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Effect of Aerobic Sludge with Increasing Level of Adaptation on Abietic Acid Biodegradation

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Extractives are low molecular weight lipophilic constituents in wood that consist chiefly of resins acids, sterols, triglycerides and free fatty acids (Kostamo et al., 2004). Although, part of these compounds are burned or recovered in the kraft process, a fraction of the wood extractives is dissolved in the effluent (Ali and Sreekrishman, 2001).

On the other hand, resin acids are highly toxic to aquatic organisms. It has been observed that wastewater pH, strongly affect the toxicity and solubility of these acids with measured 96 h LC50 for resin acids ranging from 0.4 to 1.7 mg/L for rainbow trout (Ali and Sreekrishman, 2001). Due to this, the resin acids contained in kraft mill wastewater should be removal before to be discharged in aquatic system (Kostamo et al., 2004).

Aerobic process is the most usually secondary treatment employed to treat kraft mill effluents. Resin acid removal from mill's effluent can be attributed to dilution, sorption to particles, or bacterial degradation. Moreover, Fox (1977) reported that dilution was the primary mechanism for decreasing concentrations of wood extractives and bacterial degradation was a slow process for many of these compounds. However, bacterial biodegradation is strongly dependent of the microorganisms activity, as well as level of acclimated biomass. Also, pH operation could influence the removal efficiency of the abietic acid (Werker and Hall, 1999). Recent advances in understanding resin acid biodegradation, shows that the biodegradation of resin acids contained in kraft mill effluents could be done by gram-negative bacteria like as *Pseudomonas abietaniphila* BKME-9 and *Pseudomonas sp.* strain A19-6; that systems could be below aerobic or anaerobic conditions. On the other hand, Mohn et al. (1999) have developed molecular assays for resin acid-degrading bacteria, to investigate their occurrence and activity in complex microbial communities in biological treatment systems. One important use of such assays is to better understand the normal occurrence and dynamics of resin acid-degrading populations in treatment systems. The main resin acids studied are dehydroabietic acid (DHA) and abietic acid (AbA) because they are the most abundant resin acids, representing respectively, 19 to 33% and 14 to 30% of total resin acids (Frigon et al., 1999).

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

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affect the overall rate removal of resin acids (Liver and Hall, 1996; Zhang et al., 1996, Belmonte et al., 2006). The availability of suitable cosubstrates has also been shown to enhance the biodegradation of resin acids (Liver and Hall, 1996). Although bleaching effluents contain high levels of recalcitrant compounds (eg. high molecular weight lignin fragments, chlorinated lignin and tannins compounds) (Belmonte et al., 2006), biodegradable carbon sources (eg. cellulose and hemicellulose depolymerization products) are also present that could potentially used as co-substrate by DHA-degrading bacteria (Zhang et al., 1996).

The goal of this study was to investigate the effect of aerobic sludge with increasing level of adaptation on AbA biodegradation in batch assays.

MATERIALS AND METHODS

A consortium of aerobic bacteria (4.1 g/L of volatile suspended solid (VSS), and 5.9 g/L of total suspended solid (TSS)) arises from an activated sludge system that treats bleached-kraft-mill effluent was used as microorganisms without adaptation to AbA, this sludge was denominated as non-adapted sludge (NAS) which was used as reference's sludge in the batch assays.

On the other hand, the other two kind of sludge: sludge semi-adapted (SAS) and adapted sludge (AS) to AbA was obtained by acclimation in continuous system. To obtain SAS, a continuous aerated lagoon was operated by 139 days with and AbA load rate of 0.15 g AbA/L·d (Belmonte et al., 2006). On the other hand, AS was obtained through the operation of the same aerobic system, but the time of the biomass adaptation was 266 days. In that case, the AbA load rate fed to the aerated lagoon was 0.71 AbA/L·d. A scheme of the sludge origin is shown in Figure 1.

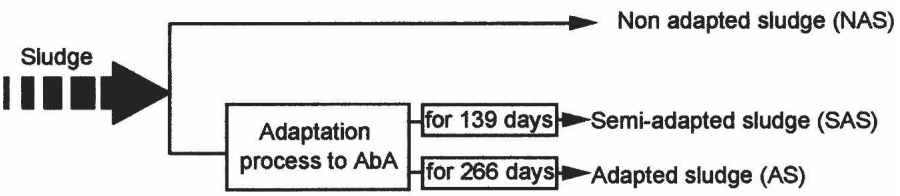

Figure 1. Origin of the: Non-adapted sludge (NAS), semi-adapted sludge (SAS), adapted sludge (AS).

The kinetics of resin acids biodegradation was measured using a batch system. Erlenmeyer flasks (250 ml) were incubated with three different biomasses: NAS, SAS and AS to AbA. Concentrations of 25, 50, 100, 250 and 500 mg AbA/L were inoculated according to Correa et al. (2003). Also, a control was considered. All of the assays were carried out in triplicate. The flasks were incubated in a shaker at 150 r.p.m., in the dark, at 25 ± 2 °C. The variation of the AbA concentration and cell concentration was analyzed according to the procedure described in analytical methods, during a period of 189 h approximately.

Bacterial viability was determined in triplicate R2A agar plates that were incubated at 25°C for 5 d according to Godoy et al. (2003). 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 biomass adaptation to AbA, considering the initial rate of the AbA degradation for each concentration evaluated as was done by Belmonte et al. (2006).

Volatile suspended solid (VSS) and total suspended solid (TSS) were measured according to Standard Methods (APHA-AWWA-WPCF, 1985).

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. (1996). Extracts were evaporated to approximately 5 mL using a rotary evaporator. 20 µL 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. (2003).

RESULTS AND DISCUSSION

In order to assess the possible adaptation of the biomass to AbA, the kinetic of AbA biodegradation were evaluated in batch assays supplemented with 25, 50, 100, 250 and 500 mg AbA/L. NAS, SAS and AS biomass were explored. Figure 2 shows AbA kinetic biodegradation at different AbA concentrations using NAS and AS as biomass.

According to Figure 2a, the kinetic biodegradation of AbA below NAS shows a lowest initial rate of biodegradation (10.2 mg AbA/L·d) for the assays 500 mg/L, compare with the initial biodegradation rate of SAS (10.6 mg gAbA/L·d) (data not shown), and AS (13.3 mgAbA/L·d) (see Figure 2b).

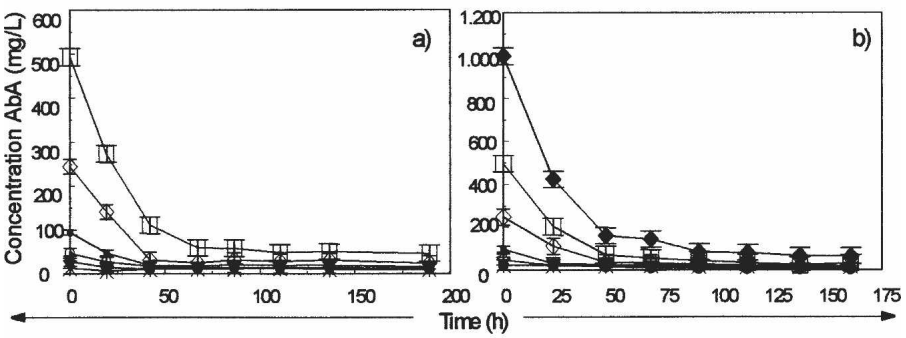

Figure 2. AbA biodegradation below different sludge adaptation; a) NAS, b) AS. Concentration of AbA: 25 (●), 50 (o), 100 (■), 250 (◊), 500 (□) and 1000 (♦) mg AbA/L.

A comparative analysis of the AbA biodegradation rate shows that AS assays biodegradation is faster 1.3 times respect to NAS assays. This is agree with continuous studies of AbA biodegradation. Moreover, Belmonte et al., (2006) show that adapted sludge can work with higher level of AbA load rate (0.71 gAbA/L·d)

and the AbA biodegradation in the system was as good as in the operation with NAS (0.15 gAbA/L·d) (over 90% of AbA removal).

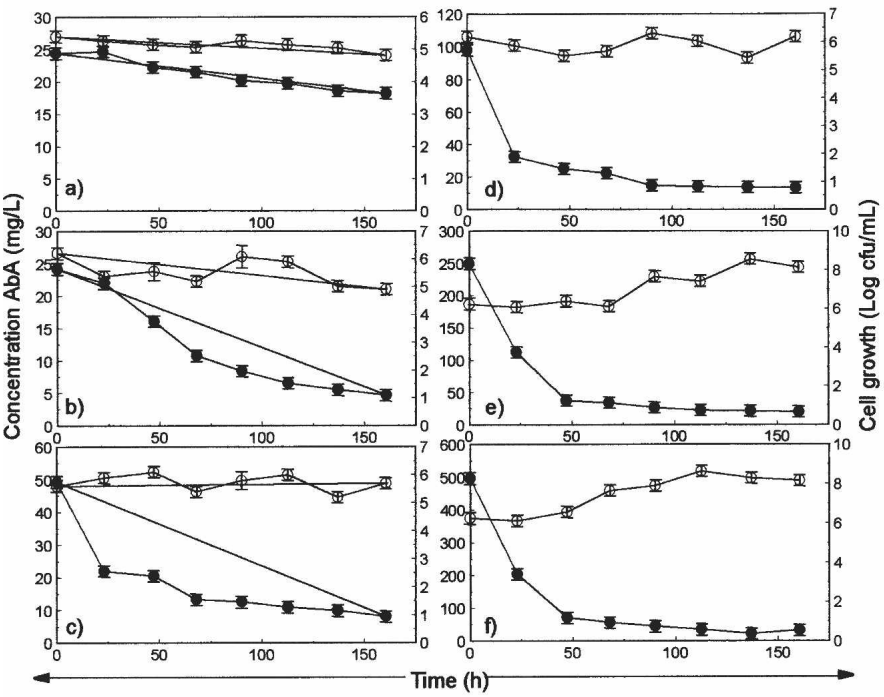

Figure 3. Kinetics of AbA concentration and bacterial viability of different batch assays using AS as biomass. a) Blank, b) 25, c) 50, d) 100, e) 250 and f) 500 mg AbA/L.

Figure 3 shows results of the batch assays with AS and AbA concentrations from 25 to 500 mg/L. The degradation of AbA in the assays with 50 and 100 mg AbA/L (Figure 3c and 3d) was higher than 75%, before 20 first hours, whereas the population density was around $1.0x10^6$ cfu/mL. However, in the case of assays with NAS population density increased from $1.0x10^5$ to $1.0x10^7$ ufc/mL, and then goes down until $1.0x10^4$ ufc/mL (data not shown). In Figure 3b (assays with 25 mgAbA/L) 75% of the total biodegradation was done before 60 hours. At higher concentrations (250 and 500 mgAbA/L, Figures 3e and 3f, respectively) most than the 80% of the total amount of AbA was degraded in the first 47 hours of assays, whereas the population density was from $1.0x10^6$ ufc/mL until 68 first hours, then it was increasing until $1.0x10^8$ ufc/mL.

Figure 4 shows global AbA removal for the different assays. Assays with NAS show the lower global degradation of AbA. On the contrary, assays with AS show values of degradation higher than 90%, for all the AbA concentrations. Moreover, if NAS, SAS and AS are compared for example at concentration of 500 mg AbA/L, the removal of AbA are 78%, 89% and 95%, respectively. Indeed, adaptation of aerobic

bacteria to AbA, was able due to it was used as the sole carbon and energy source in the assays.

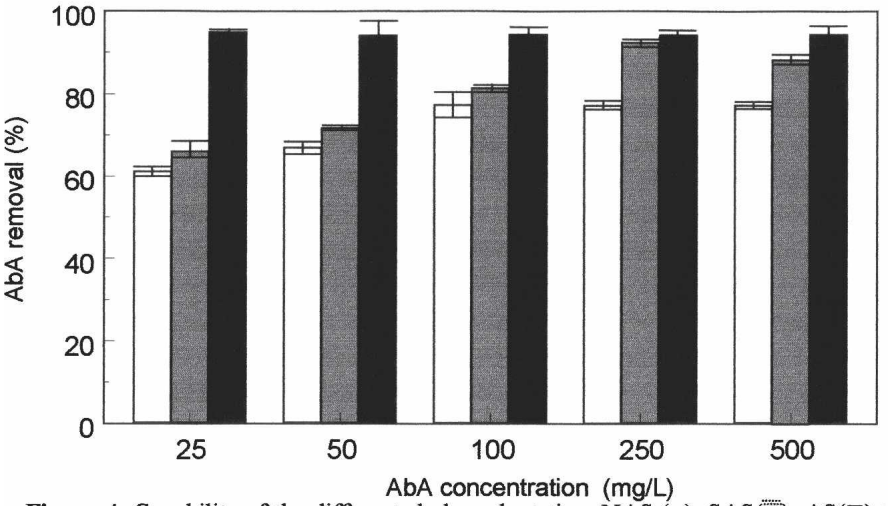

Figure 4. Capability of the different sludge adaptation: NAS (□), SAS(▒), AS(■) to AbA remove from assays with different concentration of this compounds (25, 50, 100, 250 y 500 mg AbA/L).

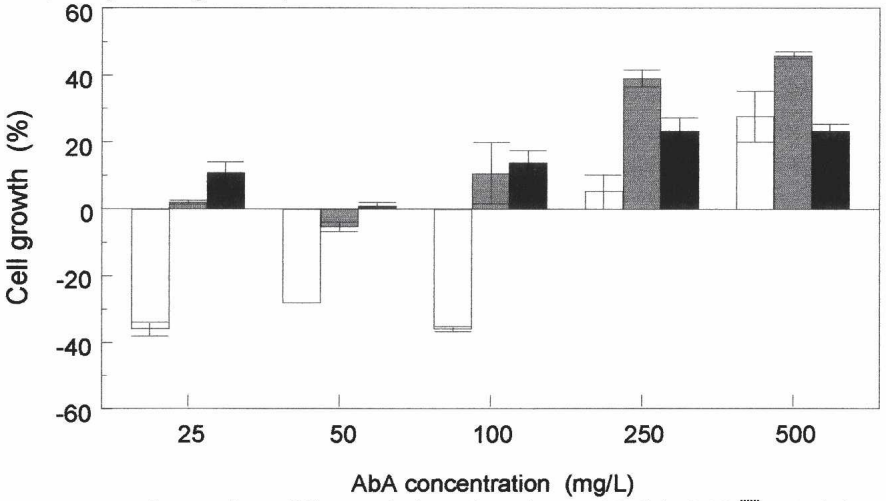

Figure 5. Cell growth at different sludge adaptation: NAS (□), SAS(▒), AS(■) to AbA in assays with different concentration of this compounds (25, 50, 100, 250 y 500 mg AbA/L).

Figure 5 shows the global percentage of cell growth for the different batch assays with NAS, SAS and AS. In the case of NAS at 25, 50 and 100 mg AbA/L, concentration of cell growth decreased and, also, a lag phase of 10 hours was observed in the kinetic of AbA biodegradation (data not shown). This indicate the toxic effect of AbA and its inhibitory effect on the specific enzymatic activities.

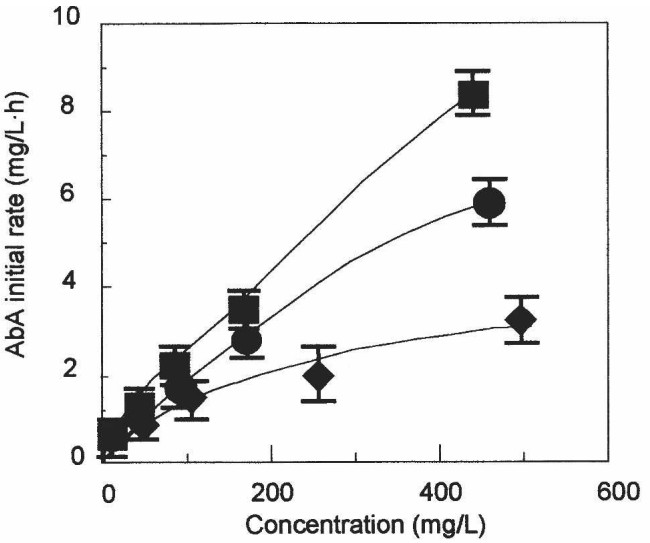

Figure 6. Effect of biomass adaptation on the degradation rate of AbA. Comparison of experimental data with results predicted by a the modified Monod model: a) NAS (■), b) SAS (●), c) AS (◆) and prediction (—).

However, in the same assays for concentrations of 250 and 500 mg/L, adaptation was observed (cell growth: 40 and 45%, respectively).

Figure 6 shows the relationship between the initial rate of AbA biodegradation and the concentration of AbA in the assays with NAS, SAS and AS. The kinetic parameters for the three different assays were calculated using modified Monod model and they show the effect of the biomass adaptation on AbA biodegradation. The Monod half saturation constant values determined for the NAS and AS were 76.7 and 1677 mg AbA/L, indicating a 21.8-fold increase in biodegradation capability.

As conclusions of the batch assays with different levels of biomass adaptation it was confirmed that the ability of aerobic bacterial consortium to degrade AbA increased with sludge adaptation.

Bacterial inhibition was detected in concentrations of 25, 50 and 100 mg AbA/L in assays with NAS. However, for concentrations over 100 mg AbA/L inhibition of aerobic bacteria was not observed.

It can be observed that the most adapted biomass (AS) resulted in the highest AbA degradation rates. AbA removal higher than 90% was observed.

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