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ARTICLE

Enzyme Production by Wood-Rot and Soft-Rot Fungi Cultivated on Corn Fiber Followed by Simultaneous Saccharification and Fermentation

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This research aims at developing a biorefinery platform to convert lignocellulosic corn fiber into fermentable sugars at a moderate temperature (37 °C) with minimal use of chemicals. White-rot (*Phanerochaete chrysosporium*), brown-rot (*Gloeophyllum trabeum*), and soft-rot (*Trichoderma reesei*) fungi were used for in situ enzyme production to hydrolyze cellulosic and hemicellulosic components of corn fiber into fermentable sugars. Solid-substrate fermentation of corn fiber by either white- or brown-rot fungi followed by simultaneous saccharification and fermentation (SSF) with coculture of *Saccharomyces cerevisiae* has shown a possibility of enhancing wood rot saccharification of corn fiber for ethanol fermentation. The laboratory-scale fungal saccharification and fermentation process incorporated in situ cellulolytic enzyme induction, which enhanced overall enzymatic hydrolysis of hemi/cellulose components of corn fiber into simple sugars (mono-, di-, and trisaccharides). The yeast fermentation of the hydrolyzate yielded 7.8, 8.6, and 4.9 g ethanol per 100 g corn fiber when saccharified with the white-, brown-, and soft-rot fungi, respectively. The highest ethanol yield (8.6 g ethanol per 100 g initial corn fiber) is equivalent to 35% of the theoretical ethanol yield from starch and cellulose in corn fiber. This research has significant commercial potential to increase net ethanol production per bushel of corn through the utilization of corn fiber. There is also a great research opportunity to evaluate the remaining biomass residue (enriched with fungal protein) as animal feed.

KEYWORDS: Lignocellulosic biomass; corn fiber; solid-substrate fermentation; simultaneous saccharification and fermentation; enzymatic hydrolysis; ethanol; fungi; *Phanerochaete chrysosporium*; *Gloeophyllum trabeum*; *Trichoderma reesei*; *Saccharomyces cerevisiae*

INTRODUCTION

The annual corn ethanol production capacity exceeded 8.5 billion gallons per year in early 2008 from 147 biorefineries in the United States. Over 55 new plants, currently under construction, will add an additional 5.1 billion gallons of ethanol annually (*1*). Needless to say, these industries also produce millions of tons of low-value feed-grade coproducts such as distiller's dried grains with solubles (DDGS) and gluten feed from dry-grind and wet-milling plants, respectively. Excess coproducts will soon saturate the feed sector, and their bulk management may pose a serious issue. These coproducts contain mainly cellulose, hemicellulose, and residual starch (*2*). The National Renewable Energy Laboratory (NREL) estimated an increase in the net ethanol yield per bushel of corn by 13% (from 2.7 to 3.1 gallon ethanol/bushel corn) via utilization of the cellulosic fiber fraction and enhanced starch saccharification (*3*). Such process also reduces the overall bulk production of coproducts.

The recalcitrance and structural complexity of the cellulose and hemicellulose (hemi/cellulose) matrix requires extensive pretreatment involving physical, chemical, and biological techniques. Mosier et al. (*4*) reported various pretreatments such as mechanical milling, pressurized steam, acids, ammonia, or enzymes in a separate or combined process. Such pretreatments break down the heterogeneous and crystalline lignocellulosic fiber matrix thereby improving downstream enzymatic saccharification of hemi/cellulose to sugars and their subsequent fermentation to ethanol. High energy and chemical costs associated with these

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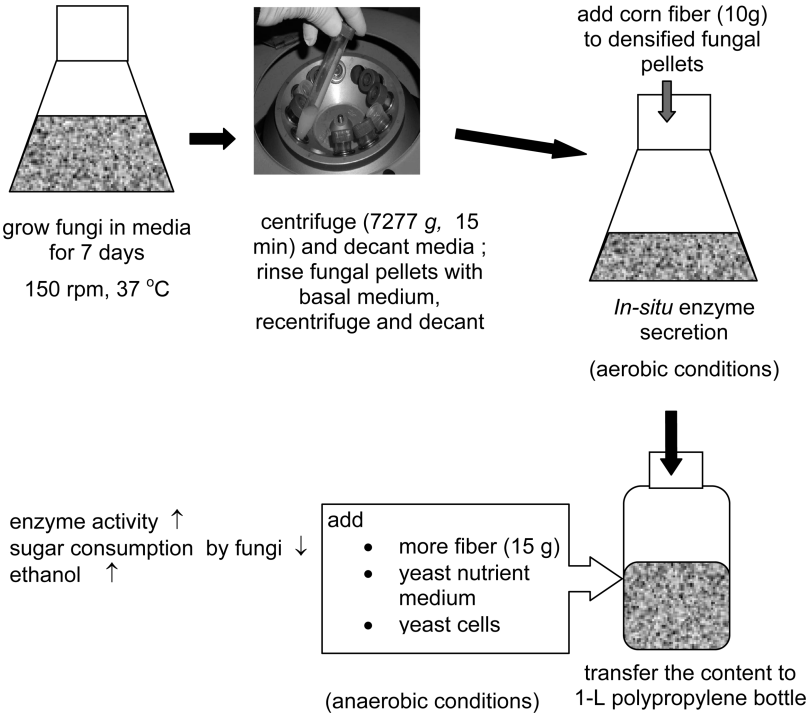

Figure 1. Schematic of bench-scale in situ fungal enzyme induction and simultaneous saccharification and fermentation of corn fiber to ethanol.

pretreatments and downstream waste management are the major drawbacks. Hydrolysis with commercial enzymes is a more favorable option compared to costly and environmentally unfriendly chemical methods. The costs of biomass pretreatment and enzyme are still the major limiting factors for the overall cost of cellulosic ethanol production.

Studies showed the potential application of indigenous fungi to break down lignocellulosic biomass. Shrestha et al. (*5*) reported the application of the white-rot fungus *Phanerochaete chrysosporium* in solid-substrate fermentation of corn fiber (coproduct from wet-milling plants) and subsequent simultaneous saccharification and fermentation to ethanol. Similar work was also examined by Rasmussen et al. (*6*) using the brown-rot fungus *Gloeophyllum trabeum*. The authors reported 3 and 4 g of ethanol production from 100 g of corn fiber via white- and brown-rot solid-substrate fermentation followed by simultaneous saccharificaiton and fermentation. These fungi were also reported to produce ethanol without yeast coculture. Wood-rot fungi, otherwise, had been studied mainly for degradation of lignocelluosic substrates (*7*, *8*), while cellulase activities have been extensively studied for *Trichoderma reesei* (*9*). These studies on wood-rot fungi open up new frontiers for biological saccharification and fermentation of lignocellulosic biomass to ethanol.

Solid-substrate fermentation, which involves developing a selected culture and enzymatic activities of microbes on selected substrates, was reported as a promising fermentation technique for in situ production of ligninolytic and cellulolytic enzymes (*10*). Previous studies examined solid-substrate fermentation using *P. chrysosporium* and *G. trabeum* for saccharification of corn fiber and conversion of hydrolyzate into ethanol using *Saccharomyces cerevisiae* in the subsequent submerged fermentation (*5*, *6*). Net ethanol yields were low (18%) in terms of the theoretical maximum yield from the cellulose and starch components of corn fiber. The objective of this research was to improve wood-rot fungal saccharification of corn fiber via enhanced enzymatic hydrolysis in submerged culture fermentation and the subsequent simultaneous saccharification and fermentation (SSF) of fermentable sugars into ethanol using *S. cerevisiae*. The performance of the wood-rot fungi was also compared under similar experimental conditions with *T. reesei* as most of the enzymatic hydrolysis studies of cellulosic feedstock were conducted with *T. reesei* and enzymes derived from its culture.

MATERIALS AND METHODS

The overall experimental procedure is presented in **Figure 1**.

Fungal Culture. Fungal cultures were obtained from the American type Culture Collection (ATCC, Rockville, MD). *Phanerochaete chrysosporium* (ATCC #24725), *Gloeophyllum trabeum* (ATCC #11539), *Trichoderma reesei* (ATCC #13631), and *Saccharomyces cerevisiae* (ATCC #24859) were separately revived by the process described in the ATCC Bulletin. Approximately 0.5 mL of sterile water was added into each of the vials containing frozen samples of the fungi. Following gentle mixing, the entire content of each vial was aseptically transferred into individual sterile tubes containing 5 mL of sterile water. The culture samples were allowed to rehydrate in the tubes for an hour. Representative cultures were then inoculated in individual flasks containing potato dextrose broth (PDB) (Difco, Becton Dickinson and Co., Sparks, MD). The flasks were incubated shaking (150 rpm) at 24 °C for 2 days. Stock cultures in 10% sterile glycerol were stored in sterile 2 mL-cryogenic vials and preserved in an ultralow temperature freezer (−75 °C, So-Low, Cincinnati, OH).

Fungal inocula for the saccharification and fermentation studies were prepared from the stock culture. The culture vials were thawed and poured aseptically, 1 vial into 1 L of sterilized yeast mold (YM) broth (Difco; composition per liter: glucose, 10.0 g; peptone, 5.0 g; yeast extract, 3.0 g; and malt extract, 3.0 g). The seed culture was incubated shaking at 150 rpm and 37 °C for rejuvenation. The mycelia grew into pellets of 2 to 3 mm size in 7 days.

Substrate. Corn fiber, obtained from a corn wet-milling plant (Archer Daniels and Midland, Decatur, IL), and was processed through hot water steeping and sulfur dioxide treatment at the beginning of the wet-milling process (*2*). The wet-milled corn fiber was oven-dried at 80 °C for 4 days followed by desiccation prior to use. Sterilization of the fiber was done by autoclaving at 121 °C for 75 min (*5*). The composition of corn fiber is given in **Table 1** (sections a,b).

Experimental Setup. *Fungal Culture Preparation.* White-rot (*P. chrysosporium*), brown-rot (*G. trabeum*), and soft-rot (*T. reesei*) fungi were grown separately in 1 L of YM broth at 37 °C in shake flasks (at 150 rpm) for 7 days and mycelia pellets of uniform diameter

Table 1.

(a) Constituent Analysis of Corn Fiber Received from Corn Wet Milling Plant[a]

ash	protein	extractives	glucan	starch	xylan	galactan	arabinan	mannan	acetyl	other
%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)
0.6	11.3	1.8	30.7	13.0	20.0	4.2	11.1	1.4	2.1	3.8

(b) Constituent Analysis of Corn Fiber using Ankom Technology (2005)

cellulose	hemicellulose	lignin	cell solubles	ash
%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)	%, (w/w)
16.4	45.3	1.3	37	0.03

[a] From personal correspondence with Kyle Beery at ADM, Decatur, IL.

(~2 to 3 mm) were formed. The media with fungal pellets were aseptically transferred into sterile 1-L polypropylene centrifuge bottles. The bottles were centrifuged at 7277*g* (5000 rpm) for 20 min. The supernatant was decanted, and the centrifuge bottle was filled aseptically to the top with basal medium (*12*), which contained 0.25 g of KH_2PO_4, 0.063 g of $MgSO_4 \cdot 7H_2O$, 0.013 g of $CaCl_2 \cdot 2H_2O$, and 1.25 mL of trace element solutions in 1 L of deionized water. The trace element solution (in 1 L deionized water) contained 3.0 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $MnSO_4 \cdot H_2O$, 1.0 g of NaCl, 0.1 g of $FeSO_4 \cdot 7H_2O$, 0.181 g of $CoSO_4 \cdot 7H_2O$, 0.082 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of $ZnSO_4$, 0.01 g of $CuSO_4 \cdot 5H_2O$, 0.01 g of $Al_2(SO_4)_3 \cdot 2H_2O$, 0.01 of H_3BO_3, and 0.01 g of $NaMoO_4$.

The pellets were resuspended in the basal medium; the centrifugation and supernatant decantation procedure was repeated to minimize the introduction of organic nutrients into the suspension.

There were duplicate sets of 2-L Erlenmeyer flasks for each of the three fungal species and controls, which had no fungal cultures. Each flask contained 600 mL of dense resuspended pellets of specific fungal species. Approximately 7.8, 5.5, and 4.4 g (dry weight (determined by drying 600 mL of dense fungal pellets at 80 °C inside a convection oven for 4 days))/L of white-, brown-, and soft-rot fungus were used, respectively, for enzyme induction and SSF.

Enzyme Induction. Sterile corn fiber (10 g) was added to each flask containing the respective fungal pellets. The control flasks had 600 mL of basal medium but without fungal cultures. These flasks, covered with sterile autoclave wraps, were placed in a shaker at 150 rpm and 37 °C for 48 h. Samples, 10 mL, from each flask were collected on the second day for the specific enzyme activity assay.

Simultaneous Saccharification and Fermentation. The content (~600 mL) of each flask was emptied into individual sterile 1-L polypropylene bottles, which contained 15 g of sterile corn fiber, 200 mL of yeast media, and 1 mL of *S. cerevisiae* culture (cell count = 2.9×10^9 cells/mL). All bottles including controls contained 600 mL of basal media, 200 mL of yeast media, 1 mL of yeast culture, and 25 g of sterile corn fiber. The bottles were then loosely capped to allow the excess CO_2 to escape. The bottles were kept static 37 °C inside an incubator for 8 days.

Analytical Methods. Every alternate day, 5 mL samples were collected aseptically from each bottle. The samples were centrifuged and syringe filtered (0.45 μm) for the following assays.

Sugar Assays. Total and reducing sugar analyses were conducted via phenol sulfuric and Somogyi−Nelson methods, respectively (*13*). The samples were analyzed for total and reducing sugars using a spectrophotometer (Spectronic 20 Genesys, Thermo Electron, Cambridge, UK) at 490 and 500 nm, respectively. The absorbance readings were then converted into equivalent sugar concentration (g/L) using a standard glucose solution curve.

Ethanol and Organic Acids Assays. Ethanol, and lactic and acetic acid concentration were measured by using a Bio Rad Aminex 87-H (78 × 300 mm) organic acid column (Waters High Pressure Liquid Chromatograph, Millipore Corporation, Milford, MA) as described elsewhere (*5*).

Specific Enzyme Activity Assays. Specific enzyme activity assays for α- amylase and glucoamylase, xylanase, endocellulase, and exocellulase were performed using the protocol described by Lee et al. (*14*). Specific enzyme activity for each enzyme was expressed as mg product/mg protein/min.

Statistical Analyses. The experimental data were validated by statistical analyses using the statistical tool, SAS (version 9.1.2, SAS Institute Inc., Cary, NC). The SSF results on sugar, ethanol, and organic assays were fitted to a two-factor fixed effects model. All assays and experiments were performed in replicates of two ($n = 2$), and a significant difference of p value 0.05 was employed. Student's t-test analyses were performed for data obtained from specific enzyme activity assays.

RESULTS AND DISCUSSION

Specific Enzyme Activity Assays. Table 2 (section a) shows the specific enzyme activity assay result of five different enzymes (α-amylase, glucoamylase, xylanase, endocellulase, and exocellulase). It is evident that all three fungal species showed activities for starch, xylan, and cellulose. The corn fiber induced enzyme secretion in all three fungal cultures during aerobic submerged culture for 2 days. The residual starch and hemi/cellulose fractions resulted in higher enzyme induction for white-rot fungus as compared to brown- and soft-rot fungi. Therefore, it is evident that both starch and hemi/cellulose fractions contributed significantly to enzyme induction and to saccharification and fermentation of corn fiber to ethanol. Similar results were also observed by Shrestha et al. (*5*) and Rasmussen et al. (*6*) for white- and brown-rot saccharification studies, respectively. There were no statistical differences between α-amylase and glucoamylase activities for all three fungal cultures (**Table 2**, section b). Xylanase, endo-, and exocellulase activities were significantly different between the fungal species. Nonenzymatic oxidative process could be one of the possible reasons for the low specific enzyme activity but higher ethanol yields in the case of the brown-rot fungus *G. trabeum*.

The difference in ratio of fungal biomass to corn fiber at the beginning of the experiment can result into performance differences during saccharification and fermentation products. The enzyme activities are normalized per unit weight of protein (**Table 2**, section a). Though *T. reesei* had higher specific enzyme activities, the ethanol yield is lower than that of SSF with *G. trabeum*, and the latter had a higher ethanol yield irrespective of low cellulolytic enzyme activities.

Sugar Release in Simultaneous Saccharification and Fermentation (SSF). During the aerobic enzyme induction phase, extracellular enzyme production resulted in the production of water-soluble simple sugars from the residual starch, cellulose, and hemicellulose fractions of corn fiber and also their consumption by the fungi. To minimize fungal sugar consumption and maximize enzymatic hydrolysis after two days of aerobic incubation, the fungi were placed in an anaerobic condition with added fiber and yeast cultures for simultaneous saccharification

Table 2. [a]

(a) Specific Enzyme Activities of Different Enzymes Expressed As Milligrams of Product per Minute per Milligram of Protein in 2-Day-Old Submerged Corn Fiber Fermentation with Three Fungal Cultures ($n = 2$)

specific enzyme activity	*P. chrysosporium*	*G. trabeum*	*T. reesei*
α-amylase (mg maltose/mg protein · min)	0.230	0.160	0.330
glucoamylase (mg glucose/mg protein · min)	0.380	0.180	0.375
xylanase (mg xylose/mg protein · min)	0.740	0.060	0.060
endocellulase (mg glucose/mg protein · min)	0.505	0.215	0.050
Exocellulase (mg glucose/mg protein · h)	1.030	0.090	0.265

(b) Comparison of Specific Enzyme Activities of Three Fungal Species Using *p* Values Obtained from Student's *t*-Test

	p values		
specific enzyme activity	*P. chrysosporium* vs *G. trabeum*	*P. chrysosporium* vs *T. reesei*	*G. trabeum* vs *T. reesei*
α-amylase	0.4341	0.4266	0.1977
glucoamylase	0.3148	0.9847	0.3817
xylanase	0.0572	0.0572	not determined
endocellulase	0.0365	0.0096	0.0422
exocellulase	0.0056	0.0101	0.1615

[a] All chemicals were purchased from Sigma Chemical Inc. (St. Louis, MO).

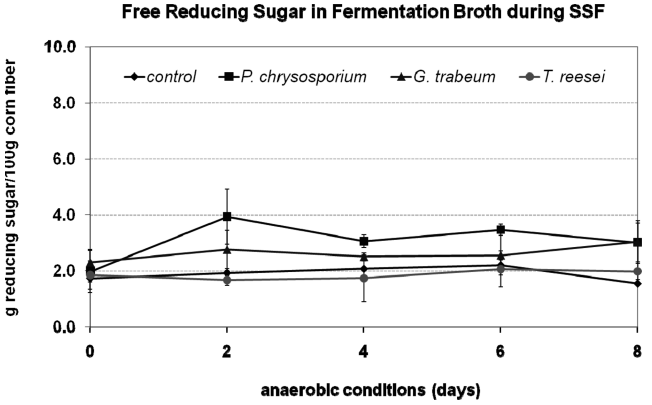

Figure 2. Residual reducing sugars present in culture broth from anaerobic simultaneous saccharification and fermentation for ethanol production in white-, brown-, and soft-rot (*P. chrysosporium*, *G. trabeum*, and *T. reesei*) fungi cocultured with *S. cerevisiae* ($n = 2$). Control is with yeast cells but no fungal culture.

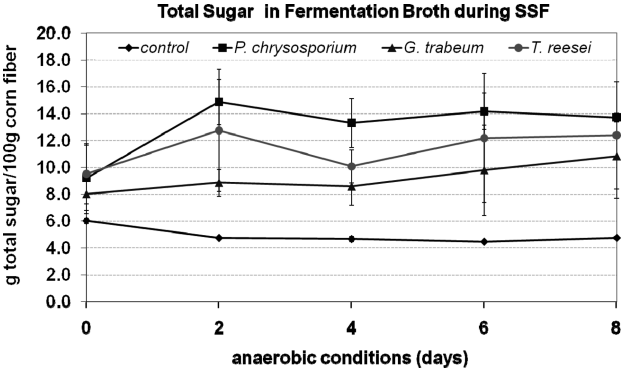

Figure 3. Total sugars present in culture broth from anaerobic simultaneous saccharification and fermentation for ethanol production in white-, brown-, and soft-rot (*P. chrysosporium*, *G. trabeum*, and *T. reesei*) fungal cocultured with *S. cerevisiae* ($n = 2$). Control is with yeast cells but no fungal culture.

and fermentation (SSF) to ethanol. The saccharification of corn fiber polysaccharides was monitored via reducing and total sugars assays. The sugar values were interpreted in terms of gram sugar produced per 100 g of initial corn fiber. Higher sugar values compared to that of the control (without fungi) confirmed active enzyme activities during the SSF process. The released fermentable sugars (especially 6-carbon glucose) could be fermented by yeast during anaerobic conditions to ethanol. The nonfermentable sugars are reported in **Figures 2** and **3**. These sugars may be cellobiose, pentoses, tri-, and oligosaccharides, which were accumulated during SSF.

The activity of cellulase enzymes depends on the microbial source, the types of substrate, and the operating conditions (i.e., pH and temperature) (*15*). Meyer et al. (*15*) reported that a pH of 5.0 and temperature of 50 °C were optimal for maximum yield of glucose from steam-pretreated barley straw using cellulase enzymes from cultures of five thermophilic fungi: *Chaetomium thermophilum*, *Thielavia terrestris*, *Thermoascus aurantiacus*, *Corynascus thermophilus*, and *Myceliophthora thermophila*, and from the mesophilic *Penicillum funiculosum*. The starting pH for SSF in this study was at 4.7 to 5.2, and the temperature was maintained at 37 °C. The pH gradually decreased to 4.2 and then remained nearly constant as the SSF progressed. A moderate temperature (32to 37 °C) was required for anaerobic yeast fermentation. The accumulation of cellulase end-products (e.g., glucose and cellobiose) suppresses enzyme activity (*16*). Thus, SSF helps to overcome the product inhibition by converting fermentable end-products into ethanol as soon as they are produced (*17*, *18*) and facilitates continuous cellulase activity. The hemicellulase activity of *P. chrysosporium* was studied by Highley and Dashek (*8*). The hydrolysis of hemicellulose releases both hexoses and pentoses. The reducing sugar measurements depend on the availability of an aldose or ketose reducing end and mono-, di-, tri-, and short-chained carbohydrates having one reducing end each. The fermentable portion of the reducing sugar can be determined by quantifying ethanol produced by yeast fermentation.

The increase in total sugars in SSF bottle cultures, compared to that of the controls, confirmed the active enzyme activities of the fungi (**Figure 3**). For control, the total sugar decreased from 6 to 4.8 g per 100 g initial corn fiber, which then remained

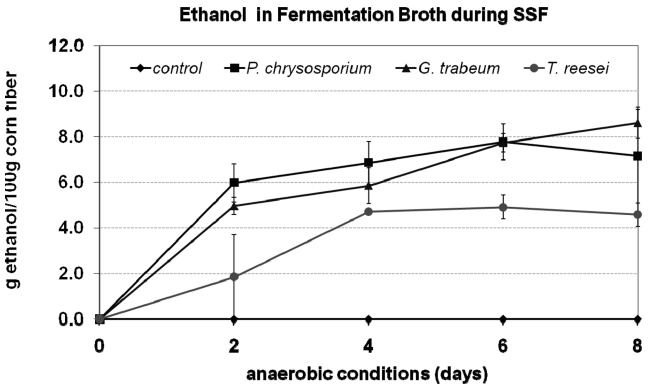

Figure 4. Ethanol yield in culture broth from anaerobic simultaneous saccharification and fermentation for ethanol production in white-, brown-, and soft-rot (*P. chrysosporium*, *G. trabeum*, and *T. reesei*) fungi cocultured with *S. cerevisiae* ($n = 2$). Control is with yeast cells but no fungal culture.

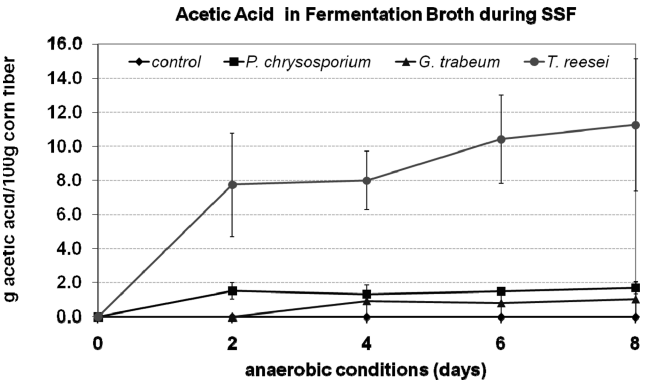

Figure 5. Acetic acid yield in culture broth from anaerobic simultaneous saccharification and fermentation for ethanol production in white-, brown-, and soft-rot (*P. chrysosporium*, *G. trabeum*, and *T. reesei*) filamentous fungi cocultured with *S. cerevisiae* ($n = 2$). Control is with yeast cells but no fungal culture.

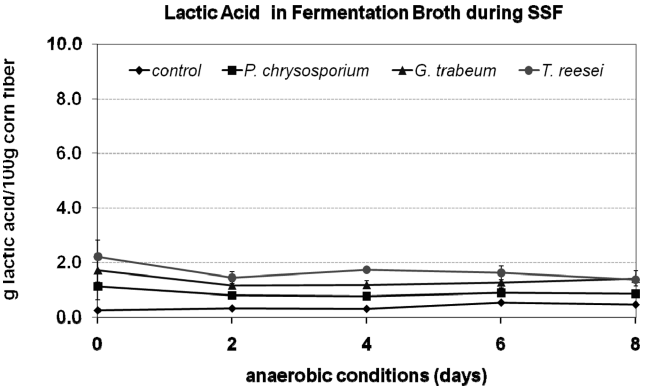

Figure 6. Lactic acid yield in culture broth from anaerobic simultaneous saccharification and fermentation for ethanol production in white-, brown-, and soft-rot (*P. chrysosporium*, *G. trabeum*, and *T. reesei*) fungal cocultured with *S. cerevisiae* ($n = 2$). Control is with yeast cells but no fungal culture.

constant throughout the experimental period. Similarly, the maximum and minimum (in parentheses) total sugar production for *P. chrysosporium* and *T. reesei* were, respectively, 14.9 (13.7) and 12.8 (12.4) g total sugar per 100 g corn fiber. The total sugar, however, showed an increasing trend for *G. trabeum* from 8 to 10.8 g total sugar per 100 g corn fiber. The overall total sugar data was not statistically different (p-value = 0.5) between three fungal species. The difference between the total and the reducing sugars also indicates that soluble sugars were not completely hydrolyzed to monosaccharides. The difference in total sugar levels was basically the differences in the enzyme activities between these fungal species. There was also no statistical difference for reducing sugar data (**Figure 2**) between fungal species and control samples (p-value = 0.29).

Ethanol Fermentation in Simultaneous Saccharification and Fermentation (SSF). One mole of glucose (C-6) is converted into 2 moles of ethanol and 2 moles of carbon dioxide during yeast fermentation. Thus, stoichiometrically, 1 g of glucose would yield 0.51 g of ethanol and 0.49 g of carbon dioxide. Fungal SSF yielded higher ethanol production compared to that of the control. The net fiber to ethanol conversion (based on initial corn fiber weight of 25 g) was as high as 8.6 g of ethanol per 100 g of corn fiber in the case of brown rot fungus (*G. trabeum*), followed by 7.1 and 4.6 g of ethanol per 100 g of corn fiber, respectively, for *P. chrysosporium* and *T. reesei* (**Figure 4**). The brown-rot saccharification and SSF of corn fiber yielded about 35% of the theoretical maximum yield (theoretical maximum ethanol yield: 25 g of ethanol per 100 g of fiber), and this would also mean that the current ethanol yield can produce 29 gallons of ethanol per dry ton of corn fiber. There was significant difference in ethanol yield data between the different fungal treatments (p-value = 0.0557); however, the white- and brown-rot ethanol yield data were not significantly different for the number of experiments (p-value = 0.8491). As seen from the contrasts output, ethanol yield following *T. reesei* treatment was interestingly different when compared with those between *P. chrysosporium* (p-value = 0.0336) and *G. trabeum* (p-value = 0.0388). The ethanol concentration would be expected to increase slightly for *G. trabeum* if the anaerobic incubation period was prolonged. However, it would not be economically sound to extend the fermentation process for such an extended time. The decreasing concentration of ethanol and sugar values indicates the low activity of saccharification and fermentation processes at a later phase. Decreasing pH trend (not reported here) was also observed in the SSF bottles with fungal biomass.

Acetic Acid Production in Simultaneous Saccharification and Fermentation (SSF). The white- and brown-rot SSF produced 1.7 and 1 g of acetic acid per 100 g of corn fiber (p-value = 0.6121), respectively. In the case of soft-rot SSF, the acetic acid yield was as high as 11.3 g per 100 g of corn fiber (**Figure 5**). Chambergo et al. (*19*) reported the paralogous gene for the enzyme aldehyde dehydrogenase (ALD1 and ALD2) responsible for converting acetaldehyde to acetate. The authors also reported that these genes are highly expressed even under higher glucose concentration unlike *S. cerevisiae*. The increasing acetic acid concentration in *T. reesei* SSF might have, therefore, affected the activity of the coculture *S. cerevisiae*, in converting glucose to ethanol and therefore leading to a lower yield of ethanol. Graves et al. (*20*) reported the inhibition of ethanol production by *S. cerevisiae* at various acetate concentrations.

Lactic Acid Production in Simultaneous Saccharification and Fermentation (SSF). Lactic acid concentration also showed valid differences between three fungal SSF (p-value = 0.043). By the end of the experiment, 0.9, 1.4, 1.4, and 0.5 g of lactic acid per 100 g of corn fiber were accumulated for white-, brown-, and soft-rot fungi and control samples, respectively (**Figure 6**). From the contrasts output, it was found that *T. reesei* was significantly different when averaged over time than *P. chrysosporium* (p-value = 0.019); but *P. chrysosporium* was not significantly different from *G. trabeum* when averaged over time (p-value = 0.08). Similar findings were observed between *G. trabeum* and

T. reesei when averaged over time (p-value = 0.137). Various conditions, such as broth composition and conditions of yeast cells during fermentation, affect lactic acid formation (*21*). There could also be the possibility of lactic acid bacteria contamination during SSF.

Corn fiber from a wet-milling plant represents cleaner lignocellulosic substrate for fungal SSF with no further pretreatment requirements. This study envisaged the concept of *on-site* enzyme induction and subsequent simultaneous saccharification and fermentation processes to further enhance the enzymatic hydrolysis in conjunction with reduced mold-sugar consumption during saccharification, and facilitate improved ethanol fermentation via the coculture of yeast. All three (white-, brown-, and soft-rot) fungi illustrated extracellular enzyme production for the hydrolysis of corn fiber. SSF with *P. chrysosporium* and *G. trabeum*, with *S. cerevisiae had* higher saccharification and ethanol fermentation yields (i.e., 35% of the theoretical maximum yield), whereas *T. reesei* had lower fermentation yields. This might be due to excess acetic acid formation compared to that in ethanol. Enzyme activities and yeast ethanol fermentation might also have been affected by sugar consumption by fungi during the enzyme induction phase, acidic pH, organic acid production, and prolonged anaerobic conditions. Submerged fermentation may have comparable benefits in optimization of experimental parameters such as temperature, pH, and oxygen diffusibility. Solid substrate fermentation, though representing a natural environment for fungi in terms of hyphal extension and improved growth, presents obstacles to maintain parameters such as temperature, pH, and oxygen diffusibility.

ABBREVIATIONS USED

SSF, simultaneous saccharification and fermentation; DDGS, distiller's dried grains with solubles; NREL, National Renewable Energy Laboratory; ATCC, American Type Culture Collection.

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