to perform a complete rheological characterization of *Scenedesmus* sp. pastes (up to 100 g/L) with or without addition of a flocculant (chitosan) in terms of viscosity and viscoelasticity. Rheological measurements were carried out using a simple, low-cost viscometer (Brookfield) and a rheometer (Physica 1000 Anton Parr), to compare the results of viscosity versus shear rate for the two systems.

MATERIAL AND METHODS

2.1 Microalgae and inocula: *Scenedesmus* sp. first inocula were grown in 1 L glass bottles and BG-11 medium, supplied with filtered air for 15 days, until culture absorbance was about 0.7. They were grown at 20 ± 2ºC and under 12:12 light periods with a light intensity of about 100 $\mu E.\ m^{-2}s^{-1}$ without any movement (static cultures).

The second inoculum was grown in a 21 L agitated tank, very similar to that reported by Torres el al.[5], using an A200 impeller, agitation of 300 rpm, and 1vvm of air. Bioreactor culture was developed as follows. Biomass (optical density and dry biomass) was evaluated along the culture. Lipid production was assessed at the beginning and end of the 15 days culture. Average light intensity in the bioreactor was 68 $\mu E.\ m^{-2}s$. The area exposed to light was 0.283m^2.

At the end of the process, the inoculum (18 L) was transferred to the 200-L raceway located in the greenhouse.

2.2 Coagulation-flocculation with and without chitosan: After culturing the microalgae, 90L of the medium was removed from the raceway using a flexible hose and a peristaltic pump; 40 mg/L of chitosan (dissolved in acetic acid-water) was added to the rest of the medium (90 L). The medium-chitosan culture was gently agitated for some 20 min and agitation was stopped. The microalgae were allowed to settle for a long time, and the supernatant was eliminated again using the flexible hose and the peristaltic pump. Once it was not possible to discard more water, the medium was removed and poured in a plastic recipient where it was allowed to sediment again and again. This procedure was continued until reaching a total volume of 0.5 L. This paste was stored at 4ºC until its use. The medium without chitosan was treated similarly and, at the end of the process, only 0.4 L of medium was recovered. This sample was kept under refrigeration until its use. The actual microalgae concentration was determined by measuring, in both pastes, the total suspended solids (in triplicate) according to Standard Methods[8].

2.3 Rheological characterization:

2.3.1 *Brookfield viscometer:* The original paste was diluted using the BG-11mediumto 80, 60, 40, and 20%. Pastes were characterized in a Brookfield viscometer (Brookfield Model DV-II+Pro, USA) at all the available speeds (0.17 to 3.33 s^{-1}). Spindle 5 was used. Raw data were adjusted to the Power law model:

$$n = K\,\dot{\gamma}^{n-1} \qquad (\ldots 1)$$

2.3.2 *Physica 1000 rheometer:* The same pastes were evaluated in two different modes, *i.e.*, viscosity versus shear rate plots and oscillatory rheology.

2.3.3 Viscosity versus shear rate: A cone and plate system was employed. Temperature was fixed at 25ºC. All measurements were carried out induplicate. Values reported are the average of these measurements. Viscosity was measured as a function of shear rate in the whole available speed range, i.e., 0.1-1000 s^{-1}.

2.3.4 Microalgae oscillatory rheology: Using more sophisticated rheological equipment (such as a Physica 1000 rheometer), it is possible to measure not only the liquid-like behavior of the microalgal suspensions (η), but also the viscoelastic behavior through parameters such as G' (the storage modulus), G'' (the loss modulus), and μ* (the complex viscosity). While G' represents the liquid behavior, G'' is related to the solid-like behavior. Finally, μ* is the combination of both parameters. These parameters have units of Pa.s (μ*) and Pa (G' and G''), respectively. They are measured as a function of the oscillation frequency (ω). The relationship between the parameters and the complex viscosity is given by:

$$\mu^* = \frac{\sqrt{G'^2 + G''^2}}{\omega} \qquad (\ldots 2)$$

Complex viscosity, G' and G'' were measured in the whole frequency range (0.1 to 1000 Hz), but the range of 0.1-100 Hz was selected, as reported by Basaca-Loya *et al.*[3] for the reported results. All measurements were carried out in duplicate and triplicate some cases. Results reported are the average of two different measurements.

Measurements were taken using the same rheometer mentioned above, from a dynamic strain sweep at constant frequency of 10 Hz. They were performed at 25 °C from 0.01% to 10% strain for all *Scenedesmus* pastes at different concentrations to determine the linear viscoelasticity zone (LVZ).

Frequency sweep tests, at 1% strain and 25 °C, were performed from 0.1 Hz to 100 Hz in triplicate. The oscillatory rheological parameters used to compare the viscoelastic properties of all *Scenedesmus* pastes at different concentrations were the storage or elastic modulus (G′), loss or viscous modulus (G″), and loss tangent (tan δ).

RESULTS AND DISCUSSION

***Scenedesmus* cells production:** The culture time was fixed to 14 days, reaching a final biomass concentration of 0.4 g/L. This means a biomass productivity of around 0.021 g/Ld. Considering a 2 W/m^3 power consumption in the raceway due to the superficial water velocity, i.e., 30 cm/s [9], 0.01036 kg biomass/ kW was obtained. **Figure 1** shows the biomass values along the 14 days of the culture process. The growth rate for this process was 0.27 g/day, giving a duplication time of 2.56 days.

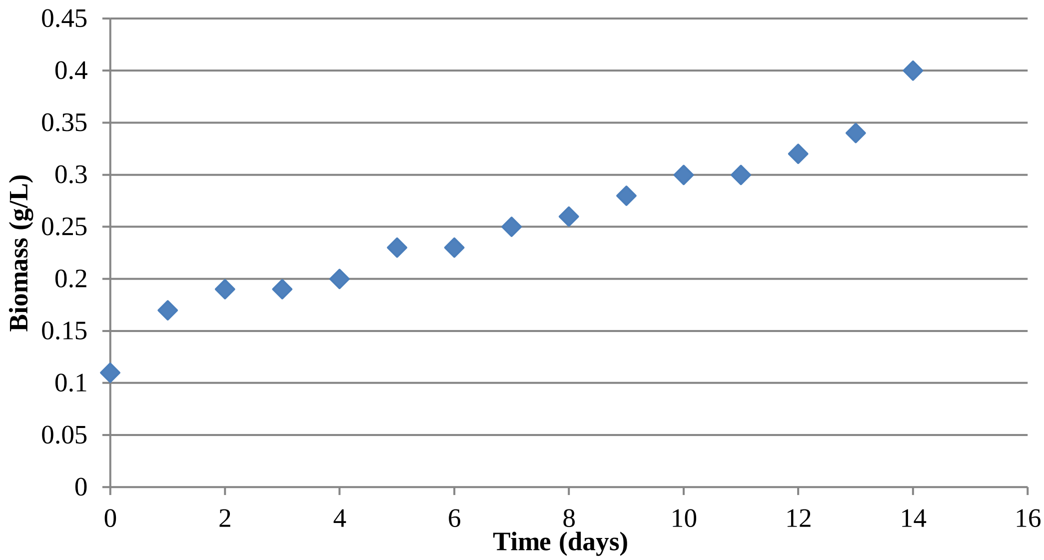

Figure 1. *Scenedesmus sp.* culture in the 200 L raceway.

Viscosity vs. shear rate for *Scenedesmus* pastes:

Brookfield viscometer

Figure 2 and 3 show the viscosity vs. shear rate rheograms for the different concentrations of *Scenedesmus* pastes without and with chitosan. The Brookfield viscometer is a simple, low cost viscometer, which can be used reliably for certain purposes. The shear rate is limited to the 0.17 to 3.33 s^{-1} range. In the case of the microalgal pastes without chitosan, three different concentrations were evaluated (100, 80, and 60), corresponding to 98.5, 78.8, and 59.1 g/L. The suspensions containing less than 59.1 g/L of microalgae were not characterized using the Brookfield system, since it was not possible. Black lines represent the Power law model and **Table 1** contains the K and n values for the 60-100% *Scenedesmus* pastes. K values ranged from 0.10 to 0.41 (Pa.s^n) and n between 0.604 and 0.626, whereas R^2 values were quite good (from 0.94 to 0.99).

Figure 3 show the rheograms for the *Scenedesmus* pastes with chitosan added. The use of chitosan for the coagulation-flocculation process incremented slightly the pastes' viscosity values. It is important to highlight that microalgal paste concentrations were slightly higher than those without chitosan (i.e., 106.7, 85.4, and 64.0 g/L). K values ranged from 0.11-0.44 (Pa.s^n) and n between 0.630 and 0.635 (non-dimensional), whereas R^2 values were quite good (from 0.95 to 0-99). These values can be compared to those reported for *Nanochloropsis oculata* with chitosan pastes, since they were measured in the same

Brookfield device reported by Torres[10] (see Table 1 for details). In comparison, these values are much higher than those reported for *Dunandiela tertiolecta* and chitosan pastes by Torres[10]. Other values of K and n reported by Basaca-Loya *et al*.[3] and Wileman *et al*.[7] for *Rhodosorus marinus* and *Nanochoropsis* sp. are included for comparison purposes, although the microalgal concentrations in those suspensions are different (in the first case, microalgal concentrations were not reported).

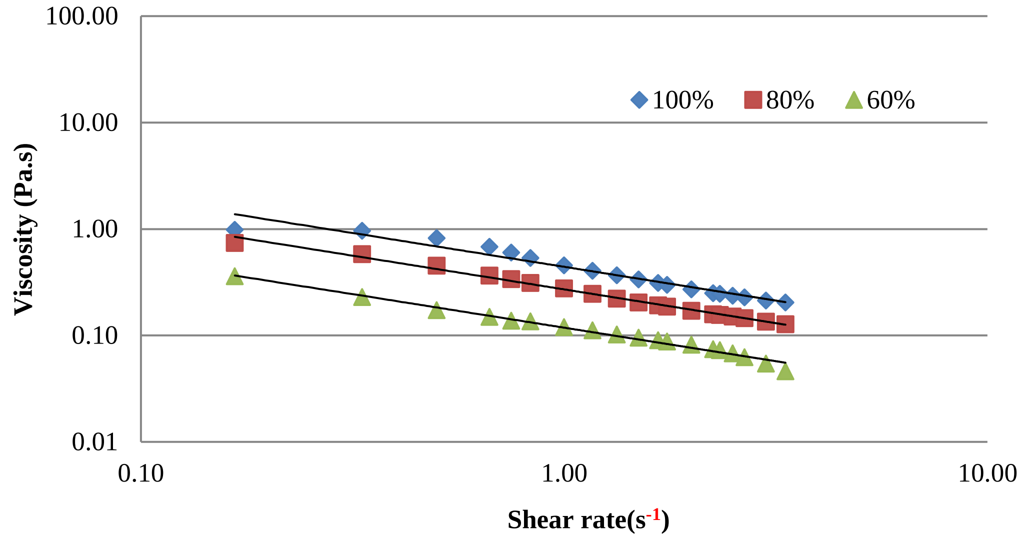

Figure 2: No chitosan *Scenedesmus* pastes rheograms, elaborated with raw data from the Brookfield viscosimeter.

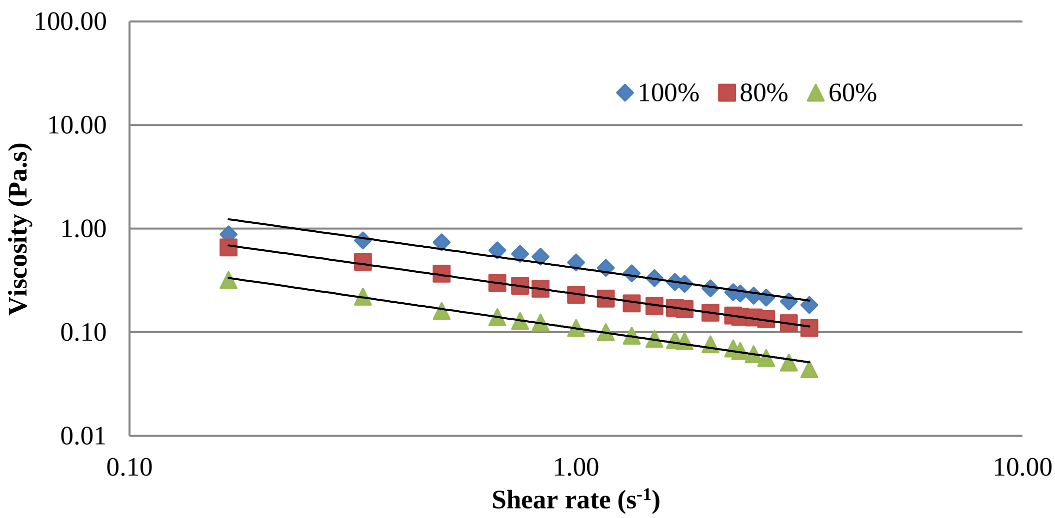

Figure 3: Chitosan added-*Scenedesmus* pastes rheograms, elaborated with raw data from the Brookfield viscosimeter.

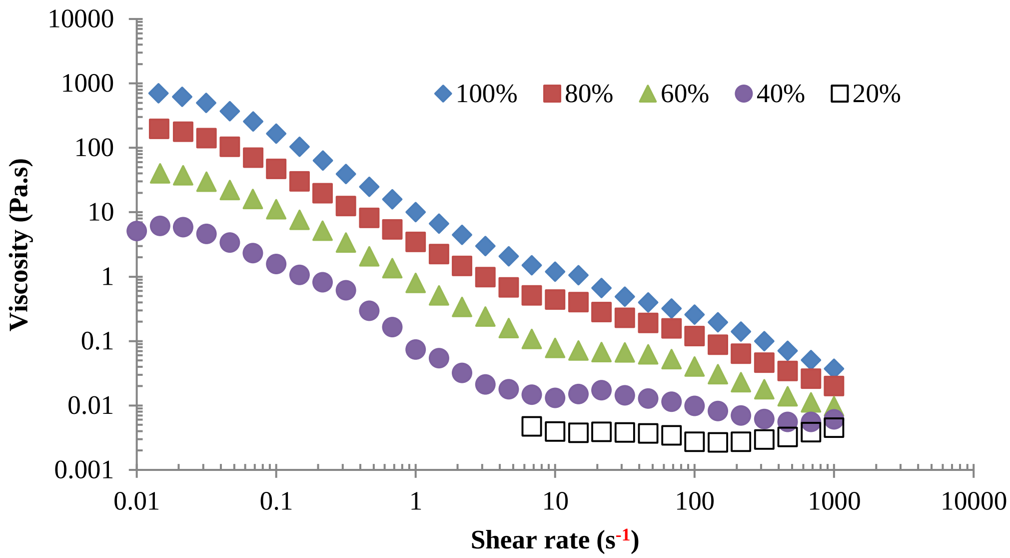

Figure 4: No chitosan added-*Scenedesmus* pastes rheograms, elaborated with raw data from the Physica 1000 rheometer.

Physica 1000 rheometer

Figure 4 shows the rheograms for *Scenedesmus* pastes (without chitosan) from 20 to 100% (i.e., 19.7 to 98.56 g/L). The rheofluidizing behavior for all microalgal concentrations can be observed. As expected, the higher the microalgal concentration, the higher the viscosity at any shear rate. It is also interesting that viscosity vs. shear rate lines seem to be divided in three sections: a) shear rates below 0.04 s^{-1}, b) shear rates between 0.04 and 6 s^{-1}, and c) shear rates up to 6 s^{-1}. This behavior is well represented by the Cross model[11]:

$$\frac{\eta-\eta_\infty}{\eta_o-\eta_\infty} = \frac{1}{(1+(\lambda\dot{\gamma})^m)} \qquad (\ldots 3)$$

Nevertheless, the Power law model (Eq. 1) can be applied with a good fitting. It is always preferable to employ simpler models, when possible. Table 1 presents the K and n Power law parameters, as well as the R^2 correlation coefficient for the *Scenedesmus* pastes without chitosan. K values ranged from 0.27 to 10.9 $Pa.s^n$, whereas n values fluctuated between 0.73 and 0.92 (non-dimensional). R^2 values were quite good for all concentrations (from 0.95 to 0.98).

Figure 5 presents the rheograms for *Scenedesmus* pastes with chitosan. Note that the addition of chitosan increased the viscosities of the microalgal pastes, especially for the lower microalgal concentrations. In fact, the rheograms for 20 and 40 g/ L overlapped.

Table 1 presents also, the Power law parameters as well as the correlation coefficient for the *Scenedesmus* pastes with chitosan. K values ranged from 0.04 to 13.9 $Pa.s^n$, whereas n values fluctuated between 0.039 and 0.90. R^2 values were quite good for most of the concentrations (from 0.89 to 0.98), except for the 21.3 g/L concentration (R^2 = 0.119). This means that the addition of chitosan produced slightly higher viscosities, and a more pronounced rheofluidizing behavior (lower n values).

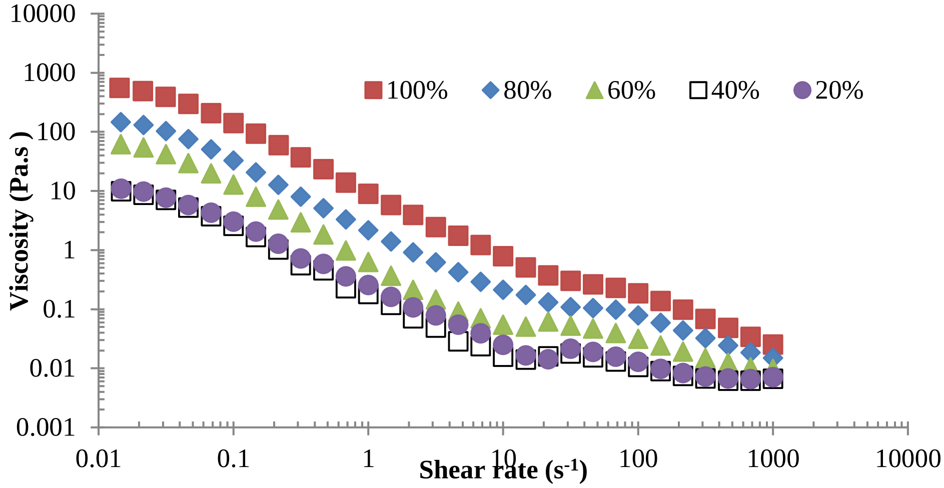

Figure 5: Chitosan added-*Scenedesmus* pastes rheograms, elaborated with raw data from the Physica 1000 rheometer.

Table 1: K, n and R^2 values form the Power law applied to different microalgae pastes.

Strain	Conc. g/L	Brookfield or other system			Physica 1000			Reference
		K (Pa s^n)	n	R^2	K (Pa s^n)	n	R^2	
Scenedesmus sp. No chitosan	98.56	0.418	0.604	0.941	10.917	0.932	0.988	This work
	78.84	0.235	0.601	0.997	3.042	0.857	0.978	
	59.13	0.109	0.626	0.988	1.060	0.836	0.949	
	39.42	-	-	-	0.345	0.740	0.954	
	19.71	-	-	-	0.276	0.735	0.953	
Scenedesmus sp.+ chitosan	106.78	0.441	0.635	0.954	13.96	0.906	0.089	This work
	85.42	0.271	0.634	0.992	4.74	0.846	0.986	
	64.06	0.118	0.630	0.985	1.17	0.789	0.977	
	42.71	-	-	-	0.191	0.693	0.908	
	21.35	-	-	-	0.004	0.039	0.119	
Dunandiela tertiolecta + chitosan	28.22	0.895	0.42	0.997	-	-	-	(10)
	25.39	0.268	0.52	0.915	-	-	-	
	22.57	0.165	0.62	0.915	-	-	-	
Nanochloropsis oculata+ chitosan	100.44	0.607	0.37	0.991	-	-	-	10
	90.39	0.197	0.26	0.998	-	-	-	
	80.35	0.136	0.29	0.997	-	-	-	
	70.38	0.091	0.31	0.993	-	-	-	
	60.26	0.097	0.38	0.970	-	-	-	
	50.22	0.062	0.48	0.921	-	-	-	
Rhodosorus marinus	Along a 30 days culture	0.0015-0.012	0.55-0.90	NR	-	-	-	3
Nanochloropsis sp.	0.5-80	0.0011-0.0040	0.89-1.0	0.999	-	-	-	6

When comparing K and n values for *Scenedesmus* pastes without and with chitosan, measured with the two rheological characterization systems, it is clear that both K and n values are quite different. It is important to remark some of the reasons for this: a) Brookfield data comprised about 20 measurements in a reduced range (0.17-3.33 s^{-1}), whereas the Physica 1000 equipment allows much more measurements in a wider shear rate (0.01 to 1000 s^{-1}) and b) geometries employed for rheological characterization are quite different. Brookfield measurements are based on a cylinder spinning inside a fluid contained in a vase with infinitum radius (sic), while measurements in the Physica 1000 equipment were obtained in a cone-plate system, with a fixed gap. There are no good or bad systems; there are different applications and necessities.

Sanchez *et al*.[12] reported the rheological characterization of xanthan fermentation broths with polymer concentrations ranging from 5.4 to 39.2 kg/m^3. They employed a simple Brookfield viscometer and a Contraves rheometer. They reported the values of K, n, and R^2 obtained using the two systems and found only small differences for the K and n values obtained using the two systems. Values of K (for a fermentation broth containing 35.6 kg/m^3) were 43.2 and 41.2 $Pa.s^n$ for the Contraves and Brookfield systems, respectively. Difference was about 4.6%.

Mouquet and Treche[13] compared the viscosities of gruels for infants using three different systems: a Haake VT550, a Brookfield RV viscometer, and a Rion VT04 viscometer. They found large differences in the apparent viscosities of maize, cassava, and rice gruels, with concentration from 1 to 12%, depending on the measurement system. They concluded that it is in vain to compare the apparent viscosity of gruels when they are measured with different apparatuses or under different measurement conditions.

The question: What is better viscometer or rheometer? Cannot be answered easily. The viscometers are cheap and easy to manage. Rheometers are in general more expensive and they tend to be more complicated. The cost of the systems is definitely very different. Rheometers are always more costly than viscometers. As McGregor[14] concludes: both viscometer and rheometer can measure viscosity vs. shear rate. The decision of choice may ultimately be determined by answering questions like the following: can I perform this set of tests as easily and quickly as the standard viscosity test? Is there a measurable payback that justifies the investment? Will this more comprehensive rheological analysis produces a more consistent product?

Results from the Physica 1000 system are more reliable. The data cover a wider range of shear stresses and this is related very much to the specific application of the viscosity values, i.e, the flow through pipes and accessories, the mixing in a cylindrical tank, the heat transfer issues when drying the microalgal pastes, etc. A rheometer can measure the viscosity as well as the viscoelasticity of the microalgal pastes, whereas the simple viscometer cannot do that.

Oscillatory rheology in the Physica 1000 rheometer

In **Figure 6** the effect of the concentration of algae on the viscoelastic behavior of the dispersions without added chitosan is presented. This figure shows that, at frequency values between 0.5 and 50 (Hz), the values of the remaining modules (constant elastic modulus) were higher, whereas at high frequencies, the two values lead to equal modules. It is also observed that as the concentration of both algal module values increases up to 100 times and the value increases to the frequency at which the modules are equal, is increased to 10 times.

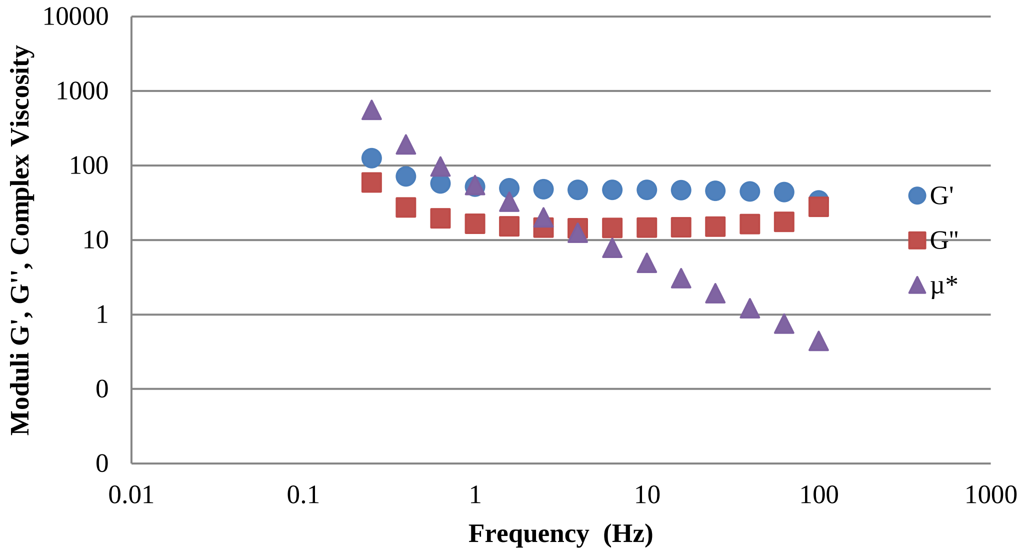

Figure 6. Typical oscillometry for *Scenedesmus* without chitosan 100% (98.5 g/L).

In *Scenedesmus* dispersions without added chitosan, the G' value for 40% dispersion, at 1 Hz frequency, was 0.47 Pa; whereas for the dispersion at 100%, at the same frequency, the G' value was 52 Pa; on the other hand, 40% dispersions at 6 Hz gave equal modules, whereas for 100% dispersions this equality was given at 100 Hz.

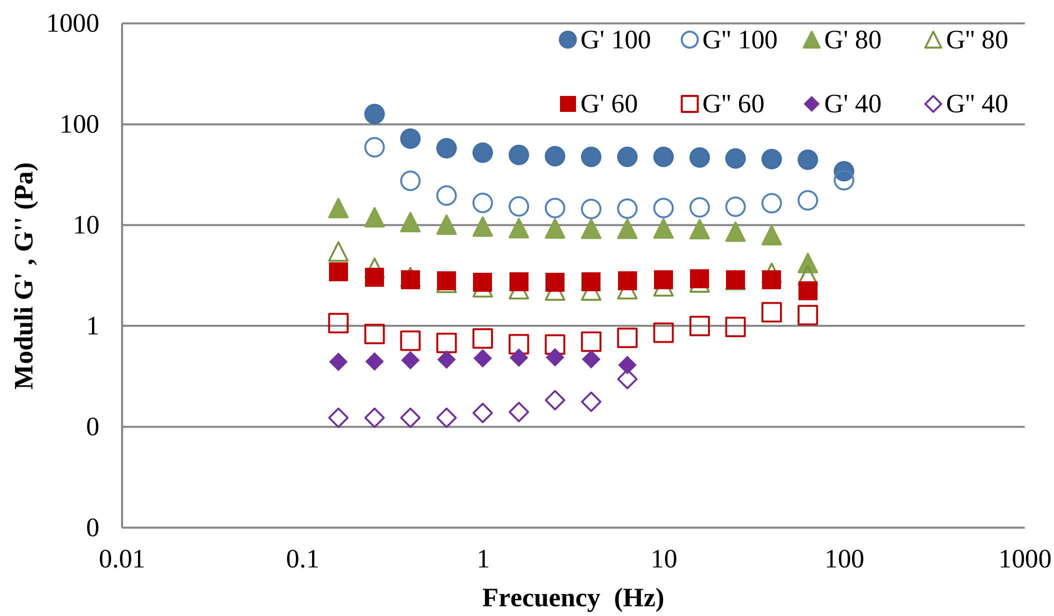

Figure 7. Oscilometry for *Scenedesmus* with chitosan. Effect of concentrations.

Figure 7 shows the viscoelastic behavior of the algal suspensions supplemented with chitosan. It can be observed that the viscoelastic properties are not different from those presented by the samples without chitosan, i.e., the elastic component was greater at low frequencies, whereas at high frequencies, the values of both components are equal.

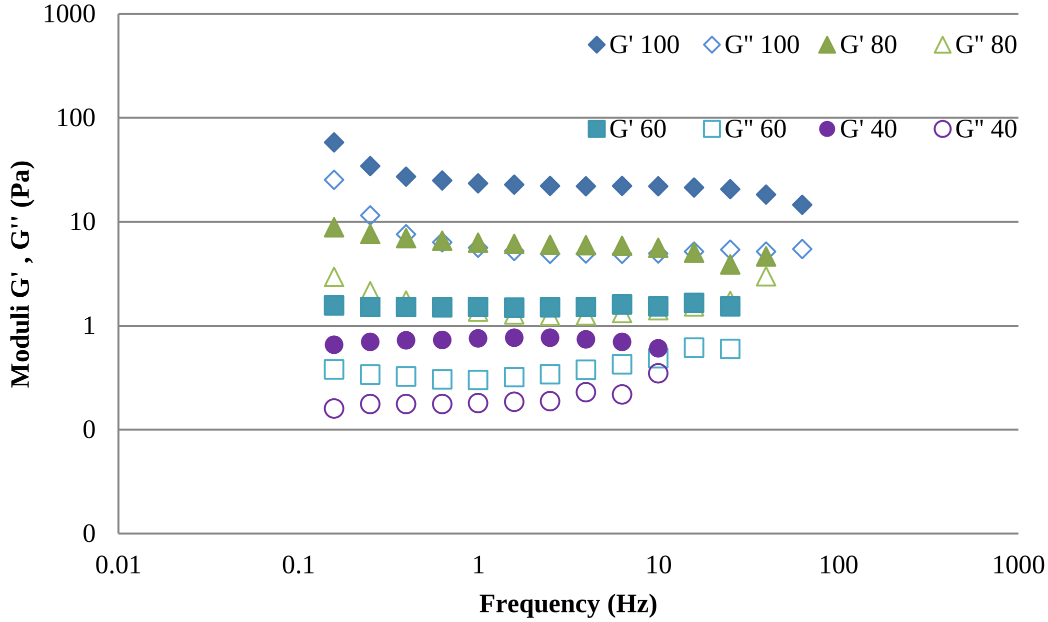

Figure 8. Oscilometry for *Scenedesmus* no chitosan. Effect of concentrations.

Changes in structure and aggregation of algal suspensions have been reported[4] when they are subjected to high shear rate values(10 to 1000 s^{-1}); as shown in **Figures 7 and 8** at low frequencies the elastic behavior is greater than the viscous behavior, indicating a degree of aggregation and structure due to the attractive forces of the algal cells, whereas at high frequencies both modules are equalized, indicating a rupture of the structure given by the aggregation of algae and making the dispersion more fluid.

CONCLUSION

With the low-cost viscometer, the shear rate determination is limited to the 0.17-3.33 s^{-1} range. In the case of the microalgal pastes without chitosan, three different concentrations were evaluated, corresponding to 98.5, 78.8, and 59.1 g/L. The suspensions containing less than 59.1 g/L of microalgae were not characterized using the Brookfield system, as this was not possible. K values ranged from 0.10-0.41(Pa.s^n) and n between 0.604 and 0.626 (non-dimensional), whereas R^2 values were quite good (from 0.94 to 0.99). In the case of the Physica 1000 rheometer, viscosity vs. shear rate lines seem to be divided in three sections: a) shear rates below 0.04 s^{-1}, b) shear rates between 0.04 and 6 s^{-1}, and c) shear rates up to 6 s^{-1}. This behavior is well represented by the Cross model. Nevertheless, the Power law model was applied with a good fitting. For the *Scenedesmus* pastes without chitosan, K values ranged from 0.27 to 10.9 Pa.s^n, whereas n values fluctuated between 0.73 and 0.92 (non-dimensional). R^2 values were quite good for all concentrations (from 0.95 to 0.98). For the *Scenedesmus* pastes with chitosan, K values ranged from 0.04 to 13.9 Pa.s^n, whereas n values fluctuated between 0.039 and 0.90 (non-dimensional). R^2 values were quite good for most of the concentrations, except for the 21.3 g/L concentration. This means that the addition of chitosan produced slightly higher viscosities, and a more pronounced rheofluidizing behavior.

Finally, viscoelastic behavior of the algal suspensions was observed at all the assessed concentrations. It was observed that the viscoelastic properties of microalgae suspensions with chitosan are not different from those presented by the samples without added chitosan, i.e., the elastic component was greater at low frequencies; whereas to high frequencies, the values of both components are equal.

NOMENCLATURE

η= viscosity, Pa.s (Eq 1)
$\dot{\gamma}$ = shear rate, s^{-1} (Eq 1)
K = consistency index, Pa.s^n (Eq 1)
n = index flow, non-dimensional (Eq 1)
G'= the storage modulus (Eq 2)
G'' =loss modulus (Eq 2)
μ*= complex viscosity (Eq 2)
η_∞ and η_o = Newtonian plateau zones (Eq 3)
λ = time constant (Eq 3)
m = dimensionless exponent (Eq 3)

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