Global NEST Journal, Vol 10, No 3, pp 426-431, 2008

TREATMENT OF THE EFFLUENT FROM A KRAFT BLEACH PLANT WITH WHITE ROT FUNGI *Pleurotus sajor caju* AND *Pleurotus ostreatus*

A. BELÉM[1]
A.V. PANTELEITCHOUK[1]
A.C. DUARTE[2]
T.A.P. ROCHA-SANTOS[1]
A.C. FREITAS[1,*]

[1]*ISEIT/Viseu - Instituto Piaget, Estrada do Alto do Gaio Galifonge, 3515-776 Lordosa, Viseu, Portugal*
[2]*CESAM & Department of Chemistry, University of Aveiro 3810-193 Aveiro, Portugal*

Received: 18/12/07
Accepted: 18/03/08

*to whom all correspondence should be addressed:
e-mail: afreitas@viseu.ipiaget.org

ABSTRACT
The final effluents from pulp and paper industry, even after biological treatment, often contain a plethora of unwanted by-products, which confer them colour and toxic characteristics. The aim of this work was to promote degradation of organic matter and remove colour by contacting fungi *Pleurotus sajor caju* or *P. ostreatus* with effluents from kraft pulp mill after treatment by an activated sludge process. Absorbance reduction of 57 and 76 % was observed after 14 days of treatment of final effluent with glucose by *P. sajor caju*, at 400 and 460 nm, respectively. Lower values of absorbance reduction were observed in final effluent with additives and inoculated with the same species (22 to 29%). Treatment with *P. ostreatus* was more efficient in the effluent with additives, 38.9 to 43.9% of reduction. Higher growth rate of *P. sajor caju* was observed in the effluent with glucose. Biological treatment resulted in 65-67% reduction of COD after 14 days revealing no differences for each effluent composition and inoculated species. Profiles of composition of organic compounds obtained by GC-MS showed no significant differences between the two effluents treated with *P. sajor caju* or *P. ostreatus*, but longer incubation time reflected higher reduction of organic compounds.

KEYWORDS: Kraft effluent, Biological treatment, Fungi, *Pleurotus*

1. INTRODUCTION

The pulp and paper industry still is an important economic activity in several countries around the world (USA, Canada, India, Portugal, etc.) although being considered as one of largest polluters (Pokhrel and Viraraghavan, 2004). Each pulp mill utilizes large amounts of water, which reappear in the form of an effluent containing large amounts of organic compounds. The higher molecular weight compounds may be biologically inactive since they cannot penetrate inside the cellular membrane of living organisms, but the degradation of such compounds results in lower molecular weight compounds which could be active and toxic to living organisms. Some fungi are able to degrade the chlorolignins, resulting in effluent decolourisation since these compounds are the main responsible for the brown colour of final effluent. Fungi have been attracted great interest for biological treatment of wastewaters (Coulibaly *et al.*, 2003), since for example wood rotting fungi are capable of degrading lignin, which is a polymeric structure with aromatic rings (Nilsson *et al.*, 2005), and have proved their potential in the lignin/phenolic wastewater treatment since they produce enzymes including lignin peroxidases, MnP-dependent peroxidases and laccases (Conesa *et al.*, 2002) which are capable of degrading lignin found in pulp bleaching effluents (Lankinen *et al.*, 1990). Peroxidases are oxidoredutases that utilize peroxide to catalyze the oxidation of a variety of organic and inorganic compounds (Conesa *et al*., 2002). White-rot fungi have been used in studies concerning biological treatment of a pulp and paper industry effluent, namely *Pleurotus* species (Ragunathan and Swaminathan, 2004; Santos *et al.*, 2002).

The aim of this work was the biological treatment of *Eucalyptus globulus* kraft pulp mill effluent, derived from the secondary treatment, by *Pleurotus sajor caju* and *P. ostreatus* in order to achieve colour reduction and decrease of organic compounds content.

2. MATERIAL AND METHODS

2.1. Source of inoculum and maintenance

Two species of *Pleurotus*: *P. sajor caju* and *P. ostreatus* were obtained from Faculdade de Ciências Agrárias – UNESP, São Paulo Brasil, subcultured and maintained on Potato Dextrose Agar (Himédia®, Índia) at 4ºC. Growth of *Pleurotus* mycelia was performed in sterilized media containing 2 g l^{-1} glucose (Riedel-Haen®, Germany), 2 g l^{-1} starch (Absolve®, Portugal), 0.1 g l^{-1} peptone (Himédia®, India) and fragments of wheat straw (1%) during 7 days at 25 ºC and 120±10 rpm. After growth, mycelia was collected by filtration with sterilized gaze and kept in sterilized plastic containers at 4ºC, for a maximum of 24h, until the biological treatment of the final effluent from the kraft pulp mill (final effluent).

2.2. Effluent source

A final effluent, from a bleached kraft pulp mill processing *Eucalyptus globulus* after secondary treatment by an activated sludge process, was collected in glass bottles, acidified at pH 2 and kept at room temperature before analysis.

2.3. Treatment of the effluent in batch reactor

The ligninolytic fungi *Pleurotus sajor caju* and *Pleurotus ostreatus* were used to treat final effluent in batch reactors with 180 ml. According to previous experiences results (Belém *et al.*, 2006), it was performed two assays to treat this effluent. In first experience the final effluent medium composition was according to Nagarathnamma & Bajpai (1992) and was denominated as final effluent with additives. In this assay 1 g l^{-1} glucose, 1.5 g l^{-1} $CaCl_2$ (Aldrich®, Steinheim), 2.0 g l^{-1} $MgSO_4$ (Panreac®, Spain), 1.5 g l^{-1} NaH_2PO_4 (Riedel-Haen®, Germany) and 0.15 g l^{-1} NH_4Cl (Sigma®, USA) were added to the effluent which was inoculated with 5-6 g of mycelia of *P. sajor caju* or *P. ostreatus*, respectively. In the second assay, it was tested the addition of 2 g l^{-1} of glucose only, to the effluent which was inoculated with 6 g of mycelia of P*. sajor caju*. In both assays, initial pH of effluent with additives or with glucose were adjusted to 5.4-5.6 and incubated at 25ºC and 120±10 rpm through 14 days.

2.4. Determination of absorbance reduction, pH, biomass and COD

Both assays were performed with two replicas where several parameters were analysed to monitories the biological treatment of final effluent with *Pleurotus* spp. Samples were withdrawn after 0, 2, 5, 7, 9 and 14 days of incubation for pH and absorbance control. Absorbance removal was followed by UV-VIS scan (200-600nm) of diluted samples at pH 5.5. Chemical oxygen demand (COD) was analyzed according to ASTM method (1994) after 0 and 14 days of incubation. Mycelia from the two species of *Pleurotus* was harvested by filtration and weighed after 7 and 14 days of treatment for biomass control.

2.5. Organic compounds analysis

Both the final effluent and the treated effluent samples, after 7 and 14 days of incubation, were characterized for organic compounds using solid phase extraction followed by analysis with Fourier transform infra-red spectroscopy (FTIR) and gas chromatography coupled to mass spectrometry (GC-MS).

After assembling the ENV1-Disk Holder with ENV1-18 Disks (C18 bonded phase), 5 ml of Dichloromethane (Labscan®, Ireland), 5 ml of methanol (Labscan®, Ireland) and 5 ml of Milli-Q Plus Water were added followed by 180 ml of sample. The sample was then extracted with 2 × 10 ml of acetonitrile (Labscan, Ireland) and dried in a rotative evaporator.

The dry extract was then derivatized by adding 250 μl of pyridin (Fluka®, Switzerland), 250 μl of BSTFA (Acros®, USA) and 50 μl of TMSCl (Aldrich®, Germany). A GC-MS Shimadzu QP1100Ex with a capillary column CPSil8CB low bleed MS (30 m × 0.25 mm × 0.25 mm film) was used for the analysis of organic compounds. The column initial temperature was 40 ºC during 4 min; program rate was 10 ºC/ min until 270 ºC and maintained during 30 min. Injector

temperature was kept at 250 ºC during all the analysis and it was injected 0.5 μl of sample after derivatisation.
Spectra of dry extracts were also collected in a FTIR (Brucker) in 4 cm^{-1} resolution.

3. RESULTS

The pH profile throughout time incubation was different in the two final effluents inoculated with *Pleurotus* spp.; higher values (4.9 to 7.6) were recorded in the effluent with glucose throughout the 14 days of incubation at 25ºC and 120±10 rpm (Figure 1). Much lower values were attained in effluent with additives and treated with *P. sajor caju* or *P. ostreatus*, which attained values of pH 3 between 5 and 7 days of incubation. This pattern is probably related with different biological pathways performed by *Pleurotus* spp resulting in different metabolic compounds or degradation products. Fungi are recognized for their superior aptitudes to produce a large variety of extra cellular proteins, organic acids and other metabolites (Palma *et al.*, 1999).

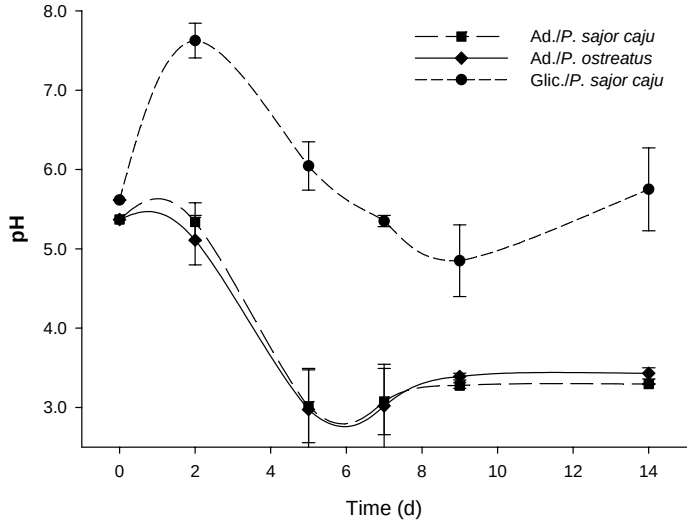

Figure 1. pH variation throughout time of final effluent with additives or glucose and inoculated with *P. sajor caju* or *P. ostreatus* at 25 ºC and 120±10 rpm

Growth rate of *P. sajor caju* was also improved in the effluent with glucose, 3.5 times against 1.3 in the final effluent with additives. In terms of COD values, biological treatment with fungi resulted in 65-67% reduction after 14 days of incubation in both experiences revealing no significant differences for each effluent composition and inoculated specie.

*Table 1***.** Biomass and COD values obtained in treated final effluent with *P. sajor caju* or *P. ostreatus,* throughout 14 days of incubation at 25ºC and120±10 rpm

Effluent composition	Fungi	Biomass (g l^{-1})				COD (g l^{-1})	
		0 d	7 d	14 d	GR[1]	0 d	14 d
Additives	*P. sajor caju*	28.7	34.3	38.4	1.3	82.3	29.1
Additives	*P. ostreatus*	34.0	33.4	42.6	1.3	92.4	31.0
Glucose	*P. sajor caju*	32.5	46.2	113.9	3.5	62.1	20.8

[1] Growth rate = total biomass (g l^{-1}) / initial biomass (g l^{-1})

Pleurotus spp. fungi were able to promote degradation of organic matter present in the effluent resulting in progressive absorbance reduction throughout time of incubation where glucose addition revealed a positive factor; 57 and 76 % of absorbance reduction were achieved after 14 days of incubation in final effluent with glucose, and treated with *P. sajor caju* at 400 and 460 nm, respectively. Lower values were observed in final effluent with

additives and treated with the same specie: 29 and 22% of absorbance reduction, respectively. In a laboratory scale treatment of effluent from a kraft bleach plant with *P. sajor caju*, the colour reduction reported after 6 days of incubation was 67% in a rotating biological contactor at 39 ºC (Ragunathan and Swaminathan, 2004).
P. ostreatus showed higher efficiency in the treatment of final effluent with additives since 38.9 and 43.9% of absorbance reduction at 400 and 460 nm was obtained after 14 days of incubation. 19.4% was the maximum removal colour in effluent from a kraft bleach plant by *P. ostreatoroseus* SING (Santos *et al.*, 2002).

*Table 2***.** Absorbance reduction (%)[1] for several wave lengths in treated final effluent with *P. sajor caju* or *P. ostreatus,* throughout 14 days of incubation at 25ºC and 120±10 rpm

Effluent composition	Fungi	λ (nm)	Time (d)				
			2	5	7	9	14
Additives	*P. sajor caju*	250	3.6	24.4	8.9	24.2	16.8
		275	6.5	27.3	12.1	25.4	19.1
		325	5.1	28.3	6.1	26.4	19.0
		400	14.1	37.7	12.3	34.8	29.4
		460	16.4	39.0	3.5	28.6	22.2
	P. ostreatus	250	12.6	15.0	13.3	17.8	9.9
		275	15.1	13.3	17.4	21.5	15.4
		325	18.6	8.5	11.8	21.9	28.3
		400	33.8	-[2]	14.5	31.3	38.9
		460	39.4	-[2]	7.6	33.9	43.9
Glucose	*P. sajor caju*	250	12.8	2.6	5.2	3.7	2.7
		275	15.2	5.4	5.6	7.6	7.0
		325	15.0	6.0	12.9	14.8	16.0
		400	19.7	29.7	37.2	49.6	57.2
		460	20.8	43.4	50.2	69.0	76.3

[1] Absorbance reduction percentage = ((Final Abs – Initial Abs)/Initial Abs)x100;
[2] No absorbance reduction.

As shown in Table 3, analysis by GC-MS detected 38 organic compounds in the final effluent prior to contacting with the fungi. Some of these compounds such as guaiacol, syringol, vanillin, vanilic acid, syringic acid, oxalic acid, hidroxypropanoic acid and aliphatic compounds with low molecular weight are probably related to lignin degradation during kraft pulping. Gaiacol, syringol and vanillin are considered the main lignin-derived markers detected in pulps (del Río *et al.*, 2001) and in black liquor from kraft pulp of Eucalyptus wood (Camarero *et al.*, 2007). In the effluent treated with *Pleurotus* spp., some of these compounds were not detected. Table 3 highlights that after 7 and 14 days of incubation, 17 to 25 organic compounds were not detected, respectively. This fact can be correlated with fungal degradation activity and it could be extrapolated that *Pleurotus* spp. have performed a complete degradation of lignin with non resulting by-products since the main lignin markers were not detected after 7 days of treatment. Several basidiomycetes fungi were found to be most efficient lignin degraders on *Eucalyptus* wood (del Río *et al.*, 2001b).

No significant differences were obtained between the two effluent compositions inoculated with *P. sajor caju* or *P. ostreatus*, but longer incubation time reflected higher reduction of organic compounds. Gaiacol, catechol, syringol, vanillin and syringic acid are examples of substances that were not detected after 7 days of effluent treatment. Other substances such as methylbutanedioic, 5-ethyl-3-metoxycatechol, vanilic acid, 1-octadecanol, lignoceric acid and β-sitostanol were not detected only after 14 days of treatment.
The FTIR results were consistent with those obtained by GC-MS, where a significant disappearance of phenols bands was recorded.
The *P. sajor caju,* a species easy to grow without special needs, has shown a high potential for treatment of effluents from bleached kraft pulp mill processing *Eucalyptus globulus,* after secondary treatment, especially with addition of glucose (2 g l^{-1}) as a co-substrate. According

to Coulibaly *et al.*, (2003), glucose and sucrose are among the co-substrates more indicated for effluent pretreatment at rates of 5 to 10 g l^{-1} which are 2.5 to 5 times superior than the rate utilized in this work.

Table 3. Organic compounds detected by GC-MS in final effluent and in treated effluent with *P. sajor caju* or *P. ostreatus,* throughout 14 days of incubation at 25ºC and 120±10 rpm

Organic compound	Final Effluent	Effluent/Additives					Effluent/Glicose		
			P. sajor caju		*P. ostreatus*			*P. sajor caju*	
		0d	7d	14d	7d	14d	0d	7d	14d
2-hidroxypropanoic acid	+	+	+	+	+	+	+	+	+
hidroxyacetic acid	+	+	-	-	-	-	+	-	-
2-furanecarboxilic acid	+	+	+	+	+	+	+	+	+
oxalic acid	+	+	+	+	+	+	+	+	+
guaiacol	+	+	-	-	-	-	+	-	-
2-hidroxybutanoic acid	+	+	+	+	+	+	+	+	+
3-hidroxypropanoic acid	+	+	+	+	+	+	+	+	+
propanedioic acid	+	+	-	-	-	-	+	-	-
catechol	+	+	-	-	-	-	+	-	-
butanedioic acid	+	+	+	+	+	+	+	+	+
syringol	+	+	-	-	-	-	+	-	-
methylbutanedioic acid	+	+	+	-	+	-	+	+	-
hidroxybutanedioic acid	+	+	+	+	+	+	+	+	+
vanillin	+	+	-	-	-	-	+	-	-
5-ethyl-3-metoxycatechol	+	+	+	-	+	-	+	+	-
NID	+	+	+	-	+	-	+	+	-
hexanodioic acid	+	+	+	+	+	+	+	+	+
dodecanoic acid	+	+	-	-	-	-	+	-	-
octanodioic acid	+	+	+	+	+	+	+	+	+
1-tetradecanol	+	+	-	-	-	-	+	-	-
vanillic acid	+	+	+	-	+	-	+	+	-
azelaic acid	+	+	-	-	-	-	+	-	-
miristic acid	+	+	-	-	-	-	+	-	-
pentadecanoic acid	+	+	+	+	+	+	+	+	+
1-hexadecanol	+	+	-	-	-	-	+	-	-
syringic acid	+	+	-	-	-	-	+	-	-
palmitic acid	+	+	+	+	+	+	+	+	+
z-9-octadecen-1-ol	+	+	+	-	+	-	+	+	-
1-octadecanol	+	+	+	-	+	-	+	+	-
1,2,4-benzenetricarboxylic acid	+	+	-	-	-	-	+	-	-
stearic acid	+	+	+	+	+	+	+	+	+
1-docosanol	+	+	-	-	-	-	+	-	-
behenic acid	+	+	-	-	-	-	+	-	-
tricosanoic acid	+	+	+	+	+	+	+	+	+
lignoceric acid	+	+	+	-	+	-	+	+	-
1-octacosanol	+	+	-	-	-	-	+	-	-
β-sitosterol	+	+	-	-	-	-	+	-	-
β-sitostanol	+	+	+	-	+	-	+	+	-

d days of incubation; + Presence; - Not detected.

4. CONCLUSIONS

Pleurotus spp. is effective and applicable for treatment of a pulp and paper mill effluent. The best results were obtained when the effluent was enriched with glucose, which indicate that this extra carbon source is important for the fungal treatment with *P. sajor caju*. The biological treatment efficiency, in terms of specific organic compounds, was confirmed by advanced analytical techniques such as GC-MS and FTIR.

ACKNOWLEDGMENTS

This work has been developed under the scope of the FCT (Portugal) funded research project, POCT/CTA/45030/2002: 'Decolourisation of effluents from pulp and paper mills: removal of organic compounds and toxicity (DECORTOX)'.

REFERENCES

ASTM (1994) Standard Test Methods for Chemical Oxygen Demand of Water, Annual Book of ASTM Standards, Report D 1252-88, American Society for Testing and Material, Philadelphia, USA.

Bajpai P. and Bajpai P.K., (1994) Biological colour removal of pulp and paper mill wastewaters, *J. Biotechnol.,* **33**, 211-220.

Belém A., Panteleitchouk A.V., Duarte A.C., Pereira R., Rocha-Santos T.A.P., Freitas A.C., (2006) Biological treatment of *Eucalyptus globulus* kraft mill effluent by *Pleurotys* spp., *International Congress on Analytical Sciences – ICAS 2006*, Moscow, *Book of Abstracts,* **1**, 371

Camarero S., Ibarra D., Martínez A.T., Romero J., Gutiérrez A. and del Río J.C., (2007) Paper pulp delignification using laccase and natural mediators, *Enz. Microbiol. Technol.,* **40**, 1262-1271.

Conesa A., Punt P.J., Hondel C.A.M.J.J., (2002) Fungal peroxidases: molecular aspects and applications, *J. Biotechnol.,* **93**, 143-158.

Coulibaly L., Gourene G. and Agathos N.S., (2003) Utilization of fungi for biotratment of raw wastewaters, *African J. Biotecnol.,* **2**, 620-630.

del Río J.C., Gutiérrez A., Martínez M.J. and Martínez A.T., (2001) Identification of residual lignin markers in eucalypt kraft pulps by Py-GC/MS, *J. Anal. Appl. Pyrol.*, **58-59**, 425-439.

del Río J.C., Gutiérrez A., Martínez M.J. and Martínez A.T., (2001b) Py-GC/MS study of *Eucalyptus globulus* wood treated with different fungi, *J. Anal. Appl. Pyrol.*, **58-59**, 441-452.

Lankinen V.P., Inkeroninen M.M., Pellinen J. and Hatakka A.I., (1990) The cost of lignin modifying enzyme, decreases of AOX and colour removal by white-rot fungi: growth on bleach plant effluent, *Water Sci. and Technol.,* **24**,189-198.

Nagarathnamma R. and Bajpai P., (1992) Decolourization and detoxification of extraction-stage effluent from Chlorine bleaching of kraft pulp by *Rhizopus oryzae*, *Applied Env. Microbiol.,* **65**, 1078-1082.

Nilsson I., Moller A., Mattiasson B., Rubindamayugi M.S.T. and Welander U., (2005) Decolourization of synthetic and real textile wastewater by the use of white-rot fungi, *Enzyme and Microbial Technol.,* **38**, 94-100.

Palma C., Moreira M.T., Mielgo I., Feijoo G. and Lema J.M., (1999) Use of a fungal bioreactor as a post treatment step for continuous decolourisation of dyes, *Wat. Sci. Tecnhol.,* **40**, 131-136.

Pokhrel D. and Viraraghavan T., (2004) Treatment of pulp and paper mill wastewater – a review, *Sci. Total Env.,* **333**, 37-58.

Ragunathan R. and Swaminathan K., (2004) Biological treatment of a pulp and paper industry effluent by *Pleurotus* spp., *World J. Microbiol. and Biotechnol.,* **20**, 389-393.

Santos A.Z, Tavares C.R.G. and Gomes-da-Costa S.M., (2002) Treatment of the effluent from a kraft bleach plant with the white-rot fungus *Pleurotus ostreatoroseus* SING, *Brazilian J. Chemical Eng.,* **19**, 371-375.