

Pretreatment studies on wheat straw and rice straw

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Abstract

Pretreatment is a necessary process for utilization of lignocellulosic materials to obtain ultimately high degree of fermentable sugars. In this work comparative study of chemical and microbiological treatments on the wheat and rice straw is carried out. Alkaline and acid treatments were selected as chemical treatments. Six fungi viz, *Aspergillus niger* A. awamori, *Trichoderma reesei*, *T. viride*, *Phanerochaete chrysosporium*, *Pleurotus sajor-caju* were used for microbial treatments. Microbial treatment resulted in release of more amount of reducing sugars (A. awamori treated wheat straw 18mg/g, rice straw 16mg/g) compared to chemical treatments (acid treated wheat straw 8mg/g, rice straw 10mg/g). Among the chemical treatments, alkali treatment gave good results (wheat straw 10mg/g, rice straw 11mg/g).

INTRODUCTION

Cellulose is the most abundant carbohydrate polymer in wastes from forest products agriculture, fruits, and vegetables. Agricultural wastes and in fact all lignocellulosics can be converted into products that are of commercial interest such as ethanol, glucose, single cell protein (Solomon et al., 1999). In these waste products, the polysaccharides, cellulose and hemicellulose are intimately associated with lignin in the plant cell wall. The lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further transformation processes (Kadam, 2000). Therefore, the pretreatment is a necessary process for utilization of lignocellulosic materials to obtain ultimately high degree of fermentable sugars. Pretreatment affects the structure of biomass by solubilizing hemicellulose, reducing crystallinity and increase the available surface area and pore volume of the substrate. There are numerous pretreatment methods or combinations of pretreatment methods available. In general, pretreatment techniques can be grouped into three categories: physical, chemical, and biological. Physical pretreatment methods include comminution, steam explosion etc. The most common chemical pretreatment methods used for cellulosic feedstocks are dilute acid, alkaline, organic solvent, ammonia, sulfur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the enzymes. Biological pretreatments are sometimes used in combination with chemical treatments to solubilize the lignin in order to make cellulose more accessible to hydrolysis and

fermentation (Ayhan Demirbas, 2005). Pretreatment of cellulosic biomass in a cost-effective manner is a major challenge of cellulose-ethanol technology research and development. In the present work comparative study of chemical and biological treatments on wheat straw and rice straw are conducted.

MATERIALS AND METHODS

Substrates: Wheat straw and rice straw were obtained from local fields of Davanagere district. Each raw material was powdered and sieved into a 1mm sieve and this was used as carbon source.

Isolation of fungi: Screening of fungi capable of degrading cellulose was done (Abdul et al 1999) from the soil of local paddy and wheat fields and fungi of interest were selected after proper identification through manuals and confirmation by MTTCC, Chandigarh.

ACID HYDROLYSIS AND SACCHARIFICATION

One hundred gram of dried sample was weighed into 2000 ml conical flasks and 1000 ml of sulphuric acid were added to the conical flask. The flasks were covered with aluminium foil and heated for two hours on flame. The flask was allowed to cool and filtered through Whatman No.1 filter paper. The pH was adjusted to 4.5 with 0.4 M sodium hydroxide (Humphrey and Caritas 2007).

ALKALINE HYDROLYSIS

One hundred gram of dried sample was weighed into 2000

ml conical flask and 1000 ml of 0.25 M sodium hydroxide solution was added to the conical flask. The flask was left for one hour, after which the mixture was neutralized with 0.1 M hydrochloric acid (HCL) to a pH of 4.5. The flask was allowed to cool at room temperature and filtered through Whatman No.1 filter paper.

CULTURE MEDIUM

Mandles medium was prepared by adding (g/l-1): urea 0.3, (NH₄)₂SO₄ 1.4, KH₂PO₄ 2, CaCl₂ 0.3, MgSO₄·7H₂O 0.3, bacto peptone 0.75, and yeast extract 0.25. Trace elements were also added, using a 1% (v/v) solution of salts(ml-1):FeSO₄·7H₂O 0.5, MnSO₄ 0.16, ZnSO₄ 0.14, CoCl₂ 2. pH was adjusted to 5.5-6.0 before sterilization (Bollok and Reczey 2000).

CULTURE CONDITIONS

10g /l of each residue was taken in conical flask containing 200ml of Mandle’s medium. The conical flasks were plugged with cotton and sterilized at 15lbs per sq.inch for 20 minutes. Each flask was inoculated with 4-5 discs of different fungi. These flasks were incubated at room temperature for 5days on an orbital shaker. After five days mycelium was separated by filtration through Whatman filter paper No.1. The filtrate was used for further studies (Harpreet et al., 1998).

Determination of total carbohydrate: The carbohydrate content of untreated and pretreated raw materials in the culture broth was measured by phenol sulphuric acid method (Thimmaiah, 1999) with glucose as standard.

Determination of reducing sugars: Reducing sugars in untreated and pretreated raw materials in the culture broth were determined by dinitrosalicylic acid (DNS) method (Miller, 1959) with glucose as standard.

RESULTS AND DISCUSSION

Initial composition of the raw materials was determined (Table 1). Pretreatment of raw materials makes it accessible for microbial treatment. So chemical treatments were conducted (table 2). Results of fungal treatments are shown in table 3.

Figure 1

Table 1 Initial composition of raw materials

Sl. No	Raw material	Total sugar (m ^g g ⁻¹)	Reducing sugar (m ^g g ⁻¹)	Non reducing sugar (m ^g g ⁻¹)	Moisture (%)	Total solids (%)	Organic carbon (%)	N ₂ (%)
1	Wheat straw	0.4	0.0175	0.685	5.265	94.735	36.18	0.126
2	Rice straw	0.7	0.0175	0.382	1.83	98.62	36.93	0.448

Figure 2

Table 2 Results of chemical treatment

Sl. No	Substrate	Total sugar (m ^g g ⁻¹)		Non reducing sugar (m ^g g ⁻¹)		Reducing sugar (m ^g g ⁻¹)	
		Acid	Alkali	Acid	Alkali	Acid	Alkali
1	Wheat straw	12	15	4	5	8	10
2	Rice straw	15	15	5	4	10	11

Figure 3

Table 3 Results of fungal treatment

Sl. No	Treatment	Wheat straw			Rice straw		
		Total sugar	Non reducing sugar	Reducing sugar	Total sugar	Non reducing sugar	Reducing sugar
1	Untreated after autoclaving	15	5	10	25	11	14
2	<i>Aspergillus niger</i>	18	3	15	28	12	16
3	<i>Aspergillus awamori</i>	20	2	18	29	13	16
4	<i>Trichoderma reesei</i>	17	3	14	27	12	15
5	<i>Trichoderma viride</i>	18	6	12	29	14	15
6	<i>Phenerochaete chrysosporium</i>	17	3	14	29	14	15

Autoclaving for sterilization has affected and resulted in increase in sugar release. With fungal treatment still increase in the yield of sugars was observed. Treatments with A. awamori and A. niger has shown better yield of reducing sugar compared to any of the two chemical treatments. Because pretreatment of cellulosic biomass in a cost-effective manner is a major challenge of cellulose-ethanol technology research and development, with the results of present work we have shown that microbial treatment, which is less costly and with less severe physical conditions, gives better yield of sugars from cellulosic residues like wheat straw and rice straw.

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