Biocatalysis and Agricultural Biotechnology 8 (2016) 66–72

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Evaluating biological pretreatment as a feasible methodology for ethanol production from paddy straw

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ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form
18 July 2016
Accepted 9 August 2016
Available online 12 August 2016

Keywords:
Paddy straw
Enzymatic hydrolysis
Steam pretreatment
Biological pretreatment
T. hirsuta
S. cerevisiae

ABSTRACT

Bioethanol has been recognized as a promising contemporary fuel. One of the most abundant renewable resources for bioethanol production is paddy straw with high carbohydrate content. A pretreatment step disintegrates the recalcitrant lignocellulosic structure in biomass, which facilitates the access of hydrolytic enzymes to the glucan macrostructure. Biological pretreatment is an eco-friendly alternative to harsh thermo-chemical pretreatment methodologies. In this study, paddy straw (rice variety Pusa 2511) was subjected to biological pretreatment with white-rot fungus, *Trametes hirsuta* and simultaneously with steam pretreatment at 121 °C. Resultant saccharification efficiencies of differentially pretreated paddy straw were compared to evaluate biological pretreatment. After pretreatment cellulose content in steam treated paddy straw was 39.5%, whereas for biological it was 37.6% and respective lignin contents were 14.2% and 4.7%. Lignin removal was substantially higher in biological pretreatment than steam pretreatment. The saccharification yields of biological pretreatment were at par with steam pretreated paddy straw. Highest saccharification efficiency was observed after 24 h, at 2% glucan loading, for both biological (76.5%) and steam pretreatment (74.1%). Maximum production of sugar (52.91 g L^{-1}) was observed in biologically pretreated biomass at 10% glucan loading after 24 h. Fermentation of biomass hydrolysates with *Saccharomyces cerevisiae*, showed low ethanol production from biologically (0.86 g L^{-1}) as well as steam pretreated biomass (1.13 g L^{-1}) with fermentation efficiency ranging from 26 to 52%, suggesting presence of inhibitory factors necessitating detoxification of hydrolysates. This study, established biological pretreatment as feasible method for pretreatment and higher sugar yields.

1. Introduction

Due to increasing demand of energy worldwide and dwindling petroleum supplies, the search of a sustainable and economically viable alternative energy source has become imperative. Renewable biofuels obtained from biomass, have potential to reduce dependence on oil, greenhouse gas emissions, help in climate change mitigation and develop rural economy. This alternative source of energy is very promising since it is inexpensive, renewable and ecofriendly, subject to which fundamental research is being focused on the utilization of lignocellulosic biomass of plant residues that are not food sources. Conventionally, such residues are produced on a much larger scale, than the edible entity (Lee et al., 2015). In fact, one of the best strategies adopted by Brazil (highest bioethanol producer around the globe) is the production of bioethanol from non-food biomasses (Vaz, 2014).

Liquid transport fuels are a substantial fraction of energy sources consumed. Blending/ replacing conventional liquid fuels with liquid biofuels i.e. bio-alcohols and biodiesel is a promising approach to cut down dependence on petroleum. However, this technologically proven energy source is facing difficulty in commercialization due to prohibitive processing costs. There are significant opportunities for improvements at every step in the overall conversion process of biomass to ethanol.

Innovative approaches can help in reducing costs through use of better quality feedstocks, better pretreatment technologies giving lesser byproducts, more active enzymes and more versatile fermenting organisms. Diverse biomass substrates can be used for energy production. In India, bioethanol is primarily produced from sugarcane molasses and sweet sorghum but due to high industrial demand, blending it with petrol is not feasible. Therefore, bioethanol production from lignocellulosic agriculture-based residues should be developed by incorporating more crop, forestry residues and herbaceous perennial feedstocks that are native to the particular land.

According to a recent study conducted by National Institute for Interdisciplinary Science and Technology (NIIST), India, paddy

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http://dx.doi.org/10.1016/j.bcab.2016.08.006
1878-8181/

straw is the major agro-residue (112 MMT) generated annually. Despite of this, management of this humungous amount of paddy straw is the cause of major concern as proper infrastructure for storage purposes is unavailable. Therefore, a large part of this straw is burnt in open fields, which not only surges the emissions of greenhouse gases, but also affects soil health (Gadde et al., 2009). As a lignocellulosic biomass, rice straw predominantly comprises of 35–55% cellulose, 20–40% hemicellulose and 10–25% lignin on weight basis (Sukumaran et al., 2010; Ghosh and Ghose, 2003). Cellulose, hemicellulose and lignin form a complex matrix, thereby forming the structural framework of the plant. These lignin and carbohydrate complexes have to be disintegrated and polysaccharides hydrolyzed into their subsequent monomers, which are converted into biofuels and other value added molecules. The removal of lignin is the prime step of conversion, as it forms a protective covering and restricts the enzymatic hydrolysis of cellulose and hemicellulose complexes (Chaturvedi and Verma, 2013; Abdelnur et al., 2014). Hence, for exposing the cellulose macrostructure to facilitate enzyme activity for hydrolysis, the selection of pretreatment method to be employed becomes imperative.

A wide range of thermal, mechanical and chemical pretreatment methods and their combinations have been reported (Hendriks and Zeeman, 2009; Saritha et al., 2012). An ideal method of pretreatment does not require size reduction of biomass, increases the yield of monomeric sugars by making the lignocellulosic biomass susceptible for quick hydrolysis, should form no or limited inhibitory compounds and reduce the energy demands and operational costs (Gupta and Verma, 2015). Without any pretreatment, hydrolysis of lignocellulosic biomass may yield less than 20% of total monomeric sugars, while after pretreatment it may reach up to ~90% (Alizadeh et al., 2005). Currently, various pretreatment strategies are available with their respective pros and cons. The efficiency of a pretreatment method also depends on the physical structure, chemical composition of the biomass and treatment conditions. Physical and chemical pretreatment methods involve varying strategies including acid, alkali, steam explosion, radiation or an amalgamation of these processes. These strategies demand specialized equipment and machinery which leads to abundant energy consumption and harsh conditions lead to production of inhibitory compounds that might hinder enzymatic hydrolysis and affect the efficiency of fermentation (Mosier, 2005).

Biological pretreatment, on the other hand, utilizes metabolites of microorganisms in nature for deconstruction of biomass and ethanol production. Despite of drawbacks like contamination and the long time period requirement, it is a promising technology due to its several advantages like eco-friendly and economically viable strategy for enhancing enzymatic saccharification rate (Mosier, 2005; Mohanram et al., 2013; Sindhu et al., 2016). Biological pretreatment does not require a high amount of energy and can be carried out under normal conditions. Lignin and hemicellulose present in agricultural waste are degraded by microorganisms like brown and white-rot fungi as they possess lignin-degrading abilities (Liong et al., 2012). A study reported (Fan et al., 1987) that brown-rot fungi, mainly targets cellulose, whereas white-rot attacks both cellulose and lignin making them the most effective basidiomycetes for biological pretreatment process. It was also seen that several white rot fungi have the capacity of selective lignin degradation and have been used for lignase production (Lee, 1997). Enzymes such as peroxidases and laccases facilitate lignin degradation through white-rot fungi (Kumar et al., 2009; Wan and Li, 2011). High delignification efficiencies have been observed with several white-rot fungi such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercoreus*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus*, *Trametes hirsuta* and *Pleurotus ostreatus* on diverse lignocellulosic biomasses (Saritha et al., 2012; Wan and Li, 2012). Also, there is no need for recycling of chemicals and no toxic compounds are released into the environment as no chemicals are used in this process (Sindhu et al., 2016). This study was aimed to assess the feasibility of ethanol production from biologically pretreated paddy straw vis-à-vis steam pretreated and determine optimal loadings for higher sugar and ethanol production.

Table 1
Enzymatic hydrolysis of α-cellulose and sugar yields.

Substrate	Enzyme	Sugar released (g L^{-1})		
α-Cellulose (%)	Accellerase (μL)	24 h	48 h	72 h
0.5	25	0.36 ± 0.04	0.49 ± 0.02	0.67 ± 0.18
1	50	0.26 ± 0.05	2.65 ± 0.03	2.4 ± 0.10
2	100	12.0 ± 0.18	19.42 ± 0.12	16.05 ± 0.02
5	250	57.93 ± 0.09	55.44 ± 0.15	55.97 ± 0.39
7	350	58.10 ± 0.09	61.3 ± 0.10	60.31 ± 0.34
10	500	59.98 ± 0.26	72.24 ± 0.15	87.23 ± 0.15
12	600	93.68 ± 0.24	102.62 ± 0.07	100.72 ± 0.15
20	1000	135.96 ± 0.10	146.93 ± 0.06	146.22 ± 0.23
SE_m (±)				6.83
CD@5%				18.85

2. Materials and methods

2.1. Substrates

Dried, ground paddy straw of the aromatic rice variety Pusa 2511 (PusaSugandh 5), cultivated in the farms of Indian Agricultural Research Institute, New Delhi, was used as substrate. α-Cellulose from Sigma Aldrich, was used as pure cellulosic substrate.

2.2. Microorganisms

Trametes hirsuta MTCC 136 used for biological pretreatment of paddy straw, was procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The fungal culture was grown on Potato Dextrose Agar at 30 ± 2 °C (see additional data Fig. 1; 1a) and stored in refrigerator. It was periodically sub-cultured.

Saccharomyces cerevisiae strain LN used in fermentation experiments was obtained from divisional culture collection of Division of Microbiology, IARI, New Delhi, India. It has ethanol production potential (Nain and Rana, 1987) and is maintained on MGYP (Malt extract: 3 g L^{-1}, Glucose: 10 g L^{-1}, Yeast extract: 3 g L^{-1}, Peptone: 5 g L^{-1}) broth. The yeast strain was grown on MGYP broth at 28 ± 2 °C. It was maintained and stored in refrigerator on MGYP slants and periodically sub-cultured.

2.3. Cellulase Enzyme

A commercially available cellulase enzyme complex Accellerase® 1500 supplied by Genencor, was used for saccharification. The cellulolytic activity of enzyme was 29 FPU (filter paper units) and endoglucanase activity ~1746 CMCase U g^{-1} (carboxymethylcellulase) as assayed by methods described by Ghose (Ghose, 1987). The reducing sugars released were measured by the DNS assay (Miller, 1959).