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Improved aerobic biodegradation of abietic acid in ECF bleached kraft mill effluent due to biomass adaptation

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Abstract

Kraft pulp mill effluents contain elevated concentrations of resin acids, chiefly abietic and dehydroabietic acid, and other lipophilic wood constituents. Resin acids, if not efficiently removed during wastewater treatment, can be responsible for chronic toxicity in aquatic systems. The objective of this study was to investigate the biological removal of abietic acid (AbA) during the treatment of elemental chlorine free (ECF) kraft mill effluents in aerobic lagoons and to assess its improvement with time as a result of biomass adaptation. Under these conditions, the average removal efficiencies of AbA and BOD_5 attained in the aerobic lagoon were high and exceeded 80% and 95%, respectively. Microbial inhibition of non-acclimated and acclimated biomass by AbA was not detected in batch bioassays. Kinetic studies showed that the K_s and V_m values equalled 76.7 mg AbA/l and 0.011 l/h, respectively, for the non-acclimated biomass, and 1678 mg AbA/l and 0.13 l/h, respectively, for the acclimated biomass.

Keywords: Resin acids; Biomass adaptation; Aerobic biodegradation; Wastewater treatment; Aerobic lagoon; ECF kraft bleached mill effluent

1. Introduction

With the replacement of bleaching sequences using elemental chlorine (Cl_2) by chlorine dioxide (ClO_2), the pulp and paper industry has reduced considerably the formation and discharge of chlorinated organic material into the aquatic environment [1]. The introduction of elemental chlorine free (ECF) processes has resulted in a decrease of the overall discharge of AOX (adsorbable organic halogens) in kraft bleaching ranging from 48% to 65% [2]. Moreover, since 1990, total chlorine free (TCF) bleaching has been introduced, largely in response to market demands for non-chlorine bleached pulp. In spite of the development of the chlorine-free bleaching processes, chronic effects (reproductive and physiological) had been observed on fish exposed to treated ECF- and TCF-kraft bleaching effluents [2,3]. Wood extractive constituents released during cooking and debarking operations are considered a main source of aquatic toxicity in chlorine-free bleaching effluents, causing chronic sublethal toxicity, genotoxicity and potential bioaccumulation in fish tissues [4,5]. Moreover, resin acids and, to a lesser extent, unsaturated fatty acids have been reported as major contributors to the toxicity of paper-industry effluents to aquatic organisms, causing chronic sublethal toxicity, genotoxicity and potential bioaccumulationin fish tissues.

Extractives are low molecular weight lipophilic constituents in wood that consist chiefly of resins acids, sterols, triglycerides and free fatty acids [2]. Although, part of these compounds are burned or recovered in the kraft process, a fraction of the wood extractives is dissolved in the effluent [5]. The proportions of resin acids: fatty acids: sterols determined in the effluent of a TCF kraft softwood mill averaged 64:30:6 (on a weight basis) [2]. AbA and dehydroabietic acid (DHA) are the most abundant resin acids representing 19–33% and 14–30% of total resin acids, respectively [6].

Concentrations of resin acids and fatty acids exceeding 11.6 mg/l have been determined in bleached kraft effluents [2]. Resin acids are highly toxic to aquatic organisms. The 96 h LC_{50} values reported for resin acids in assays with rainbow trout range from 0.4 to 1.7 mg/l for rainbow trout [3,4].

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Table 1
Total concentration of resin acids detected in effluents from pulp mill after aerobic treatment

Treatment	Raw material	Process		HRT	Resin acid		Reference
		Digestion	Bleaching		Removal (%)	Final concentration (μg/l)	
Activated sludge	SW	Kraft	ECF	12 h	50–98	4.4–200	[11]
	SW	–	ECF	–	171[a]	2400	[13]
	SW	Kraft	ECF	30 h	98–99	10–15	[2]
	SW	Kraft + TMP[b]	TCF	42 h	88–99	11–92	[2]
	SW/HW	Kraft	ECF	–	97	8.3–20	[11,13,14]
Aerated lagoon	SW	Kraft	ECF	4–9 days	96–97	8–42	[11,14]
	SW/HW	Kraft	ECF	3–6 days	97–98	2–330	[3,13,15,16]
	SW/HW	–	TCF	–	97	100	[13]
	SW/HW	Kraft	ECF	–	6–8 days	23.5	[14]

[a] Resins acids concentration increased.
[b] TMP: thermomechanical pulping process.

Literature studies suggest that the removal of resin acids during biological treatment depends on three factors: (1) the wastewater treatment process applied [2,6,7]; (2) the lignocellulosic feedstock [8]; (3) the microbial community [9,10]. The activated sludge process (AS) and aerated lagoons (AL) are the most commonly used methods for the biological treatment of pulp and paper mill effluents. ALs are operated at hydraulic retention time (HRT) values considerably longer compared to activated sludge systems (i.e., 8–18 h versus 4–9 day). Table 1 shows that the removal efficiencies attained for resin acids during the aerobic treatment of kraft bleach effluents generally vary from 87% to 98%. Based on Table 1, the effectiveness of activated sludge and AL systems to remove resin acids appear to be comparable.

Regarding the impact of lignocellulosic feedstock on resin acid levels, treated effluents from kraft mills using softwoods as feedstock and effluents from bleaching plants using elemental chlorine appear to show higher resin acids concentrations as compared to treated effluents from kraft mills utilizing only hardwoods or a mixed softwood/hardwood feedstock (Table 1). This observation is in agreement with the exceedingly higher levels of resin acids generally found in bleaching wastewaters from facilities utilizing high fractions of coniferous pulpwoods.

Acclimated biomass and the availability of suitable co-substrates could be key factors determining the effectiveness of resin acid biodegradation. Batch studies have shown that resin acid levels, biomass concentrations and nutrient availability can affect the overall rate removal of resin acids [7,9]. The availability of suitable cosubstrates has also been shown to enhance the biodegradation of resin acids [7]. Although bleaching effluents contain high levels of recalcitrant compounds (e.g. high molecular weight lignin fragments, chlorinated lignin and tannins compounds) [12], biodegradable carbon sources (e.g. cellulose and hemicellulose depolymerization products) are also present that could potentially serve as co-substrate by DHA-degrading bacteria [9].

The main goal of this study was to investigate the removal abietic acid during biological treatment of an industrial ECF effluent in an aerated lagoon and establish whether biomass adaptation would result in improved biodegradation of this toxic resin acid.

2. Materials and methods

2.1. Wastewater

The ECF effluent was obtained from a modern bleached kraft mill located in the South of Chile [12] that bleaches softwood pulp using an ECF sequence ($D_0E_0D_1D_2$) for bleaching softwood pulp. *Pinus radiata* is the raw material used in the process. The kraft mill effluent was obtained after primary treatment. The effluent was supplemented with $(NH_4)_2SO_4$ and KH_2PO_4 as a nitrogen and phosphate source (BOD_5:N:P = 100:5:1). After 73 days of AL operation, AbA (50 mg/l) (Sigma–Aldrich, St. Louis, MO, USA) was added to the influent from a concentrated stock solution adjusted to pH 11. The effluent was neutralized using a 0.1N NaOH solution.

2.2. Inoculum

The anaerobic 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.

2.3. Continuous bioreactor system

An AL with an aerated (0.44 l) and a settling zone (non-aerated zone) (0.22 l) was used as biological treatment. The system was fed by means of a peristaltic pump. 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 ECF effluent to establish steady-state performance conditions. The HRT of the system was maintained between 44.5 and 45.2 h, corresponding to organic load rates (OLR) ranging from 0.31 to 0.51 g COD/l d. In Phase II, the lagoon was fed with ECF effluent supplemented with 50 mg/l of AbA. The HRT was reduced stepwise from 47.0 to 12.4 h, resulting in an increase of the OLR from 0.55 to 2.55 g/l d and an increase of the volumetric loading rate of AbA (AbALR) from 0.15 to 0.71 mg/l d. The removal efficiencies of BOD_5, COD, total phenols, AbA and color levels

were calculated using Eq. (1) [12]:

$$\%R = \frac{F_{out}C_{out} - F_{in}C_{in}}{F_{out}C_{out}} \times 100 \quad (1)$$

where $\%R$ is the removal efficiency, F the flow (l/d), C_{in} the concentration in the influent (mg/l) and C_{out} is the concentration in the effluent (mg/l).

2.4. Kinetic of AbA biodegradation

In order to evaluate the improvement of AbA biodegradation by adapted aerobic biomass, the kinetics of AbA biodegradation were studied using shaken batch bioassays. Erlenmeyer flasks (0.25 l) were incubated with three different biomass samples: (1) biomass non adapted to AbA which was obtained from the AL on day 64, and biomass adapted to high levels of AbA obtained from the AL after: (2) 139 days and (3) 266 days of operation. Concentrations of AbA of 25, 50, 100, 250, and 500 mg/l were supplied to each inoculum according to Correa et al. [17]. Assays with biomass from day 266 also received 1000 mg AbA/l. Assays were carried out in triplicate. The flasks were incubated in a shaker at 150 rpm at 25 ± 2 °C in the dark.

Bacterial viability was determined in triplicate R2A agar plates that were incubated at 25 °C for 5 days according to Godoy et al. [18]. The AbA concentration in the liquid phase for each assay was determined by spectrophometry at 239 nm. The complete mineralization of AbA was confirmed by high performance liquid chromatography (HPLC).

The modified Monod's kinetic model was used to evaluate the possible biomass adaptation to AbA with increasing AL operation time, considering the initial rate of the AbA degradation (r_s) for each concentration evaluated [19]:

$$r_s = -\frac{dS}{dt} = \frac{X\mu_m}{Y_{X/S}} \frac{S}{K_S + S} \quad (2)$$

where S is the AbA concentration (mg/l), X the cell concentration (log cfu/ml), K_s (mg/l) the Monod half saturation constant, μ_m (h^{-1}) the maximum growth rate and $Y_{X/S}$ is the cell yield (log cfu/g).

2.5. Analytical methods

Volatile suspended solid (VSS), total suspended solid (TSS), chemical oxygen demand (COD) and biological oxygen demand (BOD_5) were measured according to Standard Methods [20]. Total phenolic compounds (UV_{215}) and color (VIS_{440}) in wastewater samples were determined spectrophotometrically at 215 and 440 nm, respectively, using a 1-cm quartz cuvette. The samples were diluted to less than 0.8 absorbance units in a 0.025 M KH_2PO_4 buffer (pH 9.0) prior to analysis. Samples for determination of COD, BOD_5 and UV_{215} and VIS_{440} were membrane filtered (0.45 μm).

Analyses of AbA by liquid chromatography were carried 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. [21]. Extracts were evaporated to approximately 5 ml using a rotary evaporator. Twenty microliters of sample was injected in a 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 1 ml/min. Calibration was performed according to Latorre et al. [22].

3. Results and discussion

3.1. Characterization of the raw wastewater

Table 2 shows the physico-chemical characteristics of the industrial kraft bleached effluent utilized in this study. The high COD/BOD_5 ratio (2.9) indicates that a high fraction of the organic contaminants present in bleach kraft mill effluents are recalcitrant to aerobic degradation. High molecular weight lignin (over 1000 Da), which is inert and does not contribute BOD_5 to the wastewater, is responsible for the typical dark color of effluents from kraft bleaching. The AbA concentration in the kraft mill effluent utilized in this study averaged 1.4 mg/l that is within the range of AbA concentrations reported for kraft bleaching effluents, 0.02–17 mg/l [2,23,27].

3.2. Performance of the aerated lagoon

The biomass inoculated in the reactor was not acclimated to AbA. Table 3 shows the control strategy during the operation of the aerated lagoon. During the start-up period (Phase I), the lagoon was fed with raw wastewater at a low organic load rate of 0.31 g COD/l d, which was progressively increased to 0.51 g COD/l d. During this period the reactor biomass was exposed to very low AbA concentrations (1.45 mg/l on the average). Phase II was initiated on day 73 by supplementing the raw wastewater effluent with 50 mg AbA/l. During this period, the AbALR was gradually increased from 0.15 to 0.71 g/l d by reducing the HRT from 47 to 12.4 h. This also resulted in an increase of the OLR from 0.55 to 2.55 g COD/l d.

Fig. 1 and Table 4 show that when the lagoon was operated with the AbA-supplemented bleaching wastewater at a HRT of approximately 2 days (47 h), high BOD_5 and COD removal efficiencies exceeding 99% and 65%, respectively, could be attained. For similar effluent and treatment method (AL with HRT = 30 h), Kostamo et al. [2] found a removal efficiency of BOD_7 and COD of 98% and 76%, respectively. In a comparable study using an AL operated at a HRT of 2 days, Liu et al. [24] reported BOD_5 and COD removal efficiencies of 98% and 78%,

Table 2
Characteristics of the kraft ECF bleaching effluent utilized in this study

Parameter	Value
pH	3.4 ± 0.2
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
Abietic acid (mg/l)	1.4 ± 0.5
Color (VIS_{440}) (1 cm × 1 cm)	0.41 ± 0.01

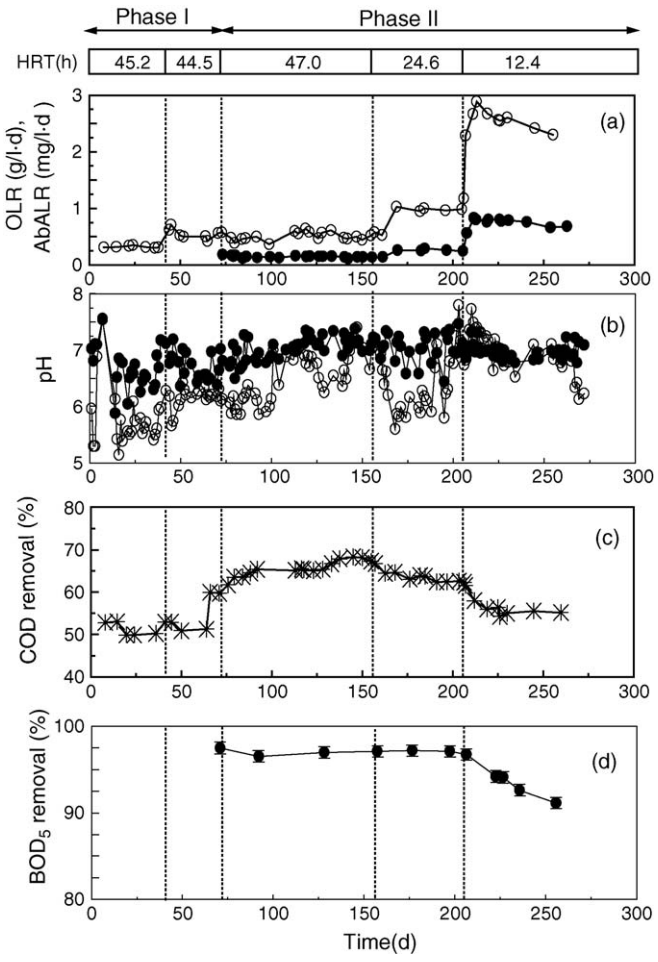

Fig. 1. Performance of AL reactor: (a) OLR (◯) and AbALR (●), (b) influent pH (◯), effluent pH (●), (c) COD removal (*), and (d) BOD_5 removal (●).

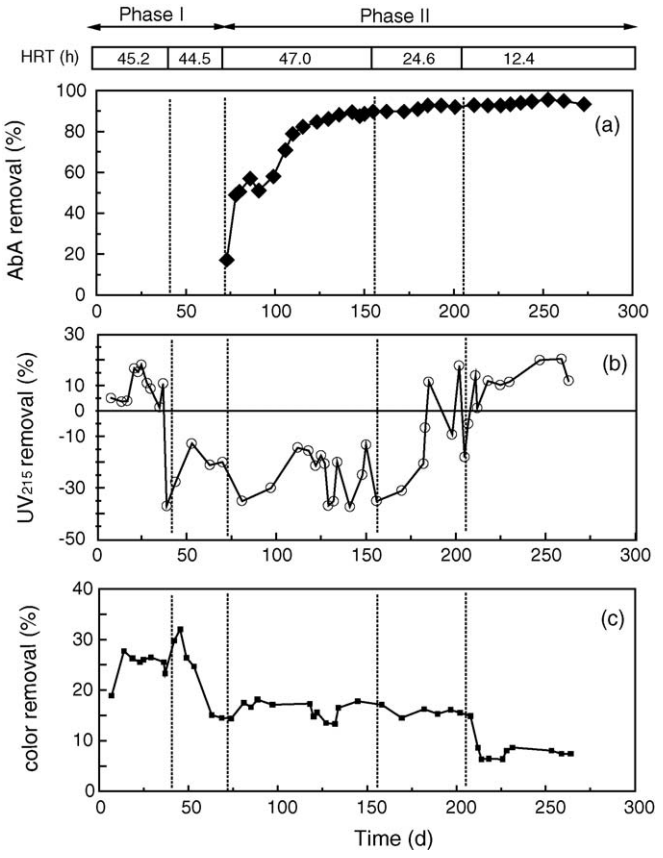

Fig. 2. Performance of AL reactor, (a) AbA removal (♦); (b) removal of total phenolic compounds (UV_{215}) (◯) and (c) removal of color-bearing compounds (VIS_{440}) (■).

respectively. The COD removal efficiency attained by the AL decreased to 63.8% when the HRT was decreased to 24.6 h, while the BOD_5 elimination remained unchanged. Further decrease of the HRT to only 12.4 h, and the concomitant increase in OLR and AbALR levels, lead to a further decrease of the COD elimination to 57.2% and a small, but noticeable, decrease in the BOD_5 removal efficiency to 94.9%. These results indicate that the treatment capacity of the system was exceeded under the new conditions of operation. In the present study, an average COD/BOD_5 ratio of 57 was determined in the treated effluent, which is somewhat higher than the COD/BOD_7 ratio (37) reported by Kostamo et al. [2]. The relatively high COD/BOD_5 ratio found indicates that the bulk of the residual organic matter in the effluent consisted of compounds that resist biological treatment. Removal of the recalcitrant COD contained in the aerobic effluent, ranging between 0.3 and 0.4 g COD/l, can be accomplished by a tertiary treatment using physico-chemical methods such as ozone oxidation or ultrafiltration [25].

Removal results for AbA, total phenolic compounds and color levels as a function of operation time are shown in Fig. 2. The AbA removal efficiency reached 80% after 110 days of operation. Following biomass acclimation to AbA (from day 150 onwards), the removal efficiency exceeded 90%. In this study

Table 3
Operation strategy of the aerobic lagoon

Period (day)	HRT (h)		OLR (g COD/l d)		AbALR (g AbA/l d)	
	Average	Range	Average	Range	Average	Range
Phase I						
0–42	45.2	42.3–47.8	0.31	0.26–0.35	0	0
43–72	44.5	42.4–46.6	0.51	0.42–0.71	0	0
Phase II						
73–161	47.0	43.5–51.9	0.55	0.37–0.64	0.15	0.12–0.18
162–205	24.6	22.4–26.4	1.02	0.95–1.17	0.25	0.20–0.29
206–270	12.4	11.7–18.4	2.55	2.29–2.88	0.71	0.54–0.8

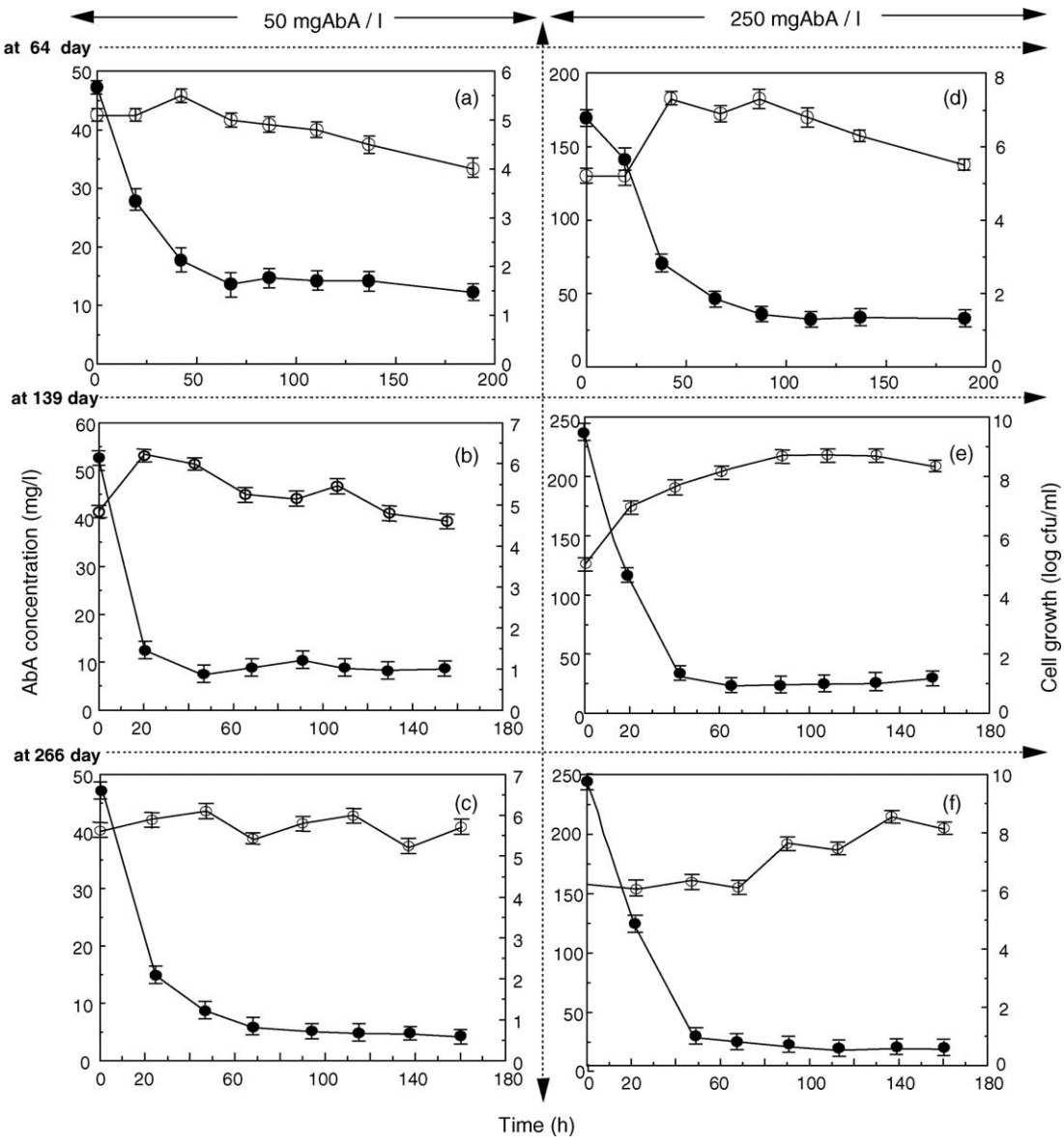

Fig. 3. Degradation of AbA (●) and cell growth (◯) by acclimated and non-acclimated biomass. The initial concentration of AbA was: 50 mg/l (graphs on the left hand side) and 250 mg/l (graphs on the right-hand side). The biomass assayed was obtained from the aerobic lagoon at different times of operation: (a) 64 days, (b) 139 days, (c) 266 days.

adaptation stage was necessary for increasing removal of AbA. Conversely, Liver and Hall [7] reported that the removal of resin acids was not dependent on biomass adaptation. The biodegradation pathway of abietane resin acids by the aerobic bacteria, *Pseudomonas abietaniphila* BKME-9 and *Zoogloea resiniphila* DhA-35, has been partially elucidated in a recent report [10]. The study shows that resin acid removal by the microbial community was stimulated when it was stressed by high AbA loading rates.

Table 4
Average removal parameters in the aerobic lagoon during the operation

Period (day)	AbA		COD		BOD_5 (%)		Color		UV_{215}	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Phase I										
0–42	0	0	44.7	29.3–55.9	–	–	−28.6	−20.3 to −32.0	12.5	5.9–16.9
43–72	0	0	58.8	50.9–68.4	98.8	98.8	−20.3	−32.7 to −15.7	11.8	7.6–15.3
Phase II										
73–161	80.7	79.3–97.8	67.3	58.6–79.2	98.2	97.9–98.5	−16.5	−17.4 to −13.1	−17.8	−9.6 to −25.7
162–205	91.2	90.3–95.6	63.8	60.0–67.6	99.0	98.6–99.5	−15.1	−19.1 to −17.7	14.6	−8.9 to 21.2
206–270	93.6	91.4–97.8	57.2	52.2–66.5	94.9	93.2–95.9	−9.6	−7.1 to −15.4	15.4	2 to −28.9

Table 5
Kinetic constants determined by Monod modified model in the degradation of AbA

Cells source	K_s (mg/l)	V_m (l/h)	OLR (mg COD/l d)	Specific rate (mg/g VSS d)	Reference
Non-acclimated biomass to AbA (at 64 days)	76.70	0.011	0.51	15.90	This study
Acclimated biomass to AbA (at 139 days)	932.90	0.088	0.55	194.40	This study
Acclimated biomass to AbA (at 266 days)	1677.90	0.131	2.55	349.60	This study
Non-acclimated biomass to resin acids[a]			11.90	11.90	[7]
			24.00	24.00	
			115.00	115.00	
			142.00	142.00	
				50.00	[24]
				1.20–16.80	[28]

[a] Total resin acids: dehydroabietic acid, abieticacid, isopimaric acid, pimaric acid and palustric acid.

On the other hand, total phenolic compounds, measured as UV215, were poorly removed (less than 15.4% elimination). Phenolic compounds were not biodegraded between days 73 and 180 of operation, likely due to the exogenous supplementation of AbA to the AL system. Similar results were reported by Diez et al. [26]. This finding is not surprising since a large fraction of the phenolic compounds in bleaching effluents are high molecular polymers, which are resistant to bacterial degradation [12]. As it was observed with the phenolic compounds, low removal efficiencies (9.6–28.6%) were observed for color-bearing constituents in this study. On the contrary, the color levels ranged between from −28.6% to −9.6% after biological treatment. Recent studies report increasing color levels following biological treatment of kraft pulp mill effluent. Aerobic treatment has been reported earlier to result in poor color removal [26,27]. In some cases, biological treatment has been observed to lead to increase in the color level of bleaching effluents. Color formation has been hypothesized to result from lignin polymerization reactions, particularly in areas with low redox conditions that receive little or no aeration [27].

3.3. Kinetics of resin acid biodegradation

In order to assess the possible adaptation of the biomass to AbA, the kinetics of AbA biodegradation were evaluated in batch assays supplemented with 25, 50, 100, 250 and 500 mg AbA/l. Kinetic parameters of three different biomass samples exposed to AbA for varying time periods were determined. AbA was used as the sole carbon and energy source in these assays. As an example, the time course of AbA degradation by non-acclimated biomass (64 days) and acclimated biomass (at 139 and 266 days) is shown in Fig. 3. A comparative analysis of the assays with 50 mg/l and different biomass acclimation (Fig. 3a–c) show that AbA biodegradation increased with biomass acclimation time. An almost complete mineralization of AbA (over 90%) was determined in the assay with adapted biomass (266 days) (Fig. 3c), whereas the AbA elimination was only 72% is the assay with non-acclimated biomass (64 days) (Fig. 3a). Moreover, the concentration of viable bacteria at the end of the experiment (170 h) was 4.0×10^5 and 5.8×10^6 cfu/ml for the non-acclimated and best acclimated (266 days) biomass, respectively. Comparative analysis of the assays with 250 mg AbA/l and biomass adapted for various time periods (Fig. 3d–f) also showed higher AbA removal rates for the acclimated biomass. Biomass growth from 1×10^5 to 2×10^8 cfu/ml was determined in the presence of AbA as sole carbon source.

These results indicate that AbA at concentrations of 50 mg/l was not toxic to bacteria in aerobic biomass, and that regardless of the length of adaptation to this chemical. Kinetic parameters for the three different assays were calculated using a modified Monod model. Table 5 shows that the specific AbA degradation rate for the non-acclimated biomass (at 64 days) was 15.9 mg AbA/g VSS d. Similar values were determined by Liver and Hall [7], 11.9 and 24.0 mg AbA/g VSS d, in batch assays utilizing 3.5 and 45 mg AbA/l, respectively. The specific AbA degradation rate determined in our study for the best-acclimated biomass was 349.6 mg AbA/g VSS d, which is 22-fold that of the non-adapted biomass. Kinetic constants show the effect of the biomass adaptation on AbA biodegradation. The K_s values determined for the non-adapted and best-adapted biomass were 76.7 and 1677.9 mg AbA/l, indicating a 21.8-fold increase in biodegradation capability. In this sense, V_m values were 0.011 l/h for non-acclimated biomass and 0.13 l/h for the acclimated biomass. From Fig. 4, that shows the relationship between

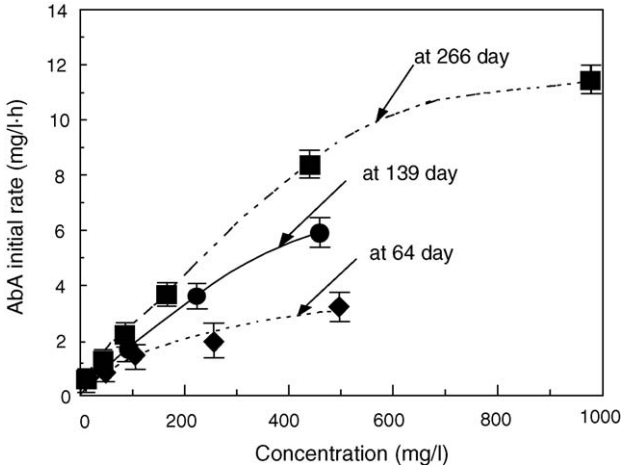

Fig. 4. Effect of biomass adaptation on the degradation rate of AbA. Comparison of experimental data with results predicted by a the modified Monod model: (a) 64 day, experimental data (■) and prediction (- · · · -), (b) 139 day, experimental data (●) and prediction (—), 266 day, experimental data (♦) and prediction (- - - -).

the initial rate of AbA biodegradation and the concentration of AbA, it is evident that the most adapted biomass was capable of the highest AbA degradation rates.

4. Conclusions

An aerobic lagoon system treating an industrial ECF kraft bleached effluent supplemented with AbA (50 mg/l) was able to remove over 98% and 80% of the BOD_5 and AbA content, respectively, when operated at a HRT of near 2 days (47 h). Under these conditions, the average COD removal efficiency was 67.3%. Results from continuous and batch bioassays confirmed that the ability of the aerobic biomass to degrade AbA increased with sludge acclimation. Bacterial inhibition by AbA was not detected for any biomass sample under and the different concentrations of AbA used in this study.

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References

[1] S.C. Stratton, P.L. Gleadow, A.P. Johnson, Pulp mill process closure: a review of global technology developments and mill experiences in the 1990s, Water Sci. Technol. 50 (2004) 183–194.
[2] A. Kostamo, B. Holmbom, J.V.K. Kukkonen, Fate of wood extractives in wastewater treatment plants at kraft pulp mill and mechanical pulp mills, Water Res. 38 (2004) 972–982.
[3] A. Oikari, B.E. Lönn, M. Castrén, T. Nakari, B. Snickars-Nikinmaa, H. Bister, E. Virtanen, Toxicological effects of dehydroabietic acid (DHAA) on the trout, *Salmo gairdneri*, in fresh water, Water Res. 17 (1983) 81–89.
[4] M. Ali, T.R. Sreekrishnan, Aquatic toxicity from pulp and paper mill effluents: a review, Adv. Environ. Res. 5 (2001) 175–196.
[5] A. Oikari, B. Holmbom, Ecotoxicological effects of process changes implemented in a pulp and paper mill: a nordic case study, in: M.R. Servos, R. Munkittrick, J.H. Carey, G. Ivan der Kraak (Eds.), Environmental Fate and Effects of Pulp and Paper Mill Effluents, St. Lucie Press/Delray Press, Florida, 1996, pp. 613–625.
[6] J.C. Frigon, R. Stephenson, S. Larabée, S. Guiot, Biotreatment of resin acid by a coupled anaerobic/aerobic integrated system, Pulp Pap. Can. 100 (1999) 131–134.
[7] S.F. Liver, E.R. Hall, Interactions of resin acids with aerobic and anaerobic biomass. I. Degradation by non-acclimated inocula, Water Res. 30 (1996) 663–671.
[8] A. Werker, E.R. Hall, Limitations for biological removal of resin acids from pulp mill effluent, Water Sci. Technol. 40 (1999) 281–288.
[9] Y. Zhang, P.A. Bicho, C. Breuil, J.N. Saddler, S.N. Liss, Resin acid degradation by bacterial strain grown on CTMP effluent, Water Sci. Technol. 35 (1997) 33–39.
[10] W.W. Mohn, V.J. Martin, Y. Zhongtang, Biochemistry and ecology of resin acid biodegradation in pulp and paper mill effluent treatment systems, Water Sci. Technol. 40 (1999) 273–280.
[11] L. Strömberg, R. Mörck, F. de Sousa, O. Dahlman, Effects of internal process changes and external treatment on effluent chemistry, in: M.R. Servos, R. Munkittrick, J.H. Carey, G.J. van der Kraak (Eds.), Environmental Fate and Effects of Pulp and Paper Mill Effluents, St. Lucie Press/Delray Press, Florida, 1996, pp. 3–19.
[12] G. Vidal, S. Videla, M.C. Diez, Molecular weight distribution of *Pinus radiata* kraft mill wastewater treated by anaerobic digestion, Bioresour. Technol. 77 (2001) 183–191.
[13] M. Verta, J. Ahtiainen, T. Nakari, A. Langi, E. Talka, The effect of waste constituents on the toxicity of TCF and ECF pulp bleaching effluents, in: M.R. Servos, K.R. Munkittrick, J.H. Carey, G.J. van der Kraak (Eds.), Environmental Fate and Effects of Pulp and Paper Mill Effluents, St. Lucie Press/Delray Press, Florida, 1996, pp. 41–51.
[14] A. Kostamo, J.V.K. Kukkonen, Fate of wood extractives in wastewater treatment plants at kraft pulp mill and mechanical pulp mills, Water Res. 37 (2004) 972–982.
[15] L.M. Hewitt, J.H. Carey, D.G. Dixon, K.R. Munkittrick, Examination of bleached Kraft mill effluent fractions for potencial inducers of mixed function oxygenase activity in rainbow trout, in: M.R. Servos, R. Munkittrick, J.H. Carey, G. Ivan der Kraak (Eds.), Environmental Fate and Effects of Pulp and Paper Mill Effluents, St. Lucie Press/Delray Press, Florida, 1996, pp. 79–94.
[16] P. Dell, F. Power, R. Donald, J. Mclntosh, S. Park, L. Pang, Monitoring environmental effects and regulating pulp and paper discharges: Bay of Plenty, New Zealand, in: M.R. Servos, K.R. Munkittrick, J.H. Carey, G. Ivan der Kraak (Eds.), Environmental Fate and Effects of Pulp and Paper Mill Effluents, St. Lucie Press/Delray Press, Florida, 1996, pp. 627–636.
[17] J. Correa, V. Dominguez, M. Martinez, G. Vidal, Interaction between 2,4,6-TCP increasing concentration content in ECF bleached effluent and aerobic bacteria degradative activity, Environ. Int. 29 (2003) 459–465.
[18] F.A. Godoy, M. Bunster, V. Matus, C. Aranda, B. Gonzales, M.A. Martínez, Poly-β-hydroxyalkanoates consumption during degradation of 2,4,6-trichlorophenol by *Sphingopyxis chilensis* S37, Lett. Appl. Microbiol. 36 (2003) 315–320.
[19] G. Vidal, C. Kennes, R. Mendez, J.M. Lema, Aerobic degradation of 2,4,6-trichlorophenols of bleaching pulp mill effluents, Afinidad 54 (1997) 207–212 (in Spanish).
[20] APHA-AWWA-WPCF, Standard Methods for Examination of Water and Wastewater, 16th ed., Washington, 1985.
[21] K. Li, T. Chen, P. Bicho, C. Breuil, J.N. Sanddler, A comparison of gas chromatographic and immunochemical methods for quantifying resin acids, in: M.R. Servos, K.R. Munkittrick, J.H. Carey, G. Ivan der Kraak (Eds.), Environmental Fate and Effects of Pulp and Paper Mill Effluents, St. Lucie Press/Delray Press, Florida, 1996, pp. 119–127.
[22] A. Latorre, A. Rigol, S. Lacorte, D. Barcelo, Comparison of gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry for the determination of fatty and resin acids in paper mill process waters, J. Chromatogr. A 991 (2003) 205–215.
[23] B.P. Quinn, M.M. Booth, J.J. Delfino, S.E. Holm, T.S. Gross, Selected resin acids in effluent and receiving waters derived from a bleached and unbleached kraft pulp and paper mill, Environ. Toxicol. Chem. 22 (2003) 214–218.
[24] H.W. Liu, S.N. Lo, H.C. Lavallée, Study of the performance and kinetic of aerobic biological treatment of a CTMP effluent, Pulp Pap. Can. 94 (1993) 172–177.
[25] L. Bijan, M. Mohseni, Using ozone to reduce recalcitrant compounds and to enhance biodegradability of pulp and paper effluents, Water Sci. Technol. 50 (2004) 173–182.
[26] M.C. Diez, G. Castillo, L. Aguilar, G. Vidal, M.L. Mora, Operational factors and nutrient effects on activated sludge treatment of *Pinus radiata* kraft-mill effluent, Bioresour. Technol. 83 (2002) 131–138.
[27] C.B. Milestone, R.R. Fulthorpe, H. Leppänen, T.R. Stuthridge, The formation of colour during biological treatment of pulp and paper wastewater, Water Sci. Technol. 50 (2004) 87–94.
[28] J.A. Zender, T.R. Stuthridge, A.G. Langdon, A.L. Wilkins, K.L. Mackie, P.N. McFarlane, Removal and transformation of resin acids during secondary treatment at a New Zealand bleached kraft pulp and paper mill, Water Sci. Technol. 29 (1994) 105–121.

Glossary

AbA: abietic acid
AbALR: abietic acid loading rate (g/l d)

AL: aerated lagoon
AOX: adsorbable organic halogens
AS: activated sludge
BOD_5: biological oxygen demand at 5 days (g BOD/l)
BOD_7: biological oxygen demand at 7 days (g BOD/l)
CFU: colony formation unit
COD: chemical oxygen demand (g COD/l)
DHA: dehydroabietic acid
ECF: elemental chlorine free
HRT: hydraulic retention time (day)
HW: hardwood
K_s: saturation constant (mg/l)
OLR: organic loading rate (g COD/l d)
SW: softwood
TCF: total chlorine free
TKN: total Kjeldahl nitrogen
TMP: thermomechanical process
TSS: total suspended solids (g TSS/l)
UV_{215}: total phenolic compounds (mg/l)
VIS_{440}: color (absorption units 1×, 1 cm)
V_m: reaction velocity (l/d)
VSS: volatile suspended solids (g VSS/l)