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Production of Xylitol by *Candida mogii* from Rice Straw Hydrolysate

Study of Environmental Effects Using Statistical Design

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ABSTRACT

The influence of aeration level, initial pH, initial cell concentration, and fermentation time on the xylitol production from rice straw hemicellulose hydrolysate by *Candida mogii* was studied. A multifactorial experimental design was adopted to evaluate this influence. A statistical analysis of the results showed that the aeration level and the initial pH had significant effects on yield factor, volumetric productivity, and xylose consumption. For the latter, fermentation time was also a significant variable. Based on the response surface methodology, models for the range investigated were proposed. The maximum values for the yield factor ($Y_{p/s}$) and volumetric productivity (Q_p) were, respectively, 0.71 g/g and 0.46 g(Lh).

Index Entries: Rice straw; *Candida mogii*; hemicellulose hydrolysate; factorial design; xylitol.

INTRODUCTION

The fermentation conditions for the biotechnological production of xylitol by several yeast strains have been the target of many investigations. The literature reports that this bioconversion is influenced by a number of variables, such as aeration, initial pH, and initial cell concentration. The aeration level interferes with the level of oxidized co-factor NAD^+, blocking or promoting the activity of the enzyme xylitol dehydrogenase, responsible for the oxidation of xylitol into xylulose. As frequently observed, decreasing the oxygen supply increases the xylitol formation. Nevertheless, the optimal oxygenation levels appear to be specific for each yeast strain *(1–4)*. Likewise, the pH level promoting the highest xylitol

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accumulation is different for each yeast strain *(1,3)* and each fermentation medium *(5)*. The influence of the initial cell concentration also showed to be dependent on the yeast strain employed *(1,6,7)*.

In a previous study, *Candida mogii* NRRL Y-17032 was selected from among 31 yeast strains as a promising xylitol producer from rice straw hemicellulose hydrolysate *(8)*. However, no investigation on the effect of the fermentation conditions has been conducted so far. In this work, a multifactorial experimental design was adopted to evaluate the influence of aeration, initial pH, initial cell concentration, and fermentation time on the xylose consumption, yield factor, and volumetric productivity during the xylitol production by this yeast strain. A response surface methodology (RSM) was used to determine a statistical model for these parameters. Statistical designs have already been employed in the evaluation of the influence of different factors on the xylitol production by yeasts *(9,10)*.

MATERIALS AND METHODS

Preparation of Hemicellulose Hydrolysate

Rice straw hemicelullose hydrolysate was obtained by acid hydrolysis of the rice straw in an AISI 316 stainless steel 25-L stirred tank reactor (10 g of H_2SO_4 0.7% w/v per g of dry matter). The hydrolysis (145°C, 21 min) was followed by vacuum filtration. The hydrolysate was then concentrated under vacuum, at 70°C, to increase the initial xylose content 5×. The pH of the concentrate was raised with NaOH pellets to 9.5, and then lowered to 5.4 with H_2SO_4 72% (w/w). Each time the pH level was changed, the precipitate was removed by centrifugation (1000*g*, 15 min). Next, the hydrolysate was autoclaved under free-flowing steam for 15 min.

Microorganism and Inoculum Preparation

C. mogii NRRL Y-17032 obtained from Northern Regional Research Laboratory (Peoria, IL) was maintained at 4°C on malt-extract agar slants. Inoculum was prepared by cultivating cells in 125-mL Erlenmeyer flasks containing 25 mL of medium composed of 20 g/L rice bran extract, 3 g/L $(NH_4)_2SO_4$, 0.1 g/L $CaCl_2{\cdot}2H_2O$, and 50% v/v rice straw hemicellulose hydrolysate. The flasks were incubated for 48 h at 30°C, under 200 rpm.

Fermentation Conditions

A fermentation medium with a composition similar to that used for the inoculum preparation was placed into 125-mL Erlenmeyer flasks, which were incubated on a rotary shaker at 30°C, under 200 rpm. Different aeration levels were obtained by varying the volume of the medium in the flasks. Three different volumes of medium (60, 40, and 20 mL), as well as three initial pH values (4.50, 5.75, and 7.00), three initial cell concentra-

tion levels (1.0, 2.5, and 4.0 g/L), and three fermentation times (44, 58, and 72 h), were employed. The minimum, intermediate, and maximum values of each variable correspond to the coded levels $-1, 0$, and $+1$, respectively.

Statistical Analysis

A 2^4 experimental design *(11)* was used to evaluate the effect of the variables studied. Statistical models for xylose consumption, yield factor, and volumetric productivity, employing the significant variables, were determined by the response surface regression procedure. A 2^3 factorial with face-centered design was performed. The models determined are expressed by the equation:

$$\hat{Y} = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j$$

where $\hat{Y}$ is the response variable; b, the regression coefficients, and X, the experimental factors coded levels.

Analytical Methods

Concentrations of D-xylose and xylitol were determined by high performance liquid cromatography (HPLC) using a Shimadzu C-R7A cromatograph (Kyoto, Japan) equipped with a refractive index (RI) detector and a Bio-Rad (Hercules, CA) Aminex HPX-87H column under the following conditions: temperature: 45°C; eluent: 0.01 *N* sulfuric acid; flow: 0.6 mL/min; sample volume: 20 μL.

RESULTS AND DISCUSSION

The effect estimates, standard errors, and Student's *t*-test for xylose consumption (X_C), yield factor ($Y_{p/s}$), and volumetric productivity (Q_P) in xylitol obtained from the 2^4 factorial design are shown in Table 1. The initial cell concentration did not show a significant effect for any of the response variables in the range studied. Only the first-order effects of aeration, initial pH, and fermentation time were significant (at 95% confidence level) for xylose consumption. For the yield factor, the most significant effects were the initial pH and the interaction between aeration and initial pH (90% confidence level). On the other hand, for volumetric productivity, the aeration and pH factors were significant at 95% confidence level. However, no interaction effect was found for this response.

The results of the 2^3 factorial with face centered design, performed using the variables aeration, initial pH, and fermentation time to determine the statistical models from RSM, are shown in Table 2. Based on these results, an analysis of variance for each response, considering all effects, was carried out (data not shown). In order to increase the degrees of free-

Table 1
Effects Estimates, Standard Errors, and Student's (t_3) test for Xylose Consumption (X_C), Yield Factor ($Y_{p/s}$), and Volumetric Productivity (Q_p) in Xylitol Obtained from 2^4 Factorial Design

	Estimates			Standard errors			t		
Effects	X_C (g/L)	$Y_{P/S}$ (g/g)	Qp g/(L h)	X_C (g/L)	$Y_{P/S}$ (g/g)	Qp g/(L h)	X_C (g/L)	$Y_{P/S}$ (g/g)	Qp g/(L h)
Average	26.668	0.620	0.301	±1.739	±0.024	±0.025	15.34	26.08	12.09
X_1: Aeration	14.200	0.070	0.169	±3.789	±0.052	±0.054	3.75[a]	1.35	3.12[a]
X_2: pH	20.025	0.103	0.246	±3.789	±0.052	±0.054	5.29[a]	1.98[b]	4.55[a]
X_3: Cell conc.	7.325	−0.035	0.076	±3.789	±0.052	±0.054	1.93	0.68	1.41
X_4: Time	12.075	−0.023	−0.021	±3.789	±0.052	±0.054	3.19[a]	0.43	0.39
X_1 X_2	−1.675	−0.110	−0.021	±3.789	±0.052	±0.054	0.44	2.12[b]	0.39
X_1 X_3	3.075	0.028	0.044	±3.789	±0.052	±0.054	0.81	0.53	0.81
X_1 X_4	2.275	0.025	−0.014	±3.789	±0.052	±0.054	0.60	0.48	0.25
X_2 X_3	−2.250	−0.005	−0.019	±3.789	±0.052	±0.054	0.59	0.10	0.35
X_2 X_4	0.200	−0.063	−0.086	±3.789	±0.052	±0.054	0.05	1.21	1.59
X_3 X_4	−0.100	−0.055	−0.031	±3.789	±0.052	±0.054	0.03	1.06	0.58

Standard error from total error with 8 d.f. 95% confidence level (t = 2.30665).
Standard error from total error with 8 d.f. at 90% confidence level (t = 1.860).
[a] Significant at 95% confidence level.
[b] Significant at 90% confidence level.

Table 2
Matrix of 2^3 with Face-Centered Factorial Design and Experimental Responses

	Coded variables			Responses		
Assay	Aeration	pH	Time	X_C (g/L)	$Y_{P/S}$ (g/g)	Q_P (g/(L h)
1	−1	−1	−1	6.0	0.46	0.06
2	+1	−1	−1	12.9	0.59	0.18
3	−1	+1	−1	20.8	0.71	0.33
4	+1	+1	−1	37.8	0.68	0.58
5	−1	−1	+1	9.0	0.45	0.06
6	+1	−1	+1	33.8	0.68	0.32
7	−1	+1	+1	37.5	0.63	0.32
8	+1	+1	+1	45.6	0.58	0.37
9	−1	0	0	19.3	0.70	0.24
10	+1	0	0	42.0	0.67	0.48
11	0	−1	0	17.0	0.53	0.16
12	0	+1	0	36.8	0.62	0.39
13	0	0	−1	17.4	0.62	0.24
14	0	0	+1	34.2	0.66	0.32
15	0	0	0	34.3	0.74	0.44
16	0	0	0	32.7	0.73	0.41
17	0	0	0	32.9	0.72	0.41
18	0	0	0	26.9	0.69	0.32
19	0	0	0	27.8	0.65	0.31

Xylose consumption (X_C) = g/L.
$Y_{P/S}$ = g xylitol produced per g xylose consumed.
Q_P = g xylitol produced per L/h.

dom for the estimate of the effects, a second analysis was made using only the terms that were significant in the first. The models determined are described by the following equations:

$$\hat{Y}_1 = 27.62 + 7.95\,X_1 + 9.98\,X_2 + 6.52\,X_3 \quad (1)$$

$$\hat{Y}_2 = 0.688 + 0.049\,X_2 - 0.052\,X_1X_2 - 0.097\,X_2^2 \quad (2)$$

$$\hat{Y}_3 = 0.352 + 0.090\,X_1 + 0.122\,X_2 - 0.042\,X_2X_3 - 0.074\,X_2^2 \quad (3)$$

where $\hat{Y}_1$ = predicted xylose consumption (g/L), $\hat{Y}_2$ = predicted yield factor (g/g), $\hat{Y}_3$ = predicted volumetric g(Lh), X_1 = coded aeration level, X_2 = coded initial pH, and X_3 = coded fermentation time.

Deriving the Eqs. 2 and 3 and making it equal to zero, the maximum values for yield factor and volumetric productivity obtained in the range studied were 0.71 g/g and 0.46 g/(Lh), respectively. The value for yield factor was calculated considering the coded levels −1 for aeration and 0.5

Table 3
Anova for Xylose Consumption as Function of Parameters Aeration, pH, and Fermentation Time

Effect	Sum of squares	Degrees of freedom	P-value
A: Aeration	632.02500	1	0.0017
B: pH	996.00400	1	0.0007
C: Time	425.10400	1	0.0035
Lack-of-fit	262.16426	11	0.2402
Pure error	44.40800	4	
Total (corr.)	2359.70526	18	

R-squared = 0.87008.

Table 4
F-values from Estimate of Effects in 2^3 Factorial with Face-Centered Design

Statistical parameter	F-value		
	X_C	$Y_{P/S}$	Q_P
Regression	33.49	10.99	13.59
Lack-of-fit	2.15	0.68	1.21

for pH. For volumetric productivity, the coded levels were +1 and 0.54 for aeration and pH, respectively. These aeration levels were employed because the points of maximum related to this variable are out of the range studied for both responses.

The analysis of variance for xylose consumption, with nonsignificant effects eliminated, is presented in Table 3. The percentage of variance explained in this analysis (R^2 = 87%) indicates that the selected model is likely to be adequate for describing the xylose consumption behavior as a function of the factors within the range studied. The significance level (*P*-values) found for aeration, pH, and fermentation time confirmed the significant effect of these factors on xylose consumption. Lack-of-fit was not found to be significant for this model. The validity of the model can be also confirmed by the F-test for regression model (Table 4).

Table 5 shows the analysis of variance for the yield factor. As can be concluded from the *P*-values, the first-order effect of the initial pH, the interaction between aeration and pH, and the second-order effect of pH were once more significant terms for the yield factor. The percentage of variance explained (R^2) was 76% out of 81% of variance explainable. This demonstrates that the model is adequate for this response, which can be confirmed by the F test using the values of Table 4, and by the *P*-value found for lack-of-fit in Table 5.

Table 5
Anova for Yield Factor as Function of Parameters Aeration and pH

Effect	Sum of squares	Degrees of freedom	*P*-value
A: Aeration	0.0057600	1	0.1527
B: pH	0.0240100	1	0.0102
AB	0.0220500	1	0.0127
B^2	0.0443650	1	0.0016
Lack-of-fit	0.0065827	4	0.6187
Pure error	0.0240429	10	
Total (corr.)	0.12681053		

R-squared = 0.758494.

Table 6
Anova for Volumetric Productivity as Function of Parameters Aeration, pH, and Fermentation Time

Effect	Sum of squares	Degrees of freedom	*P*-value
A: Aeration	0.0810000	1	0.0085
B: pH	0.1488400	1	0.0028
C: Time	0.0000000	1	1.0000
BC	0.0144500	1	0.1109
B^2	0.0260950	1	0.0518
Lack-of-fit	0.0377456	9	0.4600
Pure error	0.0138800	4	
Total (corr.)	0.32201053		

R-squared = 0.839677.

The analysis of variance for volumetric productivity is presented in Table 6. *P*-values show the significance of the first-order effects of aeration and pH, and of the second-order effect of pH at 95% confidence level. The interaction between pH and fermentation time, significant at 90% confidence, was also considered for the model of volumetric productivity. Likewise, for this response, the percentage of variance explained (R^2 = 84%), the F-test in Table 5 and the *P*-value for the lack-of-fit indicate the adequacy of the selected model.

The analysis of variance showing the value of R^2 for full regression in the three models determined is presented in Table 7.

The response surfaces for the models determined are shown in Figs. 1–3. For plotting the response surfaces for xylose consumption and volumetric productivity, the fermentation time was set at the coded value +1 because of its positive effect in the utilization of the xylose present in the

Table 7
Analysis of Variance for Full Regression of Models Determined

Statistical parameter	Xylose consumption		Yield factor		Volumetric productivity	
	Model	Error	Model	Error	Model	Error
Sum of squares	2053.13	306.572	0.0904	0.0364	0.2704	0.0516
Degrees of freedom	3	15	3	15	4	14
Mean square	684.3780	20.4382	0.0301	0.0024	0.0676	0.0037
F-ratio	33.4853		12.4259		18.3310	
P-value	0.0000		0.0002		0.0000	
R^2	0.8701		0.7131		0.8397	

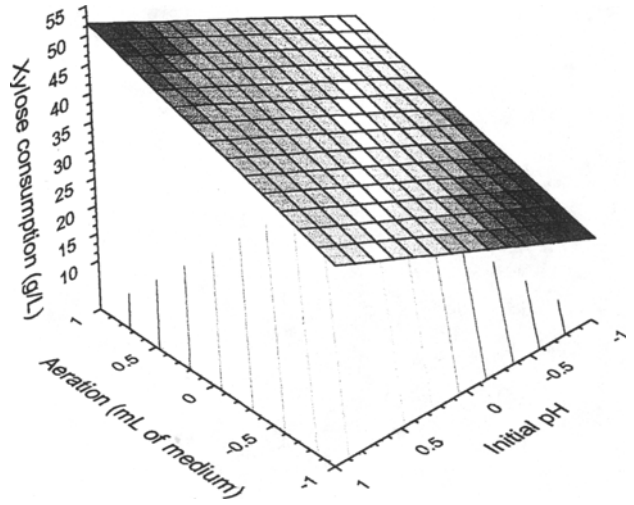

Fig. 1. Response surface and contour plot for the predicted model for xylose consumption.

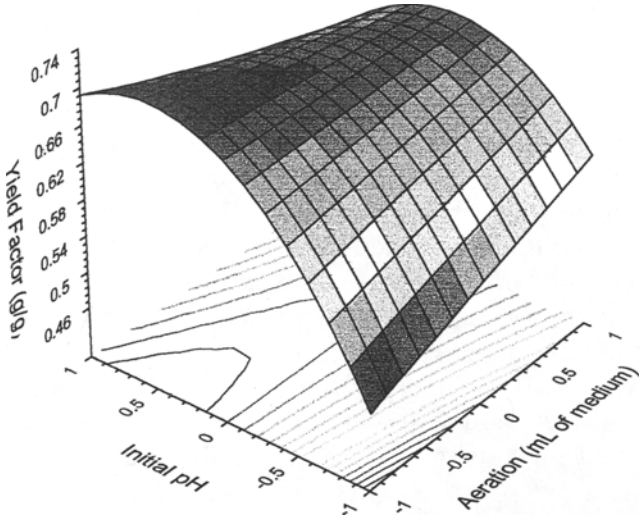

Fig. 2. Response surface and contour plot for the predicted model for yield factor.

hydrolysate. As can be seen from Fig. 1, xylose consumption augments linearly with the increase of both aeration and initial pH. Within the ranges studied, the maximum value for the yield factor was predicted with the lowest value of aeration (60 mL of medium in the flask); maximum volumetric productivity was predicted with the highest aeration (20 mL of medium in the flask, corresponding to the coded level +1). For maximum values for both responses, the initial pH was approx 6.5, corresponding to the coded level 0.5. This opposite effect of the aeration for the yield

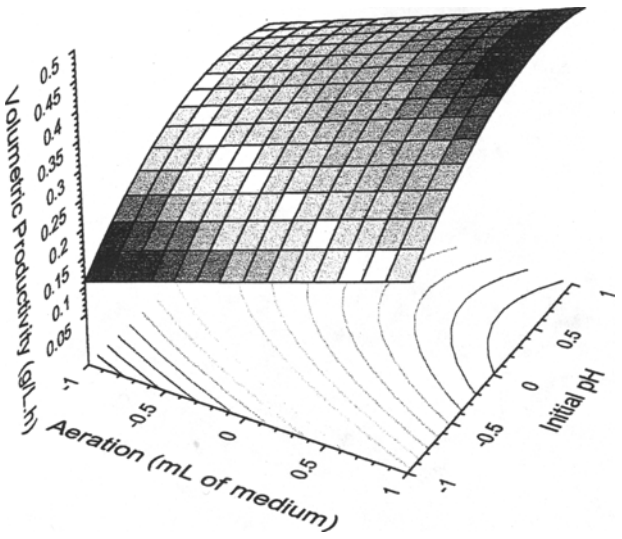

Fig. 3. Response surface and contour plot for the predicted model for volumetric productivity.

factor and productivity in xylitol was also observed for *C. mogii* ATCC 18364 by Sirisansaneyakul et al. (*12*). According to these authors, this was probably caused by the effect of accumulation of intracellular xylose, which affects its own transport across the cell membrane. The most suitable pH value for xylitol yield and productivity found in this study was also found in a study using a strain of *Candida boidinii* (*1*). Nevertheless, the influence of the initial pH on this bioconversion seems to be dependent on the yeast strain employed, and on the composition of the fermentation medium (*3,5*). The latter has been associated with the presence of acetic acid, which occurs in hydrolysate-based media. Indeed, the xylitol production using *Candida* sp B-22 in synthetic medium without acetic acid was not influenced by the initial pH (*6*). Although the initial cell concentration has been found to influence this bioconversion (*1,6*), no influence was observed in the present work.

CONCLUSION

According to the statistical analysis of the results, it can be concluded that aeration and initial pH significantly affect xylose consumption, yield factor, and volumetric productivity. The fermentation time also affects the xylose consumption, and its interaction with initial pH influences the volumetric productivity. Highest yield factor is achieved using 60 mL of medium and initial pH of 6.2, and highest volumetric productivity, using 20 mL of medium and initial pH of 6.4. Further experiments will be conducted in a bench-scale fermentor, employing the significant variables selected in the present study.

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