Carbohydrate Polymers 102 (2014) 669–673

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Xanthan from sulphuric acid treated tapioca pulp: Influence of acid concentration on xanthan fermentation

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ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form 24 October 2013
Accepted 4 November 2013
Available online 13 November 2013

Keywords:
Tapioca pulp
Acid treatment
Hydrolytic intermediates
Xanthan gum

ABSTRACT

Xanthan gum was produced by fermentation of sulphuric acid pre-treated tapioca pulp. Effect of sulphuric acid concentration (0.5%, 2.5% and 5.0%) on xanthan fermentation was investigated. Maximum xanthan yield (7.1 g/l) was obtained with 0.5% sulphuric acid pre-treatment. Further, increase in sulphuric acid concentration caused formation of inhibitory substance and lowered xanthan yield. The product was confirmed as xanthan using FTIR, ^{1}H NMR analyses. Viscosity was measured by Brookfield viscometer and the molecular weight was determined from the intrinsic viscosity. The results confirmed that the yield and quality of xanthan produced were strongly influenced by the acid concentration.

1. Introduction

Xanthan gum is an industrially important extracellular heteropolysaccharide produced by *Xanthomonas campesteris* through aerobic fermentation. It was only the second exopolysaccharide to be approved, after dextran, by Food and Drug Administration (FDA) as a safe food ingredient (Born, Langendorff, & Boulenguer, 2005).

As the cost of carbon source is one of the major factors contributing to the production cost, research on production of xanthan from low cost substrates is very important. Various agricultural wastes, consisting of complex polysaccharides like cellulose, hemicellulose, lignin and starch, had been tried as low cost substrates to reduce the raw material cost (Demirci, Arici, & Gumus, 2012; Faria et al., 2011; Stredansky & Conti, 1999; Woiciechowski, Soccol, Rocha, & Pandey, 2004). Bioconversion of low cost agro-wastes to value added products provides a solution to solid waste disposal problem (Demirci et al., 2012; Göksungur, Uzunoullari, & Dagbagli, 2011; Kalyanasundaram, Doble, & Gummadi, 2012). Cost of commercial substrates namely glucose and sucrose are in the order of US$ 400–600/Metric Tonne (ISMA, 2013; Wilke, 1999). On the other hand agricultural residues, generated as solid wastes in agro based industries, are costless. In fact disposal of these solid wastes properly in secured manner is a major problem faced by these industries. While a small fraction of them are sold out as a raw material for cattle feed, soil enrichment etc., rest of them is disposed off as land fills. The only cost involved in procuring these materials is the handling and transportation cost.

Bioconversion of solid waste to useful product, usually, is a two step process. In the first step, various pre-treatment techniques are employed to break down complex carbohydrates into simple reducing sugars. In the second step, desired products are obtained by fermentation of the reducing sugars (Lange, 2007; Lee, 1997; Sun & Cheng, 2002). Often, the composition of the intermediate influences the quality and quantity of the final product. Some of the intermediates formed during acid pre-treatment are inhibitory to product formation(Larsson et al., 1999). Though inhibitory compounds are not formed in enzymatic pre-treatment, owing to the cost of enzymes involved, hydrothermal and/or acid pre-treatments are preferred over enzymatic pre-treatment (Li & Zhu, 2011).In this work, sago industry tapioca pulp (SITP), an agro-industry waste from cassava (*Manihot esculenta*) was utilized as a low cost substrate for xanthan production. Cassava waste is preferred over other agro-industrial waste as a substrate in bioconversion processes due to its well-balanced nutritional composition (Sugumaran, Jothi, & Ponnusami, 2014). The sugar degradation products, like furfural and HMF, affect cell energetics (Taherzadeh, Gustafsson, Niklasson, & Liden, 2000). More over, they inhibit biological activities and damage microorganisms by various mechanisms like reducing enzyme activity, breaking down DNA, inhibiting protein formation and RNA synthesis. To the best of our knowledge, a systematic study on influence of intermediates on xanthan fermentation had not been reported earlier. This is the first report to investigate the influence of SITP hydrolysate composition and its sugar degraded products on xanthan fermentation.

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http://dx.doi.org/10.1016/j.carbpol.2013.11.006

2. Materials and method

2.1. Microorganism and inoculum preparation

Xanthamonas campestris (NCIM 2954) was obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. The microorganism was maintained on MGYP agar (Hi-media) slant containing malt extract 3 g/l; glucose 10 g/l; yeast extract 3 g/l; peptone 5 g/l with 2% agar adjusted to pH 7 as recommended by NCIM. It was allowed to grow for 24 h at 30 °C and then stored at 4 °C. This was sub-cultured every two weeks. The cells from freshly prepared agar slant were inoculated into the broth medium containing MGYP in 250 ml conical flask and this was used as the inoculum for fermentation. 24 h culture was used as seed for the fermentation process.

2.2. Substrate preparation and characterization

Sun dried SITP was obtained from a nearby sago industry at Salem, India. It was ground to fine size and the powder was size separated using sieve shaker. Particles of mesh size BSS # −25 + 30 were collected and stored for further use. It was then dried in hot air oven at 60 °C overnight to remove residual moisture and then stored in air-tight container at room temperature. The substrate (100 g/l) was hydrolyzed at 121 °C for 20 min with 0.5%, 2.5% and 5% (w/v) sulphuric acid. The hydrolysate was filtered using syringe filter with 0.45 μm polypropylene membrane to remove suspended particles. The hydrolysates were labelled as hydrolysate H1, H2 and H3, respectively.

Dextrose equivalent (DE) of hydrolysate was determined by Lane and Eynon constant titre method (Lane & Eynon, 1923) (Supporting file). Its composition was analyzed by high performance liquid chromatography (HPLC). Hi-plex H column, 7.7 × 300 mm (Agilent Technologies) was used to estimate the monosaccharide composition of hydrolysate at 65 °C with 5 mM of H_2SO_4 as eluent at a flow rate of 0.6 ml/min. Byproducts of pretreated SITP hydrolysate, especially HMF and furfural were analyzed using C-18 column in HPLC with UV detector at 280 nm. The solid residue was dried at 105 °C and analyzed for its starch content according to Hodge and Hofreiter (1962) and hemicellulose (Goering & Vansoest, 1975) and cellulose content according to Updegraff (1969) (Supporting file). Crude fibre, moisture and ash contents were estimated according to Maynard method and AOAC, 2000 (AOAC, 2005; Maynard, 1970).

2.3. Fermentation

Untreated SITP and the filtered hydrolysates H1, H2 and H3 with 30% initial reducing sugar were used as carbon source. The other media constituents were (in g/l) KH_2PO_4 – 5; yeast extract – 5; citric acid – 2; $MgSO_4{\cdot}7H_2O$ – 0.2; $CaCO_3$ – 0.02 and H_3BO_3 – 0.006. The media pH was adjusted to 6.8. Batch fermentation was carried out with 100 ml of production media in 250 ml conical flask as triplicates. It was inoculated with 5% *Xanthamonas campestris* (10^7 CFU/ml). The flasks were incubated at 28 °C for 72 h in refrigerated rotary shaker at 200 rpm. Samples withdrawn from the fermentation broth after 72 h were autoclaved first and then centrifuged at 10,000 rpm, 30 °C for 20 min. Ice-cold acetone at 10 °C was added to the supernatant in the ratio of 2:1 (v/v) and the mixture was left for 24 h for precipitation of xanthan. In order to ensure purity the precipitate was re-suspended in water and then filtered through filtration system (Make & Model; GE healthcare, MidGee Systems, CFP-1E-MM01A cartridge, pore size 100 nm and surface area 16 cm^2). Purified xanthan was precipitated from retentate as mentioned above by adding ice-cold acetone. The precipitate was then was dried in hot air oven at 105 °C for 6 h. The product was characterized using FT-IR and proton NMR.

Table 1
Composition of untreated and acid treated sago industry tapioca pulp hydrolysate.

Components	Untreated (%)	Acid hydrolysis (%)		
		0.5% H_2SO_4	2.5% H_2SO_4	5% H_2SO_4
DE (no unit)	–	12 ± 1	57 ± 3	71 ± 4
Maltodextrin	–	21.3 ± 1.1	3.1 ± 0.27	–
Glucose	–	5.8 ± 0.39	45.8 ± 4.1	54.1 ± 2.6
Xylose	–	0.8 ± 0.02	11.3 ± 0.97	13.4 ± 0.89
Arabinose	–	0.6 ± 0.04	2.9 ± 0.15	2.1 ± 0.099
Galactose	–	0.6 ± 0.03	8.1 ± 0.47	7.3 ± 0.23
Furfural	–	0.2 ± 0.009	0.4 ± 0.03	1.5 ± 0.07
HMF	–	0.2 ± 0.009	1.3 ± 0.06	2.4 ± 0.008
Acetic acid	–	0.07 ± 0.007	0.9 ± 0.04	1.7 ± 0.011
Formic acid	–	0.03 ± 0.002	0.06 ± 0.003	0.1 ± 0.008
Starch	57.8 ± 3.2	42.4 ± 3.1	3.4 ± 0.011	1.9 ± 0.087
Cellulose	18.1 ± 0.7	17.4 ± 1.5	14.4 ± 0.93	8.7 ± 0.27
Hemicellulose	8.6 ± 0.5	4.7 ± 0.23	1.4 ± 0.082	1.2 ± 0.075
Ash	2.3 ± 0.1	2 ± 0.17	2.1 ± 0.11	1.8 ± 0.077
Protein	1.2 ± 0.08	0.67 ± 0.04	0.4 ± 0.003	0.02 ± .001
Moisture	10.2 ± 0.7	2.5 ± 0.12	2.7 ± 0.16	2.1 ± 0.16

2.4. Determination of intrinsic viscosity and molecular weight

Viscosity of xanthan was measured using Brookfield DV (II) LDV Viscometer. The intrinsic viscosity of the polymer [η] was obtained from the *y* intercept of the plot η_{sp}/C versus xanthan concentration C (mg/l).

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{C} \quad (1)$$

Here η is the measured viscosity and η_0 is viscosity of the solvent and η_{sp} is $((\eta - \eta_0)/(\eta \cdot \eta_0))$.

Molecular weight of xanthan was determined from the intrinsic viscosity using the Mark–Houwink equation (Casas, Santos, & Garcıa-Ochoa, 2000) given below:

$$[\eta] = KM^a \quad (2)$$

where M is the molecular weight of polymer and 'K' and 'a' are Mark–Houwink constants ($K = 1.7 \times 10^{-4}$ and $a = 1.14$ (Milas, Rinaudo, & Tlnland, 1985)).

2.5. Characterization of xanthan gum

Xanthan was characterized using FT-IR spectroscopy and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. Fourier transform infrared spectra were obtained using Perkin Elmer RX I model spectrophotometer. Sample was prepared by KBr pellet method and scanned from 400 to 4000 cm^{-1}. ^{1}H NMR was done using Avance Bruker 500 MHz spectrometer by dissolving xanthan in D_2O at 80 °C.

3. Result and discussion

3.1. Composition of intermediates

Table 1 summarizes the composition of raw STIP, treated SITP and its hydrolysate. Typically, raw SITP contains 10% moisture, 58% starch and traces of free reducing sugars and proteins. When higher acid concentration was used for pre-treatment, DE and glucose content increased. Increasing acid concentration resulted in decrease in starch, cellulose, hemicellulose and maltodextrin contents of tapioca pulp. These observations indicated that increase in acid concentration favoured hydrolysis of the SITP. However, it can also be noted that pre-treatment resulted in formation of inhibitory