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Evaluation of efficient glucose release using sodium hydroxide and phosphoric acid as pretreating agents from the biomass of *Sesbania grandiflora* (L.) Pers.: A fast growing tree legume

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HIGHLIGHTS

- *Sesbania grandiflora*, a high biomass producing tree was brought into fuel line.
- Two pretreatment processes using sodium hydroxide and phosphoric acid were adopted.
- Effect of time, temperature and NaOH concentration was compared in alkali pretreatment.
- Phosphoric acid pretreatment found to release more glucose at low cellulase loadings.

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ABSTRACT

Sesbania grandiflora (L.) Pers. is one of the fast growing tree legumes having the efficiency to produce around 50 t ha^{-1} above ground dry matters in a year. In this study, biomass of 2 years old *S. grandiflora* was selected for the chemical composition, pretreatments and enzymatic hydrolysis studies. The stem biomass with a wood density of 3.89 ± 0.01 gm cm^{-3} contains about 38% cellulose, 12% hemicellulose and 28% lignin. Enzymatic hydrolysis of pretreated biomass revealed that phosphoric acid (H_3PO_4) pretreated samples even at lower cellulase loadings [1 Filter Paper Units (FPU)], could efficiently convert about 86% glucose, while, even at higher cellulase loadings (60 FPU) alkali pretreated biomass could convert only about 58% glucose. The effectiveness of phosphoric acid pretreatment was also supported by X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM) and Fourier transform infrared spectroscopy (FTIR) analysis.

1. Introduction

As the available petroleum reserve continues to decrease accompanied with being the key contributor to global warming, the energy policy need to look up an alternative which could extend the prolonged sustainability of fuel background (Demirbas, 2009). Concerns about global warming, rising cost of gasoline and its exhaustibility have oriented us (Government of India Ministry of New & Renewable Energy) to develop a renewable and environment-friendly alternative like biomass-based bioethanol (Ge et al., 2011). The biofuels produced from the renewable resources could help us reduce the fossil fuel burning and CO_2 production. First generation biofuels (biodiesel, bioethanol) exploit the food materials like edible oil and sugar, resulting in existence of concerns about availability of feedstocks and impact on biodiversity (Naik et al., 2010). In contrast, second generation biofuels broadly produced from lignocellulosic biomass, significantly reduce the chances of conflict in demand between food and energy (Naik et al., 2010; Bai et al., 2010). Besides crop residues, forest residues and municipal wastes, feedstocks from energy crops hold a significant position as the raw source for biofuels. Bioenergy crops are defined as any plant material used to produce biomass to be converted into bioenergy, but some of the important charac-

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teristics which could be bracketed; having efficiency to generate high volume of biomass in a short period of time on marginal lands not suitable for growing food crops with minimal input of fertilizer and water (Lemus and Lal, 2005). With the expansion of biofuel, there may be chances of indirect or direct land clearing, possibly minimized by adopting bioenergy crop with potency to grow on degraded lands abandoned from agricultural uses; in addition, use of such lands associated with suitable crops anticipated to reduce the conflicts among the four important dimensions – energy security, greenhouse-gas emissions, biodiversity and the sustainability of the food supply (Tilman et al., 2009).

S. grandiflora belonging to family Fabaceae is native to tropical Asia including India, Indonesia, Malaysia, Myanmar and Philippines and it is generally grown in tropical and sub-tropical regions. Green leaves of the plants because of high protein content used as a ruminant fodder (http://www.worldagroforestry.org/treedb2/speciesprofile.php?Spid=1519). Being nitrogen fixing it improves soil quality; hence, generally incorporated in agroforestry systems in India, moreover, fast growing efficiency makes it one of the sources of fuel wood in rural areas in some parts of India. In a study conducted by Desai and Halepyati (2007) on biomass partitioning of two species of *Sesbania*; *S. grandiflora* and *S. sesban*, significant biomass productivity was noticed from *S. grandiflora* stand. According to the study, after 24 months of growth with a plant density 40,000 plants ha^{-1}, *S. grandiflora* could be able to produce 60.57 dry t ha^{-1}, while 33.54 t ha^{-1} and 17.30 t ha^{-1} dry biomass were obtained from stands with densities 20,000 and 10,000 plants ha^{-1} respectively. In addition, when the density of 40,000 plants ha^{-1} were supplemented with 60 kg P_2O_5 kg ha^{-1} along with Vesicular Arbuscular Mycorrhizae, dry biomass production increased up to 100.04 t ha^{-1} after 24 months of planting (Desai and Halepyati, 2007). Though the exact coppice biomass production per hector has not thoroughly been studied for *S. grandiflora*, it is known to produce higher amount of coppice, which makes it one of the ideal short rotation woody crops. In another study conducted at Centre for Bio-resources, Institute for Coastal and Offshore Research, Visakhapatnam, India (1985) from 1985 to 1987 regarding the biomass production of different fuel wood species, *S. grandiflora* could accumulate highest amount dry matter. As per the study, after 37 months of growth of this species, about 160 t ha^{-1} dry biomass was recorded, in which around 120 t was contributed by main stem and rest 40 t was by branches.

Presence of lignin, extensive hydrogen bonds among cellulose fibrils make the lignocellulosic biomass recalcitrant, because of which the cellulose digesting enzymes cannot reach the substrate, resulting in lower cellulose conversion into glucose (Karp et al., 2015). Hence, pretreatment becomes one of the necessary steps either for the removal of lignin or for making the biomass less crystalline. Action of the pretreatment depends on the type of the chemical used, for instance, NaOH principally targets lignin fraction, while dilute H_2SO_4 acts on hemicellulose portion of the biomass (Lee et al., 2015). Various pretreatment processes are being developed to achieve these objectives. Herein, we have described recently introduced cellulose solvent and organic solvent based lignocellulose fractionation (COSLIF) pretreatment which uses concentrated phosphoric acid as the cellulose solvent and 95% ethanol as an organic solvent. Previously, COSLIF has been used in various feedstock specially grasses and a few woody feedstocks. Phosphoric acid (H_3PO_4) as a cellulose solvent in combination with 95% ethanol as an organic solvent ensure less glucose degradation and high hemicellulose removal (Moxley et al., 2008). In addition, the use of the H_3PO_4 at moderate temperature (50 °C) makes the biomass amorphous by orderly disrupting hydrogen bonds consequently facilitating the enzymatic digestion by increasing surface area (Rollin et al., 2010). Use of an organic solvent recovers the acid without dilution, while the difference in volatility among water, H_3PO_4 and ethanol makes the recycling procedure easier (Zhang et al., 2007). For the lignocellulosic woody feedstock, *S. grandiflora*, we used COSLIF as the pretreating agent on which application of even at low enzyme loading (1 FPU) significant fraction of glucan got converted into glucose, which advocates the high efficiency of the pretreatment. Alkali pretreatment mostly used for the delignification was optimised by using different percentages of dilute NaOH (0.75%, 1%, 2%) for different residence time (30, 60 and 90 min) at a fixed solid loading (1:10) and temperature (121 °C). The efficient condition was used for enzymatic digestion at different enzyme loadings. The best condition identified from the different treatments was used for the theoretical calculation of ethanol yield using the biomass of *S. grandiflora*.

2. Materials and methods

2.1. Chemicals and materials

The biomass of *S. grandiflora* (~2 years old) was collected from the plants growing at Regional Plant Resource Centre, Bhubaneswar, India and used for all the experiments. Upon receiving, % contributions of stem and bark were calculated on dry weight basis. The biomass consisting of main stem and bark were individually chopped and subjected to air drying. Air dried biomass were milled using a laboratory cutting mill (Wiley Online) fitted with a 20 mesh sieve. Milled biomass were further sieved to obtain biomass of particle size between 40 and 60 mesh for all compositional analysis and pretreatment studies. The chemicals used for the study were purchased from Merck (India) and HiMedia (India). Cellulase from *Trichoderma reesei* and glucosidase from *Aspergillus niger* were used from Sigma (C2730 and 49291 respectively).

2.2. Measurement of enzymatic activities of cellulase and glucosidase

Enzyme activities of cellulase and β-glucosidase were determined in terms of Filter Paper Unit (FPU) and International Unit (IU) respectively (Adney and Baker, 1996). FPU of cellulase was determined by using filter paper as a substrate, while IU of β-glucosidase was determined by using p-nitrophenyl-β-D glucopyranoside (pNPG) as substrate. Using the standard equation (Wood and Bhat, 1988), activity of cellulase was found to be 64 FPU ml^{-1}, while the β-glucosidase activity of glucosidase enzyme was calculated to be 683 IU g^{-1}.

2.3. Analysis of biomass composition

Moisture content (%) of raw (chopped), air dried, milled and pretreated biomass of *S. grandiflora* was estimated using the protocol described. For analysis, biomass were incubated in a hot air oven maintained at 105 °C until the constant weight was achieved. Final weight deducted from the initial weight of biomass and calculations were made for the determination of both moisture and total solid content as described elsewhere (Sluiter et al., 2008a). Using standard National Renewable Energy Laboratory (NREL) protocols, glucan, xylan and lignins were measured. About 300 mg biomass (raw and pretreated) first digested with 72% H_2SO_4 at 30 °C in a water bath for 1 h and subjected to secondary digestion with 4% H_2SO_4 (by adding 84 g DI water) at 121 °C for 60 min in an autoclave (Nat Steel, India, liquid setting). Acid soluble lignin (ASL), glucan and xylan were estimated from the 4% hydrolysate and the acid insoluble lignin (AIL) was measured from the solid residues. The monosaccharides (glucose and xylose) were analyzed by high performance liquid chromatography (HPLC, Perkin Elmer Series 200, Switzerland) using Agilent Hi-Plex H column at 60 °C with 0.01 M H_2SO_4 as a mobile phase at a flow of 0.6 mL/min. Acid insol-