Quebracho tannin treatment with Aspergillus tubingensis MUT 990 immobilized in polyurethane foam cubes in a novel submerged cage reactor

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Abstract

Quebracho tannin represents one of the most used tannin in industrial vegetable tanning process and one of the most recalcitrant compounds in tannery wastewaters. In this study treatment of a quebracho tannin solution in a 4 liters reactor (not sterile conditions) was carried out with a selected fungal strain, *Aspergillus tubingensis* MUT 990. The fungal strain was grown in polyurethane foam cubes carriers (2 cm size) submerged in a rotating cage inside an aerated reactor. The Hydraulic Residential Time (HRT) was 24 hours and the pH setpoint was 5.5. The experiment lasted two months (still ongoing) and the biofilm was maintained with about 20% removal of soluble COD. Furthermore, on-line and off-line respirometry were used to characterize fungal biomass activity.

INTRODUCTION

Tannins are the most abundant, widely distributed and water-soluble polyphenolic compounds in nature. Tannins differ from most other natural phenols in their prerogative of precipitating proteins and they are used in tanning process to bind to the collagen proteins of the animal skin and make the leather more durable and not putrescible. Tannins used in tanning processes are mainly Quebracho (*Schinopsis sp.*), Wattle (*Mimosa sp.*), Chestnut (*Castanea sp.*) and Tara (*Cæsalpinia sp.*); these tannins are one of the refractory groups of chemicals in tannery effluents. Moreover, a high concentration of tannins can inhibit biological treatment (Munz et al., 2009). Although the leather tanning industry is an important industry in several countries, there has been an increasing environmental concern regarding the release of various recalcitrant pollutants in tannery wastewater (Lofrano et al., 2013).

Despite the antimicrobial properties of tannins, many fungi, bacteria and yeast are quite resistant to tannins and can grow on them. In fact, the biodegradation of natural tannins in the environment is mainly associated with fungi rather than bacteria. In particular, *Aspergillus spp.* and *Penicillium spp.* have been observed in tannery wastewater and have been exploited in the biotransformation of tannins. *Aspergillus* tannins-degrading strains were found in quebracho phenolics-rich tannery wastewater (León-Galván et al., 2010) and *Aspergillus* section nigri are able to grow on tannic acid. Nowadays, fungi are rarely applied as biological agent in environmental biotechnologies mainly due the system instability in not sterile conditions. The aim of the present study was to evaluate the performance and system stability of Quebracho tannin removal in a novel bench scale bioreactor with fungal biofilm immobilized in polyurethane foam cubes carriers (PUF).

MATERIALS AND METHODS

Tannins, fungi and inoculum preparation

The strain tested in this study, *Aspergillus tubingensis* MUT 990, was isolated from commercial tannin powder and it is preserved at the Mycotheca Universitatis Taurinensis collection (MUT, University of Turin, Department of Life sciences and Systems Biology) on Malt Extract Agar (MEA) at 4°C. The Quebracho tannin commercially used for tanning was kindly provided by Chimont International Spa, Montopoli (Italy). All other reagents used in the present study were of analytic grade (Sigma-Aldrich). *A. tubingensis* was inoculated in a generic MEA (20 g L^{-1} malt extract, 20 g L^{-1} glucose, 2 g L^{-1} peptone, 20 g L^{-1} agar) plates (150 mm diameter) and incubated at 25°C in a dark for seven days. After incubation, the plates were used as inoculum for immobilization in PUF carriers (Anastasi et al., 2012).

Experimental set up and process operation

The experimental set up consisted of a reactor with 5 L of total volume and 4 L of effective volume. The schematic of the reactor is shown in Fig. 1. The reactor was equipped with a pH probe controlling pH set point (the pH set point was 5.5) by dosing NaOH (1 M) and HCl (1 M). There was a stone air diffuser at the bottom and a submerged plastic cylindrical cage with 100 PUF cubes (square grid with 1.25 cm size). The air flow was controlled with a rotameter and set to 100 NL h^{-1} and the outlet air were filtered at 0.2 μm. The air flow allowed a complete mixing inside the reactor. The dissolved oxygen concentration (DO) was measured with galvanic DO sensor (CellOx 325, WTW, Germany). Sensors data were continuously recorded with a home-made software developed for process monitoring and control. The process operation was designed to obtain a Hydraulic Retention Time (HRT) of 24 hours. The inlet and outlet flow were controlled with peristaltic pumps (Watson Marlow). Room temperature was kept in the range 20-25 °C. The mineral medium contained (g L^{-1}): Quebracho tannin, 1; NH_4Cl, 0.1; KH_2PO_4, 0.01 dissolved in tap water.

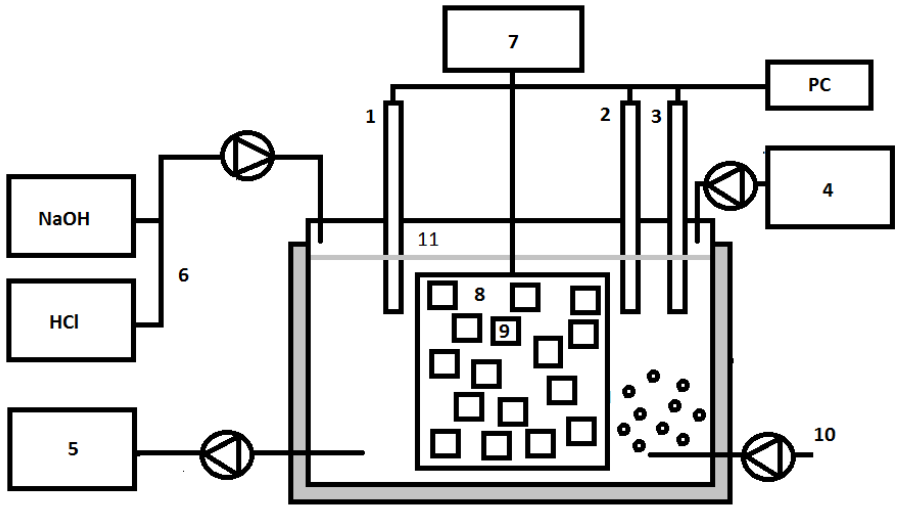

Figure 1. Reactor: 1) DO probe; 2) temperature probe; 3) temperature probe; 4) mineral medium tank and pump; 5) discharge tank and pump; 6) acid tank and caustic tank; 7) engine for cage rotation; 8) plastic cage; 9) PUF cubes, 10) air pump and diffuser; 11) reactor and water level.

Analytical methods

The monitoring of the reactor included the analysis of biological, chemical and physical parameters. Probes were installed to measure the pH, DO and temperature. TOC and COD were measured from

samples collected from the discharge tank three times per week and were determined using a TOC analyzer (Shimazdu) and Lovibond's COD tubes. Periodically, PUF cubes were removed to evaluate the dry mass and DNA was extracted with PowerBiofilm® DNA Isolation Kit MoBio for pyrosequencing analysis. Furthermore the Oxygen Uptake Rate (OUR) was used to monitor the system performance. Respirometric tests were performed in-line in the treatment reactor by periodically turning the aeration on and off and recording the decrease of DO along time. In addition, a dedicated vessel described elsewhere (Bonilla-Blancas et al., 2015) was used to perform LFS respirometry (Guisasola, 2005) with pure fungi cultures (immobilized on PUF) and PUF taken from the treatment reactor.

RESULTS AND DISCUSSION

The sCOD of the feeding medium was 1410 mg L^{-1} ± 5%, which corresponded to the total COD due the lack of particulate COD. The removal efficiency (RE) on sCOD is shown in figure 2. Variability of RE was due to the effect of several disturbances such as pH shocks, aeration stops or running of respirometric tests inside the reactors to assess the biodegradability through the on-line OUR measurements. Despite the previous issues the sCOD RE was about 20%. Biomass growth as biofilm inside the cage during the test (figure 3) demonstrated that fungi were able to grow on this substrate, while the TSS in the reactor were about 0.01 g l^{-1}.

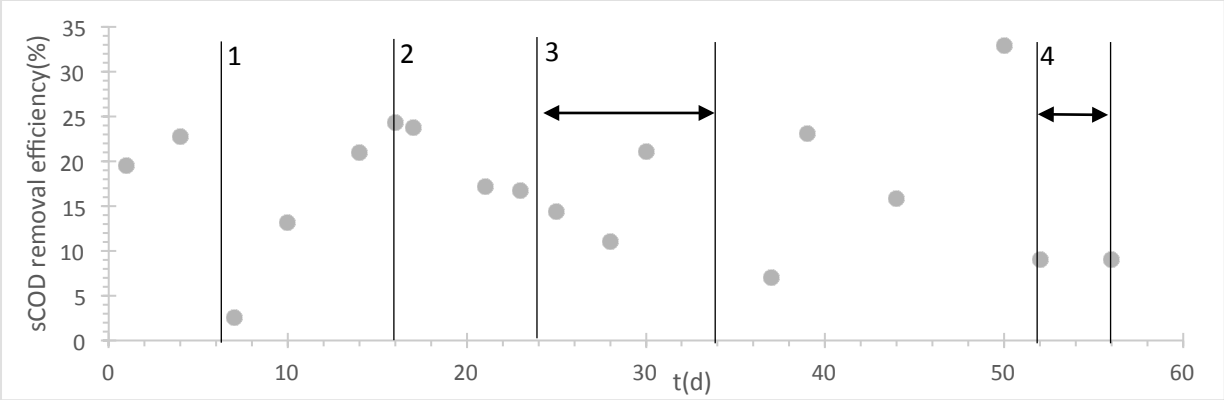

Figure 2. Removal efficiency on sCOD. 1) pH shock; 2) Aeration pump failure; 3&4) Respirometic test in the reactor

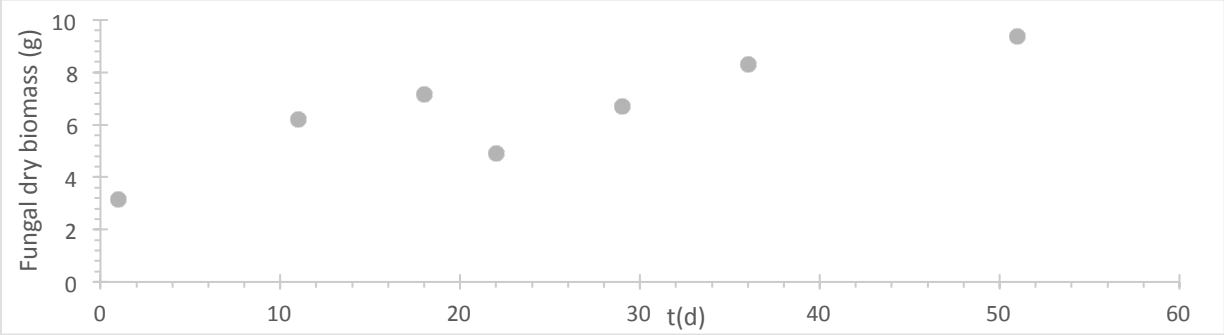

Figure 3. Fungal dry mass immobilized on PUF inside the reactor

In addition to continuous on-line OUR measurements, respirometric tests with periodic quebracho pulses in the treatment reactor (figure 4) revealed a removal capacity of quebracho four times higher than this obtained with activated sludge from a tannery wastewater treatment plant (same dry mass for volume). In addition, flask tests were performed to 1) evaluate the degradation without the fungal strain and 2) characterize the mycelium adsorption. COD of quebracho tannin did not decrease in a timespan of one week, demonstrating that removal was linked to fungi. Autoclaved PUF filled with fungi adsorbed some COD (25%), which mainly occurred within 48 hours (data not

shown). Figure 3 shows a cross section of a PUF cube taken from the reactor after 24 hours of operation and a PUF cube after a few days, showing the adsorbed tannins on the external layer of the PUF cube.

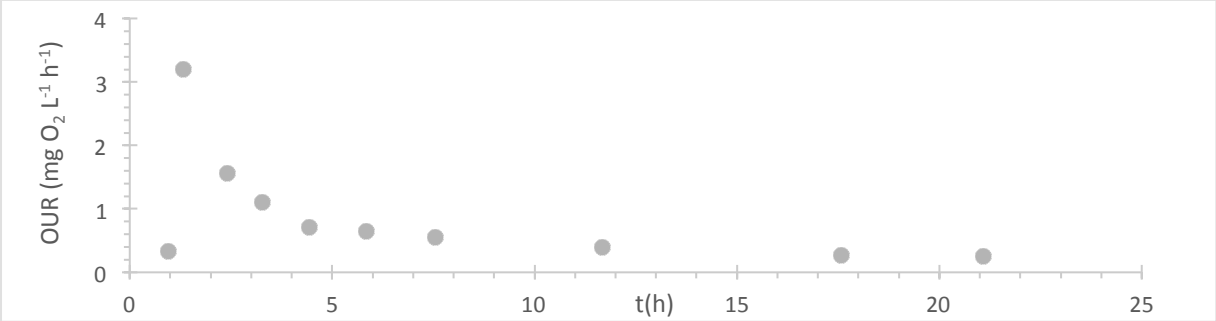

Figure 4. OUR profile in the reactor after a pulse and an endogenous phase.

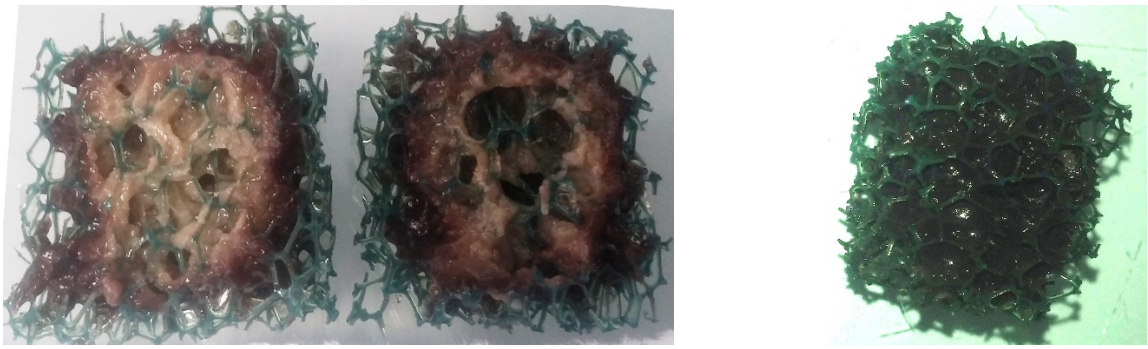

Figure 5. a) Cross section of a PUF taken from the reactor after 24 hours of operation b) PUF taken from the reactor after one week of operation.

The reactor is still ongoing and the experimental plan was designed to test cosubstrate and cage rotation as possible strategies to improve RE. In addition, the characterization of kinetic and stoichiometric parameters in dedicated respirometer with PUF taken from the reactor is ongoing.

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