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Fungal Biovalorization of a Brewing Industry Byproduct, Brewer's Spent Grains: A Review

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ABSTRACT

The beer industry is a major producer of solid waste globally, primarily in the form of brewer's spent grains (BSG), which due to its low value has historically been diverted to livestock as feed or to landfills as waste. Its high moisture content and chemical composition positions BSG as an ideal candidate for further processing with microbial fermentation, and recent research has focused on filamentous fungi and the ability of some species therein to degrade the predominant recalcitrant cellulolignin components of BSG to produce valuable compounds. Many species have been investigated to biovalorize this waste stream, including those in the genuses *Aspergillus*, *Pennicillium*, *Rhyzopus,* and *Trichoderma*, which have been used to produce a wide array of highly valuable enzymes and other functional compounds, and to increase the nutritional value of BSG as an animal feed. This review of recent developments in the application of filamentous fungi for the valorization of BSG will discuss the biochemical makeup of BSG, the biological mechanisms underlying fungi's primacy to this application, and the current applications of fungi in this realm. As the majority of these studies are at lab-scale, the challenges to scale-up and more widespread application and will be discussed as well.

Keywords: brewer's spent grains, brewing, fungal biovalorization, food waste, malt

INTRODUCTION

Though the process of brewing beer has been present in human culture for many generations, the composition of its byproducts has not changed significantly. Beer is produced using a combination of enzymatic and microbial fermentation processes to transform raw grains into a final product rich in carbohydrates, mainly in the form of oligosaccharides, and ethanol. Prior to brewing, these grains, mainly barley, are malted, where a process of partial germination begins to break down storage structures and enzymes are synthesized. At the brewery, this malt is milled and added to warm water, called the mash, where the endogenous enzymes convert starches into fermentable sugars, mostly consisting of maltose and maltotriose, and non-fermentable oligosaccharides, colloquially referred to as dextrins. Additionally, proteins are partially digested to peptides and amino acids. Following this step, the liquid portion, known as wort, is drained, separated from the insoluble materials, boiled, and finally fermented to produce

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ethanol and other byproducts before being packaged as beer [1]. The solid fraction remaining after the extraction of the wort consists primarily of barley husks and other insoluble materials [1], and is known as brewer's spent grain (BSG) (*Figure 1*). The processing of the grains in a brewery is mainly focused on the digestion of starch and extraction the of hydrolyzed products, with little focus on other compounds present in the raw materials. Due to this myopic view on ingredient usage, the solid waste stream produced by this industry is significant in its high residual composition of other functional compounds not relevant to beer production.

Figure 1. Brewer's spent grains (BSG) consisting of barley malt removed from the lauter tun at the UC Davis Pilot Brewery. Photo courtesy of Emily Newman.

The global beer industry is reported to produce over 2.3 x 10^{11} liters (2.3 x 10^{8} tonnes) of beer annually [2]. BSG represents an estimated 85 % of the solid waste produced from brewery operations, where the other 15 % are represented by trub (precipitated protein and insoluble materials after boiling), spent hops, spent yeast, and adsorbent solids from filtration, including diatomaceous earth [3]. BSG is generated from beer at an estimated rate of 19.7 % (kg BSG per kg beer produced) [4], resulting in an estimated generation of 4.5 x 10^{7} tonnes of BSG annually worldwide.

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Once the mash has had all the available wort extracted, the remaining BSG has historically been of low commercial value and is typically sold or given away as animal feed or transported to landfills as waste [5], which may incur significant costs the brewery. Neither of these processes represent the most efficient use of this byproduct and may actually produce further negative environmental impacts. As animal feed, it is primarily destined for ruminants, such as cattle, which are known to be significant producers of greenhouse gasses, and as a group are identified as the single largest anthropogenic source of methane [6]. Ruminant livestock alone in the US are estimated to produce 28 % of the total methane emitted annually [7]. Though BSG specifically has not been shown to increase methane production compared to other feeds, the diversion of this byproduct to animal feed lays the ownership of the environmental impact on the brewing industry. Similarly, the addition of highly fermentable waste to landfills increases their already unmanaged anaerobic fermentation, which also emits greenhouse gasses, such as carbon dioxide and methane [8]. At their most basic level, both methods, landfill and animal feed, are leveraging the power of microbial fermentation to transform the substrate. In the landfill, microbes are employed to reduce BSG volume, and as animal feed, the rumen microbiota act to degrade BSG into more bioavailable nutrients [9]. It is, however, through the unmanaged nature of these processes that the maximum potential commercial value of BSG is not realized, nor are the negative externalities mitigated.

Other potential avenues have been proposed for the re-routing of BSG as a byproduct, including as building materials [10], charcoal [5], material for paper manufacture [11], and energy generation through direct combustion [12], as reviewed by Mussatto *et. al.* [5]. However, none of these have been implemented widely, primarily due to the high moisture content of BSG as it exits the brewery. At 75 - 80 % dry basis moisture content [13], it has not been considered appropriate for combustion-based energy generation [12], and drying can be costly [14]. For these reasons, BSG remains of low value currently.

COMPOSITION OF BSG

BSG is produced year-round and globally, though its chemical composition can be is highly variable due to many factors. Physically, BSG is made up of the largely recalcitrant barley husk, but also portions of the grain endosperm, with contains small amounts of starch, proteins and lipids [5]. As shown in *Table 1*, BSG is mainly composed of non-starch polysaccharides (NSPs), including cellulose, beta-

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glucan, and hemicellulose, primarily represented by arabinoxylan. Combined, these compounds may contribute to over 60 % of the dry weight of BSG [13]. Arabinoxylan the most abundant NSP, at 21 - 30 %, with lignin second at 12 - 22 % [13]. Importantly, the protein content of BSG is also prominent, at 10 - 26 % [13]. The large variability in all components reflects the heterogeneity of BSG; it can vary based on the barley variety, harvest time, growing conditions, addition of other grains or starches added to the mash, and the malting and mashing conditions [13].

Table 1. Variability in composition of Brewers Spent Grain, adapted from Xiros & Christakopoulos [13]

Component	Lowest Published Measure	Highest Published Measure
protein	10.0	26.7
lipids	3.0	10.6
starch	1.0	13.0
ash	1.2	4.6
non-starch glucans (incl. cellulose)	0.3	21.9
arabinoxylan (hemicellulose)	21.0	29.6
lignin	11.9	22
phenolics	0.7	2.0

All values are expressed in g per 100 g dry matter (% w/w)

Cellulose, hemicellulose and lignin make up most of the cell walls of plants and act to maintain structural rigidity. They are collectively referred to as lignocellulosic biomass. Cellulose is the simplest of these polymers and consists of chains of glucose with β(1→4) glycosidic linkages up to hundreds of glucose monomers long [15]. Hemicellulose is a branched heteropolymer that is primarily represented by arabinoxylan in BSG. Arabinoxylan is composed of a backbone of β(1→4)-D-xylose, which can be di- or mono- substituted with α-L-arabinose. The polymer is highly heterogeneous, and may also have many substituents including uronic acids, phenolic acids, acetyl groups or proteins [16]. Lignin is a phenolic polymer of greater complexity than hemicellulose and is made up of phenylpropanoid units such as *p*-coumaryl, coniferyl, and sinapyl alcohol. Beyond its structural rigidity, lignin is specifically resistant to microbial attack through non-specific adsorption and binding of hydrolytic enzymes and through the toxicity of lignin derivatives [17]. Cellulose is frequently embedded into the lignocellulosic matrix, which increases its resistance to enzymatic hydrolysis [17]. Furthermore, because both hemicellulose and lignin

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are heterogeneous in their branching and may have huge ranges of degree of polymerization, they are generally resistant to enzymatic degradation [18].

The chemical makeup of BSG is not unique to agricultural or industrial waste streams. There are many other grain industries that produce byproducts of similar lignocellulosic makeup, including wet corn distillers grain, a byproduct of the ethanol production [19]. Furthermore, the remaining agricultural residue after the grains are harvested, such as barley straw, is high in these lignocellulosic compounds [18]. An assortment of other agricultural byproducts and their cell wall components are listed in *Table 2*. Due to their similar biochemical makeup, these biomass residues suggest an extensive range of potential substrate applications beyond BSG.

Table 2. Crop residues from plant species used for beer brewing and their cell wall components. Many of these contain contain cellulose, hemicellulose and lignin in concentrations similar to BSG. Adapted from Graminha *et. al*, 2008 [20]

Crop	Residue fraction	Cellulose	Hemicellulose	Lignin
Barley	straw	31.0 – 45.0	25.4 – 38.0	11.0 – 19.0
	bran	23.0	32.0	21.4
Wheat	straw	27.0 – 40.0	21.0 – 32.0	9.8 – 20.0
	bran	30.0	50.0	15.0
Rice	straw	28.0 – 47.0	19.0 – 28.0	4.3 – 24.0
	bran	35.0	25.0	17.0
Corn	straw	33.5	24.9	7.8
	bran	33.8	39.3	4.9
	silage	38.0 – 40.0	28.0	7.0 – 21.0
	stalk	33.6	23.7	8.7
	leaf	24.5	27.3	5.4
	cob	37.7	39.6	7.3
Oats	straw	30.0	22.0	8.5
	bran	49.3	25.0	18.0
Sorghum	stalk	27.0	25.0	11.0

All values are expressed in g per 100 g dry matter (% w/w)

ROLES FOR FUNGAL TRANSFORMATION OF BSG

Plant polymers such as cellulose, hemicellulose, and lignin, though indigestible to humans, have long been used as nutrient sources for many microorganisms [21]. Fungi, in particular, have been specifically targeted for their application to use substrates such as BSG, as they are well known to digest similar compounds in their natural environment [21]. The heteropolymeric nature of both hemicellulose and lignin requires a battery of enzymes, each with specific activity to hydrolyze the different types of

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bonds [18]. Arabinoxylan is considered difficult to degrade by microorganisms due to its structural complexity, for example, yet filamentous fungi have been shown to produce an array of enzymes that work synergistically to fully degrade the polymer. Xylanase enzymes in fungi have been reviewed by Knob *et al.* 2010 [22], the most relevant of which are endo-β-1,4-xylanase and β-D-xylosidase, but also include accessory enzymes such as α-L-arabinofuranosidase, β-glucuronidase, among others, that act to cleave subunits off of the main xylan chain.

For this reason, filamentous fungi are considered prime candidates for the degradation of lignocellulosic-rich BSG substrate and provide a more cost- and energy-efficient alternative to systems that apply a combination of heat, chemicals and purchased enzymes [23]. The most cost-effect bioreactor model, solid-state fermentation (SSF), where the media contains little to no free-flowing liquid between the solid particles and solids density is very high [24]. This is particularly well-suited for filamentous fungi [20], as their hyphae can penetrate the spaces between particles [25]. Additionally, the water activity and moisture content of untreated BSG leaving the brewery is within range of typical SSF media [24], and would not require additional drying or water addition.

Research into the use of fungi with BSG as a substrate for industrial purposes may be classified into three different categories for the purpose of this review: (1) the production of enzymes (2) the production of other valuable compounds, including bioethanol, and (3) to increase the nutritional quality of the BSG for animal feed (*Table 3*). Some of these processes can even achieve several goals simultaneously, and different species of microbes may be used in tandem to that end.

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Table 3. Fungal species and the end products of fermentation of BSG, as separated into different production categories.

Production Category	End Product	Species Used	Reference
enzymes	α-amylase	*Aspergillus oryzae*	Xu *et al.* 2008 [26], Patel *et al.* 2005 [27]
	amyloglucosidase	*Aspergillus fumigatus*	Adeniran *et al.* 2010 [28]
		Aspergillus niger	
		Helminthosporium	
		Penicillium frequestans	
	β-amylase	*Aspergillus fumigatus*	
		Aspergillus niger	
		Helminthosporium	
		Penicillium frequestans	
	cellulases	*Aspergillus flavus*	Orji *et al.* 2016 [29]
		Aspergillus fumigatus	Grigorevski-Lima *et al.* 2009 [30]
		Fusarium oxysporum	Xiros *et al.* 2008 [26]
		Neurospora crassa	
		Trichoderma reesei	Benko *et al.* 2007 [31]
		Trichoderma spp	Napolitano *et al.* 2006 [32]
	glucanases	*Trichoderma* spp	Napolitano *et al.* 2006 [32]
	hemicellulases	*Fusarium oxysporum*	Xiros & Christakopoulos 2009 [33]
		Neurospora crassa	Xiros *et al.* 2008 [26]
		Penicillium brasilianum	Panagiotou *et al.* 2006 [34]
		Penicillium janczewskii	Terrasan *et al.* 2010 [35]
		Talaromyces stipitatus	Mandalari *et al.* 2005 [36]
		Trichoderma spp	Napolitano *et al.* 2006 [32]
	proteases	*Aspergillus oryzae*	Sandhya *et al.* 2005 [37]
	xylanases	*Moesziomyces*	Faria *et al.* 2019 [38]
		Moesziomyces aphidis	
		Penicillium janczewskii	Terrasan & Carmona 2015 [39]
other products	bioethanol	*Neurospora crassa*	Deshpande *et al.* 1986 [40], Xiros *et al.* 2008 [26]
	ethanol (for bioethanol)	*Zymomonas mobilis*	Dávila *et al.* 2016[23]
	glucose (for bioethanol)	*Trichoderma reesei*	
	xylose (for bioethanol)	*Candida guilliermondii*	
	citric acid	*Aspergillus niger*	Dhillon *et al.* 2011 [41], Pathania *et al.* 2018[42]
	single cell protein	*Aspergillus niger*	Aregbesola & Omafuvbe 2014 [43]
	xylitol	*Candida guilliermondii*	Mussatto & Roberto 2005 [44]
		Trichoderma reesei	Amorim *et al.* 2019 [45]
nutrient-enhanced feed	hemicellulose digestion	*Penicillium janczewskii*	Terrasan & Carmona 2015 [39]
	increased amino acids, citric acid, antioxidants & vitamins	*Rhizopus oligosporus*	Cooray & Chen, 2018 [46]
	increased protein content	*Aspergillus awamori*	Bekatorou *et al.* 2007[47]
		Aspergillus oryzae	

Enzyme Production

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The production of enzymes has proven the most interesting and well-researched potential products of BSG fermentation, as enzymes have a myriad of applications in food, pharmaceutical, and chemical industries. A challenge for the cost effectiveness of these industrial processes is the large amounts of enzymes that are required. For example, an estimated 25 % of the cost of second generation (lignocellulosic) bioethanol production is solely from the purchasing of enzymes used to degrade the lignocellulosic material [48]. Filamentous fungi are particularly well-suited to the production of enzymes for later extraction for a few reasons. First, many of their plant cell wall-degrading enzymes are secreted extracellularly into the substrate medium, which allows for their extraction without the need to disrupt the fungal cells downstream [22]. Second, to a greater degree than yeasts and bacteria, fungi are known to produce extracellular enzymes at much higher concentrations [22], and thus their yield can be much greater.

Fungi have been used to produce enzymes industrially for many years [21], though the use of fungi with BSG for enzyme production is a newer application. Species across many genuses (*Table 3*), including *Aspergillus*, *Penicillium* and *Fusarium,* have been investigated separately for enzyme production at lab scale with BSG substrate in the past few decades with positive results [13]. The most frequently investigated enzymes are those used to break down the lignocellulosic material. *Aspergillus flavus* has been used in SSF bioreactors with BSG to produce cellulase enzymes [29], and both *Moesziomyces antarcticus* and *Moesziomyces aphidis* have been shown to produce xylanases at very high activity when using BSG as a substrate [38]. Interestingly, ligninolytic enzymes are known to be produced by many species of fungi [49], yet little research has been done on BSG as a substrate for its production and may indicate a potential avenue for future research.

Thermostability of the enzymes produced by fungi in BSG has also not been extensively researched, though it is a highly desirable trait for industrial purposes. Most enzymes exhibit enhanced activity at higher temperatures, and those higher temperatures can also inhibit unwanted microbial growth [18]. Many xylanolytic enzymes produced by fungi on different media are, however, not heat stable [21]. In general, the majority of microbial enzymes used in industrial processes are mesophilic, and used from

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35 – 60℃ [50]. For this reason, thermostability in fungal-produced enzymes should be further researched.

Bioethanol and Other Products

BSG can also be used by fungi for bioethanol production. Some species of fungi have been investigated for their ability to produce enzymes, as well as to degrade the lignocellulosic material. After BSG polymers have been broken down into smaller constituent parts, some fungi are able to further extract energy through fermentation of those remining BSG parts into ethanol. *Neurospora crassa*, a mold of the phylum Ascomycota has been known for some time to have the ability to simultaneously convert lignocellulosic material to fermentable sugars, and then ferment those sugars in to ethanol [40]. More recently, *N. crassa* has been proven applicable in BSG fermentation, with an optimized method utilizing enzyme production in SSF bioreactors, followed by lignocellulose hydrolysis and ethanol production in a submerged-state bioreactor [51].

In other studies, it has been proposed that a combined approach may be applied to produce multiple product streams, which incorporate not only fungal processes, but chemical hydrolysis and bacterial fermentations in tandem or in sequence. Dávila *et al.* [23] developed a model in which a theoretical BSG plant could incorporate the breakdown of both arabinoxylan and cellulose into the highly valuable products xylitol, ethanol and polyhydroxybuterate. In this model, the BSG is chemically pre-treated with an acid to produce a xylose-rich hydrosylate from arabinoxylan that is then fermented by *Candida guilliermondii* yeasts to produce xylitol. This yeast is an ideal candidate for the xylitol production step, as it produces xylose reductase (EC.1.1.1.21), which is well known to produce high yields of xylitol from xylose from a multitude of sources [52]. In this proposed model, the cellulose fraction is physically separated and processed with the mold *Trichoderma reesei* to produce glucose. The glucose is then routed to two separate bioreactors to produce different products from the glucose. In one of the bioreactors, the yeast *Zymomonas mobilis* ferments approximately 60 % of the glucose to produce ethanol, and the remaining glucose is processed into polyhodroxybuterate by the bacterium *Cupriavidus necatur*.

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Species in the fungal genus *Tricoderma*, including *Trichoderma reesei*, have been used for decades for bioethanol production [53], but only with their purified cellulase enzymes produced industrially. However, they have not to date been used as extensively in ethanol-producing bioreactors to break down biomass such as BSG. Furthermore, genome sequencing of this species has shown that up to ten genes for different cellulases and hemicelluases are present in the genome, though only four of them are produced at sufficient quantities for industrial production [54]. The presence of many other genes that produce these enzymes, other fungal species have also been investigated for their potential for cellulase production, or to be used in tandem with *T. reesei*, including *Aspergillus foetidus*, and a number of *Penicillium* species, including *P. verruculosum*, *P. pinophilum*, *P. funiculosum* and *P. echinulatum*, with varying success [54].

Partial BSG Degradation for Enhanced Feed Quality

Currently, the most likely disposal route for BSG utilization is as animal feed, particularly for cattle. It has also been proposed for use with pigs, poultry and even fish [55]. However, the value of BSG may increase through the bioconversion of some compounds with fungi. Feed processing methods have been developed that use feed substrates that are partially hydrolyzed by and still contain active cellulase and hemicellulose enzymes in it but no live fungi. The addition of these enzymes to feed for dairy cattle biochemically similar to BSG has been shown to increase milk yield, presumably by increasing the digestibility as the enzymes remain active in the gastrointestinal tract [20, 56]. Similarly, pre-treatment of animal feed by fungal fermentation can increase the quality of the feed itself, especially in protein content, but also in other micronutrients. The *Aspergillus* species *A. oryzae* and *A. awamori* have been shown to increase the protein content of BSG by 20 - 36 % as a result of increased fungal biomass [47]. Other studies have shown *Rhizopus oligosporus* is also capable of increasing both crude protein and soluble protein by approximately two times that of the original BSG for similar reasons [57]. More recently, fermentation using *R. oligosporus* has shown to further enhance nutritional value by increasing the concentration of some amino acids, citric acid, vitamins, and antioxidants [46].

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CHALLENGES FOR FUTURE DEVELOPMENT

The techniques involved in many of the processes reviewed herein have similar challenges. As can be seen in *Table 1*, variability exists geographically and temporally to the composition of BSG depending on a myriad of factors. A structured process must be used to account for this variability and provide guidance in the management of a highly variable substrate.

Conversely, the moisture content of BSG is consistent in that it is generally high, at approximately 75 - 80 % moisture, dry basis [14]. Coupled with the dispersed geographical nature of breweries, incurs substantial financial and energetic cost to transport to a disposal or processing facility. For this reason, drying prior to transport has been proposed, though this would increase the overall energy usage as the moisture content of BSG is roughly 90 % [14]. Additionally, the high moisture content and lack of cooling of BSG as it leaves the brewery brings with it a high microbial risk [58]. BSG held at room temperature (20 ℃) has been shown to exhibit extensive microbial activity and leads to the degradation of arabinoxylan [59]. These challenges in the processing stream have not been thoroughly investigated.

Finally, most of the studies reviewed here have only proven the viability of lab-scale processes, and scale-up of many of them will prove challenging. Scale-up of BSG fermentation tanks can lead to increased oxygen transfer into the substrate and therefore increased xylanase production for some bacterial species [60], but it is not known how this will affect filamentous fungi. Solid state fermentation at scale requires precise heat- and mass-transfer models [61] that have not been addressed.

CONCLUSION

BSG is a significant byproduct of the brewing industry that has historically been relegated to the cheapest forms of food waste disposal. Because of its high moisture and lignocellulosic content, it is most frequently diverted as low-quality animal feed or simply to landfills. However, through the use of filamentous fungi, this substrate has the potential to yield many high value products, or even increase its value as feed through bioconversion. A wide range of species have been studied for their ability to use BSG as a substrate for the production of enzymes, monosaccharides and bioethanol, among other functional compounds. Additionally, fungi have been leveraged for their ability to increase the nutrient

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quality of BSG for livestock feed. Though the techniques developed for these processes are specific to the composition of BSG, it is the functionality of the fungal species used that indicates the potential for expansion of these techniques to similar and abundant waste streams. Fungi are enzymatic powerhouses that can be used not only in the valorization of BSG, but also for other crop residues high in lignocellulosic materials. With further research into this field, as well as addressing the challenges to scaling-up, this process may be applied more widely, and beer production may become more environmentally sustainable.

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AUTHOR CONTRIBUTIONS

Andrew Marcus wrote the manuscript. Glen Fox assisted with final editing of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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