Biosynthesis of xanthan gum from nutrients extracted from sugar cane bagass

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Abstract. Xanthan gum, a microbial polysaccharide produced by certain strains of bacteria from the genus Xanthomonas, is recognized for its thickening and stabilizing properties in a variety of industrial applications. Therefore, the aim of this study was to produce and characterize xanthan gum using sucrose derived from sugar cane bagasse. The product was characterized in terms of viscosity, viscometric analysis of molar mass, and thermogravimetric analysis. Dinitrosalicylic acid (DNS) tests were conducted to measure whether the amount of sugar available in the culture medium was sufficient for the growth of the microorganism and the production of the desired product. Additionally, throughout the experimental process, specific methods were employed to purify the final product, achieving not only an optimum yield but also a high degree of purity. The results obtained were in line with data previously reported in the literature, resulting in an average yield of 2.23 g/L. Furthermore, the characterization carried out demonstrated the purity of the gum produced and the efficiency of the production process.

Keywords. Xanthan gum; Biopolymers; Bioprocesses.

1. Introduction

A utilização de polímeros convencionais se apresenta como um grande desafio a ser superado na última década. Atualmente, a utilização de microrganismos para a produção de goma xantana, que é um biopolímero muito utilizado no ramo alimentício e farmacêutico, tem sido uma boa alternativa para a futura substituição dos métodos que utilizam esses polímeros. Isso porque, as propriedades reológicas da goma são interessantes para a indústria, como a pseudoplasticidade, viscosidade elevada em baixas concentrações e a solubilidade em água (1). Xanthan gum is a polymeric substance produced by gram-negative bacteria of the genus Xantomonas sp. the vast majority of them being considered phytopathogens and capable of infecting plants, functioning as a thickener, emulsifier and dispersant (2).

Brazil, despite being an importer of xanthan gum, faces obstacles in national production. Research efforts aim to improve production, raise quality standards and reduce costs, exploring the use of agro-industrial waste as a carbon source to mitigate environmental impacts (3). Sugarcane, the cornerstone of Brazil's economy as the world's largest producer and exporter of sugar, generates substantial waste such as bagasse, comprising approximately 25 to 30% of the weight of ground sugarcane(1).

Bioprocesses involving microorganisms for the production of industrially relevant bioactives have been gaining prominence. Therefore, the production of xanthan gum assumes increasing importance as a necessary alternative to harmonize the country's social, economic, and environmental development. Thus, the present study aimed to cultivate Xanthomonas campestris using nutrients extracted from sugarcane bagasse to produce and characterize xanthan gum.

2. Methodology

2.1 Inoculum preparation

For the inoculation of the bacteria, the prepared YM medium was used, the composition of which is shown in Table 1.

Medium composition	Quantity (%m/v)
Glucose	1
Yeast extract	0,3
Meat extract	0,3
Bacteriological peptone	0,5

Table 1 - List of compounds and their respective quantities to prepare the YM medium (own authorship, 2023)

Erlenmeyer flasks were prepared with 100mL of this medium for the inoculum and then autoclaved at a temperature of 120°C for 20 minutes. These vials were cooled to room temperature. Then, 2mL of the pre-inoculum that was stored in cryotubes was added. This procedure was carried out within laminar flow in order to maintain sterile conditions of the medium. Finally, the flasks were incubated on an orbital shaker, 250 rpm, at 28°C for 24 hours.

2.2 Preparation of the growing medium

Using sugarcane bagasse, the cultivation medium was prepared by cold extraction (grinding and filtering) of the bagasse, using the ingredients listed in Table 2.

Medium composition	Quantity (%m/v)
Bagaço de cana	6
K2HPO4	0,1
Ureia	0,01

Table 2 - List of compounds and their respective quantities to prepare the cultivation medium (own authorship, 2023)

The sugarcane bagasse was ground in a mixer together with distilled water. Subsequently, the medium was filtered using organza fabric and the solid part was discarded while the filtered liquid was used as the cultivation medium. At the end, 0.1% K2HPO4 and 0.01% urea were added. After preparation, this medium was autoclaved at a temperature of 121°C for 20 minutes.

2.3 Xanthan gum production and recovery

After sterilization of the media and cooling, and reactivation of the bacteria at the end of 24 hours in the orbital shaker. 10 mL of inoculum were added to each Erlenmeyer flask, carried out inside a laminar flow hood, to guarantee the asepsis of the experiment. Subsequently, the media with the inoculum already added were taken to an Eppendorf® brand orbital shaker, refrigerated at 28°C at 250 rpm for 120 hours to produce xanthan gum. After the end of 120 hours of cultivation, centrifugation was performed at 4800 rpm at 4°C for 20 minutes to separate bacterial residues and the culture medium. At the end of the centrifugation, ethyl alcohol 92.8° GL was added to precipitate the gum at room temperature, for 1 hour in a 3:1 ratio of ethanol and medium containing gum, respectively. After the process time, a commercial sieve was used to remove the floated gum from the solution through filtration. After 24 hours, filtration was carried out again to remove traces of xanthan gum. Finally, the precipitated gum was taken to a freezer to freeze it and then it was freeze-dried and crushed for later analysis.

2.4 Characterization of xanthan gum

Solutions of 0.5% (w/v) xanthan gum were prepared in distilled water and stored. Viscosity readings were taken using a Brookfield viscometer with a water bath maintaining a temperature of 25°C. After readings, the average viscosimetric mass was determined using the Mark-Houwink solution. Additionally, a TGA analysis was conducted on a 2,073 mg sample, with temperatures ranging from 25 to 600°C in an inert N2 atmosphere, at a flow rate of 50 mL/min and a heating rate of 10°C min-1. The determination of reducing sugars and total reducing sugars was performed using the 3,5-dinitrosalicylic acid (DNS) method, with spectrophotometry according to Miller (1959). To calculate the daily production of xanthan gum, the same procedure was repeated, and the gum yield was determined for each day of cultivation (4)

3. Results and discussion

3.1 Xanthan gum recovery

To calculate the xanthan gum yield, the amount of gum recovered was rationed to the total volume of the cultivation medium. The results found are shown in Table 3.

Batch	Amount of gum (g)	Volume (L)	Yield (g/L)
1	2,24	0,97	2,31
2	4,76	2,00	2,38

Table 3 - Yields and variables found in each batch (own authorship, 2023)

Similar results were observed by Costa et al. (2016)(3), who obtained a yield of 2.81 g/L using the same substrate. The difference can be attributed to the different strains of bacteria used.

In the work of DINIZ et.al. (2012)(5), the concentrations listed in Table 4 were found for different substrates.

Culture medium	Amount of gum (g)	Standard deviation	Author
Sucrose	2,42	±0,62	DINIZ et.al. (2012)
Cocoa Shell	7,34	±0,41	
Whey	12,01	±0,65	

Table 4 - Performance found with different substrates

The productions obtained in this study (2.13 to 2.38 g/L) are comparable to the production in a study with sucrose as substrate (2.42 g/L), indicating similarity. Many studies in the literature use different substrates, but we observed that certain substrates can result in "false yields" due to the precipitation of compounds along with the gum after addition of ethanol. The methodology of this work includes efficient filtration and centrifugation processes, contributing to the reliability of the results and purity of the gum obtained.

3.2 Intrisic viscosity

The values found for intrinsic viscosity are listed in Table 5 and shown graphically in Figure 2.

			$\frac{\eta_{sp}}{\eta_0 - 1} = \eta$	$\eta = \frac{\eta_{sp}}{C}$
Concentration (g/dL)	**η(mPa)**	**Torque**	**Specific viscosity**	**Intrinsic viscosity**
0,100	18,6	62,0%	12,6764	126,7647
0,150	7,47	25,0 %	4,4926	89,8529
0,025	2,82	9,50 %	1,0735	42,9411
0,013	1,78	8,60 %	0,3088	24,7058

Table 5: Data obtained from the viscometer using 20 RPM rotation

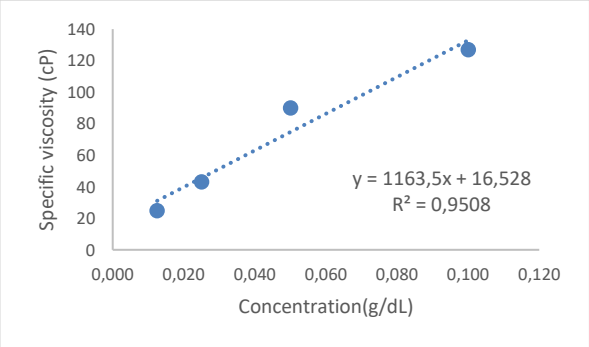

Fig. 2: Behavior of xanthan gum at different concentrations in terms of its specific specificity. (Own authorship, 2023)

With the data obtained, it was possible to analyze that the method presents an adequate degree of reliability for the analyzes carried out. Using the generated line equation, the limit viscosity value, that is, the lowest measured viscosity corresponds to the linear coefficient of the equation, which is equal to 16.528 cP.

3.3 Viscosimetric molar mass

Xanthan gum, a biopolymer produced by certain bacteria, has anionic characteristics and consists of repeating units of pentasaccharides. In this study, the viscosimetric molar mass was determined as 1,347,796 Da. These values are comparable to the results of studies carried out in the same line of research, which have not yet been published, presenting an average viscosimetric molar mass of 1,338,123 Da. It is It is important to note that factors such as cultivation temperature, type of bacterial strain and nitrogen source can directly affect the molar mass of the biopolymer, influencing the acetate and pyruvate contents (6)

3.4 Thermal analysis

Figure 3 shows the TG thermogravimetric curves and their DTG derivatives when subjected to a heating rate of 10°C min-1, between 0 and 600°C.

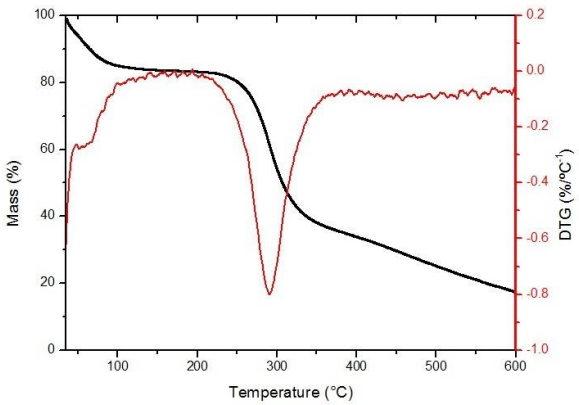

Figure 3: TGA and derivative (DTG) curves for laboratory xanthan gum (Own authorship, 2023)

The results found were very similar to those described by Brandão, 2012 (7). It is possible to observe that the first significant drop in mass, corresponding to a loss of 20%, occurred at 100°C. At this point, all liquid present evaporates, demonstrating the direct influence of temperature on the process. Subsequently, a second reduction of 60% of the total mass was recorded close to 300°C, indicating the decomposition of the organic matter present. What remained after this process were ash, constituting the remaining inorganic matter.

3.5 Determination of the amount of sugar consumed and xanthan gum produced

In Table 6 and Figure 4, total sugar concentrations (g/100mL) and gum yields (g/L) are presented over the cultivation time for 5 days.

Time (days)	Total sugars (g/100mL)	Yield (g/L)
0	28,47 ± 0,48	0
1	16,91 ± 0,10	1,8
2	12,57 ± 0,65	2
3	8,18 ± 0,38	2,1
4	6,34 ± 0,09	2,2
5	2,69 ± 0,10	1,5

Table 6: Concentration of total sugars and xanthan gum yield over the cultivation time (Own authorship, 2023)

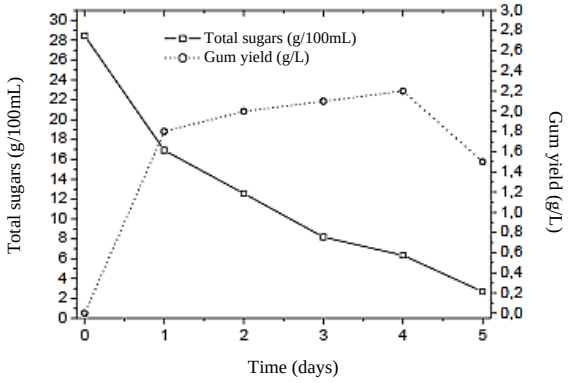

Figure 3: Curve of sugar consumption and xanthan gum production over time (Own authorship, 2023)

The results presented in Table 6 and Figure 4 demonstrate that the gum yields observed after three days of cultivation were similar to those obtained after five days, suggesting the possibility of reducing the time and cost associated with the process. This promising discovery motivates the carrying out of new experiments, aiming to optimize process conditions, including reducing cultivation time, increasing yield, sizing bioreactors and implementing fed batches.

4. Conclusions

The production of xanthan gum using sucrose obtained from sugarcane bagasse showed an average yield of 2.24g/L. Note that the sugar content decreased by 85% over 5 days of cultivation. The gum produced presented an intrinsic peculiarity compatible with gums reported in the literature, as well as an average viscosimetric molar mass close to 1 million Daltons. It can be concluded that a gum produced from nutrients extracted from sugarcane bagasse has similar physical-chemical properties to other gums produced from by-products, proving the efficiency of the methodology proposed in this work.

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