Wood contains 20 to 30 per cent lignin, which imparts firmness to the wood structure and prevents microbial penetration to the easily hydrolysable wood components cellulose and hemicellulose. Lignin composed of oxyphenyl propanoid units connected by several different C-C and COC linkages. The biodegradation of lignin has assumed through evolution a disproportionate and central importance in the cycling of terrestrial carbon. Elucidation of lignin biodegradation process is essential for establishing technology for bioconversion of plant residues and waste lignin to useful products and for protecting the environment from lignin related pollutants. The structural features of lignin dictate unusual constraints on biodegradative system responsible for initial attack. Only a few microorganisms, mainly white rot fungi and some bacteria are able to degrade or modify lignin extensively. The efficient wood destroyers are the white rot and brown rot fungi of the class basidiomycetes (Glazer and Nikaido, 1995). In the present study, three white rot fungi, *Fomes lividus*, *Thelephorace sp.* and *Trametes versicolor* were collected from Western Ghats area of Tamil Nadu, India. *F. lividus* was isolated from the *Shorea robusta* logs, *T. versicolor* from *Acacia nilotica* Del. Sp. *indica* logs and *Thelephorace sp.* from the stumps of a burnt tree by the method of Ray Watling (1971).

For the maintenance of wood rot fungi, many culture media like wood flour amended Fukazumi medium (Feniksova *et al.*, 1972), Ben 10 agar medium containing 10 ppm benomyl in 5 per cent malt agar (Hale and Savory, 1976) and Abram’s medium (Hegarty and Curran (1984, 1985) were recommended. In the present study, 2 per cent malt agar medium was used for the maintenance of white rot fungi.

Many methods have been proposed to determine the ligninolytic activity of the white rot fungi. These methods are based on the ability of the fungi to degrade or metabolise synthetic $^{14}$C lignins to $^{14}$CO$_2$ (Kirk *et al.*, 1978), crude and fractional straw lignin (Orth *et al.*, 1993), tannin and galic acid (Akhmedvoa *et al.*, 1994), unbleached hard wood kraft pulp (Iiomori *et al.*, 1994), 2 keto- 4- thiomethyl butyric acid (KTBA) to liberate ethylene (Glenn *et al.*, 1983), polymeric dyes like poly R - 481 and poly Y-
remazol brilliant blue R dye (Janshekar and Fiechter, 1983) and poly R-478 (Freitag and Morrel, 1992). Since the polymeric dyes are inexpensive, obtained commercially in high purity, stable and readily soluble having high extinction coefficients and low toxicity towards fungi, they are preferred for screening ligninolytic fungi. The decolourization of polymeric dyes is correlated with the onset of secondary metabolism and ligninolytic activity. A variety of inhibitors of lignin degradation, including thiourea, azide and 4-O-methyl isoeugenol also inhibited dye decolourization. Vyas and Molitoris (1995) reported that white rot fungus Pleurotus ostreatus produced an extracellular $\text{H}_2\text{O}_2$ requiring remazol brilliant blue R (RBBR) decolourizing enzymatic activity along with manganese peroxidase, manganese dependent peroxidase and phenol oxidase activity and ligninolytic capability. Kuwahara et al. (1984) reported two $\text{H}_2\text{O}_2$ dependent oxidases in the extracellular medium of the white rot fungi P. chrysosporium; these enzymes catalyse the oxidation of phenol red, O-dianisidine, poly R and variety of other dyes; the enzyme fractions generated ethylene from KTBA in the presence of alcohol. In the present study, ligninolytic activity of the selected fungi were tested based on their ability to decolourize the polymeric dyes, poly R and remazol brilliant blue to degrade native lignin and to generate ethylene from KTBA; for dye degradation studies, C-limited medium of Janshekar and Fiechter (1988) was used. The results revealed that all the selected fungi were capable of oxidizing the dyes indicating that all of them possessed ligninolytic activity (Table 1). The maximum degradation of poly R dye (25.19%) was brought by T. versicolor and minimum degradation (11.35%) by F. lividus. In remazol brilliant blue, maximum degradation (35.66%) was observed in T. versicolor cultures and minimum (14.09%) in Thelephorace sp.

To confirm the ligninolytic activity of the fungi, the ability of the fungi to grow and to degrade lignin was studied in lignin amended basal medium (Kirk et al., 1978). The growth was measured in terms of increase in mycelial dry weight (mg) per day. The results revealed that all fungi were capable of utilizing lignin as growth substrate. The mycelial growth rate was observed in the range of 2.17 to 2.70 mg/day. Minimum growth was observed in Thelephorace sp. (2.17mg/day) and maximum
The percent lignin degradation was higher (52.00%) in *T. versicolor* and lower (41.67%) in *F. lividus*.

The ligninolytic activities of the fungi were further confirmed by their ability to release ethylene from KTBA. Generation of ethylene from KTBA is an unique property of ligninolytic organisms (Glenn *et al.*, 1983). *T. versicolor* released high amounts of ethylene (2.58 ppm) from KTBA, followed by *Thelephorce sp.* (2.15 ppm) and least in *F. lividus* (1.73 ppm). Among the three fungi, *T. versicolor* was found to be more efficient lignin degrader.

The lignin decomposition was affected by the culture conditions like agitation, incubation period, pH, temperature, carbon and nitrogen sources. Linko and Zhong (1987) reported that immobilised mycelium of *P. chrysosporium* produced high amounts of lignin peroxidase on seventh day; similarly Willershausen *et al.* (1987) and Asther *et al.* (1988) observed maximum ligninase production on sixth and seventh day in *P. chrysosporium*. Kreitsberg *et al.* (1981) found high laccase activity in culture filtrates of *Pogonomyces hydnoides* on fourth day. Nikupaavola *et al.* (1990) observed maximum laccase production in *Phlebia radiata* on day 3 to 5 and it rapidly disappeared before the activities of MnP and LiP increased. Dhaliwal *et al.* (1992) reported that *Pleurotus florida* produced high amount of laccase (4.60 U/ml) in malt extract broth after twelve days growth under stationary conditions. In the present study, it was observed (Table 2) that in shaken condition, maximum growth in the cultures of *F. lividus* occurred on twelfth day of incubation; in *Thelephorce sp.* it was on sixth day and in *T. versicolor*, the maximum growth was observed on seventh day of incubation. In ligninase enzyme production, LiP and MnP were produced in higher amounts on seventh day and laccase on fourth day in all the three fungi.

The medium pH was critical to lignin decomposition. Kirk *et al.* (1978) observed the optimum culture pH for lignin decomposition as 4.0 to 4.5, with marked suppression above 5.5. and below 3.5 in *P. chrysosporium*. Haemmerli *et al.* (1987) reported that various oxidation products in the enzyme reaction were dependent on the
reaction pH and the oxidation was favoured between pH 4.0 and 4.5. Tran and Chambers (1987) observed the pH optimum for *P. chrysosporium* to bleach hardwood kraft pulp as 3.5. Janshekar and Fiechter (1988) reported pH 3.5 as optimum for ligninase production by *P. chrysosporium*. Niku-Paavola *et al.* (1988) reported that *Phlebia radiata* enzymes showed optimal activity at pH 3.0-4.0 and were stable in the pH range of 5.0 - 6.0; LiP of *P. chrysosporium* was also active at acid pH values. Kling *et al.* (1991) reported that in *P. chrysosporium* the optimum pH for ligninase production was 3.5. The pH optimum for lignin peroxidase synthesis by *P. chrysosporium* INA-12 was reported to be 4.5 (Asther *et al.*, 1988). Generally the optimum pH for growth was slightly higher than for ligninase production (Kirk *et al.*, 1978; Schmidt *et al.*, 1989).

In the present study it was observed (Table 3) that the optimum pH for growth of *F. lividus* was 4.0 and that of *Thelephore sp.* and *T. versicolor* were 5.0 and 5.5 respectively. As reported by Kirk *et al.* (1978) and Schmidt *et al.* (1989), the optimum pH for LiP and MnP were found to be slightly lower than that of growth. In *Thelephore sp.* the optimum pH for LiP and MnP production was 4.5; in *T. versicolor* the optimum pH for LiP production was found to be 4.0 and for MnP it was 4.5; similarly in *F. lividus* MnP production was found to be maximum at pH 4.5, but LiP production was found to be maximum at pH 4.0; laccase production was favoured by higher pH levels. In all the three fungi, laccase production was maximum at pH 6.0.

Like pH, temperature also could influence the enzyme production. Leisola *et al.* (1985) and Kirk *et al.* (1986) observed that optimal temperature for maximum lignin peroxidase production by *P. chrysosporium* BKM - F-1767 varied between 37°C and 39 to 40°C. Tran and Chambers (1987) reported that ligninase production by *P. chrysosporium* was maximum at 38°C ; similar observation was made by Janshekar and Fiechter (1988) in *P. chrysosporium* cultures. Farrell (1986) and Niku-paavola *et al.* (1988) observed temperature of 40°C as optimum for ligninase production by *Phlebia radiata* and lignin peroxidase of *P. chrysosporium* required a slightly higher temperature of about 45°C (Niku-paavola *et al.*, 1988).
Asther et al. (1988) correlated two temperature optima for lignin peroxidase synthesis by *P. chrysosporium* INA - 12; one at 37°C for the mycelium growing phase; the other at 30°C for the lignin peroxidase producing phase. In the present study it was observed (Table 4) that for *F. lividus* mycelial growth, the optimum temperature was 30°C, whereas, the ligninases production required a slightly higher temperature; for LiP and MnP production the optimum temperature was 40°C and for laccase it was 35°C. Similar trend was observed in *Thelephorce* sp. also. The mycelial growth in *Thelephorce* sp. was favoured by 35°C, but LiP and MnP productions were maximum at 40°C; laccase production was maximum at 35°C. In *T. versicolor*, both mycelial growth and LiP and MnP productions were maximum at 40°C and laccase at slightly a low temperature of 35°C. All the three enzymes were produced up to the culture temperature of 70°C.

Collins and Dobson (1995) reported that carbon at 10 g/l concentration could enhance growth and ligninolytic enzyme production and the increase in ligninase production at higher concentration was mediated at the transcription level. Gurusamy (1997) reported that glucose at 3 g/l concentration favoured mycelial growth and aryl-alcohol oxidase production in *Phanerochaete chrysosporium*; LiP and MnP productions were maximum at 2.5 g/l concentration; lignin at 3.5 g/l concentration favoured mycelial growth and 2.5 to 3.0 g/l favoured ligninase production; tannin at higher concentration (3.0 g/l) favoured mycelial growth and at lower concentrations (2.0 to 2.5 g/l) favoured enzyme production. In *Ganoderma colossum* cultures, glucose and tannin at 2.5 g/l concentration favoured mycelial growth and enzyme production, whereas, lignin at lower concentration (2.0 g/l) favoured mycelial growth and at higher concentrations (2.5 to 3.0 g/l) favoured enzyme production. In *Polyporous leucospongia* higher concentration of carbon sources favoured mycelial growth and the enzyme production was higher at slightly lower concentrations of carbons.

In the present study it was observed (Tables 5a-c) that in *F. lividus*, glucose at 2.5 g/l concentration favoured mycelial growth and LiP production; MnP and laccase
productions were maximum at 3.0 g/l concentration; in *Thelephorce sp.* growth and MnP and laccase productions were maximum at 2.5 g/l concentration; LiP production was maximum at 3.5 g/l concentration. In *T. versicolor*, growth and laccase production were favoured by 3.0 g/l concentration and peroxidases productions were favoured by slightly higher concentration (3.5 g/l).

In cellulose amendments, maximum mycelial growth in all the three fungi was observed at 3.5 g/l concentration; LiP production was also at its maximum at 3.5 g/l cellulose in *F. lividus* and MnP at 3.0 g/l concentration; but in *Thelephorce sp.* and *T. versicolor*, both LiP and MnP were produced in higher amounts at 3.0 g/l cellulose concentration; laccase production was favoured by 2.5 g/l concentration in *F. lividus*, 3.5 g/l in *Thelephorce sp.* and 2.0 g/l concentration in *T. versicolor*.

Lignin at 3.0 to 3.5 g/l concentration favoured mycelial growth of all the three fungi; 3.0 g/l lignin favoured LiP and MnP productions in *F. lividus* and LiP, MnP and laccase production in *Thelephorce sp.*; in *F. lividus*, laccase production was favoured by 3.5 g/l lignin. In *T. versicolor* lignin at 2.0 g/l concentration favoured laccase production, at 2.5 g/l concentration favoured LiP production and at 3.0 g/l favoured MnP production.

In general, it was observed that lignin favoured mycelial growth and laccase production (in *T. versicolor* laccase production was favoured by glucose); glucose favoured MnP production and cellulose favoured LiP production (except in *Thelephorce sp.*).

It was reported that nitrogen limited (growth limiting amount) medium stimulate ligninolytic enzymes production (Kirk *et al.*, 1978; Janshekar *et al.*, 1982; Rios and Eyzaguirre, 1992; Kaal *et al.*, 1995; Costa-ferriera *et al.*, 1996); but Janshekar *et al.* (1982) stated that it cannot be accepted as a general principle. There are reports stating that both low and high nitrogen media enhance ligninase production. (Shimada *et al.*, 1989; Katagiri *et al.*, 1995; Entry and Backman, 1995).
In the present study, diammonium tartrate, urea and yeast extract were used as nitrogen sources. The results revealed that only the nitrogen limited medium favoured ligninase production and nitrogen at higher amounts became inhibitory for enzyme productions (Tables 6a-c).

In *F. lividus* an increase in growth was observed with increase in diammonium tartrate content of the medium. Even the highest concentration tried (1.10 g/l) was not inhibitory for growth; but in *Thelephoraceae sp.* and *T. versicolor*, the higher concentration was found to be inhibitory for mycelial growth. In *F. lividus* and *Thelephoraceae sp.* 0.44 g/l concentration favoured LiP production; MnP production was maximum at 0.22 g/l concentration in both the fungi; but laccase production was found to be maximum at 0.66 g/l concentration in *F. lividus* and at 0.44 g/l concentration in *Thelephoraceae sp.*; in *T. versicolor*, 0.66 g/l concentration favoured LiP and MnP production and laccase production was maximum at 0.44 g/l concentration. Urea at 0.66 g/l concentration yielded maximum growth in *F. lividus* and *Thelephoraceae sp.*; in *T. versicolor* even the highest concentration (1.10 g/l) was not inhibitory to fungal growth; 0.66 g/l concentration yielded maximum growth in *F. lividus* and *Thelephoraceae sp.*; in *T. versicolor* all the three enzymes productions were at their maximum at 0.66 g/l concentration.

Yeast extract at 0.88 g/l concentration yielded maximum mycelial growth in all the fungi; but at 1.10 g/l it became toxic. In *F. lividus* the concentration optimum for mycelial growth was found to be optimum for LiP and laccase production, but MnP was produced in higher amounts at 0.44 g/l yeast extract. In *Thelephoraceae sp.* and *T. versicolor*, a slightly lower concentration of 0.66 g/l was found to be optimum for enzyme production.

From the present study, it may be interpreted that the optimum incubation period for growth was twelve days in *F. lividus*, six days in *Thelephoraceae sp.* and seven days in *T. versicolor*; LiP and MnP productions needed seven days incubation and
Laccase required four days incubation; the growth and production of LiP and MnP were found to be high in acidic pH (pH 4.0 - 5.5) and laccase was favoured by near neutral pH (pH 6.0 - 7.0); the temperature optima for growth and enzyme production were in the range of 30 - 45°C; carbon sufficient or rich medium favoured growth and enzyme production, whereas nitrogen sufficient or nitrogen limited medium favoured growth and enzyme production.

The ligninolytic enzymes produced by the white rot fungi were purified from the culture filtrates by acetone precipitation followed by dialysis and sephadex G-100 column chromatography (Tables 8 - 10; Fig. I). *T. versicolor* produced high amounts of LiP than *F. lividus* and *Thelephorce sp.;* when the enzyme preparations were subjected to sephadex G 100 column chromatography, in *F. lividus,* two fractions showed LiP activity other two fungi yielded three fractions with LiP activity (Table 8; Fig. I to III); MnP was produced in higher amounts by *F. lividus* than the other two fungi and it showed only one active peak (Table 9; Fig. I to III); *T. versicolor* produced higher amounts of laccase than *F. lividus* and *Thelephorce sp.* and the laccase activity was observed in three fractions (Table 10; Fig. IV to VI).

Niku-paavola et al. (1988) reported that LiP of white rot fungus, *Phlebia radiata* had pH optimum at 3.0-4.5, but stable in the range 5.0-6.0. Kling et al. (1991) observed that for lignin peroxidase from *P. chrysosporium,* the optimum pH was 3.5. Cui et al. (1993) reported that for lignin peroxidase of *P. chrysosporium,* the optimal pH was around 3.0 and it varies for different isoenzymes. The optimal pH for the oxidation of veratryl alcohol ranges from pH 2.0-10.0 (Kirkpatrick and Palmer, 1989). For LiP of *Phlebia radiata* the optimum pH was 3.0-5.0 (Niku-paavola et al., 1990). In the ascomycetes fungus, *Chrysonilia sitophila* also LiP was active in the pH range of 3.0-5.0. (Ferrer et al.,). Gelpke et al. (1999) reported that *P. chrysosporium* LiP had an optimal pH of 4.5. In the present study, it was observed that LiP of all the three fungi *F. lividus,* *Thelephorce sp.* and *T. versicolor* had an optimum pH of 3.5 (Table IIa; Fig. VII).
Niku-Paavola et al. (1988) observed that *Phlebia radiata* and *P. chrysosporium* LiP required slightly higher temperature of 45°C for optimal activity. LiP of *P. chrysosporium* and *Phlebia* sp. were active at 40°C (Farrell, 1986; Ferrer et al., 1992). Heinzl et al. (1998) reported that the peroxidases of *Coprinus friesii* were active up to 60°C for 60 min of incubation. Gurusamy (1997) reported that optimum temperature for LiP of *P. chrysosporium*, *Ganoderma collosum* and *Polyporous leucospongia* were 40, 45 and 50°C respectively. In the present study the optimum temperature for LiP activity was observed to be in the range of 40-50°C. For LiP from *F. lividus*, the optimum temperature was 50°C and for *Thelephorace sp.* and *T. versicolor* the optimum temperature was 45°C (Table 11a; Fig. VII).

Kirkpatrick and Palmer (1989) reported that *V* max of *P. chrysosporium* LiP isoenzymes were 3.1, 2.7 and 1.6 U/ml against veratryl alcohol and the corresponding *Km* values were 108, 121 and 269 μM. Gelpke et al. (1999) reported that recombinant lignin peroxidase of *P. chrysosporium* had a *Km* (μM) value of 89.4-92.8 μM for veratryl alcohol. *V* max observed in the present study were 1020, 917 and 820 U/mg protein (for veratryl alcohol) respectively for LiPs of *F. lividus*, *Thelephorace sp.* and *T. versicolor* and the corresponding *Km* values were 120, 60 and 100 μM (Table 11a; Fig. IX, X and XI).

Kirk (1983) reported that *P. chrysosporium* LiP had molecular weight of 42,000 Da. Niku-Paavola and Karhunen (1990) observed that *Phlebia radiata* produced three isoenzymes of LiP having molecular weights of 42,000, 45,000 and 49,000 Da and the corresponding pI values were 4.1, 3.9 and 3.2. Kirk et al. (1985) reported five isoenzymes of LiP in the molecular weight range of 38,000 to 46,000 Da from *P. chrysosporium*. Leisola et al. (1987a) reported that pI values of *Phanerochaete* lignin peroxidases ranged from 3.2 to 4.4. Niku-Paavola et al. (1988) observed that *Phlebia radiata* produced three isoenzymes of lignin peroxidases with molecular weights of 42,000, 45,000 and 44,000 Da. Palmer (1987) reported that *P. chrysosporium* ligninases had pI values of 3.0 to 5.0 and molecular weights in the range of 39,000 to 43,000 Da.
Kantilinen et al. (1989) observed two isozymes of LiP with pI values of 4.15 and 4.65 in P. radiata. Ferrer et al. (1992) reported that the ascomycete, Chrysonilia sitophila produced three isozymes of LiP with molecular weights of 68,000, 50,000 and 47,000Da. Vares et al. (1992) observed three LiPs with pI values of 3.4 to 3.5 and molecular weights of 43,000 to 47,000Da in Junghunia sepaparabilima. T. versicolor strain PRL 572 produced sixteen LiP isozymes with pI values ranging from 3.1 to 3.7 and molecular masses ranging from 41,000 to 43,000Da (Johanson and Nyman, 1993). Reddy and Souza, (1994) isolated fifteen LiP isozymes with pI values ranging from 3.3 to 4.7 and molecular weights ranging from 38,000 to 43,000Da from P. chrysosporium culture filtrates. Gold and Alic (1993) isolated two LiP isozymes with pI values of 3.2 and 4.0 and molecular weights of 38,000 to 43,000Da from P. chrysosporium. Cai and Tien (1993) reported six isozymes having pI and molecular weights in the range of 3.3 to 4.7 and 38,000 to 48,000Da respectively. Heinzkill et al. (1998) reported that Coprinus friesii produced peroxidase enzyme which had molecular weight of 45,000Da and pI value of 3.6. Similarly another species of Coprinus cinereus produced peroxidase enzyme with pI value of 3.5 and molecular weight of 39,000Da. In the present study, two active fractions of LiP was isolated from the culture filtrates of F. lividus. The first fraction had pI of 3.6 and the second 3.2. The molecular weights were 38,600 and 18,300Da. In other two fungi, Thelephorce sp. and T. versicolor, three fractions exhibited LiP activity. The pI values of Thelephorce sp. enzymes were 4.2, 3.9 and 3.4 and the molecular weights were 41,000, 39,600 and 38,400Da. The pI values of T. versicolor were 3.9, 3.7 and 3.4 and the molecular weights were 43,000, 40,000 and 41,000Da (Table 1a ;Plate Ia and Ib).

Heinzkill et al. (1998) reported that the optimum pH for the basidiomycetes fungi Panaeolus sphinctrinus and P. chrysosporium manganese dependent peroxidase was in the range of 4.0 to 5.0. Kuan et al. (1993), Chung et al. (1993) and Ruttiman et al. (1994) reported pH of 4.5 to 5.0 as optimum for MnP of P. chrysosporium. In the present study it was observed that optimum pH for MnP obtained from all the three fungi was 4.5 (Table 1b; Fig. VII).
Malteeva et al. (1991) and Golovleva et al. (1993) reported temperature of 25°C as optimum for MnP activity. Gurusamy (1997) reported temperature of 40 to 45°C as optimum for MnP activities of *P. chrysosporium*, *G. colossum* and *P. leucospongia*. In the present study MnP of *F. lividus* showed maximum activity at 55°C; whereas, *Thelephorce* sp. and *T. versicolor* at 45°C (Table 11b; Fig. VIII).

Heinziekill et al. (1998) reported that MnP of basidiomycetes fungi, *P. sphinctrinus* and *P. chrysosporium* had Km values of 53.1 and 50 μM for H₂O₂. Gelpke et al. (1999) reported that MnP of *P. chrysosporium* had Km of 47.1 μM for H₂O₂. Leontievsky et al. (1990) reported a Km of 4.35 x 10⁻⁵ M for MnP against H₂O₂; for ABTS the Km values were 5.4 x 10⁻² (Leontievsky et al., 1990) and 5.26 x 10⁻³ M. (Golovleva et al., 1993). In the present study Vₘₐₓ and Km values of MnP were determined against H₂O₂. MnP of *F. lividus* had a Vₘₐₓ of 4.40 U/mg protein and Km of 66.67 μM; *Thelephorce* sp. MnP had a Vₘₐₓ of 4.29 and Km of 50 U/mg protein; Vₘₐₓ and Km values of *T. versicolor* MnP were 5.0 U/mg protein and 33.33 μM respectively(Table 11b, Fig. XII, XIII and XIV).

Passezynoki et al. (1985) and Asada et al. (1986) reported that MnP from *P. chrysosporium* had a molecular weight of 45,000 to 47,000Da. Niku-paavola and Karhunen (1990) observed that *Phlebia radiata* MnP had a molecular weight of 49,000Da and pl of 3.8. Leisola et al. (1985a,b; 1987a, b) isolated six isoenzymes of MnP from *P. chrysosporium*; the molecular weight was 39,000Da and pl values ranged from 4.2 to 4.9. Kuwahara et al. (1984) reported that white rot fungi *P. chrysosporium* produced the MnP having a molecular weight of 46,000Da. Karhunen et al. (1990) reported that the MnP of the white rot fungus *Phlebia radiata* had a molecular weight of 49,000Da and pl of 3.8. Niku-paavola et al. (1990) isolated two MnP fractions having pl of 4.2 and 4.9 from culture filtrates of *Phlebia radiata*. The molecular weights of these fractions ranged between 45,000 to 49,000Da and 68,000 to 70,000Da. Malteeva et al. (1991) observed that *Panus tigrinus* produced manganese dependent peroxidase (MnP) having a molecular weight of 43,000 and pl values of 3.2 (minor) and 2.95
T. versicolor culture filtrates produced two MnP fractions having pI values of 3.2 and 4.9 (Addleman and Archibald, 1993). Gold and Alic (1993) observed that MnP of P. chrysosporium had molecular weight in the range of 45,000 to 47,000Da and pI values 4.2 to 4.9. Johansson and Nyman (1993) reported five MnP fractions from T. versicolor with values of 2.9 to 3.2 and molecular weights of 44,000 to 45,000Da. Phanerochaete sordida produced three MnP isoenzymes which had molecular weight of around 45,000Da (Ruttiman et al., 1994). Heinzkill et al. (1998) reported that Panaeolus sphinctrinus MnP had a molecular weight of 46,000Da. Urzva et al. (1995) isolated manganese peroxidase isoenzymes with a molecular weight of 52,500Da from Ceriporiopsis subvermispora. MnP isolated in the present study showed only one band on SDS-PAGE plates (Plate Ia and Ib). MnP of all the three fungi studied had pI values between 3.2 to 4.4. MnP of F. lividus had a pI of 4.4, Thelephorce sp. 3.9 and T. versicolor 3.2. Molecular weights of these enzymes were in the range of 45,000-48,000Da; MnP of F. lividus had a molecular weight of 48,000Da and that of Thelephorce sp. and T. versicolor were 46,000 and 45,000Da respectively (Plate Ia, and Ib).

Coll et al. (1993) reported that laccase of a new basidiomycetes strain PMI (CEAT 2971) was stable in a pH range of 3 to 9 and its optimum pH was 4.5. Fukushima and Kirk (1995) reported higher laccase activity in Ceriporiopsis subvermispora at pH 3.0. Youn et al. (1995) reported that the optimum pH for laccase activity in Pleurotus ostreatus was 6.0 to 6.5. Eggert et al. (1996) reported that purified laccase of Pycnoporus cinnabarinus was stable below pH 4.0 to 7.0. Heinzkill et al. (1998) reported that laccase purified from Panaeolus sphinctrinus and Coprinus friesii had optimum pH around 3.5. Thurston (1994) observed that ABTS laccase exhibit optimum activity in acidic pH of 3.0 to 5.0; when 2,6-DMP(dimethoxyphenol) was used as substrate, the laccase activity was maximum at pH values greater than 7.0 and the optimum pH is 7.0 to 8.0. In the present study also the optimum pH of laccase was found to be in slightly acidic range, that is, 5.0 to 6.0; for F. lividus and T. versicolor laccases the optimum pH was 5.0 and for Thelephorce sp. laccase, the optimum pH was 6.0. (Table 11c; Fig. VII).
The temperature optimum for laccase activity in the lignin degrading basidiomycete strain PMI (CECT 2971) was reported to be 80°C and the enzyme was stable for 1 h at 60°C (Coll *et al.*, 1993). Youn *et al.* (1995) observed that the temperature optimum for *Pleurotus ostreatus* laccase activity was 30 to 35°C. Eggert *et al.* (1996) reported that purified laccase enzyme of *Pycnoporus cinnabarinus* had optimum temperature of 80°C; but the enzyme was very stable below 50°C. In the present study, it was observed that laccase enzyme of *F. lividus*, *Thelephorce sp.* and *T. versicolor* had an temperature optimum of 35°C (Table 11c, Fig. VIII).

Youn *et al.* (1995) reported that the fungal laccase had Km value of 48 μM and 89 μM for ferulic acid and syringic acid. In the present study, it was observed that for guaiacol, *F. lividus* laccase had a Km of 40 μM, the Vmax was 21.67 U/mg protein; *Thelephorce sp.* had a Km of 35 μM, the Vmax was 21.67 U/mg protein; Km and Vmax values for *T. versicolor* laccase were 41 μM and 41 U/mg protein respectively (Table 11c, Fig. XV, XVI and XVII).

Niai-paavola and Karhunen (1990) isolated an extracellular oxidase (OX) from *Phlebia radiata* with pI values of 3.5 and molecular weight of 64,000Da. Maltseva *et al.* (1991) reported that *Panus trigrims* produced two groups of laccase with pI values of 3.0 (major) and 2.9 (minor compounds) and molecular weight of 64,000Da. Coll *et al.* (1993) isolated laccase from a new lignin degrading basidiomycete strain PMI (CECT 2971) having a molecular weight of 64,000Da and an isoelectric point of 3.6. Fukushima and Kirk (1995) reported that higher laccase activity in *Ceriporiospsis subvermispora* was associated with two major isoenzymes which had pI of 3.4 and 4.8 and molecular mass of 71,000 and 68,000Da. In *Monocillum indicum*, on SDS PAGE, the major laccase band was further resolved into three proteins of molecular weight 72,000, 56,000 and 24,000Da. Salas *et al.* (1995) have isolated four laccase isoenzymes with pI values in the range of 3.63 to 2.46 from the culture filtrates of *Ceriporiopsis subvermispora* and a fifth isoenzyme of high pI (4.82) in bran medium. Perez *et al.* (1996) isolated laccase from *P. flavidoalba* having a molecular weight of 94,000Da and
pl value lower than 3.55. Yaver et al. (1996) purified laccases from culture filtrates of *Trametes villosa* with molecular weight of 63,000Da and pl value in the range of 3.5 to 6.5 and 5 to 6.

Eggert et al. (1996) reported that *Pycnoporus cinnabarinus* purified laccase enzyme had only a single polypeptide with molecular mass of 81,000Da and pl of 3.7. Scherer and Fischer (1998) reported that *Aspergillus nidulans* produced laccase having molecular mass of 80,000Da. Garzillo et al. (1998) reported that *Trametes trogii* produced an extracellular phenol oxidase having molecular mass of 70,000Da and with an acidic pl value. Heinzkill et al. (1998) reported that laccase from *Panaeolus sphinctrinus* and *Coprinus friesii* had molecular weight of 60,000Da. In the present study, laccase enzyme of all the three selected fungi showed three active fractions on sephadex G 100 column chromatography. In *F. lividus*, the pl values were 4.2, 3.6 and 4.0 and the molecular weights were 67,000, 48,500 and 38,100Da. In *Thelephorce* sp. laccase, the pl values were 3.5, 3.7 and 4.1 and the molecular weights were 64,000, 46,700 and 38,700Da. *T. versicolor* laccase had pl values of 3.9, 3.8 and 3.6 and the molecular weights were 68,100, 48,500 and 34,200Da. (Plate 1a and 1b)

Ligninolytic fungi *Fomes lividus, Thelephorce* sp. and *Trametes versicolor* and their enzymes (LiP, MnP and laccase) were tried for various biotechnological processes like pretreatment of wood chips in paper manufacturing, biopulping and bleaching of hardwood kraft pulp, deinking of waste papers, degradation of azodyes and treatment of industrial effluents particularly paper and dye industry effluents; the fungi were further used for pretreatment of lignocellulosic wastes in vermicomposting.

In the pulp and paper industry for delignification of wood fibres, the wood chips are cooked either in alkaline medium (kraft pulping) or in sodium sulphite (sulphite pulping). The residual lignin was removed by pretreatment with chemical bleaching agent. This process involves high cost and it generates a lot of pollution problem. So, in recent years, biopulping is tried to avoid chemical usage and to reduce the cost. The first sustained efforts towards this goal took place in the laboratory of Karl Kirk Eriksson at
the Swedish forest product laboratory. Pretreatment of wood chips with ligninolytic fungi to decrease the energy requirement of subsequent mechanical pulping and to increase the strength of the pulp produced has been the most successful approach. Fungal pretreatment with mechanical pulping resulted in energy saving and improved pulp and paper properties (Eriksson and Mikschen, 1974). Later in 1987, biopulping consortium was created by Kent Kirk of forest products laboratory in Madison, USA and within first five years a lot of pioneering work was done on the pretreatment of wood chips for mechanical pulping. Pilon et al. (1982) examined the effect of coarse mechanical pulp after primary defibration. Treatment of such pulp with various ligninolytic fungi, notably T. versicolor, P. chrysosporium and Pleurotus ostreatus increased the tensile strength. Sachs et al. (1990) reported that fungal pretreatment of wood chips with Phlebia tremellosa increased the burst strength by 270 per cent and tear strength by 210 per cent. Leatham et al. (1990) showed the effect of incubating aspen chips for four weeks with various fungi; Phlebia brevaspora decreased the refining energy requirement by 47 per cent; in Ceriporiopsis subvermispora treatment, the energy saving was 68 per cent; Phlebia brevaspora and Dichomictus squalens also saved sustained amount of energy. Leatham et al. (1990) demonstrated that pretreatment of hardwoods with ligninolytic fungi increased the strength of paper made from the resulting biomechanical pulp. Phlebia tremellosa produced an 80 per cent increase in the tensile strength. P. chrysosporium, P. subserialis, P. brevaspora, P. mutabils, D. squalens and Perenniporia medulla and Panus sp. also increased the tensile strength of pulp significantly. Oriaran et al. (1990) reported that treating aspen chips with P. chrysosporium before kraft pulping improved the tensile and burst strength of the pulp, but decreased its tear strength, brightness and yield. Messener et al. (1992) pretreated birch wood chips with white rot fungi for four to six weeks prior to pulping; the treatment resulted in a 30 to 50 per cent reduction in kappa number, 0 to 4 per cent increase in ISO units of brightness and 10 per cent decrease in paper strength. Messener et al. (1992) worked on fungal pretreatment of wood chips for chemical pulping; P. chrysosporium decreased the kappa number by 20.5 per cent after four weeks
incubation; four species of unidentified fungi decreased the kappa number by 33.1 to 44.2 per cent.

In the present study, the *Eucalyptus grandis* wood chips were pretreated with the ligninolytic fungi for a period of one month prior to chemical pulping. In the first set of experiments (T1), the fungal pretreated pulp was subjected to conventional chemical pulping and bleaching process (Buchert *et al.*, 1992); in treatment two (T2), the pretreated pulp was subjected to conventional chemical pulping followed by 50 per cent chemical dosage of bleaching; in treatment three (T3), fungal pretreated chips were subjected to 50 per cent chemical dosage of pulping followed by conventional chemical bleaching and in treatment four (T4), it was subjected 50 per cent chemical dosage of bleaching. Pulp prepared by conventional method served as control and it had a kappa number of 20.1 and brightness of 14.0 ISO units (Flow chart). The fungal pretreatment followed by conventional pulping and bleaching (T1) resulted in 52.39 to 56.72 per cent higher reduction in kappa number when compared to the control samples; the brightness was increased by 25.0 to 37.43 per cent; in T2, the kappa number was reduced by 37.07 to 47.73 per cent and brightness was increased by 30.71 to 41.43 per cent; in T3 the kappa number reduction was 30.75 to 35.07 per cent and in T4 it was 26.42 to 30.75; both the treatments increased the brightness by 25.0 to 27.14 and 13.93 to 24.64 per cent respectively. Comparatively, kappa number reduction was higher in *T. versicolor* pretreatments; but increase in brightness was higher in T1 of *Thelephorace sp.*, T2 and T3 of *F. lividus* and T4 of *T. versicolor*.

Archibald (1992a) observed that the fungus *T. versicolor* was capable of decolourizing and delignifying the unbleached industrial kraft pulps over 2 to 5 days incubation; after three days incubation, the final pH was 4.5 and the brightness was increased by 32 to 45.8 per cent. Paice *et al.* (1993b) reported that *T. versicolor* culture increased the brightness of the Canadian kraft pulps by 28.0 per cent after one week incubation period; the kappa number was reduced by 9.56 per cent. Addleman and Archibald (1993) studied the ability of 10 dikaryotic and 20 monokaryotic strains of *T. versicolor* to bleach and delignify hard wood and soft wood kraft pulps. They have
observed an increase of 20 brightness points in hard wood kraft pulp after five days incubation and the same amount after 12 days in soft wood kraft pulps; the kappa number was reduced by 5.6 and 16.5 units respectively in hard wood and soft wood kraft pulps. Reid et al. (1994) reported that the white rot fungus T. versicolor can delignify and brighten the unbleached hard wood kraft pulp within a few days, but soft wood kraft pulps required longer treatment period; this aspect was attributed to the higher residual lignin contents (kappa number), structural differences in lignins and to the recalcitrance of soft wood kraft pulps to bleaching. Iimori et al. (1994) compared the bleaching ability of the new fungus, SKB 1152 with that of P. chrysosporium and T. versicolor; the new fungus increased the brightness of an oxygen bleached hard wood kraft pulp from 51.5 per cent to 80 per cent on seven days incubation period; typical white rot fungi P. chrysosporium and T. versicolor increased the brightness by 64 and 70 per cent respectively. Dunlop et al. (1995) screened 1200 white rot and white pocket rot fungi for their ability to biologically bleach eucalyptus kraft pulp and they observed that of the 1200 isolated fungi, 100 of them effectively bleached kraft pulp to a brightness of greater than 55 per cent. Christov et al. (1995) reported that white rot fungus Ceriportopsis subvermispora bleached the pulp effectively after 14 days of incubation; the kappa number was decreased from 6.7 to 0.8 and the brightness was increased by 47 per cent.

Nishida et al. (1995) reported that when hard wood unbleached kraft pulp (UKP) was treated with P. chrysosporium and T. versicolor in the solid and liquid state fermentation system with four different culture media, pulp brightness was increased by 15 and 30 points after five days of treatment and observed a positive correlation between the kappa number decrease and brightness increase of the fungus treated pulp. Scott et al. (1995) reported that in sodium bisulfite pulping, the fungal pretreatment reduced the kappa number by 27 per cent, but calcium based sulfite pulping reduced the kappa number by 21 to 48 per cent; in calcium based sulfite pulping, the brightness of the pulp was increased from 54 to 80 per cent, whereas, the fungal pretreatment increased the brightness of the pulp from 49 to 80 per cent. Moreia et al. (1997) reported that the white rot fungi Bjerkandara sp. strain BOS 55 extensively delignified and bleached the
oxygen delignified eucalyptus kraft pulp and brightness gains up to 14.0 ISO units were obtained.

In the present study, the white rot fungi, *F. lividus*, *Thelephorce* sp. and *T. versicolor* and their ligninases, LiP, MnP and laccase, were used for biobleaching and delignification of *Eucalyptus grandis* hard wood kraft pulp (Table 12; Fig.XVIII) *F. lividus* fungal treatment reduced the pH of the reaction mixture from 6.84 to 4.20 after six days incubation period. The kappa number was reduced from 26.97 to 16.53 ISO units (38.71%) and the brightness was increased from 20.58 to 26.74 ISO units (29.93%) after six days incubation period. In *Thelephorce* sp. treatment, the pulp pH was reduced from 6.84 to 3.64 after six days of incubation period and kappa number was reduced by 64.52 per cent; maximum increase in brightness (29.78%) was observed after five days incubation period. *T. versicolor* treatment reduced the reaction pH from 6.84 to 3.28 on sixth day incubation period; five days incubation period reduced the kappa number by 67.34 per cent and increased the brightness by 29.78 per cent.

The largest processor of renewable raw materials, the pulp and paper industry, has until now used enzymes only to a limited extent. Only a few microorganisms in nature are able to attack, modify or destroy the rigid organic polymer lignin. The main producers of ligninolytic enzymes, lignin peroxidase (LiP), manganese dependent peroxidase (MnP) and laccase, were the white rot fungi. Two of the most important fungi are *P. chrysosporium* and *Coriolus* (*Trametes*) *versicolor*. These ligninolytic enzymes could also be employed in the pulp industry for bleaching and delignification purposes. (Reinhammer, 1984; Farrell 1986; Arbeola *et al.*, 1992; Archibald and Roy 1992; Paice *et al.*,1993). Archibald (1992b) by using new lignin peroxidase inhibitor (meta-vanadateions) and a new lignin peroxidase assay using the dye azure B, showed that secreted lignin peroxidase do not play a role in the *T. versicolor* pulp-bleaching system. Paice *et al.* (1993b) reported that *T. versicolor* enzyme, MnP, played a major role in the biobleaching process. The oxido-reductase laccase from *Coriolus versicolor* and a special group of mediators especially N-OH-N-oxide, Oxime- or hydroxamic acid compounds yielded a lignin removal of 50 to 70 per cent in a short term application (1-4
h) depending on enzyme and mediator dosage and the type of pulp used (Call, 1987, 1990, 1992). Paice et al. (1993a) reported that *T. versicolor* produced both the enzymes, laccase and manganese dependent peroxidase (MnP), but not lignin peroxidase during pulp bleaching; the peak production of the enzymes occurred at the scale up time as the maximum rate of fungal culture bleaching occurred. Addleman et al. (1995) demonstrated that *T. versicolor* mutant strains which lack or have very low secretion of laccase and manganese dependent peroxidase (MnP) were unable to bleach or delignify kraft pulp. Call and Muckle (1995) reported that treatment of different kinds of pulps with LIGNOZYMS laccase - mediator system (LMS) resulted in 70 per cent reduction in kappa number. Sealy and Ragauskas (1998) reported that the laccase / N-hydroxybenzotriazole system yielded 52 per cent delignification of soft wood kraft pulp, whereas, use of ABTS yielded 35 per cent delignification. From the literature it could be speculated that LiP is not playing any role in biobleaching and delignification of wood pulp while MnP and laccase could be effective delignifying agents.

In the present study, purified LiP, MnP, laccase and a mixture of these enzymes were used for biobleaching and delignification of hard wood kraft pulp (HWKP). As reported in earlier studies, in the present study also, it was observed that laccase was very efficient in bleaching and delignifying HWKP (Table 13). The kappa number was reduced by 36.11 per cent and brightness was increased by 15.15 to 22.54 per cent by laccase of all the three fungi. Next to laccase, MnP and mixed enzyme were found to be efficient in biobleaching and delignification of HWKP. In MnP treatment, kappa number reduction was in the range of 25.10 to 33.33 per cent and in mixed enzyme treatment it was 33.33 to 36.11 per cent, similarly, the brightness was increased by 15.11 to 20.98 and 13.63 to 21.09 per cent respectively by MnP and mixed enzyme treatment. LiP had very little effect on pulp brightness (6.5 to 9.99%).

The use of enzymes or microorganisms have a major role in waste paper recycling. The enzymes process, eliminates or substantially reduces the uses of chemicals in the deinking operation and can readily be retrofitted into current industrial processes. The pulp recycled by enzymatic deinking process found to posses better
physical properties like higher brightness, lower residual ink count and improved freeness. Enzymatic deinking process represents a new approach to convert second fibres into quality products. The office waste papers are an under utilised segment of the recycled fibre market. They contain significant amount of xerographic and laser toner printed papers that are difficult to deink. Toner polymers are fused to paper fibres and remain attached following conventional fiberization and deinking processes. Moreover, because toner binding agents are synthetic thermopolymers, they do not disperse easily. Commercial cellulases added to recycled paper along with surfactants during the fiberization stage facilitates toner removal during flotation and washing. Several different enzyme preparations are effective and under optimum conditions only low dosage of enzymes are required. As little as 0.02 to 0.1 per cent is sufficient to remove most of the residual particles.

Enzyme activity is strongly affected by paper components. Most recycled papers contain calcium carbonate and have an ambient pH of about 8.5, so enzyme which are alkaline active could be used for this purpose (Jeffries et al., 1995). Successful deinking of old newspapers /old magazine (ONG/OMG) and mixed office waste papers (MOW) has been accomplished using specially formulated mixtures of enzymes. The recycled pulps were found to possess better physical properties, higher brightness and lower residual ink counts compared to chemically deinked recycled paper. More importantly, size distribution and shape of ink removed from laser / xerographic printed papers could be effectively controlled using enzymatic deinking processes to maximise the efficiency of the size based floatation process (Yang et al., 1995). Ow et al. (1995) reported that cellulase blended with hemicellulase (blended cellulase) and lipase effectively deink the old newsprint pulps. Stork and Puls (1995) reported that endoglucanase treatment could increase the drainage of mechanical pulp fibres. Miletzky et al., (1995) worked on deinking technology towards neutral pH operation, using enzymes in the case of printing inks containing vegetable oils which are normally difficult to remove. In the present study, the white rot fungi F. lividus, Thelephorce sp. and their ligninases (LiP, MnP, laccase and mixture of these enzymes) were tried for deinking of office waste papers and newspapers. Kappa number and brightness were used as quality
parameters. pH of the reaction mixture was reduced by all the fungal treatments. In ink paper and newspaper the reduction was from 6.7 to around 5.0; but in photocopy paper, the pH was reduced up to 3.1. In ink paper, the fungal treatment reduced the kappa number by 58.3 to 66.7 per cent and increased the brightness by 38.98 to 45.07 per cent; in photocopy paper also reduction in kappa number was 58.3 to 66.7 per cent, but the brightness was increased by 53.67 to 75.81 per cent; the maximum (75.81%) in *F. lividus* treatment. In newspaper, the kappa number was highly reduced (50.0 to 64.3%) when compared to ink and photocopy papers; but the brightness was increased only by 11.37 to 17.20 per cent (Table 14; Fig. XIX).

When the enzymes were used for deinking process, in ink paper, the mixed enzymes reduced the kappa number by 33.3 to 50.0 per cent (Table 15). In LiP treatment, the kappa number reduction was 50 to 66.77 per cent; in the other two enzyme treatments, a maximum of 75 per cent reduction was observed. In all the treatments, the brightness was increased to more or less same level (12.07 to 15.05 %). In photocopy papers, the kappa number was reduced by 27.32 to 72.68 per cent; the brightness was increased by 41.51 to 54.61 per cent. Laccase and mixed enzyme treatments were found to be highly efficient in deinking of photocopy papers (Table 16). In newspapers also, laccase treatment yielded maximum reduction in kappa number (64.3 to 78.6%) and increase in brightness (19.12 to 20.82%), next to laccase, mixed enzyme treatment was found to be effective with a kappa number reduction of 50.0 to 57.1 per cent; brightness increase of 16.14 to 18.44 per cent; but LiP and MnP treatments were not effective (Table 17).

The pulp industries produce large quantities of waste water during various processes. Luonsi *et al.* (1987) reported that biological methods could be employed to reduce biological oxygen demand (BOD) along with toxicity in forest industry waste water. Pellinen *et al.* (1988) reported that treatment of alkali extraction stage liquor (E1) with a white rot fungus, *P. chrysosporium* for four days, resulted in 32 per cent COD reduction, 65 per cent colour removal and increased the liberation of inorganic chloride by 34 per cent from the original concentration. Bergbauer *et al.* (1991) screened...
20 species of fungi for colour removal in a bleach plant effluent; among the 20 species *T. versicolor* strains removed 67 to 70 per cent of the colour within six days; AOX content was decreased by 45 per cent. Bryant and Barkley (1991) developed a single stage aerobic biological treatment system to convert organically bound chloride to inorganic chloride; in this system 90 per cent reduction of AOX was achieved in synthetic dichlorophenol, commercial pentachlorophenol and combined kraft waste water in less than a week. Fukui *et al.* (1992) achieved dechlorination, detoxification and decolourisation of first alkaline extraction stage (Ep) effluent from a pulp mill bleach plant by *P. chrysosporium* treatment; in this treatment, the colour removal was 65 to 80 per cent and AOX reduction was 38.57 per cent. Lackner (1992) used the MYCOPOR reactor with *P. chrysosporium* immobilised on foam cubes for removal of colour bleach plant effluent. The effluent colour removal was 80 per cent after two days incubation. Sayadi and Ellouz (1993) observed that *P. chrysosporium* could remove 65 per cent of colour and 73 per cent COD from olive mill waste water, other fungi like *Phlebia radiata*, *Dictiomitus squalens*, *Polyporus frondosus* and *T. versicolor* also removed colour and COD but to a lesser extent. Martin and Manzanares (1994) found that *T. versicolor* biomass immobilised on a plastic surface and fungal pellets could yield 70 per cent COD removal after four days. Manzanares *et al.* (1995) reported that when effluent from straw soda pulping industry was treated with *T. versicolor*, 80 per cent decolourization was obtained after four to five days incubation, phenolic content was reduced to about 90 per cent. Kang *et al.* (1995) reported that when primary treatment waste water was treated by a submerged biofilter system, maximum decrease in COD (90 to 95%) was achieved in 12 hours. Calvo *et al.* (1995) recommended eighty three ligninolytic fungi to use as alternatives for paper mill effluent treatment. Retinatto *et al.* (1995) reported that *Psychozor sanguineus* (VES-2050) and *Thermoascus auranticus* (VES-2070) were most efficient thermophilic fungi for the treatment of hard board industry effluent; the treatment reduced the colour by 70 per cent and TOC by 64 per cent and reducing sugars by 91 per cent. Lee *et al.* (1995a,b) reported that the treatment of kraft bleach effluents by *C. versicolor* could have an accelerating effect on decolourization process. The immobilised mycelium of the white rot fungi K5-62
removed 70 to 80 per cent colour from the effluent during two days incubation period. Milstein et al. (1995) found that Aspergillus sp., Penicillium sp., Aureobasidium sp. removed chloro-organics of high molecular mass from spent bleaching effluent (SBE) with a reduction of about 86 per cent in adsorbable organic chorine (AOX) and 71 per cent in chemical oxygen demand. Raghukumar et al. (1996) reported that the marine fungi Sordaria fimicola sp., Halosarpeia ratnagiriensis and an unknown basidiomycete, decolourize paper mill bleach effluent. Lu and Gau (1997) reported the effect of white rot fungi on degradation of black liquor lignin produced from pine kraft. It was observed that the fungi could degrade more than 74.5 per cent of black liquor lignin in the medium after ten days incubation. Vidal et al. (1997) studied the anaerobic biodegradability of chlorine and total chlorine-free bleaching effluent. In this method, COD was reduced by 67 to 75 per cent. Modi (1998) observed 40 per cent colour reduction in the bagasse based paper mill effluent treated with the white rot fungus T. versicolor on the second day of growth and a maximum colour removal of 60 per cent was achieved on the fourth day of culture growth. Bryant et al. (1998) and Gergov et al. (1998) reported that aerated lagoon treatment could remove 30 to 40 per cent of the organically bound chlorine and about 50 per cent of chlorinated phenolic compounds of the effluent. Activated sludge treatment has been found to remove approximately 50 per cent of organic chlorine and over 60 per cent of chlorinated phenolic compounds (Skogman and Lammi, 1998). Haggblom and Salonen (1991) found that an anaerobic fluidized bed and an aerobic trickling filter were effective in degrading the chlorinated high and low molecular weight materials; in this treatment, over 65 per cent reduction of AOX and 75 per cent reduction of chlorinated phenolic compounds were observed. Heinzle et al. (1992) reported that ozonation followed by fluidized bed bio-reactors treatment could remove more than 70 per cent of total organic chlorine, COD and adsorbable organic halogens (AOX).

In the present study, the ligninolytic fungi were used for treatment of paper mill effluents in lab scale as well as pilot scale experiments. The study (Tables 18 and 19, Fig. XX and XXI) revealed that in lab scale experiments, four days incubation reduced the effluent colour by 33.33 to 43.00 per cent and ten days incubation increased the
liberation of inorganic chlorine by 197.00 to 222.92 per cent and reduced the COD by 33.04 to 44.98 per cent. But it was observed that pilot scale experiments were not as efficient as lab scale; in pilot scale, colour removal was only at the level of 23.61 to 31.90 per cent; liberation of inorganic chlorine was 57.69 to 102.99 per cent and COD reduction was 30.83 to 40.67 revealing that pilot scale experiments needed further improved scale up processes. In paper mill effluent treatments, *Thelephoraceae sp.* was found to more efficient than other two fungi.

*Archibald et al.* (1990) reported that kraft bleachery effluent decolourization by *C. versicolor* was mainly due to laccase type phenol oxidases. *Ferrer et al.* (1991) reported that in *Chrysonila sitophila* free LiP caused 13 per cent decolourization at 48 h, whereas, lyophilised fungal culture brought only 6 per cent at 48 h from kraft effluent. Lackner (1992) found that the enzyme manganese dependent peroxidase play a major role in the break down and decolourization of chloro-lignin present in the bleach plant effluent. Manzanares *et al.* (1992) reported that the enzymes laccase and manganese depended peroxidase were involved in the decolourization of paper pulp effluents, but lignin peroxidase was not important for decolourization processes. Manzanares *et al.*, (1995) found that *T. versicolor* decolourize paper mill effluent by more than 70 per cent and detected laccase and MnP during the treatment. Dezotti *et al.* (1995) reported that immobilised horse radish peroxidase and *Crysonila sitophila* lignin peroxidase on activated silica yielded 37 and 12 per cent decolourization and 60 and 65 per cent COD reduction respectively. *Pereze et al.* (1997) attributed the decolourization efficiency of the basidiomycetes fungal strains to the extra cellular ligninases, lignin peroxidase (LiP) and manganese depended peroxidase (MnP).

Hence in the present study also, the ligninases (LiP, MnP, laccase and a mixture of these enzymes) obtained from the culture filtrates of *F. lividus, Thelephoraceae sp.* and *T. versicolor* were used for paper pulp industry effluent treatment (Table 20). It was observed that all the enzymes, except laccase, decreased the colour of the effluent by 9.43-11.23 per cent; in laccase treatment, the colour removal was slightly lower (5.67-9.43%). Mixed enzyme treatments increased the liberation of inorganic chlorine by
72.65 to 100.00 per cent, in purified LiP and MnP treatments the increase was in the order of 51.71 to 81.62 per cent; in laccase treatment it was in the order of only 27.35 to 48.29 per cent; but in COD reduction, laccase was found to be very effective; COD was reduced by 66.02 to 72.79 per cent; next to laccase, mixed enzyme treatment was found to be effective in COD removal (55.80 to 76.23%); LiP and MnP were not so effective (29.69 to 49.76%). Here again, the enzymes of Thelephorce sp. was found to be very effective in paper mill effluent treatment when compared to the enzymes of other two fungi.

Azodyes and pigments are extremely versatile colourants. Approximately one half of all known dyes are azodyes, making them a largest group of synthetic colourants. White rot fungi are a unique group among microorganisms, in that, they are the only microorganisms known to be capable of complete mineralisation of lignocellulosic polymers. Since dyes are also heterocyclic compounds, these fungi are extensively tried for the degradation of azodyes. Glenn and Gold (1983) first demonstrated that ligninolytic culture of P. chrysosporium could decolourize several polymeric dyes. Presently decolourization of the azodyes, orange II, tropeolin O, congo red, acid red 114, acid red 88, biebrich scarlet, direct blue 15, chrysophenine, teterazine and yellow 9 (Cripps et al., 1990; Rafii, 1990; Paszczynski et al., 1991) and the triphenyl methane dyes, basic green 4, crystal violet, brilliant green, cresol red, bromophenol blue and para rose anilines (Bumpus et al., 1988) by various fungi has been reported. Cripps et al. (1990) reported that P. chrysosporium could remove 8.97 per cent of tropeolin O and congo red with in five days incubation. Spadaro et al. (1992) established that P. chrysosporium are capable of mineralizing a variety of toxic azodyes and the mineralization of aromatic rings of azodyes is dependent on the nature of ring substituents. Since the dyes are aromatic compounds the ligninolytic fungi degrade these dyes during secondary metabolism. Heinfling et al. (1997) reported that Bjerkendra adusta and T. versicolor removed 95 per cent of HRB 8 dyes within four days.

In the present study, the ligninolytic fungi, F. lividus, Thelephorce sp. and T. versicolor were used for the removal of orange G, congo red and amide black 10B from
aqueous solutions. The results revealed (Table 21 to 23; Fig. XXII to XXVII) that in orange G, the fungi required nine days for 30.77 to 34.64 per cent dye removal, the K_{ad} value of the fungi for orange G ranges from 11.37 x 10^{-2} to 18.18 x 10^{-2} and the r value -1.0 to 1.0. All the fungi removed the orange G at more or less equal level (Table 21; Fig. XXII and XXIII). In congo red, a maximum of 73.41 per cent removal was observed in *F. lividus* cultures after four days incubation period; the K_{ad} and r values were 17.61 x 10^{-2} and -0.999 respectively; *Thelephorace sp.* removed 97.11 per cent dye during eight days incubation with the K_{ad} value of 98.88 x 10^{-2} and r value of -0.885; *T. versicolor* was found to be very effective in congo red removal; 97 per cent of the dye was removed within 12 h incubation (K_{ad} 16.31 x 10^{-2}; r -0.998) (Table 22, Fig. XXIV and XXV). All the three fungi were found to be very effective in amide black 10B removal; but they required different incubation periods (Table 23, Fig. XXVI and XXVII) *F. lividus* removed 98 per cent of the dye after six days incubation (K_{ad} : 22.33 x 10^{-2}; r :1.0), whereas *Thelephorace sp.* removed 98 per cent within 24 h incubation period (K_{ad} : 11.11 x 10^{-2}; r : -0.9357), *T. versicolor* removed a maximum of 78 per cent of the dye after 8 h incubation (K_{ad} : 53.20 x 10^{-2}; r : -0.9753).

Enzymatic degradation of adsorbed dyes was the major mechanism in the regeneration of the adsorption capacity of the fungal mycelium. Capalash and Sharama (1992) reported that the ligninases of *P. chrysosporium* and adsorption to biomass were responsible for decolourizing eight commercially used dyes, and 40 to 73 per cent decolourization was detected in five day old cultures after 72 h of treatment. Ollikka *et al.* (1993) showed 54 per cent decolourization of congo red in the presence of a crude preparation of lignin peroxidase and reported that these enzymes utilise congo red as substrate. Spadaro and Renganathan (1994) concluded that azodyes are cleaved by fungal peroxidases with the formation of quinone and diazene derivatives and released the azo linkage as molecular nitrogen. Pasti - Grigsby *et al.* (1994) have examined the azodyes as potential substrates for assaying lignin peroxidase and manganese dependent peroxidase of white rot fungi. Vyas and Molitoris (1995) detected manganese peroxidase, manganese independent peroxidase and phenol oxidase activities during solid state fermentation of wheat straw along with remazol decolourization and reported that
enzymes of lignin peroxidase (LiP), manganese dependent peroxidase (MnP) and laccase all of which are involved in lignin degradation, participate in the decolourization of the dyes. Young and Yu (1997) studied the process of decolourization of azodyes treated with white rot fungal cultures and by fungal peroxidase - catalysed oxidation. They have reported that the dyes were not decolourized by manganese dependent peroxidase (MnP), but 80 per cent colour was removed by ligninases catalysed oxidation. Each dye molecules contains a chromophore such as azo and anthraquinone and its colour disappears only after the chromophore structure is destroyed. Soo and Jin (1998) reported that Pleurotus ostreatus peroxidase was able to decolourize 98 per cent of bromophenol blue, but heterocyclic dyes, methylene blue and toluidine blue O were decolourized by less than 10 per cent. Wang and Yu (1998) reported that living mycelium of T. versicolor plus extra cellular enzymes gave the fastest degradation rate of the dye; T. versicolor produced little extra cellular enzymes in its primary growth phase, but released the enzymes as secondary metabolites in the stationary phase. Kim and Shoda (1999b) purified and characterized a novel peroxidase (DYP) that was responsible for the dye decolourizing activity of Geotrichum candidum Dec 1. Swamy and Ramsay (1999) reported that in white rot fungus T. versicolor the enzyme lignin peroxidase (LiP) was not detected during the decolourization of the dyes of amaranth, instead, laccase and manganese peroxidase (MnP) were detected in the decolourizing culture.

Since the extra cellular enzymes play a key role in dye degradation, in the present study, the extra cellular ligninolytic enzymes, LiP, MnP, laccase and mixture of these enzymes, were used for dye degradation. As per literatures cited above, LiP, MnP and laccase participated in dye degradation; however, in most of the preparations, laccase was found to be very effective in dye degradation. In orange G degradation, in F. lividus and Thelephorce sp. preparations, mixed enzymes (21.62 to 16.21%) and laccase (21.62 to 18.92%) were found to be effective degraders, whereas, in T. versicolor, LiP (21.62%) and MnP (13.51%) were effective; in congo red degradation, mixed enzymes (8.95%) and LiP (11.94%) were found to be effective; whereas in Thelephorce sp. and T. versicolor, mixed enzymes (14.93 and 8.95%) and laccase (11.94 and 13.43%) were effective; in amide black 10B degradation, F. lividus preparation of LiP and MnP yielded
a maximum degradation of 15 and 10 per cent respectively, whereas in *Thelephorce sp.* and *T. versicolor* preparations, maximum degradation was observed in mixed enzyme and laccase preparations; in *Thelephorce sp.* a maximum of 15 per cent degradation was observed in both of the enzymes treatments; in *T. versicolor*, the dye degradation was 12.5 per cent. The results showed that in most of the cultures, laccase played a major role in dye degradation, but the other enzymes, LiP and MnP also took part in dye degradation (Tables 24a - c).

Schiephake *et al.* (1993) reported the aerobic decolourization of the effluent from a pigment plant by white rot fungus *Pycnoporus cinnabarinus*. *P. cinnabarinus* rapidly decolourized and clarified waste water sample passed through a packed bed reactor. Spadaro *et al.* (1992) reported that *P. chrysosporium* has potential application for the clean up of textile mill effluent. Knapp *et al.*, (1995) reported that *P. chrysosporium* removed 70 per cent of colour in the oil mill waste water. Sayadi and Ellouz (1993) reported a positive role for veratryl alcohol with lignin peroxidase in decolourization of olive mill waste water by a *P. chrysosporium* culture. Kirby *et al.* (1995) reported that decolourization of effluent required seven days treatment. Wang and Yu (1998) stated that the role of lignin peroxidase in degradation of dyes was unclear; adsorption and degradation of dye molecules on living fungal hyphae might provide a mechanism for feasible application of white rot fungi in a continuous treatment of industrial effluent. Rodriguez *et al.* (1999) reported that several industrial dyes were decolourized bio catalytically by extra cellular enzymes from different strains of white rot fungi and the decolourization capacity was correlated with laccase activity levels. This enzymatic system was selected as a good candidate for immobilisation and used as a bioreactor for effluent treatment from the dye and printing industries. Swamy and Ramsay (1999) stated that in decolourization process, identification of the decolourizing enzymes is useful for optimizing process parameter and medium composition to maximise enzyme production and detected the production of extra cellular MnP and laccase but not LiP during decolourizing process.
In the present study, the dye industry effluent was treated with ligninolytic fungi for colour removal in batch mode and continuous mode. The result showed (Table 25; Fig. XXVIII) that in batch mode treatment, the fungi required four days incubation for maximum colour removal, whereas continuous flow mode required five days; moreover, batch mode was found to be more efficient than continuous flow mode. In batch mode, *T. versicolor* was found to be very effective in colour removal followed by *F. lividus* and then by *Thelephore sp.* In *T. versicolor*, the colour removal was 98.43 per cent; whereas in *F. lividus* and *T. versicolor*, the values were 84.4 and 60.93 per cent respectively. In continuous flow mode, a maximum of 40.63 per cent colour removal was obtained after five days retention in *T. versicolor* and *Thelephore sp.* treatments; *F. lividus* treatment yielded 37.5 per cent colour removal.

When the ligninases were used instead of fungi for colour removal of dye industry effluent, a maximum of only 23.52 per cent colour removal was obtained (Table 26). In *F. lividus* and *Thelephore sp.*, mixed enzyme (17.19 and 22.06%) and laccase (12.31 and 17.64%) yielded maximum colour removal; whereas in *T. versicolor*, MnP yielded 23.52 per cent colour removal followed by mixed enzymes (17.64%) and LiP (17.19%); laccase yielded only 13.20 per cent colour removal from the effluents.

Composting is the process of converting organic residues of plant and animal origin into manure rich in humus and plant nutrients. It is largely a microbiological process based upon the activities of a host of bacteria, actinomycetes and fungi. All kinds of organic residues amenable to the enzymatic activities of the microorganisms can be converted into compost by providing optimum conditions for biodegradation.

The main constituents of plant residues are the carbonaceous compounds such as cellulose, hemicellulose and lignin; nitrogenous constituents (proteins) occur to a lesser extent. Protein, cellulose and hemicellulose decompose easily. Lignin, a complex aromatic polymer, is resistant to microbial attack to a considerable extent. Most components of lignin reach the finally produced humus in the compost (Neelakantan et al., 1974, Wani and Shinde, 1977; Lynch et al., 1981). Kalekar et al. (1976) reported
that compostable organic materials are naturally inhabited by large numbers of heterotrophic microorganisms, which bring about satisfactory decomposition under appropriate environmental conditions.

Kapoor et al. (1978) and Mandmulika et al. (1993) reported *Aspergillus, Penicillium, Rhizopus, Fusarium, Chaetomonium, Trichoderma, Alternaria Cladosporium*, some species of *Paecilomyces* and *Sporotrichum* as efficient degraders of lignocellulosic wastes. Kaplan and Hartenstein (1980) and Muthukumar et al. (1983) reported white rot fungi belonging to basidiomycetes species of *Polyporus, Pleurotus, Collybia, Poria, Fomes, Trametes, Sporotrichum, Cyathus* and *Coriolus* as common lignin degraders; they degrade lignin molecule by their enzymes laccase or phenol oxidases.

Choi et al. (1988) reported that *Thermoactinomyces vulgaris* grew vigorously in a mixture of wheat straw and poultry litter at 50°C and produce good compost. Waksman et al. (1989) identified many species of these actinomycetes in compost heaps. Wani and Shinde (1977) isolated a number of bacteria from soil and used them to degrade wheat straw; many of them were found to be efficient decomposers.

Mishra et al. (1981) reported that a few thermophilic strains such as *Aspergillus fumigatus* and *Humicola lanuginosa* also proved beneficial as inoculants in compost preparations. Bhardwaj and Gaur (1985) have compiled extensive information on the isolation, selection and use of fungal cultures for rapid composting of organic wastes. Mathur et al. (1986) noticed significant improvement in the fertiliser value of rice straw compost with the employment of the inoculants of some selected fungi and phosphate solubilizing organisms. Ramat (1989) reported that inoculation with a selected species of *Trichoderma* shortened the composting time of rice straw by 20 days, but has no effect on corn stubbles and banana leaves. Pore et al. (1992) found *Trichoderma sp.* as more useful for the composting of a mixture of crop residues, grass and tree leaves than *Aspergillus* and *Paecilomyces*. Brown (1990) reported that mixed culture of some lower fungi and lignin degrading basidomycetic fungi showed better degradation of
wheat straw than individual groups of the two classes of fungi. Kakezawa et al. (1992) achieved rapid decomposition of rice straw by providing it with inoculum of *Coriolus versicolor*.

Involvement of earthworms in composting, in association with usual microorganisms, constitutes worm composting or vermicomposting as it is generally called. The role of earthworm in processing organic materials and in improving soil fertility has been known for centuries. Darwin (1881) reported that earthworms are one of the major soil macro invertebrates and are known for their contributions to soil formation. The earthworms play a role in the breakdown of organic debris in the soil surface and in the soil turnover process.

Dash et al. (1979) reported that earthworm grazing on the soil microflora enhanced microflora growth by preventing senescence. Kononova (1966) and Stevenson (1982) reported that a number of exoenzymes, secreted by the soil organisms, catalyse the hydrolytic degradation process of the litter polymers, but catalyse also a number of condensation reactions in the soil water phase leading to the formation of complex humic substances, which in addition to the lignin derived polyphenols and quinones also contain amino compounds, polysaccharide units and phosphorylated sites. These substrates were further acted upon by the earthworms to form nutrient rich manure.

Mackay et al. (1982) have speculated that earthworms would appear to stimulate phosphorous uptake from organic matter by redistribution and by increasing phosphatase activity. Similarly, earthworms can affect the cycling of nitrogen. Barley and Jennings (1959) showed that 6.4 per cent of the non-available nitrogen ingested by growing *Allolobophora caliginosa* was excreted in casts in plant available form. The available nitrogen in the worms occur as ammonium nitrogen, nitrate nitrogen and as other soluble nitrogen; 96 per cent of nitrogen in fresh casts is present as NH₄ and rapid nitrification occurred following cast production, finally improving the soil fertility (Parle, 1963).
Senapati and Dash (1981) reported that earth worms are a major component of soil fauna and they account for 80 per cent of the soil invertebrate biomass in agroecosystem of tropical and subtropical India. By shifting fine particles from coarser particles, earthworms prepare a good media that promote microbial activity essential for the fertility of most of the soil (Hayes, 1983).

Syers and Springett (1983) have shown that worms chemically influence the nutrients in the soil by direct enzyme action on organic matter in the intestine, metabolise the organic materials and release the metabolic products into the soil, particularly nitrogen. The increase in nitrogen content in worm casts is attributed to thorough mixing of the organic material by the digestive secretions (Lindquist, 1941; Lunt and Jacobson 1944; Barley and Jennings, 1959). Many factors affect the turnover of soil organic matter, the kind of organic matter, its origin, its initial distribution within the profile, the temperature and moisture regimes of the soil, its physical and chemical properties. Singh et al. (1999) reported that worm castings have more organic matter, phosphate, copper, zinc, iron manganese and low pH than that of the soils. Obviously, these casts contribute to the value of the compost. (Barley and Jenning, 1959; Parle, 1963; Mackay et al., 1982; Bano et al., 1984).

Shinde et al. (1992) analysed vermicomposts made from processing wastes of vegetable market with the help of three different species of earth worms. Nielsen (1965) reported that earthworms need the usual major and minor nutrients required for cell development; carbon compounds are required in an energy-rich form but the extent to which earthworms make direct use of plant and fungal structural polysaccharides is uncertain. It is generally assumed that more complex molecules are not broken down within the earthworm. Laverack (1963) has reported the presence of several enzymes, including cellulase, in extracts of earthworm alimentary canal tissue; nitrogen, sulphur and phosphorus are also required in organic form as proteins or amino acids (Dash et al., 1979). Kale et al. (1991) reported that earthworm aided in the decomposition of organic matters including stubbles from agricultural fields, sugarcane trash or coir waste and the
decomposition was measured in terms of reduction in levels of residual cellulose and lignin in the worm worked materials.

Hence, in the present study, the lignocellulosic wastes, vegetable waste, agricultural waste and coir pith were pretreated with the ligninolytic fungi before earthworm composting. This fungal pretreatment will degrade the complex plant polymers like cellulose, hemicellulose and lignin into simpler molecules, which otherwise could not be degraded by earthworms. The pretreatment was done in four different methods. In treatment I (T1), fungus alone was inoculated on the substrate; in treatment II (T2) the earthworm *Eudrilus eugeniae* alone was inoculated; in the treatment III (T3), the fungus and the earthworm were inoculated at the same time and in the treatment IV (T4), the earthworm was inoculated after one month of fungal inoculation. The nutrients content of the compost (carbon, nitrogen, phosphorus and potassium) were analysed at fifteen days interval and the data were statistically analysed (Tables 27 to 29 and Fig. XXIX to XXXI).

The results in general revealed that the fungal pretreatment increased the carbon and nitrogen content of the compost and also the C/N ratio, whereas phosphorus and potassium contents were decreased. In vegetable waste, in all the treatments, the carbon content was increased by 160 to 175.9 per cent and nitrogen content by 100 to 150 per cent after 105 days composting; phosphorus content was decreased by 25 to 37.5 per cent and potassium by 22.22 to 38.89 per cent; C/N ratio was increased by 9.57 to 37.94 per cent (Table 27a; Fig. XXIX). Among the treatments, the higher levels of increase (in carbon and nitrogen contents) or decrease (in phosphorus and potassium contents) were observed in treatment IV; the variations in carbon and nitrogen contents were significant at 1 and 5 per cent levels respectively, but variations observed in phosphorus and potassium contents were not significant.

Composting of agrowastes resulted in tremendous increase in carbon and nitrogen contents when compared to other two substrates. The treatments increased the carbon content by 3066 to 3583 per cent, maximum being in *F. lividus* pretreated
samples; similarly nitrogen content was also increased by 100 to 200 per cent after 105 days composting, phosphorus content was decreased by 14.29 to 28.60 per cent and potassium content by 26.09 to 34.28 per cent; C/N ratio was decreased by 47.04 - 69.3 per cent revealing an increase of nitrogen content of the compost. Statistical analysis of the data showed that the variations observed in carbon and nitrogen contents were significant at 1.0 per cent and that of phosphorus was not significant; in F. lividus treatment, potassium content was decreased significantly (1.0% level); but in the other two fungal treatments the potassium content was not significantly altered. Among fungi, alterations in all the parameters were significant at 1.0 per cent level except that of phosphorus which was significant at 5.0 per cent level (Table 28b; Fig.XXX).

Either fungal pretreatment alone or in combination with earthworm, could not decompose coir pith effectively (Table 29a; Fig. XXXI). The increase in carbon and nitrogen contents and also the decrease in phosphorus content in F. lividus and Thelephoraceae pretreatment were not significant; similarly alteration in the carbon and phosphorus content of T. versicolor pretreated samples were also not significant; but the potassium content was significantly (1% level) decreased by all the treatments; the decrease was in the range of 48.4 to 64.5 per cent; C/N ratio was decreased by 47.1 to 66.7 per cent.

The increase in carbon and nitrogen contents might be attributed to decomposition of plant polymers into simpler molecules by the fungi as well as the earthworm and decrease in phosphorus and potassium content might be due to leaching of these nutrients in to the soil.

When the compost amended soil samples were analysed, it was observed that vegetable waste compost increased the soil carbon content by 147.0 to 250.0 per cent (significant at 1.0% level); but the nitrogen content was not much affected; the phosphorus and potassium contents were significantly increased (both at 1.0% level); phosphorus content was increased by 183.3 to 250.0 per cent and potassium content by 56.9 to 109.2 per cent; C/N ratio was increased by 56.9 to 109.2 per cent; highest
nutrients contents were observed in soil samples amended with *Thelephorce sp.* pretreated and earthworm decomposed vegetable wastes.

Though amendment of soil with agrowaste compost increased the soil fertility, the level of increase is lower when compared to vegetable waste compost amendment. Here, the carbon content was increased by 11.8 to 470.6 per cent; nitrogen and phosphorus contents were not increased significantly; potassium content was increased by 4.6 to 47.7 per cent (significant at 1 % level); C/N ratio was increased by 11.2 to 323.5 per cent. Here again, the *Thelephorce sp.* pretreated and earthworm decomposed agro wastes were found to be good soil additives as manure.

The coir pith compost amendment significantly increased (1.0% level) the carbon and phosphorus contents and decreased the potassium content of the soil; but the nitrogen content was not significantly altered. However, the increase in soil nutrient content was very low when compared to the compost of vegetable waste and agrowaste. In coir pith amended soil samples, increase of 35.3 to 464.7 per cent carbon and 16.7 to 83.3 per cent phosphorus were observed; potassium content was decreased by 21.5 to 41.5 per cent; the C/N ratio was increased by 10.3-747.1 per cent. In coir pith also, *Thelephorce sp.* pretreatment with earthworm decomposition was found to be effective in preparation of compost from coir pith.