



Original Article

Acid hydrolysis optimization of cocoa pod shell using response surface methodology approach toward ethanol production

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ABSTRACT

Cocoa pod shell (CPS) is an underutilized agricultural lignocellulosic biomass. Hydrochloric acid (HCl) hydrolysis was carried out to release the reducing sugars from CPS. The conditions (CPS weight, concentration of HCl, revolutions per minute, hydrolysis period) which affect HCl hydrolysis were screened using one factor at a time approach of which determined that CPS weight, HCl concentration and hydrolysis period had a significant effect on the acid hydrolysis process. The levels of these factors were further optimized using a central composite design using response surface methodology. The optimized conditions were 8.36% (weight per volume) of CPS, 3.6 N of HCl concentration with 7.36 h of acid hydrolysis which yielded 4.09 g/L reducing sugars. A second order model was generated and validated, which was found to be a good fit (coefficient of determination = 0.914). The released reducing sugars after the acid hydrolysis under optimized conditions were subjected to alcoholic fermentation by *Pichia stipitis* to produce bioethanol. The bioethanol concentration reached 2 g/L at 2% (volume per volume) inoculum concentration after 72 h of fermentation.

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Introduction

Fluctuating oil prices, depleting petroleum reserves, global warming due to environmental pollution and greenhouse gas emission appear to have stimulated research in renewable fuels as an alternative. Biofuel produced from renewable resources such as lignocellulosic biomass is one of the options to meet the demand of fuel requirement, and it is considered to be environmental friendly compared to petroleum fuels (Saini et al., 2015). Most commonly used lignocellulosic feedstocks for the production of second-generation bioethanol include agricultural residues such as corn straw, corn stover, wheat straw, bagasse and energy crops like grasses (Sims et al., 2010). Ethanol production from lignocellulosic biomass is preferred because it does not compete with food sources such as starchy and sugary residues and hence food-versus-fuel competition can be avoided (Farrel et al., 2006). Lignocellulosic biomass has drawn worldwide attention as a viable second-

generation alternative feedstock for biofuels production, given its availability and low cost (Antonopoulou et al., 2016). Although lignocellulosic biomass is the most abundant feedstock for the manufacture of biofuels, its utilization at the commercial level for the large-scale production of ethanol has been limited because of its complex, recalcitrant nature (Kim et al., 2014). There is currently a great deal of research being carried out to convert lignocellulosic biomass to fuels (Chen et al., 2016). Low economic value biomass can be upgraded to high energy products via different routes such as chemical treatment (López et al., 2014). A wide variety of pre-treatment techniques such as dilute acid, fiber explosion by ammonia, steam explosion, alkaline hydrolysis and an organosolv approach have been employed upto date to breakdown the recalcitrant lignocellulosic biomass to various types of simple sugars (Wyman, 1994; Zhang et al., 2007; Cheng et al., 2010; Potumarthiet al., 2013). Pretreatment of lignocellulosic residues is a crucial step in the process of production of bioethanol and the cost effectiveness of the whole process of biofuel production is dependent on this step (Badhe et al., 2014). Dilute or concentrated acid can be used to hydrolyze the cellulose and hemicellulose present in the lignocellulosic biomass to sugars (Wyman, 1994). In a study conducted using cocoa pod hydrolysis with hydrochloric acid,

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sulfuric acid and nitric acid, the resulting hydrolysate was found to contain carbohydrate (Samah et al., 2011). The addition of alkali in the neutralization step facilitates detoxification of the hydrolysis product making it suitable for subsequent fermentation (Chandel et al., 2007). In India, the current annual production of cocoa is about 12,000 tons and cocoa pod shell represents 70–75% of the weight of the cocoa fruit, so that for each tonne of cocoa fruit there is 700–750 kg of waste (Cruz, 2012). Optimization of the hydrolysis of cocoa pod shell (CPS) using hydrochloric acid has not been reported so far. CPS is available abundantly in the Dakshina Kannada district of Karnataka state, India (Shet et al., 2018). Therefore, the current investigation is subjected to locally available CPS with HCl hydrolysis to release reducing sugars. A response surface methodology (RSM) was used to optimize the process under the optimized conditions. The sugars thus released were subjected to fermentation by *Pichia stipitis* to produce bioethanol.

Materials and methods

Compositional analysis of raw material

The cellulose, hemicellulose and lignin content of CPS estimated is (Sharma and Singh, 2009) as follows.

Cellulose estimation

A sample of 1g of CPS power was vortexed using 3 mL of acetic/nitric acid reagent and was placed in a water bath at 100 °C for 30 min. This was further centrifuged at 8000 revolutions per minute (rpm) for 15 min and the supernatant was discarded. The residue collected was later washed with distilled water and then 10 mL of 67% sulfuric acid was added and allowed to settle for 1 h. An additional 1 mL of the solution was diluted to 100 mL and was mixed with 10 mL anthrone reagent and placed in a boiling water bath for 10 min. The absorbance was read at 630 nm. A calibration graph using standard cellulose was determined for cellulose.

Lignin estimation

A sample of 1g of CPS powder was vortexed using 100 mL of acid detergent solution (20g of cetyltrimethylammonium bromide in 1 L of 1N H₂ SO₄). This was boiled for 10 min and the contents were filtered using a sintered funnel (G2) followed by washing twice in hot water and then an acetone wash until the filtrate turned colorless. The product was dried at 100 °C overnight and the weight was determined after cooling in a desiccator. The percentage acid detergent fiber (ADF) was calculated using Equation (1):

$$\% \text{ Lignin (acid detergent fiber)} = \frac{[(\text{weight of fiber}) / \times (\text{weight of cocoa pod shell}) \times] \times 100}{(1)}$$

Hemicellulose estimation

A sample of 1g CPS powder was mixed with 10 mL of cold neutral detergent (18.61 g disodium ethylene diaminetetracetate) and 6.81 g of sodium borate decahydrate in a reflux flask and dissolved in 200 mL of distilled water. Then 30 g of sodium lauryl sulphate and 10 mL of 2-ethoxy ethanol were added in 150 mL of distilled water and 4.5 g of disodium hydrogen phosphate dissolved in 100 mL of water was added. The pH was adjusted to 7 and the volume was raised to 1 L. Later, 2 mL of decahydronaphthalene and

500 mg of sodium sulfite was transferred to the reflux flask for 1 h at boiling temperature. The product was filtered through sintered glass (G2) with a further wash in hot water. The contents were processed using an acetone wash and dried at 100 °C for 12 h in a crucible and then weighed. The hemicellulose content was calculated using Equation (2):

$$\% \text{ Hemicellulose} = \frac{[(\text{neutral detergent fiber} - \text{acid detergent fiber}) / \text{CPS weight}] \times 100}{(2)}$$

Feedstock collection and preparation of raw material

Fresh CPS was obtained from Peruvai village, Vittla taluk in DK district, India. The CPS was washed with tap water, cut into small pieces, further sun-dried for 2 days and oven-dried at 80 °C until about 95% of the moisture in the CPS had been removed. Dried shell pieces were ground to a fine powder in a mixer-grinder and the coarse particles were removed using a Taylor number 10 sieving mesh. The fine powder was stored in an air-tight container and refrigerated until further use.

Optimization of cocoa pod shell hydrolysis process

Selection of significant parameters and their levels using one factor at a time

To select the significant physical parameters and the initial test range of the four variables of weight of CPS (X₁; % weight per volume, w/v), HCl concentration (X₂; N), hydrolysis period (X₃; hours) and agitation speed (X₄, revolution per minute) the conventional one factor at a time (OFAT) approach was used. The effect of the parameters on the acid hydrolysis process was checked by varying one parameter at a time and keeping the other parameters constant (Table 1). The experiments were carried out at room temperature in 250 mL conical flasks containing 100 mL of acid solution of appropriate strength, with an appropriate quantity of CPS (Table 1). All the experiments were conducted in triplicate. The flasks were agitated at different speeds for different intervals of time (Table 1) and the estimation of reducing sugars released was carried out using the dinitrosalicylic acid (DNSA) method (Miller, 1959). The parameter levels at which maximum reducing sugars were released, were chosen as the center point values to enhance the acid hydrolysis process using RSM.

Optimization of parameters using central composite design for release of reducing sugars by acid hydrolysis

Three experimental factors: Weight of CPS (X₁, %w/v), concentration of HCl (X₂, N) and hydrolysis period (X₃, hours) were selected for RSM optimization. A significant effect on the acid hydrolysis process during the OFAT studies was exhibited by these factors and their levels were optimized for the maximum release of reducing sugars from the CPS using the central composite design

Table 1

Selected parameters and their levels for one-factor-at-a-time studies of cocoa pod shell (CPS) hydrolysis.

Parameter	Notation	Test range
Weight of CPS (%w/v)	X ₁	1–7
HCl concentration (N)	X ₂	1–6
Hydrolysis period (hr)	X ₃	1–6
Agitation speed (rpm)	X ₄	50–150

w/v = weight per volume; rpm = revolutions per minute.

(CCD) for acid hydrolysis. The concentration of sugars released by acid hydrolysis was represented as the response and was designated as Y_1 for acid hydrolysis. Based on three factors, the CCD was designed consisting of five levels (Table 2), consisting of 20 experimental runs (Table 3). The levels of other non significant factors were kept constant at the tested center values during the OFAT studies. These optimization experiments were designed using the Statistica trial version software (Statsoft, USA.)

The data obtained from the acid hydrolysis studies were subjected to analysis of variance (ANOVA) using the same software. A second-order polynomial model was utilized to obtain the mathematical response between the response and the independent variables as shown in Equation (3):

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i \neq j} \beta_{ij} X_i X_j \quad (3)$$

where, Y is the dependent response, β_0 , β_i , β_{ii} , β_{ij} are estimates of the polynomial coefficients and X_i , X_j represent the independent variables.

Estimation of released reducing sugars

The optimization experiments were carried out in 250 mL conical flasks containing 100 mL of acid solution as shown in Table 3. Experimental run numbers from 15 to 20 represented central points of the CCD (Table 2) and were denoted by “(C)”. An agitation speed of 100 rpm determined from the OFAT study was used for experimentation. Agitation speed was not chosen as a factor, since the released reducing sugar (RRS) concentration was not changed significantly. A lower rpm would affect the distribution of CPS and a higher rpm could lead to a vortexing problem. However, maintaining a fixed value of agitation was essential, hence 100 rpm was considered for uniform distribution of the CPS and better interaction with the HCl. The flasks were taken out at the time intervals mentioned in the design and estimation of RRS was done using the DNSA method. The hydrolysate was neutralized to pH 7.0 using 5N NaOH. The expected products of hydrolysis were glucose, galactose, cellobiose and minor saccharides as xylulose and arabinose. All of these saccharides are reducing sugars (Brummer et al., 2014). Therefore, the concentration of RRS was estimated using an ultraviolet visible spectrophotometer at 540 nm using DNSA reagent as chromogen with maltose as the standard (Miller, 1959).

Validation of the second-order polynomial model

The validation of the second-order polynomial model obtained from RSM was carried out by conducting a series of experiments generated by choosing random values of parameters within the optimized levels. Experiments were conducted at the optimized conditions generated using the trial version of Statistica software. The experimental output was then compared with the values

Table 2
Independent parameters and their coded levels for acid hydrolysis of cocoa pod shell (CPS) using central composite design.

Factor	Notation	Level				
		$-\alpha$	-1	0	$+1$	$+\alpha$
Weight of CPS (%weight per volume)	X_1	1.630	3	5	7	8.36
Concentration (N)	X_2	0.318	1	2	3	3.68
Hydrolysis period (hr)	X_3	0.636	2	4	6	7.36

Table 3

Actual levels of independent variables with observed values of the response variable, released reducing sugar.

Run	X1	X2	X3	Y1 (g/L)
1	3.00	1.00	2.00	0.556
2	3.00	1.00	6.00	1.263
3	3.00	3.00	2.00	1.465
4	3.00	3.00	6.00	1.162
5	7.00	1.00	2.00	1.768
6	7.00	1.00	6.00	1.763
7	7.00	3.00	2.00	2.424
8	7.00	3.00	6.00	3.131
9	1.63	2.00	4.00	0.606
10	8.36	2.00	4.00	2.677
11	5.00	0.31	4.00	0.909
12	5.00	3.68	4.00	1.465
13	5.00	2.00	0.63	0.960
14	5.00	2.00	7.36	1.919
15 (C)	5.00	2.00	4.00	1.010
16 (C)	5.00	2.00	4.00	1.010
17 (C)	5.00	2.00	4.00	1.111
18 (C)	5.00	2.00	4.00	1.010
19 (C)	5.00	2.00	4.00	1.010
20 (C)	5.00	2.00	4.00	1.010

predicted by the second-order model obtained from the CCD, to estimate the goodness of fit of the model.

Fermentation of released reducing sugars under optimized conditions to produce bioethanol using *Pichia stipitis*.

Microorganism and inoculum preparation

P. stipitis NCIM 3498 was obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Freeze-dried culture was maintained in a medium of the composed of: glucose, 10 g/L; yeast extract, 3 g/L; malt extract, 3 g/L; peptone, 5 g/L; and pH 6.5. Stock culture of *P. stipitis* was prepared by growing the microorganism in the liquid medium for 16 h at 30 °C. Inoculum was developed from stock culture and inoculated during the log phase.

Production of bioethanol

CPS hydrolyzed under the optimized conditions was neutralized to pH 7.0 using 5N NaOH and inoculation was carried out at 2% volume per volume (v/v) using *P. stipitis*. After fermentation, the broth was distilled and the bioethanol concentration was estimated using gas chromatography (GC-2014; Shimadzu Corp.; Kyoto, Japan) fitted with a column (DB-624) of 30 m, 0.32 mm and 1.4 μ m. The temperature of the injection port was maintained at 230 °C and the column temperature was varied from 50 °C to 150 °C at a rate of 10 °C per min, which was maintained for 3min and the hydrogen flame ionization detector temperature was set at 250 °C. The flow rate of the carrier gas was maintained to 8 mL/min.

Results and discussion

Compositional analysis of raw materials

The composition of the CPS raw material was investigated and was found to be composed of cellulose (16.9%), hemicellulose (4%) and lignin (69%).

Optimization of cocoa pod shell acid hydrolysis process

Selection of significant parameters and their levels using one factor at a time analysis.

The significant physical parameters and the initial test range of the four factors in the acid hydrolysis processes obtained using the OFAT analysis are given in Table 1. When the weight of CPS was varied and the other factors were kept constant ($X_2 = 2\text{N}$, $X_3 = 1\text{hr}$, $X_4 = 100\text{ rpm}$) the maximum RRS concentration was 2.73 g/L at 5% (w/v) of CPS (Fig. 1A). The decreasing concentration of RRS with increased CPS weight may have been due to the availability of HCl for hydrolysis, since the concentration of HCl was fixed. A decrease in RRS with an increase in CPS is natural because the increase hydrolyzed material would decrease the effectiveness of the acid hydrolysis by the same acid amount. Acid concentration was varied from 1N to 6N by keeping other factors constant ($X_1 = 5\%$ (w/v), $X_3 = 1\text{hr}$, $X_4 = 100\text{ rpm}$) (Fig. 1B). The resultant decrease in the RRS concentration with an increased concentration of HCl was probably due to degradation of the RRS by the available HCl. A similar trend was reported by Samah et al. (2011) for acid hydrolysis of cocoa pod shells. Since 2N HCl was the optimum level to produce the maximum RRS of 4.73 g/L, for the subsequent step of optimization with fixed parameters ($X_1 = 5\%$ (w/v), $X_2 = 2\text{N}$, $X_3 = 1\text{hr}$), the agitation speed was varied between 50 rpm and 150 rpm (Fig. 1C). There was no significant change in the release of RRS as a result of the variation in the agitation speed. The hydrolysis time was varied by keeping the other parameters fixed ($X_1 = 5\%$ (w/v), $X_2 = 2\text{N}$, $X_4 = 100\text{ rpm}$) as shown in Fig. 1D. The maximum release of sugar of 5.13 g/L (51.3 %w/v) was reported at after 4 h of hydrolysis. Previous reports indicated that hydrolysis in 1.0 M hydrochloric acid at 75 °C for 4 hr produced the highest glucose content of 30.7% w/v Samah et al., 2011).

Optimization of parameters using central composite design for release of reducing sugars with acid hydrolysis

The influence of the weight of CPS (X_1), concentration of acid (X_2) and time required for hydrolysis (X_3) to release reducing sugars was determined from the CCD results as shown in Table 3. This table also represents the observed values for the RRS concentration

using acid hydrolysis (Y_1) at different combinations of the independent parameters. In the 20 experiments conducted, the RRS concentration varied from 0.556 g/L to 3.131 g/L for acid hydrolysis.

Table 4 indicates the values obtained using ANOVA for the release of reducing sugars on acid hydrolysis of CPS. Basically, a smaller p-value indicates higher influence and only factors having $p < 0.05$ can be considered as statistically important (Guo et al., 2009). From these values, it is imperative that there is a linear effect for the parameters X_1 , X_2 and X_3 . Similarly, X_1 and X_3 exhibited a quadratic interaction.

The response surface graphs for Y_1 as a function of the weight of CPS (X_1) and concentration of acid (X_2), of the weight of CPS (X_1) and hydrolysis period (X_3) and of the hydrolysis period (X_3) and concentration of acid (X_2) are depicted in Fig. 2A–C, respectively.

It was observed that the release of reducing sugars increased with an increase in the weight of CPS and the acid (HCl) concentration. With the X_1 and X_3 response, the concentration of RRS increased with the increase in the weight of CPS with respect to time. With the X_3 and X_2 response, RRS increased with the increase in the acid concentration. Similarly, as the hydrolysis time increased, the concentration of RRS increased. The optimum parameters determined for the maximum RRS were based on the interactive effects of X_1 , X_2 and X_3 .

The optimized levels of variables (X_1 , X_2 , X_3) for the maximum release of reducing sugars by acid hydrolysis were determined from the desirability plots (Fig. 3). On this basis, the optimized factors for obtaining maximum Y_1 were 8.36 g/L of CPS and 3.6 N of HCl after 7.36 h of treatment. The desirability profile allows inspection of the pattern of response (output produced) by fitting the observed responses using the second-order equation based on the levels of the independent variables. The level of each independent variable that generates the maximum response is chosen as the optimum level. The desirability plot to get the maximum release of reducing sugars was plotted using the least squares method. The level of variable giving the highest desirability (1.0) was selected as the optimum level. Experiments were performed at the optimized levels of the

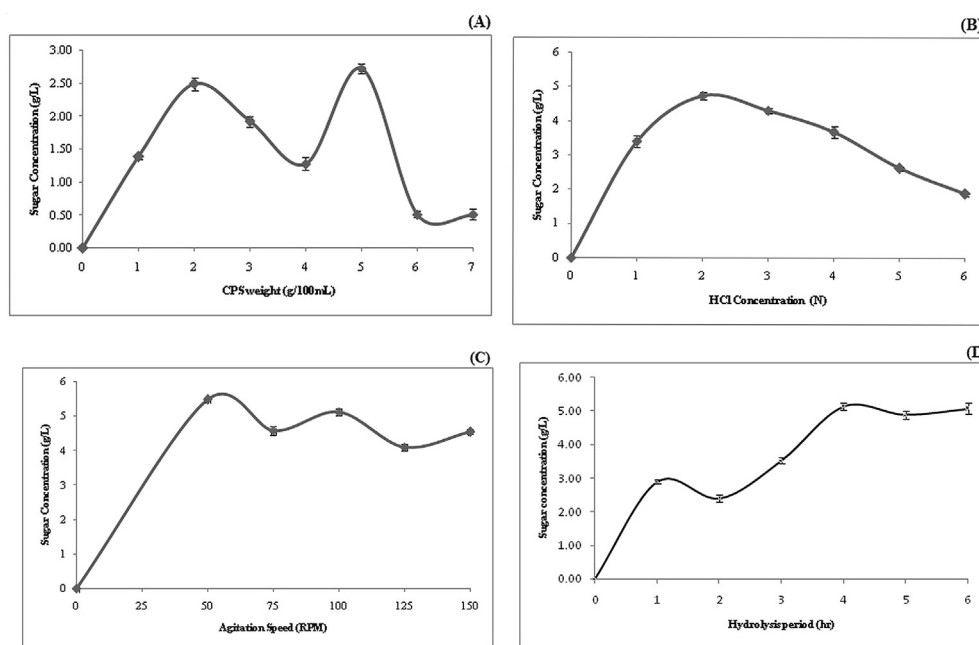


Fig. 1. (A) Release of reducing sugars on acid hydrolysis of cocoa pod shell (CPS) at different concentrations of CPS, where error bars indicate standard deviation. (B) Release of reducing sugars on acid hydrolysis of cocoa pod shell at different concentrations of HCl, where error bars indicate standard deviation. (C) Release of reducing sugars on acid hydrolysis of cocoa pod shell at different agitation speeds, where error bars indicate standard deviation. (D) Release of reducing sugars on acid hydrolysis of cocoa pod shell for different hydrolysis periods, where error bars indicate standard deviation.

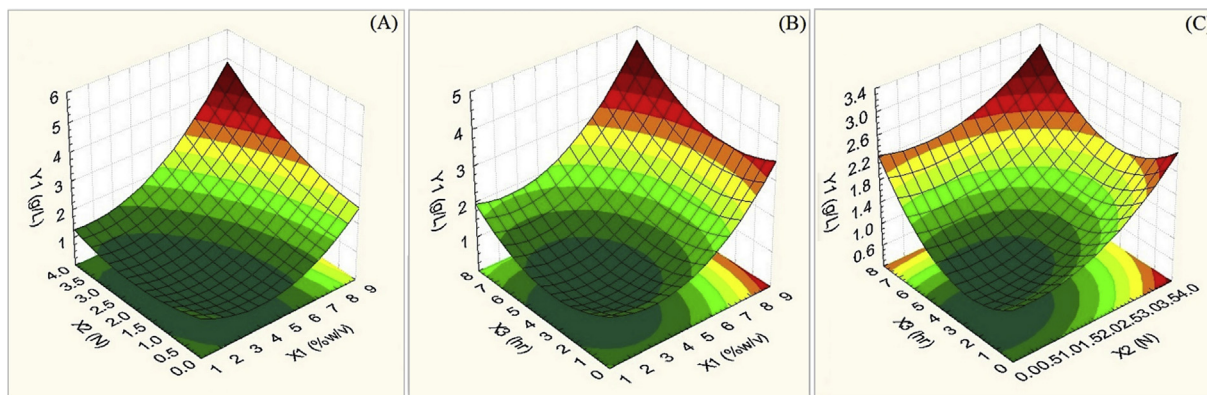


Fig. 2. Three-dimensional plot showing the effect of: (A) weight of cocoa pod shell (CPS); (X_1) and concentration of acid (X_2) on release of reducing sugars on acid hydrolysis of CPS; (B) weight of CPS (X_1) and time on release of reducing sugars on acid hydrolysis of CPS; (C) time and concentration of HCl on reducing sugars on acid hydrolysis of CPS.

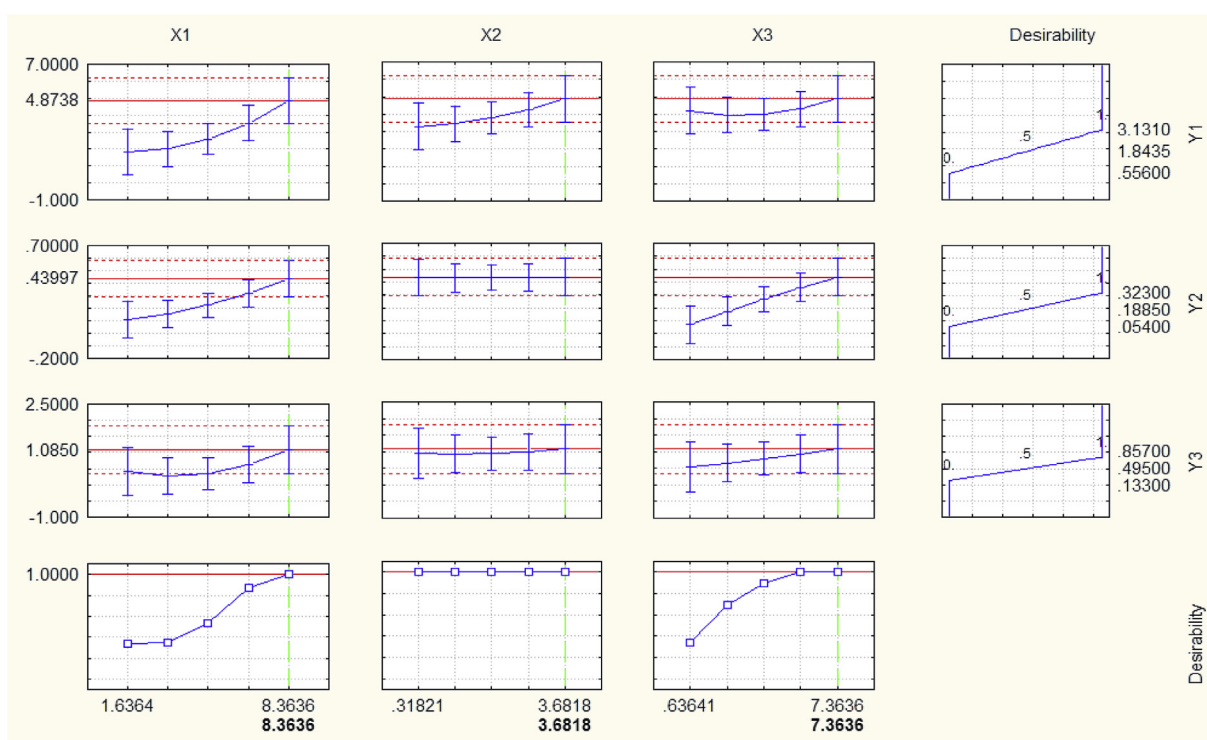


Fig. 3. Profiles for desirability levels of factors: X_1 (weight of cocoa pod shell (CPS); %weight per volume), X_2 (HCl concentration - N) and X_3 (hydrolysis period; hours) for maximum release of reducing sugars on acid hydrolysis of CPS.

variables mentioned above and the concentration of RRS was 4.09 g/L. Release of glucose from 10 g of Napier grass under optimum conditions using HCl was reported to be 2.28 g/L (Mafuleka and Kana, 2015). Hydrolysis of bagasse using 2% HCl at 128 °C for 51.1 min resulted into 3.77 g/L of glucose (Bustos et al., 2003). Furthermore, 37.6 g of sugar was extracted per 100 g of olive prunings using an RSM approach (Martin et al., 2013). From wheat straw, 50% of sugar was extracted using H_2SO_4 pretreatment (Baboukani et al., 2012). In the current investigation, 4.09 g/L of sugar was released from 8.36 g of CPS per 100 mL; hence CPS is a potential lignocellulosic material for reducing sugars.

The regression equation for the release of reducing sugars using the acid hydrolysis of CPS, as a function of the independent variables (X_1 , X_2 and X_3) and their linear and quadratic interactions, is represented by Equation (4):

$$Y1 = 2.2839 - 0.567X_1 - 0.463X_2 - 0.305X_3 + 0.067X_{12} + 0.108X_{22} + 0.049X_{32} + 0.076X_1X_2 + 0.009X_1X_3 - 0.0187X_2X_3 \quad (4)$$

The coefficient of determination (R^2) is a measure of the strength of the linear relationship between the experimental and predicted values (Sharmada et al., 2016). The value of R^2 for the correlation between the observed and predicted RRS on acid hydrolysis of CPS was 0.914.

The second-order models obtained were validated using a random set of experiments apart from the experimental runs (Table 5). The observed values of RRS were compared with the RRS values as

Table 4

Analysis of variance table for release of reducing sugar on acid hydrolysis of cocoa pod shell (CPS).

	Sum of squares (SS)	Degrees of freedom	Mean square	F-value	p-value*
Model	8.15	09	0.91	11.85	0.0003
(1) X ₁ (L)	4.83	01	4.83	63.21	1.2E-05
X ₁ (Q)	1.04	01	1.04	13.64	0.0041
(2) X ₂ (L)	1.03	01	1.03	13.59	0.0042
X ₂ (Q)	0.16	01	0.16	2.21	0.1678
(3) X ₃ (L)	0.54	01	0.54	7.08	0.0238
X ₃ (Q)	0.56	01	0.56	7.35	0.0218
1L by 2L	0.18	01	0.18	2.41	0.1509
1L by 3L	0.01	01	0.01	0.14	0.7111
2L by 3L	0.01	01	0.01	0.14	0.7111
Error	0.76	10	0.07		
Total SS	8.91	19			
Lack of Fit	0.76	05	0.15	88.81	0.0001

X₁ = weight of CPS (%weight per volume); X₂ = HCl concentration (N); X₃ = hydrolysis period (hours).Coefficient of determination (R²) = 0.91427; Adj R² = 0.8371.

*p < 0.05 is significant.

Table 5

Validation runs with observed and predicted values of reducing sugars released from coca pod shell on pre-treatment with acid.

Run	X ₁	X ₂	X ₃	Experimental concentration of RRS (g/L)	Theoretical concentration of RRS (g/L)
1	2.5	1.5	3.0	0.48	0.64
2	6.0	2.5	5.0	1.39	1.73
3	4.0	3.0	2.5	1.08	1.10
4	6.5	3.0	4.0	2.12	2.11
5	8.0	2.5	4.0	2.37	2.79
6	3.0	3.5	3.0	1.08	1.11

X₁ = weight of CPS (%weight per volume); X₂ = HCl concentration (N); X₃ = hydrolysis period (hours); RRS = released reducing sugar.

predicted by the second-order models. These results indicated that there was an excellent correlation between the experimental and predicted values and thus proved the validity of the models.

Fermentation of released reducing sugars under optimized conditions to produce bioethanol using *Pichia stipitis*

An inoculum concentration of 2% v/v was used in separate 250 mL Erlenmeyer flasks containing neutralized acid hydrolysate. An air lock system was adopted for each Erlenmeyer flask which was kept at 30 °C in a rotary shaker maintained at 100 rpm for 72 h. The recovered bioethanol was estimated using gas chromatography. The ethanol concentration was 2 g/L of CPS hydrolysate. Chandel et al. (2007) reported a concentration of 3.46 g/L ethanol from 2.5% HCl treatment followed by neutralization and fermentation of bagasse. An ethanol concentration of 3.56 g/L was reported by (Muktham et al., 2016) for 4% HCl. An ethanol yield of 17.51 g/L from *Parthenium hysterophorus* was reported with a yield of 0.41 g/g total reducing sugar (Bharadwaja et al., 2015).

The current study was designed to explore the feasibility of CPS as a source of biomass for ethanol production. The optimized conditions for HCl hydrolysis were 8.36 g CPS per 100 mL, 3.6 N HCl concentration after 7.36 h of acid hydrolysis which produced a yield of 4.09 g/L of reducing sugars. Furthermore, fermentation of CPS hydrolysate using *P. stipitis* NCIM3498 resulted in 2 g/L of ethanol production. The results clearly demonstrated that CPS can be used as a cheaper, renewable material feedstock for ethanol production.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

- Antonopoulou, G., Vayenas, D., Lyberatos, G., 2016. Ethanol and hydrogen production from sunflower straw: the effect of pretreatment on the whole slurry fermentation. *Biochem. Eng. J.* 116, 65–74.
- Baboukani, B.S., Vossoughi, M., Alemzadeh, I., 2012. Optimisation of dilute-acid pretreatment conditions for enhancement sugar recovery and enzymatic hydrolysis of wheat straw. *Biosyst. Eng.* 111, 166–174.
- Badhe, R.R., Potumarthi, R., Mekala, N.K., 2014. Influence of dilute acid and alkali pretreatment on reducing sugar production from corncobs by crude enzymatic method: a comparative study. *Bioresour. Technol.* 162, 213–217.
- Bharadwaja, S.T.P., Shuchi, S., Vijayanand, S.M., 2015. Design and optimization of a sono-hybrid process for bioethanol production from *Partheniumhysterophorus*. *J. Taiwan. Inst. Chem. Eng.* 51, 71–78.
- Brummer, V., Jurena, T., Hlavacek, V., Omelkova, J., Bebar, L., Gabriel, P., Stehlik, P., 2014. Enzymatic hydrolysis of pretreated waste paper: Source of raw material for production of liquid biofuels. *Bioresour. Technol.* 152, 43–547.
- Bustos, G., Ramirez, J.A., Garrote, G., Vázquez, M., 2003. Modeling of the hydrolysis of sugar cane bagasse with hydrochloric acid. *Appl. Biochem. Biotechnol.* 104, 51–68.
- Chandel, A.K., Kapoor, R.K., Singh, A., Kuhad, R.C., 2007. Detoxification of sugar-canebagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM3501. *Bioresour. Technol.* 98, 1947–1950.
- Chen, W., Wu, F., Zhang, J., 2016. Potential production of non-food biofuels in China. *Renew. Energy* 85, 939–944.
- Cheng, K.K., Zhang, J.A., Chavez, E., Li, J.P., 2010. Integrated production of xylitol and ethanol using corncob. *Appl. Microbiol. Biotechnol.* 87, 411–417.
- Cruz, G., 2012. Production of activated carbon from cocoa (*Theobroma cacao*) pod husk. *J. Civ. Environ. Eng.* 2, 2. <https://doi.org/10.4172/2165-784X.1000109>.
- Farrel, A.E., Plevin, R.J., Turner, R.T., Jones, A.D., O' Hare, M., Kammen, D.M., 2006. Ethanol can contribute to energy and environmental goals. *Science* 3, 506–508.
- Guo, W.Q., Ren, N.Q., Wang, X.J., Xiang, W.S., Ding, J., You, Y., Liu, B.F., 2009. Optimization of culture conditions for hydrogen production by *Ethanoligenens harbinense* B49 using response surface methodology. *Bioresour. Technol.* 100, 1192–1196.
- Kim, I., Rehman, M.S.U., Han, J.I., 2014. Enhance glucose yield and structural characterization of cornstover by sodium carbonate pretreatment. *Bioresour. Technol.* 152, 316–320.
- López, F., García, M.T., Fera, M.J., García, J.C., De Diego, C.M., Zamudio, M.A.M., Diaz, M.J., 2014. Optimization of furfural production by acid hydrolysis of *Eucalyptusglobulus* in two stages. *Chem. Eng. J.* 240, 195–201.
- Mafuleka, S., Kana, E.G., 2015. Modelling and optimization of xylose and glucose production from Napier grass using hybrid pre-treatment techniques. *Biomass Bioenergy* 77, 200–208.
- Martín, J.F.G., Sánchez, S., Cuevas, M., 2013. Evaluation of the effect of the dilute acid hydrolysis on sugars release from olive prunings. *Renew. Energy* 51, 382–387.

- Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426–428.
- Muktham, R., Ball, A.S., Bhargava, S.K., Bankupalli, S., 2016. Bioethanol production from non-edible de-oiled *Pongamia pinnata* seed residue-optimization of acid hydrolysis followed by fermentation. *Ind.Crops Prod.* 94, 490–497.
- Potumarthi, R., Badhe, R.R., Nayak, P., Jetty, A., 2013. Simultaneous pretreatment and saccharification of rice husk by *Phanerochete chrysosporium* for improved production of reducing sugars. *Bioresour. Technol.* 128, 113–117.
- Saini, J.K., Saini, R., Tewari, L., 2015. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech* 5, 337–353.
- Samah, O.A., Sias, S., Hua, Y.G., Hussin, N.N., 2011. Production of ethanol from cocoa pod hydrolysate. *J. Math. Fundam. Sci.* 43, 87–94.
- Sharma, R.K., Sangha, S.P., 2009. *Basics Techniques in Biochemistry and Molecular Biology*. I.K International Publishing House, New Delhi, India.
- Sharmada, N., Punja, A., Shetty, S.S., Shet, V.B., Goveas, L.C., Rao, C.V., 2016. Optimization of pre-treatment of de-oiled oil seed cake for release of reducing sugars by response surface methodology. *Bioethanol* 2, 94–102.
- Shet, V.B., Shetty, V.C., Siddik, A., et al., 2018. Optimization of microwave assisted H₂SO₄ hydrolysis of cocoa pod shells: comparison between response surface methodology and artificial neural network and production of bioethanol thereof. *J. Microbiol. Biotechnol. Food Sci.* 7, 473–477.
- Sims, R.E.H., Maybee, W., Saddler, J.N., Taylor, M., 2010. An overview of second generation technology. *Bioresour. Technol.* 101, 1570–1580.
- Wyman, C.E., 1994. Ethanol from lignocellulosic biomass: technology, economics, and opportunities. *Bioresour. Technol.* 50, 3–15.
- Zhang, Y.H.P., Schell, D.J., McMillan, J.D., 2007. Methodological analysis for the determination of enzymatic digestibility of cellulosic materials. *Biotechnol. Bioeng.* 96, 188–194.