



The roles of xylan and lignin in oxalic acid pretreated corncob during separate enzymatic hydrolysis and ethanol fermentation

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ABSTRACT

High yields of hemicellulosic and cellulosic sugars are critical in obtaining economical conversion of agricultural residues to ethanol. To optimize pretreatment conditions, we evaluated oxalic acid loading rates, treatment temperatures and times in a 2³ full factorial design. Response-surface analysis revealed an optimal oxalic acid pretreatment condition to release sugar from the cob of *Zea mays* L. ssp. and for *Pichia stipitis* CBS 6054. To ferment the residual cellulosic sugars to ethanol following enzymatic hydrolysis, highest saccharification and fermentation yields were obtained following pretreatment at 180 °C for 50 min with 0.024 g oxalic acid/g substrate. Under these conditions, only 7.5% hemicellulose remained in the pretreated substrate. The rate of cellulose degradation was significantly less than that of hemicellulose and its hydrolysis was not as extensive. Subsequent enzymatic saccharification of the residual cellulose was strongly affected by the pretreatment condition with cellulose hydrolysis ranging between 26.0% and 76.2%. The residual xylan/lignin ratio ranged from 0.31 to 1.85 depending on the pretreatment condition. Fermentable sugar and ethanol were maximal at the lowest ratio of xylan/lignin and at high glucan contents. The model predicts optimal condition of oxalic acid pretreatment at 168 °C, 74 min and 0.027 g/g of oxalic acid. From these findings, we surmised that low residual xylan was critical in obtaining maximal glucose yields from saccharification.

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1. Introduction

As energy consumption rises along with global population, alternatives to fossil resources must be developed. Conventional oil production outside the Middle East is likely to peak sometime between 2010 and 2030 (Greene et al., 2004). and annual global oil production has been predicted to decline dramatically by 2050 (Hallock et al., 2004). In addition to declining energy supplies, unconstrained use of fossil fuels is increasing atmospheric burdens of carbon dioxide, which results in long-term global warming (Cox et al., 2000). Therefore, many countries have great interest in exploring alternative energy sources. To solve the problem of energy exhaustion, researchers have tried to convert environmentally friendly biomass into fuel ethanol as an alternative to fossil fuels (Cheung and Anderson, 1997). While controversy has arisen over the use of food grains for fuel production and the displacement of native forests through the consequential expansion of agricultural crops, the conversion of agricultural residues to renewable

fuels can be beneficial if it is done in a sustainable manner (Tilman et al., 2009; Koh et al., 2008).

Biomass has received much attention as a resource for renewable energy. In particular, lignocellulosic material such as woody biomass and agronomic residues are regarded as promising energy sources because they do not compete with food sources and can displace fossil fuels (Sun and Cheng, 2002; Soderstrom et al., 2003). Among agronomic residues, corncobs are important by-products of the corn processing industry. Traditionally, ground corncobs have been used as an animal bedding returned to the field (Inglett, 1970). They contain approximately 70% carbohydrate 10% lignin and 2% protein (Barl et al., 1991). Therefore, they can serve as a potential source of renewable biomass for the production of fermentable sugars by chemical and enzymatic processes.

Pretreatment is an essential step for enzymatic hydrolysis in the production of ethanol from lignocellulosic biomass. Recently, oxalic acid has been used to pretreat lignocellulosics for pulp production (Akhtar et al., 2002; Swaney et al., 2003; Kenealy et al., 2007). This saves significant energy in the refining process and provides a stronger paper product because hemicellulose can be selectively removed. Oxalic acid is strong enough to catalyze hemicellulose hydrolysis, but under mild conditions it is selective enough to

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avoid extensive cellulose degradation. As a consequence, oxalic acid might be suitable as a pretreatment to release fermentable hemicellulosic sugars without extensive formation of degradation products.

Oxalic acid is secreted by wood-degrading fungi, and is thought to depolymerize cellulose and hemicellulose through non-enzymatic mechanisms (Shimada et al., 1994; Dutton and Evans, 1996; Green et al., 1991). Because oxalic acid has two pK_a's it could mimic reactions that catalyze glycoside hydrolysis through ion-pair mechanisms. Oxalic acid pretreatment could be more readily tuned or optimized for the hydrolysis of various lignocellulosic substrates than pretreatments with stronger acids such as sulfuric.

Xylan in hemicellulose of lignocellulosic biomass constitutes a barrier around cellulose, which restricts access of cellulases (Berlin et al., 2007; Garcia-Aparicio et al., 2007). Significant effort to remove xylan during pretreatment and enzymatic hydrolysis has been tried by many researchers. During severe treatments to remove residual xylan, significant amounts of cellulose are degraded. In this study, we applied oxalic acid pretreatment to corncobs to selectively remove xylan, and evaluated the effect of the pretreatment on cellulase hydrolysis and fermentation. Also we investigated role of xylan and lignin on enzymatic hydrolysis and ethanol production by fermentation based on analysis of response-surface methodology.

2. Methods

2.1. Corn cob pretreatment

The pretreatment of corn cob obtained by oxalic acid was based in the method developed by Kenealy et al. (2007). The reactor configuration has been described previously (Lee et al., 2009). Standardized corn cob pellets (Pestell, New Hamburg, Canada) contained 10% moisture. The pretreatment condition was based on 2³ full factorial design augmented with star design (six axial points) and three replicates in the central point (Table 1). The corn cob (1.5 kg dry matter) was impregnated with oxalic acid solution at different concentration (Table 1) using vacuum in semi-pilot reactor for 20 min at room temperature. The solid liquid ratio during impregnation was 1:6. The excess of oxalic acid solution after impregnation was drained out of the system. The corn cob impregnated with oxalic acid was treated at different temperature and

reaction time according to Table 1. The pretreated corn cob was washed to extract sugars and kept at 4 °C.

2.2. Chemical analysis of pretreated corn cob

The lignin content of the pretreated materials was determined by the TAPPI method (T 222-om-88). Samples were milled to pass a 1 mm screen and vacuum dried at 45 °C. Pretreated materials were hydrolyzed with 3 ml (w/w) H₂SO₄ for 1 h at 30 °C. Hydrolyzates were diluted to 4% (w/w) H₂SO₄ with distilled water and then, heated at 120 °C for 1 h. After hydrolysis, 2 ml supernatant samples were centrifuged and filtered through a 0.45 µl filter, and the solution was analyzed for monosaccharides.

The quantification of glucose, xylose and arabinose as monosaccharides in the soluble fraction was determined by HPLC (Gilson 307 system, Villiers-le-Bel, France). HPLC equipped with an Aminex HPX-87H column (Bio-Rad, 300 × 7.8 mm) and refractive index detector (RID). Analysis was performed with 5 mM H₂SO₄ as the mobile phase at 0.3 ml/min for 55 min and the analysis was done under isocratic mode. All the analyses were carried out in triplicate.

2.3. Enzymatic hydrolysis of pretreated corn cob

Enzymatic saccharification was performed by Accellerase 1000 (Genencor, NY, USA) which is a commercial cellulose enzyme complex. The pretreated material of 5 g dry weight was transferred to a 125 ml Erlenmeyer flask, and 50 ml of 50 mM sodium citrate buffer (pH 6.0) was added. Appropriate amounts of cellulase (500 CMCU/g) and β-glucosidase (80 pNPG U/g) were added. The flask was put in a shaking incubator at 50 °C and 150 rpm and incubated for 72 h (Soderstrom et al., 2003). Samples were taken at 24, 48, 72 h and the contents of released monosaccharides were analyzed by HPLC. All the determination was realized in triplicate.

2.4. Fermentation

Pichia stipitis CBS 6054 was maintained on agar malt medium and stored at 4 °C then transferred to fresh plate to be used within 24 h of incubation at 30 °C. Cells were grown in 1000 ml Erlenmeyer flasks containing 400 ml of YPD (10 g/l yeast extract, 10 g/l peptone, and 20 g/l glucose) in an orbital shaker at 30 °C and 200 rpm. Following 24 h growth, cell cultures were harvested,

Table 1
The 2³ full factorial design with six axial points and three replicates in the central point matrix employed for three independent variables.

Run no.	Variables			Coded levels		
	Temperature (°C)	Oxalic acid* (g/g)	Reaction time (min)	X ₁	X ₂	X ₃
1	132	0.015	26	-1	-1	-1
2	168	0.015	26	+1	-1	-1
3	132	0.032	26	-1	+1	-1
4	168	0.032	26	+1	+1	-1
5	132	0.015	74	-1	-1	+1
6	168	0.015	74	+1	-1	+1
7	132	0.032	74	-1	+1	+1
8	168	0.032	74	+1	+1	+1
9	120	0.024	50	-1.68	0	0
10	180	0.024	50	+1.68	0	0
11	150	0.010	50	0	-1.68	0
12	150	0.037	50	0	+1.68	0
13	150	0.024	10	0	0	-1.68
14	150	0.024	90	0	0	+1.68
15	150	0.024	50	0	0	0
16	150	0.024	50	0	0	0
17	150	0.024	50	0	0	0

* The oxalic acid concentration used during impregnation of corn cob (solid liquid ratio of 1:6). Approximately 15% of the solution was taken up by the solids (Lee et al., 2009).

Table 2

Evaluation of oxalic acid pretreatment on the generated corncob residue, enzymatic hydrolysis and fermentation (SHF) of the pretreated corncob residue.

Run no.	Pretreatment stage				Hydrolysis stage ^a		Fermentation stage ^b	
	Xylan (%)	Arabinan (%)	Lignin (%)	Xylan/lignin ratio	Total sugar produced (g/l)	Ethanol (g/l)	Q ₁ [g/l h]	
1	29.4	1.93	15.9	1.85	14.4	2.30	0.192	
2	15.4	1.03	21.2	0.73	31.2	10.2	0.283	
3	19.5	1.28	18.8	1.04	19.2	7.10	0.169	
4	10.8	0.88	22.4	0.48	35.7	13.1	0.364	
5	24.4	1.47	17.2	1.42	20.5	6.20	0.172	
6	11.3	0.70	22.0	0.51	34.2	11.7	0.325	
7	17.6	1.13	19.8	0.89	23.0	7.40	0.206	
8	8.0	0.58	23.9	0.34	42.8	16.0	0.330	
9	26.2	1.59	16.8	1.56	14.3	5.40	0.150	
10	7.5	0.58	24.4	0.31	43.4	17.9	0.370	
11	23.3	1.35	17.4	1.34	23.1	7.80	0.217	
12	13.6	0.94	22.2	0.61	26.0	9.10	0.253	
13	21.4	1.37	18.9	1.13	29.3	11.0	0.306	
14	13.0	0.82	23.1	0.56	31.4	11.5	0.319	
15	16.5	1.09	21.1	0.78	22.6	7.40	0.176	
16	15.7	1.02	22.2	0.71	27.3	8.50	0.200	
17	15.2	1.04	21.7	0.70	21.4	6.80	0.162	

^a Total sugar produced by enzymatic hydrolysis after 48 h.^b Ethanol of run 1 from samples after 12 h. Runs 3, 15, 16, 17 are after 42 h. Runs 8 and 10 are after 48 h. The remaining runs were from samples at 36 h

centrifuged (4068g, 15 min at 4 °C) and decanted to yield cell pellets, which were washed once with sterile deionized water, and adjusted to a calculated concentration of 30 g dry cell weight (DCW) per liter via standard curves relating 600 nm absorbance to DCW/I concentration. An aliquot was transferred to fresh fermentation medium for an initial cell concentration of 2.0 DCW/l.

The fermentation medium was prepared using hydrolysate after enzymatic hydrolysis obtained as above. Initially 50 ml of 50 mM sodium citrate buffer (pH 6.0) was added to different hydrolysates in 125 ml Erlenmeyer flasks followed by supplementation with 5 g/l yeast extract, 5 g/l urea, 0.5 g/l MgSO₄·7H₂O and 1 g/l KH₂PO₄. The media were autoclaved at 120 °C for 15 min.

2.5. Surface response analysis

A 2³ full factorial design was used in the optimization of oxalic acid corncob pretreatment to improve its simultaneous saccharification and fermentation for ethanol production. Temperature (X_1 , °C), oxalic acid concentration during impregnation of corncob (X_2 , g/l) and reaction time (X_3 , h) were chosen for the independent variables. Ethanol concentration (Y_1 , g/l) and ethanol volumetric productivity (Y_2 , g/l/h) were used as the dependent output variables.

For statistical calculation the variables X_i were coded as x_i according to Eq. (1):

$$x_i = (X_i - \bar{x}_i) / (\Delta x_i / 2) \quad (i = 1, 2, 3, \dots, k) \quad (1)$$

where x_i is dimensionless value of an independent variable, X_i is real value of an independent variable, \bar{x}_i is real value of the independent variable at the central point and Δx_i is step change. A 2³ full factorial design with six axial points ($\alpha = \sqrt{3}$) and three replication at the central points leading to a total number of 17 experiments was employed for the optimization of the condition of oxalic acid corncob pretreatment to improve the simultaneous saccharification and fermentation for ethanol production. The second-degree polynomials were calculated with Design-Expert Version 6.0.6 software (Stat-Ease Inc., USA) to estimate the response of the dependent variables (Eq. (2)). The response and contour plot was performed using Statistica for Windows™ release 5.1 software (StatSoft. Inc., USA).

$$Y_i = b_0 + \sum_{j=1}^n b_j x_j + \sum_{j=1}^n b_{jj} x_j^2 + \sum_{j=1}^{n-1} \sum_{k=j+1}^n b_{jk} x_j x_k \quad (2)$$

where Y_i is the response value, x_i are the coded values of the factors, b_0 is a constant coefficient, b_j are the linear coefficients, b_{jj} (i and j)

are the interaction coefficients and b_{ii} are the quadratic coefficients (Lee et al., 2009).

3. Results and discussion

3.1. The roles of pentosan and lignin contents on separate enzymatic hydrolysis and ethanol fermentation (SHF)

The chemical compositions of solid residues after pretreatment, total sugar obtained by enzymatic hydrolysis and ethanol production by fermentation were shown in Table 2. The oxalic acid pretreated corncob variation was 7.5–29.4% xylan, 0.58–1.93% arabinan, 15.9% and 24.4% lignin. The xylan–lignin ratio range was 0.31–1.85. The lignin content (13.9%) increased following severe pretreatment conditions due to hemicellulose loss during this stage. This trend has been previously reported on the lignocellulosic biomass pretreated with dilute sulfuric acid (Ohgren et al., 2007; Cara et al., 2008).

The criteria used for ethanol production and ethanol volumetric productivity optimization was based on the influence of pentosans (xylan and arabinan) and lignin contents in the oxalic acid pretreated corncobs during sugar releasing in enzymatic hydrolysis. The effects and standard errors for all the dependent variables from the experimental matrix were estimated. From a general analysis it was possible to select the factors and second-order interactions that were significant at the 95% confidence level. For all cases, except for ethanol volumetric productivity, the first-order effects of temperature, oxalic acid and reaction time were significant at a 95% confidence level. An ANOVA of the significant values from Student's t -distribution was made (data not shown) to determine if the regression equation was statistically significant. To improve the models the insignificant models terms (not counting those required to support hierarchy as reaction time for ethanol volumetric productivity) were removed from the models. The ANOVA of the quadratic model is shown in Table 3, which is adjustment to data containing only the significant independent variables. The model's F -values for xylan (160.56), arabinan (122.48), lignin (51.35), xylan and lignin ratio (90.54), total sugar produced (49.27), ethanol (24.86) and ethanol volumetric productivity (33.60) were lower than the tabular F -values indicating that the models are significant in the region studied. For all models the “Lack of Fit F -values” were between 1.78 and 6.05 (Table 3), which implied that the lack of fit was not significant relative to the pure error. To fit the models the

Table 3

Analysis of variance for the adjusted model for xylan (Y_1), arabinan (Y_2), lignin (Y_3), xylan and lignin ratio (Y_4) for pretreated oxalic acid corncob residue. Total sugar produced by enzymatic hydrolysis (Y_5) of pretreated corncob residue and ethanol production (Y_6) and ethanol volumetric productivity (Y_7) for the hydrolysate obtained after pretreated corncob residue enzymatic hydrolysis.

Source	Sum of squares	DF	Mean square	F-value	Prob > P
Y_1 model	627.7	5	125.54	160.56	<0.0001
Residual	8.6	11	0.78	–	–
Lack of fit	7.74	9	0.86	2	0.3775
Pure error	0.86	2	0.43	–	–
Cor. total	636.3	16	–	–	–
Y_2 model	2.05	4	0.51	122.48	<0.0001
Residual	0.05	12	0.00419	–	–
Lack of fit	0.048	10	0.00477	3.67	0.2332
Pure error	0.0026	2	0.0013	–	–
Cor. total	2.1	16	–	–	–
Y_3 model	102.73	4	25.68	51.35	<0.0001
Residual	6	12	0.5	–	–
Lack of fit	5.4	10	0.54	1.78	0.413
Pure error	0.61	2	0.3	–	–
Cor. total	108.74	16	–	–	–
Y_4 model	3.12	6	0.52	90.54	<0.0001
Residual	0.057	10	0.00574	–	–
Lack of fit	0.054	8	0.0067	3.53	0.23957
Pure error	0.0038	2	0.0019	–	–
Cor. total	0.38	16	–	–	–
Y_5 model	1007.66	4	251.92	49.27	<0.0001
Residual	61.36	12	5.11	–	–
Lack of fit	54.64	10	5.46	1.63	0.4401
Pure error	6.72	2	3.36	–	–
Cor. total	1069.02	16	–	–	–
Y_6 model	222.83	5	44.57	24.86	<0.0001
Residual	19.72	11	1.79	–	–
Lack of fit	18.23	9	2.03	2.72	0.2973
Pure error	1.49	2	0.74	–	–
Cor. total	242.55	16	–	–	–
Y_7 model	0.091	6	0.015	33.6	<0.0001
Residual	0.0045	10	0.00045	–	–
Lack of fit	0.00432	8	0.00054	6.05	0.1496
Pure error	0.00018	2	0.00009	–	–
Cor. total	0.095	16	–	–	–

lack of fit needs to be non-significant. The models calculated for all response (Y_1 , Y_2 , Y_3 , Y_4 , Y_5 , Y_6 and Y_7) are in Table 4, where Y_1 , Y_2 , Y_3 , Y_4 , Y_5 , Y_6 and Y_7 are the xylan, arabinan, lignin, xylan–lignin ratio, total sugar released, ethanol production and ethanol volumetric productivity, respectively. Also, a general evaluation for the “Pred R-squared” for all models showed a reasonable agreement with the “Adj R-squared”. So, those models can be used to navigate the design space.

The analysis of response surfaces showed that to enhance ethanol concentration and volumetric productivity it is necessary to increase the amount of sugar released during the enzymatic hydrolysis. To do this it is necessary to minimize the ratio of xylan to lignin, which requires reducing the amount of xylan in the

oxalic acid pretreated corncob. To achieve this, the temperature was set at a high level (+1). Fig. 1 represents the surfaces of Eqs. (1)–(7). The maximum ethanol production (14.35 g/l) and ethanol volumetric productivity (0.33 g/l/h) were obtained from maximum total sugar released (37.39 g/l) during the enzymatic hydrolysis by using oxalic acid pretreated corncob that attained the lowest xylan–lignin ratio (0.30). However, increasing the xylan–lignin ratio, which means increasing xylan content, decreases the amount of sugar released and as consequence the ethanol production and ethanol volumetric productivity is reduced.

These results were obtained by impregnating 0.027 g/g oxalic acid and holding for a reaction time of 74 min while keeping the temperature at 168 °C. In this pretreatment condition the partial oxalic acid pretreated corncob showed lowest values for xylan (8.23%) and arabinan (0.61%) contents. However, the lignin content (24.4%) was the highest of all those observed.

3.2. The conversion rate for glucan to glucose and xylan to xylose during enzymatic hydrolysis and the yields for ethanol and cells during the fermentation

The glucan and galactan variation after pretreatment were 39.4–59.9% and 0.03–0.58%, respectively (Fig. 2A). For the enzymatic hydrolysis, the glucan to glucose and xylan to xylose conversion rates variation were 26–76.2% and 21.4–63.1%, respectively (Fig. 2B). The yields variation for ethanol and cells were 0.168–0.46 g/g and 0.0979–0.262 g/g, respectively (Fig. 2C). When we used the same experimental pretreatment condition described above to improve ethanol and ethanol volumetric productivity, the oxalic acid pretreated corncob showed a high value for glucan content (58.4%) and low value for galactan content (0.064%). Also, we obtained high values for glucan to glucose conversion (76.2%) and ethanol yield (0.4 g/g) during the SHF. However, low values for xylan to xylose conversion (29.42%) and cell yield (0.13 g/g) were obtained during SHF. Similar results were achieved in experiment 4, which showed the highest values for both xylan to xylose (63.1%) and glucan to glucose (73.8%) conversion. In this case, the temperature and oxalic acid concentration were used at high levels (+1) and the reaction time at the lowest value (–1).

The cell yield (Y_x) was improved by using the xylan–lignin ratio in the range of 0.52–0.89, while the ethanol yield (Y_e) was improved at the lowest range (0.34–0.55). The xylan to xylose conversion rate during enzymatic hydrolysis did not interfere in the ethanol yield, but the glucan to glucose conversion rate needs to be at the highest level (71.39–76.2%) to improve this response. Also, a Y_x around of 0.14–0.23 g/g could favor the ethanol yield. The lowest value for Y_x (0.14 g/g) obtained could be related to the xylan to xylose conversion rate, which for this response needs to be in the range of 44.75–55.86%. The most significant results were observed for ethanol production, which could be varied from

Table 4

Polynomial models for xylan (Y_1), arabinan (Y_2), lignin (Y_3), xylan and lignin ratio (Y_4), total sugar produced (Y_5), ethanol (Y_6) and ethanol volumetric productivity (Y_7).

Polynomial models	R-squared	Adj R-squared	Pred R-squared
$Y_1 = 10.81481 - 2.99901X_2 - 2.04709X_3 + 0.67403X_2^2$	0.9865	0.9803	0.9694
$Y_2 = 0.78932 - 0.233092X_2 - 0.15870X_3$	0.9761	0.9681	0.9461
$Y_3 = 23.21266 + 1.22213X_2 + 0.85495X_3 - 0.55084X_2^2$	0.9448	0.9264	0.8854
$Y_4 = 0.449928 - 0.10375X_2 - 0.13917X_3 + 0.071280X_2^2$	0.9819	0.9711	0.9522
$Y_5 = 32.80227 + 1.85286X_2 + 1.72491X_3 + 2.21293X_2^2$	0.9426	0.9235	0.8691
$Y_6 = 12.39652 + 1.12784X_2 + 0.69203X_3 + 0.99706X_2^2$	0.9187	0.8818	0.7616
$Y_7 = 0.268857 + 0.011548X_2 + 0.003435X_3 + 0.017659X_2^2 + 0.045118X_3^2$	0.9527	0.9244	0.8666

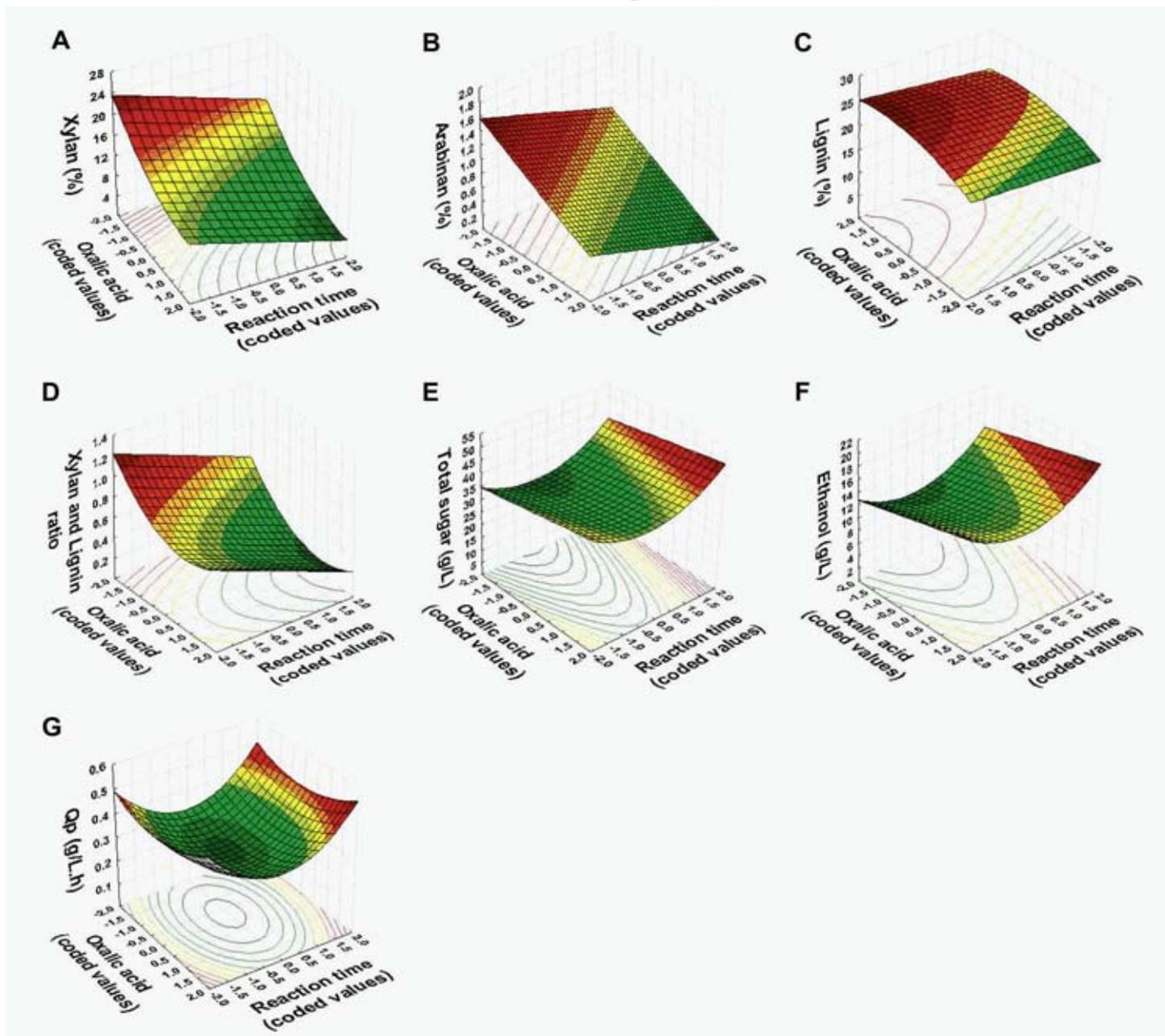


Fig. 1. Response-surface and contour plot of oxalic acid concentration used during corncob impregnation vs. pretreatment reaction time on different factors: A: xylan, B: arabinan, C: lignin contents, D: xylan/lignin ratio E: total sugar, F: ethanol, G: Q_p for the corncob residue after pretreatment.

6.3 to 13.7 g/l during the range of the best cell growth condition. In this case, the ethanol volumetric productivity range was 0.33–0.2 g/l/h and the ethanol yield was kept at high levels (~ 0.4 g/g).

Experiments 2, 4, 6, 8 and 10 (Fig. 2B) showed the highest values for glucan to glucose conversion, which were 67.9%, 73.8%, 71.4%, 75.1% and 76.1%, respectively. These results could be related to lowest values for xylan–lignin ratio, which were 0.73, 0.48, 0.51, 0.34 and 0.31, respectively. In all cases, the independent variable temperature was used at the high level (+1, +1.68). The reaction temperature had the highest effect on glucan to glucose conversion rate by reducing the xylan content in the oxalic acid pretreated corncob and the hardness of the corn residue by its association with high oxalic acid concentration and or reaction time. The improvement in the glucan to glucose conversion rate was inversely proportional to the xylan content in the oxalic acid pretreated corncob. This is indicated that xylan degradation in the pretreatment is important for degradation of cellulose during enzymatic

hydrolysis (Berlin et al., 2005, 2006). Also, experiments 8 and 10 showed the lowest values for xylan to xylose conversion rate, which were 21.4% and 29.9%, respectively. This fact could be related to the low xylan content (7.5–8.0%) concomitant with high contents of glucan (50–50.3%) and lignin (23.9–24.4%) in the oxalic acid pretreated corncob. Also, experiment 1 showed the lowest values for conversion of glucan to glucose and xylan to xylose, but in this case the mildest pretreatment condition was used. In all cases, except for experiment 9, the conversion rate of xylan to xylose was lower than that of glucan to glucose, probably because of the low value for xylanase activity in Accellerase 1000. Experiment 9 used the lowest value for reaction temperature (120 °C). During severe pretreatment conditions, the xylan, arabinan and galactan contents were reduced drastically, while the lignin and glucan contents increased in the oxalic acid pretreated corncob. This could be related to the resistance of glucan to degradation under the pretreatment conditions used in the experiments. However, glucan and lignin

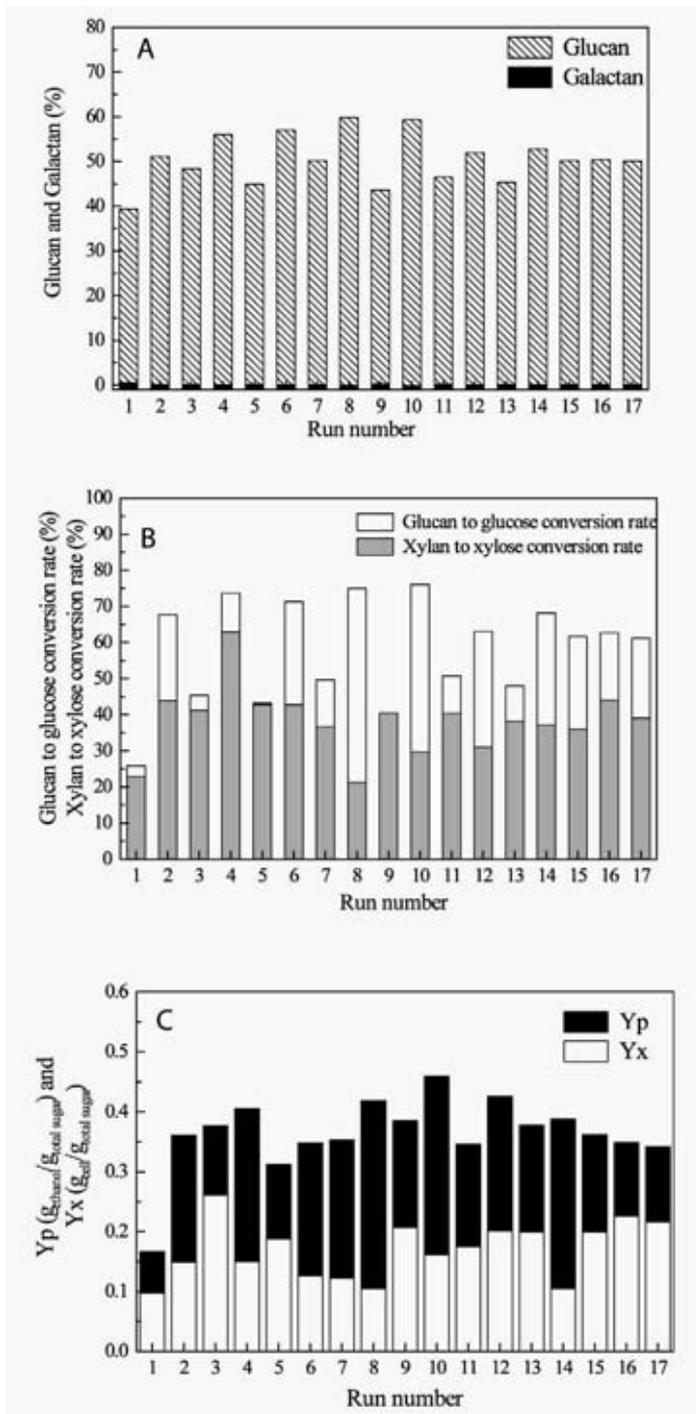


Fig. 2. Content of glucan and galactan (A) in the residue of corncob after oxalic acid pretreatment, glucan to glucose and xylan to xylose conversion ratios (B) during enzymatic hydrolysis of the pretreated corncob residue and ethanol and ethanol volumetric productivity (C) during fermentation of the hydrolysate obtained after pretreated corncob residue hydrolysis.

contents increased due to hydrolysis of the hemicellulosic fraction by oxalic acid pretreatment.

4. Conclusion

Oxalic acid pretreatment was relatively specific for xylan, and could maximize cellulase hydrolysis and ethanol production. When hemicellulose was removed, cellulase saccharification was maximal even though lignin contents were high. Under the most

severe pretreatment condition tested only 7.5% hemicellulose remained in biomass. Cellulose content in pretreated biomass was relatively high. Therefore, greater amounts of fermentable sugar and ethanol were produced by enzymatic hydrolysis and fermentation. To obtain the highest fermentable sugar, xylose should be removed. Pretreated biomass should have high glucan content and a low ratio of xylan/lignin. Oxalic acid pretreatment is suitable for these goals.

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