

## ENHANCING THE TANNINS BIODEGRADATION WITH *ASPERGILLUS TUBINGENSIS* AND *CHAETOMIUM* SP.: COSUBSTRATES BATCH TESTS

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Tannins are a class a polyphenolic compounds and they can be subdivided into natural and synthetic tannins. The ones used in tanning processes had an elevated molecular weight and represent a serious concern in tannery wastewater treatment plant for their low biodegradability and the inhibiting effect on the activated sludge processes (Munz et al. 2009). The biodegradation of natural tannins in the environment is associated with fungi rather than bacteria (Bhat et al. 1998) and in recent years fungi has been regarded with increasing interest for their potential of removing hazardous, recalcitrant and toxic pollutants. Fungi, in collaboration with bacteria, could be an important component of new wastewater biotechnologies designed to biodegrade recalcitrant compounds like tannins (Harms et al. 2011). In the first step 19 fungal strains were screened based on their ability to treat tannery wastewater. Among these 19 strains, 15 strains were collected and isolated from the mixed liquor of an Italian tannery wastewater treatment plant and 4 strains were obtained from the *Mycotheca Universitatis Taurinensis Collection* (MUT, University of Turin). Subsequently, batch tests were done with the real tannery wastewater to identify the most suitable strains. TOC, COD, pH and two enzymatic activities (tannase and laccase) were measured. On two selected strains (*Aspergillus tubingensis* MUT 990 and an autochthonous *Chaetomium* sp.) were done more batch tests to identify the operating condition for the future bench scale bioreactors. This study focused on these batch tests. The fungi were cultivated separately in Petri dishes and subsequently were immobilized in 2 cm polyurethane cubes. For each fungus three conditions were tested: real wastewater (RW) without any cosubstrate, RW supplemented with glucose ( $1 \text{ g L}^{-1}$ ) RW supplemented with cellulose ( $1 \text{ g L}^{-1}$ ). The trial was done in triplicate including three abiotic controls in 250 ml flasks containing 80 ml of tannery supernatant from secondary settler and 2 polyurethane cubes precolonized by fungi (the same report cubes/supernatant of bioreactors). The flasks were covered with a cotton taps and put in agitation with magnetic stirrers. The supernatant pH was correct from 7,8 to 6 with hydrochloric acid. The test lasted 7 days and TOC, COD, pH, phenols, glucose and two enzymatic activities (tannase and laccase) were measured. The pH of the abiotic controls and the samples without cosubstrate increased in the first day. There was a slight pH decrease in the samples with glucose. After the second day the pH increased in all sample

except the cellulose flask where the pH decreased to neutrality, maybe because the fungi started to assimilate cellulose. As shown in Table 1 the COD, in the flasks with glucose at time 0, was higher than original supernatant, due the glucose. The COD increased in the flasks with cellulose after three days, probably due also to the cellulose bioavailability. The flasks with cellulose shown the higher increase of Phenols concentrations, in the flasks with *Aspergillus tubingensis* were more than 30% in average. This may be related with the tannins degradation. This preliminary tests are promising but they are still ongoing and further studies are required.

**Table 1.** COD and Phenols concentrations at time 0 and after 3 days

Batch test (triplicate)	COD		Phenols	
	t0	t3	t0	t3
	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>
<b>Aspergillus</b>	470	459	10,1	11,5
<b>Aspergillus with glucose</b>	793	627	11,3	11,8
<b>Aspergillus with cellulose</b>	342	803	9,5	13,2
<b>Chaetomium</b>	398	467	10,7	11,4
<b>Chaetomium with glucose</b>	900	532	-	12,0
<b>Chaetomium with cellulose</b>	461	575	10,6	11,8
<b>Control</b>	500	455	-	-

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**References:**

- Bhat, T.K., Singh, B. & Sharma, O.P., 1998. Microbial degradation of tannins a current perspective. *Biodegradation*, 9(5), pp.343–357.
- Harms, H., Schlosser, D. & Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology*, 9(3), pp.177–192. Available at: <http://www.nature.com/doi/10.1038/nrmicro2519>.
- Munz, G. et al., 2009. The role of tannins in conventional and membrane treatment of tannery wastewater. *Journal of Hazardous Materials*, 164(2-3), pp.733–739.