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STATISTICAL SCREENING AND SELECTION OF SWEET SORGHUM VARIETIES FOR BIOETHANOL PRODUCTION

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Abstract

This study aims at the screening of four cultivars of sorghums as a feedstock for bioethanol production. The straw of these varieties were subjected to pretreatment (dilute sulfuric acid) followed by enzyme hydrolysis to evaluate their potential to produce sugars. Four factor full factorial experimental design ($2\times2\times2\times4=32$) was used to investigate the effects of experimental factors; sorghum varieties (84-Y-01, 85-G-86, Mr. Buster and RARI S-3), acid concentration (1 and 2%), temperature (121 and 140˚C) and pretreatment time (30 and 60 min). The tested sorghum varieties follow the order 85-G-86 (47 g/100g) > Mr. Buster (44.6 g/100g) > 84-Y-01 (42 g/100g) > RARI S-3 (36 g/100g) for their sugar yield. The factors followed given order of significance; variety > temperature > acid concentration > pretreatment time. Sorghum variety (85-G-86) was selected as an appropriate feedstock for bioethanol production due to its higher sugar yield and lower concentration of by-products and furans.

Introduction

The rapid consumption of fossil fuel reserves has not only threatened their future supply but also raised the concerns of global warming. Therefore, a continuous search for non-petroleum based renewable energy sources has already been focused (Dogaris *et al.,* 2012; Rehman *et al.,* 2013a,b). Lignocellulosic bioethanol (LCB) has emerged as one of the alternatives to these fossil fuels (Aydogan, 2012; Konur, 2012; Kim *et al.,* 2013). LCB offers several advantages which includes lower price of feedstock, sustainability and zero risk of food insecurity (Vancov & McIntosh, 2012). Several types of lignocellulosic biomasses have been explored as a feedstock for the production of bioethanol which include agriculture residue, soft and hard wood, waste paper and energy crops (Chen *et al.,* 2012; Rehman *et al.,* 2013c). Lignocellulosic biomass consists of polymers such as cellulose, hemicellulose and lignin (Sipos *et al.,* 2009; Rehman *et al.,* 2014). These polymers are arranged in such a way that they make the biomass complex and recalcitrant. Usually, a pretreatment step is required to deconstruct this complex structure for efficient enzymatic hydrolysis (Kim *et al.,* 2014; Vancov & McIntosh, 2012; Viola *et al.,* 2012). This pretreatment is the most important step in the LCB production chain because it affects the efficiency and cost of downstream processing. The performance and economics of pretreatment depends on the nature of lignocellulosic biomass (Alvira *et al.,* 2010).

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is an important biomass energy crop that is used for the production of bioethanol (Sun *et al.,* 2012; Davila-Gomez *et al.,* 2011; Han *et al.,* 2012; Rao *et al.,* 2012; Yu *et al.,* 2012) because both the juice and stalk of sorghum are used as a feedstock for bioethanol (Dogaris *et al.,* 2012). Sorghum is the fifth major cultivated crop in the world (Umakanth *et al.,* 2012). This crop offers several advantages over other energy crops, such as short growing cycle (3-5 months), higher abiotic resistance (Almodares & Hadi, 2009), higher rate of carbon sequestration (50 g/m^2.day), higher concentration of sugars in its stalks, less requirements of fertilizers (Sher *et al.,* 2012) and pesticides (Serna-Saldivar *et al.,* 2012), and cultivation under different climates (Reddy *et al.,* 2005; Iman & Capareda, 2012; Rao *et al.,* 2012; Vancov & McIntosh, 2012; Zegada-Lizarazu & Monti, 2012). Thus, it proves an economical and productive feedstock for bioethanol. The global production of bioethanol from sorghum ranges between 3-9 m^3/ha (Holou & Stevens, 2012). Recent studies show that bioethanol produced from sweet sorghum is more economical than the bioethanol produced from sugarcane in the USA (Tamang, 2010; Holou & Stevens, 2012). Several varieties of sorghum have been developed and grown in different countries around the world (Zhang *et al.,* 2010; Davila-Gomez *et al.,* 2011; Han *et al.,* 2012). Researchers are continuously striving for the development of new and improved sorghum varieties which may provide higher sugar contents and biomass (Liu *et al.,* 2012). All of these varieties yield lignocellulosic biomass that can be used as a feedstock in bioethanol production. These varieties differ in their composition, and thus, affect the entire bioethanol production chain (Zegada-Lizarazu & Monti, 2012). Thus, it is always pertinent to evaluate available sorghum varieties as a viable feedstock for bioethanol.

This study aims at screening out four sorghum varieties as a potential feedstock for bioethanol. Four sorghum varieties namely 84-Y01, 85-G-86, Mr. Buster and RARI S-3, are compared in terms of their sugar, by-products and furan yield employing dilute sulfuric acid pretreatment and enzymatic hydrolysis. The effects of four factors (sorghum verities, acid concentration, temperature and pretreatment time) are investigated using full factorial experimental design.

Materials and Methods

Biomass: The stalks of five different *Sorghum bicolor* varieties (84-Y01, 85-G-86, Mr. Buster and RARI S-3) were obtained from Millet Research Station Rawalpindi, Pakistan. The harvested biomass (stalks) was air-dried, prepared according to Laboratory Analytical Procedure (Hames, 2004), and stored at -20˚C prior to further analysis. The straw composition of four different sorghum varieties was analysed by strong acid hydrolysis method.

Dilute acid pretreatment and enzymatic hydrolysis: The biomass was subjected to dilute sulfuric acid pretreatment at a fixed solid loading of 20% (w/w). Three factors; acid concentration (1 and 2%), temperature (121 and 140˚C) and pretreatment time (30 and 60 min), were varied simultaneously according to the experimental design. Pretreatment was carried out at predetermined factor levels in an autoclave. The detailed experimental and analytical procedure can be found elsewhere in the literature (Mehmood *et al.,* 2009).

Experimental design and data analysis: A four factor full factorial design was designed to investigate the effect of experimental factors and their interaction on the yield of sugars (glucose, xylose), by-products (acetate, lactate) and furans (furfural, hydroxymeythyl furfural). Four factors were evaluated in this study; one categorical factor (sorghum varieties) and three continuous factors (acid concentration, temperature and time). Two levels were used for all the continuous factors; acid concentration (1, 2%), temperature (121, 140˚C) and pretreatment time (30, 60 min), whereas four levels (84-Y01, 85-G-86, Mr. Buster and RARI S-3) were used for categorical factor of sorghum varieties. Thus, a set of 2×2×2×4=32 experimental combinations was obtained as given in Table 1. Regression analysis was used to analyze the experimental data employing a model as given in the following equation:

$$Y\ (g/100g) = \beta_0+\sum\beta_i X_i+\sum\beta_{ij} X_i X_j+\varepsilon \qquad (1)$$

where β denotes coefficients of regression for intercept, linear and interaction terms, respectively. Y is the response vector for sugars, by-products and furans, whereas X_i and X_j are the independent factors in coded units, and ε is the error term (Chang *et al.,* 2011). Analysis of variance (ANOVA) was carried out to determine the effect each factor and its interactions at a significance level of 95%. The experimental data was compared with modelled data to evaluate the adequacy of model (Vancov and McIntosh, 2012). The experimental design and its analysis were carried out by JMP Trial version (SAS Institute Inc., Cary, North Carolina).

Table 1. Composition of *Sorghum bicolor* straw.

Component (%)	Sorghum verities			
	84-Y-01	85-G-86	Mr. Buster	RARI S-3
Cellulose	38.25 ± 1.23	42.37 ± 0.94	39.93 ± 0.17	37.35 ± 0.64
Hemicellulose	22.05 ± 0.82	23.92 ± 0.21	23.37 ± 0.29	23.43 ± 0.30
Lignin	19.65 ± 0.45	18.98 ± 0.79	18.08 ± 0.58	17.92 ± 1.13
Ash	7.24 ± 1.2	5.96 ± 1.24	7.4 ± 0.78	6.96 ± 0.85
Moisture	9.43 ± 0.002	9.50 ± 0.02	6.74 ± 0.03	4.28 ± 0.33

Results

The composition of different varieties of harvested sorghum straw is given in Table 1. Cellulose was found as one of the major component that varies from 37 to 43% among all the varieties, whereas hemicellulose and lignin were present between 22-24% and 17-20%, respectively. Sorghum variety (85-G-86) contained 42.37% cellulose compared with RARI S-3 that possesses up to 37.35% cellulose. However, both of these varieties (85-G-86 and RARI S-3) had shown opposite trend for their lignin contents (18.98% and 18.08%). Four sorghum varieties followed the given rank according to their cellulosic and hemicellulosic contents i.e. 85-G-86 > MR. Buster > RARI S-3 > 84-Y-01. Statistical analysis revealed that these sorghum varieties did not different in terms of their sugar constituents ($p > 0.05$).

Dilute sulphuric acid (DSA) process was applied to screen out sorghum varieties based on their extractable sugar yield. The effect of pretreatment, followed by enzymatic hydrolysis, on the production of sugars, by-products and furans from all of the four varieties is presented in Table 2. *Sorhum bicolor* variety (85-G-86) yielded the maximum sugar concentration, 47 g/100g of DM, employing following factor levels i.e., 121˚C, 1% acid concentration and 30 min. Moreover, this variety yielded by-products (2.04 g/100g) and furans (0.98 g/100g) at the aforementioned process conditions. The investigated sorghum varieties were ranked on the basis of their maximum sugar yield as followed: 85-G-86 (47 g/100g) > MR. Buster (44.6 g/100g) > 84-Y-01 (42 g/100g) > RARI S-3 (36 g/100g). The experimental data was further analyzed carrying out regression analysis. The codified polynomial regression models are given in the following Eqs.2-4 which correlate experimental responses (sugars, by-products and furans) with each of the experimental factor and their interactions:

Sugar (g/100g) = +40-1.022A-1.178B-0.853C+1.747D-0.190AB -0.466AC-0.978AD (2)
-0.322BD-0.397CD

By-products (g/100g) = 2.410+0.097A+0.242B+0.113C-0.022X-0.107AB+0.009AC (3)
-0.007BC-0.085156AD-0.115BD-0.068CD

Furans (g/100g) = 2.092+0.063A+0.314B+0.167C-0.079D-0.408AB-0.083AC-0.04BC(4)
-0.078AD-0.182BD-0.105CD

where A, B, C, D are the coded values of experimental factors such as acid concentration, temperature, pretreatment time and sorghum variety, whereas AB, AC, AD, BC, BD and CD are the interactions among these factors. Eqs. 2-4 are valid for sorghum variety 85-G-86 only with D=1, and the expressions for other varieties are not given. The factors, whose coefficients have positive values in above Eqs, increase the yield for an increase in their level and vice versa (Coruh *et al.,* 2011). The effect (signs) and magnitudes (value) of these factors and their interactions are presented in Table 3. Thus, it is apparent from above Eqs. 2-4 that sugar yield is a function of sorghum variety (85-G-86) only. The rest of factors (acid concentration, temperature, and pretreatment time) showed a negative effect on the sugar yield. Contrary to this, all of these factors, except sorghum variety, increased the concentration of by-products and furans. The maximum concentration of by-products (3.41 g/100g) was obtained at 140˚C, 2% acid concentration and 60 min, whereas that of furfural (4.13 g/100g) was obtained at 140˚C, 1% acid concentration and 60 min. It is apparent from Eqs 3-4 that the effect of acid concentration (A) is less pronounced than the pretreatment time and temperature level. These findings were further verified via the effect test that described the contribution, of each factor and its combinations, to the model (Table 4). The factors and their combinations were statistically significant whose Prob > F value was less than 0.05. These factors followed the given order of significance: variety > temperature > acid concentration > pretreatment time. However, no significant interaction appeared to exist among all these investigated factors. The adequacy of regression models was further verified by the closeness of experimental values of sugars, by-products and furans against their respective predicted values, as presented in Table 1. The predicted values were normally distributed along their mean value which verified the adequacy of the regression models to describe the production of sugars, by-products and furans according to the Eqs 2-4.

Table 2. Factorial design matrix for investigated factors with actual and predicted response values for sugars, by-products and furans.

Coded factors				Actual factors				Sugar (g/100g)		Byproducts (g/100g)		Furans (g/100g)	
Acid conc.	Temp.	Time	Variety	Acid conc.	Temp	Time	Variety	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
-1	-1	1	85-G-86	1	121	60	85-G-86	46	44.27	2.21	2.18	1.54	1.67
1	1	-1	85-G-86	2	140	30	85-G-86	39	39.77	2.38	2.37	2.01	1.78
-1	1	-1	85-G-86	1	140	30	85-G-86	42	43.22	2.61	2.58	2.69	2.46
-1	1	1	85-G-86	1	140	60	85-G-86	40	41.66	2.64	2.64	2.9	2.68
-1	-1	-1	85-G-86	1	121	30	85-G-86	47	45.84	2.04	2.10	0.98	1.30
1	-1	1	85-G-86	2	121	60	85-G-86	36	39.72	2.45	2.44	2.08	2.29
1	-1	-1	85-G-86	2	121	30	85-G-86	44	43.16	2.34	2.32	2.12	2.25
1	1	1	85-G-86	2	140	60	85-G-86	40	36.34	2.43	2.47	1.78	1.66
-1	1	-1	PARI S-3	1	140	30	PARI S-3	38	36.22	2.32	2.58	2.11	2.46
1	1	1	PARI S-3	2	140	60	PARI S-3	35	32.42	2.33	2.47	2.41	1.66
1	1	-1	PARI S-3	2	140	30	PARI S-3	37	36.02	2.08	2.37	1.61	1.78
-1	-1	-1	PARI S-3	1	121	30	PARI S-3	37	40.51	1.66	2.10	0.75	1.30
-1	-1	1	PARI S-3	1	121	60	PARI S-3	36	38.78	1.83	2.18	1.22	1.67
1	-1	1	PARI S-3	2	121	60	PARI S-3	37	37.48	2.08	2.44	1.77	2.29
-1	1	1	PARI S-3	1	140	60	PARI S-3	39	34.49	2.59	2.64	2.59	2.68
1	-1	-1	PARI S-3	2	121	30	PARI S-3	38	41.07	2	2.32	1.49	2.25
1	-1	1	84-Y-01	2	121	60	84-Y-01	40	39.47	2.85	2.48	2.61	2.65
-1	1	1	84-Y-01	1	140	60	84-Y-01	40	39.41	2.34	2.62	2.17	2.40
-1	-1	-1	84-Y-01	1	121	30	84-Y-01	40	41.09	1.65	1.64	1.07	0.91
-1	-1	1	84-Y-01	1	121	60	84-Y-01	41	41.02	2.915	1.75	2	1.36
1	1	-1	84-Y-01	2	140	30	84-Y-01	39	39.02	2.99	2.81	2.27	2.09
-1	1	-1	84-Y-01	1	140	30	84-Y-01	40	39.47	3.3	2.54	2.13	2.10
1	-1	-1	84-Y-01	2	121	30	84-Y-01	42	41.41	3.03	2.33	2.38	2.53
1	1	1	84-Y-01	2	140	60	84-Y-01	36	37.09	3.32	2.92	2.67	2.05
-1	-1	-1	Mr. Buster	1	121	30	Mr. Buster	44.5	45.57	1.6	1.28	1.2	1.65
1	1	1	Mr. Buster	2	140	60	Mr. Buster	35.9	35.89	3.41	3.19	1.62	2.19
1	-1	-1	Mr. Buster	2	121	30	Mr. Buster	44.4	44.46	2.06	1.72	2.57	2.38
1	-1	1	Mr. Buster	2	121	60	Mr. Buster	41.4	40.95	2.17	2.26	2.98	2.40
-1	1	1	Mr. Buster	1	140	60	Mr. Buster	38.5	39.63	3	3.15	4.13	3.43
1	1	-1	Mr. Buster	2	140	30	Mr. Buster	39	39.40	2.2	2.68	2.12	2.32
-1	-1	1	Mr. Buster	1	121	60	Mr. Buster	44.6	43.92	1.8	1.79	1.68	2.01
-1	1	-1	Mr. Buster	1	140	30	Mr. Buster	42.8	41.27	2.49	2.67	3.29	3.23

Table 3. Parameter estimates for the factors (terms) for the sugar model.

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	40.003125	0.35082	114.03	<.0001
Acid Conc.(1,2)	-1.021875	0.35082	-2.91	0.0121
Temp(121,140)	-1.178125	0.35082	-3.36	0.0051
Time(30,60)	-0.853125	0.35082	-2.43	0.0302
D[84-Y-01]	-0.253125	0.607638	-0.42	0.6838
D[85-G-86]	1.746875	0.607638	2.87	0.0130
D[Mr. Buster]	1.384375	0.607638	2.28	0.0402
Acid Conc.(1,2)*Temp(121,140)	-0.190625	0.35082	-0.54	0.5961
Acid Conc.(1,2)*Time(30,60)	-0.465625	0.35082	-1.33	0.2073
Temp(121,140)*Time(30,60)	0.078125	0.35082	0.22	0.8272
Acid Conc.(1,2)*D[84-Y-01]	0.521875	0.607638	0.86	0.4060
Acid Conc.(1,2)*D[85-G-86]	-0.978125	0.607638	-1.61	0.1315
Temp(121,140)*D[85-G-86]	-0.321875	0.607638	-0.53	0.6052
Time(30,60)*D[85-G-86]	-0.396875	0.607638	-0.65	0.5250

Table 4. Effect tests for the sugar model.

Source	Sum of squares	F-ratio	Prob > F
Acid Conc. (1,2)	33.41531	8.4845	0.0121
Temp (121,140)	44.41531	11.2775	0.0051
Time (30,60)	23.29031	5.9137	0.0302
D (84-Y01, 86-G-87, Mr. Buster, RARI S-3)	106.52594	9.0160	0.0017
Acid Conc. (1,2)*Temp (121,140)	1.16281	0.2953	0.5961
Acid Conc. (1,2)*Time (30,60)	6.93781	1.7616	0.2073
Temp (121,140)*Time (30,60)	0.19531	0.0496	0.8272
Acid Conc. (1,2)*D	13.47094	1.1401	0.3695
Temp(121,140)*D	25.42094	2.1516	0.1429
Time(30,60)*D	5.59594	0.4736	0.7059

Discussion

All of the investigated sorghum varieties were found statistically similar in terms of their compositional analysis ($p > 0.05$). These compositional values of sorghum varieties were found consistent with the reported values in literature (Mehmood *et al.,* 2009). However, Vancov & McIntosh (2012) reported contradictory results regarding carbohydrate contents of MR. Buster (Cellulose 32.4% and hemicellulose 27%) compared with our results (Cellulose 39.93% and hemicellulose 23.37%). Lignin content could discriminate all of these varieties for their utilization as a feedstock for bioethanol. Although sorghum variety 85-G-86 possessed 6% more carbohydrates than the MR. Buster, but MR. Buster contained 4.75% lower lignin content than the variety 85-G-86 (Table 1). This lignin content could affect sugar release during pretreatment and subsequent enzymatic hydrolysis steps (Han *et al.,* 2012). Thus, these varieties could not be selected as feedstock for bioethanol until these were evaluated for their extractable sugar yield. Pretreatment is an important step in the production of bioethanol from lignocellulosic biomass that deconstructs the biomass, and leads to better enzymatic hydrolysis (Phuengjayaem & Teeradakorn, 2011; Cao *et al.,* 2012). DSA is one the most common pretreatment method that is used to depolymerize lignocellulosic biomass for sugar extraction (Liu *et al.,* 2012). The results of pretreatment, followed by enzymatic hydrolysis, revealed that Sorghum variety (85-G-86) produced 5.4-27% more sugars compared with the other varieties. This result was obvious due to the higher carbohydrate content of the variety (85-G-86). Though top two sorghum varieties, 85-G-86 and MR. Buster, did not differ statistically in terms of their sugar yield ($p>0.05$) against different pretreatment conditions, the variety (85-G-86) produced 5.4% more sugars than MR. Buster employing half of the processing time (30 min). Vancov and McIntosh (2012) reported contradictory results and obtained higher sugar yield from MR. Buster compared with our results. Moreover, furans produced from MR. Buster (1.68 g/100g) were 70% higher than the variety 85-G-86 (0.98 g/100g). These furans inhibit the activity of micro-organisms in the subsequent fermentation process (Saha *et al.,* 2005), and are not desired in the hydrolyzate. Thus, it seems more economical to use sorghum variety 85-G-86 than MR. Buster as a feedstock for bioethanol production because it produces more sugars, less furans, and it requires a shorter pretreatment time.

The sugar release from tested varieties of sorghum appeared a function of cultivars itself compared with other experimental factors (acid concentration, temperature, and pretreatment time). On the other hand, all of the continuous factors were found responsible to increase the concentration of by-products and furans in the hydrolyzate. Dogaris *et al*., (2012) reported that the formation of by-products and furans, during dilute sulfuric acid pretreatment of sorghum, increased as a function of acid concentration (>1%). However, Liu *et al*., (2012) stated that elevated temperature and longer pretreatment time could degrade carbohydrates into furans. Longer processing time was dominant over elevated temperature for the formation of furans. Vancov and McIntosh (2012) also found that extended pretreatment time (>30 min) reduced sugar yield due to its degradation into by-products and furans. Thus, all of the process factors were effective up to a certain level beyond which formation of inhibitory products was noticed.

Conclusion

Sorghum bicolor is an emerging feedstock for bioethanol due to its unique characteristics. However, several sorghum varieties cannot be chosen as feedstock just on the basis of their chemical composition. These varieties may contain similar composition but they may yield variable amount of sugars and inhibitory products depending upon their agronomic, environmental and process factors. Among tested sorghum varieties, variety (85-G-86) was selected as an appropriate feedstock for bioethanol production due to its higher sugar yield and lower generation of by-products and furans.

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