Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue2-fulltext-6 *SHORT COMMUNICATION*

Use of volatile fatty acids salts in the production of xanthan gum

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Received July 9, 2012 / Accepted March 3, 2012
Published online: March 15, 2013

Abstract

Background: The aim of this study was the production of xanthan gum from salts of volatile fatty acids, which can be generated in anaerobic processes for the production of hydrogen from organic wastewaters. Xanthan gum was produced with three different acid salts used to replace the traditional citrate, which is normally used in the culture for the production of this biopolymer. The volatile fatty acids (VFA) salts used were sodium acetate 0.0328 M, sodium propionate 0.0219 M, and sodium butyrate 0.0164 M.
Results: The values of biomass yield, ($Y_{p/x}$) obtained were 9.2 g/g for acetate, 11.78 g/g for citrate, 11.80 g/g for butyrate and 14.59 g/g for propionate, while the values of the product yield ($Y_{p/s}$), were 0.92; 0.59; 0.71 and 0.72 for acetate, citrate, butyrate and propionate. As for the rheological characterization, the gums produced showed a consistency index (K) and flow index (n) of 9.8 dina.s^{-n}.cm^{-2} and 0.34 for acetate; 6.3 dina.s^{-n}.cm^{-2} and 0.39 for citrate, 5.8 dina.s^{-n}.cm^{-2} and 0.45 for butyrate, 39.2 dina.s^{-n}.cm^{-2} and 0.24 for propionate, that characterize the gums with good consistency and fluidity.
Conclusions: It is possible to produce xanthan gum from short-chain volatile acids in replacement by the citrate that is usually used in medium composition for the gum production. These results contribute to the feasibility studies for implementation of processes for treating wastewater generating products such as volatile acids, hydrogen and consequent use of these acids for the production of xanthan gum.

Keywords: rheology, volatile fatty acids, xanthan gum.

INTRODUCTION

The search for new sources of energy has fostered the development of studies that make energy generation processes possible from liquid or solid organic wastes. At the same time, the destiny of the residues generated by the industry has been a cause for concern. Conciliating these two aspects is undoubtedly a challenge. Studies on methane and hydrogen production in anaerobic reactors from wastewaters have been extensively performed (Giordano et al. 2011; Wang et al. 2011). During the production of hydrogen via anaerobic fermentation, organic fatty acids of commercial interest are produced concomitantly, such as acetic acid, butyric acid, and propionic acid among others (Leite et al. 2008).

When the acids produced by anaerobic fermentations are recovered, they should receive a strategic destination. Commercialization can be an interesting alternative, but in terms of technological development, a direct application of those compounds needs to be studied mainly for application in biotechnology process.

Xanthan gum is a well known industrial biopolymer able to thicken, disperse and stabilize different kinds of materials such as water, emulsion, and suspension among others, which justifies its presence in products of food, pharmaceutical, and petrochemical segments (García-Ochoa et al. 2000).

Due to the cost of the glucose used in the production of gums, alternative feedstocks have been investigated such as barley, corn flour, whey, and sugarcane honey (Rosalam and England, 2006). Esgalhado and Roseiro (1998) and Barboza et al. (2009) studied the production of xanthan gum from volatile acids salts.

In this sense, organic acids produced by anaerobic process from wastewaters can be interesting feedstock's for production of xanthan gum. With the development of this process, anaerobic reactors can be used for the production of hydrogen along with the production of volatile acids with the subsequent application in the production of xanthan gum. Hence, this study aimed the production of xanthan gum from short-chain volatile acids, more specifically the acetate, propionate, and butyrate, the main products in acidogenic anaerobic digestion, in replacement by the citrate that is usually used in medium composition for the gum production.

MATERIALS AND METHODS

Microorganism

A pure culture of *Xanthomonas campestris*-CBMAI 199 (ATCC 33913) enriched in Luria Bertani - glucose (LB-G) medium, composed by ($g.L^{-1}$): glucose 2,0; tryptone 10; yeast extract 5,0 e NaCl 5,0; incubated at 30ºC for 48 hrs was used. The inoculum was transferred into the specific medium (Medium I), described by García-Ochoa et al. 2000 and incubated in shaker at 150 rpm and 28ºC.

Culture media and cultivations

The composition of the culture media followed the protocol described by García-Ochoa et al. (2000). Medium II was used for xanthan gum production, in which sucrose was replaced by glucose and the citric acid was replaced by volatile acids (VA) salts. An experiment with citric acid was performed to evaluate the effect of the salts' replacement. The VA salts that replaced the citric acid were sodium acetate, sodium propionate, and sodium butyrate in respective concentrations of: 0.0328M; 0.0219M, and 0.0164M. The experiments were performed in a shaker at 150 rpm and 28ºC in 500 mL flasks in duplicate. The cultivations performances were evaluated during 80 hrs and samples were withdrawn at intervals of 10 hrs.

Determinations

The parameters monitored were: biomass concentration of xanthan gum, glucose and volatile acids.

Biomass. Biomass determinations of *Xanthomonas campestris* were done at 600 nm using a Hach DR/4000 spectrophotometer. The samples were centrifuged at 14.000 rpm for 4-8 min in 2 mL Eppendorf tubes in order to separate the cells. Then they were resuspended to be analyzed in spectrophotometer.

Xanthan gum. The determinations of xanthan gum during cultivation were made through the volume of broth centrifuged in a Falcon tube: 1.5 mL of broth with the addition of 0.9 mL of KCl (3 M) and 3 volumes of ethyl alcohol 92.5ºGL. The gum precipitate was left to rest for 2 days. The tubes containing the gum were centrifuged at 30ºC for 10 min at 5000 rpm. The resulting gum was dried in an incubator at 65ºC for 24 hrs.

Gum rheology. For the rheological characterization of the gums, a Brookfield LV - DVIII concentric cylinder viscometer was used with a SC4-18 spindle at 25ºC. The dried precipitate gum was dissolved in ultrapure water at the ratio of 0.0057 (w/w).

Glucose. The glucose concentrations were determined by the enzymatic method using glucose-oxidase (God-Pap kit-Real Lab) using a Hach DR/4000 spectrophotometer.

Volatile Acids (VA). The VA concentrations were determined by automated headspace gas chromatography - GC/FID according to the following methodology. A FID Shimadzu GC chromatograph with HP-INNOWAX column of 30 m x 0.25 mm (internal diameter) x 0.25 µm (film thickness) was used. The temperature of the oven varied as follows: 35ºC (0 min); 2ºC/min; 38ºC (0 min); 10ºC/min; 75ºC (0 min); 35ºC/min 120ºC (1 min); 10ºC/min; and 170ºC (2 min). The injector temperature was 250ºC; the detector temperature was 280ºC; carrier gas flow rate (H_2): 1.6 mL/min. Auxiliary make-up gas rate (N_2): 30 mL/min. Flame gases flow: synthetic air was of 300 mL/min and H_2 of 30 mL/min.

RESULTS AND DISCUSSION

The experimental results obtained for the cellular concentration and for the xanthan gum production are shown in Figure 1 and Table 1.

Table 1. Results of the stoichiometric yields and maximum specific growth rate obtained for each salt used in the culture medium and rheological characterization of the gums.

	$Y_{x/s}$ (g/g)	$Y_{p/s}$ (g/g)	$Y_{p/x}$ (g/g)	μ_{max} (h^{-1})	K ($dina.s^{-n}.cm^{-2}$)	n
Acetate	0.10	0.92	9.20	0.021	9.8	0.34
Propionate	0.05	0.72	14.59	0.023	39.2	0.24
Butyrate	0.06	0.71	11.80	0.024	5.8	0.45
Citrate	0.05	0.59	11.78	0.034	6.3	0.39

According to Figure 1a, it is possible to verify that the growth of *X. Campestris* CBMAI – 0199 (ATCC 3391) presents the characteristic phases of a microbial process. For the citrate and butyrate salts, cell death occurred after 50 hrs. For the medium with acetate salt, cell death was verified after 80 hrs of cultivation (data not shown in Figure1a). For the assay with propionate salt, cell death was verified after 30 hrs of cultivation indicating low cellular growth. Nevertheless, during the first 30 hrs, growth was similar for all salts used.

The production of xanthan gum was influenced by the kind of salt used in the culture medium. The results shown in Figure 1b indicate that there is a period of time in which the concentration of xanthan gum decreases coinciding with cell death for the butyrate salt. This fact can also be verified in the medium with propionate, in which cell death occurred before 50 hrs of cultivation.

The period of more than 50 hrs of cultivation may have consumed other sources of nutrients, which were not quantified in the present study, especially the dissolved oxygen level which decreases drastically with an increase in the apparent viscosity of the broth as a function of the xanthan gum present as a product. In the initial period, until 50 hrs, the quantity produced was similar for all salts used.

The glucose concentrations in the first 50 hrs of cultivation were determined for an evaluation of the initial growth period. These results allow the calculation of glucose concentration variation during this period of time for each situation. The glucose variation with the use of citrate salt was 16.7 g/L; with the acetate salt it was 12.6 g/L; with the propionate salt it was 11.4 g/L; and with the butyrate it was 11.4 g/L. From these results (Table 1), it was possible to determine the stoichiometric yield coefficients, $Y_{x/s}$ (substrate yield), $Y_{p/s}$ (product-substrate yield) and $Y_{p/x}$ (cell-product yield). The maximum specific growth rate (μ_{max}) is given by:

DOI: 10.2225/vol16-issue2-fulltext-6

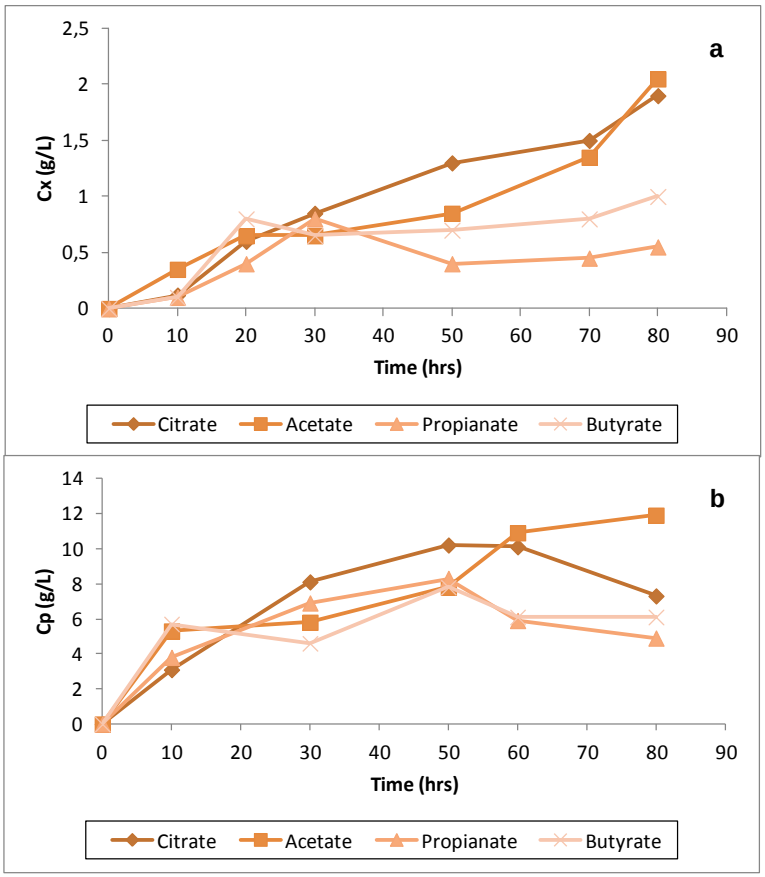

Fig. 1 (a) Growth of *Xanthomonas campestris pv campestris* - CBMAI 0199 (ATCC 33913). (b) Production of xanthan gum in the presence of different salts in culture media.

$$Ln\left(\frac{Cx}{Cx_0}\right)=\mu_{max}\cdot t$$

[Equation 1]

In Equation 1, C_x is the concentration of cells and Cx_0 is the initial concentration of the cells.

From the results of cell concentration obtained during the growth period (until 30 hrs), it was possible to determine the maximum specific growth rate for each case studied (Table 1).

According to the results shown in Table 1, it can be seen that the highest conversion of substrate into product ($Y_{p/s}$ (g/g)) and substrate ($Y_{x/s}$ (g/g)) into cells occurred with the use of acetate salt, whereas the highest conversion of product by the cell ($Y_{p/x}$ (g/g)) occurs with the use of propionate salt. The growth rate was almost the same for all. Hence, the use of propionate becomes interesting since it produces a cultivation broth with lower concentration, and consequently, a lower viscosity broth, which demands less consumption of energy from the agitators and a higher gas-liquid mass transfer coefficient, especially for the case under study, in which the product increases the apparent viscosity of the mix.

Esgalhado and Roseiro (1998) mention the increase in the gum production when the concentrations of the acetic and pentanoic acids were lower than 2.5 nM and 1.96 nM, respectively. In the present study, the uses of sodium salt were in anion concentrations higher than those reported by these authors. Moreover, the gum production (12,2 g/L) and its product-substrate yield (0.92) using acetate were higher than those found them in a continuous fermenter, reached 6.5 g/L for the gum production ($Y_{p/s}$ = 0.701 g/g). The best specific gum production result found by these authors was for the octanoic acid, $Y_{p/s}$, is 1.60 g/g. In the present study, in batch conditions, the results for $Y_{p/s}$ were 0.92; 0.72; 0.71 and 0.59 for acetate, propionate, butyrate and citrate, respectively as shown in Table 1. On the other hand, the gum production obtained by García-Ochoa et al. (2000), using batch cultivation (in similar conditions of pH, temperature and agitation) was about 6.5 g/L.

The gums produced were rheological characterized, and the results are shown in Table 1. The gums produced in this work were typically non-Newtonian pseudoplastic fluids. The gum produced with propionate salt presents higher value of consistency index; the gum produced with butyrate salt presents lower value of consistency index but higher flow index. So, gums with different characteristics can be produced for specifically uses in industries. Song et al. (2006) studied the rheology of concentrated xanthan gum solution and obtained for 1% w/w a consistence index (K) 8.5 dina.s^{-n}.cm^{-2} and flow rate index (n) of 0.14.

The apparent viscosity (η_a) can be obtained as a function of the rheological parameters (K and n) and the deformation rate (γ), as reported in the literature (García-Ochoa et al. 2000; Song et al. 2006) as Equation 2.

$$\eta_a = K \cdot \gamma^{(n-1)}$$

[Equation 2]

Similarly, it is possible to predict apparent viscosity values for same values of deformation rate. In the present study, all cultivations were made under the same stirring conditions and were performed in flasks with similar geometrical configuration to guarantee the same deformation rate. Hence, it can be said that the gum produced with propionate salt presented high consistency index value indicating an apparent viscosity value higher than those obtained with the other salts. This result corroborates that observed in the growth curve (Figure 1a), in which cell death was detected in the assay with propionate (30 hrs of growth) due to the decrease in the dissolved oxygen level necessary for the cell growth and survival. The second highest consistency index value was verified for the gum produced with acetate salt, which was also the second assay in which cell death was detected after around 80 hrs of growth. When comparing butyrate and citrate, it should be considered that despite their similar values of consistency index, the flow index (n) is different, *i.e.* for the same stirring rate imposed on the system, the apparent viscosity of medium with citrate will be lower than that of the medium with butyrate. Therefore, this result confirms the values of timing and cell death shown in Figure 1a, that is, time of cell death at 150 hrs of growth for butyrate, with higher apparent viscosity level compared to that of acetate, and time of cell death at 150 hrs of growth for acetate.

ACKNOWLEDGMENTS

The authors are grateful for the financial support (Thematic Project, Proc. no. 05/51702) and fellowship (granted to Jose Alberto Correa Leite Proc. no. 07/54930-8) provided by FAPESP.

Financial support: Thematic Project, Proc. no. 05/51702 and fellowship Proc. no. 07/54930-8 provided by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

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How to reference this article:

LEITE, J.A.C.; POZZI, E.; PELIZER, L.H.; ZAIAT, M. and BARBOZA, M. (2013). Use of volatile fatty acids salts in the production of xanthan gum. *Electronic Journal of Biotechnology*, vol. 16, no. 2. http://dx.doi.org/10.2225/vol16-issue2-fulltext-6