

Aerobic removal of stigmasterol contained in kraft mill effluents

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Abbreviations: AL: aerated lagoon
BOD: biological oxygen demand
COD: chemical oxygen demand
EDC: endocrine disrupting chemicals
HRT: hydraulic retention time
OLR: organic load rates
SLR: stigmasterol load rates
TSS: total suspended solid
VSS: volatile suspended solid

Kraft mill effluent, due to its organic matter content and acute toxicity, must be treated. A primary treatment followed by a secondary treatment is the most common system. Aerated lagoon is also considered an effective biological treatment, although this technology has some drawbacks related with operation parameters and land extension space. Moreover, the recovery efficiency for micropollutants contained in kraft mill effluent is questioned due to the anoxic zone in the

system. The goal of this work is to evaluate the performance of the aerated lagoon to remove stigmasterol contained in kraft mill effluents. Kraft mill effluent was treated by an aerated lagoon (AL), which was operated with three different stigmasterol load rates (SLR = 0.2, 0.6 and 1.1 mg/L x d) and a hydraulic retention time of 1 day. The AL's maximum Chemical Oxygen Demand (COD) removal was 65%, whereas the Biological Oxygen Demand (BOD₅) was around 95%.

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The removal efficiency of stigmasterol removal was 96% when SLR 1.1 mg/L x d, although an accumulation of stigmasterol was detected for lower SLR.

Estrogenic endocrine disruption could be produced by extractive compounds coming from the kraft pulp. High concentrations of extractive compounds (and in particular sterols) are contained in black liquor from the digestion processes, and these are generally recovered by evaporation and combustion (Xavier et al. 2005). However, there is a small part of black liquor that remains in the fiber and is dragged into the washing processes. Thus, in the discharged kraft mill effluents, concentrations ranging from 0.3 to 3.4 mg/L for sterols and 0.28 to 1.21 mg/L for resin acids can be found (Vidal et al. 2007). Moreover, physical chemical properties of stigmasterol like $\log K_{ow}$ (10.20), molecular weight (412.7 g/mol) or boiling point (140°C) show the persistent characteristics on the environment. Due to above, recent studies have demonstrated the biological effects of these compounds on fish in surface water systems (Orrego et al. 2006). The main biological effects are alteration in the

sexual steroid level (profile) in fish plasma and diminished reproductive adaptation, among others (Larsson et al. 2002).

The most commonly used biological aerobic treatments in kraft mills are aerated lagoons (Correa et al. 2003; Belmonte et al. 2006a) and activated sludge (Khan and Hall, 2003). The main characteristics, of both systems are the hydraulic conditions, biomass concentration and oxygen availability. Activated sludge and aerated lagoons are easy to operate but require large hydraulic retention time (HRT) and elevated land extensions. Biodegradable organic matter (85-95%) and acute toxicity (100%) could be removed (Kostamo and Kukkonen, 2003; Belmonte et al. 2006b). However, the operating conditions strongly influence the degradation of endocrine disrupting chemicals (EDC) (Werker and Hall, 1999). Vidal et al. 2007 demonstrated that in anoxic areas, intermediate compounds of the resin acid biodegradation can produce accumulation. Moreover, Kostamo and Kukkonen (2003) have shown that over 41% of the sterols were reduced or transformed into other

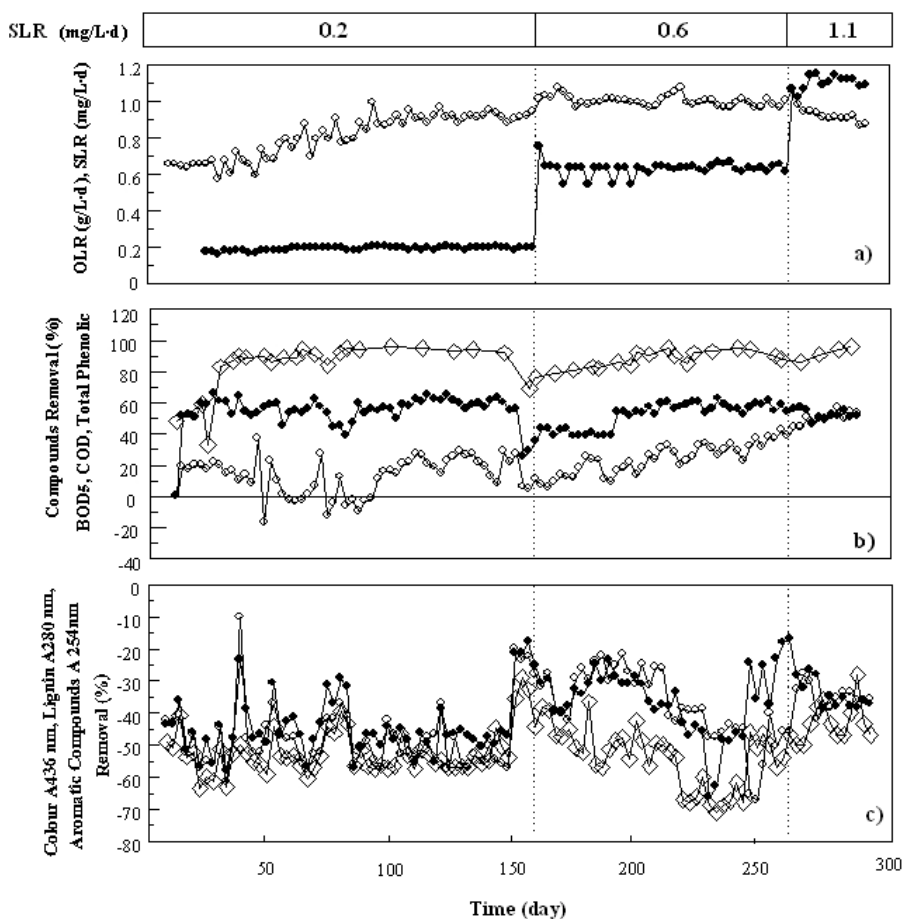


Figure 1. Performance of aerated lagoon.

(a) OLR (○), SLR (●).

(b) COD removal (●), BOD₅ removal (◇) and total phenolic compounds, UV₂₁₅ (○).

(c) Color removal (◇), Lignin removal, UV_{280 nm} (○) and aromatic compounds removal UV_{254 nm} (●).

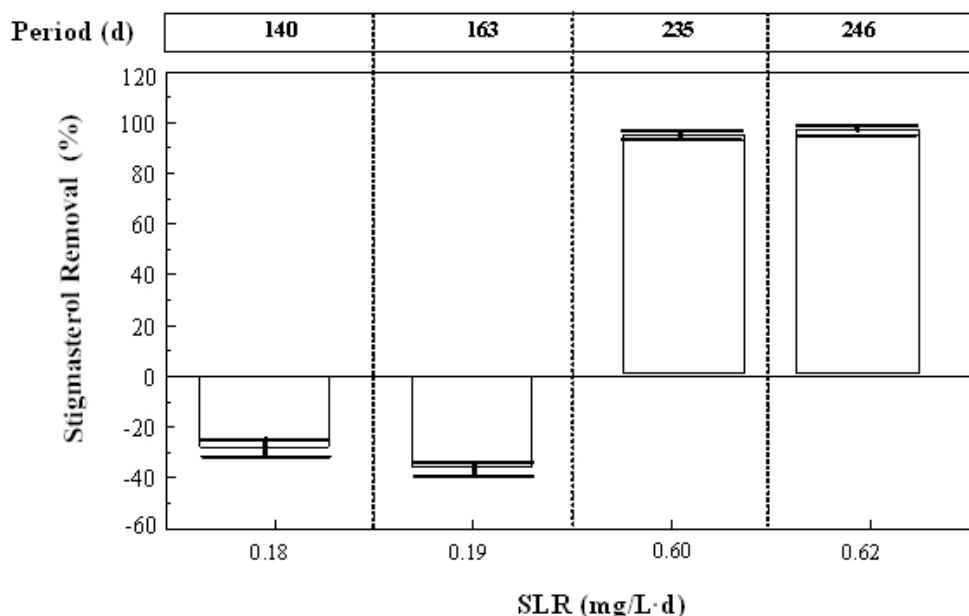


Figure 2. Stigmaesterol removal by aerated lagoon.

compounds. Furthermore, in general, less than 5% of the resin acids and over 31% of the sterols were removed in biosludge of the sludge thickener. On the other hand, Khan and Hall (2003) and Kostamo et al. (2004) have obtained percentages of phytosterol adsorption from 30 to 70 % in biomass of AS and AL systems.

Cook et al. (1997) worked with 5 kraft cellulose plants where the whitening process consisted in elementary chlorine free processes, finding that a phytosterol reduction process was produced in all cases except in the case of stigmasterol, where it could increase between 192% and 367%. In the activated sludge system, an 88% increase in stigmasterol was observed, and is probably due to the chemical or biological transformation in the precursor treatment system where it was converted into stigmasterol (Cook et al. 1997). However, few studies present results with respect to EDC behavior in aerated lagoons.

The goal of this work is to evaluate the performance of aerated lagoon in removing stigmasterol contained in kraft mill effluents.

MATERIALS AND METHODS

Wastewater

The effluent was obtained from a modern kraft mill that bleaches softwood pulp and is located in Southern Chile. *Pinus radiata* is the raw material used in the process. The kraft mill effluent was obtained after primary treatment. Table 1 shows the main physicochemical characteristics of this effluent. The effluent was supplemented with 0.022 g/L

of NH_4Cl as nitrogen source and 0.169 g/L of K_2PO_4 as phosphorus source ($\text{BOD}_5:\text{N}:\text{P} = 100:5:1$).

Inoculum

The aerobic lagoon was inoculated using an aerobic microbial consortium (5 g/L of volatile suspended solid (VSS), 9 g/L of total suspended solid (TSS)) that was obtained from an aerobic sewage treatment plant without a nitrification/denitrification process.

Continuous bioreactor system

An aerated lagoon (AL) with an aerated area (0.44 L) and a settling (non-aerated) area (0.22 L) was used as biological treatment, following Correa et al. (2003). A peristaltic pump fed the system. The oxygen concentration in the aerated zone was maintained above 6 mg/L using a diffuser system. The AL operation was divided in two phases. In Phase I, the system was fed with only kraft mill effluent to establish steady-state performance conditions. The HRT of the system was maintained for 24.0 hrs, corresponding to organic load rates (OLR) around 0.81 g COD/L \times d. In Phase II, the lagoon was fed with kraft mill effluent supplemented with an increasing concentration of stigmasterol (Merck, 95%) (concentration ranged from 0.2 to 1 mg/L). The stigmasterol was dispersed into kraft mill effluent matrix by sonication during 10 hrs. The HRT was reduced stepwise from 24.0 to 12.4 hrs, resulting in an increase of the Stigmasterol Load Rate (SLR) from 0.55 to 1.1 mg/L d. The removal efficiencies of BOD_5 , COD, total phenolic compounds, stigmasterol and color levels were calculated using equation 1. Solids were not removed from the reactor.

$$\%R = \frac{Q_{out} \cdot C_{out} - Q_{in} \cdot C_{in}}{Q_{in} \cdot C_{in}} \cdot 100 \quad [\text{Eq. 1}]$$

Where:

%R : Removal efficiency

Q : Flow (L/d)

C_{in} : Concentration in the influent (mg/L)

C_{out} : Concentration in the effluent (mg/L)

Analytical methods

VSS, TSS, COD, BOD₅, phenols, and lignin and tannins were measured following Standard Methods (APHA-AWWA-WPCF, 1985). The total phenolic compounds (UV phenols) concentration was measured by UV absorbance in a 1-cm quartz cell at 215 nm, pH 8.0 (0.2 M KH₂PO₄ buffer) and transformed to concentration using a calibration curve with phenol as standard solution. The samples for parameter determination were membrane filtered (0.45 µm). Spectrophotometric measurements of filtered samples were principally performed at wavelengths of 436 (color), 346 (lignosulfonic acids), 254 (aromatic compounds) and 280 (lignin derived compounds) in a 1 x 1-cm quartz cell using a Genesys UV-VIS spectrophotometer, and were determined according to the Çeçen (2003) procedure.

The stigmasterol present in the kraft mill effluent was first identified in samples filtered through a 0.2-µm membrane filter by gas chromatography CG-MS (HP 5890 chromatograph with mass detector HP 5972) using a column Agilent (19091s-433 HP-MS 5% phenyl-methylsiloxane, length 30 m, internal diameter 0.5 µm). The detection limit was fixed at 1 µg/L. The compounds were extracted from 100 mL of samples with 20 mL dichloromethane at pH value of 7. The extract was rota-

evaporated and re-suspended in 2 mL of chloroform and injected to the chromatograph (Cook et al. 1997; Khan and Hall, 2003). Patron solutions were prepared with stigmasterol (Sigma) using cholesterol (Carbiochem) as internal patron and a calibration curve prepared at concentrations from 4 to 130 mg/L. Samples were extracted following the procedure used for the previous qualitative analysis and spiked with 1000 mg/L of cholesterol. A volume of 1 µL was injected to the GC-FID chromatograph. Stigmasterol values were determined in 50 mL of sludge samples filtered and dried (105°C for 12 hrs). The dried sample was extracted in a Soxhlet with 200 mL of dichloromethane for 20 hrs. After this point, the same procedure used for the quantitative determination was used.

The acute toxicity of influent and effluent on *D. magna* (< 24 hrs old) was evaluated at 24 hrs. Mortality was recorded at the end of exposure, where mortality was defined as a lack of organism mobility when the vessel was shaken. Five samples with different concentrations (6.25, 12.5, 25, 50, 100%) and one control were evaluated. Four replicates of 30 mL (each one containing five organisms) were performed for each concentration and the control. The culture was not renewed during the test. Oxygen concentration, pH and conductivity were measured at the beginning and end of each test. The 24 hrs mean lethal concentrations were calculated using the Probit and the Spearman-Kärber methods, as appropriate (USEPA, 1993; NCh 2083, 1999).

RESULTS AND DISCUSSION

Figure 1 shows the performance of the AL. HRT was maintained around 1 day to evaluate the behavior of stigmasterol biodegradation and the biomass evolution. During the operation of Phase I, the OLR was on average maintained around 0.81 g COD/L x d; whereas during the

Table 1. Physical-chemical characteristics of the raw wastewater.

Parameter	Value
pH	3.4 ± 0.17
COD (mg/L)	881.5 ± 24.3
BOD ₅ (mg/L)	300.5 ± 9.5
Total phenolic compounds (UV ₂₁₅) (mg/L)	271.9 ± 14.2
β-sitosterol (mg/L)	0.333 ± 0.028
Stigmasterol (mg/L)	0.069 ± 0.014
Color (VIS ₄₄₀) (1 x 1 cm)	0.41 ± 0.01
Total suspended solids (mg/L)	2.2 ± 0.9

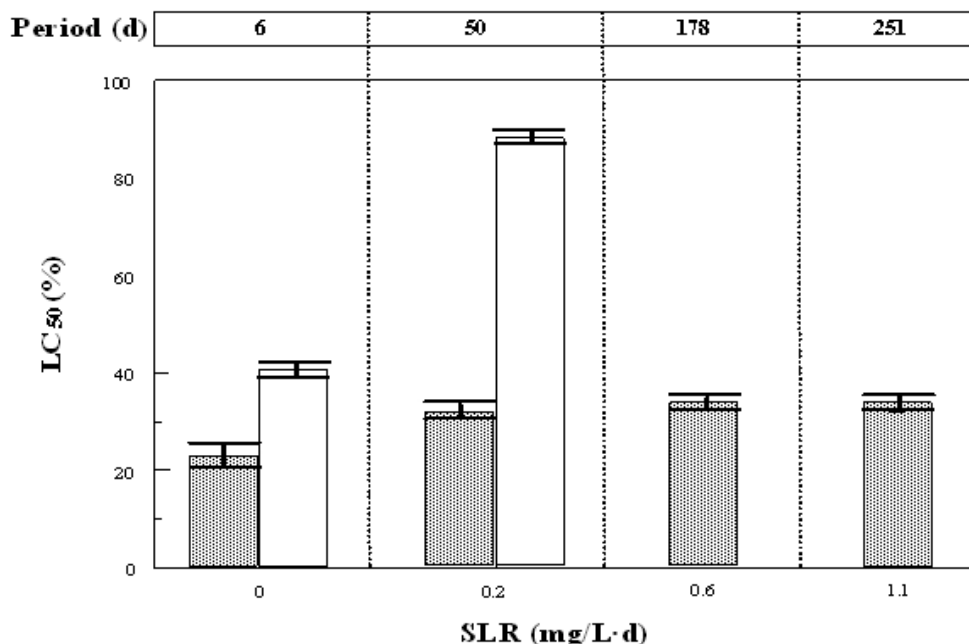


Figure 3. Acute toxicity of kraft mill Influent (▨) and effluent (□) evaluated by *Daphnia obtusa*.

second phase, the OLR increased until 0.96 g COD/L x d. On the other hand, the average SLR values during Phase II were 0.2, 0.6 and 1.1 mg/L x d, respectively.

Also, AL performance indicates removal efficiency for BOD₅ and COD ranging between 48 - 96% and 25.0 - 65.2%, respectively (Figure 1b). Simultaneously, the average removal efficiency of total phenolic compounds was 20.7% and 28.5% for Phase I Phase II, respectively.

In spite of this difference, it was observed that total phenolic compounds were removed by the AL, and that the color and aromatic compounds were polymetized in the aerobic system. Due to this, Figure 1c shows negatives removal values. These phenomena were observed in previous work. In fact, Chamorro et al. (2005) show that aromatic compounds of the higher molecular weight fraction (from 5.000 to 10.000 Da) increased after an AL biological treatment. This phenomenon is due to the oxidation-polymerization process (Milestone et al. 2004).

Table 2 shows the behavior of aromatic compounds with respect to COD during the kraft mill effluent treatment by AL assays. Most of these parameters show the degradation and non-biodegradable fraction in the kraft mill effluent (Çeçen, 1999). The behavior of lignosulfonic acid (VIS₃₄₆/COD) content in the kraft mill effluent is in a range of 0.283 - 0.448. The relationship VIS₃₄₆/COD increased, indicating low biodegradation of these compounds by aerobic bacteria. In the same way, lignin behavior (measured as UV₂₈₀/COD) shows a range between 0.026 - 0.051 in the different assays, indicating that aerobic

biomass mineralizes the lignin compounds to a lesser extent than organic matter (measured as COD).

The UV₂₅₄/UV₂₈₀ relationship is used as an indicator of lignin-derived compound presence in wastewaters, where low values indicate a higher percentage of these compounds (Çeçen, 2003). During the AL treatment process, the UV₂₅₄/UV₂₈₀ relationship in the effluent was around 1.26 - 1.28. Similarly, Çeçen (2003) shows that UV₂₅₄/UV₂₈₀ did not undergo a significant change (ranging between 1.1 - 1.13). These results suggest that the residual COD consisted in lignin compounds, which were also the major aromatic species in these effluents. The same results were found by Chamorro et al. (2005) and Milestone et al. (2004).

VIS₄₀₀ and VIS₄₃₆ show color behavior during the aerobic treatment. Low values show that recalcitrant compounds are present in the treated effluent (Çeçen, 1999; Çeçen, 2003). Color behavior in the system is also explained by the no biodegradation of lignin and aromatic compounds in general.

Figure 2 shows the stigmasterol removal in the AL system. Stigmasterol increases at low SLR, ranging from 29 - 37%. This phenomenon was also observed by Cook et al. (1997), who found that stigmasterol could increase its concentration in more than 300% in AL system. Also, Khan and Hall (2003) show that aerated systems are not efficient for stigmasterol removal. However, when SLR increases up to 0.6 mg/L x d, the removal efficiency of stigmasterol increased to 90%. The sterol biodegradation mechanisms are biotransformation and adsorption (Fernandez et al. 2007). Khan and Hall (2003) and Kostamo and Kukkonen

Table 2. Behavior of aromatic compounds during biological treatment.

	SLR	VIS ₄₄₀ /COD		VIS ₃₄₆ /COD		UV ₂₇₄ /COD		UV ₂₅₄ /UV ₂₈₀	
Period	mg/L x d	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
19-168	0.2	0.093 0.040-0.198	0.201 0.107-0.249	0.217 0.151-0.470	0.448 0.329-0.561	0.68 0.47-0.84	0.94 0.92-1.94	1.45 1.22-1.77	1.282 1.17-4.39
169-266	0.6	0.064 0.049-0.107	0.115 0.075-0.239	0.151 0.124-0.231	0.283 0.149-0.528	0.63 0.52-0.78	0.96 0.76-1.50	1.26 1.25-1.30	1.27 1.25-1.30
267-291	1.1	0.054 0.050-0.060	0.126 0.012-0.195	0.186 0.171-0.212	0.328 0.296-0.365	0.70 0.66-0.74	1.12 1.03-1.16	1.263 1.26-1.27	1.264 1.26-1.27

Color (VIS_{440 nm}, VIS₄₃₆), lignosulfonic acids (UV₂₅₄), aromatic compounds (UV₂₇₄) and lignin derived compounds (UV₂₈₀).

(2003) have obtained percentages of phytosterol adsorption from 30 to 70% in AL system biomass.

Bioassays with *D. magna* indicate that AL treatment of kraft mill effluent can only partially remove the acute toxic compounds (toxicity reduction range between 23.12% and 40.64%) at low SLR (0.2 mg/L x d) (Figure 3). However, when SLR increases up 0.6 mg/L x d, the 24 hrs LC₅₀ values of the aerobic effluent are above 100%. A similar effect was found by Priha (1996) in the evaluation of 13 industries with activated sludge and AL.

CONCLUDING REMARKS

Organic matter removal by AL ranged between 25.0 - 65.2% for COD, BOD₅ removal was above 95%, and total phenolic compounds removal was around 48%.

Under experimental conditions, stigmasterol removal was 96% when SRL increases up to 0.6 mg/L x d. Stigmasterol removal was strongly dependent on the operation rate. Stigmasterol fate needs to be studied in the future.

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