Sweet Sorghum Biorefinery for Production of Fuel Ethanol and Value-added Co-products

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ABSTRACT

An integrated process has been developed for a sweet-sorghum (*Sorghum bicolor* (L.) Moench) biorefinery in which all carbohydrate components of the feestock are used for production of fuel ethanol and industrial chemicals. In the first step, the juice is extracted from the stalks. The resulted straw (bagasse) then is pretreated using the soaking in aqueous ammonia (SAA) process, which does not result in significant loss of hemicellulose, to enhance subsequent enzyme hydrolysis for production of fermentable sugars. Following pretreatment the straw is hydrolyzed first with commercial enzyme product containing high hemicellulase activity (Accellerase XY). The xylose-rich solution obtained after solid/liquid separation is used for production of value-added co-products using suitable microorganisms. The value-added co-products produced to demonstrate the feasibility include astaxanthin and D-ribose. The residual solids then are hydrolyzed with commercial enzyme product containing high cellulase activity (Accellerase 1500) with the juice extracted in the first step being used as make-up water. By combining the sugar in the juice with the glucose released from the residual solids by enzyme hydrolysis high ethanol concentrations can be achieved, which results in lower distillation cost than if pure water is used for enzyme hydrolysis and subsequent fermentation as normally performed in cellulosic ethanol production.

Keywords: sweet sorghum, fuel ethanol, biomass bioconversion, biorefinery, value-added products

1 INTRODUCTION

Sweet sorghum was brought to the U.S. in 1853 as potential feedstock for sugar production. Its production peaked in the 1880s but then declined because of the competition from sugarcane and sugar beets [1]. Recently, sweet sorghum has attracted strong interest as a potential feedstock for ethanol production because of several reasons, which include: 1. Sweet sorghum has low water requirement, thus allows savings on irrigation cost; 2. It also has low requirement of nitrogen, about 50 to 60 lbs per acre, which is about one half for corn; 3. It matures within 100 to 120 days, thus allows multiple harvests per year; and 4. Its juice contains sucrose at concentrations as high as 150 g/L and can be fermented easily to ethanol [1], [2], [3]. The residual solid obtained after juice extraction (bagasse), because of its high carbohydrate contents, can also be converted to ethanol and industrial chemicals. We have developed an integrated process for a biorefinery, in which all carbohydrate components of sweet sorghum can be used for production of ethanol and industrial chemicals.

2 MATERIALS AND METHODS

2.1 Materials

Sweet sorghum juice and bagasse were obtained from a local farm in Clemson, SC. *Phaffia rhodozyma* ATCC 74219 (astaxanthin producer) and *Bacillus subtilis* ATCC 21951 (D-ribose producer) were stored in a glycerol medium (25% v/v glycerol) at -70^{o}C. Active Dry Ethanol Red yeast culture (*Saccharomyces cerevisiae*) was provided by Lesaffre Yeast Corporation. The biomass enzyme products Accellerase 1500 and Accellerase XY were kindly provided by Genencor International, a Danisco's division. All chemicals were of reagent grades and purchased from various suppliers.

2.2 Methods

Sweet sorghum bagasse pretreatment and hydrolysis

Ground sweet sorghum bagasse having particle size of <1 mm (70 g, dry basis) was pretreated in a closed container by soaking in aqueous ammonia (SAA) using 700 ml of 15 wt % NH_4OH (i.e. a solid:liquid loading of 1:10) at 65^{o}C for 15 h. The pretreated solids were recovered by filtration under vacuum and washed with deionized (DI) water until the pH of the wet cake was about 7. Several batches of sweet sorghum bagasse were pretreated as described. To prepare the xylose-rich hydrolysate pretreated sweet sorghum bagasse was hydrolyzed with Accellerase XY at 0.05 ml/g dry biomass in water. The solid loading was 10 wt % (dry basis). The pH of the slurry was adjusted to 5.0. Hydrolysis typically was performed in a 1-l flask incubated at 50^{o}C and 200 rpm for 72 h. The residual solids were recovered by vacuum filtration, dried in a 55^{o}C oven, and ground with a small coffee grinder for further processing. The hydrolysate was stored frozen until use.

Ethanol fermentation

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Four sets of experiments were performed on fermentation of the sweet sorghum juice for ethanol production. Set 1: The juice was used as is. Set 2: Yeast extract (Difco) and K_2HPO_4 were added to 0.4 g/l and 0.32 g/l final concentrations, respectively. Set 3: The residual solids obtained after AccelleraseXY hydrolysis were hydrolyzed in the sweet sorghum juice at 7 wt % solid loading (dry basis) and Accellerase 1500 was added at 0.05 ml/g dry solids. Set 4: Same as set 3 but yeast extract and K_2HPO_4 also were added as described. All experiments were performed in 250-ml flasks incubated at 32°C and 175 rpm. Prior to incubation the media were adjusted to pH 4.8 and each flask was inoculated with 1.0 ml of the Dry Yeast suspension (2.5 g in 50 ml DI water stirred for 30 min). Each flask of set 1 and set 2 contained 150 ml total liquid and those of set 3 and set 4 contained 100 ml each. The flasks were tightly capped with rubber stoppers punctured by a hypodermic needle. The weight loss due to CO_2 production was monitored at intervals. Ethanol concentrations in g/l were calculated using the weight loss data and adjusted for the additional liquid volume contributed by the ethanol produced. At the end of the experiments final samples were taken and ethanol concentrations were verified by high pressure liquid chromatography (HPLC - see below).

Astaxanthin fermentation

Fermentation of the hydrolysate obtained after Accellerase XY hydrolysis of the pretreated sweet sorghum bagasse for astaxanthin production using *P. rhodozyma* ATCC 74219 was performed as described previously [4].

D-Ribose fermentation

The inoculation medium contained (in g/l): glucose 5, xylose 15, yeast extract (Difco) 10, K_2HPO_4 5, KH_2PO_4 5 and $MgSO_4.7H_2O$ 1. The pH of the medium was adjusted to 7.0 with 1 N NaOH. The inoculation flask contained 25 ml medium and was inoculated with 0.2 ml thawed glycerol stock culture of *B. subtilis* ATCC 21951. The flask was incubated at 37°C and 250 rpm for 24 h. For D-ribose fermentation experiments yeast extract and inorganic nutrients were added to the Accellerase XY hydrolysate in concentrations as described for the inoculation medium. The pH of the media was adjusted to 7.0. A control experiment also was performed in which DI water was used in place of the hydrolysate. All media were sterilized by autoclaving at 121°C for 20 min. Each flask used for D-ribose fermentation study was inoculated with 2 ml inoculum. The D-ribose fermentation flasks also were incubated at 37°C and 250 rpm. Samples were taken daily for analysis.

Analytical methods

Astaxanthin was determined as total carotenoid with pure astaxanthin used as standard as described previously [5]. Samples taken for analysis of other metabolites were centrifuged on a microcentrifuged and the supernatants were filtered through a 0.2 µ filter. The metabolites were analyzed by HPLC as described previously [5]. The carbohydrate contents of the sweet sorghum bagasse were determined by the NREL method [6].

3 RESULTS AND DISCUSSION

3.1 Sweet sorghum bagasse pretreatment

The untreated sweet sorghum bagasse contained 38.31 wt % glucan, 22.36 wt % xylan, and 1.7 wt % arabinan. After pretreatment of 210 g bagasse, 145.6 g solids was recovered. The pretreated solids contained 51.60 wt % glucan, 25.29 wt % xylan, and 2.28 wt % arabinan. Therefore, 93.39 % of the original glucan, 78.42 % of the original xylan, and all of the original arabinan were preserved during the pretreatment. High retentions of glucan and xylan was the reason for choosing SAA as the pretreatment method of the sweet sorghum bagasse. The hydrolysate obtained following hydrolysis of the pretreated bagasse with Accellerase XY contained 0.19 g/l glucose and 7.97 g/l xylose. Arabinose was not detected in the hydrolysate. The residue recovered after the Accellerase XY hydrolysis contained 54.04 wt % glucan, 19.67 wt % xylan, and 1.9 wt % arabinan. Thus, the glucan content of the original (untreated) bagasse was increased from 38.31 wt % to 54.04 wt % in the final residue. The high glucan content of this residue made it suitable for use as feedstock for ethanol fermentation by the yeast *S. cerevisiae*, which is the most efficient commercial ethanol-producing organism but can only metabolize glucose.

3.2 Ethanol fermentation

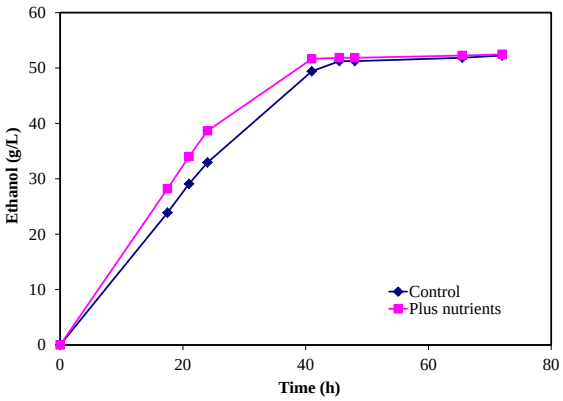

Figure 1. Fermentation of sweet sorghum juice.

The results of sweet sorghum juice fermentation are shown in Figure 1. The results showed that nutrients were not needed. The fermentations ended at about 45 h in both

cases. The results shown in Figure 1 were calculated from the weight loss data. The calculated final ethanol concentrations are 52.2 g/l (without nutrients) and 52.4 g/l (with nutrients). The corresponding HPLC results are 58.7 g/l and 58.2 g/l, respectively. The differences between calculated results based on weight loss data and HPLC results are about 11-12%. Despite these differences using the weight loss data still is a convenient and accurate way to follow the progress of the fermentations.

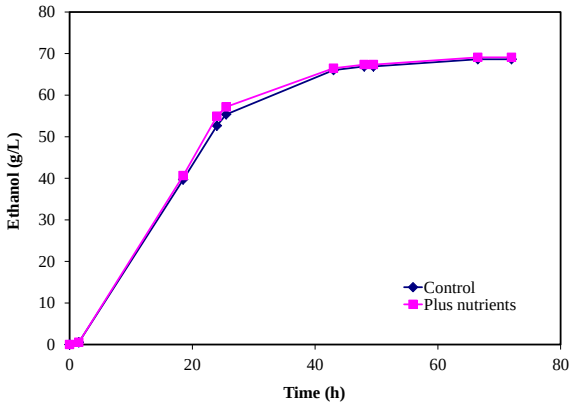

Figure 2. Fermentation of sweet sorghum juice plus enzyme hydrolysis residue of SAA pretreated bagasse

The results of fermentation of the sweet sorghum juice plus the cellulose-rich residue are shown in Figure 2. The calculated final ethanol concentrations are 68.6 g/l and 69.1 g/l without and with nutrients added, respectively. The corresponding HPLC results are 72.2 g/l and 72.3 g/l. The differenes in this case are only about 5%. Adding the cellulose-rich residue to the juice increased final ethanol concentrations to about 70 g/l. It is expected that this increase will lower distillation energy requirements and costs significantly [7]. Using the actual HPLC results the increases in ethanol production were calculated to be equivalent to 79.4% of theoretical ethanol yield from the glucan in the added residue.

3.3 Production of value-added products

The results of D-ribose fermentation are shown in Figure 3. Both D-ribose production in the hydrolysate medium and the basal medium without sugars are shown. Highest concentrations of D-ribose in the hydrolysate medium and the basal medium were 5.63 g/l and 1.14 g/l, respectively. Thus, production of D-ribose from the sugars in the hydrolysate was 4.49 g/l. Since the hydrolysate contained 0.19 g/l glucose and 7.97 g/l xylose the observed D-ribose production from the sugars in the hydrolysate was equivalent to 55.2% of theoretical yield. The low yield values probably were the results of lack of control of environmental parameters in the shakeflasks, for example, pH. The final pH's were not measured in these experiments but in other experiments using sweet sorghum bagasse hydrolysate obtained under similar pretreatment and hydrolysis conditions the pH increased to about 8.5 at the end of the experiments. Fermentations performed in fermentors with better control should result in higher yields of D-ribose. Production of D-ribose from sweet sorghum hydrolysate in fully instrumented fermentors will be investigated in our next study. Nevertheless, the results obtained in the shakeflask experiments demonstrated that the sweet sorghum hydrolysate could be used for D-ribose production.

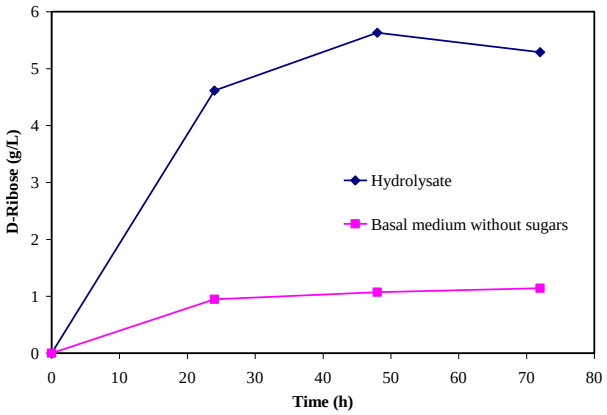

Figure 3. Production of D-ribose from sweet sorghum bagasse hydrolysate.

The results of astaxanthin fermentation are shown in Figure 4. The results clearly demonstrated that the sweet-sorghum bagasse hydrolysate was a good medium for astaxanthin production. It was previously observed that high glucose concentrations suppressed utilization of other sugars by *P. rhodozyma* [5]. The hydrolysate obtained by hydrolysis of the SAA-treated sweet-sorghum bagasse contained very low glucose concentrations, thus, qualified itself for a suitable fermentation medium for conversion of xylose to astaxanthin. The final yield was 1.92 mg astaxanthin/g total sugars consumed, which was similar to the yiled observed previously [5].

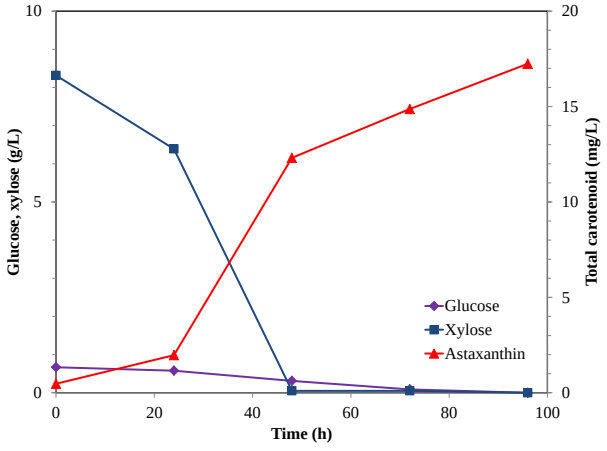

Figure 4. Production of astaxanthin from sweet sorghum bagasse hydrolysate.

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3.4 The sweet-sorghum biorefinery concept

An integrated process for production of fuel (ethanol) and chemicals (D-ribose, astaxanthin) in a sweet-sorghum biorefinery has been demonstrated. The simplified process flow diagram (PFD) is shown in Figure 5. In this process, the juice is first extracted from the sweet-sorghum stalks. The bagasse obtained after juice extraction is pretreated by the SAA process. The pretreated material is hydrolyzed with a commercial hemicellulase product to provide a solution rich in xylose and low in glucose. This solution then is used for production of value-added chemicals in fermentation processes. In this investigation we showed the results of two such chemicals, i.e. D-ribose and astaxanthin. However, other industrial chemicals, for example, xylitol, lactic acid, succinic acid, butyric acid, citric acid, itaconic acid, butanol, etc, can also be produced using the xylose-rich solution and suitable microorganisms. The residues obtained after the enzymatic hydrolysis of the pretreated bagasse, in which the glucan contents have been concentrated by extraction of lignin in the pretreatment step and conversion of xylan to xylose in the hydrolysis step, is combined with the extracted juice in a simultaneous saccharification and fermentation (SSF) process. A commercial cellulase product is used for hydrolysis of the glucan in the residue. The yeast *S. cerevisiae*, which is the most efficient ethanol-producing organism, is used for ethanol production.

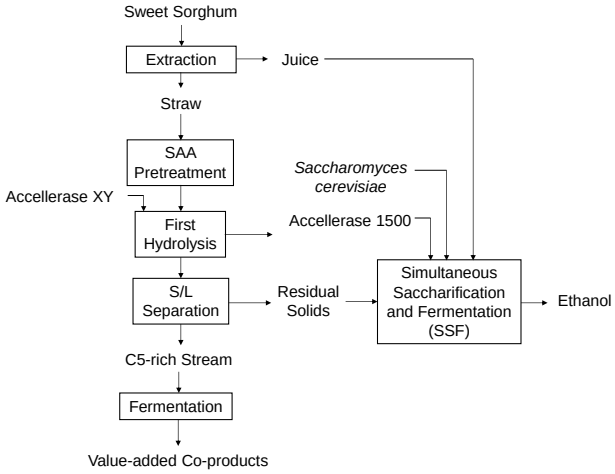

Figure 5. The integrated process for production of ethanol and value-added chemicals in a sweet-sorghum biorefinery.

4 CONCLUSIONS

An integrated process for production of fuels and chemicals in a sweet sorghum biorefinery has been demonstrated. The results shown here are only the proof of concept for such process. Further process development and optimization followed by process validation in pilot plants are needed before the developed process can be implemented in commercial practice.

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