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Removal of textile dye Novacron Yellow using the fungal biomass based on *Cladosporium sp.* genus

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Abstract. In this study we present an economically viable adsorbent material based on fungal biomass of genus *Cladosporium sp*. The molecule used as adsorbate was the commercial dye Novacron Yellow (NY). The water sample used in the fungal isolation was collected in the effluent from a textile factory. The ITS-rRNA sequence of fungal biomass used in this study match with *Cladosporium sp* genus. The FTIR spectrums of fungal biomass are compatibles with chitin structure. The maximum adsorption capacity exhibited by fungal biomass is 56mg.g^{-1}. These results can inserts the biomass based on *Cladosporium sp* as a good alternative to adsorption of organic dyes with high molecular weight such as Novacron Yellow.

Keywords: *adsorption*, *textile dye*, *fungal biomass, Cladosporium sp.*

Introduction

The scarcity of drinking water and the increasing of industrialization are factors that aggravate the environmental impact, mainly in developing countries. In this scenery, the increase of emerging chemical pollutants (ECP´s) is observed with great attention by the scientific community (LIU et al ., 2014). The ECP are classified how substances that not have well-established regulamentation however possess a potential threat to human health (DEBLONDE et al., 2011). Another threat to human health in industrialized countries is the organic dyes. This class of contaminant is widely used in the textile industry and its disposal is a controversial question (HASAN et al., 2011). Thus, many scientific articles are focused on degradation and adsorption of these pollutants. The dye biodegradation using bacteria is the most common alternative for their removal from industrial effluents (PETR et al., 2011). However, the bacterial activity depends strongly of factors such as temperature, oxygen availability and nutrient availability (YUMNA et al., 2011). By the other hand, the adsorption is a cheaper and more feasible process, especially for emerging countries [6]. Due its abundance, facility of obtaining and low cost, the biosorbents are good options as adsorbents (VAN et al, 2015). The biosorbents based on fungal biomass have, among many others substances, chitin in their composition. The presence of amino and hydroxyl groups favors the high adsorption ability (VAN et al, 2015). Biomass of different fungi genus is studied as adsorbent for organic molecules (HUA et al, 2012). Among the various fungal genus, the use of *Cladosporium* sp. is very promising because their growth is little influenced by toxic compounds and the low availability of nutrients (HUA et al, 2012).

Thus, in this paper is presented the adsorption of textile dye Novacron Yellow (NY) using dry fungal biomass of *Cladosporium* sp. genus isolated from a stream that receives effluent from a textile industry. The taxonomic affiliation of the fungus was made by partial sequencing and analysis of the ITS-rRNA region. The fungal biomass was characterized with SEM, EDS and FTIR analysis. The dye adsorption was analyzed by UV-vis analysis compared with Freundlich isotherm model.

Methods

The water sample used in the fungal isolation was collected in the effluent from a textile factory in Sete Lagoas - MG, Brazil. A water sample aliquot of 10 ml was added to 90 ml of autoclaved NaCl solution with concentration of 9mg.L^{-1}. After orbital shaking for 10 minutes at 200 rpm, a serial dilution was made until the final concentration of 10^{-4} mg.L^{-1}. An aliquot of 1ml of each dilution of 10^{-2}, 10^{-3} and 10^{-4} mg.L^{-1} was taken to be spread in Petri dishes containing Sabouraud Dextrose Agar culture medium (SDA). For the inhibition of the bacterial growth it was added 1 ml of streptomycin solution (100 mg.ml^{-1}). Incubation was done for a period of 7 days at 28°C. The fungus that experienced the fastest growth were isolated and used in the present study.

The isolated fungus was deposited in the Collection of Multifunctional and Phytopathogenic Microorganisms of the EMBRAPA Corn and Sorghum Center, Sete Lagoas, MG, Brazil under the access number BRM033655.

For DNA extraction, the fungus was previously grown in SDB. The mycelium was then collected and transferred to a porcelain melting pot with the addition of PVP (Polyvinyl pyrrolidone - Sigma) and liquid N followed by maceration. The macerated was transferred to sterilized 1.5 mL polypropylene tubes until a volume of 200 µL. Next was added 800 uL of 2% CTAB buffer (NaCl 1.4 mol.L^{-1}, EDTA 0.2 mol.L^{-1}, 2% CTAB, 2% β-Mercaptoethanol and Tris HCl 0.1 mol.L^{-1}). The tube was vortexed for 3 seconds and then incubated in a water bath at 65°C for 1h, and after cooling added to 800uL of chloroform-octanol solution (24:1). After homogenization by inversion for 15 minutes, the material was centrifuged at 14,000 rpm for 10 minutes. The supernatant was transferred to a new tube, which was added 1mL of isopropanol at -20°C. The tube contents were mixed by inversion and placed in the freezer during one hour. Then a new centrifugation was performed and the supernatant was then discarded. After, it was added 200µL of 70% ethanol at -20°C and the tube manually homogenized for 5 minutes and subsequent centrifugation under the same conditions already described. Then the supernatant was discarded and the tube remained open at room temperature until complete evaporation of the remaining ethanol. The precipitate was resuspended in 10uL of TE (10:1) at pH 8.0 and the tube was manually homogenized. Then it was added 0.2µL of RNase at 10 mg.mL^{-1} prior the incubation in a water bath for 30 minutes at 37°C. ITS-rRNA region of the fungus was amplified using the primers ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR was performed in 25 µL ultrapure water containing: 2.5 µL of DNA template solution; 2.5 µL 1× Taq DNA polymerase buffer (Fermantas); 0.125 units of Taq DNA polymerase (Fermentas Dream Taq); 2.5 µL 0.2 mM dNTP; 1.25 µL bovine serum albumine (BSA); 5 pmol of each primer. Amplification conditions were: 2 min initial denaturation at 94°C; 35 cycles of denaturation (50 seconds at 95°C), annealing (1 min at 55°C), and extention (1 min at 72°C); final extention at 72°C for 10 min. PCR product purification was performed with the kit Wizard®SV Gel and PCR Clean-UP System (Promega) according to the manufacturer's instruction.

Sequencing reaction was performed using the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and sequencing was performed in an ABI PRISM 3100 Genetic Analyzer sequencer. The electropherogram sequencing file were processed for base calling and for trimming the low quality bases (<20). The sequence was deposited in the GenBank under the accession number KT585283. The taxonomic affiliation was determined using the BLAST program provided by NCBI.

A phylogram showing the position of strain *Cladosporium* sp. BRM033655 was constructed using the six first matches of type-strains sequences provided by the BLAST-NCBI analysis and the sequences used by (HUA et al, 2012). The phylogram was calculated with MEGA 7.0 using neighbor-joining method and Jukes-Cantor model with 1000 replications of bootstrap sampling.

To obtain the adsorbent biomass, the fungus was grown during 96 hours at 28°C under constant agitation of 120 rpm in erlenmeyer flasks containing 50ml of sabouraud dextrose broth medium (SDB). The culture was centrifuged during 20min at 4000 rpm and the supernatant was discarded. The pellet containing the mycelial biomass was dried at 60°C during 72 hours and then macerated in grail. The morphology was determined using a TM-3000 scanning electron microscope (SEM) equipped with an EDS Analysis System. The results was evaluated using the Quantax70© software. The software Imagej© was used to calculate the porosity of fungal biomass. Fourier transform infrared (FT-IR) spectroscopy was recorded with a Nicolet 560 FTIR spectrometer (USA). Samples were pressed by a KBr disk.

The chemical structure of Novacron Yellow (NY) is shown in Figure 1. The 410nm wavelength value was used for detect the dye removal. To test the capacity of the fungus biomass to absorb the NY, the erlenmeyer with biomass and dye solution was maintained in contact under constant agitation of 120 rpm and constant temperature of 28°C for 24h. It was used four concentrations of textile NY dye (20ppm, 40ppm, 60ppm and 80ppm) and 100mg.L^{-1} of fungus biomass. The incubation was carried out during 24 hours. For all assays the solutions were centrifuged for 30 minutes at 4000 rpm and the supernatant was used for the absorbance determination.

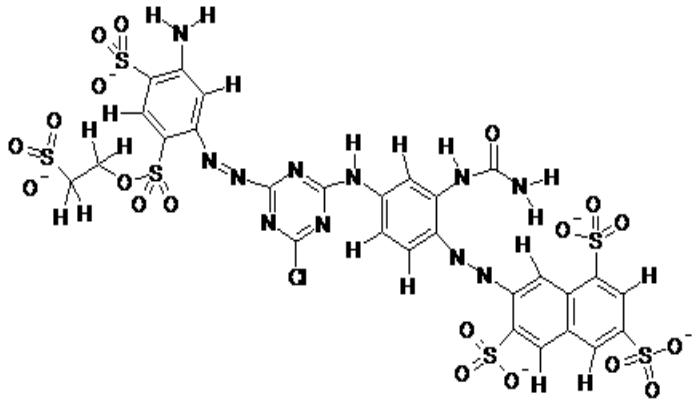

Figure 1: The structure of the Novacron Yellow (NY) dye.

Results and discussion

The analysis of ITS-rRNA nucleotide partial sequence using BLAST-NCBI showed hits with deposited sequences belonged to the genus *Cladosporium* sp. Moreover, the phylogram with the related sequences showed that our sequence match with the sequences returned by BLAST-NCBI analysis and the sequences related with *Cladosporium* sp. used by (HUA et al, 2012) in their study of dye adsorption with another *Cladosporium*-like fungus (Figure 2).

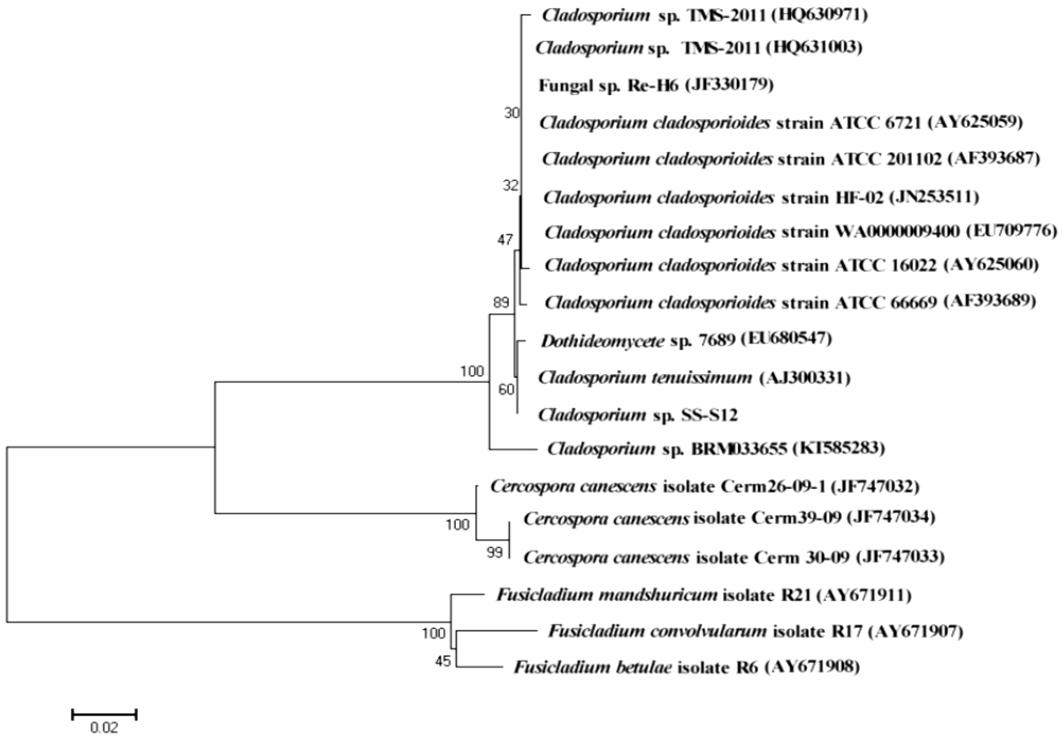

Figure 2 - Phylogram showing the position of strain *Cladosporium* sp. BRM033655, related strain sequences and sequences used by Fan et al. (2012). The phylogram was calculated with MEGA 7.0 using neighbor-joining method and Jukes-Cantor model. Numbers at the branches show bootstrap percentages after 1000 replications of bootstrap sampling.

The fungal biomass morphology was registered using scanning electron microcroscopy. The transverse cuts, caused during the maceration, exposed the internal spaces of hyphae with diameter around 3µm (Figure 3-a). The porous structure of fungal biomass is very desirable because it reduces the adsorption resistance of organic dyes. The fungal biomass porosity is about 48% (Figure 3-b). Figure 4 shows the chemical composition of the fungus biomass obtained by EDS measure.

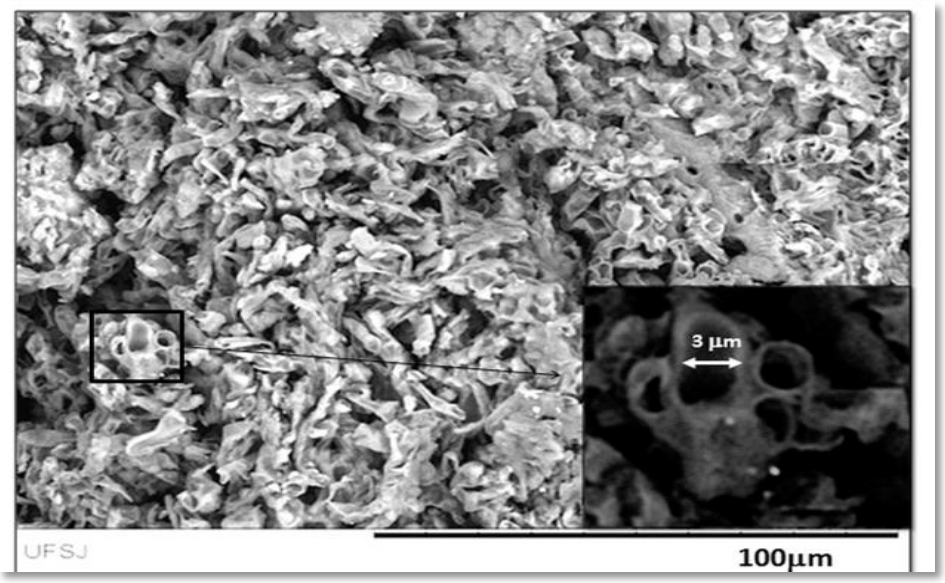

Figure 3 - The scanning electron micrograph of fungal biomass

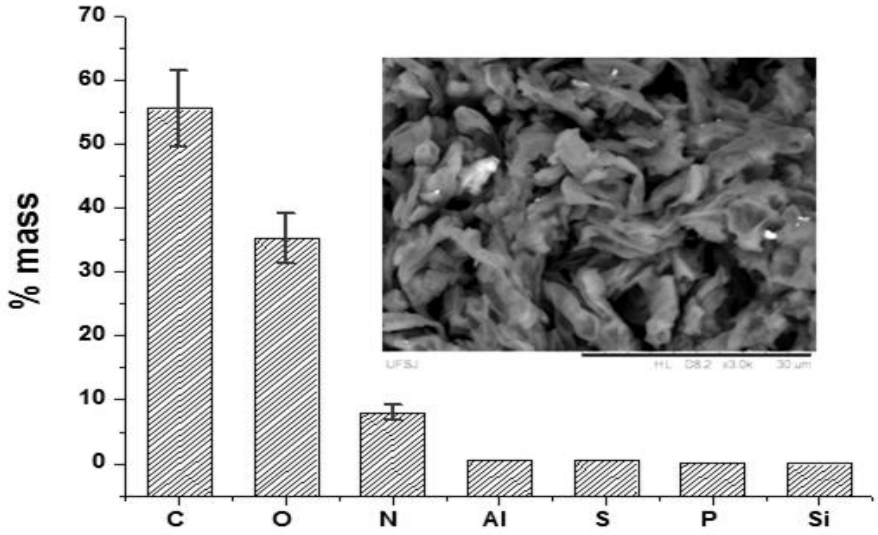

Figure 4 - Chemical composition of fungal biomass obtained by EDS measure.

The FTIR spectrum of fungal biomass (Figure 5) shows an ample absorption peak at 3274cm^{-1}, which corresponds to O-H stretching (YAKUP et al, 2007). A peak at 2926 cm^{-1} and 2800 represents the –CH symmetric stretching (TAMER et al, 2009). The peaks at around 1650, 1400 and 1240 cm^{-1} are due by the C=O stretching of carbonyl groups (YAKUP et al, 2007). The band between 610 and 535 cm^{-1} is characteristic of protein structure and represents C–N–C scissoring (YAKUP et al, 2007). There was significant decrease for the bands in the spectrum of the fungus biomass loaded with dye. This can be due the interaction between chitin and organic dye (HU et al, 2012)**.** The strongest interaction is due to the acidic hydrogen of chitin with alkoxides groups of the dye.

Figure 6 shows the quitin dimer. The WinMopac© software was used for calculation of the relative charge of hydrogen. The more acidic hydrogens are marked (Figure 6). The dynamic molecular simulation was performed with Chem3D Ultra 7.0© software and the result is show in the Figure 7. The more stable conformation is reached when the acid hydrogen of chitin is 1.78Å of alkoxides group of dye molecule. The adsorption energy was calculated.

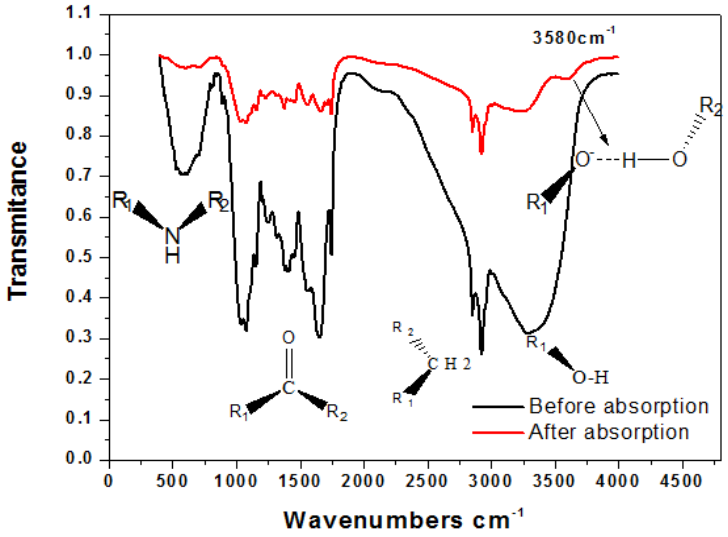

Figure 5 - The Fourier transform infrared spectral (FTIR) spectrum of fungal biomass.

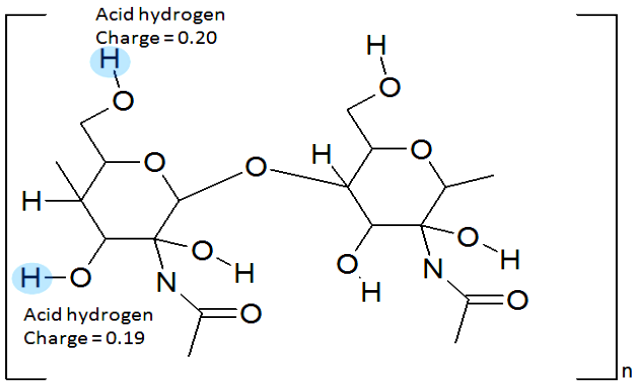

Figure 6 - Quitin dimer with acidic hydrogen calculated with WinMopac©.

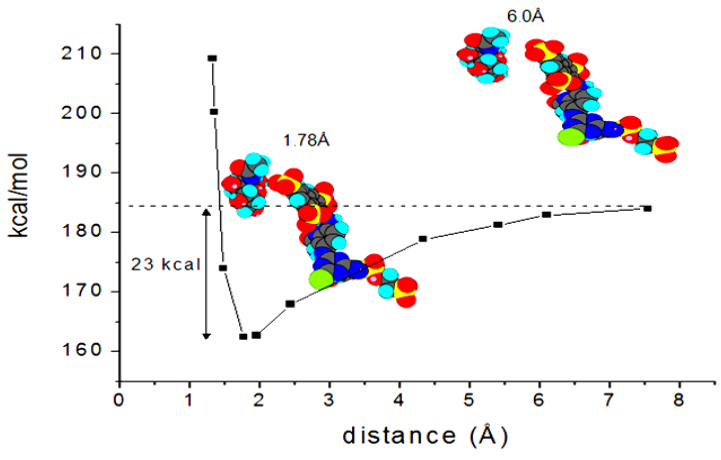

Figure 7- Molecular dynamic simulation of interaction between fungal biomass and Novacron Yellow (NY) performed with Chem3D Ultra 7.0© software.

The correlation between the dye concentration and the adsorbent ability after 24h of incubation was observed (Figure 8). At equilibrium state, the amount of adsorption (q_e) is given by equation 1, where m is the mass of adsorbent, C_0 and C_e are the initial and equilibrium concentrations of dye respectively:

$$q_e = \frac{(C_0 - C_e)}{m} \qquad \text{(Eq.1)}$$

The Freundlich isotherm model is a linear model and its mathematical representation is given by equation 2 [12,13]. The k_f is the adsorption constant and the term 1/n is a measure of the adsorption intensity (XIAO et al, 2012). This isotherm is more suitable for heterogeneous surfaces with different functional groups (HONG et al, 2012). Figure 9 shows the ln q_e versus C_e with R^2 0.99. The value found for k_f is 56 mg.g^{-1} and the n is 1.80. Values of n>1 can indicate a heterogeneous nature of dye adsorption process and a positive cooperativity in the interaction site/ligand (MANSOOR et al, 2012).

$$lnq_e = lnk_f + \left(\frac{1}{n}\right) lnC_e \qquad \text{(Eq.2)}$$

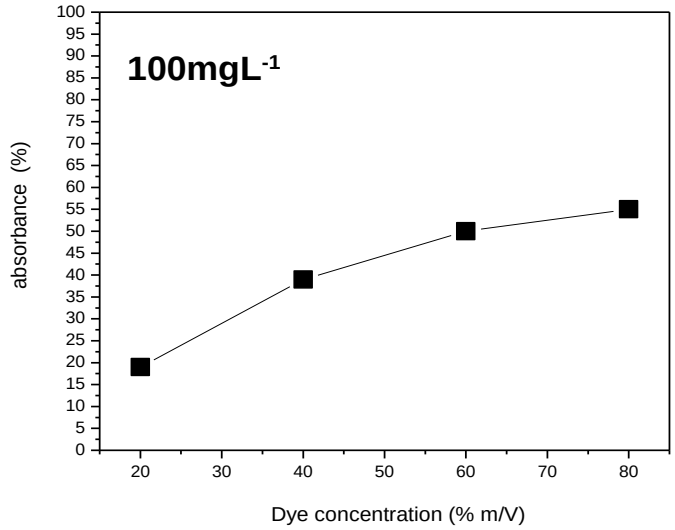

Figure 8 - Absorbance values of Novacron Yellow (NY) solution after 24h in contact with fungal biomass suspension (100mgL^{-1}).

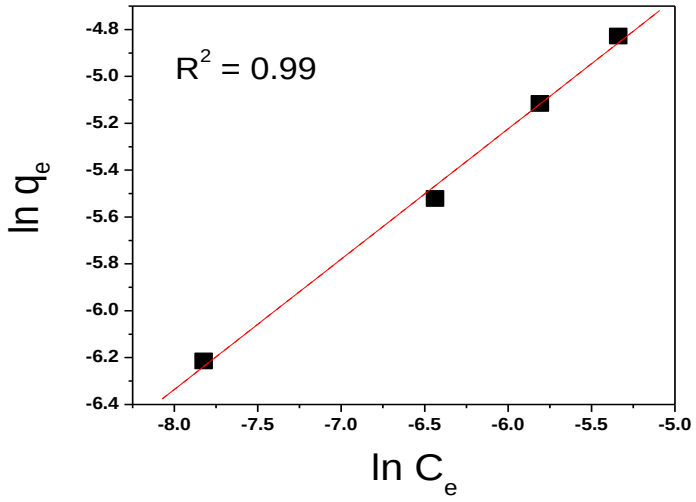

Figure 8- Freundlich isotherm model for Novacron Yellow (NY) adsorption by fungal biomass.

Table 1 shows some materials and their q_e values. It is possible to observe that the fungal biomass based on *Cladosporium* sp. is a suitable adsorbent for textile dyes. Compared to performance of waste rice hulls, the biomass based on *Cladosporium* sp is 25 times higher. The values

found for other fungal biomass is compatible with the value found in this study (Table 1). In this scenario, the biomass of *Cladosporium* sp BRM033655 is a promising adsorbent for organic dyes.

Table 1 - The values of equilibrium adsorption capacity for diverse adsorbents.

Adsorbent	Adsorbate	Equilibrium adsorption capacity (mg/g)	Reference
Waste rice hulls	Eriochrome Black T	2.02	MARK et al, 2013
Macro-fungus Agaricus bisporus and Thuja orientalis cones	Reactive Blue 49	44.37	SIBEL et al, 2009
Lignin-based activated carbon	Methylene blue	92.51	KAIFANG et al, 2013
Activated carbon from edible fungi residue	Reactive black 5	19.60	HONG et al, 2012
Cladosporium sp	Azure Blue	49.90	HUA et al, 2012

Conclusion

The morphology of fungal biomass analyzed in this paper shows the internal diameter of the hyphae around 3 µm. The chemical composition and FTIR spectrum are compatibles with quitin structure. The significant decrease in spectrum bands for fungal biomass loaded with dye can be due the interaction acidic hydrogen of chitin with alkoxides groups of the dye. The maximum adsorption capacity exhibited by fungal biomass is 56mg.g^{-1}. This result inserts the biomass based on fungi of *Cladosporium sp*. genus as a good alternative of adsorption of organic dyes with high molecular weight such as Novacron Yellow.

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