UNIVERSIDAD REGIONAL AMAZÓNICA IKIAM
FACULTAD DE CIENCIAS DE LA VIDA
CARRERA EN BIOTECNOLOGÍA

DEVELOPMENT OF IRON-DOPED BACTERIAL CELLULOSE FROM *ILEX GUAYUSA* AND ITS APPLICATION IN THE REMOVAL OF CADMIUM FROM AQUEOUS SOLUTIONS

Proyecto de investigación previo a la obtención del título de:

INGENIERA EN BIOTECNOLOGÍA

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Napo-Ecuador
2023

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AGRADECIMIENTO

Mi más sincero agradecimiento a mi tutora, Yanet Villasana, por su confianza en mí y por dedicar su tiempo y conocimientos para guiar este trabajo. Gracias a su orientación experta y su constante motivación, he podido crecer académicamente y enfrentar los desafíos con determinación.

También extender mi gratitud al docente Carlos Navas por brindarme la oportunidad de realizar mis ensayos en su universidad. Su orientación fue fundamental para enriquecer mi investigación y ampliar mis horizontes académicos.

A todos los docentes que formaron parte de mi trayectoria académica. Sus enseñanzas y conocimientos compartidos han sido la base para mi crecimiento intelectual. A mi familia y amigos, quienes han estado a mi lado en cada paso de este camino. Su presencia, consejos y ánimo han sido fundamentales para mi crecimiento personal y académico.

DEDICATORIA

Para los dos pilares fundamentales de mi vida: mi madre y mi padre. Sin su amor incondicional, apoyo constante y sacrificio, este camino no habría sido posible. Su guía, aliento y valores han forjado la persona que soy hoy. Cada paso en esta carrera ha sido gracias a su dedicación y ejemplo. Este logro es tan suyo como mío.

Este trabajo también quiero dedicarlo a Lyah, mi fiel compañera de cuatro patas. Durante todo este recorrido académico, ella ha sido una fuente constante de alegría y apoyo. Lyah, tú también te gradúas conmigo y, aunque no puedas entender completamente este logro, sé que tu amor y compañía han sido un pilar fundamental en este camino.

A mi madre, a mi padre y a Lyah, les dedico con profundo amor y gratitud esta tesis. Su amor incondicional y apoyo inquebrantable siempre serán mi mayor inspiración.

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RESUMEN

La presencia de metales pesados en las masas de agua es un problema medioambiental y sanitario mundial, que afecta especialmente a la vulnerable región amazónica. En Ecuador, el 35% de los recursos hídricos contienen niveles peligrosos de contaminantes, lo que supone una amenaza inminente para las comunidades locales. En este estudio, se obtuvieron películas de celulosa bacteriana utilizando un sustrato basado en una planta ancestral llamada guayusa (*Ilex guayusa*) como alternativa eficiente y prometedora para su producción en la región. Se desarrollaron membranas con diferentes porcentajes de hierro (Fe) para la remoción de iones cadmio (Cd^{2+}) de soluciones acuosas. Los resultados de la caracterización revelaron que los 4 tipos de muestras de celulosa bacteriana exhibieron espectros FTIR consistentes, con ligeras variaciones en la región de la huella dactilar y en la región de 700-450 cm^{-1} correspondientes a la presencia de grupos Fe-O. Además, las imágenes SEM-EDS confirmaron la presencia uniforme de partículas de hierro en la estructura de la celulosa bacteriana, con diámetros que oscilaban entre 2 µm y 2,5 µm en la superficie. Los resultados del espectrómetro de absorción atómica indican que las membranas con distintos porcentajes de hierro (1%, 3% y 5%) presentan mejores propiedades de adsorción que el control. En particular, la celulosa con un 3% de hierro demostró el mejor rendimiento en todas las dosis de Cd^{2+} evaluadas (1 ppm, 1,5 ppm, 2,5 ppm, 3,5 ppm, 5 ppm y 7,5 ppm), alcanzando porcentajes de eliminación de hasta el 70% de los iones con una capacidad de adsorción de 2,6175 mg/g en las concentraciones más altas. Las membranas de celulosa con un 3% de hierro podrían utilizarse como materiales de adsorción para la eliminación eficaz de iones de cadmio en medios acuosos contaminados con metales. Este estudio ofrece una alternativa innovadora, sostenible y eficiente para la remediación de aguas contaminadas en una región donde la minería, la extracción y otras actividades humanas han tenido un impacto ambiental significativo.

Palabras clave: celulosa bacteriana, *Ilex guayusa*, cadmio, adsorción, remediación de agua

ABSTRACT

The presence of heavy metals in water bodies is a worldwide environmental and health problem, particularly affecting the vulnerable Amazon Region. In Ecuador, 35% of water resources contain dangerous levels of contaminants, posing an imminent threat to local communities. In this study, bacterial cellulose films were obtained using a substrate based on an ancestral plant called guayusa (*Ilex guayusa*) as an efficient and promising alternative for production in the region. Membranes with different percentages of iron (Fe) were developed for the removal of cadmium ions (Cd^{2+}) from aqueous solutions. Characterization results revealed that the 4 sample types of bacterial cellulose exhibited consistent FTIR spectra, with slight variations in the fingerprint region and in the 700-450 cm^{-1} region corresponding to the presence of Fe-O groups. In addition, SEM-EDS images confirmed the uniform presence of iron particles in the bacterial cellulose structure, with diameters ranging from 2 µm to 2.5 µm on the surface. Atomic absorption spectrometer results indicate that membranes with different percentages of iron (1%, 3%, and 5%) exhibit improved adsorption properties compared to the control. In particular, cellulose with 3% iron demonstrated the best performance across all evaluated Cd^{2+} doses (1 ppm, 1.5 ppm, 2.5 ppm, 3.5 ppm, 5 ppm, and 7.5 ppm), achieving removal percentages of up to 70% of the ions with an adsorption capacity of 2.6175 mg/g at higher concentrations. Bacterial cellulose membranes with 3% iron could be utilized as adsorption materials for the efficient removal of cadmium ions in metal-contaminated aqueous media. This study offers an innovative, sustainable, and efficient alternative for the remediation of contaminated waters in a region where mining, extraction, and other human activities have had a significant environmental impact.

Keywords: bacterial cellulose, *Ilex* guayusa, cadmium, adsorption, water remediation

INTRODUCTION

Access to safe drinking water remains a global issue to date, according to information collected in 2019, approximately 2.2 billion people consume water under unsafe sanitation and hygiene conditions [1]. Among the leading causes of limited access to safe water is illegal mining, characterized by the indiscriminate extraction of metals with little regard for environmental consequences and sustainable practices. As a result, generating a high concentration of pollutants from the subsoil to the biosphere [2-4].

In the Amazon rainforest, one of the world's most vital and biodiverse regions, this threat is particularly acute [5, 6]. Mining activities have become major sources of pollution, leading to the release of toxic by-products into the region's hydrological sources [6, 7]. This indiscriminate extraction of metals generates a high concentration of pollutants, including mercury (Hg), chromium (Cr), cadmium (Cd), and copper (Cu), impacting the delicate balance of the Amazonian ecosystem [7, 8]. The consequences are severe, adversely affecting both flora and fauna, and posing a significant risk to the health of human populations relying on these water sources for their daily needs [9, 10].

The high concentration of Cd in drinking water is of particular concern, with concentrations of Cd near or above 5 µg/L have been detected [11]. This toxic metal is known to have severe adverse effects on human health, including damage to the kidneys, bones, and respiratory system, with the potential for long-term and even irreversible consequences [12]. Recognizing these serious implications, several countries and international organizations, the United Nations Children's Fund (UNICEF), the Agency for Toxic Substances and Disease Registry, and the Environmental Protection Agency, have identified it as a priority pollutant, deserving immediate attention and action [13, 14]. Hence, there is an urgent need to study and implement new alternatives to mitigate it impact on Amazonian water sources [15].

In answer to this challenge, the adsorption process is presented as a simple and effective method to remove heavy metals from polluted water [16]. It is considered a physicochemical process of accumulation of molecules on the surface of an artificial or biocompatible solid material [17]. The most outstanding characteristics of this technique are its high purification effect and low emission of harmful by-products, as well as its

bioavailability and simplicity of application [18, 19]. Hence, the development of membranes with high adsorption capacity, low toxicity and efficient separation are presented as the most suitable alternative for bioremediation.

In the last few years, the use of films with iron sulfide (FeS) and graphene oxide (Fe_3O_4) particles significantly reduces the mobility, toxicity and availability of pollutants in water [20]. However, a major problem of using them, despite requiring little energy and having low cost, is that particles can be inhaled or ingested by humans affecting their health in the long term [21]. As an alternative, it has been proposed the development of membranes based on bacterial cellulose (BC) for wastewater treatment and heavy metal removal [22, 23] with at least 93% purity, biocompatibility, and zero toxicity to humans and the environment [24].

The membrane produced from Kombucha SCOBY (a colony of acetic bacteria and yeast species) can be used for the purification of bodies of water. The advantage of this type of cellulose lays in the adsorption capacity of inorganic pollutants by the microorganisms present in the biofilm, which use the carbon source for their growth and energy [25]. According to Mousavi et al. [26], this community of bacteria and yeast has shown an efficiency of over 90% in the removal of heavy metals, making it an effective solution to contamination, and a great alternative to the use of commercial cellulose and synthetic membranes doped with FeS or Fe_3O_4.

On the other hand, the proper selection of the culture medium is a critical factor to ensure optimal growth of SCOBY during the fermentation [27]. For these reasons, we propose the use of Guayusa tea as a substrate that provides a high concentration of antioxidants and alkaloids, making it a good source of essential nutrients for cellulose production [28]. Also, as a native and ancestral plant, *Ilex guayusa* Loes. contributes to a sustainable and environmentally friendly practice, offering an efficient, cost-effective, and scalable alternative for the Amazon region and the indigenous communities involved in its production.

Moreover, several studies have confirmed that the particle doping in the matrix of the microbial consortium increases its efficiency in multiple applications [29-31]. Therefore, iron (Fe) can be used to efficiently remove toxic waste from the environment by acting as a reducing agent and cleaving harmful chemical compounds found in aqueous

solutions [32]. Authors such as Mukherjee et al. [33], Rajput et al. [34], and Sharma et al. [35] state that the use of metallic particles in cellulose increases the performance of adsorption processes.

Based on all this context, this work aims to evaluate, at the laboratory scale, the level of bacterial cellulose production on different substrates and the capacity of cadmium removal by BC doped with different contents of iron particles, comparing the performance between simple biofilm and the doped membrane. For this purpose, the adsorption capacity was analyzed and compared in terms of their spectroscopic and elemental characteristics. In fact, SCOBY presents an affordable cost, managing to increase its biomass with little funds to develop a very effective water purification system.

METHODS

Preparation and fermentation of bacterial cellulose

Production of Kombucha SCOBY was carried out on three different substrates (green tea, black tea, and guayusa tea) to compare biomass production levels and choose the most viable for application as a Cd adsorbent in the Ecuadorian Amazon. For this, the process of preparing microbial cellulose in green tea and black tea substrates was developed following the methods proposed in the research of Ahmed et al. [36] and Abou-Taleb et al. [37], and adapted for production on guayusa tea.

Two grams of each substrate (green tea, black tea, and guayusa) previously washed with distilled water were added to 500 milliliters of boiling water for 15 minutes and then removed by filtration. 70 g/L of commercial sucrose were dissolved in each filtered infusion and allowed to cool to room temperature. Each solution was then transferred to pre-sterilized 500 milliliter bottles in the oven at 100°C for 45 minutes. Every extract was inoculated with a daughter cellulose from a previous fermentation in green tea (produced under the same conditions), covered with a clean wipe, and fixed with elastic bands. Finally, fermentation was carried out under controlled temperature conditions at 25 ± 2°C for 21 days. Records of growth and proliferation of each microbial cellulose were taken every 2 days to select the substrate that showed the best performance.

Once this period was over, the data obtained were analyzed and the most competitive substrate in the production of SCOBY Kombucha for the region was chosen. The process described above was then repeated for the production of BC on the chosen substrate.

Bacterial system doping with iron particles

To functionalize the system with particles, the methods used by Pal et al. [38] were adapted to the production of iron nanoparticles. Four cellulose disks were previously separated and weighed in Petri dishes that were sterilized in advance in an oven at 100°C for 45 minutes. Next, the biofilm was pre-treated with 0.5N sodium hydroxide for five times to remove bacteria and external residues. The cellulose membranes were immersed for 24h in ferric chloride ($FeCl_3$) solutions in a 2:1 ratio of iron per mass weight, respectively. After 5 minutes of contact, the BC presented a light-yellow color, indicating the adherence of the ferric chloride. The final concentration of each solution varied from 1% Fe, 3% Fe and 5% Fe. Additionally, the membrane without $FeCl_3$ solution was immersed in 30 mL of distilled water to be used as a control sample.

After exposure to the $FeCl_3$ precursor solution, the cellulose bacterial membranes were washed and stored in distilled water. Then, for the synthesis of the iron particles, 2 drops of the reducing agent, sodium borohydride [2 mM], were added to each pretreated BC. A color change was observed, from yellow to reddish-brown, which is an indication of iron particle formation in the matrix. Lastly, the samples were washed with distilled water to remove any residue or excess solution, and then they were placed in beakers with guayusa tea prepared under the same initial conditions. The beakers were used to maintain the BC samples in fermentation until the subsequent treatments were carried out.

Characterization of bacterial cellulose

Prior to the characterization tests, the disks were washed with a 0.5N NaOH solution and then immersed in distilled water to remove any residues present in the medium. Subsequently, they were dried in an extraction chamber for 72 hours or until a constant weight was reached. The resulting dry mat had a thin, whitish leaf-like appearance. ATR-FTIR spectra were collected using a Fourier transform spectrometer (Agilent Cary 630).

Each type of dried BC (control, 1% Fe, 3% Fe, and 5% Fe) was cut into 1 cm pieces before being introduced into the equipment. Spectra were obtained in the range of 4000-480 cm^{-1} for each treatment type [39, 40]. These data analyze and represent the infrared radiation transmission through the sample as a function of frequency or wavelength. The results were compared with the spectrometer's library of known spectra to differentiate specific peaks corresponding to the chemical groups and characteristic bonds of bacterial cellulose. Generally, the predominant IR peaks identify the presence and purity of the biopolymer in the different samples.

Scanning electron microscopy (SEM) and energy dispersive X-ray microanalysis EDS (JEOL JSM-7401F microscope) were used for the morphological and elemental analysis of the BC films. Prior to observation, all samples were fixed on aluminum pins coated with a carbon stick and treated with colloidal graphite to ensure conductivity. Finally, the four types of bacterial cellulose (Control, 1% Fe, 3% Fe, and 5% Fe) were characterized using an electron bombardment at 15 kV and working at low vacuum pressure. The presence of the most common elements in cellulose, such as carbon ©, oxygen (O), sodium (Na), calcium (Ca), and potassium (K), and the detection of iron (Fe) for the treated samples, are expected to be verified through this analysis.

Evaluation of cadmium adsorption capacity

The biosorption of Cd (II) from an aqueous solution using a dried BC mat was investigated in batch adsorption experiments, according to the methodology proposed by Razmovski & Šćiban [41]. Dry cellulose samples at a rate of 50 mg per 25 ml of Cd (II) solution at 25 °C on a rotary shaker at 200 rpm for 24 hours. It should be noted that in this research, a non-adjusted pH was employed during the process, as it has been evidenced that it enhances the adsorption of the target metal ions [42]. After exposure, samples were collected and filtered using hydrophobic 0.22 µm PFTE syringe filter with a 0.984 inch diameter pore size.

The concentration of Cd (II) ions after biosorption was determined using flame atomic absorption spectroscopy (Analytik Jena, Contra 700).

The adsorption efficiency was expressed as:

$$Removal\ (\%) = \left(\frac{C_o - C}{C_o}\right) \times 100$$

where Co and C are the initial and residual concentrations of the Cd (II) ion, respectively.

In addition, the amount of Cd (II) metal ions adsorbed per unit of specific adsorbent mass was calculated as:

$$q\ (mg \cdot g^{-1}) = (C_o - C) \cdot \frac{V}{m}$$

Where V corresponds to the volume of solution and m corresponds to the mass of adsorbent.

Furthermore, for the analysis of the adsorption characteristics of Cd by the iron-doped membranes (1% Fe, 3% Fe, and 5% Fe) and the control membrane, the PUPAIM functions (version 0.3.1) in R were used [43]. This package offers the capability to fit adsorption data to 55 available isotherm models using nonlinear functions. For this study, the most commonly used mathematical models for solid surfaces were considered, including Langmuir [44], Freundlich [45], Harkins-Jura [46], and Henry [47].

RESULTS

Production and formation of cellulose

After 21 days of fermentation, the average BC yield was 6.81 g, 5.63 g, and 4.70 g for guayusa, green tea, and black tea substrates, respectively (Fig. 1A). Statistical analysis using ANOVA indicated no significant differences among the three substrate types, suggesting that guayusa can be a competitive alternative medium for BC cultivation in the Amazon region (Fig. 1B). Additionally, when the guayusa concentration increases to >7 g/L at room temperature of 25 ± 2°C, it can have an inhibitory effect by decreasing the rate of BC formation. According to this research, the general idea was to obtain 4 discs weighing at least 15 g each, exclusively produced on guayusa substrate, for subsequent iron particle doping purposes.
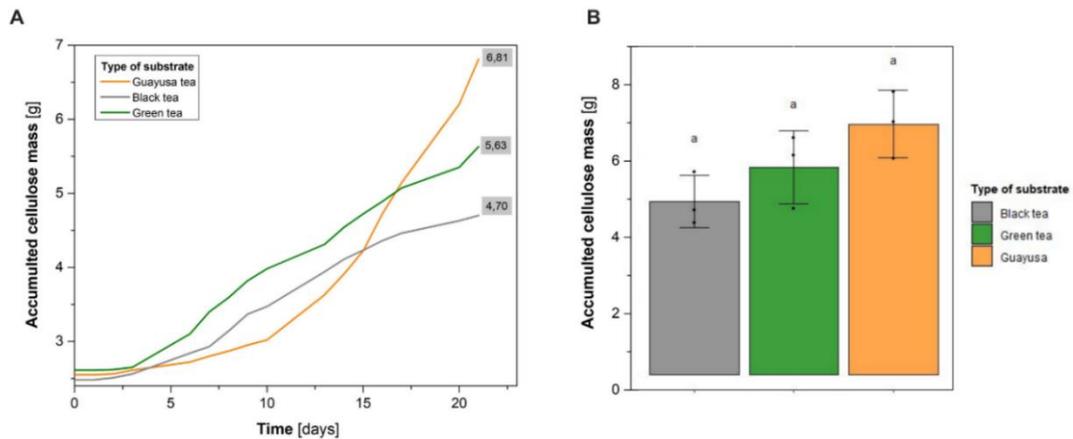
Figure 1: Amount of BC produced on the three substrates. A: Acumulative mass over 21 days;
B: Bar graph of ANOVA and Tukey's test, performed at 5% significance level.
Realizado por: Ortiz, Antonella, 2023.

Functionalization of the system with iron particles

Different types of membranes were obtained (Fig. 2). Visually, significant changes in color intensity and an increase in iron particle formation within the cellulose matrix were observed as the concentration of $FeCl_3$ increased. In contrast, the control membrane remained constant throughout the entire process. Furthermore, it was verified that after a brief inhibitory period of 72 hours, the four membranes could continue with the fermentation and BC production process, ensuring the effectiveness of microorganisms in the presence of chloride in the medium.
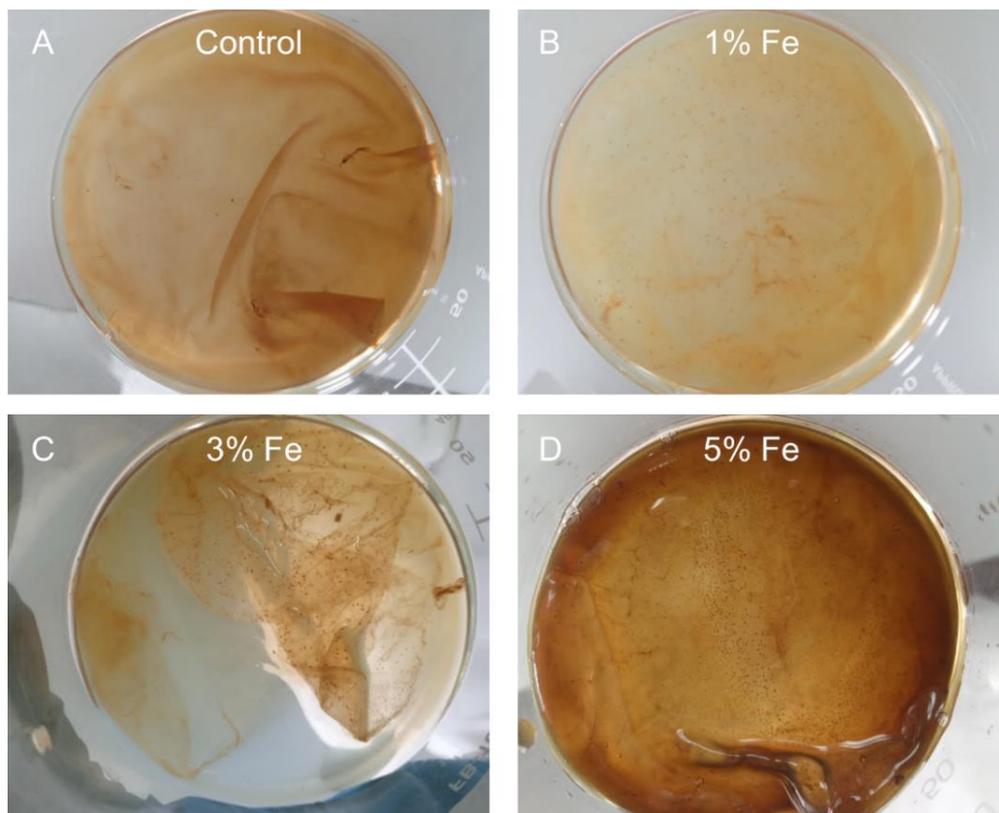
Figure 2: Membranes doped with iron particles: (A) control cellulose, (B) 1% Fe, (C) 3% Fe and (D) 5% Fe.
Realizado por: Ortiz, Antonella, 2023.

Characterization of the obtained membranes

The FTIR spectra of BC exposed to each type of treatment remained almost indistinguishable (Fig. 3), with only a slight change in the intensity of the peaks in the fingerprint region (1500-400 cm^{-1}) and the peaks in the region 700-450 cm^{-1} corresponding to the presence of Fe-O bonds obtained in the guayusa medium with the addition of ferric chloride and sodium borohydride.
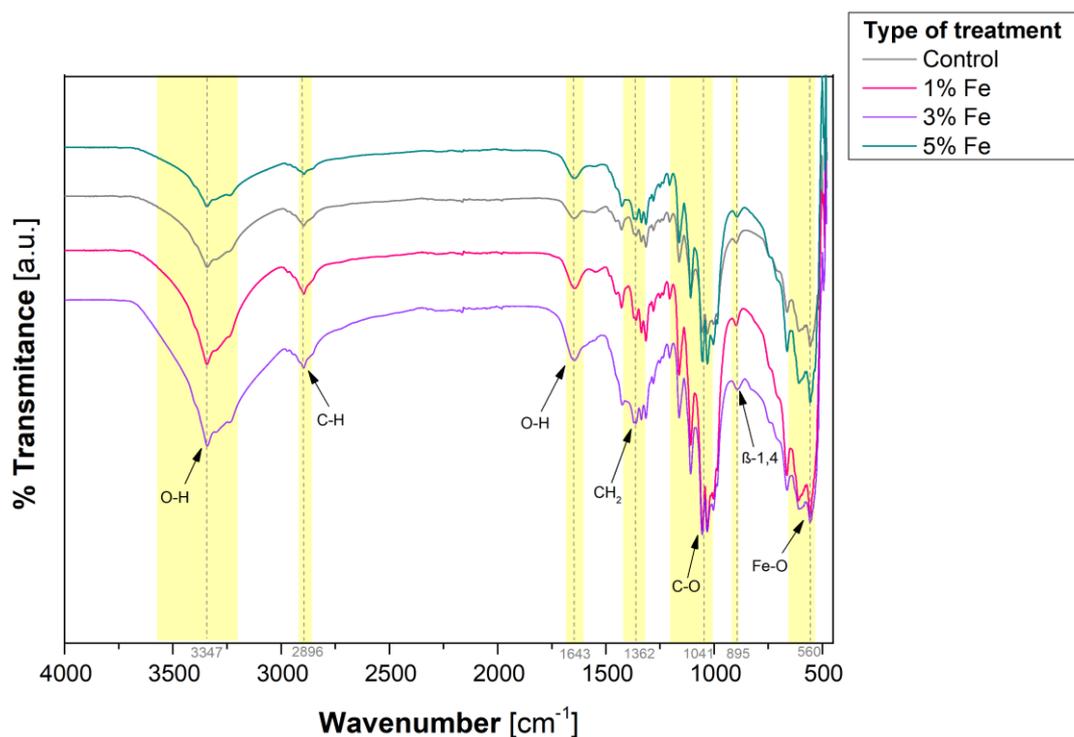
Figure 3: FTIR spectra of BC (4000 to 480 cm^{-1}). There are 4 BC sequences that were exposed to different concentrations of iron: Control, 1% Fe, 3% Fe and 5% Fe.
Realizado por: Ortiz, Antonella, 2023.

Elemental analysis of the control, 1% Fe, 3% Fe, and 5% Fe samples revealed that the main elements are oxygen (O) followed by silicon (Si), potassium (K), and calcium (Ca). Small amounts of boron and sodium are observed, indicating residues of the sodium borohydride, and since the concentrations are very low, they do not negatively affect adsorption results. Furthermore, the presence of aluminum (Al) is largely due to the pin used in the technique, while carbon (C) reflects not only the EDS stick but also the bacterial cellulose. Now, focusing on the presence of Fe in the cellulosic matrix, its specific percentage can be seen in Table S2 in supplementary information. The concentration of iron in the BC varies according to the amount with which they were initially doped, showing the efficiency in the impregnation of the fibers.
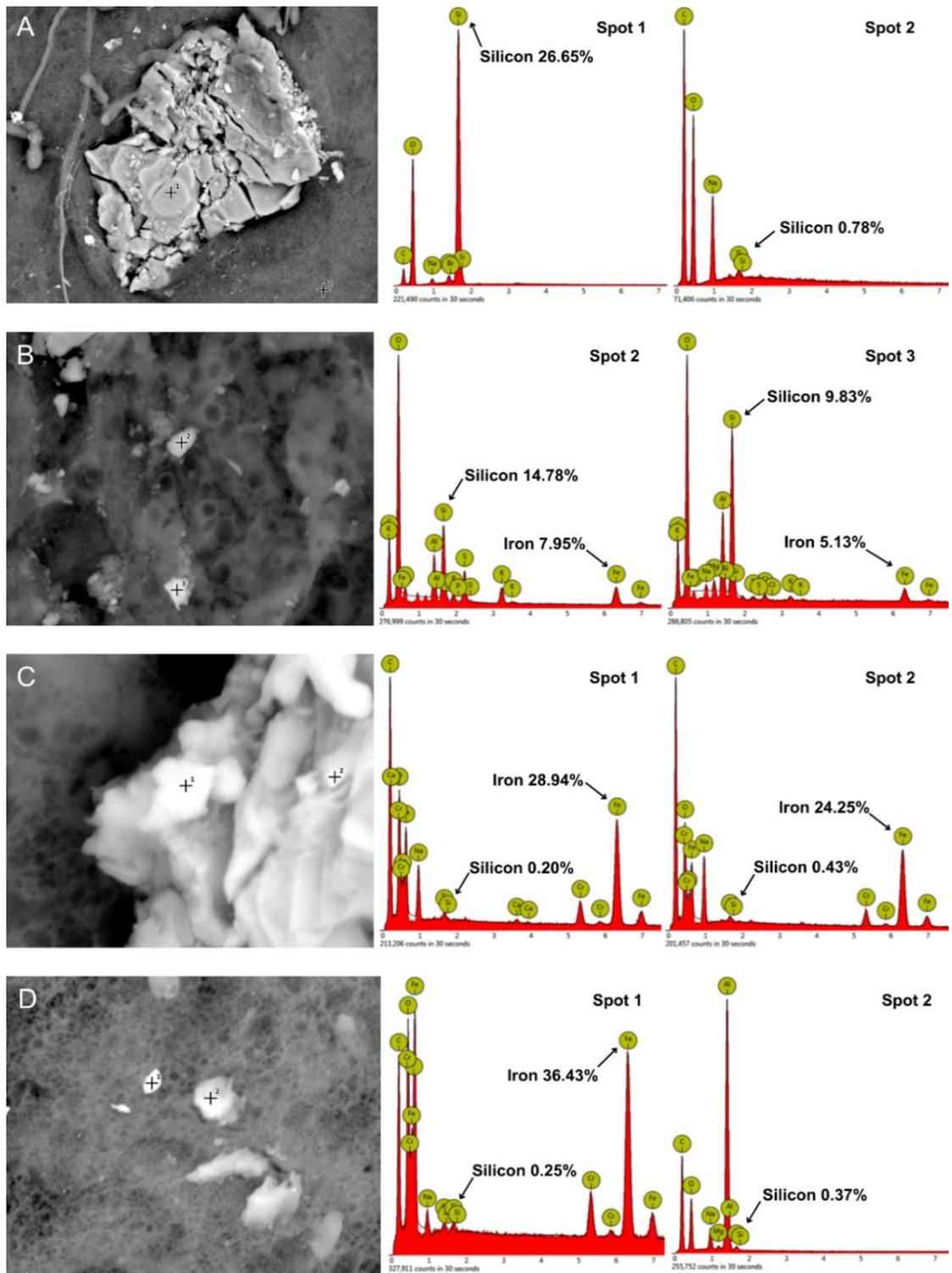
Figure 4: SEM-EDS images of: Control (A), 1% Fe (B), 3% Fe (C), and 5% Fe (D). Two points (marked with a **+**) correspond to EDS elemental analysis in each graph.
Realizado por: Ortiz, Antonella, 2023.

Cd adsorption performance

Our research showed Cd removal percentages of 97.22% for the control cellulose, 93.79% for 1% Fe, 95.52% for 3% Fe, and 96.91% for 5% Fe, confirming a high efficiency in the removal of the heavy element by BC at low concentrations of this metal (see Figure 5A). However, to understand the behavior of membrane interaction with higher metal concentrations, five additional tests were conducted to evaluate the Cd adsorption capacity.

Thus, the four types of membranes were subjected to synthetic cadmium solutions with concentrations of 1.5, 2.5, 3.5, 5, and 7.5 parts per million, values that were selected to emulate real environmental scenarios. It can be observed that the 3% Fe-doped membrane showed the best performance in all cases, achieving removal percentages of up to 70% of the ions (Fig. 5F) and an adsorption capacity of 2.748 mg/g at the highest concentration (Fig. 6). In general, the ANOVA statistical test and the Tukey's post hoc test indicate significant differences among the four treatments, resulting in a minimum of three and a maximum of four distinct groups for each Cd concentration studied. The assumptions of normality and homogeneity required for ANOVA are satisfied in all cases, see Table S3 in supporting information.
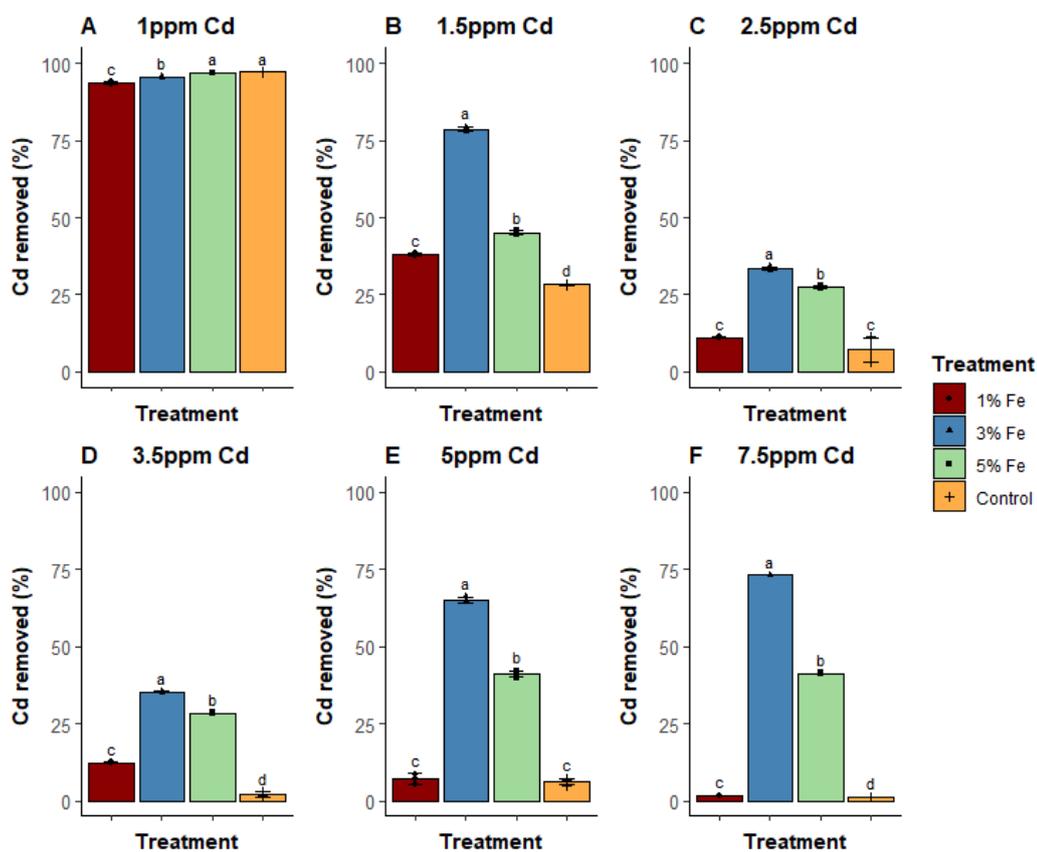
Figure 5: Removal performance as a function of treatment- A, B, C, D, E and F correspond to initial Cd concentrations of 1, 1.5, 2.5, 2.5, 3.5, 5 and 7.5 ppm, respectively.
Realizado por: Ortiz, Antonella, 2023.

Cd adsorption isotherms

The Freundlich and Harkins-Jura models stand out for exhibiting the best coefficient of determination values (R-squared = 0.82), a relative standard error below 0.27, and an adjusted R-squared value of 0.81 for 5% Fe BC (Table S4). However, the obtained data for adsorption on 3% Fe BC did not fit any of the models tested with the PUPAIM package if a significance level of 5% is considered for the models. This indicates that multiple and complex interactions between adsorbate and adsorbent may exist in this type of membrane, making it challenging to accurately describe the phenomenon using a single isotherm model.
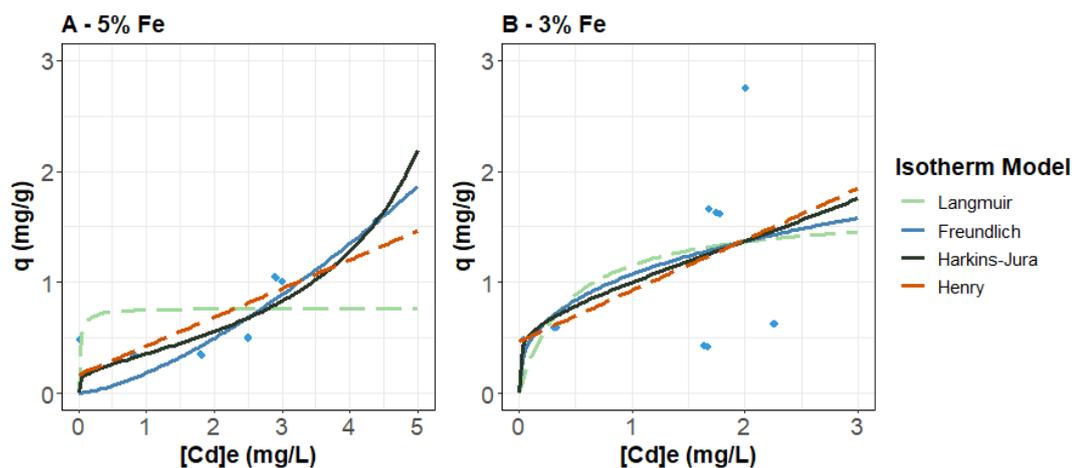
Figure 6: Isotherm models were applied to the membrane types that showed the best Cd removal performance. A: BC with 5% Fe; B: BC with 3% Fe.
Realizado por: Ortiz, Antonella, 2023.

DISCUSSION

Bacterial cellulose production

During the Kombucha fermentation process there are several physical and chemical factors that directly influence the growth of BC. Essential factors such as pH, temperature, amount of oxygen, dissolved CO_2, precursor supply, as well as the nature and composition of the medium [48-51]. Any variation in these parameters can affect the yield and amount of cellulose. Thus, different types of substrates, sugar concentration, fermentation time and composition of the parent cellulose can explain the differences in the composition and, therefore, in the type of biomaterial to be obtained.

Usually, Kombucha beverage is obtained from a carbon source such as sucrose and a nitrogen source such as green tea; however, when using guayusa tea as a precursor substrate, a remarkable production of cellulose can be observed with 6.81 g after 21 days of fermentation (Fig. 1). The main component that could determine this difference in growth on each substrate is the caffeine content in the medium [52, 53], which provides a source of energy for the bacteria and yeasts to carry out the fermentation process.

In general, green tea contains about 30-50 mg of caffeine per 8-ounce cup, whereas black tea may contain about 40-70 mg per cup [54]. Guayusa tea, on the other hand, may contain higher levels of caffeine, some sources [55, 56] suggesting up to 90 mg per cup. Thus, the specific concentration of alkaloids present in this substrate is a key factor to being competitive in BC production against the most common substrates.

As explained above, the purpose of this research was to select the cellulose production methods for the Amazon that demonstrate a satisfactory amount of biomass in the established fermentation time, but not to obtain the beverage as such. For this reason, in the following trials the cultivation and fermentation of bacteria was carried out in a medium of 4 g of guayusa per liter of distilled water, which has been shown to have a positive effect on the growth of BC due to its caffeine, theobromine and theophylline content [55]. It was also demonstrated that BC synthesis increased after 15 days in static culture, similarly to what is mentioned in several studies [57, 58] that the optimum time to ensure high cellulose production is between 10 to 15 days.

ATR-FITR spectroscopic characterization of BC

In the first region of the FTIR spectra, from 4000 to 2500cm^{-1} corresponding to single bonds two main peaks can be observed, where, the 3347 cm^{-1} peak characterizes the stretching vibrations of the free hydroxyl (-OH) functional group and the 2896 cm^{-1} peak describes the asymmetric stretching vibrations of methylene (-CH$_3$). On the other hand, in the 2000 - 1500 cm^{-1} region that mainly characterizes double bonds, there is a predominant peak at 1643 cm^{-1} that is assigned to the OH groups strongly hydrogen bonded to the adjacent cationic surface or water molecules [59].

Moreover, for the 1500 - 450 cm^{-1} region known as the fingerprint, four predominant peaks are observed. The peak at 1362 cm^{-1} indicates the methylene chain (-CH$_2$-) bending vibration, the peak at 1041 cm^{-1} is related to C-O-C and C-O stretching vibrations in glucose monomers [39]. On the other hand, the peak at 895 cm^{-1} corresponds to the vibrations of the β-1,4-glycosidic bonds and the peak at 560 cm^{-1} can be associated with the presence of Fe-O, which would confirm that the nanoparticles were successfully synthesized over the bacterial cellulose [60]. FTIR is usually a poor technique for detecting the presence of metals such as Fe in a sample, so it was necessary to perform an elemental analysis to verify the presence of iron.

The combination of both techniques allows a complete characterization of the structure and composition of the bacterial cellulose, which is essential for the development of advanced material.

SEM and elemental EDS characterization

The structure of bacterial cellulose doped with iron can be confirmed in Fig 4, in general they present a smooth and homogeneous texture, with a slightly shiny appearance. In addition, areas of higher density can be observed in the cellulose structure, indicating the presence of added substances [61]. Thus, Fe particles tend to have irregular or elongated shapes with a bright tone, and distribution is expected to be highly variable, since authors have stated that iron is usually dispersed individually in the matrix and randomly oriented within the structure of bacterial cellulose [62].

In general, the EDS elemental analysis of the samples shows a high concentration of oxygen and carbon, which are the main components of glucose molecules, the basic components of bacterial cellulose. During the synthesis of this polymer, the carbon (C), hydrogen (H), and oxygen (O) elements present in the pre-culture medium of guayusa are incorporated into the structure through glycosidic bonds, forming the BC chains [63].

The high silicon content that is verified in all the membranes may be due to the interaction between the BC and the growth source (Fig. 4). In several investigations it has been found that plants belonging to the genus Ilex can contain around 0.5 to 1.5 % of Si in dry weight, a higher concentration than most of the floral genera [56]. Consequently, the presence of this metalloid in the cellulose culture medium has proven to be an essential cofactor for its synthesis, improving the resistance and rigidity of the fibers [64].

According to the data presented in Table S2, the samples containing 1% Fe, 3% Fe, and 5% Fe exhibited concentrations of iron of 7.95%, 28.94%, and 36.43%, respectively in specific spots during the EDS analysis. The analytical results provide evidence of a direct correlation between the percentage of iron incorporation and the concentration found in the cellulose. Therefore, it is essential to ensure consistency between theoretical and experimental results in SEM-EDS analysis [65, 66].

This finding holds great importance for understanding and optimizing the loading of iron in BC for specific applications that require a high concentration of metals in the composite material.

Percentage of Cd removal by BC

Initial inspection of Fig. 5A reveals an interesting occurrence: three mean-differentiated groups are presented at the initial concentration of 1 ppm. Where the control sample and 5% Fe constitute cluster a, while the 3% Fe and 5% Fe samples represent cluster b and c, respectively. In addition, it can be observed that all four membranes present removal percentages higher than 90%. This can be explained by the low amount of Cd ions present in the solution, which is insufficient to saturate the adsorption sites of the material, allowing all Cd ions to find a place to anchor [67, 68].

In the case of Fig. 5C and 5D, with initial concentrations of 2.5 ppm and 3.5 ppm, despite the significant differences, the removal percentage drops drastically to about 35%. This is mainly due to the rapid saturation of the active sites of the cellulose membrane caused by competition from ions present in similar amounts [41]. At the beginning of the contact between the adsorbent and adsorbate, hydronium ions (H_3O^+) are released into the solution, and their presence is considered an influential factor in metal adsorption processes [20, 69, 70]. Also, considering Fig. 5E, with an initial concentration of 5 ppm, even an increase in the percentage of removal is observed, its behavior is related to the preceding cases. The decrease in competition between ions allows the active sites to become more specific in the adsorption of Cd, nevertheless the threshold necessary for normal and complete adsorption has not yet been reached [71, 72].

Now, for initial concentrations of 1.5 ppm and 7.5 ppm of Cd, significant differences were observed between the control membrane, 1% Fe, 3% Fe, and 5% Fe, respectively (Fig. 5B, 5E, and 5F). High removal percentages of approximately 70% are observed, with the 3% Fe membrane exhibiting the best performance. It is important to note that at these concentrations, the adsorbent sites of BC have started to saturate with the desired Cd^{2+} ions [73]. The main reason for this excellent performance is related to the formation of a higher proportion of Cd complexes in solution, which are more easily removable by the membranes, thus increasing the removal efficiency [74, 75].

Regarding the percentage of iron impregnated in the membrane, it can be observed that the cellulose with 3% Fe exhibits the highest removal percentage, reaching 70% at the highest evaluated concentrations. The difference in removal percentage can be attributed to the number of active adsorption sites available during the test and the uniform distribution of Fe within the BC matrix [25]. Although one might assume that a higher Fe content would result in better performance, the opposite effect is observed. Excessive addition of iron particles can lead to increased agglomeration, thereby reducing the available active surface for the adsorption of the target metal [76-78]. Furthermore, it is possible that the membrane doped with 3% Fe possesses a more homogeneous distribution of Fe particles, ensuring better exposure of the adsorption sites and, consequently, enhancing the efficiency of Cd removal [79].

Cd adsorption isotherms by BC

Adsorption isotherms are graphical representations showing the relationship between the amount of Cd adsorbed on the BC surface (q) and the equilibrium concentration of Cd with bacterial cellulose (Ce) [80]. In this sense, the results for BC doped at 5% Fe (Fig. 6A) fit the Freundlich and Harkins-Jura isotherm model, suggesting that adsorption on the BC system follows a nonlinear relationship. This behavior may be influenced by several factors, such as the heterogeneity of the adsorption sites, the variability of the adsorption energy and the interaction between the adsorbed molecules [81, 82]. In addition, the value of the constant n (Table S4), being less than 1, indicates less specific and more distributed adsorption on multiple active sites of different sizes and energies in the BC matrix. This suggests that the bacterial cellulose has a porous surface with versatile and efficient adsorption capacity for the interaction with Cd [20, 83].

On the other hand, the results of the isotherm analysis for BC at 3% Fe (Fig. 6B) show very low R-squared values, which makes it difficult to define the adsorption behavior on this membrane. It should be noted that the adsorption models are based on assumptions and simplifications that do not fully reflect the complexity of the process in the BC matrix [41]. In other words, the BC system loaded with 3% Fe can be influenced by the structure of the adsorbent, the nature of the adsorbate, the properties of the solution, the equilibrium conditions, among others [84]. With the general idea obtained from the behavior reflected by the membrane at 5% Fe, it can be deduced that cellulose has numerous adsorption sites, which makes it difficult for the main adsorption models to

fully describe the behavior in BC at 3% Fe. In these cases, research suggests the development of physics-based statistical models to fit and interpret adsorption data of liquids on solid surfaces, as they provide more useful models to explain adsorption phenomena at both the macroscopic and microscopic levels [85].

Overall, the analysis of the adsorption data using isotherm models provides valuable insights into the specific adsorption characteristics of Cd in the bacterial cellulose system (Table S4). The parameters obtained with the PUPAIM package in R for the solid-liquid isotherm types offer a comprehensive overview of the adsorption phenomenon [86, 87]. Specifically, the Langmuir model highlights the maximum adsorption capacities, while the Freundlich model reveals the heterogeneity of the active sites for adsorption [88]. Furthermore, the Harkins-Jura and Henry isotherm models describe the adsorption at the interface and the interaction between the adsorbate and adsorbent [89, 90]. In future research, investigating the mechanisms of adsorption kinetics would be intriguing to gain a more profound and detailed understanding of the process.

CONCLUSIONS

SCOBY membranes were synthesized using different substrates (green, black and guayusa tea) revealing that the use of guayusa tea as a culture medium is a viable alternative for membrane production compared to the commonly used substrates. This improvement can be attributed to the high caffeine and silicon contents present in guayusa tea leaves. Accordingly, four types of iron-doped BC membranes (Control, 1% Fe, 3% Fe and 5% Fe) were fabricated using guayusa as substrate. Characterization results of bacterial cellulose indicated consistent stability in the FTIR spectra, with slight changes in peak intensities in the fingerprint and Fe-O group regions, along with uniform distribution of iron particles in the BC structure as observed through SEM-EDS analyses. On the other hand, promising results were obtained during the evaluation of the adsorption capacity, where the initial cadmium concentration was varied while keeping the adsorbate volume and adsorbent mass constant. The biomembranes doped with 3% Fe and 5% Fe were the one that obtained the best removal capacity, reaching 73% and 41%, respectively. In the case of the 3% Fe-doped BC the Cd adsorption capacities could not be adequately explained by any well-known isotherm model, whereas for the 5% Fe-doped BC, the Freundlich and Harkins-Jura models provided a good fit to the data. These findings highlight the potential of iron-doped bacterial cellulose as a promising alternative for the removal of cadmium from aqueous media. The use of guayusa tea as a culture medium has been shown to be an interesting substrate in the region for membrane production, and the 3% Fe-doped BC membrane demonstrates remarkable cadmium adsorption efficiency. Further research is needed to elucidate the complex adsorption mechanisms involved in the 3% Fe-doped BC system and explore its practical application for cadmium remediation in the Amazon.

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SUPPLEMENTARY DATA

Table S1: ANOVA for the type of substrate in the BC production (guayusa, green, and black tea). P-values indicate that there are not significant differences.

Variable	Df	Sum Sq	Mean Sq	F value	Pr (>F)	Levene's Test		Shapiro-Wilk Test	
						F value	Pr (>F)	w	p-value
Type of Substrate	2	6.690	3.345	4.284	0.069	0.0712	0.9321	0.91149	0.3264
	6	4.684	0.781						

Realizado por: Ortiz, Antonella, 2023.

Table S2: Iron concentration in specific spots in bacterial cellulose membrane samples obtained by SEM-EDS.

Sample name	Spot number	Atomic Concentration [%]	Weight concentration [%]
1% Fe	2	2.33	7.95
	3	1.50	5.13
3% Fe	1	9.22	28.94
	2	7.32	24.25
5% Fe	1	13.61	36.43

Realizado por: Ortiz, Antonella, 2023.

Table S3: ANOVA for the 6 initial Cd concentrations (1, 1.5, 2.5, 3.5, 5, and 7.5 ppm). P-values indicate significant differences among the 4 types of membranes studied-

Cd Concentration	Df	Sum Sq	Mean Sq	F value	Pr (>F)	Levene's Test		Shapiro-Wilk Test	
						F value	Pr (>F)	w	p-value
1 ppm	3	22.086	7.362	217.3	5.29e-08	1.2261	0.3618	0.9645	0.8463
	8	0.0271	0.034						
1.5 ppm	3	4311	1437.0	4797	2.34e-13	0.214	0.8824	0.8801	0.0881
	8	2	0.3						
2.5 ppm	3	2.8e9	934464702	616	8.48e-10	0.9273	0.4707	0.9236	0.3174
	8	1.2e7	151708						
3.5 ppm	3	3.8e9	1.28e9	19490	8.62e-16	1.665	0.2507	0.8751	0.0760
	8	5.3e5	6.58e4						
5 ppm	3	7373	2457.7	1543	2.18e-11	0.2514	0.8582	0.9529	0.6809
	8	13	1.6						
7.5 ppm	3	10862	3621	498466	<2e-16	0.5765	0.6465	0.9170	0.2627
	8	0	0						

Realizado por: Ortiz, Antonella, 2023.

Table S4: Significance level of the constants, relative standard error (RSE), R2, adjusted R2, and P-value for the top four isotherm models applied to 1%, 3%, and 5% Fe.

T	Model	Constant	Value	P-value	RSE	R^2	Adjusted R^2	P-value model
5% Fe	Langmuir	Q_{max} [mg/g]	0.77	1.16×10^{-5}***	0.45	0.06	0.01	0.31
		Q_L [mg/g]	41.65	0.50				
	Freundlich	K_F	0.18	0.01*	0.26	0.82	0.81	2.72×10^{-7}***
		N	0.69	4.55×10^{-5}***				
	Harkins-Jura	A	1.36	5.35×10^{-5}***	0.21	0.82	0.81	2.65×10^{-7}***
		B	1.92	1.04×10^{-12}***				
	Henry	y0 [mg/L]	0.16	0.15	0.26	0.70	0.68	1.65×10^{-5}***
		m [L/mol]	0.25	1.65×10^{-5}***				
3% Fe	Langmuir	Q_{max} [mg/g]	1.67	0.02*	0.81	0.20	0.15	0.06
		Q_L [mg/g]	2.23	0.55				
	Freundlich	K_F	1.07	1.7×10^{-4}***	0.80	0.21	0.16	0.06
		N	2.82	0.18				
	Harkins-Jura	A	5.10	0.31	0.80	0.21	0.16	0.06
		b	2.55	0.191				
	Henry	y0 [mg/Ll]	0.47	0.21	0.80	0.21	0.16	0.06
		m [L/mol]	0.46	0.06				

Realizado por: Ortiz, Antonella, 2023.