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Integrated Process for Extraction of Wax as a Value-Added Co-Product and Improved Ethanol Production by Converting Both Starch and Cellulosic Components in Sorghum Grains

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Abstract: Grain sorghum is a potential feedstock for fuel ethanol production due to its high starch content, which is equivalent to that of corn, and has been successfully used in several commercial corn ethanol plants in the United States. Some sorghum grain varieties contain significant levels of surface wax, which may interact with enzymes and make them less efficient toward starch hydrolysis. On the other hand, wax can be recovered as a valuable co-product and as such may help improve the overall process economics. Sorghum grains also contain lignocellulosic materials in the hulls, which can be converted to additional ethanol. An integrated process was developed, consisting of the following steps: 1. Extraction of wax with boiling ethanol, which is the final product of the proposed process; 2. Pretreatment of the dewaxed grains with dilute sulfuric acid; 3. Mashing and fermenting of the pretreated grains to produce ethanol. During the fermentation, commercial cellulase was also added to release fermentable sugars from the hulls, which then were converted to additional ethanol. The advantages of the developed process were illustrated with the following results: (1) Wax extracted (determined by weight loss): ~0.3 wt % of total mass. (2) Final ethanol concentration at 25 wt % solid using raw grains: 86.1 g/L. (3) Final ethanol concentration at 25 wt % solid using dewaxed grains: 106.2 g/L (23.3% improvement). (4) Final ethanol concentration at 25 wt % solid using dewaxed and acid-treated grains (1 wt % H_2SO_4) plus cellulase (CTec2): 117.8 g/L (36.8% improvement).

Keywords: sorghum grains; fermentation process; fuel ethanol; sorghum wax; value-added co-products

1. Introduction

Ethanol has attracted attention worldwide as a clean and renewable liquid fuel. The recent low oil prices encouraged record gasoline consumption, which translated to increased demand for ethanol for use in E10 (10% ethanol, 90% gasoline) as well as higher blends such as E15 and E85. The United States currently is the largest producer of fuel ethanol in the world. Ethanol production in the United States reached a record of 57.73 billion liters (15.25 billion gallons) in 2016 [1]. More than 90% of all the ethanol produced in the United States comes from corn. Since corn prices tend to fluctuate [2], other starch-based feedstocks have been considered. Among these, grain sorghum has attracted strong interest because of high starch contents, which are equivalent to those of corn; low requirements for water and fertilizer; and high heat and drought tolerance. These characteristics allow sorghum to be grown in dry climates and regions where corn cannot thrive [3]. Currently, sorghum is used in at least nine commercial ethanol plants in the United States, mostly as an adjunct feedstock to corn [1].

The majority of commercial corn ethanol plants in the United States employ the dry-grind process. The economics of this process greatly depend on the revenue obtained by selling of the main co-product

known as distillers dried grains with solubles (DDGS). Attempts have been made to develop additional co-products such as corn oil and corn fiber. Distillers corn oil is now an established co-product of almost all corn ethanol plants in the United States. In the case of sorghum, wax has been considered as a potential high-value co-product of ethanol production [4,5]. Sorghum wax has been found to possess several characteristics very similar to carnauba wax [4,6], which is an industrial wax with a wide range of applications. Carnauba wax is derived from the leaves of the *Copernicia prunifera* tree, which is found exclusively in Brazil [7]. Because of similar physical properties, sorghum wax has been suggested as a potential replacement for carnauba wax [4]. The bulk price of carnauba wax is listed at \$6.65–\$7/kg [8]. The global market of carnauba wax in 2015 was estimated at \$246 million [9] and is expected to increase to \$335 million in 2024 [10]. If sorghum wax can be recovered and used as a replacement for carnauba wax, it will open a relatively large market for a new co-product and potentially improve the economics of ethanol production using sorghum as feedstock. Cuticular waxes extracted from stalks of the sorghum plant have been found to inhibit acetone–butanol–ethanol fermentation [11]. The effect of grain sorghum wax on fermentation processes, however, has not been reported in the literature. It is possible that sorghum grain wax also has inhibitory effects toward ethanol fermentation. If this is the case, extraction of wax from the grains prior to fermentation will serve two purposes—development of a new high-value co-product and improvement of ethanol yield.

Similar to corn and other cereal grains, sorghum grain consists of an outer seed cover or pericarp, which encloses the embryo and the starch-rich endosperm [12]. The sorghum pericarp is lignocellulosic material, which can serve as feedstock for additional ethanol production. Prior to enzymatic hydrolysis for fermentable sugar production, lignocellulosic feedstocks have to be pretreated to increase the sugar yields. The pretreatment process employs various reagents and chemicals, which include high-pressure steam, acids, bases, organic solvents, and oxidizing agents such as ozone and hydrogen peroxide. The advantages and disadvantages of these pretreatment methods have been reviewed in detail [13].

In this paper, we report the development of an integrated process for extraction of sorghum grain wax as a high-value-added co-product and production of ethanol from the dewaxed sorghum using both starch and lignocellulosic components to improve ethanol yields.

2. Materials and Methods

2.1. Materials

2.1.1. Sorghum Grains

The sorghum grains were obtained from various sources and are listed in Table 1. All grains were free of pesticides. Upon receipt, the grains were stored in closed plastic containers and kept in the laboratory at ambient temperature until use.

Table 1. Sources and descriptions of sorghum grains.

Source	Description
Bob's Red Natural Foods (BRM) (Milwaukee, OR, USA)	Commercial product; unknown variety
United Sorghum Checkoff (USC) (Lubbock, TX, USA)	Blend of two varieties, Sorghum Partner SP6929 and Terral RV9782; the majority is SP6929 but the exact proportion is unknown
DuPont Pioneer (DPP) (Johnston, IA, USA)	Pioneer 83P56
Chromatin (Chicago, IL, USA)	SP7715

2.1.2. Enzymes and Chemicals

Spezyme® XTRA (thermostable α-amylase, activity 14,000 U/g), Fermenzyme® L-400 (glucoamylase/protease mix, activity 350 glucoamylase U/g) and Accellerase® 1500 (cellulase/β-glucosidase mix, endoglucanase activity 2200–2800 carboxymethylcellulase U/g; β-glucosidase activity 525–775 pNPG U/g where 1 pNPG unit liberated 1 μmol nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside per min at 50 °C and pH 4.8) were provided by DuPont Industrial Biosciences (Palo Alto, CA, USA). Cellic® CTec2 (cellulase/β-glucosidase) was provided by Novozymes (Franklinton, NC, USA). Specific activities of CTec2 were not publicly disclosed by the manufacturer. All enzymes were kept refrigerated at 4 °C.

Active Dry Ethanol Red was provided by Lesaffre Yeast Corporation (Milwaukee, WI, USA). The dry yeast powder was kept refrigerated at 4 °C.

All chemicals were of reagent grade and purchased from various suppliers.

2.2. Methods

2.2.1. Wax Extraction

Sorghum grains were screened to remove small debris and broken pieces. Only intact and undamaged grains were used in the experiments. The moisture contents of the grains were determined prior to their use in the experiments. For wax extraction, 200.0 g (fresh weight) sorghum was placed in a 2 L flask containing 320 mL absolute ethanol. The mixture was vigorously stirred with a magnetic stir bar and heated on a heating plate. A glass condenser using laboratory cold water as a cooling medium was mounted on the top of the flask to provide total reflux of ethanol. After the ethanol started to boil, stirring and heating of the sorghum-ethanol mixture was continued for 30 min. During this time, the clear ethanol gradually became cloudy due to the presence of a milky white substance, which was later determined to be wax. The flask then was removed from the heating plate and allowed to cool to ambient temperature. The mixture was poured into a Buchner funnel to separate the wax-containing ethanol from the grains. The wax-containing ethanol was transferred to a beaker and about half of the ethanol was allowed to evaporate at ambient temperature. Samples were taken from the partially concentrated wax-ethanol mixture for use in the wax analysis. The recovered grains were washed with about 100 mL deionized (DI) water and dried in a 55 °C oven until constant weights were reached to determine the weight loss due to wax extraction.

2.2.2. Dilute H_2SO_4 Treatment

Bob's Red Mill (BRM) sorghum was selected for investigation of dilute H_2SO_4 treatment as a possible method to improve ethanol production. A batch of 200 g BRM sorghum was dewaxed as described previously. After the wax-containing ethanol was drained completely, the grains were transferred to a 500 mL glass media bottle and 180 mL of a dilute H_2SO_4 solution was added. Two acid concentrations—1 wt % and 2 wt %—were used in the study. The amount of acid solution used was sufficient to cover all the grains and still left about 100 mL of free liquid above them. The bottle was closed with the plastic cap, which was screwed into place then slightly loosened by about one tenth of a turn. The bottle was placed in an autoclave set at 121 °C for one hour. The bottle with its contents was weighed before and after autoclaving to determine the amount of water lost due to evaporation. The acid-treated grains were used directly for ethanol production without additional processing.

2.2.3. Ethanol Fermentation of Raw and Dewaxed Sorghums

Mashing

The raw and dewaxed sorghums were ground in a Krups model 203 coffee grinder (Solingen, Germany). The moisture contents of the sorghum meals were determined to calculate the amounts needed for preparation of the fermentation mash. In a 1 L stainless steel beaker, 125 g (dry weight)

sorghum meal was mixed with DI water needed to make a mash with a total weight of 500 g. The total solid content of the mash was therefore 25 wt % on dry basis. The pH of the mash was adjusted to 5.6 using 5N H_2SO_4, and 34.1 µL of Spezyme Extra (0.3 kg enzyme/MT dry solids) was added. The beaker was placed in a hot oil bath for 2 h at 60 °C then 1 h at 90 °C to complete the starch liquefaction. Mixing of the mash was provided by a mechanical agitator. DI water was added throughout liquefaction to moderate viscosity in compensation for evaporation. After liquefaction, the beaker was cooled to 40 °C and weighed to determine the amount of water lost due to evaporation. DI water then was added to bring the total weight back to 500 g. The pH of the mash was adjusted to 4.0 using 5N H_2SO_4. After pH adjustment, 0.2 g urea and 73.9 µL Fermenzyme L-400 (0.65 kg enzyme/MT dry solids) were added. In the case of the BRM sorghum, two additional sets of experiments were performed. In these experiments, the enzyme dosages were increased to 2× and 5× of the aforementioned dosages.

Simultaneous Saccharification and Fermentation

Following urea and enzyme additions, the mash was stirred thoroughly to ensure complete dissolution of urea and uniform distribution of the enzyme. The mash then was split equally into six 250 mL flasks, each containing 50 g of mash. The active dry yeast was rehydrated by addition of 2.5 g to 50 mL DI water and stirred for 30 min. Each flask was inoculated with 0.25 mL of the yeast slurry. The flasks were capped with rubber stoppers which were pierced with 18 gauge hypodermic needles to allow for the release of CO_2. The flasks were incubated in an orbital shaking incubator maintained at 32 °C and 200 rpm. Simultaneous saccharification and fermentation (SSF) was performed for 72 h. During this time, the flasks were periodically weighed to determine the weight loss due to CO_2 production. The weight loss data were used to confirm that all fermentations were complete at 72 h. Final samples were taken from each flask and centrifuged on a microcentrifuge. The supernatants were filtered through a 0.2 micron filter into closed vials and stored in a freezer for analysis of residual sugars, ethanol, and other metabolite products.

Viscosity Reduction

In a separate set of experiments performed to study the effect of viscosity, at the end of the liquefaction, the beaker was cooled to 55 °C and 3.75 mL Accellerase 1500 was added (i.e., 0.03 mL/g solid). The mash was maintained at 55 °C and continuously stirred for 1 h. The rest of the mashing and fermentation procedure then followed.

2.2.4. Ethanol Fermentation of Dewaxed and H_2SO_4-Treated Sorghums

The dewaxed and H_2SO_4-treated BRM sorghum was used directly for mash preparation and subsequent SSF without washing and grinding. After dilute H_2SO_4 treatment, the grains were transferred from the media bottle to the stainless steel beaker for mashing. It was observed that a significant portion of the grains was liquefied into a thick slurry during pretreatment. Therefore, the entire contents of the bottle was used during mashing in order to maintain an accurate solid loading. The water content of the treated grains was calculated from the initial total mass and the water loss due to evaporation, which was determined by weighing the bottle before and after the acid treatment as described previously. DI water was added to the beaker until a 25% solid loading of grains was achieved. The mashing and SSF procedures were the same as described previously for the raw and dewaxed sorghums. To generate glucose from the cellulosic component of the grains for additional ethanol production, CTec2 was added at 0.03 mL/g solid.

2.3. Analytical Methods.

2.3.1. Moisture Determination

The moisture contents of whole sorghum grains were determined by placing 10 ± 2 g of material in preweighed aluminum weight boats. The boats were then dried in a 55 °C oven overnight and reweighed to determine the moisture loss. The moisture content was determined by calculating the amount of moisture loss as a percentage of the initial weight. The moisture contents of sorghum meals were determined by drying 2–3 g of material in an Ohaus MB45 moisture balance (Parsippany, NJ, USA). All measurements were performed in triplicate.

2.3.2. Wax Characterization

The partially concentrated wax-containing ethanol extracts were dried under nitrogen and dissolved in chloroform to make 5 mg/mL solution. Not all of the material was soluble in chloroform so the insoluble particles were removed using a syringe filter. The chloroform soluble fraction was dried under nitrogen and redissolved in chloroform to make a 5 mg/mL solution. The samples were analyzed using reverse-phase High Performance Liquid Chromatography (HPLC) with an Evaporative Light Scattering Detector (ELSD). HPLC analysis was performed using a Prontosil 200-3-C30 column (3.0 µm, 2.0 mm × 150 mm; Leonberg, Germany) on an Agilent 1260 series HPLC with an Agilent 1290 Infinity II series ELSD (Santa Clara, CA, USA). The method used was similar to one previously reported by Harron et al. [6] but was shortened from 95 min to 30 min by changing solvent gradients. Solvent A contained 99.9% methanol and 0.1% formic acid (v/v) while solvent B contained 99.9% chloroform and 0.1% formic acid (v/v). The flow rate was 0.200 mL/min with a column temperature of 50 °C. The mobile phase was initially 80:20 (solvent A/solvent B, v/v) and increased linearly to 20:80 over 10 min. The mobile phase was held at 20:80 from 10 min to 20 min and then was decreased linearly back to 80:20 by 21 min. The mobile phase was held at 80:20 from 21 min to 30 min. The ELSD was operated with an evaporator temperature of 80 °C, nebulizer temperature of 50 °C, and gas flow rate of 1.6 standard liters per minute (SLM).

2.3.3. Starch Determination

Starch contents of the raw and dewaxed sorghum grains were performed using the modified Megazyme assay [14]. The assay was based on the hydrolysis of starch with thermostable α-amylase and glucoamylase to produce glucose, which subsequently was determined and used for calculation of the starch content in the sample. The only modification was that the glucose produced in the present study was determined by an YSI glucose analyzer (YSI Incorporated, Yellow Springs, OH, USA) instead of a wet chemistry method as described in the Megazyme assay. All analyses were performed in triplicate.

2.3.4. Compositional Analysis of BRM Sorghum Fiber

The composition of the BRM sorghum fiber was determined according to the National Renewable Energy Laboratory (NREL) procedure [15]. To avoid interference by glucose from the starch in the grains, starch in the samples was removed prior to the compositional analysis. About 5 g BRM sorghum meal was placed in a 50 mL centrifuge tube and 40 mL 50 mM citric acid buffer at pH 4.8 was added followed by 0.5 mL Spezyme Extra. The tube was tightly capped, thoroughly mixed, and placed in an oven at 90 °C for 3 h. Every 30 min the tube was removed, thoroughly mixed, and replaced in the oven. At the end of the incubation period, the tube was cooled to ambient temperature, the pH of the slurry was adjusted to 4.5 with 5N H_2SO_4, and 0.5 mL Fermenzyme L-400 was added. The tube was again thoroughly mixed and placed in an incubator at 55 °C overnight (about 16 h). The tube then was centrifuged at 2500 rpm for 20 min. The supernatant was discarded and the pellet was washed three times, each time with about 50 mL DI water. The washed pellet was dried in the 55 °C oven and used for the compositional analysis. The analysis was performed in triplicate.

2.3.5. Analysis of Fermentation Samples

Residual glucose, ethanol, and other fermentation minor products were determined by HPLC. The system was an Agilent Technologies (Santa Clara, CA, USA) series 1200 equipped with a refractive index (RI) detector. The column was an Aminex® HPX-87H (Bio-Rad Laboratories, Hercules, CA, USA) operated at 60 °C. The solvent was 0.5 wt % H_2SO_4 pumped at a flow rate of 0.6 mL/min. Each sample was injected twice and the average results are reported.

3. Results and Discussion

3.1. Wax Extraction and Characterization

Wax could be extracted with a wide range of solvents such as hexane, benzene, chloroform, light petroleum ether, or acetone [5]. In the present study, ethanol was selected as the solvent for wax extraction because it is the final product in an ethanol plant and is readily available. The use of ethanol will therefore eliminate the solvent cost and reduce the total operating cost. In addition, the spent ethanol can easily be recovered in the distillation unit of the plant, either as a single stream or in combination with the fermentation-derived ethanol. The results of the wax extraction are summarized in Table 2.

Table 2. Wax contents of four sorghum varieties determined by weight losses during ethanol extraction.

Sorghum Type	Initial Dry Weight (g)	Wax Extracted (% of Initial Weight)
BRM	176.64	0.29
USC	171.86	0.19
DPP	174.26	0.17
Chromatin	174.51	0.29

The relative quantities of wax shown in Table 2 are calculated from the weight losses observed during the extraction process. The results indicate that the quantities of extracted waxes are approximately 0.2–0.3% of the initial mass of the sorghum grains, which is similar to those previously reported [5].

The chromatograms of the extracted waxes are superimposed and shown in Figure 1. In this method, oils such as triacylglycerol (TAG) eluted between 6–11 min while waxes eluted between 11–16 min. The chromatograms demonstrate the similarity of the four extracted waxes. The waxes in this chromatogram are similar to those identified by Harron et al. [6] and are primarily composed of C28-C30 fatty alcohols and aldehydes. The different peaks refer to mixtures of various chain length and saturation of the wax compounds, but for this analysis were not specifically analyzed by mass spectroscopy. The extracted waxes also show high purity and are practically free of oils (indicated by the extremely small peaks eluting between 6–11 min).

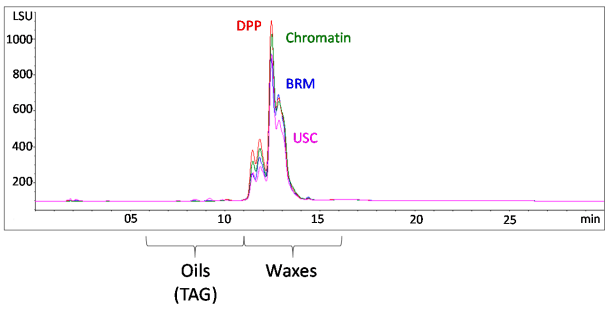

Figure 1. Chromatograms of the waxes extracted from the four sorghum grains: (1) Bob's Red Mill (BRM, blue), (2) United Sorghum Checkoff (USC, pink), (3) DuPont Pioneer (DPP, red), and (4) Chromatin (green) Oils (primarily triacylglycerol, TAG) eluted between 6-11 min and waxes eluted between 11–16 min.

3.2. Starch Contents

The results of starch content determination are shown in Table 3.

Table 3. Starch contents of raw and dewaxed sorghums.

Treatment	Starch (wt % Dry Basis)
BRM Raw	59.7 ± 3.0
BRM Dewaxed	67.4 ± 2.1
USC Raw	58.4 ± 7.8
USC Dewaxed	70.2 ± 3.5
DPP Raw	59.1 ± 1.8
DPP Dewaxed	65.3 ± 0.6
Chromatin Raw	58.7 ± 3.8
Chromatin Dewaxed	64.7 ± 1.6

From the results in Table 3 it can be seen that dewaxing resulted in significant increases in the measured starch contents, ranging from 10.1% for Chromatin to 20.0% for USC sorghum. The lower starch contents measured for the raw samples could probably be attributed to the inhibition of the starch hydrolytic enzymes used in the assay by the waxes. This is rather surprising since in the assay relatively low solid loading (about 3%) and very high enzyme dosages (about 3000 units/g solid of both enzymes) were used. The amounts of wax present in the assay mixture, therefore, seemed to be insufficient to cause the observable negative effect on the enzyme activities. Nevertheless, no other reasonable explanation could be provided. As discussed previously, there has been only one report on the negative effect of wax derived from sorghum stalk on a fermentation process [11]. There has been no report on the inhibition of either an enzymatic or fermentation process by grain sorghum wax. The result obtained in the present study is the first reported observation of a negative effect of grain sorghum waxes on an enzymatic process. The mechanism of this inhibition was not clear. Among the four sorghum grains tested, the DPP had a reddish brown color, which indicated the possible presence of tannin. To confirm its presence, whole and ground DPP grains were subjected to tannin extraction using a solution of 10 mM ascorbic acid in methanol at a solid/liquid ratio of 1:3 [16]. The extraction was performed at 55 °C for 16 h. The resultant light reddish brown color of the solvent indicated the presence of solubilized tannin. The extracted tannin was not quantified. Since ethanol extraction would remove only about 5% of tannin [16], most of the tannin was expected to remain in the DPP grains. The adsorption of proteins on tannin, which would severely limit their availability, has previously been reported in the literature [17]. The small increase of 10.5% of the measured starch content obtained for the dewaxed DPP over the raw grains indicate that the tannin in the DPP sorghum did not have significant effect on the enzymatic starch hydrolysis.

3.3. SSF of Raw and Dewaxed Sorghum Grains

The results of ethanol fermentation of the raw and dewaxed sorghum grains are shown in Table 4.

With the exception of Chromatin, dewaxing of sorghum grains resulted in increased ethanol production. These improvements can probably be linked to the higher efficiency of starch hydrolysis, which was the result of the wax removal. The highest increase of ethanol production, of 23.3%, was obtained with the BRM sorghum. For the USC sorghum, the increase of ethanol production was lower, at 5.6%. The smallest increase, of 2.8%, was observed for the DPP sorghum. As discussed in the previous section, the tannin in the DPP sorghum did not seem to have a negative effect on the starch hydrolysis. Whether the tannin had any negative effect on the fermentation is not known. The reason for no improvement of ethanol production in the case of the Chromatin sorghum is not clear. In all cases, addition of Accellerase 1500 slightly improved ethanol production. It was qualitatively observed that the addition of this cellulase enzyme formulation considerably reduced the viscosity of the mash, thus improving mixing, which would in turn improve both starch hydrolysis and fermentation.

Table 4. Final ethanol concentrations in the simultaneous saccharification and fermentation (SSF) of raw and dewaxed sorghum grains.

Feedstock	Final Ethanol (g/L)	Yield (% Theoretical)
BRM Raw	86.1 ± 2.0	71.1
BRM Dewaxed	106.2 ± 0.6	90.2
BRM Dewaxed with Accellerase	107.8 ± 1.9	91.8
USC Raw	102.2 ± 0.9	83.9
USC Dewaxed	107.9 ± 0.5	89.3
USC Dewaxed with Accellerase	108.2 ± 0.9	89.6
DPP Raw	104.1 ± 1.0	85.1
DPP Dewaxed	107.0 ± 3.8	87.8
DPP Dewaxed with Accellerase	109.6 ± 1.0	90.3
Chromatin Raw	98.7 ± 1.5	80.8
Chromatin Dewaxed	98.0 ± 5.4	80.2
Chromatin Dewaxed with Accellerase	99.7 ± 1.0	81.7

3.4. SSF of Dewaxed and H_2SO_4-Treated BRM Sorghum

Since the BRM sorghum gave the highest increase of ethanol production upon dewaxing, it was selected for investigation of potential further ethanol yield improvement by dilute H_2SO_4 treatment. To determine the potential additional ethanol yield, the compositions of the fiber obtained after destarching of the ground sorghum were determined. The results of the compositional analysis are shown in Table 5.

Table 5. Composition of the BRM sorghum fiber.

Component (wt % of Total Mass, Dry Basis)					
Glucan	Xylan	Arabinan	AI Lignin	AS Lignin	Ash
56.9 ± 1.6	4.9 ± 0.1	3.6 ± 0.0	10.7 ± 0.8	2.1 ± 0.0	0.1 ± 0.0

Notes: AI: Acid insoluble; AS: Acid soluble.

The high content of glucan in the fiber is favorable for ethanol production since upon hydrolysis it will result in high concentrations of glucose, which is the sugar most effectively fermented by the currently used commercial fuel-ethanol-producing *Saccharomyces cerevisiae* strains.

As discussed previously, the dewaxed and H_2SO_4-treated sorghum grains were used directly for mash preparation without washing and grinding. It was observed in the mashing process that the grains were sufficiently softened by the dilute acid treatment and slowly disintegrated when the mash was heated and agitated. The use of dilute acid for treatment of the sorghum grains offered an additional advantage. Prior to the start of the mashing process, the pH of the mash normally had to be adjusted to 4.5 with 5N H_2SO_4. The initial pH of the dewaxed and H_2SO_4-treated sorghum mash was found to be very close to the required pH. Therefore, only minimal pH adjustment was needed.

The final ethanol concentrations and the calculated yields obtained with the dewaxed and H_2SO_4-treated sorghums are shown in Table 6. The results obtained with the raw sorghum and dewaxed sorghum without H_2SO_4 treatment are also included for comparison. In all cases, the yields are calculated based on the total glucose available from both starch and cellulose. The results clearly demonstrate the improvements of ethanol production by treatment of the dewaxed sorghum with dilute H_2SO_4 and addition of the cellulase enzyme formulation CTec2, which resulted in the availability of more glucose for fermentation. Between the two acid concentrations used, 1 wt % H_2SO_4 gave slightly better ethanol yield than 2 wt % H_2SO_4. The higher acid concentration probably resulted in the formation of inhibitory compounds, which could have negative effects on the fermentation process. Compared with the raw sorghum, dewaxing and treatment with 1 wt % H_2SO_4 resulted in a 36.8% increase of ethanol yield.

Table 6. Final ethanol concentrations obtained in SSF of raw, dewaxed, and dewaxed plus dilute H_2SO_4-treated and cellulase-treated BRM sorghums.

Experiment	Final Ethanol (g/L)	Yield (% Theoretical) *
Bob's Red Mill raw	86.1 ± 2.0	62.7
Bob's Red Mill dewaxed	106.2 ± 0.6	77.4
Bob's Red Mill dewaxed and treated with 1 wt % sulfuric acid with CTec2 addition in SSF	117.8 ± 0.1	85.7
Bob's Red Mill dewaxed and treated with 2 wt % sulfuric acid with CTec2 addition in SSF	112.5 ± 4.5	82.0

* Based on total glucose available from starch and cellulose. A sample calculation of ethanol yield is shown in Appendix A.1.

3.5. The Proposed Integrated Process

Based on the results presented and discussed in the previous sections, an integrated process is proposed for ethanol production using grain sorghum as feedstock. In this process, glucose is obtained from both starch and fiber. The proposed process is shown in Figure 2.

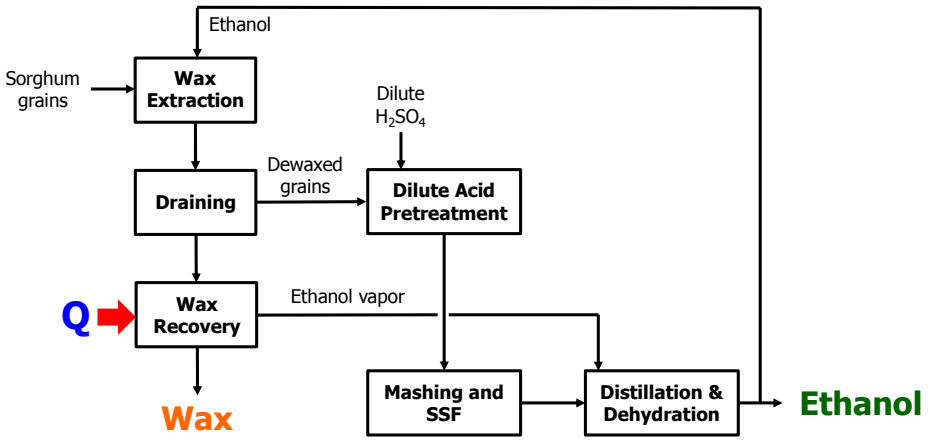

Figure 2. The integrated process for ethanol production from sorghum starch and fiber.

First, wax is extracted from the grains using ethanol, which is readily available in the plant. After a simple solid/liquid separation step—for example, by draining of the liquid—the extracted wax is recovered by evaporation of ethanol. The ethanol stream is brought to the distillation unit, where it can be fed to the distillation columns as a separate stream, or combined with other streams from the fermentation process, for ethanol recovery. The recovered ethanol is recycled and returned to the front for use in the next wax extraction cycle. The dewaxed grains are subjected to dilute H_2SO_4 treatment, then are used directly in mash preparation and subsequent SSF without washing and grinding. Omission of grain washing will result in significant savings of water consumption, whereas omission of grinding will result in significant savings of energy. The proposed process is simple and does not require expensive equipment. In addition, the key processing steps can be operated at moderate temperatures and pressures. The proposed process, therefore, can be added to an existing sorghum ethanol plant as a "bolt-on" process.

To realize the potential benefits, a simple economic analysis was performed for an ethanol plant producing 50 million gallons per year using the experimental data obtained with the BRM sorghum; the results are summarized in Table 7.

Table 7. Potential economic benefits of the integrated process for ethanol production using BRM sorghum grain as feedstock.

	Base Case	Dewaxed Only	Dewaxed/H_2SO_4 Treated
Ethanol yield (gal/bu)	2.04	2.59	2.92
Sorghum feedstock needed (million bu)	24.5	19.3	17.1
Total wax co-product (MT)	0	1252	1110
Wax co-product value (million $)	0	7.5	6.6
Sorghum feedstock savings (million $)	0	16.1	22.9

Notes: MT: metric ton; gal/bu: gallons per bushel; The value of the wax co-product is calculated using a bulk selling price of $6/kg; The feedstock savings are calculated using a unit cost of $3.10/bu. A sample calculation of potential economic benefits is shown in Appendix A.2.

The results in Table 7 indicate that if only wax extraction is performed, ethanol yield will increase from 2.04 to 2.59 gallons per bushel (gal/bu), which will translate into a saving of $16.1 million per year on reduced feedstock requirement. In addition, the wax co-product will add $7.5 million per year to the total revenue. If dilute sulfuric acid pretreatment also is performed on the dewaxed sorghum, the value of the wax co-product will decrease to $6.6 million per year but the ethanol yield will increase to 2.92 gal/bu and the saving on feedstock cost will increase to $22.9 million per year.

4. Conclusions

It has been demonstrated that wax could be extracted from four different commercial grain sorghum products by a simple process using ethanol as the solvent. The dewaxed sorghum grains gave higher ethanol yields when used as feedstock in an SSF process. Further improvements on ethanol yield were obtained when the dewaxed BRM sorghum grains were also treated with dilute solutions of sulfuric acid and a commercial cellulase was added during the fermentation to produce additional glucose from the fiber. Based on the experimental data, an integrated process for extraction of sorghum wax as a value-added co-product and production of ethanol from both starch and fiber was proposed. Using the data obtained for the BRM sorghum grains, the integrated process was shown to have significant economic benefits over the base process using raw sorghum as feedstock. The data, however, were obtained for proof-of-concept purpose only. More rigorous process optimization is needed and the benefits of dilute acid treatment must be proven with other sorghum types before the proposed integrated process is considered for commercial implementation.

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Appendix A.

Appendix A.1. Calculations of Ethanol Yields

Basis: 1000 g of 25 wt % raw BRM sorghum mash.

Total starch available: 250 g × 0.701 = 175.25 g.

Water consumed by hydrolysis of starch: 175.25 g × 0.11 = 19.28 g or 19.28 mL since the density of water is 1.

Let V_F be the final liquid volume in mL. Since the final ethanol concentration was 86.1 g/L, the additional volume (in mL) contributed by the ethanol produced was

0.0861 g/mL × V_F (mL) ÷ 0.789 g/mL = 0.109 V_F

Mass balance will give
$V_F = 750\ mL - 19.28\ mL + 0.109\ V_F$
Therefore, $V_F = 820.2$ mL.
Total ethanol production: 86.1 g/L × 0.820 L = 70.62 g.
Ethanol yield: 70.62 g ÷ 250 g = 0.283 g ethanol/g sorghum.
Assume 1 bu of sorghum weighs 56 lb and has moisture content of 15 wt%. The mass of 1 bu of BRM sorghum in g is 56 lb × 0.85 × 454 g/lb = 21610 g.
Ethanol yield per bu of sorghum is 0.283 g/g × 21610 g/bu = 6104 g ethanol/bu or 2.04 gal/bu.
Similar calculations are performed for dewaxed and dewaxed/H_2SO_4-treated BRM sorghum. The ethanol yields are 2.59 gal/bu and 2.92 gal/bu, respectively.

Appendix A.2. Calculations of Potential Economic Benefits

Design basis: A plant to produce 50 million gallons ethanol per year.
Annual feedstock requirement for raw sorghum: 50×10^6 gal ÷ 2.04 gal/bu = 24.5×10^6 bu.
Similarly, for dewaxed sorghum and dewaxed/H_2SO_4-treated sorghum, the annual feedstock requirements are 19.3×10^6 bu and 17.1×10^6 bu.
For the dewaxed sorghum, the amount of wax that can be extracted is 0.003 × 21.6 kg/bu = 0.065 kg/bu.
The total quantity of wax that can be extracted is 0.065 kg/bu × 19.3×10^6 bu = 1.251×10^6 kg or 1251 MT.
The value of the extracted wax is 1.251×10^6 kg × \$6/kg = $\$7.5 \times 10^6$.
The saving on feedstock is $(24.5 \times 10^6\ bu - 19.3 \times 10^6\ bu) \times \$3.10/bu = \$16.1 \times 10^6$.
Similar calculations are performed for the dewaxed/H_2SO_4-treated sorghum and the calculated results are shown in Table 7.

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