Journal of Environmental Biology **October 2006,** 27(4) 661-665 (2006)

Vinasse biodegradation by *Phanerochaete chrysosporium*

María F. Potentini and Antonio J. Rodríguez-Malaver
Grupo de Investigación Proyecto Fenton, Laboratorio de Bioquímica Adaptativa, Departamento de Bioquímica, Facultad de Medicina, Universidad de Los Andes, Mérida 5101, Venezuela

(Received: 11 April, 2005 ; Accepted: 26 December, 2005)

Abstract: *Vinasse is a colored recalcitrant wastewater of the distillery industry. The aim of this work was to study the use of Phanerochaete chrysosporium for the vinasse degradation under two different growth conditions. Vinasse was treated by P. chrysosporium in a liquid inoculum form, during 32 days at room temperature (approximately 25°C) and at 39°C. Chemical oxygen demand (COD), total phenol concentration and color removal were measured and there8 was a decrease in COD, phenolic concentration and color of 47.48%, 54.72% and 45.10% respectively, at room temperature and a decrease in 54.21%, 59.41% and 56.81% respectively at 39°C.*

Key words: *Biodegradation, Phanerochaete chrysosporium, Vinasse, Wastewater treatment.*

Introduction

In the distillery industry, like factories of beers, stills, alcohol and certain organic chemical compounds, there are several wastewaters and among them exists the vinasse (Lugo *et al.,* 2001). Vinasse is a liquid by-product of the ethanol production. Vinasse characteristics depend basically on the raw material used for ethanol production, which can be molasses, beet sugars, fruit sugars, cereals (wheat, rice, corn and rye), malts of cereals and wood. Vinasse is a highly colored compound which is difficult to treat by normal biological processes such as activated sludge or anaerobic lagooning (Singh and Nigam, 1995). Its recalcitrance is due to the presence of melanoidins, the brown polymers which are formed by the Maillard amine-carbonyl reaction (Wedzicha and Kaputo, 1992).

Vinasse disposal into the environment is hazardous and has a considerable pollution potential. Its high COD of approximately 40,000 mg/l and a high quantity of dissolved organic carbon (10,000 - 14,000 mg/l approx.) (Benke *et al.,* 1999) means that its disposal into natural water bodies can cause their eutrophication (a gradual increase in phosphorus, nitrogen and other nutrient concentration in an aquatic ecosystem), which induces a great increase in the concentrations of algae and microorganisms at the surface, avoiding that the solar light and the necessary oxygen get in for the subsistence of sub aquatic life.

On the other hand, the white-rot fungi have a unique capacity to degrade wood and its basic components, cellulose and lignin. These fungi use the cellulose fractions as source of carbon and they have the capacity to degrade lignins to have access to the cellulose. Basidiomycete species have been studied extensively because of their high degradation capacity (Crawford, 1981; Higuchi, 1993; Miyamoto *et al*., 2000; Vicuña, 2000; Hatakka, 2001). A wide range of compounds that these fungi are able to degrade includes many pesticides, polycyclic aromatic hydrocarbons, some dyes, TNT and other nitroexplosives, and other toxic chemicals as cyanides, azides, carbon tetrachloride and pentachlorophenol. Several species of white-rot fungi are recognized by their capacity to whiten and delignify the pulp coming from the Kraft process. Their capacity to degrade lignin has been attributed to extracellular oxidative enzymes that work together with low molecular weight cofactors (Kenneth *et al.,* 1985; De Jong *et al.,* 1994; Tekere *et al.,* 2001a,b). Although this capacity has been recognized for many years, it has been recently that many researchers have begun to understand the mechanism by means of which this degradation process is carried out (Aust and Benson, 1993). Recent developments in new treatment technologies for the wastewaters coming from the pulp and paper industry and/or the improvement of those already exist include the use of the white-rot fungi *Phanerochaete chrysosporium* (Hymenomycete) and *Trametes versicolor* (Basidiomycete) (Mehna *et al.,* 1995). The degradation of a recalcitrant compound, as lignin, is a non-specific process and oxidative that species like *P. chrysosporium* carry out with extracellular enzymes that they excrete into the medium (Kenneth *et al.,* 1985; Janse *et al.,* 1998). The most important reaction the action of these fungi, on a polymer for example as lignin, it is the fungal depolymerization that corresponds to the oxidative rupture of the propyl skeleton between the C_α and $C_ß$, by a ligninase, a ferric hemprotein that catalyzes this non steroespecific reaction with both lignin and lignin model dimmers, consuming H_2O_2 and O_2. The rupture of some dimmers occurs when oxygen is incorporated at the C_β. At the rupture of the two carbons, they originate free radicals, as intermediary products, and under anaerobic conditions these radicals react and produce aldehydes (Kenneth *et al.,* 1985). The quantity of information on the enzymatic mechanisms of the white-rot fungi is very diverse, but at the same time poor, therefore, still lack to elucidate how these species really act on compounds as complex as the environmental pollutants. Lately, the interest has grown in the application of these fungi in the bioremediation of highly polluted sites due to the low cost of its use (Aust and Benson, 1993).

A very limited experience exists about the possibility of degradation of highly polluting wastewater as vinasses.

Materials and Methods

Phenolic group concentration determination: The content of phenolic compounds were determined by the colorimetric method for Tannins and Lignins 5550B of the standard methods for the examination of water and wastewater (Clesceri *et al*., 1998) that corresponds to the spectrophotometric method at 700 nm, using the Folin-Ciocalteu's reagent (Sigma, USA) and the carbonate-tartrate reagent. The phenolic concentration was determined by a calibration curve using pure phenol as a standard.

Chemical oxygen demand (COD) determination: The COD was obtained by a colorimetric method (closed reflux 5220 D) of the standard methods for the examination of water and wastewater (Clesceri *et al*., 1998), in a reactor (COD HACH Model 45600) at 150°C for 120 min. After digestion, the COD was measured (mg/l) with the method 435 of the laboratory Spectrophotometer (Model HACH DR/2500) at 620 nm.

Color removal determination: The color removal was determined by the method described by Livernoche *et al.* (1983) that corresponds to the spectrophotometric method at 440 nm, using 20 mM sodium tetraborate buffer (pH 9.1). The reporting color removal was calculated according to the followed equation:

$$\% \text{ Color removal} = \frac{(O.D._A - O.D._B)}{O.D._{440} \text{ sample without treatment}} \times 100$$

Where $O.D._A$ = O.D. of sample without treatment and $O.D._B$= OD of treated sample

Other vinasse physicochemical characteristics: The determination of vinasse physicochemical properties; density, electric conductivity, pH, total sugar and biological oxygen demand (BOD) was carried out by following the standard methods for the examination of water and wastewater (Clesceri *et al*., 1998).

Vinasse samples: Vinasse samples were obtained from "Destilería Campo Elías" (Mérida, Venezuela), a distillery industry that uses molasse as raw material for ethanol production and generate vinasse as wastewater. Samples were taking from a container that received the still hot vinasse (70°C), were then bottled for subsequent cooling and keeping on refrigeration (4°C).

White-rot fungus *Phanerochaete chrysosporium*: *Phanerochaete chrysosporium* was obtained from a mycelial material regenerated at our laboratory (Laboratorio de Bioquímica Adaptativa, Universidad de Los Andes, Mérida-Venezuela), keeping its growth by replicating every month into a malt-agar solid medium and maintained in a sterile culture room with controlled temperature.

Biological treatment with the white-rot fungus *Phanerochaete chrysosporium*: The fungus was passed from a malt-agar solid medium into a HCM liquid medium plus glucose (Rivera *et al.,* 2002) that contained $(NH_4)_2SO_4$ (0.5 g/l), KH_2PO_4 (1.0 g/l), KCl (0.5 g/l), $MgSO_4 \cdot 7H_2O$ (0.2 g/l), $CaCl_2$ (0,1 g/l), glucose (1 g/l) and sterile distilled water, to obtain the liquid inoculum. Vinasse with an initial COD of 40,000 mg/l, a phenolic compound concentration of 15,000 mg/l and a high coloration, was treated by *Phanerochaete chrysosporium* in a liquid inoculum form for 32 days, at room temperature (25°C) and at 39°C, taking samples every 4 days. On each sample, the determinations of COD, phenolic concentration and color removal were carried out. All the handle of the biological treatment was carried out in a sterile culture room with controlled temperature on a flow laminar chamber (CITEC, Mérida, Venezuela).

Statistics: Data was analyzed using a one-way ANOVA and Newman-Keuls multiple comparison test (Graph Pad Prism package, version 2.01, 1996, GraphPad Software Incorporated).

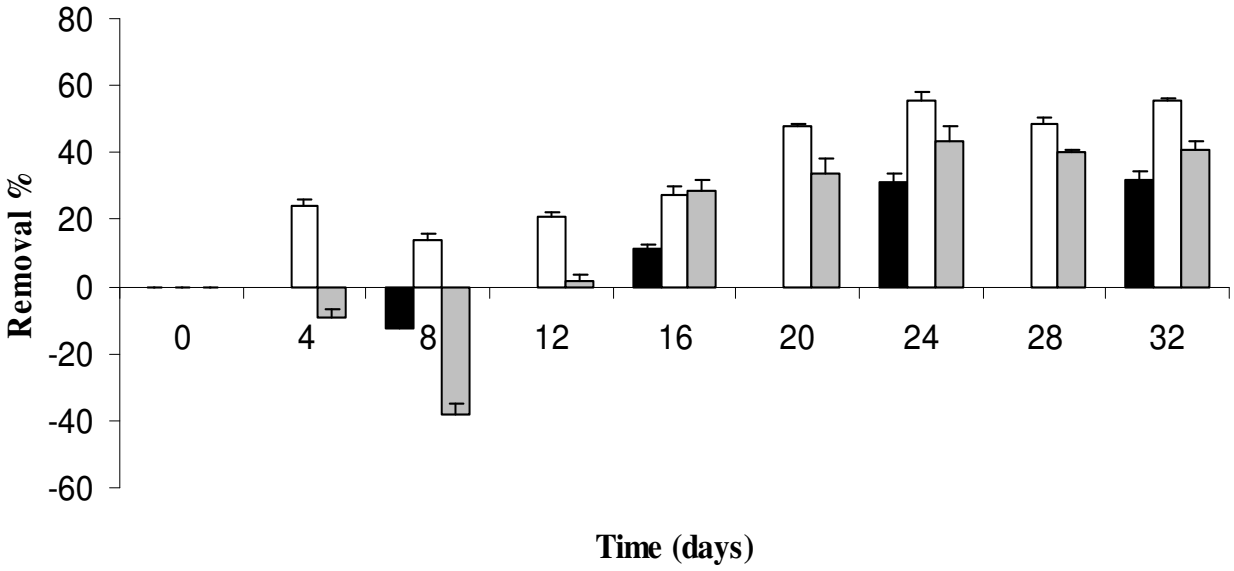
Results and Discussion

Vinasse physicochemical characteristics were summarized in Table 1. Vinasse exhibits a low value of BOD_5, a high COD, highly colored and has a high conductivity, possibly due to a high content of salts (Rodríguez *et al.,* 2002). The relationship BOD_5/COD is 0.14, indicating that this wastewater is hardly biodegradable (Abderrazik *et al.,* 2002).

Table – 1: Vinasse physicochemical characteristics.

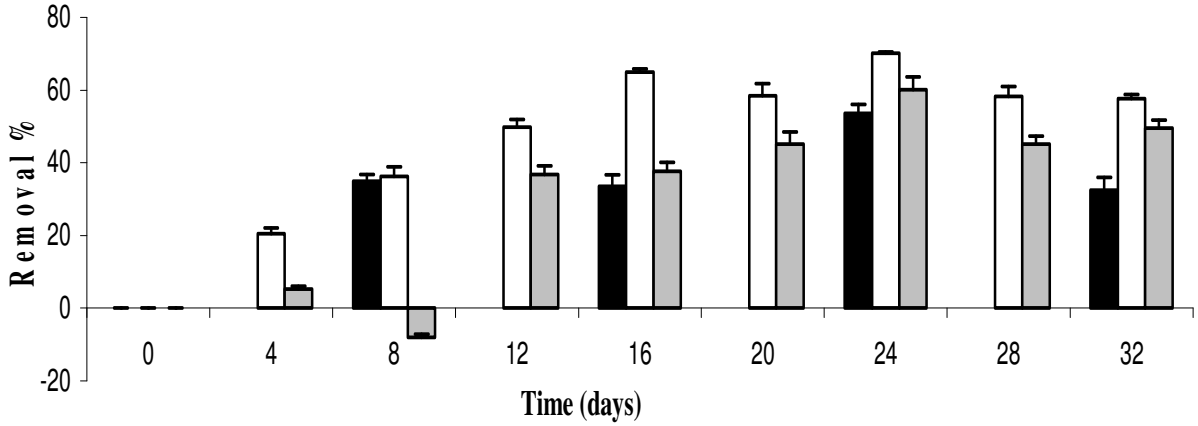
Parameter	Value
pH	4.4
Density (g/ml)	1.1
Electric conductivity (mS)	16.3
Total sugar (mg/l)	25,000.0
BOD (mg/l)	5,200.00
COD (mg/l)	40,000.00

Biological process application on vinasse: As shown in Fig. 1, the biological treatment of vinasse with *P. chrysosporium* at room temperature produced a reduction in phenolic compounds from the 4th day. On the other hand, color and COD were removed starting from the 16th day and removal reached a maximum for the three parameters after 24 days of treatment. The phenolic compound removal by white-rot fungi, specifically by *P. chrysosporium* is known and well documented. Lignolytic enzymes, that they excrete, are able to degrade complex aromatic compounds to non-polar monoaromatic, as styrene (Roldan-Carrillo *et al.,* 2001), and can act on phenolic compounds and high-molecular-weight polycyclic aromatic hydrocarbons (Boonchan *et al.,* 2000), explaining the COD and color removal reached in this treatment. The degradation system of these fungi consists of several enzymes, among them, peroxidases, laccases, cellulose dehydrogenases and trans-membrane methyl-transferases. A waste like vinasse can be attacked by this enzymatic system, due to its non-specific nature (Ishikawa *et al.,* 1963; Kenneth *et al.,* 1985; Milstein *et al.,* 1994; Janse *et al.,* 1998; D´Souza *et al.,* 1999; Cameron *et al.,* 2000; Crestini *et al.,* 2000, Tekere *et al.,* 2001b), being able to eliminate pollutants in it (Aust and Benson, 1993; Cameron *et al.,* 2000) that is compatible with the removal observed in this treatment. The application of same treatment, but at 39°C,



(■) COD, (□) phenols, (▨) color

Fig. 1: Effect of the incubation time at room temperature on vinasse COD, phenols and color removal.



(■) COD, (□) phenols, (▨) color

Fig. 2 : Effect of the incubation time at 39° C on vinasse COD, phenols and color removal.

presented a relative advantage, reaching higher removal values of the studied parameters (Fig. 2). The fungus under study has been used in many ways by other authors, varying the incubation temperature from room temperature (Nazareno *et al.,* 2000) up to 39°C (Roldan-Carrillo *et al.,* 2001). When we studied the effect of incubation temperature, it was evident that the activity of the enzymatic system of these fungi became more efficient at 39°C than at room temperature (approx. 25°C) on the decomposition of this wastewater, diminishing phenolic compounds (64%), color (37%) and COD (33%) in only 8-24 days. These results indicated that this treatment is more efficient at higher temperature than at room temperature (Table 2).

Sayadi and co-workers (1996) reported a decrease (50%) in COD with the same species acting on a wastewater from the olive oil industry. Contrastingly they were also able to reduce color up to 85%, while the maximum obtained by the conditions of this work was 60%. On the other hand, Kunz *et al.* (2001) reported a good decolorization and total phenol reduction in a wastewater from the textile industry with *P. chrysosporium.*

The action of these fungi on the phenolic compound concentration was as expected, because it is known that these species of white-rot fungi have a group of enzymes, as quinone reductases that participate in the rupture of phenols, aldehydes and polycyclic aromatic hydrocarbons (González, 1999; Boonchan *et al.,* 2000).

The differences above described in comparison to the present study were possibly due to the different and complex composition of treated wastewater. However, certainly it was

Table – 2: Removal percentages obtained with the fungal treatments applied to vinasse samples.

Parameter	Removal after fungal treatment with *P. chrysosporium*	
	at 25°C (%)	at 39°C (%)
COD	47.48 ± 0.65*a*	54.21 ± 5.38*a*
Phenolics	54.72 ± 1.85*a*	59.41 ± 3.13*a*
Color	45.10± 1.39	56.81 ± 0.56

Mean (±SE) (n=3). Means within a row sharing the letter (*a*) are not significantly different by Newman-Keuls multiple comparison test ($p<0.05$).

clear that the treatment reported here, at room temperature and at 39°C, was more efficient, comparing degradation, than those of some bacterial-fungal consortium that only could reach 12% of high-molecular-weight polycyclic aromatic hydrocarbons removal in a treatment of 56 days (Boonchan *et al.,* 2000). In the color elimination, contrastingly with what was found in this work, Dahiya *et al.* (2001) reported natural and synthetic melanoidins removal by this species, even more than 80% in 6 days at 30°C, while Robinson *et al.* (2001) found only 53.6% of color removal on an artificial textile wastewater.

For both treatments, the maximum removal of all the parameters was observed after 24 days (Figs. 1 and 2). Equally there was more degradation at 39°C than at 25°C. This period of time was longer as compared to other authors' results that obtained the elimination in 6 days (Robinson *et al.,* 2001) with the same fungus, while with the use of bacterial-fungal consortium reached the maximum after 50 days (Boonchan *et al.,* 2000). In the Table 2, there is a summary of the removal percentages obtained with the two treatment studied in this work. None of the treatments removed the phenolic concentration, color and COD completely. It has been reported that biological treatments are useful as a way to adapt the wastewater before being discharged into receiving bodies (Neyens and Baeyens, 2003). The results obtained in this investigation suggested that the application of this kind of treatments is convenient to reach pollutant removal values that agree with those demanded by law, which are at least a little more bearable for the environment.

In conclusion, the biological treatment of the vinasse with *P. chrysosporium* was better at 39°C than at room temperature. The color was most removed by the treatment at 39°C. COD and phenolic concentration removals were not significantly different in the treatment of vinasse with the fungus at room temperature and at 39°C. The maximum removal of COD, phenolic compounds and color was obtained after 24 days of treatment. The use of this kind of treatments or similar could be an effective way to treat vinasse in order to diminish the impact of this wastewater to the environment.

Acknowledgments

The authors wish to thank the help and financial support from FONACIT, Ministry of Science and Technology (Venezuela) and World Bank (Grant No.2001001363). The authors also want to thank the Destilería Campo Elías (Mérida, Venezuela) for the vinasse samples.

References

Abderrazik, N., F. Al Momani, M. Rodríguez, A. Azmani, C. Sans and S. Esplugas: Biodegradability improvement by photo fenton reaction. *Afinid*., **LIX(500)**, 391-398 (2002).

Aust, S.D. and J.T. Benson: The fungus among us: Use of white rot fungi to biodegrade environmental pollutants. *Env. H. Perspect.*, **101**, 232-241 (1993).

Benke, M., A. Mermut and H. Shariatmadari: Retention of dissolved organic carbon from vinasse by a tropical soil, kaolinite and Fe-oxides. *Geod*., **91**, 47-63 (1999).

Boonchan, S., M. Britz and G. Stanley: Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Appl. Environ. Microbiol*., **66**, 1007-1019 (2000).

Cameron, M.D., S. Timofeevski and S.D. Aust: Enzymology of *Phanerochaete chrysosporium* with respect to the degradation of recalcitrant compounds and xenobiotics. *Appl. Microbiol. Biotechnol.*, **54**, 751-758 (2000).

Caramelo, L., M. Martínez and A. Martínez: A search for ligninolytic peroxidases in the fungus *Pleurotus eryngii* involving α-keto-γ-thiomethylbutyric acid and lignin model dimmers. *Appl. Environ. Microbiol*., **65**, 916-922 (1999).

Clesceri, L., A. Greenberg and A. Eaton: Standard methods for the examination of water and wastewater. 20th Ed. A.P.H.A., Whashington D.C. 5-13, 5-52 (1998).

Crawford, R.L.: Lignin degrading microorganism. *In:* Lignin biodegradation and transformation (*Eds:* John Wiley and Sons), Wiley-Interscience, New York (1981).

Crestini, C., A. D´Annibale, G. Sermanni and R. Saladino: The reactivity of phenolic and non-phenolic residual kraft lignin model compounds with Mn(II)-peroxidase from *Lentinula edodes*. *Bioorg. Med. Chem.*, **8(2),** 433-438 (2000).

Dahiya, J., D. Singh and P. Nigam: Decolourisation of synthetic and spentwash melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40. *Bioresour. Technol.*, **78**, 95-98 (2001).

Degremont: Water Treatment Handbook. 6th Edition. Lavoisier Publishing, Paris (1991).

De Hereida, J.B., J. Torregrosa, J.R. Dominguez and J.A. Peres: Kinetic model for phenolic compound oxidation by Fenton's reagent. *Chemosph.*, **45**, 85-90 (2001).

De Jong, E., J.A. Field and J.A.M. De Bont: Aryl alcohols in the physiology of ligninolytic fungi. *FEMS Microbiol. Rev.*, **13**, 153-188 (1994).

D´Souza, T., C. Merritt and C. Reddy: Lignin-modifying enzymes of the white rot basidiomycete *Ganoderma lucidum*. *Appl. Environ. Microbiol.*, **65(12),** 5307-5313 (1999).

González, A.: *Biología molecular de hongos basidiomicetos*. Memoria del bienio 1997-1998. Centro de Investigaciones Biológicas. Departamento de Biología Molecular. España (1999).

Gonzalez, M.D., E. Moreno, J. Quevedo and A. Ramos: Studies on antibacterial activity of wastewaters from olive oil mills: Inhibitory activity of phenolic and fatty acids. *Chemosph.*, **20**, 423-432 (1990).

Hamdi, M.: Toxicity and biodegradability of olive mill wastewaters in batch anaerobic digestion. *Appl. Biochem. Biotech.*, **37**, 155-163 (1992).

Hattaka, A.: Biodegradation of Lignin. *Biopol.*, **1**, 129-145 (2001).

Higuchi, T.: Biodegradation mechanism of lignin by white-rot basidiomycetes. *J. Biotechnol.*, **30**, 1-8 (1993).

Ishikawa, H., W. Schubert and F. Nord: Investigations on lignins and lignifications. XXVII. The enzymic degradation of softwood lignin by white-rot fungi. *Arch. Biochem. Biophy.*, **100**, 131-139 (1963).

Janse, B., Gaskell Jill, M. Akhtar and D. Cullen: Expression of *Phanerochaete chrysosporium* genes encoding lignin peroxidases, manganese peroxidases and glioxal oxidase in wood. *Appl. Environ. Microbiol.*, **64**, 3536-3538 (1998).

Kenneth, E., T. Ming, B. Kalyanaraman and K. Kent: Mechanism of oxidative Cα-Cβ cleavage of a lignin model dimer by *Phanerochaete chrysosporium* ligninase. Stoiquiometric and intervention of free radicals. *J. Biol. Chem.*, **260,** 8348-8353 (1985).

Kunz, A., V. Reginatto and N. Duran: Combined treatment of textile effluent using the sequence *Phanerochaete chrysosporium*-ozone. *Chemosph.*, **44**, 281-287 (2001).

Lalov, I.G., I.I. Guerginov, M.A. Krysteva and K. Fartsov: Treatment of waste water from distilleries with chitosan. *Water Res.*, **34**, 1503-1506 (2000).

Livernoche, D., L. Jurasek, M. desrochers and J. Dorika: Removal of colour from kraft mill wastewaters with cultures of white-rot fungi and with immobilized mycelium of *Coriolus versicolor*. *Biotechnol. Bioeng.*, **25**, 2055-2065 (1983).

Mehna, A., P. Bajpai and P.K. Bajpai: Studies of decolorization of effluent from a small pulp mill utilizing agriresidues with *Trametes versicolor. Enz. Microb. Technol.*, **17**, 18-22 (1995).

Milstein, O., A. Hüttermann, R. Fründ and H. Lüdemann: Enzymatic co-polymerization of lignin with low-molecular mass compounds. *Appl. Microbiol. Biotechnol.*, **40**, 760-767 (1994).

Miyamoto, T., T. Igarashi and K. Takahashi: Lignin-degrading ability of litter-decomposing basidiomycetes from *Picea* forests of Hokkaido. *Microsci.*, **41**, 105-114 (2000).

Nazareno, M., A. Margarita, H. Tournier, M. Cabello and A. Arambarri: Extracellular ABTS-oxidizing activity of autochthonous fungal strains from Argentina in solid medium. *Rev. Iberoam. Micol.*, **17,** 64-68 (2000).

Neyens, E. and J. Baeyens: A review of thermal sludge pre-treatment processes to improve dewaterability. *J. Hazard Mater.,* **98** (1-3), 51-67 (2003).

Reid, I. and M. Paice: Effects of manganese peroxidase on residual lignin of softwood kraft pulp. *Appl. Environ. Microbiol.*, **64**, 2273-2274 (1998).

Rivera, M., R. Ferrera, V. Volke, L. Fernández and R. Rodríguez : Adaptación y selección microbiana autóctona en medios de cultivos enriquecidos con Benzo(a)Pireno. *Agroc.*, **36**, 503-514 (2002).

Robinson, T., B. Chandran and P. Nigam: Studies on the decolourization of an artificial textile-effluent by white-rot fungi in N-rich and N-limited media. *Appl. Microbiol. Biotechnol.*, **57**, 810-813 (2001).

Rodriguez, M., V. Sarria, S. Esplugas and C. Pulgarin: Photo-Fenton treatment of a biorecalcitrant wastewater generated in textile activities: Biodegradability of the photo-treated solution. *J. Photochem. & Photobiol.,* **151**, 129-135 (2002).

Roldan-Carrillo, T., R. Rodríguez-Vázquez, H. Vazquez-Torres, J. Cardozo-Martínez and A. Torres-Domínguez: Remoción de estireno por *Phanerochaete chrysosporium* en cultivo líquido. *INCI*, **26(12)**, 1-6 (2001).

Sayadi, S., F. Zorgani and R. Ellouz : Decolorization of olive mill waste-waters by free and immobilized *Phanerochaete chrysosporium* cultures. *Appl. Bioch. Biotechnol.*, **56,** 265-275 (1996).

Singh, L. and B. Nigam: Disposal and treatment of distillery effluent. *In:* Enviromental biotechnology; Principles and applications (*Eds:* M. Moo-Young, W. A. Anderson and A. M. Chakrabarty). Kluwer Academic Publishers, Holland. 735-750 (1995).

Tekere, M., A.Y. Mswaka, R. Zvauya and J.S. Read: Growth, dye degradation and ligninolytic activity studies on Zimbabwean white rot fungi. *Enz. Microb. Technol.*, **28**, 420-429 (2001a).

Tekere, M., A.Y. Mswaka and J.S. Read: Ligninolytic enzyme production in selected subtropical white rot fungi under different culture conditions. *J. Bas. Microbiol.,* **41**, 115-124 (2001b).

Vicuña, R.: Lignolysis: A very peculiar microbial process. *Molec. Biotechnol.*, **14**, 173-181 (2000).

Wedzicha, B.L. and M.T. Kaputo: Melanoidins from glucose and glycine: Composition, characteristics and reactivity towards sulphite ion. *F. Chem.*, **43**, 359-367 (1992).

Correspondence to :
Dr. Antonio J. Rodríguez-Malaver
Grupo de Investigación Proyecto Fenton
Laboratorio de Bioquímica Adaptativa
Departamento de Bioquímica
Facultad de Medicina
Universidad de Los Andes
Mérida, Mérida 5101, Venezuela
E-mail: anrod@ula.ve
Tel.: +58-274-2403094
Fax: +58-274-2403045