

Full Length Research Paper

Xanthan gum production by *Xanthomonas campestris* pv. *campestris* 8004 using cassava starch as carbon source

Qunliang Li^{1*}, Wei Yan¹, Kedi Yang¹, Yanxuan Wen¹ and Ji-liang Tang²

¹School of Chemistry and Chemical Engineering, Guangxi University, Nanning, People's Republic of China.

²The State Key Laboratory of Subtropical Bio-resource Conservation and Utilization, Guangxi University, Nanning, People's Republic of China.

Accepted 17 August, 2012

Cassava starch is a main renewable bio-resource with low price and mass production in Guangxi, China. It was used as carbon source in growing *Xanthomonas campestris* pv. *campestris* 8004 (Xcc 8004) for xanthan gum production in this study. The xanthan gum yield of gelatinized cassava starch was higher than that of raw cassava starch; the maximal reducing sugar and amylase activity reached 14.30 g/L and 1.19U/ml, respectively. The maximal xanthan gum yield of 16.95 g/L was achieved with 30 g/L cassava starch as carbon source. The concentration of residual sugar was 6.82 g/L at the end of fermentation, and the conversion efficiency of sugar reached 73.12%. Thus, Xcc 8004 should be used as a competitive strain for xanthan gum production using cassava starch in industrial applications.

Key words: Xanthan gum, cassava starch, *Xanthomonas campestris* pv. *campestris* 8004, amylase activity.

INTRODUCTION

Xanthan gum is a heteropolysaccharide with a primary structure consisting of repeated pentasaccharide units formed by two glucose units, two mannose units and one glucuronic acid unit, in the molar ratio of 2.8:2.0:2.0 (Garcia-Ochoa et al., 2000). Because of its unique rheological properties of high viscosity and pseudo-plasticity, xanthan gum has wide applications in industries such as food, pharmaceuticals, oil, etc (Garcia-Ochoa et al., 2000). However, commercially available xanthan gum is relatively expensive, since glucose is used as the substrate for its mass production (Rosalam and England, 2006). Although, productions of xanthan gum were reported using polysaccharides and their hydrolysates as carbon sources, the yield of xanthan gum was very low; 5.97 g/L by Xc-M using raw cassava starch as carbon source (Kerdsup et al., 2009) and 14 g/L by *Xanthomonas campestris* using cassava bagasse

hydrolysate as carbon source (Woiciechowski et al., 2004).

In Guangxi province of China, more than 2.94 million tons fresh cassava is produced annually, accounting for 70% of the total output of China (Huang et al., 2006), and the cassava price is cost-effective relative to other biomass for xanthan production. All these offer advantages by using cassava starch as a carbon source for xanthan gum production in Guangxi. Although, gelatinized cassava starch has a high viscosity (Marques et al., 2006) which can cause the interference of oxygen transfer in the fermentation system (Psomas et al., 2007), it was easily hydrolyzed by amylase (Snow and O'Dea, 1981). *X. campestris* pv. *campestris* 8004 (Xcc 8004) can produce amylase and xanthan gum (Tang et al., 1990), but amylase activity level and xanthan gum yield have not been reported. To this end, both raw cassava starch and gelatinized cassava starch were used by Xcc 8004 as carbon sources for xanthan gum production, the level of amylase activity and xanthan gum yield were also studied in this paper.

*Corresponding author. E-mail: qunliangli@yahoo.com.cn.

MATERIALS AND METHODS

Strains and growth media

Xcc 8004 was obtained from Ji-liang Tang Laboratory of Guangxi University. Xcc 8004 was stored in medium consisting of (w/v) 0.5% peptone; 0.3% yeast extract; 2% glycerol; 1% sucrose; 2% agar; pH 7.0, and subcultured every 2 weeks. Cells were grown in seed medium composed of (w/v) 2% sucrose, 0.5% peptone and 0.3% KH_2PO_4 . The fermentation media included gelatinized and raw cassava starch medium. The gelatinized cassava starch medium (w/v) contained 3.0% raw cassava starch; 0.5% bean flour; CaCO_3 0.2%; pH 7.5. The media were sterilized at 121°C for 20 min before the cells addition, then gelatinized cassava starch was obtained. The raw cassava starch medium was sterilized at 105°C for 12 h in drying oven to avoid gelatinization (Kazuhiro et al., 1991), and then added to the sterilized medium containing 0.5% bean flour and 0.2% CaCO_3 . The cassava starch was obtained from Guangxi State Farms Mingyang Biochemical Group, Inc., China.

Enzyme activity and reducing sugar assay

2 ml culture broth samples were centrifuged at 10,000 rpm for 15 min, and supernatants (the crude enzyme sample) were collected. For amylase activity determination, a crude enzyme sample (1 ml) was added to soluble starch solution (1% w/v, 0.5 ml) in phosphate buffer (1/15 mol/L Na_2HPO_4 and 1/15 mol/L KH_2PO_4 in volume proportion of 6:4, pH7.0), and the mixture was incubated at 35°C for 30 min (Kerdsup et al., 2009; Tseng and Peng, 1985).

An aliquot (1 ml) of the mixture was removed and quenched by the addition of dinitrosalicylic acid (DNSA) solution (1 ml). The reducing sugar released was quantified by the DNSA method (Amritkar et al., 2004). One amylase enzyme unit was taken as the amount of enzyme required to release 1 μmol of glucose per minute under the given assay conditions. 0.5 ml supernatants was used for reducing sugar assay.

Biomass detection

1 ml fermentation broth was diluted 10^7 times, and then 0.1 ml diluents were spread on a stored medium. The viable colony counts were determined after 3 days incubation at 30°C until the distinct colonies appeared with zones of xanthan gum production.

Total sugar determination

1 ml broth was hydrolyzed by 6 mol/L HCl for 30 min, the pH was adjusted to 7.0 by adding NaOH solution, then diluted to 100 ml, and 1 ml liquid was used for total sugar determination by DNSA method.

Xanthan gum production in shaking flasks

The single colony of Xcc 8004 was inoculated to 50 ml seed medium in 250 ml conical flasks (220 rpm) at 30°C for 18 h culture. This culture was inoculated at 6% (v/v) into fermentation media. Fermentation experiments were carried out in 250 ml conical flasks containing 50 ml fermentation medium and shaken at 220 rpm for 120 h.

Crude xanthan gum quantification

10 ml culture broth (diluted if necessary) was centrifuged at 8,000

rpm for 15 min. The supernatant was precipitated with two volumes of 95% ethanol to determine the xanthan gum concentration. The precipitation was filtered and dried at 60°C for 24 h. The dried sample was then weighed to calculate the crude xanthan gum yield (Zhang and Chen, 2010).

Data analysis

All measurements were carried out in triplicate. Statistical significance of differences between means was determined using Duncan test. $P \leq 0.05$ denoted significant difference.

RESULTS AND DISCUSSION

Xanthan gum production in raw and gelatinized cassava starch media

Both raw and gelatinized cassava starches were used for comparison for xanthan gum production. The result indicates that the xanthan gum yield of gelatinized cassava starch (16.98 g/L) was higher than that of raw cassava starch (14.32 g/L). Then, gelatinized cassava starch was used for further fermentation experiments. Although, researchers have reported production of xanthan gum using raw cassava starch as carbon source by Xc-M (Kerdsup et al., 2009), the highest yield was 5.97 g/L in bioreactor. The yield of xanthan gum by Xcc 8004 in shaking flasks was 2.40 times higher than that of Xc-M in a bioreactor.

Effect of gelatinized cassava starch on amylase and reducing sugar

The amylase production of Xcc 8004 grown in 3.0% gelatinized cassava starch was studied in shake-flasks. The results showed that amylase activity appeared at two peaks at 30 and 65 h; the corresponding activity was 1.10 and 1.19 U/ml, respectively (Figure 1). This was consistent with the reducing sugar concentration in Figure 2. The amylase activity was not high at 30 h, since the reducing sugar was very high (12.92 g/L) at this time; amylase activity was inhibited partly by the high concentration of reducing sugar. As the reducing sugar was consumed, the inhibition decreased slowly, more reducing sugar was released, and the highest amylase activity appeared at 65 h. Thus, the amylase and the concentration of reducing sugar reported here were higher than that of other study (Kerdsup et al., 2009). These data demonstrated that Xcc 8004 showed higher level of amylase activity than that of Xc-M.

Influence of gelatinized cassava starch on biomass and xanthan gum production

The biomass of Xcc 8004 was determined during the xanthan fermentation in 3.0% gelatinized cassava starch

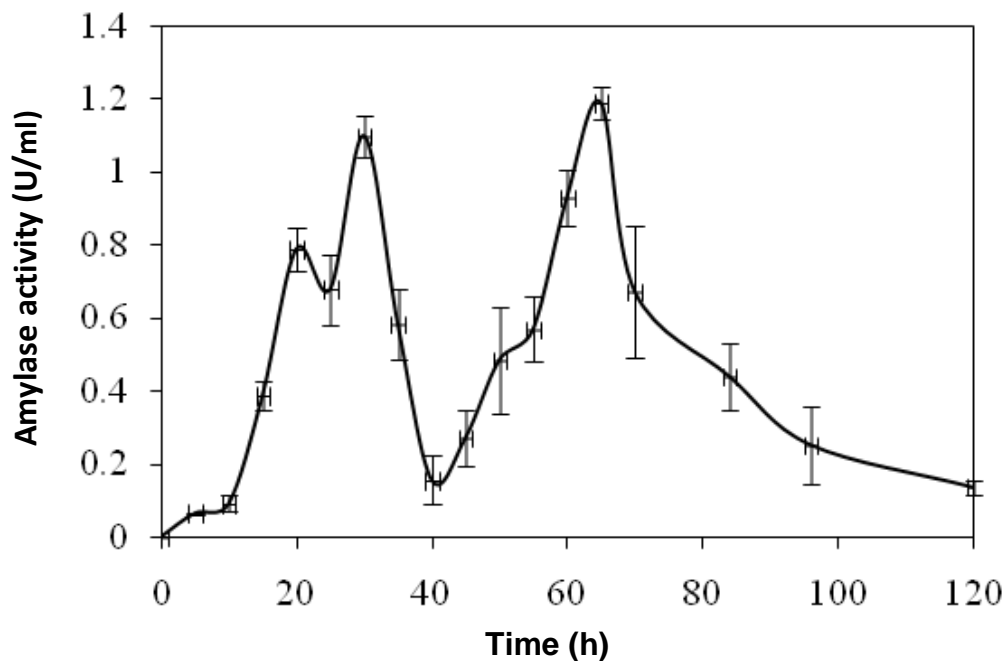


Figure 1. Amylase production of Xcc 8004 with fermentation time in shaking flasks.

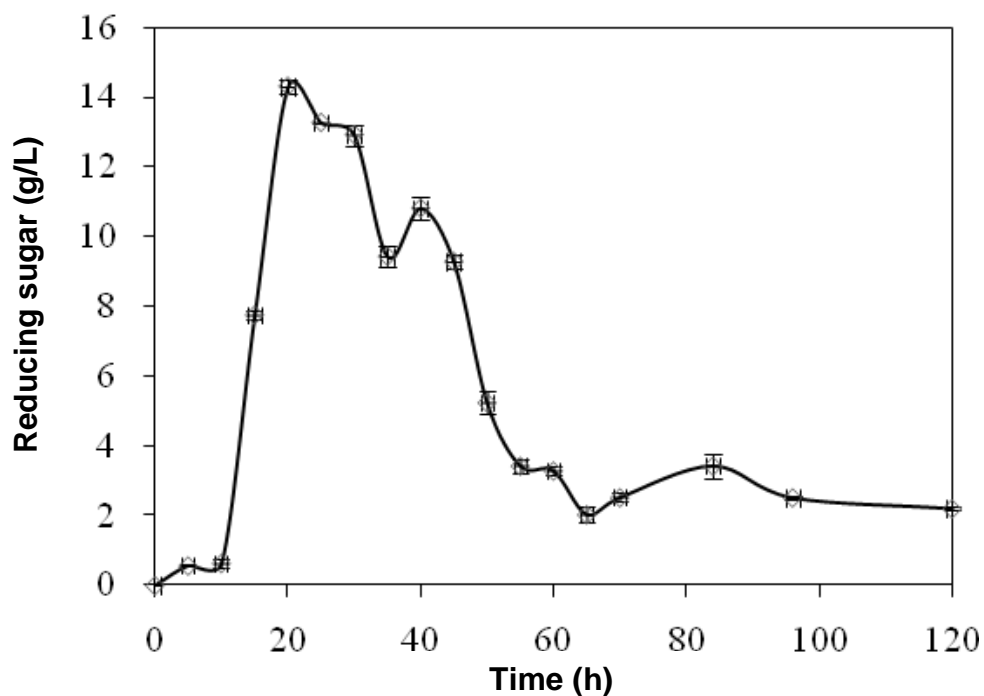


Figure 2. Reducing sugar concentration during the fermentation by Xcc 8004.

medium by the flat colony counting method. The maximal biomass was up to 4.6×10^9 cfu/ml at 25 h as shown in Figure 3. With the xanthan gum accumulation, the fermentation broth became more viscous which inhibited the

biomass increase, so the biomass reduced slowly. This was different from that of previous study (Kerdsup et al., 2009); the growth of *X. campestris* was detected by OD method in previous research which cannot reflect the

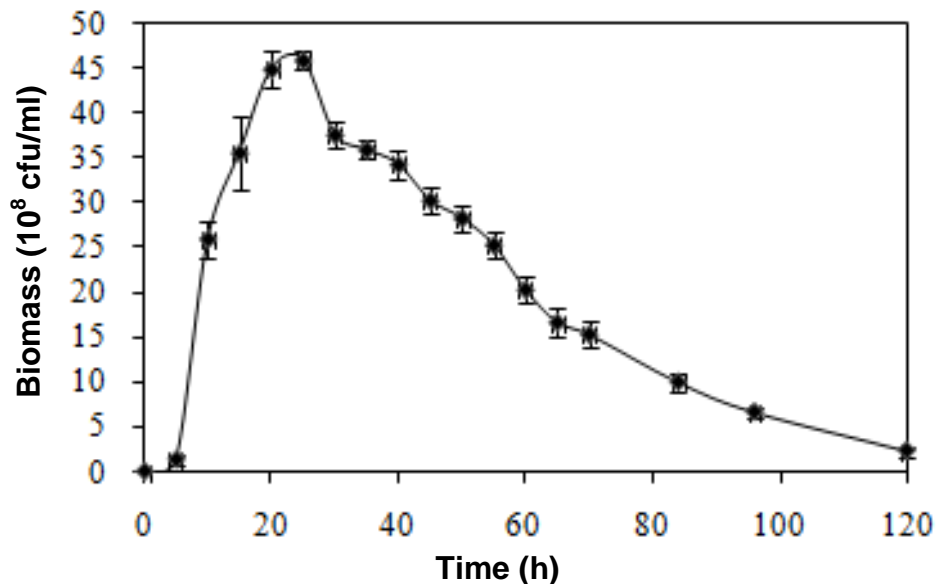


Figure 3. The biomass of Xcc 8004 during fermentation.

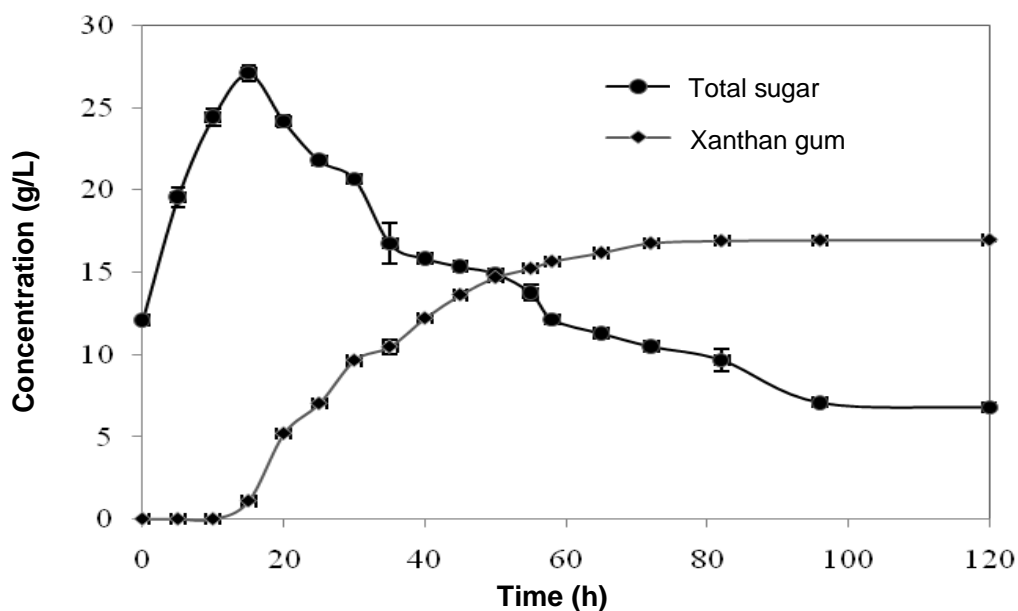


Figure 4. Fermentation curve of total sugar and xanthan gum production.

living cells, as colony counting method can reflect the counts of living bacteria.

The gelatinized cassava starch was viscous at the beginning of the fermentation; the fermentation broth was not well mixed, so the concentration of total sugar was lower than 30 g/L in some areas, but the viscosity was lower gradually due to the hydrolyzation of amylase. The greatest concentration of total sugar was 27.08 g/l at 15 h, as the sugar was consumed, the total sugar declined

slowly, and 6.82 g/L sugar was kept at the end of the fermentation. The xanthan gum started secretion to the medium at 15 h, and the maximal xanthan gum yield of 16.95 g/L was achieved at 120 h (Figure 4), and the efficiency of sugar utilization reached 73.12%. However, the concentration of xanthan gum was almost constant from 70 to 120 h; therefore, 70 h was the appropriate time point to stop fermentation from an economic viewpoint.

Conclusion

The gelatinized cassava starch was better than raw cassava starch for xanthan gum production by Xcc 8004; both amylase activity and reducing sugar concentration were high in the fermentation process when gelatinized cassava starch was used as carbon source. The maximal xanthan gum yield reached 16.95 g/L, and the efficiency of sugar utilization was 73.12%. Xcc 8004 should be a desired strain for xanthan gum production using cassava starch in industrial applications.

ACKNOWLEDGEMENTS

This study was supported by the grant Graduates Innovation Fund of Guangxi (GXU11T32533). We thank other members of our laboratory for their helpful discussion.

REFERENCES

- Amritkar N, Kamat M, Lali A (2004). Expanded bed affinity purification of bacterial [alpha]-amylase and cellulase on composite substrate analogue-cellulose matrices. *Process Biochem.* 39(5):565-570
- García-Ochoa F, Santos VE, Casas JA, Gómez E (2000). Xanthan gum: production, recovery, and properties. *Biotechnol. Adv.* 18(7):549-579.
- Huang J, Li K, Ye J, Chen X (2006). The Developing Countermeasure for Cassava Commercialization in China. *Chin. Agric. Sci. Bull.* 22(05):421-426
- Kazuhiro H, Masayuki T, Hideji M, Kazuyuki S, Michihiro F (1991). Continuous Lactic Acid Production from Raw Starch in a Fermentation System Using a Reversibly Soluble-autoprecipitating Amylase and Immobilized Cells of *Lactobacillus casei* (Microbiology & Fermentation Industry). *Agric. Biol. Chem.* 55(2):479-485.
- Kerdsup P, Tantratian S, Sanguandekul R, Imjongjirak C (2009). Xanthan Production by Mutant Strain of *Xanthomonas campestris* TISTR 840 in Raw Cassava Starch Medium. *Food Bioprocess Technol.* pp. 1-4. doi:10.1007/s11947-009-0250-7.
- Marques PT, Pérégo C, Le Meins JF, Borsali R, Soldi V (2006). Study of gelatinization process and viscoelastic properties of cassava starch: Effect of sodium hydroxide and ethylene glycol diacrylate as cross-linking agent. *Carbohydr. Polym.* 66(3):396-407.
- Psomas SK, Liakopoulou-Kyriakides M, Kyriakidis DA (2007). Optimization study of xanthan gum production using response surface methodology. *Biochem. Eng. J.* 35(3):273-280.
- Rosalam S, England R (2006). Review of xanthan gum production from unmodified starches by *Xanthomonas campestris* sp. *Enzyme Microb. Technol.* 39(2):197-207.
- Snow P, O'Dea K (1981). Factors affecting the rate of hydrolysis of starch in food. *Am. J. Clin Nutr.* 34(12):2721-2727.
- Tang JL, Gough CL, Daniels MJ (1990). Cloning of genes involved in negative regulation of production of extracellular enzymes and polysaccharide of *Xanthomonas campestris pathovar campestris*. *Mol. Gen. Genet.* 222(1):157-160.
- Tseng Y, Peng K (1985). Characterization of α -amylase from phytopathogenic *Xanthomonas campestris* pv. *campestris*. Part B. *Life Sci.* 9(2):103-109
- Woiciechowski A, Soccol C, Rocha S, Pandey A (2004). Xanthan gum production from cassava bagasse hydrolysate with *Xanthomonas campestris* using alternative sources of nitrogen. *Appl. Biochem. Biotechnol.* 118(1):305-312.
- Zhang ZG, Chen HZ (2010). Xanthan Production on Polyurethane Foam and Its Enhancement by Air Pressure Pulsation. *Appl. Biochem. Biotechnol.* 162(8):2244-2258.