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Production and Rheological Characterization of Biopolymer of *Sphingomonas capsulata* ATCC 14666 Using Conventional and Industrial Media

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Abstract

This work was aimed at the production and rheological characterization of biopolymer by *Sphingomonas capsulata* ATCC 14666, using conventional and industrial media. The productivity reached the maximum of 0.038 g/L·h, at 208 rpm and 4% (w/v) of sucrose. For this condition, different concentrations of industrial medium were tested (2.66, 4, 6, and 8%). The best productivity was obtained using pretreated molasses 8% (w/v) (0.296 g/L·h), residue of textured soybean protein 6% (wt/v) (0.244 g/L·h) and crude molasses 8% (w/v) (0.192 g/L·h), respectively. Apparent viscosity presented similar results when compared with those in the literature for other biopolymers.

Index Entries: Agroindustry waste; biopolymers; experimental design; rheology; *Sphingomonas capsulata*.

Introduction

The production of microbial biopolymers, also known as extracellular polymeric substances, is an alternative to gums extracted from plants, because they present physico-chemical properties of high industrial interest, which are essential to define their applications *(1)*. In the past two decades significant progress was observed, concerning the identification, characterization, and utilization of microbial polysaccharides *(2)*. These

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compounds have the ability to form gels and viscous solutions in aqueous medium, even at low concentrations *(3)*.

Bacteria of the genus *Sphingomonas* produce biopolymers, like gelan, welan, ramsan, and diutan, which present gelation characteristics, high viscosity, and better thermal stability than other gums. Thus, such gums are interesting for the food, pharmaceutical, and petrochemical industries *(4–6)*.

Glucose and sucrose are preferentially used as carbon sources for biopolymer production. However, the use of low-cost substrates, such as agroindustry wastes and byproducts in fermentation processes, may favor the reduction of production costs and minimize environmental problems *(7)*. Some alternative sources have been suggested, such as sugarcane molasses, soybean industry wastes, cheese whey, and others *(8–11)*. Molasses is a byproduct of sugar production and a very economical carbon source in bioprocesses *(8,10)*. A byproduct of soybean processing is a protein rich source and is sold as a low-value residue. Depending on the process, it may contain a considerable amount of carbohydrates. Cheese whey has been studied as an alternative source of xanthan gum production *(12,13)*.

Industrial media are very complex, and some of their components may be responsible for inhibiting polysaccharide production or hindering its recovery and purification *(14)*. Contaminants, such as, heavy metals and specific inhibitors may be removed with special pretreatments *(10)*. These pretreatments may clarify the medium without affecting fermentation performance and then guarantee better product extraction and purification *(14)*.

The chemical structures of each biopolymer determine its rheological characteristics and thus, their applications *(1)*. Solutions of microbial polysaccharides present pseudoplastic behavior, i.e., the viscosity decreases with the increase in shear rate *(15)*. In this context, the aim of this work was to study the production and characterization of biopolymers produced by *Sphingomonas capsulata* ATCC 14666, using conventional and industrial media (raw and pretreated molasses and textured soybean protein waste).

Material and Methods

Microorganism

S. capsulata ATCC 14666 was maintained at 4°C, in Yeast Malt (YM) medium containing 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, and 20 g/L agar.

Fermentation

The production of cells was carried out in 50 mL liquid YM medium in 300-mL Erlenmeyer flask, in two steps. First a preinoculum was prepared, inoculating a loopful of stock culture in 50 mL of YM medium and incubating at 120 rpm, 28°C ± 2°C, for 17 to 19 h. The inoculum was prepared by the addition of 1 mL of preinoculum culture to 50 mL of YM

Table 1
Values of Coded Levels and Real Values Used in the Experimental Design With Conventional Medium

Coded variable levels	–1.41	–1	0	+1	+1.41
Sucrose (%)	1.18	2	4	6	6.82
Stirring rate (rpm)	152	160	180	200	208

Table 2
Industrial Media

Industrial media	Concentrations
Molasses	4, 6, and 8%
Pretreated molasses	4, 6, and 8%
Aqueous extract of STP waste	2.66, 4, and 6%

medium incubated in an orbital shaker, at 120 rpm, 28°C ± 2°C, for 21 to 23h, when cell concentration reached 10^8 CFU·m/L.

The conventional medium contained sucrose in concentrations according to the experimental design, adding salts to the medium to reach the following concentrations, 1 g/L KNO_3, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L K_2HPO_4, 0.1 g/L $CaSO_4$, 0.05 g/L $NaMoO_4$ (*16*). The production medium was added to the medium containing cells and incubated in an orbital shaker at 28°C ± 2°C, 72 h. The stirring rate was defined by the experimental design. After inoculation, work volume was 150 mL. Table 1 presents the factors and levels investigated in the complete experimental design 2^2, with two axial points and a central point. Productivity was the response variable and the results were statistically analyzed using Statistica 5.1® (StatSoft Inc).

Biopolymer production was carried out using raw and pretreated molasses and an aqueous extract of soybean textured protein waste (STP) (*17*) in different concentrations, according to Table 2. Production medium was added to the medium containing cells and incubated in an orbital shaker at 208 rpm, 28°C ± 2°C. Experimental runs were performed in triplicate, and productivity was used as the response. Results were statistically evaluated using Tukey's test.

Biopolymer Recovery

After fermentation, the broth was centrifuged at 4700*g* for 40 min at 4°C, for cell separation. The polysaccharide was precipitated from the supernatant with the addition of isopropyl alcohol (1:3), followed by refrigeration for 12 h. After, samples were centrifuged at 4700*g* for 40 min at 4°C. The precipitate was dried at constant weight at 50°C ± 5°C, dialyzed for 48 h against sterile Milli-Q® water, lyophilized, and stored in hermetical flasks until further analyses.

Table 3
Matrix of the Experimental Design With the Coded and Real Values (in Parentheses) for the Response Productivity

Run	Sucrose (%)	Stirring rate (rpm)	Productivity (g/L·h)
1	–1 (2)	–1 (160)	0.003
2	+1 (6)	–1 (160)	0.004
3	–1 (2)	+1 (200)	0.004
4	+1 (6)	+1 (200)	0.015
5	–1.41 (1.18)	0 (180)	0.001
6	+1.41 (6.82)	0 (180)	0.007
7	0 (4)	–1.41 (152)	0.005
8	0 (4)	+1.41 (208)	0.038
9	0 (4)	0 (180)	0.019
10	0 (4)	0 (180)	0.016
11	0 (4)	0 (180)	0.015

Rheological Analysis

Aqueous solutions (3% w/v) of the gum were prepared for apparent viscosity analysis, at 25°C and 60°C. A digital rheometer (Brookfield, LVDV III+) coupled to a water bath (Brookfield, TC-502P) was used for viscosity determinations. Readings of viscosity and shear stress were taken at 10 s intervals, varying shear rate (0-264-0/s), based on each of the sample characteristics. Viscosity data was fitted for increasing and decreasing shear rate, using the Ostwald-de-Waele model ($\sigma = K\gamma^n$), where σ is shear stress, and γ is shear rate, K and n are the consistency index and power law exponent, respectively. For Newtonian flow n is equal to one and K equals the viscosity. For shear-thinning fluids n is less than one whereas it is bigger than one for shear-thickening fluids *(8)*.

Results and Discussion

Experimental Design for Conventional Medium

Table 3 presents the matrix of the experimental design and the response in terms of productivity. The highest productivity (0.038 g/L·h) was obtained at 28°C ± 2°C, 208 rpm, in a medium containing 4% of sucrose at 72 h (run 8). Regression coefficients and standard deviations were calculated using the data of Table 3, and are presented in Table 4. Second order coefficient for sucrose concentration and the first order coefficient for stirring rate were statistically significant ($p < 0.05$). Nonsignificant parameters were added to lack of fit for analysis of variance (ANOVA) shown in Table 5.

The correlation (R) was 0.85 and F calculated was 2.43 times higher than F tabulated, what permits the formulation of a nonlinear coded second order model (Eq. 1). This model describes productivity as a function

Table 4
Regression Coefficients for Biopolymer Productivity

Parameter	Regression coefficients	Standard deviation
Mean	0.017[a]	0.001
Sucrose (Linear parameter)	0.005	0.001
Sucrose (Quadratic parameter)	–0.016[a]	0.002
Stirring rate (Linear parameter)	0.015[a]	0.001
Stirring rate (Quadratic parameter)	0.002	0.002
Interaction sucrose × Stirring rate	0.005	0.002

[a]Statistically significant for productivity at the 95% confidence level.

Table 5
Analysis of Variance to Biopolymer Productivity

Source of variation	Sum of squares	Degrees of freedom	Mean squares	*F*-test
Regression	0.00084	2	0.00042	10.85
Residual	0.00031	8	3.85×10^{-5}	
Lack of fit	0.0003	6		
Pure error	7.92×10^{-6}	2		
Total	0.00114	10		

Regression coefficient: R = 0.85.
$F_{0.95;2;8} = 4.46$.

of the independent variables (stirring rate and sucrose concentration), inside the investigated range.

$$P = 0.017 - 0.008 \cdot (C_s)^2 + 0.007 \cdot A \quad (1)$$

where P is the productivity; C_s the sucrose concentration; A the stirring rate.

The coded model was used to plot the surface presented in Fig. 1. The concentration of sucrose that resulted in higher productivity was 4%, which corresponds to the central point. Upper levels of stirring rate showed a tendency to increase gum yields.

To check if productivity would increase with stirring rate, as indicated by statistical data analysis, two experimental runs were carried out. The first run repeated the maximized experimental condition, which corresponds to run 8 of the experimental design. The second run was carried out in the same experimental conditions as before, except for the stirring rate, which was increased to 236 rpm in order to evaluate its influence on productivity.

Average productivity decreased from 0.041 g/L·h at 208 rpm to 0.009 g/L·h at 236 rpm. Tukey's test showed that these two results are statistically different ($p < 0.05$). Thus, the maximization of biopolymer production by *S. capsulata* ATCC 14666 was achieved, for conventional medium, inside

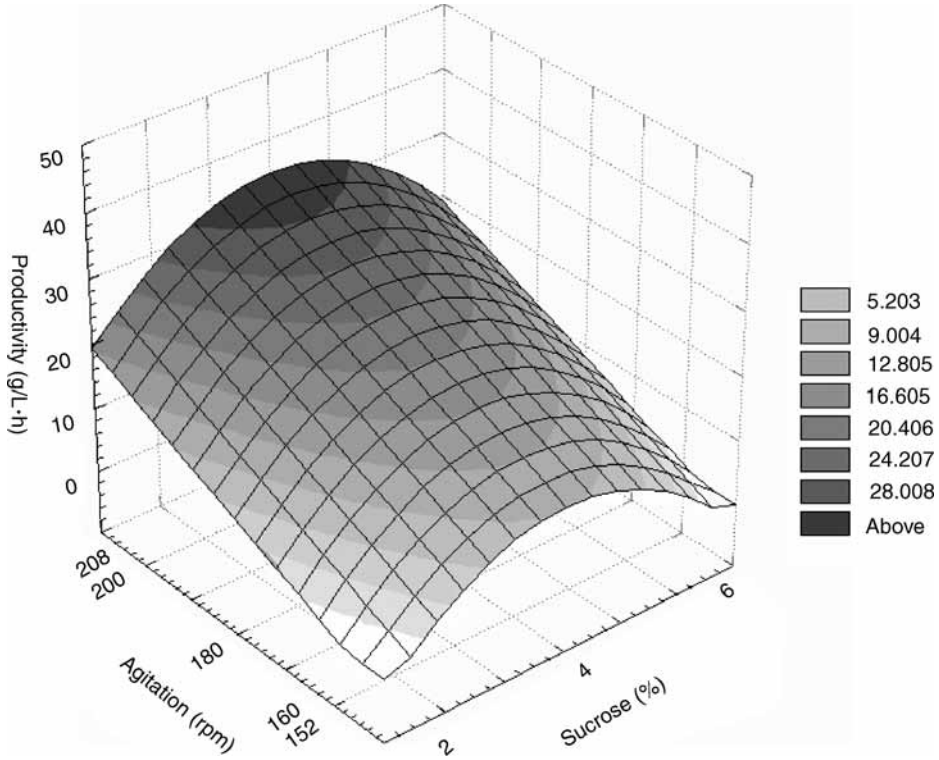

Fig. 1. Response surface for productivities of biopolymer.

the range investigated. It was also possible to note, through the repetition of run 8 of the first experimental design, that a good reproducibility is obtained.

Industrial Media

Tukey's test was carried out to compare the best results (that showed higher gum productivity) for each substrate, considering the different substrates (Table 6). The results for the three different concentrations of the STP waste extract are significantly ($p < 0.05$) different.

Gum productivity increases with the increase in the concentration of this substrate. The highest productivity was obtained with 6% of the STP extract. The productivities obtained with molasses (raw and pretreated) at 4% and 6% were not statistically different ($p < 0.05$), although the addition of 8% of this substrate showed a result significantly higher than the other two.

The statistical analysis of the highest productivity for each substrate showed that the STP waste extract at 6%, raw molasses at 8%, and pretreated molasses at 8%, yielded the same productivities ($p < 0.05$). However, pretreated molasses 8% showed higher productivity than raw molasses at 8% (Table 6).

Table 6
Productivities Obtained Using Industrial Media

Condition	Productivity and standard deviation
Aqueous extract of STP waste 6 %	0.2437[a] ± 0.0158
Molasses 8%	0.1917[a] ± 0.048
Pretreated molasses 8%	0.2957[a] ± 0.0403

[a]Means with different letters in the same column differ significantly ($p \leq 0.05$) in Tukey's test.

The average biopolymer productivity obtained in this work was 0.24 g/L·h, using 6% of aqueous extract of STP waste, without any supplementation. In the production of gelan using soybean sauce residue Jin et al. *(9)* achieved 0.1 g/L·h, using a 7-L bioreactor and a medium containing 2% (w/v) glucose and 2% (w/v) soybean residue.

Using raw molasses the average productivity was 0.19 g/L·h, and when molasses was pretreated it increased to 0.29 g/L·h. In both cases, molasses was used as the sole source of carbon and nitrogen, without further supplementations. Kalogiannis et al. *(10)* obtained higher polymer productivity (2.21 g/L·h), but they used a concentration of molasses almost two times higher than in our work (175 g/L), and supplemented the medium with salts.

Biopolymer Rheology

Experimental results showed that aqueous solutions of the biopolymer produced in this work presented pseudoplastic behavior. Viscosity also decreases with a temperature increase. Table 7 presents the Ostwald-de-Waele model parameters (power law exponent and consistency index). All correlation coefficients were higher than 0.99, indicating a good fit of experimental data to the model. The power law exponents were less than one, indicating the pseudoplastic behavior. The slight tixotropic behavior was observed at lower shear rates at 60°C (data not shown).

Viscosity data obtained in our work are comparable to those reported in literature for other polymers. At 10/s we obtained 176 cP, for 3% solution of the biopolymer at 25°C. Navarrete and Shah *(6)* obtained apparent viscosity of 100 cP, for 1.4×10^{-4}% solutions of diutan, at 24°C. Ashtaputre and Shah *(16)* obtained 200 cP for 0.5% solution of biopolymer at 30°C.

The biopolymer produced by *S. capsulata* showed lower viscosity than those reported in literature, because we worked at higher concentrations. This biopolymer may find application when the product requires lower thickener ability. Interaction studies of this gum with other biopolymers should still be studied, aiming to induce synergetic increase in viscosity, as it occurs with xanthans and galactomannans.

Table 7
Ostwald-de-Waele Model Parameters (Behavior and Consistency Indexes)

Biopolymer obtained from	*T* (°C)	*n*	*K*	R^2
Sucrose 4%	25	0.894	2.2898	0.9995
	60	0.9023	0.7436	0.9999
Pretreated molasses 8%	25	0.8781	1.4973	0.9996
	60	0.8848	0.5299	0.9999
Aqueous extract of TSP waste 6%	25	0.8109	0.1955	0.9996
	60	0.8121	0.1148	0.9999

Conclusions

The best experimental condition for biopolymer production by *S. capsulata* ATCC 14666 was at 28°C ± 2°C, 208 rpm, in a medium containing 4% of sucrose for 72 h of fermentation, in which an average productivity of 0.038 g/L·h was obtained. It is possible to use industrial media like raw and pre-treated molasses and STP aqueous extracts to produce biopolymers using *S. capsulata*, which may reduce production costs and minimize environmental problems. The best productivities were achieved with pretreated molasses 8% (0.296 g/L·h), STP 6% (0.244 g/L·h), and raw molasses 8% (0.192 g/L·h). Aqueous solutions of the biopolymer presented pseudoplastic behavior, confirmed by the good fit of the experimental data to the Ostwald-de-Waele model.

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