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Degradation Potential Of Free And Immobilized Cells Of White Rot Fungus Phanerochaete chrysosporium On Synthetic Dyes

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Abstract: There is a growing public concern over toxicity and carcinogenicity of dyes which usually are of synthetic origins made up of complex aromatic molecular structures and highly recalcitrant. Over 10,000 dyes are commercially available and around 15% of the global dye production is being discharged to the aquatic environment untreated or with partial treatment. Direct disposal of these toxic dyes causes deterioration of groundwater along with disruption of aquatic ecosystems and indirectly it enters into our food chain. Various treatment techniques such as physical, chemical, biological and advanced oxidation methods are available to treat the textile effluents and each has its own pitfalls and benefits depend on the techno-economical characteristics. Besides all techniques, biological methods are highly versatile, cost effective and minimal sludge production. Now considerable research efforts have been devoted to the biological treatment processes with the use of microorganisms such as bacteria and fungi. White rot fungi is one such well-known fungal family proven for effective dye decolourization. The evaluation of decolourization potential of free and immobilized cells of basidomycetes *phanerochaete chrysosporium* has been investigated in this study.
Keywords: Biological methods; Dyes; Microbial decolourization; White rot fungi; Phanerochaete chrysosporium.

1.0 Introduction

Textile industries are one of the key water and chemical intensive industries that consumes water and chemicals extensively for wet processing of textile dyes. Approximately 10,000 different types of dyes are available commercially and over 0.7 million tons of synthetic dyes are produced annually, worldwide[1]. Out of this massive quantity, around 10-15% of the dyestuffs reached environment through the release of industrial effluents from manufacturing and processing activities[2]. In a typical textile industry approximately 1000 kiloliters of water is utilized per ton kilogram of cloth processed. Dye wastewater usually consists of a number of contaminants including acids, bases, dissolved solids, toxic compounds, and colored materials which are noticeable even at very low concentrations and need to be removed before the wastewater can be discharged. Since the presence of color and its

compounds in wastewater at low concentration itself is visible, it has always been undesirable and has recently become an area of major public concern and scientific interest[3].

The conventional physical and chemical effluent treatment methods such as adsorption, chemical precipitation and flocculation are inefficient as they result either in large volumes of sludge or in the release of other harmful chemicals[4]. All the methods possess significant differences in color removal results, volume capability, operating time and capital costs. Physical and chemical methods of dye removal are effective only if the effluent volume is small otherwise they are infrastructure intensive, expensive and generate secondary sludge. On the other hand, biotechnological approaches were proven to be potentially effective in treatment of this pollution source in an eco-friendly manner and at low cost, better effectiveness and environmental benignity[5,6].

In recent years, numerous studies have focused on exploring the bacterial capabilities of dye removal from wastewater. Yet, relatively very limited studies have focused on the possible exploration of fungal capabilities in dye removal. Most currently used laboratory methods for biodegradation involve aerobic microorganisms like fungi, which utilize molecular oxygen as reducing equivalent acceptor during the respiration process biodegradation under anaerobic conditions are also reported[7]. White-rot basidiomycetes are a group of fungi capable of depolymerizing and mineralizing lignin with their extracellular, non-specific ligninolytic enzymes and this fact stimulated research on the ability of ligninolytic fungi to degrade various organic pollutants[8]. Another important advantage for degradation of azo dyes using white rot fungi is that ligninolytic enzymes degrade synthetic dyes by oxidation, in contrast to the reduction pathway in bacterial degradation. The lignolytic enzymes of *P. chrysosporium* (due to their oxidative mechanism) are considered responsible for the aerobic degradation of dyes that not only decolorize but have also shown to detoxify the effluents completely[9]. Recently, the application of immobilized cells for biosorption of dyes has been gaining attention in the field of wastewater decolourization. Many researchers have studied the effect of immobilized whole cells and enzymes on decolourization characteristics since immobilization provides distinct stability over free cells[10].

Therefore, the present study is directed to investigate the decolourization ability of free and immobilized cells of white rot fungus *phanerochaete chrysosporium* on reactive azo dye solutions of Amido black B and Procion blue-2G at various concentrations.

2.0 Materials and Methods

Amido black B, Procion blue-2G, Potato-Dextrose (PD) Agar and broths, glucose, Manganese Sulphate ($MnSO_4$), Ammonium hydroxide (NH_4OH), Sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), boric acid (H_3BO_3) are all laboratory grade chemicals. Chemicals are used without any purification unless otherwise stated.

2.1 Microorganisms and Preparation of Culture

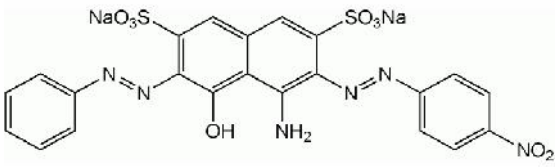
The white rot fungus *P. chrysosporium* MTCC 787 was obtained from the culture bank of Institute of Microbial Technology (IMTECH), Chandigarh, India. The stock cultures were maintained by periodic subculture on PD agar plates at 4°C. The fungus *P. chrysosporium* was inoculated on malt agar and incubated at 35°C until extensive spore growth occurred. Then the fungal culture were inoculated into 250 ml Erlenmeyer flasks having 100 ml of PD broth medium containing (g/L): peeled potatoes (200), glucose (20), yeast extract (0.1) and incubated for 96 h at 30°C at static condition. This medium was kept at 4°C for two days in an incubator and this was further utilized as a inoculants in biodegradation studies.

The medium was prepared by using 20 ml of PD agar in petridish and sterilized. The culture was grown in that PDA broth in an incubator at a constant temperature of 32°C for fifteen days. After 15 days the mycelium was taken and used for sub culturing. A periodic subculture is done for every five days from fully grown culture and the variation in colour intensity was measured by UV visible spectrophotometer.

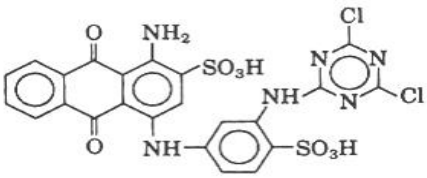
2.2 Preparation of Nutrient Solution

The Biodegradation studies were done in a 250 ml conical flask. The following nutrients were taken in a 100 ml standard flask and make up with distilled water. This nutrient solution was autoclaved at 121°C for 30 minutes. The nutrient solution contained the following chemicals (g/L in distilled water): Glucose (10 g); KH_2PO_4 (2 g); $MgSO_4.7H_2O$, $CaCl_2.2H_20$ and $NH_4H_2PO_4$ each 0.5 g; NH_4Cl, $FeSO_4.5H_2O$ and MnSO4 each 0.1 g; $CoSO_4$, $ZnSO_4$, $CuSO_4.5H_2O$ and Na_2HPO_4 each 0.05 g.

2.3 Structure of the Dye



Structure of Amido black dye



Structure of procion blue 2G

2.4 Decolourization Studies Amido Black by *Phanerochaete Chrysosporium*

20 ml of 0.01% Amido black dye was mixed with 10 ml of nutrients solution and made up to 100 ml with distilled water in a conical flask and covered with sterilized cotton. The conical flask was kept in a sterilizer at 121° C for 15 minutes. This solution was allowed to cool to room temperature. *Phanerochaete chrysosporium* mycelium from the subculture in Petri dish was cut into pieces of 10 mm × 10 mm. This mycelium was added to the conical flask and the cotton was plugged. The conical flask was kept in an orbital shaker and shaken continuously for sufficient oxygen transfer. 10 ml of the solution was periodically taken and centrifuged to separate the biomass for the analysis of solution. The optical density of the solution was measured in the UV visible spectrophotometer. The pH of the solution was measured using a pH meter.

The fungal mycelium of *P.Chrysosporium* is added to 100 ml of the dye solution containing the growth nutrients in a conical flask. Then the solution is subjected to place in an orbital shaker for effective growth. The solution becomes turbid as time passes due to the growth of mycelium. The dye first gets adsorbed on the mycelium and subsequently, the adsorbed dye is degraded by the fungi. The degradation is inferred from the decrease in the optical density of dye solution.

$$\%\ Decolourization = \left(\frac{Initial\ Absorbance - Final\ Absorbance}{Initial\ Absorbance}\right) \times 100$$

2.4.1 Studies of Decolourization for various substrate concentrations

The rates of biodegradation for various substrate concentrations were measured. Five conical flasks were prepared to make 100%, 80%, 60%, 40%, and 20% with the addition of nutrient solution and distilled water. 10 mm x 10 mm mycelium was agitated in an orbital shaker. The rate of decolourization was measured by measuring the optical densities by an UV–visible spectrophotometer. The calibration graph between dye concentration and optical density is shown in Fig. 1.

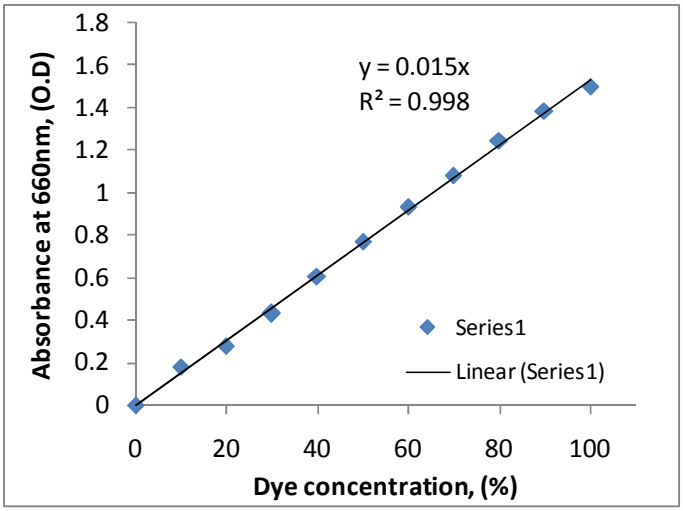


Fig. 1. Calibration graph between dye concentration and optical density

2.4.2 Preparation of Immobilized cells

Biosorption of textile effluent containing procion blue-2G using immobilized *P.chrysosporium* was carried out in two sets.

P.chrysosporium was grown into the stationary phase in PD Broth. 0.5 g of polyvinyl alcohol (PVA) and 0.5 g of sodium alginate were heated to dissolve in which 10 ml of fungal cell suspension was mixed. Similarly another set was prepared using 20 ml fungal cell suspension. The final mixtures were added drop by drop through syringe, into 5% of $CaCl_2$ respectively to yield PVA gel beads with 2mm mean diameter. The beads were stored in $CaCl_2$ solution at 4°C for 24h to complete the gel formation. The insoluble and stable immobilized *P.chrysosporium* alginate beads thus obtained were further used for the decolourization studies.

2.4.3 Ability of dye adsorption in textile effluent by immobilized P. chrysosporium

Batch biosorption studies were conducted at room temperature by varying biobeads weight of 5 g and 10 g and two concentration of textile containing procion blue-2G such as 50 ppm and 100 ppm were used for this study. The ability of immobilized cells to decolourize the dye was tested using shaking the culture at 120 rpm in a rotary shaker. 5 g and 10 g of biobeads containing 10ml of fungal cell suspension were placed in two 250 ml Erlenmeyer flasks each containing 200ml of textile effluent containing the procion blue at the concentration of 50 ppm respectively. Similarly 5g and 10 g of biobeads were taken and suspended into two 250 ml Erlenmeyer flasks each containing 200 ml of textile effluent and procion blue dye at the concentration of 100 ppm respectively. A 20 ml of fungal cell suspension containing biobeads was suspended in both concentration of procion blue in textile effluent. Then the flasks were shaken at room temperature in orbital shaker (130 rpm). Samples were withdrawn at 1 h intervals of time up to 5 h and centrifuge at 5000 rpm in cooling centrifuge for 20 min to sediment the suspended particle. Adsorption was calculated by following formula:

$$Q = \left(\frac{C_o X V}{m_b}\right)$$

where, Q → Amount of dye adsorption capacity of beads, mg/g

Co → initial concentration of dye, mg/l

V → throughput volume of the textile effluent,

m_b → mass of biobeads, g

X → Extent of adsorption

3.0 Results and Discussion

3.1 Growth of Mycelium

The fungal mycelium of *Phanerochaete chrysosporium* when cultured on the petridish from the mother culture (sealed tube) showed slight growth on the third day. Then the mycelium started growing in radial direction to fill the entire surface of the PD agar medium. The fungus did not produce any spores for the period under study.

3.2 Effect of Dye Concentration

The effect of decolourization for different substrate concentration was studied by varying the concentration of dye solution. The study reveals that the rate of decolourization decreases with increase in concentration from 20% to 100% for amido black-B dye solution. The growth of biomass also decreased with increase in concentration of dye. The inhibiting factors such as salt, pH increases with increase in concentration of dye, thus decreasing the growth of the fungal mycelium and also the rate of decolourization. The effect of Amido black-B dye concentration on rate of decolourization of textile effluent is shown in Fig. 2.

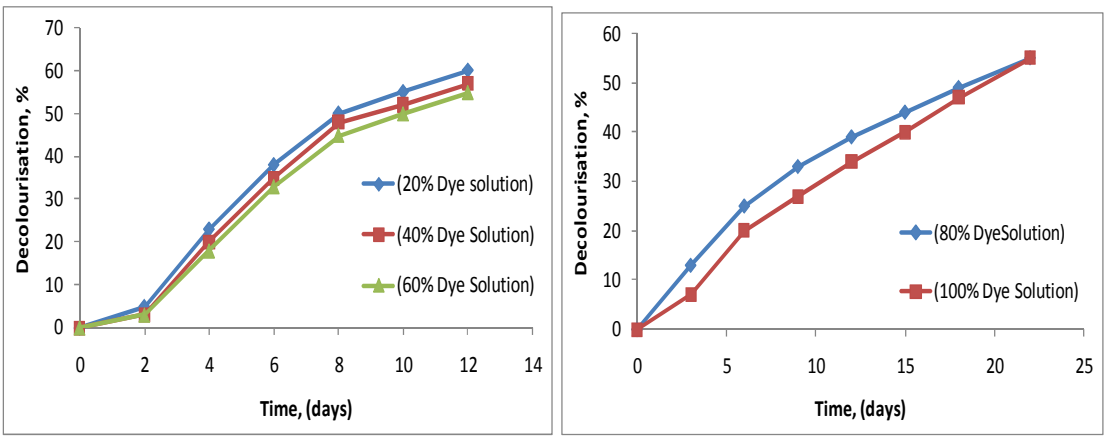


Fig. 2. Effect of Amido black-B dye concentration on rate of decolourization of textile effluent

3.3 Effect of immobilized P. chrysosporium on dye adsorption in textile effluent

Batch biosorption studies were conducted at room temperature by varying biobeads weight of 5 g and 10 g and two concentration of textile effluent containing procion blue such as 50 ppm and 100 ppm. The ability of immobilized cells to decolourize the dye was tested using shake culture situation. 5 g and 10 g of biobeads containing 10ml of fungal cell suspension were placed in two 250 ml Erlenmeyer flasks each containing 200ml of textile effluent containing the procion blue at the concentration of 50 ppm and 100 ppm respectively. In 20 ml of inoculum containing 5 g of biobeads, the amount of dye adsorption (Q) was 1.2 (mg/g) and 0.263 (mg/g) (Table 1 and Fig. 3) in the textile effluent containing the procion blue at concentration of 50 ppm and 100 ppm respectively. In case of 10 g of beads in the textile effluent containing the procion blue at concentration of 50 ppm and 100 ppm, the amount of dye adsorption (Q) was 0.972 (mg/g) and 1.242 (mg/g) respectively. In 10 ml of inoculum containing 5g biobeads, the amount of dye adsorption (Q) was 1.094 (mg/g) and 0.3 (mg/g) in the textile effluent containing the procion blue at concentration of 50 ppm and 100 ppm respectively. The amount of dye adsorption (Q) is 0.669 (mg/g) and 0.058 (mg/g) (Table 1 and Fig. 3) in 10 g of beads in the textile effluent containing the procion blue at concentration of 50 ppm and 100 ppm respectively.

Decolorization depends upon on the enzyme secretion, media and dyes. Similar trends of observation regarding the dye degradation by the white rot fungi have been reported by earlier researchers[6-10]. It is well known that most of the white-rot fungi produce at least two of the three highly nonspecific enzymes like lignin peroxidase (LiP) and manganese peroxidise (MnP) and laccases (Lac), which enable the generation of free radicals during the course of reactions. The structure of dyes strongly influences their degradability by pure cultures and isolated enzymes. The fungal growth and enzyme production, and consequently, decolourization and degradation are influenced by numerous factors, e.g. media composition, pH value, agitation and aeration, temperature and initial dye concentration. Thus, depending on the culture characteristics, the degradation potential for dyes will vary upon the environmental conditions.

Table. 1. Effect of cell concentration (in terms of volume of inoculum) in dye adsorption for the initial dye concentration

Volume of Inoculum (mL)	*Time (hr)*	*Amount of dye adsorbed Q (mg/g)*			
		P. chrysosporium (50 ppm)		*P. chrysosporium (100 ppm)*	
		5 g of beads	*10 g of beads*	*5 g of beads*	*10 g of beads*
10	0	0	0	0	0
	1	0.23	0.131	0.24	0.066
	2	0.484	0.352	0.688	0.116
	3	0.820	0.589	0.956	0.084
	4	1.094	0.668	0.300	0.058
20	0	0	0	0	0
	1	0.314	0.468	0.042	0.047
	2	0.484	0.672	0.105	0.788
	3	0.842	0.904	0.184	1.058
	4	1.200	0.972	0.263	1.242

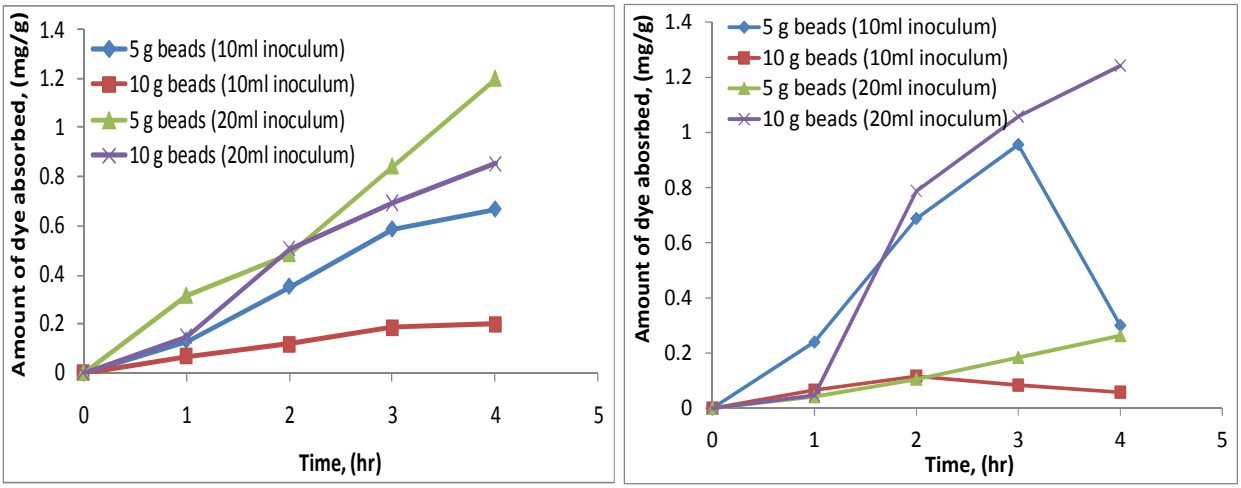


Fig. 3. Amount of procion blue dye-2G (50 ppm and 100 ppm) adsorbed with fungal beads

4.0 Conclusion

The present study clearly demonstrates the decolorization capabilities of the fungi *P. chrysosporium* on Amido black B and procion blue-2G dye solutions. A different decolorization capability was exhibited by the fungi, *P. chrysosporium* based on the nature of dye characteristics as well as whether it existed as free or immobilized cells. In general, the concentration of the dye solution has inverse relationship to rate of degradation. Manganese peroxides, lignin peroxides and laccase might be the main reason for the decolorization by *P. chrysosporium*, and the different profiles of lignin modifying enzymes produced by the fungi can explain their different decolorization specificities. Biotreatment of textile effluent with fungi *P. chrysosporium* seems to be low-cost effective viable options for the textile wastewater treatment. The potential of these fungal cultures can be exploited further to remove residual dyes in textile wastes. Additional investigation is needed to optimize the process parameters for bioremediation of textile effluents using the fungal isolates.

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