

Fabrication and characterization of a bacterial cellulose-chitosan and ethylenediaminetetraacetic acid composite for adsorbing lead and cadmium in solution and achieving sustainable development goals

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ABSTRACT

The studied with efficiency of bacterial cellulose (BC) combined with chitosan (CS) and ethylenediaminetetraacetic acid (EDTA) for the absorption of heavy metals, focusing on lead (Pb) and cadmium (Cd). The CS and EDTA were deposited on the BC under 12 different conditions. A physical analysis was performed using scanning electron microscopy, the chemical structure was determined using Fourier-transform infrared spectroscopy (FTIR), and a heavy metal analysis was conducted using inductively coupled plasma–optical emission spectroscopy. Minimal differences were observed in the FTIR spectra of the BC mixed with CS or EDTA and the BC mixed with CS and EDTA, the major spectral groups identified being C=C, C=O, C–H, and –NH₂. The adsorption efficiencies of the Pb and Cd were found to be 2.89 ± 1.69 and 1.07 ± 0.086 mg kg⁻¹, respectively, for the BC combined with CS at a ratio of 1:1.25 volume per weight unit (v/w). This led to a significantly higher adsorption of Pb ($p < 0.05$) than under any other condition. For the BC combined with CS at a ratio of 1:5 v/w, Cd was absorbed more high significantly ($p < 0.05$) than under any other condition. The results of this study address several sustainable development goals (SDGs) related to the environment and human quality of life, including SDGs 3, 6, 9, 14, and 15. However, this study is only preliminary, and the most appropriate conditions need to be further developed to be applicable to real-world environments.

Keywords: bacterial cellulose, heavy metals, absorption, sustainable development goals (SDGs).

INTRODUCTION

Bacterial cellulose (BC) is a natural form of cellulose produced or synthesized by bacteria (Zhong, 2020; Deshpande et al., 2023; Potočník et al., 2023). Bacterial cellulose is used in diverse fields, such as biomedicine, the food industry, materials science and engineering, the cosmetics industry, and the textile industry, and has environmental applications (Augimeri et al., 2015; Picheth et al., 2017; Ul-Islam et al., 2020; Wahid et al., 2021; Pendey et al., 2024; Absharina et al., 2025). Bacterial cellulose consists of an ultra-fine network of cellulose nanofibers approximately 3–8 nm (Saavedra-Sanabria et

al., 2021; Zeng et al., 2021; Jose et al., 2025) and is synthesized by various bacterial species, the most notable producers being *Komagataeibacter*, *Agrabacterium*, *Rhizobium*, *Gouconacetobacter*, and *Sarcina* (Krishnamachari et al., 2011; Costa et al., 2017; Lahiri et al., 2021).

The BC from *Komagataeibacter intermedius* BE073, isolated from bio-extracts during fruit fermentation (Singhaboot et al., 2022, 2023), has been shown to hold water up to an average of $91.15 \pm 3.68\%$ and has a water absorption index averaging 5.305 (Luo et al., 2018; Kroeksakul et al., 2023). Based on its ability to accumulate and absorb large amounts of liquids, it was found that if

BC was supplemented with an adsorption aid, such as chitosan (CS) or ethylenediaminetetraacetic acid (EDTA) (Jia et al., 2017; Strnad and Zemljič, 2023; Sayago et al., 2024; Fujita et al., 2025), it could be used to store or absorb heavy metals.

Here, we focused on the heavy metals lead (Pb) and cadmium (Cd) in relation to Thailand, which has suffered mine collapses that have led to Pb leaking into natural water sources (Phupinyokul, 2001; Pongboonjun and Trong-ngam, 2021) and problems with the removal of Cd from waste (Songpasert et al., 2016; Inthawan and Chunnual, 2017). Consequently, we aimed to study the efficiency of BC combined with CS and EDTA for the absorption of heavy metals, with a future goal of developing products for use in real-world situations. This study supports Sustainable Development Goal 6 (SDG6), relating to clean water and sanitation, because it addresses pollution in natural water sources, key goals being access to clean water and sustainable water management. It is also related to SDG12, focusing on responsible consumption and production through the management and treatment of toxins in the environment, increasing efficiency from agricultural waste materials to add more value, and developing products to treat environmental pollution in the future, which is one of the components of the BCG model.

METHODOLOGY

Producing bacterial cellulose

Production of the *K. intermedius* BE073 isolate, taken from a village in Nakhon Nayok province, Thailand (Singhaboot et al., 2022), involved 3 kg of fruit, 3 kg of sugar, and 5 L of water, the mixture fermented in covered containers (Kroeksakul et al., 2023). After approximately 2–3 months, BC develops on the surface of the bio-extract, as illustrated in Figure 1A. The yield amount is proportional to the width of the container (Figure 1C). After harvesting the BC, an energy source (e.g., fruit) is added to the bacteria. Here, mango was used as the energy source (Figure 1B).

After harvesting, the BC was cleaned by washing it in water before moving it to the laboratory. There, it was purified by boiling in 0.5 M sodium hydroxide (NaOH) at temperatures of 120–150°C for approximately 1–2 h (Robelo et al., 2017; El-Gendi et al., 2022). The BC was then centrifugated at speeds of approximately 45,000–50,000 rpm to



Figure 1. Illustration of stages in the preparation of the BC

remove the moisture, using a Silver Crest blender (2 L, 4.500 W; Thailand), before being crushed to a powder and mixed with the CS and EDTA, using a food mixer (imaflex IF-163; Japan).

The BC was then combined with CS under three conditions: (1) 1 L BC + 0.625, 1.25, 2.5, and 5 g CS (ratios of 1:0.625, 1:1.25, 1:2.5, and 1:5 volume per weight unit [v/w] [L g⁻¹]); (2) 1 L BC + 0.625, 1.25, 2.5, and 5 g EDTA (ratios of 1:0.625, 1:1.25, 1:2.5, and 1:5 v/w [L g⁻¹]); and (3) 1 L BC + 0.625, 1.25, 2.5, and 5 g CS+EDTA (ratios of 1:0.625:0.625, 1:1.25:1.25, 1:2.5:2.5, and 1:5:5 v/w [L g⁻¹]). These conditions are presented in Table 1, and illustrations of the different samples are presented in Figure 2.

The average moisture of the BC was 96.0 (±1.01)%, which corresponds to the general humidity level of BC (Cazón et al., 2020; Bodea et al., 2021). The BC+EDTA at 1:2.5 had a moisture percentage that was less than significant ($p < 0.05$) relative to the other conditions, while the BC+CS at 1:5 had a significant moisture percentage ($p < 0.05$) relative to the other conditions, as shown in Table 1. After making the different BC mixtures, a BC mixed volume of 60 mL was pouring onto a plate, smeared, and left in the sun to dry. The characteristics of the BC mixtures after drying are presented in Figure 3.

The selected ratios aimed to investigate the extent to which EDTA can effectively bind to bacterial cellulose and chitosan tissue components, hence enhancing attributes such as microbiological activity and heavy metal adsorption (Rostami et al., 2023; Kaczorowska and Bożejewicz, 2024;

Table 1. Conditions of the BC mixed with CS and EDTA

Sample	Condition	v/w (L g ⁻¹)	Moisture content (%)	Sample code
1	BC+CS	1:0.625	96.4(±0.216) ^a	A
2	BC+CS	1:1.25	96.3(±0.119) ^a	B
3	BC+CS	1:2.5	96.8(±0.335) ^a	C
4	BC+CS	1:5	97.1(±0.425) ^c	D
5	BC+EDTA	1:0.625	95.5(±0.825) ^{ab}	E
6	BC+EDTA	1:1.25	96.4(±0.283) ^a	F
7	BC+EDTA	1:2.5	94.5(±1.99) ^b	G
8	BC+EDTA	1:5	95.9(±0.853) ^a	H
9	BC+CS+EDTA	1:0.625:0.625	96.1(±1.07) ^a	I
10	BC+CS+EDTA	1:1.25:1.25	95.3(±0.877) ^{ab}	J
11	BC+CS+EDTA	1:2.5:2.5	96.4(±0.632) ^a	K
12	BC+CS+EDTA	1:5:5	95.4(±0.839) ^a	L
Average	96.0(±1.01)			

Note: ^{abc} difference significant at $p < 0.05$ (honest significant difference [HSD]).

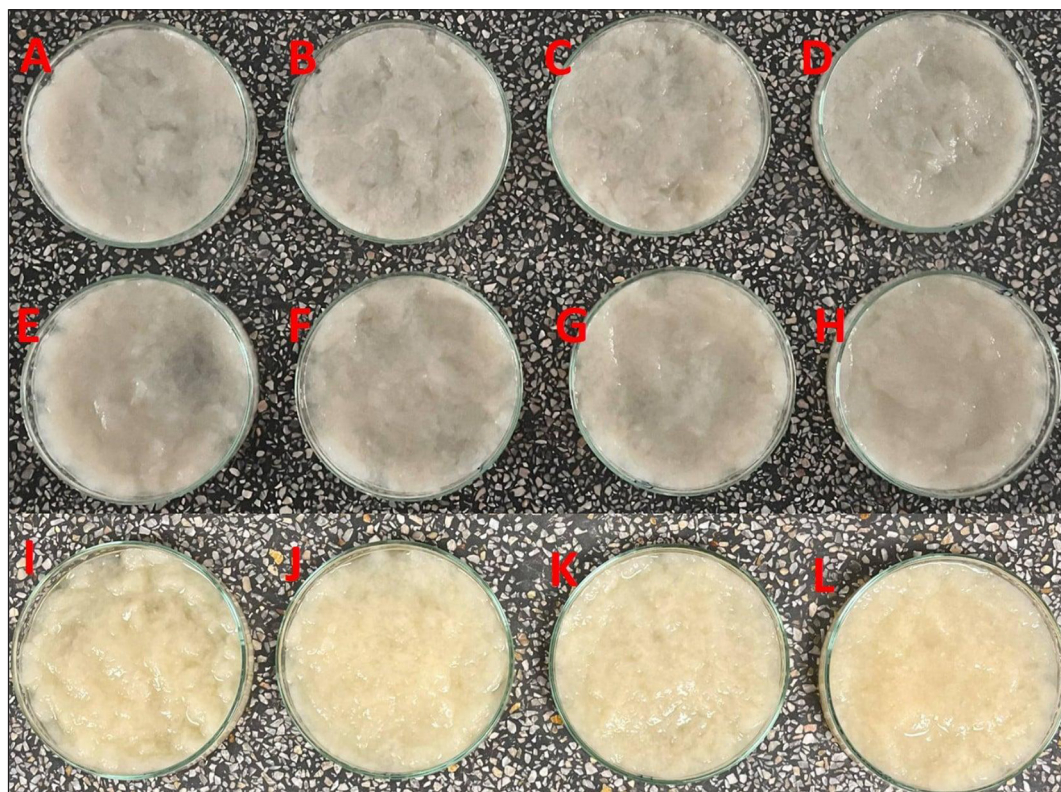


Figure 2. Different BC conditions. Sample codes A–L refer to the conditions before drying, as provided in Table 1

Begum et al., 2024). However, the crucial aspect is the equilibrium between performance and material characteristics. Lower ratios (about 1:0.625) facilitate the evaluation of the minimum quantity of EDTA necessary, whilst larger ratios (up to 1:5) enable the assessment of the impact of surplus EDTA on attributes such as durability, flexibility, or microbiological efficacy (Bhandary et al., 2017; Pinheiro et al., 2021). These ratios are frequently

derived from previous research indicating that this range yields substantial results while maintaining the composite's durability, in addition to serving as an experimental range for identifying the ideal value. Examining a broad spectrum of ratios facilitates the determination of the ideal CS/EDTA ratio for achieving specific attributes, such as peak cross-linking capability or microbiological efficacy, while ensuring material stability.

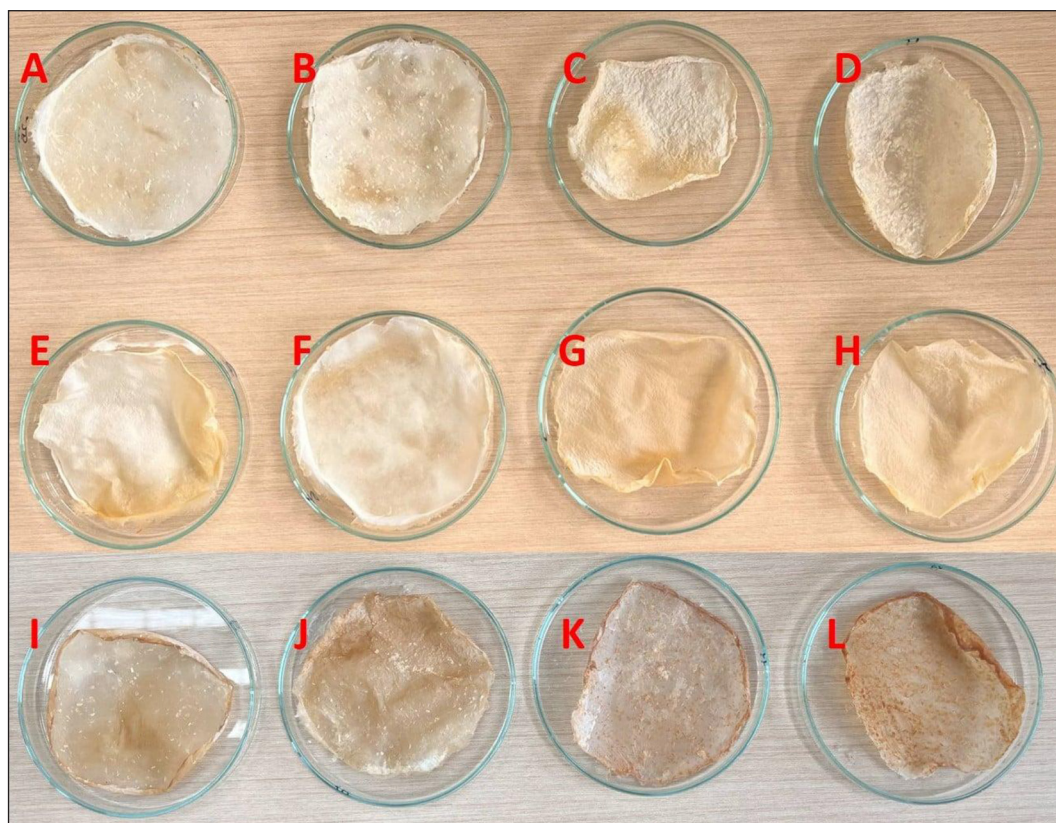


Figure 3. Different BC conditions. Sample codes A–L refer to the conditions after drying, as provided in Table 1

Fourier-transform infrared spectroscopy

The chemical structure of the films was analyzed using Fourier-transform infrared (FTIR) spectroscopy, using a SpectrumTwo (Perkin Elmer, USA) FTIR instrument in the region 4.000–500 cm^{-1} at a spectral resolution of 4 cm^{-1} .

Characterization of the BC film surface morphology

The surface morphology of all the BC films was recorded using scanning electron microscopy (SEM) (JSM-6610, JEOL Ltd., Japan). The film was coated with gold before examination and examined at an accelerating voltage of 15 kV.

Heavy metal analysis

The BC from *K. intermedius* BE073 absorbs reagents optimally after 120 min (Kroeksakul et al., 2023). Thus, in our experiments, 1.5 g of BC (all mixtures) was soaked for 120 min in Pb and Cd solutions at concentrations of 10 ppm, after which the samples were air dried. Samples (0.5 g) were digested in pure reagents (12 ml), including nitric acid (HNO_3), hydrofluoric acid

(HF), and hydrochloric acid (HCl), at a ratio of 2:1:1, using a TANK ECO microwave digestion/extraction band Hanon (SINEO, Shanghai, China) to produce samples for inductively coupled plasma–optical emission spectroscopy (ICP-OES) analysis (PlasmaQuant 9100 series, Germany). Quality assurance and quality control procedures ensured that all 20 samples, as well as their duplicates and blanks, were collected, processed, and examined to standard conditions. These samples were compared against ICP-OES multi-element standard solutions (AccuStandard, USA). However, the reason for using a 10 ppm concentration is frequently employed in laboratory research as it represents the levels of concerning heavy metals in the environment, including pollutants in contaminated water sources (Tchounwou et al., 2012; Operacz et al., 2022). This concentration also aligns with the detectability and analytical sensitivity of ICP-OES instruments, thereby mitigating the risk of rapid sample saturation or damage.

Statistical analysis

The data were analyzed using a one-way analysis of variance and differences in the data

were compared using the post hoc Tukey's honestly significant difference (HSD) test for $p < 0.05$ between datasets. All analyses were conducted using SPSS software.

RESULTS AND DISCUSSION

Morphological characterization of BC+CS

In Figure 4, Con_1 and Con_2 show the basic structure of BC with no CS added, with the unmodified appearance of the BC and the natural fiber density being apparent. Figure 4(A1) and (A2), in which 1:0.625 v/w CS was added, some densification of the structure and interconnection between the fibers was observed, which is more obvious in Figure 4(A2). Figure 4(C1) and (C2), showing the CS at a ratio of 1:2.5 v/w, shows a finer surface texture, suggesting that the CS may have formed a denser and more continuous structure. Figure 4(D1) and (D2), with CS at a ratio of 1:5 v/w, illustrates the highest structural density and the highest penetration and strengthening of the CS (Rezazadeh et al., 2024; Cetin et al., 2025). The addition of different amounts of CS thus significantly influenced the surface structure of the BC.

The SEM images show that increasing the ratio of CS in the BC resulted in structural and superficial modifications, which are important factors influencing heavy metal adsorption efficiency. Understanding these structures is key to developing materials with optimal properties and performance for environmental applications.

Morphological characterization of the BC+EDTA

As shown in Figure 5, Con_1 has a relatively smooth and hard surface, whereas Con_2 clearly has cavities and a fibrous structure, depending on the magnification used. In Figure 5(E1), the fiber structure appears complex and systematic, and differences in the structure were noted. In Figure 5(E2), the details of the fine structure are clearer. Figure 5(F1) has a fiber structure that is smooth and simple, but an increasing concentration may cause the structure to deteriorate or become harder. Figure 5(F2) shows fibers with thinner and more complex structures. In Figure 5(G1) the structure appears complex and orderly and the fibers are higher in number and resolution. In Figure 5(H1), the structure appears to be degraded, potentially

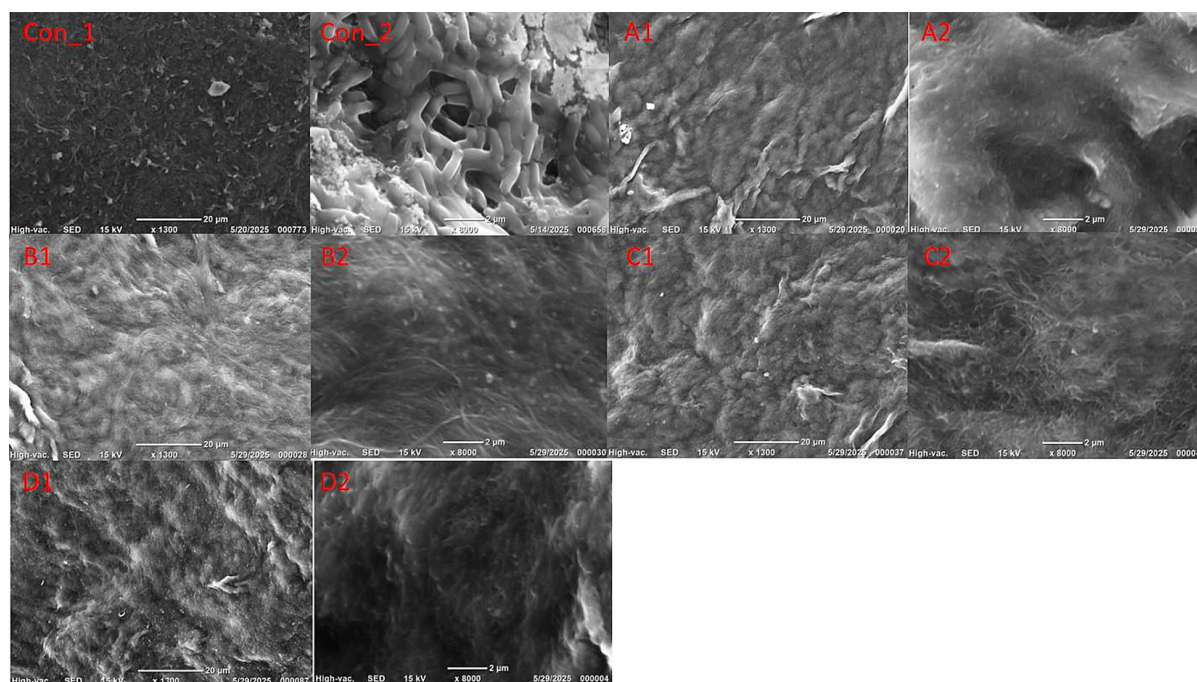


Figure 4. Morphology of BC+CS. (Con_1) BC with no addition at 1.500x magnification; (Con_2) BC with no addition at 8.000x magnification; (A1) BC+CS at 1:0.625 v/w, 1.500 x magnification; (A2) BC+CS at 1:0.625 v/w, 8.000x magnification; (B1) BC+CS at 1:1.25 v/w, 1.500x magnification; (B2) BC+CS at 1:1.25 v/w, 8.000x magnification; (C1) BC+CS at 1:2.5 v/w, 1.500x magnification; (C2) BC+CS at 1:2.5 v/w, 8.000x magnification; (D1) BC+CS at 1:5 v/w, 1.500x magnification; and (D2) BC+CS at 1:5 v/w, 8.000x magnification

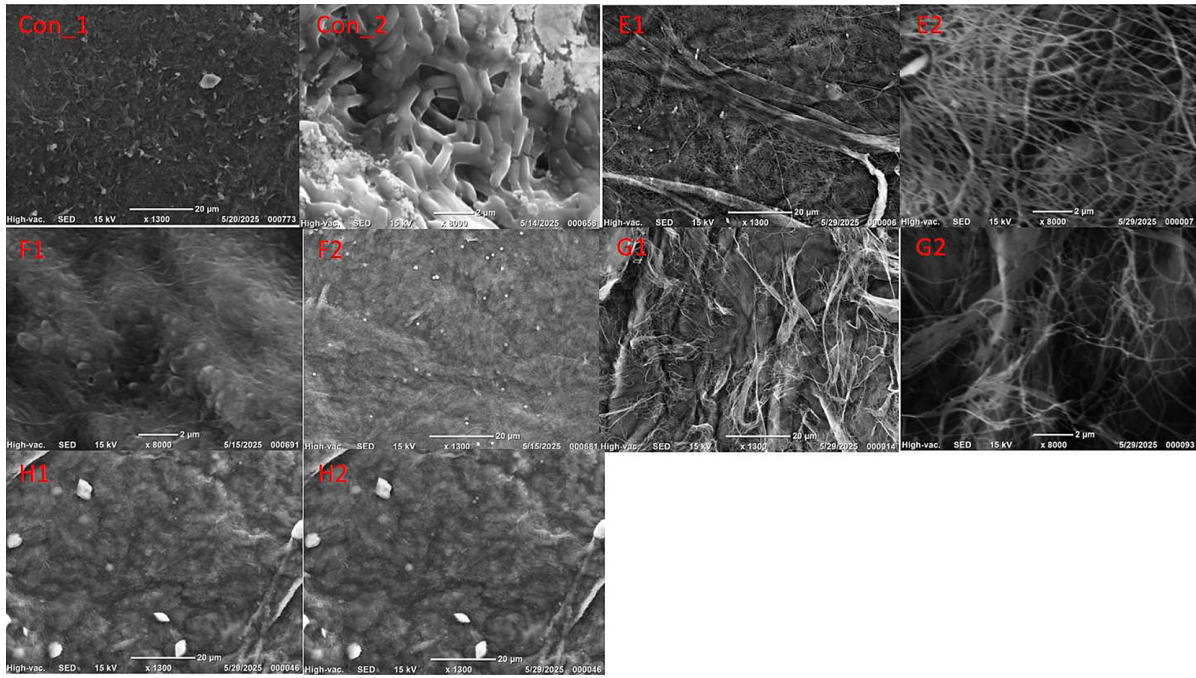


Figure 5. Morphology of BC combined with EDTA. (Con_1) BC with no additions at 1.500x magnification; (Con_2) BC with no additions at 8.000x magnification; (E1) BC+EDTA at 1:0.625 v/w, 1.500x magnification; (E2) BC+EDTA at 1:0.625 v/w, 8.000x magnification; (F1) BC+EDTA at 1:1.25 v/w, 1.500x magnification; (F2) BC+EDTA at 1:1.25 v/w, 8.000x magnification; (G1) BC+EDTA at 1:2.5 v/w, 1.500x magnification; (G2) BC+EDTA at 1:2.5 v/w, 8.000x magnification; (H1) BC+EDTA at 1:5 v/w, 1.500x magnification; and (H2) BC+EDTA at 1:5 v/w, 8.000x magnification

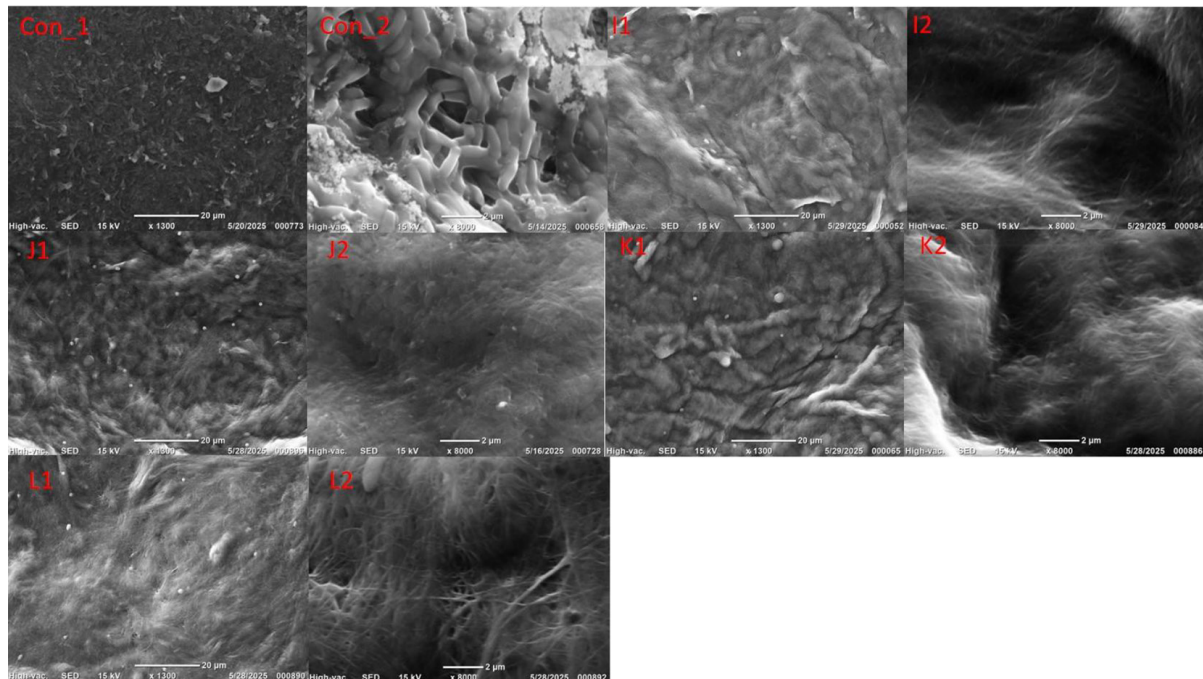


Figure 6. Morphology of BC combined with CS and EDTA. (Con_1) BC with no additions at 1.500x magnification; (Con_2) BC with no additions at 8.000x magnification; (I1) BC+CS+EDTA at 1:0.625:0.625 v/w, 1.500x magnification; (I2) BC+CS+EDTA at 1:0.625:0.625 v/w, 8.000x magnification; (J1) BC+CS+EDTA at 1:1.25:1.25 v/w, 1.500x magnification; (J2) BC+CS+EDTA at 1:1.25:1.25 v/w, 8.000x magnification; (K1) BC+CS+EDTA at 1:2.5:2.5 v/w, 1.500x magnification; (K2) BC+CS+EDTA at 1:2.5:2.5 v/w, 8.000x magnification; (L1) BC+CS+EDTA at 1:5:5 v/w, 1.500x magnification; and (L2) BC+CS+EDTA at 1:5:5 v/w, 8.000x magnification

due to the highest concentration of EDTA. In Figure 5(H2), the structure appears less complex and less degraded, potentially being affected by the highest concentrations of EDTA. The SEM images helped to clarify that: (1) the addition of EDTA at a low concentration (1:0.625) resulted in fibrous and hollow structures, which were still maintained to some extent; (2) increasing the concentration of EDTA (1:1.25, 1:2.5) produced more complex and fine fibrous structures that influenced the internal structure of the BC; and (3) the highest concentration (1:5) may cause the structure to start deteriorating, disintegrating or degrading, the addition of too much EDTA potentially leading to the destruction of the structure. Indeed, EDTA is known to be a metal and inorganic binding agent that can alter the structure of BC, in terms of its strength, complexity or degradation (Song et al., 2020; Fujita et al., 2025).

Morphological characterization of BC+CS+EDTA

In Figure 6, Con_1 and Con_2 are the control samples (i.e., untreated BC), showing a well-preserved fiber network and a porous structure, considered the baseline morphology. Figure 6(I1) and (I2) show fibers with wavy, fibrous or tissue-like features that possibly denote biological tissue or processed fiber structures. Figure 6(J1) and (J2) show granular features and rough surfaces with granular particles and surface irregularities, possibly indicating degradation from the treatment. Figure 6(K1) and (K2) show fibrous structures with some smooth areas, the fibers sometimes intertwined, denoting the effects of the different treatments or different biological materials. Figure 6(L1) and (L2) show granular and rough regions with fibers, and granular surfaces with fibrous elements. In summary, it is possible to distinguish between both material degradation and the effects of specific treatments. The SEM images in Figure 6 illustrate several significant differences relating to: (1) size and porosity, such that the control samples (Con_1 and Con_2) mostly present fibrous and porous structures typical of BC; (2) surface features, where samples present varying textures, which could be due to the different treatments, biological states or material modification; and (3) magnification effects, with higher-magnification imaging revealing finer details, such as interconnections between fibers, surface granularity, and degradation.

FTIR analysis

The FTIR spectra acquired from the BC are shown in Figure 7. There is little difference between the FTIR spectra from the BC+CS [Figure 7(A)], BC+EDTA [Figure 7(B)], and BC+CS+EDTA. In the FTIR spectra, the bands at $2.925\text{--}2.930\text{ cm}^{-1}$ denote the C–H stretching vibrations of $-\text{CH}_2$ of the hydroxymethyl group (Schneider et al., 1979; Ghozali et al., 2021), whereas the C=C group typically absorbs in the region of $1.600\text{--}1.670\text{ cm}^{-1}$ (Almohareb et al., 2020), and C=O carbonyl group in the range of $1.680\text{--}1.760\text{ cm}^{-1}$ (Ellerbrock and Gerke, 2021; Nandiyanto et al., 2023). The bands at 3.225 cm^{-1} are interpreted as $-\text{NH}_2$ from primary amides, with secondary amines appearing at $1.000\text{--}1.350\text{ cm}^{-1}$ (Smith, 2019; Pasieczna-Patkowska et al., 2025). The results of the FTIR analysis indicate that, despite the addition of the various modifiers, the basic structure and main functional groups of the BC remained similar in all samples, reflecting their structural stability and ability to retain important functional groups.

Potential of BC to absorb heavy metals

The adsorption efficiencies of the heavy metals Pb and Cd were found to be $2.89(\pm 1.69)$ and $1.07(\pm 0.086)\text{ mg kg}^{-1}$, respectively. In the Pb adsorption of the control samples, the average value was $2.83(\pm 0.259)\text{ mg kg}^{-1}$. The BC+CS at 1:1.25 v/w could adsorb up to $8.33(\pm 1.30)\text{ mg kg}^{-1}$ (i.e., significant levels) ($p < 0.05$). These data are presented in Table 2 and Figure 8(A). For Cd adsorption by the control samples, the average value was $1.08(\pm 0.002)\text{ mg kg}^{-1}$, with BC+CS at 1:5 v/w adsorbing up to $1.21(\pm 0.030)\text{ mg kg}^{-1}$. This is again highly significant ($p < 0.05$). These data are presented in Table 2 and Figure 8(B). The addition of CS and EDTA cause electrostatic interactions with the metals and different functional groups, such as $-\text{OH}$, $-\text{NH}_2$, and $-\text{COOH}$ (Feng et al., 2021; Verma et al., 2022; Zhang et al., 2022). However, although the addition of CS and EDTA is effective for heavy metal adsorption, it must be added in the right proportions to achieve maximum efficiency. Nevertheless, the study results indicated no synergistic effect on heavy metal adsorption capacity between CS and EDTA, particularly at the ratio of BC, CS, and BC at 1:5:5 L g⁻¹, which considerably reduced heavy metal adsorption efficiency.

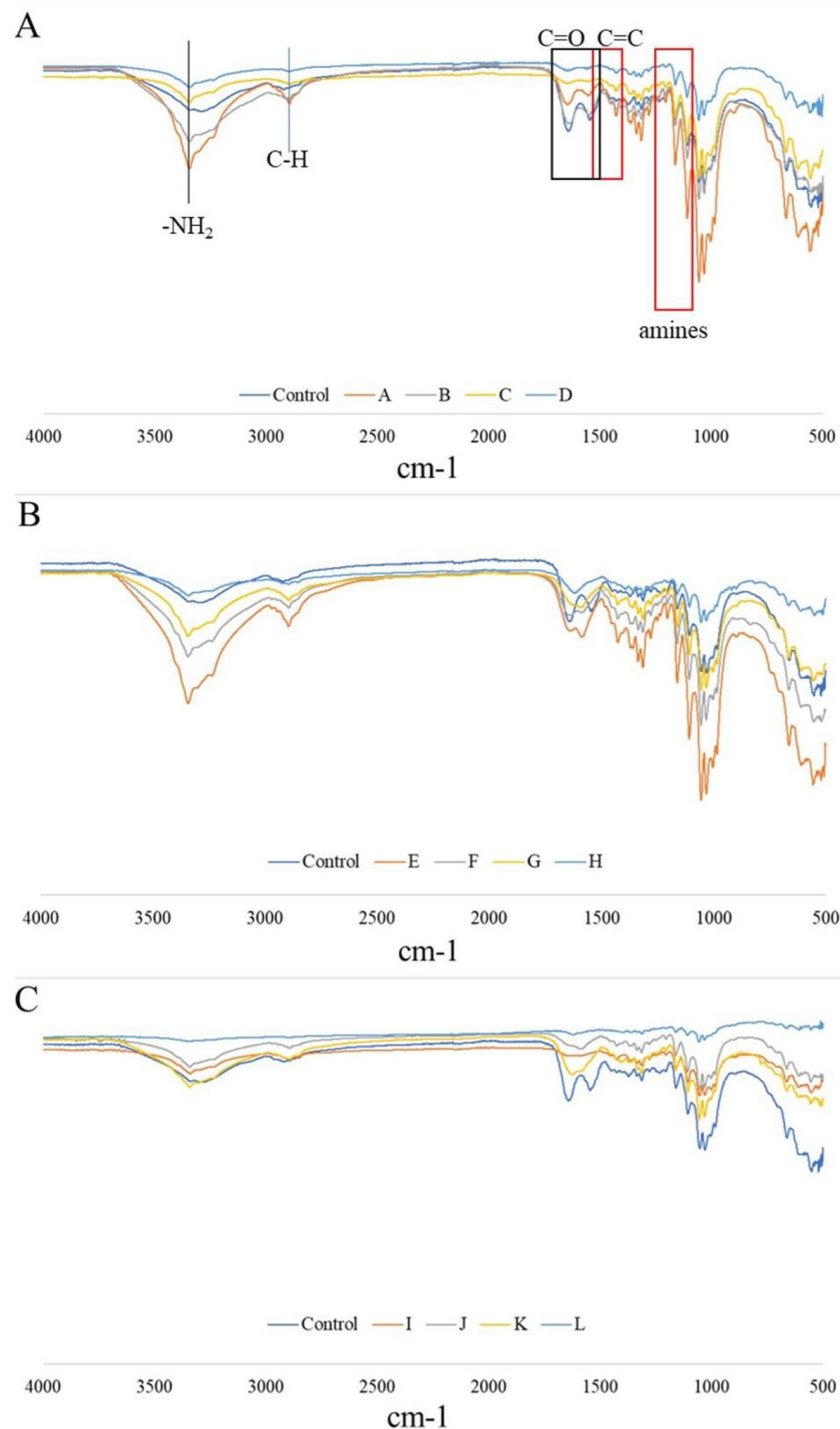


Figure 7. FTIR spectra of the BC. (A) BC+CS; (B) BC+EDTA; and (C) BC+CS+EDTA

Potential of BC+CS+EDTA to absorb heavy metals for SDGs

Bacterial products, such as biofilm or dried biomass, including cellulose, have advantageous chemical properties for capturing heavy metals such as Cd^{2+} and Pb^{2+} (Genué et al., 2025; Oziegbe et al., 2025) because functional groups, such as -COOH , -OH , -NH_2 , and -SH , on the surface

of cells or microbial products have the ability to capture the positive charge of the metal (Gu and Lan, 2023; Tavan et al., 2025). The addition of CS, a natural polymer derived from chitin, which has amino groups (Aranaz et al., 2021; Piekarska et al., 2024) that can bind heavy metals, allows the binding of metals through chelation and ion-exchange mechanisms, and CS also has a porous network structure that is suitable for use as an

Table 2. Elemental contents under different BC conditions

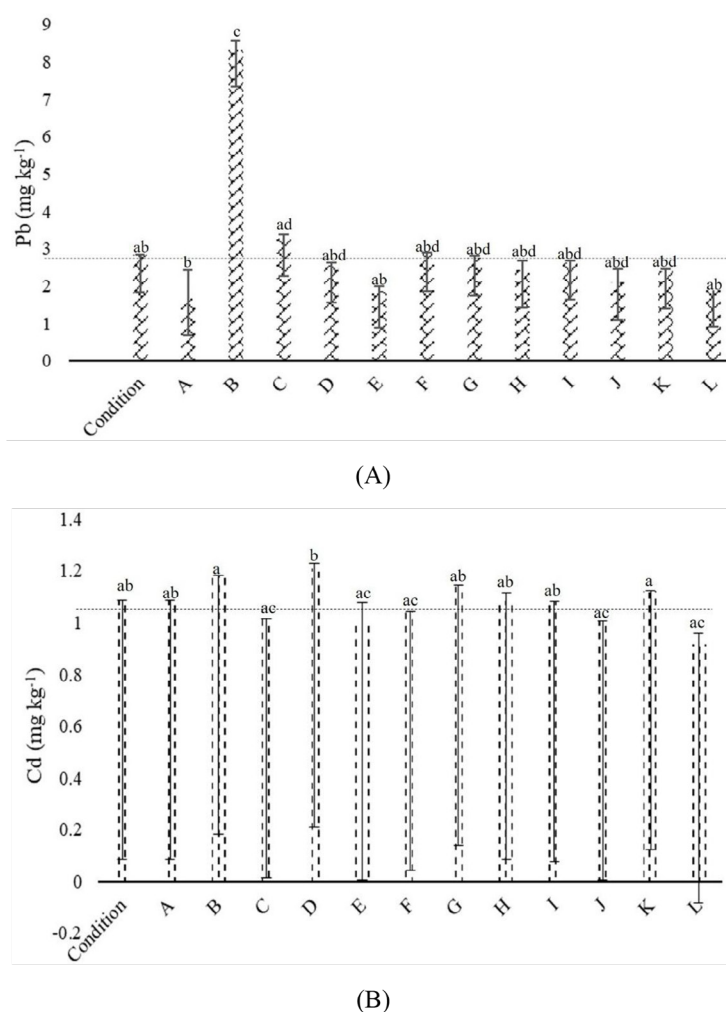
Condition	Pb	Cd
	(mg kg ⁻¹ dry weight)	(mg kg ⁻¹ dry weight)
Control	2.83±0.259 ^{ab}	1.08±0.002 ^{ab}
A	1.68±0.026 ^b	1.08±0.001 ^{ab}
B	8.33±1.30 ^c	1.18±0.004 ^a
C	3.27±0.399 ^{ad}	1.01±0.004 ^{ac}
D	2.55±0.219 ^{abd}	1.21±0.030 ^b
E	1.86±0.136 ^{ab}	1.00±0.127 ^{ac}
F	2.86±0.214 ^{abd}	1.04±0.002 ^{ac}
G	2.75±0.080 ^{abd}	1.13±0.009 ^{ab}
H	2.42±0.083 ^{abd}	1.08±0.055 ^{ab}
I	2.63±0.455 ^{abd}	1.07±0.005 ^{ab}
J	2.10±0.094 ^{abd}	1.00±0.004 ^{ac}
K	2.40±0.601 ^{abd}	1.12±0.006 ^a
L	1.90±0.083 ^{ab}	0.915±0.07 ^c
Average	2.89±1.69	1.07±0.086

Note: ^{abcd} Mean is column differences significant at $p < 0.05$ (HSD).

adsorbent. When mixed with bacterial products, it increases both the surface area (Figure 4) and the adsorption efficiency.

In addition, EDTA is a chelating agent with excellent abilities to bind several heavy metals, such as Pb²⁺ and copper (Cu²⁺) (Dong et al., 2023; Genua et al., 2025), and is used to enhance the metal binding efficiency of adsorption systems. When combined with BC and CS, it can promote faster and more efficient metal binding. It also helps to move heavy metals from soils or other environments into a form that can be easily absorbed.

The use of environmentally friendly biotechnology for heavy metal removal has a direct link to the targets of several SDGs (Kumar et al., 2023; Meftah et al., 2025), including SDG3 (Good Health and Well-being) in terms of reducing the heavy metal contamination from water, soil, and food, protecting human health, SDG6 (Clean Water and Sanitation) in restoring water quality by

**Figure 8.** Elemental contents under different BC conditions. (A) Pb; and (B) Cd

safely removing heavy metals, SDG9 (Industry, Innovation, and Infrastructure) in developing bio-sorbent materials as a green industrial innovation, and SDGs 14 and 15 (Life Below Water and Life on Land) in reducing the impact of heavy metals on aquatic and terrestrial life, including the correlation of initial product forms of BC in relation to SDG objectives.

CONCLUSIONS

The results of this study show that BC was effective at absorbing heavy metals, especially when CS was added to the BC at a ratio of 1:1.25 v/w, which resulted in a significant ($p < 0.05$) Pb adsorption capacity. The addition of CS to BC at a ratio of 1:5 v/w produced a significant ($p < 0.05$) Cd adsorption capacity. We also found that the addition of CS to BC gave better adsorption efficiency for Pb and Cd than the addition of just EDTA or CS+EDTA. Adding CS to BC in different proportions prompted different responses in terms of the adsorption efficiency of the different heavy metals. Our findings relate to SDG targets that involve the environment and human quality of life, including SDGs 3, 6, 9, 14, and 15. However, this is only a preliminary evaluation of the best ratios needed for heavy metal removal in real-world environments.

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