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Bioremediation and decolorization of Distillery effluent by novel Microbial Consortium

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ABSTRACT

Distillery effluent is perilous and hazardous to human race by polluting the water bodies and soil. In whole world every year the total production of distillery products is less then the production of distillery effluents. And due to this the ecological balance continuously disturbing and the food web and food chain of organism also ill effected with the toxic pollutants released from distillery effluents in environment. In present time there are lots of microorganism used to bioremediate the pollutants from industries. In this research work we had used the microbial consortium for the bioremediation of distillery effluents. Consortium was made by the white rot fungus (Phanerochaete chrysosporium)MTCC787. which produced maximum levels of ligninolytic enzymes along with isolated organism i.e. Pseudomonas aeruginosa and Aspergillus niger from distillery effluents. Phanerochaete chrysosporium in respect to Pseudomonas aeruginosa and Aspergillus niger showed a maximum decolorization zone (31.0 mm) inoculated with brilliant blue dye plates with in 15 days of incubation. It also shows the maximum ligninolytic activity on 13th day in flask studies as compared to other isolates. This WRF was used in combination with the other isolated organism i.e. Pseudomonas aeruginosa and Aspergillus niger from distillery effluent showed significantly better variations in reducing color (87.8%),Dissolved sulphites (96.8%), COD (60.7%) ,BOD (59.4%) and Sulphates (63.5%), respectively on 15 days of incubation when compared to these organism used alone.

Keywords: Distillery effluents, bioremediation, microbial consortium, *Phanerochaete chrysosporium*, BOD, COD, Decolorization.

INTRODUCTION

In whole world, cane molasses base distilleries are included under one of the polluting industries in concern to water pollution. These distilleries producing enormous amount of effluent in respect to the production of alcoholic products. Wastewater discharge from these distilleries are one of the major component of water pollution, contributing to coloration ,BOD, COD and nutrient loading of the water bodies, promoting toxic algal blooms and leading to a destabilized aquatic ecosystem [1,2]. The change in pH values of water bodies have been reported to affect aquatic life and alter toxicity of other pollutant in one form or the other[3]. Low pH values in a water bodies impair recreational uses of water and effect aquatic life. Besides this, the highly colored nature of the effluent also affects the aquatic life. Due to the effluent, the color of water bodies changes and blocks out the sunlight. Due to this oxygenation of water by photosynthesis gets reduced and results in to the death of aquatic life. The coloration is due

to the water soluble recalcitrant coloring compound called melanoidin. Melanoidin are the natural condensation product of sugar and amino acid due to the non enzymatic activity. It is dark brown to black in color and the reaction involved in formation of compound is called maillard reactions.[4] Due to this, the management of effluent is a necessary task for the distillery industries in sake of environmental pollution. The physico chemical method suggested for the effluent treatment are not eco-friendly and lots of drawbacks with them.[5,6] On the other hand , Biological processes like anaerobic digestion reduce BOD load of spent wash but the considerable amount of organic compounds and dark brown color left behind which requires secondary treatment .[7]

Due to the modernization and development, demand of ethanol is continuously increasing due to its various applications including sterilization agent in laboratories and as fuel for the transportation .Due to this the production of alcohol increases which will generate more and more distillery effluent.[8] Keep a lookout for this , it was envisaged to detect out suitable microorganisms capable of remediate the distillery effluent. Since few decades , application of bacteria [9,10,11,12] yeast [13,8], fungi[14,15,16] etc used for the bioremediation of distillery effluent. At present time, Microorganism having lignolytic activity with enzymatic potential for Bioremediation has been extensively studied . The major enzymes like Lignin Peroxidase(LiP), Manganese Peroxidase (MnP) and Laccase are the key lignin degrading enzymes with great potential in industrial applications.

In this research study, we focused on the decolorisation ability of *Phanerochaete chrysosporium* MTCC787 which is a white rot fungus with *Psedomonas aeruginosa* and *Aspergillus niger* which isolated from the contaminated sites and checks the isolates on synthetic dyes medium by enzymatic activity that resulted in Bioremedation of Effluents.

MATERIALS AND METHODS

A lyophilized culture of *Phanerochaete chrysosporium* (MTCC 787) collected from the IMTEC Chandigarh. The organism was revived on the malt extract agar (Blakeslee's formula) and in malt extract broth under aseptic conditions as described by MTCC, IMTECH Chandigarh India. The other microorganism i.e. *Pseudomonas aeruginosa*. and *Aspergillus niger*. isolated from the distillery effluents by serial dilution and identified by conformation test [17].

Test for the Antagonistic and synergistic:-
The three microorganism were tested for the antagonistic and synergistic test under different media by streaking on the sterilized media plates and then after conformation used for the purpose of bioremediation as consortium.

Screening in solid media:-
For Intial screening of *Phanerochaete chrysosporium*, *Pseudomonas aeruginosa* and *Aspergillus niger*,the dye decolorizing method on solid medium was adopted . The screening was performed in the petridishes (90 mm diameter) with Brilliant blue dye medium. In prepration of 1000 ml Brilliant Blue Dye medium we require Glucose (2.0 g), KH_2PO_4 (0.26 g), Na_2HPO_4 (0.26 g), $MgSO_4.7H_2O$ (0.5 g), $CuSO_4.5H_2O$ (0.019 g), $CaCl_2.2H_2o$ (0.0066 g), $FeSO_4$ (0.005 g), $ZnSO_4.7H_2O$ (0.0005 g), Na_2MoO_4 (0.02 mg), $MnCl_2.2H_2O$ (0.09 mg), $H_{23}Bo_3$ (0.07 mg), Brilliant Blue Dye (0.19 g) and Agar (20.0 g) pH was adjusted at 5.6 and medium was autoclaved at 121^0C for 15 minutes.

Sterilized plates of BBD medium were Inoculated with Agar Discs (6.0 mm diameter) of actively growing mycelium was maintained in Malt Extract Agar plates (Blakeslee's formula) for WRF and *Aspergillus niger*. And for *Pseudomonas aeruginosa* also the same agar discs method was used. Then plates were incubated at 30^0C for 15 days. Colony that turned Brilliant Blue in to Yellow or decolorized it was considered positive lignolytic.

Screening in liquid media :-
For Lignin peroxidase and Manganese peroxidase activity determination , the fungal and bacterial cells were grown in 250 ml Erlenmeyer flask containing 50 ml of (SMM) medium composed of Glycerol (10 g/l), Ammonium tartarate (1.84 g/l),Sodium tartarate (2.3 g/l), Di hydrogen Phosphate (2.0 g/l), Calcium chloride (0.14 g/l), Ferrous sulphate (0.07 g/l), Magnesium sulphate (0.7 g/l), Zinc sulphate (0.046 g/l), Magneous sulphate (0.035 g/l), Copper sulphate (0.007 g/l), Thiamine (0.0025 g/l), Yeast Extract (1.0 g/l) , Veratrylic alcohol (0.067 g/l) and Tween 80 (0.5 g/l). The culture were incubated for 15 days at 30 ± 2^0C in static condition.

For Determination of Laccase activity , the fungal and bacterial culture were grown in 250 ml Erlenmeyer flasks containing 50 ml of a (LMM) medium composed of Glucose (10 g/l) , Pottasium di hydrogen Phosphate (1.0 g/l),

Ammonium tartarate (2 g/l), Pottasium chloride (0.5 g/l), Magnesium sulphate (0.5 g/l), Yeast Extract (1.0 g/l), Soya tone (5.0 g/l), $CuSO_4$ (150 mM) and 10 mg/l of trace elements containing per litre of Distilled water.

Assaying of enzyme

LiP activity was determined by monitoring the oxidation of Veratryl alcohol to Veratraldehyde at 37^0C as indicated by an increase in A_{310} . The reaction mixture contain 500 µl extract of enzyme , 500 µl Hydrogen peroxide (2 mM/l), 500 µl veratyl alcohol solution (10 mM/l) and 1.0 ml sodium tartarate buffer ,pH 3.0 (0.1 mM/l). One unit of enzyme activity defined as the amount and enzyme oxidizing 1 µM of substrate /min.

MnP activity was measured with phenol red as the substrate at A_{610} . Reaction mixture contained 500 µl enzyme extract , 100 µl phenol red solution (1.0 g/l), 100 µl Sodium tartarate, pH 4.5 (250 mM/l)activity is defined as the amount of enzyme oxidizing 1 µ mol of substrate per litre.

Laccase activity is determined by the oxidiation of 2,2'-azino bis (3- ethyl thiazoline -6-sulphonate), i.e. ABTS at 37^0C. The reaction mixture contained 600 µl enzyme extract , 300 µl sodium acetate buffer pH 5.0 (0.1M) and 100 µl ABTS solution (1mM). Oxidation was followed via the increase in absorbance at 420 nm. One unit of enzyme activity is defined as the amount of enzyme oxidizing 1m mol of ABTS per minute.

Effluent decolorization

*Phanerochaete chrysosporium*MTCC787,Aspergillus *niger* and Pseudomonas *aerigunosa*. were used as inoculums alone per consortium at 4% (w/v) to decolorize the distillery spent wash.

The distillery spent wash collected from a local distillery was used at the different concentrations as fermentation medium to reduce the pollution loads in term of decolorisation, BOD, COD, Sulphates and Sulphites. Spent wash was supplemented with 0.01% yeast extract. The potent microbes included *Phanerochaete chrysosporium*, *Pseudomonas aeruginosa*, and *Aspergillus niger* .

All the experiments carried out in a triplicates in 100 ml glass tubes containing 50 ml medium and revised two times. The tubes were incubated at room temperature for 15 days. Decolorisation was measured as decrease in optical density of supernatant at 475nm against 0 hrs sample by UV-Visible Spectrophotometer (Hitachi2900). The BOD, COD, sulphates, sulphites were determined by standard methods .[18]

Calculations:

Percentage decolorisation was calculated by (A - B/A) ×100. Where A is initial absorbance, B is final absobance and COD measured by dichromate method.[18]

RESULTS AND DISCUSSION

***Dye Decolorisation method*:-**

Table 1. Dye Decolorization in medium by microorganisms.

Microorganism	Radial growth (mm)	Radial growth (mm)
Phanerochaete chrysosporium	26.0±2.2	31.0±2.4
Pseudomonas aeruginosa	12.0±1.0	15.0±0.8
Aspergillus niger	24.0±2.2	28.0±2.2

The table 1 shows that, all the three microorganism shows positive results for the dye decolorisation they actively decolorized the brilliant blue dye plates within 15 days of incubation as the medium was turned from blue to yellow or decolorized were considered as ligninolytic positive and also carried out for further studies in liquid culture for ligninolytic property i.e. MnP, LiP and laccase. On other hand *Phanerochaete chrysosporium* which required period of between 7-20 days to achieve 90% decolorisation in different range of synthetic dyes [19].

Enzyme activity

All the microorganism showed the positive ligninolytic activity of three enzymes tested i.e. laccase, MnP, LiP. Among them highest enzyme activity was showed by *Phanerochaete chrysosporium* MTCC787 on 14th day. Where as *Aspergillus niger* shows slight less then *P.chrysosporium* and *Pseudomonas* shows very less activity on 14th day .
In this study the production of ligninolytic enzymes i.e. laccase , MnP, LiP by *Phanerochaete chrysosporium* MTCC787,*Pseudomonas aeruginosa*. and *Aspergillus niger* and their subsequent use for decolorisation of distillery effluent is reported . In earlier studies, Kahraman and Yesilada in 2003 reported the molasses decolorisation in solid state cultivation with white rot fungus[20]. Since decades the white rot fungus are used for decolorisation of distillery waste waters by Kumar et al., Dahiya et al.[21,22], The correlation between decolorizing and lignolytic abilities of white rot fungus has been commented upon by several authors like Banat et al. and Zhang et al.[23,24]
Essentially the main color causing compound in distillery effluent is melanoidin , which has a chemical structure quite similar to Humic acid, another recalcitrant compound found in soils and lignin [4]. The lignolytic activity of white rots is thought to be responsible for degradation of melanoidin.

Effluent treatment

Isolates of distillery effluent i.e. *Pseudomonas aeruginosa, Aspergillus niger* and *Phanerochaete chrysosporium* MTCC787 and their consortium were used to analyse the chemical characteristics of distillery effluent .
Untreated distillery effluent showed dissolved Sulphite (14,556 mg/lit.), sodium hydrogen sulphite (0.187g), Sulphate (1575 ppm), COD (74230 ppm), and BOD (3608 ppm) respectively.

In present study , the percentage reduction of coloration by three microorganism alone in respect to consortium shows less results . Here, *Phanerochaete chrysosporium* shows 78.30% , *Aspergillus niger* shows 52.5%, *Pseudomonas aeruginosa* shows 70.8% and the Consortium of all microorganism shows 87.80%. (Fig.1)

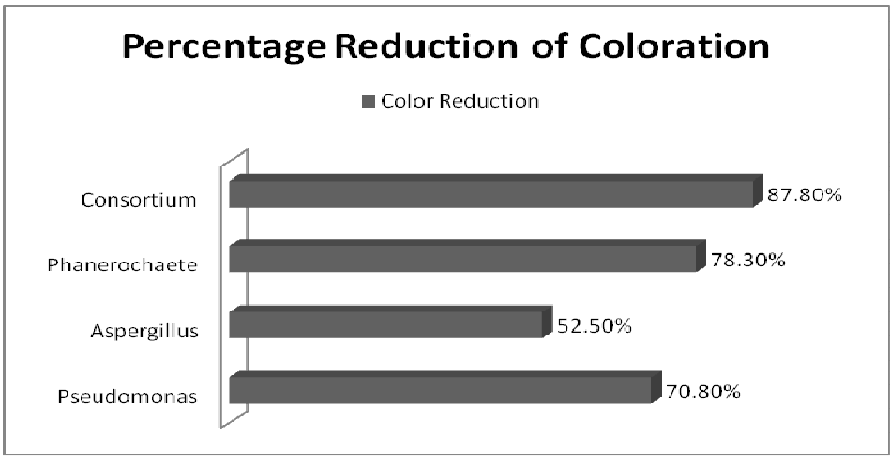

Fig. 1 Percentage reduction of coloration.

In case of dissolved sulphites, *Phanerochaete chrysosporium* shows 92.50%, *Aspergillus niger* shows 87.70%,*Psedomonas aeruginosa* shows 69% and Consortium of microorganism in combine shows 96.80% reduction of dissolved sulpites from effluents.(Fig.2)

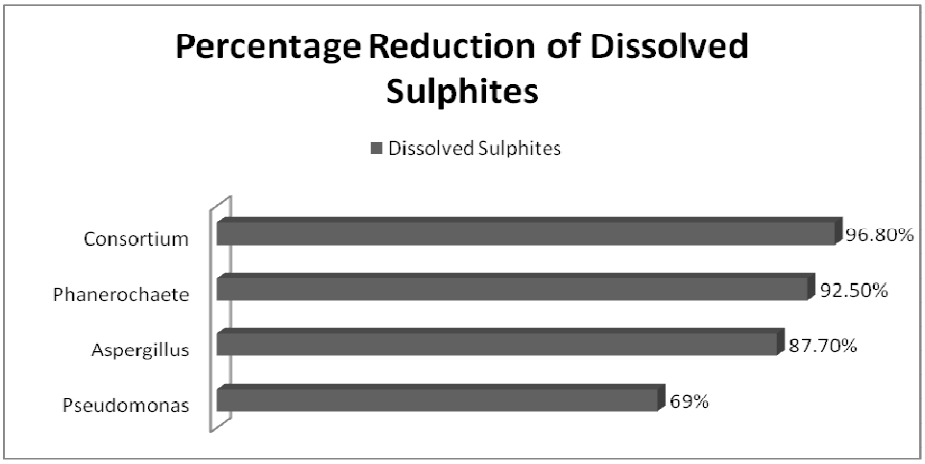

Fig. 2 Percentage reduction of dissolved sulphites

While in case of COD, *Phanerochaete chrysosporium* shows 55.50% reduction, *Aspergillus niger* shows 53.40% ,*Pseudomonas aeruginosa* shows 29.20% and consortium shows 60.70% reduction of COD from effluents.(Fig.3)

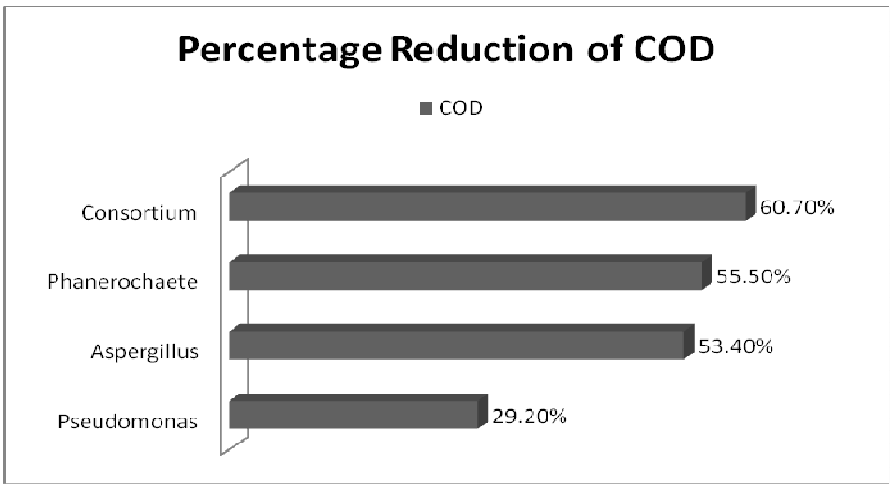

Fig.3 Percentage reduction of COD

In case of Reduction of BOD , *Phanerochaete chrysosporium* shows 56.30% ,*Aspergillus niger* shows 58.20%,*Pseudomonas aeruginosa* shows 53.30% and microbial consortium shows 59.40% reduction in BOD level in effluents.(Fig.4)

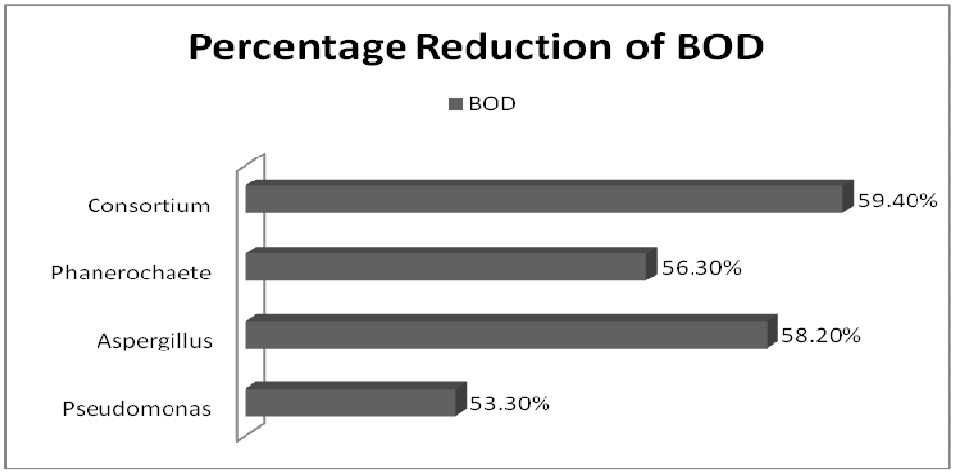

Fig. 4 Percentage reduction of BOD

In Fig. 5 , the percentage reduction of sulphates by *Phanerochaete chrysosporium* is 69.30%, *Aspergillus niger* shows 80%,*Psedomonas aeruginosa* shows 49.20% and consortium of microorganism containing the above three microorganism shows 63.50% reduction of sulpahtes from effluents.

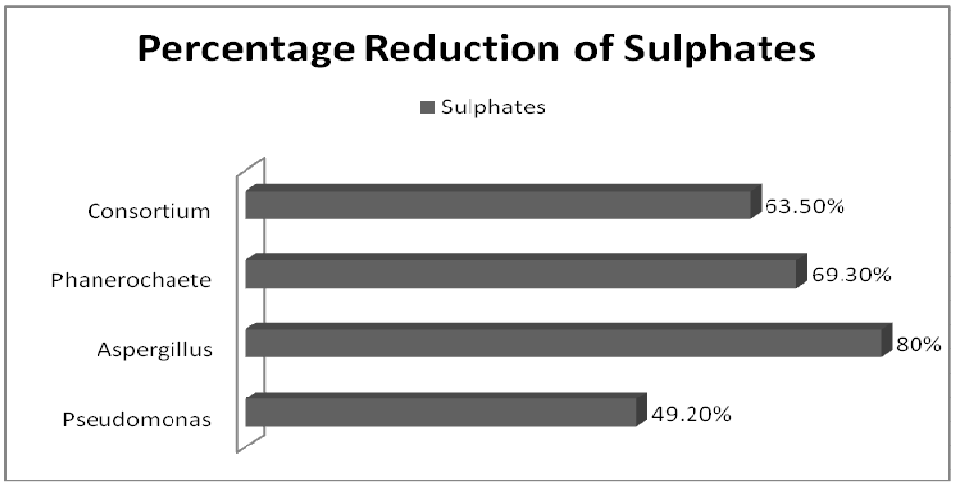

Fig. 5 Percentage reduction of Sulphates.

In earlier studies, *Aspergillus terreus* was used as a bioremediant and it shown 40% removal of COD in the effluents. And the similar research done on the microorganisms like *Neurospora crassal* [25], *Trichoderma viride* [26] and *Aspergillus foetidus* [27]. Also degradation of azo dyes in textiles wastewater by crude enzymes of *Aspergillus* fungus was reported [28,29].

In one of earlier studies[30] it was shown that the enzymes of this fungus could degrade fiber in pulp and sugar beet wastewater, pH (5–7), temperature (30–50°C) and addition of inoculated enzymes (up to 3 ml) did not have any significant effects on biodegradation of cellulose in wastewater. It was also shown [30] that *Aspergillus terreus* had peroxidase and laccase activities when grown on wheat straw and removed COD of textile dyes effluent.

Decarboxylation of vanillic acid and oxallic acid by laccase and peroxidase is reported by Akamatsu et al.and Agemata et al.[31,32] Dezotti and co-workers showed that lignin peroxidase catalyzed 65% removal of COD from kraft effulent[33].

Also Itavara and co-workers reported that a mixture of *Trichoderma reesei* culture filtrate, purified endoglucanase and *β*-glucosidase of *Aspergillus niger* degraded 70% of cellulose material to carbon dioxide. Also in a non-sterile system the dissolved organic carbon (DOC) released by the enzymes is subsequently utilized by the microbial community.[34] Therefore in this study we have concluded that 60.7% COD removal of wastewater is due to the presence of microbial consortium.

Although other fungi representatives of different taxonomic and ecophysical groups are able to degrade lignocelluloses substrates and produce lignolytic enzymes. Manganese peroxidase activity was reported in Alternaria alternate with a possible role in humic acid degradation[35].

Penicillium has also been reported to produce lignolytic enzymes[36],through its degrading mechanism is considered to be different from that of the WRF [37].

Table 2- Microbial study so far done for the decolorization and COD removal

Microorganism	% COD removal	% Color removal	References
Trametes versicolor	75	80	[49]
Phanerochaete chrysosporium	73	53.5	[21]
Phanerochaete chrysosporium	NIL	85(free) 59(immobilized)	[50]
Phanerochaete chrysosporium	57	37	[20]
Phanerochaete Chrysosporium1557	NIL	75	[51]
Phanerochaete chrysosporium ATCC 24725	48	55	[52]
P. chrysosporium NCIM 1106	NIL	82	[53]
P. chrysosporium NCIM 1197	NIL	76	[54]
Coriolus versicolor	49	63	[20]
Coriolus hirsutus	NIL	45	[55]
Flavodon flavus	NIL	80	[14,39]
Penicillium decumbens	50.7	41	[38]
Pleurotus pulmonarius	34	43	[20]
Bacillus sp.	NIL	35.5	[56]
Pseudomonas fluorescence		76	[49]
Pseudomonas putida U	44	60	[12]
Mixed culture of six microorganism i.e. Pseudomonas, Enterobacter, Stenotrophomonas, Aeromonas, Acinetobacter and *Klebsiella*	44	NIL	[11]
Bacillus cereus	81	75	[57]
Funalia trogii	62	57	[20]
Microbacterium hydrocarbonoxydans *Achromobacter xylosoxidans* *Bacillus subtilis* *Bacillus megaterium* *Bacillus anthracis* *Bacillus licheniformis* *Achromobacter xylosoxidans* *Achromobacter* sp. *Bacillus thuringiensis* *Bacillus licheniformis* *Bacillus subtilis* *Staphylococcus epidermidis* *Pseudomonas migulae* *Alcaligens faecalis* *Bacillus cereus*	86.14	75.5	[58]
Microbial consortium*(Phanerochaete chrysosporium,Aspergillus niger,Pseudomonas aeruginosa)*	60.70	87.80	Present study

In case of beet molasses alcoholic fermentation waste water 41% decolorisation by penicillium decreases has been repeated [38]. Using Flavodan flavus ,a white rot fungus isolated from a marine habitat,73% decolorisation of 10% diluted molasses spent wash was achieved in a week[39]. In one of studies, individual and consortium of fungal starins used for the purpose of bioremediation of Azo dye containing textile effluents and the percentage reduction of color was 67.24% and reduction in COD was 26%.[40] In another similar studies Enterococcus faecalis strain YZ 66 was used for the biodegradation of sulphonated azo dye C.I. reactive orange 16.[41]

So, the microorganisms exhibited lignolytic enzyme activity production which was enhanced by submerged fermentation. Phenol tolerance of under varying conditions of nitrogen sufficiency by Pleurotus florida [42]. A study done for the Range determination for resistance and tolerance and growth kinetic of indigenous bacteria isolated from lead contaminated soils near gas stations [43].A study of an electrocoagulation (EC) unit for the treatment of industrial effluent of Ouagadougou done for the bioremediation [44]. A study on Chemcial oxygen demand reduction of Aqueous Active Pharmaceutical Ingredient - Furosemide waste water streams using Advanced OxidationFenton process based on H2O2/Fe+2 Salt done for the effluents degradation and bioremediation [45].A study on removal of BOD contributing components from Sugar Industry Waste water using Bagasse Fly Ash-Waste material of Sugar Industry for the removal of COD and other components[46]. A study on the adsorption study of BOD content from Sugar Industry waste water by low cost material Fly ash done by the scientist for the purpose of bioremediation[47]. In one of earlier studies a preparation and characterization of activated carbon from Thevetia peruviana for the removal of dyes from textile waste water done for the removal of COD and other components for the bioremediation of effluents[48].Thus, providing a hope for the further application in different purposes of safe bioremediation. A comparative study table of different microorganism used earlier for the decolorization and bioremediation of effluents. (Table.2)

CONCLUSION

In present research study, individual organism shows not so good results but in form of consortium, microorganism shown better results for the reduction of color, sulphates, sulphites, COD, BOD in the distillery effluents. So, in future aspects the present consortium could be boon for the field of environmental toxicology and the microbial consortium used in the research is a eco friendly , economically sound and can be used for the removal of toxic compounds from the effluents and contaminated sources which are hazardous for the environment and human being.

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