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Original research article

Biodegradation of Reactive Black 5 by *Aeromonas hydrophila* strain isolated from dye-contaminated textile wastewater

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ABSTRACT

Aeromonas hydrophila isolate showed the best decolorization of Reactive Black 5 (RB5) at the concentration of 100 mg L^{-1} on modified mineral salt medium among 42 bacterial isolates. Optimization of parameters for RB5 dye decolourization was studied under static condition. Under optimized condition, decolorization efficiency of RB5 by *A. hydrophila* was found to be 76% at 100 mg L^{-1} within 24 h. The optimum pH and temperature for the decolorization was 7 and 35 °C respectively. Biodegradation and decolorization of RB5, was monitored by UV–Vis spectrophotometry, Thin Layer Chromatography, Fourier Transform Infrared Spectroscopy and Gas Chromatography Mass Spectrometry analysis. The study has confirmed the potential of *A. hydrophila* isolated from textile effluent in degradation of RB5 and opened scope for future analysis in the treatment of textile effluent.

1. Introduction

One of the important classes of the pollutants is dyes that are considered to be particularly dangerous organic compounds for the environment [1]. Once the dyes entered the water, it is difficult to treat such as dyes have a synthetic origin and a complex molecular structure which makes it more stable and difficult to be degraded. Dye molecules comprise of two key components: the chromophores, responsible for producing the color, and the auxochromes, which can not only supplement the chromophore but also render the molecule soluble in water and so give enhanced affinity toward the tissue [2,3]. Dyes may be classified on the basis of their solubility: soluble dyes which include acid, mordant, metal complex, direct, basic and reactive dyes; and insoluble dyes including azoic, sulfur, vat and disperse dyes.

Significant proportions of these dyes enter in the environment through wastewater. Discharge of colored textile effluents into drainages and lakes results in reduced dissolved oxygen concentration and creates toxic conditions to aquatic flora and fauna [4]. Among many classes of synthetic dyes used in the textile and dyeing industries, reactive dyes are used widely in many industries due to their bright color, excellent colorfastness and ease of application [5]. Reactive dyes are typically azo-based chromophores combined with different reactive groups. They differ from all other dye classes in that they bind to the textile fiber, such as cotton, through covalent bonds; and thus are highly recalcitrant to conventional wastewater treatment processes. One of the main sources with severe pollution problems worldwide is the textile industry and its dye-containing wastewaters. In particular, the discharge of dye-containing effluents into the water environment is undesirable, because of their color, and se huge amount of dyes dissolved in large volumes of water both in the dye bath and also during the rinsing step. Without adequate treatment these dyes can remain in the environment for a long period of time. However, even lesser concentrations of dyes may have significant environmental impacts [6]. Therefore, the treatment of dye contaminated effluents is currently an environmental concern [7].

Reactive Black 5 (RB5) dye is one of the most common used synthetic reactive dyes in the dyeing industry. This type of dye is highly soluble in water and has reactive groups which can form covalent bonds between dye and fiber [2,3]. RB5 is the most commonly used to dye cotton and other cellulosic fibers, wool and nylon [8]. RB5 forms a covalent bond with the fiber and contains chromophoric groups such as azo, anthraquinone, triarylmethane, phthalocyanine, formazan, oxazine, etc. Large amounts of these

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dyes are discharged in the water courses in developing countries such as Egypt that can have adverse effects on water and human bodies. Thus removing color from wastewater is as important as treating other colorless organics. There is an urgent need for textile industry to develop effective methods of treatment because small amounts of dye are clearly visible and detrimental to the water environment [8].

RB5 was selected as a model compound, due to the incomplete fixation reaction on cellulose. The reason is the competition between the reaction of the reactive vinylsulphone groups with the fiber and the hydrolysis of the vinylsulphone groups yielding the 2-hydroxyethylsulphone groups. The 2-hydroxyethylsulphone groups do not react with the fibers resulting in low efficiency of the dyeing process [9].

Although there are many methods for the removal of the dyes, it is difficult to treat the wastewater by using traditional methods, because most of the synthetic dyes are stable to light, chemicals and biological treatment [10]. Among these methods, biodegradation can be thought to be the most efficient process for treating industrial effluents due to it is a cost-effective and eco-friendly technique as well as its ease of operation. For this reason, biodegradation has the ability not only to decolorize dyes but also to detoxify [11].

In this study, biodegradation was used for the bacterial decolorization of RB5 in batch mode studies. The effects of experimental conditions such as initial dye concentration, temperature and pH were investigated to obtain information on dye removal.

2. Materials and methods

2.1. Chemicals

The RB5 dye used for this decolorization study was obtained from the local textile industry in Nasr Company for Spinning and Dyeing, El Mahala El Koubra, Egypt. Heavily dye-contaminated wastewater samples were obtained from surrounding areas of the textile industries and wastewater treatment plant in Nasr Company for bacterial isolation. Solutions of RB5 were simulated from the commercial product (Sigma–Aldrich, St. Louis, USA) according to real concentrations found in textile effluents. Generally, dye concentrations in textile effluents vary from 10 to 25 mg L^{-1}, although concentrations about 100 mg L^{-1} have also been found. A concentration of 100 mg L^{-1} was selected as the initial concentration of dye solutions for this work. Fig. 1 shows the structure of RB5 (Color index: 20505, formula: $C_{26}H_{21}N_5Na_4O_{19}S_6$, molecular weight: 991.8 and λ_{max}: 597 nm), where the main functional groups of the molecule, reactive groups and chromophore groups, are marked. All chemicals were of the highest purity and of an analytical grade.

2.2. Isolation of bacteria by enrichment method

The wastewater samples collected were subjected to enrichment culture technique. The enrichment was carried out by adding 10 mL of wastewater sample separately in 100 mL nutrient broth medium (5 g L^{-1} peptone, 1 g L^{-1} meat extract, 2 g L^{-1} yeast extract and 5 g L^{-1} NaCl at pH 7), to prepare concentration of RB5 dye to be 100 mg L^{-1} in 250 mL Erlenmeyer flasks. The flasks were then incubated in a rotary shaker at 50 rpm. After 3 d of incubation, a loop-full of medium was streaked onto sterile nutrient agar plates with same concentrations of ingredients and incubated 35 °C for 24 h. At the end of incubation the individual different isolated colonies were noted and re-streaked on nutrient agar plates for identification.

2.3. Screening of efficient dye decolorizing isolates

Screening was done to find out the most efficient bacterial isolates capable of decolorizing RB5 dye using modified mineral salt medium (MSM) (3 g L^{-1} glucose, 2 g L^{-1} $(NH_4)_2SO_4$, 1 g L^{-1} KH_2PO_4, 10 g L^{-1} K_2HPO_4, 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$ and 5 g L^{-1} NaCl) containing 100 mg L^{-1} RB5 dye [12]. For this, the morphologically different isolates isolated from the effluent were inoculated in modified MSM and incubated at 35 °C for 24 h. The decolorization activity was expressed in term of decolorization percentage (%) of dye. Aliquot (3 mL) was withdrawn aseptically and centrifuged at 10,000 rpm for 10 min and residual dye content in the supernatant was measured at λ_{max} 597 nm using UV–Vis spectrophotometer (Perkin Elmer Lambda 35). All assays were performed in triplicate and uninoculated mineral salt media supplemented with same concentration of dye was used as control [13]. Decolorization percentage was expressed by Eq. (1):

$$Decolorization(\%) = \frac{(I - F)}{I} \times 100 \quad (1)$$

where I = initial absorbance; F = final absorbance of decolorized medium. *Aeromonas hydrophila* isolate was found to possess more than 60% decolorization of the selected dye (RB5) in modified MSM containing 100 mg L^{-1} RB5 dye and incubated at 35 °C for 24 h. This isolate was selected for further study and stored at −20 °C in nutrient broth containing 20% (v/v) glycerol. Working culture was maintained by sub-culturing every two weeks on nutrient agar slants.

2.4. Phylogenetic analysis

The identification of *A. hydrophila* was based on standard morphological and biochemical methods and 16s rRNA gene sequence analysis. The polymerase chain reaction (PCR) amplification and DNA sequencing of the 16s rRNA gene was carried out as described earlier [14]. Almost the full length of 16S rRNA gene was amplified by PCR using universal primers forward 5′-AGAGTTTGATMTGGCTCAG-3′ and 5′-CGGYTACCTTGTTACGACTT-3′ corresponding to the positions 9 to 27 and 1525 to 1545, respectively, in the 16S rRNA gene sequence. PCR products were sequenced directly using ABI PRISM Big Dye Terminator Cycle Sequencing Kit on an ABI 3100 DNA sequencer following the manufacturer's instruction. Performed multiple alignments of the sequences, and a neighbor

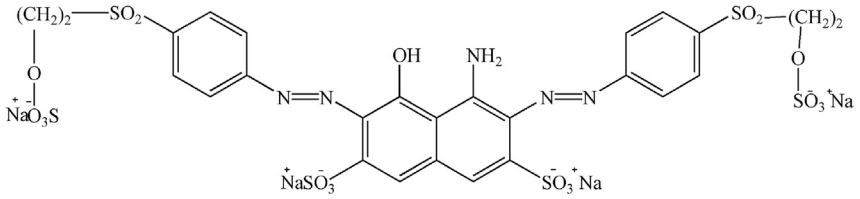

Fig. 1. Chemical structure of Reactive Black 5.