

Unlocking the Potential of Mushroom for Industrial Applications

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ABSTRACT

Farms, food processing facilities, numerous factories, and other industries are producing alarming amounts of agro-industrial waste, which necessitates immediate action to prevent the negative environmental effects of its disposal and incineration. The waste, which primarily comprises cellulose, hemicellulose, and lignin, collectively known as lignocellulosics, holds significant untapped potential for various agro-based applications and industrial processes. In particular, mushrooms use this waste as a substrate. By producing lignocellulolytic enzymes, mushrooms break down lignocellulosic substrates and utilize them in the formation of their fruiting bodies. Consequently, mushroom farming has emerged as a leading biotechnology strategy. It addresses and benefits from agro-industrial byproducts in environmentally friendly, and sustainable alternative approach. It biotransforms low-value agricultural byproducts into protein-rich nutritious foods that significantly enhances human health and contributes to the development of rural economies. Moreover, it serves as a bioremediation strategy that is less intrusive than other methods of environmental restoration. Recently, the substratum of mushrooms has been acknowledged as an invaluable source of biofuels and a plethora of enzymes that have significant vital functions in various industrial operations and are of substantial commercial value. This article offers a summary of recent scientific understanding regarding the mushroom substratum as a resource of industrially significant enzymes and biofuel.

Keywords: Agro-industrial waste; Biofuels; Enzymes; Mushroom mycelium; Spent mushroom substrate; Sustainable industrial applications.

INTRODUCTION

Mushrooms are macrofungi with a vegetative subterranean mycelium and a large visible sporocarp (Yang *et al.*, 2021). The vegetative form of mushrooms, known as mycelium, is composed of intertwined, branched, multicellular, filamentous underground root-like threads, called hyphae, that grow profusely in the substrate (Yang *et al.*, 2021). Being saprotrophic fungi, they grow on dead, decaying substratum that is rich in organic matter and perform an indispensable task in regulating the nutrient cycle of the earth's ecosystems (Falandysz and Borovička, 2013; Bell *et al.*, 2022). Fungi are the primary recyclers in nature and contribute significantly to decomposing organic matter due to their powerful enzymes (Deshmukh *et al.*, 2016). Additionally, edible mushrooms provide a unique umami taste (Zhang *et al.*, 2013), nutritional benefits that match up to those of meat, dairy products, eggs (Wang *et al.*, 2014; Zhang *et al.*, 2014; Grimm and Wosten, 2018; Viana, 2021; Okuda, 2022) and numerous medicinal benefits (Valverde, 2015; Chugh *et al.*, 2022).

Mushroom farming is more carbon-neutral than conventional farming because it can recycle organic material by transforming farm waste into

compost (Gupta *et al.*, 2019; Small Farms, 2020; Rosenberg, 2021; Small Farms, 2022; Sustainability, 2022; Beyer, 2023). Mushrooms could potentially usher in a circular resource-efficient economy that improves environmental outcomes and modifies the manner in which we generate and consume goods, by lowering emissions, maximizing land use, and decreasing waste generation by upcycling agro-waste (Grimm and Wosten, 2018; Cunha *et al.*, 2020; Meyer *et al.*, 2020). With the growing interest in sustainable solutions, industrial enzymes are increasingly being sourced from microbes. Fungal enzymes have numerous applications because they are released extracellularly and can be easily separated from mycelium by filtration (Cunha Zied, 2020; Kumla *et al.*, 2020; El-Gendi *et al.*, 2021).

Complex enzymatic combustion of wood, made possible due to the evolution of microbial ligninolytic enzymes, is primarily accountable for the degradation and detoxification of environmental biomass (Janusz *et al.*, 2017; Kumar and Chandra, 2020). Among all the microorganisms, mushrooms have the most potent lignocellulolytic enzymes (Da Luz, 2012; Kumla *et al.*, 2020). The oxido-reductive degradation of lignin by mushroom ligninolytic proteins makes

them a promising biocatalyst. The major enzymes responsible for depolymerizing highly intricate lignin compounds are laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase. These enzymes have found innumerable commercial applications in various industries (Kumla *et al.*, 2020; Cunha Zied, 2020; El-Gendi *et al.*, 2021). The mushrooms used for the production of enzymes include *Pleurotus* spp., *Lentinula edodes*, *Hericium erinaceum*, and *Trametes versicolor* (Kuhad *et al.*, 2011; Li *et al.*, 2012; Phan and Sabaratnam, 2012). The transition from chemical-based processes to biological processing will undeniably go a long way in mitigating the environmental impact (Lange, 2014). Further research on enzymes has a huge potential to open new commercial possibilities for industries while reducing the carbon footprint caused by their chemical counterparts (Bell *et al.*, 2022).

MUSHROOM BIOTECHNOLOGY: DECOMPOSING ORGANIC MATTER

The essential component of the cell walls of woody plants, lignocellulose, evolved to strike a balance between mechanical functionality and physical protection from herbivorous animals and saprobionts. From the primitive pteridophytes and gymnosperms to the angiosperms, the chemical complexity of lignin has risen over time. Higher plants necessitate rigid tubular structures to move water and nutrients; additionally, cellulose microfibrils, lignin, and hemicellulose amalgamate to create a bio-composite material, capable of imparting unique properties to trees such that it enables them to support the massive weight, withstand strong winds and thrive for thousands of years (Janusz *et al.*, 2017).

Wood has a considerable amount of organic carbon and is exceptionally resistant to degradation because of its lignin content. The only known organism capable of considerable lignin breakdown are fungi. Molecular clock studies imply that the rapid fall in the rate of organic carbon buried at the end of the Carboniferous era may have coincided with the emergence of lignin breakdown (Floudas *et al.*, 2012).

Historically, the utilization of lignocellulosic-biomass for obtaining feedstock or biofuel has been one of the most challenging endeavors. Numerous extracellular hydrolytic enzymes (e.g., cellulases, xylanases, and tanases) and oxidative enzymes (e.g., laccase and peroxidase) are secreted by the fungi in order to degrade the composted substrate throughout the mushroom growth process (Gavrilescu, 2004; Shea, 2018; Kumla *et al.*, 2020; Beyer, 2023). These hydrolytic enzymes reduce the complexity of organic compounds to simpler ones, so that the

fungus can acquire nutrition for its growth and development through saprotrophic mode (Erjavec *et al.*, 2012; Phan and Sabaratnam, 2012; Shea, 2018). In order to directly bioconvert softwood polysaccharides into fermentable sugar, most mushrooms are naturally adapted to growing in soil or on wood and include lignocellulolytic enzyme machinery (Cohen *et al.*, 2002; Ghorai *et al.*, 2009; Goyal and Soni, 2011; Dehariya and Vyas, 2013; Kumar *et al.*, 2015; Altaf *et al.*, 2016; Mata *et al.*, 2016; Davis, 2017; Oyedele *et al.*, 2018; Debnath *et al.*, 2020; Chang *et al.*, 2021; Rosenberg, 2021; Ghorai, 2022; Mlambo and Maphosa, 2022; Okuda, 2022). Endoglucanase, laccase, and phenol oxidase are some of the enzymes found in *Pleurotus* and *Lentinus* (Elisashvili *et al.*, 2008). They degrade and digest important plant materials like lignin and cellulose, and restore nitrogen from the air as ammonium nitrate to the soil, where plants can use it to grow and thrive (Ganguly *et al.*, 2022). Lignocellulose is a natural bioresource that is used to make biofuel and chemical compounds. The waste byproducts of soft vegetation, timbers, and crops contain polysaccharides, making lignocellulosic biomass a rich and sustainable asset (Kim, 2021).

Mushrooms are commonly cultivated on a substrate comprised of composted agricultural waste (Stamets and Chilton, 1983; Ahlawat and Tewari, 2007; Yang *et al.*, 2013; Feeney *et al.*, 2014; Grimm and Wosten, 2018; Kumla *et al.*, 2020; Raman *et al.*, 2020). Spawn is used to "seed" the compost prepared for mushroom cultivation (Krishnadubey, 2012; Mathur and Gunwal, 2022; Commercial Mushroom, 2023).

Mushroom farming has a significant global economic impact in the food production sector (Okuda, 2022). Ease of cultivation and effective enzymatic degradation of a wide variety of pollutants are the two most important reasons for mushrooms to be used more frequently in biodegradation processes (Kumla *et al.*, 2020).

INDUSTRIAL APPLICATIONS: MUSHROOM-BASED ENZYME AND BIOFUEL PRODUCTION USING AGRO- INDUSTRIAL WASTES

The conventional chemical processes in industries exacerbate pollution, habitat loss, environmental degradation, generation of organic waste, and public health and safety concerns. This indiscriminate use of chemicals in most industrial applications also results in environmental contamination by heavy metals, which do not biodegrade but rather biomagnify in the food chain, endangering human health. These metals can impair many biological functions. Their effects typically depend on concentration. Even in low concentrations, they are hazardous. Prior to

being released into the environment, these compounds must be eliminated or detoxified. Microbial enzymes have the potential to eliminate the negative environmental impact associated with chemical industries (Menk *et al.*, 2019; Cunha Zied *et al.*, 2020; Kumla *et al.*, 2020; El-Gendi *et al.*, 2021). Microbes flourish in relatively mild temperature, pH, and environmental conditions and can also survive harsh production conditions. They are readily available, grow quickly, and can be genetically-engineered to synthesize enzymes that are effective in different manufacturing industries (Hyde *et al.*, 2019). Enzymes produced by microorganisms are indispensable as metabolic catalysts in the development of modern bioprocessing technologies and consequently find widespread application across an extensive spectrum of industries.

Enzymes are effectively replacing chemicals in an ecologically friendly manner in a wide variety of industrial production procedures because they use resources very economically, do not generate any greenhouse emissions or hazardous wastes, reduce energy consumption, and are economical and recyclable (Sadh *et al.*, 2018; Adejumo and Adebisi, 2021; Alemu *et al.*, 2022). Enzymes are becoming increasingly important in many different industries, and microbial enzyme synthesis is the quickest and most effective means of addressing this requirement.

MUSHROOM SUBSTRATUM IS A SOURCE OF INDUSTRIALLY SIGNIFICANT ENZYMES

Plant and algal cell walls are primarily composed of cellulose, an organic polymer. Industrial research faces complications tackling the complexity of lignocellulosic-biomass (Bell *et al.*, 2022). Wood, straw, and sawdust are decomposed by the enzymes secreted by certain fungi (Veloz Villavicencio *et al.*, 2020). This capability of mushrooms is significant in reducing the environmental pollution that would otherwise arise from untreated or incinerated biomass.

Mushrooms have enzyme technology that enables them to effectively utilize lignocellulosic biomass. The substrate on which mushrooms grow contains a plethora of enzymes with diverse functionalities. Once the substratum is utilized for the cultivation of mushrooms, the soil-like residue remaining after harvesting, or the "spent-mushroom substrate," or SMS, is typically regarded as worthless (Owaid, 2017; Cunha Zied, 2020; Becher *et al.*, 2021; Huang, 2022). The SMS could, however, be utilised as potting soil, as animal-feed, as a source of degradative enzymes and biofuel, and even as a casing-layer for growing other mushroom species (Stamets and Chilton, 1983; Yi *et al.*, 2023). Many enzymes of

commercial significance, including cellulase, protease, mannanase, lipase, xylanase, amylase, β -galactosidase, phytase, lignin peroxidase, cellulase, hemicellulase, and laccase, are present in the spent mushroom substrate (SMS). Rather than being dumped in a landfill, these enzymes and bioactive molecules can be extracted at a low cost from the substratum remaining after harvesting the mushrooms. Peroxidase, laccase, cellulase, monooxygenases, transferases, etc. are also just a few examples of the many enzymes found in fungi that help them break down harmful compounds (Fen *et al.*, 2014; Kulshreshtha *et al.*, 2014; El-Gendi *et al.*, 2021).

In nature, fungi secrete the enzyme laccase to degrade the polymer lignin and acquire access to the cellulosic and hemicellulosic carbohydrates in the wood. Laccase is referred to as the "Green Tool," because in the presence of molecular oxygen (as the sole co-substrate), it catalyzes the oxidation of several different substrates (including phenolic compounds) to water, making it a promising and potent enzyme. The low substrate specificity makes it appropriate for a broad spectrum of industrial uses. The abundantly available non-food lignocellulosic materials represent a renewable raw material to be utilized in biorefineries for producing biofuel. This solves the issue of finding a suitable feedstock for making biofuel. The enzyme is also suitable for numerous industrial applications such as remediation of food industry waste water, composting, hair coloring, and poison ivy dermatitis treatment (Elkhateeb, 2022). Feedstock digestibility enhancement is yet another attribute of the enzymatic process to make animal food nutritious and highly digestible (Yi *et al.*, 2023).

The pulp and paper industry utilizes the polymer-degrading activities of enzymes for the production of papers from wood pulp and also from waste paper. Lignocellulolytic-enzymes have piqued interest due to their possible application in innumerable agro-industrial-bioprocesses, such as paper and pulp delignification. Cutting and crushing raw wood generates a pulp with high granularity, volume, and stiffness, whereas biomechanical pulping using cellulases saves 20–40% energy during refining and improves the sheet strength. Enzyme treatments remove dissolved and colloidal contaminants, which cause significant complications in the drainage systems in the pulp and paper factories. The enzyme cellulase has greatly improved paper mill performance (Kuhad *et al.*, 2011). One of the most remarkable functions of enzymes in the pulp and paper sector is the elimination of ink from recycled paper. Waste papers can be de-inked and recovered using microbial enzymes such as amylase, xylanase,

cellulase, hemicellulase, laccase, mannanase, lipase, and pectinase (Balda *et al.*, 2020). Enzymes either hydrolyze the ink carrier or release the molecules of ink from the outermost layer of the fiber. Enzymatic deinking and recycling of paper is an effective alternative that not only prevents deforestation but also lessens the burden on the environment due to toxic bleaching agents. Enzymatic treatment of pulp for bleaching enhances paper quality and brightness (Elisashvili *et al.*, 2006; Enshasy *et al.*, 2013).

The enzyme xylanase catalyzes the hydrolysis of hemicellulose. Xylan is a vital constituent of hemicellulose. The enzyme is one of the lignocellulolytic enzymes mandatory for the complete degradation of plant biomass to obtain biofuels (bioenergy) and other relevant value-added products. It is utilised to bleach and deink used paper pulp in the paper and pulp sector, for preparing feed for animals and fish, to clarify fruit juices, and to improve bread quality. Probiotics, anti-inflammatory, anti-hyperlipidemic, anti-oxidant, anti-allergic, anti-cancer, and antibacterial formulations are some of the pharmaceutically significant substances synthesized with the help of xylanase (Valverde *et al.*, 2015; Elkhateeb, 2022; Ghorai, 2022).

Food processing industries are enzymatically performing green-processing of some foods and beverages. In terms of taste perception, enzymes improve the sensory assessment of food products. The juice and wine making industries utilize enzymes such as xylanase, pectinase, and cellulase, which have a synergistic effect on the reaction process when administered simultaneously. This suggests that a combination of hydrolases is required for effective cell wall disintegration. This softens the raw fruits and vegetables by hydrolyzing cellulose and hemicellulose in them, thereby facilitating extraction and enhancing their transparency, stabilization, and yield. The untreated juice contains floating cellulose, hemicellulose, and pectin particles, which make it extremely thick, cloudy, and less appealing (Inácio *et al.*, 2015). In nature, enzymes break down pectic substances. Pectinases are great biocatalysts for clarifying juices because of their low toxicity and low environmental impact (Patel *et al.*, 2022). The enzyme is not very stable and is also not reusable. Immobilization of the enzyme on a membrane, however, improves its processing performance.

Cellulase helps with the maceration process in terms of improving cloud stability and imparting a smoother texture and thinner consistency to tropical fruit pulp and juices. This enhances yield as well as productivity without capital input in fruits like mango, peach, papaya, plum, and apricot (Kuhad *et al.*, 2011). Preconditioning of

fodder with the enzyme-cellulases or the enzyme-xylanases has the potential to boost nutritional value and the performance of livestock (Kuhad *et al.*, 2011).

Proteases make cow's milk products less likely to cause allergic reactions and are therefore used in the dairy industry and in the baking industry. Proteases are also used in the brewing, tenderizing meat, and photography industries because they can hydrolyze amide linkages (Naeem *et al.*, 2022). Protease and lipase make cheese taste better.

In the **beverage and baking industries**, hydrolysis of maize starch with glucoamylase and α -amylase yields glucose, and then glucose is partially isomerized into fructose to yield high-fructose corn syrup (HFCS). In the beverage industry, HFCS is more popular due to its liquid form for easy miscibility. HFCS is a directly fermentable form of yeast nutrition and is preferred in the fermented product baking industry because it reduces the proof time. HFCS is used as a humectant to safeguard the food from drying out and prolong the freshness and shelf life of bread and crackers in the unfermented product baking industry. Since HFCS is a liquid, it can be easily incorporated into dairy products in the dairy industry (Zargaraan *et al.*, 2016).

Enzyme catalase is used in the baking industry to remove glucose from egg whites before drying them to obtain egg whites in powdered form (Kaushal *et al.*, 2018). H_2O_2 , or hydrogen peroxide, is a strong oxidant that is extremely harmful to living organisms. However, when manufacturing cheeses like Swiss cheese, instead of pasteurization (which would raise the temperature to 140 degrees Celsius, thereby denaturing milk enzymes), FDA-approved cold pasteurization using H_2O_2 is preferred. This maintains the inherent milk enzymes that improve the overall quality of the finished product. During the subsequent steps, however, any traces of hydrogen peroxide left in the milk would inhibit the growth of the bacterial cultures necessary to make the cheese. Catalase enzymes are introduced to prevent any damage due to H_2O_2 after cold pasteurization as a very cost-effective and environmentally benign process (Geciova *et al.*, 2002).

Catalase enzymes have been effectively applied in many sectors; some examples are food processing, textiles, paper, pharmaceuticals, and bioremediation because of their antioxidant property of scavenging free radicals. It has been implemented for biological remediation of wastewater from the textile industry, particularly to remove H_2O_2 from bleaching discharges and recycle the water for subsequent dyeing stages.

Active packaging (using catalase in wrappers) can potentially give a longer shelf life to food and pharmaceutical products, requiring protection from moisture, oxidation, atmospheric degradation, etc. (Kaushal *et al.*, 2018).

The process of making cheese involves adding proteases to milk to hydrolyze kappa casein, which prevents coagulation and stabilizes micelle formation. In the baking industry, proteases help partially hydrolyze gluten so that dough can be prepared more quickly. Since the enzyme is heat-sensitive, fungal protease is inactivated early in baking (Razzaq *et al.*, 2019).

In the textile industry, enzymes are utilized to improve the fabric's properties. Processing techniques for natural fibers that are sourced from a plant or an animal are undergoing extensive transformation. The endeavor is to make all processes eco-friendly, starting from obtaining the fiber to the ready-to-use fabric form and further on to the fabric aftercare. Microbial enzymes are used at various steps of fabric preparation such as: for eliminating pectin and hemicellulose from fibers, using enzyme preparations with xylanases and pectinases; bio-bleaching the fibers with laccases and xylanases as the ligninolytic enzymes; proteases for getting rid of microbial biofilms; sizing agent such as starch is used to join the fibers to make the thread strong enough to be woven into fabric, followed by desizing using α -amylases and β -amylases, to breakdown the starch; scouring using an enzyme preparation containing pectinases, proteases, lipases and cellulases, along with surfactants to reduce the water requirement during washing and rinsing; bleaching agent hydrogen peroxide (H_2O_2) which is subsequently removed by the enzyme catalase (El-Gendi *et al.*, 2021; Nyanhongo *et al.*, 2022).

Traditional stonewashed jeans involve amylase-mediated desizing and pumice stone abrasion (1-2 kg/pair) in huge washing machines. Jeans and other cellulosic materials can successfully undergo biostoning and biopolishing with cellulases. Cellulose-based treatment not only reduces fiber damage and labor requirements but also minimizes the environmental impact of the textile industry and improves productivity.

The traditional method for degumming raw silk in an alkaline soap solution involves removing a proteinaceous material called "sericin or silk gum." The most effective method for removing sericin without damaging the silk fiber is alkaline protease. It has been established that with the enzyme treatment, the possibility of fiber breakage is minimized, and silk threads become far more resilient than they were when using earlier conventional methods.

Laundry detergent industry: Traditionally, proteases and catalases were the first to have been used in laundry detergents (Kaushal *et al.*, 2018; Omrane Benmradi *et al.*, 2019). The commercial potential of developing enzyme-based detergents with proteases, amylases, or lipases for minimizing textile wear and tear is substantial. It lessens the environmental impact of chemical detergents and allows crop waste to be utilized. As extracellularly secreted enzymes, proteases play several important physiological roles, notably in protein catabolism. Fungi are able to acquire the nutrition they require from both organic and inorganic nitrogen sources by secreting extracellular enzymes to aid in the breakdown of natural organic matter. Mushroom protease is a combination of endopeptidases and exopeptidases, with the former creating numerous free C- and N-terminal fragments and the latter acting on the fragmented-peptide to disintegrate proteins. Proteolytic enzymes that degrade protein, such as those released by fungi, possess a significantly wide range of substrates (Kumar Chandrawanshi *et al.*, 2022).

The hydrolytic biocatalytic properties of proteases are utilized in the chemical manufacturing, pharmaceutical industry, food processing, laundry detergent, and leather goods industries because of their thermal versatility over a broad temperature range. The enzyme is used as a detergent in the cell isolation step for producing cell-free enzyme formulations (Kumar Chandrawanshi *et al.*, 2022).

Leather processing involves the elimination of non-collagenous particles for softness and durability. Proteases can replace the numerous poisonous chemicals and thereby regulate pollution (Kumar Chandrawanshi *et al.*, 2022). Although protease is the enzyme with the widest commercial availability on the world market, there are a lot of opportunities to conduct cutting-edge, innovative research in the future on the protease gene (Li *et al.*, 2012).

Pharmaceutical and healthcare industries: As a defense mechanism against potential pathogens or competing microorganisms in their natural habitat, mushrooms produce antimicrobial enzymes. These enzymes enable mushrooms to ensure their own survival and protect themselves from pathogens. The strategy of anti-microbial fungal enzymatic action may be either a direct or an indirect approach (El-Gendi *et al.*, 2021). Fungal-enzymes such as cellulases, amylases, and lipases have been shown to have a direct means of action by rupturing the cell membrane of the pathogenic microorganism, thus leading to the depletion of integral components that are essential to its survival. Oyster mushroom, *P. ostreatus* purified laccase demonstrated potential antiviral activity

against hepatitis C virus (HCV) (EL-Fakharany *et al.*, 2010).

Researchers have shown that proteases have numerous applications in the healthcare sector. The therapeutic potential of alkaline proteases has been applied in numerous medical preparations, featuring surgical gauze and ointment formulations. Possible potential uses of fibrinolytic enzymes (Fibrin breakdown by alkaline fibrinolytic proteases) include cancer treatment and thrombolytic therapy. Therapeutic uses for the slow-release dosage forms, comprising collagenases and alkaline proteases, are widespread (Razzaq *et al.*, 2019). Whereas indirect antimicrobial activity could be achieved by using fungal enzymes to produce intermediate molecules with antibacterial activity (El-Gendi *et al.*, 2021).

There is a rise in infections that are resistant to commonly used antibiotics, which in turn leads to longer hospital stays, costlier treatment, and more fatalities. This spread of infectious diseases due to a lack of effective treatments is a major threat to public health. Mushrooms such as *Agaricus blazei* Murrill, *Ganoderma lucidum*, and *Lentinula edodes* have multiple bioactive compounds that promote important functional qualities, such as antibacterial activity (Valverde *et al.*, 2015; Lima *et al.*, 2016; Garcia *et al.*, 2021; Bhambri *et al.*, 2022; El Sheikh, 2022). Antimicrobial metabolites produced by mushrooms, and indeed fungi, can be used to explore the possibility of curing infections.

As opposed to synthetic products, natural remedies for the treatment of wounds are in high demand because their use is not associated with negative consequences. Medicinal mushrooms possess bioactive substances that aid in several biological processes essential for wound healing (Chugh *et al.*, 2022). The tiger milk mushroom, or *Lignosus rhinocerus*, has the potential to prevent inflammation as a result of additional infections by bacteria by controlling the production of cytokines that trigger inflammation at an early stage, thereby shortening the inflammatory phase of wound healing (Yap *et al.*, 2023). This prevents inflammation from lasting too long and causing tissue damage. Most mushrooms typically have a significant role in promoting wound healing due to their antibacterial, immunomodulatory, and anti-inflammatory properties. Traditional antibacterial and antifungal products may be included to help keep wounds clean and prevent them from getting infected. Research on the effectiveness of macrofungi as wound healers is now underway (Yap *et al.*, 2023). Application of *Fomes fomentarius* promotes wound healing, and consumption of the mushroom *Phallus*

impudicus enhances wound healing (Malik *et al.*, 2017).

Biopolymers like chitin and chitosan have great bioactive qualities. They degrade quickly, are non-toxic, biocompatible, promote blood clotting, and exhibit antimicrobial properties. Chitin and chitin derivatives have naturally beneficial properties and a promising potential in wound treatment, wherein they help the skin regenerate quickly and hasten up the healing process after injuries. The dressings for wounds made from chitin and chitosan have been developed and employed successfully (Malik *et al.*, 2017). Mushroom-based substitutes offer promises, but further research and development are needed to enhance performance, ensure safety, and fulfill regulatory criteria so that the environmental impact caused by single-use healthcare goods' is reduced.

Mycoremediation: In nature, bioremediating microorganisms could be aerobic or anaerobic (Kaushal *et al.*, 2018). Petroleum hydrocarbons and other fuel oxygenates can be effectively metabolized through aerobic metabolism. The introduction of H₂O₂, hydrogen peroxide (which decomposes into oxygen (O₂) and water (H₂O), in the presence of catalase, can supply the bioremediation microorganisms with the oxygen that the aerobic bacteria need to decompose the organic pollutants in a wastewater treatment plant. The bioremediation process is boosted by using hydrogen peroxide as an oxygen source (Zappi *et al.*, 2000). Peroxidase enzyme is employed in the textile industry to remove dyes and in water treatment facilities to break down chemicals and other contaminants.

Spent *Agaricus bisporus* substrate (SAS), which is a common agro-industrial waste, is used to bioremediate soil contaminated with polycyclic aromatic hydrocarbons (PAH), which are pervasive organic toxic, mutagenic, and carcinogenic contaminants. Many kinds of organic pollutants, including PAH, can be degraded because of the fungal ability to secrete enzymes with low substrate specificity, lignin-degradation enzymes, such as heme-peroxidases (Mn-peroxidase (MnP), versatile peroxidase, and lignin peroxidase), and multi-copper oxidases like laccase (Covino *et al.*, 2010; García-Delgado *et al.*, 2015).

Some varieties of mushrooms have the capacity to denature chemical toxins like dioxin, petroleum hydrocarbons, polychlorinated biphenyls, and many others, thereby contributing to mycoremediation (Adenipekun and Lawal, 2012). Mushroom mycoremediation (or fungal remediation) refers to a bioremediation strategy that exploits the potential of mushrooms for waste

management (Kulshreshtha *et al.*, 2014). It takes advantage of the powerful mushroom enzymes that are secreted extracellularly and are very effective at breaking down many different types of substrates and contaminants (Kulshreshtha *et al.*, 2014). Fungi break down pollutants in contaminated soil or water, turning them into less-harmful or non-harmful substances, thereby rehabilitating the environment. (Anand *et al.*, 2006; Suseem and Mary, 2014; Akpasi *et al.*, 2023). Fungal cell walls contain chitin (Lenardon *et al.*, 2010), a compound that is comprised of N-acetyl D-glucosamine that facilitates the absorption, binding, and endurance to high metal concentrations, thereby exhibiting a potential for mycoremediation (Latha *et al.*, 2012).

Mycelium secretes enzymes that degrade a multitude of substrates and contaminants, making mushrooms ideal for use in mycoremediation (Hyde *et al.*, 2019). Heavy metals such as lead, nickel, and chromium are readily removed by the biosorbent, *P. ostreatus* (Elekes *et al.*, 2010; Kulshreshtha *et al.*, 2014; Siric *et al.*, 2016; Kapahi and Sachdeva, 2017).

Fungal filters formed by the mycelium of oyster mushrooms filtered harmful contaminants like *E. coli* from the Chicago River and other water sources. Over the course of 96 hours, oyster mushroom mycelia were effective in removing over 99% of *E. coli*. Thus, fungi contribute to cleaning the environment of potentially dangerous microorganisms (Pini and Geddes, 2020). The escalating issue of water and soil contamination can be productively addressed, economically, sustainably, and effectively with the use of mushrooms (El-Ramady *et al.*, 2022). According to Mosa *et al.* (2016) and Kapahi and Sachdeva (2017), mushrooms are among the most important mycoremediators because of their filamentous nature, which consists of highly branching mycelial biomass.

Mushroom-based biosorption is an environmental technology to get rid of heavy metal pollution due to its sequestration ability (Vimala and Das, 2009; Dhankhar and Hooda, 2011; Kapahi and Sachdeva, 2017; Kariuki *et al.*, 2017; Akpasi *et al.*, 2023).

Phthalates, such as benzyl butyl phthalate (BBP), di-n-butyl phthalate (DBP), and diethyl phthalate (DEP), are plasticizers that are widespread in the environment and urban wastewater but are a challenge to eliminate using traditional wastewater treatment methods. The enzyme extracts from SMS from four edible mushrooms (*P. eryngii*, *P. djamor*, *P. ostreatus*, and *A. polytricha*) were found to be effective at degrading these phthalates (Chang *et al.*, 2021; Kosre *et al.*, 2023). The potential extracellular

fungal enzymes in the SMS, i.e., esterases, oxygenases, and oxidases/dehydrogenases, are responsible for the degradation of these phthalates from wastewater by adsorption and biodegradation.

Additionally, the mycoremediation potential of *Auricularia* sp. (with extremely low levels of laccases) has been used to remove organic contaminants that are resistant to degradation by laccases. Mycoremediation-based phthalate elimination using SMSs can be a green and sustainable approach (Chang *et al.*, 2021).

Ganoderma is useful for bioremediation and bioenergy production as it has lignocellulose-degrading enzyme machinery that helps break down woody plant components to release nutrients (Bijalwan *et al.*, 2020).

MUSHROOM SUBSTRATUM IS A SOURCE OF BIOFUEL

The emissions of greenhouse gases from fossil-fuel combustion, particularly petroleum and natural gas, are projected to rise. The pressing demand to alleviate the global issues due to the overuse of fossil fuels and the pressing need for a substitute sustainable energy source has accelerated the search for a source of fuel. Since cellulosic material is abundantly available and is a potentially clean and sustainable energy source, the possibility of commercially obtaining fuel from cellulose by microbial-enzymatic degradation is being studied and applied extensively (El-Gendi *et al.*, 2021).

Biofuels made from organic waste substances could offer a promising solution to meet this demand for energy. The large surplus of these cellulosic materials is both environmentally and economically viable (Gorey, 2018). Biofuel production involves the polymer-degrading activities of a number of enzymes. *Thermoanaerobacterium thermosaccharolyticum* (strain-TG57, discovered in 2015) has been reported to directly convert cellulose present in the spent mushroom substrate into biobutanol (Li *et al.*, 2018). Biofuels can be made in an environmentally friendly and economically viable way using the metabolic engineering of SMS, a non-food, lignocellulose-rich renewable biomass. Biobutanol, with its highest energy density and best characteristics of all the known biofuels, has the potential to replace gasoline in automobiles without requiring any mechanical adjustments, making it an alternative fuel that is both more affordable and more viable. The potential of other microorganisms to convert the cellulosic substratum into biofuels is being explored, aiming to address the global issues due to the overuse of fossil fuels (Leong *et al.*, 2022).

Butanol production from cellulose represents a key milestone toward the eventual objective of developing a streamlined bioprocess for the economical production of biofuels that are sustainable and other compounds from lignocellulosic-biomass (Leong *et al.*, 2022).

MYCELIUM-BASED ALTERNATIVES INDUSTRIES **ECO-FRIENDLY REVOLUTIONISING INDUSTRIES**

Fungi are not very substrate-specific (Suwannarach *et al.*, 2022). The fungal inoculants colonize and create densely interwoven chitosan mycelium, which is like the exoskeleton of insects, over a substrate rich in carbohydrates like cereal straw and wood shavings (Yang *et al.*, 2021). Variations in fungal species, substrate, nutritional additions, bioprocess design, container shape, carbon dioxide concentration, humidity, temperature, and post-production processing can all have a bearing on the characteristics and functionality of the resultant mycelium (Philippoussis, 2009; Yang *et al.*, 2021). The fungal mycelium is organic, compostable, and biodegradable (Hyde *et al.*, 2019). Once it has cured, it becomes extremely strong mold and fire resistant (Boyer, 2017; Taylor, 2018; Sidhu, 2020; Zou and Gao, 2020; Robinson, 2022; Sydor *et al.*, 2021). As opposed to synthetic materials, which could take hundreds of years to decompose, mycelium naturally decays following its intended product cycle (Marchant, 2020). Innovative concepts and experimentation have led to the creative designing of mycelium-based products like packaging, furniture, household items, and building supplies.

For over two decades, designing objects based on mycelium has been the focus of attention (Meyer *et al.*, 2020). Ingenious ideas and experimentation have brought about the creation of mycelium-based objects such as packaging, household items, furniture, and construction materials (Attias *et al.*, 2017; Ghazvinian *et al.*, 2019). Mycelium-based leather, fabrics and textiles have been manufactured for clothing, accessories and apparel by amalgamating studies pertaining to renewable assets with insights on biodegradable plastic and other forms of biotechnology, while ongoing developments have extended to encompass the creation of new types of foods, cosmetics, and medications (Karana *et al.*, 2018).

Mycelium bricks are one of the most fascinating new materials currently being researched. Composite materials made from mycelium cultivated on farm waste have the potential to lessen the demand for fossil fuels in the building sector. Items made from mycelium are economical and environmentally friendly (Sidhu, 2020; Wandosell *et al.*, 2021). Packaging made

from raw materials or recycled materials minimizes carbon footprint and is referred to as "green packaging" (Asore, 2021).

Rising demands for zero waste production and reduced expenditures pertaining to packaging procedures are driving the mushroom mycelium-based packaging industry in the forward direction (Mushroom Packaging Industry Market, Analysis, Report 2027 | IGR).

CONCLUSION

Technological advancements have enhanced mushroom quantity and quality and also decreased production expenses, contributing to the market's steady progression. Mushrooms requirement for a rich organic substrate can be met with crop waste, such that we get the organic waste sustainably utilized and converted into a range of useful items such as nutritious, high-quality food, numerous eco-friendly composite materials, enzyme molecules, and biofuel, while also protecting the environment through mycoremediation from the adverse impacts of improper industrial waste disposal.

Crop residue and industrial waste management are critical for long-term sustenance. It is important to implement effective measures to overcome this escalating issue. Mushroom cultivation is a feasible solution with multiple benefits. Sustainability of the environment and ecological recycling can be linked to growing mushrooms since it is a low-impact, resource-efficient way to produce high-quality edible proteins and medicines from the recycling of organic agricultural wastes and their by-products. Mushrooms have enormous potential to provide long-term food security and also create opportunities for employment and entrepreneurship. The properties of certain mushrooms that are suitable contenders for the mycoremediation process, which aims to restore damaged environments by using fungi or their derivatives to eliminate environmental pollutants as a green, clean, and effective method, are now being worked on. Mycelium-based innovations and biofabrication add economic and pragmatic significance to agro-industrial waste that would otherwise be destroyed. An analysis of the material-based applications of fungi unequivocally indicates that the unique recycling and repurposing domain holds immense possibilities for expansion and development, making mushroom farming a profitable venture for the growers. Products made from mushroom mycelium have the potential to establish a circular economy. Mushroom-based materials have found several creative uses. Mushroom substratum contains a variety of degrading enzymes produced by the fungus to obtain nutrition. Enzyme research

will open new commercial possibilities for industries and further reduce the carbon footprint. However, it will only become apparent in the future to what extent and in which areas these innovative products can substitute existing products and the extent of their commercial importance. There are obstacles and constraints that must be overcome before this technology can be scaled up for widespread industrial use. Further research is required to explore various possibilities, as mushrooms can surely contribute significantly to eco-restoration, agricultural sustainability, and industrial applications, leading to the benefits of a circular economy.

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