STUDY ON THE GROWTH AND YIELD OF MUSHROOM WHITE ROT FUNGI – PLEUROTUS SPECIES

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INTRODUCTION

Mushrooms, the biological potential of these white rot fungi are far from exploited. In addition to the traditional bioconversion of organic wastes into edible protein and animal feedstock, there are other fields where the lignocellulolytic potential of oyster mushrooms may also achieve economic relevance.. First there is an increasing demand from agriculture, industry and medicine for hydrolytic and oxidizing enzymes manufactured by . inexpensive processes. Second numerous data presented in the literature in favor of utilizing lignolytic basidiomyctes as a tool for the remediation of contaminated soils and industrial waste waters **(Pointing 2001; Semple et ai., 2001)**. Among these Basidiomycetes, Pleurotus mushrooms are good candidates to use in bioremediation.

Enzymes secreted by microorganisms play a key role in biodegradation, bioconversion and bioremediation **(Singh *et al.*, 2007 a & b)**. Several microorganisms including bacteria, actinomycetes, fungi, yeast and algae have been reported for the production of enzymes. The capability of white rot fungi to secret extracellular enzymes which have low or no substrate specificity has attracted the attention of scientist around the globe. This property can be exploited for various applications particularly the degradation of vast variety of recalcitrant compounds resembling lignin structure. According **to Erikkson (1993)**, white rot fungus are the only microorganisms which degrade lignin to any substantial degree.

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OBJECTIVES

Keeping in view the extensive applications of enzymes, the proposed research work has been taken up with following objectives:

1. To assess the enzymatic production under *in vivo* condition at different stages of growth of *Pleurotus* species during cultivation.
2. The ultimate aim of these studies is to get enhanced production of extracellular enzymes.

REVIEW OF LITERATURE

***Pleurotus,* a basidiomycetes:**

Fungi secrete enzymes which play a role in the degradation of lignocelluloses material and other recalcitrant materials. Various elements (i.e. carbon, nitrogen, hydrogen, oxygen, potassium, phosphorus etc.) present in the substrates are released by the enzymatic hydrolytic activity of the microorganism and are used in their food chain leading to their further multiplication. Lignocelluloses wastes degradation maintains environmental and nutritional balance in ecosystem **(Eveleigh, 1987)**. Lignocelluloses materials serve as substrate for further fermentation to fuels and chemicals.

Cellulolytic enzymes have gained great importance in recent past in view of their role in degradation of lignocelluloses **(Eriksson, 1993; Ortega *et al.*, 1993; Singh and Kaushal, 1996).** Numerous microorganisms produce celluloses in nature, of which fungi have been the most studied **(Eriksson, 1993).** Cellulose acts on cellulose which is the first most abundant single organic compound in biosphere followed by lignin In nature, cellulose is always associated with variety of hemicelluloses along with phenolic polymers e.g. lignin. Among the microorganisms studied so far there is a complete set of hydrolytic (cellulolytic) enzymes minimally composed of following enzymes involved in cellulose degradation.

MATERIAL AND METHOD

Spawn is a white fibrous matter that forms the matrix when fungus grow and is referred to as the vegetative mycelium of the fungus, which is grown on cereal grains i.e,grains of barley, maize, bajra, wheat, rice, oat etc. The preparation of spawn is done by soaking of buffers. After sterilization, inoculation with pure culture of appropriate Pleurotus app. under aseptic conditions. For preparation of spawn, 500 ml of dextose bottles or polypropylene bags were used. The fungal mycelium started spreading on the grains after 3 to 4 days, and appear like white net web. In 10-12 days the bottles on bags were half filled and it took 18-20 days for the bottles to be completely filled with white mycelium growth.

SUBSTRATES PRETREATMENTS

Plants extract treatment

The lignocelluloses wastes (sugar cane leaves, paddy straw and wheat straw) were treated with neem oil (*Azadirachta indica*) and aqueous extract of Ashoka leaves (*Saracca indica*) (1:3.5 w/v). The aqueous extract of Ashoka leaves was prepared as follows:

1:5w/v oven dried (50-60*c) ashoka leaves powder was mixed in distilled water. The mixture was boiled for 2-3 hours and filtered through muslin cloth. The aqueous extract of ashoka leaves was kept in oven at 80*c for 30minutes to prevent contamination while the supernatant was discarded. After taking out from oven, the aqueous extract of ashoka leaves was used for substrate treatment. The extract has (1:3.5 w/v) of leaves and water.

Neem oil was obtained from market and for pretreatment of substrates. different concentrations i.e.10,15,20,30,40 and 60ml/l of both plants extracts were used. These were used to check the efficiency of lignocelluloses wastes for growth of mycelia. The conclusion was then drawn that 20ml/l concentration of ashoka leaves (1:3.5w/v) and neem oil treated wastes sholved better growth of fungus species. The plants extracts of this concentration were then mixed in 1 liter of water at the time of soaking of substrates. The excess water was drained out after 24 hours and substrates were spread on clean platform for about 30 minute to further shed free water. These treated substrates were now ready for further spawning.

Hot water treatment

Hot water was used for sterilization of substrates. the substrates were immersed in water completely for 20 hours. After this, the excess water was chained out and the substrates were now dipped in water at temperature 70 - 80*C for one hour. the excess water was then again drained out and the substrates were spread on platform evenly till they cooled and were ready for spawning.

Chemical sterilization

In this, each substrate was separately soaked in water (50 l for every 10 kg dry chopped substrates) and 50 ppm each of nuvan and bavistin were added.This were kept for 24 hours after which excess water was drained and the substrates were spread on platform evenly for 1one hour. These sterilized substrates were now ready for spawning.

Table 1

In vitro Polyphenol oxidize (unit/ml/ Days) activity of Pleurotus species

Su/Sp	5	10	15	20	25
SP1	5.00	4.41	2.32	2.00	1.25
SP2	3.52	2.96	2.13	1.82	1.09
RP1	0.15	0.76	0.32	0.10	0.04
RP2	0.15	0.72	0.32	0.09	0.03
WP1	0.14	1.11	0.77	0.43	0.08
WP2	0.10	1.01	0.59	0.41	0.07

S= sugarcane leaves P1=P.florida

R=paddy straw

W=wheat straw P2=P.sajor-caju

Table 2

In vitro poly phenpl oxidize activity (unit/ml/ Days) of Pleurotus species on plant extract treated substrates

Su/Sp/T	5	10	15	20	25	30	35	40	45	50	55	60
SP1A	2.12	2.05	1.86	1.60	1.51	1.33	1.31	1.24	1.24	1.00	0.70	0.45
SP2A	2.21	2.08	2.07	1.81	1.53	1.36	1.25	1.25	1.05	0.80	0.61	0.67
SP1N	3.74	3.50	3.25	3.00	2.16	1.82	1.66	1.50	1.46	1.15	0.79	0.47
SP2N	3.85	3.85	3.75	3.50	3.35	2.39	1.50	1.32	1.26	1.16	0.85	0.59
RP1A	2.21	3.21	3.45	3.40	3.30	3.15	3.07	3.02	2.05	1.50	0.69	0.50
RP2A	2.21	2.85	5.76	5.50	5.45	5.30	4.11	3.40	2.60	2.02	1.41	0.82
RP1N	2.41	2.96	3.82	3.70	3.63	3.40	3.14	3.02	2.15	1.91	1.55	0.85
RP2N	2.25	2.93	6.82	6.49	5.63	5.40	4.25	3.76	3.25	2.26	1.46	1.20
WP1A	2.02	2.75	3.20	2.90	2.87	2.83	2.79	2.69	2.02	1.35	1.85	0.85
WP2A	2.15	2.68	4.85	4.74	4.65	4.64	3.87	3.14	2.35	1.93	1.30	1.15
WP1N	2.23	2.90	3.46	3.40	3.37	3.25	2.81	2.79	2.00	1.75	1.20	0.84
WP2N	1.95	2.65	7.50	6.47	5.50	4.82	3.55	3.50	2.95	2.15	1.71	1.15

S= sugarcane leaves P1=P.florida A=aqueous extract of ashoka leaves

R=paddy straw N=neem oil

W=wheat straw P2=P.sajor-caju

Table 3

Activity of exo-100,4-β-glucanase (unit/ml/ Days) of pleurotus species on hot water and chemical treated Substrates under in vivo condition

Su/Sp/T	5	10	15	20	25	30	35	40	45	50	55	60
SP1H	5.18	5.60	5.46	4.15	3.70	3.00	2.00	1.91	1.70	1.46	1.11	0.75
SP2H	4.07	4.45	4.18	4.00	3.60	3.31	2.14	1.91	1.50	1.08	1.00	0.71
RP1H	4.01	4.44	5.32	6.31	9.10	8.15	6.14	5.50	5.00	4.44	2.95	1.92
RP2H	3.02	3.33	4.00	5.53	10.20	8.68	5.48	4.75	3.55	2.82	2.44	1.61
WP1H	3.31	4.21	5.00	6.08	8.65	7.07	6.00	5.10	4.44	3.60	2.20	1.51
WP2H	2.85	3.32	3.96	5.32	8.60	8.87	5.34	4.51	3.55	2.52	2.21	1.54
SP 1C	5.11	5.61	5.41	4.11	3.60	2.92	1.93	1.60	1.50	1.42	1.11	0.72
SP2C	4.02	4.32	4.10	3.94	3.51	3.21	2.09	1.61	1.47	1.22	0.90	0.65
RP1C	3.92	4.21	5.29	7.48	8.50	8.09	6.11	5.52	4.87	4.22	2.80	1.81
RP2C	3.00	3.21	3.96	5.49	10.00	8.50	5.42	4.61	3.48	2.76	2.42	1.61
WP1C	3.28	4.09	4.96	7.10	8.41	7.01	5.92	5.02	4.39	3.52	2.12	1.48
WP2C	2.78	3.28	3.92	5.30	8.62	8.21	5.20	4.41	3.50	2.46	2.15	1.50

S= sugarcane leaves P1=P.florida H=hot water

R=paddy straw C=chemical

W=wheat straw P2=P.sajor-caju

RESULT

Rice husk gave the fastest mycelial growth rate; however, this did not correspond with yield, indicating that mycelial growth and yield of mushrooms have different

requirements . Also, this substrate is very susceptible to drying, which affected sporophore formation .Given the physical nature and high porosity of the rice husk, and also the fact that it dries up very fast, it will be advisable to use it as an additive to sawdust for use as mushroom substrate. In an experiment in which 2% (w/ w) of rice husk was added to composted sawdust, there was an 11% increase in mushroom yield Among the different lignocelluloses by-products tested as substrates for the cultivation of P. ostreatus, composted sawdust and rice straw were found to best support growth of the fungus, with the mycelium fully colonizing the substrates at 33 and 28 days, respectively. The mycelium density was very thick and dense in the two substrates.

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