Xanthan gum production using jackfruit-seed-powder-based medium: optimization and characterization

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Abstract Xanthan gum (XG) production by *Xanthomonas campestris* NCIM 2961 using jackfruit seed powder (JSP) as a novel substrate was reported. Central composite design (CCD) of response surface method (RSM) was used to evaluate the linear and interaction effects of five medium variables (JSP, peptone, citric acid, K₂HPO₄ and KH₂PO₄) for XG production. Maximum XG production (51.62 g/L) was observed at the optimum level of JSP (4 g/L), peptone (0.93 g/L), citric acid (0.26 g/L), K₂HPO₄ (1.29 g/L) and KH₂PO₄ (0.5 g/L). K₂HPO₄ and KH₂PO₄ were found as significant medium components, which served as buffering agents as well as nutrients for *X. campestris* growth. The obtained biopolymer was characterized as XG by XRD and FTIR analysis. Results of this study revealed that JSP was found to be a suitable low cost substrate for XG production.

Keywords Jackfruit seed powder · Xanthan gum · Optimization · RSM · XRD · FTIR

Introduction

Xanthan gum (XG), a commercially important exopolysaccharide has been obtained naturally by the plant pathogenic bacterium, *Xanthomonas* sp. by aerobic submerged fermentation (García-Ochoa et al. 2000). XG is a pentasaccharide repeat unit, comprising glucose, mannose and glucuronic acid in the molar ratio of 2:2:1. XG is an anionic polyelectrolyte because of pyruvic acid and acetic acid in its structure (Petri 2015). The percentage composition of D-glucose, D-mannose, D-glucuronic acid, pyruvate and acetate in the polysaccharide may vary according to different species of *Xanthomonas* bacteria used for production. The pseudoplasticity and high viscosity of XG depend on the association between chains forming aggregates and the molecular weight distribution ranges (García-Ochoa et al. 2000). The physicochemical properties of XG can be altered by several methods such as carboxymethylation, free radical, microwave-assisted, chemo enzymatic and plasma assisted chemical grafting for biological applications (Badwaik et al. 2010). XG is widely used as an emulsion stabilizer, suspending agent, dispersant, lubricant or friction reducer and rheology modifier in food, agriculture, textile, cosmetics medical, biomedical and pharmaceuticals industries (Habibi and Khosravi-Darani 2017; Petri 2015). It has been proven that XG has tremendous applications in the delivery of drugs, proteins or biosolutes, and acts as a scaffold in cell adhesion, proliferation and differentiation (Vania et al. 2015).

Many studies reported that the process cost is highly influenced by several factors like type of microbial strain used, substrate cost, unit operations involved in product recovery and physicochemical parameters, etc. (Kennedy and Krouse 1999). Researchers are focused on cost-cutting strategies such as strain development and identification of

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Table 1 CCD for XG production by *X. campestris* using JSP-based medium

Run order	A Jackfruit seed powder (% w/v)	B Peptone (%, w/v)	C Citric acid (%, w/v)	D K ₂ HPO ₄ (%, w/v)	E KH ₂ PO ₄ (%, w/v)	Xanthan gum (g/L)	
						Observed	Predicted
1	5	1.5	0.2	1	1	14.7	9.41
2	4	1.5	0.1	0.5	0.5	18.96	20.93
3	6	0.5	0.3	0.5	0.5	46.22	43.90
4	4	0.5	0.1	1.5	0.5	39.87	35.92
5	4	0.5	0.1	0.5	0.5	19.67	19.30
6	4	1.5	0.1	1.5	0.5	41.2	36.30
7	4	0.5	0.1	0.5	1.5	18.84	11.90
8	6	1.5	0.1	1.5	1.5	25.26	27.34
9	4	0.5	0.1	1.5	1.5	20.02	23.01
10	4	0.5	0.3	1.5	0.5	42.45	46.35
11	4	1.5	0.3	0.5	1.5	22.12	21.57
12	5	0.5	0.2	1	1	9.32	10.09
13	6	0.5	0.1	1.5	0.5	32.89	36.59
14	6	1.5	0.1	0.5	1.5	29.74	28.64
15	5	1	0.2	1	0.5	33.8	29.77
16	4	1.5	0.3	1.5	0.5	42.01	38.88
17	5	1	0.2	0.5	1	14.74	13.71
18	4	1	0.2	1	1	34.8	44.01
19	4	1.5	0.3	0.5	0.5	23.4	29.03
20	5	1	0.2	1	1.5	19.48	18.99
21	4	0.5	0.3	0.5	0.5	38.1	35.25
22	5	1	0.3	1	1	26.3	18.37
23	6	0.5	0.3	1.5	0.5	46.18	43.83
24	5	1	0.2	1	1	19.35	21.10
25	5	1	0.2	1	1	31.08	21.10
26	5	1	0.2	1.5	1	22.1	18.61
27	5	1	0.1	1	1	13.74	17.15
28	4	1.5	0.1	0.5	1.5	27.6	26.38
29	6	0.5	0.3	0.5	1.5	17.28	22.39
30	6	0.5	0.3	1.5	1.5	16.96	16.81
31	6	1.5	0.3	0.5	0.5	32.82	29.32
32	6	1	0.2	1	1	57.62	43.89
33	6	1.5	0.3	0.5	1.5	17.94	20.65
34	6	0.5	0.1	0.5	1.5	20.04	22.54
35	4	0.5	0.3	1.5	1.5	18.54	20.53
36	6	1.5	0.3	1.5	0.5	18.52	28.00
37	4	1.5	0.3	1.5	1.5	31.8	25.92
38	5	1	0.2	1	1	18.9	21.10
39	4	1.5	0.1	1.5	1.5	31.24	36.24
40	6	1.5	0.1	1.5	0.5	28.7	28.60
41	6	0.5	0.1	0.5	0.5	26.38	31.14
42	6	1.5	0.3	1.5	1.5	13.1	13.83
43	6	0.5	0.1	1.5	1.5	28.36	22.47
44	6	1.5	0.1	0.5	0.5	26.3	24.40
45	4	0.5	0.3	0.5	1.5	15.82	14.94
46	5	1	0.2	1	1	17.98	21.10

Table 1 continued

Run order	A Jackfruit seed powder (% w/v)	B Peptone (%, w/v)	C Citric acid (%, w/v)	D K ₂ HPO ₄ (%, w/v)	E KH ₂ PO ₄ (%, w/v)	Xanthan gum (g/L)	
						Observed	Predicted
47	5	1	0.2	1	1	16.52	21.10
48	5	1	0.2	1	1	17.58	21.10
49	5	1	0.2	1	1	15.46	21.10
50	5	1	0.2	1	1	18.34	21.10
51	5	1	0.2	1	1	18.14	21.10
52	5	1	0.2	1	1	19.6	21.10

alternate low cost substrate for obtaining maximum yield of XG. Various cheap substrates like cheese, whey (Niknezhad et al. 2015), tapioca pulp (Gunasekar et al. 2014), grape juice (Ghashghaei et al. 2016), molasses (Gilani et al. 2011), and palm date juice (Moshaf et al. 2014; Farhadi et al. 2012; Khosravi-Darani et al. 2011) were utilized for the effective production of XG through a fermentation process. In this study, jackfruit seed powder (JSP) was used as a cheap substrate for XG production. The seeds of jackfruit are starchy with protein and rich in minerals such as calcium, magnesium, zinc, potassium, iron, etc. (Swami et al. 2012). In the literature, JSP was reported as a low cost alternative for biopolymer production (Sharmila et al. 2013; Sugumaran et al. 2013; Ramadas et al. 2009).

The versatility of XG applications in various fields mainly depends on the composition of polysaccharide, structural arrangement and the type of organism used. It is important to highlight that optimization of the production conditions plays a significant role in deciding the properties of XG produced. Optimizing the media composition and process parameters is an essential step to enhance the XG production with less time and process cost. The study of mutual interactions between parameters is a key factor in optimization studies. The statistical optimization approach using response surface methodology (RSM) clearly explains the interactive effects of parameters and minimizes the number of experiments. RSM has been reported widely in literature for optimization of media composition aimed at maximizing the fermentative production of biopolymers (Khani et al. 2016; Padmanaban et al. 2015; Srikanth et al. 2014; Zafar et al. 2012).

The aim of this study is to optimize medium components for XG production using JSP as a natural cheap substrate. RSM was employed to identify the optimal level of chosen medium variables to achieve the maximum XG yield. Further, the obtained biopolymer was characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectrometry (FTIR).

Materials and methods

Microorganism and chemicals

Xanthomonas campestris NCIM-2961 was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. The culture was maintained in nutrient agar plates and sub-cultured at regular intervals. All chemicals used in this study were of analytical grade and procured from Himedia Chemicals.

Jackfruit seed powder (JSP) as a natural substrate

Jackfruit seeds were purchased from the local market and chopped into small pieces. The chopped pieces were dried in the sunlight and ground to fine powder. The obtained fine powder was used as a natural carbon source in the medium.

Response surface optimization

Five significant variables (JSP, peptone, citric acid, K₂HPO₄ and KH₂PO₄) were selected based on the two-level screening by the Plackett–Burman method (data not shown). These variables were further optimized by response surface methodology (RSM) to obtain maximum XG yield. Based on the literature review and from PBD screening study, the levels of the variables were selected. The chosen level of five variables and the experiments were carried out according to the central composite design (CCD) as given in Table 1. The ANOVA statistics involves an *F* test, which assesses the coefficient of determination, related probability values, significance of model and goodness of fit. The model was further validated using 3-D response surface plots.

Fermentation conditions

Experiments were carried out in 250 mL Erlenmeyer flasks with 100 mL sterile production medium prepared

Table 2 Estimated regression coefficients for XG production by *X. campestris* using JSP-based medium

Term	Coefficient
Constant	21.1029
JSP (% w/v)	−0.0626
Peptone (% w/v)	−0.3391
Citric acid (% w/v)	0.6103
K ₂ HPO ₄ (% w/v)	2.4479
KH ₂ PO ₄ (% w/v)	−5.3921
JSP (% w/v)*JSP (% w/v)	22.8472
Peptone (% w/v)*peptone (% w/v)	−11.3528
Citric acid (% w/v)*citric acid (% w/v)	−3.3428
K ₂ HPO ₄ (% w/v)*K ₂ HPO ₄ (% w/v)	−4.9428
KH ₂ PO ₄ (% w/v)*KH ₂ PO ₄ (% w/v)	3.2772
JSP (% w/v)*peptone (% w/v)	−2.0922
JSP (% w/v)*citric acid (% w/v)	−0.7966
JSP (% w/v)*K ₂ HPO ₄ (% w/v)	−2.7928
JSP (% w/v)*KH ₂ PO ₄ (% w/v)	−0.3016
Peptone (% w/v)*citric acid (% w/v)	−1.9616
Peptone (% w/v)*K ₂ HPO ₄ (% w/v)	−0.3116
Peptone (% w/v)*KH ₂ PO ₄ (% w/v)	3.2122
Citric acid (% w/v)*K ₂ HPO ₄ (% w/v)	−1.3797
Citric acid (% w/v)*KH ₂ PO ₄ (% w/v)	−3.2272
K ₂ HPO ₄ (% w/v)*KH ₂ PO ₄ (% w/v)	−1.3772

according to the combination of medium components represented in the CCD (Table 2). The initial pH of the medium was adjusted to 7 and inoculated with 5% (v/v) *X. campestris* culture. The flasks were stirred for 72 h in an orbital shaker (37 °C, 120 rpm).

XG extraction

A sample of 5 mL was collected from the broth in a 15 mL Falcon tube and centrifuged at 4400×g for 20 min. The supernatant was carefully collected in 50 mL Falcon tubes. Precipitation of XG was achieved by adding three volumes of absolute ethanol per volume of the broth and keeping it overnight at 4 °C (Zhang and Chen 2010). The precipitated gum was obtained by centrifuging the sample at 7800×g, and the pellet was dried in hot air oven at 60 °C until constant weight was obtained.

Characterization of XG

The morphology of XG was examined by SEM, and the functional groups present in the XG were characterized by FTIR analysis using a Bruker Equinox 55 spectrometer in the scan range 4000–500 cm^{−1} (Ahuja et al. 2012). The crystallinity of XG was analyzed by XRD analysis (Ahuja et al. 2012).

Results and discussion

Response surface modeling and optimization

The central composite design (CCD) of RSM utilizing five factors, each at three levels, was considered to determine the interactive effect of independent significant factors on the XG production. Several reports support the method of optimising the process parameters for enhanced production of XG using CCD (Silva et al. 2009). The process variables employed in this study were distributed at three levels for each factor in the range of low (−1), mid (0) and high (+1). Based on the levels of distribution, the factors assigned in the production of XG comprised A: JSP (4, 5 and 6%, w/v), B: peptone (0.5, 1 and 1.5%, w/v), C: citric acid (0.1, 0.2 and 0.3%, w/v), D: K₂HPO₄ (0.5, 1 and 1.5%, w/v) and E: KH₂PO₄ (0.5, 1 and 1.5%, w/v), respectively. To the given input data software generated a table consisting of 52 experimental runs with 7 replicates (runs 46, 47, 48, 49, 50, 51 and 52) around the centre point for optimizing the XG production (Table 1). The relationship between the dependent (Y, XG production) and independent variable (A, B, C, D and E), is generally represented by a second-order polynomial equation (Eq. 1) as given below:

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_4D + \beta_5E + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{44}D^2 + \beta_{55}E^2 + \beta_{12}AB + \beta_{13}AC + \beta_{14}AD + \beta_{15}AE + \beta_{23}BC + \beta_{24}BD + \beta_{15}BE + \beta_{34}CD + \beta_{35}CE + \beta_{45}DE. \quad (1)$$

After performing the experiments, the obtained data gave an intelligible idea about the role of independent variables in the enhancement of XG production. The estimated regression coefficient given in Table 2 was employed to express the second-order polynomial model (in coded units) (Eq. 2) for XG production:

$$Y = 21.1029 - 0.0626A - 0.3391B + 0.6103C + 2.4479D - 5.3921E + 22.85A^2 - 11.3528B^2 - 3.3428C^2 - 4.9428D^2 + 3.2772E^2 - 2.0922AB - 0.7966AC - 2.7928AD - 0.3016AE - 1.9616BC - 0.3116BD + 3.2122BE - 1.3797DE. \quad (2)$$

The ANOVA statistics are given in Table 3. The *F* and *p* values were used to determine the significance of the individual effect, the interaction effect of the employed process variables and the overall quadratic model. The *p* value determines the significance (*p* < 0.05) and insignificance (*p* > 0.1) of coefficient terms. The *p* value

Table 3 ANOVA statistics for XG production by *X. campestris* using JSP-based medium

Source	DF	SS	MS	F	p
Regression	20	4338.59	216.93	6.27	<0.001
Linear	5	1208.98	241.80	6.99	<0.001
JSP (% w/v)	1	0.13	0.13	0.00	0.951
Peptone (% w/v)	1	3.91	3.91	0.11	0.739
Citric acid (% w/v)	1	12.66	12.66	0.37	0.550
K ₂ HPO ₄ (% w/v)	1	203.74	203.74	5.89	0.021
KH ₂ PO ₄ (% w/v)	1	988.53	988.53	28.57	<0.001
Square	5	1805.45	361.09	10.44	<0.001
JSP (% w/v)*JSP (% w/v)	1	1010.59	1292.28	37.35	<0.001
Peptone (% w/v)*peptone (% w/v)	1	679.41	319.08	9.22	0.005
Citric acid (% w/v)*citric acid (% w/v)	1	43.37	27.66	0.80	0.378
K ₂ HPO ₄ (% w/v)*K ₂ HPO ₄ (% w/v)	1	45.48	60.48	1.75	0.196
KH ₂ PO ₄ (% w/v)*KH ₂ PO ₄ (% w/v)	1	26.59	26.59	0.77	0.387
Interaction	10	1324.17	132.42	3.83	0.002
JSP (% w/v)*peptone (% w/v)	1	140.07	140.07	4.05	0.053
JSP (% w/v)*citric acid (% w/v)	1	20.30	20.30	0.59	0.449
JSP (% w/v)*K ₂ HPO ₄ (% w/v)	1	249.59	249.59	7.21	0.012
JSP (% w/v)*KH ₂ PO ₄ (% w/v)	1	2.91	2.91	0.08	0.774
Peptone (% w/v)*citric acid (% w/v)	1	123.13	123.13	3.56	0.069
Peptone (% w/v)*K ₂ HPO ₄ (% w/v)	1	3.11	3.11	0.09	0.766
Peptone (% w/v)*KH ₂ PO ₄ (% w/v)	1	330.18	330.18	9.54	0.004
Citric acid (% w/v)*K ₂ HPO ₄ (% w/v)	1	60.91	60.91	1.76	0.194
Citric acid (% w/v)*KH ₂ PO ₄ (% w/v)	1	333.27	333.27	9.63	0.004
K ₂ HPO ₄ (% w/v)*KH ₂ PO ₄ (% w/v)	1	60.69	60.69	1.75	0.195
Residual error	31	1072.44	34.59		
Lack of fit	22	903.97	41.09	2.20	0.111
Pure error	9	168.46	18.72		
Total	51	5411.03			

R-Sq = 0.801

of the model (<0.05) suggests that the model is found to be statistically significant for the experimental results (Liu et al. 2013). According to the conditions of *p* value (<0.05), ANOVA statistics was concluded to pinpoint that *D* (K₂HPO₄), *E* (KH₂PO₄), *AD* (JSP and K₂HPO₄), *BE* (peptone and KH₂PO₄) and *CE* (Citric acid and KH₂PO₄) are found to be significant model terms in this study (Table 2). Similarly, the interaction effects of *AC* (JSP and citric acid), *AE* (JSP and KH₂PO₄), *BD* (peptone and K₂HPO₄), *CD* (citric acid and K₂HPO₄) and *DE* (K₂HPO₄ and KH₂PO₄) are insignificant model terms.

The graphical representation of the regression model as 3-D response surface plots was used to depict the interactions amongst the process variables and to determine the optimal conditions for the maximum XG production (Fig. 1a–j). The 3-D plot of *AB* proposed the interaction between JSP and peptone, indicating that a high concentration level of JSP (6%, w/v) and a mid-level

concentration of peptone (1%, w/v) showed an increase in XG production (Fig. 1a). Similarly, the 3-D plots of *AC* (Fig. 1b) and *AD* (Fig. 1c) suggested that a high level concentration of JSP (6%, w/v) and a mid-level concentration of citric acid (0.2%, w/v) and K₂HPO₄ (1%, w/v) induced the yield of XG to a maximum. The surface plot, *AE*, represented the combined effects of JSP with KH₂PO₄ in the production of XG (Fig. 1d) and explained that high level concentration of JSP (6%, w/v) and low concentration of KH₂PO₄ (0.5%, w/v) are required to enhance the yield. With further increase in KH₂PO₄ concentration, the yield of XG declined. The results indicate that JSP significantly increases the XG yield by interacting with other process variables. An interesting outcome was observed in evaluating the interaction effects of *BC* (peptone and citric acid); the results indicated that maximum XG production was achieved at mid-level concentration of both peptone (1%, w/v) and citric acid (0.2%, w/v), respectively (Fig. 1e).

Fig. 1 3-D surface plots showing the interaction effects of **a** JSP and peptone, **b** JSP and citric acid, **c** JSP and K_2HPO_4 , **d** JSP and KH_2PO_4 , **e** peptone and citric acid, **f** peptone and K_2HPO_4 , **g** peptone

and KH_2PO_4 , **h** citric acid and K_2HPO_4 , **i** citric acid and KH_2PO_4 , **j** K_2HPO_4 and KH_2PO_4 on XG production by *X. campestris*

Fig. 2 Response optimizer plot representing the optimal level of selected variables (JSP, peptone, citric acid, K_2HPO_4 and KH_2PO_4) for maximized XG production

The interaction effects of BD (peptone and K_2HPO_4) suggested that the mid concentration of both the variables showed maximum response in productivity (Fig. 1f). The interaction effect of BE (peptone and KH_2PO_4) indicates that the peptone at mid-level concentration and KH_2PO_4 at low concentration 0.5%, w/v secured the maximum yield of XG (Fig. 1g). The data obtained from the plots (Fig. 1e–g) altogether give a clear idea about the interaction effect of peptone with other process variables indicates that peptone consistently showing a significant effect at mid level concentration (1%, w/v). Figure 1h shows the surface plots of CD (citric acid and K_2HPO_4), infer that the productivity decreases when both the variables interacting at the high and low level concentration and attains maximum production at mid level concentrations of corresponding variables. The graphical view of interaction effects of CE (citric acid and KH_2PO_4) and DE (K_2HPO_4 and KH_2PO_4), respectively, shown in (Fig. 1i, j). The observed interaction results clearly indicate that at low level of KH_2PO_4 (E) XG yield was maximum and increasing its concentration reduces the XG yield. Similar studies reports that increase in concentration of KH_2PO_4 above 1%, w/v reduces the production of XG (Niknezhad et al. 2015).

Model validation

The optimal level of medium variables for maximum XG production (55.83 g/L) was predicted at JSP (4 g/L), peptone (0.93 g/L), citric acid (0.26 g/L), K_2HPO_4 (1.29 g/L) and KH_2PO_4 (0.5 g/L) using the response optimizer tool of Minitab 14.0 software (Fig. 2). The regression model for XG production using JSP was validated by performing batch fermentation using the predicted optimal values of medium components and 51.62 (g/L) of XG was obtained. The experimental result was fitted 92.45% with the predicted results and concluded that obtained model was valid for XG production using JSP. The yield achieved in this study comparably higher than the previous studies involved

Fig. 3 SEM image showing the morphology of produced XG

in the production of XG (de Sousa Costa et al. 2014; Casas et al. 2000; Chaitali et al. 2003).

Characterization

The XG obtained from the validation experiment was characterized by SEM, FTIR and XRD. Scanning electron microphotographs (SEM) of the XG sample are shown in Fig. 3 and revealed a non-distinct, amorphous, fibrous-shaped structure. The morphological characteristics of XG can be improved by using acid or alkali curing process to achieve a compact sustainable structure has been reported (de Mello et al. 2016; Latifi et al. 2016). The morphology observed in SEM analysis in this study showed a cementitious fibrous smooth amorphous structure with less pore volume and stronger attractive forces indicates the enhanced stability of the XG and recommended as an eco friendly and sustainable gelling agent.

Fig. 4 XRD pattern of **a** JSP-derived XG and **b** commercial XG

Fig. 5 FTIR spectrum of **a** XG produced by *X. campestris* and **b** commercial XG

The FTIR spectra of the JSP-derived XG and commercial XG are shown in Fig. 4a, b. A peak at 3300 cm^{-1} depicts OH stretching vibrations, 2881 cm^{-1} shows stretching vibrations due to CH groups, 1730 cm^{-1} denotes the vibration frequencies attributed to C=O stretching, 1600 cm^{-1} frequency vibration related to the presence of COO^- functional group. The results obtained from FTIR analysis inferred that the cumulative stretching vibrations of all functional groups closely resemble the

structure of XG as reported (Anjum et al. 2015; Faria et al. 2011).

The XRD pattern of the XG showed an important crystallographic reflection between two different scattering angles ($2\theta = 15^\circ\text{--}35^\circ$). It is observed that a wide single diffraction peak is recorded for both JSP-derived XG and commercial XG (Fig. 5a, b). In close association with our results, two crystalline peaks was observed in 2θ range of $32^\circ\text{--}35^\circ$ in previous studies related to XRD

studies of XG (Anjum et al. 2015). Hence, the results of XRD suggest that the structure is semi-crystalline, amorphous, and correlated with earlier studies (Bhattacharya et al. 2013; Mudoi et al. 2013). Characterization by FTIR and XRD analysis confirmed that the biopolymer obtained in this study as XG.

Conclusion

The production of XG using JSP was optimized successfully and reported. Five medium components were optimized using CCD of RSM. ANOVA statistics revealed that linear terms (K_2HPO_4 and KH_2PO_4), square terms (JSP, peptone) and interaction term ($JSP \times K_2HPO_4$, $peptone \times KH_2PO_4$ and $citric\ acid \times KH_2PO_4$) had significant effect on XG production. The salts K_2HPO_4 and KH_2PO_4 was found as significant component in this study since it act as both buffering agent and phosphate source in the production medium. Maximum XG yield of 51.62 g/L was predicted by model and it was fitted with the validation experimental results. FTIR and XRD characterization results confirmed the fermented biopolymer as XG. The results obtained in this study can be useful for economical XG production using a low cost substrate, as well as to reduce the environmental impact caused by disposal of residue on the environment.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest in the publication of this article.

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