Acid hydrolysis of barley straw: study of lignocellulosic pretreatment conditions and batch reactor

Hidrólise ácida da palha de cevada: estudo das condições de pré-tratamento lignocelulósico e do reator de lote

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RESUMO
O cultivo da cevada cresceu no Brasil nos últimos anos, atingindo uma produção anual de 1,5 milhão de toneladas. Este estudo teve como objetivo projetar e construir um reator para hidrólise ácida de materiais lignocelulósicos e investigar a liberação de açúcares fermentáveis da palha da cevada. Para orientar o desenvolvimento de um reator viável, foi realizado um planejamento experimental para investigar as condições de pré-tratamento com ácido sulfúrico. As variáveis independentes foram a relação palha de cevada / solução (5,0: 10, 1,0: 10, 1,5: 10 e 2,0: 10% p / v) e concentração de ácido sulfúrico (0,5, 0,8, 1,0, 1,5, 2,0, 2,5 e 3,0 % v / v). O pré-tratamento foi realizado por 40 min em autoclave vertical a 121 ° C e 1 kgf / cm^2. As variáveis de resposta foram sólidos solúveis (° Bx) e o rendimento de glicose (razão em peso de glicose liberada para biomassa inicial de palha de cevada). Os resultados mostraram que as condições ótimas para a liberação de glicose e sólidos solúveis foram uma relação palha de cevada / solução de 7:10 (p / v) e 2,6% (% v / v) de ácido sulfúrico.

Palavras-chave: Açúcar fermentável, Palha de cevada, Pré-tratamento ácido, Biorreator.

ABSTRACT
Barley cultivation has grown in Brazil in recent years, reaching an annual production of 1.5 million tons. This study aimed to design and build a reactor for acid hydrolysis of lignocellulosic materials and investigate the release of fermentable sugars from barley straw. To guide the development of a feasible reactor, we used experimental design techniques to investigate sulfuric acid pretreatment conditions. The independent variables were barley straw/solution ratio (5.0:10, 1.0:10, 1.5:10, and 2.0:10 %w/v) and sulfuric acid concentration (0.5, 0.8, 1.0, 1.5, 2.0, 2.5, and 3.0 %v/v). Pretreatment was conducted for 40 min in a vertical autoclave at 121 °C and 1 kgf/cm^2. The response variables were soluble solids (°Bx) and glucose yield (weight ratio of released glucose to initial barley straw biomass). The results showed that the optimal conditions for the release of glucose and soluble solids were a barley straw/solution ratio of 7:10 (w/v) and 2.6% (%v/v) sulfuric acid.

Keywords: Fermentable sugar, Barley straw, Acid pretreatment, Bioreactor.

1 INTRODUCTION

There is a worldwide tendency towards the scientific and technological development of "environmentally friendly" fuels, among which stand out those coming from the reuse of agro-industrial waste (MOURA *et al*., 2020). There is considerable and growing interest from several countries that explore monocultures, such as Brazil, The

United States and Canada, in the development of ethanol production technologies from the use of lignocellulosic materials and the reuse of agro-industrial waste, since there is no need to increase planting area. (ROCHA *et al., 2019)*

Brazil, has extensive areas cultivated with monoculture crops, such as sugarcane, wheat, rice, and barley. Barley monoculture systems have expanded greatly in recent years, generating large amounts of lignocellulosic materials rich in fiber, cellulose, hemicellulose, and lignin. Brewing companies discard barley straw, which is often used as soil mulch or animal feed. (MAPA, 2018)

Nature provides several plant species that present sugars for fermentation processes (ROSA *et al.*, 2021). Such species are classified according to their biological and taxonomic structure, biochemical composition and complexity, being divided into: Sugary raw materials are water-soluble and easily extracted by pressure and diffusion (sucrose, glucose, fructose, galactose and lactose); Starchy raw materials, provide polysaccharides that need physical pre-treatment, grinding, and enzymatic hydrolysis to obtain fermentable sugars; Lignocellulosic raw materials that are agro-industrial and forest waste, require physical, chemical and enzymatic pre-treatments to hydrolysis to convert lignin into monosaccharides. (ROCHA *et al., 2019)*

Barley (Hordeum vulgare) is a small grassy cereal, originally from the Middle East, which ranks fifth in the world grain harvest. Being characterized by developing better in cultures of cold and dry climate, but demanding in relation to soil fertility. The product resulting from the artificial germination of grains, malt, is used mainly in the manufacture of beers. Barley grains consist of about 65% to 68% starch, 10% to 17% protein, 4% to 9% β-glucan, 2% to 3% lipids, and 1.5% to 2.5% minerals. Barley straw is a potential source of fermentable sugars and secondary products, including resins, alcohols, and essential oils. (QUINDE *et al.*, 2004)

For the extraction of these reducing sugars present in the vegetable structure, it is necessary to use a pre-treatment process, which has as main objective to break the interactions and chemical bonds between the lignin, hemicellulose and cellulose molecules, removing the fiber unit (SILVA *et al.*, 2021. By breaking the lignin, which protects and surrounds the substrate, there is a greater reaction surface area for chemical attack, thus separating the three main components of the lignocellulosic materials. (QUINDE *et al.*, 2004).

Acid hydrolysis can occur with concentrated or diluted acids. When hydrolysis with concentrated acid is carried out, the process takes place in a reduced time; however, there is a high level of degradation of the fermentable sugars formed, exothermically, and corrosion of the metal parts of the equipment. The method using diluted acids, on the other hand, can be considered more viable, since it makes the same break efficiently and promotes less corrosion of the equipment; however, the use of diluted acid demands more reaction time, but it has a number of advantages, such as the degradation content of the sugars formed, generating fermentation inhibitor products to be significantly lower, with a partial dilution of these sugars in solution, and the high penetrability in the fiber. (NAKASU, 2015)

The present work is justified with a transdisciplinary proposal that aims to build the bridge between the academy and society through the applicability of research in the rural and industrial sector, promoting awareness of the use of agro-industrial waste. Thus contributing to ease the environmental impact and add value to the discarded materials. (QUINDE *et al.*, 2004)

This study comprises two steps aimed at obtaining reducing sugars by hydrolysis of barley straw. The first step was the development of a reactor for depolymerization of plant fibers. The second step was the study and optimization of barley straw acid hydrolysis.

2 METHODS

Raw material

About 5.3 kg of barley straw was kindly donated by a microbrewery in São João da Boa Vista, São Paulo State, Brazil. The material was thoroughly mixed in 100 ppm sodium hypochlorite solution for 20 min and aliquoted to ensure sample homogeneity in the experiments. After homogenization, samples were dried and ground in a 6 L stainless-steel knife mill (Poli®,Metalúrgica Siemsen Ltd.) at 60 Hz for 15 min. This procedure was used to reduce the particle size of plant fibers. It is known that particle size has a great influence on the efficiency of chemical pretreatment of different lignocellulosic materials. The smaller the fiber length, the greater the solvent penetrability for acid hydrolysis. (MUSSATO *et al.*,2010)

Study of pretreatment conditions

All chemicals used were of analytical grade. First, different quantities of ground barley straw (5.00, 7.18, 12.50, 17.82, and 20.00 g) were transferred to 250 mL Schott Duran glass bottles. Then, 100 mL of sulfuric acid at different concentrations (0.50, 0.86, 1.75, 2.63, and 3.00 %v/v) was added. Samples were pretreated in a vertical autoclave (model A50, BIOENG) at 120 °C and 1.0 kgf/cm^2 for 40 min. Upon completion of the pretreatment, the reaction was stopped by placing the bottles in an ice water bath. The mixture was filtered under vacuum, and the solid fraction was washed with 60 mL of distilled water to extract the remaining reducing sugars. (VIEIRA *et al.*, 2020) The liquor was evaluated for glucose concentration by the 3,5-dinitrosalicylic acid colorimetric method, proposed by Miller (1959). Quantification was performed against a standard curve of glucose. Analyses were conducted in triplicate.

Experimental design and statistical analysis

Statistica® software version 5.0 (StatSoft Inc., USA) was used for analysis of variance (ANOVA) and generation of the experimental design. The level of significance was set at $p \leq 0.05$. A 2^2 full factorial design with three center points was chosen to study the effects of sulfuric acid (H_2SO_4) concentration and barley straw loading, each at five levels. Star points were included in the experimental design to obtain a second-order model (Table 1). Factor levels were defined on the basis of preliminary tests. Response surface models were fitted to experimental data to describe two dependent variables (y): soluble solids (ºBx) and glucose yield (ratio of released glucose to initial barley straw biomass). The general second-order response function fitted to experimental data is given by Eq. (1).

$$y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 \quad (1)$$

Where A is the coded value of sulfuric acid concentration (%v/v), B is the coded value of barley straw loading (g/100 mL), and β_0, β_1, β_2, β_{12}, β_{11}, and β_{22} are the estimated coefficients.

Table 1: Experimental design matrix showing coded and actual values of independent factors

Run	Points	Coded values		Actual values	
		H_2SO_4 concentration	Barley straw loading	H_2SO_4 concentration (%)	Barley straw loading (g/100 mL)
1		1	1	2.63	17.82
2		1	−1	2.63	7.18
3	Star points	−1	−1	0.86	7.18
4		−1	1	0.86	17.82
5		−1.41	0	0.5	12.5
6	Factorial	1.41	0	3	12.5
7	points	0	−1.41	1.75	5
8		0	1.41	1.75	20
9	Center points	0	0	1.75	12.5
10		0	0	1.75	12.5
11		0	0	1.75	12.5

Characterization of barley straw

Table 2 presents the cellulose, hemicellulose, lignin, and ash contents of the barley straw used in this study. The results were similar to literature values for cellulose, hemicellulose, lignin, and ash contents in barley straw. (ALBINI *et al.*, 2015)

The natural polymers present in barley straw (Table 2) can be hydrolyzed into fermentable sugars and account for about 60% of the gross weight of the raw material. Concentrations of structural components in plants may vary according to the place of cultivation, climate, humidity, soil moisture and nutrient conditions, and plant developmental stage. (SLUITER *et al*., 2005)

Table 2: Chemical composition of barley straw

Component	Concentration (% w/w)
Cellulose	37.45 ± 3.18
Hemicellulose	20.31 ± 2.61
Lignin	18.85 ± 1.78
Ash	8.45 ± 1.57

Reactor design and testing

We opted to design a batch-type reactor for acid hydrolysis because of its low cost and versatility. (SLUITER *et al.*, 2005) The materials used for manufacture were either recycled or obtained by donation. The system is composed of a cylindrical reaction vessel (34.5 cm height and 25 cm diameter), a support plate (1.27 cm thickness), a conical base (12 cm height), and a lug valve at the base of the vessel for sanitization and removal of contents. An industrial-type ceramic heating element (250 mm diameter, 200 mm height, 1 cm thickness, and 9400 W; RR Resistências Industriais®) was placed inside the reactor vessel. The upper part of the bioreactor is equipped with a lid (33 cm diameter) and a mechanical stirrer (120 to 5000 rpm; model 713, Fisatom). The lid contains a concentric

inlet (7 cm diameter) and four holes for fixing screws. Four screws connect the lid to a flange and a 407 stainless steel vessel (103 cm height) packed with silica beads. There is also a support for coupling the stirring motor.

Reactor experiments were planned on the basis of the results of laboratory-scale experiments. The sulfuric acid concentration was 2.6% (v/v), and the barley straw loading was 7 g/100 mL. (LYND *et al.*, 1996) The total volume of the reaction vessel was 13.5 L, and the working volume was 10.8 L. Tests and readings were performed in triplicate. Table 3 shows the results of the reactor test.

Table 3: Results of the reactor test

Barley straw loading (g/100 mL)	H_2SO_4 concentration (%)	Absorbance	Glucose concentration (g/L)	Total glucose released (g)	Glucose yield (g/g straw)
756.6	2.6	0.053	8.179	81.794	0.108

Acid hydrolysis of barley straw in the reactor resulted in a glucose yield of 0.108 g/g straw. This result was similar to that obtained in bench-scale experiments (0.137 g/g straw), showing that mechanical stirring not enhanced hydrolysis efficiency despite probably by promoting contact between plant fibers and sulfuric acid, not influencing the acid's penetrability in the fiber. Glucose, xylose, and arabinose concentrations were determined by high-performance liquid chromatography. Table 4 compares the sugar concentrations obtained by acid hydrolysis of barley straw in the batch reactor and in bench-scale experiments.

The results of Table 4 demonstrate that the batch reactor was more efficient or as efficient as bench-scale reactions in hydrolyzing barley straw lignocellulosic biomass.

Table 4: Sugar concentrations obtained by hydrolysis of barley straw by different methods

Method	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)
Batch reactor	29.2727	10.7407	3.8306
Bench-scale reaction	20.8541	8.5699	3.0131

3 RESULTS AND DISCUSSION

Chemical composition of barley straw

The chemical composition of barley straw was similar to that of other straws evaluated in previous studies (Table 5). The minor differences between barley straw composition and that of sugarcane, wheat, and rice straws can be attributed to differences in cultivation region, climate, relative humidity, soil moisture and nutrient content, and

crop age. (FOGLER, 2002) A high cellulose content is desirable, as cellulose is hydrolyzed to glucose and cellobiose (reducing sugars that are essential for fermentation). Barley straw also contained high levels of hemicellulose (the polymer of main interest in the bioconversion of xylose to xylitol), lignin, and ash. Natural polymers that release fermentable sugars when hydrolyzed (cellulose, hemicellulose, and lignin) accounted for up to 75% of the total weight of barley straw.

Table 5: Chemical composition of lignocellulosic materials

Raw material	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**	**Ash (%)**	**Reference**
Barley straw	37.45 ± 3.18	20.31 ± 2.61	18.85 ± 1.78	8.45 ± 1.57	This study
Sugarcane straw	35.42	25.02	22.5	5.83	Rúbio Mattos *et al*. (2002)
Wheat straw	33.81	31.83	20.12	8.02	Cândido *et al*. (2003)
Rice straw	43.50	22.01	17.20	11.40	Mussatto (2002)

Soluble solids

An experimental design was used to assess the soluble solids content of barley straw pretreated under different conditions. The highest soluble solids content (3.58 °Bx) was obtained by pretreating 7.18 g/100 mL barley straw with 2.63% sulfuric acid in an autoclave for 40 min (Table 6). Dilute acid pretreatment affords a liquid fraction rich in hemicelluloses (separated from the fibrous cellulosic matrix), a solid fraction consisting of lignin, and cellulose, which is further broken down to glucose in the hydrolysis step. (SCHULTZ *et al.*, 2014) The lowest soluble solids concentration was obtained using 0.5% sulfuric acid and 12.5 g/100 mL barley straw. These results show that a high concentration of acid is needed to promote hemicellulose solubilization and fiber opening. (SLUITER *et al.*, 2008)

Table 6: Soluble solids content of barley straw subjected to dilute acid pretreatment

Run	Factor		Soluble solids (°Bx)
	H_2SO_4 concentration (%)	Barley straw loading (g/100 mL)	
1	1 (0.86)	1 (7.18)	1.38
2	1 (2.63)	−1 (7.18)	3.58
3	−1 (0.86)	−1 (17.82)	1.58
4	−1 (2.63)	1 (17.82)	3.18
5	−1.41 (0.5)	0 (12.50)	0.28
6	1.41 (3.00)	0 (12.50)	2.58
7	0 (1.75)	−1.41 (5.00)	2.48
8	0 (1.75)	1.41 (20.00)	2.48
9	0 (1.75)	0 (12.50)	2.58
10	0 (1.75)	0 (12.50)	2.68
11	0 (1.75)	0 (12.50)	2.58

Data normality was confirmed by the Shapiro–Wilk test, with a calculated *W*-value of 0.88771 and a *p*-value of 0.13018. The calculated test statistic was greater than the critical value for a dataset of 11 samples (W_c = 0.85). Statistical analysis showed that only the linear and quadratic terms of sulfuric acid concentration were significant ($p < 0.05$) at the 95% confidence interval (CI) (Table 7).

The linear term of sulfuric acid concentration had the greatest effect on soluble solids content, followed by the quadratic term. Linear and quadratic effects of barley straw loading were not significant. Thus, linear and quadratic terms of straw loading and the linear interaction term of acid concentration and straw loading were treated as residuals for mathematical modeling and ANOVA and regression analyses.

Table 7: Regression coefficients of soluble solids content of barley straw subjected to dilute acid pretreatment

Factors	Regression coefficient	Standard error	t(5)	p-value	Lower 95% CI	Upper 95% CI
Mean	−1.30573	0.217006	−6.0170	0.026527	−1.93938	−0.672074
Linear term of acid concentration	3.50204	0.134861	25.9678	0.001480	3.10825	3.895828
Quadratic term of acid concentration	−0.60318	0.031064	−19.4177	0.002642	−0.69389	−0.512478
Linear term of straw loading	0.00239	0.024378	0.0979	0.930948	−0.06880	0.073571
Quadratic term of straw loading	0.00194	0.000862	2.2507	0.153277	−0.00058	0.004459
Acid concentration straw loading	−0.03183	0.006131	−5.1912	0.035162	−0.04973	−0.013926

Table 8 shows the results of ANOVA for soluble solids content. The effects of significant variables and residuals (nonsignificant variables) were calculated. The coefficient of determination (R^2) was 99.65%, and the *F*-value (regression and residual) was 48.43 times higher than the *F*-critical, confirming the significance of the proposed model. Thus, it was possible to construct response surface and contour plots.

Table 8: ANOVA for soluble solids content of barley straw subjected to dilute acid pretreatment

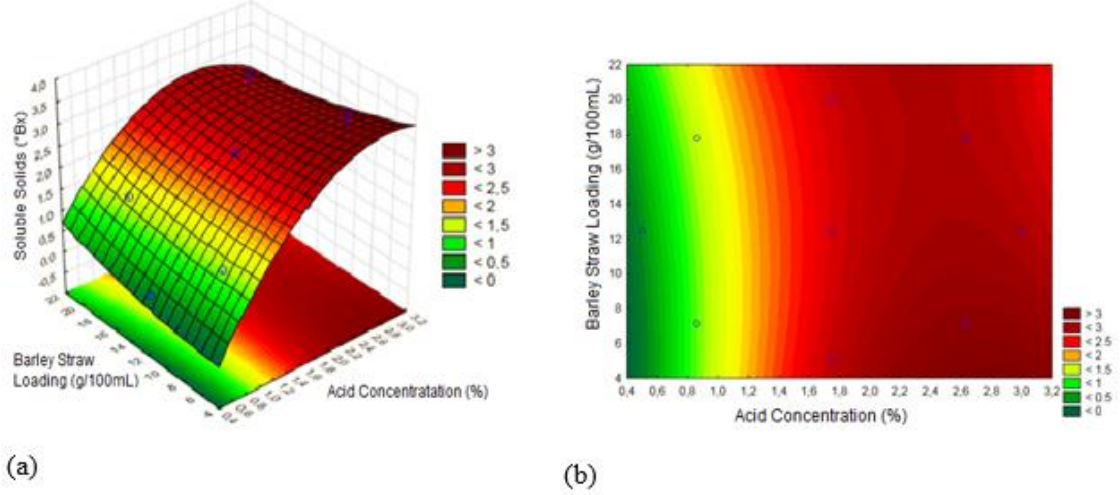
Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*-value	*p*-value
Regression (R)	8.273418	6.0	1.378903	194.208951	0.000143
Residual (r)	0.028400	4.0	0.007100		
Lack-of-fit	0.501552	3.0	0.167184		
Pure error	0.006667	2.0	0.003333		
Total	8.301818	10.0	0.830182		
R^2	99.657901				
F-critical (R,r)	4.01				

Response surface and contour plots were used to optimize barley straw pretreatment. The curves show that the response variable is maximized by pretreatment of 15 g/100 mL barley straw with 2% (v/v) sulfuric acid (Figure 1). Eq. (2) describes the statistical model for estimating soluble solids as a function of sulfuric acid concentration and barley straw loading.

$$SS = 1.305 + 3.502C - 0.603C^2 + 0.0238L + 0.0194L^2 \quad (2)$$

where SS is the soluble solids content (°Bx), C is the sulfuric acid concentration (% v/v), and L is the barley straw loading (g/100 mL).

Figure 1: (a) Response surface and (b) contour plots of soluble solids as a function of sulfuric acid concentration and barley straw loading



(a) (b)

Glucose yield

Glucose concentration was calculated from absorbance values obtained in each experimental run, as follows: Glucose concentration = (2.2545 × Absorbance + 0.0441) × 50. In the equation, 50 represents the dilution factor. Glucose released (g) was determined by multiplying glucose concentration (expressed as g/L) by the volume (L)

of liquor recovered after separation of the solid fraction by filtration through a cotton cloth. Then, glucose yield (g/g straw) was calculated as glucose released divided by initial barley straw weight. Results are presented in Table 9. The highest glucose concentration was obtained by using 2.63% (v/v) sulfuric acid and 7.18 g/100 mL barley straw (run 2). However, the highest glucose yield was obtained in center point runs (1.75% v/v sulfuric acid and 12.5 g/100 mL barley straw, runs 9–11) because of the higher liquor volume.

Table 9: Glucose yields obtained by dilute acid pretreatment of barley straw under different conditions

Run	H_2SO_4 concentration (%)	Absorbance	Glucose concentration (g/L)	Liquor volume (L)	Glucose released (g)	Straw weight (g)	Glucose yield (g/g straw)
1	0.86	0.016	4.01	0.093	0.373	7.18	0.0519
2	2.63	0.048	7.62	0.098	0.746	7.18	0.1039
3	0.86	0.029	5.47	0.093	0.509	17.82	0.0286
4	2.63	0.083	7.95	0.104	0.827	17.82	0.0675
5	0.5	0.001	2.32	0.105	0.243	12.50	0.0195
6	3.00	0.052	8.07	0.096	0.774	12.50	0.0620
7	1.75	0.025	5.02	0.098	0.492	5.00	0.0985
8	1.75	0.047	7.50	0.095	0.713	20.00	0.0356
9	1.75	0.063	7.95	0.103	0.819	12.50	0.0767
10	1.75	0.062	7.84	0.103	0.808	12.50	0.0758
11	1.75	0.063	7.95	0.103	0.819	12.50	0.0767

Experimental data were subjected to the Shapiro–Wilk test to assess the normality assumption. The calculated W-value (0.95404) was higher than the critical W (0.85) for a dataset of 11 samples. Statistical analysis revealed that linear and quadratic terms of sulfuric acid concentration and barley straw loading were significant at the 95% CI ($p < 0.05$) for glucose yield (Table 10).

Table 10: Regression coefficients of glucose yield obtained by dilute acid pretreatment of barley straw

Factor	Regression coefficient	Standard error	t(5)	p-value	Lower 95% CI	Upper 95% CI
Mean	0.076272	0.000310	246.3444	0.000016	0.075368	0.077176
Linear term of acid concentration	0.037931	0.000379	99.9642	0.000100	0.036823	0.039039
Quadratic term of acid concentration	−0.031147	0.000452	−68.9125	0.000211	−0.032467	−0.029827
Linear term of straw loading	−0.037194	0.000380	−97.9292	0.000104	−0.038303	−0.036085
Quadratic term of straw loading	−0.004702	0.000453	−10.3707	0.009170	−0.006026	−0.003378
Acid concentration × straw loading	−0.006602	0.000536	−12.3110	0.006533	−0.008168	−0.005036

The linear term of sulfuric acid concentration was the most significant, followed by the quadratic term of sulfuric acid concentration and the linear term of barley straw loading. For ANOVA and mathematical modeling, the linear interaction term between sulfuric acid concentration and barley straw loading and the quadratic term of straw loading were excluded and treated as residuals.

According to the ANOVA results shown in Table 11, the R^2 was 99.99% and the F-value was 345.22 times higher than the F-critical, representing satisfactory data dispersion and good agreement between values.

Table 11: ANOVA for glucose yield obtained by dilute acid pretreatment of barley straw

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Regression (R)	0.00744233	8.0	0.00093029	3234.78427507	0.00034
Residual (r)	0.00000058	2.0	0.00000029		
Lack-of-fit	0.00039068	3.0	0.00013023		
Pure error	0.00000058	2.0	0.00000029		
Total	0.00744291	10.0	0.00074429		
F-critical (R,r)	9.370000				
R2	99.992272				

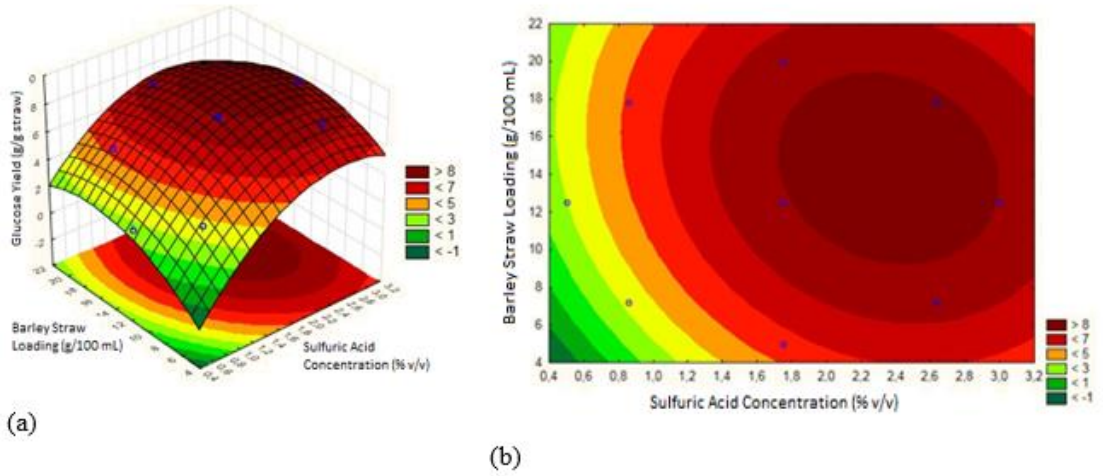
Response surface analysis demonstrated that the best experimental condition is 2.63% sulfuric acid and 7 g/100 mL barley straw, as shown in Figure 2. Eq. (3) describes the statistical model for estimating glucose yield as a function of acid concentration and straw loading.

$$\text{Glucose yield} = -0.0408 + 0.1203C - 0.2581C^2 + 0.0392L - 0.000247L^2 \quad (3)$$

where C is the sulfuric acid concentration (% v/v) and L is the barley straw loading (g/100 mL).

Schultz, 2014 carried out an experimental design similar to that applied in the present study but used sugarcane bagasse at a solid loading of 1:10. Pretreated solids were subjected to enzymatic hydrolysis at 50 °C for 48 h using an enzyme loading of 15 FPU/g biomass (Cellic® CTec3, Novozymes). The authors found that the optimal acid concentration and reaction time for sugarcane bagasse pretreatment were 1.5% sulfuric acid and 56 min, respectively. Optimized conditions were similar to those obtained in this study for barley straw (2.6% sulfuric acid, 7 g/100 mL barley straw, and 40 min). Sugarcane biomass and barley straw are quantitatively similar in structural components.

Figure 2: (a) Response surface and (b) contour plots of glucose yield as a function of sulfuric acid concentration and barley straw loading



Experimental validation

Experiments were carried out under optimal conditions (2.6% v/v sulfuric acid and 7 g/100 mL barley straw) to validate the proposed model. The resulting liquor had a mean absorbance of 0.063 ± 0.0005 at 540 nm (coefficient of variation = 0.75%) (Table 12).

Table 12: Absorbance at 540 nm of liquor obtained by dilute acid pretreatment of barley straw under optimal conditions

Run	Absorbance			Mean ± SD	Coefficient of variation (%)
Center point (Validation Run)	0.063	0.062	0.063	0.063 ± 0.0005	0.75

The absorbance was used to calculate glucose concentration, glucose released, and glucose yield, as shown in Table 13. Glucose yield was 0.137 g/g straw, representing about 90% of the predicted value (0.14% g/g straw).

Table 13: Glucose yield obtained by dilute acid pretreatment of barley straw under optimal conditions

Run	**Absorbance**	**Glucose concentration (g/L)**	**Liquor volume (L)**	**Glucose released (g)**	**Glucose yield (g/g straw)**
Center point (Validation Run)	0.063	9.31	0.103	0.959	0.137

The soluble solids content of liquor obtained under optimal pretreatment conditions was 2.61 °Bx, similar to the predicted value of 3 °Bx (coefficient of variation = 0.275%).

A further experiment was carried out to confirm the results of both experimental designs by evaluating the effects of barley straw loading on glucose yield and soluble solids. Experiments were conducted using the optimal acid concentration (2.3% sulfuric acid v/v) and barley straw loadings ranging from levels −1 to +1, that is, 7.18, 12.5, and 17.82 g/100 mL barley straw.

Comparation in Reactor test

Glucose yield was 0.108 g/g straw, similar to that obtained in bench experiments (0.137 g/g straw), demonstrating that mechanical stirring increases the efficiency of hydrolysis by promoting contact between fibers and acid. Table 14 compares the concentrations of reducing sugars obtained by batch reactor and bench experiments. Similar results were obtained, which shows that reactor experiments promote biomass digestibility.

Table 14: Sugar concentrations obtained under different methods

Method	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)
Batch reactor	29.2727	10.7407	3.8306
Bench experiment	20.8541	8.5699	3.0131

4 CONCLUSIONS

Physicochemical characterization of barley straw revealed the following composition: 8.19% moisture, 37.45% cellulose, 20.31% hemicellulose, 18.85% lignin, and 8.45% ash. Acid pretreatment conditions were studied using an experimental design. Statistical analyses showed that sulfuric acid concentration (linear and quadratic terms) and straw loading (linear term) were significant at the 95% CI for glucose yield, whereas only sulfuric acid concentration (linear and quadratic terms) was significant for soluble solids. Optimal conditions for the pretreatment of barley straw in an autoclave (121 °C, 40 min) were found to be 2.3% (%v/v) sulfuric acid and 7 g/100 mL barley straw, affording a glucose yield of 0.137 g/g straw and a soluble solids content of 2.61 °Bx. These conditions were used for acid hydrolysis in the batch reactor. The hydrolysate was found to contain 29.27 g/L glucose, 10.74 g/L xylose, and 3.83 g/L arabinose. Batch reactor and bench-scale experiments afforded similar concentrations of reducing sugars, showing that acid hydrolysis in the developed reactor promoted biomass digestibility. The results demonstrate that barley straw has great potential as a substrate for second-generation ethanol production, xylose extraction, and furfural production.

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