Indian Journal of Chemical Technology
Vol. 23, September 2016, pp. 353-361

Optimization of fermentation media for xanthan gum production from *Xanthomonas campestris* using Response Surface Methodology and Artificial Neural Network techniques

Selvi Velu*, Vijayagopal Velayutham & Sathiyamoorthy Manickkam[1]

Department of Chemical Engineering, Annamalai University, Annamalai Nagar 608 002 Tamilnadu, India.

[1]Department of Chemical Engineering, Defence University, Debrezeit, Ethiopia

E-mail: selviraja26@yahoo.in

Received 20 March 2014; accepted 3 August 2015

Xanthan gum produced by the bacterium ***Xanthomonas campestris*** ATCC 29497(NCIM 5028) using synthetic substrate has been studied. The nutritional requirements for xanthan gum production have been optimized using Response Surface Methodology (RSM) and Artificial Neural Network. The medium components considered are glucose, yeast extract, peptone, malt extract, KH_2PO_4, $MgSO_4.7H_2O$, Citric acid, $(NH_4)_2SO_4$, H_3BO_3, $ZnCl_2$, $CaCO_3$, Na_2SO_4, $FeCl_3.6H_2O$, NH_4NO_3 and $MgCl_2$. Initial screening using Plackett-Burman statistical design identified the following five components glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$, and $FeCl_3.6H_2O$ as significantly influencing the xanthan gum production. RSM-Central Composite Design has been applied to determine the mutual interactions between these five media components and its optimal levels for xanthan gum production. The optimal concentrations for enhanced production of xanthan gum are found to be: glucose, 40.72g/L; peptone, 9.84g/L; KH_2PO_4, 4.976 g/L; $(NH_4)_2SO_4$, 3.024 g/L and $FeCl_3.6H_2O$, 0.1134 g/L. Artificial neural network (ANN) is employed for the modelling of xanthan gum production by ***Xanthomonas campestris***. A feed forward back propagation neural network tool is used to optimize media components for xanthan gum production. ANN predicted and RSM predicted values are compared with the experimental values.

Keywords: Artificial neural network, Batch fermentation, FTIR, Response surface methodology, ***Xanthomonas campestris***, Xanthan gum

Xanthan gum is an important commercial biopolymer. It was discovered in the 1950s at the Northern Regional Research Laboratories (NRRL) of the United States Department of Agriculture[1]. Xanthan is composed of pentasaccharide repeating units, containing d-glucose, d-mannose, d-glucoronic acid (at a ratio 2:2:1), acetal-linked pyruvic acid and d-acetyl groups[2].

Xanthan has excellent solubility and stability under both acidic and alkaline conditions. It is a water-soluble hetero-polysaccharide[3]. It is widely used in a broad range of industries, such as in toiletries, oil recovery, cosmetics, as water-based paints, etc., due to its superior rheological properties and is also used as a rheological control agent in aqueous systems and as stabilizer for emulsions and suspensions. The annual worldwide production of xanthan gum is 30,000 tons. Xanthan gum is also used in food industries as thickening, suspending and stabilizing agent[4].

It is produced economically by gram-negative bacteria of the genus ***Xanthomonas***. This polysaccharide produced by the bacterium ***Xanthomonas campestris*** ATCC 29497(NCIM 5028) was expansively studied[5]. ***Xanthomonas campestris*** cells are gram-negative, aerobic, straight rods (usually 0.4–0.7 wide × 0.7–1.8 µm long) with a single polar flagellum. Colonies are usually yellow, smooth and butyrous or viscid. It can be cultured at different temperatures ranging between 25 to 35°C in neutral ***p***H[6].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques widely used to determine the effects of several variables and has already used to optimize different bioconversion processes. Plackett–Burman (PB) design tool of RSM has been reported previously for the screening of media components in biological conversions[7]. But this tool cannot determine the exact quantity but can provide suggestion and propensity regarding the necessity of each factor in relatively few experiments, where as a foundation experiment conducted by Plackett–Burman design had been performed prior to the present investigation[8].

Artificial neural networks (ANN) have emerged as an attractive tool for developing non-linear empirical models especially in situations where the development of conventional empirical models becomes impractical. The most widely utilized ANN paradigm is the feed forward back propagation neural network (newff). The first layer has weights coming from the input. Each subsequent layer has a weight coming from the previous layer and the last layer is the network output[9].

In the present study, optimization of xanthan gum production by ***Xanthomonas campestris*** in batch experiments was endeavoured using response surface methodology. Media composition for the production of xanthan was screened using plackett-Burman design and its corresponding concentrations were optimized by central composite design combined with RSM.

Experimental Section

Microorganism, growth conditions and inoculums preparation

Xanthomonas campestris ATCC 29497 (NCIM 5028) was obtained from the National collection of industrial microorganisms, Pune, India. It was cultivated in MGYP medium containing (g/l); glucose 10, peptone 5, yeast extract 3, malt extract 3 and agar 20. The inoculums was prepared in MGYP medium without agar and incubated for 24 h at 30°C. Sub culturing was carried out once in every 2 weeks and culture was stored at 4°C in refrigerator.

Production media and culture conditions

Production medium consist of (g/L); glucose, 40.72, peptone, 9.84, KH_2PO_4, 4.976, $(NH_4)_2SO_4$, 3.024 and $FeCl_3.6H_2O$, 0.1134. The initial ***p***H of the medium was adjusted to 7.0 with 1 N NaOH and incubated for 4 days at 30°C in an orbital shaker.

Fermentations

Experiments were carried out in 500 mL Erlenmeyer flasks containing 90 mL of medium and 10 mL of the inoculums (grown for 24 h on MGYP medium). The ***p***H of the culture medium was adjusted to 7 before sterilization and was not controlled throughout. Production medium was incubated for 4 days at 30°C in an orbital shaker at 200 rpm (constant agitation was maintained throughout the experiments). The constant airflow rate of 1 vvm was maintained.

Determination of bacterial growth

Biomass determination was done by dry cell-weight estimations. The cells were collected after centrifugation at 5000 rpm for 10 min. After discarding the supernatant, the biomass was washed with distilled water and re-centrifuged. Cells were dried in an oven at 65°C for 2 h and weighted.

Determination of xanthan gum concentration

After fermentation, 10 mL of broth sample was centrifuged at 10000 rpm for 30 min at 4°C. Xanthan gum in the supernatant was precipitated using absolute ethanol (1:2 v/v). The solution was maintained at 0°C for 24 h and re-centrifuged at 10000 rpm, for 15 min at 4°C. The precipitate was diluted in distilled water[10]. Xanthan samples were subjected to repeated precipitations (twice) for further purification. Then the polysaccharide was diluted in distilled water and lyophilized using lyophilizer (-80°C, Southern Scientific Instruments, India).

Spectroscopy of Fourier transform infrared (FTIR)

Fourier transform infrared spectroscopic analysis was performed at the Facility of Chemical Engineering at Annamalai University, Tamil Nadu, India. Samples of commercial xanthan gum (CX) and produced xanthan gum (PX) were analysed using a Fourier Transform Infrared Spectrophotometer (ALPHA, Germany) in the spectral window of 400 to 4000 waves/cm using KBR pellets.

Experimental methodology

Response surface methodology was selected for the present study to maximize xanthan production. The Plackett–Burman experimental design was used to evaluate the relative importance of various nutrients for xanthan gum production by commercial software Minitab 16. The fifteen factors selected based on some correlative literature and on our own experience gained had been screened, they are: glucose, yeast extract, peptone, malt extract, KH_2PO_4, $MgSO_4.7H_2O$, Citric acid, $(NH_4)_2SO_4$, H_3BO_3, $ZnCl_2$, $CaCO_3$, Na_2SO_4, $FeCl_3.6H_2O$, NH_4NO_3 and $MgCl_2$. Through the initial screening experiments glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$ and $FeCl_3.6H_2O$, variables have proved to be the significant factors affecting the yield of xanthan gum. The major factors mentioned above were further investigated by Central Composite Design (CCD) of the software Minitab 16.

The Plackett–Burman experimental design with twenty experimental runs for screening media components is shown in Table 1. Each variable is represented at two levels, a high level denoted by (+1) and a low level designated by (-1). The Plackett–

Burman considers insignificant dummy variables, whose number should be one-third of all variables. The dummy variables, which are not assigned any values, experiments are performed at various combinations of high and low values of the process variables and analyzed for their effect on the process[11].

The effect of five variables (glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$ and $FeCl_3.6H_2O$) on xanthan production was studied using a central composite design at five experimental levels: −a, −1, 0, +1, +a where a = $2^{n/4}$; n is the number of variables and 0 corresponds to the central point[12]. The experimental levels of these variables were selected on the basis of our preliminary experimental work, which indicated that an optimum could be found within the level of the parameters used in this study. Table 2 shows the variables and their coded levels used in this study. The actual level of each factor was calculated using the following equation:

$$\textit{Coded value} = \frac{\textit{actual level} - (\textit{high level} + \textit{low level})/2}{(\textit{high level} - \textit{low level})/2} \quad ...(1)$$

Fifty two experiments were carried out each at five levels (Table 3) in a batch experiment. Xanthan gum concentration was analyzed using second-order polynomial equation and the data were fitted to the equation by multiple regression. The model equation for the analysis is given below:

Table 1 — Level and code of variables chosen for central composite design

Variable (g/L)	Symbols		Coded levels				
	Uncoded	Coded	−2.378	−1	0	1	+2.378
Glucose	X_1	A	20	30	40	50	60
Peptone	X_2	B	6	8	10	12	14
KH_2PO_4	X_3	C	3	4	5	6	7
$(NH_4)_2SO_4$	X_4	D	1	2	3	4	5
$FeCl_3.6H_2O$	X_5	E	0.08	0.09	0.1	0.10	0.11

Table 2 — Plackett–Burman experimental design matrix for screening of media components for xanthan gum production using ***Xanthomonas Campestris sp***

S.No	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	Xanthan gum yield (g/L)	
																Experimental	Predicted
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	2.6	2.45
2	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	5.8	5.95
3	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	11.6	11.96
4	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	9.1	9.2
5	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	8.2	8.19
6	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	9.4	9.65
7	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	10.5	10.3
8	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	12.3	11.99
9	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	12.9	13.21
10	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	11.3	11.31
11	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	7.4	7.36
12	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	7.2	7.1
13	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	6.9	6.89
14	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	12	11.75
15	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	11.2	10.89
16	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	12.6	12.64
17	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	14.8	14.65
18	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	6.4	6.76
19	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	11	10.75
20	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	13.9	14.1

Table 3 — Central composite design (CCD) of five variables with experimental and predicted values of xanthan gum

S.No	A	B	C	D	E	Xanthan gum yield (g/L)		
						Experimental	Predicted	ANN- Predicted
1	1	1	-1	1	-1	9.7	10.20964	9.7013
2	-1	-1	1	1	1	10.5	10.68671	13.3059
3	-1	-1	1	-1	-1	9.7	9.605318	9.6905
4	1	-1	-1	1	1	12.5	12.48238	10.7776
5	0	0	0	0	0	16.5	16.44085	16.4833
6	-1	1	-1	1	-1	10	9.836951	9.1197
7	-1	-1	-1	-1	-1	9.1	9.378291	9.0733
8	1	-1	1	1	1	11	11.1344	10.5256
9	-1	1	1	1	1	10.3	10.53072	10.2987
10	1	1	1	-1	1	11.2	11.13876	13.0526
11	-1	1	1	-1	1	10.8	11.04107	10.7887
12	1	-1	1	1	-1	11	11.16766	13.9841
13	0	0	0	0	2.378	12.5	12.11824	12.4955
14	0	0	0	0	0	16.5	16.44085	16.4833
15	0	0	0	-2.378	0	13	12.47155	12.9662
16	0	0	0	0	0	16.5	16.44085	16.4833
17	1	-1	-1	1	-1	11.9	11.79063	11.8852
18	0	0	0	2.378	0	13	12.62529	16.3412
19	1	1	1	1	-1	10.5	10.68667	10.5019
20	1	1	-1	1	1	9.9	10.35139	10.4465
21	0	0	-2.378	0	0	12.1	11.36087	12.0904
22	0	-2.378	0	0	0	11.1	10.67032	9.5069
23	-1	-1	1	-1	1	10.2	10.42206	10.1811
24	-1	-1	-1	-1	1	11	10.92004	11.8151
25	1	1	-1	-1	-1	10.7	10.34499	10.6922
26	0	0	0	0	-2.378	11.5	10.9786	12.8513
27	0	0	0	0	0	16.5	16.44085	16.4833
28	0	2.378	0	0	0	10	9.526523	9.1565
29	-1	-1	-1	1	-1	10	10.54294	9.9942
30	1	-1	-1	-1	1	11.3	11.74273	11.9867
31	-1	1	-1	-1	-1	8.8	9.447301	8.8083
32	2.378	0	0	0	0	5.7	5.298248	8.5829
33	0	0	0	0	0	16.5	16.44085	16.4833
34	1	-1	1	-1	1	11.3	11.39475	12.9063
35	1	1	-1	-1	1	10.1	10.38673	10.0993
36	0	0	0	0	0	16.5	16.44085	16.4833
37	1	-1	1	-1	-1	11.4	11.52801	11.3922
38	0	0	0	0	0	16.5	16.44085	16.4833
39	-1	1	1	1	-1	10.1	10.16398	10.0971
40	-1	1	-1	1	1	10.8	10.9287	9.2254
41	0	0	0	0	0	16.5	16.44085	16.4833
42	1	1	1	-1	-1	11.4	11.82202	10.7693
43	0	0	0	0	0	16.5	16.44085	16.4833
44	0	0	2.378	0	0	11.5	11.33597	10.6693
45	-1	1	1	-1	-1	10.7	10.77433	10.69
46	1	-1	-1	-1	-1	10.8	11.15098	9.7531
47	-1	-1	-1	1	1	12	12.18469	11.9928
48	-2.378	0	0	0	0	4.2	3.698594	10.336
49	-1	1	-1	-1	1	10.3	10.43905	10.3137
50	0	0	0	0	0	16.5	16.44085	16.4833
51	1	1	1	1	1	10.3	10.10341	10.3042
52	-1	-1	1	1	-1	9.7	9.769969	9.1109

$$Y = {}_0 + {}_1X_1 + {}_2X_2 + {}_3X_3 + {}_4X_4 + {}_5X_5 + {}_{11}X_1^2 + {}_{22}X_2^2 + {}_{33}X_3^2 + {}_{44}X_4^2 + {}_{55}X_5^2 + {}_{12}X_1X_2 + {}_{13}X_1X_3 + {}_{14}X_1X_4 + {}_{15}X_1X_5 + {}_{23}X_2X_3 = {}_{24}X_2X_4 + {}_{25}X_2X_5 + {}_{34}X_3X_4 + {}_{35}X_3X_5 + {}_{45}X_4X_5 \quad ...(2)$$

where X_1, X_2, X_3, X_4 and X_5 are the levels of the factors and β_1, β_2, β_3, β_4, and β_5 are linear coefficients, β_{11}, β_{22}, β_{33}, β_{44} and β_{55} are quadratic coefficients, and β_{12}, β_{13}, β_{14}, β_{15}, β_{23}, β_{24}, β_{25}, β_{34}, β_{35}, and β_{45} are interactive coefficient estimates with β_0 playing the role of scaling ***constant***. Analysis of variance (ANOVA), and regression analysis were carried out and 3D plots were drawn using the software design expert 8.

Artificial neural network

Neural network feed forward back propagation, which is widely used ANN, consists of three layers namely input, output and hidden layer. Neurons in input layer simply direct input data to the neurons of hidden layer without any processing. The processing in hidden layers consists of collecting the data from previous layer, multiplying them by their corresponding weights, summing the values, putting the results in a nonlinear or linear activation function (f), and finally adding a constant value called bias, mathematically:

$$y_i = \sum_{i=1}^{n} f\left(w_{ij}x_i\right) + b_j \quad ...(3)$$

where x and y are input and output of neuron, respectively, n is number of inputs to the neuron, w_{ij} is the weight of the connection between neuron i and neuron j, and b_j is the bias associated with j^{th} neuron.

In this work, tangent sigmoid transfer function (TANSIG) is used in hidden layer, while a linear transfer function (PURELIN) is applied in the output layer. The two layers of hidden neurons are used in this study. The input layer consists of the nutrients screened in the experimental values of CCD design, and the output layer contains the xanthan gum production.

In this study, experimentally collected data (RSM-CCD) are divided into two groups. Alternative data can be used for training (50%) and testing (50%). The first partition is used to perform the training of the network and the last partition is utilized for estimating the performance of the trained network on new data, which never was seen by the network during the training.

Back-propagation algorithm with the momentum-learning rule is used to implement supervised training of the network. Back propagation is based on searching an error surface using gradient descent for point(s) with minimum error[14]. In this algorithm, training starts with randomly initialized connection weights. The response to each neuron in the output layer is then calculated and is compared with the corresponding desired output. Errors associated with the output neurons are propagated from output layer to the input layer through the hidden layer to modify the weights. Correlation coefficient (R^2) is calculated based on testing data and applied to study the performance of ANN in prediction of xanthan gum production.

Results and Discussion

Screening of medium components for xanthan gum production by *Xanthomonas Csampestris*

The experiments are carried out based on Plackett–Burman design and the results obtained are given in Table 2. From the table, it is observed that there is a broad deviation in xanthan gum production. This deviation reflected the importance of optimization to attain higher productivity. From the Pareto chart (Fig. 1) the nutrients glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$ and $FeCl_3.6H_2O$ are found to be significant for the production of xanthan gum by ***Xanthomonas campestris***. Hence these nutrients are selected for further optimization using CCD design to maximize the production of xanthan gum.

Optimization of screened medium constituents for xanthan gum production by *Xanthomonas Campestris*

Using CCD method a total of Fifty two experiments were conducted using the media components glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$ and $FeCl_3.6H_2O$. Fifty two experiments are performed at different combinations. The experimental and

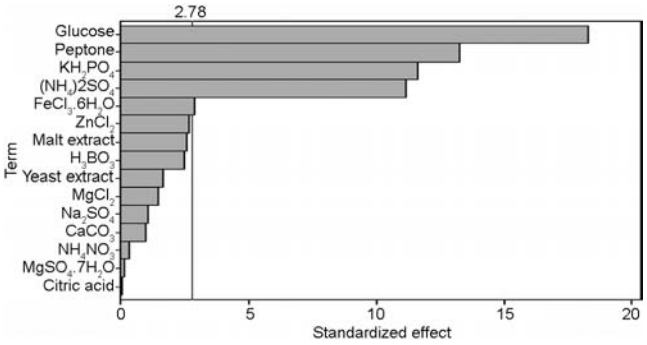

Fig.1 – Pareto chart screening the effect of media components on xanthan gum production using ***Xanthomonas campestris sp***.

predicted values of xanthan gum along with design matrix are presented in Table 3. The theoretical values of xanthan gum were obtained from quadratic model fitting techniques using the software Minitab 15. There was a substantial variation in the xanthan gum depending upon the medium composition. Nearly the centre point conditions resulted in higher xanthan gum than at other levels. The results are analysed by ANOVA. The second order regression equation provides the xanthan gum production as the function of glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$ and $FeCl_3.6H_2O$. This can be presented in terms of coded factors as:

$$Y=16.43 +0.34A -0.24B -0.032D +0.24E-0.22AB +0.038AC -0.13AD -0.24AE +0.27BC -0.19BD -0.14BE -0.25CD -0.18CE +0.25DE -2.11A^2 -1.12B^2 -0.90C^2 -0.69D^2 -0.86E^2 \quad ...(3)$$

where Y is the xanthan gum production (g/l), A, B, C, D and E are concentrations of glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$ and $FeCl_3.6H_2O$ respectively.

ANOVA for the response surface is shown in Table 4. The model F-value of 104.04 implies that the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. Values greater than 0.1 indicate the model terms are not significant. In the present work A, B, E, AB, AE, BC, BD, CD, CE, A^2, B^2, C^2, D^2, E^2 are significant model terms for production of xanthan gum. The coefficient of determination (R^2) for xanthan gum production is calculated as 0.9863, which is very close to 1 and can explain up to 98.63% variability of the response. The predicted R^2 value of 0.9427 is in reasonable agreement with the adjusted R^2 value of 0. 9768. An adequate precision value greater than 4 is desirable. The adequate precision value of 48.770 indicates an adequate signal and suggests that the model can be used to navigate the design space.

Equation 3 can be used to predict the xanthan gum production within the limits of the experimental factors. Figure 2 shows that the actual response values agree well with the predicted response values.

Three dimensional surface plots are drawn to determine the optimum values of the five variables and are shown in Figs. 3a to 3j. The three dimensional surface plot shown in Fig. 3(a) explains the interactive effect of glucose and peptone on the production of xanthan gum. The maximum value of xanthan gum

Table 4 — Analysis of Variance (ANOVA) for response surface quadratic model for the production of xanthan gum

Source	Coefficient factor	Sum of squares	DF	F	P > F
Model	16.43	337.35	20	104.04	0.0001
A-Glucose	-0.34	4.90	1	30.22	0.0001
B-Peptone	-0.24	2.50	1	15.45	0.0005
C-KH2PO4	-0.00523	0.001188	1	0.007326	0.9324
D-(NH4)2SO4	0.032	0.045	1	0.28	0.6013
E-Fecl3.6H2O AB	0.24	2.49	1	15.34	0.0005
AC	-0.22	1.53	1	9.44	0.0046
AD	0.038	0.045	1	0.28	0.6023
AE	-0.13	0.55	1	3.40	0.0754
BC	-0.24	1.81	1	11.13	0.0023
BD	0.27	2.42	1	14.93	0.0006
BE	-0.19	1.20	1	7.41	0.0109
CD	-0.14	0.60	1	3.73	0.0632
CE	-0.25	2.00	1	12.34	0.0015
DE	-0.18	1.05	1	6.48	0.0165
A^2	0.025	0.020	1	0.12	0.7280
B^2	-2.11	247.04	1	1523.74	0.0001
C^2	-1.12	69.51	1	428.76	0.0001
D^2	-0.90	44.76	1	276.07	0.0001
E^2	-0.69	26.10	1	160.98	0.0001
Residual	-0.86	41.30	1	254.74	0.0001
Lack of fit		4.70	29		
Pure Error		4.70	22		
Cor Total		0.00	7		
		342.05	49		

Std. Dev. = 0.40; R^2 = 98.63%; Mean = 11.51; Adj R^2 = 97.68%; C.V. % = 3.5;
Pred R^2 = 94.27%; Adeq Precision = 48.77

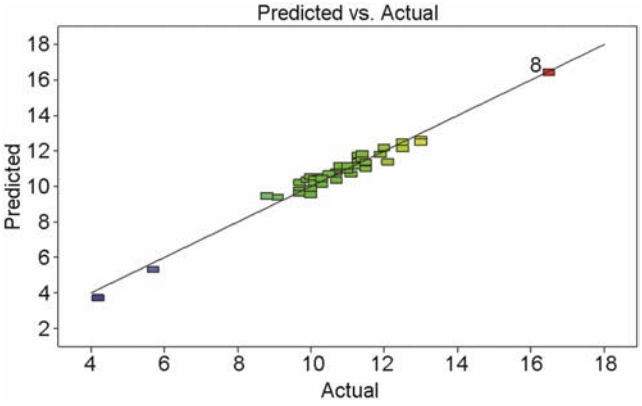

Fig. 2 – Predicted responses versus actual value of xanthan gum production

was obtained between 39 g/L to 40 g/L and 10 g/L to 11 g/L of glucose and peptone respectively.

Figure 3(b) explains the interaction between glucose and KH_2PO_4. The trend observed that maximum production of xanthan gum was obtained between 39 g/L to 40 g/L and 4.9 g/L to 5 g/L of glucose and KH_2PO_4 respectively.

Figure 3(c) explains the interactive effect of glucose and $(NH_4)_2SO_4$ on the production of xanthan gum. The maximum value of xanthan gum was obtained between 39 g/L to 40 g/L and 5 g/L to 5.1 g/L of glucose and $(NH_4)_2SO_4$ respectively.

Figure 3(d) explains the interactive effect of glucose and $FeCl_3.6H_2O$ on the production of xanthan gum. The maximum value of xanthan gum was obtained between 39g/L to 40 g/L and 0.1 g/L to 0.03 g/L of glucose and $FeCl_3.6H_2O$ of glucose and $FeCl_3.6H_2O$ respectively.

Figures 3 (e), (f),(g), (h), (i) and (j) also showed similar trends as that of previous ones. The following components are the optimum values obatained by solving the second degree polynomial equation: glucose, 40.72g/L; peptone, 9.84g/L; KH_2PO_4, 4.976 g/L; $(NH_4)_2SO_4$, 3.024 g/L and $FeCl_3.6H_2O$, 0.1134 g/L. These optimum values were maintained for all further studies.

Artificial neural network based modelling for xanthan gum production by *Xanthomonas campestris*

The data obtained from RSM experiments are used for modelling the xanthan gum production. 50% of the data are used to train the ANN. The model is trained using different combinations of the parameters like so as to achieve maximum determination coefficient values (i.e., 100% correlation between measured and predicted values). This is achieved by a vigorous trial and error approach. Xanthan gum production is predicted using various nutrient concentrations as the input variables. ANN predicted values are compared with remaining 50% data. It can be observed that all the data points for xanthan gum production are predicted accurately by the ANN model. The Absolute Standard Deviation (ABSD) and % Root Mean Square Error (RMSE) for the ANN model are found to be 0.1347 and 3.67% respectively. The correlation coefficient R^2 value is found to be 0.99, which is very close to 1 and can explain up to 99% variability of the model. Figure 4 shows the comparison of predicted values of ANN and RSM with experimental values. From the figure it is clear that, ANN fits the data well to experimental values from RSM.

Spectroscopic analysis (FT-IR)

The Fourier Transform-infrared spectrum (FT-IR) is a methodology to detect similarities or differences in chemical structures of compounds. The functional groups present in commercial xanthan (CX) gum and produced xanthan gum (PX) were analyzed and compared. The region studied included all the spectral bands located in the window between the wave numbers 400 and 4000 cm^{-1}.

The infrared spectra of PX and CX shows that the most important bands recorded in the range of 3500-500 cm^{-1} were: 3400-3450 cm^{-1}: axial deformation of –OH; 2850-2950 cm^{-1}: axial deformation of C–H (may be due to absorption of symmetrical and asymmetrical stretching of CH_3 or even groups of CH_2) and CHO; 1700-1600 cm^{-1}: axial deformation of C=O ester, acid carboxylic, aldehydes and ketones; 1430-1650 cm^{-1}: axial deformation of C=O of enols (β-diketones); 1410-1430 cm^{-1}: deflection angle C–H; and 1050-1100 cm^{-1}: axial deformation of C–O)[15]. The infrared spectrum of the CX is very similar to that obtained for the PX using the strain of ***Xanthomonas campestris*** ATCC 29497(NCIM 5028). Based on the results obtained from FTIR, the remote polysaccharide was found to follow the same spectral behavior as the standard.

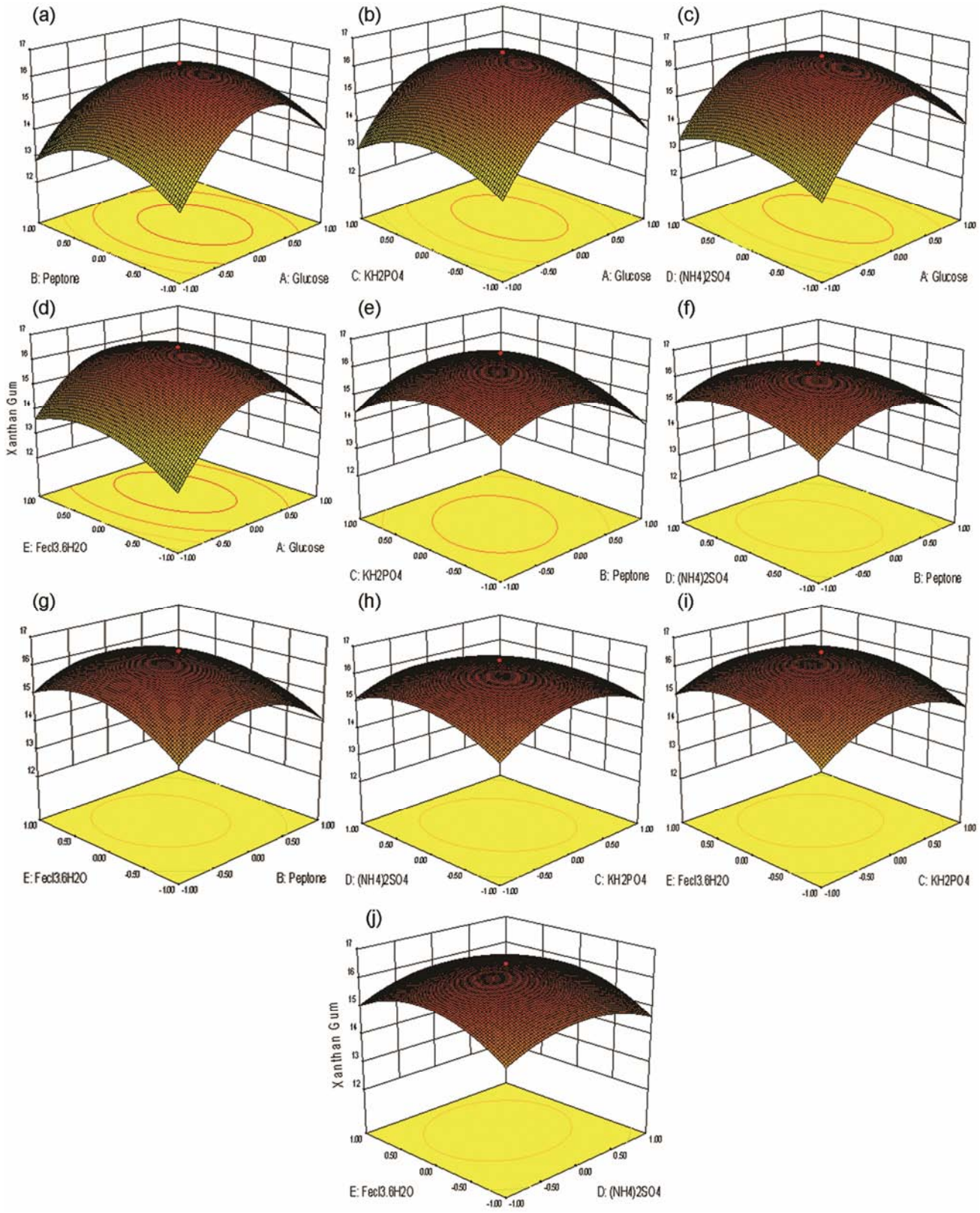

Fig. 3 — (a-j) 3D plots showing the interactive effects between the significant media components on xanthan gum production using ***Xanthomonas campestris sp***.

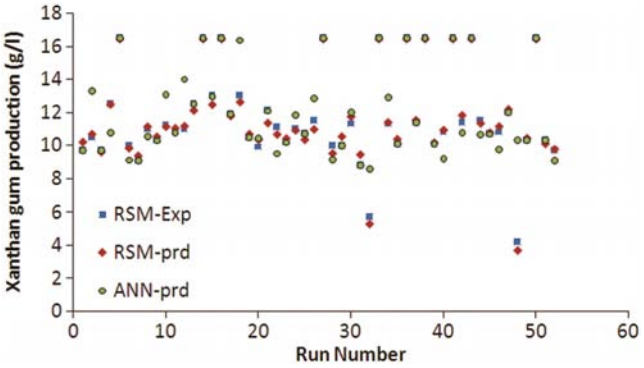

Fig. 4 – Comparison of experimental values with RSM and ANN predicted values for the production of xanthan gum production using ***Xanthomonas campestris sp***

Conclusion

This current work optimized the media composition developed for the production of xanthan gum using the bacterium ***Xanthomonas campestris*** ATCC 29497(NCIM 5028) by response surface methodology and artificial neural network modelling. Plackett–Burman statistical design technique is found to be useful for identifying the most influential components of the system. Using CCD the selected optimal media composition for xanthan gum production (glucose, 40.72g/L; peptone, 9.84g/L; KH_2PO_4, 4.976 g/L; $(NH_4)_2SO_4$, 3.024 g/L and $FeCl_3.6H_2O$, 0.1134 g/L) have been checked and confirmed by supplementary experiments. The experimental yield of xanthan gum was found to be in good agreement with that of predicted (16.5 g/L versus 16.44 g/L, respectively). The ANN model was constructed on the basis of data from experiments. This ANN model was found to possess excellent prediction accuracy and generalization ability (16.5 g/L versus 16.48 g/L respectively). Functional group of the produced xanthan gum is compared with that of the commercial xanthan gum by FTIR spectra.

References

1 Gils P S, Ray D & Sahoo P K, ***Int J Biol Macromol***, 45 (2009) 364.
2 Jansson P E, Kenne L & Lindberg B, ***Carbohydr Res***, 45 (1975) 275.
3 Rosalam S & England R, ***Enz Microbiol Technol***, 39 (2006) 197.
4 Bradford P A, Baid J***, The Polysaccharides***, ***edited by*** G O Aspinall, (Academic Press, New York), 1983, 411.
5 Kurbanoglu E B & Kurbanoglu N I, ***Process Biochem***, 42 (2007) 1146.
6 Bradbury J F, ***Bergey's Manual of Systematic Bacteriology***, vol.1. edited by N R Krieg & J G Holt, (Williams and Wilkins, Balimore), 1984, 199.
7 Plackett R L & Burman J P, ***Biometrika,*** 33 (1946) 305.
8 Moshaf S, Hamidi-Esfahani Z & Azizi M H, ***Appl Food Biotechnol***, 1 (2014) 17.
9 Nandi S, Ghosh S, Tambe S S & Kulkarni B D, ***AIChE J***, 47 (2001) 126.
10 Shu C H & Yang S T, ***Biotechnol Bioengg,*** 35 (1990) 454.
11 Haaland P D, ***Experimental Design in Biotechnology*** (Marcel Dekker, New York), 1989.
12 Khuri A I & Cornell J A, ***Response Surface Methodology***, (ASQC Quality Press, New York), 1987, 116.
13 Rumelhart D, Hinton G & Williams R, ***Nature***, (1986) 323.
14 Freitas F, Alves V D, Pais J, Costa N, Oliveira C & Mafra L, ***Bioresour Technol***, 100 (2009) 859.
15 Sandra F, Carmen L, de Oliveira P & Sérgio Antônio Lemos de Morais, ***Carbohydr Polym***, 86 (2011) 469.