Journal of Hazardous Materials 167 (2009) 660–663

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

In situ biodecolorization kinetics of Acid Red 66 in aqueous solutions by *Trametes versicolor*

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ARTICLE INFO

Article history:
Received 29 November 2008
Received in revised form 7 January 2009
Accepted 7 January 2009
Available online 19 January 2009

Keywords:
Acid Red 66
Decolorization
Trametes versicolor
Azo dyes

ABSTRACT

The biological decolorization methodology and the mechanism involved in the degradation of Acid Red 66 was chosen as a model of azo dye using the white rot fungi *Trametes versicolor* was studied. Biodecolorization of Acid Red 66 using white rot fungi *T. versicolor* was demonstrated by the decolorization of the culture medium, the extent of which was determined by monitoring the decrease in absorbance at 506 nm. The biodecolorization kinetics of Acid Red 66 (100 ppm) was found to be first order and the degradation rate coefficient is 1.312×10^{-2} min^{-1} ($R = 0.96683$, $n = 8$) at 30 °C. The effects of independent variables such as carbon sources, nitrogen sources pH, and temperature on the biodecolorization of Acid Red 66 was also investigated.

1. Introduction

Tannery and textile industries are the country's largest industries earning large amount of foreign exchange and attracts public attention from the viewpoint of pollution. Synthetic dyes are released in to the environment. Over 100,000 commercially available dyes exist and more than 7×10^5 metric tons of dyestuffs are produced world wide annually [1]. Azo dyes are the most widely used dyes in industry with a world market share of 60–70%. These dyes are characterized by nitrogen to nitrogen (N = N). The color of azo dyes is due to the azo bond and associated chromophores [2]. Reactive azo dyes are very soluble by design and as a result, not all are exhausted by textile fibers during the dyeing process and therefore end up in the discharge from dye houses [3]. Color is usually the first contaminant to be recognized in wastewater. A very small amount of dye in water (10–50 mg/l) is highly visible and affects the aesthetic merit, water transparency and gas solubility of water bodies [4]. The discharge of those colored wastewater into rivers and lakes leads to reduction of sunlight penetration in natural water bodies which in turn decreases both photosynthetic activity and dissolved oxygen concentration and also toxic to living beings [5].

Many physical and chemical methods including adsorption, coagulation, precipitation, filtration and oxidation have been used for the treatment of azo dye-contaminated effluents [6]. Physical treatment alters the hazardous material to a more convenient form for further processing or disposal. Chemical treatment uses chemical reactions to alter the hazardous chemicals to less hazardous form. These methods however may generate a significant amount of sludge or may easily cause secondary pollution due to excessive chemical usage [7]. Moreover their municipal treatment costs are high. Therefore it may be economical to develop alternative means of dye decolorization such as bio-remeadiation due to its reputation as an environmentally friendly and publicly acceptable treatment technology.

To overcome these difficulties fungi are being investigated for their potential to decolorize effluents [8]. Among them the most widely studied are the white rot fungi, *P. chrysosporium* and *Trametes versicolor*. Lignin degrading [9] cultures of the white rot basidiomycete *P. chrysosporium* are capable of mineralizing a number of sulphonated and non-sulphonated azo dyes to CO_2. Lignin and manganese peroxidases are two extra cellular peroxidases produced by ligninolytic cultures of *T. versicolor* are involved in the degradation of lignin [10,11] and various other environmental pollutants by this fungus. Both LiP and MnP can depolymerize lignin, dehalogenate chloro phenols, and eliminate nitro groups from the nitro phenol or nitro aniline intermediates formed in nitrotoluene degradation [12]. The focus of this review therefore concerns the use of fungi to remove or degrade various wastewater constituents.

2. Materials and methods

2.1. Materials

Reactive azo dyes used in this study are Acid Red 66 (AR66), (C.I.26905) received from Aldrich Chem. USA. NaOH, HCl, fructose,

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doi:10.1016/j.jhazmat.2009.01.032

glucose, lactose, cellulose, ammonium sulfate, ammonium chloride, urea, diammonium mono sodium phosphate, mono ammonium disodium phosphate, sulfuric acid, silver sulfate, mercuric sulfate, potassium dichromate, ferroin indicator and ferrous ammonium sulfate were supplied by s.d.Fine-Chem limited, Mumbai, India. Potato dextrose agar and Czapek-Dox broth received from Hi-media Laboratories, Mumbai.

2.2. Collection of soil samples

Soil and water samples for the isolation of elite microorganisms were collected from drenched areas of dye house sludge at Central Leather Research Institute, Chennai. Soil and water samples were collected in polyethylene bags and sterilized containers, and stored at 4 °C in a refrigerator for further analysis. The microbes were isolated from the soil.

2.3. Isolation of fungi

In order to ascertain certain microbial biosystem for dye effluent decolorization, the fungal sp were isolated from contaminated soil samples and same were collected from Central Leather Research Institute, Chennai. The acclimatized microorganisms were isolated by employing standard serial dilution plating technique [13]. A quantity of 10 g of soil samples with 10 ml of double distilled water were taken in 90 ml sterile water blanks and serially diluted to 10^4 times using sterile water blank. After thorough shaking, 1 ml of aliquot from 10^4 dilutions was drawn and pour plated in potato dextrose agar medium (PDA) amended with 50 ppm of streptomycin to control bacteria [14]. The plates were incubated at 30 °C for 7 days. The isolated microorganisms were further purified by subsequent sub-culturing and maintained in the slant culture at 4 °C.

2.4. Composition and preparation of potato dextrose agar medium

Potato tubers peeled 250 g, dextrose 10 g, double distilled water 1000 ml, pH 5.5–6.5, and agar 15 g. Well-sliced potato tubers were cooked for 5 min in 250 ml tap water over a water bath. After cooling, the slices were macerated and filtered through two layers cheesecloth. The volume of the extract was made up to 1000 ml with water.

2.5. Composition of Czapek-Dox broth

Sodium nitrate 3.00 g, dipotassium hydrogen phosphate 1.00 g, magnesium sulfate 0.50 g, potassium chloride 0.50 g, ferrous sulfate 0.01 g, sucrose 30.00 g, distilled water 1000 ml, and pH 6.5–6.8.

2.6. Screening of elite microbes for decolorization

Simulated aqueous dye solutions and dye factory effluent were scanned in UV spectrophotometer to ascertain the wavelength and the maximum absorbance was observed at 506 nm for AR66. The rate of decolorization was monitored at this respective wavelength. The fungal cultures *Trametes* sp. isolated from contaminated soil were used for the decolorization of dye effluent. The *Trametes* sp. isolated from the contaminated soil will suppress the growth of other organism because it shows elite growth when compared to other organisms during isolation process.

2.7. Identification of isolated fungi

The isolated fungal cultures were grown on the potato dextrose agar medium for 5–7 days. A glass slide was taken and a drop of lacto phenol plus cotton blue fluid was mounted on center of a glass slide.

Table 1
Morphological characteristics of the isolated fungi.

Culture name	Morphology
Trametes sp.	Colony morphology was flesh thick, white tough and fibrous. Spores are slightly curved, cylindrical, smooth, hyaline, white to pale yellow in deposit. The hyphae are hyaline and basidia are two or four spored, clavate. They are trimitic hyphal system.

A portion of mycelia mat from the colony was transferred into the drop of lacto phenol plus cotton blue with the help of flamed and cooled needle [15]. With the help of two needles the propagules were gently spread, so that the mycelia were mixed with the strain. The slides were observed under low and high power objectives of a microscope (Nikon, Japan) and the types of conidia; hyphae and their arrangement were noted [16–18].

2.8. Decolorization of dye effluent using isolated fungal cultures

In order to test the potentiality of *Trametes* sp. on the decolorization of AR66, an experiment was conducted. Treatments were replicated thrice with control. Five milliliters of samples were drawn from the inoculated treatments and centrifuged at 7000 rpm for 10 min [19]. The supernatant was collected and the OD values were measured for 7 days at 1 day intervals at 506 nm in the spectrophotometer.

2.9. Color removal percentage

The percent of color reduction was measured as follows:

$$\text{color removal } (\%) = \frac{(\text{A})-\text{absorbance of residual broth}}{\text{absorbance of uninoculated broth (A)}} 100$$

3. Results and discussion

The fungus was identified and the growing conditions were optimized. The taxonomic position of the fungi was determined (Table 1) based on the conidial morphology, size and shape and was identified as *Trametes* sp.

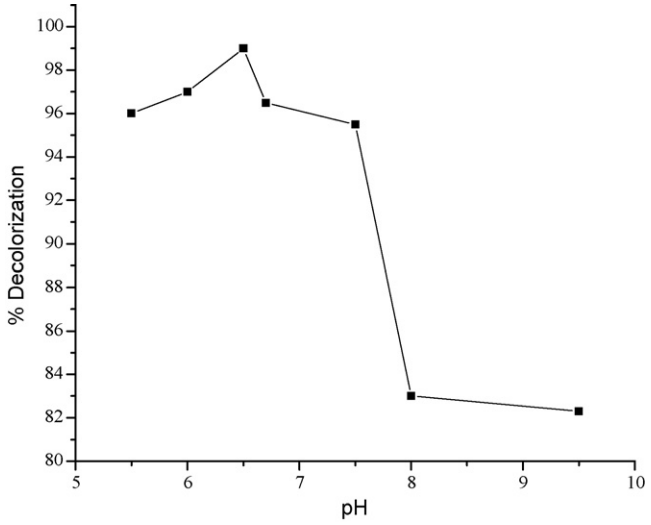

Fig. 1. Effect of pH on biodecolorization of AR66 by *Trametes versicolor*.

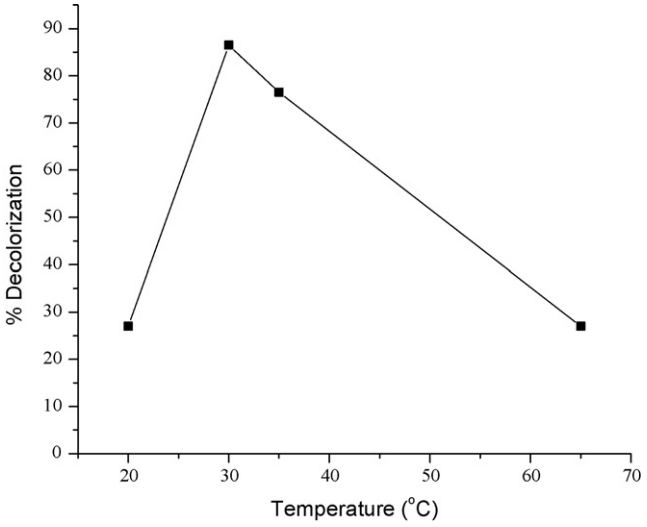

Fig. 2. Effect of temperature on biodecolorization of AR66 by *Trametes versicolor*.

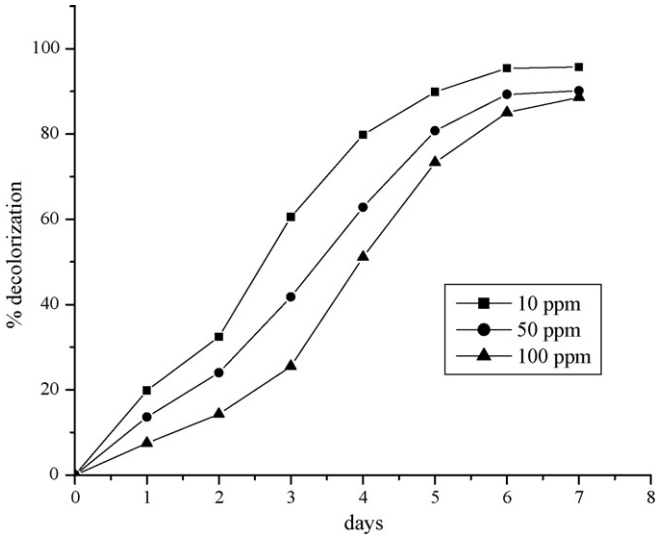

Fig. 4. Biodecolorization kinetics of AR66 by *Trametes versicolor*.

3.1. Effect of pH on decolorization of azo dye (AR66)

In general, the dyes set at 6.5 supported higher color reduction of AR66 as 99% (Fig. 1). At pH 9.5 the color reduction of AR66 by *Trametes* sp. recorded the lowest value of 82.3%. Slightly acidic pH shows better growth for this fungus and leads to the maximum color removal of AR66. The biomass production was estimated for different pH. The maximum biomass production was observed at pH 6.5 when compared with the neutral pH.

3.2. Effect of temperature on decolorization of azo dye (AR66)

In general, 30 °C appeared optimum for the color reduction of AR66 by *Trametes* sp. and it was expressed in Fig. 2. At temperature of 20 °C and 65 °C the *Trametes* sp. recorded the lowest value. The ambient temperature was found to be suitable for the optimum growth and the maximum degradation occurred at this temperature [20].

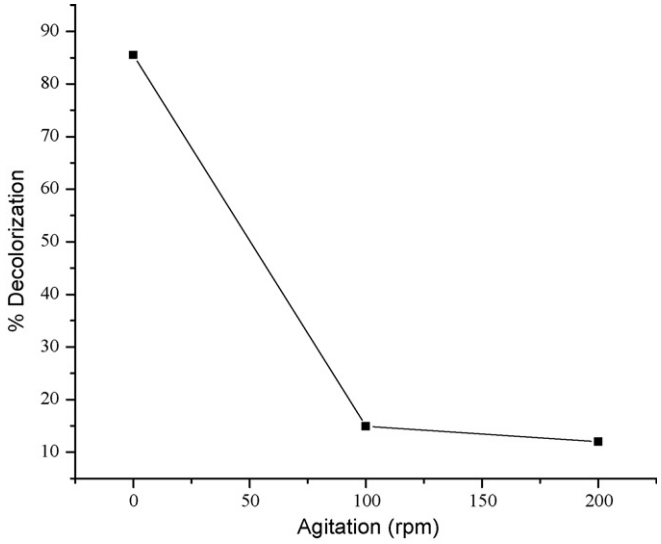

Fig. 3. Effect of agitation on biodecolorization of AR66 by *Trametes versicolor*.

3.3. Effects of agitation on decolorization of azo dye (AR66)

The fungi *Trametes* sp. recorded highest color reduction at static condition for AR66 as 85.5%. Further increase in agitation tended to decrease the color reduction. During agitation the cell walls get ruptured [21] and ultimately affect the growth of the fungi (Fig. 3). The increase in agitation speed will suppress the growth of the organisms due to the rupture of cell wall.

3.4. Decolorization of azo dye (AR66) by Trametes sp.

The color reduction increased with increase of incubation time was expressed in Fig. 4. The maximum color reduction for 10 ppm, 50 ppm and 100 ppm of AR66 was 95.7%, 90.1%, and 88.6%, respectively. The color reduction was mainly due to the production of degradative peroxidase enzyme produced by *Trametes* sp.

4. Conclusion

The biological degradation of Acid Red 66 that was chosen as a model of azo dye in aqueous solution was studied. It was found that the biodecolorization kinetics of Acid Red 66 in water is first order and the degradation rate coefficient is $1.312 \times 10^{-2}\ \text{min}^{-1}$ ($R = 0.96683$, $n = 8$) at 30 °C. with pH 6.5 for 100 ppm. It was observed that with increasing initial concentration, the degradation rate coefficient decreased. The influence of the acidity of the reaction medium on biodecolorization was that the biodecolorization rate of Acid Red 66 in slightly acidic water are higher than those obtained in acidic and the k1 obtained at basic medium (pH 10) are the lowest.

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