2) Research Project Plan outline

TREATMENT OF TANNERY DYE-CONTAINING EFFLUENTS USING NATIVE FUNGAL STRAINS

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Introduction:

In the final stages of leather processing in drums, dyestuffs are added to the wet process to impart the sensory characteristics of the final product, such as colour shade and intensity, colour penetration and uniformity and chemical and physical stability. This step has a great importance in post-tanning operations because these features of appearance are one of the first to be visually judged by the consumer (Gomes et al., 2016). Conventionally, the dyeing step is carried out in an aqueous medium during the wet finishing process, requiring high volume of water. Dyes have different rates of exhaustion from the medium and different manners of fixation. On the other hand, leather is a difficult substrate to dye due to the heterogeneous nature of skin/hide. Problems with diffusion and affinity cause incomplete exhaustion of the dyestuffs, and a portion of dye remains in the effluent (Kanth et al., 2008). The volume of effluent generated in the dyeing process is about 7 L per kg of processed leather with dye concentrations of over 0.5 g L^{-1} (Piccin et al., 2012). The discharge of dye-containing effluents without adequate treatment is a matter of serious concern, not only for the undesirable visual pollution associated with the colour in wastewater, but also by the negative impacts on the environment, such as inhibition of aquatic photosynthesis, reduction of water reoxygenation capacity, depletion of dissolved oxygen and acute and chronic toxicity affecting flora, fauna and humans (Rodríguez-Couto, 2015).

Currently, physical, physicochemical and biological treatment technologies are used to handle dye-containing effluents, for example adsorption with activated carbon materials, coagulation, flocculation, ion exchange and biological activated sludge processes. However, these traditional technologies are not ever completely suitable for the treatment of dyes because of their high cost, low efficiency to treat toxic and recalcitrants compounds and the large amounts of sludge remaining, which involve handling and disposition of the residues produced and/or regeneration of adsorbent materials generated (Baccar et al., 2011). The treatment of dye-containing effluents remains a challenge that motivates the application of new and more advanced technologies. Biological strategies are presently earning importance in the treatment of dye-containing wastewaters and appear as an environmental/eco-friendly and less expensive alternative. Several studies have demonstrated a variety of different types of microorganism, including fungi, yeasts, algae and bacteria, able to biodecolorize and to biodegrade a broad range of industrial dyes (Ali, 2010). Among these microorganisms, white-rot fungi were demonstrated to be by far the most efficient group in degrading synthetic dyes, which have been the subject of intensive research. The great ability of these basidiomycetes to remove dyes and other recalcitrant pollutants from effluent is attributed to their highly oxidative, non-specific and non-stereoselective enzyme system consisting of laccases, lignin peroxidases and manganese peroxidases. In addition to enzymatic biodegradation, fungi present alternative mechanisms that are involved during dye removal processes, such as biosorption and bioaccumulation (Kaushik and Malik, 2015). However, publications dealing with the treatment of leather dyes and real tannery effluent from dyeing operations by white-rot fungi are scarce. Besides, most studies developed to treat dye-containing effluents involving microorganisms were mainly focused on the removal of textile dyes, and only few researches tested leather dyes decolorization. It should be noted that leather dyes may be structurally different from textile dyes since they are synthesized for dyeing different types of texture (leather and cotton fibres).

Therefore, searching for new white-rot fungi strains with the ability to biodecolorize and to detoxify specific dyes for leather and real effluents from post-tanning operations is also needed for the possible industrial application in the treatment of real tannery effluents.

Objectives:

The general objective of this project is to find/test native fungal strains with potential to decolorize and to detoxify real tannery dye-containing effluents, contributing to the development of technologies for treatment of wastewater of leather industry. Specific aims:

- Find white-rot fungal strains natives of south Brazil with potential to decolorize pure tannery dyes;
- Asses the ability of the new native strains to decolorize dyeing effluents produced in pilot scale and real tannery effluents, as well as to intensify the research with the fungal previously found;
- Reduce pollution parameters in the effluent such as toxicity, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) and total organic carbon (TOC) by application of fungal strains;
- Elucidate the factors involved in the decolorization and detoxification processes by performing several analytic procedures;
- Optimize the effect of different operational conditions such as sterilization, addition of nutrients, temperature, pH and agitation on the fungal decolorization and detoxification of effluents;

Methods:

- Local: This research will be performed at the Laboratory for Leather and Environmental Studies (LACOURO) at the Federal University of Rio Grande do Sul (UFRGS), under the advice of Prof. Mariliz Gutterres.
- Microorganism: New fungal strains will be collected from pieces of rotted wood as well as strains of *Trametes villosa*, previously isolated and studied by Ortiz-Monsalve (2015), will be used in the current project.
- Dyes and effluents: Different tannery dyes were provided by Lanxess, industry which has partnership with the LACOURO and has contributed with technical support and material contribution for research purposes. Dye containing effluents will be produced in pilot scale (Gomes et al., 2016). Real effluents will be collected in local tannery companies which also cooperate with the LACOURO, such as JBS-Couros, Indústria de Peles Pampa, Curtume Nimo and Curtume Minuano.
- Screening in solid and liquid media: All isolates will be subjected to a screening based on the ability of each fungus to produce ligninolytic enzymes. The enzymatic action will be considered positive with a visible reaction with indicator substrates. Liquid media containing dyes will be inoculated with each fungus and will be incubated under submerged fermentation conditions (Ortiz-Monsalve, 2015).
- Analytical procedures: To study the kinetics of biodecolorization related to dye removal, enzyme activity and biomass production, samples will be withdrawn from cultures every 24 h, centrifuged and the supernatant will be used for analysis. Dye decolorization will be determined by UV-Vis spectral analysis and will be expressed in terms of biodecolorization efficiency as follows: BE (%) =(Ao-At)/Ao ×100, where A_o and A_t represent the initial and observed absorbance post-treatment with at the λ_{Max} of each dye. Enzyme production will be assessed by spectrophotometry. Extracellular laccase activity will be determined monitoring the oxidation of ABTS (Wang and Ng, 2006). Lignin peroxidase activity will be determined by using veratryl alcohol as substrate (Arora and Gill, 2001). Manganese peroxidase activity will be measured by catalysis of DMP (Máximo et al., 2003). Biomass production will be determined by dry weight, drying the mycelia at 105 °C for 24 h until constant weight was obtained. To assess the biosorption ability and chemical stability after decolorization, fungal biomass will be suspended in different solvent solutions (Plácido et al., 2016). Changes in morphological characteristics will be observed using light microscopy, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX) attached to SEM.
- Detoxification assays: Detoxification will be based on the measurement some parameters such as pH, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), phosphorus (P), ammonia nitrogen and total Kjeldahl nitrogen (TKN). These assays will be realized according to Brazilian standards and International Standard Methods (Gutterres et al, 2015)

- Optimization: In order to optimize the percentage of decolorization and detoxification will be performed a response surface methodological approach, where the effect of different operational conditions such as sterilization, nutrients, temperature, pH and agitation will be investigated.
- Viability studies and tests for real application and scaling up: Aiming the real application in treatment processes will be studied techniques for fungal cell and enzyme immobilization by using substrates such as PUF (polyurethane foam), nylon sponge, orange peelings or rice straws. Also will be studied different ways of operation and scaling up (batch, air fluidized, expanded-bed or fixed-bed bioreactors).

Hypothesis/Expected Results:

Preliminary studies performed at the LACOURO and presented at the XXXIII IULTCS Congress in 2015, showed the efficiency of native fungal strains to decolorize tannery dyes in aqueous solutions, reaching high decolorization values up to 90%. Based on this ability, the hypothesis envisages the possibility of treatment of real dye-containing effluents by using these strains. We are expecting to find suitable fungal species with ability to decolorize and to detoxify real tannery dyeing wastewaters.

Research benefit for the local or global leather industry:

Considering that most tanneries in Rio Grande do Sul work in post-tanning operations and that most of them don't reach the legal standards for removal of toxic substances such as dyes, this research will contribute to improve the current treatment processes of the local tanneries, as well as to expand the global scientific and technology knowledge about the treatment of industrial effluents by white-rot fungi.

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