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Abstract:	In this study, an innovative approach is proposed for the integral valorization of all sugars (cellulosic and hemicellulosic) contained in a lignocellulosic residue, as is brewer's spent grain (BSG), through the production of an advanced biofuel such as biobutanol. For this purpose, the entire slurry obtained in the microwave assisted dilute sulfuric acid pretreatment under optimized conditions (147 ºC, 2 min and 1.26% H 2 SO 4) at a biomass loading as high as 15% (w/v) was enzymatically hydrolyzed without previous solid-liquid separation and the highly concentrated solution of sugars recovered was fermented to butanol by Clostridium beijerinckii . In this way, all sugars (pentoses and hexoses) contained in BSG could be fermented using a single bioreactor, leading to 11 g/L of butanol. The mass balance revealed than an overall yield of 91 kg butanol/t BSG and 138 kg ABE/t BSG could be reached.
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Integral valorization of cellulosic and hemicellulosic sugars for biobutanol production: ABE fermentation of the whole slurry from microwave pretreated brewer`s spent grains

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ABSTRACT

In this study, an innovative approach is proposed for the integral valorization of all sugars (cellulosic and hemicellulosic) contained in a lignocellulosic residue, as is brewer's spent grain (BSG), through the production of an advanced biofuel such as biobutanol. For this purpose, the entire slurry obtained in the microwave assisted dilute sulfuric acid pretreatment under optimized conditions (147 ºC, 2 min and 1.26% H_2SO_4) at a biomass loading as high as 15% (w/v) was enzymatically hydrolyzed without previous solid-liquid separation and the highly concentrated solution of sugars recovered was fermented to butanol by *Clostridium beijerinckii*. In this way, all sugars (pentoses and hexoses) contained in BSG could be fermented using a single bioreactor, leading to 11 g/L of butanol. The mass balance revealed than an overall yield of 91 kg butanol/t BSG and 138 kg ABE/t BSG could be reached.

Keywords: brewing industry waste; lignocellulosic biomass; microwave pretreatment; slurry; bioenergy; *Clostridium beijerinckii.*

1. Introduction

The development of renewable energy sources which allow emissions of greenhouse gases and the risks related to dependence on fossil fuels in the transport sector to be reduced is essential. In this sense, Directive (EU) 2015/1513 of the European Parliament and of the Council, of 9 September [1], aims to encourage research into so-called advanced biofuels, which can be obtained from lignocellulosic biomass.

Biobutanol, which can be obtained from lignocellulosic residues by anaerobic fermentation with *Clostridia* strains in what is known as acetone-butanol-ethanol (ABE)

fermentation, nowadays has increased importance due to its application as an industrial chemical and advanced biofuel [2,3]. Biobutanol has an energy content comparable to gasoline and higher than ethanol, a low corrosive nature and is safer to handle due to its lower vapor pressure in comparison with ethanol. Therefore, gasoline might be partial or totally replaced by butanol, since existing engines do not need any modifications. In addition, butanol can be used as a chemical commodity in different industries, such as enamels, lacquers, antibiotics, pharmaceuticals, food and flavoring [4,5].

Brewer spent grain (BSG) is an interesting lignocellulosic residue, accounting for 85% of the total waste generated in breweries [6,7]. Considering the European Union and the world, the production of beer in 2014 was 37.4 and 180.3 million tonnes, respectively [8]. The typical ratio of wet BSG is 20 kg produced per 100 L beer. Although BSG can be used as feed for livestock, nowadays its commercial application is limited. Therefore, it could be used to produce liquid biofuels such as butanol through biological processes due to its carbohydrate content, about 50% [9].

BSG has a complicated structure, mainly formed by cellulose, hemicellulose and lignin [10]. Then, in order to produce biobutanol from BSG, different steps (pretreatment, enzymatic hydrolysis and fermentation) have to be carried out. The most essential step is the pretreatment, since it is necessary to overcome the recalcitrance of lignocellulose and reduce the cellulose crystallinity for improving sugar released in the subsequent enzymatic hydrolysis. A great number of pretreatments have been developed, such as liquid hot water, dilute acid, alkaline, ionic liquids, ozone, microwave, steam explosion or fungal, among others [11,12]. In industries, the most usually applied process is the dilute acid pretreatment, as it is able to obtain hemicellulose recoveries of about 85-95%; the pretreated solid is enriched in cellulose, which is more accessible to enzymes, reducing enzyme loads; and it is economically

feasible [13,14]. However, dilute acid pretreatment has the disadvantage that it is necessary to use high temperatures and long process times [15]. In order to mitigate these long process times and get a higher homogeneity in the heating process, the dilute acid pretreatment can be used in combination with microwave, which is an interesting emerging technology that is substituting conventional heating. Microwave pretreatment is able to induce heat at the molecular level by the direct transformation of microwave irradiation into energy. Therefore, energy can be homogeneously dispersed through the material, while an overheating of the outside surface with some cooler inside areas can occur in conventional heating. Thus, in comparison with the simple dilute acid, dilute acid pretreatment assisted by microwave is simpler, more homogeneous, more energy efficient, profitable, environmentally friendly and is able to withdraw larger amounts of acetyl groups from the hemicellulose. What is more, unlike the single dilute acid pretreatment, the dilute acid pretreatment combined with microwave is faster [16–18]. The combined acid-microwave pretreatment has been applied to different feedstocks (such as maize distillery stillage, macroalgal *Laminaria japonica*, or water hyacinth) to produce bioethanol and biohydrogen [19–21]. No previous references about butanol production from lignocellulosic biomass after microwave pretreatment catalyzed by dilute acid have been found.

It is worth mentioning that, after the lignocellulosic biomass pretreatment, the solid and liquid fractions are usually separated, fermenting only the sugars from the pretreated solid and throwing away the liquid fraction due to its low sugar concentration. However, the use of higher sugar concentrations is essential and this can be achieved by using the slurries generated in the pretreatment of lignocellulosic biomass. In addition, there are many other reasons that considerably increase the importance of using slurries; for instance, their use allows a single bioreactor to be used,

avoiding filtration processes to separate the solid and prehydrolysate originated, and preventing independent fermentation stages. In this way, a liquor containing both pentoses (xylose, arabinose) and hexoses (glucose, galactose) can be obtained as a result of using the whole slurry in the pretreatment and subsequent enzymatic hydrolysis process, which can be used as the substrate in ABE fermentation [22].

This work aims to propose a process based on microwave assisted dilute sulfuric acid pretreatment to recover sugars from BSG and to obtain a slurry which is highly concentrated in pentoses and hexoses and which could be transformed to biobutanol in a single fermenter. So, the operating conditions of the microwave pretreatment in dilute sulfuric acid were firstly optimized to maximize the recovery of fermentable sugars from both the hemicellulose and cellulose fractions. Secondly, the whole slurry from the pretreatment was enzymatically hydrolyzed to obtain a solution rich in sugars that could be further fermented to butanol by *Clostridium beijerinckii* DSM 6422. Then, in order to increase the concentration of fermentable sugars, and therefore to improve the butanol concentration, the pretreatment was conducted under optimal conditions at two different solid loadings (10 and 15% w/v). The main novelty of the work is the use of an emergent pretreatment as microwave for the integral valorization of hemicellulosic and cellulosic sugars in low value lignocellulosic biomass through the production of an advanced biofuel such as biobutanol.

2. Materials and methods

2.1. Raw material

BSG was kindly provided by a local brewery (Cerveza Milana, Valladolid) and stored frozen at -20ºC until being used. Prior to its use, the BSG was water washed, dried at 50°C, ground (particle size lower than 1 mm) and homogenized. The feedstock

showed the following composition (g/100 g dry matter): cellulose: 17.9 ± 0.3; hemicellulose: 28.7 ± 0.8; starch: 2.1 ± 0.0; acid lignin: 25.8 ± 1.2; extractives: 2.3 ± 0.1; ash: 2.7 ± 0.1 [23].

2.2. Microwave assisted dilute sulfuric acid pretreatment

Microwave pretreatment of BSG was carried out in a Multiwave PRO SOLV reactor 50 Hz (Anton Paar GmbH, Austria, Europe) at 10% w/v as described elsewhere [23].

After pretreatment, the pretreated BSG was separated from the liquid fraction by vacuum filtration, water washed, dried at 40 ºC and weighed to calculate the solid recovery (g pretreated solid per 100 g untreated BSG). The pretreated BSG was used in enzymatic hydrolysis assays, and its composition in carbohydrates and lignin was determined. The pretreatment liquids were characterized for fermentable sugars and potential inhibitors (organic acids, furans and phenolic compounds). The recovery of carbohydrates in the pretreatment liquid was calculated as previously explained [23].

2.3. Experimental design

A central composite experimental design was planned ($\alpha = 1.414$) to determine the optimum experimental conditions that maximize sugar recovery from BSG. Temperature (120-170 ºC), time (2-10 min) and sulfuric acid concentration (0.5-1.5%, w/v) were selected as factors (Table 1). The intervals of the variables were selected on the basis of previous results [24]. 20 experimental runs were performed, including one point and five replications. Statgraphics Centurion XVIII was used to plan the design and analyze the experimental data.

The Combined Severity Factor (CSF) was calculated as proposed by MacAskill et al. [25] (Eq. 1) as indicator of the severity of the pretreatment conditions:

$$\text{Combined Severity Factor (CSF)} = \text{Log}\left[t \times exp\left(\frac{T-100}{14.75}\right)\right] - \text{pH} \quad (1)$$

where t is time (min), T is temperature (ºC) and the pH is that of the initial sulfuric acid solution used in each run.

2.4. Enzymatic hydrolysis

Pretreated solids obtained in the experimental design were used as substrate in the enzymatic hydrolysis (EH) assays, which were carried out at a solid loading of 5% (w/v) at 50ºC for 48 h in an orbital shaker as described elsewhere [23]. The enzyme complex used was Cellic CTec2, kindly provided by Novozymes (Denmark), being the enzyme load employed of 15 Filter Paper Units (FPU)/g solid. Samples were taken at 24 and 48h, centrifuged and analyzed for monosaccharides and degradation products. Glucose recovery in enzymatic hydrolysis (referred to pretreated or untreated BSG) was calculated considering the glucose in the enzymatic hydrolysates and the glucose in the pretreated or non-pretreated lignocellulosic material, as previously described [23].

In order to confirm optimization results, enzymatic hydrolysis essays were carried out with the pretreated BSG obtained under optimal conditions. In addition, to increase the sugar concentrations in hydrolysates, the solid loading in the pretreatment was also increased to 15% w/v. What is more, in order to obtain a sugar solution rich in pentoses and hexoses which can be used in ABE fermentation, the whole slurry obtained under optimal pretreatment conditions (at 10 and 15% solid loading of pretreatment) was enzymatically hydrolyzed using 1 L flasks with 400 mL of slurry (4.8 and 7.9% insoluble solid concentration for 10 and 15% solid loading in pretreatment, respectively). Moreover, sodium citrate buffer was not added, and water was used as the solvent at pH 4.8, which was adjusted with solid NaOH. After saccharification, vacuum

filtration was used to separate liquid and solid phases. The solid phase was water washed, dried at 40 °C and analyzed for residual sugars and lignin. For the liquid phase (slurry enzymatic hydrolysate), monosaccharides and degradation products were determined, as well as its suitability as substrate in ABE fermentation.

2.5. Microorganism, detoxification and ABE fermentation

C. beijerinckii DSM 6422, which is a microorganism acquired from the German collection (DSMZ, Leibniz, Germany), was maintained and grown as previously described [23].

Slurries from the microwave acid pretreatment under optimal conditions at 10 and 15% (w/v) BSG concentrations were enzymatically hydrolyzed as described in section 2.4. Then, the resulting slurry enzymatic hydrolysates rich in sugars from hemicellulose and cellulose were transformed into biobutanol with *C. beijerinckii*.

Before fermentation, the enzymatic hydrolysates were detoxified with powder activated charcoal or ion-exchange resins (Lewatit S4528) under conditions selected from previous experimental runs (data not shown). The enzymatic hydrolysates were mixed with activated charcoal or ion-exchange resins at a ratio of 2%, 5% or 10% (w/v) in an orbital shaker (Comecta Optic Ivymen system) at 35 ºC. The activated charcoal detoxification was carried out at 130 rpm and 1.5 h, whereas the ion-exchange resin treatment was performed at 150 rpm for 24 h. Prior to the detoxification, the ion-exchange resin was conditioned with a 70 g/L NaOH solution for 24 h, recovered by vacuum filtration, washed with distilled water and dried at 40 °C. After detoxification, the mixtures were vacuum filtered and the hydrolysates were analyzed. Afterward, ABE fermentation with *C. beijerinkii* was conducted at 35 ºC and 135 rpm for 120 h. The initial pH of the fermentation was 5.5, and that was not controlled during the

fermentation process (for more details, see Plaza et al. [26]). Fermentation runs were performed in triplicate.

2.6. Analytical methods

In order to determine the composition of BSG before and after pretreatment the analytical methods of the National Renewable Energy Laboratory (NREL) [27,28] were applied.

High Performance Liquid Chromatography (HPLC) based on refractive index (Waters 2414) and photodiode array detection (DAD Waters 996) was the analytic technique used to measure the concentrations of monosaccharides (glucose, xylose and arabinose), potential fermentation inhibitors (acetic acid, formic acid, furfural and hydroxymethylfurfural (HMF)) and ABE products (acetone, butanol, ethanol and butyric acid). The description of the analytical procedure can be found in a previous work [23].

On the other hand, an acid hydrolysis process (120 °C, 3% w/v H_2SO_4, 30 min) was applied to quantify the oligomeric sugar concentration in the liquid fractions obtained as a consequence of the BSG microwave pretreatment. Oligomer composition was calculated through the difference between the total free monosaccharides in the hydrolysates before and after the acid hydrolysis step. The total content of phenolic compounds was measured by the Folin-Ciocalteu method [29]. Analytical determinations were carried out in triplicate and the average results are shown.

3. Results and discussion

3.1. Effect of microwave pretreatment assisted by dilute sulfuric acid on BSG

First, an experimental design was planned to select the operating conditions that could maximize the recovery of sugars (hemicellulosic and cellulosic). The CSF factor, which takes into account the influence of such operating factors as temperature, time and acid concentration, was employed to analyze the results.

Solid recoveries corresponding to the experimental runs carried out are shown in Table 2. In general, the solid recovery decreased when the CSF factor increased. Then, solid recoveries ranged from 37% to 87%, corresponding to one of the most severe (CSF=2.71, run 14) and less severe (CSF=0.37, run 9), respectively. As a result of the pretreatment, all pretreatment conditions assayed led to pretreated solids enriched in cellulose. This fact is due to the solubilization of extractives and hemicellulosic components in the liquid fraction. On the other hand, Table 2 also shows glucose recoveries in the pretreated solids (GR_s). The highest recovery (GRs = 76%) corresponded to soft pretreatment conditions (CSF = 0.37, run 9). However, when the pretreatment was carried out at harshness conditions (CSF=2.84, run 19), a GR_S as low as 54% was achieved. The lignin in the pretreated solid fractions also increased, which might be due to formation of lignin-like structures from condensation reactions [30]. Regarding the hemicellulose content in the pretreated solids (Table 2), its complete solubilization (HSR_s = 0%) was achieved for the highest combined severity pretreatment (CSF > 2, runs 14, 18 and 19). Nevertheless, when the CSF was lower than 1, a considerable content of hemicellulose fraction was observed in the pretreated BSG (15-19%), which could negatively influence the subsequent enzymatic hydrolysis process, due to the greater difficulty for the enzymes to get into contact with the cellulose [31].

A pH ranging from 0.3 (runs 6, 11 and 13) to 2.3 (run 1) was measured in the liquid fractions (Table 3), corresponding to the highest and the lowest sulfuric acid

concentrations (1.5-1.84 and 0.16%), respectively. The monosaccharide concentrations (glucose, xylose and arabinose), as well as the sugar recoveries (GR_L, HSR_L) in the liquid fractions are summarized in Table 3. In this way, it can be seen that the sugar concentrations ranged from 7.3 g/L (run 9) to 33.2 g/L (runs 5, 6 and 17), mainly in monomeric form (Table 3). Glucose was detected in the liquid fractions, even at the softest pretreatment conditions (runs 1, 4, 7, 9, 13 and 15, CSF < 1), due to the content of non-structural glucose (glucose in extractives and starch) and amorphous cellulose, which is easily solubilizable [32]. On the contrary, arabinose and xylose were the majority sugars, being measured the highest concentrations for CSF = 1.60 (run 5). This same behavior was also observed for hemicellulosic sugar recoveries in the liquid fraction (HSR_L). Nevertheless, due to hemicellulosic sugar degradation reactions, xylose concentrations and hemicellulosic sugar recoveries diminished when the pretreatment was carried out at CSF > 2 (runs 2, 14, 18 and 19). It should be noted that when the pretreatment was performed at low CSF (CSF = 0.02 and 0.37, runs 15 and 9), pretreatment conditions were not severe enough to get the solubilization of the hemicellulose contained in BSG.

Compounds such as acetic and formic acids, furfural, HMF and phenolic compounds originate in the pretreatment (Table 4). At low pretreatment severities (CSF < 1, runs 1, 4, 7, 9, 13 and 15), inhibitor concentrations (except acetic acid and total phenols) were very low or even not detected. Furfural and total phenol concentrations of up to 3.84 and 2.43 g/L were detected in the liquid fractions at the highest CSF (run 19, CSF = 2.84). In general, inhibitor compound concentrations generated were lower than those reported by Rojas-Chamorro et al. [32] in the phosphoric acid pretreatment of BSG, which could be more suitable for the subsequent fermentation process.

3.2. Enzymatic hydrolysis experiments of BSG pretreated by microwave

The pretreated solid fractions obtained in the experimental design were used in enzymatic hydrolysis assays (at 5% w/v solid load) to assess the effectiveness of the microwave pretreatment assisted by dilute sulfuric acid in the release of glucose from cellulose.

Table 5 shows glucose and xylose concentrations obtained in the enzymatic hydrolysates, which range from 3.4 to 18.1 g/L and 0.3 to 3.0 g/L, respectively. In this way, the highest glucose concentration and glucose recovery (referred to pretreated BSG) (18.1 g/L and 100%, respectively) were obtained for a CSF of 2.01 (run 18: 170 ºC, 2 min, 1.5% H_2SO_4), the recovery of glucose being four times higher than those achieved when the enzymatic hydrolysis was applied to non-pretreated BSG (25.6%) [23]. However, when the CSF was higher than 2.01, lower glucose concentrations and EH glucose recovery (referred to pretreated BSG) were obtained, probably due to glucose degradation [33].

Rojas-Chamorro et al. [32] also observed an almost complete conversion of cellulose to glucose in BSG pretreated under acid conditions (155 °C, 0 min and 2% H_3PO_4). Fernández-Delgado et al. [34] pretreated BSG with peroxide alkaline, obtaining an glucose recovery (referred to pretreated material) of 98% (50 ºC, 60 min, 5% H_2O_2, pH 11.5). However, lower values (60-69%) were obtained when the BSG was pretreated with NaOH (120 °C, 30 min) or ozone (2.7 % O_3, 30 min). Microwave pretreatment, assisted by alkaline or deep eutectic solvent, has also been applied with other lignocellulosic materials (wheat straw, *Miscanthus*, switchgrass or corn stover), yielding lower saccharification values (about 70%) [35,36], probably due to the use of limited domestic microwave ovens instead of multiwave closed reactors, as multiwave closed

reactors allow a better control of the pretreatment conditions, as well as the use of higher temperatures and pressures.

Table 5 also summarized the EH glucose recovery referred to the glucose in the non-pretreated BSG. As BSG contains starch and non-structural glucose, which are effortlessly hydrolyzed in the pretreatment, only the glucose contained as cellulose has been considered to determine the EH glucose recovery (referred to untreated BSG) [37]. Although high recoveries (average 67%) were found around the central point (145 ºC, 6 min, 1% H_2SO_4), it can be said that the highest EH glucose recovery (referred to untreated BSG) (72%) was obtained when the pretreatment was carried out at a combined severity of 1.93 (run 3). However, for CSF > 1.93, EH glucose recovery (referred to untreated BSG) decreased, probably due to glucose degradation.

A similar maximum EH glucose recovery (referred to untreated BSG) (about 74-75%) was obtained in the microwave assisted hydrothermal pretreatment of BSG (at 192.7 ºC for 5.4 min) [23] and in the microwave-assisted dilute sulfuric acid pretreatment of maize distillery stillage (300 W, 3.7 atm, 15 min, 1.96% H_2SO_4) [19].

The overall sugar recovery (Table 5) takes into account the concentration of sugars in the liquid fractions and the glucose and xylose in enzymatic hydrolysates, with regard to the total sugar content in the untreated BSG. The highest recovery (87.4%) was achieved at the central point (145 ºC, 6 min, 1% H_2SO_4). In conclusion, it can be said that microwave pretreatment catalyzed by dilute sulfuric acid led to the recovery of 87% of the sugars contained in BSG (49 g of sugars from 100 g of untreated BSG). This overall sugar recovery was higher than those previously reported for this raw material after pretreatment with H_3PO_4, 78% [32], or HCl plus HNO_3, 72% [38].

3.3. Microwave pretreatment assisted dilute sulfuric acid: optimization of operating conditions

As previously explained, this work aims to recover sugars from both cellulose and hemicellulose in BSG through a microwave assisted dilute sulfuric acid pretreatment. In addition, inhibitory compounds should be as low as possible so as not to interfere in the ABE fermentation. Thus, in order to optimize the pretreatment, the responses chosen were the hemicellulosic sugar recovery in the liquid fraction (HSR_L) and the glucose recovery in enzymatic hydrolysis (referred to untreated BSG), which were maximized simultaneously, as well as the total inhibitor concentration in the liquid fraction, which was minimized at the same time. The optimization was carried out using a method known as the desirability function, which allows different responses to be simultaneously optimized [39]. Polynomial equations of second order (Eqs. 2, 3 and 4) were proposed in order to calculate the responses (HSRL, EH glucose recovery and total inhibitor concentration in the liquid fraction):

$$HSR_L = 80.87 + 9.79\,T + 1.74\,t + 5.31C - 5.35\,T\,t - 7.08\,T\,C - 16.19\,T^2 - 4.36\,C^2 \quad (2)$$

$$\text{EH glucose recovery} = 68.36 + 9.37\,T + 1.91\,t + 2.48C - 6\,T\,t - 8.35\,T^2 - 4.62\,C^2 \quad (3)$$

$$\text{Total inhibitor in liquid fraction} = 2.34 + 2.40\,T + 0.42\,t + 0.50\,C - 0.33\,T\,t + 0.74\,T\,C + 0.71\,t\,C + 0.68\,T^2 - 0.22\,C^2 \quad (4)$$

where T is the temperature (°C), t is the time (min) and C is the sulfuric acid concentration (% w/v).

The variance analyses (ANOVA) for HSR_L, EH glucose recovery and total inhibitor concentration in the liquid fraction are summarized in Supplemental Table 1S. As can be seen, the three models were predictive, as suggested by their values of R^2 and adjusted R^2 and the confidence level (95%, $p < 0.05$).

Concerning the HSR_L response (Eq. 2), the three factors (temperature, time and acid concentration) exerted a positive effect, the influence of the temperature being higher

than that of the acid concentration and time. The combined effect of temperature and time, or temperature and acid concentration, lead to a decrease in the HSR_L, probably due to a lack of hemicellulose solubilization or sugar degradation at very soft or severe pretreatment conditions, respectively. This behavior can be observed in Fig. 1(a,b), which depicts the response surface showing the influence of temperature and time (Fig. 1a), or of temperature and acid concentration (Fig. 1b) on the HSR_L. In this way, as can be observed, it is in the area close to the central point (145 ºC, 6 min, 1% H_2SO_4) where the highest HSR_L was achieved.

Regarding glucose recovery, the three factors have a positive effect (Eq. 3). However, in this case, the temperature factor has a much higher influence than time or sulfuric acid concentration. What is more, there is a considerable negative interaction between temperature and time. Fig. 1(c,d) plots the 3D response surface for glucose recovery in enzymatic hydrolysis, considering temperature and time (Fig. 1c), or temperature and sulfuric acid concentration (Fig. 1d). The glucose recovery increases as the temperature and time rise, until a certain level (near the central point conditions) is reached, where it begins to decrease (Fig. 1c). This is due to the negative interaction between temperature and time, as explained above. On the other hand, the interaction between the temperature and sulfuric acid concentration was insignificant, as can be appreciated in Eq. (3) and Fig. 1d.

Considering the total inhibitor concentration in the liquid fraction (Eq. 4), all the variables exerted positive effects, including the interactions between temperature and sulfuric acid concentration and between time and acid concentration, the influence of temperature being slightly higher. On the contrary, a very slight negative interaction can be appreciated between temperature and time. Therefore, the total inhibitor

concentration increases when temperature and time (Fig. 1e), or temperature and sulfuric acid concentration (Fig. 1f), increase simultaneously.

As a result, 147 ºC, 2 min and 1.26% (w/v) H_2SO_4 were the optimal conditions which the model predicted. Under these optimal pretreatment conditions, the best results found by the model were 81.6%, 67.8% and 2.0 g/L for the HSR_L, recovery of glucose in enzymatic hydrolysates (referred to non-pretreated BSG) and total concentration of inhibitors in the liquid fraction, respectively. In order to confirm the optimization results, an experimental run was performed under the optimal conditions for pretreatment (CSF = 1.26) (Table 6). As was expected, a cellulose-enriched solid was obtained, as well as a liquid fraction with 33.5 g/L of monomeric sugars, which is equivalent to 81% hemicellulosic sugar recovery. Additionally, inhibitory compounds in the liquid fraction were about 2.4 g/L, mainly due to the presence of acetic acid, furfural and phenolic compounds. The pretreated solid fraction resulting from the pretreatment conducted under optimal conditions was enzymatically hydrolyzed, yielding a glucose recovery (referred to untreated BSG) of 64.7%. Thus, in general, a good agreement was found between the predicted and the observed values (HSR_L 81.6 vs 81%; EH glucose recovery 67.8 vs 64.7%; total inhibitor concentration, 2.0 vs 2.4 g/L). Under these optimal conditions, an overall sugar recovery of 85.3% was achieved (47.3 g of sugars from 100 g of BSG), considering sugars in the liquid fraction and the glucose and xylose obtained from the pretreated solid by enzymatic hydrolysis.

3.4. Use of the whole slurry in enzymatic hydrolysis

The use of the whole slurry obtained after the lignocellulosic biomass pretreatment, without solid-liquid separation, is very interesting as it leads to a unique hydrolysate which contains both cellulosic and hemicellulosic sugars, and these can be fermented

together. Thus, in order to analyze the effect of the use of the slurries on the effectiveness of the enzymatic hydrolysis stage, the whole slurry obtained after the pretreatment of BSG at two solid loadings (10 and 15% w/v) under optimal conditions (147 ºC, 2 min and 1.26% w/v H_2SO_4) was subjected to enzymatic saccharification. Higher sugar concentrations can be found in the hydrolysates resulting from higher solid concentrations in the pretreatment, which could increase the butanol concentration in the further fermentation step. This fact is profitable for the downstream stage, as it is necessary to obtain butanol with a purity higher than 99% for industrial uses [40].

Considering the sugars from the enzymatic hydrolysis and the prehydrolysate, the total sugar concentrations in the whole slurries were 47.6 and 73.9 g/L at 10 and 15% (w/v) of solid load in pretreatment, respectively, under optimal conditions (Table 7).

3.5. ABE fermentation of the slurry enzymatic hydrolysate

The hydrolysates corresponding to the enzymatic hydrolysis of the whole slurries obtained at 10% and 15% solid load in the pretreatment (slurry enzymatic hydrolysates, SEH_10% and SEH_15%, respectively), were fermented with *C. beijerinckii*. However, none of these hydrolysates produced butanol. This was probably due to the presence of phenolic compounds and furfural. According to Klinke et al. [41], a highly negative synergistic effect of furfural and phenols can take place.

Thereby, both SEH_10% and SEH_15% were detoxified with activated charcoal (SEH-ACD) or ion-exchange resins, SEH-RD (Lewatit S4528), to decrease the inhibitor compounds. Table 7 shows the carbohydrate and inhibitor concentrations measured in the different SEH before and after detoxification. Regarding the detoxification by activated charcoal, which is a process that is highly effective, economical and proficient at withdrawing inhibitor compounds [42], furfural and phenols were eliminated in high

percentages (Table 7) in both SEH_10%-ACD and SEH_15%-ACD (100 and 70-83%, respectively), as was also reported by other authors [33,43,44], It is worth mentioning that, although only a 2% activated charcoal load was necessary for the SEH_10%, a slightly higher charcoal load (5%) was employed in the case of SEH_15%, since this hydrolysate contained higher inhibitor concentrations.

Concerning the detoxification with ion-exchange resins (Table 7), this method also shows a high capacity for removing furfural and phenols (100 and 40-61%, respectively) in both SEH_10%-RD and SEH_15%-RD, its effect being negligible for the other inhibitor compounds, as was previously reported [45,46]. In the case of SEH_10%, a resin concentration of 2% was necessary, whereas the resin concentration has to be increased to 10% for SEH_15%.

Regarding ABE fermentation, Fig. 2 shows the initial and final monosaccharide concentrations, as well as the butanol and ABE concentrations obtained for SEH_10%-ACD and SEH_15%-ACD (Fig. 2a), and for SEH_10%-RD and SEH_15%-RD (Fig. 2b). In this way, as can be seen, in the case of SEH_10%, 8.2 and 8.0 g/L butanol were obtained when the hydrolysate was detoxified with activated charcoal (Fig. 2a) or ion-exchange resins (Fig. 2b), respectively, which resulted in high butanol yields (0.26 and 0.24 g/g sugars consumed, respectively) (Table 8). It is worth noting that only 2% (w/v) activated charcoal or resin-liquid was employed in this case. ABE concentrations achieved were also high from both SEH_10%-ACD and SEH_10%-RD (11.8 and 12.0 g/L ABE, respectively), which corresponds to ABE yields of 0.37 and 0.35 g/g sugars consumed, respectively, and ABE productivities of 0.285 and 0.291 g/L·h, respectively. Almost all sugars were used by *C. beijerinckii* in both SEH_10%-ACD and SEH_10%-RD (sugar uptake = 97-97.9%, Table 8).

Concerning SEH_15%, as can be seen in Fig. 2, butanol concentrations as high as 10.8 and 11.0 g/L were achieved for SEH_15%-ACD and SEH_15%-RD, respectively (16.0 and 16.9 g/L ABE, respectively). Therefore, it can be said that the use of more concentrated hydrolysates in sugars allowed fermentation broths to be obtained with higher butanol and ABE concentrations. On the other hand (Table 8), although fermentation with SEH_15% also resulted in high yields of butanol (0.21 and 0.22 g/g for SEH_15%-ACD and SEH_15%-RD, respectively) and ABE (0.32 and 0.33 g/g for SEH_15%-ACD and SEH_15%-RD, respectively), these yields were slightly lower than those obtained for SEH_10%. On the other hand, as can be observed in Figure 2, in SEH_15%, the sugars were not completely consumed, with 7.4 and 5.1 g/L of unconsumed total sugars remaining at the end of fermentation for SEH_15%-ACD and SEH_15%-RD, respectively. According to Gu et al. [47], the presence of unconsumed sugars at the end of the ABE fermentation is due to final product inhibition (butanol). A model medium with the same concentration of sugars present in SEH_15% (58 g/L), but without the presence of inhibitors was also fermented (data not shown), resulting in similar butanol and ABE concentrations (10 and 14.3 g/L, respectively), butanol and ABE yields (0.20 and 0.28 g/g sugars consumed, respectively) and 7.9 g/L unconsumed sugar remaining at the end of the fermentation. Therefore, recovery processes which allow butanol and ABE solvents to be recovered from the fermentation broth should be used, such as gas stripping separation, liquid-liquid extraction, adsorption or pervaporation techniques [48].

On the other hand, as Table 8 shows, butyric acid concentrations at the end of fermentation were low (< 0.3 g/L), which is adequate, since butyric acid is generated during the acidogenic phase and later consumed during the solventogenic phase to produce butanol [5].

Experimental results compare favorably with those reported by Nguyen et al. [49], who fermented with *C. acetobutylicum* the non-detoxified whole slurry of green macroalgae *Enteromorpha intestinalis*, pretreated by sulfuric acid pretreatment (121 °C, 60 min, 270 mM H_2SO_4), obtaining 8.5 g/L ABE. Nimbalkar et al. [50] reached butanol and ABE concentrations of only 4.43 and 6.69 g/L, respectively, after ABE fermentation with *C. acetobutylicum* of hydrolysates obtained after sulfuric acid pretreatment (121ºC, 15 min, 1.5% H_2SO_4) of sugarcane industry waste. Microwave assisted hydrothermal pretreatment (192.7 °C and 5.4 min) of BSG [23] led to 8.3 g/L butanol and a butanol yield of 0.26 g/g from the fermentation of the enzymatic hydrolysate of the pretreated solid. Therefore, the process based on microwave assisted dilute sulfuric acid pretreatment developed in this work allows a lower temperature and process time to be used (147 ºC and 2 min, respectively) in the presence of dilute sulfuric acid (1.26% H_2SO_4) for the production of biobutanol from pentoses and hexoses in a single bioreactor.

3.6. Overall process material balance

The material balance of the overall process for ABE production from BSG with *C. beijerinckii*, regarding the process configuration developed in this work, is shown in Fig. 3. BSG was submitted to an acid pretreatment under optimal conditions (147 ºC, 2 min, 1.26% H_2SO_4) at 10 and 15% w/v solid load, resulting in a slurry (with 4.8 and 7.9% of pretreated solid concentration), which was enzymatically hydrolyzed and fermented with *C. beijerinckii* after detoxification by activated charcoal. In this way, regarding the pretreatment at a solid load of 10% (w/v), a total production of 113 g butanol/kg BSG (dry matter) and 162 g ABE/kg BSG (dry matter) can be obtained (Fig. 3a). Nevertheless, 91 g butanol/kg BSG and 138 g ABE/kg BSG were achieved when

the pretreatment solid load used in the pretreatment was increased to 15% (w/v) (Fig. 3b). Although the use of a higher solid load in the pretreatment resulted in lower global butanol and ABE yields, the final concentration of the ABE solution was 36% higher, which allows the downstream process to be more feasible economically. The consumption of water and sulfuric acid was also lower.

Plaza et al. [26] reported a lower butanol and ABE production (75 g butanol/kg BSG and 95 g ABE/kg BSG, respectively) after dilute sulfuric acid pretreatment and fermentation with *C. beijerinckii*. Fernández-Delgado et al. [34] achieved a much lower butanol and ABE production after pretreating BSG with NaOH (44 g butanol/kg BSG and 54 g ABE/kg BSG) or H_2O_2 (45 g butanol/kg BSG and 56 g ABE/kg BSG). Therefore, it is worth mentioning that the microwave assisted dilute sulfuric acid pretreatment process carried out in this work allowed the combined valorization of cellulosic and hemicellulosic sugars, through their biotransformation to butanol.

4. Conclusions

This work shows that microwave pretreatment assisted by dilute sulfuric acid is an interesting choice to recover all sugars contained in BSG, the optimal conditions being 147 ºC, 2 min and 1.26% H_2SO_4 at 10% biomass loading. In addition, the complete valorization of cellulosic and hemicellulosic sugars contained in BSG is possible by fermentation to biobutanol of the highly concentrated slurry enzymatic hydrolysates, using a single fermenter. Thus, when a biomass load of 15% (w/v) was used in the pretreatment, this process configuration allowed a butanol concentration as high as 11 g/L to be reached, yielding 91 kg butanol/t BSG and 138 kg ABE/t BSG. Future work will focus on the optimization of fermentation system, such as gas stripping separation

technique, which allow butanol and ABE solvents to be recovered from the fermentation broth.

Conflict of interest

Declarations of interest: none

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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TABLES

Table 1. Microwave pretreatment assisted by dilute sulfuric acid pretreatment of BSG. Experimental design (coded and real factors) and Combined Severity Factor (CSF).

Run	Temperature (ºC)		Time (min)		H_2SO_4 conc. (%)		CSF
	Coded	Real	Coded	Real	Coded	Real	
1	0	145	0	6	-1.41	0.16	0.74
2	+1	170	+1	10	-1	0.50	2.19
3	0	145	+1.41	12.73	0	1	1.93
4	0	145	-1.41	0	0	1	-0.70
5	0	145	0	6	0	1	1.60
6	0	145	0	6	+1.41	1.84	1.84
7	-1	120	+1	10	-1	0.50	0.72
8	+1	170	-1	2	-1	0.50	1.49
9	-1.41	102.96	0	6	0	1	0.37
10	0	145	0	6	0	1	1.60
11	-1	120	+1	10	+1	1.50	1.24
12	0	145	0	6	0	1	1.60
13	-1	120	-1	2	+1	1.50	0.54
14	+1	170	+1	10	+1	1.50	2.71
15	-1	120	-1	2	-1	0.50	0.02
16	0	145	0	6	0	1	1.60
17	0	145	0	6	0	1	1.60
18	+1	170	-1	2	+1	1.50	2.01
19	+1.41	187.04	0	6	0	1	2.84
20	0	145	0	6	0	1	1.60

Table 2. Microwave pretreatment of BSG assisted by dilute sulfuric acid. Recovery of total solids (%), and composition of the solid fraction after pretreatment. Recovery (%) of glucose (GR_S) and hemicellulosic sugars (HSR_S) in the solid fraction.

Run	CSF	Solid Recovery (%)	Cellulose (g/100 g pretreated BSG)	Hemicellulose (g/100 g pretreated BSG)	Lignin (g/100 g pretreated BSG)	GR_S (%)	HSR_S (%)
1	0.74	59.29	21.61 ± 0.67	14.59 ± 0.37	36.32 ± 0.18	60.94	30.18
2	2.19	42.74	33.11 ± 0.26	1.28 ± 0.47	46.09 ± 0.33	67.29	1.91
3	1.93	40.74	33.73 ± 0.81	3.27 ± 0.02	44.70 ± 0.13	65.36	4.64
4	-0.70	47.08	30.24 ± 0.56	5.72 ± 0.09	37.58 ± 0.49	67.70	9.40
5	1.60	45.76	32.35 ± 0.76	4.50 ± 0.17	41.58 ± 0.16	70.40	7.19
6	1.84	42.87	30.03 ± 1.87	1.99 ± 0.20	43.70 ± 0.31	61.22	2.97
7	0.72	68.80	21.57 ± 0.47	14.63 ± 0.24	30.69 ± 0.64	70.57	35.13
8	1.49	46.40	26.83 ± 0.80	3.12 ± 0.08	43.23 ± 0.51	59.19	5.05
9	0.37	86.78	18.44 ± 0.01	18.84 ± 0.13	27.35 ± 1.15	76.11	57.03
10	1.60	45.27	32.92 ± 0.17	3.97 ± 0.08	39.82 ± 0.16	70.86	6.27
11	1.24	54.83	25.73 ± 0.93	6.57 ± 0.38	36.93 ± 1.64	67.10	12.57
12	1.60	46.69	25.82 ± 0.26	3.30 ± 0.03	43.52 ± 1.56	57.33	5.37
13	0.54	75.93	19.00 ± 0.57	15.29 ± 0.52	31.82 ± 0.07	68.60	40.51
14	2.71	36.84	32.04 ± 0.46	n.d.	54.64 ± 1.59	56.13	0
15	0.02	76.81	19.47 ± 0.89	17.78 ± 0.86	30.67 ± 0.29	71.11	47.65
16	1.60	45.80	28.71 ± 0.97	2.93 ± 0.12	44.09 ± 0.60	62.53	4.68
17	1.60	45.60	27.72 ± 0.34	2.05 ± 0.22	44.66 ± 0.30	60.11	3.27
18	2.01	37.64	32.67 ± 1.76	n.d.	52.65 ± 1.25	58.48	0
19	2.84	38.68	29.54 ± 0.43	n.d.	57.32 ± 0.19	54.34	0
20	1.60	45.74	28.00 ± 1.44	2.17 ± 0.04	44.88 ± 0.74	60.90	3.47

GR_S (glucose recovery in solid fractions): g glucose in solid fraction/100 g glucose in BSG

HSR_S (hemicellulosic sugar recovery in solid fractions): g hemicellulosic sugars in solid fraction/100 g hemicellulosic sugars in BSG

n.d.: not detected

Table 3. Microwave pretreatment of BSG assisted by dilute sulfuric acid. Composition of liquid fractions: carbohydrates (g/L), oligomeric sugar (%) and pH. Recovery (%) of glucose (GR_L) and hemicellulosic sugars (HSR_L) in the liquid fractions.

Run	CSF	pH	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Oligomeric sugars (%)	GR_L (%)	HSR_L (%)
1	0.74	2.30	5.57 ± 0.08	9.90 ± 0.04	7.33 ± 0.11	65.29	24.10	53.19
2	2.19	1.31	6.33 ± 0.09	16.41 ± 0.12	7.49 ± 0.01	1.26	27.35	73.79
3	1.93	0.93	4.77 ± 0.01	18.39 ± 0.04	8.33 ± 0.08	0.23	20.64	82.51
4	-0.70	0.84	4.78 ± 0.00	16.20 ± 0.15	8.52 ± 0.12	10.69	20.66	76.30
5	1.60	0.60	4.94 ± 0.03	19.20 ± 0.17	9.04 ± 0.00	1.34	21.34	86.92
6	1.84	0.34	7.12 ± 0.11	17.80 ± 0.09	8.24 ± 0.05	n.d.	30.78	80.38
7	0.72	0.88	4.89 ± 0.04	8.76 ± 0.05	7.12 ± 0.08	63.31	21.15	49.00
8	1.49	1.06	6.98 ± 0.08	16.93 ± 0.10	8.02 ± 0.06	3.86	30.19	77.01
9	0.37	0.52	1.53 ± 0.00	2.45 ± 0.00	3.27 ± 0.02	65.02	6.60	17.67
10	1.60	0.53	4.61 ± 0.02	18.22 ± 0.15	8.59 ± 0.01	2.41	19.92	82.79
11	1.24	0.35	5.49 ± 0.07	14.12 ± 0.12	7.87 ± 0.04	28.12	23.73	67.88
12	1.60	0.53	6.35 ± 0.12	16.11 ± 0.18	8.22 ± 0.08	3.15	27.46	75.11
13	0.54	0.28	3.35 ± 0.04	5.83 ± 0.10	5.68 ± 0.06	58.21	14.49	35.54
14	2.71	0.50	7.33 ± 0.08	13.52 ± 0.04	6.62 ± 0.07	n.d.	31.70	62.17
15	0.02	0.77	3.74 ± 0.06	5.02 ± 0.01	5.67 ± 0.04	65.77	16.15	33.00
16	1.60	0.58	6.77 ± 0.06	17.20 ± 0.17	8.11 ± 0.03	2.19	29.28	78.15
17	1.60	0.53	6.64 ± 0.03	18.29 ± 0.10	8.21 ± 0.09	1.48	28.71	81.80
18	2.01	0.52	7.33 ± 0.01	16.41 ± 0.12	7.45 ± 0.10	n.d.	31.70	73.68
19	2.84	0.87	6.50 ± 0.15	10.47 ± 0.09	5.41 ± 0.08	n.d.	28.10	49.02
20	1.60	0.65	6.67 ± 0.01	17.88 ± 0.17	8.19 ± 0.11	1.10	28.85	80.49

GR_L (glucose recovery in liquid fractions): g glucose in liquid fractions/100 g glucose in BSG

HSR_L (hemicellulosic sugar recovery in liquid fractions): g hemicellulosic sugars in liquid fractions/100 g hemicellulosic sugars in BSG

n.d.: not detected

Table 4. Microwave pretreatment of BSG assisted by dilute sulfuric acid. Composition of liquid fractions: potential inhibitor compounds (g/L).

Run	CSF	Acetic acid (g/L)	Formic acid (g/L)	Furfural (g/L)	HMF (g/L)	Total phenols (g GAE/L)
1	0.74	0.10 ± 0.00	0.01 ± 0.00	n.d.	n.d.	0.84 ± 0.07
2	2.19	0.85 ± 0.02	0.02 ± 0.00	0.49 ± 0.03	0.17 ± 0.01	1.71 ± 0.01
3	1.93	1.02 ± 0.11	0.06 ± 0.01	0.67 ± 0.01	0.05 ± 0.00	1.27 ± 0.09
4	-0.70	0.75 ± 0.08	0.02 ± 0.00	0.02 ± 0.00	n.d.	0.95 ± 0.02
5	1.60	0.98 ± 0.04	0.01 ± 0.00	0.23 ± 0.04	0.02 ± 0.00	1.19 ± 0.08
6	1.84	0.97 ± 0.10	0.02 ± 0.00	0.40 ± 0.01	0.05 ± 0.00	1.21 ± 0.02
7	0.72	0.19 ± 0.01	0.04 ± 0.00	n.d.	n.d.	0.49 ± 0.05
8	1.49	0.68 ± 0.04	0.02 ± 0.00	0.60 ± 0.06	0.08 ± 0.00	1.46 ± 0.04
9	0.37	0.11 ± 0.02	0.05 ± 0.01	n.d.	n.d.	0.15 ± 0.00
10	1.60	0.92 ± 0.03	0.02 ± 0.00	0.21 ± 0.01	0.01 ± 0.00	1.14 ± 0.08
11	1.24	0.66 ± 0.09	0.06 ± 0.00	n.d.	n.d.	0.82 ± 0.06
12	1.60	0.81 ± 0.12	0.05 ± 0.00	0.16 ± 0.00	0.02 ± 0.00	1.10 ± 0.11
13	0.54	0.25 ± 0.10	0.05 ± 0.01	n.d.	n.d.	0.37 ± 0.04
14	2.71	1.22 ± 0.04	0.05 ± 0.01	3.19 ± 0.15	0.35 ± 0.05	2.45 ± 0.05
15	0.02	0.12 ± 0.05	0.03 ± 0.00	n.d.	n.d.	0.33 ± 0.01
16	1.60	0.84 ± 0.08	0.02 ± 0.00	0.19 ± 0.01	0.02 ± 0.00	1.18 ± 0.04
17	1.60	0.91 ± 0.16	0.06 ± 0.00	0.23 ± 0.00	0.03 ± 0.00	1.20 ± 0.06
18	2.01	1.12 ± 0.15	0.17 ± 0.02	1.99 ± 0.12	0.24 ± 0.04	2.01 ± 0.04
19	2.84	1.24 ± 0.21	0.30 ± 0.03	3.84 ± 0.15	0.56 ± 0.08	2.43 ± 0.00
20	1.60	0.91 ± 0.07	0.02 ± 0.00	0.29 ± 0.01	0.04 ± 0.00	1.22 ± 0.09

n.d.: not detected

Total phenols (g GAE/L): expressed as g gallic acid equivalent/L.

Table 5. Microwave pretreatment of BSG assisted by dilute sulfuric acid. Enzymatic hydrolysis of the pretreated solids obtained in the pretreatment. Monosaccharides concentration (g/L) and glucose recoveries (EH glucose recovery, %) referred to pretreated or untreated BSG. Overall sugar recoveries (%) referred to untreated BSG.

		Carbohydrate concentration (g/L)		EH glucose recovery (%)		Overall sugar recovery (%)
Run	CSF	Glucose	Xylose	referred to pretreated BSG	referred to untreated BSG	
1	0.74	8.1 ± 0.2	3.0 ± 0.1	68.1	48.8	64.7
2	2.19	16.0 ± 0.5	1.2 ± 0.0	88.1	69.7	80.9
3	1.93	17.5 ± 0.3	1.8 ± 0.0	94.1	72.3	84.9
4	-0.70	14.0 ± 0.0	2.3 ± 0.0	84.3	67.1	80.8
5	1.60	15.0 ± 0.2	1.9 ± 0.0	84.2	69.7	87.4
6	1.84	14.2 ± 0.6	1.3 ± 0.1	85.8	61.7	83.6
7	0.72	7.1 ± 0.2	3.0 ± 0.1	59.6	49.4	62.3
8	1.49	14.1 ± 0.3	1.7 ± 0.1	95.7	66.6	83.9
9	0.37	3.4 ± 0.1	2.1 ± 0.1	34.0	30.4	30.2
10	1.60	15.8 ± 0.3	2.0 ± 0.1	87.3	72.7	85.6
11	1.24	10.3 ± 0.4	2.5 ± 0.2	72.5	57.2	74.7
12	1.60	12.7 ± 0.0	1.9 ± 0.2	89.5	60.3	79.8
13	0.54	4.9 ± 0.2	2.2 ± 0.0	46.8	37.7	46.3
14	2.71	16.7 ± 0.8	0.3 ± 0.0	94.5	62.3	71.9
15	0.02	4.6 ± 0.0	2.1 ± 0.0	43.3	36.2	44.6
16	1.60	14.2 ± 1.1	1.7 ± 0.1	89.6	65.9	83.9
17	1.60	14.1 ± 0.2	1.5 ± 0.0	92.1	65.1	85.3
18	2.01	18.1 ± 0.7	0.5 ± 0.0	100.0	69.2	81.4
19	2.84	15.6 ± 0.2	0.3 ± 0.0	96.1	61.3	62.4
20	1.60	14.6 ± 0.0	1.5 ± 0.0	94.6	67.7	85.5

EH glucose recovery, % (referred to pretreated BSG): g glucose by enzymatic hydrolysis/100 g glucose (contained as cellulose) in pretreated BSG.
EH glucose recovery, % (referred to untreated BSG): g glucose by enzymatic hydrolysis/100 g glucose (contained as cellulose) in untreated BSG.
Overall sugar recovery (%): sum of glucose and xylose grams in enzymatic hydrolyzates and pretreatment liquid /100 g total sugars in untreated BSG.

Table 6. Pretreatment of BSG by microwave assisted dilute sulfuric acid under optimal conditions (147 ºC, 2 min, 1.26% H_2SO_4) at 10% solid loading. Confirmatory experimental run: composition of solid and liquid fractions.

Component	Concentration
Solid fraction (%)	
Cellulose	24.54 ± 0.23
Xylan	1.35 ± 0.02
Arabinan	n.d.
Lignin	47.82 ± 1.25
Liquid fraction (g/L)	
Sugars	
Glucose	7.32 ± 0.15
Xylose	17.74 ± 0.22
Arabinose	8.50 ± 0.19
Inhibitors	
Furfural	0.27 ± 0.04
HMF	0.04 ± 0.00
Formic acid	0.02 ± 0.00
Acetic acid	0.90 ± 0.09
Total phenols	1.19 ± 0.14

Table 7. Composition of slurry enzymatic hydrolysates (SEH) before and after detoxification individually with activated charcoal or ion-exchange resins.

	Carbohydrates (g/L)			Inhibitors (g/L)				
	Glucose	Xylose	Arabinose	Acetic acid	Formic acid	Furfural	HMF	Total phenols
SEH_10%	20.7 ± 0.1	18.9 ± 0.1	7.9 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d.	1.0 ± 0.1
SEH_10%-ACD (2%)	19.4 ± 0.2	17.7 ± 0.0	7.5 ± 0.1	0.9 ± 0.0	0.1 ± 0.0	n.d.	n.d.	0.3 ± 0.0
SEH_10%-RD (2%)	19.0 ± 0.1	17.8 ± 0.1	7.5 ± 0.1	1.0 ± 0.0	0.1 ± 0.0	n.d.	n.d.	0.6 ± 0.0
SEH_15%	33.7 ± 0.3	28.4 ± 0.2	11.8 ± 0.0	1.6 ± 0.1	n.d.	0.7 ± 0.0	0.1 ± 0.0	1.8 ± 0.1
SEH_15%-ACD (5%)	33.4 ± 0.1	28.0 ± 0.0	11.6 ± 0.0	1.4 ± 0.1	n.d.	n.d.	n.d.	0.3 ± 0.0
SEH_15%-RD (10%)	30.7 ± 0.0	27.6 ± 0.2	11.2 ± 0.1	1.6 ± 0.0	n.d.	n.d.	n.d.	0.7 ± 0.0

SEH: slurry enzymatic hydrolysate
ACD: activated charcoal detoxification
RD: ion-exchange resin detoxification
n.d.: not detected

Table 8. ABE fermentation of the slurry enzymatic hydrolysates detoxified with activated charcoal or ion-exchange resins, resulting from the enzymatic hydrolysis of the whole slurry obtained under optimal pretreatment conditions. Initial monosaccharide concentration (g/L), acetic and butyric acid concentrations (g/L) at the end of fermentation, butanol and ABE yields ($Y_{BUT/sugars}$, $Y_{ABE/sugars}$ expressed as g/g sugars consumed), and butanol and ABE productivities (P_{BUT}, P_{ABE} expressed as g/L·h) at the time of maximum production of butanol and ABE.

	t (h)	Sugar uptake (%)	Acetic acid (g/L)	Butyric acid (g/L)	$Y_{BUT/sugars}$ (g/g)	$Y_{ABE/sugars}$ (g/g)	P_{BUT} (g/L·h)	P_{ABE} (g/L·h)
Activated charcoal detox								
SEH_10%-ACD (2%)	48	97.0 ± 0.4	0.4 ± 0.0	0.2 ± 0.0	0.26	0.37	0.198	0.285
SEH_15%-ACD (5%)	72	87.2 ± 0.3	0.4 ± 0.0	0.0 ± 0.0	0.21	0.32	0.175	0.259
Ion-exchange resins detox								
SEH_10%-RD (2%)	48	97.9 ± 0.6	0.5 ± 0.0	0.3 ± 0.0	0.24	0.35	0.194	0.291
SEH_15%-RD (10%)	72	90.9 ± 0.7	0.5 ± 0.0	0.1 ± 0.1	0.22	0.33	0.178	0.273

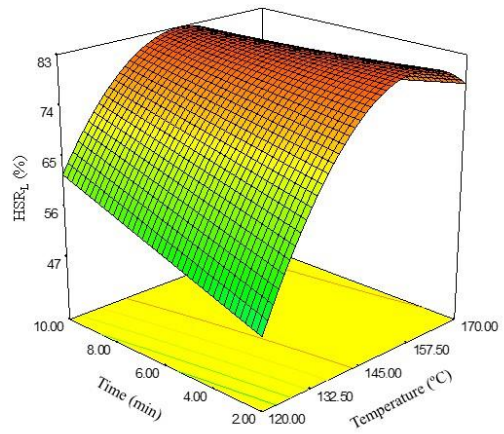
SEH: slurry enzymatic hydrolysate
ACD: activated charcoal detoxification
RD: ion-exchange resin detoxification

Figure captions

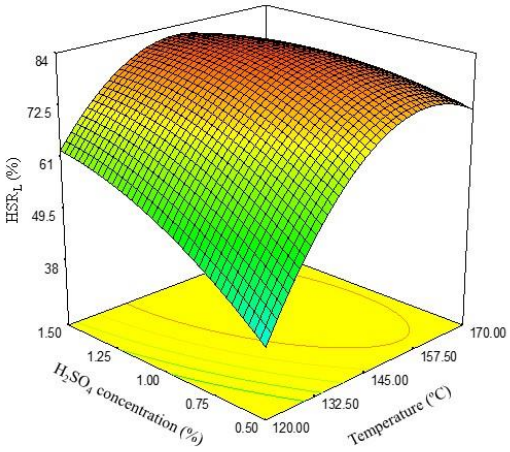
Fig. 1. Response surface plots representing the interactive effect of temperature and pretreatment time at 1% H_2SO_4 on the hemicellulosic sugar recovery (HSR_L) (a), EH glucose recovery (referred to untreated BSG) (c) and total inhibitor concentration in the liquid fraction (e). Response surface plots representing the interactive effect of temperature and sulfuric acid concentration for 6 min on the hemicellulosic sugar recovery (HSR_L) (b), EH glucose recovery (referred to untreated BSG) (d) and total inhibitor concentration in the liquid fraction (f).

Fig. 2. ABE fermentation of the slurry enzymatic hydrolysate (SEH) detoxified with activated charcoal (a) or ion-exchange resins (b).

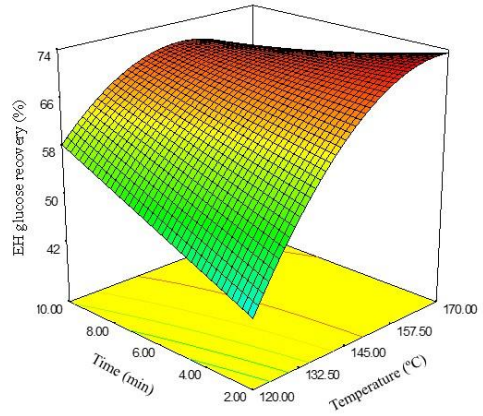
Fig. 3. Mass balance flow diagram of the overall ABE production process from slurry enzymatic hydrolysates (SEH) detoxified with activated charcoal, using a pretreatment solid load of 10% (w/v) (a) and 15% (w/v) (b).


HSR_L (%)
83
74
65
56
47
10.00
8.00
6.00
4.00
2.00
Time (min)
120.00
132.50
145.00
157.50
170.00
Temperature (°C)

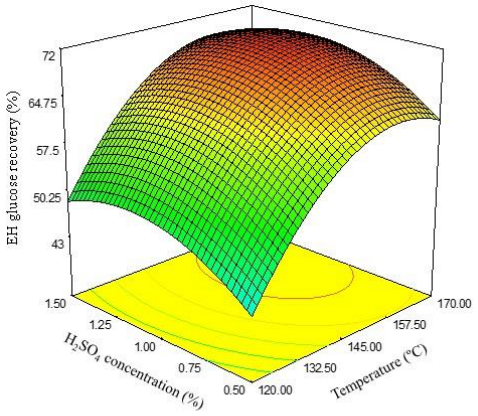
a)


HSR_L (%)
84
72.5
61
49.5
38
1.50
1.25
1.00
0.75
0.50
H2SO4 concentration (%)
120.00
132.50
145.00
157.50
170.00
Temperature (°C)

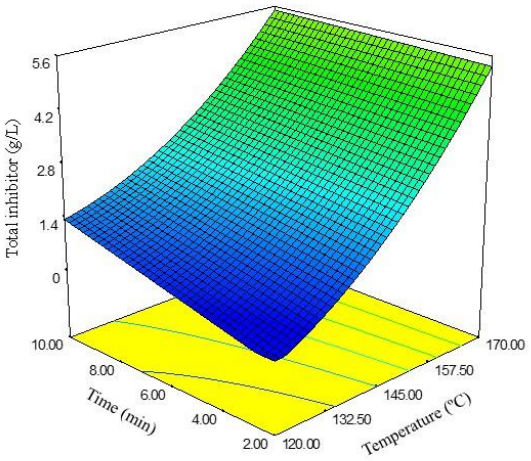
b)


EH glucose recovery (%)
74
66
58
50
42
10.00
8.00
6.00
4.00
2.00
Time (min)
120.00
132.50
145.00
157.50
170.00
Temperature (°C)

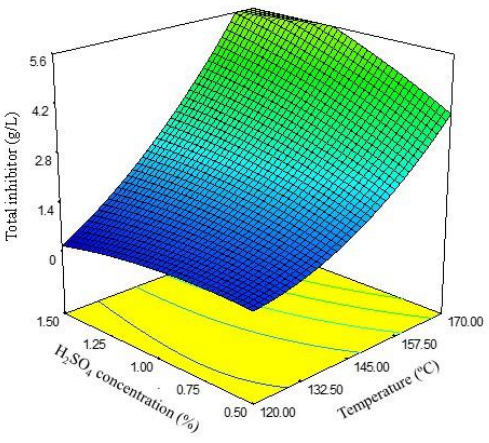
c)


EH glucose recovery (%)
72
64.75
57.5
50.25
43
1.50
1.25
1.00
0.75
0.50
H2SO4 concentration (%)
120.00
132.50
145.00
157.50
170.00
Temperature (°C)

d)

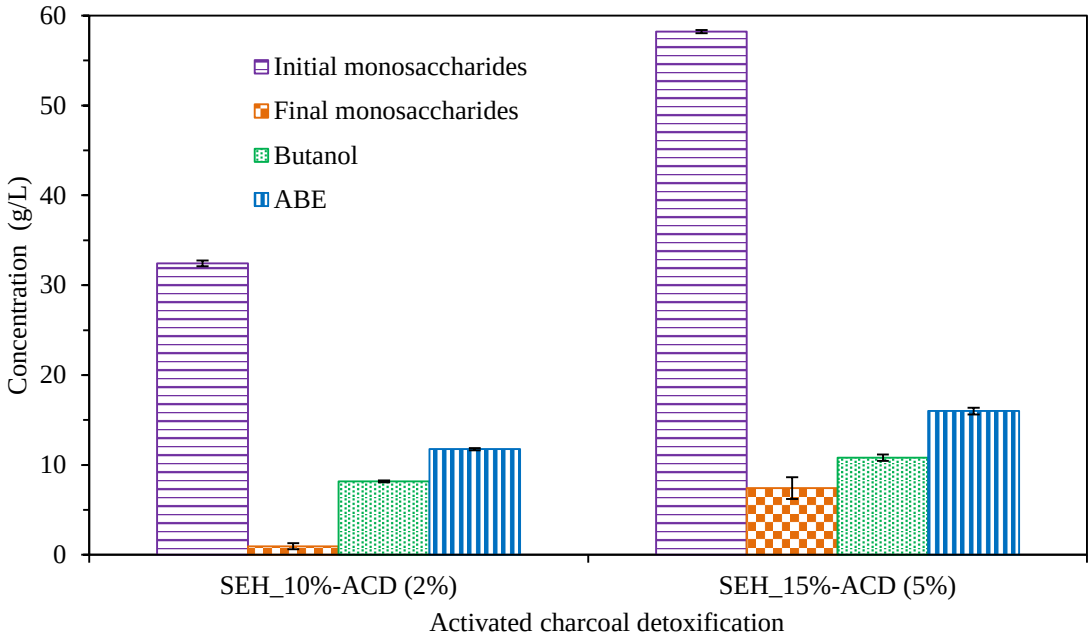

Total inhibitor (g/L)
5.6
4.2
2.8
1.4
0
10.00
8.00
6.00
4.00
2.00
Time (min)
120.00
132.50
145.00
157.50
170.00
Temperature (°C)

e)

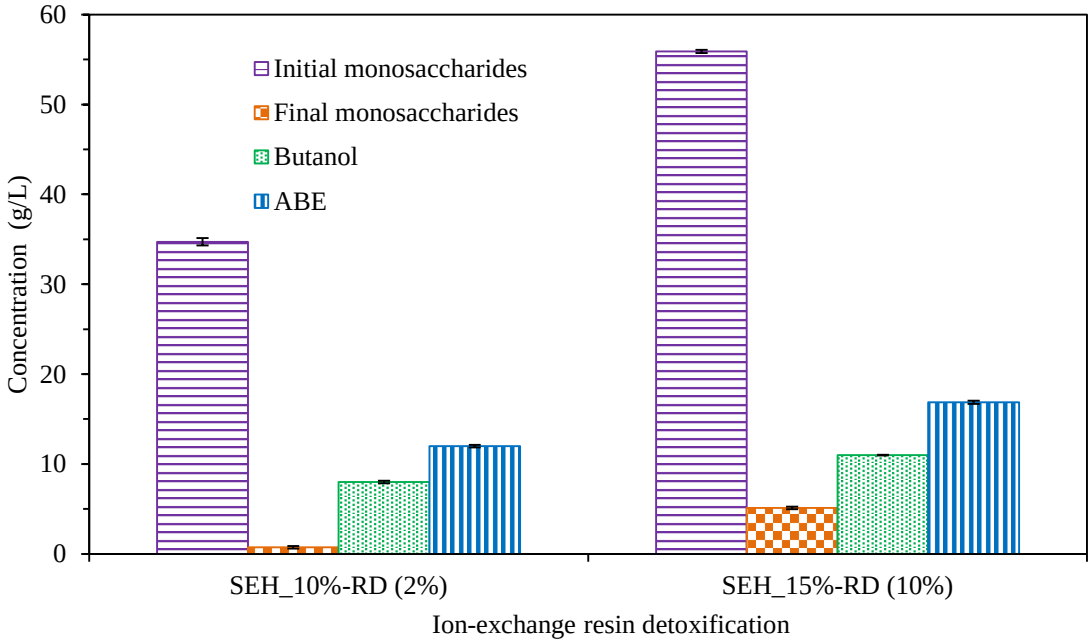

Total inhibitor (g/L)
5.6
4.2
2.8
1.4
0
1.50
1.25
1.00
0.75
0.50
H2SO4 concentration (%)
120.00
132.50
145.00
157.50
170.00
Temperature (°C)

f)

Fig. 1.



a)

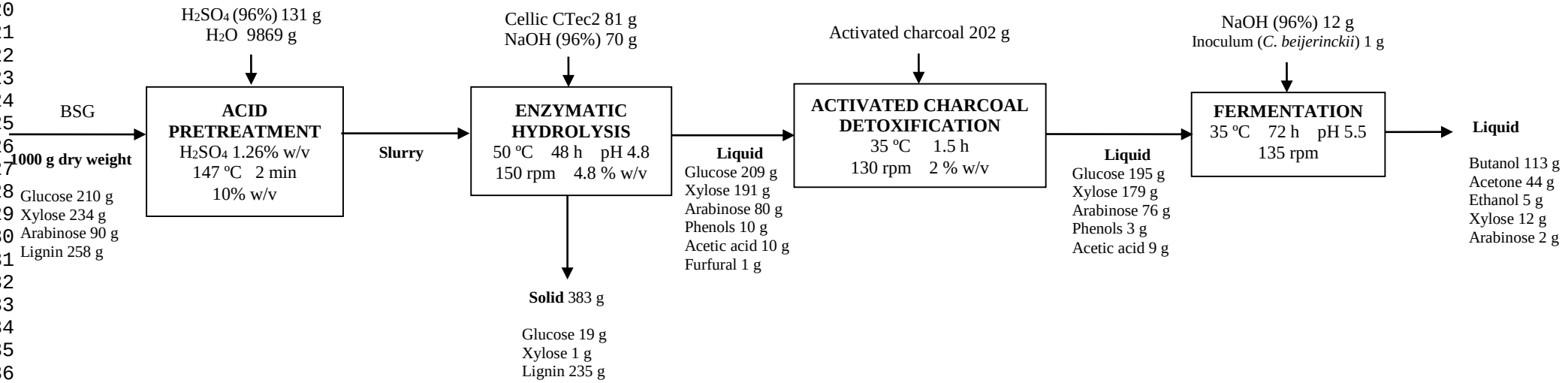


b)

SEH: slurry enzymatic hydrolysate
ACD: activated charcoal detoxification
RD: ion-exchange resin detoxification

Fig. 2.

a)


H2SO4 (96%) 131 g
H2O 9869 g
BSG
1000 g dry weight
Glucose 210 g
Xylose 234 g
Arabinose 90 g
Lignin 258 g
ACID PRETREATMENT
H2SO4 1.26% w/v
147 ºC 2 min
10% w/v
Slurry
Cellic CTec2 81 g
NaOH (96%) 70 g
ENZYMATIC HYDROLYSIS
50 ºC 48 h pH 4.8
150 rpm 4.8 % w/v
Solid 383 g
Glucose 19 g
Xylose 1 g
Lignin 235 g
Liquid
Glucose 209 g
Xylose 191 g
Arabinose 80 g
Phenols 10 g
Acetic acid 10 g
Furfural 1 g
Activated charcoal 202 g
ACTIVATED CHARCOAL DETOXIFICATION
35 ºC 1.5 h
130 rpm 2 % w/v
Liquid
Glucose 195 g
Xylose 179 g
Arabinose 76 g
Phenols 3 g
Acetic acid 9 g
NaOH (96%) 12 g
Inoculum (C. beijerinckii) 1 g
FERMENTATION
35 ºC 72 h pH 5.5
135 rpm
Liquid
Butanol 113 g
Acetone 44 g
Ethanol 5 g
Xylose 12 g
Arabinose 2 g

b)

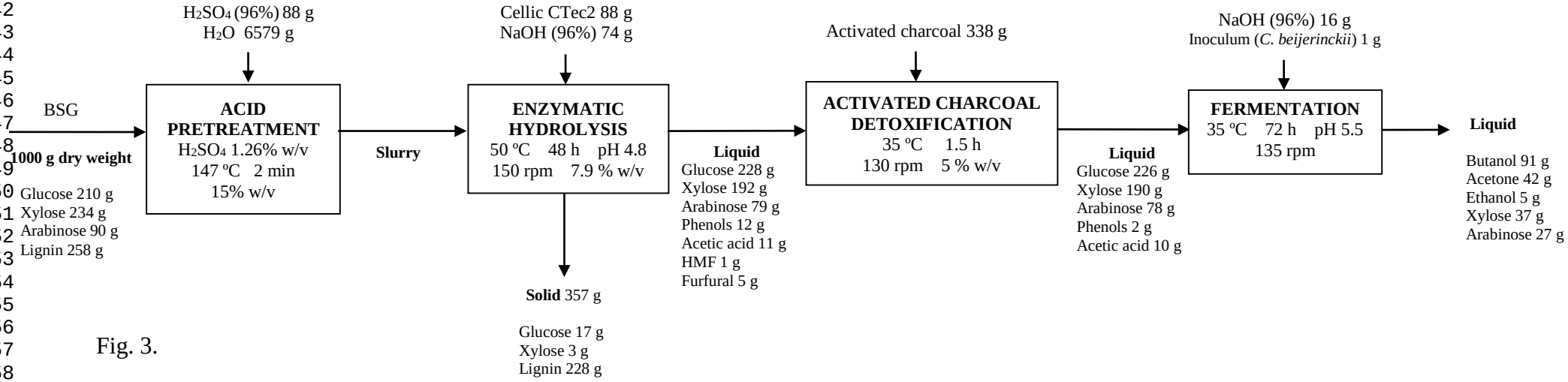

H2SO4 (96%) 88 g
H2O 6579 g
BSG
1000 g dry weight
Glucose 210 g
Xylose 234 g
Arabinose 90 g
Lignin 258 g
ACID PRETREATMENT
H2SO4 1.26% w/v
147 ºC 2 min
15% w/v
Slurry
Cellic CTec2 88 g
NaOH (96%) 74 g
ENZYMATIC HYDROLYSIS
50 ºC 48 h pH 4.8
150 rpm 7.9 % w/v
Solid 357 g
Glucose 17 g
Xylose 3 g
Lignin 228 g
Liquid
Glucose 228 g
Xylose 192 g
Arabinose 79 g
Phenols 12 g
Acetic acid 11 g
HMF 1 g
Furfural 5 g
Activated charcoal 338 g
ACTIVATED CHARCOAL DETOXIFICATION
35 ºC 1.5 h
130 rpm 5 % w/v
Liquid
Glucose 226 g
Xylose 190 g
Arabinose 78 g
Phenols 2 g
Acetic acid 10 g
NaOH (96%) 16 g
Inoculum (C. beijerinckii) 1 g
FERMENTATION
35 ºC 72 h pH 5.5
135 rpm
Liquid
Butanol 91 g
Acetone 42 g
Ethanol 5 g
Xylose 37 g
Arabinose 27 g

Fig. 3.

SUPPLEMENTARY MATERIAL

Table 1S. Results obtained from the analysis of variance for the responses a) HSR_L, b) EH glucose recovery (referred to untreated BSG) and c) total inhibitor concentration in the liquid fraction.

a)

Source	Sum of squares	Degrees of freedom	Mean square	F-value	p-value (Prob > F)	Remarks
Model	6174.52	7	882.07	46.64	< 0.0001	Significant
Temperature (T)	1130.27	1	1130.27	59.77	< 0.0001	
Time (t)	35.72	1	35.72	1.89	0.1967	
H_2SO_4 conc. (C)	332.46	1	332.46	17.58	0.0015	
Tt	180.29	1	180.29	9.53	0.0103	
TC	316.35	1	316.35	16.73	0.0018	
T^2	3747.75	1	3747.75	198.17	< 0.0001	
C^2	272.48	1	272.48	14.41	0.0030	
Residual	208.03	11	18.91	-	-	
Lack of Fit	126.15	6	21.03	1.28	0.4009	Not significant
Pure Error	81.87	5	16.37	-	-	
Cor Total	6382.54	18	-	-	-	
R-squared	0.9674	-	Adj R-squared[a]	0.9467	-	
Mean	67.41	-	Pred R-squared[b]	0.8612	-	
C.V. %[c]	6.45	-	Adeq Precision[d]	23.095	-	

b)

Source	Sum of squares	Degrees of freedom	Mean square	F-value	p-value (Prob > F)	Remarks
Model	2704.70	6	450.78	66.09	< 0.0001	Significant
Temperature (T)	1041.81	1	1041.81	152.75	< 0.0001	
Time (t)	43.42	1	43.42	6.37	0.0302	
H_2SO_4 conc. (C)	41.64	1	41.64	6.11	0.0331	
Tt	228.71	1	228.71	33.53	0.0002	
T^2	939.33	1	939.33	137.72	< 0.0001	
C^2	151.02	1	151.02	22.14	0.0008	
Residual	68.20	10	6.82	-	-	
Lack of Fit	30.63	6	5.11	0.54	0.7597	Not significant
Pure Error	37.57	4	9.39	-	-	
Cor Total	2772.90	16	-	-	-	
R-squared	0.9754	-	Adj R-squared[a]	0.9606	-	
Mean	59.55	-	Pred R-squared[b]	0.9293	-	
C.V. %[c]	4.39	-	Adeq Precision[d]	25.420	-	

c)

Source	Sum of squares	Degrees of freedom	Mean square	F-value	p-value (Prob > F)	Remarks
Model	81.88	8	10.24	554.29	< 0.0001	Significant
Temperature (T)	45.89	1	45.89	2485.06	< 0.0001	
Time (t)	1.80	1	1.80	97.59	< 0.0001	
H_2SO_4 conc. (C)	2.03	1	2.03	110.13	< 0.0001	
Tt	0.40	1	0.40	21.92	0.0011	
TC	2.85	1	2.85	154.19	< 0.0001	
tC	1.81	1	1.81	98.02	< 0.0001	
T^2	6.56	1	6.56	355.03	< 0.0001	
C^2	0.65	1	0.65	35.32	0.0002	
Residual	0.17	9	0.018	-	-	
Lack of Fit	0.08	4	0.020	1.18	0.4201	Not significant
Pure Error	0.09	5	0.017	-	-	
Cor Total	82.05	17	-	-	-	
R-squared	0.9980	-	Adj R-squared[a]	0.9962	-	
Mean	2.77	-	Pred R-squared[b]	0.9835	-	
C.V. %[c]	4.90	-	Adeq Precision[d]	83.838	-	

A: Temperature (ºC).
B: Time (min).
C: Sulfuric acid concentration (%).
[a] Adjusted R^2.
[b] Predicted R^2.
[c] Coefficient of variation.
[d] Adequate precision.