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Decolorization of dye Reactive Black 5 by newly isolated thermophilic microorganisms from geothermal sites in Galicia (Spain)

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ABSTRACT

In this study, thermophilic microbial strains from thermal spots in northwestern Spain displaying excellent decolorization capability were isolated. The research work tackled: (i) the ability of consortia to degrade a model di-azo dye Reactive Black at different pHs in flask cultures, obtaining that just neutral pHs licensed degradation levels near to 70%, (ii) the isolation of tree of the bacteria, which rendered possible reaching high levels of decolorization (80%) after just 24 h in aerobic conditions, and which were identified through 16S rRNA sequencing to possess high homology (99%) with *Anoxybacillus pushchinoensis*, *Anoxybacillus kamchatkensis* and *Anoxybacillus flavithermus*, and (iii) the cultivation of the isolates in a bench-scale bioreactor, which led to a decolorization rate two-fold higher than that obtained in flask cultures. Therefore, this work makes up the first time that a decolorization process of an azo dye by thermophilic microorganisms in aerobic conditions is investigated.

1. Introduction

Management of water pollution is currently one of the major challenges for environmentalists. More than 10,000 different textile dyes, with an estimated annual production of 8×10^5 metric tonnes, are commercially available worldwide; about 50% of these are azo dyes [1,2].

Highly colored synthetic dye effluents from the textile, food, paper and cosmetic industries have contaminated water resources, and these contaminants are easily identifiable to the naked eye. The complex aromatic structures of the dyes are resistant to light, biological activity, ozone and other environmental degradative conditions [3]. The effluents may significantly affect photosynthetic activity in aquatic life due to reduced light penetration and increased chemical oxygen demand. Concerns have arisen because many of the dyes are made from known carcinogens, toxins and mutagens, such as benzidine and other aromatic compounds, and often involve the presence of metals, chlorides, and aromatic compounds [4].

Dyes can be classified according to several features, but one typical consideration is whether they are ionic or nonionic, as reported by Robinson et al. [5]. Ionic dyes are direct, acid and reactive dyes. Nonionic dyes refer to disperse dyes because they do not ionise in an aqueous medium. Direct dyes are the most popular class of dyes, owing to their easy application, wide color range, and availability at modest cost. Most direct dyes have di-azo and tri-azo structures. Azo dyes are the largest class (60–70%) of dyes, with the greatest variety of colors [6].

Importantly, conventional wastewater treatment remains ineffective in decolorizing these compounds. Several methods for the removal of dyes in textile wastewater have been implemented to overcome this problem. These methods have been classified into three categories, physical, chemical and biological, and they have been extensively reviewed [5,7–10]. Although physico-chemical techniques are commonly used [11–13], their major disadvantages are high cost, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste generated. Microbial decolorization, being cost-effective, is receiving more attention for the treatment of textile dye wastewater [14].

Dye-bath effluent compositions vary depending on the type of fibers to be dyed. For instance, while wool dyeing involves effluents with acid pH, cotton dye entails neutral or alkaline conditions. Besides, these effluents, with a temperature range of 30–60 °C, exhibit high concentrations of dye stuff, biochemical oxygen demand, total dissolved solids, sodium, chloride, sulphate, hardness, heavy metals and carcinogenic dye ingredients. For this reason, the use of biological processes for their treatment requires the presence of microorganisms thriving in extreme conditions. Thermophilic microorganisms are amongst the most studied extremophiles and are gaining wide industrial and biotechnological interest due to the fact that they are well suited for harsh industrial processes. For this reason, thermal springs, solphataric fields,

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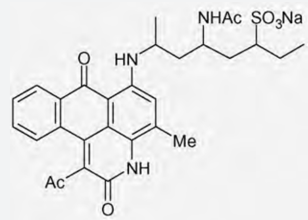
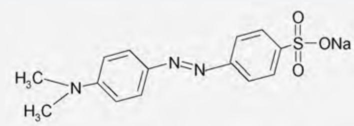
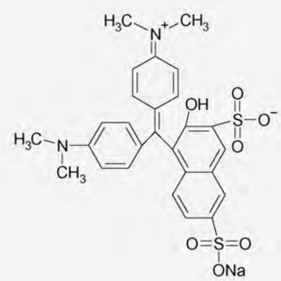
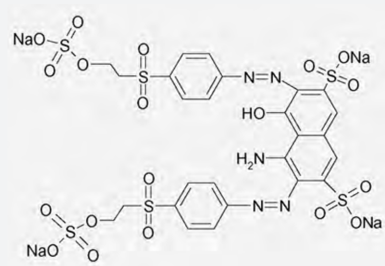
Table 1
Chemical structure, color index (C.I.) and wavelength at maximum absorbance of dyes.

Class	Dye	Structure	C.I.	λ_{max} (nm)
Poly R-478	Anthraquinone		–	520
Methyl Orange	Azo		13,025	466
Lissamine Green	Diphenylnaphthyl-methane		44,090	633
Reactive Black 5	Di-azo		20,505	597

abyssal hot vents (“black smokers”), active seamounts, smouldering coal refuse piles and hot outflows from geothermal and nuclear power plants have been screened worldwide to find the right metabolite for every application [15–19]. However, the use of thermophilic strains for textile dye decolorization is yet to be deeply investigated. Willets et al. [20] was the first to report on this issue, and since then, few papers have been found in the literature. Recently, Boonyakamol et al. [21] have reported the benefits of using thermophiles instead of mesophiles to decolorize a model anthraquinone dye, and dos Santos et al. have widely reported the necessity of introducing redox mediators to achieve high decolorization efficiencies of several azo dyes by using anaerobic thermophiles [22–27]. However, as the anaerobic degradation of azo dyes usually produces aromatic amines, which are carcinogenic and mutagenic, the aerobic treatment is the only safe method for the biodegradation of textile azo dyes, and thermophilic aerobic treatments is yet to be studied [28].

There are some benefits of working at high temperatures, such as reduced cooling costs, increased solubility of most compounds (except gases), decreased viscosity and a lower risk of contamination. However, there are also disadvantages, such as higher equipment corrosion problems, liquid evaporation, and substrate decomposition. Therefore, one of the most challenging and less studied aspects of culturing extremophilic microorganisms is the scaling-up of the process. Nevertheless, there are a few papers in which large-scale operations using thermophilic organisms have been addressed [24–26,29–31].

In this work, the model di-azo dye Reactive Black 5, one of the most commonly used reactive dyes for textile finishing, was selected as the target compound representing a dye pollutant of industrial wastewaters. Up to our knowledge, there are almost no reports dealing with thermophilic aerobic decolorization processes of azo dyes, so several hot springs in the northwest of Spain were screened to find thermophilic aerobic bacteria and consortia that could efficiently decolorize an effluent containing the model dye in aerobic conditions. The process was then scaled up from shake flasks to bench-scale bioreactors.

2. Experimental

2.1. Dyes

Poly R-478, Methyl Orange, Lissamine Green B and Reactive Black 5 were purchased from Sigma. The structure and the main characteristics of these dyes are shown in Table 1.

2.2. Sampling for strain isolation

The samples containing mud and water were collected during October (wet season) 2008 in four different locations of the