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Pilot scale conversion of wheat straw to ethanol via simultaneous saccharification and fermentation ☆

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HIGHLIGHTS

- Conversion of wheat straw to ethanol was scaled up at pilot scale.
- Dilute acid pretreated wheat straw was bioabated by growing a fungus aerobically.
- Recombinant bacterium fermented all sugars to ethanol.
- Maximum ethanol produced from 124 g wheat straw was 36 g in 83 h.
- Ethanol yield was 0.29 g/g wheat straw which is 86% of theoretical ethanol yield.

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ABSTRACT

The production of ethanol from wheat straw (WS) by dilute acid pretreatment, bioabatement of fermentation inhibitors by a fungal strain, and simultaneous saccharification and fermentation (SSF) of the bioabated WS to ethanol using an ethanologenic recombinant bacterium was studied at a pilot scale without sterilization. WS (124.2 g/L) was pretreated with dilute H_2SO_4 in two parallel tube reactors at 160 °C. The inhibitors were bio-abated by growing the fungus aerobically. The maximum ethanol produced by SSF of the bio-abated WS by the recombinant *Escherichia coli* FBR5 at pH 6.0 and 35 °C was 36.0 g/L in 83 h with a productivity of 0.43 g L^{-1} h^{-1}. This value corresponds to an ethanol yield of 0.29 g/g of WS which is 86% of the theoretical ethanol yield from WS. This is the first report on the production of ethanol by the recombinant bacterium from a lignocellulosic biomass at a pilot scale.

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1. Introduction

Ethanol is the most dominant biofuel. In the USA, nearly 200 operating plants churned out an estimated 13.3 billion gallons of ethanol from corn starch in 2013 (2014 Ethanol Industry Outlook, www.ethanolrfa.org). Various agricultural residues [corn stover, wheat straw (WS), rice straw, barley straw, sugar cane bagasse], processing byproducts (corn fiber, rice hulls), and energy crops (switchgrass, miscanthus) are available as low cost lignocellulosic feedstocks for conversion to fuel ethanol (second generation biofuel). WS is one of the most abundant agricultural residues in the world. The average yield of WS is 1.3–1.4 kg/kg of wheat grain (Montane et al., 1998). The world production of wheat grain in 2013/14 is estimated to be 683 million metric tons (http://www.igc.int/downloads/gmrsummary/gmrsumme.pdf). WS contains 35–45% cellulose, 20–30% hemicellulose, and 8–15% lignin. This makes WS an attractive feedstock to be converted to ethanol and other value-added products.

The production of ethanol from WS generally involves four main steps – feedstock pretreatment, enzymatic saccharification, fermentation, and product recovery. Integration of two or more process steps is important for simplification of the process and reduction of production cost. To this effect, simultaneous saccharification and fermentation (SSF) of the pretreated lignocellulosic feedstock is considered to be an ideal integrated process for ethanol production. It offers distinct advantages over separate hydrolysis and fermentation (SHF) in the production of ethanol from lignocellulosic feedstock. It can improve the ethanol yield by eliminating end-product inhibition of cellulose hydrolysis. The microorganism can utilize the sugars for growth and ethanol

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production as they are formed. Moreover, SSF does not require separate reactors for enzymatic saccharification and fermentation of generated sugars to ethanol. There are a number of studies available related to SSF of pretreated lignocellulosic biomass using *Saccharomyces cerevisiae* at 30–35 °C (Alfani et al., 2000; Ohgren et al., 2006, Olofsson et al., 2008; Tomas-Pejo et al., 2008; Saha et al., 2013).

WS, upon pretreatment and enzymatic saccharification, produces a mixture of pentose (xylose and arabinose) and hexose sugars (glucose and galactose) (Saha, 2003). The utilization of all sugars generated from WS is essential for economical production of ethanol (Saha, 2004). The conventional ethanol fermenting yeast (*S. cerevisiae*) or bacterium (*Zymomonas mobilis*) cannot ferment xylose and arabinose to ethanol. A number of recombinant microorganisms such as *Escherichia coli*, *Klebsiella oxytoca*, *Z. mobilis*, and *S. cerevisiae* have been developed over the last 25 years with a goal of fermenting both hexose and pentose sugars to ethanol (Saha, 2003). Our research unit has developed a recombinant *E. coli* (strain FBR5) that ferments mixed multiple sugars to ethanol (Dien et al., 2000). The strain carries the plasmid pLOI297, which contains the genes for pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase (*adh*) from *Z. mobilis* necessary for efficiently converting pyruvate into ethanol (Alterthum and Ingram, 1989). The plasmid also contains the genes for ampicillin and tetracycline resistance. It selectively maintains the plasmid when grown anaerobically and is capable of fermenting both hexose and pentose sugars to ethanol. In our previous papers, we reported about the production of ethanol from WS by dilute acid, lime, alkaline peroxide and microwave pretreatments, enzymatic saccharification, and fermentations of the hydrolyzates by both SHF and SSF using this recombinant *E. coli* strain FBR5 (Saha et al., 2005, 2008, 2011a,b, 2013; Saha and Cotta, 2006, 2007, 2011) at laboratory scale (350 ml in a 500 ml fleaker). The minimum and maximum ethanol produced in these studies were 13.0 ± 2.0 and 41.8 ± 0.0 g/L from pretreated WS (86–150 g/L) which are equivalent to 0.17 and 0.28 g ethanol per g straw, respectively. The yields varied between 0.37 and 0.50 g per g of available sugars depending on the type of pretreatment used. The fermentation time also varied greatly from 17 to 136 h which was also highly dependent on the type of pretreatment and the inhibitory compounds present in the pretreated hydrolyzate. We also studied the long term performance of this recombinant bacterium in a series of continuous culture runs (16–105 days) using alkaline peroxide pretreated and enzymatically saccharified wheat straw hydrolyzate (WSH) as feedstock (Saha and Cotta, 2011). During these studies, no loss of ethanol productivity was observed which indicates that the strain showed stability and robustness in performance. We were thus interested to study the ethanol production from WS at a pilot scale by SSF. In this paper, we report the production of ethanol from WS by the recombinant bacterium at pilot scale.

2. Methods

2.1. Materials

WS, supplied by Dr. Matthew Digman, U.S. Dairy Forage Research Center, Madison, WI, was dried in a forced-air oven at 55 °C for 24 h and milled in a hammer mill to pass through a 1.27 mm screen. The milled WS was stored at room temperature. Celluclast 1.5 L (cellulase) and Novozym 188 (β-glucosidase) were purchased from Brenntag Great Lakes, Milwaukee, WI, USA. Aminex HPX 87P column (300 × 7.8 mm), Aminex HPX 87H column (300 × 7.8 mm), De-ashing cartridge (30 × 4.6 mm), Carbo-P micro-guard cartridge (30 × 4.6 mm), and Cation H micro-guard cartridge (30 × 4.6 mm) were purchased from Bio-Rad Laboratories, Inc., Hercules, CA, USA. Membrane Filter Unit (0.2 μm) was purchased from Nalge Nunc Int., Rochester, NY, USA. Lactoside V™ (Virginiamycin) was supplied by Lallemand Biofuels and Distilled Spirits, Milwaukee, WI, USA. Yeast extract and casein peptone type M were obtained from Marcor Development Corp., Carlstadt, NJ, USA. Biospumex 153K antifoam was from Cognis Corp., Tucson, AZ, USA. Hydrated lime was obtained from Mississippi Lime Co., St. Louis, MO. All other chemicals used were of standard analytical grades.

2.2. Enzyme assays

The cellulase activity in terms of filter paper activity was assayed and expressed as filter paper unit (FPU) by the procedure described by Ghose (1987). Carboxymethyl cellulase (CMCase), β-glucosidase, xylanase, β-xylosidase, α-L-arabinofuranosidase, and ferulic acid esterase activities were assayed by the procedures described previously (Saha et al., 2005). All enzyme assays were performed at pH 5.0 and 45 °C and the activities were expressed in terms of international units (IU, μmole product formed per min).

2.3. Dilute acid pretreatment of wheat straw

Two steam heated jacketed parallel tube reactors (each 10 L working volume) were used. Milled WS (124.2 g/L, dry basis) was slurried in 0.75% (v/v) H_2SO_4 and pretreated in the tube reactors at 160 °C for 20 min holding time. The heating and cooling times of the reactors were around 15 min each. The reactors were cooled using chilled tap water. One set of pretreatment generated 20 L of pretreated material.

The severity factor (SF) for a gradually heating, holding and gradually cooling process was determined by the following equation (Rubio et al., 1998):

$$\mathrm{SF} = \mathrm{Log}[R_0] = \log_{10}\left\{\int_0^t \exp\frac{T(t) - 100}{14.75}\,\mathrm{dt}\right\}$$

where t is the residence time in min and T is the temperature of pretreatment in °C at one min residence intervals during heating, holding and cooling. This is necessary due to long heating and cooling times. R_0 originally designated by Overend and Chornet (1987) as reaction ordinate or severity parameter is commonly used to represent SF.

The combined severity factor (CSF) for dilute acid pretreatment takes into account of temperature, time and acid concentration (pH) and is calculated using the following equation (Nguyen et al., 2000):

$$\mathrm{CSF} = \mathrm{Log}[R_0] - \mathrm{pH}$$

where pH of the reaction mixture for use in CSF was measured after pretreatment.

The pH of the pretreated WS was adjusted to 6.5 using commercial grade hydrated lime.

2.4. Bioabatement of dilute acid pretreated wheat straw hydrolyzate

The fungus *Coniochaeta ligniaria* NRRL 30616 was used to bioabate the dilute acid pretreated WS (Nichols et al., 2005). The detailed procedure for biobatement of pretreated WS by the fungal strain was described previously (Saha et al., 2011a). For bioabatement at 2 L scale, the seed culture was grown aerobically in a 500 ml baffled flask containing 125 ml of the liquid portion of pretreated WS, 0.1% $(NH_4)_2SO_4$ and 2 ppm Virginiamycin at pH 6.5, 30 °C and 225 rpm. For bioabatement at pilot scale, the seed culture was grown in a 10 L fermentor (Biostat B, B. Braun Biotech., Inc., Sartorius Stedium North America, Inc., Behima, NY) with 6 L of the liquid portion of pretreated WS, 0.1% $(NH_4)_2SO_4$ and 2 ppm