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Integration of Coagulation–flocculation(with Natural Coagulant) to Constructed Wetlands for Color Removal from Tequila Vinasses

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Abstract: The aim of this study was to evaluate a natural coagulant, Moringa oleifera seeds (MOC), to reduce the color concentration in treated tequila vinasses (TVs). TV-A was the effluent of horizontal subsurface flow wetlands (HSSFW); TV-B was the effluent of vertical up-flow wetlands (VUFW); and TV-C was the effluent of vertical down-flow constructed wetlands (VDFW). Raw TVs were also evaluated with MOC. Jar tests were performed to find the optimal dose and pH value for apparent color (AC) removal. With the optimal dose and pH for each type of TV, tests were performed in triplicate to evaluate the removal of apparent color (AC), true color (TC), turbidity, total suspended solids (TSS), chemical oxygen demand (COD), and electrical conductivity (EC). For TV-A and TV-B, the optimal values were 1 g/L of MOC and pH 8, and the removals were 52%, 43%, 50% and 72% of AC, turbidity, TC, and TSS, respectively. For TV-C, the optimal values were 2.5 g/L and pH 5, with removals of 66%, 73%, and 98% for AC, TC, and TSS, respectively. For TV-D, the MOC had no coagulant effect in any of the experimental conditions evaluated, probably due to the high concentration of turbidity and TSS in the raw vinasses, which prevented the interaction between MOC and melanoidins. Deeper studies are required to understand and evaluate those factors that influence MOC efficiency so that the coagulation–flocculation process can be optimized.

Keywords: tequila beverage; *Moringa oleifera*; apparent color; true color; melanoidins; jar tests; optimal dose; optimal pH

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1. Introduction

Tequila is an alcoholic beverage produced in Mexico, and its production has increased over the years. In the last decade (2013–2023), tequila production increased from 226.5 to 598.7 million liters per year [1]. During tequila production, a liquid waste named vinasse is generated. Tequila vinasse (TV) is a dark brown-colored liquid characterized by a high content of organic matter nutrients, total solids, and an acidic pH [2]. Due to its physicochemical characteristics, the environmental impacts of TV are very negative if discharged untreated. Unfortunately, it has been documented that TVs are frequently discharged into soils as a disposal method or discharged directly into water bodies [2,3]. The pollution problems associated with these disposal practices are diverse and range from a decrease in the quality of water bodies until the total destruction of aquatic ecosystems occurs [4], to alterations in the physicochemical characteristics of soil, causing sodicity, salinity, or modifications to the composition and microbial activity, which, together, lead to soil infertility [5].

One of the most distinctive characteristics of TV is its color (the most difficult to remove) due to melanoidins produced during the feedstock cooking. Melanoidins are high molecular weight polymers [6] that are formed in the Maillard reaction that occurs between sugars and amino acids at high temperatures [7,8]. Several studies have shown that melanoidins, although chemically diverse, are negatively charged [9].

TV coloration remains even after anaerobic treatment due to the inability to degrade melanoidins [10]. Anaerobic treatment is a very common technology for vinasse treatment [8]. When TVs are disposed of without treatment or partially treated, the high coloration of TV reduces the penetration of sunlight and, consequently, the photosynthetic activity in water bodies. This, as a result, causes dissolved oxygen concentrations to decrease, thus affecting aquatic life [11]. In addition, the presence of coloration in treated TVs limits their possible reuse. One technology recently evaluated by this research group for the treatment of TVs is constructed wetlands (CWs). CWs, an apparently simple but internally complex technology, can be adequately designed for the treatment of TVs [8]. Horizontal subsurface flow wetlands (HSSFWs) and vertical up-flow wetlands (VUFWs) were capable of reducing 95.4 and 95.8% of COD, and 86.5 and 86.4% of true color, respectively, along with other pollutants from TVs mixed with domestic wastewater [12]. Despite the high removal of true color by means of the two types of CWs, the remaining color was still high. Therefore, additional technologies are required to increase the removal of color.

CWs have also been evaluated for the removal of color from other effluents. For example, Yamamoto et al. [13] evaluated lab-scale CWs planted with *Typha orientalis* and *Cyperus isocladus* for the removal of the Reactive Yellow 86 dye, widely used in the textile industry. They found that cells planted with Cyperus achieved removals between 34% and 81% and cells planted with *Typha* achieved removals between 22% and 71%—removals significantly greater than those of non-planted cells (<12%). The mechanisms that explain these removals are adsorption in the roots and assimilation by the plants, in addition to adsorption in the filter medium (gravel), although to a lesser extent. There was also degradation of the dye by microorganisms. In addition, Faisal and Nasif [14] evaluated vertical wetlands with different substrates (filter sand and waste foundry sand) and plants (*Phragmites australis* and *Canna indica*) for the removal of methylene blue, a dye also widely used in industrial processes. They reported that both planted and unplanted wetlands were able to remove more than 98% of the dye, attributing these results to the effects of the filtering material and the presence of biofilms.

Other technologies that have been studied for the treatment of colored effluents include the use of ligninolytic fungi, which degrade color-causing phenolic compounds through enzymatic activity or through adsorption onto their mycelium [15–17]; or the use of acetogenic bacteria [18] or lactic acid bacteria [19,20], which are capable of biotransforming the substances that cause color [19], among others. One common technology for color removal is the coagulation–flocculation process, whose operating principle is based on neutralizing negatively charged particles using hydrolyzed cationic products [21,22]. Coagulants made from metal salts of iron and aluminum are used, mainly aluminum sulfate, as well as synthetic flocculants commonly derived from acrylamides [23]. However, some disadvantages of the use of inorganic coagulants are their high costs and production of toxic sludges. An option to replace metallic salts are natural coagulants that have the advantages of being easy to manipulate, do not modify the pH and, most importantly, allow the formation of non-toxic biodegradable sludge [24–26]. Natural coagulants have proven to be efficient in removing color from different effluents. Husen et al. [27] reported that flaxseed-based natural coagulant was effective in treating surface water, with high color (94%) and turbidity (95%) removal at pH 7 and a coagulant dose of 2.5. The authors highlight that the color of the water was reddish-brown, and after treatment it became completely clear. In addition, Moltot et al. [28] evaluated *Acanthus sennii* C., *Moringa stenopetala* B., and *Aloe vera* L., in combination, for the removal of color and turbidity from coffee wastewater, and found a removal higher than 98% for both parameters. Furthermore, one of the best-known natural coagulants is obtained from the seed of *Moringa oleifera* which is a cosmopolitan tropical tree tolerant to drought and, therefore, available all year round in some regions [29]. The mechanisms of the coagulant activity of *Moringa oleifera* seeds include adsorption and neutralization of charges as well as the formation of bridges between particles [29]. This natural coagulant belongs to the group of cationic coagulants because it has polymeric molecules with a net positive charge and has proven to be effective in removing turbidity in water treatment [30]. In addition, there are some studies that report the evaluation of *M. oleifera* seeds for the elimination of color from distillery effluents. Prasad [31] reported color removals of more than 50% from diluted distillery spent wash, while [32] evaluated the use of this natural coagulant as an aid to inorganic coagulants for color removal from distillery effluents and found that efficiencies were lower when the effluent was treated with chemical coagulants alone.

Due to the need to eliminate color from tequila vinasses and the proven capacity of *Moringa oleifera* seed as a coagulant, the aim of this study was to evaluate this natural coagulant to reduce the color concentration in treated tequila vinasses (with CWs). The ultimate goal was to increase color removal with a treatment train composed of CWs and coagulation–flocculation with a natural coagulant. A greater reduction in the color of tequila vinasses could allow them to diversify their reuse after being treated in CWs.

2. Materials and Methods

2.1. Types of Tequila Vinasses

The study was carried out at the Environmental Quality Research Center, in the Centro Universitario de la Ciénega (a campus of the University of Guadalajara) in Ocotlán, Jalisco, México, during June to October 2022. Treated tequila vinasses were evaluated. The treated vinasses were the effluents of three types of pilot-scale constructed wetlands (CWs) in operation for more than a year. Horizontal subsurface flow wetland effluent (HSSFW) was identified as TV-A; vertical up-flow wetland effluent (VUFW) as TV-B, and vertical down-flow wetland effluent (VDFW) as TV-C. Raw tequila vinasses (TV-Ds) were also evaluated to compare the efficiency of the coagulation–flocculation process with TVs with and without previous treatment. TV-D was only subjected to a sedimentation process, while the treated vinasses (TV-A, TV-B and TV-C) were pretreated with a sedimentation and neutralization (with $Ca(OH)_2$) process before being fed to the CWs. A complete description of the CWs in which TV-A and TV-B were treated can be found in Montoya, Tejeda, Sulbarán-Rangel and Zurita [12]. Briefly, the HSSFWs (125 cm long, 110 cm high and 50 cm wide) and the VUFWs (50 cm long, 110 cm high and 50 cm wide) were constructed of fiberglass, and the filter medium was volcanic rock of a size 2–4 cm in diameter. *Arundo donax* and *Iris sibirica* were used as emergent vegetation. The tequila vinasses were fed continuously with a flow rate of 10 L/d and 5 L/d, in the HSSFW and VUFW, respectively. Regarding VDFWs, they are fully described in Zurita, et al. [33]. Briefly, the VDFWs were built with PVC pipes (25 cm in diameter and 120 cm in height) and were planted with one individual of *I. sibirica*. The filter medium was also volcanic rock of a size 1–4 cm in diameter. The flow rate was 5 L/day, with 4 pulses in 24 h. The filter medium, which is very common in Mexico, is known as tezontle and is used in the construction industry. It is a very porous material with a porosity of 0.5 (or higher) and is composed of goethite (FeO(OH)), quartz (SiO_2), and hematite (Fe_2O_3). Other structural and textural characteristics of this material can be found in Tejeda et al. [34].

2.2. Preparation and Characterization of Moringa oleifeira Coagulant

The husks of the *M. oleifera* seeds were removed manually and subsequently the seeds were ground with a porcelain mortar. Then, they were dried in an oven (Yamato, Shanghai, China, DVS402 model) at 55 °C for two hours to reduce humidity. After that, the crushed and dried seeds underwent an oil extraction process with hexane in a Soxhlet extractor (Glas-Col, Terre Haute, IN, USA). The procedure described by Zurita, et al. [35] for oil extraction from *M. oleifera* was taken as a reference. Briefly, 10 g of dried and ground seeds were weighed and placed in an extraction cartridge which was placed in the extraction chamber of a Soxhlet extractor in which 250 mL of hexane was added in order to completely cover the cartridge. Four cycles were carried out, each lasting 25–30 min. After the extraction process, the *M. oleifera* powder was left for 24 h under a fume hood to completely evaporate the hexane, ground again, and sieved (500 μm). It was then stored in clean, dry plastic bottles to later be used as the coagulant (MOC) in the following tests.

Furthermore, the MOC was characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). FTIR analysis was conducted by Bucker Alpha equipment with ATR module, with a spectral range of 400–4000 cm^{-1} and a resolution of 2 cm^{-1}. XRD analysis was carried out by a powder X-ray diffractometer D500 kristalograph with a cooper Kα radiation using a wavelength of 0.1542 nm. High-angle XRD data were collected from 5° to 70° at 0.02° increments and 1 min count times.

2.3. Jar Test to Evaluate Color Removal from Tequila Vinasses

2.3.1. General Description

Samples of 1 L of tequila vinasses (TV-A, TV-B, TV-C and TV-D) were placed in 1 L beakers and taken to jar test apparatus (Prendo, AM-3 model) at room temperature (approximately 25 °C); the corresponding doses of MOC were added simultaneously to each beaker. The tests were performed with the following conditions: rapid mixing at 200 RPM for 3 min, followed by slow mixing at 30 RPM for 30 min, and a sedimentation time of 45 min. The settling time was selected from preliminary tests of three different settling times (30, 45 and 60 min). When necessary, the pH values of the samples were adjusted with H_2SO_4 and NaOH.

2.3.2. Test with TV-A and TV-B

To begin the evaluation of MOC with TV-A and TV-B, the two effluents were mixed because they had very similar characteristics [12]. To the best of our knowledge, MOC has not been evaluated for color removal from TVs. Because of this, for mixed TV-A and TV-B, tests were performed at a fixed pH value of 7 with a wide range of MOC concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.9, 1, 2, 3 and 5 g/L). For these first tests, apparent color (AC) was used as a control parameter. Based on the results, the MOC dose range was subsequently shortened. These doses were tested at pH values of 6, 7 and 8, to find the optimal dose of MOC. Following this, this optimal dose of MOC was tested at pH 7, 8 and 9, in order to find the optimal pH value. Finally, the jar tests were performed in triplicate with the selected optimal pH value and optimal MOC dose for TV-A and TV-B samples separately. The parameters analyzed in the samples in the triplicate tests were AC, turbidity, true color (TC), total suspended solids (TSS), chemical oxygen demand (COD), and electrical conductivity (EC). All the water quality parameters were determined according to the Standard Methods for the Analysis of Water and Wastewater [36].

2.3.3. Test with TV-C

In this case, the optimum MOC dose found for TV-A and TV-B was used as a point of beginning. This MOC dose was evaluated with different pH values: 5, 6, 7, 8 and 9, to find the optimal value. After finding the optimal pH value, different doses of MOC were evaluated (1.5, 2.5, 3, 3.5, 4, 5 and 6 g/L) to find the optimal dose. Similar to TV-A and TV-B, once the optimal values of MOC dose and pH value were found, the jar tests were performed in triplicate analyzing the same water quality parameters.

2.3.4. Test with TV-D

In the case of TV-D, turbidity was also analyzed along with AC. The tests began with the original pH value (pH 4.5) of the unneutralized vinasse in order to know the behavior of the samples with that acidic pH value with different doses of MOC (1.5, 2.5, 3.5 and 9 g/L). Subsequently, a dose of MOC was set and was evaluated with different values of pH (6, 7, 8, 9, 10 and 11).

3. Results

3.1. MOC Preparation

A total of 240.3 g of *M. oleifera* seeds was used to obtain 159 g of MOC (66 ± 4%). The percentage of MOC obtained in this study is similar to that reported by Boukandoul, et al. [37] who obtained 30–40% oil (60–70% of solid waste) from *M. oleifera* seeds. Furthermore, another study by Vilaseca, et al. [38] focused on the recovery of the residue after oil extraction and its use as a coagulant due to its water-soluble protein content; the authors reported a recovery of 65 to 75% of seed weight, which is also consistent with this study.

3.2. MOC Characterization

The MOC functional groups were determined by FTIR analysis. The spectrum (Figure 1) shows several broadbands that can be related to a protein structure that is responsible for flocculant–coagulant activity. The narrow and intense band located at 1646 cm^{-1}, by the presence of C=O stretching vibrations, is related to the N-H bending of the amide I, and the medium intensity band at 1533 cm^{-1} is caused by the C-N stretching vibrations of amide II [39,40]. In addition, there is another important band related to amide II, that is 3280 cm^{-1}, originated by the deformation of N-H in the NH_2 molecules as well as by the vibration of N-H in amide I. In the same line, the spectrum shows characteristic amide bands between 300–700 cm^{-1} [40]. Finally, the broadbands at 2919 and 2841 cm^{-1} correspond to symmetric and asymmetric C-H stretching that may be related to the original lipid content of moringa seed [41].

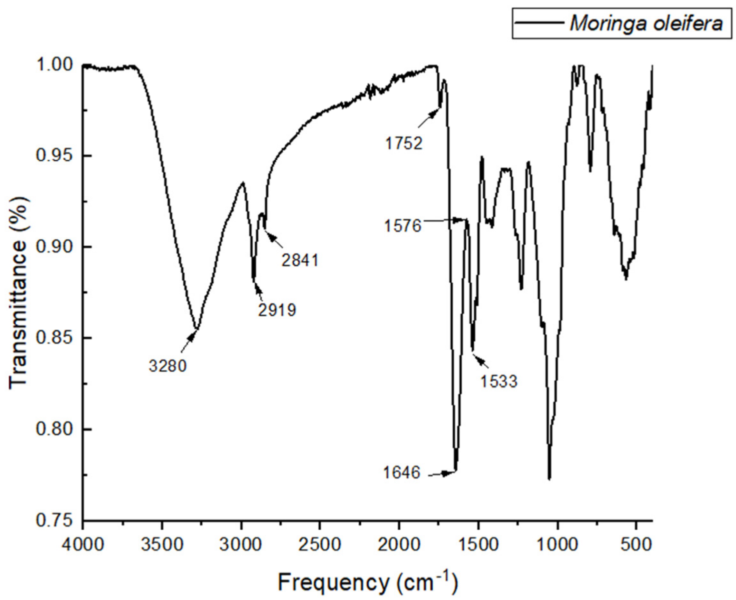

Figure 1. FT-IR spectrum of *Moringa oleifera* Coagulant.

With regard to X-ray diffraction analysis, the pattern presents a wide peak at an angle of 20.18° (Figure 2). It can be interpreted as a low degree of crystalline structure due to the broad pick on the baseline that is related to less ordered or amorphous phases [27,42]. It is important to highlight that the amorphous nature of MOC is a desirable characteristic since it improves its solubility in water [43], facilitating its dispersion as a coagulant [44].

3.3. MOC Performance in TV-A and TV-B

The two key parameters for an efficient coagulation–flocculation process are coagulant dose and pH [45]. The results of the AC removal percentages for the mixture of TV-A and TV-B for all evaluated doses of MOC at pH 7 are shown in Figure 3. It can be seen that the highest values were achieved with 0.8 and 2 g/L of MOC. In both tests, the final AC value was 780 Pt-Co Units (from an initial value of 1650 Pt-Co Units). However, the 2 g/L dose is more than double that of the 0.8 mg/L dose of MOC (which would imply a higher cost).

Furthermore, it is noted in Figure 3 that between 0.8 and 2 g/L, the 1 g/L dose showed an AC removal close to that achieved with 2 mg/L. Therefore, 0.8 g/L and 1 g/L were selected to perform the next set of tests.

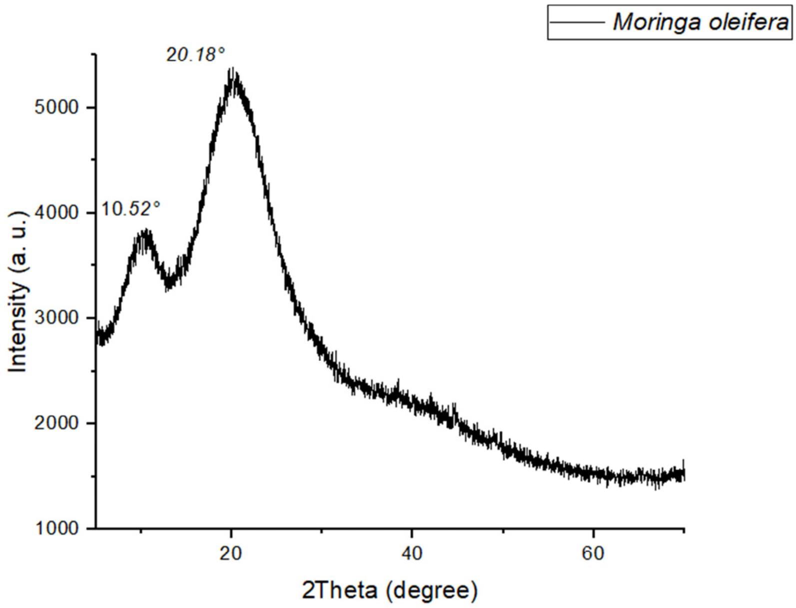

Figure 2. X-ray diffractogram of *Moringa Oleifera* Coagulant.

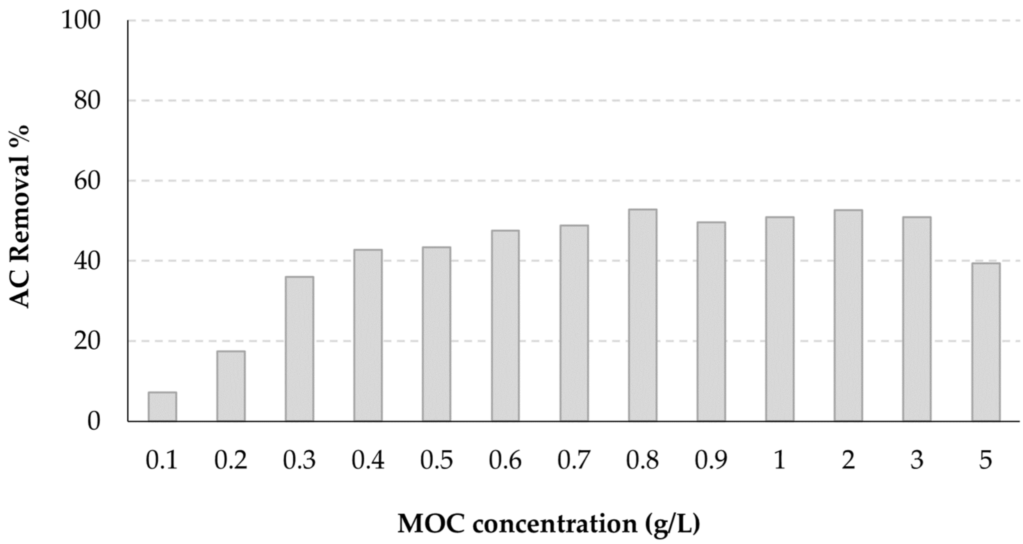

Figure 3. Apparent color removal percentage at different MOC doses at pH 7.

The doses of 0.8 and 1 g/L were evaluated at pH values of 6, 7 and 8. The results are shown in Figure 4, where it can be seen that for the two doses, the higher the pH, the greater the removal of AC, with greater influence of the increase in pH at the dose of 0.8 g/L. Also, it can be seen that the optimal dose of MOC was 1 g/L.

The observed increase in AC removal with increasing pH was taken as a basis to analyze more basic conditions in order to find the optimal pH value by setting the MOC dose at 1 g/L. The results with pH 7, 8 and 9 are shown in Figures 5 and 6. These tests allowed us to find that pH 8 was the optimal value to remove the AC with a dose of 1 g/L of MOC. By finding the optimal values of coagulant dosage and pH value, it can be assumed that the highest efficiency has been found in the coagulation–flocculation process [45].

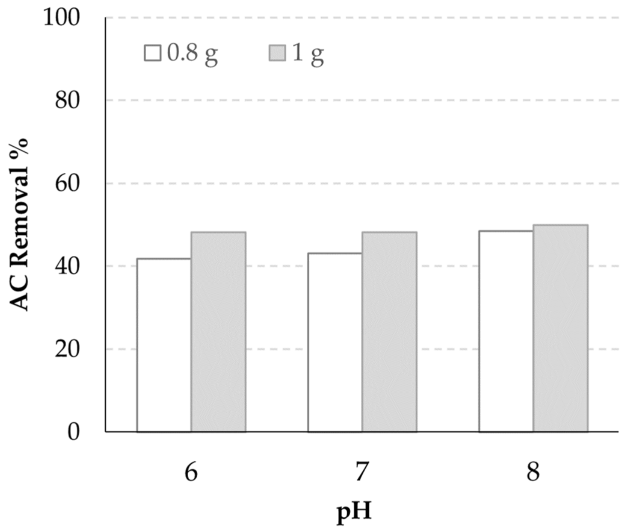

Figure 4. Apparent color removal percentage at 0.8 g/L and 1 g/L of Moringa Oleifera Coagulant.

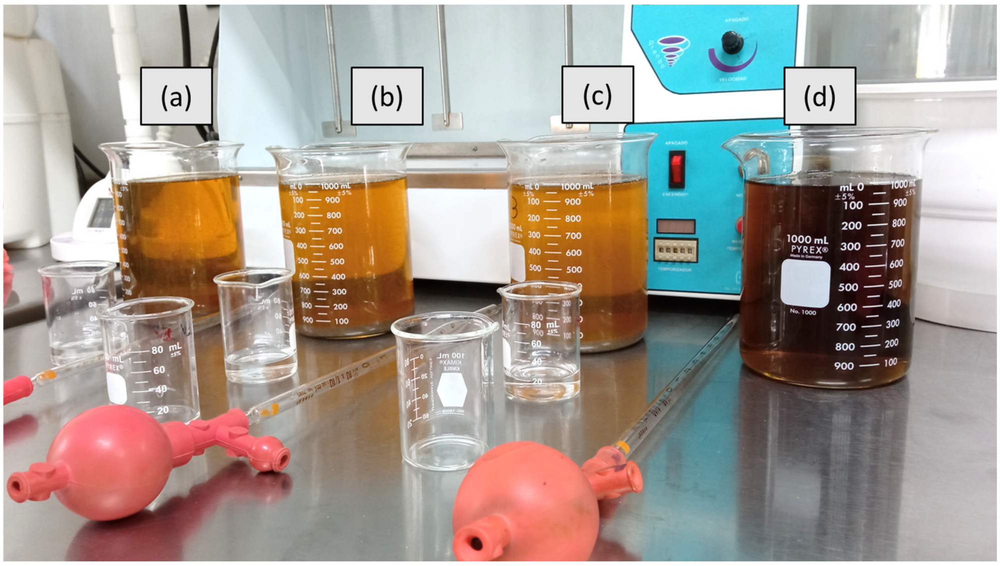

Figure 5. Color reduction in the mixture of TV-A and TV-B at a dose of 1 g/L of MOC and pH (**a**) 7, (**b**) 8, (**c**) 9, and (**d**) tequila vinasses without MOC (control).

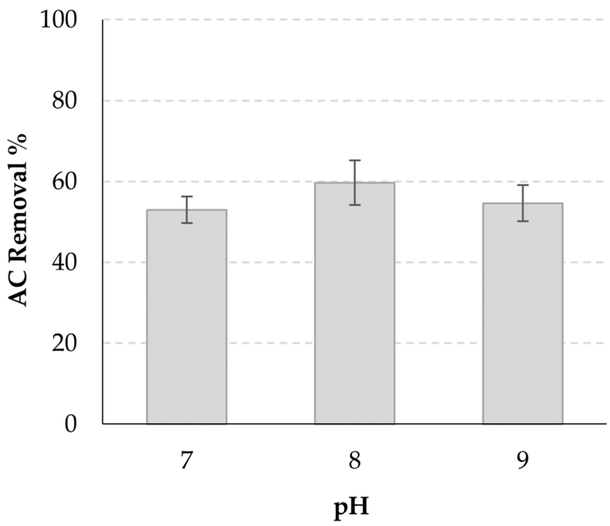

Figure 6. Percentages of apparent color removal at different pH values with a dose of 1 g/L.

It can be seen in Figure 6 that the removal percentages of AC at pH 7 and 8 were notably higher than those of the preliminary tests in Figure 4; as will be seen, the final value for pH 8 was more similar to that observed in Figure 4. These higher efficiencies were probably due to the fact that the TVs tested with MOC were different samples taken from the CW effluents. The CWs were in continuous operation, fed with tequila vinasses whose characteristics are variable over time. However, despite this discrepancy, the results allowed us to find the optimal pH of 8 that would be applicable in a continuous treatment on a real scale.

Finally, the results of the tests for TV-A and TV-B with the optimal value of pH (8) and optimal dose of MOC (1 g/L) are shown in Figure 7 and Table 1. The tests were performed separately but the results, as expected, were very similar with around 52% of AC removal and around 43% of true color removal. Such results are very similar to those reported by [31] who reported a maximum color removal of 56% with 0.8 g/L of MOC (20 mL of a 4% MOC solution; pH 7) extracted with NaCl, from diluted distillery spent wash.

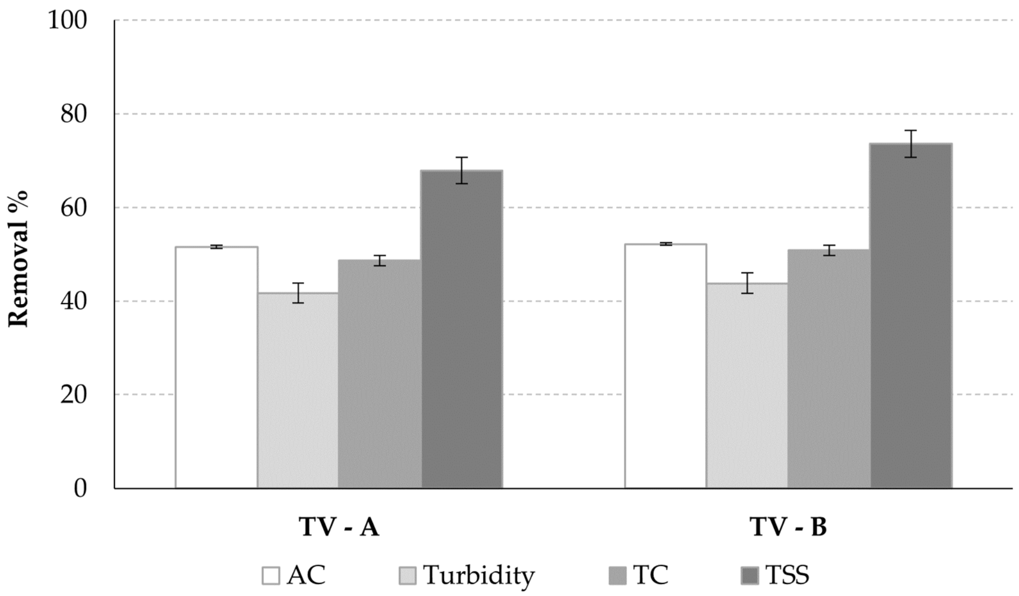

Figure 7. Removal percentage of apparent color, turbidity, true color and total suspended solid using 1 g/L of MOC at pH 8 in tests with TV-A and TV-B.

Table 1. Characteristics of TV-A and TV-B before and after coagulation–flocculation tests with MOC.

Parameter	TV-A	TV-A *	TV-B	TV-B *
Apparent Color (Pt-Co Units)	2440 ± 0.0	1180 ± 55.7 (50 ± 2%)	2520 ± 0.0	1203.3 ± 49.3 (52 ± 2%)
True Color (Pt-Co Units)	2340 ± 0.0	1200 ± 60.8 (48 ± 2%)	2580 ± 0.0	1266.7 ± 28.9 (51 ± 1%)
Turbidity (NTU)	23.3 ± 0.0	13.6 ± 2.8 (41 ± 12%)	26.3 ± 0.0	14.8 ± 1.6 (43 ± 6%)
COD (mg/L)	789 ± 0.0	1082.3 ± 19.8	869 ± 0.0	1158.7 ± 11.0
TSS (mg/L)	58.3 ± 0.0	18.7 ± 4.6 (68 ± 7)	95 ± 0.0	25.1 ± 4.3 (73 ± 4%)
EC (µS/cm)	4932 ± 0.0	4933.7 ± 5.5	5100 ± 0.0	5096.7 ± 11.5
pH	8.02 ± 0.0	8.0 ± 0.0	8.04 ± 0.0	8.0 ± 0.0

Note: * After coagulation–flocculation treatment. The removal percentages for TV-A * and TV-B * are shown in parenthesis.

As can be seen in Table 1, during the coagulation–flocculation process other water quality parameters may be affected, such as EC (which showed a slight increase) and COD that increased from an average value of 829 mg/L to 1120 mg/L. As expected, no increase in pH value was observed after coagulation–flocculation treatment with MOC. This is one of the advantages of natural coagulants since they do not consume alkalinity, unlike inorganic coagulants [28].

It is important to mention that TVs are highly colored effluents, so despite the high efficiencies of CWs for color removal, additional processes are required. In the two types of CWs, the removal of AC was greater than 80% while the removal of TC was greater than 64% (unpublished data).

3.4. MOC Performance in TV-C

Based on the previous results with TV-A and TV-B, the experiments began with a concentration of 1 g/L with different values of pH. Unlike TV-A and TV-B, in this case the acidic pH values showed a higher efficiency for color removal as shown in Figure 8. Specifically, pH 5 showed approximately 40% of apparent color removal.

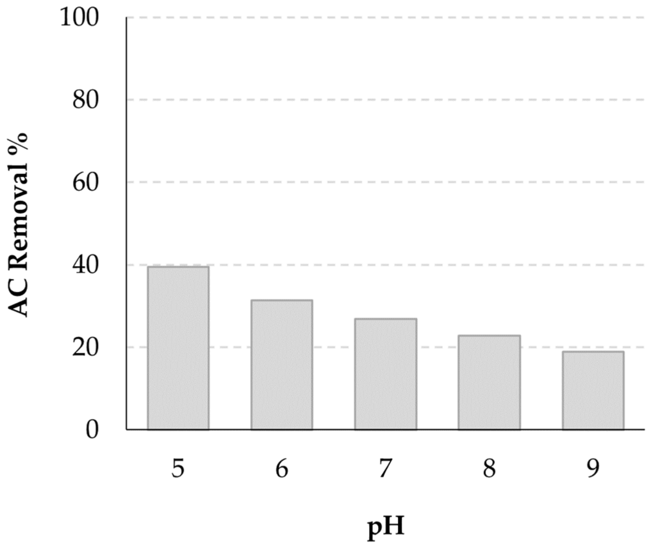

Figure 8. Removal percentage of apparent color in TV-C using 1 g/L of MOC at different values of pH.

Due to the aforementioned results, pH 5 was fixed to evaluate different doses of MOC (1.5, 2.5, 3, 3.5, 4, 5 and 6 g/L). The results (Figure 9) showed that the AC removal efficiencies were around 70% in a range between 2.5 and 4 g/L of MOC. Such behavior of MOC is similar to that reported by Pritchard et al. [46], who reported that MOC performance is very balanced when experimental conditions are in the optimum range. In addition, although 3 and 3.5 mg/L tended to be slightly more effective than 2.5 mg/L, this last dose was selected as the optimal value to minimize the increase in other parameters as COD with higher doses of MOC. Furthermore, a lower dose is considered more economically and technically adequate for large-scale application.

The results obtained in the tests in triplicate with pH 5 and a dose of 2.5 mg/L showed a very efficient performance of MOC (Figure 10). Similarly, Prasad [31] reported a 67% color removal from diluted distillery spent wash with a MOC dose of 2.4 g/L (60 mL of 4% MOC solution, pH 8.5) when extracting MOC with KCl salt. The values of other water measured parameters are shown in Table 2, where it can be seen that a final value of 2940 ± 103 g/L of COD was reached from the initial value of 2220 ± 262 mg/L, justifying the selected doses of 2.5 g/L rather than 3–3.5 mg/L. Other authors have also reported an increase in COD concentrations when high doses of natural coagulant are used because a large amount of coagulant mass contributes to the addition of organics in wastewater [47]. Furthermore, it is important to mention that even with the increase, the COD value after MOC treatment is still very low compared to the value in raw vinasses before treatment in VDFWs (around 20,000 mg/L). A slight increase was also observed in EC. On the other hand, the removal percentages of AC, TC and TSS showed higher efficiencies than those achieved with TV-A and TV-B.

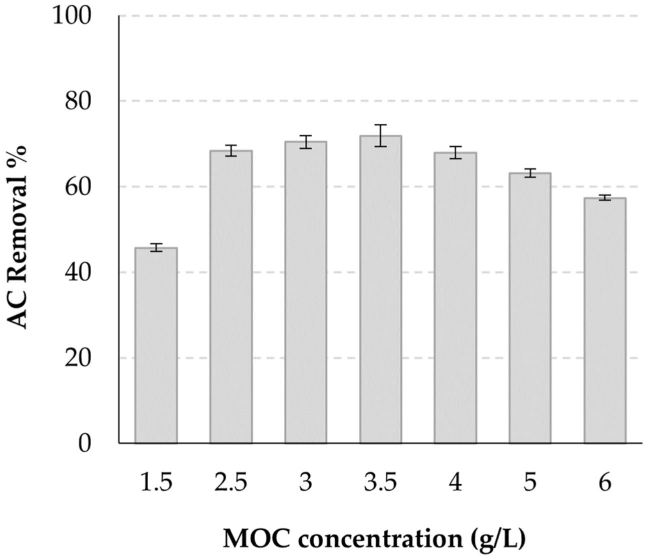

Figure 9. Removal percentage of apparent color using 1 g/L of MOC at different pH.

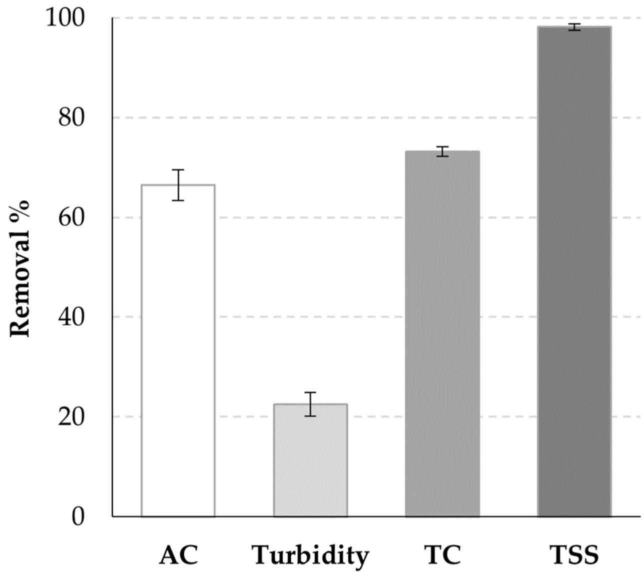

Figure 10. Removal percentage of apparent color, turbidity, true color and total suspended solid using 2.5 g/L of MOC and pH 5, in TV-C.

The noticeable difference for the optimal conditions for the coagulation–flocculation tests for each type of TV is understandable since the treated vinasses came from completely different types of wetlands, which implies different internal conditions and hydraulic retention times (HRT). In HSSFWs (TV-A) and VUFWs (TV-B), the HRT was approximately 30 d [12], thus the removal of pollutants was higher due to a longer contact between them and microorganisms developed on the filter medium and roots. However, in VDFWs which were fed in the typical way by pulses (4 pulses/day), the free flow after each pulse allowed a very short retention time in the system, impacting the effluent characteristics which were evaluated for color removal. Therefore, a different optimal pH was expected. The pH value not only modifies the charge and structure of the natural-polymeric coagulants (depending on the pH, their functional groups accept protons or dissociate), but also modifies the structure of the contaminants to be eliminated [45].

Table 2. Characteristics of TV-C before and after coagulation–flocculation tests at optimal pH and dose with MOC.

Parameter	TV-C	TV-C *
Apparent Color (Pt-Co Units)	8260 ± 0.0	2773.3 ± 257.1 (66 ± 3%)
True color (Pt-Co Units)	7300	1960.0 ± 69.2 (73 ± 1%)
Turbidity (NTU)	151 ± 11	117.0 ± 3.6 (22 ± 2%)
COD (mg/L)	2220 ± 262	2940 ± 103.9
TSS (mg/L)	4038 ± 25	74.7 ± 25.8 (98 ± 1%)
EC (µS/cm)	3899 ± 0.0	4090.3 ± 4.2
pH	5.0 ± 0.0	4.9 ± 0.03

Note: * After coagulation–flocculation treatment; TV-C * shows removal percentage between parenthesis.

3.5. MOC Performance in TV-D

The MOC performance with raw vinasses was completely different in comparison to the treated TVs. As can be seen in Figure 11, tests carried out at the original pH of 4.5 with increasing doses of MOC showed an increase in apparent color (from an initial value of 5955 Pt-Co units) and turbidity (from an initial value of 344.5 NTU). These results showed that MOC activity was not occurring but was only contributing to an increase in dissolved solids [46] and organic matter [48].

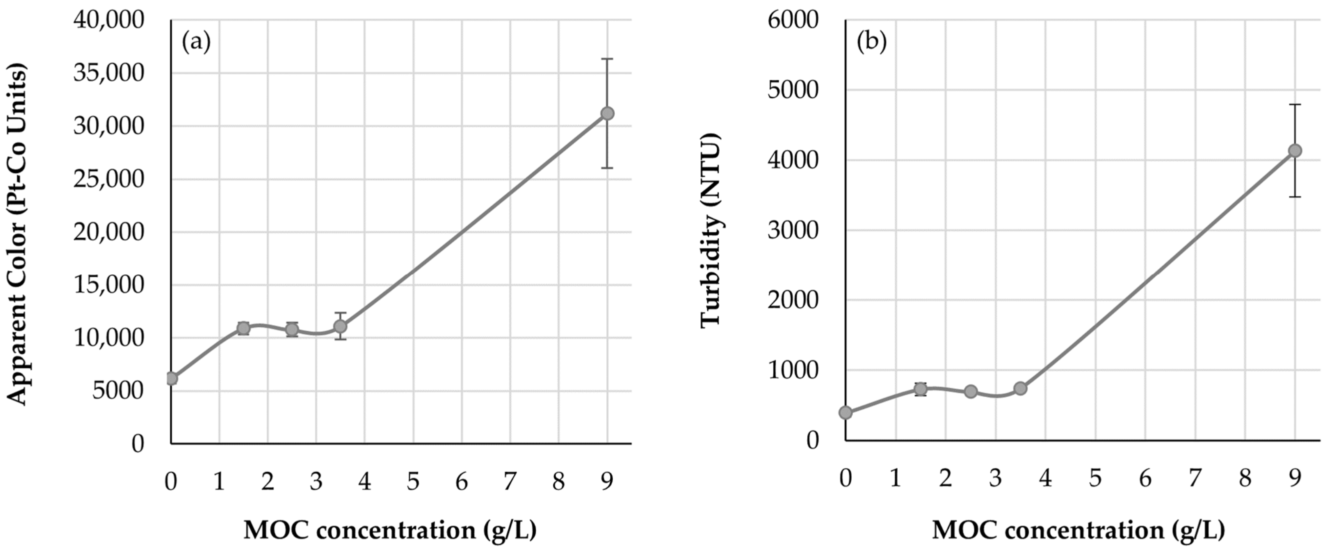

Figure 11. Increase in (**a**) apparent color and (**b**) turbidity concentrations when performing coagulation–flocculation tests with raw vinasses at pH 4.5 and different MOC doses.

After the obtained results with the original pH of the raw vinasses, the doses of MOC were fixed at 2 g/L. This is because it is a concentration close to those with outstanding performance for the treated vinasses. However, apparent color and turbidity results showed a similar behavior with an increase at pH 6 and a trend to diminish after pH 7 (Figure 12). While turbidity was reduced until a value of 52 NTU at pH 11, AC reached a value of 5070 Pt-Co units. In these tests, the initial concentration of AC was 6360 Pt-Co units, while the turbidity concentration was 419 NTU. With these poor results at pH 11 for AC removal (20.3%), it was impractical to continue the evaluation of MOC at higher values of pH. Furthermore, it is important to mention that during the experimental tests it was observed that the samples suffered a coagulation–flocculation process with only NaOH addition, which was used to increase the values of pH; this was more noticeable at pH 12 before the addition of MOC.

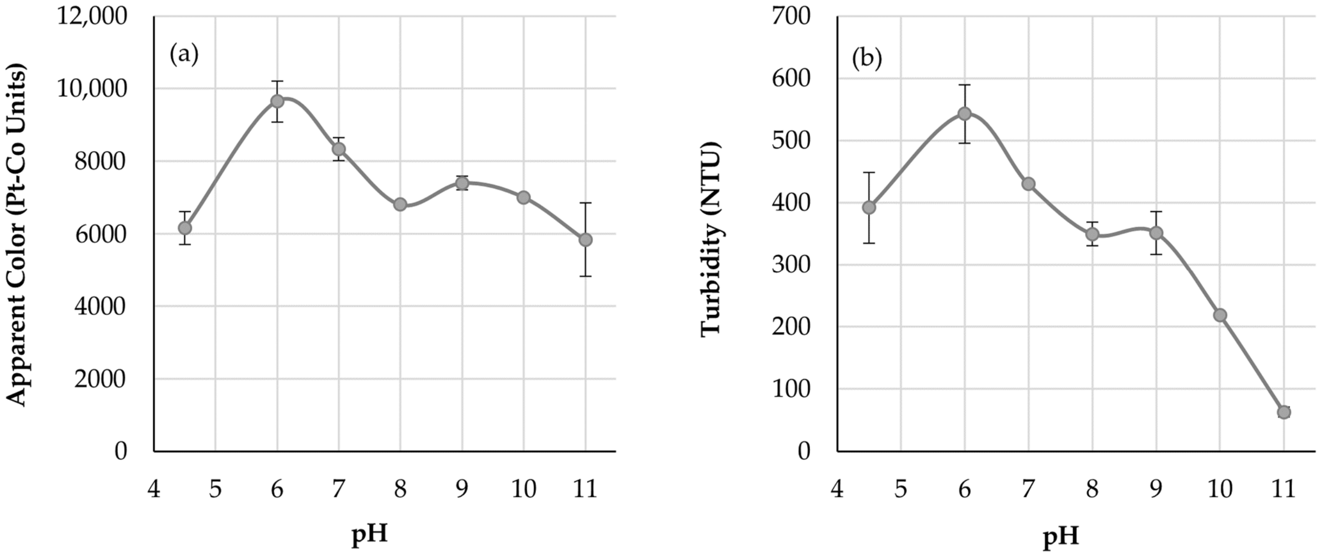

Figure 12. Behavior of (**a**) apparent color and (**b**) turbidity concentrations when performing coagulation–flocculation tests with raw vinasses using 2 g/L of MOC at different pH values.

4. Discussion

Melanoidins are mainly responsible for the color of distillery effluents, such as tequila vinasses. Therefore, technologies for color removal must be based on the elimination of melanoidins. Some authors have already reported that the use of coagulation–flocculation as post-treatment of biologically treated distillery effluents could achieve high color removal [49,50].

Although melanoidins' degradation pathways are not fully understood [51], several strains of fungi and bacteria have the capacity to remove them [52] through enzymatic activity [53]. In addition, some microorganisms have also been reported to have an adsorption mechanism that can occur alone or in conjunction with a biodegradation pathway [54]. Therefore, due to the biological treatment in the CWs, the high concentration of melanoidins in the TVs was decreased. This lower concentration allowed the coagulation–flocculation process with MOC to be efficient, unlike the coagulation–flocculation process with raw vinasses. Other studies have reported similar findings. For example, [55] reported a removal of 25–30% of color in raw vinasses and 80–90% in anaerobically treated vinasses when using inorganic coagulants for the treatment of sugarcane vinasses. They concluded that the removal of soluble organic compounds during the anaerobic treatment enhanced colloid/metal interaction. In addition, Prasad [31] found 56–67% color removal in a highly diluted raw distillery effluent.

On the other hand, the treated TVs had a comparatively lower concentration of TSS and turbidity than the raw vinasses, due to the high efficiency of CWs for the elimination of these contaminants [12] which have been found to affect the efficiency of a coagulation–flocculation process. Low turbidity improves the process due to the high collision frequency between coagulants and dissolved colloidal solids [56]. Additionally, the coagulation–flocculation process between MOC and remained melanoidins is presumably based on the cationic protein of MOC [57] and negative charge of melanoidins due to ketone or hydroxyl functional groups from pyranone or pyridone into its high molecular amino-carbonyl structure [52]. It is important to mention that an optimal dose of coagulant can enhance the removal of colloidal particles. In contrast, an overdose promotes the recuperation of TSS [58] that increases turbidity and organic load [59] causing an efficiency reduction in coagulant performance.

Finally, in this study, significant color removals were achieved in TVs treated in three types of CWs. These results are promising because they demonstrate that the use of nature-based technologies consisting of CWs + coagulation–flocculation with MOC can work for the treatment of tequila vinasses, considered a high-strength effluent [8]. CWs are well known as a low-cost technology compared to conventional wastewater treatment technologies, and natural coagulants can also be cost-effective due to their renewability and biodegradability (easy disposal of the sludge produced) [28]. However, it seems that so far, this natural coagulant is not offered on the market as a coagulant but as a food supplement (because healing properties are attributed to it). Therefore, currently its price could be higher than that of inorganic coagulants. We hope to see changes in the short term due to the many studies that have demonstrated its efficiency for water and wastewater treatment. Furthermore, it is clear that there are several factors that can affect the performance of MOC: on the one hand, the concentration of contaminants in TV, the pH, and the doses of coagulant; and on the other hand, the method of obtaining MOC as well as its particle size.

5. Conclusions

- The efficiency of MOC for color removal in treated and raw vinasses showed a clear difference. While in treated vinasses the removal efficiencies were 52–66% for AC and 49–73% for TC with the optimal doses and pH values, in the raw vinasses the MOC had no coagulant effect in any of the experimental conditions evaluated in this study.
- This last result was probably due to the high concentration of turbidity and TSS in the raw vinasses, which prevented the interaction between MOC and melanoidins. Furthermore, the high concentration of melanoidins in the raw vinasses probably made the coagulation process between MOC and melanoidins imperceptible.
- These results emphasize the role of CWs and their implementation before the coagulation–flocculation process in a tequila vinasse treatment train.
- Further studies are required to fully understand and evaluate those factors that influence the efficiency of MOC (particle size, extraction method, combination with flocculants, etc.) for removing color from treated TVs in order to increase the efficiency of the coagulation–flocculation process.
- For real applications, the implementation of CWs + coagulation–flocculation with MOC will allow an effluent with less color to be obtained, with greater possibilities of reuse.

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