International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No. (03), January 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Study of Biodegradation Potential of Fungal strains isolated from distillery effluent affected soil.

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ABSTRACT

Fungal strains from distillery effluent affected soil were isolated and studied for their biodegradation potential.P. chrysosporium was taken as control in the investigation to see comparative performance of different isolates obtained from soil. Decolourization decreased with increasing effluent concentration for all the fungal isolates after 5 days of treatment. Maximum decolourization was observed in the range of 5 -10% effluent concentration for most of the fungal isolates. At 10% effluent concentration, the reduction in colour was 76.67, 77.96, 72.56, 73.63, 73.40, 73.82, 72.29, 75.70, 74.44, 75.12 and 74.94%, respectively by P. chrysosporium , F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8 , F_9 and F_{10} isolates, respectively. Among the 11 fungal isolates including P. chrysosporium F_1, F_7, F_8 and F_9 were more efficient in colour removal compared to the rest of the isolates. F_1 isolate was most efficient among all the isolates at all the concentrations. The reduction in colour by F_1 was 77.60, 77.96, 73.60, 64.16, 58.05 and 46.32%, respectively at 5, 10, 20, 30, 40 and 50% effluent concentration, respectively.Fungal stain F_1 isolated from effluent affected soil was found to be most effective in terms decolourization (77.96 %) and COD reduction (81.40%) at 10% effluent concentration supplemented with 2% glucose after 5 days of treatment.

Key words :Biodegradation, COD, Colour, Decolourization, Distillery effluent, Fungus

I.INTRODUCTION

Paper manufacturing, distilleries and dairy industry play a major role in polluting water courses as most of them discharge large volumes of waste waters carrying huge pollution load. Distilleries are one of the major agro based polluting industries. The portion of waste generated from distilleries is nearly 15 times of the total alcohol production. Distillery effluent also known as spentwash is one of the most complex, caramalized, and cumbersome waste having very high BOD (35,000-50,000 ppm) and COD (85,0000-1, 30,000 ppm)[1]. Molasses waste water (MWW) or spentwash is one of the most difficult waste products to dispose off because of low pH and dark brown colour.The characteristic recalcitrant nature is due to presence of brown melanoidins polymers that are formed in Mailland reaction between amino acids and carbohydrates [2].Distillery waste water with high BOD and COD values creates toxic conditions in the receiving water body by immediate depletion of oxygen. The problem of disposal is further accentuated by the fact that the effluent is very hot, highly coloured and acidic with a strong and objectionable odour[3].Physiochemical methods to treat distillery effluent have been found to be unsatisfactory compared

to biological methods. Biological treatment of spent wash by bacteria and fungi have been found to be satisfactory to certain extent therefore the present study was under taken to isolate indigenous fungal strains well adapted to distillery effluent for its effective and potential treatment in terms of COD and colour.

II.MATERIALS AND METHODS

The distillery effluent for present investigation was collected from Kesar Enterprises, Baheri (U.P.) after anaerobic treatment.

2.1 MICROBIAL COMMUNITY PROCUREMENT

The fungi used in present study were all local isolates, they were isolated from soil collected from the near by sides of drainage discharging distillery effluent. The serial dilution technique was adopted to isolate microorganisms [4]. The white rot fungi *Phanerochaetechrysosporium*(MTCC-787), used as control, was procured from IMTECH., Chandigarh.

2.2 PREPARATION OF FUNGAL INOCULUM (PELLETS)

Forthe treatmentof effluent the fungal inoculum was prepared as method by Benito [5]. For the preparation of fungal inoculum in the form of pellets, the fungal isolates were individually grown and cultured on potato dextrose agar plates and, these were incubated at 30°C for 4 days. When the fungal mycelium developed and covered the plate, mycelial discs of about 1 cm diameter were cut from the zone of active growth. Sterilized Erlenmeyer flask containing 100 ml potato dextrose broth and streptoamphicillin (100 ppm) was taken, and then 6 mycelial discs were added to each flask. These flasks were allowed to shake for 4 days at 30°C. The pellets of 1.5-2.0 mm size were observed in the suspension. Pellets were used as inoculum for colour removal after washing with saline solution (0.85% NaCl), and distilled water. As the pellets were highly variable in size, it was not possible to use them by count method. Thus, in the experiments, the technique was modified, and 10 ml of the inoculum was added to 100 ml of effluent for treatment.

2.3 SCREENING OF POTENTIAL STRAINS

The fungal isolates were screened for their decolourization potentiality. 25 set of triplicate flasks were filled with 100 ml of distillery effluent. To 2 sets of flasks 10 ml of fungal inoculum was added. Streptoamphicillin (100ppm) was added to flasks inoculated with fungus to prevent bacterial contamination. One set of flasks was maintained as control in which no fungus was added. The flasks were then incubated at 30^0C in a rotatory shaker (120 rpm) for 5 days, and then colour, and COD were measured after 5 days period as per methods [6] and [7]. On the basis of comparative reduction in colour and COD by different isolates, the first four most potential isolates were screened out and selected for further studies. Using these four isolates the experiment was again performed in same way as above with addition of 2% glucose as carbon source in the effluent. Again the observations were taken after 5 days for pH, colour and COD to screen out best potential fungal and bacterial strain for effluent treatment.

III. RESULTS AND DISCUSSIONS

3.1 SCREENING OF FUNGAL ISOLATES FOR DECOLOURIZATION POTENTIAL

Decolourization of anaerobically treated distillery effluent (spentwash) of different concentrations using different isolates of fungi and *P. chrysosporium*was studied. Changes in colourof distillery effluent by fungal isolated from

effluent affected soil after 5 days of incubation are represented in **TABLE1** and Fig 1. In general, decolourization decreased with increasing effluent concentration for all the fungal isolates. Maximum decolourization was observed in the range of 5 -10% effluent concentration for most of the fungal isolates. At 10% effluent concentration, the reduction in colour was 76.67, 77.96, 72.56, 73.63, 73.40, 73.82, 72.29, 75.70, 74.44, 75.12 and 74.94%, respectively by *P. chrysosporium*, F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8 , F_9 and F_{10} isolates, respectively. The white rot fungi *P. chrysosporium*was taken as control in the investigation to see comparative performance among the different isolates. Among the 11 fungal isolates including *P. chrysosporium* F_1, F_7, F_8 and F_9 were more efficient in colour removal compared to the rest of the isolates. F_1 isolate was most efficient among all the isolates at all the concentrations. The reduction in colour by F_1 was 77.60, 77.96, 73.60, 64.16, 58.05 and 46.32%, respectively at 5, 10, 20, 30, 40 and 50% effluent concentration, respectively.Again in order to find out the best isolate, the above selected 4 isolates were further tested for decolourization ability. During this screening the effluent was supplemented with 2% glucose to enhance decolourization. Changes in colour, COD and pH of distillery effluent (10 and 20%) by *P. chrysosporium* and 4 selected fungal isolates after 5 days of incubation are presented in **TABLE 2** and Fig 2. At both the concentrations, F_1 was most efficient among all the isolates including *P. chrysosporium*. At 10% effluent concentration, the reduction in colour and COD by F_1 was 81.40 and 79.10%, respectively; however at 20% effluent concentration it was 74.54 and 67.34%, respectively. The pH of effluent treated with F_1 declined from 7.35 to 7.21 at 10% effluent concentration; however, it decreased from 7.45 to 7.26 at 20% effluent concentration.

Table 1: Changes in colour of distillery effluent by *P. chrysosporium*and different fungal isolates after 5 days of incubation

Treatment with fungal isolates	Effluent Concentration (%)					
	5	**10**	**20**	**30**	**40**	**50**
Without fungus (control)	4570.50 ± 3.25	8750.50 ±1.25	15673.52 ± 1.55	23241.45 ± 2.12	28572.25 ±4.13	34950.70 ±2.95
P. chrysosporium	1075.23 ±2.250	2041.23 ±2.25	4652.32 ±11.25	8562.12 ± 22.25	133516.21 ±50.25	19236.36 ±21.25
F_1	1023.5 ±10.25	1928.30 ±3.41	4136.35 ±12.21	8327.5 ±23.50	11985.5 ±42.50	18760.8 ±14.85
F_2	1175.30 ±1.25	2400.72 ±5.41	5115.32 ±2.25	8812.5 ±40.60	12850.25 ±39.25	19725.5 ±17.25
F_3	1123.5 ±10.75	2306.75 ±2.50	5200.5 ±6.25	9012.75 ±23.70	12912.30 ±25.60	19925.23 ±16.25
F_4	1345.62	2327.55	5012.5	9237.23	13872.5	20125.23 ±

	±11.25	±10.25	±7.25	±21.50	±27.80	17.25
F_5	1326.40 ±21.25	2290.25 ±1.25	5213.345 ± 9.25	9873.5 ±17.25	13921.75 ±30.21	20728.23 ±19.25
F_6	1456.30 ±1.72	2425.5 ±0.935	6001.50 ±1.02	10350.0 ±1.93	14210.5 ±4.13	22560.50 ± 1.73
F_7	1245.36 ± 1.82	2125.75 ±7.25	4975.25 ±7.25	9985.25 ±3.01	13723.20 ±4.15	20928.36 ± 1.92
F_8	1092.36 ±21.25)	2195.25 ±6.25	4768.23 ±6.450	9623.5 ±21.25	13621.5 ±38.25	20472.63 ±16.70
F_9	1176.20 ±20.25	2177 ±5.50	4752.35 ±7.35	9756.35 ±40.40	14000.50 ±29.50	22760.25 ±18.25
F_{10}	1300.50 ±30.21	2236.5 ±6.25	5312.36 ±0.925	10210.5 ±17.25	13431.5 ±30.15	22325.50 ±31.45

F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9 and F_{10} are fungi isolated from effluent affected soil.

Each reading is the mean of three replication with ± SE

Figure 1 : Reduction in colour of different concentration of distillery effluent using *P. chrysosporium* (PC) and different fungal isolates after five days of incubation

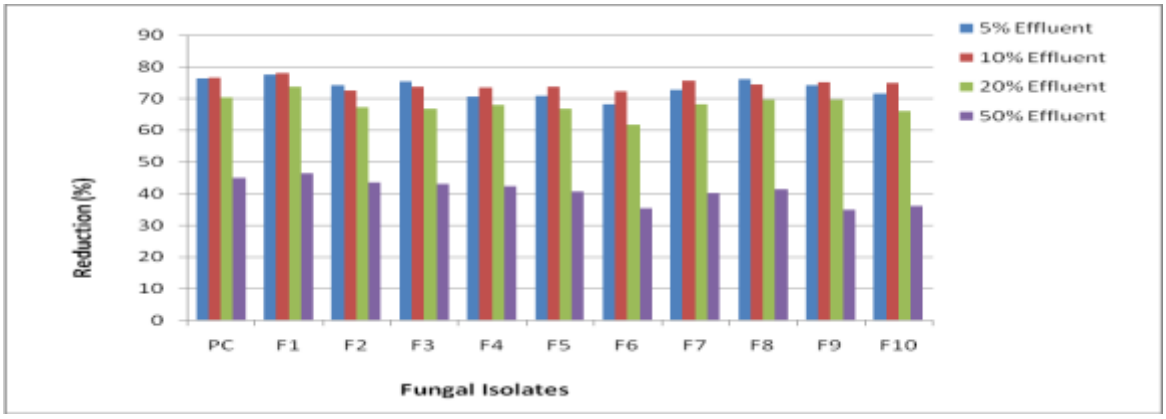

Table2: Changes in Colour, COD and pH of distillery effluent, supplemented with 2% glucose, by *Phanerochaetechrysosporium*and fungal isolates after 5 days of incubation.

Treatment with fungal isolates	Parameters		
	Colour (CU)	COD (ppm)	pH
10% Effluent			
Without fungus (control)	8765.50±12.25	4785.75±3.25	7.35±0.02
P. chrysosporium	1756.50±2.250	1023.5±0.628	7.25±0.012
F_1	1630.23±1.27	1000.00±1.250	7.21±0.021
F_7	2062.50±2.60	1176.30± 10.25	7.30±0.012
F_8	2120.5±4.71	1245.50± 3.41	7.27±0.023
F_9	2115.45±2.75	1175.15±2.75	7.32±0.05
20% Effluent			
Without fungus (control)	15654.52±16.6	9265.50±5.250	7.45±0.001
P. chrysosporium	4325.75±11.25	3245.40±12.25	7.30±0.005
F_1	3985.50±12.50	3025.60±11.25	7.26±0.062
F_7	4758.50±4.70	4078.23±12.50	7.42±0.001
F_8	4655.50±3.25	3845.50±17.25	7.32±0.025
F_9	4855.75±1.25	4256.36±13.25	7.50±0.027

F_1, F_7, F_8, and F_9 are fungi isolated from effluent affected soil

Each reading is the mean of three replication with ±SE

Figure 2 : Reduction in colour and COD of distillery effluent (10%) using *P. chrysosporium* (PC) and selected fungal isolates after five days of incubation

International Journal of Advance Research in Science and Engineering
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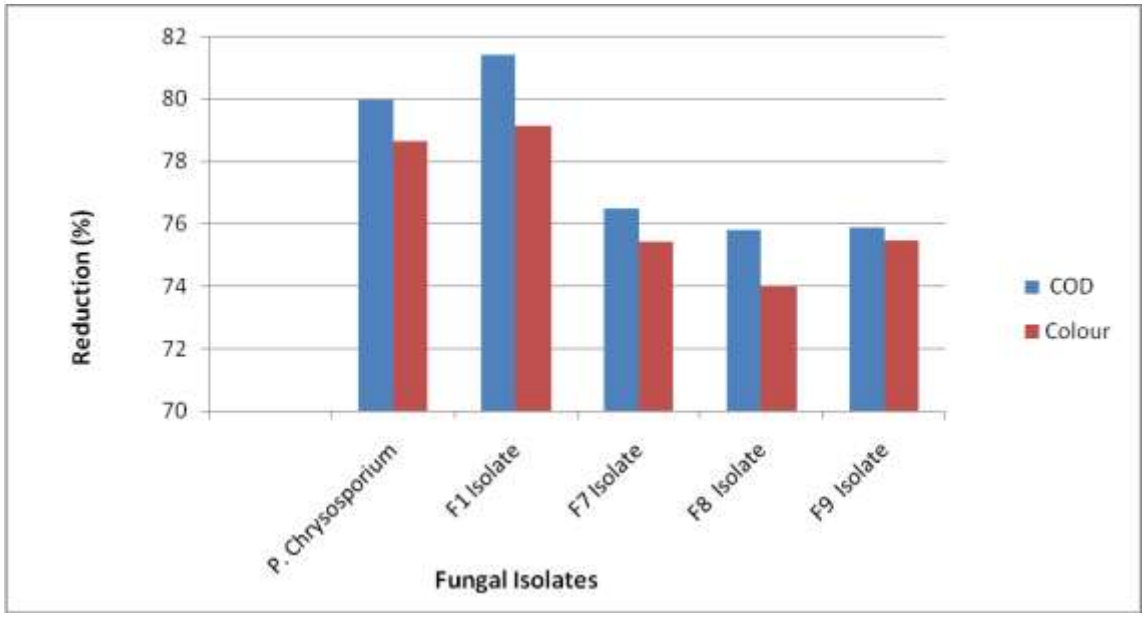
www.ijarse.com
IJARSE
ISSN: 2319-8354



The white rot fungi *P. chrysosporium*used as control yielded 76.67% reduction in colour. Thus, the most efficient fungal isolate F_1 and *P. chrysosporium* showed nearly the same reduction in colour. Similar results have been reported earlier. Reference [5] reported 82% decolourization of molasses waste water using *Trametes versicolor*. Reference [8]reported 71.5 and 53.5% decolourization of anaerobicallydigested molasses spentwash.using*P. chrysosporium*and *Coriolos versicolor*.Using *Aspergillus* niger UM2 65-85% decolourization of biomethylated distillery effluent was reported [9]. Using *P. chrysosporium* JAG-40 80% decolourization of synthetic and spentwashmelanoidins was reported after 6 days [10].*P. chrysosporium* is capable ofdecolourization as the white rot fungi produced extracellular enzymes causing pigment decolourization in a similar fashion to lignolytic enzymes produced by white rot fungi in lignin degradation. Fungi are capable of breaking down the chromophore bearing polymers to colourless and soluble volatile low molecular weight products [11].

Colour removal may be due to the degradation of smaller molecular weight fractions of melanoidins [8]. Reference [12] explained that fungal decolourization of melanoidins is due to two decomposition mechanisms; in the first the smaller molecular weight melanoidins are attacked and in the second the larger molecular weight melanoidins are attacked. Fungal degradation and decolourization of melanoidins is mediated by three enzymes, sorbose oxidase, sugar oxidase and manganese dependent peroxidase [8]. Reference [13] reported an extracellular manganese dependent enzyme catalyzing the mineralization of melanoidin. Reference [14] also reported two extracellular peroxidases a manganese independent peroxidase (MIP) and a manganese peroxidase (MnP) involved in decolourization of melanoidins.

In the present study, COD reduction was observed for all the selected fungal isolates and *P. chrysosporium*; F_1 fungal isolate was found to be most effective (Table 2 and Fig. 2). The reduction in COD of effluent (10%) was 79.10 and 78.61%, respectively by F_1 fungal isolate and *P. chrysosporium*, respectively. The reduction in COD of fungus containing effluent may be due to depletion of organic matter in effluent which may be either due to utilization of organic matter by fungi for growth and/or due to decomposition of organic matter by enzymes released by white rot fungi [15]. Decolourization and COD reduction are growth related process [8].

IV.CONCLUSIONS

Based on present investigation, indigenous fungal stains isolated from effluent affected soil are efficient in reducing pollution in terms of decolourization and COD reduction. The isolated stains can be utilized for treatment of distillery effluent as are easily available, well adapted and cost free. Bioremediation with indigenous fungal strains could be opted as a cost effective method for treatment of distillery effluent.

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