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Enhanced saccharification efficiency of lignocellulosic biomass of mustard stalk and straw by salt pretreatment

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ABSTRACT

Cost effective technologies for bio-ethanol production from lignocellulosic biomass are important to meet India's need of bioenergy. Mustard stalk and straw (MSS) is an abundant agroresidue of India for enzyme production and saccharification. This study demonstrates the effects of pretreatment processes on MSS with common salt (NaCl) in comparison to hot water and alkali. SEM images revealed loosening of fibers with some distortion of cellular arrangements, increased external surface area and porosity in NaCl treated MSS. In XRD analysis crystallinity index of salt pretreated MSS (61.95%) was higher than untreated (36.84%) biomass. FTIR spectroscopy analysis further supported SEM and XRD data. Saccharification efficiency of salt treated MSS increased to 82% as compared to untreated biomass (16%) with decrease in total lignin content by 26% in comparison. Salt pretreatment appeared as a cheaper and nontoxic alternative to alkali and other expensive and toxic pretreatment processes for bioethanol production from lignocellulosic biomass.

1. Introduction

Modern world still heavily depends on fossil fuel as main energy source and thus it controls the economy of the modern world (Uihlein and Schebek, 2009). The energy demand of the world is increasing every day due to the growing population and industrial revolution. At the same time the fossil fuel reserve of this world is decreasing steadily, which make an urgent call for a renewable, non-polluting and cleaner alternative fuel. An ideal alternative energy sources need to be renewable, safe, sustainable, efficient, cost effective and convenient (Chum and Overend, 2001). Thus, it is necessary to develop bio-based novel products and other groundbreaking technologies to overcome the widespread dependence on fossil fuels.

Biofuel research not only depends heavily on both the right type of biomass (Saidur et al., 2011; Hamelinck et al., 2005) and their subsequent conversion, but also along its economic sustainability when operated on a large-scale (Parmar et al., 2011). India causes a pressing demand for renewable transportation fuels and bio-ethanol is seen as one of the most important choices. The ethanol for this is primarily sourced from molasses feedstock, but this is hardly sufficient to satisfy the current need. Improvements in industrial biotechnology offer potential opportunities for economic usage of agro-industrial residues. Lignocellulosic biomass is the most suited alternative but its availability is a big challenge (Sukumaran et al., 2010). Limited reserves of fossil fuels necessitate the search for appropriate alternative sources of fuel obtained from resources available locally in respective locations across the globe. These may include ethanol, vegetable oils or biodiesel. The preceding century experienced a significant change in the environment and natural resources due to extensive industrial revolution and misuse of resources have been carried out by human civilization (Karl and Trenberth, 2003). Along with depletion of fossil fuel, its combustion also adversely affected the earth's climate. The overall average temperature of the earth is increasing due to the change in land usage policies and formation of green house gases upon burning of fossil fuels (Ekpeni and Olabi, 2013). In these circumstances, ethanol poses as an attractive alternative to fossil fuel due to its bio-based resources that are renewable and it gets oxygenated thus have the potential to decrease the emissions of particulates in compression-ignition types of engines (Christensen et al., 1997).

In this regard bioethanol has the potential to be the ideal alternative and renewable fuel source. The worldwide use of ethanol as a substitute for conventional motor fuel is increasing substantially (Agarwal, 2007; Farrell et al., 2006). Domestic bioethanol produc-

Abbreviations: MSS, mustard stalk and straw; SEM, scanning electron microscopy; XRD, X-ray diffraction; FTIR, fourier transform infrared spectroscopy.

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tion will further reduce the dependence on foreign oil, produce job openings in rural sectors and decrease the trade deficits. The cleaner composition and lead free nature of the carbon dioxide produced as a result of combustion of bioethanol—means it will also help in reducing environmental pollution and carbon dioxide concentration in air (Badger, 2002). Ethanol, being an excellent transportation fuel can also be used as a blend with gasoline. USA and Brazil are already using 10 and 22% blends of ethanol with gasoline, respectively. Brazil is even using 100% ethanol as motor fuel (E100) (Li et al., 2003), an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO*x* emission during combustion. It may also be used directly (95% ethanol and 5% water) as a fuel (Wyman, 1994). It is observed that blending up to 15% of ethanol with diesel causes less visible smoke and the good thing is it can be used without any modification of the existing engine. Smoke and smog are the greatest problems that diesel vehicles cause in congested cities.

Ethanol produced from sugar cane or starchy crops after fermentation is known as first generation (1G) fuel and produced from lignocellulosic wastes are known as second generation (2G) fuel. The generation of 1G ethanol from edible plant parts and their subsequent use as fuel has led to several controversies as they have alternative uses as food crops. Agro-residues or agro-wastes can be the ideal alternative substrate for the production of 2G lignocellulosic ethanol. Annually over 40×10^9 kg of inedible plant material, including wheat stems, corn stover, wood-shavings from logging are produced along with many other conventional agro wastes (Koçar and Civaş, 2013). Mustard stalk and straw (MSS) is one such agrowaste which has very little utility left over after removing the seeds for oil extraction. It is unsuitable as cattle feed due to its characteristic pungent smell, thus left behind unused in the field itself and later burnt out. It is important to note that MSS does not compete with food crops for cultivation. The mustard stalk and straw (MSS) constitutes 70% of the total mustard plant, which is considered as agricultural waste having no specific use (Binder and Raines, 2009; Wyman, 1994). The cellulose (48.5%) and hemicellulose (29.6%) present in MSS (Maiti et al., 2007) is considerably higher than common agro-wastes such as rice straw (cellulose 28.5%, hemicellulose 24.7%), wheat bran (cellulose 30%, hemicellulose 27.2%), that are utilized as substrate for bio-ethanol production. Moreover, it is economically much cheaper than the other agro-wastes (Gawande and Kamat, 1999).

In India the total cultivation of mustard in 2010 is 6.81 million ha that produced oilseeds of 6.43 million tons (28.3 and 19.8% of global share respectively). Thus, annual production of MSS in India is approximately 22 MT MSS (Tripathi et al., 2008).

The woody material, i.e., the lignocellulosic part gives plants their rigidity and the structure comprises of three main types of carbon based polymer-cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses of lignocellulosic biomass are ideal substrates for bioethanol production. Cellulose is a polymer of glucose, and makes the perfect substrate for fermentation to produce ethanol or butanol (long chain alcohol) (Maiti et al., 2007). Hemicellulose contains polymers of various sizes with a range of different sugars incorporated at different positions. Lignin, on the other hand has a polymeric backbone made of ring shaped phenolic groups (carbon based structures). Apart from these three main components, lignocellulosic biomass has several useful chemicals such as furans, which could serve as alternative high energy-density fuels. Most of the current effort in second generation biofuel production focuses on ethanol with furans and butanol at earlier stages of development (Gawande and Kamat, 1999).

The major bottleneck of these processes is that the lignocellulosic biomass are hard to degrade because of its complex polymeric structure. The sugar present in the complex is insoluble and inaccessible. The cellulose micro fibrils remains attached to hetero-polysaccharide hemicellulose, containing a variety of sugars, thus saccharification of this heterogeneous polymer and subsequent saccharification to a single product like ethanol becomes difficult. The cellulose–hemicellulose complex is surrounded by lignin, which protects the accessibility to cellulose and hemicelluloses (Fahn, 1982). Thus to obtain the degradation of lignocellulosic biomass, i.e., pretreatment to break through the lignin counterpart and expose the cellulose and hemicelluloses polymers inside is an essential step in the production of utilization of the biomass to bioethanol (Vallejos et al., 2015; Xu et al., 2015).

Pretreatment of the biomass by various procedures to break apart these lignocellulosic materials to its building blocks involve heat and strong chemicals (Khowala and Pal, 2011). Enzymes can then saccharify the biomass and liberate sugars. The sugar hydrolysate is subsequently fermented to produce ethanol. The pretreatment step could represent up to 20% of the total costs of cellulosic ethanol production. An effective pretreatment should (a) improve cellulose digestibility, (b) produces low concentrations of degradation products and (c) have low energy demand. The other criteria always remain to minimize the environmental pollution load (Gastaldi et al., 1998; Khowala and Pal, 2011).

Recently we introduced MSS as a cheap agro-residue substrate for production of lignocellulolytic enzymes by *Termitomyces clypeatus*. MSS can also be used to generate bioethanol after saccharification by lignocellulolytic enzymes or other processes (Pal et al., 2013). In the present study we report salt pretreatment of MSS using common salt as a cheap, non toxic, non-corrosive chemical agent. Structural assessment of physico-chemical changes in MSS resulting from salt pretreatment in comparison to other pretreatment methods by hot water and alkali has been carried out using FTIR, SEM and XRD method. The study shows that NaCl treatment may be considered as a favorable method of MSS pretreatment with respect to lignin removal and increased porosity. The saccharification efficiency of salt pretreated biomass increased significantly and was comparable to that of alkali pretreated biomass.

2. Methodology

2.1. Materials and methods

Chemicals such as *p*-nitrophenyl-*d*-glucosidase (*p*NPG), *p*-nitrophenyl-xylopyranoside (*p*NPX), carboxyl methyl cellulose (CMC), DNSA and xylan were purchased from Sigma–Aldrich. Novozyme was purchased from Sigma–Aldrich and cellulase preparations was obtained from Zytex. All other chemicals and NaCls (analytical grade) were purchased locally unless otherwise mentioned.

Mustard and other chemicals: Mustard (*Brassica juncea*) stalk and straw residue (MSS) (without leaves and seeds) were collected from fields of West Bengal, India (22.56°N, 88.36°E) where they were dumped after harvesting (harvesting usually done after 40–60 days of sowing). After collection the waste crop was taken to the laboratory, washed, cut and dried overnight in oven at 40 °C. It was further cut into small pieces ~2.5 cm. The chopped MSS was powdered in mixer (Remi laboratory grade) and sieved to ~1 mm in size.

2.2. Enzymes and assays

Xylanase produced by *T. clypeatus* was obtained and assayed using birch wood xylan as substrate (Pal et al., 2013). The solution of 0.9 ml xylan (stock solution of 10 g l^{-1}) and the 0.1 ml of enzyme at appropriate dilution were incubated at 50 °C for 10 min, and the reducing sugar was determined by the DNS method at 540 nm with xylose as standard. Enzyme activity unit was defined as 1 μmol of reducing sugar released in 1 min under the assay conditions.