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Biodegradability of kraft mill TCF biobleaching effluents: Application of enzymatic laccase-mediator system

P.G. Monje [a], S. González-García [a,*], D. Moldes [b], T. Vidal [b], J. Romero [c], M.T. Moreira [a], G. Feijoo [a]

[a] Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] Textile and Paper Engineering Department, ETSEIAT, Universitat Politècnica de Catalunya, Colom 11, E-08222 Terrassa, Spain
[c] ENCE, Marisma de Lourizán s/n, 36153 Pontevedra, Spain

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ABSTRACT

The great amount of pollutants released from kraft pulp processes, mainly from cooking and bleaching stages, is one of the most relevant environmental problems in this type of industry. New bleaching sequences are being studied based on the use of oxidative enzymes from fungal cultures. In this study, the bleaching systems consisting of Laccase and different mediators such as 1-hydroxybenzotriazole, violuric acid, syringaldehyde and methyl syringate in the bleaching sequence of *Eucalyptus globulus* kraft pulp were applied. The main objective of this study is to evaluate the aerobic and anaerobic biodegradability and toxicity to *Vibrium fischeri* of generated L-stage and total bleaching sequence effluents. The highest levels of aerobic and anaerobic degradation of the generated effluents were achieved for treatments with laccase plus violuric acid, with 80% of aerobic degradation and 68% of anaerobic biodegradation. *V. fischeri* toxicity was remarkably reduced for all the effluents after aerobic degradation.

1. Introduction

Pulp and paper industry is traditionally known to be a large contributor to the environmental impact due to its large consumption of energy and chemicals, and also to the generation of effluents with high concentrations of suspended solids, organic load, as well as toxicity (Berube and Kahmark, 2001; Kahmark and Unwin, 1999; Srinivasan and Unwin, 1995). Pulp bleaching sequences are especially problematic due to the presence of organohalogens and wood extractives in the effluents (Xavier et al., 2005; Jokela and Salkinoja-Salonen, 1992). Chemical bleaching of pulp was initially carried out by using chlorine (Cl_2), later replaced by elemental chlorine-free reagents (ECF): ClO_2 and NaOCl (Folke et al., 1993), or by totally chlorine-free (TCF) reagents, such as H_2O_2, O_2 and O_3 (Bajpai, 2004; Nelson et al., 1998). Regarding toxicity levels, softwood and hardwood TCF effluents are less toxic than ECF effluents, being Cl_2 bleaching effluents the most toxic wastewaters based on EC_{50} values (Tarkpea et al., 1999; Cates et al., 1995).

Aerobic and anaerobic treatments of the bleaching effluents have been applied to reduce their environmental impact, in particular, their organic load and toxicity (Freitas et al., 2009; Mounteer et al., 2002; Tarkpea et al., 1999; Ahtiainen et al., 1996). Aerobic processes ranging from activated sludge to aerated lagoons were successfully applied in the treatment of high strength wastewaters and chlorinated bleaching kraft

* *Corresponding author*. Tel.: +34 981563100x16020; fax: +34 981547168.
E-mail address: sara.gonzalez@usc.es (S. González-García).
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Nomenclature

L-stage	pulp bleaching step with laccase and/or mediator
LMS	laccase-mediator system
$L_{Mediator}$	LMS-stage bleaching effluent
$L^{t}_{Mediator}$	LMS-stage combined with TCF bleaching sequence effluent (L-O-Q-PoP)
HBT	1-hydroxybenzotriazole
VA	violuric acid
SyAl	syringaldehyde
MetSyr	methyl syringate
O-stage	oxygen delignification
Q-stage	treatment with chelating agents
PoP-stage	oxygen-reinforced hydrogen peroxide bleaching followed by a depressurization to remove the oxygen
COD_t, COD_s	total and soluble chemical oxygen demand
COD_{CH4}	methanized chemical oxygen demand
BOD_5, BOD_{30}	biochemical oxygen demand after 5 and 30 d respectively
SS, TSS, VSS	suspended solids, total and volatile suspended solids respectively
VFA	volatile fatty acids
EC_{50}	effective concentration of the sample that causes a 50% reduction in the light output of the Microtox test microorganism.
UASB reactor	upflow anaerobic sludge blanket reactor

effluents (Pokhrel and Virarahghavan, 2004). Anaerobic biodegradability and toxicity to methanogens are strongly dependent on the wastewater characteristics, which depend on the processing technology, the feedstock used as raw material and the internal wastewater recycling (Vidal and Diez, 2003; Cates et al., 1995). In theory, the anaerobic process presents significant advantages in comparison with the aerobic alternative, such as considerably lower energy consumption, production of biogas and low production of sludge. However, the anaerobic treatment presented limited efficiency when applied to the decontamination of pulp mill effluents (Bajpai, 2000). The organohalogens and extractive compounds presented in bleaching wastewaters showed to be inhibitory to methanogenic bacteria (Sierra-Álvarez et al., 1994). The effluents from Cl_2 and ECF bleaching effluents presented high methanogenic toxicities and only TCF bleaching effluents were less toxic (Vidal et al., 1997).

Nowadays, the enzymatic bleaching could be an alternative for a cleaner pulp production (Fu et al., 2005; Skals et al., 2008). Treatment with xylanases boosts overall bleaching process by improving subsequent stages (Valls and Roncero, 2009; Shatalov and Pereira, 2007; Roncero et al., 2003) and has even been incorporated into bleaching sequences in some pulp mills (Bajpai, 2004; Popovici et al., 2004). The enzymatic treatment with oxidoreductases such as laccases is a promising alternative under intensive research. These enzymes are capable of oxidizing phenolic units and amine compounds in lignin (Higuchi, 2004). In combination with redox mediators, laccases can expand their action to non-phenolic substrates (Freudenreich et al., 1998). Several studies have confirmed the potential of the so-called laccase-mediator system (LMS) for the bleaching of different types of pulp (Ibarra et al., 2006; Sigoillot et al., 2005; Camarero et al., 2004; Nelson et al., 1998). Laccase-mediators can be synthetic compounds, such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1-hydroxybenzotriazole (HBT), violuric acid (VA) or natural ones, such as syringaldehyde (SyAl), methyl syringate (MetSyr) and *p*-coumaric acid. Laccase-HBT was found to be one of the most promising combinations for delignification of kraft pulp in mill applications (Sigoillot et al., 2005; Camarero et al., 2004; García, 2003) as well as laccase-VA (Moldes et al., 2008). The scale-up of this technology must overcome two main challenges: (i) the efficient production of enzyme and mediators at low cost and (ii) the use of eco-friendly mediators in the biobleaching stage, which assures minimal environmental pollution.

The goal of this paper is to determine the anaerobic and aerobic biodegradabilities as well as the Microtox® toxicity of effluents from L-stage and from the combination of the LMS with the entire TCF bleaching sequence.

2. Materials and methods

2.1. *Effluents*

Eucalyptus (*Eucalyptus globulus*) kraft pulp was washed and enzymatically treated (L-stage) at lab-pilot scale according to the following conditions: laccase (20 U/g of dry pulp) with mediators (1.5% of dry pulp) were added to 200 g of dry pulp at 10% consistency (dry pulp mass/total mass), pH 5, 50 °C and 6 bar O_2 pressure for 2 h. The mediators used to boost the laccase effect were: 1-hydroxybenzotriazole (HBT), violuric acid (VA), syringaldehyde (SyAl) and methyl syringate (MetSyr). In addition, control experiments were performed without mediator and without mediator and enzyme.

After L-stage, the following bleaching step was an oxygen delignification (O-stage) with the addition of a solution of NaOH (1.5%) and a solution of $MgSO_4$ (0.5%) at 6 bar O_2 and 98 °C for 1 h. The third step corresponded to a Q-stage, a treatment with chelating agents in order to avoid metal pollution, with the addition of 0.3% DTPA solution at 40%, pH 5.5–6, 85 °C and 1 h. The last step of the TCF sequence was the PoP-stage, an oxygen-reinforced hydrogen peroxide bleaching followed by a deppressurization where oxygen is removed. The PoP-stage included the addition of NaOH (1.5%), $MgSO_4$ (0.1%), SiO_3Na_2 (0.5%), H_2O_2 (3%), Busperse (0.033%) at a pressure of 6 bar O_2 and 105 °C for 140 min. Thereafter, pressure was released and the temperature was maintained at 98 °C for 3 additional hours.

The conventional TCF bleaching sequence referred as O-O-Q-PoP was also compared with the combination of the enzymatic stage in replacement of the first oxygen stage in the conventional TCF sequence, that is, L-O-Q-PoP, where L