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Enzyme Profiles of *Pleurotus HK-37* During Mycelia Vegetative Growth and Fruiting on Solid Sisal Waste Fractions Supplemented with Cow Manure

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Abstract: The utilization of agro-industrial residues to cultivate edible mushrooms is of great environmental importance. However, the use of lignocellulose as carbon source depends on the capacity of the mushroom to produce lignocellulolytic enzymes and to secrete them to the extracellular substrate. Thus, the profile of lignocellulolytic enzymes produced during different phases of cultivation of *Pleurotus* HK-37 on sisal waste fractions supplemented with cow dung manure was determined. Mushroom cultivation was performed in plastic bags using substrates formulated by mixing various proportions of sisal leaf residues and sisal boles and supplementing with cow dung manure on dry weight basis. A total of three hydrolytic (carboxymethyl cellulase, pectinase and xylanase) and two oxidative (laccase and lignin peroxidase) enzymes produced by *Pleurotus* HK-37 were analyzed. Among these enzymes assayed, laccase was found to be predominant and highly expressed. After 30 days of incubation, its activity was 158.75 ± 7.66 Ug^{-1}wet spent mushroom substrate (SMS) on 30% supplemented sisal leaf decortication residues: sisal boles (25:75) substrate formulation. The highest lignin peroxidase activity observed was 4.01 ± 1.12 Ug^{-1}wet SMS during full mycelia colonization on unsupplemented sisal leaf decortication residues: sisal boles (25:75) substrate formulation. Meanwhile, for the hydrolytic enzymes; the highest carboxymethyl cellulase activity (5.45 Ug^{-1}wet SMS) was observed on unsupplemeted sisal leaf decortication residues: sisal boles (75:25) substrate formulation 50 days after of substrate inoculation, that of xylanase (3.73 ± 0.98 Ug^{-1}wet SMS) was found on 10% supplemented sisal leaf decortication residues: sisal boles (0:100) substrate formulation after 20 days and that of pectinase (8.28 ± 2.14 Ug^{-1}wet SMS) was observed 20 days after substrate inoculation on 30% supplemented sisal leaf decortication residues: sisal boles (100:0) substrate formulation. The present investigation indicates the utilization of solid sisal wastes as support-substrate for production of both edible mushrooms and extracellular enzymes during solid state fermentation; it also provides an alternative approach and value-addition to these agrowaste residues.

Keywords: Oxidative Enzymes, Hydrolytic Enzymes, *Pleurotus* HK-37, Sisal Wastes

1. Introduction

The cultivation of edible mushrooms is a key example of the bioconversion of many types of low-value lignocellulosic wastes, mostly from agricultural activities, into a higher-value crop. For commercial mushroom production, mainly cereal straws, baggase, sawdust, cotton wastes, banana leaves and coffee grounds is used as cheap basic substrate nevertheless other agricultural wastes may also be used [1]. These agricultural wastes are mainly rich in lignocellulose. Lignocellulose is primarily composed of cellulose, hemicelluloses and lignin. Cellulose is a linear biopolymer consisting of D-glucose subunits linked by β-1,4-glycosidic bonds forming cellobiose molecules whereas hemicelluloses are heterogeneous polymers of pentoses (including D-xylose and L-arabinose), hexoses (mainly D-mannose, less D-glucose and D-galactose) and sugar acids [2, 3]. Lignin normally contains three precursor aromatic alcohols, these are; coniferyl, sinapyl and *p*-coumaryl alcohol which acts as a barrier by linking to both hemicellulose and cellulose [4, 5]. Consequently, growth

and fruiting are dependent on the ability of the particular mushroom to attack these components as nutrient sources. This in turn depends on the ability of the fungus to produce the necessary hydrolytic and oxidative enzymes required to degrade these materials into smaller molecules for assimilation [6].

Naturally, white rot fungi have ability to grow on such lignocellulosic substrates by secreting different types enzymes for lignin degradation [7, 8]. *Pleurotus* sp. is a white rot fungus that produces a set of lignocellulolyitc enzymes, which allow it to grow on lignocellulosic substrates rich in lignin [9, 10, 11]. The versatile set of lignocellulolytic enzymes produced by this white-rot fungus made it possible to be cultivated in a wide range of lignocellulosic substrates, including different agro-industrial residues [12, 13]. The mushroom yields were very different depending on the substrate employed for cultivation [9]. The chemical nature of the substrate used to cultivate *Pleurotus* sp. has striking influence on the yields of mushrooms, in addition, the phenotype of the different strains regarding to their ability of expressing lignocellulolytic enzymes is directly relating to the potential of the white-rot fungi to colonize and convert a lignocellulosic substrate into edible mushrooms [14, 15].

Fruiting body production in higher basidiomycetes has been linked with ligninolytic enzymes but timings of high enzyme production appear to differ among species and there is no clear-cut picture on functional relevance of the enzymes in fruiting [11]. Previous studies using oyster mushroom, *Pleurotus ostreatus*, reported that production of ligninolytic enzymes increases with vegetative biomass production on solid growth substrates and that it drops during the sexual fruiting stage [11, 16]. However, so far information on production of these enzymes by *Pleurotus* HK-37 during solid state cultivation on sisal waste fractions supplemented with cow manure is totally non-existent. Therefore, the aim of this work was to determine the profile of the oxidative and hydrolytic enzymes produced by *Pleurotus* HK-37 during mycelia colonization and growth over three flushes of fruiting body production on solid sisal waste fractions supplemented with cow manure.

2. Material and Methods

2.1. Fungal Source and Culture Maintenance

Pleurotus HK-37 was obtained from strain bank of Department of Molecular Biology and Biotechnology, University of Dar es Salaam in Tanzania. This oyster mushroom strain originated from South Africa is among the oyster mushrooms grown in Tanzania [17]. The fungi stock culture was maintained on malt extract agar (MEA). Cultures on plates were incubated at ambient temperature, which ranged from 25-30 °C for 5 to7 days. Maintenance of pure mycelia cultures is a necessity for ultimate spawn preparation. Subcultures were made routinely every one month.

2.2. Substrate Preparation and Formulation

Sisal boles and fresh sisal decortications residues were collected from Kidugalo sisal decortications factory at Morogoro region, in Tanzania and were sun dried for 5 days. Fresh cow dung manure was obtained from local husbandry keeper at Ubungo Kibangu, Dar es Salaam, Tanzania and was sun dried for five days and ground to fine powder using a laboratory blender (Snijders Scientific Tilburg, Holland, Waring Blender, Torington, CT, USA). The sisal boles were chopped into 3 to 4 cm lengths using a locally made manual chopper followed by grinding using forage cutter machine. The dried fibres from sisal boles were soaked in water for 2 hours to moisten them and were subjected to a composting process to allow fermentation of free sugars for 14 days by covering with black polythene sheet. In order to search for the best substrate combination which will lead to highest mushroom yield, the following proportions of the sisal bole compost (abbreviated as SB in the rest of the paper) and sisal leaf decortications residues (also referred as sisal leaves and abbreviated as SL in the rest of the paper) were mixed: SL:SB (100:0); (75:25); (50:50); (25:75) and (0:100). These fractions and dry cow dung manure were pasteurized using an autoclave (Koninklijke AD Linden JR BN-Zwijinderect, Holland) at 70 °C for three hours, thereafter were left to cool before they were mixed as reported in [18].

2.3. Spawn Production and Mushroom Cultivation Experiments

Spawn of *P.* HK-37 were prepared using sorghum grains. The grains were washed thoroughly, and 1 kg was boiled in 2 litres of water until they became semi soft. They were then decanted over a sieve to remove excess water and cooled to room temperature. One hundred grams wet weight grains were packed in 250 ml glass bottles, and then they were covered with aluminium foil and were sterilized at 121 ºC for 15 minutes. Thereafter, each cooled bottle of sterilized grains was aseptically inoculated with three mycelia agar fragment (1 cm^2 each) taken from 5 day old cultures of *P.* HK-37. The grains were shaken to evenly distribute the mycelia, and then incubated at 25±2 ºC until when fully colonized by mycelia. Mushroom cultivation was carried out on different sisal waste formulations supplemented with cow dung manure as per procedure outlined, as in [18] and the spawn to substrate ratio was (1:6 w/w). After inoculation, both openings of the bags were tied with a sisal string and the inoculated substrates were then incubated at 28 ±2 ºC and at a relative air humidity of 78 ± 2 %. The spawning room was kept humid by pouring 20 litres of tap water per day on the floor that was laid with mattress to retain water for long period. Vegetative growth was monitored by observing the inoculated substrates until they were fully colonized. Fruiting body formation was triggered by shifting environmental variables namely moisture, air exchange, temperature and light in the cropping room. When mycelia colonized the whole substrate mass, random holes were

made on the bags to initiate pinheads or primordia formation by lowering carbon dioxide concentration in the substrate. Curtains on the windows were removed to allow ventilation and more light. Relative humidity in the room was increased to 86 ± 4 % and temperature decreased to (26.5± 0.05) °C by pouring 20 litres of ice-cold water twice a day on the floor. The bags were also sprayed with ice cold water twice a day to keep them moist. Mushroom fruiting bodies were harvested when the in-rolled margins of the basidiomes began to flatten and the aspects of crop yield were evaluated and reported in [18].

2.4. Sampling and Enzyme Detection in the Substrate

Sampling for quantitative evaluation of enzymatic activity was done on the colonized substrate at different stage of spawn run such as active colonization, primordial initiation, fructification and after each mushroom harvest up to third flush harvest. The crude enzyme extract was prepared by soaking 20 g (± 2 g) of substrate (with active mycelia) in 50 ml of the subsequent extraction buffer in 250 ml Erlenmeyer flasks then agitated on orbital shaker (Edmund Bühler, 7400 Tübingen SM 25, Germany) for one hour at 120 rpm. During this step, all flasks containing the substrate-buffer mixture were kept on ice throughout the entire shaking period. These mixtures were then filtered through double layered cheesecloth followed by centrifugation at 6000 rpm for 10 minutes (Mikro 22R centrifuge, Hettich, England) at 4 °C to remove residual particles. The clear supernatants obtained were regarded as crude enzymes which were later used for enzyme activity assays.

Laccase production in the crude enzyme extract was determined as described by Risdianto *et al.* [19], by monitoring the absorbance change at 420nm (A_{420nm}) related to the rate of oxidation of 1 mM 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulfonate] (ABTS) in 50 mM sodium acetate buffer (pH 5.5). Assays were performed in 1.5 ml cuvette containing 0.5 mM of ABTS in 50 mM sodium acetate buffer (pH5.5) and 50 µl of crude enzyme [20]. The oxidation of ABTS was monitored by the formation of intense blue-green colour which was observed by measuring the increase in the absorbance at 420 nm, with a molar extinction coefficient of 36,000 $M^{-1}cm^{-1}$ using UV-Visible spectrophotometer (Jenway, Genova, Bibby Scientific Ltd, England). One unit activity was defined as the amount of enzyme which leads to the oxidation of 1 µmol of ABTS per minute.

Lignin peroxidase activity was determined at 30 °C using the method described by Sugiura *et al*. [21], a reaction mixtures (1.5 ml) contained 20 mM citrate buffer (pH 3.0), 10 mMveratryl alcohol (= 3,4-dimethoxybenzyl alcohol) and 0.1 ml crude enzyme extract. The reaction was started by adding 10 mM H_2O_2 in the assay mixture after incubation at 30 °C for 5 minutes, and the increase in absorbance was followed spectrophotometrically using Jenway UV-Visible spectrophotometer, (Bibby Scientific Ltd, United Kingdom) at 310 nm (extinction coefficient, ε310 = 9300 $M^{-1}cm^{-1}$,) due to oxidation of veratryl alcohol to veratraldehyde (= 3,4-dimethoxybenzaldehyde). One unit (1 U) of LiP activity was defined as the amount of enzyme that oxidized 1.0 µmole of veratryl alcohol per minute at pH 3 and 30°C.

Carboxymethyl cellulase (CMCase), pectinase and xylanase activities were estimated using the DNS method described by Miller [22], by measuring the amount of reducing sugars (as glucose for CMCase/ pectinase or xylose in case of xylanase) liberated in reaction mixtures containing 0.5 % carboxymethyl cellulose (Sigma), 0.5 % pectin (Citrus pectin, Sigma) or 0.5 % birch wood xylan (Sigma) and supernatant, respectively. The incubation times were 30 and 60 minutes at 30°C, respectively [23]. Standard curves were obtained using glucose or xylose. Enzyme activities (U) were expressed as the amount of enzyme releasing 1 µmole of glucose or xylose equivalent per mL per min. All enzyme assays were carried out in triplicate.

3. Results and Discussion

3.1. Spawn Running (Vegetative Growth/Mycelia Development), Pinheads and Fruiting Bodies Formation

Table 1 illustrates the number of days in which various growth stages of *P*. HK-37 took during cultivation on solid sisal waste formulations supplemented with cow dung manure. Depending on the substrate formulation the mycelia vegetative growth (spawn running) took an average of 14±2 days of incubation for fully colonization while pinheads took an average of 3 to 31 days to appear after substrates being full colonized. Induction for pinheads formation was done by subjecting all substrates to cold shock for about one hour daily. The pinheads grew into mushrooms, which were harvested 2to3 days later. For the growth and penetration of the mycelium into basal substrates, which ultimately influences fruiting of mushrooms, the structure and porosity levels of substrate are important factors to be considered [24].

From the results obtained in this study, mycelia run of 12 to16 days were in the range similar to those obtained by Shah *et al*. [25] of 16-25 days of *Pleurotus ostreatus* on wheat straw, leaves and saw dust. On the other hand, Baysal [26], reported the fastest spawn running (15.8 days) in waste paper as substrate. Mshandete [17] and Tripathy *et al*. [27] observed that the duration of different growth stages of cultivated mushrooms are affected by several factors which would include, but not limited to, type of substrates and supplements used, the type of species and/or the strain employed, spawn type and the rate of inocula/spawn applied, spawning method, spawning/cropping containers as well as on the prevailing mushroom growing conditions. After completion of the spawn run, the bags were moved to the cultivation room where there was higher light intensity. Pinheads/primordia formation was observed after 3-31 days on the substrates depending on the substrate formulation and supplementation rate [18]. The pinheads developed to mature fruiting bodies which were harvested 3±1 days later, the

subsequent 2nd and 3rd flushes followed 8±3 days thereafter. The aspects of mushroom crop yield and productivity are reported by Raymond *et al*. [18].

Table 1. *Days for completion of spawn running, pinheads and fruiting bodies formation.*

Substrate formulation	Supplement level	Days for completion of spawn running	Days for pinheads formation	Days for fruiting bodies formation
SB	0%	13±1	43±2	46±3
	10%	12±1	17±3	20±2
	20%	12±1	22±1	27±3
	30%	12±2	18±4	22±3
SB:SL (75:25)	0%	13±1	37±3	41±2
	10%	13±1	38±5	41±1
	20%	12±1	37±2	41±4
	30%	12±1	38±3	42±3
SB:SL (50:50)	0%	13±1	34±6	38±3
	10%	12±1	34±3	37±2
	20%	12±1	34±4	38±5
	30%	12±1	35±2	36±3
SB:SL (25:75)	0%	13±1	44±3	48±4
	10%	13±1	42±1	46±2
	20%	12±1	40±3	44±5
	30%	12±1	41±5	45±6
SL	0%	14±2	42±4	46±3
	10%	13±1	39±3	42±2
	20%	12±1	38±1	42±4
	30%	12±1	38±2	44±3

3.2. Enzyme Detection and Their Profiles During Vegetative and Fruiting Stages

Like other basidiomycetes, *Pleurotus* HK-37 exhibit two-phase life cycle; the vegetative (mycelium or colonization phase) followed by a second phase of fruiting body (reproductive phase that bears the spores). A maximum of three flushes were obtained and enzyme detection and their activities were followed during cultivation of *Pleurotus* HK-37 on solid sisal waste formulations supplemented with different levels of cow dung manure. The utilization of insoluble sisal wastes which are of lignocellulosic nature by edible *Pleurotus* HK-37 depended upon the simultaneous production of enzymes (oxidative and hydrolytic enzymes) that brought about hydrolysis of the macro molecules of cellulose, hemicellulose and lignin components, thereby, liberating low molecular weight nutrients. Production of enzymes by the fungal mycelium is a crucial part of the hydrolysis of complex lignocellulosic materials into monomers, which facilitate colonization process and ultimately determine mushroom yield [28]. In the present study, to better understand the production of enzymes by *Pleurotus* HK-37, a total of three hydrolytic (CMCase, pectinase and xylanase) and two oxidative (laccase and lignin peroxidase) enzymes were analyzed.

3.2.1. Oxidative Enzymes - Laccase and Lignin Peroxidase Activities (Ug^{-1}SMSwet wt)

Laccase activities are presented on Table 2 while those of lignin peroxidase on Table 3. These results revealed that lignin peroxidase activities were detected in all substrate formulations except laccase which was not detected after third flush harvests on SL:SB (50:50) supplemented with 30 % cow dung manure and on unsupplemented SL:SB(0:100) substrates. Generally, both enzyme activities showed a periodical alteration with mushroom production in all substrate formulation regardless of supplementation levels. The activities were high during vegetative growth phase before and during fully mycelia colonization and later declined during the fruiting body production phase. Among five substrate formulations used, highest laccase activity (158.75±7.66 Ug^{-1}SMSwet wt) was detected on SL:SB (25:75) substrate formulation with 20 % supplement of cow dung manure after first flush harvest while that of lignin peroxidase (4.01±1.12 Ug^{-1}SMSwet wt) was found on unsupplemented SL:SB (25:75) substrate formulation during fully mycelia colonization. Laccase activity observed to diminish drastically during the formation and development of the fruiting bodies.

Similar results were observed by Patrick *et al*. [10] in a previous experiment on *Pleurotus* spp cultivation with sugar cane baggase. Moreover, Elisashvili *et al*. [29] reported that, the production of oxidative enzymes increases with vegetative biomass production on solid growth substrates and that it drops during the sexual fruiting stage an observation that is in agreement with the results of this study. The study of Rühl *et al*. [11] on the ligninolytic activities with mushroom production during cultivation of two strains of *Pleurotus ostreatus* on wheat straw-based substrate found high laccase, MnP and LiP activities during vegetative growth phases which dropped upon initiation of fruiting body formation to be hardly detectable at the harvest of the mature mushrooms; later on there was a sharp increase in all three enzymatic activities in the substrate after harvest at all observed flushes for both strains. Muthangya *et al*. [9] and Patrick *et al*. [10] investigated enzyme activity profiles from *Pleurotus sapidus* P969 and

Pleurotus HK 37 on sugarcane residues and sisal wastes, respectively. Their studies indicated that all ligninolytic enzymes from all substrates, displayed a similar pattern along the cultivation time in which activities increased during the colonization phase, reaching a peak when the substrate was fully colonized at days 20-30. They gradually declined afterwards, but decreased markedly during fruiting body formation. Thus, for these species, it is very likely that the enzymes are produced to digest lignocellulosic substrates in order to provide nutrients for the growing organism.

Table 2. *Laccase activity (Ug^{-1}SMSwet wt) profiles of Pleurotus HK-37at different stages during cultivation on solid sisal wastes supplemented with cow dung manure.*

		Substrate formulations				
Supplement level	**Days of incubation**	**SL:SB (100:0)**	**SL:SB (25:75)**	**SL:SB (50:50)**	**SL:SB (75:25)**	**SL:SB (0:100)**
0 %	10	20.95±6.79	8.93±2.17	5.93±1.17	2.91±0.62	4.03±1.04
	20	**120.03±20.54**	109.17±23.54	61.67±12.96	57.17±15.32	67.50±4.71
	30	60.29±25.76	71.67±9.43	27.08±2.95	24.58±2.95	49.58±1.77
	40	74.75±14.14	83.50±10.61	41.67±13.57	27.08±11.85	38.75±2.95
	50	7.97±1.45	32.50±9.42	32.50±9.42	4.97±0.99	19.17±5.89
	60	Nd	3.82±1.03	12.97±3.92	1.03±0.43	4.17±1.43
10 %	10	10.07±2.98	19.28±4.13	12.92±3.91	4.22±1.19	2.61±0.94
	20	88.37±12.37	**131.67±4.71**	81.68±35.35	62.50±14.9	29.17±2.36
	30	81.67±28.93	94.58±12.94	46.25±11.20	83.52±16.6	53.33±9.95
	40	79.12±5.30	92.92±23.74	30.83±7.39	24.17±3.54	28.75±7.76
	50	42.33±8.99	55.01±12.96	55.00±14.09	15.33±2.54	21.67±3.53
	60	17.98±3.16	8.73±1.05	23.94±6.92	2.95±0.73	8.75±1.69
20 %	10	5.03±1.17	8.19±1.94	13.78±2.64	9.73±0.84	17.03±2.69
	20	80.08±33.64	26.25±0.59	55.42±18.92	58.54±15.50	77.08±11.77
	30	141.83±20.04	**158.75±7.66**	81.25±17.68	79.58±7.12	45.83±3.85
	40	89.84±40.07	112.50±31.80	37.50±11.78	42.92±11.20	55.83±13.35
	50	80.3±22.39	55.42±15.89	31.69±5.89	25.83±10.36	24.75±3.67
	60	39.84±5.03	11.98±3.95	10.75±2.34	6.97±2.04	3.89±0.78
30 %	10	28.16±4.91	16.79±5.83	6.38±1.73	19.64±4.01	8.64±1.78
	20	**154.17±24.75**	75.83±27.11	67.04±5.73	85.58±8.92	22.50±2.04
	30	50.28±17.68	116.67±23.57	**134.83±17.21**	106.25±5.30	43.33±6.89
	40	93.25±20.67	**155.83±21.21**	126.25±18.83	71.67±27.14	29.58±14.12
	50	23.05±1.97	17.92±4.12	38.75±7.66	54.20±12.55	7.50±2.07
	60	8.05±2.49	3.94±1.15	Nd	19.94±3.94	3.19±1.23

* nd = no activity detected; bolded values represent high activity

Table 3. *Lignin peroxidase (LiP) activity (Ug^{-1}SMSwet wt) profiles of Pleurotus HK-37at different stages during cultivation on solid sisal wastes supplemented with cow dung manure.*

		Substrate formulations				
Supplement level	**Days of Incubation**	**SL:SB (100:0)**	**SL:SB (25:75)**	**SL:SB (50:50)**	**SL:SB (75:25)**	**SL:SB (0:100)**
0 %	10	3.02±1.03	2.24±0.43	1.32±0.52	0.97±0.23	2.97±0.61
	20	2.13±0.74	**4.01±1.12**	2.63±1.01	2.13±0.97	2.18±0.92
	30	1.61±0.47	1.32±0.32	0.76±0.22	1.92±0.17	1.44±0.37
	40	2.63±0.88	**3.21±0.57**	0.95±0.34	2.04±1.01	1.73±0.81
	50	1.03±0.27	0.74±0.13	0.35±0.04	0.83±0.13	1.12±0.32
	60	0.07±0.01	0.17±0.03	0.08±0.02	0.36±0.09	0.56±0.10
10 %	10	**3.49±0.89**	2.81±0.91	1.53±0.91	2.39±1.04	1.86±0.63
	20	2.21±0.56	1.94±0.76	1.11±0.57	1.92±0.46	2.18±0.92
	30	0.89±0.23	1.05±0.53	0.64±0.16	1.01±0.38	1.45±0.73
	40	1.03±0.47	1.13±0.81	0.83±0.13	0.67±0.13	1.07±0.46
	50	1.10±0.26	0.69±0.21	0.32±0.01	0.46±0.06	0.54±0.12
	60	0.78±0.11	0.22±0.04	0.06±0.01	0.15±0.04	0.13±0.05
20 %	10	**3.28±1.12**	**3.21±0.79**	2.19±1.07	3.13±0.87	2.87±0.82
	20	2.93±0.83	2.47±1.03	1.97±0.89	2.84±0.74	2.09±0.79
	30	2.04±0.69	1.89±0.88	1.08±0.56	2.49±0.96	1.43±0.31
	40	0.56±0.12	0.93±0.27	0.72±0.23	1.06±0.23	0.95±0.14
	50	0.79±0.13	0.63±0.19	0.23±0.03	0.74±0.12	0.34±0.13
	60	0.21±0.14	0.09±0.02	0.06±0.02	0.18±0.07	0.12±0.04
30 %	10	**3.58±1.16**	**3.11±0.99**	2.01±0.69	3.89±1.11	2.18±0.75
	20	3.07±0.76	2.89±1.15	2.36±0.83	3.21±0.82	2.67±0.89
	30	1.43±0.83	2.03±0.89	1.85±0.61	2.64±0.57	1.72±0.27
	40	1.92±0.95	1.48±0.58	1.18±0.49	1.95±0.48	1.08±0.42
	50	0.98±0.32	0.83±0.18	0.89±0.27	1.04±0.27	0.56±0.13
	60	0.34±0.09	0.37±0.12	0.16±0.04	0.67±0.19	0.23±0.08

bolded values represent high activity

The increase in enzyme activity during vegetative growth and during mycelia regenerative phase (after mushroom harvest) is possibly associated with morphogenesis and often the energetic requirements for initiating fruitification where enzymes are secreted to digest the substrate to provide carbon and other nutrients [11, 30, 31]. On a practical level, laccase could be used as a morphogenetic marker, a rapid drop indicating that maximum anamorphic growth has been achieved. This would be useful to mushroom growers in timing the opening of their spawn-run bags for fruiting.

3.2.2. Hydrolytic Enzymes – Carboxymethyl Cellulase (CMCase), Xylanase and Pectinase Activities (Ug^{-1}SMSwet wt)

A time course for *Pleurotus* HK-37 hydrolytic enzymes production is shown as below. As shown for oxidative enzymes, the activities of hydrolytic enzymes were also found to be dependent on the stage of mushroom development.

Cellulase enzyme is responsible for releasing cellobiose from long cello-oligomers and cellulose, to be further converted to glucose by the action of beta-glucosidases [32]. Generally, cellulase activity (Table 4) was low in vegetative phase, sharply increased after first flush when pinheads appeared to initiation of fruiting body formation and a decline in later mushroom harvests. The pattern observed is typical for white rot fungi which exhibit low cellulolytic activity in the anamorphic stage and are characterized for attacking mainly highly lignified substrates such as wood and sawdust and produce enzymes targeted at lignin polymerization [6]. Similar observations have been reported by Geetha and Sivaprakasam [33], suggesting a relation between the morphogenesis of the fungi and its cellulolytic activity, and it is known that the function of CMCase along with other enzymes is to provide the mycelium with the necessary energy for the formation of fruiting bodies.

Furthermore, similar levels and pattern of CMCase productivity was reported for other *Pleurotus* sp., grown on rice straw, banana biomass as well as sawdust [34, 35, 36]. The highest CMCase activity (5.45 Ug^{-1}SMSwet wt) was observed on unsupplemeted SL:SB (75:25) substrate formulation 50 days after of substrate inoculation (Table 4). However, twin peaks of CMCase activities were observed in some substrate formulations, which was similar to the results of some white rot fungi [37, 38]. Reappearance of enzyme activity during the later days of mushroom growth might be ascribed to fungal autolysis resulting in the release of intracellular enzymes into the substrates.

In contrast to CMCase, xylanase activity (Table 5) was high during colonization stage up to 30 days and declined during the first primodia and fruiting body formation stages. The highest activity (3.73±0.98 Ug^{-1}SMSwet wt) was found on 10% supplemented SL:SB (0:100) substrate formulation after 20 days. Sherief [35], reported an early increase in xylanase production by *P. ostreatus* cultivated on sawdust and rice straw with the highest activity being recorded after 20 and 40 days of incubation in the substrates, respectively. However, these results are contrary to those reported by Muthangya *et al*. [9]; Isikhuemhen and Mikiashvilli [39], whereby higher xylanase productivity from *P.* HK 37 and *P. ostreatus* were observed in fruiting than mycelia growth period when cultivated on solid wastes.

Table 4. *CMCase activity (Ug^{-1}SMSwet wt) profiles of Pleurotus HK-37 at different stages during cultivation on solid sisal wastes supplemented with cow dung manure.*

		Substrate formulations				
Supplement level	**Days of Incubation**	**SL:SB (100:0)**	**SL:SB (25:75)**	**SL:SB (50:50)**	**SL:SB (75:25)**	**SL:SB (0:100)**
0 %	10	0.10±0.04	0.07±0.02	0.05±0.01	0.28±0.09	0.73±0.30
	20	0.19±0.06	0.68±0.10	2.23±0.08	0.73±0.20	1.37±0.74
	30	0.37±0.10	0.38±0.04	1.05±0.36	1.04±0.43	1.14±0.50
	40	0.80±0.11	0.41±0.16	0.36±0.10	0.68±0.12	2.32±0.98
	50	0.23±0.03	**3.59±1.02**	2.88±0.98	**5.45±0.30**	0.17±0.05
	60	0.85±0.24	0.27±0.09	0.47±0.12	0.16±0.04	0.36±0.02
10 %	10	0.06±0.01	0.10±0.03	0.47±0.10	0.65±0.09	0.99±0.25
	20	1.94±0.53	2.11±0.27	2.38±0.78	1.43±0.78	1.86±0.18
	30	0.87±0.34	0.89±0.20	2.19±0.39	0.80±0.30	0.73±0.30
	40	0.59±0.10	1.04±0.10	0.91±0.29	2.75±0.91	1.99±0.15
	50	2.25±0.97	2.45±0.10	2.78±1.03	1.84±0.10	1.28±0.21
	60	0.28±0.09	0.58±0.12	0.28±0.03	0.16±0.05	0.25±0.03
20 %	10	0.20±0.08	0.15±0.06	0.78±0.25	0.93±0.40	0.86±0.16
	20	0.98±0.37	0.78±0.20	0.89±0.25	2.54±1.12	1.31±0.22
	30	0.72±0.23	1.04±0.43	0.97±0.30	1.05±0.20	1.09±0.10
	40	0.53±0.17	1.52±0.02	0.85±0.10	**3.30±0.98**	0.65±0.18
	50	1.41±0.82	2.63±1.03	**3.74±0.93**	1.07±0.38	2.13±0.28
	60	0.70±0.19	0.83±0.30	0.09±0.04	0.08±0.02	0.56±0.14
30 %	10	0.19±0.09	0.07±0.02	0.61±0.40	0.91±0.31	0.83±0.09
	20	0.83±0.29	1.03±0.42	1.05±0.31	1.34±0.57	2.17±0.36
	30	0.57±0.11	0.79±0.27	0.81±0.20	0.87±0.25	0.73±0.20
	40	0.61±0.08	2.12±0.98	0.94±0.28	1.28±0.11	0.92±0.30
	50	1.37±0.82	2.58±1.12	**3.22±0.89**	1.72±0.92	2.63±0.40
	60	0.36±0.13	0.31±0.11	0.43±0.05	0.84±0.16	0.58±0.10

bolded values represent high activity.

Table 5. *Xylanase activity (Ug^{-1}SMSwet wt) profiles of Pleurotus HK-37at different stages during cultivation on solid sisal wastes supplemented with cow dung manure.*

		Substrate formulations				
Supplement level	**Days of Incubation**	**SL:SB (100:0)**	**SL:SB (25:75)**	**SL:SB (50:50)**	**SL:SB (75:25)**	**SL:SB (0:100)**
0 %	10	0.39±0.20	0.46±0.08	0.52±0.19	0.37±0.14	1.07±0.04
	20	0.33±0.04	0.56±0.12	1.44±0.64	1.17±0.29	2.03±1.00
	30	0.36±0.18	0.12±0.02	0.72±0.12	0.43±0.12	0.74±0.20
	40	0.06±0.01	1.27±0.19	1.01±0.23	0.59±0.06	1.45±0.36
	50	0.18±0.06	0.63±0.17	0.68±0.02	0.11±0.08	0.92±0.14
	60	0.11±0.04	0.08±0.02	0.19±0.04	0.08±0.02	0.26±0.09
10 %	10	0.75±0.08	0.39±0.09	0.89±0.12	0.16±0.04	0.98±0.04
	20	0.38±010	**2.64±0.37**	2.21±0.59	1.05±0.30	**3.73±0.98**
	30	0.32±0.09	0.34±0.12	0.46±0.07	0.34±0.09	**2.20±0.25**
	40	0.13±0.03	0.22±0.10	1.06±0.33	0.50±0.15	1.08±0.44
	50	0.17±0.04	0.41±0.10	0.14±0.06	0.29±0.09	0.65±0.20
	60	0.15±0.05	0.09±0.02	0.07±0.01	0.12±0.03	0.22±0.07
20 %	10	0.17±0.07	0.83±0.14	0.26±0.11	0.29±0.03	0.48±0.10
	20	0.34±0.13	2.02±0.16	1.09±0.04	0.97±0.13	**2.68±0.64**
	30	0.20±0.08	**3.31±1.03**	0.92±0.16	0.49±0.07	1.57±0.25
	40	0.15±0.06	0.96±0.02	0.49±0.09	0.65±0.10	0.89±0.26
	50	0.21±0.09	0.48±0.14	0.42±0.12	0.37±0.01	0.42±0.19
	60	0.09±0.04	0.23±0.03	0.24±0.05	0.18±0.08	0.12±0.04
30 %	10	0.25±0.11	0.99±0.08	0.27±0.03	0.10±0.03	0.45±0.08
	20	0.55±0.10	**2.27±0.93**	2.16±0.04	0.98±0.19	1.56±0.20
	30	0.91±0.07	1.19±0.07	1.34±0.20	0.48±0.01	1.22±0.10
	40	0.26±0.12	0.73±0.18	1.13±0.17	0.28±0.11	0.38±0.13
	50	0.35±0.09	0.37±0.12	0.87±0.08	0.11±0.05	0.44±0.15
	60	0.17±0.04	0.05±0.01	0.23±0.12	0.09±0.03	0.19±0.08

bolded values represent high activity

Table 6. *Pectinase activity (Ug^{-1}SMSwet wt) profiles of Pleurotus HK-37at different stages during cultivation on solid sisal wastes supplemented with cow dung manure.*

		Substrate formulations				
Supplement level	**Days of incubation**	**SL:SB (100:0)**	**SL:SB (25:75)**	**SL:SB (50:50)**	**SL:SB (75:25)**	**SL:SB (0:100)**
0 %	10	0.31±0.06	0.76±0.08	0.32±0.07	0.77±0.09	0.78±0.14
	20	**7.52±1.48**	5.45±1.04	4.22±1.33	3.54±1.11	2.25±1.40
	30	1.57±0.59	1.98±0.29	2.01±1.04	0.93±0.07	0.13±0.05
	40	2.98±0.93	1.28±0.11	3.22±1.07	2.54±0.71	1.96±0.11
	50	0.67±0.20	0.89±0.18	1.07±0.20	0.97±0.12	1.13±0.20
	60	0.14±0.04	0.27±0.09	0.43±0.19	0.09±0.02	0.34±0.10
10 %	10	1.52±0.21	2.29±0.74	1.62±0.18	2.14±0.98	3.99±1.09
	20	3.46±0.96	**6.47±1.33**	**6.39±1.07**	**6.21±2.03**	4.53±1.03
	30	0.44±0.03	0.38±0.04	0.49±0.01	0.67±0.05	0.59±0.15
	40	1.78±0.15	2.82±0.23	1.93±0.54	1.49±1.14	0.50±0.19
	50	0.97±0.26	1.23±0.17	0.89±0.12	0.63±0.18	0.17±0.04
	60	0.26±0.09	0.47±0.12	0.16±0.04	0.15±0.04	0.08±0.02
20 %	10	0.12±0.07	1.77±0.19	1.99±0.59	2.35±0.60	4.68±1.85
	20	5.46±1.02	**6.08±0.37**	**7.04±1.26**	**8.04±1.33**	3.17±1.85
	30	0.28±0.05	0.44±0.03	0.67±0.03	0.97±0.03	0.57±0.29
	40	1.01±0.78	2.48±1.82	1.22±0.26	1.47±0.15	0.86±0.37
	50	0.45±0.11	1.32±0.20	0.63±0.10	1.05±0.09	0.34±0.10
	60	0.19±0.08	0.41±0.09	0.11±0.04	0.43±0.17	0.11±0.03
30 %	10	0.18±0.05	1.27±0.44	1.48±0.82	2.12±1.06	1.28±0.20
	20	**8.28±2.14**	5.47±1.26	**7.33±1.48**	5.37±1.19	3.54±0.74
	30	0.34±0.10	1.01±0.05	0.18±0.06	0.31±0.05	0.48±0.17
	40	2.57±0.68	1.20±0.15	0.72±0.94	1.06±0.33	1.30±0.30
	50	1.04±0.19	0.96±0.10	0.29±0.09	0.56±0.10	0.67±0.06
	60	0.32±0.10	0.47±0.09	0.10±0.02	0.23±0.08	0.16±0.03

bolded values represent high activity

The activity of pectinase (Table 6) showed the increase in activity during full mycelia colonization, followed by decrease after first flush harvest and further increase after second flush harvest i.e. with a characteristic wave-like pattern (Table 6). It was found to increase up to 30 days and decline after mushroom harvest. The highest pectinase activity (8.28±2.14 Ug^{-1}SMSwet wt) was observed 20 days after substrate inoculation on 30% supplemented SL:SB (100:0) substrate formulation. Low activity shortly after inoculation is probably due to low content of pectin present during those stages

compared to the content of other lignocellulosic materials. Higher pectinase titres have been reported for ascomyceteous fungus (e.g., *Botryosphaeria rhodina*) grown on various fruit processing waste materials by solid state fermentation that included orange bagasse [40].

4. Conclusion

These results indicate that, changes in the level of both oxidative and hydrolytic enzyme activities are closely linked to the development of the fruiting body and physiological state of the mushroom culture. However, enzyme profiles differ depending on the mushroom species, the size of the waste used and, probably on the nature of substrate used for cultivation. In this study, it was found that the levels of enzyme production vary to a large extent during the three flushes of the oyster mushroom. For oxidative enzymes, the highest laccase activity was 158.75±7.66 Ug^{-1}wet SMS on 30% supplemented SL:SB (25:75) substrate formulation after 30 days while that of lignin peroxidase was 4.01±1.12 Ug^{-1}wet SMS during full mycelia colonization on unsupplemented SL:SB(25:75) substrate formulation. Meanwhile, for hydrolytic enzymes; the highest carboxymethyl cellulase activity (5.45 Ug^{-1}wet SMS) was observed on unsupplemeted SL:SB (75:25) substrate formulation 50 days after of substrate inoculation , that of xylanase (3.73±0.98 Ug^{-1}wet SMS) was found on 10% supplemented SL:SB (0:100) substrate formulation after 20 days and that of pectinase (8.28±2.14 Ug^{-1}wet SMS) was observed 20 days after substrate inoculation on 30% supplemented SL:SB (100:0) substrate formulation. From the findings of this work it may, therefore, be concluded that the solid sisal wastes can be used as a cheap source of both lignocellulolytic enzymes and edible mushrooms.

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References

[1] J. A. Buswell, S. T. Chang, "Edible mushrooms: attributes and applications. In Genetics and breeding of edible mushrooms," eds S. T. Chang, J. A. Buswell, and P. G. Miles, Philadelphia. Gordon and Breach Scientific Publishers, 1993 pp. 197-324.

[2] Y. Hong, M. Dashtban, S. Chen, R. Song, and W. Qin, "Enzyme production and lignin degradation by four Basidiomycetous fungi in submerged fermentation of peat containing medium," International Journal Biology 4(1), pp. 172 – 180. doi:10.5539/ijb.v4n1p172. 2012.

[3] T. W. Jeffries, "Biodegradation of lignin and hemicelluloses," In: C. Ratledge (ed.) Biochemistry of microbial degradation. Kluwer, Dordrecht, 1994, pp 233–277.

[4] M. Dashtban, H. Schraft, T. Syed, and W. Qin, "Fungal degradation and enzyme modification of lignin," International Journal of Biochem. Mol. Biol., vol 1, pp. 36-50, 2010.

[5] G. Y. Wei, W. Gao, I. H. Jin, S. Y. Yoo, J. H. Lee, C. H. Chung, and J. W. Lee, "Pretreatment and saccharification of rice hulls for the production of fermentable sugars," Biotechnology and Bioprocess Engineering, 14(6), pp. 828-834, 2009.

[6] Y. H. Tan, M. N. Wahab, "Extracellular enzyme production during anarmorphic growth in the edible mushroom, *Pleurotus sajor-caju*," World Journal of Microbiology and Biotechnology, vol 13, pp. 613-617, 1997.

[7] P. J. Hoegger, A. Majcherczyk, R. C. Dwivedi, K. Svobodová, S. Kilaru, and U. Kües, "Enzymes in wood degradation. In: Kües U. (Ed.), Wood Production, Wood Technology, and Biotechnological Impacts," Universitätsverlag Göttingen, Göttingen, Germany, http://webdoc.sub.gwdg.de/univerlag/2007/wood_production.pdf, pp. 389-438, 2007.

[8] A. Leonowicz, A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wojtas-Wasilewska, N. S. Cho, M. Hofrichter, and J. Rogalski ,"Biodegradation of lignin by white rot fungi," Fungal Genetics and Biology, vol. 27, pp.175-185, 1999.

[9] M. Muthangya, A. M. Mshandete, S. O. Hashim, M. J. Amana, A. K. Kivaisi, "Evaluation of enzymatic activity during vegetative growth and fruiting of *Pleurotus* HK 37 on *Agave sisalana* saline solid waste," Journal of Chemical, Biological and Physical Sciences 4(1), pp. 247-258, 2014.

[10] F. Patrick, G. Y. S. Mtui, A. M. Mshandete, and A. K. Kivaisi, "Ligninolytic enzymes activities of *Pleurotus sapidus* P969 during vegetative growth and fruit development on sugarcane residues-based substrates," International Journal of Biotechnology 3(4), pp.58-71, 2014.

[11] M. Rühl, C. Fischer, and U. Kües, "Ligninolytic enzyme activities alternate with mushroom production during industrial cultivation of *Pleurotus ostreatus* on wheat straw-based substrate," Current Trends in Biotechnology and Pharmacy 2(4), pp. 478 -492, 2008.

[12] J. M. Savoie, D. Salmones, and G. Mata, "Hydrogen peroxide concentration measured in cultivation substrates during growth and fruiting of the mushrooms *Agaricus bisporus* and *Pleurotus* spp," Journal Science of Food and Agriculture 87(7), pp. 1337-1134, 2007.

[13] N. Hatvani, and I. Mécs I, "Production of laccase and manga-nese peroxidase by *Lentinus edodes* on malt containing by product of the brewing process," Proc. Biochemistry, vol 37, pp. 491-496, 2001.

[14] B. E. Lechner, and V. L. Papinutti, "Production of lignocellulosic enzymes during growth and fruiting of the edible fungus *Lentinus tigrinus* on wheat straw," Process Biochemistry, 41(3), pp. 594-598, 2006.

[15] S. Ohga, "Adaptability of *Lentinus edodes* strains to a sawdust- based cultivating procedure," Mokuzai Gakkaishi, vol. 38, pp. 301-309, 1992.

[16] G. Mata, and J. M. Savoie, "Extracellular enzyme activity in six *Lentinula edodes* strains during cultivation in wheat straw," World Journal of Microbiology and Biotechnology 14(4), pp. 513–519, 1998.

[17] A. M. Mshandete, "Cultivation of *Pleurotus HK-37* and *Pleurotus sapidus* (oyster mushrooms) on cattail weed (*Typha domingesis*) substrate in Tanzania," International Journal Research of Biological Sciences 1(3), pp. 35-44, 2011.

[18] P. Raymond, A. M. Mshandete, and A. K. Kivaisi, "Cultivation of Oyster Mushroom (*Pleurotus* HK-37) on solid sisal waste fractions supplemented with cow dung manure," Journal of Biology and Life Scienc*e,* 4(1), pp. 273-286, 2013. doi:10.5296/jbls.v4il.2975.

[19] H. Risdianto, S. H. Suhardi, T. Setiadi, and T. Kokugan, "The influence of Temperature on Laccase Production in Solid State Fermentation by using White Rot Fungus *Marasmius* sp," Proceeding of the 1st International Seminar on Fundamental and Application of Chemical Engineering. Bali 3-4 November 2010.

[20] F. Patrick, G. Mtui, A. M. Mshandete, G. Johansson, and A. Kivaisi, "Purification and characterization of a laccase from the basidiomycete *Funalia trogii* (Berk.) isolated in Tanzania," African Journal of Biochemistry Research 3(5), pp. 250-258, 2009.

[21] M. Sugiura, H. Hirai, and T. Nishida, "Purification and characterization of a novel lignin peroxidase from white-rot fungus *Phanerochaete sordida* YK-624," FEMS Microbiology Letters, vol. 224, pp. 285-290, 2003.

[22] G. L. Miller, "Use of Dinitrosalicylic acid reagent for determination of reducing sugar", Analytical. Chemistry 31(3), pp. 426-428, 1959. doi:10.1021/ac60147a030.

[23] T. K. Ghose, "Measurement of cellulase activities," Pure and Applied Chemistry. 59(2), pp. 257-268, 1987.

[24] A. Philippoussis, P. Diamantopoulou, G. Zervakis, "Monitoring of mycelial growth and fructification of *Lentinula edodes* on several lignocellulosic residues," Mushroom Biology and Mushroom Products, (eds. J. E. Sanchez , G. Huerts , and E. Montiel) Universidad Autonoma del Estado de Morelos, Mexico, 2002, pp. 279-287.

[25] Z. A. Shah, M. Ashra, and M. C. Ishtiaq, "Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (Wheat straw, Leaves and Sawdust)," Pakistan Journal of Nutrition 3(3), pp. 158-160, 2007. http://dx.doi.org/10.3923/pjn.2004.158.160.

[26] E. Baysal, H. Peker, M. K. Yalinkilic, and A. Temiz, "Cultivation of oyster mushroom on waste paper with some added supplementary materials," Bioresource. Technology, 89(1), pp. 5-7, 2003. http://dx.doi.org/10.1016/S0960-8524(03)00028-2.

[27] [27]A. Tripathy, T. K. Sahoo, S. R. Begera, "Yield evaluation of paddy straw mushrooms (*Volvoriella spp*.) on various lignocellulosic wastes," Botanical Research International 4(2), pp. 19-24, 2011.

[28] J. A. Buswell, Y. J. Cai, and S. T. Chang, "Fungal- and substrate-associated factors affecting the ability of individual mushroom species to utilize different lignocellulosic growth substrates," In: Mushroom biology and mushroom products eds. S. T. Chang, J. A. Buswell, S. W. Chiu, SW. Hong Kong: Chinese University Press, 1993, pp. 141-150.

[29] V. Elisashvili, E. Kachlishvili, M. J. Penninckx, "Lignocellulolytic enzymes profile during growth and fruiting of *Pleurotusostreatus*on wheat straw and tree leaves," Acta Microbiologica et Immunologica Hungaria, vol.55 pp.157-168, 2008.

[30] S. Kurt, and S. Buyukalaca, "Yield performances and changes in enzyme activities of *Pleurotus* spp. (*P. Ostreatus* and *P. Sajor-Caju*) cultivated on different agricultural wastes," Bioresource Technology, 101(9), pp. 3164–3169, 2010.

[31] R. M. Gaitán-Hernández, A. Esqueda, Gutiérrez, and M. Beltrán-García, "Quantitative changes in the biochemical composition of lignocellulosic residues during the vegetative growth of *Lentinula edodes*," Brazilian Journal of Microbiology, 42(1): pp. 30-40, 2011.

[32] K. Kirk, and D. Cullen D, "Enzymology and molecular genetics of wood degradation by white-rot fungi," In: R. A. Young, and M. Akhtar, (eds). Environmentally friendly technologies for the pulp and paper industry. Wiley, New York, 1998. pp 273 -307.

[33] D. Geetha, and K. Sivaprakasam, "Enzyme and sporophore production potential of oyster mushroom (*Pleurotus* spp)," Mushroom Resource, vol 7, pp 39-42, 1998.

[34] R. D. Rai, and S. Saxena, "Extracellular enzymes and non structural compounds during growth of *Pleurotus sajor-caju* on rice straw," Mushrooms Journal. Tropical, vol. 10, pp. 69-73, 1990.

[35] A. A. Sherief, A. B. El-Tanash, and A. M. Temraz, "Lignocellulolytic enzymes and substrate utilization during growth and fruiting of *Pleurotus ostreatus* on some solid wastes," Environmental Science Technology vol. 3, pp. 18-34, 2010.

[36] G. V. Reddy, P. Ravindra Babu, P. Komaraiah, K. R. R. M. Roy, and I. L. Kothari, "Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus and P. sajor-caju*)," Process Biochemistry, vol. 38, pp. 1457-1462, 2003.

[37] D. S. Arora and P. K. Gill, "Laccase production by some white-rot fungi under different nutritional conditions," Bioresource. Technology, vol. 73, pp. 283–285. 2000.

[38] C. Raghukumar, T. M. Dsouza , R. G. Thorn, and C. A. Reddy, "Lignin-modifying enzymes of *Flavodon flavus*, a basidiomycete isolated from costal marine environment," Applied Environmental Microbiology, vol. 65, pp. 2103–2111, 1999.

[39] O. S. Isikhuemhen, and N. A. Mikiashvilli, "Lignocellulolytic enzyme activity, substrate utilization and mushroom yield by *Pleurotus ostreatus* cultivated on substrate containing anaerobic digester solids," Journal of Indian Microbial Biotechnology, vol. 32, pp. 1353-1362, 2009.

[40] E. C. Giese, R. F. H. Dekker, and A. M. Barbosa, "Orange bagasse as substrate for production of pectinase and laccase by *Botryosphaeria rhodina* MAMB-05 in submerged and soild state fermentation," BioResources 3(2), pp. 335-345, 2008.