Ethanol production from wheat straw by recombinant *Escherichia coli* strain FBR5 at high solid loading

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**A B S T R A C T**

Ethanol production by a recombinant bacterium from wheat straw (WS) at high solid loading by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) was studied. The yield of total sugars from dilute acid pretreated WS (150 g/L) after enzymatic saccharification was 86.3 ± 1.5 g/L. The pretreated WS was bio-abated by growing a fungal strain aerobically in the liquid portion for 16 h. The recombinant *Escherichia coli* strain FBR5 produced 41.1 ± 1.1 g ethanol/L from non-abated WS hydrolyzate (total sugars, 86.6 ± 0.3 g/L) in 168 h at pH 7.0 and 35 °C. The bacterium produced 41.8 ± 0.0 g ethanol/L in 120 h from the bioabated WS by SHF. It produced 41.6 ± 0.7 g ethanol/L in 120 h from bioabated WS by fed-batch SSF. This is the first report of the production of above 4% ethanol from a lignocellulosic hydrolyzate by the recombinant bacterium.

**1. Introduction**

Ethanol is a renewable oxygenated fuel. In the USA, the production of fuel ethanol from corn starch reached 13 billion gallons in 2010, replacing the gasoline produced from some 445 million barrels of imported oil. In many countries including the USA, wheat straw (WS) is an abundant byproduct from wheat production. The average yield of WS is 1.3–1.4 kg/kg of wheat grain (Montane et al., 1998). The world production of wheat in 2011 is estimated to be 672 million metric tons (http://www.igc.int/downloads/gmr-summary/gmrsummary.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 steps – feedstock pretreatment, enzymatic saccharification, fermentation, and product recovery. Further, WS, upon pretreatment and enzymatic saccharification, produces a mixture of sugars such as glucose, xylose, arabinose, and galactose (Saha, 2003).

The utilization of all the sugars generated from WS is essential for the economic production of ethanol (Saha, 2004). The conventional ethanol fermenting yeast (*Saccharomyces cerevisiae*) or bacteria (*Zymomonas mobilis*) cannot ferment pentose sugars to ethanol. One major technical hurdle to converting any lignocellulosic feedstock to ethanol is developing an appropriate microorganism for fermentation of both hexose and pentose sugars. 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 can ferment 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 (Aelterthum 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.

As mentioned earlier, the conversion of any lignocellulosic feedstock to ethanol involves four steps. 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 pretreated lignocellulosic feedstock is an excellent choice for process integration. It offers certain 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 production as they are formed. Moreover, SSF does not require separate reactors for saccharification and fermentation. There are a number of studies available related to SSF of pretreated lignocellulosic biomass; most of these studies used S. cerevisiae as the fermentative organism at 30–35 °C (Alfani et al., 2000; Ohgren et al., 2007; Olofsson et al., 2008; Tomás-Pejo et al., 2008). Stirring is a significant problem at high solid loading due to mass and heat transfer problems (Pimenova and Hanley, 2003). This becomes less pronounced with fed-batch SSF, due to gradual hydrolysis of added fibers (Rudolf et al., 2005). An additional advantage with fed-batch fermentation is that the glucose level can be kept lower during cofermentation of xylose and glucose, which promotes xylose uptake (Ohgren et al., 2006).

In our previous papers, we have reported about the production of ethanol from WS by dilute acid, lime, and alkaline peroxide pretreatments, enzymatic saccharification, and batch fermentations of the hydrolyzates by both separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) using this recombinant E. coli strain FBR5 (Saha et al., 2005a, 2008; Saha and Cotta, 2006, 2007a). The minimum and maximum ethanol produced in these studies were 13.0 ± 2.0 and 22.5 ± 0.6 g/L from pretreated WS (86 g/L), respectively. The yields varied between 0.37 and 0.50 g/g 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 of the type of pretreatment and the inhibitory compounds present in the hydrolyzate. Also, we obtained a maximum 18.7 ± 0.6 g/L ethanol in 64 h from dilute acid pretreated rice hulls (150 g/L) (Saha et al., 2005b; Saha and Cotta, 2007b) and 11.9 ± 0.0 g/L in 17 h from alkaline peroxide pretreated barley straw (100 g/L) by SHF (Saha and Cotta, 2010). Recently, we 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 WSH as feedstock (Saha and Cotta, 2011). During these studies, no loss of ethanol productivity was observed which indicates that the strain showed robustness in performance. Dian et al. (2000) reported that the recombinant E. coli strain FBR5 produced a maximum of 30–42 g/L ethanol in 60 h from xylose (95 g/L) with ethanol yields of 86–92% of theoretical in batch culture. Another study indicates that the maximum ethanol that could be produced by the strain from xylose in batch culture was 43.5 g/L (Qureshi et al., 2006). Increasing the ethanol titer in the fermentation broth is crucially important for cost reduction of cellulose ethanol production due to the high energy demand for ethanol recovery by distillation (Galbe et al., 2007). An ethanol concentration of 4% (v/v) or above in the fermentation broth could be considered as a bench mark for economically viable distillation (Zacchi and Axellson, 1989). We were thus interested to increase the ethanol production level by the recombinant strain from 22.5 to 40–43 g/L. In this paper, we report the production of ethanol at the stated level by the recombinant bacterium using dilute acid pretreated WS by both SHF and fed-batch SSF.

2. Methods

2.1. Materials

WS was purchased from a local farmer. It 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. Cellulclast 1.5 L (cellulase) and Novozym 188 (β-glucosidase) were purchased from Brenntag Great Lakes, Milwaukee, WI, USA. ViscoStar 150 L (hemicellulase) was supplied by Dyadic Corp., Jupiter, FL, USA. Aminex HPX 87P column (300 × 7.8 mm), Aminex HPX 87H column (300 × 7.8 mm), De-asching 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 mm) was purchased from Nalge Co., Rochester, NY, USA. All other chemicals used were of standard analytical grades.

2.2. Enzyme assays

The cellulase activity using filter paper as substrate 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., 2005b). All enzyme assays were carried out at pH 5.0 and 45 °C and the activities were expressed in terms of international units (IU, µmol product formed/min).

2.3. Dilute acid pretreatment

Milled WS (150 g/L) was slurried in 0.75% (v/v) H2SO4 and pretreated in a rotating stainless steel reactor with infrared heating (Labomat BFA-12, Mathis USA Inc., Concord, NC) at 160 °C for 10 min. The heating and cooling times of the reactors were not considered part of the reported pretreatment time, even though the heat-up ramp took about 27 min to reach the final temperature. The cooling time was around 20 min. The reactors were rotated at 50 rpm during pretreatments for mixing.

2.4. Enzymatic hydrolysis

The enzymatic saccharification of the pretreated WS was performed by shaking slowly (100 rpm) at 45 °C for 72 h after adjusting the pH to 5.0 with 10 M NaOH and adding filter sterilized cocktail of three commercial enzyme preparations. The residual solid was separated from the liquid by centrifugation (12,000 × g, 10 min) before using the liquid portion as enzymatically saccharified WS hydrolyzate (WSH). The commercial enzyme preparations used for enzymatic saccharification contained small quantities of glucose. For simplification purpose, the quantity of glucose present in the enzyme cocktail was subtracted from the measured glucose in each case.

2.5. Bioabatement of pretreated wheat straw

The ascomycete Coniochaeta ligniaria NRRL 30616 was used to bioabate the pretreated WS (Nichols et al., 2005). The strain was grown aerobiocally at 225 rpm at pH 6.5 and 30 °C for 16 h in the liquid portion of the dilute acid pretreated WS after separating it from the solid according to the procedure described recently (Saha et al., 2011). The culture broth was then pasteurized at 60 °C for 30 min. Additional sugars (exactly in the same sugar composition related to lost sugars) were then added to the liquid portion in order to compensate for the sugar loss (5–7%) due to bioabatement before adding it back to the solid portion.

2.6. Ethanologenic bacterial strain and preparation of inoculums

Recombinant E. coli strain FBR5 was provided by Dr. Bruce S. Dien (USDA-ARS, NCAUR, Peoria, IL, USA) and was maintained in glycerol vials at −80 °C for use as a working stock. It was plated on Luria broth (LB, 10 g tryptone, 5 g yeast extract, and 5 g NaCl) containing 4.0 g xylose and 20 mg tetracycline solidified with 15 g agar/L (pH 6.5). Plates were incubated at 35 °C. Cells from a single well-isolated colony were inoculated into a 125 mL flask containing 100 mL of LB with 20 g xylose and 20 mg tetracycline/L. Cultures were incubated at 37 °C and 100 rpm for 24 h. This
grown culture was used as seed culture for fermentation experiments. The inoculum size was always 5% (v/v).

2.7. Batch and fed-batch fermentations (SHF, SSF) with recombinant E. coli FBR5

Batch SHF experiments were carried out in pH-controlled 500 mL fleakers with a working volume of 350 mL at pH 6.5 and 35 °C essentially as described previously (Bothast et al., 1994) except that 4 M NaOH was used instead of 4 M KOH for pH control. A diagram of the fleaker system was presented previously by Beall et al. (1991). Rubber fleaker caps were drilled to allow the insertion of a pH probe, CO2 vent, sampling needle, and a port for addition of base. A magnetic stirrer was located beneath the water bath and maintained at 130 rpm. No attempts were made to exclude oxygen. SSF experiments were carried out at pH 6.0 instead of pH 6.5 adding the cocktail of three enzyme preparations. Fed-batch SSF experiments were conducted in the same way except that the pretreated WS was divided into four equal portions, starting the SSF with one portion and then adding the other three portions at 16, 21, and 24 h. Samples were withdrawn periodically, centrifuged to remove cells, and kept at −20 °C prior to high pressure liquid chromatography (HPLC) analysis. The reactor performance was monitored by quantifying unutilized sugars and fermentation products (ethanol, succinate). Base consumption and pH were recorded. Duplicate parallel fermentation experiments were run for reproducibility and comparison. Additional sugars (essentially in the same composition) were added to the fermentation medium in order to compensate for the volume increase due to dilution effects of the medium ingredients and inoculum addition. For simplification purpose, the quantity of ethanol produced from the additional sugars (glucose, fructose) present in the enzyme cocktail was subtracted from the measured ethanol yield in each case.

2.8. Analytical methods

The cellulose, hemicelluloses, and lignin content of WS, yields of each sugar as well as total sugars were calculated by the procedure provided by NREL (Sluiter et al., 2008). Sugars, ethanol, succinic acid, acetic acid, furfural, and HMF were analyzed by HPLC (Saha and Bothast, 1999). The separation system consisted of a solvent delivery system (P2000 Pump, Spectra-Physics, San Jose, CA) equipped with an autosampler (717, Waters Chromatography Division, Millipore Corp., Milford, MA), a refractive index detector (410 Differential Refractometer, Waters), a dual λ absorbance detector (2487, Waters), and a computer software based integration system (Chromquest 4.0, Spectra-Physics). Two ion moderated partition chromatography columns (Aminex HPX-87P with Deashing and Carbo-P micro-guard cartridges, Aminex HPX 87H with Cation H micro-guard cartridge) were used. The Aminex HPX-87P column was maintained at 85 °C, and the sugars were eluted with filtered (Milli-Q, Millipore Corp., Bedford, MA, USA) deionized water at a flow rate of 0.6 mL/min. The Aminex HPX-87H column was maintained at 65 °C, and the sugars, organic acids, and ethanol were eluted with 10 mM HNO3 prepared using Milli-Q filtered water at a flow rate of 0.6 mL/min. Peaks were detected by refractive index or UV absorption (277 nm) and were identified and quantified by comparison to retention times of authentic standards (glucose, xylose, arabinose, galactose, ethanol, succinic acid, acetic acid, furfural, and HMF).

3. Results and discussion

3.1. Pretreatment and enzymatic hydrolysis of wheat straw

The WS (moisture, 5.5 ± 0.0%) used in this study contained 32.0 ± 0% cellulose, 32.1 ± 1.3% hemicellulose (total carbohydrate content, 64.1 ± 1.3), 11.2 ± 0.4% lignin, and 6.5 ± 0.0% ash. It (150 g/L) was slurried in 0.75% (v/v) H2SO4 and then pretreated at 160 °C for 10 min. The liquid portion of the pretreated WS contained 4.8 ± 0.1 g glucose, 30.8 ± 0.3 g xylose, 5.6 ± 0.5 g arabinose, 1.7 ± 0.3 g galactose (total sugars, 42.9 ± 1.0 g), 152 ± 3 mg HMF, and 1230 ± 4 mg furfural/L. Three commercial enzyme preparations (cellulase, β-glucosidase, and hemicellulase) were used for saccharification of the pretreated WS. Cellulast had 34 ± 2 FPU, 1,045 ± 93 U CMCase, 21 ± 0 U β-glucosidase, 1227 ± 57 U xylanase, 37 ± 3 U β-xylosidase, 9 ± 0 U α-L-arabinofuranosidase, and 0 ± 0 U fericulic acid esterase activities. These activity Units for Novozym 188 were 0.0 ± 0.0, 48 ± 2, 581 ± 6, 47 ± 0, 2 ± 0.3, and 0.3 ± 0.1, respectively. For ViscoStar 150 L, these unit values were 10 ± 1, 1135 ± 18, 42 ± 1, 13,878 ± 216, 35 ± 0, 17 ± 0, and 0 ± 0, respectively. The effect of three enzyme doses on the hydrolysis of WS at pH 5.0 and 45 °C for 72 h is shown in Fig. 1. It is obvious that dilute acid pretreatment under the conditions used solubilized and degraded the hemicellulose present in the WS fully as the yield of xylose, arabinose, and galactose did not increase at all upon enzymatic hydrolysis at pH 5.0 and 45 °C for 72 h using the commercial hemicellulase preparation even at the highest dose level (0.15 mL/g WS). On the other hand, the glucose yield increased from 4.8 ± 0.1 to 48.6 ± 0.8 g/L (11.7-fold) upon enzymatic hydrolysis at 45 °C and pH 50 for 72 h at each enzyme dose level of 0.15 mL/g WS. The yields of total sugars from pretreated WS (150 g/L) were 80.70 ± 1.3, 83.2 ± 1.9, and 86.3 ± 1.5 g/L using each enzyme dose levels of 0.0375, 0.075, and 0.15 mL/g WS, respectively. So, it was decided to use each enzyme dose level of 0.15 mL/g WS for SHF studies.

The WS used in this study contained 641 ± 13 g total carbohydrates/kg which upon hydrolysis should yield about 705 ± 14 g sugars (Sluiter et al., 2008). The maximum sugar yield of 86.3 ± 1.5 g from 150 g WS gives a sugar yield of 82% of the theoretical yield. Component-wise, assuming that all glucose generated was from cellulose, about 92% of cellulose was converted to glucose. The sugar (xylose, arabinose, and galactose) yield from hemicellulose was only 7% of the theoretical yield. However, the dilute acid pretreatment under the conditions used also generated 1230 ± 4 mg furfural and 152 ± 3 mg HMF/L. These fermentation inhibitory compounds need to be removed from the fermentation broth for efficient production of ethanol from WS (Saha, 2003).

![Fig. 1. Effect of enzyme dose on the saccharification of dilute acid (0.75% H2SO4, w/v) pretreated (160 °C, 10 min)/wheat straw (150 g/L) at pH 5.0 and 45 °C for 72 h. The enzyme cocktail contained three commercial enzyme (cellulase, β-glucosidase, and hemicellulase) preparations. The data presented are averages of two separate experiments. 1, pretreated wheat straw; 2, 0.0375 mL of each enzyme preparation/g straw; 3, 0.075 mL of each enzyme preparation/g straw; 4, 0.15 mL of each enzyme preparation/g straw.](image-url)
Overliming of the hydrolyzate has become a state of the art technology to remove the inhibitors, but it generates salts and also decreases the sugar concentration due to loss of sugars during treatment (Purwadi et al., 2004). In this study, we have chosen instead to use a fungal bioabatement process for removal of inhibitors (Nichols et al., 2005). The procedure worked well in removing the inhibitory compounds (furfural, HMF) under controlled fermentation time.

3.2. Separate hydrolysis and fermentation

In our previous paper, we have reported that the recombinant *E. coli* strain FBR5 could grow and produce ethanol from non-abated dilute acid (0.5% H$_2$SO$_4$, v/v) pretreated (160 °C, 10 min) WSH (86 g/L) after a lag period of 24 h at pH 7.0 instead of pH 6.5 commonly used for such fermentations (Saha et al., 2011). Fig. 2 shows the patterns of disappearances of furfural and HMF, utilization of total sugars (86.6 ± 0.3 g/L), and production of ethanol by the recombinant *E. coli* FBR5 from non-abated dilute acid (0.75% H$_2$SO$_4$, v/v) pretreated (160 °C, 10 min) and enzymatically hydrolyzed (pH 5.0, 45 °C, 72 h) WSH (150 g/L) at pH 7.0 and 35 °C. As expected, there was a lag time of about 30 h in case of non-abated hydrolyzate. The utilization of sugar started after the furfural level decreased from 1225 ± 9 to 67 ± 9 mg/L and HMF level decreased from 151 ± 25 to 6 ± 0 mg/L in about 30 h. In 48 h, 26.8 ± 2.8 g total sugars was utilized and 14.1 ± 2.9 g ethanol was produced. The bacterium utilized 60.6 ± 3.9 g sugars and produced 29.7 ± 1.7 g ethanol/L in 72 h. At the end of 168 h fermentation, there was only 2.0 ± 1.8 g sugars left unutilized and the ethanol production level reached 41.1 ± 1.1 g/L. This provides a very useful and practical option of running the fermentation at pH 7.0 for SHF process without detoxifying the hydrolyzates. It appears that the *E. coli* was able to metabolize furfural, HMF, and acetic acid under these conditions.

Fig. 3 shows the time courses of utilization of glucose, xylose, arabinose, and total sugars and ethanol production by the recombinant *E. coli* strain FBR5 from the dilute acid pretreated, bioabated and enzymatically hydrolyzed WSH at pH 6.5 and 35 °C. In 30 h, 42.6 ± 2.7 g total sugars was utilized and 20.0 ± 1.7 g ethanol was produced. This was equivalent to the lag time of fermentation of non-abated WSH at pH 7.0. Within 48 h, 65.8 ± 3.2 g of the total sugars was utilized and 30.7 ± 1.3 g ethanol was produced. The bacterium utilized 80.5 ± 3.5 g sugars and produced 38.8 ± 1.5 g ethanol/L in 72 h. In 120 h, only 0.7 ± 0.2 g sugars remained unutilized and the ethanol production reached 41.8 ± 0.0 g/L. In our previous paper, we have shown that ethanol productivity (g L$^{-1}$ h$^{-1}$) by the recombinant bacterium decreased from 1.24 to 0.58 and 0.37 during the first 24 h period of growth in the presence of 1.5% and 2.5% ethanol, respectively (Saha and Cotta, 2011). That is the reason the fermentation took a long time to finish in this case.

As expected, the fermentation of the bio-abated WSH started without a lag period even at pH 6.5 and was 93% complete within 72 h (Fig. 3). The furfural and HMF levels in the bioabated WSH were 275 ± 2 and 114 ± 0 mg/L, respectively and in 7 h of fermentation at pH 6.5, these values decreased to 116 ± 4 mg and 32 ± 3 mg/L, respectively (data not shown). About 48% of the ethanol was produced within 30 h during which time 79% of the glucose was already utilized. Complete utilization of glucose was observed in 56 h. On the other hand, both xylose and arabinose
were slowly utilized in comparison to glucose and only 9% xylose and 20% of arabinose were utilized by the bacterium in 30 h. This pattern of sugar utilization (glucose > arabinose > xylose) was also observed in ethanol production from pure mixed sugars (glucose, xylose, and arabinose) by the bacterium (Dien et al., 2000).

3.3. Simultaneous saccharification and fermentation (SSF)

Integration of process steps is very important in lowering the cost of the production of ethanol from any lignocellulosic feedstock (Saha, 2004). SSF is an option that is often considered a necessary step in this regard (Wingren et al., 2003). The optimal pH and temperature for enzymatic hydrolysis of pretreated WS were actually 5.0 and 45–50 °C (Saha and Cotta, 2007a). The recombinant E. coli strain FBR5 performed well at pH 6.5 and 35 °C (Dien et al., 2000; Qureshi et al., 2006). In this study, we have used a compromised pH of 6.0 and 35 °C. The SSF experiments were conducted using bioabated WS (150 g/L), enzyme dose levels of 0.15, 0.75, and 0.0375 mL of each enzyme/g of WS and recombinant E. coli FBR5 as fermenting organism. In order to solve the problem of mixing due to high solids level, we have decided to divide the solids at four equal portions. The SSF experiment was started with one-fourth of the pretreated solid material in the fermentation broth and the remaining three portions of solid were added at 16, 21, and 24 h. This approach helped greatly with the mixing problem. Fig. 4 shows the time courses of ethanol production by the recombinant E. coli using three levels of enzyme doses – at 0.15, 0.075, and 0.0375 mL of each enzyme/g substrate. The data show that 41.6 ± 0.7, 40.8 ± 1.7, and 40.7 ± 0.8 g ethanol were produced by the bacterium at 104 h of fermentation using an enzyme dose level of 0.15, 0.075, and 0.0375 mL of each enzyme/g straw, respectively. Further continuing the fermentation up to 168 h did not increase the ethanol production level. Even though similar levels of ethanol were produced by the three enzyme doses, the fermentation rate was faster with the higher dose level of enzyme. Similar finding of higher ethanol production with higher dose of enzyme in SSF of hydrothermally pretreated switchgrass (200 °C, 10 min) was reported by Faga et al. (2010). However, the data also clearly demonstrate that the enzyme dose level can be easily reduced by 4-fold from 0.15 to 0.0375 mL of each commercial enzyme preparation/g WS by using this SSF approach as the overall ethanol yield at 104 h was decreased by only 2.17% using the low dose of each commercial enzyme preparation (Fig. 4).

The SSF experiments were conducted with the non-abated WSH under the same conditions except that the SSF was performed at controlled pH 7.0 instead of pH 6.0 used for bio-abated WSH failed. There are at least two reasons for this: (i) all the substrates were added during the lag period of the recombinant bacterium thus providing very high concentration of solid residues and (ii) the enzymes were only 30% active at pH 7.0 (Saha and Cotta, 2007a).

Table 1 summarizes the fermentation activity of the recombinant E. coli FBR5 from dilute acid pretreated WSH at 35 °C. The maximum yield of ethanol was 0.48 g/L available sugar mixtures. In comparison, a newly developed glucose, xylose and arabinose fermenting recombinant S. cerevisiae strain 424A(LNH-ST) produced ethanol with a yield of 72.5% from a mixture of sugars containing glucose, xylose, arabinose, galactose and mannose – each at a concentration of 20 g/L (Bera et al., 2010). An improved recombinant S. cerevisiae strain 424A(LNH-ST) containing overexpressed glyceraldehyde 3-phosphate dehydrogenase enzyme produced 82.2% ethanol from xylose (70 g/L) (Bera et al., 2011). The maximum ethanol produced by the recombinant E. coli FBR5 was 41.9 ± 0.3 g from 150 g of WS by SSF. This corresponds to 0.28 g of ethanol from 1 g of WS which is about 80% of the theoretical yield of ethanol.

This is the first report of the production of above 4% ethanol from a lignocellulosic hydrolyzate by the recombinant bacterium. The final residue containing mainly lignin can be used to supply solid biofuel to the power station that is needed to produce steam and power for the process (Larsen et al., 2008).

4. Conclusions

This research demonstrates that it is possible to produce over 40 g/L ethanol from dilute acid pretreated and detoxified WS (150 g/L) using both the hexose and pentose sugars utilizing recombinant E. coli strain FBR5 in either SHF or SSF. SSF offers distinct advantages over SHF with respect to reduction of total time and enzyme dose level required to produce ethanol from WS.

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References


