SCIENTIFIC DATA BASE

Text

for SPORADIC's 2023 Field Experiment

in Nouvelle-Aquitaine

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About

SPORADIC is a french non-profit organization whose main purpose is to spread fungi-cultures.

We function according to three core principles:

1. **Scientific rigor**: our actions advocate for change based on rigorous science
2. **Open data**: all our data is publicly available and accessible
3. **Inclusive action**: we work to passerelle scientific knowledge and local communities

1. Introduction

The generation of agriculture and agro-industrial waste worldwide is considerable and the elimination and burning of this waste have generated various environmental impact problems around the world (Dhull, 2022). Generally, agriculture and agro-industrial residues are water-insoluble materials reported to be rich in the lignocellulosic components that include cellulose, hemicellulose, and lignin, collectively referred to as “lignocellulosic biomass residue” or LBR, a most common natural polymer on Earth. In this regard, the so-called agriculture and agro-industrial “waste” for disposal represents a source of energy and carbon and can be utilized for different high value-added products such as biofuels, fine chemicals, and cheap energy sources for microbial fermentation and enzyme production).

This study focuses on this form of waste as a source of the necessary nutrients for the growth of edible and lignocellulolytic fungi and production of enzymes by solid-state fermentation. Located in Europe’s leading agricultural region, Nouvelle Aquitaine, this study will experiment mushroom cultivation on a variety of LBR generated by local farming and industrial productions.

Mushroom cultivation seeks to recreate natural processes in controlled conditions. This applies to a high-tech approach as much as for a simple and small production. The better the growing chamber is able to simulate nature, the better the final result will be, the better the quality and quantity of the fruiting bodies – or mushrooms – will be. This is the secret of a successful production. But what does this term, successful production, entail? Is it the ability to create fruiting bodies on some substrate under controlled conditions? Or is it rather the opportunity for a sustainable business generated by this product?

2. Goals & Methodology

The overarching goal or ambition of this experiment is to *gather and spread data and* knowledge on mushroom cultivation as a feasible waste management biotechnology from and to established farming and industrial communities in agricultural region Nouvelle Aquitaine. Main participants are volunteering citizen-scientists from SPORADIC organization and producers of lignocellulosic biomass residues. The field experiment is to be carried over a period of nine months, fragmented in two phases of four month and a half each.

Phase I consists in a preliminary and contextual study of the LBR productions in Nouvelle-Aquitaine. Phase II studies the changes in digestibility of LBR during the vegetative and fructification phases of fungal growth.

(1) Phase I is initiated with the realization of a cartography of the productions of LBR from economical activities, namely crop-based and processing-based (Figure 1), that are suitable

substrate materials for the growth of white-rot fungi. The data will be collected from AREC, the regional agency of environmental evaluation and climate.

Second, citizen-scientists will inquire, survey and compare, directly on agricultural and industrial production sites, current waste management practices and knowledge about fungi as a waste-treatment biotechnology. Currently in discussion is the production of a numerical, interactive cartography or repertoire of LBR productions and producers. Possible partnership for this include the organization Artisan Numérique, which, in collaboration with the Chambre des Métiers et de l'Artisanat (Chamber of Trades and Crafts), has experience in creating such interfaces for the use of the public.

The statistical and journalistic materials collected during this preliminary study may be published as a podcast and be exhibited at diverse events organized by SPORADIC in collaboration with broadcoasting organizations, such as Campus Radio and Radio Tournesol. According to the results of phase I, namely good relationship with LBR producers, we may move on to the second phase of the experiment.

(2) Phase II begins with the selection of the raw, uncomposted form of lignocellulosic biomass residues and its matching white-rot species. Accordingly, we select a control substrate (i.e., oak sawdust for shiitake, wheat straw for pleurotus etc.). Once logistics are established, we begin testing mushroom growth, applying EkoFungi's cultivation techniques.

We will evaluate the changes in digestibility of LBR substrates during the spawn running period and fruitbody production, and investigate the relationship between the nitrogen and lignocellulosic contents of the substrates and their productivity. To do so, we conduct substrates analysis for nitrogen (N), cellulose, hemicellulose and lignin content at three different growth stages : prior to spawning, during spawn running and during fruit body production. We will measure the effect of LBR on spawn run time, time to first primordia initiation, time to first harvest, yield, C:N ratio, biological efficiency (BE) and average mushroom weight. In addition, some of the chemical properties of fruit bodies and spent mushroom substrate (SMS) will be determined.

According to these quantitative and qualitative analysis, we will evaluate the most appropriate mushroom cultivation protocol for the tested LBR and the most suitable purpose(s) for the end-of-cycle product (fruitbodies and SMS): edible mushrooms, compost, animal feed, medicinal extracts, myco-materials, cosmetic products etc. (Figure 2; 5-6).

Ultimately, mushroom cultivation is a small commercial activity involving the agricultural community and technical experts, which fulfills three sustainable development goals, i.e., nutrition (proteinaceous food), good health and well-being (medicinal value), and lignocellulosic biomass-based waste management, making Earth a better place for human survival with less environmental and nutritional challenges.

3. Environmental Considerations[1]

The fungi are unique among the living organisms and are omnipresent in the biosphere. They are eukaryotic, heterotrophic, absorbotrophic, a-bi-flagellate spore-bearing and they consist of a ramified, diffused and tubular vegetative apparatus with a chitinous cell wall (Heckman, 2001; Lutzoni, 2004; Munnecke, 2010).

Recently, many studies showed that wild mushrooms are exploited for decomposition of forest litter, cellulose, hemicelluloses, and lignin compounds, and other biodegradation of environmentally hazardous materials (Dhull, 2022, 435-451). These organisms had the biochemical and ecological function to degrade organic chemicals, by secreting extracellular enzyme which help in the assimilation of complex carbohydrates without prior hydrolysis. This leads them to having the ability to degrade a wide range of pollutants by chemical modification or by influencing chemical bioavailability (Barh et al., 2019). There are several wood decaying basidiomycetes, i.e. *Ganoderma sp, Lentinus tigrinus, Panellus stipticus, Phanerochaete chrysosporium, Abortiporus biennis, Inonotus hispidus, Pleurotus sp., Bjerkandera adusta, Trametes hirsuta, Irpex lacteus, and Dichomitus squalens* that are potent agents to degrade and/or detoxify a broad range of pollutants, i.e.,. chlorinated compounds, textile or industrial effluents, aromatic hydrocarbons, preservatives, agro-industrial waste (fungicides, herbicides, and insecticides, etc.) into non-toxic simple organic compounds (Anastasi, 2010; Da Silva Coelho et al., 2010; Inoue et al., 2010; Ntougias et al., 2012).

The aforesaid mushroom species could be eventually exploited by many significant biotechnological processes meant for providing eco-friendly and economical approaches related to waste management. Macrofungi can be considered both remarkable degrading agent of waste by-products and a significant element of the food web. They cannot degrade forest litter directly; first of all, they cover the entire forest floor with their mycelial mat and then disintegrate the waste material by means of an arsenal of enzymes (Rhodes, 2012). Degradation speed generally depends upon the nutrients present in the soil. There is an increase in recent literature reporting that these properties are exploitable for the transformation of various lignocellulosic waste to produce edible and medicinal mushrooms (Kumla et al., 2020) or to obtain diverse biologically active components i.e.,. enzymes (Dhull, 2022).

A successful production must provide those conditions during the vegetative phase that promote fungal enzymatic function. Contrastingly, the fructification phase does not have specific nutritive requirements. In effect, all that we want and need to do is to efficiently simulate the natural processes typical for lignocellulosic-degrading species from the Basidiomycetes orders.

Nutrient availability, C:N ratio, pH, compaction, oxygen, and carbon dioxide concentrations, and temperature of the substrates also influence mushroom growth; however, they are strongly influenced by the type of base substrate and supportive supplement percentage along with precise timing and proper method of application.

4.1 Sources, Composition & Availability of Lignocellulosic Biomass Residues

Commonly, about 85-90 % (dry weight) of LBR are made of cellulose, hemicellulose and lignin, whereas the rest are balanced between ash and minor components such as nitrogen, phosphorus, potassium, calcium, magnesium, manganese, iron, zinc, copper, boron, etc.(Philippoussis, 2009; Cherubin, 2018). However, the quantitative and qualitative aspects of

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[1] The following considerations are the so-far-compiled blend between SPORADIC's study of recent literature and insights kindly shared by scientists/producers from EkoFungi

these components are defined by the nature of the source of the residues (i.e., crop type, part of the plant, season of growth etc.), harvesting techniques, processing techniques, storage conditions etc., (Pasangulapati, 2012; Kumula, 2020).

On a dry weight basis, softwood comprises 33–42% of cellulose, 22–40% of hemicellulose, and 27–32% of lignin (Nhuchhen, 2014; Tarasov, 2018). Hardwood contains 38–51% of cellulose, 17–38% of hemicellulose, and 21–31% of lignin (Menon & Rao, 2012; Tarasov et al., 2018). Herbaceous crops contain 25–95% of cellulose, 20–50% of hemicellulose, and 0–40% of lignin (Smit & Huijgen, 2017; Tarasov, 2018). Therefore, based on quantity, cellulose is one of the prominent ubiquitous organic polymers on Earth in LBR like plant wood, cotton, sugarcane, cereals, etc.

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Cellulose is a relatively stable homopolymer made up of several hundred to many thousands of β-anhydro-glucose units interconnected by β-1,4 glycosidic bonds (Klemm, 2005). Hemicellulose is a heteropolymer based on polysaccharide backbones which structure depends on the sugar units, the chain length, and the way the side chains are connected. Typical binding sugars involved in the construction of hemicellulose are pentose (arabinose and xylose), hexose (glucose, galactose, and mannose), hexuronic acids (glucuronic, galacturonic, and 4-O-methyl-d-glucuronic), as well as small amounts of fucose and rhamnose and an acetyl group. Lignin is a highly branched, amorphous, hydrophobic, and rigid polymer containing various functional groups such as carboxyl, methoxy, aliphatic, phenol hydroxyl, and carbonyl. These functional groups contribute to the very complex structure characteristic of lignin. In complex polymerization reactions, a unique, three-dimensional, highly branched lignin configuration is formed. The composition of lignin depends on the respective plant species and the environment in which it is formed (Dhull, 2022).

Complex lignocellulosic material in nature is broken down by organisms that possess lignocellulosic enzymes such as bacteria, fungi, earthworms, and woodlice, which ensure the circulation of carbon in nature through these activities (Bredon et al., 2018). Due to the extremely complex binding between lignocellulose polymers, degradation is performed by the synergistic action of several carbohydrate-active enzymes (Lombard et al., 2013). Complete degradation of lignocellulose is achieved by the combined action of hydrolytic enzymes involved in the degradation of cellulose and hemicellulose as well as oxidative enzymes responsible for the degradation of lignin.

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Numerous works of research and production have demonstrated the particularly efficient ability of white-rot fungi, by controlled solid-state fermentation, to utilize diverse ranges of LBR in individual or combination as a substrate, most commonly straw or stover from crops (wheat, oat, barley, rye, soybeans, rice, corn, bean, oil palm, potato, cassava) are used, but sawdust, woodchips, bark and branches, sugarcane bagasse, husks of soya, cotton waste, peanuts, grape seeds, byproducts from the brewing industry (brewery spent grain), byproducts from the coffee industry (chaff, pulp and coffee waste) and other materials rich in lignin and cellulose can also be used *(Table 1-3).*

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4.2 Indicators for selection

The main criteria to select a potential substrate candidate is the amount of cellulose, hemicellulose and nitrogen it contains. They are the key components of any agricultural and agro-industrial residue, and their degradation depends on their respective amount in the residue and mushroom species. In order to help our estimate of substrate conversion efficiency, two indicators are important: the biological efficiency and the C:N ratio.

Biological efficiency (BE) is a function of the genotype of mushrooms, nature, and concentration of nutrients and minerals in the substrate (Sardar, 2017; Chawla et al., 2019). The maximum values of biological efficiency labels the quantifiable degradation of lignocellulosic components of the LBR by lignocellulolytic enzymes. In other words, it validates the ability of a particular mushroom species to utilize substrate productively. A higher BE value indicates a higher potential for waste utilization.

$$\text{BE} = \frac{\text{Weight of fresh mushroom harvested per bag x 100 \%}}{\text{Weight of dry substrate per bag before inoculation}}$$

For instance, Attila (2019) reported highest yield and BE with the substrate having lower cellulose to lignin ratio and vice versa for sunflower head residue (2:1) and chickpea straw (3:1) and associated maximum total yield (g/kg) (233.7 and 228.1 respectively) and BE (51.0% and 50.6% respectively) for *Lentinula edodes.* Moreover, Lentinula edodes (white rot fungus) proved their high selectivity for lignin degradation, (Philippoussis, 2009; Ritota & Manzi, 2019). Contrastingly, the corn stalk substrate with high cellulose to lignin ratio (8:1) resulted in a lower yield (87.9 g/kg) and BE (20.1%).

Another relevant parameter in the selection of components for mushroom cultivation is ratio of carbon and nitrogen (C:N). Most LBR are defined as low nitrogen content materials and have variable C:N ratios (Dhull, 2022). This C:N directly affects mycelium growth, mushroom weight, yields and protein content in the fruiting body of mushrooms (Sardar, 2017; Zied 2011).

Each mushroom species has its optimum C:N ratio that ensures maximum yield in minimum time (Zied et al., 2011; Atila, 2019). During the production phase, the breakdown of organic matter in substrate causes a reduction in carbon content and increment in nitrogen content that ultimately decline the C:N ratio. The higher nitrogen content in the lignocellulosic biomass during the production cycle the higher the development of high-protein content (Koutrotsios et al., 2014; Dhull, 2022). Inversely, the nitrogen supply of certain LBR, such as traditional cereal straw or sawdust, is exhausted during the mycelium formation, which results in the decline of yield and protein content. Hoa et al. (2015) reported high protein content in *Pleurotus ostreatus* and *Pleurotus cystidiosus* with corncob substrate (C:N = 34.57) and low protein value with sawdust substrate (C:N = 51.7). Koutrotsios et al. (2014), Sardar et al. (2017), and Atila (2019) made similar observations using different varieties of LBR.

One way to balance the hurdle of nutrient availability is to combine with other low C:N ratios LBR in order to obtain the most favorable C:N ratio. Reported processed-based residues that are low cost and easily available include wheat bran, rice bran, mustard cake, cottonseed cake, soya cake powder, carrot pulp, molasses, dry nuts, fruits peels, grape pomace, cottonseed meal, chicken manure, urea (Pardo-Giménez et al., 2018; Salama et al., 2019).

Control Substrates

i.e., *Sawdust*

Sawdust consists of cellulose, hemicellulose, and lignin as lignocellulosic components that vary with the types and parts of the tree. The hardwood sawdust consists of 38–51% of cellulose, 17–38% of hemicellulose, and 21–31% of lignin (Table 2.1). Softwood sawdust consists of 33–42% cellulose, 22–40% hemicellulose, and 27–32% lignin (Table 2.1). Sawdust produced from wood or forest waste is a plentiful source of lignocellulosic biomass that is proficiently utilized for mushroom cultivation. *Ganoderma lucidum* is one of the most efficient wood biomass degrader mushrooms (Rashad et al., 2019).

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3. Environmental Considerations

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