Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 2, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

FUNGAL BIOTECHNOLOGY OF LIGNOCELLULOSIC WASTE CONVERSION - A REVIEW

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Abstract

Lignocellulosic materials are the constituent elements of all plants and are most renewable feedstock available for most regions of our world. Lignocellulosic waste represents huge amounts of unutilized plant-based bioresurces, difficult to degrade for industry. Very important components of natural lignocellulosic materials are carbohydrate polymers represented by cellulose and hemicellulose, and lignin an aromatic polymer. In its natural lignocellulose state, cellulose is protected from microbial degradation, mainly due to the lignin and hemicellulose polymer components. The biotechnology of lignocellulosic material conversion into bio-products normally requires multistep processes. The focus of this article is a study on the potential cultivation of edible and medicinal mushrooms, using different types of residues as natural substrates: fruit tree wastes, winery and vine wastes, agricultural and agro-industrial wastes. A large number of fungi are capable for selectively degrading lignin. Proper management of lignocellulose biodegradation and utilization can serve to improve the quality of the environment.

Key words: *environment biotechnology, cellulose, hemicellulose, lignin, lignocellulose, biodegradation.*

INTRODUCTION

Lignocellulose is the most abundant source of biomass on earth, originating from fruit tree, forestry, agricultural, and agro-industrial wastes, causing environmental problems.
Lignocellulose is mainly composed from carbohydrate polymers: cellulose and hemicellulose, and they are tightly bound to an aromatic polymer, lignin (Figure 1).

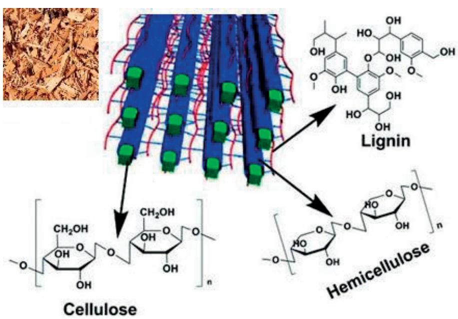

Figure 1. Structure of the lignocellulosic biomass
Source: http://www.iitbmonash.org

Some variety of edible and medicinal fungi can fragment these macromolecules. In natural environments, breakdown of lignin is brought by filamentous fungi belonging to the class of basidiomycetes that secrete an array of enzymes, such as lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases for this purpose (Vicuña Errázuriz, 2000).
Huge amounts of residual plant biomass can potentially be converted into various different value-added products like human nutrients, improved animal feedstuffs, and cheap energy sources for fermentation, chemicals compounds, and high-quality biofuels.
Lignocellulosic enzymes also have significant potential applications in various industries including: chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture (Howard et al., 2003).
Main focus in this review is to highlight significant aspects of fungal biotechnology of lignocellulose waste bioconversion.

MATERIALS AND METHODS

Online research was conducted using PubMed, ScienceDirect, Cochrane, and Embase data.

BIODEGRADATION AND UTILIZATION OF LIGNOCELLULOSIC RESIDUES BY FUNGI

White and brown rot fungi produce extracellular enzymes or exoenzymes, which are synthesised inside the cell and then secreted outside the cell. These enzymes are employed in a number of industries for pollution control. For example, laccases which can be produced by white rot fungi are capable of oxidising several environmental pollutants. Cellulases, represent a mixtures of hydrolytic enzymes produced by basidiomycetes fungi, which catalyses the conversion of carbohydrates and is widely used in pharmaceutical, food and beverage industries. Cellulases include endo-acting (endoglucanases) and exo-acting (cellobiohydrolases) enzymes. The use of laccases as enzymes for biotechnological applications has increased since their discovery in basidiomycetes white-rot fungi. There are many additional technologies that include selective delignification to produce cellulose, the conversion of lignocellulose into feed and biofuels, and also new technologies for treating environmental pollutants and toxic agents generated in vary industrial processes. As a result, laccases enzymes have been extensively studied for various biotechnological applications, including functionalization of lignocellulose materials, modification of wood fibres and remediation of contaminated soil and effluents, also to use in various biosensors. These studies show that laccases are enzymes of interest for utilization in environmental protection applications (Viswanath et al., 2014).

Very often cultivated mushroom in the world are in the following order *Agaricus bisporus* (button mushroom) followed by *Lentinus edodes* (shiitake), *Pleurotu*s spp. (oyster mushrooms), *Auricularia auricula* (wood ear mushroom), *Flammulina velutipes* (winter mushroom), and *Volvariella volvacea* (straw mushroom). Others species of fungi successfully produced on diverse substrates include *Hericium erinaceus*, *Ganoderma* spp., *Agrocybe aegerita*, *Grifola frondosa, Lepista nuda, Hypsizygus marmoreus, Coprinus comatus, Pholiota nameko* and *Stropharia* spp. Even if the mentioned mushroom species, have the capacity to degrade lignocellulosic residues, there are some differences regarding production of enzymes necessary to degrade lignocellulosic substrates. So these fungi present different abilities to grow and fruit on the substrate consisting of bio-residues. White-rot mushroom degrade lignin, leaving decayed wood whitish in colour (bleaching of the wood), and include widespread cultivated species like: *Pleurotus* spp., *Ganoderma* spp. L. *edodes* etc. They are the most efficient lignocellulosic biomass degraders, capable to produces wide variety of hydrolytic enzymes (cellulases and hemicellulases) and a unique oxidative and extracellular ligninolytic system (advanced lignin depolymerisation). Expression of many fungal laccases is influenced by many culture parameters, such as: concentration of carbon and nitrogen sources; media composition; pH varying and temperature; or presence of inducers and lignocellulosic materials (Philippoussis A.N., 2009).

Ram and Kumar (2010) examine morphological parameters of *Agaricus bisporus* fruiting bodies and total cultivation productivity on different agricultural waste. Six nutrient medium formulations were analysed for studies. Maximum weight of fruiting body was obtained with application of casing coconut coir pith + vermin compost + sand.

Pleurotus species (Oyster mushrooms) is the second most commercially cultivated edible mushroom worldwide. Optimal results for *P. ostreatus* growth, yield, biological efficiency and mushroom size have been studied at large-scale, by a big number of authors. Notable results have obtained in Romania from winery + apple wastes (1:1) (cellulose degradation 0.9 g %) (Petre and Petre, 2012) and from apple wastes + 1.5 % barley (cellulose degradation 0.9 g % d.w.) (Petre and Petre, 2013).

The strong enzyme system of *Pleurotus* spp. increase biodegradation of the wide spectrum of substrates, not only sawdust and cereal straw.

Philippoussis et al. (2004), examined the influence of oak-wood sawdust substrates on *Lentinula edodes* mushrooms, and observed colonization rate is much faster on substrates enhanced with wheat straw or corn-cobs in a ratio of 1:2. Higher sporophore yields were observed on oak-wood sawdust substrates and corn-cobs mixtures, especially in the supplementation ratios 1:1 and 1:2. Substrates with high oak-wood sawdust content (2:1 ratio) appeared to promote mushroom quality and high protein content of the sporophores.

Lentinula edodes cultivation on hard wood saw-dust, rice straw, crushed corn cobs and crushed bagasse supplemented with 20% wheat bran, 1% soy bean flour, 2% gypsum has been investigated by Hassan (2011). Incubation period and early of harvesting yield were estimated. Sawdust produced the highest maximum yield 297 g/kg with wet media while bagasse recorded the lowest values. Sawdust recorded the shortest incubation time and first harvesting day time, while bagasse showed the longest ones.

The effect of pH and temperature variations on the growth of *Volvariella volvacea* cultivated on various agricultural wastes, single and in various combinations has been studied by Akinyele and Adetuyi (2005). A pH range of 5.5 to 8.5 recorded the maximum mycelia yield and the highest mycelia weight was recorded at pH 6.5. High mycelia growth of the mushroom was also observed between 25°C and 30°C. The researcher also evaluated effect of mycelial growth on diverse substrates of *Volvariella* spp. (*V. diplasia* and *V. volvacea*).

Paddy straw, oil palm fibre, sawdust, and a mixture of oil palm fibre and sawdust were screened for the cultivations of *V. volvacea* (Tripathy et al., 2011). Growth and production of fruit bodies on oil palm fibre was similar to that of paddy straw (Onuoha et al., 2009). *V. volvacea* showed that it is an very good agro waste destructor (Barshteyn & Krupodorova, 2016).

Akavia et al. (2009) investigated the cultivation of five *Hypsizygus marmoreus* strains on 24 substrates. Average number of colonized particles per day, number of mushrooms and weight of mushrooms harvested during one month have been studied. The best substrate in terms of 85.6%. biological efficiency, was corn cob with bran and olive press cake. Without olive press cake was only 67.5% biological efficiency.

Researchers are interested not only in *Agaricus* spp., *Pleurotus* spp. and *Volvariella* spp. fruit bodies cultivation on cheap substrates, but also in others edible and medicinal mushrooms, such as:

Ganoderma lucidum cultivated on sawdust and rice bran + 10% of food waste compost, with good biological efficiency;

Hericium erinaceus Good results was obtained from: sawdust (yield = 184 g/kg); wheat straw (protein); sawdust + wheat straw + 20% wheat bran + 1% $CaCO_3$ + 1% sugar (fat*)* investigated cultivation by Hassan (2007);

Auricularia auricula-judae Best results was obtained from dry olive mill residue, by increases peroxidase secretion and produced a sharp decrease in total phenolic content of growing substrate (Reina et al., 2013);

Flammulina velutipes cultivated on rice bran, wheat bran (Peng, 2010) and paddy straw + palm empty fruit bunches (25:75). Biological efficiency was 185.09% (Harith et al., 2014).

Another current and future resource for biotechnology research opportunities is a group of filamentous fungi.

Companies such as AB Enzymes, BASF, Bayer, DuPont, Novozymes, Puratos are global leaders in using filamentous fungi as cell factories in white and red biotechnology. This group of microorganisms is often superior to bacterial and yeast based production systems, in terms of metabolic versatility, robustness and secretory capacity.

Large-scale manufacturing processes have been developed for the production of organic acids, proteins, enzymes and small molecule drugs including antibiotics, statins and steroids. Fungal biotechnology plays a very important role for many industries including: food and feed, pharmaceutical industries, paper and pulp, detergents, textiles and bio-fuels (Meyer et al., 2016).

Trichoderma reesei are the most widely used strains of filamentous fungi for the production of cellulolytic enzymes and recombinant proteins. Several species of *Aspergillus* as well as other fungi, *Myceliophthora thermophila*, are important for industrial enzyme production. The metabolic diversity of fungi and the broad-range of ecological niches they inhabit, mean that many species, especially the basidiomycetes, have significant potential as sources of novel enzymes for future exploitation. Intensification of research studies on valorisation of hemicellulose and lignin-rich fractions for high-value applications. These wastes could be used for the growth of filamentous fungi with the capability to convert both hexose and pentose sugars into ethanol, carbon dioxide, and fungal biomass with a relevant nutritional composition (high protein and fat contents, essential amino acids, polyunsaturated fatty acids, cell wall compounds with immunostimulant properties, antioxidants) as an alternative to fishmeal (Karimi et al., 2018).

Another area of research includes filamentous fungi as large-scale producers of pigments and colorants for the food industry with exceptional biological roles (anti-oxidative, free radical killing, anti-carcinogenic, immunostimulation, protection against viruses and bacteria). They produce a wide range of pigments such as carotenoids, citrinin, melanins, flavins, phenazines, quinones, and sometimes monascins, or indigo.

Produced commercially and supplied to the market are: lycopene, β-carotene, astaxanthin, canthaxanthin, lutein, and capxanthin. Red and yellow pigments from *Monascus* sp. are produced in large scales and used as food colorants (Figure 3).

Figure 2. *Monascus purpureus* colony and red pigment Manan M.A. (2017)

The most frequent immunostimulants found in filamentous fungi cell wall are glucans (30-80%), chitin and chitosan (1-15%), mannans and/or galactomannans, and glycoproteins. These compounds enhance the immune system capabilities, stress related responses and resistance to diseases and are present in mycelia, stalks and spore tissues. Filamentous fungi can synthesize water-soluble vitamins such as C (ascorbic acid), B6 (pyridoxine), B2 (riboflavin), nicotinic acid, and nicotinamide. There are reports on the production of pantothenic acid (B5) and β-carotene (pro-vitamin A) by *Fusarium* sp. and *Neurospora* sp. (Karimi et al., 2018).

RESULTS AND DISCUSSIONS

According to data provided by the Food and Agriculture Organization of the United Nations, this statistic illustrates the production in tonnes of mushrooms in the last four years. Asia is in first place, followed by Europe, America, Oceania and Africa. Mondial annual average production is approx. 9 million tons (Figure 3; Table 1).

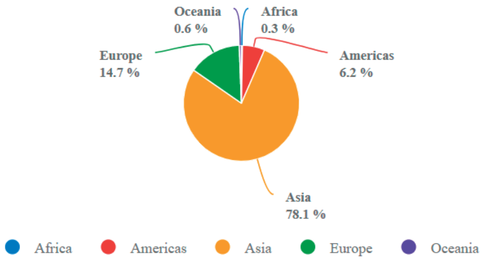

Figure 3. Average production share of edible mushrooms and truffles by region (2015-2018) Source: FAOSTAT (2015-2019)

Table 1. World production of edible mushrooms (tons) Source: FAOSTAT (2015-2019)

World production of edible mushrooms tons	**2015**	**2016**	**2017**	**2018**	**2018/ 2014 (%)**
ASIA	7038694	7087944	6956812	7031724	**78.1**
EUROPA	1363833	1307406	1318614	1324198	**14.7**
AMERICA	547269	558782	553764	554462	**6.2**
OCEANIA	45642	53178	47946	54128	**0.6**
AFRICA	25510	26367	27068	28767	**0.3**
Total	**9020948**	**9033677**	**8904204**	**8993279**	

CONCLUSIONS

Lignocellulosic waste being a major pollutant of the environment, containing the complex composition of cellulose, hemicellulose, and lignin along with plant resins and fatty acids. Due to its complex structure; lignin, resin and plant fatty acid they are not easily degradable by microbial communities.

The natural capabilities of microorganisms to degrade lignocellulosic waste efficiently due to highly effective enzymatic systems are attractive as new strategies for the development of industrial processes. Many factors may be involved in the difference of nutritional composition of mushrooms cultivated in different substrates.

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MISCELLANEOUS