Proceedings of the 16th International Mushroom Congress on the Science and Cultivation of Edible and Medicinal Fungi.

Held on 14th – 17th March, 2004, Miami, Florida.

Bioconversion of Some Agro-processing Waste through Pleurotus Production.

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ABSTRACT

Agro-processing waste materials (cocoa husk, rice husk, rice bran, corn cobs, oil palm fiber, kernel cake and spent pito malt) were formulated into five different media. These materials were pre-treated by milling and wetting before being used to used to formulate substrates for the cultivation of oyster mushrooms (*Pleurotus ostreatus* and *P. eous*). Growth characteristics and yield from the various formulations were compared to that from a commercially prepared substrate composed of composted sawdust. Both species displayed a higher rate of colonization and yield on corncob-based formulations than on cocoa- and rice husk-based media. The biodegradation characteristics of cellulose, hemicellulose and lignin by these two fungal species and their correlation to yield were determined throughout the growing cycle. Water content, C/N ratio, pH and protein content was monitored at spawning, after spawn run, after the first flush and at the completion of cropping for each substrate. *Pleurtous eous* produced higher yields than *P. ostreatus* for all media tested.

INTRODUCTION

Lignocelluloses are the most abundant materials present on earth, comprising 50% of all biomass with an estimated annual production of 5 x 10^{10} tonnes (Goldstein 1981). It also has been estimated that about one- half the total production of plant residues from agriculture and industrial processes remains unused and burdens the environment (Zadrazil & Grabbe 1983). Chang (1989) noted that all agricultural production for plant crops generated enormous waste, because little of each crop was actually used; typically 80-90% of the total biomass of agricultural production is discarded as waste. This is due to the fact that only part of the organic matter synthesized through photosynthesis every year is directly edible in the form of fruits, vegetables and food grains and assumes various forms, such as inedible sugarcane bagasse and corn cobs (Savalgi & Kaulkarnis 2001). The handling and disposal of these lignocellusic residues are often problematic due to their chemical structure and decomposition properties (Philippoussis et al. 2001). With the advent of biotechnology, attempts have been made globally to make potential use of agro-industrial residues for added value by production of enzymes, organic acids, bioactive secondary metabolites, single cell protein, etc. (Pandey et al. 1988, 1999a, b). Solid-state fermentation (cultivation) is promising in this regard (Pandey 1992a, 1994; Soccol & Krieger 1998; Pandey et al. 2000).

According to Smith (1993), mushroom cultivation represents the only current economically profitable biotechnology process for the conversion of plant residues (lignocelluloses) from forestry and agriculture. Further, it is the only microbiological system that can bio-convert all of the major plant polymers; lignin, cellulose and hemicellulose (Wood & Smith 1987). Other microbial treatment systems, natural or manmade, utilizing lignocelluloses, such as waste digesters or the feeding of ruminant farm animals, do not utilize lignin. Mushroom cultivation exploits the natural ability of fungi to bio-convert solid waste generated by industry and agriculture into food (Martinez et al. 1991; Tripothi & Yadar 1992; Chiu et al. 2000).

Two mushroom species, *P. ostreatus* and *P. eous*, are typically cultivated commercially on substrates composed of sawdust (Obodai 1992; Sawyer 1994). Other agro-processing waste (e.g., cocoa husk and corn cobs) has shown potential for *Pleurotus* cultivation (Obodai 1992; Darker 1997; Aborbar 1998). In the present study, we evaluated six agro-processing waste materials: corn cobs, cocoa husks, rice husk, rice bran, oil palm fiber, kernel cake and spent pito malt (i.e., spent grain after brewing of pito, an alcoholic beverage produced from malted sorghum) for their suitability as substrates for the production of *Pleurotus* mushrooms

MATERIALS AND METHODS

Cultures

Pleurotus ostreatus strain EM_1 and *P. eous* strain OT_3 were obtained from the Mycology unit of The Food Research Institute (FRI) of the Council for Scientific and Industrial Research (CSIR), Ghana.

Analytical procedures

Composition of formulated media at various stages of growth (spawning, after spawn run, after the first flush and at the end of cropping) and the proximate composition of mushrooms harvested from each formulated media were determined. Samples for the determinations (except for moisture and pH) were oven-dried overnight at 70°C and then ground with a hammer mill. Analyses were done in duplicate and the means and standard deviations noted. Proximate analysis was determined according to procedures described in the AOAC (1994). Organic carbon was determined as described by Black and Walker (1984). Cellulose, hemicellulose and lignin were determined according to the procedures of Van Soest and Robertson (1985).

Pre-treatment of substrates

Cocoa husks were obtained with sizes ranging between 0.5-1 cm^2. The samples were soaked for 10 min and the excess water drained. Corncobs were shredded in a crushing mill and then ground in a disc attrition mill to obtain particle sizes of up to 0.4 cm. These were soaked for 30 min and then drained.

Media formulations

Media were formulated by blending pretreated samples based on their predetermined chemical characteristics (Table 1) (Youri 2003). The availability of the waste materials also was considered so that the medium could be produced on a commercial scale. All ingredients were thoroughly mixed and moistened (Oei 1992) to give a uniform mixture of substrates.

Yield and statistical analysis

Yield was measured as total number and weight of mushrooms per bag. An analysis of variance (ANOVA) was performed and Duncan's Multiple Range Test (DMRT) was used to separate treatment means.

Table 1. Media formulations for six substrates used to produce *Pleurotus ostreatus* and *P. eous*

Formula	Substrate	Ratio
A	Cocoa husk:rice bran	90:10
B	Cocoa husk:oil palm	90:10
Di	Oil palm fiber:rice husk:kernel cake	20:60:20
I	Corncob:rice bran	90:10
J	Corncob:pito mash	90:10
K (Control)	Composted sawdust:rice bran	90:10

RESULTS AND DISCUSSION

Moisture

For all formulated substrates, moisture content increased after the spawn run, decreased after the first flush and was lowest in the spent substrate (Fig. 1). This trend was attributed to the presence of abundant mycelia in the media as a result of the colonization process. According to Flegg (2001), mushroom tissue is *ca.* 90% water. The progressive drying out of the substrate results from the release of water during fungal metabolism (Rajaratham & Bano 1989), evaporation and absorption by the mushroom (Flegg 2001).

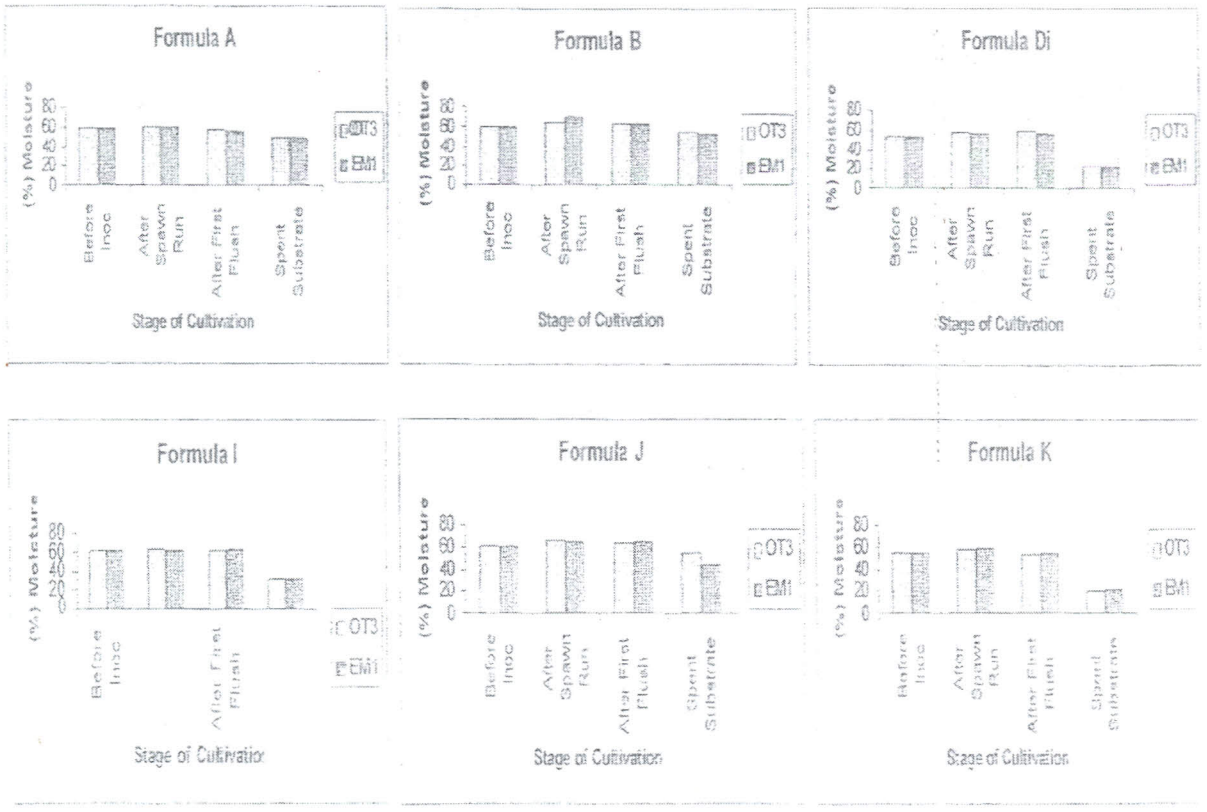

Figure 1. Moisture content of six formulated media at different stages of cultivation.

pH

The pH profiles during cultivation are presented in Fig 2. The pH decreased after the spawn run and increased after the first flush and in the exhausted media. pH drift according to Bridson and Brecker (1972), is not uncommon in solid culture. This initial drop, according to Gray (1967), is due to the fact that the decomposition products of the easily attacked components of the substrates are simple acids, and as the degradation continues the media becomes alkaline. In all, the initial pH varied from 5.1 to 7.4. This was within the optimal pH range for the growth of *Pleurotus* spp. (pH 5.5-7) as reported by Kurtzman and Zadrazil (1982).

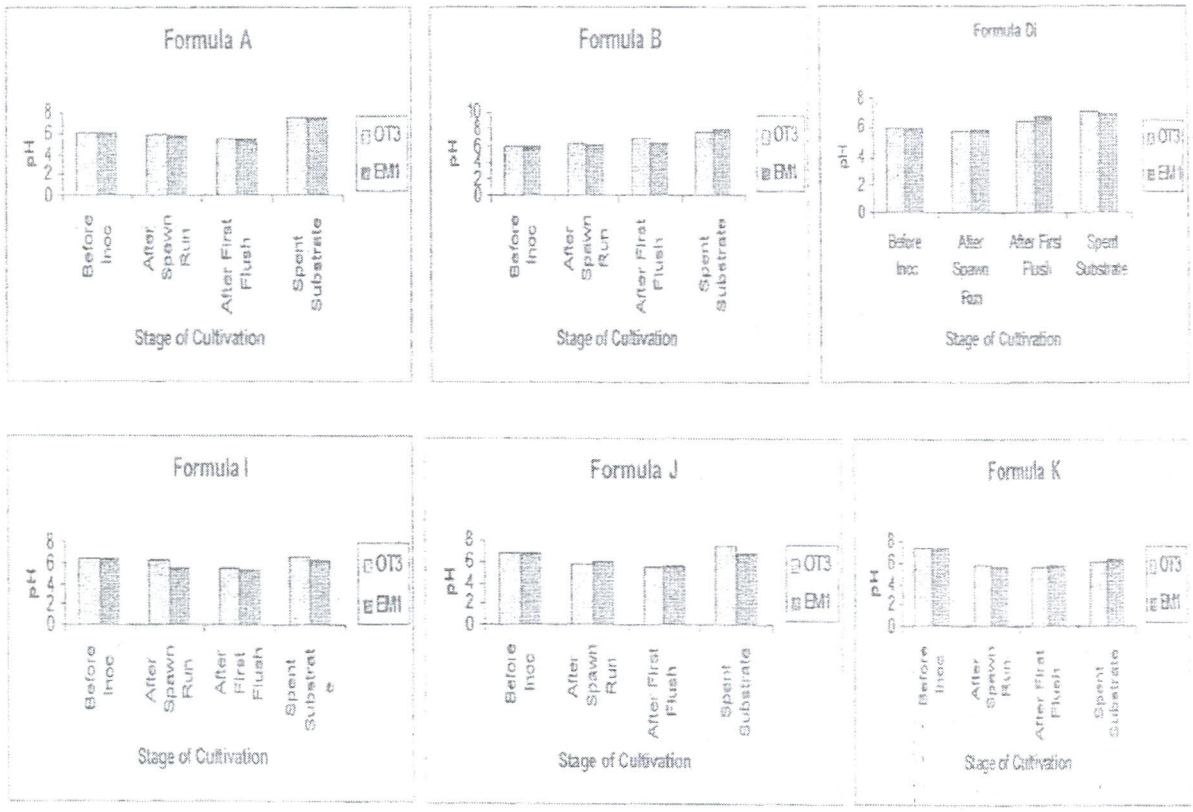

Figure 2. pH of media at different stages of cultivation.

C/N ratio

The C/N ratio (Fig. 3) decreased after the spawn run, increased thereafter and peaked in the spent substrate (but not as high as in the original medium). The presence of mycelial tissue in the media after the spawn run increased the nitrogen content. The carbon content was generally higher compared to the non-inoculated media, but the C/N ratio overall decreased dramatically. However, with fructification and subsequent flushes, the C/N ratio increased as more nitrogen than carbon was used by the fungi (Oei 1992). At the end of the cropping cycle, the C/N was higher, but not as high as in the original media.

Protein

As shown in Fig. 4, the changes in protein content were quite different from that of moisture, pH and C/N. After the first flush and in subsequent flushes, the protein content in the substrates gradually

decreased due to the transfer of nitrogen from the substrate to the fruiting bodies (Xiujin et al. 2001). In the end, however, the protein content of the spent substrate was higher than the initial values for all media. This increase could be attributed to the fact that more carbohydrates than protein were utilized during cultivation (Leifa 2001). These modifications according to Leifa (2001) were due to the total wt. loss during solid-state fermentation, degradation of lignocelluloses and the liberation of carbon dioxide. The largest increase after the spawn run was noted in formula I for EM1, which increased from 3.9 to 25.7% and the lowest in B (7.6 to 16.8%). Formula A colonized by OT_3 had the highest protein content (9.4%) in the spent substrate, while the lowest value (5.3%) was observed for Di, also colonized by OT_3.

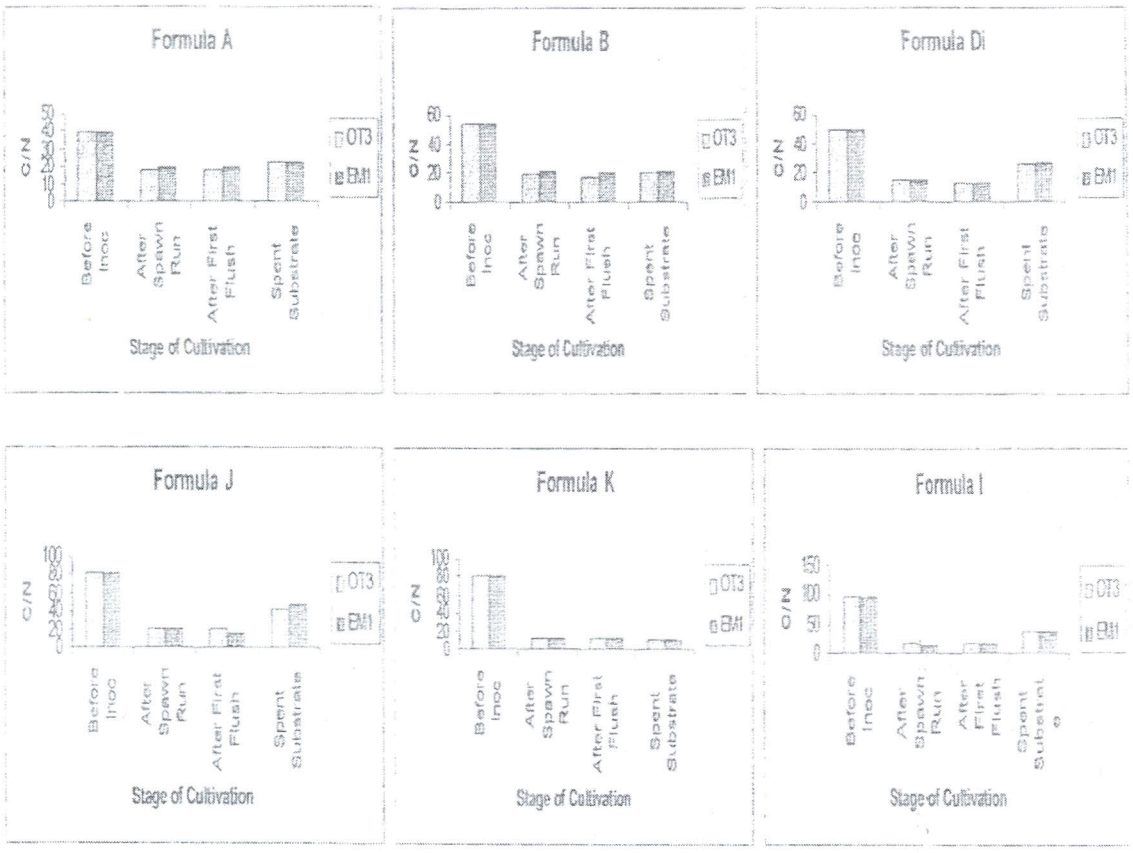

Figure 3. C/N ratio of media at different stages of cultivation.

Lignocellulose degradation

Degradation and utilization of cellulose, hemicellulose and lignin greatly affects the growth of the *Pleurotus* spp. and the feed value of the spent substrate (Xiujin et al. 2001). Generally these components are continuously degraded. However, the profile for each component was different, reflecting their different degradation characteristics (Xiujin et al. 2001).

Fig. 5 depicts the cellulose degradation ability of *Pleurotus* spp. for the various substrate formulations. The degradation pattern indicated that the rate of cellulose breakdown or utilization was greater after the first flush compared to spawn run and the spent media. This is because the first flush is the most productive and hence, utilizes the most cellulose (Anderson 2001). Xiujin et al. (2001) reported that ca. 75% of the total mushroom yield was obtained at first flush for *P. pulmonarius* on compressed hull substrate.

Datta and Chakravarty (2000) observed a similar pattern for the degradation of paddy straw during the cultivation of *Tricholoma lobayense*.

The least cellulose-degraded substrate was the rice husk-based formulation (Di) in which the cellulose content was reduced by 13.7% for OT_3 and 12.8% for EM_1. The most degraded substrate was the corn formulation (I) in which the cellulose content was reduced by 33.4% for OT_3 and 29.4% for EM_1. In general, it was noted that the degradative ability of OT_3 was higher than EM_1 and this was correlated to

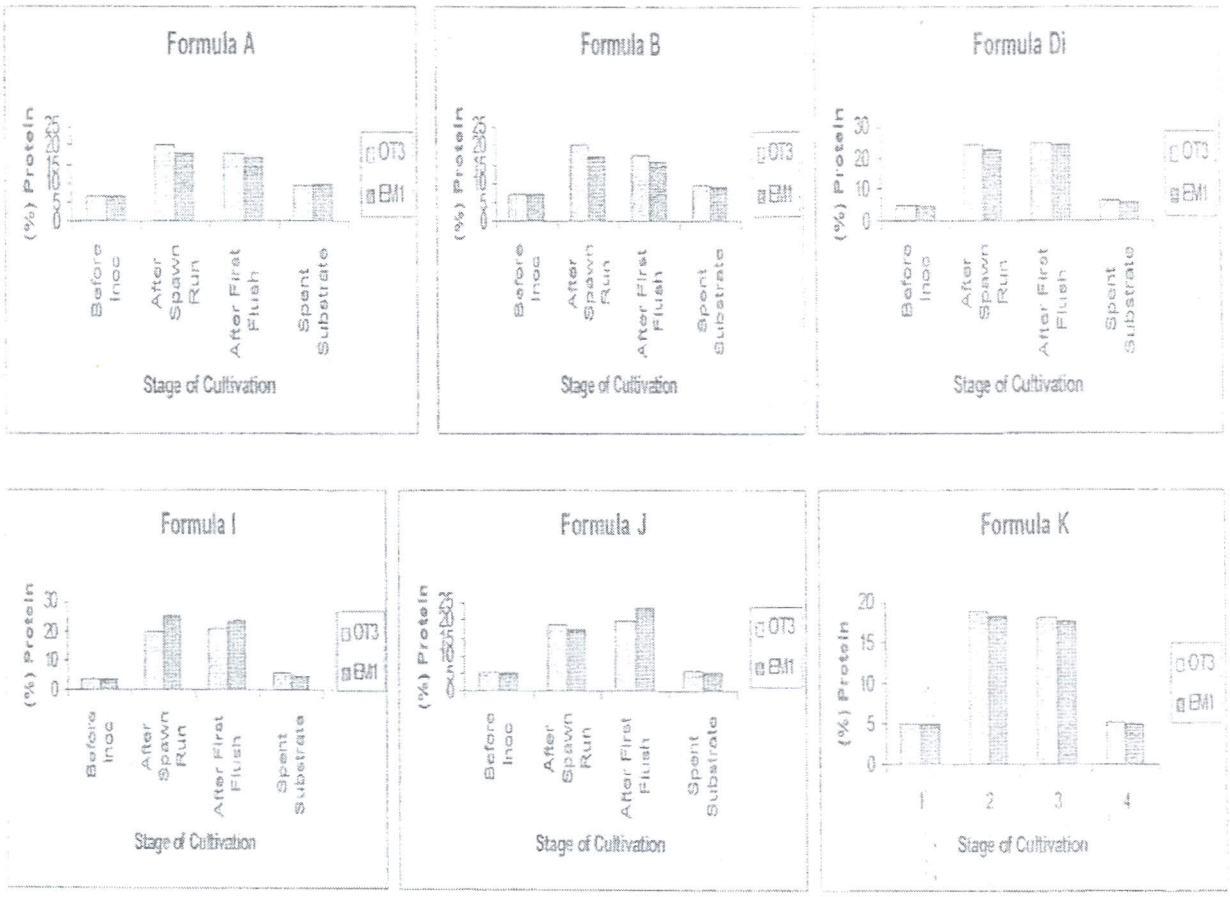

Figure 5. Protein content of media at different stages of cultivation.

the yield profile. The ability to degrade cellulose has been attributed to the synergistic action of three types of hydrolases, collectively called cellulases (Datta & Chakravarty 2001).

Hemicellulose

Fig. 6 represents the hemicellulose degradation profile by the mushroom species for the various media. The profile was similar to that of cellulose except that during the spawn run the degradation rate for hemicellulose was higher. Xiujin et al. (2001) observed a similar trend during the cultivation of *P. ostreatus* on cottonseed hull substrate. They concluded that this was probably due to the fact that hemicellulose is more easily degraded than cellulose and lignin and so more rapidly assimilated. The least degraded substrate was the whole rice husk-based substrate, Di. Here, the hemicellulose content was reduced by 36.8% for OT_3 and 35.7% for EM_1. The most degraded substrate was the cocoa husk-based substrate (B) in which the hemicellulose content was reduced by 74.5% for OT_3 and 73.8% for EM1.

Pleurotus spp. produce enzymes that will hydrolyze a variety of 2-(1-4) linked glucan substrates as well as various glycosides (Algren & Eriksson, 1967; Kelleck et al. 1969; Highley, 1975).

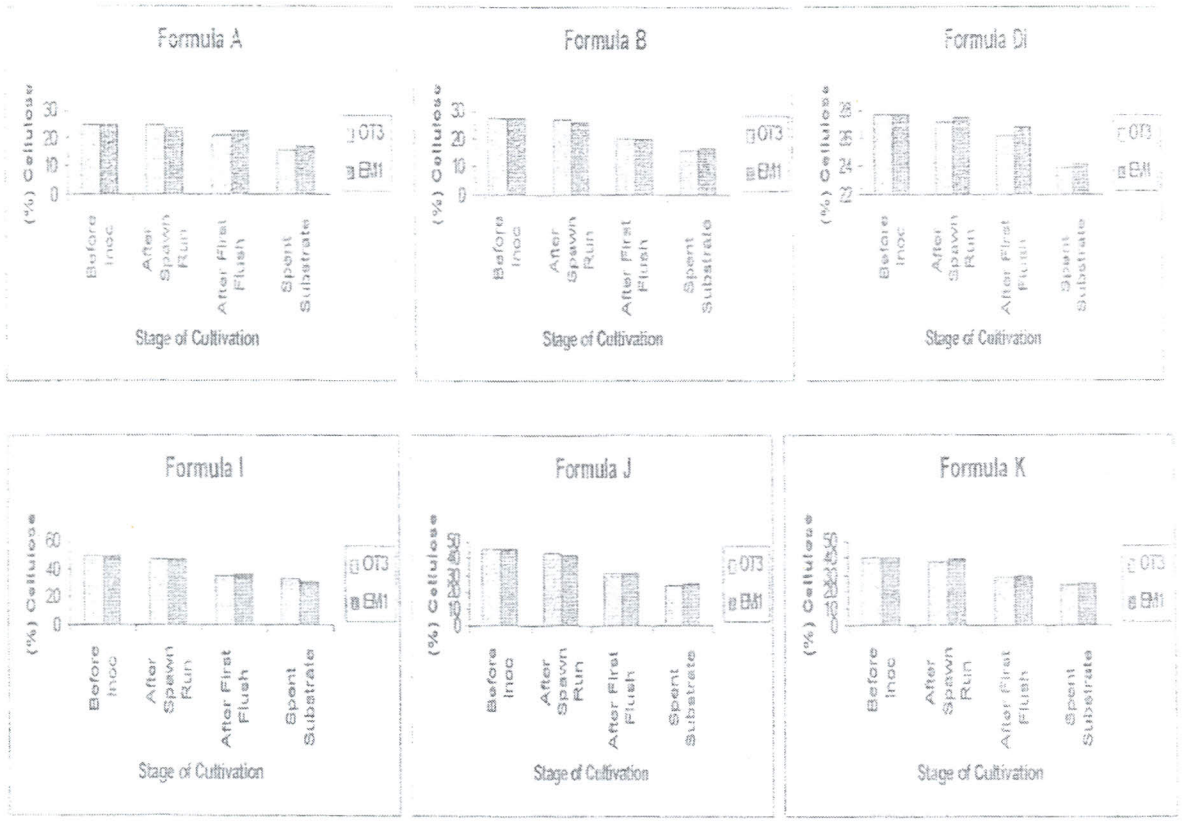

Figure 6. Cellulose degradation of various media.

Lignin

Fig. 7 indicates the lignin degradation of the *Pleurotus* spp. for the various substrates. The most extensive degradation of lignin occurred after the spawn run, implying that most of it was degraded during the colonization period. Little degradation was observed thereafter. These pattern was similar to that observed by Xiujin et al. (2001). Platt and Hader (1983) also observed a similar trend in lignin degradation and noted that during incubation, *P. ostreatus* mycelia had a greater capacity to digest lignin, and that the degradation of lignin played an important role in mycelial development. This degradation ability was diminished when primodia began to develop into fruiting bodies. Lo et al. (2001) explained that the lignin moiety of lignocelluloses can act as a barrier to cellulose and hemicellulose degradation, and thereby restricts the availability of nutrients required for fungal growth. Therefore, the degradation of lignin during the spawn run period serves to increase the availability of cellulose (Datta & Chakravarty 2001). The lignin content of whole rice husk substrate, Di, was least degraded, being reduced by 35.3% for OT_3, and 34.7% for EM_1 by the end of cultivation. The highest rate degradation was exhibited in corn cob-based media I, where the lignin content was reduced by 71.5% for OT_3 and 71.1% for EM_1.

Degradation of lignin is accomplished by the production of extracellular enzymes that oxidize both the aromatic rings and the aliphatic side chains to produce low-molecular weight products that can be absorbed by the fungus (Garaway and Evans 1984). Phenol oxidizing enzymes, such as laccase, also are

[illegible] in this respect and are produced in quantity by white rot fungi (Kirk 1977; Datta & Chakravarty [illegible])

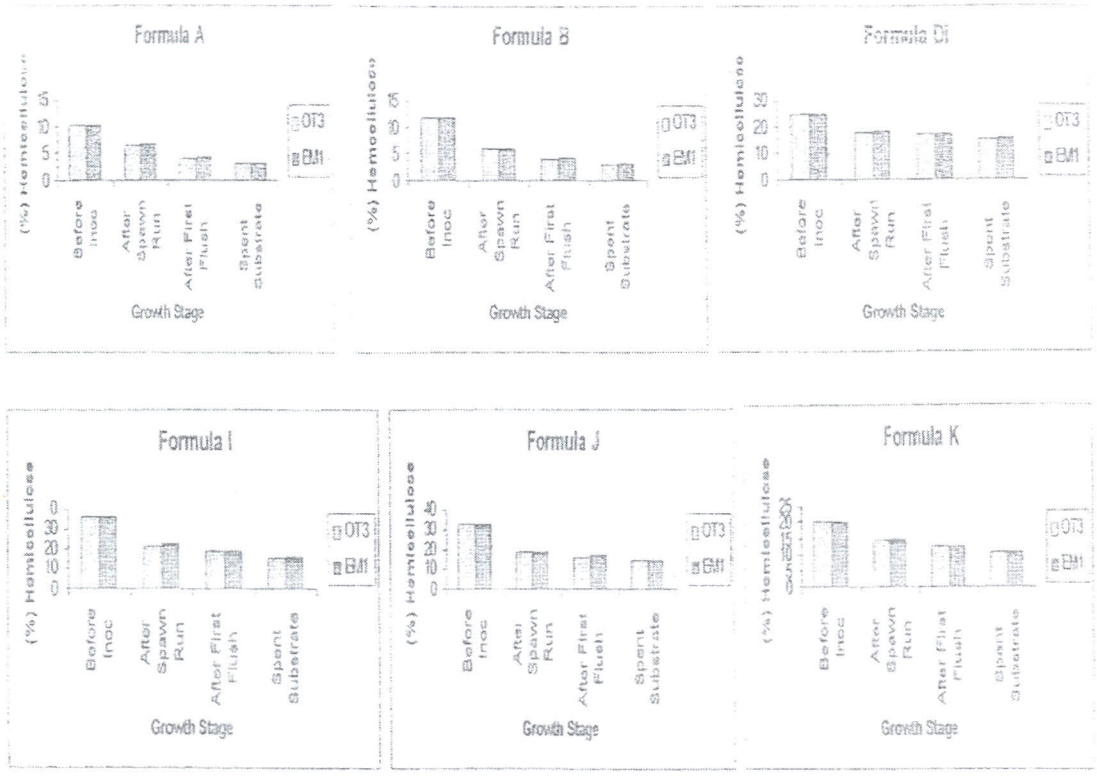

Figure 7. Hemicellulose degradation of various media.

Patterns of fruiting and productivity evaluation

The ave. numbers of fruiting bodies harvested from each bag per flush and total number per bag a shown in Table 1. The ave. number of fruiting bodies for EM_1 and OT_3 was lowest on medium I recording a total of 21 and 12 fruiting bodies, respectively. The highest number of fruiting bodies f EM_1 was recorded on medium I with an ave. of 50 and for OT_3 on media A with 45. Generally, the low the number of fruiting bodies, the higher was the mean wt.

Fruiting bodies appear in breaks or cycles, also called flushes (Wood & Smith 1987). The ave. snumb of flushes ranged from three in media B and J to five in medium Di. In general, the number of flust was positively correlated with yield.

The shortest interval between flushes was observed in medium A, with 7 days for EM_1 and 8 days OT_3. The longest interval was recorded for medium J, with 14 days for EM_1 and 18 days for OT_3 general, a shorter cropping cycle reduces the turnaround time and, hence, more production cycles can realized. Furthermore, according the Stamets (1992), a rapid turnaround reduces the risk contamination.

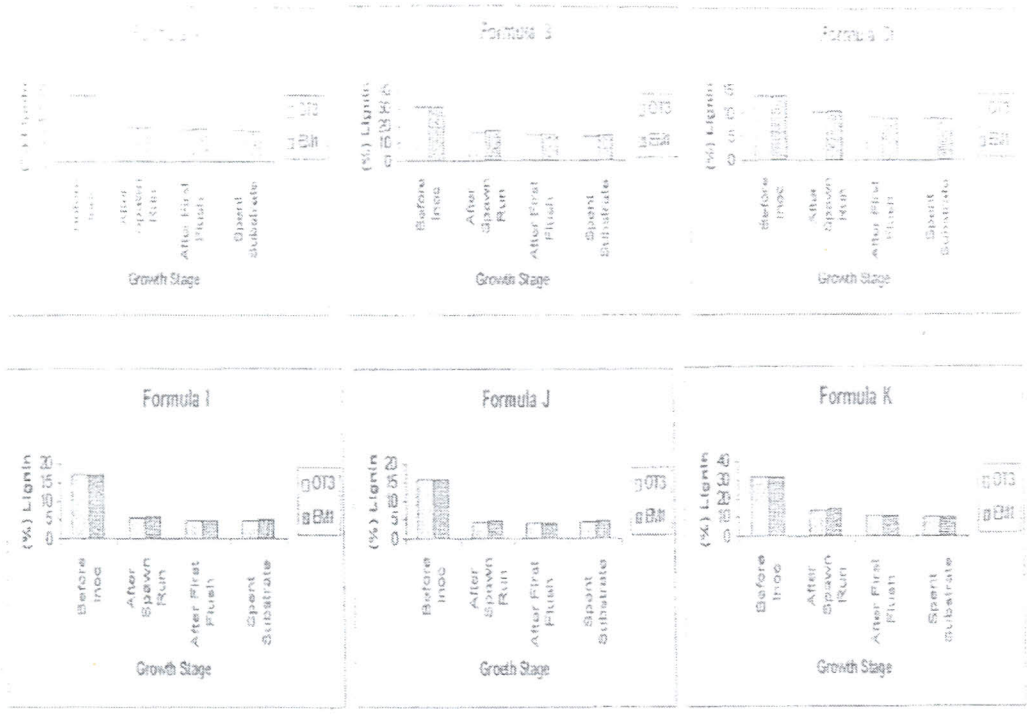

Figure 8. Lignin degradation of various media.

Table 2. Patterns of fruiting and yield

Medium	Strain	No. of Fruiting Bodies/Flush	Total No. of Fruiting Bodies	No. of Flushes	Interval Between Flushes (days)	(%) Yield
A	EM_1	17	48	4	7	20.7
	OT_3	15	44	4	8	28.9
B	EM_1	13	40	3	10	11.1
	OT_3	11	31	3	10	14.7
Di	EM_1	7	40	5	10	15.7
	OT_3	6	32	5	10	18.8
I	EM_1	10	50	5	9	33.9
	OT_3	8	39	5	9	53.2
J	EM_1	8	32	3	14	30.9
	OT_3	4	21	4	18	35.7
K	EM_1	6	24	4	14	22.5
	OT_3	4	19	4	16	30.1

In closing, corncobs supplemented with rice bran or pito mash produced higher yields than the commercial sawdust substrate supplemented with rice bran. Yield from cocoa husk-based substrate (A) was comparable to the control substrate. A rice husk-based medium provided lower yields, but with further optimization, the yield might be increased. Using these agro-processing wastes, alternative media for commercial production of mushrooms appeared feasible. These wastes could be used for the production of edible mushrooms, while simultaneously reducing the amount of raw materials destined for disposal.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Dr. Jeff F. Smith, Treasurer and Information Officer of the ISMS, for his constant encouragement and valuable contributions.

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