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Effect of Citric Acid Inducer on Xanthan Gum Production from Cassava Bagasse, a Potential Agro-Industry Waste

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ABSTRACT

In this study cassava bagasse, an agro-industrial solid-waste, was converted to xanthan gum. Glucose obtained by the acid pre-treatment of cassava bagasse was used as the substrate for the production xanthan gum. The production rate of xanthan gum was improved by the addition of citric acid. The analytical studies had shown a gradual increase in xanthan gum production when citric acid was added in fed batch mode to the fermentation media. Higher citric acid concentration was inhibitory to xanthan gum synthesis. The polymer obtained was then analyzed using FTIR spectrum for the identification of functional groups.

Keywords: Xanthan gum, *Xanthomonas campestris,* citric acid.

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INTRODUCTION

Xanthan gum is the first microbial extracellular heteropolysaccharide produced by the gram negative bacterium *Xanthomonas campestri*s. Xanthan gum consists of D-glucosyl, D-mannosyl, and D-glucoronyl acid residues in a molar ratio of 2:2:1 small proportions of O-acetyl and pyruvate groups [1-4]. The genome of the bacterium consists of two genes, the gum cluster and a gene cluster gene. The genes present in the gum cluster are responsible for releasing the xanthan gum in the growth medium. In *X.campestris*, xanthan gum is produced by the metabolism of glucose via Entner-Doudoroff pathway in conjunction with tricarboxylic acid cycle. The molecular weight of xanthan gum is approximately 2 million, but it can go as high as 13-50 million [5]. Due to its physical and rheological properties xanthan gum can be used as an additive in animal feed and pesticide formulations, petroleum production, slurry explosives, pharmaceuticals, and textile printing [6].

Different kinds of carbon sources like glucose, sucrose, maltose, corn starch, dextrose, sorbose, galactose, rhamnose, mannose, lactose, arabinose and ribose were used for the production of xanthan gum. The nutritional requirements for optimal xanthan production were studied earlier. Glucose and sucrose were proved to be the best substrates (carbon source) and glutamate as the best nitrogen source [7, 8]. The addition of organic acids (succinic acid, acetic acid, citric acid) to the culture medium had been reported to improve xanthan gum synthesis during the fermentation process. Citric acid is used as a chelating agent to prevent the precipitation of salts in the medium [9]. Xanthan gum is soluble in acetic acid, and therefore it provides a good medium to dissolve the xanthan gum [8, 10, 11].

The use of glucose as a sole carbon source is uneconomical due to its high cost and hence glucose obtained from the naturally available sources like molasses, cheese whey, cassava bagasse were used for xanthan gum production [12,13]. Cassava bagasse (*Manihot esculenta)* contains high amount of starch and fibre content. Glucose is obtained from the cassava by different pre-treatment methods namely acid, thermal and enzymatic treatment [14].

The objective of this study is to improve the xanthan gum production by addition of citric acid.

MATERIALS AND METHODS

Microorganism and Media

Xanthomonas campestris was obtained from The Microbial Type Culture Collection (MTCC) Chandigarh, India. The strain was maintained on glucose yeast extract agar (20 g/l glucose, 10 g/l yeast extract, 20 g/l $CaCO_3$, 17 g/l agar). Inoculated slants were grown at 28-30º C for 48 hrs and stored at 4°C.

Inoculum Preparation

Inoculum was prepared in the same medium without agar by placing 100 ml of sterile medium in a 250 ml Erlenmeyer flask and incubated in a rotary orbital shaker (28-30 ºC and 150 rpm) for 24 hrs. Five percent (v/v) of this suspension was used as an inoculum for fermentation process.

Acid Hydrolysis of Cassava Bagasse

Acid hydrolysis of cassava bagasse was carried out by taking 100 g of dry cassava bagasse/L of 1% HCl and autoclaving at 121°C for 12min. After cooling, contents were neutralized (pH 7.0) by adding 1.0 N NaOH. The content was then filtered using Whatmann No. 1 filter paper and was assayed for reducing sugars by DNS method [14]. The concentration of glucose was adjusted to 2% by dilution.

Production of Xanthan Gum

The xanthan gum production was carried out in a 500 ml Erlenmeyer flask with 100 ml of the production media composed of (g/L) glucose 20, potassium nitrate 5, magnesium chloride 0.6, sodium sulphate 0.1, boric acid 0.06, ferric chloride 0.02, calcium carbonate 0.02. Citric acid was added in fed batch mode in the media after 24 hrs.

Estimation of Biomass concentration and recovery of xanthan gum

The fermentation medium was centrifuged at 10,000 rpm for fifteen minutes to recover the cells. After centrifugation the cells get sediment at the bottom. The cells were then dried at 95ºC in conventional oven overnight. Xanthan gum in the supernatant was then separated by the addition of isopropyl alcohol (2:1v/v) and then the solution was then centrifuged at 12,000 rpm for 20 mins at 4°C and the pellet was dried overnight in hot air oven. The dried powder of xanthan was then analyzed for its functional groups using FTIR.

RESULTS AND DISCUSSION

Fig.1 shows the FT-IR spectra of xanthan, a broad absorption peak at 3430 cm^{-1} indicate the hydrogen bonded OH groups. Two peaks one at 614 cm^{-1} and other at 1424 cm^{-1} are attributed to –COO groups (16).

The improvement of xanthan gum production from cassava bagasse hydrolysate by *Xanthomonas campesteris* has been studied. The experiment was carried out in shake flasks. The agitation rate of the medium was 150 rpm. Fig.2 represents the variation of biomass and glucose concentration in the fermentation broth. The experiments were carried out in shake flasks with the addition of different concentrations of citric acid (2.2 g/L, 3.3 g/L, 4.4 g/L, 5 g/L) in fed batch mode after 24 hrs (when all the citric acid in the medium was consumed). Fig.3 represents the addition of citric acid increased the production of xanthan gum and biomass

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markedly. The maximum yield of xanthan (20.82 g/L) was obtained when the citric acid in the medium was 4.4 g/L. Further addition of citric acid to the medium was inhibitory to xanthan gum production.

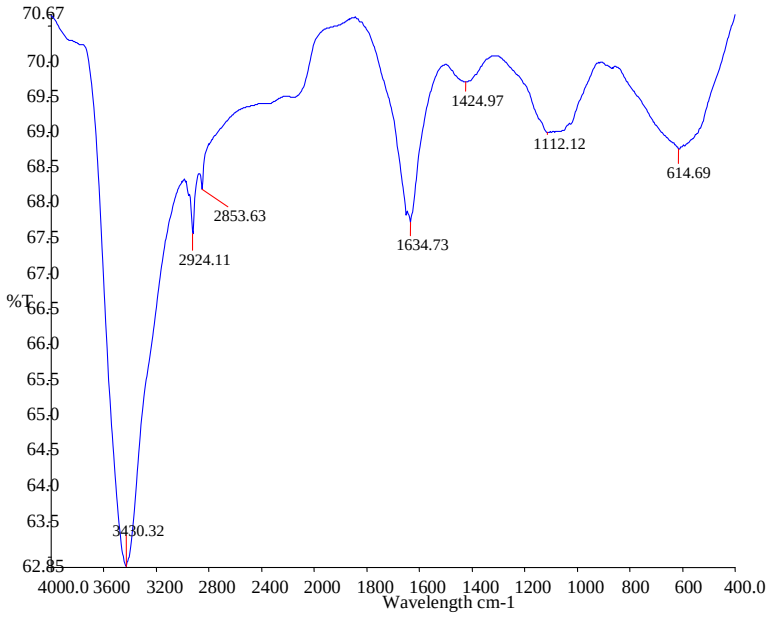

Fig 1. FTIR SPECTRA OF THE SYNTHESISED XANTHAN GUM

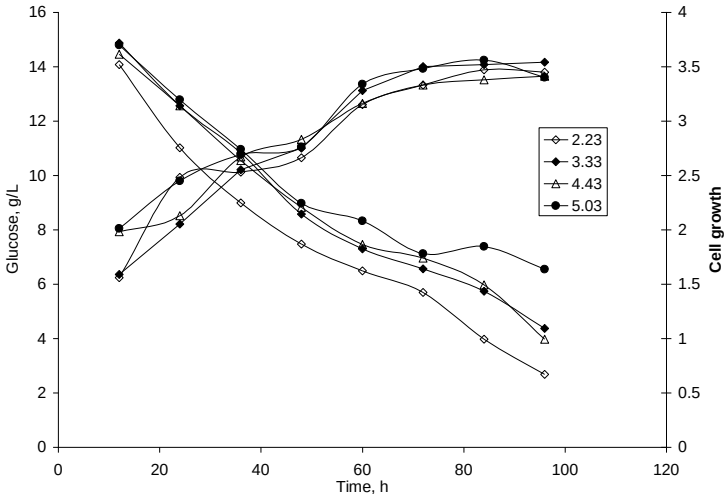

Fig 2: Effect of glucose on addition of different concentrations of citric acid

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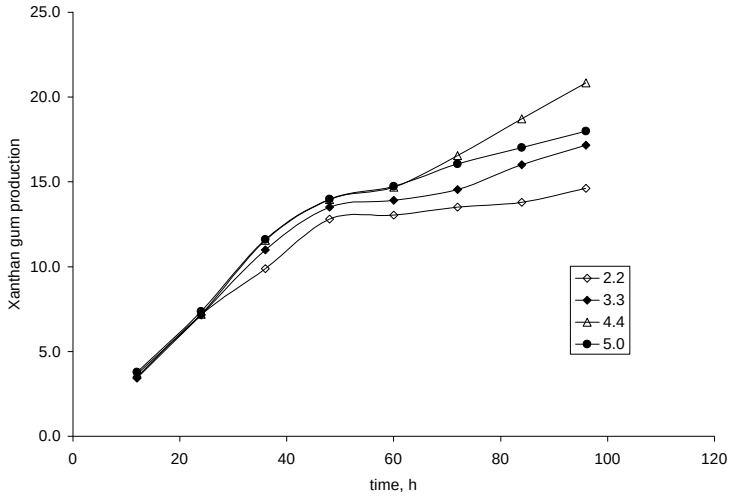

Fig 3. Xanthan yield obtained by the addition of different concentrations of citric acid

Normally citric acid acts as an energy source in the biosynthesis of xanthan. In the metabolism of citric acid utilizing bacteria citric acid is first split into oxaloacetate and acetate and finally gives pyruvic acid. Pyruvic acid is further converted to lactic acid or it can enter the TCA cycle through acetyl CoA. When citric acid is metabolized by the organisms through the TCA cycle ATP is produced which is required by the micro organism for the export of xanthan from the cytoplasmic membrane of the cells to the extracellular environment.

CONCLUSION

Cassava bagasse is one of the cheapest sources that can be used for the production of xanthan gum industrially. From the present study it was clear that the addition of citric acid improve xanthan gum production. Higher concentration of acids might cause inhibitory effect on polymer synthesis.

REFERENCES

[1] Jeanes A, Pittsley JE, Senti FR. J App Polymer Sci 1961; 17:519-526.
[2] Rogovin SP, Anderson RF, Cadmus MC. J Biochem Microbiol Technol Eng 1961; 3:51-63.
[3] Rogovin P, Albrecht W, Johns V. Biotech Bioeng 1965; 7:161-169.
[4] Morris ER, Rees DA, Walkinsaw MD, Darke A. J Mol Bio 1977; 110;1-16.
[5] Becker A, Katzan F, Puhler A, Ielpi L. Appl Microbiol Biotechnol 1998; 50:145-52.
[6] Garcia-Ochoa F, VE Santosa, JA Casasb, E Goameza. Biotechnol adv 2000; 18: 549-579.
[7] Souw P, Demain AL. Appl Environ Microbiol 1979; 37:1186-92.
[8] Souw P, Demain AL. J Ferment Tech 1980; 58 :411-16.
[9] Peters HU, Ghosh P, Zaidi A, Schumpe A, Deckwer WD. Fifth European Congress on Biotechnology 1990; 2:1049-1052.
[10] Jana AP, Ghosh W. J Microbio Biotech 1997; 13: 261-264.

[11] Sara Alaei Shehni, Mohammad Reza Soudi, Saman Hosseinkhani, Niloufar Behzadipour. Afr Journ Biotech 2011; 10:9425-9428.
[12] Adenise L Woiciechowski, Carlos R Soccol, Saul N Rocha, Ashok Pandey. Appl Biochem Biotech 2004; 118:305-312.
[13] SL Gilani, HD Heydarzadeh, N Mokhtarian, A Alemian, M Kolaei. Aus J Basic App Sci 2011; 5(10): 855-859.
[14] Sugumaran KR, Kumar BK, Mahalakshmi M, Ponnusami V. International Journal of Chem Tech Research 2013; 5(1): 394-400.
[15] Miller GL. Anal Chem 1959; 31: 426-428.
[16] SN Yuen, SM Choi, DL Phillips, CY Ma. Food Chem 2009; 114: 1091–1098.