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Article

Chlorella sp Microalgae Suspensions—Rheological Behaviour Analyzes at Different Culture Times

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Abstract: Taking into account the growing interest in microalgae to be used as raw material for biodiesel production, this research is aimed at analyzing the rheological behaviour of microalgae suspensions (*Chlorella* sp*)* at different culture times under eight different conditions (temperature, salinity and CO_2, NO_3 and PO_4 levels) in order to estimate the energy demands of each step, with the purpose of optimizing a continuous feed tubular bioreactor construction. For each condition, it was calculated the biomass and oil yields, so as to correlate these results with rheological parameters. The suspension results indicated that the microalgae *Chlorella* sp is a non-Newtonian material with dilatant characteristics; the processing time hardly exerted an influence on the rheograms of the suspension of the microalgae *Chlorella* sp, except for the simultaneous conditions of low salinity and low CO_2 content; NO_3 and PO_4 contents and the amount of supplements influenced the rheological parameters of the suspension of the microalgae *Chlorella* sp, when in low concentration of CO_2 and low salinity levels.

Keywords: *Chlorella*; rheology; culture time

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1. Introduction

The oil crisis in the 70's and 80's has elicited several studies on the pyrolysis of triglycerides. At the risk of depletion of a few energy sources, especially fossil sources that offer no prospect of renewal, there is strong motivation for developing technologies which enable the use of renewable energy sources. Furthermore, society's growing concern about environmental issues must influence the decisions of leaders about the possible use of renewable energy sources. In this context, fossil fuels, such as diesel oil and gasoline, are those which are the most criticized due to the production of a quantity of CO_2 that the planet is not able to assimilate on a long-term, thus producing the so-called greenhouse effect, and also on account of the possibility of releasing sulphur oxides. Such fuels belong to a category of non-biodegradable fuels that release polluting compounds into the atmosphere during combustion [1].

Biodiesel, a fuel derived from vegetable oils and animal fats, is a biodegradable fuel with low emission of pollutants into the atmosphere, which can be regarded as a futuristic type of fuel. Triggered by a catalyst, such oils chemically react with alcohol. There are different kinds of nuts in Brazil that can be used to produce biodiesel. Among them are castor bean, palm kernel oil, canola, sunflower, peanut, soy bean and cotton. Raw materials that originate from animals, such as beef tallow and pork fat can also be used for producing biodiesel [2, 3, 4, 5, 6, 7, 8,9,10,11].

However, experimental projects conducted around the world have highlighted microalgae as a promising source for biodiesel production. These organisms present numerous advantages over traditional terrestrial crops for biofuel production, such as high efficiency in the conversion of sunlight into biomass, the possibility of cultivation on unsuitable land for food crops, the use of non-potable water for biomass production, lipid accumulation, sorption capacity of heavy metals (Hg, Zn, Pb and Cu , for example), easy and fast cell growth, and for being able to be grown in media with high CO_2 concentrations [12,13, 14].

Despite being a process which has still been little exploited on a large scale, obtaining energy from microalgae has aroused the interest of many researchers from many parts of the world, such as the United States, Germany, Israel, Belgium, France and New Zealand. The encouragement of using microalgae as raw material source for producing biofuels (such as biodiesel, biogas and bioethanol), results from the great advances of bio-systems engineering on this area of expertise, but there are still great gaps yet to be filled. Besides other challenges, the use of continuous bioreactors for cell growth is still regarded as a field which has not been exhaustively studied. Culture in a continuous operation mode is of great advantage when compared to batch and semi batch modes as regards cell productivity [15, 13].

Over the years, a series of studies involving the production of biofuels from microalgae have been conducted, which predict the development of continuous flow bioreactors, where the most convenient feeding system was selected after physical and chemical analyses of power supply chains. In this line of research, the rheological behavior has a prominent position, as all materials involved in the process have no Newtonian behavior, and should then be subject to all sorts of unit operations, such as agitation, mixing, heat exchanges, handling by special pumps, separations and others that may arise from the design and development of the entire process [16].

From previous observations, it can be noted the importance of a rigorous rheological characterization for different ways of feeding the reactor, given that the viscosity data used in the equipment projects should be determined with the most amazing degree of accuracy, so as to avoid under- or overdimensioning. Furthermore, rheological data are of fundamental importance in energy economy which, in recent decades, has become so important that the operations of heat and mass exchange are increasingly being addressed more exhaustively [17, 18, 19].

Given the paucity of information about rheological data in scientific literature, the purpose of this study was to analyze the rheological behavior of *Chlorella* sp microalgae suspensions at different culture times, in order to estimate the energy demands of each step and optimize the construction of a continuous feed tubular bioreactor for the aforementioned microalgae's cellular culture.

2. Results

2.1 Experiment conditions effect

The graphics of the apparent viscosity as a function of shear rate obtained from different conditions of the experiment can be found in Figure 1. In this figure, the presented results are averages of the experimental triplicates.

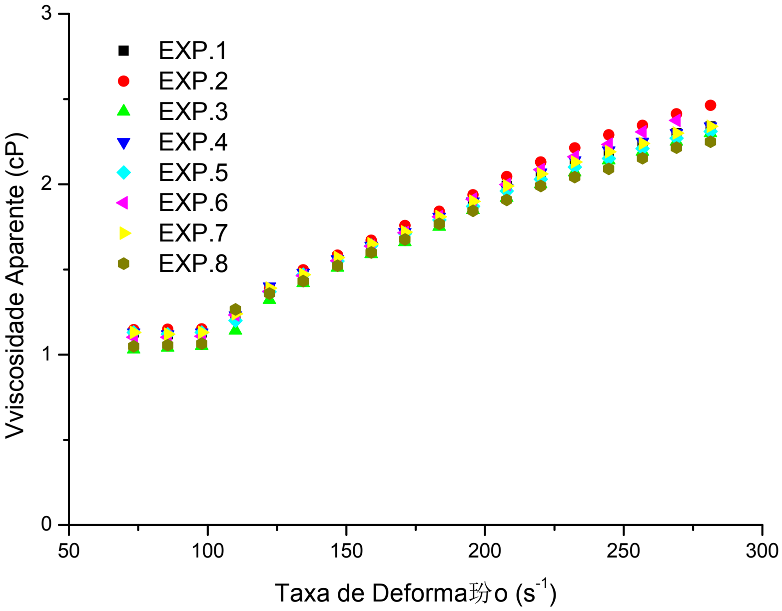


Figure 1: Apparent viscosity of the suspension of *Chlorella* sp under different conditions.

The graphics of shear stress as a function of shear rate obtained from different conditions of the experiment can be found in Figure 2. As in Figure 1, the presented results are averages of the experimental triplicates.

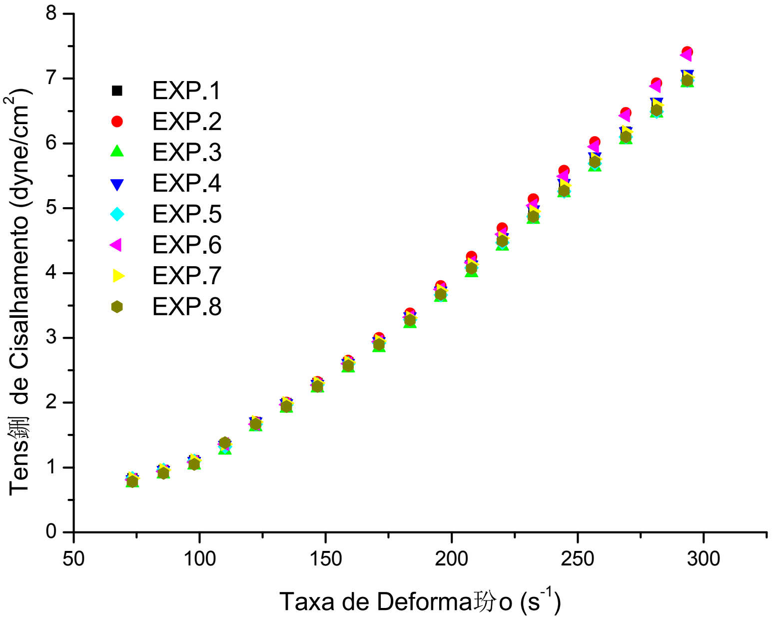


Figure 2: Rheograms of the suspension of *Chlorella* sp under different process conditions.

In order to verify the influence of culture time on the rheological behavior during the *Chlorella* sp microalgae suspension, the graphics of the shear stress as a function of shear rate were obtained daily under different experimental conditions, which can be found in Figures 3 to 10.

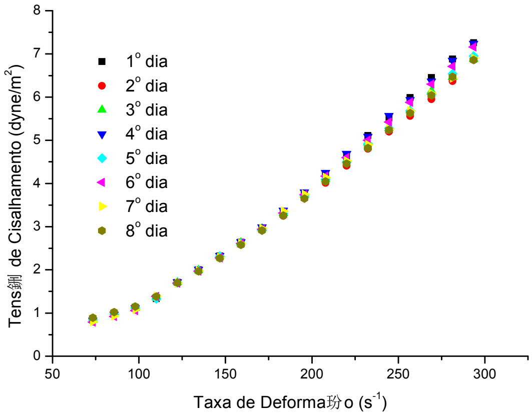


Figure 3: Rheogram (experiment 1).

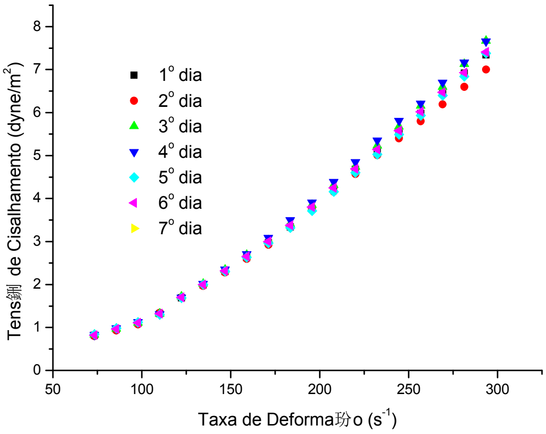


Figure 4: Rheogram (experiment 2).

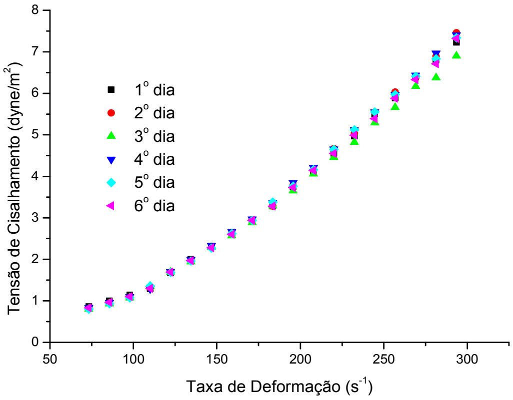


Figure 5: Rheogram (experiment 3).

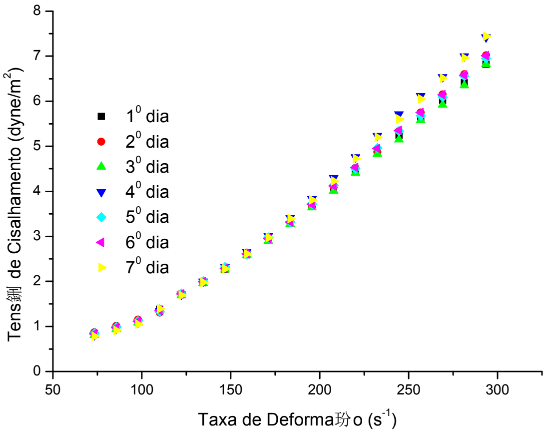


Figure 6: Rheogram (experiment 4).

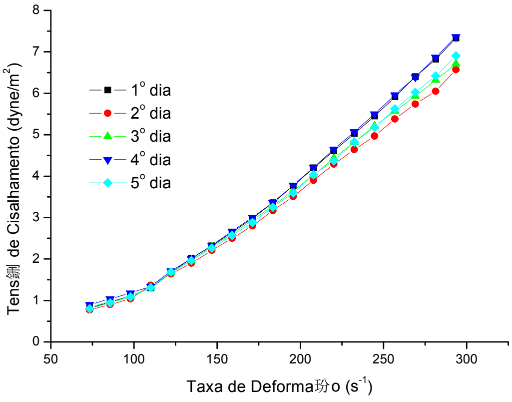


Figure 7: Rheogram (experiment 5).

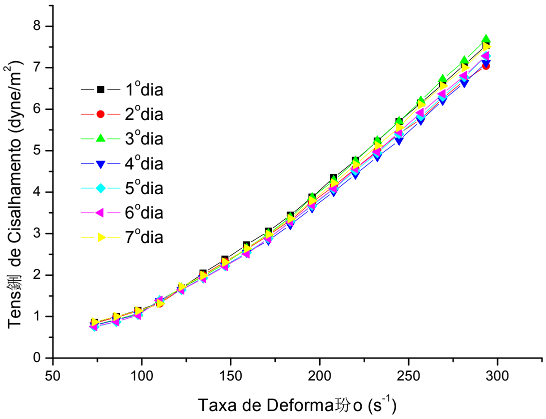
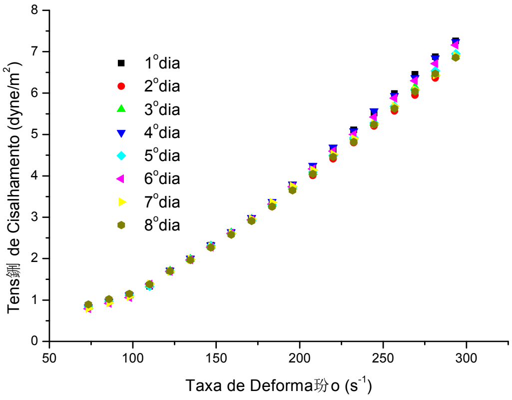


Figure 8: Rheogram (experiment 6).



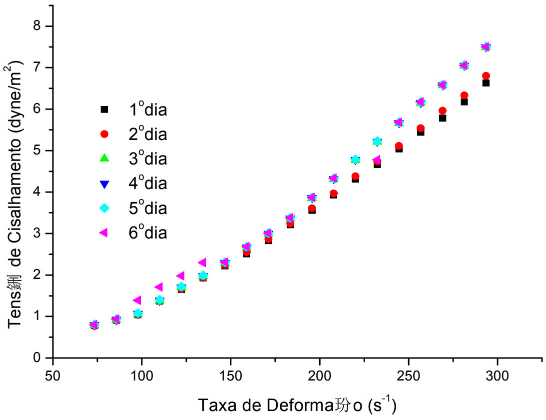


Figure 9: Rheogram (experiment 7).

Figure 10: Rheogram (experiment 8).

Table 1 presents the values of the parameters obtained by adjusting the models of Bingham, Casson and the Power law in the rheograms of Figure 2 to the process conditions of the experiments. On this table, each parameter corresponds to an average of the values at different culture times, and the values between brackets, standard deviation.

Table 1: Rheological parameters of the *Chlorella* sp microalgae suspension.

Experiment	Bingham			Casson			Power		
	η	τ_0	R^2	K_C	K_{0C}	R^2	n	K	R^2
1	2,82	0	0,98	4,33	0,97	0,99	1,61	0,075	0,99
	(0,13)	(0)		(0,19)	(0,07)		(0,01)	(0,01)	
2	3,08	0	0,98	4,92	1,27	0,99	1,68	0,056	0,99
	(0,11)	(0)		(0,24)	(0,12)		(0,017)	(0,01)	
3	2,89	0	0,99	4,61	1,18	0,99	1,68	0,053	0,99
	(0,19)	(0)		(0,25)	(0,05)		(0,03)	(0,01)	
4	2,94	0	0,98	4,59	1,10	0,99	1,62	0,07	0,99
	(0,149)	(0)		(0,33)	(0,18)		(0,04)	(0,01)	
5	2,93	0	0,99	4,46	1,00	0,99	1,61	0,08	
	(0,146)	(0)		(1,23)	(0,23)		(0,08)	(0,09)	
6	3,04	0	0,98	4,84	1,25	0,99	1,67	0,06	0,99
	(0,12)	(0)		(0,25)	(0,14)		(0,04)	(0,01)	
7	2,92	0	0,99	4,53	1,07	0,99	1,63	0,07	0,99
	(0,10)	(0)		(0,21)	(0,03)		(0,03)	(0,01)	
8	2,89	0	0,99	4,60	1,16	0,99	1,67	0,06	0,99
	(0,08)	(0)		(0,34)	(0,09)		(0,07)	(0,00)	

where: ⇒ η Plastic Viscosity (cP); τ_0 ⇒ Initial Voltage (dyne/cm^2); K_C ⇒ Plastic Viscosity of Casson (cP); K_{0C} ⇒ Initial Voltage of Casson (dyne/cm^2);n ⇒ behavior index; K ⇒ consistency index (cP)

2.2 Analysis of the correlation of rheological parameters with the oil percentage obtained

The coefficients of Pearson and Pvalues resulting from the response surface analysis can be found in Table 2.

Table 2: Correlation coefficients between the rheological parameters with the yield in the oil extraction process

	η	Kc	K0C	n	K	%Oil
Kc	0,919 0,001					
Koc	0,773 0,025	0,958 0,000				
n	0,489 0,209	0,781 0,022	0,916 0,001			
K	-0,356 0,387	-0,685 0,061	-0,856 0,007	-0,976 0,000		
% Oil	0,544 0,164	0,693 0,057	0,710 0,049	0,62 0,101		
Biomass Conc	-0,127 0,765	-0,069 0,872	-0,034 0,936	0,009 0,984		
% Oil					-0,635 0,091	
Biomass Conc					0,027 0,949	-0,204 0,628

From the results shown in Table 2, it is noted strong correlations between parameters K0C with η, n with Kc, and n with K0C. With respect to oil percentage, it is observed a correlation between parameters Kc and K0C, whose graphics are presented in figures 11 and 12.

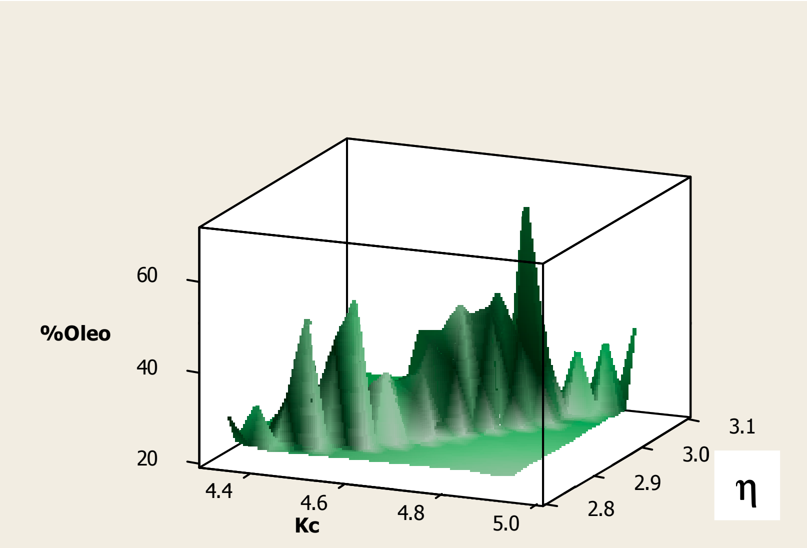


Figure 11: Correlations between apparent viscosity and Casson plastic viscosity with oil extracted.

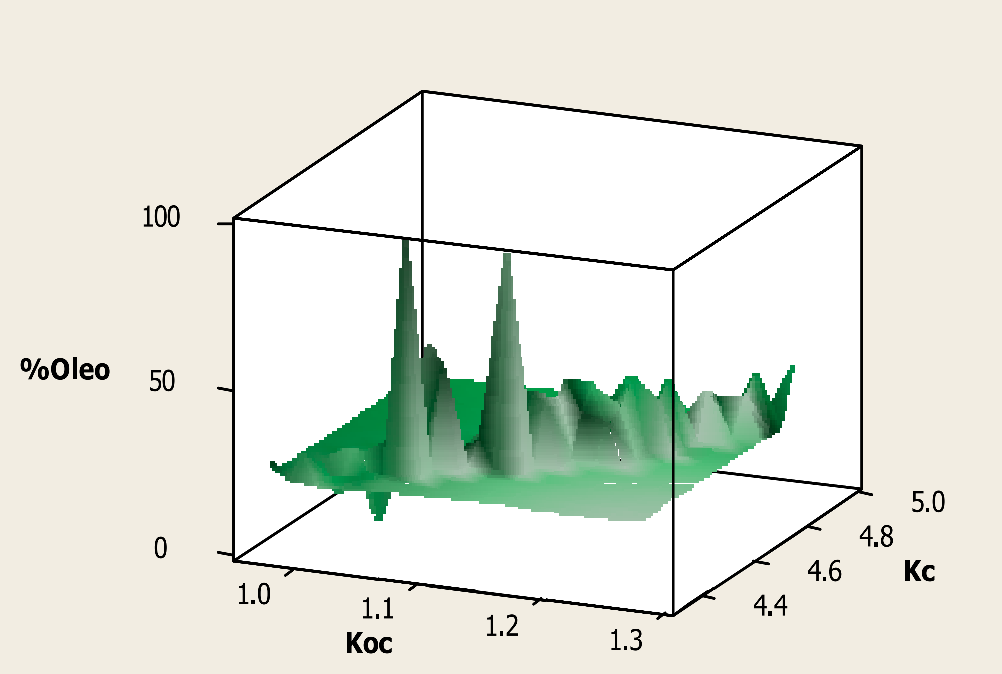


Figure 12: Correlations between the rheological paramenters of Casson with the percentage of oil extracted.

3. Discussion

Figure 1 shows that, for the *Chlorella* sp microalgae suspension obtained from different process conditions, their apparent viscosity has influenced the increase in the rotation frequency (which is directly proportional to shear rate) in all cases. On the other hand, during the implementation of the three repetitions for measuring viscosity, for the same process condition, it could be observed that the apparent viscosity values were not so close, which indicates that there is evidence that such suspension presents a certain thixotropy, i.e. their apparent viscosity decreased over time for a given rotation frequency, which is possibly due to the sedimentation of suspended particles. Such observation was evidenced by analyzing the degree of thixotropy of the *Chlorella* sp microalgae suspension: for all process conditions, the thixotropy index resulted in 0.5. These results are consistent with several authors works on algae suspensions in general: the same show the rheological behavior of shear-thickening fluid and an explanation for this behavior is that, as the shear stress increases, the interstitial fluid that lubricates the friction between the particles is unable to fill the spaces, due to an increase in the volume that often accompanies the phenomenon, where there is direct contact between the solid particles, thus causing the apparent viscosity increase [20]. There are few studies that discuss the rheological properties of hydrocolloids, with the vast majority being undertaken on kappa-carrageenan [20, 21, 22, 23, 24].

With respect to eight different process conditions, Figure 1 shows that there is a certain tendency of the fluid to become less viscous when the *Chlorella* sp microalgae suspension is treated with low NO_3 contents, supplementation and lower salinity of the medium (experiments 3 and 8). On the other hand, it can be observed in Figure 1 that the curves are next to each other, suggesting that the different process conditions exert little influence on the viscosity of *Chlorella* sp suspension, regardless of the treatment. Therefore, a more cautious correlation analysis of these three parameters on the material's rheological behavior must be made.

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From Figure 2, it is observed curves with typical features of a shear-thickening fluid [25]. Figure 2 results were also consistent with the comments made by [25], who had reported a similar rheological behavior by analyzing the rheological properties of sulfated galactans of red algae *Meristiella gelidium* and *Gymnogongrus griffithsiae* sp. The graphics in figures 3 to 10 show that the processing time hardly exerted an influence on the rheograms of the *Chlorella* sp microalgae suspension, except for the simultaneous conditions of low salinity and low CO_2 content (experiments 5 and 8), which contribute to a greater sedimentation of suspended particles.

From Table 2 it can be observed that both the Bingham and Casson and Ostwald-de-Waelle (Power Law) models provided good adjustment parameters, showing high coefficient of determination (r^2) values. The results presented in the tables confirm the theory that the *Chlorella* sp microalgae suspension features a shear-thickening fluid rheological behavior, since the behavior index values were higher than 1. In addition, the initial voltage values were null when the data were adjusted to the rheological model of Bingham; when adjusted to the rheological model of Casson, such values were close to zero. At this point, it is worth mentioning that the process did not influence the type of rheological behavior, despite exerting an effect on the consistency index.

With respect to the consistency index, plastic viscosity of Casson and Bingham, and the results of experiment 5 (Table 2) showed a slight variation over process time where, generally speaking, they decreased at first (between the first and second day) which was possibly due to the fact that a shear-thickening behavior is seen in solid particles mixed with highly concentrated suspensions of fluids, and that, in the early days of culturing ,there was no time for the microalgae to multiply to a point where the suspension concentration increased significantly. In experiment 8, it was observed a similar relation between culture time and the rheological behavior of the suspension; however, in this case, there was a greater time interval required for the concentration of biomass to increase significantly in order to change the rheological behavior. In the latter case, biomass has multiplied significantly between the second and third day of culture, which evidences the influence of NO_3 and PO_4 levels and of the amount of supplements on the rheological parameters of the *Chlorella* sp microalgae suspension.

Figure 11 shows that there are several great points between the oil percentage and parameters K_C and K_{0C}, being the best situation represented in point ($K_C = 4,5$; $K_{0C} = 1,1$). In Figure 12, it is evident that point ($n=1,67$; $0,05<K<0,06$) represents the best situation.

4. Materials and Methods

For the study in question, the microalgae *Chlorella* sp from Cabo Frio, RJ (chlor-CF), were used, which were kindly donated by the Biological Oceanography Department in the Oceanographic Institute of the University of São Paulo.

The project is aimed at analyzing the rheological behavior of *Chlorella* sp microalgae suspensions at different culture times in batch reactors in order to optimize all stages of biodiesel production from during its cultivation in photobioreactors.

4.1 Microalgae cultivation for biodiesel production

For each cultivation series, all glassware to be used was washed in running water, rinsed with distilled water and autoclaved at 121ºC for 20 minutes. For the Erlenmeyer flasks, foil sheets were used as cover [26].

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Microalgae inoculation was performed around the flame of a lamp, and they were cultivated in an air-conditioned room. Illumination was provided with 40 W fluorescent lamps. The luminous intensity was measured with a lux meter (AKZO, model AK 308) and the room's temperature was kept constant by a heat & cool air conditioner. The cultivation was conducted in an Environment Laboratory, which consisted of a room that was approximately 8 m^2 with no diffuse light containing a portable heat & cool air conditioner for complete control of artificial lighting and temperature. All reagents used preparing the culture media were of an analytical standard and basic for maintaining the main culture with the following composition: sea salt (1 g/L), $NaNO_3$ (75 g/L), $NaH_2PO_4{\cdot}H_2O$ (5 g/L), $FeCl_3{\cdot}6H_2O$ (3,15 g/L), Na_2EDTA (4,3 g/L), $ZnSO_4{\cdot}7H_2O$ (22,2 mg/L),$MnCl_2{\cdot}4H_2O$ (180 mg/L),$Na_2MoO_4{\cdot}2H_2O$ (6,3 mg/L), $CoCl_2{\cdot}6H_2O$ (10 mg/L),$CuSO_4{\cdot}5H_2O$ (9,8 mg/L),Thiamine (100 mg/L), Cyanocobalamin (0,5 mg/L) and Biotin (0,5 mg/L).

The stock solutions used in the preparation of the culture medium were autoclaved at 110° C with the exception of vitamins (Thiamin, Cyanocobalamin and Biotin), which were filtered through a 0.22 μm mesh. The strain of the microalgae *Chlorella* SP. used in this study was kept in a wooden incubator during a photoperiod which was set by a timer. A luminous intensity of 15 W was provided by a fluorescent lamp.

For maintaining a cell bank, a subculturing was conducted in 125 mL Erlenmeyer flasks with working volume of 100 mL for a photoperiod of 12 h: 12 h (light/dark) and average illuminance of 4.8 klux. The subcultures were made in periods of 10 to 15 days at a ratio of 10 mL of the preceding culture to 90 mL of the new culture medium, and the flasks were shaken manually once a day.

Microalgal growth was accompanied by an analysis of absorbance in a UV-Vis Bel Photonics spectrophotometer, where a wavelength of 570 nm was selected for obtaining the readings, according to [27]. It was set an analytical curve which related the dry biomass concentration (g/L) with absorbance at 570 nm, where the microalgae development was analyzed under different conditions of temperature, salinity, supplemental volume and mass concentration of carbon dioxide, nitrate and phosphate, as shown in Table 1 as follows.

4.2 Rheological analyses of the material

Experimental measurements were carried out with a Brookfield LVDV-3T rheometer with temperature control through a thermostatic bath which circulates around the shirt where the container for storing the fluid during the assay is inserted.

The assays were carried out in triplicate at 25°C, and a new sample was used for each repetition. The measurement system used in this determination was that of concentric cylinders, which consists of two cylinders where only one of them turns to a certain angular velocity while the other one remains motionless. This device is kept at constant rotation speed that corresponds to a certain shear rate, and the shear stress is obtained by measuring the torque on the measuring cylinder, which remains fixed. Consequently, by setting various angular velocities for the rotational cylinder and detecting the corresponding torque on the measuring cylinder, rheological curves could be obtained for a given fluid. As in the initial assays, it was found that the *Chlorella* sp microalgae suspension showed the rheology behavior of a non-Newtonian fluid, thus the analysis of variation of the shear stress as a function of shear rate was deemed interesting. For such a purpose, it was used a cylindrical coaxial SC4-31 spindle which is compatible with the Thermosel and an adapter for small SSA samples with a SC4-13R chamber in LV-Series rheometers made of # 304 stainless steel. This adapter uses 10.0 ml samples.

The device includes software which makes it possible to set time intervals between each reading, while at the same time recording data on shear rate, shear stress, viscosity and temperature. The software increasingly runs the variation of $\dot{\gamma}$ (or decreasingly) up to the highest value (or lowest), then it returns to the values read in the form of a table.

The results were processed with the software ORIGIN 7.0, and the curves obtained were adjusted to the rheological models of Casson, Ostwald-de-Waelle (Power Law) and Bingham, where it is also determined the degree of thixotropy of the material, besides the following statistical parameters:

R^2 - determination coefficient – it measures the proportion of the total average variation explained by the regression, defined as the total quadratic sum.

X^2 - Chi-square – it express the difference between the predicted values for the model and the values obtained experimentally.

The verification of thixotropy existence was made through the evaluation of flow curves obtained by varying the shear rate (or shear stress) over a period of time, by keeping a constant temperature. After the assay, if the fluid is thixotropic or rheopectic, it is noted the presence of hysteresis: when the curve related to the increase in shear rate does not match the curve of its decrease in the rheogram.

The quantification of the thixotropy value was made through direct reading on the equipment, which was set to follow the steps proposed by [28] and followed by [29], which consist in:

i. increasing the shear rate from 0 to γ_M for a period t_1;
ii. Keeping the shear rate γ_M for a period t2;
iii. Reducing the shear rate from γ_M to 0 for a period t1.

4.3 Analysis of the correlation of rheological parameters with the obtained oil percentage

In order to correlate the rheological parameters with the yield obtained from the oil extraction or biomass concentration, a 2^3 response surface analysis was conducted (6 factors with 8 experimental conditions), through the Minitab 17 software.

5. Conclusions

Based on the presented results, it can be concluded that:

- the suspension of the microalgae *Chlorella* sp obtained from different process conditions presented a typical of rheological behavior of a shear-thickening fluid, showing also a certain thixotropy;
- processing time hardly exerted an influence on the rheograms of the suspension of the microalgae *Chlorella* sp, except for the simultaneous conditions of low salinity and low CO_2 content;
- NO_3 and PO_4 contents and the amount of supplements influenced the rheological parameters of the suspension of the microalgae *Chlorella* sp, when in low concentration of CO_2 and low salinity levels.

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