International Journal of Science, Environment and Technology, Vol. 4, No 2, 2015, 285 – 292

ISSN 2278-3687 (O)
2277-663X (P)

OPTIMIZATION OF RICE BRAN HYDROLYSIS AND KINETIC MODELLING OF XANTHAN GUM PRODUCTION USING AN ISOLATED STRAIN

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Abstract: In the present investigation the isolated strain producing Xanthan gum was studied. The strain was isolated from the extract of infected banana petioles. Using Response Surface methodology with a Box- Behnken design, three major independent variables including hydrolysis time, H_2SO_4 concentration and rice bran weight were evaluated for reducing sugar extraction from acid hydrolysis of powdered rice bran. Optimized values from the second order polynomial equation were found to be hydrolysis time 45 minutes; H_2SO_4 concentration 1% (v/v) and rice bran weight (substrate concentration) 10g. The maximum reducing sugar extraction of 4.2g/10g of rice bran was achieved. The maximum Xanthan gum production of 24.5 g/l was achieved at 96 h using isolated strain by rice bran hydrolysate as a carbon source. The produced Xanthan gum was compared with the commercial Xanthan gum by FTIR spectra. The capability of the various growth models, namely, Logistic, Monod, Herbert, Shehata & Marr, Tessier and Haldane models in representing the batch kinetic data of the present work were analyzed, while Logistic model is best suited in describing the Xanthan gum production of the present work. Substrate utilization and product formation kinetics were also studied.
Keywords: Acid Hydrolysis, Banana petioles, Response surface methodology, Rice bran hydrolysate and Xanthan gum.

1. INTRODUCTION

Rice bran is obtained as a by-product during the rice milling process, is one of the major agro-industrial by-products. It serves many important usages for both humans & animals as well for commercial purposes, which provide a low cost material and bio-resource for the production of bio-plastics, bio-fuels, bio-chemicals and biomaterials Furthermore [1]. Rice bran is a rich material of carbohydrates and starch that can be converted to glucose which has been applied to be a carbon source in fermentation process [2]. While, carbohydrates and starch of rice bran can be readily hydrolyzed to monosaccharide in kind of reducing sugars by dilute sulphuric acid [3]. However, the concentration of reducing sugar relative with many variables such as; hydrolysis time, % of sulphuric acid concentration and

*Received Feb 20, 2015 * Published April 2, 2015 * www.ijset.net*

the weight of substrate [4]. Therefore, optimization of reducing sugar production from acid hydrolysis is an important investigation to apply in many researches of energy [5 & 6].

Xanthan gum is a water-soluble hetero-polysaccharide [7]. This polymer is used in a wide variety of foods for a number of important reasons. Xanthan gum is used in pharmaceutical formulations, cosmetics and agricultural products. It is used in textile printing, ceramic glazes, slurry explosive formulations, and rust removers. It is also used in drilling fluids and in enhanced oil recovery [8 & 9]. In this present work, Xanthan gum is produced by submerged aerobic fermentation by using locally isolated strain extracted from infected banana petioles and rice bran hydrolysate as a carbon source optimized by Response Surface methodology with a Box- Behnken design.

2. MATERIALS AND METHODS

2.1. Isolation of strain and maintenance

Isolation of strain and maintenance as described in previous publication [10].

2.2. Acid hydrolysis

To study the effect of acid concentration on the hydrolysis, the experiments were carried out by treating the powdered rice bran with dilute 1N H_2SO_4 at different acid concentrations of 0.5, 1and 1.5% (v/v).

2.3. Batch Fermentations Studies

Batch fermentation studies as described in previous publication [11].

2.4. 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^{o}C for 2 h and weighted.

2.5. Determination of xanthan gum concentration and reducing sugars concentration: Xanthan gum and reducing sugars were determined as described in previous publication [10].

2.6. Spectroscopy of Fourier transform infrared (FTIR)

Fourier transform infrared spectroscopic analysis was described in previous publication [11].

2.7. Experimental methodology

RSM is a very effective and most popular statistical tool to optimize the variables having equal importance and influence on each other. Box- Behnken design with three factors were used to test the effect of significant process parameters such as Hydrolysis time, H_2SO_4 acid concentration and rice bran weight on reducing sugar extraction using commercial software Minitab 16 [12]. The data obtained from RSM on reducing sugar extraction are subjected to the analysis of variance (ANOVA).

2.8. Kinetics and Modelling.

The logistic, Substrate consumption and Product formation kinetics can be described in previous publication [11].

3. RESULTS AND DISCUSSION

3.1. Process Parameter Optimization Using Box- Behnken Design for Reducing Sugar Extraction from Acid Hydrolysis of Rice bran

The design matrix and their corresponding experimental and the predicted values are given in Table 1. The experimental results suggest that the maximum values of reducing sugar extraction were obtained for the runs with the central points. The experimental runs of 10, 12, 14, 16 and 17 produced the highest reducing sugar (42 g/l). The results were analyzed using the analysis of variance (ANOVA) and the estimated coefficients are presented in Table 2.

ANOVA results of the data indicate that the model terms, A, B, AB, AC, A^2, B^2 and C^2 are significant ($P < 0.05$). Values greater than 0.1000 indicate the model terms are not significant. The reducing sugar extract from acid hydrolysis of rice bran can be expressed in terms of the following regression equation 1:

$$Y = 42.00 + 1.00A + 1.5B + 0.25C - 1.25AB - 1.25AC + 0.75BC - 6.13A^2 - 17.62B^2 - 7.62C^2 \quad \ldots\ldots(1)$$

where, A, hydrolysis Time; B,Acid concentration; C,Substrate Concentration.

The regression equation obtained from the ANOVA showed that the R^2 (multiple correlation coefficient) value was 0.9963 (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 99.63% of the variation in response. Three dimensional surface plots are drawn to determine the optimum values of the three parameters and are shown in Figure 1(a - c). The following are the optimum values obatained by solving the second order polynomial equation hydrolysis Time 45 minutes, Acid concentration 1% and Substrate Concentration 10 g. These optimum values were maintained for all further studies.

3.2. Xanthan gum production from rice bran hydrolysate using an isolated strain in batch cultures

The experiments were performed at concentrations ranging 4% (w/v) (glucose equivalent). The time profile of cell mass, Xanthan gum and reducing sugar concentration are presented in table 3. The maximum Xanthan gum of 24.5 g/l was achieved at 96 h.

3.3. Model evaluation

Various growth, substrate utilization and product formation kinetic models were analyzed using the experimental data. The models which showed maximum fit are represented in Table 4 along with the estimated parameters. The optimized model parameter values are evaluated using MATLAB coding. The regression coefficient values (R^2) were estimated for cell mass concentrations are presented in Table 5. From the regression coefficient values, it is clear that the correlation between the experimental data and the theoretical predictions are good. Thus, the Logistic model represents Xanthan gum production very well using isolated strain.

3.4. 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. Figure 2(a-b) shows that the infrared spectrum of the CX is very similar to that obtained for the PX using rice bran hydrolysate. Based on the results obtained from FTIR, the remote polysaccharide was found to follow the same spectral behavior as the standard.

4. CONCLUSION

The production of Xanthan gum by the locally isolated strain was carried out. The process parameters namely Hydrolysis time, Acid concentration and Substrate concentration were optimized using Box- Behnken Design of RSM. The optimum conditions were found to be hydrolysis Time 45 minutes, Acid concentration 1% and Substrate Concentration 10 g for reducing sugar extract from rice bran. The maximum Xanthan gum production of 24.5 g/l was achieved using the isolated strain from extracted reducing sugar. The batch kinetic data obtained for substrate utilization growth kinetics and product formation and fitted into various models. The substrate utilization data best fits with substrate utilization kinetics with yield co efficient of 0.209, the growth kinetic data fits with the Logistic model with a correlation coefficient of 0.9837 and Luedeking-Piret model fits for the product formation with yield co efficient of 3.48. Functional group of the produced Xanthan gum was confirmed with that of the commercial Xanthan gum by FTIR spectra.

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Table 1. Box- Behnken design matrix for for Reducing Sugar Extraction from Acid Hydrolysis of Rice bran

Run No.	Time (minutes)	H_2SO_4 Conc. (%) (v/v)	Substrate Conc.(g)	Reducind Sugar Concentration (g / 100g of rice bran)	
				Experimental	Predicted
1	1	-1	0	18	18.875
2	-1	0	1	28	28.625
3	0	-1	1	16	14.000
4	0	1	1	19	19.250
5	0	1	-1	16	17.000
6	1	0	-1	31	30.375
7	-1	0	-1	26	25.875
8	1	1	0	20	19.625
9	1	0	1	28	28.125
10	0	0	0	42	42.000
11	-1	-1	0	14	14.375
12	0	0	0	42	42.000
13	0	-1	-1	16	15.750
14	0	0	0	42	42.000
15	-1	1	0	21	20.125
16	0	0	0	42	42.000
17	0	0	0	42	42.000

Table 2. Results of the ANOVA of the process parameter optimization data Reducing Sugar Extraction from Acid Hydrolysis of Rice bran by Box- Behnken design matrix

Source	Coefficient	Sum of Squares	Degrees of Freedom (DF)	Mean Square	F Value	P-Value Prob > F
Model	42	1898.06	9	210.9	314.84	0.0001
A-Hydrolysis time	1.00	8.00	1	8.00	12.79	0.0255
B-H_2SO_4 Concentration	1.50	18.00	1	18.00	28.00	0.0038
C-Substrate Concentration	0.25	0.5	1	0.5	0.74	0.5024
AB	-1.25	6.25	1	6.25	9.21	0.0410

AC	-12.5	6.25	1	6.25	10.51	0.0410
BC	0.75	2.25	1	2.25	4.14	0.1773
A^2	-6.13	157.96	1	157.96	221.07	0.0001
B^2	-17.62	1307.96	1	1307.96	1963.17	0.0001
C^2	-7.62	244.80	1	244.80	368.49	0.0001
Residual		7.00	7	1.00		
Lack of Fit		7.00	3	2.33		
Pure Error		0.000	4	0.000		
Total		1905.06	16			

Table 3.The time profile of cellmass, Xanthan gum and glucose concentration using rice bran hydrolysate

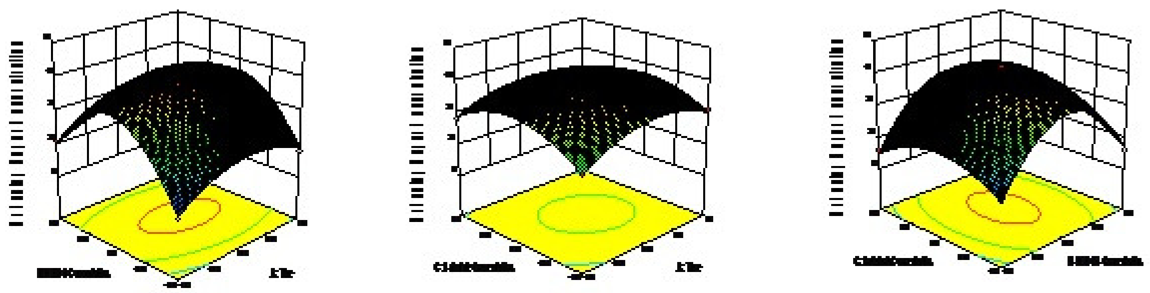
Time (hours)	Cell Mass Concentration (g/L)	Substrate Concentration (g/L)	Xanthan gum production (g/L)
0	0.2	40	0
12	0.7	34.3	3.5
24	1.8	29.7	7.3
36	3.1	24.8	11.4
48	4.4	18.7	15.1
60	5.3	13.2	18.1
72	6.2	9.7	20.7
84	6.9	7.3	22.9
96	7.3	6.5	24.5
108	7.3	6.3	24.1
120	7.3	6.2	23.9

Table 4: Values of the kinetic parameter and regression coefficient (R^2) for Xanthan gum production using isolated strain from rice bran extract

Model	Parameter Estimation	Regression coefficient (R^2)
Logistic	$k=0.08hr^{-1}$, $\beta=0.136$ L/g	0.9837
Substrate utilization	$Y_{X/S} = 0.2$	-
Product formation	$Y_{P/X} = 3.48$	-

Table 5: Growth kinetic parameters and regression obtained for different models during Xanthan gum production using isolated strain from rice bran extract

Model	Parameter estimation	Regression coefficient (R^2)
Monod model	μ_m = 0.096, k_s = 11.74	0.2586
Herbert model	μ_m =8.222, k_s =3.619, m = -3.522	0.2518
Shehata & marr model	μ_m =0.09808, μ_1 = 6.614, k_1 =1.622, k_2 = 1.535	0.356
Tessier model	μ_m =0.05045, T = 0.235	0.111
Haldane model	μ_m = 10.16, k_i = 2.861 k_s = 1702	0.4171



(a) **(b)** **(c)**

Figure 1 (a-c) 3D plots showing the interactive effects between the significant process parameters on Reducing Sugar Extraction from Acid Hydrolysis of Rice bran

(a)

(b)

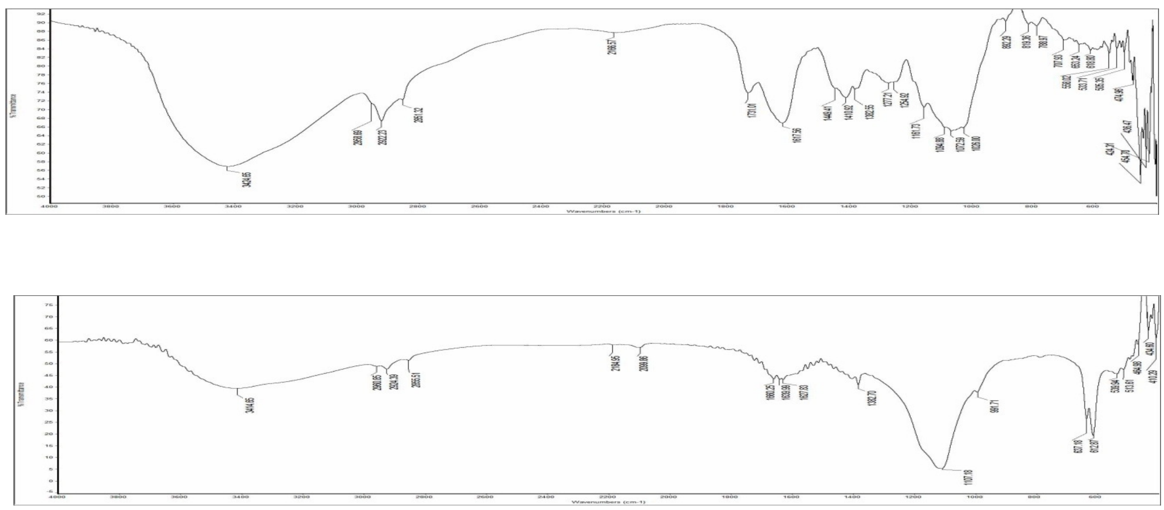


Figure 2 (a-b) FTIR spectra of commercial Xanthan gum and produced Xanthan gum from rice bran extract using isolated strain