Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 Spl Issue III 2014: 229-233

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

Full Length Article

Optimization of Fermentation Conditions of *Xanthomonas campestris* to Increase the Production Efficiency of Xanthan Gum via the Response Surface Methodology

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ABSTRACT
Xanthan gum is an extracellular heteropolysaccharide created by various species of the genera of Xanthomonas such as Xanthomonascampestris. The present study aimed at an investigation into the effect of different carbon, nitrogen and phosphate sources on the efficiency of the production of xanthan via Response Surface Methodology. The effects of the factors on the efficiency of the production of xanthan were initially identified using a two-level Fractional Factorial Design (FDD) and their effects were then investigated using response surface methodology. To determine the biomass, the fermentation medium was centrifuged at 5000 rpm for 15 minutes at 105°C until it reached aconstant weight and was dried. To measure thexanthan production rate, the aforementioned liquid, after being centrifuged, was mixed with two volumes of ethanol and 100 ml potassium chloride 1%; it was then stored for 12 hours at 4°C and re-centrifuged until it reached aconstant weight and dried at 50°C. The results indicate that the optimization of the factors of xanthan gum production can considerably enhance the efficiency of production, which, in turn, results in a decrease in theproduction costs.
Keywords: *Xanthomonascampestris*, Xanthan, Response surface methodology, Carbon sources and Nitrogen sources

Received 17.03.2014 Revised 13.05.2014 Accepted 20.06. 2014

INTRODUCTION

Xanthan gum is an extracellular heteropolysaccharide formed by the aerobic fermentation of the gram-negative bacterium *Xanthomonas campestris*. The structure of xanthan gum includes a main chain of beta-D glucose 1-4 beta-D glucose (like a cellulose) and an accessorial chain which is a tri-saccharide with the formula of D-mannose 1-4 D gluconic acid 1-2 D-mannose and is linked to the main chain on links 1 and 3[1].The production rate of xanthan is affected by the stirring speed in a way that an increase in the speed leads to an increase in the production of xanthan and biomass. In the present study, increasing the stirring speed from 100 to 600 rpm resulted in the production of a considerable amount of xanthan gum (Papagianni et al., 2001) .Additionally, it is possible to produce xanthan gum utilizing substrates of waste and scrap materials. In the experiments conducted, date juice was used to produce xanthan via the bacterium NRRLB-1459 *Xantomonas campestris,* and the resultant xanthan was analyzed using TLC and HPLC methods; its contents were also subjected to analysis [2, 3]. The results indicated that it contained glucose, gluconic acid and mannose, which means that the method used in the present study could be an innovative and economical way of producing xanthan gum (Bensalah et al., 2010).Optimization of the fermentation conditions of *Xantomonas campestris* was carried out in order to enhance the efficiency of xanthan gum via response surface methodology. Additionally, FFD (2-level Fractional Factorial Design) was used to identify the factors effective in the efficiency of production [5, 4].

MATERIALS AND METHODS

Microorganisms

*Xantomonascampestris*PTCC-1473micro-organismwas purchased from the Iranian Organization for Research and Technology. It was maintained on a medium consisting of 20g/l glucose, 20g/l calcium carbonate, 10g/l yeast extract, 17g/l agar and was transferred to a new medium every two weeks to protect the strain of the bacterium [6].

Preparation of the Inoculums

The inoculums consisted of 25g/l glucose, 3g/l yeast extract, 2g/l potassium di-hydrogen phosphate and low-value materials including ammonium nitrate, sodium acetate and salt. They were placed in a rotary shaker for 24 hours at 150 rpm and the inoculation rate was %10v/v [7].

Medium

The optimized medium mainly consisted of 40g/l glucose, 1g/l ammonium nitrate, 10g/l phosphate di-hydrogen potassium,2g/l citric acid and low-value materials including 0.0089g/l NaCl ,0.507g/l magnesium sulfate,0.01g/l boric acid ,0.065g/l zinc sulfate ,0.0027g/l ferric chloride ,0.024g/l calcium carbonate , 0.13ml hydrochloric acid and the pH of the medium was 13.7[8].

Measuring the Biomass

The intended material, after centrifugation, included some sediment and upper material. The sediment was separated and dried it using a 100°C oven for 3 hours. To determine the weight of the biomass, the flask containing the material was weighed and then washed and dried in the oven until it reached a constant weight. The clean flask was subjected to weighing again. The difference between the weight of the clean flask and the one containing the material was the weight of the biomass:

Weight if the flask containing biomass – weight of empty flask = weight of biomass [9].

Determination of Xanthan Gum

To obtain the gum, supernatant centrifuged zinc solvent was used in the following way. First, 15 ml of supernatant was diluted with ethanol 70% and twice isopropranol and then it was mixed with 100ML pure potassium chloride. Then, it must have been re-centrifuged at 10000 rpm and after centrifugation the resultant sediment must be dried in a 105°Coven [10].

RESULTS AND DISCUSSION

Table one presents different amounts of the used nutrients in the medium throughout the 16 experiments of the research (each row represents one experiment).The amount of the produced xanthan gum in each experiment is shown in the last column. The purpose of this action has been to identify and introduce the best composition for the maximum production of xanthan gum [11, 12].

As can be seen in the model amounts in the Table2, the f ratio is significant at a p value of $P<0.05$ for the production of xanthan and therefore is asterisked. The values reported for other substances show no significant effect on the production of xanthan gum and therefore, are not asterisked [13, 14]. In addition, the effect of these substances on the production of xanthan has been displayed in response surface A and B.

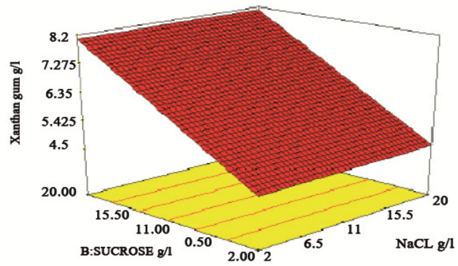
Table1: Amount of produced xanthan at different composition of various substances

Row no.	Glucose g/l	Sucrose g/l	Yeast extract g/l	$(NH_4)_2SO_4$ g/l	$MgSo_4$ g/l	K_2HPO_4 g/l	NaCl g/l	Xanthan gum g/l
1	2	2	20	1	3	3	3	10.58
2	20	20	2	1	1	3	3	10.31
3	2	20	2	1	3	3	1	12.39
4	20	2	2	3	3	3	1	3.22
5	2	2	20	3	3	1	1	2.72
6	2	20	20	3	1	3	1	6.91
7	20	20	20	1	3	1	1	8.54
8	2	2	2	3	1	3	3	3
9	20	2	20	3	1	1	3	3.22
10	20	2	20	1	1	3	1	6.57
11	2	20	20	1	1	1	3	7.41
12	20	20	20	3	3	3	3	11.58
13	20	2	2	1	3	1	3	5.58
14	2	20	2	3	3	1	3	5.63
15	20	20	2	3	1	1	1	1.95
16	2	2	2	1	1	1	1	2.02

Table 2: Analysis of variance of the effect of substances on xanthan gum production

Source	Sum of Squares	df	Square	Value	Prob>F	Significant
Model	179.20	7	25.60	26.57	<0.0001	*
A-Glucose	0.01	1	0.01	0.01	0.001	*
B-Sucrose	48.34	1	48.34	50.17	0.0001	*
C-Yeast extract	11.27	1	11.27	11.70	0.0091	*
D-$(NH_4)2SO_4$	39.60	1	39.60	41.10	0.0002	*
E-Mg.SO_4	22.21	1	22.21	23.05	0.3114	
F-K2HPO_4	47.23	1	47.23	49.03	0.9390	
G-NaCl	10.55	1	10.55	10.95	0.0207	
Residual	7.71	8	0.96			
Cor Total	186.90	15				

Response surface A:



Response Surface B:

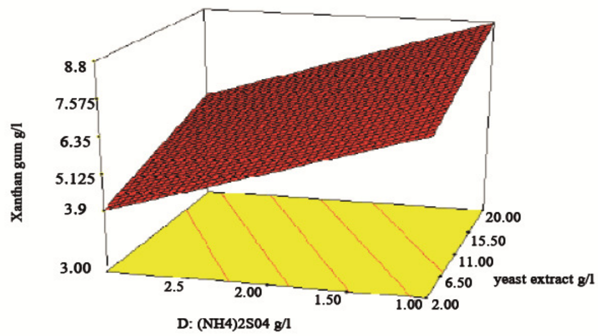


Fig1: Effect of NaCl, sucrose, $(NH_4)_2SO_4$ and yeast extract on xanthan gum production

As can be seen in the response surface A, NaCl has no significant effect on the production of xanthan, whereas sucrose has increased the production of this gum. It is also shown in Table2 that $(NH_4)_2SO_4$has not affected the production of xanthan; the yeast extract, however, is shown to increase the production rate of xanthan gum.

Table2 indicates that $(NH_4)_2SO_4$ has hadonly a marginal effect on xanthan gum production. Therefore, it was replaced with malt extract and its effect on the production of biomass and xanthan gum was investigated [15]. As it is shown in Table3, the malt extract hashad a considerable effect on xanthan gum production, as compared with yeast extract, sucrose and glucose.

Table3: Effect of yeast and malt extracts, glucose and sucrose on xanthan gum production and Biomass.

Run no.	Yeast extract (g/l)	Glucose (m mol)	Sucrose (m mol)	Malt extract (g/l)	Xanthan gum (g/l)	Biomass (g/l)
1	7.5	41.62	27.75	10	9.89	1.56
2	11.25	20.81	41.62	7.5	10.47	1.58
3	11.25	20.81	41.62	2.5	10.22	2.21
4	7.5	41.62	27.75	5	11.38	2.95
5	7.5	41.62	27.75	5	11.48	2.91
6	0	41.62	27.75	5	10.57	2.33
7	7.5	41.62	0	5	10.23	1.68
8	3.75	20.81	41.62	7.5	10.45	2.32
9	3.75	20.81	13.87	7.5	10.18	1.65
10	11.25	62.43	41.62	7.5	10.87	2.19
11	3.75	62.43	13.87	2.5	10.23	2.18
12	3.75	62.43	41.62	7.5	10.41	1.85
13	7.5	41.62	27.75	5	11.32	2.76
14	11.25	52.43	13.87	7.5	11.06	2.65
15	11.25	62.43	13.87	2.5	10.84	2.89
16	7.5	41.62	55.5	5	10.45	2.11
17	7.5	83.25	27.75	5	10.89	2.51
18	7.5	0	27.75	5	10.37	2.21
19	3.75	62.43	41.62	2.5	11.02	2.73
20	3.75	20.81	41.62	2.5	10.89	2.58
21	15	41.62	27.75	5	10.77	2.41
22	3.75	20.81	13.87	2.5	10.11	1.45
23	11.25	20.81	13.87	7.5	10.75	2.25
24	11.25	20.81	13.87	2.5	10.13	2.02
25	7.5	41.62	27.75	5	11.45	2.86
26	7.5	41.62	27.75	5	11.53	2.71

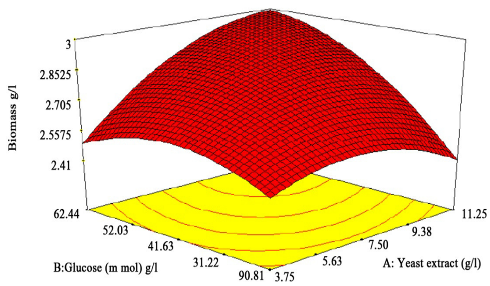
27	11.25	62.43	41.62	2.5	11.15	2.93
28	3.75	62.43	13.87	7.5	9.59	1.56
29	7.5	41.62	27.75	0	10.63	2.81
30	7.5	41.62	27.75	5	11.59	2.83

The effect of glucose, fructose, malt extract, yeast extract and their binary blendson xanthan gum production was investigated (Table4). As indicated in Table4, the binary blend of glucose and malt extract as well as fructose and malt extract did not affect xanthan production significantly, which is clearly seen in response surface C, D, and E.

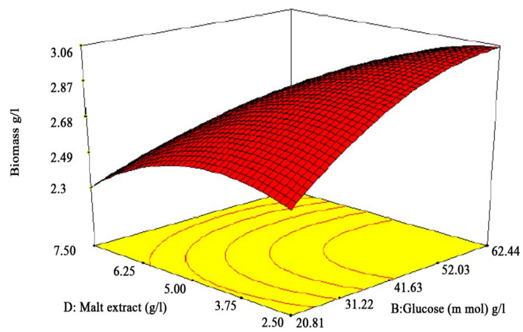
Table4: The effect of glucose, fructose, malt extract, yeast extract and their binary blends on xanthan gum production

Source	Sum of Squares	df	Mean Square	F- Value	P-Value Prob>F	Significant
Model	7.57	13	0.58	24.16	<0.0001	*
A-Yeast extract	0.38	1	0.38	15.66	0.0011	*
B-Glucose	0.38	1	0.38	15.66	0.0011	*
C-Fructose	0.38	1	0.38	15.86	0.0011	*
D- Malt extract	0.22	1	0.22	9.06	0.0083	*
AB	0.47	1	0.47	19.32	0.0005	*
AC	0.47	1	0.47	19.32	0.0005	*
AD	0.37	1	0.37	15.31	0.0012	*
BD	0.20	1	0.20	8.49	0.0101	
CD	0.11	1	0.11	4.72	0.0451	
A^2	0.98	1	0.98	40.66	<0.0001	*
B^2	1.09	1	1.09	45.08	<0.0001	*
C^2	2.02	1	2.02	83.89	<0.0001	*
D^2	2.33	1	2.33	96.70	<0.0001	*
Residual	0.39	16	0.02			
Lack of fit	0.34	11	0.03	3.18	0.1061	not significant
Pure Error	0.05	5	0.01			
Cor Total	7.96	29				

Response surface C:



Response surface D:



Response surface E:

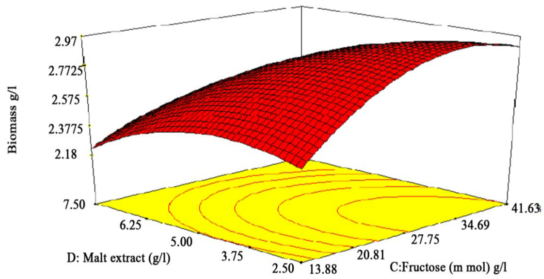


Fig2: The effect of glucose, fructose, malt extract, yeast extract and their binary blends on xanthan gum production

Table5: The best combinations of the four major substances used in the medium.

Substance g/l	Glucose g/l	Yeast g/l	malt g/l	Fructose g/l
Biomass	41.63	8.1	4.1	30.75
xanthan	53.75	7.8	5.6	27.8

According to the diagrams, Table5 shows the best combinations of the four major substances used in the medium.

CONCLUSION

The present research was an attempt at the optimization of xanthan gum production via the bacterium *xanthomonas campestris* using the response surface methodology. A two-level Fractiona Factorial Design (FFD) was also used to identify the effective factors in the production of xanthan gum. Furthermore, the best combinations of nutrients for the medium were found. Glucose and the binary blend of glucose and fructose proved to be the best combinations.

On xanthan gum production but the binary blend of glucose and malt extract as well as fructose and malt extract did not effect on xanthan gum production. This can considerably reduce the costs of the production of xanthan as a widely-used gum.

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