PRETREATMENT AND CONVERSION OF DISTILLER'S DRIED GRAINS WITH SOLUBLES FOR ACETONE-BUTANOL-ETHANOL (ABE) PRODUCTION

B. Wang, T. Ezeji, Z. Shi, H. Feng, H. P. Blaschek

ABSTRACT. *Distiller's dried grains with solubles (DDGS) is a low-value co-product from dry grind ethanol production. Due to its high fiber content, DDGS is used primarily as a feed for ruminant animals. Conversion of the carbohydrate components in DDGS to biofuel would improve the overall economics of dry grind ethanol production. The effect of solids loading on pretreatment of DDGS and on subsequent acetone-butanol-ethanol (ABE) fermentation was examined. DDGS samples were subjected to six pretreatments, involving acidic and alkaline electrolyzed water, at three solids loadings (20%, 30%, and 40% w/w). After a 72 h hydrolysis, the highest glucose yield was obtained from the sulfuric acid pretreated samples. In fermentation tests using* Clostridium acetobutylicum *P260 for ABE production, the highest ABE concentration was ~17 g* L^{-1}*, which was achieved from alkaline electrolyzed water pretreated samples at 30% (w/w) solids loading. The solids loading in a pretreatment significantly affected both the sugar yield from enzymatic hydrolysis and the ABE yield from fermentation of DDGS hydrolysates. The hydrolysate from alkaline electrolyzed water pretreatment at 30% (w/w) solids was found to be most favorable to ABE production, while that from sulfuric acid pretreatment was the least fermentable, although it produced the highest sugar yield.*

Keywords. *ABE fermentation, DDGS, Electrolyzed water, Enzymatic hydrolysis, Pretreatment, Solids loading.*

Distiller's dried grains with solubles (DDGS) is the dried fermentation residue from dry grind ethanol production, mainly consisting of germ, corn fiber, unused starch, protein, and ash. Given the rapid expansion of the corn-based ethanol industry, as well as the saturation of DDGS in the ruminant feed market, the utilization of low-value DDGS has become an issue important to the overall economics of dry grind ethanol facilities.

To reduce the volume of DDGS and diversify its markets, new process modifications to conventional dry grind ethanol production have been exploited. The new methods that have been investigated include those based on the quick germ and/or quick fiber concept (Singh and Eckhoff, 1997; Singh at al., 1999; Wahjudi et al., 2000; Taylor at al., 2001), enzymatic milling (Singh et al., 2005), and the dry degerm, defiber (3D) process (Rajagopalan et al., 2005; Murthy at el., 2006). Efforts have also been made utilizing DDGS and other types of distiller's grains to produce value-added products (Romero et al., 2007). Recently, an elutriation process has been developed to separate DDGS into a fiber-rich portion and an enhanced DDGS portion (Srinivasan et al., 2005; Srinivasan et al., 2006).

DDGS has approximately 16% cellulose, 13.5% hemicellulose, and 5.2% starch, which can be converted to fermentable sugars for the production of ethanol or butanol (Tucker et al., 2004; Ladisch and Tyner, 2005; Bals et al., 2006; Srinivasan et al., 2007). Butanol has a calorific value (29.2 MJ L^{-1}) higher than that of ethanol (19.6 MJ L^{-1}) and can be produced in a biological process known as acetone-butanol-ethanol (ABE) fermentation (Qureshi and Blaschek, 1999; Ezeji et al., 2003, 2004, 2007a).

The first step to convert the carbohydrates in DDGS to fermentable sugars is pretreatment. Numerous pretreatment concepts have been proposed and tested over the years. Steam treatment (or steam explosion) with and without the addition of a catalyst is one of the oldest methods (Söderström et al., 2004; Palmarola-Adrados et al., 2004; Sassner et al., 2005; Varga et al., 2004). A coordinated study supported by a USDA Initiative for Future Agricultural and Food Systems grant has examined the performance of five promising biomass pretreatment methods. Using a single feedstock (corn stover), common analytical protocols, and consistent data interpretation, five research teams documented the technical and economical feasibility of five pretreatment techniques (Wyman et al., 2005; Eggeman and Elander, 2005). Almost all five pretreatment methods, i.e., dilute acid (Lloyd and Wyman, 2005), hot water (neutral pH) (Mosier et al., 2005a, 2005b), ammonia fiber/freeze explosion (AFEX) (Teymouri et al., 2005), ammonia recycle

Submitted for review in October 2008 as manuscript number FPE 7739; approved for publication by the Food & Process Engineering Institute Division of ASABE in April 2009.

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  ISSN 0001–2351

percolation (ARP) (Kim and Lee, 2005), and lime (Kim and Holtzapple, 2005), can produce high sugar yield. Nevertheless, it is found that those pretreatments with a relatively low-cost reactor are often counterbalanced by the higher costs associated with either pretreatment catalyst recovery or higher costs for ethanol product recovery (Eggeman and Elander, 2005). There is still a need for the development of new pretreatment methods.

Electrolyzed water is produced by the electrolysis of tap water containing dissolved sodium chloride (0.1%, w/w). The acidic water from the anode of an electrolysis unit normally has a pH of $\leq$2.7 and oxidation reduction potential (ORP) of >1100 mV. The alkaline water produced from the cathode has a pH of $\geq$11.4 and ORP of < −795 mV. The acidic electrolyzed water (AEW) has been used in the decontamination of food and agricultural products (Wang et al., 2004). Because of its high hydrogen ion (H^+) concentration, AEW might be used as an environmentally friendly alternative to dilute sulfuric acid for biomass pretreatment. Likewise, the alkaline electrolyzed water (ALEW) may play a role similar to the bases used in ammonia fiber expansion (AFEX) (Teymouri et al., 2005), ammonia recycle percolation (ARP) (Kim and Lee, 2005), and lime (Kim and Holtzapple, 2005) pretreatments.

Solids loading is an important factor in biomass conversion. For example, a low solids loading in enzymatic hydrolysis would lower the sugar concentration in hydrolysate, resulting in a low biofuel concentration in fermentation broth and a high energy cost for biofuel recovery, while a high loading may reduce the rate of enzymatic hydrolysis (Kim et al., 2008). In this work, the use of electrolyzed water as a new pretreatment method for conversion of DDGS to ABE at three solids loadings (20%, 30%, and 40% w/w) was explored. Pretreated samples were hydrolyzed by enzyme preparations followed by fermentation using *Clostridium acetobutylicum* P260 for ABE production. Qureshi et al. (2006) showed that *C. acetobutylicum* P260 supported efficient ABE production using corn fiber arabinoxylan hydrolysate as a substrate, and in particular, a high productivity was observed in an integrated hydrolysis-fermentation-recovery system (Qureshi et al., 2006). It is thus of interest to use *C. acetobutylicum* P260 as a microbial host to assess the production of ABE based on DDGS hydrolysate fermentation.

Materials and Methods

Raw Material and Reagents

The DDGS was obtained from Big River Resources (West Burlington, Iowa) and dried to 11.5% moisture content (wet basis). The initial composition of the DDGS with a 95% confidential interval was 16.0% ±6.6% cellulose, 8.2% ±3.3% xylan, 5.3% ±0.7% arabinan, 5.2% starch, and 26.4% protein (Bals et al., 2006). Glucoamylase was supplied by Enzyme Development (New York, N.Y.). Spezyme CP (cellulase) was provided by Genencor International (Rochester, N.Y.). Novozym 188 (β-glucosidase) and protease were purchased from Sigma (St. Louis, Mo.). Other chemicals were obtained from either Sigma or Fisher Scientific (Hanover Park, Ill), and they were of analytical grade. A saturated NaCl solution and tap water from the laboratory supply line were simultaneously introduced into an electrolyzed water generator (ROX-20TA, Hoshizaki, Nagoya, Japan). AEW and ALEW w ere generated and collected from the electrode outlets of the generator with beakers. The pH values of the solutions were measured with an AR15 pH meter (Accumet Research, Pittsburgh, Pa.).

Pretreatment and Enzymatic Hydrolysis of DDGS

DDGS samples were subjected to six pretreatments in duplicates, i.e., hot water, acidic electrolyzed water (pH 2.7), alkaline electrolyzed water (pH 11.7), diluted H_2SO_4 (pH 2.7), NaOH (pH 11.7), and H_2SO_4 (0.25% v/v, pH <1.0) at three solid-to-liquid loadings (20%, 30%, and 40% w/w). The pretreatments were conducted with stainless steel tubular reactors of 2.54 cm (1 in.) OD × 17.78 cm (7 in.) length. An SBL-2D fluidized sand bath (4000 W, Techne, Inc. Burlington, N.J.) was used to heat the reactors. The temperature control was facilitated with a TC-8D temperature controller. DDGS containing 17 g dry matter was mixed with different amounts of pretreatment liquid to reach a desired solids loading (20%, 30%, or 40%), and then the slurry was loaded into the reactors, which were submerged in the fluidized sand bath and heated to 160°C for 30 min for all pretreatments. Right after a pretreatment, the reactors were removed from the sand bath and submerged immediately in an ice bath to stop the reaction. The pretreated DDGS was adjusted to pH 5.0 by ammonium hydroxide and 0.05 M citrate buffer enzyme solutions. The solid-to-liquid ratio of the pretreated slurry including both solids and liquid was brought to 15% (w/w) before enzymatic hydrolysis. Spezyme CP (9 FPU g^{-1} glucan of raw DDGS), Novozyme 188 (20 IU g^{-1} glucan of raw DDGS), glucoamylase (2.7 U g^{-1} dry basis DDGS), and protease (5 U g^{-1} dry basis DDGS) were then added. The enzymatic hydrolysis was performed in 250 mL Pyrex flasks in a shaker water bath (Tecator, Inc., Höganäs, Sweden) at 50°C and 100 rpm. During the course of hydrolysis, aliquots of 2 mL were collected at 12, 24, 48, and 72 h, boiled in 100°C water to deactivate the enzymes, and frozen at −20°C for later HPLC analysis.

ABE Fermentation

After hydrolysis, hydrolysates were centrifuged at 10,000*g*. The solids residue was discarded, and the sugar containing supernatant was used for fermentation. The volume of fermentation broth was 100 mL. Laboratory spore stocks of *C. acetobutylicum* P260 were maintained as spore suspensions in sterile water at 4°C. Spore samples (200 μL each) were heat-shocked at 65°C for 3 min, followed by cooling on ice for 10 min. The heat-shocked spores were inoculated into 100 mL TGY medium containing 3% tryptone, 2% glucose, 1% yeast extract, and 0.1% L-cysteine. The cells were grown at 35°C for approximately 12 h in an anaerobic chamber maintained under a gas mixture of 85% N_2, 10% CO_2, and 5% H_2. Subsequently, an aliquot of 5 mL actively growing TGY culture was used to inoculate the hydrolysates in 175 mL Pyrex screw-capped bottles for solvent production. Fermentation was carried out in P2 medium (Ezeji et al., 2003) with filter-sterilized DDGS hydrolysates as the carbon source. The culture was grown anaerobically at 35°C. During the course of fermentation, 2 mL culture aliquots were collected to quantify cell, ABE, and acid concentrations.

Scanning Electron Microscopy

Scanning electron microscopy was used to observe morphological changes of the DDGS samples before and after a

selected pretreatment. The DDGS samples were dehydrated in a graded series of ethanol solutions (25%, 50%, 70%, 95%, and 100%) and dried with a CO_2 critical-point drier (Samdri-PVT-3D, Tousimis Research Corp., Rockville, Md.). The dried samples were mounted on stubs and sputter-coated with gold/palladium for 80 s by a Desk II TSC turbopumped sputter coater (Denton Vacuum, Moorestown, N.J.). Scanning electron micrographs (SEM) were obtained by an environmental scanning electron microscope (Philips XL30 ESEM-FEG, FEI Co., Eindhoven, The Netherlands).

Analytical Procedures

Glucose concentration was determined by an HPLC system that consisted of a Waters 2695 separation module, a Waters 717 plus autosampler, and a Waters 410 refractive index detector (Waters Corp., Milford, Mass.), monitored by an HP Chem Station computer program (Agilent Technologies, Germany). A Bio-Rad HPX-87P column (Bio-Rad Laboratories, Inc., Hercules, Cal.) equipped with a guide column (30 × 4.6 mm) was used. The column temperature was kept at 85°C. The temperature of the refractive index detector was set at 35°C. The mobile phase was ultrapure water (18.1 MΩ cm) at a flow rate 0.6 mL min^{-1}. The correlation coefficient of the glucose standard curve was greater than 0.99.

The cell density of *C. acetobutylicum* P260 in the fermentation broth was measured using a Beckman Coulter spectrophotometer and a pre-determined correlation between OD540 nm and cell dry weight. ABE, acetic acid, and butyric acid concentrations were quantified using gas chromatography (Hewlett Packard, Avondale, Pa.). The gas chromatography system was equipped with a flame ionization detector (FID) and a 1829 × 2 mm glass column (10% CW-20M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW). The standard used was composed of 2 g L^{-1} acetone, 5 g L^{-1} butanol, 2 g L^{-1} ethanol, 2 g L^{-1} acetic acid, and 2 g L^{-1} butyric acid in distilled water. The internal standard contained 50 g L^{-1} isopropanol. The samples (250 µL) and the standard were mixed with 25 µL of internal standard and injected into the GC.

Statistics

All experiments were performed in duplicate. Using SAS software (version 9.1.3, SAS Institute, Cary, N.C.), the experimental data were analyzed by two-way ANOVA followed by LSD (t) pairwise comparisons of means. The statistical significance level (P-value) was set at 0.05.

Results and Discussion

DDGS Surface Morphology Changes

SEM images of the untreated DDGS are shown in figure 1. A large number of starch granules can be observed on the surfaces of the DDGS (fig. 1a). The surface under observation exhibited a smooth and textile-like structure (fig. 1b). After a hot water pretreatment at 160°C for 30 min, only a few starch granules remained on the DDGS (fig. 2a). A close look at 20,000× magnification revealed that swarms of tiny pores had formed on the surfaces of the DDGS and starch granules after the pretreatment (fig. 2b). These porous surfaces may provide more access area to enzymes, resulting in increased enzyme digestibility. Compared to the pore sizes of 1 µm × 4.5 µm on the corn stover surfaces generated by a 190°C hot water pretreatment (Mosier et al., 2005a), the pores created by the hot water pretreatment (160°C and 30 min) on the DDGS surfaces were much smaller (<1 µm). The much higher temperature used by Mosier et al. (2005a) may have contributed to the larger pore sizes observed in their report.

The images in figure 3 show that the crystalline structures of the DDGS were disrupted by the pretreatments with AEW (pH 2.7) and H_2SO_4 (pH 2.7) (fig. 3c). Large cracks can be observed on the fiber matrix of the H_2SO_4-pretreated samples. Compared to the hot water pretreatment (fig. 2a), the pretreatments in figure 3 are distinguished by more structural alterations or disintegration in the DDGS. The surfaces of hot water pretreated DDGS (fig. 2) and DDGS pretreated by the other methods (fig. 3) thus exhibited different morphologies. It is not clear why the DDGS samples treated with different methods exhibited different surface morphologies. The acid and alkaline may have reacted and/or dissolved different substances of the biomass during the pretreatment. In all pretreatments, fewer starch granules were observed on the DDGS surfaces compared to the untreated DDGS samples. The disappearance of most of these starch granules from the DDGS fiber matrix may be due to the solubilization of the starch granules into maltodextrans (Mosier et al., 2005b).

Pretreatment and Enzymatic Hydrolysis of DDGS

Generally, sugar concentrations in DDGS hydrolysates increased with an increase in enzymatic hydrolysis time (fig. 4). For 20% solids loading pretreatment, the highest glucose concentration (23.9 g L^{-1}) was obtained when DDGS was treated with 0.25% H_2SO_4 (v/v) for 72 h (fig. 4f). The NaOH-pretreated DDGS produced the least (<18 g L^{-1})

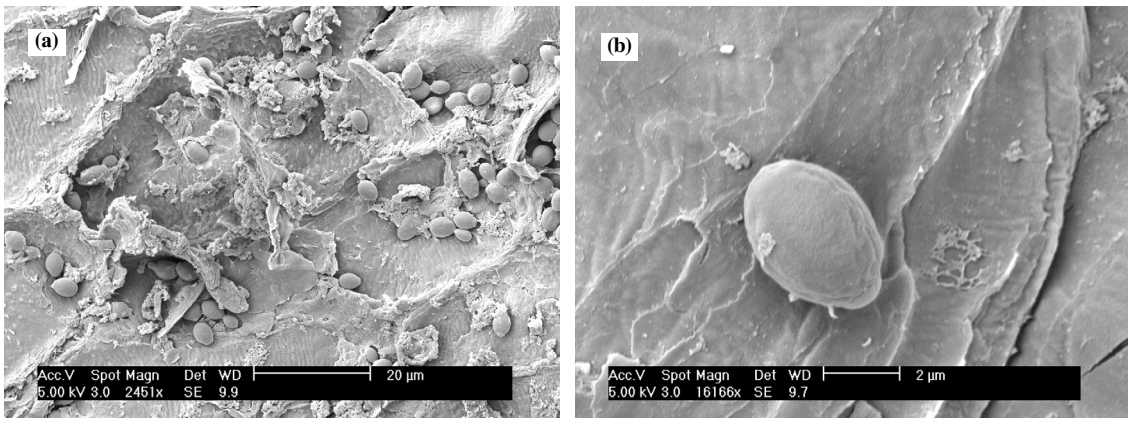

Figure 1. DDGS samples before pretreatment.

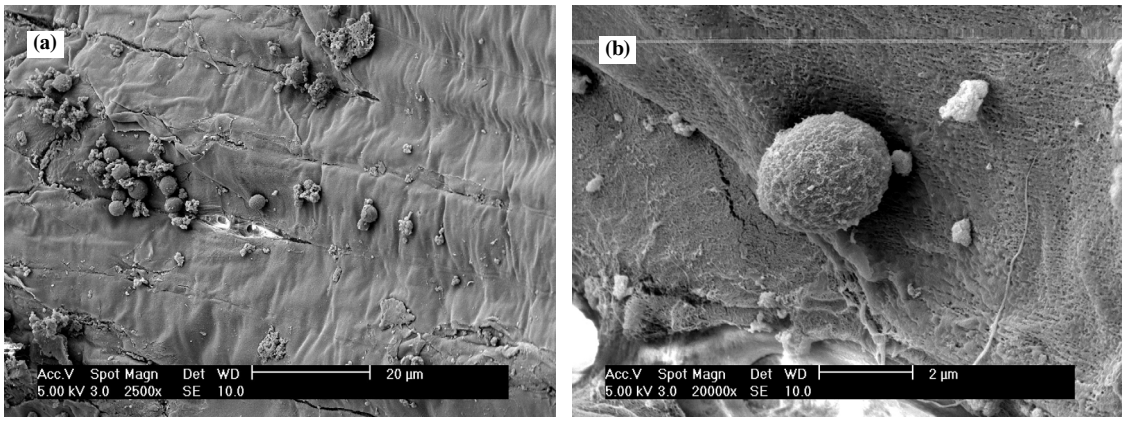

Figure 2. DDGS samples pretreated by hot water at 160˚C for 30 min (solids loading 20% w/w).

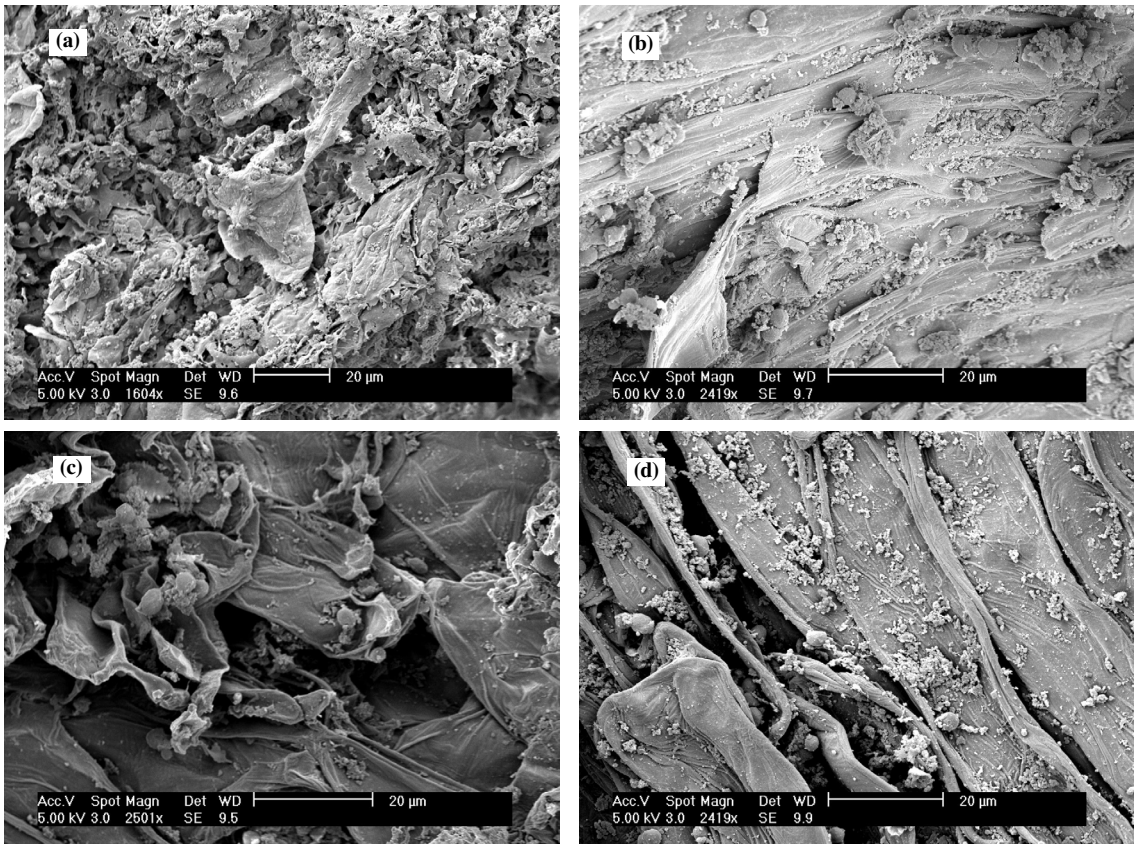

Figure 3. SEM images of DDGS pretreated at 160˚C for 30 min (solids loading 20% w/w) in: (a) AEW (pH 2.7), (b) ALEW (pH 11.7), (c) H_2SO_4 (pH 2.7), and (d) NaOH (pH 11.7). The magnification of (a) is 1600×, and that of (b), (c), and (d) is 2400× to 2500×.

amount of glucose after 72 h (fig. 4e). None of the glucose concentrations in figure 4 reached a plateau after a 72 h hydrolysis. Figure 4 also shows the sugar concentration from DDGS samples pretreated at 30% and 40% solids loads. At 30% solids loading, after a 12 h enzymatic hydrolysis, for the pretreatments shown in figures 4a, 4b, 4c, and 4d, the glucose concentrations were higher for samples pretreated at 30% solids loads than for those at 20% solids. A level-off in glucose concentration can be observed in figures 4a, 4c, 4e and 4f, indicating that a 72 h hydrolysis was sufficient for DDGS saccharification under the described conditions. Around 23.5 g L^{-1} glucose was obtained by hydrolyzing DDGS treated by 0.25% H_2SO_4. For the electrolyzed water pretreatments (AEW and ALEW), the highest glucose concentration was about 22 g L^{-1}, achieved for samples pretreated at 40% solids loading (figs. 4b and 4c).

Pretreatment of biomass by steam explosion can allow for $\geq$50% solids, while in a typical hot water pretreatment process, $\leq$20% solids loading is employed (Laser et al., 2002). In this study, three solids loadings in the range of 20% to 40% were used in the pretreatments, while in the enzymatic hydrolysis, a single solids concentration of 15% was employed. Therefore, the difference in the sugar yield during hydrolysis should be due to variation in solids loads in the pretreatments. In figure 4, it appears that the effect of solids loading on sugar yields is dependent on both solids content and pretreatment,

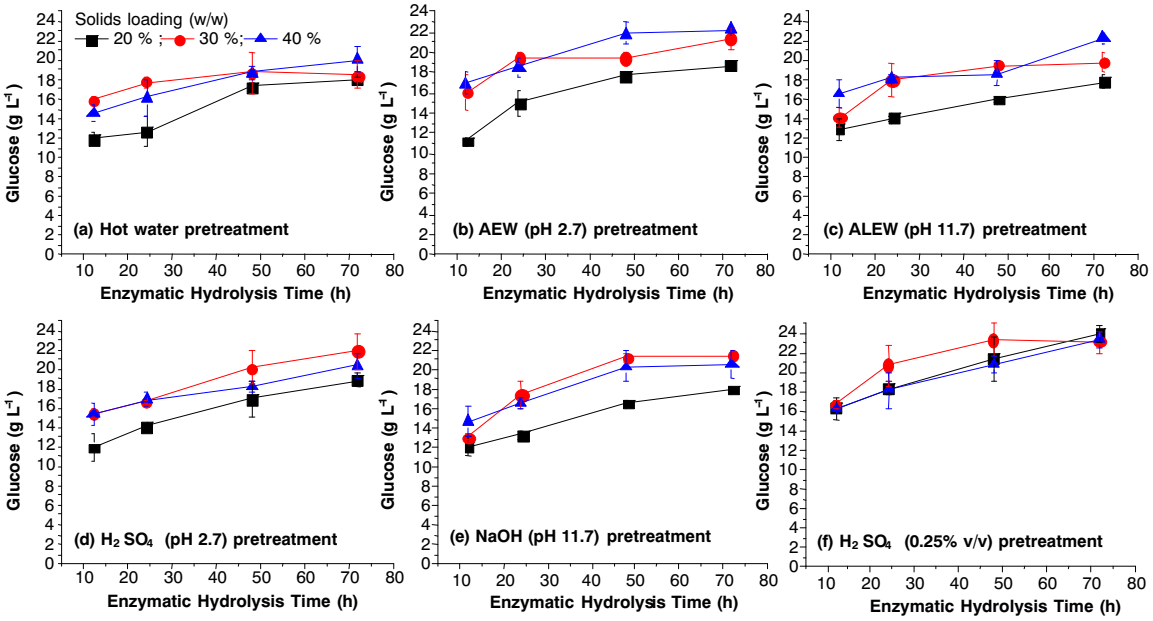

Figure 4. Glucose yield in DDGS hydrolysis for samples pretreated at 160°C for 30 min. The six pretreatments are: (a) hot water, (b) acidic electrolyzed water (pH 2.7), (c) alkaline electrolyzed water (pH 11.7), (d) H_2SO_4 (pH 2.7), (e) NaOH (pH 11.7), and (f) H_2SO_4 (0.25% v/v).

although a high solids content resulted in slightly higher sugar yields during the early stages of hydrolysis (up to 12 h). For the traditional acid or base pretreatments (figs. 4d, 4e, and 4f), after a 72 h saccharification, the solids loading at 40% did not result in higher sugar concentrations than the samples pretreated at 30% solids concentration. In a high-solids digestion test, Kim et al. (2008) also reported that the highest solids loading (30%, w/w) in controlled pH hot water pretreatment of wet distiller's grains did not result in the highest glucose yield. For the two electrolyzed water pretreatments (AEW and ALEW), an increase in glucose concentration was observed when the solids loading was increased from 20% to 40%. From the SAS analysis, solids loadings at 30% and 40% during pretreatment led to significantly higher glucose yields ($P < 0.0001$) than that of 20% solids loading, but no significant difference between the two solids levels (30% and 40%). Pretreatment (f) (0.25% H_2SO_4) produced a significantly higher amount of glucose compared with all other pretreatments. Pretreatment (b) (AEW) was the second highest, which produced significantly more glucose than pretreatments (c) (ALEW), (e) (NaOH, pH 11.7), (d) (diluted H_2SO_4, pH 2.7), and (a) (hot water). The glucose concentrations among pretreatments (c), (e), (d), and (a) were not significantly different.

Cell Concentration

The ALEW pretreatment (pH 11.7) was found to be the most favorable to *C. acetobutylicum* cell growth at 30% solids, where the cell mass reached a maximal level (fig. 5). In contrast, the dilute acid pretreatment (H_2SO_4 0.25% v/v) recorded the least amount of cell growth, with the cell mass only reaching approximately 50% of that obtained with the ALEW method. Interestingly, the cell growth was found to be sensitive to solids loading. A higher cell growth was attained at 30% solids loading than that for either 20% or 40% solids for all the pretreatments except NaOH (fig. 5e). Since the sugar concentrations of the samples pretreated at 30% solids were not significantly different from those of the samples pretreated at 40% solids (fig. 4), there might be more degradation products in 40% solids samples that inhibited the cell growth, again with NaOH the only exception.

ABE Production

At 20% solids loading, the ABE concentration for each treatment was low (<8 g L^{-1}), with butanol produced at 4.2 to 5.4 g L^{-1} and acetone at 2.0 to 2.5 g L^{-1} after 72 h (fig. 6). Acetic acid produced was 2.3 to 3.2 g L^{-1}, and butyric acid was 1.4 to 2.8 g L^{-1}. It appeared that 20% solids samples were not effective in acetone and butanol production, which may be due to the low sugar concentrations in the hydrolysates.

For the samples pretreated at 30% solids loading, the total ABE production from the hydrolysate from the pretreatment with ALEW (fig. 6c) reached 16.9 ±0.06 after 72 h, the highest concentration among all pretreatments. In comparison, for pretreatments (a), (b), (d), and (e) in figure 6, ABE was

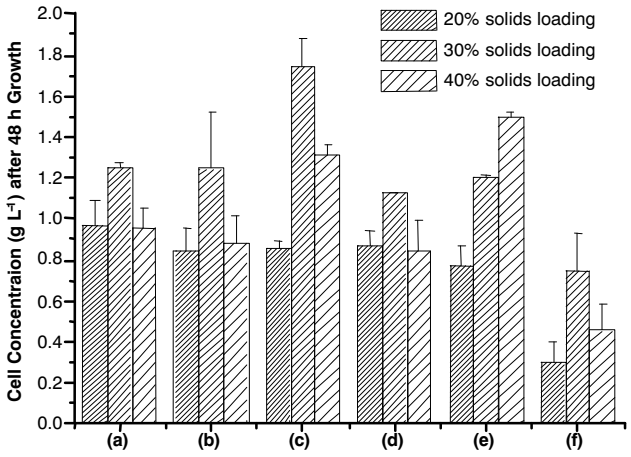

Figure 5. Cell concentration of *Clostridium acetobutylicum* 260 in the hydrolysates at 48 h with six pretreatments at three DDGS loadings: (a) hot water, (b) AEW (pH 2.7), (c) ALEW (pH 11.7), (d) H_2SO_4 (pH 2.7), (e) NaOH (pH 11.7), and (f) H_2SO_4 (0.25% v/v).

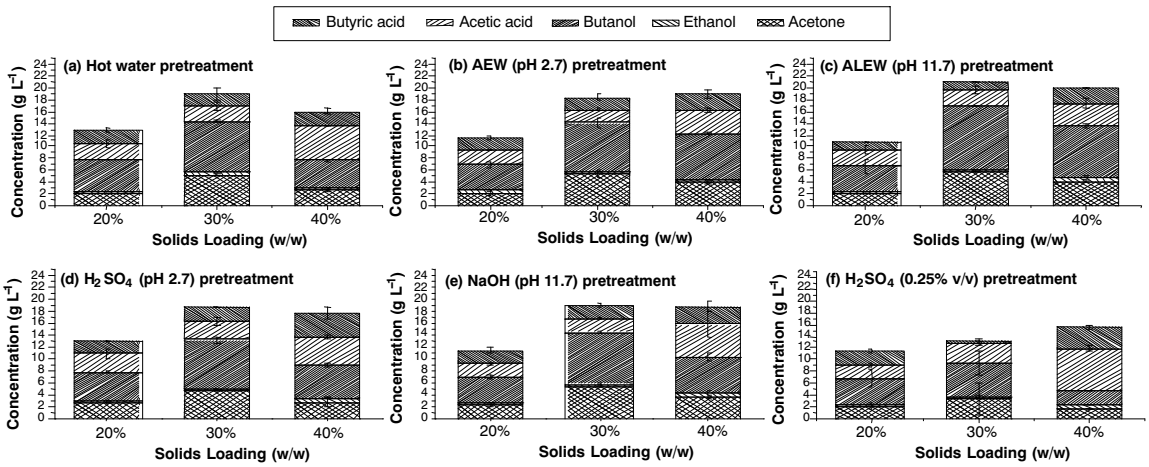

Figure 6. Solvents and acids production after 72 h fermentation from DDGS hydrolysates pretreated at solids loadings of 20%, 30%, and 40% by: (a) hot water, (b) AEW (pH 2.7), (c) ALEW (pH 11.7), (d) H_2SO_4 (pH 2.7), (e) NaOH (pH 11.7), and (f) H_2SO_4 (0.25% v/v).

produced at 14 to 15 g L^{-1} in 72 h (figs. 6a, 6b, 6d, and 6e). Most of the differences were due to butanol production. Butanol was produced at the highest level under pretreatment (c), reaching 10.7 ±0.12 g L^{-1} after 72 h, whereas it was produced at 5.7 to 8.6 g L^{-1} under the other pretreatments.

Acetone yield was typically at ~5 g L^{-1} at 72 h and was unaffected by pretreatments (a) through (e) in figure 6. Acid production was comparable for pretreatments (a) through (e), with acetic acid produced at 2 to 3 g L^{-1} and butyric acid at 1 to 2 g L^{-1} after 72 h (fig. 6). The pretreatment with ALEW appeared to result in a favorable outcome for ABE formation, which may be linked to the better cell growth observed under this condition (fig. 5). When compared to the solvent production using a model system where a pure sugar mixture that was representative of sugars present in hydrolzyed DDGS was used for ABE fermentation by *C. acetobutylicum* P260, the ABE yield from the ALEW hydrolysate reached 83% of the yield of the model system (Ezeji and Blaschek, 2008).

The most drastic effect of a pretreatment on fermentation was observed with the H_2SO_4 0.25% v/v pretreatment (fig. 6f). For this dilute acid pretreatment, the butanol concentration only reached ~5.5 g L^{-1} after 72 h, half of the maximal level observed with the ALEW pretreatment (fig. 6c). The acetone production was also minimal (fig. 6f). With regard to acid formation, the acetic acid production was similar to that obtained with other solids loadings, whereas butyric acid was produced at ~25% of the average level found under other conditions. Since the sugar yield under this condition was the highest (fig. 4f) and the cell density was the lowest (fig. 5), the low solvent yield for samples pretreated by this method may be attributed to the presence of inhibitory compounds in the hydrolysate. It should be noted that during pretreatment and hydrolysis of fiber-rich agricultural biomass, compounds such as salts; acetic, ferulic, glucuronic, ρ-coumaric acids; and phenolic compounds are produced. These compounds have been shown to have negative effects on growth and ABE production by solventogenic clostridia (Ezeji et al., 2007b).

For the 40% solids loading pretreatment (fig. 6), the highest yield of solvents was again observed with the ALEW pretreatment (fig. 6c), with an ABE production of ~14 g L^{-1} after 72 h. All other pretreatments produced much less solvents compared to that from fermentation at 30% solids. However, the concentrations of acids for the 40% solids loading samples were higher. For instance, the acetic acid yield was in the range of 4 to 7 g L^{-1} at 40% solids loading and in the range of 2 to 6 g L^{-1} at 30% solids loading. These results suggest that pretreatments at 40% solids may be associated with generation of compounds that regulate solventogenesis in a concentration-dependent manner (Ezeji et al., 2007b). This is also supported by the low cell concentrations seen in figure 5.

According to the statistical analysis, 30% solids loading during a pretreatment led to a significantly higher ($P < 0.0001$) butanol yield than that from 40% and 20% solids loadings. The butanol yield from 40% solids loading was significantly higher than that of the 20% solids loading. With ALEW pretreatment (c), the fermentation process produced significantly more butanol than with the other pretreatments except AEW (b). Pretreatment with 0.25% H_2SO_4 (f) led to significantly less butanol than the other pretreatments. The butanol yields among pretreatments (b), (e), (a), and (d) were not significantly different. The favorable butanol fermentation from pretreatment with ALEW may be attributed to ALEW's strong reducing property (with an ORP of < −795 mV). An unfavorable oxidation-reduction potential (ORP) has been cited as a cause of poor fermentability (Leonard and Hajny, 1945). Collingsworth and Reid (1935) found that the addition of reducing agents to fermentation media improved fermentation. Improved fermentation was also observed when Na_2SO_3, $NaHSO_2$, $Na_3SO_3 \cdot 5H_2O$, $Na_2S_2O_3$, $Na_2S_2O_5$, $KHSO_3$, Na_2S, sulfite waste liquor, alkali-decomposed sugar, ascorbic acid, cysteine, and reduced iron filings were added to wort hydrolysate to overcome unfavorable ORP (Leonard and Hajny, 1945; Leonard and Peterson 1947). No significant difference in ethanol production was found for any of the pretreatments.

Overall, the solids loadings in a pretreatment exhibited a greater impact on solvent production than on enzyme hydrolysis. There appears to be a solids loading at which an optimal production of ABE can be achieved. Further studies are needed to examine the generation of fermentation inhibitors in DDGS as affected by pretreatments and solids loadings.

Conclusion

The dilute acid (0.25% v/v) pretreatment was the most effective for monomeric sugar production, but it was also the least fermentable. The fermentation of electrolyzed water pretreated DDGS was successful. The solids loading during a pretreatment significantly affected both the sugar yield from enzymatic hydrolysis and the ABE production during fermentation of DDGS hydrolysates. Among the three solids loadings investigated in this work, pretreatments with 30% solids resulted in the highest ABE production. Under this condition, up to 0.10 g ABE or 0.06 g butanol can be expected per gram of DDGS. The ALEW pretreatment was superior to other pretreatments, and the DDGS hydrolysates from this treatment supported optimal *C. acetobutylicum* P260 growth and ABE production.

Acknowledgements

This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (Grant No. 2006-35504-17419) and the U.S. Department of Energy (DOE) DE-FG36-046014220 grant to the *Midwest Consortium for Biobased Products and Bioenergy.* We thank Dr. Nasib Qureshi for the *C. acetobutylicum* P260 culture.

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