Rheological Characterization of Algal Suspensions for Bioethanol Processing

DOINITA ROXANA CIOROIU[1,2], OANA CRISTINA PARVULESCU[1]*, CLAUDIA IRINA KONCSAG[2], TANASE DOBRE[1], CRISTIAN RADUCANU[1]

[1]University Politehnica of Bucharest, Chemical and Biochemical Engineering Department, 1-3 Gheorghe Polizu Str., 011061, Bucharest, Romania[2] Ovidius University of Constanta, 124 Mamaia Str., 900527, Constanta, Romania

The paper has aimed at studying the rheology of macroalgae aqueous suspensions in the presence of cellulase enzyme relevant to bioethanol processing by a subsequent fermentation. Rheological measurements of aqueous suspensions of Ceramium virgatum and Cladophora vagabunda macroalgae species were performed using a Couette geometry rotational viscosimeter. The effects of operation temperature (t=25, 50 °C), cellulase/dried algae ratio (R=0, 16 U/mg$_{da}$), and algal suspension mass concentration (c=5-15%) on rheological behaviour and parameters were evaluated. Algal suspensions behaved as non-Newtonian fluids obeying either a Bingham plastic linear relationship or an Ostwald-de Waele power law corresponding to a pseudoplastic fluid. Characteristic dynamic viscosity of Bingham plastic fluids were in the range 0.045-0.115 Pa×s for C. virgatum suspensions and 0.021-0.114 Pa×s for C. vagabunda ones, whereas apparent viscosity varied from 0.138 Pa×s to 43.551 Pa×s for C. virgatum and from 0.181 Pa×s to 45.417 Pa×s for C. vagabunda. Data obtained in 8 rheological tests corresponding to a Bingham plastic behaviour of C. vagabunda suspensions, which were processed according to a 2^3 factorial experiment, emphasized an increase in suspension viscosity with all process factors. The results could be useful for optimization of enzymatic hydrolysis process in order to develop efficient and cost effective saccharification and fermentation strategies.

Keywords: macroalga, seaweed, algal suspension, rheology, non-Newtonian fluid, Bingham plastic, power law fluid

Nowadays, in the context of fossil fuel depletion, concern over energy security and environmental degradation due to carbon dioxide emissions, the replacement of conventional fuels with biomass-based ones is an imperative issue. Considerable research has recently focused on the third-generation algal biomass, since the first-generation biomass (edible crops, sugars, and starches) has received criticism considering the food *vs.* fuel debate, whereas the second-generation feedstock (lignocellulosic vegetal material including non-edible crops, forestry and agriculture wastes) commonly involves a high cost for lignin removal and a low biofuel yield [1-9]. Algae are divided into unicellular or simple-multicellular microalgae and multicellular macroalgae (seaweeds). Macroalgae and most microalgae are able to convert photosynthetically the sunlight, carbon dioxide, and nutrients from water to various compounds. Algae species offer some considerable advantages over terrestrial biomass, *e.g.*: (i) lower risk of competition with food/feed, (ii) higher values of photosynthetic efficiency, biomass production rate per unit area, and biofuel yield, (iii) very low percentage of lignin, (iv) no land, fresh water, fertilizer, and pesticides for cultivation, (v) easy adaptability in a wide range of climatic conditions [1-13].

Microalgae are rich in lipids (commonly 10-20% dry wt.) and are valuable sources of biodiesel, whereas macroalgae mainly consist of carbohydrates (commonly 42-77% dry wt.) and are suitable to be used in fermentation process for bioethanol production after enzymatic hydrolysis [3-13]. Algae-based bioethanol and biodiesel are renewable, sustainable, effective, and eco-friendly liquid biofuels which can replace gasoline and diesel, respectively, and are able to meet the global fuel demand [8,9].

Rheological characterization of algal suspensions is essential for a large scale production of economic liquid biofuels, because the rheological properties of suspensions affect the pumping and mixing power in the system [2,10,14]. Studies on rheology of microalgae suspensions emphasized that, below a critical biomass concentration, most suspensions exhibited a Newtonian behaviour, whereas above a certain concentration, some suspensions behaved as non-Newtonian fluids [10,14,15]. There are no studies in the related literature on rheological characterization of macroalgae suspensions for the production of bioethanol.

Macroalgae are classified into three major groups depending on their pigmentation, *i.e.*, red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyta). They contain high amounts of polysaccharides, as follows [3,5,8]: (i) red macroalgae contain mainly agar (up to 52% dry wt.) and carrageenan (up to 75% dry wt.) but also cellulose, (ii) green algae are rich in cellulose (up to 52% dry wt.) but are also composed of starch, ulvan, and mannan (*i.a.*), and (iii) brown algae are rich in alginate (up to 40% dry wt.) but contain also other compounds including laminarin and cellulose. Polysaccharides conversion to bioethanol generally requires three steps, *i.e.*, pretreatment, enzymatic hydrolysis, and fermentation. The first step is commonly a thermal acid pretreatment which aims at increasing the enzymatic digestibility of biomass prior to enzymatic hydrolysis. By hydrolysis with specific enzymes, commonly cellulase and commercial enzyme complexes containing cellulase (*e.g.*, Celluclast 1.5 L, Viscozyme L, Cellulase 22119, Ultraflo L), the polysaccharides are converted to simple sugars which are further used as substrate for fermentation process in the presence of suitable microorganisms, usually *Saccaromyces cerevisiae* [3-8,11-13]. Optimization of enzymatic hydrolysis process is essential for developing efficient and inexpensive saccharification and fermentation strategies [12]. The yield

* email: oana.parvulescu@yahoo.com

Table 1

EFFECT OF PROCESS FACTORS ON RHEOLOGICAL PARAMETERS OF ALGAL SUSPENSIONS

Exp.	t (°C)	R (U/g_{da})	c (%)	τ (Pa)	Non-Newtonian fluid model: BINGHAM η (Pa·s)	BINGHAM τ_0 (Pa)	POWER LAW K (Pa·s^n)	POWER LAW n	POWER LAW η_{app} (Pa·s)	R^2	RMSE (Pa)
C. virgatum											
1	25	0	5	0.876-7.575	0.045	1.146	-	-	-	0.994	0.146
2			10	0.751-14.022	0.092	0.529	-	-	-	0.995	0.259
3			15	3.756-11.268	-	-	4.304	0.218	0.138-7.404	0.995	0.143
4		16	5	2.316-11.644	0.115	2.227	-	-	-	0.997	0.162
5			10	2.128-10.517	0.107	2.244	-	-	-	0.995	0.185
6			15	2.066-10.767	0.108	2.109	-	-	-	0.998	0.130
7	50	0	5	20.533-31.738	-	-	21.016	0.092	0.388-39.444	0.985	0.391
8			10	20.846-32.928	-	-	20.959	0.102	0.405-39.051	0.981	0.495
9			15	20.658-32.928	-	-	20.862	0.102	0.403-38.884	0.982	0.465
10		16	5	20.658-29.798	0.111	21.013	-	-	-	0.995	0.198
11			10	22.223-26.918	-	-	22.408	0.041	0.332-43.551	0.981	0.202
12			15	22.035-28.045	-	-	22.377	0.051	0.346-43.203	0.993	0.156
C. vagabunda											
13	25	0	5	0.689-10.016	0.021	1.181	-	-	-	0.991	0.203
14			10	2.504-5.822	0.039	2.734	-	-	-	0.986	0.113
15			15	20.846-33.178	-	-	4.081	0.291	0.181-6.669	0.993	0.147
16		16	5	1.878-10.517	0.114	1.749	-	-	-	0.993	0.237
17			10	1.941-10.955	0.109	2.052	-	-	-	0.995	0.195
18			15	2.504-11.143	0.104	2.446	-	-	-	0.996	0.162
19	50	0	5	23.287-30.236	0.083	23.591	-	-	-	0.991	0.171
20			10	23.851-31.926	0.100	24.069	-	-	-	0.996	0.156
21			15	22.536-29.735	-	-	23.547	0.052	0.366-45.417	0.988	0.232
22		16	5	20.032-25.416	0.061	20.332	-	-	-	0.986	0.175
23			10	21.034-26.542	0.064	21.295	-	-	-	0.990	0.159
24			15	22.849-29.359	0.078	22.992	-	-	-	0.997	0.103

of fermentable sugars obtained by enzymatic hydrolysis depends on various factors including type and concentration of biomass and enzyme, incubation time, process temperature and *p*H [4,6-8,12].

This paper has aimed at studying the influence of biomass concentration and operating temperature on the rheological properties of macroalgae aqueous suspensions with and without cellulase. Two macroalgae species, *i.e.*, *Ceramium virgatum* (red) and *Cladophora vagabunda* (green), which are abundant along the Romanian coast of Black Sea [16-20], were selected as biomass.

Experimental part

Materials

C. virgatum and *C. vagabunda* species were harvested on the Black Sea shore after a summer storm, washed with distilled water in order to remove the sand and impurities from the surface, then dried to a constant weight at 45°C and ground into a powder using a grinder. A commercial *Aspergillus niger* cellulase purchased from Sigma (Sigma-Aldrich, Germany) was used for enzymatic hydrolysis.

Procedure

Three aqueous suspensions of dried algae (da) at mass concentrations of 5, 10, and 15% (g_{da}/100g) were prepared for each algal species. Each suspension was halved into 2 parts and cellulase at a concentration of 16 U/mg_{da} was added into one of them, resulting in 3 suspensions with cellulase and 3 without cellulase. One unit of cellulase (U) represents the amount of enzyme which liberates 1μmole of glucose from cellulose in 1 hr at *p*H 5.0 and 37 °C (2 h incubation time) [21]. The samples with cellulase were kept in the dark at 25°C for 24 h.

Samples from all 6 suspensions with and without cellulase were rheological tested at two levels of temperature, *i.e.*, 25 and 50°C, using a Rheotest 2 rotational viscosimeter (MLW, Germany) equipped with coaxial cylinders. Prior to rheological measurements, the suspensions were mixed in the dark at 25°C for 2 h and then analyzed by means of an IOR ML-4M optical microscope (IOR, Romania).

Rheological tests were conducted at two values of operation temperature (t=25, 50 °C), two values of cellulase/dried algae ratio (R=0, 16 U/mg_{da}), and three levels of algal suspension mass concentration (c=5, 10, 15%), resulting in 12 tests for each alga species (table 1).

Results and discussions

Optical microscopy tests

Microscopic images of *C. virgatum* and *C. vagabunda* species suspensions without cellulase at 25°C and different values of algal suspension mass concentration are depicted in figures 1 and 2. Particles less than 500 μm can be identified in figures 1a and 2a for 5% algae concentration, whereas fine crowded particles are observed in figures 1b

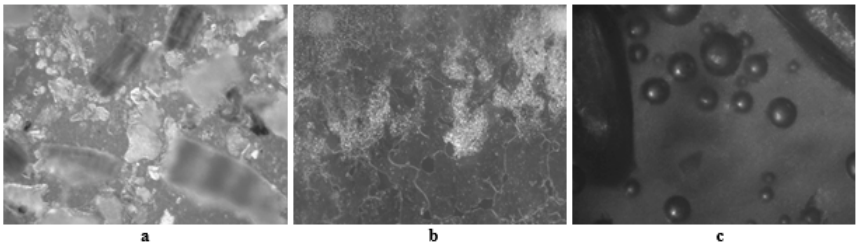

Fig. 1. Microscopic images (100x magnification) of *C. virgatum* suspensions without cellulase at 25 °C:(c) (a) 5%; (b) 10%; (c) 15%.

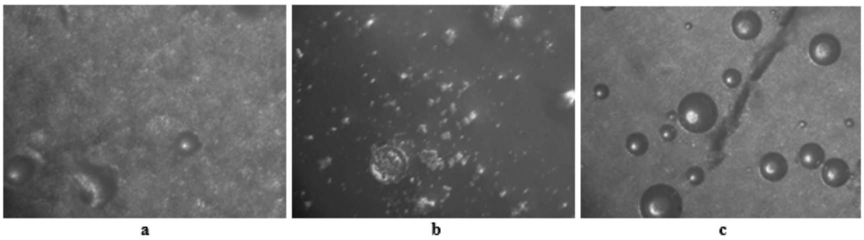

Fig. 2. Microscopic images (100x magnification) of *C. vagabunda* suspensions without cellulase at 25 °C: (a)5%; (b) 10%; (c) 15%

and 2b for 10% concentration, probably as an effect of extraction process. Some extracted compounds, possibly lipids, can be seen in figures 1c and 2c for the highest concentration of algal suspension.

Rheological tests

Rheological curves depicted in figures 3 and 4, describing the variation of shear stress, τ(Pa), depending on shear rate, γ (s^{-1}), for suspensions of both algal species emphasize a non-Newtonian behaviour under various operation conditions. The dependence between τ and γ was expressed either by the linear relationship corresponding to a Bingham plastic (eq. (1)), where η (Pa×s) is the dynamic viscosity and τ_0 (Pa) the yield stress, or by the Ostwald-de Waele power law (eq. (2)) corresponding to a pseudoplastic fluid (n<1), where K (Pa×s^n) represents the flow consistency index and n the flow behaviour index. Apparent viscosity of a pseudoplastic fluid, η_{app} (Pa×s), was calculated depending on γ using eq. (3). Values of characteristic rheological parameters of Bingham plastic and power law models at various levels of process factors are summarized in table 1, where γ=0.5-146 s^{-1} for exp. 1 and exp. 2 (5 and 10% suspensions of *C. virgatum* without cellulase), γ=0.5-437 s^{-1} for exp. 13 (5% suspension of *C. vagabunda* without cellulase), and γ=0.5-81 s^{-1} for the other experiments.

$$\tau = \eta\gamma + \tau_0 \qquad (1)$$

$$\tau = K\gamma^n \qquad (2)$$

$$\eta_{app} = K\gamma^{n-1} \qquad (3)$$

Plots in figure 3 and data specified in table 1 for *C. virgatum* suspensions emphasize the following aspects: (i) for rheological measurements performed at 25 °C, the suspensions without cellulase containing 5% and 10% dried biomass behave as Bingham plastics, whereas the most concentrated suspension (15%) obeys the Ostwald-de Waele power law; the dynamic viscosity of 5% suspension (0.045 Pa×s) is about two times lower than that of 10% suspension (0.092 Pa×s), whereas its yield stress (1.146 Pa) is about two times higher than that of 10% suspension (0.529 Pa); the apparent viscosity of 15% suspension decreases from 7.404 Pa×s (at γ=0.5 s^{-1}) to 0.138 Pa×s (at γ=81 s^{-1}) corresponding to K=4.304 Pa×s^n and n =0.218; (ii) all suspensions prepared with cellulase obey a Bingham plastic linear relationship at 25 °C and have almost identical values of dynamic viscosity (≈0.1 Pa×s) and yield stress (≈2 Pa); (iii) for rheological measurements conducted at 50 °C, the suspensions without cellulase obey the Ostwald-de Waele power law and have almost identical values of K (≈21 Pa×s^n), n (≈0.1), and η_{app} (from about 0.4 Pa×s to 39 Pa×s); the values of η_{app} at 50 °C for 15% suspension are 3-6 times higher than those obtained at 25 °C (fig. 5a); (iv) the suspension containing 5% biomass and cellulase behaves as a Bingham plastic (η=0.111 Pa×s and τ_0=21.013 Pa) at 50 °C, whereas the more concentrated suspensions are power law fluids (K=22.408 Pa×s^n, n=0.041, η_{app}=0.332-43.551 Pa×s for 10% suspension with cellulase, and K=22.377 Pa×s^n, n=0.051, η_{app}=0.346-43.203 Pa×s for 15% suspension with cellulase).

The effects of process factors on rheological behaviour and parameters of *C. vagabunda* suspensions, evaluated based on data presented in figure 4 and table 1, are as follows: (i) for rheological measurements performed at 25 and 50 °C, the suspensions without cellulase containing 5 and 10% dried biomass behave as Bingham plastics, whereas the most concentrated suspension obeys the Ostwald-de Waele power law; (ii) the values of rheological parameters of 5% suspension without cellulase (η=0.021 Pa×s and τ_0=1.181 Pa) at 25 °C are about two times lower than those of 10% suspension without cellulase (η=0.039 Pa×s and τ_0=2.734 Pa); the dynamic viscosities of 5% and 10% suspensions without cellulase are about two times lower and than those obtained for *C. virgatum* suspensions under the same conditions; the values of apparent viscosity of 15% suspension without cellulase at 25°C decreases from 6.669 Pa×s at γ=0.5 s^{-1} to 0.181 Pa×s at γ=81 s^{-1} (corresponding to K=4.081 Pa×s^n and n=0.291) and these values are almost similar to those of *C. virgatum* suspension under the same conditions (fig. 5); (iii) characteristic rheological parameters of 5% suspension without cellulase (η=0.083 Pa×s and τ_0=23.591 Pa) at 50 °C are slightly lower those of 10% suspension without cellulase (η=0.100 Pa×s and τ_0=24.069 Pa) and these values of η and τ_0 are significantly larger than those obtained at 25 °C (up to 4 times and 20 times, respectively); the values of apparent viscosity of 15% suspension without cellulase at 50 °C decrease from 45.417 Pa×s at γ=0.5 s^{-1} to 0.366 Pa×s at γ=81 s^{-1} (corresponding to K=23.547 Pa×s^n and n =0.052) and these values, that are 2-7 times higher than those obtained at 25 °C (fig. 5b), are almost similar to those of *C. virgatum* suspension under the same conditions (fig. 5); (iv) for rheological measurements performed at 25 and 50 °C, all suspensions prepared with cellulase behave as Bingham plastics; (v) the suspensions with cellulase have almost identical values of dynamic viscosity (≈0.1 Pa×s) and yield stress (≈2 Pa) at 25 °C, which are almost similar with those obtained for *C. virgatum* suspensions under the same conditions, whereas the values of their rheological parameters (η=0.061-0.078 Pa×s and τ_0=20.332-22.992 Pa) at 50 °C slightly increase with an increase in suspension concentration.

An increase in algal suspension viscosity at higher value of operation temperature, revealed by results presented in table 1 and figure 5, is the effect of an increase in concentration of extracted compounds. The values of determination coefficient (R^2=0.981-0.998) and root mean square error (RMSE=0.103-0.495 Pa) specified in table 1 indicate a good agreement between experimental and predicted data.

Table 2
EXPERIMENTAL MATRIX FOR 2^3 FACTORIAL EXPERIMENT

Exp.	*t* (°C)	*R* (U/mg$_{da}$)	*c* (%)	x_1	x_2	x_3	$y_1=\eta$ (Pa·s)	$y_2=\tau_0$ (Pa)
13	25	0	5	-1	-1	-1	0.021	1.181
14	25	0	10	-1	-1	1	0.039	2.734
16	25	16	5	-1	1	-1	0.114	1.749
17	25	16	10	-1	1	1	0.109	2.052
19	50	0	5	1	-1	-1	0.083	23.591
20	50	0	10	1	-1	1	0.100	24.069
22	50	16	5	1	1	-1	0.061	20.332
23	50	16	10	1	1	1	0.064	21.295

Statistical model

The results obtained in 8 rheological tests, *i.e.*, 13, 14, 16, 17, 19, 20, 22, and 23 in table 1, corresponding to a Bingham plastic behaviour of *C. vagabunda* suspensions, were processed according to the characteristic procedure of a 2^3 factorial experiment [22-27]. Operation temperature (*t*=25, 50°C), cellulase/dried algae ratio (*R*=0, 16 U/mg$_{da}$), and algal suspension mass concentration (*c*=5, 10%) were selected as process factors, whereas the dynamic viscosity of algal suspension ($y_1=\eta$) and yield stress ($y_2=\tau_0$), were the process dependent variables (responses). The dimensionless values of process factors (x_1, x_2, and x_3) were determined by eqs. (4)-(6), where t_{cp}=37.5 °C, R_{cp}=8 U/mg$_{da}$, and c_{cp}=7.5% are natural factor values within the centre of experimental plan (centre-points). The values of process factors and responses are presented in table 2.

$$x_1 = \frac{t-37.5}{12.5} \quad (4) \qquad x_2 = \frac{R-8}{8} \quad (5) \qquad x_1 = \frac{c-7.5}{2.5} \quad (6)$$

The statistical model is described by the system of regression equations (7), where $\beta_{i,j}$ (*i*=0, 1, 2, 3, 12, 13, 23, 123, and *j*=1, 2) are regression coefficients. The coefficients β_{ij} were determined based on the characteristic procedure of a 2^3 factorial experiment and eqs. (8) and (9), expressing the dependency between the process responses and dimensionless factors, were obtained.

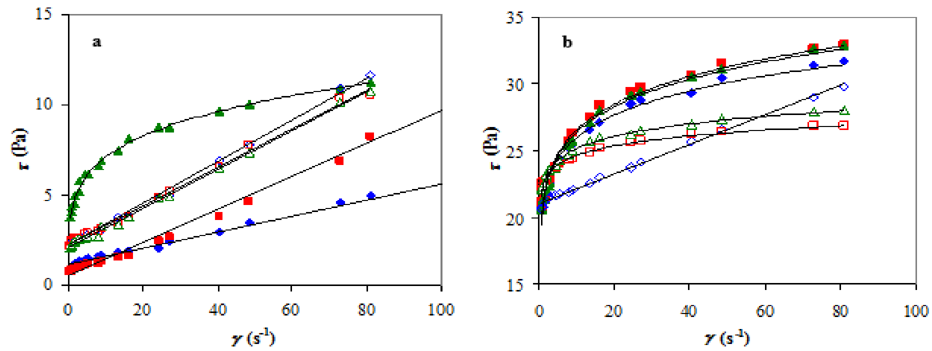

Fig. 3. Shear stress *vs.* shear rate at 25 °C (a) and 50 °C (b) for *C. virgatum* suspensions (bullets: experimental, line: predicted): 5% (♦), 10% (■), 15% (▲), 5%+cellulase (◊), 10%+cellulase ( ), 15%+cellulase (△).

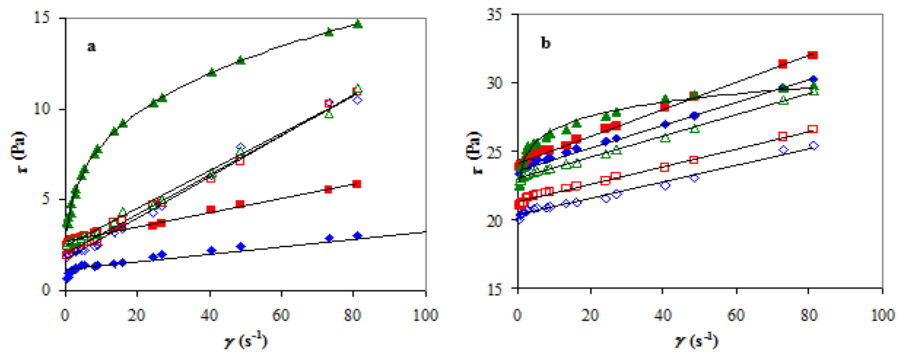

Fig. 4. Shear stress *vs.* shear rate at 25 °C (a) and 50 °C (b) for *C. vagabunda* suspensions (bullets: experimental, line: predicted): 5% (♦), 10% (■), 15% (▲), 5%+cellulase (◊), 10%+cellulase ( ), 15%+cellulase (△).

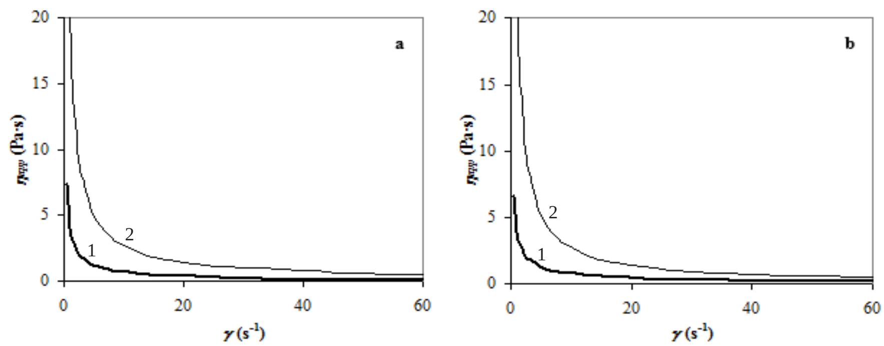

Fig. 5. Apparent viscosity *vs.* shear rate at 25 °C (1) and 50 °C (2) for 15% *C. virgatum* (a) and 15% *C. vagabunda* (b) suspensions without cellulase

$$y_j = \beta_{0,j} + \beta_{1,j}x_1 + \beta_{2,j}x_2 + \beta_{3,j}x_3 + \beta_{12,j}x_1x_2 + \beta_{13,j}x_1x_3 + \beta_{23,j}x_2x_3 + \beta_{123,j}x_1x_2x_3 \quad (7)$$

$$y_1 = \eta = 0.074 + 0.003x_1 + 0.013x_2 + 0.004x_3 - 0.028x_1x_2 + 0.001x_1x_3 - 0.005x_2x_3 + 0.001x_1x_2x_3 \quad (8)$$

$$y_2 = \tau_0 = 12.125 + 10.196x_1 - 0.768x_2 + 0.412x_3 - 0.740x_1x_2 - 0.052x_1x_3 - 0.096x_2x_3 + 0.217x_1x_2x_3 \quad (9)$$

In order to test the significance of statistical model regression coefficients, four centre-point runs (N_{cp}=4) were selected, for which the response values were assumed as follows:

$y_{j,1,cp} = \beta_{0,j} + 3\sigma_{y_j,cp}$ (10), $y_{j,2,cp} = \beta_{0,j} + \sigma_{y_j,cp}$ (11), $y_{j,3,cp} = \beta_{0,j} - \sigma_{y_j,cp}$ (12), and

$y_{j,4,cp} = \beta_{0,j} - 2\sigma_{y_j,cp}$ (13) for j=1; $y_{j,1,cp} = \beta_{0,j} + 0.5\sigma_{y_j,cp}$ (14), $y_{j,2,cp} = \beta_{0,j} + 0.2\sigma_{y_j,cp}$ (15),

$y_{j,3,cp} = \beta_{0,j} - 0.1\sigma_{y_j,cp}$ (16), and $y_{j,4,cp} = \beta_{0,j} - 0.4\sigma_{y_j,cp}$ (17) for j=2.

Standard deviation of $y_j=y_j(c,R,t)$ response function, $\sigma_{y_j,cp}$, was estimated depending on standard deviations of independent variables, $\sigma_{u,cp}$ ($u=c$, R, t), using Kline-McClintock correlation [24,28,29]:

$$\sigma_{y_j,cp} = \sqrt{\left(\left(\frac{\partial y_j}{\partial t}\right)_{t_{cp}} \sigma_{t,cp}\right)^2 + \left(\left(\frac{\partial y_j}{\partial R}\right)_{R_{cp}} \sigma_{R,cp}\right)^2 + \left(\left(\frac{\partial y_j}{\partial c}\right)_{c_{cp}} \sigma_{c,cp}\right)^2} \quad (18)$$

Based on relationships (4)-(6) and considering that $\sigma_{u,cp}=0.05u_{cp}$, eq. (18) turns into:

$$\sigma_{y_j,cp} = \sqrt{\left(\frac{\beta_{1,j}}{12.5}0.05t_{cp}\right)^2 + \left(\frac{\beta_{2,j}}{8}0.05R_{cp}\right)^2 + \left(\frac{\beta_{3,j}}{2.5}0.05c_{cp}\right)^2} \quad (19)$$

The mean value of response, $y_{j,mn,cp}$, reproducibility standard deviation, $\sigma_{rp,j}$, and number of degrees of freedom, ν_1, associated to centre-point runs, were determined using eqs. (10)-(17), (19)-(22).

$$y_{j,mn,cp} = \frac{\sum_{k=1}^{N_{cp}} y_{j,k,cp}}{N_{cp}} = \beta_{0,j} - \frac{\sigma_{y_j,cp}}{N_{cp}} \quad (20)$$

$$\sigma_{rp,j} = \sqrt{\frac{\sum_{k=1}^{N_{cp}} \left(y_{j,k,cp} - y_{j,mn,cp}\right)^2}{\upsilon_1}} \quad (21)$$

$$\upsilon_1 = N_{cp} - 1 = 3 \quad (22)$$

Standard deviation associated to regression coefficients, $\sigma_{\beta j}$, was calculated depending on reproducibility standard deviation, $\sigma_{rp,j}$, and characteristic tests number of 2^3 factorial plan (N=8) by eq. (23).

$$\sigma_{\beta,j} = \frac{\sigma_{rp,j}}{\sqrt{N}} \quad (23)$$

The parameters needed to test the significance of statistical model regression coefficients are summarized in table 3. Values of Student's random variable, $t_{\beta 1,\varphi}$, estimated by eq. (24), were compared with the theoretical value of Student's variable, $t_{\alpha,\nu i}$ = 3.176, obtained by solving eq. (25), where α=0.05 represents the significance level and Γ function is defined by eq. (26) [24].

$$t_{\beta_{i,j}} = \frac{\left|\beta_{i,j}\right|}{\sigma_{\beta,j}} \quad (24)$$

$$\int_{-t_{\alpha,\upsilon_1}}^{t_{\alpha,\upsilon_1}} \frac{\Gamma\left(\frac{\upsilon_1+1}{2}\right)}{\sqrt{\pi\upsilon_1}\,\Gamma\left(\frac{\upsilon_1}{2}\right)} \left(1+\frac{t^2}{\upsilon_1}\right)^{-\frac{\upsilon_1+1}{2}} dt = 1-\alpha \quad (25)$$

$$\Gamma(z) = \int_0^\infty x^{z-1} e^{-x} dx \quad (26)$$

Regression coefficients satisfying the condition $t_{\beta_{i,j}} > t_{\alpha,\upsilon_1} = 3.176$, which were considered as significant, were as follows: $\beta_{0,j}$, $\beta_{1,j}$, $\beta_{2,j}$, $\beta_{3,j}$, $\beta_{12,j}$, and $\beta_{23,j}$ for j=1; $\beta_{0,j}$, $\beta_{1,j}$, $\beta_{2,j}$, and $\beta_{12,j}$ for j=2. Accordingly, the statistical model described by eqs. (8) and (9) becomes:

$$y_1 = \eta = 0.074 + 0.004x_1 + 0.013x_2 + 0.003x_3 - 0.028x_1x_2 - 0.005x_2x_3 \quad (27)$$

$$y_2 = \tau_0 = 12.125 + 10.196x_1 - 0.768x_2 - 0.740x_1x_2 \quad (28)$$

Regression equations (27) and (28) indicate the following aspects: (i) suspension viscosity (η) increases with all process factors, *i.e.*, temperature (x_1), cellulose/algae ratio (x_2), and suspension concentration (x_3), as well as decreases with an increase in x_1x_2 and x_2x_3 interactions, the effect of x_2 factor and x_1x_2 interaction being significant; (ii) yield stress (τ_0) heavily increases with x_1 and decreases with an increase in x_2 and x_1x_2. Statistical model described by eqs. (27) and (28) may be applied to estimate the values of η and τ_0 rheological parameters of *C. vagabunda* species suspensions at levels of process factors in the ranges considered in the statistical analysis, *i.e.*, t=25-50 °C, R=0-16 U/mg$_{da}$, and c=5-10%.

Table 3
CHARACTERISTIC PARAMETERS OF STATISTICAL MODEL

y_j	$\beta_{0,j}$	$\sigma_{y_j,cp}$	$y_{j,mn,cp}$	$\sigma_{rp,j}$	$\sigma_{\beta j}$
units of y_j					
$y_1=\eta$ (Pa·s)	0.074	0.001	0.074	0.002	0.001
$y_2=\tau_0$ (Pa)	12.125	1.531	12.202	0.593	0.210

Conclusions

Rheological tests of aqueous suspensions of red *C. virgatum* and green *C. vagabunda* macroalgae species were performed using a rotational viscosimeter equipped with coaxial cylinders. The effects of shear rate (γ=0.5-437 s^{-1}), operation temperature (t=25, 50 °C), cellulase/dried algae ratio (R=0, 16 U/mg$_{da}$), and algal suspension mass concentration (c=5-15%) on shear stress were evaluated. Both algal suspensions behaved as non-Newtonian fluids obeying either a Bingham plastic linear relationship or an Ostwald-de Waele power law corresponding to a pseudoplastic fluid.

The effects of process factors on rheological properties of algal suspensions were as follows: (i) for rheological measurements of *C. virgatum* suspensions performed at 25 °C, the most concentrated suspension (15%) without cellulase obeyed the Ostwald-de Waele power law and its apparent viscosity (η_{app}) ranged from 0.138 Pa×s to 7.404 Pa×s, whereas the other algal suspensions with and without cellulase behaved as Bingham plastics characterized by dynamic viscosity (η) in the range of 0.045-0.115 Pa×s and yield stress (τ_0) from 0.529 Pa to 2.244 Pa; (ii) for measurements of *C. virgatum* suspensions conducted at 50 °C, the suspension containing 5% biomass and cellulase behaved as a Bingham plastic (η=0.111 Pa×s and τ_0=21.013 Pa), whereas the other algal suspensions obeyed the Ostwald-de Waele power law (η_{app}=0.332-43.551 Pa×s); the values of η_{app} at 50 °C for 15% suspension are 3-6 times higher than those obtained at 25°C; (iii) for rheological tests performed at 25°C and 50°C, the suspensions with and without cellulase containing 5 and 10% *C. vagabunda* dried biomass behaved as Bingham plastics (η=0.021-0.114 Pa×s and η_0=1.181-24.069 Pa), whereas the most concentrated suspension of *C. vagabunda* obeyed the Ostwald-de Waele power law (η_{app}=0.181-6.669 Pa×s at 25 °C and η_{app}=0.366-45.417 Pa×s at 50 °C). Data obtained in 8 rheological tests corresponding to a Bingham plastic behaviour of *C. vagabunda* suspensions (t=25, 50 °C; R=0, 16 U/mg_{da}; c=5, 10%) were processed according to a 2^3 factorial experiment. Regression equations expressing the variation of rheological parameters with the process factors indicated an increase in suspension viscosity (η) with all process factors and a significant increase in yield stress (τ_0) with operation temperature (t). The results could provide useful information applicable to further studies on the use of red *C. virgatum* and green *C. vagabunda* macroalgae species as renewable, sustainable, effective, and eco-friendly feedstocks for bioethanol production.

Acknowledgments: This work was supported by the project PN-III-P3-3.1-PM-RO-FR no. 79 BM. The authors are grateful to Mariana Bucsoiu, Rodica Anghel and Oana Marin for experimental assistance, kindness, patience, and time.

References

1. ADENLE, A.A., HASLAM, G.E., LEE, L., Energy Pol., **61**, 2013, p. 182.
2. ADESANYA, V.O., VADILLO, D.C., MACKLEY, M.R., J. Rheol., **56**, no. 4, 2012, p. 925.
3. CHEN, H., ZHOU, D., LUO, G., ZHANG, S., CHEN, J., Renew. Sust. Energ. Rev., **47**, 2015, p. 427.
4. CHO, Y.K., KIM, M.J., KIM, S.K., Korean Soc. Biotechnol. Bioeng. J., **28**, no. 6, 2013, p. 366.
5. JUNG, K.A., LIM, S.R., KIM, Y., PARK, J.M., Bioresour. Technol., **135**, 2013, p. 182.
6. SIRAJUNNISA, A.R., SURENDHIRAN, D., Renew. Sust. Energ. Rev., **66**, 2016, p. 248.
7. TRIVEDI, N., GUPTA, V., REDDY, C.R.K., JHA, B., Bioresour. Technol., **150**, 2013, p. 106.
8. TRIVEDI, N., GUPTA, V., REDDY, C.R.K., JHA, B., Marine Macroalgal Biomass as a Renewable Source of Bioethanol (Chapter 11) in Marine Bioenergy Trends and Developments (Eds. S.K. Kim and C.G. Lee), CRC Press, 2015, p. 197.
9. VASSILEV, S.V., VASSILEVA, C.G., Fuel, **181**, 2016, p.1.
10. ZHANG, X., JIANG, Z., CHEN, L., CHOU, A., YAN, H., ZUO, Y.Y., ZHANG, X., Bioresour. Technol., **139**, 2013, p. 209.
11. CHO, H., RA, C.H., KIM, S.K., J. Microbiol. Biotechnol., **24**, no. 2, 2014, p. 264.
12. NEIFAR, M., CHATTER, R., CHOUCHANE, H., GENOUIZ, R., JAOUANI, A., MASMOUDI, A.S., CHERIF, A., AIMS Bioengineering, **3**, no. 3, 2016, p. 400.
13. TRIVEDI, N., REDDY, C.R.K., RADULOVICH, R., JHA, B., Algal Res., **9**, 2015, p. 48.
14. WILEMAN, A., OZKAN, A., BERBEROGLU, H., Bioresour. Technol., **104**, 2012, p. 432.
15. SOULIES, A., PRUVOST, J., CASTELAIN, C., BURGHELEA, T., J. Nonnewton. Fluid Mech., **231**, 2016, p. 91.
16. BECHIR, A., SIRBU, R., PACURAR, M., PODARIU, A.C., MONEA, M., BECHIR, E.S., GHERGIC, D.L., Rev. Chim. (Bucharest), **65**, no. 3, 2014, p. 362.
17. HORINCAR, V.B., PARFENE, G., TYAGI, A.K., GOTTARDI, D., DINICA, R., GUERZONI, M.E., BAHRIM, G., J. Appl. Phycol., **26**, no. 1, 2014, p. 551.
18. MARIN, O., SAVA, D., SAMARGIU, M., Scientific Studies and Research, Series Biology, **24**, no.1, 2015, p. 27.
19. MARIN, O., ABAZA, V., SAVA, D., Marine Research, **43**, 2013, p. 197.
20. MARIN, O., TIMOFTE, F., Atlas of Macrophytes on the Romanian Sea Shore, Boldas, Constanta, 2011.
21.*** http://www.sigmaaldrich.com/catalog/product/sigma/c1184?lang=en&region=RO.
22. DOBRE, T., PARVULESCU, O.C., RODRIGUEZ RAMOS, I., CEATRA, L., STROESCU, M., STOICA, A., MIREA, R., Rev. Chim. (Bucharest), **63**, no. 1, 2012, p. 54.
23. DOBRE, T., STOICA, A., PARVULESCU, O.C., STROESCU, M., IAVORSCHI, G., Rev. Chim. (Bucharest), **59**, no. 5, 2008, p. 590.
24. ION, V.A., PARVULESCU, O.C., DOBRE, T., Appl. Surf. Sci., **335**, 2015, p. 137.
25. PARVULESCU, O.C., DOBRE, T., CEATRA, L., IAVORSCHI, G., MIREA, R., Rev. Chim. (Bucharest), **62**, no. 1, 2011, p. 89.
26. PARVULESCU, O.C., GAVRILA, A.I., DOBRE, T., CEATRA, L., Rev. Chim. (Bucharest), **67**, no. 11, 2016, p. 2254.
27. SANDU OHREAC, B., DOBRE, T., PARVULESCU, O.C., DANCIU, T.D., Rev. Chim. (Bucharest), **65**, no. 5, 2014, p. 582.
28. DOBRE, T., PARVULESCU, O.C., SANCHEZ-MARCANO, J., STOICA, A., STROESCU, M., IAVORSCHI, G., Sep. Purif. Technol., **82**, 2011, p. 202.
29. KLINE, S.J., MCCLINTOCK, F.A., Mech. Eng., **75**, no. 1, 1953, p. 3

Manuscript received: 22.12.2016