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Optimization of Brewery's Spent Grain Dilute-Acid Hydrolysis for the Production of Pentose-Rich Culture Media

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Abstract

Dilute-acid hydrolysis of brewery's spent grain to obtain a pentose-rich fermentable hydrolysate was investigated. The influence of operational conditions on polysaccharide hydrolysis was assessed by the combined severity parameter (*CS*) in the range of 1.39–3.06. When the *CS* increased, the pentose sugars concentration increased to a maximum at a *CS* of 1.94, whereas the maximum glucose concentration was obtained for a *CS* of 2.65. The concentrations of furfural, hydroxymethylfurfural (HMF), as well as formic and levulinic acids and total phenolic compounds increased with severity. Optimum hydrolysis conditions were found at a *CS* of 1.94 with >95% of feedstock pentose sugars recovered in the monomeric form, together with a low content of furfural, HMF, acetic and formic acids, and total phenolic compounds. This hydrolysate containing glucose, xylose, and arabinose (ratio 10:67:32) was further supplemented with inorganic salts and vitamins and readily fermented by the yeast *Debaryomyces hansenii* CCMI 941 without any previous detoxification stage. The yeast was able to consume all sugars, furfural, HMF, and acetic acid with high biomass yield, 0.68 C-mol/C-mol, and productivity, 0.92 g/(L·h). Detoxification with activated charcoal resulted in a similar biomass yield and a slight increase in the volumetric productivity (11%).

Index Entries: Brewery's spent grain; dilute-acid hydrolysis; pentose-rich media; *Debaryomyces hansenii*.

Introduction

Lignocellulosic agroindustrial byproducts are renewable, widespread, and generally an inexpensive source of biomass. Brewery's spent grain (BSG) is a disposal residue generated throughout the year in large amounts, for

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which new uses are needed, owing to its low value and negative environmental impact. Like other hemicellulose-rich agroindustrial residues, it may be used for the production of added-value products such as functional oligosaccharides by selective hydrolysis *(1,2)*, and fuel or chemicals by fermentation processes *(3–6)*. In the latter case, complete polysaccharide hydrolysis is required to ensure an efficient fermentation process. During hydrolysis several potential inhibitory compounds are cogenerated, resulting in a decrease in yield and productivity. To overcome this well-documented effect, several physicochemical detoxification treatments have been reported, such as overliming, sulfitation, extraction with organic solvents, activated charcoal adsorption, ion-exchange resins, and evaporation or biologic treatments using peroxidase and laccase enzymes from fungi *(5,7)*, which are the main reason for the increase in the overall bioprocessing cost.

Thus, it is important to look for hydrolytic processes to overcome the need for detoxification. Mild processes using very dilute sulfuric acid, water, or steam alone have been mainly used as a pretreatment in the biomass-to-ethanol process *(8–11)*. Although these processes allow a high solubilization of hemicellulose to be obtained *(11–13)*, the high quantity of sugars in the oligomeric form *(10,11,14)* or the reduced sugar recoveries *(15)* are drawbacks that limit the use of these processes for producing hydrolysates for yeast fermentation, at least without subsequent post-hydrolysis *(16–18)*.

Dilute-acid hydrolysis is therefore the main choice for the hydrolysis of hemicellulose to monosaccharides once it is a fast and efficient method *(19)*. However, a careful optimization of operational conditions used for hydrolysis is important to ensure high monosaccharide recovery and minimize coformation of microbial inhibitors.

In the present work, we studied the hydrolysis of BSG using the combined severity concept *(20)*, which combines the effects of time, temperature, and acid concentration into a single parameter (combined severity factor [*CS*]), with the aim of establishing the operational conditions leading to the maximum yield of pentoses. The inhibitory byproducts recovered in the hydrolysates, such as acetic acid, furfural, hydroxymethylfurfural (HMF), formic acid, levulinic acid, and total phenolics, were also evaluated. The pentose-rich hydrolysate obtained was further used as culture medium for the pentose-assimilating and xylitol-overproducing yeast *Debaryomyces hansenii*. Hydrolysate toxicity was assessed by comparing the yeast growth kinetic parameters on detoxified vs nondetoxified dilute acid hydrolysates.

Materials and Methods

Preparation of Feedstock

BSG, kindly supplied by Sociedade Central de Cervejas (Vialonga, Portugal), was pretreated in an autoclave (liquid-to-solid ratio of 8 [w/w]) at 100°C for 1 h in order to remove the residual starch. The solid was fil-

tered, washed, and dried at 50°C until reaching a moisture content <10% (w/w). The feedstock material was screened (>0.5 mm retained) and stored in polyamide-polyethylene vacuum-sealed bags prior to use. On average, the pretreated BSG contained 19.6% glucan, 19.8% xylan, 9.4% arabinan, 1.4% acetyl groups, 22.8% Klason lignin, 23.5% protein, and 1.0% ash.

Dilute-Acid Hydrolysis

The feedstock was mixed with 3% (w/w) sulfuric acid solution in 500-mL closed universal flasks with a liquid-to-solid ratio of 8 (w/w). The moisture content of the samples was included as water in the material balances. The mixtures were allowed to stand for 10 min at room temperature in order to equilibrate the acid concentrations between the bulk phase and biomass. Hydrolysis was performed in an autoclave at 130°C for preestablished isothermal periods ranging from 2 to 240 min. The flasks were placed inside the autoclave at 100°C, and the heating time to reach 130°C was recorded. After the reaction time had elapsed, the autoclave was rapidly cooled down and the hydrolysate and solid phase were recovered by filtration (Whatman no. 1 filter paper). All experiments were done at least in duplicate.

The solid phase was washed with water, dried at 40°C, and the yield and composition (xylan, arabinan, glucan, Klason lignin) were determined.

The severity of the treatments was measured using the *CS (20)*, which relates the effect of acid catalysts with severity factor R_0 (Eq. 1) *(21)* into a single parameter (Eq. 2):

$$R_0 = \int_0^t \exp\left(\frac{T-100}{14.75}\right) \cdot dt \tag{1}$$

$$CS = \text{Log } R_0 - \text{pH} \tag{2}$$

in which t is time (min) and T is the temperature (°C). The heating periods from 100 to 130°C were considered for R_0 calculations. The pH was calculated from the amount of sulfuric acid added *(22,23)*.

Detoxification of Dilute-Acid Hydrolysate

For CS 1.94, several treatments were performed as described above in order to obtain enough volume of hydrolysates for fermentation experiments. Thereafter the hydrolysates were mixed, stored at 4°C and several detoxification methods, including activated charcoal and anion-exchange resin treatments, were applied. Nondetoxified hydrolysate was adjusted to pH 5.5 by adding $Ca(OH)_2$. After 1 h the precipitate was removed by centrifuging (Avanti J-25i; Beckman Coulter, Fullerton, CA) at 7500*g* for 25 min.

The detoxification treatments were done as follows.

Activated Charcoal Treatment

Granular activated charcoal about 2.5 mm from Merck (Darmstadt, Germany) was washed with water and equilibrated with HCl (0.4 *M*),

washed again with water and dried at room temperature. The charcoal was mixed with the pH 5.5 hydrolysates (10% [w/v]) and stirred for 1 h at room temperature. The detoxified hydrolysate was recovered by filtration (Whatman no. 1 filter paper). Thereafter, the pH was adjusted to 5.5 with $Ca(OH)_2$ or H_2SO_4 and, if needed, centrifuged at 7500*g* for 25 min.

Anion-Exchange Resin Treatment

Weak anion-exchange resins (Dowex Marathon WBA, 25–50 mesh) obtained from Aldrich (Milwaukee, WI) were equilibrated with 0.1 *M* NaOH and then washed with water and filtered until a neutral effluent was obtained. The hydrolysate was treated with resins in a batch process at room temperature. The amount of resins added to the hydrolysate was sufficient to obtain pH 5.5. Thereafter, the resin beads were removed by filtration (Whatman no. 1 filter paper).

Microorganism and Growth Conditions

D. hansenii CCMI 941 was used in all experiments. Stock cultures were maintained at 4°C on YM-xylose agar slants containing 20 g/L of D-xylose, 3 g/L of yeast extract, 3 g/L of malt extract, 5 g/L of peptone, and 20 g/L of agar.

Prior to fermentation, the hydrolysates (obtained at CS 1.94) were sterilized using 0.22-µm Gelman membrane filters (Ann Arbor, MI). pH-adjusted and detoxified hydrolysates were supplemented with salts, vitamins, nitrogen, phosphate, and magnesium sources. The nutrients were added in concentrated stock solutions to final concentrations as described before *(24)*, except citric acid, which was replaced with EDTA (final concentration of 0.19 g/L). In the nonsupplemented medium, the supplements were replaced with sterile water.

D. hansenii YM-xylose slants incubated at 30°C for 24 h were used to inoculate 1-L baffled Erlenmeyer flasks containing 80 mL of pH-adjusted (to 5.5) and nonsupplemented hydrolysate. Inocula cultures were grown in an Unitron orbital incubator (Infors, Bottmingen, Switzerland) at 150 rpm and 30°C for 16 h. Ten milliliters of inoculum culture was used to seed 1-L baffled Erlenmeyer flasks containing 80 mL of nondetoxified or detoxified hydrolysates, both nonsupplemented and supplemented with nutrients. Cultivations were run for 24 h at 30°C and 150 rpm. All experiments were carried out at least in duplicate.

Analytical Methods

Feedstock material and processed solids were analyzed for glucan, xylan, arabinan, and acetyl groups by quantitative acid hydrolysis according to standard methods *(25)*. The acid-insoluble residue was considered as Klason lignin, after correction for the acid-insoluble ash (determined by igniting the contents at 575°C for 5 h).

Protein content in the feedstock was estimated by the Kjeldahl method *(26)* using the $N \times 6.25$ conversion factor. Moisture content of the samples was determined by oven drying at 105°C to constant weight.

Glucose, xylose, arabinose, acetic acid, formic acid, levulinic acid, glycerol, ethanol, HMF, and furfural were analyzed by high-performance liquid chromatography (HPLC) (Waters, Milford, MA) using an Aminex HPX-87H (Bio-Rad, Hercules, CA) column operating at 50°C in combination with a cation H^+-guard column (Bio-Rad). The mobile phase was 5 m*M* H_2SO_4, and the flow rate was 0.4 mL/min. Detection was performed using an RI detector (Waters 2410) except in the case of furfural and HMF, in which an ultraviolet detector (Waters 486) set at 280 nm was used.

In fermentation samples, owing to the partial overlap of arabinose, xylitol, and arabitol, in the column Aminex HPX-87H, those components were also analyzed using a Sugar-Pak I column (Waters) operating at 90°C in a Merck-Hitachi HPLC system (Merck) equipped with an RI detector (L-7490; Merck). The mobile phase was 50 mg/L of calcium EDTA at a flow rate of 0.5 mL/min.

The total soluble phenolics were determined by a modification of the Prussian blue method *(27)* using tannic acid as standard. Cell mass was determined by optical density measurements at 600 nm using an LKB spectrophotometer (Biochrom II, Cambridge, UK) and gravimetrically at the beginning and end of fermentations as follows: Five-milliliter samples were vacuum filtered through 0.45-µm dried filters (Gelman), washed with 10 mL of ultrapure water, and dried overnight at 100°C to constant weight.

The elemental composition (C, H, N) of the cell mass was measured in a Leco CHN-2000 analyzer (St. Joseph, MI), while the oxygen content was obtained by the difference after correction for ash. Total organic carbon (TOC) of fermentation medium at the beginning and end of fermentations was analyzed with a TOC analyzer (Shimadzu 5050A; Kyoto, Japan) by the combustion technique with nondispersive infrared detection.

Results and Discussion

Dilute-Acid Hydrolysis of BSG

The effects of the operational conditions in the hydrolysates and solid composition were compared using *CS* in the range of 1.39–3.06. Sulfuric acid concentration and reaction temperature were kept constant for all runs. The severity was varied by setting the isothermal period from 2 to 240 min.

Figure 1 shows the variation in monosaccharide yield obtained after hydrolysis as a function of *CS*. Xylose and arabinose yield increased to a maximum at *CS* 1.94 and 2.04, respectively. Because only a slightly higher arabinose yield was obtained for *CS* 2.04 compared with *CS* 1.94, it was considered that maximum recovery of pentoses was obtained at CS 1.94. The highest pentose yields were 18.9 g/100 g for xylose and 9.0 g/100 g for arabinose. At these conditions, 95% of the feedstock xylan was recovered as xylose and 96% of the feedstock arabinan as arabinose. Further increases in *CS* resulted in lower recoveries of pentoses owing to their degradation. Note that a large part of the feedstock hemicellulose was released to the medium for the lowest severity assayed. At the highest severity, xylose and

arabinose recoveries were only 59 and 69% of the original feedstocks xylan and arabinan, respectively.

Glucose concentrations increased with the severity up to *CS* 2.65. A further increase in *CS* led to a slight decrease in glucose concentration. The highest glucose concentration obtained corresponds to a yield of 4.7 g/100 g.

The high pentose yields obtained in the current study are favorable when compared to those previously reported for dilute-acid hydrolysis in batch processes *(28–31)*. However, the hemicellulosic sugar yield reported in the literature is often expressed as xylose equivalents, which is difficult to compare to monosaccharide recovery. The highest monosaccharide recovery was obtained with low *CS* values, which is in agreement with previous findings for sulfuric acid–catalyzed steam explosion, in which also better hemicellulosic sugar recoveries were obtained at relatively low severities *(28,32)* of <2.2 *(22)*.

Since we aim to use the BSG acid hydrolysate for yeast growth, special attention was paid to the compounds that can have a potential inhibitory effect on fermentation. These are generated both from the hydrolysis of lignocellulosic components, such as acetic acid and phenolic compounds, and by degradation reactions, such as furfural, HMF, formic acid, and levulinic acid. The concentrations of all measured inhibitory byproducts increased with severity, with the exception of acetic acid, which reached a maximum at *CS* 2.04 (Fig. 2), where 100% of the acetyl groups present in the feedstock were recovered as free acetic acid in the hydrolysate. For *CS* >2.04, acetic acid concentration remained quite stable. The maximum yields of furfural and HMF were 2.3 and 0.26 g/100 g, respectively. However, in the conditions leading to the highest recovery of pentoses (*CS* 1.94), the concentrations of both furan derivatives were quite low: 0.35 g/100 g for furfural and 0.02 g/100 g for HMF, which corresponds to 1.6% of feedstock xylan and 0.12% of feedstock glucan, respectively.

Levulinic acid was detected only for *CS* values >1.94, and the maximum yield obtained was 0.37 g/100 g for the severest conditions. The maximum yields of formic acid and total soluble phenolics found were 0.45 and 1.43 g/100 g. However, at *CS* 1.94 (maximum recovery of pentoses), those yields were much lower: 0.19 g/100 g for formic acid and 0.75 g/100 g for total phenolics.

The low yields of all inhibitory byproducts obtained here for BSG dilute-acid hydrolysates compare favorably with those of other agroindustrial residues for which higher inhibitory byproducts recoveries were obtained *(33)*, even when lower sulfuric acid concentrations were used *(34)*.

Note that near the maximal recovery of pentoses, inhibitor concentrations were quite stable, except for furfural, whose concentration increased steadily with *CS*, which is an advantage since it allows a broader range in operating conditions.

Since dilute-acid hydrolysis mainly solubilizes the hemicellulose components, the changes in solid yield (a measure of dry weight loss) are useful for monitoring the progress of the treatment. Table 1 shows the yield and

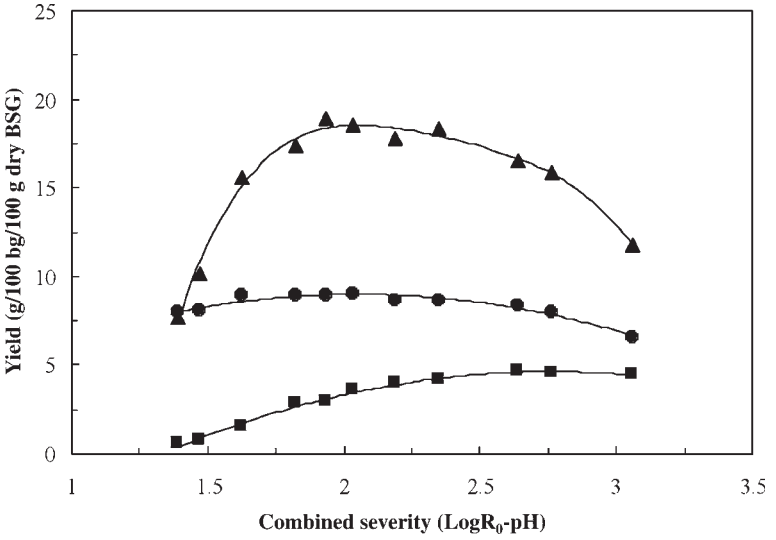

Fig. 1. Yield of monosaccharides after dilute-acid hydrolysis of BSG as a function of *CS*. (▲), Xylose; (●), arabinose; (■), glucose. The lines only show trends.

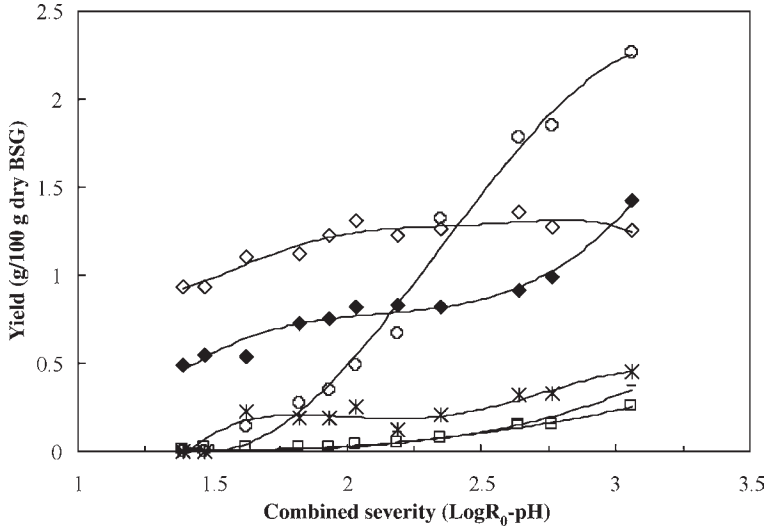

Fig. 2. Yield of inhibitory byproducts after dilute-acid hydrolysis of BSG as a function of *CS*. (◇), Acetic acid; (○), furfural; (□), HMF; (∗), formic acid; (—), levulinic acid; (◆), total phenolic compounds. The lines only show trends.

composition of the processed solids as a function of hydrolysis severity. The amount of solubilized xylan increased with *CS* reaching 100% for the severest operating conditions assayed, *CS* 3.06. Despite being solubilized slightly faster than xylan at low severities, traces of arabinan (0.8%) were still found in the solid at *CS* 3.06.

Table 1
Effect of *CS* Parameter on Solid Yield and Polymeric Composition of Processed Solids Obtained After Dilute-Acid Hydrolysis of BSG

	CS (Log R_0 – pH)[a]										
	1.39 (2)	1.48 (3.5)	1.63 (5)	1.83 (10)	1.94 (15)	2.04 (20)	2.19 (30)	2.36 (45)	2.65 (90)	2.77 (120)	3.06 (240)
Solid yield (%)[b]	70.2	69.3	64.4	61.4	60.7	59.2	56.2	50.2	48.2	49.3	46.8
Xylan (%)[c]	9.4	8.1	6.7	5.9	6.2	5.7	5.0	4.2	1.6	1.6	0.0
Arabinan (%)[c]	3.1	3.1	2.0	1.9	2.0	2.1	1.7	1.3	1.1	1.2	0.8
Glucan (%)[c]	25.5	26.5	29.2	28.8	32.9	31.1	33.3	36.5	38.4	38.4	37.1
Klason lignin (%)[c]	37.5	38.8	37.0	38.8	39.1	40.5	41.9	43.9	47.2	46.7	53.8

[a] Values in parentheses indicate the duration of the isothermal period (min).
[b] (g/100 g feedstock).
[c] (g/100 g processed solids).

Table 2
Composition of BSG Dilute-Acid Hydrolysate Obtained at *CS* Factor of 1.94

Compound	Concentration (g/L)
Xylose	26.7
Arabinose	12.8
Glucose	4.0
Acetic acid	1.5
Formic acid	0.23
Furfural	0.29
HMF	0.02
Total phenolics	0.91

Glucan was not significantly affected by the hydrolytic treatment, and the maximal glucan solubilization was only 11% for the severest conditions. Torget et al. *(11)* also reported a low solubilization of glucan when *CS* is relatively low.

Under the present conditions of acid hydrolysis, no significant removal of lignin was expected to occur. However, for all the experiments performed, the percentage of Klason lignin (measured as the acid-insoluble residue) increased with the increase in *CS* and its recovery ranged from 97 to 118%. The greater than 100% yield could be owing to the condensation of lignin with sugar and/or sugar degradation products such as furfural *(15,35,36)* to give insoluble reaction products, causing an increase in apparent Klason lignin yield. Moreover, since the Klason lignin procedure allows direct estimation of all components that are insoluble in both concentrated and hot dilute sulfuric acid, the Klason lignin value could also include part of the protein *(29)*.

From the reported data (Figs. 1 and 2, Table 1) the optimum condition to obtain a pentose-rich hydrolysate from dilute-acid hydrolysis of BSG at 130°C was 15 min (*CS* 1.94). Such hydrolysate contains about 43.5 g/L of glucose, xylose, and arabinose (ratio of 10:67:32), together with a low content of furfural, HMF, acetic acid, formic acid, and total phenolic compounds (Table 2). This condition was chosen for subsequent production of hydrolysates for fermentation.

Detoxification and Fermentation of BSG Acid Hydrolysates

The inhibitory effect caused by the acid hydrolysis byproducts depends mainly on the chosen microorganism and on the overall composition of the hydrolysate. Moreover, interaction effects involving several inhibitors are most likely implicated *(37)*.

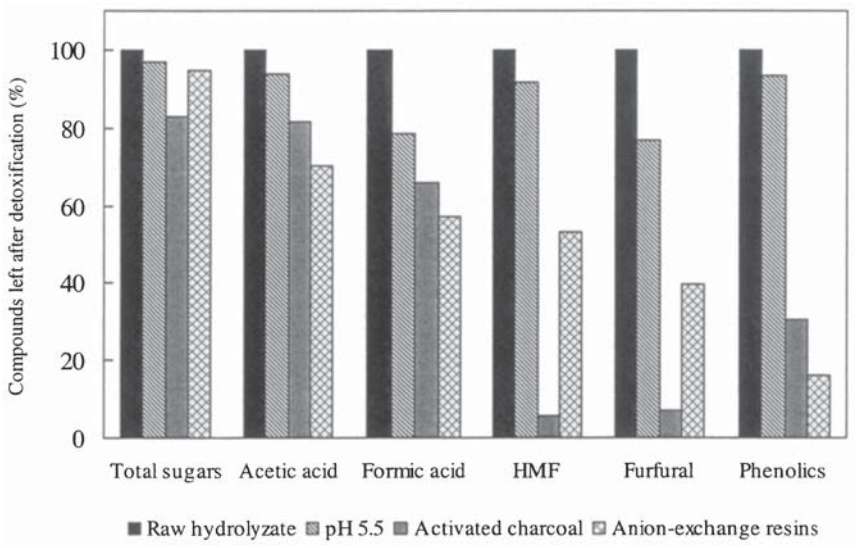

Fig. 3. Influence of detoxification methods on composition of BSG hydrolysates. Values are expressed as the percentage of the concentration of compounds left after treatment relative to the raw hydrolysate.

To evaluate the degree of microbial growth inhibition caused by the hydrolysate used, *Debaryomyces hansenii* was grown in detoxified hydrolysates and in a hydrolysate only subjected to a pH correction, both nonsupplemented and supplemented. As detoxification methods, anion-exchange resins and activated charcoal treatments were chosen because both enable the reduction of most of the fermentation-inhibiting compounds *(7,38,39)*.

Figure 3 shows the effect of pH correction to 5.5 and of the detoxification treatments assayed (anion-exchange resins and activated charcoal) on hydrolysate composition relative to the raw hydrolysate. Activated charcoal treatment was the most effective method for removal of furan derivatives, whereas the anion-exchange resin treatment enabled the highest removal of phenolics, which is in agreement with previous studies *(40–42)*. Resins were also slightly better than activated charcoal for the removal of aliphatic acids. Contrary to what happened with activated charcoal treatment, no significant loss of sugars was obtained with anion exchangers, which is in agreement with our previous findings *(43)*. Both detoxification treatments removed more inhibitors than the simple pH adjustment, which had only a minor effect on inhibitor removal.

Figure 4 shows the growth profiles of *D. hansenii* in nondetoxified (pH 5.5) and detoxified hydrolysates, both nonsupplemented and supplemented with several nutrients. Similar specific growth rate and biomass yield were observed for all nonsupplemented hydrolysates regardless of the utilization of a detoxification step (Table 3). A similar pattern occurred

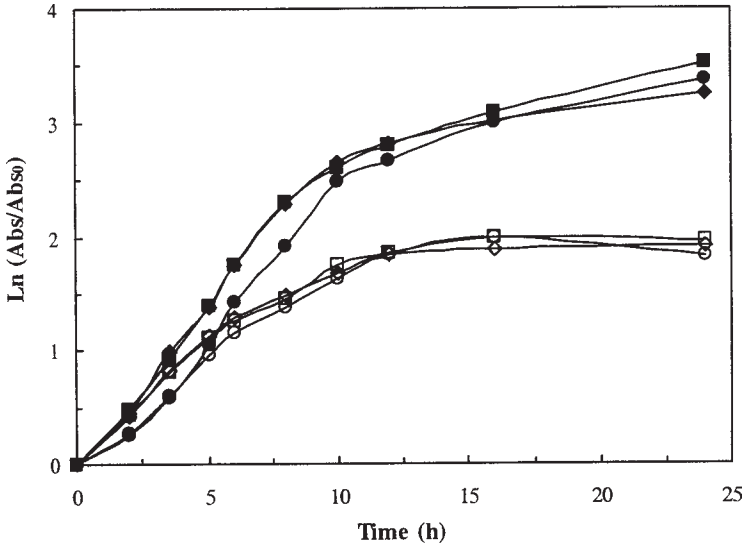

Fig. 4. Effect of detoxification and/or supplementation of BSG dilute-acid hydrolysates on growth profile of *D. hansenii* CCMI 941. Nonsupplemented: (○), pH 5.5 hydrolysate; (◇), activated charcoal detoxified hydrolysate; (□), anion-exchange resins detoxified hydrolysate; supplemented: (●), pH 5.5 hydrolysate; (◆), activated charcoal detoxified hydrolysate; (■), anion-exchange resins detoxified hydrolysate.

Table 3
Kinetic and Stoichiometric Parameters of *D. hansenii* CCMI 941 Batch Growth in Nonsupplemented and Supplemented Nondetoxified, Activated Charcoal Detoxified and Anion-Exchange Resins Detoxified BSG Dilute-Acid Hydrolysates[a]

	Nondetoxified		Activated charcoal		Anion-exchange resins	
	NS	S	NS	S	NS	S
μ (h^{-1})	0.22	0.29	0.22	0.31	0.21	0.31
Q_S (g/[L·h])	0.28	1.24	0.30	1.32	0.38	1.31
Q_X (g/[L·h])	0.14	0.92	0.15	1.04	0.14	0.66
Y_{XS} (g/g)	0.50	0.74	0.49	0.79	0.37	0.50
Xyl cons. (%)	10	90	15	99	17	93
Ara cons. (%)	1	44	2	100	3	49
Ac cons. (%)	90	100	96	100	91	100
Form cons. (%)	0	14	15	100	5	100

[a]μ, specific growth rate; Q_S, overall volumetric sugar consumption rate; Q_X, volumetric biomass production rate; Y_{XS}, biomass yield from total monosaccharides; Xyl cons., Ara cons., Ac cons., and Form cons. are the percentages of consumption of xylose, arabinose, acetic acid, and formic acid, respectively. All parameters, except μ, were calculated after 24 h. NS, nonsupplemented; S, supplemented.

for supplemented hydrolysates. Therefore, the use of a previous detoxification step before yeast growth seems to be unnecessary. Conversely, hydrolysate supplementation by itself greatly improved the specific growth rate and biomass yield. Supplementing the hydrolysates increased the specific growth rate and biomass yield by 1.3–1.5 and 4.7–6.9 fold, respectively.

The final biomass yield increased for the supplemented hydrolysates and was higher than expected, even when taking into account the consumption of compounds other than monosaccharides, such as furfural, HMF, acetic acid, and formic acid. High biomass yields (0.53–0.58 g/g) have already been ascribed to *D. hansenii* in xylose-limited chemostat cultures *(24)*. Since the concentration of total soluble phenolics remained unchanged at the end of fermentation, and the sugar oligomers present in the hydrolysate (about 1.3 g/L) were not consumed (data not shown), our results suggest that other compounds in the hydrolysate, which are not quantified by the current analytical methods used, contributed to the high biomass yields. To elucidate this statement, TOC in the experiments with the nondetoxified and supplemented media was measured at the beginning and end of fermentation. Based on these results, and on the carbon content of biomass as determined by elemental analysis (46.5%), the new, adjusted biomass yield obtained, 0.68 C-mol/C-mol, was close to the values reported in the literature for this yeast, 0.67 C-mol/C-mol *(24)*.

The overall volumetric sugar consumption rate showed the same trend as specific growth rate, since much lower values were obtained in nonsupplemented hydrolysates (Table 3). The highest final biomass concentration was obtained in the supplemented hydrolysates treated with activated charcoal, corresponding to a volumetric productivity of 1.04 g/(L·h). Only a slight decrease in biomass productivity (11%) was observed in the nondetoxified hydrolysates. Furthermore, xylose was almost completely exhausted when the hydrolysate was supplemented, whereas a slight tendency to increase xylose consumption rate was noticed when the hydrolysate was detoxified but not supplemented. In the nonsupplemented hydrolysates, arabinose consumption was almost negligible whereas in supplemented hydrolysates it increased from 44 to 100%.

For all conditions tested, *D. hansenii* was able to consume all furfural and HMF, regardless of the type of hydrolysate (data not shown). Acetic acid consumption ranged from 90 to 100%. Formic acid was only fully consumed for detoxified and supplemented hydrolysates. The complete consumption of acetic acid in hydrolysates has already been reported by other investigators *(44,45)*.

Besides biomass and CO_2, no significant amount of extracellular products was detected. Xylitol and arabitol were only accumulated in small amounts, with a maximum of 2.0 and 3.8 g/L, respectively. Other metabolic products usually associated with the pentose metabolism of *D. hansenii*, such as ethanol and glycerol *(46,47)*, were only found in trace amounts, less than 0.5 and 0.3 g/L, respectively.

Conclusion

Dilute-acid hydrolysis of BSG enables a high solubilization of hemicellulose at a low severity (*CS* 1.94) with almost total pentose recovery (close to 96%). This pentose-rich hydrolysate was found to exhibit a low level of fermentation-inhibiting compounds, namely aliphatic acids, furan derivatives, and total phenolics. In fact, the use of nondetoxified hydrolysate as culture medium for *D. hansenii* gave similar growth performances compared with detoxified hydrolysates. This strongly suggests that a detoxification procedure is not required for BSG dilute-acid hydrolysate. This is a significant advantage over other hemicellulosic hydrolysates and can lead to lower operative costs at the industrial level.

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References

1. Gírio, F. M., Carvalheiro, F., Esteves, M. P., Amaral-Collaço, M. T., Parajó, J. C., Domínguez, H., et al. (2003) Portuguese patent no. 102563.
2. Kabel, M. A., Schols, H. A., and Voragen, A. G. J. (2002), *Carbohydr. Polym.* **50,** 191–200.
3. Roberto, I. C., Felipe, M. G. A., Lacis, L. S., Silva, S. S., and Mancilha, I. M. (1991), *Bioresour. Technol.* **36,** 271–275.
4. Roberto, I. C., Felipe, M. G. A., Mancilha, I. M., Vitolo, M., Sato, S., and Silva, S. S. (1995), *Bioresour. Technol.* **51,** 255–257.
5. McMillan, J. D. (1994), in *Enzymatic Conversion of Biomass for Fuels Production*, Himmel, M. E., Baker, J. O., and Overend, R. P., eds., ACS Symposium Series 566, American Chemical Society, Washington, DC, pp. 411–437.
6. Saha, B. C. and Bothast, R. J. (1996), *Appl. Microbiol. Biotechnol.* **45,** 299–306.
7. Palmqvist, E. and Hahn-Hägerdal, B. (2000), *Bioresour. Technol.* **74,** 17–24.
8. van Walsum, G. P., Allen, S. G., Spencer, M. J., Laser, M. S., Antal, M. J., and Lynd, L. R. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 157–170.
9. Allen, S. G., Schulman, D., Lichwa, J., Antal, M. J., Jennings, E., and Elander, R. (2001), *Ind. Eng. Chem. Res.* **40,** 2352–2361.
10. Allen, S. G., Schulman, D., Lichwa, J., Antal, M. J., Laser, M., and Lynd, L. R. (2001), *Ind. Eng. Chem. Res.* **40,** 2934–2941.
11. Torget, R., Hatzis, C., Hayward, T. K., Hsu, T. A., and Philippidis, G. P. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 85–101.
12. Allen, S. G., Kam, L. C., Zemann, A. J., and Antal, M. J. (1996), *Ind. Eng. Chem. Res.* **35,** 2709–2715.
13. Mok, W. S. L. and Antal, M. J. (1992), *Ind. Eng. Chem. Res.* **31,** 1157–1161.
14. Saska, M. and Ozer, E. (1995), *Biotechnol. Bioeng.* **45,** 517–523.
15. Heitz, M., Capek-Ménard, E., Koeberle, P. G., Gagne, J., Chornet, E., Overend, R. P., Taylor, J. D., and Yu, E. (1991), *Bioresour. Technol.* **35,** 23–32.
16. Garrote, G., Domínguez, H., and Parajó, J. C. (2001), *Appl. Biochem. Biotechnol.* **95,** 195–207.
17. Duarte, L. C., Carvalheiro, F., Lopes, S., Marques, S., Parajó, J. C., and Gírio, F. M. (2004), *Appl. Biochem. Biotechnol.* **113–116,** 1139–1056.
18. Vázquez, M. J., Alonso, J. L., Domínguez, H., and Parajó, J. C. (2001), *World J. Microbiol. Biotechnol.* **17,** 817–822.

19. Domínguez, J. M., Cao, N. J., Gong, C. S., and Tsao, G. T. (1997), *Bioresour. Technol.* **61,** 85–90.
20. Chum, H. L., Johnson, D. K., Black, S. K., and Overend, R. P. (1990), *Appl. Biochem. Biotechnol.* **24/25,** 1–14.
21. Overend, R. P. and Chornet, E. (1987), *Phil. Trans. R. Soc. Lond.* **A321,** 523–536.
22. Nguyen, Q. A., Tucker, M. P., Keller, F. A., and Eddy, F. P. (2000), *Appl. Biochem. Biotechnol.* **84–86,** 561–576.
23. Tengborg, C., Stenberg, K., Galbe, M., Zacchi, G., Larsson, S., Palmqvist, E., and Hahn-Hägerdal, B. (1998), *Appl. Biochem. Biotechnol.* **70–72,** 3–15.
24. Nobre, A., Duarte, L. C., Roseiro, J. C., and Gírio, F. M. (2002), *Appl. Microbiol. Biotechnol.* **59,** 509–516.
25. Browning, B. L. (1967), in *Methods in Wood Chemistry*, Sarkanen, K. V. and Ludwig, C.H., eds., John Wiley & Sons, New York, NY.
26. AOAC. (1975), *AOAC Official Methods of Analysis*, AOAC International, Washington, DC.
27. Graham, H. D. (1992), *J. Agric. Food Chem.* **40,** 801–805.
28. Carrasco, J. E., Sáiz, M. C., Navarro, A., Soriano, P., Sáez, F., and Martínez, J. M. (1994), *Appl. Biochem. Biotechnol.* **45/46,** 23–34.
29. Torget, R., Werdene, P., Himmel, M., and Grohmann, K. (1990), *Appl. Biochem. Biotechnol.* **24/25,** 115–126.
30. Torget, R., Walter, P., Himmel, M., and Grohmann, K. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 75–86.
31. Grohmann, K., Torget, R., and Himmel, M. (1985), *Biotechnol. Bioeng. Symp.* **15,** 59–80.
32. Taherzadeh, M. J., Eklund, R., Gustafsson, L., Niklasson, C., and Liden, G. (1997), *Ind. Eng. Chem. Res.* **36,** 4659–4665.
33. Martín, C., Galbe, M., Nilvebrant, N. O., and Jönsson, L. J. (2002), *Appl. Biochem. Biotechnol.* **98–100,** 699–716.
34. Neureiter, M., Danner, H., Thomasser, C., Saidi, B., and Braun, R. (2002), *Appl. Biochem. Biotechnol.* **98–100,** 49–58.
35. Aoyama, M., Seki, K., and Saito, N. (1995), *Holzforschung* **49,** 193–196.
36. Montané, D., Salvadó, J., Farriol, X., Jollez, P., and Chornet, E. (1994), *Wood Sci. Technol.* **28,** 387–402.
37. Palmqvist, E., Grage, H., Meinander, N. Q., and Hahn-Hägerdal, B. (1999), *Biotechnol. Bioeng.* **63,** 46–55.
38. Domínguez, J. M., Gong, C. S., and Tsao, G. T. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 49–56.
39. Parajó, J. C., Domínguez, H., and Domínguez, J. M. (1996), *Bioresour. Technol.* **57,** 179–185.
40. Miyafuji, H., Danner, H., Neureiter, M., Thomasser, C., Bvochora, J., Szolar, O., and Braun, R. (2003), *Enzyme Microb. Technol.* **32,** 396–400.
41. Larsson, S., Reimann, A., Nilvebrant, N. O., and Jönsson, L. J. (1999), *Appl. Biochem. Biotechnol.* **77–79,** 91–103.
42. Nilvebrant, N. O., Reimann, A., Larsson, S., and Jönsson, L. J. (2001), *Appl. Biochem. Biotechnol.* **91–93,** 35–49.
43. Carvalheiro, F., Duarte, L. C., Lopes, S., Parajó, J. C., Pereira, H., and Gírio, F. M. (2003), submitted.
44. Roberto, I. C., Mancilha, I. M., Souza, C. A., Felipe, M. G. A., Sato, S., and Castro, H. F. (1994), *Biotechnol. Lett.* **16,** 1211–1216.
45. Pessoa, A., Mancilha, I. M., and Sato, S. (1996), *J. Biotechnol.* **51,** 83–88.
46. Roseiro, J. C., Peito, M. A., Gírio, F. M., and Amaral-Collaço, M. T. (1991), *Arch. Microbiol.* **156,** 484–490.
47. Tavares, J. M., Duarte, L. C., Amaral-Collaço, M. T., and Gírio, F. M. (2000), *Enzyme Microbiol. Technol.* **26,** 743–747.