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Selection of suitable mineral acid and its concentration for biphasic dilute acid hydrolysis of the sodium dithionite delignified *Prosopis juliflora* to hydrolyze maximum holocellulose

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HIGHLIGHTS

- Acid hydrolysis of delignified substrate with three different mineral acids.
- Selection based on maximum hydrolysis with release of fewer amounts of inhibitors.
- Variation of reaction time and temperature for maximum saccharification.

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ABSTRACT

Two grams of delignified substrate at 10% (w/v) level was subjected to biphasic dilute acid hydrolysis using phosphoric acid, hydrochloric acid and sulfuric acid separately at 110 °C for 10 min in phase-I and 121 °C for 15 min in phase-II. Combinations of acid concentrations in two phases were varied for maximum holocellulose hydrolysis with release of fewer inhibitors, to select the suitable acid and its concentration. Among three acids, sulfuric acid in combination of 1 & 2% (v/v) hydrolyzed maximum holocellulose of 25.44 ± 0.44% releasing 0.51 ± 0.02 g/L of phenolics and 0.12 ± 0.002 g/L of furans, respectively. Further, hydrolysis of delignified substrate using selected acid by varying reaction time and temperature hydrolyzed 55.58 ± 1.78% of holocellulose releasing 2.11 ± 0.07 g/L and 1.37 ± 0.03 g/L of phenolics and furans, respectively at conditions of 110 °C for 45 min in phase-I & 121 °C for 60 min in phase-II.

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1. Introduction

Bioethanol is considered as one of the important alternative clean fuel since emissions of CO, N_2O, CO_2, hydrocarbons and particulate matter are less by 35%, 42%, 79%, 43% and 39%, respectively, when compared with petroleum based fuels. Moreover, bioethanol has got the adaptability to existing engines and if used in transportation can replace 30% of gasoline in use (Krishnan et al., 2010). At the foremost, large scale production of bioethanol and its commercialization mainly depends on the choice of feedstock. As lignocellulosic feedstocks which has no fuel vs. food demand (Brown, 2006), no negative impact on regional water resources, biodiversity, soil quality, and increased green house gas emissions has attracted worldwide attention (Wi et al., 2011) and has become the major focus of intensive research and development for bioethanol production (Lynd et al., 1999).

Worldwide, annually about 200 billion metric tons and in India about 600 million metric tons of cellulosic waste biomass is generated (Abhay, 2012). Utilization of these feedstocks for bioethanol production not only generates income, employment but also solves the problem of their disposal. Moreover, lignocellulosic biomass is a source of energy with neutral carbon, as bioethanol produced from these substrates on combustion produces no net carbon dioxide into atmosphere thus avoiding global warming (Abo-State et al., 2013). But the commercialization of lignocellulosic bioethanol has been limited due to lack of suitable technologies, efficient pretreatment strategies, cost of enzymes (accounting as high as 25–50% of the total ethanol production cost) and efficient strain for bioethanol production.

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Among all the methods of pretreatment chemical pretreatment shows higher degree of selectivity towards biomass for the component it is degrading but uses harsh reaction conditions. Chemical method of pretreatment involves the use of acids, alkali, ammonia, organic solvents, SO_2, CO_2, reducing agents or other chemicals. The advantage associated with chemical method of pretreatment is easy to operate and have good conversion yields in short period of time (Sun and Cheng, 2002). Moreover, it solubilizes and separates one or more components of biomass *viz.* hemicellulose, cellulose and lignin to make the remaining solid biomass more susceptible to further chemical or enzymatic treatment steps (Sarkar et al., 2012).

Due to low cost and easy availability of acids, among all the chemical methods of pretreatment, dilute acid treatment is considered as a cheapest and effective method (Chaturvedi and Verma, 2013). Dilute acid hydrolysis depolymerizes more hemicellulose fraction at mild temperature and cellulose fraction at severe temperature. Among all the acids, sulfuric acid is commonly used (Mosier et al., 2005). The hemicellulose fraction of biomass is hydrolyzed into xylose and other sugars using acid in the concentrations of 1–5% (v/v) in a steam reactor or in a bioreactor such as autoclave (Kumar et al., 2009) in the temperature ranging from 110 °C to 220 °C (Agbor et al., 2011). Dilute acid hydrolysis at low severity releases oligomers and monomeric sugars by hydrolyzing hemicellulose and at high severity could hydrolyze cellulose (Naseeruddin et al., 2013). Depending on the severity of the conditions used during the acid hydrolysis, sugars can be converted into inhibitory compounds such as furfural and hydroxyl methyl furfural (HMF). The removal of hemicellulose increases the porosity of the biomass, which improves the digestibility of cellulose by cellulolytic enzymes (Agbor et al., 2011). To maximize sugar recovery and enhance biomass digestibility of feedstocks by biphasic dilute acid treatment, in the first phase hemicellulose hydrolysis is promoted and in the second phase remaining portion of cellulose is converted to dextrose (Nguyen et al., 2000). Biphasic acid hydrolysis is preferred in the concentration range of 1–5% (v/v) (Lee et al., 1997) leads to less reduced sugar degradation and less inhibitor formation in the hydrolysate (Taherzadeh and Karimi, 2007). Moreover, acid hydrolysis is strongly affected by the ratio of solid to liquid, temperature, reaction time, type of acid used and its concentration (Zhuang et al., 2009). Release of sugars during acid hydrolysis also depends directly upon the type of lignocellulosic material, its composition and the reactors employed in the process (Lenihan et al., 2010). Therefore, to get maximum yield of sugars and release of minimum amount of inhibitors optimization of above parameters is of fundamental importance as the hydrolysate obtained during acid hydrolysis will be used further for fermentation (Gírio et al., 2010). Temperature and time are crucial factors that affect directly the degradation of sugars into toxic compounds (inhibitors), which eventually affect microbial metabolism (Chandel et al., 2007). In general, it is observed that mild temperature led to maximum recovery of sugars while higher temperatures caused more sugar degradation aiding the formation of inhibitors.

Hence in the present work, acid hydrolysis of delignified *Prosopis juliflora* was carried out by using three mineral acids *viz.* phosphoric acid, hydrochloric acid, and sulfuric acid at different concentrations. The main aim of using three different acids in different concentrations was to check and select suitable acid and its concentration to hydrolyze maximum holocellulose from sodium dithionite delignified *P. juliflora*. Further acid hydrolysis at variable reaction time and temperature with selected acid was carried out for maximum holocellulose hydrolysis. Later, enzymatic hydrolysis of acid hydrolyzed substrates (at various time periods and temperatures) carried out to compare saccharification percentage/recalcitrance of the substrate towards enzymatic hydrolysis.

2. Methods

2.1. Raw material

Dried *P. juliflora* stem was taken as raw experimental material for bioethanol production. The stem was chopped into pieces of 4–5 cm. Later the substrate was ground in a laboratory pulverizer and sieved through standard mesh to attain a particle size of 2–3 mm. The grinder used had the provision to fix a sieve with desired pore size in front of the sample outlet such that the sample could be retained till ground to the desired mesh size. The ground substrate was collected in plastic bags and again sieved through standard mesh of 2–3 mm to get uniform starter material. Later the substrate was washed with tap water to make it dust-free and then it was dried in hot air oven at 65 ± 2 °C overnight to constant weight.

2.2. Cellulase enzymes

Commercial advanced cellulase enzyme powder (FPase, 29.2 IU/g, CMCase 43.0 IU/g and β-glucosidase 18.10 IU/g of enzyme) was procured from Advance Enzyme Technologies, Mumbai.

2.3. Delignification

Powdered *P. juliflora* in 1:10 ratio (solid: liquid) was delignified with 2% (w/v) sodium dithionite (hydro) at 30 ± 2 °C for 18 h to remove maximum lignin, as reported previously (Naseeruddin et al., 2013). The pretreated biomass was then neutralized with tap water and dried at 65 ± 2 °C for overnight and used for subsequent acid hydrolysis.

2.4. Biphasic dilute acid hydrolysis of the sodium dithionite delignified substrate with phosphoric acid, hydrochloric acid, and sulfuric acid

Two grams of delignified substrate at 10% (w/v) level were taken in 100 ml Erlenmeyer flask and was subjected to biphasic dilute acid saccharification in autoclave (Biocare) by varying the combinations of acid concentrations in two phases between 1% and 5% (v/v). The different combinations of acid used in two phases were 1 & 2%, 2 & 3%, 3 & 4%, 4 & 5%, 5 & 1%, 1 & 5%, 5 & 4%, 4 & 3%, 3 & 2%, and 2 & 1% (v/v). The phase-I of acid hydrolysis was carried out at 110 °C for 10 min and phase-II was carried out at 121 °C for 15 min. The hydrolysates obtained from both the phases were mixed and sugars, phenolics and furans were estimated. The percent of holocellulose hydrolyzed and the amount of inhibitors released in the hydrolysate were used as parameters to select the suitable acid and its combination of concentrations in the two phases of hydrolysis.

2.5. Acid hydrolysis at variable reaction time and temperature with selected acid for maximum holocellulose hydrolysis

After the selection of sulfuric acid at concentration of 1% (v/v) for phase I and 2% (v/v) for phase II for the hydrolysis of *P. juliflora*, reaction parameters like time and temperature were varied for the release of maximum sugars from the holocellulose.

2.5.1. Acid hydrolysis at variable reaction times

Ten grams of the delignified substrate at 1:10 ratio (solid: liquid) was taken in 500 ml Erlenmeyer flask and subjected to biphasic acid hydrolysis by keeping the reaction temperatures constant i.e. 110 °C for phase-I and 121 °C for phase II of acid hydrolysis using 1% (v/v) and 2% (v/v) of sulfuric acid, respectively, by varying the reaction time of the two phases. Two different reaction times

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