DECOLORIZATION OF DYES FROM TEXTILE WASTEWATER BY *Trametes versicolor*

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Abstract

The white rot fungus *Trametes versicolor* was applied to the decolourisation of three synthetic textile dyes in the presence and absence of glucose. Different initial dye concentrations were tested and approximately 97% decolourisation was achieved. It was found that fungal metabolism induced by the glucose as well as the pH play an important role in the decolourisation process. This treatment was also applied to a real wastewater from a textile industry-dyeing sector leading to 92% decolourisation.

Keywords: *Trametes versicolor*; decolourisation; textile wastewater; white-rot fungi.

INTRODUCTION

The wastewater from the textile industry is known to be strongly colored, presenting large amount of suspended solids, pH broadly fluctuating, high temperature, besides high chemical oxygen demand (COD). Colour is the first contaminant to be recognized in this wastewater. A very small amount of dye in water (10 – 50 mg l^{-1}) is highly visible and reduces light penetration in water systems, thus causing a negative effect on photosynthesis [1, 2].

Recently, dye removal became a research area of increasing interest, as government legislation concerning the release of contaminated effluent becomes more stringent. Physical and chemical methods such as adsorption, coagulation-flocculation, oxidation, filtration, and electrochemical methods may be used for wastewater decolourisation [3]. Unfortunately, these methods are quite expensive and show operational problems such as development of toxic intermediates, lower removal efficiency, and higher specificity for a group of dyes, among others

Over the past decade, white rot fungi have been studied for their ability to degrade recalcitrant organo-pollutants such as polyaromatic hydrocarbons, chlorophenols, and polychlorinated biphenyls [4]. The low specificity of the lignin-degrading enzymes produced by these fungi suggests that they may be suitable for the degradation of textile dyes wastewater. Although dye degradation by white rot fungi has been focused on *Phanerochaete chrysosporium* results of another work [5] showed that *Trametes versicolor* displayed the greatest ability for decolorisation by evaluating five white rot fungi on six different dyes.

T. versicolor releases laccase as its major extracellular enzyme, a copper-containing polyphenol oxidase (benzenediol: O_2 oxidoreductase, EC 1.10.3.2) which catalyses the oxidation of phenolic compounds [6]. Laccase can also catalyse the oxidation of organic pollutants through molecular oxygen reduction, even in the absence of hydrogen peroxide [7]. Moreover, environmental conditions such as pH, type of carbon source, among others seem to play an important role in decolourisation by *T. versicolor* [8].

Most studies have been limited to the decolourisation of a single dye [8, 9] or even to mixtures of dyes [3, 5, 10]. Nevertheless, a biodecolourisation system must sustain its ability upon exposure to real wastewater conditions. Thus, this study investigates not only the decolourisation of a synthetic wastewater

but also faces the problem of dealing with a real dyeing wastewater. The effects of pH, presence or absence of carbon source and different initial colour concentrations on the decolourisation process performed by *T. versicolor* are determined.

MATERIALS AND METHODS

Fungi Culture

The white-rot fungus, *Trametes versicolor* was obtained from the National Institute of Industrial Engineering and Technology (INETI, Portugal) and was maintained on Tien and Kirk medium [11] at 4ºC.

Inoculum preparation and growth

T. versicolor grown for 7 days at 28ºC in agar plate [11] was used to inoculate 250 ml of *Trametes* defined medium (TDM) [12]. The growth was carried out in 500 ml Erlenmeyer flasks for three days at 28ºC and 180 rpm. Initial cell concentration was 70 mg dry weight l^{-1}.

Synthetic Wastewater

SENAI / CETIQT, Rio de Janeiro, Brazil, generously provided the reactive dyes, Procion Orange MX-2R (C.I. Reactive Orange 4), Remazol Red 3B (C.I. Reactive Red 23) and Remazol Black GF (C.I. Reactive Black 5). A mixture of dyes containing each dye in equal amounts, in a 1g l^{-1} solution, was taken as the synthetic wastewater.

Experiments with synthetic wastewater

The experiments carried out with synthetic wastewater were performed in 500 ml Erlenmeyer flasks with 250 ml TMD medium with the synthetic wastewater for 10 days at 28ºC and 180 rpm. All the experiments were realized at least twice and the characteristic profiles are present in this article.

Experiments with glucose

After three days of cell growth, a synthetic wastewater was added to Erlenmeyer flasks containing TDM medium to obtain different initial colour concentrations of 0, 50, 100 and 300 mg l^{-1} of the mixture of dyes. In all cases an initial glucose concentration of 9 g l^{-1} was present.

Experiments without glucose

Cell growth was carried out for three days, in the same conditions described before Afterwards, the biomass was filtrated and disposed in other Erlenmeyer flasks containing the synthetic wastewater and TDM medium, without glucose. Different synthetic wastewater volumes were considered to reach initial concentrations of 0, 50, 100 and 300 mg l^{-1}, as for the experiments with glucose.

Real Wastewater

A real wastewater was collected from the dyeing sector from SENAI / CETIQT, Rio de Janeiro, Brazil, and some of its properties were: pH = 8.0 – 8.5, COD = 36,000 – 36,100 mg.l^{-1} and colour: 343 Pt-Co units.

Experiments with the real wastewater

The experiments carried out with the real wastewater were performed in 500 ml Erlenmeyer flasks with 250 ml TMD medium with the real wastewater for 10 days at 28ºC and 180 rpm. All the experiments were carried out at least twice and the characteristic profiles are presented.

These experiments were conducted as described above for the synthetic wastewater with glucose. For the real wastewater, two different concentrations were tested. It was attempted to achieve similar conditions to those previously employed in the synthetic wastewater studies. Therefore, based on the real wastewater absorbance measured at 509 nm (maximum wavelength for the synthetic wastewater), the volumes of real wastewater added to the media were determined in order to achieve the same absorbance obtained initially for the experiments with 50 and 300 mg l^{-1} of the synthetic wastewater. As for the experiments with synthetic wastewater, an initial glucose concentration of 9 g l^{-1} was used.

Analytical Methods

Glucose and pH Quantifications

The extra cellular glucose was measured through the 3,5-dinitrosalycilic acid method [13] and the pH was obtained with a specific electrode (Orion) previously calibrated with standard buffers.

Laccase Activity

The enzyme activity was assayed in extra cellular medium based on the oxidation of a laccase substrate, 2,2`-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), ABTS [14]. A sample was added to a citrate-phosphate buffer (0.05 M/0.1 M, pH 4.5) containing 0.4 mM ABTS. Laccase activity was determined through the monitoring of the product formation rate of enzymatic oxidation of ABTS spectrophotometrically at 420 nm with an extinction coefficient ε of 3.6 x 10^4 $M^{-1}.cm^{-1}$. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μM of ABTS per minute.

Colour Determination

Absorbance measurements were performed using a HACH DR/4000 UV spectrophotometer. The wavelength of maximum absorbance ($ë_{máx}$) of the dyes studied were: 488 nm for Procion Orange MX-2R, 511 nm for Remazol Red 3B and 555 nm for Remazol Black G. Neverthless, the wavelength of maximum absorbance of the synthetic wastewater was determined as 509 nm through a scan absorbance presented in Figure 1a, and this wavelength was used for the colour measurements of all samples. The decolourisation was determined as follows:

$$Decolouris\,ation, i = \frac{(Abs,0 - Abs,i)}{Abs,0} * 100 \qquad \text{(i)}$$

where *Decolourisation,i* is the colour removal efficiency until day *i*, *Abs,*0 is the absorbance (509 nm) determined immediately after the dye addition, and *Abs,i* is the absorbance (509 nm) determined in the *i*th day.

For the real wastewater, the colour measurements were carried out using the Platinum-Cobalt Standard Method adapted from Hongve and Åkesson [15]. The decolourisation was determined as follows:

$$Decolouris\,ation, i = \frac{(Colour,0 - Colour,i)}{Colour,0} * 100 \qquad \text{(ii)}$$

where *Decolourisation,i* is the colour removal efficiency until day *i*, *Colour,*0 is the colour in units of Pt-Co (455 nm) determined immediately after the synthetic wastewater addition, and *Colour,i* is the colour in units of Pt-Co (455 nm) determined in the *i*th day.

RESULTS AND DISCUSSION

Experiments with Synthetic Wastewater

Figures 2a and 2b present the kinetic profiles obtained for enzyme production in systems with and without glucose, respectively. It can be observed that the absence of glucose led to higher enzymatic activities, indicating that carbon source starvation may be an interesting strategy for laccase production. Dye addition seems to induce enzyme excretion in both conditions, in the absence and presence of glucose, as they show the same trend. Nevertheless, it seems that enzyme production are submitted to an inhibition when dye concentrations are higher 100 mg l^{-1}.

The pH in systems with glucose was more stable than in those without this carbon source. In the presence of glucose it remaind at a value between 3 and 4.5 until the tenth day, whereas in other cases, a considerable pH increase, from $3.0 - 4.5$ to $6 - 8$ was noticed after the fifth day. These results seem to be in agreement with results presented elsewhere [8], where it was reported that the optimal pH for *T. versicolor*

growth and decolourisation was 4.5. It was also reported that the decolourisation efficiency decreased to nearly 50% for pH around 6.

Results for the decolourisation treatment with and without a carbon source are shown in Figures 3a and 3b, respectively. These results indicate that decolourisation is more effective in the presence of glucose; reaching 97% for 50 and 100 mg dye l^{-1} and 87% for 300 mg dye l^{-1}. Although the absence of glucose seemed to enhance the laccase production, the presence of glucose was necessary for the decolourisation performed by *T. versicolor*. These results indicate that decolourisation rates were not proportional to the laccase activity as shown in Figure 4. Similar results for azo and indigo dyes are presented elsewhere [6]. This behaviour may have two explanations. One hypothesis could be that, other enzymes rather than laccase are responsible for the decolourisation, as lignin and manganese peroxidase, but low activity levels of these enzymes were measured for this specific fungus. The other hypothesis could be that laccase, although the main oxidative enzyme, requires the presence of other metabolites in the medium to act as mediators in the decolourisation process.

The profile of glucose utilization during the decolourisation treatment presented a reduction of more than 50% of glucose concentration after eight days in all cases. Figures 5a to 5c depict the decolourisation profile in batch experiments. Considering these experimental profiles the decolourisation rates ($\Delta Abs / t$) could be determined by a linear model in two phases since the decolourisation performance was more effective until the eight day of treatment. After glucose concentration had reached exhaustion, the removal rate decreased drastically. In such conditions, the fungal metabolism seemed to change for the production of secondary metabolites with a consequent increase in the pH and a decrease in treatment performance. Such profile indicated a possible saturation in the enzymatic system. Similar observations were described elsewhere [16], and it was showed that consumption of the primary growth substrate played an important role in the decolourisation performance.

It should also be considered that the synthetic wastewater was composed by three different dyes, which might have shown different degradation rates according to the dependency of the initial decolorization rates on the phenolic rings substituents of the dyes, as it was reported elsewhere [17]. It is possible to notice in

Figures 1a and 1b that the absorbance peak diminished a little (from 509nm to 478 nm) and a possible explanation for that is that the dye Procion Orange might have a lower decolourisation rate as it presents a maximum absorbance wavelength of 488 nm. Other study [5] reported that Remazol Orange 3R ($ë_{máx}$ = 473 nm) showed the lowest decolourisation rate among other dyes when treated individually with white rot fungi. Another spot in the absorbance scan presented in Figure 1b for both 4^{th} and 10^{th} day of treatment was a reduction in the colour peak intensity along process time for the synthetic wastewater, which demonstrated a global decolourisation in spite of the different decolourisation rates of the individual dyes.

Experiments with Real Wastewater

After studying the performance of *T. versicolor* catalysis for a mixture of synthetic dyes, an investigation with an industrial textile wastewater was performed. Since the results presented before indicated that decolourisation was more effective in the presence of glucose, this carbon source was added to the medium used for the experiments with the real wastewater. Figure 5d shows the decolourisation for the experiments with real wastewater. Low decolourisation (40%) was obtained for the more concentrated effluent (7 times diluted). This result differed from the dye removal efficiency obtained for the synthetic dye experiment with the same colour (300 mg l^{-1}). Thus, the effluent was diluted 42 times, which corresponded to a dye concentration of 50 mg l^{-1}, allowing a better performance and reaching a decolorization of 92%.

The profiles for pH change and glucose consumption (data not shown) are similar for both synthetic and real wastewater, denoting that these parameters were not responsible for the differences observed in the decolourisation. The presence of other recalcitrant wastewater compounds could be responsible for the lower decolourisation achieved in real systems, which caused a reduction in the performance of more than 50% when compared to the synthetic effluent. Due to a lower inhibition of the fungus enzymatic system, the dilution of these compounds might have been responsible for the improvement in the decolourisation performance.

CONCLUSIONS

Decolourisation of synthetic and real wastewaters was performed by *Trametes versicolor*. A decolorization of 97% was achieved for initial dye concentrations up to 100 mg l^{-1}. The pH and the presence of glucose were identified as important parameters for an adequate decolourisation performance. The results reported in this study showed that the decolourisation rates were not proportional to the laccase activity corroborating the data obtained from systems with and without glucose, since higher enzymatic activities were obtained in *T. versicolor* cultures without glucose. For a real wastewater, decolourisation reached efficiencies of about 92% in a diluted system (approximately 50 mg dye l^{-1}). Thus, the behaviour demonstrated by the real wastewater, when compared to the synthetic one, induced the usage of a dilution step to minimize such effects.

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FIGURE CAPTIONS

Figure 1. Absorbance scan for the initial condition of the synthetic wastewater (a) and for a sample from 4^{th} and 10^{th} day (b) of decolourisation process.

Figure 2. Kinetic profiles for laccase activity during synthetic wastewater treatment with *T. versicolor* in the presence (a) and absence (b) of glucose for different initial dye concentrations.

Figure 3. Decolourisation of the synthetic wastewater by *T. versicolor* in the presence (a) and absence (b) of glucose.

Figure 4. Relationship between laccase activity and decolourisation rate for a synthetic wastewater (300 mg l^{-1}) with glucose.

Figure 5. Colour along the experiment with synthetic wastewater in the presence of glucose for initial dye concentrations of 50 mg l^{-1} (a), 100 mg l^{-1} (b), 300 mg l^{-1}(c) and decolourisation of the real wastewater (d).

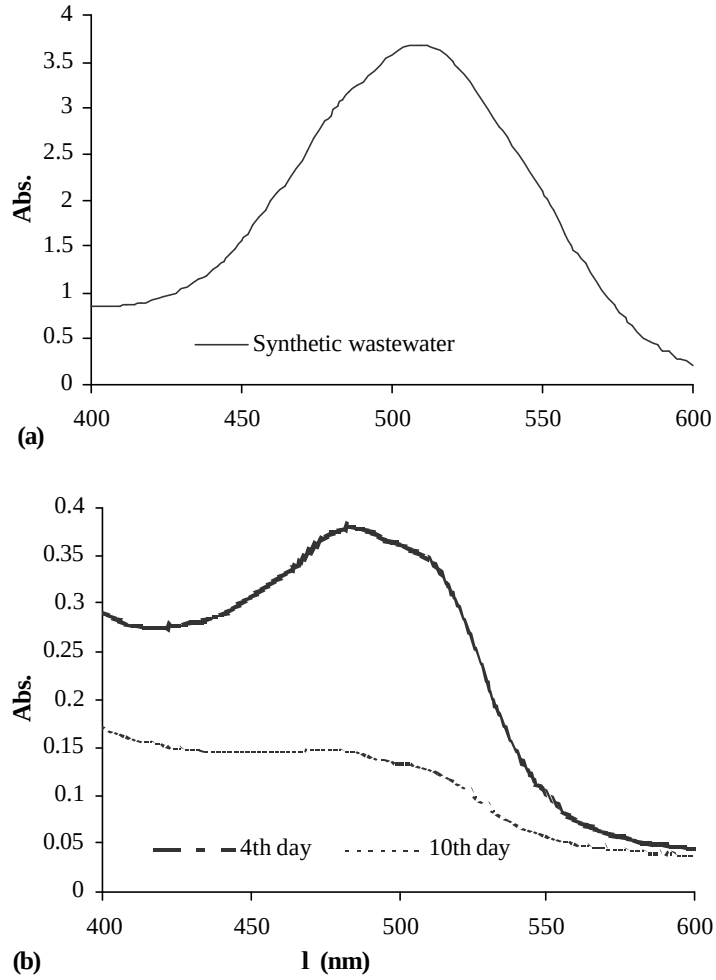

FIGURE 1

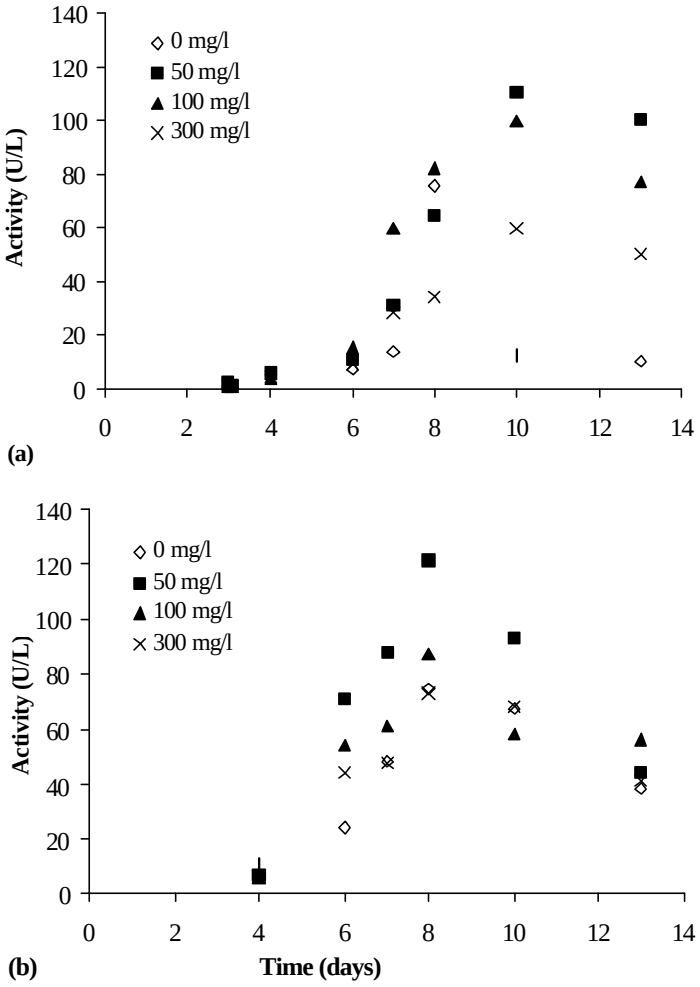
◇ 0 mg/l
■ 50 mg/l
▲ 100 mg/l
× 300 mg/l
Activity (U/L)
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
(a)
◇ 0 mg/l
■ 50 mg/l
▲ 100 mg/l
× 300 mg/l
Activity (U/L)
Time (days)
(b)

FIGURE 2

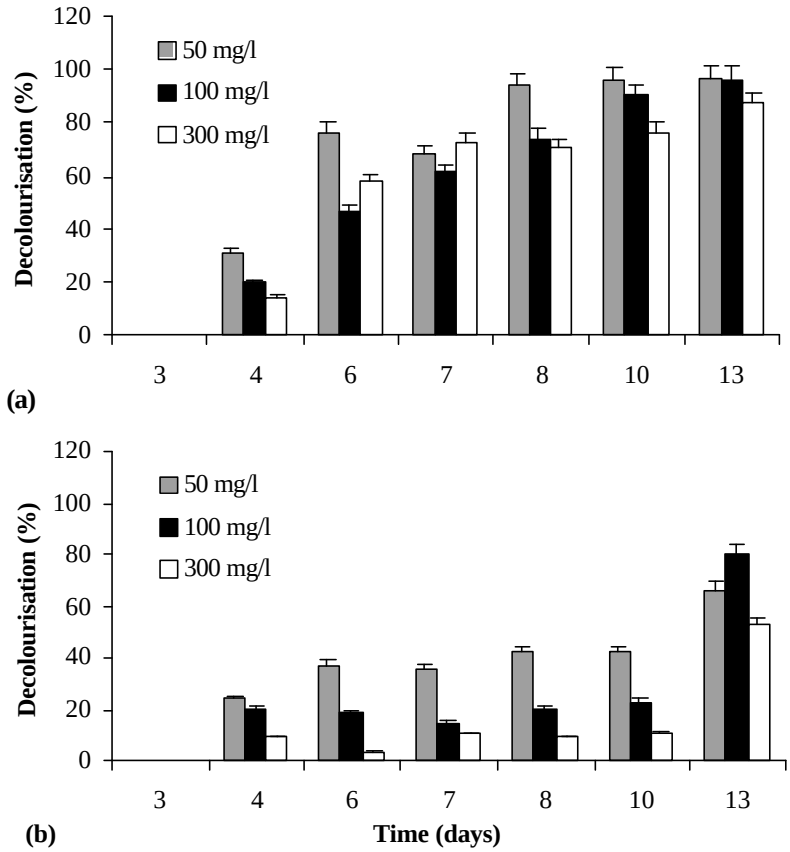
120
100
80
60
40
20
0
Decolourisation (%)
50 mg/l
100 mg/l
300 mg/l
3
4
6
7
8
10
13
(a)
120
100
80
60
40
20
0
Decolourisation (%)
50 mg/l
100 mg/l
300 mg/l
3
4
6
7
8
10
13
(b)
Time (days)

FIGURE 3

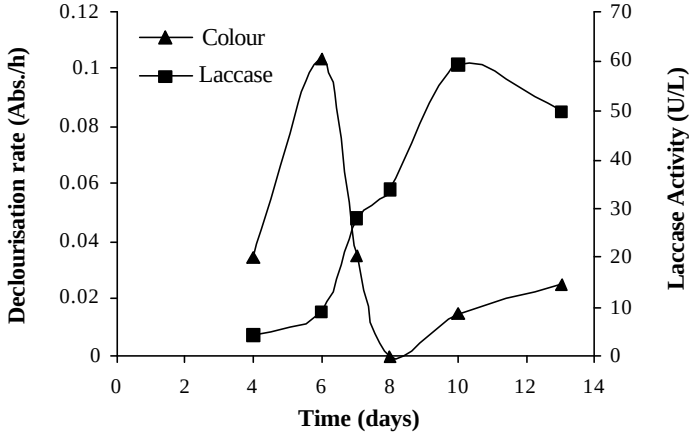
Colour
Laccase
Declourisation rate (Abs./h)
Laccase Activity (U/L)
Time (days)
0.12
0.1
0.08
0.06
0.04
0.02
0
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14

FIGURE 4

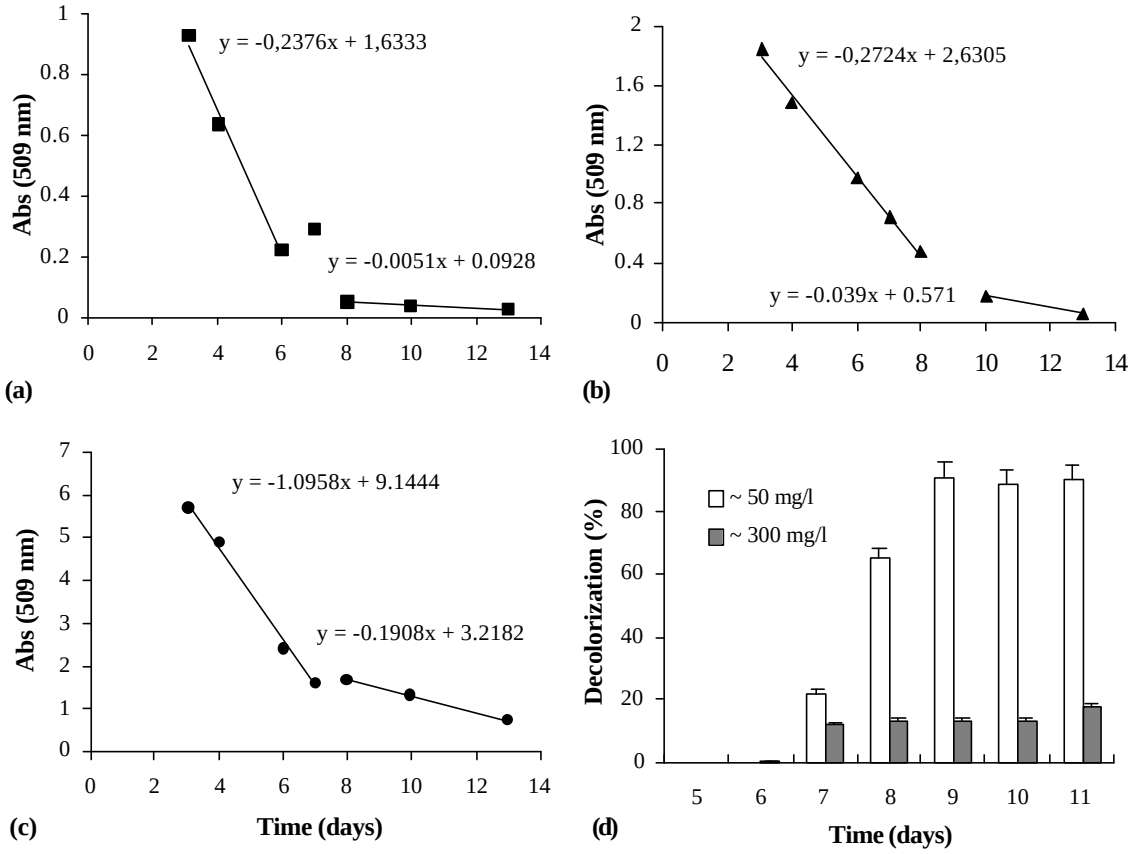
y = -0,2376x + 1,6333
y = -0.0051x + 0.0928
Abs (509 nm)
(a)
y = -0,2724x + 2,6305
y = -0.039x + 0.571
Abs (509 nm)
(b)
y = -1.0958x + 9.1444
y = -0.1908x + 3.2182
Abs (509 nm)
Time (days)
(c)
~ 50 mg/l
~ 300 mg/l
Decolorization (%)
Time (days)
(d)

FIGURE 5