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Saccharification of alkali treated biomass of Kans grass contributes higher sugar in contrast to acid treated biomass

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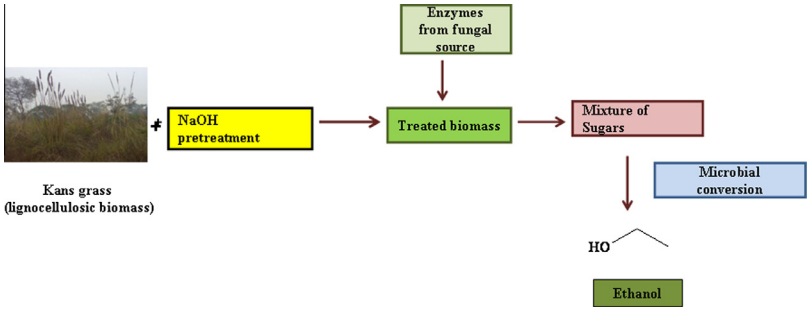
HIGHLIGHTS

- In present study as Kans grass biomass was used as a potential substrate for bioethanol production.
- Alkali pretreatment of Kans grass was followed by the saccharification by using crude cellulase enzyme.
- Resulted Sugar after enzymatic hydrolysis was used for ethanol production by *Saccharomyces cerevisiae* and *Pichia stipitis*.
- A high yield of ethanol was obtained.
- On site produced crude enzyme may be more cost effective for bioethanol production process.

GRAPHICAL ABSTRACT



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ABSTRACT

Economical production of biofuel is prerequisite to depletion of fossil fuel. In recent years, biomass of numerous food crops was used as a feedstock for bioethanol production. Unfortunately, due to limited availability as well as confliction with food, these sources may hold back for continuous production of bioethanol. Therefore, in the present study a waste land crop "Kans grass" was utilized as feedstock for microbial production of bio-ethanol. The Kans grass biomass obtained after NaOH pretreatment at optimum conditions (in term of lignin removal) was subjected to enzymatic saccharification by using crude enzyme (obtained from *Trichoderma reesei*) to total reducing sugars (TRSs), which was further fermented for bioethanol production using yeast strains. Different time (30, 60, 90 and 120 min), concentrations of NaOH (0.5%, 1%, 1.5% and 2%) as well as temperatures (100, 110 and 120 °C) were used for pretreatment study. At 120 °C, approximately more than 50% of delignification was observed. Moreover, subsequent enzymatic saccharification contributed 350 mg g^{-1} dry biomass of total reducing sugar (TRS) production. Interestingly, TRS was approx. fivefold higher than enzymatic saccharification of acid pretreated biomass (69.08 mg g^{-1}) as reported previously (Kataria et al., 2011) and fermentation of enzymatic hydrolysate using microbes resulted in the 0.44–0.46 g g^{-1} ethanol yield which is a high yield when compared to the other existing literature. Another advantage of alkali pre-treatment was without production of toxic compounds in comparison to acid pre-treatment method. In conclusion, Kans grass was shown as potential feedstock for biofuel production via alkali and enzymatic saccharification in contrast to acid pre-treatment.

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1. Introduction

The overall energy consumption is increasing with the growing world population and rapid industrial growth, as a result resources of non-renewable energy are depleting very fast and that results in increase the price. Bio ethanol is one of the alternative clean liquid fuels that can be produced by fermentation of sugars or simple starch such as sugarcane, maize etc. However, those sources may have adverse effect on farmland or forest diversity and as well as on soil, water and food resources. Among potential alternative bio-energy resources, lignocellulosics have been accepted for production of next generation fuel [1]. Lignocellulosic substances contain cellulose, hemicellulose and lignin. The different Sources of lignocellulosics including wood, agricultural residues, water plants, grasses, and other plant substances are well known for the starting material for bioethanol. Kans grass (*Saccharum spontaneum*) is one of the novel and potential non food source of lignocellulosic material that can be cultivated on waste lands without conflicting the food crop. This plant biomass is available throughout the year without much cultivation efforts and with low supply of water. Addition to this, it is composed of sufficient amount of holocellulose content (cellulose and hemicellulose, 64.67%) and can be utilized for bioethanol production [2]. For conversion of lignocellulosic biomass to ethanol, there are majorly three steps: pretreatment, hydrolysis (sachharification) and fermentation. Pretreatment is one of the important steps where the three dimensional cell wall is disrupted for sufficient availability of cellulose by removing lignin, which is the main obstacle for cellulase action (for monomer sugar production).Cellulose (a polymer of glucose sugar) and hemicellulose (polymer of Xylose, mannose and arabonise sugar) portion of plant biomass first depolymerised in to monomer sugars and further these monomer sugar are utilized by microorganisms (yeast and bacteria).

Two approaches have been developed in parallel for conversion of lignocelluloses to fermentable sugars that may be acid based and enzyme based. The enzyme based technology is advantageous over acid based treatment (conc. H_2SO_4) due to higher conversion efficiency, absence of substrate loss due to chemical modification, lack of inhibitory compounds production, low cost, no need of recycling of acid and the use of more moderate and non-corrosive conditions like low temperatures, neutral pH [3]. Use of biodegradable and non toxic reagents is more economical than any other method. The substrate usually requires a pretreatment process before being subjected for enzymatic breakdown which is aimed at increasing the susceptibility of cellulose to enzyme. The overall performance of cellulase enzyme is highly dependant on the residual lignin present along with cellulose.

Pretreatment by sodium hydroxide is one of the conventional methods among the all pretreatment methods that received high interest over the years. It is a low energy demanding and relatively inexpensive technique which has beet studied with various lignocellulosic materials. Sodium hydroxide disrupt the structural linkages, affect the lignin barriers, reduce the cellulose crystallinity, and increase the cellulose accessibility by exposing up the structure and make it more accessible to the cellulase enzyme, which results in a sharp increase in sugar yield.

As cellulase enzyme production accounts for 40% of total cost in overall production of bioethanol [4], hence to reduce cost of production, on site production of crude enzyme is more viable than commercial cellulase due to their reasonable cost (exclusion of enzyme purification step), high enzyme production capacity, etc. The reduction in cost paves cost effective way for ethanol production [5]. *Trichoderma reesei* has been used for industrial cellulase production since 1960s [6]. But it was mainly used in food, pulp and paper and textile industry. Due to increase cost of fermentation process for bioethanol, cellulase production is one of the key steps for hydrolysis of the lignocellulosic materials. Several different strains have been developed since then to enhance the production of cellulase from the fungal strain *QM6a* [7] that is the first industrially used strain and nearly all strains have been obtained by mutating this strain in some way [8].

In the present investigation, dilute NaOH pretreatment was optimized for lignin removal for a novel lignocellulosic material Kans grass and delignified biomass was saccharified by using crude cellulase enzyme [2] to obtain reducing sugars, which further utilized for bioethanol production by using yeast strains *Pichia stipitis* and *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Kans grass biomass

Kans grass biomass was obtained from various parts of Uttrakhand, India was chopped into small pieces (0.5–1.0 cm), washed and dried at 60 °C overnight and finally stored at room temperature. As plant biomass is composed of carbohydrate (cellulose and hemicelluloses), lignin and other components hence composition estimation for cellulose, hemicelluloses, lignin, ash as well as moisture content was done on dry weight basis [2].

2.2. Dilute NaOH pretreatment

Dilute alkali (NaOH) pretreatment of Kans grass biomass (5% w/v) was done by different sodium hydroxide concentrations (0.5, 1, 1.5 and 2% w/v) for variable residence time (30, 60, 90 and 120 min) at different temperatures (100, 110 and 120 °C). After pretreatment, solid residue obtained was separated from the liquid portion. The liquid fraction (hydrolysate) was kept at −20 °C for the analysis of total reducing sugars, xylose. However, the collected solids were washed with distilled water to obtained neutral pH, dried at 60 °C. These portions of the solid residues were used for determination of total residual solid, holocellulose and lignin content. The reduction in lignin following pretreatment was calculated based on the initial dry-weight of lignin in the untreated sample and the dry-weight of lignin in the remaining solids after pretreatment. All experiment were carried out in triplicates.

2.3. Cellulase enzyme production

The crude enzyme used in this hydrolysis study was obtained from *T. reesei*, (procured from NCIM) was cultivated in enzyme production media by inoculating the fungal spores at 28 °C at pH 5 for 8 days with cellulose as carbon source (optimized condition, [2]). The fungal biomass was separated from media by centrifugation (20 min at 3220 g), the clear supernatant was analyzed for the CMCase, xylanase and total cellulase activity (filter paper activity) and stored at −80 for further use in saccharification step.

2.4. Enzymatic saccharification/hydrolysis of (NaOH) pretreated Kans grass biomass

Dilute NaOH pre-treatment as carried out with different NaOH concentrations, time and temperatures and the conditions were optimized for maximum removal of lignin. At 120 °C the maximum removal of lignin (14–79.30%) was observed with solubilization of sugars. As more the lignin was removed the solubilization of total reducing sugars was also observed thereby the reduction of holocellulose content of the biomass. Hence the solid residue remained (all NaOH concentration and pretreatment duration at 120 °C) after