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Characterization and Isolation of Fungi for Removal of Color from Pulp and Paper Mill Effluent, Meerut (India)

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Abstract

In this research paper, use of biological agents for the treatment of effluent is more environmental friendly and can lead to the production of more value added products like biogas and compost etc. Pulp and paper mill is the major industrial hub in our country. The heavy demand for the paper helps in steady expansion of paper industries. Pulp and paper industry is one of the largest and most notorious sources of industrial pollution. The Ministry of Environment and Forest, Govt. of India has categorized the pulp and paper industry as one of the twenty most polluting industries.

Keywords: Effluent; Pulp and paper; Biogas; Industry; Pollution

Introduction

India is one of the first ten industrialized countries in the world. We have good industrial infrastructure in core industries like chemicals, fertilizers, petroleum, pulp and paper mill and leather industries etc. Though three fourth volume of the waste water is generated from municipal sources, industrial waste water contribute over half of the pollution load and major portion of this originates from large and medium scale industries. Pulp and paper mill is the major industrial hub in our country. The heavy demand for the paper helps in steady expansion of paper industries. In 1951, there were 17 paper mills in country which producing 0.13 million tons paper per annum. The number has gone up to 406 in 2002 producing 1.9 million tons paper per annum. Pulp and paper mills are utilizing huge amount of lingo-cellulosic components of plants and using chemicals during manufacturing regarded as polluting industries because of huge amount of waste material enter into the environment.

Pulp production from wood (10^6 metric tons day^{-1} worldwide) not only requires large amount of fresh water but also is responsible for the discharge of a considerable volume of effluents (200 m^3/ metric ton of pulp). Since the pulp production by plant materials corresponds to only about 40-50% of the original weight of the wood, these effluent are heavily loaded with organic material. Over 100 organic chemicals most of them chlorinated have been identified in spent bleaching liquor. These compounds are chlorinated lingnosulphonic acid, chlorinated resin acid, chlorinated phenol and chlorinated hydrocarbon. Although the pulp industry has made a considerable effort to reduce the residual organic matter but organic compounds generated during pulping and bleaching performed by using chlorine, chlorine dioxide and sometimes hydro chlorite formed recalcitrant xenobiotics which are not removed by treatment method [1-6].

Biological decolourisation methods use several classes of microorganism including bacteria, algae and fungi to degrade the polymeric lignin derived chromophoric material. Among these wood degrading white rot fungi have been shown to efficiently and completely degrade and metabolize lignin resulting in rapid decolourisation of the effluents. *Schizopyllum commune*, *Tinctoporia borbonia*, *Phanerochaete chrysosporium* and *Trametes versicolor* have been found to degrade lignin and metabolize it along with carbohydrates. *Aspergillus niger* with *Trichoderma sp.* one of the fungi are also capable of degrading lignin and decolourizing El stage effluent of hard wood pulp bleaching.

Bacterial cultures have been marketed for decolonization or kraft mill effluent. *Pseudomonas aeruginosa* is capable of reducing kraft mill effluent color by 26-54% or more under aerobic conditions. Color was primarily removed by adsorption with little depolymerization. During microbial attack of lignin a number of sample aromatic compound like vanilic acid, p-hydroxy benzoic acid, ferulic acid, syringic acid and coniferaldehyde are produced.

Profile of the pulp and paper industry in India

The Pulp and paper industry is one of the largest and oldest industries in India. It has most notorious sources of industrial pollution. The Ministry of Environment and Forest, Govt. of India has categorized the pulp and paper industry as one of the twenty most polluting industries. The first paper mill was commissioned in 1812 in the eastern state of West Bengal. Today are about 406 pulp and paper industries with an annual installed capacity of 6.2 million tons. The capacity utilization is estimated at around 60-65% of the total installed capacity (Table 1).

Pulp and paper industry and its status

Worldwide pulp production from wood is 10^6 metric tons per day which is also responsible for the discharge of considerable volume of effluent (200 m^3/metric tons). The world population used over 214 million tons of paper and board products in 1987 and all estimates show that paper consumption is going to increase in the seeable future. In India, it is about 288 pulp and paper mill which manufacture 27.5 $\times 10^5$ tons of paper per year. Pulp and paper industry in India is quite old and it has an installed capacity of about 3.0 million ton per annum. The large paper mills with capacity greater than 55 tons per day and numbering 34 account for about 50 percent of total installed capacity.

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State	Number of mills	Installed capacity (tons per year-TPY)
Andhra Pradesh	22	414550
Assam	2	220500
Bihar	4	25000
Gujarat	68	935800
Haryana	15	149150
Himachal Pradesh	6	53200
Jammu and Kashmir	1	5000
Karnataka	14	34530
Kerala	5	215600
Madhya Pradesh	21	290650
Maharashtra	71	1034050
Nagaland	1	33000
Odissa	1	27050
Pondicheri	1	9000
Punjab	37	375162
Rajasthan	7	12195
Tamil Nadu	31	639250
Uttar pradesh	73	870780
West Bengal	18	222600
Total	**406**	**6121327**

Table 1: Geographical spread of the pulp and paper mill in India.

The rest mills are small and have a production capacity of less than 30 tons per day of over 300 mills producing about 2.5 million tons of pulp about 10 percent is pulp for viscous rayon grade industry. The pollution potential of small paper mills is greater than that of large mills as they lack proper effluent treatment system and chemical recovery units.

Characteristics of pulp and paper mill effluent

The paper making processes produces an effluent which contains a substantial quality of cellulose "fines" and others additives. This can be up to 50% of the total mass. This contaminated water is frequently referred to as "white water". Reclamation of the effluent is economically essential as the gross usage of water in the industry is very high and cost of effluent treatment for all water assigned to drain would be too expensive and would also involve a loss of raw materials [6-13].

Materials and Methods

Sampling

Sampling was started for the purpose of isolation of fungal strains. The sediment was collected from the Kaccha nala outside the Centaury Pulp and Paper Mill (Ghanshyam Dham, Lalkuan, Nainital and Uttaranchal) premises. Second sampling was done near Anand Tissue Paper Mill, Meerut. Sediments samples were collected from the drain near the industry. The Sediment was collected in clean plastic containers and brought to the laboratory of the department and immediately stored in refrigerator at 4°C unit used for further analysis.

Microbial community procurement

Culture media: The composition of the culture media which was used in the present investigation has been given as under:

Potato Dextrose Agar (PDA): The sterilized potato dextrose agar media used for culturing the fungal strains was used at the rate of 39 gm per liter of distilled water and the pH of the media was adjusted to 5.0 ± 1.0.

Potato Dextrose Broth (PDB): The sterilized potato dextrose broth was used for the continuous enrichment of the fungal strain has been used at the rate of 24 gm per liter of distilled water. The pH of the media was adjusted to 5.0 ± 1.0.

Minimal Salt Medium (MSM): The sterilized minimal salt medium was used for the continuous enrichment of the microbial strains in this investigation. The composition is described above.

Antibiotic: Antibiotic streto penicillin was used for the prevention of any contamination of the media used in the investigation. It has been used at the rate of 100 mg per liter sterilized media.

Isolation of fungus from the sediments: 1.0 gm of sediments was dissolved in 10 ml of autoclaved water. It was shaken vigorously to mix them properly. It was kept standing at room temperature for 2 hr. Then the supernatant was decanted and centrifuged at 1000 rpm for 5 minute. The same process was repeated with the sediments of both the industry. Serial dilution of the supernatant in the order of 10^{-3}, 10^{-4}, 10^{-5} were done using 0.1 ml autoclaved double distilled water from each dilution was spread on the PDA plats and incubated at 30°C for 4 days. Spreading was done in triplicate.

Preparation of fungal inoculums (Pellets): For the preparation of fungal inoculums in the form of pellets initially the fungal isolates were individually grown on potato dextrose agar plates by incubation at 30°C for 4 days. Fungal mycelium disc (of about 1 cm diameter) were cut for the zone of active growth and inoculated at the rate of mycelia discs inn sterilized Erlenmeyer flasks containing potato dextrose broth (100 ml) and steriptopenicilliini (100 ml). The flasks were incubated at 30°C for 4 days under shake conditions in orbital shaker. The pellets of approximately 1.5-2.0 mm size were observed suspended in the medium ready for use in the treatment of pulp and paper mill effluent.

Screening of potential strains

MSM effluent (150 ml) in a sterilized Erlenmeyer flasks inoculated with individual fungal isolates and a control were then inoculate with individual fungal isolates and a control were then incubated at 30°C in a rotary shaker for 15 days. The parameter such as color and lignin were observed and measured at an interval of 0, 1, 3, 5, 7, 10, 15 days. The experiment was repeated on the basis of comparative analysis of reduction percentage of different parameters studied by the individual isolates along with control; the potential strains were screened out.

Decolonizing assay

Color was estimate by the method 212°C (APHA). The sample was filtered and then centrifuged at 10,000 rpm for 30 minutes to remove all the suspended matter. The supernatant was taken and volume of sample was maintained at 50 ml. The pH of the sample was adjusted to 7.0 with 2M NaOH. Then the absorbance was taken at the wavelength of 456 nm using Varian Cary Spectrophotometer. The value of color was calculated in coloring unit by making standard curve [3-8].

Preparation of standard curve

Dissolved 1.246 gm of potassium chloroplatinate and 1.0 gm crystallized cobaltous chloride in ware with 100 ml core HCl and diluted to 1 liter. This stock solution has color of 500 CU. The suitable dilution was made from this stock solution to prepare standard curve.

Measurement of Lignin

The lignin of the samples was estimated according to Pearl and Benson. In this method the sample was centrifuged at 10,000 rpm for 30 minutes to remove all the suspended matter. The pH of the

supernatant was then adjusted to 7.0 with 2M NaOH. The sample (50 ml) was mixed with 1 ml of CH_3COOH (10%) and 1 ml of $NaNO_2$ (10%). After 15 minutes, 2 ml of NH_4OH was added. It was then left for five minutes and absorbance (OD) was measured at 430 nm. For blank, 1 ml of CH_3COOH (10%) was added in 50 ml of distilled water and 2 ml of NH_4OH. After 15 minutes, 1 ml of $NaNO_2$ (10%) was added. After waiting for 5 minutes optical density (OD) was taken at 430 nm. The absorbance value was transformed into lignin content (ppm) using the following formulate [3-8].

Lignin (ppm)=Absorbance/0.000247

Enzyme analysis

Finally the three most potent strains were selected for enzyme analysis. Two enzymes Lignin *peroxidase* and Manganese *peroxidase* were studied [3-8].

Lignin *peroxidase* (Lip): Veratryl alcohol (VA) assy. Lip can be measured photometrically through the oxidation of VA to veratryl aldehyde at 310 nm. The reaction mixture will contain 2 mM VA, 35 mM sodium titrate buffer (pH 3.0) and enzyme. The reaction starts with the addition of 0.36 mM H_2O_2.

Manganese *peroxidase* (MnP): MnP activity can be measured directly through the oxidation of Mn (II) to Mn (III) at 270 nm. The reaction mixture consists of 0.5 mM $MnSO_4.H_2O$ and enzyme, 45 mM sodium malonate buffer (pH-4.5) and enzyme. The reaction starts with the addition of 0.1 mM H_2O_2.

Microscopic study and identification of fungal strains

In this the isolated fungal strains were grown on PDA plate. A very small amount of mycelium was picked up from plate and kept on clear glass slide having a drop of water. Then observing under microscope (2X) the mycelium was teased and the fungal hyphae were separated from each other. Finally this mycelium was transferred on second glass slide having a drop of water. The cover slip was placed to make a temporary slide. This slide was observed under a microscope, Olympus and Magnus MLX-TR at 40X and 100X having camera attached with it. Fungal mycelium, spores and the spore attachment was observed and photographs were taken for the purpose of identification.

Results

Isolation of fungal strains

Sampling was done from two industries namely as centrally pulp and paper mill, Lalkuan and Anand tissue paper mill, Meerut where sediment samples were collected. Fungal strains were isolated by serial dilution and grown on Potato Dextrose Agar (PDA) pates. Three fungal strains F1, F2 and F3 were isolated from century pulp and paper mill, Lalkuan and three fungal strains 1, 5 and 7 were isolated from Anand tissue paper mill, Meerut. One fungal strain AF3 was available in laboratory. Hence total seven fungal strains were used in this study. Figures 1 and 2 shows the isolated and purified fungal strains. The characteristics of these strains are given in the Tables 2 and 3 [1-4].

Microscopic study of fungal strains 1, F1 and AF3

The fungal strains were inoculated on the PDA plates. They were allowed to mature and form spores. Then temporary slides were made and observed under microscope and photographs were taken for the purpose of identification. The morphological characteristics of the three fungal strains are in the Table 3, Figures 3-5.

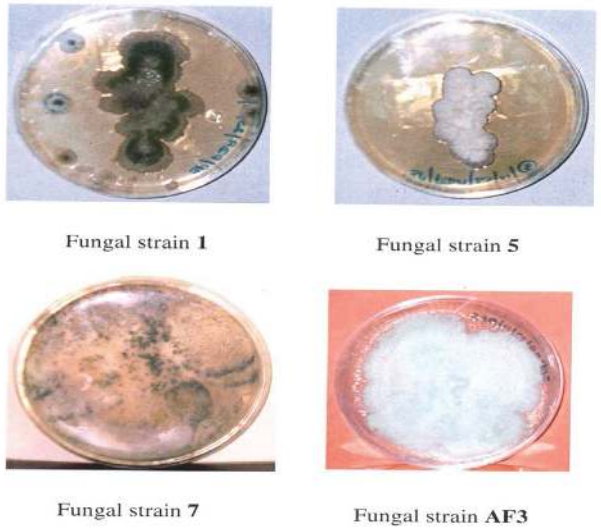

Figure 1: The isolated and purified fungal strains 1, 5, 7 and AF3.

Figure 2: The isolated and purified fungal strains F1, F2 and F3.

The three fungi on the basis of their morphology have been identified as

1: *Aspergillus sp.*

Fungal Isolates	Source	Growth	Characteristics
1	Anand Paper Mill	Slow	White mycelia, light green spores
5	Anand Paper Mill	Slow	White mycelium with dark colour spores, mycelia becomes dark from behind
7	Anand Paper Mill	Fast	White cottony mycelia with green spores
AF3	Previously isolated	Medium	White snowy cottony mycelia with yellowish pigmentation
F1	Century Paper Mill	Slow	Dirty white mycelia with green spores
F2	Century Paper Mill	Fast	White mycelia with excess spores production of green color
F3	Century Paper Mill	Fast	White mycelia with light brown spores produced in excess

Table 2: Source and characteristics of isolated fungal strains.

Fungal isolate	Morphological characteristics
1	White mycelium, light green spores, mycelium septate, branched mycelium, branching at right angles, spores spherical and single-cell attached on sporangiophore, sporangiophore bulbous bearing bottle shaped phialides spores in chain.
F1	White mycelium, green spores, mycelium septate, branched mycelium, branching at right angles, spores spherical and single cell, attached on sporangiophore, sporangiophore bulbous bearing botted-shaped phialides, and spores in chain.
AF3	White cottony mycelium, dirty yellow spores, septate, mycelium branched, uninucleate spore, spore globules, spores attached at the tip of mycelium.

Table 3: Morphological characteristics of 1, F1 and AF3.

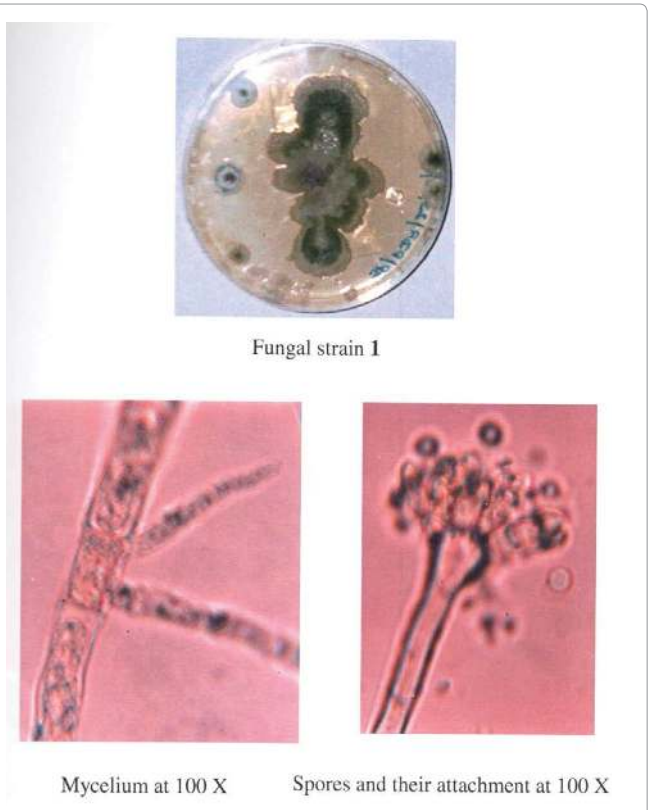

Figure 3: Shows the morphology of 1.

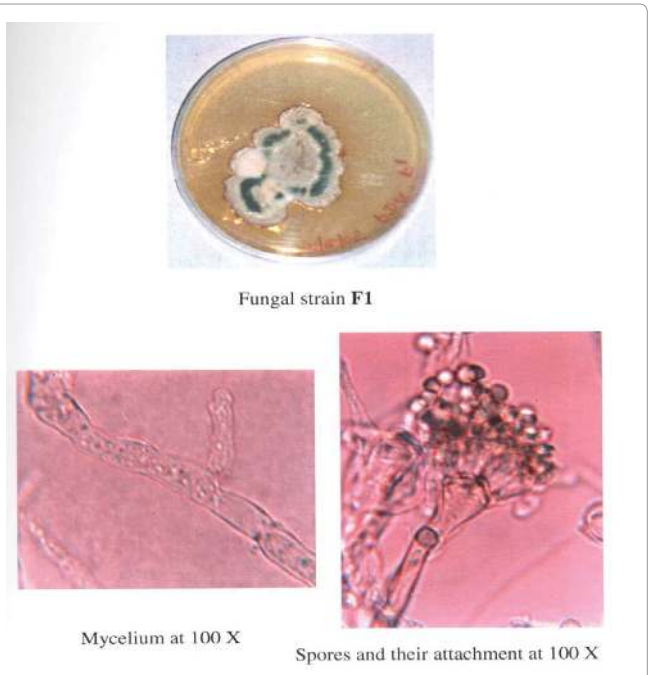

Figure 4: Shows the morphology of F1.

F1: *Aspergillus sp.*

AF3: *Basidioclamyces sp.*

Enrichment of fungal isolated in potato dextrose broth

The fungal isolate were enriched for the preparation on fungal beads to be used as inoculums in the subsequent screening experiment. The purified fungal isolates in the form of disc of 1 cm^2 diameter cut from the active growth zone of the PDA plate was enriched in the PDB containing 100 ppm streptopenicilin and pH was adjusted to 5.2 ± 1. It was observed that beads like structure were formed after 3 days of incubation in shake flask condition at 30°C. The fungal beads were used as inoculums at the rate of 10 percent (w/v) in experiments conducted.

Screening experiment for decolourisation of pulp and paper mill effluent

The seven fungal isolates were purified and enriched in the PDB and were used to test their efficiency in the decolourisation of pulp and paper mill effluent. Pulp and Paper mill effluent is highly colored. The color is mainly due to lignin present in it. Hence, color and lignin content were used as parameters to evaluate the efficiency of different fungal isolates in decolorizing pulp and paper mill effluent.

Minimum salt medium of pulp and paper mill effluent was prepared. All seven fungal strains were screened for their capacity to decolorize effluent. Inoculums size was 10 percent (w/v). The experiment was set for 10 days. Regular sampling was done on zero hour, first day, second

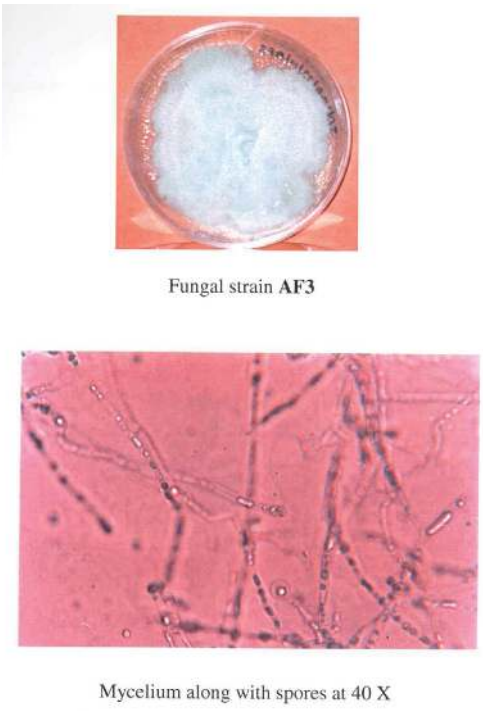

Figure 5: The morphology of AF3.

day, third day, fifth day, seventh day and tenth day. The results are given in the Table 3.

Confirmatory experiment and enzymatic study

It was found that fungal strain 1 and F1 were most efficient in decolourisation of pulp and paper mill effluent. AF3 was kept as a reference. The experiment was repeated with three fungal strains 1, F1 and AF3. Further, enzyme assay was done to check the presence of enzymes lignin *peroxidase* and manganese *peroxidase*. The results are given in Table 3. From Table 4, it is clear that 1 and F1 are quite efficient in decolourising pulp and paper mill effluent as compared to AF3. Among 1 and F1, F1 more efficient than 1. Lignin *peroxidase* was found in 1 and F1 but absent in AF3 and manganese *peroxidase* was absent in all three strains [8-13] (Table 5).

Where, changes in pH, Color (C.U.), Lignin (ppm), Lignin *peroxidase* and Manganese *peroxidase* by 1, F1 and AF3 fungal strains at different time intervals in Pulp and Paper mill effluent; ND: Not Detectable.

Discussion

Fungal strains have been shown to be efficient in the decolourisation of pulp and paper mill effluent. Some of the popular fungi used are *Phanerachaete chrysosporium*, *Trametes versocolor*, *Aspregillus sp.*, *Cariolus cersicolor* etc.

All the fungal isolates were capable of decolourizing the pulp and paper mill effluent. However, the efficiency of decolourisation varied from fungi to fungi. Among all, F1 strain was most efficient in decolourisation and least effective was strain 7. Figure 4 shows the percentage decrease in the color content of the pulp and paper mill effluent by different fungal isolates. The relative efficiency of decolourization all the fungal strains is F1>1>F3>F2>AF3>5>7.

Days	Strains	pH	Color (C.U.)	Lignin (ppm)
0 Day		5.96	27806.63	75060.73
1 Day	1	6.17	15893.03	51983.81
1 Day	5	6.20	11611.58	54939.27
1 Day	7	6.29	14701.67	57489.88
1 Day	F1	6.28	6271.17	33157.89
1 Day	F2	6.32	10941.44	49190.28
1 Day	F3	6.45	11537.12	44736.84
1 Day	AF3	6.18	12505.10	53765.18
2 Day	1	6.80	4223.52	33562.75
2 Day	5	6.81	12393.41	49190.28
2 Day	7	6.81	13286.93	57975.71
2 Day	F1	6.83	2659.86	28076.92
2 Day	F2	6.20	9787.31	42550.61
2 Day	F3	6.93	9452.24	44412.96
2 Day	AF3	6.72	11983.88	52672.06
3 Day	1	6.90	3869.83	31133.60
3 Day	5	6.90	12132.80	44210.53
3 Day	7	6.90	12802.94	53036.44
3 Day	F1	6.89	2492.32	25668.02
3 Day	F2	6.09	10606.37	44858.30
3 Day	F3	6.92	8298.11	41012.15
3 Day	AF3	6.80	10941.44	47004.05
5 Day	1	6.94	4202.81	35991.90
5 Day	5	6.90	12616.79	46032.39
5 Day	7	6.90	11537.12	50080.97
5 Day	F1	6.92	3309.29	25991.90
5 Day	F2	6.14	9415.01	35587.04
5 Day	F3	6.93	9340.55	40323.89
5 Day	AF3	6.86	11946.65	43562.75
7 Day	1	7.03	9005.48	37287.45
7 Day	5	6.97	11648.81	45101.21
7 Day	7	6.94	9638.39	48623.48
7 Day	F1	6.95	5133.56	31376.52
7 Day	F2	6.20	10680.83	42145.75
7 Day	F3	6.98	8558.72	37570.85
7 Day	AF3	6.90	12207.26	45101.21
10 Day	1	6.94	21589.22	55344.13
10 Day	5	6.90	15334	50364.37
10 Day	7	6.94	8968.25	46761.13
10 Day	F1	6.78	9750.08	37732.79
10 Day	F2	6.15	16451.48	45546.56
10 Day	F3	6.93	8744.87	41781.38
10 Day	AF3	6.90	20286.17	56599.19

Table 4: Changes in pH, Color (C.U.) and Lignin content (ppm) by different fungal strains at different time intervals in Pulp and Paper mill effluent.

The efficiency to remove lignin from the effluent was also observed in all the strains. Among all the strains F1 was most efficient in removing lignin and 7 were least effective. Figure 4 shows the percentage decrease in the lignin content of the pulp and paper mill effluent by different fungal isolates. The trend for lignin removal among different strains is F1>1>F3>F2>AF3>5>7.

The trend for lignin removal is similar to decolonization probably because the most important color causing compound in the pulp and paper mill effluent is lignin. Hence the removal of lignin is translated into color reduction of the effluent. The decolorizing capacity of different fungi is maximum in the beginning of the experiment, increase till 2nd

Days	Strains	pH	Color (C.U.)	Lignin (ppm)	Lip	MnP
0 hour		6.93	24493.16	65587.04	ND	ND
6 hours	1	7.10	10308.53	45789.47	ND	ND
6 hours	F1	7.10	8521.48	42550.61	0.012	ND
6 hours	AF3	7.09	11015.90	46518.22	ND	ND
1 Day	1	7.22	5096.33	36720.65	0.043	ND
1 Day	F1	7.26	4575.11	37004.05	0.075	ND
1 Day	AF3	7.25	7590.74	41578.95	ND	ND
2 Day	1	7.80	4537.88	28218.62	0.054	ND
2 Day	F1	7.84	3756.05	32348.18	0.091	ND
2 Day	AF3	7.90	6213.23	39797.57	ND	ND
3 Day	1	8.26	4351.73	30161.94	--------	-------
3 Day	F1	8.39	3793.28	27854.25	--------	-------
3 Day	AF3	8.25	4575.11	32105.26	-------	-------
5 Day	1	8.32	5692.01	33117.41	0.018	ND
5 Day	F1	8.46	4575.11	28380.57	0.01	ND
5 Day	AF3	8.48	8186.42	38016.19	ND	ND
7 Day	1	8.67	6920.60	35222.67	--------	--------
7 Day	F1	8.76	7292.90	33360.32	-------	--------
7 Day	AF3	8.56	14180.45	49190.28	--------	--------
10 Day	1	8.68	7516.28	35870.45	0.012	ND
10 Day	F1	8.76	6585.53	31700.40	0.007	ND
10 Day	AF3	8.63	10718.06	42348.18	ND	ND
15 Day	1	8.70	13361.39	48056.68	ND	ND
15 Day	F1	8.87	10122.38	39635.63	ND	ND
15 Day	AF3	8.74	12914.63	47246.9	ND	ND

Table 5: Changes at different time intervals in Pulp and Paper mill effluent.

day and then becomes almost constant till 5th day. After 5th day there is decrease in the efficiency. This may be due to the death and decay of the fungal mycelium. From these screening experiments two most efficient fungal strains F1 and 1 were selected for further experiments and AF3 was also used [8-13].

The experiment was repeated with F1, 1 and AF3. Results for the decolourisation and lignin reduction were same as above (Figure 4). F1 was most effective followed by 1 and finally AF3.

Enzyme assay was done for lignin *peroxidase* and manganese *peroxidase*. F1 and 1 produced lignin *peroxidase*. AF3 did not produce any of the enzymes. Maganese *peroxidase* was absent in all three. Maximum enzyme activity was observed on 2nd day of the experiment then it declined sharply till 5th day and finally become almost negligible (Figure 4). This shows that the lignin *peroxidase* plays an important role in the decolourization of effluent as the maximum reduction was found on 2nd day of experiment. In AF3, physical adsorption / absorption seems to play the major role. Physical adsorption/absorption also takes place with F1 and 1 as the fungal mycelium became colored at the end of the experiment [8-13] (Figures 6-10).

Conclusions

The use of biological agent for the treatment of effluent is more environmental friendly and can lead to the production of more value added products like biogas, compost etc. Fungus has been shown to be efficient in the decolonization of the pulp and paper mill effluent. Among all the fungal isolates tested F1 is most efficient in decolorizing the pulp and paper mill effluent followed by 1. Both the strains performed better than AF3, a strain previously isolated. The maximum decolourization was on the 2nd day though the process of decolourization started within the 6 hours. Lignin *peroxidase* also seems to play an important role in

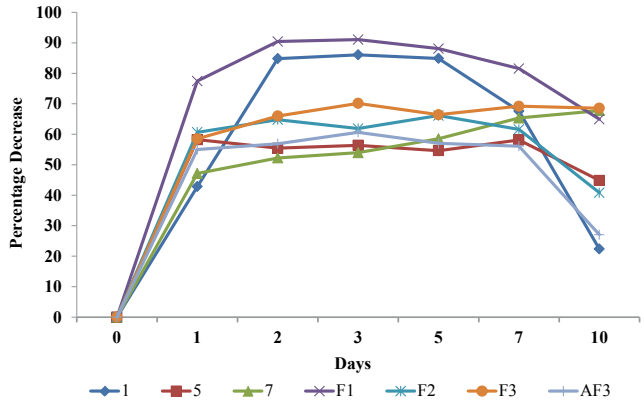

Figure 6: Changes in color at different time intervals by different fungal strains in pulp and paper mill effluent.

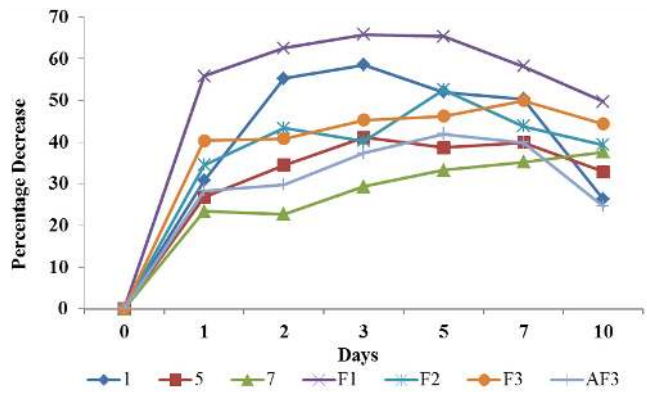

Figure 7: Changes in lignin content at different time intervals by different fungal strains in pulp and paper mill effluent.

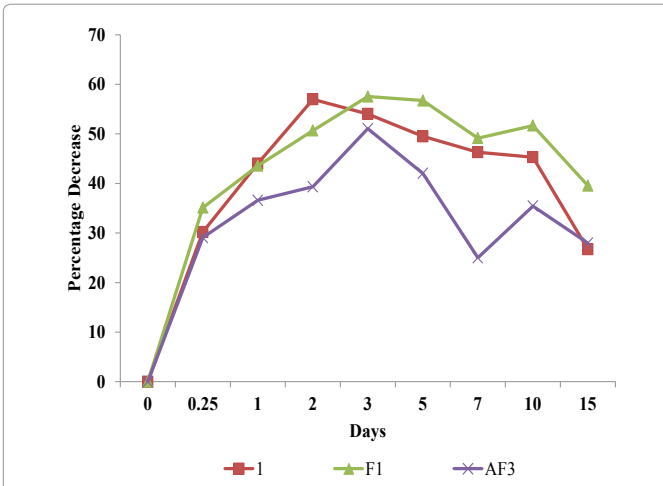

Figure 8: Changes in color at different time intervals by 1, F1 and AF3 fungal strains in pulp and paper mill effluent.

the decolourization of the pulp and paper mill effluent as it is produced by both F1 and 1. Maganese *peroxidase* was absent in the case of all three fungi that is 1, F1 and AF3. The quick response shown by two fungi 1 and F1 in the treatment of the effluent will reduces the retention time for the treatment which on applied on the industrial scale makes

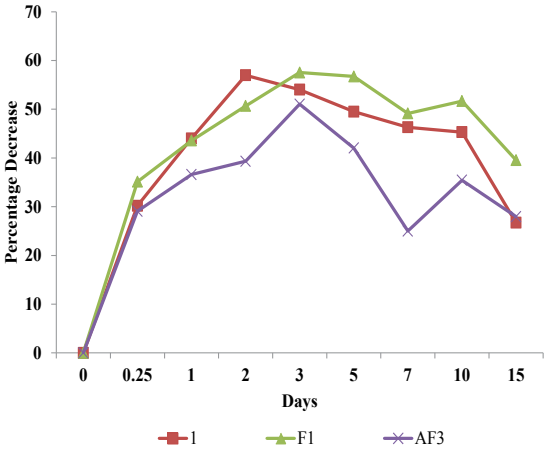

Figure 9: Changes in lignin content at different time intervals by 1, F1 and AF3 fungal strains in pulp and paper mill effluent.

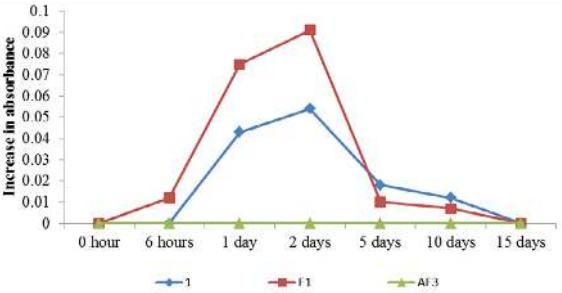

Figure 10: Increase in absorbance during the enzyme assay of lignin *peroxidase* present in pulp and paper mill effluent treated by 1, F1 and AF3 at different time intervals.

the process more feasible and economical. Thus the strains F1 and 1 has the potential for industrial application for decolourisation of pulp and paper mill effluent.

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