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BIOCONVERSION FROM CRUDE GLYCERIN BY *Xanthomonas campestris* 2103: XANTHAN PRODUCTION AND CHARACTERIZATION

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Abstract - The production and rheological properties of xanthan gum from crude glycerin fermentation, a primary by-product of the biodiesel industry with environmental and health risks, were evaluated. Batch fermentations (28 °C/250 rpm /120 h) were carried out using crude glycerin, 0.01% urea and 0.1% KH_2PO_4, (% w/v), and compared to a sucrose control under the same operational conditions, using *Xanthomonas campestris* strain 2103 isolate from Brazil. Its maximal production by crude glycerin fermentation was 7.23±0.1 $g{\cdot}L^{-1}$ at 120 h, with an apparent viscosity of 642.57 mPa·s, (2 % w/v, 25 °C, 25 s^{-1}), 70% and 30% higher than from sucrose fermentation, respectively. Its molecular weight varied from 28.2 to 36.2×10^6 Da. The Ostwald-de-Waele model parameters (*K* and n) indicated a pseudoplastic behavior at all concentrations (0.5 to 2.0 %, w/v) and temperatures (25-85 °C), while its consistency index indicated promising rheological properties for drilling fluid applications. Therefore, crude glycerin has potential as a cost-effective and alternative substrate for non-food grade xanthan production.
Keywords: Xanthan; Biodiesel waste; *Xanthomonas* isolate from Brazil; Drilling fluid.

INTRODUCTION

Microbial polysaccharides, known as gums or exopolysaccharides (EPS), represent an important class of polymers because of their ability to form gels and viscous solutions that are industrially produced by fermentation techniques (Chaitali *et al*., 2003; Ruffing and Chen, 2006, Morris *et al.*, 2012).

Among these, xanthan gum is a high molecular weight, pseudoplastic and water-soluble hetero-polysaccharide that consists of polymerized penta-saccharide repeating units composed of glucose, mannose and glucuronic acid in a ratio of 2:2:1. It is commonly produced by bacteria of the genus *Xanthomonas* by a strictly aerobic process. The unusual rheological properties of xanthan gum are affected by several factors such as *Xanthomonas* strain, batch variability, cultivation media and down-stream processing. The chemical composition of the fermentation medium affects the properties of xan-than solutions (Mulchandani *et al.*, 1988; Sutherland, 1998; García-Ochoa *et al.*, 2000; Desplanques *et al.*, 2012).

Commercially, xanthan is the most important

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microbial polysaccharide, with a worldwide production of approximately 30,000 $t.y^{-1}$. It has widespread commercial applications in food, pharmaceutical formulations, cosmetics, and agricultural products for a number of reasons, including emulsion stabilization, viscosity enhancement, its stability over a wide range of pH and temperature, its compatibility with many salts, and its pseudoplastic rheological properties (García-Ochoa *et al.*, 2000; Moraes *et al.*, 2011).

The ability of xanthan to form viscous aqueous solutions has led to important applications in the petroleum industry, where it is commonly used in drilling fluids and in enhanced oil recovery (EOR) processes (García-Ochoa *et al.*, 2000).

In fact, xanthan gum is the most appropriate biopolymer for EOR, but commercially available xanthan gum is relatively expensive due to glucose or sucrose being used as the sole carbon source. However, several reports have demonstrated that xanthan can be obtained from agricultural and industrial wastes (López *et al.*, 2004; Brandão *et al.*, 2008; Nery *et al.*, 2008; Brandão *et al.*, 2010; Salah *et al.*, 2011).

Worldwide, there are abundant alternative substrates to produce this polysaccharide on an industrial scale that are less expensive than corn glucose, including other sugars, sugar molasses and polyols. Xanthan gum produced from glucose or sucrose is still economically unviable based on the relative costs of the process (García-Ochoa *et al.*, 2000; Kojima *et al.*, 2007), but the use of cheap substrates to produce xanthan gum for non-food applications (*e.g.*, drilling fluids and EOR) should lower the cost of the final product (Shah and Ashtaputre, 1999; Thompson and He, 2006).

It is estimated that, between 2008 and 2013, the Brazilian biodiesel industry will have overproduced glycerin, an industrial residue with a high environmental impact, by 80,000-150,000 $t.y^{-1}$ (Pachauri and He, 2006; da Silva *et al.*, 2009; Albarelli *et al.*, 2011). As the demand and production of biodiesel grow exponentially, the utilization of the glycerol becomes an urgent topic.

The glycerin derived from biodiesel production has high impurity content, and significant cost of purification prevents its use in the food and pharmaceutical industries (Thompson and He, 2006; Elik *et al.*, 2008). A promising alternative use of this by-product is the microbial conversion of crude glycerin through biotechnological processes (Johnson and Taconi, 2007; da Silva *et al.*, 2009) into value-added products, like poly-3-hydroxybutyrate polymers (Zhu *et al.*, 2010); clavulanic acid (Teodoro *et al.*, 2010); recombinant human erythropoietin (Elik *et al.,* 2008), and citric acid (Papanikolaou *et al*., 2002; Rywińska *et al.*, 2011).

Thus, *Xanthomonas* can also be employed to convert crude glycerin and this strategy was patented by our research group (Brandão *et al*., 2007). The objective of this work was to produce and characterize the xanthan gum synthesized by *X. campestris mangiferaeindicae* 2103 in batch fermentation in a shaker from crude glycerin, which is an co-product of biodiesel with lower cost and higher availability compared to sucrose. The present study is the first paper presented in the literature that explores the use of crude glycerin as a feedstock to produce non-food-grade xanthan of lower cost for applications in drilling fluids and EOR.

MATERIAL AND METHODS

Reagents

Analytical reagent grade components purchased from VETEC (São Paulo, Brazil) were used both to prepare all solutions for growth media and to perform all measurements.

Microorganisms and Maintenance

The Tropical Collection Culture of the Biological Institute (Campinas - SP, Brazil) supplied the *X. campestris mangiferaeindicae* 2103 strain isolated in Brazil used in the study. The strain was grown and maintained in yeast extract malt agar (YMA) with the following composition ($g \cdot L^{-1}$): glucose 10.0, yeast extract 3.0, malt extract 3.0, peptone 5.0, and agar 15.0. The medium pH was adjusted to 7.0 and then sterilized (121 °C, 20 min). After growing for 48 h at 28 °C, the culture was maintained under sterile conditions at 4 °C and transferred at 2-week intervals.

Residual Crude Glycerin

The residual crude glycerin was supplied by the Biodiesel Pilot Plant of the Universidade Estadual de Santa Cruz (Ilhéus, BA, Brazil). The following analyses, in triplicate, were carried out on the crude glycerin: acidity (pH), volatiles at 105 °C, residual crude protein (Kjeldahl method), ash (AOAC, 1997) and total lipids content (Bligh and Dyer, 1959). The carbohydrate content (glycerol) was calculated by

difference [100 - (protein + lipid + volatiles + ash) percentages].

Inoculum Preparation

Inoculum cultures were prepared in YM broth, containing (w/v) 1.0% glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract (Garcia-Ochoa *et al.*, 2000). The medium pH was adjusted to 7.0 before autoclaving (121 °C/15 min). *X. campestris* cells were incubated in 250 mL Erlenmeyer flasks containing 50 mL of YM broth at 28±2 °C for 48 h. The flasks were placed in an orbital shaker (Tecnal mod. TE-424, São Paulo, Brazil) at 150 rpm.

Xanthan Gum Production

Experiments were developed in 250 mL shake flask cultures with 80 mL of medium containing 2% crude glycerin supplemented with 0.01% urea and 0.1% KH_2PO_4 (w/v) according to patent by Brandão *et al*. (2007). After adjusting the pH to 7.0 and autoclaving, the media were inoculated with *X. campestris mangiferaeindicae* 2103, using 10% inoculum. Aerobic cultures were grown in triplicate in a batch fermentation process using an orbital shaker (Tecnal mod. TE-424, São Paulo, Brazil) at 250 rpm and 28±2 °C for 120 h. The control fermentation was carried out under identical operating conditions, except that conventional medium containing 2% sucrose supplemented with urea and phosphate was used instead of crude glycerin. All experiments were performed in triplicate.

Broth samples were collected in centrifugation tubes immediately after inoculation and at regular intervals to evaluate cell growth, xanthan production, and glycerin consumption. Cell growth was estimated by measuring the absorbance of cell suspensions at 620 nm in a Perkin Elmer model Lambda 35 spectrophotometer (Norwalk, U.S.A.).

Polymer Recovery

Cells were removed from the fermentation broth by centrifugation at 9626 × g, 5 °C for 60 min (Eppendorf, mod. 5702R, Hamburg, Germany). The xanthan gum product was recovered from the supernatants by precipitation with 98% ethanol at a 3:1 ratio (v/v). Precipitated xanthan was collected, dried in an oven (Tecnal mod. TE 394/2, São Paulo, Brazil) at 30±2 °C for 72 h, ground to a homogeneous powder and then stored (Brandão *et al*., 2007). The production was expressed as $g \cdot L^{-1}$ (grams per liter of fermentation broth).

Xanthan Molecular Weight and Crude Glycerin Consumption During Fermentation

Xanthan average molecular weight was estimated by size-exclusion chromatography (GPC HPLC system, PerkinElmer Serie 200, Shelton, U.S.A) with Shodex OHpak SB 803, 804, 805, 806 columns in series (Kawasaki-ku, Japan), using 50 mM $NaNO_3$ as eluent at a flow rate of 1 $mL \cdot min^{-1}$. The detector used was a Refractive Index (RI) PerkinElmer Series 200 (Shelton, U.S.A.). The calibration of the column was done with dextran standards (molecular weights between 1.02×10^5 Da and 5.9×10^6 Da) (American Polymer Standards, U.S.A.). An aliquot of 80 µL (0.3% m/v) of dextran standards and xanthan gum aqueous solutions was injected.

Xanthan MW determination was performed using the calibration curve of log MW (dextran standards molecular weight) × RT (retention time). The molecular weight of xanthan gum from crude glycerin or sucrose was compared with the molecular weight of commercial xanthan (applied in drilling fluid by the Carboflex Company).

The consumed crude glycerin was also determined by GPC HPLC-IR. Aqueous solutions of crude glycerin before fermentation (2% w/v) and of the broth collected during the fermentation were analyzed under the same chromatographic conditions used to determine the MW. The crude glycerin consumption was obtained by area normalization (decrease of the area of the crude glycerin peak during fermentation), and the percentage reduction calculated by attributing 100% to the maximum value.

Statistical Analysis

All values are presented as mean ± SD. For the statistical analysis of data the software STATISTICA 7 was employed, using the Tukey test for comparison of the means, at the level of 5% of probability.

Apparent Viscosity

To study the rheological characteristics of the gums, xanthan solutions ranging from 0.5 - 2.0% were prepared using distilled water, stirred for 5 min to complete gel formation, and then maintained at room temperature for 12 h before testing. The apparent viscosities of the gum solutions from the alternative and the standard media were measured in a concentric cylinder rheometer (Haake Rheotest mod 2.1, Medingen, Germany) coupled to a water bath for temperature control (25, 45, 65 and 85 °C), with at a shear rate of 25 to 1000 s^{-1}.

To evaluate the performance of xanthan gum as viscosifier in drilling fluid, the viscosity was determined according to Petrobras standards with a base fluid saline preparation containing ($g.L^{-1}$) NaCl (60), $CaCl_2$ (0.2), $MgCl_2$ (0.08) and xanthan (22.5 $g.mL^{-1}$) (Petrobras, 2003, 2009). Their rheological parameters were evaluated after rolling in aging stainless steel cells in a rotatory hot-rolling oven (50 °C for 16 h, 60 rpm) by a direct reading rotating viscometer (Fann mod. 35A Fann, Houston, Texas, U.S.A) according to API RP 13B-1 (API, 2003) at 100, 200, 300, 600 rpm and 28 ± 2 °C.

Rheological data were fitted to the Oswald-de-Waele model: $\mu = K (\gamma)^{n-1}$, where μ is the apparent viscosity, K is the consistency index, γ is the shear rate and n is the flow behavior index. Regression analyses were performed to describe the relationship between each tested parameter and the apparent viscosity (K and n values as well as the regression coefficients R^2).

RESULTS AND DISCUSSION

Xanthan Gum Production and Molecular Weight

Table 1 presents the composition of the crude glycerin used in this work, which was determined to compare samples of glycerin from different batches. The standard deviations of the measurements were low. The RCG composition includes minerals, organic nitrogen and total lipids from biodiesel processing.

Table 1: Chemical composition of residual crude glycerin.

Parameter	Mean value ± SD (%)
Acidity (pH)	6.02 ± 0.03
Volatiles at 105 °C	53.50 ± 0.01
Ash	3.40 ± 0.01
Total lipids	6.70 ± 0.02
Crude protein	2.71 ± 0.03
Carbohydrate (glycerol)	33.69 ± 0.02

It is worth noting that C and N compounds supplied as nutrients for bacterial growth are known to affect xanthan production, depending on their composition and relative amounts. The C:N ratio in RCG is approximately 15:1, corresponding to 0.55 g of glycerol, 0.10 g of lipids, and 0.04 g of protein per 80 mL of fermentation medium. The glycerin chemical composition found here is in agreement with fermentation conditions that indicate that a non-limiting nitrogen concentration is required for rapid cell growth, while excess carbon and low nitrogen concentrations are essential for the production of polymers with suitable rheological properties (Sutherland, 1996). Usually industrial processes are conducted under conditions chosen to optimize both cell growth and xanthan gum production (García-Ochoa *et al.*, 2000).

Batch culture profiles of *X. campestris mangiferaeindicae* 2103 for cell growth, xanthan production, and crude glycerin consumption obtained by GPC-HPLC-IR are shown in Figures 1 and 2. The xanthan concentration continued to increase during 120 h of fermentation until all crude glycerin was completely consumed. In general during the fermentation, an accentuated increase in production of xanthan occurs, followed by a decline due to lack of carbon source and consequent degradation of the polymer by depolymerases released into the medium (Druzian and Pagliarini, 2007; Diniz *et al*., 2012).

Therefore, it was observed that the presence of this polyol (glycerin) in the batch culture stimulated polysaccharide production, even though glucose and sucrose have so far been considered the best carbon sources for xanthan production. It has been reported that the yeast *Yarrowia lipolytica* produces the same amount of citric acid when grown on glucose or on crude glycerin (Papanikolaou *et al*., 2002). Several reports have also demonstrated that xanthan gum can be obtained from agricultural and industrial wastes using less expensive carbon sources to produce it, such as citric acid (Jana and Ghosh, 1995), cassava serum (Brandão *et al.*, 2010) and milk whey (Nery *et al.*, 2008; Silva *et al.*, 2009), since the utilization of glucose or sucrose is a critical factor in the production cost of this polysaccharide (Garcia-Ochoa *et al.*, 2000).

To achieve a high xanthan production, it is necessary to regulate both cell growth and polysaccharide biosynthesis throughout the process. A concentration of 2.0% crude glycerin in the fermentation media was optimal for xanthan biosynthesis during fermentation, since this alternative medium provided better conditions for both growth and xanthan gum accumulation than sucrose. This is consistent with different studies on the nutritional requirements for fermentation with *Xanthomonas*, aiming at the sustainability of the process regarding the cost-effectiveness of the production (Casas *et al.*, 2000; García-Ochoa *et al.*, 2000; Druzian and Pagliarini, 2007).

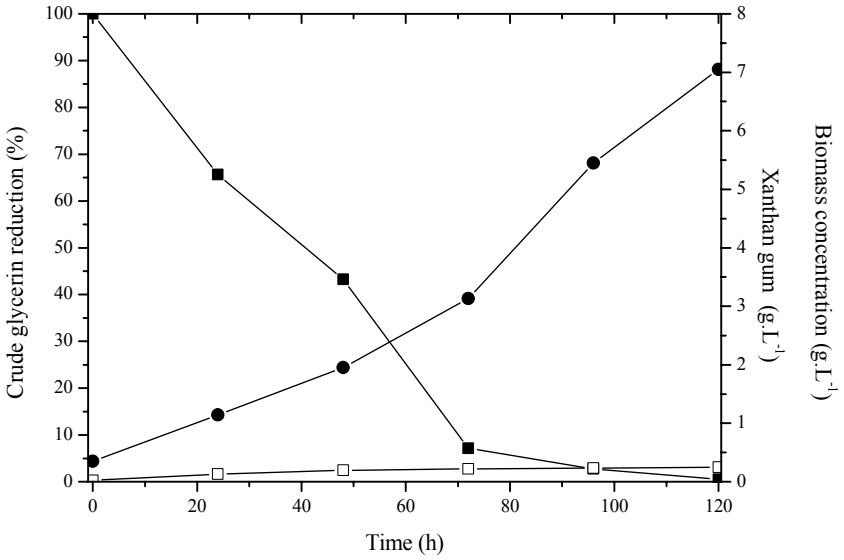

Figure 1: Batch culture profiles for *X. campestris mangiferaeindicae* 2103 showing the effects of the crude glycerin decrease and biomass increase during fermentation. (■) crude glycerin (100% corresponds to 20 $g.L^{-1}$), (□) cellular concentration, and (●) xanthan gum concentration.

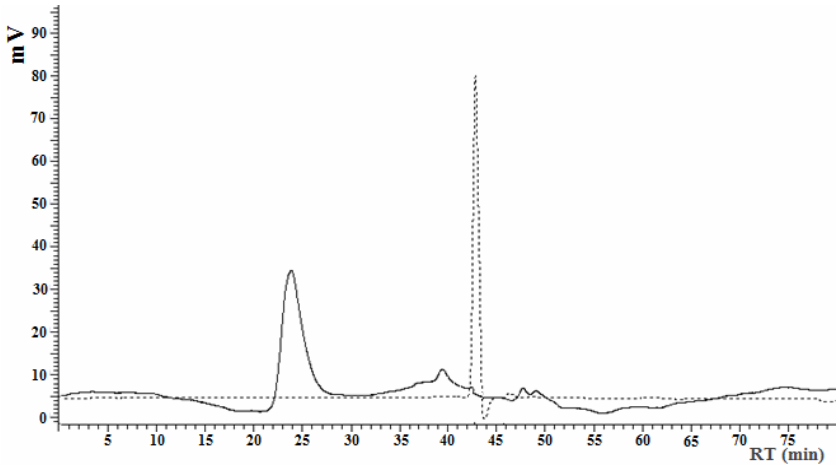

Figure 2: GPC-HPLC-IR chromatograms of the aqueous solution of crude glycerin from biodiesel at 2% w/v before fermentation (—) with retention time of 43.0 min. and the broth collected after 120 h of fermentation in shaker (----) xanthan gum retention time of 23.55 min.

Table 2 shows the effects of the two different carbon sources on xanthan production and the maximum yield of polymer per amount of crude glycerin or sucrose ($Y_{P/S}$) produced by the *X. campestris mangiferaeindicae* 2103 isolate from Brazil. The xanthan gum production and the maximum $Y_{P/S}$ using the alternative medium containing crude glycerin were approximately 1.7 fold higher than the xanthan production and maximum yield with the conventional medium containing sucrose as carbon source. For xanthan gum produced by *X. campestris* PTCC 1473 from molasses, Gilani *et al.* (2011) reported maximum values of $Y_{P/S}$ of 0.57 $g.g^{-1}$ with 30 $g.L^{-1}$ of substrate in a batch process in a shaker. With different *Xanthomonas* strains different and sucrose (19 to 50 $g.L^{-1}$) Rottava (2005) obtained values of 0.15 to 0.42 $g.g^{-1}$ by the same process.

Table 2: Xanthan gum production in alternative and conventional media by fermentation with *X. campestris mangiferaeindica*e 2103 strain at 28 ºC and 250 rpm agitation for 120 h in shaker.

Fermentation medium	Production (gL^{-1})	Maximum $Y_{P/S}$ ($g.g^{-1}$)
Alternative [1]	7.23 ± 0.10 [a]	0.36
Conventional [2]	4.21 ± 0.20 [b]	0.21

Means followed by the same letter in the column do not differ significantly at the level of 5% probability by Tukey test.
[1] 2.0% crude glycerin, 0.01% urea and 0.1% K_2HPO_4, all w/v.
[2] 2.0% sucrose, 0.01% urea and 0.1% K_2HPO_4, all w/v.
$Y_{P/S}$ = yield of xanthan per amount of crude glycerin or sucrose.

According to Umashankar *et al.* (1996), these results can be explained by considering the influence of the nutrient source on the polymer synthetic pathway. Glycerol and free fatty acids (soaps) are the two major components in crude glycerin (Table 1), but according to Thompson and He (2006) it also contains a variety of elements such as calcium (3-15 ppm), magnesium (1-2 ppm), phosphorous (8-13 ppm), and sulfur (22-26 ppm), independent of the feedstock source (such as canola, rapeseed, and soybean). Hence, a richer fermentative medium in terms of nutrients and micronutrients and a possible bacterial adaptation to an alternative medium may contribute to this increase in xanthan gum production (Table 2).

The calibration curve of log MW (molecular weight) × RT (retention time) was linear: log MW = - 0.301×RT + 14.56 with a correlation coefficient of $R^2 = 0.97$. Table 3 shows the MW of xanthan gum obtained from crude glycerin as compared to a xanthan from sucrose and to commercial xanthan gum. The observed differences can be explained based on the influence of medium composition, *Xanthomonas* strain, and the operational conditions, which have a significant impact on the synthesized gum molecular structure, generating polysaccharides with different molecular weights (Garcia-Ochoa *et al.*, 2000). The differences found can be ascribed to the use of glycerol as carbon source, since commercial gums are obtained from glucose fermentation.

Table 3: Retention time (RT), average molecular weight (MW), and apparent viscosity (μ) of xanthan gum synthesized from either sucrose or crude glycerin by *X. campestris mangiferaeindica*e 2103, compared to commercial xanthan gum.

Xanthan gum	RT (min)	$MW \times 10^6$ (Da)	μ[1] (mPa.s)
Xanthan from crude glycerin	23.55	29.6	69.50
Xanthan from sucrose	23.62	28.2	49.20
Commercial xanthan	23.26	36.2	140.80

[1] 0.5% xanthan gum aqueous solutions at 25° C and 25 s^{-1}.

Rheological Properties

The measurements of viscosity at different shear rates showed that the xanthan samples from the RCG and sucrose fermentations by *X. campestris mangiferaeindicae* 2103 had similar rheological behaviors (Figure 3). The relationship between the viscosity and the shear rate at various concentrations of polymer and at temperatures ranging from 25 to 85 °C was described by the Ostwald-de-Waele model. The apparent viscosity decreased as the shear rate increased, indicating that xanthan from glycerin also exhibited a pseudoplastic behavior.

The temperature and the polysaccharide concentration of the solution are known to affect the viscosity and the degree of pseudoplasticity (Milas and Ranudo, 1979; Sutherland, 1994). Figure 4 indicates that the viscosity increased strongly with increasing polymer concentration but decreased with increasing temperature, independent of the xanthan gums employed.

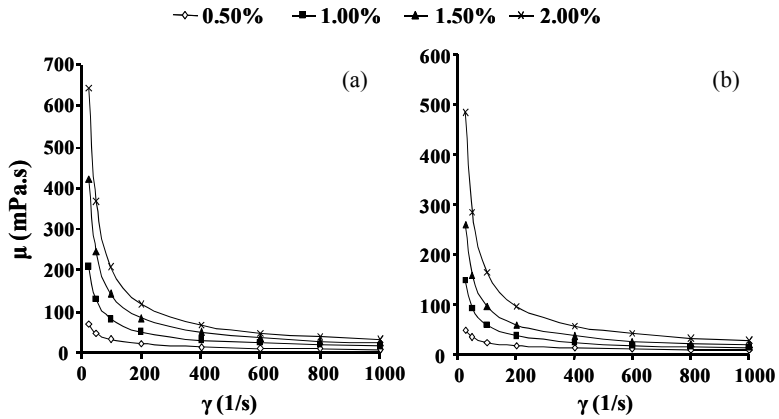

Figure 3: The effect of concentration (%, w/v) and shear rate (ranging from 25 to 1000 s^{-1}) on the apparent viscosity of aqueous solutions of xanthan gum synthesized by *X. campestris mangiferaeindicae* 2103 by fermentation of residual crude glycerin (a) and sucrose (b).

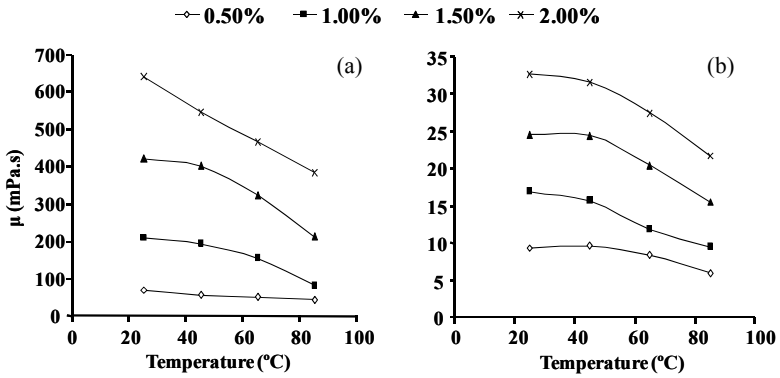

Figure 4: The effect of temperature on the apparent viscosity of different aqueous solutions of xanthan gum synthesized by *X. campestris mangiferaeindicae* 2103 from residual crude glycerin at a shear rate of 25 s^{-1} (a) and 1000 s^{-1} (b).

The parameters estimated by the Ostwald-de-Waele model are presented in Table 4. The measurement of the viscosity at different shear rates showed that the polymers had similar pseudoplastic rheological behavior since the flow index was less than 1. This model accurately described the experimental data because the regression coefficient R^2 was 0.99. However, both apparent viscosity and the pseudoplasticity of the gum derived from RCG were better than those obtained from the sucrose-derived gum, according to data cited in the literature (Casas *et al.*, 2000; Mulchandani *et al.*, 1988).

Table 4: Rheological parameters of 0.5% xanthan solutions at 25 °C and 400 s^{-1} for xanthan obtained by fermentation of sucrose or residual crude glycerin compared with commercial xanthan and the desired values for EOR and drilling fluids.

Xanthan	μ (mPa.s)	n	R^2
Xanthan from crude glycerin	15.44 ± 0.10 [a]	0.45	0.9986
Xanthan from sucrose	12.83 ± 0.12 [b]	0.52	0.9991
Commercial xanthan	18.47 ± 0.15 [c]	0.23	0.9993
Desired value*	>15	<0.40	-

*Value for EOR and drilling fluids according to Shah and Ashtaputre (1999).
n = 3, values expressed as mean±standard deviation.
Means followed by the same letter in a column do not differ significantly at the level of 5% probability by Tukey test (ANOVA, p<0.05).

The rheological properties of xanthan gum from residual crude glycerin shown in Table 4 revealed the requirements to impart desirable rheological behavior to drilling fluids, as described by Shah and Ashtaputre, (1999). The rheological properties of xanthan gum at high temperatures are of great practical interest, especially for EOR applications. The flow index (*n*) should be less than 1 to facilitate the injection of the fluid and to improve the flow rate of drilling fluids (Shah and Ashtaputre, 1999).

The consistency index (K) indicates the degree of resistance to the flow of fluid, *i.e.*, higher K values indicate a higher degree of pseudoplasticity and inherent suspension properties. As seen in Table 5, similar results for K were observed for the RCG-derived and the commercial xanthans. The samples exhibited the desired rheological behaviors for application in drilling fluid for oil when used in a saline solution. This finding indicates that xanthan gum obtained from biodiesel-derived crude glycerin has the necessary apparent viscosity and other characteristics required in drilling fluid applications. From Table 5, it can be seen that the apparent viscosities (μ) were the same both before and after the rolling period. A similar behaviour was observed before and after the rolling period for both the xanthan obtained from glycerin and the commercial xanthan. High values of K (> 1000 mPa.s) and n < 0.5 are desirable in drilling fluids because a high viscosity is required to maintain the suspended cuttings generated during drilling, which improves the pump flow rates in coiled tubing applications. These results, performed according to Petrobrás Standard for drilling fluid (Petrobras, 2003, 2009), are promising for fluids used in oil well perforation with saline solutions, because results for the xanthan consistency index in saline solutions, shown in Table 5, corroborate the values for drilling fluids, in accordance with those obtained by Borges *et al.* (2009), and with the rheological properties described by Shah and Ashtaputre (1999) for the exopolysaccharide of *Sphingomonas paucimobilis* for application in oil exploration.

Table 5: Rheological parameters of xanthan gum from residual crude glycerin, according to Petrobrás standard N-2604 (2009) for drilling fluids at 28 ± 2 °C.

Xanthan	Parameters			
	K (mPa.s^n)		*n*	
	B.R.[1]	A.R.[2]	B.R.[1]	A.R.[2]
Xanthan from crude glycerin	5822.8 ± 0.10 ^{Aa}	6342.6 ± 0.08 ^{Ab}	0.21 ± 0.10 ^{Ac}	0.21 ± 0.01 ^{Ac}
Commercial xanthan	7149.4 ± 0.09 ^{Ba}	7849.3 ± 0.09 ^{Bb}	0.23 ± 0.11 ^{Ac}	0.23 ± 0.09 ^{Ac}
Desired value for drilling fluids	minimal 1500		maximal 0.5	

[1]B.R. = before rolling; [2]A.R. = after rolling. Rolling at 50 °C during 16 h.
n = 3, values expressed as mean±standard deviation.
Means followed by the same uppercase letters in each column and lowercase letter in each row do not differ significantly at the level of 5% probability by Tukey test (ANOVA, $p<0.05$).

A branched structure and a high molecular weight are responsible for a high-viscosity xanthan gum. Many of the rheological properties of xanthan gum derive from its double-helix conformation and its molecular weight is crucial to its properties (Garcia-Ochoa *et al.*, 2000).

However, both xanthan gums obtained experimentally showed MW ranging from 28.2 to 36.2×10^6 Da. Regarding these results, the apparent viscosity of xanthan gum (0.5% concentration) at 25 °C and 25 s^{-1} (Table 4 and Table 3) is directly correlated with the molecular weight of the polymer with a correlation coefficient $R^2 = 0.99$. This can be attributed to the intermolecular interactions in solution (García-Ochoa *et al*., 2000; Higiro *et al*., 2007).

It is worth noting that the rheological analyses indicate that the xanthan gum produced with glycerol as an alternative substrate is promising for EOR and drilling fluid applications. Therefore, there is motivation for research with other *Xanthomonas* strains in order to obtain xanthan gum with even higher viscosity using crude glycerin as a carbon source.

CONCLUSIONS

The current work investigated the possibility of using residual crude glycerin from biodiesel production as an alternative substrate for xanthan gum biosynthesis by *X. campestris* using a fermentation medium supplemented with 0.01% urea and 0.1% K_2HPO_4. The production of xanthan obtained using residual crude glycerin as substrate was 7.23 gL^{-1}, approximately 70% higher than the production obtained from the conventional substrate sucrose under the same operating conditions. The xanthan gum from residual crude glycerin exhibited a pseudoplastic rheological behavior characteristic of xanthan solutions (2.0% w/v), with a viscosity of up to 642.57 mPa·s at 25 °C and a shear rate of 25 s^{-1}. The viscosity was 30% higher than the viscosities found for the gums obtained from the sucrose media. The xanthans gum molecular weight (MW) varied from 28.2 to 36.2×10^6 Da, the upper limit being commercial xanthan. The apparent viscosity of xanthans was directly proportional to the MW. In addition, the molecular weight of xanthan gum produced from glycerin presented a similar value to gum obtained from sucrose, although smaller than that observed for commercial xanthan gum (from glucose).

Overall, the findings of this study indicate that residual crude glycerin shows great promise as a substrate for the efficient production of cost-effective xanthan gum. Thus, further studies are needed to establish parameters to improve yield and productivity.

The present study is the first in the literature to explore and report the use of this by-product to produce xanthan for EOR applications. The gum obtained showed pseudoplastic rheological behavior with values of K and n that are satisfactory for use as a drilling fluid.

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