Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 13	No. 3	pp. 535-540	2014

Original Research Paper

Microbial Decolourisation of Rathiline Navy Blue Dye Using Immobilized Fungal and Actinomycetal Biomass

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 23-11-2013
Accepted: 16-1-2014

Key Words:
Dye decolorisation
Immobilized biomass
Aspergillus terreus
Rathiline navy blue dye

ABSTRACT

Textile industry generates large quantities of dyes like azo dyes, direct dyes, reactive dyes, basic dyes, synthetic dyes which are very toxic and difficult to dispose. Many dyes are toxic, carcinogenic or prepared from known carcinogens such as benzidine or other aromatic compounds. Considering adverse effect of aniline dye and their product (present in textile effluent), decolourisation of aniline dye is essential before releasing it in environment. Immobilised fungal cells have several advantages over dispersed cells such as simple reuse of the biomass, easier liquid-solid separation and minimal clogging in continuous-flow systems. Cell immobilization lowers the apparent broth viscosity and makes the rheological features more favourable for oxygen supply and mass transfer. In present dye decolourisation process, we have used live biomass of two different fungi (*Aspergillus terreus* and one unknown fungus) and Actinomycete (*Streptomyces*). Live biomass of the two fungi and one actinomycetes gives maximum decolourisation of dye as compared to dead biomass. We have also assessed the effect of decolourised water on seed germination.

INTRODUCTION

Dye usually have a synthetic origin and complex aromatic molecular structure which make them more stable. Dye molecules are comprised of two key components the chromophore which is responsible for producing colour and auxochrome which not only supplement the chromophores but also render the molecule soluble in water and give enhanced affinity to attach (Gupta 2009). More than 1000 different dyes are used in industries such as paper and pulp, leather, paint, textiles, etc. This has resulted in the discharge of highly polluted effluents in the environment. In these effluents dye concentration higher than 1mg/L has been reported from textile manufacturing process (O'Neill et al. 1999). Physical and chemical treatments of textile dyes are expensive and may generate a large volume of sludge (Dubrow et al. 1996), while biological treatment has low removal efficiency (Dubrow et al. 1996, Kapdan et al. 2000a).

Immobilised fungal cells have several advantages over dispersed cells such as simple reuse of the biomass, easier liquid-solid separation and minimal clogging in continuous-flow systems (Arica et al. 1993, Tieng & Sun 2000). In addition, immobilised cultures have a higher level of activity and they are more resistent to environmental perturbations such as pH, or exposure to toxic chemical concentrations than suspension cultures (Shin et al. 2002). Immobilisation protects the cells from shear damage (Abraham et al. 1991, Fiedurek & Ilczuk 1991, Vassilev & Vassileva 1992). Moreover, cell immobilization lowers the apparent broth viscosity and makes the rheological features more favourable for oxygen supply and mass transfer (Thongchul & Yang 2003). When applied to recombinant strains, cell immobilization can also alleviate strain genetic stability problems (Caunt et al. 1988, Dincbas et al. 1993). Another advantage of cell immobilisation is a reduction in the protease activity and contamination risk.

MATERIALS AND METHODS

Collection of dye sample: Rathiline navy blue dye (aniline) sample was brought from a textile industry in Aurangabad.

Isolation of microorganisms: For the isolation of microorganisms garden soil was used as a source of microorganisms. For the isolation of fungi, potato dextrose agar was used, and for isolation of actinomycetes starch casein nitrate agar was used.

Along with this, one fungus (*Aspergillus terreus*), which was procured from National Chemical Laboratory, Pune was also used for the decolourisation studies.

Determination of λ max and estimation of dye: The dye concentration used for determination of λ max was 0.01% (w/v). The absorbance of dye at various wavelengths was noted and the same was plotted on graph to determine the λ max.

Different concentrations (10-100µg/mL) of dye were used for the determination of standard graph of dye absorbance. The absorbance was noted at λ max value, obtained earlier.

Biomass production: Decolourisation experiment needs a biomass of isolates. So for the production of biomass the isolates were grown in respective nutrient medium. Potato dextrose broth with yeast extract was used for the growth of fungi (*A. terreus,* ADA 1 and ADA 2), and starch casein nitrate broth was used for the growth of actinomycetes (BJ1, BJ2, BJ3, BJ4). The cultures were grown in a 500 mL capacity Erlenmeyer flask containing 250 mL of sterile nutrient medium. The flasks were then incubated in a rotary incubator shaker adjusted at 120 rpm at 37°C for 72 h. After incubation, the flasks were observed for the produced biomass.

Screening of microorganisms for dye decolourisation: The live biomass of isolates *viz.,* three fungi and four actinomycetes was inoculated in 250 mL capacity Erlenmeyer flask containing 100 mL dye (0.01%) solution. One uninoculated (without biomass) flask containing same concentration of dye was kept as control. The flasks were then incubated in a rotary incubator shaker adjusted at 120 rpm at 37°C for 48 to 96 h. After every 24 h the sample was taken from each flask and used for the determination of absorbance of dye at 550nm. The flasks were also observed visually for dye decolourisation. Percent (%) dye decolourisation was determined using following formula:

$$\% \text{ Dye decolourisation} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

Initial OD refers to the OD of control set.

Comparison between live and dead biomass: Dye decolourising ability of live and dead biomass was assessed. For this, autoclaved biomass was used as dead biomass. The biomass (2g) was added in a flask containing 100 mL of dye (0.01%). The flasks were then incubated in rotary incubator shaker adjusted to 120 rpm at 37°C for 72 h. After every 24 h, the sample was taken from each flask and used for the determination of absorbance of dye at 550nm and finally percent (%) dye decolourisation was determined.

Dye decolourisation using immobilized biomass: Different materials (gunny bag, sugar cane, coconut coir and scotchbrite) were used for immobilizing the biomass. The immobilizing material was first washed with ethanol for the removal of dust and colour and then dried in oven. The material was sterilized by autoclaving before use.

For immobilization of biomass on material, each culture (*A. terreus*, ADA2 and BJ4) was inoculated separately in 500mL capacity Erlenmeyer flask containing 250 mL nutrient medium (potato dextrose yeast extract broth for fungi and starch casein nitrate broth for actinomycetes). Equal amount of gunny bag material (5 pieces of 2 cm^2) was added in each flask. Same procedure was followed for other immobilizing materials. The flasks were then kept for incubation in rotary incubator shaker adjusted to 120 rpm at 37°C for 72 h.

The obtained immobilized biomass of each organism was then added in a 100mL dye solution containing 0.01% dye. One flask containing unimmobilized biomass was kept as a control. The flasks were then incubated in rotary incubator shaker adjusted to 120 rpm at 37°C for 72 h. After every 24 h the sample was taken from each flask and used for the determination of absorbance of dye at 550nm and finally percent (%) dye decolourisation was determined.

Effect of pH on dye decolourisation by immobilized biomass: Different initial pH was tested for dye decolourisation experiment. For this, the immobilized (gunny bag) biomass was inoculated in 100 mL dye solution (0.01%) of varying initial pH *viz.,* 5, 7 and 9. The flasks were then incubated in rotary incubator shaker adjusted to 120 rpm at 37°C for 72 h. After every 24 h the sample was taken from each flask and used for the determination of absorbance of dye at 550nm and finally percent (%) dye decolourisation was determined.

Effect of decolourised water on seed germination: For assessing the effect of decolourised water on seed germination, first the decolourized water from the flasks of each organism was collected. Equal amount of the decolourised water from each flask was then added in respective Petri plate containing pea seeds kept on filter paper. The control was kept by following the same procedure but by replacing the decolourized water with the dye solution (0.01%). After 72 hrs the length of shoot was measured. Then comparison of the shoot length of pea seeds of fungal and actinomycetes dye adsorbed water was done with the control containing dye concentration 0.01g/100 D/W.

RESULTS AND DISCUSSION

Isolation of microorganisms: After the incubation of 48 h the growth microorganisms was observed both on PDA plate and on starch casein agar. Two fungus and four actinomycetes were isolated. They were labelled as ADA1, ADA2 (fungi) and as BJ1, BJ2, BJ3 and BJ4 (actinomycetes).

Determination of maximum absorbance (λ max) of rathiline navy blue dye: The absorbance of Rathiline navy blue dye was noted at different wavelength (350-1000 nm) and the same was plotted on graph. It was found that, the Rathiline navy blue dye has maximum absorbance (λ max) at 550nm.

Biomass production: After the incubation of 72h, the microbial biomass was observed (fungal biomass in potato dextrose broth and actinomycetal biomass in starch casein nitrate broth).

Screening of microorganisms for dye decolourisation: Three fungi (*A. terreus*, ADA1 and ADA2) and four actinomycetes (BJ1, BJ2, BJ3, BJ4) were screened to check their dye decolourising ability. For this, the biomass was added in dye solution and incubated in rotary shaker adjusted to 120 rpm at 37°C for 72-96 h. During incubation, the sample was taken after each 24 h, absorbance was noted and subsequently the % dye decolourisation was determined. The values of % dye decolorization are given in Table 1. It was observed that *A. terreus* gives maximum dye decolourisation. Among the isolated actinomycetes, BJ4 gives maximum dye decolourisation.

The fungal isolate ADA2 gives more % decolorization than ADA1 in 72h and the bacterial isolate BJ4 (actinomycete) gives more % decolorization than BJ1, BJ2 and BJ3. So for further decolorization process we used fungal biomass of ADA 2 and bacterial biomass of actinomycete BJ4.

Dye decolourisation by live and dead biomass: Dye decolourisation by live and dead biomass was studied. During incubation the sample was taken after each 24 h, absorbance was noted and subsequently the % dye decolourisation was determined. The values of % dye decolorization are depicted in Table 2.

After 72 hours of incubation, the complete dye decolorization was observed in a flask containing live biomass, however, there was less dye decolourisation in the flask containing dead biomass. Shin et al. (2002) have used live biomass of *Trametes versicolor* for removal of dye. Yuzhu Fu & Viraraghavan (2002) have used live biomass of *Aspergillus niger* for removal of dye from an aqueous solution.

Dye decolourisation using immobilized biomass: Dye decolourisation was assessed using different immobilizing materials *viz.,* gunny bag, sugar cane, scrotch brite and coconut coir (Figs. 1A, 2A, 3A and 4A). After the incubation of 72hr, the growth of biomass was observed on immobilizing materials. The observed biomass was attached and/or entrapped in the immobilizing materials. These materials were then inoculated in the dye solution and incubated in an orbital rotary shaker adjusted at 120 rpm at 37°C for 72-96 h.

During incubation, the sample was taken after each 24 h, absorbance was noted and subsequently the % dye decolourisation was determined. The values of % dye decolorization are shown in Figs. 1B, 2B, 3B and 4B.

It was found that, there was an increase in % dye decolorization when immobilized biomass was used. It was found that out of the four immobilizing materials tested, gunny bag gave better results of dye decolorization. So we have decided to use gunny bag as an immobilizing material for further experiments.

Shin et al. (2002) found that jute (gunny bag) to be the best support material as *T. versicolor* grew well on it without colour leaching from the support and without loss of jute's integrity. The fungus immobilized on jute decolourise the dye more efficiently.

Effect of pH on dye decolourisation by immobilized biomass: Various pH *viz.,* 5, 7 and 9 of dye solution was tested for decolourisation experiments. The organisms [ADA 2, *A. terreus* (NCIM 657) and BJ4 (actinomycete)] were inoculated in the dye solution of each pH and the flasks were incubated in an orbital rotary shaker adjusted at 120 rpm at 37°C for 72-96 h. Optimization of pH was done for adsorption of Rathiline navy blue dye at different pH for the fungus ADA 2, *A. terreus* (NCIM 657) and BJ4 (actinomycete). After incubation the absorbance was checked at 550nm and the % decolourisation was calculated.

From the results of pH optimization, it was concluded

Table 1: % dye decolourization by isolates.

Sr. No.	Organism	% Dye decolorisation		
		24h	48h	72h
1	ADA1	66.33	67.21	68.53
2	ADA2	71	74.22	76.30
3	*A. terreus* (NCIM 657)	71.33	72.65	77.05
4	BJ1	29.87	31.76	46.17
5	BJ2	31.76	35.17	48.82
6	BJ3	40.48	46.17	55.23
7	BJ4	51.47	63.98	66.33

Table 2: Percent dye decolourization by using live and dead biomass.

Sr. No.	Organism	% Dye decolorization (Live biomass)			% Dye decolorization (Dead biomass)		
		24h	48h	72h	24h	48h	72h
1	ADA2	66.71	67.09	71.26	55.07	56.10	62.16
2	*A.terreus* (NCIM 657)	71.94	72.40	77.06	55.42	58.07	62.24
3	BJ4	57.61	62.05	65.01	54.58	57.31	63.76

Table 3: Effect of decolourized water on shoot length.

Sr. No.	Organisms	Shoot length in average (cm)
1	Control	0.5
2	ADA2	3.05
3	*A. terreus*	3.65
4	BJ4 (Actinomycete)	2.4

Using gunny bag

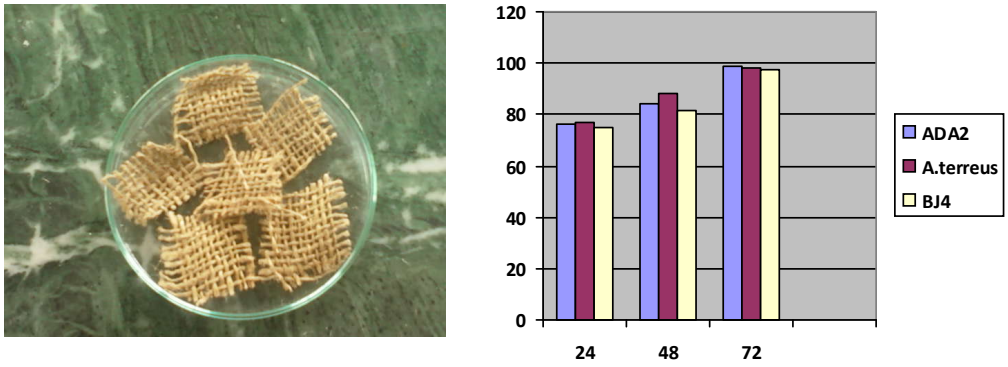

Fig. 1A: Gunny bag as an immobilizing material; 1B: % dye decolorisation by organism immobilized in gunny bag.

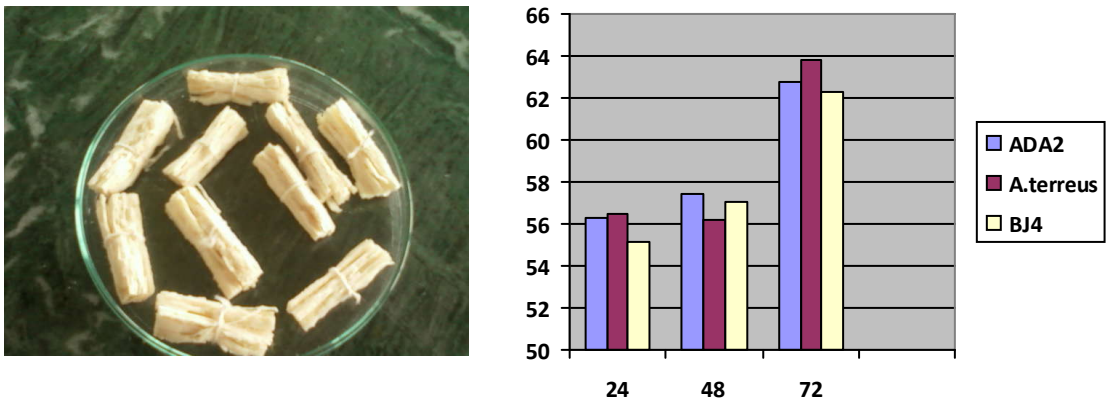

Fig. 2A: Bagasse as an immobilizing material; 2B: % dye decolorisation by organisms immobilised in bagasse.

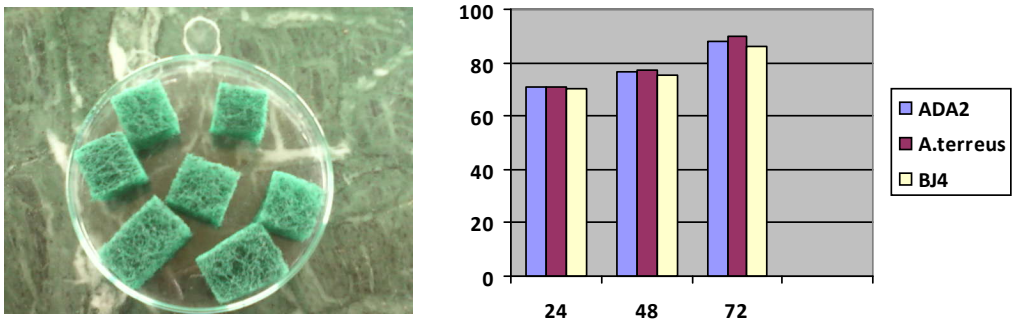

Fig. 3A: Scotchbrite as an immobilising material; 3B: % dye decolorisation by organisms immobilised in scotchbrite.

that pH 5 was optimum pH for dye decolourisation by ADA2 and *A. terreus* (NCIM 657), whereas pH 7 was optimum for dye decolourisation by BJ4.

Iqbal & Saeed (2007) observed that pH 2 was optimum pH for dye decolourisation by *Phanerochaete chrysosporium* and decolourisation declined sharply with further increase in pH.

Effect of decolourised water on seed germination: The decolourised and dye adsorbed water of each fungus ADA 2, and *A. terreus* (NCIM 657) and BJ4 (actinomycete) was added in Petri plates containing filter paper and pea seeds on it. After 72 hrs, it is observed that the seeds get germinated (Fig. 5). After germination shoot length was measured and mentioned in Table 3.

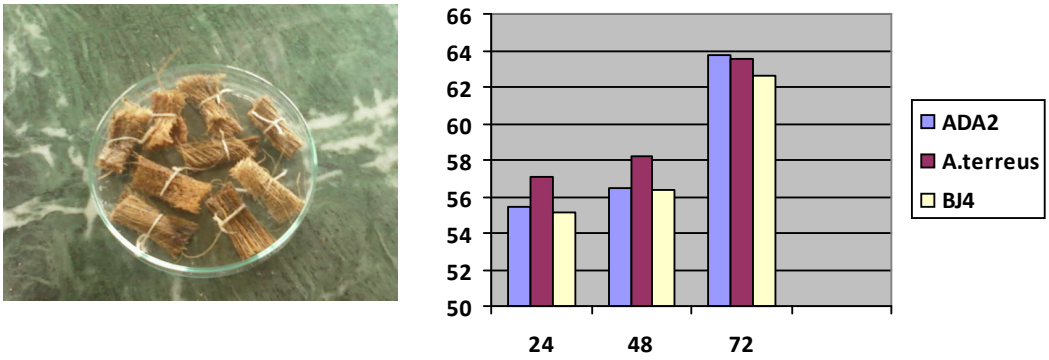

Fig. 4A: Coconut coir as an immobilizing material; 4B: % dye decolorisation by organisms immobilised in coconut coir.

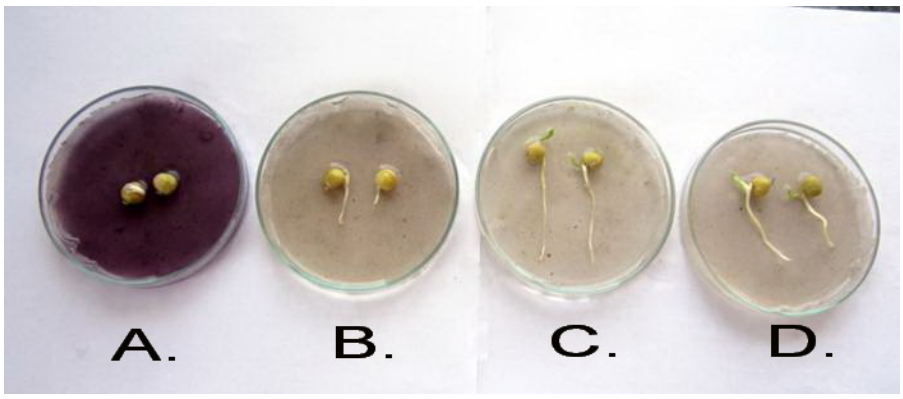

Fig. 5: Effect of decolourized water on seed germination. A. Control, B. BJ4, C. *A. terreus*, D. ADA2.

So by using dye adsorbed water for seed germination, it gives higher growth of seeds than that of control.

The decolourised water from the flasks of individual organism was used for the germination studies. After 72 h of incubation, it was observed that there was a better seed germination when the decolourized water obtained from *A. terreus* (NCIM 657) was used.

Kathirvel (2012) also observed that the seed germination rate was significantly higher than control. It shows that organic substances present in the dye supports the growth of plant with some limitations. The excess amount of organic matter serves as toxic material and prevents the growth of the experimental plant. The similar results have been reported in different crops (Vijayakumari & Kumudha 1990, Tiwari et al. 1993, Vijayarengan & Lakshmanachary 1993, Albino Wins & Murugesan 2010).

CONCLUSIONS

Fungal biomass of *A. terreus*, ADA2 and bacterial biomass of BJ4 (actinomycete) was found to be effective in removing Rathiline navy blue dye from an aqueous solution. Live biomass was found to have better dye decolourising ability than dead biomass. Out of the four tested immobilizing materials gunny bag was found to have greater decolourising ability. The optimum pH for dye decolourisation by ADA2 and by *A. terreus* was 5.0. The optimum pH for dye decolourisation by BJ4 was 7.0. The decolourised solution from the flask of *A. terreus* was found to be less inhibitory to the seed germination.

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