Asian Journal of Chemistry; Vol. 24, No. 9 (2012), 3887-3890

ASIAN JOURNAL OF CHEMISTRY

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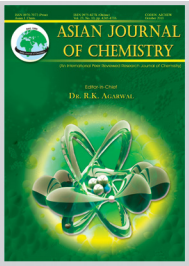

Enhancement of Xanthan Production on Date Extract Using Response Surface Methodology

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(*Received*: 3 October 2011; *Accepted*: 18 April 2012) AJC-11262

Evaluation of process variables (concentration of carbon, nitrogen and phosphorus sources) impact on xanthan production by *Xanthomonas campestris* from date extract were investigated using response surface methodology. Batch fermentation was carried out at 28 ºC (cell growth phase, 24 h) and 32 ºC (gum production phase, 48 h) in the medium containing date extract, NH_4NO_3 and K_2HPO_4 as carbon, nitrogen and phosphorus sources, respectively. The main and interaction effects of three variables on xanthan production were studied using a face-centre composite design. A quadratic equation was fitted for gum production. Maximum xanthan concentration 14.61 g/L was obtained at 40, 9, 0.1 g/L carbon, phosphorous and nitrogen sources, respectively.

Key Words: Xanthan gum, *Xanthomonas campestris*, Date extract, Response surface methodology.

INTRODUCTION

Xanthan gum is a hetero-polysaccharide, which is produced by the aerobic fermentation of *Xanthomonas campestris*[1]. Special rheological properties of xanthan gum water solutions (pseudo-plastic behaviour, stable viscosity in wide ranges of temperature, pH and saline concentration and synergistic effect with solutions of galactomannans) are caused widely usage as emulsifier, suspending agent and thickener in many different industries such as food, cosmetic, pharmaceutical and oil industry[2].

The results of research show that the production and properties of xanthan gum are influenced by bacterial strain[3,4], culture medium[5-8], substrate[9-17], temperature[18,19], pH[20] and time of fermentation[21], as well as agitation rate[22,23], impeller type[24-26] and aeration[27,28]. Both batch[29,30] and continuous[31,32] fermentations have been applied in different types of bioreactors[33,34] for the production of this valuable biopolymer. There are several works focused on the optimization of xanthan gum production by using statistical analysis[5,17,35]. Among all, batch fermentation of *X. campestris* with glucose as a substrate is still the most economical process for xanthan gum production. Increase in both price and demand for this product indicates the necessity of application of an economic glucose substrate.

Iran is ranked as the second date producer country in the world by production of 20 % of the total date. Unfortunately a large amount of this product is wasted, while it is rich in carbohydrates and other required metabolites for microbial growth and production. This syrup can be utilized fairly as a domestic and available industrial medium for preparation of a medium culture for the growth of *X. campestris* and the production of a metabolite with an important industrial interest like xanthan[35].

The aim of this study is to evaluate the effects of three important medium components (phosphorous, nitrogen and carbon source concentration) on the growth of *X. campestris* and gum production by using response surface methodology. Also a two-stage heating strategy for enhancement of growth and gum production was conducted.

EXPERIMENTAL

Microorganism and inoculum preparation: In this study, *X. campestris* (PTCC-1473) was obtained from Persian Type Culture Collection; Iranian Research Organization for Science and Technology (IROST) (Tehran, Iran) used as the microorganism for xanthan gum production. The bacterial cells were grown on complex solid medium slants (YM) for 24 h at 28 ºC; maintained at 4 ºC and were transferred to fresh media every 14 days. The inoculum development was done in a medium with a composition similar to growth medium to minimize lag phase. Inoculum was incubates at 28 ºC and shaking of 200 rpm till 72 h and then it was added to fermentation medium by 10 % (v/v).

Production medium and conditions: Experiments were conducted using 500 mL Erlenmeyer flasks containing 200 mL of production medium. Date extract, ammonium nitrate

and phosphate hydrogen di-potassium were used as the carbon, nitrogen and phosphorous sources, respectively, as shown in Table-1. Other components of fermentation medium were (g/L) H_3BO_3 2.1, $MgCl_2$ 0.507, Na_2SO_4 4.6, H_3BO_3 0.006, ZnO 0.006, $Fe_2Cl_3.6H_2O$ 0.020, $CaCO_3$ 0.020, $FeSO_4$ 0.008, HCl 0.13 mL. Flasks were transferred to shaker incubator at 200 rpm and a two-stage temperature strategy were applied at 28 ºC and 32 ºC for promotion of cell growth (24 h) and xanthan production (48 h), respectively.

TABLE-1
CENTRAL COMPOSITE DESIGN FOR EVALUATION OF THREE INDEPENDENT VARIABLES ON XANTHAN PRODUCTION AS THE RESPONSE

Run	Carbon concentration coded	Carbon concentration uncoded (g/L)	Nitrogen concentration coded	Nitrogen concentration uncoded (g/L)	Phosphorous concentration coded	Phosphorous concentration uncoded (g/L)	Xanthan concentration (g/L)
1	0	30	1-	0.1	0	5	8.40
2	1	40	1	0.7	1	9	13.10
3	-1	20	1-	0.1	1-	1	6.67
4	-1	20	1-	0.1	1	9	7.69
5	-1	20	1-	0.1	1-	1	6.70
6	-1	20	1-	0.1	1	9	7.90
7	-1	20	0	0.4	0	5	7.25
8	0	30	1	0.7	0	5	7.32
9	1	40	0	0.4	0	5	13.47
10	-1	20	1	0.7	1-	1	5.42
11	-1	20	1	0.7	1	9	7.04
12	1	40	1-	0.1	1-	1	12.88
13	0	30	1	0.7	0	5	7.317
14	0	30	0	0.4	1	9	8.20
15	0	30	0	0.4	1	9	8.80
16	0	30	0	0.4	0	5	8.40
17	-1	20	1	0.7	1	9	7.00
18	1	40	1-	0.1	1	9	14.10
19	1	40	1	0.7	1-	1	11.70
20	-1	20	1	0.7	1-	1	5.40
21	1	40	1-	0.1	1	9	14.61
22	1	40	1	0.7	1	9	13.20
23	1	40	1	0.7	1-	1	11.80
24	0	30	1-	0.1	0	5	8.42
25	0	30	0	0.4	1-	1	7.37
26	0	30	0	0.4	1-	1	7.40
27	1	40	1-	0.1	1-	1	12.8
28	0	30	0	0.4	0	5	8.20
29	1	40	0	0.4	0	5	13.60
30	-1	20	0	0.4	0	5	7.80

Determination of cell growth (biomass): Biomass determination was done gravimetrically. Production medium was diluted 4 times and cells were collected by centrifugation for 40 min at 21000 ×g. Then pellet (biomass) was resuspended in isopropanol (IPA) twice to wash out xanthan gum residues. Cells were dried in an oven for 24 h at 80 ºC and weighted.

Determination of xanthan gum: Determination of xanthan gum was also done gravimetrically. For separation of xanthan gum, isopropanol was added to supernatant of culture medium (at 300 % v/v) containing 1 g/L NaCl and centrifuged for 20 min at 21000 × g. Precipitated gum was dried in an oven for 48 h at 80 ºC and weighted.

Experimental design: A central composite design (CCD) (at three levels) for three independent variables of glucose, NH_4NO_3 and K_2HPO_4 concentration was used to predict a quadratic equation for xanthan production and evaluate the interaction effects of these variables. According to this methodology each variable at three coded value of -1, 0 and 1 were calculated. The coded values of variables were estimated by the eqn. 1[3]:

$$\text{Coded value} = \frac{\left(\text{Actual level} - \frac{[\text{High level} + \text{Low level}]}{2}\right)}{\left(\frac{[\text{High level} - \text{Low level}]}{2}\right)} \quad (1)$$

Range finding (selection of uncoded values) for three levels of variables were based on our previous experience[13,35] and also literature review.

RESULTS AND DISCUSSION

Fifteen experiments were conducted twice to eliminate the interferential errors caused by passing of the time. The results of trials are presented in Table-1.

According to the results given in Table-1 a quadratic equation (eqn. 2) with all (main and interaction) effects was proposed to predict xanthan gum concentration.

$$Y=\beta_0 + \beta_1 C + \beta_2 N + \beta_3 P + \beta_4 C^2 + \beta_5 N^2 + \beta_6 P^2 + \beta_7 C \times N + \beta_8 C \times P + \beta_9 N \times P + \beta_{10} C \times N \times P \quad (2)$$

where; Y: Xanthan gum concentration (g/L); C: Carbon concentration; P: Phosphorous concentration; N: Nitrogen concentration; βi: i=0-10 statistical coefficients.

Statistical and numerical analyses were carried out by using Minitab[14] software. The t and P-values of estimated regression coefficients for xanthan production (results are not presented) showed that the interaction effects of carbon, nitrogen and phosphorous concentrations are not significant ($P < 0.05$). So, these parameters were neglected for next stage and recalculated results for regression coefficients are shown in Table-2.

TABLE-2
ESTIMATED REGRESSION COEFFICIENTS FOR EVALUATION OF PROCESS VARIABLES IMPACT ON XANTHAN PRODUCTION

Term	Coefficient	Standard error coefficient	*t*	P
Constant	8.2373	0.07714	106.789	0.000
Carbon	3.0895	0.04538	68.076	0.000
Nitrogen	-0.5137	0.04538	-11.318	0.000
Phosphorous	0.6450	0.04538	14.212	0.000
Carbon × carbon	2.3084	0.08950	25.793	0.000
Nitrogen × nitrogen	-0.3574	0.08950	-3.993	0.001
Phosphorous × phosphorous	-0.2791	0.08950	-3.119	0.005

S = 0.2030, R-Sq = 99.6 %, R-Sq (adj) = 99.5 %

The analysis of variance (Table-3) showed that the regression models, as well as the linear and square terms are highly significant. There is no evidence of 'lack-of- fit', due to the P-value of 0.702, which is >>0.05. The quadratic model

TABLE-3
ANALYSIS OF VARIANCE FOR QUADRATIC MODEL OBTAINED FROM THE CCD FOR XANTHAN PRODUCTION

Source	Degree of freedom	Sum of squares (SS)	Adjusted (Adj) SS	Adj mean squares	F	P
Regression	6	234.089	234.089	39.0148	947.14	0.000
Linear	3	204.497	204.497	68.1658	1654.82	0.000
Square	3	29.591	29.591	9.8637	239.46	0.000
Residual error	23	0.947	0.947	0.0412		
Lack-of-Fit	8	0.252	0.252	0.0315	0.68	0.702
Pure Error	15	0.695	0.695	0.0463		
Total	29	235.036				

for the xanthan concentration (Y) as a function of carbon (C), nitrogen (N) and phosphorous (P) concentrations was derived from uncoded values as following (eqn. 3):

$$Y=18.5515-1.07608C+1.46438N+0.335694P+0.0230839C^2 - 3.97068N^2 - 0.0174444\ P^2 \quad (3)$$

According to the response surface plot (Fig. 1), increase in carbon concentration of medium causes an increase in xanthan production. Phosphorous concentration has the same but very slight influence on xanthan production (Fig. 2). But, increased nitrogen concentration causes a decrease in gum production. The magnitudes of coefficients in Table-2 also indicate that concentration of carbon source has more positive effect on gum production than phosphorous source (about five times more) (Fig. 2), whereas nitrogen source concentration has a negative and very less effect on xanthan gum production in compare to carbon and phosphorus source initial concentration (Figs. 2 and 3). In fact, high concentration of nitrogen source is not suitable for xanthan production because it is not a participant component in polysaccharide structure. In another word, presence of nitrogen source is necessary only mainly for cell growth and enzyme production for catabolic and anabolic pathways of bacterial cells. Totally, the maximum amount of product can be achieved at 40, 0.1 and 9 g/L of carbon, nitrogen and phosphorous concentration, respectively.

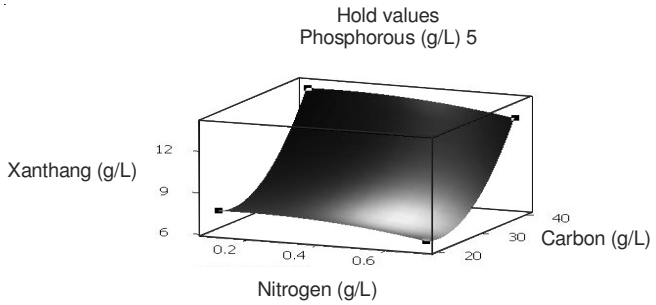

Fig. 1. Surface plot of xanthan production *versus* carbon and nitrogen initial concentration

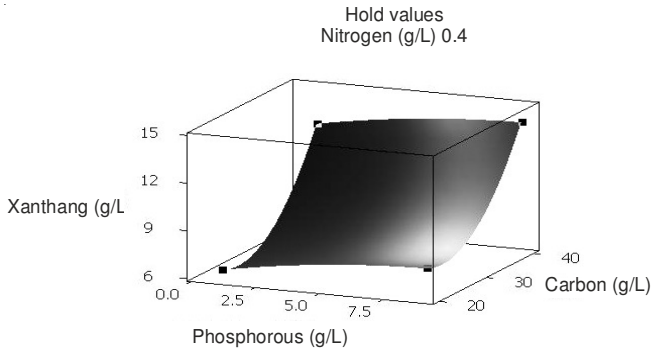

Fig. 2. Surface plot of xanthan production *versus* carbon and phosphorous initial concentration

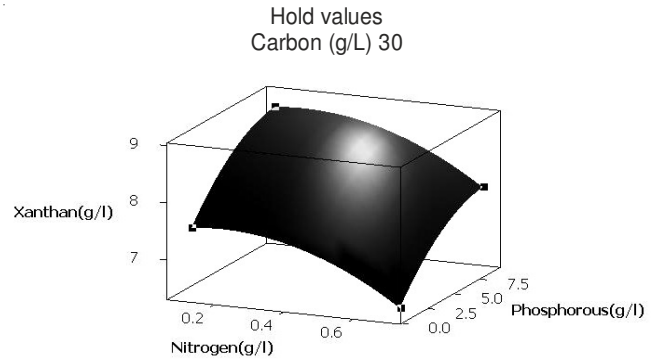

Fig. 3. Surface plot of xanthan production *versus* nitrogen and phosphorous initial concentration

Results obtained from change in carbon and phosphorous concentrations are similar to those reported by Taher[17] and Khosravi[35]. However, they reported the maximum xanthan production at 3 g/L of nitrogen source. In this research by applying temperature change strategy during the cell growth (28 ºC) and gum production (32 ºC) phases, the maximum of product achieved at lower level of nitrogen source. This report is the first and the only one, which apply temperature strategy as well as using response surface methodology to enhance the medium component of xanthan production grown on date extract.

Conclusion

This study was conducted to evaluate the effects of three important medium components (phosphorous, nitrogen and carbon source initial concentration) on the growth of *X. campestris* and gum production by using response surface methodology. The results show increased carbon and phosphorous initial concentration in medium, respectively, in the range of 20-40 and 1-9 g/L causes an increased gum production. Reversely, increasing of nitrogen source concentration has negative and slight effect on xanthan production in the range of 0.1-0.7 g/L. Also, applying two-stage temperature strategy during the growth and production phases reduces the final consumption of nitrogen source and increases the maximum produced gum by 11 % in compare with the same process. Lower consumption of nitrogen source may lead to decreasing medium cost in large scale and can promote commercialization of the xanthan production from date syrup.

ACKNOWLEDGEMENTS

The authors thank the National Nutrition and Food Technology Research Institute (NNFTRI) of the Iran for financial support of this research project.

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