



Removal of textile dye reactive green-19 using bacterial consortium: Process optimization using response surface methodology and kinetics study



Adya Das*, Susmita Mishra

Biochemical Engineering Laboratory, Department of Chemical Engineering, National Institute of Technology, Rourkela 769008, India

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ABSTRACT

In the present work, removal of a textile dye Reactive Green-19 from the aqueous medium was investigated using a developed bacterial consortium. Optimal combinations of three significant process parameters pH (5–10); incubation temperature (25–45 °C); and Yeast extract concentration (0–1.5 g/100 mL) were determined using response surface methodology. Experiments were based on Box–Behnken surface statistical design, performed at initial dye concentration of 100 mg/L as a fixed input parameter. Regression analysis displayed good fit of the experimental data to the second-order polynomial model with the coefficient of determination (R^2) = 0.9974 and Predicted (R^2) = 0.9677. The adequacy of the model was established with various descriptive statistics like lack of fit, coefficient of variation, predicted residual sum of squares, and adequate precision. The optimal decolorization conditions were as follows: temperature, 32.04 °C; pH, 8.3 and Yeast Extract concentration, 1.16 g/100 mL. Under optimized conditions, more than 97% removal efficiency was achieved within 24 h. First order kinetic fitted well with experimental data for different concentrations of dye Reactive green-19. Michaelis–Menten kinetics was used to define the apparent relationship between the decolorization rate and the dye concentration at optimized condition. The double reciprocal plot for kinetics yield maximum decolorization rate, $V_m = 120.48 \text{ h}^{-1}$ and half saturation constant, $K_m = 1674 \text{ mg/L}$. Biodegradation of dye was confirmed through UV–vis spectroscopy, Fourier transform infra-red spectroscopy, and High-performance liquid chromatography analysis. Reactive green-19 degradation products were identified through GC–MS analysis.

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1. Introduction

Since the discovery of first synthetic dye in 1856, advancement in synthetic chemistry together with rapid industrialization of textile production have done colossal damage to the aquatic environment in the form of toxic textile dye effluent. Due to continuous release of synthetic textile dye in to the water sources various toxic and hazardous chemical compounds have been introduced in to the aquatic life as well as humans and animals. Azo dyes are one such group of compounds which are used extensively in industrial dyeing and printing processes. Textile industries consume maximum percentage of the synthetic dyes (around 56%) of world total annual production [1]. Reactive azo dyes which are

characterized by —N=N— bonds, are the largest and most diverse group of synthetic dyes which are widely used in textile industries. Apart from their negative aesthetic effects, these substances are known for their toxic effect towards aquatic life, can be responsible for allergenic effects and are known to have carcinogenic and mutagenic effects to living organisms [2–5]. Textile industries uses large amount of water in every textile processing operations and waste water generated during these processes contains 5–10% of unfixed dyes [6]. Colored effluents, when released into the nearby water bodies without proper treatment affects the light and oxygen penetration which ultimately affects the aquatic ecosystem. Therefore, it is very essential to effectively treat these colored effluents before discharging them into the environment.

Various conventional physicochemical treatment methods for the textile dye containing effluents have been found to be insufficient for treatment. Moreover other physicochemical methods like coagulation–flocculation, adsorption, membrane filtration and advance oxidation process (AOP) are effective for

* Corresponding author at: Chemical Engineering Department, National Institute of Technology, Rourkela, 769008, India.

E-mail addresses: das.aadyasha@gmail.com, 511ch110@nitrrkl.ac.in (A. Das), smishra@nitrrkl.ac.in (S. Mishra).

treatment of textile dye, but unattractive for many reasons at industrial level. Coagulation-flocculation is largely ineffective in treatment of textile dyes. Advance processes like AOP requires high maintenance cost, frequent replacement of oxidizing chemicals, energy inputs for UV treatment and still toxic intermediate compounds may form. While membrane filtration requires high cost of maintenance because of fouling, [5,7,8]. Biological treatment method for textile dye effluent is technically attractive, eco-friendly and provides a cost effective alternative to other methods. A wide variety of microorganisms have been reported for their potential to decolorize and degrade these toxic dyes effluent [9–11]. Although decolorization with pure cultures have been reported as effective tool by various researchers, but these are not found to be so effective for the treatment of actual industrial effluent in comparison to consortium of indigenous microorganisms [12]. Since, pure bacterial cultures are found to be less efficient (not capable of complete decolorization) or time consuming, use of bacterial consortium may provide more realistic and efficient solution rather than pure culture. Hence trend must be shifted towards the use of bacterial consortium compared to pure cultures. Several mixed microbial cultures have been reported to be more efficient for color removal from dye effluent as compared to pure cultures [12–15]. However, comprehensive solutions for sulfonated azo dyes removal are far from reality, which requires continued search for new organisms and technologies. These findings motivated the rationale for the current study. Moreover, as we know most municipal WWTPs rely on the microbial component of the activated sludge process, this study will facilitate application of potential bacterial consortium in existing facilities where segregated colored waste can be targeted for bioremediation.

Process optimization requires thorough evaluation of different parameters affecting the desired output. Conventional single-factor optimization technique is usually time consuming and costly. Apart from that, the combined synergistic effect of various parameters cannot be determined through this exhaustive procedure. Hence, statistical design method including response surface methodology (RSM) are used to establish a relationship between response and a set of design variables and predicts a model for the response with a minimum number of experiments [16,17]. This design method could be used as an effective optimization tool to achieve enhanced decolorization of dyes at industrial level.

The present work deals with the evaluation of a developed bacterial consortium for its potential to degrade a commonly used textile azo-dye Reactive Green-19 (RG-19). Though, very few reports are available on decolorization of Reactive green-19 dye using different microorganisms (i.e. algae, fungi, and bacteria) most of them incorporate pure cultures [18–21]. Since, use of consortium is more realistic and efficient in application rather than pure culture [22–26]. Our study has been comprehensively focused on the same to fill this research gap in the field of bio-

decolorization by using a novel developed bacterial consortium for efficient removal of azo-dye from the aquatic systems. However to best of our knowledge, this could be the first report covering decolorization study, process optimization, kinetics and metabolite identification using a developed bacterial consortium, providing comprehensive solution for removal of sulfonated azo dye Reactive green-19. This study will provide a rational and scalable solution for microbial removal of textile dyes from water which will be relevant to broader environmental and health impacts, as the removal of dye from aqueous environment directly affects the mobility and transport of these compounds in soil and groundwater systems.

2. Material and methods

2.1. Chemicals and dyestuff used

The textile dye, RG-19 used in this study was procured from a textile dye wholesaler, Kolkata. Textile waste water sample used as the source of dye degrading bacteria was collected from Viwandi, Mumbai. All the media components and chemicals used were obtained from Himedia (Mumbai, India). A stock solution of RG-19 dye was prepared (10,000 mg/L) and desired concentrations of the dye were obtained by further subsequent dilutions. The structure and properties of RG-19 dye are shown in Table 1.

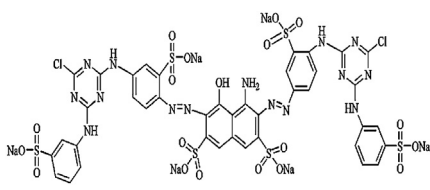
2.2. Medium used for culture maintenance and decolorization study

For initial isolation and screening of potent dye degrading bacteria, nutrient medium (containing Yeast Extract, NaCl & peptone) was used. All the decolorization and optimization experiments were done in minimal salt media (MSM). The MSM used had the following composition per liter: NaCl (0.5 g), Na₂HPO₄ (1.0 g), KH₂PO₄ (1.0 g), MgSO₄·7H₂O (0.5 g), CaCl₂·2H₂O (0.1 g) and glucose (in form of Dextrose) at 1.5% (w/v) at pH 7.2. The bacterial strains were maintained on nutrient agar slants at 4 °C prior to use. The organisms from stock cultures were used for decolorization study after pre-culturing them in nutrient medium.

2.3. Isolation, screening and identification of dye degrading microorganisms

Potent dye degrading bacterial strains were isolated from textile wastewater sample collected from Viwandi, Mumbai, India, by enrichment culture technique. The textile wastewater sample was acclimatized with increasing concentration of mixture of three different textile dyes (i.e. Reactive Green-19, Reactive Red and Remazol Navy blue). A small amount of the acclimatized culture was spread on nutrient agar plates containing dye. After 24 h of incubation, the microbes showing zone of decolorization were isolated and screened based on their dye decolorization capacity. The isolated strains were further screened and best two strains

Table 1
Structure, properties, and maximum absorbance of dye Reactive Green-19.

Dye	Molecular structure	Molecular weight	Absorption Maxima
Reactive Green-19 (anionic di-azo dye)		1418.93 g/mole	635 nm

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