

Review Article : Open Access

Assessing the potential of various lignocellulosic waste as substrate for mushroom cultivation

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Article Info

Article history

Received 14 September 2024

Revised 28 October 2024

Accepted 29 October 2024

Published Online 30 December 2024

Keywords

Lignocellulosic waste

Cellulose

Hemicellulose

Lignin

Lignocellulolytic enzymes

Abstract

Lignocellulosic waste materials, such as agricultural residues, and forestry wastes represent abundant and renewable resources with potential for use as substrates in mushroom cultivation. Mainly, these agricultural residues left in the field itself for burning, lead to the emission of methane and nitrous oxide, causing health hazards. Utilizing these waste materials not only addresses the challenge of waste management, but also offers economic and environmental benefits. This review examines the potential of various lignocellulosic waste materials as substrates for mushroom cultivation, focusing on their chemical composition and enzymes for degradation.

1. Introduction

Lignocellulose wastes consist of cellulose and hemicellulose, it includes agricultural residues (straw, stover, peelings, cobs, stalks, nutshells, non-food seeds, bagasse), domestic wastes, etc. (Nagendra *et al.*, 2011). The estimated global production of crop residues was five billion metric tons in 2020-2021, which is three times greater than the production levels in 1960-1961, using a grain-residue conversion factor (Shinde *et al.*, 2022). Every year, India generates over 686 million tonnes of crop waste, of which 368 million tonnes come from cereals. Among the many crops, rice accounts for 40% of burning residue, followed by wheat (21%), and sugarcane (19%) (Jain *et al.*, 2014). It was predicted that India has a surplus of 234 million tonnes (34% of gross) of crop residues for variable management choices (Hiloidhari *et al.*, 2014).

Worldwide availability of paddy straw is substantial, with an annual production of approximately 731 million tons across Africa, Asia, Europe, and America (Binod *et al.*, 2010). Countries like China, Pakistan, Bangladesh, Vietnam, Thailand, Japan, Sri Lanka, and India are following new adaptive methods for the management of surplus paddy straw produced in their country which include biochar production, soil amendments (Mohammadi *et al.*, 2016), rice straw-

based stoker boiler (Suramaythangkoor *et al.*, 2010), bioethanol production for power generation (Diep *et al.*, 2015), paper pulp, poultry litter, mats (Bhattacharyya *et al.*, 2021). Due to its easy availability and affordable price as compared to other substrates, paddy straw become preferable for cultivation of *Volvariella* spp. in the North-eastern region of India (Tripathy *et al.*, 2011; Kumar *et al.*, 2016; Samantaray *et al.*, 2024).

Sugarcane cultivation produced nearly 80% of global sugar demand, after its industrial processing, large quantities of sugarcane bagasse (SB) as a by-product was produced (Anukam *et al.*, 2016; Dontaniya *et al.*, 2016). The most common solid waste generated in the production of sugar are press mud cake (PMC), sugarcane trash, sugarcane bagasse (SCB), and fly ash (Konde *et al.*, 2021). Mainly, it consists of cellulose, hemicellulose, lignin, wax, and ash which make it a suitable ingredient to be applied and utilized for reinforcement fiber (Walford, 2018). The use of bagasse improves the physical condition of soil by decreasing bulk density and increasing macro-spore for better root growth (Patel and Shingate, 1981). SB ash is considered an alternative replacement for cement material (Prabath *et al.*, 2022). To remove heavy metals such as Pb, Zn, Cu, Cd, and others, bagasse is also utilized as a biosorbent (Al Arni, 2018).

Maize yields the highest residues nearly 8.9 t/ha, which include stalks, leaves, and cobs remaining after the grain harvest used as an energy source (Zang *et al.*, 2012; Garcia *et al.*, 2019). By using pyrolysis, maize residues can serve as a significant feedstock for the production of biochar (Intaniya *et al.*, 2018). Around the world, 529 million tons of wheat straw is generated annually (Govumoni *et al.*, 2013). Wheat straw have a fibrous structure, low bulk density, and high C/N ratio (Wang *et al.*, 2012).

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Nearly 13.5 million hectares across India, Pakistan, Nepal, and Bangladesh, the Gangetic plain is a hotspot for air pollution owing to crop waste burning (Ghosh *et al.*, 2019). Continuous burning leads to many health hazards for both humans and animals through the emission of methane and nitrous oxide (Koppmann *et al.*, 2005). Globally, crop residue burning contributes approximately 0.07 tetragram of nitrous oxide emissions per year (Akagi *et al.*, 2011). The emission factors for greenhouse gases vary depending on the crop type, residue composition, and burning conditions. For instance, the emission factor for CO from burning wheat straw is estimated to be 1,370 g/kg, while for methane, it is 2.7 g/kg (Andrea and Merlet, 2001). Massive burning of crop residues leads to a decline in air quality, smog, heat waves, and many health issues (Barbier *et al.*, 2009; Bakhsh *et al.*, 2018). The heat produced by burning increases

the temperature of the soil and reduces the population of bacteria and fungi (Gupta *et al.*, 2004). Smoke released from burning residues increases the incidence of lung problems, asthma, and eye problems (Grace *et al.*, 2003; Aswathi *et al.*, 2010).

2. Utilization of lignocellulosic wastes

Traditionally, crop residues are used for purposes that have high demand such as animal feed, fodder, fuel, thatching roofs, packaging, and composting (Figure 1) (Kumar *et al.*, 2014). Cereal residues, in particular, are predominantly employed as cattle feed. In West Bengal, paddy straw and husk are used as domestic fuel and in boilers for parboiling rice. Farmers either utilize the residue themselves or sell it to landless households or intermediaries, who subsequently market it to industries (Mehta *et al.*, 2021).

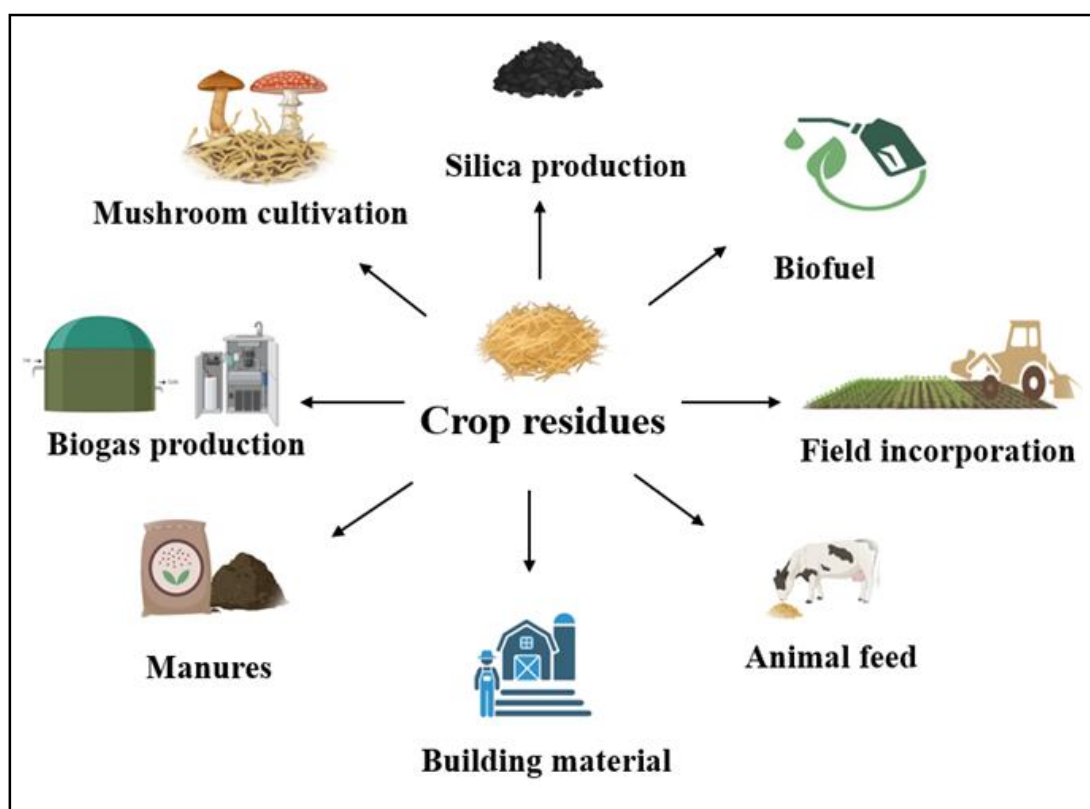


Figure 1: Utilization of crop residues.

Cellulose and hemicellulose, comprise approximately two-thirds of the dry lignocellulosic biomass which includes polysaccharides that can be hydrolyzed to yield sugars, which can then be fermented to produce bioethanol (Hamelinck *et al.*, 2005). The incorporation of mixed fruit peel with paddy straw undergoes simultaneous saccharification and co-fermentation at 35°C after 72 h producing the maximum amount of ethanol (4.4%) (Malik *et al.*, 2020). The most economically viable and conflict-free second-generation renewable alternative is the conversion of lignocellulosic waste to biofuel (Rubin, 2008). It provides a favourable growing environment for mushrooms which are the good source of protein and pharmaceutical products (Ayimbila *et al.*, 2023). Mainly, *Lentinula edodes*, *Tremella fuciformis*, *Ganoderma lucidum*, *Schizophyllum commune* and *Pleurotus* spp. consist of bioactive compounds that have many medicinal properties which include antitumor, anti-

inflammatory, antidiabetic, antiviral and antibacterial polysaccharides (Philippoussis *et al.*, 2007; Roja *et al.*, 2022). Bioactive compounds derived from medicinal mushrooms are widely used for controlling pathogenic diseases of crops and trees (Gayathiri *et al.*, 2021). Fourteen edible mushroom species were tested against various fungal pathogens, among them mycelial development of *Alternaria brassicicola* was moderately inhibited by *Agaricus bisporus* (Akshaya *et al.*, 2021). Oyster mushrooms grown from these wastes can be used for preparing extract from which biosynthesis of nano silver particles can be done (Mistry *et al.*, 2022). In addition to mushroom cultivation, the spent mushroom substrate is used to produce various value-added products (Martin *et al.*, 2023). Lignocellulosic biomass has natural polymers that can be utilized in the preparation of bioplastics (Chang and Holtzapfel, 1999; Inyang *et al.*, 2022).

3. Chemical composition of lignocellulosic wastes

The three main components of lignocellulosic wastes are lignin, hemicellulose, and cellulose (Figure 2). Hemicellulose and cellulose are macromolecules with different sugar compositions, whereas lignin is an aromatic polymer that is produced from precursors that are phenylpropanoids (Sanchez, 2009). Straw from traditional paddy

cultivars contains more cellulose and hemicellulose (Vellaiyan *et al.*, 2024). Crystalline zones are formed by regular bundles of cellulose molecules, while amorphous regions are formed by random geometry. Hemicellulose and lignin act as protective barriers for cellulose polymer microfibrils, which are connected by hydrogen and van der Waals connections (Figure 3) (Yosuf *et al.*, 2020).

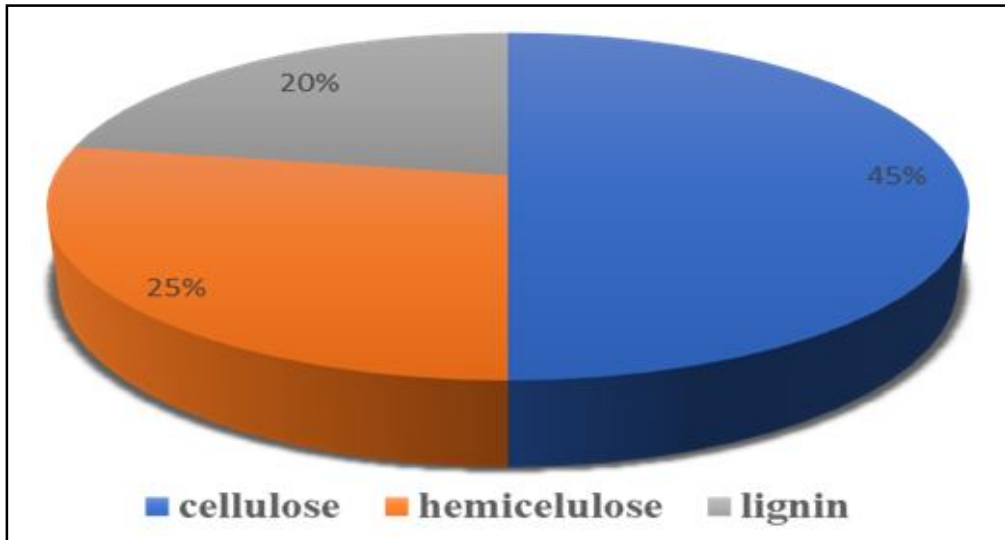


Figure 2: Chemical composition of lignocellulosic wastes.

It has a variety of natural polymers such as starch, lipids, glycogen, elastin, collagen, keratin, chitin, and lignocelluloses, as well as synthetic polymers such as polyesters, polyethylene, and

polypropylene (Taherzadeh and Karimi, 2008). Other constituents of these wastes are: silica, CaO, MgO, and K₂O, and some trace elements (Bondioli *et al.*, 2010; Cornejo *et al.*, 2014).

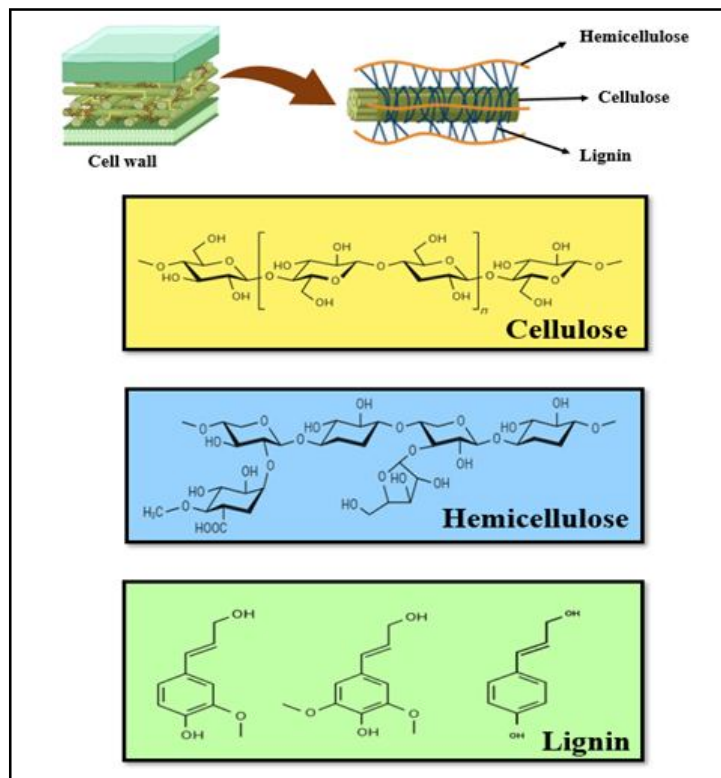


Figure 3: Structure of cellulose, hemicellulose and lignin.

3.1 Lignocellulosic wastes as a substrate for mushroom cultivation

Utilizing lignocellulosic substrates for mushroom cultivation serves as a better way of extracting sugars from the cellulosic substances for mushroom production (Ponmurugan *et al.*, 2007). These substrates act as a reservoir for cellulose, hemicelluloses, and lignin that support luxuriant mycelial growth and fructification process of mushrooms (Yildiz *et al.*, 2002).

The fruiting bodies have a high protein content, ranging from 22.89% to 259.7% on a dry weight basis, and also explained fruiting bodies grown on paddy straw had the highest recorded levels of protein, fiber, carbohydrates, and energy (Sharma *et al.*, 2011). *G. lucidum*, often known as lingzhi or reishi medicinal mushrooms, are typically grown on hardwood logs or sawdust/woodchips, but Veena *et al.* (2011) mentioned paddy straw as a suitable substrate for growth and production of *Ganoderma*. Kumar *et al.* (2023) used wheat straw as substrate material with different doses of micronutrients

for enhancing the *P. florida* production. Different substrate materials have varying degrees of effectiveness in promoting biological efficiency (Table 1).

Zhang *et al.* (2002) conducted an experiment in which they found that ground substrate enhanced the mushroom growth more than chopped substrate. They next compared the oyster mushroom yield parameter with wheat and paddy straw results proved that paddy straw yields 10% more mushrooms than wheat straw. When compared to other lignocellulosic wastes, paddy straw was found to be the most common and effective substrate for oyster mushroom cultivation (Bandopadhyay, 2013; Jayantibhai *et al.*, 2024). Additionally, a combination of paddy straw with vetiver straw is also commercially utilized to enhance oyster mushroom production (Thiribhuvanamala *et al.*, 2018). In future, paddy straw may be successful in an entire economic process and it has the good potential to yield high-value products such as fatty acids, polysaccharides or sugars, amino acids, polyphenols, *etc.* (Gummert *et al.*, 2020).

Table 1: Lignocellulosic waste as substrate for mushroom cultivation and its biological efficiency

S.No.	Mushroom species	Substrate	Biological efficiency (%)	Reference
1.	<i>Pleurotus cystidiosus</i>	Sawdust	36.2	Hoa <i>et al.</i> , 2015
2.	<i>Pleurotus ostreatus</i>	Sawdust	46.4	Hoa <i>et al.</i> , 2015
3.	<i>Pleurotus ostreatus</i>	Paddy straw	95.4	Sharma <i>et al.</i> , 2013
4.	<i>Pleurotus ostreatus</i>	Sugarcane bagasse	67.04	Sharma <i>et al.</i> , 2013
5.	<i>Pleurotus sajor caju</i>	Paddy straw	65.2	Rawte and Diwan, 2019
6.	<i>Auricularia polytricha</i>	Corn stalk	54.05	Liang <i>et al.</i> , 2019
7.	<i>Ganoderma lucidum</i>	Oat straw	2.3	de Carvalho <i>et al.</i> , 2015
8.	<i>Agaricus bisporus</i>	Composted wheat straw	47.2-100.3	Andrade <i>et al.</i> , 2008; Toker <i>et al.</i> , 2007
9.	<i>Lentinus edodes</i>	Barley straw	64.1-88.6	Gaitán-Hernández and Norberto Cortés, 2014
10.	<i>Calocybe indica</i>	Cotton waste	21.96	Maurya <i>et al.</i> , 2019

Several substrates were used, including sawdust from mangoes, jackfruits, jams, coconuts, kadom, mahogany, and shiris. Among these, sawdust from mahogany showed the highest running rate (0.765 cm/day), followed by shiris (0.76 cm/day). The largest biological yield (150 g) was observed in a substrate based on mango sawdust, whereas the lowest yield (83 g) was found in coconut sawdust (Islam *et al.*, 2009). Saw dust is suitable for yielding maximum fruiting bodies of *Pleurotus* spp. compared to others (Neupane *et al.*, 2018). The fruiting bodies with the highest levels of N, P, and K were formed by sugarcane bagasse, straw, and coconut coir (Johnnie *et al.*, 2023). *C. indica* obtained maximum fruiting bodies in paddy straw substrate followed by wheat + paddy straw (Maury *et al.*, 2019).

3.2 Lignocellulolytic enzymes of mushroom in substrate utilization

Lignocellulolytic fungal which includes mushrooms can be exhibited as multiple hydrolytic enzyme activities of laccase, cellulase, and xylanase (Figure 4) (Tanwar *et al.*, 2020). The breakdown of lignocellulosic materials is accomplished by decomposers, which include bacteria, micro-fungi, mushrooms, *etc.* (Eichorst and Kuske, 2013; Bredon *et al.*, 2018). Effective biological delignification process

depends on ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase, which are secreted by basidiomycetes (Mondal *et al.*, 2010). To induce the production of cellulase and laccase enzymes, several substrates are used such as pulse husk, paddy straw, rice bran, sawdust, sesame oil cake, wheat bran, *etc.* Solid-state fermentation showed the production of laccase by *P. sajor-caju* was enhanced by paddy straw, whereas cellulase enzyme was enhanced by wheat bran (Table 2) (Ramachandran *et al.*, 2013).

Ligninases can be broadly classified into two categories: (a) peroxidases, which include manganese peroxidase and lignin peroxidase, and (b) phenol oxidases, also known as laccases (Bredon *et al.*, 2018). Many different species of mushrooms can degrade cellulose present in the substrate by enzymes including both endo-cleaving (endoglucanases) and exo-cleaving (cellobiohydrolases) (Mondal *et al.*, 2010). Hemicellulose is a heterogeneous polymer containing sugar acids, pentoses (such as arabinose and xylose), and hexoses (glucose, galactose, and mannose) (Radhika *et al.*, 2013) which is degraded by many enzymes including mannanase, arabinase, and xylanase (Abella *et al.*, 2014).

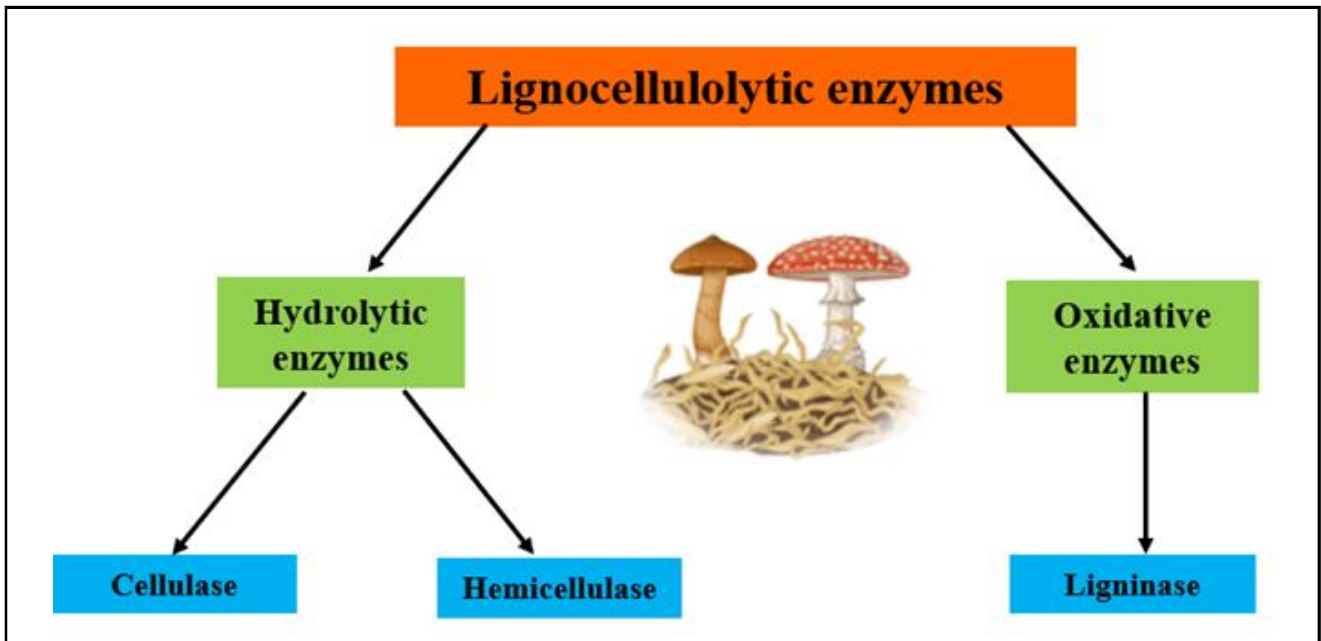


Figure 4: Lignocellulolytic enzyme involved in substrate utilization.

Table 2: Lignocellulolytic enzymes involved in mushroom cultivation

S.No.	Enzyme	Mushroom species	Reference
1.	Cellulase	<i>Lentinus, Pleurotus</i>	Elisashvili <i>et al.</i> , 2008
2.	Laccase	<i>Pleurotus sajor-caju, Volvariella volvacea</i>	Chen <i>et al.</i> , 2003
3.	Lignin peroxidase	<i>Pleurotus ostreatus, Ganoderma lucidum</i>	Arora and Gill, 2001; Moilanen <i>et al.</i> , 2015
4.	Manganese peroxidase	<i>Pleurotus pulmonarius</i>	Rajan <i>et al.</i> , 2010
5.	Lignin peroxidases	<i>Ganoderma lucidum</i>	da Silva <i>et al.</i> , 2019
6.	Xylanase	<i>Pleurotus tuber-regium</i>	Elisashvili <i>et al.</i> , 2003
7.	Endoglucanase	<i>Ganoderma applanatum</i>	Levin <i>et al.</i> , 2008
8.	Exoglucanase	<i>Pleurotus ostreatus, Lentinus sajor-caju</i>	Pandit and Maheshwari, 2012

Table 3: Composition of different substrate materials for mushroom cultivation

S No.	Substrates	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
1.	Paddy straw	35-50	25-35	10	Chen <i>et al.</i> , 2020
2.	Wheat straw	35-45	20-30	15	de Rio <i>et al.</i> , 2012
3.	Corn cob	38	27	22	Akhtar <i>et al.</i> , 2021
4.	Sugarcane bagasse	46	27	23	Pippo and Luengo, 2013
5.	Cotton stalk	45	20	21	Li <i>et al.</i> , 2022
7.	Sawdust	48	18	39	Boadu <i>et al.</i> , 2023
8.	Sorghum straw	42	24	10	de Rossi <i>et al.</i> , 2022

The leftover agricultural residues have a high potential and stimulate the growth and generation of cellulases in a variety of mushroom species, including *Pleurotus*, *Ganoderma*, *Grifola*, *Lentinula*, etc. (Ahlawat *et al.*, 2008; Bibi *et al.*, 2009). Enzymes like laccase and peroxidase activities by *A. bisporus* increase in the substrate from vegetative growth to the early stages of fruiting body development and decrease during fruiting body maturation (Wood and

Goodenough, 1977). These enzymes can be employed in a variety of biotechnological processes, such as the bioconversion, bioremediation, and detoxification of refractory pollutants (Adebayo and Martinez, 2015). *P. pulmonarius* and *P. sajor-caju* have higher lignocellulolytic activity that could degrade agro waste effectively contributing for higher yield (Thiribhuvanamala *et al.*, 2017).

3.3 Influence of cellulose, hemicellulose, and lignin on mushroom growth

Carbon source includes cellulose, hemicellulose, and lignin; nitrogen, and inorganic substances are the three main nutritional sources necessary for the growth of mushroom cultivation (Porselvi and Vijayakumar, 2015). The primary source of carbohydrates is the paddy straw which contains lignin, then it is transformed into the nitrogen-rich lignin-humus complex that offers the source of protein for mushroom mycelium (Scrase, 1996). Consequently, greater cellulose content in the substrate material can accelerate the mycelium growth in mushrooms (Table 3) (Ety *et al.*, 2006).

Hericium erinaceus needs hemicellulose as a source of energy for mycelial development because its structure and physical and physicochemical characteristics of hemicellulose are easier to remove than cellulose and lignin (Sanchez, 2009). Lignin was highly degraded during spawn running, the primordia formation period, and that its degradation decreased after the primordia stages in *P. ostreatus* cultivation (Li *et al.*, 2001).

4. Future perspectives

The global population needs nutrient-rich foods particularly protein which causes a demand in mushroom cultivation. This could be achieved by utilizing the abundant lignocellulosic waste as substrate for mushroom production thereby meeting this demand sustainably. Future research should concentrate on improving the pretreatment methods and substrate formulations for specific mushroom species and waste materials. Exploiting the novel qualities of lignocellulolytic enzymes secreted by specific mushroom species that would serve as to degrade this waste efficiently. Incorporating genetic engineering and strain development techniques to develop the mushroom strain enhanced with the secretion of these enzymes production will pave way for identification of elite strains.

Interlinking mushroom cultivation with other biorefinery processes could be more beneficial for industrial applications. To maximize the overall utilization of these waste materials, residual lignocellulosic waste from mushroom cultivation can be used for the production of biofuels, biochemicals, or other value-added products. In addition, the development of eco-friendly, low-cost techniques for the purification extraction of important substances from spent mushroom substrates, including proteins, antioxidants, and polysaccharides. Addressing obstacles to the large-scale implementation of lignocellulosic waste-based mushroom cultivation will require collaboration among researchers, industry, and policymakers. In the future, it will have plenty of opportunities that contribute to food security and waste management.

5. Conclusion

Lignocellulosic wastes are abundant renewable resources that can be utilized sustainably. Evaluating the qualities of these wastes for mushroom cultivation by assessing their chemical composition, particularly cellulose, hemicellulose, and lignin. Optimizing the potential of lignocellulosic waste materials as substrate needs a deeper understanding of lignocellulolytic enzyme and its activity modification based on the substrate material. Mushrooms can secrete lignocellulolytic enzymes during their growth phase which makes the continuous breakdown of the substrate material for supply of nutrients throughout the cultivation cycle. Lignocellulolytic enzymes

secretion pattern plays pivot role in effective degradation of agro-wastes for better production of mushroom.

Acknowledgments

We duly acknowledge the financial support rendered by Altum Agrotechnologies Pvt Ltd, Coimbatore, the laboratory facilities by Tamil Nadu Agricultural University, Coimbatore and the use of BioRender (BioRender.com) for creating some of the scientific illustrations used in this work (Created in BioRender. A, A. (2024) BioRender.com/p13d449).

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation

A. Arundathi, D. Amirtham, G. Thiribhuvanamala, B. Rajagopal, N. Bharathi, T. Praveen and Vibin Perumalsamy (2024). Assessing the potential of various lignocellulosic waste as substrate for mushroom cultivation. Ann. Phytomed., 13(2):298-307. <http://dx.doi.org/10.54085/ap.2024.13.2.29>.