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Valorization of Spent Mushroom Substrate: Establishing the Foundation for Waste-Free Production

Andrew Ravlikovsky [1], Maria Nazaré Coelho Pinheiro [2], Lucian Dinca [3], Vlad Crisan [3,*] and Lyudmyla Symochko [1,4,5,*]

[1] Faculty of Biology, Uzhhorod National University, Voloshyna Str. 32, 88000 Uzhhorod, Ukraine; andrew@naturegreen.eu
[2] Coimbra Institute of Engineering, Polytechnic Institute of Coimbra, 3030-199 Coimbra, Portugal; mnazare@isec.pt
[3] Romanian National Institute of Research and Development in Forestry "Marin Dracea" Brasov Branch, 13 Closca Street, 500040 Brasov, Romania; dinka.lucian@gmail.com
[4] Institute of Agroecology and Environmental Management, Metrologichna Str. 12, 03143 Kyiv, Ukraine
[5] Department of Life Sciences, University of Coimbra, Martim de Freitas Str., 3000-456 Coimbra, Portugal
* Correspondence: vlad_crsn@yahoo.com (V.C.); lyudmilassem@gmail.com (L.S.)

Abstract: Spent mushroom substrate (SMS), often disregarded as waste despite its abundance in organic matter and mineral micronutrients, has emerged as a valuable resource for diverse applications. While Europe and Asia have witnessed extensive research in this field over the past decade, Ukraine's exploration remains limited. This study conducts a thorough investigation into SMS recycling and reutilization over a 4-year period. Employing experimental and comparative methods, this research unveils compelling insights into the potential of SMS for reintroduction into the primary production cycle and secondary activities. The main conclusions reveal the success of SMS valorization in the production of additives, fertilizers, and alternative fuels. Furthermore, the application of SMS in agroecosystems significantly enhances the soil biological activity. The integration of these methods into production chains not only yields economic benefits for companies but also fosters environmental stewardship, aligning with waste-free practices and the principles of bioeconomy and sustainability.

Keywords: sustainability; agroecosystem; spent mushroom substrate; recycling; additive; solid fuel

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1. Introduction

Worldwide mushroom and truffle production has increased from 42.9 million tons in 2020 to 48.3 million tons in 2022 [1], driven by the growing demand for healthy food options. Edible mushrooms are valued as a nutritious source of protein, fiber, vitamins, and minerals, making them an appealing alternative. Additionally, mushrooms contain abundant bioactive compounds with anti-cancer, antioxidant, and anti-diabetic properties [2].

Mushroom cultivation involves not only the mushrooms themselves but also a significant amount of spent mushroom substrate (SMS). For 1 kg of mushrooms cultivated, approximately 5 kg of SMS is produced [3], which means that the generation rate of SMS was about 242 million tons worldwide in 2022. Traditionally, SMS has been treated as waste, leading to environmental problems [4], despite its richness in organic matter and mineral micronutrients. Over the past decade, overcoming this challenge has become increasingly important to the mushroom production industry, emerging a new line of research aimed at identifying the most environmentally and economically sustainable methods for its reuse [5–8].

Several efforts have been made to develop efficient and sustainable strategies for the SMS valorization, including its application as an alternative fertilizer and soil amendment agent [9,10], as a supplement to enrich nutrient levels in subsequent mushroom cultivation cycles [11], as a soil bioremediation agent [12–14], and as a feedstock for biofuel production [15–18].

The physical properties and nutritional value of SMS have led to several studies where it has been used as a fertilizer [19]. SMS has been successfully used as a component of growing media for the germination and growth of horticultural plants, indicating that it can be applied in professional horticulture and contribute to its disposal in an environmentally friendly way, thereby reducing the need for peat [19]. In particular, SMS has been tested as a bioadditive and organic fertilizer for lettuce and leek crops, generally resulting in high harvest percentages compared to mineral fertilizers [20]. Crops with short growing cycles are more suitable for mixed feeding, as organic substrates typically mineralize slowly, while organic fertilization is more effective for crops with long cycles. In addition, the prolonged use of spent substrate can contribute to an increase in soil organic matter content [20].

The potential of replacing peat with SMS in the composition of Chinese biobeds was also evaluated, showing that SMSs from different mushrooms (*Pleurotus eryngii*, *Flammulina velutipes,* and *Lentinus edodes*) are suitable for the preparation of the biomixture based on the biological activity and pesticide degradation and can therefore be used as substitutes for peat [21]. Additionally, the stabilization of SMS was investigated for application as a plant growth-promoting organic amendment. When the maturity and quality of the stabilized SMS were assessed in a horticultural growing trial, the results showed that the product has great potential as an alternative to reduce the use of peat in horticulture, thereby reducing the environmental impact on peatland ecosystems [22]. Experiments have also been conducted on the growth medium for tomato and pepper seedlings based on the addition of composted biogas residue with SMS, providing nutrition for plant growth comparable to that of chemical fertilizers [23].

Furthermore, SMS has shown potential as a disease control agent for agriculture. The use of different SMSs to inhibit Fusarium wilt of cucumber was evaluated [24]. The greenhouse experiment revealed that the options using SMS from *Flammulina velutipes*, *Lentinus edodes*, and *Pleurotus ostreatus* cultivations reduced the disease incidence by 53.3%, 25.7%, and 37.9%, respectively, compared to the unamended situation and simultaneously promoted the plant growth [24]. This approach to the control of Fusarium wilt in cucumber resulted from the manipulation of the soil microbial community in the rhizosphere with SMS application, but further studies need to be conducted to confirm it as a cost-effective amendment for practical applications.

With the energy crisis and the search for new energy resources, the evaluation of the possibility of reusing SMS as a biofuel has become a current and relevant research topic. From the comparison of two thermal treatments (combustion and pyrolysis) for energy recovery using conventional fuel and SMS and coal tailings wastes, it was concluded that fluidized-bed combustion was the most efficient (91.7%) compared to packed-bed combustion or the use of unpelletized SMS [3]. In addition, minimal acid gas emissions (NO_x, SO_x, and HCl) were produced, providing a sustainable management solution for diverting SMS from landfill and assisting in the reclamation of contaminated land, which is both practical and environmentally sound [3].

The use of SMS in biogas production has also been evaluated with promising results, since the raw material is previously digested by fungal metabolism as a pre-treatment prior to anaerobic digestion [25]. The residual digestive from the biogas process can also be reused, and these productive processes could be integrated into a sustainable virtuous circle, where the residues produced by one activity become the substrate for another one [25]. The performance of the batch thermophilic anaerobic digestion of SMS has also been investigated, indicating that this technology can increase methane yields (177.69 mL/g), reduce fermentation times, and improve the cellulose and hemicellulose degradation rates (47.53 and 55.08%, respectively) [26].

In Ukraine, the cultivation of exotic mushroom is currently on the rise, but limited research has been conducted on the use of SMS [27,28]. Therefore, it is necessary to study this topic in more detail. The article presents the results of a 4-year study exploring potential methods for recycling and reusing spent substrate, including its use as a component in new substrate production cycle, as a soil additive, and as an alternative solid fuel.

2. Materials and Methods

2.1. Preparation of Spent Shiitake Mushroom Substrate

Fresh spent shiitake mushroom substrate (SMS) received immediately after the completion of mushroom picking in blocks (20 cm × 10 cm × 15 cm) with an average weight of 1.3 kg each. In order to be reused as a component in substrate production cycle, blocks were crushed to small particles (2–10 cm) and stored on a flat concrete surface, under a roof, in small heaps for 14 days (Figure 1). During the storage period, fermentation took place. To make this process uniform, heaps were periodically turned over. It was also performed to prevent the spreading of *Trichoderma* spp. (green mold) and *Neurospora* spp. (orange mold), as well as the accumulation of volatile and flammable compounds that are formed during fermentation. Average humidity and acidity levels were measured. The average humidity was in the range 66.1–71.3% and acidity between 3.5 and 4.

Figure 1. Preparation of spent substrate for reuse as a component in shiitake substrate production cycle.

For use as an additive to the soil for blueberry cultivation, fresh SMS was stored in heaps 2.5–3 m high in the open air for 3–6 months (Figure 2). In everything else, the same processes took place as described above.

Figure 2. Preparation of spent shiitake substrate for use as an additive to the soil.

To determine suitability for use as an alternative solid fuel, fresh SMS was kept in blocks stacked in several rows, not tightly, on a flat concrete surface, under a roof. In this form, the natural drying process took place during the summer period (the average air temperature was within 26 °C).

2.2. Reuse as a Component in Substrates' Production Cycle

The experiment took place at a local shiitake farm "Nature Green Ukraine LLC".

The original substrate recipe includes 70% aged beech sawdust, 10% cereals (such as cord or barley), 5% oil seeds, and 5% wheat bran. The humidity is between 65 and 70% and pH of 6–7. It was used as a control (CS).

In the tested recipes only sawdust was replaced. The following recipes were used:

EX1—40% aged beech sawdust, 30% aged SMS, 10% cereals (such as cord or barley), 5% oil seeds, 5% wheat bran. The humidity was between 65 and 70% and pH 6–7.

EX2—35% aged beech sawdust, 35% aged SMS, 10% cereals (such as cord or barley), 5% oil seeds, 5% wheat bran. Humidity in the range 65–70% and pH 6–7.

EX3—50% aged beech sawdust, 50% aged SMS. The humidity was between 65 and 70% and pH 6–7.

Substrates were sterilized for 13 h (9 h under 95 °C followed by 4 h at 110 °C). Cooling down took place in a sterile room. As soon as the inner temperature of substrates dropped below 28 °C, they were inoculated with 1% of spawn.

Incubation took place in the same room with the following climate conditions: temperature 21–25 °C, relative humidity 60–80%, CO_2 concentration 2000–10,000 ppm (0.2–1%), and exposure to light—twice a day for 1 h (50–100 lux).

Fruiting took place in the same room with the following climate conditions: temperature 16–18 °C, relative humidity 80–95%, and CO_2 concentration 1000–1700 ppm (0.1–0.17%).

Substrates evaluation was carried out based on the yield data after 14th, 16th, and 18th weeks of incubation and organoleptic evaluations of the picked mushrooms (color, texture, taste).

The yield is considered good if it is $\geq$20%, and the organoleptic evaluation of the picked mushrooms is classified as bad, good, satisfactory, or excellent.

2.3. Additive to the Soil for Blueberry Cultivation

The experiment took place at a local blueberry farm "Green FE" where the following blueberry varieties were grown, i.e., Duke, Bluecrop, and Elliott. The weather conditions of the area where this farm is located are characterized by mainly dry weather with minimal precipitation in the form of short-term rains and thunderstorms. The average daytime air temperature during summer period was in the range of 21–28 °C, and the average night temperature was 12–16 °C.

The obtained soil analysis showed that it is umbrisol. In the words of the owner of the farm "Green FE"(the text quoted below), for blueberry cultivation, umbrisol is not the best option, and for consistently good growth and yield, it needs additives to improve its physical characteristics, maintain humidity, and provide an adequate environment for bioactivity of the soil and enrich it with nutrients. Peat with the addition of agrochemicals is mainly used for these needs. Therefore, the comparison of efficiency of SMS as an additive was carried out in comparison with peat.

The chemical composition of additives, chemical composition of soil, number of microorganisms, content of total microbial biomass, and direction of microbiological processes were determined. The experiments were performed with four-year repetitions.

The chemical compositions of spent shiitake mushroom substrate and peat are described in Table 1. A comparison reveals that spent shiitake mushroom substrate exhibits higher organic matter content of 48.44% compared to peat. Additionally, the substrate demonstrates higher percentages of potassium, magnesium, and phosphorus relative to peat.

Table 1. Chemical composition of additives.

Indicators	SMS	Peat
relative humidity, %	58.04	59.32
acidity, pH	3.70	4.50
organic matter in the calculation of carbon, %	48.44	44.95
mass fraction of total nitrogen, %	1.04	1.99
ratio C:N	44.7:1	45.2:1
total potassium, %	0.43	0.07
total phosphorous, %	0.37	0.31
calcium content, %	0.42	0.50
magnesium content, %	0.17	0.10

Soil samples were collected during the spring–summer period before blueberry fruiting began. Sampling occurred annually for four years after the application of spent mushroom substrate. Samples were taken from the top 0–20 cm layer of soil during stable system conditions. After collection, all samples were air-dried, sieved to remove particles larger than 3 mm, and meticulously cleared of visible plant and mesofauna residues. For accuracy, each sample was taken with a four-fold repetition (Figure 3).

Figure 3. Selection of soil samples.

The chemical composition was determined using standard methods:

- DSTU 7882:2015 "Peat and its processing products for agriculture. Methods of determining metabolic and active acidity" [29] for determination of the level of acidity;
- DSTU EN 12048:2005 "Solid fertilizers and liming materials. Determination of moisture content by gravimetric method. Drying at a temperature of (105 ± 2) °C" (EN 12048:1996, IDT) [30] to determine moisture content;
- DSTU 7942:2015 "Soil quality. Determination of ash content of peat and peat soil" [31] to quantify the level of ash;
- DSTU 8454:2015. "Organic fertilizers. Methods of determination of organic matter" [32] to obtain the content of organic matter in terms of carbon;
- DSTU 7911:2015 "Organic and organo-mineral fertilizers. Methods of determining the total mass fraction of nitrogen and the mass fraction of ammonium nitrogen" [33] for determination of the mass fraction of total and ammonium nitrogen;
- DSTU 7949:2015 "Organic fertilizers. Method for determining the mass fraction of total potassium" [34] for determination of total potassium;
- DSTU EN 15956:2015 "Fertilizers. Method for extracting phosphorus soluble in mineral acids" (EN 15956:2011, IDT) [35] to obtain total phosphorus;
- DSTU 7670:2014 method. "Raw materials and food products. Preparation of samples. Mineralization to determine the content of toxic elements" [36] for calcium, magnesium, and sodium content quantification.

For the microbiological analyses, soil samples were selected from each variant in four-fold replication and prepared as an average sample. Batches of 10 g each were put on a sterile mortar, and then, the microorganisms were separated from the soil particles using the method of D. Zviahyntsev [37]. The quantitative compound of the microorganisms of the main ecological-trophic and taxonomic groups in soil was determined using the methods of inoculating the soil suspension into standard growth medias, which are generally accepted in soil microbiology [37]: streptomyces and bacteria that use mineral nitrogen in starch and ammonia agar (SAA); pedotrophs on soil agar (SA); oligotrophs on purified agar (PA); micromycetes on Czapek-Dox agar; and bacteria that use organic nitrogen in meat infusion agar (MIA). After the inoculation into the media, the bacteria were incubated at the temperature of 28 °C during 5–14 days. The colonies that grew in these media were calculated assuming that one colony is formed from one vital cell. The results of the assessments of the number of microorganisms grown on the nutrient media were expressed in Colony Forming Units (CFUs) per 1 g of dry soil. For this purpose, the moisture of the soil samples was determined for the experiments using the thermostat-gravimetric analysis, and the obtained number of colonies was recalculated taking into consideration the coefficient of moisture and solution of the soil suspension. The inoculations were repeated three times, and the obtained data were analyzed using mathematical statistics, calculating the confidence interval in the number of microorganisms.

The direction of microbiological processes in the soil was determined by the appropriate coefficients [38]:

- Coefficient of mineralization (C_{min}) calculated by the ratio of the number of microorganisms immobilizing the mineral forms of nitrogen (N_{SAA}) to the number of organotrophs (N_{MIA}) as follows:

$$C_{\mathrm{min}} = \frac{N_{\mathrm{SAA}}}{N_{\mathrm{MIA}}}; \tag{1}$$

- Coefficient of oligotrophity (C_{ol}) calculated by the ratio of the number of microorganisms, which are able to absorb nutrients from very rarefied solutions (N_{PA}), to the total number of eutrophic microorganisms (N_{SAA} + N_{MIA}) as follows:

$$C_{\mathrm{ol}} = \frac{N_{\mathrm{PA}}}{N_{\mathrm{SAA}} + N_{\mathrm{MIA}}}; \tag{2}$$

- Coefficient of pedotrophity (C_{ped}) calculated as the ratio of the number of pedotrophic microorganisms (N_{SA}) to the number of microorganisms using organic nitrogen (organotrophs) as follows:

$$C_{\mathrm{ped}} = \frac{N_{\mathrm{SA}}}{N_{\mathrm{MIA}}}. \tag{3}$$

2.4. Alternative Solid Fuel

Firewood, sawdust, woodchips, or wood pellets are the types of solid fuels that are widely used by the average rural resident during the heating season, which lasts from mid-autumn to mid-spring. The same statement is true for most agro-industrial complexes, the production of which does not depend on the season (greenhouses, poultry houses, mushroom houses, etc.). Since the spent substrate consists of 70% of sawdust with the addition of 5% of oil seeds, it was decided to test it as a solid fuel using the following indicators: total humidity, ash, sulfur, carbon content, release of volatile substances, and heat of combustion. The obtained data on heat of combustion were compared with data for fuel properties of firewood, sawdust, wooden pallets, and wood chips from literature sources [39].

Combustion and analysis of SMS were carried out using standard methods:

- DSTU EN 14774-1 "Solid biofuel. Determination of moisture content. Method of drying in a drying cabinet" [40] for determination of sample moisture content, drying of samples to a constant weight at a temperature of 105 °C;
- Ash content was determined by a combined method. First, dried samples were burned according to the EN 15403 method "Solid recovered fuels—Determination of ash content" at a temperature of 550 °C [41], and then, the ash was additionally calcined under the conditions corresponding to the ISO 1171 method "Solid mineral fuels—Determination of ash" at a temperature of 815° WITH [42];
- Carbon and sulfur content determinations were carried out on a Leco CS 230 analyzer according to its instructions, which correspond to the methods of DSTU EN 15104:2013 "Solid biofuel. Methods for determining the content of total carbon, hydrogen and nitrogen" [43] and DSTU EN 15289:2013 "Solid biofuel. Methods for determining the total content of sulfur and chlorine" [44];
- DSTU EN 15148:2012 "Solid biofuel. Method for determining the content of volatile substances" by mass loss during the rapid heating of the sample to 900 °C without air access for 7 min [45] for volatile substance determination;
- Determination of the caloric parameters of the sample was performed on the IKA C2000 calorimeter according to its instructions, which meet the requirements of DSTU ISO 1928 [46].

2.5. Statistical Analyses

The statistical software Statistica 10.0 was used to analyze the data from the bioassays. The results were expressed as mean values (±standard deviation (SD) and smallest significant difference (SSD_{05})) of experiments conducted four times. The level of significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Reuse as a Component in New Substrates' Production Cycle

Substrate results based on the yield data after 14th, 16th, and 18th weeks of incubation are presented in Table 2. Our study outcomes demonstrated that, among the three experimental substrate recipes tested with SMS, the most favorable yield was observed with EX1 as it presents values greater than 20 for incubation periods longer than 14 weeks. This blend consisted of 40% aged beech sawdust, 30% aged SMS, and 10% cereals (such as oats or barley), resulting in an average yield of 25.80% greater than the one obtained with the original substrate recipe for shiitake mushroom cultivation. These results underscore the effectiveness of this specific combination in optimizing crop output.

Table 2. Yield of substrates after 14th, 16th, and 18th week of incubation.

Substrate	Yield after 14th Week (%)	Yield after 16th Week (%)	Yield after 18th Week (%)	Average Yield (%)
CS	20.83	21.91	22.50	21.75
EX 1	24.80	26.41	26.2	25.80
EX 2	16.73	20.63	23.91	20.42
EX 3	10.71	17.98	18.20	15.63

The recipe with equal proportion (35%) of aged beech and aged SMS (EX2) showed a stable average yield of 20.42%, but with an unsatisfactory performance during the first 14 weeks. When the substrate is made up of half of aged beech and half of aged SMS (EX3) and without any cereal additives, it does present stable yield values.

The organoleptic evaluation of picked mushrooms from all types of substrates (Figure 4) was excellent: brown color with white spots, fleshy closed cap, and average size of 45–50 mm.

Figure 4. Shiitake mushroom quality from experimental substrates.

From the results obtained, we conclude that SMS can be reused in substrate production for shiitake mushroom production. For a stable average yield of ≥20%, cereal grain components must be added, with the amount of SMS not exceeding 35%.

3.2. Additive to the Soil for Blueberry Cultivation

The evaluation of the chemical composition of the soil subsequent to the application of both types of additives (see chemical composition in Table 1), crucial for the growth and fruiting of blueberries, was conducted and is depicted in Table 3, revealing noteworthy findings. Specifically, in agroecosystems where SMS was applied, the soil exhibited a higher organic matter content of 49.00% compared to 34.31% for peat. This disparity is expected to positively impact the soil microbiome, fostering conducive conditions for blueberry growth and fruiting [47–49]. Furthermore, Lipiec et al. [50] confirmed that the application of spent mushroom substrate, particularly over the long term, augmented the organic matter content within the soil. The application of SMS contributed to the increase in soil organic carbon content and microbial biomass. Usually, microbial biomass responds more promptly to management practices compared to the total organic carbon content in the soil [51–53].

Table 3. Chemical composition of soil samples after adding SMS and peat.

Indicators	Soil Added with SMS	Soil Added with Peat
acidity, pH	5.16	5.60
hydrolytic acidity, mmol-eq/100 g	44.46	34.65
electrical conductivity mS m^{-1}	56.83	34.73
relative humidity, %	49.39	51.90
organic matter, %	49.00	34.31
nitrate nitrogen, mg/100 g	103.7	31.19
ammonium nitrogen, mg/100 g	2.47	5.20
mobile compounds of potassium, mg/100 g	78.73	73.17
mobile compounds of phosphorous, mg/100 g	75.75	74.75
water-soluble calcium, mg kg^{-1}	435.00	264.50
water-soluble magnesium, mg kg^{-1}	118.50	69.92

Note: data are statistically significant ($p < 0.05$).

It is known that microbial biomass is an important indicator in assessing the ecological status of ecosystems, as well as a sensitive indicator for the influence of applied agricultural technologies and fertilizers [54–56]. The microbiological analysis of soil from the selected agroecosystems, where blueberries were cultivated with the addition of SMS and peat, yielded notable results as can be observed in Table 4. Specifically, the utilization of SMS resulted in a significant increase in the overall microbial biomass, averaging 157.1 $\pm$ 1.82 μg. In contrast, when peat was used, the microbial biomass averaged 143.18 $\pm$ 1.71 μg. These findings underscore the beneficial impact of SMS on soil microbial activity compared to peat application. In the realm of microbial communities, delving into the species composition and the presence of ecological functional groups is crucial. Each microbe plays a distinct role within the community structure, ultimately influencing soil health and plant productivity. The intricate dynamics of microbial communities form complex networks, where various interactions occur among microbes. These interactions encompass resource competition, metabolic dependencies, spatial organization, including the production of biofilms, signaling mechanisms, and horizontal gene transfer [57–59]. Understanding these multifaceted interactions offers profound insights into the functioning and resilience of ecosystems. The investigation of different ecological and functional groups in soil from the agroecosystems studied demonstrated that the application of SMS led to an increase in the functional diversity of the soil microbiome (Table 4). Specifically, there was a notable and significant increase in the number of representatives across practically all functional groups. A more than threefold increase in bacteria utilizing mineral nitrogen in soil (15.6 $\pm$ 1.1 $\times$ 10^3 CFU g^{-1}) compared to bacteria that use mineral nitrogen in agroecosystems with peat (4.3 $\pm$ 0.28 $\times$ 10^3 CFU g^{-1}) can have a positive effect on soil fertility. Also, a significantly increased number of bacteria that use organic nitrogen (11.61 $\pm$ 2.12 $\times$ 10^3 CFU g^{-1}) was observed. Such an increase suggests a potentially heightened capacity for nitrogen cycling and enhanced nutrient availability, thereby benefiting plant growth and productivity. The increased microbial activity in nitrogen utilization may indicate improved soil fertility and overall ecosystem functioning. Previous research has demonstrated that biodiversity, particularly microbial diversity and activity, has the potential to enhance soil ecosystem multifunctionality [60–65].

Table 4. Number of soil microorganisms and the content of total microbial biomass.

Indicators	Soil Added with SMS	Soil Added with Peat
Micromycetes ($\times 10^{-3}$ CFU g^{-1})	8.19 ± 1.69	1.99 ± 0.17
Bacteria which use organic nitrogen ($\times 10^{-3}$ CFU g^{-1})	11.61 ± 2.12	1.34 ± 0.22
Bacteria which use mineral nitrogen ($\times 10^{-3}$ CFU g^{-1})	15.6 ± 1.1	4.3 ± 0.28
Oligotrophs ($\times 10^{-3}$ CFU g^{-1})	1.32 ± 0.13	0.54 ± 0.06
Streptomyces ($\times 10^{-3}$ CFU g^{-1})	7.82 ± 0.23	2.68 ± 0.14
Pedotrophs ($\times 10^{-3}$ CFU g^{-1})	11.53 ± 0.89	1.88 ± 0.14
Total microbial biomass (μg)	157.1 ± 1.82	143.18 ± 1.71

As can be seen from the results, the number of microorganisms was much higher in soil samples with SMSs. Research conducted by Joniec et al. [51] demonstrated that the application of spent mushroom substrate (SMS) led to improvements in various microbiological, enzymatic, and biochemical parameters. These enhancements translated into higher overall soil fertility and quality. Therefore, the application of spent mushroom substrate could indeed enhance soil quality indicators, a finding corroborated by our own results as well. It can be assumed that spent substrate creates favorable conditions for the development of the soil microbiome. Based on the number of soil microorganisms, coefficients of mineralization, oligotrophity, and pedotrophity were calculated (Table 5).

Table 5. Direction of soil microbiological processes.

Samples	Coefficient of Mineralization (C_{min})	Coefficient of Oligotrophity (C_{ol})	Coefficient of Pedotrophity (C_{ped})
Soil added with SMS	1.46	0.27	0.99
Soil added with peat	3.60	0.43	1.40

Note: data are statistically significant ($p < 0.05$).

The application of the spent mushroom substrate into the soil contributed to the activation of the mineralization–immobilization process. The coefficient of mineralization (C_{min}) increased by 2.46 times compared to the soil of the agroecosystem where peat was applied. Additionally, the use of SMS led to a decrease in oligotrophy and pedotrophy coefficients, indicating an improvement in the biological activity of the soil microbiome [38,55,66] and the ecological status of soil.

3.3. Alternative Solid Fuel

In well-managed mushroom industries, following the harvest of mushroom fruit bodies, approximately 70% of the substrate remains as waste, referred to as spent mushroom substrate (SMS). Currently, SMS could be utilized also as fuel [67]. The data obtained during burning of SMS are presented in Table 6, and for comparison. data for firewood and other usual solid fuels are also indicated. Some other parameters for SMS were measured: release of volatile compounds (ROV) was 86.1%, the ash content obtained was 2.44%, sulfur was 0.11%, and total carbon content was 48.5%.

The heat of combustion of SMS is of the order of magnitude of the other solid fuels commonly used. However, it can be observed that the best for use as fuel is spent substrate with a humidity ≤ 20% presenting comparable heat of combustion of wood pallets. Such a level of humidity for SMS can only be achieved with artificial drying (at an average temperature of 105 °C) or naturally but for longer period (2–3 years).

Table 6. Heat combustion of SMS and some commonly used solid fuels.

Samples	Relative Humidity %	Heat of Combustion kcal kg^{-1}	MJ kg^{-1}	kW h kg^{-1}
SMS (naturally dried)	34	2568	10.76	2.99
SMS (artificially dried)	18	4042	16.94	4.71
Firewood (one year under the canopy)	30	2875	12.05	3.35
Firewood (dried)	20	3381	14.17	3.94
Wood pallets	≤10	4100	17.17	4.7
Sawdust	20–30	2000	8.37	2.3
Woodchips	20–30	2610	10.93	3

Note: data are statistically significant ($p < 0.05$).

Based on the results obtained, it can be concluded that the spent shiitake substrate is a potential alternative to the commonly used solid fuel; however, further studies must be conducted to optimize its utilization. For example, an alternative approach involves combining the spent shiitake substrate with other types of solid fuels, such as firewood or pallets. With this combination, it is expected not only to enhance the overall efficiency of combustion but also to present a commendable alternative from both economic and ecological perspectives. Each kilogram of mushroom produced generates approximately 5 kg of wet mass spent mushroom substrate (SMS), with an average water content of around 65%. This indicates a significant potential, totaling almost 8 million tons of dry mass SMS, suitable for biofuel production after shiitake cultivation [67,68].

Therefore, considering the need for pre-drying and potential synergies with other solid fuels, the spent shiitake substrate demonstrates promise as a valuable and sustainable energy source.

4. Conclusions

The potential to valorize spent mushroom substrate by its reintroduction into the primary production cycle and secondary activities, including the production of additives, fertilizers, and alternative fuels, was studied.

The study was conducted to evaluate the potential to reuse SMS for shiitake mushroom production and showed positive results. To obtain a stable average yield, an amount of SMS not exceeding 35%, with cereal grain components and aged beech sawdust, must be used. The organoleptic evaluation of picked mushrooms produced with this was excellent.

The chemical composition of SMS was determined and compared with peat when its potential use of fertilizer was assessed in blueberries cultivation. The SMS is not inferior to peat, presenting higher organic matter and contents of potassium, magnesium, and phosphorus. Based on the results of the microbiological study, it was also possible to conclude that the SMS has a positive effect on the soil microbiome. Moreover, the production of blueberries increased when SMS was used instead of peat, showing that it can be used as an additive to the soil for blueberry cultivation. Further studies are also needed to find out the behavior of SMS on other agricultural crops.

In each concern, the use of SMS as an alternative solid fuel, based on the results obtained from the comparative heat combustion analysis, presents a great potential. However, it is crucial to note that its effective use requires prior drying, either through artificial or natural means, albeit for an extended period. Without proper drying, the substrate may not be suitable as a standalone fuel source.

The main conclusions about the proposed methods of SMS valorization point to economic and environmental benefits for companies, in line with zero waste and green principles. The use of SMS as a raw material for agroindustry and energy production follows the circular economy model and the industrial symbiosis approach.

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