

*Full Length Research Paper*

# Evaluation of three immobilization supports and two nutritional conditions for reactive black 5 removal with *Trametes versicolor* in air bubble reactor

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Accepted 14 October, 2011

**Polyurethane foam, *Luffa cylindrica* sponge and Ca-alginate (3% w/v) were evaluated as immobilization supports for removing reactive black 5 dye using the white rot fungus *Trametes versicolor* at 1, 4 and 8 days of colonization. According to statistical results, the *L. cylindrica* sponge was the best support at 4 days of colonization (90% color removal). Using a factorial design 2<sup>2</sup>, two nutritional conditions were evaluated with free and immobilized biomass in air bubble reactor, determining that this microorganism requires a high ratio of C/N (50:1) associated with the addition of 2 g·L<sup>-1</sup> of glucose and 0.05 g·L<sup>-1</sup> of NH<sub>4</sub>Cl to get the maximum decolorization (86 and 87%) and enzymatic activity (1.1 and 2.1 U·L<sup>-1</sup>) with free and immobilized biomass, respectively. The removal kinetics were evaluated in the same reactor showing a 98% of color removal obtained at 7 days with 0.84 U·L<sup>-1</sup>, 383 U·L<sup>-1</sup> of laccase and lignin peroxidase activities. The bioreactor operated in three successive batches for 12 days with immobilized biomass, was maintained throughout the fermentation; the color removal was 70% with 1.3 and 300 U·L<sup>-1</sup> of laccase and lignin peroxidase. Finally, the UV/VIS spectrum showed that the transformation of dye was associated with biochemical and physical mechanisms.**

**Key words:** Reactive Black 5, *Trametes versicolor*, immobilization supports.

## INTRODUCTION

Textile industries have used a variety of synthetic dyes with structural diversity as a consequence of the presence of different chromophores and auxochromics groups in the molecule (Zille et al., 2005). Azo dyes represent the biggest group of all the synthetic dyes and are equivalent to 70% of usage in the textile industry (Fernández et al., 2009). The chemical structure of this type of dyes, is characterized by the presence of the azo group (-N=N-), associated to amino or hydroxyl groups (Figure 1) (Urra et al., 2006). The increasing use of dyes has generated serious environmental problems by affecting the water bodies since the dye is dyeing

process effluents, thus conferring color. Also, because the dyes absorb light strongly, they block photosynthesis process and thereby diminish the concentration of dissolved oxygen up to critical levels, causing the death of species of different trophic levels. In addition, they can be toxic for aquatic organisms, present bioaccumulation capacity and resistance to biological degradation (Pereira and Freire, 2006).

The use of white rot fungi for the degradation of dyes has become a very effective alternative due to capacity of these microorganisms to degrade compounds similar to lignin, by unspecific enzymes action (laccase, manganese peroxidase, lignin peroxidase, etc.) and the mechanism of adsorption to the fungal biomass (Swamy and Ramsay, 1999; Rodríguez, 2009). The efficiency of these microorganisms is complemented by the

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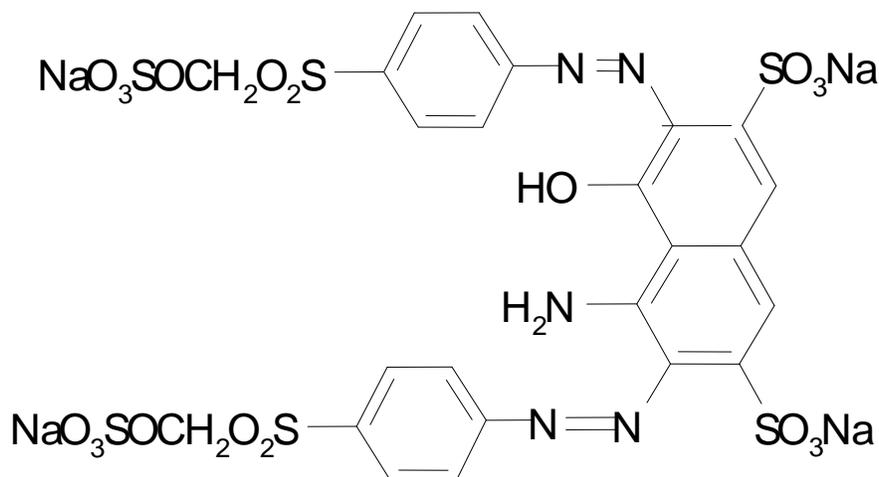


Figure 1. Reactive Black 5 structure.

colonization capacity that the hyphae have allowing the total biomass to be immobilized in different inert and non inert materials (Morales et al., 2010). On the other hand, many of the supports used for the immobilization of white rot fungi like lignin cellulosic materials, do not generate additional costs because they are by-products of different process that do not have additional uses. In addition, these supports do not need chemical reagents for the immobilization. Another advantage also, is the adsorption of the polluting agent to the structure of the support, generating an additional adsorbent that would increase the removal efficiency of the system.

The textile industry uses high volumes of water for its processes, thereby generating polluted water that is commonly disposed without treatment. A lot of researches have been focused on solving this problem, but most of them are expensive methods that are not viable or rentable for this kind of industries, hence it is necessary to use biological treatments and improve microorganisms' effects in these treatments. It is also important to highlight the advantage to use a natural support as biocarrier, thus diminishing the cost of the treatment and improving the dye decolorization. The aim of this work was to evaluate the effect of three immobilization supports on the capacity of *Trametes versicolor* to remove the dye reactive black 5 under two nutritional conditions.

## MATERIALS AND METHODS

### Microorganism

The fungus, *T. versicolor*, used in this study was maintained on wheat bran extract agar at 4°C. For the reactivation, *T. versicolor* was grown in a wheat bran extract agar plates at 30°C for 8 days (Ha et al., 2001).

### Immobilization of *T. versicolor* in inert and non-inert support

The supports used in this study were *Luffa cylindrica* sponge (LCS), polyurethane foam (PF) and Ca-alginate (CaA). These materials were characterized to determine their density (Sampat, 1991), porosity percentage (Nava-Hernández, 2007) and saturation volume (Shaolin and Wilson, 1997). The immobilization by colonization was made in solid medium for LCS cubes and PF cubes (30 cubes of each one) using the technique described by Fernández et al. (2009). The Petri dishes were incubated at 30°C varying the time of colonization according to the treatment of the factorial design.

In the reactor tests, a great quantity of colonized support was necessary, and 200 ml of agar wheat bran extract was used, adding 100 cubes (0.5 × 0.5 × 0.5 cm) and 30 agar disks (5 × 5 mm) with the fungus.

For the immobilization by entrapment in alginate, *T. versicolor* was cultivated for 8 days in 50 ml of broth WBE to 120 rpm, later the biomass was recovered by filtration using filter paper. The biomass (10 g) was washed twice with 50 ml of saline solution 0.85% (w/v), and then aseptically macerated using a homogenizer for 2 min. The paste was transferred to an Erlenmeyer flask of 250 ml with 50 ml of saline solution 0.85% (w/v). The entrapment of biomass suspension in CaA was carried out as follow: 3% (w/v) Na-alginate was dissolved in distilled water and was mixed with the fungal mycelium. The mixture was introduced into 20 ml syringe sterile and dropped into a solution containing 0.2 M CaCl<sub>2</sub> at 4°C. The biomass entrapped was stored at 4°C until their use in the factorial design (Ramsay et al., 2005).

### Experimental design 3<sup>2</sup>

The experimental design 3<sup>2</sup> was used to determine the effects of operating parameters such as immobilization support and the entrapment time on % decolorization. The design was applied using the statistical program Design Expert (p = 0.05) (Montgomery, 1943). Three different supports (*L. cylindrica* sponge, polyurethane foam and Ca-alginate) and different times of immobilization (1, 4 and 8 days) were chosen as the critical variables and called as X<sub>1</sub> and X<sub>2</sub>, respectively, as shown in Table 1. The design generated nine treatments. For each combination, 50 ml of Radha medium

**Table 1.** Treatments and controls evaluated during the experimental design 3<sup>2</sup>.

Treatment	Factor X <sub>1</sub> support	Factor X <sub>2</sub> immobilization time (days)
1	LCS	1
2	PF	1
3	CaA	1
4	LCS	4
5	PF	4
6	CaA	4
7	LCS	8
8	PF	8
9	CaA	8
Control	Immobilized inactivated biomass Thermal treatment at 1 h to 120°C 15lbs	
Depending variable	Color removal (%); laccase activity (U l <sup>-1</sup> )	
Operational conditions	Agitation, 120 rpm; Temperature, 30°C.	

modified by the authors supplemented with 300 mg·L<sup>-1</sup> of reactive black 5 (RB5) in 250 ml Erlenmeyer flasks and final pH of 4.5 (Radha et al., 2005) were used. The inocula used were biomass entrapped in each support (0.5 g); approximately 5 LCS cubes, 5 PF cubes and 30 CaA beads. The cubes had a dimension of 0.5 cm<sup>3</sup> and the alginate beads had 5 mm of diameter, approximately. All the Erlenmeyer flasks were incubated using a shaker (120 rpm) for 4 days of treatment at 30°C.

The controls used were immobilized inactivated biomass (thermal treatment of 1 h to 123.4°C) and support without biomass. The dependent variables evaluated were: color removal (%) (Livernoche et al., 1983) and laccase activity (Lac) (EC 1.10.3.2) (Tinoco et al., 2001). The statistical analysis was carried out by means of variance analysis of ANOVA with the statistical program Design Expert 6.0.

### Biological reactor and operational conditions

The bubble column reactors were glass tubing (5 cm inner diameter, 50 cm long) with 750 ml of capacity and were used with 525 ml of Radha medium supplemented with RB5. The experiments in the reactor were carried out in two stages; the first one consisted of a factorial design 2<sup>2</sup> and the final removal curves using the best results in the experimental design.

### Experimental design 2<sup>2</sup>: Effect of the biomass (free and immobilized) and carbon-nitrogen supplement over the decolorization and enzymatic activities

The effect of the free and immobilized biomass and the carbon-nitrogen supplement on color removal and enzymatic activity was evaluated by means factorial experiment 2<sup>2</sup>. The factors evaluated were biomass (level +1: Immobilized biomass, level -1: Free biomass) and carbon-nitrogen supplement (level +1: 2 g·L<sup>-1</sup> of glucose and 0.05 g·L<sup>-1</sup> of ammonium chloride, level -1: without supplement). For each treatment, 1.3% (w/v) of free or immobilized biomass was inoculated into the reactor, the biomass was obtained by difference between the weight of the alone cube and the weight of the cube after immobilized. The carbon-nitrogen supplement used was the Radha medium with 300 mg·L<sup>-1</sup> of RB5 and the treatment without supplement was 300 mg·L<sup>-1</sup> of RB5 just dissolved

in distilled water and final pH 4.5. The temperature was 25°C, with 500 cc·min<sup>-1</sup> of aeration (Fernández et al., 2009), for the 4 days of treatment. The dependent variables were color removal (%) (Livernoche et al., 1983), laccase activity (EC 1.10.3.2) (Tinoco et al., 2001), manganese peroxidase (MnP) (EC 1.11.1.13) (Lu et al., 2008) and lignin peroxidase (LiP) (EC 1.11.1.7) (Fernández et al., 2009). Statistical analysis was carried out by means analysis of variance ANOVA with the statistical program Design Expert 6.0. (p = 0.05).

### Kinetics of removal

The final treatment was performed in bubble column reactors packed with 7 g of the immobilized biomass of *T. versicolor* and 525 ml of non-sterilized RB5 solution at 300 mg·l<sup>-1</sup>, supplemented with carbon and nitrogen (2.0 g·L<sup>-1</sup> of glucose and 0.05 g·L<sup>-1</sup> of inorganic nitrogen) at pH 4.5. The operating conditions were the same as earlier mentioned. The process along the time was evaluated for 8 days. The parameters were the same evaluated in the factorial design. Additionally, the immobilized biomass was observed by scanning electron microscopy (SEM) (Pedroza et al., 2006). The abiotic control was the support without biomass and this treatment was evaluated under the same conditions as that of biotic system. The comparison between the treatment was carried out using the statistical program SAS 9.0 for Windows (p = 0.05). Dye biotransformation was followed using a spectrophotometric method which recorded the UV-VIS spectrum in every sample (0, 1, 4 and 8 days) in the wavelength range 200 to 800 nm (Borchert and Libra, 2001).

### The sequencing batch experiments

To determine the number of cycles that the immobilized biomass supported, the biological reactor was inoculated with the same biomass concentration (7 g / 525 ml) and 300 mg·L<sup>-1</sup> of RB5 at pH 4.5. The process was left until 4 days, then the effluent was removed and the system was loaded with a new batch of RB5 white supplement. These experiments were carried out for 5 cycles with 3 repetitions. The parameters were the same as evaluated in the removal curves.

## RESULTS AND DISCUSSION

### Characterization of supports

According to the results for density, the values obtained were  $0.008 \pm 0.002$ ,  $0.009 \pm 0.001$  and  $0.7 \pm 0.017 \text{ g}\cdot\text{cc}^{-1}$ , for LCS, PF and CaA, respectively. The difference between supports could be related to physical characteristics of the materials; the ionotropic gelification of the CaA absorbed water that increases the density. On the other hand, the LCS and PF did not occur through a previous hydration process (Mofidi et al., 2000). The density is an important parameter for the operation of aerobics fluidized reactors; already there is guarantee that the biomass immobilized moves so long the column, stays in permanent contact with the wastewater, and in this way removes the pollutants compounds.

The support with the better porosity (%) was LCS ( $91 \pm 2$ ) followed by PF ( $73 \pm 1$ ) and CaA ( $35 \pm 2$ ). This characteristic favors the diffusion of the oxygen and the nutrients towards the fungal cells, increasing the enzymatic and biomass production. Additionally, these pores allow the fungal colonization for the apical growth of the hyphae (Pedroza et al., 2006). Furthermore, the support with mayor volume of saturation was PF ( $87 \pm 5$ ), followed by LCS ( $39 \pm 1.1$ ) and CaA ( $2 \pm 0.8$ ). The foam had greater volume of saturation due to its structural conformation, which generates pores of small diameter with greater compaction, thus preventing the exit of the water. In addition, it is an adsorbent by nature.

Besides, the LCS retains less quantity of water, because its support is composed of lignin, cellulose and hemicellulose polymers. For these chemical characteristics, the support has a certain hydrophobicity degree by the presence of aromatic and aliphatic hydroxyl groups (Corrales et al., 2007). As for CaA, the volume of saturation and the percentage of porosity were low due to its structural conformation which does not allow water to penetrate easily within pearls. Hence, the best support to use after the characteristics determined is the LCS, because of the low density, high porosity and low saturation volume.

### Effect of the support and time of colonization of *T. versicolor* over the decolorization and laccase production

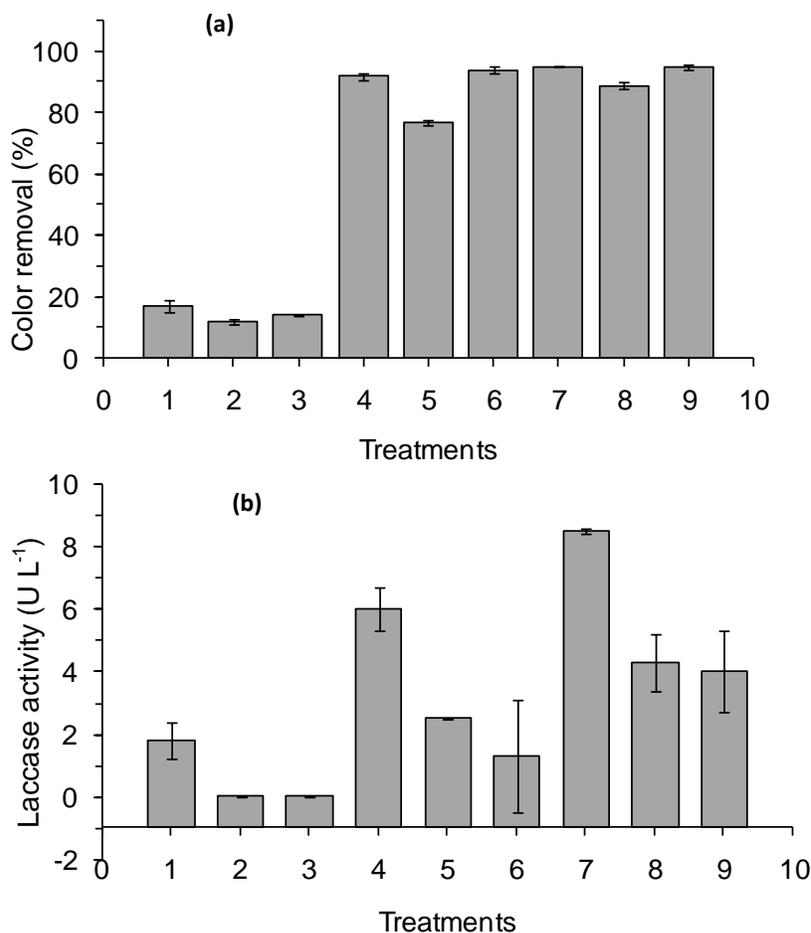
According to the analysis of variance (ANOVA), the time of entrenchment ( $X_1$ ) and the support used for the *T. versicolor* immobilization ( $X_2$ ), had a highly significant effect on the decolorization and enzymatic production ( $p < 0.0001$ ). It was observed that when the fungus was immobilized in LCS and CaA to 4 and 8 days, the decolorization obtained was 95, 92, 95 and 94%, respectively. On the other hand, the PF with 4 days of immobilization was the material with less decolorization

potential (77%) and Lac activity ( $2.4 \text{ U}\cdot\text{L}^{-1}$ ) (Figure 2). Mazmanci and Ünyayar (2005) reported that the mycelium age is important for decolorization rate and demonstrated that the immobilized mycelium was effective in 3 days old culture.

For the laccase activity, the interaction of the factors  $X_1$  and  $X_2$  in their high level had a significant effect on the production of the enzyme ( $p < 0.0001$ ). Results indicate that when the biomass was immobilized for 8 days on LCS, the enzymatic activity was  $8.5 \text{ U}\cdot\text{L}^{-1}$ . On the other hand, when the biomass was incubated for 4 days, the maximum activity was  $6 \text{ U}\cdot\text{L}^{-1}$ ;  $2.5 \text{ U}\cdot\text{L}^{-1}$  less than that for the high level of  $X_2$ . However, this value was superior to that obtained in PF and the CaA; hence the selected time for the immobilization of the biomass was 4 days. The enzyme synthesis probably was stimulated for the dye and for the chemical composition of the supports, due to its natural origin and compounds that are lignin (11.2%), cellulose (63%), holocellulose (82.4%), ashes (0.4%) and hemicellulose (19.4%) (Valcineide et al., 2005). Enayatzamir et al. (2009) using *Trametes pubescens* immobilized on stainless steel sponges, showed that laccase production increases with the immobilization and was related to the decolorization of RB5 obtaining more than 70% of decolorization in 120 h. However, they have used small amounts of dyes that allow a faster decolorization and increase the enzyme production.

In the controls, corresponding to the inactivated biomass and single support (LCS), the decolorization obtained were 8 and 5% respectively. According to these results, the color removal for absorption was low compared with the active biomass. Hence, the decolorization reached about *T. versicolor* in the different supports, was related mainly to the enzymatic activity and in low proportions to adsorptive capacity of the biomass. Also, according to our results, *L. cylindrica* sponge was selected for immobilization of *T. versicolor* in the degradation process of azo dyes due to their adsorptive properties, biodegradability, low cost, and low toxicity after the dye treatment with this bio-support ( $\text{DL}_{50} 5.5 \text{ mg}\cdot\text{L}^{-1}$ ) (Henao-Jaramillo et al., 2011). This material had good characteristics that favor the colonization process reaching a maximum of biomass per cube of  $0.47 \text{ mg}\cdot\text{mg}^{-1}$  LCS. Additionally, we observed by SEM that the colonization of the fungal biomass was inside the LCS and an extensive mycelium network recovered completely the sponge surface, generating biosorption of the dye and biodegradation of the support (Figure 3).

The CaA has been widely used in decolorization studies, nevertheless, the methodology entails to a greater risk of contamination, and the implementation of a system to a greater scale is expensive and undergoes processes of fragmentation by gaseous metabolites ( $\text{CO}_2$ ) produced by the immobilized microorganism. On the other hand, the PF only acts as physical matrix so that the hyphae of *T. versicolor* colonized the holes of the support. In the SEM microphotography, the vegetative



**Figure 2.** Color removal (a) and laccase activity (b) during the experimental design 3<sup>2</sup>. LCS-1 day (T1); PF-1 day (T2); CaA-1 day (T3); LCS-4 days (T4); PF - 4 days (T5); CaA-4 days (T6); LCS-8 days (T7); PF-8 days (T8); CaA-8 days (T9), 30°C.

mycelia are located in the superficial areas of the polyurethane foam cubes (Figure 4A to C).

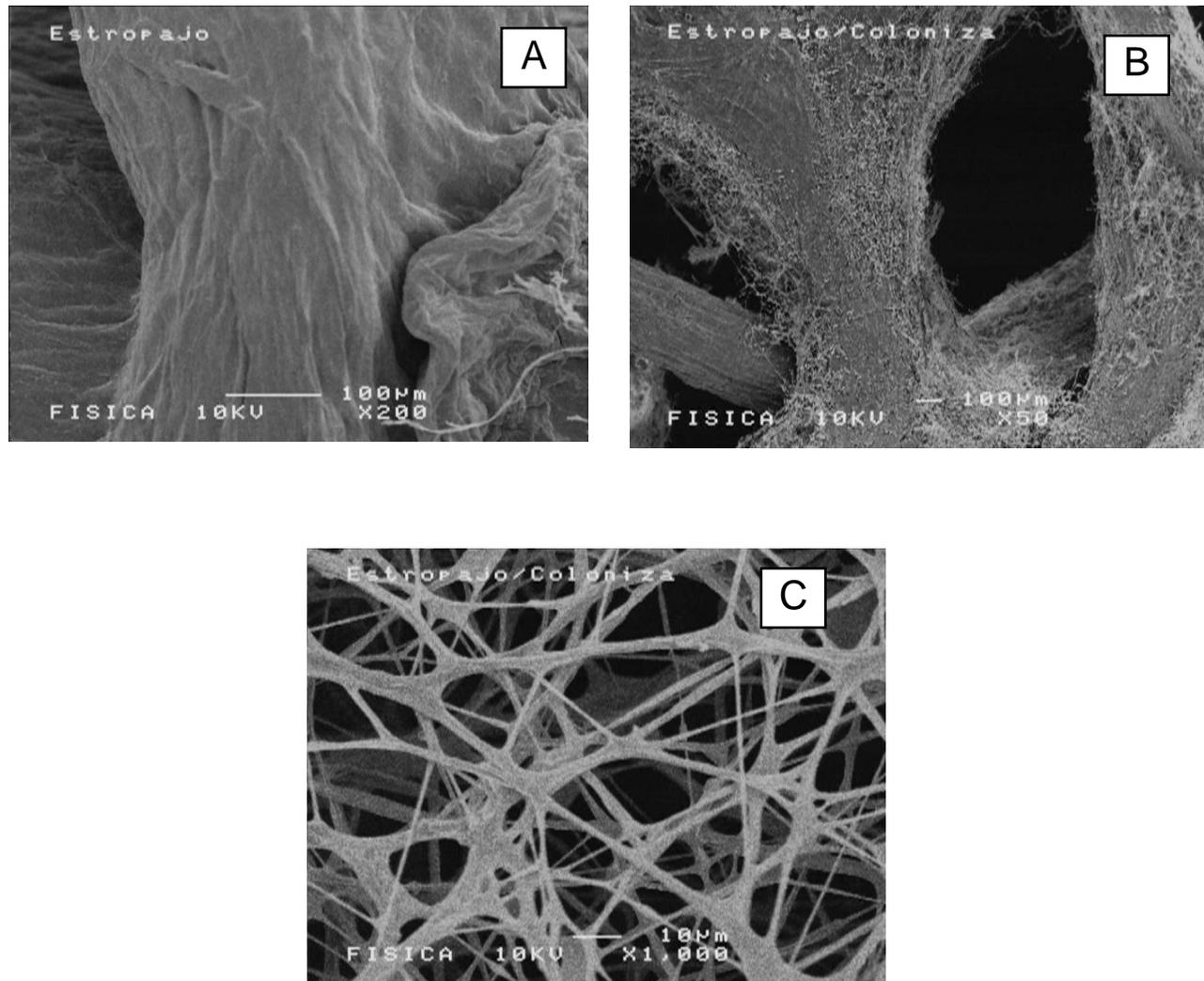
### Removal in biological reactor

#### Experimental design 2<sup>2</sup>: Effect of the biomass (free and immobilized) and carbon-nitrogen supplement over the decolorization and enzymatic activities

In the first experiment, the analysis of variance (ANOVA) showed that the free and immobilized biomass ( $X_1$ ) and nutritional supplementation ( $X_2$ ), did not have a significant effect on decolorization ( $p = 0.6711$ ) and enzyme production ( $p = 0.2893$ ). However, when the water was supplemented with simple carbon and inorganic nitrogen source, the decolorization was high (87%) with the free and immobilized biomass ( $p < 0.0001$ ) (Table 2). The results suggest that the addition of carbon and nitrogen was necessary to maintain the primary metabolism during

the process of biotransformation of the dye. In the treatments without nutritional supplement, the decolorization was low and it could be related with dye adsorption to immobilized biomass. Shin et al. (2002) found that the fungus in a long period of time without nitrogen source may result in a decrease in the enzyme production for decolorization and cell death, causing a decline in decolorization rate. They also showed that the decolorization rate increases when 1 g L<sup>-1</sup> of glucose is added, and also indicated that carbon substrates in textile dye effluents could be the cotton fibers or starch used in the process. The main mechanisms involved could be ionic force between the solubilizers groups of the dye and functional groups like carbonyl, amino and hydroxyl, which is present in the chitin (Urrea et al., 2006).

Carbon and nitrogen sources are necessary to maintain the metabolism of the fungus and to maintain the enzyme production. If these amounts had variations, the lignolytic enzymes productions will be modified (Mikiashvili et al., 2005). About the Lac activity in the 4 experiments



**Figure 3.** SEM microphotographs of *L. cylindrica* sponge without fungal biomass (A), *L. cylindrica* colonized with fungal biomass at 1 day (B), and *L. cylindrica* colonized with fungal biomass at 4 days (C).

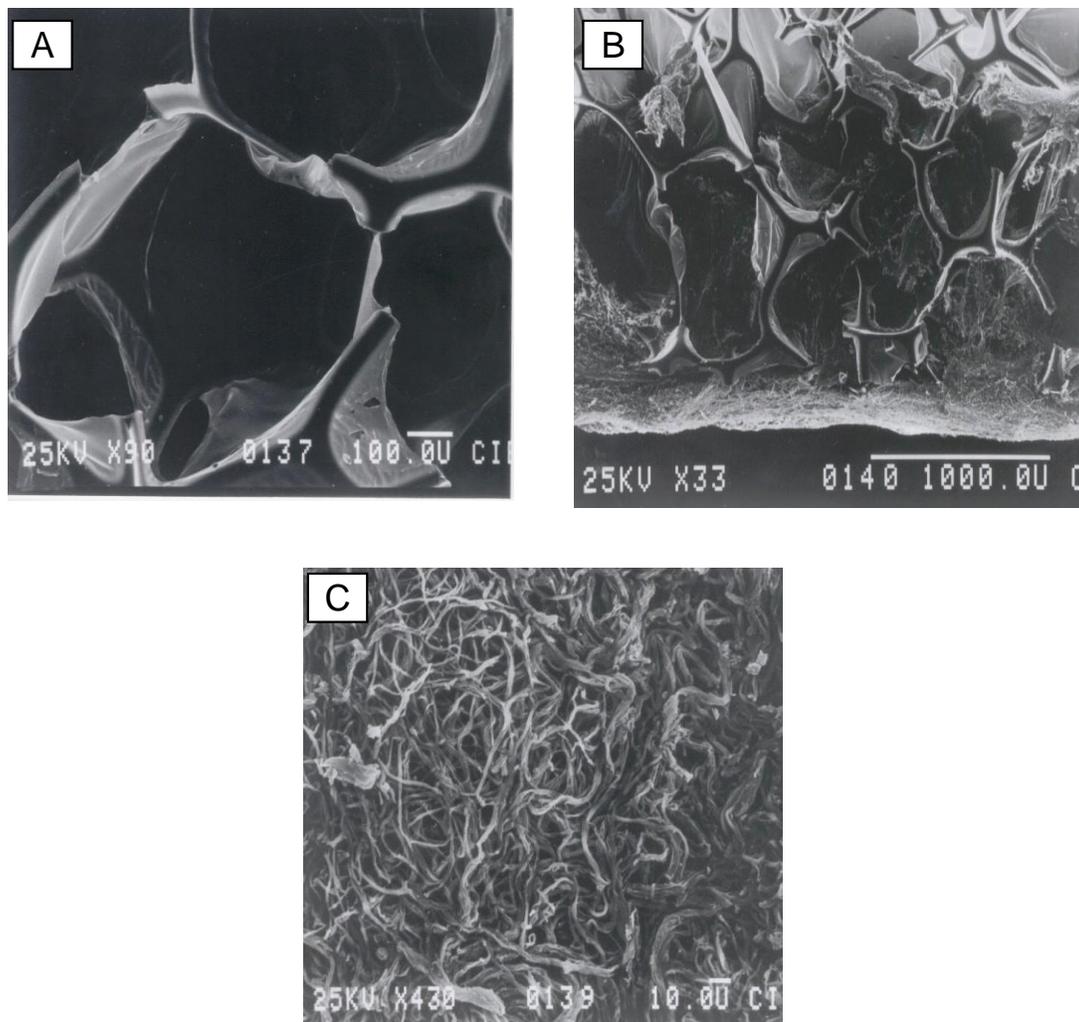
there were no significant differences ( $p = 0.2893$ ). Nevertheless, the variation between the levels of activity in the free and immobilized biomass in supplementation absence ( $0.085 \text{ U}\cdot\text{L}^{-1}$  and  $1.16 \text{ U}\cdot\text{L}^{-1}$ ), could have an induction by the lignin of the support that must be degraded to assimilate polymers of *Luffa* sponge and to maintain the associated growth metabolism (Ramsay et al., 2005).

### Dye removal in bubble column reactor

During the first 24 h, an accelerated increase of the decolorization (48%) was observed, possibly associated to physical mechanisms of biosorption (Kasikara et al., 2005; Papinutti et al., 2006), then the decolorization was increased gradually until it reached a maximum removal of 99% (296.6 ppm) at 7 days (Figure 5). The RB5 had

affinity for the *Luffa* sponge without biomass (62%) probably due to the cellulose polymer because this dye is designed to color the cellulose fibers such as cotton. This value is higher compared with the support removal in Erlenmeyer experiments due to the support amount increases generating more union sites for the dye in the LCS. However, the decolorization associated to the support adsorption did not surpass the removal and the degradation obtained in the biological treatment. About the time of maximum removal, the comparison of means showed that significant differences did not exist between the 4 and 7 days of treatment; according to this result the time chosen for valuing cycles was 4 days.

Considering the peroxidases and polyphenol oxidases production, the enzymes that were related with the decolorization were LiP and Lac, attaining maximum values of  $383 \text{ U}\cdot\text{L}^{-1}$  and  $0.84 \text{ U}\cdot\text{L}^{-1}$  at 7 days. The two enzymes could act synergistically on the dye like



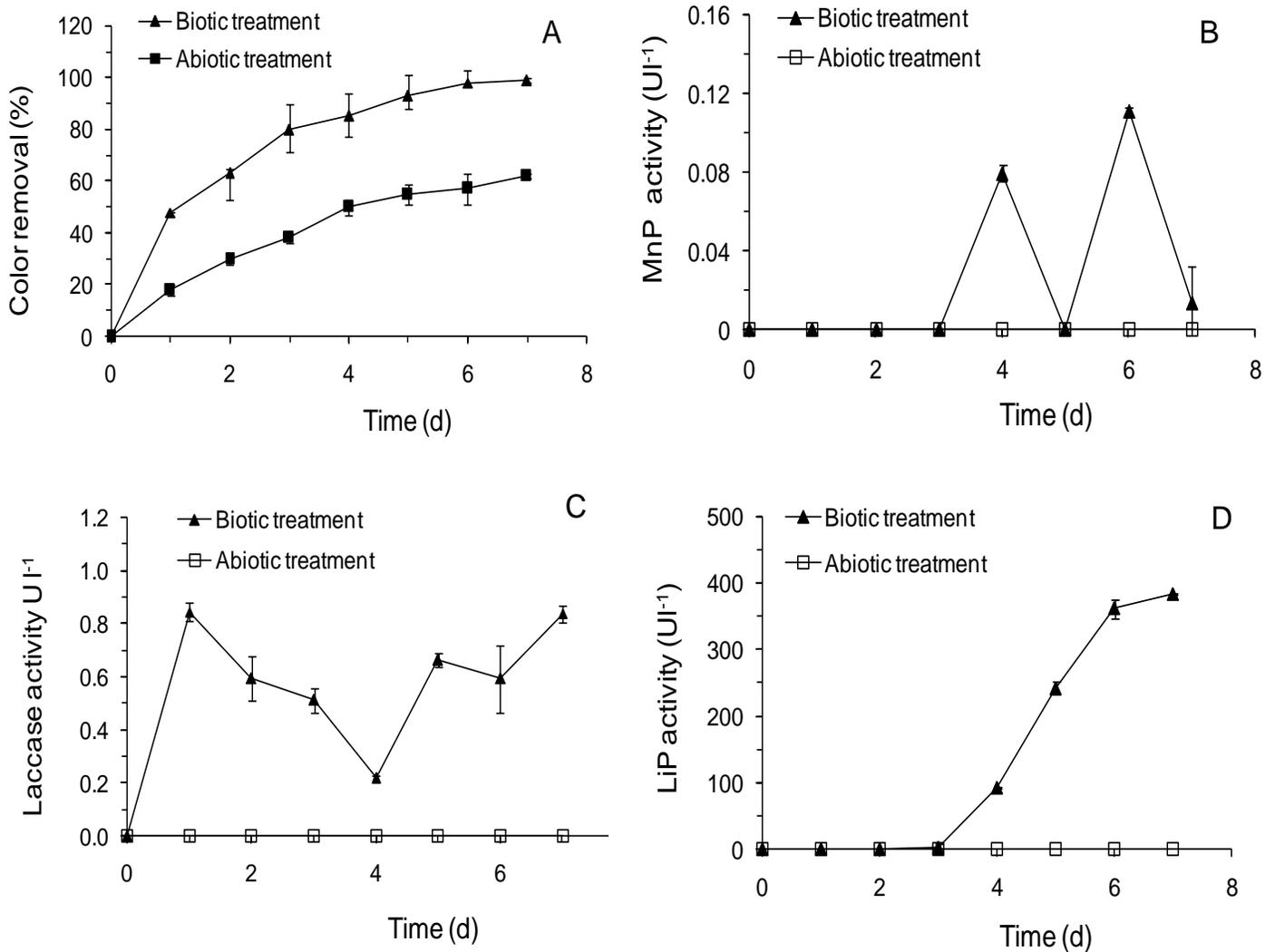
**Figure 4.** SEM microphotographs of polyurethane foam without fungal biomass (A), polyurethane foam colonized with fungal biomass at 1 day (B), and polyurethane foam colonized with fungal biomass at 4 days (C).

**Table 2.** Experimental design  $2^2$  results.

Treatment	Factor $X_1$	Factor $X_2$	Color removal (%)	Laccase activity ( $U l^{-1}$ )
1	-1	-1	$7.2 \pm 2.1$	$0.085 \pm 0.08$
2	+1	-1	$4.1 \pm 0.94$	$2.5 \pm 2.1$
3	-1	+1	$87 \pm 7.04$	$1.1 \pm 0.4$
4	+1	+1	$87.2 \pm 7.3$	$2.1 \pm 0.7$
Factor $X_1$ (Biomass)	Free biomass (-1) ; Immobilized biomass (+1)			
Factor $X_2$ (C/N addition)	Without carbon and nitrogen (-1); With carbon and nitrogen (+1)			

response to a mechanism of induction by the dye and phenols derived of lignin present in the support (Camarero et al., 2005). Additionally, the LiP had a very interesting behavior because it led after the 3rd day, obtaining a peak maximum at 7 days, coinciding with low levels of glucose, thus indicating that the enzyme is

expressed in response to the transition to secondary metabolism. Moreover, this could be because the LiP enzyme is one of the most important enzymes in lignin degradation (Shah and Nerud, 2002; Martínez et al., 2005) and the principal content of the support is lignin which activates the fungus enzyme production after the



**Figure 5.** Removal kinetics in bubble column reactor. (A) Color removal; (B) MnP activity; (C) laccase activity; (D) LiP activity, 8 day, 30°C, air flow of 500 cc·min<sup>-1</sup> in non-sterilized conditions.

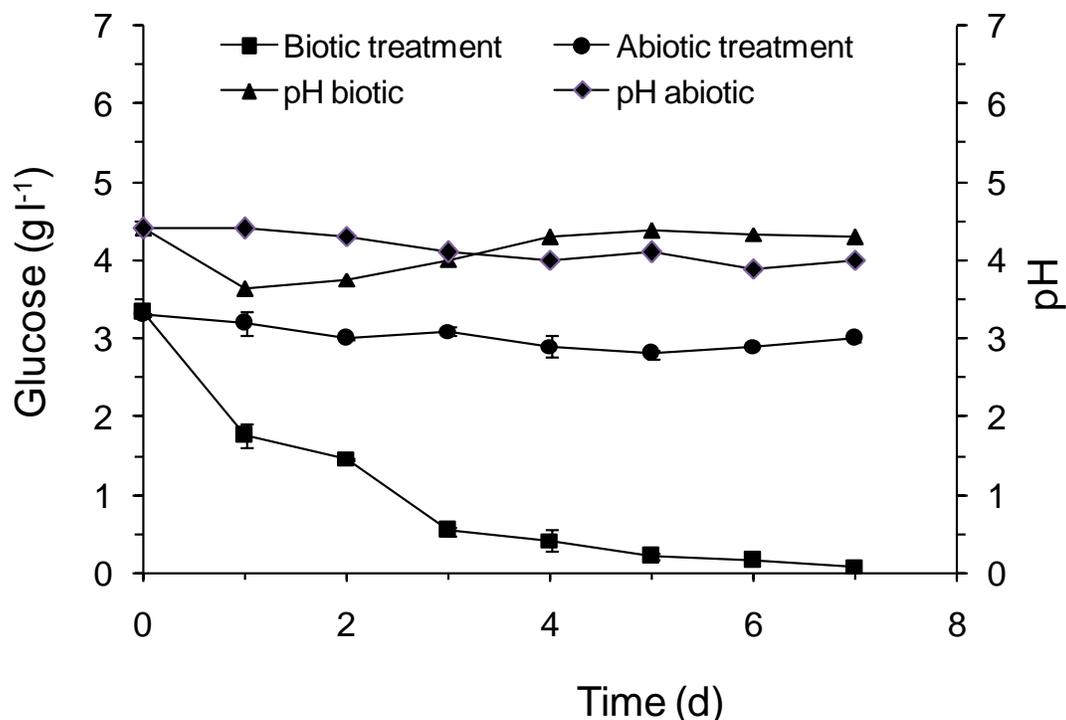
glucose consumption, since the fungus is a ligninolytic fungus. It is important to note that *T. versicolor* is not a strain that produce high quantities of LiP (Novotny et al., 2001, Viral et al., 2005), although, in this study, it displayed high values. Previous studies (Karimniaae-Hamedani et al., 2007) suggested that manganese peroxidase is the main degrader enzyme of lignin and it has great ability of decolorization. The *Luffa* sponge, as a lignocelulosic material can provide precursors for the production of the MnP enzyme, which in this study presented a very irregular expression that it does not correlate with the reduction of color units.

The reducing sugars, which decreased as a function of time by using the same primary metabolism, for this reason generated a slight decrease in the average pH of the production of acids. Under these conditions, the enzymes carried the catalytic reaction to pH near the optimum favoring the removal color (Figure 6).

### The sequencing batch experiments

With the purpose of overcoming the limitations inherent in multiples cycles of operation, this study analyzed a 5 sequential decolorization cycles. The continuous enzymatic production and decolorization were restricted by physiological and physical characteristics of the fungus. A high level of decolorization (69%) was shown during the 3 cycles (12 days), which diminished until 20% in the last cycles (Table 3). The enzymatic activity with similar tendency showed that in the first 3 cycles, the values oscillated between 1.3, 0.25 and 206.4  $U \cdot L^{-1}$ , for Lac, MnP and LiP, respectively. In the 4th and 5th cycles, the Lac production diminished and the decolorization percentage decreased, demonstrating that this enzyme is closely related with the chromophore group biotransformation.

On the other hand, the LiP and MnP were present in all



**Figure 6.** Consumption of glucose and pH in the air bubble column reactor (8 day, 30 °C, air flow: 500 cc·min<sup>-1</sup>, non-sterilized conditions).

**Table 3.** Results of the sequencing batch experiments.

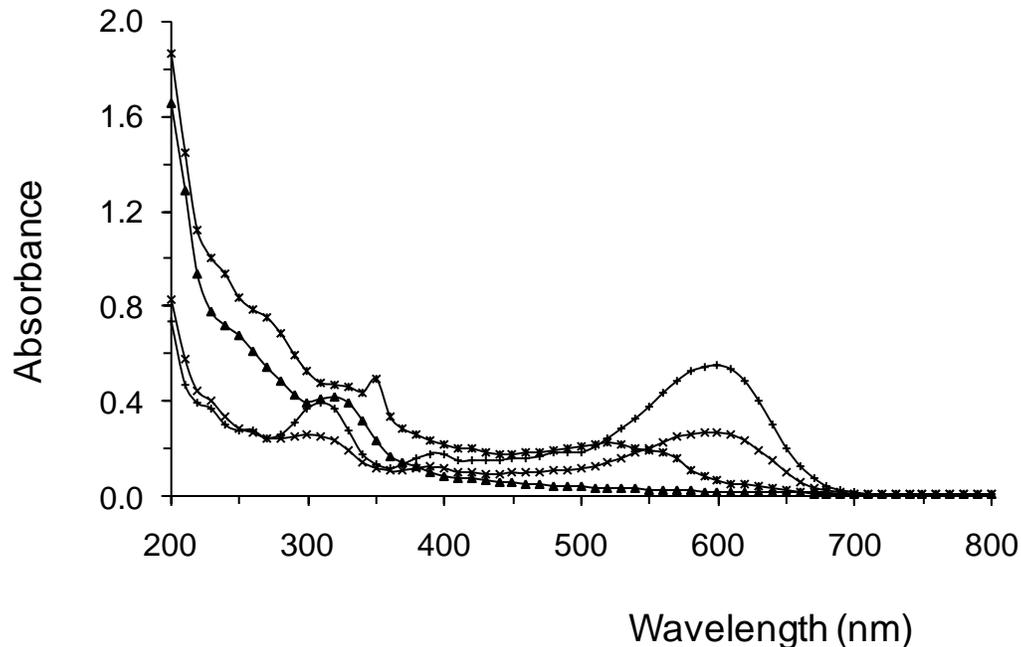
Variable	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Color removal (%)	76.7 ± 0.1	75.7 ± 0.6	58.2 ± 2.6	20 ± 0.8	16 ± 1.3
Laccase activity (U L <sup>-1</sup> )	1.5 ± 0.02	1.5 ± 0.001	1.7 ± 0.003	0.48 ± 0.002	0.23 ± 0.001
MnP activity (U L <sup>-1</sup> )	0.21 ± 0.003	0.32 ± 0.002	0.26 ± 0.004	0.36 ± 0.001	0.36 ± 0.002
LiP activity (U L <sup>-1</sup> )	392.3 ± 1.0	371 ± 1.6	357.5 ± 5.6	271.5 ± 4.1	274 ± 0.9

the cycles; probably the peroxidase oxidized the other functional group into the dye or produced the lignin degradation present in the support. The reduction in the ability to remove color could be associated with the formation of by-products, saturations of the biomass and an uncontrolled growth of the mycelium. Under these conditions, the systems can have anaerobic or micro-aerophilic areas that could cause death of the microorganism already known to be a fungus strict aerobic (Pedroza et al., 2006).

### UV/VIS spectrophotometric analysis

The ultraviolet and visible absorbances (200 to 800 nm) of RB5 dye samples were monitored to evaluate the biodegradation or the bioadsorption. This dye is composed of auxochrome-chromophore complex, consisting of two azo groups, four aromatic rings and the

sodium sulphate. The visible portion of the spectrum shows a major peak at 600 nm, and in the UV portion, the major peak was 310 nm (Figure 7). The high color removal occurred in the first 24 h in the visible spectrum and the removal was related with adsorption to biomass and the support. The area of the major peak decreased gradually until very low absorbances (0.067 and 0.02) were recorded at 4 and 8 days, respectively. For the UV region, the peak at 310 nm decreased over time and new peaks were observed at 350 and 200 to 250 nm. These peaks may be by-products such as benzenes (255 nm) or aromatic rings (205 to 260 nm). Similar results were published by other researchers in their studies, reporting that in the azo biodegradation with white rot fungus, the by-products that can be formed are benzenesulfonic acid, 4-hydroxy-benzenesulfonic acid, 4-hydroxy-benzenesulfonic, 3-methyl-4-hydroxy-benzenesulfonic, 3-hydroxybenzil alcohol, 1,2-naftoquinone, 4-nitroaniline, 4-nitrobenzene, 4-nitrophenol and 4-nitroanisole (Sharma



**Figure 7.** UV/VIS spectral analysis of Reactive Black 5 biodegradation process. (+) 0 hours; (○) 1 day (x) 4 days (\*) and 8 days (▲).

et al., 2004, Zhao and Hardin, 2007).

## Conclusion

The LCS showed the best characteristics to be a suitable support because it has desirable porosity and saturation volume that allows the immobilization of microorganisms, as well as improving the pollutants removal. The time of immobilization was reduced to 4 days, thereby improving the efficiency of the system and reducing the treatment time. Also, the addition of a simple carbon and a nitrogen source actively maintained the primary metabolism of the fungus and increased the color removal; in addition, the enzymatic production increases in treatments with immobilized biomass because the support components act like inductors.

Furthermore, the bioport presents high adsorption to the structure at the first 24 h, and the removal was due to the enzymatic activity and metabolism process. More also, the established time for the treatment with high removal percentages was 4 days in bubble reactor. Decreasing the time used in other researches and supporting 3 cycles of 4 days (12 days) showed good results of color removal and enzymatic production.

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