Use of Response Surface Methodology Analysis for Xanthan Biopolymer Production by *Xanthomonas campestris*: Focus on Agitation Rate, Carbon Source, and Temperature

Zakeri, Ali; Pazouki, Mohammad*+

Environmental Group, Energy Department, Material, and Energy Research Center, Karaj, I.R. IRAN

Vossoughi, Manouchehr

Chemical and Petroleum Engineering Department, Sharif University of Technology, Tehran, I.R. IRAN

ABSTRACT: *The current study is an attempt to contribute for efficient and cost-effective substrates for xanthan gum production. In this context, the sugar cane molasses wastes can be used as a cheap substrate for xanthan gum production. Xanthan biopolymer production by a novel Xanthomonas campestris strain IBRC-M 10644 was optimized with statistical approaches. Based on the results of Response Surface Methodology (RSM) with Central Composite Design (CCD) technique, a second-order polynomial model was developed and evaluated the effects of variables on the maximum xanthan production. Agitation rate (X1: 200-500 rpm), sugar cane molasses concentration (X2: 30-90 g/L) and operation temperature (X3: 25-35 °C) were the factors investigated. The optimal conditions for maximum yield of xanthan production were derived from agitation rate 500 rpm, carbon source concentration 65 g/L and operation temperature 30°C. Under these conditions, xanthan and biomass production were found to be 16.03 g/L and 1.37 g/L, respectively. Our results signify that the sugar cane molasses can be used as a cheap substrate for xanthan biopolymer production.*

KEYWORDS: *Xanthan; Sugar cane molasses; Optimization; Central composite design.*

INTRODUCTION

Xanthan biopolymer is a water-soluble polysaccharide produced industrially by fermentation with the bacteria *Xanthomonas campestris* and can be used as a thickener, stabilizer, emulsifier, or suspending agent. This pseudoplastic biopolymer has a high viscosity and is resistant to wide variations in pH, temperature, and salt [1, 2]. The chemical structure of xanthan is composed of a main chain comprising beta-1, 4 D-glucose units. Trisaccharide side chains on alternating anhydroglucose units are composed of a glucuronic acid residue between two mannose units [3]. Xanthan gum is widely used in a broad range of industries, such as in foods, oil recovery, pharmaceutical formulations, cosmetics, water-based paints, etc., due to its superior rheological properties and is used as a rheological control agent in aqueous systems and as a stabilizer for emulsions and suspensions [4].

* *To whom correspondence should be addressed.*
+ E-mail: mpazouki@merc.ac.ir
1021-9986/2017/1/173 11/$/6.10

Xanthan molecular structure is often reported to be heavily affected by the composition of the production medium and environmental parameters [5-8]. In this respect, several studies have so far focused on a variety of nutrients, particularly the nitrogen and carbon sources, with glucose and sucrose as the most frequently used carbon sources [9]. In fact, the cost of the fermentation medium has always been a major concern in the commercial production of xanthan. For this reason, recent research in the field has particularly focused on the search for cheaper natural alternatives for the currently used substrates, namely glucose or sucrose, so as to control the cost of the production process as well as of the final product [10, 11]. The current study is an attempt to contribute to this current search for efficient and cost-effective substrates for xanthan gum production. In this context, the sugar cane molasses can be used as a cheap substrate for xanthan gum production.

Accordingly, the present work was undertaken to explore and further optimize xanthan gum production by *X.campestris* in batch experiments on sugar cane molasses using response surface methodology with central composite design technique. In the extraction processes, where there are multiple independent variables affecting the responding factors, it is likely to use an optimization method that can determine all the factors. In addition, the possibility of interactions between the independent variables should be considered in order to determine the optimal experimental conditions. Response Surface Methodology (RSM) has been reported to be an effective tool for optimization of a process when the independent variables have a combined effect on the desired response. RSM is a collection of a statistical and mathematical system that has been successfully used for developing, improving and optimizing such processes [12, 13]. A favorite model in RSM is the Central Composite Design (CCD), which is efficient and flexible in providing adequate data on the effects of variables and overall experiment error even with a fewer number of experiments. The CCD model offers useful data on direct, pairwise interaction, and curvilinear variable effects [14-16]. It evaluated three main independent variables, namely agitation rate, carbon sources and temperature values, in terms of their individual and combined effects on data analysis, quadratic model fitting and optimum xanthan and biomass production.

EXPERIMENTAL SECTION

Microorganism and culture condition

Xanthomonas campestris IBRC-M 10644 was used throughout this study. Inoculum preparation was by means of microorganism transfer from the stock culture to yeast extract-malt agar slants, which contained 20 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 15 g/L agar, and incubate for 48 h at 30°C [17, 18]. Following this period, single colonies were transferred into the yeast extract-malt broth inoculum medium, which contained 20 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract, and 5 g/L peptone. 100 mL inoculum medium was sterilized in 250 mL Erlenmeyer flasks at 121°C for 20 min. The cells were grown in inoculum at 28°C and 200 rpm for 24 h [19]. The medium pH has to be adjusted to 7 by the addition of HCl. Fermentation was carried out in 250 mL Erlenmeyer flasks, each of which contained 100 mL of the sterile culture medium. The culture medium was inoculated for 72 h with 5% (v/v) of the inoculums culture. The exact composition of the culture medium was based on sugar cane molasses with the following materials: Sugar Cane Molasses (at 30, 60 and 90 g/l), KH_2PO_4 (5g/L), $MgSO_4 .7H_2O$ (0.2 g/L), Citric Acid (2g/L), H_3BO_3 (0.006g/L), $FeCl_3.6H_2O$ (0.002 g/L), $CaCO_3$(0.02 g/L), Glutamate(2 g/L)[20]. The agitation rate, the concentration of the carbon source (sugar cane molasses) and the temperature were changed in several experiments to study their influence on the xanthan production (see Table 1) by the response surface methodology based on a three variable central composite design.

Xanthan and Biomass extraction

The final fermented medium was processed in a centrifuge (Funke Gerber super varrio) at 6000 g for 30 min to remove the cells. The sediment cells was washed twice with distilled water, dried in an oven at 60°C for 24 h to a constant weight and reported as biomass yield in g/L [21].

Xanthan biopolymer was extracted from the culture supernatant after addition of 1% KCl with two volumes of Isopropanol solvent. The mixture was stored under refrigeration at 4°C for 24 h and then centrifuged again at 6000 rpm for 30 min to recover the precipitated xanthan biopolymer. The precipitated xanthan was dried in an oven at 60°C for 24 h and weighted [22].

Table 1: Levels and codes of variables tested in face central composite design.

Factor	Ranges and Level		
	High (1)	Center (0)	Low (-1)
X_1, Agitation rate (rpm)	500	350	200
X_2, carbon source concentration (g/L)	90	60	30
X_3, temperature (°C)	35	30	25

Experimental Design

Response Surface Methodology (RSM) is an experimental technique used to find the optimal response within the specified ranges of the factors. In this work, a three level face centered Central Composite Design (CCD), which is the standard RSM, was applied for the optimization and evaluated the interaction of the parameters effects. Agitation rate (X_1), carbon source (sugar cane molasses) concentration (X_2), and temperature (X_3) were chosen as three independent variables in the xanthan biopolymer and biomass production [23]. All factors and levels were given in Table 1. Each parameter in the design was studied at three different levels (−1, 0, 1). All variables were taken at a centrally coded value considered as zero [24].

Xanthan (Y_1) and biomass (Y_2) production were selected as the dependent and response variables. The experimental data were fitted by a second order model in the form of quadratic equation, which has the following form:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_i X_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} b_{ij} \quad (1)$$

Where Y is the predicted response variable to be modeled, X_i and X_j are the independent variables which influence Y; b_0 is the intercept term; b_i is the main effects for each variable and b_ig is the interaction effects. Finally, the response surfaces of the variables inside the experimental area were analyzed by analysis of variance (ANOVA) using Design Expert v.7.0 software package (Stat-Ease, Inc.). Comparisons of means were performed by one-way ANOVA) followed by Tukey's test (p-value < 0.05) [24].

RESULTS AND DISCUSSION

Results

Experimental Results

RSM is a consecutive method with an initial purpose to conduct the experiments quickly and efficiently along a path of progress towards the optimal response. In this case, a three level face centered composite design by six replicates at the center points, resulting in a total number of 20 experiments, was used to evaluate both the main and the interaction effects of the operating conditions and xanthan biopolymer extraction. The actual design of this work is presented in Table 2.

The highest amount of xanthan production was obtained 16.82 g/L, at 500 rpm agitation rate, 60 g/L sugar cane molasses as the carbon source and 30 °C operating temperature. The highest biomass production was obtained 1.55 g/L at 500 rpm agitation rate, 60 g/L sugar cane molasses as the carbon source and 30 °C operating temperature.

Statistical Data Analysis

The significant contribution of each coefficient was determined by p-value of F-test ($p < 0.05$). The fitness of the model is generally assessed based on lack-of-fit test ($p > 0.05$). The p-value obtained for the xanthan biopolymer and biomass production indicated the suitability of the model to accurately predict the variations (Table 3). Lack-of-fit compares the residual error to the pure error. Lack-of-fit is not desirable, so a small F value and probability greater than 0.1 are desired. If a model shows lack-of-fit, it should not be used to predict the response [25, 26]. As indicated in Table 3, the lack-of-fit of models in xanthan and biomass production was 0.166 and 0.169 respectively, have a probability more than 0.1, and second order models are highly significant with very low probability values (<0.0001). The quality of fitting the second order equation can be determined based on the coefficient of determination R^2-values which was at 0.9494 and 0.9891 for xanthan and biomass respectively. The R^2-values provide a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The R^2-values is always between 0 and 1. The closer the R^2-value is to 1,

Table 2: CCD and response results for the study of three experimental variables in the coded units.

Run	Factors			Response	
	Agitation rate (X_1)	Carbon source (X_2)	Temperature (X_3)	Xanthan production (g/L)	Biomass production (g/L)
1	-1	-1	-1	5.84	0.56
2	1	-1	-1	7.11	1.45
3	-1	1	-1	6.12	0.63
4	1	1	-1	9.39	1.55
5	-1	-1	1	6.32	0.37
6	1	-1	1	6.85	0.46
7	-1	1	1	7.21	0.39
8	1	1	1	9.61	0.69
9	-1	0	0	9.84	0.54
10	1	0	0	16.82	1.21
11	0	-1	0	8.42	0.91
12	0	1	0	11.25	1.05
13	0	0	-1	11.82	0.85
14	0	0	1	11.91	0.41
15	0	0	0	14.23	0.87
16	0	0	0	14.12	0.86
17	0	0	0	14.39	0.84
18	0	0	0	13.98	0.83
19	0	0	0	14.32	0.81
20	0	0	0	14.11	0.80

Table 3: Summary of the analysis of variance result for the responses quadratic model.

	Responses	
	Xanthan	Biomass
R^2	0.9494	0.9891
R^2 adjusted	0.9030	0.9794
p-value Prob. > F	< 0.0001	< 0.0001
Lack of fit	0.166	0.169
PRESS	183.14	382.12

Table 4: Regression coefficient of polynomial functions of response surfaces of xanthan and biomass content.

	P-value	F value	Coefficients
Xanthan (g/l) Constant	14.10	20.85	< 0.0001
X_1	1.45	18.24	0.0016
X_2	0.90	7.14	0.0234
X_3	0.16	0.23	0.6424
X_1^2	-0.62	0.93	0.3579
X_2^2	-4.12	40.71	< 0.0001
X_3^2	-2.09	10.46	0.0090
X_1X_2	0.48	1.64	0.2298
X_1X_3	-0.20	0.28	0.6063
X_2X_3	0.14	0.13	0.7262
Biomass (g/L)	0.84	101.25	< 0.0001
X_1	0.29	374.19	< 0.0001
X_2	0.056	14.25	0.0036
X_3	-0.27	336.10	< 0.0001
X_1^2	0.21	0.55	0.4769
X_2^2	0.13	19.80	0.0012
X_3^2	-0.22	62.73	< 0.0001
X_1X_2	0.03	3.27	0.1006
X_1X_3	-0.18	114.50	< 0.0001
X_2X_3	0.010	0.36	0.5600

the better the model predicts the response. for example, the value of the determination coefficient (R^2=0.9494) for xanthan response, indicating that 94.94% of the variability in the response could be explained by the model. As well, the adjusted determination coefficient (adjusted R^2=0.9030) is also very high to advocate for a high significance of the model. These ensured a satisfactory adjustment of the quadratic polynomial model to the experimental data. Significant adequacy of the model was confirmed at the 0.0001% level of probability with the R^2 and adjusted R^2 of >90%. No evidence for lack of fit of the model for all the responses indicated with the p-value > 0.05. Moreover, the predicted sum of squares (PRESS) is a degree of how a particular model fitted each point in the design [27, 28].

The calculated coefficients of all factors are shown in Table 4. The predicted quadratic models in terms of coded variables for xanthan (Y_1) and biomass (Y_2) production are presented in Eq. (2) and Eq. (3), respectively:

$$Y_1 = 14.1 + 1.45X_1 + 0.9X_2 + 0.16X_3 + 0.48X_1^2 - 0.2X_2^2 + 0.14X_3^2 - 0.62X_1X_2 - 4.12X_1X_3 - 2.09X_2X_3 \quad (2)$$

$$Y_2 = 0.84 + 0.29X_1 + 0.056X_2 - 0.27X_3 + 0.03X_1^2 - 0.18X_2^2 + 0.01X_3^2 - 0.021X_1X_2 - 0.13X_1X_3 - 0.2X_2X_3 \quad (3)$$

As shown in Table 4, agitation rate (X_1) was found to play an important role for xanthan production (p=0.0016 < 0.05). The results showed that the main effect of carbon source concentration (X_2) (p=0.0234< 0.05) and the interaction effect between carbon source (X_2^2) and temperature (X_3^2) was significant to the xanthan production.

As a result shown in Table 4, agitation rate (X_1) and temperature (X_3) were played a much more important role for biomass production, although carbon source concentration (X_2) and the interaction effect between temperature (X_3^2) and agitation rate, temperature (X_1X_3) were in the next degree of importance. The other term coefficients had no significant effect on the xanthan extraction and biomass production ($P > 0.05$).

Response surface plots provide a method to predict the xanthan and biomass production efficiency for different values of the tested variables and the contours plots help in recognition of the type of interactions between these variables [28]. Each contour plot shows an infinite number of combinations of two tested variables with the other two maintained at their respective zero level. A circular contour of response surfaces indicates that the interaction between the corresponding variables is negligible. In contrast, an elliptical contour plot indicates that the interaction between the corresponding variables is significant. The response surface contour plots for the effect of each pair of variables for xanthan and biomass production are shown in Figs. 1 and 2, respectively. Fig. 1A represents the contour plots for xanthan production by the interaction between the agitation rate and carbon source. The temperature was held at zero level. The results are in agreement with other reports [29] showing that agitation rate has a positive effect on xanthan production. In addition, this high yield at 500 rpm is also associated with better aeration of the culture medium [30, 32]. According to Figs. 1B-C higher xanthan production was obtained at temperatures between 30 and 32 °C by increasing the carbon source concentration and the results are in agreement with other reports [33, 34]. According to Figs. 2A-C agitation rate has a positive effect on biomass production and maximum yield can be obtained at 500 rpm. In addition, at the temperature from 25 to 28 °C, with increasing the carbon source concentration, the maximum yield of biomass was produced.

Figs. 3 and 4 show the normal probability of the experimental results for xanthan and biomass production. These plots indicate that none of the individual residuals exceeded the residual variance and that an excellent adequacy of the regression model was utilized [35]. The results of these curves show that the experimental values are in good agreement with the predicted values and in this study, the method of optimization the operation

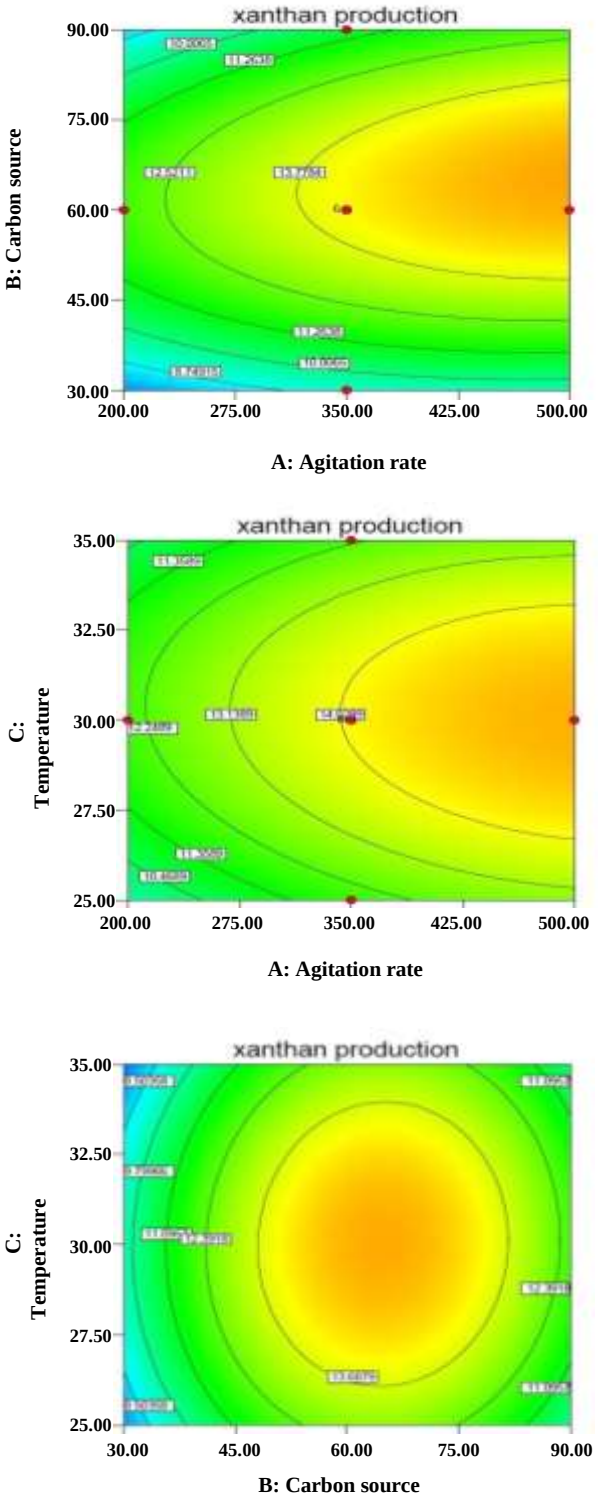

Fig. 1: 2D contour plots for xanthan production:(a) the effect of agitation rate and carbon source on xanthan production; (b) the effect of agitation rate and temperature on xanthan production; (c) the effect of carbon source and temperature on xanthan production.

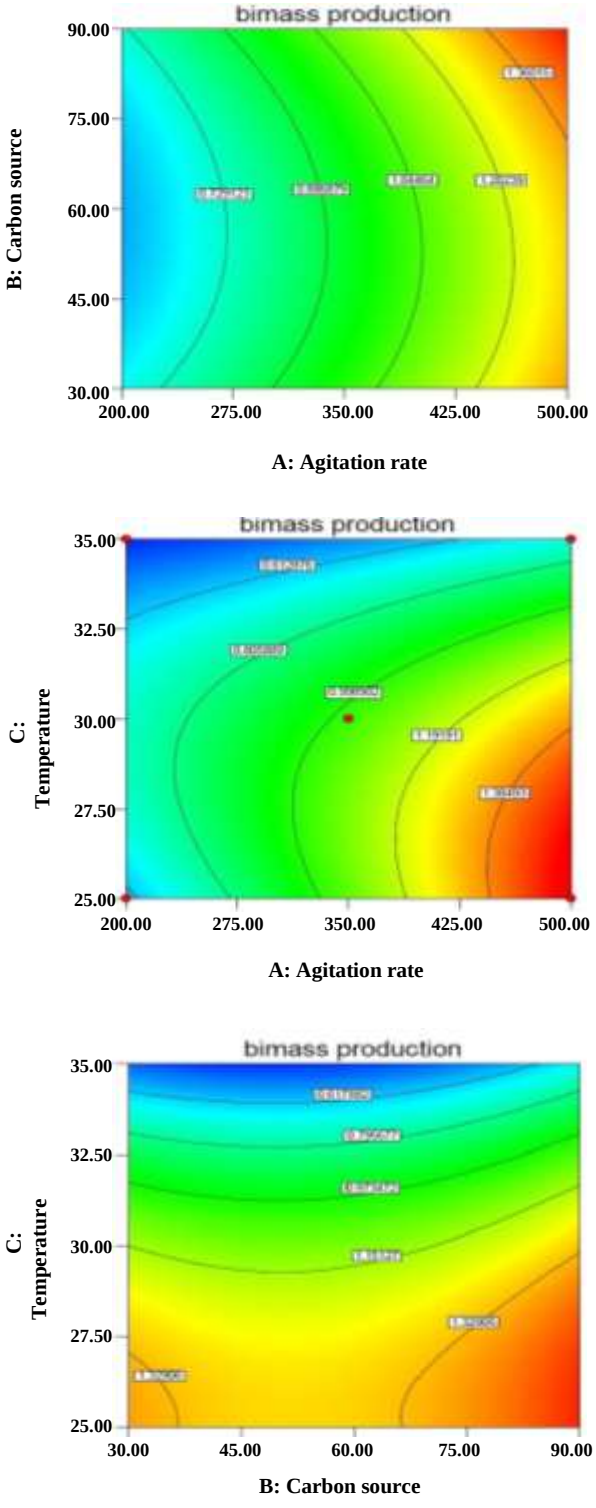

Fig. 2: 2D contour plots for biomass production: (a) the effect of agitation rate and carbon source on biomass production; (b) the effect of agitation rate and temperature on biomass production; (c) the effect of carbon source and temperature on biomass production.

conditions to obtain the maximum xanthan and biomass production by RSM, are successful.

From the model, the optimum condition for xanthan production was at 500 rpm, 65 g/L, and 30 °C, and the xanthan yield was 16.03 g/l. At this condition, the model was validated with a set of experiments (3 replicates) which gave the extraction yield of 16.03±0.2 g/L, which agreed reasonably with the model prediction.

Validation of model

The optimized conditions were Agitation rate (X1) 500 rpm, sugar cane molasses concentration (X2) 65 g/L and operation temperature (X3) 30°C. Under the optimal conditions, the model predicted xanthan yield is 16.03 g/L. The results of the analysis indicated that the experimental value (16.23 g/L) were in good agreement with predicted ones (not significant at the 5% confidence level) and consequently, indicated that the RSM model is satisfactory and accurate. Different xanthan production yields have been reported by other reports those obtained in the literature using agro-industrial residues such as carob extract, 17 g/L [36]; citrus waste, 14.5 g/L [37]; olive mill waste waters, 7 g/L [20]; ram horn hydrolysate, 25.6 g/L [7]; and cheese whey, 26.35 g/L [38]. Different extraction sources could explain the significantly different yields from various sources. Therefore sugar cane molasses the efficiency of xanthan extraction, resulting in an increased yield with cheap carbon source material.

It is worth noting in this context that the cost of the fermentation medium has often been considered one of the principal factors that determine the viability of xanthan production. In fact, the substrates presented in the current study (sugar cane molasses) offer cheap and promising alternatives that can substitute the currently used substrates, namely glucose or sucrose, and can, therefore, help lower the cost of the final product. From the model, at this condition, the biomass production was 1.37 g/L, which agreed with experiment results and indicating the efficacy of the model for prediction of the amount of biomass production under different situations of the medium.

FT-IR Spectra of Xanthan biopolymer

The FT-IR spectra of produced and commercial xanthan biopolymer recorded under the same conditions.

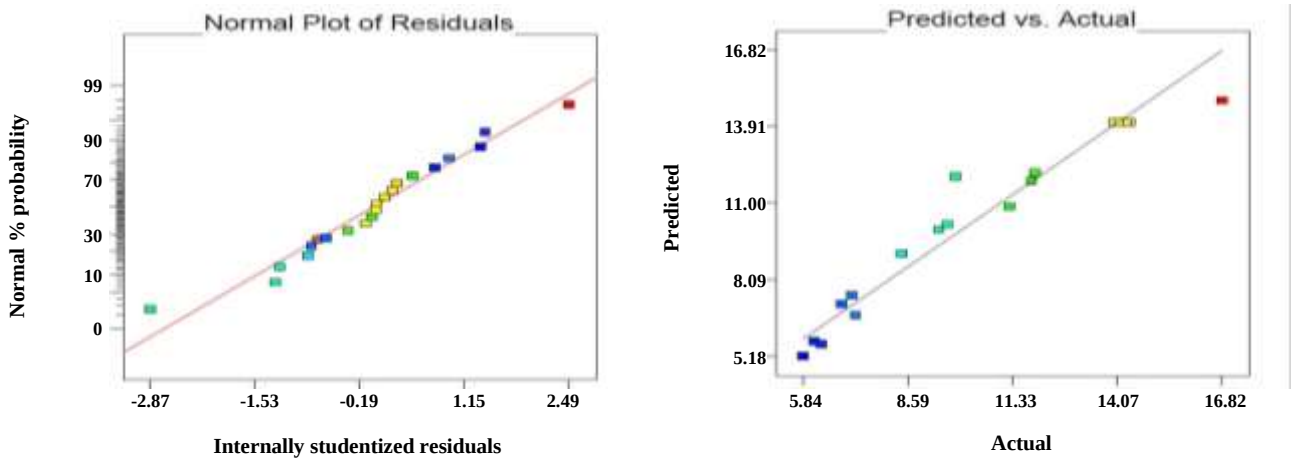

Fig. 3: Design expert plot: (a) normal probability plot of the internally studentized residuals for Xanthan production and (b) Actual values versus predicted response by CCD.

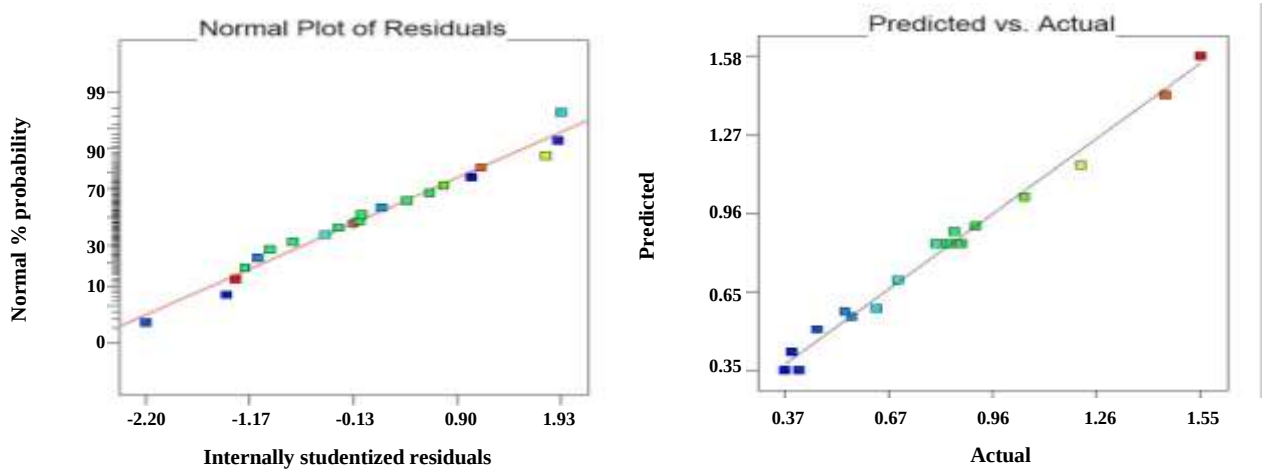

Fig. 4: Design expert plot: (a) normal probability plot of the internally studentized residuals for Biomass production and (b) Actual values versus predicted response by CCD.

The FT-IR spectra of the commercial xanthan, a broad absorption peak at 3414cm^{-1} indicate the hydrogen bonded OH groups. Two peaks, one at 601cm^{-1} and the other at 1418 cm^{-1}, are attributed to −COO groups. Additional characteristic absorption bands of xanthan biopolymer appeared at 1040cm^{-1} and 2912cm^{-1} due to O–H and -CH_2 bending vibrations, respectively. The peaks at 1612 cm^{-1} (C-O asymmetric stretching) and 1418 cm^{-1} (C-O symmetric stretching) are due to the carboxylate anion [39]. Fig.5a and Fig.5b show the FT-IR spectra of the commercial and produced xanthan biopolymer. The results indicate the accommodating between produced and commercial xanthan biopolymer.

Discussion

In this study, the response surface methodology based on a three variable central composite design was adopted to determine the significant effects and polynomial functions that describe the effects of agitation rate, sugar cane molasses as the carbon source and operation temperature on the xanthan and biomass production. The high correlation of the model showed that second-order polynomials could be used to optimize the fermentation condition for maximizing the xanthan production. The significant effects for the xanthan yields were the main effect of agitation rate, carbon source concentration, and the interaction effect between carbon source, carbon source, and temperature, temperature. Likewise,

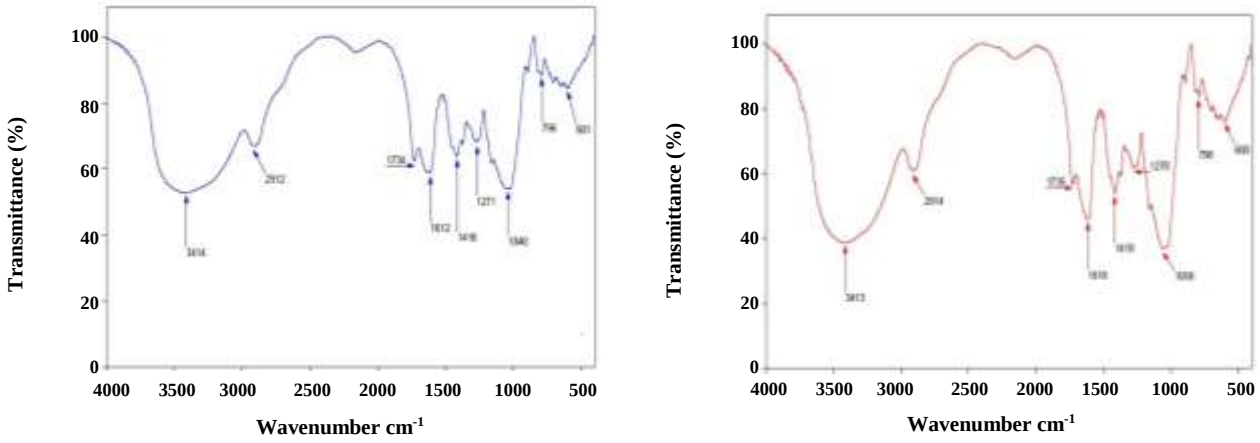

Fig. 5: FT-IR spectrum of commercial (a) and produced xanthan biopolymer (b).

the significant effects for the biomass yields were the main effect of agitation rate, temperature, carbon source concentration, and the interaction effect between agitation rate, temperature, and temperature, temperature. The optimized parameters for maximum xanthan production in this study were set as follows: agitation rate, 500 rpm; carbon source concentration, 65 g/l; temperature, 30 °C and the xanthan and biomass yield were 16.03 g/L and 1.37 g/L, respectively. These values were further validated by actually performing the experiment at the optimized values of these parameters. The methodology as a whole has been proved to be appropriate for the design and optimization of the xanthan production process. Based on these results, it was recommended that the fermentation process is carried out at optimal conditions for maximum xanthan production. Moreover, the functional groups of produced xanthan biopolymer were compared with the commercial xanthan by FT-IR spectra. The results indicate the accommodating between produced and commercial xanthan biopolymer.

Received: June 9, 2016 ; Accepted: Jan. 31, 2017

REFERENCES

[1] Rosalam S., England R., Review of Xanthan Gum Production from Unmodified Starches by *Xanthomonas Campestris sp*, *Enzyme Microb. Technol.*, **39**:197-207 (2006).

[2] Bacelo K.L., Hartwig D.D., Seixas F.K., Schuch R., Moreira A.D.S., Amaral M., Xanthan Gum As an Adjuvant in A Subunit Vaccine Preparation Against Leptospirosis, *BioMed Res. Int.*, **6**:1-14 (2014).

[3] Ahlgren J.A., Purification and Characterization of A Pyruvated-Mannose-Specific Xanthan Lyase from Heat-Stable, Salt-Tolerant Bacteria, *Appl. Environ. Microbiol.*, **57**:2523-2528 (1991).

[4] Garcia-Ochoa F., Santos V.E., Casas J.A., Gomez E., Xanthan Gum: Production, Recovery, and Properties, *Biotchnol. Adv.*, **18**:549-579 (2000).

[5] Afendra A.S., Yiannaki E.E., Palaiomylitou M.A., Kyriakidis D.A., Drainas C., Co-Production of Ice Nuclei and Xanthan Gum by Transformed *Xanthomonas Campestris* Grown in Sugar Beet Molasses, *Biotechnol. Lett.*, **24**: 579-583 (2002).

[6] Shu C.H., Yang S.T., Effects of Temperature on Cell-Growth and Xanthan Production in Batch Cultures of *Xanthomonas Campestris*, *Biotechnol. Bioeng.*, **35**:454-568 (1990).

[7] Basaran-Kurbanoglu E., Izzet-Kurbanoglu N., Ram Horn Hydrolysate as Enhancer of Xanthan Production in Batch Culture of *Xanthomonas Campestris* EBK-4 Isolate, *Process Biochem.*, **42**:1146-1149 (2007).

[8] Gilani S.L., Najafpour G.D., Heydarzadeh H.D., Zare H., Kinetic Models for Xanthan Gum Production Using *Xanthomonas Campestris* from Molasses, *Chem. Ind. Chem. Eng. Q.*, **17**(2):179-187 (2011).

[9] Thonart P., Paquot M., Hermans L., Alaoui H., d'Ippolito P., Xanthan Production by *Xanthomonas Campestris* NRRL B-1459 and Interfacial Approach by Zeta Potential Measurement, *Enz. Microb. Technol.*, **7**:235-238 (1985).

[10] Ashraf S., Soudi M.R., Sadeghizadeh M., Isolation of A Novel Strain of *Xanthomonas Campestris* for Xanthan Gum Production Using Whey as the Sole Substrate, *Pak. J. Biol. Sci.*, **11**(3):438-442 (2008).

[11] Moshaf S., Hamidi-Esfahani Z., Azizi M.H., Statistical Optimization of Xanthan Gum Production and Influence of Airflow Rates in Lab-Scale Fermentor, *Appl. Food Biotechnol.*, **1**(1):17-24 (2014).

[12] Eslami A., Asadi A., Meserghani M., Bahrami H., Optimization of Sonochemical Degradation of Amoxicillin by Sulfate Radicals in Aqueous Solution Using Response Surface Methodology (RSM), *J. Mol. Liq*., **222**:739-744 (2016).

[13] Yousefi N., Pazouki M., Alikhani H.F., Alizadeh M., Statistical Evaluation of the Pertinent Parameters in Bio-synthesis of Ag/MWf-CNT Composites Using Plackett-Burman Design and Response Surface Methodology, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **35**(2):51-62 (2016).

[14] Krishna-Ganduri V.S.R., Unstructured Modeling of Aureobasidium Pullulans Fermentation for Pullulan Production Mathematical Approach, *Int. J. Eng. Res. Appl.*, **3**:1076-1079 (2014).

[15] Wang X., Xu P., Yuan Y., Liu C., Zhang D., Modeling for Gellan Gum Production by Sphingomonas Paucimobilis ATCC 31461 in a Simplified Medium, *Appl. Environ. Microbiol.*, **72**(5):3367-3374 (2006).

[16] Rajendran A., Thangavelu V., Application of Central Composite Design and Artificial Neural Network for the Optimization of Fermentation Conditions for Lipase Production by *Rhizopus Arrhizus* MTCC 2233, *J. Bioproces Biotechniq.*, **2**(3):1-9 (2012).

[17] Nakajima S., Funahashi H., Yoshida T., Xanthan Gum Production in A Fermentor with Twin Impellers, *J. Fermen. Bioengineer.*, **70**(6):392-397 (1990).

[18] Souw P., Demain A.L., Nutritional Studies on Xanthan Production by *Xanthomonas Campestris* NRRL B-1459, *Appl. Environ. Microbiol.*, **37**:1186-1192 (1979).

[19] Serrano-Carren L., Corona R.M., Snchez A., Galindo E., Prediction of Xanthan Fermentation Development by A Model Linking Kinetics, Power Drawn and Mixing, *Process. Biochem.*, **33**:133-146 (1998).

[20] Lopez M.J., Moreno J., Ramos-Cormenzana A., *Xanthomonas Campestris* Strain Selection for Xanthan Production from Olive Mill Wastewaters, *Water. Res.*, **35**:1828-1830 (2001).

[21] Papagianni M., Psomas S.K., Batsilas L., Paras S.V., Kyriakidis A., Liakopoulou-Kyriakides M., Xanthan Production by *Xanthomonas Campestris* in Batch Cultures, *Process. Biochem.*, **37**:73-80 (2001).

[22] Moreno J., Lo´pez C., Vargas-Garcı´ M.J., Va´zquez R., Use of Agricultural Wastes for Xanthan Production by *Xanthomonas Campestris*, *J. Ind. Microbiol. Biot.*, **21**:242-246 (1998).

[23] Mason R., Gunst F., Hess L., "Statistical Design and Analysis of Experiments with Application to Engineering and Science", 2nd ed., John Wiley and Sons, Canada (2003).

[24] Montgomery D.C., "Design and Analysis of Experiments", 8th ed., John Wiley and Sons, New York (2013).

[25] Haaland P.D., "Experimental Design in Biotechnology", Marcel Dekker, New York (1989).

[26] Liu H.L., Chiou Y.R., Optimal Decolorization Efficiency of Reactive Red 239 by UV/TiO_2 Photocatalytic Process Coupled with Response Surface Methodology, *Chem. Eng. J.*, **112**:173-179 (2005).

[27] Isar J., Agarwal L., Saran S., Saxena R.K., A Statistical Method for Enhancing the Production of Succinic Acid from *Escherichia Coli* under Anaerobic Conditions, *Bioresour. Technol.*, **97**:1443-1448 (2006).

[28] Aghaie E., Pazouki M., Hosseini M.R., Ranjbar M., Ghavipanjeh F., Response Surface Methodology (RSM) Aanalysis of Organic Acid Production for Kaolin Beneficiation by *Aspergillus Niger*, *Chem. Eng. J.*, **147**:245-251 (2009).

[29] Peters H.U., Herbst H., Hesselink P.G.M., Lunsdorf H., Schumpe A., Deckwer W.D., The Influence of Agitation Rate on Xanthan Production by *Xanthomonas Campestris*, *Biotechnol. Bioeng.*, **34**:1393-1397 (1989).

[30] Amanullah A., Serrano L.C., Castro B., Galindo E., Nienow A.W., The Influence of Impeller Type in Pilot Scale Xanthan Fermentations, *Biotechnol. Bioeng.*, **57**(1):95-108 (1998).

[31] Garcia F.O, Gomez E., Mass Transfer Coefficient in Stirred Reactors for Xanthan Gum Solutions, *J. Biochem. Eng.*, **1**:1-10 (1998).

[32] Sanchez A., Martinez A., Torres L., Galindo E., Power Consumption of Three Impeller Combinations in Mixing Xanthan Fermentation Broths, *Process. Biochem.*, **27**:351-365 (1992).

[33] Kalogiannis S., Iakovidou G., Maria L.K., Kyriakidis D.A., Skaracis G.N., Optimization of Xanthan Gum Production by *Xanthomonas Campestris* Grown in Molasses, *Process. Biochem.*, **39**:1-8 (2003).

[34] Shu C.H., Yang S.T., Kinetics and Modeling of Temperature Effects on Batch Xanthan Gum Fermentation, *Biotechnol. Bioeng.*, **37**:567-574 (1991).

[35] Box G.E.P., Hunter W.G., Hunter J.S., "Statistics for Experimenters", John Wiley and Sons, New York (2005).

[36] Roseiro J.C., Costa D.C., Collaco M.T.A., Batch and Fed-Batch Cultivation of *Xanthomonas Campestris* in Carob Extracts, *LWT-Food Sci. Technol.*, **25**:289-293 (1992).

[37] Green M., Shelef G., Bilanovic D., The Effect of Various Citrus Waste Fractions on Xanthan Fermentation, *Chem. Eng. J.*, **56**:37-41 (1994).

[38] Fernandez-Silva M., Fornari R.C.G., Mazutti M.A., De-Olivera D., Ferreira-Padhila F., Jose-Cichoski A., Production and Characterization of Xantham Gum by *Xanthomonas Campestris* Using Cheese Whey as Sole Carbon Source, *J. Food Eng.*, **90**:119-123 (2009).

[39] Gils P.S., Ray D., Sahoo P.K., Characteristics of Xanthan Gum-Based Biodegradable Superporous Hydrogen, *Int. J. Biol. Macromol.*, **45**: 364-371 (2009).