Agriculture and Natural Resources 52 (2018) 280–284

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Optimization of reducing sugars production from agro-residue coconut leaflets using autoclave-assisted HCl hydrolysis with response surface methodology

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ARTICLE INFO

Article history:
Received 11 February 2017
Accepted 10 July 2017
Available online 4 October 2018

Keywords:
Acid hydrolysis
Coconut leaflets
Optimization
Reducing sugar

ABSTRACT

The utilization of coconut leaflets, an agro residue generated from coconut farming, was investigated to develop a process to release reducing sugars using hydrochloric acid (HCl)-assisted, autoclave-based hydrolysis. The variables affecting HCl hydrolysis (duration of autoclaving, concentration of HCl and weight of coconut leaflets) were screened using a one factor at-a-time approach. The duration of autoclaving and concentration of HCl both had significant effects on the hydrolysis process and the levels of these factors were further optimized using a central composite design with response surface methodology. The optimized conditions were 3.17% of HCl and 44.14 min of autoclaving that released the maximum reducing sugars of 29 g/L. A second order model was generated, which was a good fit with a coefficient of determination value of 0.83.

Introduction

Coconut palm (*Cocos nucifera L.*) is an important agro-industry plant and it generates residues continuously throughout the year, which are a burden to the coconut orchard owner (Brígida et al., 2010). With the increase in environmental awareness, more attention should be been paid to utilizing coconut residues effectively for value-added and environmentally friendly products. The coconut residues are lignocellulosic material with renewable, biodegradable, biocompatible characteristics and should be an ideal raw material with wide application (Xu et al., 2015). Conversion of lignocellulosic feedstock into fermentable sugars can be achieved through hydrolysis and acid hydrolysis is advantageous over the enzymatic process because of its shorter reaction time and its ability to breakdown the cellulose compared to enzymatic breakdown of cellulose (Asoaka et al., 2011; Kosarik et al., 2011). Acid hydrolysis can be accomplished using dilute sulfuric acid (0–4%) at low temperature (20–40 °C), as well as using weak acids at high temperatures (100–250 °C) (Kim et al., 2008). Dilute acid treatment can be used to hydrolyze the lignocellulosic biomass to sugars (Wyman and Charles, 1994). There is scant literature on the conversion of coconut leaflets into reducing sugars. The current investigation explored coconut leaflets as a biomass source for the conversion process and autoclave-assisted hydrochloric acid hydrolysis was carried out to release the reducing sugars. The experimental design consisted of the one factor at-a-time (OFAT) approach to screen the variables affecting the release of reducing sugars during acid hydrolysis and optimization of hydrolysis for the screened variables to release the maximum amount of reducing sugars was achieved through response surface methodology (RSM).

Materials and methods

Chemicals and raw material

The chemicals required for the study were purchased from Sigma Aldrich. Coconut leaflets (CL) were collected from Nitte village of Karkala Taluk situated in Udupi district, Karnataka, India.

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https://doi.org/10.1016/j.anres.2018.09.015

Processing of raw material

The collected CL was cut into small pieces and sun dried. After sun drying, leaflets were placed in a hot-air oven at 90 °C for the removal of 92% of moisture. After drying, 5 g of CL were weighed in a glass crucible and noted as W2. The weight of the empty glass crucible was noted as W1. A crucible with CL was placed in muffle furnace for 2 h at 600 °C and then the crucible was weighed, and the weight was noted as W3. The moisture content of 8% was determined based on Equation (1):

$$\frac{[(w2 - w1) - (w3 - w1)]}{(w3 - w1)} \quad (1)$$

Then, the leaves were powdered using a domestic mixer. The powder was sieved using Tyler mesh number 10. The powder was stored in a refrigerator until further use.

Compositional analysis of raw material

The cellulose, hemicelluloses and lignin content of CL were estimated (Sharma and Singh, 2009) as follows.

Cellulose estimation

A sample of 3 mL acetic/nitric reagent was added with 1 g of coconut leaflet powder in a test tube and mixed using vortex mixer. The test tube was placed in a water bath at 100 °C for 30 min. The solid particles were separated using centrifugation at 8000 revolutions per minute for 15 min. The supernatant was discarded and the residue was washed with distilled water. Then, 10 mL of 67% sulfuric acid was added to the residue and allowed to stand for 1 h. A sample of 1 mL of the solution was diluted to 100 mL. A sample of 1 mL of the diluted solution was mixed with 10 mL of anthrone reagent and placed in the boiling water bath for 10 min. After cooling, absorbance was read at 630 nm. Quantification of cellulose estimation was determined using a calibration graph with standard cellulose.

Lignin estimation

Lignin was determined as acid detergent fiber. A sample of 1 g of coconut leaflet powder was mixed with 100 mL of acid detergent solution (20 g of cetyl trimethylammonium bromide in 1 L of 1 N H_2S0_4). The contents were boiled for 10 min and then filtered through a sintered funnel (G2) before washing twice with hot water. Acetone washing was used until the filtrate was colorless. Drying was carried out at 100 °C overnight. After cooling, the weight was determined in a desiccator. The percentage acid detergent fiber was calculated using Equation (2):

$$\%\ \text{Lignin (acid detergent fibre)} = \left[\frac{(\text{Weight of the fibre})}{(\text{Weight of the coconut leaflets})}\right] \times 100 \quad (2)$$

Hemicellulose estimation

A sample of 1 g of coconut leaflet powder was transferred to a refluxing flask and mixed with 10 mL of cold neutral detergent (18.61 g disodium ethylene diamine tetracetate and 6.81 g of sodium borate decahydrate dissolved in 200 mL of distilled water; 30 g of sodium lauryl sulphate was transferred in 150 mL of distilled water and 10 mL of 2-ethoxy ethanol was added; 4.5 g of disodium hydrogen phosphate was dissolved in 100 mL of water; all these solutions were mixed and volume was made up to 1 L and the pH adjusted to 7.0). Then, 2 mL of decahydronaphthalene and 500 mg of sodium sulfite were transferred to the refluxing flask and the contents were refluxed for 1 h at boiling temperature. The contents were filtered through sintered glass (G2) and then washed with hot water and then with acetone before placing in a crucible to dry at 100 °C for 12 h. After cooling, the crucible was placed in a desiccator and contents were weighed. The hemicellulose content was calculated using Equation (3):

$$\%\text{Hemicellulose} = \left[\frac{(\text{Neutral detergent fibre} - \text{Acid detergent fibre})}{\text{Weight of coconut leaflets}}\right] \times 100 \quad (3)$$

Optimization of coconut leaflet hydrolysis

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

Hydrolysis of the CL was carried out using hydrochloric acid. The OFAT approach was adopted to select the significant physical parameters and the levels for the hydrolysis process are given in Table 1. After neutralizing the hydrolysate, estimation of the released reducing sugars was carried out using dinitrosalicylic acid (Miller, 1959). The parameters at which maximum released reducing sugars (RRS) were produced were chosen as the center point values to enhance the hydrolysis process using RSM. A calibration graph was prepared using standard glucose by measuring absorbance at 540 nm. A sample volume of 0.1 mL was withdrawn to make up the volume to 1 mL; hence there was 10 times dilution of the sample. After measuring the absorbance, the dilution factor was multiplied to the concentration based on the calibration graph (Fig. 1).

Central composite design

The factors (X1 and X2) that had a significant effect during the OFAT studies were selected and their levels were optimized for the maximum release of reducing sugars as response (Y) using central composite design (CCD) for the HCl hydrolysis. The CCD trials were designed for RSM optimization using the trial version of STATISTICA software (Statsoft, 1999) with two factors and five levels (Table 2) consisting of 12 experimental runs (Table 3). The data obtained from the study of HCl hydrolysis were subjected to analysis of variance (ANOVA) using the same software. A second order polynomial model was utilized to obtain the relationship between the response and the independent variables (Equation (4)):

$$Y = \beta o + \sum \beta iXi + \sum \beta iiXi^2 + \sum_{i \neq j} \beta ijXiXj \quad (4)$$

where, Y is the dependent response, βo, βi, βii, βij are estimates of polynomial coefficients and Xi, Xj represent the independent variables.

Table 1
Parameters and their levels for one factor at-a-time study.

Parameter	Notation	Test range
HCl concentration (%v/v)	X1	2–10
Weight of coconut leaflets (%w/v)	X3	2–18
Time (min)	X2	10–60

w/v = weight per volume; v/v = volume per volume.

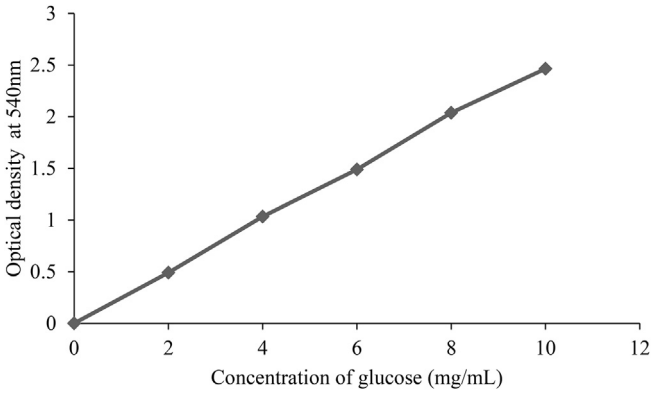

Fig. 1. Glucose calibration graph.

Table 2
Coded parameters and their actual levels for optimization using central composite design.

Factor	Notation	Level				
		$-\alpha$	−1	0	+1	$+\alpha$
Concentration of HCl (% v/v)	X_1	0.34	2	6	10	11.66
Time (min)	X_2	1.715	10	30	50	58.28

v/v = volume per volume.

Table 3
Central composite design for optimization of coconut leaflets hydrolysis.

Run	X1	X2	OD @540 nm	Y (mg/mL)
1	2	10	0.232	9.24
2	2	50	0.668	26.63
3	10	10	0.410	16.33
4	10	50	0.252	10.05
5	0.34	30	0.190	7.57
6	11.65	30	0.323	12.86
7	6	1.71	0.034	1.35
8	6	58.28	0.519	20.69
9	6	30	0.578	23.02
10	6	30	0.594	23.68
11	6	30	0.576	22.94
12	6	30	0.56	22.58

X1 = concentration of HCl (% volume per volume); X2 = time (hours); OD = optical density; Y = concentration of released reducing sugars from coconut leaflets after hydrolysis using HCl.

The optimization experiments were carried out with 100 mL acid solution taken in 250 mL conical flasks (Table 3).

Estimation of released reducing sugars using the dinitrosalicylic acid method

The acid hydrolysates were neutralized to pH 7.0. The expected products of hydrolysis are glucose, galactose, cellobiose and minor saccharides such as xylulose and arabinose. All these saccharides are reducing sugars (Brummer et al., 2014). Therefore, the concentration of released reducing sugars (RRS) was estimated using an ultraviolet visible spectrophotometer at 540 nm using DNS reagent (Miller, 1959).

Validation of the second order polynomial model

The second order polynomial model obtained from RSM was validated by conducting experiments at random values of parameters within the optimized levels. Goodness of fit of the model was obtained by comparing the experimental output with the values predicted by the second order model from the CCD.

Results and discussion

Compositional analysis of raw materials

The raw material of coconut leaflets was composed of: cellulose (7.19%), hemicellulose (1%), lignin (63.5%) and ash (13.4%). Perennial grass consists of cellulose (37–45%), hemicellulose (19–25%), lignin (17–21%) and 1–3% ash (Haffner et al., 2013). Pine leaf is composed of cellulose (25–42%), lignin (18–26%), hemicellulose (21–30%) and 0.3–2% ash (Sorek et al., 2014). The distribution of cellulose, hemicellulose and lignin are not uniform within the cell walls and the composition of biomass varies widely among species depending on the cell type or in response to environmental conditions. Even though pine leaf and perennial grass have been reported to contain higher levels of cellulose and hemicelluloses, in the current investigation, coconut leaflet was explored since it is locally available in abundance throughout the year and it is a cheap and underutilized form of biomass.

Optimization of coconut leaflet hydrolysis

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

The significant parameters for the pre-treatment process were obtained using the OFAT study. When the HCl concentration was varied (X1), keeping other factors constant (X2 = 20 min, X3 = 5% (w/v)), the maximum amount of reducing sugars released was at 6% HCl concentration (Fig. 2). Values of X2 and X3 were chosen randomly within the selected range (Table 1). The weight of the CL (X3) was varied between 2 and 18% (w/v) by keeping X1 = 6% volume per volume (v/v) HCl and X2 = 20 min constant (Fig. 3). Beyond 14% weight per volume (w/v) of loading, slurry formation takes place and the volume of hydrolysate was reduced significantly, hence 14% w/v of CL was fixed for the next step of OFAT. Similarly, when the time of hydrolysis (X2) was varied by keeping X1 = 6%v/v HCl concentration and X3 = 14% (w/v), the maximum reducing sugar was obtained at 30 min (Fig. 4). Based on these results, the significant parameters (X1 and X2) were subjected to optimization using CCD.

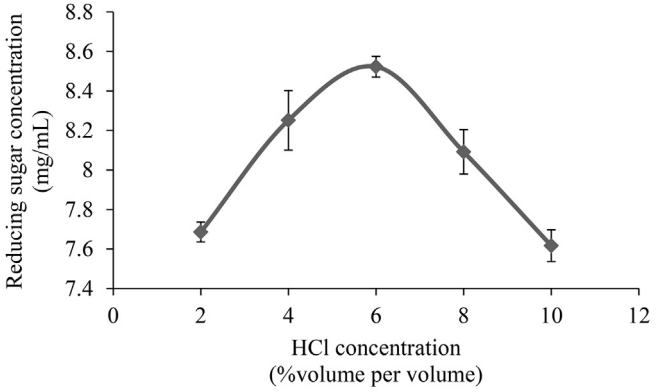

Fig. 2. Effect of HCl concentration on release of reducing sugar, where error bars indicate standard deviation.

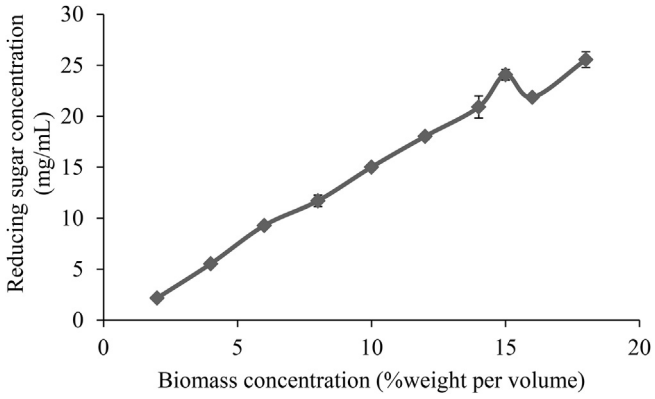

Fig. 3. Effect of coconut leaflets concentration on release of reducing sugar, where error bars indicate standard deviation.

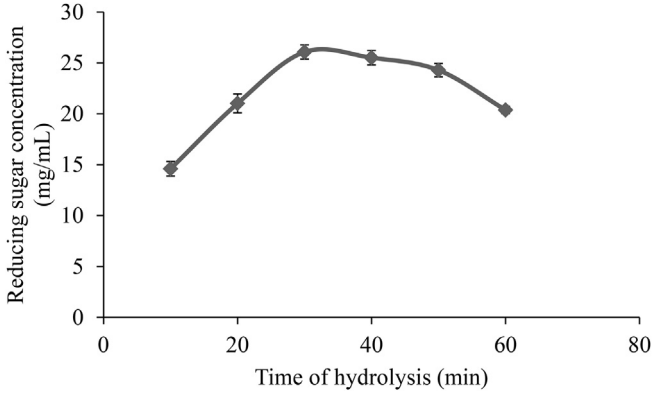

Fig. 4. Effect of duration of hydrolysis on release of reducing sugar, where error bars indicate standard deviation.

Optimization of parameters for release of reducing sugars using central composite design

The effect of the significant factors X1 and X2 on the release of reducing sugars was determined using CCD as indicated in Table 3 along with the observed values for RRS concentration using acid hydrolysis (Y) at different combinations of the independent parameters. The variation in the concentration of RRS was between 1.35 g/L to 26.63 g/L.

Table 4 indicates the data obtained from the ANOVA for the release of reducing sugars on acid hydrolysis of CL. Basically, a smaller p-value indicates a higher influence and only factors having $p < 0.05$ can be considered as statistically important (Guo et al., 2009). With increased time of autoclaving, the concentration of released reducing sugars also increased (Fig. 5). The release of reducing sugar increased continuously with the increase in HCl concentration. This was probably due to the lignin removal with the increase in acid concentration (Sukri et al., 2014). The optimized levels of variables X_1 and X_2 for the maximum release of reducing sugars using hydrolysis were determined by the desirability profiles (Fig. 6). The level of variable giving the highest desirability (1.0) was selected as the optimum level. Based on the desirability plots, the optimized factor for releasing maximum sugars (Y_1) was 3.17% of HCl with 44.12 min of autoclave treatment. An experiment was conducted under the optimized conditions and the concentration of reducing sugar produced was 29.08 g/L. Microwave-assisted HCl hydrolysis of coconut leaflets released a maximum reducing sugar amount of 16.6 g/L from 15.12 g/L of biomass according to Varun

Table 4
Analysis of variance table for release of reducing sugar on acid hydrolysis of coconut leaflets.

	SS	DF	MS	F value	p value[a]
(1) X1 (L)	0.522967	1	0.522967	0.026495	0.876041
X1 (Q)	172.025000	1	172.025000	8.715254	0.025541
(2) X2 (L)	184.805700	1	184.805700	9.362759	0.022229
X2 (Q)	146.179700	1	146.179700	7.405859	0.034580
1 L by 2 L	140.067200	1	140.067200	7.096185	0.037331
Error	118.430300	6	19.738380		
Total SS	709.317700	11			
(1) X1 (L)	0.522967	1	0.522967	0.026495	0.876041

SS = sum of squares; DF = degrees of freedom; MS = mean square; X1 = concentration of HCl (% volume per volume); X2 = time (minutes).
[a] p values less than 0.05 indicates significance at 95% confidence interval; $R^2 = 0.83$.

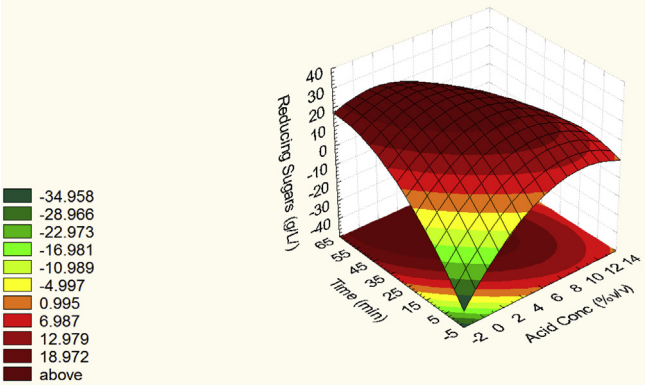

Fig. 5. Response surface plot showing effect of HCl concentration and duration of hydrolysis on coconut leaflets for the release of reducing sugar.

et al. (2016). The release of 16.6 g/L reducing sugar compared to 29.08 g/L was significantly less, probably due to degradation of released reducing sugar by the available excess acid. The release of glucose from 10 g of Napier grass under optimum conditions using HCl was 2.28 g/L (Mafuleka et al., 2015). Hydrolysis of bagasse using 2% HCl at 128 °C for 51.1 min resulted in 3.77 g/L of glucose (Guadalupe et al., 2003). The regression equation for the release of reducing sugars using autoclave-assisted acid hydrolysis of CL, as a function of the two independent variables (X_1 and X_2) and their linear and quadratic interactions, is represented by Equation (5):

$$Y = -19.51 + 6.04X1 + 1.40X2 - 0.32X1^2 - 0.119X2^2 - 0.074X1X2 \quad (5)$$

The coefficient of determination (R^2) is a measure of the strength of the linear relationship between the predicted and experimental values (Sharmada et al., 2016). The R^2 value for the correlation between the predicted and observed RRS for acid hydrolysis of CL was 0.83. The second order model was validated using a set of random experiments other than the experimental runs (Table 5). The predicted values of RRS were compared with the experimental RRS values and indicated that there was a correlation between the predicted and experimental values, which, in turn, proved the model valid. The significance of the current work is that agro residue hitherto underutilized could be used for the production of reducing sugars. The hydrolysis process was cost effective because it used HCl. In this study, the acid hydrolysis produced 29.08 g/L reducing sugars in 44.14 min. Moreover, the optimized

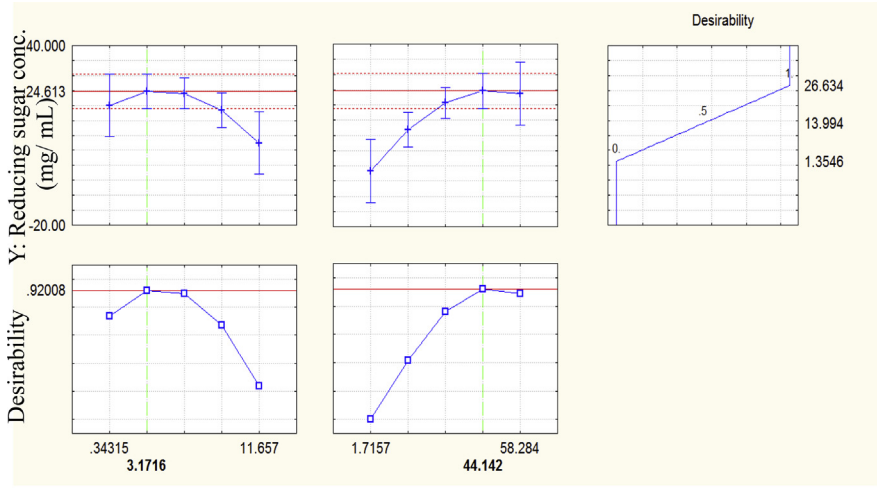

Fig. 6. Profiles for predicted HCl hydrolysis process and the desirability levels for different parameters for optimum hydrolysis.

Table 5
Validation runs with observed and predicted values of reducing sugars released from CL on hydrolysis with acid.

Run number	X_1	X_2	Experimental yield (g/L)	Theoretical yield (g/L)
1	6	45	22	23
2	9	25	18	19
3	10	34	14	17

X1 = concentration of HCl (% volume per volume); X2 = time (minutes).

conditions obtained from this study can be used for large scale hydrolysis of coconut leaflets to ensure utilization of agro residue.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank the SPFU, Karnataka State for partial financial support under the TEQIP phase 2 programme.

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