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The Potential Biosorption of Copper and Manganese by Bacterial Cellulose in the Environment

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ABSTRACT
This study investigated the efficiency of copper and manganese adsorption by bacterial cellulose (BC) produced from *Komagataeibacter intermedius* BE073. BC was collected from production processes in a village in Nakhon Nayok province. BC had high moisture content of 91.15±3.68%, an average water absorption index (WAI) of 5.30±0.362, an average tensile strength of 99.1 ±6.18 MPa, average elongation at break of 6.41±0.67%, and an average Young modulus of 1445±177 MPa. Structural analysis of the BC material shows that it is a cellulose powder with a main group. Measurements show that the Mn content in BC rapidly decreased after soaking in solution, and that the highest Cu absorption efficiency of BC during a 120 minute period was 15469 mg kg^{-1}. The results of this study show that BC may be successfully used to absorb various heavy metal residues from leachate, particularly Cu solutions. BC cannot absorb Mn from solution, so it cannot be used to absorb Mn from leachate. However, studies have shown that BC can release Mn into solution. Therefore, BC may be effective for use in agriculture, as Mn is a micronutrient for plants.

Keywords: bacterial cellulose, biosorption, heavy metal, bio-extract, *Komagataeibacter intermedius* BE073.

INTRODUCTION

Komagataeibacter intermedius BE073 was isolated from a bio-extract taken from a productive village in the Nakhon Nayok province (Singhaboot et al., 2022; Singhaboot et al.,2023). The microorganism group uses carbon energy from various sources available in the environment, such as sugar or ripe fruit. Currently, bacterial cellulose (BC) has a number of applications, including the development of special food combinations and the creation of bioplastics for use in medicine, and is safe for use in these specific purposes. BC is a by-product of farmers' bio-fermentation systems. Farmers prefer using bio-fermented water, and it is simple to produce BC in the bio-fermentation process. The BC produced by farmers can to be used for environmental protection applications that have no bearing on human health.

BC was found to absorb 150 mg·g^{-1} and 217.8 mg·g^{-1} of Cu^{2+} and Pb^{2+} ions, respectively, in a Freundlich kinetic model (Song et al., 2020), and BC produced from *Rhodococcus sp.* MI2 has been found to absorb Fe (III) in solution at an average of 20 mg·L^{-1} and Cu (II) in solution at an average of 75 mg·L^{-1} (Yingkong & Tanskul, 2019). Because BC can absorb the Cu, Pb, and Fe heavy metals, this paper aimed to answer the research question: "Can BC absorb heavy metals from leachate?". The heavy metals found in the leachate at waste dump sites include As, Cr, Se, Fe, Cu, and Mn (Agbeshie et al., 2020; Akanchise et al., 2020). Manganese is particularly significant, in part due to the decomposition of certain types of waste which contain these heavy metals as components (Essien et al., 2022). The purpose of this study was to investigate the efficiency of copper and manganese adsorption by the BC produced from *Komagataeibacter intermedius* BE073. This study gathered the data on BC performance, which can be utilized to create standards for landfill leachate treatment. Additionally, these results will contribute to the development new heavy metal storage and adsorption materials, which will help to direct the next steps for future leachate treatment.

METHODOLOGY

Preparation of the BC

The villagers' bio-extract production formula is 3 kg of ripe fruit, 3 kg of sugar and 5 L of water. They ferment biological extracts in covered containers, which are easily available in the community (Figure 1A). After about two months, the farmers will begin using the bio-extract for cultivation and agriculture, but do not use the BC produced in the bio-fermentation process, either discarding it or feeding it to chickens. The BC yield is proportionate to the cross-sectional size of the container (Figure 1D), and the microorganisms produce BC in a thick layer (see Figure 1).

Drying BC

Fresh BC mats are large, and must be cut into smaller sizes before drying (Figure 2A). The fresh BC was dried in a hot air oven at 40°C for 120 hours (Figure 2B), then the BC film was trimmed to a size of 1–2 cm (Figure 2C) before being placed in a desiccator. The characteristics of BC during drying are presented in Figure 2.

Mechanical properties test

The tensile strength of the BC was tested using an EZ-X tensile machine no. I30835735007 (Shimadzu, USA) with a 100N capacity at an ambient temperature of 21.3°C, humidity of 53.2% RH, speed of 25 mm·min^{-1}, and displacement origin force of 0.05 N. The determination of

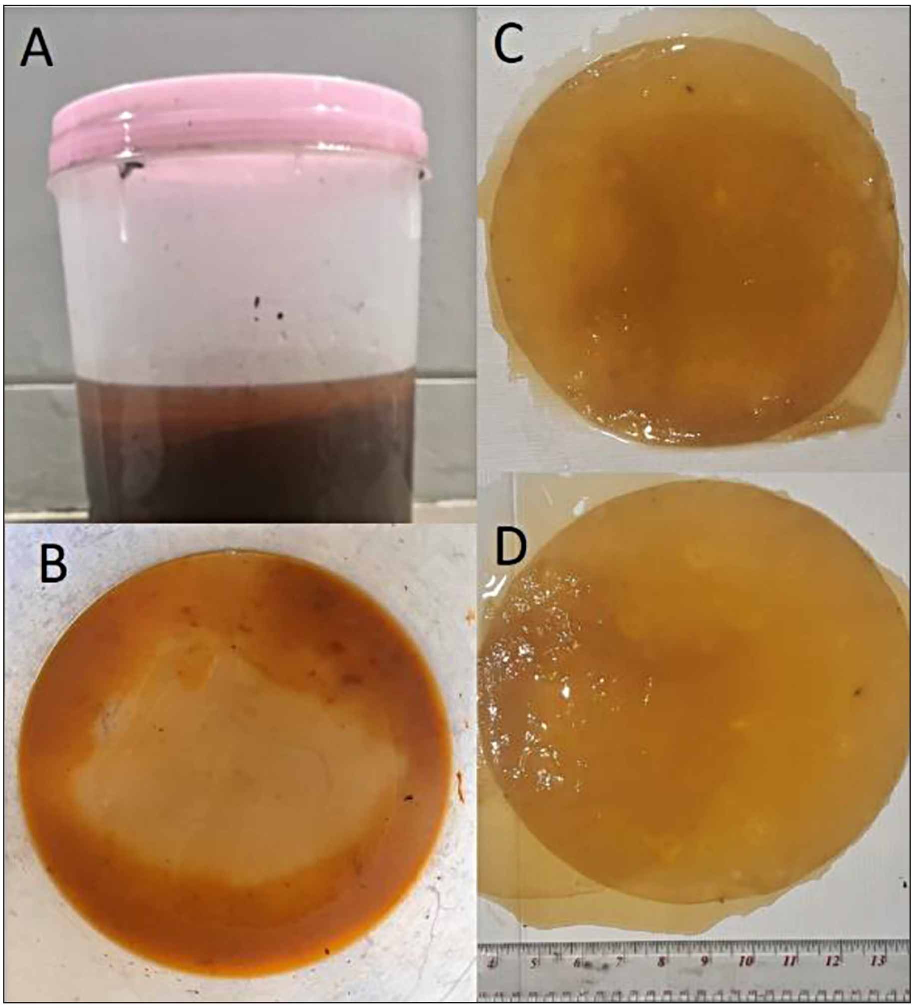

Figure 1. Bio-extract and BC from biofermentation: (A) the container has an easy-to-source lid; (B) BC after 90 days of fermentation; (C) BC yield; and (D) BC yield size is proportionate to container width

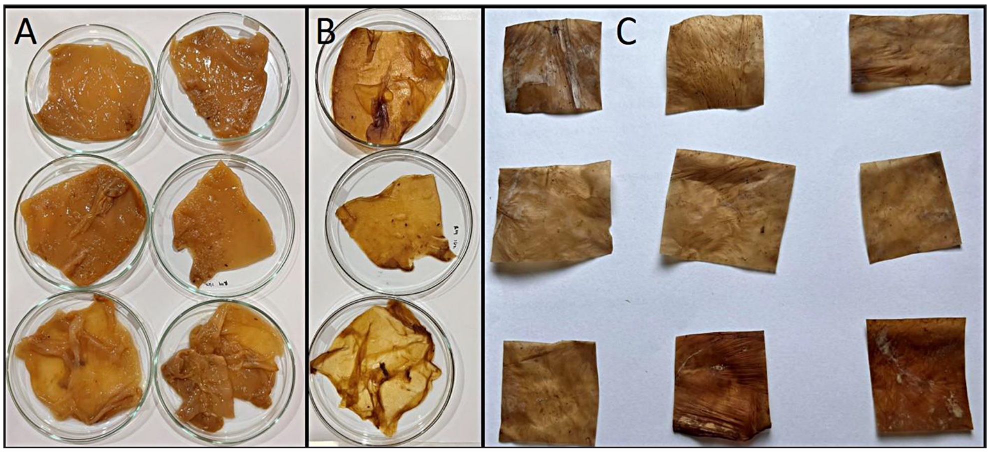

Figure 2. The characteristic of BC film: (A) fresh BC before drying; (B) BC film after drying; and (C) trimmed BC before being added to a desiccator

elongation at break, tensile strength, and Young's modulus was performed by educing of BC films in strip-shaped specimens of 10 mm in width and 30 cm in gauge length. The mechanical properties recorded for each sample were the average values determined from three specimens.

Water absorption index

Samples were prepared by washing them with distilled water (DI) and weighing them. They were dried at 40°C for 120 hours in a hot air oven and then weighed again to calculate the water content in bacterial cellulose, as follows:

$$WC\% = (W2 - W1) \cdot 100\% \quad (1)$$

where: *WC%* is the percentage of water content in BC,
W1 is the sample weight before drying,
W2 is the sample weight after drying.

A sample BC film weighing 2.5 g was placed into a test tube, and 30 ml of distilled water was added. This was left at room temperature of 30°C for 30 minutes, centrifuged at 3000 rpm for 15 min, and then drained. The remaining sample was weighed, and the value was used to calculate the Water Absorption Index (WAI) as the ratio of the weight of the sediment to the dry weight of the product, as follows (Aderson et al., 1969; Schrecker & Gostomski, 2005; Carvalho et al., 2016):

$$WAI = Weight_{sed} / \text{Weight}_{dry} \quad (2)$$

where: *WAI* is the water absorption index,
$Weight_{sed}$ is sediment precipitate after centrifugation,
$Weight_{dry}$ is BC film weighing 2.5 g.

The percentage of water held by the BC was then calculated as follows:

$$WH\% = [(\text{Weight}_{sed} - \text{Weight}_{dry}) / \text{Weight}_{dry}] \cdot 100\% \quad (3)$$

where: *WH%* is the percentage of water content in BC.

Element retention from bacterial cellulose

To examine heavy metal retention by BC, standard Cu and Mn concentrations were used: 1000 ppm of heavy metal in solution with 2% hydrochloric acid (HCl) (Laboratory Reagents & Fine Chemicals, India). Heavy metal retention was better than water retention capacity in 0 – 3 houses (Top et al., 2021; Amorim et al., 2023). Measurements were taken at time points of 0, 15, 30, 45, 60, 90, 120 and 180 min.

Bacterial cellulose extraction and element analysis

One gram of BC from each sample was mixed with 50 ml of aqua regia, a solution of nitrohydrochloric acid (Cheng et al.,2019; Deng et al.,2023) which was composed of a 3:1 mixture of hydrochloric acid (HCl, Beker Analyzed, Taiwan) and nitric acid (HNO_3, QRëC, New Zealand), and heated to 500°C in the SpeedDigester K-425 BUCHI (Switzerland) until it was dry (Kroeksakul et al.,2021). The residue was rinsed with 1% HNO_3, then sieved through Whatman No.1 paper. The supernatant was then transferred to a 50 mL volumetric flask and 1% HNO_3 was added prior to inductively coupled plasma optical emission spectroscopy analysis (ICP-OES). A PlasmaQuant 9100 series (Germany) was used for this analysis; quality assurance and quality control (QA/QC) procedures ensured that all 48 samples, as well as duplicates and blanks, were collected, processed, and examined in the laboratory. The samples were compared an ICP multielement standard solution from AccuTrace™ Stands (USA). The concentration of each studied element in the samples was calculated using Equation 4:

$$\text{Element concentration } (\text{mg}\cdot\text{kg}^{-1}) = Cx(v/w) \quad (4)$$

where: Cx is the concentration value given by the instrument ($\mu g \cdot L^{-1}$),
v represents the soluble volume of the sample (L),
w is the weight of the sample (g).

Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structures of the films were analyzed and recorded by FTIR using a Spectrum Two™ (Perkin Elmer, USA) instrument in the region of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

Statistical analysis

Data were analyzed using one-way ANOVA for variance. Differences in the data were compared using a Least Significant Difference (LSD) test at $p < 0.05$ between data components. All analyses were performed using the SPSS V.22 and Sigmaplot 12.0 programs (free trial versions).

RESULT AND DISCUSSION

Bacterial cellulose capacity

In the fresh BC, water made up 91.15±3.68% of the material weight. The mass of water extracted during the determination of water holding capacity was divided by the dry weight of cellulose, giving an average water absorption index (WAI) value of 5.30±0.362; this means that the BC can absorb water up to 430.5% of its dry weight. The mechanical properties of BC were calculated: the average value for maximum force was 51.9±6.04 N, the average tensile strength was 99.1±6.18 MPa, average elongation at break was 6.41±0.67%, and the average Young modulus was 1445±177 MPa. The BC properties are presented in Table 1.

Copper in bacterial cellulose

The Cu content of the BC averaged 79.82±0.871 $mg \cdot kg^{-1}$ of dry weight, and the highest Cu stock in the BC was found to be 19379% at 120 min (p<0.05), which was statistically significant compared to other stage. However, the BC had the potential to absorb Cu at average rates of 12155±2075% or 15229±2600% of BC dry weight, as presented in Table 2 and Figure 3. The BC from *Rhodococcus sp.* MI 2 could store Cu at about 16000 $mg \cdot kg^{-1}$ of BC dry weigh at the 180 min stage, and at the final 210 min stage could store Cu at 18000 $mg \cdot kg^{-1}$ of BC dry weight (Yingkong & Tanskul, 2019). BC can absorb Cu at 25000 $mg \cdot kg^{-1}$ at a pH of 4.5 (Shen et al., 2008). However, the performance of BC in terms of Cu absorption depends on other factors, such as pH, Cu concentration in solution, temperature, time and type of BC.

Table 1. Bacterial cellulose properties

Properties of BC	Average	SD
Moisture of bacterial cellulose (%)	91.15	3.68
Water absorption index (WAI)	5.305	0.362
Water absorption (%)	430.5	33.13
Thickness (mm)	0.052	0.008
Maximum force (N)	51.9	6.04
Tensile strength (MPa)	99.1	6.18
Elongation at break (%)	6.41	0.67
Young modulus (MPa)	1445	177

Manganese in bacterial cellulose

The quantity of Mn in BC averaged 2289 ±12.8 mg·kg^{-1} of dry BC weight, and the Mn content in BC was found to be released to environment (Figure 3). The manganese was released to environment after soaking in solution; at the 0 min stage, The Mn in BC decreased by 40%. The average Mn release rate was 85.5% of BC dry weight. The Mn content in the BC at the 60, 120 and 180 min stages was not significant, but it was significant ($p<0.05$) at the 0, 15, 30, 45, 90 min stages, as presented in Table 2. This reflects the fact that there is a quantity of Mn in raw materials like mango products (Agarwala et al., 1988; Maldonado-Celis et al., 2019; Lebake et al., 2021).

FTIR analysis

The samples were classified into three groups; (1) BC film, (2) BC film soaked in element solution for 30 or 120 min, and (3) BC film soaked in element solution for 30 or 120 min after the extraction process. The BC structure was analyzed by a FTIR spectrophotometer: the samples were scanned in the range of 400 – 4000 cm^{-1} due to the functional group of BC, and the results are presented in Figure 4. In the whole group, the major group of the structure was found in the cellulose powder. However, the structure of BC reveals a functional group of peaks between 665–670 cm^{-1} related to a carbon-hydrogen bond (C-H) (Mohammadkazemi et al., 2014; Hishiwawa et al., 2017; Wohlert et al., 2022), and a bending of the carbon bond of carbon to the hydroxy group (δ(C-OH) (Sighaboot et al, 2022; Movasaghi et

Table 2. Copper and manganese content in BC

Condition	Cu (mg kg^{-1} of dry weight)	%	Mn (mg kg^{-1} of dry weight)	%
control	79.8 (±1.06)^{a}	100	2289 (±15.7)^{a}	100
0 min	9588 (±27.7)^{b}	12012	1384 (±12.5)^{b}	60
15 min	11707 (±13.6)^{c}	14667	161 (±3.05)^{c}	7
30 min	10264 (±39.8)^{d}	12858	497 (±3.31)^{d}	21
45 min	10865 (±35)^{e}	13611	178 (±3.51)^{e}	7.8
60 min	14681 (±25.9)^{f}	18393	26.6 (±1.15)^{f}	1.2
90 min	10755 (±54.5)^{g}	13474	344 (±4.16)^{g}	15
120 min	15469 (±27)^{h}	19379	32.5 (±2.17)^{f}	1.4
180 min	13917 (±99.6)^{i}	17435	42.6 (±13.6)^{f}	1.8
Average	12155	15229	333	14.5
SD	2075	2600	426	18.6

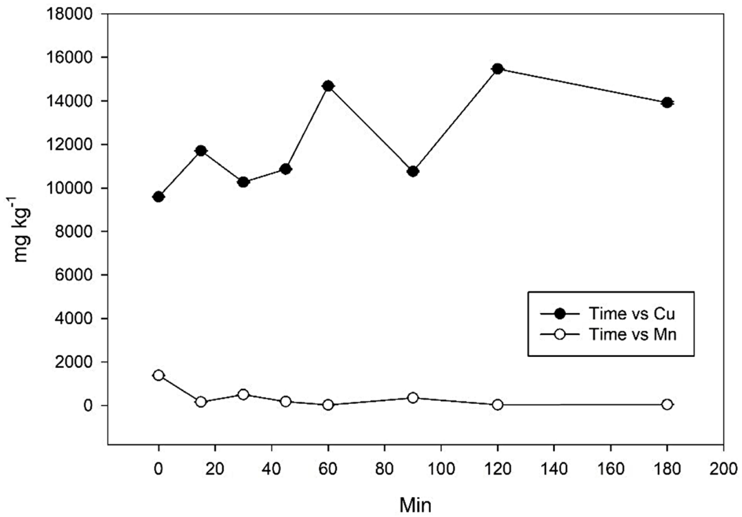

Figure 3. Element content in BC varies over time

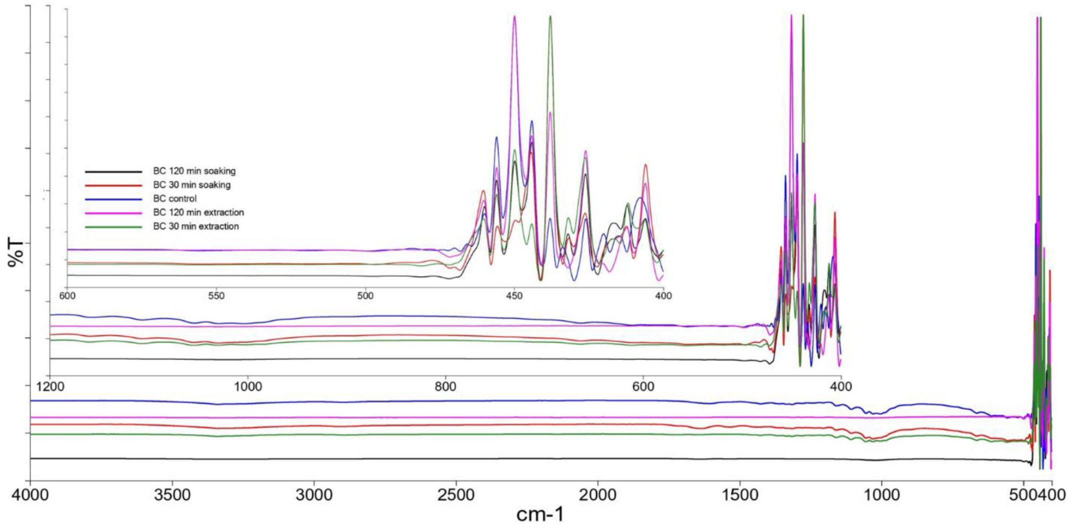

Figure 4. FTIR spectra of the BC film produced by *Komagataeibacter intermedius* BE073

al., 2008). The results indicate that BC film from *Komagataeibacter intermedius* BE073 performs well in the maintenance of cellulose, and the extraction process can succeed at a high temperature of 500°C.

CONCLUSIONS

The BC produced by *Komagataeibacter intermedius* BE073 has the potential for use in the community. Fresh BC has water content of 91.15±3.68% of its dry weight, an average WAI value of 5.30 ±0.362. BC can absorb water up to a content of 430.5%, has an average tensile strength of 99.1±6.18 MPa, average elongation at break of 6.41±0.67%, and an average Young's modulus of 1445±177 MPa. The highest Cu absorption efficiency of BC within a 120 minute period was measured at 15469 mg·kg^{-1}. The Mn content of the BC was released to the environment; the Mn content in BC decreased rapidly after soaking in solution. Structural analysis of BC shows that it is a cellulose powder with a main group and a carbon-hydrogen bond (C-H), bending the carbon bond of carbon to the hydroxy group (C-OH), and the structure is capable of withstanding the conditions of digestion at a temperature of 500°C. The findings of this study demonstrate that BC can be successfully developed into a substance which can be used to absorb some heavy metal residues from leachate, particularly Cu solutions. BC cannot absorb Mn from the solution, so it cannot be used to remove Mn from leachate. However, studies have shown that BC can release Mn into solution. Therefore, BC may be effective for use in agriculture, as Mn is a micronutrient for plants.

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