Catalytic Hydrolysis of Cotton Stalk Biomass Using a Reusable Solid Carbon Acid Catalyst

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ABSTRACT

The aim of this work was to evaluate a glycerol-based carbon acid catalyst for the hydrolysis of acid and alkali pre-treated and cotton stalk when it released 23 and 67mg/g of total reducing sugars respectively on reaction at 160 °C for 6h with a biomass to catalyst ratio of 1:0.5. Increase in biomass to catalyst ratio resulted in a doubling of sugar yield Optimization of the conditions for hydrolysis using a response surface method resulted in further increase of sugar yield from the alkali treated cotton stalk to 179mg/g, which corresponded to 21.01% conversion of sugar polymers. The catalyst could be reused albeit with some loss of efficiency. Catalytic hydrolysate of cotton stalk was fermented to alcohol with 41 % efficiency. Results demonstrated the potential use of the catalyst in biomass hydrolysis to generate fermentable sugars for bioethanol production.

1. Introduction

Solid acid catalysts show unique advantages in the hydrolysis of lignocellulosic biomass. Various solid acid catalysts such as carbon acid catalysts, resins, metal oxides, zeolites, and others have been explored as potential heterogeneous catalysts for this purpose (Guo et al., 2012; Huang and Fu, 2013). The use of solid acid catalysts may address some of the challenges in biomass conversion to fermentable sugars due to their reusability and easy separation of sugars from reaction mixtures (Goswami et al., 2015).

The hydrolysis of lignocellulosic biomass is usually carried out using either acid or enzymes (Gustsch et al., 2012; Lee and Jeffries, 2011). The use of concentrated mineral acids is efficient at high temperature (170–240ºC), but controlling the further degradation of monomers, corrosion and generation of large amounts of acid wastewater are the major drawbacks of acid hydrolysis (Taherzadeh and Karimi, 2007). In the enzymatic hydrolysis, the notable disadvantages include low activity, high cost of enzymes and separation issues restricting reusability.

The development of new, green and economical process (es) for the conversion of lignocellulosic biomass into glucose and xylose under mild conditions with high selectivity is therefore highly desired. Solid acid catalysts, which have favorable characteristics such as efficient activity, high selectivity, longer life and easiness in recovery and reuse, have great potential for efficiently transforming lignocellulosic biomass into biofuels

and could replace many conventional liquid acids for hydrolysis and pre-treatment. Recently, solid acid-catalyzed hydrolysis has attracted increasing attention with several types of solid acid catalysts being reported (Huang and Fu, 2013).

Carbonaceous solid acid catalysts (CSAC) are by far, considered as the most promising catalysts for cellulose hydrolysis, since they provide good access of reactants to the acidic sites of SO_3H groups and offers different reactive moieties on the graphene like structure which can participate in reaction or form bonds with the carbohydrate polymers aiding in the hydrolysis (Pang et al., 2010; Guo et al, 2012; Hara, 2015). While upto 75 % glucose yield has been obtained with sulfonated carbon catalyst (Pang et al., 2010), majority of the studies have used pure cellulose as the model substrate for hydrolysis and not lignocellulosic biomass. The behavior of catalyst with a lignocellulosic substrate can be entirely different from when cellulose is used. The major challenges for a catalytic process include low cellulose/liquid ratios (as low as 1:100) and the inability to convert the lignin.

Use of a glycerol-based solid carbon acid catalyst was previously demonstrated for acid catalyzed biodiesel production (Prabhavathi Devi et al., 2009), and later in the hydrolysis of rice straw (Goswami et al, 2015). In the current study the catalyst was evaluated for hydrolysis of Cotton stalk (CS), which is a post harvest residue generated in significant quantities in several countries and which is particularly resistant to enzymatic hydrolysis.

2. Materials and methods

2.1. Carbon acid catalyst, biomass and pretreatment

The glycerol based solid carbon acid catalyst was synthesized at CSIR-IICT as previously described (Prabhavathi Devi et al., 2009). Cotton stalk (CS) was a kind gift from Reliance Industries, Mumbai, India. Cotton stalk was knife milled and sieved to a maximum particle size of 2 mm. The milled biomass was pretreated using dilute alkali (4% w/v NaOH at 120 °C for 60 min), or dilute acid (2.5 % w/v H_2SO_4 at 120°C for 60 min). The biomass loading was 15% (w/w). After autoclaving, the biomass slurry was neutralized by either 10 N H_2SO_4 or 10N NaOH followed by water wash. Pretreated materials were sun dried and stored in air tight containers until used. The compositional analysis of native and pretreated rice straw (RS) was carried out by the two stage acid hydrolysis protocol which was developed by National Renewable Energy Laboratory (Ruiz and Ehrman, 1996).

2.2. Hydrolysis of cotton stalk using the carbon acid catalyst

Catalytic hydrolysis was carried out as described by Goswami et al (2015). Hydrolysis was performed in acid digestion bombs having Teflon inserts. Specified quantity of feed stock and catalyst were mixed together and were placed inside the Teflon container inside the bomb and the lids were screwed in to seal the container. The bombs were heated to required temperature in a hot air oven and were held at that temperature for the specified duration. After completion of the reaction, the bombs were allowed to cool and then the hydrolysate was recovered after addition of enough water to make up the volume to 25ml. The contents were then centrifuged at 6000 x g for 10 min to separate the debris. The supernatant was recovered and analyzed for the presence of total reducing sugars by DNS method (Miller 1959) and individual sugars by HPLC (Sluiter et al, 2008) method and used for fermentation for ethanol production.

2.3. Optimization of the catalytic hydrolysis of cotton stalk

A response surface Box-Behnken design (Box and Behnken, 1960) was used for optimizing the hydrolysis of alkali pretreated cotton stalk. Biomass to catalytic ratio was kept constant at 1:1 and 0.2% Tween 80 wa added into the reaction mixture for all experimental runs. The parameters studied were biomass loading (2, 4 and 6 % w/v), Temperature (160, 180 and 200 °C) and reaction time (4, 5 and 6h) and combinations of these parameters as per the experimental design matrix generated using Design Expert v 8.05(Statease Inc, USA) were evaluated experimentally. The design matrix which included a total of 17 runs with four replicates of the midpoint is given in Table 2. The model constructed as a response function of the variables on sugar yield was a second order polynomial as follows:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j + \varepsilon$$

Where, Y is the predicted response; $\hat{a}_0$ is the offset term; $\hat{a}_i$ is the linear effect; $\hat{a}_{ii}$ is the squared effect, $\hat{a}_{ij}$ is the interaction effect, X_i and X_j are coded terms for independent variables under study and e is the error factor. Regression analysis and estimation of the coefficients were performed using Design Expert software. Three dimensional response surfaces were generated using the software. The ideal levels and combinations of parameters were identified by numerical optimization functions in the Design Expert software and were experimentally validated.

2.4. Catalyst Reusability

The reusability study was studied with 6% (w/w) of biomass loading and 100% (w/w) of catalyst loading at a temperature of 160 °C for 4 h. The supernatant containing sugars were separated by centrifugation after the first cycle. The slurry containing undigested biomass and catalyst was suspended in excess volume of water and was mixed in a vortex mixer. The suspension was allowed to stand for few minutes when the catalyst settled down. It was collected by decanting the top layer and the process was repeated to remove all the undigested material. The catalyst was then given a water wash followed by methanol wash and was used for the next cycle as above. Amount of sugar released was monitored for each cycle and the efficiency was expressed as percentage of sugar yield in the first cycle which was taken as 100%.

2.5. Fermentation

A preliminary evaluation of the fermentation of catalytic hydrolysate of cotton stalk was performed with hydrolysate concentrated to a glucose concentration of 22.8 g/L by vacuum centrifugation. Enzymatic hydrolysate of CS with a glucose concentration of 120g/L was generated using commercial cellulase and its fermentation was performed parallel to that of the catalytic hydrolysate under similar conditions for comparison. The hydrolysates were supplemented with yeast extract (0.25%), $(NH_4)_2SO_4$ (0.5%), $MgSO_4$- $7H_2O$ (0.1%), KH_2PO_4 (0.2%) and pH was adjusted to 5.0. The hydrolysates were inoculated with *Saccharomyces cereviseae* NCIM 3059 at a concentration of 5.0mg dry cells/ml and were incubated at 30 ºC for 36h. Alcohol estimation was performed by NREL method (Templeton, 1994).

3. Results and Discussion

3.1. Hydrolysis of pretreated cotton stalk

The hydrolysis acid or alkali pretreated cotton stalk (CS) was performed using a biomass to catalyst ratio of 1:0.5 at 140 °C for 4h. Control experiments were performed with just biomass and no catalyst under the same conditions. The results presented in Table 1 indicated that alkali pretreated CS was hydrolyzed more efficiently by the solid acid catalyst compared to the acid pretreated CS. The sugar yields were 67.78 mg/g and 23 mg/g respectively for alkali and acid pretreated CS. These represented 4.1 and 7.9 % of the theoretical maximum conversion of available polysaccharides since the cellulose and hemicellulose content of native, acid pretreated and alkali pretreated CS were 33.88 and 23.93 %, 46.23 and 4.21 % and 59.4 and 17.3 % respectively. Carbonaceous solid acid catalysts (CSACs) are considered as highly efficient among heterogeneous catalysts for biomass hydrolysis and efficiencies as high as 65% has been reported for lignocellulose (milled bagasse) hydrolysis (Namchot et al, 2014). Previously we had reported 31% conversion

Table 1: Catalytic hydrolysis of native, acid and alkali pretreated cotton stalk

Biomass Type	Biomass Loading (% w/v)	Catalyst (% of Biomass)	Temp (°C)	Time (h)	Sugar Yield (mg/g)
Acid	5	*	140	4	6.88
Alkali	5	*	140	4	3.94
Acid	5	50	140	4	23.00
Alkali	5	50	140	4	67.78

* Without catalyst

Table 2: Box Behnken design matrix for optimization of catalytic hydrolysis of cotton stalk with responses for experimental runs

Run	(A)Biomass Loading (% w/w)	(B)Temp (°C)	(C) Time (h)	Sugar Yield (mg/g)
1	4	200	4	13.06
2	4	200	6	23.67
3	6	180	6	53.44
4	4	180	5	73.15
5	6	180	4	179.46
6	4	180	5	99.87
7	6	160	5	144.10
8	4	180	5	55.12
9	4	160	4	155.83
10	4	180	5	67.13
11	4	180	5	28.86
12	2	180	4	87.58
13	2	200	5	21.39
14	4	160	6	137.67
15	2	180	6	47.58
16	2	160	5	71.70
17	6	200	5	20.48

Table 3: Analysis of variance (ANOVA) for the response surface quadratic model

Source	Sum of Squares	DF	Mean Square	F Value	p-value
Model	36284	9	4032	4.2	0.037
A-Biomass Loading	3580	1	3580	3.7	0.096
B-Temperature	23188	1	23188	24.0	0.002
C-Time	3766	1	3766	3.9	0.089
AB	1344	1	1344	1.4	0.277
AC	1850	1	1850	1.9	0.209
BC	207	1	207	0.2	0.658
A^2	86	1	86	0.1	0.774
B^2	102	1	102	0.1	0.754
C^2	2163	1	2163	2.2	0.179
Residual	6773	7	968		
Lack of Fit	4083	3	1361	2.0	0.253
Pure Error	2690	4	673		
Cor Total	43058	16			

Table 4: Validation of conditions

Biomass (%)	Temp (°C)	Time (h)	Predicted (mg/g)	Experimental (mg/g)
6	160	5.0	150.29	144.0
6	140	4.0	225.82	155.0
6	160	4.9	151.80	139.0

Correlation coefficient: 0.9467

efficiency for alkali pretreated rice straw with the same catalyst (Goswami et al, 2015) but in that study the biomass to catalyst ratio used was 1:1. So an increased catalyst ration of 1:1 was evaluated for cotton stalk hydrolysis which improved the sugar yield to 14.46% (123.32 mg/g). It was therefore decided to optimize the hydrolysis conditions for the alkali pretreated CS so as to further improve the sugar yields.

3.2. Optimization of the hydrolysis of alkali prtetreated CS

Optimization of the parameters affecting total reducing sugar yield in the catalytic hydrolysis of cotton stalk (CS) was studied by following a response surface Box-Behnken design (Box and Behnken, 1960). The parameters studied were biomass loading, temperature of treatment and retention/holding time. Biomass to catalyst ratio was kept constant at 1:1 and 0. 2% w/v Tween 80 was also used in the hydrolysis experiments. Table 2 shows the experimental design matrix and the sugar yields obtained.

$$Y = 64.83 + 21.15A - 53.84B - 21.7C - 18.33AB - 21.51AC + 7.19BC + 4.52A^2 - 4.93B^2 + 22.66C^2$$

The maximum sugar yield obtained (179.46 mg/g, 21.01% conversion efficiency) was for run number 5 which used 6 % biomass loading and a time and temperature of 4h and 180 °C respectively. The data was analyzed by multiple regression analysis and the regression coefficients were determined. A second order polynomial equation (equation 1) was derived to represent the sugar yield as a function of the independent variables tested.

Where Y is the predicted sugar yield, and A, B and C are coded values of biomass loading, temperature and time respectively. The adequacy of the model was tested by Fischer's statistical test for the analysis of variance (ANOVA) using design expert software and the results are shown in Table 3. ANOVA suggested that the model was significant with a p value of 0.04 and an F value of 4.17. The only parameter with statistically significant effect was temperature with a p value of 0.0018 and none of the interactions had significant effects on sugar yield. Nevertheless all interactions had p values less than 1.0 indicating that these were not insignificant and hence their analyses were performed by plotting response surface curves. Figure 1a-c represent the response surfaces obtained for interaction effects of parameters tested. Increase in temperature had a negative impact on sugar yield at all the biomass loadings evaluated. However, the effect was more pronounced at higher biomass loadings (Fig 1a). Similarly, regardless of the temperature of hydrolysis higher sugar yields were obtained at higher biomass loadings. Increase in sugar yields with increase of biomass loadings can be attributed to the higher content of sugar polymer per unit reaction volume. Rapid decrease in

Figure 1: Response Surface Plots showing interactive effect of parameters on sugar yields for catalytic hydrolysis of cotton stalk

a) Interactive effects of biomass loading and temperature

b) Interactive effects of biomass loading and time

c) Interactive effects of biomass loading and time
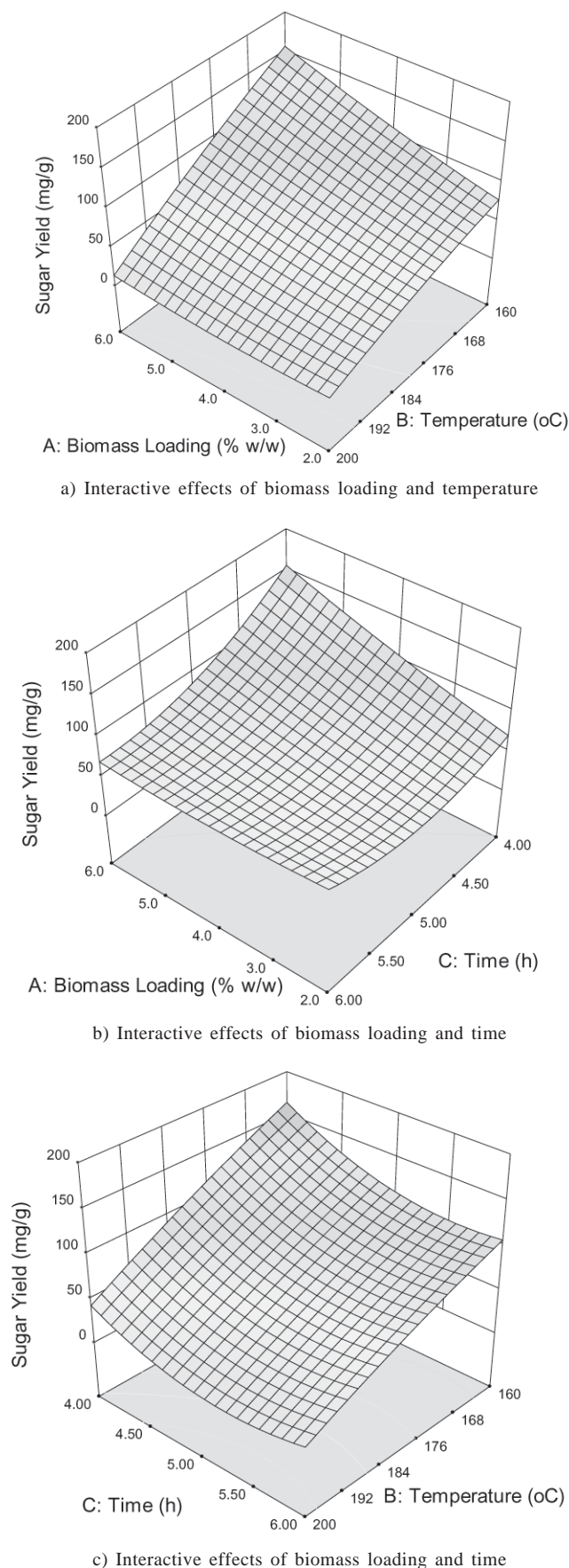
sugar yields with increase in temperature from 160 -200°C could be due to the increase degradation of the sugars released at high temperature.

Longer holding times at elevated temperatures can result in sugar degradation and this was evident at higher and lower biomass loadings. The decrease in sugar yield with increase in reaction time was more pronounced at higher biomass loadings (Fig 1b). This was also evident in the response surface of reaction time vs temperature, where again the highest sugar yields were obtained at lower temperature and lower reaction time (Fig 1c). The results indicated that the best conditions for maximal sugar yield were a biomass loading closer to 6.0 %, temperature near 160 °C and a reaction time of approximately 4h. Repetition of the experiment for run number 5 could result in a sugar yield of 219mg/g (25.67 %).

The numerical optimization function in Design Expert was used to predict the optimal conditions for maximal sugar production. Three randomly selected solutions and their predicted and experimental responses (sugar yields) are given in Table 4. A correlation analysis of the experimental and predicted response gave a value of 0.9467 for correlation coefficient validating the model. Nevertheless, the maximum yield was only 155mg/g (18.17%) against the predicted yield of 225.82mg/g (26.87%).

3.3. Catalyst hydrolyzes hemicellulose more efficiently

HPLC analysis of the hydrolysate indicated that the catalyst liberated more of hemicellulose derived sugars than glucose. The glucose and xylose concentrations in the hydrolysates of alkali pretreated cotton stalk were 4.07mg/ml (101.75mg/g) and 4.63 mg/ml (115.75 mg/g) respectively, which corresponded to conversion efficiencies of 15 % and 60 % respectively of cellulose and hemicellulose. Better efficiency in hydrolysis of hemicellulose by the catalyst was confirmed by hydrolysis of pure cellulose and hemicellulose using the catalyst. Experiments performed with microcrystalline cellulose and xylan independently under same conditions, resulted in sugar yields of 17.5mg/g (1.75%) and 850.1mg/g (85 %) demonstrating the highly efficient hydrolysis of hemicellulose by the catalyst. Hydrolysis of native cotton stalk however resulted only in 17% and 21 % conversion of cellulose and hemicellulose respectively which indicated the possible inhibition of reaction by presence of lignin. Alkali pretreatment which removes lignin therefore was a better pretreatment option for catalytic hydrolysis as was evident from the hydrolysis results in Table 1.

3.4. Reusability of catalyst

Studies were made on the catalyst separation and its reusability. After first cycle of hydrolysis, the catalyst was recovered by filtration and washed with distilled water, followed by methanol washing. This was used for the second and subsequently after similar treatment for the third batch of the hydrolysis. The results showed good stability of the catalyst after second cycle with ~ 85% of the original hydrolysis ability retained while in the third cycles it was reduced to ~60%. Previously we had reported the repeated reusability of the same catalyst for hydrolysis of rice straw (Goswami et al., 2015). The loss in efficiency on reuse in this case might be due to the use of a higher temperature at which leaching of the sulfonyl groups might have occurred. Increase in the water to solids ratio could also negatively affect the acidity and catalytic activity. Reusability is the major advantage of solid acid catalysts since it reduces pollution and lower the operation costs (Guo et al., 2012). It is speculated that the optimization of these parameters might address the reduced yield and loss of efficiency in repeated re-use.

3.5. Fermentability of catalytic hydrolysate

A preliminary evaluation of the fermentation of catalytic hydrolysate of cotton stalk was performed with hydrolysate concentrated to a glucose concentration of 22.8 g/L by vacuum centrifugation. Enzymatic hydrolysate of CS with a glucose concentration of 120g/L was generated using commercial cellulase and its fermentation was performed parallel to that of the catalytic hydrolysate under similar conditions for comparison. Maximum ethanol yield obtained for catalytic hydrolysate of cotton stalk was 0.6% v/v which was 41 % of the theoretical maximum based on initial glucose concentration. In comparison, the maximum ethanol yield from enzymatic hydrolysates was 59.2 % indicating that the catalytic hydrolysate could have had some inhibitors which also were concentrated

along with the sugars. Cotton stalk contains relatively higher amounts of lignin (~35%) compared to other feedstock and also it contains other resins and tannins etc (~5.4 % extractives) which makes it a difficult substrate to hydrolyze. Apparently, better methods of pretreatment which can remove these components efficiently may improve both the hydrolysis and fermentation of this feedstock.

4. Conclusions

Glycerol based carbon acid catalyst hydrolyzed alkali treated cotton stalk with an efficiency of 7.9% at a biomass to catalyst ratio of 1:0.5. Increase in the biomass to catalyst ratio to 1:1 doubled the sugar yield and efficiency (14.46%). Optimization of hydrolysis conditions using response surface method resulted in the improvement of sugar yield to 179.46 mg/g (21.04). Results validated by random experiments at predicted conditions gave a correlation coefficient of 0.94 indicating the validity of the model used. The catalyst could be reused without much loss of activity for second cycle, but there was significant activity loss after the second cycle and the efficiency was only 60% during third cycle. Catalytic hydrolysate of the cotton stalk was fermented with 41 % efficiency, which although was less compared to enzymatic hydrolysate (59.2% efficiency). It could be concluded that the catalyst held promise in the hydrolysis of lignocellulosic biomass.

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