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Effect of Extrusion Pretreatment on Enzymatic Hydrolysis of *Miscanthus* for the Purpose of Ethanol Production

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Abstract

Lignocellulosic biomass can be converted to energy via several routes. One of them is hydrolysis to sugars with subsequent transformation to fuels and chemicals. Due to the crystalline structure of lignocellulose, pretreatment is a prerequisite to achieving increased enzymatic hydrolysis' rates. The objective of this study was to determine the optimum extrusion operating conditions for glucose and xylose production from *Miscanthus*. Extrusion was conducted in a high shear extruder (single screw type) with compression ratio 3:1. Barrel temperature and screw speed, along with sample moisture content and particle size were the parameters evaluated using Response surface methodology (RSM). Conversion rate to glucose and xylose was monitored after enzymatic hydrolysis with low enzyme loadings (5 FPU g^{-1} of cellulase complex and 18 CBU g^{-1} of β-glucosidase). The optimum conditions for the glucose production (3.63 g L^{-1}) were: barrel temperature 150 °C, screw speed 2.5 Hz, moisture content 20% and particle size 2 mm; the optimum conditions for the xylose production (0.78 g L^{-1}) were: barrel temperature 150 °C, screw speed 1.67 Hz, moisture content 15% and particle size 2 mm. Hence, under controlled conditions, extrusion resulted in better digestibility of *Miscanthus* and as such it can be utilized as a source of glucose and xylose in ethanol production.

Keywords: extrusion, pretreatment, *Miscanthus*, enzymatic hydrolysis, ethanol

1. Introduction

Ethanol is a well-established liquid biofuel, which can be produced from different biomass feedstocks and conversion technologies. Biomass is a complex resource that can be processed in many ways leading to a variety of products (Chum & Overend, 2001). Nowadays, biomass resources have become very important for their use as bioenergy supplies, and research and development efforts directed towards commercial production of ethanol have increased (Voca, Kricka, Janusic, & Matin, 2007). Today, ethanol is the most widely used biofuel either as a total fuel or as a gasoline blend (Fukuda, Kondo, & Tamalampudi, 2009; Demirbas, 2011). However, on commercial basis, it is still produced from sugar and starch-based materials, such as sugarcane and corn; a concern for the continued growth of this first-generation biofuel is the availability of raw feedstock at a reasonable cost (Voca et al., 2009), and, more importantly, food versus fuel dilemma regarding the risk of diverting farmland or crops for liquid biofuels production in detriment of the food supply on a global scale. On the other hand, due to their abundance and low costs, lignocellulosic biomass is especially interesting as a source of fermentable sugars.

The potential for lignocellulosic biomass to supply the feedstock for larger amounts of useful bioenergy with reduced environmental impacts, compared to fossil fuels, has stimulated substantial research and development of systems to grow, harvest, handle, process, and convert biomass to heat, electricity, solid, liquid and gaseous fuels, and other chemicals and products (Taherzadeh & Karimi, 2007). Biomass energy technologies use waste or plant matter to produce energy with a lower level of greenhouse gas emissions than fossil fuel sources (de Vrije, de

Haas, Tan, Keijsers, & Claassen, 2002). Due to the growing trend towards employing modern efficient bioenergy conversion technologies to produce a range of biofuels, they are becoming cost competitive with fossil fuels (Sheehan, Cambreco, Duffield, Gaborski, & Shapouri, 1998). However, since (enzymatic) hydrolysis of lignocellulose is limited by several factors - crystallinity of cellulose, degree of polymerization, moisture content, available surface area, and lignin content (Puhan, Vedaraman, Rambrahaman, & Nagarajan, 2008; Chang & Holtzapple, 2000), efficient biological conversion of biomass depends strongly upon pretreatment processing of raw materials to produce a feedstock which can efficiently be fermented by microorganisms (Taherzadeh & Karimi, 2007; Kahr, Wimberger, Schurz, & Jaeger, 2013). Thus, efficient de-polymerization of cellulose and hemicellulose to fermentable soluble sugars must be assessed in comparison with established sugar- or starch-based ethanol production (Laureano-Perez, Teymouri, Alizadeh, & Dale, 2005).

Limited resources and sustainability of the biomass production system led to intensive investigation of many alternative crops, and the expectation is that high yielding biomass crops would play a critical role (Johnson, Clementson, Mathanker, Grift, & Hansen, 2012). One of these is *Miscanthus*, a woody rhizomatous C4 perennial grass species, which grows rapidly and produces high yields per hectare (Taherzadeh & Karimi, 2007), and which is emerging as one of the most promising crops suitable for biomass production (Heaton, Flavell, Mascia, Thomas, Dohleman, & Long, 2008). Similar to all lignocellulosic biomass, *Miscanthus* is composed primarily of cellulose, hemicelluloses, lignin, and smaller amounts of pectin, protein, extractives and ash. It can be used directly as fuel for production of heat and electric power, or for conversion to other useful products such as ethanol. *Miscanthus* can be converted via several routes, one of them being hydrolysis to sugars with subsequent transformation to fuels and chemicals by chemical conversion or fermentation. However, since raw, untreated biomass is extremely recalcitrant to enzymatic digestion (Hahn-Hagerdal, Galbe, Gorwa-Grauslund, Liden, & Zacchi, 2006), the first steps in the conversion of biomass to ethanol are size reduction and pretreatment (Gray & Zhao, 2006). Pretreatment disrupts the plant cell wall and improves enzymatic access to the polysaccharides (Kim & Holtzapple, 2006). Its efficiency depends upon the particle size. Various pretreatment methods have been explored which promote the accessibility of polysaccharides, in a lignocellulose complex, for enzymatic hydrolysis. They are steam explosion and wet oxidation under alkaline conditions, supercritical CO_2 expansion, mild and concentrated acid hydrolysis and solvent extractions (Gray & Zhao, 2006). These methods often involve high temperature which may lead to the formation of degradation products which act as inhibitors during fermentations (Olsson & Hahn-Hagerdal, 1996). Extrusion is a possible physical pretreatment alternative method, which can be used in the process of ethanol production (Karunanithy & Muthukumarappan, 2010; Liu et al., 2013).

Extrusion is a thermo-chemical processing operation in which the raw material is fed into a hopper and forced down a passage between a rotating screw and a stationary barrel (Gopalakrishna & Jaluria, 1992). The high shear and temperature environment inside the screw channel result in mechanical disruption of the raw material structure, seen as an increase in solubility, loss of water holding capacity, reduced paste viscosity, and softer product texture (Harper, 1992). Therefore, extrusion processing has the potential to disruption the biomass crystalline structure and further improvement enzymatic access to the cellulose and hemicellulose components.

The objective of this research was to determine the optimum operating conditions for the extrusion of *Miscanthus* biomass. Independent variables were biomass moisture content and particle size, and extruder temperature and screw speed. Response surface methodology was used to determine the effect of these independent variables on the enzymatic hydrolysis of extruded *Miscanthus* to fermentable sugars.

2. Materials and Methods

2.1 Raw Material

Miscanthus sacchariflorus, grown in one of the experimental fields (44.315297N − 96.770175W) of the Agricultural Experiment Station, SDSU, Brookings, SD was used as raw material in the investigation. After the harvest, biomass was ground into smaller particles by using a hammer mill (Speedy King, Winona Attrition Mill Co, MN) before storage in environmental conditions, and air-dried before use. Considering the application of such process on industrial scale, and its subsequent feasibility in terms of the raw material, different parts of biomass stalk were mixed and used. Dried biomass (moisture content = 5.6%) was again fed into a hammer mill and was ground to desired particle sizes, for the purpose of further experiments. Compositional analysis of raw *Miscanthus* was conducted prior to the extrusion processing according to NREL protocols (Sluiter et al., 2008a; Hames et al., 2008; Sluiter et al., 2008b; Sluiter et al., 2008c; Sluiter et al., 2006).

2.2 Pretreatment

Twenty-nine pretreatments were performed on *Miscanthus* samples. Pretreatment was conducted in a high shear

extruder (Brabender, PL-2000 Plasti-Corder) of a single screw type with screw compression ratio 3:1. Samples of various particle sizes (0.67, 1.00, 1.50, 2.00, 2.33 mm) and moisture contents, which were achieved by rehydration, (13.34, 15.00, 17.50, 20.00, 21.66%) were conditioned and extrusion processed at different barrel temperatures (83.39, 100.00, 125.00, 150.00, 166.61 °C) and screw speeds (1.39, 1.67, 2.09, 2.5, 2.78 Hz). Extrusion temperature and screw speed of the extrusion process were predefined using PC; feeding was done manually.

2.3 Enzymatic Hydrolysis

Enzymatic hydrolysis of pretreated *Miscanthus* samples was performed using a commercial cellulase complex (NS50013) and β-glucosidase (NS50010) (Novozymes, Bagsvaerd, Denmark). The cellulase complex contained ~70 FPU g^{-1} of cellulose, and the β-glucosidase activity was 250 CBU g^{-1}. One CBU (cellobiase unit) is the amount of enzyme needed to release 2 μmol of glucose per minute under standard conditions with cellobiose as substrate, while 0.185 FPU is that quantity of enzyme activity that will produce reducing sugar equivalent to 2 mg of glucose. Prior to enzymatic hydrolysis, moisture content of the pretreated samples was determined according to NREL protocol (Sluiter et al., 2008a).

Enzymatic hydrolysis was conducted according to the NREL protocol (Selig & Weiss, 2008). Hydrolysis experiments were carried out in 50 mL Erlenmeyer flasks. Untreated *Miscanthus* of the same particle sizes and pretreated *Miscanthus* without enzyme addition were used as controls. Standard conditions of a *Miscanthus* hydrolysis sample were as follows: substrate concentration 30 g L^{-1}, enzyme loadings – the cellulose complex had an activity of 5 FPU g^{-1} of cellulose, and β-glucosidase had 18 CBU g^{-1}, citrate buffer (conc. = 50 mM, pH 4.8). Sodium azide was added as a preservative. Flasks were incubated for 72 hours at 50 °C and 2.5 Hz in an orbital shaker (Thermo Scientific, Forma Orbital Shaker). After incubation, samples were chilled on ice, boiled, and centrifuged (Fisher Scientific, accuSpinTM 400) at 13,000 g for 15 min. Supernatants were collected for further analysis.

Conversion of cellulose and hemicellulose to glucose and xylose during the enzymatic hydrolysis was calculated according to the following equation:

$$\%Conversion = \frac{conc.of\ product\ gL^{-1}}{conc.of\ substrate\ gL^{-1}} \times 100 \quad (1)$$

2.4 Analytical Methods

Total solids, ash, extractives, sugar, acid soluble lignin, and acid insoluble lignin content of the untreated *Miscanthus* were determined according to the NREL Laboratory Analytical Procedures (LAP) (Sluiter et al., 2008a; Hames et al., 2008; Sluiter et al., 2008b; Sluiter et al., 2008c; Sluiter et al., 2006). Total carbon, nitrogen and hydrogen were determined according to the CEN/TS 15104:2005 method, total sulphur according to the CEN/TS 15289:2006 method, while higher heating value was determined according to the CEN/TS 14918:2005 method.

Carbohydrate contents of both, untreated and pretreated *Miscanthus* samples were determined by measuring the hemicellulose (xylose) and cellulose (glucose) derived sugars according to the NREL (Sluiter et al., 2006). Composition of samples after enzymatic hydrolysis was determined using the modified NREL protocol (Sluiter et al., 2008b). Glucose and xylose levels were measured using a HPLC system (Agilent Technologies, 1200 series) equipped with a gradient pump, an automated autosampler, thermostat compartment, BIO-RAD Aminex HPX-87P Column (300 mm × 7.8 mm) and appropriate deashing and guard columns, and refractive index detector (RID). HPLC analysis conditions were as follows: injection volume 20 μL, HPLC grade water as mobile phase, 0.6 mL min^{-1} flow rate, column temperature of 85 °C and run time of 20 min. Monomeric sugar solutions of glucose and xylose were used as standards in concentrations from 0.05 g L^{-1} to 10.0 g L^{-1}. Prior to HPLC analysis, all samples were neutralized with addition of 3 M NaOH.

2.5 Scanning Electron Microscopy (SEM)

To observe the disruption in cell wall structure of the raw material, scanning electron microscope (SEM) pictures of both, untreated and pretreated *Miscanthus* (dried biomass of size 0.67 mm) samples were captured at magnification of approx. 31 mm × 400 SE, at 30 kV (Hitachi, S-3400 N).

2.6 Data Analysis and Modeling

A central composite experimental design (CCD) is one of the most efficient classes of designs capable of generating a response surface. Since it both, (1) identifies independent variables affecting the final product, and (2) studies its effect on dependent variables, it was found suitable for this type of study. A four-level

four-factorial design with five replicates at the center points leading to 29 samples was employed for the optimization of the pretreatment processing conditions. Sample particle size (X_1) and moisture content (X_2), and barrel temperature (X_3) and screw speed (X_4) of the extruder were chosen for the independent variables and are shown in Table 1. The levels chosen for each variable in this study were based on previous research (Xu, Wang, Jiang, Yang, & Ji, 2007; Karunanithy & Muthukumarappan, 2010); the dependent responses observed were glucose and xylose yields.

Table 1. Variables and experimental design levels for response surface

Variables	Symbol	Range and Levels				
		-1.66 (-δ)	-1	0	1	1.66 (A)
Particle size, mm	X_1	0.67	1.00	1.50	2.00	2.33
Moisture content,% wb*	X_2	13.34	15.00	17.50	20.00	21.66
Temperature, °C	X_3	83.39	100.00	125.00	150.00	166.61
Screw speed, Hz	X_4	83.39	100.00	125.00	150.00	166.61

Note. * wb - wet basis.

Experimental data were analyzed according to the response surface methodology using Design-Expert software (Design Expert, 2008). The quadratic model for predicting the optimal point was expressed as:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} x_i x_j \quad (2)$$

The success of the response surface methodology (RSM) depends on the approximation of Y by a low order polynomial in some region of the independent variables (Özer, Gürbüz, Çalimli, & Körbahti, 2009). In Equation (2), Y is the response, xi and xj are variables, k is the number of independent variables (factors), β is the constant coefficient, β_j's, β_{jj}'s and β_{ij}'s are interaction coefficients of linear, quadratic and the second-order terms, respectively.

Regression analyses, statistical significances, and response surfaces of obtained data were evaluated by ANOVA by using Design-Expert 7.1.6. software.

3. Results and Discussion

Raw *Miscanthus* samples used in the study were found to have 34% of cellulose, and 37% of hemicellulose (dry matter basis) (Table 2), which was somewhat different than already reported (Sheehan, Cambreco, Duffield, Gaborski, & Shapouri, 1998; Le Ngoc Huyen, Rémond, Dheilly, & Chabbert, 2010). This might be due to early harvesting date, which was shown to mainly influence the biomass content (Le Ngoc Huyen, Rémond, Dheilly, & Chabbert, 2010; Karunanithy & Mutukumarappan, 2011a).

Table 2. Composition of raw *Miscanthus*

Component	Percentage,% db*
Cellulose	34
Hemicellulose	37
Lignin	24
Ash	5

Note. * db - dry basis.

In order to obtain the maximum hydrolysis yield, *Miscanthus* samples were conditioned and pretreated in the extruder, according to the CCD experimental design parameters (Table 1). Enzymatic hydrolysis was conducted on the latter samples while raw, untreated *Miscanthus* was used as control. Since glucose and xylose are predominant sugars in lignocellulosic biomass (Dien et al., 2006), yields of these sugars were used as reference in evaluating the enzymatic hydrolysis efficiency.

Table 3. Experimental design showing both coded and actual values of extrusion variables and experimental, experimental glucose and xylose responses

Run	Variables*				Glucose response (mg/mL)		Xylose response (mg/mL)	
	X_1	X_2	X_3	X_4	Y_{exp}	Y_{pred}	Y_{exp}	Y_{pred}
1	1 (2.00)	1 (20.00)	1 (150.00)	1 (2.50)	3.63	3.69	0.60	0.59
2	-1 (1.00)	-1 (15.00)	-1 (100.00)	1 (2.50)	1.41	1.60	0.34	0.36
3	1 (2.00)	-1 (15.00)	-1 (100.00)	1 (2.50)	1.88	1.86	0.58	0.53
4	0 (1.50)	0 (17.50)	-1.66 (83.39)	0 (2.09)	2.46	2.41	0.54	0.57
5	0 (1.50)	1.66 (21.66)	0 (125.00)	0 (2.09)	2.10	2.08	0.48	0.46
6	-1 (1.00)	-1 (15.00)	1 (150.00)	-1 (1.67)	2.55	2.59	0.68	0.68
7	0 (1.50)	-1.66 (13.34)	0 (125.00)	0 (2.09)	2.23	2.43	0.46	0.52
8	0 (1.50)	0 (17.50)	1.66 (166.61)	0 (2.09)	3.17	3.39	0.73	0.75
9	0 (1.50)	0 (17.50)	0 (125.00)	0 (2.09)	2.47	2.15	0.55	0.48
10	-1 (1.00)	1 (20.00)	1 (150.00)	1 (2.50)	3.10	3.18	0.52	0.55
11	-1 (1.00)	1 (20.00)	1 (150.00)	-1 (1.67)	1.64	1.57	0.49	0.49
12	0 (1.50)	0 (17.50)	0 (125.00)	0 (2.09)	2.13	2.15	0.47	0.48
13	-1 (1.00)	-1 (15.00)	1 (150.00)	1 (2.50)	3.04	2.85	0.64	0.61
14	-1 (1.00)	1 (20.00)	-1 (100.00)	-1 (1.67)	0.99	1.21	0.43	0.44
15	-1 (1.00)	1 (20.00)	-1 (100.00)	1 (2.50)	1.69	1.52	0.55	0.53
16	0 (1.50)	0 (17.50)	0 (125.00)	1.66 (2.78)	2.74	2.90	0.56	0.57
17	1 (2.00)	1 (20.00)	-1 (100.00)	-1 (1.67)	2.22	2.32	0.55	0.54
18	1 (2.00)	1 (20.00)	1 (150.00)	-1 (1.67)	2.48	2.25	0.51	0.51
19	0 (1.50)	0 (17.50)	0 (125.00)	0 (2.09)	2.16	2.15	0.50	0.48
20	0 (1.50)	0 (17.50)	0 (125.00)	-1.66 (1.39)	2.56	2.57	0.50	0.53
21	-1 (1.00)	-1 (15.00)	-1 (100.00)	-1 (1.67)	2.80	2.63	0.43	0.40
22	1 (2.00)	-1 (15.00)	-1 (100.00)	-1 (1.67)	3.18	3.08	0.56	0.55
23	1.66 (2.33)	0 (17.50)	0 (125.00)	0 (2.09)	1.51	1.73	0.46	0.50
24	0 (1.50)	0 (17.50)	0 (125.00)	0 (2.09)	2.12	2.15	0.42	0.48
25	0 (1.50)	0 (17.50)	0 (125.00)	0 (2.09)	2.09	2.15	0.50	0.48
26	-1.66 (0.67)	0 (17.50)	0 (125.00)	0 (2.09)	0.99	0.95	0.34	0.34
27	1 (2.00)	-1 (15.00)	1 (150.00)	1 (2,50)	2.93	2.68	0.68	0.70
28	1 (2.00)	-1 (15.00)	1 (150.00)	-1 (1.67)	2.52	2.59	0.78	0.75
29	1 (2.00)	1 (20.00)	-1 (100.00)	1 (2.50)	2.54	2.46	0.64	0.66

Note. X_1 – particle size (mm), X_2 – moisture content (%), X_3 – extruder barrel temperature (°C), X_4 – extruder screw speed (Hz); Y_{exp} – experimental value, Y_{pred} – predicted value.

From the experimental design data and its corresponding glucose and xylose yields (Table 2), quadratic predictive polynomials were determined to be:

$$Y_1\left[gL^{-1}\right] = 2.18 + 0.24X_1 + 0.30X_3 - 0.29\left(X_1\right)^2 + 0.27\left(X_3\right)^2 + 0.21\left(X_4\right)^2 + 0.18X_1X_2 + 0.35X_2X_4 + 0.32X_3X_4 \quad (3)$$

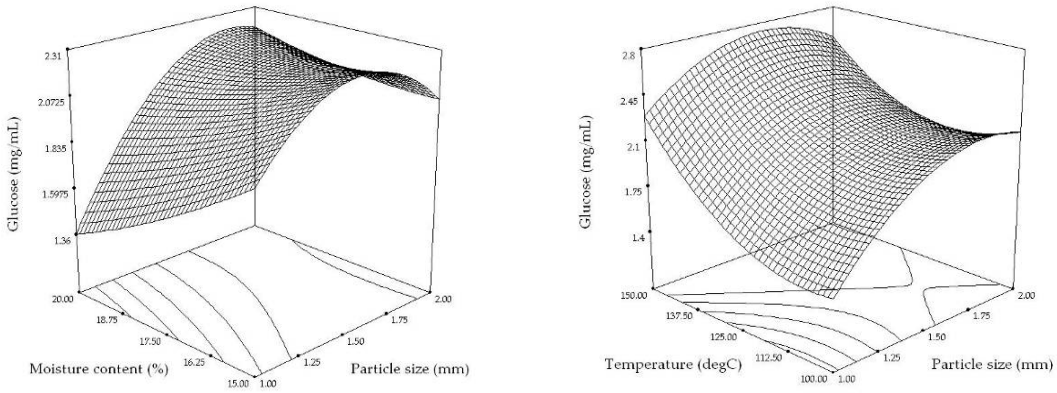
$$Y_2\left[gL^{-1}\right] = 0.47 + 0.048X_1 + 0.054X_3 + 0.065\left(X_3\right)^2 + 0.026\left(X_4\right)^2 - 0.058X_2X_3 + 0.034X_2X_4 \quad (4)$$

Where, Y_1 is glucose yield, Y_2 xylose yield, X_1 particle size (mm), X_2 moisture content (%), X_3 barrel temperature (°C), and X_4 extruder screw speed (Hz). Equation (2) describes significant effects of independent variables on glucose yield for pretreated *Miscanthus*, after a 72-hour enzymatic hydrolysis, in terms of coded values. Stepwise elimination regression with Alpha to Enter = 0.050, Alpha to Exit = 0.050 was used. Glucose yield

significantly depended upon 8 model terms, that is particle size ($p = 0.0003$), barrel temperature ($p < 0.0001$), interactions between particle size and moisture content ($p = 0.0107$), moisture content and screw speed ($p < 0.0001$), and between barrel temperature and screw speed ($p < 0.0001$), and all quadratic terms but moisture content ($p < 0.01$). Coefficient of determination, R2 was determined to be 90.46% ($p < 0.0001$), which suggested that the model was suitable to adequately represent relationship among the selected independent variables, and was in agreement with the reported data for big bluestem (Karunanithy & Mutukumarappan, 2011a). Glucose yield varied from 0.99 g L^{-1} to 3.63 g L^{-1}, with max. yield and cellulose conversion rate of 34.05% when reaction was carried out at 150 °C, 2.5 Hz, particle size of 2 mm, and moisture content of 20% (Table 2). From Equation (3), it can be perceived that the yield was increased with an increase in barrel temperature, and decreased with particle size.

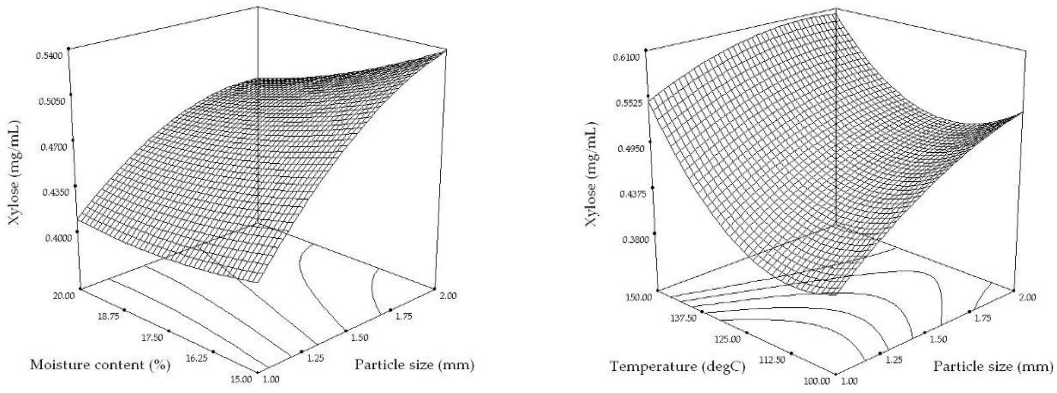
Equation (4) describes significant effects of independent variables on xylose yield for pretreated *Miscanthus*, after a 72-hour enzymatic hydrolysis, in terms of coded values. Again, stepwise elimination regression with Alpha to Enter = 0.050, Alpha to Exit = 0.050 was used. Xylose yield significantly depended upon 6 model terms, that is particle size ($p = 0.0001$), barrel temperature ($p < 0.0001$), interactions between moisture content and barrel temperature ($p < 0.0001$), and moisture content and screw speed ($p = 0.0090$), and quadratic terms of barrel temperature ($p < 0.0001$) and screw speed ($p = 0.0370$). Coefficient of determination, R2 was found to be 86.24% ($p < 0.0001$), whereas adjusted and predicted coefficients were 80.74% and 73.75%, respectively. Xylose yield varied from 0.34 g L^{-1} to 0.78 g L^{-1}, with the highest release (Yield = 0.78 g L^{-1}; hemicellulose conversion rate = 6.82%) being observed when operating in the following conditions: 150 °C, 1.67 Hz, particle size 2 mm, and moisture content 15%; it increased with an increase in particle size and barrel temperature; this was in accordance with the investigation conducted by Karunanithy and Muthukumarappan (2011a, 2011b) on extrusion of big bluestem and switchgrass.

Response surface figures demonstrating interaction effects between chosen test variables are shown in Figure 1 (for glucose) and Figure 2 (for xylose).



(a) (b)

Figure 1. Response surface of glucose formation as a function of (a) moisture content and particle size (barrel temperature = 150 °C; screw speed = 2.5 Hz); (b) barrel temperature and particle size (moisture content = 15%; screw speed = 2.5 Hz)



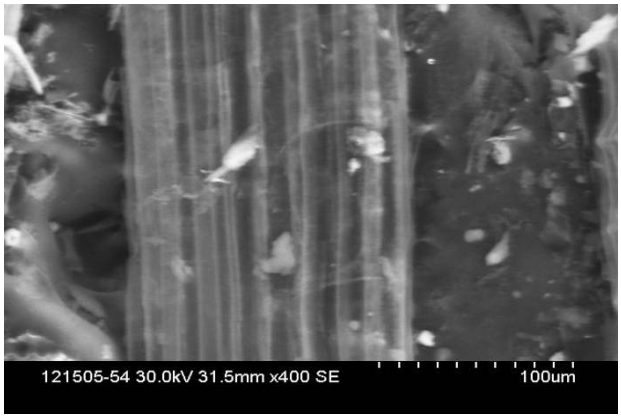
(a) (b)

Figure 2. Response surface of xylose formation as a function of (a) moisture content and particle size (barrel temperature = 150 °C; screw speed = 2.5 Hz); (b) barrel temperature and particle size (moisture content = 15%; screw speed = 2.5 Hz)

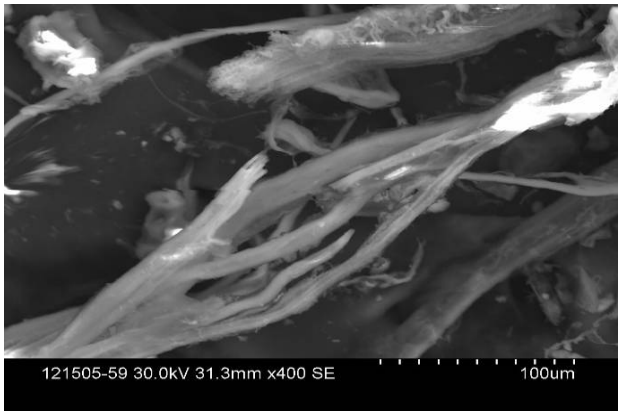
The interactive effects of moisture content and particle size at constant barrel temperature (150 °C) and screw speed (2.5 Hz) on glucose yield are shown in Figure 1a, whereas interactive effect of barrel temperature and particle size at constant moisture content (15%) and screw speed (2.5 Hz) on glucose yield are presented in Figure 1b. From Figure 1a, it can be observed that both, increases in moisture content and particle size led to an increase in glucose yield. Quadratic impact of the observed variables was most significant in the extremes. Figure 1b shows a quadratic impact of the tested variables, with maximum glucose values (2.78%) obtained at the highest observed barrel temperature. This might be due to an increase in barrel temperature that introduce more energy to the material in the barrel, and subsequently enhance the moisture evaporation at the exit (Yu, Ramaswamy, & Boye, 2012).

Figures 2a and 2b depict interaction effect of moisture content and particle size at constant barrel temperature (150 °C) and screw speed (2.5 Hz) on xylose yield, and effect of barrel temperature and particle size at constant moisture content (15%) and screw speed (2.5 Hz) on xylose yield, respectively. It can be perceived that in terms of xylose yield, increase in moisture content had negative effect, whereas increase in particle size had a significant positive effect on the yield after a 72-hour enzymatic hydrolysis of pretreated *Miscanthus* (Figure 2a), which was, again, in accordance with the previous research (Blechschmidt, Engert, & Stephan, 2004; Hu & Wen, 2008; Lee, Teramoto, & Endo, 2009). Moreover, from Figure 2b it can be observed that the xylose yield increased with an increase in both, barrel temperature and particle size. The observed increase in sugar yield with an increase in barrel temperature and particle size was similar to the increase in glucose yield, and again can be explained with the enhancement of the moisture evaporation at the exit.

Furthermore, scanning electron microscope was used to observe changes in microfibril structure due to pretreatment, and compared to untreated ones. Figure 3 shows the cross-section of a *Miscanthus* fiber before (a) and after (b) pretreatment. Breakdown of cell-wall structure caused by extrusion is clearly visible in pretreated samples, since distortion occurred due to mechanical forces. Moreover, it can be seen that microfibrils were separated in pretreated, in comparison to untreated structure, which lead to an increase in external surface area and porosity of the sample, and subsequently to higher enzymatic hydrolysis efficiency as observed in previous studies (Xu, Wang, Jiang, Yang, & Ji, 2007).



(a)



(b)

Figure 3. Cross-section of a Miscanthus fiber (a) before and (b) after extrusion

4. Conclusions

Due to its structure, pretreatment of the lignocellulosic biomass is a prerequisite to achieve higher yields in ethanol production. Therefore, extrusion of *Miscanthus* and its subsequent enzymatic hydrolysis was conducted in order to determine the digestibility of raw material in this study. RSM was adopted in order to optimize the extrusion process. Following, enzymatic hydrolysis was carried out under various conditions. It was found that barrel temperature was the most important variable in terms of its effect; the highest sugar yields were reached at the highest barrel temperature (150 °C). Following, the highest particle size chosen (2 mm), and higher screw speeds resulted in better sugar yields due to better mechanical disruption of lignocellulosic material. Since extrusion process includes operational temperatures which prevent formation of degradation and oxidation products, as an important advantage over other pretreatment methods, and having in mind that the obtained results were comparable to other pretreatment techniques, it can be concluded that under controlled conditions, *Miscanthus* can be extruded and utilized as a source of glucose and xylose, especially as raw material in ethanol production.

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