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Optimization of Xanthan Gum Production from Grape Juice Concentrate Using Plackett-Burman Design and Response Surface Methodology

Tahereh Ghashghaei[1], Mohammad Reza Soudi[1], Saman Hoseinkhani[2]*

1. Department of Microbiology, Faculty of Biological Science, Alzahra University, Vanak, Tehran, Iran
2.Department of Biochemistry, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran

Abstract

Low grade grape juice concentrate was used as carbon source for xanthan production. Significant factors affecting xanthan concentration, productivity and viscosity were investigated using Plackett-Burman Design. Based on the obtained results, carbon and nitrogen concentrations, inoculum size and agitation rate, were assumed as significant factors. Broth culture viscosity and xanthan concentration were optimized using Response Surface Methodology with four independent variables: carbon source (30, 40, 50 g l^{-1}), ammonium sulfate as nitrogen source (0.5, 1.25, 2 g l^{-1}), agitation (150, 200, 250 rpm) and inoculum size (5, 10, 15% v v^{-1}). Optimum level for each factor was obtained by desirability function approach. The average of xanthan gum production and its viscosity under optimized conditions were recorded as 14.35 g l^{-1} and 1268 cP, respectively. The average yield of production and productivity of xanthan within 72 h under optimized conditions were 35% and 0.19 g l^{-1} h^{-1}, respectively. The current study showed the potential of low-grade grape juice concentrate as an economic carbon source for xanthan gum production.

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Correspondence to:
Mohammad Reza Soudi
Department of Microbiology
Faculty of BiologicalScience,
Alzahra University, Tehran Iran,
Tel: +98-21-88044051(2733)
Fax: +98-21-88058912
E-mail: msoudi@alzahra.ac.ir

1. Introduction

Xanthan gum, a greatly viscous bacterial exopolysaccharide with a roughly estimated global production of 100,000 metric tons, has recently found different applications in food, petroleum and many other industries [1,2]. Some species of the genus, such as *Xanthomonas campestris*, can produce xanthan gum as a secondary metabolite and the end product of aerobic growth [3]. Xanthan gum is a GRAS (generally recognized as safe) product, and its applications in food and pharmaceutical industries have been approved by UD Food and Drug Administration [4]. As a suspending, emulsifying and thickening agent, xanthan has important physico-chemical properties including high shear stability [5], pseudo-plastic features [6-8] and stability on a wide variety of temperatures and pH values [9-11]. Similar to many other microbial biopolymers, xanthan can be produced on chemically defined semi-synthetic and completely complex culture media [12]. Providing an appropriate and cheap carbon source is the main challenge [13]. After decades of introducing different carbon sources to xanthan fermentation industry‘ now these feedstocks can be classified into five categories: i) pure simple sugars [14,15], ii) agro-industrial by-products containing directly consumable ingredients such as whey and molasses [16,17], iii) agro-industrial wastes demanding previous hydrolytic processes like acid hydrolysates of fruit pulps [18], iv) pulps in solid state fermentation including apple pumice, and potato peels [19], and v) low grade juices from fruits such as date [20].

Syrups containing simple sugars (glucose and suc-

rose) are usually used as carbon source in xanthan production [4]. These substrates are expensive, and have not been usually used as carbon source at industry level [13]. After solvent extraction and gum purification procedures, feedstock is the next item, which affects the price of xanthan gum product [4]. Thus, using economic substrates is much important at industrial production, and remarkably decreases the cost of xanthan production. Agro-industrial wastes are the first choice to decrease the expenses (e.g. date extract, and agricultural wastes such as melon) [13,18, 20].

Wild type grapes of the world have been originated from the eastern countries such as Iran, Turkey and Georgia [21]. Substandard grape is used to produce juice concentrate, which is composed of different ingredients mainly including sugars, as well as phenolic and acidic compounds. Depending on climate, cultivar and soil, the composition can be different in various kinds of grapes [22].

In the present study, four important factors out of six discriminated by Plackett-Burman Design (PBD), including carbon source, nitrogen source, agitation and inoculum size, were selected to optimize xanthan gum production and viscosity using Response Surface Methodology (RSM).

2. Materials and Methods

2.1. Grape juice preparation

Grape juice concentrate was purchased from a domestic company in Takestan, Qazvin province, Iran. Mohammadi Sani reported grape juice concentrate composition [22]. Total sugar of the juice was measured using phenol sulfuric acid method [23]. The juice concentration was diluted to 30, 40 and 50 g l^{-1} using distilled water and adjusted to sugar concentrations equal to the dextrose equivalent of 26, 35, and 43 g l^{-1}, respectively. The solutions were passed from ordinary filter papers and separately steam sterilized and then added to production media.

2.2. Microorganism, media and fermentation

A strain of *Xanthomonas campestris* pv. *campestris* (b82) obtained from the culture collection at Alzahra University (Tehran, Iran) was used in this study. Pure cultures of the bacteria were maintained on Yeast Malt (YM) agar slants at 4°C, and transferred into a fresh medium every 14 days to prevent strains from losing their production capability [4]. Actively growing cells from 24 h slant cultures of each isolate were inoculated to test the tubes containing YM broth. The cultures were incubated at 28°C overnight, and then transferred into 100-ml flasks containing 20 ml of YM broth. After incubation at 28°C on an orbital shaker at 150 rpm, the inocula were added to separate 500-ml flasks each containing 80 ml of the production medium. The composition of the medium was the same as the synthetic medium previously introduced by Roseiro [14], other than using the grape juice as the main carbon source.

2.3. Xanthan gum viscosity and production

After incubation at 28°C for 72 h, the apparent viscosity of fermentation broth was measured at room temperature using a Brookfield system viscometer (Anton Paar, DV1, USA) and spindle number 3 at 60 rpm. Raw product was precipitated with 1.5 volumes of isopropyl alcohol and 0.5 g l^{-1} NaCl and dried in an oven. The experiments were carried out in duplicate.

2.4. Experimental methodology

2.4.1. Plackett-Burman Design

Carbon source concentration ranging from 30 up to 50 g l^{-1} has been defined for xanthan production culture broth in different studies [20]. PBD was used to determine significant factors affecting xanthan production and its viscosity. The experimental range of PBD for each factor was selected on the basis of results obtained from the preliminary experiments carried out using one factor at a time [24]. Twenty four runs in duplicates were carried out. PBD analysis was evaluated by 6 factors in two levels including carbon source: grape juice (30 and 50 g l^{-1}), nitrogen source (1and 3 g l^{-1}), phosphate (2.5, 5 g l^{-1}), agitation rate (150 and 250 rpm), inoculum size (5 and 10%), and initial pH (6.5 and 7.2).

2.4.2. Response surface methodology (RSM)

Optimization was performed by central composite design and desirability function approach using 4 factors in three levels including carbon source (30, 40, 50 g l^{-1}), nitrogen source (0.5, 1.25, 2 g l^{-1}), agitation rate (150, 200, 250 rpm) and inoculum size (5, 10, 15%). The experimental range of central composite design for each factor was selected on the basis of results obtained from preliminary experiments carried out by one factor at a time design [24]. Trials in 56 runs including two replicates for central composite design were carried out.

2.5. Determination of sugar content and residual sugar

The sugar content and residual sugar under optimum conditions were determined by phenol sulfuric acid method. A 2 ml sample was centrifuged for 10 min at 16,000 rpm [15], and the supernatant was used for the determination of sugar concentration. The supernatant was mixed with 1 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of concentrated sulfuric acid was added rapidly to the mixture [23].

2.6. Desirability function

Desirability function is a general and recognized technique to concurrently determine of input variables that can give the optimum presentation levels for response. The desirability 1 is for maximum and desirability 0 is for minimum (or non-desirable) [25].

2.7. Statistical analysis

The experimental design and statistical analysis of the data were performed using MINITAB software (ver. 16.2.0), and the level of significance was 95%.

3. Results and Discussion

3.1. Determination of significant factors by PBD

In the present study, it was shown that growth of *Xanthomonas campestris* strain *b82* occurs in a range of 20-50 g sugar l^{-1}. The growth was restricted in sugar concentrations greater than 60 g l^{-1}. Levels of the substrate concentrations were selected according to our findings and those presented in the literature.

The results from PBD are shown in Table 1. Evaluation of the p-value showed four influencing factors as the most important ones in increasing the xanthan viscosity. These factors include nitrogen source, agitation, inoculum size and carbon source. The latter was found to possess the greatest importance in increasing xanthan production. Analysis of variance for PBD is shown in Table 2.

3.2. Optimization by RSM

The results from RSM according to uncoded values are given in Table 3. Y_1 (xanthan viscosity) and Y_2 (xanthan production) are response values in all experiments. The average amounts of xanthan production and viscosity were 13.03 g l^{-1} and 1008 cP, respectively. Analysis of RSM is shown in Table 4.

3.3. Analysis of experimental data

The equations for xanthan viscosity (Y_1) and xanthan production (Y_2) were second order polynomial equations as Eq. 1:

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_4X_4+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2+b_{44}X_4^2+b_{12}X_1X_2+b_{13}X_1X_3+b_{14}X_1X_4+b_{23}X_2X_3+b_{24}X_2X_4+b_{34}X_3X_4 \quad \text{Eq. 1}$$

(b_0: constant, $b_{1, 2, 3}\ldots$: coefficient, X_1: carbon, X_2: nitrogen, X3: agitation, and X_4: inoculum size)

$$Y_1=-4417-91.24X_1+867.01X_2+60.98X_3+136.25X_4+1.14X_1^2-166.42X_2^2-0.14X_3^2-2.14X_4^2-4.15X_1X_2+0.05X_1X_3-0.65X_1X_4-1.15X_2X_3-12.97X_2X_4-0.19X_3X_4 \quad \text{Eq. 2}$$

$$Y_2=-18.9427+0.1589X_1+0.9595X_2+0.2381X_3+1.0924X_4-0.0039X_1^2+0.3720X_2^2-0.0006X_3^2-0.0202X_4^2+0.0267X_1X_2+68.30X_1X_3+22.20X_1X_4+160.95X_2X_3+187.43X_2X_4+159.48X_3X_4 \quad \text{Eq. 3}$$

R^2 value showed variations in experimental response with the regression model [26]. In the present study, these data for xanthan production and viscosity were 0.98 and 0.97, respectively. Adjusted R^2 for Y_1 and Y_2 are 0.96 and 0.98, respectively, indicating that the model is good and thus capable of explaining 98 % (Y_1) and 97% of the variations in response and (Y_2). The F-value and p-value showed that all selected factors and their interactions were significant ($p<0.05$). The ANOVA analysis of the optimization study indicated the significance of the model ($p<0.05$) (Table 4). Model F-value for Y_1 and Y_2 was 99.34 and 195.10, and lack of fit for Y_1 and Y_2 was 1.54 and 3.31, respectively. The non-significant lack of fit indicates that the models are fit. Lack of fit indicates the error in the description model and that the p-value more than 0.05 for lack of fit represents a good model. P-values for lack of fit in Y_1 and Y_2 were 0.31 and 0.069, respectively, which are more than 0.05 ($p>0.05$). These results show the sufficient validation of the model.

3.4. Estimated residual sugars

At the end of fermentation, residual sugar under 40 g l^{-1} grape juice concentrate (equivalent to 35 g sugar l^{-1}), 1.25 g l^{-1} nitrogen source, 200 rpm, and 10% inoculum size was 15 g l^{-1}.

Validity evaluation was assayed under optimum conditions, in triplicate. The results confirmed the suitability of the model as well as the results of desirability function assay.

3.5. Optimum range of xanthan production and viscosity

Contour plot of xanthan viscosity and production optimization by RSM are shown in Figures 1 and 2, respectively. It is to be noted that in each plot, two factors are constant, and are in the central point, i.e. carbon 40 g l^{-1}, nitrogen 1.25 g l^{-1}, agitation 200 rpm, and inoculum size 10%. The results showed that optimum xanthan viscosity and production occur when all factors are in the central point.

3.6. Desirability function approach

The desirability values for the responses are shown in Figure 3. The number in bracket indicates the optimal level of the related parameter. The desirability value varies between 0 and 1, depending on the closeness of the outputs towards the target.

3.7. Validity evaluation

The magnitude of coefficients showed the importance of factors. The regression Y_1 revealed that nitrogen source is the most effective factor affecting on xanthan viscosity. Nitrogen has negative effect on xanthan viscosity and production. Cadmus and Knutson reported that type and amount of nitrogen source effect on xanthan pyruvate content, and consequently, xanthan viscosity [27].

Enough amount of nitrogen is necessary for biomass production and bacterial growth but when bacterial cells starts xanthan production, the nitrogen source no longer plays a role in xanthan production as it does not take part in xanthan structure and can cause negative effect on xanthan viscosity and production by promoting growth and inhibition of xanthan.

The regression Y_2 showed that inoculum size is the most effective factor in xanthan production, and by increasing the inoculum size, xanthan production and viscosity could be increased.

Table 1. Plackett-Burman Design and response for xanthan gum production after 72 h of fermentation for *Xanthomonas campestris*

Run number	Carbon source, g l^{-1}	Nitrogen source, g l^{-1}	PO_4, g l^{-1}	Agitation Rate, rpm	Inoculm size, %	Initial pH	Xanthan Viscosity, cP	Xanthan production, g l^{-1}	Yield: Product: carbon, %	Productivity, g l^{-1} h^{-1}
1	30	3	2.5	150	5	7.2	455	11.00	36	0.15
2	30	1	2.5	150	5	6.5	1105	12.75	42	0.18
3	30	3	5.0	150	10	6.5	723	13.40	44	0.19
4	30	1	2.5	250	10	7.2	1209	13.60	45	0.19
5	30	3	5.0	150	10	6.5	830	12.90	43	0.18
6	50	3	5.0	150	10	7.2	639	9.40	18	0.13
7	50	1	5.0	150	5	6.5	268	9.60	19	0.13
8	50	1	5.0	250	5	7.2	770	11.40	22	0.15
9	30	3	5.0	250	5	7.2	491	12.90	43	0.18
10	50	1	2.5	150	10	7.2	617	9.80	19	0.14
11	30	1	2.5	250	10	7.2	1237	14.50	48	0.20
12	50	3	2.5	250	5	6.5	918	14.00	28	0.19
13	30	1	5.0	250	10	6.5	1035	9.00	30	0.12
14	50	1	2.5	150	10	7.2	667	9.80	19	0.14
15	50	1	5.0	250	5	7.2	770	11.40	22	0.16
16	30	3	2.5	150	5	7.2	432	11.40	38	0.16
17	50	3	2.5	250	10	6.5	1087	14.50	29	0.20
18	50	1	5.0	150	5	6.5	268	9.60	19	0.13
19	50	3	2.5	250	5	7.2	483	9.50	19	0.13
20	30	3	5.0	250	5	6.5	586	12.50	41	0.17
21	50	3	2.5	250	10	6.5	483	7.30	14	0.10
22	30	1	5.0	250	10	6.5	1151	13.16	43	0.18
23	50	3	5.0	150	10	7.2	692	9.40	18	0.13
24	30	1	2.5	150	5	6.5	1033	12.00	40	0.17

In PBD for xanthan production, only carbon source was the most significant factor but, in RSM, all of the four factors including carbon source, nitrogen source, agitation and inoculum size were significant; it was because of the different number of levels studied in PBD and RSM (i.e. using 2 levels against 3 levels).

In this study, carbon source was significant in xanthan production, and the mentioned four factors were significant in xanthan viscosity; thus, we selected all of these significant factors for optimization by RSM and desirability function assay.

Leela 3. reported that lower concentrations of glucose in fermentation media were not sufficient to give maximum cell growth. Although high concentrations of glucose had no adverse effect on growth, there was no enhancing in xanthan production with the increase of glucose concentration, possibly due to the reciprocal effect of catabolite repression. Higher concentrations of glucose were inhibitory to xanthan production [28]. Consistent with the present study, the optimum levels of xanthan production were obtained at 40 g l^{-1} grape juice, and 30 g l^{-1} or 50 g l^{-1} had not suitable xanthan production efficiency. Agitation is a significant factor in the batch fermentation of *Xanthomonas campestris.*

The valuable effects of increased agitation have been qualified by some investigators to a thinning slime layer, enhancing this way the transfer of nutrients and oxygen for xanthan formation. Agitation effects include both hydrodynamic shear and better aeration [29]. This could explain the different values of xanthan production at various speeds of agitation.

Maximum xanthan production was obtained at 200 rpm agitation rate. More and less agitation rate had no suitable effect on xanthan gum production.

The increased amount of inoculum hadn't positive effect on xanthan production. [28]. Maximum xanthan production, in this study, was obtained at 10% inoculum size. During the microbial fermentation, the nitrogen source was just needed to organize the growth conditions and produce enzymes used in the synthesis of biological xanthan production .

Among the synthesized bacterial exopolysaccharide, xanthan production is greater in the media containing higher ratios of carbon to nitrogen [30]. Cell concentration and xanthan concentration continued to increase when adequate nitrogen and carbon were supplied [31].

According to the desirability func-tion, the optimum xanthan production was obtained in 0.69 g l^{-1} of the nitrogen source. The results of the present study are in agreement with previous reports [20,32-34] showing that enough concentration of nitrogen source has a positive effect on xanthan production [34].

The results of the present study are in agreement with previous reports [20,32-34] showing that enough concentration of nitrogen source has a positive effect

Table 2. Analysis of variance in Plackett-Burman Design

Factors	Xanthan production		Xanthan viscosity	
	F-value	p-value	F-value	p-value
Carbon source	6.29	0.007	6.40	0.022
Nitrogen source	4.86	0.023	0.03	0.866
Inoculum size	7.12	0.016	0.02	0.891
Initial pH	0.67	0.425	0.00	0.948
Phosphorsource	2.03	0.172	0.35	0.561
Agitation rate	5.66	0.029	1.89	0.188

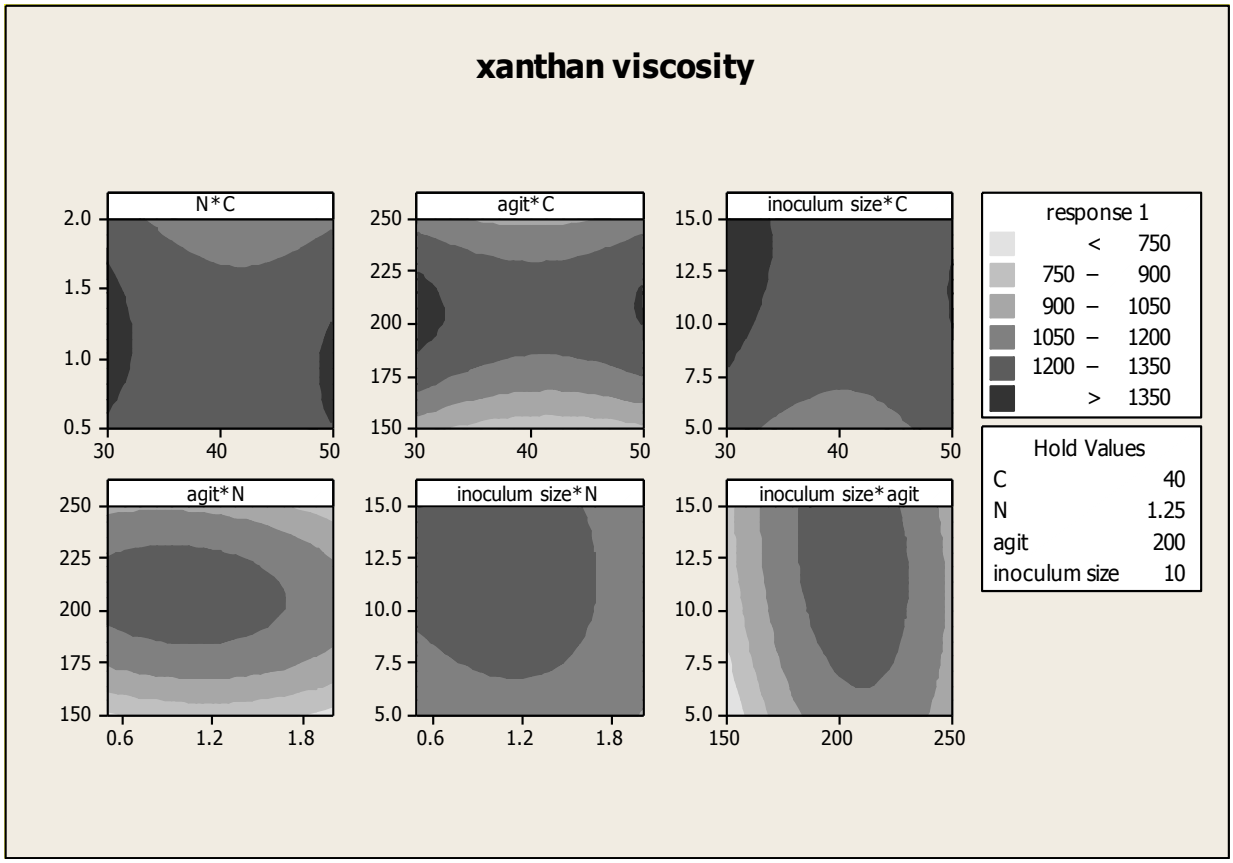

Figure 1. Contour plot of xanthan viscosity optimization by RSM. In each plot, two factors are varied, and two factors held constant at the central point. Two factors that have been written above each plot are variable factors

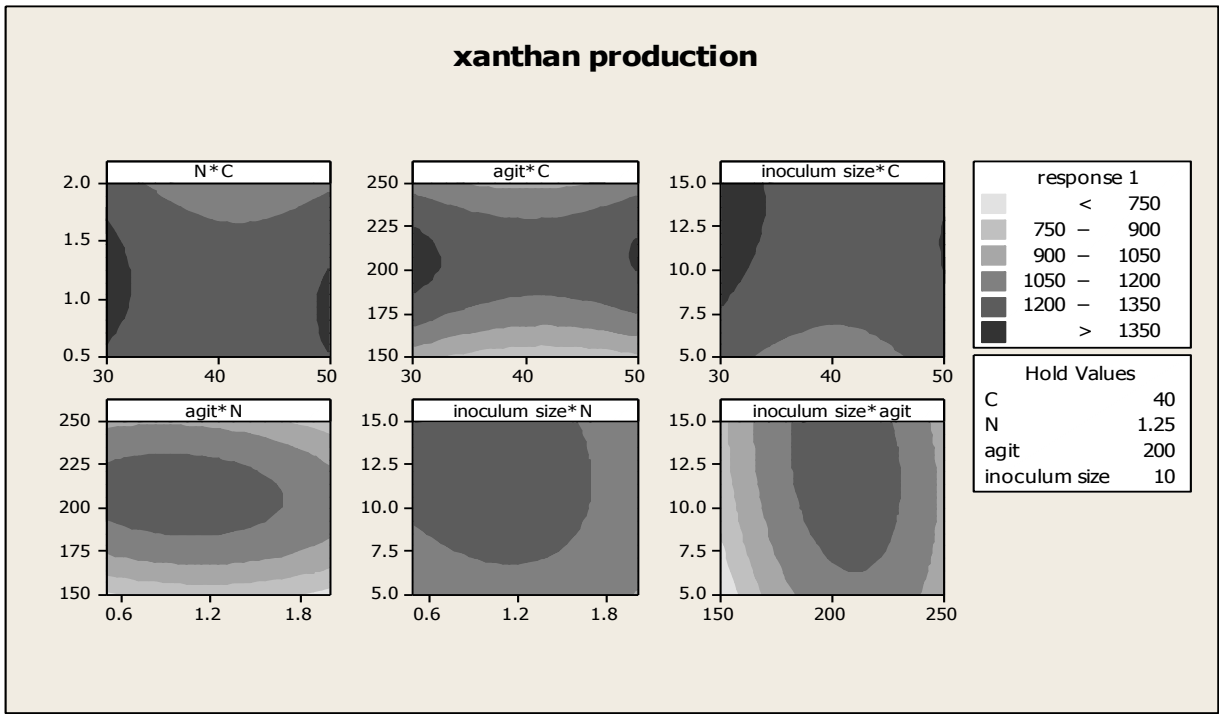

Figure 2. Contour plot of xanthan production optimization by RSM. In each plot, two factors are varied, and two factors held constant at the central point. Two factors that have been written above each plot are variable factors

Table 3. Central composite design with responses for xanthan gum production after 72 h of fermentation for *Xanthomonas campestris*

Run number	Carbon source, g l^{-1}	Nitrogen source, g l^{-1}	PO_4, g l^{-1}	Agitation Rate, rpm	Inoculm size, %	Initial pH	Xanthan Viscosity, cP	Xanthan production, g l^{-1}
1	50	2.00	150	5	689	11.25	22	0.15
2	40	1.25	200	10	1300	14.39	35	0.19
3	50	0.50	250	5	1121	12.85	25	0.17
4	30	0.50	150	5	617	11.21	37	0.15
5	50	2.00	150	15	668	11.80	39	0.16
6	50	0.50	150	5	605	9.88	19	0.13
7	40	0.50	200	10	1198	14.70	36	0.20
8	40	2.00	200	10	1100	14.49	36	0.20
9	40	1.25	250	10	983	13.30	33	0.18
10	30	2.00	250	5	844	12.10	40	0.16
11	40	1.25	150	10	800	12.46	31	0.17
12	40	1.25	200	15	1263	14.12	35	0.19
13	40	1.25	200	10	1273	14.30	35	0.19
14	40	1.25	200	10	1204	14.38	35	0.19
15	30	2.00	150	5	690	11.86	39	0.16
16	40	1.25	200	5	1150	13.34	33	0.18
17	30	0.50	250	15	1086	14.39	47	0.19
18	30	0.50	150	15	1002	14.33	47	0.19
19	30	2.00	150	15	900	12.80	42	0.17
20	30	2.00	250	15	960	11.77	39	0.16
21	50	2.00	250	15	733	11.61	23	0.16
22	50	1.25	200	10	1450	13.60	27	0.18
23	30	1.25	200	10	1381	14.32	47	0.19
24	30	0.50	250	5	977	13.12	43	0.18
25	50	0.50	150	15	902	11.99	23	0.16
26	50	0.50	250	15	1201	13.66	27	0.18
27	50	2.00	250	5	949	12.30	24	0.17
28	40	1.25	200	10	1210	14.50	36	0.2
29	40	1.25	150	10	780	12.60	31	0.17
30	40	1.25	200	10	1261	14.40	36	0.2
31	40	0.50	200	10	1208	14.83	37	0.2
32	30	2.00	250	5	940	12.35	41	0.17
33	40	2.00	200	10	1135	14.25	35	0.19
34	50	2.00	150	5	689	11.20	22	0.15
35	50	2.00	250	5	845	12.60	25	0.17
36	50	2.00	150	15	672	11.90	23	0.16
37	50	0.50	250	5	1005	12.94	25	0.17
38	40	1.25	200	10	1320	14.38	35	0.19
39	40	1.25	200	10	1280	14.36	35	0.19
40	50	0.50	250	15	1120	13.20	26	0.18
41	50	1.25	200	10	1320	14.01	28	0.19
42	40	1.25	200	10	1301	14.13	35	0.19
43	30	0.50	150	15	1001	14.31	47	0.19
44	40	1.25	200	15	1260	14.50	36	0.2
45	30	2.00	150	15	960	12.85	42	0.17
46	30	1.25	200	10	1321	13.96	46	0.19
47	50	0.50	150	15	900	11.90	23	0.16
48	40	1.25	250	10	1018	13.55	33	0.18
49	50	0.50	150	5	583	9.50	19	0.13
50	30	2.00	150	5	733	12.01	40	0.16
51	50	2.00	250	15	780	11.30	22	0.15
52	30	0.5	150	5	617	11.15	37	0.15
53	30	2.00	250	15	935	11.83	39	0.16
54	30	0.50	250	15	1088	14.34	47	0.19
55	30	0.50	250	5	990	13.10	43	0.18
56	40	1.25	200	5	1128	13.45	33	0.18

Table 4. Estimated coefficients of multiple determinations (R^2) for xanthan production and viscosity using uncoded values

Terms	Xanthan viscosity			Xanthan production		
	Coefficient	F-value	p-value	Coefficient	F-value	p-value
Constant	-4417.00	-	0.000	-18.9427	-	0.000
Carbon source	-91.24	30.07	0.000	0.1589	5.73	0.021
Nitrogen source	867.01	60.72	0.000	0.9595	4.67	0.037
Agitation	60.98	335.77	0.000	0.2381	321.52	0.000
Inoculum size	136.25	52.27	0.000	1.0924	211.07	0.000
Carbon × Carbon	1.14	31.97	0.000	-0.0039	22.90	0.000
Nitrogen × Nitrogen	-166.42	21.47	0.000	0.3720	6.74	0.013
Agitation × Agitation	-0.14	315.03	0.000	-0.0006	293.39	0.000
Inoculum size × Inoculum size	-2.14	7.04	0.011	-0.0202	39.36	0.000
Carbon × Nitrogen	-4.15	14.72	0.000	0.0267	38.42	0.000
Carbon × Agitation	0.05	8.26	0.006	0.0005	68.30	0.000
Carbon × Inoculum size	-0.65	15.87	0.000	-0.0031	22.20	0.000
Nitrogen × Agitation	-1.15	28.10	0.000	-0.0109	160.95	0.000
Nitrogen × Inoculum size	-12.97	35.93	0.000	-0.1182	187.43	0.000
Agitation × Inoculum size	-0.19	35.65	0.000	-0.0016	159.48	0.000
Lack- of- fit	-	1.54	0.31	-	3.31	0.069

Table 5. Different carbon sources for xanthan production

Substrate	Xanthan production g l^{-1}	Yield: product to source%	Fermentation process	Reference
Palm date	43.35	0.51	Flask culture	[13]
Whey	16.4	0.42	Flask culture	[17]
Molasses	53	0.30	Flask culture	[16]
Waste sugar beet pulp	20	0.77	Flask culture &solid state	[36]
Melon waste	1.3	1.30	Flask culture	[18]
Sucrose	12.74	0.42	Flask culture	[15]
Grape juice	14.35	0.35	Flask culture	Present study

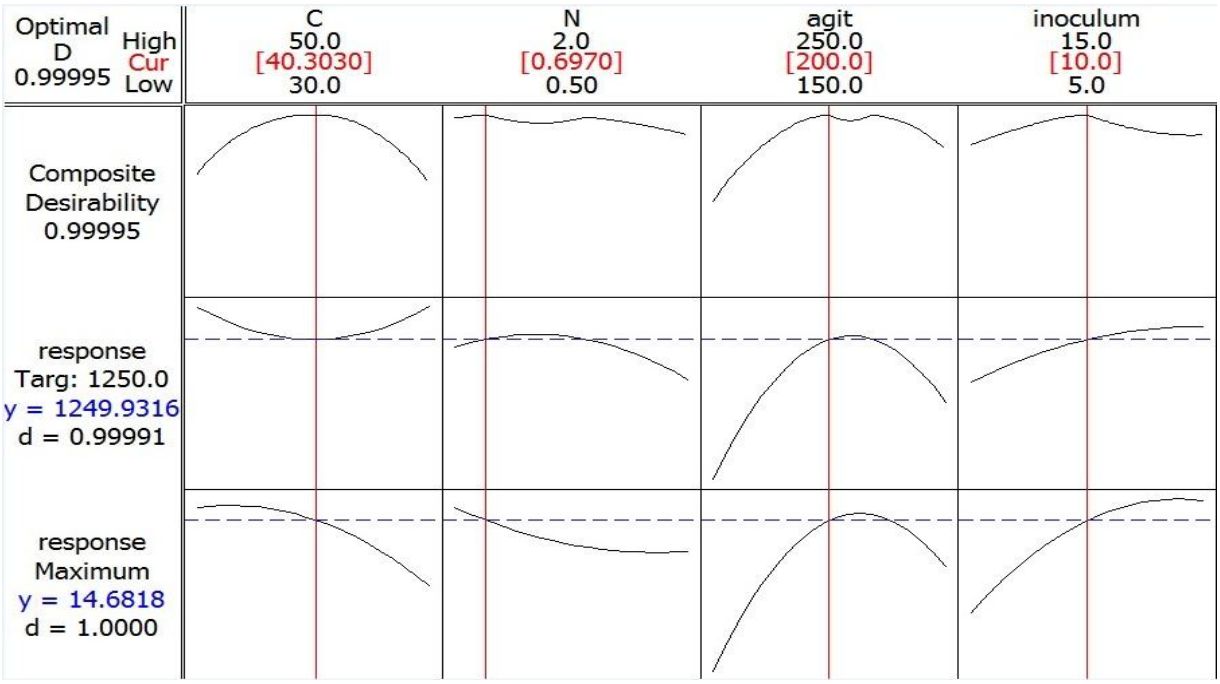

Figure 3. Maximized desirability for response 2 and target desirability for response 1 were presented. The number in bracket indicates the optimal level of the related parameter. The desirability value varies between 0 and 1 depending on the closeness of the outputs towards the target.

on xanthan production [34].

Cadmus and Knutson reported that organic nitrogen sources are disadvantageous in that they are not constantly available, and sometimes, fail to stimulate production of high-pyruvate polysaccharides. They further decrease the gum feature by means of their residual insoluble and dark coloration. The purification procedures for obtaining an acceptable product from this gum are complicated and costly. Inorganic ammonium nitrate is proper substitute for the organic nitrogen source. Although this was a rather successful way, both polysaccharide yields and pyruvate content were undesirably low. They suggested diammonium phosphate as a nitrogen source for xanthan production. This inorganic source could increase production of high-pyruvate polysaccharides [27]. There are several reports about using different substrates for xanthan production (Table 5).

4. Conclusions

The present study showed that grape juice concentrate can be used as carbon source for xanthan production by *Xanthomonas campestris.* Optimum conditions for increasing xanthan production and viscosity were developed by RSM. Primary experiments were carried out by PBD for determining the significant factors. The results also indicated that carbon source, nitrogen source, agitation and inoculum size were important factors in xanthan production and viscosity.

The current work investigated the possibility of using grape juice concentrate as substrate for xanthan gum production by *Xanthomonas campestris*. The influence of nitrogen source, agitation rate, inoculum size and carbon source was determined using RSM. The average of xanthan gum production and viscosity under optimized conditions was recorded as 14.35 g l^{-1} and 1268 cP, respectively.

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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