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Response surface methodology (RSM) to evaluate moisture effects on corn stover in recovering xylose by DEO hydrolysis

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ABSTRACT

Response surface methodology (RSM), based on a 2^2 full factorial design, evaluated the moisture effects in recovering xylose by diethyloxalate (DEO) hydrolysis. Experiments were carried out in laboratory reactors (10 mL glass ampoules) containing corn stover (0.5 g) properly ground. The ampoules were kept at 160 °C for 90 min. Both DEO concentration and corn stover moisture content were statistically significant at 99% confidence level. The maximum xylose recovery by the response surface methodology was achieved employing both DEO concentration and corn stover moisture at near their highest levels area. We amplified this area by using an overlay plot as a graphical optimization using a response of xylose recovery more than 80%. The mathematical statistical model was validated by testing a specific condition in the satisfied overlay plot area. Experimentally, a maximum xylose recovery (81.2%) was achieved by using initial corn stover moisture of 60% and a DEO concentration of 4% w/w. The mathematical statistical model showed that xylose recovery increases during DEO corn stover acid hydrolysis as the corn stover moisture level increases. This observation could be important during the harvesting of corn before it is fully dried in the field. The corn stover moisture was an important variable to improve xylose recovery by DEO acid hydrolysis.

1. Introduction

Corn stover (the stalks, leaves, and cobs that remain after corn grain is harvested) is a great source of lignocellulosic biomass. In the United States, about 75 million dry matter (DM) tons of corn stover residue is produced yearly (Perlack et al., 2005). With technology and land-use improvements, the estimated future production potential of corn stover is greater than 170 million DM tons (Perlack et al., 2005), thus making corn stover a resource of interest for the emerging lignocellulosic biofuels industry (Hess et al., 2009). Its composition is about 70% cellulose and hemicellulose and 15–20% lignin (Montague, 2003). The hemicellulose components can be converted to monomeric and oligomeric sugars by various hydrolysis processes, to form a xylose-enriched liquid hydrolysate fraction (Rodrigues et al., 2003). Kenealy et al. (2007) recently developed a process to treat wood chips of pine, spruce, aspen, and maple at 135–140 °C with vapor-phase diethyloxalate (DEO). Under the conditions used, DEO enters the lumen of the cell where water de-esterifies it to ethanol and oxalic acid (OA). Oxalic acid hydrolyzes the hemicellulose, and the sugars are then washed from the chips, concentrated and fermented (Kenealy et al., 2007). The treated cellulose can be used for paper fiber or hydrolyzed to glucose. The amount and identity of carbohydrates released from the chips depends on the wood species (Kenealy et al., 2007). Increasing the DEO, reaction time, or temperature results in greater carbohydrate release. Mild treatment of wood chips with DEO can release carbohydrates suitable for fermentation and leave the cellulose largely intact. With more severe treatments, several by-products are formed or released in the hydrolysis process. The most important are furans (furfural and 5-hydroxymethylfurfural), carboxylic acids (i.e. levulinic, formic and acetic acids) and phenolics (for example phenol, vanillin, acid vanilic, coumaric acid, syringaldehyde, syringic acid, 4-hydroxybenzaldehyde, etc.) (Palmqvist and Hahn-Hagerdal, 2000; Rodrigues et al., 2010). Cornstalks pretreated only with water at a log severity of 3.5 achieved maximum yields of about 53% of potential xylose with no more than 8% glucose yield (Rubio et al., 1998; Schultz et al., 1984). Also for, corn stover, the highest xylose yields varied from 55% to over 70% (Carrion et al., 1990; Lloyd and Wyman, 2003) and for corn cobs, the highest xylose yield reported was 72% (Garrote et al., 2001). The xylose yields from hemicelluloses were significantly improved by adding small amounts of a sulfuric acid (Lu et al., 2008; Rodrigues et al., 2010; Weiss et al., 2009). Subsequent

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enzymatic saccharification of the corncob residual cellulose was strongly affected by the oxalic acid pretreatment condition with cellulose hydrolysis ranging between 26.0% and 76.2% (Lee et al., 2010). However, although substantially better xylose yields were observed when acid was added for pretreatment (Lu et al., 2008; Rodrigues et al., 2010; Weiss et al., 2009), capital and operating costs would be reduced and other processing simplified if less acid could be used while maintaining good yields. A techno-economic comparison of four pretreatment technologies (dilute-acid, 2-stage dilute-acid, hot water, and ammonia fiber explosion or AFEX) to biochemical ethanol production from corn stover showed the dilute-acid pretreatment process has the lowest ethanol product value (PV) among all process evaluated (Kazi et al., 2010). Corn stover, a major crop-based lignocellulosic biomass feedstock, needs to be at optimal moisture content for efficient bioconversion processes. Environmental conditions surrounding corn stover; as in storage facilities, affect its moisture because of hygroscopic sorption or desorption (Igathinathane et al., 2009). In the present studies, we examined the corn stover moisture and DEO concentration by response surface methodology (RSM) to evaluate moisture effects on corn stover and their efficacy in recovering xylose by DEO hydrolysis.

2. Methods

2.1. Preparation of corn stover for pretreatment

The pretreatment of corn stover obtained by vapor phase diethyl oxalate (DEO) was based on the method developed by Kenealy et al. (2007). Before pretreatment, the corn stover was cut in a knife mill and ground to 3 mm particle size in a Wiley Mill Grinder (model 2).

2.2. Corn stover pretreatment

Pretreatment experiments were carried out in laboratory reactor vials (10 mL glass ampoules) containing corn stover (0.5 g) properly ground. The initial corn stover moisture was determined by gravimetric method and different amounts of water were added to the reactor to alter the initial corn stover moisture content (12%). A small core containing DEO was set-up inside the ampoule, which was closed and placed in the holes of a heating block. The ampoules were kept at 160 °C for 90 min. After the hydrolysis, the material was drained and the remaining liquid analyzed by HPLC for xylose, glucose, arabinose, acetic acid and ethanol contents. The diethyl oxalate was purchased from Sigma–Aldrich Chemical Company (St. Louis, Missouri).

2.3. Variation of corn stover moisture and DEO concentration

Preliminary experiments were conducted at initial DEO concentrations of 0%, 1%, 2% and 4% (w/w), with different corn stover moisture contents corresponding to 12%, 20%, 30%, 40%, 50% and 60% (w/w). After, a factorial design experiments was done using different corn stover moisture and DEO concentration according to Table 1.

2.4. Statistical analysis: response surface methodology (RSM)

A factorial design approach was used to determine the optimal parameters for the model (Box et al., 1978; Montgomery, 2001). The specific design chosen in this case is referred to a 2^2 full factorial design, which was applied to DEO concentration and corn stover moisture to improve xylose extraction from corn stover. Initial DEO concentration (X_1, % of DEO/g of dry matter) and corn stover moisture (X_2, M%) were chosen for the independent variables. Xylose extraction (Y_1, g/L) was used as the dependent variable.

Table 1
The 2^2 full factorial design with 4 centered face points and 3 replicates in the central point employed for xylose recovery by corn stover DEO acid hydrolysis.

Run No.	Variables		Coded levels		Xylose (g/L)
	M (%)	DEO (g/L)	X_1	X_2	
	X_1	X_2			
1	12	0	−1	−1	0.00000
2	60	0	+1	−1	0.00000
3	12	4	−1	+1	0.06841
4	60	4	+1	+1	0.41836
5	12	2	−1	0	0.16919
6	60	2	+1	0	0.38676
7	36	0	0	−1	0.00000
8	36	4	0	+1	0.24209
9	36	2	0	0	0.25000
10	36	2	0	0	0.23000
11	36	2	0	0	0.26000

M = moisture; DEO = diethyloxalate; Xylose (g/L) = xylose recovery.

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

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

where x_i is dimensionless value of an independent variable, X_i is real value of an independent variable, $\bar{x}_j$ is real value of the independent variable at the central point and Δx_j is step change.

Experiments were based on a 2^2 full factorial design with 4 centered faced points and 3 replicates in the central point leading to a total number of 11 experiments.

Statistical tests performed to determine the appropriateness and significance of the response surface models were as follows: (1) normal probability plot of Studentized residuals, e_i, (2) predicted χ^2_{pred} vs. χ^2_{exp} and (3) e_i vs. χ^2_{pred} (Box et al., 1978; Montgomery, 2001). Additional statistical tests performed included calculation of the model p-values and a lack of fit test. The p-values indicate quantitatively the statistical significance of how well the response surface models describe the 2^2 full factorial design with centered faced points and 3 replicates in the central point data sets (Box et al., 1978; Montgomery, 2001).

The second-degree polynomials and overlay plot (graphical optimization) 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).

$$Y_i = b_0 + \sum_{i=2}^{n} b_i x_i + \sum_{i=2}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j \tag{2}$$

where Y_i is the response value, x_i are the coded values of the factors, b_0 is a constant coefficient, b_i are the linear coefficients, b_{ij} (i and j) are the interaction coefficients and b_{ii} are the quadratic coefficients (Box et al., 1978; Montgomery, 2001). Design-Expert determines model parameters using a graphical optimization technique based on user optimization goals and the expected parameter ranges. The program uses multiple starting points of the parameters between the regions values studied. The response surface models were then assessed using statistical tests to determine their appropriateness and significance for describing the 2^2 full factorial design with centered faced points and 3 replicates in the central point data sets (Box et al., 1978; Montgomery, 2001).

2.5. Determination of sugars, acetic acid and ethanol concentrations

Xylose, arabinose, glucose, ethanol and acetic acid were determined by high performance liquid chromatography (HPLC) (Rodrigues et al., 2008) using a refractive index detector (Hitachi High-Technologies Corporation model L-2490, Japan) equipped with an online degasser and Bio Rad (Hercules, CA) Aminex HPX-87H column (300 × 7.8 mm) at 55 °C using 0.005 M H_2SO_4 as, at a flow rate of 0.3 mL/min and an injection volume of 20 μL. Samples were appropriately diluted in deionized water, and then filtered through PrepSEP C18 (Fisher Scientific) filters before injection (20 μL).

3. Results and discussion

3.1. Corn stover moisture efficacy in recovering sugars by DEO hydrolysis: preliminary experiments

Variation in temperature, acid concentration and residence time during lignocellulosic acid hydrolysis promote a chemical change in the hydrolysate composition related to sugar concentration (D-xylose, D-glucose, L-arabinose, mannose, etc.), and release other compounds which are considered inhibitors to microorganisms (Larsson et al., 1999; Lavarack et al., 2002; Rodrigues et al., 2003).

The Figs. 1A–C and 2A show the results for DEO corn stover acid hydrolysis related to recovery of xylose, glucose and arabinose sugars and acetic acid, respectively. Also, ethanol was produced (Fig. 2B) as function of DEO reaction with water in the reactor environment.

During the experiments corn stover moisture ranged from 10% to 60% and DEO concentration from 0% to 4% (w/w). The reaction time as well as reaction temperature was kept constant at 90 min and 160 °C, respectively. The results showed a clear evidence of increasing sugars recovery by increasing the corn stover moisture during the DEO acid hydrolysis (Fig. 1A–C), which was independent of DEO concentration. The maximum xylose and glucose recovery was obtained in experiments 12, 18 and 24, which used 60% corn stover moisture and 1%, 2% and 4% of DEO, respectively (Fig. 1A and B). However, no xylose and glucose was released at 10% corn stover moisture using 1% DEO. Arabinose was not recovered at the same corn stover moisture (10%) independent of DEO concentration. The arabinose started to be released effectively only at 30% corn stover moisture or above (Fig. 1C) independent of DEO initial concentration. This observation could be related to a lack of ethanol production at 10% corn stover moisture (Fig. 2B), which could mean insufficient oxalic acid from DEO to conduct the hydrolysis of the corn stover at this corn stover moisture level.

Ethanol production increased with increasing DEO concentration and corn stover moisture (Fig. 2B), which is related also to the oxalic acid release. During biomass hydrolysis with vapor-phase DEO, water in the cell lumen de-esterifies it to ethanol and oxalic acid. In the presence of a little water and heat the oxalic acid hydrolyzes hemicellulose, and sugars can be washed from the biomass, concentrated and fermented (Kenealy et al., 2007). Overall, no sugar extraction occurred without DEO (Figs. 1A–C and 2A and B). The negative effect of the highest DEO concentration (4%) could be associated with sugar degradation during hydrolysis. This can be seen by comparing the results of sugars at 2% and 4% of DEO at the same corn stover moisture. In this case, the highest or very similar results were found for 2% compared with 4% of DEO. However, this was not noted in comparing 1% and 2% of DEO, which showed the highest results at 2% of DEO concentration at the same corn stover moisture. In general, we obtained the highest concentration for xylose followed by glucose and arabinose (Fig. 1A–C). D-Xylose was the major product in the hemicellulosic hydrolysate, due to the high xylan content in corn stover. In addition, D-glucose and L-arabinose were found in low concentrations. D-Glucose could be released from the cellulose fraction and from the hemicellulose fraction (Aguilar et al., 2002). The mild hydrolysis condition was more than likely not enough to release D-glucose from the cellulose fraction. The L-arabinose could be formed from the arabinoxylan, which is a hemicellulosic heteropolymer that contains more xylose than L-arabinose (Aguilar et al., 2002).

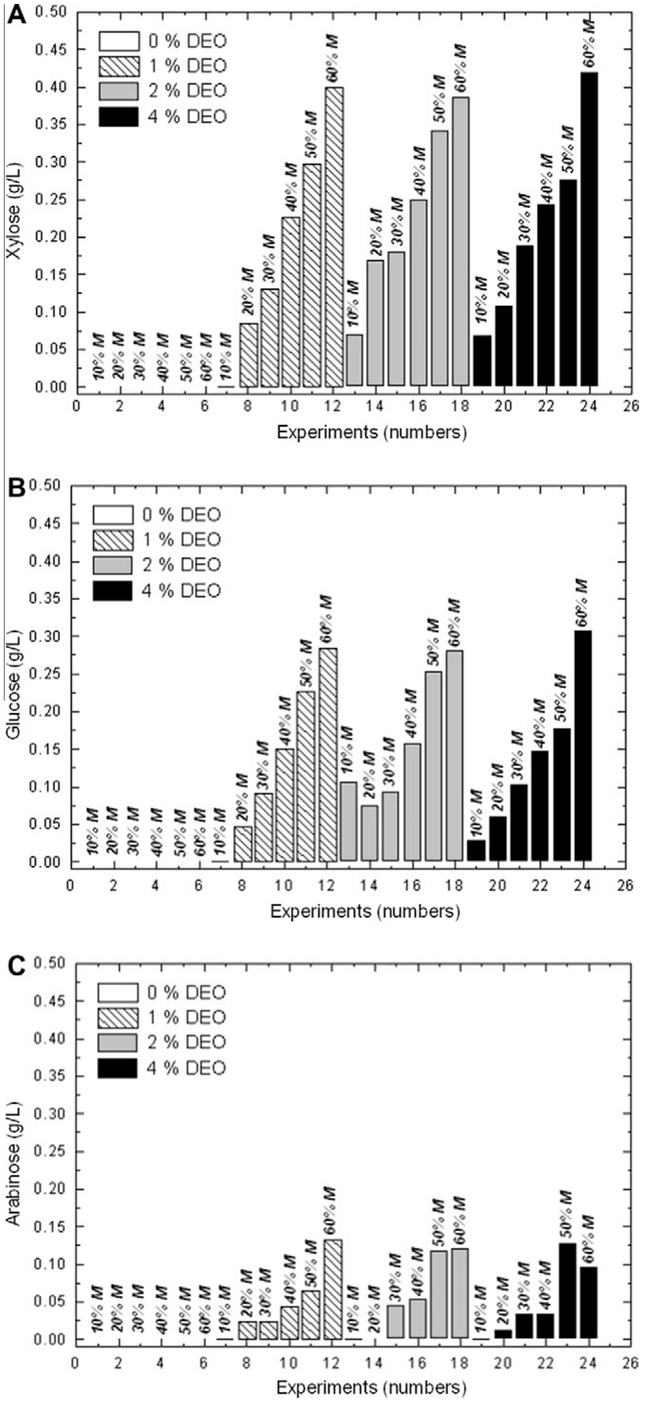

Fig. 1. Xylose (A), glucose (B), and Arabinose (C) volumetric concentrations obtained after corn stover DEO acidic hydrolysis at different acid concentrations and corn stover moistures.

In the field, corn stover moisture is monitored under ambient conditions to aid biomass collection decisions. Timing to collect

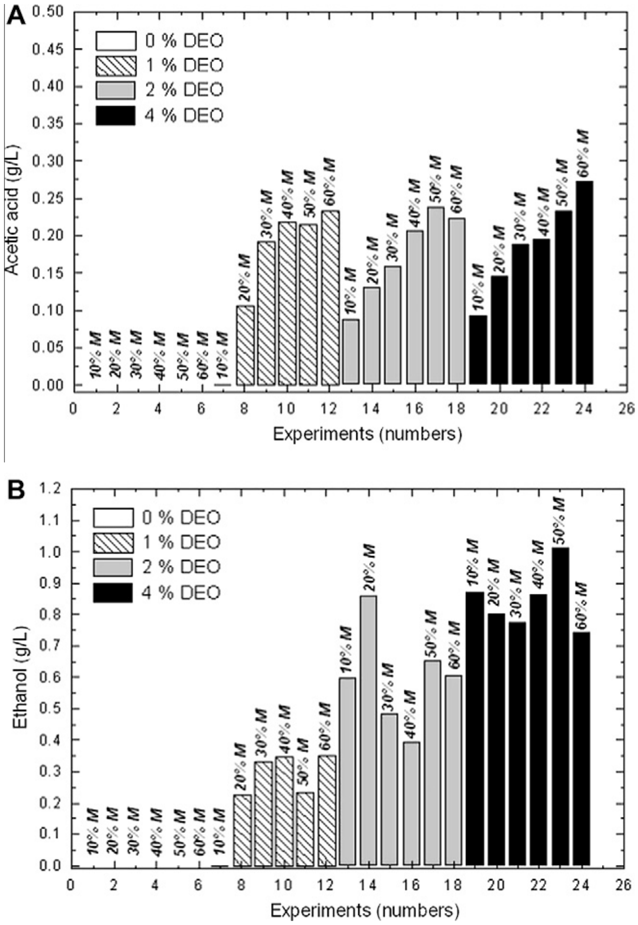

Fig. 2. Acetic acid (A) and ethanol (B) volumetric concentrations obtained after corn stover DEO acidic hydrolysis at different acid concentrations and corn stover moistures.

stover at low moisture depends on elapsed time on field, elapsed time after precipitation, daytime and soil contact (Igathinathane et al., 2009; Womac et al., 2005). However, the corn stover moisture needed to conduct an efficient biomass acid hydrolysis to recover xylose from its hemicellulosic fraction without sugar degradation during DEO hydrolysis needs to be better understood. So, we applied response surface methodology (RSM) to determine the optimum conditions for xylose recovery from corn stover at different DEO concentrations and moisture contents. Additional experiments were done to perform a 2^2 full experimental design.

3.2. Response surface methodology (RSM) to evaluate moisture change on corn stover in recovering xylose by DEO hydrolysis

Initially, for RSM analysis, we carried out the screening analysis involving experiments 01–04 (Table 1) and three replicates of the central point, which were experiments 9, 10 and 11. Effects of corn stover moisture (M) and DEO concentration were statically significant at the 95% confidential level. Also, we obtained significant curvature and no significant lack of fit for the xylose recovery response (data not shown). For that reason, we advance the experiments 05–08 (Table 1), which agreed to face-centered experiments to compose our factorial design. The model aimed to recovery xylose from the corn stover at a proper corn stover moisture content and DEO concentration. Table 2 shows the analysis of variance (ANOVA) for all the factors and interactions. From a general analysis it was possible to select the independent variables and second-order interactions that were significant in the range of 90–95% confidence level. The first-order effects, as initial corn stover moisture (X_1, M) and initial DEO concentration (X_2, DEO) were significant at 95% confidence level together with the interactions X_1X_2 and X_2X_2.

Table 2
Analysis of variance for corn stover xylose recovery (Y_1) by DEO acid hydrolysis as a role of initial corn stover moisture (X_1) and DEO concentration (X_2).

Effects	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	*p*-Value
X_1	0.0536798	1	0.0536798	161.13	0.0001
X_2	0.0885395	1	0.0885395	265.77	0.0000
X_1X_1	0.000583074	1	0.000583074	1.75	0.2431
X_1X_2	0.0306163	1	0.0306163	91.90	0.0002
X_2X_2	0.0509089	1	0.0509089	152.81	0.0001
Total error	0.00166574	5	0.000333149	–	–
Total (corr.)	0.226807	10	–	–	–

R-squared = 0.992656; *R*-squared (adjusted for d.f.) = 0.985311.

Table 3
Analysis of variance after removing factors and interactions not significant at the 95% confidence level for corn stover xylose recovery (Y_1) by DEO acid hydrolysis as a function of initial corn stover moisture (X_1) and DEO concentration (X_2).

Effects	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	*p*-Value
X_1	0.0536798	1	0.0536798	143.22	0.0000
X_2	0.0885395	1	0.0885395	236.23	0.0000
X_1X_2	0.0306163	1	0.0306163	81.69	0.0001
X_2X_2	0.0517226	1	0.0517226	138.00	0.0000
Total error	0.00224882	6	0.000374803	–	–
Total (corr.)	0.226807	10	–	–	–

R-squared = 0.990085; *R*-squared (adjusted for d.f.) = 0.983475.

Table 3 shows ANOVA after removing the independent variables not significant at 90% and 95% of confidential level. The interaction of corn stover moisture versus corn stover moisture (M × M) was removed from the statistical analysis (Table 2).

For the proposed model, the analysis of residues (data not shown) showed a disposed random way in arranging the predicted values by the curve and the average of theirs residues. This does not suggest a geometric pattern that may result from an abnormality in scattering residuals. Thus, the curve satisfactorily represents the points because the errors followed a normal distribution.

The model equation for xylose recovery was based on the regression coefficients obtained from Table 3 related to significant independent variables at 95% of confidential level associated with their respective standard errors.

To test whether the regression equation was statically significant it was performed an analysis of variance (ANOVA) (Table 3), which uses the method of least squares adjustment, including mean squares and interactions of second order. The results showed a 95% confidence level for the independent variables and their interactions showing the estimated *F* was greater than the tabular *F*. Thus, there is evidence of a quadratic relationship between the independent variables and their interactions with the xylose recovery response (dependent variable), which means that all independent variables were statistically significant with good confidence interval. Table 4 shows the analysis of variance for the quadratic model from Table 3.

The total residue was separated into lack of fit and pure error (Table 4). This procedure is valid since replicates were carried out at the central point to get a random error estimative (pure error). Thus, it is possible quantitatively judge if the model represents satisfactorily the observations. The coefficient of determination

Table 4
Analysis of variance for the adjusted model for corn stover xylose recovery (Y_1) by DEO acid hydrolysis as a function of initial corn stover moisture (X_1) and DEO concentration (X_2).

Statistical parameters	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	*p*-value
Model	0.220000	4	0.05600	149.78	<0.0001
Residual	0.002249	6	0.0003748	–	–
Lack-of-fit	0.00178215	4	0.000445538	1.91	0.3720
Pure error	0.000466667	2	0.000233333	–	–

R-squared = 0.990085.

(Table 3) showed that 99% of total variation about the mean is explained by the regression. The *p*-value of the variance model is less than 0.01, which suggests a statistically valid relationship between variables at the 99% confidence level. Eq. (3) represents the mathematical model for maximum xylose recovery during DEO corn stover acid hydrolysis.

$$Y_1 = 0.25919 + 1.0945867x_1 + 0.121477x_2 + 0.0874875x_1x_2 - 0.137713x_2^2 \qquad (3)$$

where Y_1 matches to recovery xylose (g/L) and X_1 and X_2 match to coded values for corn stover moisture (M) and diethyloxalate acid concentration (DEO), respectively.

The magnitude of the independent variables can be noted from the absolute value of the regression coefficient preceding the independent variable in Eq. (3). From the equation, it is evident that the two factors examined alone have a positive impact on xylose recovery. The positive sign in front of the interaction effect X_1X_2 signifies that the combination of initial corn stover moisture (X_1) with either initial DEO concentration (X_2) causes a significant increase in xylose recovery compared to either independent variables examined alone. This can be seen in Fig. 3a, which shows the highest values for xylose recovery were obtained by increasing the values for both independent variables. However, the negative sign in front of the interaction effect X_2X_2 signifies that the quadratic combination of initial DEO concentration (X_2) did not cause a significant increase in xylose recovery (Y_1) compared to either initial DEO concentration examined alone.

The response surface model used Eq. (3) to display the tendency to increase the xylose recovery during DEO corn stover acid hydrolysis at different corn stover moisture levels. Maximum xylose recovery as estimated by the response surface methodology (Fig. 3a) was achieved employing both DEO concentration and corn stover moisture at near their highest levels. We amplified this area by using an overlay plot, as a graphical optimization support for RSM (Fig. 3b).

With multiple responses it is necessary to find regions where requirements simultaneously meet the critical properties, which is known as the "sweet spot". By superimposing or overlaying critical response contours on a contour plot we can visually search for the best compromise. As we are dealing with only two input variables, we can do a direct graphical instead of a numerical optimization to find a feasible region. Graphical optimization displays the area of feasible response values in the factor space. In Fig. 3b, the area that satisfies the constraints is shaded, while the area that does not meet the criteria is blank. Also, we planted flags (inside the "acceptable" area of the graph) that show predictions for all responses at that location in space (Fig. 3b). We gave numeric limits for the shaded area above than 0.4 g/L of xylose recovery, which equaling more than 80% of xylose recovery from DEO corn stover hydrolysis (Fig. 3b).

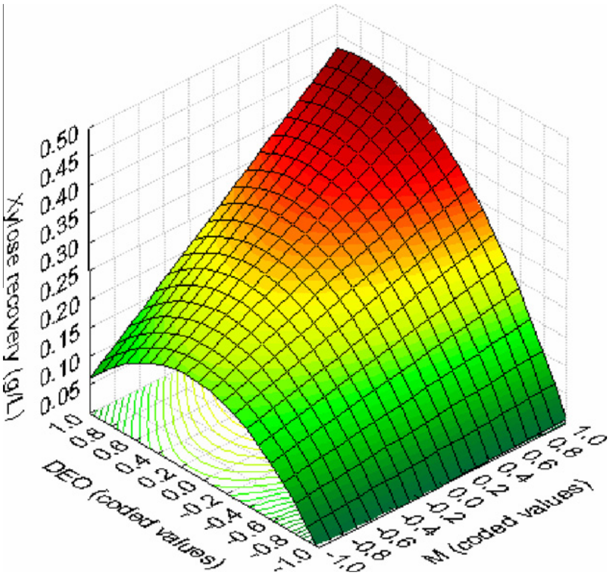

Fig. 3a. Analysis of response surface methodology for corn stover xylose recovery by DEO acid hydrolysis as function of change in the initial corn stover moisture and DEO concentration.

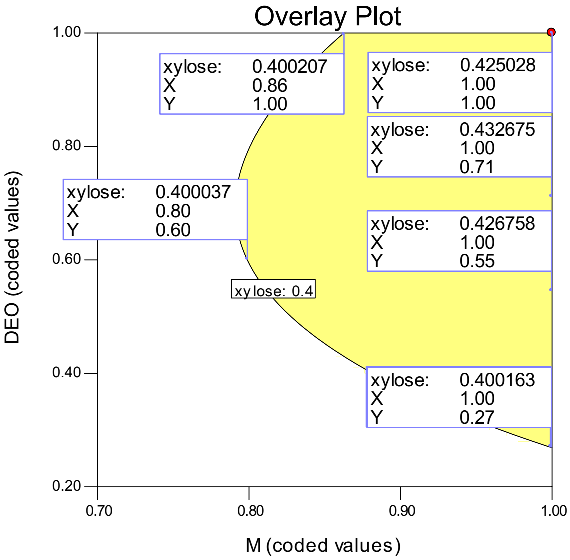

Fig. 3b. Overlay plot revealing window of operability (shaded region) to improve corn stover xylose recovery by DEO acid hydrolysis as function of change in the initial corn stover moisture and DEO concentration.

However, the model predicted a greater xylose recovery value (0.433 g/L) (Fig. 3b) using the coded values 1 and 0.71 for corn stover moisture and DEO concentration, respectively. The uncoded values were 60% and 3.42% DEO, respectively. This prediction was in accord with the preliminary experiments where we detected possible xylose degradation between 2% and 4% DEO.

Model validation could be carried out in any condition above the line corresponding to more than 80% g xylose mass recovery per g of dry matter (0.4 g/L xylose). We chose to validate the model, the highest level for both corn stover moisture (60%) and DEO concentration (4% w/w). By Eq. (3) we obtained 0.425 g of xylose/L as an estimated value for xylose recovery. Experimentally, we obtained a value of 0.418 g/L, which shows that the model can represent mathematically the recovery of xylose from corn stover by DEO acid hydrolysis at coefficient of 0.99. The value for volumetric xylose recovery obtained experimentally to validate the model agreed to 81.16% of g of xylose mass recovery by g of dry matter.

4. Conclusions

Response surface methodology (RSM), based on a mathematical statistical model, showed that xylose recovery increases during DEO corn stover acid hydrolysis as the corn stover moisture level increases. This observation could be important during the harvesting of corn before it is fully dried in the field. The maximum xylose recovery by the response surface methodology was achieved employing both DEO concentration and corn stover moisture at near their highest levels. Experimentally, a maximum xylose recovery (81.2%) was achieved by using initial corn stover moisture of 60% and a DEO concentration of 4% w/w.

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