International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.4, pp 2475-2483, July-Aug 2014

Comparative Study of Xanthan Gum production using Syntheic substrate by *Xanthomonas campestris* and Local isolated Strain

Selvi Velu[1,*], Vijayagopal Velayutham[1]

[1]Department of Chemical Engineering, Annamalai University, Chidambaram, Tamilnadu, India.

Corres.author: selviraja26@yahoo.in, vevevin@yahoo.co.in
Tel: +918012011222

Abstract: In the present investigation xanthan gum production by the bacterium *Xanthomonas campestris* (ATCC 29497) and a local isolated strain was compared. The process parameters for xanthan gum production were optimized using Response Surface Methodology and Artificial Neural Network. RSM-Central Composite Design was applied to determine the mutual interactions between the four parameters like pH, temperature, fermentation time and inoculums size and its optimal levels for xanthan gum production using *Xanthomonas campestris* and local isolated strain. The optimum parameter values are found to be pH; 6.98, temperature; 29.88^{o}C, fermentation time; 4.06 day and inoculums size; 9.95ml using *Xanthomonas campestris* and pH; 6.9, temperature; 30.04^{o}C, fermentation time; 4.1 days and inoculums size; 9.3ml using local isolated strain. Artificial neural network (ANN) is also employed for the modelling of xanthan gum production by *Xanthomonas campestris* and local isolated strain. A feed forward back propagation neural network tool is used for the optimization. ANN predicted and RSM predicted values are compared with the experimental values for both organisms.
Keywords: Xanthan gum, *Xanthomonas campestris*, Banana petioles, Response surface methodology, Artificial neural network and FTIR.

1. Introduction

Xanthan gum, presently the most important and second commercialized microbial polysaccharide [1] is produced by microbial fermentation with the bacterium *Xanthomonas campestris* [2]. It was discovered in 1963 at Northern Regional Research Center (now called The National Center for Agricultural Utilization Research) of the United States Department of Agriculture (USDA) [3]. *Xanthomonas campestris* is being used for the production of xanthan gum. *Xanthomonas* cells are gram-negative, aerobic, straight rods (usually 0.4–0.7 µm wide×0.7–1.8µm long) with a single polar flagellum. Colonies are usually yellow, smooth and butyrous or viscid [4]. It can be cultured at different temperatures ranging between 25 and 35°C in neutral pH [5].

Xanthan gum is widely used in a broad range of industries, such as in foods, toiletries, oil recovery, cosmetics, as water-based paints, etc., due to its superior rheological properties and is used as a rheological control agent in aqueous systems and as stabilizer for emulsions and suspensions. The xanthan gum now become one of the widely used ingredients in food product [6 7 8]. Commercial production of xanthan gum uses glucose as the substrate, and generally batch production instead of continuous production due to the batch process having been proven to work successfully. It has been found that the production and the properties of

xanthan gum are influenced by pH, temperature, fermentation time and inoculums size used for xanthan gum production.

The present study was undertaken to compare xanthan gum production by *Xanthomonas campestris* and local isolated strain in batch experiments using response surface methodology where, the instantaneous effects of the four independent variables (pH, temperature, time of fermentation and inoculums size) were investigated for optimum xanthan gum production. Response surface methodology is a very effective and most popular statistical tool to optimize the variables having equal importance and influence on each other in the xanthan gum production. Recently, artificial neural network (ANN) is gaining importance as the most popular artificial learning tool in biotechnology. An artificial neural network (ANN) trained by back propagation algorithm with three layers is used to predict the yield of xanthan gum. The first layer has input neurons, which send data via synapses to the second layer of neurons, and then via more synapses to the third layer of output neurons.

2. Materials and Methods

2.1. Isolation of strain

A local strain was isolated from infected banana petioles. The diseased petioles tissues were cut into small pieces, soaked in 10ml sterile distilled water and 10^{-5} dilution of the suspension was streaked on to the agar plates (10 g/l glucose, 3 g/l yeast extract, 3 g/l malt extract, 5 g/l peptone and 20 g/l agar). The plates were incubated at 30^{o}C for 24h. Individual bacterial colonies developed were purified by re-plating on selected medium and stored at 4^{o}C for further use. The inoculum was prepared in that medium without agar and incubated for 24 h at 30^{o}C.

2.2. *Xanthomonas campestris* - growth conditions and inoculums preparation

Xanthomonas campestris (ATCC 29497) was obtained from the National Collection of Industrial Microorganisms (NCIM), 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 the medium without agar and incubated for 24 h at 30^{o}C. Sub culturing was carried out once in every 2 weeks and culture was stored at 4^{o}C in refrigerator.

2.3. Production media and culture conditions

The production of Xanthan gum was carried out in 500ml Erlenmeyer flasks with 100ml medium containing (g/l) : glucose 40, yeast extract 3, peptone 5, KH_2PO_4 5, $MgSO_4.7H_2O$ 0.2, $(NH4)_2SO_4$ 2.7, citric acid 2, H_3BO_3 0.06, $ZnCl_2$ 0.06 and $CaCO_3$ 0.02. The initial pH of the medium was adjusted to 7.0 with 1 N NaOH and incubated for 4 days at 30°C in an orbital shaker.

2.4. Fermentations

Experiments were carried out in 500ml Erlenmeyer flasks containing 90ml of medium and 10 ml of the inoculums (grown for 24 h on MGYP medium). Production medium was incubated for 4 days at 30°C in an orbital shaker at 200 rpm (constant agitation was maintained throughout the experiments) and a constant airflow rate of 1 vvm was maintained.

2.5. Determination of xanthan gum concentration

Xanthan gum was recovered from the samples by centrifugation at 10000g for 30 minutes at 4^{o}C. Xanthan gum in the supernatant was precipitated using ethanol (1:2 v/v). The solution was maintained at 5^{o}C for 24h and re-centrifuged at 10000g for 30 minutes at 4^{o}C. The centrifuged precipitate was collected which is to be the required product, xanthan gum [9]. The moisture was removed from precipitate by lyophilization.

2.6. Determination of residual sugar concentration

The supernatant of samples centrifuged at 5000 rpm for 10 min was used for the determination of residual sugar concentration by the dinitrosalicylic acid method.

2.7. 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 analyzed by operating in the spectral window from 400 to 4000 waves/cm using KBR pellets.

2.8. Experimental methodology

Central composite factorial plan with four factors and five levels was used to test the effect of significant process parameters such as pH, temperature, fermentation time and inoculums size on xanthan gum production by *Xanthomonas campestris* and local isolated strain using commercial software Minitab 16 [10]. Coded and Uncoded levels and experimental design are given in Table 1. The actual level of each factor was calculated using the following equation:

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

Thirty one experiments were carried out each at five levels (Table 2 & 3) in a batch experiment for the production of xanthan gum using *xanthomonas campestris* and isolated strain. Xanthan gum concentration was analyzed using second-order polynomial equation and the data were fitted to the equation by multiple regressions [11]. The model equation for the analysis is given below:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 \quad (2)$$

where X_1, X_2, X_3 and X_4 are the levels of the factors and β_1, β_2, β_3 and β_4 are linear coefficients, β_{11}, β_{22}, β_{33} and β_{44} are quadratic coefficients, and β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} 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. The design allows to vary the factors simultaneously and to give the individual, interactive and square effects of the parameters associated with the xanthan gum production through a second order polynomial equation.

Table 1 Level and code of variables chosen for Central Composite Design

Variable	Symbols		Coded levels				
	Uncoded	Coded	−2	−1	0	1	2
pH	X_1	A	5	6	7	8	9
Temperature (^oC)	X_2	B	26	28	30	32	34
Fermentation time (day)	X_3	C	2	3	4	5	6
Inoculums size, ml (v/v)	X_4	D	5	7.5	10	12.5	15

2.9. 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.

Table 2. Five level Central-Composite design matrix for the optimization of significant process parameters in Xanthan gum production using *Xanthomonas campestris*

Run No.	pH	Temperature (^oC)	Fermentation time (day)	Inoculums Size, ml (v/v)	Xanthan gum production (g/l)	
					Experimental	Predicted
1	-1	1	-1	-1	6.5	6.204167
2	0	0	0	0	18.3	18.3
3	-1	1	-1	1	7.2	7.191667
4	0	0	0	0	18.3	18.3
5	1	-1	1	-1	7.4	8.025
6	0	0	-2	0	10.3	10.45417
7	-1	-1	-1	-1	6.9	7.208333
8	1	1	1	1	5.8	6.108333
9	0	0	0	0	18.3	18.3
10	0	0	0	0	18.3	18.3
11	1	-1	-1	-1	6.2	6.320833
12	0	2	0	0	4.3	3.654167
13	2	0	0	0	2.5	1.520833
14	-1	-1	-1	1	7.9	8.070833
15	0	0	0	2	8.3	8.220833
16	-1	-1	1	1	7.7	7.375
17	1	-1	1	1	5.5	6.4875
18	1	-1	-1	1	4.9	4.808333
19	0	0	0	-2	10	8.770833
20	-1	1	1	1	6	6.570833
21	0	0	0	0	18.3	18.3
22	0	0	2	0	13	11.5375
23	-2	0	0	0	3.2	2.870833
24	-1	1	1	-1	4.9	5.608333
25	0	0	0	0	18.3	18.3
26	-1	-1	1	-1	5.7	6.5375
27	1	1	-1	-1	4.8	5.741667
28	1	1	-1	1	4.5	4.354167
29	0	0	0	0	18.3	18.3
30	1	1	1	-1	7	7.520833
31	0	-2	0	0	5.7	5.0375

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 [12]. 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.

3. Results and Discussion

3.1. Process parameter optimization using Central-Composite Design for Xanthan gum production using *Xanthomonas campestris*

The design matrix which consists of 31 experimental runs was constructed, in order to arrive at a second order polynomial equation for the prediction of xanthan gum production. The design matrix and their

corresponding experimental and the predicted values are given in Table 2. The experimental results suggest that the maximum values of xanthan gum production were obtained for the runs with the central points. The experimental runs of 2, 4, 9, 10, 21, 25, and 29 produced the highest xanthan gum (18.3 g/l). The results were analyzed using the analysis of variance (ANOVA) and the estimated coefficients are presented in Table 3.

Table 3. Results of the ANOVA of the process parameter optimization data of Xanthan gum production using *Xanthomonas campestris* by Central-Composite design of experiments

Source	Coefficient	Sum of Squares	Degrees of Freedom (DF)	Mean Square	F Value	P-Value Prob > F
Model	18.3	781.43	14	55.82	82.79	0.0001
A-pH	-0.34	2.73	1	2.73	4.06	0.0623
B-Temperature	-0.35	2.87	1	2.87	4.26	0.0568
C-Fermentation time	0.27	1.76	1	1.76	2.61	0.1269
D-Inoculums size	-0.14	0.45	1	0.45	0.67	0.4248
AB	0.11	0.18	1	0.18	0.27	0.6123
AC	0.59	5.64	1	5.64	8.37	0.0112
AD	-0.59	5.64	1	5.64	8.37	0.0112
BC	0.019	0.0056	1	0.0056	0.00834	0.9284
BD	0.031	0.016	1	0.016	0.023	0.881
CD	-0.00625	0.000625	1	0.000625	0.000927	0.9761
A^2	-4.03	444.59	1	444.59	659.47	0.0001
B^2	-3.49	333.8	1	333.8	495.14	0.0001
C^2	-1.83	91.46	1	91.46	135.66	0.0001
D^2	-2.45	164.78	1	164.78	244.42	0.0001
Residual		10.11	15	0.67		
Lack of Fit		10.11	10	1.01		
Pure Error		0.000	5	0.000		
Total		791.55	29			

ANOVA results of the data indicate that the model terms, A, B, AC, AD, A^2, B^2, C^2 and D^2 are significant ($P < 0.05$). Values greater than 0.1000 indicate the model terms are not significant. Thus, it is clear that the linear effects of pH and temperature, square effects of pH, temperature, microbial load and effluent concentration and interactive effects of pH and fermentation time & pH and inoculums size are significant. The model F-value was 82.79. The high F-value and non-significant lack of fit indicate that the model is a good fit. The P-values for the model (<0.0001) from the analysis also suggested that the obtained experimental data was in good fit with the model. The regression equation coefficients were calculated and the data was fitted to a second-order polynomial equation. The xanthan gum production using *xanthomonas campestris* can be expressed in terms of the following regression equation:

$$Y = 18.3 - 0.34A - 0.35B + 0.27C - 0.14D + 0.11AB + 0.59AC - 0.59AD + 0.019BC + 0.031BD - 0.00625CD - 4.03A^2 - 3.49B^2 - 1.83C^2 - 2.45D^2$$

where, A, pH; B, temperature; C, Fermentation Time and D, Microbial Load.

The regression equation obtained from the ANOVA showed that the R^2 (multiple correlation coefficient) value was 0.9872 (a value > 0.1 indicates the fitness of the model). This is an estimate of the fraction of overall variation in the data accounted by the model, and thus the model is capable of explaining 98.72% of the variation in response. The 'adjusted R^2' (0.9753) and the 'predicted R^2' (0.9264) are in reasonable agreement, which indicates that the model is good. The 'adequate precision value' of the present model was 28.9, and this also suggests that the model can be used to navigate the design space. The 'adequate precision value' is an

index of the signal-to-noise ratio, and values of higher than 4 are essential prerequisites for a model to be a good fit.

The following are the optimum values obatained by solving the second degree polynomial equation: pH, 6.98; temperature, 29.88^{o}C; fermentation time, 4.06 days and inoculums size, 9.95 ml. These optimum values were maintained for all further studies.

3.2. 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 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 correlation coefficient R^2 is found to be 0.99, which is very close to 1 and can explain up to 99% variability of the model. Figure 1 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.

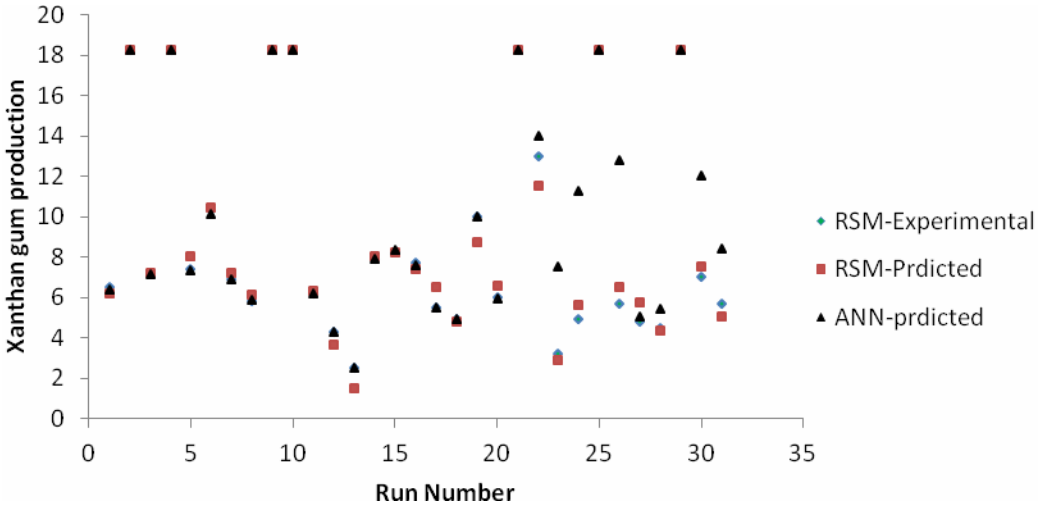

Figure 1: Comparison of experimental values with RSM and ANN predicted values for the production of Xanthan gum production using *xanthomonas campestris*

3.3. Process Parameter Optimization using Central-Composite Design for Xanthan Gum Production by Local Isolated Strain

The design matrix which consists of 31 experimental runs was constructed, in order to arrive at a second order polynomial equation to predict the xanthan gum production system. The design matrix and their corresponding experimental and the predicted values are given in Table 4. The experimental results suggest that the maximum values of xanthan gum production were obtained for the runs with the central points. The experimental runs of 6, 10, 15, 18, 20, 21, and 27 produced the highest xanthan gum production (21.8 g/l). The results were analyzed using the analysis of variance (ANOVA**)** and the estimated coefficients are presented in Table 5.

ANOVA results of the data indicate that the model terms, A, C, D, AB, AC, AD, BC, A^2, B^2, C^2, D^2 are significant ($P < 0.05$). Values greater than 0.1000 indicate the model terms are not significant. Thus, it is clear that the linear effects of pH, fermentation time and inoculums size, square effects of all the terms and interactive effects of pH and temperature, pH and fermentation time, pH and microbial load & temperature and inoculums size are significant. The high F-value (94.01) and non-significant lack of fit indicate that the model is a good fit. The P-values for the model **(**<0.0001) from the analysis also suggested that the obtained experimental data was in good fit with the model. The regression equation coefficients were calculated and the

data was fitted to a second-order polynomial equation. The xanthan gum production using local isolated strain can be expressed in terms of the following regression equation;

$$Y = 18.3–0.63A+0.058B+0.95C–0.725D–0.762AB+0.237AC–0.55AD +0.675BC–0.062BD–0.112CD – 4.245A^2 –4.42B^2 – 1.408C^2 – 1.83D^2 \quad (5)$$

where, A, pH; B, temperature; C, Fermentation Time and D, Microbial Load.

The regression equation obtained from the ANOVA showed that the R^2 (multiple correlation coefficient) value was 0.9981 (a value > 0.1 indicates the fitness of the model). This is an estimate of the fraction of overall variation in the data accounted by the model, and thus the model is capable of explaining 99.81% of the variation in response. The 'adjusted R^2' (0.9963) and the 'predicted R^2' (0.9888) are in reasonable agreement, which indicates that the model is good. The 'adequate precision value' of the present model was 30.018, and this also suggests that the model can be used to navigate the design space. The 'adequate precision value' is an index of the signal-to-noise ratio, and values of higher than 4 are essential prerequisites for a model to be a good fit.

Table 4. Five level Central-Composite design matrix for the optimization of significant process parameters in Xanthan gum production using local isolated strain

Run No.	pH	Temperature (^oC)	Fermentation time (day)	Inoculumn Size, ml (v/v)	Xanthan gum production (g/l)	
					Experimental	Predicted
1	-1	1	1	1	13.0	13.1583
2	-1	1	-1	1	10.5	10.6083
3	-1	-1	1	1	10.4	10.0417
4	1	1	-1	1	6.7	6.2417
5	0	-2	0	0	4.2	4.0000
6	0	0	0	0	21.8	21.8000
7	0	0	2	0	18.0	18.0667
8	1	-1	1	-1	13.0	12.5750
9	0	0	0	-2	18.3	18.5167
10	0	0	0	0	21.8	21.8000
11	1	1	1	1	9.6	9.7417
12	1	-1	1	1	9.5	9.6750
13	0	2	0	0	4.0	4.2333
14	1	-1	-1	1	8.7	8.8750
15	0	0	0	0	21.8	21.8000
16	2	0	0	0	3.4	3.5500
17	-1	1	1	-1	14.1	13.6083
18	0	0	0	0	21.8	21.8000
19	0	0	-2	0	14.3	14.2667
20	0	0	0	0	21.8	21.8000
21	0	0	0	0	21.8	21.8000
22	1	-1	-1	-1	11.2	11.3250
23	-1	-1	-1	-1	10.9	10.4417
24	-2	0	0	0	6.2	6.0833
25	0	0	0	2	15.8	15.6167
26	1	1	1	-1	12.5	12.3917
27	0	0	0	0	21.8	21.8000
28	-1	1	-1	-1	10.5	10.6083
29	-1	-1	-1	1	9.8	10.1917
30	1	1	-1	-1	8.4	8.4417
31	-1	-1	1	-1	10.0	10.7417

Table 5. Results of the ANOVA of the process parameter optimization data of Xanthan gum production using local isolated strain by Central-Composite design of experiments

Source	Coefficient	Sum of Squares	Degrees of Freedom (DF)	Mean Square	F Value	P-Value Prob > F
Model	21.8	959.28	14	68.52	94.01	0.0001
A-pH	-0.63	7.82	1	7.82	10.73	0.0001
B-Temperature	+0.058	0.034	1	0.034	0.046	0.0436
C-Fermentation time	0.95	21.09	1	21.09	28.94	0.0001
D-Inoculums size	-0.725	11.9	1	11.9	16.33	0.0001
AB	-0.762	5.88	1	5.88	8.07	0.0001
AC	0.237	0.33	1	0.33	0.45	0.017
AD	-0.55	5.88	1	5.88	8.07	0.0001
BC	0.675	7.43	1	7.43	10.19	0.0001
BD	0.062	0.14	1	0.14	0.19	0.495
CD	-0.112	0.005625	1	0.005625	0.0077	0.227
A^2	-4.245	494.22	1	494.22	678.09	0.0001
B^2	-4.42	526.75	1	526.75	722.73	0.0001
C^2	-1.408	59.25	1	59.25	81.3	0.0001
D^2	-1.18	46.88	1	46.88	64.32	0.0001
Residual		10.93	15	0.73		
Lack of Fit		10.93	10	1.09		
Pure Error		0.000	5	0.000		
Total		970.21	29			

3.4. Artificial neural network based modelling for Xanthan gum production by local isolated strain

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 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 2 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.

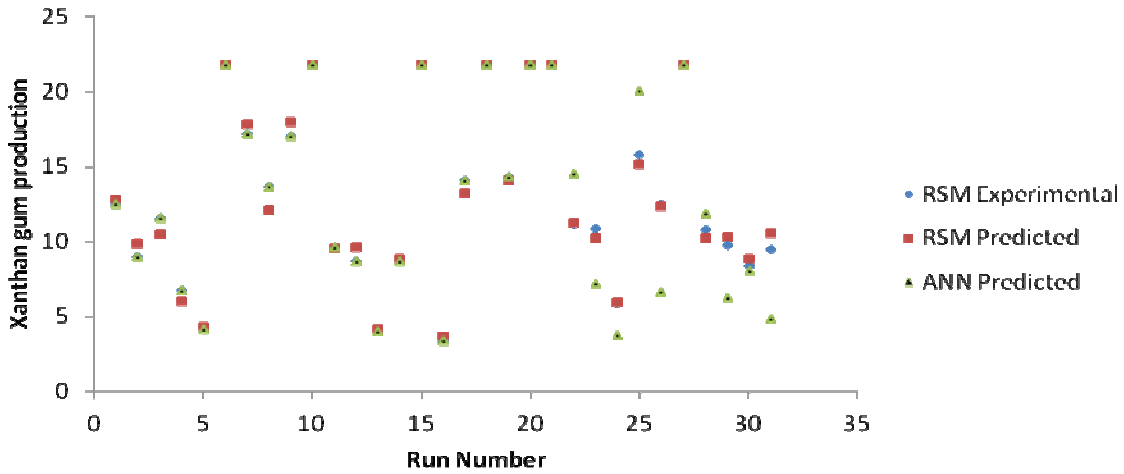

Figure 2 : Comparison of experimental values with RSM and ANN predicted values for the production of Xanthan gum production using local isolated strain

3.5. 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 spectrum of the CX is very similar to that obtained for the PX using the strains of *Xanthomonas campestris* (NCIM 5028) and local isolated strain. Based on the results obtained from FTIR, the remote polysaccharide was found to follow the same spectral behavior as the standard.

4. Conclusion

The current work optimized the parameters for the production of xanthan gum using the bacterium *Xanthomonas campestris* (ATCC 29497) and local isolated strain by response surface methodology and artificial neural network modelling. Central composite design technique was found to be useful for identifying the most influential variables of the system. Optimum values of pH, temperature, fermentation time and inoculums size for Xanthomonas campestris and local isolated strains were found to be 6.98, 29.88^{o}C, 4.06 days and 9.95ml and 6.9, 30.04^{o}C, 4.1 days and 9.3ml respectively. Under the optimum conditions, the maximum xanthan gum production of 21.8 g/l was achieved using the local isolated strain which is found to be better than that of *Xanthomonas campestris* 18.3 g/l. Functional group of the produced xanthan gum was compared with that of the commercial xanthan gum by FTIR spectra.

References

1. Paul F, Morin A, Monsan P (1986). Microbial polysaccharides with actual potential industrial application. Biotechnol. Adv. 4: 245-259
2. Galindo E (1994). Aspects of the process for xanthan production. Trans. I. Chem. E. 72: 227-237.
3. Margaritis, A., Zajic, J.E., 1978. Biotechnology review: mixing mass transfer and scale-up of polysaccharide fermentations. Biotechnology and Bioengineering 20, 939–1001
4. Sandvic EL, Maeker JM (1977). Application of xanthan gum for enhanced oil recovery. Extracellular microbial polysaccharides. Am. Chem. Soc. Symp. Ser. 45: 242-264.
5. F.-O. Garcia,V.E. Santos, J.A. Casas, E. Gomez, Xanthan gum: production, recovery, and properties, Biotechnol. Adv. 18 (2000) 549–579.
6. Bradford PA, Baid J (1983). Industrial utilization of polysaccharide. In: Aspinall GO (ed) The polysaccharides, Academic Press, New York, 2: 411-490.
7. Cottrel IW, Kang KS (1978). Xanthan gum a unique bacterial polysaccharide for food applications. Dev. Ind. Microbiol. 19: 117-131.
8. J.F. Bradbury, Genus II: *Xanthomonas*, in: N.R. Krieg, C.G. Holt (Eds.), Manual of Systematic Bacteriology, Williams & Wilkins, Baltimore, MD, 1984, pp. 199–210.
9. Shu, C. H., & Yang, S. T, Effects of temperature on cell growth and xanthan production in batch cultures of Xanthomonas campestris. *Biotechnology and Bioengineering*, 35(1990) 454–468.
10. Haaland PD. Experimental design in biotechnology NY: Marcel Dekker(1989).
11. StatSoft, Inc. (1995). Statistica for windows, release 5.0. Tulsa, OK. Tuinier, R., ten Grotenhuis, E., & de Kruif, C. G. (2000). The effect of depolymerised guar gum on the stability of skim milk. Food Hydrocolloids, 14, 1–7.
12. Rumelhart D, Hinton G, Williams R. Learning representations by back propagating errors. Nature (1986) 323:533.

*****