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Evaluation of Amberlyst15 for hydrolysis of alkali pretreated rice straw and fermentation to ethanol

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ABSTRACT

Solid acid catalysts are potent alternatives to enzymes or mineral acids currently used in the hydrolysis of lignocellulosic biomass to generate sugars for further applications in biofuel or chemicals manufacturing. They are considered green, environmentally safe, and are reusable. In the present work, Amberlyst 15 was evaluated as a solid acid catalyst for the hydrolysis of alkali pretreated biomass. A response surface methodology was used to optimize the hydrolysis of pretreated rice straw. Optimized conditions of 7.0% biomass and catalyst loading resulted in a sugar yield of 255 mg/g of pretreated biomass in 4.0 h. The catalytic hydrolysate generated lesser amount of fermentation inhibitors compared to similar treatment by mineral acids. Recovery and reusability of the catalyst was also studied. The catalytic hydrolysate could be used for fermentative production of bioethanol. Results indicate the possibility to use solid acid catalysts as an alternative strategy for biomass hydrolysis.

1. Introduction

Biomass conversion to sugars forms the basis of future biorefineries, as the sugars can then be converted to fuels, chemicals or materials through fermentative or chemical routes. While biomass digestion can be achieved by acid or enzymatic hydrolysis, solid acids with anionic charges on their surface present an interesting and potential opportunity to be used as catalysts in the hydrolysis. The typical biomass conversion steps include pretreatment and hydrolysis which are distinct unit operations. In lignocellulosic biomass, cellulose and hemicelluloses are present in thick bundles which are covered by layers of impermeable lignin, so, initial treatment has to be harsh enough to remove the protective covering. The first step in biomass hydrolysis to sugars is the pretreatment, which is currently done with dilute acids, alkali, microwave, steam explosion, AFEX etc., which helps in the initial conditioning of the biomass. This is achieved primarily by lignin or hemicellulose removal, reducing the crystallinity of cellulose and by increasing the surface area which can be acted upon by enzymes or other agents for hydrolysis [1]. Hydrolysis is currently accomplished by either acid or enzymatic digestion. This results in the formation of monomeric sugars like hexoses (glucose, galactose etc.) and pentoses (xylose, arabinose etc.). Dilute acid at higher temperature is typically used for hydrolysis which results in corrosion of vessels, and there is a need to handle acid waste waters in addition to the generation of sugar break down products [2]. Enzymatic digestion is currently considered as the most suitable method for hydrolysis. Several enzymes like cellulase, xylanase, β-glucosidase etc., play important roles in enzymatic hydrolysis. These enzymes act on the pretreated biomass at relatively milder conditions to release considerably large amount of sugars [3]. Leading enzyme manufactures like Novozymes and Genencor already have enzymes that are specific for biomass hydrolysis in the market. Nevertheless, studies are continued in this area as the costs of enzymes are still high which makes the final product (e.g., ethanol) costly. In either acid or enzymatic digestion, reuse of the hydrolysis agent is difficult, primarily due to the technicalities and cost of separation.

The hydrolysis step is the most significant in biomass conversion to ethanol or any value added products in a biorefinery, where any reductions in cost achieved will impact the overall cost of biofuels. A re-usable solid acid catalyst could be a possible option for hydrolysis of biomass in addition to mineral acids or enzymes. Solid acids with anionic charges on their surface can overcome some of the disadvantages mentioned above and thus present an interesting and potential opportunity to be used as catalyst in biomass hydrolysis. The catalytic sites present on the surfaces could be Bronsted

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acids or Lewis acids or a combination of both. These include sulfonic acids, carboxylic acids, phosphonic acids etc., which are either bound to organic polymers or to inorganic support material. They can donate protons or accept electrons and thus catalyze biomass hydrolysis. The catalytic activity is dependent upon the strength of the acidic sites and stronger the sites, the more active are the catalysts. The acidity of a catalyst can be determined by titration. There are many solid acid catalysts which have been used in different chemical reactions such as H-form zeolites, transition-metal oxides [4], cation exchange resins, carbanacoeous solid acid catalyst etc., [5–7]. They are considered green, environmentally safe, and recoverable. One of the main reasons solid acid catalysts can replace the mineral acids in biomass hydrolysis is the ease of its recovery and recyclability.

Amberlyst 15 is a strongly acidic cation exchange resin with styrene–divinyl benzene matrix that can perform heterogeneous acid catalysis. Its structure allows permeability of liquid or gaseous reactants to the site of hydrogen ions that is present throughout the bead, thus ensuring successful performance of the resin in catalytic reactions. Studies have been done on various chemical reactions being catalyzed by Amberlyst 15, like synthesis of levulinic acid from fructose [8], synthesis of pyrazolines [9], conversion of cellulose into cellulose acetate [10], conversion of cellulose into biodegradable surfactants like octyl-α,β-glucoside and octyl-α,β-xyloside [11] etc. Amberlyst 15 has also been known to aid the hydrolysis of $\beta(1\rightarrow4)$ linkage of the cellulose backbone [12]. Very few studies have been done on biomass hydrolysis using Amberlyst though it has been reported for hydrolysis of cellulose. The objective of the study was to evaluate Amberlyst 15 for hydrolysis of alkali pretreated rice straw and to demonstrate the feasibility of using the hydrolysate for ethanol production.

2. Materials and methods

2.1. Alkali pretreatment of rice straw and hydrolysis using Amberlyst 15

Rice straw was purchased locally and was milled using a knife mill to a maximum size of 1–2 mm and was pretreated using 2% (w/w) NaOH, at a biomass loading of 15% (w/w). The pretreatment was carried out at 120 °C for 60 min in an autoclave. After pretreatment, the biomass was neutralized with 10N H_2SO_4, washed with tap water, and was then air dried at room temperature and was powdered using a laboratory mill. This sample was used as substate for hydrolysis using the solid acid catalyst. The biomass loading was 5% (w/w), catalyst loading was 100% (5% w/w), temperature was 120 °C and time was 60 min. Hydrolysis reaction was performed in Teflon lined acid digestion bombs, in a total volume of 25 ml. The bombs were heated to the required temperature and were held at that temperature for 60 min. After the reaction, the hydrolysate was centrifuged at 5076 × *g* for 10 min to recover the supernatant and the total reducing sugars (TRS) were analyzed by DNS method [13].

2.2. Hydrolysis optimization

A Response Surface Hybrid experimental design was used for optimizing hydrolysis of alkali pretreated rice straw with Amberlyst® 15 DRY (Sigma Aldrich, India) as catalyst. As increasing the biomass loading beyond 7.0% w/w results in reduced interaction between biomass and catalytic sites thereby resulting in lesser efficiency of hydrolysis, this study was carried out at biomass loading lesser than 7.5%. When the catalyst was used at lesser concentrations of 10–50% w/w at 120 °C, the sugar yield was comparatively less, which indicated that increased amount of catalyst was required for release of maximum sugars. In this study, effect of biomass loading (5.0–7.5% w/w), catalyst to biomass ratio (50–100%), residence time (1–4 h) and temperature of treatment (110–140 °C) was studied following an experimental design matrix designed using Design Expert® (Statease Inc., USA). This design included 19 runs with 3 replicates of the midpoint and all the runs were carried out in duplicates. Hydrolysis reactions were carried under the conditions specified in Table 1, as described above.

Table 1
Design matrix for response-surface hybrid design for catalytic hydrolysis of biomass.

Run	Biomass (% w/w)	Catalyst (% w/w of biomass)	Time (h)	Temp (°C)	Sugar yield (mg/g)
1	7	50	1	135	92
2	7.5	75	2.5	110	110
3	6	75	2.5	125	203
4	6	75	4.8	110	144
5	7	100	1	135	175
6	5	50	1	135	79
7	6	75	2.5	120	163
8	6	75	0.22	110	5
9	6	75	2.5	125	195
10	6	75	2.5	125	208
11	4.5	75	2.5	110	99
12	5	100	4	135	243
13	6	37	2.5	110	99
14	7	50	4	135	242
15	5	100	1	135	146
16	6	113	2.5	110	110
17	5	50	4	135	227
18	6	75	2.5	150	250
19	7	100	4	135	255

2.3. Inhibitor analysis

Identification and quantification of inhibitors like organic acids, furfural and HMF in the hydrolysates generated at the optimal temperature (135 °C) was compared against those generated at higher (150 °C) and lower (110 °C) temperatures while the other parameters were maintained constant. Inhibitor analyses were performed by HPLC. A Rezex® ROA column (Phenomenex) and photodiode array (PDA) detector was used with 0.05M H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min. The oven temperature was set at 50 °C.

2.4. Catalyst reusability

The reusability study was studied with 7.0% (w/w) of biomass loading and 100% (w/w) of catalyst loading at a temperature of 135 °C for 4 h. The supernatant containing the sugars were separated by centrifugation after the first cycle. For the 2nd round of hydrolysis, the un-hydrolyzed biomass along with catalyst and fresh biomass (3.5% w/w) was also added to it and hydrolysis was carried out at 135 °C for 4 h. The supernatant was separated by centrifugation. For the 3rd round of hydrolysis, the unhydrolysed biomass and catalyst from the second round of hydrolysis was taken and another 3.5% biomass was added and hydrolysis was done at 135 °C for 4 h. The amount of total reducing sugars was estimated for each cycle.

2.5. Fermentation

Hydrolysis was performed under the optimized conditions (i.e., with 7.0% (w/w) biomass loading, 7.0% (w/w) catalyst loading at 135 °C for 4 h. The hydrolysate supernatant was separated by centrifugation and was concentrated by vacuum evaporation. As small amounts of inhibitors were present which could inhibit the growth of yeast, these were removed by over liming followed by neutraliza-