BAOJ Microbiology

Bwee Houweling-Tan, et al., BAOJ Microbio 2016 2: 3
2: 013

Research Article

Barley Distillers Dried Grains with Solubles (DDGS) as Feedstock for Production of Acetone, Butanol and Ethanol

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Abstract

Distillers dried grains with solubles (DDGS) represent important co-product from commercial yeast fermentations, including bioethanol, from grains. In view of the current expansion of the bioethanol fermentation process, with the concomitant increase in production of DDGS, alternative applications to their main current use as animal feed are being explored. In this study, DDGS from a bioethanol facility which uses barley as feedstock have been characterized and used as feedstock for biobutanol production. These DDGS contained, per kg of dry matter, 250 grams of protein and 390 grams of sugars, being glucose, xylose and arabinose the main sugar components. DDGS were hydrolyzed by alkaline pre-treatment followed by enzymatic hydrolysis resulting in the solubilization of approx. 80 % of the sugars in the feedstock and contained 57 g/L total sugars. The fermentation of 20 % (w/v) DDGS suspensions and of the hydrolysate of DDGS by two acetone, butanol and ethanol (ABE)-producing bacterial strains is described. Both strains utilized the sugars in these suspensions and in the hydrolysate to produce ABE. In these cultures, the strains only utilized soluble mono- or oligosaccharides. The hydrolysate was fermentable without addition of extra nutrients, being *C. acetobutylicum* the best-performing strain, producing 8.3 g/L ABE. In addition, DDGS were used as nutrient for the fermentation of wheat straw hemicellulosic syrup (C5-syrup) with low nutrient content. This C-5 syrup was a side stream obtained from steam-exploded wheat straw, and was subjected to overliming to make it fermentable. The supplementation of the C5-syrup with DDGS eliminated the need for addition of nutrients for the fermentation.

Keywords: DDGS; Acetone-Butanol-Ethanol Fermentation; ABE Fermentation; Second Generation Feedstocks; Steam Explosion

Introduction

Distillers dried grains with solubles (DDGS) refer to the product obtained after the removal of ethyl alcohol by distillation from yeast fermentation of grain or grain mixtures by condensing and drying at least three fourths of the solids of the resultant whole stillage. The composition of DDGS depends on different factors, such as the variety of substrate (barley, wheat, etc) or the geographical location and growing conditions of the crop. One of the main components in DDGS is protein, which accounts to between 20- 45 % of the dry weight, depending on the source. For DDGS derived from corn to ethanol fermentation, a protein content between 24-27 % (w/w) and total carbohydrates (including cellulose, starch and hemicelluloses) content of 53 % (w/w) have been reported [1]. Currently, DDGS resulting from commercial ethanol fermentation plants are mainly sold as animal feed, given their high content in proteins and other valuable nutrients.

In view of the current expansion of the bioethanol fermentation process, with the concomitant increase in production of DDGS, alternative applications for these are being explored. The polysaccharides (mainly cellulose and hemicelluloses) in DDGS are indigestible in monogastric livestock (e.g. swine and poultry) and are of limited value as feed components for cattle. Therefore, the carbohydrates present in the fiber component of DDGS have potential value as a source of fermentable sugars. The most abundant sugars in DDGS from corn are glucose, xylose and arabinose [1]. An interesting process for the valorization of this feedstock is the production of acetone, butanol and ethanol (ABE). The ABE process is nowadays being commercially re-introduced for the production of biologically derived butanol (biobutanol) to be used as biofuel or to replace petrochemically produced butanol in the bulk chemical market [2]. Recently the conversion of acetone, butanol and ethanol mixes into C_7-C_{15}-long alkanes has been reported. These alkane mixes can be deoxygenated, yielding alkanes compatible with current refinery infrastructures and suitable for blending with current fuels [3]. Most ABE-producing bacteria ferment a wide variety of carbohydrates, including some sugar polymers (starch, xylan) and different mono- and di-saccharides [4,5], although they are not able to degrade lignocellulose as other Clostridial species do [6]. The successful fermentation of different lignocellulosic biomasses to ABE has been described in literature, including

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Sub Date: May 6, 2016, **Acc Date:** May 25, 2016, **Pub Date:** May 26, 2016.

Citation: Bwee Houweling-Tan, Bram LHM Sperber, Hetty van der Wal, Rob R Bakker and Ana M López-Contreras (2016) Barley Dried Distillers Grains and Solubles (DDGS) as Feedstock for Production of Acetone, Butanol and Ethanol. BAOJ Microbio 2: 013.

hydrolysates from corn-derived DDGS [7-12]. Since DDGS are rich in sugars and other nutrients, their use as nutrient supplement, not only as carbon source, is another potential application. The addition of corn-derived DDGS to corn stover during pre-treatment to produce hydrolysates for ethanol fermentation [13], or during simultaneous saccharification and fermentation [14] has been reported to have beneficial effects on the fermentation. Most current literature on uses of DDGS as fermentation feedstock concern corn-derived DDGS (as they are the most abundant), however cereal-derived DDGS are getting attention as the use of cereals for ethanol production is increasing [15].

In this study, DDGS from a bioethanol facility which uses barley as feedstock have been characterized and used as fermentation nutrient for biobutanol production. The fermentation of DDGS suspensions by two acetone, butanol and ethanol (ABE)-producing bacterial strains is described, which show different fermentation performances. In addition the use of DDGS as nutrient component for the fermentation of a xylose-rich hemicellulosic fraction produced as by product of steam explosion pre-treatment of wheat straw is described for the first time for ABE production.

Material and Methods

Strains and Cultivation Conditions

C. acetobutylicum ATCC 824 and *C.beijerinckii* NCIMB 8052 were laboratory strains. They were cultivated under anaerobic conditions and stored as spore suspensions in glycerol (at -20 °C) as previously described [16]. For the preparation of pre-cultures, spores were heat-shocked in a water bath (10 min. at 80 °C for *C. acetobutylicum* and 1 min. at 100 °C for *C. beijerinckii*) and placed into CM2 medium. As carbon sources, stock solutions of glucose, mannitol or their mixes were prepared, sterilized separately, and added to the medium at the indicated concentrations. The pH of the media was adjusted to 6.0-6.4 with 1 M NaOH prior fermentation if needed.

For growth experiments, the semi-synthetic medium CM2 was used. CM2 contains, per liter: 2.5 g yeast extract, 1 g KH_2PO_4, 0.8 g K_2HPO_4. $3H_2O$, 2.9 g (NH_4) $C_2H_3O_2$, 0.1 g p-aminobenzoic acid, 1 g $MgSO_4.7H_2O$, 6.6 mg $FeSO_4$. $7H_2O$. Sugars were sterilized separately, and added, per liter: 30 g glucose and 30 g xylose, unless otherwise indicated. All solutions were flushed with N_2 gas to remove oxygen. Cultures were inoculated with 2 % (v/v) of an overnight pre-culture and grown in anaerobic serum flasks with working volume of 50 mL, and incubated at 37 °C in a stove. Fermentation experiments were carried out in duplicate, and values of concentrations of metabolites and other parameters described in the results section correspond to average values.

Source and Composition of DDGS

DDGS were originated in a Spanish commercial ethanol plant based on barley grains. They were supplied in the form of pellets with a dry matter content of approx. 90 % (w/w) and stored at room temperature.

Sugars, extractives, uronic acids and ash in DDGS where determined as described previously [16]. Total nitrogen (N) was determined using the method of Kjeldahl AACC 46-12. Protein was estimated by multiplying the total N content by 6.25.

Cultivation Media Based on DDGS

For fermentation of untreated DDGS, these were re-suspended in demineralised water at a concentration of 20 % (w/v). DDGS suspensions were supplemented with nutrients, salts and/or sugars as mentioned in the results sections. For this, stock solutions of nutrients were prepared separately, sterilized and stored at 4 °C until use. Nutrients were added to the DDGS suspensions or to the C5-syrup to reach the same final concentration as in CM2 medium.

The pH of the DDGS suspensions was adjusted with 4 M NaOH during the preparation of the media to reach a starting pH of the cultures between 6.0 and 7.0.

Preparation of DDGS Hydrolysates

Alkaline pre-treatment of DDGS was performed in a Terlet Stirred vessel of approximately 15Liters volume. Approx. 4 kg DDGS (containing 88.8 % dry matter) were resuspended in 8 en liters of hot water (70 °C) and the pH was adjusted with 25 % (w/w) NaOH to 9.0. The suspension was then incubated at 85 °C for 4 hours.

The pre-treated DDGS was cooled down and stored overnight at 4 °C. For enzymatic hydrolysis the pre-treated DDGS was diluted 1:1 with water to obtain slurry with a dry matter loading of approx. 15 % (w/w). The pH was then adjusted to 5.0 using 25 % (v/v) acetic acid. Subsequently, a commercial enzyme preparation GC220 (Genencor, Palo Alto, USA) was added to a concentration of 128 mL of GC220/kg dry matter. Enzymatic hydrolysis was carried out at 50 °C during 24 h., after which the slurry was cooled and stored at -20 °C until further use. Samples were taken at every step for sugar analysis by means of HPAEC. Hydrolysates were centrifuged to remove solid particles and sterilized prior fermentation.

Preparation of Pre-Treated Wheat Straw Supernatant (C5-Syrup)

Steam-exploded wheat straw slurry was a gift from an ethanol-producing company and contained 34.5 % dry matter. Upon reception, tap water was added (at 60 % (w/w) of the initial weight) to the pre-treated straw and the material was then soaked overnight at 4 °C. The slurry was pressed using a manual piston press, yielding a press-cake and a supernatant rich in sugars, mostly xylose. The press-cake had a dry matter content of 31.9 %. The supernatant, which had a pH of 2.2 and had a dark brown colour, was centrifuged to remove insoluble particles and stored at -20 °C.

The supernatant was overlimed as described by Mohagheghi *et al* [17]. Solid $Ca(OH)_2$ was added to adjust the pH to 10. This mixture was incubated at 50 °C for 30 min. The precipitate was separated by centrifugation at 15000 xg, 10 °C for 10 min. The pH of the supernatant was then adjusted to pH=6.3 with 5 M H_2SO_4. The resulting precipitate was removed by centrifugation at 15000 xg, 10 °C for 10 min. Supernatants were sterilized prior fermentation.

Analytical Procedures

Sugars and fermentation products were determined in clear culture supernatants from samples taken during the growth experiments and stored at -20 °C. Organic acids, furfural, solvents and sugars in

fermentation cultures were analyzed by High Performance Liquid Chromatography (HPLC). Sample preparation: samples were diluted (1:1) with internal standard solution (250 mM propionic acid in 1M H_2SO_4) and centrifuged for 5 minutes at 14,000 rpm (Centrifuge 5417 C Eppendorf desktop). The supernatant was filtered through a 13 mm GHP acrodisc 0.2 µm filter (Spartan 13/0.2 RC) and transferred to HPLC test vials. Separation was carried out in a Waters HPLC system equipped with an autosampler (Waters model 717) and a HPLC column Shodex KC-311 (Shodex, Tokyo, Japan). The column was kept at 80 °C, with 3 mM H_2SO_4 as eluent at a flow rate of 1 mL/min. A refractive index detector (Waters model 2414) and a UV absorbance detector (Waters model 2487) were used in series. The concentrations of most of the metabolites were determined from the refractive index chromatograms; with exception of furfurals and butyric acid, which were determined from the UV chromatograms.

Sugars in DDGS and DDGS fractions were determined using HPAEC. Sulfur in the C5-syrup samples was determined using element analysis by ICP-IAS technique.

Results

Chemical Analysis of DDGS and Preparation of Hydrolysate for Fermentation

In Table 1, the composition of the barley DDGS used in this study is shown. During analysis using standard protocols for lignocellulosic materials, where organic solvent (ethanol and ethanol/toluene mix)- and hot water-soluble components are extracted from the biomass as a first step, it was observed that DDGS contained a high percentage of both organic solvent and hot water extractives, accounting for 387 g/kg of the dry matter. In the hot water extractives, free glucose, maltose and residual starch were most likely to be present. In addition, components of the yeast cells, such as proteins, lipids and metabolites, present in the DDGS are also expected to contribute considerably to the solvent and hot water extractives. The insoluble sugar fibres, representing mostly barley glucans, cellulose and related polymers, represented 288 g/kg dry matter in the DDGS.

In order to determine the total sugar content of the DDGS, including all sugar polymers, a second analysis was performed where no extractions with organic solvents or hot water took place. When treating the whole DDGS with sulphuric acid for hydrolysis of all sugar polymers, the total sugars in the material represented the 390 g/kg dry matter, indicating that approx. 100 g/kg Dry matter of the DDGS corresponded to water soluble saccharides, composed mainly by glucose and arabinose (Table 1).

As expected, the DDGS were rich in protein, 250 g/kg Dry matter, mostly originating from the yeast-biomass in the ethanol fermentation. Further, lignin (163 g/kg Dry matter), ash and a small amount of uronic acids were determined in the DDGS (Table 1).

Most sugars in DDGS are in present in polymeric form and therefore not available as substrate by the ABE-producing-clostridial strains, as well as the best known organisms for fermentation of sugars to ethanol or other products. Therefore a hydrolysate was prepared by pre-treating the DDGS with NaOH followed by enzymatic hydrolysis using commercial cellulases (GC220). The pre-treatment conditions used were mild (temperature was 85 °C, pH 9) to prevent sugar degradation and the formation of fermentation inhibitors, as normally occurs during pre-treatment of lignocellulosic material at high temperatures [18].

The solubilisation of sugars in DDGS during the pre-treatment and the subsequent hydrolysis steps is shown in Table 2 and Figure 1. During the alkaline pre-treatment, approximately 25 % of the total sugars present in the DDGS became soluble in oligomeric form, since very low concentrations of free monomeric sugars were detected. The pre-treated DDGS slurry was very viscous and after cooling down, it formed a thick paste most probably due to the presence of polymerized starch. During enzymatic hydrolysis, the amount of monomeric sugars in the supernatant increased, as a result of amylase activity in the GC220 cellulase [19], although still some oligomers were present. At the end of the enzymatic hydrolysis treatment, approximately 80 % of the total sugars in DDGS were solubilised to mono- and oligosaccharides (Figure 1). During the preparation of this enzymatic hydrolysate, a dilution step was included which resulted in a total concentration of sugars in the hydrolysate of 57 g/L. If this dilution step could be avoided, the resulting hydrolysate might show a higher sugar concentration.

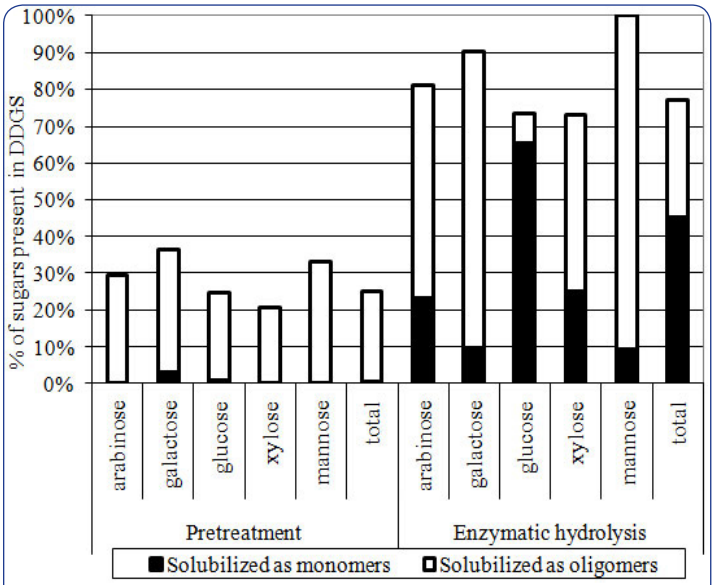

Figure 1: Solubilization of individual and total sugars in barley DDGS, as percentage of the sugars present in the DDGS, after alkaline pretreatment and subsequent enzymatic hydrolysis by GC220 cellulase.

Fermentation of DDGS Suspensions by *C. acetobutylicum* and *C. beijerinckii*

For fermentation tests, DDGS were resuspended in demineralised water at a concentration of 20 % (w/v). The DDGS suspensions were supplemented with glucose at 1 % (w/v) and/or nutrients, to determine fermentability. Two different Clostridial strains have been used in our studies: *C. beijerinckii* NCIMB 8052 and *C. acetobutylicum* ATCC 824, which are both well-known solvent (acetone, butanol and ethanol) producers and show different fermentation patterns and substrate utilization preferences.

Table 1: Composition of DDGS

	g/kg dry matter													
	Extractives			Sugars						Uronic ac.	Lignin		Protein	Ash
Sample	EtOH/ toluene	EtOH	H_2O	Ara	Xyl	Man	Gal	Glu	Rha		Ail	Asl		
DDGS[1]	155	20	212	44	111	11	6	115	1	8	107	54		
DDGS[2]				52	103	16	11	208	nd				250	60

[1] Standard analysis of the insoluble components in DDGS, after extraction of soluble components with solvents and water; [2]Analysis of whole DDGS, without solvent or water extraction steps. Abbreviations: Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose; Rha, rhamnose; Ail, acid insoluble lignin; Asl, acid soluble lignin; nd, not detectable

Table 2: Sugar composition of supernatants of DGGS slurry after alkaline pre-treatment and subsequent enzymatic hydryolysis. See materials and methods for details. Rhamnose was not detected in any of the samples.

		Sugars (g/L)					
		Ara	Gal	Glu	Xyl	Man	Total
NaOH pre-treated DDGS	Monosaccharides	0.1	0.1	0.6	0.1	nd	0.9
	Total sugars*	5.6	1.5	18.6	7.8	1.9	35.4
After GC220 enzyme hydrolysis	Monosaccharides	2.3	0.2	25.7	5	0.3	33.5
	Total sugars*	8.1	1.9	28.9	14.6	3.8	57.3

***** After hydrolysis with 1 M H_2SO_4. Abbreviations: Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose; Rha, rhamnose;nd, not detectable

In the DDGS suspension, the concentration of soluble monomeric sugars was low, approx. 2 g/L of glucose, 2 g/L of xylose and 0.5 g/L arabinose. This low sugar content did not support solvent production, and the strains produced low levels of acids on these suspensions. When the DDGS suspensions were supplemented with monomeric glucose at 10 g/L, both strains produced ABE as major fermentation products (Tables 3 and 4). Cultures of the strain ATCC 824 did not required pH adjustment prior fermentation. On the other hand, in cultures of NCIMB 8052 the pH of the suspensions needed to be adjusted to approx. 6-6.5 prior inoculation. In Tables 3 and 4, the results of the fermentations by *C. beijerinckii* and *C. acetobutylicum*, respectively, are shown.

In all fermentations on DDGS suspensions supplemented with glucose, the yields of solvents produced per gram of monosaccharide sugars consumed were higher than the ones determined in fermentations in control medium and sometimes higher than the theoretical maximum yield, reported to be approx. 0.4 grams ABE/gram sugar consumed (Tables 3 and 4). This indicates that these suspensions, in addition to the monosaccharides measured, contain other carbon sources such as free maltose and starch and other oligosaccharides, which are utilised as substrate for solvent production. Therefore the consumption of total sugars (both soluble and insoluble sugars) present in the DDGS suspension during fermentation by *C. acetobutylicum* was determined. In Table 5, the total sugar content in DDGS suspension supplemented with glucose before and after 198 hours of fermentation is shown. In the insoluble fraction, the sugars are present as fibres, and they were not significantly degraded by the bacteria (see Table 5), indicating that the bacteria grew mainly on soluble saccharides and did not degrade polysaccharides.

Fermentation of Alkaline-Pre-treated DDGS Hydrolysate

The DDGS hydrolysate contained 33.5 g/L of monosaccharide sugars and 57.3 g/L of total sugars. These concentrations of sugars are sufficient to support solvent production, and therefore the hydrolysate was used as fermentation medium without addition of other nutrients. In addition to the sugars, the hydrolysate contained 4.1 g/L of lactic acid and 10.7 g/L of acetic acid. The lactic acid was most probably originated during the hydrolysis steps, due to the growth in the hydrolysate of lactic acid –producing microorganisms. The high acetic acid concentration results from the use of this acid for pH adjustment during the pre-treatment (as described in Materials and Methods).

Both strains produced a significant concentration of solvents in this medium (Tables 3 and 4). *C. acetobutylicum* produced significantly more ABE than *C. beijerinckii* on the hydrolysate, 8.3 g/L vs 5.8 g/L, as a result of higher sugar consumption. *C. acetobutylicum* utilized all sugars in the hydrolysate, including arabinose, while *C. beijerinckii* mainly fermented glucose in the medium. Both strains consumed partially lactic and acetic acids in the media.

Fermentation of the C5-Rich Fraction of Steam-Exploded Wheat Straw

The liquid stream resulting from diluted acid hydrolysis of steam-exploded wheat straw contained mainly sugars from the hemicellulose fraction of the straw and was rich in xylose and therefore it was referred to as C5-syrup.

This initial syrup was not fermentable by any of the strains tested,

Table 3: Fermentation of DDGS-based media by *C. beijerinckii*. End fermentation times varied from 160 to168 hours. Media: DDGS+G, DDGS resuspended at 20 % (w/v) in water supplemented with 10 g/L glucose; DDGS hydrolysate, DDGS hydrolysate without supplements and pH adjusted by addition of NaOH; C5-S, C5 supernatant with pH adjusted by addition of NaOH; C5-S + DDGS, C5-S supplemented with 20 % DDGS; C5-S+N, C5-S medium supplemented with nutrients as in control medium CM2.

	Cultivation media				
Metabolites and fermentation parameters	**CM2 (control)**	**DDGS+G**	**DDGS hydrolysate**	**C5-S + DDGS**	**C5-S +N**
***Soluble monosaccharides t=0h* (g/L)**					
Glucose	24.8	12.1	19.2	7.4	7.6
Xylose	25.8	2.9	3.4	31.4	34.3
Arabinose		nd	1.8	nd	nd
***Soluble monosaccharides t=end*(g/L)**					
Glucose	4.2	0.6	7.6	2.1	0.9
Xylose	13.3	0.8	2.7	12.2	18.0
Arabinose		nd	1.7	nd	nd
***Organic acids t=end*(g/L)**					
Lactic acid	0	0	1.3	0	0
Acetic acid	0.2	1.4	7.1	2.1	3.6
Butyric acid	0.1	0.8	2.2	0.5	1.2
***Solvents t=end*(g/L)**					
Acetone	2.6	2.1	2.0	3.4	3.2
Butanol	8.8	6.7	3.6	6.9	6.5
Ethanol	0.3	0.2	0.2	0.3	0
Total ABE(g/L)	11.7	9.1	5.8	10.6	9.7
pH t=0/pH t=end	6.3/6.5	6.6/5.7	5.8/6.3	6.0/6.5	6.0/6.5
Yield ABE/monosacc.(g/g)	0.3	0.7	0.5	0.4	0.4

nd, not determined

even when supplemented with nutrients at the same concentration as in then control medium, indicating the presence of inhibitory substances for the bacteria. In the syrup, no furfurals (HMF or furfural) were detected (results not shown). The concentration of sulphur (S) in the syrup was relatively high, approx 2.9 g/L, which is expected to be in the form of SO_4^{2-} added during the pre-treatment of the wheat straw. High concentrations of sulphate are known to be inhibitory of bacterial growth [20]. Therefore, the syrup was subjected to detoxification by precipitating toxic components by addition with $Ca(OH)_2$, a method known as overliming [17]. In the resulting overlimed syrup the concentration of Sulphur was significantly lower than in the syrup, 0.8 g/L.

The overlimed C5-syrup was not fermentable as such, but when it was supplemented with nutrients or with DDGS, significant growth and solvent production was observed by both strains (Tables 3 and 4). The total ABE concentration produced on media supplemented with DDGS was higher than that in media supplemented with nutrients as in the control medium, indicates a positive effect of the DDGS on the fermentation. In contrast to the results on DDGS-based cultures, *C. beijerinckii* showed higher sugar consumption and ABE production on the C5-syrup cultures than *C. acetobutylicum*, which produced mainly acetic and butyric as end products of the fermentation.

Discussion

The chemical composition of the DDGS used in this study corresponded well with that of other cereal-derived DDGS reported in literature [21,22]. DDGS have a high content in nutrients, since they correspond to the grain lacking the most part of the starch, which was removed by enzymatic hydrolysis and microbial fermentation, resulting in a concentration factor of approx. 3 compared to the original grain [23]. In addition they are rich in proteins, accounting for an average of approx. 300 g/kg Dry matter, derived from the microbial biomass, making DDGS good animal feed ingredients, their major current commercial application. However, other applications of DDGS are taking more importance due to the on-going expansion of the ethanol fermentation process.

The most abundant sugar in these DDGS was glucose, followed by xylose and arabinose. The concentration of free sugars in the hydrolysate was higher than that reported for corn-derived DDGS hydrolysates using other pre-treatments [7, 12]. Both glucose and xylose represent good carbon sources for fermentation, while the fermentation of arabinose has been less studied in solvent-

Table 4: Fermentation of DDGS-based media by C. acetobutylicum. End fermentation times varied between 160-190 h. Media: DDGS+G, DDGS resuspended at 20 % in water supplemented with 10 g/L glucose; DDGS hydrolysate, DDGS hydrolysate without supplements and pH adjusted by addition of NaOH ; C5-S, C5 supernatant with pH adjusted by addition of NaOH; C5-S + DDGS, C5-S supplemented with 20 % DDGS; C5-S+N, C5-S medium supplemented with nutrients as in control medium CM2.

	Cultivation media				
Metabolites and fermentation parameters	**CM2 (control)**	**DDGS+G**	**DDGS hydrolysate**	**C5-S+ DDGS**	**C5-S+N**
***Soluble monosaccharides t=0h* (g/L)**					
Glucose	27.8	12.4	21.2	8.2	8.5
Xylose	27.6	2.8	3.8	31.1	35.6
Arabinose		nd	2	nd	nd
***Soluble monosaccharides t=end* (g/L)**					
Glucose	0.1	0.2	0	0.4	0.4
Xylose	15.9	1.5	0	17.6	22.3
Arabinose		nd	0.7	nd	nd
***Organic acids t=end*(g/L)**					
Lactic acid	0.1	0.1	1.8	0.0	0.0
Acetic acid	1.6	1.1	7.0	6.3	6.9
Butyric acid	1.7	1.2	4.2	5.0	4.3
***Solvents t=end*(g/L)**					
Acetone	2.7	3.8	3.1	1.4	2.3
Butanol	7.0	5.8	4.7	2.2	2.5
Ethanol	0.7	0.8	0.6	0.3	0.1
Total ABE(g/L)	10.4	10.4	8.3	3.9	4.9
pH t=0/pH t=end	6.4/4.7	4.7/4.8	5.2/5.5	6.1/5.1	6/5.8
Yield ABE/monosacc.(g/g)	0.3	0.8	0.4	0.2	0.2

nd, not determined

Table 5: Total sugars (both as soluble and insoluble) present in DDGS-suspensions before (t=0 h) and after fermentation (t=198 h) by *C. acetobutylicum*.

	concentration (g/L)					
	glucose	**xylose**	**arabinose**	**mannose**	**galactose**	**total sugars**
*Soluble sugars at t=0h**						
DDGS	20.0	4.6	3.9	1.5	1.5	31.5
DDGS+G	28.3	5.8	3.4	1.4	1.5	40.4
*Soluble sugars at t=198h *						
DDGS	13.6	9.1	5.9	2.0	1.6	32.2
DDGS+G	4.4	8.0	3.1	1.4	1.5	18.4
*Insoluble sugars at t=0h***						
DDGS/DDGS+G	18.2	13.6	7.2	1.4	1.1	41.5
*Insoluble sugars at t=198h **						
DDGS	15.6	11.3	4.2	1.2	0.7	33.0
DDGS + G	15.5	11.6	4.2	1.0	0.6	32.9

*Sugars determined in culture supernatants after hydrolysis with 1 M H_2SO_4,

** Sugars in insoluble fraction determined as described in materials and methods. Legend: DGGS, DDGS suspension at 20 % (w/v) in demineralised water, DDGS+G corresponds to suspension of DDGS supplemented with 1 % (w/v) glucose.

producing strains.

The potential of using DDGS and alkaline-pre-treated DDGS hydrolysate as fermentation substrates for ABE production has been studied using two of the best studied solventogenic strains, which show different fermentation pattern and sugar utilization. *C. acetobutylicum* utilized all sugars in the DDGS hydrolysates, including arabinose (Table 4), while *C. beijerinckii* utilized the sugars only partially (Table 3). The lower sugar consumption by C. *beijerinckii* might be due to inhibitory effects of components in the DDGS hydrolysate. Interestingly, hydrolysates of DDGS produced under mild pre-treatment conditions were fermentable as such without addition of extra nutrients of further treatments for production of ABE.

Suspensions of DDGS in water were only poorly fermentable, and solvents were not produced by any of the strains on these suspensions (results not shown). When glucose was added to the suspensions at a relatively low concentration (10 g/L) both strains were able to grow and produce ABE at yields (0.7 and .8 g ABE/g monomeric sugar consumed for *C. beijerinckii* and *C. acetobutylicum*, respectively) higher than that in control media (0.3 g ABE/g sugar consumed). This indicates that fermentable sugars are present in the DDGS suspensions, and that these are only utilized when extra monosaccharides are present in the media. Most probably these sugars are taking part in oligosaccharides that can be utilized only in cultures that have a certain optical density and are able to produce the necessary enzymes for degradation and utilization, or in cultures that have been adapted previously to the use of these oligosaccharides. The precise mechanisms involved in this differential substrate use need to be further investigated.

Very few reports are available on the fermentation of mixed substrates for ABE production, since most studies focus on the use of single feedstock's. A good example of the use of mixed substrates for AB production is described by Zverlov and co-workers [24]. In their overview of the commercial process operated in the Soviet Union in the last century, up to the 1980s, it is described in detail how hydrolysates of agricultural wastes (i.e. corn cobs, sunflower shells and hemp waste) were mixed with molasses and rye flour in order to reduce the use of food-grade substrates in the process. Fermentation of mixed substrates containing up to 75 % (w/w) of hydrolysate, depending on the feedstock's, were reported to be successful. In the present study, the DDGS were added to a C5-syrup resulting from steam-exploded wheat straw in a pilot plant. Steam explosion is a pre-treatment widely investigated for lignocelluloses [25,26]. The sugar streams resulting from steam explosion of straws normally require the addition of extra nutrients (nitrogen sources, etc) to be fermentable and be detoxified due to the presence of fermentation inhibitors [27-29]. Because the C5-syrup was not fermentable, it was subjected to overliming to remove inhibitors. The overlimed syrup showed lower sulfate content compared to the original syrup, showing that the treatment was effective in salt reduction. However, the overlimed syrup was not fermentable as such. When this overlimed C5-syrup was supplemented with DDGS in a ratio in the culture media similar to that described in the example above, approx. 80 % hydrolysate with 20 % of DDGS, with no further addition of nutrients, fermentation was achieved, indicating that DDGS can be used as nutrient supplement. The successful use of DDGS as nutrient supplement for C5-rich lignocellulosic syrup produced from wheat straw, one of the biomasses with big potential as biorefinery feedstock in Europe, has been described for the first time for ABE production.

These results described in this study demonstrate new uses of barley-derived DDGS as feedstock and as nutrientfor second generation fermentation feedstocks for the production of ABE, replacing expensive nutrient supplements and possibly improving process economics.

Acknowledgements

The authors wish to thank Mr. T. (Tao) Jing for help with fermentations, Dr. L. A. M. (Ben) van den Broek for help with analysis, and Mr. J. C. (Hans) van der Kolk for preparation of the C5-syrup. This work was financially supported by the European Comission, by the BIOSYNERGY project through the Sixth Framework Programme for Research and Technological Development under contract number 038994 – SES 6.

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