ABSTRACT

The industry is a major source of pollution for water ecosystems. Industrial production of textile, cellulose and various chemicals is connected with synthetic dyes usage. The discharged effluents could have a hazardous influence on the environment. The biological treatment for synthetic dyes removal is a very perspective, environmentally protective and low cost approach for solution of such problems. One of the often used and very important in dyeing of cellulosic fabrics and textile industry dyes is the anthraquinone-base chlorotriazine dye, known as Reactive Blue 4.

Decolorization of Reactive Blue 4 by Trametes versicolor strain 1 was investigated. The experiments were carried out with different concentrations of dye (50mg/l and 125mg/l) and glucose (1, 2 and 3%) in a medium. The enzyme activity of laccase (EC 1.10.3.2) was measured during the process of decolorization. It was shown that there was a direct correlation between the observed enzyme activity and the investigated process effectiveness. It was established that the best conditions for laccase production and decolorization of 125mg/l Reactive Blue 4 dye are in a medium containing 3% glucose. In these conditions 90% Reactive Blue 4 was decolorized for 384 hours.

Keywords: decolorization, laccase, Reactive Blue 4, Trametes versicolor

Introduction

The industry is a major source of pollution for water ecosystems. Industrial production of textile, cellulose and various chemicals is connected with synthetic dyes usage. The discharged effluents could have a hazardous influence on the environment. The biological treatment for synthetic dyes removal is a very perspective, environmentally protective and low cost approach for solution of such problems (9).

Reactive dyes are synthetically produced and are increasingly used in the textile industries because of their ease and cost effectiveness in synthesis, firmness and variety in color. The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength. Most of the traditional methods such as adsorption, coagulation and biological treatments have been tried for treatment of textile dye wastewater, but none of these gave satisfactory results because of the effluent’s high degree of polarity and complex molecular structure (10).

Decolorization of dye wastewater by white rot fungi which are known as efficient producers of extracellular oxidizing enzymes is the subject of various studies. They are able to degrade a wide variety of recalcitrant pollutants including various types of dyes (10). Laccase based decolorization treatments are potentially advantageous to bioremediation technologies since the enzyme is produced in larger amounts. Laccase (EC 1.10.3.2) belongs to the group of copper containing oxidative enzymes detected in many plants and secreted by numerous fungi (7, 15).

Most information on the biodegradation of synthetic dyes by ligninolytic fungi has been obtained with Phanerochaete chrysosporium (5, 13). Recently, a lot of other white rot fungi members are subject of similar investigations. Strains of Trametes hirsuta and Pleurotus florida have shown decolorizing activity toward some reactive dyes such as Blue CA, Black B133 and Corazol Violet SR.

One of the often used and very important in dyeing of cellulosic fabrics and textile industry dyes is an anthraquinone-base chlorotriazine dye, known as Reactive Blue 4 (Fig. 1). Fixation of the dye molecule onto the fiber occurs via covalent bonding by nucleophilic displacement of the halide substituents at a hydroxyl group on cellulose. Stable bonds are formed, but during the alkaline dyeing process hydrolysis of the dye occurs as a side reaction (4).

In the present study the ability of Trametes versicolor strain to decolorize Reactive Blue 4 dye was studied in a medium with different concentrations of dye and glucose. Laccase enzyme assay during the decolorization processes was performed as well.

Materials and Methods

Microorganism, growth conditions and analytical methods

The strain used in this study was Trametes versicolor 1. The microbial cultivation was carried out in Chapek-Dox medium (pH 6.5) with varied concentrations of glucose as a carbon source (17). The medium was supplemented with 50 mg/l or 125 mg/l Reactive Blue 4 dye (Sigma). The flasks containing 50 ml inoculated culture medium were agitated at
240 rpm, 26°C. Samples were taken at intervals of 24 hours and centrifuged at 5000 rpm for 20 min. The dry weight of the cells was determined by ULTRA X apparatus for drying (17). The initial biomass dry weight was 0.02 g/l in all experiments. The decrease of color intensity in cell free supernatant was analyzed spectrophotometrically at \( \lambda = 492 \) nm. The specific rates of decolorization (Q) were calculated as:

\[
Q = (E_0 - E)/TX,
\]

where \( E_0 \) and \( E \) are the initial and respectively final solution extinctions at \( \lambda = 492 \) nm; \( T \) – time (h); and \( X \) – accumulated biomass.

The laccase activity assay was performed spectrophotometrically (LKB UV-Vis Ultraspec 1000). The oxidation of ABTS (2,2’-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) at 420 nm was measured. One unit of enzyme activity was defined as the amount of enzyme that oxidizes 1 mmol ABTS in 1 min (2, 10).

**Results and Discussion**

The degradations of the reactive blue 4 (RB4), has been studied by different methods of oxidation as: wet air oxidation (WAO), wet peroxide oxidation (WPO), photocatalytic oxidation and electro Fenton (EF) advanced oxidation. The RB4 oxidation was evaluated by the decrease in total organic carbon (TOC) content and concentration in these studies. It has been established that the most efficient method for mineralization of RB4 was WPO, but in all methods TOC removal efficiency has been above 75% after 60 min of treatment (8). However, the application of WAO under rigorous conditions (high temperature and oxygen pressure) is limited due to the high apparatus requirements, which result in high running costs. The biological treatment is known as mild and low cost procedure which could replace or complement the chemical methods usually used.

The process of decolorization was investigated with two different concentrations of the Reactive Blue 4 dye. The results are demonstrated on **Fig. 2**.

![Fig. 2. The laccase activity and Reactive Blue 4 dye decolorization by *Trametes versicolor* strain 1 in a medium with 1% glucose: laccase activity values during 50 mg/l dye decolorization (◊); laccase activity values during 125 mg/l dye decolorization (○); color intensity alteration of 50 mg/l dye (♦); color intensity alteration of 125 mg/l dye (●)](image)

At lower concentration of 50 mg/l detectable decolorization by *Trametes versicolor* strain 1 was observed within 24 h. Maximal decolorization was found to be 73% within 144 h. The laccase activity arose at the beginning of the process and reached its maximum value – 70.6 U/ml at 48 h. Thereupon the enzyme activity has slowly gone down until 144 h. The disappearance of the laccase activity leaded to the end of the decolorization process.

At 125 mg/l, the 45% decolorization within 144 h was measured. The maximum value of laccase activity (62 U/ml) was observed at 72 h. It could be seen that comparable enzyme activities did not give the same rate of decolorization. The data shown on **Fig. 1** helped us to improve the microbial growth by increased glucose concentrations in the culture medium. It led to increased period of laccase production by the investigated strain’s cells.

It is conventionally accepted that C limitation triggers ligninolytic activity in white rot fungi and is required for pollutant degradation (1, 3, 6, 11, 14). The present study examines the effects of glucose concentrations on sequential dye and dye mixture decolorization by *T. versicolor*.

The obtained results indicated that dye decolorization required a minimal amount of glucose. The glucose depletion corresponded to cessation of decolorization of the medium. At the other hand it seemed that in our experiments the process of decolorization was not stimulated by C limitation. The total decolorization was improved by an increase in the initial glucose concentration in the medium. The higher concentrations of glucose maintained more percentage of decolorization due to better growth of *Trametes versicolor*. For example, the experiments carried out with 125 mg/ml Reactive Blue 4 dye showed the following maximal biomass measurements: in the media with 1% glucose – 0.35 g/l dry cells; with 2% glucose...

![Fig. 1. Structure of Reactive Blue 4 dye](image)
– 0.92 g/l dry cells; and with 3% glucose – 1.44 g/l dry cells. Nevertheless it was established that maximal laccase activity was comparably equal in all described experiments (58 U/ml – 65 U/ml). The observed contradiction with published by other authors’ data was resolved by the estimation of specific rate of decolorization (see Materials and Methods). It could be seen that the calculated rate of decolorization in a medium completed with 1% glucose was the highest. The total Decolorization (%) measured in the carried out experiments and the relevant rates of Reactive Blue 4 dye decolorization (Q) are shown in Table 1.

<table>
<thead>
<tr>
<th>Glucose (%)</th>
<th>Specific decolorization rate (Q h⁻¹)</th>
<th>Total decolorization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.66</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>0.26</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>0.16</td>
<td>90</td>
</tr>
</tbody>
</table>

The presented data gave proof of the fact that higher glucose concentration actually lowered the specific decolorization rate. The highest percent of decolorization observed in a medium containing 3% glucose is due to the longer time of growth and development of microbial culture. Logically, the laccase synthesis was realized for longer time. In this way the investigated process continued correspondingly longer and reached better rate of decolorization. The data are shown on Fig. 3.

**Fig. 3.** Decolorization of 125 mg/l Reactive Blue 4 dye by *Trametes versicolor* strain 1 in a culture medium supplemented with: 2% glucose (♦) and 3% glucose (●); laccase activity in a culture medium supplemented with: 2% glucose (◊) and 3% glucose (○)

**Conclusions**

The experimental data received in this study proved that the strain *Trametes versicolor* 1 can be grown in a presence of Reactive Blue 4 in the culture medium. Additionally, because of its ability to produce extracellular laccase the strain can successfully decolorize the dye included in the medium. The results clearly demonstrated that in a medium with 3% glucose the decolorization continued more than twice as long as in a medium supplemented with 1% or 2% glucose. The established correlation between glucose concentration, laccase activity and the rate of decolorization gave us a reason to conclude that Reactive Blue 4 dye can not serve as an alternative carbon source for *Trametes versicolor* 1. The observed decolorization was due most likely to the enzyme transformation of the tested dye chemical structure.

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**REFERENCES**