Valorization of brewers' spent grain (BSG) by submerged edible filamentous fungi cultivation

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Introduction

As the most popular alcoholic beverage and third most popular drink overall after water and tea, the beer retains a considerable market value. In 2018, more than 39 billion liters of beer (beer from malt containing more than 0.5% v/v alcohol), with a value of 32.5 billion Euro, were manufactured in European Union (EU) (Cook, 2019). The main by-product of the brewing is brewers' spent grain (BSG), accounting for 85% of total by-products and 30% of the initial malt. For every liter of beer, around 0.2 kg wet BSG (70 to 80% moisture) is generated, meaning that about 8 million tons of BSG are produced in 2018. High moisture and nutrient contents render BSG susceptible to biological deterioration result in various environmental problems (El-Shafey et al., 2004). BSG has limited application as low-quality animal feed due to high fibre and low protein content, making landfilling the primary handling method. Therefore, improving the nutritional value of BSG could broaden its application. For this purpose, submerged cultivation of edible filamentous fungi (*Aspergillus oryzae, Neurospora intermedia, and Rhizopus delemar*) was introduced to enhance the BSG protein content. The effects of fungal strain, medium supplementation, and cellulase addition on the amounts of recovered solids and protein content were evaluated.

Material and method

The BSG was kindly donated by Göteborgs Nya Bryggeri AB (Gothenburg, Sweden). The collected wet BSG was stored in sealed bags at room temperature after air-drying at room temperature for three days.

The submerged fungal cultivation was carried out in batch mode using cotton-plugged Erlenmeyer flasks (250 mL) containing 100 mL medium (30 g/L BSG) at initial pH 5.2 and 35 °C. The cultivation was done in a water bath shaker at 125 rpm for three days. For the medium supplementation, a mixture of yeast extract, salts, trace metals, and vitamins was added to the cultivation medium (Ferreira et al., 2014).

Results and discussion

BSG is a nutritionally rich food-grade by-product that has excellent potential for producing food and feed-grade products; however, most research attention is focused on the production of chemicals. Thus, this work is focused on the valorization of BSG through submerged edible fungal cultivation and protein-rich biomass production. Additionally, the process industrial applicability was another factor considered for the process design. Fungal cultivation was chosen since it is currently applied at an industrial scale (Ferreira et al., 2016). Submerged fermentation was selected due to a more straightforward scale-up than solid-state fermentation, and it is the technology used in the brewing process. Moreover, the proposed process was designed with a minimum number of stages and was very simple to improve the probability of integration into an already established brewery.

The BSG mainly consisted of polysaccharides (64.9%), proteins (22.65%), and lignin (15.68%). The main polysaccharides were cellulose, hemicellulose, and starch.

The submerged fungal cultivation of the BSG without medium supplementation resulted in 15-20 g/L solids recovery with a protein content of 24-27% (percentage of solids dry weight). For the three fungal strains, the protein content of recovered solids after fermentation was increased 10-20% compared to the initial protein content of BSG. The highest increase in protein content was obtained for the cultivation with *A. oryzae* with a value of 20%.

The medium supplementation significantly increased the recovered solids concentration and protein content for the three fungal cultivation compared with those obtained from cultivation without medium supplementation. These results indicate that either fungus could not access the nutrients or nutrient content of BSG was not sufficient to support optimal fungal growth. The concentration of recovered solids and protein content for the cultivation

with medium supplementation were 24 g/L and 31%, respectively. There was no significant difference between the values obtained from cultivation with different fungal strains.

Compositional analysis of the recovered solids after fungal cultivation with medium supplementation showed a decrease in polysaccharides content in the form of starch, cellulose, and hemicellulose ranging from 40% to 50% compared with the initial composition of the BSG. This polysaccharides decrease was followed by a lignin increase on a percentage weight basis.

From the assessment of ethanol yield for cultivation with and without medium supplementation, it was obtained that the current state of BSG was more in favour of biomass production rather than ethanol production. The cultivation with N. intermedia and medium supplementation yielded the highest ethanol yield (17% of theoretical yield).

BSG is a lignocellulosic material and maintains a recalcitrant structure, limiting fungal growth. Typically, a pre-treatment step is required to overcome this recalcitrance; however, pre-treatment is resource-intensive and increases the process's complexity. Therefore, no pre-treatment was performed, but cellulase enzyme was added to the cultivation medium to boost fungal growth and BSG conversion. Although the compositional analysis of the solids after cultivation showed no considerable improvement in polysaccharides consumption. Moreover, the addition of enzymes directed the process toward ethanol production. The highest ethanol yield was 30% of the theoretical yield obtained for the cultivation with *N. intermedia.* In addition, the concentration of recovered solids for cultivation with medium supplementation and cellulase for the three fungi was significantly lower than thereof obtained during cultivation with only medium supplementation, while the protein contents were similar.

Simple cultivation with filamentous fungi on BSG led to 47% weight reduction while increasing the protein content by 40%, which could provide significant implications. The 8 million tons of generated BSG in 2018 hold 1.8 million tons of protein which with simple fungal cultivation could be increased to 2.5 million tons while reducing the solid size to 4.2 million tons. These results make BSG an ideal candidate for producing protein-rich products and proposing various valorization scenarios such as integration to breweries and bio-refinery platforms.

Conclusion

1. Protein-rich biomass production from BSG by submerged edible fungal cultivation was presented as a novel method for BSG valorization.
2. The growth of all strains on BSG successfully increased the protein content (up to 40%), upgrading the nutritional value of BSG; however, medium supplementation was necessary for higher protein recovery.
3. Simple submerged fungal cultivation reduced the weight of the solids up to 47%, indicating this method effectively reduces solid by-product size.
4. The addition of cellulase enzyme resulted in a 24-32% reduction in recovered solids concentration compared to the cultivation with only medium supplementation.

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