

XANTHAN GUM FROM SUGAR CANE RESIDUE

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ABSTRACT: We focused on the usefulness of agricultural waste, we produced artificial paper from agricultural waste such as pineapple peel and sugar cane residue. We found that water used to boil sugar cane residue was contained sugar and we thought that this might be used as a raw material to produce other products. Xanthan gum is a polysaccharide that is used as an additive to increase the viscosity and stability of food product and other products. Some micro-organisms could produce xanthan gum. This project focused on the xanthan gum production by using sugar cane residue extraction as raw material, and *Xanthomonas campestris TISTR 840*. We found that the incubation time of *Xanthomonas campestris TISTR 840* at room temperature was 48 hours. Sugar cane residue extraction with a sugar concentration of 1.5g/100 mL and pH 7, incubated with *Xanthomonas campestris TISTR 840* for 144 hours at room temperature and 200 rpm shaker speed could produce xanthan gum in highest amount, 1.5% yield. Addition of KCl at 4 g per 100 mL of incubation media removed the micro-organism. We dried xanthan gum for 30 minutes at 100^o C and collected 1.5 g of xanthan gum. The produced xanthan gum was soluble in water, 95% ethanol, 5% NaOH, 8% HCl, isopropyl alcohol and glycerol. It was stable in 5% NaOH, 8% HCl and 2% NaCl. The produced xanthan gum was soluble in water at a concentration of 2 g/100mL and was stable at 120^oC. Infrared spectroscopy showed that both produced and commercial xanthan gum had a similar IR spectrum. The results indicated that xanthan gum could be produced from agricultural waste.

Keywords: Xanthan gum, *Xanthomonas campestris TISTR 840*, Sugar cane residue, Polysaccharide, Microbial gum, Agricultural waste

1. INTRODUCTION

Our research about the production of artificial paper, found that fiber from some plant and agricultural waste was used as a raw material. One of raw material was sugar cane residue. We found that boiling the residue before paper production could reduce the amount of NaOH needed to extract cellulose and left sugar in the boiled water as a waste. Xanthan gum is one of the microbial gums that The FDA (Food and Drug Administration) approved in 1996 for use as an additive in food production [1]. Xanthan gum is use as an additive in foods, cosmetics and medicines due to its suitable properties, such as a high degree of pseudoplasticity, and a high viscosity at low concentration [2]. Xanthan gum is exopolysaccharide produced by *Xanthomonas campestris*. *Xanthomonas campestris* can grow on rice, barley, corn flour, acid whey, molasses and sugar cane, etc, [1] and produce xanthan gum [2], [3].

The objective of this project focused on the usefulness of agricultural waste such as sugar cane residue or bagasse. Therefore, we used sugar cane residue extraction containing sugar as raw material for production of xanthan gum from *Xanthomonas campestris TISTR 840*.

2. MATERIALS AND METHODS

2.1 Sugar Cane Residue Extraction

1 kilogram of sugar cane residue was cut into small pieces and boiled in 1,000 mL of water for 3 hrs. The pH of the resulting light yellow extraction was measured by pH meter and the amount of reducing sugar was determined by DNSA using a glucose (100-500 mg/dm³) as the standard [6].

The sugar cane residue extraction was sterilized and stored at low temperature under aseptic conditions throughout the entire experiment.

2.2 Growth Rate of *Xanthomonas campestris TISTR 840* in Sugar Cane Residue Extraction

The activated *Xanthomonas campestris TISTR 840* was inoculated in 50 mL sterile sugar cane residue extraction and incubated at room temperature with a shaking rate of 200 rpm for 144 hrs. Five mL of this inoculated extraction was inoculated in 250 mL of sterile sugar cane residue extraction and incubated at the same conditions. The growth rate was determined by measuring turbidity at 600 nm.

2.3 Factors Affecting Xanthan Gum Production

2.3.1 The reducing sugar content

The reducing sugar content of the sugar cane residue extraction was adjusted to 0.5, 0.75, 1.5 and 3.0 g/100 mL, to pH 7 using NaOH and sterilized. The activated *Xanthomonas campestris TISTR 840* was inoculated in 100 mL of this extraction and incubated for 24 hours at room temperature with a shaking rate 200 rpm. Five mL of this inoculated extraction was inoculated in 95 mL of sterile sugar cane residue extraction and incubated at the same conditions. At 0, 48, 96, 144 hours., we measured pH and viscosity by using 4 cm probe at 100 rpm. The micro-organism was separated from the media by centrifuge. The amount of reducing sugar of sterilized media was measured. Xanthan gum was precipitated using 1% KCl.

2.3.2 Acid- base condition

The reducing sugar content of the sugar cane residue extraction was adjusted to 1.5 g /100mL, and the pH was adjusted to 5, 7and 9 using NaOH. The primary pH and adjusted pH extraction were then sterilized. The activated *Xanthomonas campestris TISTR 840* was inoculated in 300 mL of each extraction and incubated for 24 hours at room temperature, with a shaking rate of 200 rpm Ten mL of each inoculated extraction was inoculated in 90 mL of sterile sugar cane residue extraction and incubated at the same conditions. At 0, 48, 96, 144 hours., we measured pH and viscosity using 4 cm probe at 100 rpm. The micro-organism was separated from the media by centrifuge. The amount of reducing sugar was measured. Xanthan gum was precipitated using 1% KCl.

2.3.3 Shaking rate

The sugar content of the sugar cane residue extraction was adjusted to 1.5 g/100mL, and the pH was adjusted to 7 using NaOH. The extraction was then sterilized. The activated *Xanthomonas campestris TISTR 840* was inoculated in 120 mL of this extraction and incubated for 24 hours at room temperature, with shaking rates of 150, 200 and 250 rpm. Ten mL of each inoculated was inoculated in 90 mL of sterile sugar cane residue extraction and incubated at the same conditions. At 0, 48, 96, 144 hours, we measured pH and viscosity using 4 cm probe at 100 rpm. The micro-organism was separated from the media by centrifuge. The sterilized media was measured amount of reducing sugar. Xanthan gum was precipitated using 1% KCl.

2.3.4 Concentration of KCl

The sugar content of the sugar cane residue extraction was adjusted to 1.5 g/100mL, and the pH was adjusted to 7 using phosphate buffer. The extraction was then sterilized. The activated *Xanthomonas campestris TISTR 840* was inoculated in 120 mL of this extraction and incubated for 24 hours at room temperature, with shaking rate of 150, 200 and 250rpm. Ten mL of each inoculated extraction was inoculated in 90 mL of sterile sugar cane residue extraction and incubated at the same conditions. At 0, 48, 96, 144 hours, we measured pH, and viscosity using 4 cm probe at 100 rpm. The micro-organism was separated from media by centrifuge. The amount of reducing sugar in the sterilized media was measured and 2%, 3% and 4% KCl were used to coagulate the xanthan gum.

2.4 Solubility and Stability of Produced Xanthan Gum

2.4.1 Solubility in solvent

We compared the solubility of 2% xanthan gum 2% starch and 2% gum arabic in various solvents : H₂O, ethyl alcohol, 5% NaOH, 8% HCl , isopropyl alcohol and glycerol.

2.4.2 Stability in solutions

We compared the viscosity of 2% xanthan gum, 2% starch and 2% gum arabic in various solvent, 2% NaCl, 5% NaOH and 8% HCl using a 4 cm probe viscometer(Brookfield), at room temperature in the interval 24, 48,72 and 96 hours.

2.4.3 Stability at temperature

The viscosity of 2% xanthan gum, 2% starch and 2% gum arabic in 2% NaCl, 5% NaOH and 8% HCl were measured using a 4 cm probe viscometer(100rpm), at 40, 60, 80 and 100°C.

2.5 Properties Comparison of Produced and Commercial Xanthan Gum

2.5.1 Viscosity

The viscosity of 1% of each produced and commercial xanthan gum was measured using a 4 cm probe at 100 rpm.

2.5.2 Acid-base value

The pH of each 1% produced and commercial xanthan gum was measured at room temperature.

2.5.3 Melting Point

The melting point of produced and commercial xanthan gum was determined using Stuart Melting Point Apparatus (SMP3).

2.5.4 Infrared spectrum

We compared infrared spectrum of produced and commercial xanthan gum.

3. RESULT AND DISCUSION

3.1 Growth Rate of *Xanthomonas campestris* TISTR 840 in Sugar Cane Residue Extraction

Figure 1 showed that the stationary phase of the activated *Xanthomonas campestris* TISTR 840 in sterile sugar cane residue extraction was 48-144 hrs.

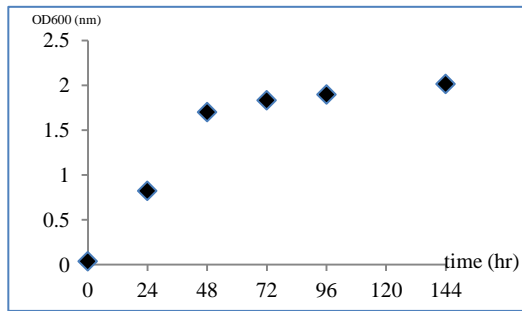


Fig.1 Growth curve of activated *Xanthomonas campestris* TISTR 840

3.2 Factors Affecting Xanthan Gum Production

3.2.1 The Reducing sugar content

Figure 2 showed that a higher amount of reducing sugar content resulted in a higher growth rate of *Xanthomonas campestris* TISTR 840.

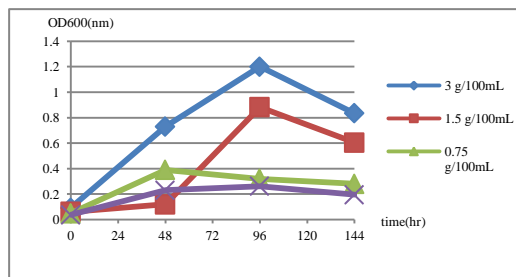


Fig. 2 Effecting of reducing sugar content on growth rate of *Xanthomonas campestris* TISTR 840.

Figure 3 showed that the reducing sugar content in all of the sugar cane residue extractions had the same profile in the growth of

Xanthomonas campestris TISTR 840. This profile indicated that *Xanthomonas campestris* TISTR 840 used the sugar cane residue extraction as its carbon source.

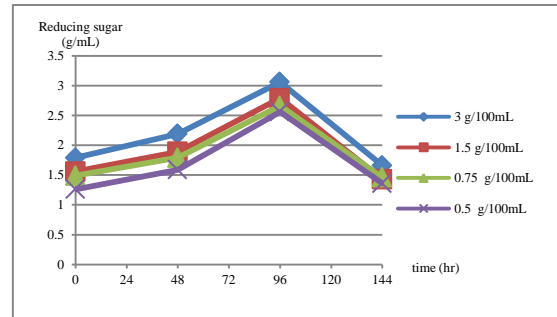


Fig. 3 Profile of reducing sugar content in growth rate of *Xanthomonas campestris* TISTR 840

Figure 4 showed that the *Xanthomonas campestris* TISTR 840 produced the highest amount of xanthan gum with 1.5 g/100mL of reducing sugar in the sugar cane residue extraction.

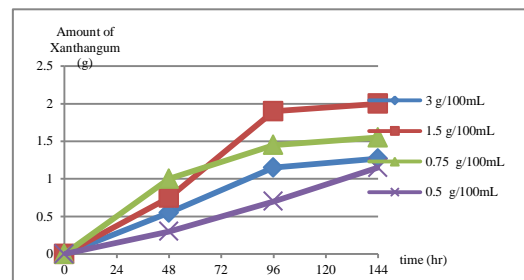


Fig. 4 The highest xanthan gum production

3.2.2 Acid-base condition

The adjusted pH of the sugar cane residue extraction was adjusted to pH 5 by acetate buffer, to pH 7 by phosphate buffer and to pH 9 by Tris buffer. The origin sugar cane residue extraction was designated the primary pH. Figure 5 showed that the sugar cane residue extraction with 1.5 mg/100mL of reducing sugar; had similar growth rate at both primary pH and pH 7.

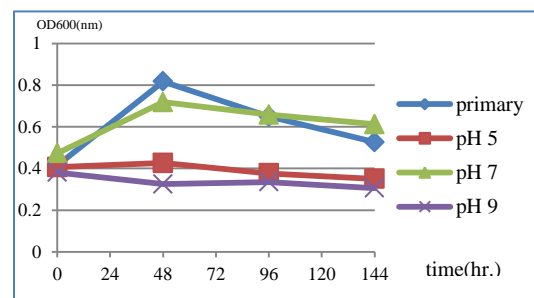


Fig.5 Effect of pH on the growth rate of *Xanthomonas campestris* TISTR 840

Figure 6 showed that the primary pH sugar cane residue extraction had the highest viscosity.

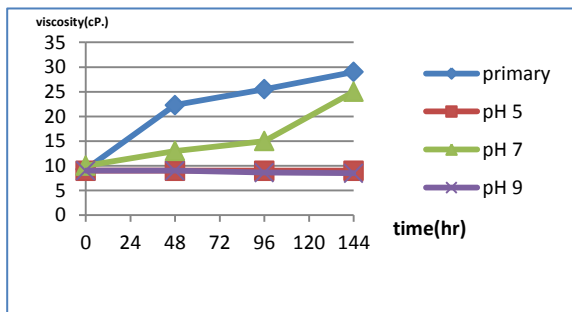


Fig. 6 Effect of pH on viscosity of sugar cane residue extraction

Figure 7 showed that the adjusting the primary pH sugar cane residue extraction to pH 7 produced the highest amount of xanthan gum.

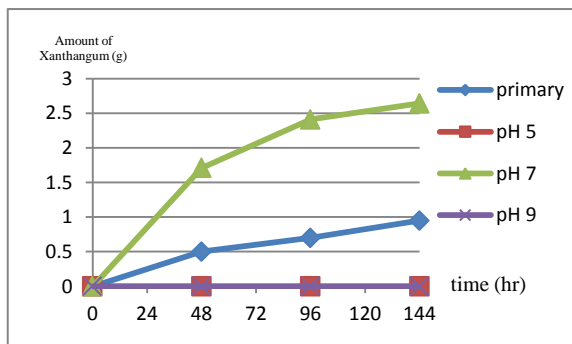


Fig. 7 Effect of pH on the amount of xanthan gum produced

3.2.3 Concentration of KCl

Figure 8 showed that the 4% KCl precipitated the highest amount of xanthan gum from the sugar cane residue extraction.

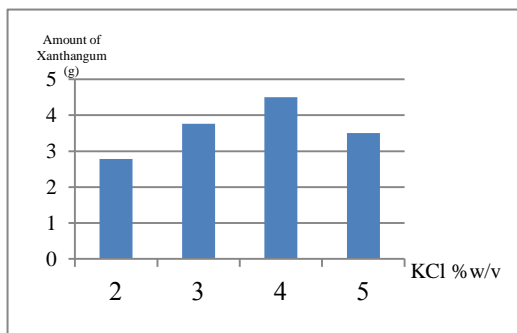


Fig. 8 Effect of KCl concentration on the precipitation of xanthan gum.

3.3 Suitable Conditions for Production of Xanthan Gum from Sugar Cane Residue Extraction

From the results showed in figure 1-8 we concluded that optimum production of xanthan gum from the 1.5 g/100mL of reducing sugar in the sterilized sugar cane residue extraction could be achieved by adjusting the pH to 7 using phosphate buffer [4] because phosphate was energy source [5], shaking at a rate of 200 rpm, at room temperature and precipitating the xanthan gum using 4% KCl. These conditions produced a 1.5% yield of xanthan gum.

Xanthomonas campestris TISTR 840 used sucrose as carbon source in the initial stage and then glucose in the stationary phase to produce xanthan gum, Fig.3, 4 and 5. The research of Infee Sherley,R.D. [7] showed that xanthan bacteria could utilize both glucose and sucrose as carbon source and that glucose was the best carbon source. They found that sugar cane and cassava bagasse could produce a good range of yield.

3.4 Properties of Produced Xanthan Gum

The xanthan gum we produced was a light yellow powder had similar properties to the commercial xanthan gum.



Fig.9 Powder of produced xanthan gum

3.4.1 Solubility in solvent

2% produced xanthan gum, 2% starch and 2% gum arabic could dissolve well in H₂O, ethyl alcohol, 5%NaOH, 8%HCl, isopropyl alcohol and glycerol.

3.4.2 Stability of solutions

Figures 10-12 showed the viscosity of 2% produced xanthan gum, 2% starch, 2% gum arabic dissolved in 2% NaCl, 5% NaOH and 8% HCl The results indicated that the solutions of produced xanthan gum were more stable than the others.

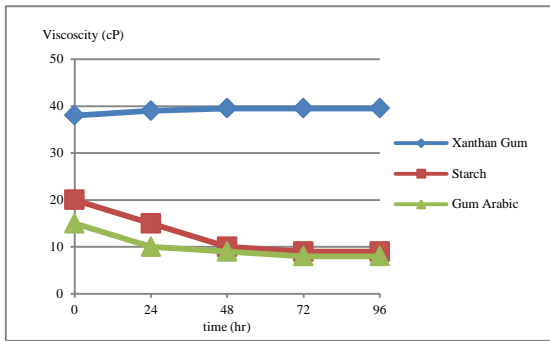


Fig.10 Stability of 2% of xanthan gum, 2% starch and 2% gum arabic in 2% NaCl

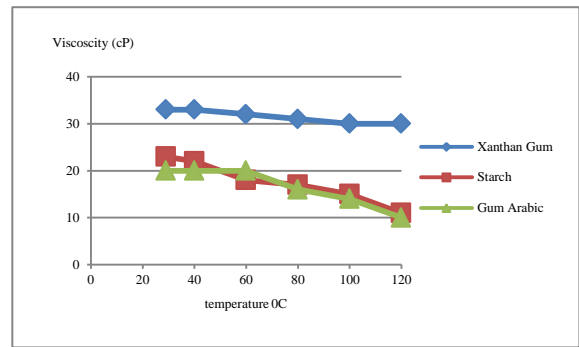


Fig.13 Stability of 2% of xanthan gum, 2% starch and 2% gum arabic in 8% HCl at 40, 60, 80 and 100°C.

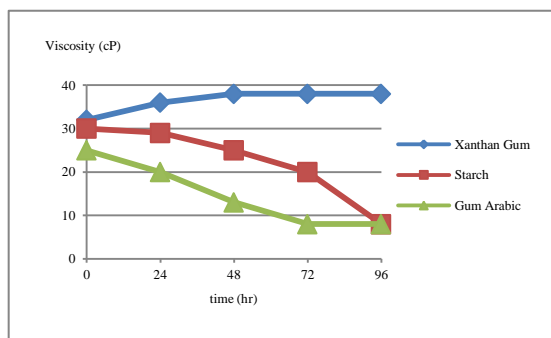


Fig.11 Stability of 2% of xanthan gum, 2% starch and 2% gum arabic in 5% NaOH

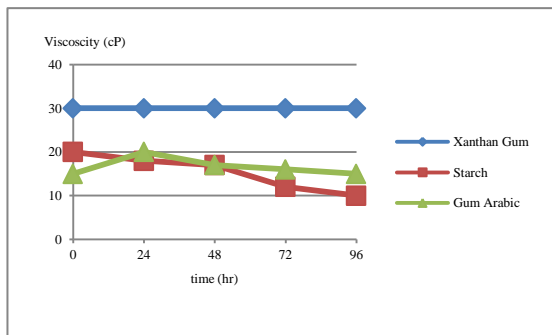


Fig.12 Stability of 2% of xanthan gum, 2% starch and 2% gum arabic in 8% HCl

3.4.3 Stability at temperature

Figure 13 showed the viscosity of 2% produced xanthan gum, 2% starch and 2% gum arabic in 8% HCl measured over a range of temperatures. The results indicated that the 2% produced xanthan gum was more stable than the others.

3.5 Properties Of Produced And Commercial Xanthan Gum

3.5.1 Viscosity, acid-base value and melting point

Table 1 Properties of produced and commercial xanthan gum

Property	Produced xanthan gum	Commercial xanthan gum
Viscosity(cP.)	600	580
pH	7	7
Melting Point(°C)	170	>145

Table 1 indicated that the properties of produced and commercial xanthan gum were similar.

3.5.2 Infrared spectrum

Figure 14 and 15 indicated that the infrared spectra of produced and commercial xanthan gum were similar. Both spectra had a band around 3,440 cm^{-1} corresponds to hydroxyl stretching vibration, and both had a band at 2,822 cm^{-1} that corresponds to weak C-H stretching vibration. These bands indicated a polysaccharide such as xanthan gum, which is an exopolysaccharide produced from a micro-organism. Therefore, xanthan gum can be produced from agricultural waste sugar cane residue extraction.

Sandra, F, et.al. [2] also showed that the infrared spectra of produced and commercial xanthan gum were similar. Sandra's process produced xanthan gum by *Xanthomonas campestris* pv. *Campestris* NRRLB-1459 using diluted sugar cane broth at last 24 hours. The following component 27.0 g/L sucrose, 2.0 g/L Brewer's yeast, and 0.8 g/L NH_4NO_3 were added to the sugar cane broth and then fermented at 750 rpm. Production of xanthan gum was confirmed by comparing its spectrum with the infrared spectrum

of commercial xanthan gum. The infrared spectra bands recorded in the range of 4,000-400 cm^{-1} and xnthan gum spectra bands were found at 3,400, 2,939 and 990-1,200 cm^{-1} that are common to all polysaccharide. These bands represented OH-bonds, C-H bonds of CH_2 groups and saccharides, respectively.

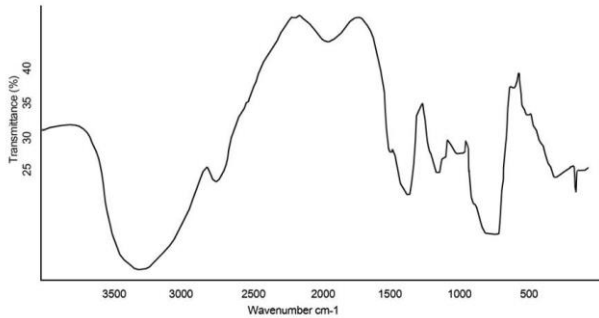


Fig.14 Infrared spectra of produced xanthan gum.

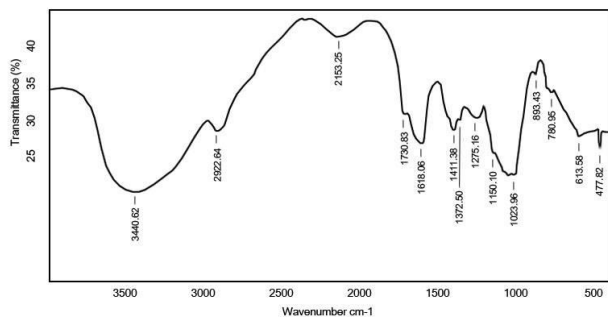


Fig.15 Infrared spectra of commercial xanthan gum.

4. CONCLUSION

The results identified suitable conditions for production of xanthan gum from the sugar cane residue extraction. We could produce xanthan gum from sterilized sugar cane residue extraction by adjusting the reducing sugar content to 1.5 g/100 mL, adjusting pH to 7 using phosphate buffer, shaking at a rate 200 rpm, at room temperature and precipitating the xanthan gum using 4% KCl. The solubility, stability of produced xanthan gum was similar to the other gums, i.e. starch and gum arabic. The viscosity, pH, melting point and IR spectrum of both produced and commercial xanthan gum were similar.

This study showed that production of xanthan gum from *Xanthomonas campestris* TISTR 840

using sugar cane residue extraction as raw material was possible and confirmed that the agricultural waste was a substrate capable of producing a useful product. The objective of this project focused on finding on a use for agricultural waste to reduce garbage. We hope that it will be possible to use all parts of a plant to reduce agricultural waste and to preserve the environment, too.

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