THE APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR THE OPTIMIZATION OF AUTOCLAVE ASSISTED HCL HYDROLYSIS OF AN AGRO RESIDUE COCOA POD SHELLS FOR RELEASING REDUCED SUGARS

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ABSTRACT

Cocoa pod shells are the agro waste generated causing problem during the disposal. In the present study, possibility of utilizing the cocoa pod shells to release the reducing sugars has been explored. Hydrochloric acid hydrolysis assisted with autoclave was adopted to obtain reducing sugars. The one parameter at a time approach was implimented to screen the significant parameters influencing the hydrolysis among weight of cocoa pod shells, concentration of HCl and duration of autoclave. Parameters such as concentration of HCl and duration of autoclave were found to be significant. These parameters were chosen for the central composite design comprising of five levels using response surface methodology to optimize the hydrolysis process. At optimized condition of 54.15 minutes of autoclave treatment and 4.41% of HCl, 21.11 g/L of maximum reducing sugar was released. A second order polynomial equation was generated having a good fit with R^2 of 0.84.

Keywords: Acid hydrolysis, Cocoa pod shell, Optimization, Reducing sugar

INTRODUCTION

The alarming issues related to the economic, social and environment has created an atmosphere of intensive research to substitute the raw materials for energy and chemical production in the coming years. Fossil fuel derived transportation fuels offer disadvantages such as pollution, greenhouse gas emission, unbalanced supply demand relations and resource depletion. This has created a necessity to explore for alternative energy sources such as, lignocellulosic biomass (**Hamelinck *et al.,* 2005**). The demand for energy is steadily increasing due to an increase in population throughout the world. Currently non-renewable sources such as oil, coal and natural gas are used as the primary source of energy. It has been used for the production of electricity, fuel and other goods (**Uihlein *et al.,* 2009**). Lignocellulosic feedstock, dedicated energy crops, wood chips, sawdust, grasses, forestry residues and crop wastes are inexpensive and thus are available abundantly in nature. This does not compete with food, hence offers a promising source for biofuels in the next generations (**Sarkar *et al.,* 2012, Brethauer *et al.,* 2015**). The supply of potential global bioenergy is estimated in the range of less than 100 to over 400 EJ per year for 2050 (**Berndes *et al.,* 2003**). Thus, naturally available biomasses on the earth are being explored to produce bioethanol. After petroleum, natural gas and coal biomass is found to be the fourth largest source of energy (**Sheehan *et al.,* 1999**). Concentrated or dilute acid can be used for hydrolysis of hemicellulose and cellulose existing in the lignocellulosic residues into sugars (**Wyman, 1994**). Monosaccharide yield upto 90% can be attained by making use of concentrated acid pretreatment of residues since the acid pretreatment is much quicker in comparison with enzymatic hydrolysis (**Heinonen *et al.,* 2011**). The annual generation of cocoa fruit in India is concerning 12,000 metric tonnes. Between 70 to 75 % of entire weight of the fruit is represented by cocoa pod shell, *i.e.* 700 to 750 kg of residue is generated per each ton of cocoa fruit (**Cruz *et al.,* 2012).**
Cocoa pod hydrolysate obtained using nitric acid, sulphuric acid and hydrochloric acid reported the presence of carbohydrates (**Samah *et al.,* 2011**). The neutralization step enables detoxification of hydrolysate making it favourable for the successive fermentation (**Chandel *et al.,* 2007**).
In the current investigation, conversion of biomass for releasing reducing sugar using an agro residue cocoa pod shell (CPS) was explored. Due to the lack of availability of relevant literature on CPS hydrolysis, the design of experiments

one parameter at a time (OPAT) approach was adopted for autoclave assisted hydrolysis by considering following parameters for screening such as, weight of CPS, time taken for hydrolysis and concentration of hydrochloric acid to identify the parameter level. The parameter at which maximum reducing sugar was released during OPAT studies were chosen as centre points for central composite design (CCD) to optimize the hydrolysis process.

MATERIAL AND METHODS

Chemicals and raw material

The chemicals used in the current investigation were procured from Sigma-Aldrich. Raw material cocoa pod shells were procured from a farmer of Peruvai village situated in Bantwal taluk, Karnataka, India.

Processing of cocoa pod shell

Cocoa pod shells were collected from Peruvai village of Dakshina Kannada, India. CPS was sun dried for 2 days and further placed in hot air oven (Borg scientific) at 90^0C for overnight in order to remove 95% of the moisture. Size reduction unit operation was adopted and sieving was carried out using Taylor number 10 mesh to bring about uniformity in the particle size of 1.7 mm. It was stored in the air tight container and placed in the refrigerator maintained at 4^0C until further use.

Optimization of Pre-Treatment Process

Screening of parameters

OPAT method was adopted to screen the significance of the parameters. One parameter was varied by maintaining rest of the parameters at fixed value. Parameters selected and its range for the OPAT experiments of autoclave assisted CPS hydrolysis is given in Table 1. All the OPAT experiments were performed in triplicate using Erlenmeyer flask containing 100 mL working volume and the resulting hydrolysate was neutralised to pH 7.0 using sodium hydroxide. 3, 5-Dinitrosalicylic acid (DNSA) method was implemented to determine the

concentration of released reducing sugar (RRS) (**Miller, 1959**). As the parameters varied at one of its level, maximum sugars were released. The values of the parameters responsible for maximum RRS were maintained as centre point for CCD using response surface methodology (RSM).

Table 1 Parameters and its range chosen for the OPAT experiments of autoclave assisted CPS hydrolysis

Parameter	Notation	Range
Time (min)	X_1	20-60
Concentration of HCl (% v/v)	X_2	1-10
Weight of CPS (%w/v)	X_3	4-20

Optimization of acid hydrolysis

The two parameters *viz.* time (X_1) and concentration of acid (X_2) were optimized to obtain maximum sugar concentration using central composite design (CCD) with 12 runs.

Table 2 Coded parameters and its levels used in the central composite design.

Parameters	Notations	Levels				
		$-\alpha$	-1	0	+1	$+\alpha$
Time (min)	X_1	25.85	30	40	50	54.15
Concentration of HCl (%v/v)	X_2	0.17	1	3	5	5.83

Table 3 Central Composite Design for two parameters

Run No.	X_1	X_2	Y_1
1	30.0	1.00	12.89
2	30.0	5.00	19.52
3	50.0	1.00	21.95
4	50.0	5.00	23.29
5	25.9	3.00	18.78
6	54.2	3.00	23.11
7	40.0	0.17	04.15
8	40.0	5.83	19.87
9	40.0	3.00	21.2
10	40.0	3.00	20.94
11	40.0	3.00	22.14
12	40.0	3.00	20.94

Central composite design

Time (X_1, minutes) and concentration of HCl (X_2, %v/v) were the two experimental parameters screened for RSM optimization. OPAT studies revealed significant effect of these factors on the hydrolysis process. CCD was incorporated to obtain maximum RRS from the raw material. The concentration RRS during autoclave assisted hydrolysis was estimated as the response and denoted as "Y". Based on the two significant parameters, the CCD with five levels generated (Table 2) 12 experimental runs (Table 3) using trial version of statistica.

The analysis of variance (ANOVA) was carried for the experimental data obtained from CCD. The polynomial model with second order consisting linear and quadratic effect on the response was obtained

$$Y = \beta o + \sum \beta i X i + \sum \beta i i X i^2 + \sum_{i \neq j} \beta i j X i X j \qquad (1)$$

The response is denoted as "Y" and the polynomial coefficients as βo, βi, $\beta i i$, $\beta i j$. Independent parameters were represented as Xi, Xj.

The experiments were conducted to perform optimization of CPS acid hydrolysis in 250 ml Erlenmeyer flask containing 100mL acid solution (Table 3). The weight of CPS (%w/v) was maintained same for all the CCD experimental run.

Estimation of RRS

The following saccharides such as glucose, arabinose, xylulose , cellobiose, and galactose are expected to be the products of hydrolysis and reported to be reducing sugars (**Brummer et al., 2014**). Hence DNSA method was adopted to determine, the RRS concentration using UV Visible spectrophotometer (Systronics) at 540 nm (**Miller, 1959**).

RESULTS AND DISCUSSION

**Identifying the significant parameters based on OPAT**

Time duration of hydrolysis was varied from 20 to 60 minutes by maintaining rest of the parameters at fixed value [(X_3=4 % (w/v) and X_2=4% (v/v)]. At 40 min of hydrolysis, a maximum of 18.2 mg/mL reducing sugar was released. Hence, 40 min (X_1) was chosen as a significant value (Fig.1). Weight of raw material CPS was increased from 4 to 20% (w/v) by keeping other parameters fixed [(X_1= 40 min and X_2=4% (v/v)] (Fig.2). At 10 %w/v, maximum sugar of 37 mg/mL was released. Beyond 10% w/v of CPS even though concentration increased, the volume of hydrolysate decreased. Concentration of HCl was varied from 1 to 10% (v/v) by maintaining other parameters constant (X_1=40 min and X_3=10 %w/v). At 3% (v/v) acid maximum concentration of 38.54 mg/mL of reducing sugar was released (Fig.3). Beyond 3% (v/v) of acid, released sugars were probably degraded slowly up to 9 % (v/v), hence the reduction of reducing sugar concentration was not significant. The increased reducing sugar concentration is due to the effect of hydrolysis and decreased sugar concentration is because of degradation of released sugars by the remaining HCl used in the hydrolysis. The parameter at which maximum RRS was obtained during OPAT studies were chosen as centre points for CCD.
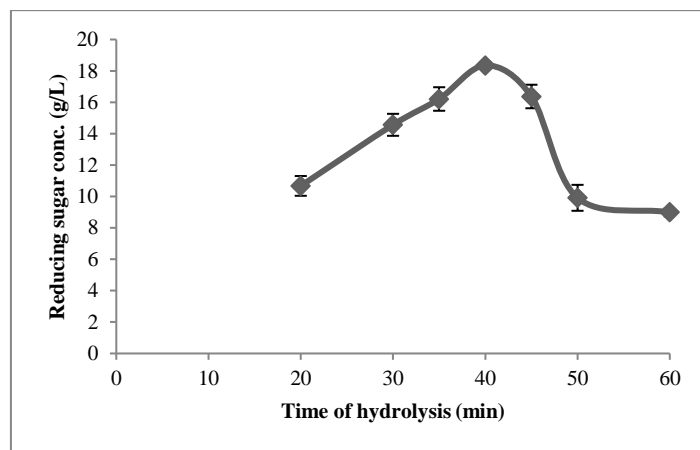
Figure 1- Effect of time of hydrolysis on the RRS
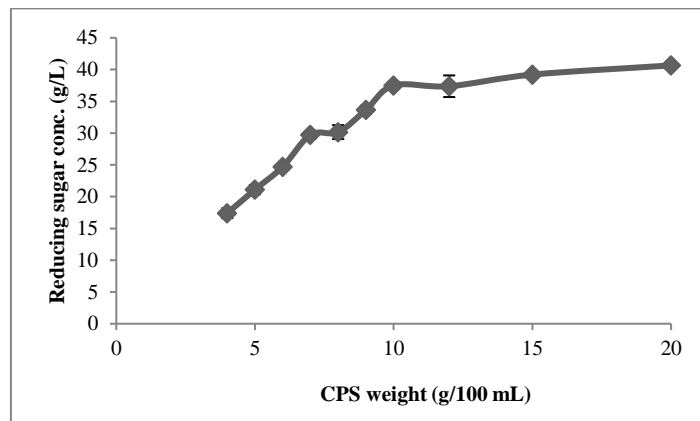
Figure 2- Effect of biomass weight on the RRS
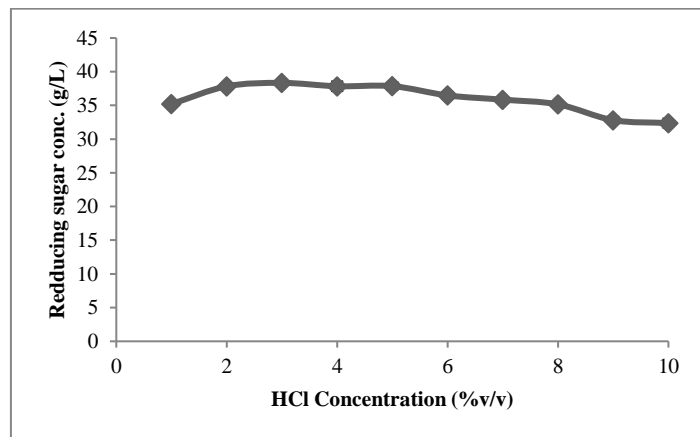
Figure 3- Effect of acid concentration on the RRS

Central composite design

The effect of X_1 (time) and X_2 (concentration of acid) on RRS was obtained by CCD results (Table 3). Experimental values of RRS concentration obtained for the CCD is denoted as Y_1

The regression equation for autoclave assisted acid hydrolysis process using two significant parameters and its linear and quadratic interactions to achieve RRS from CPS, is represented by following equation:

$$Y_1 = -1.75898 - 0.00508 * X_1 + 0.005504 * X_1^2 + 10.40058 * X_2 - 0.97804 * X_2^2 - 0.06612 X_1 * X_2 \qquad (2)$$

The values obtained from ANOVA for the RRS on HCl hydrolysis of CPS and the linear effects of the independent factors on RRS due to hydrolysis is represented in Table 4. The parameters having p-values less than 0.05 is considered as statistically significant (**Guo et al., 2009**).
Table 4 shows that the linear effect (L) of time and quadratic effect (Q) of HCl concentration was found to be statistically significant.

**Table 4** ANOVA table for RRS on HCl hydrolysis of CPS

	SS	Df	MS	F	P-Value*
X_1 (L)	**44.89577**	**1**	**44.89577**	**5.48**	**0.057815**
X_1 (Q)	1.941525	1	1.94152	0.24	0.643745
X_2 (L)	**114.0458**	**1**	**114.04578**	**13.91**	**0.009736**
X_2 (Q)	**98.10562**	**1**	**98.10562**	**11.97**	**0.013474**
1L by 2L	6.996025	1	6.99603	0.85	0.3912
Error	49.18021	6	8.19670		
Total SS	325.1239	11		R^2=0.84873	

L: Linear, Q: Quadratic, X_1: Time, X_2: Concentration of HCl
*P Values < 0.05 indicate significance

The 3D surface plot for Y_1 as a function of X_1 (time) and X_2 (HCl concentration) is represented in Figure 4. It clearly shows that increase in concentration of RRS with increase in time of autoclaving. As the HCl concentration was increased, concentration of RRS increased continuously. The trend depicted in Fig. 4 of RRS may be probably because of lignin removal with the increase in acid/alkali concentration (**Sukri et al., 2014**). The desirability plot was used to determine the optimized levels of significant parameters X_1 and X_2 for the maximum concentration of RRS by the hydrolysis (Fig.5). The optimized factor for maximum RRS (Y_1) were 4.41% of HCl and 54.15 minutes of autoclave treatment with predicted RRS concentration of 25.14 g/L as obtained from desirability plots. Validation experiment was conducted at optimized condition and RRS concentration was determined to be 21.11 g/L. Hydrolysis of CPS using 1.0 M of HCl at 75°C for 4 h released the maximum glucose concentration of 30.7% w/v (**Samah et al., 2011**). Reducing sugar concentration of 4.9g/L was released from wheat straw (**Saha et al., 2015**). CPS hydrolysis using H_2SO_4 at 80 °C for 150 min of treatment released 45.08mg/ml of reducing sugar (**Awolu and Oyeyemi 2015**). The HCl treated CPS at 75 °C for 4 hour released 135g/L of reducing sugar (**Mbajiuka et al., 2015**). The concentration of RRS from 8.36% w/v of CPS hydrolysis using 3.6 N HCl in room temperature was found to be 4.09g/L (**Shet et al., 2018**).
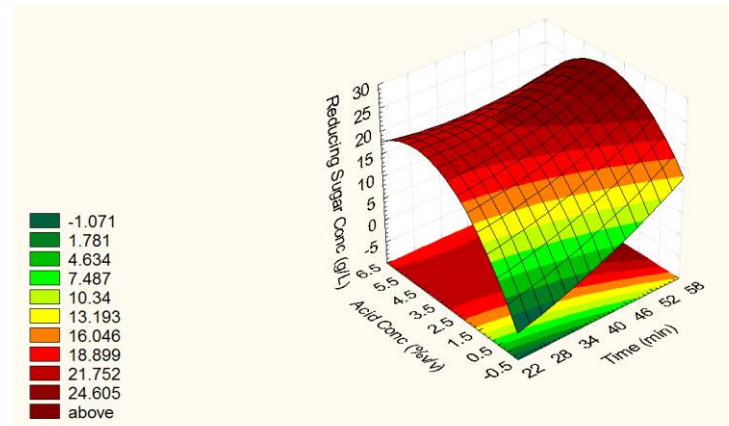
Figure 4- 3D surface plot exhibiting effect of time duration and acid concentration on hydrolysis of cocoa pod shell on RRS.
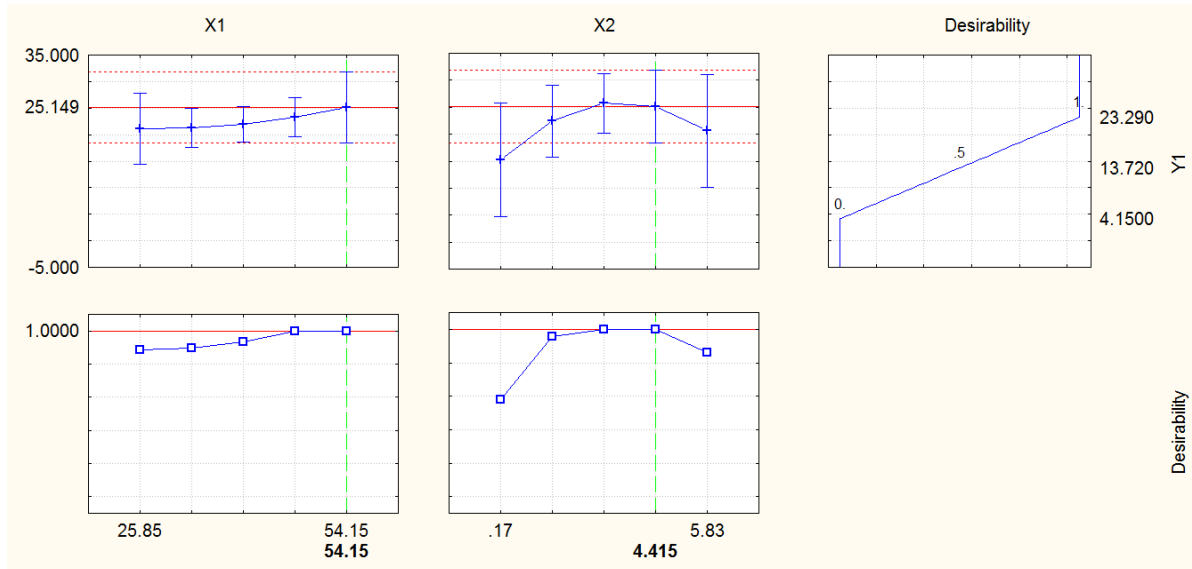
Figure 5- Desirability plot representing optimum parameters of hydrolysis process

CONCLUSION

In the current study, CPS hydrolysis was carried out using HCl. A second order model was obtained for the hydrolysis and revealed the good fit of R^2 with 0.84.Maximum RRS concentration of 21.11g/L at optimum conditions was achieved. The results are promising in terms of converting agro residue CPS into the reducing sugars; it could be used as precursor for the production of spectrum of biochemicals through fermentation. CPS is an environmentally and economically benign renewable source for the releasing reduced sugar since it is available abundantly in Bantwal taluk, Karnataka, India.

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