Journal of Chromatography A, 1208 (2008) 223–231

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of the degradation products of selected sulfonated phenylazonaphthol dyes treated by white rot fungus *Pleurotus ostreatus* by capillary electrophoresis coupled with electrospray ionization ion trap mass spectrometry

Yiping Lu [a,*], Dennis R. Phillips [b], Lude Lu [c], Ian R. Hardin [a]

[a] *Department of Textiles, Merchandising, & Interiors, University of Georgia, Athens, GA 30602-3622, USA*
[b] *Chemical and Biological Sciences Mass Spectrometry Facility, Department of Chemistry, University of Georgia, Athens, GA 30602, USA*
[c] *College of Chemical Engineering, Nanjing University of Science & Technology, Nanjing, Jiangsu 210094, China*

ARTICLE INFO

Article history:
Received 15 July 2007
Received in revised form 13 August 2008
Accepted 15 August 2008
Available online 28 August 2008

Keywords:
Sulphonated phenylazonaphthol dye
White rot fungus *Pleurotus ostreatus*
Degradation
Capillary electrophoresis (CE)
Electrospray ionization mass spectrometry (ESI)

ABSTRACT

The removal of water-soluble sulphonated phenylazonaphthol dye effluents generated by textile industries is an important issue in wastewater treatment. Microbial treatment of environmental pollutants including dyes, with white rot fungi has received wide attention as a potential alternative for conventional methods in wastewater treatment. Three sulphonated phenylazonaphthol dyes with similar molecular structures Acid Orange 7, Acid Orange 8 and Mordant Violet 5 were selected and degraded by the white rot fungus *Pleurotus ostreatus*. Chemical instrumental analysis methods such as high-performance liquid chromatography (HPLC) and capillary electrophoresis combined with electrospray ionization mass spectrometry (CE-ESI-MS) were used to identify the degraded dyes. Mordant Violet 5 had two degradation pathways when degraded by *P. ostreatus*. The first degradation pathway for Mordant Violet 5 was for *trans* structure and the *cis*-Mordant Violet 5 followed the second pathway. Acid Orange 8 and Acid Orange 7 had the same degradation mechanism as the first degradation mechanism for Mordant Violet 5, that is cleavage of azo bond at the naphthalene ring side where benzenesulfonic acid and 1,2-naphthoquinone are formed.

1. Introduction

Textile industries consume large volumes of water and chemicals for wet processing of textiles. This wastewater has a very diverse chemical composition, ranging from inorganic compounds to polymers and organic compounds [1–3]. Among industrial wastewaters, dye wastewater is one of the most difficult to treat. Color in wastewater is highly visible and undesirable, even if at a very low dye concentrations.

Their synthetic origin and complex aromatic molecular structures make dyes stable and difficult to be biodegraded. Due to the low biodegradability of dyes, conventional biological wastewater treatment systems are inefficient in treating dye wastewater. Dye wastewater is usually treated by physical or chemical treatment processes. Physical and chemical methods of dye removal are effective only if the effluent volume is small. This limits the use of physio-chemical methods, such as membrane filtration and cucurbituril, to small-scale *in situ* removal. A limiting factor of these methods is cost. This is true even in laboratory-scale studies; therefore they are unable to be used by large-scale industry. The microbial treatment of environmental pollutants, including dyes, by the use of white rot fungi has received wide attention as a potential alternative to conventional methods in wastewater treatment. White rot fungi are able to decompose lignin extensively and simultaneously degrade all major components of wood to CO_2 and water. This property is based on the capacity of white rot fungi to produce one or more extracellular lignin-modifying enzymes, which are able to degrade a wide range of xenobiotic compounds. The decolorization efficiency ranged from 70% to 90% when investigating nine dyes with different structure degraded by white rot fungi *Pleurotus ostreatus* [4]. White rot fungi *P. ostreatus* could decolorize Orange II more than 90% for the concentration of dyes as high as 2×10^{-4} g/L, which gave a highly visible color to the solution [5]. Generally, decolorization by white rot fungi is a promising alternative to replace or supplement present treatment processes [6].

* Corresponding author. Tel.: +1 704 530 8663; fax: +1 704 735 2641.
E-mail addresses: yiping73@gmail.com, yipinglu@uga.edu (Y. Lu).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.08.080

Among the many structural varieties of dyes these, azo dyes constitute a major class of environmental pollutants accounting for 60–70% of all dyes and pigments used. These compounds are characterized by aromatic moieties linked together with azo groups (–N=N–). The release of azo dyes into the environment is a concern due to the coloration of natural waters, and the toxicity, mutagenicity and carcinogenicity of these dyes and their biotransformation products. Therefore, considerable attention has been given to evaluating the fate of azo dyes during wastewater treatment and in the natural environment [7].

Sulphonated phenylazonaphthol dyes are sodium salts of organic sulphonic acids. They consist of an aromatic structure containing a chromogen and a solubilizing group(s), almost always a sulphonic acid salt. Sulphonated phenylazonaphthol dyes are soluble ionic compounds, where the color is contained in the anionic portion. Acid Orange 7 and Acid Orange 8 are the most widely used acid dyes in the world, so they were chosen in this study. In addition, the structure of Mordant Violet 5 is very similar with Acid Orange 7 and Acid Orange 8, it also was selected to investigated in this paper.

Due to the different properties of azo dyes and their metabolites, a variety of spectroscopic methods, including spectrophotometry, chromatography, mass spectrometry, and capillary electrophoresis, are employed for structural identification of dyes and their breakdown fragments [4,8,9]. Recently, the technical maturation of soft ionization techniques (e.g. ESI) has made the analysis of nonvolatile ionic dyes possible. The sulphonic groups are strongly acidic and are therefore completely dissociated in the aqueous solution. Hence, negative ion MS is much more sensitive than positive ion MS. Negative ion ESI mass spectra have been published for some chemical compounds which contain multi-sulphonic groups, indicating that the negative ion (ESI) is probably the best method to identify nonvolatile sulphonated dyes.

High-performance liquid chromatography (HPLC) has been used for analysis of various dyes in wastewater and metabolites from various degradation procedures [4,8]. HPLC is a powerful analytical tool that can provide reproducible and precise analytical results. Capillary electrophoresis (CE) has been used for analysis of dyes in recent decades and can be more suitable than HPLC for the analysis of charged dyes because of its separation principle, higher separation efficiency and simple method development [9].

The objectives of this study are: to use analytical methods such as CE–MS, HPLC, and UV–vis to separate and identify the degradation products. To establish a possible mechanism of the dye degradation pathway for the dye molecular model by *P. ostreatus*.

2. Experimental

2.1. Chemicals

Three sulphonated azo dyes were obtained from Sigma–Aldrich (St. Louis, MO, USA): (1) Mordant Violet 5; (2) Acid Orange 7; and (3) Acid Orange 8. Compounds used as standards, benzenesulfonic acid, 4-hydroxybenesulfonic acid, veratryl alcohol, veratraldehyde, 1,2-naphthoquinone, and other possible products were analytical reagents and obtained from Sigma–Aldrich. Unless otherwise specified, all chemicals were analytical grade.

Acetonitrile (Sigma–Aldrich) used in analysis and sample preparation was of HPLC grade. Ammonium acetate was obtained from Aldrich and ammonium hydroxide (28%) was purchased from J.T. Baker (Phillipsburg, NJ, USA). All the solutions and buffers were filtered with a 0.22 μm, 13 mm, polyvinylidene difluoride (PVDF) membrane (FisherBrand, Pittsburgh, PA, USA).

2.2. Microorganism and culture conditions

P. ostreatus (strain Florida) was used in this work. This fungus was originally obtained from the laboratory of Dr. Karl-Erik Eriksson at the University of Georgia. The white rot fungus was transferred into the agar plate and incubated at 30 °C for 5 days until the fungus colonized the entire plate. A whole piece of agar with fungus growing on it was cut and put into a flask containing 200 ml Kirk's medium [10]. The whole medium with agar and Kirk's medium was mixed with a blender (Fisher Scientific, Suwance, GA, USA). An aliquot of 5 ml of homogenous dispersion solution was inoculated into a flask with 120 ml of autoclaved Kirk's medium. The flask was covered with eight layers of cheesecloth to avoid dust falling in and placed in a water-bath shaker (New Brunswick Scientific, Edison, NJ, USA) at 30 °C and 200 rpm.

2.3. HPLC analysis

Three milliliters of supernatant were taken from the fungal culture by pre-autoclaved pipette each day for 7 days. The same amount of liquid medium containing 1×10^{-4} g/L dye solution was added after each sampling to keep a constant volume in the culture flask. Five replicate flasks with the same dye concentration were used for the study and results were reported as an average of the five samples. No significant variation of dye concentration was induced by photo-degradation and no degradation products were detected in control samples (that is, samples with no fungal culture included). The samples were filtered through a 0.22 μm, 13 mm, PVDF membrane filter prior to HPLC analysis.

Sample was analyzed using Hewlett-Packard 1100 series HPLC system (Hewlett-Packard, Waldbronn, Germany), consisting of a model G1311A quaternary pump, G1322A degasser, and a diode array detector (Model G1315A). HP ChemStation software (version 3.1) was used for data processing and reporting.

HPLC analysis was performed under ambient conditions using a RP-C18 guard column and a stainless steel ODS column with 5 μm packing (Phenomenex Ultracarb 150 × 4.6 mm I.D., Torrance, CA, USA). The mobile phase was acetonitrile/water (20/80, v/v). The injection volume was 100 μl each time and the flow rate was 1 ml/min. Identification of separated degradation compounds was confirmed by comparison of both the retention times and spectra with those of standard compounds.

2.4. Analysis of degradation products by CE–MS

The CE equipment used was Hewlett Packard^{3D} CE (Palo Alto, CA, USA). The capillaries for CE separation (75 μm I.D., 360 μm O.D., 80 cm length) were preconditioned with acetonitrile, 1 M NaOH, 0.1 M HCl, and water for 10 min each, and conditioned with running buffer for 20 min before the first run and for 3 min between runs. The running buffer was 0.1 M pH 9.0 ammonium acetate. Hydrodynamic injection was performed with a pressure of 5 kPa for 30 s. The voltage applied in separation was +30 kV. The mass spectrometer used was an Esquire 3000 plus ion trap equipped with an electrospray ionization (ESI) interface (Bruker Daltonics, Billerica, MA, USA), which was operated in negative ionization mode. CE and MS were connected through a commercial interface based on coaxial sheath flow. The interface was a triple-tubular configuration, with the separation capillary inside a liquid sheath tube, surrounded by a second coaxial tube supplying the nebulizing gas. The position of the CE capillary with ESI needle was adjusted during optimization. Mass spectrometric parameters used in the analysis were listed as follows: full scan data acquisition from m/z 50 to 400 with maximum accumulation time 50 ms; average 8; compound stability 100%; scan range normal; trap drive level 100%;