



## Biological treatment of model dyes and textile wastewaters



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### HIGHLIGHTS

- *Bacillus aryabhatai* shows ligninolytic enzymes such as laccases and/or peroxidases.
- *B. aryabhatai* efficiently decolorizes some model dyestuffs.
- *B. aryabhatai* reduces Chemical Oxygen Demands on real effluents.
- *B. aryabhatai* shows potential application on the bioremediation of real effluents.

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### ABSTRACT

Previous works conducted in our laboratory, revealed that *Bacillus aryabhatai* DC100 produce ligninolytic enzymes such as laccases and/or peroxidases, opening new applications in different bioprocesses, including the treatment of disposal residues such as dyestuffs from textile processing industries. This work described the degradation of three commercial model dyes Coomassie Brilliant Blue G-250 (CBB), Indigo Carmine (IC) and Remazol Brilliant Blue R (RBBR) under different culture media and operational conditions. The process was optimized using a Central Composite Rotatable Design, and the desirability predicted complete decolorization of 150 mg/L CBB at 37 °C, 304.09 rpm and salt concentration of 19.204 g/L. The model was validated with concentrations up to 180 mg/L CBB and IC, not being able to remove high amount of RBBR. The procedure here developed also allowed Chemical Oxygen Demands (COD) reductions in CBB of about 42%, meanwhile tests on real effluents from a local textile industry involved COD reductions of 50% in a liquid wastewater and 14% in semi-liquid sludge. Thus, allow the authorized discharge of wastewater into the corresponding treatment plant. Decolorization efficiencies and COD reductions open on the potential application of *B. aryabhatai* DC100 on the bioremediation of real effluents from textile industries.

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## 1. Introduction

Pigments have been used since prehistoric times with esthetic, artistic and communicative purposes (Berke, 2002; García-Fernández et al., 2015). Initially, these pigments were obtained from plants, insects, animals and minerals, being considered as renewable, eco-friendly, and biodegradable (Shahid et al., 2013). However, these natural dyes showed some stability problems, which were solved with the discovery of synthetic dyes. At the same time, these synthetic dyes highly reduced the costs of

production, thus fulfilling the growing industrial demand (Yamjala et al., 2016). Literature compiles a large number of synthetic dyes, which represent a very large and complex group of organic compounds, which differ in their origin, chemical and/or physical properties, and characteristics related to the application process (Yamjala et al., 2016; Abbas et al., 2015; Rayaroth et al., 2016; Neill et al., 1999; Sarıkaya et al., 2012; Holkar et al., 2014). For instance, Coomassie Brilliant Blue G-250 (acid blue 90) is a triphenylmethane dye previously used in the textile industry, but nowadays it is commonly used for staining proteins in analytical biochemistry with applications in pharmaceutical and medical fields (Abbas et al., 2015; Rayaroth et al., 2016). Indigo Carmine (acid blue 74) is a dye belonging to indigo class and thoroughly used as food colorant, but also finds application in the textile industry and as indicator in analytical and biological chemistry (Yamjala et al.,

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2016; Sarikaya et al., 2012; Li et al., 2015). Meanwhile, Remazol Brilliant Blue R (Reactive Blue 19) is a dangerous anthraquinone dye used in textile industries (Holkar et al., 2014).

However, the strong development of synthetic dyes has several detrimental effects on the environment and human health. Firstly, low concentrations of dyes in effluents are highly visible and undesirable (Neill et al., 1999; Ayed et al., 2011; Robinson et al., 2001). Secondly, improper discharge of strongly colored effluents and their metabolites in aqueous ecosystems reduce sunlight penetration, causing inhibitory effect on photosynthesis (Li et al., 2015; Solís et al., 2012). Thirdly, aromatic amines which are toxic, carcinogenic and mutagenic, can be generated, if dyes are broken anaerobically (Yamjala et al., 2016; Sarikaya et al., 2012; Robinson et al., 2001).

Nowadays, over 100,000 different dye structures have been synthesized and more than 0.7 million tons of dyestuff with applications in textile, paper, leather, cosmetics, food or pharmaceutical industries are produced annually (Shahid et al., 2013; Yamjala et al., 2016; Šekuljica et al., 2015; Tehrani-Bagha and Holmberg, 2013). More than 11% of these synthetic dyes produced worldwide are lost in effluents during manufacture and application processes (Alhassani et al., 2007). The textile industries use more than 7000 different compounds and additives consuming large volumes of water (Hessel et al., 2007), having effluents concentrations ranging from 10 to 200 mg/L, high salinity (sodium chloride and sodium sulfate), detergents, oil and suspended solids, which results in strongly colored, saline and alkaline effluents (Rayaroth et al., 2016; Hessel et al., 2007; Vandevivere et al., 1998; Deive et al., 2010).

Considering that traditional processes of municipal sewerage systems are not able to degrade these compounds, wastewaters need special treatments to be spilled. Consequently, control organisms and more restrictive legislation were promoted to supervise the discharge of wastewater. In spite of the fact that each country has a specific legislation and control organisms, the most important parameters to be considered in general are: Biochemical Oxygen Demand (BOD5), Chemical Oxygen Demand (COD), Suspended Solids (SS), salinity (chloride or sulfate), color, and detergents or oil (Neill et al., 1999; Robinson et al., 2001; Hessel et al., 2007).

Current methodologies to remove dyes are classified into chemical, physical or biological processes. Chemical methods include those processes using strong oxidizing agents (e.g.  $H_2O_2$ ,  $O_3$

and Fenton's reagent) or advanced oxidation processes as photochemical and photocatalytic (e.g.  $H_2O_2/UV$  or electrochemical oxidation). However, they involve some handicaps as short lifetime, inefficiency against some dyes and high costs. On the other hand, physical methods are based on coagulation, flocculation, filtration and adsorption procedures, but produce high amount of sludge, and have low effectiveness and high costs (Holkar et al., 2014; Robinson et al., 2001; Vandevivere et al., 1998; Anjaneyulu et al., 2005). Finally, biological methods consist in dye degradation by metabolic pathways or adsorption by living/dead biomass including bacteria, fungi, yeasts, algae and plants. In general these processes are inexpensive, ecofriendly and could be applied to a wide range of dyes, although they also show some drawbacks particularly the time required for the processes (Solís et al., 2012; Saratale et al., 2011).

Preliminary studies demonstrated the ability of the specie *Bacillus aryabhatai* to degrade commercial dyes (Paz et al., 2016). Consequently, this work aims to study three commercial model dyes: triphenylmethane Coomassie Brilliant Blue G-250 (CBB), Indigo Carmine (IC) and anthraquinone Remazol Brilliant Blue R (RBBR). The decoloration process was optimized by Central Composite Rotatable Design (CCRD), and optima conditions were assayed on real effluents from the textile industry.

## 2. Material and methods

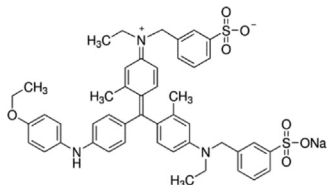
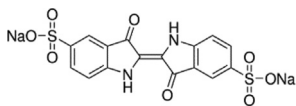
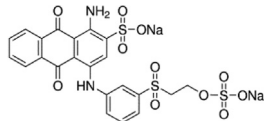
### 2.1. Dyestuff and chemicals

Coomassie Brilliant Blue G-250 (CBB), Indigo Carmine (IC) and Remazol Brilliant Blue R (RBBR) were purchased from Sigma–Aldrich (St Louis, MO). Table 1 shows the chemical structure and main characteristics of these dyes. Panreac Química SAU (Barcelona, Spain) and Pronadisa (Madrid, Spain) supplied all the components used to formulate the culture media. A local textile company in San Cibrao das Viñas (Ourense, Spain) kindly provided the real effluents (a liquid wastewater and a semi-liquid sludge).

### 2.2. Microorganism and inoculum preparation

*Bacillus aryabhatai* DC100 was previously isolated in our laboratory and stored in cryovials with 20% (v/v) glycerol solution at  $-80^\circ C$  until use. The strain has been deposited in the Spanish Type Culture Collection (CECT) with accession number CECT 9226.

**Table 1**  
Chemical structure, wavelength (range and maximum absorbance) and type of dyes used in this work.

Dye	Structure	$\lambda_{range}$ (nm)	$\lambda_{max}$ (nm)	Type of dye
Coomassie Brilliant Blue R-250 (Acid Blue 90) (CBB)		460–720	580	Triphenylmethane
Indigo Carmine (Acid Blue 74) (IC)		470–720	612	Indigo
Remazol Brilliant Blue R (Reactive Blue 19) (RBBR)		470–720	594	Anthraquinone

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