

BIODEGRADATION OF ACACIA AND CHESTNUT TANNINS BY NATIVE ISOLATES OF THE GENUS *Penicillium* AND *Aspergillus*

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Abstract - In the present work, the potential of native isolates of fungi strains to biodegrade vegetable tannins used in the tanning industry was evaluated. *Penicillium citrinum* showed to be more efficient for consumption of acacia tannin, reaching 94.85%. *Aspergillus chevalieri* needs a greater adaptation phase (48 h) in both acacia and chestnut medium, evidenced by the slow growth (0.022 h⁻¹) and low biomass productivity (0.31 g.L⁻¹. h⁻¹). The acacia tannin presented a higher COD/BOD ratio (2.97) and lower total phenol content (68%) when compared to chestnut tannin. In addition, there was greater consumption of this tannin in the cultivations, which contradicts previous reports and corroborates the results obtained with FTIR analysis that suggests the biodegradation of acacia by *Penicillium citrinum* and *aethiopicum*. The expressive results obtained demonstrated that the biodegradation of condensed tannins may be a promising alternative, with the potential to minimize tannery waste.

Keywords: Microbial degradation; Condensed tannin; Tannery waste.

INTRODUCTION

Vegetable tannins can be easily extracted with water from almost all plants (Tondi and Petutschnigg, 2015; Sundar and Muralidharan, 2017). They occur in bark, wood, fruits, fruit pods, leaves, roots, and plant galls and can accumulate in large quantities in certain organs or tissues of plants, being found mainly in the vacuoles of plants (Ricci et al., 2015). Vegetable tannins have the molecular weight between 500 and 3,000 Da and differ from most other phenolic compounds by their ability to form water-insoluble complexes with proteins, polysaccharides and alkaloids (Grasel and Ferrão, 2016). Considering their property to precipitate proteins, vegetable tannins have been traditionally explored to transform animal hides into leather (Haroun et al., 2013). This specific reactivity with proteins is called astringency, being the basis for their use in the tanning industry (Khanbabaee and Ree, 2001).

Raw hides are susceptible to attack by microorganisms. The collagen must be stabilized by

the tanning process in which tanning agents react with the collagen matrix, stabilizing it and avoiding its degradation (Fuck et al., 2011). Due to the stringent requirements imposed on tanning industry, the development and implementation of cleaner technologies for environmental protection becomes increasingly imperative (Plavan et al., 2009; Pillai and Archana, 2012). For tanning this involves effort for the substitution of chromium by other tanning substances, making use of a combination of materials of inorganic origin (aluminum, silica, zinc, etc.) and organic (vegetable tannins, synthetic tannins, resins, aldehydes, etc.) to provide the required leather characteristics like stiffness, UV resistance. (Covington and Lampard, 2004; Saravanabhavan et al., 2007; Maier et al., 2017).

After the tanning process, liquid effluent is generated containing high organic loads, phenolic compounds, proteins, fat and neutral salts. It is estimated that 15% of the tannin used in tanning is discarded in the effluent. Vegetable tanning effluents show high chemical oxygen demand (COD) and bio-

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chemical oxygen demand (BOD) (Guanamani et al., 2001; Song and Burns, 2005). Due to the presence of tannins, the wastewaters are usually highly colored, which is very difficult to eliminate by common methods (He et al., 2007). They also exhibit long-term negative environmental effects, due to their toxicity for microorganisms (He et al., 2007; Teng et al., 2016). The toxicity of tannins can be associated with several mechanisms such as substrate deprivation, loss of metal ions, and inactivation of microbial adhesins, enzymes, and cell envelope transport proteins (Bhoite and Murthy, 2015).

Tannins are a heterogeneous group of polyphenols widely present in the plant kingdom (pteridophytes, gymnosperms and angiosperms), secondary metabolites for protective purposes (Falcão and Araújo, 2014). The chemical classification of tannins divides them into the groups of hydrolysable and condensed (Adamczyk et al., 2011). The hydrolyzable tannins are readily hydrolyzed by acids, alkalis or enzymes into a sugar or a related polyhydric alcohol (polyol) and a phenolic carboxylic acid (Haroun et al., 2013). The condensed tannins, also called proanthocyanidins, are oligomers and polymers composed of flavonoid units (Ricci et al., 2015). These compounds are used for many different applications, such as flocculants, anti-corrosion, tanning, adhesives, cement super plasticizers, pharmaceutical agents and foams (Grasel et al., 2016; Maier et al., 2017). A variety of vegetable tannins from different plants with different chemical constitutions and properties are used for tanning.

Tannin antimicrobial activity can be explained by their ability to complex irreversibly with proteins and therefore inhibit any activity (Maier et al., 2017). When vegetable tannins are present in sludge, anaerobic digestion does not establish due to their high toxicity and concentration (Agustini et al., 2018). Tannins are very recalcitrant; the presence of aromatic rings constitutes the main obstacle for the treatment of effluents that present phenolic compounds in their composition. However, some microorganisms are resistant to tannins and have the ability to degrade them by the action of enzymes (Silva et al., 2010). The main sources of enzymes are microorganisms such as bacteria, yeast and filamentous fungi. However, the interesting aspect to use fungi in the treatment of effluents containing compounds of this nature is the great ability of these microorganisms to produce enzymes (tannases, cellulases, ligninases, peroxidases) which make them more accessible to biodegradation (Luke and Burton 2001). Among the filamentous fungi, *Aspergillus* and *Penicillium* are important tannase producers (Valera et al., 2015).

The elimination of wide ranges of pollutants and wastes from the environment is a prerequisite for promoting sustainable development of our society

with low environmental impact. Environmental sustainability in leather processing is possible with the use of biotechnology and clean technologies in tanneries through organic tannins and adequate environmental management of the solid wastes and liquid effluents generated. In this context, the biodegradation of condensed and hydrolysable tannins by native *Penicillium citrinum*, *Penicillium aethiopicum*, *Aspergillus chevalieri* and *Aspergillus oryzae* was investigated in this paper.

METHODOLOGY

Microorganisms

Fungal strains were collected from stored corn (*Penicillium citrinum*, *Penicillium aethiopicum*, *Aspergillus chevalieri*) and rice flour (*Aspergillus oryzae*) sampled in Porto Alegre, RS, Brazil. The fungi were isolated from inoculation of a tissue plug obtained from mycelia identified as described by Ortiz-Monsalve et al. (2017) and gently provided by the Laboratory of Mycology of the Federal University of Rio Grande do Sul. The strains were maintained on potato dextrose agar slants at 4 °C until use. Subcultures were made every three months on potato dextrose agar slants with a inoculation loop and incubated at 25 °C for 4 a 5 days. After this time, the tubes were kept under refrigeration. The fungi are registered in GenBank with the following access numbers: *Penicillium citrinum* (MH532414), *Penicillium aethiopicum* (MH675468), *Aspergillus chevalieri* (MH532415) and *Aspergillus oryzae* (MH569333).

Shaken Flasks Cultivation

Five agar plugs (7 mm of diameter), taken from the edge of an actively growing colony (Anastasi et al., 2011), were used to aseptically inoculate each 250 ml shake flask containing 50 ml of culture medium (g/l): tannin (40.0), KH_2PO_4 (4.38), $(\text{NH}_4)_2\text{SO}_4$ (8.76), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.088), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.88), $\text{Na}_2\text{MnO}_4 \cdot 2\text{H}_2\text{O}$ (0.0088), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.018), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.012) (Chávez-González et al., 2014). The flasks were incubated in an orbital shaker (200 rpm) at 25 °C for 120 h. Vegetable tannins powder from black acacia and chestnut provided by Tanac S.A and Silvateam S.A respectively, were used as the main source of carbon. The assays were performed in triplicate.

Analytical methods

The determination of the tannin concentration in the medium was carried out on a spectrophotometer (T80 + UV / Vis Spectrometer (PG Instruments Ltd.) using a wavelength of 278 nm obtained by scanning. The absorbance values were converted to concentration (g/L) through a calibration curve previously plotted for each tannin.

The biomass dry weight was determined after vacuum filtration through reweighed glass microfiber filters (Whatman, 45 µm pore and 50 mm diameter) and drying at 105 °C to a constant weight (Baccar et al., 2011).

The determinations of total organic carbon (TOC) and total nitrogen (TN) of the culture medium were performed in a total organic carbon analyzer (TOC-L Shimadzu) equipped with a total nitrogen measuring unit (TNM-L Shimadzu) and 8-port sampler (OCT-L Shimadzu). Total phenols were estimated as tannic acid equivalents, according to the Folin-Ciocalteu assay (Sartori et al., 2014). The physicochemical analyses of chemical oxygen demand (COD) and bio-chemical oxygen demand (BOD) were performed according to the procedure described by Eaton et al. (2005). These determinations were performed at the beginning of cultivations.

Biodegradation was assessed using Fourier transform infrared spectroscopy (FTIR). FTIR analysis was carried out (MIR-FTIR, Perkin Elmer, Frontier™ spectrometer) in the mid-IR region of 500-4000 cm⁻¹ with 16 scan speed.

The experiments were performed in triplicate. The data were analyzed by analysis of variance and the Tukey test to verify the significant differences between the microorganisms under study, at a 95% confidence level ($p \leq 0.05$), using Statistica 5.0 software (Stat Soft Inc., USA).

Calculation of growth parameters

Maximum specific growth rate (μ_{\max}) was calculated at the exponential phase of growth using the following equation:

$$\mu_{\max} = \frac{(\ln X_2 - \ln X_1)}{(t_2 - t_1)} \quad (1)$$

in which X_2 and X_1 are the mean of cell dry weights at the times t_2 and t_1 , respectively.

Maximum biomass productivity ($P_{X_{\max}}$) was calculated by:

$$P_{X_{\max}} = \frac{(X_2 - X_1)}{(t_2 - t_1)} \quad (2)$$

in which X_2 and X_1 are the mean of biomass dry weights at the times t_2 and t_1 , respectively.

Biomass yield on substrate ($Y_{x/s}$) was calculated by:

$$Y_{x/s} = \frac{\Delta X}{\Delta S} \quad (3)$$

in which ΔX and ΔS are the amount of biomass production and the total amount of substrate consumed, respectively.

RESULTS AND DISCUSSION

Shake flask cultivation

Tannin concentration, biomass and pH in fungi cultivations containing acacia and chestnut tannin are shown in Figure 1. A previous evaluation of the influence of pH on tannin consumption and biomass production was carried out using pH of 3.5, 4.5 and 5.5 (data not shown). As no significant differences were found, it was decided to use the pH of the medium, without adjustment, around 4.5.

It was observed that the pH of the culture medium decreased during the first 72 h (Figure 1a and 1d) and from this point onwards, remained practically constant except for the cultivation of *Aspergillus chevalieri* where a constant decline of the pH was coupled with the increase in biomass production until the end of the cultivation. This can be explained by the fact that *Aspergillus chevalieri* requires a greater adaptation phase to the culture medium while the other microorganisms since the beginning of the cultivation presented intense microbial activity, resulting in a faster decrease of pH. The pH changes of the medium throughout the culture are due to the metabolic activity of these microorganisms that have the capacity to produce organic acids. In this way the medium assumes acid values which represents a competitive advantage over other microorganisms (Kyriacou et al., 2005).

The growth curves (Figure 1b e 1e) demonstrated that the maximum biomass values were reached at 72 h for both acacia and chestnut cultivations, except for *Aspergillus chevalieri*, as also occurred for pH. By observing the biomass concentration obtained for the different fungi, it appears that, in the cultures with acacia tannin, there was greater growth of fungi of the genus *Penicillium*. There were no significant differences between the species (*citrinum* and *aethiopicum*). The best results were obtained with these fungi using acacia tannin as substrate. In chestnut cultivations, however, the highest concentrations of biomass were reached by fungi of the genus *Aspergillus*. There was also no significant difference between *Aspergillus* species. It was observed, however, that *Aspergillus chevalieri* needs a greater adaptation phase (48 h) for both acacia and chestnut medium. In acacia medium this is evidenced by the slow growth (0.022 h⁻¹) and low biomass productivity (0.31 g.L⁻¹. h⁻¹), as shown in Table 1. According to Kyriacou et al. (2005) the microbial activity is directly influenced by the amount of biomass, observing better removal of COD.

Lipid accumulation in oleaginous fungi has been demonstrated to occur when a nutrient in the medium becomes limited and the carbon source is present in excess (high C/N ratio) (Khot et al., 2012). Nitrogen limitation is the most efficient condition for inducing lipogenesis. The results (Table 1) indicate that the C/N

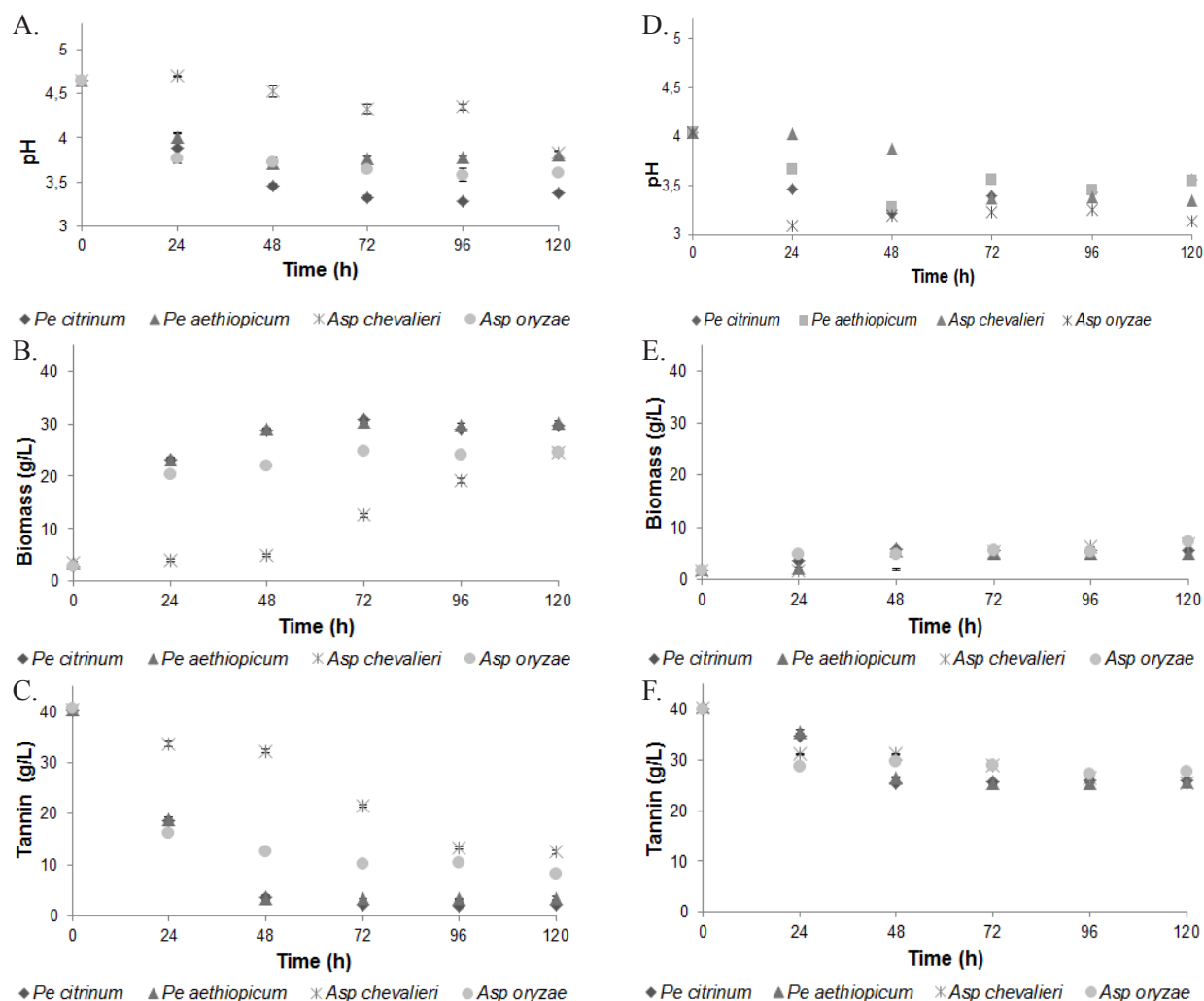


Figure 1. (a) pH, (b) Biomass and (c) Tannin concentration in medium containing acacia tannin and (d) pH, (e) biomass and (f) tannin concentration in medium containing chestnut tannin along the cultivation time of *Penicillium citrinum* (◆), *Penicillium aethiopicum* (▲), *Aspergillus chevalieri* (*) and *Aspergillus oryzae* (●).

Table 1. Cultivation parameters, total phenols, total carbon and nitrogen, COD and BOD of the cultivations containing acacia and chestnut tannin as substrate.

Fungi	X max (g.L ⁻¹)	Tannin consumption (%)	μ _{max} (h ⁻¹) ^A	P _{Xmax} (g.L ⁻¹ .h ⁻¹) ^B	Y _{x/s} (g.g ⁻¹) ^B
Acacia tannin					
<i>Penicillium citrinum</i>	30.86 ± 0.11 ^a	94.85 ± 0.45 ^a	0.033 ^a	0.84 ^a	0.74 ^b
<i>Penicillium aethiopicum</i>	30.39 ± 0.38 ^a	91.45 ± 0.75 ^b	0.029 ^b	0.81 ^a	0.73 ^b
<i>Aspergillus chevalieri</i>	24.42 ± 0.21 ^b	68.90 ± 0.68 ^c	0.022 ^c	0.31 ^c	0.76 ^a
<i>Aspergillus oryzae</i>	24.77 ± 0.04 ^b	79.78 ± 0.04 ^d	0.029 ^b	0.72 ^b	0.68 ^c
Medium*	Total phenols (%) 68 ± 0.9 ^b	C/N ratio 8:1	COD (mgO ₂ .L ⁻¹) ^B 63840 ^a	BOD (mgO ₂ .L ⁻¹) ^B 21500 ^a	COD/BOD ^B 2.97 ^a
Chestnut tannin					
<i>Penicillium citrinum</i>	5.72 ± 0.29 ^b	35.15 ± 0.57 ^a	0.024 ^a	0.09 ^b	0.28 ^{ab}
<i>Penicillium aethiopicum</i>	5.45 ± 0.10 ^b	35.51 ± 0.23 ^a	0.023 ^b	0.13 ^a	0.25 ^b
<i>Aspergillus chevalieri</i>	6.69 ± 0.01 ^a	36.28 ± 0.51 ^a	0.017 ^c	0.14 ^a	0.35 ^a
<i>Aspergillus oryzae</i>	7.14 ± 0.19 ^a	30.77 ± 0.47 ^b	0.015 ^d	0.12 ^{ab}	0.43 ^a
Medium*	Total phenols (%) 73 ± 0.6 ^a	C/N ratio 6:1	COD (mgO ₂ .L ⁻¹) ^B 36480 ^b	BOD (mgO ₂ .L ⁻¹) ^B 13250 ^b	COD/BOD ^B 2.76 ^b

* Total phenols, total carbon and nitrogen, COD and BOD: determined at the beginning of cultivation.

μ_{max} is the maximum specific growth rate; P_{Xmax} is the maximum biomass productivity; Y_{x/s} is the biomass yield on substrate.

Different superscript letters in the same column indicate a statistically significant difference (p < 0.05).

^A Standard deviation less than 10⁻⁴; ^B Standard deviation less than 10⁻².

ratio ranged from 8:1 to 6:1 for acacia and chestnut tannin, respectively. For lipid production, it would be necessary to optimize the culture medium in order to increase the C/N ratio of the medium. Lipid accumulation per liter of culture is usually optimal at molar C:N ratios exceeding 50 and near 100 (Ageitos et al., 2011). For instance, when optimum nutritional conditions for lipid production were studied for microbial lipid production by fungi, C/N= 31:1 and C/N= 52:1 were found to be optimum, reaching 12.75% and 21.71% lipids for *Penicillium citrinum* and *Aspergillus niger* respectively (El-haj et al., 2015).

Biodegradation potential of acacia and chestnut tannin

The total phenols of tannins (Table 1) show that chestnut tannin presents a higher phenols content (73%) when compared to acacia tannin (68%), which corroborates the higher consumption of acacia tannin in cultivations. Mohanty and Jena (2017) reported that with the increase in the initial phenol concentration, a decrease in the percentage of phenol degradation was observed in cultures of *Pseudomonas* sp. NBM11. The presence of vegetable tannin in the effluent cause pollution problems that are usually toxic and xenobiotic. The residual amount of phenolic compounds is one of the major criteria of biodegradability. It has been found that the decomposition of phenolic compounds may significantly improve the biodegradability of preozonized phenolic compounds (Kanagaraj and Mandal, 2012). *Aspergillus* species have demonstrated efficiency in the degradation of phenolic compounds and has been used in the treatment of wastewater. Fungi of this genus use aromatic compounds through the production of catabolic enzymes (Rodrigues et al., 2007). It may be due to this efficiency that in the chestnut cultivations, where there was a higher content of total phenols, fungi of the genus *Aspergillus* and *Penicillium* had similar performance.

Vegetable tannins are usually regarded as biodegradable chemicals because they are natural products. Nevertheless, biodegradation behaviors of vegetable tannin extracts might vary greatly with their sources, molecular structures and molecular weights. A common property of tannins is that they can be employed as carbon and energy sources by many microorganisms (Shi and Di, 2000). Some microorganisms resistant to tannins have developed mechanisms and pathways for tannin degradation. Earlier reports suggest that *Aspergillus niger* and *Penicillium* ssp are capable of growing in tannic acid as sole carbon source (Silva et al., 2010). It was verified (Table 1) that all fungi assimilated acacia and chestnut tannin as a carbon source. The range of tannin consumption varied from 94.85% for *Penicillium citrinum* cultivated in acacia tannin

to 30.77% for *Aspergillus oryzae* cultivated in chestnut tannin. These species have been reported as microbial sources of tannase. Filamentous fungi of the *Aspergillus* genus have been widely used for tannase production because it can occur in the absence of tannic acid. Phenolic compounds such as gallic acid, pyrogallol, methyl gallate, and tannic acid induces tannase synthesis. For instance, gallic acid such as tannic acid, has been reported as an inducer of tannase synthesis under submerged fermentation (Aguilar et al., 2007).

A particular property of vegetable tanning extracts is their hygroscopicity, miscible with water to form polydisperse solutions, partly of a colloidal type (Cassano et al., 2003). They are usually extensively used in processes of tanning and retanning so as to ensure full penetration of tannins and complete reaction between tannins and hides. Consequently, a part of tannins and of most non-tannins will inevitably remain in solution, which will lead to high loads of total solids and chemical oxygen demand (COD) in wastewater (Arana et al., 2001). During the tannage, tannins react with the hide fraction faster than non-tannin compounds. However, at the end of the process the concentration of tannin components is still significant. So the discharge of the bath represents an economical and environmental damage due to low biodegradability of tannins (Cassano et al., 2003). Compared with the aromatic syntans, aldehydic tanning agents and acrylic tanning agents, vegetable tannins are much easier to be biodegraded by tannery-activated sludge as reported by He et al. (2008). Meanwhile, hydrolysable tannin extracts exhibit better biodegradability than condensed tannin extracts. In this study, higher consumption of condensed tannin (acacia) was observed in relation to the hydrolysable tannin (chestnut) for all evaluated fungi. These results contradict the literature, which states that the condensed tannins are more resistant to degradation than hydrolysable tannins. Condensed tannins are not hydrolysed by classical tannases, with initial degradation steps carried out by mono- or di-oxygenases (Contreras- Dominguez et al., 2006). However, further studies are needed to further characterize the degradation of condensed tannin.

Earlier reports show that a concentration of tannins higher than 0.2 g/L in wastewater strongly inhibits microbial activity, while 2 g/L of tannins will completely inhibit it (Kalyanaraman et al., 2015). Besides that, the organic load removal is more sensitive to condensed tannin concentrations than to hydrolysable tannins (Dhayalan et al., 2007). According to Agustini et al. (2017), vegetable tannin in leather shavings showed an inhibitory effect for anaerobic biodegradation, where much of the residue underwent hydrolysis, but only a small part was mineralized to methane, resulting in an increase of organic matter.

It is possible to estimate the biodegradability of an effluent and its environmental impact on a hydrous body. Traditionally, the concentration of organic matter in wastewater is measured by total organic carbon (TOC), dissolved organic carbon (DOC), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) (Yang et al., 2014). The COD/BOD ratio expresses a lot about what kind of oxidation will be effective in the degradation of the organic load. If $\text{COD/BOD} < 2.5$, the compound is easily biodegradable. If $5.0 > \text{COD/BOD} > 2.5$ this effluent will require care in choosing the biological process so that there is a desirable removal of the organic load (Braga et al., 2012). As can be seen in Table 1, the acacia tannin presented a slightly higher COD/BOD ratio than that presented by chestnut tannin, 2.97 and 2.76, respectively. With values greater than 2.5 for the COD/BOD ratio, both are considered more difficult to be biodegraded.

Traditionally, the concentration of organic matter in wastewater is measured by total organic carbon (TOC), dissolved organic carbon (DOC), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) (Yang et al., 2014).

The characterization of tannin extracts by FTIR has been published by several authors with the main aim of characterizing the extracts of different plants (Falcão and Araujo, 2013; Tondi and Petutschnigg, 2015; Grasel et al., 2016). In this context, FTIR was employed to evaluate the biodegradation of vegetable tannin by filamentous fungi (Figure 2). Phenols are organic compounds characterized by a hydroxyl groups bonded directly to an aromatic ring (benzene ring) (Blainski et al., 2013). Analysis of the spectra obtained shows peaks at 3000 to 3500 cm^{-1} due to the sum of the OH stretching that is characteristic of polyphenolic extracts. In addition, it could correspond to O-H stretch of the organic acids formed during the cultivation (Grasel et al., 2016).

The region of the spectra between 950 and 600 cm^{-1} features aromatic torsions, but mainly C-H bending out-of-plane (Tondi and Petutschnigg, 2015). The condensed tannins show a more structured profile with a more intense absorption in the region between 1116 to 1110 cm^{-1} (Figure 2A), while the hydrolysable tannins present a major absorption at around 1731-1704 cm^{-1} and 1325-1317 cm^{-1} (Figure 2B) (Falcão and Araújo, 2014; Ricci et al., 2015). The peak at 1609 cm^{-1} for chestnut and 1612 cm^{-1} for acacia tannin correspond to C=C of aromatic rings (Falcão and Araújo, 2014). The disappearance or reduction of peaks in this region suggests the breakage of C=C bonds indicating the biodegradation of the condensed tannin, being more pronounced in the cultivations of *Penicillium citrinum* and *Penicillium aethiopicum*. The FTIR spectrum of the cultivations in chestnut tannin showed no significant change in the positions

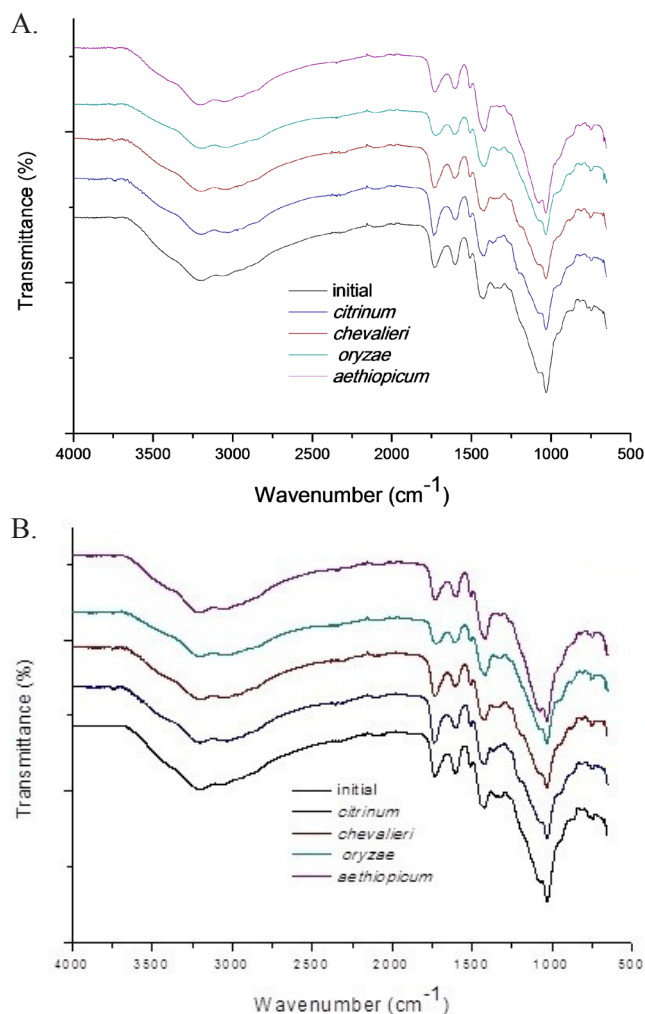


Figure 2. (A) FTIR spectrum of acacia and (B) FT-IR spectrum of chestnut cultivation of *Penicillium citrinum*, *Penicillium aethiopicum*, *Aspergillus chevalieri* and *Aspergillus oryzae*.

or intensity of peaks (Figure 2B). This is probably due to the fact that there was low production of biomass and consumption of tannin in these cultivations.

Studies on enhancing the biodegradation of tannins by ozonation and Fenton's oxidation process (Kalyanaraman et al., 2015) have shown that the pre-ozonation improved the biodegradability and subsequent aerobic treatment carried out in batch aerobic reactors resulted in BOD₅ and COD of 60 and 350 mg/L in the treated effluent. FT-IR results showed the presence of biodegradable organics like carboxylic acids, aldehydes and quinones in pre-ozonated wastewater. Fenton pre-treatment resulted in a considerable increase in biodegradability with mineralization.

CONCLUSIONS

The present investigation was carried out in order to determine the potential of the fungi of the genera

Penicillium and *Aspergillus* to biodegrade condensed and hydrolysable tannins. *Penicillium citrinum* and *Penicillium aethiopicum* were it to be more efficient, reaching 94.85% and 91.45% respectively for consumption of acacia tannin. In the chestnut cultivations the tannin consumption was low, not exceeding 36.28%, there being no differences between the genera of the fungi. The acacia tannin presented a slightly higher COD/BOD ratio than that presented by chestnut tannin, 2.97 and 2.76, respectively. On the other hand, chestnut tannin present a higher total phenols (73%) compared to acacia (68%). In this study, a higher consumption of condensed tannin (acacia) was observed in relation to the hydrolysable tannin (chestnut) for the evaluated fungi. The disappearance of peaks around 1600 cm⁻¹ suggests the biodegradation of the condensed tannin by *Penicillium citrinum* and *Penicillium aethiopicum*. The expressive results obtained from the consumption of acacia evidently suggest that the native isolates identified as *Penicillium citrinum* and *Penicillium aethiopicum* are efficient strains for the biodegradation of the tannin most used in tannery industry. Though some reports about tannin degradation by filamentous fungi are available, literature showing expressive consumption of a condensed tannin was not found. Therefore, the biodegradation of vegetable tannins by *Penicillium citrinum* and *aethiopicum*, *Aspergillus chevalieri* and *oryzae* may be a promising alternative.

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