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Research Article

Decolourization and Detoxification of an Azo Dye Direct Red 81 by *Pseudomonas aeruginosa* Strain MZA 85

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Abstract

The textile and dye industry is an important sector of the chemical industry. These industries generate large volume of waste water. Largest user of azo dyes is textile industry. These recalcitrant dyes are extensively used in cosmetics, pharmaceuticals, printing etc. The waste water generated from these industries has been treated by physical, chemical and biological methods. These effluents show variation in their composition according to the dye used or manufactured which makes the treatment intricate. Treatment of dye industry effluent is a challenging task before its disposal into water bodies. Direct Red 81 was selected for decolourization and detoxification studies by *Pseudomonas aeruginosa* strain MZA 85. Dye decolorization and optimization of parameters were carried out under stationary condition. Under optimum conditions the isolate was found to decolorize 94.66% in 13 hrs at pH 7and at 28°C under static condition. Degradation products were analyzed by UV-Visbile spectroscopy, TLC and HPLC. The isolate was also able to decolourize and degrade five dyes under study.

Hence this indigenous isolate could be a potential organism for bioremediation of wastewater carrying dyes.

Keywords: Azo Dyes; Direct Red 81 (DR81); Degradation; Decolourization; HPLC Analysis; Oxidoreductases

Abbreviation

DR 81; ABTS; DCM; TLC; HPLC

Introduction

A variety of synthetic dyes are used in textile industries producing large volumes of waste water containing dyes. Dye industries use several chemicals containing acids. Amongst different types of dyes, azo dyes find widest applications in different industries specially textile, cosmetics, printing etc. Because of ease of application reactive dyes are used on large scale in textile industry. During the dying process approximately 10-15% of dyes are lost in water and comes in environment through effluent of these industries [1].

Azo dyes and their metabolites are found to induce mutations and thus also induce cancer production. These dyes act as an allergen. These are found to inhibit growth of different types of microorganisms [2,3].

Many microorganisms were known to decolourize and mineralize dyes under certain environmental conditions. The bacterial decolorization and degradation of these dyes have received increasing attention, since it can achieve a high degree of biodegradation and mineralization, in addition it is inexpensive and environmentally friendly [4,5].

Direct Red 81 is a toxic sulphonated azo-based dye which makes it easily soluble in water. It is a widely used anionic or acidic dye among its category for colouring cellulosic fibres like cotton, rayon and jute. It is also used as a dyestuff for colouring leather and paper materials [6]. It is known for its carcinogenic nature and toxicity towards animals and humans and is selected as a synthetic model dye solution for experimentation which is widely used in many industries. It also has harmful effects on the skin and eyes [7].

Hence the present study is undertaken to degrade the dye DR 81 under study and decolourize the dye containing wastewater.

Material and Methods

Isolation of the bacterium

The bacterium is isolated by enrichment culture technique by using soil contaminated with the dye industry effluent. The isolate was stored under refrigeration on using Hi Media Nutrient agar.

Dyestuff and chemicals

The dye under study was procured from Spectrum Dyes and Chemicals PVT LTD, Surat, India. Chemicals used in all experiments were of analytical grade.

Decolourizationstudies

Pseudomonas aeruginosa strain MZA 85 was grown for 24 h at 37°C on nutrient agar. Decolourization studies were carried out by using inoculum of 1.0 absorbanceat 600nm [8,9]. Throughout decolourization studies medium used was sterile nutrient broth. Addition of the dye was done after medium sterilization. Sterilization of the dye was carried out by filtration using 0.2 µm filter. Decolourization study was carried out at a dye concentration 50 mg/L under stationary condition at 28°C. The samples were withdrawn at definite time intervals and observed for decolourization at maximum absorbance of the dye 511 nm.Percent decolourization was calculated as -% Decolorization ¼ A0 – At/ A0 X 100 [10].

Decolourization performance at different dye concentration

The decolourization ability of the isolate was studied at different dye concentration ranging from 50-500 mg/L under static anoxic condition. The% of dye decolourized was measured hourly at 511 nm. Percentage dye decolourizedwas calculated as mentioned in Sahasrabudhe and Pathade, 2012 [10].

Decolourization performance at different pH and temperature

Dye decolourization studies were carried out in sterile nutrient broth at different pH ranging from 3 to 8 at 28°C. For detecting optimum temperature for dye decolourization, the experiment was carried out in same manner but tubes were incubated at different temperatures ranging from 25 to 50°C. Each experiment was done in set of three. To avoid the effect of inanimate factors experiment was carried out without the isolate under study. Decolourization was monitored till complete decolorization of the medium. Extent of decolourization was calculated from initial and observed absorbance.

Preparation of the cell free extract

Pseudomonas aeruginosa strain MZA 85 was grown in nutrient broth at 28°C for 24 hours and centrifuged at 10,000 rpm for 20 minutes. The cells were subjected to ultra sonication and the supernatant was obtained. The supernatant acted as a source of enzymes. Ultrasonication was carried out in phosphate buffer at 4°C. The parameters and conditions used for ultrasonication were as per method used by Sartale., *et al*. 2009.

Oxidative and reductive enzyme assays

The enzymes involved in degradation are Oxidoreductases. The enzymes studied were laccases, lignin peroxidase and. azoreductase and NADH-DCIP reductase. All enzymes assay were carried out spectrophotometrically by using cell free extracts. Assay of the enzymes were carried out as method mentioned in Sahasrabudhe., *et al*. 2012. All enzyme assays were carried out at in triplicate 37°C with reference blanks that contained all components except the enzyme to be assayed. Enzyme activity was calculated in terms of absorbance U/ml/min of the enzyme. Sartale., *et al*. and Kalyani., *et al*. [11,12] methods were used in the evaluation of the enzymes.

Analytical Methods

The products of biodegradation of the dye were extracted in dichloromethane after 13 h. The DCM extracts were evaporated at 40°C in a rotary evaporator and the same was used for further studies [13]. For HPLC analysis small amount of the extract were dissolved in analytical grade methanol. The parameters used in HPLC analysis were as mentioned in Parshetti., *et al*. 2009. Spectrophotometric analysis was done by using UV-Visible spectra and change in absorption of the control dye solution and the decolourized medium. TLC was carried out on silica gel with a solvent

system composed of methanol: ethyl acetate: n-propanol: water : acetic acid(1:2:3:1:0.2 v/v). The plates were developed in iodine chamber [8,12].

Toxicity studies

Toxicity of the metabolites produced in biodegradation of DR 81 were studied by using Sorghum vulgare. The test was carried out at room temperature and by using dye concentration of 400mg/L. Control was also kept [14].

Statistical analysis

Data was analyzed by ANOVA and treated to be significancant at P was ≤ 0.05.

Results and Discussion

Isolation and identification of the microorganism

Amongst the microorganisms isolated, efficient decolourizer was isolated. The isolate was identified by morphological, cultural, biochemical and genetic characters. The gene Ombio Technologies, Pune provided 16s rRNA sequencing. The sequence was submitted to GenBank and identified to be *Pseudomonas aeruginosa* strain MZA 85 with accession number JN 234859.

Decolourization experiment

The isolated *Pseudomonas aeruginosa* strain MZA 85 was able to decolourize DR 81 94.66% in 13 hrs at pH 7 and at 28°C at a dye concentration of 50 mg/L(Fig 1). The shift in absorption maxima confirms the degradation of the dye [15]. New peaks were observed at 200, 371 and 400nm. Absence of the abiotic loss confirmed biodegradation of the dye. Wet weight of the isolates was found to be increased indicating growth of the organism in presence of the dye.

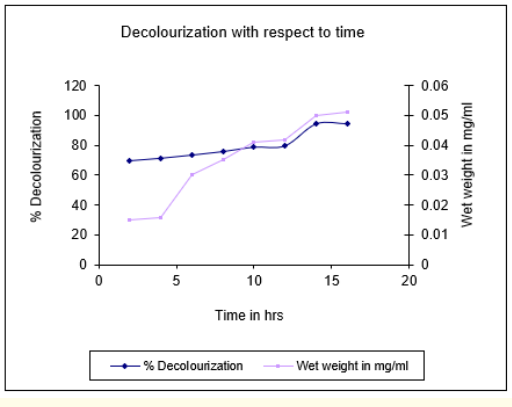

Figure 1: Decolourization with respect to time.

Effect of physicochemical conditions on the decolourization performance

All parameters were studied at 28°C under stationary condition. 10% inoculum A_{600} 1.0 was used at a dye concentration 50 mg/L.

Effect of pH

DecoIourization and degradation of the dye was found to be maximum around neutral pH. It was observed that strong acidic pH rate of decolourization adversely. This is probably due to enzymatic activity is dependent on pH [16,17]. *Pseudomonas aeruginosa* strain MZA 85 showed 94.66% decolourization of DR 81 at pH 7.0 within 13 h. It showed decolourization in the pH range of 5 – 8 (Figure 2) while at pH 3and 4 about 40% decolourization was observed.

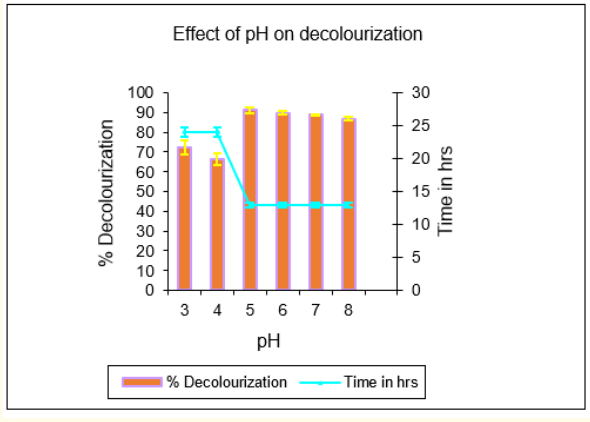

Figure 2: Effect of pH on decolourization of DR 81 by *P. aeruginosa.*

Effect of temperature

The rate of colour removal increases with increasing temperature. The temperature required to produce the maximum rate of colour removal tends to correspond with the optimum cell culture growth temperature of 35 - 45°C. Temperature affects microbial growth and enzyme production and, consequently, the percentage of decolouration [17]. Decolouization depends on the activity of enzymes. At higher temperature the enzymes involved in decolourization may undergo denaturation reducing the rate of decolourization. Decolourization was found to be in the range of 25-50°C [9]. *Pseudomonas aeruginosa* strain MZA 85 decolourized the dye under study in the range of 84-94% within a temperature of 28-40°C. (Figure 3) At 28°C, 94.66% decolourization was observed

while at 40°C, 89.41% decolourization was seen. At 45°C and 50°C, 10.68 and 11.48% decolourization was observed, respectively.

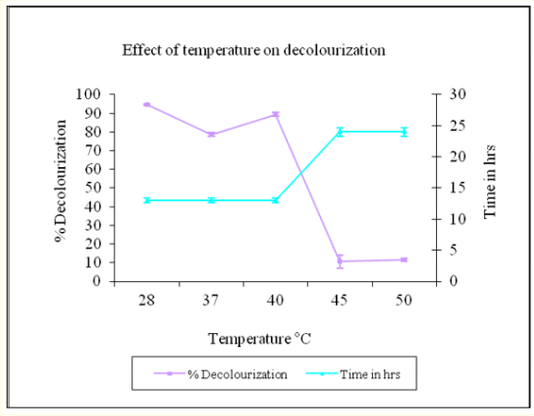

Figure 3: Effect of temperature on decolourization of DR 81 by *P.aeruginosa.*

Effect of initial dye concentration

Decolourization of different initial concentrations of the dye from 50 - 500mg/L was studied under stationary condition. It was reported by various researchers that at low dye concentration, the decolourization was rapid. It was observed that as the dye concentration increases, the% decolourization declines. This may be due to temperature affect enzyme activity. The *Pseudomonas aeruginosa* strain MZA 85 showed faster decolourization up to 350mg/L after which the rate of decolorization decreased (Figure 4). The isolate was able to decolourize DR 81 up to 500 mg/L concentration with an average efficiency ranging from 89 - 92% in maximum 72h. Dehghani., *et al*. reported removal of DR 81 up to 98.29% efficiency by using Fenton process [1].

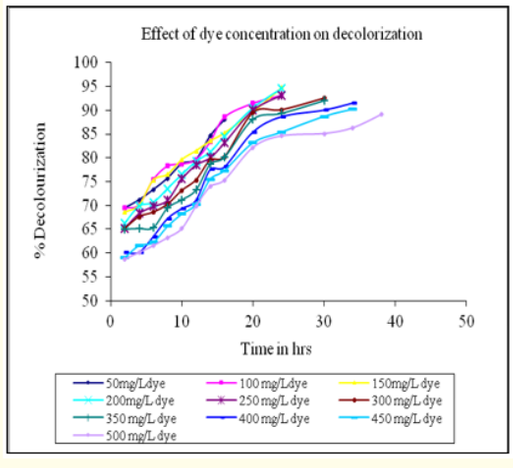

Figure 4: Decolourization of different concentration of DR 81 by *P.aeruginosa.*

Effect of initial dye concentration

Dye decolourization is mainly due to cleavage of azo bond. Enzymes involved in decolourization are Oxidoreductases. Remarkable changes were observed in enzyme activity of the induced cells as compared with enzyme profile of the biocontrol. It was observed that lignin peroxidase and laccase activity was enhanced in induced cells while there was no significant increase in the activity of NADH DCIP reductase. Cell free extract showed absence of enzyme activity (Figure 5). Coates., *et al*. 2002 reported that lignin peroxidase is the only enzyme for degradation of azo dyes [18]. The isolate in the present study exhibited raised levels of lignin peroxidase in induced cells which confirms biodegradation of the dye.

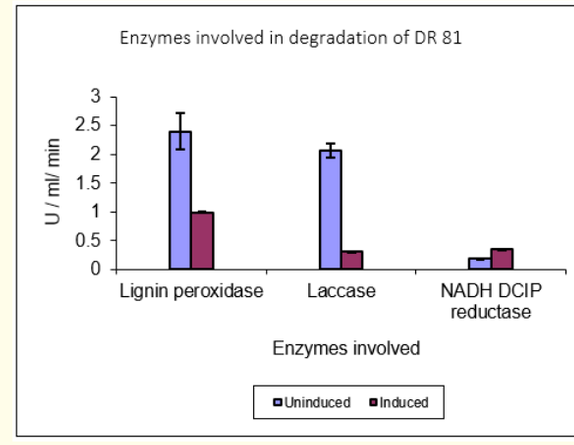

Figure 5: Enzymes involved in degradation of DR 81.

Analysis of metabolites resulting from decolourization

To analyse the metabolites produced in biotransformation of the dye and to confirm biodegradation, the products of DR 81 were studied by UV visible spectral analysis, TLC and HPLC. UV visible scan of the culture supernatant withdrawn at different time intervals indicated the decolourization and decrease in dye concentration from batch culture. There is a hypsochromic shift in the decolourized supernatant sample (Figure 6). TLC chromatogram showed one spot in the sample containing the extracted metabolites of completely decolourized medium with Rf value 0.87 where as Rf value of DR 81 was noted as 0.97 confirming the biodegradation of DR 81 by *Pseudomonas aeruginosa* strain MZA 85.

HPLC elution profile of DR81 showed a distinct single peak at retention time of 1.71. min. Three peaks at 3.008, 3.861 and 4.021min showed the degradation products of *Pseudomonas aeruginosa* strain MZA 85. (Figure 7 and Figure 8).

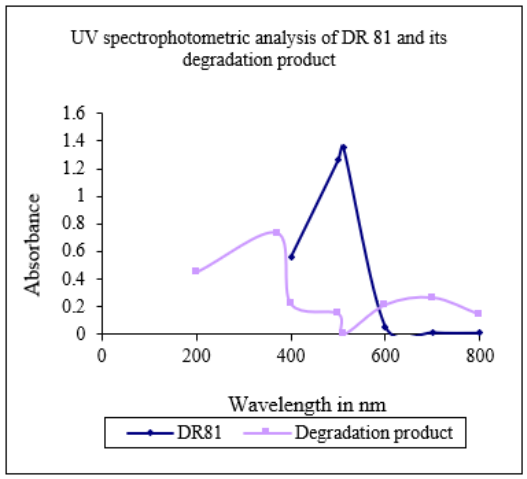

Figure 6: UV spectrophotometric analysis of DR 81 and its degradation products.

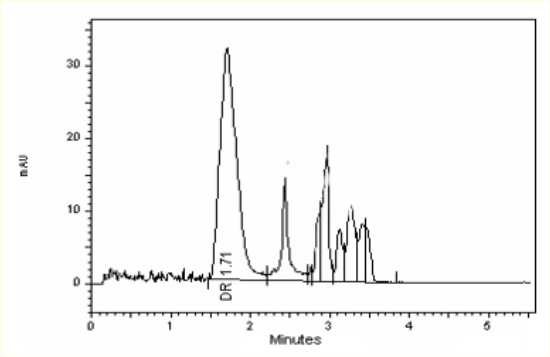

Figure 7: HPLC of DR 81.

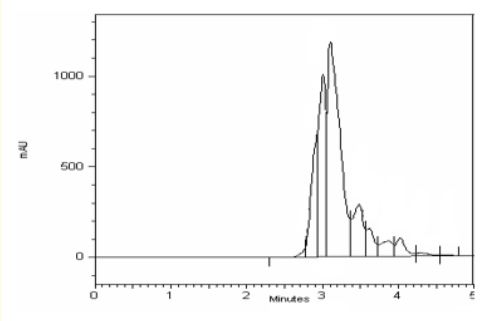

Figure 8: HPLC of the degraded product.

Phytotoxicity studies

Many industries dispose untreated or partially treated effluents into nearby water bodies. The same water is used for irrigation purposes. Thus it was found necessary to study phytotoxicity of the dye before and after degradation. Phytotoxicity was studied by using seeds of Sorghum vulgare. Seeds were irrigated by the dye, the metabolite after complete decolourization and tap water as control. Root and shoot length of the metabolite irrigated showed significant increase as compared to control. Dye irrigated seeds (Figure 9). This indicates the detoxification of DR 81 by *Pseudomonas aeruginosa* strain MZA 85.

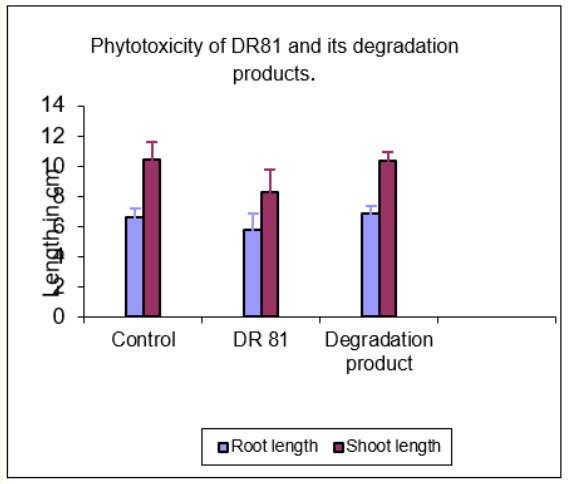

Figure 9: Phytotoxicity of DR81 and its degradation products.

Conclusions

This study demonstrates that isolated *Pseudomonas aeruginosa* strain MZA 85 showed shift in the peak from the λ_{max} of the dye indicating degradation of the dye. Enzyme analysis indicated prime involvement of oxidoreductive enzymes in the decolorization process. and phytotoxicity studies shows nontoxic residual metabolites. Analytical studies of extracted products confirmed the biodegradation of Direct Red81 by *Pseudomonas aeruginosa* strain MZA 8. This strain could be potential strain for the treatment of recalcitrant dyes used in textile and dye industry effluent by using appropriate bioreactor. Thus the use of the isolate *Pseudomonas aeruginosa* strain MZA 85 was found to be eco-friendly, inexpensive and for rapid biological treatment of dyes present in the textile effluent containing DR 81.

Conflict of Interest

Author declares no conflict of interest.

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