Setas para una agricultura sostenible – el concepto *MUSA*

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Resumen:

El proyecto ***MUSA*** – **MU**shrooms for **S**ustainable **A**griculture [Setas para una Agricultura Sostenible] es un esfuerzo para utilizar procesos basados en setas comestibles con el fin de mejorar la sostenibilidad de la agricultura en los países nórdicos y bálticos. El proyecto abarca tanto la producción de esporocarpos de hongos comestibles como el aprovechamiento del sustrato agotado resultante del cultivo de dichos hongos. Se investiga el uso de residuos de la agricultura nórdica y flujos subutilizados del manejo forestal, así como subproductos del procesamiento de la madera como sustrato para la producción de los hongos comestibles shiitake (*Lentinula edodes*) y pleuroto ostra (*Pleurotus* spp.). El proyecto explora el potencial del sustrato agotado de setas (SMS por sus siglas en inglés) para su uso en apoyo a la producción de alimentos. Se evalúa el potencial del SMS como fuente de compuestos bioactivos y de azúcares. Además, ***MUSA*** investiga el uso de los hidrolizados de SMS como fuente de carbono para la producción de aceite microbiano de calidad alimentaria cultivando levaduras oleaginosas. También se evalúa el uso del SMS para sustituir fertilizantes minerales y proporcionar soluciones de biorremediación de aguas residuales.

Palabras clave: Setas comestibles, sustrato agotado del cultivo de setas, biorefinería, agricultura sostenible.

Mushrooms for enhanced agriculture sustainability – the *MUSA* concept

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Abstract:

The project ***MUSA*** – **MU**shrooms for **S**ustainable **A**griculture is an effort to use mushroom-based processes to enhance agriculture sustainability in Nordic and Baltic countries. The project covers both the production of fruitbodies of edible fungi and the upgrading of the exhausted substrate from mushroom cultivation. The suitability of residues generated locally for producing edible mushrooms is investigated. Residues from Nordic agriculture and sub-utilized streams from forestry management, as well as wood processing by-products, are evaluated as the substrate base for producing shiitake (*Lentinula edodes*) and oyster (*Pleurotus* spp.) mushrooms. The project explores

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the potential of spent mushroom substrate (SMS) to support food production. SMS prospective as source of bioactive compounds and sugars is evaluated. ***MUSA*** investigates the suitability of SMS hydrolysates as carbon sources for cultivating oleaginous yeast to produce microbial oil suitable for human consumption. Using SMS for substituting mineral fertilizers and providing wastewater bioremediation solutions is also assessed.

Key Words: Mushroom, spent mushroom substrate, biorefinery, sustainable agriculture.

1. INTRODUCTION

The generation of crop residues, e.g., straws of wheat or barley, is continuously increasing as a result of the expansion of the agricultural production necessary to support global population growth. Correctly managing the generated residues poses significant challenges (Carvalho et al., 2017). Crop residues are extensively considered as wastes. Although the environmental impact of disposing agricultural wastes has not been as mediatic as the discussion on impacts of industrial and vehicular emissions, burning crop residues has a major contribution to air pollution in many countries. Therefore, the economical use of crop residues within new recycling models is crucial for agriculture sustainability (Donner et al., 2021). Using agricultural residues as raw materials for bioconversion processes in biorefineries is a valid alternative considering their availability, low cost, and renewable nature (Martín, 2021; Passoth and Sandgren, 2019).

Mushrooms are the macroscopically visible reproductive structures of fungi; many are edible. Mushrooms grow well on plant biomass, including crop wastes and underutilized wood residues, such as remnants of early thinning of young forests and sawdust from the sawmilling industry. Edible mushrooms are protein-rich food sources that can partially substitute meat, whose production has a significant climate impact (Lynch and Pierreumbert, 2019). Furthermore, edible mushrooms are rich in vitamins, minerals, and other health-promoting ingredients beneficial for human nutrition and wellbeing (Carrasco-González et al., 2017). They exert health benefits associated with their immunomodulatory, antibacterial, cytostatic, and antioxidant properties.

Unlike regular agriculture, which is season-dependent, mushroom production can be performed throughout the year independently of the climatic conditions. Mushroom cultivation is mainly an indoor activity carried out under controlled conditions, resulting in a highly efficient continuous-flow process. The land area required for growing mushrooms, which is operated as vertical cultivation, is remarkably lower than that required in traditional agriculture. Therefore, mushroom cultivation is a way of diversifying agricultural activities, allowing the farmers to keep the production cycle during the long winter of the Nordic region and to ensure continuous income year around.

The mushroom industry is dynamic, but a few species dominate the world market. *Lentinula edodes* (shiitake) and *Pleurotus* spp. (oyster mushrooms) are among the main edible mushrooms commercialized globally (Royse et al., 2017). Due to the combined food, medicinal, and nutraceutical values of shiitake and oyster mushrooms, their popularity has been pushed forward in Europe. Anyway, the annual production of shiitake and oyster mushrooms in Europe, around 55 000 and 10 500 tons, respectively, is still a minor fraction of the 1.2 MT produced globally (European Mushroom Growers' Group, 2022). Most of European production is concentrated in the south and center of the continent, while in the Nordic countries the production is unsatisfactorily low. For example, in Sweden the champignon, shiitake, and oyster mushroom market relies heavily on imports (Svenska Svampodlarföreningen, 2022).

2. SPENT MUSHROOM SUBSTRATE – PROBLEM AND OPPORTUNITIES

A problem associated with mushroom cultivation is handling the large amount of waste left after harvesting the fungal fruitbodies. Around 3-5 kg of "spent" substrate is generated per kg of produced mushrooms (Zisopoulos et al., 2016). That material, known as "Spent Mushroom Substrate" (SMS), is a residual substrate unsuitable for continuing another cultivation cycle due to the depletion of nutrients. SMS accumulation is a major environmental concern because of the emission of greenhouse gases from spontaneous anaerobic digestion, offensive odors, and runoff drainage ending at water sources (Xiong, personal communication). Furthermore, SMS disposal is an economic problem due to the transport costs because of its high moisture content and low bulk density.

However, SMS should be considered a promising source of valuable products and starting material for several uses rather than valueless waste (Leong et al., 2022). Recent publications show that SMS is a potential source of feed, food, bioactive compounds, biofuels, and enzymes, and it can be used as a biofertilizer, soil amendment, bioremediation agent, and several other uses (Martín et al., 2023). Valorization of SMS is crucial for the sustainability of the mushroom industry. Its development will contribute to agricultural sustainability and to the transition to a circular economy.

3. *MUSA* PROJECT AND VALORIZATION CONCEPT

MUSA (**MU**shrooms for **S**ustainable **A**griculture) is a project, where scientists from Norway, Sweden, and Estonia join efforts to develop solutions contributing to enhanced agriculture sustainability in Nordic and Baltic countries. ***MUSA*** researchers are convinced that developing an economically efficient and environmentally sustainable production of mushrooms in the Nordic region, including the highly appreciated shiitake and oyster mushrooms, is possible. The large availability of crop and forest residues in the region poses a high potential as a substrate base for mushroom cultivation. By growing edible mushrooms on residues from local agriculture and indigenous forestry plantations, waste streams can be converted into high-quality food. For the Nordic societies, that would decrease food imports, leading to economic profits and food security and reducing greenhouse gas emissions in transportation. Furthermore, it would add value to materials currently considered residues, promoting bioeconomy and rural development.

The ***MUSA*** concept envisages growing mushrooms as a sustainable process because it converts residual biomass into high-quality food and produces SMS for various applications. The rationale behind the valorization concept is the combination of (i) presence of bioactive compounds in SMS, (ii) pretreating effect of fungal cultivation, which activates polysaccharides to enzymatic saccharification, (iii) suitability of SMS cellulosic hydrolysates as substrate for microbial cultivation, (iv) ability of *Rhodotorula* spp. yeasts to produce oils and bioactive compounds in hydrolysates, (v) potential of green solvents for extracting lignin from lignocellulosic residues, (vi) SMS feasibility as biofertilizer and soil amendment, and (vi) potential of SMS for wastewater bioremediation.

The general aim of the project is to develop a novel approach for enhancing agriculture and forestry sustainability in the Nordic region by producing edible mushrooms on substrates based on local bioresources in combination with upgrading the SMS in applications supporting food production, the substitution of mineral fertilizers and providing wastewater bioremediation solutions. The ***MUSA*** concept is unique and novel in its holistic approach, which combines mushroom cultivation on plant residues with a broad palette of SMS applications. In contrast, previous research has often focused on mushroom cultivation on a specific type of substrate, with limited attention to SMS valorization. Furthermore, previous valorization efforts seldom included different alternative uses as ***MUSA*** does.

4. METHOD/DEVELOPMENT OF INNOVATION EXPERIENCE

The project is organized into five work packages (WP) listed below.

- WP1. Mushroom production
- WP2. SMS biorefining
- WP3. Use of SMS as biofertilizer and soil amendment
- WP4. Use of SMS for the conservation of water ecosystems
- WP5. Management, dissemination, exploitation, and communication

The research activities in the technical WPs (WP1-4) are discussed in subsections 4.1 – 4.4. A schematic representation is given in Figures 1 and 2.

4.1. Mushroom production

Previous work by ***MUSA*** researchers has shown the suitability of underused streams of Nordic forestry for producing shiitake (Xiong et al, 2019) and oyster mushrooms (Chen et al., 2022a). That experience is used in the WP1 of the current project for cultivating edible mushrooms and generating SMS on substrates based on residues of Nordic agriculture and forestry (Fig. 1, top). The effect of the substrate composition on the quantity and quality of fruitbodies of several gourmet mushroom species (mainly shiitake and various oyster mushrooms) and SMS characteristics are being evaluated.

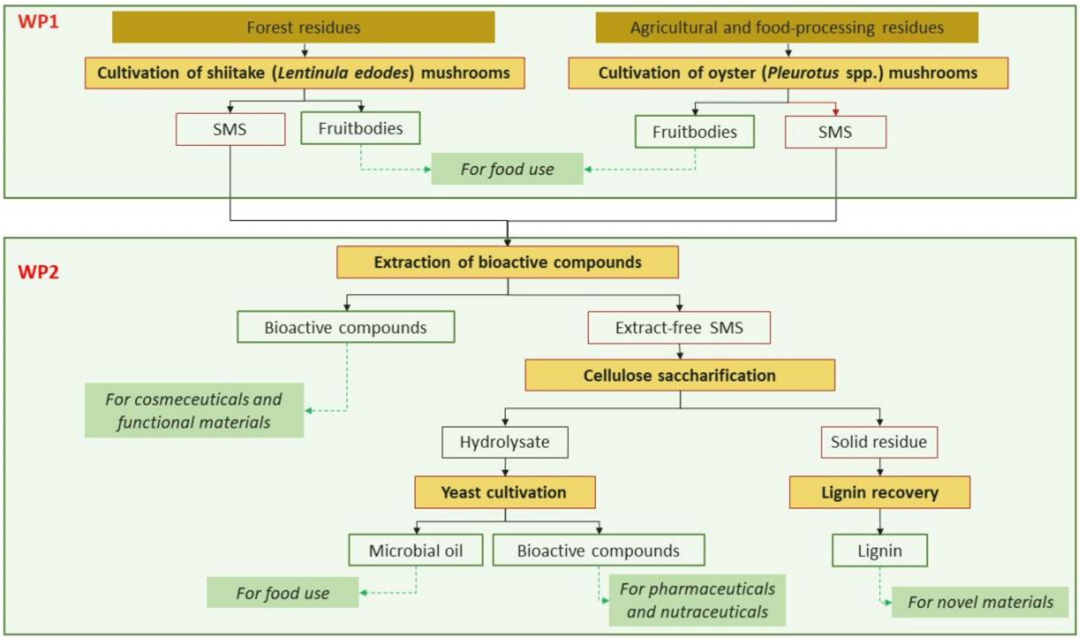

Figure 1. Schematic representation of the research activities in WP1 (top) and WP2 (bottom). **Source:** Own work

The feasibility of a novel automated system for reaching high-quality fruitbodies within shorter periods than in state-of-the-art methods will be assessed. Factorial experiments, with the substrate components and their weight fraction as independent variables, mycelial colonization rate, mushroom yield, and compositional changes as response factors, will be carried out under controlled temperature, humidity, light, and CO_2. Analytical acid hydrolysis combined with high-performance liquid chromatography, and pyrolysis-gas chromatography/mass spectrometry are used for compositional analysis.

4.2. SMS biorefining

Using SMS as a biorefinery feedstock is an important research line in the ***MUSA*** concept. The WP2 envisages a biorefining scheme including four connected processes, namely, (i) extraction of bioactive compounds, (ii) enzymatic saccharification of cellulose and hemicelluloses contained in extract-free SMS, (iii) production of microbial oil and carotenoids by yeast cultivation in SMS sugars, and (iv) lignin recovery from saccharification residue (Fig. 1, bottom).

4.2.1. SMS as a source of bioactive compounds

SMS is a promising source of bioactive compounds of either fungal or plant origin, e.g., polyphenols, polysaccharides, and proteins. That includes (i) molecules contained in the mycelium, (ii) substances secreted by fungal growth, (iii) phytochemicals of the lignocellulose extractives, and (iv) products of partial degradation of plant cell wall polymers. However, the bioactive molecules of the SMS, differently from those of the sporocarps of edible fungi, have so far not received enough attention.

MUSA actions are directed to extract bioactive phenolics, β-glucans, proteins, and ergosterol from the SMS resulting from cultivatiing *L. edodes* and *P. ostreatus*. Fruitbodies of those fungal species have high pharmaceutical and culinary interest (Parola et al., 2017), but their SMSs are largely underexploited. ***MUSA*** researchers will apply smart extraction procedures for recovering bioactive compounds from SMS. Novel smart-extraction protocols leading to high recovery of bioactive molecules without affecting their functional properties and avoiding the degradation of non-targeted compounds are now under development. The first experiments within the project allowed recovering polyphenolics from SMS of different *Pleurotus* spp. using ultrasound and subcritical water for intensifying the extraction (Klausen et al., 2023). So far, the research has identified phenolic acids contained in SMS extracts and correlated the antioxidant activity with the content of total phenolic compounds and with individual phenolic acids. A good correlation between the FRAP antioxidant capacity and the concentration of caffeic acid in extracts was elucidated. Experiments on optimization of the subcritical-water extraction of *Pleurotus* spp. SMS and extending it to *Lentinula edodes* (shiitake) SMS are underway. The produced extracts will be concentrated, refined, and evaluated as source of ingredients for cosmeceuticals and functional packaging.

4.2.2. SMS as a source of sugars

Even if a part of the cell wall components is consumed during fungal growth, the SMS still contains considerable amounts of cellulose, hemicelluloses, and lignin (Chen et al., 2022a). Those components remain in the solid residues after extraction of bioactive compounds from SMS. Processing them via sugar-platform routes allows for obtaining additional bioactive substances and other valuable compounds.

During mushroom cultivation, the growing fungi partially degrade lignin and a part of hemicelluloses, thus enhancing the enzymatic saccharification of cellulose (Chen et al., 2022b). Therefore, mushroom cultivation can be considered a bio-pretreatment for lignocellulose bioconversion. We have previously shown that cellulose contained in shiitake SMS is highly susceptible to enzymatic saccharification (Xiong et al., 2019; Chen et al., 2022a). ***MUSA*** project will investigate how the extraction processes applied to recover bioactive compounds affect the enzymatic saccharification of SMS cellulose. The initial experiments have shown that the extract-free material remaining after the recovery of bioactive compounds from *Pleurotus* spp. SMS displays an enhanced saccharification compared to raw SMS (Klausen et al., 2023).

4.2.3. SMS sugars as sources of lipids, carotenoids, and β-glucans

The sugars released from enzymatic saccharification serve as carbon sources for microbial fermentations. Furthermore, we have previously shown that SMS hydrolysates contain nutrients that allow fermentation with baker's yeast (*Saccharomyces cerevisiae*) without additional supplementation (Chen et al., 2022c). The current project intends to assess the fermentability of SMS hydrolysates with oleaginous yeasts. We have previously cultivated oleaginous yeasts in hydrolysates of other lignocellulosic materials (Passoth et al., 2023), and we expect that SMS hydrolysates will also be suitable substrates.

Oleaginous yeasts can convert hydrolysates into valuable substances like oils, carotenoids, and β-glucans. The lipid content of oleaginous yeasts, e.g., *Rhodotorula toruloides*, can be above 60% of cell dry matter, which can be reached within 3 – 4 days of cultivation (Brandenburg at al. 2021; Chmielarz et al., 2021). Vegetable oils (VO) are among the products causing the largest environmental impact (Kathri & Jain, 2017). The greenhouse gas potential per ton of produced palm-, soybean- and rapeseed oil is above 2000 kg CO_2 (Uusitalo et al., 2014). Furthermore, rainforest clearing for soybean and oil palm plantation is a major problem in VO-producing countries. Thus, oil production from SMS hydrolysates will decrease the environmental impact of food production and increase the security of supply. Fungal oil produced from lignocellulose was used as food in Germany during the first world war (Lundin 1950). There are also examples of using yeast oil in fish feed (Blomqvist et al. 2018, Brunel et al. 2022).

In a recent study it has been shown that yeast oil produced by *Rhodotorula* spec. from wheat straw requires 10-38% fossil resources less than producing the same amount of rape seed oil. VO for feeding salmonid fish feed can be replaced by yeast oil without requiring additional agricultural land (Sigtryggsson et al. 2023).

Rhodotorula spp. yeasts are also a source of bioactive compounds, such as carotenoids (β-carotene, torulene, and torularhodine) and β-glucans. Carotenoids exhibit antioxidant properties and are important for pharmaceutical and food industries (Rapoport et al., 2021). β-Glucans have immunostimulant activity (Zhu et al., 2016).

4.2.4. Lignin recovery from SMS saccharification residue

Lignin contained in the initial substrate used for mushroom cultivation is majorly degraded by fungal growth. The lignin fraction not degraded during fungal growth remains in the treated solids even after extraction and saccharification. The SMS saccharification residue is a lignin-rich material with good valorization potential, provided lignin is appropriately recovered and purified. Based on our previous experience in upgrading hydrolysis lignin resulting from softwood saccharification in a biorefinery (Momayez et al., 2022), we intend to use green solvents to extract lignin from SMS saccharification residue. ***MUSA*** envisages recovering high-purity lignin from SMS and directing it to biomedical applications, energy-storage solutions, and other value-added uses.

4.3. SMS as biofertilizer and soil amendment

SMS can result in agronomic benefits when applied to farmlands. Previous reports show that SMS contributes to repairing soil quality and enhancing the growth of selected plants, and its application allows for diminishing the use of mineral fertilizers (Martín et al., 2023; Leong et al., 2020). SMS contains a high percentage of carbon, leading to nitrogen and other nutrients immobilization during the first phases of the decomposition (Medina et al., 2012). Although SMS's suitability as a biofertilizer or soil amendment has previously been shown, the full potential of the nutrients captured in the fungal mycelium and its substrate is still poorly explored. By quantifying the long-term agronomic benefits of using SMS as a peat substitute in horticulture or as a soil amendment in agriculture (WP3, Fig. 2), the ***MUSA*** project expects to fill the existing knowledge gap.

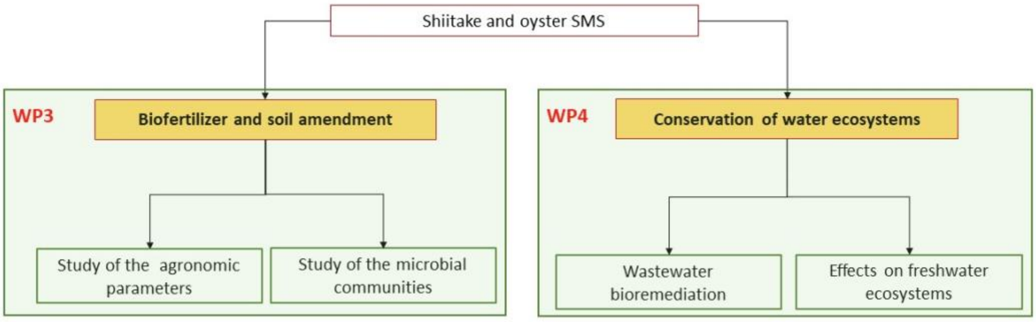

Figure 2. Schematic representation of the research activities in WP3 (left) and WP4 (right). **Source:** Own work

Since microbes inhabiting SMS hold enzymatic systems that degrade polymeric compounds into simpler substances accessible to plants, knowledge on the microbial communities in the SMS are important for the use as a biofertilizer and soil amendment (Xu et al., 2022). Previous studies have mainly focused on microbes with cellulolytic and ligninolytic capacity (Hu et al., 2019), whereas chitinolytic bacteria have been underestimated. However, the promotion of chitinolytic microbes is a promising strategy to improve crop yield and to control diseases considering that chitin biodegradation generates products that can act as nutrients and that chitin-degrading microbes are often biocontrol agents of plant pathogens (Hui et al., 2019). In the WP3 of the current project, the structure of microbial communities in SMS will be elucidated and manipulated to enhance the bioavailability of nutrients and facilitate their uptake by selected crops (Fig. 2). Chitinolytic and N-fixing bacteria will be isolated, and possible correlations between specific microbes and SMS effectivity as biofertilizer and soil amendment will be assessed. The research on SMS as a biofertilizer and soil amendment is backed by the previous studies by the team on the impact of organic fertilizers (Mousavi et al., 2022) and on studying mushroom-inhabiting microorganisms (Gohar et al., 2022).

4.4. SMS for conservation of water ecosystems

The direct reuse of treated wastewater for irrigation has attracted much attention to reduce the demand for freshwater supplies in agriculture. However, due to incomplete removal by conventional water treatment, organic micropollutants (OMPs) can be detected in treated wastewater (Golovko et al., 2021). Sustainable and cost-efficient treatment options are needed to prevent the introduction of OMPs into the agricultural ecosystem and food chain, and SMS has gained attention in this context (Leong et al., 2022). ***MUSA*** researchers have previously observed high removal of OMPs by treatment with fungal substrates, which was explained by the release of enzymes, such as laccases and peroxidases, in parallel with adsorption of the micropollutants to lignocellulose (Hultberg et al., 2020).

SMS-based bioremediation technology is still in an early phase, and further research is still required. Furthermore, the impact of exposing water ecosystems to SMS needs to be assessed. Despite the potential of direct use of SMS as an *in-situ* bioremediation tool in polluted water bodies, the possible leakage of nutrients into the ecosystem might increase the eutrophication risk. Therefore, the SMS effects on freshwater ecosystems need to be evaluated. WP4 includes using SMS in wastewater bioremediation and evaluating the effects on selected water systems (Fig. 2).

5. DISCUSSION / CONCLUSIONS

Production of edible mushrooms based on local crop and forest residues is a promising commercial activity contributing to the sustainability of Nordic agriculture and forestry. The generation of high quantities of spent mushroom substrate (SMS) is a consequence of an active mushroom business. Therefore, the efficient valorization of SMS is highly relevant for a sustainable mushroom industry.

The ***MUSA*** concept focuses on valorizing SMS towards valuable products and services. The project will develop novel protocols for green extraction of bioactive compounds from SMS. The extracted bioactive molecules could be used as bio-based ingredients in socially sensitive commercial areas. SMS-derived nutraceuticals, cosmeceuticals, food supplements, and active ingredients in functional materials might be the base of a new "*next-generation mycoderivatives*" sector.

Saccharification of SMS polysaccharides provides carbon sources for microbial fermentations. ***MUSA*** will investigate in detail the production of microbial oil and bioactive compounds by cultivating oleaginous yeasts in SMS hydrolysates. Our results will be of interest for extending the experience to producing biofuels, biopolymers, and platform chemicals using yeasts and bacteria. **MUSA** project also includes a pioneering study on recovering lignin from SMS saccharification residue. Characterization of the recovered lignin will help elucidate potential applications.

Investigating the benefits of SMS application on soil fertility and structure will clarify its potential for substituting a part of the currently used mineral fertilizers, which would contribute to mitigating soil salinization and acidification and avoiding nutrient imbalances and accumulation of toxic compounds. The ***MUSA*** researchers will also generate valuable knowledge on the structure of microbial communities inhabiting SMS and on their effect on SMS properties as biofertilizer and soil amendment.

Using SMS in the bioremediation of wastewater to be used in irrigation is a possible sustainable treatment for preventing the introduction of organic micropollutants into the food chain. ***MUSA*** expects to generate knowledge on an efficient SMS-based bioremediation technology that removes pollutants from contaminated water and avoids detrimental effects on selected water ecosystems.

MUSA has an interdisciplinary team allowing for an interdisciplinary approach. That is crucial for setting a technically feasible and environmentally sustainable method for valorizing plant residues via mushroom production. That interdisciplinary approach will also allow for efficiently upgrading SMS within a circular bioeconomy scenario.

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