Biomass and Bioenergy 94 (2016) 46–56

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Sweet sorghum as a whole-crop feedstock for ethanol production

Carolina A. Barcelos [a], Roberto N. Maeda [a], Lídia Maria M. Santa Anna [b], Nei Pereira Jr. [a, *]

[a] Department of Biochemical Engineering, School of Chemistry, Laboratories of Bioprocess Development, Federal University of Rio de Janeiro, Av. Athos da Silveira Ramos 149, Rio de Janeiro, Brazil
[b] Leopoldo Américo Miguez de Mello Research and Development Center, PETROBRAS, Av. Horácio Macedo 150, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 2 August 2014
Received in revised form
24 July 2016
Accepted 25 August 2016

Keywords:
Sweet sorghum
Ethanol
Enzymatic hydrolysis
Pretreatment
Simultaneous saccharification and fermentation
Lignocellulose

ABSTRACT

The potential of sweet sorghum as an alternative crop for ethanol production was investigated in this study. Initially, the enzymatic hydrolysis of sorghum grains was optimized, and the hydrolysate produced under optimal conditions was used for ethanol production with an industrial strain of *Saccharomyces cerevisiae*, resulting in an ethanol concentration of 87 g L^{-1}. From the sugary fraction (sweet sorghum juice), 72 g L^{-1} ethanol was produced. The sweet sorghum bagasse was submitted to acid pretreatment for hemicellulose removal and hydrolysis, and a flocculant strain of *Scheffersomyces stipitis* was used to evaluate the fermentability of the hemicellulosic hydrolysate. This process yielded an ethanol concentration of 30 g L^{-1} at 23 h of fermentation. After acid pretreatment, the remaining solid underwent an alkaline extraction for lignin removal. This partially delignified material, known as partially delignified lignin (PDC), was enriched with nutrients in a solid/liquid ratio of 1 g/3.33 mL and subjected to simultaneous saccharification and fermentation (SSF) process, resulting in an ethanol concentration of 85 g L^{-1} at 21 h of fermentation. Thus, from the conversion of starchy, sugary and lignocellulosic fractions approximately 160 L ethanol.ton^{-1} sweet sorghum was obtained. This amount corresponds to 13,600 L ethanol.ha^{-1}.

1. Introduction

Brazil is one of the world's largest agricultural producers and is known for its agricultural expansion potential. Brazil is among the world's five largest agricultural producers and exporters and ranks second worldwide in bioethanol production. Currently, bioethanol is the most promising alternative renewable energy source to petroleum production. Bioethanol has the potential to provide sustainable, cost effective energy while reducing greenhouse gas emissions. Sweet sorghum [*Sorghum bicolor* (L.) Moench] has been widely recognized as a promising energy crop for bioethanol production due to its relative high biomass productivity and low input requirements. Sweet sorghum is a drought and heat tolerant, multipurpose crop that can be cultivated on a wide range of soils. As the only crop that provides grain and stems, sweet sorghum can be used for the production of sugar, alcohol, syrup, jaggery, fodder, fuel, bedding, roofing, fencing, paper and chewing. Sweet sorghum is also known for its easy cultivation from seeds, possibility of multiple crops per season and its large breeding potential [1–6]. In addition, its short growth period (3–5 months) makes this crop suitable as a complementary feedstock to sugarcane in marginal areas or in crop rotations. Therefore, sweet sorghum can expand production capacity, offering existing sugar-based biofuel industries a flexible, low-cost crop that can be utilized within existing harvest and processing infrastructures. Moreover, sweet sorghum can be used as an alternative crop in areas where sugarcane does not perform well.

Given all these advantages of sweet sorghum as a potential source of biofuels, the aim of this study was to evaluate ethanol production from whole sweet sorghum (i.e., juice, grains and bagasse) and the potential of this flexible crop as an alternative feedstock for sustainable and renewable ethanol production.

Although several studies highlight the potential of sweet sorghum for ethanol production, the results reported in this work prove that sweet sorghum must also be seen as an ideal energy crop and viable feedstock for a productive industrial scale since it has

Abbreviations: AC, Acid Cellulignin; dw, dry weight; CCRD, Central Composite Rotatable Design; PDC, Partially Delignified Cellulignin; RSM, Response Surface Methodology; SSB, Sweet Sorghum Bagasse; SSF, Simultaneous Saccharification and Fermentation.

* Corresponding author.
E-mail addresses: ninabarcelos@gmail.com (C.A. Barcelos), rmaeda76@gmail.com (R.N. Maeda), lidia@petrobras.com.br (L.M.M. Santa Anna), nei@eq.ufrj.br (N. Pereira).

http://dx.doi.org/10.1016/j.biombioe.2016.08.012
0961-9534/

higher ethanol productivity compared with other crops. Additionally, its utilization would not require major modifications in the existing industrial units (ethanol production worldwide must diversify feedstock), particularly in countries (as Brazil) subjected to harvest seasons.

Moreover, as far as hydrosability and fermentability (of all fractions – starch, hemicellulose and cellulose) are concerned, there are very few results in literature which depict high results of sugar and ethanol concentrations as we reported in this work. When compared with the Brazilian ethanol 1G production that is a mature technology, the volumetric productivity values herein obtained with starch hydrolysate fermentation as well as with cellulose hydrolysate fermentation are quite similar. It is also important to point out that high concentrations of sugars were obtained in the enzymatic hydrolysis of sweet sorghum grains, using low enzyme loads and with hydrolysis times shorter than those reported in the literature. Equally important, in the fermentation process of enzymatic hydrolysate of sweet sorghum grains, the ethanol productivity was higher when compared with other studies describes in literature. Besides, most studies show the conversion of only two fractions of sweet sorghum (juice/bagasse, juice/grains). Thus, the income generated from grain yield as a coproduct and other coproducts from processing, such as ethanol, will cover the cost of producing the crop making whatever income generated from ethanol a profit.

Finally, the energy biomass of the future should consider developing crops that deliver more than just cellulose. This way, the refineries that utilize integrated biochemical conversion and thermochemical conversion processes could use such crops to sustainably produce fuels and coproducts. This way, sweet sorghum, with its multi-platform of resources of starch, sugar and cellulose, is considered a very promising energy crop to the biorefinery approach.

2. Materials and methods

2.1. Feedstock, enzymes and enzymatic assays

Sweet sorghum grains, juice and bagasse samples were kindly supplied by Monsanto do Brasil Ltda (Uberlândia - MG, Brazil). The commercial enzymes used in this study were α-amylase from *Bacillus licheniformis* and glucoamylase from *Aspergillus niger*, which were donated from LNF Latino Americana (Bento Gonçalves - RS, Brazil), and cellulase from *Trichoderma reesei*, which was obtained from Novozymes Latin America Ltda. The enzymatic activities of α-amylase, glucoamylase and cellulase were measured using the methods described by Fuwa [7], Summer [8] and Ghose [9], respectively.

2.2. Yeast strains, culture media and inoculum preparation

The fermentative agent employed in this study was an industrial strain of *Saccharomyces cerevisiae* (JP1) isolated from a Brazilian distillery (Japungu Agroindustrial, Santa Rita - PB) and a flocculant strain of *Scheffersomyces stipitis* (CBS5774) from the "Central Bureau voor Schimmelcultures - CBS".

S. cerevisiae JP1 was maintained at 4 °C on YED medium containing 1% yeast extract, 2% glucose and 1.5% agar at pH 6.0. Cellular growth was performed in two steps. First, the yeast cells were cultured aerobically in a rotatory shaker (New Brunswick Scientific – Edison N. J., U.S.A.) at 37 °C, pH 4.5 and 200 rpm in a medium containing 20 g L^{-1} glucose, 1.25 g L^{-1} urea, 1.1 g L^{-1} KH_2PO_4, 2 g L^{-1} yeast extract and 40 mL L^{-1} of mineral salts solution, as described by Pereira Jr. and Bu'lock [10]. Then, the inoculum was grown aerobically in conical flasks with 200 mL of the same medium used in the previous cultivation and was inoculated with a yeast suspension of 3.0 g dw.L^{-1} of cells. The inoculated flasks were incubated in a rotatory shaker at 37 °C and 200 rpm. After this step, the cells were aseptically centrifuged (5000 g/40 min), and the biophase was used to inoculate the fermentation medium.

S. stipitis CBS5774 was maintained at 4 °C in a medium of the following composition: 1% yeast extract, 2% xylose and 1.5% agar at pH 6.0. A preculture was grown aerobically in a rotatory shaker at 30 °C, pH 6.0 and 200 rpm in a medium containing 20 g L^{-1} xylose, 1.25 g L^{-1} urea, 1.1 g L^{-1} KH_2PO_4, 2 g L^{-1} yeast extract and 40 mL L^{-1} of mineral salts solution. The inoculum was performed in two stages using the hemicellulosic hydrolysate obtained from acid pretreatment to adapt the cells for the fermentation process. In the first stage, the cells (5% v/v) were transferred to a liquid medium containing 25% hydrolysate, and in the second stage, the hydrolysate content was increased to 50%. For these two stages, the liquid medium was supplemented with urea (2.5 g L^{-1}), KH_2PO_4 (2.2 g L^{-1}), yeast extract (4 g L^{-1}) and 40 mL L^{-1} of salts solution. All cells were aseptically centrifuged (5000 g for 20 min), and the biophase was used to inoculate the fermentation medium.

2.3. Sweet sorghum juice: evaluation of fermentability

Batch fermentation was performed in a 2 L Biostat B reactor (B. Braun Biotech International – Germany) with a working volume of 0.8 L to evaluate the fermentability of sweet sorghum juice with no supplementation. First, the juice was centrifuged (4000 g/15 min) and sterilized at 0.5 atm for 20 min. The initial cell concentration (*S. cerevisiae* JP1) was approximately 12 g L^{-1} (dw.L^{-1}). Fermentation was conducted at 37 °C and 200 rpm, and the pH was maintained at 4.5.

Fermentation efficiency was calculated as: (actual weight of ethanol produced/theoretical weight of ethanol produced from juice) × 100%. Ethanol productivity was expressed as g of ethanol. $L^{-1}.h^{-1}$. Ethanol yield and volumetric ethanol productivity were calculated according to Eqs. (1) and (2), respectively:

$$Y_{P/S} = \frac{P - P_0}{S_0 - S} \quad (1)$$

$$Q_P = \frac{P - P_0}{t_f} \quad (2)$$

where: $Y_{P/S}$ is the ethanol yield ($g.g^{-1}$), P denotes the final ethanol concentration ($g.L^{-1}$), P_0 represents the initial ethanol concentration ($g.L^{-1}$), S_0 defines the initial substrate concentration ($g.L^{-1}$), S is the final substrate concentration ($g.L^{-1}$), Q_P represents the volumetric ethanol productivity ($g.L^{-1}.h^{-1}$) and T_f is the total fermentation time (h).

2.4. Sweet sorghum grains: compositional analysis

The starch content of dried sorghum grains was determined via quantitative enzymatic hydrolysis using commercial cellulases, α-amylase and glucoamylase, as described by Barcelos et al. [11].

2.5. Sweet sorghum grains: enzymatic hydrolysis

Table 1 outlines the optimal reaction conditions for the enzymatic hydrolysis of sweet sorghum grains was previously determined by Barcelos et al. [11].