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Biodegradation and detoxification potential of rotating biological contactor (RBC) with *Irpex lacteus* for remediation of dye-containing wastewater

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ARTICLE INFO

Article history:
Received 23 March 2013
Received in revised form
26 June 2013
Accepted 5 July 2013
Available online 20 October 2013

Keywords:
Liquid textile wastes
Dye decolorization
Genetic toxicity
Biological toxicity
Rotating biological contactor
Irpex lacteus

ABSTRACT

Use of fungal organisms in rotating biological contactors (RBC) for bioremediation of liquid industrial wastes has so far been limited in spite of their significant biodegradation potential. The purpose was to investigate the power of RBC using *Irpex lacteus* for decolorization and detoxification of industrial dyes and dyeing textile liquors. Recalcitrant dye Methylene Blue (150 mg L^{-1}) was decolorized within 70 days, its mutagenicity removed, and the biological toxicity decreased more than 10-fold. *I. lacteus* biofilm in the RBC completely decolorized within 26 and 47 days dyeing liquors containing disperse or reactive dyes adjusted to pH4.5 and 5-fold diluted with the growth medium, respectively. Their respective biological toxicity values were reduced 10- to 10^4-fold in dependence of the test used. A battery of toxicity tests comprising *Vibrio fisheri*, *Lemna minor* and *Sinapis alba* was efficient to monitor the toxicity of textile dyes and wastewaters. Strong decolorization and detoxification power of RBC using *I. lacteus* biofilms was demonstrated.

1. Introduction

Textile industry produces large volumes of dye-containing effluents that are ineffectively remediated in wastewater treatment plants and are responsible for coloration of streams that negatively affects water life. Biological and genetic toxicity of dyes for bacteria, protozoa, aquatic animals, plants and mammals has been widely documented (Gottlieb et al., 2003; Soni et al., 2006; etc.). Textile wastewaters are extremely variable in composition due to the presence of various dyes, desizing and scouring agents, detergents, finishing agents and inorganic salts that all can contribute to their toxicity (Dubrow et al., 1996). Consequently, efficient remediation must result in both decolorization and detoxification of the wastewater.

Decolorization of dyes with ligninolytic fungi has been proven to be an efficient, cheap and environment-friendly process but their detoxification power has been studied less frequently (e.g. Knapp et al., 2008). A number of chemically-different types of persistent dyes have been shown to be

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0043-1354/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2013.07.050

effectively degraded by various fungal organisms (e.g. Singh, 2006). Methylene Blue (CAS No. 61-73-4, C.I. 52015, MB), a heterocyclic phenothiazine dye, is widely used for dyeing leather and textile materials. Its decolorization strongly depends on the conditions of fungal culture and the efficiency of various ligninolytic fungi is quite variable (Tychanowicz et al., 2004; etc.). Partial to weak decolorizations were reported by various ligninolytic fungi in liquid- and solid-state cultures, by bacteria and the aerobic activated sludge (e.g. Ma et al., 2011). Anaerobic sludge is able to remove the dye but due to re-oxidation by air only a low color removal effect was achieved (Ong et al., 2005).

Broader adverse effects of MB include eye injury, breathing problems, methemoglobinemia, bacteriostatic and fungicidal activities, and a significant toxicity to aquatic plants, crustaceans and fish (Wainwright et al., 1999 etc.). Because of the persistence and toxicity, MB was chosen in this study as a model industrial dye for testing the efficiency of RBC reactor using *Irpex lacteus* to decolorize and detoxify dyes and textile effluents. RBC reactors operable in repeated-batch or continuous mode offer advantages in bioremediation of industrial wastewaters due to great surface per unit volume, low power requirement and limited flow clogging (Anderson, 1983). Ligninolytic fungi behave well in RBC but so far few studies have been conducted, comprising only a limited number of fungal strains (e.g. Guimaraes et al., 2005; Axelsson et al., 2006). Biodegradation and detoxification power of *I. lacteus* in this type of reactor has not been thoroughly investigated.

Ecotoxicity of pollutants is usually measured with standard toxicity tests, i.e. bacterial, crustacean, algal and seed germination tests. For instance, *Vibrio fischeri* bioluminiscence test was used to measure both a decrease and increase of toxicity resulting from degradation of various textile dyes by *Trametes versicolor* (Ramsay and Nguyen, 2002) or formation of toxic products in the course of anaerobic decolorization of Reactive Black 5 by *Enterococcus faecalis* and *Clostridium butyricum* (Gottlieb et al., 2003). Tests with *Daphnia* spp. were used to monitor decolorization-linked detoxification of phthalocyanine- and azo dyes obtained with *Penicillium simplicissimum* or of a raw textile effluent treated with horseradish peroxidase (Bergsten-Torralba et al., 2009). A reduction of mutagenicity of Reactive Orange 16 and Disperse Blue 3 dyes in a two-step treatment with activated sludge and a static culture of *I. lacteus* was monitored by the Ames test (Malachová et al., 2006).

Our study was undertaken to investigate the dye decolorization and detoxification capacity of *I. lacteus* under the conditions of RBC reactor using MB and two different textile dyeing liquors containing mixtures of reactive or disperse dyes to test the decolorization and detoxification efficiency. Biological toxicity changes during the treatment were measured with a battery of standard bacterial and plant tests and the change of genetic toxicity with Ames test.

2. Material and methods

2.1. Chemicals

The dyeing liquors were obtained from INOTEX a.s., Czech Republic. Wastewater I contained Sumifix Black B 150% (C.I. Reactive Black 5) (9.82 g L^{-1}), Inosin Yellow V-GR 160% (C.I. Reactive Yellow 15) (2.47 g L^{-1}), NaCl (75 g L^{-1}) and the fixation agent Texalkon MS (7.87 g L^{-1}). Wastewater II contained Itosperse Yellow RAP dye mix (5.47 g L^{-1}), Itosperse Red RAP dye mix (3.75 g L^{-1}), Itosperse Blue RAP dye mix (2.47 g L^{-1}), the disperging agent Nicca Sunsolt™ RF-557 (1 g L^{-1}) and acetic acid (0.3 ml L^{-1}).

Malt extract and agar were purchased from Oxoid, UK, Disperse Blue 3 (DB3, anthraquinone) and Methylene Blue (MB, phenothiazine) dyes from Sigma–Aldrich, Czech Republic. Other chemicals were of analytical grade.

2.2. Microorganism

Irpex lacteus 931 was provided by the Culture Collection of Basidiomycetes, Institute of Microbiology ASCR, Prague and maintained on malt extract-glucose (MEG) medium containing 2% (w/w) agar at 4 °C.

2.3. Biodegradation in RBC reactor

The rotating biological contactor (RBC) reactor consisted of a glass vessel and a horizontal driving axis with six 1-cm thick polyurethane foam (PUF) discs (diameter 8 cm, rotation speed 2 rpm, 40% of disc volume immersed). The experiments were carried out aseptically in MEG medium (per litre: 5 g malt extract, 10 g glucose, pH 4.5) at 22 °C and forced aeration with air (50 L h^{-1}).

Sterile PUF discs were put horizontally in MEG and inoculated with a homogenate (Ultra-Turrax T25 mixer, IKA Werk, Germany, 20 s) of a 7-d-old, static MEG culture grown at 28 °C (10% v/v inoculum). The discs were colonized with the fungus (7 d, 28 °C) and then mounted aseptically in the reactor containing one litre of MEG medium with DB3 or MB dyes dissolved at a concentration of 150 mg L^{-1} representing the respective dye concentrations of 0.56 and 0.47 mM. Their decolorization was measured spectrophotometrically at 645 nm and 505/580 nm, respectively. Wastewaters I and II were adjusted to pH 4.5 and used 5-fold diluted with MEG. Their decolorization was measured at respective maxima of 575 and 425 nm. The fungal biomass on the discs was estimated gravimetrically at the end of the experiment as dry biomass.

2.4. Biological toxicity tests

The acute biological toxicity was estimated using bacterial luminiscence, aquatic plant growth and seed germination as the endpoints. *V. fischeri* test (ISO 11348-3, 2007) measured bioluminiscence inhibition after a 30-min exposition using a LUMIStox300 luminometer (Hach-Lange, Düsseldorf, Germany). *Lemna minor* test (ISO CD, 20079, 2005) determined growth inhibition of fronds, the exposition time was 7 days. The Phytotoxkit *Sinapis alba* test (ISO 11269-1, 1993) determined the inhibition of root growth after a 3-d exposure. The test was considered to be valid if the germination of the control was ≥90%. The stimulation effect of endproducts was evaluated by using a linear model.

A positive toxic effect was evaluated in the tests against negative controls containing only the culture medium. Positive controls using toxicants recommended in the corresponding ISO standard were also measured to check the sensitivity of the