

Review

Green Biotechnology of Oyster Mushroom (*Pleurotus ostreatus* L.): A Sustainable Strategy for Myco-Remediation and Bio-Fermentation

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Abstract: The field of biotechnology presents us with a great chance to use many organisms, such as mushrooms, to find suitable solutions for issues that include the accumulation of agro-wastes in the environment. The green biotechnology of mushrooms (*Pleurotus ostreatus* L.) includes the myco-remediation of polluted soil and water as well as bio-fermentation. The circular economy approach could be effectively achieved by using oyster mushrooms (*Pleurotus ostreatus* L.), of which the substrate of their cultivation is considered as a vital source for producing biofertilizers, animal feeds, bioenergy, and bio-remediators. Spent mushroom substrate is also considered a crucial source for many applications, including the production of enzymes (e.g., manganese peroxidase, laccase, and lignin peroxidase) and bioethanol. The sustainable management of agro-industrial wastes (e.g., plant-based foods, animal-based foods, and non-food industries) could reduce, reuse and recycle using oyster mushrooms. This review aims to focus on the biotechnological applications of the oyster mushroom (*P. ostreatus* L.) concerning the field of the myco-remediation of pollutants and the bio-fermentation of agro-industrial wastes as a sustainable approach to environmental protection. This study can open new windows onto the green synthesis of metal-nanoparticles, such as nano-silver, nano-TiO₂ and nano-ZnO. More investigations are needed concerning the new biotechnological approaches.

Keywords: agro-industrial residues; waste recycling; pollutants; bio-fermenter; myco-remediator; white-rot fungi

1. Introduction

Green biotechnology is the use of scientific techniques and tools, including molecular markers, genetic engineering, molecular diagnostics and tissue culture, on plants; it is called red biotechnology in the medical field and white biotechnology in the industrial field [1]. Fungi have several applications in agricultural and environmental sustainability, which can support the growth of plants, such as mycorrhizal association [2], for human nutrition, including edible mushrooms [3,4]. Arbuscular mycorrhizal fungi can also be utilized to sustain and improve soil health [5]. Fungi, such as mushrooms, are important plants, which can be consumed due to their nutritional benefits (as edible mushrooms) and their

medicinal values (as medicinal mushrooms) since time immemorial [6]. Many applications of mushrooms were confirmed by researchers, such as their valuable biotechnological properties [7,8], the sustainability of the mushroom industry through a zero waste and circular bioeconomy [9], and myco-remediation [10].

The genus *Pleurotus* is considered as the second most cultivated and distributed edible mushroom all over the world after the champignon mushroom (*Agaricus bisporus*), because of its adaptation capability [11–13]. The mushrooms of *Pleurotus* are characterized by several valuable medical, biotechnological, and nutritional attributes. Numerous studies have reported the many relevant features of the *Pleurotus* genus, which confirmed their attractive low-cost industrial tools that resolve the pressure of ecological issues [7,11,12,14–17]. These issues may include the production of enzymes (oxidases and hydrolases) and biomass from fruit residues using *Pleurotus* spp. [18], bioethanol production [19], the biodegradation of pollutants [20,21], and medicinal attributes [6]. This genus is also characterized by its high content of fatty acids, steroids, and polysaccharides, which can produce a lot of bioactive molecules and has become a popular functional food [15]. Additionally, a number of *Pleurotus* species are highly adaptive, possess specific resistance to pests and polluted diseases, and they do not require any specific conditions for their growth [7]. *P. ostreatus* is an important member of the *Pleurotus* species, which has significant medicinal importance and nutritional values [22] due to its anti-oxidative, anti-carcinogenic, anti-inflammatory, anti-hypercholesteremic, anti-viral, and immune-stimulating properties [11].

Therefore, this review is an attempt to highlight *Pleurotus* as an important genus of mushrooms. Different features of *Pleurotus* genus are discussed with special focus on *Pleurotus ostreatus*. Many biotechnological features of *P. ostreatus* are debated, including its role as a myco-remediator, a bio-degrader of organic solid wastes, and its use in bioethanol and enzyme production.

2. Methodology

The present review was created by collecting the required information from different databases, including ScienceDirect, Springer link, and PubMed. The strategy for this review depended on the collection of information from different databases during certain periods, especially during the last 15 years, for obtaining the latest information. To obtain the required information, we selected the suitable keywords, which we inserted into the website of each database, and then selected the suitable literatures based on three criteria: the high-impacted journals, the up-to-date publications (with a preference for the publication years of 2022, 2021, and 2020), and the reputation of the scientist or researcher of the published materials. The most important aim of the present review is to create a strong and attractive table of contents (TOC), which mainly depends on the main objective of this review. After preparing the TOC, which can be changed according the availability of information, different sections of the review can be created. The main keywords depend on the section, for example, for the section titled “Food importance of *Pleurotus* genus”, the key words are “food”, “*Pleurotus*”, and “nutritional value”. The second aim in editing the review is the presence of Tables and Figures, which need the precise evaluation of the information, summaries, and presentation of the output of the authors. A significant amount of experience is needed to edit the review, as well as the knowledge of how to support the perspectives of the readers using the correct arguments. Harmony between the structure of the review’s TOC is also needed. The building of survey tables for discussing the ideas presented in the review is also required. The review should depend on all published materials, including original articles, reviews and book chapters. The total number of cited literatures should be significant, and some journals require at least 50 references in a review, but a larger quantity is preferable.

3. The Genus *Pleurotus* and Its Potential Uses

3.1. Taxonomy and Botanical Description

The *Pleurotus* species belongs to the Kingdom of Fungi, Phylum of Basidiomycota, Class of Agaricomycetes, Order of Agaricales, Family of Pleurotaceae, and the Genus of *Pleurotus* [23] (Figure 1). *Pleurotus* species, such as many species of mushrooms, include cultivated and wild mushrooms, which are dominant in many forests worldwide. *Pleurotus* was first scientifically described in 1775 and, in 1871, the German mycologist Paul Kummer transferred the oyster mushroom to the genus *Pleurotus*. This is a new genus defined by Kummer himself in 1871 and is the currently accepted scientific name. *Pleurotus ostreatus* grows throughout the United Kingdom, Ireland, and most parts of Europe. It is also widely distributed in many parts of Asia, including Japan, and is located in parts of North America [24].

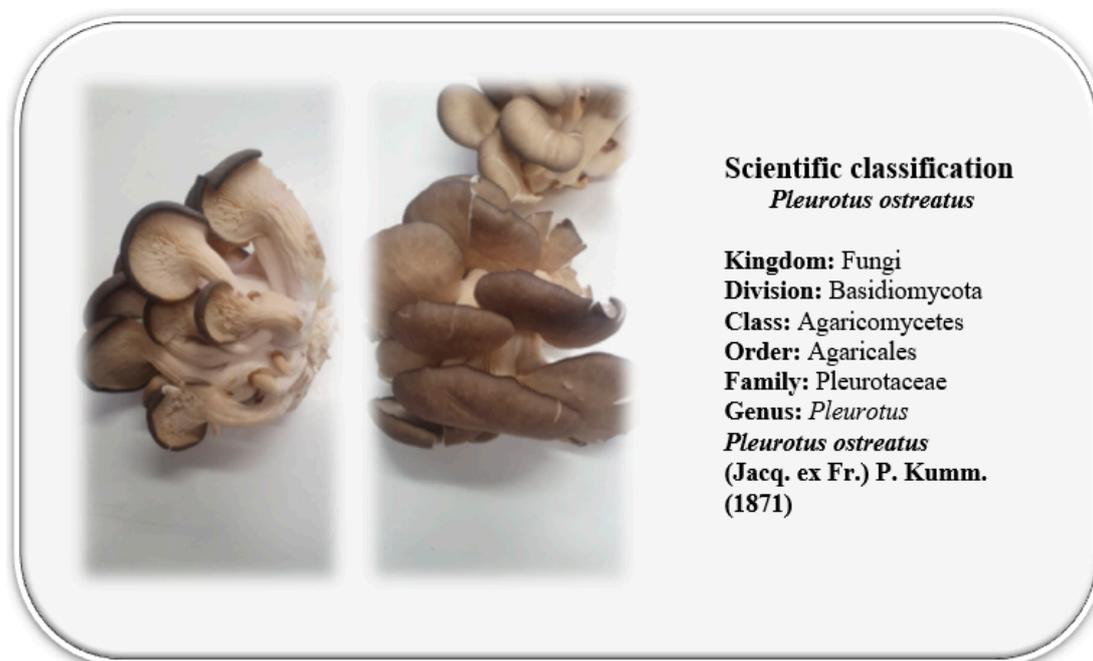


Figure 1. Photos of *Pleurotus ostreatus* and the scientific classification of this mushroom. (Photos were taken by Gréta Törös, Debrecen University, Hungary).

3.2. Food Importance of the *Pleurotus* Genus

The genus *Pleurotus* includes more than 200 species, which are consumed worldwide as edible mushrooms with an annual increase of 15%, and *Pleurotus* is considered as the second most commonly consumed mushrooms [25]. Some representatives of this genus (e.g., the oyster mushroom or *P. ostreatus*) are well known for their odor, flavor, nutraceutical value, and gastronomic properties, which are important to several consumers [26]. Thus, the edible mushroom of *P. ostreatus* has been used as a source of food additives due to its high content of antioxidants, bioactives, and β -glucans [25,27]. Due to their contents of minerals, fibers, lipids, and vitamins, *Pleurotus* mushrooms have become increasingly appealing as functional foods [16]. Different species of *Pleurotus* mushrooms and the chemical composition of their nutritional values, including moisture, proteins, carbohydrates, fats, ash and fiber, are presented in Table 1. Based on this Table, the highest content of crude proteins (30 and 35.5%, respectively) was recorded for the *Pleurotus citrinopileatus* and *P. djamor var. roseus* mushrooms, whereas the *P. eryngii* mushroom had the highest fiber content (28.29%).

Table 1. The nutritional content (as % or g/100 g of dried mushrooms) of some *Pleurotus* spp. mushrooms from different sources.

<i>Pleurotus</i> spp.	Moisture (%)	Proteins (%)	Carbohydrates (%)	Fats (%)	Ash (%)	Fiber (%)	Refs.
<i>Pleurotus ostreatus</i>	90.7	18.3	71.25	2.58	7.82	14.31	[28]
<i>Pleurotus eryngii</i>	91.0	11.9	39.85	7.50	4.89	28.29	[29]
<i>Pleurotus eryngii</i>	88	20	53	2.8	7.5	7.5	[30]
<i>Pleurotus eryngii</i>	88	18.8	57	2.3	5.5	10	[31]
<i>P. citrinopileatus</i>	88.9	30.0	42.50	3.90	7.65	20.78	[32]
<i>Pleurotus flabellatus</i>	91.0	21.6	57.40	1.80	10.7	11.90	[33]
<i>P. djamor var. roseus</i>	79.5	35.5	44.75	1.72	5.90	14.60	[34]
<i>Pleurotus pulmonarius</i>	78.8	20.3	34.00	2.62	7.33	9.00	[35]
<i>Pleurotus djamor</i>	86.8	24.1	45.59	4.73	9.84	15.91	[36]
<i>Pleurotus tuber-regium</i>	87.1	22.1	63.03	1.06	2.97	10.86	[37]
<i>Pleurotus florida</i>	87.5	20.5	42.83	2.31	9.02	11.50	[38]
<i>Pleurotus sajor-caju</i>	87.0	24.6	39.82	2.29	8.28	10.90	[39]
<i>Pleurotus cystidiosus</i>	91.1	15.6	55.92	2.05	6.30	20.05	[22]

In 2017, the world production of *P. ostreatus* was approximately 4.1 million tons. Similar to many oyster mushrooms, *P. ostreatus* is cultivated for foods and medicinal purposes. It can be cultivated on different lignocellulosic substrates, including maize cobs, wheat straw, sawdust, or cotton waste [40]. *Pleurotus* spp. can colonize and bio-degrade a large variety of lignocellulosic wastes, as a result of their ability to produce many ligninolytic enzymes [40]. *Pleurotus* mushrooms have been used for their high nutritive content and their potential biotechnological and environmental applications.

3.3. Medicinal Importance of the *Pleurotus* Genus

This genus includes more than 40 species, commonly referred to as the “Oyster mushroom”, including *P. ostreatus* and *P. eryngii*, which has attracted special attention because of its high nutritional values and medicinal attributes [11,12]. It is well known for its anti-oxidative, anti-carcinogenic, anti-inflammatory, anti-viral, anti-hypercholesteremic, and immune-stimulating properties, as well as its ability to regulate glucose levels and blood lipids [11,12]. Table 2 lists the bioactive compounds and their activities, which are dominant in *Pleurotus ostreatus* and their mode of actions or mechanisms [41].

Table 2. Different bioactive compounds of *Pleurotus ostreatus* and their mode of actions.

Activity	Bioactive Compound	Mode of Action	Refs.
Anti-oxidative	Lectins	The dendritic cells were activated using the pathway of “Toll-like receptor 6 signal”	[42]
	Polysaccharides	Increasing the activities of SOD, CAT, GST, GR, APx and reducing superoxide radicals, and the activity of GPx	[43]
	Phenols	Inhibits the growth of HL-60 cells by inducing apoptosis	[44]
	Flavonoids, ascorbic acid and β -carotene	Induces apoptosis by inhibiting HL-60 cell growth	[44]
	Vitamin E	Lipid peroxidation is prevented in cell membranes	[43]
Immuno-modulatory	Polysaccharides	The toxicity of cyclophosphamide in mice was decreased due to the immune-modulatory activity	[43]
Anti-inflammatory	Polysaccharides (β -glucans)	Methotrexate may have a synergistic effect on the arthritis of rats	[45]

Table 2. Cont.

Activity	Bioactive Compound	Mode of Action	Refs.
Anti-hypercholesterolemic	Statins (lovastatin)	In the cholesterol synthesis pathway, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase is inhibited due to the conversion of enzymes to mevalonic acid	[46]
	Flavons (chrysin)	Non-enzymatic antioxidant parameters in hypercholesterolemic rats, the blood/serum levels of lipid profile parameters and hepatic marker enzymes decreased	[47]
Anti-cancer and anti-tumor	Polysaccharides	In HeLa cell lines, cytotoxic activity inhibited the development of Ehrlich Tumor and Sarcoma 180 (S-180)	[45,48]
	Pleuran (β -glucan)	Anti-neoplastic properties of different cells (breast, colorectal and prostate cancers)	[48]
	Proteins	In cell line SW 480, therapeutic effects on colorectal cancer and monocytic leukemia by inducing apoptosis	[48]
	Lectins	Tumor burden in Sarcoma S180 reduced by 88.4% and hepatoma H-22 by 75.4% in mice; increase in survival time	[45]
Anti-viral and anti-microbial	Laccase	Anti-viral effects against hepatitis C	[48]
	Ubiquitin-like protein	Anti-viral effects in human immunodeficiency viruses, such as HIV-1	[45]
	Nanoparticles mixed with aqueous extract	Inhibiting the growth of Gram-negative bacteria	[45]
	Ribonucleases	Degradation of viral genetic materials to neutralize HIV	[49]
Hepatoprotective	Poly-saccharopeptides	Thioacetamide is alleviated, inducing alterations in inflammation, steatosis, fibrosis and necrosis	[49]
Anti-aging	Mushroom powder	Significant bifidogenic and then strong lactogenic effects	[50]

Abbreviations: HIV-1 (human immunodeficiency viruses), SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase), GR (glutathione reductase), GST (glutathione S-transferases).

4. General Features of *Pleurotus ostreatus*

Pleurotus ostreatus is found in dead and living tree branches, especially hornbeam (*Carpinus* sp.), beech (*Fagus* sp.), willow (*Salix* sp.), poplar (*Populus* sp.), birch (*Betula* sp.) and the common walnut (*Juglans regia*) trees [51]. This species produces grouped fruiting bodies of various sizes, similar to oyster mushroom colonies. The fruiting body is pink, gray-to-dark brown, and is 4 to 15 cm in size. In the wild, its offspring generally appear between October and November (Figure 2). However, they can be encountered in mild winters or in early, warm springs. Their cap is 3–15 cm in diameter; broadly convex, flat or depressed flat; kidney shaped-to-fan shaped in outline, or nearly spherical when growing on tree trunks; young and fresh and somewhat greasy; bald; pale-to-dark brown; fade to buff; sometimes slowly fading and becoming two-toned; and the edge is slightly curled when young [52]. Gills can run down the trunk (or pseudo stem); close, short gills are common; and can be whitish or grayish, turning yellowish with age and sometimes with brownish edges [52]. A salient feature of these gilled mushrooms is their ability to capture and feed nematodes to the gills using a “lasso” made of hyphae. Their stem is whitish, hairy to velvety, and hard. Moreover, their flesh is thick, white and unchanging when cut.

These mushrooms are edible, in addition to having several important applications in biotechnological [7], nutraceutical [15,22,53], medicinal [54–57], and environmental fields [12,58]. Due to their exceptional ligninolytic attributes, the *Pleurotus* genus is considered as one of the most extensively investigated types of white-rot fungi. These species also have a significant role in the global initiative towards the “zero waste economy”, as a result of their ability to convert or biodegrade waste for biomass production using various enzyme

properties, such as endoglucanase and laccase [12]. This distinguished role of *Pleurotus* spp. in the biodegradation of agro-industrial residues has been confirmed by many published reports, such as those published by Kumla et al. [59], Mahari et al. [60], Durán-Aranguren et al. [18], Ogidi et al. [61], Caldas et al. [15], and Melanouri et al. [11,12,30,31,62].



Figure 2. The cultivation of oyster (*Pleurotus ostreatus*) mushrooms, the mushroom-fruiting basic processes, and how to produce a *Pleurotus* inoculant on a millet substrate are presented in photos 1 to 6. The mushroom fungi (*Pleurotus ostreatus*) culture should first be ready on the surface of the agar plate (photo 1); the cultivation substrates of oyster mushrooms are ready (photo 2); tools for the propagation of the mushroom and poured media (heat treated at 95 °C for 1 day); inoculant should be prepared, and a jar with boiled millet and oyster culture (photo 3); the millet spawn in the jar and culture media (photos 4 and 5); and finally we obtain the oyster mushroom. (These steps were photographed in the factory of “Magyar Gomba Kertész Kft.”, whereas all photos were taken by Gréta Törös, Debrecen University, Hungary).

In the *Pleurotus* mushroom cultivation industry, it is important to meet the increased demands of human consumption of *Pleurotus* mushrooms, for which new methods of mushroom cultivation are needed to reduce global waste and increase mushroom productivity [34,60]. The cultivation of *Pleurotus* mushrooms depends on both intrinsic factors (i.e., substrate type, pH, C:N ratio, levels of spawning, surfactants, the content of N, C and moisture) and extrinsic factors, which include temperature, relative humidity, luminosity, air composition, and light [30,31,36,58,60,62,63]. The growth and cultivation of mushrooms can be achieved using growth media or substrates, which have a vital impact on the functional, chemical, and sensorial characteristics of mushrooms [58,64,65]. Solid substrates can be used traditionally in cultivating many species of mushrooms for fruiting body formation, which usually needs several months with a non-stable quality of harvested materials [66]. The technology of solid phase cultivation in the bioreactors of mushrooms can reduce the required time for producing the biomass, depending on the control of several physical process parameters, such as the aeration temperature and pH [66]. This technology may include the production of mushrooms in submerged liquid culture or sterilized solid substrates inside static or mixed chambers with a reduced amount of water, compared to submerged liquid cultivations, which allows for a nature-like growth of mycelium on the substrate [66].

5. Myco-Remediation by *Pleurotus ostreatus*

Environmental pollution is a global unsolved health issue, which requires advanced strategies through the remediation of soil, water, sediment, and air. This pollution can result from organic pollutants, such as petroleum [67], or inorganic pollutants, such as metal/metalloids (i.e., lead) [68]. These pollutants represent a potential threat to human health, which needs bioremediation by micro-organisms (bacterial, fungi, and algae), phytoremediation by plants, or nano-bioremediation by micro-organisms in the presence of nanoparticles. Myco-remediation is the process that can remediate or bio-degrade pollutants using fungi, such as mushrooms, through many mechanisms, such as biosorption, bioaccumulation, bioconversion, and biodegradation. Myco-remediation is also eco-efficient and an ecologically sound approach to counter the escalating crisis of terrestrial and aquatic pollution [69]. Myco-remediation has many advantages of biodegradation, such as the ability to oxidize pollutants, a safe and low cost, the production of diverse coupling products, the efficient production of biodiesel, and a tolerance under conditions of salinity, whereas the disadvantages include the necessity for nutrient addition, its inability to remove toxicity, its long depletion period, and the necessity for immobilization [70]. The previous advantages of mushrooms are mainly to the result of their immense hyphal networks, which strengthen growth through the production of multi-purpose extracellular enzymes, increasing the surface area-to-volume ratio, increasing the capabilities towards complex contaminants, enhancing the adaptability to fluctuating temperatures and pH, and possessing a metal-binding protein [71]. It is worth mentioning that the myco-remediation of different environmental pollutants can be applied for the removal or biodegradation of polluted soil and water via certain mechanisms, including biodegradation, biosorption, biotransformation, bioaccumulation, bioconversion, precipitation, and surface sequestration [69,72,73]. More details concerning these mechanisms and a comparison between them is presented in Table 3.

Table 3. A comparison between the different myco-remediation mechanisms.

Approach or Mechanism	Kinds of Pollutants	Advantages	Disadvantages	Refs.
Biosorption	Mainly metal pollutants	Simple process; highly cost-effective way to produce biomass; removes various HMs at the same time without using chemicals	Many adsorbent types are required; reversible sorption of metals on biomass; suffers from the saturation and clogging of reactors; expensive regeneration	[74]
Bioaccumulation or precipitation	All pollutants	To remediate wastewater, it is the simplest and cheapest method; very efficient for removing sulfides and metals; non-selective of metals; does not require chemicals	A difficult method to maintain; the key for success by precipitation is the genetic engineering; the oxidation step is required for complex metals	[75]
Biotransformation or bioconversion	Agro-industrial wastes by biological catalysts	It is a time-saving technology and has low operational control; produces biodegradable compounds by green chemistry	Depending on enzymes (high cost), biocatalysts require narrow operation parameters, which are susceptible to the inhibition of products or substrates	[76]
Biodegradation	Pollutants from human activities	High reduction pollutant rate; can be used in entirely polluted areas depending upon its characteristics; economically viable; can clean-up with time	Removing other beneficial elements during the natural attenuation of pollutants, the mobility and toxicity of pollutants may be too high; monitoring and groundwater controls are required	[77]

Source: extracted from Kumar et al. [69] and Yadav et al. [72].

Several studies reported about the myco-remediation of the *Pleurotus* genus for different pollutants, such as petroleum solid wastes [78], industrial wastes [79], perfluoroalkyl substances, pharmaceuticals [80], chlorinated pesticides [81], and sulfonamides by myco-degradation [82]. Several mushrooms as fungi have the ability to bioremediate the polluted environments through their ubiquitous nature and their efficient enzymatic machinery for the biodegradation and biotransformation of toxic pollutants [81]. Furthermore, myco-remediation can be achieved by biosorption through the removal of dyes, metals, or organic pollutants by the process presented in [81]. The ability of *Pleurotus ostreatus* in the myco-remediation of pollutants in different media has been reported in many studies, as presented in Table 4. Different factors controlling myco-remediation by *Pleurotus ostreatus* are presented in this table, including the substrate, growth conditions, and the kinds of pollutants, and their concentrations. The biodegradation of pollutants by *Pleurotus ostreatus* mainly depends on many factors, including the growth conditions and the kinds of pollutants, and the mechanism of this process is linked to certain enzymes, such as manganese peroxidase, lignin peroxidase, and laccase [21]. Many studies discussed the role of *Pleurotus ostreatus* in the myco-remediation process via the consideration of different perspectives, such as the myco-remediation of chlorinated pesticides [81], the accumulation of metals in fruiting bodies [83], and the biodegradation of decabromodiphenyl ethane [21], as well as including some reviews that include those published by Akhtar and Mannan [84], Pini and Geddes [85], Kumar and Dwivedi [86], and Yadav et al. [72].

Table 4. The myco-remediation of pollutants by the fungi of *Pleurotus ostreatus* in different media.

Pollutant Details	Growth Conditions	The Main Findings or the Mechanism	Refs.
Decabromo-diphenyl ethane (5, 20 mg L ⁻¹)	Biodegradation after 120 h	Biodegradation of pollutants by enzyme P450, manganese peroxidase, lignin peroxidase, and laccase	[21]
Cytostatic drugs include vincristine and bleomycin (5, 10 and 15 mg L ⁻¹)	Cultivated in liquid medium for 30 days before the test	Studied drugs as anticancer treatments can be removed by biosorption on fungal biomass during wastewater treatment	[87]
Sulfonamide antibiotics (0.1 mM)	Biodegradation after 14 d in polluted wastewater	Mushrooms as biofilters removed sulfonamides by up to 83–91% of the applied doses over 14 d from polluted wastewater	[88]
Cadmium at doses ranging from 0.5 to 20 mg L ⁻¹ Cd	Removal rate up to 54.6% for 7 days	Cd detoxification pathways included 26 enzymes, including catalase, superoxide dismutase, and peroxisomal enzymes	[89]
Petroleum hydrocarbons in soils (339 g kg ⁻¹ dry weight) for 90 days	Mushroom spawn (10 g) added to pot (1.5 kg soil)	Myco-remediation efficiency was 85% from polluted soil, depending on the type of substrates and application method	[90]
Organic micro-pollutants, such as diclofenac and bicalutamide	Substrate content was 200 g L ⁻¹ during 14 and 36 d	Removal efficiency of bicalutamide, lamotrigine, and metformin was 43%, 73%, and 59%, respectively, from water	[91]
Chloro-hydroxyl-actones	Culture medium for 72 h	Mushroom bio-transformed bicyclic halolactones to chlorolactones	[92]
Triclosan (5, 10, 20, 30, and 50 mg L ⁻¹)	Biodegradation at 4, 7, and 10 days in liquid medium	Complete biodegradation within the first day of sampling through manganese peroxidase and laccase activity	[93]
Pesticide of carbendazim residue (up to 25 days) using wheat straw	Biodegradable in spawned bags (at 22–26 °C)	Mushroom can bioremediate both thiophanate-methyl (up to 60 ppm) and fungicides with a similar chemistry	[94]
Polychlorinated biphenyls (PCBs at 0.1–1.0 µg L ⁻¹)	Contaminated groundwater for 30–71 days	Spent oyster substrate degraded PCBs and aerobic and/or anaerobic bacteria (87 %)	[95]
Lamotrigine, C ₉ H ₇ Cl ₂ N ₅ (100 mg L ⁻¹)	Transformation on culture medium within 20 days	Oxidation of cytochrome P450, where, after 10 days, ~50% of the pollutant was removed	[96]
Polycyclic aromatic hydrocarbons (50 mg L ⁻¹)	Biodegradative effect up to 14 d in liquid medium	Naphthalene was completely degraded within 5 days (86.47%) by laccase or dioxygenase and ligninolytic enzymes	[97]
Applied cobalt (Co) of up to 20 mg kg ⁻¹ to the soil	Spent mushroom substrate for 30 d in fluvo-aquic soil	Mushroom reduced Co phyto-availability if added to cultivated soil at a range of 8.86 to 9.51 g kg ⁻¹ with pakchois plants	[98]
Lead (Pb) from liquid media	Removal rate of Pb was 53.7%	Mushroom removed Pb by biosorption, precipitation, and bioaccumulation	[99]

6. Recycling of Organic Solid Wastes by *Pleurotus ostreatus*

Agro-industrial wastes are defined as any residue that results from the agricultural and industrial activities. These wastes have been successfully used as growth substrates in the cultivation of mushrooms. Several agro-industrial wastes have low N-content materials, which represents an important factor in the growth and cultivation of mushrooms on these wastes [59]. The protein content in a mushroom-fruiting body depends upon both the C:N ratio of the substrates and the chemical composition, in addition to the mushroom species that is being cultivated [59,100,101]. This group of mushrooms could also be artificially cultivated in different phenolic wastes and lignocellulosic substrates (under both the solid-state and liquid-submerged fermentation processes) through their complex enzymatic system, which includes cellulolytic (e.g., cellulase and xylanase) and ligninolytic (e.g., laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase) enzymes, which have different applications in the beverage and food industry [12]. Several fermentation factors can control the activity of these lignocellulosic enzymes, such as the pH, medium composition, C:N ratio, air composition, and temperature [58]. The C:N ratio that is required for the substrate to obtain the highest yield of *Pleurotus ostreatus* should be (40:1) at the minimum, (45–60:1) as the optimum, and (90:1) at the maximum, as reported by Kumla et al. [59]. The chemical composition and biological efficiency of *Pleurotus ostreatus* grown on different kinds of agro-industrial wastes are presented in Table 5. It is evident from Table 5 that the chemical composition of harvested mushrooms on agro-wastes mainly depends on the type of waste; the highest biological efficiency was recorded for soy stalks (85.2%).

Table 5. Chemical composition and biological efficiency of *Pleurotus ostreatus* grown on different kinds of agro-industrial waste (as % or g g⁻¹ dry weight).

Agro-Industrial Wastes	Biological Efficiency (%)	Crude Proteins (%)	Carbohydrates (%)	Fats (%)	Fiber (%)	Ash (%)	Refs.
I. Applied individual agro-industrial waste							
Wheat straw	37.6	13.6	60.5	2.3	22.7	10.3	[102]
Barley straw	21.3	12.8	54.7	29.9	0.90	1.2	[103]
Rice straw	55.6	17.9	56.4	8.4	4.30	9.6	[104]
Maize cob	46.4	23.4	50.8	3.1	22.0	7.6	[105]
Soya stalk	85.2	24.7	53.2	2.8	7.2	6.7	[104]
Cotton stalk	44.3	30.1	40.2	2.1	17.2	8.4	[106]
Cotton seed hull	8.9	17.5	65.9	1.2	10.2	5.2	[107]
Rice husk	9.5	5.9	48.5	30.9	0.3	14.3	[103]
Sugarcane bagasse	65.7	27.1	34.9	2.0	29.3	6.7	[105]
Sugarcane bagasse	52.3	17.1	-	1.18	12.1	4.5	[108]
Cassava peel	25.1	10.6	73.7	2.2	8.7	7.6	[109]
Acacia sawdust	46.4	19.5	51.3	1.3	22.0	5.9	[105]
Beech sawdust	46.8	16.1	73.6	3.5	15.8	6.2	[102]
Birch sawdust	42.5	21.0	67.6	1.0	6.4	6.4	[110]
II. Applied combined agro-industrial wastes							
Soya stalk + rice straw	81.7	23.0	50.5	2.7	7.7	6.4	[111]
Soya stalk + wheat straw	77.7	21.1	52.0	2.6	7.4	6.2	[111]
Wheat and rice straw	71.8	20.3	56.0	2.6	7.5	5.9	[111]
Cotton stalk + cottonseed hull	20.2	22.8	58.0	2.9	10.8	5.5	[107]
Acacia sawdust + maize cob	58.8	18.7	46.9	3.3	24.5	6.7	[105]
Acacia sawdust + sugarcane bagasse	58.9	24.2	37.8	2.5	28.8	6.7	[105]
Wheat straw + olive pruning residues	56.8	19.9	71.7	1.9	16.5	6.5	[111]
Cassava peel + maize cobs	32.4	10.7	73.8	2.2	8.7	7.6	[109]

Source: extracted from Kumla et al. [59] and the ratio of the combined agro-industrial wastes is 50% for each one or (1:1).

Many reports have discussed the many purposes of oyster mushrooms through their cultivation on agro-wastes or agro-industrial wastes, which include mushroom production, and the biodegradation of agro-wastes, producing many bioactive compounds and creating a healthy environment for humans, as well as these mushrooms possessing pharmaceutical attributes for medical applications, such as anti-diabetic, anti-carcinogenic, anti-oxidative, and immune suppressor attributes (e.g., [11,12,61,112–114]). Furthermore, oyster mushrooms have a great ability to convert the agro-wastes into bioenergy, bio-compost and biofertilizers [60,115]. The cultivation of oyster mushrooms can be also carried out on coffee waste substrates or spent coffee grounds [116].

7. Enzyme Production by *Pleurotus ostreatus*

The mushrooms of genus *Pleurotus* have a great tolerance to diverse conditions, such as high temperatures or growing on hard woods in their natural ecosystem or forests [11]. The distinguished feature of these fungi is represented in their ability to cultivate on different lignocellulosic agro-industrial by-products, as a result of their complex enzymatic system [11]. The cultivation of *Pleurotus ostreatus* and the collection of spent mushroom substrates following cultivation have attracted great attention due to their potential applications in the production of enzymes, biomass, bioethanol, feed ingredients, and functional foods, such as xylo-oligosaccharides [117]. Table 6 presents the different applications of *Pleurotus ostreatus* in the production of some cellulolytic enzymes and other by-products. Three main factors are responsible for controlling this production process, including the media, the source of carbon used in the substrate, and the solid-state fermentation conditions and its purpose, as listed in Table 6.

Table 6. Some applications of solid-state fermentation using *Pleurotus ostreatus* and the used substrate for the production of some cellulolytic enzymes and other by-products.

Media	Fermentation Conditions and Its Purpose	Substrate (Source of Carbon)	Refs.
Malt extract agar (up 36 days) 26 °C, RH 75%	Production of laccases and endoglucanases were recorded for oat straw, rice bark, and poplar wood sawdust (26–51 days)	Oat straw, rice bark, poplar wood sawdust, olive pulp, and wheat straw	[12]
Malt extract agar (up to 36 days) 25 °C, RH 80%	Rice bark presented the highest productivity, with the highest biological efficiency > 70% (during a cropping period of 51 days)	Rice bark, wheat straw, coffee residue, barley and oat straw	[11]
Potato dextrose agar for 5 days	Incubated at 25 °C and sampled after 77 days for improved ruminant animal feed	Maize stover and kudzu (<i>Pueraria montana</i>)	[118]
Potato dextrose agar for 7 days	Incubated at 27 °C for 15 days to produce phenolics and flavonoids	Cocoa pod husk and kolanut pod	[61]
Fungi stock culture for 12 days	Inoculation at 25°C and 60% humidity for 12 d for proteinaceous animal feed	Brewer's spent grain	[119]
Bacteriological agar for 5 days	Solid culture media at 30 °C, carbon source (sugarcane bagasse), to produce cellulases, pectinases, and xylanase	Pineapple wastes (fruits, leaves, and stalks)	[120]
Potato dextrose agar for 28 days	Inoculated straw with fungus at 28 °C, humidity 80% for rumen degradability	Naked oat straw	[121]
Potato extract for 5 days	Produce exopoly-galacturonases after 7 or 10 days at 30 °C	Peel of pomelo (<i>Citrus maxima</i>)	[122]
Potato dextrose agar for 8 days	Production at 28–32 °C of cellulases, hemi-cellulases, and reducing sugars	Leaf-and-stem mixture of <i>Alstroemeria</i> sp.	[123]
Potato dextrose broth for 6 days	Incubated at 28 °C to produce lovastatin at a rate of 34.97 mg g ⁻¹	Barley, wheat bran, rice husk, and oat grains	[124]
Culture media (50 °C for 72 h)	Butanol production through treatment by laccase (saccharification yield 99%)	Brewer's spent grain	[125]

The cultivation of *Pleurotus ostreatus* and its benefits can be explained from different studies as follows:

1. Studies on the role of spent mushroom substrates after this cultivation produces many important materials, such as enzymes [12,19,126,127], biomass [118,128,129], bioethanol [19,127,130], feed ingredients, and functional foods [61,131,132]. Spent mushroom substrates can be recycled as a substrate for the “new cultivation cycle” of mushrooms, a feedstock for producing the second generation of biofuels, a bio-control agent, a biofertilizer, and for soil amendment [133–135];
2. Studies on the biodegradation of agro-wastes or agro-industrial by-products through the solid-state fermentation or submerged fermentation by *P. ostreatus*, such as (a) using the deinking sludge as a substrate to produce lignocellulolytic enzymes (Vodovnik et al. [136]), (b) producing exo-polygalacturonases using pomelo peel powder under submerged fermentation by *P. ostreatus* [122], and (c) producing laccases by white-rot fungi under solid-state fermentation conditions [137];
3. Studies on the production of ligninolytic enzymes and their potential [129,138–140]. The most important ligninolytic enzymes, which can be produced by *Pleurotus* include manganese peroxidase, laccase, and lignin peroxidase through biodegradation, which varied from species to species. The main factors controlling the *Pleurotus* species and their ability to produce enzymes or to degrade wastes or pollutants include the pH, pollutant/waste concentration, and C:N ratio of the substrate [141];
4. Studies on the role of nano-mycology, including the applications of *Pleurotus* spp. to the green or myco-synthesis of nano-silver [142–144], nano-TiO₂ [145,146], nano-ZnO [147], and the production of fluorescent carbon quantum dots as a C-based nanomaterial [148]. The myco-synthesis of nano-nutrients (nano-Ag, nano-TiO₂, and nano-ZnO) has been investigated for medical attributes, such as controlling mosquito larvae, and anti-cancer activities [145–147]. Nanomaterials conjugated lignocellulosic wastes for producing biofuels using immobilized enzymes [149];
5. Sustainable management of agro-industrial wastes could be achieved by the reduction and conservation of wastes as well as different utilizations of wastes, including reuse and recycling [132,150]. The main agro-based industries that produce large amounts of waste may include plant-based foods (e.g., cereals, fermentation, sugar, food and fruit processing), animal-based foods (e.g., milk, dairy, fish and poultry products), and non-food industries, such as paper and textiles [132].

8. Bioethanol Production by *Pleurotus ostreatus*

Bioethanol is considered an important source of bio-based fuel, which has the ability to mitigate global warming and conserve fossil fuels [151]. Nowadays, the production of bioethanol is manufactured by fermenting different agro-industrial wastes. These substrates may include fermentable sugars (mainly sugarcane or by-products of the sugar industry), amylaceous feedstocks (e.g., maize, barley, potatoes, and wheat), and cellulosic substrates, such as bagasse, post-harvest agricultural residues, and wood wastes [151]. In other words, biofuel is a type of fuel of which the energy is derived from biological C-fixation via different processes, including gasification, liquefaction, pyrolysis, supercritical fluid extraction, supercritical water liquefaction, and biochemical processes [152]. Therefore, there is an urgent need for more intensive research to be conducted on the utilization of waste and agricultural biomass to produce the required energy for the following generations. The sustainable production of bioethanol can be achieved using fermentable sugars without decomposition through a pretreatment technology [153]. The correct selection of this pretreatment technology has a vital role in determining the cost of the whole technology, which contributes to about 30–35% of the overall production costs [153]. In general, the main production techniques of oyster mushrooms may include both solid substrate fermentation and submerged liquid fermentation using solid and liquid spawn [154]. The different species of mushrooms are listed in Table 7, which have the ability to produce bioethanol compared to the *Pleurotus ostreatus* mushroom and used substrate.

Table 7. The role of different kinds of fungi in the production of bioethanol compared to the *Pleurotus ostreatus* mushroom and used substrate.

Fungi Species	Fermentation Conditions	Biofuel Production	Refs.
<i>Pleurotus florida</i>	Cotton-spinning waste mixture using solid-state cultivation for 14 d; hydrolysis at 32 °C for 72 h	Ethanol at 1.18 g L ⁻¹ (64 % at 60 h)	[19]
<i>Pleurotus ostreatus</i>	Mushroom compost derived from millet and sorghum produced ethanol by saccharification and fermentation (applied substrate at 5–30% w/v)	Ethanol at 45.8 g L ⁻¹ dry weight (70%)	[155]
<i>Pleurotus ostreatus</i>	Rice straw was biodegraded by ligninolytic enzymes (cellulase and xylanase) up to 45 days	Biomethane yield 269 mL·g ⁻¹ (at 25 d)	[156]
<i>Pleurotus ostreatus</i>	Using 181 g of mushrooms per wet 2 kg waste of banana leaves with a biological efficiency of 37%	Biogas yield (282 mL g ⁻¹ VS ⁻¹)	[157]
<i>Pachysolen tannophilus</i>	Spent mushroom substrate of <i>Agaricus bisporus</i> was enzymatic hydrolysis (30 °C after 48 h) incubation	Ethanol at 6.41 g L ⁻¹ (76.13%)	[156]
<i>Saccharomyces cerevisiae</i>	Spent mushroom substrate of <i>Pleurotus florida</i> was enzymatic hydrolysis (30 °C after 48 h) incubation	Ethanol yield was 5.8 g L ⁻¹ (58.12%)	[158]
<i>Saccharomyces cerevisiae</i>	Cultivation wastes of <i>Aspergillus tubingensis</i> , which produced cellulolytic enzymes (at 30 °C for 10 days)	Ethanol yield was 17.3 g L ⁻¹ (48 h)	[159]
<i>Saccharomyces cerevisiae</i>	Rice straw was biodegraded by <i>Trichoderma reesei</i> and mushroom for 7 days and incubated at 32 °C	Bioethanol produced by <i>S. cerevisiae</i>	[160]
<i>Kluyveromyces marxianus</i>	Pine needle wastes were catalyzed by xylanase from <i>Bacillus</i> sp., fermented by fungi (at 40 °C for 96 h)	Bioethanol was 5.34 g L ⁻¹ (3.89% yield)	[161]
<i>Ganoderma lucidum</i>	Substrate of old newspapers was alkali (4% NaOH) or enzymatic fermented using <i>Trichoderma harzianum</i> (30 °C for 5 d)	Ethanol production: 17.8 and 20.4 g L ⁻¹ , respectively, for 2 methods	[162]
<i>Aspergillus niger</i> and <i>Saccharomyces cerevisiae</i>	Rice straw was pretreated (4% NaOH; incubated 40 °C for 24 h), saccharified, and fermented at 37 °C by <i>S. cerevisiae</i>	Ethanol yield was 31.9 g L ⁻¹ after the incubation	[163]
<i>Aspergillus niger</i> and <i>Saccharomyces cerevisiae</i>	Potato wastes were pretreated, saccharified, and fermented at pH 5.8, 35 °C, and no-aeration by co-cultures of <i>A. niger</i> and <i>S. cerevisiae</i>	Ethanol yield was 37.93 g L ⁻¹	[163]
<i>Saccharomyces cerevisiae</i>	Maize husks, peanut husks, and husks of coffee cherry were pretreated using 3 methods (acid or H ₂ SO ₄ , alkaline or NaOH and steam)	Ethanol yield was 20.61, 18.21 and 6.86 g L ⁻¹ , respectively, for each one	[164]
<i>Ganoderma lucidum</i>	Spent substrate of <i>G. lucidum</i> was acid-pretreated by H ₂ SO ₄ using <i>Saccharomyces cerevisiae</i> (30 °C for 5 d)	Ethanol yield was 0.91 g L ⁻¹	[165]

Abbreviation: volatile solids (VSs).

It is well known that the production of bioethanol from wastes or non-crop-based lignocellulosic materials attracted a lot of attention on a commercial scale, as a possible sustainable solution for “the decarbonization of the transport sector” [166]. The world is facing a great challenge to save enough fuel for transport by the exploitation of global crop residues. Instead of being burnt, which can pollute the environment and increase global warming [158], crop residual wastes are important sources that can be used to produce bioenergy or bioactive compounds. These crop residues can also be exploited for producing several value-added products, such as lignocellulolytic enzymes, biogas, bioethanol, biohydrogen, and biofertilizers [158]. Mushrooms and their waste may be

considered as a suitable source for bioethanol production. Mushrooms of *Pleurotus* can produce many ligninolytic enzymes (laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase) during the biodegradation of agro-industrial wastes through solid-state or submerged-state fermentation. Therefore, bioethanol can be produced from the lignocellulosic biomass and/or spent mushroom substrate [158].

Many studies reported the production of bioethanol using the mushrooms of *Pleurotus ostreatus*, which can be highlighted as follows:

1. The chemical composition of agro-wastes (cellulose, hemicellulose, and lignin) or spent mushroom substrates (SMSs) is considered an important factor controlling the biodegradation of these wastes, as reported in Table 5. Every 1 kg of grown mushroom generates nearly 5 kg of SMSs, establishing a promising industry for SMSs [158]. The biodegradation mechanism of these SMSs by mushrooms (e.g., *Pleurotus* spp.) may include the enzymatic degradation of various substrates (mainly cellulose, hemicellulose, and lignin) into soluble compounds of a low molecular weight. A partial degradation of the lignocellulosic biomass by saccharification during the pre-treatment is needed;
2. The condition of fertilization and its kind (solid-state fermentation or submerged), using media are the substrates as a source of carbon, and are the main factors that control the applications of *Pleurotus ostreatus* for the production of some cellulolytic enzymes and other by-products, such as reducing sugars, biofertilizers, and animal feeds, as reported in Table 6;
3. The production of biofuel from saccharification and/or fermentation in the presence or absence of *Pleurotus ostreatus* or other mushrooms depends on the kind of applied substrate, mushroom species, and the oxidation or fermentation conditions, as reported in Table 7;
4. Based on about 998 million tons of agro-wastes produced per year from agricultural practices [167], new approaches are needed to overcome the resistant nature of lignocellulosic wastes, to convert them into valuable products in an economical and eco-friendly manner [168];
5. The general structure of the lignocellulosic agro-wastes includes cellulose (30–50%), lignin (10–20%), and hemicelluloses (15–35%), in addition to some components, including minerals, extractives, and ash, in tiny amounts [167]. These agro-wastes are still underutilized, especially in developing countries. The unwise utilization of these wastes (mainly the burning of them) causes many environmental crises, such as global warming due to an increase in the emissions of gases, particularly carbon dioxide and sulfur dioxide, as well as underground water pollution [169];
6. It is evident from Table 7 that the oyster mushroom (*Pleurotus ostreatus*) is a mushroom distinguished for its ability to produce of bioethanol compared to other mushrooms, even in the case of the genus *Pleurotus*. This rate of production is higher in *Pleurotus ostreatus* (up to 46 g L⁻¹ with efficiency up to 70%), which is higher than *Pleurotus florida* or other species, such as *Ganoderma lucidum*.

9. General Discussion

In this general discussion, it is necessary to present the answers, in brief, for the main following questions:

What is the importance or potential of this study?

This central aim of the present review concerns the genus *Pleurotus* and more details about the most famous mushroom in this genus (*Pleurotus ostreatus*). This species of mushroom has brilliant features, which appear in their applications in several fields, including food, myco-remediation, the production of bioethanol and enzymes, as well as bio-degradation and the conversion of agro-industrial wastes into organic fertilizers.

What are the main ideas presented in this review?

The main points presented in this review include the many biotechnological applications of *Pleurotus ostreatus* in several fields, such as the food sector, pollution and the

remediation of polluted environments, agro-industrial wastes and their recycling, the production of enzymes and bio-organic fertilizers from the biodegradation of different wastes, the cultivation and production of mushrooms for human foods, the energy sector, and the production of bioethanol.

Why is this work important for readers?

This work is important for the readers because it includes information concerning an important source of human food and an alternative form of energy production and enzyme. These aspects are crucial to our lives, and they especially help to sustain our activities as well.

What are the open questions that still need to be answered?

The relationship between the *Pleurotus ostreatus* mushroom and nanotechnology can lead to many questions, which need to be investigated in the future, and this will be the subject of our next review.

10. Conclusions

Mushrooms are fungal plants that represent an important source of human foods because of their high nutritional value, bioactive compounds, and many medicinal uses. Recently, these mushrooms have new applications, particularly in the biotechnology sector. These applications mainly depend on the plant of mushrooms and their green biotechnological tools in the field of myco-remediation, bio-fermentation, bioethanol, and enzyme production. The use of oyster mushrooms is considered a sustainable strategy in the bioremediation of polluted environments, the biodegradation of agro-wastes or agro-industrial wastes, and the bio-fermentation of ligninolytic wastes to produce enzymes. The oyster mushroom (*Pleurotus ostreatus* L.) can also be applied to produce biomass, feed ingredients, and functional foods. The spent substrate of the oyster mushroom has multiple benefits, which include recycling as a substrate for the new cultivation cycle of mushrooms, producing biofuels, a bio-control agent, a biofertilizer, and soil amendment. The oyster mushroom has also penetrated the field of nanotechnology through the myco-synthesis of nano-silver, nano-TiO₂, and nano-ZnO. Therefore, several environmental problems can include the issue of sustained management concerning the use of oyster mushrooms, especially the accumulation of large amounts of agro-industrial waste, removing the bioremediation or the bio-degradation of pollutants, cultivating nutritive mushrooms for the purpose of human nutrition. Similar to any scientific topic, many questions are still require an answer, including “what is the expected role of oyster mushrooms in producing other nanoparticles of different nutrients in addition to Ag, Ti, and Zn?”; “to what extent can these nano-nutrients be used in the agricultural sector?”; and “is there any possibility to establish a global standard criterion for the toxic levels of nutrients or other toxic nutritional compounds of oyster mushrooms (*Pleurotus ostreatus* L.) and other species?”.

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