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Article

Second Generation Ethanol Production from Brewers' Spent Grain

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Abstract: Ethanol production from lignocellulosic biomasses raises a global interest because it represents a good alternative to petroleum-derived energies and reduces the food *versus* fuel conflict generated by first generation ethanol. In this study, alkaline-acid pretreated brewers' spent grain (BSG) was evaluated for ethanol production after enzymatic hydrolysis with commercial enzymes. The obtained hydrolysate containing a glucose concentration of 75 g/L was adopted, after dilution up to 50 g/L, for fermentation by the strain *Saccharomyces cerevisiae* NRRL YB 2293 selected as the best producer among five ethanologenic microorganims. When the hydrolysate was supplemented with yeast extract, 12.79 g/L of ethanol, corresponding to 0.28 g of ethanol per grams of glucose consumed (55% efficiency), was obtained within 24 h, while in the non-supplemented hydrolysate, a similar concentration was reached within 48 h. The volumetric productivity increased from 0.25 g/L·h in the un-supplemented hydrolysate to 0.53 g/L h in the yeast extract supplemented hydrolysate. In conclusion, the strain *S. cerevisiae* NRRL YB 2293 was shown able to produce ethanol from BSG. Although an equal amount of ethanol was reached in both BSG hydrolysate media, the nitrogen source supplementation reduced the ethanol fermentation time and promoted glucose uptake and cell growth.

Keywords: brewers' spent grain; lignocellulosic conversion; second generation bioethanol; enzymatic saccharification; ethanologenic microorganisms

1. Introduction

The depletion of fossil feedstock and climate changes, such as global warming, due to green house gas emissions (GHGs), have caused increasing interest in alternative renewable sources of energy, pushing the worldwide trend to produce and use bio-based products and biofuels in substitution of the fossil-based ones [1].

The most abundant renewable resource produced all around the world is represented by lignocellulosic biomasses [2], which include agricultural residues, food farming wastes, "green-grocer's wastes", tree pruning residues and the organic and paper fraction of urban solid wastes. A global interest in using the lignocellulosic residues and waste as source of added value bioproducts is rising due to their renewability, low cost, abundance, and non-competitiveness with food [3], boosting the development of the biorefinery concept and advancing sustainable waste management [4].

Among the lignocellulosic residues, a great interest has been focused on brewers' spent grain (BSG). BSG is a solid residue of breweries consisting of exhausted grain husks, obtained after mashing and lautering. Its composition changes based on the operative conditions adopted during harvest, malting and mashing time [5].

Until now, BSG was employed as animal feed or deposited in landfill. However, recent studies revealed its potential application for the production of a wide range of high added value bio-products, such as organic acids, biogas, bioethanol, biopolymers and molecules for the food and pharmaceutical industries [6,7]. The feasibility of these production processes should be evaluated on the basis of local availability of BSG, its current utilization for animal feed production and the environmental and economical advantages that could be gained by changing its use to generate higher added value products. Second generation ethanol, produced from non-food renewable lignocellulosic resources, has been recognized as a good alternative to petroleum-derived transportation fuels, with several known advantages, such as high octane number, low cetane number and high heat of vaporization [8]. Furthermore, it reduces the food *versus* fuel conflict created by first generation ethanol, mainly produced in USA and Brazil by using corn and sugar cane, respectively, as a feedstock [9]. The complex structure of lignocellulosic biomasses requires a pretreatment step to remove the recalcitrant lignin, followed by the enzymatic saccharification prior to sugar fermentation into ethanol.

Several techniques, such as physical treatment, chemical (alkaline and/or acid) treatment, biological treatment, physicochemical treatment, *i.e.*, steam explosion, liquid hot water (LHW), ammonia fiber expansion (AFEX), supercritical fluid (SCF) treatment and thermochemical treatment have been explored for removing lignin [10]. Once lignin has been removed, saccharification of the free accessible (hemi)cellulose portions of the biomass is carried out to obtain a hydrolysate rich in fermentable monosaccharides [11].

In this manuscript, the screening of five ethanologenic microorganisms for their ethanol production ability was performed. The most productive strain was then tested for its ability to grow and produce

ethanol using the sugar mixture obtained by enzymatic hydrolysis of chemically pretreated BSG. Compared to the few other works so far reported on ethanol production from BSG, this study allowed achieving a higher or similar ethanol yield and a higher productivity.

2. Results and Discussion

2.1. Screening of Ethanologenic Microorganisms for Ethanol Production in a Synthetic Medium

The strains *Saccharomyces cerevisiae* NRRL Y 12,908, *S. cerevisiae* NRRL YB 2293, *S. cerevisiae* NRRL Y 11,878, *S. cerevisiae* NRRL Y 2034 and *Zigosaccharomyces rouxi* NRRL Y 2547 were investigated for their ethanol production ability in a synthetic growth medium containing 40 g/L glucose. Initial glucose was completely consumed by the five strains after 72 h of fermentation at 30 °C, while the final ethanol concentration varied from a minimum value of 9.01 ± 0.05 g/L to a maximum one of 10.82 ± 0.03 g/L (Table 1). Among the investigated strains, *S. cerevisiae* NRRL YB 2293 showed the highest fermentation yield, with a maximum value of ethanol production of 10.82 ± 0.03 g/L after 72 h and a productivity of 0.15 g/L h (Table 1). It gave an ethanol yield of 0.27 g/g, equivalent to 53% of the theoretical ethanol yield by *S. cerevisiae*.

Table 1. Glucose and ethanol concentration after 72 h of fermentation in synthetic medium of the yeast species strains. Data represent the mean of two independent experiments.

Strains	Glucose (g/L)	Ethanol (g/L)
	40.0	**0.0**
Saccharomyces cerevisiae NRRL Y 12,908	0.99	10.52 ± 0.02
Saccharomyces cerevisiae NRRL YB 2293	0.0	10.82 ± 0.03
Saccharomyces cerevisiae NRRL Y 11,878	0.0	9.25 ± 0.05
Saccharomyces cerevisiae NRRL Y 2034	0.0	9.56 ± 0.03
Zigosaccharomyces rouxi NRRL Y 2547	0.0	9.01 ± 0.05

2.2. Chemical Pretreatment of BSG and Saccharification of the Pretreated BSG by a Cocktail of Commercial Enzymes

BSG, as well as other lignocellulosic wastes, represents a renewable source of fermentable sugars that can be employed as a feedstock for biofuels and chemicals production.

In this study, the BSG was chemically pretreated and saccharified with a cocktail of commercial enzymes.

A chemical pretreatment of BSG was carried out following the operative conditions reported by Mussatto *et al.* [12]. The analyses of the macromolecular composition of BSG before and after the pretreatment are reported in Table 2. The untreated BSG consisted of about 14.42% (*w*/*w*) cellulose, 34.21% (*w*/*w*) hemicelluloses, 3.93% (*w*/*w*) lignin and 47.43% (*w*/*w*) other materials (ash, protein and extractives). The chemical composition of BSG varies according to barley variety, harvest time, malting, and mashing conditions [13]. It is worth noting that Murdock *et al.* [14] reported a BSG with a similar lignin and hemicellulose content of 29.9% and 3.8% (*w*/*w*), respectively. Moreover, the high content of other materials, such as proteins and fibers, detected in our study is typical for BSGs, in which those materials account for 20% to 70% of total composition [7].

Table 2. Chemical Composition (% *w*/*w*) of untreated, pretreated BSG.

Component	Untreated BSG	Pretreated BSG
Cellulose	14.42	86.49
Hemicellulose	34.21	3.87
Lignin	3.93	2.31
Others (ash, protein and extractives)	47.43	7.33

A first treatment with sulfuric acid was performed to solubilize the hemicellulosic fraction and increase the diffusion of sodium hydroxide into the lignocellulosic structure, thus enhancing soda pulping. The alkaline pretreatment, performed to liberate cellulose fibers from lignin [15], is not totally selective for lignin, and the carbohydrates, including cellulose, can also be degraded [16]. The immediate cooling of the reaction medium in an ice bath allowed reducing the sugar loss and obtaining a solid residue with high cellulose content [17].

After the pretreatment, a total biomass recovery yield of 7.89% (*w*/*w*) was obtained. Considering an initial amount of 100 g of dried BSG, the hemicellulose was the main removed fraction, with a recovery of only 3.87 g from the 34.21 g present in the initial biomass, while 2.31 g of lignin were recovered from the 3.93 g treated. The pretreatment also promoted a high hydrolysis of the other compounds (ash, protein and extractives) with a recovery of 7.33 g from the 27 g treated.

The composition of BSG after the complete pretreatment consisted of about 86.49% (*w*/*w*) cellulose, corresponding to the main fraction, 3.87% (*w*/*w*) hemicelluloses, 2.31% (*w*/*w*) lignin and 7.33% (*w*/*w*) other materials (Table 2).

The cellulose pulp obtained after pretreatment was saccharified with a cocktail of commercial enzymes. At the end of the reaction, a hydrolysate with a final glucose concentration equal to 75 g/L, a value higher than those obtained in the other few studies so far reported on the use of hydrolysates from agricultural residues as fermentation media for ethanol production (Table 3). In particular, the value of 97% efficiency for cellulose conversion into glucose attained in the saccharification step was higher than the efficiency of 72% obtained by Mussatto *et al.* on a similarly pretreated BSG [12]. The glucose yield was calculated according to the following equation:

$$\text{Glucose yield (\%)} = (\text{Glucose} \times 0.9 \times 100)/(\text{cellulose content in the substrate}) \quad (1)$$

Table 3. Ethanol production from BSG hydrolysates.

Waste Type	Waste Pretreatment	Enzymatic Hydrolysis	Yield of Glucose after Enzymatic Hydrolysis	Microorganism Used in Fermentation Step	Ethanol Production	References
BSG	1.25% (*v*/*v*) H_2SO_4 in a ratio of 1:8 (*w*/*w*) at 120 °C for 17 min following by treatement with 2% (*v*/*v*) NaOH in a 1:20 (*w*/*w*) ratio at 120 °C for 90 min	2.24% (*v*/*v*) cellulase (Novozymes) and 1% (*v*/*v*) β-glucosidase (Novozymes), using 8% (*w*/*v*) * substrate at 45 °C and 120 rpm for 72 h	75 g/L glucose corresponding to 97% efficiency of cellulose conversion into glucose	*Saccharomyces cerevisiae* NRRL YB 2293	12.0 g/L ethanol, corresponding to 0.26 g ethanol/g substrate in BSG hydrolysate without adding any nutrients supplementation and 12.79 g/L ethanol, corresponding to 0.28 g ethanol/g in the yeast extract-supplemented BSG	This study
BSG	20% (*w*/*v*) BSG, pretreated with 0.16 N HNO_3 at 121 °C for 15 min, partially neutralized to pH 5–6 with NaOH	Novozymes Biomass sample kit (cocktail of 42.0 (U/g) ** cellulase, 1.5 (U/g) β-glucosidase, 3.0 (U/g) hemicellulase and 2.5 (U/g) xylanase) for 18 h at 50 °C, 130 rpm	27 g/L glucose, 16.7 g/L xylose and 11.9 g/L arabinose	*Pichia stipitis* NCYC 1540 and	8.3 g/L corresponding to ethanol conversion yields of 0.32 g ethanol/g substrate	[18]
BSG	12.5% (*w*/*v*) BSG, pretreated with 2.5 M NaOH at 121 °C for 30 min, neutralized to pH 5–6 with H_2SO_4	17.0 (U/g) of xylanase and 3.21 (U/g) of endoglucanase from *Fusarium oxysporum* for 24 h at 30 °C, 1400 rpm	52 g/L glucose	*Fusarium oxysporum* F3	Ethanol conversion yields of 0.065 g ethanol/g substrate	[19]
BSG	20% (*w*/*v*) BSG, pretreated with 0.16 N HNO_3 at 121 °C for 15 min, partially neutralized to pH 5–6 with NaOH	Novozymes Biomass sample kit (cocktail of 42.0 (U/g) cellulase, 1.5 (U/g) β-glucosidase, 3.0 (U/g) hemicellulase and 2.5 (U/g) xylanase) for 18 h at 50 °C, 130 rpm	27 g/L glucose, 16.7 g/L xylose and 11.9 g/L arabinose	*Pichia stipitis* NCYC 1540	14.8 g/L ethanol	[20]
BSG	25% (*w*/*v*) BSG, pretreated with 5% (*w*/*v*) NaOH at 50 °C for 12 h	51 Filter Paper Uunit (FPU)/g Cellic®CTec2 (Novozymes) for 24 h	41.7 g/L glucose and 14.6 xylose	*Saccharomyces cerevisiae* strain NCYC479	17.3 g/L ethanol, corresponding to *ca.* 81% of theoretical ethanol yield	[21]
BSG	BSG treated with 7% (*w*/*v*) H_2SO_4 at 96 °C for 3 h	Enzymatic cocktail produced by *Fusarium oxysporum* F3 under submerged conditions	-	*Fusarium oxysporum* F3	109 g/Kg of substrate, corresponding to 60% of theoretical ethanol yield	[22]

Notes: * (*w*/*v*): weight/volume; ** (U/g): enzymatic unit per gram of substrate.

This result could be explained by a different composition of the BSG used in the two studies. Before and after the pretreatment, the BSG employed in this work and in that of Mussatto *et al.* had a similar cellulose and hemicellulose composition [12], but they differed in the content of lignin, which was higher in the material investigated by Mussatto *et al.* [12]. It is possible that the lignin content of 8.2% (*w*/*w*) for the BSG used by Mussatto *et al.*, compared to a value of 2.31% (*w*/*w*) for the BSG employed in this work, had a negative effect (i) during the alkaline pulping process, decreasing the lignin loss and (ii) during the saccharification, reducing the efficiency of cellulose conversion into glucose [12]. Moreover, it was reported that different pretreatment methods could influence the saccharification yield. The decrease of the BSG particle size to the micron level allowed improving the carbohydrate solubilization yield from 23% up to 45% by using a multi-enzyme mixture, as reported by Niemi *et al.* [23]. Xiros *et al.* [24] demonstrated that the yield of enzymatic hydrolysis of alkali pre-treated BSG, through the enzyme extract from *Neurospora crassa*, increased by about 50% in comparison with the non-pretreated material, achieving about 50% and 60% yield of pentose and glucose, respectively.

2.3. Ethanol Production Using BSG Hydrolysate as Fermentation Medium

The strain *S. cerevisiae* NRRL YB 2293, previously selected among the investigated ethanologenic strains, was analyzed for its ability to grow on the sugar mixture obtained by enzymatic hydrolysis of chemically pretreated BSG. The BSG hydrolysate was adopted as growth medium with or without 1.25% yeast extract supplementation. The medium was diluted to a glucose concentration of 50 g/L and the pH was adjusted to 6.0.

The strain was able to grow in both media, showing that the BSG hydrolysate is a potential fermentation medium for ethanol production. The ethanol yield per substrate consumed ($Y_{P/S}$) was almost the same in the two different media: 0.26 g/g (51% efficiency) and 0.28 g/g (55% efficiency) when the BSG hydrolysate was used without adding any additional element and in the yeast extract-supplemented formulation, respectively (Table 4). However, the cell biomass increased when the yeast extract was added to the BSG hydrolysate. Hence, the ethanol yield per cell mass ($Y_{P/X}$) obtained in the no supplemented medium was much higher than that reached in the yeast extract supplemented BSG hydrolysate (2.8 g/g *versus* 1.7 g/g, respectively) (Table 4).

Table 4. Fermentative parameters of ethanol production by *Saccharomyces cerevisiae* NRRL YB 2293 from BSG hydrolysate and BSG hydrolysate + yeast extract.

Medium composition	Glucose Consumption (g/L)	Ethanol (g/L)	$Y_{P/S}$ (g/g) [a]	$Y_{P/X}$ (g/g) [b]	Q_P (g/L h) [c]	η (%) [d]
BSG hydrolysate	45.0	12.0	0.26	2.8	0.25	51
BSG hydrolysate + yeast extract	45.0	12.79	0.28	1.7	0.53	55

Notes: [a] g-ethanol produced/g-glucose consumed; [b] g-ethanol/g-dry cell weight; [c] (g/L) ethanol/(h) fermentation time; [d] $Y_{P/S}$/maximum theoretical value (0.51 g/g).

Even though all the glucose was consumed by the cells in both media, and the same ethanol concentration was reached, it is worth noting that the yeast extract supplementation allowed increasing the volumetric productivity: the cells produced 12.79 ± 1.2 g/L of ethanol, completed the glucose

uptake and reached the highest cell dry biomass (7.01 ± 1.3 g/L) within 24 h, while in the BSG hydrolysate without any nutrient supplementation, all the glucose was used by the cells, achieving almost the same ethanol concentration of 12.0 ± 1.2 g/L, and reaching the highest cell dry biomass (4.25 ± 1.1 g/L) only after 48 h (Figure 1) (Table 4). In fact, the volumetric productivity in BSG hydrolysate was 0.25 g/L·h, two-fold lower than the value reached in the yeast supplemented BSG hydrolysate (0.53 g/L·h) and the ethanol yield per cell mass ($Y_{P/X}$) obtained in the no supplemented medium was 2.7 g/g, much higher than that reached in the yeast extract supplemented medium (1.7 g/g) (Table 4).

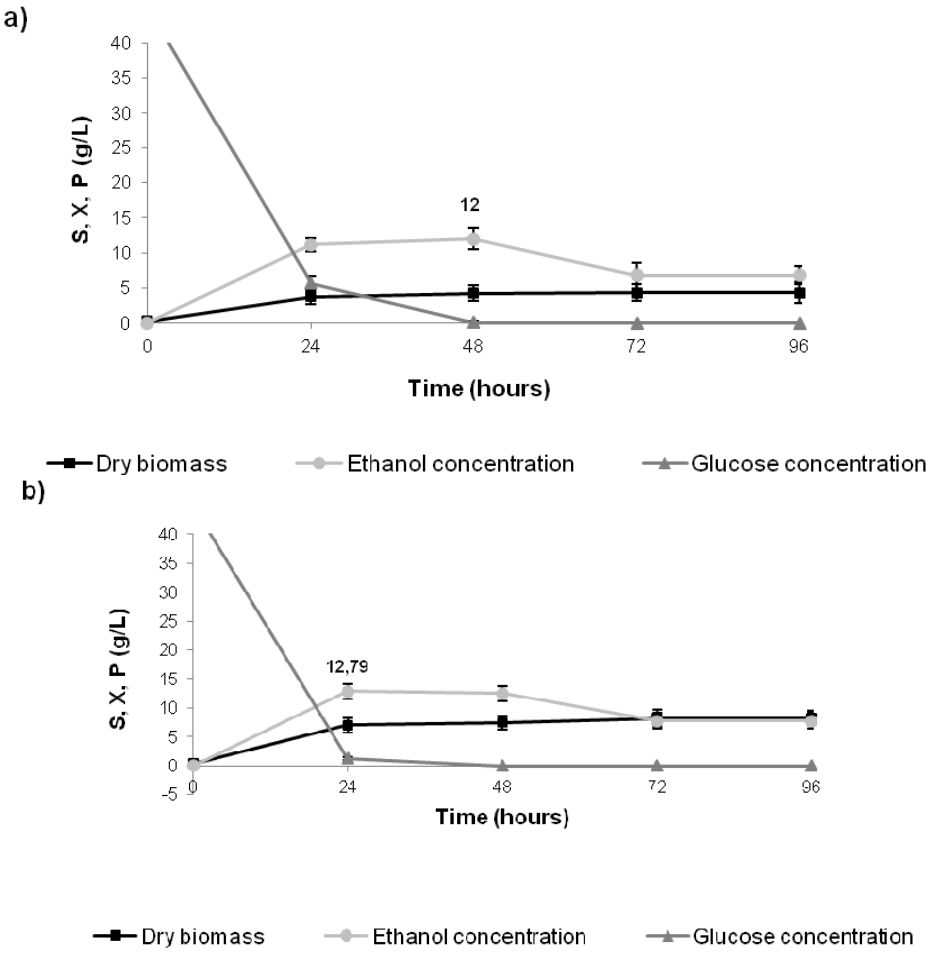

Figure 1. Fermentation of glucose to ethanol by *Saccharomyces cerevisiae* NRRL YB 2293 in (**a**) BSG hydrolysate and (**b**) BSG hydrolysate + yeast extract; glucose concentration (*S*, Δ), dry biomass concentration (*X*, □) and ethanol concentration (*P*, ○). All values are mean of two independent experiments.

These results showed clearly that the nitrogen source supplementation allowed a better growth of the cells, which completed the glucose uptake and reached the fermentation ethanol rate in 24 h instead of 48 h, as reported by Kolothumannil and Ingledew [25]. They demonstrated that the yeast extract supplementation to the wheat mashes hydrolysate reduced the ethanol fermentation time and promoted the glucose uptake and cell growth: an ethanol yield of 17.1% (*v*/*v*) was obtained within three days when yeast extract was added, while in the absence of nutrient supplementation, a final ethanol yield of 16.9% (*v*/*v*) was still achieved but after eight days.

Few works on BSG conversion into ethanol are so far reported and the results of these studies are summarized in Table 3. The different pretreatment methods, enzymatic cocktails for the saccharification step, ethanologenic microorganisms and growth conditions employed in these works influenced the ethanol yield.

Recently, Wilkinson *et al.* performed a study targeted at establishing the best BSG treatment, among the NaOH- or alkaline peroxide-based methods [21], to maximize the release of sugars during enzymatic hydrolysis. Pre-treatment with 5% NaOH at 50 °C for 12 h at 25% (*w*/*v*) solids, followed by a saccharification with an excess commercial enzyme resulted in the best pretreatment method for the BSG. The operative conditions of each step were completely different from our work, influencing the fermentation rate, but not the final ethanol concentration (Table 3). As a matter of fact, in 10 days of fermentation, they obtained almost the same ethanol yield reached in only one day by our strain *S. cerevisiae* NRRL YB 2293 (17.3 g/L *vs.* 12.79 g/L).

An ethanol yield production of 0.065 g/g from dry BSG, very low in comparison to 0.28 g/g obtained by *S. cerevisiae* NRRL YB 2293, was reported by Xiros *et al.* [19] (Table 3). However, the authors adopted very different operative conditions to produce ethanol by employing the mesophilic fungus *Fusarium oxysporum* and coupling alkali BSG pretreatment with solid-state (for enzymes production by the fungus) and submerged fermentation (for ethanol production).

The concentration of ethanol obtained from pretreated BSG by the strain *Pichia stipitis* NCYC 1540 was lower than that reached by *S. cerevisiae* NRRL YB 2293 (8.3 g/L *vs.* 12.79 g/L, respectively) (Table 3), although the ethanol yield is almost the same (0.32 g/g *vs.* 0.26 g/g, respectively) [18]. Even after optimization (effect of pH, other toxic inhibitors or lack of nutrients), performed by Yohannan *et al.* [20], the same strain gave a comparable ethanol production (~14.8 g/L) (Table 3) to that obtained in this work (12.79 g/L). Recently, Xiros and Christakopoulos [22] obtained a high ethanol yield from BSG by using the fungus *Fusarium oxysporum*. The (hemi)cellulolytic fungal enzymes, produced under submerged fermentation on BSG, were used for the saccharification of alkali-pretreated BSG; the obtained hydrolysate was converted into ethanol by the fungus in a consecutive submerged fermentation. The optimized-process allowed obtaining an ethanol yield of 109 g/Kg dry BSG, corresponding to 60% of the theoretical ethanol yield.

In conclusion, compared to the few other works so far reported on ethanol production from BSG, this study achieved a higher or similar ethanol yield and a higher productivity.

3. Experimental Section

3.1. Microorganisms and Cultivation Conditions for Screening in Synthetic Medium

The microorganisms investigated in this study include the strains *Saccharomyces cerevisiae* NRRLY 12,908, *S. cerevisiae* NRRL YB 2293, *S. cerevisiae* NRRL Y 11,878, *S. cerevisiae* NRRL Y 2034 and *Zigosaccharomyces rouxi* NRRL Y 2547, all belonging to the strain collection of the Bioprocess and Biotechnology Division of the Department of "Engenharia de Bioprocessos e Biotecnologia" (University Federal do Paraná, Brasil). Cell cultures were maintained in 10 g/L glycerol at −20 °C. One milliliter of stock culture cells was transferred to 25 mL test tubes containing 10 mL sterile YM broth medium with the following composition: 10 g/L glucose, 5 g/L peptone,

3 g/L malt extract and 3 g/L yeast extract. After incubation at 30 °C and 120 rpm for 24 h, 5 mL of pre-inoculum were inoculated in 125 mL Erlnemeyer flask containing 50 mL of synthetic fermentation broth with the following composition: 40 g/L glucose, 5 g/L peptone, 3 g/L malt extract and 3 g/L yeast extract. The flasks were incubated at 30 °C and 120 rpm for 72 h. Samples collected at time zero and after 72 h were subjected to analytical tests.

3.2. Chemical Pretreatment of BSG and Enzymatic Hydrolysis of Pretreated Material

The BSG, kindly provided by the brewery Bier Hoff Curitiba-PR (Brazil), was pretreated by using 1.25% (*v*/*v*) H_2SO_4 in a ratio of 1:8 (*w*/*w*) at 120 °C for 17 min [15]. After washing with water until neutral pH and drying over night at 50 ± 5 °C, the solid residue was treated with 2% (*v*/*v*) NaOH in a 1:20 (*w*/*w*) ratio at 120 °C for 90 min [26]. The residual cellulose pulp was washed and dried over night at 50 ± 5 °C. The chemical composition of the untreated and treated BSG was determined by the Animal Nutritional Laboratory of the Federal University of Paraná, using the method of Van Soest [27,28]. Saccharification was performed with 2.24% (*v*/*v*) cellulase (Novozymes) and 1% (*v*/*v*) β-glucosidase (Novozymes), using 8% (*w*/*v*) substrate at 45 °C and 120 rpm for 72 h. The hydrolysate was collected after centrifugation.

3.3. Inoculum and Fermentation Conditions for Cultivation in BSG Hydrolysate

The strain selected as the best producer of ethanol in the synthetic fermentation broth, *S. cerevisiae* NRRL YB 2293, was pre-inoculated in 25 mL tubes containing 10 mL of YM broth. After incubation at 120 rpm for 24 h at 30 °C, 1 mL of this culture was transferred to a new tube with 10 mL of YM broth, and incubated at the same conditions. After 24 h, cells were collected by centrifugation at 10,000 rpm for 15 min and washing three times in sterilized water. Ten percent (*v*/*v*) of this suspension was added to 10 mL of BSG hydrolysate with or without the addition of 1.25% (*w*/*v*) yeast extract. The fermentation media were adjusted to a pH of 6.0 by adding 5 M NaOH and diluting up to 50 g/L of glucose concentration. Incubation was performed at 30 °C and 120 rpm for 96 h. Samples for High-Performance Liquid Chromatography analyses were collected every 24 h and all of the assays were performed in duplicate.

3.4. Analytical Methods

Samples taken during the fermentation were centrifuged at 13,000 rpm for 15 min. Glucose and ethanol concentrations were measured by High-Performance Liquid Chromatography (HPLC). The analyses were performed in a Shimadzu LC-10-AD equipped with a C-RSA Integrator Chromatopac Chromatography Unit (Shimadzu, Kyoto, Japan) set to 210 nm and (300 mm × 7.8 mm) column Aminex HPX-87-4 (Bio-Rad Labs, Richmond, CA, USA), and a refractive index detector. Before the injection into the chromatograph, samples were diluted with deionized water and filtered through 0.2 μm cellulose acetate filters (Sartorius Biolab Products, Goettingen, Germany). The conditions of chromatography used were: a column temperature of 60 °C, a mobile phase of 5 mM sulfuric acid at a flow rate of 0.6 mL/min, and an injection volume of 50 μL. Calibration curves obtained using standard solutions were utilized to calculate the glucose and ethanol concentration. Cell optical density (OD)

was spectrophotometrically measured at 600 nm by using the spectrophotometer SP-2000 (Shanghai Spectrum Instruments Co., Shanghai, China). The OD values were correlated with the cell concentration (g/L) by means of a standard calibration curve previously established. One unit of optical density at 600 nm corresponded to approximately ~0.75 g dry cell weight/L.

3.5. Analysis of Fermentative Parameters

The ethanol yield per substrate consumed ($Y_{P/S}$ g/g), the volumetric productivity (Q_p g/L h), the ethanol yield per cell mass ($Y_{P/X}$ g/g), and the efficiency (η, %) were evaluated for each sample collected during the fermentations.

4. Conclusions

Among the investigated strains, *Saccharomyces cerevisiae* NRRL YB 2293, belonging to the collection of the Bioprocess and Biotechnology Division of the Department of "Engenharia de Bioprocessos e Biotecnologia" (University Federal do Paraná, Brasil), was selected for its higher ability to produce ethanol in a synthetic medium. It was then applied for conversion of BSG hydrolysate into ethanol.

BSG was subjected to a chemical pretreatment by acid-alkali method and saccharified with a cocktail of commercial enzymes, obtaining 97% efficiency of cellulose conversion into glucose.

The strain *S. cerevisiae* NRRL YB 2293 was able to grow and to produce ethanol on the BSG hydrolysate with and without adding yeast extract. The ethanol yield per substrate consumed ($Y_{P/S}$) was almost the same in both media: 0.26 g/g (51% efficiency) and 0.28 g/g (55% efficiency) when the BSG hydrolysate was used without adding any additional element and in the yeast extract-supplemented BSG, respectively.

Yeast extract supplementation promoted cell growth, glucose uptake and ethanol fermentation at a higher rate. When the BSG was supplemented with yeast extract, the cells produced 12.79 g/L ethanol, completed the glucose uptake and reached the highest cell dry biomass (7.01 g/L) within 24 h, while in the BSG hydrolysate without any nutrient supplementation, all the glucose was consumed by the cells, achieving almost the same ethanol concentration of 12.0 g/L and reaching the highest cell dry biomass (4.25 g/L), within 48 h.

The volumetric productivity in the unsupplemented BSG was 0.25 g/L h, twofold lower than the value reached in the yeast extract supplemented BSG (0.53 g/L h) and the ethanol yield per cell mass ($Y_{P/X}$) obtained in the non-supplemented medium was 2.7 g/g, much higher than that reached in the yeast extract supplemented medium (1.7 g/g).

The application of the strain *S. cerevisiae* NRRL YB 2293 on BSG hydrolysate allowed obtaining a higher or equal ethanol yield compared to the few other works so far reported.

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Author Contributions

Rossana Liguori carried out the experiments of screening of the ethanologenic microorganisms; chemical pretreatment of BSG and enzymatic saccharification of the solide residue remaining after pretreatment; and test of the most productive strain in ethanol production from BSG hydrolysate; Carlos Ricardo Soccol contributed to conceiving the study and participated in its design and coordination for the part of BSG pretreatment and saccharification; Luciana Porto de Souza Vandenberghe contributed to conceiving the study and participated in its design and coordination for the part of BSG conversion and analyzed the data; Adenise Lorenci Woiciechowski carried out the HPLC analyses; Vincenza Faraco contributed to conceiving the study and participated in its design and coordination for the part of screening of microorganisms and conversion of BSG hydrolysate and drafted the manuscript; All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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