A COMPARATIVE STUDY ON RHEOLOGICAL BEHAVIOUR OF AQUEOUS SUSPENSIONS OF *CYSTOSEIRA BARBATA* AND *ULVA LACTUCA* MACROALGAE

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Rheological behaviour of aqueous suspensions of C. barbata and U. lactuca was studied at different values of temperature (25, 50 °C), cellulase/dried macroalgae ratio (0, 16 U/mgdma), and suspension mass concentration (5-15%). At 25 °C, the suspensions without cellulase obeyed the Ostwald-de Waele power law and their apparent viscosities (0.25-15.31 Pa·s for C. barbata and 0.04-5.35 Pa·s for U. lactuca) increased with macroalga concentration, whereas those containing cellulase behaved as Bingham plastics and their viscosities (0.113-0.141 Pa·s) were invariant with macroalga species and concentration. At 50 °C, all suspensions were power law fluids with similar apparent viscosities (0.35-50.09 Pa·s).

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

1. Introduction

Macroalgae (seaweeds) form the main vegetation in the Black Sea and establish a biological balance by fixing carbon and feeding aquatic animals. They are renewable and inexpensive sources of food, feed, soil fertilizers, biofuels, as well as of biochemicals and biomaterials for the food industry, cosmetics, pharmaceuticals, nutraceuticals, and medicine [1-8]. Selection of suitable macroalga species is a key step for specific applications. Besides their composition there are also other factors which should be considered, *e.g.*, photosynthetic efficiency, biomass production rate per unit area, adaptation to different environmental conditions, valuable co-products.

High amounts of polysaccharides are present in the structure of macroalgae, *i.e.*: (i) galactans as carrageenans (up to 75% dry wt.) and agar (up to 52% dry wt.) as well as glucans (cellulose, floridean starch) and xylans in the red

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macroalgae; (ii) cellulose (up to 52% dry wt.), starch (up to 4% dry wt.), ulvans (up to 29% dry wt.), mannan, xylans, and sulphated galactans in the green macroalgae; (iii) alginates (up to 47% dry wt.), laminarans (up to 35% dry wt.), fucoidans (up to 20% dry wt.), and cellulose in the brown ones [1,2,4,5,7].

Biochemical (alcoholic fermentation and anaerobic digestion) and thermochemical (pyrolysis and hydrothermal liquefaction) routes are usually applied to produce biofuels from macroalgae. Bioalcohols (bioethanol, biobuthanol) and biogas (methane-rich gas) are obtained by alcoholic fermentation and anaerobic digestion, respectively, whereas bio-oil, bio-char, and biogas can be produced by pyrolysis of dried algal biomass or hydrothermal liquefaction of wet feedstock.

Macroalgae-based bioethanol is renewable, sustainable, effective, and eco-friendly liquid biofuel which can replace gasoline and is able to meet the global fuel demand [7,8]. Bioethanol can be biochemically produced from red, green, and brown macroalgae by converting their polysaccharides to fermentable sugars and fermentation using suitable microorganisms. The process generally requires three steps, *i.e.*, physico-chemical pretreatment, enzymatic hydrolysis, and fermentation. Pretreatment step, which aims at increasing the enzymatic digestibility of algal biomass, usually consists in thermal acid pretreatment (0.1-0.9 N H_2SO_4 at 100-140 ℃ for 15-120 min). After pretreatment, polysaccharides are converted by enzymatic hydrolysis to simple sugars which are further used as substrate for fermentation process. Cellulase and commercial enzyme complexes (*e.g.*, Celluclast 1.5 L, Cellulase 22119, Viscozyme L) designed for lignocellulosic materials are widely used for enzymatic saccharification process, especially [3,4,6,7,9,10]. Fermentation process occurs in the presence of appropriate microorganisms, usually *Saccaromyces cerevisiae* for red and green macroalgae and *Pichia angophorae* or *Pichia stipitis* for brown ones [1,4,6,7,9,10]. Enzymatic hydrolysis and fermentation occurs either separately or simultaneously.

In order to develop effective saccharification and fermentation strategies, optimization of enzymatic hydrolysis process is needed [10]. Type and concentration of algal species and enzyme, process temperature and pH, can heavily affect the yield of fermentable sugars produced during the enzymatic hydrolysis [6,7,9,10]. Moreover, the pumping and mixing power in the equipments used to produce bioethanol can be considerably influenced by the rheological properties of algal suspensions [11,12]. Accordingly, an analysis of rheological behaviour of algal suspensions is imperative to design, scale up, operate, and optimize the process.

This paper contains a study on the rheological behaviour of aqueous suspensions of *Cystoseira barbata* and *Ulva lactuca* species at different levels of macroalga concentration, operating temperature, and cellulase content. Both algal

species are abundant along the Romanian coast of Black Sea [13,14]. *C. barbata* (Fig. 1a) is a strongly branched brown macroalga with a great adaptability to natural and anthropic factors, which can have a height up to 1.5 m [14]. *U. lactuca* (Fig. 1b) is a green macroalga species that is mainly found on the rocks from sheltered areas of the seaside and can reach sizes ranging from 5 to 40 cm [14].

Fig. 1. Images of macroalgae: (**a**) *C. barbata*; (**b**) *U. lactuca*.

2. Experimental

Materials

Cystoseira barbata and *Ulva lactuca* were collected on the Romanian seaside coast in September 2015. The fresh algal species were washed with seawater and distilled water in order to remove the sand and epiphytes. Then, the samples were dried in an UNB 200 Universal Oven (Memmert, Germany) for 48 hr at 45 °C and further ground at a particle size less than 630 μm. A commercial *Aspergillus niger* cellulase (0.8 U/mg) produced by Sigma-Aldrich (Germany) was used for the biochemical treatment of algal biomass.

Equipment and procedure

Six aqueous suspensions of dried macroalgae (dma) at different mass concentrations (c=5, 10, 15%) were prepared for each macroalga species, three of them with cellulase at a concentration of 16 U/mg$_{dma}$ and three without cellulase. The algal suspensions were introduced in a shaker at 25 °C for 24 hr and further were rheological tested by means of a Rheotest 2 rotational viscosimeter (MLW, Germany) equipped with coaxial cylinders. Rheological measurements were performed at different levels of temperature (t=25, 50 °C), cellulase/dried macroalga ratio (R=0, 16 U/mg$_{dma}$), and algal suspension mass concentration (c=5, 10, 15%), as shown in Table 1. Microscopic structure of powders and suspensions of *C. barbata* and *U. lactuca* was examined at 25 °C using an Olympus Microscope CX21 (OLYMPUS, Germany).

3. Results and discussion

Optical microscopy measurements

Microscopic images of algal powders are shown in Fig. 2, where the dark parts represent the macroalga thalli, whereas those of aqueous suspensions of *C. barbata* and *U. lactuca* prepared without cellulase (t=25 °C, c=5-15%) are illustrated in Figs. 3 and 4, respectively. Fine crowded particles as well as some extracted compounds, possibly lipids, are observed in Figs. 3 and 4 for macroalga concentrations of 10% and 15%.

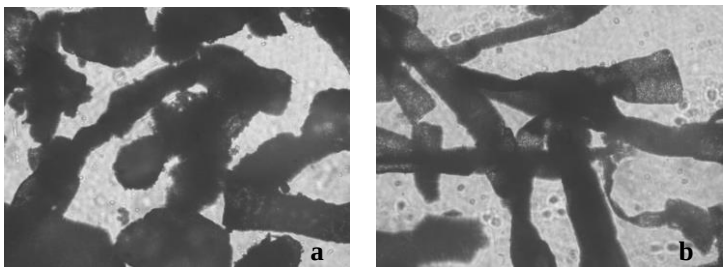

Fig. 2. Microscopic images (100x magnification) of algal powders: (**a**) *C. barbata*; (**b**) *U. lactuca*.

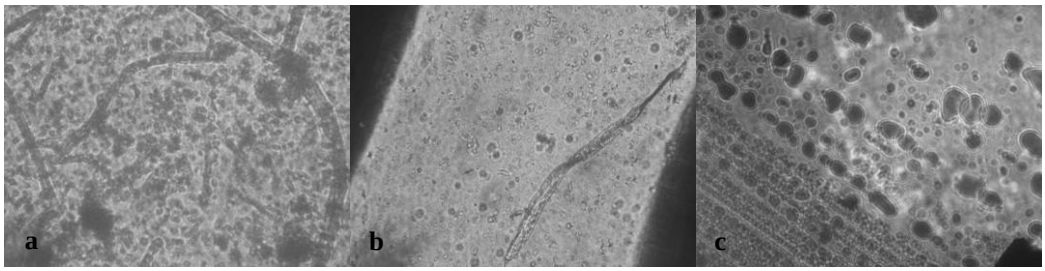

Fig. 3. Microscopic images (100x magnification) of *C. barbata* suspensions without cellulose at 25 °C: (**a**) 5%; (**b**) 10%; (**c**) 15%.

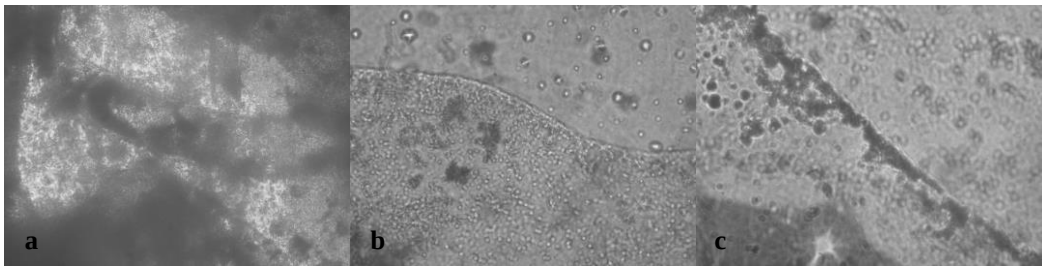

Fig. 4. Microscopic images (100x magnification) of *U. lactuca* suspensions without cellulase at 25 °C: (**a**) 5%; (**b**) 10%; (**c**) 15%.

Rheological measurements

The results of rheological tests performed under various operation conditions, which are shown in Figs. 5 and 6, indicate a non-Newtonian behaviour of *C. barbata* and *U. lactuca* suspensions. The dependence between the shear stress, τ (Pa), and shear rate, γ (s^{-1}), was described either by Eq. (1), which is the Ostwald-de Waele power law for a pseudoplastic fluid, or by Eq. (2)

corresponding to a Bingham plastic, as we have previously reported for aqueous suspensions of *Cladophora vagabunda* and *Ceramium rubrum* macroalgae [15]. Rheological parameters in Eqs. (1) and (2) are as follows: K (Pa·s^n) is the flow consistency index, n the flow behaviour index, η (Pa·s) the dynamic viscosity, and τ_0 (Pa) the yield stress. Values of characteristic rheological parameters of power law and Bingham plastic models are specified in Table 1, where the apparent viscosity of a pseudoplastic fluid, η_{app} (Pa·s), was determined by Eq. (3).

Table 1

Effect of process factors on rheological parameters of algal suspensions

Exp.	t (°C)	R (U/g$_{dma}$)	c (%)	τ (Pa)	Non-Newtonian fluid model				
					BINGHAM		POWER LAW		
					η (Pa·s)	τ_0 (Pa)	K (Pa·s^n)	n	η_{app} (Pa·s)
C. barbata									
1	25	0	5	0.31-35.96	-	-	1.628	0.625	0.25-2.11
2			10	2.57-32.18	-	-	2.399	0.595	0.40-3.18
3			15	8.14-25.66	-	-	9.092	0.248	0.33-15.31
4		16	5	2.00-13.46	0.141	1.934	-	-	-
5			10	2.00-12.02	0.129	2.016	-	-	-
6			15	2.07-11.58	0.117	2.159	-	-	-
7	50	0	5	21.28-28.17	-	-	22.42	0.052	0.35-43.28
8			10	21.53-29.92	-	-	22.55	0.056	0.36-43.38
9			15	22.10-30.49	-	-	22.67	0.066	0.37-43.32
10		16	5	21.28-30.60	-	-	21.21	0.082	0.38-40.08
11			10	23.35-31.49	-	-	25.85	0.045	0.39-50.09
12			15	22.72-30.55	-	-	24.29	0.053	0.38-46.82
U. lactuca									
13	25	0	5	0.31-5.82	-	-	0.722	0.395	0.04-1.10
14			10	0.50-12.21	-	-	0.935	0.534	0.09-1.29
15			15	3.13-10.08	-	-	3.157	0.239	0.11-5.35
16		16	5	1.94-12.02	0.126	2.073	-	-	-
17			10	1.88-10.71	0.113	1.907	-	-	-
18			15	1.88-11.71	0.127	1.846	-	-	-
19	50	0	5	20.91-29.43	-	-	21.02	0.077	0.36-39.87
20			10	21.22-29.66	-	-	21.36	0.075	0.37-40.57
21			15	21.41-30.86	-	-	21.53	0.076	0.37-40.86
22		16	5	20.72-37.50	-	-	21.18	0.130	0.46-38.71
23			10	21.35-30.80	-	-	21.92	0.061	0.35-42.03
24			15	21.91-31.05	-	-	22.35	0.070	0.37-42.58

$$\tau = K\gamma^n,\ n<1 \tag{1}$$

$$\tau = \eta\gamma + \tau_0 \tag{2}$$

$$\eta_{app} = K\gamma^{n-1} \tag{3}$$

Data presented in Table 1 and Figs. 5-7 highlight the following issues: (i) for rheological measurements performed at **25 °C**, the suspensions of *C. barbata* (*Cb*) and *U. lactuca* (*Ul*) **without cellulase** obey the Ostwald-de Waele power law (R^2=0.938-0.991) and their apparent viscosity ($\eta_{app,Cb}$=0.25-15.31 Pa·s and

$\eta_{app,Ul}$=0.04-5.35 Pa·s) increases with an increase in suspension concentration (c=5-15%); (ii) for rheological tests conducted at **25 °C**, the suspensions prepared **with cellulase** behave as Bingham plastics (R^2=0.981-0.995), and the values of dynamic viscosity (η_{Cb}=0.117-0.141 Pa·s and η_{Ul}=0.113-0.127 Pa·s) and yield stress ($\tau_{0,Cb}$=1.934-2.159 Pa and $\tau_{0,Ul}$=1.846-2.073 Pa) are almost invariant with c; (iii) the suspensions **with** and **without cellulase** obey the Ostwald-de Waele power law (R^2=0.962-0.994) at **50 °C**, and they have similar values of apparent viscosity ($\eta_{app,Cb}$=0.35-50.09 Pa·s and $\eta_{app,Ul}$=0.35-42.58 Pa·s) irrespective of c; these values of η_{app} are higher (up to about 20 times for *C. barbata* and 36 times for *U. lactuca*) than those obtained at **25 °C**, for suspensions prepared **without cellulase** (Fig. 6).

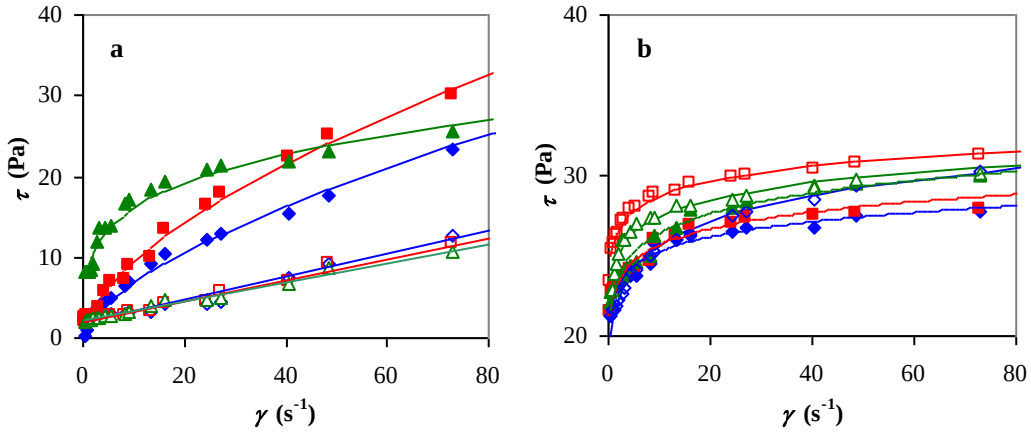

Fig. 5. Shear stress *vs.* shear rate at 25 °C (**a**) and 50 °C (**b**) for *C. barbata* suspensions (bullets: experimental, line: predicted): 5% (♦), 10% (■), 15% (▲), 5%+cellulase (◊), 10%+cellulase (□), 15%+cellulase (Δ).

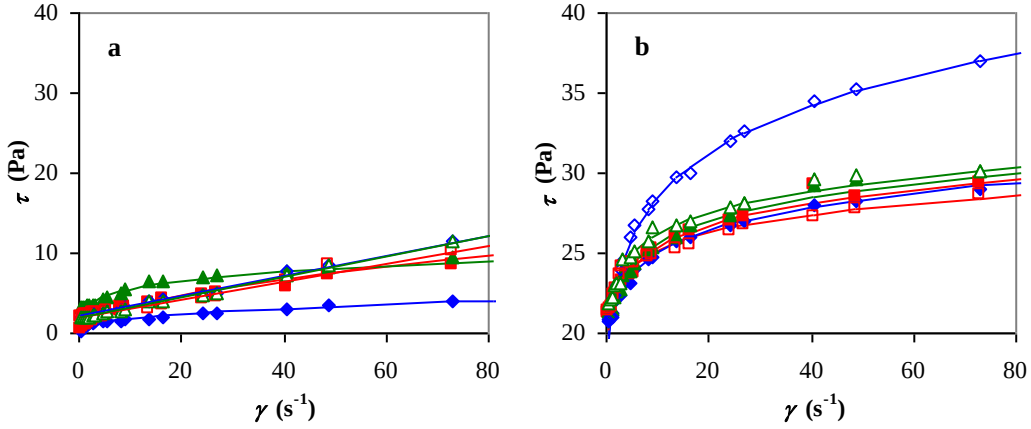

Fig. 6. Shear stress *vs.* shear rate at 25 °C (**a**) and 50 °C (**b**) for *U. lactuca* suspensions (bullets: experimental, line: predicted): 5% (♦), 10% (■), 15% (▲), 5%+cellulase (◊), 10%+cellulase (□), 15%+cellulase (Δ).

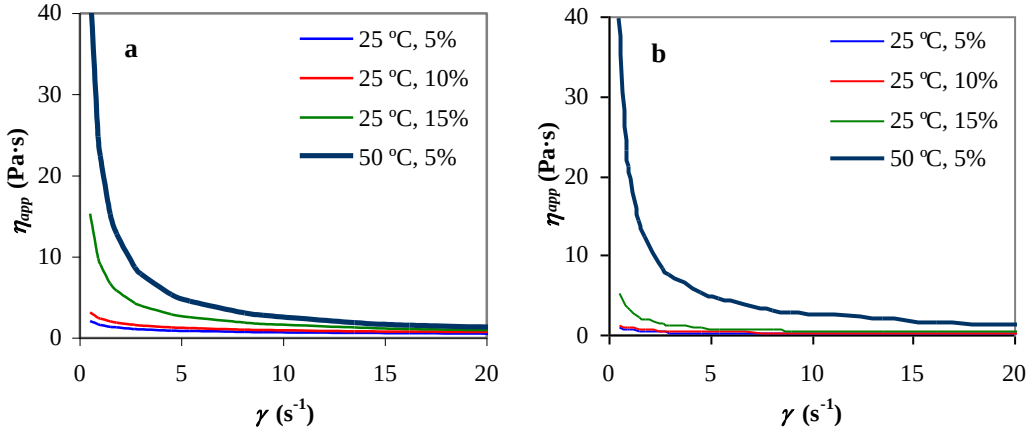

Fig. 7. Apparent viscosity *vs.* shear rate for *C. barbata* (**a**) and *U. lactuca* (**b**) suspensions without cellulase.

4. Conclusions

Rheological behaviour of aqueous suspensions of two types of macroalgae from Black Sea, *i.e.*, *C. barbata* and *U. lactuca*, was studied. The influence of shear rate (0.5-146 s^{-1}), operation temperature (25, 50 °C), cellulase/dried macroalgae ratio (0, 16 U/mg_{dma}), and algal suspension mass concentration (5-15%) on shear stress was analyzed. Algal suspensions behaved as non-Newtonian fluids under the conditions considered in the experimental tests.

For rheological measurements performed at **25 °C**, algal suspensions **without cellulase** obeyed the Ostwald-de Waele power law and their apparent viscosity (0.25-15.31 Pa·s for *C. barbata* and 0.04-5.35 Pa·s for *U. lactuca*) increased with suspension concentration, whereas the suspensions prepared **with cellulase** behaved as Bingham plastics and the values of dynamic viscosity (0.113-0.141 Pa·s) and yield stress (1.846-2.159 Pa) were almost invariant with macroalga species and suspension concentration. Algal suspensions **with** and **without cellulase** obeyed the Ostwald-de Waele power law at **50 °C** and they had similar values of apparent viscosity (0.35-50.09 Pa·s) irrespective of macroalga species and suspension concentration. These findings could provide helpful information for further studies on the use of *C. barbata* and *U. lactuca* macroalgae as renewable and green feedstocks for bioethanol production.

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