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Solid state fermentation of maize *(Zea mays)* cob by *Pleurotus ostreatus* strain EM-1: Biopolymer profiles and cellulose degradability

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ABSTRACT

The low digestibility and low protein content of maize cob are major limitations to its use as animal feed in Ghana. The possibility of enhancing the feed potential of maize cob through solid state fermentation by *Pleurotus ostreatus* strain EM-1 was investigated. At the end of spawn run, lignin, cellulose and hemicellulose content had decreased by 42.3, 5.6% and 41.0% respectively. No further reduction in lignin content occurred thereafter. In contrast, after 28 days, cellulose and hemicellulose had been degraded by 36.0% and 58.5% respectively. A biphasic protein profile, characterized by a 6-fold increase by day 14, followed by a dramatic decline was observed. The rate of release of reducing sugars from spent maize cobs during incubation with exogenous cellulase was 400% greater than that of untreated maize cobs. The present findings indicate that the positive effects of *P. ostreatus* strain EM-1 on the nutritive value of maize cob appear to be optimal after complete colonization by mycelia. At this stage, maximum biodegradation of lignin had occurred, protein content was markedly elevated and the reduction in cellulose content was negligible. Thus, solid state fermentation by *Pleurotus ostreatus* strain EM-1 may be an efficient means of transforming maize cob into nutritive animal feed.

Keywords: Oyster mushroom, delignification, animal feed, biodegradation, cellulose, hemicellulose.

INTRODUCTION

Maize cob, an abundant but underutilized bioresource in Ghana, is a potential solution to the country's perennial problem of inadequate dry season feed for livestock. Although it is rich in cellulose, a good source of energy for ruminants (Vizitier, 2004; Kuan and Liong, 2008; Israel et al., 2008), there are two major limitations to its use as animal feed. These are its extremely poor digestibility and low protein content. It has been reported that the growth of animals is adversely affected when dietary concentrations of maize cob meal exceed 10% (Ndubuisi et al. 2008). Owing to a strong association between lignin and cellulose, the rate of biological degradation of the cellulose fraction of lignocellulosic materials, including maize cob, is inversely proportional to the extent of lignification (Huttermann et al., 2000; Besombes and Mazeau, 2005). Although treatment with concentrated alkali or acid is reported to enhance the digestibility of maize cob by disrupting lignocellulosic bonds**,** the use of such chemicals by largely illiterate peasant farmers would pose

numerous safety and environmental challenges, would not be cost effective, and could be harmful to livestock.

If found to be effective, the biodegradation of lignin might be a non-toxic, affordable and environmentally-friendly alternative to the use of chemical methods (Perez et al., 2002; Dashtban et al., 2009). *Pleurotus ostreatus*, an edible mushroom, is capable of growing on a variety of lignocellulosic substrates because it produces extracellular ligninolytic enzyme complexes as well as cellulase (Elisashvili et al., 2008; Olfati and Peyvast, 2008; Moussa, 2009; Isikhuemhen and Mikiashvilli, 2009). The use of mushroom biotechnology for converting maize cobs into nutritive animal feed would only be feasible if delignification could be achieved while avoiding a considerable reduction in cellulose content. The purpose of the present study was to determine the suitability of *Pleurotus ostreatus* strain EM-1 as a tool for the delignification of maize cobs. Specifically, the study sought to determine the effect of solid state fermentation of maize cob by *P. ostreatus* strain EM-1 on the profiles of substrate biopolymers, and on the rate of *in vitro* cellulose degradation. The EM-1 strain is widely accepted and cultivated in commercial quantities in Ghana because of its high biological efficiency, shelf-life, taste and yield. It is cultivated all year round and readily adapts to changes in environmental conditions (Obodai and Vowotor, 2002).

MATERIALS AND METHODS

Maize cobs were obtained from farms in Jumapo and Accra in the Eastern and Greater Accra Regions of Ghana.

Spawn preparation

P. ostreatus (Jacq. ex. fr) Kummer strain EM-1, was maintained on potato dextrose agar slants and spawn was prepared on sorghum grains (Zadrazil, 1978). Both cultures and spawn were incubated in the laboratory at 26–28 ^{o}C and 60–65% relative humidity.

Substrate preparation

Dried maize cobs were shredded in a hammer mill and then ground in a disc attrition mill to a particle size of approximately 4mm. The milled maize cob was soaked in distilled water for 30 min, sun-dried until the moisture content was approximately 65-70%. This was determined by the squeeze test (Buswell, 1984). Samples of 250 g of maize cobs were put into heat-resistant, polypropylene bags. Each bag was closed with a plastic neck, steam-sterilized for 2.5 and inoculated with 5 g sorghum spawn.

Mushroom cultivation

The colonization of maize cobs by the mycelia was monitored till the end of spawn run (14 days). The bags were cut open to initiate the formation of pinheads. First flush of fruiting bodies were harvested on day 21, while second flush were harvested on day 28. The fruiting bodies were harvested by holding the base of the fruiting body and gently twisting the mushroom out of the substrate. In each study, samples of substrate were taken from six different bags on days 14, 21 and 28, dried at 70 ^{o}C to a constant weight, and stored in sealed bags at 4 ^{o}C. Three separate studies were conducted.

Analytical procedures

The detergent system of analysis was employed in determining the lignin, hemicellulose and cellulose content of the growth substrate before inoculation and 14, 21 and 28 days after inoculation (Van Soest and Robertson, 1980). Protein content was estimated using the Kjeldahl method (AOAC, 1990).

In vitro cellulose digestibility

The *in vitro* digestibility of cellulose present in untreated maize cobs or growth

substrate was determined using exogenous cellulase. Briefly, an aliquot of 10 mL potassium hydrogen phosphate buffer, pH 5.0 containing 0.05 U/mL cellulase was added to 0.2 g of untreated maize cobs or growth substrate, and incubated at 37 ^{o}C for 0.5 – 3 h. The reaction was terminated and deproteinized simultaneously by the addition of Carrez I (0.219 g zinc acetate dehydrate, 0.3 mL acetic acid) and Carrez II reagents (0.1 g potassium ferrocyanide trihydrate). The contents were filtered through Whatman No.1 filter paper and the amount of reducing sugar present was measured spectrophotometrically (Folin and Wu, 1919).

Statistical analysis

Analysis of variance (ANOVA) tests along with least significant difference (LSD) post-hoc comparisons were conducted using Excel Data Analysis Statistical Software (2007 version) and Statgraphics-plus Software Programme (Version 3.0). The level of significance was set to $p < 0.05$. Differences among means with $p<0.05$ were accepted as representing statistically significant differences.

RESULTS

Pleurotus ostreatus strain EM-1 was successfully cultivated on milled maize cob. The colonization of the maize cob substrate by mycelia was completed 14 days after inoculation, while first and second flush fruiting bodies were harvested on days 21 and 28 respectively. At each of these stages of cultivation, samples of growth substrate were analyzed for lignin, hemicellulose and cellulose to determine the extent to which these biopolymers were degraded by the extracellular enzymes. The lignin profile, depicted in Figure 1, showed that the extent of lignin degradation was 42.3% ($p < 0.05$) after complete colonization of the substrate by mycelia. In the present study, no significant reduction in lignin content was observed during the development of fruiting bodies (Figure 1).

Although the extent of hemicellulose degradation (41.0%) at the end of spawn run was similar to that observed for lignin (Figure 2), there was a difference in profile, as hemicellulose content continued to decrease during the development of fruiting bodies, culminating in a 58.5% reduction by day 28 ($p < 0.05$). The magnitude of cellulose degradation was only 5.6% at the end of spawn run (Figure 3). By the end of the 28 day period, the cellulose content had decreased by 36.0%.

In sharp contrast to the profiles of the lignocellulosic biopolymers, the protein content of the maize cob substrate exhibited a biphasic pattern (Figure 4). A dramatic 6-fold increase in protein content of the substrate was recorded after spawn run. This subsequently declined to a 2-fold increase (relative to untreated maize cobs) by the end of the 28 day period.

As shown in Figure 5, the amount of reducing sugars released from samples taken on days 14, 21 and 28 was similar - approximately 4-fold greater than that of untreated maize cobs ($p<0.05$).

DISCUSSION

Delignification and *in vitro* digestibility

The objective of the present study was to investigate whether *P. ostreatus* strain EM-1 would be a useful tool for the bioconversion of maize cob into a more nutritive animal feed ingredient.

Although the successful delignification of maize cob by *P. ostreatus* strain EM-1 was not unexpected, the extent of lignin depolymerization (42.3%) far surpassed values reported for other strains, suggesting

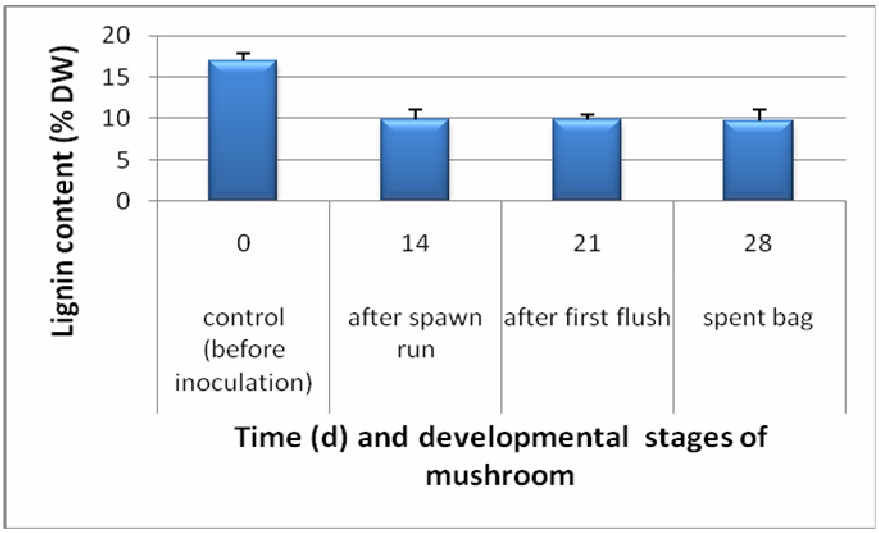

Figure 1: Lignin content of maize cobs during the growth of *Pleurotus ostreatus.* Lignin content of maize cobs at each developmental stage of the mushroom was determined by the detergent system of gravimetric analysis. A significant ($p<0.05$) reduction in lignin was observed after 14 days. Values in the figures are mean ± SEM of at least six determinations.

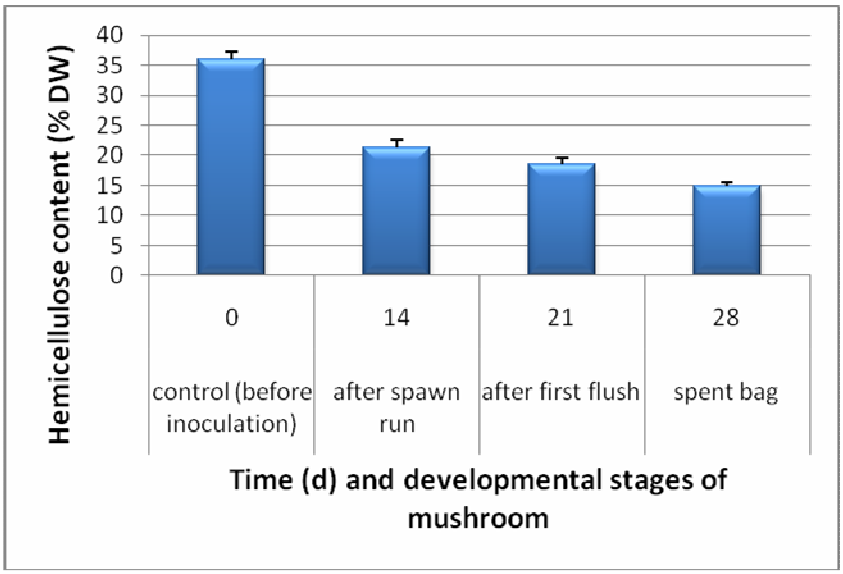

Figure 2: Hemicellulose content of maize cobs during the growth of *Pleurotus ostreatus*. Hemicellulose content of maize cobs at each developmental stage of the mushroom was determined by the detergent system of gravimetric analysis The reduction in hemicellulose content at each developmental stage was significant ($p<0.05$). Values in the figures are mean ± SEM of at least six determinations.

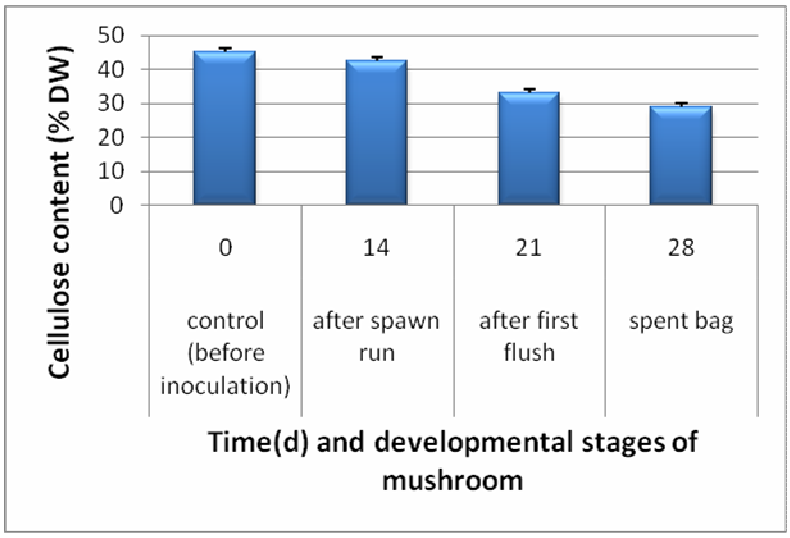

Figure 3: Cellulose content of maize cobs during the growth of *Pleurotus ostreatus.* Cellulose content of maize cobs at each developmental stage of the mushroom was determined by the detergent system of gravimetric analysis The reduction in cellulose content at each developmental stage was significant ($p<0.05$). Values in the figures are mean ± SEM of at least six determinations.

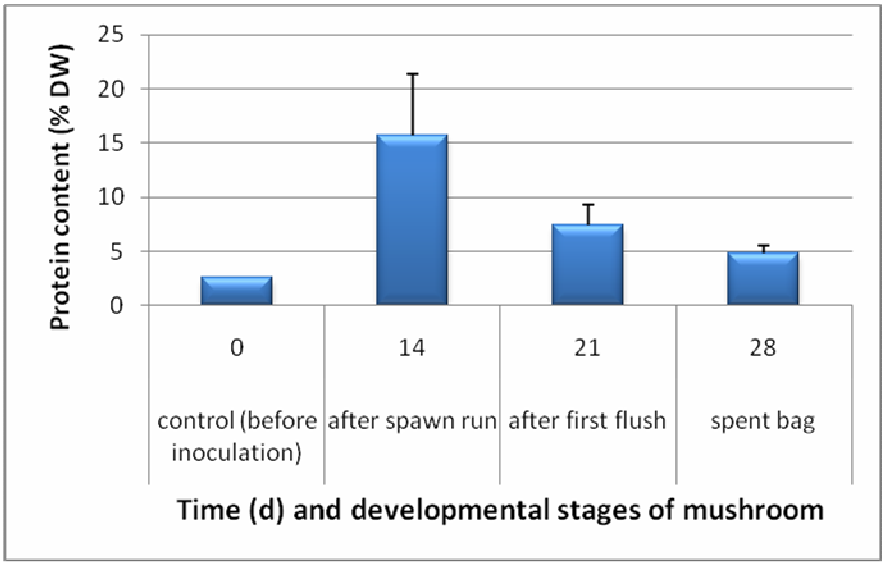

Figure 4: Protein content of maize cobs during the growth of *Pleurotus ostreatus.* Protein content of maize cobs at each developmental stage of the mushroom was determined using the Khedahl method. The changes in protein at each developmental stage was significant ($p<0.05$). Values in the figures 5 are mean ± SEM of at least six determinations.

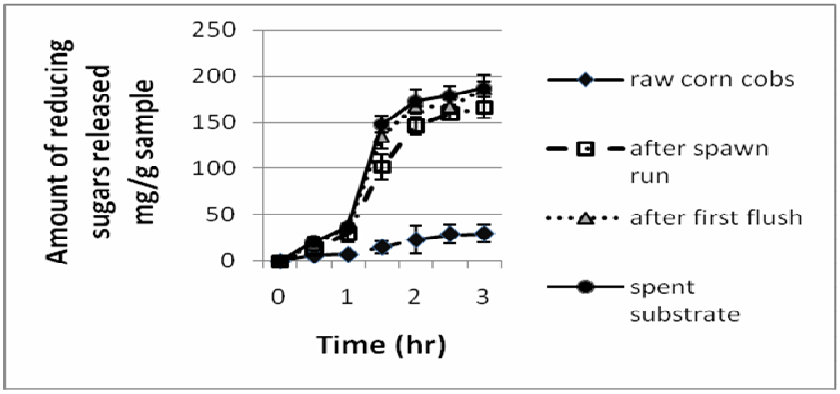

Figure 5: *In vitro* cellulose digestibility. *In vitro* digestibility of 0.2 g maize cobs was determined with 0.5U cellulase in 10ml phosphate buffer, pH 5.0. Reducing sugar determination was done by Folin Wu method using glucose as standard. The amount of reducing sugars released from spent maize cobs was significantly greater than that of untreated maize cobs ($p<0.05$). Values in the figures are mean ± SEM of three determinations.

that its ligninolytic enzymatic machinery is particularly efficient. For instance, after treatment of various lignocellulosic materials with *P. ostreatus*, the lignin content decreased by only 23-30% (Rani et al., 2008). Our findings are consistent with the well-documented ability of mycelia of *Pleurotus* species to synthesize hydrolytic and oxidative extracellular enzymes including laccase and manganese peroxidase (Olfati and Peyvast, 2008). The observation that no significant reduction in lignin content occurred during the development of fruiting bodies is in agreement with the findings of Elisashvili et al. (2008) who reported that laccase and manganese peroxidase activities of *P. ostreatus* strains were high during substrate colonization, but declined rapidly during fruiting body development. The massive (four-fold) improvement in the *in vitro* rate of enzymatic cellulolysis induced by *P. ostreatus* strain EM-1 is attributable to the fact that the degradation of lignin caused cellulase to gain greater access to its substrate. The present findings are consistent with those of Jafari et al. (2007) who reported that four *Pleurotus* species, including *P. ostreatus*, increased the *in vitro* dry matter and organic matter digestibility of rice straw.

Cellulose/hemicellulose profiles during fermentation

The feasibility of the application of *P. ostreatus* strain EM-1 biotechnology to animal feed production in Ghana depends not only on improved cellulose degradability, but also, to a large extent, on the degree to which polysaccharides are depleted during treatment of maize cobs with *P. ostreatus* strain EM-1. It was, therefore, of interest to determine the profiles of non-starch polysaccharides, particularly cellulose. The 5.6% reduction in cellulose content by the completion of spawn run is almost negligible, and indicates that the use of this fungus for the bioconversion of maize cob is highly desirable (Figure 3). It is important to note that spent maize cob contained approximately 64% cellulose. Thus, in terms of both cellulose degradability and cellulose content, *P. ostreatus* strain EM-1-treated maize cob should be suitable for use as an animal feed constituent.

The observed profiles of the components of lignocellulose gave an indication of the patterns of enzyme secretion by *P. ostreatus* EM-1. Cellulolytic enzyme production appears to have been minimal during mycelia colonization and higher during mushroom fruiting, while ligninolytic and

hemicellulolytic activities were higher before the development of fruiting bodies. These results are in agreement with those of Isikhuemhen and Mikiashvilli (2009) on laccase, peroxidase, and carboxymethyl-cellulase activities during cultivation of *P. ostreatus* on anaerobic digester solids. However, the present findings are at variance with the low activity of xylanase observed by Elisashvili et al. (2008) during substrate colonization by *P. ostreatus*. Differences in the patterns of enzyme secretion by different strains may account for this.

Protein profile during fermentation

The protein value of 15% recorded after treatment with *P. ostreatus* strain Em-1 far exceeds the 2.4-3.4% range for untreated maize cob (Adeyemi and Familade, 2003), and is much higher than values reported by Akinfemi et al. (2009) following treatment of maize cob with *Pleurotus pulmonarius* (10.05%) and *Pleurotus sajor caju* (10.37%). It is important to note that this protein value satisfies the 7-12% maintenance requirement for ruminants (National Research Council, 2000). Thus, consumption of *P. ostreatus* strain Em-1-treated maize cob would ensure that both energy and protein requirements of livestock are met. Ideally, for purposes of upgrading maize cob, the cultivation of *P. ostreatus* strain EM-1 should be terminated when mycelia colonization has been completed because, at this stage, protein content is high, the hydrolysis of cellulose is negligible, and maximal lignin depolymerization has been achieved.

In conclusion, the present study has amply demonstrated, for the first time, that *P. ostreatus* strain EM-1 is an efficient decomposer of the lignin fraction of maize cob. Furthermore, it retains considerable amounts of cellulose in the growth substrate, making it an ideal biological tool for purposes of upgrading of maize cob. Treatment of maize cob with this fungus has enormous potential to benefit both maize and livestock farmers in Ghana. The vast quantities of maize cob generated and discarded in the country annually, usually through burning, can be transformed into nutritive animal feed using this biotechnology.

REFERENCES

Adeyemi OA, Familade FO. 2003. Replacement of maize by rumen filtrate fermented corn-cob in layer diets. *Bioresource Technol*., **90**: 221-224.

Akinfemi A, Adu OA, Doherty F. 2009. Assessment of the nutritive value of fungi treated maize cob using *in vitro* gas production technique. *Livestock Research for Rural Development. 21, Article # 188.* Retrieved November 28, 2009, from http://www.lrrd.org/lrrd21/11/akin21188.htm

AOAC. 1990. Protein determination in animal feed: copper catalyst Kjeldahl method. In *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists. 984.13.

Besombes S, Mazeau K. 2005. The cellulose/lignin assembly assessed by molecular modeling. Part 1: adsorption of a threo guaiacyl β-O-4 dimer onto a Iβ cellulose whisker. *Plant Physiol. Biochem.*, **43**: 299-308.

Buswell JA. 1984. Potentials of spent mushroom substrate for bioremediation purposes. *Compost*, **2**: 31-35.

Dashtban M, Schraft H, Qin W. 2009. Fungal bioconversion of lignocellulosic residues; opportunities and perspectives. *Int. J. Biol. Sci.*, **5**: 578-595.

Elisashvili V, Kachlishvili E, Penninckx MJ. 2008. Lignocellulolytic enzymes profile during growth and fruiting of *Pleurotus ostreatus* on wheat straw and tree leaves. *Acta Microbiol. Immunol. Hung*., **55**: 157-168.

Folin O, Wu H. 1919. A system of blood analysis. *J. Biol. Chem*., **38**: 81-110.

Huttermann A, Majcherczyk A, Braun-Lullemann A, Mai C, Fastenrath M, Kharazipour A, Huttermann J, Huttermann AH. 2000. Enzymatic activation of lignin leads to an unexpected copolymerization with carbohydrates. *Naturwissenschaften,* **87**: 539-541.

Israel AU, Obot IB, Umoren SA, Mkpenie V, Asuquo JE. 2008. Production of Cellulosic Polymers from Agricultural Wastes. *E-J. Chem.*, **5**: 81-85.

Isikhuemhen OS, Mikiashvilli NA. 2009 Lignocellulolytic enzyme activity, substrate utilization, and mushroom yield by *Pleurotus ostreatus* cultivated on substrate containing anaerobic digester solids. *J. Ind. Microbiol. Biotechnol.*, **36**: 1353-1362.

Jafari MA, Nikkhah A, Sadeghi AA, Chamani M. 2007. The effect of *Pleurotus* spp. fungi on chemical composition and *in vitro* digestibility of rice straw. *Pak. J. Biol. Sci*., **10**: 2460-2464.

Kuan YH, Liong MT. 2008. Chemical and physicochemical characterization of agrowaste fibrous materials and residues. *J. Agric. Food Chem.*, **56**: 9252-9257.

Moussa TA. 2009. Molecular characterization of the phenol oxidase (pox2) gene from the ligninolytic fungus *Pleurotus ostreatus*. *FEMS Microbiol Lett.*, **298**: 131-142.

National Research Council (NRC). 2000. *Nutrient Requirements of Beef Cattle* (7th edn). Washington, D.C.: National Academy Press; 243.

Ndubuisi EC, Iheukwumere FC, Onyekwere MU. 2008. The effect of varying dietary levels of maize cob meal on the growth and nutrient digestibility of grower pigs. *Res. J. Anim. Sci*., **2**: 100-102.

Obodai M, Vowotor KA. 2002. Performance of different strains of *Pleurotus* species under Ghanaian conditions. *J. Food Technol. Afr.*, **7**: 98-100.

Olfati JA, Peyvast GH. 2008. Lawn Clippings for Cultivation of Oyster Mushroom. *Int. J. Veg. Sci.*, **14**: 98-103.

Perez J, Munoz-Dorado J, Rubia T, Martinez J. 2002. Biodegradation and biological treatments of cellulose, hemicelluloses and lignin: An overview. *Int. Microbiol*., **5**: 53-63.

Rani P, Kalyani, N, Prathiba, K. 2008. Evaluation of lignocellulosic wastes for production of edible mushroom. *Appl. Biochem. Biotechnol*., **151**: 151-159.

Van Soest PJ, Robertson JB. 1980. Systems of analysis for evaluating fibrous feeds. In: Standardization of Analytical Methodology in Feeds, Pigden WJ, Balch CC, Graham M (eds). International Research Development Center: Ottawa, Canada; 49-60.

Vizitier G. 2004. Oyster mushroom cultivation. In *Mushroom Growers Handbook*. Agarikan Press: Romania; 50-57.

Zadrazil F. 1978. Cultivation of *Pleurotus*. In *The Biology and Cultivation of Edible Mushroom*, Change ST, Hayes WA. (eds). Academic Press: New York; 512-558.