Tequila vinasses treatment with basidiomycetes fungi

Alvillo-Rivera A.*, Garzón-Zúniga M.**, Estrada-Arriaga E.***, Esperanza-Ramírez M.***, Buelna G.****, Díaz-Godínez G.*****

* Universidad Nacional Autónoma de México, campus IMTA. Paseo Cuauhnáhuac 8532, Progreso, Jiutepec, Mor. (E-mail: angjulieta1@hotmail.com)
** Instituto Politécnico Nacional. CIIDIR Unidad Durango. Sigma # 119, 20 de Noviembre II, Durango, Dgo. C.P. 34220 (E-mail: mgarzon@ipn.mx)
***Instituto Mexicano de Tecnología del Agua. Paseo Cuauhnáhuac 8532, Progreso, Jiutepec, Mor. (E-mail: edson_estrada@tlaloc.imta.mx, eramirez@tlaloc.imta.mx)
****Industrial Research Center of Quebec, 333 rue Franquet, Quebec, Canadá (E-mail: Gerardo.Buelna@criq.qc.ca)
***** Centro de investigación en ciencias biológicas, UAT. Km 10.5 autopista Tlaxcala-Texmelucan Ixtacuixtla, Tlaxcala (E-mail: diazgdo@hotmail.com

Abstract
This study focused on the application of a system of aerobic biofiltration (BF) inoculated with two fungus basidiomycete *Phanerochaete chrysosporium* (BF 1) and *Trametes versicolor* (BF 2) for the treatment of Tequila vinasses. Ficus chips were used as means of support. The systems were compared with a biofilter without inoculum (BF W) in order to determine the influence of fungi on degradation of contaminants contained in the vinasses. Were evaluated three different dilutions of vinasse/water (30/40, 40/60 and 50/50). Removals of chemical oxygen demand (COD) obtained during each operation stage for BF 1, 2 and were 72, 72 and 8% (30/40); 72, 73 and 66% (40/60) and 22, 20 and 18% (50/50). Lacasas enzyme activity was generated during the operation of the biofilters, even at the stage of maximum concentration of vinasses in which is removal of the COD fell in all BFs which proves that it was not inhibited by substrate concentration. The effluent of the treatment requires a treatment of polishing since even presents high concentrations of pollutants recalcitrant.

Keywords
Tequila vinasses, basidiomycetes, biofiltration. Lacasas enzyme

INTRODUCTION

The Tequila is a traditional alcoholic beverage of Mexico, its production in the year 2015 was 248.3 million liters (Regulatory Council of Tequila, 2016), during which it generated large quantities of solid waste (bagasse) and liquids (vinasses) (Rodríguez et al., 2013). In the year 2014 were recorded 159 companies (Regulatory Council of Tequila, 2016), of whom 60% are small producers who do not have systems in place for wastewater treatment, while only 50% of large industries is their waste (Iñiguez-Covarrubias and Hernandez, 2010). According to López-López et al. (2010) it is estimated that by 1 L of tequila are generated from 10 to 15 liters of vinasse tequila, which have a very high organic load and a chemical oxygen demand between 20,000 and 40,000 mg L^{-1}, in addition to having an acid pH and color dark brown due to the presence of melanoidinas, which have a chemical structure similar to the structure of the lignin.

There are strategies of management that have been studied for give a treatment to the vinasses of Tequila, among which are: 1) the stabilization ponds, which serve as storage of the vinasses with this is achieved removals of more than 90% of suspended solids, however do not receive any additional treatment and its design does not encompass the impermeability of the ground (Iñiguez-Covarrubias et al., 2010); 2) the flotation with air dissolved, that shows as advantages the removal

of suspended solids 80%, however the removal of dissolved solids and organic matter are not significant (López-López et al., 2010); 3) ozonation, Goyes and Bolaños (2005) obtained organic matter removals of 97% using ozone,while, Siles et al. (2011), obtained 40% of phenol removals with hydraulic retention times (HRT) of 15 min,. However, this type of treatment is expensive and not showed significant reductions in other types of compounds; 4) the mixture of Tequila vinasses with animal feed has been studied by Fernández et al. (2009) showing that when mixed in a ratio of 10% in ruminants, 30% in pigs and 13% in sheep there is no organoleptic differences in their food or differences in digestibility, however there are no studies on the long-term effect; 5) the coagulation-flocculation has shown removals 70% of color and 30% of suspended solids, when working with $Al_2(SO_4)_3$ as a coagulant and a pH of 6, which leads to the increase of the cost in its treatment due to the continuing need for the acquisition of coagulants, as well as chemicals, as are CaO and NaOH for the increase of pH, without forgetting the generation of sludge and its respective treatment (Iñiguez-Covarrubias and Hernandez, 2010; López-López et al. , 2010); 6) the anaerobic digestion in which Jáuregui-Jáuregui et al. (2014) obtained removals of 90% of chemical oxygen demand (COD) with HRT of 4 days, in addition to the production of biogas whose composition was found conformed by 75% of methane. In spite of the removals reached the slow start-up, the influence of temperature on the production of both methane and hydrogen and the existence of dead zones when operating at low speeds are considered their main disadvantages. 7) the hydrogen production by acidogenesis stage has been studied by Dávila-Vázquez et al. (2008), which showed that when working reactors semi-continuous at HRT of 5 d, pH of 5.5 and temperature of 55°C was obtained 1 kg of hydrogen. On the other hand, studies by Buitron and Carvajal (2010) showed that it is possible to encourage the production of hydrogen from the Tequila vinasses, at a HRT short, while increasing the HRT is favors the simultaneous production of hydrogen and methane.

On the other hand, have carried out studies on the use of fungi basidiomycetes for the degradation of recalcitrant organic matter (Robles-González et al., 2012), which are based on the conversion of the lignin to lignocellulose by not specific extracellular enzymes, which to degrade lignin can also degrade similar molecules and generate byproducts that can be exploited as a source of energy and carbon by other agencies. The studies have been carried out at laboratory level in-vitro and with the use of supplemental sources of carbon, as are the glucose in the majority of cases. The main objective of the treatments with basidiomycetes fungi is oxidize organic substances, reducing the COD and biological oxygen demand (BOD), also basidiomycetes have a lower sensitivity that microorganisms to temperature changes, nutrients, aeration (screen and Adholeya, 2007; Spain-Gamboa et al., 2011) and pH, since they can withstand pH's from 2 to 9 units, being the optimum of 5.6 (Carranza-Diaz, 2006).

For the treatment of vinasses few studies have been studied. The basidiomycetes fungi in in-vitro assays, as for example *Trametes pubescens* (Melamane et al., 2007; Strong and Burgees, 2008) and *Phanerochaete chrysosporium* (Potentini and Rodriguez-Malaver, 2006), which demonstrated that the removal efficiency of colour and phenols is not affected by temperature (25 and 39 °C). On the other hand, Ferreira et al. (2011) used *Pleurotus sajor-caju* for the treatment of vinasses from the sugar industry, with which obtained COD removals of 83%, 75% of BOD and 99% of colour and turbidity; also carried out toxicity bioassays with which showed a reduction of it after your treatment.

The biofiltration process on organic bed are presented physical-chemical mechanisms (filtration, adsorption and ion exchange) and biological (biological degradation) that allow the degradation of easily biodegradable organic matter and complex and toxic compounds (Garzon-Zúñiga et al., 2008). This type of treatment has been studied for the degradation of dyes, pig waters,

petrochemical and to treat wastewater from small communities and industries. There are very few studies using biofiltration inoculated with fungi, most are studies are realized in-vitro assays. The biofiltration on organic bed has been used with a fungus as a major component of biological degradation for the removal of azo colorants (Davila-Solano,2004 and Garcia-Sanchez, 2007). The biofiltration on organic bed is characterized by having as the filter medium materials such as straw, pieces of wood, peat, among others, on which can grow basidiomycetes and feed them through the degradation with extracellular enzymes which simultaneously degrade the complex molecules pollutants of interest. Because the mechanisms that present the biofilters for the removal of pollutants and to the characteristics of basidiomycetes for the transformation of compounds difficult to biodegrade in compounds more easily assimilated it is important to study the effect of these two processes as a whole for the removal of contaminants in industrial waste waters of Tequila. The main objective of this study was determine the degradation of contaminants present in the waste water of the Tequila industry through a system of biofiltration inoculated with two strains of basidiomycetes fungi.

MATERIALS AND METHOD

Selection of a strain of basidiomycetes fungi

To select a species of basidiomycetes to inoculate the biofilters were made two tests of growth of three different species (*Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus*). The tests consisted in prepare solid media with different dilutions of vinasse for evaluate the tolerance of fungi in-vitro (Petri) to contaminants in the vinasse. For which it prepared three solid media culture, with Potato-Dextrose-Agar (PDA), in two of them was used vinasse to 10% and 20%. The third was used as a target and was prepared only with distilled water. Each of fungi was sown in the different media prepared and its growth was measured daily. The test was performed in triplicate. The second test of mycelium growth was conducted in a liquid medium, since it is the form in which becomes massively grow the fungus to be able to inoculate the reactors. In this case, the fluid medium was prepared with malt extract (García-Sanchez, 2007) and a solution of vinasse with ascending concentration (20, 40 and 100% of vinasse) and a witness with distilled water. Was planted each of the strains and remained in orbital shaker, at a temperature of 32°C during 15 days, according to the methodology described by Garcia-Sanchez (2007). This test was also performed in triplicate. The fungal strains were donated by the Mycological Herbarium of Morelos (HEMIM), which is located in the Autonomous University of the State of Morelos. The strains were delivered in culture media made from wholemeal wheat flour (HIT) and are classified as described below: *Phanerochaete chrysosporium* (HEMIM 5); *Trametes versicolor* (HEMIM 9) and *Pleurotus ostreatus* (HEMIM 50).

Experimental setup

Three biofilters at laboratory-scale with dimensions of 60 cm in height and 9.3 cm in diameter were worked (Figure 1). The reactors were operated in continuous flow, with a flow of 2 L d^{-1} and flow aeration of 1,000 mL min^{-1}, which were regulated by peristaltic pumps mark Masterflex and a rotameter brand mark Gilmont, respectively. It is operated at ambient temperature with a pH of 3.5 units and without artificial lighting, due to the natural lighting was considered sufficient to carry out the growth of fungi Each of the three reactors packed with 3.5 L of splinters of ficus, same which were characterized in terms of the grain size and porosity according to Garzon-Zúñiga et al. (2003).

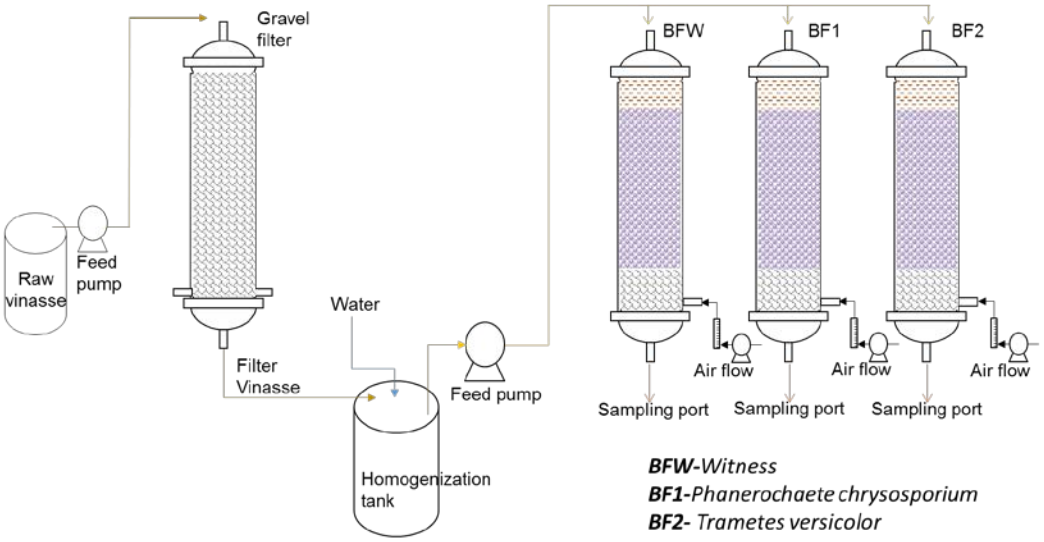

Figure 1. Experimental setup for treatment of Tequila vinasses.

The splinters of ficus moistened to 65% were mixed perfectly with 1 L of two basidiomycetes fungi selected (*Phanerochaete chrysosporium* (BF 1) and *Trametes versicolor* (BF 2)) propagated in liquid medium. Subsequently, the filter material inoculated was introduced to each one of the reactors on a layer of gravel which served as support material, while the third biofilter (BF W) was not inoculated with fungi and served as a witness.

Tequila vinasses

The biofilters were fed with Tequila vinasses at the Jalisco factory (Mexico). Tequila vinasses were collected every 4 months and were stored at 3°C before being used. **Table 1** shows the physico-chemical characteristics of Tequila vinasses.

Table 1. Physico-chemical characteristics of Tequila vinasses

PARAMETER	VINASSES (Value±S.D.)	PARAMETER	VINASSES (Value±S.D.)
pH	3.7±0.55	Total nitrogen (mg L^{-1})	250±188
Temperature (°C)	19.6±1.91	Ammoniacal nitrogen (mg L^{-1})	331±179
Conductivity (mS cm^{-1})	2.01±0.35	Sulphides (mg L^{-1})	15,695±14,000
Aparent color (Pt-Co)	45,400±5,000	Total phosphorus (mg L^{-1})	805±520
Turbidity (NTU)	4,408±4,000	Suspended solids (mg L^{-1})	6,220±3,480
Total COD (mg L^{-1})	25,257±4,262	TOC (mg L^{-1})	12,4350±5,448

Due to the high concentration of suspended solids present in the vinasses, was installed a slow sand filter as pretreatment, which is packed with gravel of three different particle sizes (0.46, 1.00 and 1.19 mm) and are operated at a rate of filtration of 0.1 $m^3 m^{-2} d^{-1}$ (**Figure 1**).

The Tequila vinasses were mixed with water of the key to decrease the concentration of toxic and recalcitrant compounds present in this, because this type of compounds affect directly the biological treatments (**Figure 1**). It began with a low concentration of the Tequila vinasses and it was increasing to know the maximum concentration that the system can handle. The relations vinasse/water used were 30/70 (stage I), 40/60 (stage II) and 50/50 (stage III).

Analytical measurements

The biofilter influent and effluent from each stage were analyzed according to the following parameters: pH with a multiparameter brand ORION, ammoniacal nitrogen, sulphides, color, turbidity, suspended solids (by the techniques of Hach using a spectrophotometer DR 2400), proteins according to the methodology described by Lowry et al. (1951), reducing sugars using the method of Nelson (1944) modified by Somogyi (1952), COD based in Standard Methods for Examination of Water and Wastewater (APHA, 1992) and the determination of activity of extracellular fungal enzymes laccases described by Diaz et al. (2013).

RESULTS

Selection of strains

To grow in-vitro strains in the culture media solids, *Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus* had a growth rate of 0.32, 0.87 and 3.05 in the whitness, while in the middle of culture prepared with 10% of vinasses was of 2.24, 1.28, and 1.05 respectively, showing a positive effect of the vinasse except for *Pleurotus ostreatus*. In the prepared culture media with 20% of vinasses, growth rates were 1.00, 0.67 and 1.73 in the whitness, while in the middle with vinasse were 0.85, 0.39 and 0.19 respectively. In this case the effect was negative in all cases with a higher effect for *Pleurotus ostreatus*. With regard to the growth of the strains in liquid culture medium was able to observe that there is a percentage of vinasse of Tequila that cause inhibition. It was noted that *Phanerochaete chrysosporium* grew in all media (malt extract with distilled water, 20, 40 and 100% vinasse), *Trametes versicolor* only grew up in whitness and in the medium prepared with 20% of vinasse, while *Pleurotus ostreatus* only grew in the whitness (**Figure 2**). With the above mentioned results, the strains chosen for the inoculation of biofilters were *Phanerochaete chrysosporium* and *Trametes versicolor* due to its capacity of adaptation to the contaminants present in the vinasses and its capacity to massive growth in liquid culture medium with vinasses.

Figure 2. In-vitro growth (flask) in liquid medium prepared with 0, 20, 40 y 100% of vinasses for A: *Phanerochaete chrysosporium*, B: *Trametes versicolor*, C: *Pleurotus ostreatus*

Biofiltration perfomance

During the biofilter operations, it was observed that the fungi inoculated in both biofilters grew in acidic conditions as designates Bitton (1994). Couillard (1994) mentions that during the first days of operation in biofilters packed with peat, the pH's descend due to washing of the humic and fulvic acids of organic materials, in addition according to Strong and Burgess (2008) fungi treatment decreases the pH of the treated water, however in this study was not submitted such behavior. **Figure 3** shows a tendency to increase the pH, this may be due in the three biofilters alkalization of the medium due to the loss of organic acids and the formation of humic compounds that have properties buffer (Moreno-Casco and Moral-Herrero, 2007) and in the biofilters inoculated with fungi to the liberation of anions product of the biotransformations of complex molecules of organic matter.

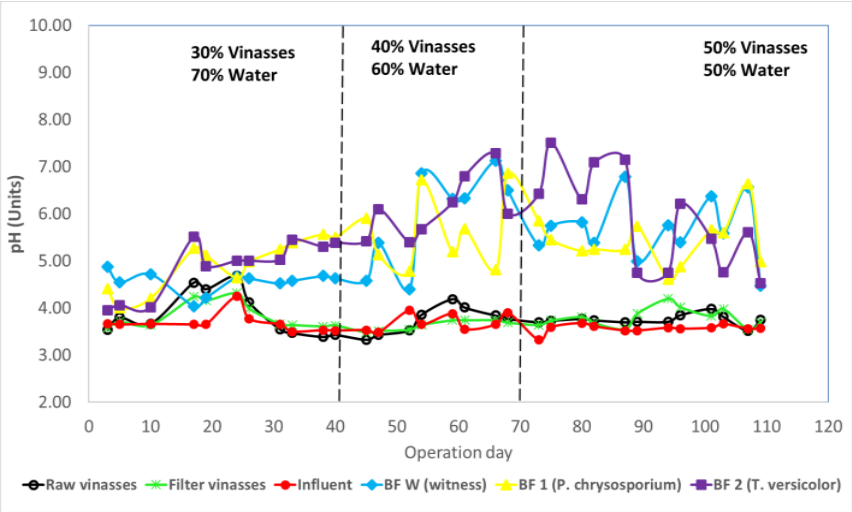

Figure 3. pH of the biofilters during operation

The content of organic matter in raw vinasses was found in the range from 20,000 to 60,000 mg COD L^{-1}, due to the presence of suspended solids. Once that the solids were removed, the COD decreased 34±25% (19,469±3,492 mg L^{-1}). **Figure 4** shows the behavior of the COD in the influent and effluent from each one of the reactors during the different operation stages. It can be observed that during the first 25 days of operation, the three biofilters presented high removal efficiencies, which can relacionated with a biodegradation and adsorption processes, but of the day 25 onwards, in the witness the removal efficiency decreased until disappearing, which could be due to the saturation of the filter material, while in the BF 1 and 2, inoculated with basidiomycetes, removals were increasing as the system was renovating and the mycelium was spreading in the organic material. COD removals obtained from the day 31 to 40, were for the BF W (witness) of 10±6%, for the BF 1 (*Phanerochaete chrysosporium*) of 69±7% and for the BF 2 (*Trametes versicolor*) of 66±12%, which showed that the action of the extracellular enzymes excreted by each one of the fungi permitted an increase between 23 and 26% removal of organic matter present in the Tequila vinasse with regard to the witness (BF W).

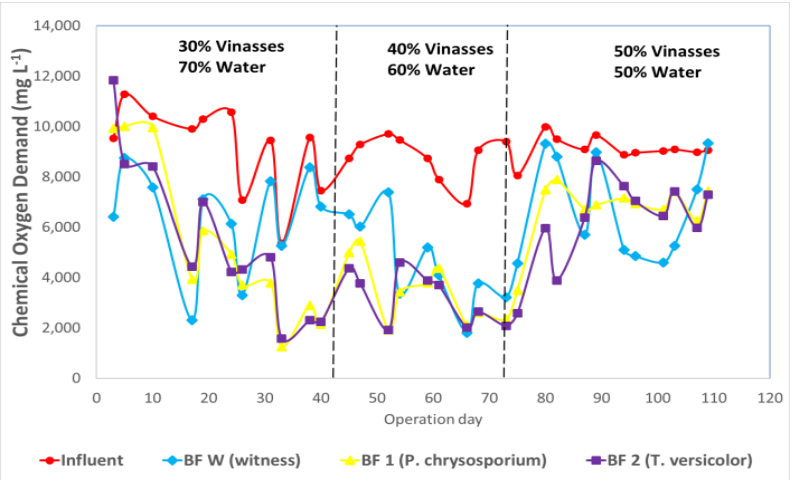

Figure 4. Concentration of organic matter during operation

In the second stage of operation, to change the concentration of the vinasse of entry is presented a period of adjustment in the reactors but taking into account the removals obtained from the day 66 to 73, these were for the BF W 62±11%, for the BF 1 of 65±14% and to the BF 2 of 68±11%. During this stage the organic matter removal obtained for the witness biofilter (BF W), approaching the biofilters inoculated with fungi (BF 1 and 2), which is attributed to the fact that during the first

stage the microorganisms present in the vinasse supplied at the BF W began to adapt and colonize the organic medium (period of stabilization of the reactor), while fungi began its action from the first stage. In the case of the inoculated BFs by increasing the concentration of vinasses, the removal efficiency remained almost the same. But in the third stage, to again increase the concentration of vinasses in the influent, presented a steady trend toward a decrease in the efficiency of COD removal during the first 15 days (adjustment stage) and subsequently began to pick up and stabilize in the BFs inoculated with fungi, but in the witness, the efficiency is completely lost. The averages of mine in the effluent obtained from the day 101 to 109, were 8±12 for BF W, 24±9 (BF 1) and 26±10% (BF 2) which is associated with the increase of contamintantes recalcitrant and difficult to biodegrade both for the microorganisms present in the BF W as for the consortium created between basidiomycetes fungi and microorganisms in the biofilters 1 and 2 by wich the removal was less.

The best COD removals owere obtained during the last days of each stage of operation, these were 72, 72 and 22% in the BF 2 (*Phanerochaete chrysosporium*), 72, 73 and 20%, in the BF 3 (*Trametes versicolor*) and of 8, 66 and 18% in the BF W (witness) respectively. In-vitro studies made by Benito et al. (1997) showed COD removals from 77% when work with *Trametes versicolor* and with a supplement of carbon for vinasses from a brandy destillery, while Potenini and Rodriguez-Malaver (2006), also in-vitro wastewater, treat vinasses from destillery industries and obtained removals 48% of COD with *Phanerochaete chrysosporium* after 32 days of HRT , on the other hand, Kumar et al. (1998) used vinasses from fermented sugar molasses, which in turn were previously digested and achieved removal efficiencies of COD in-vitro of 90% but with a diluición (75%) with *Trametes versicolor* and 73% with *Phanerochaete chrysosporium*. In the present work, the best efficiencies were obtained in the two biofilters inoculated with strains of basidiomycetes fungi when working with a 40% of vinasse in the influent (second stage), achieving effluents with concentrations of 2350 ±245 mg L^{-1} of COD in the BF 2 (*Phanerochaete chrysosporium*) and 2.243 ±348 mg L^{-1} of COD in the BF 3 (*Trametes versicolor*).

Enzymatic activity (laccases)

The results showed that the laccasse activity during the operation of biofilters with a low concentration of vinasses (20%) was clearly increased, for *Trametes versicolor* that for *Phanerochaete chrysosporium*, both at a pH of 4.5 and 6.5. Which is unexpected because in the experiments of growth in liquid culture medium with 10% and 20% of vinasses observed a greater growth of *Phanerochaete chrysosporium* than *Trametes versicolor*. But by increasing the concentration of the solution of vinasses to 40% in the BFs, *Phanerochaete chrysosporium* increased the laccase activity at pH 4.5 to levels similar to those that showed the *Trametes versicolor*, but activity at pH 6.5 behaved in an unstable manner increased and decreasing so cyclical (**Figure 5**). The again increase the concentration of vinasses in the influent of the BFs, the enzymatic activity of *Phanerochaete chrysosporium* increased slightly for both pHs and for *Trametes versicolor* was stable the lacasa activity at pH 4.5, but the activity at pH 6.5 increased markedly. These results suggest that the capacity of adaptation of fungi to the conditions of the biofilters is balanced with a greater or less production of extracellular enzymes.

On the other hand, it can be seen that despite that the removals of organic matter decreased during the third stage (when working with a 50% of vinasse in the influent) in both biofilters, the enzymatic activity continues to be favorable and in a similar way that the work in previous stages. This differs from the one reported by Strong (2010), in whose study was not observed the production of laccases to treat sewage from an industry producing alcohol through the use of *Phanerochaete chrysosporium*. The relationship between the production of laccases and organic matter removal allows assume that by increasing the concentration of the recalcitrant compounds

and difficult of biodegrade to fungi gives them time only to transform them into compounds more easily assimilated, but not of biodegrading. For this reason the COD does not decrease as efficiently as in the other stages.

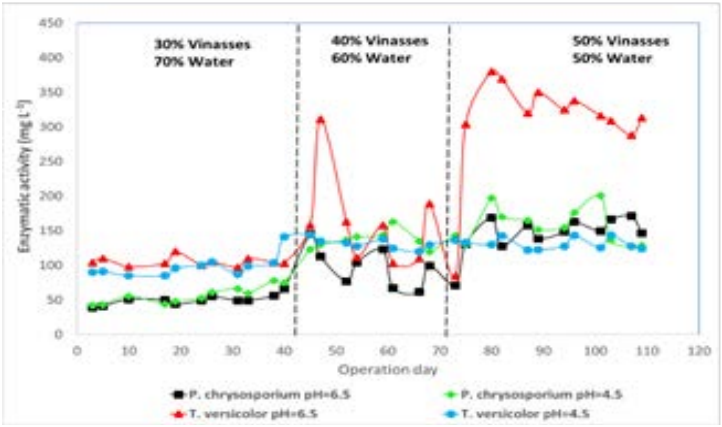

Figure 5. Behavior of the laccases during operation

Reducing sugars

The amount of glucose contained in the Tequila vinasse was measured as reducing sugars, which indicates the concentration of glucose that is being consumed in the middle, which in this case is the vinasse of Tequila (Rodríguez-Couto, 2002). According to what was reported by Cabrera-Soto (2011), the concentration of the reducing sugars in the vinasses of tequila is 2 mg mL^{-1} however in the results obtained were reached maximum concentrations of 0.0060 mg mL^{-1} in raw vinasse. When working with 40% of vinasse in feed water the concentration of reducing sugars was 0.0055 mg mL^{-1} and when working with a 50% of 0.0058 mg mL^{-1}. The reduction of the sugars was greater in the BFs inoculated with fungi (BF 1 and 2) than in the witness biofilter (BF W). This could be due to the fact that there is a balance between the formation of sugars by the action of extracellular fungal enzymes and their use by microorganisms while in the BF W sugars consumed less. To increase the concentration of vinasses to 50% it was observed an accumulation of sugars in the BF 1 and 2 while the BF W is more low, which tells us that the sugars are being produced at a speed greater than that of its consumption mainly in the BFs with fungi, perhaps by the increase in the initial concentration of vinasses or because the rigid structure of the organic material has been weakening and its degradation is more easy to contribute more to the concentration of reducing sugars. While in the BF without inoculum has been forming a microbial biocenosis that is responsible for consuming the reducing sugars (**Figure 6**).

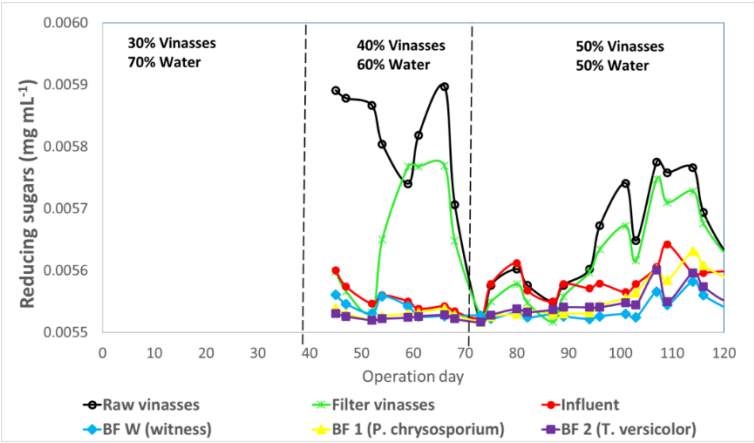

Figure 6. Behavior of the reducing sugars during operation

CONCLUSIONS

The solid culture media prepared with vinasse can be used as part of the substrate for the growth of the three strains of basidiomycetes fungi (*Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus*). The inhibition of the growth of fungi in the liquid culture media occurred when working with 40% of vinasse for *Trametes versicolor* and 20% for the *Pleurotus ostreatus*, while the *Phanerochaete chrysosporium* were grew without inhibition in the middle prepared with 100% of vinasse. The packaging material used, Ficus, provided a solid and stable support and a source of organic matter for the growth of the two strains of basidiomycetes fungi. The degradation of contaminants present in the waste water of the Tequila industry was favored when working with a 40% of vinasse in the feed water. The organic matter removal as COD, was higher in the biofilters inoculated with *Phanerochaete chrysosporium* and *Trametes versicolor* with a 72% and 73% respectively, in comparison with the biofilter witness with a 66%. The generation of laccases is not adversely affected by the increase in concentration of vinasse in the feed water. On the contrary, by increasing the concentration of vinasses the concentration of the laccasse activity also increased. The reducing sugars removal in the biofilters was better in the inoculated with basidiomycetes fungi and the increase in the concentration of vinasses decreased slightly the efficiency of the witness.

REFERENCIAS

APHA, AWWA, WEF, Standar methods for examination of water and wastewater. 1999. Washington, American Public Health Association.

Bitton G. 1994**.** Wastewater microbiology. Wiley-Liss. Estados Unidos. 29-31. ISBN: 0-471-30985-0.

Buitrón G. y Carvajal C. 2010. Biohydrogen production from Tequila vinasses in an anaerobic sequencing batch reactor: Effect of initial substrate concentration, temperature and hydraulic retention time. Bioresource Techonology. 101, 9071-9077.

Cabrera-Soto M. (2011) Tesis maestría: Producción de lacasa de Pleurotus ostreatus utilizando los residuos de Agave tequilana weber como sustrato. Instituto Politécnico Nacional.

Carranza-Díaz Z. 2006. Tesis licenciatura: Selección e identificación de especies de hongos ectomicorrizógenos del estado de Hidalgo más competentes en medio de cultivo sólido. Universidad Autónoma del Estado de Hidalgo.

Consejo Regulador del Tequila (Acceso Enero 2016). Estadísticas. Disponible en: http://www.crt.org.mx/

Couillard D. 1994. The use of peat in wastewater treatment. Water Research. 28,1261-1274.

Dávila-Solano V. (2004). Tesis maestría: Degradación fúngica del colorante diazo: naranja ácido 24. Universidad Nacional Autónoma de México.

Davila-Vazquez G., Arriaga S., Alatriste-Mondragón F., de León-Rodríguez A., Rosales-Colunga L., Razo-Flores E. 2008. Fermentative biohydrogen production: trends and perspectives. Environmental Science Biotechnology. 7, 27–45

Díaz R., Téllez-Téllez M., Sánchez C., Bibbins-Martínez M., Díaz-Godínez G., Soriano-Santos J. 2013. Influence of initial pH of the growing médium on the activity, production and genes expression profiles of laccase of *Pleurotus ostreatus* on submerged fermentations. Electronic Journal of Biotechonoly. 16(4), 1-6. ISSN: 0717-3458.

España-Gamboa E., Mijangos-Cortes J., Barahona-Perez L., Dominguez-Maldonado J., Hernández-Zarate G. y Alzate-Gaviria L. 2011. Vinasses: Characterization and treatments. Waste Management and Research. 0,1-16.

Fernández B., Bodas R., López-Campos O., Andrés S., Mantecón R. y Giráldez F. 2009. Vinasse added to dried sugar beet pulp: Preference rate, voluntary intake and digestive utilization in sheep. Journal of animal science. 87, 2055-2063.

Ferrerira L., Aguilar M., Messias T., Pompeu G. López A., Silvia D., Monteiro R. 2011. Evaluation of sugar-cane vinasse treated with *Pleurotus sajor-caju* utilizing organisms as toxicologial indicators. Ecotoxicology and Environmental Safety. 74 (1), 132-137.

García-Sánchez L. 2007. Tesis maestría: Decoloración fúngica de efluentes industriales con colorantes azo en sistemas de biofiltración con diferentes empaques orgánicos. Universidad Nacional Autónoma de México.

Garzón-Zúñiga M. A., Lessard P., Buelna G. 2003. Determination of the hydraulic residence time in a trickling biofilter filled with organic matter. Environmental Techonology. 24(5), 605-614.

Garzón-Zúñiga M. A., Tomasini-Ortíz A., Moeller-Chávez G., Hornelas-Uribe Y., Buelna G. y Mijaylova-Nacheva P. 2008. Enhanced pathogen removal in on-site biofiltration systems over organic filtration materials. Water Practice and Technology. 3, 1-8.

Goyes A. y Bolaños G. 2005. Un estudio preliminar sobre el tratamiento de vinazas en agua supercrítica. In Proceedings of the XXIII Congreso Colombiano de Ingeniería Química. Manizales, Colombia

Iñiguez-Covarrubias G. y Hernández R. 2010. Estudio para la rehabilitación de una planta de tratamiento de vinazas tequileras mediante un floculante polimérico de poliacrilamida. Revista Internacional de Contaminación Ambiental. ISBN: 0188-4999. México.

Jáuregui-Jáuregui J., Méndez-Acosta H., González-Álvarez V., Snell-Castro R., Alcaraz-González V. y Godon J. 2014). Anerobic treatment of tequila vinasses under seasonal operating conditions: Start-up, normal operation and restart-up after a long stop and starvation period. Bioresource Techonology. 168, 33-40.

López-López A., Dávila-Vázquez G., León-Becerril E., Villegas-García E., Gallardo-Valdez J. 2010. Tequila vinasses: Generation and full scale treatment processes. Reviews in Environmental Science Biotechnology. 9,109-116.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with Folin-phenol reagent. Journal of Biological Chemistry. 1936, 265-275.

Melamame X., Tancliich R., Burgess J. 2007. Anaerbic digestion of fungally pre-treated wine destillery wastewater. African Journal of Biotechnology 6 (17), 1990-1993.

Moreno-Casco J. y Moral-Herrero R. 2007. Compostaje. Madrid: Ediciones Mundi-Prensa. ISBN: 13-978-84-8476-346-8.

Nelson, N. 1944. A photometric adaption on the Somogyi method for the determination of glucose. Journal of Biological Chemistry. 153, 375–380.

Pant D. y Adholeya A. 2007. Biological approaches for treatment of distillery wastewater: A review. Bioresource Technology. 98, 2321-2334.

Potentini M. y Rodríguez-Malaver A. 2006. Vinasse biodegradation by *Phnerochaete chrysosporium*. Journal Environmental Biotechnology. 27 (4), 661-665.

Robles-González V., Galíndez-Mayer J., Rinderknecht-Seijas N., Poggi-Varaldo H. 2012. Treatment of mezcal vinasses: A review. Journal of Biotechnology. 157, 524-548

Rodríguez-Couto S., Gudín M., Lorenzo M., Ángeles-Sanromán M. 2002). Screening of supports and inducers for laccase production by Trametes versicolor in semi-solid-state conditions. Process Biochemistry. 38, 249-255.

Siles J., García-García A., Martín M. 2011. Integrated ozonation and biomethanization treatments of vinasse derived from etanol manufacturing. Journal of Hazardous Materials. 188, 247-253.

Somogyi, M. 1952. Notes on sugar determination. Journal of Biological Chemistry 1952, 195, 19-23.

Strong P., Burgess J. 2008. Fungal and enzimatic remediation of wine lees and five wine-related distillery wastewater. Bioresource Technology. 99 (14), 6134-6142.

Strong, P.J., 2010. Fungal remediation of Amarula distillery wastewater. World Journal of Microbiology and Biotechnology. 26 (1), 133-144.