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Research Paper

Effects of pretreatment conditions and post–pretreatment washing on ethanol production from dilute acid pretreated rice straw

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Rice straw pretreatment was examined in a full factorial study at temperatures of 120 °C and 160 °C and 0% and 1% [H_2SO_4]. Pretreatment efficacy was assessed by measuring hydrolysate composition and reducing sugar yield after enzymatic hydrolysis. Pretreatment with 1% [H_2SO_4] and 160 °C yielded the highest amount of reducing sugar, 259 mg g^{-1} [dry matter], during enzymatic hydrolysis corresponding to 57% glucose conversion based on cellulose content of the pretreated solid. Under this pretreatment condition hydroxymethylfurfural and furfural were 0.19 and 0.68 g l^{-1}, respectively. Rice straw pretreated with 1% [H_2SO_4] at 160 °C was subjected to simultaneous saccharification and fermentation (SSF) using either *Saccharomyces cerevisiae* D_5A or recombinant *Escherichia coli* KO11. Solid and hydrolysate separation and washing techniques were evaluated for their effect on ethanol production during SSF. Pretreated rice straw without liquid–solid separation or washing had the highest 7-d ethanol yield of 0.2 g [ethanol] g^{-1} [dry substrate] for *E. coli* KO11. This finding has economic implications on the processing of rice straw to bioethanol.

1. Introduction

In recent years there has been an interest in ‘second generation’ biofuels which originate from biomass that does not have competing value as a food source; such biomass is often produced in great quantity and on a more regular basis. Lignocellulosic biomass such as grasses, agricultural residues, industrial wastes, and food processing byproducts are a few examples from which second generation biofuels can be derived.

Rice production worldwide generates the highest amount of agriculture residue each year with as much as 1.5 kg straw produced per kg of rice crop (Kim & Dale, 2004). Because of this

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Nomenclature

CBU	cellobiase unit
DI	deionised
DM	dry matter
FPU	filter paper unit
HMF	5-hydroxymethylfurfural
HPLC	high-performance liquid chromatography
LB	Luria–Bertani
NREL	National Renewable Energy Laboratory
OD	optical density
PTFE	polytetrafluoroethylene
SSF	simultaneous saccharification and fermentation
YP	yeast–peptone
YPD	yeast–peptone–dextrose

large production scale, rice straw is an attractive source for energy needs; each year as much as 100 billion gallons of ethanol could be produced worldwide from over 600–900 million tonnes of rice straw (FAOSTAT, 2007; DOE, 2009). Rice straw can gain additional value as a potential fuel. Its use for biofuels can also reduce some negative agricultural practices. For instance, rice straw contains a substantial amount of lignin; instead it is disposed of by burning in the open fields. Field burning of rice straw is a concern to human health due to air pollutant emissions (Pathak, Singh, Bhatia, & Jain, 2006). If rice straw is directly re-incorporated into the soil it is shown to increase the likelihood of crop diseases (Gadde, Bonnet, Menke, & Garivait, 2009).

Rice straw, as a lignocellulosic biomass, is primarily composed of cellulose, hemicellulose and lignin. Typically, the steps to ethanol from lignocellulose begin with pretreatment, followed by fermentable sugar production through addition of hydrolytic enzymes, finishing with simultaneous or separate fermentation. Dilute acid pretreatment is one approach which subjects biomass to temperatures in the range of 160–220 °C, with sulphuric or hydrochloric acid acting to hydrolyse a large portion of hemicellulose for periods in the range from seconds to minutes (Mosier et al., 2005). Although, dilute acid pretreatment aids the removal of the hemicellulose-lignin matrix, it can produce an assortment of inhibitory compounds along with sugars in the hydrolysate. These compounds may include organic acids, furans derived from carbohydrate degradation, and phenolic compounds derived from lignin (Palmqvist & Hahn-Hagerdal, 2000). These compounds act in a variety of ways to reduce enzymatic activity, inhibit cell growth and reduce ethanol yields during fermentation. There are methods to eliminate such inhibitors after pretreatment and prior to fermentation. Extensive washing is sometimes included to remove inhibitors, although sugars may be extracted as well (Rajan & Carrier, 2014; Zheng et al., 2013). It could be more economical to integrate the liquid fraction from the pretreatment step into fermentation; however it would be necessary to account for both the amount of sugars and inhibitors solubilised, in addition to understanding the composition of the pretreated solid fraction. Acknowledging this issue, recombinant microorganisms have been engineered such that cell growth is maintained in the presence of inhibitors while having increased ethanol yield from fermentation (Dien, Cotta, & Jeffries, 2003).

In this study, we explored four different pretreatment conditions on rice straw and determined the effectiveness of each pretreatment based upon the sugars solubilised after pretreatment and reducing sugar yields after enzymatic hydrolysis. The rice straw pretreated under the optimal conditions was used as the substrate for a batch simultaneous saccharification and fermentation (SSF) process, which was carried out for one week. Two fermenting organisms were compared: *Escherichia coli* KO11 and *Saccharomyces cerevisiae* D$_5$A. Additionally, two methods to remove pretreatment inhibitors to improve ethanol yield during SSF were tested: (1) separation of pretreatment solid and hydrolysate, and (2) water washing. These methods were evaluated based on the final ethanol production after SSF.

2. Materials and methods

2.1. *Biomass preparation*

Fresh rice straw (*Oryza sativa* L., California rice M206) was collected a few hours after rice harvest from a field in the Central Valley of California (38°43′21″N and 121°35′39″W). Straw was dried by forced aeration at room temperature for 1 week to 6.3% moisture content. Air-dried rice straw was milled through a 2-mm mesh screen using a knife mill (Pulverisette 19, Fritsch, Germany). Straw particles were stored in plastic bags at room temperature until used for pretreatment.

The chemical composition of dried rice straw is shown in Table 1. The major components included carbohydrates, which were 61.4% (dry basis) with 40.4% consisting exclusively of glucan. The ash content was 12.0%, whereas the lignin was 22.1% with 16.2% defined as acid-insoluble and 5.9% as acid-soluble.

2.2. *Dilute acid pretreatment*

The methods used for pretreatment, enzymatic hydrolysis and SSF were similar to the methods in our previous work (Zheng et al., 2013). Dilute acid pretreatment was performed in a 1-l reactor (Carpenter 20 Cb-3, Parr Co., Moline, IL, USA), equipped with an impeller mixer. A full factorial experiment was run containing four treatments at 120 °C and 160 °C with

Table 1 – Chemical composition of rice straw.

Component	Percentage (%, dry basis)
Glucan	40.4
Xylan	14.1
Galactan	2.7
Arabinan	4.2
Acid-insoluble lignin	16.2
Acid-soluble lignin	5.9
Ash	12.0