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Biodegradation of Lignocellulosic waste by *Agaricus bisporus*

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Abstract: *Lignocellulose is a renewable resource or organic matter. It consists of three types of polymers viz., cellulose, hemicelluloses and lignin that are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkage. Many fungi are capable of degrading cellulose and hemicelluloses and utilize as chief carbon and energy sources. However, some filamentous fungi have evolved with their ability to degrade lignin. These are known as white-rot fungi, which possess the unique ability to degrade lignin into CO_2. The Lignocellulosic material is composed of cellulose, hemicelluloses, pectin and lignin. Cellulose is a polysaccharide composed of linear glucan chains that are linked together by β-1, 4-glycosidic linkages with cellobiose residues as the repeating units.*

In the present investigation, biodegradation of lignocellulosic waste by Agaricus bisporus isolated from rotting wood was studied. For this purpose Agaricus bisporus was cultivated on axenic and non-axenic mushroom compost in 20 cm X 3 cm tubular growth vessels. The composition of compost was determined before, during and after the cultivation of mushroom. The chemical analysis of the compost wheat straw before, during and after the cultivation of Agaricus bisporus indicated that the cellulose, hemicelluloses and lignin were degraded preferentially after the addition of the casing layer and the emergence of the basidiocarps, the fruiting bodies. Total culture nitrogen decreased to almost nil after 80 days of incubation. The mineralization of various radiolabelled lignin preparations was also monitored to investigate the preferential degradation of lignin by Agaricus bisporus. In all the cases, the preparations were mineralized preferentially to $^{14}CO_2$ during the mycelial growth and also during the formation of fruiting bodies.

The lignocellulolytic microbial consortium can be used for the conversion of biomass feedstock to useful bio-based products. The techniques can be exploited to separate cellulose, hemicelluloses and lignin from polymeric compounds. This approach comes under sustainable "Green Biotechnology".

Key Words: *Lignocellulose, Agaricus bisporus, Hemicellulose, Cellulose, Lignin, Biodegradation, Mineralization.*

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I. Introduction

Lignocellulose is a renewable organic matter in nature. It is the major component of plant biomass, comprising half of the plant matter synthesized by photosynthesis. It is therefore also called photomass. Different industries such as forestry, paper, paper pulp and agriculture produce a large amount of Lignocellulosic waste (Kim and Dale, 2004) [1]. These materials are treated as waste and either left or incarnated causing serious environmental problems (Mehdi Dashtban *et al*., 2009) [2]. Lignocellulose consists of three types of polymers viz., cellulose, hemicelluloses and lignin that are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages (Perez *et al*., 2002) [3]. Only a small amount of these polymers are used in industrial sectors, and the rest is considered as waste. Many fungi are capable of degrading cellulose and hemicelluloses and utilize as chief carbon and energy sources. However, some filamentous fungi have evolved with their ability to degrade lignin. These are known as white-rot fungi, which possess the unique ability to degrade lignin into CO_2. Some lignocelluloses degrading fungi are called brown-rot fungi which rapidly depolymerize cellulosic materials while only modifying lignin. These wood and litter-degrading fungi collectively play an important role in the carbon cycle. White-rot fungi are also able to degrade a variety of persistent environmental pollutants, such as chlorinated aromatic compounds, heterocyclic aromatic compounds, various dyes and synthetic polymers (Bennet *et al*., 2002) [4]. This degradative ability of

white-rot fungi is due to the strong oxidative activity and low substrate specificity of their lignocellulolytic enzymes.

The Lignocellulosic material is composed of cellulose, hemicelluloses, pectin and lignin. Cellulose is a polysaccharide composed of linear glucan chains that are linked together by β-1,4-glycosidic linkages with cellobiose residues as the repeating unit at different degree of polymerization, and packed into microfibrils which are held together by intramolecular hydrogen bonds as well as intermolecular van der waals forces. Hemicellulose is the second most important biopolymer. Xylan is the main type of hemicelluloses. It is a branched heteropolysaccharide consisting of β-1,4-linked xylose units with side branches of α-arabinofuranose, α-glucuronic acid or other monosachharides. Pectin is a structural acidic heteropolysachharide consisting of galacturonic acids joined together by α-1, 4-glycosidic linkages. Similarly, Lignin is the most abundant and complex biopolymer composed of phynylpropane units, which is synthesized by radical polymerization of guaiacyl units (G), syringyl units (S), and p-hydroxyphenyl units (H) from precursor coniferyl, sinapyle and p-coumaryl alcohol.

The biodegradation process of lignocelluloses is facilitated by several fungi viz., *Phanerochaete chrysosporium* strain RP8 (Martinez *et al*., 2004) [5], *Coprinus cinerea* (Walti *et al*., 2006) [6], *Postia placenta* (Stajich, 2007) [7], *Pleurotus ostreatus* (Irie *et al*., 2000) [8], *Schizophyllum commune* (Horton and Raper, 1991) [9], *Serpula lacrymans* (Bruce, 2007) [10] etc.

In the present investigation, biodegradation of lignocellulosic waste by *Agaricus bisporus* isolated from rotting wood was studied.

II. Materials and Methods

Agaricus bisporus (Lange) was collected from Rajendra Agriculture University, Pusa, Samastipur. This was a commercial strain of A. bisporus. This strain was maintain in on Malt extract agar medium consisted of malt extract (2% w/v) and 1.5% (w/v) agar. The pH was adjusted to 7.0 prior to autoclaving at 15lb/square inch pressure for 20 minutes. The culture was incubated at 25 $\pm 2^0$C and sub-cultured at the interval of six weeks.

Agaricus bisporus was cultivated on axenic and non-axenic mushroom compost in 20 cm X 3 cm tubular growth vessels as suggested by Long and Jacob (1974) [26]. The commercial mushroom compost (30 gm) was packed into each tube. For axenic culture, the compost was autoclaved at 15lb/square inch pressure for 30 minutes on three successive days. The axenic and non-axenic composts were then inoculated with four cubes (5 X 5 X 5 mm cubes) of malt agar colonized with mycelia of *Agaricus bisporus*. The sterilized and humidified delivered from a compressor were passed through the compost at a flow rate of 30 ml/minutes. The culture was incubated at 25^0C by the process suggested by Wood and Smith (1987) [27]. After the mycelium had colonized the compost in 15-20 days, a sterile layer of peat/chalk/activated charcoal (1:1:1 by wt) was added to the surface of the compost (casing layer) and the incubation temperature reduced to 18^0C to induce the formation of fruiting bodies (basidiocarps).

The contents of culture vessels along with basidiocarps were removed. The compost was dried at 105^0C overnight. The composition of compost was determined before, during and after the cultivation of mushroom. Cellulose and hemicelluloses content was determined following the methods of van Soast, (1960, 1967) [11, 12]. Cellulose, hemicellulose, lignin and total nitrogen content the lignin content was determined by the method of Johansson *et al*., (1986) [13]. Total nitrogen content was determined following the method of macro-Kjeldahl.

For radiolabelling of lignin, samples of [ring-^{14}C], [side-chain ^{14}C] and [methoxyl-^{14}C] DHPs were used. These were dissolved in dimethylformamide and filter sterilized using an autoclaved BAS microfilter fitted with a 0.2 pm PTFE filter. A sterilized solution of Lignocellulose labelled with [lignin-^{14}C] was used for synthetic lignin.

The sterilized solution of radiolabelled lignin were added to sterilized 5 ml aliquots of water which gave 50000 d.p.m. each aliquot was added to a 30 gm sample of mushroom compost. Each tube was sealed, and the radioactive suspension was dispersed throughout the column of compost by forcing a gentle flow of sterile air for about 2 hrs.

After inoculating the tubes with *Agaricus bisporus*, the air outlet was then inserted into a an air-tight air trap and the outlet from this was immersed into a $^{14}CO_2$-trapping cocktail of 7 ml 2-ethoxyethanol and 1 ml ethanolamine (Hatakka and Uusi-Rauva, 1983) [14]. Sterile humidified air was forced through the compost (30 ml//minute) and into the trapping fluid. Vials were changed every day, and the radioactivity was determined by adding 5 ml of scintillant and assessed by liquid scintillation counting. All tubes used were Butyl-XX, 1.6 mm wall X 5 mm bore which were non-permeable to CO_2. The tubing and CO_2 traps were tested regularly for leakage by examining the recovery of $^{14}CO_2$ from the known amounts of acidified [^{14}C] sodium bicarbonate. Bacterial air filters were incorporated into the air inlet lines. The results obtained have been presented in Table-1 and Figure: 1-3.

Table-1: Biodegradation of hemicelluloses, cellulose, lignin and losses of nitrogen by *Agaricus bisporus* growing in compost (Concentration of compost in 120 mg (g dry wt/l)

Biodegradation of lignocellulosic waste	Incubation time in days								
	10	20	30	40	50	60	70	80	90
Hemicellulose	118	105	95	90	55	35	30	0	0
Cellulose	93	75	58	45	38	35	30	0	0
Lignin	71	59	48	41	40	38	35	0	0
Nitrogen losses	22	17	16	11	8	5	3	0	0

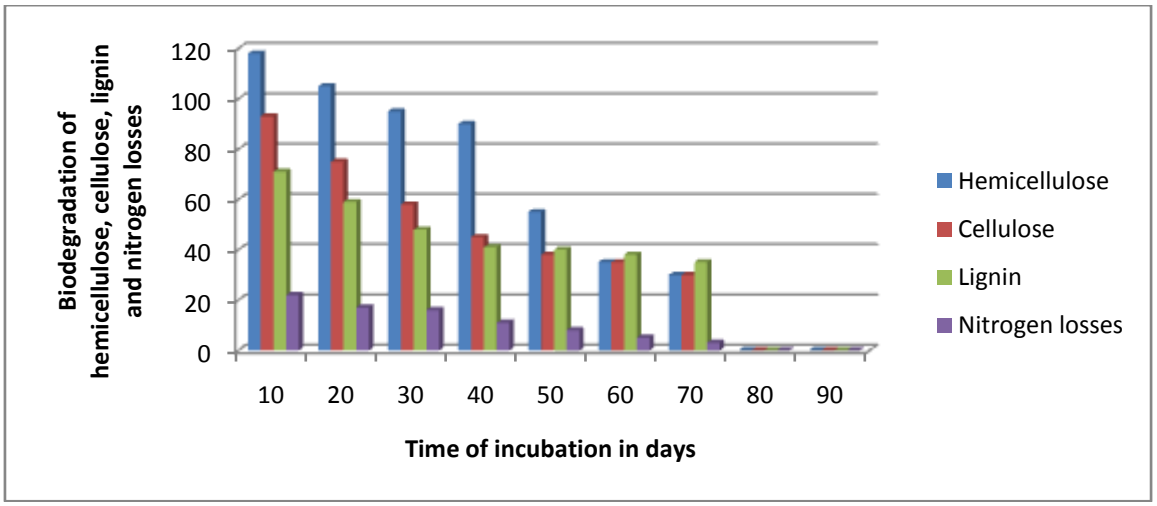

Figure-1: Biodegradation of hemicelluloses, cellulose, lignin and nitrogen losses during decomposition of 120 mg of compost (concentration of compost in mg (g dry wt/l).

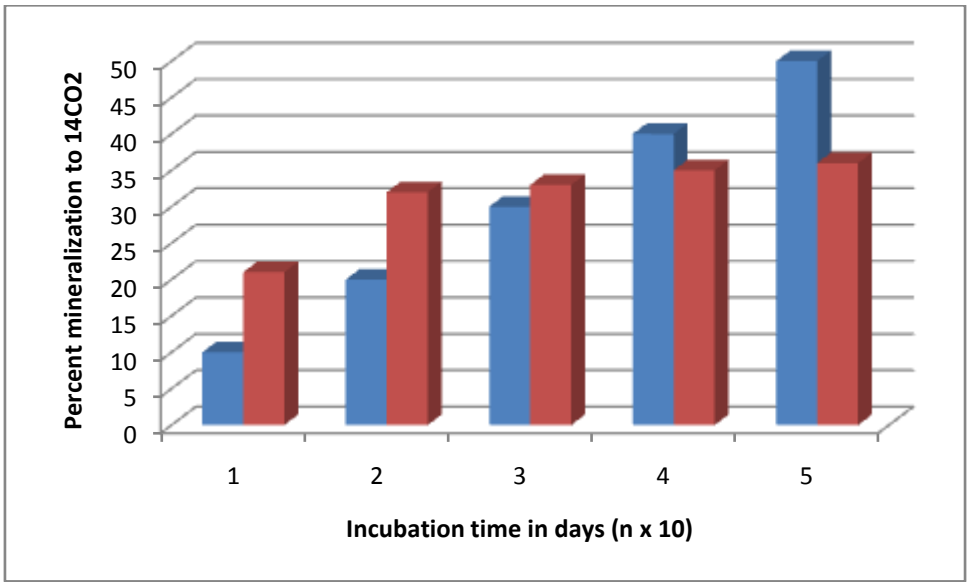

Figure-2a

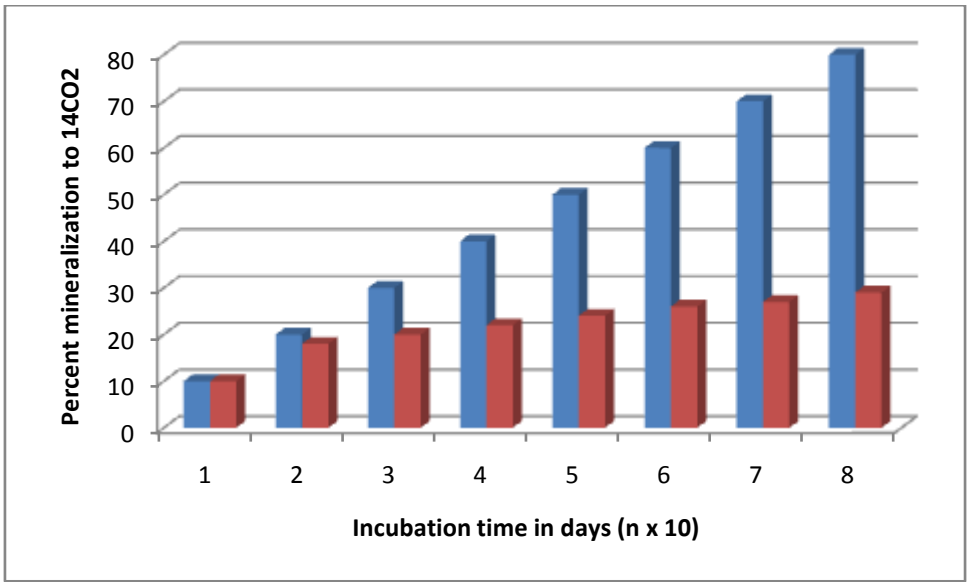

Figure-2b

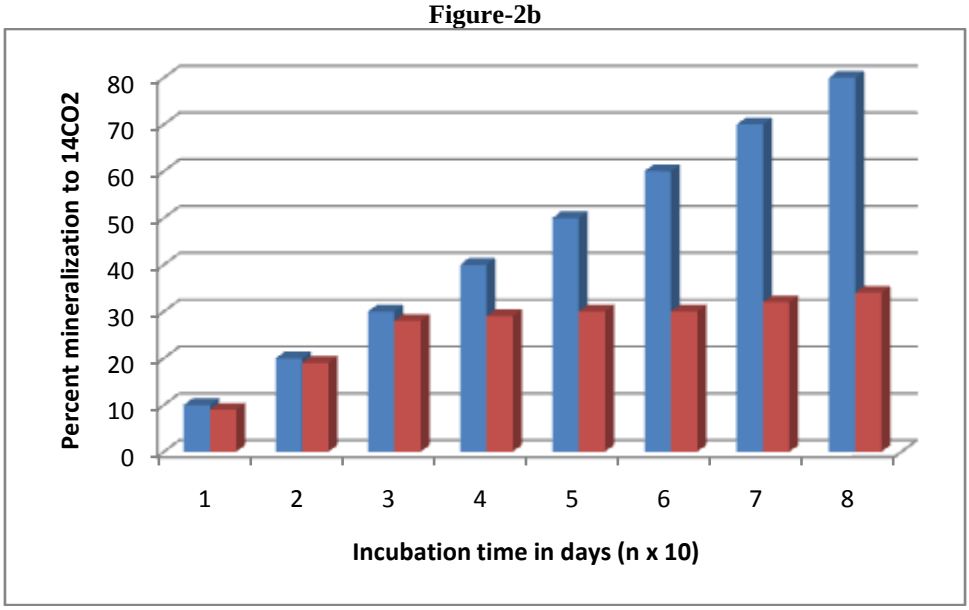

Figure-2c

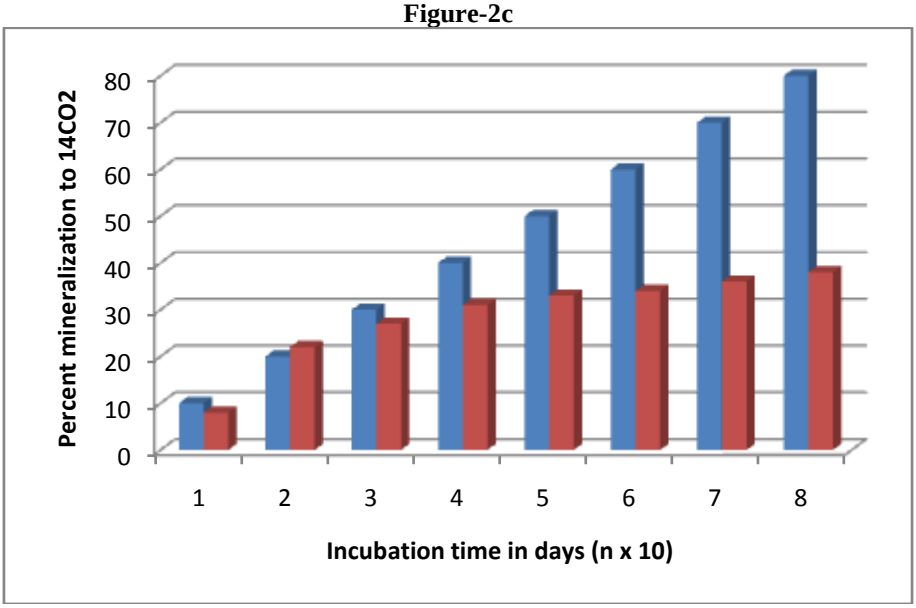

Figure-2d

Figure-2: Mineralization of DHPs and a model lignin dimer by *A. bisporus* during axenic solid state culture. The radiolabelled compounds and specific activities were (a) [ring-TC] DHP. 48600 d.p.m; (b) [side-chain TC] DHP d.p.m; (c) [ring A-^{14}C] B-O 4 dimer, 52000 d.p.m; (d) Wheat [lignin-^{14}C]lignocelluloses, 38000 d.p.m

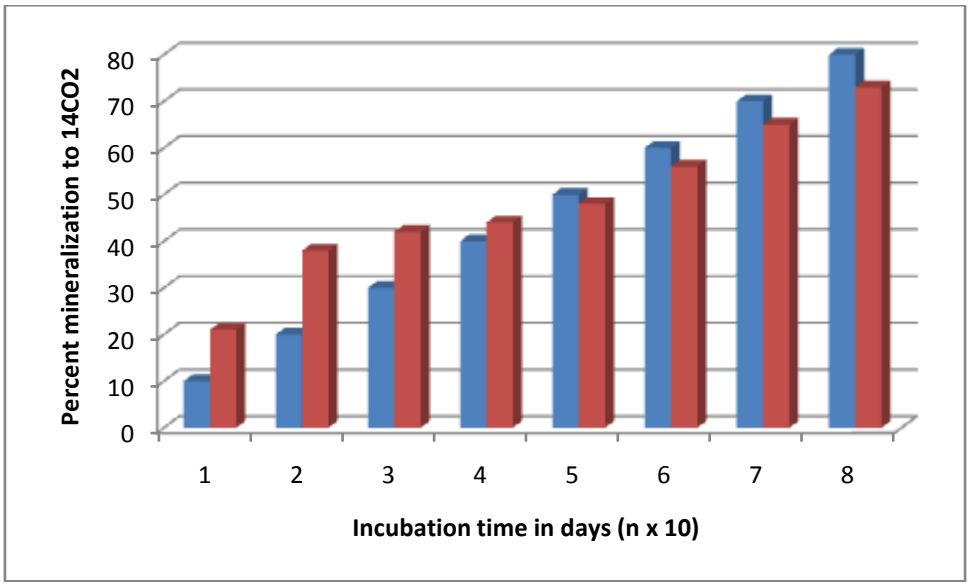

Figure-3a

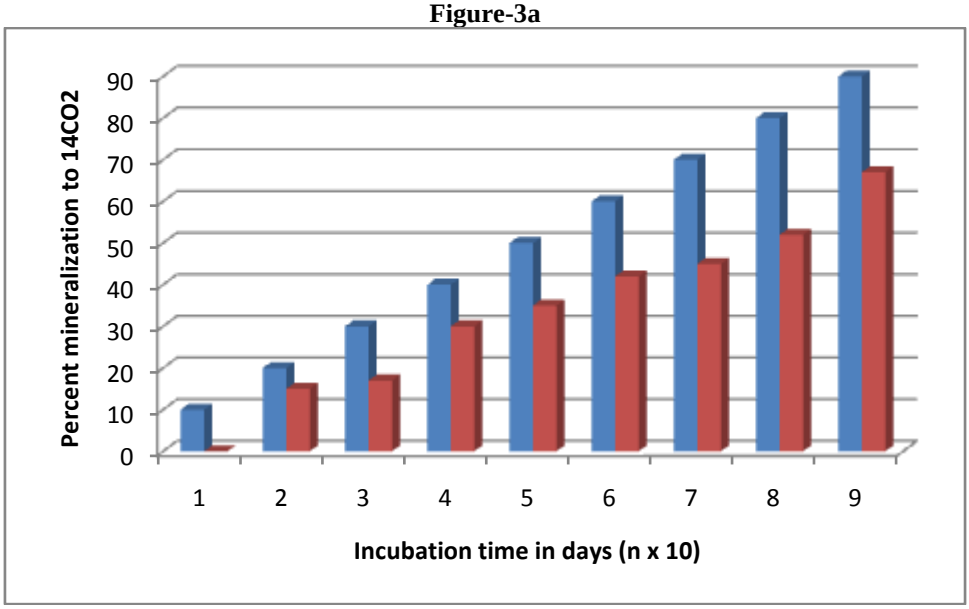

Figure-3b

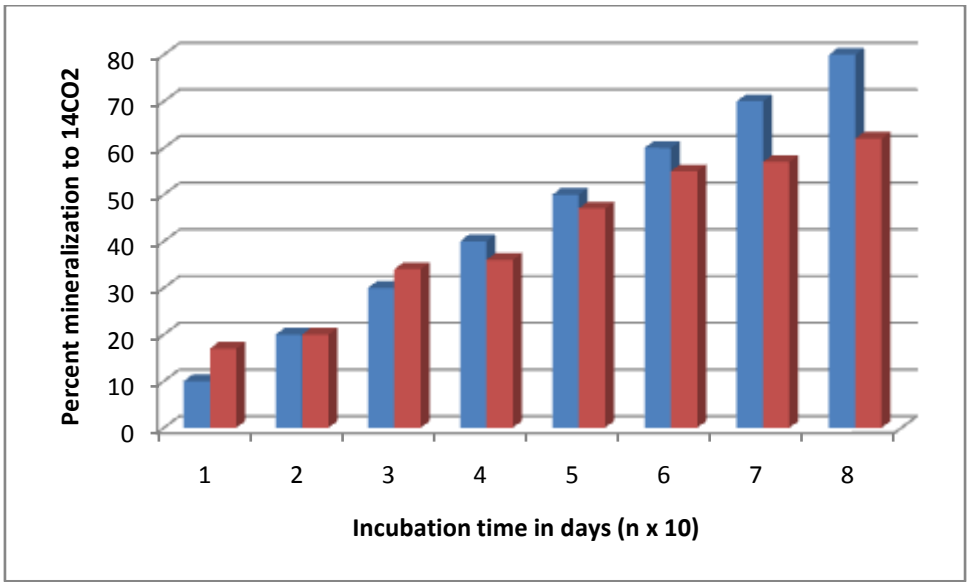

Figure-3c

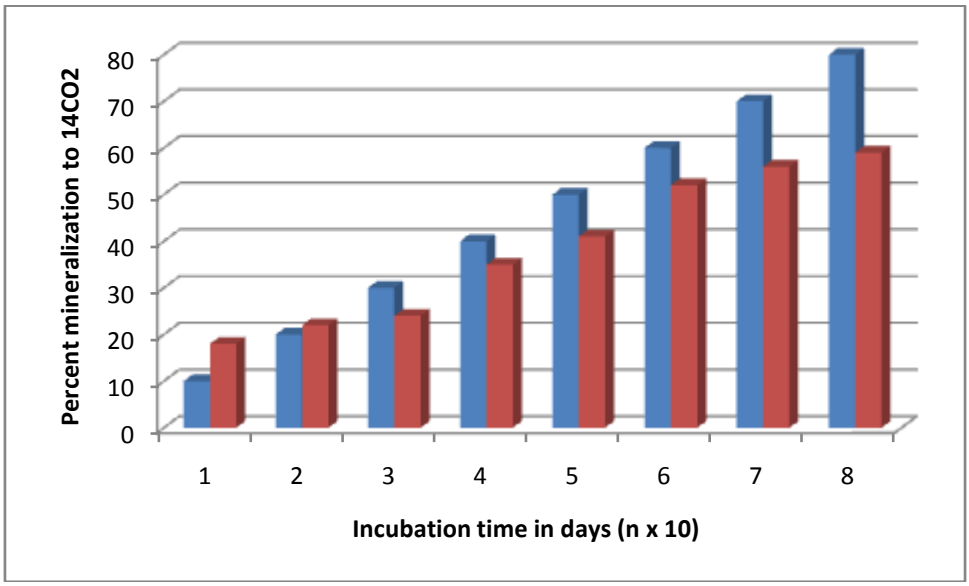

Figure-3d

Figure-3: Mineralization of [ring-^{14}C] DHP (54200 d.p.m per vessel) (a, c) and Wheat [lignin-^{14}C] lignocelluloses (38400 d.p.m per vessel) (b, d) by a fruiting body-deficient mutant of A. bisporus during axenic solid-state culture; (a and b) Uncased; (c and d) cased.

III. Results

Agaricus bispirus was successfully grown on axenic mushroom compost within tubular fermentation chamber. The basidiocarps formation was induced by the addition of a layer of peat-chalk-activated charcoal as a casing layer only after 20 days of vegetative growth. The chemical analysis of the compost wheat straw before, during and after the cultivation of *Agaricus bisporus* indicated that the cellulose, hemicelluloses and lignin were degraded preferentially after the addition of the casing layer and the emergence of the basidiocarps, the fruiting bodies. Total culture nitrogen decreased to almost nil after 80 days of incubation (Table-1; Figure-1). The degradation rate did not change even after the formation of fruiting bodies. The lignin fraction was also degraded preferentially before the addition of the casing layer.

The mineralization of various radiolabelled lignin preparations was also monitored to investigate the preferential degradation of lignin by *Agaricus bisporus*. In all the cases, the preparations were mineralized preferentially to $^{14}CO_2$ during the mycelial growth and also during the formation of fruiting bodies (Figure-2a-d). However, addition of the casing layer markedly reduced the lignolytic activity towards all preparations. The mycelium of this mushroom grew well on the lignocellulosic substrate. This mushroom was also cultivated in the presence of radiolabelled DHP and wheat lignocelluloses (Figure-3a-d). It was observed that the addition of

the casing layer had no significant influence on the mineralization of the preparation to $^{14}CO_2$. It was observed that the process of mineralization continued rapidly even after the formation of basidiocarps (fruiting bodies).

IV. Discussion

In the present observation it was found that the vegetative and fruiting bodies of *Agaricus bisporus* caused concomitant increase in the endo-lignocellulase secretion. These findings gain support from the work of Wood and Goodenough (1977) [15]. The increase in enzyme secretion is reflected by the enhanced rates of lignocellulose degradation within the mushroom compost. The total nitrogen losses in the fermentation process were attributed to the release of ammonia and to the exclusion of mushroom crop during the Kjeldahl digestion. The lignin fraction of lignocellulosic substrates was also degraded during both vegetative and reproductive phases. This is in accordance with the findings of Durrant *et al*., (1991) [16]. Wood (1980) [17] reported that the vegetative growth phase of *Agaricus bisporus* is dominated by the secretion of an extracellular enzyme laccase. It has been estimated that more than 2% of all the enzyme proteins produced by *Agaricus bisporus* is in the form of laccase. In general, laccases have been implicated in the degradative capacities of many mushrooms (Ander and Eriksson, 1976; Kawai *et al*., 1988) [18, 19].

The mineralization of lignocellulosic substrates also confirmed these preliminary observations. In the present investigation it was found that all of the radiolabelled preparations were mineralized to $^{14}CO_2$ during the vegetative as well as reproductive phases of growth of *Agaricus bisporus*. Wheat [lignin-^{14}C] liognocellulose was mineralized to a greater extent in comparison to the amount of lignin lost from compost in the biopolymer experiment. The present findings gain support from the work of Johansson *et al*., (1986) [13]. The side chain-labelled and the ring-labelled DHPs were also mineralized extensively to give [ring-14C] DHP and the [ringA-14C] dimer. This indicated that the β-O-4 linkage was the one of the sites where *Agaricus bisporus* is able to cleave the lignin polymer. The biodegradation of lignocellulosic waste by fungi has also been studied by several workers viz. Abd-Elsalan and Hanafy (2009) [20, Alessi *et al*., (2018) [21], Cortes-Tolalpa *et al*., (2017, 2018) [22, 23], Gontikaki *et al*., (2015) [24], Ransom-Jones *et al*., (2017) [25] etc.

V. Conclusion

Lignocellulosic wastes are most abundant agricultural residues. The lignocellulosic substrates can be utilized by fungi, bacteria and Actinomycetes. Mushrooms grow well on the lignocellulosic substances mostly during humid conditions. The lignocellulolytic microbial consortium can be used for the conversion of biomass feedstock to useful bio-based products. The techniques can be exploited to separate cellulose, hemicelluloses and lignin from polymeric compounds. This approach comes under sustainable "Green Biotechnology".

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Conflict of interest: Authors declare no conflict of interest directly or indirectly.

References

[1]. Kim S, Dale BE (2004): Global potential bioethanol production from wasted crops and crop residues. **Biomass and Bioenergy**, 26:361-375.
[2]. Mehdi Dashtban, Heidi Schraft, Wensheng Qin (2009): Fungal Bioconversion of Lignocellulosic Residues; Opportunities & Perspectives **Int. J. Biol. Sci. 5(6):**578-595
[3]. Pérez J, Muñoz-Dorado J, De-la-Rubia T, Martínez J. (2002): Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. **Int Microbiol**, **5:**53–63.
[4]. Bennett JW, Wunch KG, Faison BD (2002): Use of fungi in biodegradation. In: Hurst CJ, editor. Manual of Environmental Microbiology.Washington DC: AMS press; p. 960–71.
[5]. Martinez G, Larrondo N, Putman N, Gelpke MDS, Huang K, Chapman J, et al., (2004): Genome sequence of the lignocellulose degrading fungus Phanerochaete chrysosporium strain RP78. **Nature Biotechnol**; 22:1–6.
[6]. Walti AM, Villalba C, Buser MR, Grunler A, Aebi M, Kunzler M. (2006): Targeted gene silencing in the model mushroom Coprinopsis cinerea (Coprinus cinereus) by expression of homologous hairpin RNAs. **Eukaryot Cell**; **5:**732–44.
[7]. Stajich J. (2007): Genome of Postia placenta. **Joint genome institute**; http://fungalgenomes. org/blog/2007/06/genome-of-postia-placenta/).
[8]. Irie T, Honda Y, Ha H-C, Watanabe T, Kuwahara M. (2000): Isolation of cDNA and genomic fragments encoding the major manganese peroxidase isozyme from the white rot basidiomycete Pleurotus ostreatus. **J Wood Sci**; **46:**230–3.
[9]. Horton JS, Raper CA.(1991): A mushroom-inducing DNA sequence isolated from the basidiomycete, **Schizophyllum commune**. **Genetics**; **3:**707–16.
[10]. Bruce D. (2007): Sequencing for the DOE Microbial Genome Program. Joint genome institute; 2007 (http://www.jgi.doe.gov/sequencing/DOEmicrobes2007.html).
[11]. VAN SOEST, P. J. (1963): The determination **of** acid detergent fibre. **Journal of the Association** of' **OjKcial Agricultural Chemists 46:** 829- **835.**
[12]. VANS OESTP, . J. (1967): The determination of neutral detergent fibre. **Journal** of **the Association of Oficial Agricultural Chemists 50:** 50-55.

[13]. JOHANSSOMN,. B., KOGEL,I . & ZEICH,W . (1986): Changes in the lignin fraction of spruce and pine needle litter during decomposition as studied by some chemical methods. **Soil Biology and Biochemistry 18, 611-619.**
[14]. HATAKK&A UUSI-RAUVAU, . (1983): Degradation 14C-labelled poplar wood lignin by selected white rot fungi. **Applied Microbiology and Biotechnology 17,** 235-242.

[15]. WOOD, D. A. & GOODENOUGHP.. **W.** (1977). Fruiting of **Agaricus bisporus.** Changes in extracellular enzyme activities during growth and fruiting. **Archives** of' **Microbiolog J, 114,** 161-165.
[16]. **A. J . DURRANTD, A. J**, **A. wood** and R. **B. CAI (1991): Lignocellulose biodegradation by Agaricus bisporus during solid substrate Fermentation, Journal of General Microbiology, 137, 75 1-75.**
[17]. WOOD, D. A. (1980): Inactivation of extracellular laccase during fruiting of **Agaricus bisporus. Journal** of **General Microhiologj. I 17.** 339-345.
[18]. ANDERP, . & ERIKSSONK, . E. (1976). The importance of phenol Oxidase activity in lignin degradation by the white rot fungus **Sporotrichum pulverulentum. Archives of Microbiology 109,** 1-8.
[19]. KAWAI,**S .,** UMEZAWAT, . & HIGUCHI,T . (1988). Degradation mechanisms of phenol B-1 lignin substructure model compounds by laccase of **Coriolus versicolor. Archives** of' **Biochemistry und Biophysics, 262, 99-110.**
[20]. Abd-Elsalam H.E. and El-Hanafy A. A. (2009): Lignin Biodegradation with Ligninolytic Bacterial Strain and Comparison of Bacillus subtilis and Bacillus sp. isolated from Egyptian Soil. **American-Eurasian J. Agric. & Environ. Sci**. **Vol. 5 No 1: Pages** 39-44.
[21]. Alessi A. M., Bird S. M., Oates N. C. et al. (2018): Defining functional diversity for lignocellulose degradation in a microbial community using multi-omics studies. **Biotechnology for Biofuels**. **Vol. 11:** Page166.
[22]. Cortes-Tolalpa L., Salles J. F. and van Elsas J. D. (2017): Bacterial Synergism in Lignocellulose Biomass Degradation – Complementary Roles of Degraders As Infl uenced by Complexity of the Carbon Source. Front. Microbiol. Vol. 8: Pages1628. doi: 10.3389/fmicb.2017.01628
[23]. Cortes-Tolalpa L., Norder J., van Elsas J. D. & Falcao Salles J. (2018): Halotolerant microbial consortia able to degrade highly recalcitrant plant biomass substrate. **Applied Microbiology and Biotechnology** Pages 2913- 2927. DOI: 10.1007/s00253-017-8714-6
[24]. Gontikaki E., Thornton B., Cornulier T. and Witte U. (2015) Occurrence of Priming in the Degradation of Lignocellulose in Marine Sediments. **PLoS ONE**. **Vol. 10 No 12**: Pages e0143917. doi:10.1371/journal.pone.0143917.
[25]. Ransom-Jones E., McCarthy A. J., Haldenby S., Doonan J. and McDonald J. E. (2017): Lignocellulose-degrading microbial communities in landfi ll sites represent a repository of unexplored biomass-degrading diversity. mSphere 2:e00300-17. https://doi .org/10.1128/mSphere.00300-17.
[26]. LONG, P. E. & JACOBS, L. (1974): Aseptic fruiting of the cultivated mushroom **Agaricus bisporus. Transactions** of **the British M.vcological Society 67,** 99-107.
[27]. WOOD, D. A. & SMITH, J. F. (1987). The cultivation of mushrooms. In **Essays in Agricultural and Food Microbiology,** pp. 309-343. Edited by J. R. Norris & G. L. Pettipher. Chichester: John Wiley.

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