e-ISSN: 2319-9849
p-ISSN: 2322-82

Research and Reviews: Journal of Chemistry

Biodegradation of dyes by Basidiomycetes fungi using HPLC and UV –Visible Spectrophotometer

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Research Article

Received: 03/02/2015
Revised: 15/02/2015
Accepted: 20/03/2015

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Keywords: Azo Dyes, Laccase, Biodegradation, Decolorization

ABSTRACT

Basidiomycete's fungi are used for biodegradation of azo dyes, which is mainly employed in textile, food and pharmaceutical industry. In addition they have some medicinal uses like anti-tumor effect due to their polysaccharide; they can reduce cholesterol level as they produce Lovastatin. They are rich in microelements as well as minerals. Basidiomycete's fungi can decolorize and degrade Eriochrome black T and Methyl orange. Since these dyes has an ability to depolymerize and mineralize because it cannot degrade lignin with their ligninolytic and extracellular enzymes. The extent of degradation for these dyes by fungi can be evaluated with the help of UV-Visible Spectrophotometer and High Performance Liquid Chromatography. According to our UV Spectroscopic methods, biodegradation of dyes occur by the fungi used in the Kirks medium. Decolorization study showed that the methyl orange dye was removed by more than 50% in 3 days, Eriochrome Balck T (removed by more than 33%) and HPLC analysis determined several degradation products. These results suggest that this fungi body has potential in color removal from textile waste water containing various azo dyes.

Introduction

A huge variety of synthetic dyes are used in textile, food and Pharmaceutical industries. Azo dyes are the largest class of synthetic dyes with a wide variety of color and structure which are used extensively in many industries. Almost 10,000 various dyes and pigments are used commercially [1]. Due to the presence of sulfonic groups and nitrogen-nitrogen double bond, azo dyes are categorized under highly recalcitrant compound and it is very difficult to degrade these compounds. Since these dyes are highly colored compounds they cause evidential environment threat by reducing the transparency of water bodies. In textile industries, during dyeing process nearly 10-15% of used dyes are released in waste water which is toxic for environment and aquatic life [2]. Before disposing off the waste water of these industries into water body it is required to treat them. Moreover, precursors of some dyes or their products which are results of biotransformation are highly carcinogenic and toxic. Acute toxicity of textile dyes leads to skin irritation and skin sensitization. Many technologies such as physical and chemical are implemented for decolorization and degradation of dyes. Physical methods which are employed for treating wastewater containing dyes of textile industries produce huge amount of sludge and disposal of these sludge creates problem. On the contrary chemical methods are very expensive to carry out. As a result both of these methods are commercially disfigured [3-55].

Biodegradation is another procedure used for these technologies because they are eco-friendly, lead to thorough mineralization of organic and toxic pollutants at low cost and do not produce huge amount of sludge. Microorganisms such as bacteria or fungi are used for treating waste water of several industries [56-70].

Basidiomycetes fungi is an area of interest because they are lignin degrading fungi, synthesize lignin peroxidase, manganese peroxidase and laccase and thus are able to degrade broad range of recalcitrant, carcinogenic and toxic organic compounds including several dyes [71-100].

Materials and Methods

Chemicals

Methyl orange dyes were purchased from Rankem (Product code-M0301) and Eriochrome Black T from Qualigens Limited (Product no.-39952). These dyes were used as such. Methanol (Sigma Aldrich) used in HPLC analysis was of HPLC grade. 0.025M Phosphate buffer (1.7g of potassium hydrogen phosphate, 1.7g of anhydrous disodium hydrogen phosphate in 500 ml water) was of analytical grade. Purified water was also used for all determinations.

Microorganism

From spawns, Pink oyster mushroom was obtained at dried conditions. Strains were obtained from dried powder.

Culture Preparation

First Malt Agar plates were prepared using (malt extract 30g/L, Peptone 5g/L, Agar 15g/L) and placed in an incubator for 3 days. Strains obtained from above culture, inoculated into Kirk's Medium5 (D glucose 10g, KH2PO4 2 gm, MgSO4.7H2SO4 0.5gm, CaCl2.2H2O 0.1gm, L- Asparagine monohydrate 93 mg, NH4NO3 50mg, 6M KOH, Nitrogen supplements, Trace element solution 1ml,thiamine 100 µg, dialyzed poly acrylic acid 0.72gm/L of glass distilled water) maintaining pH 5.0 in the 250 ml Erlenmeyer flask. Culture formed now placed in shaking incubator at the rate of 200 rpm for 3 days. Dyes were added on fourth day in the culture simultaneously controls were also maintained in the same conditions without the addition of inoculum.

Spectrophotometric Analysis

Using distilled water as a blank, Aliquots of sample 5-6ml volume of clear dye solution were prepared and absorbance was analyzed using UV- Visible Spectrophotometer (HITACHI, Model no-U2800). Decolorization can be determined within absorbance of wavelength 200-800 nm and by the reduction in area of peak for each dye.

HPLC Analysis

HPLC (WATERS, Model no.1525) having column CAT 4.6 × 150 millimeter operated at 254nm were employed for the analysis. 0.025M Phosphate buffer and Methanol (HPLC grade) were used as mobile phase. Injection volume of 20µl, run at flow rate of 1 ml/min maintaining 37ºC for column separation.

Results and Discussion

UV-Visible Spectrophotometer analysis

Since the absorbance of UV-Visible regions comes in between 200-800 nm were examined by UV-Visible Spectrophotometer for the decolorization of azo dyes. A clear decolorization was observed for both the dyes. For methyl orange most color removal was observed in the first day due to absorption of dye by fungi's mycelium. But for Eriochrome Black T the most decolorization was observed on the third day (Figure 1 a &1b and Figure 2a & 2b)

Methyl orange contain two substituted aromatic ring with an azo group containing sulfonyl group with sodium salts and dimethyl amino group whereas Eriochrome Black T contains 4 substituted aromatic ring ,two hydroxyl group, one sulfonyl group, one nitro group and one azo group. Both dyes show maximum absorbance in between 400-500 nm (Table 1 and Table 2).

e-ISSN: 2319-9849
p-ISSN: 2322-82

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Following graphs obtained were given below.

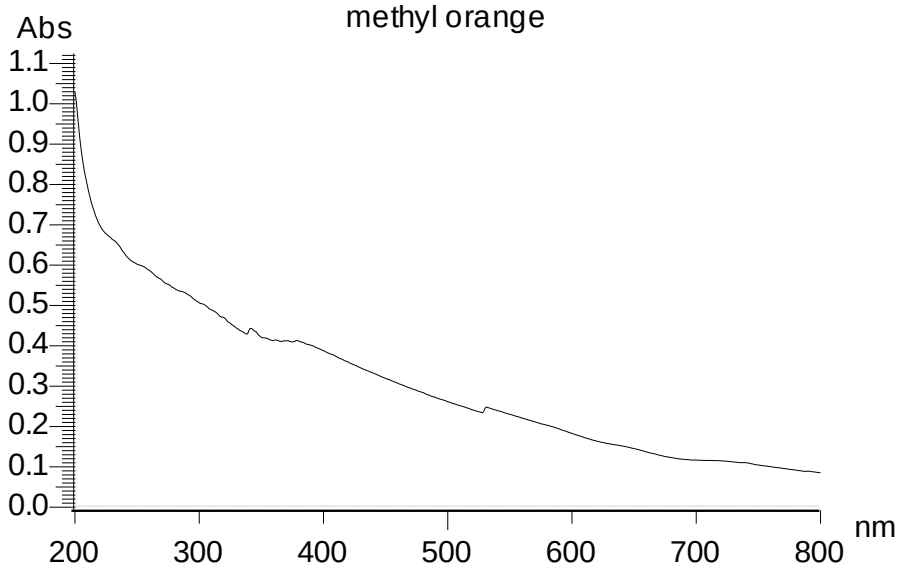

Figure 1(a): UV-Visible spectra of methyl orange with sample.

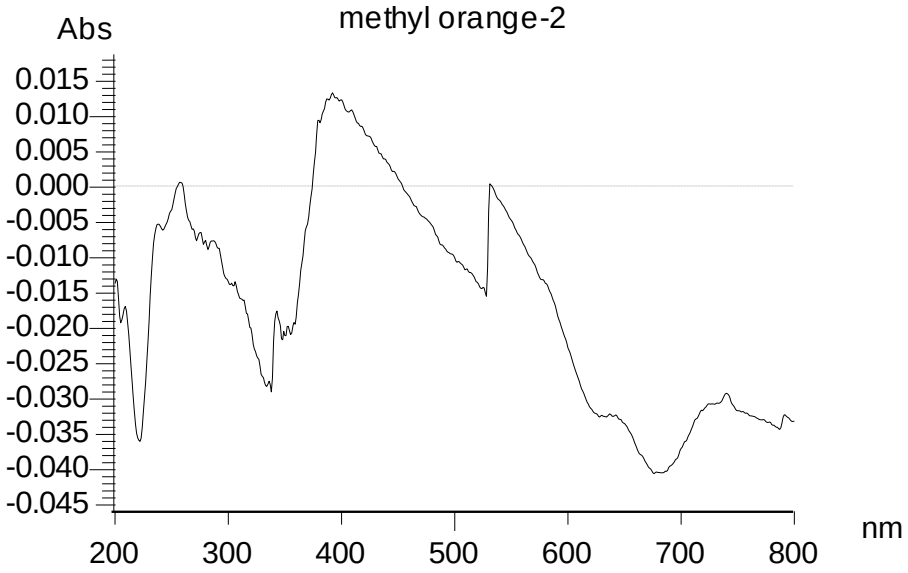

Figure 1(b): UV-Vis spectra of methyl orange without sample.

e-ISSN: 2319-9849
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Table 1 : Showing decrease in Degradation (%) of Methyl Orange.

Wavelength(nm)	Absorbance of Methyl orange with sample	Absorbance of Methyl orange without sample	Standard solution	Degradation %
200	1.030	-0.014	2.046	51.02
300	0.507	-0.013	1.395	37.27
400	0.388	-0.012	0.881	42.67
500	0.262	-0.010	0.969	28.07
600	0.183	-0.023	0.002	NA
700	0.117	-0.037	-0.016	NA
800	0.085	-0.033	-0.025	NA

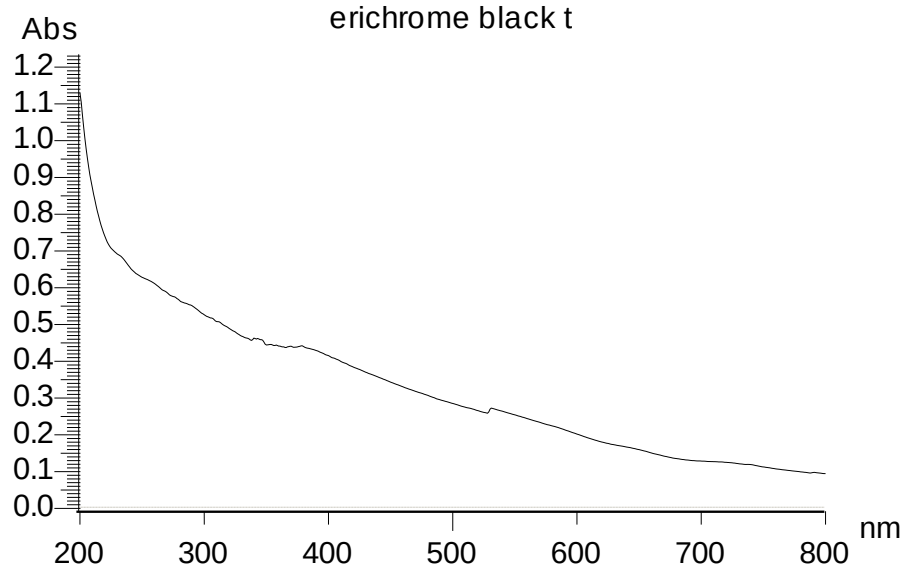

Figure 2(a): UV-Vis spectra of Eriochrome Black T with sample.

e-ISSN: 2319-9849
p-ISSN: 2322-82

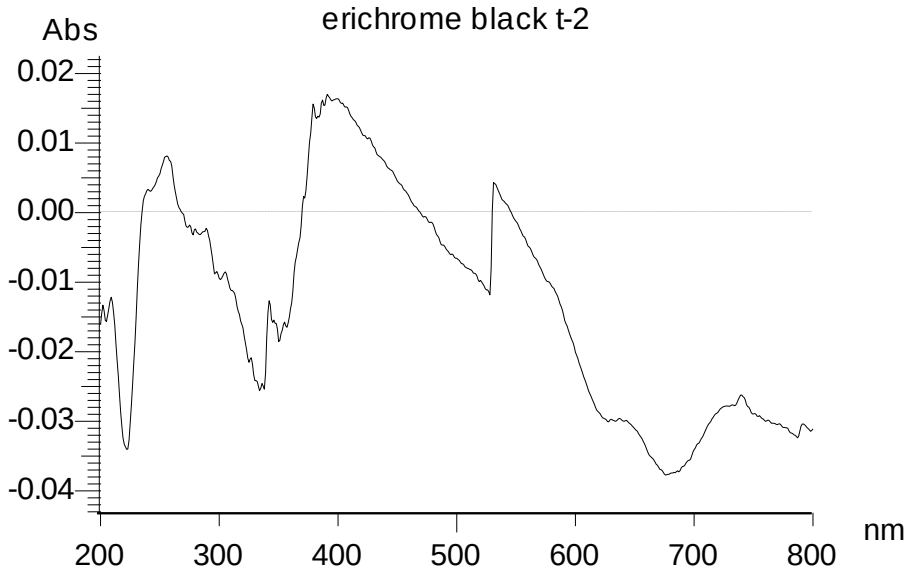

Figure 2(b): UV-Visible spectra of Eriochrome Black T without sample.

Table 2: Showing decrease in degradation (%) of Eriochrome Black T.

Wavelength(nm)	Absorbance of Eriochrome Black T with sample	Absorbance of Eriochrome Black T without sample	Standard solution	Degradation %
200	1.130	-0.016	3.398	33.72
300	0.527	0.009	2.921	17.73
400	0.415	0.016	10.000	3.99
500	0.286	-0.007	10.000	2.93
600	0.202	-0.020	10.000	2.22
700	0.129	-0.034	0.464	35.12
800	0.095	-0.031	0.117	NA

Discussion

According to UV-Visible spectra the sample degraded maximally (51.02%) at 200nm for methyl orange. For Eriochrome Black T, sample degraded maximally (35.12%) at 700nm and 33.72% at 200nm.

HPLC Analysis

Using HPLC various degraded products were analyzed. For Eriochrome Black T degradation products found were Nitrobenzene, 1-amino 2-hydroxyl naphthalene, 4-amino -3- hydroxy benzyl sulfonyl by comparing of retention time and UV-Visible spectrum.

e-ISSN: 2319-9849
p-ISSN: 2322-82

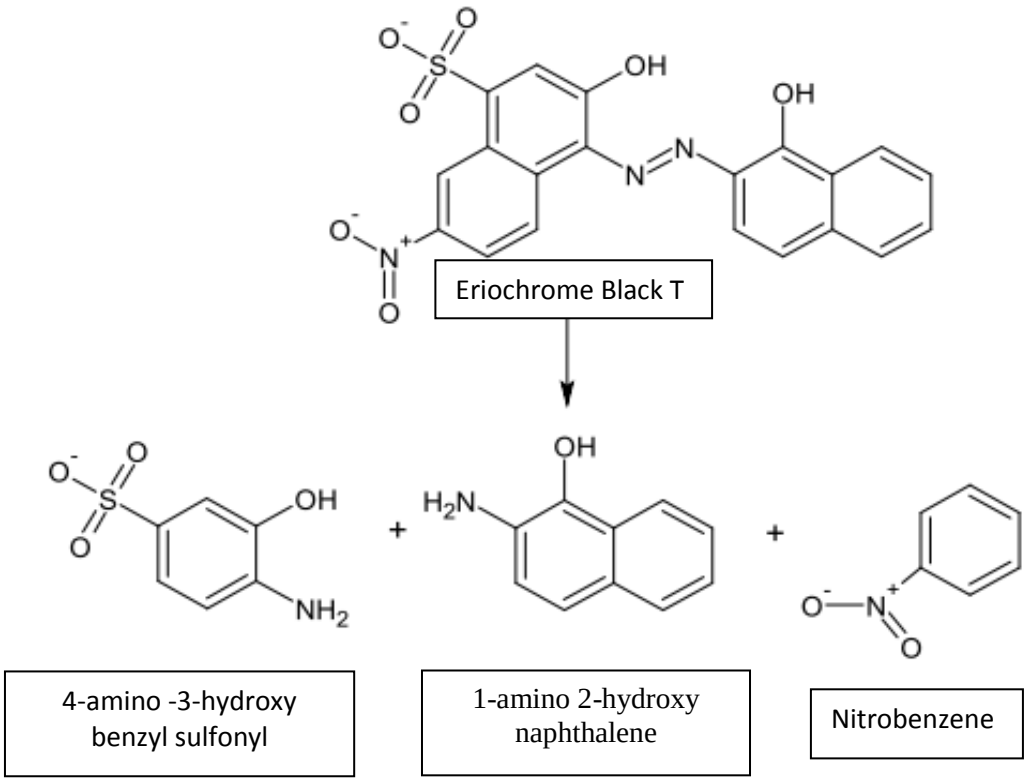

Figure 3a: Biodegradation product of Eriochrome Black T.

For Methyl Orange degradation products were Para–amino benzyl sodium sulphonate and 4-N,N dimethyl aniline.

e-ISSN: 2319-9849
p-ISSN: 2322-82

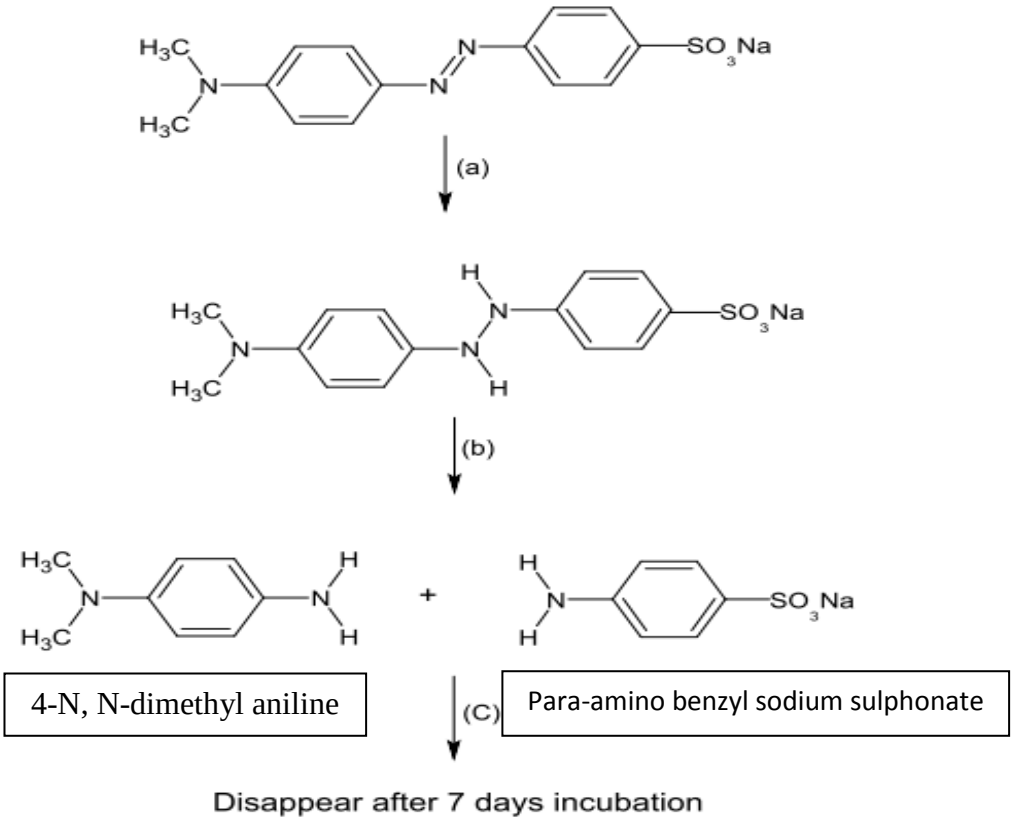

Figure 3b: Biodegradation product of methyl orange.

Conclusion

Our present study demonstrates that this edible mushroom could degrade both the azo dyes with enzymatic systems involved in the decolorization of these dyes in liquid medium. Further study of the mechanism of the degradation of Methyl Orange and Eriochrome Black T with similar structures by this mushroom is underway. So that this macro fungi can be used for the treatment of waste water in textile industries.

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