GENOTOXIC EFFECTS OF KRAFT PULP MILL EFFLUENTS TREATED BY BIOLOGICAL AEROBIC SYSTEMS

Claudia R. Xavier, Elizabeth Oñate, María A. Mondaca, José L. Campos and Gladys Vidal

SUMMARY

Kraft mills are responsible for massive discharges of highly polluted effluents containing high COD concentration, and also exert toxicity on aquatic organisms. This toxic effect is generally related to the presence of abietic acid and sterols. The present work focuses on the search of a possible correlation between the presence of these compounds and the toxic effect of both untreated and treated bleached kraft mill effluents (BKME). An activated sludge system and an aerated lagoon, fed with BKME, were operated at organic loading rates (OLR) of 0.20, 0.40 and 0.60g COD/L·day, and the genotoxicity of both untreated and treated BKME was evaluated using the Bacillus subtilis 'rec' assay. The results indicate that toxicity of BKME could not be explained by the presence of abietic acid and sterols. Moreover, only a partial removal of the chronic toxicity could be achieved by the biological system used.

Introduction

Pulp mill effluents are characterized by their high concentrations of biochemical oxygen demand (up to 13.3g BOD_5/L), chemical oxygen demand (up to 39.8g COD/L) and suspended solids (up to 6.1g SS/L) (Bajpai, 2000) and their genotoxic effect on aquatic organisms (Salem *et al*., 1995; Gravato and Santos, 2002; Maria *et al*., 2003; Fernández *et al*., 2007). In order to avoid the environmental effects of this kind of effluents, biological aerobic technologies such as activated sludge systems (AS) or aerated lagoons (AL) are widely used (Thompson *et al*., 2001; Chamorro *et al*., 2009; Xavier *et al*., 2009). Removal efficiencies >80% and >40% for BOD_5 and total COD, respectively, were reported for both technologies (Xavier *et al*., 2009) but their capacity to reduce genotoxicity was scarcely studied (Houk, 1992; Kostamo and Kukkonen, 2003; Xavier *et al*., 2009).

Some authors attribute the genotoxic effects mainly to resin acids (abietic and dehydroabietic acid; Fernández *et al*., 2007) and sterols (Lehtinen *et al*., 1999) contained in the kraft mill effluents. For this reason, recent work has focused on the fate of these compounds during biological treatment (Chamorro *et al*., 2010a, b). Belmonte *et al*. (2006) and Chamorro *et al*. (2009) observed that 98% of the resin acids and 96% of

KEYWORDS / Activated Sludge / Aerated Lagoon / *Bacillus subtilis* / Genotoxicity / Kraft Pulp Mill Effluent /

Received: 10/12/2009. Modified: 04/19/2011. Accepted: 04/25/2011.

Claudia R. Xavier. Doctor in Environmental Science, Universidad de Concepción (UDEC), Chile. Professor, Universidade Tecnológica Federal do Paramá, Brazil.

Elizabeth Oñate. M.Sc. Student in Chemical Engineering, UDEC, Chile.

María A. Mondaca. Doctor in Biological Sciences, UC, Chile. Professor, UDEC, Chile

José L. Campos. Doctor in Chemical Science, Universidad de Santiago de Compostela (USC), Spain. Professor, USC, Spain.

Gladys Vidal. Doctor in Chemical Science, USC, Spain. Professor, UDEC, Chile. Address: Environmental Science Center, EULA-Chile, UDEC, Chile. P.O. Box 160-C. Concepción, Chile. e-mail: glvidal @udec.cl

EFECTOS GENOTÓXICOS DE EFLUENTES PROVENIENTES DE CELULOSA KRAFT TRATADOS POR SISTEMAS BIOLÓGICOS AERÓBICOS

Claudia R. Xavier, Elizabeth Oñate, María A. Mondaca, José L. Campos y Gladys Vidal

RESUMEN

Los molinos de papel kraft son responsables de descargas masivas de efluentes altamente contaminantes con una alta demanda química de oxígeno (DQO), los cuales también son tóxicos para organismos acuáticos. Este efecto tóxico se relaciona generalmente con la presencia de ácido abiético y esteroles. El presente trabajo se enfoca en la búsqueda de una posible correlación entre la presencia de estos compuestos y el efecto tóxico de efluentes blanqueados de molinos de papel kraft (EBMK), bien sea tratados o no. Un sistema de lodos activados y una laguna de aeración alimentados con EBMK fueron operados a niveles de carga orgánica de 0,20; 0,40 y 0,60g DQO/L·día, y la genotoxicidad de los EBMK tratados o no fue evaluada utilizando el ensayo de Bacillus subtilis *'rec (+/-)'. Los resultados indican que la toxicidad de los efluentes no puede ser explicada por la presencia de ácido abiótico y esteroles. Más aún, solo se logró una remoción parcial de la toxicidad con los sistemas biológicos utilizados.*

EFEITO GENOTÓXICO DE EFLUENTES A PARTIR DE CELULOSE KRAFT TRATADOS POR SISTEMAS BIOLÓGICOS AERÓBICOS

Claudia R. Xavier, Elizabeth Oñate, María A. Mondaca, José L. Campos e Gladys Vidal

RESUMO

Os moihnos de papel kraft são responsáveis de descargas massivas de efluentes altamente contaminantes com uma alta demanda química de oxígênio (DQO), os quais também são tóxicos para organismos aquáticos. Este efeito tóxico se relaciona geralmente com a presença de ácido abiético e esteróis. O presente trabalho está focado na procura de uma possível correlação entre a presença destes compostos e o efeito tóxico de efluentes de celulose branqueada de moihnos de papel kraft (EBMK), sejam tratados ou não. Um sistema de lamas ativadas e uma lagoa de aeração alimentados com EBMK foram operados a níveis de carga orgânica de 0,20; 0,40 e 0,60g DQO/L·dia, e a genotoxicidade dos EBMK tratados ou não foi avaliada utilizando o ensaio de Bacillus subtilis *'rec (+/-)'. Os resultados indicam que a toxicidade dos efluentes não pode ser explicada pela presença de ácido abiótico e esteróis. Mais ainda, somente se conseguiu uma remoção parcial da toxicidade com os sistemas biológicos utilizados.*

stigmasterol were removed, respectively, when the pulp mill effluent was treated in an aerated lagoon. Kostamo and Kukkonen (2003) also found removal efficiencies >97% for resin acids and sterols in an activated sludge system. These authors detected that resin acids were mostly degraded, while sterols were removed by both biodegradation (41-67%) and absorption to sludge (31-57%).

The objective of this work is to study the capacity of an activated sludge system and an aerated lagoon to reduce the genotoxicity of kraft mill effluents and relate it to the removal efficiency of resin acids and sterols.

Materials and Methods

Raw wastewater

The effluent was obtained from a modern kraft mill located in Southern Chile, which bleaches softwood pulp of *Pinus radiata* using an elemental chlorine-free sequence. The kraft mill effluent was collected after a primary treatment and stored in the dark at 4 ±1°C (Table I). The raw wastewater was supplemented with $(NH_4)_2SO_4$ and KH_2PO_4 as nitrogen and phosphate sources (BOD_5:N:P = 100:5:1) and pH was adjusted to 7.0 ±0.1.

TABLE I
PHYSICOCHEMICAL CHARACTERISTICS OF BLEACHED KRAFT MILL EFFLUENT

Parameter	Value
pH	3.4 ±0.2
COD ($mg \cdot l^{-1}$)	881.5 ±24.3
BOD_5 ($mg \cdot l^{-1}$)	300.5 ±9.5
Total phenolic compounds (UV_{215}) ($mg \cdot l^{-1}$)	271.9 ±14.2
Color (VIS_{440}) (1×1cm)	0.41 ±0.01
Aromatic compounds (UV_{254}) (1×1cm)	6.69 ±0.07
Lignin derived (UV_{272}) (1×1cm)	5.90 ±0.08
Lignin derived (UV_{280}) (1×1cm)	5.37 ±0.06
Lignosulphonic acid (VIS_{346}) (1×1cm)	1.73 ±0.04
Resin acid (abietic acid) ($mg \cdot l^{-1}$)	1.45 ±0.93
β-sitosterol ($mg \cdot l^{-1}$)	0.33 ±0. 03
Stigmasterol ($mg \cdot l^{-1}$)	0.07 ± 0.01

TABLE II
OPERATIONAL PHASES

Phase	Operation time (days)	OLR (g COD/L·day)	HRT (days)
I	0-87	0.20	4.4
II	88-108	0.40	2.2
III	109-175	0.60	1.1

Experimental set-up and operational strategy

An activated sludge reactor and an aerobic lagoon with a useful volume of 0.9L and a settling zone of 0.45L were used. Both systems were operated at dissolved oxygen (DO) concentration >2mg O_2/L, room temperature and without pH control. Each reactor was inoculated with biomass from an industrial aerated lagoon treating BKME (5g VSS/L).

The AS and AL reactors were operated during 175 days (Table II). During this period, the organic loading rate (OLR) was increased stepwise from 0.20 to 0.60g COD/L·day by decreasing the hydraulic retention time (HRT). Removal efficiencies for COD, BOD_5, color, total phenolic compounds and lignin derived were measured for the AS and AL

systems during these three different phases.

Genotoxicity determination

Genotoxicity of β-sitosterol (0-32mg·l^{-1}), stigmasterol (0-32mg·l^{-1}), abietic acid (0-25mg·l^{-1}), effluent (0-0.5mg·l^{-1}) and treated effluent (0-0.5mg·l^{-1}) was determined by using *Bacillus subtilis* PB 1791 (rec-) and *Bacillus subtilis* PB 1652 (rec+) (Mazza, 1982). To test the toxic effects, stock solutions of β-sitosterol (Calbiocem, 95%), stigmasterol (Sigma, 95%) and resin acid (Sigma, 99%) were prepared, while samples of effluent and treated effluent (pH= 7.0) were previously filtrated through a 0.45µm membrane and sterilized by using a filter syringe (0.20µm). All 'rec' assays were done in tubes containing 2ml of soft agar maintained on a dry batch at 45°C. Each experiment was performed in duplicate. Each tube was supplemented with: i) a sample of the tested compound or treated effluent, and ii) 0.1ml of a 10^4 CFU/ml dilution of an overnight culture nutrient broth (Difco). Then, each tube was mixed in a vortex and distributed onto an agar plate surface. Plates were incubated at 37°C during 24h. Plate efficiency (A) is provided by the survival ratio (N/N_0) of *Bacillus subtilis* rec- and *Bacillus subtilis* rec+ according to

$$A = \frac{N_{rec-} / N_0}{N_{rec+} / N_0}$$

where N_0: reference bacterial growth, N_{rec+}: rec+ bacterial growth within different effluent or influent doses, and N_{rec-}: rec- bacterial growth within different effluent or influent doses. When A<1.0, the tested compound yields genotoxicity to the cells.

Statistix for Windows 2.2 software was used to evaluate sample variance analysis (ANOVA- Bonferroni test).

Analytical methods

Chemical oxygen demand (COD) and biological oxygen demand (BOD_5) were measured following APHA (1985). The total phenolic compound (UV phenol) concentration was measured by UV absorbance in a 1cm quartz cell at 215nm, pH 8.0 (0.2M KH_2PO_4 buffer), and then transformed to concentration using a calibration curve with phenol as a standard solution. Spectrophotometric measurements of filtered samples were principally performed at wavelengths of 440 (color), 346 (lignosulfonic acids), 254 (aromatic compounds) and 280 (lignin derived compounds) in a 1×1cm quartz cell using a Genesys UV-VIS spectrophotometer, and were determined according to the Chamorro *et al.* (2005) procedure. COD, BOD_5, color, total phenolic compounds and resin acid were measured after membrane (0.45µm) filtration.

Analyses of resin acid were performed by liquid chromatography using an HPLC system (Shimadzu model LC-10 ATVP, Kyoto, Japan) coupled to a mass spectrometry detector (DAD Shimadzu model SPD-M 10 AVP). Liquid samples were extracted with dichloromethane using the procedure developed by Li *et al.* (1996). Extracts were evaporated to ~5ml using a rotary evaporator. Of the sample, 20µl were injected in an RP-18-Lichrospher-60 column (Darmstadt, Germany) thermostated at 20°C. The liquid phase was methanol:water (70:30, v/v) at a flow rate of 1ml·min^{-1}. Calibration was performed according to Latorre *et al.* (2003). β-sitosterol and stigmasterol were determined according to the analytical method described by Cook *et al.* (1997).

Results

Removal of organic compounds

Figure 1 shows the average removal of COD, BOD_5, phenolic compounds (UV_{215}) and color (VIS_{440}) during the three successive operation phases. The activated sludge system achieved a COD removal efficiency of 63.3 ±10.3% which was slightly higher than that obtained by the aerated lagoon (57.1 ±7.1%). However, BOD_5 removal was similar in both reactors, with values up to 99.2 ±0.2%. The fate of total phenolic compounds was very different in both systems. A low degradation was observed in the aerated lagoon while generation of these compounds was detected in the AS reactor. Color removal was higher in the activated sludge reactor but decreased when the OLR was increased to 0.6g COD/L·day.

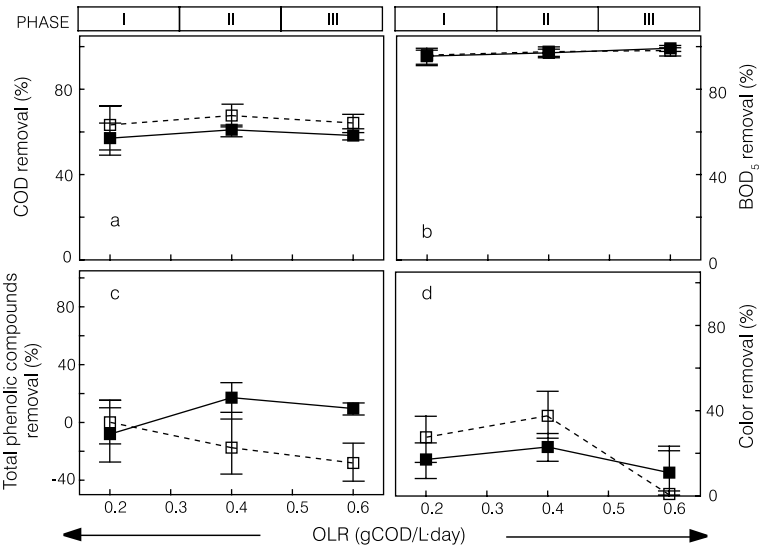

Figure 1. Performance of activated sludge (□) and aerated lagoon (■) reactors. A: COD, b: BOD_5, c: total phenolic compounds (UV_{215}), d: color (VIS_{440}) removal.

Removal efficiencies of aromatic compounds (UV_{254}) and lignin derivatives (UV_{272} and UV_{280}) were between 20-40% during whole operation period for both systems, AS being slightly more efficient than AL (Figure 2). A decrease of lignosulfonic acid (VIS_{346}) removal efficiency was observed when the OLR was increased to 0.6g

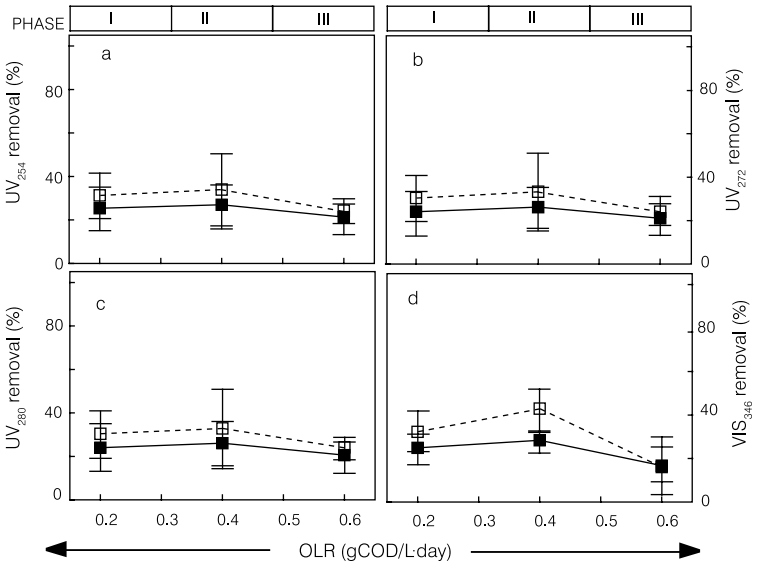

Figure 2. Performance of activated sludge (□), and aerated lagoon (■) reactors. a: aromatic compounds (UV_{254}), b: lignin derived (UV_{272}), c: lignin derived (UV_{280}), d: lignosulfonic acid (VIS_{346}).

COD/L·day. Both the activated sludge system and the aerated lagoon were able to totally remove abietic acid. However, removal of sterols only achieved an efficiency of ~100% in the AS reactor, the AL system reaching an efficiency of 40%.

Genotoxicity removal

Firstly, the genotoxic effects of different concentrations of β-sitosterol, stigmasterol and abietic acid were determined (Table III). Stigmasterol caused genotoxicity at the lower concentration assayed, while β-sitosterol and abietic acid show a clear genotoxic effect at concentrations higher than 8 and 15mg·l^{-1}, respectively.

TABLE III
EFFICIENCY OF PLATE (A) VALUES FOR β-SITOSTEROL, STIGMASTEROL AND ABIETIC ACID

Concentration (mg·l^{-1})	A		
	ß-sitosterol	Estigmasterol	Abietic acid
0	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00
1	1.10 ±0.02	0.54 ±0.10	1.12 ±0.15
2	1.17 ±0.58	0.67 ±0.10	-
4	1.28 ±1.06	0.72 ±0.03	-
5	-*	-	-
8	0.93 ±1.03	0.68 ±0.05	-
15	-	-	0.95 ±0.26
16	0.81 ±0.17	0.80 ±0.28	-
25	-	-	0.80 ±0.09
32	0.44 ±0.96	0.72 ±0.07	-

* Genotoxicity was not determined at this concentration.

Table IV shows the efficiency of plate values of the effluent and the treated effluent when the systems operated at OLRs of 0.2 and 0.6g COD/L·day. Only the AS reactor was able to achieve a partial removal of genotoxicity (A values close to 1) when operated at 0.2g COD/L·day. At this OLR, the effluent of the aerated lagoon still presented citotoxic effects at all the doses tested but it was lower than that exerted by the BKME. Nevertheless, when an OLR of 0.6g COD/L·day was applied to both systems, an increase of genotoxicity was observed during the aerobic treatment, this increase being higher for the aerated lagoon. This fact could be explained by the generation of intermediate products during biodegradation that may be more toxic than the compounds contained in the kraft mill effluents (Kostamo *et al*., 2004).

TABLE IV
EFFICIENCY OF PLATE (A) VALUES FOR THE KRAFT MILL EFFLUENT AND TREATED EFFLUENT

OLR (g COD/L·day)	Dose (ml)	Efficiency of plate (A) value		
		Kraft mill effluent	AS effluent	AL effluent
0.2	0.1	1.017 ±0.052	0.971 ±0.156	0.887 ±0.290
	0.3	0.828 ±0.017	0.925 ±0.166	0.881 ±0.092
	0.5	0.792 ±0.132	1.188 ±0.029	0.893 ±0.100
0.6	0.1	1.017 ±0.052	0.731 ±0.020	0.561 ±0.116
	0.3	0.828 ±0.017	0.880 ±0.085	0.639 ±0.136
	0.5	0.792 ±0.132	0.692 ±0.144	0.740 ±0.196

Discussion

Both systems tested achieved COD and BOD_5 removal efficiencies ~60 and 99%, respectively, which are similar to values reported for this kind of systems (Pokhrel and Viraraghavan, 2004) and for aerobic moving bed bioreactors (Villamar *et al*., 2009), although for this type of effluents removal of COD may be improved up to values higher than 90% by using SBR systems (Tsang *et al*., 2007). The performance of both systems was quite different with regards to the degradation of total phenolic compounds: while the aerated lagoon, in general, removed only slightly these compounds, the activated sludge system generated them. This tendency was also observed by Xavier *et al.* (2009). However, the fate of total phenolic compounds could be more affected by the operational conditions than by the technology applied (Belmonte *et al*., 2006, Villamar *et al*., 2009). Color in pulp and paper mills is largely due to lignin and lignin derivates. For this reason, the poor removal of color could relate to the resistance of these compounds to be biodegraded. In fact, similar profiles were observed for color and lignosulfonic acid (VIS_{346}) (Figures 1d and 2d).

The high efficiencies of abietic acid removal obtained in this work agree with those found by other authors using either aerobic lagoons (Zender *et al*., 1994; Belmonte *et al*., 2006) or activated sludge reactors (Kostamo and Kukkonen, 2003). Sterols were completely removed when the effluent was treated in the activated sludge reactor, as was also found by Kostamo *et al.* (2004), but a removal of only 40% was achieved by the aerated lagoon. Fernández *et al.* (2007) also found that both aerated lagoons and trickling filters showed a lower capacity than activated sludge systems to remove sterols.

In this work, an increase of the genotoxic effect was observed in the treated effluent. This was also found by Chamorro *et al.* (2009), who evaluated the acute toxicity, using *Daphnia obtuse*, of both raw and treated (with an aerated lagoon) kraft mill effluents. Similar results were also obtained by Pintar *et al.* (2004) when they treated this kind of wastewater by catalytic wet-air oxidation and found that the end product solutions were all more toxic to *Daphnia magna* than the starting effluents by factors ranging from 2 to 33, although the TOC removal efficiency achieved was ~95%.

This means that intermediate products even more toxic than the original compounds could be generated during aerobic biodegradation (Pintar *et al*., 2004). For example, resin acids are not mineralized by aerobic bacteria, but only transformed into forms like decarboxylated resin acid hydrocarbons, or oxygenated resin acids or retene (Zender *et al*., 1994). Similar results have been observed for sterols (Mattsson *et al*., 2001).

On the other hand, the genotoxic effects of the kraft mill effluent were higher than those obtained from assays with solutions containing similar concentrations of sterols and resin acid. Therefore, these genotoxic effects may not be directly associated to such compounds. In fact, the treated effluent contains lower concentrations of sterols and resin acid than the raw effluent but its toxicity is higher. This agrees with results obtained by Chamorro *et al.* (2009), who observed an increase of toxicity after the biological treatment, in spite of the fact that their system removed 96% of the inlet stigmasterol. Moreover, Chamorro *et al.* (2010b) showed that a biological system treating *Pinus radiata* kraft mill effluent was able to remove 80-83% of estrogenic activity at HRTs of 1 and 2 days.

The use of either activated sludge system and aerated lagoon appear not to be efficient systems to remove chronic toxicity. Therefore, the use of hybrid systems (Kennedy *et al*., 2000) or a post-treatment of the kraft mill effluent, such as ozonation (Yeber *et al*., 1999) or activated carbon (Reyes *et al*., 2009), is recommended.

Conclusions

The activated sludge system and aerated lagoon was able to remove COD and BOD_5

around 60% and 99%, respectively. Only the activated sludge reactor was able to achieve a partial removal of genotoxicity which was operated at 0.2g COD/L·day. The results indicate that toxicity of BKME could not be explained by the presence of abietic acid and sterols.

ACKNOWLEDGEMENTS

This work was supported by FONDECYT grant 1070509. José L. Campos thanks Dirección de Investigación (UDEC) of the Universidad de Concepción, Chile, for supporting his stay at the Environmental Science Center EULA-Chile.

REFERENCES

APHA (1985) *Standard Methods for Examination of Water and Wastewater*. 16th ed. APHA-AWWA-WPCF Washington, DC, USA. 1268 pp.

Bajpai P (2000) *Treatment of Pulp and Paper Mill Effluents with Anaerobic Technology*. Pira International. Leatherhead, UK. 129 pp.

Belmonte M, Xavier C, Decap J, Martínez M, Sierra-Álvarez R, Vidal G (2006) Improved aerobic biodegradation of abietic acid in ECF bleached kraft mill effluent due to biomass adaptation. *J. Hazard. Mat. 135*: 256-263.

Chamorro S, Xavier C, Vidal G (2005) Behaviour of aromatic compounds contained in the kraft mill effluents measurements by UV-VIS. *Biotechnol. Progr. 21*: 1567-1571.

Chamorro S, Xavier C, Hernández V, Becerra J, Vidal G (2009) Aerobic removal of stigmasterol contained in kraft mill effluents. *E J. Biotech. 12*: 1-7.

Chamorro S, Pozo G, Jarpa M, Hernández V, Becerra J, Vidal, G (2010a) Monitoring endocrine activity in kraft mill effluents treated by an Aerobic moving bed bioreactor system. *Wat. Sci. Technol. 62*: 157-161.

Chamorro S, Monsalvez E, Hernández V, Becerra J, Mondaca MA, Piña B, Vidal G (2010b) Detection of estrogenic activity from kraft mill effluents by yeast estrogen screen. *Bull. Env. Contam. Toxicol. 84*: 165-169.

Cook DL, LaFleur L, Parrish A, Jones J, Hoy D (1997) Characterization of plant sterols form 22 us pulp and paper mills. *Water Sci. Technol. 35*: 297-303.

Fernández MP, Ikonomou MG, Buchanan I (2007) An assessment of estrogenic organic contaminants in Canadian wastewaters. *Sci. Total Env. 373*: 250-269.

Gravato C, Santos MA (2002) Juvenile sea bass liver biotransformation and erythrocytic genotoxic responses to pulp mill contaminants. *Ecotoxicol. Env. Saf. 53*: 104-112.

Houk VS (1992) The genotoxicity of industrial wastes and effluents. *Mutat. Res. 277*: 91-138.

Kennedy KJ, Graham B, Droste RL, Fernandes L, Narbaitz R (2000) Microtox and *Ceriodaphnia dubia* toxicity of BKME with powdered activated carbon treatment. *Water SA 26*: 205-216.

Kostamo A, Kukkonen JVK (2003) Removal of resin acids and sterols from pulp mill effluents by activated sludge treatment. *Water Res. 37*: 2813-2820.

Kostamo A, Holmbom B, Kukkonen JVK (2004) Fate of wood extractives in wastewater treatment plants at kraft pulp mills and mechanical pulp mills. *Water Res. 38*: 972-982.

Latorre A, Rigol A, Lacorte S, Barcelo D (2003) Comparison of gas chromatographic-mass spectrometry and liquid chromatographic-mass spectrometry for the determination of fatty and resin acids in paper mill process waters. *J. Chromatogr. A 991*: 205-215.

Lehtinen KJ, Mattsson K, Tana J, Engström C, Lerche O, Hemming J (1999) Effects of wood-related sterol on the reproduction egg survival and offspring of brown trout (*Salmo trutta lacustrus* L.). *Ecotox. Env. Saf. 42*: 40-49.

Li K, Chen T, Bicho P, Breuil C, Saddler JNA (1996) Comparison of gas chromatographic and immunochemical methods for quantifying resin acids. In Servos MR, Munkittrick KR, Carey JH, van der Kraak GJ (Eds.) *Environmental Fate and Effects of Pulp and Paper Mill Effluents*. St. Lucie Press. Delray Beach, FL, USA. pp. 119-127.

Maria VL, Correia AC, Santos MA (2003) Genotoxic and hepatic biotransformation responses induced by the overflow of pulp mill and secondary-treated effluents on *Anguilla anguilla* L. *Ecotox. Env. Saf. 55*: 126-137.

Mattsson K, Lehtinen KJ, Tana J, Härdig J, Kukkonen J, Nakari T, Engström C (2001) Effects of pulp mill effluents and restricted diet on growth and physiology of rainbow trout (*Oncorhynchus mykiss*). *Ecotox. Env. Saf. 49*: 144-154.

Mazza G (1982) *Bacillus subtilis* "*rec* assay" test with isogenic strains. *Appl. Env. Microbiol. 43*: 177-184.

Pintar A, Besson M, Gallezot P, Gibert J, Martin D (2004) Toxicity to *Daphnia magna* and *Vibrio fischeri* of Kraft bleach plant effluents treated by catalytic wet-air oxidation. *Water Res. 38*: 289-300.

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

Reyes F, Chamorro S, Yeber MC, Vidal G (2009) Characterization of E1 kraft mill effluent by toxicity identification evaluation methodology. *Water Air Soil Pollut. 199*: 183-190.

Salem S, Rao B, Quinn A, Burnison BK, Hayes MA, Metcalfe CD (1995) Assessment of the genotoxic potential of pulp mill effluent using bacterial, fish and mammalian assays. *Chemosphere 31*: 3553-3566.

Thompson G, Swain J, Kay M, Forster CF (2001) The treatment of pulp and paper mill effluent: a review. *Bioresour. Technol. 77*: 275-286.

Tsang YF, Hua FL, Chua H, Sin SN, Wang YJ (2007) Optimization of biological treatment of paper mill effluent in a sequencing batch reactor. *Biochem. Eng. J. 34*: 193-199.

Villamar CA, Jarpa M, Decap J, Vidal G (2009) Aerobic moving bed bioreactor performance: a comparative study of removal efficiencies of kraft mill effluents from *Pinus radiata* and *Eucalyptus globulus* as raw material. *Wat. Sci. Technol. 59*: 507-514.

Xavier CR, Mosquera-Corral A, Becerra J, Hernández V, Vidal G (2009) Activated sludge versus aerated lagoon treatment of kraft mill effluents containing β-sitosterol and stigmasterol. *J. Env. Sci. Health A Tox. Hazard. Subst. Env. Eng. 44*: 327-335.

Yeber MC, Rodríguez J, Freer J, Baéza J, Durán N, Mansilla HD (1999) Advanced oxidation of a pulp mill bleaching wastewater. *Chemosphere 39*: 1679-1688.

Zender JA, Struthridge TR, Langdon AG, Wilkins AL, Mackie KL, McFarlane PN (1994) Removal and transformation of resin acids during secondary treatment at a New Zealand kraft pulp and paper mill. *Water Sci. Technol. 29*: 105-121.