Ecotoxicology and Environmental Safety 150 (2018) 232–239

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Efficient degradation of Azo dyes by a newly isolated fungus *Trichoderma tomentosum* under non-sterile conditions

Xiao-ling He[a,*], Chao Song[a], Yuan-yuan Li[a], Ning Wang[a], Lei Xu[a], Xin Han[b], Dong-sheng Wei[c,*]

[a] a State Key Laboratory of Separation Membranes and Membrane Processes, Tianjin Engineering Center for Safety Evaluation of Water Quality & Safeguards Technology, School of Environment and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300387, China
[b] Tianjin Rongtai Water Corporation, Tianjin 300000, China
[c] Key Laboratory of molecular microbiology and technology, Ministry of education, College of Life Sciences, Nankai University, Tianjin 300071,China

ARTICLE INFO

Keywords:
Acid Red 3R
Trichoderma tomentosum
Response surface methodology
Biodegradation
Phytotoxicity

ABSTRACT

A fast-growing fungus with remarkable ability to degrade several azo dyes under non-sterile conditions was isolated and identified. This fungus was identified as *Trichoderma tomentosum*. Textile effluent of ten-fold dilution could be decolorized by 94.9% within 72 h before optimization. Acid Red 3 R model wastewater with a concentration of 85.5 mg L^{-1} could be decolorized by 99.2% within the same time after optimization. High-level of manganese peroxidase and low-level of lignin peroxidase activities were detected during the process of decolorization from the culture supernatant, indicating the possible involvement of two enzymes in azo dye decolorization. No aromatic amine products were detected from the degradation products of Acid Red 3 R by gas chromatography–mass spectrometry (GC/MS) analysis, indicating the possible involvement of a special symmetrical oxidative degradation pathway. Phytotoxicity assay confirmed the lower toxicity toward the test plant seeds of the degradation products when compared to the original dye.

1. Introduction

Azo dyes have been extensively used in textile, food, plastic, printing, leather, cosmetics and pharmaceutical industries (Prasad and Rao, 2013). About 10–15% of these dyes are not attached to the substrates and discharged into environment as wastewater (Asad et al., 2007). Azo dyes contain at least one azo group (–N=N–) linked to benzene or naphthalene rings (Mnif et al., 2016). Due to the complex structure and synthetic origin, azo dyes are difficult to be degraded by simple exposure to light and water. The persistence of color in natural receiving water affects light penetration and reduces photosynthesis (Robinson et al., 2001; Singh et al., 2015a, 2015b). The toxic, mutagenic and carcinogenic nature of azo dyes is also harmful to higher living organisms (Ozer et al., 2005; Chen et al., 2009; Tan et al., 2009). Therefore, degradation of azo dyes into colorless and harmless products are an urgent need for azo dye-containing wastewater treatment sectors in the above industries.

Methods for treatment of azo dye-containing wastewater are broadly divided into three categories: physical, chemical and biological (Kaushik and Malik, 2009; Bafana et al., 2011). Biological method has received special attention owing to its high efficiency, versatility, eco-friendliness and low costs (dos Santos et al., 2009). However, degradation of azo dyes by bacteria often leads to the formation and accumulation of more recalcitrant and toxic aromatic amine substances, which restricts their wide use in azo dye-containing wastewater treatment plant (Davies et al., 2006).

Fungi, especially those could secrete non-specific oxidases and mineralize azo dyes into carbon dioxide, have been investigated (Singh et al., 2015a, 2015b). Degradation by these fungi often leads to complete detoxification and avoids the problems of sludge disposal and secondary pollution. These investigations are mainly focused on white rot fungi (WRF) (Chagas and Durrant, 2001; Selvam et al., 2003; Kasinath, 2004; Zhao and Hardin, 2007; Yang et al., 2009). Although WRF can degrade azo dyes with high efficiency, there are still some difficulties to be overcome before their practical application in wastewater treatment. WRF often grows slowly and it will take a long time for them to degrade azo dyes. They are also liable to be contaminated by bacteria and other fungi in wastewater treatment, leading to instability of the system (Hai et al., 2009). Moreover, fermentation conditions for WRFs are also harsh. Because expression of laccase and perxoidases, which have been confirmed to be responsible for the degradation of azo dyes (Falade et al., 2017) usually occurs under low carbon or nitrogen conditions (Janusz et al., 2013). It is difficult to meet these conditions due to the complex compositions in actual wastewater

* Corresponding authors.
E-mail addresses: hexiaoling301@163.com (X.-l. He), weidongsheng@nankai.edu.cn (D.-s. Wei).

https://doi.org/10.1016/j.ecoenv.2017.12.043
Received 21 July 2017; Received in revised form 17 December 2017; Accepted 19 December 2017
Available online 04 January 2018
0147-6513/

(Levin et al., 2010). Therefore, it is necessary to screen other fungi with strong adaptability and high efficiency in degrading azo dyes in wastewater.

Ascomycetes typically grow faster than WRFs and have been selected for the decolorization of several azo dyes (Tan et al., 2016). Several yeast genuses, such as *Pichia, Candida, Magnusiomyces and Scheffersomyces*, have been confirmed to possess ability to decolorize different azo dyes (Saratale et al., 2011; Tan et al., 2016). Although some yeast species grow faster and possess higher adaptability to harsh environment than WRF, decolorization mechanisms by yeast are often regarded as biosorption rather than biodegradation (Sen et al., 2016). Filamentous fungi other than white rot basidiomycetes have also been found to be able to degrade several azo dyes. *Penicillium oxalicum* strain SAR-3 has been shown to degrade Acid Red 183, Direct Blue15 and Direct Red 75 (Saroj et al., 2014). A fast-growing *T. atroviride* F03 could degrade bisazo dye, Reactive Black 5 (RB5) (Adnan et al., 2015). *P. simplicissimum* has been observed to degrade triphenylmethane (Chen and Ting, 2015). However, the performances of these fungi in degrading azo dyes in azo dye-containing wastewater under non-sterile conditions have rarely been reported, which is a crucial factor before their practical application in azo dye-containing wastewater treatment plants.

In this study, we screened a fast-growing fungus that could efficiently degrade several azo dyes and real textile effluent under non-sterile conditions. As far as we know, there are very few reports about degradation of azo dyes and decolorization of textile wastewater by ascomycetes under non-sterile conditions.

2. Material and methods

2.1. Chemicals

Azo dyes were obtained from Tianjin Polytechnic University (Tianjin, China) and their characteristic wavelengths of maximal absorbance and structures were provided in Supplemental Table S1. Textile wastewater without any pretreatments was provided by Tianjin Rongtai Water Corporation (Tianjin, China) with the following physicochemical characteristics : Chemical oxygen demand 3000 mg L^{-1}; pH 4; BOD/COD 0.05; Chroma 3550. 2, 2-Azinobis (3-ethylbenzothiazolin-6-sulfonic acid)(ABTS) and 3, 4-dimethoxybenzyl alcohols were obtained from Sigma Chemical Company (St. Louis, MO, USA). Other chemicals with analytical grade were purchased from Tianjin Guangfu Technology Development Co., Ltd (Tianjin, China).

2.2. Fungal source and culture media

Degraded wood wafers used for screening fungi were collected from forest in Liupanshui City (Guizhou Province, China). All fungi were maintained on Potato Dextrose Agar (PDA) plates. Mycelia pellets used for degradation of Azo dyes were prepared in liquid medium (LM) containing 1% glucose, 0.5% peptone, 0.1% KH_2PO_4, and 0.05% $MgSO_4{\cdot}7H_2O$. Another minimal liquid medium (MLM) containing 1% glucose, 0.4% $(NH_4)_2SO_4$, and 0.2% KH_2PO_4 were used for the dye degradation with or without sterilization.

2.3. Isolation of dye-degrading fungi

About 5 g wood wafers were washed with 100 mL sterilized distilled water by stirring for 30 min at room temperature and the suspension was used for subsequent screening. 5 mL original textile wastewater was mixed with 45 mL distilled water and pH of the diluted suspension was adjusted to 6.0 with NaOH (4 M). After 1 g agar was added, the mixture was sterilized by autoclave at 105 °C for 20 min and poured into sterile Petri dishes to obtain wastewater-containing plates. After solidification, 100 μL of the above suspension was spread on the surface of plates and incubated at room temperature. Plate without spreading suspension was used as a control. After incubation at room temperature for 72 h, fungal colonies that could decolorize dyes in wastewater-containing plates were purified with single spore isolation method for further investigations (Choi et al., 1999).

2.4. Decolorization of several azo dyes and real textile effluent by one of the selected fungus under non-sterile conditions

About 100 mg (wet weight) mycelia pellets made from the purified colonies were inoculated into three kinds of azo dye solutions (Direct Fast Scarlet 4BS, Direct Turquoise Blue 5B and Acid Red 3 R) and real textile effluent with a volume of 100 mL. Azo dyes were dissolved in MLM medium with concentration of 100 mg L^{-1} and the effluent was 10-fold diluted with MLM medium without sterilization. The treatment was performed by incubating the solutions at room temperature with shaking speed of 150 rpm for 72 h.

2.5. Molecular identification of the screened fungus

For molecular identification of the selected fungus, genomic DNA was extracted with glass bead protocol (Griffin et al., 2002). Amplification of Internal Transcribed Spacer (ITS) fragments and identification was done according to the method described (Saroj et al., 2014).

2.6. Confirming the ability of the selected fungus to decolorize the real effluent under non-sterile conditions

Spores of the selected fungus were inoculated into sterilized LM medium and incubated under shaking at 150 rpm for 72 h to form mycelia pellets. Mycelia pellets were recovered by vacuum filtration and rinsed with sterile distilled water twice. After that, about 100 mg (wet weight) mycelia pellets were inoculated into one flask containing 10 mL wastewater and 90 mL MLM medium. Meanwhile, two other flasks (without mycelia) containing 10 mL wastewater plus 90 mL non-sterile water or 90 mL non-sterile MLM medium seperately were used as controls. The characteristic wavelengths of the maximal absorbance of the effluent was analyzed with UV–Vis spectrophotometer (Mapada UV-1100, Shanghai, China) by scanning from 200 to 900 nm and determined to be 632 nm. After incubation at room temperature with shaking speed of 150 rpm for 72 h, the changes of the characteristic wavelengths of the maximal absorbance were recorded. Decolorization rate of the real effluent was calculated as the following Eq. (1):

$$Decolorization\% = (Initial\ \ absorbance - Final\ \ absorbance) / Initial\ \ absorbance \times 100\%. \quad (1)$$

2.7. Optimization of decolorization conditions by RSM

The decolorization conditions were optimized with model wastewater containing Acid Red 3 R. Experiments were designed by using Design Expert 8.0 Trial version. Decolorization rate was set as the response value (Y). The initial pH value (X_1), the mycelial biomass concentration (X_2) and the concentration of dye (X_3) were set as the main factors (independent variables), as primary tests showed that these parameters dramatically affected the decolorization rate. All the experiments were performed with shaking speed of 150 rpm at 26 °C for 72 h. Initial pH of the media was all adjusted with NaOH (4 M) or HCl (6 M). The ranges and levels of the variables were given in Table S2.

A three-factor, five-level Central Composition Design (CCD) at the center points leading to 20 runs was employed to optimize the dye decolorization rate. Supplemental Table S3 shows the experimental design points and the test results for the response variables.