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By
K. Parani and M. Eyini

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K. Parani* and M. Eyini

Department of Botany with specialization in Plant Biotechnology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi - 626123, Tamil Nadu, India*

Research Centre and P.G. Department of Botany, Thiagarajar College (Autonomous), Madurai – 625009, Tamilnadu, India.

ABSTRACT

In our study, pH of the coffee pulp colonized by the white rot, brown rot and soft rot fungal monocultures and their corresponding co-cultures continuously decreased during the course of biodegradation of the coffee pulp substrate to a very low level of 5.2 - 5.5 from the initial 7.2. pH of coffee pulp colonized by the co-cultures containing Phanerochaete chrysosporium with a brown rot fungus or a soft rot fungus or by the antagonistic co-culture of P. eous + F. badius decreased by a minimum value (final pH 5.9 – 6.2) indicating the lower efficiency of these co-cultures in this group in coffee pulp biodegradation. The combination P. eous + P. flabellatus gave the maximum lignin loss 73.4% which was statistically significant when compared to the lignin loss caused by either of the two organisms in monoculture. The lignin loss caused by the association between the different soft rots and white rots was significantly higher when compared to the lignin loss caused by the soft rot fungal monocultures.

Keywords: White roters, Brown roters, Soft roters, coffee pulp degradation

INTRODUCTION

Coffee pulp is one of the most abundant agro industrial wastes and pollutants generated by the coffee industry. The utilization of coffee pulp as food, animal feed and compost has been investigated for several years (Pandey et al., 2000), but its chemical composition is a great limiting factor due to the presence of anti-physiological factors such as caffeine, poly phenolic compounds and tannins (Hakil et al., 1998) which include tannic acid in high concentrations (3-4%).
Later it was proposed that desirable biochemical changes in terms of higher nitrogen content and degradation of cellulose, hemi cellulose, lignin and other toxic components can be effected in these substrates through mushroom growth (Arora and Kahlon, 1992; Ramamoorthy et al., 1999). Early reports on lignocellulosic conversion into fungal food and feed indicated *Chaetomium cellulolyticum* as the most promising organism of choice (Chahal et al., 1983). Later, several workers demonstrated the lingo cellulose degradation potential of *Phanerochaete chrysosporium* and *Pleurotus sp.* which have been found to be the most efficient lignocellulose decomposing types of the white rot fungi (Kannan et al., 1990; Masaphy and Levanon, 1992).

The study on degradation of lignin from fibres by *Chrysosporium pruinosum* (Rosenberg, 1980) and from wheat straw by *Pleurotus ostreatus* (Detroy and Rhodes, 1980) indicated that it took a long time (30-60 days) to obtain about 40% degradation of lignin. Lignin degradation was found to be inhibited by CO$_2$ and increasing air or oxygen flow over the substrate reversed the inhibition (Zadrazil and Kamra, 1989).

Lignin is found in all vascular plants, a major fraction being distributed throughout the secondary walls of woody cells and also in the middle lamella between the secondary cell walls (Eriksson et al., 1990). Whilst cellulose and hemi cellulose are the supporting components of plants, lignin provides the essential rigidity and durability. Lignin is a natural polymer with a high molecular mass of up to 100 KDa or more (Kastner, 2000) and can make up 20-30% of the lingo cellulose in trees (Kuhad et al., 1997). It is the most abundant aromatic carbon form and after cellulose, the second most abundant natural organic compound on earth. Lignin is deposited as an encrusting and protecting material on the cellulose or hemi cellulose matrix and acts as a kind of glue that cements the fibrous cell walls together. Due to its unique structure, lignin is highly resistant and forms a barrier to microbial attack. In general, only white rot fungi are considered to be efficient degraders of lignin (Griffin, 1994).

Most biodegradation studies have been carried out by using monocultures though a few workers have shown that synthetic lignins or their model compounds are best degraded by consortia (Federle and Vestal, 1980). The variation in degradation success can perhaps be explained by the fact that under field conditions the fungus must compete with the indigenous soil microflora. On the one hand, degradation of contaminants may be enhanced by synergistic interaction between the inoculated wood-rotting fungus and the native soil microflora (in der Wiesche et al., 1997) while on the other hand, degradative activity could be negatively influenced by soil microorganisms antagonistic to the fungus (Ali and Wainwright, 1994).

Biological delignification has been targeted towards production of animal feeds (Al-Ani and Smith, 1986), edible mushrooms (Tautorus, 1985) and composting processes (Peerally, 1981). A major constraint to commercial exploitation of biological systems for delignification is the time factor required to achieve appreciable degradation. Earlier efforts concentrated on the use of monocultures for delignification (Al-Ani and Smith, 1986). However, lingo cellulose de polymerization using monocultures may not be a perfect simulation of natural lignocellulose fermentation, which may explain reasons for poor feed conversion ratios with such processes.
Mixed cultures of aerobic fungi are known to perform certain catabolic operations not known to occur with monocultures in laboratory fermentations (Asiegbu et al., 1996). Keeping these in mind, the present study focused on the effect of co-fungal cultures on fibre biodegradation of coffee pulp waste.

MATERIAL 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.

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 co-culture 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 measuring pH and fiber by the gravimetric method of Chesson (1978). All the experiments were carried out in triplicates and were replicated twice.

RESULTS AND DISCUSSION

Solid state fermentation has scored high among other fermentation types used in large scale biodegradation and bioconversion processes, due to its economically viable and practically acceptable design (Pandey et al., 2001). Garcha et al. (1995) have proposed solid state fermentation as a promising technology for bio-processing of agricultural residues into quality feed. One of the current approaches in improving the efficiency of biodegradation and bioconversion of agricultural or agro-industrial residues in SSF is the use of co cultures or mixed cultures of lignocellulolytic microorganisms.
Many of these co cultures or mixed cultures were reportedly more efficient in lingocellulolytic biodegradation in producing high activity enzymes due to their synergistic action (Ahlawat and Verma, 2001; Bajpai et al., 2001).

**pH**

pH of the coffee pulp colonized by the white rot, brown rot and soft rot fungal monocultures and their corresponding co cultures continuously decreased during the course of biodegradation. Co cultures involving the white rot or mushroom fungi brought down the pH of the coffee pulp substrate to a very low level of 5.2 - 5.5 from the initial 7.2. A second group of co cultures comprising the mushroom fungi, *Pleurotus eous* or *Pleurotus flabellatus* with the selected brown rot or soft rot fungi brought down the pH to an intermediate range of 5.5 - 5.8.

pH of coffee pulp colonized by the co cultures containing *Phanerochaete chrysosporium* with a brown rot fungus or a soft rot fungus or by the antagonistic co culture of *Pleurotus eous* + *Fomes badius* decreased by a minimum value (final pH 5.9 – 6.2) indicating the lower efficiency of these co cultures in this group in coffee pulp biodegradation.

The drop in pH by two degrees of magnitude as observed in the white rot + white rot co cultures indicated the higher rate of biodegradation achieved by these co cultures through the efficient production of hydrolytic enzymes and the acids (Kumaran et al., 1997). Similar views in support of our results on the better performance of co cultures involving white rot fungi have been given by Friteg and Marrell (1992), who suggested that enzyme activities of white rot fungi might be responsible for regulating the pH of the substrate at a suitable range for fungal colonization. Similar results were reported by Safari Sinegani et al. (2000) who found that *Aspergillus terreus* produced the highest biomass utilizing various aromatic compounds resulting in a significant reduction in the pH of the culture media. Ofuya and Nwajuba (1990) recorded a similar drop in pH from 5.6 to 4.4 in cassava peel degraded by *Rhizopus sp*, and they associated this drop in pH with increasing concentration of soluble reducing sugars and crude protein of the peel indicating a high degree of biodegradation achieved by this fungus than the other organisms.

A significant drop in pH of coffee pulp caused by the white rot + white rot co cultures compared very well with the higher crude protein content of the substrate. Similar growth associated decrease in pH was observed by Singh et al. (1989) during degradation of wheat straw by *Coprinus fimetarius* monocultures. Further co cultures of *Coprinus fimetarius* with *Azotobacter chroococum* reportedly resulted in a steeper decrease in pH and a higher degree of degradation (Singh et al., 1994).

**Fibre**

The fibre content of coffee pulp was 17.3% dry weight. Among the different fungal monocultures, *Phanerochaete chrysosporium* degraded the maximum 50.2% fibre from coffee pulp in 40 days followed by *Pleurotus flabellatus* and *Pleurotus eous* (48.0% and 46.2% fibre loss respectively).
Comparatively, the brown rot fungi *Fomes badius* and *Ganoderma lucidum* caused only 36.4% and 38.7% loss in fibre content respectively during the same period. The fibre degrading potential of *Chaetomium globosum* was higher than that of the *Ganoderma lucidum* (40.4% loss in fibre content), while *Aspergillus terreus* had the least fibre degrading potential showing the minimum 23.1% loss in fibre content (Table 1).

Basidiomycetous were reported to be the most efficient lignin degraders, but other ascomycetes and deuteromycetes also were reported to degrade lignin slowly (Rai et al., 1993). Significant fibre degradation observed in *Chaetomium globosum* was in agreement with findings of other workers (Arora and Kahlon, 1992). The results agreed well with the ligninase positive character and laccase activity of the individual organisms on plate assays. *Aspergillus terreus*, the poor lignin degrader neither showed the brown zone in MEA – tannic acid medium nor the purple blue color in the MEA – lignin medium treated with α - naphthol.

Singh et al. (1992) attributed the decrease in neutral detergent fibre of wheat straw to the degradation of cell wall constituents by co cultures and mixed cultures. Choudhary et al. (1998) and Ramamoorthy et al. (1999) found that different *Pleurotus* spp., were able to decrease the lignin content of coir pith by 62.0 to 78.0% over control. Our results on fibre degradation by mushroom monocultures corroborate with this report. Further the results showed that the coffee pulp fibre degradation potential of both the brown rot fungi was considerably less than that of the white rot fungi and *Chaetomium globosum*. Even though brown rot fungi are known colonizers of wood, the percentage of lignin degradation by the brown rot and white rot fungi was found to vary in different substrates by Ortega et al. (1992) and Saravanan et al. (2002).

Fibre loss in coffee pulp caused by the white rot and brown rot monocultures was low at initial stages of incubation, but was found to increase gradually with time. The results agree favorably with those of Ghosh and Nandhi (1995). Different schools of research reported that the maximum lignolysis occurred between 21 and 28 days of incubation (Choudhary et al., 1998; Arora and Kahlon, 1992) or between 30 and 40 days of incubation (Ramamoorthy et al., 1999; Garcha et al., 1995; Ghosh and Nandi, 1995). It was found in this study that maximum fibre loss occurred between 20-30 days in white rot monocultures and between 30-40 days in brown rot monocultures.

The fungi with longer life duration and higher biological efficiency (*P.flabellatus, F.badius* and *G.lucidum*) continued to degrade the various components of coffee pulp with greater efficiency during the experimental period and finally ended up with achieving higher mineralization of the substrate. Lignin degradation had frequently been associated with biomass production. *Phanerochaete sp.* which degraded raw fibres of apple distillery sample by 20% was found to have increased the crude protein content of the filter cake by 17-20% (Kahlon et al., 1990). Substantial reduction in lignin and phenols through solid state fermentation by *Pleurotus ostreatus* was reported by Martinez et al. (1985). Variable responses were observed in the extent of ligninolysis in the different combinations tested. The combination *P.eous + P.flabellatus* gave the maximum lignin loss 73.4% (Table 1 & 2).
which was statistically significant when compared to the lignin loss caused by either of the two organisms in monoculture. The lignin loss caused by the association between the different soft rots and white rots was significantly higher when compared to the lignin loss caused by the soft rot fungal monocultures. The results are in agreement with those of Adhikary et al. (1992) who reported that mixed cultures of fungi, actinomycetes and bacteria could substantially improve lignin degradation compared with the control fungal monocultures. The reason for the enhanced degradation by mixture of organisms could be due to their synergistic activity through utilization of intermediate degradation products.

Table 1. Percent degradation of fibre in coffee pulp during solid state biodegradation of selected fungal monocultures.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organisms</th>
<th>Fibre – Percent degradation (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td><em>P. chrysosporium</em></td>
<td>11.5</td>
</tr>
<tr>
<td>2</td>
<td><em>P. eous</em></td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td><em>P. flabellatus</em></td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td><em>F. badius</em></td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td><em>G. lucidum</em></td>
<td>5.7</td>
</tr>
<tr>
<td>6</td>
<td><em>A. terreus</em></td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td><em>C. globosum</em></td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05) = 1.57</td>
<td></td>
</tr>
</tbody>
</table>

Initial fibre content: 17.3 % dry wt

In co cultures, depending upon the type of association, the maximum fibre loss was found to be induced earlier. Similar promotion in substrate lignin degradation due to the synergistic lingo cellulolytic activity of the organisms in a co culture had been reported by Asiegbu et al. (1996) in sawdust colonized by co-fungal cultures. The results of our studies corroborate with earlier findings of Arora (1995) and Puniya and Singh (1995) where lignin degradation was better in co cultures and mixed cultures. These studies showed that certain associations particularly between white rot fungi can be useful in enhancing the biodegradation of coffee pulp.
Table 2. Percent degradation of fibre in coffee pulp during solid state biodegradation of white rot and their fungal associations.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organisms</th>
<th>Degradation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>I.</td>
<td>White rot + White rot</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>P. chrysosporium</em> + <em>P. eous</em></td>
<td>15.6</td>
</tr>
<tr>
<td>2</td>
<td><em>P. chrysosporium</em> + <em>P. flabellatus</em></td>
<td>19.0</td>
</tr>
<tr>
<td>3</td>
<td><em>P. eous</em> + <em>P. flabellatus</em></td>
<td>19.0</td>
</tr>
<tr>
<td>II</td>
<td>White rot + Brown rot</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>P. chrysosporium</em> + <em>F. badius</em></td>
<td>15.6</td>
</tr>
<tr>
<td>2</td>
<td><em>P. eous</em> + <em>F. badius</em></td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td><em>P. flabellatus</em> + <em>F. badius</em></td>
<td>15.6</td>
</tr>
<tr>
<td>4</td>
<td><em>P. chrysosporium</em> + <em>G. lucidum</em></td>
<td>17.3</td>
</tr>
<tr>
<td>5</td>
<td><em>P. eous</em> + <em>G. lucidum</em></td>
<td>15.6</td>
</tr>
<tr>
<td>6</td>
<td><em>P. flabellatus</em> + <em>G. lucidum</em></td>
<td>15.6</td>
</tr>
<tr>
<td>III</td>
<td>White rot + Soft rot</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>P. chrysosporium</em> + <em>A. terreus</em></td>
<td>17.3</td>
</tr>
<tr>
<td>2</td>
<td><em>P. eous</em> + <em>A. terreus</em></td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td><em>P. flabellatus</em> + <em>A. terreus</em></td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td><em>P. chrysosporium</em> + <em>C. globosum</em></td>
<td>19.0</td>
</tr>
<tr>
<td>5</td>
<td><em>P. eous</em> + <em>C. globosum</em></td>
<td>15.6</td>
</tr>
<tr>
<td>6</td>
<td><em>P. flabellatus</em> + <em>C. globosum</em></td>
<td>13.2</td>
</tr>
</tbody>
</table>

Initial fibre content: 17.3 % dry wt

The suppression in fibre loss caused by *Pleurotus eous* + *Fomes badius* association was significant in comparison with that caused by *Pleurotus eous* and *Fomes badius* monocultures. Asiegbu et al. (1996) working with cocultures involving *P.chrysosporium*, *P.sajor-caju* and *T.versicolor* found that coculture of *P.chrysosporium* + *P.sajor-caju* similarly had only a lower potential for delignification. Co cultures in which the mushroom fungus overgrew its partner at a later stage (*P.flabellatus* + *A. terreus*; *P.eous* + *A.terreus*; *P.flabellatus* + *F.badius* and *P.flabellatus* + *G.lucidum*) showed higher lignin removal after this stage. The nitrogen from the mycelial biomass of the coculture partner might have kept the synthesis of lignin degrading enzymes under a de-repressed state as reported by Bisaria et al. (1997) and thus could have aided in enhancing lignin degradation.
REFERENCES


**Corresponding author:** Dr. K. Parani, Department of Botany with specialization in Plant Biotechnology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi - 626123, Tamil Nadu, India.

Email: parani_k@yahoo.co.in