Evaluation of *Ficus benjamina* wood chip-based fungal biofiltration for the treatment of Tequila vinasses

Garzón-Zúñiga Marco Antonio, Alvillo-Rivera Angélica Julieta, Ramírez Camperos Esperanza, Buelna Gerardo, Díaz-Godínez Gerardo and Estrada-Arriaga Edson Baltazar

**ABSTRACT**

This study was focused on the application of an aerobic biofiltration (BF) with *Ficus benjamina* wood chips as support medium, inoculated with two basidiomycete fungi, *Phanerochaete chrysosporium* (BF 1) and *Trametes versicolor* (BF 2), to treat Tequila vinasses from a Tequila industry. The biofiltration system was compared with a biofilter system without basidiomycete fungi (BF W), in order to determine the influence of fungi on the treatment of vinasses. Three different vinasses/water ratios (30/70, 40/60, and 50/50) were evaluated. The maximum removals of chemical oxygen demand (COD) obtained during each operation step were 72% (BF 1), 72% (BF 2), and 8% (BF W) for 30 vinasses/70 water; 72% (BF 1), 73% (BF 2), and 66% (BF W) for 40 vinasses/60 water; and 22% (BF 1), 20% (BF 2), and 18% (BF W) for 50 vinasses/50 water. The total organic carbon (TOC) removal was significantly increased using a volumetric organic load of 5.5 kg COD m⁻³ d⁻¹. During the operation of the biofilters, the enzymatic activity of laccase was present, even at the step of highest concentration of vinasses.

**Key words** | basidiomycete fungi, biofiltration, *Ficus benjamina* wood chip, laccase enzyme activity, tequila vinasses

**INTRODUCTION**

Tequila is a traditional alcoholic beverage from Mexico. It is generated from the distillation of fermented *Agave tequilana* Weber var. *azul* juice, previously cooked. The production of Tequila in Mexico, in 2015, was 248.3 million liters (Regulatory Council of Tequila 2016), which generated large quantities of solid waste (bagasse) and liquids (vinasses). According to Moran-Salazar et al. (2016) and Iñiguez et al. (2001), the bagasse generated from Tequila production represents 40% of the total weight of agave hearts. Thus, approximately 350,000 tons of bagasse were generated in 2015, during Tequila production. In 2014, 159 companies were recorded (Regulatory Council of Tequila 2016), of which 60% are small producers who do not have wastewater treatment systems in place; only 50% of the large industrial plants treat their waste (Iñiguez-Covarrubias & Hernández 2010). According to López-López et al. (2010), it is estimated that for each liter of Tequila produced, 10 to 12 L of vinasses and 1.4 kg of bagasse are generated. The range of the chemical oxygen demand (COD) contained in vinasses is between 20,000 and 100,000 mg L⁻¹. In addition to the COD, the vinasses have an acidic pH and a dark-brown color, due to the presence of melanoidins, high total suspended solids (TSS) concentration, polyphenols, polycyclic aromatic compounds, cellulose, lignin, furans, nitrogen, sulfur compounds, and long-chain and short-chain fatty acids (Retes-Pruneda et al. 2014). The annual production of Tequila generated 90.12 m³ · s⁻¹ of vinasses, of which only 15% was treated (España-Gamboa et al. 2011).

There are different strategies studied for the management of Tequila vinasses, such as: 1) Stabilization ponds, which serve as storage for the vinasses and the
neutralization of pH. However, they do not receive any additional treatment (Iñiguez-Covarrubias & Hernández 2010); 2) Dissolved air flotation (DAF), which has the advantage of removing suspended solids up to 80%. However, the removal of dissolved solids and organic matter was not significant when applying DAF (López-López et al. 2010); 3) The mixture of Tequila vinasses with livestock food has been studied by Fernández et al. (2009), showing that when mixed in a ratio of 10% of vinasses in ruminant food, 30% in pig food, and 13% in sheep food, there are no organoleptic differences in their food or their digestibility. However, there are no studies on the long-term effect; 4) The coagulation-flocculation has shown removal of 70% of COD and color, and 30% of suspended solids, when using Al₂(SO₄)₃, NaOH, and alginate (Iñiguez-Covarrubias & Hernández 2010; López-López et al. 2010; Retes-Pruneda et al. 2014); 5) Anaerobic digestion has been an excellent biological process for vinasses treatment. Jáuregui-Jáuregui et al. (2014) and Méndez-Acosta et al. (2010) obtained removals of COD of up to 95% during the treatment of Tequila vinasses; 6) Hydrogen production from Tequila vinasses in an anaerobic sequencing batch reactor has been studied by Buitrón & Carvajal (2010), indicating that it is possible to enhance the production of hydrogen from Tequila vinasses with a short hydraulic retention time (HRT).

On the other hand, studies on the use of basidiomycete fungi for the degradation of recalcitrant organic matter have been carried out (Strong 2010). These studies are based on the conversion of lignin to lignocellulose by unspecified extracellular enzymes, which by degrading lignin could also degrade similar molecules and generate byproducts that can be exploited by other microorganisms as a source of energy and carbon. Studies have been carried out at laboratory level in-vitro and with the use of supplemental sources of carbon such as glucose (in most cases). The main objective of the treatments with basidiomycete fungi is to oxidize organic substances, reducing COD and the biological oxygen demand (BOD). Additionally, basidiomycete fungi have a lower sensitivity to temperature changes, nutrients, aeration, and pH compared with microorganisms, since they tolerate pH from 2 to 9, 5.6 being the optimum pH (España-Gamboa et al. 2011).

Fungal techniques for the treatment of vinasses or molasses from an alcohol distillery as carbon source have not been fully investigated. Many experiments have been carried out using fungi. However, these experiments use dyes, textile wastewater treatments, or phenols as carbon source (Hai et al. 2003; Novotny et al. 2011; Pradeep et al. 2015; Carabajal et al. 2016). There are a few studies reported about the treatment of high strength wastewater using basidiomycete fungi. These reports are based only on the decolorization and COD removal of the vinasses on pure-culture experiments and have not been investigated in continuous stirred-tank reactors or in packed reactors (immobilized fungi). Basidiomycete fungi in-vitro assays – for example, Trametes pubescens and Phanerochaete chrysosporium – demonstrated that the removal efficiency of color and phenols contained in vinasses is not affected by these fungi (Potentini & Rodríguez-Malaver 2006; Melamane et al. 2007; Strong & Burgess 2008). Ferreira et al. (2011) used Pleurotus sajor-caju for the treatment of vinasses from the sugar industry, which showed COD removals of 85%, BOD removals of 75%, and color and turbidity removals of 99%. Retes-Pruneda et al. (2014) used numerous fungal strains for the bioremediation of tequila vinasses-based solid media. With Pleurotus ostreatus 7992 and Trametes trogii 8154, both COD and BOD were reduced, by 88.7 and 89.7%, respectively, during the bioremediation of Tequila vinasses.

The organic bed biofiltration system is an emerging technology for high strength wastewater treatments (Día et al. 2016). The organic bed biofiltration system involves both physical-chemical (filtration, adsorption, and ion exchange) and biological mechanisms (biological degradation), which allowed the degradation of easily biodegradable organic matter and of complex and toxic compounds (Garzón-Zúñiga & Buelna 2011). The biofiltration system used wood chip as support medium on which bacteria and fungi grow as biofilms. These bacterial communities that grow on support media excrete extracellular enzymes, which can also degrade molecules that are hard to degrade. According to these physical-chemical removal mechanisms of the biofilters, and to the metabolic characteristics of basidiomycete fungi for the transformation of compounds difficult to biodegrade to readily digestible compounds, it is important to study the effect of these two processes as a whole for the removal of contaminants in industrial Tequila wastewaters. This type of treatment has been studied for the degradation of dyes, pig water, and petrochemicals, and to treat wastewater from small communities and industries (Zhidong et al. 2010; Garzón-Zúñiga & Buelna 2011). There are a few very studies using biofiltration inoculated with a fungal strain for wastewater treatment (Dávila-Solano 2004; García-Sánchez 2007). There have been no studies to determine the removal of organic matter of Tequila vinasses by biofiltration systems inoculated with basidiomycete fungi. The main objective of this study is to determine the performance of fungal biofiltration using Ficus wood chips as a support medium for the treatment of vinasses from a Tequila factory.
MATERIALS AND METHODS

Selection of basidiomycete fungal strains

In order to select a species of basidiomycete fungi to inoculate the biofilters, two growth tests of three different species (*Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus*) were carried out. The tests consisted on preparing solid media with different dilutions of vinasses to evaluate their tolerance to Tequila vinasses of fungi *in-vitro* (Petri dishes). Three solid culture media with Potato-Dextrose-Agar (PDA) were prepared. In two of them, the vinasses were used at 10% and 20%. The third one was used as a blank and was prepared only with distilled water. The test was performed in triplicate. The second test for the mycelium growth was conducted in a liquid medium, since this is the form in which the fungus massively grows after inoculation of the biofilters. In this case, the liquid medium was prepared with malt extract and a solution of vinasses with ascending concentration (20, 40, and 100%) and a blank with distilled water. Each strain was maintained in an orbital shaker at a temperature of 32 ºC for 15 days, according to the methodology described by García-Sánchez (2007). This test was also performed in triplicate. The fungal strains were obtained from the Mycological Herbarium of Morelos, Autonomous University of the State of Morelos. The strains were delivered in culture media made from wholemeal wheat flour (HIT) and are classified as: *Phanerochaete chrysosporium* (HEMIM 5); *Trametes versicolor* (HEMIM 9); and *Pleurotus ostreatus* (HEMIM 50).

Experimental setup

Three laboratory-scale biofilters (BFs) with dimensions of 60 cm (height) and 9.3 cm (diameter) were employed (Figure 1). The working volume of the biofilters was 3.5 L. The reactors operated in a continuous flow of 2 L d⁻¹ and an aeration flow of 1,000 mL min⁻¹, which were regulated by peristaltic Masterflex pumps and a Gilmont rotameter, respectively. They were operated at room temperature with a pH of 3.7 (vinasses) and without artificial lighting, since natural lighting was considered sufficient to carry out the growth of fungi. The three reactors were packed with 3.5 L of *Ficus benjamina* wood chips as support medium. The *Ficus benjamina* wood chips were mixed perfectly with 1 L of two basidiomycete fungi selected (*Phanerochaete chrysosporium* (BF 1) and *Trametes versicolor* (BF 2)), which were propagated in liquid media. Subsequently, the two strains were introduced to each biofilter. One biofilter (BF W) was not inoculated with a fungi strain and worked as a control biofilter. The biofiltration systems were operated with different vinasses/water ratios (30/70 (step I), 40/60 (step II) and 50/50 (step III)). The HRT in both biofilters was 0.3 d, which was determined according to Garzón-Zúñiga et al. (2005). Table 1 shows the operating condition of the biofilters through different vinasses/water ratios. The water content and bed porosity for biofilters were

![Figure 1](https://iwaponline.com/wst/article-pdf/77/5/1449/249394/wst077051449.pdf)

**Figure 1** | Experimental setup for the treatment of tequila vinasses.
65%. These parameters were calculated according to Garzón-Zúñiga et al. (2005).

Tequila vinasses

The biofilters were fed with Tequila vinasses at the factory in Jalisco, Mexico. Tequila vinasses were collected every month and stored at 3°C before being used. Due to the high TSS concentration found in raw vinasses, a slow filter was installed for the removal of TSS. The slow filter was packed with gravel using three different particle sizes (0.46, 1.00, and 1.19 mm) and operated at a filtration rate of 0.1 m³ m⁻² d⁻¹ (Figure 1). Table 2 shows the physicochemical properties of the raw and filtered vinasses.

Analytical measurements

The influents and effluents from the biofilters were analyzed according to the following parameters: COD, total organic carbon (TOC), reducing sugars, and phenols. Reducing sugars were analyzed according to Nelson (1944). The COD was determined based on standard method No. 5220 (APHA 2005). The TOC was measured by thermo catalytic oxidation using a high temperature and a Shimadzu TOC-5000A TOC analyzer. The enzyme activity of fungi during the performance of the biofilters was measured through the extracellular fungal laccases. The laccases were determined according to Diaz et al. (2013). The quantification of phenols in Tequila vinasses was measured using a HPLC C18 reversed phase column (5 μm, 4.6 × 250 mm), coupled with UV/vis detector (wavelength between 254 and 270 nm).

RESULTS AND DISCUSSION

Selection of strains

During the growth of strains in-vitro in solid culture media, Phanerochaete chrysosporium, Trametes versicolor, and Pleurotus ostreatus had a growth rate of 8.35, 6.98, and 6.62 cm² d⁻¹, respectively, on PDA medium, without vinasses. Meanwhile, in the medium of culture prepared with 10% of vinasses, the growth rates were 12.18 cm² d⁻¹ for Phanerochaete chrysosporium, 11.77 cm² d⁻¹ for Trametes versicolor, and 8.33 cm² d⁻¹ for Pleurotus ostreatus, showing a positive effect of vinasses as a growth substrate, except for Pleurotus ostreatus. In the culture media prepared with 20% of vinasses, the growth rates were 5.96, 7.43, and 4.72 cm² d⁻¹ for Phanerochaete chrysosporium, Trametes versicolor, and Pleurotus ostreatus, respectively. In this case, the growth rates were reduced by 40–50% in all fungi strains (Figure 2). It was noted that Phanerochaete chrysosporium grew in all media (malt extract with distilled water, 20, 40, and 100% vinasse), Trametes versicolor only grew in the blank and in the medium prepared with 20% of vinasses, while Pleurotus ostreatus only grew in the blank (Figure 3). With the results mentioned above, the strains chosen for the inoculation of biofilters were Phanerochaete chrysosporium and Trametes versicolor, due to their adaptation capacity to the contaminants present in the vinasses and their capacity to grow massively in a liquid culture medium with vinasses.

Table 1 | Experimental periods and operational conditions of aerated biofiltration systems packed with Ficus benjamina wood chips

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinasses/water ratio</td>
<td>30/70</td>
<td>40/60</td>
<td>50/50</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Superficial hydraulic load (m² m⁻² d⁻¹)</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
</tr>
<tr>
<td>Volumetric hydraulic load (m³ m⁻³ d⁻¹)</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Volumetric organic load (kg COD m⁻³ d⁻¹)</td>
<td>4.5</td>
<td>5.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Solid load (kg TSS m⁻² d⁻¹)</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Table 2 | Physicochemical composition of vinasses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw vinasses</th>
<th>Filtered vinasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg L⁻¹)</td>
<td>32.900 ± 11,400</td>
<td>19.500 ± 3,500</td>
</tr>
<tr>
<td>TOC (mg L⁻¹)</td>
<td>18.950 ± 4,000</td>
<td>10,000 ± 2,500</td>
</tr>
<tr>
<td>TSS (mg L⁻¹)</td>
<td>13.600 ± 4,400</td>
<td>1,000 ± 650</td>
</tr>
<tr>
<td>Reducing sugar (mg L⁻¹)</td>
<td>6.5 ± 0.5</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Phenols (mg L⁻¹)</td>
<td>32 ± 5</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>pH</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
</tbody>
</table>
During biofilter operations, it was observed that the fungal strains in both biofilters grew in acidic conditions. Couillard (1994) mentions that during the first days of operation in biofilters packed with peat (without fungi), the pH effluent decreased due to the washing off of the humic and fulvic acids contained in organic materials. In addition, according to Strong & Burgess (2008), the wastewater treatment with fungi decreased the pH of the treated water. However, in this study such behavior was not observed. Figure 4 shows a tendency to increase the pH, which may be due to the alkalization of the three biofilters due to the release of anions, products of the biotransformation of complex molecules from organic matter in Tequila vinasses in the biofilters inoculated with fungi (Moreno-Casco & Moral-Herrero 2007).

The content of organic matter in raw vinasses was found to be in a range of 20,000 to 60,000 mg COD L$^{-1}$. The solids were removed before entering the biofilters. When the TSS were eliminated, the COD decreased by 40% and average values of 19,500 mg L$^{-1}$ were maintained. Figure 5 shows the COD profiles in the influent and effluent from each one of the reactors, during the different operation steps. During the first 25 days of operation, the three biofilters presented high removal efficiencies, which can be related to the biodegradation and adsorption processes. Nevertheless, from day 25 onwards, the removal efficiency in the BF W decreased until reaching zero, which could be due to the saturation of the filter material, while in BF 1 and BF 2, the removal efficiencies increased and mycelial spread in the support media was observed. COD removals obtained from days 31 to 40 were: 10 ± 6% for BF W, 69 ± 7% for BF 1, and 66 ± 12% for BF 2. These values showed that the action of extracellular enzymes excreted by each fungus allowed an increase between 30 to 75% for removal of the organic matter present in Tequila vinasses.

When changing the percentage of vinasses in the biofilters’ influent (second step), the COD removal rates for BF 1 and BF 2 were not impacted. During this stage, the organic matter removal obtained for the control biofilter increased

**Figure 2** | Growth rates of basidiomycete fungi strains on solid culture media.

**Figure 3** | In-vitro growth (flask) in liquid medium prepared with 0, 20, 40 and 100% of vinasses for (a) Phanerochaete chrysosporium, (b) Trametes versicolor and (c) Pleurotus ostreatus.
Figure 4 | Evolution of pH from biofiltration systems.

Figure 5 | Evolution of COD in biofiltration systems inoculated with basidiomycete fungi.
up to 66%, which was attributed to the fact that, during the first stage, the support medium from the control biofilter was contaminated by some bacteria and began to develop a biofilm on its surface. The support media in all the biofilters were not sterilized. In the case of the fungal biofilters, the removal efficiency increased slightly. In the third step, the removal efficiencies of the COD decreased in the biofiltration systems, obtaining removals of 8 ± 2% for BF W, 24 ± 9% for BF 1, and 26 ± 10% for BF 2. These low removals were related to the increase of TSS in the biofilters’ influents, thus reducing the filtration capacity and generating short circuits inside the biofilters. The accumulation of solids in the porous spaces of the biofilters generated a smaller effective area available for reaction volume. When the 50/50 ratio was used, the phenols concentration in the influent increased from 13 ± 3 mg L⁻¹ to 18 ± 5 mg L⁻¹. Perhaps, this increase of phenols could generate an inhibitory effect on basidiomycete fungi. However, the phenols under aerobic biodegradation generate an inhibition at concentrations of 100 mg L⁻¹ (Christen et al. 2012). In this study, the phenols concentrations detected in raw vinasses were low. For anaerobic biodegradation, the phenol concentrations higher than 500 mg L⁻¹ can be successfully treated (Pradeep et al. 2015). Various types of fungi involved in the biodegradation of phenols have been reported to remove high concentrations of phenols and other recalcitrant pollutants, without showing inhibition problems (Pradeep et al. 2015; Carabajal et al. 2016). Phenol tolerance, degradation of phenol, and phytotoxicity bioassay were determined using Trametes versicolor, indicating that a high concentration of phenols (15 mM) can be efficiently removed using the fungi previously mentioned, and decreasing the phytotoxicity of the phenols (Carabajal et al. 2016). According to this, the low removal of COD observed in step 3 was generated by short circuits inside the biofilter. Maximum COD removals obtained during the steady stage for each operation step were: 72% (50/70), 72% (40/50), and 22% (50/50) in BF 1; 72% (50/70), 73% (40/60), and 20% (50/50) in BF 2; and 8% (50/70), 66% (40/60), and 18% (50/50) in BF W.

In vitro studies by Benito et al. (1997) obtained COD removals of up to 77% with fungus Trametes versicolor in the presence of vinasses from a brandy factory. Potentini & Rodríguez-Malaver (2006) obtained COD removals of 48%. After a contact time of 32 days, Potentini and Rodríguez-Malaver obtained a COD removal of 48% from wastewater Tequila industry (COD 40,000 mg L⁻¹) with in vitro testing using Phanerochaete chrysosporium. Kumar et al. (1998) used vinasses from fermented sugar molasses, which were previously pre-treated in an anaerobic digester. After the effluent was submitted to white-rot fungi, it achieved removal efficiencies of 90% of COD in in vitro assays, using Trametes versicolor and Phanerochaete chrysosporium. In the present study, the best efficiencies were obtained in two fungi biofilters using Ficus benjamina as support media when working with 40% of vinasses in the influent (5.5 kg COD m⁻³ d⁻¹). COD in the effluents of 2,350 ± 245 mg L⁻¹ for BF 1 and 2,243 ± 348 mg L⁻¹ for BF 2 was achieved.

During the treatment in biofilters systems, the mineralization of organic compounds contained in vinasses was measured using a TOC analysis. The TOC in the influent during steps 1, 2, and 3 was 3, 400 ± 400 mg L⁻¹, 3,900 ± 300 mg L⁻¹ and 4,300 ± 200 mg L⁻¹, respectively. The maximum TOC removal generated in the biofilters occurred during step 2, with a removal of up to 81% (TOC concentration in the effluent of 770 mg L⁻¹). BF 2 inoculated with Trametes versicolor was the biofilter with a higher carbon removal, under a volumetric organic load of 5.5 kg COD m⁻³ d⁻¹. The biofilters inoculated with the two basidiomycete fungi showed a high mineralization of organic compounds compared with the biofilter without fungi (Figure 6). The TOC analysis indicated that several organic compounds contained in vinasses were mineralized through the biofiltration system. When the organic load was increased (step 3), the TOC removal in all biofilters decreased to 22%, similar to the COD.

The lignin and cellulose contents of different species of Ficus wood (F. natalensis, F. thonningii and F. glumosa), similar to those used in this study, have been reported in a range of 54% to 59% and 0.4% to 7.2%, respectively. The water absorption rate and the tensile strength of the different species of Ficus are from 134 to 145 and from 52.2 to 64.3 N mm⁻². According to Mwanja et al. (2016), these physico-chemical and mechanical properties of different species of Ficus indicate that the Ficus species wood is not suitable for the production of paper pulp. However, the high lignin content and high tensile strength in Ficus wood could be used for different applications (e.g. dye and paint production as well as biofilm support material for wastewater treatment).

A statistical analysis of variance (ANOVA) was performed to see if the organic matter removal between three biofilters was statistically significant or not. All the statistical analyses had a confidence interval of 95%. Statistical analysis according to the F-test results showed that there were no significant differences in the COD and TOC removals between BF 1 and BF 2. However, significant differences
were observed between the control biofilter and the biofilters inoculated with basidiomycete fungi, regarding COD and TOC removal.

**Enzymatic activity (laccase)**

Figure 7 shows the enzymatic activities' profiles of *Phanerochaete chrysosporium* and *Trametes versicolor*. The results showed that the laccase activity during the operation of biofilters with a low concentration of vinasses (30%) clearly increased for *Trametes versicolor* but not for *Phanerochaete chrysosporium*, at the pH values of 4.5 and 6.5, respectively. This was unexpected, because in the growth experiments in liquid culture media with 10% and 20% of vinasses, a higher growth of *Phanerochaete chrysosporium* was observed, which was higher than that of *Trametes versicolor*. When the concentration of the vinasses solution was increased to 40% in the BF, the laccase activity for *Phanerochaete chrysosporium* increased at a pH of 4.5 to levels similar to those showed by *Trametes versicolor*. By contrast, at a pH of 6.5, the activity was unstable, increasing and decreasing cyclically. When the concentration of vinasses in the biofilters increased again, the enzymatic activity for *Phanerochaete chrysosporium* increased slightly for both pHs. For *Trametes versicolor*, the laccase activity at a pH of 4.5 was stable, but the activity at a pH of 6.5 increased noticeably. These results suggest that the capacity of fungi to adapt to the conditions of the biofilters is balanced with a higher or lower production of extracellular enzymes. On the other hand, it can be observed that although the removals of organic matter decreased during the third step of the operation in both biofilters, the enzymatic activity of basidiomycete fungi was not affected. The occurrence of laccase inside
the biofilters in every step of the operation indicated that the vinasses and the *Ficus* wood chips served as a carbon source to the basidiomycete fungi. Laccases were thought to play an important role during the biodegradation of lignin contained in woodchips (Madhavi & Lele 2009). Strong (2010) did not observe the production of laccase using *Phanerochaete chrysosporium* at high COD during the treatment of wastewater from an industry wine-related distillery. According to Retes-Pruneda et al. (2014), *Pleurotus ostreatus* and *Trametes tropii* have the capacity to produce laccase and other lignin-modifying enzymes during the bioremediation of Tequila vinasses, which degrade the high content of organic matter that could not be bio-transformed by bacteria.

**Reducing sugars**

The amount of glucose or fructose contained in Tequila vinasses was measured as reducing sugars, which indicates the concentration of any sugar containing a hemiacetal group (Rodríguez-Couto et al. 2002). According to Cabrera-Soto (2011), the concentration of reducing sugars during the biofiltration of Tequila vinasses.
in Tequila vinasses is 2 mg mL⁻¹. However, in this study, maximum concentrations of 0.0065 mg mL⁻¹ in raw vinasses were obtained. When working with 40% and 50% of vinasses, the concentration of reducing sugars was 0.0055 mg mL⁻¹ and 0.0058 mg mL⁻¹, respectively. The reducing sugars were higher in the BFIs inoculated with fungi than in the control biofilter. This observation indicates a balance between the formation of sugars by the action of extracellular fungal enzymes and their use by microorganisms, while in BF W, sugars were less consumed. By increasing the concentration of vinasses to 50%, accumulation of sugars was observed in BF 1 and BF 2, but it was lower in BF W, indicating that the sugars were produced faster than their consumption, mainly in the BFIs with fungi. Perhaps this is due to the increase in the initial concentration of vinasses, or due to the weakening of the rigid structure of the organic material, causing its degradation. In the BF W, a microbial biocenosis was formed that was responsible for the consumption of the reducing sugars (Figure 8).

**CONCLUSIONS**

The solid culture media prepared with vinasses can be used as part of the substrate for the growth of three strains of basidiomycete fungi (*Phanerochaete chrysosporium, Trametes versicolor* and *Pleurotus ostreatus*). The inhibition of fungi growth in the liquid culture media occurred when working with 40% of vinasses for *Trametes versicolor* and 20% for *Pleurotus ostreatus*, while *Phanerochaete chrysosporium* grew in the medium prepared with 100% of vinasses. The *Ficus benjamina* wood chips used as support media and source of organic matter for the biofiltration systems provided a solid and stable support for the growth of *Phanerochaete chrysosporium*, and *Trametes versicolor*. The maximum degradation of contaminants and mineralization of organic compounds contained in Tequila vinasses were favored when the biofilters were operated with a volumetric organic load of 5.5 kg COD m⁻³ d⁻¹. The percentages of COD removals were higher in the biofilters inoculated with *Phanerochaete chrysosporium* and *Trametes versicolor*, showing 72% and 73%, respectively. The production of laccase was observed in the biofilters inoculated with basidiomycete fungi. The generation of laccase was not affected by the increase in concentration of vinasses in the biofilter’s influent.

**ACKNOWLEDGEMENTS**

This research was financially supported by Centre de Recherche Industrielle du Québec (CRIQ) of Canada AC.01.02.4.0.2013.-000092.

**REFERENCES**


