OPTIMIZATION OF BIOETHANOL PRODUCTION FROM CORN COBS BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION USING RESPONSE SURFACE METHODOLOGY

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The present study was carried out to optimise the simultaneous saccharification and fermentation process for bioethanol production from corn cobs. Ten (10) different corn genotypes (hybrids) were characterized in terms of chemical composition, including total solid, moisture, cellulose, hemicelluloses, lignin and ash contents. Among different corn genotypes, milled cobs of corn genotype PMH10 were found to have significantly high cellulose (34.05%) and low lignin content (11.87%). With sodium hydroxide pretreatment, the relative proportion of cellulose (56.70%) increased, while that of hemicelluloses, lignin and ash substantially decreased (11.87, 8.61 and 0.6%) in the treated cob residues. The optimization of the simultaneous saccharification and fermentation (SSF) process of pretreated cob residues through response surface methodology showed that maximum ethanol concentration of 3.64 mg/mL could be achieved when SSF was performed at 28.58 FPU/g enzyme dosage, solid loading of 14.95% and yeast inoculum of 9.56%.

Keywords: corn cobs, pretreatment, cellulase, simultaneous saccharification and fermentation, bioethanol

INTRODUCTION

Maize is the 'Queen of Cereals' and ranks second in terms of global acreage. India produced 31.51 million tonnes of maize on 9.9 million hectares in 2020-2021.[1] The United States of America is the major producer of maize and contributes 30 percent of the total production in the world. Energy production from corn can meet the future fuel demands and encourage the farmers to use maize as an alternative kharif crop to reduce water table depletion and straw burning.[2] The corn grain is harvested along with the husk, shank and silk. The other corn parts are left in the soil for *in-situ* deterioration.[3] Every 100 kg of corn grains produces 18 kg of cobs.[4] The cobs represent about 20 per cent by weight of the corn residues and are either buried or left as such in the field.[5]

Corn cobs are considered as one of the most potential lignocellulosic feedstocks for bioethanol production. They have a peculiar chemical composition, with high hemicelluloses, as well as low lignin and ash contents, as compared to other biomass types.[6] Specifically, corncobs are mainly composed of cellulose (39–65%), hemicelluloses (25–35%) and lignin (17–21%), with small amounts of ash and extractives.[8] They have higher bulk density and thus are easy to collect and transport.[7] The economically viable utilization of corn cobs for the production of bioethanol and other bio-based products is a great challenge today due to their structural complexity. The cellulose, hemicelluloses and lignin in the cob are intricately associated, which makes them difficult to be degraded by enzymes and microbes.[9] The three major steps involved in the ethanol production from corn cob include pretreatment, saccharification and fermentation. The pretreatment is a key process to break the cob

biomass and to make it accessible to hydrolysing enzymes for the release of monomeric sugars that can be eventually converted into ethanol or other valuable bio-products. The pretreatment increases the surface area of the biomass and breaks the lignin seal in order to release hemicelluloses and decrease the crystallinity of cellulose.[10]

Pretreatment can be accomplished through various methods, such as physico-chemical, physical, biological, chemical or combined treatments. Among different types of pretreatments that could be used, alkaline pretreatment is the most appropriate for corn biomass and other monocots due to their peculiar cell wall composition. The cell wall of monocotyledonous plants contains lignin with high phenolic hydroxyl groups, as well as alkali-labile ferulate ester cross-links within hemicelluloses. This renders increased solubility of their walls in an alkaline solution and makes them more susceptible to delignification.[11] Sodium hydroxide treatment increases the internal surface area of the biomass, disrupts the lignin structure and decreases the crystallinity of cellulose, thereby, enhancing the reactivity of polysaccharides with the hydrolytic enzymes. Alkaline treatment of biomass also causes the minimal generation of sugar degradation products, although the extent of hemicellulose hydrolysis has been reported to be low as compared to acid treatment.[12]

The hydrolysis of the pretreated biomass into simple sugars is a cost-intensive step for bioethanol production. The major ethanol production cost (20-25%) is due to the enzymatic saccharification step that requires high-cost enzymes for the hydrolysis of biomass.[13] The enzymatic hydrolysis and fermentation is generally performed sequentially and is referred to as a separate hydrolysis and fermentation (SHF) process. The main drawback of this process is the inhibition of cellulase activity by the released sugars, mainly cellobiose and glucose.[14] To scale up the industrial ethanol fermentation process, it becomes pertinent to identify the major limiting factors that are involved during the saccharification and fermentation process. There is a need to develop an efficient biomass saccharification and fermentation processes that could not only enhance the yields of ethanol with short fermentation time, but also minimize the operation cost.[15] The process of simultaneous saccharification and fermentation (SSF) is considered as a constructive operational strategy to minimize the cost of production and increase the concentration of ethanol in relatively shorter time due to the elimination of long saccharification steps. The SSF process is usually carried out at the same working temperature within a single reactor and there is continuous removal of sugars that would otherwise inhibit cellulases or β-glucosidases.[16] Several studies have been focused on optimization of the SSF process. Zhang *et al.*[17] subjected corn cobs to batch SSF process and ethanol concentration as high as 69.2 g/L was achieved with 19% dry matter (DM), thereby, resulting in 81.2% overall ethanol yield. Similarly, McIntosh *et al.*[18] reported high ethanol titer and glucan to ethanol yields of 56 g/L and 90%, when pretreated *Eucalyptus grandis* was subjected to SSF.

Many agricultural crops have unique biomass composition, but the knowledge about differences in biomass composition within a particular crop is either lacking or only superficial. Saccharification yields or degradability of cell walls can also vary from 0.1% to 0.5%, depending on the type and composition of the agricultural biomass and the reaction to different pretreatments and hydrolytic processes. The biomass of different crops may respond differentially to the similar pretreatment process or have different cell wall characteristics that facilitate differential saccharification or biomass digestibility.[19] Keeping these points in view, the present study focused on the compositional analysis of cobs of different corn genotypes to identify the most promising genotype with desirable traits for ethanol production. Further, optimization of simultaneous saccharification and fermentation of corn cobs was carried out to identify the right combinations of various process parameters required to enhance the ethanol production.

EXPERIMENTAL

Raw material

Ten (10) corn genotypes (hybrids) were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The grains were separated from the cobs. The cobs were ground with an electric grinder, and sieved using a sieve shaker. The milled cobs with particle size ranging from 0.35-0.17 mm were used for the further studies.

Chemical pretreatment of corn cob

The cob of the selected corn genotype with low lignin content was subjected to alkali pretreatment. The pretreatment of milled cob with sodium hydroxide

was carried out as per the method of Kaur *et al*.[20] For this, 5 g of cob was soaked into 1M sodium hydroxide solution overnight. The solid loading was kept at 1:20 (w/v). The contents pretreated at 121 °C and 15 psi pressure in an autoclave for 60 minutes. The solid and liquid parts of the pretreated slurry were separated by filtration through double layered cheese cloth. The solid residues thus obtained were repeatedly washed with distilled water and the dried in an oven at 60±2 °C and analyzed for various biochemical parameters.

Simultaneous saccharification and fermentation

Procurement and maintenance of culture

An industrial strain of *Saccharomyces cerevisiae* NCIM3078 provided by the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune, Maharashtra, was used. The strain was maintained on malt-glucose yeast extract (MGYE) agar medium containing (in g/L) malt extract (3.0), glucose (10.0), yeast extract (3.0), peptone (5.0) and agar (20.0), at pH 6.5.

Procedure for simultaneous saccharification and fermentation of pretreated corn cob residue

Simultaneous saccharification and fermentation of pretreated cob was carried out with 12 h pre-hydrolysis, as per the procedure described by Sewsynker-Sukai and Kana.[16] The reaction mixture contained pretreated corn residue with a solid loading of 5–15%, enzyme loading of 10–30 FPU/g, 0.05 M sodium citrate buffer (pH 4.8), 10 g/L peptone and 5 g/L yeast extract. The mixture was incubated at 50±2 °C in an orbital shaking water bath for 12 h prehydrolysis. The enzyme and substrate loadings were worked out based on a working volume of 25 mL. The enzymatic hydrolysate from the prehydrolysis stage was further inoculated with the inoculum *S. cerevisiae*. The latter was prepared by transferring a loopful of the respective stock cultures into 100 mL of GYE broth contained in 250 mL conical flasks. The cultivations were performed at 28±2 °C with constant shaking at 100 rpm in an orbital shaking incubator until the absorbance (A_{600}) of inoculum reached 0.6. The simultaneous saccharification and fermentation was carried out at 28±2 °C for 72 hours in an orbital shaking incubator, with initial shaking at 100 rpm for 12 hours, and then static conditions were maintained for the rest of the fermentation hours. The fermented broth was analyzed for ethanol content after 72 hours of fermentation.

Optimization studies for simultaneous saccharification and fermentation of pretreated corn cob by Response Surface Methodology (RSM)

Process optimization studies were carried out to evaluate optimum parameters for simultaneous saccharification and fermentation of pretreated corn cob. A Box-Behnken Design (BBD), with three factors [solid loading (%), enzyme units (FPU/g) and yeast inoculum (%)] and three levels, which included three replicates at the centre point, was selected for optimization of reaction conditions. BBD was used to study the major and interaction effects of different parameters, like solid loading (A), enzyme loading (B) and yeast inoculum (C) on ethanol concentration. The range and levels of variables investigated have been mentioned in Table 1. The design matrix was obtained with 15 experimental runs in one block with three replicates.

Table 1
Coded level of variables selected using Box-Behnken design

Coded factor	Parameter name	Coded levels		
		-1	0	+1
		Actual levels		
A	Solid loading (%)	5	10	15
B	Enzyme units (FPU/g)	10	20	30
C	Yeast inoculum (%)	5	7.5	10

A quadratic polynomial equation was fitted in the model to identify the effect of each independent variable on ethanol concentration. The following regression equation was obtained for maximum response value to identify the optimum SSF conditions with the help of Design Expert Software 13 (Stat-Ease, Inc. Minneapolis, USA):

$$Y = \beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{12}AB+\beta_{13}AC+\beta_{23}BC+\beta_{11}A^2+\beta_{22}B^2+\beta^{33}C^2 \quad (1)$$

where Y is the predicted response; β_0 is a constant; β_1, β_2, β_3 are the linear coefficients; β_{12}, β_{13}, β_{23} are the cross coefficients; β_{11}, β_{22}, β_{33} are the quadratic coefficients. The analysis of variance was used to study significant parameters. The predicted values were calculated from the regression model derived from the coefficients of the model. The variation in results was explained by the coefficient of determination value (R^2 value).

Validation of simultaneous saccharification and fermentation of pretreated corn cob

The validation experiment was conducted by taking 25 g of cob residues. The validation experiment

included simultaneous saccharification and fermentation of pretreated cob residue under optimized reaction conditions, determined as per the model. Fermented broth was analyzed for ethanol concentration as per the procedure mentioned above.

Analytical methods

Total solid content of the milled cobs was determined as per NREL (National Renewable Energy Laboratory) protocol by the method of Sluiter *et al*.[21] The milled sample was weighed and dried in a hot air oven at 105 °C till constant weight. The difference in weight and percentage of total solids was calculated on the basis of the dry weight of the sample. The moisture content of the sample was determined by subtracting the percentage of total solids from 100. The cellulose content of the cobs was determined as per the method of Crampton and Maynard.[22] For this, one gram of dried sample was placed in a 250 mL round bottomed flask fitted with a reflux condenser. Then, 25 mL of acidic solution prepared by mixing acetic acid, nitric acid and distilled water in the ratio of 65:8:15 (v/v) was added to the flask. The contents were boiled for 20 min and then cooled. The mixture was then transferred into a dried pre-weighed sintered crucible and the residue was washed several times with water, alcohol and acetone by using suction. Subsequently, the sample was dried in an oven and the loss on ignition was recorded as the cellulose content of the sample. To estimate the hemicellulose and lignin contents of the cobs, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as per the method of Goering and Vansoest.[23] The hemicellulose content was calculated as the difference between NDF and ADF contents. The lignin content of the cob was determined based on the amount of loss upon ignition at 500 °C after treating the acid detergent residue with 72% (v/v) sulfuric acid for 3 h. To determine the ash content, the milled cob sample was weighed and ignited in the muffle furnace at 630 °C for 2 h. The difference in weight and percentage of ash was calculated on the basis of the dry weight of the sample.[24] The ethanol content of the cellulosic hydrolysate was determined by the method of Caputi *et al.*[25]

Statistical analysis

The data pertaining to the chemical composition of corn cob were analysed by applying one-way analysis of variance (ANOVA) using SPSS software (IBM SPSS Statistics 20.0 version). The Box-Behnken Design in Response Surface Methodology was used for optimization of simultaneous saccharification and fermentation of pretreated corn cob residue using Design Expert Software 13 (Stat-Ease, Inc. Minneapolis, USA).

RESULTS AND DISCUSSION

Chemical composition of cobs of different corn genotypes

The milled cobs of different corn genotypes were evaluated for various parameters, such as total solids, moisture content, cellulose, hemicelluloses, lignin and ash content. The data pertaining to the total solid and moisture content of milled cobs of different corn genotypes have been presented in Table 2. The total solid and moisture content of milled cobs of different corn genotypes ranged from 91.70 to 96.10%, and from 3.90 to 8.30%, respectively. The total solid content was observed to be significantly high (96.10%) and moisture content was observed to be significantly low (3.90%) for the milled cobs of corn hybrid PMH1. The polysaccharide components of milled cobs of different corn genotypes varied significantly, as can be noted in Table 2. The cellulose content of milled cobs of different corn genotypes ranged from 28.65 to 34.05%. Significantly high cellulose content of 34.05% was recorded in the milled cobs of corn genotype PMH 10, followed by PMH1 with cellulose content of 32.95%. The cellulose content was found to be significantly low, *i.e.* 28.65% in the milled cobs of corn genotype DKC, and was significantly at par with the cellulose content of PSC (29.80%), PMH8 (29.90%) and P1844 (30.30%). Significant differences were also observed in the hemicellulose content of milled cobs of different corn genotypes, and the content ranged from 13.57 to 35.63% (Table 2) The hemicellulose content was found to be significantly high in the milled cobs of corn genotype PMH2 (35.63%), while the content was reported to be significantly low in the corn genotype PMH8 (13.57%).

Significant variation was observed in the lignin content of milled cobs of different corn genotypes. The lignin content ranged from 11.87 to 24.33% (Table 2). Among all the corn genotypes tested, significantly low lignin content of 11.87% was observed in the milled cobs of corn genotype PMH10, which was statistically at par with the lignin content of other corn genotypes. However, the lignin content was reported to be significantly high, *i.e.* 24.33% in the milled cobs of corn genotype DKC. The ash content varied significantly and ranged from 0.60 to 2.40% (Table 2). The ash content was found to be significantly low, *i.e*., 0.60% in the milled cobs of corn genotype P1844 and was statistically at par with the ash content of corn genotype PSC

(0.80%) and PMH1 (1.00%). However, it was significantly high in the milled cobs of corn genotypes PMH2 and PMH11 (2.40%).

Table 2
Chemical composition of milled cobs of different corn genotypes

Corn genotypes	Total solids (%)	Moisture content (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
DKC9 108	92.60^{d} (±0.12)	7.40^{b} (±0.12)	28.65^{e} (±0.14)	20.93^{cde} (±1.30)	24.33^{a} (±0.85)	1.50^{c} (±0.12)
PMH1	96.10^{a} (±0.06)	3.90^{e} (±0.06)	32.95^{ab} (±0.26)	26.67^{bcd} (±3.00)	18.60^{ab} (±0.96)	1.00^{d} (±0.06)
PMH2	93.40^{c} (±0.12)	6.60^{c} (±0.12)	32.00^{abc} (±0.06)	35.63^{a} (±0.85)	14.03^{b} (±2.00)	2.40^{a} (±0.12)
PMH10	91.70^{e} (±0.06)	8.30^{a} (±0.06)	34.05^{a} (±0.03)	28.03^{abc} (±1.67)	11.87^{b} (±0.35)	2.10^{ab} (±0.12)
Parkash	93.30^{c} (±0.12)	6.70^{c} (±0.12)	30.90^{bcd} (±1.04)	30.37^{ab} (±0.75)	13.67^{b} (±1.05)	1.40^{c} (±0.06)
PMH7	93.60^{c} (±0.06)	6.40^{c} (±0.06)	32.55^{ab} (±0.32)	18.60^{de} (±1.19)	17.33^{b} (±1.29)	2.10^{ab} (±0.06)
PMH8	92.20^{d} (±0.12)	7.80^{b} (±0.12)	29.90^{cde} (±0.40)	13.57^{e} (±1.82)	16.83^{b} (±1.32)	1.90^{b} (±0.17)
PMH11	91.70^{e} (±0.06)	8.30^{a} (±0.06)	32.05^{abc} (±0.03)	21.93^{bcde} (±1.88)	14.90^{b} (±2.54)	2.40^{a} (±0.06)
P1844	94.80^{b} (±0.12)	5.20^{d} (±0.12)	30.30^{cde} (±0.69)	23.40^{bcd} (±3.37)	15.57^{b} (±1.77)	0.60^{d} (±0.06)
PSC	93.40^{c} (±0.17)	6.60^{c} (±0.17)	29.80^{de} (±0.58)	27.27^{abcd} (±1.81)	12.40^{b} (±1.28)	0.80^{d} (±0.12)

*Each value represents the mean ± standard error of triplicate; Mean values within a column followed by different superscripts are significantly different at $p < 0.05$

The present study on the compositional analysis of different corn genotypes revealed that the milled cobs of corn genotype PMH10 were found to have high cellulose and hemicellulose content of 34.05 and 28.03%, respectively. The lignin content of this genotype was also reported to be significantly low (11.87%). High carbohydrate, along with low lignin content, could be beneficial as the enzymatic hydrolysis of pretreated cob residue of this corn genotype might lead to the release of considerable amounts of sugars that could be subsequently fermented into ethanol with high yields.[26] The low lignin content of corn genotype might help to overcome the high-cost pretreatment process and reduces the recalcitrance of cellulose, thereby, leading to improved saccharification of cellulose by the cellulolytic enzyme complex.[27] According to Pointner *et al.*[28] (2014), no significant differences were reported for the fibre content of different cob varieties and proportions of cellulose, hemicelluloses and lignin were found to be 38.8±2.5%, 44.4±5.2% and 11.9±2.3%, respectively. The content of hemicelluloses was found to be higher, as compared to the results published by Wang *et al.*,[29] who reported that the corncob raw material contained 40–44% cellulose, 31–33% hemicelluloses, 16–18% lignin and 3–5% ash.

The compositional analysis of different maize hybrids could form the basis for various maize breeding strategies to be undertaken for a particular trait that leads to specific effects on cell wall composition. The selection of inbred lines with reduced lignin content could improve saccharification efficiency and increase glucose release for ethanol fermentation. Further, through up and down regulation of various enzymes directly involved in the lignin synthesis and monolignol composition, saccharification efficiency of the biomass could be increased for enhanced ethanol yield.

Effects of alkali pretreatment on cob chemical composition

The milled cobs of corn genotype PMH10 with low lignin content were subjected to alkali pretreatment. The chemical composition of the

cob residue after alkali pretreatment has been given in Figure 1. The cellulose, hemicellulose, lignin and ash contents of milled cobs were reported to be 34.05, 28.03, 11.87 and 2.1%, respectively. The relative proportion of cellulose (56.70%) increased, while that of hemicelluloses (11.87%), lignin (8.61%) and ash (0.6%) drastically reduced in the sodium hydroxide-treated cob residues. The results thus showed that alkaline sodium hydroxide substantially removed hemicelluloses and lignin from the milled cobs, leaving a high proportion of complex carbohydrate, *i.e.*, cellulose, in the treated residue that could become more accessible for the further attack by hydrolytic enzymes. Sodium hydroxide preferentially cleaves intermolecular ester bonds between hemicelluloses and lignin through saponification reactions, thereby leading to the solubilization of lignin, as well as hemicellulose fragments.[30] Sahare *et al.*[31] reported that after alkaline pretreatment (1% sodium hydroxide) of corn cobs, the lignin content was reduced from 14.8 to 7.4%, whereas the cellulose content of the cobs increased from 36.4 to 50.4%. The total glucan and xylan content of the remaining solid were found to be 82%. About 21% in weight loss was reported, when the cobs were treated with alkali at 50 °C for 4 h.

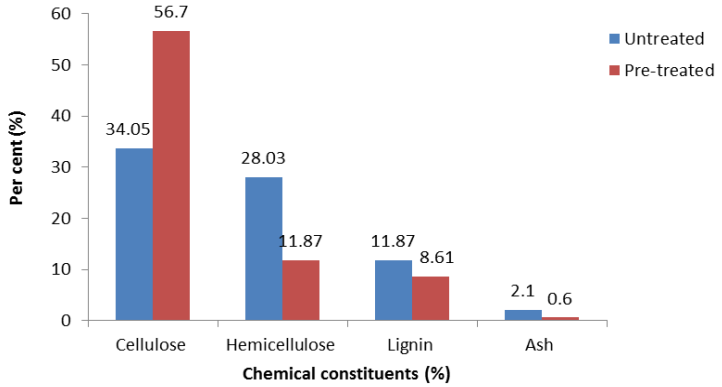

Figure 1: Chemical composition of alkali-treated cob of corn genotype PMH10

Optimization of simultaneous saccharification and fermentation of pretreated corn cobs

To optimize the simultaneous saccharification and fermentation process of pretreated cob residues, the effect of three independent variables, *viz.* solid loading (A), enzyme dosage (B) and yeast inoculum (C) on ethanol concentration was studied. The results deduced from fifteen experimental runs as per the Box-Behnken Design of Response Surface Methodology (RSM) have been summarized in Table 3. Under different simultaneous saccharification and fermentation conditions, the measured ethanol concentration ranged from 0.35 to 3.58 mg/mL. The maximum ethanol concentration of 3.58 mg/mL was observed, when the treated cob residues were saccharified and fermented with 30 FPU/g enzyme dosage and 10% yeast inoculum at a solid loading of 10% (Run 4, Table 3). The experimental data given in Table 3 was used to develop a three variable quadratic polynomial regression equation to predict the ethanol concentration (Y, mg/mL) as a function of the three process parameters, including solid loading (A, %), enzyme dosage (B, FPU/g) and yeast inoculum (C, %), which was given by:

$$Y = 2.34+0.3462A+1.17B+0.2188C-0.1150AB+0.0900BC-0.4037B^2+0.1037C^2 \quad (2)$$

The fitted model was evaluated by the analysis of variance (ANOVA) given in Table 4. It showed that the response model was highly significant as the F-value was 428.78 and the probability value was very low (P model > F= 0.0001). The p-values less than 0.05 indicated that the model terms were significant. According to this, the most significant terms were observed to be A (solid loading), B (enzyme dosage), C (yeast inoculum), AB (solid loading and enzyme dosage), BC (enzyme dosage and yeast inoculum). The "Lack of Fit F-value" of 5.62 implied that there was insignificant lack of fit. The high F-value and non-significant lack of fit suggested that the obtained experimental data presented a good fit to the model. The coefficient of variation (CV) obtained was 2.67 per cent (Table 4), which indicated the degree of precision of the experimental runs conducted. The high reliability and reproducibility of the design was

attributed to a low CV value. An adequate precision value of 67.57 obtained indicated an adequate signal and that the model can be used to navigate the design space. The coefficient of determination (R^2) of the model obtained (0.9987) indicated the degree to which the model was able to predict the response *i.e*., 99.87% of variation in ethanol concentration was explained by solid loading, enzyme dosage and yeast inoculum. The high values of R^2 and adjusted R^2 of this model (Adj R^2 = 0.9964) depicted the close agreement between experimental results and model predicted theoretical values.

Table 3
Box-Behnken design for simultaneous saccharification and fermentation of pretreated cobs of corn genotype PMH10

Run	Factor 1	Factor 2	Factor 3	Response	
	Actual values			Ethanol concentration (mg/mL)	
	A: Solid loading (%)	B: Enzyme dosage (FPU/g)	C: Yeast inoculum (%)	Observed values	Predicted values
1	10	10	5	0.68	0.74
2	15	30	7.5	3.30	3.34
3	15	20	10	3.03	3.01
4	10	30	10	3.58	3.47
5	5	10	7.5	0.35	0.31
6	5	20	10	2.26	2.20
7	5	20	5	1.93	1.88
4	15	10	7.5	1.26	1.23
9	10	20	7.5	2.37	2.34
10	10	20	7.5	2.34	2.34
11	5	30	7.5	2.85	2.88
12	10	20	7.5	2.31	2.34
13	15	20	5	2.57	2.58
14	10	30	5	2.92	2.90
15	10	10	10	0.98	1.00

Table 4
ANOVA for Response Surface Quadratic model

Source	Ethanol concentration					
	Sum of squares	Df	Mean square	F value	p-value Prob>F	Inference
Model	13.10	9	1.46	428.78	< 0.0001	Significant
A-Solid loading	0.9591	1	0.9591	282.51	< 0.0001	Significant
B-Enzyme dosage	11.00	1	11.00	3239.48	< 0.0001	Significant
C-Yeast inoculums	0.3828	1	0.3828	112.76	0.0001	Significant
AB	0.0529	1	0.0529	15.58	0.0109	Significant
AC	0.0042	1	0.0042	1.24	0.3153	Not significant
BC	0.0324	1	0.0324	9.54	0.0272	Significant
A^2	0.0001	1	0.0001	0.0153	0.9064	Not significant
B^2	0.6019	1	0.6019	177.29	< 0.0001	Significant
C^2	0.0397	1	0.0397	11.71	0.0188	Significant
Residual	0.0170	5	0.0034			
Lack of Fit	0.0152	3	0.0051	5.62	0.1548	Not significant
Pure Error	0.0018	2	0.0009			
Cor. Total	13.12	14				
R-Squared	0.9987					
Adj R-Squared	0.9964					
C.V. %	2.67					
Adeq. Precision	67.57					

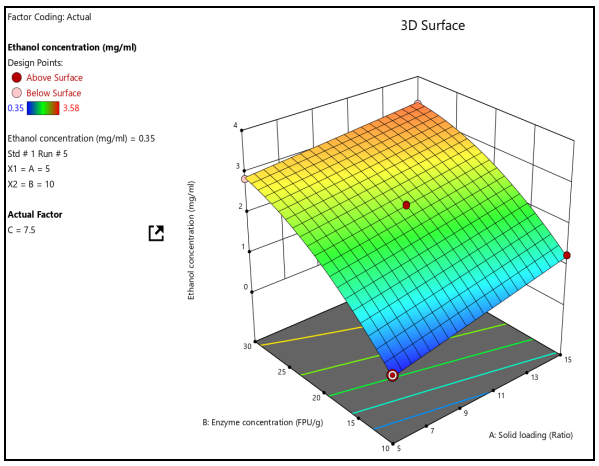

Figure 2: Response surface plot showing the effect of solid loading and enzyme dosage on ethanol concentration during SSF of pretreated corn cob

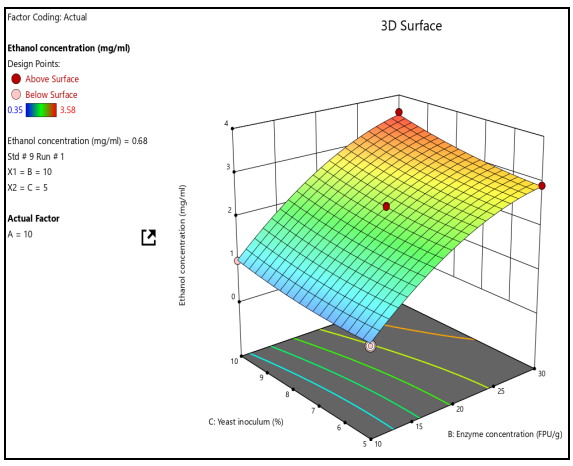

Figure 3: Response surface plot showing the effect of enzyme dosage and yeast inoculum on ethanol concentration during SSF of pretreated corn cob

Response surface plots and optimization for simultaneous saccharification and fermentation of pretreated corn cob

Response surface curves were plotted in order to study the interactions between independent variables and determine the ideal number of variables. The study on the effect of the interaction between solid loading and enzyme dosage on ethanol concentration was studied at a yeast inoculum of 7.5 per cent and is shown in Figure 2. The study showed that the enzyme loading should be in the range from 15 to 30 FPU/g to achieve high ethanol concentration. Further, high solid concentration required high enzyme loading to catalyze the hydrolysis. Further, it can be seen from Figure 3 that the percent yeast inoculum should be between 8 to 10%, to achieve high ethanol concentration. Under specified yeast inoculum percent, too low enzyme loading (*e.g.*, <25 FPU/g) would limit the supply of soluble sugars for fermentation by yeast and might decrease the ethanol concentration. On the other hand, high enzyme dosage leads to over-supply of sugars that would inhibit the yeast performance and also increase the cost of SSF process.

The optimal conditions for simultaneous saccharification and fermentation of treated cob residues were selected by Design Expert software through a numerical (best treatment conditions shown in Table 5) and graphical optimization (Fig. 4). The optimization results showed that maximum ethanol concentration of 3.64 mg mL^{-1} was obtained during simultaneous saccharification and fermentation of cob residues at 28.58 FPU/g cellulase, 9.56% yeast inoculum and solid loading of 14.95%. Wang *et al.*[32] similarly studied the response of ethanol concentration to enzyme loading, solid concentration and yeast concentration during simultaneous saccharification and fermentation of sweet sorghum bagasse. The maximum ethanol concentration of 39 g/L was achieved at an enzyme loading of 29 FPU/g, solid concentration of 10% and yeast concentration of 1.4 g/L. Similarly, Sharma *et al.*[13] optimized simultaneous saccharification and fermentation of pretreated corncobs by Box-Behnken design and optimum reaction conditions were found to be 8% (w/v) substrate loading, 11 FPU/gds enzyme loading at a temperature of 38 °C and pH 3.0. The maximum theoretical ethanol yield of 48.8% was achieved through simultaneous saccharification and fermentation (SSF) of pretreated corncobs.

Validation of statistical model of simultaneous saccharification and fermentation of pretreated corn cobs

The confirmation experimental run was performed under the identified optimum SSF conditions to confirm the validity of the statistical model. The results showed that the maximum experimental ethanol concentration of 3.42 mg mL^{-1} was observed, which was quite close to the predicted value of 3.64 mg/mL. The correlation between the predicted and measured values of these experiments was observed to be excellent. This showed the validity of the statistical model.

Table 5
Solutions for numerical optimization of simultaneous saccharification and fermentation of pretreated corn cob

Solutions						
Number	Solid loading (%)	Enzyme units (FPU/g)	Yeast inoculum (%)	Ethanol concentration (mg/mL)	Desirability	
1	14.95	28.58	9.56	3.64	1.00	Selected
2	14.68	29.01	9.38	3.61	1.00	
3	14.93	26.18	9.98	3.59	1.00	
4	14.18	28.43	9.65	3.61	1.00	
5	11.79	29.56	9.96	3.59	1.00	

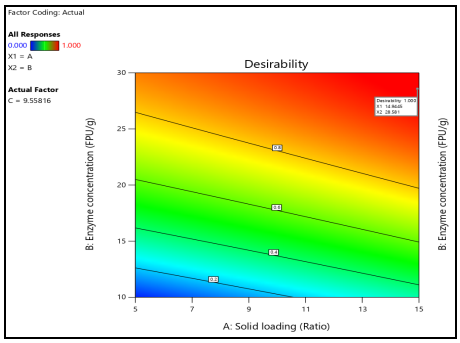

Figure 4: Overlay plot showing optimization of SSF of pretreated corn cob

CONCLUSION

The present study showed that among corn genotypes tested, milled cobs of corn genotype PMH10 were found to have significantly high cellulose content (34.05%) and low lignin content (11.87%). Alkali pretreatment (4% NaOH for 60 min) resulted in efficient delignification of cobs, resulting in considerably low lignin content of 8.61% in the treated residue. The response surface model was an effective method to optimize the operating conditions, including solid loading, enzyme dosage and yeast concentration during SSF of the alkali-pretreated cob residues, to attain maximum ethanol concentration in the fermented hydrolysate. The maximum ethanol concentration of 3.64 mg/mL was achieved at a solid loading of 14.95%, enzyme dosages 28.58 FPU/g loading and yeast inoculum of 9.56%

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