African Journal of Biotechnology Vol. 11(18), pp. 4216-4224, 1 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1030
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Decolorization of laundry effluent by filamentous fungi

Rita de Cassia M. Miranda[1, 2]*, Edelvio de Barros Gomes[3], Ester Ribeiro Gouveia[2], Katia Maria G. Machado[4] and Norma Buarque de Gusmao[1, 2]

[1]Post-Graduation in Biology of Fungi, Federal University of Pernambuco, Center of Biological Sciences, Recife, PE, Brazil.
[2]Department of Antibiotics, Federal University of Pernambuco, Center of Biological Sciences, Recife, PE, Brazil.
[3]Department of Chemical Engineering, Laboratory of Bioprocess Development, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
[4]Center for Educational Sciences, Course of Biological Sciences, Catholic University of Santos, Santos, SP, Brazil.

Accepted 24 September, 2010

This study aimed to select fungi with the potential to decolorize effluent and optimize culture conditions using the methodology of experimental design. Twenty fungi were inoculated into flasks containing the liquid synthetic medium every 24 h; aliquots were over 10 days. The culture conditions and stationary stirring of 130 rpm were evaluated. After selecting, the best fungi were subjected to an experimental design and evaluation of the production of the ligninolytics enzymes. Fungi *Phanerochaete chrysosporum* CCT 1999*, Lentinula edodes* CCT 4519 and *Curvularia lunata* UFPEDA 885 reduced 100% the color of the effluent during growth under agitation while the fungus *Aspergillus* sp. F 75 reduced 98% the color of the effluent under the same condition. Statistical analysis confirmed a significant difference between the culture conditions evaluated, with greater efficiency of decolorization of textile effluent under agitation for most fungi evaluated. The experiment 19 was noted for facilitating discoloration in 99% of the effluent. The kinetics of discoloration shows that the fungus *P. chrysosporum* CCT 1999 and *C. lunata* UFPEDA 885 stand out for discoloration among the fungi studied. The four selected fungi proved to be good producers of the enzyme laccase.

Key words: Effluent, decolorization, fungi.

INTRODUCTION

Industrial growth has contributed to economic and social development but its interference in the increase of environmental problems has become increasingly critical and frequent (Cotter et al., 2006). The textile industry adds value economically and socially, but on the other hand generates large volumes of complex effluents which have color intensity, variation in organic matter concentration and high levels of salts, thus potentially contributing to environmental degradation (Oliveira and Souza, 2003; Santos et al., 2005). In the northeast of Brazil, the expansion of textile industries in Pernambuco resulted in high production of clothing in the cities of Caruaru, Toritama and Santa Cruz of Capibaribe. Among these the city of Caruaru is an important center of laundry that are responsible for environmental degradation, especially the river Capibaribe PE, which receives the waste chemicals from the processing of parts in jeans (Sebrae PE, 2009). This pollution which is easily visible cause changes in the biological cycle and the presence of dyes and by-products that are mutagenic and carcinogenic (Kunz et al., 2002).

Bioremediation is a set of techniques where organisms are used to degrade organic compounds by removing

*Corresponding author. E-mail: ritamend@hotmail.com. Tel: (81) 2126 8866. Fax: (81) 2126 8346.

Abbreviations: PDA, Potato dextrose agar; **SAB,** sabouraud; malt agar; **MEA,** malt agar; **MgP,** manganese peroxidase; **LiP,** lignin peroxidase; **ABTS,** 2.2-azino-bis-ethylbenthiazolina.

Table 1. Values set out in factorial design experiment.

Variable	Level				
	- α	**-1**	**0**	**+1**	**+ α**
Yeast extract	0	0.01	0.05	0.1	0.5
pH	5.0	5.5	6.5	7.5	8.0
$CuSO_4$	0.01	0.02	0.04	0.06	0.08
With agitation	0	30	80	130	180

them from the environment. Among these, bacteria and fungi are extremely versatile in degrading recalcitrant substances (Barr and Aust, 1997). Fungi mitosporic are also described as potentially degrading organic compounds, mainly due to the activity of the enzyme laccase. For these reasons, this work is aimed at optimizing the parameters for cultivation of fungi in the biological treatment of effluent generated by laundry textile at Caruaru PE.

MATERIALS AND METHODS

Fungi

In this work twenty (ten basidiomycetous and ten mitosporic) fungi cultures assigned by the Culture Collection of the Department of Mycology, Federal University of Pernambuco (URM Culture Collection), Culture Tropical Collection, Andre Toselo Fundation (CCT Culture Colletion) and Microorganisms Culture Collection (Antibiotics Departament) were used. Basidiomycete fungi were maintained on potato dextrose agar (PDA) at 4°C, while the fungi were maintained on Sabouraud (SAB) under the same conditions. It is worth noting that the fungi were isolated from environments contaminated with petroleum derivatives.

Sampling and maintenance of effluent

The effluent used throughout the experiment was obtained from the storage tank of waste laundry "Stomp", belonging to the Textile Complex Industrial Caruaru PE. The effluent was collected in various parts of the dump tank and stored in cold chamber.

Decolorization of effluent by fungi

The fungi were first grown on malt agar (MEA) and incubated at 30°C for 10 days. Three discs of fungal growth (6 mm) were transferred to a 500mL flask containing 200 mL of liquid synthetic medium (Yamanaka et al., 2008) modified. The modification consisted of replacing the distilled water by the effluent. The vials were kept under static conditions or with shaking at 130 rpm for basidiomycetes and static or shaking at 150 rpm for mitosporic fungi. Every 24 h for 10 days, 2 ml of aliquots were used for reading the absorbance at 670 nm in spectrometry HP – 8453/UV-Visible. All experiments were performed in triplicate. The percentage of discoloration was calculated according to the formula below:

$$\%D = \frac{AbsT_0 - AbsTx \times 100}{AbsT_0}$$

Where, %D, percentage of discoloration; $AbsT_0$, initial absorbance; AbsTx, absorbance at each time

Experimental design

Using the strains which have reported discoloration of the effluent of more than 97%, experimental design was carried out to obtain the best operating conditions. For this a central composite rotational design (DCCR) was applied through a complete factorial design (24) with levels -1 and +1, eight axial points (-2 and +2) and four central points (zero). In the variables studied, pH, concentration of yeast extract, agitation and concentration of copper sulphate ($CuSO_4$) were independent variables and percentage of discoloration at the end of the tenth day was the dependent variable. The plan consisted of 27 experiments (Table 1) and its implementation was carried out using Statistic ™ 6.0 SOFTWARE.

Activity of ligninolytic enzymes in textile effluent

To determine the production of the three major ligninolytic enzymes, manganese peroxidase (MgP), lignin peroxidase (LiP) and laccase, selected fungi were cultured in flasks under the conditions established in the experimental design (0.05 g yeast extract, KH_2PO_4 0.2 g, $MgSO_4$ 0.05 g, $CuSO_4$ 0.02 g, $MnSO_4$ 0.016 g, pH 7.5, 1 L of laundry effluent) at 28°C for ten days without agitation. After this time, the enzyme extract was obtained by membrane filtration of 0.45 μm.

Enzymes assays

All enzymatic activities were measured spectrophotometrically (HP - 8453/UV-Visible). The laccase activity was determined using 2.2-azino-bis-ethylbenthiazolina (ABTS) in accordance with Buswell et al. (1995). The mixture consisted of 0.1 ml of 0.1 M sodium acetate buffer (pH 5.0), 0.8 ml ABTS solution in a 0.03% (w/v) and 0.1 ml of enzyme extract. The oxidation of ABTS was measured by monitoring the increase in absorbance at 420 nm. MgP activity was measured by phenol red oxidation method at 610 nm as determined by Kuwahara et al. (1984). The reaction mixture consist of 500 μL enzymatic extract, 100 μL phenol red (0.01%, w/v), 100 μL sodium lactate (0.25 M), 200 μL albumin bovine (0.5%, w/v), 50 μL (MnSO4 (2 mM) and 50 μL hydrogen peroxide in sodium succinate buffer (20 mM, pH 4.5). The mixture was incubated at 30°C for 5 min and the reaction was interrupted by the addition of 40 μL NaOH (2N). LiP activity was determined by the oxidation of veratryl alcohol as described by Buswell et al. (1995). The mixture reaction consist of 1 mL sodium tartrate buffer 125 mM pH 3.0, 500 μL veratryl alcohol 10 mM; 500 μL hydrogen peroxide 2 mM and 500 μL enzyme extract. The reaction was started by adding hydrogen peroxide and

Table 2. Percentage of decolorization of the effluent by fungi evaluated after ten days of cultivation under both conditions tested.

Fungi	Reduction of color (%)	
	Without agitation	With agitation
F. supina	88	80
P. sanguineus	72	72
L. critinus	92	88
F. fasciatus	94	81
C.caperatus	76	81
T. villosa	70	78
S.commune	88	90
G. applanalum	88	89
P. chrysosporum	97	100
L. edodes	95	100
Abiotic control *	2	3
Aspergillus sp.	95	98
C. clavata	81	88
Curvularia sp.	75	75
A. tamarii	83	83
P. griseofulvum	94	94
P. aurantiogriseus	81	81
C. lunata	98	100
F45	79	79
F48	93	90
F112	90	90
Abiotic control *	2	1

the appearance of veratraldehyde was determined at 310 nm. One enzyme unit was defined as 1.0 µmol product formed per minute under the assay conditions.

Statistical analysis

To obtain the rate of discoloration, a constant linearity of each sample was obtained. The significant variance between the treatment conditions for both groups of fungi was performed using the Student t test using the Statistic Software™ 6.0, where the change was considered significant when $p < 0.05$.

RESULTS

Decolorization waste water by fungi

Table 2 shows the percentage discoloration of effluent by the 20 fungi evaluated after 6 days of cultivation under both conditions tested.

Basidiomycetes

Among the ten fungi group of basidiomycetes, two stood out for their ability to decolorize textile effluent in the two culture conditions tested. *Phanerochaete chrysosporum* CCT 1999 reduced the color of the effluent by 97 and 100%, under static and stirred conditions, respectively. *Lentinula edodes* CCT 4519 reduced the color of the effluent by 95 and 100% under both conditions. Other fungi showed the ability of discoloration in the two conditions of the fungus. *Fomes fasciatus* URM 2676 and *Lentinula crinitus* URM 2672 demonstrated ability to reduce color in 92 and 94% in static condition while stirring decolorized the effluent by 81 and 88% respectively. Fungi *Fomitella supine* URM 2675, *Schizophylum commune* and *Ganoderma applanatum* presented a potential discoloration of 88% in static condition while the condition agitated decolorized up to 80, 90 and 89% respectively. Among the group of basidiomycetes, fungi which were less promising as potential for discoloration of the effluent were *Pycnoporus sanguineus* URM 2540, *Coriollus caperatus* URM 2673 and *Trametes villosa* CCT 5567 which gave percentage of discoloration of 72, 76 and 70% under static condition and 72, 81 and 78% under condition of agitation.

Mitosporic

Among the ten fungi tested, only mitosporic *Curvularia lunata* UFPEDA 885 bleached 100% of the agitated effluent and 98% under static condition, followed by the fungus *Aspergillus* sp. F75 that showed discoloration of 95 and 98% under static and agitated conditions, respectively. The fungus *Penicillium griseofulvum* UFPEDA 880 showed 94% of bleaching effluent in both conditions tested while the unidentified fungi F94 and F112 decolorized the effluent by 93 and 90% in static condition while stirring both decolorized 90% of the effluent. The *Aspergillus tamari* UFPEDA 870, *Curvullaria* sp. F45, *Penicillium aurantiogriseus* UFPEDA 884 and *Curvularia clavata* F111 showed the percentage of decolorization of 83, 81, 81 and 75% under static condition and 83, 88, 81 and 75% under rough conditions while the unidentified fungus F48 showed a percentage of discoloration of 75% in both conditions.

The student *t test* showed a reliability level of 95%, statistically significant ($p < 0.05$) between the mean values of percentage of discoloration of the different textile effluent treatment when performed in a stationary and agitated (130 rpm) form as shown by the results shown in Table 3. Several statistical methodologies are employed in order to validate the techniques used during study.

Experimental design

After completion of the experimental design, it was found that among the 27 experiments, 19 (99%) discoloration of

Table 3. Statistical analysis within groups of fungi tested.

Fungi	Average groups of fungi in culture condition		*P*- value
	Without agitation	With agitation	
Basidiomycets	0.481550	0.852410	0.000000
Mitosporics	0.415620	0.566390	0.040053

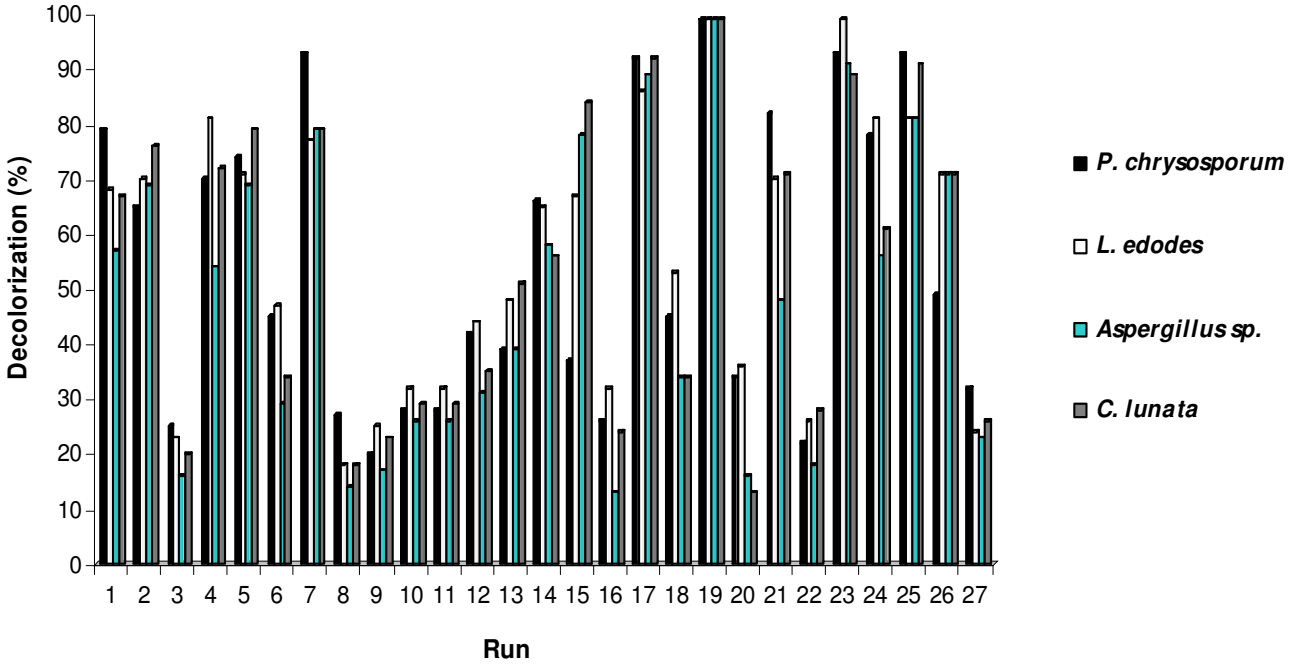

Figure 1. Percentage of decolorization of effluent from the laundry of jeans in the experimental design by the fungi *P. chrysosporum* CCT 1999, *L. edodes* CCT 4519, *Aspergillus* sp. F75 and *C. lunata*.UFPEDA 885.

the effluent at the end of the tenth day of the four fungi used was observed (Figure 1). However, the kinetics of discoloration shows that the fungi *P. chrysosporum* CCT 1999 and *C. lunata* UFPEDA 885 were more promising. From the fourth day where there was 40% of dye in the effluent, reaching 1% of residual dye at the end of the sixth day unlike the other two fungi that were still residual dye in the same period of time (Figure 2). When the fungus used was *P. chrysosporum* CCT 1999, the Pareto chart (Figure 3) generated after the execution of the experimental design shows that the interaction between the variables agitation and yeast extract showed a confidence level of 98.7%. The surface chart in Figure 4 can be observed as a trend of excellent conditions for both static and shaken (150 rpm) conditions and the concentration of yeast extract about 0.03 g/L, is maximized by the interference of the central points. In the case of the fungus *C. lunata* UFPEDA 885, it can be seen that only agitation significantly influence the process, with a confidence level of 3.8% (Figure 5). This can be seen from the surface chart of *P. chrysosporum* CCT 1999 (Figure 6).

Activity of ligninolytic enzymes in textile effluent

The quantification total values of the three major ligninolytic enzymes are shown in Table 4. In the culture conditions established by the experimental design, it was shown that the four fungi are producers of the enzyme laccase; the fungus *C. lunata* UFPEDA 885 presented the greatest potential, followed by *Aspergillus* sp F75. These results demonstrated the importance of the source of copper in the medium due to the dependence of this enzyme on this mineral. Fungi also demonstrated potential in the production of manganese-dependent peroxidase. For this enzyme the fungus that had a greater potential in the production was *Aspergillus* sp. F75, followed by *C. lunata* UFPEDA 885. The four fungi did not prove to be good producers of the enzyme lignin peroxidase. This may be due to the absence of an

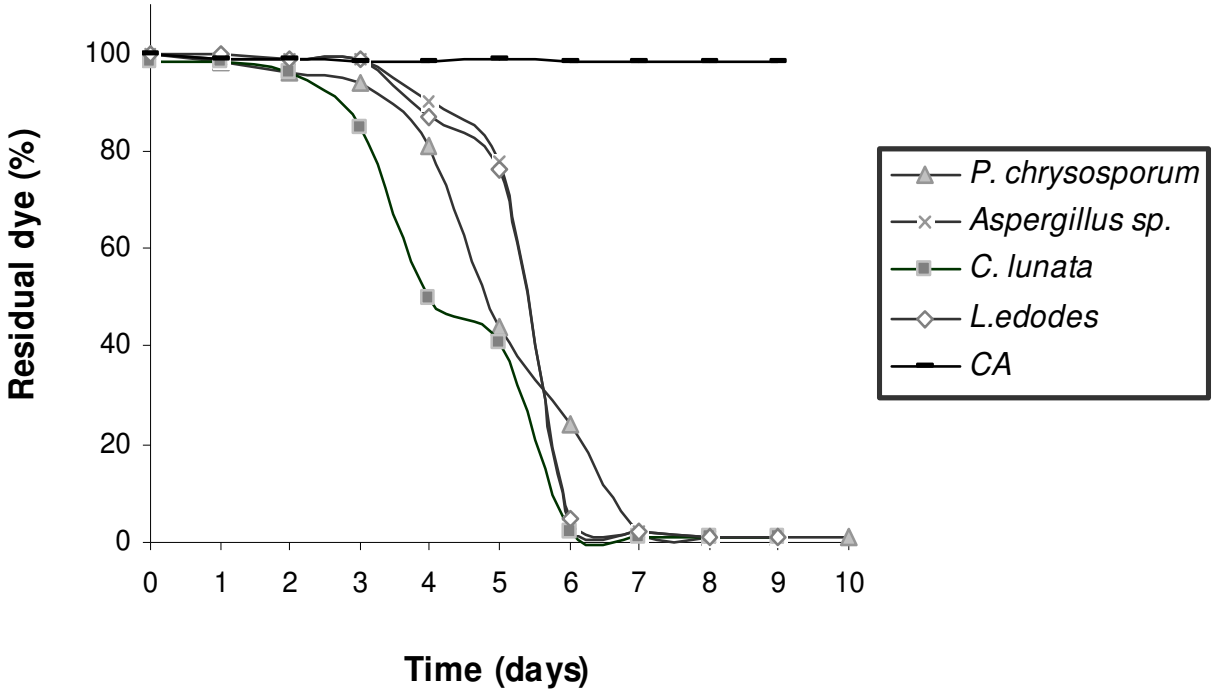

Figure 2. Kinetics of bleaching effluent by fungi *P. chrysosporum* CCT 1999, *Aspergillus* sp. F75, *C. lunata* UFPEDA 885 and *L edodes* CCT 4519 over ten days in the treatment of experimental design 19.

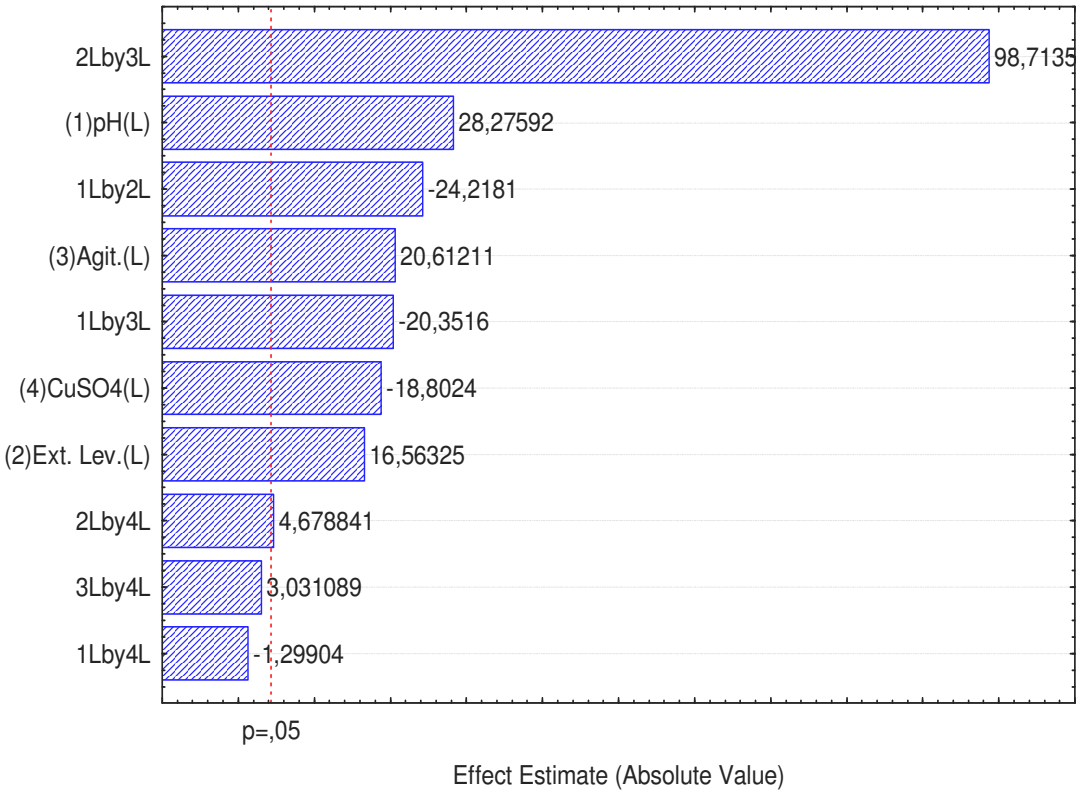

Figure 3. Pareto chart in the process of textile effluent decolorization by the fungus *P. chrysosporum* CCT 1999.

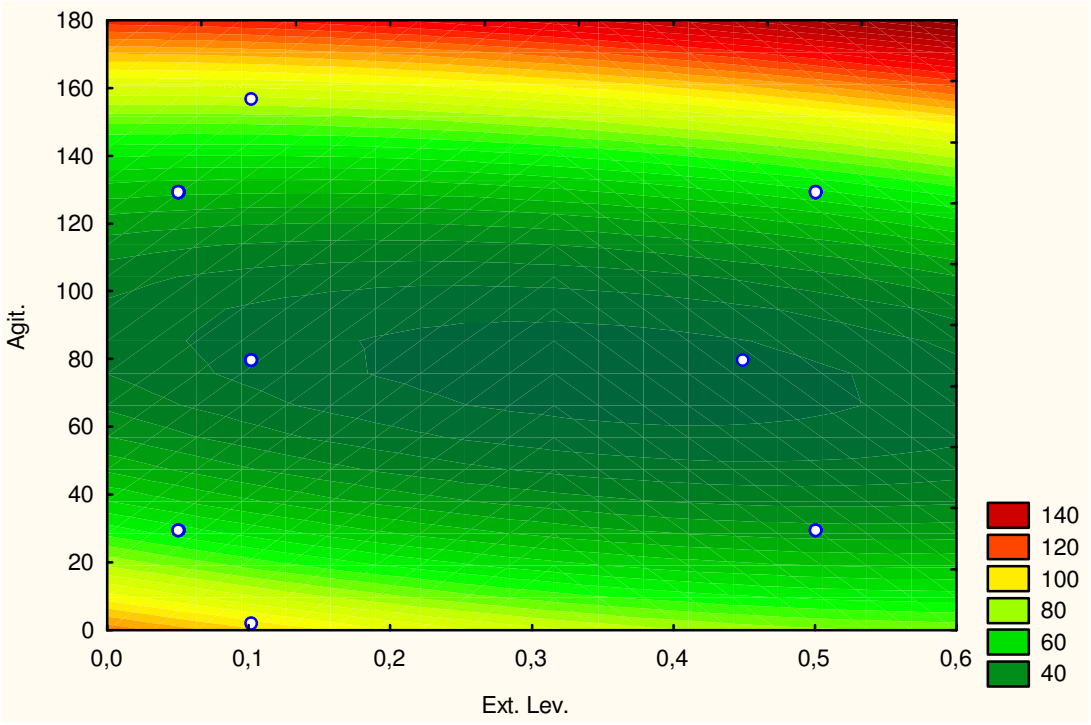

Figure 4. Response surface of agitation and concentration of yeast extract (g / L) with dependent variable and percentage of degradation (%) of *P. chrysosporum* CCT 1999. Fitted surface; Variable: % degradation; four factors, one block, 27 runs; MS residual = 435,7829; DV: % degradation.

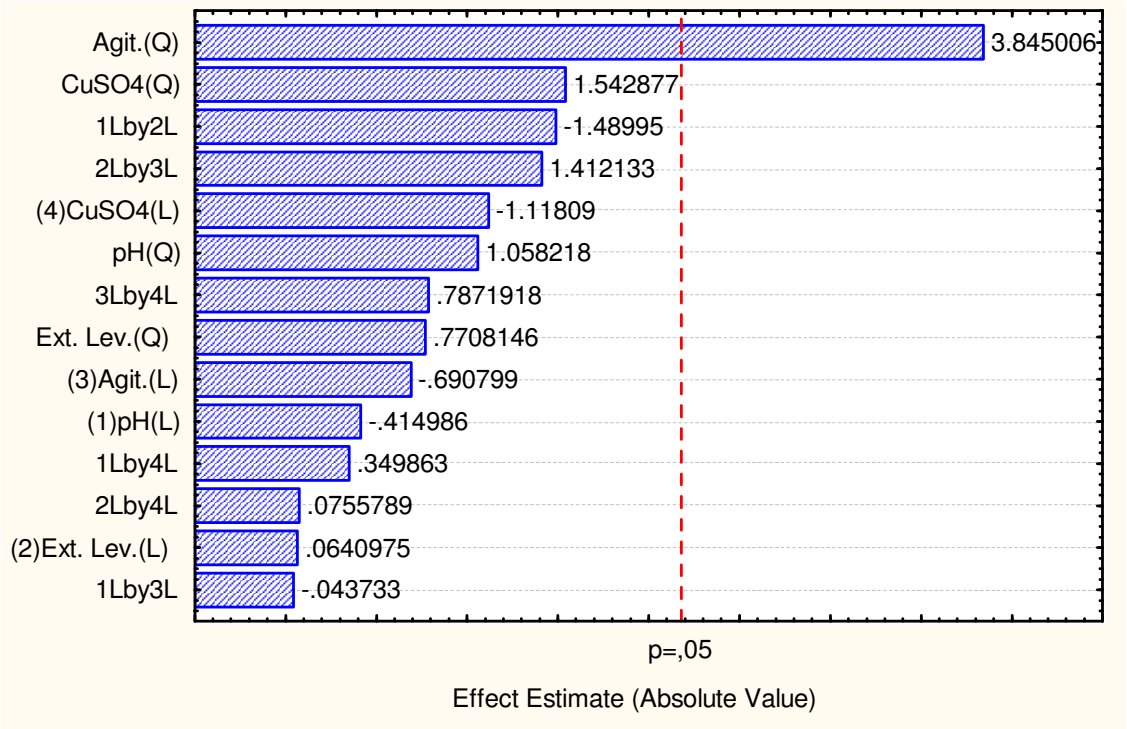

Figure 5. Pareto chart in the process of textile effluent decolorization by the fungus *C. lunata* UFPEDA 885. Four factors, one block, 27 runs; Ms residual = 522,8582.

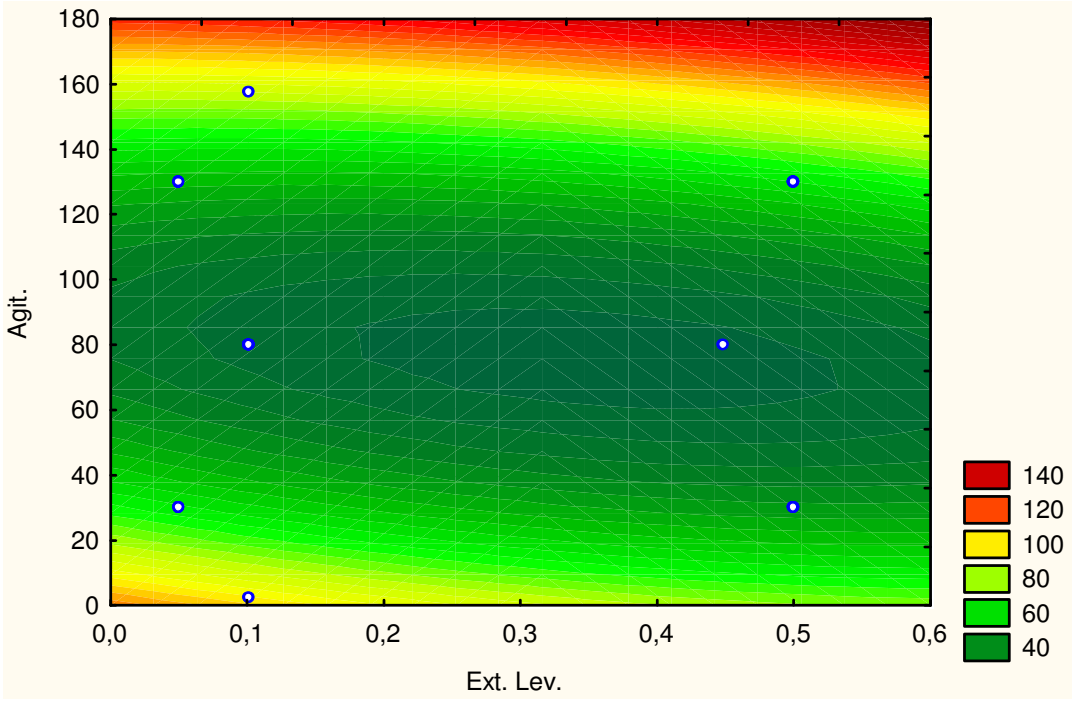

Figure 6. Response surface of agitation and concentration of yeast extract (g / L) with dependent variable the percentage of degradation (%) of *C. lunata* UFPEDA 885. Four factors, one block, 27 runs; Ms residual = 522,8582.

Table 4. Quantification of total activity of three major enzymes ligninolytics by fungi *Aspergillus* sp. F75, *C. lunata* UFPEDA 885, *P. chrysosporium* CCT 1999 and *L. edodes* CCT 4519.

Fungi	Enzymes (U/L)		
	Mn Peroxidase	Laccase	Li Peroxidase
Aspergullus sp. F75	552	1950	111
C. lunata UFPEDA 885	474	2100	50
P. chrysosporium CCT 1999	466	1506	30
L. edodes CCT 4519	466	1835	96

additional source of iron in the culture medium. These results demonstrated that the major producers were found among fungi mitosporic group with easier cultivation and rapid growth.

DISCUSSION

Decolorization of textile effluent by fungi

The fungi causing white rot in wood are reported as potential degraders of dyes used by the textile industry due to its set of ligninolytic enzymes. Martins et al. (2001) observed that *P. chrysosporum* bleached a mixture of eight azo dyes during eight days of growth in liquid medium. Using the same fungus Radha et al. (2005) reported that *P. chrysosporum* discolorized 99% of color "Methyl Violet", "Orange" and "Vat Majenta" in initial concentrations of 0.05 g/L at pH 4.5 and 35°C temperature. The mitosporic fungi also have the potential to decolorize textile effluents by enzymes lignolitics and substances of low molecular weight, such as reactive oxygen species, hydroxyl radical and Fe ions (Vitali et al., 2006). An efficient mechanism for removal of dyes in textile effluents by fungi mitosporic is biosorption. Some reports in the literature show the efficiency of this mechanism. Fahl et al. (2004) reported that *Aspergillus oryzae* in the form paramorphogenic, had the capacity to adsorb the dye "acid yellow 25" in different pH conditions. *Aspergillus niger* showed potential in discoloring aquatic environments contaminated with reactive dye sinazol by adsorption, after 18 h of exposure of their biomass

(Khalaf, 2008). Other mitosporic fungi also demonstrate the ability to remove color from textile effluents. Shedbalkar et al. (2008) reported that *Penicillium ochrochloron* MTCC 517 has demonstrated its ability to decolorize 93% of triphenylmethane dye on condition of stationary cultivation, pH 6.5 at 25°C in two and a half hours of contact. Ambrosio and Campos-Takaki (2004) reported that *Cunninghamella elegans* bleached 83% of the color orange II in medium containing sucrose and peptone after 96 h of treatment. Omission of sucrose reduced the discoloration of dye to 48% over the same period of time. Growing conditions are essential for satisfactory performance of organisms. *Aspergillus ochraceus* has shown promise in the degradation of the dye "Reactive blue 25" in medium containing only distilled water and glucose Parshetti et al. (2007). In this paper, the authors related the presence of enzymes laccase, lignin peroxidase and tyrosinase in the degradation of this dye and the data were corroborated by analysis on high performance liquid chromatography (HPLC) and cromatography gas accolade mass spectrometric (CGMS) that had peaks of the compounds phitalimidin and di-iso-butilphitalate intermediate metabolites of the dye.

The authors used the test of Turkey to establish the existence of significant differences between the means of three processes employed and observed greater efficiency of clearing in bioassays using cultures in the consortium.

Experimental design

Some authors have used a factorial design to optimize the conditions used in the degradation of dyes and effluents by microorganisms. Srinivan and Murthy (2009), conducted a complete experimental design of central-type compound to optimize the initial concentrations of glucose, dye and ammonium chloride in culture medium in which it is used to test the ability of the fungus *Trametes versicolor* to decolorize azo dyes. The dye-Reactive orange 16 (RO-16) was downgraded to 94.5% when the optimal concentrations of glucose, dye and ammonium chloride were 17.50, 0.66 and 2.69 g/L, respectively, while for the dye-Reactive red 35 (RR-35) these concentrations were 16.67, 0.68 and 2.13 g/L, respectively. These values were studied and optimized after the observation of the surface chart response. Evangelista-Barreto *et al*. (2009) after performing a complete factorial design with two levels and four variables reported the degradation of 96-98% of the azo dye Orange II by *Geobacillus stearothermophillus* when grown in Luria Bertani medium under condition of stirring of 150 rpm; a phenomenon attributed to the need for co-metabolism by bacteria. After observing the Pareto chart, it could be said that the turmoil is the variable that positively influence the process.

Activity of ligninolytic enzymes in textile effluent

Yamanaka et al. (2008) observed the production of laccase enzyme by the fungus *T. villosa* throughout their growth and under different growing conditions. A higher yield was observed when the medium was supplemented with copper. The same authors also observed that the enzyme activity of manganese dependent peroxidase was induced when the medium was supplemented with vegetable oil emusilfying with surfactant. Bonugli-Santos et al. (2010) studied the production of enzymes ligninolytics in fungi isolated from saline environment and observed the production of three key enzymes, laccase, manganese-dependent peroxidase and lignin peroxidase when the *Aspergillus sclerotiorum* CBMAI 849, 857 *Cladosporium cladosporioides* CBMAI and *Mucor racemosus* CBMAI 847 were cultured in malt extract. When the same fungus was cultivated in basal medium containing glucose and wheat bran, there was inhibition in the production of lignin peroxidase, while the enzymes laccase and manganese-dependent peroxidase production increased.

REFERENCES

Ambrosio ST, Takaki GMC (2004). Decolorization of reactive azo dyes by *Cunninghamella elegans* UCP 542 under co-metabolic conditions. Bioresour. Technol. 91: 69-75.

Barr DP, Aust SD (1997). Mechanism White Rot Fungi Use to Degrade Polluants. Environ. Sci. Technol. 28(2): 78-87.

Bonugli-Santos RC, Durrant LR, Silva M, Sette LD (2010). Production of laccase, manganese peroxidase and lignin peroxidase by brazilian marine-derived fungi. Enzyme Microb. Technol. 46: 32-37.

Buswell JK, Cai YJ, Chang ST (1995). Effect of nutrient nitrogen on manganese peroxidase and lacase production by Lentinula (Lentinus) edodes. FEMS Microbiol. Lett. 128: 81-88.

Cotter JA, Rezende MOO, Piovani MR (2006). Avaliação do teor de metais em sedimento do rio Betari no Parque Estadual Turístico do Alto Ribeira-PETAR. Quím. Nov. 29: 40-45.

Evangelista Barreto NS, Albuquerque, CD, Vieira RHSF, Campos-Takaki GM (2009). Cometabolic Decolorization of the Reactive Azo Dye Orange II by *Geobacillus stearothermophilus* UCP 986. Text. Reser. 79: 1266-1273.

Fahl P, Vitor V, De Jesus GJ, Corso CR (2004). Biosorção do Corante Azóico Acid Yellpw 25. por *Aspergillus oryzae* Paramorfogênico, Arq. Do Inst. De Biol. 71: 132-134.

Khalaf MA (2008). Biosorption of Reactive Dye from Textile Wastewater by non-viable biomass of *Aspergillus niger* and *Spirogyra sp*. Bioresour. Technol. 99: 6631-6634.

Kunz A, Peralta-Zamora P, Moraes SG, Duran N (2002). Novas Tendências no Tratamento de Efluentes Industriais. Quim. Nov. 25: 78-82.

Kuwahara M, Glenn JK, Morgan MA, Gold MH (1984). Separation and characterization of two extracellular H2O2 dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium*. FEBS Lett. 169: 247-250.

Martins MAM, Ferreira IC, Santos IM, Queiroz MJ, Lima N (2001) Biodegradation of bioaccessible textile azo dyes by *Phanerochaete chrysosporium*, J. Biotechnol. 89: 91-98.

Oliveira JR, Souza RR (2003). Biodegradação de efluentes contendo corantes utilizados na indústria têxtil. In: Seminário de Pesquisa, Aracajú SE.

Parshetti GK, Kalme SD, Gomare SS, Govindwar SP (2007). Biodegradation of Reactive Blue-25 by *Aspergillus ochraceus* NCIM-

1146. Bioresour. Technol. 98 3638-3642.
Radha KV, Regupathi I, Arunagiri A, Murugesan T (2005). Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics. Proc. Biochem. 40: 3337-3345.
Santos DAB, Traverse J, Cervantes FJ, Van Lier JB (2005). Enhancing the electron transfer capacity and subsequent color removal in bioreactors by applying thermophilic anaerobic treatment and redox mediators. Biotechnol. Bioeng. 89(1): 42-52.
SEBRAE/PE; Confecção em Pernambuco. Disponível em http:// WWW.sebraepe. com.br; acesso em 13 de junho de 2009
Shedbalkar U, Dhanve R, Jadhav J (2008). Biodegradation of Triphenylmethane dye cotton blue by *Penicillium ochrochloron* MTCC 517. J. Hazard. Mater. 157: 472-479.
Srinivan SV, Murthy DVS (2009) Statistical Optimization for Decolorization of Textile Dyes Using *Trametes versicolor*. 165: 909-914.
Vitali VMV, Machado KMG, Andrea MM, Bononi VLR (2006). Screening Mitosporic Fungi for Organochlorides Degradation. Braz. J. Microb. 37: 256-261.
Yamanaka R, Soares SF, Matheus DR, Machadp KMG (2008). Lignolytic Enzymes Produced by *Trametes Villosa* CCB176 Under Different Culture Conditions. Braz. J. Microb. 39: 78-84.