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ORIGINAL ARTICLE

Bioremediation of Azo Dye Amido Black 10B by Bacterium *Lysinibacillus* sp. strain AK2

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ABSTRACT
A toxic diazo dye, Amido Black 10B was decolorized and partially degraded by bacterium Lysinibacillus sp. strain AK2. Various parameters such as dye concentration, pH, temperature and NaCl concentration were standardized for optimum decolorization of the dye. The strain decolorized 90% of the 200 mg/L dye at temperature 35°C and pH 7.0 in 36 h of incubation. Decolorizing ability over a wide range of pH (5-11), temperature (25-55 °C), and NaCl concentration (5-30 g/L) was observed with only slight decrease in the decolorization at lower and higher sides of pH and temperatures. Further, the strain decolorized up to 600 mg/L of the dye in 72 h. The cell free extract of the strain AK2 grown on Amido Black 10B exhibited the azoreductase activity of 1.5736 µM/min/mg protein. UV–Vis spectroscopic analysis of the dye decolorized sample indicated the formation of breakdown products of azo bond.

Keywords: *Azo dye, Amido Black 10B, Decolorization, Lysinibacillus, Azoreductase*

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INTRODUCTION

Azo dyes constitute the largest group of mutagenic and carcinogenic xenobiotic pollutants [1], and disposal of these dyes and remediation of associated contaminated sites remains a worldwide concern. Adsorption over activated [2], H_2O_2, heterogeneous photocatalysts such as TiO2, ZnO, Pt/ZnO, iron salts [3], ozone or the photo-Fenton reaction [4] are also employed. Biological approaches such as microbial degradation including the use of genetically modified bacteria [5], immobilized enzymes [6], and phytoremediation [7] are expected to be more environmentally friendly, as they can lead to complete mineralization or formation of non-toxic residues of noxious organic waste at low cost. Metabolites formed during biodegradation were analyzed by UV-Vis amd HPLC to elucidate a probable degradation pathway.

Amido black 10B is an amino acid staining diazo dye (Figure 1). It is applied to all kinds of natural fibers such as wool, cotton, and silk as well as to synthetics like polyesters, acrylics, and rayon. It not only damages the human respiratory system but also causes skin and eye irritations.

In this context, the possibility of decolorization Amido Black 10B by the bacterum *Lysinibacillus* sp. strain AK2 was explored. Degradation was evaluated with respect to pH, concentration of dye, salinity and temperature. In addition, the activity of enzymes involved in the degradation of dye was determined. Further, activity of azoreductase, an enzyme involved in the cleavage of azo bond was determined in the cell free extract of this strain. The dye decolorized sample was subjected to UV-Vis spectroscopic analysis for identification of decolorization products.

MATERIAL AND METHODS

Dye and chemicals

Amido Black 10B was purchased from SDFCL, Mumbai, India. NADH was procured from Sigma, Steinem, Germany. The media supplements peptone and yeast extract and other chemicals of culture media were

obtained from Hi-Media and SDFCL, India. Chemicals of scientific grade and of the maximum lucidity are used in the study.

Microorganisms and culture media

The bacterial culture *Lysinibacillus* sp. strain AK2 used in this study was previously isolated in this laboratory [8]. The mineral salts (MS) medum contained K_2HPO_4; 6.3 g/L, KH_2PO_4; 1.8 g/L, NaCl; 5 g/L, $MgSO_4.7H_2O$; 0.1 g/L, $MnSO_4$; 0.1 g/L, $CaCl_2.2H_2O$; 0.1 g/L, $FeSO_4.7H_2O$; 0.1 g/L, $Na_2MoO_4.7H_2O$; 0.006 g/L. The medium pH was adjusted to 7. The MS medium was supplemented with yeast extract (2.5 g/L) and peptone (5 g/L).

Decolorization experiment

The decolorization experiment was carried out at 37 ^oC in Erlenmeyer flask (250 ml) containing 50 ml of MS medium with Amido Black 10B (200 mg/L). After sterilization at 121 ^oC and 15 lb pressure for 20 min, the flask was inoculated with bacterial cells. Percent decolorization of the dye was recorded by checking the optical density (620 nm) of the supernatant of the culture samples withdrawn at regular time intervals using UV-Vis spectrophotometer (Specord 50, Germany). The percent decolorization of the dye was computed by the following formula [9].

$$\% \text{ Decolorization} = \frac{\text{Ao} - \text{A}_1}{\text{Ao}} \text{x } 100$$

Where, A_0 is initial O.D. and A_1 is final O.D.

Effect of different parameters on decolorization

The effect of different concentration of dye (200-600 mg/L), pH of medium (5-11), temperature (25-55 ^oC) and NaCl concentration (5-30 g/L) on the decolorization of Amido Black 10B by *Lysinibacillus* sp. AK2 was studied.

Preparation of cell free extract

The bacterial cells grown in 1000 ml of MS medium containing 200 mg Amido Black 10B were harvested by centrifugation at 12,000 rpm for 10 min at 4 ^oC. The cells were washed and suspended in 50 mM potassium phosphate buffer (pH 7). Then the cell suspension was subjected to sonication in cold condition (Vibra Cell Ultrasonicator VC 375, USA). The sonicated cells were centrifuged at 12,000 rpm for 10 min at 4 ^oC and the resulting supernatant was used as crude enzyme.

Assay of azoreductase

Azoreductase was assayed by the modified method of Zimmerman *et al*. [10]. For the assay of azoreductase, 1 mM Amido Black 10B (40 µL) and crude enzyme (40 µL) was taken in 50 mM phosphate buffer (600 µL, pH 7.0). To this mixture, 1 mM NADH (40 µL) was added and the enzyme activity was monitored by decrease in optical density of NADH at 340 nm spectrophotometrically. A difference in absorbance unit/min/mg protein was taken as one unit of enzyme activity. The enzyme assay was carried in triplicate.

RESULTS AND DISCUSSION

Time course for Amido Black 10B decolorization

At 200 mg/L dye concentration the strain AK2 decolorized 67% of the dye in first 12 h and reached maximum decolorization of 90% in 36 h (Figure 1). Abhishek *et al*. [11] showed the adsorptive removal of 20 mg/L Amido Black 10B. Selvam *et al*. [12] demonstrated decolorization of only 78 % of Amido Black 10B by using fungus *Trametes versicolor* with initial 25 µM concentration.

Effect of dye concentration on decolorization

The effect of dye concentration (200, 400, 600, 800 mg/L) on the decolorization potential of the bacterium was investigated (Table 1). The strain decolorized 65% of the dye at 600 mg/L concentration. However the percentage of decolorization slightly decreases with increase in the dye concentration. This bacterial strain exhibited relatively higher decolorization competence than those of the other reported bacterial strains. Poornima *et al*. [13] reported similar results for decolorization of Amido Black by laccase from *Rigidoporus ulmarius.* Rajguru *et al*. [14] reported the biodegradation of Amido Black 10B up to a maximum dye concentration of only 100 mg/L.

Effect of media pH on decolorization

The strain AK2 showed maximum decolorization the dye at pH 7 (Table 2). The strain showed good decolorizing ability at wide pH range 5-11. Maximum decolorization was obtained at pH 7. At pH 5 and 11, the decolorization decreased slightly to 68 and 73% respectively. This property of the bacterium is most desirable as the textile wastewaters have varying pH from acidic to alkali. Senthilkumar *et al*. [15] reported that a white-rot fungus *Phanerochaete chrysosporium* was able to decolorize Amido Black 10B

only in the acidic pH 3-7. Modi *et al*. [16] reported that Reactive Red 195 was decolorized by *Bacillus cereus* M1 at optimum pH between 6 and 7.5. On the contrary, the strain AK2 is able to decolorize the dye at different pH ranges.

Effect of temperature on decolorization

The dye decolorization was studied at the temperature range from 25 to 55 ^{o}C (Table 2). The optimum temperature for the highest decolorization of the dye is 35^{o}C. This strain has the capability to decolorize the dye in the temperature range from 25 to 55^{o}C. The decolorizing capacity increased gradually from 15 to 35^{o}C and then declined due to thermal denaturation. Wong *et al*. [17] observed that the *Klebsiella pneumonia* RS-13 could not degrade Methyl Red above 40^{o}C.

Effect of salt concentration on decolorization

The decolorization potential of this strain for Amido Black 10B at increasing NaCl concentrations (5-30 g/L) was assessed. The results suggested that the strain is resistant to the NaCl concentrations up to 30 g/L (Table 2). However, the decolorization time increased with increase in the NaCl concentration. Inhibitory effect of NaCl on dye decolorization was noticed on increasing the concentration of salt beyond 30 g/L. Anjaneya *et al*. [1] observed decrease in the percent decolorization of metanil yellow at NaCl concentration above 15 g/L. Guo *et al*. [18] stated that salt concentration above 3 g/L can cause obstruction of bacterial metabolism.

Assay of azoreductase

The azo dye decolorization involves the breakage of the N=N bond reductively by azoreductase. There was a significant decrease in O.D. at 340 nm, when NADH was added to the cell free extract in the presence of Amido Black 10B. The crude enzyme showed 1.5736 µM/min/mg protein of azoreductase activity for Amido Black 10B. The induction of azoreductase showed that Amido Black 10B can be decolorized by the enzymatic action.

Table 1. Percent decolorization of Amido Black 10B by *Lysinibacillus* sp. strain AK2 at increasing dye concentrations.

Time	Concentration of dye			
	200 mg/L	400 mg/L	600 mg/L	800 mg/L
12 h	64	51	31	12
24 h	78	61	42	19
36 h	90	74	57	25
48 h	90	77	65	36

Table 2. Percent decolorization of Amido Black 10B by AK2 at different (a) pH values (b) temperatures (c) NaCl concentrations.

Time	pH				Temperature				Salt concentration			
	5	7	9	11	25°C	35°C	45°C	55°C	10 g/L	20 g/L	30 g/L	40 g/L
12 h	41	67	58	43	48	65	57	34	60	54	43	24
24h	56	78	71	64	62	79	71	45	72	64	55	38
36h	68	90	82	73	71	90	80	58	83	73	66	45
48h	68	90	82	73	71	90	80	58	83	81	74	51

At 200 mg/L dye concentration

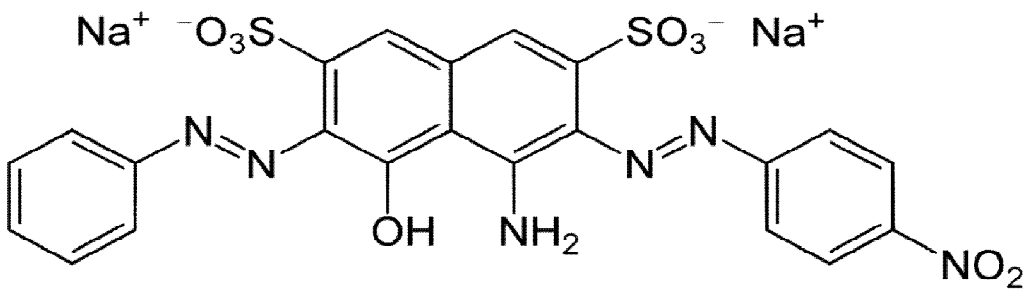

Figure 1. Chemical structure of Amido Black 10B

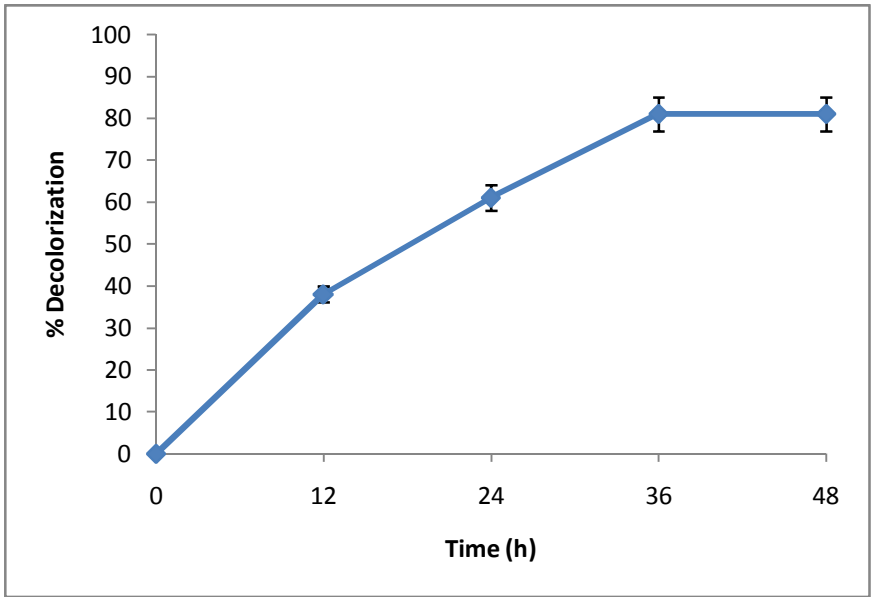

Figure 2. Decolorization of Amido Black 10B by *Lysinibacillus* sp. strain AK2 at different time intervals.

CONCLUSION

The bacterial strain *Lysinibacillus* sp. AK2 is capable of decolorizing higher concentrations of Amido Black 10B in a short incubation time. The strain exhibited good decolorization efficiency at pH from 6-11 and temperatures from 25 to 45 ^{0}C. The strain showed decolorization potentiality even at higher NaCl concentrations of up to 30 g/L. These results suggest the potential use of Lysinibacillus sp. Strain AK2 in the treatment of textile dye effluents.

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