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Articles

Mezcal vinasses treatment: A review of assessed processes

Tratamiento de vinazas de mezcal: revisión de procesos evaluados

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Abstract

Mezcal is an alcoholic beverage made in Mexico. This industry produces mezcal vinasses, which are complex wastewater with a high concentration of organic matter (35 000 to 122 860 mg COD/l), low pH (3.60-3.94) and recalcitrant compounds such as phenols (478-1 460 mg gallic acid/l) and sulfates (308-947 mg/l), its disposal without treatment to the environment poses a danger to soil and water bodies.

The present study seeks to present and discuss the current technological development in the treatment of mezcal vinasses and to identify the systems with the most significant potential for its application.

The mezcal vinasses treatments with the best COD removal efficiencies are fungal and anaerobic (80 %). Ozonation has potential as a pretreatment, which can remove more than 80 % of phenols. Some hybrid systems can remove > 90 % COD. However, these treatments do not include the concept of "circular economy", so its implementation is seen as an expense that sometimes cannot be afforded by producers. One option includes the revalorization for generating an economic benefit to producers. Biorefinery treatment, in which various types of energy have been obtained, such as biohydrogen, methane, and bioelectricity by applying different bioprocesses sequentially, was identified as the treatment with the best potential.

Keywords: Mezcal vinasses, vinasses biorefinery treatment, agro-industrial wastewater revalorization, mezcal vinasses hybrid treatment, mezcal vinasses biological treatment.

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Resumen

El mezcal es una bebida alcohólica elaborada en México. Esta industria produce vinazas de mezcal, que son aguas residuales complejas con alta concentración de materia orgánica (de 35 000 a 122 860 mg DQO/l), pH bajo (3.60-3.94) y compuestos recalcitrantes como fenoles (478-1460 mg ácido gálico/l) y sulfatos (308-947 mg/l), cuya disposición sin tratamiento al medio ambiente representa un peligro para cuerpos de agua y suelo.

El presente estudio busca presentar y discutir el actual desarrollo tecnológico en el tratamiento de las vinazas de mezcal e identificar los sistemas con mayor potencial para su aplicación.

Los tratamientos de vinaza de mezcal con las mejores eficiencias de remoción de DQO son fúngicos y anaeróbicos (80 %). La ozonización tiene potencial como pretratamiento, eliminando más del 80 % de los fenoles. Algunos sistemas híbridos pueden eliminar más del 90 % DQO. Sin embargo, estos tratamientos no incluyen el concepto de economía circular, por lo que su implementación se ve como un gasto que en ocasiones no puede ser asumido por los productores. Una opción incluye la revalorización para generar un beneficio económico para los productores. El tratamiento de biorrefinería, en el que se han obtenido diversos tipos de energía, como biohidrógeno, metano y bioelectricidad mediante la aplicación secuencial de diferentes bioprocesos, fue identificado como el tratamiento con mayor potencial.

Palabras clave: vinazas de mezcal, tratamiento de biorrefinería de vinazas, revalorización de aguas residuales agroindustriales, tratamiento híbrido de vinazas, tratamiento biológico de vinazas.

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Introduction

Mezcal is an alcoholic beverage made in Mexico, in the States of Oaxaca, Durango, Guerrero, San Luis Potosí, Zacatecas, Guanajuato and Tamaulipas, with a production of 7 145 039 l in 2019 (CRM, 2020). According to the Official Standard to produce mezcal (NOM-070-SCFI-2016), it is defined as "Mexican distilled alcoholic beverage, 100 % made of maguey or agave, obtained by distillation of fermented juices with spontaneous or cultivated microorganisms, extracted from mature heads of agaves or cooked agaves, harvested in the territory comprised by the previously mentioned States". The mezcal production consists of four stages: cooking the agave hearts, grinding the cooked heads, fermenting the juices obtained from the crushed agave fiber, and finally distillation. However, the mezcal production brings with it the generation of highly polluting liquid waste known as "vinasses" (Robles-González, Galíndez-Mayer, Rinderknecht-Seijas, & Poggi-Valardo, 2012). Vinasse is the liquid waste from the entire production process and includes the by-products of fermentation, distillation and washing of the facilities.

Vinasses are wastewater from the production of different liquor, composed of particulate material, yeast, and higher alcohols. One liter of mezcal generates 7 to 11 liters of vinasses wastewater (Acosta & Iñiguez, 2009). The vinasses of different origins present high concentrations of organic matter (5 340-122 860 mg COD/l), acid pH (3.7-6), phenols (478-1 460 mg $_{\text{gallic acid}}$/l) and sulfates (308-947 mg/l), and temperatures above 70 °C (Table 1). These wastes are highly harmful to the environment, so their disposal without treatment in the soil generates the following damages: deteriorates the soil structure, increases phytotoxicity (due to the increase in the concentration of acetic acid, lactic acid, glycerol, and ammoniacal nitrogen); reduces porosity (generating anaerobic zones in the soil) and inhibits the germination capacity of the seeds (due to the presence of phenols) (Gómez-Guerrero, Caballero-Caballero, & Hernández-Gómez, 2014; Robles-González *et al*., 2012). When the vinasses are discharged into water bodies, its high concentration of organic matter (OM) and nutrients cause water eutrophication, decreasing the content of dissolved oxygen (DO) (Retes-Pruneda, Jáuregui-Rincón, & Lozano-Álvarez, 2014; Robles-González *et al*., 2012). In addition, the vinasse's high temperature and acidity create a hostile environment for various organisms (Robles-González *et al*., 2012). It has also been reported that vinasse's brown color and turbidity can affect the penetration of light, reducing the presence of photosynthetic organisms (Mostafa, Mostafa, & Keikhosro, 2019; Retes-Pruneda *et al*., 2014; Robles-González *et al*., 2012; López-López, Davila-Vazquez, León-Becerril, Villegas-García, & Gallardo-Valdez, 2010).

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Table 1. Comparative physicochemical characterization of vinasses of different origin.

Parameter	Tequila[1]	Ethanol[2]	Beer[3]	Wine[4]	Mezcal[5]
pH	3.9	4.83	6.0	3.76	3.78 ± 0.17
Alkalinity (mg $CaCO_3$/l)	510	-	2 450	-	-
Volatile Fatty Acids (mg/l)	-	1 360	-	-	9 615
COD (mg /l)	37 000	65 180	5 340	15 000-16 500	61 169 ± 12 091
BOD_5 (mg /l)	15 076		3 215		29 169 ± 6 513
Kjeldahl Nitrogen (mg NH_3/l)	243	-	-	-	-
Nitrites (mg/l)	-	-	0.37	-	70
Nitrates (mg/l)	-	-	4.30	-	90
Total Solids (mg/l)	16 920	56 230	5 698	-	265 726 ± 454 346
Total suspended solids (mg/l)	338	3 240	1 826	-	41 106 ± 38 226
Volatile Suspended Solids (mg/l)	297	43 330	1 090	-	2 966 ± 3 364
Orthophosphates (mg/l)	-	-	23	-	-
Conductivity (mS/cm)	4.1	-	1.52	-	4.2 ± 1.6

Sources: 1) Ferral-Pérez, Torres-Bustillos, Méndez, Rodríguez-Santillan and Chairez (2016; 2) Barrera, Spanjers, Romero, Rosa and Dewulf (2019); 3) Enitan, Adeyemo, Kumari, Swalaha and Bux (2015); 4) Beltran-de-Heredia, Dominguez and Partido (2018); 5) average values of different characterizations of mezcal vinasses presented in Table 2.

There are few references to works on the characterization of mezcal vinasses. These vinasses have high concentrations of organic matter (BOD_5: 22 500-35 000 mg/l and COD: 35 000-122 860 mg/l), electrical conductivity (2.6-5.81 mS/cm), phenols (58-542 mg/l), total solids (45 000-96 000 mg/l), total suspended solids (8 400-83 130 mg/l), acidity (pH 3.5-3.94) and an elevated temperature of discharge (70 to 90 °C) (Table 2).

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Table 2. Physicochemical characterization of mezcal vinasses from different regions.

Parameter	Mezcal (Oaxaca)[1]	Mezcal (Oaxaca)[2]	Mezcal (Oaxaca)[3]	Mezcal (Durango)[4]
pH	3.6-3.8	-	-	3.94
Temperature (°C)	90	-	-	70
Color (475 nm)	4.6-10.6	-	-	
Volatile Fatty Acids (mg/l)	-	-	-	9 615
COD (mg /l)	56 230-122 860	42 000	35 000-50 000	60 925
BOD_5 (mg /l)	26 500-33 600	25 576	35 000	22 500
Phenols (mg Galic acid/l)	478-542	-	-	58
Nitrites (mg/l)	-	70	-	-
Nitrates (mg/l)	-	91	-	-
Total solids (mg/l)	26 830-94 130	45 860	-	43 084
Total suspended solids (mg/l)	8 400-83 130	-	-	31 788
Volatile Suspended Solids (mg/l)	1 130-6 850	-	-	920
Settling solids (ml/l)	-	260	44 000-106 000	-
Phosphate (mg/l)	290-1 705	-	-	-
Sulfate (mg/l)	308-947	-	-	
Fructose (mg/l)	14-50	-	-	-
Hardness (mg $CaCO_3$/l)	-	312	-	-
Conductivity (mS/cm)	2.6-4.2	-	-	5.81

Sources: 1) Robles-González *et al*. (2012); 2) Retes-Pruneda *et al*. (2014); 3) Gómez-Guerrero *et al*. (2014); 4) mezcal from Durango.

Unfortunately, most of the mezcal producing industries —and in Durango state all of them— do not have a treatment system for the vinasses they generate. This is due to the ignorance on the part of the producers of the harmful effects of these wastes towards the environment and due to the little development of technological options and the high costs of treatment systems used in other industries, like tequila industry. Therefore, the present work aims to present a compilation of the published studies regarding the treatment of the mezcal vinasses, to discusses the removal of contaminant efficiencies reported and to emphasize which systems are more promising and should be studied further to allow the development of technological options for this industry.

The mezcal industry has few technical studies regarding the treatment of its wastes. On the other hand, mezcal vinasses share similarities with tequila vinasses. The vinasses generated by both present high acidity (pH < 4), discharge temperatures higher than 50 °C and although they have high concentrations of OM, the tequila vinasses reach lower concentrations (100 000 mg COD/l) compared to the mezcal vinasses (122 860 mg COD/l). However, tequila vinasses have a lower content of volatile fatty acids (VFAs) than mezcal vinasses, associated with a lower concentration of organic matter, probably because tequila is produced industrially and mezcal is produced mainly by hand (Table 3). The tequila industry has had greater commercial success and expansion due to its industrialized production that allows it to generate significantly greater amounts of tequila (173.42 million liters per year) than mezcal (7.14 million liters per year), as well as attracting more academic interest, this is reflected in different characterizations and studies dedicated to the treatment of its vinasses (CRM, 2020).

Table 3. Comparative characterization of tequila vinasses with mezcal vinasses.

Parameter	Tequila[1]	Tequila[2]	Tequila[3]	Mezcal[4]
pH	3.35	3.4–4.5	3.6 ± 0.1	3.6-3.8
Temperature (°C)	50.4	-	-	90
Color (475 nm)	-	-	-	4.6-10.6
Volatile Fatty Acids (mg/l)	2 500-3 400	-	-	-
COD (mg/l)	28 000-50 000	60 000-100 000	63 100 ± 6 500	56 230-122 860
BOD_5 (mg/l)	13 000-24 000	35 000-60 000	29 200 ± 7 900	26 500-33 600
Phenols (mg Gallic acids/l)	-	-	1 460.6 ± 128.2	478-542
Total solids (mg/l)	-	25 000-50 000	43 800 ± 3 600	26 830-94 7130
Total suspended solids (mg/l)	-	2 000-8 000	-	8 400-83 130
Volatile Suspended Solids (mg/l)	-	1 990-7 500	-	1 130-6 850
Phosphate (mg/l)	-	-	-	290-1 705
Sulfate (mg/l)	-	-	-	308-947
Fructose (mg/l)	-	-	-	14-50
Conductivity (mS/cm)	-	-	-	2.6-4.2

Sources: 1) García-Depraect and León-Becerril (2018); 2) Robles-González *et al*. (2012); 3) Méndez-Acosta, Snell-Castro, Alcaraz-González, González-Álvarez and Pelayo-Ortiz (2010); 4) López-López, Davila-Vazquez, León-Becerril, Villegas-García and Gallardo-Valdez (2010).

The mezcal industry, as previously mentioned, has only begun to attract greater interest in the last decade, which may explain the lack of studies focused on the treatment of its residues. The studies are mostly approached locally at the laboratory level (Robles-González, Poggi-Valardo, Galíndez-Mayer, & Ruíz-Ordaz, 2017; Retes-Pruneda *et al*., 2014; Villalobos-Castillejos, Robles-González, & Poggi-Valardo, 2009). These studies constitute the first efforts to provide a solution to the management of this waste. Among the technologies evaluated are physicochemical and biological treatments and both accoupled (Robles-González *et al*., 2017; Retes-Pruneda *et al*., 2014; Villalobos-Castillejos *et al*., 2009). The objective of the present is to provide an overview of the current situation of the technological development of mezcal vinasses treatment and to identify sustainable treatment options that the producers could accept to implement on a real scale.

Information search

The search for information on the different treatments of mezcal vinasses was carried out in various databases using as keywords: mezcal vinasses, mezcal wastewater, mezcal waste treatment, mezcal vinasses treatment, mezcal vinasses revalorization and mezcal industry. However, the information found was scarce, so after presenting the technologies focused on the management of mezcal vinasses found, the search was extended to the treatment of a similar residue "tequila vinasses" to compare it and take some examples of the treatment of tequila vinasses that can be applied to the treatment of mezcal vinasses.

Treatment of mezcal vinasses

As previously mentioned, the mezcal industry has attracted less academic interest compared to other alcoholic beverages, such as tequila. Due to this, when carrying out an exhaustive bibliographic search in different databases, it was found that the works focused on the treatment of their vinasses are scarce (Table 4). These works are reviewed, compared, and discussed in the following sections.

Table 4. Available research focused on the treatment of mezcal vinasses.

Treatment	Type of treatment	Initial concentration of organic matter (mg COD/l)	Hydraulic retention time	COD removal efficiency (%)	Author
Fungal	Biological	31 500	21 d	67.9 ± 0.4	Retes-Pruneda *et al*. (2014)
Activated sludge	Biological	24 263	13 d	69.6 ± 1.3	Robles-González *et al*. (2017)
Anaerobic treatment	Biological	952	25 d	81 ± 1	Villalobos-Castillejos *et al*. (2009)
Flocculation with alginates	Physiochemical	42 000	5 min	39	Retes-Pruneda *et al*. (2014)
Ozonation	Physiochemical	42 000	120 min	10.8 ± 0.2	Robles-González *et al*. (2017)
Flocculation with fungal treatment	Hybrid	31 500	25 d	92.5	Retes-Pruneda *et al*. (2014)
Ozonation with activated sludge	Hybrid	42 000	13 d	85	Robles-González *et al*. (2017)

Biological treatment

Activated sludge

An activated sludge process takes advantage of microorganisms under aerobic conditions for the degradation of OM in wastewater (Varila-Quiroga & Díaz-López, 2008). This type of biological process for the treatment of mezcal vinasses has been evaluated only at the laboratory level, in a 1 l capacity system with an operating volume (Vop) of 800 ml, with an initial concentration of OM of 24 263 mg COD/l, which was aerated with a flow rate of 3 l/min and operated at 23 °C, with a pH of 7 and a hydraulic retention time (HRT) of 13 days, reaching an average COD removal efficiency of 69.6 ± 1.3 % (Robles-González *et al*., 2017).

Because aerobic wastewater treatments require the inclusion of equipment such as aerators, which can raise the operating cost, these processes are recommended for the treatment of influents with OM concentrations lower than 1 000 mg COD/l, such as municipal wastewater (290 mg COD/l), reaching COD removal efficiencies close to 90 % (Varila-Quiroga & Díaz-López, 2008), while for influents with OM concentrations higher than 1 000 mg COD/l, it is preferable anaerobic treatments, which operate without aeration equipment (Acharya, Mohana, & Madamwar, 2008).

Due to the high OM concentration of mezcal vinasses, the activated sludge treatment proposed by Robles-González *et al*. (2017) may not be the most suitable for decontaminating this waste compared to other treatments. More efficient technology is anaerobic digestion that can achieve COD removal efficiencies greater than 85 % for tequila vinasses without the need of aeration equipment, for example for tequila vinasses (Arreola-Vargas, Snell-Castro, Rojo-Liera, González-Álvarez, & Méndez-Acosta, 2017; Arreola-Vargas *et al*., 2016).

Fungal treatment

Ligninolytic fungi (LF) are a group of heterotrophic organisms commonly found on tree bark; these LF degrade the cellulose, hemicellulose, and lignin through lignocellulosic enzymes (Retes-Pruneda *et al*., 2014). This non-specific extracellular ligninolytic system allows this type of fungi to degrade through peroxidases and oxidases, a wide variety of organic components (phenolic and non-phenolic compounds), such as those present in vinasse. Some fungi previously used to treat pollutants such as pesticides, ammunition residues, polycyclic aromatic hydrocarbons, colorants, and wood preservatives belong to the genera *Bjerkandera, Phaanerochaete, Pleurotus* and *Trametes* (Asif, Hai, Hou, Price, & Nghiem, 2017). This type of treatment has been studied with vinasses of different origin, such as those generated in the production of alcohol from sugar cane or the drink "Crema de amarulla", removing 55 to 74 % of organic matter (COD), respectively, reaching a color removal of up to 98 % (Strong *et al*., 2010).

Retes-Pruneda *et al*. (2014) studied in the laboratory the ability of two species of ligninolytic fungi, *Trametes troggi* and *Pleurotus ostreatus*, to remove OM in mezcal vinasses, using as a reactor a 250 ml flask with an operational volume of 100 ml, pH 5.5, 28 ± 2 °C, with 120 rpm orbital

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stirring, a 21-day HRT and with a mixing of 75 % mezcal vinasses diluted with 25 % of distilled water (COD: 31 500 mg/l; BOD_5: 19 182 mg/l). Under these operating conditions, *T. troggi* reached a BOD_5 average removal of 62.6 ± 4.6 % and 67.9 ± 0.4 % of COD. *P. ostreatus* reached an average removal of 61.4 ± 4.4 % of BOD_5 and 67.3 ± 1.01 % of COD. The authors of this research report that exposing ligninolytic fungi to high concentrations of mezcal vinasses, the activation of their enzymatic system was favored. However, when using vinasses undiluted, the fungi could not to develop in this medium or remove the OM content in the vinasse, but by reducing its concentration to 75 % the LF was not inhibited. In the work of Retes-Pruneda *et al*. (2014) a reactor was inoculated with a concentration of 100/g/l of ligninolytic fungi which grew inside a container with mezcal vinasses. However, as the fungi developed, they occupied more space within the flask, absorbing part of the vinasses, which could cause the decrease observed in the concentration of COD at the end of the treatment cycle. So, another aspect that can be improved in this treatment is how LF is used to induce a biodegradation process instead to only an absorption process. In this regard, for example, Garzón-Zúñiga, Sandoval-Villasana and Moeller-Chávez (2011) used reactors with the fungi immobilized on organic support (wood chips) on which an active fungal film performing biotransformation of complex compounds was formed, and the reactor was operated as a continuous treatment. In another work, Garzón-Zúñiga *et al*. (2018) evaluated the capacity of two species of fungi, *P. chrysosporium* and *T. versicolor,* for the removal of organic matter in tequila vinasses using laboratory-scale aerated biofilters of 3.5 l of useful volume, packed with *Ficus benjamina* wood chips operated at a continuous flow of 2 l/d of 60 % tequila vinasses

(5.5 kg COD/l d). A control reactor was also operated in the absence of fungi. When fungal reactors were operated under these conditions the efficiencies increase in 10 %.

The treatment of mezcal vinasses with ligninolytic fungi in a reactor with suspended biomass, as proposed by Retes-Pruneda *et al*. (2014), presents encouraging removal efficiencies (62 %). However, this required a prolonged HRT (21 d) and a high fungal biomass concentration (100 g/l). These conditions can be challenging to achieve in a traditional factory of mezcal where 9 600-24 000 $l_{vinasses}$/month can be generated, considering a production of 1 200-1 600 l_{mezcal}/month and production ratio of 8 to 15 l of vinasses for each liter of mezcal (Secretaría de Turismo de Durango, 2019; Robles-González *et al*., 2012). On the other hand, the fungal technology evaluated by Garzón-Zúñiga *et al*. (2018), which uses reactors packed with organic support, where the formation of fungal biomass (*Phanerochaete chrysosporium* and *Trametes versicolor*) that reaches a removal of 72.5 ± 0.5 % of the COD in the treatment of tequila vinasses, presents as advantages that it can operate continuously higher flow rates without the need for fungal re-inoculation. However, further studies are still required to scale up the system to a pilot plant and industrial scale.

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Anaerobic treatment

Anaerobic digestion (AD) is a biological process in which organic matter is assimilated and transformed in by-products for many different microorganisms in the absence of oxygen that gives rise to a mixture of gases usually known as "biogas" (40-70 % methane, and 60-30 % carbon dioxide) with an aqueous suspension containing microorganisms (suspended biomass) responsible for the degradation of organic matter and the treatment of the liquid effluent. The microorganisms present in these processes are generally: acidogenic (or fermentative) bacteria, acetogenic bacteria and methanogenic archaea, and sulfate-reducing bacteria (Moraes, Zaiat, & Bonomi, 2015).

Villalobos-Castillejos *et al*. (2009) studied an anaerobic treatment for the removal of OM in mezcal vinasses at the laboratory level, in a 1 l flask with an operating volume (OpV) of 400 and an HRT of 25 days, using as biomass inoculum from an activated sludge system, with a concentration of 952 mg COD/l (90 % diluted vinasses). Under these conditions, an average OM removal efficiency of 81 ± 1 % was achieved. However, by increasing the vinasses concentration to 40 % (2 272 mg COD/l), only 9 % of the COD was removed. This poor removal efficiency is probably due to the lack of an acclimatization strategy that would allow the biomass to adapt to take advantage of this substrate without being inhibited.

On the other hand, other works have been able to achieve removal efficiencies close to 70 %, without the need to apply such high dilutions after biomass acclimatization. For example, Alvillo-Rivera, Garzón-

Zúñiga, Estrada-Arriaga, Buelna and Bahena-Bahena (2015) achieved average COD removal efficiencies of 71 ± 1 % in tequila vinasses diluted 40 % (12 638 ± 3 805 mg COD/l), using sequential up-flow batch reactors (UASB) with fluidized bed, with two types of inoculums (activated sludge and activated carbon activated sludge), with an HRT of 7.16 d, 24 °C and neutral pH.

It is known that when only a biological treatment is used to purify wastewater with a complex and recalcitrant composition, medium or low removal efficiencies of these pollutants are to be expected. So, it could be advisable to apply a pretreatment to reduce the content of toxic compounds that inhibit microbial activity in these processes (Robles-González *et al*., 2017).

One of the advantages that anaerobic digestion offers is the ability to generate biogas with a methane content ranging between 40 and 70 %, which is a product of energy interest, from substrates with high concentrations of organic matter (> 10 000 mg COD/l) (Robles-González *et al*., 2017; Buitrón, Prato-Garcia, & Zhang, 2014a). Some studies on methane production from tequila vinasses as Toledo-Cervantes, Guevara-Santos, Arreola-Vargas, Snell-Castro and Méndez-Acosta (2018), produced biogas with 82.6 ± 1.2 % methane, with a COD removal efficiency of 95.2 ± 1.2 %, using an 8 l packed bed reactor, with PVC pipes as support material, operating at a pH of 7.7 and 35 °C. López-López, León-Becerril, Rosales-Contreras and Villegas-García (2015) produced biogas composed of 60-65 % methane with a yield of 335 ml CH_4/g COD in a UASB reactor with pH 7 at 35 °C with a COD removal greater than 75 %; Arreola-Vargas *et al*. (2017) reached yields of 280 ml

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CH_4/g COD, together with a COD removal of 89.2 ± 0.52 % in a packed bed reactor, with ½ in PVC tubes as support material on a pilot scale. One recent research (Gómez-Guerrero *et al*., 2018) produced biogas with a maximum methane concentration of 35 %, with a yield of 3 Nml/gSV$_{added}$ in an anaerobic co-digestion of mezcal vinasses, and bagasse of *Agave angustifolia* Haw (in a proportion 12/88 V/V), in a 1 000 ml reactor with an operational volume of 700 ml. However, by increasing the vinasses concentration in the reactor, the yield of methane production decreased. Again, it should be noted that the biomass used was not previously acclimatized to the mezcal vinasses.

Physicochemical treatment

Until now, only two types of physicochemical processes have been reported for the treatment of mezcal vinasses: flocculation with alginates and ozonation.

Flocculation with alginates

Alginates are organic polymers that can be obtained from various species of marine algae of the genus *Phaeophyceae* and bacteria of the genus *Pseudomonas* and *Azotobacter*, which are used as flocculants in the pharmaceutical, cosmetic industry and for the preparation of dental gels due to their capacity to retain metals and immobilize cells and biomolecules in liquid media. Retes-Pruneda *et al*. (2014) evaluated sodium alginate and flocculant for the treatment of mezcal vinasses at the laboratory level. Using a flask with a working volume of 100 ml, he treated crude mixture vinasses with initial concentrations of COD and BOD_5 of 42 000 and 25 576 mg/l, respectively, and applying 3 g alginates/l, pH 9, 30 °C and HRT of 5 minutes, obtaining an organic matter removal efficiency of 21.6 % (BOD_5) and 39 % (COD). Among the advantages of the use of flocculants as wastewater pretreatment, compared to other systems such as sedimentation or screening, are the reduction of HRT, as well as the generation of a more homogeneous effluent (Retes-Pruneda *et al*., 2014).

Other studies support the use of alginates in the wastewater treatment other than vinasses and with lower concentrations of organic matter. For example, Wu, Wang, Gao, Zhao and Yue (2012), achieved a COD removal efficiency of 80.1 %, from synthetic wastewater (2 860 mg COD/l) treated with 1 000 mg/l of sodium alginate and 1 000 mg/l of aluminum sulfate. However, the use of alginates as the only treatment is not very efficient, compared to anaerobic systems, but as a pretreatment in mezcal vinasses it represents an improvement greater than 20 % in the total OM removal efficiency (Retes-Pruneda *et al*., 2014).

Ozonation

The use of ozone in water treatment is due to its oxidizing capacity, which allows breaking the structure of complex biomolecules into simpler structures, which increases their biodegradability and allows the removal of recalcitrant compounds present in mezcal and tequila vinasses such as melanoids and polyphenols (Robles-González *et al*., 2017; Muñoz & Orta, 2013). This technology has been studied at the laboratory level in 3 l reactors with a useful volume of 500 ml, to treat mezcal vinasses with an initial concentration of 42 000 mg COD/l, neutral pH, ozone flow of 50 ml/s, mass flow of 0.5 g ozone/h, and HRT of 120 minutes. Under these conditions, the following removal efficiencies were obtained: 10.8 ± 0.2 % of organic matter; 83.8 ± 0.6 % of phenols, and 31.4 ± 1 % of aromatic compounds (Robles-González *et al*., 2017). Ozonation in this type of vinasses presented a relatively low OM removal efficiency (10.8 ± 0.2 %) compared than that achieved by biological methods (close to 80 %). However, the most remarkable about this type of treatment is the ability to remove 83.8 ± 0.6 % of phenols, compounds that in high concentrations can inhibit microbial activity. So, the ozonation potential is as a pretreatment to a biological process.

Hybrid treatment systems

An alternative to improve the efficiency of pollutant removal in complex wastewater is the combination of treatment processes, such that the effluent from a first process serves as a substrate for a later stage. So far, the reported studies focused on using different combined technologies to treat mezcal vinasses are scarce. Some process that has been evaluated are Flocculants in combination with a fungal treatment and ozonation in combination with an activated sludge process. These studies are the first efforts to identify combined treatment options and have been only performed on a laboratory scale.

Flocculation with fungal treatment

Retes-Pruneda *et al*. (2014) evaluated both treatment processes separately and subsequently tested a combined system with the best conditions found in each process, as follows: For flocculation with sodium alginate, the raw vinasses were treated with 3 g alginate/l, pH 9, 30 °C, HRT for 5 minutes, followed by a biological treatment inoculated with 100 g/l of ligninolytic fungi (*T. troggi* and *P. ostreatus*), mezcal vinasses solution 75 % (pretreated by flocculation), pH 5.5, 28 ± 2 °C, stirred at 120 rpm, with HRT for 21 days. In this study, an increase in COD removal of 26 % was observed when including the flocculant pretreatment in the process with both species of fungi. With *T. troggi*, organic matter removal efficiencies of 82.8 % (BOD_5) and 92.5 % (COD) were obtained, and with *P. ostreatus* 80 % (BOD_5), and 91.4 % (COD). However, it is important to mention that the sodium alginate used in this work is a commercial reagent with a cost of $15.01 USD/kg (Dental Packs, 2020), whose use on a large scale may not be affordable. Another drawback of these flocculants is the absence of treatment information for the generated sludge.

Ozonation with activated sludge

Robles-González *et al*. (2017) separately evaluated the efficiency in the removal of OM from a physicochemical treatment by ozonation and biological treatment of activated sludge in mezcal vinasses (42 000 mg COD/l). A 3 l reactor, 500 ml OpV, and HRT of 120 min was used in the ozone process, achieving a COD removal of 10.8 %. In the activated sludge process under aerobic conditions, using reactor flask 1 l, with an operational volume of 800 ml, at 23 °C, pH 7, and HRT of 13 days, and efficiency of 69 % COD removal was obtained. When evaluating these technologies in a combined system to treat mezcal vinasses, 85 % of the COD, 83 % of phenols, and 32 % of aromatic compounds were removed.

The COD removal efficiency achieved in this treatment train is like that achieved by other technologies such as anaerobic digestion (Villalobos-Castillejos *et al*., 2009), but with the advantage of removing toxic and recalcitrant compounds. However, the use of ozone for treat mezcal vinasses has only been evaluated in the laboratory (Robles-González *et al*., 2017), so it is necessary to study the efficiency and economic feasibility of this system on a larger scale. It is also necessary to consider other complementary processes that can be incorporated into this treatment train, such as anaerobic digestion and fungal treatments, because a limiting factor to increase the concentration of vinasses in the influent is the concentration of recalcitrant compounds (Villalobos-Castillejos *et al*., 2009; Retes-Pruneda *et al*., 2014).

Current development status of mezcal vinasses treatments

Currently, the development of treatment systems for mezcal vinasses has advanced and has diversified compared to the situation 8 to 10 years ago, when most of the treatment options were still theoretical because they only had been tested, with tequila vinasses or other types of vinasses. As has been presented in this document, different studies have been conducted with biological, physical, and chemical processes and combined processes. However, despite the notable progress, these studies can be considered the first efforts in the developing of applicable treatment systems to the mezcal industry since many of them have been carried out on a flask scale. So, much more research remains to be done, including the validation of technologies in pilot and full-scale. The development of treatment systems for tequila vinasses and other vinasses is an important reference that can provide valuable information on where future efforts to treat mezcal vinasses could be directed.

Treatment of tequila vinasses

As previously mentioned, tequila vinasses share important similarities with mezcal vinasses. The most studied technology for treating these vinasses is anaerobic digestion (Toledo-Cervantes *et al*., 2018; Arreola-Vargas *et al*., 2016; Méndez-Acosta *et al*., 2010).

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As seen in Table 5, OM removal efficiencies higher than 95 % can be achieved from tequila vinasses (30 000-33 000 mg COD/l), in continuous stirred tank reactors (CSTR) and packed bed reactors (PBR) operating at 35 °C, with a pH between 7.4-7.7 and HRT of 5-6 d (Toledo-Cervantes *et al*., 2018; Méndez-Acosta *et al*., 2010). However, at the pilot plant level there is the problem of maintaining the temperature at 35 °C, for this reason, Arreola Vargas *et al*. (2017) operated at room temperature (25 °C) a PBR, with an initial OM concentration of 25 000 mg COD/l, reaching removal efficiencies close to 90 % (Arreola-Vargas *et al*., 2017). Other operational problems that the treatment of these residues may present is the presence of inhibitory compounds, such as phenols, lactic acid, acetic acid, formaldehyde and furfual, which can inhibit more than 60 % of microbial activity in various biological processes, despite acclimatization, but even do these systems can remove until 70 to 80 % of COD (Kumar & Ram, 2021; Weber, Estrada-Maya, Sandoval-Moctezuma, & Martínez-Cienfuegos, 2019; Palomo-Briones *et al*., 2018). Anaerobic systems have the potential to be used as a highly efficient treatment in mezcal vinasses. However, studies using this substrate are still needed.

Table 5. Anaerobic treatment of tequila vinasses.

Reactor	**PBR[1]**	**PBR[2]**	**UASB[3]**	**CSTR[4]**	**AnSBR**
OpV	445 l	4 l	1.2	5 l	5.1
Packing	PVC de ½ in	PVC Cloisonyle©	-	-	-
HRT (d)	5	6	1.5	5	7
Temperature (°C)	25	35	35	35 ± 1	32
pH	5.5	7.7	6.5-6.8	7.4	7
COD $_{initial}$ (mg/l)	25 000	30 000	7 500	33 000	8 100±100
COD $_{Removal}$ (%)	89.2±0.52	95.2±1.2	70	95	85.4±0.1

Sources: 1) Arreola Vargas *et al*. (2017); 2) Toledo-Cervantes *et al*. (2018); 3) Marino-Marmolejo *et al*. (2015); 4) Méndez-Acosta *et al*. (2010); 5) Arreola-Vargas *et al*. (2016).

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Revalorization of vinasses

A trend of interest in the treatment of organic waste is its revalorization to obtain energy products. From tequila vinasses (7 500-33 000 mg COD/l), it is possible to generate biogas with high methane content (60-72 %) that can be used as fuel (Arreola-Vargas *et al*., 2016; Marino-Marmolejo *et al*., 2015; Méndez-Acosta *et al*., 2010). However, one way to obtain more than one energy compound of interest is using "treatment trains" such as, for example, a dark fermentation (DF) stage for the generation of biohydrogen followed by a methanogenic process to produce methane. In this regard, Buitrón, Kumar, Martinez-Arce and Moreno (2014b) obtained the production of 57.4 ± 4.0 ml H_2/l-h in an SBR (dark fermentation) and a production of 11.7 ± 0.7 ml CH_4/l-h in a UASB (methanogenic process).

The DF process is characterized by a low COD removal efficiency (5.78-13.3 %). However, it allows obtaining biogas rich in biohydrogen with a yield that ranges from 714 to 833 mg COD/l H_2 (Guo, Yang, Xiang, Wang, & Ren, 2010; Wang & Zhao, 2009). This technology has been studied in tequila vinasses (57 000 mg COD/l), generating biogas composed of 70 ± 5 % hydrogen, with a production rate of 72 ± 9 ml H_2/l-h, removing up to 7.32 % of the COD (García-Depraect & León-Becerril, 2018). The OM that remains after DF, which is in the form of by-products such as alcohols and VFAs, can be used as a substrate in other bioprocesses, such as anaerobic digestion (Guo *et al*., 2010) or microbial fuel cells (Wang & Zhao, 2009) for the generation of other energetic products.

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Microbial fuel cells (MFC) are systems where it is possible to use the electrons released by the degradation of organic substrates in the presence of microorganisms for the generation of electricity (Li, Sheng, Liu, & Yu, 2011). The MFC have not been evaluated with mezcal vinasses, but with other types of vinasses, for example, those generated in the production of sugarcane alcohol (800 mg COD/l), removing 97 % of the OM and generating up to 554 mV (Chi-Wen, Chih-Hung, Wan-Ting, & Shen-Long, 2015). Other works such as Ottoni *et al*. (2018) achieved OM removal efficiencies of 54.5 % from this same type of vinasses with an initial concentration of 24 000 mg COD/l producing up to 800 mW/m^2. Therefore, it is important to evaluate the application of MFC in the treatment of mezcal vinasses.

However, a concept that has not been applied to the treatment of tequila or mezcal vinasses is that of "biorefinery". In these processes, a series of treatments are coupled in such a way that the effluent from one stage serves as a substrate for the next, generating products of interest and minimizing the generation of waste (Schievano *et al*., 2016; Demirbas, 2009). This type of process allows the obtaining of various energy compounds of interest from complex diversified residues, using different microbial populations that allow different metabolites coming from a single original substrate.

Currently, at the National Polytechnic Institute (IPN) of Mexico, a study is being carried out that seeks biofuels from mezcal vinasses by the focus of biorefinery that includes two bioprocesses: a dark fermentation followed by a MFC process. Preliminary results show that in the dark fermentation of mezcal vinasses operated with an initial concentration of

18 367 ± 1 200 mg COD/l, an OM removal efficiency of 20 ± 3 % have been obtained with a biogas production of 1 041 ± 97 ml/l-reactor, composed by both biohydrogen and methane with a proportion varying between 79 and 5 % of H_2, and between 11 and 28 % of CH_4 (Díaz-Barajas, Garzón-Zúñiga, Moreno-Andrade, Vigueras-Cortés, & Barragán-Huerta, 2021). The effluent of the DF reactor with a concentration of 3 765±519 mg VFA/l was used for feeding the MFC, have been generated up to 29.55 ± 20 mV with an OM removal efficiency of 76 ± 9 % (Díaz-Barajas, Garzón-Zúñiga, Moreno-Andrade, Vigueras-Cortés, & Barragán-Huerta, 2019). These results suggest that this biorefinery treatment train can achieve significant OM removal efficiencies, and the generation of three sustainable energy sources. Therefore, it is needed to carry out more research work with the biorefinery approach to obtain various compounds of high energy value that can benefit mezcal producers, reducing the discharge of highly polluting effluents into the environment.

Future perspectives

Regarding the mezcal vinasses treatments published, activated sludge is not a good option as a single treatment since it only achieves 69 % COD removal and has an HRT of 13 days, which implies enormous energy consumption and high operating costs. The fungal tests have presented good results for removal efficiency of COD (74 %) and color (98 %). However, these are discontinuous tests where the main mechanism is adsorption. Therefore, much more research is required, such as that carried out by Garzón-Zúñiga *et al*. (2018), where fungi immobilized in an organic packaging medium are used to allow the biotransformation and biodegradation of tequila vinasses. This is achieved by the action of non-specific extracellular enzymes of basidiomycetes fungi. Regarding the anaerobic digestion work of mezcal vinasses, a good COD removal efficiency was achieved (81 %) but applying a high dilution. However, in works with tequila vinasses better efficiencies have been obtained and applying initial concentrations up to 10 times higher (27 g COD/l). Therefore, anaerobic digestion studies with mezcal vinasses should also focus on being able to work with higher concentrations, for which an acclimatization of the reactor biomass to recalcitrant substances, present in the mezcal vinasses, such as phenols and furfurals, is required. The physicochemical processes of flocculation and ozonation showed low COD removal efficiencies between 10 and 39 %, being the least efficient technologies. And neither are they recommended as a single technology due to the cost of supplies (alginates and ozone) that make the treatment more expensive. The combination of physicochemical with biological process increases the removal efficiencies but increases the treatment costs. Considering all the above and the fact that mezcal producers see treatment costs as an expense, this being an important reason why they do not treat their vinasses, what seems to be more appropriate is to revalue the waste. In other words, develop bioprocesses that generate by-products with commercial value to interest producers in investing in them and at the same time reduce environmental pollution. This is beneficial both from the economic point of view and from the sustainability of the treatment. Some work has been done in this area in the case of tequila vinasses, but very little has been studied in the case of mezcal vinasses. Therefore, we propose that this is where treatment efforts should be focused and finally within the line of revalorization of mezcal waste, we propose that it should be done with the biorefinery approach. The high concentration of organic matter and the diversity of organic molecules in the mezcal vinasses allow different bioprocesses to be carried out to generate more than one product with value, for example bio-hydrogen, methane, bio-hythane, volatile fatty acids, and electrical energy, all of them with high value and potential to even be reused in the same production facilities. That is, take advantage of the contaminants from the mezcal industry as substrates under the concept of circular economy.

Conclusions

In the last decade, the first works on the treatment of mezcal vinasses have been presented, including activated sludge, fungal treatments, anaerobic systems, flocculation and ozonation systems, and some hybrid systems that combine a biological and a physicochemical treatment. Of these treatments, those with the most encouraging results for large-scale application are the fungal treatment in biofilters with organic support material and anaerobic treatment, which has high organic matter removal efficiencies, in addition to being able to generate biogas with potential as fuel.

The presence of toxic compounds, such as phenols in vinasses, can inhibit microbial activity in biological treatment systems, reducing the efficiency in the removal of organic matter. Therefore, the inclusion of a physicochemical pretreatment as ozonation that removes these

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compounds or performs the biomass´s acclimatization to these compounds can improve the performance of a biological treatment system.

Due to the high concentrations of organic matter present in the mezcal vinasses, its revalorization is proposed. Indeed, few investigations has been done by applying treatment systems that allows to obtain high-energy compounds such as biohydrogen and methane, and simultaneously improving the efficient removal of pollutants in these effluents.

However, considering that the high content of organic matter in mezcal vinasses is found in the form of compounds of different complexity, different groups of microorganisms are required to be bio-transformed. In this work, it is proposed to develop biorefinery type treatment trains, in which several types of energy can be obtained by applying different bioprocesses in a sequential way in which the products of one process are the substrate of the next. Few recent works have demonstrated that it is possible to apply biorefinery treatment to mezcal vinasses and simultaneously to produce biohydrogen, methane and electrical energy. However, much more research must be done to develop biorefinery treatment from organic wastes of mezcal production.

Conflict of interests

The authors declare that they have no conflict of interests.

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