Xanthan gum production from acid hydrolyzed broomcorn stem as a sole carbon source by *Xanthomonas campestris*

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Abstract

Xanthan gum is an exo-polysaccharide industrially produced by fermentation using simple sugars. In this study, broomcorn stem was introduced as a low-cost- and widely available carbon source for xanthan gum fermentation. Broomcorn stem was hydrolyzed using sulphuric acid to liberate reducing sugar which was then used as a carbon source for biosynthesis of xanthan gum by *Xanthomonas campestris*. Effects of hydrolysis time (15, 30, 45 and 60 min), sulphuric acid concentration (2, 4, 6 and 8% v/v) and solid loading (3, 4, 5 and 6% w/v) on the yield of reducing sugar and consequent xanthan production were investigated. Maximum reducing sugar yield (55.2%) and xanthan concentration (8.9 g/L) were obtained from hydrolysis of 4% (w/v) broomcorn stem with 6% (v/v) sulphuric acid for 45 min. The fermentation product was identified and confirmed as xanthan gum using Fourier transform infrared spectroscopy analysis. Thermogravimetric analysis showed that thermal stability of synthesized xanthan gum was similar to those reported in previous studies. The molecular weight of the produced xanthan (2.23×10^6 g/mol) was determined from the intrinsic viscosity. The pyruvate and acetyl contents in xanthan gum were 4.21 and 5.04%, respectively. The chemical composition results indicated that this biopolymer contained glucose, mannose and glucuronic acid with molecular ratio of 1.8:1.5:1.0. The kinetics of batch fermentation was also investigated. The kinetic parameters of the model were determined by fermentation results and evaluated. The results of this study are noteworthy for the sustainable xanthan gum production from low-value agricultural waste.

Keywords Xanthan gum · *Xanthomonas campestris* · Broomcorn stem · Acid hydrolysis

Introduction

Xanthan gum (XG) is an important extracellular heteropolysaccharide produced by *Xanthomonas* species (Demirci et al. 2012). Bacterial XG is a suitable alternative for the replacement of traditional gums extracted from plant or algal sources (Bhatia et al. 2015). Owing to its superior rheological properties along with reliable viscosity at low concentrations, pseudo-plasticity, and high stability over a wide range of temperature and pH, XG is widely used in pharmaceuticals, paper, water-based paints, foods, cosmetics, textile and oil recovery industries (Pooja et al. 2014; Palaniraj and Jayaraman 2011).

XG is one of the major industrial biopolymers with annual worldwide production of approximately 100,000

metric tons (Ghashghaei et al. 2016). Commercial xanthan is relatively expensive, since it is manufactured by fermentation using glucose and sucrose as non-renewable carbon sources (Palaniraj and Jayaraman 2011). Increasing market price and demand of these raw materials suggest that they may not be economic and viable choices for long-term production of XG. Therefore, it is of great importance to search for cheap and renewable feedstocks which are readily available (Papagianni et al. 2001). Various low-cost materials have been used for xanthan production, such as kitchen waste, sugar cane, palm date juice, tapioca pulp and cheese whey, etc (Papagianni et al. 2001; Faria et al. 2010; Salah et al. 2010; Niknezhad et al. 2015; Li et al. 2016). Several methods have been studied to enhance the yield and properties of XG using different substrates (Kalogiannis et al. 2003; Demirci et al. 2012; Jazini et al. 2017). Nowadays, lignocellulosic materials are in focus as renewable and abundant substrates for production of various bio-based products including polysaccharides, e.g., dextran, curdlan and xanthan (West and Nemmers 2008; Jazini et al. 2017).

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However, due to their recalcitrant structure, they cannot be utilized directly as a carbon source by microorganisms. Bioconversion of lignocellulosic materials to useful product consists of three steps, namely pretreatment, hydrolysis and fermentation (Kumar and Murthy 2011). In the first step, the complex structure of lignocellulosic materials is disrupted to make cellulose more accessible for hydrolysis process (Kim et al. 2010). To date, several processes including acid, alkali, oxidation and other treatments have been applied for lignocelluloses pretreatment prior to hydrolysis. Pretreatment with sodium hydroxide is one of the most effective methods among all with respect to lignin removal and susceptibility of resultant material for hydrolysis (Mirahmadi et al. 2010). The effectiveness of alkaline method depends on the lignin content of feedstocks; therefore, it is a suitable pretreatment for agricultural residues. Alkaline pretreatment can largely improve enzymatic hydrolysis of the cellulose and sugar degradation to inhibiting compounds is less than acid treatment (Talebnia et al. 2010). Acid hydrolysis is a cost-effective method in which a high proportion of carbohydrates could be converted to fermentable sugars which is crucial for process commercialization (Talebnia et al. 2007), although the composition of intermediates formed during acid hydrolysis influences the product formation (Papagianni et al. 2001).

Broomcorn is a type of sorghum grown in tropical and subtropical regions. The fibrous panicle of this plant is used for fabrication of broom, brush and wooden decorative items, leaving the leftover stem as waste with little forage value (Farahi et al. 2012). A survey of the literature shows a few research in regard to the application of this agricultural residue (Nikzad et al. 2014; Riazi et al. 2015). In the current study, the feasibility of XG production using broomcorn stem (BS) as a cost-effective substrate was investigated. The optimization of XG production from BS in terms of acid hydrolysis conditions was performed. The main effects of acid concentration, solid loading and hydrolysis time on the yields of liberated glucose and xanthan gum were studied. In addition, batch kinetic models were proposed for modeling of substrate consumption, cell growth and XG formation for better understanding of the fermentation process. The rheological analysis and physico-chemical characterization of produced XG were also conducted.

Materials and methods

Substrate preparation and pretreatment

The broomcorn stem free from leaves and husks was prepared from a local market in Amirkola, Babol, Iran. It was dried at 55–60 °C overnight to remove residual moisture and then ground to fine size in a rotary mill. The milled

material was separated using sieve shaker and particles of mesh size BSS# – 30 + 40 were collected and stored in tightly sealed containers at room temperature until further use.

The broomcorn-prepared sample was pretreated with dilute NaOH solution. The determination of solid loading, NaOH concentration and heating time was based on our previous study on the optimal conditions for alkali pretreatment of BS (Nikzad et al. 2014). A solid–liquid ratio of 4.2% (w/v) of screened BS was pretreated with a 1.7% (w/v) NaOH solution in an autoclave (Hirayama, HV-25, Japan) for 60 min at 121 °C. After cooling down, the pretreated substrate was separated by filtration through a porcelain Buchner funnel and washed with deionized water until the filtrate registered a neutral pH. Finally, the pretreated solid samples were sealed in plastic bags and stored at 4 °C for subsequent hydrolysis. To understand the effects of alkaline pretreatment on the chemical and structural composition of broomcorn stem, untreated and pretreated substrates were analyzed according to the standard biomass analytical procedures developed by National Renewable Energy Laboratory (NREL) (Sluiter et al. 2005, 2008; Ruiz et al. 2011).

Microorganism and inoculum preparation

Xanthomonas campestris (PTCC 1473) was purchased from Persian Type Culture Collection (PTCC) and used for XG production. The microorganism was maintained on YPD agar medium made from glucose 20 (g/L), peptone 10 (g/L), yeast extract 10 (g/L) and agar 10 (g/L). For the pre-inoculum cultures, a loop of cells was transferred from the YPD agar plate into the 50 mL of liquid YPD broth in a 250 mL conical flask and incubated at 28 °C with the speed of 200 rpm for 24 h. The grown cells were employed as seed culture for the fermentation process.

Acid hydrolysis

For acid hydrolysis, pretreated BS samples were added into 250 mL flask and diluted with distilled water to obtain 100 mL of BS/water slurry with various solid fractions of 3, 4, 5 and 6% (w/v). By adding sulfuric acid (98%) to the slurries, the final acid concentration was adjusted to 2, 4, 6 and 8% (v/v). Then, the slurries were heated in an autoclave at various residence times of 15, 30, 45 and 60 min at 120 °C. All samples were then cooled down to 50(±2) °C before removing from the autoclave. The solid fraction of cooled samples was quickly separated by vacuum filtration. The liquid fraction was stored at –20 °C for glucose, furfural and 5-hydroxymethylfurfural (HMF) determination.

Fermentation

Batch fermentation was carried out with 100 mL of production media in a 250 mL flask. The filtered hydrolysate of BS was used as a sole carbon source and supplemented with the following chemicals per liter of media: 0.2 g of MgSO_4 , 2 g of $\text{C}_6\text{H}_8\text{O}_7$, 0.0006 g of H_3BO_3 , 0.0006 g of ZnCl_2 , 0.0006 g of FeCl_3 , 0.02 g of CaCO_3 and 3 g of KH_2PO_4 . Carbon and nitrogen are critical nutritional parameters in the production of xanthan gum. The nitrogen source was NH_4NO_3 and the effect of carbon to nitrogen (C/N) ratio on the xanthan gum production was studied by varying it from 5 to 40. To investigate the effect of C/N ratio on xanthan production, filtered hydrolysate of BS at optimum condition of hydrolysis was used as a sole carbon source. The quantity of nitrogen content in the medium was varied whereas the quantity of carbon content (filtered hydrolysate of BS) was fixed. In all experiments, the medium pH was adjusted to 7.0 ± 0.1 . It was inoculated with 5% (v/v) *X. campestris* culture (10^7 CFU/mL). The experiments were conducted in shaker incubator (IKA, Germany) at 28 ± 0.1 °C and 200 rpm. Samples were taken from the fermentation broth after 72 h for further analysis.

Analytical method

Scanning electron microscopy (SEM)

The morphology of untreated and pretreated BS was analyzed using a KYKY-EM 3200 scanning electron microscope (China). Prior to taking pictures, the specimens were coated with a thin layer of gold and palladium using a SCD 005 sputter coater (BAL-TEC, Switzerland) with conductive materials to improve the quality of micrograph.

Determination of reducing sugar

All liquid samples from the acid hydrolysis and cell-free supernatant from fermentation broth were used for the determination of reducing sugar by the dinitrosalicylic acid method (Miller 1959). The yield of hydrolysis, Y_H was calculated using the following equation (Chen et al. 2009):

$$Y_H(\%) = \frac{\text{Reducing sugar (g)} \times 0.9}{\text{Polysaccharide in substrate (g)}} \times 100 \quad (1)$$

Determination of furfural and 5-hydroxymethylfurfural

All liquid samples from the acid hydrolysis tests were filtered using a 0.45 µm Millipore filter and then analyzed by high performance liquid chromatography (HPLC). A HPLC unit (Knauer, Germany) equipped with a UV

absorbance detector (Knauer, Smartline UV Detector 2500, Germany) was applied to detect Furfural and HMF. These main byproducts of pretreated BS hydrolysate were analyzed using Eurospher II column (C18, 4×150 mm) in HPLC with UV absorbance detector at 275 nm. The analytical column was operated at 25 °C with water and methanol (20 and 80%) as eluent at flow rate of 1 mL/min.

Determination of biomass and xanthan production

Biomass determination was carried out by cell dry weight estimation. All samples from fermentation broth were diluted properly. Cells were collected after centrifugation at 10,000 rpm for 20 min and the supernatant was discarded. The precipitates were subsequently washed twice with aseptic distilled water to remove traces of xanthan and recentrifuged at 6000 rpm for 10 min. Finally, the cells were dried in an oven at 105 °C (Binder, Germany) for 6 h and weighed. After determination of biomass, the cell-free supernatant was mixed with two volume of isopropanol per volume of the broth and then centrifuged for 15 min at 10,000 rpm. The precipitate was dried in a hot-air oven at 60 °C for 24 h. The product was used for further analyses. The production of XG was evaluated by measuring the weight of the dry product per volume of fermented broth.

$$\text{Xanthan production (g/L)} = \frac{\text{Dry weight of precipitate (g)}}{\text{Volume of fermented broth (L)}} \quad (2)$$

Determination of pyruvate and acetyl content

The content of pyruvate attached to the xanthan gum was determined by a colorimetric method (Salah et al. 2010). The percentage of pyruvate was measured by a lactate dehydrogenase assay (Pyruvate kit, Sigma Diagnostics) after the xanthan sample hydrolysis in 0.1 M HCl for 4 h at 100 °C. The percentage of acetyl acid was determined by hydroxamic acid method as described by McComb et al. (1957).

Monosaccharide composition

The monosaccharide composition of the biopolymer synthesized by *X. campestris* was analyzed according to the method of Faria et al. (2011). The hydrolyzed sample was analyzed by HPLC (Knauer, Germany) equipped with an RI detector (Knauer, Smartline RI Detector 2400, Germany). Eurokat Pb column, 300×8 mm was utilized for the analyses of monosaccharide composition at 75 °C with water as eluent at a flow rate of 0.5 mL/min.

Fourier-transformed infrared (FTIR) spectroscopy

Functional groups of commercial (Sigma-Aldrich, St. Louis, United States, CAS Number: 11138-66-2) and isolated xanthan gum samples were recorded using a (Shimadzu 4100, Japan) FTIR spectrophotometer. Samples were prepared by KBr pellet method and scanned in the transmittance (%) mode with a resolution of 4 cm^{-1} in the range of $400\text{--}4000\text{ cm}^{-1}$.

Thermogravimetric analysis (TGA)

The thermal stability of biosynthetic xanthan gum from BS was determined using TGA/DTA (differential thermal analysis). Non-isothermal experiments were performed on a Shimadzu TG50 (Japan) thermogravimetric analyzer. The dynamic differential scanning calorimetry (DSC) experiments were carried out in the temperature range of $50\text{--}800\text{ }^{\circ}\text{C}$ at a ramping up rate of $10\text{ }^{\circ}\text{C}/\text{min}$ under nitrogen atmosphere. TGA/DTA was used to determine mass loss, temperature ranges, and the maximum temperature of thermal degradation, respectively.

Determination of intrinsic viscosity and molecular weight

Viscosity of isolated and commercial XG was measured using BROOK-FIELD DV-II viscometer at $25\text{ }^{\circ}\text{C}$. To obtain the intrinsic viscosity $[\eta]$, the specific viscosity, η_{sp} of original solution (0.5 g/L) and different dilutions ($0.25, 0.2, 0.15, 0.10, 0.05$ and 0.01 g/L) of xanthan was measured and then $[\eta]$ was determined by extrapolating the relationship between reduced viscosity, η_{red} and xanthan concentration, C (mg/L) (Li and Feke 2015). In fact, $[\eta]$ was obtained from the y intercept of the plot reduced viscosity versus xanthan concentration. The relative (η_{rel}), specific, reduced and intrinsic viscosity equations are given below, respectively (Faria et al. 2011):

$$\eta_{\text{rel}} = \frac{\eta}{\eta_0} \quad (3)$$

$$\eta_{\text{sp}} = \frac{\eta - \eta_0}{\eta_0} = \eta_{\text{rel}} - 1 \quad (4)$$

$$\eta_{\text{red}} = \frac{\eta_{\text{sp}}}{C} \quad (5)$$

$$[\eta] = \lim_{C \rightarrow 0} \frac{\eta_{\text{sp}}}{C} \quad (6)$$

where η is the measured viscosity, and η_0 is viscosity of the solvent.

Molecular weight of xanthan was calculated using the Mark–Houwink equation. The intrinsic viscosity is related to the average molecular weight of polymer, M_v through this equation as following (Gunasekar et al. 2014):

$$[\eta] = KM_v^\alpha \quad (7)$$

where, K and α are the Mark–Houwink constants. Based on the previous reports, the value of K and α was considered 1.7×10^{-4} and 1.14 for xanthan solutions, respectively (Casas et al. 2000; Gunasekar et al. 2014).

Kinetic models

Design and scale up of xanthan gum production require a deep understanding of the process kinetics. Both unstructured and structured kinetic models have been studied by authors to describe the biosynthesis of xanthan gum in a batch process (Garcia-Ochoa et al. 2004; Faria et al. 2010). In this work, unstructured kinetic models were used for the cells mass (X), the product concentration (P) and the substrate concentration (S) during XG fermentation. Logistic, Luedeking–Piret and modified Luedeking–Piret kinetic equations were applied for cell growth, xanthan production, and substrate consumption, respectively. The logistic equation was commonly applied to simulate microbial growth in polysaccharide fermentation systems (Rosalam and England 2006; Gilani et al. 2011; Li et al. 2016). According to Malthus's population theory, logistic equation can be given as:

$$\frac{dX}{dt} = \mu_{\text{max}} X \left(1 - \frac{X}{X_{\text{max}}} \right) \quad (8)$$

where X_{max} is the maximum cell dry weight concentration (g/L), μ_{max} is the maximum specific growth rate (h^{-1}) and t is fermentation time (h). The integration of the above equation using $X = X_0$ at $t = 0$ results in the following Logistic model equation for cell concentration (Li et al. 2016):

$$X(t) = \frac{X_0 e^{\mu_{\text{max}} t}}{\left[1 - \left(\frac{X_0}{X_{\text{max}}} \right) (1 - e^{\mu_{\text{max}} t}) \right]} \quad (9)$$

The kinetic of product formation was based on Luedeking–Piret equation which describes both growth- and non-growth-associated models (Faria et al. 2009, 2010). According to this model, the product formation rate linearly depends on the growth rate and biomass concentration as follows:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (10)$$

where the parameters α and β are empirical constants which may differ under different fermentation conditions

(Garcia-Ochoa et al. 2004). By incorporating Eq. (8) into (10), integration of Eq. (10) with initial condition ($t=t_0$, $P=P_0$) results in:

$$P(t) = P_0 + \alpha X_0 \left[\frac{e^{\mu_{\max} t}}{1 - \left(\frac{X_0}{X_{\max}} \right) (1 - e^{\mu_{\max} t})} - 1 \right] + \beta \left(\frac{X_{\max}}{\mu_{\max}} \right) \ln \left[1 - \left(\frac{X_0}{X_{\max}} \right) (1 - e^{\mu_{\max} t}) \right] \quad (11)$$

In addition, modified Luedeking–Piret equation was used to describe substrate utilization rate (Faria et al. 2009). This kinetic model considers substrate utilization for cell growth and maintenance. The relation is given by the following equation:

$$-\frac{dS}{dt} = \gamma \frac{dX}{dt} + \varepsilon X \quad (12)$$

where γ and ε are considered as growth and non-growth associated constants, respectively (Gilani et al. 2011). Similarly, the integration of the above equation using initial condition $S=S_0$ at $t=0$, implementing Eq. (9) into (12) yields:

$$S(t) = S_0 + \gamma \left[\frac{X_0 e^{\mu_{\max} t}}{1 - \left(\frac{X_0}{X_{\max}} \right) (1 - e^{\mu_{\max} t})} - X_0 \right] + \varepsilon \left(\frac{X_{\max}}{\mu_{\max}} \right) \ln \left[1 - \left(\frac{X_0}{X_{\max}} \right) (1 - e^{\mu_{\max} t}) \right] \quad (13)$$

Thus, non-linear curves of the Eqs. (9), (11) and (13) fitted to experimental data, using Curve Fitting Toolbox of MATLAB version 3.1 (R2011a) to study the behavior of cell growth, XG production and broomcorn hydrolysate substrate consumption.

Results and discussion

Effect of pretreatment on broomcorn stem

To evaluate the influence of alkali pretreatment on the composition of BS, the samples were analyzed before and after

Table 1 Chemical composition of broomcorn stem before and after pretreatment (% w/w on a dry basis)

Components	Untreated (%)	Pretreated (%)
Cellulose	44.19	65.34
Hemicellulose	25.52	22.18
Lignin	23.13	10.63
Ash	2.83	1.05

pretreatment. The weight percentages of the main constituents, i.e., cellulose, hemicellulose and lignin of the raw and pretreated materials are listed in Table 1 to provide an insight into the variation of the structural components after pretreatment.

Cellulose (44.19%) was the main component of broomcorn stem, suggesting this agricultural waste as a promising alternative feedstock for xanthan production. As shown in Table 1, the composition of BS has been changed after the pretreatment. The cellulose content of the treated sample was increased from 44.19 to 65.34%, whereas the hemicellulose content was reduced from 25.52 to 22.18%. Additionally, the lignin content of untreated BS was 23.13%, which was reduced to 10.63% after the pretreatment. The findings show that lignin removal and hemicellulose dissolution after pretreatment increased the percentage level of cellulose.

Scanning electron microscope images of the untreated and pretreated broomcorn stems are represented in Fig. 1. For untreated BS, an ordered and compact structure is clearly observed (Fig. 1a). The raw stem has a smooth and continuous surface whereas pretreated stem has a rough surface. The structure of broomcorn stem has been disrupted and a lot of debris is obtained (Fig. 1b). This comparison shows that pretreatment induced discernible structural changes in broomcorn stem and removed external fibers. Similar structural changes were reported for alkali-pretreated rice straw (Jazini et al. 2017) and for cotton stem pretreated with NaOH (Wang et al. 2016a).

Acid hydrolysis of pretreated broomcorn stem

The pretreated broomcorn stem was subjected to acid hydrolysis at different conditions. The acid hydrolysis conditions could influence the reducing sugar and inhibitory compound concentrations. Furfural and HMF, as two main inhibitors to cells in subsequent fermentation, are formed due to decomposition of liberated sugars during the hydrolysis. Formation of furfural and HMF during acid hydrolysis is a sequential reaction where hemicellulose and cellulose are initially hydrolyzed to their monomers, followed by decomposition of released sugars to furfural and HMF. These two reaction rates are affected by different factors such as acid concentration, hydrolysis time, solid loading and temperature (Talebnia et al. 2007). Table 2 summarizes the concentration of reducing sugar, furfural and 5-hydroxymethyl furfural in hydrolysate of treated BS at different hydrolysis conditions. The highest concentration of furfural and HMF obtained was 1.6 (g/L) and 2.5 (g/L) when time, acid concentration and solid loading were 45 min, 8% (v/v) and 4% (w/v), respectively. Results show time and acid concentration have more impact than solid loading on the furfural and HMF formation.

Fig. 1 Scanning electron microscopic images of **a** native broomcorn stem; **b** pretreated broomcorn stem

Table 2 Reducing sugar and inhibitory compound concentration in BS hydrolysate after different acid hydrolysis conditions

Time (min)	H ₂ SO ₄ conc. (% v/v)	Solid loading (% w/v)	Reducing sugar conc. (g/L)	Hydrolysis yield (%)	Furfural conc. (g/L)	HMF conc. (g/L)
15	4	4	13.22 ± 0.17	34.00 ± 0.27	0.20 ± 0.02	0.3 ± 0.004
30	4	4	16.37 ± 0.19	42.00 ± 0.18	0.41 ± 0.03	0.48 ± 0.006
45	2	4	17.20 ± 0.13	44.00 ± 0.15	0.26 ± 0.06	0.36 ± 0.002
	4	4	18.86 ± 0.21	48.50 ± 0.35	0.52 ± 0.04	0.61 ± 0.001
	6	3	11.72 ± 0.12	40.18 ± 0.19	0.61 ± 0.01	0.90 ± 0.005
	6	4	21.47 ± 0.18	55.20 ± 0.11	0.84 ± 0.02	1.43 ± 0.002
	6	5	23.02 ± 0.11	47.35 ± 0.21	1.05 ± 0.03	1.81 ± 0.005
	6	6	24.85 ± 0.16	42.60 ± 0.16	1.12 ± 0.06	1.92 ± 0.003
	8	4	20.38 ± 0.22	52.40 ± 0.19	1.60 ± 0.03	2.50 ± 0.004
60	4	4	17.15 ± 0.26	44.11 ± 0.16	1.44 ± 0.07	1.21 ± 0.006

The hydrolysis yield was calculated for each experiment to compare the performance of hydrolysis. The yield of hydrolysis was obtained at different time when NaOH and H₂SO₄ concentrations were selected, 4%. The maximum hydrolysis yield, $48.5 \pm 0.35\%$ was obtained at 45 min, while the corresponding furfural and HMF concentrations were 0.52 ± 0.04 (g/L), and 0.61 ± 0.001 (g/L), respectively. The minimum yield of $34 \pm 0.27\%$ was achieved at 15-min hydrolysis time. In general, yield of hydrolysis reached a maximum value with increase in hydrolysis time from 15 to 45 min and then declined with further increase in time, certainly due to sugar decomposition to furfural and HMF. The kinetic of lignocellulosic material hydrolysis and sugar decomposition reactions were studied and the results indicated that both are first-order reactions. The time is related to constant values of both sugar liberation and sugar

decomposition reactions. The rate of sugar decomposition reaction enhances at the time of hydrolysis longer than the optimal value (Talebnia et al. 2007) leading to lower hydrolysis yields.

The effect of acid concentration on hydrolysis yield was evaluated when 4% pretreated BS hydrolyzed for 45 min. The results show that increase in acid concentration from 2 to 6 (%v/v) favored hydrolysis of BS but further increase declined hydrolysis yield. It can also be noted that the rise of acid concentration resulted in the formation of inhibitory compounds. The effect of H₂SO₄ concentration at the lower values on the formation of furfural and HMF was negligible, but the sugar decomposition rate increased with enhancing acid concentration (Table 2). Maximum hydrolysis yield, $55.20 \pm 0.34\%$ was achieved when 6% sulphuric acid was used for hydrolysis of 4% BS for 45 min.

Fig. 2 Effect of **a** hydrolysis time, **b** acid concentration, and **c** solid loading on the xanthan concentration

The effect of variation of solid loading on the hydrolysis yield, when pretreated BS hydrolyzed with 6% H_2SO_4 for 45 min, is shown in Fig. 2c. The hydrolysis of pretreated BS showed that increasing hydrolysis yield was correlated with an increase in solid loading up to 4% (w/v) under applied conditions which might be explained by mass transfer limitation. At higher solid loading, lack of appropriate substrate

Table 3 Effect of C/N ratio on the *X. campestris* and xanthan gum production

C/N ratio	Cell dry weight (g/L)	Xanthan gum concentration (g/L)
5	1.48	6.02
10	1.86	7.66
20	2.04	8.90
40	1.75	7.13

and acid mixing lowers the conversion rate. Based on the results, the optimum condition that yielded the highest liberation of sugars ($55.20 \pm 0.34\%$) achieved at 45 min, 6% acid concentration, and 4% (w/v) solid loading.

Xanthan production

Figure 2a shows the xanthan concentration from hydrolysates of pretreated broomcorn stem under different time of hydrolysis. This figure reveals the fact that the hydrolysis time has an influence on the xanthan concentration. The xanthan concentration enhanced from 3.9 to 6.17 (g/L) with increase in hydrolysis time from 15 to 45 min. However, further increasing of the hydrolysis time showed negative effect on the xanthan concentration. This was obviously due to the reducing sugar content reduction and the inhibitory substance formation. The presence of inhibitor compounds would be detrimental to the fermentation process (Li et al. 2016). As mentioned in an earlier study, the inhibition effect of these compounds on the growth rate of *X. campestris* is an important factor affecting the final product yield (Gunasekar et al. 2014). At present, investigations of effects of inhibiting compounds on *X. campestris* have been rarely reported in the literature. But, the inhibitory action of furfural and HMF on various microorganisms such as yeast had been widely investigated (Bhatia et al. 2016, 2017; Taherzadeh et al. 2000).

Figure 2b shows the effect of acid concentration on xanthan concentration. The xanthan concentration increased with the acid concentration rising from 2 to 6% (v/v) which is due to increase of the sugar content of hydrolysate. However, after 6% (v/v), further increase in acid concentration decreased the xanthan concentration most likely due to the inhibitory action of HMF and furfural on *X. campestris* and decrease of reducing sugar as carbon source. The effect of solid loading on xanthan production is shown in Fig. 2c. Though the sugar content of hydrolysate increased from 11.72 to 24.85 (g/L) with enhancement in solid loading, xanthan concentration did not increase proportionately. But the concentration of inhibitors in hydrolysate increased when solid loading enhanced from 3 to 6%. The maximum xanthan concentration, 8.9 (g/L), was achieved when 4% (w/v) pretreated BS hydrolyzed by 6% sulphuric acid for 45 min.

The xanthan and biomass concentration at different carbon/nitrogen (C/N) ratio are listed in Table 3. The results show that maximum xanthan concentration (8.9 g/L) was achieved when C/N ratio was 20. The xanthan gum production increased from 6.02 to 8.9 g/L when C/N ratio was raised from 5 to 20 and then decreased to 7.13 g/L at C/N ratio 40. Nitrogen is one of the major nutrients affecting the biomass growth and xanthan production but its high concentration reduces the flux of nitrogen in medium and is thus detrimental to xanthan production. As can be observed in Table 3, C/N ratio (20) was preferred for both *X. campestris* growth and XG production.

Characterization of xanthan

Figure 3 shows the FTIR spectra of the commercial and biosynthetic XG. The most representative peaks can be summarized as follows. A strong broad adsorption peak of hydroxyl stretching (OH) was found at $3426\text{--}3437\text{ cm}^{-1}$. The peak at $2917\text{--}2925\text{ cm}^{-1}$ was assigned to CH stretching which might be related to symmetric and asymmetric stretching of CH_2 or CH_3 and aldehyde groups. The characteristic peak was observed between the wave numbers $1625\text{--}1632\text{ cm}^{-1}$ was mainly due to carbonyl group. CH deflection angle was detected in the wave number region of $1389\text{--}1415\text{ cm}^{-1}$. The vibration peak of CO group was identified at $1059\text{--}1113\text{ cm}^{-1}$ (Salah et al. 2010; Pooja et al. 2014). The peaks at $604\text{--}616\text{ cm}^{-1}$ in the spectrum were assigned to vibrations of CH bending (Wu et al. 2013). The FTIR spectra of xanthan synthesized in this study were in agreement with the commercial xanthan and those reported in the literatures (Faria et al. 2011; Jazini et al. 2017).

The biosynthetic XG was also subjected to TGA/DTA analysis. This analysis illustrates the thermal decomposition mechanism of biosynthetic XG from hydrolysate of BS. The

Fig. 3 FTIR spectrum of biosynthetic and commercial xanthan gum

Fig. 4 Simultaneous TGA-DTA curves of biosynthetic xanthan gum

TGA and DTA curves are presented in Fig. 4 for produced xanthan. There were two mass losses for XG sample. The first mass loss ($60\text{--}140\text{ }^{\circ}\text{C}$) corresponds to approximately 20% is attributed to the hydration of XG. Water absorption by desiccative XG is easily due to the presence of hydrophilic groups especially --OH grouping in its structure. The second clear mass loss took place at $230\text{--}320\text{ }^{\circ}\text{C}$ with mass loss of 60% due to xanthan gum degradation. Based on the DTA curve, the thermo grams also indicted the predominance of exothermic processes in the second degradation events. According to the DTA curves, the maximum temperature of biosynthetic XG degradation was $280.00\text{ }^{\circ}\text{C}$, similar to $298\text{ }^{\circ}\text{C}$ shown in a previous study (Li et al. 2016; Jazini et al. 2017).

Intrinsic viscosity $[\eta]$ of commercial and biosynthesized XG was determined by extrapolating the specific viscosity results to zero concentration (Fig. 5). The obtained intrinsic viscosity of biosynthesized and commercial XG were 2945.21 and 3707.89 (mL/g), respectively. Intrinsic viscosity value of xanthan samples was in agreement with results reported by Faria et al. (2011).

Molecular weights (M_w) of commercial and biosynthesized XG were calculated by Eq. (7). The molecular weights of the biosynthetic and commercial XG were 2.23×10^6 and 2.73×10^6 (g/mol), respectively. Values of molecular weight of xanthan samples that were synthesized from tapioca pulp and sugar cane broth were on the same order of magnitude as those obtained in this study (Faria et al. 2011; Gunasekar et al. 2014). The range of molecular weight of XG was reported from 5×10^5 to 1.3×10^7 (g/mol) in the literature (Li et al. 2016). It showed that the xanthan gum obtained from BS had a normal molecular weight.

The xanthan gum produced in this work consists of glucose, mannose, glucuronic acid with no rhamnose. Silva et al. (2009) found a biopolymer without rhamnose by *X. campestris* pv. *Manihotis* 1182. The molecular ratio of the

Fig. 5 Linear regression of reduced viscosity versus concentration curve for **a** biosynthesized and **b** commercial xanthan gum

Fig. 6 Time course of biomass, reducing sugar and xanthan gum during fermentation of *X. campestris*

main monomers i.e., glucose, mannose and glucuronic acid was 1.8:1.5:1.0 in the present study. This result was very close to those found by Faria et al. (2011). Several studies have shown that different strains of *Xanthomonas* and culture conditions produce biopolymers with different compositions (Salah et al. 2010; Silva et al. 2009; Wang et al. 2016b). In many cases, the proportion of glucose to mannose is 1:1, but small variations can occur among different strains (Silva et al. 2009; Wang et al. 2017). Other monosaccharides, such as arabinose, xylose, galactose and rhamnose were also detected at lower concentrations (Salah et al. 2010).

Differences in chemical structure of xanthan gum tend to be mostly due to a change in pyruvate and acetyl content. The content of pyruvate and acetyl groups depends on the fermentation conditions and the microorganism strain (Faria et al. 2011). The variation of these component effects on the rheological properties of XG. The pyruvate and acetyl percentages were determined 4.21 and 5.04%, respectively, which is aligned with results reported by other researchers (Wang et al. 2017, 2016b).

Kinetic analysis

To validate the kinetic models, experimental data were collected from batch fermentation of BS hydrolysate with initial reducing sugar of 21.5 (g/L). Figure 6 shows the time course of cell growth, carbon source consumption and xanthan production. As shown in Fig. 6, during the XG fermentation, increasing the biomass concentration was accompanied by a decrease in reducing sugar concentration. The consumption of sugar was to supply cell growth, cell maintenance, and product formation. The cell growth of *X. campestris* entered exponential phase after 2 h of initial lag phase. The exponential growth phase took along for 28 h and then cells entered the stationary phase with the maximum cell growth

Table 4 Comparison of xanthan gum concentration from various carbon sources

Substrate	Xanthan gum concentration (g/L)	References
Tapioca pulp	7.10	(Gunasekar et al. 2014)
Raw starch-based media (cassava)	6.00	(Kerdsup et al. 2011)
Date extract	11.2	(Khosravi-Darani et al. 2011)
Shrimp shell	4.64	(de Sousa Costa et al. 2014)
Kitchen waste	11.73	(Li et al. 2016)
Broomcorn stem	8.90	This study

Table 5 Values of the kinetic model parameters from experimental data

Equation	Parameter	Value	Correlation coefficient (R^2)
Logistic	X_0 (g/L)	0.2	0.9941
	X_m (g/L)	2.1	
	μ_m (h^{-1})	0.125	
Luedeking–Piret	α (g product/g cell)	1.71	0.9920
	β (g product/g cell. h)	0.03	
Modified Luedeking–Piret	γ (g substrate/g cell)	2.8	0.9984
	ε (g substrate/g cell. h)	0.095	

rate of $0.125\ h^{-1}$. After 48 h, the cell concentration reached about 2.04 (g/L) and remained constant. The XG concentration increased rapidly in the exponential phase of cell growth and the highest amount of XG reached to 8.9 (g/L) at the end of fermentation. A comparison of XG concentration from various carbon sources reported in the literatures is listed in Table 4. It can be concluded that acid hydrolysis could be effectively applied for conversion of pretreated broomcorn stem to XG.

To obtain kinetic parameters, all experimental data were fitted to the proposed kinetic model by non-linear regression using MATLAB (2014a). The model parameters are listed in Table 5. The values of growth-associated- and non-growth-associated constants (α and β) were 1.71 (g product/g cell) and 0.03 (g product/g cell h), respectively. Very small value of β indicates that XG production was a growth-associated process. Thus, influencing factors on the cell growth such as mineral salts, by-products and C/N ratio would affect the xanthan production (Li et al. 2016). Similar to XG production, comparison between value of γ and ε shows that the value of growth-associated parameter was about 30 times higher than the value of non-growth-associated parameter. Therefore, the reducing sugar consumption was growth associated and mainly related to the cell concentration. The correlation coefficient (R^2) was higher than 0.992 suggesting

the kinetic models were appropriate for describing the XG fermentation using BS as the sole carbon source.

Conclusion

In this study, the feasibility of xanthan gum production using acid hydrolyzed broomcorn stem as the sole substrate was demonstrated. The hydrolysis time, acid concentration and solid loading influenced reducing sugar yield and xanthan gum concentration. The maximum xanthan concentration achieved was 8.9 (g/L) while the yield and concentration of reducing sugar obtained were 55.2% and 21.5 (g/L), respectively. The comparison of Fourier transform infrared spectroscopy analysis of synthesized product with commercial xanthan, confirmed that chemical and structural characteristics of xanthan gum as the main fermentation product. Molecular weight and viscosity results of synthesized xanthan from BS were similar with those of commercial xanthan. Hence, acid hydrolysis of broomcorn can provide a renewable carbon source for XG production. The logistic and Luedeking–Piret kinetic models could describe xanthan production, catabolism of reducing sugar and cell growth. The obtained results demonstrated that XG biopolymer was growth associated. This study provides an effective bioconversion route for XG production from BS as a renewable raw material.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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