BIODEGRADATION OF COFFEE PULP WASTE BY DIFFERENT FUNGAL ASSOCIATIONS

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ABSTRACT

Substrate protein content increased from the initial value of 12.0% dry weight to the maximum 15 - 15.1% dry weight in *Pleurotus flabellatus* and *Ganoderma lucidum* monocultures on the 20th day. Both the soft rot fungi, *C.haetomium globosum* and *Aspergillus terreus* showed a similar pattern of protein turn over.Cocultures involving *Ganoderma lucidum* and *Pleurotus eous* (16.9 % dry weight protein) or *Pleurotus flabellatus* (17.1% dry weight protein) or *Phaerochaete chrysosporium* (16.5% dry weight protein) were more efficient in increasing the substrate protein content compared to the cocultures involving *Fomes badius* and any one of the three white rot fungi.The maximum 24.7% loss in organic matter was caused by *Pleurotus eous* monoculture, followed by the monocultures of *Phaenerochaete chrysosporium, Ganoderma lucidum* and *Fomes badius* which caused 23.4%, 22.8% and 22.6% of loss in organic matter respectively.But *Pleurotus flabellatus* in association with *Aspergillus terreus* caused the maximum 33.5% LOM on the 40th day, which was significantly higher than the LOM, caused by the monocultures. On the other hand significant suppression in total weight loss was observed in two association's *viz., Phaenerochaete chrysosporium* + *Fomes badius* and *Pleurotus eous* + *Fomes badius* which was lower than that caused by their corresponding monocultures

KEY WORDS: Protein content, Loss in organic matter, Monocultures, Co cultures, biodegradation.

INTRODUCTION

Coffee pulp is one of the most abundantly available agro industrial wastes produced, during the pulping operation of the coffee cherries to obtain coffee beans in many coffee- producing areas of the tropics (Roussos *et al*., 1995). Coffee (*Coffea sp*.) is one of the most important agricultural commodities in the world. *Coffea arabica* and *Coffea robusta* are the two principal varieties of the genus cultivated all over the world for commercial production (ICO, 1998). Coffee production around the world is an economically important factor for more than fifty under-developed countries in America, Asia and Africa located in the tropical area between the tropics of Cancer and Capricorn.

Several workers reported that the use of mixed cultures of lignocellulolytic microorganisms looked promising in increasing the protein content compared to pure cultures and many of them were reported to be more efficient in degrading lignocellulolytic substrates and in producing high activity enzymes (Duff *et al*., 1987).

Though rich in carbohydrate content, lignocellulosic substrates are poor in protein content. A more efficient way to use these annually available large quantities of lignocellulosics is the cultivation of edible fungi (or) mushroom for human consumption and upgrading of nutritionally poor residues into a protein-rich material for animal consumption. Application of agro-industrial residues in bioprocesses on the one hand provides alternative substrates and on the other helps in solving pollution problems, which their disposal may otherwise cause.

Continuous efforts have been made to improve the efficiency of utilization of the lignocellulosic crop residue through physical, chemical and biological treatments. However, physical methods are energy intensive and uneconomical on a larger scale. The chemical treatments which have been used to improve the feeding value include the aqueous treatment and urea-ammonia treatment, which have shown much promise (Singh *et al.,* 1989).

A microbial delignification process is considered to have potential applications in the conversion of lignocellulosics into food, feed and fuel.

The importance of biological delignification has been amply demonstrated frequently in various fields of biotechnology. Ligninolytic strains not only prevent pollution but have also been used for the bioconversion of industrial wastes into useful energy yielding chemicals (Hammel, 1989). With the removal of lignin barrier, cellulose becomes easily accessible for bioconversion. Delignification of forage crop residues enhances their digestibility and also improves their nutritive value (Reid, 1989).

Due to the presence of these compounds (caffeine, tannins and polyphenols), these organic solid residues show toxic nature and thus have not been utilized beneficially. This has also led to the problem of environmental pollution (Pandey *et al.,* 2001). In spite of the toxic components, coffee husk and pulp are very rich in organic components and could be used as substrates in bioprocesses to produce enzymes, aroma compounds, plant hormones, edible mushrooms and feeds (Soccol, 2001).

MATERIALS AND METHODS

Substrate:

Coffee pulp, the solid waste of coffee industry, processing the coffee beans by wet processing method was used as the substrate for biodegradation studies. It was sun dried, coarsely ground to uniform size (2mm) and was stored in gunny bags. The material was used within three months after procurement.

Organisms:

The selected white rot fungi were *Phanerochaete chrysosporium, Pleurotus eous, Pleurotus flabellatus;* brown rot fungi namely *Ganoderma luciderm* and *Fomes badius*; Soft rot fungi namely *Chaetomium globosum* and *Aspergillus terreus* are used for biodegradation of coffee pulp. These fungal cultures were maintained on malt extract (2%) agar medium.

Biodegradation Studies:

Biodegradation of coffee pulp was studied in solid state in Erlenmeyer flasks (250ml) using the selected mushroom fungi and their fungal associations. Ten g of coffee pulp containing 60% moisture was taken in individual Erlenmeyer flasks (250ml). The flasks were plugged with cotton and autoclaved at 121°C for 15 min. Single mycelial agar block (8 mm) from seven days-old cultures of the selected fungi was used as inoculum for monoculture experiments. For coculture studies, two agar blocks of the test white rot fungus and its respective fungal association were used as inocula. The conical flasks were incubated at 28±2°C for a period of 40 days in the culture room.

At each ten days interval of study, the entire content of each flask was withdrawn, dried at 60°C overnight and was used in the analyses for mycelial protein usingLowry *et al.* (1951) method, Loss in organic matter (LOM) was measured by the method of Arora(1995). All the experiments were carried out in triplicates and were replicated twice.

RESULTS AND DISCUSSION

With the increasing awareness of the conservation of the earth's ecosystems, numerous studies have appeared on the development of efficient and cost-effective biodegradation techniques based on the exploitation of the collective metabolic efforts of the microbial populations (Betts, 1991; Singleton, 1994). The diverse microbial pathways involved in the biodegradation process, the methodologies and techniques used by various schools of research on biodegradation and bioconversion were reviewed by Chahal (1991).

Coffee pulp is essentially rich in carbohydrates, proteins and minerals (especially potassium) and it also contains appreciable amounts of tannins, polyphenols and caffeine (Bressani, 1979). Owing to the presence of the anti - nutritional factors, its use as an animal feed has been restricted to a large extent. For want of practical and economical avenues, coffee pulp has not been commercially exploited. As this product of the coffee industry does not find any commercial application, it has become the major polluting agent of rivers and lakes located near the coffee-processing regions.

The presence of proteins, sugars and minerals in coffee pulp and its high humidity favours the rapid growth of microorganisms and if it is not utilized immediately, it causes environmental pollution (Roussos *et al*., 1995).

Mycelial protein

The substrate protein content increased by 25.0% over the initial value on the 30th day and this protein content was maintained

or slightly increased during the course of biodegradation in *P. flabellatus, F.badius* and *G.lucidum* monocultures (Fig 1). Protein enrichment of coffee pulp in SSF with these organisms showed a linear increase with the vegetative growth.

Table 1: Percent increase or decrease in protein content in coffee pulp during solid state biodegradation of selected fungal monocultures.

S.No.	Organisms	Protein content – Percent increase or decrease			
		Degradation (Days)			
		10	20	30	40
1.	*P. chrysosporium*	6.66	12.5	19.1	-6.6
2.	*P. eous*	8.33	19.1	22.5	4.1
3.	*P. flabellatus*	12.5	25.0	27.5	29.1
4.	*F. badius*	10.8	21.6	25.8	26.6
5.	*G. lucidum*	14.2	25.8	29.1	29.1
6.	*A. terreus*	6.66	15.8	5.0	15.0
7.	*C. globosum*	10.8	22.5	0	-10.0
LSD (0.05) = 0.766					

Initial protein content: 12.0 % dry wt.

Table 2: Percent increase or decrease in protein content in coffee pulp during solid state biodegradation of white rot and their fungal associations.

S.N	Organisms	Degradation (days)			
		10	20	30	40
I	White rot + White rot				
1	P. chrysosporium + P. eous	20.8	39.1	28.3	-1.6
2	*P. chrysosporium + P. flabellatus*	18.3	27.5	45.0	30.0
3	*P. eous + P. flabellatus*	25.8	45.8	55.0	31.6
II	White rot + Brown rot				
1	P. chrysosporium + F. badius	17.5	30.8	8.3	-15.8
2	*P. eous + F. badius*	22.5	28.3	1.6	-18.3
3	*P. flabellatus + F. badius*	25.0	35.8	12.5	-15.0
4	*P. chrysosporium + G. lucidum*	24.1	37.5	23.3	-5.0
5	*P. eous + G. lucidum*	29.1	40.8	16.6	-12.5
6	*P. flabellatus + G. lucidum*	31.6	42.5	15.0	-19.1
III	*White rot + Soft rot*				
1	*P. chrysosporium + A. terreus*	14.1	34.1	-10.0	-20.0
2	*P. eous + A.. terreus*	20.8	34.1	-15.0	-21.6
3	*P. flabellatus + A. terreus*	25.0	37.5	44.1	-3.33
4	*P. chrysosporium + C. globosum*	23.3	37.5	-1.60	-15.8
5	*P. eous + C. globosum*	25.8	40.0	-3.33	-13.3
6	*P. flabellatus + C. globosum*	27.5	39.1	23.30	-11.6
	LSD (0.05) = 0.865				

Initial protein content: 12.0 % dry wt.

The positive correlation between crude or soluble protein content of the substrate with the biomass of the fungal mycelium had been reported in white rot fungi by several workers (Natarajan *et al.,* 1993). Many white rot and brown rot fungi like *Ganoderma applanatum* (Hatakka and Pirhonen, 1985) and *P.sajor – caju* have been used for improving the digestibility and feed value of agricultural residues (Kahlon *et al*., 1990).

Table 3: Loss in organic matter (%) in coffee pulp during solid state biodegradation of selected fungal monocultures.

S. No	Organisms	Loss in organic matter – Percent degradation			
		Degradation (Days)			
		10	20	30	40
1	*P. chrysosporium*	10.5	16.9	21.7	23.4
2	*P. eous*	10.4	19.6	23.7	24.7
3	*P. flabellatus*	6.0	10.7	14.4	18.2
4	*F. badius*	7.4	14.4	18.0	22.6
5	*G. lucidum*	11.4	15.7	20.4	22.8
6	*A. terreus*	7.8	4.9	19.6	19.7
7	*C. globosum*	8.5	14.0	18.7	20.7
LSD (0.05) = 0.298					

Table 4: Loss in organic matter (%) in coffee pulp during solid state biodegradation of white rot and their fungal associations.

S. No	Organisms	Degradation (days)			
		10	20	30	40
I	White rot + White rot				
1	P. chrysosporium + P. eous	17.0	25.8	30.5	31.0
2	*P. chrysosporium + P. flabellatus*	17.7	27.4	34.7	35.7
3	*P. eous + P. flabellatus*	19.0	26.8	35.7	39.7
II	White rot + Brown rot			`	
1	P. chrysosporium + F. badius	10.7	16.4	19.7	20.0
2	*P. eous + F. badius*	10.6	15.6	18.4	20.4
3	*P. flabellatus + F. badius*	10.9	18.4	27.4	33.6
4	*P. chrysosporium + G. lucidum*	11.4	19.4	23.0	25.0
5	*P. eous + G. lucidum*	11.0	23.0	31.7	33.6
6	*P. flabellatus + G. lucidum*	15.7	26.9	36.9	38.8
III	*White rot + Soft rot*				
1	*P. chrysosporium + A. terreus*	15.7	20.4	24.4	26.4
2	*P. eous + A.. terreus*	11.7	16.4	20.5	24.6
3	*P. flabellatus + A. terreus*	14.4	18.0	25.0	33.5
4	*P. chrysosporium + C. globosum*	12.7	23.7	27.7	29.0
5	*P. eous + C. globosum*	15.4	21.8	27.0	32.0
6	*P. flabellatus + C. globosum*	14.7	22.8	24.0	27.7
LSD (0.05) = 0.297					

A progressive increase in the production of protein was observed till 18 days of fermentation in *A.niger* (Singh *et al.,* 1989) while in *Sporotrichum pulverllentum*, protein production reportedly increased till 30th day of fermentation on rice straw (Kahlon *et al*., 1990). Our results on protein production by *A.terreus* and *P.chrysosporium* monocultures agreed favourally with these reports. The ability of *G.lucidum* to convert several substrates into biomass and protein had been reported by Saravanan *et al*. (2002). Several mushrooms like *Pleurotus sp*. and *Lentinus sp*.were found to increase appreciably the concentration of cell solubles and crude protein content of the substrate during their growth (Zadrazil *et al*., 1996 and Leifa *et al*., 2000).

In other moncultures, substrate protein content was increased by 15.8 to 22.5%, but it was lost after 20th or 30th day of biodegradation in the soft rot or white rot fungal treatments respectively. Singh *et al.* (1989) reported the increase in crude protein from 11.71 to 15.0% on day 8th day of the SSF of wheat straw with *Coprinus fimetarius*, while the crude protein content increased appreciably with the growth of *Pleurotus sp*. after 35 days of growth in paddy and wheat straw (Singh *et al.* 1994) and in potato peels (Arora and Kahlon, 1986).

Substrate protein content was positively correlated with biomass produced by the fungi in both the monocultures and cocultures. The maximum substrate protein content of 18.6% dry wt was observed in *P.flabellatus* + *P.eous* colonized coffee pulp, followed by *P.flabellatus* + *A.terreus* coculture which showed the highest 17.3% dry wt substrate protein content on 30th day of biodegradation (Fig 2). Similary, increase in protein content was correlated with increase in sugar content during the growth of *P.citrinopileatus* on wheat straw by Natarajan *et al*. (1993).

The results showed that *P.flabellatus* monoculture and *P.flabellatus* + *P.eous*, *P.flabellatus + A.terreus* coculture which achieved the highest biomass and a maximum sugar production also showed a higher crude protein content of the substrate (Fig 2). Zabala *et al*. (1994) observed an increase in biomass content with a higher production of extracellular proteins in coculture fermentations involving *T.reesei* and *Monosporium* species.

Similarly several authors (Zabala *et al*., 1994) found that the use of mixed cultures of lignocellulolytic microorganisms looked promising in increasing the protein content compared to pure cultures and many of them were reported to be more efficient in degrading lignocellulosic substrates and producing high activity enzymes (Duff *et al*., 1987). These reports substantiate the results of this study where *P.flabellatus* + *P.eous*, the coculture which produced the maximum biomass and the highest crude protein content was also the most efficient degrader of coffee pulp, as assessed by their enzyme production potential and the loss in organic matter of coffee pulp during the biodegradation.

Loss in organic matter

The maximum 24.7% loss in organic matter of coffee pulp was caused by *P.eous* monoculture followed by the monocultures of *P.chrysosporium*, *G.lucidum* and *F.badius* which caused a LOM of 20.6 to 23.4% respectively (Fig 3).

Considering that loss in organic matter (LOM) represented the efficient colonization of substrate (Sharma *et al*., 2002) and the efficiency of degradation by the fungus (Ouseph *et al.,* 2001; Leifa *et al*., 2000), *P.eous* and *P.chrysosporium* scored over *P.flabellatus.*

But *P.flabellatus* in association with *A.terreus* caused the maximum 33.5% LOM on the 40th day, which was significantly higher than the LOM, caused by the monocultures (Fig 3). The results are in agreement with those of Arora (1995) and Puniya and Singh (1995) who reported the highest LOM of wheat straw colonized by cocultures of *P.chrysosporium* + *F.moniliforme* and *P.chrysosporium* + *A. chroococcum* and those of Singh *et al.* (1994) who studied the coculture of *C.fimetatius* and *A.chroococcum* on wheat straw.

The association of various fungi resulted in enhancement in total weight loss in few combinations and decline in other combinations (Fig 4). The enhancement was statistically significant in combinations of *P.eous* + *P.flabellatus*, *P.flabellatus* + *G.lucidum, P. flabellatus* + *A. terreus.* On the other hand significant suppression in total weight loss was observed in two associations *viz.,P. chrysosporium* + *F.badius* and *P.eous* + *F.badius* which was lower than that caused by their corresponding monocultures (Fig 4). Similar observations were recorded by Arora (1995).

The present investigation was designed to study the biodegradation potential of selected microbial strains of mushroom / white rot, brown rot and soft rot fungi in cocultures. The work was directed towards identifying the best synergistic coculture which could successfully colonize and degrade coffee pulp in solid state fermentation.

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