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Hydrotropic pretreatment on rice straw for bioethanol production

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The aim of this study was to investigate the effect of hydrotrope as a pretreatment process on rice straw for bioethanol production. Sodium cumene sulfonate and sodium xylene sulfonate (Na—X) were used as hydrotropes to delignify rice straw. Effects of biomass loading, time, temperature and hydrotrope concentration were studied for both the hydrotropes. There was no loss of cellulose & hemicellulose in the hydrotrope solution. Sodium cumene sulfonate could remove more than 50% of lignin from rice straw with 5% biomass loading at 121 °C for 1 h. Reusability of aqueous hydrotrope solution was demonstrated. Enzymatic digestibility of the hydrotrope pretreated rice straw was studied and evaluated for subsequent bioethanol production. The use of the hydrotrope for biomass pretreatment has the potential to mitigate the environmental impact of chemical pretreatment.

1. Introduction

Biofuels produced from biomass have taken the lead position as a viable option to petroleum-derived fuels. The production of cellulosic ethanol through biological route has garnered extensive interest over the past decade with one of its primary advantages being that it is based on non-food lignocellulosics [1]. Lignocelluloses are the most abundant raw materials on Earth comprised of cellulose, hemicelluloses, and lignin. Due to the rigid structure of lignocellulosic biomass, very few microorganisms can use the biomass directly for growth and production. A pretreatment-hydrolysis step is carried out to break down the structure of lignocellulosic biomass and hydrolyze the exposed polysaccharides into monomers. Rice straw is one of the most abundant agricultural wastes. According to FAO points, 600–900 million tones of rice straw is produced each year globally [2]. Asia has over 90% of worldwide rice straw production. Most of the biomass remains unused and destroyed just by burning which increases the air pollution and consequently affects public health [1]. Therefore, rice straw is believed to have the potential as the preferred feedstock for fuel ethanol production in Asia [3]. Rice straw contains 32–47% cellulose, 19–27% hemicellulose and 5–24% lignin [4]. There have been several reports to improve its digestibility to reduced sugar units by applying different pretreatment methods such as microwave pretreatment [5,6], electron beam irradiation [7], dilute acid hydrolysis [8,9] and alkaline pretreatment [10]. Recently researchers have attempted to use a new class of cellulose solvents called ionic liquid [11,12] for pretreatment of rice straw at atmospheric pressure, but at elevated temperatures. Biological pretreatment using white rod fungi has been reported for rice straw [13]. Ammonia fiber/freeze explosion/expansion (AFEX) is reported as an effective pretreatment process for rice straw as it resulted in 3% sugar loss during pretreatment [14]. The major drawback of these methods, however, is that it is not possible to separate lignin without any alteration of its chemical structures. These literature reports reveal that a significant research has already been conducted to remove lignin as a pretreatment process.

Hydrotropic treatment has several attractive features that make it an attractive alternative for biomass refineries. Hydrotrope pretreatment methods are known or suspected to affect the biomass lignin, and thus, presumably facilitate enzymatic hydrolysis [15]. Lignin, a phenolic polymer, is hydrophobic and essentially insoluble in water. Thus the use of aqueous hydrotrope solutions to solubilize lignin is potentially attractive. A Hydrotrope solution is a greener solvent as it is water based and hence safe to handle. In this study, aqueous solutions of alkyl benzene sulfonates were used to isolate lignin from rice straw. Alkyl benzene sulfonates act as hydrotropic agents which are capable of improving the aqueous solubility of water insoluble components. The main advantage of hydrotrope is the quick recovery of the solute from hydrotrope solutions by simple dilution with water and reuse of the hydrotrope solution after reconcentration. However, the study of the hydrotropic method as a pretreatment for successful fermentolysis in the

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biofuel technology has not received enough consideration so far. There have been only a few reports on biomass pretreatment using hydrotropes. Hydrotropic pretreatment of Spruce chips, Sawdust, common reed, Birchwood and pine wood have been reported using sodium xylene sulfonate (Na–X) at elevated temperatures [14,16,17]. Khursheed and Gaikar (2014) have studied delignification of sugarcane bagasse wherein they have reported 85% of delignification using Na–X [18]. To our knowledge, this is the pioneer report on hydrotropic pretreatment of rice straw for bioethanol production. There have been no reports on hydrotropic pretreatment discussing fermentation for bioethanol production.

2. Materials and methods

2.1. Hydrotrope and feedstocks

Rice straw was purchased from the local market and was milled and homogenized to 1–2 mm. The hydrotropes Sodium cumene sulfonate (NaCS) and sodium xylene sulfonate (Na–X) were obtained from Navdeep Chemicals Pvt Ltd, Mumbai, India. Enzyme Cellulase was procured from Zytex India Pvt Ltd. HPLC analysis of sugars was performed on Shimadzu LC-2010 unit using degassed Milli-Q water as mobile phase at a flow rate of 0.6 ml/min and Phenomenex Rezex RPM-Monosaccharide Pb^{+2} (300 × 7.8 mm) column was used. Oven temperature was maintained at 80 °C and the sugars were detected using (Refractive Index) RI detector. Glucose, xylose, cellobiose, mannose, galactose and arabinose were used as standards for HPLC analysis.

2.2. Hydrotropic pretreatment of rice straw

Before pretreatment, rice straw was ground to a maximum size of 1–2 mm and dried in an oven at 50 °C for 6 h. Sodium cumene sulfonate (NaCS) and sodium xylene sulfonate (Na–X) were used as hydrotropes for the study. An aqueous solution of hydrotrope with known concentration was prepared. Based on the weight of hydrotrope solution 5% weight of ground rice straw was added to it. The suspension was then stirred in a shaking water bath at predetermined time and temperature. After the pretreatment, the suspension was filtered under the same temperature conditions and washed with dilute hydrotrope solution followed by a water wash in 1:6 ratios to remove the adhering hydrotrope from the solid cake if any. After thorough washing, the solid mass was dried and weighed to verify the extent of extraction. A series of experiment was conducted to investigate the effect of temperature, time, biomass loading, hydrotrope concentration, and type of hydrotrope on pretreatment.

2.3. Characterization of native and pretreated biomass

Compositional analysis of native and pretreated biomass were analyzed by a two-stage acid hydrolysis protocol developed by NREL [19]. The difference in the lignocellulosic structure of rice straw before and after hydrotropic pretreatment was evaluated by scanning electron microscopy (JEOL JSM-5600). The images of native and hydrotrope pretreated rice straw were acquired with a 10–15 kV accelerating voltage at a magnification of 700×. The crystallinity of native and pretreated rice straw was analyzed using a PANalytical (Netherlands) X-pert pro diffractometer [20]. Crystallinity Index (CrI), was calculated as per protocols adopted by Segal et al., 1959 [21].

2.4. Recovery of lignin

The hydrotrope solution after separation of the residual rice straw is diluted four times with its original volume for the recovery of lignin. The diluted hydrotrope solution is kept on standing for overnight so as to have complete precipitation of lignin. The precipitated lignin was then separated by centrifugation at 8500 rpm for 10 min. The extent of delignification was calculated based on its initial lignin content which was determined according to National Renewable Energy Laboratory (NREL) analytical methods.

2.5. Reusability of hydrotrope solution

The reusability of hydrotrope solution with 20% concentration was verified with 5% loading of biomass as the amount of lignin extracted in the single stage was not sufficient to saturate the hydrotrope solution. The hydrotrope solution after completion of one cycle is diluted to four times with its original volume for isolation and recovery of lignin. The diluted hydrotrope solution is then concentrated by rotavac under reduced pressure to its initial volume for the next pretreatment cycle with fresh biomass.

2.6. Enzymatic hydrolysis

Pretreatment effect was evaluated on enzyme hydrolysis, the pretreated rice straw was subjected to enzyme hydrolysis using commercial Zytex enzyme with an enzyme loading of 20 FPU/g. The hydrolysis was conducted in a 150 ml hydrolysis flask by suspending 3 g of hydrotrope pretreated rice straw in 1 M sodium citrate buffer (pH 4.5). The samples were incubated at 50 °C for 48 h in shaking water bath. The hydrolysate was subjected to reducing sugar analysis by 2,5-dinitrosalicylic acid method [22].

2.7. Fermentation

The enzymatic rice straw hydrolysate obtained after hydrotropic pretreatment was used for ethanol fermentation. It was supplemented with yeast extract (0.005% w/v), Ammonium Sulfate (0.2% w/v), Magnesium sulfate (0.05% w/v), Sodium chloride (0.02% w/v) and pH was adjusted to 5.00. Fermentation was performed in 15 ml screw capped vials containing 10 ml hydrolysate. *Saccharomyces cerevisiae* was cultivated in YPD, centrifuged and the biomass cake was used as inoculum with 4% wet weight basis. The vials were capped and were allowed to ferment for 72 h at the end of which samples were withdrawn and centrifuged at 10,000 RPM to recover the supernatant which was analyzed for ethanol by gas chromatography as outlined in NREL Laboratory Analytical protocol #011 [23].

3. Results and discussion

3.1. Pretreatment of rice straw

Pretreatment of rice straw was carried out using aqueous hydrotrope solution of sodium cumene sulfonate and sodium xylene sulfonate. During the pretreatment process, the color of the hydrotrope solution darkened, indicating the dissolution of the material from the rice straw. Lignin, a phenolic polymer, is hydrophobic and essentially insoluble in water. It was believed that the solubilization of lignin in the hydrotrope solution is due to hydrophobic interactions between the aromatic ring of phenolic lignin with the aromatic ring of the hydrotrope which is leading to preferential solubilization of lignin compared to cellulose and hemicellulose. The hydrotropic solution with the extracted lignin was diluted below the minimum hydrotrope concentration (MHC) for separation of lignin. The solid residue obtained after pretreatment has to be thoroughly washed with 15% aqueous hydrotrope solution