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Evaluation of the adsorption capacity of glyphosate in a microbial cellulose composite

Evaluación de la capacidad de adsorción de glifosato en un compuesto de celulosa microbiana

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Abstract

The objective of this project was to develop a composite based on microbial cellulose (CM), chitosan (Qs) and citric acid (AC) as a crosslinker at two different concentrations (4 and 6% w/v). The CM was obtained from the fermentation of fruit residues with a SCOBY (symbiotic consortium of bacteria and yeast). The composites were morphologically characterized by SEM and spectroscopy techniques (FTIR-ATR) as well as UV-VIS spectrophotometry with ninhydrin were used to evaluate the composite as an adsorbent for glyphosate in water; in the spectra obtained by FTIR, the representative vibrational bands of the functional groups belonging to chitosan and microbial cellulose were observed; for cellulose and chitosan composites with 4% citric acid in 1579 cm^{-1}, 1386 cm^{-1}, 1046 cm^{-1} and 853 cm^{-1}; and for the 6% cellulose-chitosan-citric acid composite at 1573 cm^{-1}, 1380 cm^{-1}, 1056 cm^{-1} and 503 cm^{-1}, thus attributing cross linkage between the biopolymers. Post- adsorption FTIR-ATR analysis revealed significant changes possibly attributable to the adsorption of glyphosate in the cellulose composites. Further research is still being conducted to better understand this interaction and the involved mechanism; the goal is to comprehend how microbial cellulose can effectively adsorb glyphosate, which could have practical and efficient applications in the remediation of environments contaminated with this herbicide.

Glyphosate, Microbial Cellulose, Composite

Resumen

El objetivo de este proyecto fue desarrollar un composito a base de celulosa microbiana (CM), quitosano (Qs) y ácido cítrico (AC) como entrecruzante a dos diferentes concentraciones (4 y 6%p/v). La CM se obtuvo de la fermentación de residuos de frutas con *SCOBY* (por las siglas en inglés de consorcio simbiótico de bacterias y levaduras). Los compositos se caracterizaron morfológicamente por SEM y se usaron las técnicas de espectroscopia (FTIR-ATR) y espectrofotometría de UV-VIS con ninhidrina para evaluar al composito como adsorbente de glifosato en agua; en los espectros obtenidos se observaron las bandas vibracionales representativas de los grupos funcionales pertenecientes al quitosano y celulosa microbiana; para los compositos de celulosa y quitosano con ácido cítrico al 4% en 1579 cm^{-1}, 1386 cm^{-1}, 1046 cm^{-1} y 853 cm^{-1}; y para el composito celulosa-quitosano-ácido cítrico al 6% en 1573 cm^{-1}, 1380 cm^{-1}, 1056 cm^{-1} y 503 cm^{-1} atribuyendo entonces un entrecruzamiento entre los biopolímeros. Los análisis de FTIR-ATR posterior al proceso de adsorción revelan cambios significativos posiblemente atribuibles a la adsorción de glifosato en los compositos de celulosa. Aunque se sigue investigando para comprender mejor esta interacción y los mecanismos involucrados, se busca comprender cómo la celulosa microbiana puede adsorber eficazmente el glifosato, lo que podría tener aplicaciones prácticas y eficientes en la remediación de ambientes contaminados con este herbicida.

Glifosato, Celulosa microbiana, Composito

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Introduction

The excessive use of pesticides represents a risk to human health and the environment, since they pollute soil, water, sediments and air (ONU, 2016). In Mexico, agricultural activities are the main source of the economy and the extraction of water can generate high contamination with metals in alarmant concentrations of iron, arsenic, etc. (Moola *et al.*, 2023). Glyphosate is the main active ingredient of a group of commercial pesticides that are widely used worldwide due to its high efficiency in the non-selective elimination of weeds. Its components include different types of salts and surfactants that are dangerous for human health. The commercialization of this herbicide began worldwide in 1974 (Ramírez, 2021 & Salguero, 2023). For this reason, the scientific community has dedicated resources to find alternatives and ways to remove pesticide residues that reach water bodies and soils, which can cause irreversible damage.

Biopolymers are considered a viable option for the formation of hydrogels, since they have biodegradation capacity and microorganisms that contribute to the formation of adsorbed ions through a biotechnological process. On the other hand, composites are formed of two or more different materials, the use of reinforced materials that are cheaper and with less environmental impact is currently being studied. Therefore, many biopolymers such as cellulose derivatives, carbon, chitin and chitosan are being reinforced (Khattak *et al.*, 2019 & Kushwaha *et al.*, 2023,). Therefore, microbial cellulose is an alternative to be used as a composite matrix, the latter arises from the sense of innovation in biotechnology and is a material that allows metals and microorganisms to be removed from water (Baghdad & Hasnaoui, 2020). Chitosan is a biopolymer that also has adsorbent capacity for some contaminants such as heavy metals (Alonso-Segura *et al.*, 2021).

Thus, this work seeks to develop and validate a compound based on microbial cellulose crosslinked with chitosan and citric acid, with which it seeks to achieve an ester bond between both biopolymers (Alonso-Segura *et al.*, 2009) in order to increase the active sites of the compound and thus adsorbent the glyphosate present in water.

Methodology

1. Synthesis of Microbial Cellulose

Organic residues were collected at the Universidad Tecnológica de Corregidora, mainly fruit peels rich in fructose such as banana, mango, papaya and apple, for the preparation of the modified Hestrin-Schramm, HS culture medium: 120g of peels were crushed with 250 mL of distilled water. This mixture is placed in previously washed and disinfected glass jars. The reagents were added, all of them described in Table 1. Subsequently, it was homogenized with the help of a spatula and covered, ensuring that there were no leaks. This was sterilized at 121°C and 15 psi for 15 minutes.

Reagents	Mass in grams
Fruit peels	120
Yeast extract (Mcd lab, Oaxaca, México)	2.5
Peptone (Materiales y abastos especializados, S.A de C.V., Jalisco, México)	2.5
Na_2HPO_4 (Materiales y abastos especializados, S.A de C.V., Jalisco, México)	1.35
Citric acid (Diquitra, China)	0.575
Saccharose (Materiales y abastos especializados, S.A de C.V., Jalisco, México)	2.5

Table 1. Reagents for the preparation of the modified HS culture medium

After the sterilization process of the modified HS culture medium, the flasks were allowed to cool before inoculating them with 12.5 mL of the SCOBY consortium broth and 10g of the solid matter in a laminar flow hood (Tecnolab S.A de C.V, Querétaro, México). in each of the prepared jars.

They were left to ferment for 7 days sheltered in a cool and dry place until the formation of the cellulose film as a cream formed on the surface of the modified HS medium, with a viscous and firm appearance at the air-liquid interface of the containers; these were carefully removed inside the laminar flow hood with the help of clean and sterile forceps. They were washed with distilled water until the remains of the culture medium were removed.

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Followed by a washing with a 0.5 N KOH solution (Meyer Chemical Reagents, Edo. de México, México) and they were heated to 70°C for 15 min. After this, the MC film was washed again with distilled water. These are placed in an oven at 45°C for 24 h to remove moisture. Subsequently, the MC was crushed until a fine-textured powder was obtained, with the help of a commercial coffee grinder (LONGLIV B09NW2VV32, Mexico City, Mexico), finally it was passed through a 1mm sieve (Lacor Accesorios, Mexico City, Mexico). and stored until further use.

2. Preparation of the MC composite with Qs and citric acid

This was carried out by modifying the crossover method reported by Alonso *et al.,* (2009) described in annex 1, the MC was functionalized with food grade chitosan (Encapsuladoras de México, CdMx, México). 100 mL of aqueous solution of chitosan with food grade citric acid (Diquitra, China) at 4% w/v and 6% w/v respectively were prepared, the pH was measured with the help of a potentiometer (LAQUA PH1100, Japan) making sure that this was close to or less than 3.

To these solutions, 2.5g of MC powder were added, stirring at 200 rpm for 24h. Subsequently, 2.5% w/v of NaH2PO (Materiales y abastos especializadas, S.A de C.V., Jalisco, México) were added and the shaking was maintained until the solution was homogeneous. This solution was heated at 70°C for 15 min. After time, 30 mL of this solution was poured into Petri dishes until uniformly distributed, these were dry-cured in an oven at 50°C for approximately 24 h.

3. Characterization of the MC and the composites by FTIR-ATR and SEM

In order to observe the physicochemical characteristics of the microbial cellulose powder and the elaborated compounds, tests were carried out using Fourier transform infrared spectroscopy and Attenuated Total Reflectance (Bruker VERTEX 70v, Karlsruhe, Germany) and Scanning Electron Microscopy (Philips, XL20 SEM, Europe).

A small powder sample of MC, compounds (with 4 and 6% w/v citric acid), was taken and each was placed on the FTIR-ATR spectrometer to be read in the mid-infrared range.

To obtain SEM images, small samples of each of the microbial cellulose and chitosan compounds, as well as cellulose powder, were taken, covered with carbon and observed.

4. Glyphosate quantification by UV-Vis spectrophotometry with ninhydrin and sodium molybdate

For the quantification of glyphosate, a commercial brand named Enemigo (Agricam, CdMx, México) was purchased. A calibration curve was made using UV-VIS spectrometry following the ninhydrin and sodium molybdate technique described by Xu *et al.,* (2018).

A commercial glyphosate solution was prepared starting from an initial concentration of 410,000 ppm until a stock 2 of 25,000 ppm is obtained. A determined volume of distilled water and the stock solution were added to screw cap tubes. 1 mL of sodium molybdate and ninhydrin solution (Beckman Coulter, USA) at 2.5% v/v was added. The tubes were placed in a water bath at 80°C for 7 min until a purple color was observed (annex 2).

5. Glyphosate adsorption with MC and CM-AC-Qs composites

To evaluate the adsorption of glyphosate with microbial cellulose and with the composites, polypropylene columns were packed with 2.5g of the adsorbents (CM and composites, respectively), 20 mL of the stock solution with commercial glyphosate were added to each column. (25,000 ppm), and it was left to rest for 48 and 72 h at room temperature. Columns were mounted with a bosshead clamp and covered with aluminum foil to prevent light exposure. After this, the samples are stored for later analysis.

6. Quantification of adsorption by FTIR-ATR

After the rest time, aliquots of the solids (adsorbents) were taken for analysis in the Fourier Spectrometer and Total Attenuated Reflectance (FTIR-ATR; Bruker VERTEX 70v, Karlsruhe, Germany), with 50 scans, a resolution of $2cm^{-1}$, from 4000- $350cm^{-1}$.

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Results

1. Microbial cellulose

A fine textured microbial cellulose powder was obtained as shown in Figure 1, which was used for the preparation of cellulose-chitosan-citric acid compounds (CM-AC-Qs).

Figure 1 Microbial Cellulose Powder (PCM).

2. Composites of microbial cellulose-citric acid-chitosan, CM-AC-Qs

Figures 2 and **3** show the compounds designed with microbial cellulose, citric acid and chitosan.

Figure 2 Microbial Cellulose-Chitosan-4% Citric Acid Composite (CCM4).

Figure 3 Microbial Cellulose-Chitosan-6% Citric Acid Composite (CCM6)

Comparing the compounds in relation to the concentration of citric acid used, at 4 and 6% w/v, it is possible to highlight the homogeneity on the surface of the film when AC was used at 6% w/v, this due to the reduction of pH, which favored the dissolution of both QS and CM, prior to the crosslinking process.

3. Glyphosate quantification by ninhydrin and sodium molybdate

Glyphosate was quantified using the ninhydrin and molybdate technique in the liquid after the adsorption process with both the MC and the compounds. Figure 4 shows the dilutions made with the aliquots after adsorption.

Figure 4 Test tubes for commercial glyphosate calibration curve

Table 2 shows the concentrations of glyphosate quantified by UV-Vis, being the compound of CM-Qs and 4% w/v of citric acid with which the lowest concentration was obtained.

Sample	Glyphosate concentration (ppm)	Standard deviation
PCM	125.125	±0.005
CCM4	89.75	0
CCM6	115.05	0

Table 2 Final concentration of glyphosate present in the samples after UV-VIS adsorption

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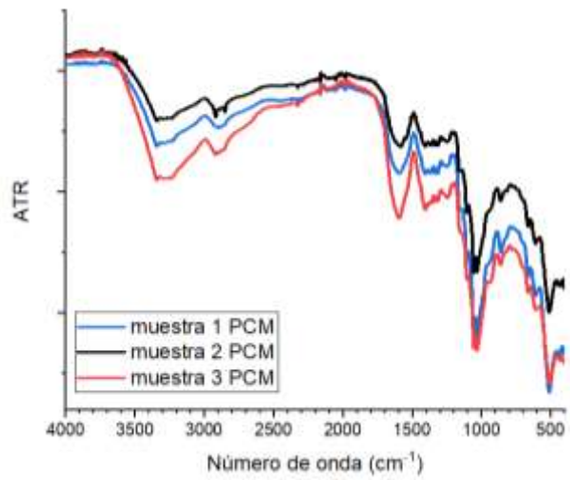
4. Characterization of microbial cellulose by FTIR-ATR, before and after adsorption

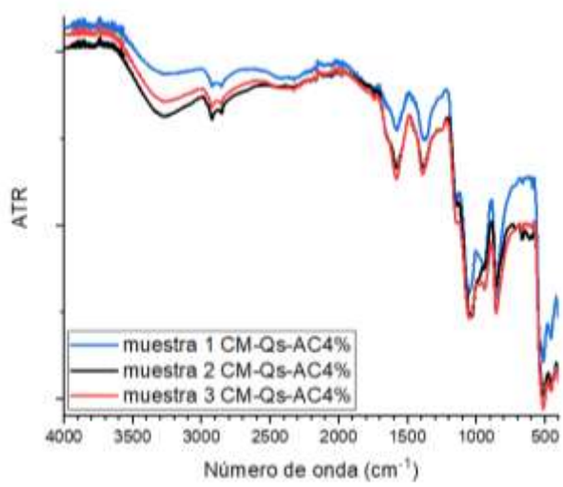
The spectra obtained from powdered microbial cellulose and from the compounds with Qs and citric acid at 4% w/v and 6% w/v are shown in graphs 1, 2 and 3 respectively. The FTIR spectrum shows the position and intensity of the vibrational bands of the functional groups found in the microbial cellulose powder (MCP). The spectrum obtained from MCP (Graph 1) shows bands similar to those previously reported (Avcioglu, Birben & Bilkay, 2021), confirming the basic structure of microbial cellulose. Replications of the sample were taken, averaging 50 scans three times for each sample, demonstrating that no significant variations were observed between each of the spectra, demonstrating homogeneity in the microbial cellulose as well as in the designed compounds. The spectra obtained from the compounds designed with chitosan, microbial cellulose and citric acid show peaks similar to those reported by Urbina *et al.,* (2018), thereby demonstrating crosslinkage of this polymers.



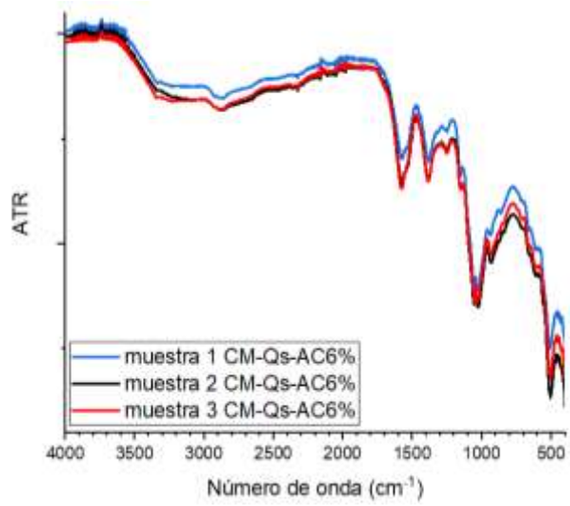
Graph 1 Powdered microbial cellulose spectrum

In graph 1, a broad band can be highlighted around 3300 cm^{-1} attributed to the presence of hydroxyl groups (-OH) related to a stretching of OH groups with hydrogen bonds (Ghozali *et al.*, 2021). Around 2900 cm^{-1} C-H stretching vibrations of the CH2 groups of hydroxymethyl (Barshan et al, 2019). The band around 1600 cm^{-1} and 1400 cm^{-1} indicates the carboxylic and carboxylate groups (Kiziltas *et al.*, 2015). At 1036 cm^{-1} a band associated with C-O-C and C-OH ether stretching vibration of the glucose ring is observed (Ghozali *et al.*, 2021) and the vibrational band located at 897 cm^{-1} is the beta glycosidic bond of cellulose.

In graph 2 we can see the spectrum of the cellulose compound with chitosan and 4% citric acid. The band located around 3300 cm^{-1} attributed to the O-H stretching of the hydroxide groups can be highlighted. At 2900 cm-1 and 2800 cm^{-1} the bands are attributed to C-H asymmetric stretching vibrations of the methylene group. Around 1650 cm^{-1} there is a vibration associated with the C=O stretching of the acetyl and carbonyl groups (Stanescu *et al.*, 2021). The band at 1370 cm^{-1} corresponds to the C-H bending deformation of methyl and methylene. An intense band at 1089 cm^{-1} which can be associated with a C-O stretching vibration and C-O-C ether bonds (Fernández Queiroz *et al.*, 2014). In the vibrational band located at 897 cm^{-1} is the beta glycosidic bond of cellulose.



Graph 2 Microbial cellulose spectrum, chitosan and 4% citric acid



Graph 3 Microbial cellulose spectrum, chitosan and 6% citric acid

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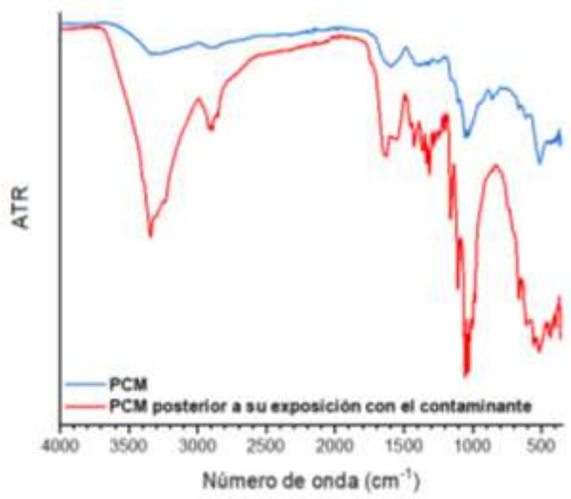
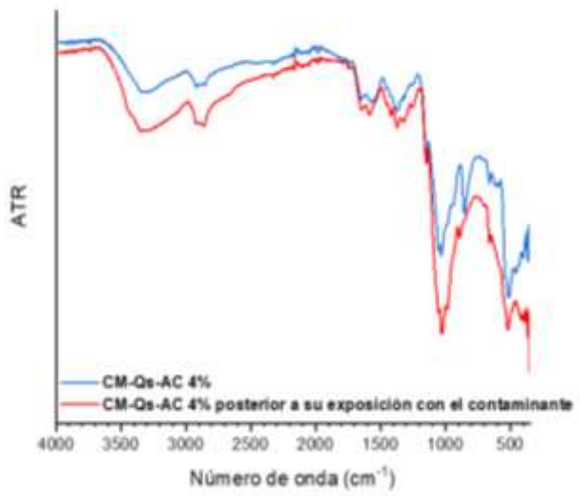
The spectrum of graph 3 shows the treatment with 6% citric acid. Vibrational bands located at 1600 cm^{-1} can be highlighted, which can be associated with asymmetric COO- stretching, 1460 cm^{-1} symmetrical COO-, and 1012 cm^{-1} C-C doubling (Stanescu *et al.,* 2021). Comparing the compound in relation to the concentration of AC used 4% w/v and 6% w/v, we can highlight that each of the repetitions shows greater homogeneity in which 6% w/v was used compared to 4% w/ v.

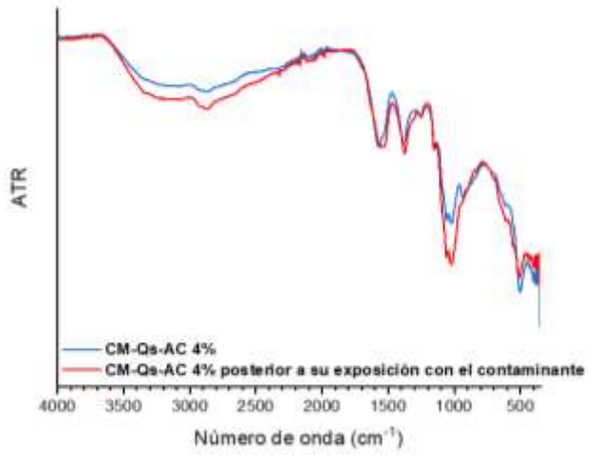
In graphs 4, 5 and **6** the spectra after adsorption are shown. We can observe a remarkable difference between the spectra of MC and compounds before and after being exposed to the pollutant. An increase in the intensity of the band is observed around 3500 cm^{-1}, especially in the MCP spectrum (MC after adsorption). This is because the cellulose begins to hydrate when coming into contact with the solution of water and glyphosate. Characteristic peaks of amino groups (NH2) were also observed in the compounds at 1483 cm^{-1} and some phosphate groups in the area of 1223 cm^{-1}, however the amino groups can also be from chitosan.



Graph 4 Post-adsorption spectrum of microbial cellulose glyphosate



Graph 5 Spectrum after commercial glyphosate adsorption with the composite CCM4



Graph 6 Spectrum after commercial glyphosate adsorption with the composite CCM6

5. Microscopy, SEM of microbial cellulose and composites

It was used scanning electron microscopy, SEM (Philips, XL20 SEM, Europe), in figures 5 and **6**, we can appreciate the micrographs of the microbial cellulose powder at 100X and 5000X in magnitude, in these it is observed that the powdered MC presents a irregular morphology and is amorphous.

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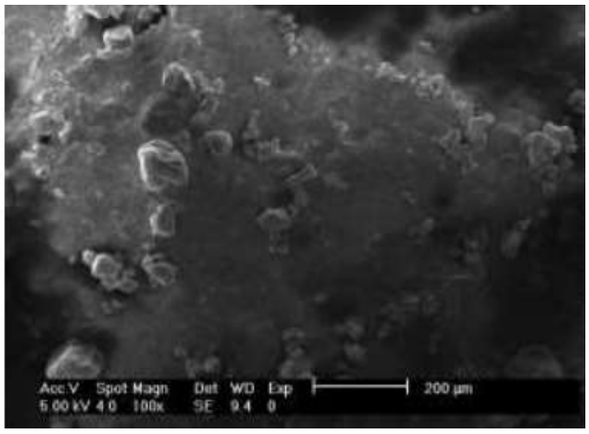

Figure 5 Micrograph of microbial cellulose powder at 100X

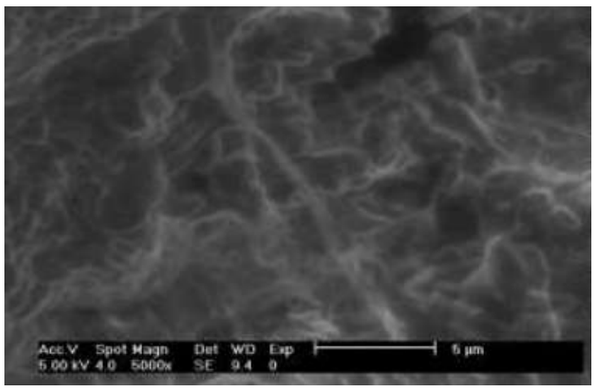

Figure 6 Micrograph of microbial cellulose powder at 5000X

In figure 7, we observe a micrograph of CCM4 powder at a magnitude of 1500X in magnitude, in which it is observed that the composite presents an amorphous morphology and a type of fibers that are characteristic of chitosan.

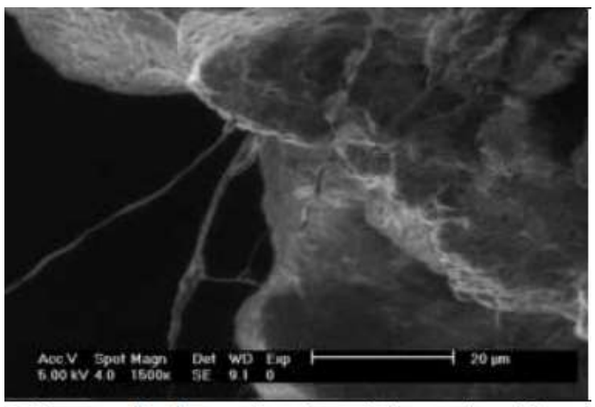

Figure 7 Micrograph of compound CCM4 a 1500X

In figure 8, we observe the micrographs corresponding to CCM6 with a magnitude of 2500X and it is observed that this compound presents a greater quantity of characteristic chitosan fibers.

Which indicates a greater quantity of this biopolymer due to a greater concentration of crosslinking, AC, Chitosan mayor intertwined with the MC.

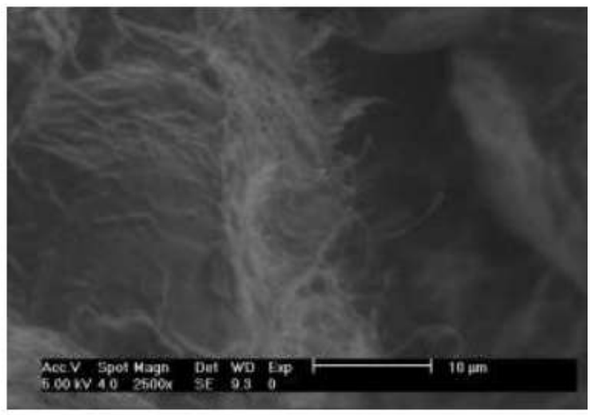

Figure 8 Micrograph of microbial cellulose casting with 6% citric acid at 2500X

Conclusions

The SEM analysis showed differences in the physical characteristics of the MC compared to the compounds developed here, likewise it was demonstrated that the characteristic chitosan fibers are distinguished with greater precision in the compound with 6% w/v of AC. On the other hand, the FTIR spectra show that the treatments present the characteristic bands of microbial cellulose and chitosan, so we have the expected crosslinking. However, there is still work to be done to achieve a complete interpretation of the spectra obtained after the adsorption process where the cellulose was already in contact with glyphosate. Further research is required to fully understand the interaction between the commercial glyphosate molecule, water, and microbial cellulose compounds. With this work we can then propose, with the investigation of these compounds based on microbial cellulose with chitosan and citric acid, to evaluate the adsorption capacity with glyphosate solutions at different pH values close to neutrality and even with other pesticides.

Annexes

Annex 1. Modified casting method

An esterification reaction was carried out by means of a heat treatment where citric acid was used as a crosslinking agent and sodium phosphate as a catalyst.

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A 1.5% chitosan solution with citric acid in distilled water was prepared and 2.3% w/v NaH2PO4 was added as a catalyst for the esterification reaction. Subsequently, a heat treatment was carried out at 70°C/5 min, during which the phosphate was added and it was dry-cured in an oven at 130°C/3 min.

Annex 2 Calibration curve

The registered absorbances of the UV/Vis spectrophotometer were used to create a calibration curve reported in graph 7, where we observed a coefficient of determination of 0.9958.

Graph 7 Calibration curve of commercial glyphosate concentration

Annex 3. Spectroscopy: FTIR-ATR

The region of infrared radiation is frequently divided into three zones: near-infrared of 1.0-2.5 μm (10,000 - 4,000 cm-1), mid-infrared of 2.5-50 μm (4,000 - 200 cm- 1) and far infrared of 50-1000 μm (200 - 10 cm-1). The result obtained from the analysis by infrared spectroscopy is an infrared spectrum of the analyzed sample.

Annex 4. SEM

The images of an electron microscope are obtained by detecting, processing and visualizing the signals resulting from the interactions between a beam of high energy electrons with matter. In a scanning electron microscope, the image is obtained from the signals emitted by the sample and is formed as the electron beam moves over a portion of its surface.

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