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Xanthan Gum Production From Cassava Bagasse Hydrolysate With *Xanthomonas campestris* Using Alternative Sources of Nitrogen

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Abstract

Cassava bagasse was hydrolyzed using HCl and the hydrolysate was used for the production of xanthan gum using a bacterial culture of *Xanthomonas campestris*. Cassava bagasse hydrolysate with an initial concentration of approx 20 g of glucose/L proved to be the best substrate concentration for xanthan gum production. Among the organic and inorganic nitrogen sources tested to supplement the medium—urea, yeast extract, peptone, potassium nitrate, and ammonium sulfate—potassium nitrate was most suitable. Ammonium sulfate was the least effective for xanthan gum production, and it affected sugar utilization by the bacterial culture. In media with an initial sugar concentration of 48.6 and 40.4 g/L, at the end of fermentation about 30 g/L of sugars was unused. Maximum xanthan gum (about 14 g/L) was produced when fermentation was carried out with a medium containing 19.8 g/L of initial reducing sugars supplemented with potassium nitrate and fermented for 72 h, and it remained almost the same until the end of fermentation (i.e., 96 h).

Index Entries: Cassava bagasse; fermentative process; xanthan gum; nitrogen sources; *Xanthomonas campestris*.

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Introduction

Xanthan gum is the common name of a complex microbial exopolysaccharide produced by fermentative process using *Xanthomonas campestris*, which is a Gram-negative and pathogenic bacterium for some plants. The structure of the xanthan gum is formed by a linear chain of D-glucopyranose, linked by β-(1,4) bonds, like cellulose, and its lateral chains are formed by residues of D-glucosyl, D-manosyl, and D-glucuronyl at a molar proportion of 2:2:1 and variable proportions of *O*-acetyl and pyruvyl substitutions *(1–3)*. Because of its physical properties, xanthan gum is used as a stabilizing agent in a wide range of emulsions, suspensions, and foam products *(4)*. Since 1964, the industrial application of this polymer has found more and more applications as a thickening, stabilizing, and gelling agent in a large range of industries and in food products *(1)*. It is used as an additive in textile industries, paint and automotive oils, ceramic coats, and polish and has vast application in pharmaceutical, cosmetic, and food industries. In food industries, it finds application in the manufacture of jams, puddings, sauces, canned and frozen food, and drinks *(5)*. Several species of *Xanthomonas*, including *X. campestris*, *X. phaseoli*, *X. malvacearum*, and *X. carotae*, produce extracellular polysaccharides, generating viscous colonies with gum *(3,5,6)*.

Different kinds of carbon sources have been reported for the production of xanthan gum *(1,7,8)*. Generally, the concentration of carbon source affects the conversion yield of sugar to polysaccharide *(1,7)*. Xanthan gum has also been produced in solid medium. Apple bagasse and malt grains were used (impregnated with a saline solution of micronutrients supplemented with nitrogen sources). The gum production was about 30–35 g/kg of solid substrate, independent of the culture time and sugar concentration of the medium *(8)*.

Cassava (*Manihot esculenta* Crantz) is a crop of tropical countries. It is considered the sixth most important crop in the world and forms the basis of food for more than 700 million people in many countries. It is used in part to produce starch. Industries that process cassava roots to obtain starch produce huge quantities of cassava bagasse during the processing to isolate the starch. Cassava bagasse contains fibers and residual starch that cannot be extracted during the process (this could vary between 30 and 60%). It has a high moisture content (approx 75%). Generally, it is used as animal feed or discharged in the surrounding environment, causing serious pollution problems. Cassava bagasse, however, is rich in organic matter, which could make it a potential raw material as substrates for bioprocesses *(9–12)*. Natural polymers are considered potential raw material for hydrolysis processes to produce syrups of its monomers or the basic units of the original polymer. Acid or enzymatic hydrolysis of starch from many sources has been studied to optimize the hydrolysis conditions *(11,12)*.

The objective of the present investigation was to hydrolyze cassava bagasse and use the hydrolysate for xanthan gum production using a bac-

terial strain of *X. campestris*. Experiments were conducted to study the influence of supplementation of fermentation medium with organic and inorganic nitrogen sources for gum production.

Materials and Methods

Microorganism, Strain Conservation, and Inoculum

A bacterial culture of *X. campestris* from the Culture Collection of the Biotechnological Process Laboratory of Federal University of Parana was used. The strain was maintained on a medium containing 10 g/L of glucose, 5 g/L of yeast extract, 0.1 g/L of KH_2PO_4, 10 g/L of $CaCO_3$, and 17 g/L of agar. Inoculated slants were grown at 28–30°C for 48 h and stored at 4°C. Inoculum was prepared in this same medium (but without agar) by placing 40 mL of sterile medium in a 250-mL Erlenmeyer flask and incubating it at 28–30°C for 24 h. Two percent (v/v) of this cell suspension was used as inoculum for fermentation.

Acid Hydrolysis of Cassava Bagasse

Acid hydrolysis of the cassava bagasse was carried out by taking 100 g of dry cassava bagasse/L of 1% HCl and autoclaving at 121°C for 12 min. After cooling, the contents were neutralized (pH 7.0) using NaOH (1.0*N*) and filtered. The filtrate was assayed for reducing sugars *(13)*.

Fermentation

For fermentation, hydrolysate obtained as just described was used in which the initial reducing sugar concentration was adjusted to 2% by dilution. It was supplemented with 1% organic and inorganic nitrogen sources (yeast extract, peptone, urea, potassium nitrate, ammonium sulfate, and KH_2PO_4 [0.01%]). The fermentation was carried out by placing 50 mL of sterile medium in 250-mL Erlenmeyer flasks kept in a shaker at 200 rpm and 28–30°C for 96 h. All the tests were done in duplicate, and the results are the average of the two values.

Analyses

Reducing sugar was analyzed by the Somogyi-Nelson method *(14)*. Biomass was measured by centrifuging a known volume of the fermented broth at 10,000 rpm and 10°C for 10 min. The solid residue obtained was washed with distilled water and recentrifuged at the same conditions, and the biomass was dried at 80°C for 24 h, cooled, and weighed. The supernatant obtained after centrifuging the fermented broth was used to assay xanthan gum. The gum was precipitated by treating the supernatant with ethanol (2 vol of ethanol/vol of supernatant), and the mixture was centrifuged at 14,000 rpm and 10°C for 15 min. The solid fraction was washed with ethanol followed by absolute ethanol and acetone, and then it was dried under vacuum or at 80°C for 24 h and weighed. The dried matter was milled to make powder.

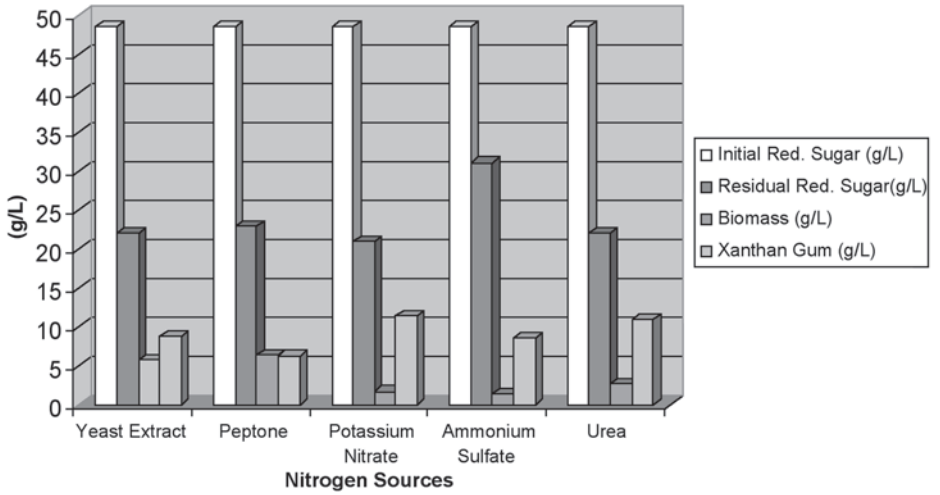

Fig. 1. Fermentation of cassava bagasse hydrolysate with initial reducing sugar concentration of 48.6 g/L by *X. campestris* to produce xanthan gum.

Results and Discussion

Acid hydrolysis of cassava bagasse was done by autoclaving 100 g/L of bagasse in acid medium (1% HCl) at 121°C for 12 min. After neutralization and filtration, the hydrolysate was obtained with a concentration of reducing sugars of 55 g/L from the starch present in the cassava bagasse (data not shown). This was diluted with distilled water to set different initial reducing sugar concentrations (48.6, 40.4, 24.4, and 19.8 g/L) and fermentation was carried out. The results are presented in Figs. 1–4, which show initial and residual (final) concentrations of reducing sugars, and the xanthan gum and biomass produced in each case.

Figures 1–3 show that the reducing sugar concentration at the end of the fermentation decreased in a typical relationship to the decrease in the initial reducing sugar concentration in the hydrolysate. This was probably owing to physical limitations of the liquid fermentation system, where the medium viscosity limited the gas exchange and mixing of the medium. An increase in the viscosity of the fermentation medium (owing to the increase in the concentration of xanthan gum produced) interfered with the oxygen transfer, and this could reduce or stop microbial activity, even if the carbon source is available in the medium. Hence, irrespective of the nitrogen source used, xanthan gum production and reducing sugar consumption were almost similar; only the fermentation with ammonium sulfate was less efficient. In general, biomass production was higher with organic nitrogen sources and lower with inorganic nitrogen sources. Ammonium sulfate was least effective for sugar utilization by the bacterial culture; at the end of fermentation, maximum residual sugars were found in all cases with different initial sugar concentrations when ammonium sulfate was added as nitrogen source in the medium, and this effect was

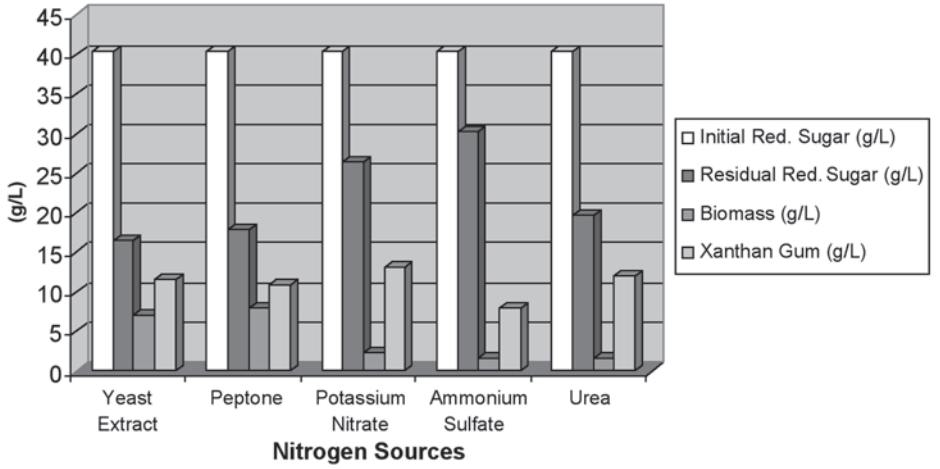

Fig. 2. Fermentation of cassava bagasse hydrolysate with initial reducing sugar concentration of 40.4 g/L by *X. campestris* to produce xanthan gum.

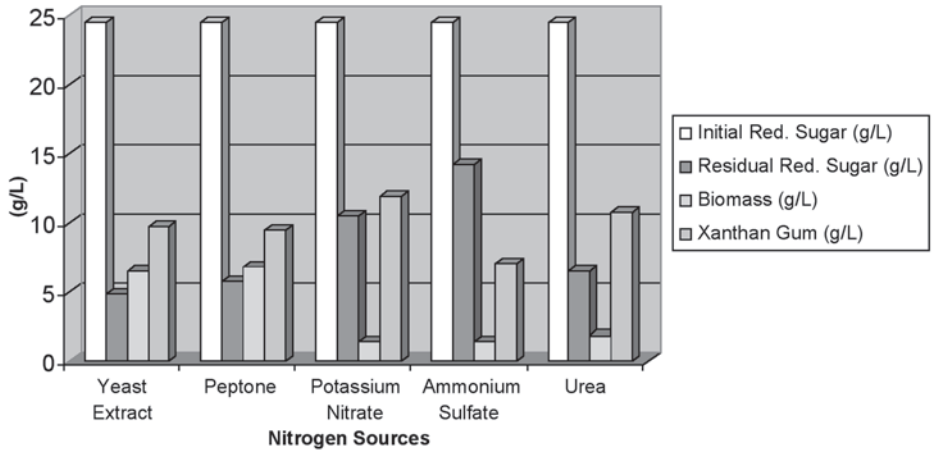

Fig. 3. Fermentation of cassava bagasse hydrolysate with initial reducing sugar concentration of 24.4 g/L by *X. campestris* to produce xanthan gum.

higher with higher initial sugar concentrations (with sugar concentrations of 48.6 and 40.4 g/L, residual sugars was about 30 g/L). Xanthan gum production was the best when potassium nitrate was used as nitrogen source, among all the compounds tested. The results showed that a convenient medium dilution must be done, because a high reducing sugar concentration at the beginning might not necessarily correspond to a high xanthan gum production and, thus, would lead to a waste of carbon source. In addition, the results showed that a suitably diluted initial sugar concentration also led to high consumption efficiency by the bacterial culture (approx 90%; Fig. 4).

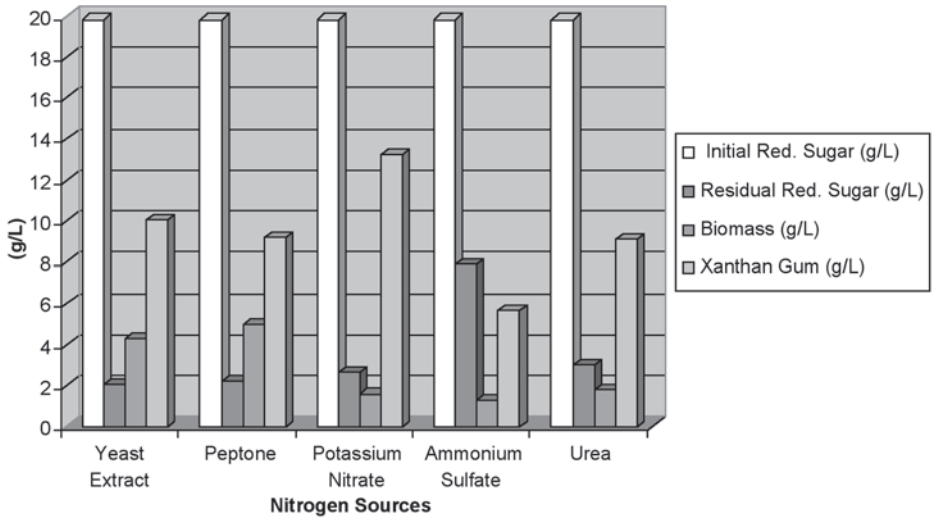

Fig. 4. Fermentation of cassava bagasse hydrolysate with initial reducing sugar concentration of 19.8 g/L by *X. campestris* to produce xanthan gum.

Fermentation Kinetics

The fermentation kinetics of cassava bagasse hydrolysate supplemented with potassium nitrate as nitrogen source were studied. The results are shown in Fig. 5. The initial and final reducing sugar concentrations were 19.5 and 1.0 g/L, respectively, showing good substrate consumption by the bacterial culture. Biomass production was maximum after 48 h (1.8 g/L), which remained more or less constant until 72 h, and then started to decline (varied between 1.7 and 1.8 g/L during 48 and 96 h). Maximum xanthan gum was produced after 72 h (about 14 g/L), which remained almost the same until the end of fermentation (i.e., 96 h). However, sugar consumption continuously declined until the end of 96 h.

Conclusion

Because of their richness in organic matter content, residues from agroindustrial processing, such as cassava bagasse, could be potentially used for value addition through microbial means. Our studies showed that cassava bagasse hydrolysate prepared by acid hydrolysis and supplemented with nitrogen source could be a suitable substrate for the production of xanthan gum by fermentative process using *X. campestris*. All the tested nitrogen sources—yeast extract, peptone, potassium nitrate, ammonium sulfate, and urea—showed positive results on xanthan gum production. Regarding the initial concentrations of sugars, approx 20 g/L was found to be ideal for this process. Concentrations higher than this were not suitable because a good amount of the sugars were left unused in the fermentation medium. Xanthan gum produced was accumulated in the

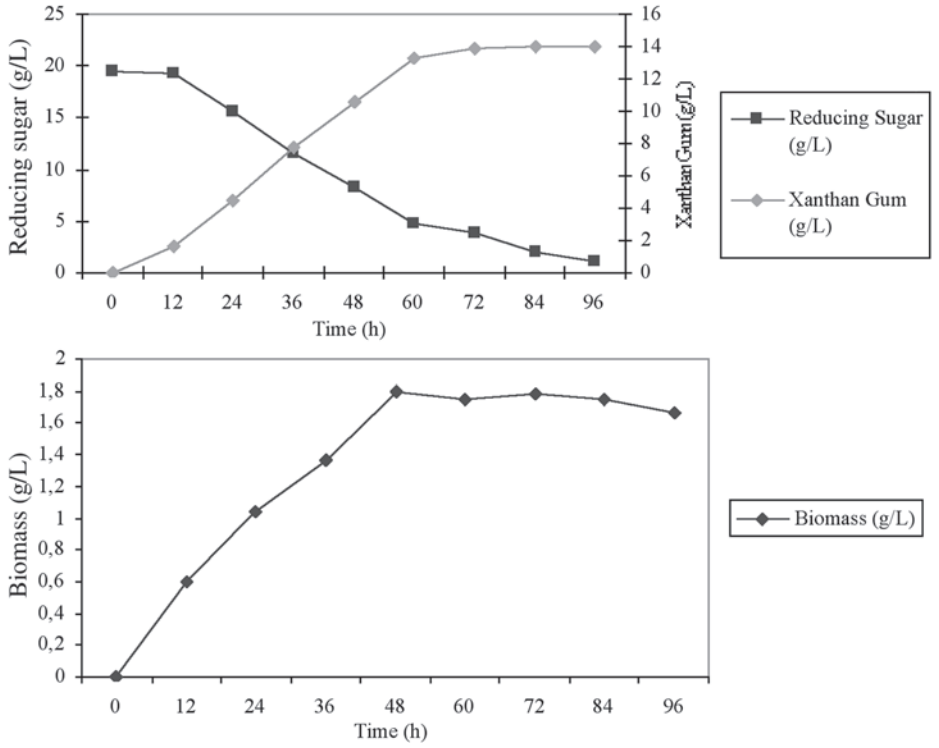

Fig. 5. Fermentation kinetics for xanthan gum production using cassava bagasse hydrolysate with initial reducing sugar concentration of 19.5 g/L by *X. campestris* and potassium nitrate as nitrogen source. **(A)** Reducing sugar and xanthan gum concentration along the fermentation line. **(B)** Biomass concentration along the fermentation line.

medium, which increased the medium's viscosity. This caused difficulty in mass transfer of the substrate and product and oxygen diffusion in the medium, which limited assimilation of the substrate by the microorganism. The best xanthan gum production was obtained when potassium nitrate was used as nitrogen source.

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