



Sustainability of Mushroom Cultivation Systems

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In the European Union alone, about 700 million tons of agricultural waste is generated annually [1], and many times more is generated in the rest of the world. The growing environmental pressures from the disposal of waste materials and the shortage of natural resources has stimulated the implementation of sustainable solutions, and the transfer to a circular economy with an emphasis on the valorizing, minimizing and recycling of wastes [2]. Since vast volumes of agro-forestry residues and agro-industrial by-products are rich in organic compounds, mushroom cultivation, as a biotechnological process, is a method of transforming and effectively utilizing those wastes which are worthy of being recovered. Many kinds of substrates have already been investigated as suitable for mushroom cultivation; however, climate change has fomented new types of crops and cultivation systems, generating new potential mushroom growing media. To be fully sustainable, wastes from mushroom cultivation, such as spent mushroom substrate (SMS) and waste water, must also be a part of the loop and be reused or utilized. Also, since mushrooms are very well known for their bioaccumulation abilities, the cultivation occurring on re-used substrates must be monitored, together with sustainable disease management. All of these aspects are discussed in ten papers published in this Topical Collection on ‘Sustainable Mushroom Cultivation’.

Generally, mushroom farming is considered to be carbon-positive, with lower GHG emissions than other sectors such as crops, fruits and animal rearing for meat production. It is estimated that 9 kg of freshwater is needed to produce 1 kg of mushrooms: some of the water is consumed by the mushroom itself; however, part of it is wasted. Širić et al. [3] described the novel possibility of the utilization of carbon dioxide (CO₂) and wastewater released from a mushroom farm for the cultivation of *Chlorella vulgaris* microalga. Their study described a microcontroller-based aided CO₂ capture and mixing prototype, which was constructed for the cultivation of *C. vulgaris* under varying concentrations of mushroom-farm wastewater (0 as control, 50 and 100%). The results showed that the constructed prototype was helpful in maintaining desirable CO₂ levels (6000 ppm) in the mushroom cultivation chamber with a constant CO₂ supply to the algal culture, i.e., 0.6% at an airflow rate of 50 mL/min. After 16 days of algal cultivation, it was observed that the maximum significant ($p < 0.05$) algal biomass production of 2.550 ± 0.073 mg/L was recorded in a 50% wastewater concentration, followed by 100% and control. Also, the maximum removal of selected mushroom-farm wastewater pollutants, such as the total dissolved solids ($84.00 \pm 1.37\%$), biochemical oxygen demand ($90.17 \pm 2.42\%$), chemical oxygen demand ($91.53 \pm 0.97\%$), total nitrogen ($86.27 \pm 1.60\%$) and total phosphorus ($94.19 \pm 2.33\%$), was achieved in the 50% concentration of wastewater treatment, with maximum first-order rate constant (k) values. The post-harvest characterization of algal biomass revealed that the proximate, biochemical, ultimate elements (carbon, oxygen and nitrogen) and structural properties were significantly higher in the 50% treatment than those in the 100% and control treatments. These findings give significant insight into the synergistic use of CO₂ and wastewater produced by mushroom farms for algal cultivation and biological wastewater treatment.



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New substrates in commercial mushroom cultivation can be introduced by many different channels, such as the expansion of production of a new crop in the region, an attempt to utilize the problematic waste of a local energy production facility, or inadequate amounts of conventionally used substrate. Three articles in this TC aimed to find a sustainable answer to the abovementioned questions. As the processing of hazelnuts, of which Italy is one of the world's largest producers, generates large amounts of waste, especially woody pericarps due to the cracking process, which are generally used for domestic heating that causes air pollution, Puliga et al. [4] investigated the effect of the cultivation of edible and medicinal mushrooms on the high lignin content present in the pericarps. *Ganoderma lucidum*, *Lentinula edodes*, and *Pleurotus cornucopiae* were grown and cultivated on different hazelnut-shell-based substrates—Hazelnut Shell (HS), Hazelnut Shell and Wheat Straw (HS-WS), and Wheat Straw mixed with Beech Chips (WS-BC) as control—showing the decay of the lignocellulosic fraction of the HS. However, cultivation trials showed a similar biological efficiency but a different Fruiting Body Production (FBP) in the presence of HS with respect to the control, which provides attractive perspectives both for more sustainable management and for the improvement of mushroom cultivation efficiency.

Energy in Norway is produced mainly by water plants; however, considerable amounts are also generated by the anaerobic co-digestion of dairy manure and food waste. AD leaves an effluent called 'digestate'. This nitrogen- and organic-matter-rich product has been investigated as an ingredient in growing substrates, together with the straw of Norwegian grains, barley, oat, or a mixture of different straw types, as suitable for mushroom cultivation [5]. All the investigated experimental mushroom compost (EMC) types worked well during the composting process, reaching the desired moisture of 65–75%, N content of 1.43–1.93%, and a C/N ratio ranging from 21.5 to 29.1, supporting the growth of mycelium and producing mushrooms of *Agaricus subrufescens*. Supplementation with barley straw resulted in a better EMC structure with the highest yield and biological efficiency (BE) (157.9 g kg⁻¹; 64%), whereas oat addition gave the lowest yield and BE (88.6 g kg⁻¹ and 38%). Precociousness (the yield at the mid-cycle of the crop development) was higher for the oat substrates (68.9%), while earliness (the days to harvest from casing) was lower for barley EMC. The above-mentioned findings support the assumption that original digestate can be used directly in mushroom cultivation. If a mushroom farm could be situated close to an anaerobic digestion plant, the sustainable use of assets could be achieved.

When the limited availability of the conventional cultivation substrate prohibits local mushroom production, the utilization of the potential new substrate is one way of dealing with this problem. In Malaysia, the palm oil industry generates large volumes of organic by-products that have caused environmental concerns. Aubrey et al. [6] researched the possibility of using empty fruit bunches (EFBs), oil palm fronds (OPFs), and oil palm trunks (OPTs) as growing substrates for *Pleurotus ostreatus*. Generally, using 100% EFB showed a better agronomic performance, and mushroom growth was 1.9 times faster compared to the control, with a comparable mushroom yield. The crude protein and beta glucan content of mushrooms grown on oil palm using product-formulated substrates were significantly higher than those grown using the control. Additionally, the number of fruiting bodies and the crude protein and beta glucan content of the mushrooms were positively correlated with potassium levels in the substrate. Therefore, 100% EFB could be used as a potential substitute for RWS for the cultivation and production of *P. ostreatus*.

As the spent mushroom substrate (SMS) or spent mushroom compost (SMC) are produced in vast amounts—on average, 5 kg of SMS/SMC is generated for 1 kg of mushrooms—there is an urgent need for the utilization of this waste. Although many studies have proven the value of SMS/SMC as a soil amendment, plant growing substitution or animal feed, still not enough of this valued by-product is reused in a sustainable way. This TC brings up four novel approaches for SMS/SMC recycling. Lisiecka et al. [7] discuss the feasibility of utilizing spent mushroom substrates (SMSs) as a growing medium component for *Pleurotus ostreatus* cultivation. SMSs from *Pholiota nameko* (N-SMS), *Hypsizygus marmoreus* (M-SMS), and *Hericium erinaceus* (E-SMS) in varying supplementation rates (10%, 20%, and 30%) were

investigated as a possible supplement for wheat straw substrate. Significant differences in the yield, biological efficiency BE, protein content, and dry matter of *P. ostreatus* were found among the studied substrates. The highest yield was recorded in 20% E-SMS (254.33 g), 20% N-SMS (253.43 g), and 10% E-SMS (251.67 g). The biological efficiency ranged from 66.48% (30% M-SMS) to 72.67% (20% E-SMS) and followed a similar trend to the yield. The highest protein content was recorded in 30% M-SMS (29.93 g·100 g dry weight⁻¹). The highest dry matter of *P. ostreatus* was observed in 30% of M-SMS (23.74 g) and 10% of M-SMS (23.06 g). The research presented the potential of those SMSs as a low-cost, sustainable alternative (10–30%) and as a renewable component of traditional growing media for *P. ostreatus* cultivation. A different approach is proposed by the team of Kumar et al. [8]: their study investigates the biotransformation of SMS obtained after Shiitake mushroom cultivation into biogas and the attendant utilization of slurry digestate (SD) in tomato (*Solanum lycopersicum* L.) crop fertilization. The results on biogas production revealed that the SMS 50% treatment yielded the highest biogas volume (8834 mL or 11.93 mL/g of organic carbon) and methane contents (61%) along with maximum reduction of the physicochemical and proximate parameters of the slurry. Furthermore, the biogas digestate from 50% treatment further helped to increase the seed germination (93.25%), seedling length (9.2 cm), seedling root length (4.19 cm), plant height (53.10 cm), chlorophyll content (3.38 mg/g), total yield (1.86 kg/plant), flavonoids (5.06 mg/g), phenolics (2.78 mg/g), and tannin (3.40 mg/g) contents of tomato significantly ($p < 0.05$) in the 10% loading rate. The findings of this study suggest the sustainable upcycling of SMS inspired by a circular economy approach through the synergistic production of bioenergy and secondary fruit crops, which could potentially contribute to minimizing the carbon footprints of the mushroom production sector.

With the inevitable growth of the mushroom industry, the production of SMS/SMC will be even bigger, thus forming an agricultural waste which requires proper management other than dumping or burning. Pyrolysis has become, lately, the newest tactic for problematic wastes, producing a valuable product—biochar—which not only captures carbon but, when of good quality, could be used in numerous ways. Also, SMC/SMS from the cultivation of shiitake fungus (SF) and black fungus (BF) could provide highly porous biochars, which was proven by the investigation of Chen et al. [9]. Their results showed that the pore properties of the biochar products indicated a significant increase with the increase in the pyrolysis temperature from 400 to 600 °C. The data on the maximal Brunauer–Emmett–Teller (BET) surface area for the biochar products produced at 800 °C (i.e., SMC-SF-BC-800 and SMC-BF-BC-800) were found to be 312.5 and 280.9 m²/g, respectively. Based on the EDS and FTIR, plenty of oxygen-containing functional groups were found on the surface of the resulting biochar products.

Good-quality biochar is of a high value, and its most common use is for soil quality improvements. Deng et al. [10] reported that biochar derived from SMS could be used as soil amendment while providing a solution for SMS disposal. They found that the application of biochar derived from SMS to moso bamboo forest soils decreased soil N₂O emissions. The authors of the next article from this TC, Širić et al. [11], investigated the impact of the combined use of spent mushroom substrate (SMS) biochar and plant-growth-promoting rhizobia (PGPR) on the growth, yield, and biochemical response of cauliflower (*Brassica oleracea* var. *botrytis*). The results of their study showed that the addition of SMS biochar aids the improvement of soil nutrient properties. The application of SMS biochar and PGPR also significantly ($p < 0.05$) improved the selected growth, yield, and biochemical parameters of cauliflower. In particular, the highest cauliflower yield (550.11 ± 10.05 g), fresh plant biomass (1.66 ± 0.04 kg), dry plant biomass (149.40 ± 4.18 g), plant height (22.09 ± 0.14 cm), root length (11.20 ± 0.05 cm), plant spread (28.35 ± 0.18 cm), and the number of leaves (12.50 ± 0.50) were observed when 10 g/kg of biochar and PGPR were used. Also, this treatment obtained the best values for biochemical parameters and enzyme activities, such as total chlorophyll (TC: 3.13 ± 0.07 mg/g), superoxide dismutase (SOD: 79.12 ± 1.29 µg/g), catalase (CAT: 55.70 ± 2.52 µg/g), peroxidase (POD 30.18 ± 0.37 µg/g),

total phenolics (TP: 19.50 ± 0.31 mg/g), ascorbic acid (AA: 14.18 ± 0.55 mg/g), and total carotenoids (TCT: 150.17 ± 8.20 μ g/100 g). The findings of this study suggest the efficient recycling of mushroom-industry waste for biochar production and the use of PGPR to improve nutrient utilization in sustainable agriculture.

A novel approach for sustainable Shiitake mushroom cultivation using agro-industrial wastes, such as those from a secondarily treated dairy plant and sugar mill wastewaters (DPW and SMW), was presented by Kumar et al. [12]. The results revealed that DPW and SMW moistening significantly ($p < 0.05$) increased the nutrient levels of the formulated substrate, which later gave better mushroom yield. The highest Shiitake mycelial coverage (90.70 ± 1.47 and $88.65 \pm 1.82\%$), yield (186.00 ± 3.10 and 176.09 ± 4.12 g/kg fresh substrate), biological efficiency (80.00 ± 0.58 and $75.73 \pm 0.93\%$), total phenol (2.84 ± 0.03 and 2.69 ± 0.03 mg/g), ascorbic acid (0.34 ± 0.03 and 0.32 ± 0.02 mg/g), and β -carotene (2.48 ± 0.06 and 2.29 ± 0.02 μ g/g) contents with the minimum time taken for spawn running (60 ± 1 days) was observed using a 50% concentration treatment of both DPW and SMW, respectively. Besides this, the kinetic studies using a first-order-based model showed acceptable accuracy in predicting the rate constant for substrate delignification and heavy-metal uptake by Shiitake mushroom. The concept can be used for the production of high-quality mushrooms for edible and medicinal purposes while contributing toward the United Nations' Sustainable Development Goals (SDGs 12) on the responsible consumption and production of superfoods.

After the successful application of the sustainable, circular substrates, and mushroom-cultivation waste reuse, it is also crucial to attend to the sustainable management of diseases, which unfortunately can touch every mushroom grower independently of how careful and precise the production is. This topic was touched upon by Altaf et al. [13] in their research, where they investigated the sustainable management of green mold disease (*Trichoderma harzianum*) of white button mushroom using botanicals and biocontrol agents under temperate conditions. Their study revealed that the integration of botanical and bacterial antagonists in pathogen-infested white-button-mushroom casing reduces green mold infection with corresponding yield gains. The in vitro evaluation of an ethanol extract of botanicals against the mycelial growth of *T. harzianum* at 1, 2, and 3% concentrations showed that *Juglans regia* and *Allium sativum* exhibited the maximum mycelial growth inhibition of 84.9 and 79.8%, respectively. When the same botanicals were tested against the mycelial growth of *A. bisporus*, it was observed that *J. regia*, *Curcuma longa*, and *Azadirachta mellea* were least inhibitory (4.66–7.4%). From the in vivo evaluation of plant botanicals at 2% concentration, *J. regia* and *C. longa* had the highest average weight (11.8–11.9 g) of a single fruit body and a combined button yield of 11.3–11.9 kg/quintal compost. Among the bacterial bioagents evaluated in vitro, *Pseudomonas fluorescens*, *Azotobacter* sp., and *Bacillus subtilis* displayed stimulatory effects of varying degrees on the mycelial growth of *A. bisporus*, but exhibited antagonistic effects on *T. harzianum*. *B. subtilis*-38, and *P. fluorescens*-104. *Azotobacter*-108 caused the highest mycelial growth inhibition of 97.6, 97.4, and 90.3% of *T. harzianum*, respectively.

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