

Decolorization and Degradation of Direct Blue 6 from textile wastewaters by *Pseudomonas aeruginosa* HS-DY012

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Abstract. Wastewater discharged from printing and dyeing plants on both sides of the Yangtze River seriously threatens the ecological and water security. Direct Blue 6 is a synthetic chemical dye and widely used in textile and dyeing industry. In this study, *Pseudomonas aeruginosa* HS-DY012 was used as a original strain in the decolorization of Direct Blue 6. Results indicated that HS-DY012 strain could completely decolorize and degrade 30 mg/L Direct Blue 6 dye in MSM medium within 18h culturing at 35 °C on a shaker at 120 rpm. The optimum condition of decolorization and degradation were determined, i.e. temperature at 35°C and pH7.0, and the optimum concentration was 30 mg/L. HS-DY012 strain could effectually decolor selected two type dyes both Reactive Black 5 and Congo red with a high degradation rate, as well as Acid scarled GR and Vat Yellow 2 could be decolored partially. These results indicated that HS-DY012 strain was a great degrader with a broad-spectrum dye decolorization character and it has a potential application.

Introduction

Azo dyes are the majority of all textile dyestuffs and are the most commonly synthetic dyes in the textile, printing and dyeing industries, because of their low biodegradability, some of these dyes have been reported to accumulate in surface water and sediments [1]. As the largest type of dyes, azo dyes have differences structural based on one or more azo bonds (-N=N-) bearing aromatic ring and consequently give out a great variety of colors [2], and dominates the worldwide market of dyestuffs with a share of about 70% [3]. Approximately 10–15% of the azo dyes are released into the environment during manufacturing and usage, and poses a potential health hazard to all forms of life in river and lake Basin, majority of these dyes is either toxic or mutagenic and carcinogenic and threaten the life in much aspects in environment [4].

Since they are highly recalcitrant to conventional wastewater treatment processes, azo dyes bearing effluents to the regulatory levels usually cannot be efficiently decolorised and degraded. The release of azo dyes in effluent from relative painting and dyeing plants into the environment has become a major concern in wastewater treatment [5]. The recalcitrance of azo dyes has been attributed to the presence of azo bonds, which generally considered as xenobiotic. In addition, some azo dyes or their metabolites of decoloration and biodegradation may be carcinogens or mutagens [6]. Therefore, it is very important not only to environment protection but also to human health to decolorization and degradation completely of azo dyes remained in environment. Several anaerobic and aerobic microbial treatments have been suggested to enhance the decolorization and degradation of azo dyes [2,5]. Up to date, biological degradation of some azo dyes such as Azo dye 4BS [8], Direct Black-38 [1], Methyl Orange [9], and so on, have been reported. Other research suggested that the decolourisation mechanism occurs mainly by co-metabolism in the presence of a co-substrate used as carbon and energy source [4,7,10].

In the present study, Direct Blue 6, a benzidine based azo dye with water soluble (Fig. 1), was selected as substance for carrying out microbial decolorisation and biodegradation studies. Microbe

capable of mineralizing Direct Blue 6 was isolated and characteristics of decolorisation and biodegradation was advanced investigated.

Materials and methods

Microorganism and culture medium

The original strain HS-DY012 was isolated from a wastewater treatment pool of ShenGang Textile and Dyeing Plant in Huanggang city, Hubei province, and it was identified and belonged to *Pseudomonas aeruginosa* HS-DY012 (data not show). Luria-Bertani medium (LB) and Mineral salt medium (MSM) was used in the tests and the composition of MSM was as follows (g/L): KH_2PO_4 0.5, K_2HPO_4 1.5, NaCl 0.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5. All media were adjusted pH to 7.0 and autoclaved for 30 min. HS-DY012 was incubated into Luria-Bertani liquid medium at 30°C for 15h, then the cells were harvested, centrifuged (5000 r/min for 10 min at 4°C), washed for 3 times and suspended with sterile MSM medium as prepared inoculums.

Decolouration and biodegradation assay

Direct Blue 6 and other dyes were supplied by Huanggang Shenggang Textile and Dyeing Company CO., LTD. Samples containing dyes were analyzed by Uv-vis spectrophotometer with Varian Cary 100 (USA). Bacterial growth was measured spectrophotometrically by optical density at 600 nm (OD₆₀₀). The absorbance of Direct Blue 6 was measured spectrophotometrically at 571 nm.

Dyes were added to the liquid MSM medium as an aqueous solution to a final concentration of 30 mg/L for decolorization study. On the process of dye decolorization and degradation in MSM medium, the samples were collected every 6 h interval.

Results and discussion

Dynamic decolorisation and biodegradation analysis

HS-DY012 strain was inoculated into MSM liquid medium containing 30 mg/L Direct Blue 6 dye and cultured at 35 °C on a shaker at 120 rpm. Sample was taken every 6 hour and scanned with UV-Vis spectrophotometer. As showed from Figure 1, an absorption peak at 571 nm was observed at 0 h, with the Direct Blue 6 dye degradation, the absorption peak was gradually decreased at 6 h, and the peak was disappeared at 12 h and 18h. Meanwhile, the blue colour of solution was disappeared obviously with the degradation occurred. These results indicating the Direct Blue 6 dye was degraded completely by HS-DY012 strain and it is an effective degrader in Direct Blue 6 decolorization.

Optimization of decolorization conditions

The different decolorization and degradation condition of Direct Blue 6 by HS-DY012 strain were optimized with single factor and multi level experiment. As showed from figure 2A, HS-DY012 strain could effectively decolorize Direct Blue 6 at a wide range temperature from 25°C to 43°C, and degradation rates of Direct Blue 6 in range of these temperatures were exceed 50%, indicating HS-DY012 strain could adapt to a wider temperature range and have potential application value in printing and dyeing wastewater treatment. Comprehensive consideration the decolorization and bacteria growth, the optimum temperature was 35°C.

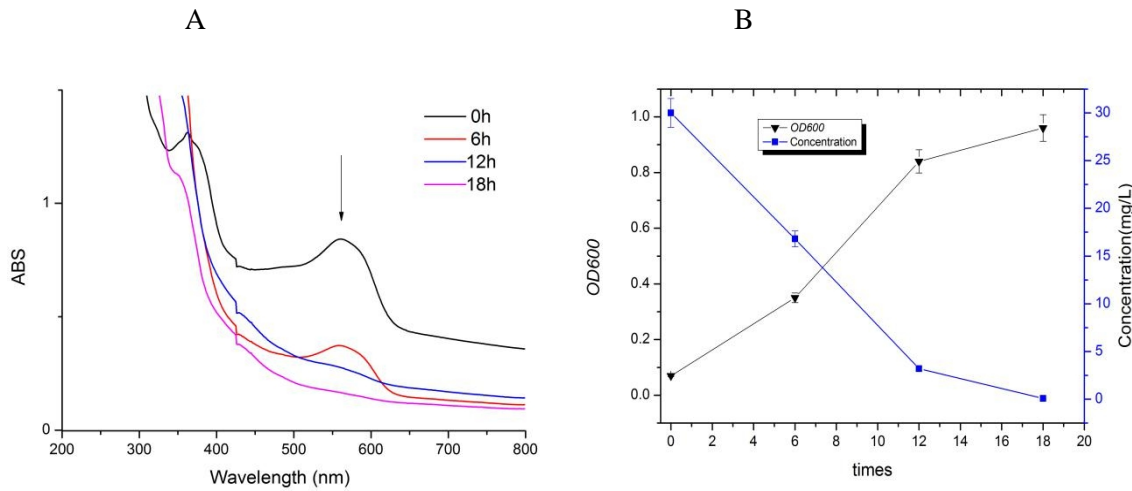


Figure 1. Degradation of Direct Blue 6 by HS-DY012 strain. (A) analysis results with UV-vis spectrophotometer, sample was scanned at 0 h, 6 h, 12 h, 18 h respectively in MSM medium containing 30 mg/L Direct Blue 6 dye; (B) Change of Direct Blue 6 dye concentration and bacteria growth (OD600 nm) at different time.

The pH value effected on not only the bacteria growth but also dyes solution. In our research, pH of aqueous solution medium was adjusted from 3.0 to 10.0, which including 30 mg/L Direct Blue 6 and cultured at 35°C with 120 rpm shaking for 24 h. Results indicated that HS-DY012 strain had a better degradation rates in the range of pH 5.0 to 8.0, and the optimum pH was determined at 7.0 (Figure 2B).

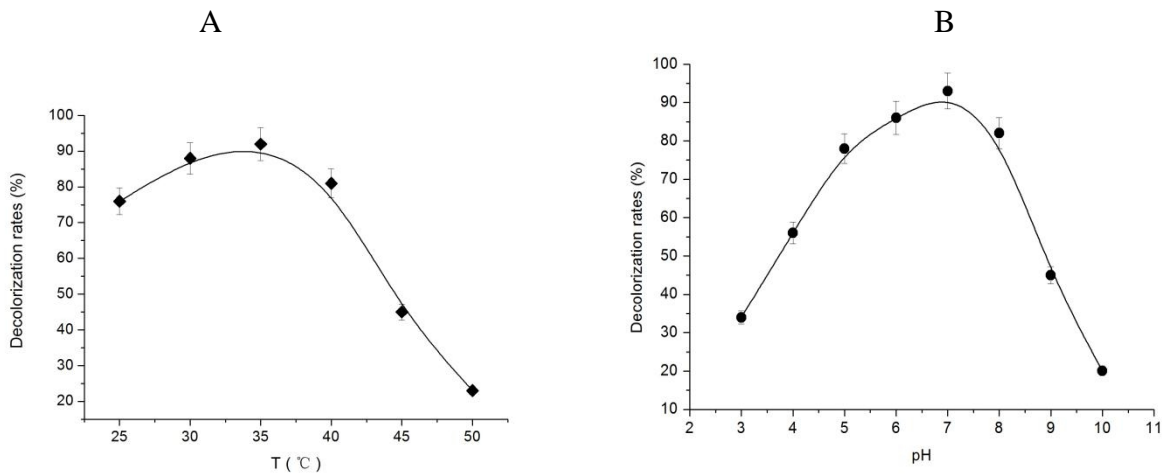


Figure 2. Results of optimization of decolorization condition. A: temperature; B: pH

Decolorization in different concentration of Direct blue 6

To determine the effect of initial concentration on Direct Blue 6 degradation, HS-DY012 strain was cultured in liquid MSM medium containing different concentration of Direct Blue 6, and adjusted pH 7.0, 150 rpm shaking at 35 °C. As showed from Figure 3, with the concentration increased, the dye biodegradation capabilities of HS-DY012 was decreased. The degradation rate was less than 50% when the concentration exceed 90 mg/L, and the degradation rate was above 90% when the concentration was under 40 mg/L. Whereas, the biomass was lower at 20 mg/L than that of 30 mg/L, meaning HS-DY012 strain could effectually degrade dye of Direct blue 6 at 20 mg/L, but the sources of nutrition from degradation metabolins were not enough for bacteria growing. High

concentration of Direct blue 6 was prevent decoloring and growing of bacteria , suggesting high concentration of dye was harmful to bacteria as a xenobiotic[10].

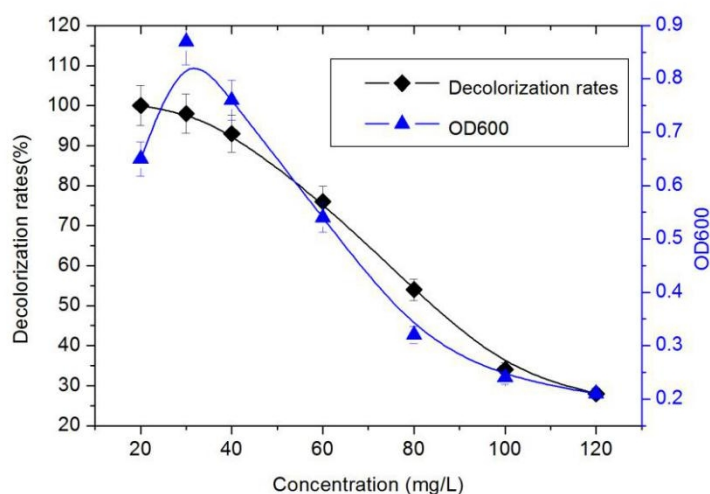


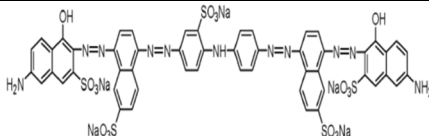
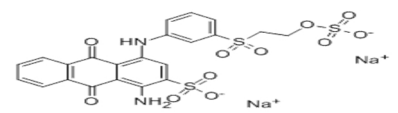
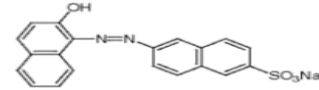
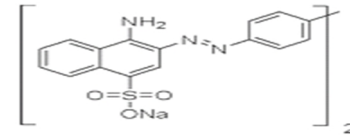
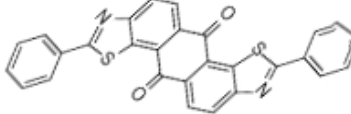
Figure 3. Decolorization and bacteria growth at different concentration

Broad-spectrum dye degradation characteristics

Broad-spectrum dye degradation was benefit to application in waste water treatment in painting and dyeing plant. Herein five different type dyes were selected as substrate of decolorization and degradation to measure broad-spectrum degradation characteristics of HS-DY012 (Table 1). They are belonged to Direct dye, Reactive dye, Acid dye and Vat dye respectively.

As showed in Figure 4, HS-DY012 strain could effectually decolor three dyes of the five, and the decolorization rates were over 90% respectively in the MSM medium at the concentration of 20 mg/L, two of them, i.e. Acid scarled GR and Vat Yellow 2, were decolorization at a lower lever. These results indicated that HS-DY012 strain have a high ability to decolor and degrade direct blue 6 with broad-spectrum dye decolorization and degradation characteristics.

Table 1 Molecular structure and properties of different types of dyes

Dye	type	Structural formula	$\lambda_{max}(nm)$
Direct blue 6	Direct dye		571
Reactive black 5	Reactive dye		600
Acid scarled GR	Acid dye		510
Congo red	Direct dye		526
Vat yellow 2	Vat dye		428

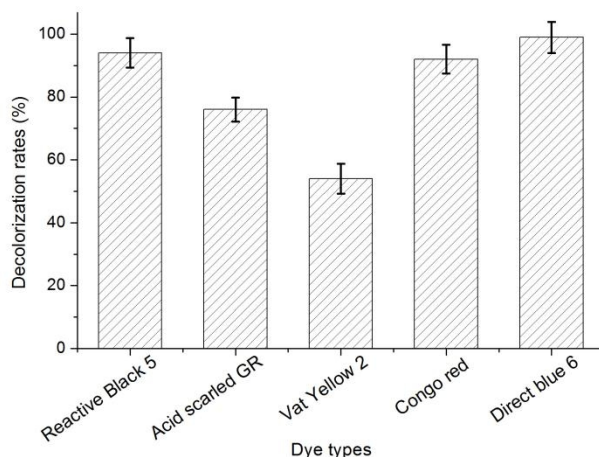


Figure 4. Decolorization rates of different dyes by HS-DY012 strain

Conclusions

HS-DY012 strain was original isolated from sludge samples in a dyeing plant and used to decolor and degrade Direct blue 6 dye. Dynamic analysis indicated that the Direct blue 6 dye with concentration of 30 mg/L was degraded completely by HS-DY012 strain within 12h culturing at 35 °C on a shaker at 120 rpm. The optimum condition of decolorization and degradation were determined, i.e. temperature at 35°C and pH7.0, and the concentration was below 40 mg/L with a high gradation rate. HS-DY012 strain could effectually decolor three of the five dyes selected from four common types of dyestuffs, indicating HS-DY012 strain was a great degrader with a broad-spectrum dye decolorization and a high degradation rate.

Acknowledgements

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