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## Spent Mushroom Substrate: From Agricultural Waste to Value Added Bio-Resource

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The benefits of mushroom consumption on human health and wellbeing are well recognized. As a result, pertinent demand has considerably increased in all continents, and edible mushroom commercialization has now a days become a worldwide business. Hence, mushroom production has increased more than 30 times since 1978, and it is a fast-expanding industrial activity. The global mushroom market achieved a notable volume of around 13 million tons in 2018, with projections indicating a substantial growth to 21 million tons by 2026.

As the mushroom industry advances, it yields a consequential by-product known as spent mushroom substrate (SMS). Comprising residual fungal mycelium, lignocellulosic biomass and enzymes. The composition of raw SMS can vary, with contents of upto 48.7% cellulose, 34% hemicellulose, and 39.8% lignin, contingent upon the source of the mushroom cultivation medium. SMS also serves as a source of essential vitamins and minerals, including iron, magnesium, zinc, and calcium. Moreover, it is remarkably rich in bioactive compounds, encompassing polysaccharides, polypeptides, and phenolics. SMS additionally harbors valuable enzymes; research has indicated that SMS from *Lentinula edodes*, *Agaricus bisporus*, and *Pleurotus eryngii* possesses enzymes such as  $\beta$ -glucanase, xylanase, laccase, and phytase.

SMS has garnered significant attention as a substantial waste product. However, as the edible mushroom sector thrives, SMS accumulates at a remarkable rate, approximately 5 kg for every kilogram of freshly harvested mushrooms, culminating in a staggering 60 million tons over a decade. Regrettably, SMS frequently encounters disposal as agricultural waste, incurring substantial costs. The absence of sustainable disposal strategies emerges as a significant hindrance to the continued expansion of the mushroom industry.

### Agricultural sustainability

In the realm of agriculture, spent mushroom substrate (SMS) emerges as a compelling sustainable alternative to conventional chemical fertilizers and soil amendments, offering solutions to both energy consumption and environmental concerns. Remarkably, even after undergoing multiple cycles of mushroom production, SMS retains its robust nutrient content and organic matter, rendering it an ideal choice for biofertilization and soil enhancement. The distinctive qualities of SMS, including excellent air permeability, water and nutrient retention capabilities, and a loose texture, foster an improved ecological environment for soil microorganisms and enhance the physical structure of the soil, thereby minimizing plant stress and bolstering nutrient bioavailability.



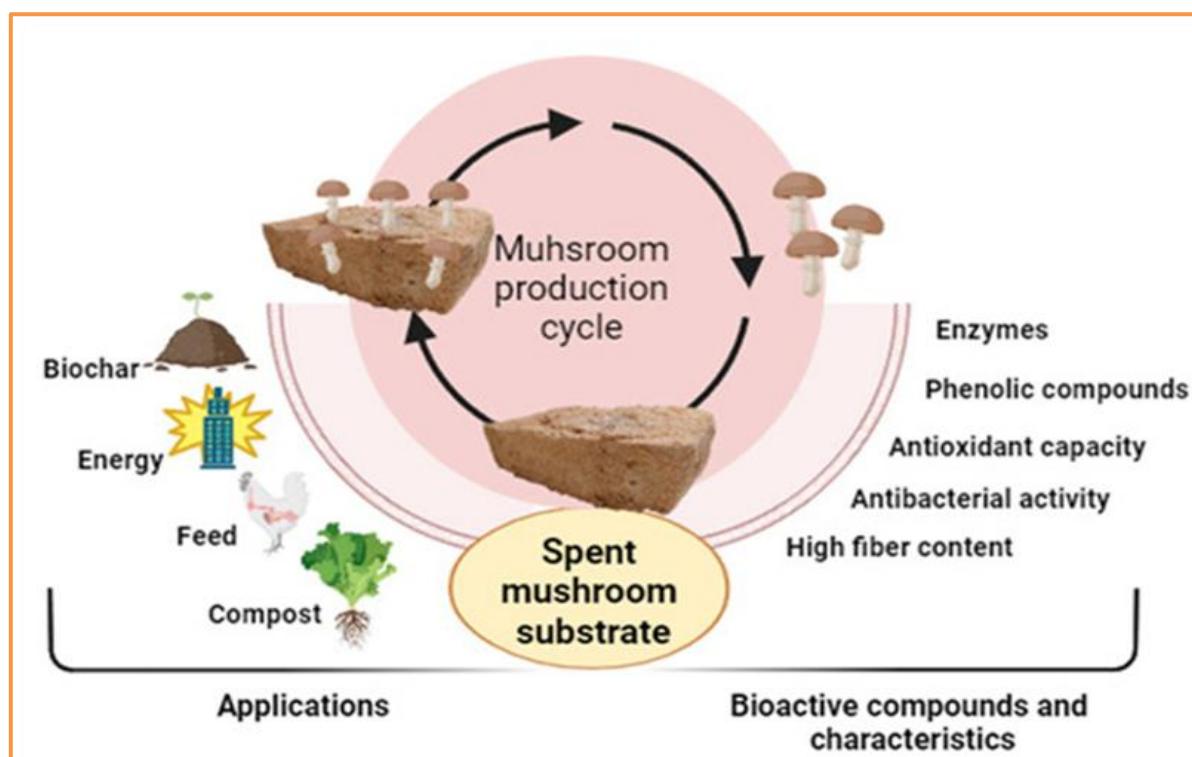


Figure 1. Mushroom production cycle and spent mushroom substrate bioactive compounds and main applications

## Bioremediation

SMS exhibits the capacity to fine-tune soil pH levels and mitigate soil contamination by various pollutants such as heavy metals, pesticides, and polycyclic aromatic hydrocarbons. It seamlessly integrates into various bioenergy production methods, bolstering the generation of biomass fuels, biofuels, bioethanol, and biogas. Expanding its role, SMS emerges as a bioremediation contender, targeting environmental contamination through living organisms. SMS from various mushroom species effectively removes pollutants like H<sub>2</sub>S, volatile compounds, and heavy metals, even degrading harmful compounds like polycyclic aromatic hydrocarbons and pesticides. This underscores SMS's potential for remediating polluted soils and waters.

## Spent mushroom substrate as feed

The need for animal feed production is predicted to increase significantly, and the feed industry must look for additional/alternative means to cover the respective demand. Exploiting suitable bio-resources (e.g. SMS) could contribute toward this direction by readily providing material to be used as feed supplement. The main raw materials used in mushroom cultivation are rich in cellulose, hemicelluloses and lignin, while their protein content is generally low. During solid-state fermentation by mushroom-forming fungi, the substrate polymers are enzymatically degraded, and the digestibility of plant residues is considerably improved. Concomitantly, the growth of mycelial biomass upgrades the substrate by increasing its content in proteins and bioactive compounds, e.g. polysaccharides and ergosterol. The high nutritional value of SMS is the main factor for its inclusion in the diets of poultry, ruminants, and monogastric animals, and, recently, in fish and edible insects.

## Recovery of enzymes from SMS

SMS is a source of various enzymes that can be recovered by extraction with different solvent systems. Furthermore, SMS can be used as substrate for the cultivation of enzyme-producing microorganisms. Upon the end of cultivation, SMS contains extracellular fungal enzymes, such as ligninases, cellulases, and hemicellulases, that can be recovered using different extraction procedures. The level of enzyme activities and their corresponding titers depend on the growth substrate and the fungal species' ability to degrade different

lignocellulose components. For example, since white-rot fungi degrade lignin and hemicelluloses preferentially, extracts of their spent substrates are rich in ligninases and xylanases, while cellulase activity is hardly detected. The enzymatic systems present in SMS of various fungal species make possible their application for different purposes. For example, *P. ostreatus* SMS can be applied for decolorizing textile effluents because it contains oxidoreductases that degrade the dye molecules. Similarly, the laccase and manganese peroxidase activities of *P. pulmonarius* SMS allow its direct application to remove polycyclic aromatic hydrocarbons from contaminated soil samples. However, rather than directly using the bulk SMS, many applications require using isolated enzymes that can be recovered from SMS.

### Bioactive compounds

SMS contains bioactive compounds of different functionality and origin. The fungal mycelium contains polysaccharides, sterols, proteins, poly phenols, vitamins, and other bioactive molecules. Mycelial growth throughout the surrounding environment also results in the secretion of potentially useful bioactive compounds. In addition, the extractive fraction of the lignocellulosic substrate and the oligomeric products from fungal degradation of polysaccharides and lignin might also be sources of bioactive substances. However, while the bioactive molecules of the sporocarps of edible fungi have been extensively investigated, the information on the bioactive potential available in SMS is still limited. Recovery of bioactive compounds is a promising direction for valorizing SMS.

### Conclusion

In conclusion, SMS stands as a valuable resource with myriad environmentally sustainable applications. As a biofertilizer and soil enhancer, SMS offers a cost-effective, nutrient-rich alternative to chemical fertilizers, resulting in improved soil health and a diminished ecological footprint. SMS exhibits versatility by serving as a key player in renewable energy production, utilizing agro-industrial biomass and thereby reducing our dependence on non-renewable energy sources. In the realm of environmental remediation, SMS proven its worth as a bioremediation agent, facilitating the removal and degradation of contaminants in air, soil, and water. SMS has garnered attention as a promising ingredient in poultry nutrition due to its high fiber content, protein content, and bioactive compounds. These attributes have been shown to enhance poultry, ruminants, and monogastric animal's growth, health, and overall performance. By harnessing the nutrient-rich properties of SMS by-products, the poultry and cattle industry can simultaneously reduce its reliance on chemical fertilizers, promote soil health, and contribute to the overall sustainability of the sector.

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