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Simultaneous saccharification and fermentation of alkali-pretreated corncob under optimized conditions using cold-tolerant indigenous holocellulase

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Abstract−The present investigation was aimed towards pretreatment optimization of corncob to maximize cellulose and hemicellulose recovery, followed by substrate selection for holocellulase production using psychrotolerant *Aspergillus niger* SH3. Dilute alkali pretreatment (1.5% NaOH) resulted in higher recovery of cellulose (59.66%) and hemicellulose (28.34%) from corncob, while corn stover proved to be the best substrate for holocellulase production. Further, saccharification was optimized by Box-Behnken design to select the suitable conditions for maximum sugar release from pretreated corncob. The optimum conditions for maximum sugar release were 8% (w/v) substrate loading, 11 FPU/gds enzyme loading at temperature 38 °C and pH 3.0 which resulted in 114.5% higher sugar yield (912 mg/gds of pretreated biomass) as compared with un-optimized conditions (425.35 mg/gds). Theoretical yield of 48.8% ethanol was achieved through simultaneous saccharification and fermentation (SSF) using pretreated corncob. This study illustrates the potential of different corn residues as a promising substrate for bioethanol production.

Keywords: Bioethanol, Corn residues, *Aspergillus niger*, Saccharification, Fermentation, Box-Behnken

INTRODUCTION

Petroleum based global energy consumption will reach up to 60% by 2030 due to increasing industrialization and population growth [1]. Depleting fossil fuels, fluctuating petroleum prices and concurrent climate change have led to global attention on renewable and sustainable energy alternatives [2]. Biomass-based biofuel production is the most lucrative alternative energy source for the fast growing economy of a developing country like India where more than 700 MT biomass is available annually for recycling [3]. Currently, all commercial ethanol production in India is based on molasses as feedstock, but these limited resources cannot fulfill the huge demand of ethanol for targeted 10% blending with gasoline in 2017. Lignocellulosic biomass can be a promising feedstock for bioethanol production [4], as a large amount of crop residues are generated globally.

Corn, predominantly a kharif crop, is grown throughout the year in India. Corn is the third most abundant cereal crop after rice and wheat with 9% of total grain yield [5]. The compound annual growth rate (CAGR) of corn production has continuously increased in the last decade and production reached 24.7 MT in 2014-15 from 14 MT (2004-05) [6]. The post harvested residues of corn are either burnt or wasted in the field. Corn plant produces approximately 4.96 MT cobs annually which can be used to generate nearly 2.22 million liters of ethanol.

Second generation bioethanol production involves three major steps: pretreatment, saccharification and fermentation [4]. The pretreatment step involves the removal of lignin, which improves the accessibility of polysaccharides by reducing cellulose crystallinity, increasing the surface area and pore volume of the substrate [7,8]. But the generation of inhibitors and high energy intensive nature of pretreatment process are of major concern in the development of cost and energy efficient pretreatment process [9]. The loss of hemicellulose content during the pretreatment process is one of the major bottlenecks which reduces the biomass to ethanol conversion rate [10]. Although the solubilized hemicellulosic fraction can be fermented by engineered strains, their efficiency is low due to the presence of various inhibitors. Therefore, optimization of pretreatment process is essential to improve the overall sugar yield and bioethanol productivity.

On the other hand, enzymatic hydrolysis of the pretreated biomass into monomeric sugars is the second most crucial and cost intensive process for bioethanol production [8]. Around 20-25% of the total cost of bioethanol production is attributed to the enzymatic saccharification step because of high cost of commercial enzyme [11]. Enzymatic saccharification is based on polysaccharide (cellulose and hemicellulose) enzyme synergy; hence, substrate based holocellulase production has to be undertaken for developing substrate specific enzyme cocktail [12]. Moreover, low enzyme doses with high substrate loading at lower saccharification temperature would make the process more attractive for bioethanol production [13]. Larsen et al. [14] and Hodge et al. [15] used high substrate

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concentration with low enzyme loading to achive better sugar yield in enzymatic hydrolysate for ethanol production. Similar studies are lacking on these aspects especially for corncob; therefore, efforts were made to utilize all parts of corn efficiently to develop a cost effective protocol for ethanol production.

Biological conversion of biomass to bioethanol employing simultaneous saccharification and fermentation, which combines cellulose hydrolysis and fermentation in a single step, has the potential to lower the process cost significantly [16]. This method prevents end production inhibition hydrolases through rapid consumption of sugars by fermenting microbes. However, disparate incubation temperature requirements for saccharification (approx. 50-55 °C) and fermentation (approx. 30-35 °C) are the major bottlenecks. Therefore, exploring cold active novel hydrolytic enzyme system with high saccharification efficiency at low temperature can provide solutions for economically viable consolidated bioprocessing or development of a simultaneous saccharification and fermentation process for bioethanol production [17].

In this study, the pretreatment step was optimized to recover maximum polysaccharides, including hemicellulose from corncob. The structural and chemical changes were also studied through scanning electron microscopy (SEM), Fourier transform infrared (FTIR) and X-ray diffraction (XRD) analysis. The hemicellulosic fraction was extracted to evaluate the effect of pretreatment processes on the conformational changes by FTIR and nuclear magnetic resonance (NMR). Further, indigenous holocellulase was produced from a psychrotolerant *Aspergillus niger* SH3 with lower temperature optima and high xylanase activity. This enzyme cocktail was further used to optimize the saccharification process to achieve maximum sugar yield from pretreated corncob biomass, followed by simultaneous saccharification and fermentation (SSF) to generate bioethanol.

MATERIAL AND METHODS

1. Materials

Corncobs (var. PEHM-3) were obtained from the ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. The collected biomass was dried and ground to a mesh size of (0.2 cm) and stored at room temperature until further use. All other chemicals and media components used in the present study were of analytical grade obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India and Sigma-Aldrich (St. Louis, MO, USA).

2. Corncob Pretreatment Optimization and its Compositional Analysis

Corncob was chemically pretreated with different concentrations of dilute acid and alkali. Steam, acid and alkali pretreatments were performed with four different concentrations of H_2SO_4 and NaOH (0.5%, 1%, 1.5% and 2%) at 121 °C for 20 min in screw capped bottles with 20% w/v solid loading in autoclave. Cold alkali and acid pretreatments were also performed with the above mentioned H_2SO_4 and NaOH concentrations with same solid loading at room temperature for 1 h under static condition. After pretreatments, samples were washed with distilled water until the pH of the washed water reached to neutral. All pretreated substrates were air dried and stored at room temperature for further studies. Raw and pretreated samples were also analyzed for cellulose [18], pentosans and klason lignin [19].

3. Microorganism and Enzyme Production

Previously isolated psychrotolerant holocellulolytic fungi, *A. niger* SH3 (GenBank accession no. KP193130) was grown on potato dextrose agar (PDA) plate at 30 °C and used for holocellulase production by solid state fermentation method. For hydrolytic enzyme production from *A. niger* SH3, six different substrates were used. *Eichhornia crassipes* (EC) was collected from ponds of IARI, New Delhi. Wheat straw (WS), paddy straw (PS), corncob (CC) and corn stover (CS) were collected from IARI. Wheat bran (WB) was purchased from a local market.

3-1. Holocellulase Production Under Solid State Fermentation

Each of the substrates PS, EC, CS, WS, WB and CC (5 g) were suspended in 15 mL Reese's minimal media (RMM) [20] in 500 mL Erlenmeyer flasks and sterilized at 121 °C for 20 min. SH3 was inoculated by using 3 mg fresh weight of fungal mycelium scraped from seven days old fungal culture grown in PDA. Flasks were incubated at 30 °C for ten days in static condition. After incubation, crude enzyme was extracted by adding 150 mL of 50 mM citrate buffer (pH 4.8) from each flask followed by shaking at 150 rpm for 1 h. The crude extracellular enzyme was collected by centrifugation at 10,000 g for 10 min at 4 °C and assayed for hydrolytic enzymes by standard protocols as described below.

3-2. Quantitative Assay of Hydrolytic Enzyme

Endoglucanase (CMCase; EC: 3.2.1.4), Filter paperase (FPase) (EC: 3.2.1.91), and xylanase (EC: 3.2.1.8) were estimated in the crude enzyme filtrate by standard assay methods [21,22]. β-Glucosidase (cellobiase; EC: 3.2.1.21) and β-1,4-xylosidase (EC: 3.2.1.37) activities were quantified by the method of Wood and Bhat [23]. One unit of enzyme activity was expressed as the amount of enzyme required to release 1 μmol of end product per minute under the standard assay conditions.

4. Optimization of Saccharification Process

Saccharification of cold dilute alkali (1.5%) pretreated corncob was optimized by the Box-Behnken design experiment (Design Expert, Stat-Ease Inc., Minneapolis, USA). A set of four factors--substrate loading (%), pH, enzyme loading, and temperature were selected to identify the most conducive saccharification conditions leading to optimal sugar yield. Variables generated for all the factors including substrate loading, enzyme loading, pH and tempera-

Table 1. Experimental range and coded levels employed in the Response surface methodology (RSM) using the Box-Behnken design to increase the sugar yield after 48 h saccharification with indigenous hemicellulase enzyme

Factor	Independent variables	Range and levels		
		−1	0	1
A	Substrate loading (%), (w/v)	5	6.5	8
B	pH	3	4.5	6
C	Enzyme loading (FPU/gds)*	5	10	15
D	Temperature (°C)	30	35	40

*FPU/gds refers to FPU per gram of dry substrate (pretreated corn cob)

ture from experimental design (Table 1) were employed for saccharification in a shaking water bath (150 rpm) for 48 h. Saccharification was performed in 100 mL screw capped bottles, and sodium citrate buffer (50 mM) was used to provide variable pH conditions. A total of 27 experiments were conducted along with the selected range of described variables. Table 1 describes the low, middle and high concentration levels of selected variables as −1, 0 and +1 (coded values), respectively. Each experiment was performed in triplicate and the mean used as response value. The complete experimental design with coded variables is described in Supplementary Table 1 (SI). Samples were taken periodically for the estimation of total reducing sugar by DNSA method [24]. Monomeric sugars were quantified by high performance liquid chromatography (Waters pump 515 model) equipped with Waters 2414 refractive index (RI) detector. The Aminex HPX-87H column was used with mobile phase (5 mM H_2SO_4) at a flow rate of 0.5 mL/min. The oven temperature was held at 50 °C. The extent of saccharification was expressed as mg reducing sugar per g of dry substrate of pretreated biomass (mg/gds).

To evaluate and study the interactions of independent variables on the dependent variable, statistical and mathematical analyses were done using Design expert 8.0.7.1. All the experiments were in triplicate and data were expressed as average values. An experiment was also conducted to confirm the predicted optimum response using the selected optimum values of all the four variables.

5. Structural Analysis of Raw and Pretreated Corncob

Raw and pretreated corncob was subjected to SEM, XRD, FTIR and ^{1}H-NMR spectroscopy analysis (details are available in Fig. SI) to decipher the changes in the biomass during pretreatment and saccharification process.

6. Simultaneous Saccharification and Fermentation (SSF)

The SSF process was executed using *Saccharomyces cerevisiae* LN under optimized hydrolysis conditions as described earlier. Briefly, SSF was performed in 250 mL screw capped plastic bottles containing alkali pretreated corncob (8%), yeast extract (0.1%), $MgSO_4{\cdot}7H_2O$ (0.5%) and $(NH_4)_2SO_4$ (0.5%) dispensed in citrate buffer (pH 4.8). A measured amount of 11 FPU/gds of cold active holocellulase from SH3 was added into the medium. For the primary inoculum, LN was grown in MGYP medium containing malt extract (3 g/L), yeast extract (3 g/L), peptone (5 g/L) and glucose (10 g/L) at pH 6.8 and incubated at 38 °C under shaking condition (150 rpm) for 72 h. This culture broth was inoculated at 10% (v/v) after 16 h of enzymatic hydrolysis for fermentation process. The bottles were kept at 38 °C under shaking condition (150 rpm). The samples were withdrawn periodically for the estimation of sugars and ethanol by HPLC.

RESULTS AND DISCUSSION

1. Pretreatment of Corncob

Chemical pretreatment of biomass leads to disruption of cell wall components and sequential decrease in the cellulose crystallinity. Acid and alkali pretreatments lead to swelling of the substrate, which increases its internal surface area and facilitates the removal of lignin molecules by disrupting its structure; this enhances the enzymatic hydrolysis of biomass [25]. Since the structure of lignocellulose varies according to biomass/crop varieties, it is necessary to standardize the pretreatment method for each type of biomass to obtain higher saccharification efficiency. In this study, pretreatment for corncob was optimized with different concentrations of NaOH and H_2SO_4, with and without steam sterilization, so as to retain maximum holocellulose and remove lignin. Based on the results of this experiment, we concluded that both alkali and acid pretreatment without steam sterilization were more promising for the retention of high hemicellulose and cellulose content in corncob (Supplementary Table 2). However, alkali pretreatment (1.5%) without autoclaving was found to be most promising in terms of the total carbohydrate content among all the pretreatments. Moreover, lignin removal (34.68%) was observed in alkali pretreated substrate, with only 4.27±0.42% lignin as compared to 12.31±1.23% in control. As reported for many other agro residues [26,27] acid pretreatment resulted in higher loss of hemicellulose. Kumar and Parikh [28] also reported that mild alkali pretreatment of rice straw and sugarcane bagasse increased the cellulose content (40-60%) with a marginal loss of hemicellulose, while acid pretreatment resulted in complete removal of hemicellulose resulting in enrichment of cellulose. The results indicated that a mild pretreatment condition (1.5% NaOH at room temperature) was not only able to retain most of holocellulose (88.0%) but also removed lignin, thereby facilitating high sugar yield from corn cob. Also, the energy savings from omission of autoclaving step can help in improving the overall economics of the process.

2. Structural Analysis of Raw and Pretreated Corncob

Chemical pretreatment induces many structural changes, which

Table 2. Effect of various substrates on hydrolytic enzyme production (IU/g of substrate) from *A. niger* SH3 under solid state fermentation at 30 °C (* alphabets a, b, c, d, e and f denotes the DMRT ranking)

Substrate	β-Glucosidase	Endoglucanase	FPase	Xylanase	β-Xylosidase
Eichornia	425.61^b	358.64^b	42.76^a	1392.71^d	68.10^f
Wheat Straw	207.44^e	233.52^d	11.36^d	940.29^e	156.16^e
Wheat bran	876.01^a	362.54^a	21.26^c	2758.84^c	439.48^a
Paddy Straw	314.21^c	262.73^c	22.48^b	2838.67^a	230.35^d
Corn cob	271.76^d	163.09^e	9.09^e	736.25^f	248.01^c
Corn stover	424.55^b	232.71^d	22.31^b	3317.71^b	336.49^b
SEM	0.67	0.45	0.32	2.35	0.75
CD	1.85	1.23	0.88	6.49	2.08

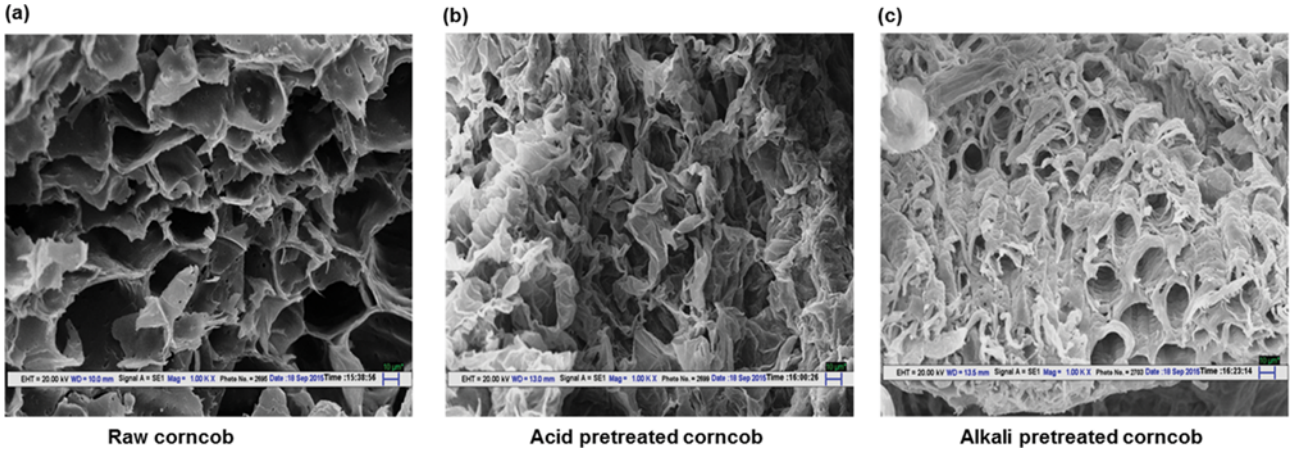

Fig. 1. SEM images of raw corncob (a), acid pretreated corn cob (b) and alkali pretreated corncob (c).

removes lignin and decreases cellulose crystallinity, resulting in increased internal surface area for enzymes to act. Structural changes taking place in the corncob during different pretreatments were studied by SEM, FTIR and XRD. The extracted hemicellulose from alkali pretreated corncob was also subjected to FTIR and ^{1}H-NMR for the structural identification of polysaccharides.

Raw corncob (Fig. 1(a)) displays a rigid, regular and intact surface, while acid (Fig. 1(b)) and alkali (Fig. 1(c)) pretreated corncob exhibit considerable changes in the heterogeneous layer with shattering and swelling of corncob resulting in increased internal surface area. This facilitates the holocellulases to easily diffuse and hydrolyze the biomass, improving the saccharification yield (details in SI).

The FTIR spectra indicated that pretreatment resulted in a significant plunge in the intensities of polysaccharide bands with a consequent emergence of functional groups (Fig. 2). A reduction in the transmittance observed at 1,166 cm^{-1} and 836 cm^{-1} in pretreated corncob displayed the removal of Syringyl-Guaiacyl-Hydroxyphenyl (SGH) units of lignin [29]. The data revealed that pretreatment process selectively removed lignin, making cellulose and hemicellulose more accessible for hydrolytic treatment. The FTIR-based cellulose crystallinity was 0.82 for raw corncob, which was decreased to 0.73 and 0.64 after alkali and acid pretreatment, respectively. This result was consistent with the XRD analysis, verifying the decrease in cellulose crystallinity after pretreatment. The CrI of raw corncob was observed as 43.33, which decreased to 39.79 and 38.14, respectively, after alkali and acid pretreatment (Fig. 3). This reduced cellulose crystallinity of pretreated corncob revealed the expansion of amorphous region, which improved the accessibility towards substrate binding modules of cellulase enzyme system [30] (details in SI).

The FTIR spectra of hemicellulose fraction extracted after alkali pretreatment confirm the presence of glycosidic linkages, arabinofuranosyl and 1-4 glycosidicbonds between xylopyranose. The signals obtained in ^{1}H-NMR spectra were assigned as per the literature [30-32]. In the ^{1}H-NMR spectrum, a chemical shift of 3.0 ppm-4.2 ppm originated from the equatorial proton and anhydroxylose units of hemicellulose, which is the typical signal pattern expected

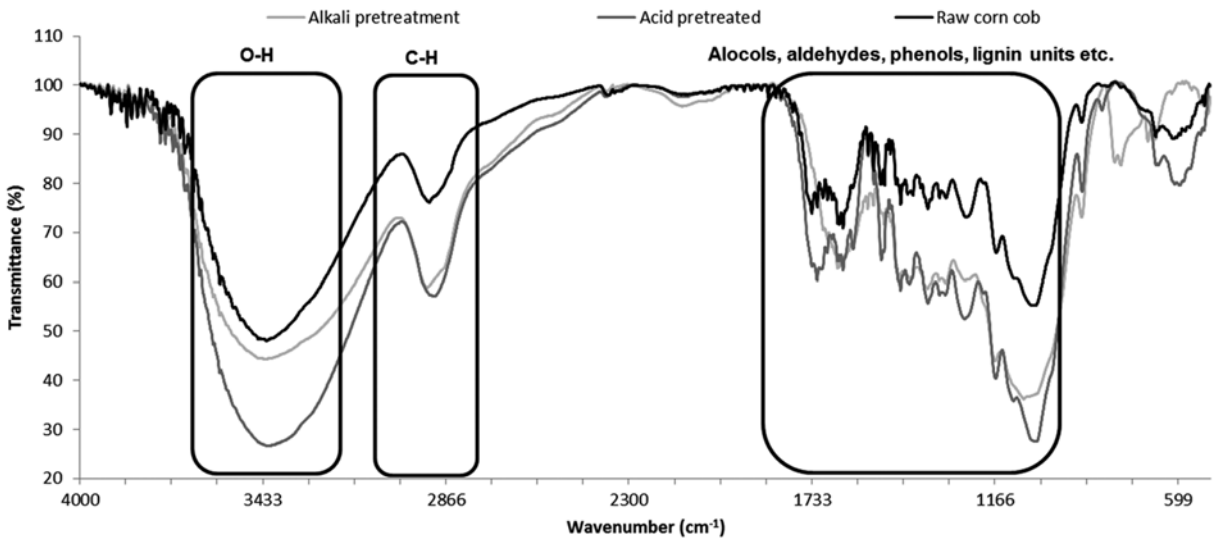

Fig. 2. FTIR analysis of raw, acid and alkali pretreated corncob.

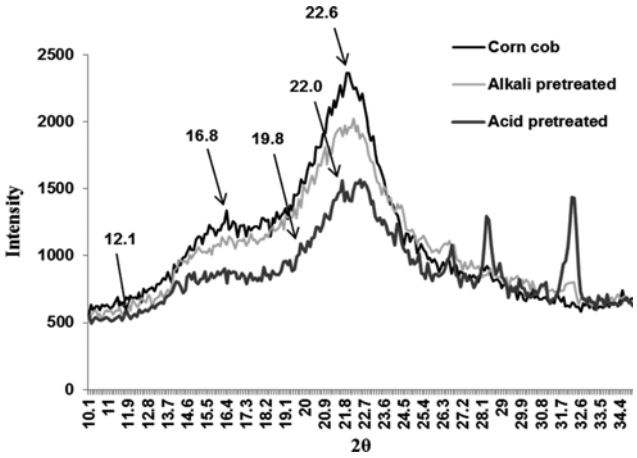

Fig. 3. XRD pattern of raw and chemical (1.5% NaOH and 1.5% H_2SO_4) pretreated corncob to deduce the changes in cellulose crystallinity.

for the hemicellulose moiety (details in SI). On the basis of ^{1}H-NMR and FTIR spectral analysis of extracted hemicellulose from alkali pretreated corncob, the hemicellulosic fraction of corncob was structurally defined as L-arabino-4-*O*-methyl-D-gluurono-D-xylan.

3. Production of Enzyme Cocktail from *A. niger* SH3

Hydrolytic enzyme production from cheap agro residues under SSF is considered as a major step to cut down the overall cost of bioethanol production. Depending upon the composition of lignocellulosic component of the plant material, the induction and secretion of extracellular enzyme varies [33]. Different agro residues were evaluated for enzyme cocktail production using psychrotolerant *A. niger* which was isolated from Kargil, (India) and capable of growth between 5-35 °C [17]. Various species of *Aspergillus* are known to be a potential producer of holocellulase from various agro-residues. Extracellular hydrolytic enzymes from various sp. of *Aspergillus* and *Neocallimastix patriciarum* belong to different glycosyl hydrolases groups and are reportedly active at low temperature and pH [17,28,34]. In the present investigation, SH3 was used as the potential microbe for production of enzyme cocktail containing 125 different glycosyl hydrolases (data not shown). For selection of best substrate for holocellulase production by SH3 under SSF, various substrates (EC, WS, PS, WB, CC and CS) were evaluated and the crude enzyme extract was used for the quantification of hydrolytic enzymes (Table 2). The results revealed that among all the substrates, corn stover produced highest xylanase (3317.71 IU/g), β-xylosidase (336.49 IU/g) followed by paddy straw. Corn stover has high hemicellulose content, which induces better level of xylanase and β-xylosidase in the secretome.

4. Optimization of Saccharification Process

Box-Behnken design-based response surface methodology was used to select the most suitable interaction of selected variables for maximizing the sugar release during saccharification using indigenous holocellulase from SH3. Different statistical tools have been widely used to optimize saccharification parameters with various raw materials for improving their sugar release [35,36]. Based on previous studies, four variables which play an important role in saccharification--substrate loading, pH, enzyme loading and temperature--were investigated for statistical optimization. The experimental response along with actual and predicted values of total sugar release is given in Supplementary Table 1. The results indicated that experimental response data well matched the predicted response values. Furthermore, ANOVA was performed with the polynomial equation to obtain a second-order response surface model (Supplementary Table 3). The following polynomial equation of a quadratic model for response describes the relationship between sugar yield from corncob using indigenous hemicellulase of SH3.

$$\text{Sugar release (Response)} = +5977.72 - 272.92*A + 83.86*B - 72.57*C - 259.44*D - 12.66*A*B + 10.95*A*C + 0.30*A*D + 1.37*B*C + 2.55*B*D + 1.12*C*D + 18.78*A^2 - 18.32*B^2 - 2.35*C^2 + 3.59*D^2$$

High model F-value (98.26%) showed that the model is very much significant. All the variable interactions were significant at $P<0.05$ level, except for the two interactions between the variables AD and BC. Moreover, coefficient of determination (R^2) was 0.9913, which showed that the model is 99% suitable for the variability in the samples. The insignificance of the lack of fit was described by the compound F value. The small model P-value ($P<0.05$) showed the high significance of the model and also revealed the meaningful relationship between the selected variable range and the experimental response values (Supplementary Table 3).

The 3-D contour plots described the interactive effect of selected variables including substrate loading and pH (Fig. 4(a)), substrate loading and enzyme loading (Fig. 4(b)), pH and temperature (Fig. 4(c)), and enzyme loading and temperature (Fig. 4(d)) on the sugar release. The model proposed substrate loading of 8%, pH of 3.0,

Table 3. Comparison of total sugar release during saccharification using corncob as substrate

S. no.	Pretreatment	Substrate loading	Enzyme source	Hydrolysis temperature (°C)	Enzyme loading (FPU/gds)	Sugar release (mg/gds)	Reference
1.	Dilute acid	5%	*Trichoderma reesei* QM 9414	50	224	170	[41]
2.	Physical (size reduction)	0.2%	Novozyme 188 with crude enzyme of *Streptomyces* sp. ssr-198	60	20	210	[42]
3	Alkali	5%	*Aspergillus aculeatus* BCC199	50	7.5	800	[43]
4	Ionic liquid	1%	Trichoderma reesei	50	30	425	[44]
5	Mild alkali	8%	*Aspergillus niger* SH3	38	11	912	Present study

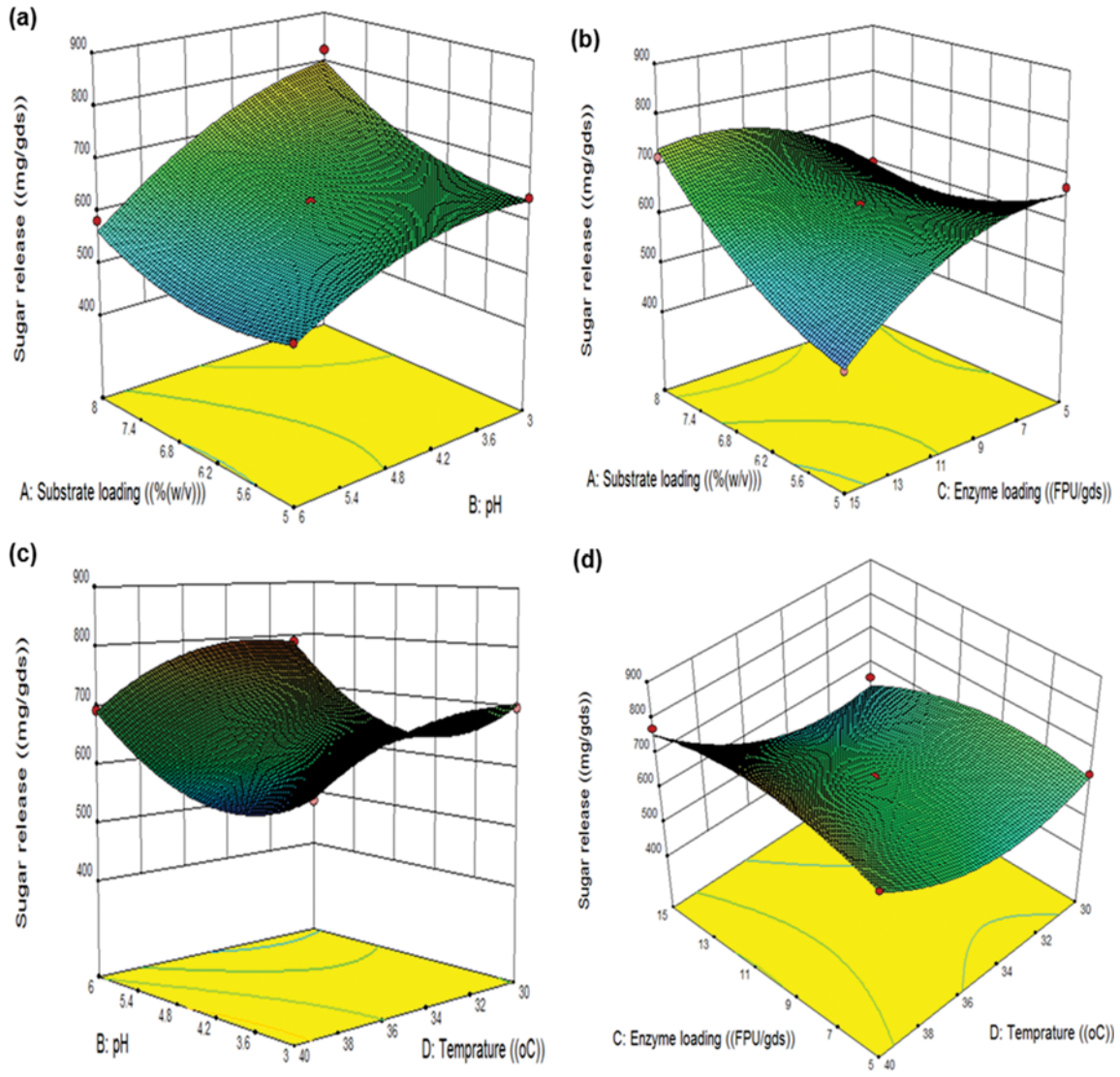

Fig. 4. Response surface plots based on Box-Behnken design for optimization of saccharification parameters by indigenous holocellulase enzyme.

enzyme loading of 11 FPU/gds and temperature 38 °C as the optimum conditions with predicted sugar release of 899.97 mg/gds of pretreated biomass. The model was validated by performing the experiment under optimized condition and higher sugar release 912 mg/gds (glucose: 598.87; xylose: 308.32 and arabinose: 44.81 mg/gds) was observed from the pretreated corncobs. Similar effect of high substrate loading with minimal enzyme load on sugar release was observed by other researchers [37,38]. The major highlight of this study is that the holocellulase used in the study has lower temperature optima for saccharification (38 °C) as compared to other commercial enzymes, which functions optimally at 50 °C. A comparison of the total sugar release from differently pretreated corncob after saccharification is given in Table 3, which validates the superiority of our process to produce maximum sugar yield at lower temperature of saccharification. All these characteristics of enzyme are suitable for simultaneous saccharification and fermentation and may help to lower the cost of biofuel production.

5. Simultaneous Saccharification and Fermentation (SSF) of Pretreated Corncob

The alkali pretreated corncob under the optimized pretreatment condition was further used for bioethanol production through simultaneous saccharification and fermentation. The cold active holocellulase from SH3 was employed to hydrolyze the pretreated corncob into monomeric sugars, which was simultaneously fermented into the bioethanol through *S. cerevisiae* LN. The SSF process has many advantages over separate hydrolysis and fermentation, such as prevention of end-product inhibition and also offers an economical solution by saving on incubation time and energy. However, unmatched incubation temperature requirements for saccharification (approx. 45-55 °C) and fermentation (approx. 30-35 °C) is a major bottleneck for this process [17]. To overcome this obstacle, for the first time, cold active holocellulase from SH3 was used for SSF process. As the optimization of saccharification experiment suggested optimum temperature to be 38 °C for the efficient hydro-

lysis of pretreated corncob, which allows the mesophilic yeast strain for ethanol fermentation at this temperature. The results suggested that maximum ethanol production (13.05±0.32 g/L) ~48.85% theoretical yield was achieved after 72 h of SSF process. Li et al. [39] also obtained nearly 48% of ethanol yield from pretreated corncob. Further improvement needs to focus on the removal of various phenolics, organic acids and lignin derivatives generated by chemical pretreatment, which inhibits the enzymatic action and yeast cell growth [40]. Interventions for the removal of these toxins and inhibitors can further enhance the bioethanol production and make it more cost and energy efficient.

CONCLUSIONS

Mild alkali pretreatment of corncob at room temperature resulted in the highest recovery of holocellulose (88.0%) with significant lignin removal. Furthermore, indigenous holocellulase enzyme was produced by psychrotolerant *A. niger* SH3 using corn stover as carbon source. Saccharification process was statistically optimized, and hydrolysis condition at high substrate loading (8%), low enzyme loading (11 FPU/gds), low temperature (38 °C) and pH (3.0) was established to achieve maximum sugar release. Moreover, SSF of pretreated corncob resulted in good ethanol yields with 48.8% fermentation efficiency, which can be further increased by refinement of fermentation related parameters. The present study highlighted the promising utilization through bioconversion of corn crop residues, including corn stover and corncob into biocatalyst and biofuel, respectively. Further research needs to focus on refining and upscaling the process for industrial purpose.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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