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Citrobacter freundii mediated degradation of textile dye Mordant Black 17

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ABSTRACT

One of the important worldwide environmental problems is the improper discharge of colored effluents to aqueous ecosystems which causes acute toxic effects on aquatic flora and fauna. The present study concentrates on efficiency and potentiality of a bacterium isolated from a textile effluent to degrade the azo dye Mordant black 17. Phenotypic characterization and phylogenetic analysis using 16S rRNA sequence indicated that the bacterial strain was *Citrobacter freundii*. This organism was able to decolorize the azo dye, mordant black 17 (at a concentration of 100 mg L^{-1}) efficiently bringing about 82% and 89% decolourization under shake and static conditions, respectively, at a temperature of 35 °C and at pH 6.5. Instrumental analysis confirmed the presence of naphthoquinone and salicylic acid as break-down products of the dye, indicating that the dye can be completely degraded by this organism.

1. Introduction

Color is the first contaminant to be recognized in wastewater and has to be removed before discharging into water bodies or on land. The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies [1]. The World Bank estimates that 17–20 percent of industrial water pollution comes from textile dyeing and treatment. 72 toxic chemicals have been listed in our water solely from textile dyeing, 30 of which cannot be removed [2]. This represents an appalling environmental problem for the clothing designers and other textile manufacturers. The textile effluents contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes [3]. The release of colored wastewaters represents not only a serious environmental problem, but also causes a public health concern [4].

Although there are several wastewater treatment processes, such as chemical oxidation/coagulation, advanced oxidation, photocatalysis and adsorption that can be used to treat the dye containing effluent [5,6], they possess inherent limitations such as high cost, formation of hazardous by-products and intensive energy requirements [7,8]. Conversely, bio-processing can overcome these defects because it is cost saving and environmentally benign. Fungi [9], algae [10,11] and bacteria [12] have been extensively studied with reference to dye decolorization. Adsorption, rather than degradation, plays a major role during the decolorization process by fungi and algae. As a result, the dyes remain in the environment. It is well-known that bacteria can degrade and even completely mineralize many dyes under certain conditions [13–16]. Even the products of intermediate metabolism produced during the degradation process like aromatic amines, can be degraded by the hydroxylase and oxygenase produced by bacteria [17]. In our previous study, an aerobic consortia was constructed to degrade MB17 and the capability of *Moraxella osloensis*, one of its constituents, was checked under various physical and nutritional conditions [18]. In this study, the dye degradation potential of *Citrobacter freundii*, an aerobic bacterium and another constituent of the constructed consortia [19] isolated from textile effluent, was evaluated for its ability to degrade an azo dye Mordant Black 17 and the mechanism by which it carries out this process was also studied.

2. Materials and methods

2.1. Dyes and chemicals

Mordant black 17 (MB17) [CAS Registry Number 2538-85-4] was obtained from Dynasty Chemicals (Ningbo) Co., Ltd., China. The Chemicals, growth media and solvents were purchased from Hi-Media, SISCO Research, Mumbai and Merck Pvt., Ltd., respectively.

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2.2. Microorganism and culture conditions

The bacteria present in the effluent were isolated by serial dilution method and their dye degrading ability was checked by pour plate method on nutrient agar containing MB17. All the bacterial cultures were maintained on nutrient agar slants and were used for biodegradation studies after preculturing in nutrient broth for 12 h. The bacterial culture showing appreciable dye degrading ability was cultivated in nutrient broth for 24 h and the cells after centrifugation were resuspended in 20% glycerol and stored at −20 °C as stock cultures. The purity of the glycerol stocks were checked on nutrient agar plates before sub culturing on nutrient agar slants for inoculums preparation. The bacterial culture was identified using various biochemical tests, followed by molecular characterization techniques.

For the degradation studies, mineral salt medium having following composition was used (g/l): 1.73 K_2HPO_4, 0.68 KH_2PO_4, 0.1 $MgSO_4{\cdot}7H_2O$, 0.1 NaCl, 0.03 $FeSO_4$, 1.0 NH_4NO_3, 0.02$CaCl_2{\cdot}2H_2O$ and 5.00 glucose (18).

The medium was inoculated with 12 h old 2% inoculum containing approximately 2×10^8 cells and incubated in an incubator shaker at 200 rev min^{-1} at 30 °C. Decolorization of MB17 was monitored spectrophotometrically by reading the *n*-butanol extract of the culture medium containing degradation product at 520 nm [20].

Media optimization experiments were carried out by changing the glucose and ammonium nitrate in the medium with different carbon sources (lactose, sucrose, fructose and maltose) and nitrogen sources ($(NH_4)_2SO_4$, $NaNO_3$, potassium nitrate, yeast extract and peptone) respectively. The influence of pH (6–8), temperature (30–50 °C) and dye concentration (100–1000 mg L^{-1}) on MB17 degradation by *C. freundii* were also studied.

2.3. Identification of metabolites

The culture supernatant containing the degradation products was lyophilized and kept in a desiccator. Thin layer chromatography (TLC) analysis for the breakdown products was performed on fluorescent silica plates (Polygram Sil G/UV, 40 × 80 mm, Germany). For identification of naphthoquinone, the solvent system used was isopropanol:acetic acid:water in the ratio of 19:9:1. The compound was identified by comparing the R_f values with that of standard. Fourier Transform Infra Red (FTIR) spectroscopic analysis of the spot from TLC plate (eluted using ethyl acetate) was carried out in Perkin Elmer RX1 FTIR spectrophotometer [18,19].

The culture medium containing the degradation products after the incubation period was centrifuged and the supernatant was extracted thrice with equal volume of ethyl acetate, dried

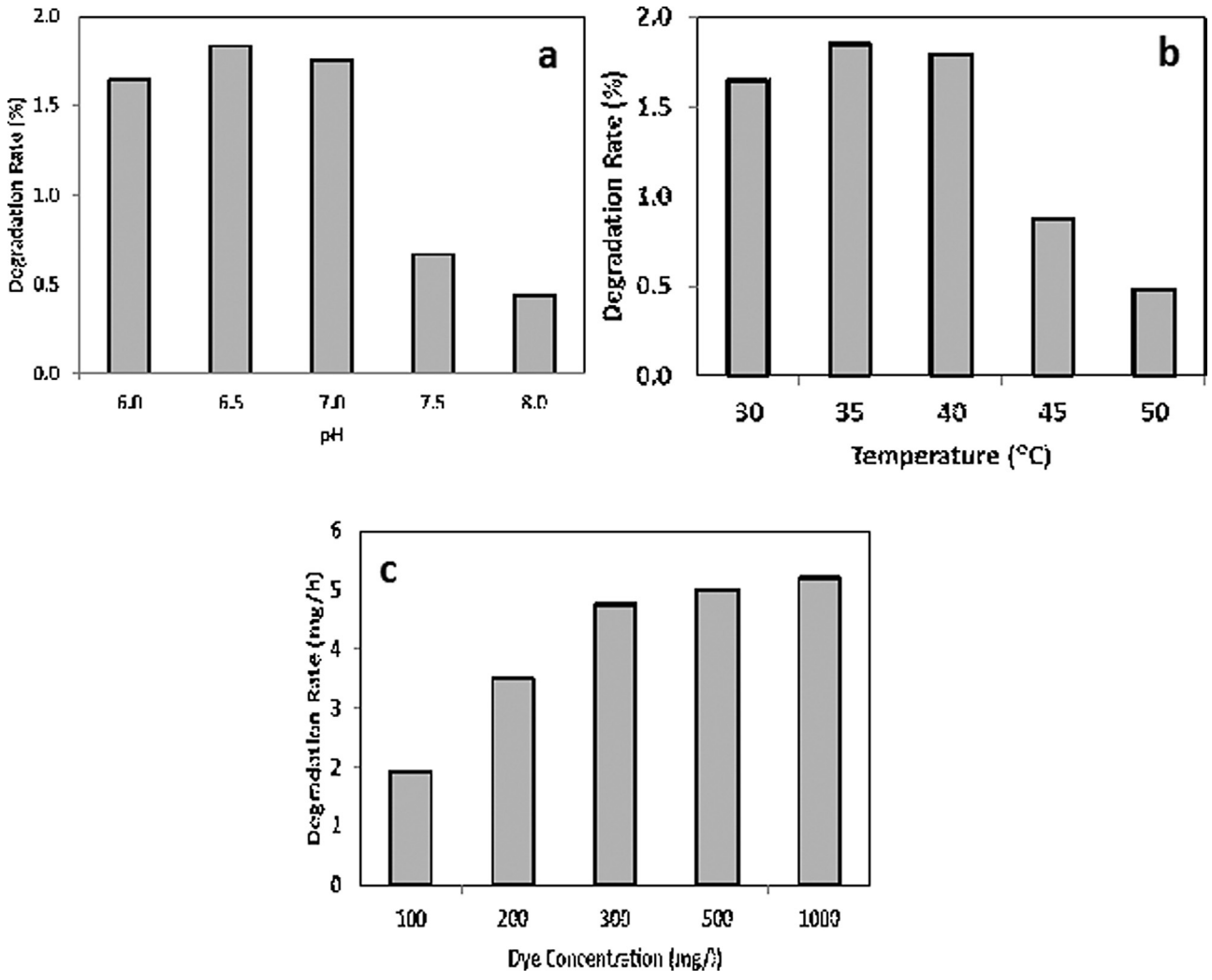

Fig. 1. Effect of different (a) pH (b) temperature and (c) initial dye concentration on MB 17 degradation by *Citrobacter freundii*.