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Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol

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Abstract: The hemicellulosic fraction of brewer's spent grain (BSG) was hydrolysed with diluted acid under different conditions of liquid/solid ratio (8–12 g g^{-1}), sulfuric acid concentration (100–140 mg g^{-1} dry matter) and reaction time (17–37 min) in order to produce a liquor with a large amount of xylose and good fermentability to produce xylitol. Results showed that all the evaluated reaction conditions were able to hydrolyse xylan and arabinan with efficiencies higher than 85.8 and 95.7% respectively, and even under the mildest reaction condition a considerable amount (92.7%) of the hemicellulosic fraction could be extracted. The hydrolysates presented different fermentabilities when used as fermentation media for xylitol production by *Candida guilliermondii* yeast, owing to the differences in their composition. Based on statistical analysis, the best condition for BSG acid hydrolysis was the use of a liquid/solid ratio of 8 g g^{-1}, 100 mg H_2SO_4 g^{-1} dry matter and a reaction time of 17 min. Under this condition a high extraction efficiency of hemicellulosic sugars (92.7%) and good fermentation results ($Y_{P/S} = 0.70$ g g^{-1} and $Q_P = 0.45$ g dm^{-3} h^{-1}) were attained.

Keywords: brewer's spent grain (BSG); hemicellulose; acid hydrolysis; xylose; *Candida guilliermondii*; xylitol

INTRODUCTION

Brewer's spent grain (BSG), the barley malt residue obtained after wort manufacture, is the brewery by-product produced in the largest quantity, corresponding to around 85% of the total generated.[1] Although it is produced in large quantities during the whole year, BSG has received little attention as a marketable commodity and is mainly used as animal feed.[2] Nevertheless, BSG is a material that presents in its composition sugars polymerised into cellulose and hemicellulose. Thus, if submitted to a fractionation process under adequate conditions, BSG may produce a liquor rich in xylose, a sugar that can be used as a carbon source for xylitol or ethanol production.[3,4]

Hemicellulose-rich materials can be hydrolysed by various processes, among which diluted acid hydrolysis stands out as one of the most efficient to selectively release hemicellulosic sugars (xylose and arabinose), leaving a residue containing the cellulose and lignin fractions almost unaltered.[5] The major problem of acid hydrolysis is that the decomposition of monomeric sugars produced during the reaction takes place simultaneously with the hydrolysis of polysaccharides (xylose and arabinose are decomposed into furfural, and glucose into hydroxymethylfurfural (HMF)).[6] To prevent sugar decomposition, it is very important to conduct the process under adequate reaction conditions. Other compounds that may often be formed during an acid hydrolysis process include acetic acid and lignin degradation products (LDPs). Furfural, HMF, acetic acid and LDPs are potent inhibitors of yeast growth. For this reason, before carrying out a successful fermentation, it is necessary to establish the best operational conditions for hydrolysis of the raw material in order to produce a liquor with large amounts of fermentable sugars and few toxic compounds.

Xylitol is a sweetener that stands out among others because it can be used to combat dental caries, to treat illnesses such as diabetes, disorders in lipid metabolism and parenteral and renal lesions and to prevent lung infection, otitis and osteoporosis.[7] Another advantage of xylitol when compared with other sweeteners is the fact that it can be produced by a biotechnological process, which has economic potential because it is very specific and requires less energy than a chemical process.[8] Besides, xylitol production by fermentation also has great environmental impact owing to the use of low-cost lignocellulosic wastes that can be found in large quantities in nature.

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This study deals with the acid hydrolysis of BSG hemicellulose to produce a liquor with a large amount of xylose and good fermentability to produce xylitol. Reactions were performed in a batch reactor under different conditions of liquid/solid ratio, sulfuric acid concentration and reaction time, which were selected based on values used for the acid hydrolysis of other lignocellulosic materials.[9–11] Xylitol production was evaluated for each hydrolysate produced. The best hydrolysis condition to extract the hemicellulose-derived sugars, generating a liquor with good fermentability to produce xylitol, was established by statistical analysis.

MATERIALS AND METHODS

Brewer's spent grain

The BSG used in this work was obtained from a process employing 100% malt (without addition of other cereal adjuncts) and contained (% dry weight) cellulose (16.8), hemicellulose (28.4), lignin (27.8), acetyl groups (1.35), ash (4.6), proteins (15.25) and extractives (5.8). The material, supplied by the microbrewery of the Faculty of Chemical Engineering of Lorena, was first washed with water until neutral pH was achieved, then dried at $50 \pm 5\,^{\circ}C$ to 10% moisture content and stored until required for processing or analysis.

Hydrolysis reactions

Different conditions of liquid/solid ratio (8, 10 and $12\,g\,g^{-1}$), sulfuric acid concentration (100, 120 and $140\,mg\,g^{-1}$ dry matter) and reaction time (17, 27 and 37 min) were employed in the hydrolysis process. Reactions were carried out at $120\,^{\circ}C$ in a $1.5\,dm^3$ stainless steel batch reactor (made in the Faculty of Chemical Engineering of Lorena), which was filled with 90 g of BSG (containing 90% dry matter) and the required amount of acid solution. The average time required to reach the reaction temperature was 1.5 h and a similar time was necessary to cool the reactor. After hydrolysis the resulting solid material was separated by filtration with Gennapaper (50 cm × 50 cm, 80 g; Hipperquímica, Santo André, SP, Brazil) and the filtrate (hemicellulosic hydrolysate) was analysed for solubilised sugars (glucose, xylose and arabinose), degradation products (furfural, HMF and LDPs) and acetic acid. The hydrolysates obtained had an average pH of 1.25. Prior to fermentation, their pH was adjusted to 6.5 by addition of NaOH pellets (PA-ACS, Labsynth, Diadema, SP, Brazil), the precipitate being removed by centrifugation ($1100 \times g$, 20 min).

Micro-organism and inoculum

Candida guilliermondii FTI 20 037, maintained at $4\,^{\circ}C$ on malt extract agar slant, was grown in $250\,cm^3$ Erlenmeyer flasks containing $100\,cm^3$ of a medium composed of ($g\,dm^{-3}$) xylose (20), $(NH_4)_2SO_4$ (3.0), $CaCl_2{\cdot}2H_2O$ (0.1) and 20% (v/v) rice bran extract. Solutions prepared with each component separately were sterilised at $121\,^{\circ}C$ for 20 min, except for the xylose solution, which was autoclaved at $112\,^{\circ}C$ for 15 min. To use rice bran as a nutrient, a 10% (w/v) suspension of rice bran was sterilised at $121\,^{\circ}C$ for 20 min, cooled to room temperature and centrifuged aseptically at $1100 \times g$ for 20 min. The inocula were incubated at $30\,^{\circ}C$ in a rotary shaker (TE-420, Tecnal, Piracicaba, SP, Brazil) at 200 rpm for 24 h and the cells were subsequently separated by centrifugation ($1100 \times g$, 20 min) and directly resuspended in the fermentation medium.

Fermentation conditions

Fermentations were performed in $250\,cm^3$ Erlenmeyer flasks containing $100\,cm^3$ of medium (hydrolysate at pH 6.5) and inoculated with an initial cell concentration of $1\,g\,dm^{-3}$. Flasks were agitated at 200 rpm in an orbital shaker at $30\,^{\circ}C$. The fermentation runs lasted 24 h and were monitored through periodic sampling to determine cell growth, glucose and xylose uptakes and xylitol production.

Analytical procedures

Glucose, xylose, arabinose, xylitol and acetic acid concentrations were determined by high-performance liquid chromatography (HPLC) in a chromatograph with a refractive index (RI) detector and an HPX-87H column (300 mm × 7.8 mm; Bio-Rad, Hercules, CA, USA) at $45\,^{\circ}C$, using $0.005\,mol\,dm^{-3}$ sulfuric acid as eluent, a $0.6\,cm^3\,min^{-1}$ flow rate and a $20\,mm^3$ sample volume. HMF and furfural were also determined by HPLC, but with a UV detector (at 276 nm) and a Resolve C_{18} 5 μm column (3.9 mm × 300 mm; Waters, Milford, MA, USA) at room temperature, using acetonitrile/water (1:8 with $10\,g\,dm^{-3}$ acetic acid) as eluent, a $0.8\,cm^3\,min^{-1}$ flow rate and a $20\,mm^3$ sample volume.

LDPs were estimated by UV spectroscopy at 280 nm according to the methodology described by Rocha.[12] Cell concentration was determined at 600 nm in a DU 640B spectrophotometer (Beckman, Fullerton, CA, USA) by means of a calibration curve (dry weight *vs* optical density (OD)) obtained from cells grown in hydrolysate medium agitated in a rotary shaker at 200 rpm for 24 h at $30\,^{\circ}C$. Samples were diluted to a reading band from 0.05 to 0.5 OD units.

Recovered sugar yield Y_S (g of substance that can be obtained from 100 g of BSG dry matter) and hydrolysis efficiency η (%) were calculated using Eqns (1) and (2) respectively, where C is the concentration of the component in the liquid phase ($g\,dm^{-3}$), M is the amount of BSG (dry matter) employed in the experiment (g), V is the volume of liquid solution employed (dm^3) and Y_{max} is the maximum yield of recovered sugars that can be attained (g per 100 g dry matter):

$$Y_S = (C \times V/M) \times 100 \quad (1)$$

$$\eta = (Y_S/Y_{max}) \times 100 \quad (2)$$

Experimental design

Experiments were carried out according to a 2^3 statistical experimental design to evaluate the effects of the variables liquid/solid ratio (8, 10 and 12 g g^{-1}), sulfuric acid concentration (100, 120 and 140 mg g^{-1} dry matter) and reaction time (17, 27 and 37 min) on BSG hemicellulose hydrolysis. Temperature was fixed at 120 °C. The experimental error was estimated at the centre point (10 g g^{-1}, 120 mg g^{-1}, 27 min), which was replicated fourfold. The hemicellulose hydrolysis efficiency and the fermentative parameters xylitol yield factor, cell yield factor and xylitol volumetric productivity were taken as the dependent variables or responses of the experimental design. All the experiments were performed in duplicate (means are given) in randomised run order. The design allowed the estimation of the significance of the parameters and their interactions using Student's *t* test. Furthermore, the data could be used to calculate contour line models. Statistica 5.0 (StatSoft, Tulsa, OK, USA) was the commercial software employed for regression and graphical analyses of the results obtained.

RESULTS AND DISCUSSION

BSG hydrolysate composition

BSG is a lignocellulosic material with around 70% of its hemicellulose represented by a xylan backbone, while the other 30% is basically an arabinan structure (Mussatto SI and Roberto IC, unpublished). During diluted acid hydrolysis, pentose sugars (produced from xylan and arabinan) are the main compounds formed, and, for this reason, xylose and arabinose were the monosaccharides present in highest quantities in BSG hydrolysates (xylose being the main monosaccharide released, followed by arabinose) (Table 1). The hydrolysis efficiencies of xylan into xylose and of arabinan into arabinose were calculated (Table 2) and the results obtained were higher than 85.8% for all the experiments. Nevertheless, the efficiency of arabinan hydrolysis was always higher than that of xylan hydrolysis. Similar behaviour was also observed during BSG autohydrolysis to produce xylo-oligosaccharides.[13,14] According to Carvalheiro *et al*,[14] arabinose exhibits a higher thermal sensitivity than xylose, and for this reason it is released first from the hemicellulose structure.

Table 1. Experimental conditions for brewer's spent grain acid hydrolysis, and composition of hydrolysates obtained in each experiment

	Variables levels			Concentration (g dm^{-3})						
Experiment	Liquid/solid ratio (g g^{-1})	Acid conc. (mg g^{-1})	Reaction time (min)	Glucose	Xylose	Arabinose	Acetic acid	Furfural	HMF	LDPs
1	8	100	17	1.19	21.88	10.71	1.20	0.63	0.09	4.22
2	12	100	17	0.57	14.28	7.17	0.77	0.31	0.05	2.66
3	8	140	17	1.72	21.44	10.10	1.19	0.99	0.08	2.70
4	12	140	17	1.08	15.11	7.21	0.76	0.48	0.04	2.73
5	8	100	37	1.25	20.96	10.12	1.34	0.87	0.07	3.22
6	12	100	37	0.57	14.36	6.81	1.12	0.28	0.03	2.26
7	8	140	37	1.89	22.62	10.51	1.39	1.02	0.07	2.56
8	12	140	37	0.96	15.48	7.12	0.81	0.46	0.03	2.20
9	10	120	27	1.33	18.16	8.65	1.12	0.58	0.04	3.40
10	10	120	27	1.30	17.69	8.40	1.03	0.65	0.05	3.50
11	10	120	27	1.36	17.60	8.23	1.08	0.5	0.04	3.16
12	10	120	27	1.12	16.94	8.03	0.99	0.60	0.05	3.13

HMF = hydroxymethylfurfural; LDPs = lignin degradation products.

Table 2. Efficiency of brewer's spent grain acid hydrolysis under different operational conditions

	Variables levels			Hydrolysis efficiency (%)		
Experiment	Liquid/solid ratio (g g^{-1})	Acid conc. (mg g^{-1})	Reaction time (min)	Xylan	Arabinan	Hemicellulose
1	8	100	17	88.7	100	92.7
2	12	100	17	87.1	99.0	91.8
3	8	140	17	86.7	96.1	89.5
4	12	140	17	91.7	100	95.1
5	8	100	37	87.2	96.6	90.0
6	12	100	37	87.5	97.6	90.5
7	8	140	37	91.5	100	94.1
8	12	140	37	94.2	100	96.5
9	10	120	27	92.0	100	95.3
10	10	120	27	89.6	100	92.7
11	10	120	27	89.1	98.1	91.8
12	10	120	27	85.8	95.7	88.7

Hemicellulose = xylan + arabinan.

The high hydrolysis efficiencies obtained for xylan and arabinan resulted in high values for hemicellulose hydrolysis efficiency (varying from 88.7 to 96.5%; Table 2). Statistical analysis of these results showed that there were no significant differences among treatments ($P > 0.05$).

Glucose was another monosaccharide also produced in BSG hydrolysates, but in smaller quantities than xylose and arabinose (Table 1). The glucose/xylose/arabinose ratio varied in each hydrolysate produced and the highest ratios were observed in assays 2 and 6, corresponding to 1:25.05:12.58 and 1:25.19:11.95 respectively. The glucose released in the hydrolysates can originate from both hemicellulose and cellulose. However, the glucose from cellulose is not usually hydrolysed under the range of operational conditions commonly used for diluted acid hydrolysis.[15] Therefore, as the obtained glucose and HMF (product of glucose degradation) concentrations were very low (<1.9 and <0.09 g dm^{-3} respectively), it is probable that almost all the released glucose originated from hemicellulose, while the cellulose remained practically unhydrolysed.

Besides monomer sugars, the resulting liquors from BSG acid hydrolysis also contained other by-products, including acetic acid (compound released from acetyl groups of hemicellulose), sugar decomposition products (furfural and HMF) and LDPs.

Acetic acid is a compound that, when present in the hydrolysates in concentrations higher than 3 g dm^{-3}, acts as a potent micro-organism inhibitor if the hydrolysate is employed as a fermentation medium.[16] According to Lawford and Rousseau,[17] this acid can pass through the cellular membranes and decrease the intracellular pH, thus affecting the metabolism. In the present work the acetic acid concentration in the hydrolysates varied from 0.76 to 1.39 g dm^{-3} (Table 1), the maximum concentration being reached under the most drastic condition studied (8 g g^{-1} liquid/solid ratio, 140 mg H_2SO_4 g^{-1} dry matter, 37 min reaction time, i.e. assay 7). Probably these concentration values are not able to affect the micro-organism's metabolism during BSG hydrolysate fermentation.

Furfural, generated as a degradation product from pentoses, was also produced only in small quantities, reaching values in the range 0.28–1.02 g dm^{-3}. As xylose and arabinose were almost completely recovered in the hydrolysates and low furfural concentrations were obtained, this means that a low degradation of pentose sugars occurred during the hydrolysis processes. The HMF formed from decomposition of hexoses is another compound that was found in BSG hydrolysates, but it was only present in trace amounts, 0.09 g dm^{-3} being the maximum concentration obtained in the experiments. These results suggest that, under the evaluated hydrolysis conditions, pentose sugars were more susceptible to degradation than hexose sugars.

According to Pessoa *et al*,[9] sugar degradation during acid hydrolysis can occur if a high acid concentration is employed or when acid homogenisation in the reactor is inadequate, creating regions with high acidity. In this sense, several authors have reported the influence of acid concentration on the formation of furfural and HMF. Téllez-Luis *et al*[15] observed an increase in furfural concentration during the acid hydrolysis of sorghum straw when the acid concentration and reaction time were increased. Lavarack *et al*[18] also observed in the acid hydrolysis of sugarcane bagasse that the higher the acid concentration (from 0.25 to 8 wt% of liquid) the faster was the degradation of xylose into furfural. Neureiter *et al*[19] verified during the acid hydrolysis of silage and grass that the degradation of sugars was strongly affected by temperature (160–180 °C) and acid concentration (0.4–0.6%) but unaffected by dry matter concentration. The furfural and HMF concentrations obtained in BSG hydrolysates were not influenced by any variable employed in the hydrolysis process, owing to the low acid concentration (<1.5%) and temperature (<120 °C) employed in this work.

Other compounds, besides acetic acid, furfural and HMF, also considered as toxic to micro-organisms and normally found in hemicellulosic hydrolysates are LDPs. BSG hydrolysates presented LDP concentrations varying from 2.2 to 4.22 g dm^{-3} (Table 1). When compared with the other by-products present in BSG hydrolysates, LDPs were the compounds produced in highest concentrations. According to Parajó *et al*,[20] LDPs are more toxic to micro-organisms than acetic acid, furfural or HMF, even when present in low concentrations.

A significant increase (92%) in the LDP concentration in the hydrolysate was obtained as a function of the employed hydrolysis conditions (Table 1). Table 3 shows that all independent variables (liquid/solid ratio, acid concentration and reaction time) were statistically significant at 95% confidence level, all of them presenting a negative effect. This means that LDP formation is increased when the liquid/solid ratio, acid concentration and reaction time are decreased. Besides, the curvature was also significant for this

Table 3. Estimated effects, standard errors and Student's *t* test for lignin solubilisation in brewer's spent grain acid hydrolysis

Independent variable or interaction	Estimated effect	Standard error	*t* value
Average	2.819	±0.083	33.92*
Curvature	0.957	±0.288	3.33*
A: liquid/solid ratio	−0.712	±0.166	4.29*
B: acid concentration	−0.542	±0.166	3.26*
C: reaction time	−0.517	±0.166	3.11*
Interaction AB	0.547	±0.166	3.29*
Interaction AC	0.052	±0.166	0.32
Interaction BC	0.182	±0.166	1.10

* Significant at 5% probability level.

response, suggesting that LDP formation during acid hydrolysis varies according to a quadratic equation that involves the independent variables A, B and C and the interaction AB.

The effect of acid concentration on lignin solubilisation was reported by Fengel and Wegener.[21] According to those authors, a small fraction of the lignin is solubilised during the treatment of lignocellulosic materials with diluted mineral acids. Nevertheless, when the acid concentration is increased, the lignin becomes more insoluble, because condensation reactions occur in major proportion, modifying its structure and properties. As a result, a rigid structure is formed which is difficult to solubilise and decreases the possibility of lignin removal from raw material in a later step.

The effect of reaction time on lignin solubilisation is also related to lignin condensation. According to Allen *et al*,[22] two consecutive reactions occur when lignin is hydrolysed with dilute acid: (1) depolymerisation of the native lignin by acid and (2) condensation/repolymerisation of the partially hydrolysed lignin. Initially, the hydrolysis reaction predominates, resulting in higher lignin solubility. However, at longer reaction times the repolymerisation reactions predominate, resulting in larger amounts of insoluble residual lignin. Recently, Carvalheiro *et al*[14] evaluated BSG autohydrolysis and observed that the Klason lignin recovery in the solid residue was higher when the reaction time was increased.

BSG hydrolysate fermentability

The hydrolysis efficiency and the solubilised lignin are not the only responses that should be considered when studying hydrolysates. For practical applications, their fermentability is also very important and must be evaluated. In the present work, BSG hydrolysates were employed as fermentation media for xylitol production by *C. guilliermondii* yeast. It is expected that the hydrolysates will show good fermentability owing to the presence of low levels of toxic compounds, and also because BSG has a high protein content (15.25%) that can be solubilised during hydrolysis, providing a good nitrogen source for micro-organisms. Confirming this idea, Table 4 shows that *C. guilliermondii* yeast was able to grow and produce xylitol in all BSG hydrolysates, but the fermentation results varied for each medium as a function of the hydrolysis conditions.

Glucose was totally consumed by the yeast in all hydrolysates. Nevertheless, xylose consumption ranged from 67 to 96.9% for each hydrolysate (Table 4), and arabinose was not assimilated by *C. guilliermondii* during the considered fermentation time (24 h).

Cell growth was almost the same in all hydrolysates, but xylitol production varied significantly for each one (from 5.32 to 10.76 g dm^{-3}). As xylitol production tends to increase with increasing initial xylose concentration,[23,24] these results must be compared in terms of the fermentative parameters $Y_{P/S}$, Q_P and $Y_{X/S}$. It can be observed in Table 4 that the $Y_{P/S}$ and Q_P values varied by up to 100% in BSG hydrolysate fermentation, depending on the conditions used to produce the hydrolysate. Nevertheless, when compared with the fermentation of hydrolysates produced from other raw materials, all BSG hydrolysates presented good fermentability to produce xylitol. Parajó *et al*[24] obtained a $Y_{P/S}$ of 0.38 g g^{-1} and a Q_P of 0.055 g dm^{-3} h^{-1} during the fermentation of untreated wood hydrolysates containing 17 g xylose dm^{-3}, supplemented with nutrients. Higher results ($Y_{P/S}$ = 0.66 g g^{-1} and Q_P = 0.41 g dm^{-3} h^{-1}) were attained by Cruz *et al*[25] during the fermentation of a hydrolysate of untreated barley bran with and without supplemented nutrients. In the present work, $Y_{P/S}$ and Q_P values

Table 4. Effect of hydrolysis conditions of brewer's spent grain on xylose yield, cell growth, xylitol production and fermentative process parameters ($Y_{P/S}$, Q_P and $Y_{X/S}$)

	Variables levels			Fermentation results						
Experiment	Liquid/solid ratio (g g^{-1})	Acid conc. (mg g^{-1})	Reaction time (min)	Initial xylose (g dm^{-3})	Xylose consumed (%)	Cells (g dm^{-3})	Xylitol (g dm^{-3})	$Y_{P/S}$ (g g^{-1})	Q_P (g dm^{-3} h^{-1})	$Y_{X/S}$ (g g^{-1})
1	8	100	17	21.88	78.7	3.86	10.76	0.70	0.45	0.23
2	12	100	17	14.28	89.3	3.97	6.30	0.46	0.26	0.28
3	8	140	17	21.44	75.2	3.65	9.28	0.53	0.39	0.19
4	12	140	17	15.11	86.1	3.61	6.98	0.50	0.29	0.24
5	8	100	37	20.96	82.5	4.15	9.07	0.50	0.38	0.21
6	12	100	37	14.36	94.5	4.51	5.32	0.38	0.22	0.31
7	8	140	37	22.62	67.0	3.18	9.08	0.55	0.38	0.17
8	12	140	37	15.48	87.2	3.98	6.43	0.43	0.27	0.24
9	10	120	27	18.16	79.1	3.27	7.87	0.55	0.33	0.21
10	10	120	27	17.69	88.1	4.46	8.05	0.51	0.33	0.26
11	10	120	27	17.60	88.1	4.28	7.76	0.48	0.32	0.24
12	10	120	27	16.94	96.9	4.57	7.45	0.41	0.31	0.24

$Y_{P/S}$ (g g^{-1}) = xylitol yield factor (ratio between g of xylitol produced and g of xylose consumed at the end of each fermentation); $Y_{X/S}$ (g g^{-1}) = cell yield factor (ratio between g of cells formed and g of substrate (xylose + glucose) consumed at the end of each fermentation); Q_P (g dm^{-3} h^{-1}) = xylitol volumetric productivity (ratio between concentration of xylitol produced at the end of each run and fermentation time).

Table 5. Estimated effects, standard errors and Student's *t* test for xylitol yield factor ($Y_{P/S}$), xylitol volumetric productivity (Q_P) and cell yield factor ($Y_{X/S}$) obtained during brewer's spent grain hydrolysate fermentation

	$Y_{P/S}$ ($g\,g^{-1}$)			Q_P ($g\,dm^{-3}\,h^{-1}$)			$Y_{X/S}$ ($g\,g^{-1}$)		
Independent variable or interaction	Estimated effect	Standard error	*t* value	Estimated effect	Standard error	*t* value	Estimated effect	Standard error	*t* value
Average	0.506	±0.022	22.65*	0.330	±0.004	85.65*	0.234	±0.006	35.50*
Curvature	−0.037	±0.077	0.48	−0.015	±0.013	1.12	0.007	±0.023	0.33
A: liquid/solid ratio	−0.127	±0.045	2.85*	−0.140	±0.008	18.17*	0.067	±0.013	5.12*
B: acid concentration	−0.007	±0.045	0.17	0.005	±0.008	0.65	−0.047	±0.013	3.61*
C: reaction time	−0.082	±0.045	1.84	−0.035	±0.008	4.54*	−0.002	±0.013	0.19
Interaction AB	0.052	±0.045	1.17	0.035	±0.008	4.54*	−0.007	±0.013	0.57
Interaction AC	0.007	±0.045	0.17	0.005	±0.008	0.65	0.017	±0.013	1.33
Interaction BC	0.057	±0.045	1.29	0.020	±0.008	2.59*	−0.007	±0.013	0.57

* Significant at 10% probability level.

up to $0.70\,g\,g^{-1}$ and $0.45\,g\,dm^{-3}\,h^{-1}$ respectively were obtained from untreated BSG hydrolysate containing $21.9\,g$ xylose dm^{-3}, without nutrient supplementation.

Owing to the large differences observed in the fermentation results of BSG hydrolysates, a statistical analysis was carried out to evaluate the effects of the operational variables employed in the hydrolysis process on the obtained $Y_{P/S}$ and Q_P values. According to this analysis, the liquid/solid ratio was the variable with the highest influence on both $Y_{P/S}$ and Q_P, presenting a negative effect (Table 5). The contact time also showed statistical significance at 90% confidence level for Q_P, with a negative effect. This means that the fermentation results were favoured when *C. guilliermondii* was cultivated in hydrolysates produced at the lowest liquid/solid ratio ($8\,g\,g^{-1}$) and reaction time (17 min). The acid concentration presented no main significant effect for these parameters, but its interactions with the liquid/solid ratio (AB = +4.54) and with the reaction time (BC = +2.59) were significant at 90% confidence level for Q_P. The positive sign of these interactions suggests that Q_P was favoured in hydrolysates produced with the lowest values of acid concentration, liquid/solid ratio and reaction time. The curvature was not significant for both $Y_{P/S}$ and Q_P, suggesting that the values of these parameters increased linearly when the values of the hydrolysis operational variables decreased. These behaviours can be observed in Fig. 1, which shows the $Y_{P/S}$ and Q_P values as a function of the liquid/solid ratio and reaction time (the two main significant variables).

It is interesting to note that the hydrolysate produced under conditions that favoured $Y_{P/S}$ and Q_P (experiment 1) also had the highest LDP concentration. In a previous work where xylitol was produced from rice straw hydrolysate, the highest values of these fermentative parameters were also not obtained from the hydrolysate with the lowest LDP concentration.[3] This suggests that there is no relation between a high LDP concentration and a high inhibition of the fermentative process. Probably the lignin contains compounds that do not interfere in the microbial metabolism or that still enhance the fermentative process, presenting some stimulant effect. Such compounds could have been solubilised in greater quantities in the BSG hydrolysate of experiment 1 than in the hydrolysates obtained under other conditions. As lignin is composed of a large variety of phenolic and aromatic compounds such as catechol, hydroquinone, coniferyl, 4-methylcatechol, guaiacol, vanillyl alcohol, syringic, vanillic, ferulic and palmitic acids, etc.,[26] a study on the effect of each of these compounds on the xylose-to-xylitol bioconversion could be useful to explain the results observed here.

Cell yield factor $Y_{X/S}$ is another fermentative parameter that was evaluated during BSG hydrolysate fermentation (Table 4) and showed significant differences for each medium. The statistical analysis for this response showed that $Y_{X/S}$ was mainly affected by the liquid/solid ratio (main effect) and acid concentration, which presented positive and negative effects respectively (Table 5). This means that $Y_{X/S}$ was favoured in hydrolysates produced at the highest liquid/solid ratio and lowest acid concentration. In general, the statistical analysis of the fermentative parameters disclosed that the BSG hydrolysates produced from a low liquid/solid ratio favoured product formation ($Y_{P/S}$ was increased), while those obtained from a high liquid/solid ratio favoured cell growth ($Y_{X/S}$ was increased), i.e. an increase in liquid/solid ratio produced hydrolysates that showed a deviation in the microbial metabolism from product formation to cell growth.

Based on the statistical analysis results, the optimal condition for BSG acid hydrolysis with sulfuric acid was established with the use of a liquid/solid ratio of $8\,g\,g^{-1}$, 100 mg $H_2SO_4\,g^{-1}$ dry matter and a reaction time of 17 min. It must also be noted that, besides the good results obtained, this condition was the most economically viable among all those evaluated, because it needed less acid and a shorter reaction time, thus requiring a lower energy input.

CONCLUSIONS

BSG is a biomass with great potential for xylitol production by fermentation, because the acid hydrolysate

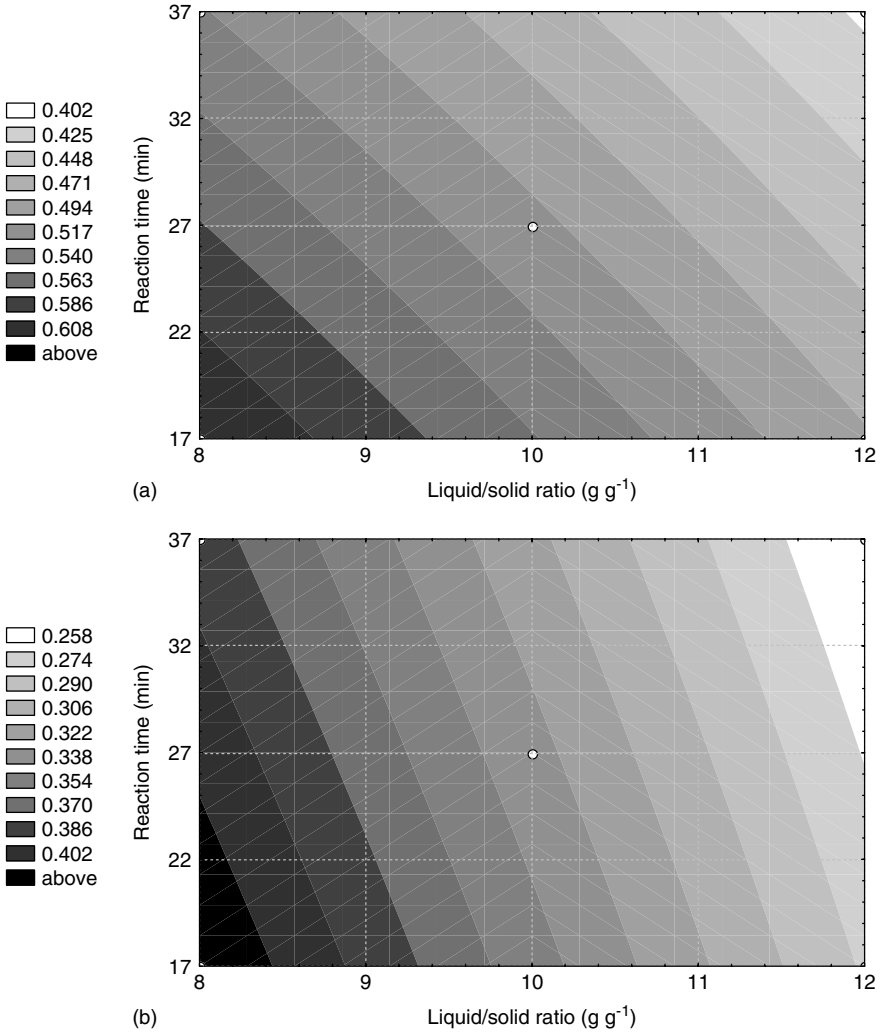

Figure 1. Contour lines representing (a) xylitol yield factor ($Y_{P/S}$) and (b) xylitol volumetric productivity (Q_P) during brewer's spent grain hydrolysate fermentation.

obtained from its hemicellulosic fraction is rich in fermentable sugars (especially xylose) and presents nutritional characteristics suitable for xylitol production, since good results for $Y_{P/S}$ and Q_P ($0.70\,g\,g^{-1}$ and $0.45\,g\,dm^{-3}\,h^{-1}$ respectively) were obtained without nutrient supplementation.

Employing BSG hydrolysate to produce xylitol can be an alternative use for this agro-industrial by-product, with the advantage of producing a value-added product. Besides, the solid residue obtained, which is rich in cellulose and lignin, can also be useful for other applications, e.g. to produce cellulosic paste or to generate glucose solutions by enzymatic hydrolysis.

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