BRAZILIAN JOURNAL OF MICROBIOLOGY xxx (2016) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

Degradation of textile dyes by cyanobacteria

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ARTICLE INFO

Article history:
Received 31 October 2015
Accepted 20 July 2016
Available online xxx
Associate Editor: Valeria Maia de Oliveira

Keywords:
Dyes
Toxicity
Discoloration
Cyanobacteria
Degradation

ABSTRACT

Dyes are recalcitrant compounds that resist conventional biological treatments. The degradation of three textile dyes (Indigo, RBBR and Sulphur Black), and the dye-containing liquid effluent and solid waste from the Municipal Treatment Station, Americana, São Paulo, Brazil, by the cyanobacteria *Anabaena flos-aquae* UTCC64, *Phormidium autumnale* UTEX1580 and *Synechococcus* sp. PCC7942 was evaluated. The dye degradation efficiency of the cyanobacteria was compared with anaerobic and anaerobic–aerobic systems in terms of discolouration and toxicity evaluations. The discoloration was evaluated by absorption spectroscopy. Toxicity was measured using the organisms *Hydra attenuata*, the alga *Selenastrum capricornutum* and lettuce seeds. The three cyanobacteria showed the potential to remediate textile effluent by removing the colour and reducing the toxicity. However, the growth of cyanobacteria on sludge was slow and discoloration was not efficient. The cyanobacteria *P. autumnale* UTEX1580 was the only strain that completely degraded the indigo dye. An evaluation of the mutagenicity potential was performed by use of the micronucleus assay using *Allium* sp. No mutagenicity was observed after the treatment. Two metabolites were produced during the degradation, anthranilic acid and isatin, but toxicity did not increase after the treatment. The cyanobacteria showed the ability to degrade the dyes present in a textile effluent; therefore, they can be used in a tertiary treatment of effluents with recalcitrant compounds.

Introduction

Synthetic dyes are recalcitrant molecules that constitute the main residue found in the effluent of the textile dyeing industry. Acute toxicity tests showed that most textile dyes are not particularly toxic.[1] Nevertheless, their persistence and resulting long exposure time is of particular concern for the discharge of waste dye effluent, since these substances may exhibit chronic effects such as mutagenic damage and carcinogenicity towards biota.[2,3] Several authors reported that dye discharges from textile processing plants were the major

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http://dx.doi.org/10.1016/j.bjm.2016.09.012

Please cite this article in press as: Dellamatrice PM, et al. Degradation of textile dyes by cyanobacteria. *Braz J Microbiol*. (2016), http://dx.doi.org/10.1016/j.bjm.2016.09.012

contributors to the mutagenic activity found in Brazilian rivers.[4,5]

In some textile dyeing operations, as much as 15% of the dyes used do not attach to the fibres, so they are lost to wastewater,[6] and the resulting coloured effluents can represent a serious water pollution problem due to their colour content and toxic components. The usual effluent treatment involves biological systems like activated sludge; however, conventional treatment has not efficiently removed the effluent dye due to the recalcitrant nature of the dyes and the diverse composition of the effluent.[7] A tertiary treatment of effluents is recommended for the removal of nutrients and recalcitrant substances, which is done mainly by several algae species. Studies have shown the ability of some algae to degrade dyes,[8–11] making tertiary treatment a viable possibility for the efficient degradation of these compounds. Vyayakuma and Manoharau[12] studied the degradation by indigenous cyanobacterium species of a textile effluent containing the dyes remazol and venyl sulfone and observed not only colour removal, but also reduction in the levels of the inorganic compounds such as nitrites, phosphates, ammonia, calcium and magnesium. The main cyanobacterium strains reported to be responsible for the removal of nutrients and chemicals from the industrial effluents were *Westiellopsis* sp., *Lynghya* sp., *Oscillatoria* sp. and *Chlorella* sp.[12–15]

The environmental problem caused by hazardous dyes is particularly important in the Municipal Treatment Station of Americana, SP, Brazil, which is responsible for the treatment of effluents from 43 textile plants as well as city sewage. Periodic releases of liquid effluent from this treatment station were carried to the Piracicaba River, a domestic water source for cities downstream. The biological treatment employed is efficient for BOD removal, but the colour persists after the treatment, making this treatment insufficient to remove the contaminants from the effluent. In the present study, three strains of cyanobacteria, *Anabaena flos-aquae* UTCC64, *Phormidium autumnale* UTEX1580 and *Synechococcus* sp. PCC7942 were evaluated for their ability to degrade three different dyes: RBBR (Remazol Brilliant Blue R), indigo and sulphur black, and also the dye-containing liquid effluent and solid waste from the Municipal Treatment Station of Americana (SP, Brazil) containing those dyes.

Materials and methods

Cyanobacterial strains

The cyanobacteria were filamentous heterocystous *A. flos-aquae* UTCC64, the filamentous non-heterocystous *P. autumnale* UTEX1580, and unicellular *Synechococcus* sp. PCC7942, all from the Culture Collection of the Centro de Energia Nuclear na Agricultura – CENA/USP. These strains were chosen based on a study by Fiore and Trevors[16] on the bioremediation of metals by the cyanobacterium, in which the three selected strains had the highest detoxification capability. The organisms were maintained in flasks containing 50 mL of AA medium[17] for *A. flos-aquae* UTCC64, or BG-11 medium[18] for *P. autumnale* UTEX1580 and *Synechococcus* PCC7942; 1 mL from these seven day cultures was used to inoculate the different treatments.

Textile waste sampling

Liquid effluent was sampled (3 L) after biological treatment (biological filters) and the sludge also was sampled (3 kg) after aerobic-anaerobic treatment in the Municipal Treatment Station of Americana, SP, Brazil. Samples were kept at 4 °C until use. Both of these residues still contained a high amount of organics after the treatment, and remained strongly-coloured.

Dye discoloration by cyanobacteria

The synthetic dyes RBBR [(Remazol Brilliant Blue R) purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA)], indigo and sulphur black (Bayer, São Paulo, SP, Brazil), were added at a concentration of 0.02% (m/v) to Erlenmeyer flasks containing 50 mL of AA or BG-11 culture media. Each flask was inoculated with 1 mL of the cyanobacterium culture medium, in triplicate. The effluent and the solid waste, which were diluted to a concentration of 10% in an AA or a BG-11 media, were treated in the same way as the dyes. All of the treated flasks were incubated for 14 days at 25 °C with constant fluorescent illumination (4000 ± 10% lux). Control experiments were conducted in light and dark conditions in the absence of microorganisms to evaluate the effect of photodegradation. The discoloration was evaluated by absorption spectroscopy. The absorption maximum for each dye was 680 nm (indigo), 595 nm (RBBR) and 454 nm (sulphur black). The samples were centrifuged at 10,000 × *g* for five min and the colour reduction was based on a standard curve comparing dye concentration in the treatments in relation to non-inoculated control flasks.

An anaerobic–aerobic degradation system was also evaluated for dye discoloration in the liquid wastewater. The anaerobic conditions were obtained by completely filling the triplicate 150 mL penicillin bottles with the effluent, and sealing them with rubber tops in a CO_2 atmosphere. The bottles were kept for 15 days at 28 °C in the dark. Then, the bottles were opened and 50 mL of effluent was transferred to sterilized Erlenmeyer flasks and re-enriched with 1 mL of inoculum obtained from a fresh effluent. This inoculum was prepared through centrifuging (2000 × *g*) 100 mL of fresh effluent and resuspending the pellets in salt solution (0.85% NaCl). The flasks were incubated aerobically for 15 days at 28 °C.

Toxicity evaluation of the treated effluent and sludge

The toxicity of the effluent and sludge was evaluated using the bioindicator organisms *Hydra attenuata*, the green algae *Selenastrum capricornutum* and root growth of *Lactuca sativa* seedlings. These organisms are maintained at CENA/USP and bioassays were conducted as described by Trottier et al.,[19] Blaise et al.,[20] and Dutka,[21] respectively. The 50% inhibitory concentrations (IC_{50}) were determined for each sample using the program EcoTox-Statistics Version 1.1.

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