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Cultivation of *Pleurotus* spp. on a combination of anaerobically digested plant material and various agro-residues

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ABSTRACT

Two species of *Pleurotus*, *Pleurotus florida* and *Pleurotus flabellatus* were cultivated on two agro-residues (paddy straw; PS and coir pith; CP) singly as well as in combination with biogas digester residue (BDR, main feed leaf biomass). The biological efficiency, nutritional value, composition and nutrient balance (C, N and P) achieved with these substrates were studied. The most suitable substrate that produced higher yields and biological efficiency was PS mixed with BDR followed by coir pith with BDR. Addition of BDR with agro-residues could increase mushroom yield by 20–30%. The biological efficiency achieved was high for PS + BDR (231.93% for *P. florida* and 209.92% for *P. flabellatus*) and for CP + BDR (148.31% for *P. florida* and 188.46% for *P. flabellatus*). The OC (organic carbon), TKN (nitrogen) and TP (phosphate) removal of the *Pleurotus* spp. under investigation suggests that PS with BDR is the best substrate for growing mushroom.

Introduction

Pleurotus spp. is the most extensively cultivated mushroom totaling a production of 9 Mt/year. China alone produces 8 Mt/year. Such extensive cultivation is because of its flexible range of requirements of temperature and other environmental conditions (Swaminathan et al., 1995). These as primary wood rot fungi are able to colonize various types of agricultural waste as substrates. Generally *Pleurotus* spp. are cultivated on single substrates such as paddy or wheat straws. Various groups around the world have tried to utilize other agro-residues such as maize stover, coir pith, ground nut husk, sugarcane bagasse and mixes of these wastes as substrates (Bisaria et al., 1987; Chang et al., 1981; Ragunathan et al., 1996; Ramamoorthy et al., 1999; Ragunathan and Swaminathan, 2003; Sangwan and Saini, 1995).

Production and cultivation of mushroom are major industries in many countries where raw materials and the preparation of selective compost for mushroom production constitute the major cost inputs (Royse and Sanchez, 2008; Van Roestel, 1988; Wuest, 1983). Therefore, growers seek new ways to lower production costs by increasing bio-efficiency, i.e., producing greater mushroom yield from lesser raw materials (Royse, 2010). Though India's present share in the world production and trade of oyster mushroom is meager, being only an estimated 2000 t, the potential for the future is rated as high for a variety of reasons. Large quantities of various primary substrates such as wheat straw, paddy straw, bagasse, chicken manure, tea waste, and de-oiled cakes are easily available all over India and can therefore be most suitable and inexpensive substrates for expanding future cultivation. Agro-residue production in India estimated at around 1200 Mt (550 Mt surplus) out of which paddy straw is 217 Mt and coir pith is 6.9 Mt (Chanakya and Malayil, 2012a;). Even though paddy straw has a variety of uses such as animal fodder, animal bedding, domestic fuel, raw material for paper and board making or building material, there is still a surplus in India. Coir pith on the other had has very few uses, mainly used as compost, and can be used as a potential feedstock for cultivation of mushroom. Coir pith is difficult to compost under normal conditions and requires a very long time. During the rainy season, the tannins and phenols of the coir pith are leached out into the soil and into the irrigation canals, thereby causing water pollution. Their conversion to mushroom has the potential to avoid this environmental problem.

Anaerobic digestion of plant biomass in a plug-flow reactor (PFR, Chanakya and Malayil, 2012a, 2012b) gives two by-products — digested plant material (henceforth called biogas digester residue, BDR) and biogas digester liquid (BDL) along with biogas — the main output. Both the digester liquid and the digested biomass are rich in nutrients like N, P, K and trace elements (Chanakya and Malayil, 2013). The digested biomass is commonly used as fertilizer and the digester liquid as pest repellent (Rivard et al., 1995; Tiwari et al., 2000; Yamamoto et al., 2006). The anaerobically digested biomass contains 25% more accessible ammonium (NH_4^+–N) than untreated liquid manure (Monnet, 2003). However, when its is applied directly to soil as fertilizer, it leads to leaching into ground water and volatilization of major nutrients like ammonia takes place which will decrease the efficiency

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of nutrient recycling (Möller et al., 2008; Rivard et al., 1995). Mushrooms such as *Pleurotus* spp. are a promising possibility to recycle nutrients as they have been used for centuries to produce protein rich food from agro-residues. Spent biomass from biogas plants retains about 40% to 60% of the cellulose and higher extent of lignin (from original feedstock) thus providing adequate nitrogen rich substrates for the cultivation of edible mushrooms with potential to generate cash outflow along with the use of biogas plants in the rural areas. BDR, by virtue of its high nitrogen content, when applied to soil directly as fertilizer, leads to rapid nitrification and denitrification of the added organic nitrogen. This in turn leads to lower N-efficiency. Further, basidiomycetes are the only class of organisms which can feed on the partially digested material from the biogas plant containing high lignin and therefore are organisms of importance in managing efficiency of N-recycling processes.

Spent mushroom compost (SMC) has been explored for a diversity of potential commercial applications such as amendment for plant growth media (Medina et al., 2009; Ribasa et al., 2009) and bioremediation agent (Chiu et al., 2009). The use of SMC as feedstock for bioethanol production has been examined by a few groups around the world (Kapu et al., 2012; Zhu et al., 2013). Many white-rot fungi growing on agro-residues exhibit high levels of lignin degrading enzymes like laccase and manganese peroxidase (Mishra and Kumar, 2007). The genera of *Pleurotus* have the ability to produce extracellular ligninolytic enzymes: laccase and two peroxidases: manganese dependent peroxidase, versatile peroxidase and aryl-alcohol oxidase which modify and degrade lignin (Stajic et al., 2006). This ability of *Pleurotus* has led to their cultivation on various lignin-rich lignocellulosic materials such as saw dust, paper byproducts and many agricultural residues (Rodriguez et al., 2004). This preference of *Pleurotus* in achieving higher lignin degradation (Ganguli and Chanakya, 1994) can also be used to recover hemicellulose and cellulose for other applications such as bioethanol.

In the context of embedding biogas plants into sustainable energy, rural life and livelihoods, conversion of the BDRs to value added products such as mushrooms could be of great importance in the widespread acceptance of biogas plants and renewable energy programs of countries like India and China (Chanakya and Malayil, 2012b). The process of using biomass as a substrate for biogas in a plug flow reactor thus provides three outputs such as i. biogas for cooking, ii. digester liquid (as pest repellent; Chanakya and Malayil, 2012a, 2012b) and iii. digested biomass residue which can be converted to mushroom and many other value added products. This also leads to efficient resource recovery and nutrient recycling (Chanakya and Malayil, 2012b). Biogas residue as a single substrate for mushroom cultivation has been tested by Ganguli and Chanakya (1994) and found to be poor due to the physical collapse of mushroom substrate after pretreatment and consequent infestation by insects. BDR contains many undesirable microorganisms and therefore it needs to be sterilized before spawning. When autoclaved this BDR softens, becomes a pulpy mass and its structure collapses. In this process there is very little air space left and it thus impedes mycelial growth and fruiting body formation. When deployed as the sole substrate for mushroom cultivation, the 100% biogas residue substrate collapsed after a 14 day spawn run. Therefore to avoid collapsing of biomass bed during spawn run (with BDR) and to provide better aeration, the BDR needs to be mixed with other agro-residues. When BDR was mixed with paddy straw in the order of 0%, 50% and 100%, it was found that 50% mix gave better yields of 1.25 kg mushroom per kg of substrate (bioefficiency = 248%, Ganguli and Chanakya, 1994).

The aim of this research was to study the process requirements and yield of *Pleurotus florida* and *Pleurotus flabellatus* on feed stocks like paddy straw and coir pith and in combination with BDR. The degradation patterns of these feedstocks were monitored to examine the potential of *P. florida* and *P. flabellatus* to degrade lignin for utilizing the digested BDR and potentially enhance returns from deploying modern biogas plants and make available potential feedstocks for bioethanol production.

Materials and methods

Collection of substrates and spawn

The mushroom substrates (PS and CP) for growing *P. florida* and *P. flabellatus* were obtained from Hassan District, Karnataka, India. Anaerobically digested biomass waste was recovered from a plug flow type biogas plant developed by CST-ASTRA, IISc fed with leafy biomass as feedstock (mainly banana leaf, *Musa* sp.). The primary inocula of *P. flabellatus* and *P. florida* were obtained from Indian Institute of Horticultural Science (IIHR), Bangalore.

Pretreatment of substrate

The substrates thus collected were dried in an oven at 90 °C for 24 h. Oven dried PS was chopped into 5–7 cm length pieces. The chopped material was filled in gunny bags and soaked in fresh tap water for 12 h. Excess water was then allowed to drain off and substrates were pasteurized by dipping in hot water at 75 °C for 30 min. BDR was sun dried and solarized for seven days. Further, CP and BDR were steam sterilized in order to prevent disintegration of the material during pasteurization in hot water (Ganguli and Chanakya, 1994). Later the moisture content of these substrate mixes was adjusted to 50% and spawning was carried out. Anaerobically digested biomass material and fresh feedstock were mixed in a ratio of 3:7 — a ratio chosen based on previous studies (Ganguli and Chanakya, 1994) where it was reported that a 50% substitution gave high yields (>2.4 kg mushroom/kg dry wt of mix). The mixtures and single biomass feedstocks were filled in perforated polythene bags (Bano et al., 1962) after spawning (@10% overall weight). The cultivation was carried out in a humid chamber covered with jute cloth in laboratory conditions. The poly-bag cultures were frequently sprayed with water every day till the stage of fruiting body initiation in order to maintain an adequate moisture content and high humidity.

Sampling of substrates

A composite sample of 50 g was collected from different bags at various intervals i.e., before spawning, 10th day after spawning, after complete mycelia colonization, at the first harvest and final harvest. These samples were dried in a hot air oven at 90 ± 2 °C. The dried material was later powdered and samples were stored in paper bags for further analysis. These samples were used to determine various parameters in order to estimate residual nutrient status in substrates and consequent uptake efficiencies.

Physico-chemical analysis

The organic carbon fraction, TKN and TP reduction among substrates at various stages of the process were monitored. Organic carbon (OC) fraction of the sample was estimated by dry ashing (Jackson, 1973). Total Kjeldhal Nitrogen (TKN) and total phosphate (TP) were determined by the method outlined by Tandon (1993) and APHA (1975). In order to study the degradation pattern of these feedstocks, estimations of cellulose, hemicellulose and lignin contents were carried out by a sequential extraction procedure outlined by Chesson (1978). The harvested mushrooms were analyzed for moisture and crude protein. The protein content was obtained by Lowry's method. Total weight of all the fruiting bodies harvested from the three subsequent pickings was measured and reported as the total yield of mushroom. The biological efficiency (yield of mushroom per kg dry substrate) was calculated using the formula given by Chang (1978). The statistical analysis was

carried out using R-Studio (version 2.15.2 (2012-10-26)) and other worksheet packages.

Results and discussion

Mushroom cultivation was attempted on 2 feedstocks (PS and CP singly as well as in combination with BDR) carried out in a jute-cloth covered humid chamber under laboratory conditions (21–25 °C). Various groups around the world have tried to grow *Pleurotus* species on various agro-wastes like PS, wheat straw, etc. and have reported maximum yields to occur with PS (Kalmis and Sargin, 2004; Liang et al., 2009; Ragunathan et al., 1996; Yildiz et al., 2002). The addition of BDR to agro-residues was not only expected to increase the yields but also hasten the process of mushroom production (Ganguli and Chanakya, 1994). The growth and production stages of *Pleurotus* sp. are characterized by defined time required to reach the stages of pin-head formation, fruit body emergence, duration required for the first harvest, and subsequent harvests, etc.

Pin-head initiation and its rapidity

The pin head formation process of mushroom production was enhanced by admixture with BDR (in this case digested banana leaf biomass; PS amended with BDR; Figs. 1 and 2). A 3 day advancement in the time required for pin head formation for both the *Pleurotus* species was observed in the case of PS and CP mixed with BDR when compared to their use as a single substrate. This suggests that the addition of BDR to agro-residues could hasten the fruiting process of mushroom production. Many authors report primordia initiation to take place around 24–30 days with single feedstocks like PS, CP, maize stover, sugarcane bagasse, wheat straw, etc. while using various *Pleurotus* species such as *Pleurotus ostreatus*, *Pleurotus sajor-caju*, and *Pleurotus citrinopileatus* (Kalmis and Sargin, 2004; Liang et al., 2009; Ragunathan et al., 1996; Yildiz et al., 2002). Figs. 1 and 2 show the growth stages of the two *Pleurotus* spp. cultivated on PS and CP mixed with BDR. The pin-head formation for *P. florida* was faster for both the feedstocks mixed with biogas residue when compared to *P. flabellatus*.

Time for fruiting body emergence

Fruiting body formation for *Pleurotus* spp. cultivated on both the substrates occurred between 20 and 30 days (Figs. 1 and 2). In this study the fruiting body initiation for PS with both *P. florida* and *P. flabellatus* was 21 days which was early when compared to previous studies (Ganguli and Chanakya, 1994; Kalmis and Sargin, 2004; Liang et al., 2009; Ragunathan et al., 1996; Yildiz et al., 2002). The addition of BDR to PS hastened the fruiting body initiation process by 1–2 days among the two *Pleurotus* spp. studied. A fruiting body initiation time of 28–30 days for PS and 22–27 days for CP with various *Pleurotus* spp. has been reported (Ragunathan et al., 1996). In the case of CP the fruiting body formation occurred on 24 days for *P. florida* and 30 days for *P. flabellatus*. The addition of biogas residue to CP reduced fruiting body initiation by 3–4 days among the two *Pleurotus* spp. BDR alone, as reported earlier (Ganguli and Chanakya, 1994) does not serve as a good substrate as a single feedstock and with regard to the time for fruiting body formation the time requirement by both the *Pleurotus* spp. (29 days for *P. florida* and 31 days for *P. flabellatus*, Fig. 2). From the above observations we conclude that with regard to fruiting body formation time with PS + BDR, *P. florida* performed better while in the case of CP + BDR, *P. flabellatus* performed better.

The mushroom yields are reported as fresh weight of mushroom harvested in 80 days (3 flushes) per unit fresh weight of feedstock. It is evident from Fig. 2 that PS along with BDR gave the highest mushroom productivity. When comparing the two *Pleurotus* spp., *P. florida* gave a higher yield of 2.32 kg/kg substrate. Previous study in this lab showed that *P. flabellatus* gave a maximum yield of 1.25 kg/kg when grown on a mixture of PS + BDR (Ganguli and Chanakya, 1994, with weed biomass feedstock derived BDR at 50% substitution). In the current study a better yield was obtained with a 30% substitution of PS with BDR (2.32 kg/kg by *P. florida* and 2.09 kg/kg by *P. flabellatus*).

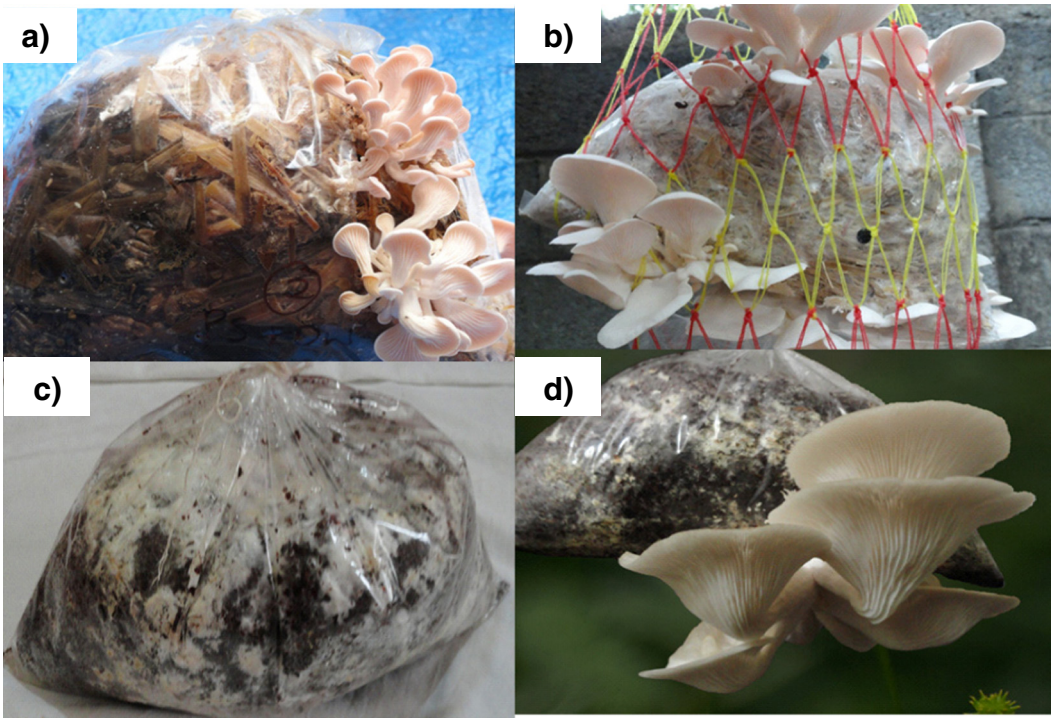

Fig. 1. (a) Early stages of mushroom bodies *P. flabellatus* on PS + BDR. (b) Ready to harvest stage of *P. flabellatus* grown on PS + BDR. (c) CP + BDR with extensive mycelial network of *P. florida*. (d) Ready to harvest mushroom — *P. florida* grown on CP + BDR.

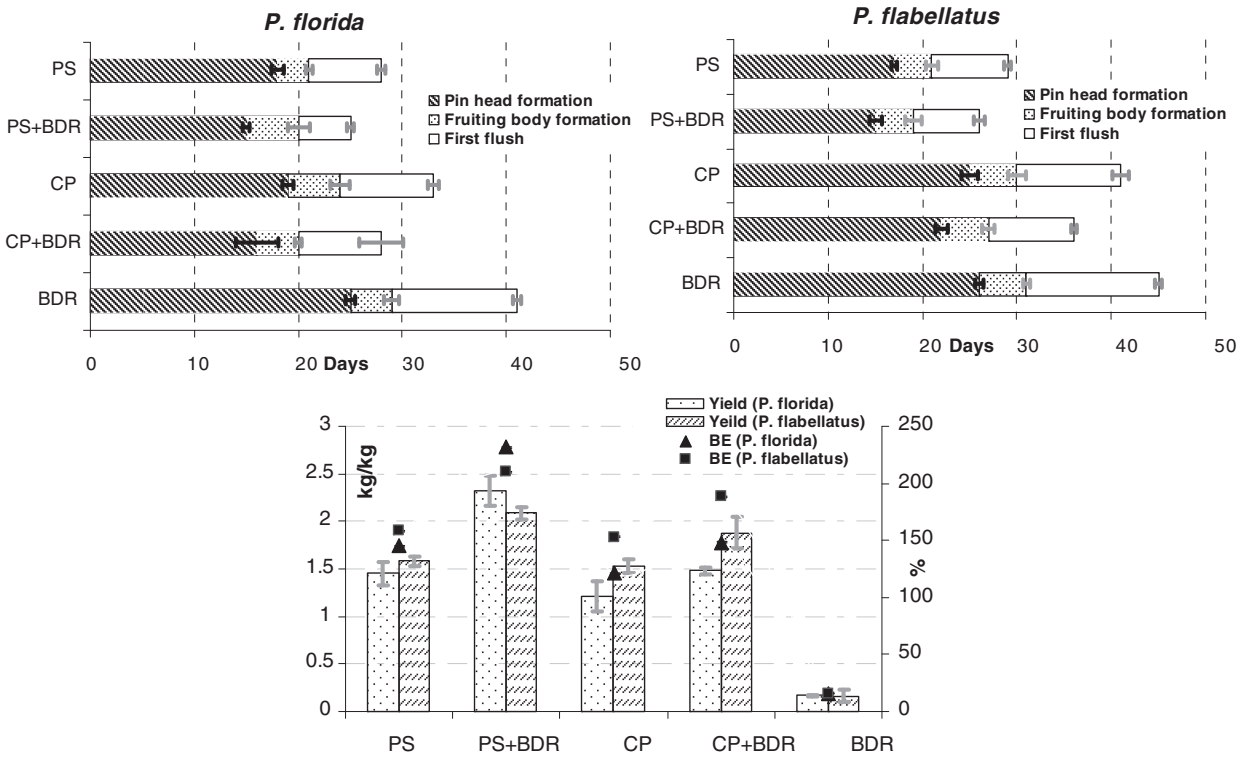

Fig. 2. Effect of substrate combinations on onset of various growth stages such as time required for pin head, fruiting body formation and first flush with two species of *Pleurotus*. The yields of mushroom from three harvests and bioefficiency values for different combinations of feedstocks and *Pleurotus* spp. increased yields and efficiency by supplementing with BDR.

Therefore a 30% substitution was found be more efficient compared to earlier studies (Ganguli and Chanakya, 1994). Further, it is observed that in this study, firstly the baseline productivity using single feedstocks was higher than reported earlier (PS 2.2 kg/kg with *P. ostreatus*; Yildiz et al., 2002; and for CP 0.279 kg/kg for *P. sajor-caju*, *Pleurotus platypus* 0.327 kg/kg and *P. citrinopileatus* 0.252 kg/kg for 3 flushes; Ragunathan et al., 1996). Secondly, the addition of BDR significantly enhanced the mushroom yields when mixed with PS or CP.

In the case of CP as feedstock, when comparing the overall yield of the two mushroom species, *P. flabellatus* produced higher yield compared to *P. florida* and this has not been reported before. In the case of PS, the addition of BDR gave a 37% increase in yield for *P. florida* and 24% for *P. flabellatus*. Similarly, for CP, the addition of BDR increased mushroom yields by 18.2% for *P. florida* and 18.6% for *P. flabellatus*. However, BDR used singly was found to be a poor substrate with a mushroom yield of 0.16–0.17 kg/kg substrate for both species of *Pleurotus*. The increased level of compaction leading to low air availability is believed to be the cause of low mushroom yields (Ganguli and Chanakya, 1994).

In the case of PS as feedstock, the biological efficiency of conversion expressed as the fresh weight of mushroom harvested in 80 days (3 flushes) per unit dry weight of the substrate was the highest for PS + BDR (209.92% for *P. flabellatus* and 231.93% for *P. florida*). This is the highest biological efficiency reported so far in literature. In the case of CP + BDR a high biological efficiency was achieved with *P. flabellatus* (188.46%) and *P. florida* showed a biological efficiency of 148.31%. Similar biological efficiency for CP has been reported by various other authors (Ragunathan et al., 1996). The important conclusion is that the biological efficiency could be increased by about 10–30% when single feedstocks are mixed with ca. 30% BDR. Data recorded during the study showed that single feedstocks supplemented with BDR held higher moisture content. This could lead to rapid mycelial growth in supplemented substrates and its impact needs to be investigated further.

The varied productivity and bio-efficiency of different substrate combinations may be attributed to differences in both their physical properties and nutritional composition. The poor growth and low yield of mushroom in the BDR have been reported due to the collapse of their bulk, low air movement, high water content potentially leading to anaerobic conditions, etc. Proper gaseous exchange in the mushroom substrate packed in bags is essential to acquire more oxygen and remove respired gases and potentially harmful volatiles (Ganguli and Chanakya, 1994).

Dry matter loss and efficiency

In the present investigation it was found that, the dry matter loss of the substrates is in agreement with the mushroom yield and biological efficiency for both the *Pleurotus* spp. (Figs. 4 and 5). Dry matter loss is directly proportional to the carbon loss in the substrate. In the present study, higher mushroom yields and biological efficiencies correspond to higher dry matter loss. The dry matter lost was partly assimilated into the mushroom fruit bodies and partly lost into the atmosphere as carbon dioxide due to mushroom respiration. There was around 51% TS removal by *P. florida* in PS, 54% in PS + BDR, 40% in CP and 37% for CP + BDR in 40 days. Total TS loss in 40 days by *P. flabellatus* was 50% for PS and PS + BDR, and 35% for both CP and CP + BDR (Fig. 4). This shows that the efficiency of conversion of substrate TS to mushroom weight was higher for *P. florida* with PS + BDR as the substrate.

A correlation was attempted between the % TS lost and weight of *Pleurotus* spp. harvested among various substrate combinations. A reasonable trend was observed for *P. florida* raised on various substrate combinations (Fig. 3). In the case of *P. florida*, a straight line fit suggested a good correlation between %TS lost and mushroom yield. For yields obtained on coir pith, a straight line trend obtained suggested a 23% TS being compartmentalized for hyphal growth the mushroom harvested being proportional to %TS lost. A poor correlation was obtained between mushroom yields and %TS lost for all other combinations of mushroom species and substrate mixes.

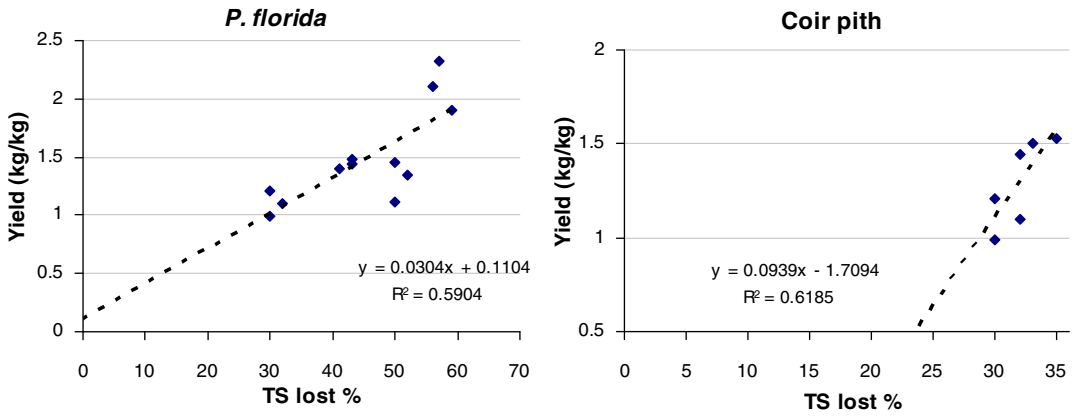

Fig. 3. Correlation of TS lost (%) with yield for *P. florida*, *P. flabellatus* and coir pith.

Organic carbon removal and efficiency

The changes of organic carbon in *P. florida* and *P. flabellatus* on various substrates during stages of the process are shown in Fig. 4 and the mass balance for the process is shown in Fig. 5. The organic carbon content of substrates gradually reduced over the period of mushroom growth from the initial to final harvest stage. This is possible due to the accumulation of C in mushroom biomass as well as by loss due to respiration. Under normal composting processes, the organic C content of digesting feedstock gradually increases with the extent of decomposition due to the accumulation of lignin rich substances in the residue (Kausar et al., 2011). However, in this study there is a gradual decrease

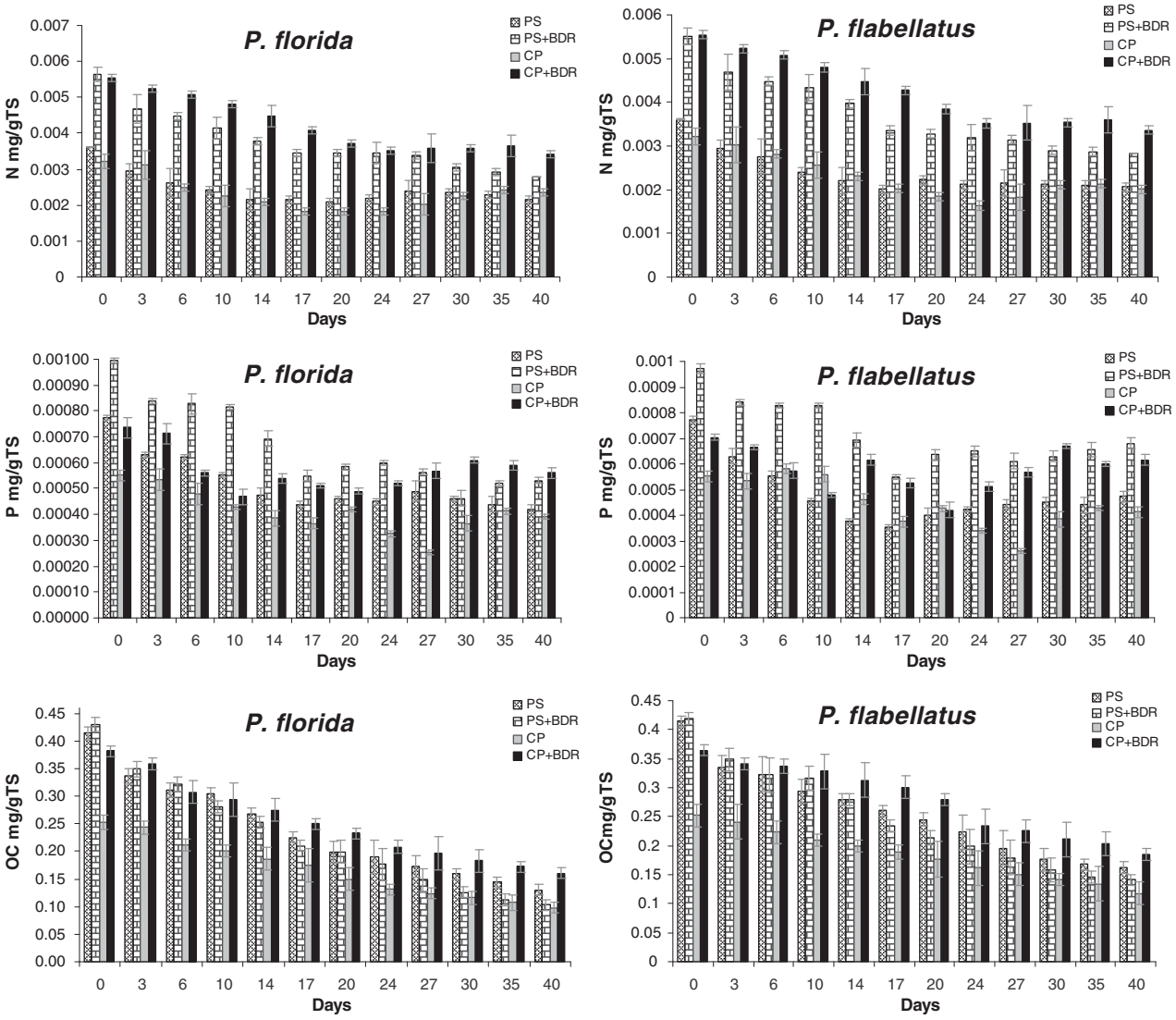

Fig. 4. Dry matter, nitrogen, phosphorus and organic carbon removal from various substrates by *Pleurotus* spp.

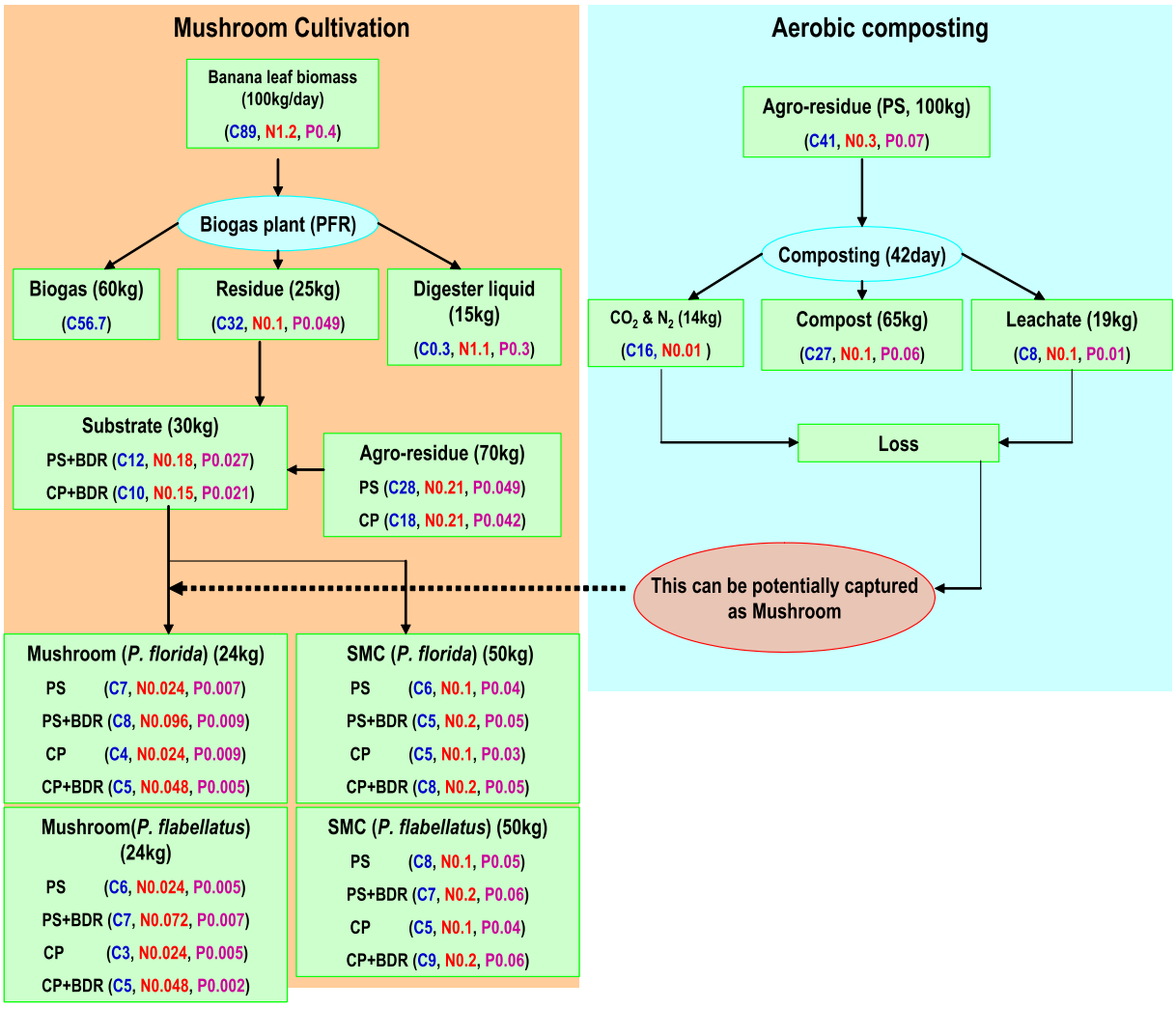

Fig. 5. Carbon, nitrogen and phosphorus balance of the system.

in organic C suggesting a higher level of degradation of lignin compared to the other material in the feedstock as well as due to the presence of >10% recalcitrant silica in PS that is expected to further depress the measured organic carbon content (Kausar et al., 2011).

In the present investigation, there was a drastic reduction of organic carbon content in PS + BDR (65% for *P. florida* and 60% for *P. flabellatus*) and CP + BDR (57% for *P. florida* and 50% for *P. flabellatus*). This indicated that the carbon utilization by *P. florida* was higher and in the case of substrate PS + BDR was found to allow a higher level of organic carbon removal. The rate of organic carbon removal by *P. florida* was maximal in the first 20 days with more than 50% carbon removal occurring with both PS and PS + BDR. This C-removal process was delayed in the case of CP and CP + BDR where it took 27 days to achieve a 50% carbon removal (*P. florida*). The corresponding time for 50% C removal for *P. flabellatus* was 27 days for PS, 20 days for PS + BDR, 35 days for CP and 40 days for CP + BDR (Figs. 4 and 5). The reduction in the organic carbon contents reflects the rate and extent of microbially induced degradation. The extent of carbon removed (mineralized) by *P. florida* (respired + harvested biomass + loss in leachate) is around 68% for PS, 76% for PS + BDR, 60% for CP and 57% for CP + BDR (Fig. 4). The carbon fixed by *P. florida* is higher for PS + BDR which correlates with its high yield (Fig. 2). The carbon mineralized by *P. flabellatus* on the other hand was 60% for PS, 67% for PS + BDR, 56% for CP and 52% for CP + BDR. This suggests that under conditions of the study, *P. florida* could utilize a higher fraction of carbon compared to *P. flabellatus*.

TKN removal and efficiency

The changes in the TKN of various substrates under study are shown in Fig. 4 and the mass balance of the overall process is computed in Fig. 5 and summarized in Table 1. The highest increase in nitrogen percentage was observed in PS (42% to 51%), CP (35% to 42%) with the cultivation of *P. florida*. Variations of TKN in different supplemented and non-supplemented substrates for *P. florida* and *P. flabellatus* were observed and graphically presented in Figs. 4 and 5. *P. florida* utilized 40% of the initial TKN from PS, 50% in PS + BDR, 26% in CP and 38% in CP + BDR in 40 days (Figs. 4 and 5). These results appear to implicate the higher yield of mushrooms in BDR supplemented agro-residues to the higher TKN uptake (Figs. 4 and 5).

P. flabellatus showed an N utilization of 42% of initial TKN in PS. Corresponding values for PS + BDR is 48%; 37% in CP and 39% in CP + BDR in 40 days. *P. flabellatus* did not show much efficiency in CP + BDR which explains the low yield with this substrate when compared to PS. The ammonia that remains in spent biomass is usually transformed into nitrate and escapes as nitrogen gas via nitrification and denitrification reactions during the maturation stage of spent mushroom compost (Bisaria et al., 1987; Prabhu Desai and Shethy, 1991). As a result, inorganic nitrogen content in the matured compost is usually low in the spent mushroom compost. Nitrogen is converted to ammonia nitrogen and Beyer and Wilkinson (2002) found a direct correlation between substrate ammonia content and subsequent

Table 1
Effect of C:N ratio of different substrates on the growth of *Pleurotus* spp.

Agricultural waste	Carbon %		Nitrogen %		C:N ratio	
	Substrate	Spent substrate	Substrate	Spent substrate	Substrate	Spent substrates
P. florida						
PS	48.17	31.10	0.42	0.51	114.69	60.98
PS + BDR	51.79	27.02	0.68	0.73	76.16	37.01
CP	27.41	22.12	0.35	0.42	78.31	50.29
CP + BDR	44.14	28.60	0.63	0.62	70.06	46.13
BDR	54.01	42.33	0.79	0.76	68.37	55.70
P. flabellatus						
PS	48.17	35.20	0.42	0.48	114.69	73.33
PS + BDR	51.79	37.83	0.68	0.71	76.16	53.28
CP	27.41	20.02	0.35	0.34	78.31	58.88
CP + BDR	44.14	33.07	0.63	0.60	70.06	55.12
BDR	55.01	43.20	0.86	0.84	63.97	51.43

growth of mushrooms. At the end of the mushroom cultivation, when the substrate was analyzed without removing the mycelia, the content of N was approximately the same as the untreated substrate, suggesting that nitrogen was incorporated into the mycelial mass, whereas in a few substrates N content increased appreciably. This is due to the ability of *Pleurotus* species to fix atmospheric nitrogen which might have contributed to this increment (Rangaswamy et al., 1975). The final TKN content in spent mushroom compost is dependent on the initial nitrogen present in the feed material as well as the degree of decomposition and this is observed in our study also.

Effect of C:N ratio

The C:N ratio of the substrate (mixture and single feedstock) before cultivation and after cultivation of *Pleurotus* spp. is shown in Table 1. From Fig. 4 it is observed that the pattern of N removed occurs quicker than C removal wherein a fall in N levels occurs till 14 days while a fall in C levels occurs till 17–20 days and then gradually the C and N increase. The addition of BDR to the feedstocks narrowed the C:N ratio considerably. In the case of PS, the addition of BDR narrowed the C:N ratio by 44% and in the case of CP the addition of BDR narrowed the C:N ratio by 11% suggesting an increased nitrogen for the growth of *P. florida* and *P. flabellatus*. *P. florida* utilized 54 carbon for every nitrogen and addition of BDR to PS, this was reduced to 39 carbon for every nitrogen utilized (Table 1). For *P. flabellatus* uptake of C is 41 for every unit of N and in the case of the admixture with BDR this was reduced to 23. In the case of CP, *P. florida* utilized 44 carbon for one nitrogen and in the admixture it was narrowed to 24. *P. flabellatus* with CP as substrate utilized 20:1 (C:N) and the addition of BDR reduced this ratio to 15:1 (Table 2).

Table 2
Moisture and protein content of *Pleurotus* spp. on various substrates.

Substrates	Moisture (%)	SD	Crude protein (%)	SD
P. florida				
PS	89.64	0.10	22.50	0.08
PS + BDR	90.40	0.06	23.67	0.08
CP	88.42	1.21	20.25	0.10
CP + BDR	88.97	0.48	21.33	0.17
BDR	92.22	0.13	21.97	0.35
P. flabellatus				
PS	91.88	0.63	19.56	0.64
PS + BDR	91.55	0.46	20.87	0.01
CP	90.89	0.01	19.12	0.05
CP + BDR	91.21	0.52	19.73	0.02
BDR	91.22	0.03	20.16	0.03

The changes in the C:N ratio showed that the C:N ratios for all supplemented and non-supplemented substrates narrowed rapidly. One of the main problems with organic residues with wider C:N ratio is that it will lead to immobilization of N and slow down microbial decomposition of organic matter. With an increase in the N component of the substrate, a proportionate decrease in carbon was observed. These results are in accordance with those reported by Shetty and Moorthy (1981), Thilahavathy et al. (1991) and Theradimani and Marimuthu (1991). They reported that the C:N ratio of CP could be narrowed down by cultivation of mushroom. Jadhav et al. (1998) also reported that the concentration of nitrogen in spent substrates increased with narrowing in the C:N ratio and thus showed possibility of using spent mushroom as a quality cattle feed.

Nitrogen conservation/temporary immobilization

It has been established that both mycelial growth and fruiting body development depend on lignocellulosic materials particularly with reference to their C:N ratio (Theradimani and Marimuthu, 1991; Banik and Nandi 2004). The N content in most of the substrates employed for mushroom cultivation ranges between 0.5 and 0.8% (C:N = 50–60:1), hence the addition of nitrogen to the substrate helps in getting higher mushroom yield (Theradimani and Marimuthu, 1991; Banik and Nandi 2004). Most of the primary colonizers/decomposing micro-organisms cannot utilize the N in BDR as the N in BDR is degraded anaerobically in a biogas plant. Thus much of the N is utilized in the production of fungal biomass and is usually efficient. Basidiomycetes are among the few micro-organisms that can effectively convert this N present in BDR to protein which is edible (Leisola et al., 2012; Rodriguez et al., 2004). From Fig. 5 it is seen that supplementing agro-residues with BDR increased the nitrogen content of the substrate and therefore availability of N for the *Pleurotus* spp. under study. When the process of mushroom cultivation is compared to aerobic composting it can be seen that there is a higher level of better conservation of N in mushroom cultivation (Fig. 5). If agro-residue (PS) is composted the loss of nitrogen through nitrification, denitrification and as leachate would account for 66% of the initial nitrogen present. In Table 1 it may be observed that supplementing the agro-residues with BDR narrowed the C:N ratio which suggests higher availability of N. The nitrogen content of the agro-residue + BDR had increased N in the order of 0.2% for both *P. florida* and *P. flabellatus*. In the case of PS, *P. florida* used 68% of the initial carbon and *P. flabellatus* used 60%. The utilization of carbon by *P. florida* from PS + BDR was 76% and by *P. flabellatus* was 67%. This suggests that the utilization of carbon from the substrate by *P. flabellatus* was lower than *P. florida*. This pattern was repeated with CP and CP + BDR whereas the carbon uptake by *P. florida* and *P. flabellatus* was similar when the feedstock was BDR. It may therefore be concluded that conversion of BDR to mushroom is the most efficient biological route for i. recovery of N resource present in BDR, ii. bringing it back to the food chain without sacrificing the compost potential of the digested feedstock and iii. creating livelihoods around biogas plants and making their use economically attractive.

TP removal and efficiency

The TP removal among the two feedstocks and by *Pleurotus* spp. is reported in Figs. 4 and 5. The TP removal by *P. florida* in 40 days for PS was 45%, PS + BDR is 46%, CP is 28% and CP + BDR is 23%. In the case of *P. flabellatus* the TP removal within 40 days for PS is 38%, PS + BDR is 30%, CP is 25% and CP + BDR is 12.5%. The removal efficiency by *P. florida* was higher in PS whereas in other feedstocks it seems to be lesser. In *P. flabellatus* the removal efficiency for feedstocks emended with BDR was lower. However this lower removal efficiency of TP has not affected the total yield and this needs further investigation.

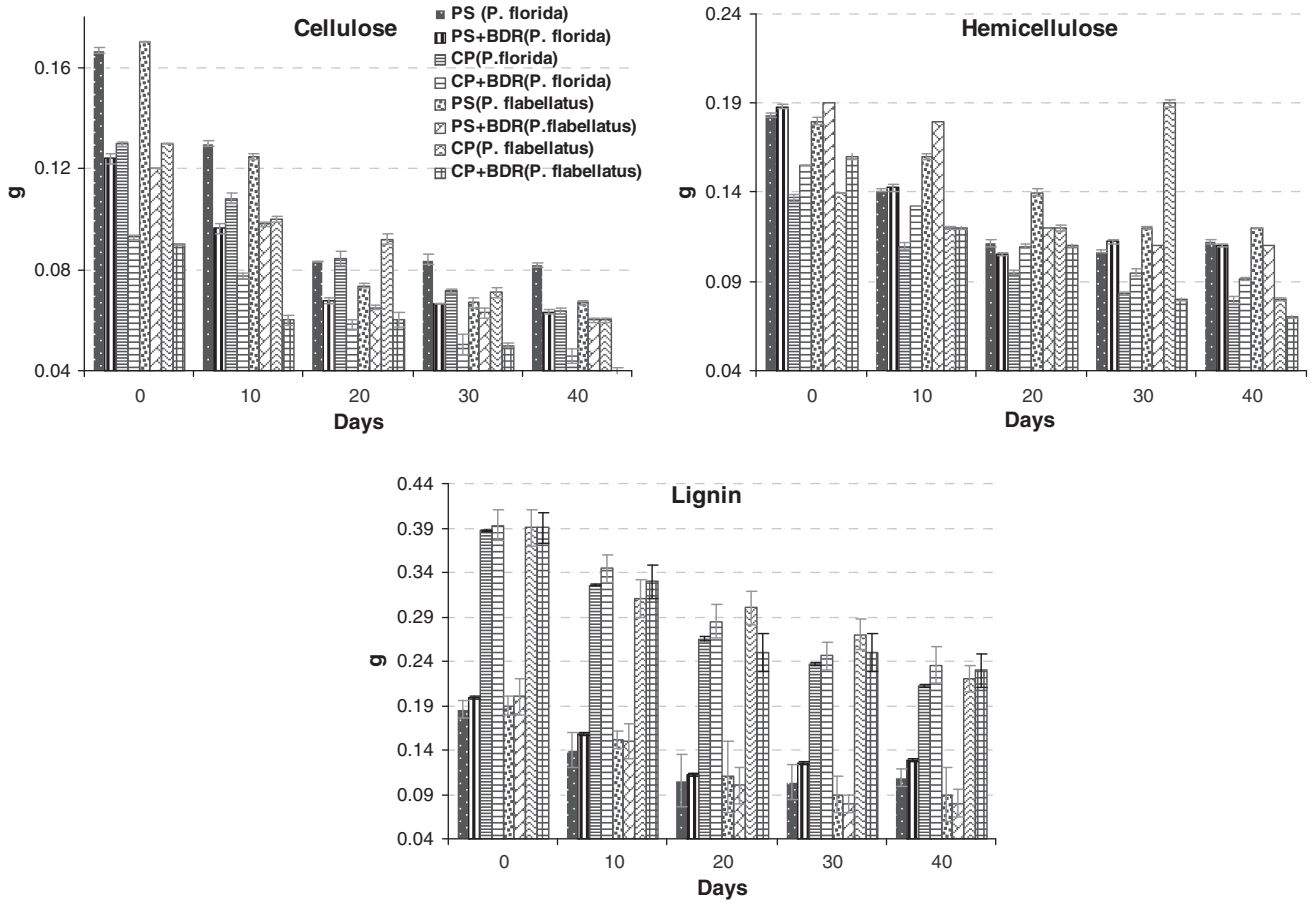

Fig. 6. Decomposition pattern of the feedstocks with the *Pleurotus* spp.

Decomposition pattern of feedstock

The decomposition pattern of lignin, cellulose and hemicellulose by *P. florida* and *P. flabellatus* on various supplemented and non-supplemented substrates is presented in Fig. 6. Degradation of lignin, cellulose and hemicellulose was noticed from an early stage both in *P. florida* and *P. flabellatus* (Fig. 6). The extent of cellulose degradation was in the range of 50–70% of the dry weight in both supplemented and non-supplemented substrates of CP and PS (Fig. 6). The maximum lignin degradation was observed in the case of CP wherein from an initial value of 43% it fell to 31% (Fig. 6). The general observation has been that the cellulose content decreased as the days of spawning increased, for *P. florida* in both the two supplemented and non-supplemented substrates. The decrease in hemicellulose was relatively greater than cellulose in CP supplemented with spent biomass implying that the fungi utilized a greater extent of hemicellulose than cellulose. The maximum extent of initial lignin content observed (40–45%) was in CP + BDR and CP as a single feedstock.

P. florida was more effective in degrading the lignocellulosic content of agricultural residue among both the feedstocks. Basidiomycetes have a unique ability of degrading lignin while retaining most of the hemicellulose and cellulose intact (Henriksson et al., 2000). This property of basidiomycetes may be utilized for bioethanol production from lingo-cellulosic biomass where the major difficulty is removing the lignin component without affecting the hemicellulose and cellulose removal efficiency (Dent and Brown 1978). Ganguli and Chanakya (1994) have reported a similar pattern in degradation where the free sugars and pectins increased in substrate after mushroom cultivation while hemicellulose and cellulose were similar and lignin content reduced significantly.

During mushroom cultivation, agricultural residues provide a reservoir of carbon in the form of cellulose, hemicellulose and lignin, which is utilized during the growth of spawn and during fructification (Ganguli and Chanakya, 1994). Materials with higher lignin concentrations (>15%) lead to slow decomposition and N immobilization. Because lignin is an aromatic, branched and complex compound, it requires a long period to be broken down by conventional soil microorganisms. Lignin contributes to the recalcitrance of agro-wastes to decomposition by occluding the more easily decomposable polysaccharides — cellulose and hemicellulose. *Pleurotus* spp. are known to produce a wide range of hydrolytic and oxidative enzymes that enable them to successfully colonize, degrade and convert many lignocellulosic substrates to their biomass (Bano et al., 1993). Such degradation of lignocellulosic materials results from the concerted and synergistic action of many enzymes including endoglucanases, exoglucanases, b-glucosidases, xylanases, laccases and polyphenol oxidases (Buswell et al., 1996; Diaz-Godinez et al., 2008). Previous studies on wood ear cultivation suggest that the cellulose content of the substrate and enzyme production of the mushrooms are important in determining the yield of a mushroom crop (Onyango et al., 2011). The variations observed in yield may also be attributed to the complexity of substrates in terms of their cellulose content resulting in a difference in the rate of degradation by the mushroom enzymes. The present study shows a decline in the cellulose, hemicellulose and lignin contents of the spent substrates and is within the normal understanding of this process (Ganguli and Chanakya, 1994).

Protein content of mushroom

The protein content of harvested mushrooms is presented in Table 2. The addition of BDR to PS increased the protein content of *P. florida*

marginally (1.17%) when compared to PS as a single feedstock. In the case of CP + BDR the increase in protein content for *P. florida* was also marginally higher (1.08%) than CP as a single feedstock. Similarly for *P. flabellatus* the increase in protein content by the addition of BDR to PS was marginally higher (1.31%) and for CP + BDR was 0.61% higher than CP alone. From the above data it appears that supplementation of BDR marginally improves the protein content in mushroom when compared with the use of agricultural residue as single feedstocks.

Conclusion

The present study was carried out to understand the effects of the addition of BDR to agro-residues (PS and CP) used as substrate for growing mushroom. Laboratory results indicated that the addition of BDR to agro-residues could enhance the yield of mushroom in the range of 20–30%. The addition of BDR enhances the N and P contents of the substrate and the uptake of N, P and C by the *Pleurotus* spp. used. Between the two feedstocks tried, PS with BDR supplement gave higher yields while among the two mushroom species *P. florida* gave better performance. The higher mushroom yields from BDR supplementation could substantially improve the economics and value addition by use of biomass based biogas plants and would enhance the spread of biogas technology. However, identifying and optimizing the causes of higher yields need further investigation.

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