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Removing reactive dyes using fungus *Bjerkandera adusta*[1]

Remoción de colorantes reactivos empleando el hongo Bjerkandera adusta[1]

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Abstract

The manufacture of textiles in Colombia represents an important economic activity, with participation in 6 % of exports and generates sales of non-traditional products around 13 %. This industry uses about 15 % of the total water used for industrial work, discarding about 170,000 tons of wastewater per year in Colombia. The contamination caused in the dyeing stages is highlighted since the dyes used have a fixing range between 68 and 80 %. Initially, in the development of this project, the physicochemical and microbiological composition of the affluents from the dyeing process of the textile sector was determined, for which parameters such as alkalinity, hardness, BOD, COD, conductivity, solids and dissolved oxygen were taken into account. At the same time, the removal of dyes from synthetic wastewater was evaluated, using the white rot fungus Bjerkandera adusta. The removals obtained for the family of reactive dyes from the Bezaktiv family (vinyl sulfonic dyes) were 30 % for the blue V-2B 133 dye Bezaktiv, for the yellow dye V-5 GL was 91 % for the red dye Bezaktiv V-5B was 74 % using for the three media the Park-Robinson medium.

Keywords: dyeing reactive; hongos ligninolíticos; degradación.

Introduction

The manufacture of textiles for Colombia has been along with the history an important economic activity, in the year 2014, it represented 9,2 % of the manufacturing industry. For your part, the EBITDA (Earnings Before Interest, Taxes, Depreciation, and Amortization), increased in 12,4 % in the year 2014 opposite to 2013, when $ go on from 279.322

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million to 313.955 $ million, which indicates a major efficiency of the income for sales. (Superintendence of Companies, 2015)

The above mentioned industry uses 15 % of the total water used for industrial labors. The obtaining of the textile with his diverse tonalities and ended it generates approximately 170.000 tons of residual water per year in Colombia. These waters contain waxes, fats, own pectins of the fiber, gluings added in the stages of thread and fabric, you will help and colorings of the stages of preparation and dyeing of the substratum (Of Jager, Sheldon & Edwards, 2014). In Colombia the resolution 0631 of March 17, 2015, regulates the unload of the affluent of the textile industry on the water bodies (Department of Environment and Sustainable Development, 2015), nevertheless, this one doesn´t do specific mention to the values demanded of unloading of colorings. It is worth a sorrow mentioning that in the resolution 2016 of 26 of October of the year 2012 sent by the Metropolitan Area of Medellin, it is mentioned and the concentrations of color are indicated allowed (50-75 units Pt-Co) in the affluent from ones the textile sector (Metropolitan Area, 2012).

The colorings not fixing in the stage of dye, represent an important factor in the pollution of the water since in the process of dyeing with colorings reagents it is fixed between 60-80 % (Kumar, Raut, Bandyopadhyay & Raut, 2016; Ruiz, 2011). The presence of colorings in the water reduces the step of the solar light, which can generate an imbalance in the aquatic ecosystems and provoke disagreeable visual effects in the natural landscape painting (Osorio, Vidal, & Quintero,2011).

To reduce the problems mentioned previously, they have proposed alternatives of treatment that involve biological processes which have chowed a great potential due to the fact that they are efficient, friendly with the environment and attractions economically, (Anastasi *et al.*, 2010). Different studies show the capacity that there have the fungi of the white rotting of the wood due to the fact that they possess a system oxidative that exhibits a high degree of specificity, allowing them to degrade a wide number of recalcitrant compounds besides the lignin (Anastasi *et al.*, 2010; Barrasa *et al.*, 2014). Nowadays there are in use aerobic and anaerobic systems, with fungi and bacteria for the discoloration and mineralization of the present colorings in the above mentioned waters (Kumar *et al.*, 2016; Senthilkumar, Perumalsamy & Prabhu, 2014). On there having been applied this type of biotic Treatment the pollutants generally are metabolized by the microorganisms by means of biochemical reactions of type oxide - reduction, though also they can realize reactions of hydroxylation, hydrolysis, dehalogenation, and dealkylation (Kumar *et al.*, 2016).

The fungi can degrade organic complex compounds by means of the production of enzymes of type ligninolytic extracellular, most brought they are: laccase, manganese - peroxidase and peroxidase (Singh, Singh, & Singh, 2015; Tuomela & Hatakka, 2011); it has been confirmed that many species of fungi like Pleurotus ostreatus, Pichia sp., Penicillium sp. and Candid tropicalis, realize an adsorption of colorings on their surface but not a chemical degradation (Kumar *et al.*, 2016; Taha, Adetutu, Shahsavari, Smith, & Ball, 2014). Between the different types of fungi, Phanerochaete chrysosporium and Bjerkandera, *Phanerochaete chrysosporium* and *Bjerkandera adusta* are effective microorganisms to realize processes of degradation of colorings, both have been brought widely as models in the remediation of affluent of textiles due to the fact that they can remove more than 75 % of the colorings reagents of the affluent ones (Knapp, Newby, & Reece, 1995; Osorio *et al.*, 2011).On the other hand, P. chrysosporium possesses the aptitude to degrade complex compounds as the starch, the cellulose, the pectin, the lignin, which they are substances that they find in the affluent ones of the textile industry (Podgornik, Grgić, & Perdih, 1999; Kirk *et al.*, 1990). Therefore, for the textile sector it continues being a need to raise alternatives and environmental solutions that tend to control the pollutant load of his dumping, is for this that in this work realized an evaluation of the potential that has the mushroom Bjerkandera adusta to remove colorings reagents, due to the fact that it is capable of degrading complex substrate across a specific enzymatic system for every family of coloring, according to they it bring (Anastasi *et al.*, 2010; Eichlerova, Homolka, & Nerud, 2007; Heinfling, Martínez, Martínez, Bergbauer, and Szewzyk, 1998; Tuomela & Hatakka, 2011; Valentin *et al.*, 2007).

This evaluation of the future will be able to contribute to the decrease of the environmental impact caused by the water sources of the region for the affluent textiles.

Materials and methods

Colorings

For the development of this work were colorings in use reagents blue Bezaktiv V-2B 133 (? = 600 nm), yellow Bezaktiv V-5 GL (? = 410 nm), red Bezaktiv V-5B (? = 510 nm), belonging to the family of colorings vinylsulphonics, these they were supplied by the company CHT BEZEMA, which indicated us that they are used widely by the industries of the textile sector in the city of Medellin, Colombia.

Analytical methods

• Quantification of growth and degradation of color in a solid way

There were took measurements of the diameters of haloes of degradation and growth every 12 hours with a graduated rule. This procedure followed up to the complete settling or discoloration of the box, the information was obtained by triplicate for every coloring.

• Determination of biomass

There was used the technology of dry weight, there took samples of 4 mL which filtered and dried to 105 °C up to obtain constant weight.

• Determination of the discoloration degree

Every sample was centrifuged to 10000 rpm during 10 minutes, in the supernatant, it was read absorbance in a spectrophotometer, according to the length typical of every coloring; the concentration of the coloring obtained by means of a curve of calibration concentration of coloring (g/L) vs absorbance elaborated before. The information was obtained by triplicate for every coloring.

• Physicochemical parameters determined to the samples of wastewater

To realize the choice of parameters to determine in the affluent ones, the brought was born in mind by Kumar *et al.*, (2016) and the resolution 0631 of March 17, 2015, these mention parameters as: oxygen dissolved (OD), Biological Demand of Oxygen (DBO), Chemical Demand of Oxygen (DQO), solid total (ST), solid suspended total (SST), pH, temperature, alkalinity, conductivity and salinity. The determination of these parameters was realized according to the protocols of the IDEAM TP 0436, TP0088, TP0080, TP0211, TP0084, TP 0082.

• Microorganisms

Bibliographical reviews were realized in order to establish the potential of different types of microorganisms, which include from aerobic, anaerobic bacteria and fungi of white rotting (Knapp, 1995) the above mentioned present a great capacity of degradation of Azo type colorings (Ghodake, Jadhav, Dawkar, & Govindwar, 2009), triphenylmethane, colorings sulphonic polymers (Joshi, Iyengar, Singh, & Garg, 2008), anthraquinones (Kabbout & Taha, 2014). These properties and described previously in the introduction they were those that we take as parameters of selection to work with the mushroom *Bjerkandera adusta,* this one was donated by the group of bioprocesses, assigned to the department of Chemical engineering of the University of Antioch. For the activation of the vine-stocks the half modified Kirk was used (Kirk *et al.*, 1990), which consists (g L-1): glucose 10, tryptone, thiamine 2, chloride of calcium 0,1, traces of you go out 100 mL L-1 for a pH of 4,5. The way of maintenance of the vine-stock was the Agar-Papa-Dextrose (PDA) of the house Merck ®, for both means the temperature of incubation was 30 °C.

• Selection of degradation media:

There were evaluated three means, which have been used for the degradation of colorings by different authors: I happen Kirk (Cardona, Osorio, & Farmer, 2009; Kirk *et al.*, 1990; Osorio *et al.*, 2011), media Zouari (Zouari - Mechichi *et al.*, 2006) isolated from decayed acacia wood (from Northwest of Tunisia, and the middle Park-Robinson (Robinson, McMullan, Marchant, & Nigam, 2001). The effect that has the way on the degradation of three colorings was realized in a solid way. The evaluation consisted of evaluating every coloring in every way of culture to a concentration of 125 ppm, according to Cardona *et al.,* (2009) to major concentrations the mushroom can keep out, every box was inoculated by a disc of 7 mm of mycelium with the mushroom B. austere, this mycelium was located in the center of the box Petri. Also, the presence of sawdust was evaluated in the way as agent elicitation due to the fact that the enzymes ligninase aren´t constitutive and need to be induced by the way of growth (Kirk *et al.,* 1990; Robinson *et al*., 2001). All of this was realized in conditions of sterility, asepsis, and triplicate.

• *Inoculum of degradation*

The inoculum for every Erlenmeyer consisted of 4 circles of 7 mm of diameter, taken of Petri's box incubated to 30 °C in a time of 7 days.

• *Evaluation of degradation in liquid synthetic way, batch system*

Once selected the way of culture, there decided the kinetic one of degradation of three colorings for the mushroom B. austere in liquid way. The assemblies I cultivate they were realized in an Erlenmeyer of 250 mL by a volume of work of 200 mL, to a temperature of 30 °C, one agitation of 150 rpm, a pH of 4,5 and took 125 as a concentration of coloring ppm; this value has been brought by different authors as an inhibitory concentration for the mushroom (Cardona *et al.,* 2009; Osorio *et al.,* 2011; Senthilkumar *et al.,* 2014; Taha *et al.,* 2014); samples took every two days, for each one the concentration of coloring decided by means of the technology of degree of discoloration mentioned previously

Results and discussion

Physicochemical parameters determined to the samples of waste wáter

The characterization was realized on samples of the affluent of the textile process obtained in the laboratory of dry cleaner's shop of the CTGI. The physicochemical parameters can observe in Table 1.

Table 1. Physicochemical parameters of the effluent one that goes out of the processes of dyeing Selection of the way of culture

Afluene textile Characterization	
Alkalinity (ppm)	744
O.D. (ppm)	3,51
pH	11,78
Temperature (°C)	37
Conductivity	45.700
%O.D.	47
Salinity	29,73
ST (ppm)	22.808
SST (ppm)	207
DBO (mgO_2/L)	574
DQO (mgO_2/L)	2048

Source: the authors

Selection of the way of culture

There were evaluated the means Park-Robinson, Kirk, and Zouari-Mechichi. In Figure 1. They present the results of the evaluations of the means of culture and his behavior in presence or absence of the elicitation (sawdust 2 gL-1). It was observed that for the second day the degradation of coloring was approximately 80 % in the way Park-Robinson, this was calculated by the diameter of degradation measured. The total degradation of color agent appeared for the third day of culture in the boxes with the way Park-Robinson more elicitation. The boxes that did not have agent elicitation were late 7 days to start showing activity degrader. The presence of the agent elicitation favors the degradation of the coloring on having stimulated, in a natural way, the production of enzymes. This behavior agrees with the brought for Barrasa *et al.,* 2014; Hatvani & Mécs, 2002; Osorio *et al.,* 2011, which affirm that this type of agents induces the production of enzymes extracellular.

From Figure 1 can infer to him that the way that favors the degradation of coloring for the mushroom B. austere is the way Park-Robinson. On having analyzed the components of the way, we find substances that play an important paper in the degradation of colorings and that have been discussed by some authors:

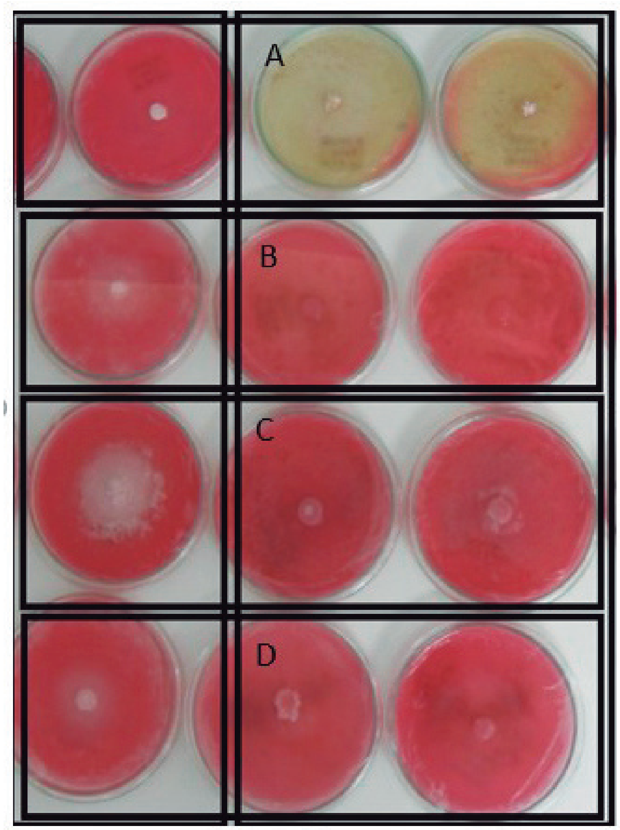

Figure1. Degradation of the coloring red Bezaktiv V-5B on the part of the mushroom B. austere on the second day of culture.
To: Park - Robinson, with sawdust, B: Kirk with sawdust,
C: Zouaori-Mechichi with sawdust

- ***Glucose:*** Kumar *et al., (*2016) recommend top concentrations of glucose to 5 g/L, since the glucose is the source of carbon that sustains the microorganism while the process of degradation, this due to the fact that the coloring is not consumed by the mushroom in the process of degradation. Senthilkumar *et al.,* (2014) bring that an increase in the concentration of glucose generates increase in the growth of the biomass and the discoloration increases in 0,3 %

- ***Nitrogen:*** an essential element for the growth micellar and the production of enzymes, which are the persons in charge of the break of the links azo. The speed of discoloration of the molecules of colorings depends on the speed of break of the links azo (-N=N-) in the molecule of coloring. With the increase in the concentration of nitrogen of 0,01 % to 0,5 %, the rate of discoloration increases by 40 % (Senthilkumar *et al.,* 2014). The composition of the way Park-Robinson shows itself in Table 2.

Table 2. Way of culture Park-Robinson used for the supplementation of the way of degradation

Medium	Components	Concentratión [gL^{-1}]
Park-Robinson	Glucose	0,7
	KH_2PO_4	0,2
	NH_4NO_3	0,1
	$MgSO_4$*$7H_2O$	0,5
	Agar-Agar	15
	Sawdust	2

Source: the authors

With the selected way Park-Robinson there was evaluated the degradation of the colorings blue Bezaktiv V-2B 133 and yellow Bezaktiv V-5 GL to an initial concentration of 125 ppm in solid way. The degradation of these colorings had a very similar behavior to finding for the coloring red Bezaktiv V-5B; nevertheless, the degradation I am late one more day (the third day) on having been in presence of the agent elicitor.

Figure 2 presents the degradation of the colorings blue Bezaktiv V-2B 133 and yellow Bezaktiv V-5 GL to the third day of culture and in presence of sawdust.

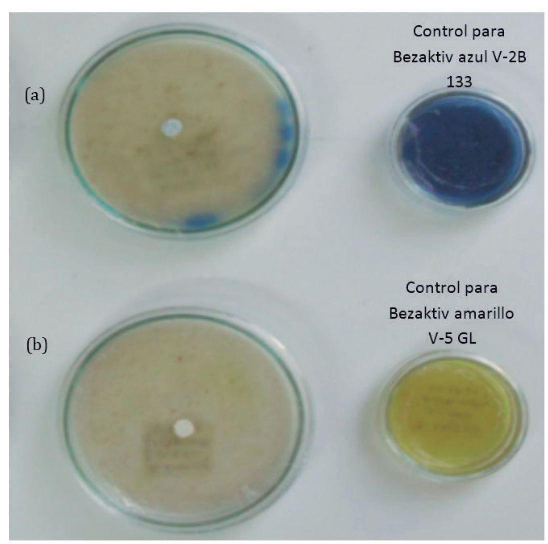

Figure2. On the 3rd of the degradation of the colorings blue Bezaktiv V-2B 133 (a), yellow Bezaktiv V-5 GL (b) in way Park-Robinson with sawdust

Evaluation of degradation in liquid synthetic way Batch System

• Yellow dye Bezaktiv V-5GL

During the evaluation of degradation of coloring for the mushroom B. austere, we could demonstrate the capacity of adjustment and of degradation that would have the microorganism opposite to the present coloring in the affluent ones. Since result of this evaluation thought that the microorganism was capable of growing and degrading the coloring to concentrations of 125 ppm.

From Figure 3 it is possible to affirm that the maximum percentage of degradation of coloring is given after passed 18 days of treatment and his value was 92 %. Anastasi *et al.*, (2010) bring values of degradation of 90 % for affluent synthetic of colorings reagents yellow and degraded with B. austere; Eichlerova *et al.*, (2007) bring values of degradation of 76 % in a time of 28 days when there is in use the mushroom B. *adusta*.

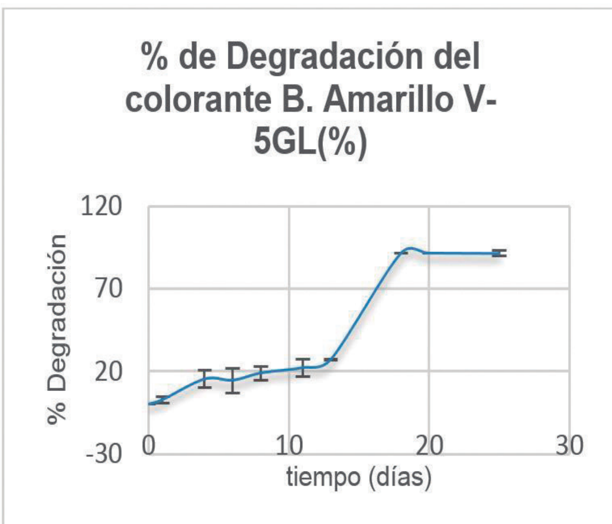

Figure 3. Kinetic of degradation of the yellow dye Bezaktiv V-5 GL, with the mushroom B. austere, on the way Park-Robinson

• Blue dye Bezaktiv V-2B-133

Since the result of this evaluation can steady that the microorganism was capable of growing and degrading the coloring to an initial concentration of 125 ppm.

Figure 4, it is possible to observe that the percentage of degradation of maximum coloring happens after passed 13 days of treatment and the maximum percentage of degradation was 30 %.

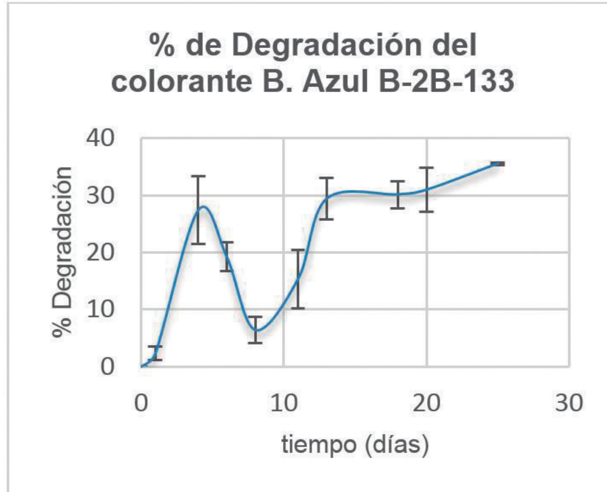

Figure 4. Kinetic of degradation of the blue dye Bezaktiv V-2B 133, with the mushroom B. austere, on the way Park-Robinson

In Figure 4 it reveals that in the 4th one presents a maximum beak of degradation and in the 8th a minimal value of degradation, during the same days there was demonstrated in the mushroom a blue coloration, which later disappeared. Several authors have tried to explain this by means of two phenomena: the first one is the absorption of coloring on the biomass, in this one the coloring reacts chemically with the functional groups OH - and C=O of the present chitin in the cellular wall of the fungi (Tuomela & Hatakka, 2011), the other fraction of coloring that doesn´t react with the chitin is liberated again to the way (Kabbout & Taha, 2014; Robinson *et al.*, 2001). Another phenomenon owes principally to the trend that the colorings have reagents for being adsorbed on the surface of the microorganism, Osorio *et al.*, (2011) they argue that the degree of adsorption of the coloring on the biomass of the mushroom is an important mechanism principally in presence of you go out, where it can reach up to 36 % of the removal of the coloring.

• Red dye Bezaktiv rojo V-5B

This evaluation is realized for the vine-stock B. austere, it is possible to say that the microorganism was capable of degrading the coloring when the initial concentration of coloring was of 130 ppm.

In Figure 5, it is possible to think that the percentage of degradation of maximum coloring is given after passed 18 days of treatment and the maximum percentage of degradation was 75 %.

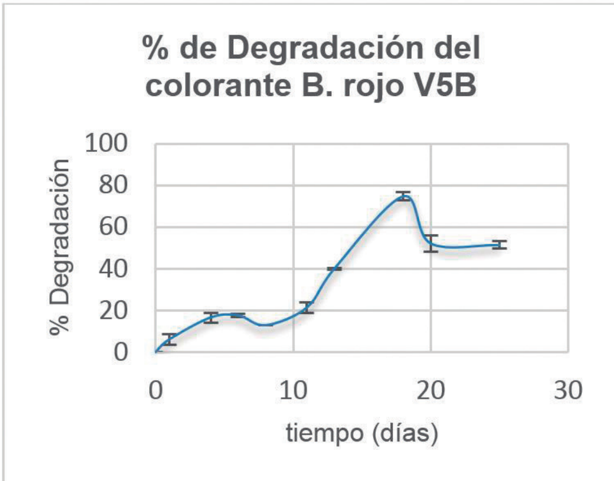

Figure 5. Kinetic of degradation of the red dye Bezaktiv V-5B with the mushroom B. adusta, on the Park-Robinson

The time of adjustment in the affluent one was 8 days. The change of earring that is given in the exponential phase (between the 13th and 18th) has been brought as a change in the mechanism of degradation that has the microorganism, this owes principally to the production of another group of enzymes that would realize the same effect of degradation on the coloring as it is reported by Ghodake *et al.*, (2009), Barrasa *et al.*, (2014).

Conclusions

The mushroom *Bjerkandera adusta*, has the aptitude to bleach affluent of the textile industry, in this particular case; colorings reagents of type vinylsulphonics. Of the three evaluated colorings, the one that presented major percentage of degradation was the yellow Bezaktiv V-5GL with a percentage of 91 %.

Bearing in mind that the concentrations to which the microorganism presented growth and degrading capacity think far below of the concentrations that it can have an affluent royal textile, a process recommended is to use combined (physical or chemical treatment) first; so that the concentration of coloring is diminished up to values near to the 150 ppm and later a biotechnological process.

The process of adsorption of the coloring in the surface of the mushroom can increase the percentage of degradation by 25 %, this can meet favored by the presence of inorganic salts.

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