Review

Received: 15 April 2013 Revised: 15 September 2013 Accepted article published: 19 November 2013 Published online in Wiley Online Library: 24 January 2014

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6486

Brewer's spent grain: a valuable feedstock for industrial applications

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Abstract

Brewer's spent grain (BSG) is the most abundant by-product generated from the beer-brewing process, representing approximately 85% of the total by-products obtained. This material is basically constituted by the barley grain husks obtained as solid residue after the wort production. Since BSG is rich in sugars and proteins, the main and quickest alternative for elimination of this industrial by-product has been as animal feed. However, BSG is a raw material of interest for application in different areas because of its low cost, large availability throughout the year and valuable chemical composition. In the last decade, many efforts have been directed towards the reuse of BSG, taking into account the incentive that has been given to recycle the wastes and by-products generated by industrial activities. Currently, many interesting and advantageous methods for application of BSG in foods, in energy production and in chemical and biotechnological processes have been reported. The present study presents and discusses the most recent perspectives for BSG application in such areas.

Keywords: brewer's spent grain (BSG); chemical composition; ethanol; adsorbent; fermentation; biotechnological processes

INTRODUCTION

Briefly, the beer-brewing process starts with the production of the wort, a sugar-rich solution that will be used in the subsequent fermentation stage to produce ethanol. For the wort elaboration, the milled barley malt is mixed with water in a mash tun where the temperature is slowly increased from 37 to 78 °C in order to convert the malt starch into fermentable (mainly maltose and maltotriose) and non-fermentable (dextrins) sugars. Proteins from the barley malt are also partially degraded during this stage into polypeptides and amino acids. At the end of this process, the insoluble undegraded part of the barley malt grain, also known as brewer's spent grain (BSG), is obtained in mixture with the wort. The wort is filtered through the BSG bed formed at the bottom of the mash tun and is transferred to the fermentation tank, while BSG is obtained as a by-product of this process. More details on the beer-brewing process and the by-products generated during this process can be found in previously published studies.[1,2]

BSG is constituted by the husks that covered the original barley malt grain in mixture with part of the pericarp and seed coat layers that are obtained as residual solid material after the wort elaboration step. In some cases, according to the kind of beer that will be produced, other cereals such as corn (maize), rice, wheat, oats, rye or sorghum can be used in mixture with the barley malt for the wort elaboration. In such cases, the insoluble part of these grains after the mashing process is separated with BSG as a unique fraction. Therefore BSG can be derived from barley malt only or from a mixture of barley malt with adjuncts (other cereal grains).

The worldwide annual production of BSG has been estimated as approximately 38.6×10^6 t. This is a significant amount considering that BSG is obtained only from the barley husks and that not all the produced barley is used for the production of beer. Compared with the total amount of by-products generated from the most important agricultural crops,[3–6] the amount of BSG produced annually is much lower (Table 1). However, the values reported for the other by-products correspond to the sum of husks, straws, leaves and stems derived from these crops, while BSG is derived only from the barley husks. Owing to the significant amount in which it is produced annually, efforts must be made to valorize this agro-industrial by-product derived from breweries.

BSG COMPOSITION

BSG is mainly composed of the barley malt grain husks in mixture with part of the pericarp and seed coat layers of these grains. Although it is well known that BSG is rich in sugars, proteins and minerals, the chemical composition of this material may suffer significant variations due to a variety of factors, which include the variety of the barley used in the process as well as its harvest time and the conditions under which it was cultivated, the conditions used for malting and mashing and the amount and type of the adjuncts added in mixture with the barley malt for the wort elaboration. Table 2 summarizes the chemical composition of BSG reported in different studies, which used barley malt produced in different countries, including Brazil,[7] Japan,[8] Portugal[9–11] and Ireland.[12] This table clearly shows variations in the chemical composition of this raw material as a consequence of the above-mentioned factors. For example, Mussatto and Roberto[7] and Waters *et al.*[12] used BSG derived from a brewing process without addition of adjuncts (i.e. using 100% barley malt), but the former used Brazilian barley malt while

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Table 1. Annual worldwide production of brewer's spent grain (BSG), some of the most important agricultural crops and their by-products in 2011

Item	Total annual production (10^6 t)	By-products (10^6 t)
Agricultural crops		
Wheat	694.02[a]	763.42[b]
Rice	465.40[a]	698.10[b]
Corn	864.96[a]	1729.92[b]
Soybean	245.07[a]	416.62[b]
Cotton	126.92[a]	107.13[b]
Barley	124.07[c]	144.88[d]
Agro-industrial residue		
BSG		38.60[e]

[a] USDA.[3]
[b] Zhang *et al.*[4]
[c] De Mori and Minella.[5]
[d] Estimated considering a residue/crop ratio of 1.2.[6]
[e] Estimated considering the annual production of beer in 2011 (1930×10^6 hL) and the generation of 20 kg BSG hL^{-1} beer produced.

the latter used barley malt from Ireland. Differences can also be observed when comparing the results of these authors with those reported by Meneses *et al.*,[11] who used BSG derived from a process using barley malt with adjuncts. Carvalheiro *et al.*[9] and Meneses *et al.*[11] used BSG derived from barley malt with adjuncts, obtained from two different Portuguese breweries. The variations observed in the results of these authors suggest that the brewing process conditions affected the composition of the residual BSG material. In fact, the conditions used for the brewing process were not reported in any of the studies presented in Table 2, and this is probably another factor with significant influence on the results.

Independently of the variations reported in the amount of each constituent in the BSG composition, this material is mainly composed of fibres (cellulose, hemicellulose and lignin) and protein (Table 2). Hemicellulose and cellulose are fractions constituted by sugars, among which xylose, arabinose and glucose are the most abundant in BSG. Sugars correspond to approximately half of the BSG composition on a dry weight basis, thus representing a very important fraction. Besides sugars, lignin (a polyphenolic macromolecule of complex structure) and proteins are also present in significant amounts in BSG. Essential amino acids represent approximately 30% of the total protein content, with lysine being the most abundant (14.3%), followed by leucine (6.12%), phenylalanine (4.64%), isoleucine (3.31%), threonine (0.71%) and tryptophan (0.14%). The non-essential amino acids in BSG (corresponding to 70% of the total protein content) include mainly histidine (26.27%) and glutaminc acid (16.59%), with minor amounts of aspartic acid (4.81%), valine (4.61%), arginine (4.51%), alanine (4.12%), serine (3.77%), tyrosine (2.57%), glycine (1.74%), asparagine (1.47%), γ-aminobutyric acid (0.26%) and glutamine (0.07%).[12]

Finally, BSG also contains a variety of minerals elements, among which silicon, phosphorus and calcium are the most abundant (Table 2). Silicon is also the most abundant mineral element in other cereals such as rice, oat and wheat straw, but the content of phosphorus and calcium in BSG is particularly higher than those commonly reported for these cereals.

Compared with other agro-industrial by-products (Table 3),[13–18] BSG presents lower cellulose content but similar lignin content, which would allow its use as raw material for the production of valuable compounds such as activated charcoal, phenolic compounds, dispersant, emulsificant and chelant agents, pesticides, fertilizers, polymers, adhesives, components for resins, etc.[19] On the other hand, BSG presents elevated hemicellulose content, which is higher than that present in several other crop by-products, including rice straw, wheat straw, rice husks, barley straw and oat straw. Protein in BSG is also present at elevated levels, while in other crop by-products it is usually found in minor proportions. The presence of hemicellulose sugars and protein in high amounts in BSG makes this material very interesting for food and biotechnological applications, as will be discussed in the next sections.

TECHNOLOGIES FOR BSG USE

As mentioned above, BSG is rich in sugars and proteins. Therefore the main and quickest alternative that has been used for its elimination is as animal feed. In the past, most of the BSG generated by brewing activities was disposed of to the environment. Currently, breweries usually supply this material at low cost to local farmers for use as cattle feed. However, BSG is a raw material of interest for application in different areas because of its low price, large availability throughout the year and valuable chemical composition. The following sections summarize and discuss the most recent efforts that have been made towards BSG use in food, in energy production and in chemical and biotechnological processes.

BSG USE IN FEED AND FOOD, AND BENEFITS TO HEALTH

The possibility of BSG application in the food area, both as animal feed and in the human diet, has been evaluated for a long time, and, increasingly, health benefits arising from the ingestion of BSG compounds have been found. As a consequence, many research centres continue investigating the possibility of using BSG in the manufacture of different food products. More recently, investigations have also been performed aimed at recovering compounds of interest from this material, which could be added to food products to promote differential characteristics and possible beneficial effects on health.

Use as animal feed

The main current application of BSG is as cattle feed, where it can be utilized directly in wet form (as separated from the mash tun) or as dry material. The high contents of fibre and protein in BSG together with the low cost of this by-product make it a substrate of great interest for use as animal feed. When combined with inexpensive nitrogen sources such as urea, for example, BSG is able to provide all the essential amino acids needed for animal nutrition.[20] Additionally, the inclusion of BSG in the cow's diet increases milk production and the content of total solids while decreasing the content of fat in the milk produced.[21,22]

The important advantages resulting from the inclusion of BSG in cattle feed encouraged the application of this material in the feeding of other animals such as poultry,[23–25] pigs[26,27] and fish.[28–31] Again, positive effects such as body weight gain were reported as a consequence of BSG supplementation of the diet

Table 2. Chemical composition of brewer's spent grain (BSG)

Item	Mussatto and Roberto[7]	Kanauchi *et al.*[8]	Carvalheiro *et al.*[9]	Silva *et al.*[10]	Meneses *et al.*[11]	Waters *et al.*[12]
Components (g kg^{-1} dry weight basis)						
Cellulose (glucan)	168	254	219	253	217	260
Hemicellulose	284	218	296	419	192	222
Xylan	199	NR	206	NR	136	NR
Arabinan	85	NR	90	NR	56	NR
Lignin	278	119	217	169	194	NR
Acetyl groups	14	NR	11	NR	NR	NR
Proteins	153	240	246	NR	247	221
Ashes	46	24	12	46	42	11
Extractives	58	NR	NR	95	107	NR
Minerals (mg kg^{-1} dry weight basis)						
Silicon	10740	NR	NR	NR	NR	1400
Phosphorus	5186	NR	NR	NR	6000	4600
Calcium	3515	NR	NR	NR	3600	2200
Magnesium	1958	NR	NR	NR	1900	2400
Sulfur	1980	NR	NR	NR	2900	NR
Potassium	258.1	NR	NR	NR	600	700
Sodium	309.3	NR	NR	NR	137.1	100
Iron	193.4	NR	NR	NR	154.9	100
Zinc	178.0	NR	NR	NR	82.1	100
Aluminium	36.0	NR	NR	NR	81.2	NR
Manganese	51.4	NR	NR	NR	40.9	NR
Cobalt	NR	NR	NR	NR	17.8	NR
Copper	18.0	NR	NR	NR	11.4	NR
Strontium	12.7	NR	NR	NR	10.4	NR
Iodine	NR	NR	NR	NR	11.0	NR
Barium	13.6	NR	NR	NR	8.6	NR
Chromium	5.9	NR	NR	NR	$<$0.5	NR
Molybdenum	NR	NR	NR	NR	1.4	NR
Boron	NR	NR	NR	NR	3.2	NR

NR, not reported.

Table 3. Chemical composition of different agricultural by-products

Component (g kg^{-1} dry weight basis)	Agricultural by-products									
	Rice straw[13]	Wheat straw[14]	Barley straw[15]	Oat straw[15]	Rice husks[16]	Barley husks[16]	Corn cobs[16]	Corn stover[14]	Sugarcane bagasse[17]	Spent coffee[18]
Cellulose	434	450	401	398	367	214	344	407	431	86
Hemicellulose	229	161	222	234	173	325	343	266	252	367
Lignin	172	241	194	182	213	192	188	260	229	NR
Acetyl groups	NR	NR	17	18	16	17	31	NR	NR	22
Proteins	NR	NR	34	25	25	59	43	NR	NR	136
Ashes	114	68	35	39	143	155	13	43	28	16
Extractives	NR	80	NR	NR	88	14	5	23	43	NR

NR, not reported.

of these animals.[28] Nowadays, BSG is considered as a low-cost alternative to the feed ingredients used in poultry diets. However, most of the cell wall polysaccharides, including arabinoxylan and β-glucan, present in BSG are not digested in the gastrointestinal tracts of these birds, because they do not have the enzymes needed for hydrolysis of the polymer chains.[32] Therefore xylanase and β-glucanase enzymes have been commonly added to BSG to overcome this problem.

Incorporation in human diet

Apart from being a material of interest for animal feed, BSG has also been considered as an ingredient of value for use in the human diet. In fact, the high content of fibre, protein and minerals present in BSG is very attractive to improve the nutritional value of foods. Additionally, the ingestion of BSG provides several health benefits, such as accelerated transit time, increased faecal weight and fat excretion (alleviating both constipation and diarrhoea), decreased gallstone incidence and reduced plasma cholesterol and postprandial serum glucose levels.[33,34] These beneficial effects are attributed to the presence of glutamine-rich protein, non-cellulosic polysaccharides and soluble dietary fibres, including $(1 \rightarrow 3,1 \rightarrow 4)$-$\beta$-glucan, in BSG. When ingested in the form of a protein-rich fibrous foodstuff, BSG is able to improve the clinical condition and endoscopic score of patients with mild to moderate ulcerative colitis, which is associated with an increase in stool butyrate concentration.[8,35]

Owing to all the above-mentioned benefits, the possibility of BSG application in food products has been extensively evaluated. There are many studies reporting, for example, the incorporation of BSG in the manufacture of bakery products such as breads, biscuits, cookies, muffins, cakes, waffles, pancakes, tortillas, snacks, doughnuts and brownies. To be incorporated in these products, BSG is firstly converted to flour, because its original form is too granular for direct application.[36–39] The incorporation of BSG in bakery products increases the contents of protein, fibre and amino acids while decreasing the calorie content of the final product.[12,20,36,40] Additionally, BSG incorporation in foods imparts higher water absorption and lower fat absorption capacities to the final product. However, some care must be taken when incorporating BSG in human food. Since BSG has a brownish colour, it is only recommended for application in coloured products such as some breads, cookies, cakes, etc. in order not to affect the final colour of the product. Moreover, the incorporation of only small amounts (up to 100 g kg^{-1}) in food formulations has been recommended, with the objective of avoiding alterations in the flavour and texture of the final product.[36–38,40,41]

Although BSG has been mainly used to prepare bakery products, the use of this material to produce other food products has also been evaluated recently. One example is the use of BSG for the production of frankfurters, where the addition of BSG allowed the fibre content in these products to be increased without negatively affecting their sensory parameters. Since BSG promotes these characteristics in the final product, it was suggested for use as a fat substitute for producing high-fibre and low-fat meat products.[42] Another recent application of BSG is for the production of tarhana, a fermented wheat flour/yoghurt product. The utilization of milled BSG in tarhana production resulted in acceptable soup properties in terms of most of the sensory properties. The slightly lower values of some sensory properties (colour, odour and taste) were considered to be compensated by the health benefits provided by the increased content of fibre in the product.[43]

Although the incorporation of BSG in food products contributes important properties, further research is still needed to improve the quality (appearance, texture and taste) of the final product as well as to evaluate consumer acceptance.

Source of ingredients for food applications

In recent years, bioactive compounds, including phenolic compounds, have received much attention for incorporation in food products owing to their potential to promote human health, such as antioxidant, antiallergenic, anti-inflammatory and antimicrobial effects and reduction of the incidence of diabetes and cancer as well as of the risk factors of cardiovascular diseases.[44] Owing to these important beneficial effects on human health, research aimed at finding natural resources rich in bioactive phenolic compounds has intensified.

BSG is rich in bioactive phenolic compounds with antioxidant activity, which can be recovered by various extraction methods, including solid–liquid extraction, microwave-assisted extraction, enzymatic reactions and alkaline reactions. Among these methods, solid–liquid extraction using 60% (v/v) acetone as solvent for 30 min at 60 °C proved to be highly efficient to extract antioxidant phenolic compounds from BSG.[11] Ferulic and *p*-coumaric acids are the phenolic acids present in higher amounts in BSG and may contribute significantly to the antioxidant potential of this material. Flavonoids have also been suggested to be strongly correlated with the antioxidant capacity of BSG.[11]

Antioxidant phenolic compounds extracted from BSG represent a promising alternative for application in the food industry in order to avoid the use of synthetic antioxidants. This would be an interesting alternative for application of BSG, since antioxidant phenolic compounds are commercially expensive and have many industrial applications not only in food but also in the pharmaceutical area.

BSG USE IN ENERGY PRODUCTION

Several alternatives have been proposed for BSG use in energy production, including thermochemical conversion (pyrolysis, combustion), biogas production and ethanol production. The production of energy from BSG is motivated by the energy crisis that the world is currently experiencing. Additionally, the large availability of this material together with its chemical composition and low cost makes BSG a raw material of great interest for application in this area.

Thermochemical conversion

One of the possible alternatives to generate energy from biomass materials like BSG is by using thermochemical conversion technologies such as pyrolysis and combustion. BSG presents important net and gross calorific values of 18.64 and 20.14 MJ kg^{-1} dry mass respectively[45] and therefore can be considered as an interesting raw material to produce energy by combustion. To be used in combustion processes, BSG must be initially dried to moisture content lower than 550 g kg^{-1}, which can be done by pressure, for example.[46] The heat generated by combustion of the BSG could be used to support the energetic demand of breweries in a brewery-integrated system. However, BSG combustion generates the emission of particles[47] and toxic gases that contain nitrogen and sulfur dioxide at 1000–3000 and 480 mg m^{-3} respectively.[48] For these reasons, it is very important to take special care when performing the combustion of BSG in order to avoid or minimize these problems.

Another interesting alternative is using BSG to produce charcoal bricks. The production process consists of an initial step where BSG is dried, followed by pressing and carbonization in a low-oxygen atmosphere.[49] The charcoal bricks produced by this method have high calorific value (27 MJ kg^{-1}), which is higher than the calorific value of the original BSG and similar to the calorific value of charcoals produced from other raw materials such as wood, sugarcane, grape bagasse, olive bagasse and hazelnut shell.[50–52] On the other hand, charcoal bricks produced from BSG present burning properties inferior to those reported for sawdust charcoal, for example, because the ignition temperature is higher and the burning period is longer.[53]

Intermediate pyrolysis of BSG at 450 °C using a twin coaxial screw reactor known as a Pyroformer gives 29% yields of char, 51% of bio-oil and 19% of permanent gases. Bio-oil, which is the most abundant fraction obtained by this process, contains a complex mixture of low- to intermediate-molecular-weight hydrocarbons, including benzene, cyclooctatetraene, hexene, toluene, xylene, phenolic-derived aromatic compounds, undecanoic acid and methyl esters.[54]

Biogas production

Several studies have reported the possibility of producing biogas from BSG. This application can be considered especially suitable for obtaining thermal energy in breweries with few adverse environmental effects. Biogas contains approximately 55–65% methane, 30–45% carbon dioxide, traces of hydrogen sulfide and fractions of water vapour. Biogas production processes by anaerobic fermentation can be divided into two steps: (1) an initial hydrolytic step to promote complete degradation of the material; (2) a methanogenic step in which acidogenic microorganisms convert the macromolecules released from the previous hydrolytic stage to volatile fatty acids, acetate, butyrate and propionate, and subsequently methanogenic bacteria convert these volatile acids to methane.[55] The hydrolysis step can be performed by using different methodologies such as chemical/thermal treatment, crushing (by wet rotor grinding or ball milling) or enzymatic treatment. The most important factor is to use conditions able to promote complete degradation of the material structure so that the next stage of conversion can be performed with elevated yields.

Anaerobic batch fermentation of BSG resulted in the production of biogas with a total yield of 3476 cm^3 per 100 g BSG after 15 days of digestion.[55] The theoretical potential of methane production per tonne of BSG is estimated at 98 N m^3 of methane.[56] However, the biogas production from BSG is inhibited by intermediate lignocellulosic biodegradation products, mainly *p*-cresol.[57] The inhibition is not prevented even by submitting BSG to mechanical, thermochemical or chemical pre-treatments. The biogas production from BSG is therefore a research area that merits great attention in order to develop a stable production process. The adaptation of anaerobic microbial biomass to elevated concentrations of *p*-cresol as well as submitting BSG to a biological pre-treatment stage with fungi or bacteria could be possible alternatives to overcome this problem. Currently, biogas production from BSG is limited to larger breweries owing to the economic constraints of smaller units.

Ethanol production

In the last decade, ethanol has experienced unseen levels of attention owing to its value as a fuel alternative to gasoline, the increase in oil prices, and climatic changes, as well as being a renewable and sustainable energy source that is efficient and safe to the environment. Currently, worldwide ethanol production is at high levels, and corn is the main raw material used for this purpose. However, as this kind of feedstock is essentially food, this scenario is expected to change owing to the incentive that has been given for the production of second-generation ethanol, i.e. ethanol produced from lignocellulosic waste materials.[58] Various lignocellulosic raw materials have been studied for this purpose, among which BSG has also been considered owing to its high content of hemicellulose and cellulose fractions. In the last decade, interest in establishing a competitive process for second-generation ethanol production led to an intensification of research in this area, and BSG was considered as a promising raw material for this application, being evaluated in different kinds of processes using different microbial strains.

Both the hemicellulose and cellulose fractions of BSG can be used for ethanol production. In a recent study, ethanol was produced with 86.3% conversion efficiency by fermentation of the xylose-rich hemicellulosic hydrolysate produced by dilute acid hydrolysis of BSG with *Pichia stipitis*. This result was obtained without supplementing the BSG hydrolysate with nutrients,[59,60] which is an important aspect for the economy of the process and makes BSG different from other raw materials used for second-generation ethanol production, such as sugarcane bagasse, which require the addition of nutrients to the fermentation medium for the efficient conversion of sugars to ethanol.[61,62]

In order to produce ethanol from cellulose, some pre-treatment of the raw material is required to make the extraction of glucose from cellulose easier, for subsequent use as a carbon source for fermentation. Pre-treatment may involve, for example, acid treatment, microwave digestion, ultrasonication or enzymatic hydrolysis, among others. Some recent literature data[63–66] concerning ethanol production from BSG cellulose using different pre-treatment and fermentation conditions are presented in Table 4. The ethanol yield varied significantly according to the process utilized. For example, the hydrolysate prepared by enzymatic digestion of BSG pre-treated with 0.16 mol L^{-1} HNO_3 was fermented to ethanol by *P. stipitis* NCYC 1540 and *Kluyveromyces marxianus* NCYC 1425, resulting in ethanol conversion yields of 63 and 45% of the maximum theoretical value respectively.[63] An ethanol yield of 74 g kg^{-1} dry BSG was obtained by fermentation with *Neurospora crassa* DSM 1129 under aeration of 0.1 vvm using alkali-pre-treated BSG as raw material.[64] This result was improved to 109 g kg^{-1} dry BSG (corresponding to 60% of the theoretical yield) when the fermentation was carried out under similar process conditions but using the fungus *Fusarium oxysporum* F3[65] (Table 4). Such results demonstrate that BSG has great potential for use in ethanol production, but establishment of the best fermentation conditions is fundamental to maximize the conversion results.

An alternative system that has been evaluated in order to improve the production of ethanol from BSG is a consolidated bioconversion process using the mesophilic fungus *F. oxysporum* F3. Using this system, the cellulolytic and hemicellulolytic enzymes are produced under solid state cultivation, while the alkali-pre-treated BSG is converted to ethanol by *F. oxysporum* in a consecutive submerged fermentation. A yield of 65 g kg^{-1} dry BSG was obtained using alkali-pre-treated BSG under microaerobic conditions (0.1 vvm), which corresponded to 30% of the theoretical ethanol yield based on the total glucose and xylose composition of BSG[66] (Table 4).

Recently, a method for the production of fuel ethanol from BSG was patented. The method consists of the following steps: (1) pre-treatment of the BSG with acid and heat to degrade the hemicellulosic fraction; (2) hydrolysis of the pre-treated BSG with enzyme to convert starch and cellulosic material to simple sugars; (3) fermentation of the simple sugars to ethanol with an ethanol-producing microbe (which can be *Zymomonas mobilis*, *Saccharomyces cerevisiae*, *Escherichia coli*, *Bacillus subtilis* or *Pichia pastoris*); (4) distillation of the ethanol produced by fermentation; (5) dehydratation of the ethanol to remove water.[67]

Table 4. Technologies reported for ethanol production from brewer's spent grain (BSG) and results obtained

Pre-treatment method	Microorganism	Production conditions	Ethanol yield	Ethanol efficiency (% of theoretical yield)	Reference
Acid pre-treatment with 0.16 mol L^{-1} HNO_3 at 121 °C for 15 min, followed by enzymatic digestion (cellulase + hemicellulase) at 50 °C, 150 rpm for 18 h	*Pichia stipitis* NCYC 1540	Erlenmeyer flasks containing 50 mL of fermentation medium, 30 °C, 100 rpm	0.32 g g^{-1} sugars	63	White *et al.*[63]
	Kluyveromyces marxianus NCYC 1425		0.23 g g^{-1} sugars	45	
Alkali pre-treatment using 1 g NaOH per 10 g BSG at 121 °C for 30 min	*Neurospora crassa* DSM 1129	2 L bioreactor containing 1 L of fermentation medium, 30 °C, 0.1 vvm, 180 rpm	74 g kg^{-1} BSG (equivalent to 0.17 g g^{-1} sugars)	36	Xiros *et al.*[64]
Alkali pre-treatment using 1 g NaOH per 10 g BSG at 121 °C for 30 min	*Fusarium oxysporum* F3	2 L bioreactor containing 1 L of fermentation medium, 30 °C, 0.1 vvm, 180 rpm	109 g kg^{-1} BSG	60	Xiros and Christakopoulos[65]
Alkali pre-treatment using 1 g NaOH per 10 g BSG at 121 °C for 30 min	*Fusarium oxysporum* F3	Consolidated process: solid state fermentation at 30 °C for enzyme production, followed by submerged fermentation in a 2 L bioreactor containing 1 L of fermentation medium at 30 °C, 0.1 vvm for ethanol production	65 g kg^{-1} BSG	30	Xiros *et al.*[66]

BSG USE IN CHEMICAL PROCESSES

BSG is a raw material of interest for chemical processes either to obtain (by extraction) or produce (by chemical reaction) compounds of industrial interest. Several possible applications have been proposed for BSG use in this area, among which its use as an adsorbent material for wastewater treatment appears to be one of the most promising applications.

Extraction of valuable compounds by chemical processes

As mentioned above, BSG is rich in cellulose and hemicellulose polysaccharides, which are added value compounds with many industrial applications. In order to extract these compounds from the BSG structure, several chemical processes can be employed, among which acid and hydrothermal hydrolysis have been widely used. By chemical hydrolysis, cellulose can be converted into glucose, while hemicellulose can be converted into xylose and arabinose, which are the most abundant sugars present in this fraction of BSG.[7,11]

Depending on the conditions used for hydrolysis, the polysaccharide structure may not be completely hydrolysed and low-molecular-weight oligosaccharides are then obtained in the final reaction medium. The molecular weight of the oligosaccharides released from the BSG structure varies according to the temperature and reaction time used during the pre-treatment. For example, a hydrolysate containing a variety of arabino-oligoxylosides with different structural features can be obtained by hydrothermal treatment of BSG at 150 °C for 60 and 120 min.[68] On the other hand, dilute acid hydrolysis of BSG at 120 °C using a liquid/solid ratio of 8 g g^{-1}, 100 mg H_2SO_4 g^{-1} BSG and a reaction time of 17 min promotes the hydrolysis of the hemicellulose structure to the corresponding monomeric sugars with 92.7% efficiency.[69] Heating by microwave radiation at 160 °C in the presence of 0.1 mol L^{-1} HCl is also an efficient method to hydrolyse BSG polysaccharides, providing an 80% yield.[70]

Alkaline hydrolysis using NaOH solution is efficient to extract lignin from BSG.[71] The BSG lignin solubilized in the alkaline liquor can be separated by precipitation with H_2SO_4[19] and used as starting material for a series of useful products such as dispersant, emulsificant and chelant agents, activated charcoal, polymers, adhesives and fertilizers, among others.

Hydroxycinnamic acids can also be recovered from the BSG structure by means of chemical treatment. Ferulic and *p*-coumaric acids are the most abundant hydroxycinnamic acids in BSG and can be efficiently recovered by alkaline hydrolysis using 20 g L^{-1} NaOH solution in a solid/liquid ratio of 1 g per 20 g at 120 °C for 90 min.[72] Microwave-assisted extraction is also a technology with potential to extract polyphenols, including ferulic acid, from BSG.[73] Considering that ferulic and *p*-coumaric acids have antioxidant properties and important industrial applications, the extraction of these acids from BSG offers new possibilities for the reuse of this industrial by-product in food, cosmetic and/or pharmaceutical areas.

Antioxidant phenolic compounds can also be recovered from BSG by extraction with solvents. Various solvents, including methanol, ethanol, acetone, hexane, ethyl acetate, water and mixtures of methanol/water, ethanol/water and acetone/water, can be used for this purpose. Nevertheless, acetone/water mixtures, especially at 60% (v/v), are more efficient to release these compounds from the BSG structure.[11] Antioxidant phenolic compounds extracted from BSG could be used as a natural and inexpensive alternative to synthetic antioxidants.

Raw material for pulp and paper production

Owing to its fibrous nature, BSG is considered as a suitable raw material for use in the production of papers. BSG has already been used to prepare paper towels, business cards and coasters, conferring high-grade texture on these products.[74] A more recent study reported the production of bleached cellulose pulp from BSG. A cellulose-rich pulp (904 g kg^{-1}) with low hemicellulose and extractive contents (79 and 34 g kg^{-1} respectively) was obtained by soda pulping of acid-pre-treated BSG. Subsequently, the pulp was bleached by a totally chlorine-free sequence carried out in three stages, using 5% (v/v) hydrogen peroxide in the first two stages and 0.25 mol L^{-1} NaOH solution in the last stage. Using this procedure, a bleached pulp presenting a kappa number of 11.21, viscosity of 3.12 cP, brightness of 71.3%, cellulose content of 957 g kg^{-1} and residual lignin of 34 g kg^{-1} was obtained from BSG.[75]

Adsorbent material

One of the most interesting and promising applications of BSG in chemical processes is as an adsorbent for removing either organic compounds from waste gases or dyes from wastewater. The adsorption capacity of volatile organic compounds on pyrolysed BSG is similar to that on coconut shell charcoal.[76]

In aqueous solution, BSG is able to adsorb cadmium, lead and chromium with sorption capacities of 17.3, 35.5 and 18.94 mg g^{-1} respectively.[77,78] However, previous treatment of the BSG with NaOH is needed to enhance its metal sorption capacity. The capacity of BSG to adsorb cadmium, lead and chromium from aqueous solution is similar to the capacity reported for other materials such as tree fern, wheat bran, sugarcane bagasse, sugar beet pulp and coconut shell.[79–82] However, a novel adsorbent consisting of esterified BSG was recently demonstrated as having improved ability to remove cadmium from aqueous solution. Esterified BSG presented very high efficiency for cadmium removal in a wide pH range of 4–8, with a maximum adsorption capacity of 473.93 mg g^{-1}, and showed good reusability during nine cycles of sorption/desorption.[83]

Another important and potential application of BSG as an adsorbent is for the removal of dye from wastewater. BSG presents elevated capacity to adsorb acid orange 7 dye (AO7), which is commonly used in the paper and textile industries and whose presence in effluents causes serious environmental problems. BSG is able to adsorb this dye with an adsorption capacity of 30.5 mg AO7 g^{-1} BSG at 30 °C, without requiring any previous treatment to improve the adsorption.[10,84] This result opens up real possibilities for BSG application in this area, since adsorption processes must be efficient, fast and use cheap adsorbents to compete with other techniques.

BSG can also be used as a raw material to produce activated carbons, which are widely used for the purification of water and gases, among many other applications. The process to produce activated carbon from BSG lignin consists of the following steps: recovery of the lignin from the BSG structure by alkaline treatment, followed by its precipitation with H_2SO_4 and the impregnation/activation of the recovered lignin with 3 g H_3PO_4 g^{-1} lignin at 600 °C. The activated carbon produced from BSG presents an adsorption capacity similar to or even better than that of some commercial products and shows potential for use on wastewater treatment, since it is able to absorb phenolic compounds and metallic ions (mainly Ni, Fe, Cr and Si) from liquid media.[85]

BSG USE IN BIOTECHNOLOGICAL PROCESSES

BSG is a substrate of high potential for use in biotechnological processes, since it is rich in polysaccharides, proteins and minerals. Therefore many studies have been focused on the use of BSG as a raw material to extract or to produce valuable compounds by fermentation or enzymatic processes. The most recent advances on the use of BSG in biotechnological processes are discussed below.

Substrate for microorganism cultivation/enzyme production

The main evidence that BSG is a good substrate for cultivation of microorganisms lies in the fact that, as separated from the lauter tun, it is a very unstable material able to deteriorate rapidly via microbial activity. Such rapid deterioration occurs mainly as a consequence of the elevated content of water that it possesses when separated from the lauter tun (800 g kg^{-1}) and the presence of proteins and sugars in its composition. Up to eight different fungal strains from the genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* were found in BSG when it was stored in wet form in gunnysacks for 30 days.[86] Therefore it is strongly recommended to reduce the moisture content of BSG to less than 100 g kg^{-1} in order to safely store it.

Taking this important property of BSG into account, several studies have been proposed using this material as a low-cost substrate for cultivation of fungal strains. Species of *Aspergillus*, *Pleurotus*, *Lentinus*, *Trametes*, *Neurospora* and *Agrocybe*, for example, are able to grow successfully when cultivated in BSG. However, not only the elevated moisture content and the presence of proteins and sugars favour the fungal growth in BSG, but some physical properties of this material such as particle size, volume weight, specific density, porosity and water-holding capacity also influence the growth of these microorganisms.[87] Besides fungi, *Streptomyces* bacteria are also able to grow and show enhanced sporulation when cultivated in a protein fraction isolated from BSG.[88]

In many cases the production of enzymes by cultivation of microorganisms in BSG has been reported. Some examples include the production of α-amylase by cultivation of *Aspergillus oryzae*,[89–92] *B. subtilis*,[93] *Bacillus licheniformis*[94] and *Bacillus* sp. KR-8104;[95] cellulases by *Trichoderma reesei*[96] and *N. crassa*;[64] xylanases by *Streptomyces avermitilis*,[97] *Aspergillus awamori*,[98] *N. crassa*,[64] *Aspergillus fumigatus*,[99] *Talaromyces stipitatus* and *Humicola grisea*;[100] feruloyl esterase by *S. avermitilis*,[97] *N. crassa*,[64] *T. stipitatus* and *H. grisea*;[100] laccases by *Trametes versicolor*;[101] and arabino-furanosidases and acetyl esterase by *N. crassa*.[64] In most cases it is not necessary to supplement the BSG with any nutritional source to obtain efficient enzyme production, but in some cases the addition of nutrients such as sources of nitrogen, amino acids, vitamins and/or inorganic compounds may improve the enzyme yield.

Extraction of valuable compounds by enzymatic processes

As mentioned above, some chemical methods can be used to extract valuable compounds from the BSG structure. However, some biological methods may also be used for this purpose, with the additional advantage that they do not generate toxic effluents, therefore being considered more environmentally friendly. In this sense, several enzymes have been used to extract valuable compounds from BSG. Mixtures of polysaccharide hydrolases from *Aspergillus japonicus*, *A. versicolor* and *T. reesei*, for example, are able to hydrolyse more than 42% of the total polysaccharides in BSG to sugars in only 1 day.[102] Some commercial microbial proteases are also able to release the non-cellulosic glucose, a portion of feruloylated arabinoxylan and over 50% of the protein from BSG after 24 h of hydrolysis.[103] Nevertheless, it is known that lignocellulosic materials such as BSG present a rigid structure that makes the action of enzymes difficult. Additionally, the presence of lignin in these materials decreases the efficiency of enzymatic hydrolysis. The negative effect of lignin on the enzymatic hydrolysis of BSG has been reported recently.[104]

Taking this into account, an alternative to obtain elevated yields of soluble sugars after enzymatic hydrolysis of BSG is to pre-treat this material (with dilute acid or base, for example) so as to make the polysaccharide structure more accessible to the enzymes. In order to avoid extra costs for the process, the pre-treatment stage should be planned in a biorefinery concept, where the liquid fraction obtained from the pre-treatment stage is also utilized for a valuable application. For example, Mussatto *et al.*[104] pre-treated BSG by sequential processes of dilute acid hydrolysis and alkaline hydrolysis in order to extract glucose by enzymatic hydrolysis of the cellulose fraction, attaining a conversion yield of cellulose to glucose of 85.6%. This value was further improved to 93.1% when the conditions of agitation, enzyme loading and substrate concentration used for enzymatic hydrolysis were optimized.[105] The liquid fraction obtained from the acid pre-treatment of BSG was used for the production of xylitol,[69,106–108] while the liquid fraction obtained from the alkaline pre-treatment was used for the production of activated carbon.[85]

Besides extracting sugars, enzymes can also be used to extract other compounds from the BSG structure, such as hydroxycinnamic acids (ferulic and *p*-coumaric acids). A combination of esterase from *Aspergillus niger* and xylanase from *Trichoderma viride* was able to extract 30% of the total ferulic acid present in BSG.[109] An enzyme preparation from the thermophilic fungus *Humicola insolens* released almost all the ferulic acid and 9% of the *p*-coumaric acid present in BSG.[110]

Raw material for fermentation processes

Several alternatives have been proposed to use BSG as a raw material for the production of value-added compounds by fermentation. The possibility of using BSG as a carbon and/or nutrient source has been demonstrated in both solid state and submerged fermentation systems for the production of a variety of compounds of industrial interest.

As mentioned above, the sugar-rich hydrolysate produced by dilute acid hydrolysis of BSG can be used for ethanol production by fermentation using different microorganisms. BSG hydrolysate can also be successfully used for the production of xylitol by yeasts such as *Candida guilliermondii*[69,106–108] and *Debaryomyces hansenii*.[111,112] Optimization of the conditions for dilute acid hydrolysis of BSG is fundamental to produce a hydrolysate rich in xylose but with low amounts of inhibitory compounds (furfural, hydroxymethylfurfural, acetic acid and phenolic compounds). By optimizing the hydrolysis conditions, xylitol was produced by *C. guilliermondii* with good yield (0.70 g g^{-1}) and productivity (0.45 g L^{-1} h^{-1}).[69] Based on experimental results, the xylitol production from BSG is estimated as approximately 0.107 g g^{-1} raw material.[106] However, the xylitol yield and productivity can be improved by increasing the xylose content available for conversion into xylitol.[107,108] Xylitol can be produced by *C. guilliermondii* from concentrated BSG hydrolysate (70 g L^{-1} xylose) with a yield of 0.78 g g^{-1} and productivity of 0.58 g L^{-1} h^{-1}. An important advantage of this process compared with the processes using hydrolysates produced from other raw materials is that BSG hydrolysate does not require supplementation with nutrients to be efficiently used as a fermentation medium for xylitol production.[108]

Debaryomyces hansenii has the ability to simultaneously produce xylitol and arabitol when cultivated in a pentose (xylose and arabinose)-rich hydrolysate obtained by autohydrolysis followed by H_2SO_4 hydrolysis of BSG.[111,112] This is a very interesting fact, because BSG has a significant amount of arabinose sugar in its composition, and this pentose sugar is not easily assimilated by many microorganisms. Conversion of arabinose to value-added products such as arabitol contributes to complete utilization of BSG.

BSG can also be used in fermentation processes for the production of lactic acid. The fermentation of a molasses/BSG/coconut water medium by *Lactobacillus plantarum* resulted in highly efficient (93–95%) production of lactic acid after 4 days of processing.[113] In more recent studies, lactic acid was produced by *Lactobacillus delbrueckii* from a glucose-rich medium obtained by enzymatic hydrolysis of BSG cellulose with a commercial cellulase preparation.[114,115] The best bioconversion performance occurred when the hydrolysate was supplemented with nutrients and the pH was controlled at 6. Under these conditions, lactic acid was produced with a yield and productivity of 0.99 g g^{-1} glucose and 0.59 g L^{-1} h^{-1} respectively. The nutritionally enriched BSG hydrolysate was considered as an efficient substrate for lactic acid production by *L. delbrueckii*.[115]

An interesting and novel application that has been reported for BSG is as raw material for the production of a distilled beverage.[59] In order to produce this distillate, BSG is initially submitted to a hydrothermal process that aims to extract aroma compounds, and the produced extract is submitted to fermentation and distillation processes. The produced distillate contains 38 volatile compounds contributing to the aroma and flavour and presents organoleptic quality acceptable for human consumption.

Finally, the possibility of using BSG as an additive for fermentation processes has also been reported. The addition of a neutralised acid extract of BSG to the wort enhanced the yeast performance during the fermentation for beer production, and the produced beer presented similar quality to that fermented without BSG addition.[116]

Carrier for cell immobilization

Fermentation systems using immobilized cells are being increasingly utilized owing to the several advantages that they present compared with traditional fermentation systems using free cells. Such systems are eco-friendly and the immobilized cells completely maintain their biological functions with increased stability, leading to increased cell productivity. As a consequence of the high cell concentration in the fermentation medium, the efficiency and productivity of the process are also improved. In addition, immobilized cell systems make the separation of the cells from the fermented broth easier for subsequent use in repeated batch operations.[117,118] Nevertheless, the use of a suitable immobilization carrier is fundamental to obtain an effective system for each particular application.

A variety of inorganic and organic materials can be used as supports for cell immobilization; however, those of natural origin are commonly preferred, since they are renewable, biodegradable, non-toxic, inexpensive and readily available. In this sense, several studies have evaluated the possibility of using BSG as a carrier for cell immobilization. BSG can be successfully used for immobilization of *S. cerevisiae* in order to produce beer by a high-gravity continuous process.[119,120] It is also a promising biocatalyst for use in batch winemaking at low fermentation temperatures.[121] Additionally, BSG is a good material for use as a cell immobilization carrier for the production of pectinase by *K. marxianus* CCT 3172[122] and for the production of fructooligosaccharides and β-fructofuranosidase by *A. japonicus*.[118] As a whole, BSG can be considered as a material with great potential for use for cell immobilisation, since it has an irregular structure and a non-homogeneous chemical composition, which provide active sites that are easily colonised by microorganisms. Although BSG can be used as an immobilization carrier without any previous processing, treatment of this material with acid and/or base solutions may be useful to improve its immobilization capacity.

ECONOMIC AND ENVIRONMENTAL ASPECTS RELATED TO BSG USE

Many possibilities for BSG valorization have been reported, but none of them has been implemented on an industrial scale up till now. Currently, supplying this wet material to local farmers for use as cattle feed continues to be the main solution of the breweries for its elimination, since this is a cheap alternative that avoids the energy spend needed for drying the BSG. However, the costs of transporting BSG are significant, corresponding to an average of U$16 per tonne of wet BSG transported a distance of 5 miles (~8 km). Therefore BSG is usually supplied to local farmers (preferentially no further than 5 miles from the brewery) in order to minimize the costs involved in its elimination. Nevertheless, in some cases, the BSG produced may surpasses the demand for cattle feed required by the nearby farmers. In other cases, there are no farms close to the breweries. Both situations result in increased amounts of BSG, with nowhere to send it. The most common option that has been used in these cases is disposal to the environment. However, more recently, some breweries have surpassed this problem by using BSG as an energy source for their own companies. By installing special combustion equipment for the purpose of burning BSG to produce steam energy, the breweries are able to recover over 50% of their energy costs. Using spent grain as a source of fuel allows breweries to conserve energy and also benefit the environment, as it reduces leftover grain waste (100 000 t of BSG leaves only 2000 t or less of ash as a result of the process) and eliminates the need to transport BSG to nearby farmers, with consequent reduction of carbon emissions.

Although the burning of BSG for energy generation can be an interesting and fast solution for the breweries to eliminate this by-product, other promising alternatives for BSG reuse have been proposed, as discussed above. Some of them could be of interest for the brewing industry directly, such as using BSG for the production of fuel ethanol for transportation, for the production of paper for the labels of their packaging, as a nutrient source for yeast cultivation and as a carrier for cell immobilization. In most of these cases, successful implementation of such technologies in breweries would be expected owing to their technical nature (equipment, facilities, laboratories and so on). However, BSG can also be a raw material of interest for other industries, as discussed above. Independently of the application that will be given to the BSG (in the brewery or in other industries), a technological and economic analysis would be useful in order to verify the possibility of implementation of the proposed technologies on an industrial scale. In this sense, a recent study was carried out in order to draw recommendations on the technological, economic and environmental feasibility for implementation of a biorefinery for BSG utilization as raw material for the production of xylitol, lactic acid, activated carbon and phenolic acids, taking into account the Brazilian case. Full mass integration of water plus full energy integration was the configuration with the best economic and environmental performance, proportioning an economic margin of 62.25%, with a potential environmental impact of 0.012 potential environmental impact (PEI) kg^{-1} products, and the carbon footprint of the processing stage representing 0.96 kg CO_2-equivalent emission (e) kg^{-1} BSG.[123]

CONCLUSIONS

BSG is an important agro-industrial by-product in terms of quantity generated and valuable chemical composition. Studies have been focused for decades on the reuse of this material for valuable applications, but in the last decade the research in this area has been intensified, possibly motivated by environmental and economic concerns. The current literature suggests a number of possible applications for e BSG reuse in food, energy, chemical and biotechnological processes. Important advantages and benefits resulting from these applications have been demonstrated. The advances that have been observed in this research area suggest that the implementation of industrial processes for BSG reuse may be a close reality.

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