



Recycling potential of brewer's spent grains for circular biorefineries

Deepti Agrawal^{1,a}, Deeksha Gopaliya^{2,a},
Nicholas Willoughby³, Sunil K. Khare² and Vinod Kumar⁴

Abstract

Brewer's spent grain (BSG) is the major by-product of the brewing industry. BSG is principally composed of carbohydrates and proteins, with substantial amount of lipids. Presently, BSG usage is restricted to low-grade applications such as ruminant feed or landfills. The high volume, nutrient-rich composition, low cost (€35/ton), abundance, and around the year availability, makes it a promising and renewable feedstock for biorefinery development. The current review begins with beer production process, where BSG is produced. Further, it appraises emerging biotechnological advancements and green processes targeting BSG valorisation ensuring maximal resource recovery. Particularly, it illustrates diverse marketable products obtained by repurposing carbohydrate and protein fraction of BSG using either isolated or cascading approach. We believe that this review will encourage more research groups to work on developing innovative technologies for integrated and holistic valorisation of BSG. Inclusive efforts towards reduced water consumption and waste minimisation is further advocated, which are presently primary challenges associated with beer industry. It will leave a significant imprint on environmental sustainability and pave a way for developing circular bio-based economy.

Addresses

¹ Biochemistry and Biotechnology Area, Material Resource Efficiency Division, CSIR-Indian Institute of Petroleum, Dehradun, India

² Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, 110016, India

³ Institute of Biological Chemistry, Biophysics and Bioengineering, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, EH14 4AS, UK

⁴ School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK

Corresponding author: Kumar, Vinod (Vinod.Kumar@cranfield.ac.uk)

^a Co-first authors.

Introduction

Brewer's spent grain (BSG) is an abundant, highly nutritious by-product of the brewing industry, holding special significance by virtue of its specific biochemical composition. This lignocellulosic material accounts for 85% (w/w) of the total by-products generated in beer production and is formed from grains leftover after the mashing and filtration process [1]. Statistically, every litre of beer produced, generates ~0.2 kg of BSG. Considering this data, in the year 2021, 37.2 million tonnes of BSG was produced globally from 1.86 billion hectolitres beer, demonstrating the abundance of this valuable bioresource [2]. Currently, BSG is primarily used as a low-value feed ingredient suitable for ruminants; or otherwise ends up in landfill posing a potential environmental threat [3]. However, BSG is emerging as an ingredient in functional foods, owing to relatively high levels of dietary fibers in the form of β -glucan and arabinoxylan, proteins, and the presence of polyphenols (such as hydroxy-cinnamic acids) which display high antioxidant or other nutritional properties. All these components are considered to offer potential health benefits [4,5]. Furthermore, depolymerisation of the carbohydrate fraction produce simple sugars, which are precursors for the production of several industrially important products using either chemical or biochemical routes. However, the high degree of biomass recalcitrance, along with limited shelf life and increased risk of susceptibility to microbial attack due to high moisture content act as major roadblocks in harnessing the true potential of BSG.

These opportunities have shifted the attitude towards BSG, and researchers are adopting a holistic approach and targeting maximum resource recovery, thereby showcasing its value as biorefinery feedstock [6,7]. This review begins with brief description on how this renewable waste originates and its distinct chemical composition. From the perspective of emerging global interests in implementing the concept of sustainable circular economy, the review then considers technological developments in the past five years where inexpensive carbon-rich BSG has been explored for the production of marketable and bio-based materials including green fuels and chemicals. Particularly, the review highlights the green approaches used for waste mining from BSG, which targets either recovery of its carbohydrate fraction or protein or have fractionated each of these valuable biologically important compounds

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Keywords

Brewer's spent grain, Valorisation, Carbohydrates, Protein, Circular economy.

in a cascading mode and attempted their valorisation. Furthermore, the existing challenges of beer industry are showcased and thrust on developing end-to end processes are discussed, which can leave everlasting impact on environmental sustainability besides developing bio-based economy.

A brief overview of the brewing process and chemical composition of BSG

Traditionally beer is fermented from malted barley. However, as beer styles and demand changed and, in recent times the use of other grains such as maize, wheat, and oats as adjuncts has escalated, thereby changing the chemical makeup of BSG. A typical process of beer production is outlined in Figure 1, showing various stages such as malting, milling, mashing, brewing, and fermentation [8].

The first step of brewing involves soaking of barley in warm water (steeping) before being allowed to rest and partially germinate. This process activates starch degrading enzymes within barley to produce metabolisable sugars, which yeast can metabolise. The barley is then kiln-dried. The combined step of steeping, germination and kilning is termed as malting. The dried malted barley is then subjected to roller or hammer mill to produce a coarse powder known as grist. If other grains are being used in addition to barley, they are usually added either directly before or directly after the mill, depending on the character required. The grist is soaked again in warm water in a process known as mashing. This releases soluble, fermentable sugars into the liquid. The residual barley solids (the spent grains)

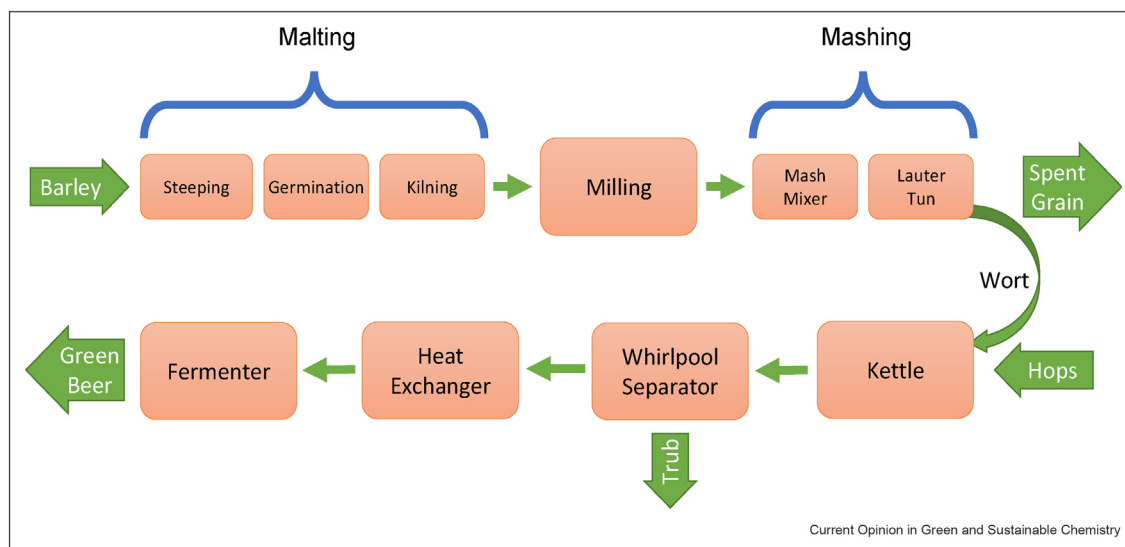
are removed, usually by filtration, to leave a liquid rich in soluble sugars (wort). The wort is then boiled for sterilisation prior to fermentation, causing protein coagulation and its precipitation, which may otherwise negatively affect the flavour or appearance of the beer. These precipitates (known as trub) are removed in a whirlpool and the cooled wort is transferred to a fermenter, where yeast ferments sugars to produce ethanol and other volatile organic compounds, conveying desired beer flavor profile. The 'green' beer from the finished fermentation can then be matured in various ways. Thus, BSG is the waste produced during the intermediate step of beer production.

Based on the compositional analysis, BSG essentially comprises of 60%–70% fibre and 15%–30% protein [9]. The detailed constituents of BSG as depicted in Figure 2, reveals its high nutritive nature and heterogeneous distribution of functional organic carbon compounds [10]. Considering its diverse chemical composition, recent research has primarily focused on either fractionating the key components in their pure form or hydrolysing its constituents followed by transformation to commercially viable products. Whilst some approaches chose to focus on carbohydrate or protein as their primary targets, others have attempted holistic utilisation of both key fractions of the biomass.

Carbohydrate fractionation of BSG and its valorisation

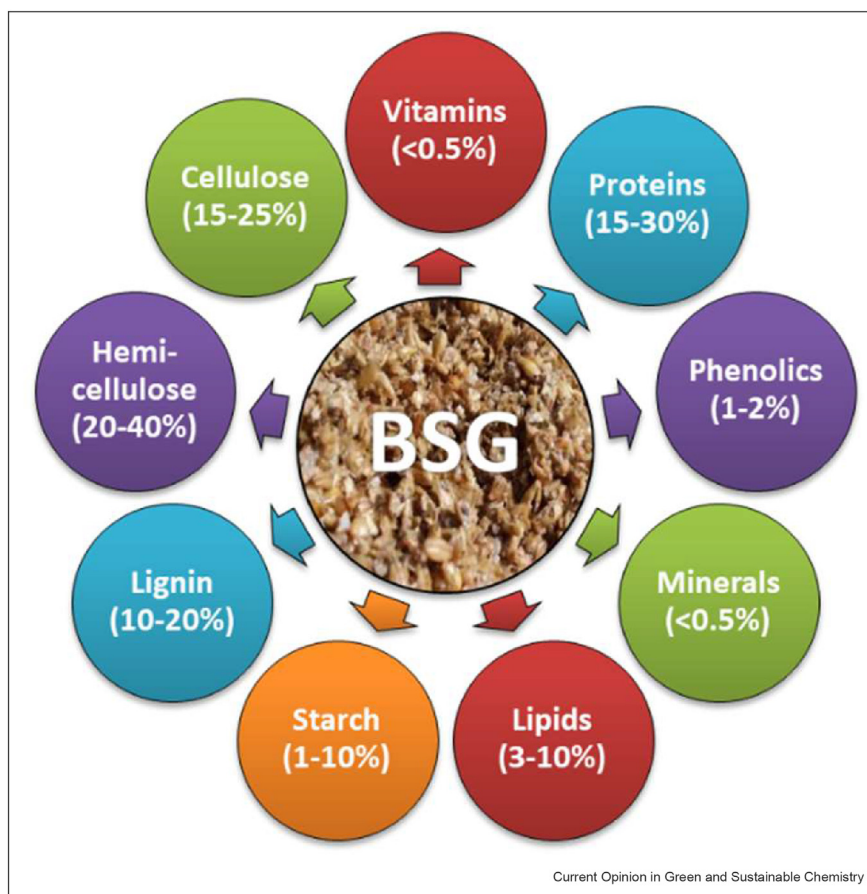
Being a rich source of the structural and storage polysaccharides, cellulose, hemicellulose, and starch, BSG serves as an inexpensive and efficient feedstock for the

Figure 1



Schematics of beer production in a typically barley-based brewery.

Figure 2



Detailed chemical composition of BSG.

production of a plethora of bio-based energy and non-energy products. Some of the commercially scalable products, produced from its carbohydrate fraction are shown in Table 1, covering the salient features of each process.

One area of research draws special attention to Table 1, where the researchers demonstrated the accumulation of > 100 g/L 2,3-butanediol (BDO) from the hydrolysed glucan fraction of BSG [17]. The group further conducted a detailed techno-economic analysis of the process, where they redirected the fermentation residue and the acid hydrolysate (which is rich in soluble sugars) for biogas production [23]. Process integration using pinch technology reduced the hot and cold utility consumption by 34 and 18%, respectively. A techno-economic assessment (TEA) revealed that the plant size critically affected the minimum selling price (MSP) of BDO. This price reduced significantly from 3.36 USD/kg to 2 USD/kg when the BSG processing capacity was raised from 100 to 2000 MT/day and BDO titre was assumed to be 100 g/L [23].

Complete valorisation of storage and structural carbohydrate fraction of BSG to ethanol was earlier attempted by Rojas-Chamarro *et al.* [24]. The authors developed an optimised dilute acid pretreatment strategy (12.5% solids; 1% H₂SO₄; 130 °C; 26 min) that recovered 94% carbohydrates in the resultant solid residue. Simultaneous saccharification and fermentation was performed using Cellic CTec3 and *Scheffersomyces stipitis*. Parallely, the acid hydrolysate comprising of variegated sugars and inhibitors was also fermented to produce ethanol using recombinant *E.coli* strain. The authors demonstrated production of 18.1 kg ethanol from 100 kg BSG [24]. Along similar lines, glucose-rich enzymatic hydrolysate of BSG was explored for BDO, lactic acid (LA) and ethanol production by *Bacillus polymyxa* DSM 742. The said process produced 15.43, 1.22, and 2.61 g/L of LA, BDO, and ethanol, respectively. However, when the same organism was chosen for the valorisation of detoxified acid hydrolysate rich in pentose sugars in a stirred tank bioreactor, LA was the major product (30.07 g/L) with minor quantities of acetate (3.96 g/L) [25].

Table 1

Commercially important and valorised products from carbohydrate fraction of BSG.

Product Type	Salient features of the study	Fermentative organism	Product			Reference
			Conc g/L	productivity g/L/h	Yield (g/g)	
Lactic acid	<ul style="list-style-type: none"> Maltose and malto-oligomer rich liquor produced by pressing BSG Yeast extract produced by yeast autolysis used during beer production 	<i>Lactobacillus delbrueckii</i> subsp.lactis	79.06	4.93	0.89	[11]
	<ul style="list-style-type: none"> Hydrolysate from BSG and malt rootlets generated by sequential enzymatic hydrolysis with α-amylase, α-amylase + amylase followed by cellulase Soy lecithin and Brewer's spent YE assessed as potential substitute of commercial yeast extract and their concentrations optimised using RSM 	<i>Lactobacillus rhamnosus</i> ATCC 7469	70.17	1.22	0.946	[12]
Ethanol	<ul style="list-style-type: none"> BSG pretreatment with H_2SO_4 & H_3PO_4 followed by Cellic CTec2 hydrolysis 	<i>Escherichia coli</i> SL100	37.2 [#]	0.547 [#]	0.84 [#]	[13]
	<ul style="list-style-type: none"> Fermentation ability of ethanologenic <i>E.coli</i> assessed 		38.6 ^{\$}	0.62 ^{\$}	0.81 ^{\$}	
	<ul style="list-style-type: none"> Submerged atmospheric air pressure plasma pretreatment in a dielectric barrier discharge (DBD) plasma reactor followed by enzymatic hydrolysis 	<i>S.cerevisiae</i>	25	0.347	0.16	[14]
	<ul style="list-style-type: none"> High solids hydrothermal pretreatment followed by Cellic CTec2 hydrolysis 	<i>S.cerevisiae</i> BLGII 1762	42.27	—	0.94	[15]
	<ul style="list-style-type: none"> Two yeast strains evaluated for hybrid saccharification and fermentation 	<i>S.cerevisiae</i> PE-2	40.3	—	0.82	
	<ul style="list-style-type: none"> On-pot sequential hydrolysis, saccharification and fermentation 	<i>Escherichia coli</i> MS04	29.5	0.983	—	[16]
	<ul style="list-style-type: none"> Use of recombinant bacterial ethanol fermenting strain capable of utilizing glucose, xylose and arabinose under non-detoxified conditions 					
2,3 Butanediol	<ul style="list-style-type: none"> Microwave assisted alkali pretreatment followed by cellulase hydrolysis optimised through Taguchi method Fed-batch fermentation with bacterial strain obtained after ethyl methane sulphonate (EMS) mutagenesis under pH controlled and aerated conditions 	Mutant strain of <i>Enterobacter ludwigii</i>	118.5	1.65	0.43	[17]
Itaconic acid	<ul style="list-style-type: none"> Hydrothermal organosolv pretreatment followed by enzymatic hydrolysis using commercial enzyme containing xylanase, endo β-glucanase 	<i>Ustilago maydis</i>	—	0.11	0.38	[18]
Butanol	<ul style="list-style-type: none"> Arabinoxylans selectively removed from BSG by microwave-assisted alkali pretreatment followed by ethanol precipitation 	<i>C. beijerinckii</i> DSM 6422	9.9	0.24	0.2	[19]
	<ul style="list-style-type: none"> Residual solid residue enzymatically hydrolysed with cellulase 					
	<ul style="list-style-type: none"> Dilute acid hydrolysis followed by Cellic CTec2-mediated saccharification 		13.8	0.11	0.17	[20]
	<ul style="list-style-type: none"> Fed-batch fermentation coupled with continuous gas stripping and pulsed feeding of concentrated glucose 					
Ascorbic acid	<ul style="list-style-type: none"> Alkaline peroxide pretreatment followed by Cellic CTec2-mediated hydrolysis 		11	0.118	0.3	[21]
	<ul style="list-style-type: none"> Fermentation media containing 0.6% brewery waste, 2% D-glucose 	<i>Aspergillus flavus</i> <i>Aspergillus tamaraii</i>	6.25 7.25	0.065 0.075	— —	[22]

Note: # Detoxified hydrolysate generated from H_2SO_4 pretreatment; \$ Undetoxified hydrolysate generated from H_3PO_4 pretreatment.

Recently, Kavalopoulos et al. investigated three valorisation pathways for BSG. Initially 70% of the BSG's lipid content was fractionated by solid-liquid extraction using hexane. Following this, 45% ethanol yield was obtained from BSG-derived sugars by fermentation after carrying out acid hydrolysis with 0.7N H₂SO₄ followed by saccharification with Cellic CTec2 on the residual solid post solvent extraction. Simultaneously, the biogas formation potential of raw and defatted BSG was assessed as well [26]. Likewise, Sganzerla et al. assessed the techno-economic feasibility of a BSG-based bio-refinery with fertiliser and biomethane as two major products. The authors concluded that a plant processing 137 tonnes BSG/day, selling 100% of its fertiliser and biomethane produced during anaerobic digestion (AD) for agricultural applications and natural gas, respectively, would have a pay-back time of 3.67 years, with return on investment (ROI) and internal rate of return (IRR) being 23.95% and 20.12%, respectively [27]. Swart et al., introduced a preliminary screw-press dewatering step to obtain BSG with 25% dry matter. Steam explosion (180 °C; 10 min) of the said biomass at pilot scale produced 73% xylo-oligosaccharides (XOS) [28]. Later, when three scenarios were evaluated for TEA of BSG valorisation, the most favourable scenario was the co-production of xylitol and XOS > XOS > xylitol [29]. Likewise, the hemicellulosic liquor fraction of BSG was evaluated for xylitol and ethanol production using *S. stipitis* and *Pachysolen tannophilus*. It was found that the former yeast produced better ethanol while the latter strain produced high xylitol under oxygen-limited conditions [30].

BSG-derived sugar hydrolysates have also been explored for the cultivation of oleaginous microbes, as their lipid composition is typically suitable for conversion to bio-diesel. For instance, recently, *Rhodospiridium toruloides* was grown on BSG hydrolysate (~72 g/L of monomeric sugars) generated by sequential organosolv process and enzymatic saccharification [31]. A maximum biomass of 18.44 ± 0.96 g/L was accumulated on a dry weight basis containing 56.45 ± 0.76% lipids. In another study, the suitability of sugar-rich BSG-derived acid hydrolysate was assessed for the cultivation of *Trichosporonoides spathulata* JU4-57, *Rhodotorula mucilaginosa* G43 and *Yarrowia lipolytica* TISTR 5151. *T. spathulata* emerged as the best oleaginous yeast and produced 62.9 mg lipid/g substrate [32]. Further isolated study confirmed that the unsaturated fatty acids constituted 78.4% of the total lipids and were predominant in oleic acid, followed by linoleic acid and palmitic acid [33].

Unlike more conventional products discussed in the preceding sections, recently BSG was simultaneously evaluated for lignocellulolytic enzyme production and its viscozyme L treated sugar-rich fraction was assessed for polyhydroxyalkanoate production. Three fungal strains (*Aspergillus niger*, *Thermoascus aurantiacus*, and

Trichoderma reesei) were grown on BSG using solid state fermentation (SSF) which displayed high xylanase activities with side activity of cellulases. Additionally, *Cupriavidus necator* produced a maximum of 9.0 ± 0.44 mg of PHA/g BSG, demonstrating the feasibility of BSG for the production of multiple bioproducts [34]. In a later work, the same group showed high polyhydroxy-3-butyrate (yield: 23 ± 1 mg PHA/g BSG) by *Burkholderia cepacia* when grown on enzymatically hydrolysed BSG under submerged conditions [35]. Similarly, *T. reesei* strain 938 (CBS 836.91) was grown on BSG as solid substrate for lignocellulolytic enzyme production. Later the same enzyme cocktail, liberated 193.35 mg sugars/g dry BSG from BSG pretreated with H₂O₂ [36].

Protein fractionation of BSG and its valorisation

Proteins constitute a significant fraction of BSG and are mainly present as hordien, glutelin, globulin, and albumin. These proteins offer numerous health benefits (eg. anti-inflammatory, anti-oxidant properties) over and above simple nutritional value, if extracted in a suitable fashion [37].

In a recent study, combinatorial protease pretreatment with 0.5% protamex and 0.1% flavourzyme (50 °C; pH 8.5; 3h) resulted in the production of protein hydrolysates (PH). In-depth techno-functional analysis revealed that these PH's had high protein content (31.4%). Further they showed high oxygen radical absorbance capacity (ORAC), and antioxidant properties (ABTS assay), with enhanced water solubility index, emulsifying activity, foaming capacity and stability. Additionally, the sediments from the process got enriched with Flavon-3-ols [38]. In a separate study, when the same group fortified yogurt with enzymatically hydrolysed (0.5% Protamex) BSG, it not only improved the survival rate of *Lactobacillus bulgaricus* but increased LA production also. Further, the texture, surface and functional properties of the yoghurt enhanced significantly, especially during long-time storage [39]. In yet another study BSG was fermented by *Rhizopus oligosporus* and later the fermented BSG was extracted with ethanolic-alkali mixture followed by protein precipitation at isoelectric point. Fermentation with *R. oligosporus* enriched protein from 25.2 to 34.1% [40]. The fermentation enhanced levels of essential amino acids (valine, methionine, lysine, and histidine) in the protein extract, but at the expense of proline and glutamine levels. In addition to significant improvements in foaming, emulsifying, water, and oil binding capacities, the protein displayed high anti-oxidant properties and no cytotoxic effect. The protein's application as a plant emulsifier and as an excellent substitute for egg during mayonnaise preparation was also demonstrated [40]. In the same year, Alcalase pretreatment of BSG improved the antioxidant or anti-oxidative properties of hydrolysed protein and enhanced emulsion capacity by 2.4 fold (from

30% to 72%). This study claims the potential of BSG-derived protein hydrolysate for food applications [41].

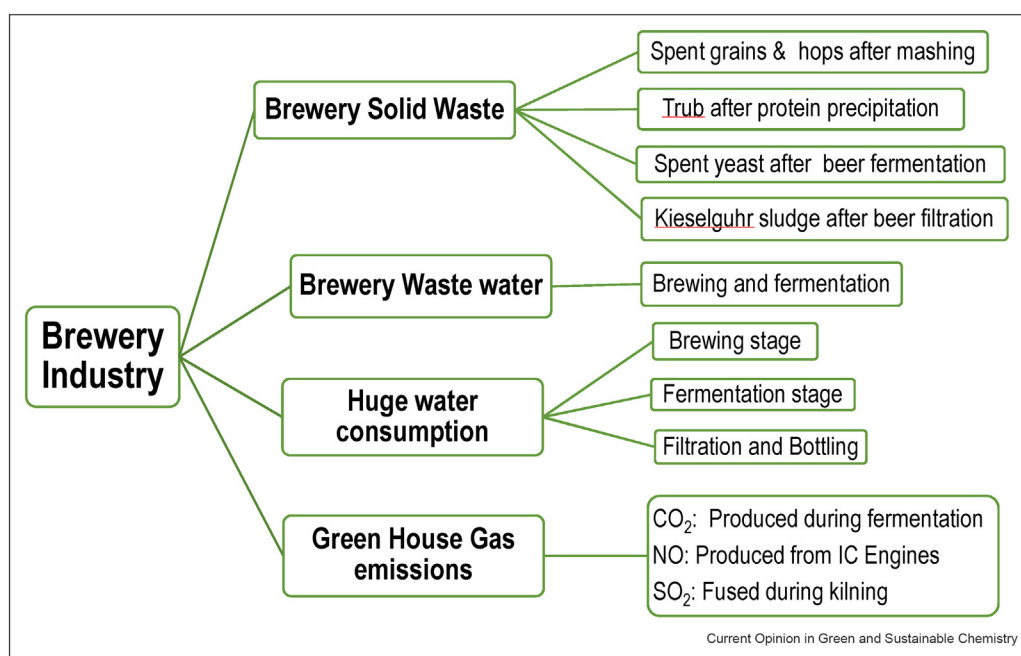
BSG fractionation in a cascading manner and valorisation

Complete fractionation of BSG has been employed by a few research groups, with intent of maximising the recovery of diverse functionally active components. For instance, Rojas-Pérez et al. [42] employed a sequential enzymatic deproteinisation followed by acid catalysed steam explosion (ACSE). ACSE hydrolysed the hemicellulosic fraction of BSG. The research reported recovery of ~64% protein, with 30% and 93% of xylose and arabinose, respectively, extracted as monomers in the acid hydrolysate. Hydrolysis of the glucan-rich residue resulted in 72.2% glucose yield. This investigation opens further avenues for the valorisation of the separated BSG fractions [42]. In another study, 85.65% lignin was recovered from BSG along with 90% hemicellulose removal by optimising an ethanol organosolv process. Simultaneously a highly digestible glucan-rich (59.68% cellulose yield with 37.6% purity) pulp with a low total crystallinity index (TCI) was generated. Further processing of recovered lignin reduced its yields to 58% but attained 95% purity [43]. Earlier, the same group performed hydrothermal pretreatment of BSG (180 °C; 30 min) leading to 83% and 48% solubilisation of starch and protein, respectively. When *Neurospora intermedia* was cultivated separately on the liquid and solid fraction post-pretreatment, this edible fungus accumulated

50.42 and 30.86%(w/w) protein with ethanol as a by-product, demonstrating the feasibility of BSG for the development of a brewery-based biorefinery [44].

Earlier, He et al. [45] took a holistic approach and in the first stage enzymatically separated the protein fraction of BSG, and later subjected the carbohydrate fraction to acid pretreatment followed by cellulase-mediated hydrolysis. Alcalase (20 µL/g) pretreatment led to the generation of hydrolysate with a protein concentration of 41.4%. On the other hand, sugars produced in Stage II were assessed for BDO production using thermophilic *Bacillus licheniformis* YNP5-TSU under sterile and non-sterile conditions. A maximum BDO concentration of 20.4–21 and 12.2–12.3 g/L was attained from sugar-rich enzymatic hydrolysates and mixed sugars (acid and enzymatic hydrolysates) respectively, with productivity being 0.285 and 0.17 g/L/h, respectively [45]. These examples elucidate the emerging trends and opportunities which are yet to be unleashed fully, targeting fractionation of important constituents of BSG and their valorisation. However, there are various processes during brewing which pose critical environmental threats and need to be simultaneously addressed. These issues are dealt in forthcoming section, citing remedies and path forward. The authors anticipate that an integrated approach can further optimise both carbon and water footprint of breweries and contribute towards sustainability development goals of nations where it is a pre-dominant revenue generating industry.

Figure 3



Environmental challenges associated with brewing industry.

Current challenges and path forward

Huge water consumption, production of solid waste, waste water and greenhouse gas (GHG) emissions at various stages of brewing (Figure 3), pose severe environmental challenges as per the extensive review done by Olajire [46]. Reduced water consumption and waste minimisation are presently high-priority areas for the brewing industry. Hence, a holistic approach is highly desirable and should not be underestimated. To accomplish these targets, brewing industries should accelerate their R&D efforts and work cohesively with academia to address these critical issues. A rapid upsurge in recycling, reusing or valorising their important by-product streams like “BSG” shows a clear path of biorefinery development. The present review already demonstrates the rationale repurposing of BSG-derived carbohydrates and proteins to numerous product such as ethanol, butanol, biomethane, xylitol, BDO, fertilisers, enzymes, prebiotics (XOS, ArXOS), functional foods and nutritional supplements. It further illustrates cases where spent yeast has been successfully used as an excellent substitute of yeast extract during microbial fermentation. This organic nitrogen source, rich in water soluble vitamins can be used for food fortification as well. Side by side the breweries should identify areas where water recycling/reuse is possible. They should strive for reducing the water footprint during the overall process of beer production. Recovery of gases such as carbon dioxide especially during fermentation and its subsequent sequestration can be other useful approaches. A relevant example to cite is Earthly Labs US, who launched CiCi carbon-capture system, which can capture, purify and recycle gases especially carbon dioxide and offers sustainability solutions to various breweries, wineries and distilleries [47]. Eddystone Brewery in New Zealand, Maine Beer Company, Proof Beer Company and Big Grove Brewery in US are few examples who have implemented their CiCi technology [48–51]. Presently innovations in carbon capture, utilisation and storage (CCUS) is an uptrend, as they play a vital role in decarbonisation, and promoting environmental sustainability. Such an integrated approach would greatly add to developing circular economy and would offer transformational benefit to the brewing industry. It would be advantageous especially within large brewery sites, where a biorefinery approach can be applied *in-situ*, further aiming carbon dioxide capturing and its valorisation including clean water recovery and its re-use in the brewing process.

Concluding remarks

The present review demonstrates the potential of BSG for biorefinery development with multiple renewable energy and non-energy products as examples. However, more focus is needed to develop a cost-competent, environmentally benign, green, and sustainable end-to end processes targeting maximum recovery of valuable

carbonaceous components from side waste streams, including solids, water and gaseous streams. Since BSG accounts majorly among all the solid waste streams, its pivotal role in diversifying product portfolio, generating huge revenues and enhance profitability of brewing industries is undeniable. The work discussed above add significant value to science around BSG as the feedstock, but none standing alone offer the end-to-end processing, crucial for a commercial biorefinery. Thus, future work should be directed toward the development of pretreatment technology to ensure efficient, benign, and optimal resource recovery, robust bio-transforming strains with high TYP metrics matching industrial requirements, process intensification, and carrying out sustainability (techno-economic and environment) assessment of promising technologies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this article.

Data availability

No data were used for the research described in the article.

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- * of special interest
- ** of outstanding interest

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This recent article highlights the challenges and opportunities associated with Brewer's spent grains for developing a biorefinery. Techno-economic evaluation of this feedstock for prospective applications

and production of various marketable products (energy and non-energy) is described in an elaborative manner.

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This review exclusively emphasizes on resource recovery from some undermined but high potential food and industrial waste streams like breweries, crude glycerol, organic fraction of municipal solid waste etc. Further it evaluates the production of various bio-based chemicals and polymers by valorising these streams in a sustainable manner, benefiting environment and society using integrated biorefining approach.

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It is the first kind of its article where the liquor rich in glucose, maltose and its oligomers is separated from wet Brewer's spent grains and valorised to lactic acid using autolysed yeast obtained from beer production. High titre of lactic acid is demonstrated with high productivity, using *Lactobacillus delbrueckii* strain.

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Agrawal, Deepti; Gopaliya, Deeksha; Willoughby, Nicholas; Khare, Sunil Kumar; Kumar, Vinod

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