Microbial Degradation Of Reactive Dye Using Mixed Consortium

C.Nivetha, S.P.Sangeetha, Haleel Kaleemullah, Umar Ibin Aziz, Adarsh J S

Abstract: Synthetic chemicals have been widely used as dyes by the textile industries. Since a large amount of different dyes are used by textile industries, wastewater discharged out of them poses a threat to the surroundings. These dyes enter into the surrounding water bodies in the form of colored wastewater, unless degraded properly. Micro organisms have the ability to convert colored dyes to non-colored liquid under defined environmental conditions. In this study, dye degradation ability for mixed consortia (Bacterial and fungal) of Pseudomonas putida and Phanerocheate chrysosporium is studied for synthetic dye Reactive Red 198. Results show that mixed consortia of Pseudomonas putida and Phanerocheate chrysosporium has an efficiency of 91% in decolorization and 87% in COD removal within 3 days of dynamic condition.

Key Words: Decolorization, Degradation, mixed consortium, reactive dyes, Pseudomonas putida, Phanerocheate chrysosporium

1 INTRODUCTION:

Synthetic chemicals are being used as dyes by the textile industries in the recent years. Weaving industries wastewater poses a serious threat to the environment since they contain a large amount of chemical dyes. Natural dyes have been replaced by synthetic reactive dyes because of their economic effectiveness. These dyes when disposed into the environment cause serious problems like influencing the penetration of light which affects the photosynthetic activity of aquatic plants. Sheng H. Lin and Chi F. Peng[1] stated that the physical and chemical methods of treatment require a large amount of expenditure and produce more sludge. An alternative method of Bioremediation has been emerging as a economical, low sludge production and environmental friendly method for the treatment of textile industry wastewater. According to Pooja Upadhyay[2] ,the microbial degradation of artificial azo dyes starts with the depletion of azo bond and produces carcinogenic aromatic amines in the absence of oxygen. But in the presence of oxygen, fungi produce enzymes like laccase which decolorize dyes using free radical mechanism avoiding the formation of toxic aromatic amines and other byproducts can be easily degraded by bacterial enzymes. Thus, mixed consortium (bacterial and fungal species) degrades the dye and the midway compounds.

2 MATERIALS & METHODS:

2.1Dye & Chemicals:

The water-soluble Reactive Red 198 dyes were taken for degradation studies. They are mixed bi-functional reactive dyes where vinyl sulphone group is linked to chromophore through a mono-chloro tri-azine group as a bridge link. The dyes were used at a quality identical to that being used in the textile industry. These dyes were of industrial grade and were a generous gift from local textile industry located in Tirupur, India. Reactive Red 198 dye was used as a model dye for all the degradation experiments. All other chemicals used were of an analytical grade.

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2.2 Pure Culture Collection

Pure cultures were collected from MTCC, Chandigarh.

2.3 Microorganisms And Culture Conditions

2.3.1 Pseudomonas putida

Pure culture was maintained on nutrient agar slants at 4^0C. Subcultures are made routinely on Nutrient Agar (NA) medium. The culture of P.putida was grown in 250 mL Erlenmeyer flask, containing nutrient broth medium of following composition (g/100 mL): 0.5 g peptone, 0.5 g sodium chloride and 0.3 g beef extract at room temperature. 2.3.2 Phanerocheatechrysosporium Pure culture was maintained at 4^0C. Subcultures can be made routinely on Potato Dextrose Agar (PDA) medium. The culture of P.chrysosporium grown in 250 mL Erlenmeyer flask, containing nutrient broth medium of following composition (g/100 mL): 5g Potato Extract , 2 g Dextrose, 3 g Agar at room temperature for 5 days of incubation in aerobic condition.

2.3.3 Mixed Consortium

Mixed culture containing Pseudomonas putida and Phanerocheatechrysosporium were grown on nutrient potato dextrose agar medium.

2.3.4 Synergestic Activity Of Both Microorganisms

The bacterial culture and fungal culture was grown on nutrient agar and potato Dextrose agar respectively. Nutrient potato dextrose agar was used for the detection of synergism between these two microorganisms. About 20 mL of molten nutrient potato dextrose agar was poured aseptically in a sterile 100mm Petri plate. Dual culture plating method was employed for the assay. A single streak of bacterial line was made on agar plate and adjacently 5mm fungal agar plug was placed. After 4 days of inoculation both culture was seen. Inhibition between organisms was checked. No inhibition occurred between the organisms. Therefore, both organisms have the synergic nature.

Fig 1 *Synergic test for P.putida and P.chrysosporiumas a mixed culture*

2.3.5 Growth Curve For Mixed Consortia

Since the growth phase of the organisms determines the rate of microbial growth, death rate and the extent of secondary metabolic activity in a specific medium with dye, the growth curve is found to be an important characteristic of the strain used for biodecolorization studies. The growth was analyzed by inoculating colonies of P.putida and P.chrysosporium in a fresh NB medium with 500 mg/l concentration of dye at a pH of 6.0 and observing the cell density for every day at 600 nm, which is the maximum absorbance of microbial cells.

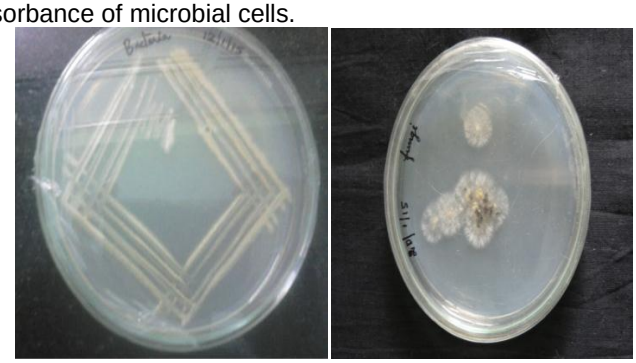

Fig 2 *Streaked cultures of Pseudomonas putida & Phanerchaetechrysosporium*

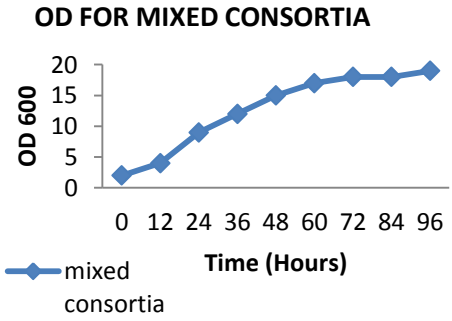

Fig 3 *Growth Curve for mixed consortia*

The values of time and absorbance over a period of growth were monitored and growth curve was plotted as given in figure . From the results, it has been inferred that consortia reached the exponential growth phase in the period of first 2 days, in which the cell concentration tends to increase logarithmically. After that the stationary period was observed, in which the performance of enzymes will be more.

3 EXPERIMENTAL SET UP

250 mL Erlenmeyer flask is used for the decolorization and degradation studies. 250 mL nutrient media with dye was inoculated with both microorganisms. Depending on the experimental conditions pH and concentration of dye were changed.

4 RESULTS & DISCUSSIONS

4.1 Study Under Static And Dynamic Conditions

In order to check the biodecolorization and degradation mechanism of mixed consortia, the studies on the effect of shaking and static incubation on growth rate and removal rate has been found to be necessary. To study the growth and degradation pathway of mixed consortia under static and shaking condition, the dry cell weight was plotted against the time period under both the incubation conditions. The results are given in fig 5 and fig 6. From the results, it is observed that agitated cultures grew well compared to static condition. As the agitation was continued during the stationary phase, decolorization and degradation was gradually increased with respect to time. But in stationary phase, Decolorization was high at initial stage because of the bacterial activity and minimal DO level but it will produce aromatic amines as intermediate components. It will be more carcinogenic than original dye.

Table 1 *Color and COD removal efficiency at Static and Dynamic conditions*

	CONTROL COD (mg/l)	1st day		2nd day		3rd day		4th day	
		COD (mg/l)	Efficiency (%)	COD (mg/l)	Efficiency (%)	COD (mg/l)	Efficiency (%)	COD (mg/l)	Efficiency (%)
Static	1820	1490	18	1290	29	1130	38	1090	40
Shaking	1900	1430	25	1000	47	170	91	170	91

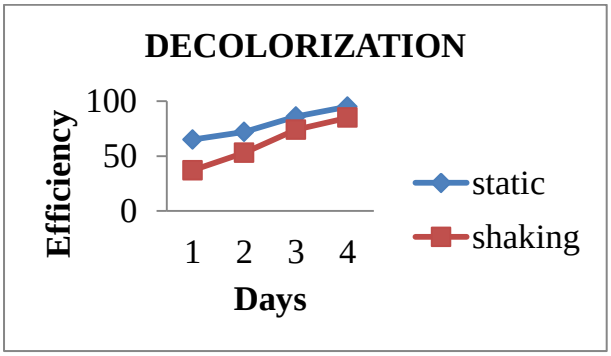

Fig 4 *Efficiency of Decolorization at static and dynamic conditions*

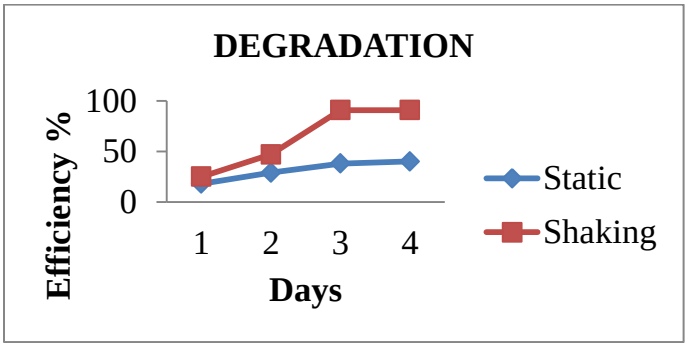

Fig 5 *Efficiency of Degradation at static and dynamic conditions*

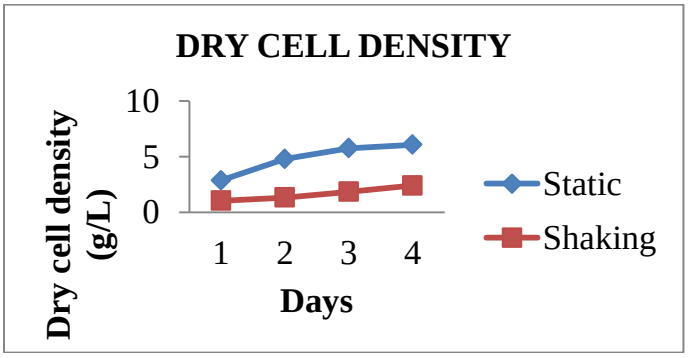

Fig 6 *Dry cell density at static and dynamic condition*

Fig 7 *Synthetic dye solution at initial stage and after 3 days of incubation*

4.2 pH Optimization Study

The variation of initial pH on the decolorization of dye is presented in fig 8. This seems to indicate that pH 5 would be more favorable for decolorization of the dye. As can be seen from fig, the maximum color removal efficiency of 73% was achieved at 3rd day of biodecolorization process.

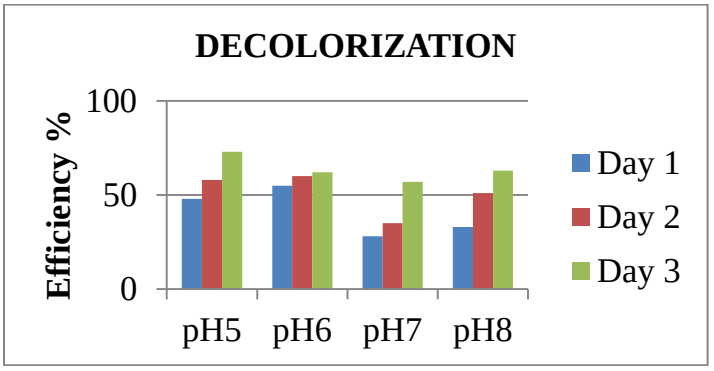

Fig 8 *Efficiency of Decolorization for different pH*

The variation of initial pH on the degradation of dye is presented in fig 9. This seems to indicate that pH 5 would be more favorable for degradation of the dye. As can be seen from Fig, the maximum COD removal efficiency of 80% was achieved at 3rd day of biodegradation process.

Table 2 *Color removal efficiency at different pH*

	CONTROL COD (mg/l)	1st day		2nd day		3rd day	
		COD (mg/l)	Efficiency (%)	COD (mg/l)	Efficiency (%)	COD (mg/l)	Efficiency (%)
pH 5	1600	1140	29	830	48	290	82
pH 6	2000	1720	14	1300	35	580	71
pH 7	1520	1230	19	850	44	640	58
pH 8	2080	1140	45	1000	52	790	62

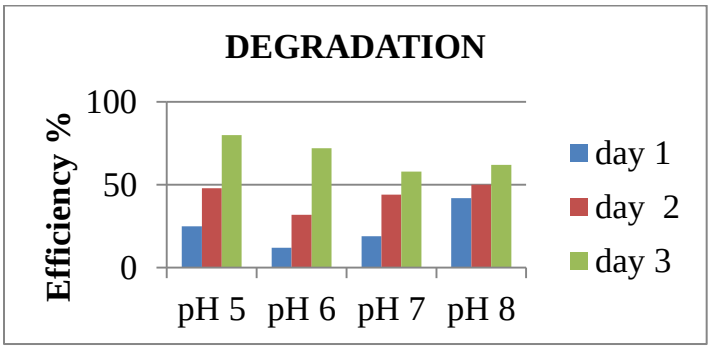

Fig 9 *Efficiency of degradation for different pH*

4.3 Effect Of Dye Concentration

The biodegradation and decolorization activity of mixed consortia was studied at dye concentrations ranging of 50,100,250,500,750 and 1000mg/L. The results are

presented in Fig 10 and Fig 11 respectively. It shows the high concentration of dye inhibits the microbial growth and removal efficiency.

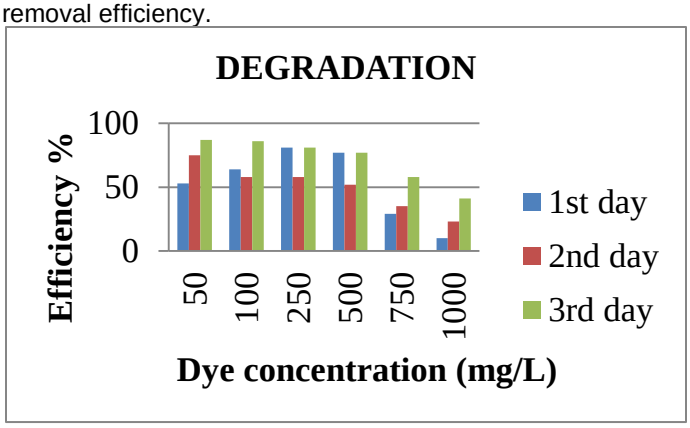

Fig 10 *Efficiency of Degradation at different initial dye concentration*

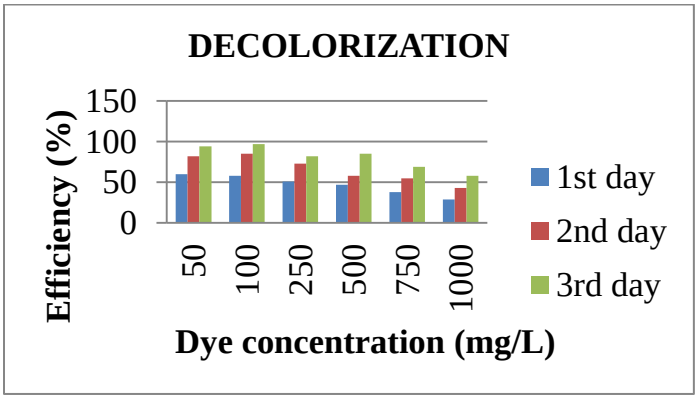

Fig 11 *Efficiency of Decolorizationfor different initial dye concentrations*

5 CONCLUSION

In this investigation water soluble Reactive Red dye was taken for biodecolorization and degradation studies. The experiments were conducted in batch mode to study the influence of operational parameters such as pH and dye concentration on biodecolorization and biodegradation performance of P.putida and P.chrysosporium as mixed consortia. Results obtained from this work shows that mixed consortia possesses high decolorization and degradation efficiency to a maximum of 91 % and 87 % respectively within 3 days of dynamic condition. The degradation rate decreased in high dye concentration and the overall removal percentage was high up to 500 mg/L. Hence, the consortium is proven to have ability to degrade up to 500 mg/L of Reactive Red effectively under normal conditons. From the studies carried out with a variation in pH, it is observed that pH 5.0 was found to be optimum for decolorization and degradation activity to occur.

6 REFERENCES:

[1] Sheng H. Lin and Chi F. Peng. (1994), “Treatment of Textile Wastewater by Electrochemical Method”, Wat.Res.Vol.28, No.2, pp.277-282.

[2] Pooja Upadhyay, Rahul Shrivastava and Pavan Kumar Agrawal (2016), “ Bioprospecting and Biotechnological applications of fungal laccase”, 3 Biotech 6 : 15.

[3] Rena Mehta &KanikaYadav (2013), “Soil contamination due to textile effluent- case study on the printing cluster of Jaipur”. Journal of the textile association.

[4] Maulin P Shah, Patel K.A, Nair S.S and Dari A.M, “Microbial degradation of Textile Dye (Remazol Black B) by Bacillus spp. ETL-1012”, BioremedBiodeg 2013, Volume 4, Issue 2,1000180

[5] Joshni T Chacko, KalidassSubramaniam, (2011), “Enzymatic Degradation of Azo Dyes – A Review”International Journal of Environmental Sciences Volume 1, No 6.

[6] Ehlers GA, Rose PD , (2005), “ Immobilized white rot fungal biodegradation of phenol and chlorinated phenols in trickling packed - bed reactors by employing sequencing batch operations”, Bioresour. Technol. 96:1264-1275.

[7] Bhaskar M, Gnanamani A, Ganeshjeevan R J, Chandraseka R, Sadulla S, (2003),“Analysis of carcinogenic aromatic amines released from harmfullazo colorants by Streptomyces”SP SS07. J. Chromatogr. A. 1018:117-123.

[8] Azeem Khalid, Muhammad Arshad, David E. Crowley (2009), “Biodegradation potential of pure and mixed bacterial cultures for removal of 4-nitroaniline for textile dye wastewater research 43.

[9] Claus H., Faber G. &Konig H., (2002), “Redox-mediated decolorization of syntheticdyes by fungal laccases”. Appl. Microbiology & Biotechnology, 59, 672-678.

[10] JoycelinLeebana V (2008), “Biodegradation of Direct Golden Yellow, A Textile Dye by Pseudomonosputida”, Center for Environmental Studies, Anna University, M.E (Environmental Engineering) thesis.P-58.

[11] Padmavathy.S, Sandhya.S, Swaminathan.K, Subramanian Y. V., Chakrabarti.T, and S. N. Kaul, (2003), “Aerobic Decolorization of Reactive Azo Dyes in Presence of Various Cosubstrates”,Chem. Biochem. Eng. Q. 17 (2) 147–151 (2003).