Renewable Energy 52 (2013) 16–22

Contents lists available at SciVerse ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Biobutanol production from fiber-enhanced DDGS pretreated with electrolyzed water

Xiaojuan Wang [a,b], Yi Wang [c,d], Bin Wang [b], Hans Blaschek [b,c], Hao Feng [b,d,*], Zhiyi Li [a]

[a] Department of Chemical Machinery, Dalian University of Technology, Dalian, China
[b] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[c] Center for Advanced BioEnergy Research, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[d] Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA

ARTICLE INFO

Article history:
Received 2 September 2011
Accepted 9 October 2012
Available online 13 November 2012

Keywords:
Fiber-enriched DDGS
Butanol
Electrolyzed water
Pretreatment
Enzymatic saccharification
Fermentation

ABSTRACT

DDGS (distiller's dried grains with solubles) is a major co-product in dry-grind ethanol production from corn. A recently developed physical process separates DDGS into two value-added components: a fiber-enriched DDGS and a portion that is rich in oil and protein. Electrolyzed water, a new pretreatment catalyst was employed to pretreat fiber-enriched DDGS. Four temperatures (130, 145, 160, and 175 °C) and three treatment times (10, 20, and 30 min) were examined in the pretreatment with a solid loading of 20% w/w. Other pretreatment methods, such as diluted sulfuric acid, alkaline solution, and hot water, were also tested for comparison purposes. Fifteen FPU cellulase/g cellulose, 40 units β-glucosidase/g cellulose, and 50 units xylanase/g dry biomass were used in the enzymatic hydrolysis at 50 °C and 10% solid loading. The hydrolyzates were fermented by *Clostridium beijerinckii* BA 101 at 35 °C in an auto-controlled Six-fors fermentor with continuous mixing. The highest sugar yield was achieved when using the acidic electrolyzed water treatment at 175 °C for 10 min, with 23.25 g glucose, xylose and arabinose released from 100 g fiber-enriched DDGS. The *C. beijerinckii* fermentation produced 5.35 g ABE (acetone, butanol, and ethanol) from 100 g dry fiber-enhanced DDGS. This study demonstrated that DDGS pretreated with electrolyzed water and hydrolyzed with commercial enzymes could be used to produce biobutanol without detoxification.

1. Introduction

The exhaustion of fossil oil has stimulated considerable interest in fuel and chemical production from renewable resources. Bio-ethanol has been used as an alternative energy source for replacing fossil fuel in both the U.S. and other countries. Currently, bio-ethanol production in the U.S. is largely from corn starch fermentation. For instance, in 2010, over 13 billion gallons ethanol was produced from 4.65 billion bushels of corn in the U.S., as reported by the Renewable Fuels Association [1]. In a typical dry-grind ethanol process, considerable amounts of distillers dried grains with solubles (DDGS) are produced. For every bushel (56 pounds) of corn converted into ethanol (2.7 gallons), 18 pounds (8.2 kg) of DDGS are generated. As a result, 38 million metric tons of DDGS was produced in 2010. According to the U.S. Grains council, because of the mandate of the Renewable Fuels Standard II, DDGS production is expected to continue to grow through 2015 [2]. DDGS has high protein content and is mainly used as a food supplement for beef and swine. It currently has a value of approximately \$0.04 lb^{-1}. As the supply of DDGS increases, its price is anticipated to decrease. Therefore, measures must be taken to increase the value of DDGS in order to keep dry-grind ethanol production competitive and sustainable.

Efforts have been made in recent years to produce value-added products utilizing DDGS as a feedstock. A new method using aspiration to enhance the value of DDGS has recently been developed by Singh et al. [3,4]. There are two end-products obtained from this process. One is rich in oil and protein, and can be used for animal feed. The other is fiber-enriched DDGS, containing mainly fibers that can be converted to fermentable sugars.

In recent years, a renewed and growing interest for the production of acetone, butanol and ethanol (ABE) has been stimulated by the increasing demand in biofuels production. ABE fermentation was industrialized in the United States during the first

* Corresponding author. Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 382F-AESB, 1304 W Pennsylvania Ave, Urbana, IL 61801, USA. Tel.: +1 11 217 244 2571; fax: +1 11 217 333 9329.
E-mail address: haofeng@illinois.edu (H. Feng).

0960-1481/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.renene.2012.10.011

half of last century, but was unable to continue due to unfavorable economic conditions brought by competition from the petrochemical industry [5]. Butanol is the main product of ABE fermentation, and has some attractive properties. The advantages of butanol include 30% higher energy content over ethanol, low vapor pressure, no sensitivity to water, less volatile, less flammable and mixable with gasoline at any proportion [6]. ABE production from different feedstocks employing various processing methods has been examined [7–12].

A key step in biofuel production from lignocellulose biomass is pretreatment. The purpose of a pretreatment is to alter the physical and chemical structure of a feedstock as well as its chemical composition so that the carbohydrate fraction can be easily accessed and converted into fermentable sugars during enzymatic hydrolysis [13]. Five promising pretreatment techniques were selected and evaluated using a single feedstock (corn stover) in a project funded by USDA [14,15]. The five methods included the dilute acid, hot water, ammonia fiber explosion (AFEX), ammonia recycle percolation (ARP), and lime pretreatments [16–20]. The results showed that a pretreatment with a higher sugar conversion yield was often accompanied by either harsh pretreatment conditions or a higher cost in equipment and downstream separation. Due to the low lignin content, corn fiber was considered to be a low cost feedstock that can be pretreated with hot water under mild pretreatment conditions [21].

The use of electrolyzed water in biomass pretreatment was first proposed and tested at University of Illinois in 2006. This method has been used for the pretreatment of selected biomass, such as *Miscanthus* and DDGS [12,22]. There are two types of electrolyzed water: acidic electrolyzed water (AEW) with a pH of $\leq$2.7 and an oxidation reduction potential (ORP) of 1123–1170 mV and alkaline electrolyzed water (ALEW) having a pH of $\geq$11.4 and ORP of <-795 mV. Compared with traditional pretreatment methods, AEW and ALEW may provide a new and environmentally friendly alternative for pretreatment of biomass.

In this paper, the electrolyzed water was used as the pretreatment agent for the production of ABE from fiber-enriched DDGS. The pretreated-samples were hydrolyzed by commercial enzyme solutions. At a fixed solid loading, a maximum mono-sugar yield was obtained. Finally, the fermentability of the hydrolyzates from electrolyzed water pretreated-samples and those pretreated with other methods was compared.

2. Materials and method

2.1. Materials

The fiber-enriched DDGS was provided by Dr. Vijay Singh at University of Illinois at Urbana-Champaign and stored at −20 °C. The enzyme loading and sugar conversion yields were calculated based on the following DDGS composition in Table 1, which was determined by the Experiment Station Chemical Laboratories, University of Missouri. The moisture content of DDGS was determined from weight loss after drying at 105 °C till a constant weight was attained.

Table 1
Composition of fiber-enhanced DDGS.

Composition%	Sample
Cellulose	10.36
Hemicellulose	33.28
Lignin	1.05
ADF	11.07
NDF	44.45
Crude protein	16.10
Crude fat	8.27
Ash	3.88

Cellulase (Spezyme CP) and β-glucosidase (Novo 188) were purchased from Sigma–Aldrich (St. Louis, MO), and xylanase was supplied by Enzyme Development Corporation (New York, NY, USA). The activities of the enzymes were determined using published methods or assays provided by the enzyme companies [23,24]. The release of reducing sugars in the cellulase and xylanase assays was determined as described in reference [25]; while the release of glucose in the β-glucosidase assay was determined using a glucose assay kit purchased from Sigma–Aldrich.

2.2. Electrolyzed water pretreatment and enzymatic hydrolysis

The fiber-enriched DDGS was pretreated with acid electrolyzed water (AEW) and alkaline electrolyzed water (ALEW) at four temperatures (130, 145, 160, and 175 °C) and three times (10, 20, and 30 min). The solid loading was set at 20% w/w, which was achieved by adding 8 g DDGS (dry weight) in each tubular reactor to get a total slurry of 40 g. All the pretreatments were conducted in tubular reactors (1 inch OD × 7 inch L). A SBL-2D fluidized-bed sand bath (4000 W, Techne Inc. Burlington, NJ, USA) equipped with a TC-8D temperature controller was used for heating. The DDGS samples were presoaked in AEW or ALEW for 4 h in the reactors. When the bath reached the set temperature, the tubular reactors with DDGS slurry were inserted into the sand bath. Following the treatment, the reactors were immersed into ice water to terminate the reaction.

Three other pretreatments, i.e., sulfuric acid, sodium hydroxide and hot water, were also carried out for comparison purposes. The treatment conditions for these methods were chosen and modified based on published data. The 1% wt sulfuric acid and 2.5% sodium hydroxide pretreatments were conducted at 140 °C with a treatment time of 20 min [27]. For the hot water pretreatment, the conditions were 160 °C and 20 min [28]. Since the microorganism employed in this paper can use both glucose and xylose to produce ABE, the pretreatment conditions for the three pretreatments were chosen based on the highest glucose and xylose combine yields. A solid loading of 20% w/w was used for these pretreatments. The pretreated slurries were washed into 100 ml VITLAB flasks using a sodium citrate buffer (pH 5.0, 100 mM). The pH of the slurry was adjusted to pH 5.0. The enzyme loading was: 15 FPU cellulase/g cellulose, 40 U β-glucosidase/g cellulose; and 50 U xylanase/g dry biomass. After the enzymes were loaded, the total weight of the slurry was adjusted to 80 g with the sodium citrate buffer to achieve a solid loading of 10% w/w.

Enzymatic hydrolysis was performed in flasks in a shaker water bath (Aquatherm water bath shaker, New Brunswick Scientific Co. INC, NJ, USA) at 50 °C and 225 rpm. During the hydrolysis, aliquots of 2 ml were sampled at 6, 12, 24, 48, and 72 h. The samples were immersed into boiling water for 5 min to deactivate the enzymes and then transferred to icy water to cool down in preparation for HPLC analysis.

2.3. ABE fermentation

The ABE fermentation was carried out using *Clostridium beijerinckii* BA 101 grown from laboratory stocks of spores. The fiber-enhanced DDGS hydrolyzates were centrifuged at 10,000 g in a Sorvall RC 5B Superspeed Centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA). The sugar containing supernatant was used for fermentation. Laboratory stocks of *C. beijerinckii* BA 101 spores were heat-shocked at 80 °C for 10 min, followed by cooling on ice for 5 min. The heat-shocked spores were inoculated into

a tryptone–glucose–yeast extract (TGY) medium (in 50 ml screw capped Pyrex bottles at an inoculum ratio of 1%) and incubated at 35 ± 1 °C for 16–18 h in an anaerobic chamber maintained under a gas mixture of 85% N_2, 10% CO_2, and 5% H_2. Subsequently, 15 ml actively growing TGY culture was used to inoculate the fermentation broth containing a total volume of 300 ml for solvent production. Fermentation was conducted in an auto-controlled Sixfors benchtop bioreactor (Infors AG, Bottmingen, Switzerland). Before inoculation, the DDGS hydrolyzate supplemented with yeast extract of 1 g/L final concentration was autoclaved at 121 °C for 15 min. On cooling, oxygen-free nitrogen was flushed through the broth overnight for anaerobiosis. Filter-sterilized P2 medium stock solutions were added and the pH was adjusted to 6.5 using filter-sterilized 2 M NaOH or HCl [29,30]. The temperature of the medium was controlled at 35 °C. During fermentation, 2 ml culture aliquots were collected to quantify cell, ABE, acid and sugar concentrations. The pH values were continuously recorded by the Sixfors controlling program Iris V5.

2.4. Analytical methods

Monosaccharide concentrations were determined using high pressure liquid chromatography (HPLC). The HPLC system consisted of a Waters (Milford, MA, USA) 2659 Separation Module, a Waters 717 plus auto sampler, and a Waters 410 refractive index detector. A Bio-Rad HPX-87P column (Bio-Rad Laboratories Inc., Hercules, CA, USA) with a guide column (30 × 4.6 mm) was used. The column temperature was 85 °C and that of the guide column was 30 °C. The mobile phase was ultrapure water at a flow rate of 0.6 mL/min.

ABE, acetic acid, and butyric acid concentrations were quantified by gas chromatography (Hewlett Packard, Avondale, PA, USA). The GC was equipped with a flame ionization detector (FID), an 1829 × 2 mm glass column (10% CW-20 M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW), and an Agilent 7683 series automatic liquid sampler (Agilent Technologies, Inc. Palo Alto, CA, USA). 5 g/L of 1-proponal was used as the internal standard. The liquid samples were first centrifuged at 10,860 g for 3 min using a 5415 R Centrifuge (Eppendorf North America, Westbury, NY, USA), and then filtrated through 0.2 μm membrane and finally measured on the GC.

3. Results and discussion

3.1. Sugar yield from enzymatic hydrolysis of DDGS

Fig. 1 shows the sum of glucose, xylose, and arabinose yield, referred to as total sugar in the hydrolyzates for the samples pretreated by AEW and ALEW under different conditions. For both pretreatments, the yield of total sugar increased with an increase in pretreatment temperature. At a lower temperature (130 °C or 145 °C), an increase in the total sugar yield can be observed when the pretreatment time was increased. At high temperatures (160 and 175 °C), however, extended pretreatment time may not result in a higher total sugar yield. When treating the samples at 175 °C, increasing the treatment time from 10 to 30 min was accompanied by a decrease in total sugar yield. This might be caused by the degradation of five carbon sugars (xylose) at high temperatures. Similar xylose degradation was observed in hot water pretreatment of corn stover and dilute acid pretreatment of switchgrass [17,26]. In the AEW-treated samples, the highest total sugar yield was obtained at 175 °C and 10 min, with 23.25 g sugars (glucose, xylose, and arabinose) released from 100 g (dry weight) fiber-enriched DDGS (Fig. 1a). A similar sugar release from the ALEW pretreatment could be observed. The highest yield for ALEW was 21.32 g of total sugar from 100 g (dry weight) fiber-enriched DDGS at 175 °C and 10 min (Fig. 1b). It can also be seen that the sugar yields from ALEW-pretreated-samples are lower than those obtained from the AEW-pretreatment.

Table 2
Hydrolyzates from different pretreatment methods (72 h enzymatic hydrolysis).

g/l	AEW 175 °C 10 min	Hot water 160 °C 20 min	NaOH 140 °C 20 min	Sulfuric acid 140 °C 20 min
Glucose	15.65	12.87	10.23	14.96
Xylose	3.44	2.14	2.55	6.87
Arabinose	3.79	2	2.23	4.34
Total sugars	23.25	17.01	15.01	26.16

The sugar concentrations in the hydrolyzates obtained from DDGS samples treated with different methods are listed in Table 2. The highest sugar yield (26.16 g/L) was from the sulfuric acid pretreated-samples, while that from the NaOH (15.01 g/L) had the lowest. In the hot water pretreatment, 17.01 g/L total sugar was released, slightly higher than that obtained following alkaline pretreatment. These hydrolyzates were subjected to fermentation without any concentration and detoxification.

3.2. ABE fermentation of hydrolyzates from different pretreatment methods

The fermentability of the hydrolyzate obtained following the AEW-pretreatment of DDGS was compared with those from hot water, sulfuric acid, and NaOH pretreatments, and the results are summarized in Table 3.

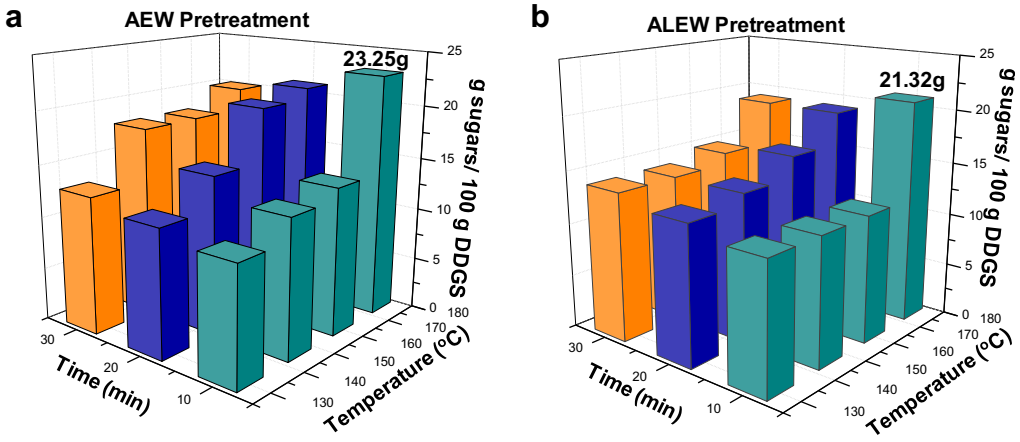

Fig. 1. Sugar yield in hydrolysis for samples pretreated by AEW and ALEW at different conditions.

Table 3
ABE fermentation of hydrolyzates from different pretreatment methods (final data after 96 h fermentation).

g/l	AEW	Hot water	NaOH	Sulfuric acid
Acetone	1.54	1.33	1.21	0
Butanol	3.62	3.64	2.09	0
Ethanol	0.30	0.17	0.16	0
ABE	5.46	5.14	3.46	0

The hydrolyzate from the AEW-pretreatment was obtained using DDGS treated at 175 °C and 10 min that gave the highest total sugar yield. Fig. 2 shows the ABE production, sugar consumption, and OD values during the fermentation with the hydrolyzates from the AEW, sodium hydroxide, and hot water pretreated-samples; while Fig. 3 shows the pH changes during the fermentation with each hydrolyzate. The fermentation of the hydrolyzate from the sulfuric acid pretreatment did not produce detectable ABE and was hence not included in Fig. 2. For the AEW-pretreated DDGS, there was no ABE production during the first 22 h (Fig. 2a). However, a rapid cell growth as measured by optical density increase (Fig. 2b) and a reduction of sugar concentration from 23.3 g/L to 18.4 g/L were observed during this period (Fig. 2b). Subsequently, an increased ABE production was observed until the concentration reached 4.33 g/L, while the sugar concentration deceased to 5.5 g/L at 45 h. The ABE production after 45 h was less pronounced, and at 96 h, the ABE concentration was 5.46 g/L and that for butanol was 3.62 g/L. The ABE fermentation described by Ezeji and Blaschek using a mixed sugar solution and produced up to 20 g/L ABE for a total sugar concentration of 60 g/L [31]. In this work, the concentration of ABE produced from the hydrolyzate of AEW-pretreated DDGS was 5.47 g/L (0.23 g ABE/g sugar), or 56%–64% of the yield reported by Ezeji and Blaschek [31]. Since the total sugar concentration used in the fermentation was only 23.25 g/L, the relatively low ABE yield may be attributed to the fact that the fermentation was not conducted at the optimal sugar concentration.

The ABE yield, OD value, and sugar consumption for the hot water hydrolyzate are shown in Fig. 2c, d, and Fig. 3b, where the solvent production exhibited a different pattern compared to that from the AEW hydrolyzate (Fig. 2a & b). There was no sugar consumption, OD and pH changes, or ABE production in the first 25 h (Fig. 2c & d). Following that, a rapid cell growth was observed, which was accompanied by moderate sugar consumption and ABE production. The highest ABE concentration (5.19 g/L) was obtained after 45 h fermentation, and a slight reduction in the ABE concentration was seen thereafter.

The fermentation of the NaOH hydrolyzate showed a significant delay in both cell growth and ABE production (Fig. 2e & f). No

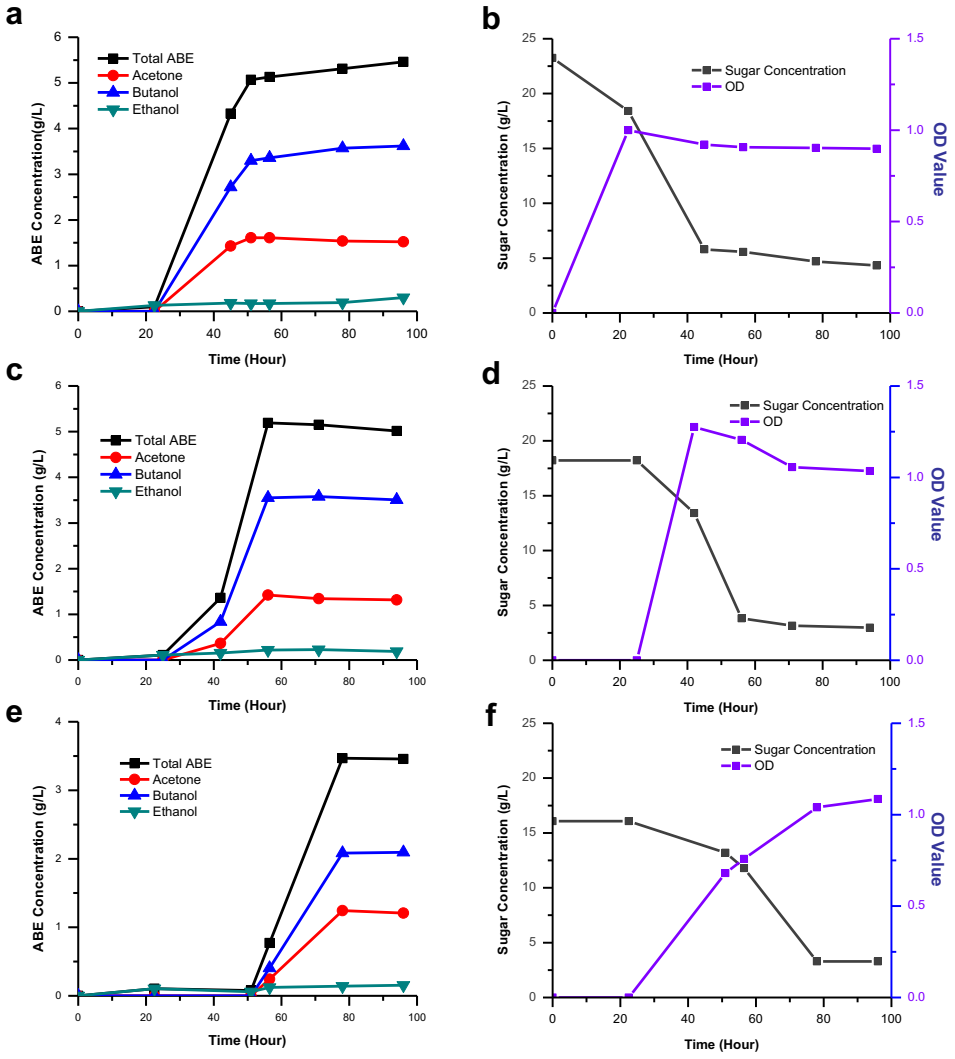

Fig. 2. ABE production, sugar consumption, and OD in fermentation of hydrolyzates from different pretreatments: AEW – (a) and (b); hot water – (c) and (d); NaOH – (e) and (f).

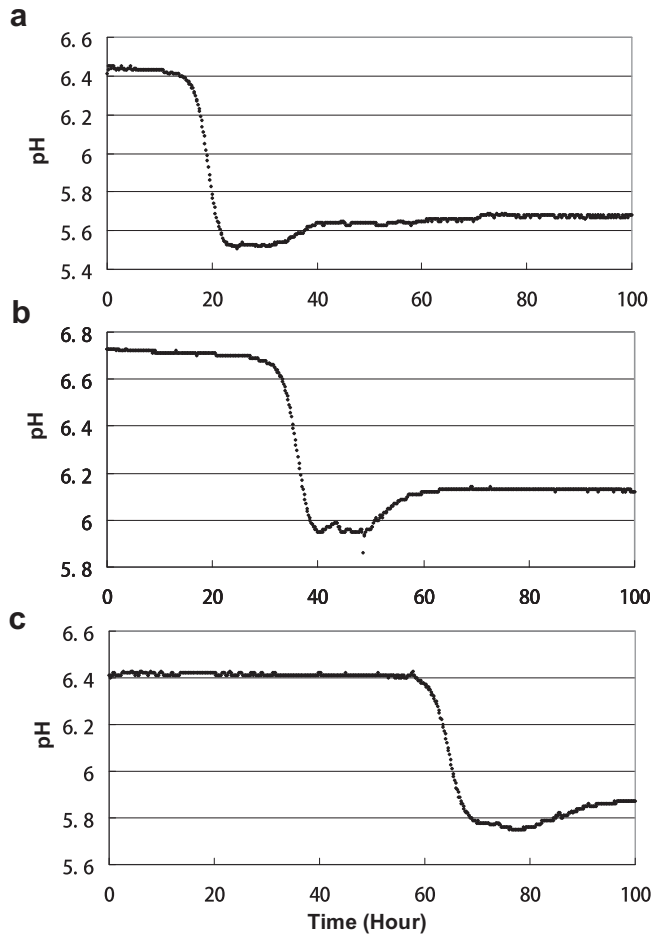

Fig. 3. Changes of pH in fermentation of hydrolyzates from different pretreatments: AEW – (a); hot water – (b); NaOH – (c).

ABE production was observed till 45 h. The cell growth was also negligible in the first 22 h with minimal sugar consumption. Since *C. beijerinckii* BA 101 is sensitive to the existence of inhibitors, the absence of ABE and lack of growth of the cells is an indication of the existence of inhibitory compounds in the NaOH hydrolyzate [32]. This is also true for the hydrolyzate obtained from the hot water pretreatment. The delay in ABE production from the NaOH pretreated-samples was 45 h and that for the hot water treated was 25 h. Some inhibitors should have also presented in the AEW-pretreated-samples as no ABE production was detected for 22 h. However, the rapid cell growth during the first 22 h suggested that the inhibition only had an impact on ABE production and not cell growth. In addition, the highest ABE concentration was obtained in AEW-pretreated DDGS, showing a better fermentability when compared to samples pretreated by other methods.

In this study, the delay in cell growth was less than 22 h in the fermentation of the hydrolyzate from AEW-treated samples (Fig. 2b). In contrast, there was no cell growth in the first 22 h in the fermentation for the hydrolyzates from the hot water and NaOH pretreated DDGS. It is likely that the inhibitor concentration was relatively low in the AEW DDGS hydrolyzates and once the cells adapted to the inhibitor-containing environment, cell growth and production of ABE was initiated. The inhibitors in the hydrolyzates might include compounds like furfural, HMF and other sugar degradation products with low concentrations. The most toxic compounds for ABE fermentation are those in the phenolic group [33]. Ezeji and Blaschek [31] reported that the addition of selected phenolic compounds, such as syringaldehyde, ρ-coumaric acid, and ferulic acid, significantly impacted the growth of *C. beijerinckii* BA 101, and hence ABE production. Furfural and HMF did not show any inhibitory to *C. beijerinckii* BA 101, but showed a stimulatory effect on growth and ABE production at concentrations up to 2.0 g/L. In another research, syringaldehyde, ρ-coumaric acid, and ferulic acid were found to be inhibitory to *C. beijerinckii* BA 101 at concentrations as low as 0.3 g/L [34].

3.3. DDGS surface morphology

The SEM micro-images of the fiber-enriched DDGS samples before pretreatment are shown in Fig. 4a, b and c. Fig. 4a shows a piece of corn bran from untreated DDGS, with the bran surface and the cross-section or edge in the view. On the bran surface, the alignment of some fibrous matter could be seen underneath a dense and relatively smooth surface, while at the edges, some fragments are packed together and formed a porous structure. Fig. 4b and c are a close view of the edges and surface. Fig. 4d, e, and f are images of fiber-enriched DDGS following an AEW-pretreatment at 175 °C and 10 min. Some breakage or tiny holes can be observed on the bran surface (Fig. 4d). This becomes more evident in the close-up shown in Fig. 4f. Compared to the SEM images before pretreatment (Fig. 4b & c), most of the fragments packed on the bran surface and the edge were removed during pretreatment (Fig. 4e & f). Since a high concentration of hemicellulose depolymerization products (e.g. xylose and arabinose) were detected in the liquid following the pretreatment, the fragments covering the DDGS samples should mostly be hemicellulose in nature. There are some small granules on the bran surface and at the edge (Fig. 4d and 4f). From their sizes (<1 μm) and location, they may not be starch granules. The corn starch granules in DDGS as observed by Wang et al. were on the surface with a size of about 2 μm, while those in Fig. 4f are much smaller and embedded in the sub-surface [12]. The small particulates embedded or semi-embedded on the fiber surface might be proteins and a further analysis is needed to ascertain this.

3.4. Mass balance

A mass balance using 100 g fiber-enriched DDGS (dry basis) was performed in this study, as shown in Fig. 5.

The pretreatment for the mass balance calculations was carried out at 175 °C and 10 min with AEW. As can be seen from Fig. 5 52.7 g DDGS feedstock were dissolved during pretreatment. Some of this was fat that can be washed into the liquid portion after the pretreatment. A small amount of cellulose was also degraded as glucose was detected by HPLC of the liquid fraction with a concentration of 1.71 g/L. The xylose and arabinose concentrations in the liquid portion were 1.14 g/L and 2.38 g/L, respectively. There may be oligomers of 5 carbon sugars present in the liquid portion as well. Following the pretreatment, the solid portion (47.3 g) was used for enzymatic hydrolysis. After saccharification, 21.7 g insoluble solids remained which mainly contained protein and ash (19.98 g protein and ash were in the untreated 100 g fiber-enriched DDGS). Almost 1 L hydrolyzate was obtained from 100 g fiber-enriched DDGS, which contained 15.65 g/L glucose, 3.44 g/L xylose, and 3.79 g/L arabinose. Finally, 5.46 g/L ABE was produced after fermentation from 100 g dry DDGS. In the work reported by Wang et al., 10 g/L ABE was produced from 100 g (dry basis) traditional DDGS [12]. Since Wang et al. performed detoxification and concentration to increase the sugar concentration in the hydrolyzate before fermentation, a direct comparison in terms of ABE yield is not possible [12].

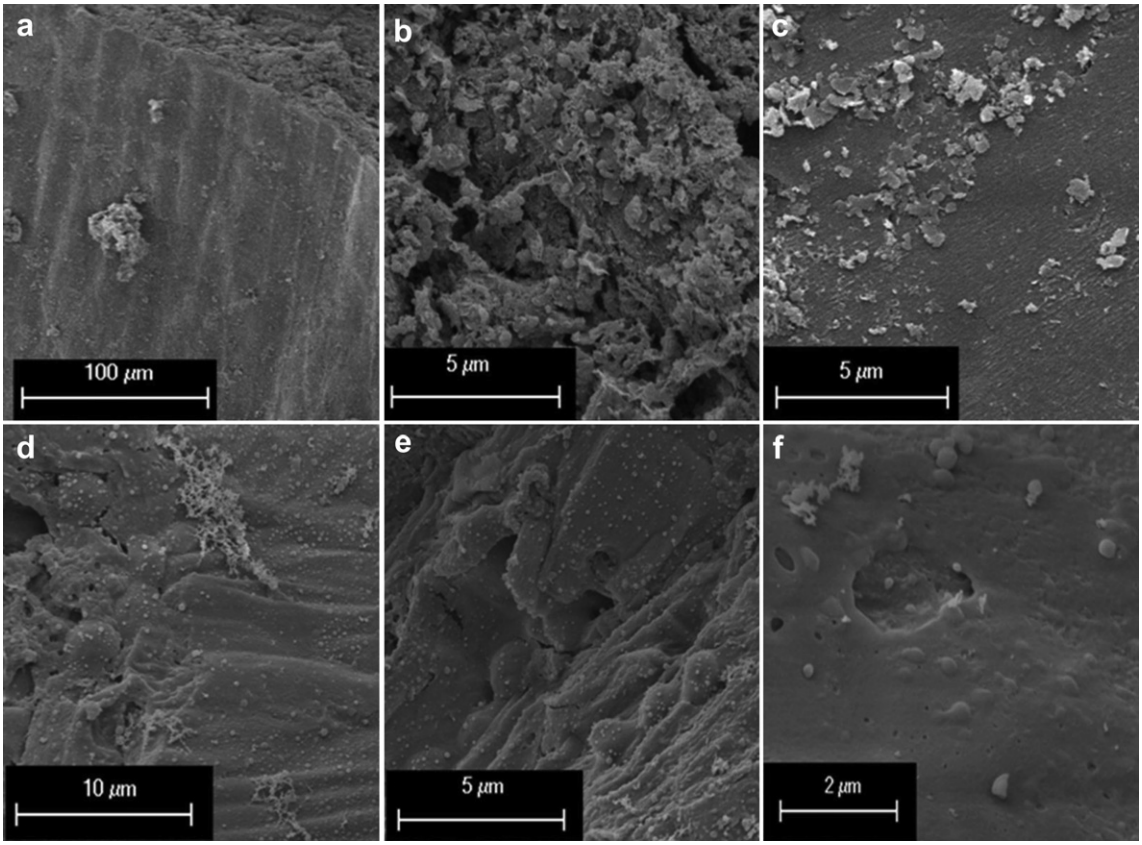

Fig. 4. SEM of AEW-pretreated fiber-enhanced DDGS samples at 175 °C and 10 min: (a), (b), and (c) were non-pretreated samples; (d), (e), and (f) were samples after pretreatment.

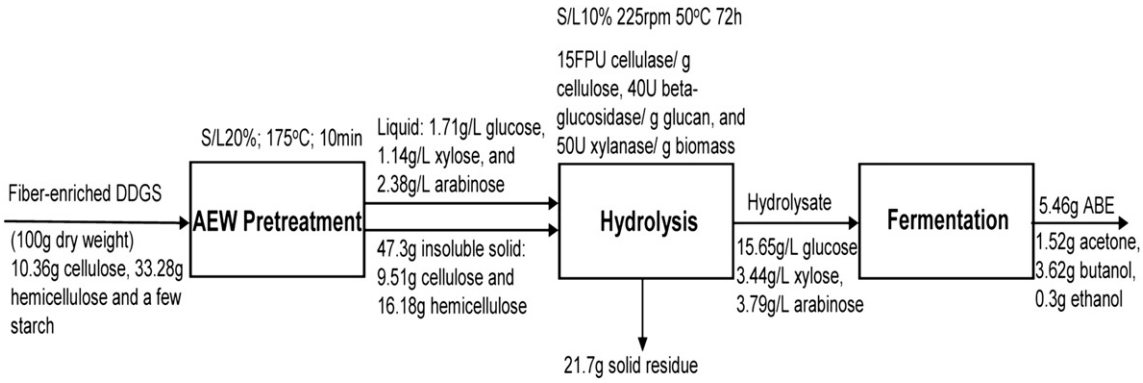

Fig. 5. Mass balance: AEW-pretreatment at 175 °C for 10 min.

In the current study, no detoxification was performed. The pretreated-samples were hydrolyzed with enzymes and then used directly in ABE production. Butanol fermentation from biomass hydrolyzates with *Clostridium* can sometimes be very sensitive to the presence of inhibitory compounds. Qureshi et al. [35] reported that even after overliming, the switchgrass hydrolyzate fermentation with *C. beijerinckii* P260 was not successful. Since the fiber-enriched DDGS pretreated with ABE can be directly used for fermentation without detoxification and concentration, it will no doubt contribute to lower the total cost for ABE production.

4. Conclusions

With a pretreatment solid loading of 20% w/w, the highest sugar yield was obtained from the AEW-pretreated samples at 175 °C and 10 min. Under the conditions tested in this study, 23.25 g total sugars (glucose, xylose, and arabinose) were released from 100 g (dry basis) fiber-enriched DDGS after enzymatic hydrolysis. The ABE fermentation with *C. beijerinckii* BA 101 was successful with 5.35 g ABE and 3.55 g butanol produced from 100 g fiber-enriched DDGS. Since no detoxification or concentration was performed before fermentation, the AEW-pretreatment may have generated fewer inhibitors than other pretreatment methods. This work suggests that electrolyzed water may be used as an alternative pretreatment catalyst for biofuel production from biomass.

Acknowledgments

The project was partially supported by the National Research Initiative of the USDA Cooperative State Research, Education and

Extension Service (Grant No. 2006-35504-17419). We thank Mr. John Jerrell for his assistance on GC analysis. Thanks are also sent to the China Scholarship Council for supporting Xiaojuan Wang.

References

[1] Renewable Fuels Association. http://www.ethanolrfa.org/pages/ethanol-facts-agriculture [accessed 02.09.11].
[2] U.S. Grain Council. http://www.agprofessional.com/news/us_grain_council_exports_the_only_solution_to_saturated_ddgs_market_120033249.html [accessed 02.09.11].
[3] Srinivasan R, Moreau R, Rausch K, Belyea R, Tumbleson M, Singh V. Separation of fiber from distiller's dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chem 2005;82:528–33.
[4] Srinivasan R, Singh V, Belyea R, Rausch K, Moreau R, Tumbleson M. Economics of fiber separation from distiller's dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chem 2006;83:324–30.
[5] DÜrre P. New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. Appl Microb Biotech 1998;49:639–48.
[6] D'Aquino R. Biobutanol on the horizon. Chem Eng Progr 2007;103:10.
[7] Qureshi N, Ezeji T. Butanol 'a superior biofuel' production from agricultural residues (renewable biomass): recent progress in technology. Biofuel Bioprod Bior 2008;2:319–30.
[8] Qureshi N, Ezeji T, Ebener J, Dien B, Cotta M, Blaschek H. Butanol production by *Clostridium beijerinckii*. Part I: use of acid and enzyme hydrolyzed corn fiber. Bioresour Technol 2008;99:5915–22.
[9] Qureshi N, Saha B, Cotta M. Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*. Bioprocess Biosyst Eng 2007;30:419–27.
[10] Qureshi N, Saha B, Hector R, Cotta M. Removal of fermentation inhibitors from alkaline peroxide pretreated and enzymatically hydrolyzed wheat straw: production of butanol from hydrolysate using *Clostridium beijerinckii* in batch reactors. Biomass Bioenerg 2008;32:1353–8.
[11] Qureshi N, Sahaa B, Hector R, Hughes S, Cotta M. Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: part I-batch fermentation. Biomass Bioenerg 2008;32:168–75.
[12] Wang B, Ezeji T, Shi Z, Feng H, Blaschek H. Pretreatment and conversion of distiller's dried grains with solubles for acetone–butanol–ethanol (ABE) production. TASABE 2009;52:885–92.
[13] Mosier N, Wyman C, Dale B, Elander R, Lee Y, Holtzapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 2005;96:673–86.
[14] Eggeman T, Elander R. Process and economic analysis of pretreatment technologies. Bioresour Technol 2005;96:2019–25.
[15] Wyman C, Dale B, Elander R, Holtzapple M, Ladisch M, Lee Y. Coordinated development of leading biomass pretreatment technologies. Bioresour Technol 2005;96:1959–66.
[16] Lloyd T, Wyman C. Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids. Bioresour Technol 2005;96:1967–77.
[17] Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch M. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresour Technol 2005;96:1986–93.
[18] Teymouri F, Laureano-Perez L, Alizadeh H, Dale B. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresour Technol 2005;96:2014–8.
[19] Kim T, Lee Y. Pretreatment and fractionation of corn stover by ammonia recycle percolation (ARP) process. Bioresour Technol 2005;96:2007–13.
[20] Kim S, Holtzapple M. Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresour Technol 2005;96:1993–2006.
[21] Saha B. Hemicellulose bioconversion. J Ind Microbiol Biotechnol 2003;30:279–91.
[22] Wang B, Wang XJ, Feng H. Deconstructing recalcitrant *Miscanthus* with alkaline peroxide and electrolyzed water. Bioresour Technol 2010;101:752–60.
[23] Adney B, Baker J. Measurement of cellulase activities. LAP-006 NREL analytical procedure. Golden, CO: National Renewable Energy Laboratory; 1996.
[24] Ghose T. Measurement of cellulase activities. Pure Appl Chem 1987;59:257–68.
[25] Miller G. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426–8.
[26] 2009 ASABE annual international meeting. https://elibrary.asabe.org/azdez.asp?JID=5&AID=27175&CID=reno2009&T=2, Paper number 096026.
[27] Tuchker M, Nagle N, Jennings E, Ibsen K, Aden A, Nguyen Q, et al. Conversion of distiller's grain into fuel alcohol and a higher-value animal feed by dilute-acid pretreatment. Appl Biochem Biotechnol 2004;113:1139–59.
[28] Mosier N, Hendrickson R, Brewer M, Ho N, Sedlak M, Dreshel R, et al. Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. Appl Biochem Biotechnol 2005;125:77–97.
[29] Qureshi N, Blaschek H. Butanol recovery from model solution/fermentation broth by pervaporation: evaluation of membrane performance. Biomass Bioenerg 1999;17:175–84.
[30] Ezeji T, Qureshi N, Blaschek H. Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J Microb Biot 2003;19:595–603.
[31] Ezeji T, Blaschek H. Fermentation of dried distillers' grains and solubles (DDGS) hydrolyzates to solvents and value-added products by solventogenic clostridia. Bioresour Technol 2008;99:5232–42.
[32] Lee J, Mitchell W, Tangney M, Blaschek H. Evidence for the presence of an alternative glucose transport system in *Clostridium beijerinckii* NCIMB 8052 and the solvent-hyperproducing mutant BA101. Appl Environ Microbiol 2005;71:3384–7.
[33] Wang B, Feng H. Detoxification of lignocellulosic hydrolysates. In: Blaschek H, Ezeji T, Scheffran J, editors. Biofuels from agricultural wastes. Ames, IA: Wiley-Blackwell; 2010.
[34] Ezeji T, Qureshi N, Blaschek HP. Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinck*ii growth and butanol fermentation. Biotechnol Bioeng 2007;97:1460–9.
[35] Qureshi N, Saha B, Hector R, Dien B, Hughes S, Liu S, et al. Production of butanol (a biofuel) from agricultural residues: part II – use of corn stover and switchgrass hydrolysates. Biomass Bioenerg 2010;34:566–71.