Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-00918-4

ORIGINAL ARTICLE

Brewer's spent grain biotransformation to produce lignocellulolytic enzymes and polyhydroxyalkanoates in a two-stage valorization scheme

Jordi Llimós[1] · Oscar Martínez-Avila[1] · Elisabet Marti[1] · Carlos Corchado-Lopo[1] · Laia Llenas[1] · Teresa Gea[2] · Sergio Ponsá[1]

Received: 11 May 2020 / Revised: 17 July 2020 / Accepted: 23 July 2020

Abstract

Lignocellulolytic enzymes from low-cost sources are gaining attention as a tool to reduce production costs. Such enzymes can be obtained sustainably by diverse fungal strains via solid-state fermentation (SSF) of lignocellulosic-derived residues as substrates. Besides, these enzymes allow hydrolyzing the same residue, releasing fermentable sugars that can be transformed into value-added products. This study shows a two-stage valorization approach for the lignocellulosic leftover brewer's spent grain (BSG): first, by producing lignocellulolytic enzymes through the SSF of BSG using three fungal strains and, second, by using the self-produced enzymes to hydrolyze the same BSG and obtaining sugar-rich hydrolysates that serve as an alternative carbon source for polyhydroxyalkanoates (PHA) production. From the evaluated set, *Aspergillus niger* and *Thermoascus aurantiacus* produced the highest xylanase activities compared with *Trichoderma reesei* (268 ± 24, 241 ± 10, and 150 ± 24 U per gram of dry BSG, respectively). Also, *A. niger* extracts resulted in the most effective for releasing sugars from BSG, obtaining up to 0.56 g per gram of dry BSG after 24 h without any pretreatment needed. Thus, the sugar-rich hydrolysate obtained with *A. niger* was used as a source for producing PHA by using two bacterial strains, namely, *Burkholderia cepacia* and *Cupriavidus necator*. Maximum PHA yield was achieved by using *C. necator* after 48 h with 9.0 ± 0.44 mg PHA·g^{-1} dry BSG. These results show the significant potential of BSG as raw material for obtaining value-added bioproducts and the importance of multiple valorization schemes to improve the feasibility of similar residue-based systems.

Keywords Solid-state fermentation · Enzymatic hydrolysis · Bioplastics · Agro-industrial residues · Waste to product

1 Introduction

Commercial lignocellulolytic enzymes are mainly produced through submerged fermentation (SmF) systems. Typically, such enzymatic solutions are obtained starting from high-purity substrates implying a significant increase in the total processing costs [1]. In this context, the need for more economical and sustainable substrates, along with the development of more efficient processes for producing these enzymes, has gained attention recently. One of the most attractive bio-based materials with potential for being a source of enzymes is the lignocellulosic materials [2]. Lignocellulosic-derived wastes are abundant carbon sources produced in many industrial processes, reaching up to 10^{11} tons of biomass leftovers per year globally [2]. Although most of the lignocellulosic-derived wastes are either used as an energy source (by burning) or directly disposed into the environment, their characteristics make them potential raw materials for the development of different biotechnological applications [3]. In general, these wastes are attractive due to their renewable nature, abundance, and low cost, constituting one of the most studied materials in the biorefinery sector for producing value-added products [3, 4].

✉ Oscar Martínez-Avila
Oscarmauricio.martinez@uvic.cat

[1] BETA Tech Center. (TECNIO Network), University of Vic-Central University of Catalonia, Carrer de la Laura 13, 08500 Vic, Spain

[2] Composting Research Group, Department of Chemical, Biological and Environmental Engineering, School of Engineering, Carrer de Les Sitges Edifici Q, Universitat Autònoma de Barcelona 08193 Bellaterra, Barcelona, Spain

Published online: 08 August 2020

However, the biodegradability of lignocellulosic biomass depends on the particular composition of its organic components: cellulose (9%–80%), hemicellulose (10%–50%), and lignin (5%–35%) [5, 6]. Hence, to efficiently process lignocellulosic materials, a hydrolysis step is usually needed. Hydrolysis allows transforming cellulose and hemicellulose into fermentable sugars that can be used to produce value-added products [3, 5]. Although this process can be carried out by using chemical hydrolysis or other physicochemical treatments, the current trend is to use lignocellulolytic enzymes such as cellulases and xylanases [7]. In the hydrolysis process, enzymes act as catalysts accelerating various reaction steps. However, differently than their chemical counterparts, enzymatic processes tend to work under mild conditions making them more attractive from diverse points of view [6, 8].

Based on this context, different authors have proposed the solid-state fermentation (SSF) as a potential alternative technology to SmF for producing lignocellulolytic enzymes more sustainably and economically [9, 10]. Compared with SmF, SSF usually achieves higher productivities and yields, it requires lower water and energy consumption, and it limits the production of waste streams [11, 12]. For instance, Castilho et al. [13] have analyzed both, SSF and SmF, on the annual production of 100 m^3 of *Penicillium restrictum* lipase concentrate economically. The study concluded that SmF requires a total capital investment about 78% higher than the SSF process, and the unitary product obtained from the SSF process was more economical than the actual selling price (47% lower). Such an outcome suggests that the economic differences between the two approaches are mainly influenced by the raw material cost. Nevertheless, one of the most interesting traits of SSF resides in the ability to process a wide range of solid organic residues as raw materials to obtain an extensive set of marketable bioproducts [12]. Thus, apart from industrial enzymes, SSF has been successfully used to produce other value-added products such as biosurfactants [14], base chemicals [15], or biopesticides [16], starting from solid organic wastes, showing that SSF is a versatile and fruitful technology.

On the other hand, the brewery industry is one of the most productive industries of beverages in the world [17], and it generates large quantities of brewer's spent grain (BSG), a solid leftover obtained after the fermentation process which is primarily used as animal feed [18]. This lignocellulosic-derived waste has significant amounts of proteins and fibers that could be used as a source for producing value-added products [19].

Although SSF has been previously used to obtain different lignocellulolytic enzymes using lignocellulosic materials [9, 20], just a few reports show BSG as raw material to obtain fermentable sugars [21–23] from those enzymes. Furthermore, many of the reports focus on the enzyme production, but not on the application of such enzymes to obtain other value- added products. In this sense, it is evident that the enzymes are not final products per se, but intermediates required to produce other marketable products [24]. Thus, determining both the efficiency of the produced enzymes and their application on the production of final value-added products becomes of prime importance [24].

One of the fields requiring an efficient process of lignocellulosic biomass is the biosynthesis of biodegradable bioplastics [25]. Among them, the polyhydroxyalkanoates (PHA) production from lignocellulosic-derived materials has gained remarked attention as an alternative to producing these bioplastics economically and sustainably [25]. PHA resemble several properties of the traditional petroleum-based plastics while being non-toxic, biocompatible, and biodegradable. Such characteristics make these compounds highly-valued as potential substitutes of petroleum-based plastics [25]. Typically, PHA are produced via SmF [26, 27]. However, one of the main constraints of these processes is the high cost of the substrates, representing between 40 and 60% of the total fermentation cost [28]. Hence, Brigham et al. [29] have proposed coupling the PHA production to the food industry, building the PHA production plants next to large landfills of food waste or agro-industrial facilities. This way, transportation costs could be reduced, process efficiency is improved, and the valorization of the produced leftovers in such industries is promoted. Having this in mind, the lignocellulolytic enzymes produced from the SSF of BSG could serve to hydrolyze the same leftover sustainably as previously done with other lignocellulosic wastes [30]. Then, the released fermentable sugars could be used as a source for PHA production by specific microorganisms, consequently obtaining value-added products from a low-cost substrate, achieving a two-stage valorization of the residue, and presenting an advance on the development of this bio-based industry.

This study aimed to assess three different fungal strains, namely, *Aspergillus niger*, *Trichoderma reesei,* and *Thermoascus aurantiacus*, for producing lignocellulolytic enzymes (mainly cellulases and xylanases) from the leftover BSG via SSF in a batch-SSF system. Besides, after an initial assessment for determining the maximum enzyme activity from the evaluated group, the produced enzymes have been tested to perform the enzymatic hydrolysis of BSG. Finally, the sugar-rich hydrolysates which resulted from the hydrolysis step were evaluated as a source for the PHA production via SmF using the PHA producers *Burkholderia cepacia* and *Cupriavidus necator*. Thus, proposing a two-stage valorization scheme for BSG by producing enzymes and valuable bioplastics.

2 Material and methods

2.1 Strains and inoculum preparation

Aspergillus niger (ATCC 16888) and *Trichoderma reesei* (ATCC 26921) were obtained from "Colección Española de Cultivos Tipo" (CECT, Spain). *Thermoascus aurantiacus (*ATCC 26904) from American Type Culture Collection (ATCC, Virginia). *B. cepacia* (CCM 2656) was purchased from the Czech Collection of Microorganisms, Brno, Czech Republic, and *Cupriavidus necator* (DSM428) from the *German Collection of Microorganisms and Cell Cultures (*DSMZ*, Braunschweig, Germany).* Strains were maintained at − 80 °C in cryovials (Brand, Germany). Inoculum preparation for the fungal strains consisted of adding one pearl into a 100-mL Erlenmeyer flask containing a glucose-starch media (GS) (peptone 5 $g{\cdot}L^{-1}$, starch 20 $g{\cdot}L^{-1}$, and glucose 20 $g{\cdot}L^{-1}$). Flasks were placed in an orbital shaker at 120 rpm for 96 h, and the temperature was set at 30 °C for *A. niger* and *T. reesei* and at 45 °C *T. aurantiacus*.

For *B. cepacia* and *C. necator,* the same procedure was performed but using LB (Lysogeny Broth) medium. Flasks were placed in an orbital shaker at 120 rpm, 30 °C, keeping an aerobic environment for 24 h. All reagents and materials had been previously sterilized at 121 °C for 15 min.

2.2 Substrate

Brewer's spent grain was provided by Companyia Cervesera del Montseny (Catalunya, Spain). It was dried at 60 °C overnight, and then it was stored at room temperature until further use. Preparation of the BSG for solid-state fermentation consisted of adding 2 × 2 cm sponge pieces as a bulking agent (3% *w*/w) as suggested by [31]. The sponge was selected as an alternative to lignocellulosic bulking agents, due to its ability to increase the porosity of the mixture without providing a biodegradable carbon source. Then, distilled water was added to the mixture to reach 78 ± 2% of moisture content (MC) (similar level to the original moisture content of the received BSG). The prepared BSG was sterilized at 121 °C for 15 min, and after cooling, it was inoculated with 10% (*v*/*w*) of each of the evaluated fungi strains.

2.3 SSF experiments for enzyme production

SSF experiments were carried out (triplicates) in 0.5-L glass Erlenmeyer where 100 g of the prepared substrate (Section 2.2) was added. The flasks were placed in a temperature-controlled water bath, and each reactor was connected to an air mass flowmeter that provided a controlled flow such that the air entered at the bottom of the solid bed as detailed elsewhere [32]. The system was sealed such that the exhausted gases were conducted to an oxygen sensor, connected to an online acquisition system (Arduino-based). With this data, a respirometric monitoring was made as suggested by Ponsá et al. [32] by computing the specific oxygen uptake rate (sOUR). Experiments were followed up to 216 h. The temperature for the experiments was set at 37 °C for *A. niger* and *T. reesei* as recommended by Díaz-Godínez et al. [10] and at 45 °C for *T. aurantiacus* as proposed by dos Santos et al. [33]. Besides, the specific airflow rate (sAFR) was set at 0.05 L h^{-1} g^{-1} of dry matter of the BSG used in the experiments (DM), to provide enough oxygen to the solid substrate, and therefore, avoiding oxygen limitations in the system [34]. Experiments with the fungal strains were conducted in different runs (not simultaneously) due to capacity limitations of the respirometric system.

2.4 Enzyme extraction from SSF

For each strain, enzymes were extracted from the fermented BSG in 24-h intervals. Extraction consisted of putting 10 g of solid sample in a 250-mL flask and mixing with citrate buffer (pH 4.8) in a 1:15 ratio. The flasks were shaken for 30 min at 25 °C. Then, the mixture was centrifuged at 5000 rpm for 10 min at 4 °C, and the supernatant was filtered using a 0.45-μm membrane filter. The obtained filtrate was used as a crude enzymatic extract for the hydrolysis experiments and to determine the enzyme activity.

2.5 BSG hydrolysis with enzymatic extracts

These experiments consisted of mixing 2 g of dried and sterile BSG with each of the following solutions: the crude enzymatic extract obtained after the SSF process (Section 2.4), a commercial enzymatic cocktail Viscozyme L (Novozyme Inc., Copenhagen, Denmark) (1% dose), or a citrate buffer 0.05 M as a blank control. In all cases, a 5% (*w*/*v*) BSG:extract ratio was used (40 mL of citrate buffer in the case of the blank control). Hydrolysis was followed by measuring the reducing sugar content in the media up to 48 h.

2.6 PHA production in liquid culture

Sugar-rich hydrolysates obtained from the hydrolysis of BSG (Section 2.5) were used to produce PHA via SmF. First, 20 mL of the hydrolysates was placed into 100-mL Erlenmeyer flasks, and they were complemented by adding two mineral media as described by Kucera et al. [35]. Both substrates and materials have been sterilized at 121 °C for 15 min. After cooling, the liquid substrate was inoculated by using 5% (*v*/v) of *B. cepacia* or *C. necator*. Fermentation was conducted at 30 °C and 120 rpm in an orbital shaker assuring an aerobic environment. The system was monitored up to 72 h as suggested by Kucera et al. [35], and the cells were

harvested after 48 h for *C. necator* and after 72 h for *B. cepacia* for PHA analysis.

2.7 Analytical methods

2.7.1 BSG characterization

BSG was characterized carrying out (triplicate) the following analyses: total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), ammonium (NH_4^+), and oxidizable carbon (OXC) according to standard procedures [36]. Also, cellulose, hemicellulose, and lignin content were determined by the enzymatic-gravimetric method [37] based on the utilization of the three enzymes (heat-stable α-amylase, protease, and amyloglucosidase).

2.7.2 Reducing sugars quantification

The reducing sugars content in the BSG was determined by using the supernatant obtained after a solid-liquid extraction of the solid material. The extraction consisted of adding distilled water in a 1:10 (w:v) ratio at 30 °C. The mixture was mixed for 25 min at 180 rpm. The obtained supernatant was used to quantify the reducing sugars following the DNS method [38]. Briefly, 1 mL of sample was taken in a test tube, and 3 mL of DNS reagent was added. The solution was placed in a bath with boiling water for 5 min. After cooling, its absorbance was measured at 540 nm. The reducing sugars were calculated from a calibration curve of known concentrations using glucose as standard.

2.7.3 Xylanase activity

Xylanase activity was determined (triplicates) by using the supernatant obtained after a solid-liquid extraction of the solid material following the method presented by Ang et al. [39]. The extraction was conducted using citrate buffer (pH 4.8, 0.05 M) at 25 °C, 120 rpm, and a 1:15 (w:v) ratio. The final product, xylose, was measured with the DNS method. The substrate was xylan from beechwood (Apollo Scientific, England) and the enzymatic reaction ran for 20 min at 50 °C. One unit of xylanase activity (U) was expressed as 1 mol of xylose released from xylan from beechwood per minute. Xylanase activity was expressed as $U \cdot g^{-1}DM$.

2.7.4 Cellulase activity

Total cellulase activity was measured (triplicate) using filter paper assay (FPase), as recommended by IUPAC, according to Ghose [40]. The extraction ratio for cellulases activity was the same as performed for xylanases. Final reducing sugars were measured using the DNS method. The substrate was 1 × 6 cm Whatman filter paper in 0.05 M citrate buffer (pH 4.8), and the enzymatic reaction was run for 1 h at 50 °C. One unit of FPase (FPU) was expressed as the amount of enzyme that releases 1 mol of reducing sugars from Whatman filter paper per minute. Cellulase activity was expressed as $FPU \cdot g^{-1}DM$.

2.7.5 Biomass and PHA extraction from liquid culture

The biomass produced during the bacterial fermentation was quantified by centrifuging 10 mL of the fermented culture at 5000 rpm for 5 min at 10 °C. The cells were washed with distilled water and centrifuged again at 5000 rpm for 5 min at 10 °C. The biomass pellet was dried at 60 °C for 48 h and then weighed to obtain the cell dry weight (CDW) and used to determine the PHA content. PHA extraction was performed as described by Brandl et al. [41]. Briefly, 8–15 mg of the dry pellet was placed in a 2-mL vial, and 1 mL of chloroform and 0.8 mL of methanol-sulfuric solution were added. Benzoic acid ($0.1\ g \cdot mL^{-1}$) was also added as the internal standard. Vials were adequately sealed and placed in a thermostatic bloc at 94 °C for 3 h. After cooling, the vial content was transferred into 4-mL vials to be mixed with 0.5 mL NaOH 0.05 M through inversion for 5 min. The organic phase produced was used to quantify the PHA content of the biomass.

2.7.6 PHA quantification

PHAs were quantified by GC-FID using the extracts obtained from the biomass (Section 2.7.5). The GC system (Agilent 7820A) consisted of a flame ionization detector with an HP-Innowax column (30 m × 0.53 mm × 1 μm). The injection port was set at 250 °C in splitless mode, and oven temperature was set at 70 °C for 2 min, then temperature reached 190 °C at $10\ °C\ min^{-1}$ and hold for 7 min. The detector temperature was set at 300 °C. Identification and quantification were performed using calibration curves (internal standard) by comparing retention times of analytical grade standards (Sigma-Aldrich) P(3HB-co-3 HV-12% mol PHV) processed using the same conditions than the samples.

2.7.7 Inhibitory compounds quantification

Inhibitory compound content on the liquid hydrolysates was determined by HPLC using a modified method from [42]. Briefly, the HPLC system consisted of an Agilent 1920 Infinity UHPLC equipped with a UV-Vis Diode Array Detector G4212A and a Nucleosil 120C18 (3μm × 125mm × 4mm) column. The mobile phase ($1\ mL\ min^{-1}$) was a mixture of 0.05% H_3PO_4 and acetonitrile: H_2O (90:10). A gradient allowed changing 100% of 0.05% H_3PO_4 until 100% acetonitrile: H_2O after 38 min. Then this condition was held for 10 min. Quantification was performed by comparing samples from the analytical standards of the selected inhibitory

compounds at the same conditions by using external standard calibrations.

2.8 Statistical analysis

Statistical differences of the experiments were evaluated by using one-way ANOVA ($p < 0.05$) using the Tukey test. Experiments were conducted in triplicates, and values were presented as mean ± standard deviation. Data were analyzed using SPSS 21.

3 Results and discussion

3.1 Substrate characterization

Table 1 summarizes the characterization made on the used BSG. As it can be seen, this substrate contains a significant amount of hemicellulose and cellulose, making this leftover a potential source of sugars after a hydrolysis process. Besides, lignin content is particularly low, which could be considered a positive aspect since this would limit the adverse effects of lignin on the availability of hemicellulose and cellulose [43]. In general, these values are in accordance with those previously found by other authors such as del Río et al. [44] but slightly different compared with Mussatto et al. [43] and Paz et al. [23] wherein they found higher lignin contents for BSG.

Besides, in other studies such as in Russ et al. [45], cellulose, hemicellulose, and lignin of the used BSG were 23–25%, 30–35%, and 7–8% respectively, while in Mussatto and Roberto [46] they were 16.8%, 28.4%, and 27.8% respectively. Thus, compared with the characterization presented here, some differences can be attributed to the inherent dependence on factors such as the quality of the used cereal, or the specific conditions of the beer processing [47]. Hence, it is expected that BSG could be a potential source of fermentable sugars after the hydrolysis of its cellulose and hemicellulose fractions.

Table 1 Initial brewer's spent grain characterization

Parameters	Value
Moisture content (%)	79.0 ± 1.3
Volatile Solids (% DM)	96.0 ± 0.1
pH	5.8 ± 0.1
Reducing sugars (g g^{-1}DM)	0.03 ± 0.00
TKN (g kg^{-1}DM)	30.4 ± 4.3
Ammoniacal nitrogen (g kg^{-1}DM)	3.6 ± 0.4
OXC (g kg^{-1}DM)	585 ± 92
C/N	19.2
Cellulose (% DM)	21.6 ± 1.6
Hemicelluloses (% DM)	40.1 ± 1.4
Lignin (% DM)	7.7 ± 1.4

DM dry matter

3.2 Fungal strain assessment for enzyme production

The initial experiments consisted of assessing the ability of each of the selected strains to produce xylanases and cellulases through SSF. Table 2 summarizes the main results obtained for each fungal strain. As observed in Table 2, it was found that *A. niger* reached the maximum xylanase activity of the evaluated set (268 ± 24 U·g^{-1}DM) after 168 h of fermentation. However, this strain presented a second peak of xylanase activity (217 ± 30 U·g^{-1}DM) after 48 h of fermentation with no significant difference compared with the first one (p 0.086) (Fig. 1). It can be observed that, from the productivity standpoint, the peak at 48 h (4.5 ± 0.6 U·g^{-1}DM·h^{-1}) was better than the 168 h peak (1.6 ± 0.1 U·g^{-1}DM·h^{-1}), making the first more interesting to produce xylanases. In terms of cellulase activity, *A. niger* reached up to 1.9 ± 0.1 FPU·g^{-1}DM after 48 h of fermentation. Regarding *T. aurantiacus*, the maximum xylanase activity was reached after 72 h (241 ± 10 U·g^{-1}DM) and the maximum cellulases activity (3.0 ± 0.2 FPU·g^{-1}DM) after 168 h. Similarly, with *Trichoderma reesei*, the maximum xylanase activity was reached after 168 h (150 ± 24 U·g^{-1}DM) and the maximum cellulases activity (3.0 ± 0.1 FPU·g^{-1}DM) after 120 h of fermentation.

Comparing the enzyme activities found here against a commercial multi-enzyme complex (Viscozyme L) with a standard dose for enzymatic hydrolysis (1%) [35, 48], the produced extracts reached similar activity levels (Table 2). As observed, *A. niger* (p 0.986) and *T. aurantiacus* (p 0.584) extracts reached the same xylanase activity levels than Viscozyme L (1%) (p 0.986). Only *T. reesei* was significantly different (p 0.008), but in terms of cellulases, all extracts resulted similarly to Viscozyme L (1%) (p 0.183). Although the obtained cellulase activities were not as high as in other studies using similar feedstocks, they were in the same order of magnitude of Viscozyme L (1%). Such a result suggests that there was enough potential for using these enzymes for the hydrolysis processes. This aspect could be highly dependent on another factor such as the substrate selection. For instance, Dhillon et al. [49] have used apple pomace as a substrate with *A. niger* reaching cellulase activities as high as 384 FPU·g^{-1}DM, while authors such as Ortiz et al. [50] using wheat bran and *T. reesei* have reached activities around 96 FPU·g^{-1}DM. Also, Paz et al. [23] reached cellulase activities of 14.8 FPU·g^{-1}DM using BSG as substrate, while Leite et al. [21] reached between 50 and 60 FPU·g^{-1}DM using *A. niger* and *A. ibericus* with the same leftover. Although variability among substrates is significant, the obtained results were at the same levels as previous reports using BSG such in Leite et al. [21].

Table 2 Maximum enzymatic activities obtained from the evaluated fungal strains and commercial Viscozyme L (1%)

Parameter	*A. niger*	*T. aurantiacus*	*T. reesei*	**Viscozyme L (1%)**
Max xylanase activity (U g^{-1}DM)	217 ± 30^{A} (48 h fermentation)	241 ± 10^{A} (72 h fermentation)	150 ± 24^{B} (168 h fermentation)	277 ± 58^{A}
Max cellulase activity (FPU g^{-1}DM)	1.9 ± 0.1^{A} (48 h fermentation)	3.0 ± 0.2^{B} (168 h fermentation)	3.0 ± 0.1^{B} (120 h fermentation)	$2.4 \pm 0.4^{A,B}$
Max sOUR (mgO_2 g^{-1}DM h^{-1})	4.7	10.2	5.6	–

sOUR specific oxygen uptake rate, *DM* dry matter, *FPU* filter paper activity unit, *U* xylanase activity unit. Capital letters (A, B) in superscripts denote significant differences between the evaluated groups ($p < 0.05$) on each row based on the Tukey test analysis

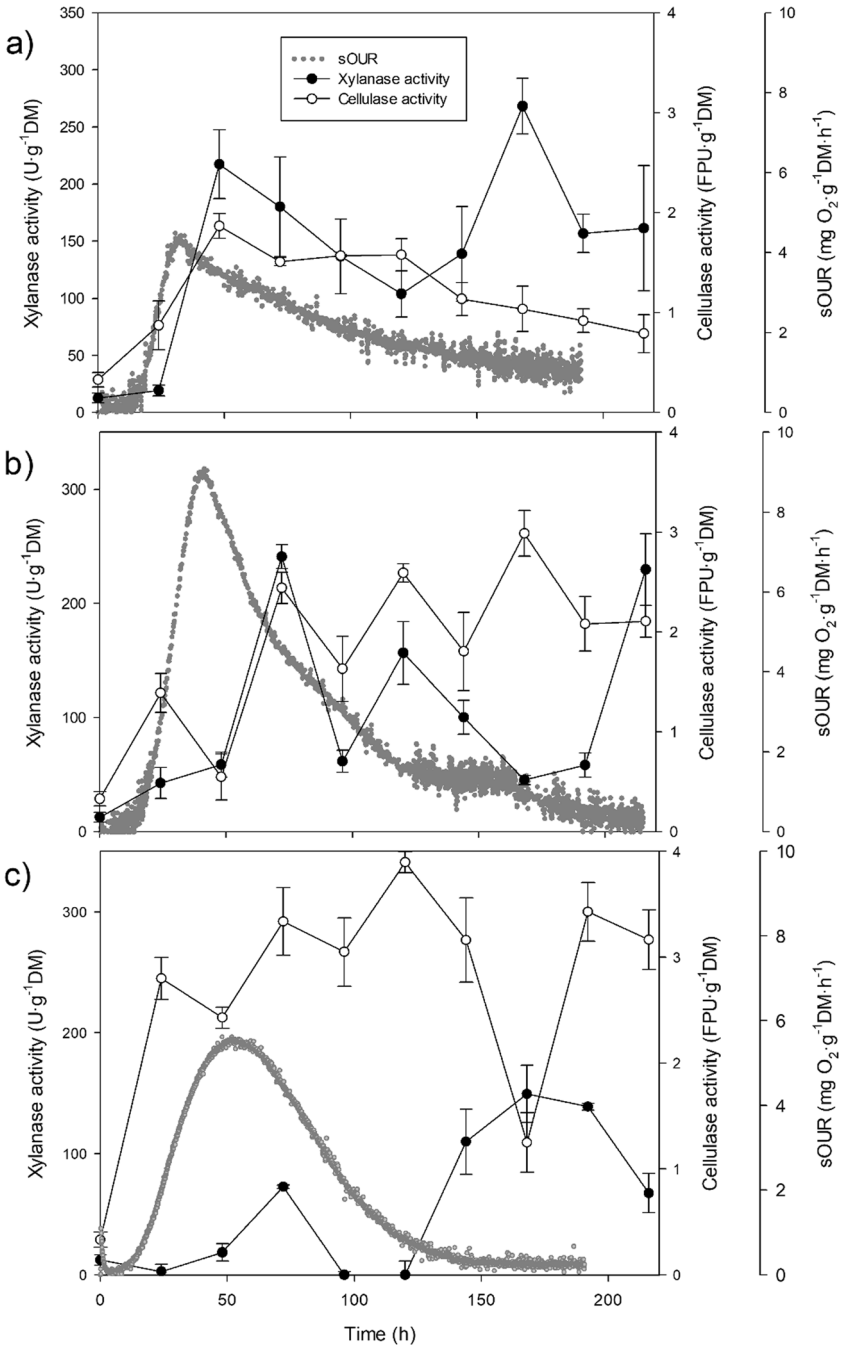

Fig. 1 Time-course of the solid-state fermentation of brewer's spent grain (BSG) for producing xylanases and cellulases with **a** *A. niger*, **b** *T. aurantiacus*, and **c** *T. reesei*. sOUR, specific oxygen uptake rate; DM, dry BSG; FPU, filter paper activity unit; U, xylanase activity unit

Similarly, by using other substrates, the xylanase activity levels could be as high as 1000 U·g^{-1}DM [49], but typical values have been found between 200 and 800 U·g^{-1}DM [51] as occurred here. Also, with BSG as a substrate, Leite et al. [21] reached xylanase activities between 250 and 310 U·g^{-1}DM with *A. niger* and *A. ibericus*. These values are in concordance with the results obtained in this study using the same fungal strain. Moreover, the xylanase activity obtained by Leite et al. [21] with BSG resulted similarly to those obtained in this study with *T. aurantiacus*.

Given these results, it could be stated that *T. aurantiacus* and *A. niger* were the fungal strains with higher potential to produce lignocellulolytic enzymes from BSG from the evaluated group. Figure 1 details the time-course of the xylanases and cellulase activities from the SSF of the BSG of the three fungal strains. With *A. niger* (Fig. 1a), the maximum enzyme activities were obtained after the peak of maximum microbiological activity (indirectly measured as sOUR) (32 h), and the maximum cellulases activity coincided with the first peak of xylanases activity. Similarly, with *T. aurantiacus* (Fig. 1b), it can be observed that, as occurred with *A. niger*, the peaks of activities have occurred some hours after the peak of maximum sOUR, which suggests that there could exist a relationship among enzyme activities and the respirometric parameter as it was found in other SSF processes [52]. Hence, sOUR could be potentially used in further development stages as a monitoring and control parameter for producing these enzymes [53].

From Fig. 1, it can also be seen that, in the evaluated period, xylanases activity had a pseudo-cyclic behavior for both strains. For *A. niger*, there were two characteristic peaks separated by 120 h, and the levels achieved in both points were significantly higher than in the rest of the fermentation. Similar behavior can be found when using *T. aurantiacus*. This time, peaks were split by 144 h, and there were no significant differences among them (p 0.585). Finally, for *T. reesei,* the same pseudo-cyclic behavior was found (Fig. 1c). However, in this case, the xylanase activity levels were significantly lower, and the activity of the second peak was higher than the first one, leading to a decrease in the productivity of the process. During the fermentation of the three fungal strains, no significant change in the MC was detected (MC changes were below 3%), assuring that neither drying of the bed nor the leaching of the products affects the process. According to these results, fermentation time for maximum productivity corresponds to 48 h for *A. niger* and 72 h for *T. aurantiacus* and *T. reesei*. Thus, the extracts obtained from these fermentations were selected for further hydrolysis tests.

3.3 Hydrolysis tests

In these experiments, the enzymatic extracts obtained from the SSF have been used to hydrolyze BSG to determine their efficiency. As seen in Fig. 2a, these tests have been conducted at 37 and 45 °C [23, 43] using the extracts from the three fungal strains, the commercial enzyme cocktail Viscozyme L (1%), and a blank control. From these tests, it was clear that there was a significant difference among the obtained extracts and the commercial enzymes during the first 12 h (p 000). Independently of the hydrolysis temperature, the commercial enzymes reached the highest reducing sugar release after 12 h reaching almost 35 g·L^{-1}. On the other hand, *A. niger* extract at 45 °C showed the best performance among the evaluated extracts and temperatures, reaching 86–90% of the reducing sugar release obtained by the commercial enzymes at 45 °C and 37 °C, respectively, after 24 h. In general, these results suggest that working at 45 °C is better than at 37 °C for both the commercial enzymes and those produced from BSG.

On the other hand, after 12 h, a small decrease in reducing sugar release was detected. In this sense, it was found that after 24 h of hydrolysis, the extracts from BSG started to present fungal growth (samples were inoculated in Petri dishes, and fungal growth was confirmed), suggesting that these could be consuming part of the sugars released during the process. Thus, based on these results, the second set of experiments was conducted at 45 °C using only *A. niger* extracts in an extended period to identify the effect of this reduction due to the potential fungal growth. As Fig. 2b details, the maximum reducing sugar release in this scenario was obtained after 24 h with 28.1 ± 1.0 g·L^{-1}, which was 36% lower than the maximum achieved by the commercial enzymes at the same conditions. Hence, the maximum sugars released corresponded to 0.56 g of reducing sugar g^{-1}DM. After reaching the maximum, a significant decrease in reducing sugar content occurred, reaching up to 51% after 48 h of hydrolysis.

Consequently, it is expected that, at the evaluated conditions, the hydrolysis could be conducted just for 24 h such that the potential loss of sugars can be minimized. Besides, due to the potential loss of sugars, future developments using such an enzymatic extract should include a purification step to limit this adverse effect, for instance, by using ultrafiltration processes with 30 kDa membranes capable of retaining the contaminants [54].

In general, the reducing sugar levels found here (28.1 ± 1.0 g·L^{-1}) are similar to those found by other authors such as Mussatto and Roberto [46] that reached concentrations between 21 and 35 g·L^{-1} by using acid hydrolysis of the BSG. Paz et al. [23] using enzymatic hydrolysis by *A. niger* reached a total sugar content of almost 23 g·L^{-1} with raw BSG. Besides, the performance of BSG hydrolysate was competitive compared to other residues used to obtain fermentable sugars. For instance, Buzała et al. [55], using a commercial

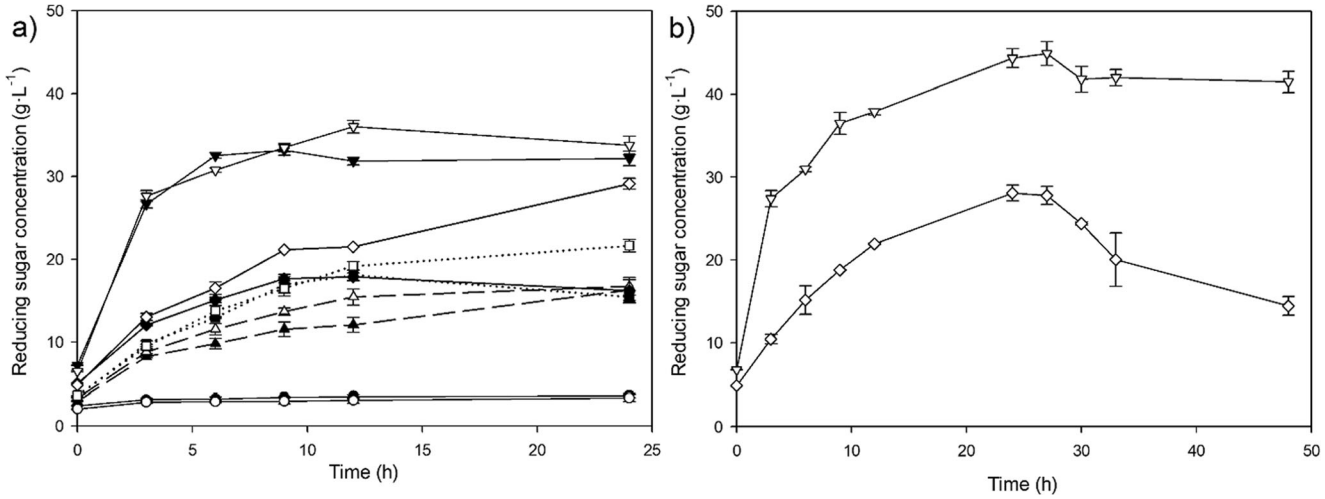

Fig. 2 Hydrolysis tests to release fermentable sugars from dried brewer's spent grain (BSG) using the enzymatic extracts obtained via solid-state fermentation. **a** BSG hydrolysis at two temperature levels (37 °C, 45 °C) during 24 h, **b** BSG hydrolysis at 37 °C during 48 h using *A. niger*. —●—: Blank control 37 °C; —○—: blank control 45 °C; —▲—: *T. aurantiacus* 37 °C; —△—: *T. aurantiacus* 45 °C; ···■···: *T. reesei* 37 °C; ··□··: *T. reesei* 45 °C; —◆—: *A. niger* 37 °C; —◇—: *A. niger* 45 °C; —▼—: Viscozyme L 37 °C; —▽—: Viscozyme L 45 °C

multi-enzyme preparation NS-22086, reached concentrations of 16.5 g·L^{-1} using poplar pulp, 14.5 g·L^{-1} for pine pulp, 16.9 g·L^{-1} for beech pulp, 15.9 g·L^{-1} for birch pulp, 17.7 g·L^{-1} for wheat straw pulp and 11.1 g·L^{-1} for hemp hard pulp. Similarly, Saucedo-Luna et al. [56], using a commercial multi-enzyme mixture from *A. niger and T. reesei*, reached 17.5 g·L^{-1} of fermentable sugars after 48 h of hydrolysis of agave bagasse. Also, Wei et al. [57], using the commercial enzymatic mixture Celluclast (Novozyme Inc., Denmark), reached 17.3 g·L^{-1} of fermentable sugars after 48 h of hydrolysis of rice hull. However, in many of these studies, chemical pretreatments have been performed before the enzymatic hydrolysis to enhance the sugar release, or synthetic substrates have been added as substrates, making these alternatives more expensive. Thus, by using BSG without any pretreatment appears as an interesting option for obtaining fermentable sugars economically and sustainably.

3.4 PHA production from sugar-rich hydrolysates

In these experiments, the BSG sugar-rich hydrolysates obtained from the enzymatic extracts of *A. niger* and the commercial enzyme cocktail Viscozyme L (1%) have been used to evaluate the PHA production in liquid cultures. The solution obtained after the hydrolysis of BSG with citrate buffer was also tested as control (blank). The PHA production was analyzed by quantifying the produced poly-3-hydroxybutyrate (P3HB), and the polyhydroxyvalerate (P3HV) as the most common forms of polyhydroxyalkanoates produced starting from sugars. In this case, the P3HV levels were negligible (data not shown), so the results only considered the produced P3HB.

As seen from Fig. 3a, the P3HB accumulation was higher when using *C. necator* compared with using *B. cepacia* independently of the enzymatic extract used. With *A. niger* extracts as hydrolysis agent, 0.15 ± 0.03 and 0.13 ± 0.01 g P3HB·g^{-1} CDW with *C. necator* and *B. cepacia*, respectively, were obtained (0.41 ± 0.05 g P3HB·L^{-1} and 0.35 ± 0.03 g P3HB·L^{-1}). On the other hand, by using the commercial enzymes as hydrolysis agent, the P3HB contents were higher, achieving 0.25 ± 0.03 and 0.21 ± 0.03 g P3HB·g^{-1} CDW with *C. necator* and *B. cepacia*, respectively (1.19 ± 0.14 g P3HB·L^{-1} and 1.01 ± 0.17 g P3HB·L^{-1}).

In all cases, *C. necator* had slightly higher production per biomass than *B. cepacia*. This result suggests that *C. necator* is a better PHA producer than *B. cepacia* from hydrolyzed BSG. Previously, Rodrigues et al. [58] compared the PHA production using *C. necator* and *B. cepacia* from soybean as a substrate. In that study, *C. necator* resulted better than *B. cepacia* to produce PHA, reaching up to 0.84 g P3HB·L^{-1}. Also, the PHA content in the biomass was higher with *C. necator*, between 20 and 23%, compared with *B. cepacia*, which only reached 7–10%, suggesting that *C. necator* is better accumulating PHA from this kind of hydrolysates.

Figure 3b summarizes the reducing sugars content of the BSG sugar-rich hydrolysates at time zero of the fermentation and the end of the fermentation. The reducing sugars content obtained after 24 h of hydrolysis with each enzymatic extract was considered as the initial value (corresponding to time zero of fermentation). As seen, after hydrolysis of the BSG, the commercial enzymes and *A. niger* extract released 38.9 g·L^{-1} and 21.8 g·L^{-1} of reducing sugars, respectively, in accordance with the results found before. As detailed, it was found that *B. cepacia* consumed more sugars than *C. necator* in all

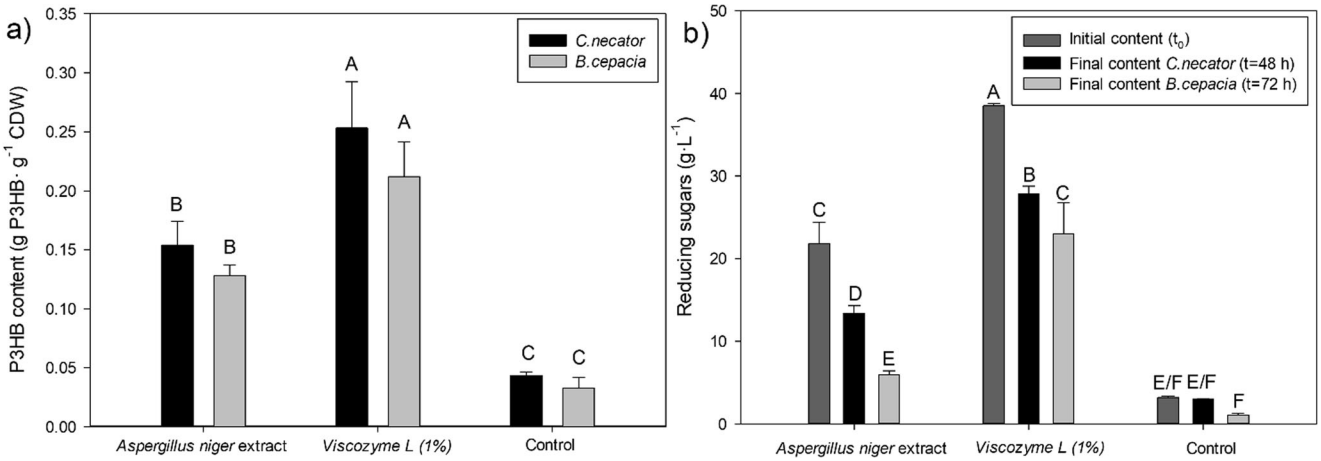

Fig. 3 **a** P3HB production in liquid culture using different enzymatic extracts and **b** initial and final reducing sugars content during P3HB production. CDW, cell dried weight; P3HB, poly-3-hydroxybutyrate. Different capital letters indicate significant differences between the evaluated groups ($p < 0.05$) based on the Tukey test analysis

the evaluated scenarios: *A. niger* extract (72.9% and 38.7% of consumed sugars, respectively), the commercial enzymes (40.2% and 27.4% of consumed sugars, respectively) and control (68.3% and 6.3% of consumed sugars, respectively) (Fig. 3b). However, P3HB production with *C. necator* resulted better than using *B. cepacia*. Thus, even though reducing sugars consumption was higher for *B. cepacia*, that consumption was not reflected in higher P3HB production.

A higher sugar consumption was observed in *A. niger* extracts than in the commercial enzyme's samples, and it could be attributed to the potential fungal growth observed before. Comparing *A. niger* extracts and the commercial enzymes as hydrolysis agents on the *C. necator and B. cepacia* P3HB production, *A. niger* samples obtained 9.0 ± 0.4 and 7.0 ± 0.6 mg P3HB·g^{-1} DM respectively, and the commercial enzymes samples 23.4 ± 1.8 and 19.7 ± 3.5 mg P3HB·g^{-1} DM respectively.

Additionally, *A. niger* extracts reached between 35 and 40% of P3HB production reached with the commercial enzymes with both strains. This result suggests that an optimization of the hydrolysis step to obtain higher sugar levels and, then, potentially increase the production of P3HB from the BSG is required. However, the obtained P3HB levels could also be attributed to the presence of inhibitory compounds produced during the hydrolysis step. In this sense, some authors [59] suggested that the formation of furan aldehydes as well as some soluble substances like hemicellulose-derived and cellulose-derived carbohydrates could interfere with the cellulolytic enzymes altering the hydrolysis step. Other compounds such as acetate, furfural, vanillin, and levulinic acid could also affect the bacterial fermentation, decreasing the PHA content [60]. Table 3 summarizes the concentration of some of the most common inhibitory compounds found in the hydrolysates used here (from Viscozyme L and *A. niger*). In general, it could be stated that the commercial enzymes produced hydrolysates with a higher content of inhibitory compounds such as 5-HMF, furfuraldehyde, vanillic acid, or syringic acid compared with hydrolysates obtained from *A. niger*. Although the levels found in both hydrolysates allowed the growth of both bacterial strains used, these could be limiting the transformation of the available sugars into PHA. In future developments, a detoxification step could reduce the presence of such compounds, promoting a higher sugar consumption.

On the other hand, the results obtained here were in line with other reports using alternative raw material for producing PHA. For instance, Kucera et al. [35] reached 0.30 g PHB·g^{-1} CDW using *B. cepacia* and up to 0.88 g

Table 3 Concentration (mg L^{-1}) of some potential inhibitory compounds in the evaluated hydrolysates

Inhibitory compound	Hydrolysate	
	Viscozyme L (1%)	*A. Niger* extract
Formic acid	< 50	< 50
Acetic acid	150 ± 1	157 ± 2
Levulinic acid	< 100	< 100
Furfuryl alcohol	< 0.5	< 0.5
5-HMF	0.64 ± 0.04	0.48 ± 0.03
Furfuraldehyde	4.9 ± 0.1	3.5 ± 0.1
Vanillic acid	0.53 ± 0.03	0.42 ± 0.02
Syringic acid	0.65 ± 0.04	0.51 ± 0.03
Vanillin	< 0.5	< 0.5
Syringaldehyde	< 0.2	< 0.2
Coumaric acid	0.98 ± 0.05	0.80 ± 0.04

Mean values are presented as the mean ± the standard deviation. "<" means below the detection limit of the method

PHB·g^{-1} CDW with *Burkholderia sacchari* starting from spruce sawdust as a substrate. Also, Tripathi et al. [61] using sucrose as a carbon source obtained 0.35 g PHA·g^{-1} CDW with *Pseudomonas aeruginosa.* Thus, to the best of our knowledge, this is the first report showing a valorization strategy in two stages for BSG, by using a combined approach of obtaining self-produced enzymes (via SSF) and the further PHA production with the same BSG hydrolysates.

4 Conclusions

A. niger produced the highest enzymatic activities from the SSF of BSG reaching up to 268 ± 24 U·g^{-1}DM, obtaining an enzymatic extract capable of producing significant fermentable sugars contents. Although *A. niger* reached similar xylanase and cellulase activities than Viscozyme L (1%), its efficiency to hydrolyze the BSG resulted significantly lower. However, the BSG hydrolysates obtained from *A. niger* enzyme extract have supported the P3HB production using *C. necator* and *B. cepacia* reaching 9.0 ± 0.4 and 7.0 ± 0.6 mg P3HB·g^{-1} DM, respectively. Future work will be needed to optimize the SSF and hydrolysis steps to increase the enzymatic activities as well as the release of sugars from BSG. Furthermore, adding a pretreatment/detoxification strategy coupled with the hydrolysis step could serve to improve the overall performance of the proposed approach. These results suggest that BSG can be used as a low-cost raw material for obtaining both lignocellulolytic enzymes and PHA as value-added products resulting in a valorization approach in two stages.

Acknowledgment EM acknowledges the Juan de la Cierva research fellowship (FJCI-2015-24,616) from the Spanish Ministry of Economy and Competitiveness.

Availability of data and material It will be available upon request.

Authors' contributions Jordi Llimós: data collection, data analysis and wrote the paper. Oscar Martínez-Avila and Elisabet Marti and: data analysis and critical revision of the manuscript. Carlos Corchado-Lopo: data collection. Teresa Gea, Laia Llenas, and Sergio Ponsá: Financial support acquisition, study conception, and critical revision of the manuscript.

Funding information This work was supported by the Spanish Ministerio de Economía, Industria y Competitividad (CTM2016–81038-R), VALORA Project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Dodge T (2009) Production of industrial enzymes. In: Enzymes in food technology. 2nd ed; Amauri, A.B., Ed.; Wiley-Blackwell: Hoboken, NJ, USA. pp 44–58
2. De Bhowmick G, Sarmah AK, Sen R (2018) Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products. Bioresour Technol 247:1144–1154. https://doi.org/10.1016/j.biortech.2017.09.163
3. Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal HMN (2017) Lignocellulose: a sustainable material to produce value-added products with a zero waste approach—a review. Int J Biol Macromol 99:308–318. https://doi.org/10.1016/j.ijbiomac.2017.02.097
4. Bilal M, Asgher M, Iqbal HMN, Hu H, Zhang X (2017) Biotransformation of lignocellulosic materials into value-added products—a review. Int J Biol Macromol 98:447–458. https://doi.org/10.1016/j.ijbiomac.2017.01.133
5. Lin L, Xu F, Ge X, Li Y (2019) Biological treatment of organic materials for energy and nutrients production—anaerobic digestion and composting, 1st edn. Elsevier Inc.
6. Verma N, Kumar V, Bansal MC (2020) Comparative view on microbial consumption of agro-based lignocellulosic waste biomass in sustainable production of cellulases. Biomass Convers Biorefinery. https://doi.org/10.1007/s13399-020-00617-0
7. Verma N, Kumar V (2020) Valorization of waste biomass in fermentative production of cellulases : a review. Waste and Biomass Valorization. https://doi.org/10.1007/s12649-020-01048-8
8. Singh R, Kumar M, Mittal A, Mehta PK (2016) Microbial enzymes: industrial progress in 21st century. 3. Biotech 6:1–15. https://doi.org/10.1007/s13205-016-0485-8
9. de Castro RJS, Sato HH (2015) Enzyme production by solid state fermentation: general aspects and an analysis of the physicochemical characteristics of substrates for agro-industrial wastes valorization. Waste and Biomass Valorization 6:1085–1093. https://doi.org/10.1007/s12649-015-9396-x
10. Díaz-Godínez G, Soriano-Santos J, Augur C, Viniegra-González G (2001) Exopectinases produced by *Aspergillus niger* in solid-state and submerged fermentation: a comparative study. J Ind Microbiol Biotechnol 26:271–275. https://doi.org/10.1038/sj.jim.7000113
11. Soccol CR, da Costa ESF, Letti LAJ et al (2017) Recent developments and innovations in solid state fermentation. Biotechnol Res Innov 1:52–71. https://doi.org/10.1016/j.biori.2017.01.002
12. Yazid NA, Barrena R, Komilis D, Sánchez A (2017) Solid-state fermentation as a novel paradigm for organic waste valorization: a review. Sustainability 9:1–28. https://doi.org/10.3390/su9020224
13. Castilho LR, Polato CMS, Baruque EA, Sant'Anna GL Jr, Freire DMG (2000) Economic analysis of lipase production by *Penicillium restrictum* in solid-state and submerged fermentations. Biochem Eng J 4:239–247. https://doi.org/10.1016/S1369-703X(99)00052-2
14. Jiménez-Peñalver P, Gea T, Sánchez A, Font X (2016) Production of sophorolipids from winterization oil cake by solid-state fermentation: optimization, monitoring and effect of mixing. Biochem Eng J 115:93–100. https://doi.org/10.1016/j.bej.2016.08.006
15. Martínez-Avila O, Sanchez A, Font X, Barrena R (2019) Fed-batch and sequential-batch approaches to enhance the bioproduction of 2-Phenylethanol and 2-Phenethyl acetate in solid- state fermentation residue-based systems. J Agric Food Chem 67:3389–3399. https://doi.org/10.1021/acs.jafc.9b00524
16. Sala A, Artola A, Sánchez A, Barrena R (2020) Rice husk as a source for fungal biopesticide production by solid-state fermentation using *B. bassiana* and *T. harzianum*. Bioresour Technol 296:122322. https://doi.org/10.1016/j.biortech.2019.122322

17. Aliyu S, Bala M (2011) Brewer's spent grain: a review of its potentials and applications. African J Biotechnol 10:324–331. https://doi.org/10.5897/AJBx10.006
18. Ibarruri J, Cebrián M, Hernández I (2019) Solid state fermentation of brewer's spent grain using *Rhizopus sp.* to enhance nutritional value. Waste and Biomass Valori 10:3687–3700. https://doi.org/10.1007/s12649-019-00654-5
19. Mussatto SI, Dragone G, Roberto IC (2006) Brewers' spent grain: generation, characteristics and potential applications. J Cereal Sci 43:1–14. https://doi.org/10.1016/j.jcs.2005.06.001
20. Carrillo-Nieves D, Saldarriaga-Hernandez S, Gutiérrez-Soto G, Rostro-Alanis M, Hernández-Luna C, Alvarez AJ, Iqbal HMN, Parra-Saldívar R (2020) Biotransformation of agro-industrial waste to produce lignocellulolytic enzymes and bioethanol with a zero waste. Biomass Convers Bior. https://doi.org/10.1007/s13399-020-00738-6
21. Leite P, Silva C, Salgado JM, Belo I (2019) Simultaneous production of lignocellulolytic enzymes and extraction of antioxidant compounds by solid-state fermentation of agro-industrial wastes. Ind Crop Prod 137:315–322. https://doi.org/10.1016/j.indcrop.2019.04.044
22. Outeiriño D, Costa-Trigo I, Pinheiro de Souza Oliveira R et al (2019) A novel approach to the biorefinery of brewery spent grain. Process Biochem 85:135–142. https://doi.org/10.1016/j.procbio.2019.06.007
23. Paz A, Outeiriño D, Pérez Guerra N, Domínguez JM (2019) Enzymatic hydrolysis of brewer's spent grain to obtain fermentable sugars. Bioresour Technol 275:402–409. https://doi.org/10.1016/j.biortech.2018.12.082
24. Xiros C, Christakopoulos P (2012) Biotechnological potential of brewers spent grain and its recent applications. Waste and Biomass Valori 3:213–232. https://doi.org/10.1007/s12649-012-9108-8
25. Gironi F, Piemonte V (2011) Bioplastics and petroleum-based plastics: strengths and weaknesses. Energy Sources, Part A Recover Util Environ Eff 33:1949–1959. https://doi.org/10.1080/15567030903436830
26. Rodriguez-Perez S, Serrano A, Pantión AA, Alonso-Fariñas B (2018) Challenges of scaling-up PHA production from waste streams. A review J Environ Manage 205:215–230. https://doi.org/10.1016/j.jenvman.2017.09.083
27. Tan GYA, Chen CL, Li L et al (2014) Start a research on biopolymer polyhydroxyalkanoate (PHA): a review. Polymers (Basel) 6:706–754. https://doi.org/10.3390/polym6030706
28. Du C, Sabirova J, Soetaert W, Ki Carol Lin S (2012) Polyhydroxyalkanoates production from low-cost sustainable raw materials. Curr Chem Biol 6:14–25. https://doi.org/10.2174/2212796811206010014
29. Brigham CJ, Riedel SL (2019) The potential of polyhydroxyalkanoate production from food wastes. Appl Food Biotechnol 6:7–18. https://doi.org/10.22037/afb.v6i1.22542
30. Frassatto PAC, Casciatori FP, Thoméo JC et al (2020) β-Glucosidase production by *Trichoderma reesei* and *Thermoascus aurantiacus* by solid state cultivation and application of enzymatic cocktail for saccharification of sugarcane bagasse. Biomass Convers Biore. https://doi.org/10.1007/s13399-020-00608-1
31. Puyuelo B, Ponsá S, Gea T, Sánchez A (2011) Determining C/N ratios for typical organic wastes using biodegradable fractions. Chemosphere 85:653–659. https://doi.org/10.1016/j.chemosphere.2011.07.014
32. Ponsá S, Gea T, Sánchez A (2010) Different indices to express biodegradability in organic solid wastes. J Environ Qual 39:706–712. https://doi.org/10.2134/jeq2009.0294
33. dos Santos E, Piovan T, Roberto IC, Milagres AMF (2003) Kinetics of the solid state fermentation of sugarcane bagasse by *Thermoascus aurantiacus* for the production of xylanase. Biotechnol Lett 25:13–16. https://doi.org/10.1023/A:1021709610251
34. Mejias L, Komilis D, Gea T, Sánchez A (2017) The effect of airflow rates and aeration mode on the respiration activity of four organic wastes: implications on the composting process. Waste Manag 65:22–28. https://doi.org/10.1016/j.wasman.2017.04.008
35. Kucera D, Benesova P, Ladicky P et al (2017) Production of polyhydroxyalkanoates using hydrolyzates of spruce sawdust: comparison of hydrolyzates detoxification by application of overliming, active carbon, and lignite. Bioengineering 4:53. https://doi.org/10.3390/bioengineering4020053
36. Thompson WH, Leege PB, Millner PD, Watson ME (2001) Test methods for the examination of composting and compost
37. Cunniff P (1995) Official methods of analysis of AOAC international, 16th edn Assoc Off Anal Chem Int Arlingt
38. Miller GL (1959) Use of dinitrosaliicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030
39. Ang SK, Shaza EM, Adibah YA et al (2013) Production of cellulases and xylanase by *Aspergillus fumigatus* SK1 using untreated oil palm trunk through solid-state fermentation. Process Biochem 48:1293–1302. https://doi.org/10.1016/j.procbio.2013.06.019
40. Ghose T (1987) Measurement of cellulose activities. Pure Appl Chem 59:257–268. https://doi.org/10.1016/0076-6879(88)60109-1
41. Brandl H, Gross RA, Lenz RW, Fuller RC (1988) *Pseudomonas oleovorans* as a source of poly (β-hydroxyalkanoates) for potential applications as biodegradable polyesters. Appl Environ Microbiol 54:1977–1982
42. Chen S-F (2006) High-performance liquid chromatographic methods for quantitative assessment of degradation products and extractives in pretreated lignocellulose
43. Mussatto SI, Fernandes M, Milagres AMF, Roberto IC (2008) Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. Enzym Microb Technol 43:124–129. https://doi.org/10.1016/j.enzmictec.2007.11.006
44. del Río JC, Prinsen P, Gutiérrez A (2013) Chemical composition of lipids in brewer's spent grain: a promising source of valuable phytochemicals. J Cereal Sci 58:248–254. https://doi.org/10.1016/j.jcs.2013.07.001
45. Russ W, Mörtel H, Meyer-Pittroff R (2005) Application of spent grains to increase porosity in bricks. Constr Build Mater 19:117–126. https://doi.org/10.1016/j.conbuildmat.2004.05.014
46. Mussatto SI, Roberto IC (2005) Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol. J Sci Food Agric 85:2453–2460. https://doi.org/10.1002/jsfa.2276
47. Santos M, Jiménez JJ, Bartolomé B, Gómez-Cordovés C, del Nozal MJ (2003) Variability of brewer's spent grain within a brewery. Food Chem 80:17–21. https://doi.org/10.1016/S0308-8146(02)00229-7
48. Min JY, Kang SM, Park DJ, Kim YD, Jung HN, Yang JK, Seo WT, Kim SW, Karigar CS, Choi MS (2006) Enzymatic release of ferulic acid from *Ipomoea batatas* L. (sweet potato) stem. Biotechnol Bioprocess Eng 11:372–376. https://doi.org/10.1007/BF03026256
49. Dhillon GS, Kaur S, Brar SK, Verma M (2012) Potential of apple pomace as a solid substrate for fungal cellulase and hemicellulase bioproduction through solid-state fermentation. Ind Crop Prod 38:6–13. https://doi.org/10.1016/j.indcrop.2011.12.036
50. Ortiz GE, Guitart ME, Cavalitto SF, Albertó EO, Fernández-Lahore M, Blasco M (2015) Characterization, optimization, and scale-up of cellulases production by *trichoderma reesei* cbs 836.91 in solid-state fermentation using agro-industrial products. Bioprocess Biosyst Eng 38:2117–2128. https://doi.org/10.1007/s00449-015-1451-2
51. Mansour AA, Arnaud T, Lu-Chau TA, Fdz-Polanco M, Moreira MT, Rivero JAC (2016) Review of solid state fermentation for lignocellulolytic enzyme production: challenges for environmental

applications. Rev Environ Sci Biotechnol 15:31–46. https://doi.org/10.1007/s11157-016-9389-7

52. Cerda A, Mejías L, Gea T, Sánchez A (2017) Cellulase and xylanase production at pilot scale by solid-state fermentation from coffee husk using specialized consortia: the consistency of the process and the microbial communities involved. Bioresour Technol 243:1059–1068. https://doi.org/10.1016/j.biortech.2017.07.076
53. Puyuelo B, Gea T, Sánchez A (2010) A new control strategy for the composting process based on the oxygen uptake rate. Chem Eng J 165:161–169. https://doi.org/10.1016/j.cej.2010.09.011
54. Liu J, Xia W (2006) Purification and characterization of a bifunctional enzyme with chitosanase and cellulase activity from commercial cellulase. Biochem Eng J 30:82–87. https://doi.org/10.1016/j.bej.2006.02.005
55. Buzała KP, Kalinowska H, Przybysz P, Małachowska E (2017) Conversion of various types of lignocellulosic biomass to fermentable sugars using kraft pulping and enzymatic hydrolysis. Wood Sci Technol 51:873–885. https://doi.org/10.1007/s00226-017-0916-7
56. Saucedo-Luna J, Castro-Montoya AJ, Martinez-Pacheco MM, Sosa-Aguirre CR, Campos-Garcia J (2011) Efficient chemical and enzymatic saccharification of the lignocellulosic residue from Agave tequilana bagasse to produce ethanol by *Pichia caribbica*. J Ind Microbiol Biotechnol 38:725–732. https://doi.org/10.1007/s10295-010-0853-z
57. Wei GY, Gao W, Jin IH et al (2009) Pretreatment and saccharification of rice hulls for the production of fermentable sugars. Biotechnol Bioprocess Eng 14:828–834. https://doi.org/10.1007/s12257-009-0029-8
58. Rodrigues PR, Nunes JMN, Lordelo LN, Druzian JI (2019) Assessment of polyhydroxyalkanoate synthesis in submerged cultivation of *Cupriavidus necator* and *Burkholderia cepacia* strains using soybean as substrate. Brazilian J Chem Eng 36:73–83. https://doi.org/10.1590/0104-6632.20190361s20170267
59. Jönsson LJ, Martín C (2016) Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199:103–112. https://doi.org/10.1016/j.biortech.2015.10.009
60. Pan W, Perrotta JA, Stipanovic AJ, Nomura CT, Nakas JP (2012) Production of polyhydroxyalkanoates by *Burkholderia cepacia* ATCC 17759 using a detoxified sugar maple hemicellulosic hydrolysate. J Ind Microbiol Biotechnol 39:459–469. https://doi.org/10.1007/s10295-011-1040-6
61. Tripathi AD, Yadav A, Jha A, Srivastava SK (2012) Utilizing of sugar refinery waste (cane molasses) for production of bio-plastic under submerged fermentation process. J Polym Environ 20:446–453. https://doi.org/10.1007/s10924-011-0394-1