

Bacterial cellulose-charcoal composite gels: A sustainable material for arsenic and methylene blue adsorption

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ABSTRACT

The goal of the present study was the preparation of bacterial cellulose-charcoal composite gels for the adsorption of arsenic and methylene blue. A bacterial cellulose gel was produced from a high-performance strain of *Komagataeibacter intermedius* BE073. The bacterial cellulose-charcoal gels (BC-C gels) were successfully prepared by *in situ* method. Charcoal at concentrations of 0.005–1.50% (w/v) was used to prepare the BC-C gels, and the efficiency of the BC-C gels for arsenic as well as methylene blue adsorption was investigated. The results showed that the BC-C gels could retain charcoal, and the FTIR signal followed the increasing charcoal content, indicating the adhesion of charcoal powder within the bacterial cellulose structure. For arsenic adsorption, the 0.05% charcoal concentration showed the highest binding value (~3, 200 mg/kg), significantly higher than other conditions, possibly due to the opening of the pore structure and the increase in suitable charcoal surface adhesion sites. In term of methylene blue adsorption, the gels containing 0.05 to 0.10% charcoal showed the highest efficiency, with adsorption per gram of gel (q_t) increasing with the initial methylene blue concentration. While increasing the gel mass increased the removal percentage, the q_t value decreased at the equilibrium of the adsorption system. The results confirmed that the BC-C gels can be produced using an environmentally friendly synthetic process, thereby reducing chemical use and providing significant adsorption capacity for both arsenic and methylene blue, with an optimum carbon addition level of 0.05 – 0.10%. The findings indicate the potential of this new adsorbent material for wastewater treatment applications, addressing sustainable technology and supporting the Sustainable Development Goals (SDGs) of clean water, health, and the environment.

Keywords: bacterial cellulose, charcoal, adsorption, arsenic, methylene blue.

INTRODUCTION

Wastewater problems have increased in severity in recent years. Common wastewater issues, such as contamination by heavy metals (Ayangbenro and Babalola, 2017), oil, dyes (Dixit et al., 2015; Tonato et al., 2019; Garg and Chopra, 2022), organic compounds (Kinigopolou et al., 2022), and metalloids (Rai et al., 2019). This has resulted in increasing hazards to living organisms. Many human activities contribute to heavy metal contamination. For example, the use of chemical fertilizers in agricultural production leads to contamination by cadmium (Cd), copper

(Cu), and lead (Pb) (Atafar et al., 2008; Wang and Li, 2014). In addition, contamination by manganese (Mn), zinc (Zn), and Cu, components of pesticides, has been detected in various agricultural areas (Chopra et al., 2009; Alloway, 2013; Alengebawy et al., 2021). Moreover, industrial manufacturing releases heavy metals, such as Zn, Cu, Cd, Pb, and mercury (Hg) into the environment (Guan et al., 2014; Yuan and Wang, 2023; Liu et al., 2025).

Arsenic (As), as a heavy metal, is a highly toxic substance. It one of the poisons of heavy metal contamination and a serious carcinogen (Kamnev and Lelie, 2000). Arsenic has a complex effect on

metabolism among the “big four” toxic non-essential elements: arsenic, mercury, cadmium, and lead (Ghosh et al., 2019). Arsenic contamination can arise from many sources, such as mining and metallurgical industries, petroleum refining, agricultural chemicals, and wastewater; As has been found at high levels in both surface water and groundwater (Viraraghavan et al., 1999; Ungureanu et al., 2015). A recent report stated that the arsenic contamination in groundwater has been documented in about 108 countries worldwide (Shaji et al., 2021). This is particularly concerning in water sources used for daily consumption and household activities, as well as for agricultural purposes. Moreover, accumulation of arsenic is harmful even at very low concentrations, increasing the risk of skin, lung, and kidney cancers (Chen et al., 1992). In humans, arsenic affects the central nervous system and liver (Monrad et al., 2017). Therefore, removing arsenic from water is essential to prevent environmental problems and ensure ecosystem sustainability.

In addition to heavy metal contamination, wastewater has also been found to contain dyes, such as methylene blue. Methylene blue is an organic dye with an aromatic structure and a benzene ring (Fernandes et al., 2021). It is highly soluble in water (Water, 2022). Methylene blue dye is among the most harmful dyes to human health, causing cyanosis, methemoglobinemia, dyspnea, and tachycardia, and it can irritate the skin as well as induce an allergic reaction (Zainol et al., 2022). Contamination by methylene blue dye is generally associated with the use of coloring products in paper, temporary hair colorants, and the dyeing of cotton, wood, and silk, as well as activities in dye manufacturing and the textile industry (Baloo et al., 2021).

Several chemical and physical methods are currently being researched for arsenic removal, including membrane technology (Johnston and Heijnen, 2001), flocculation (Zakhar et al., 2018), and coagulation (Nidheesh and Singh, 2017). For methylene blue removal, methods such as membrane technology (Nowik-Zajac et al., 2024) and photocatalytic degradation are employed (Yao and Wang, 2010; Hou and Zhu, 2018). Adsorption is a widely used method due to its low cost, high efficiency, ease of use, and post-harvest separation (Yang et al., 2018). Various adsorbents have been used for arsenic and methylene blue, including activated carbon (Hadi et al., 2015), biochar (Abid et al., 2025), cellulose (Kim, 2025), resin

(Li et al., 2015), clay materials (El-Habacha et al., 2023), and nano-based materials (Badawi et al., 2021), etc. However, for many adsorbents, synthesis requires the use of reagents and chemical methods, as well as significant amounts of energy. Furthermore, some adsorbents are non-biodegradable, resulting in environmental pollution.

Previous research has shown that bacterial cellulose can be used as an adsorbent. Bacterial cellulose can be produced from many strains of bacteria, including the genera *Acetobacter*, *Agrobacterium*, *Pseudomonas*, and *Rhizobium* (Pereira et al., 2020) and most strains of *Komagataeibacter* (formerly known as *Gluconacetobacter*) (Yamada et al., 2012). The fiber structure consists of β -D-glucopyranose units that are linked by β -1,4 glycosidic bonds (Lahiri et al., 2021). Bacterial cellulose has the molecular formula $(C_6H_{10}O_5)_n$, similar to plant cellulose (Jamsheera and Pradeep, 2021). However, bacterial cellulose fibers differ from plant cellulose fibers due in that they do not contain lignin or hemicellulose (Klemm et al., 2001). In addition, bacterial cellulose has many desirable properties, such as good water-holding and retention capacity, stability, as well as high swelling value. In addition, bacterial cellulose is a biodegradable and environmentally friendly biopolymer. Therefore, bacterial cellulose has been developed for various applications, including packaging, medical materials, biofilms, stability modifiers, adhesion agents, and water-holding agents (Czaja et al., 2006; Esa et al., 2014; Ruka et al., 2014; Cacicedo et al., 2016). Furthermore, bacterial cellulose has a key ability to adsorb heavy metals (Mohite and Patil, 2014; Ashjaraan and Zare, 2019; Song et al., 2020; Kroeksakul et al., 2023) and methylene blue (Amaro-Reyes et al., 2025).

Therefore, based on the aforementioned properties, the present study aimed to prepare bacterial-cellulose gels for adsorbing arsenic and methylene blue. This involved using bacterial cellulose as the main component in the gel synthesis, along with structural modifications by charcoal for enhanced adsorption efficiency. The potential applicability of arsenic and methylene blue adsorption by the bacterial-cellulose gels (BC-C gels) as adsorbents was investigated. The research results represent an alternative solution to mitigating the environmental impacts of arsenic and methylene blue, and thus align with the United Nations Sustainable Development Goals (SDGs). This research supports SDG 6 (Clean water and sanitation) and SDG 13

(Climate action), as it is relevant to developing the wastewater treatment technology that is both efficient and low-cost. Additionally, improving the access to safe water helps protect human health and ecosystems, reinforcing SDG 3 (Good health and well-being) and SDG 14 (Life below water) by reducing toxic pollutant discharge.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation

The bacterial cellulose produced by *Komagataeibacter intermedius* BE073 was isolated from bioextract (Singhaboot and Kroeksakul, 2022) and cultured in Hestrin and Schramm medium (HS medium) (Hestrin and Schramm, 1954) for preparation of the inoculum. The HS medium was supplemented with 2% (weight (w)/volume (v)) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.27% (w/v) g of Na_2HPO_4 , and 0.115% (w/v) citric acid. The method for preparing the inoculum was adapted from the procedures described by Singhaboot and Kroeksakul (2022). The pH was adjusted to 4.2 with acetic acid, and the medium was sterilized at 121 °C for 15 minutes. Subsequently, 10% (v/v, volume/volume) of the stock culture was transferred to the HS medium and incubated at 30 °C for 7 days under static conditions. The bacterial cellulose inoculum was used to produce the BC-C gels.

Preparation of the BC-C gels

The BC-C gels were prepared *in situ* method; charcoal was added simultaneously with the bacterial culture from the initial stage of the gel production process. The bacteria were responsible for synthesizing the bacterial cellulose polymeric framework of the gel, while the charcoal was embedded within the gel network as it formed. The inoculum (10 mL) was transferred into 100 mL of HS medium and incubated at 30 °C, with shaking at 100 rpm. The charcoal powder (DC Fine Chemicals SLU, Spain; Figure 1) at concentrations ranging from 0.005% to 1.50% (w/v) was added to the bacterial cellulose-producing culture medium and incubated with shaking.

The preparation conditions of the BC-C gels, expressed as weight per volume (w/v) of charcoal relative to the culture medium, are summarized in Table 1.



Figure 1. Charcoal powder

In the first *in situ* method for the preparation of the BC-C gels, charcoal powder as an adsorption agent was added to the initial step, along with the *K. intermedius* BE073 culture, to produce bacterial cellulose gel. Charcoal was added at concentrations of 0.005, 0.01, 0.03, 0.05, 0.70, 0.10, 0.25, 0.50, 0.75, 1.00, and 1.50% (w/v), with a control treatment with no charcoal powder added or with a charcoal powder concentration of 0%. Then, the culture was incubated with shaking at 100 rpm and 30 °C for 7 days. The obtained BC-C gels had an oval shape and were large, but only one or two gels were obtained. When the charcoal concentration exceeded 0.25% (w/v), no gel formation occurred.

Table 1. The charcoal concentration (% w/v) used in the preparation of the BC-C gels

Symbol	Charcoal concentration (% w/v)
BC-C0	0
BC-C0.005	0.005
BC-C0.01	0.01
BC-C0.03	0.03
BC-C0.05	0.05
BC-C0.07	0.07
BC-C0.10	0.10
BC-C0.25	0.25
BC-C0.50	0.50
BC-C0.75	0.75
BC-C1.00	1.00
BC-C1.50	1.50

Therefore, the conditions for the *in situ* preparation of the BC-C gels were modified by cultivating *K. intermedius* BE073 in the HS medium and then growing the bacteria at 30 °C with shaking at 100 rpm for 4 days. After that, charcoal powder at concentrations of 0.005–1.50% (w/v) was added, and the cultivation continued for another 3 days. Although the medium remained predominantly liquid at the time of charcoal addition, the formation of gel nuclei was already observed. During bacterial cultivation, a continuous gel network subsequently developed *in situ*, incorporating the charcoal within the matrix. The BC-C gels were then harvested, boiled and washed with deionized water. The diameter of the BC-C gel was measured by a micrometer vernier caliper. After the BC-C gels were collected, the culture was centrifuged at 10,000 rpm for 10 minutes and washed with distilled water. The charcoal residue was dried until a constant weight was reached and weighed by the gravimetric method.

The gel production was better than during the initial attempt. The BC-C gels could be produced when charcoal powder was added at concentrations between 0.005–0.25% (w/v), but when the concentration of charcoal was higher than 0.25% (w/v), no gels were produced. The gels were oval to round, with an average size of 10.22 ± 4.00 mm in length and a width average of 8.53 ± 3.54 mm. The appearance of the BC-C gels was oval to round and the average weight of the gels produced under each condition was approximately 9.14 ± 3.14 g. The BC-C gels were black because the charcoal powder was incorporated into the

bacterial cellulose. The black color increased with charcoal concentration (Figure 2).

The chemical structure of the BC-C gels was analyzed using a Fourier transform infrared (FTIR) spectrophotometer (PerkinElmer, Inc., USA) at wave numbers ranging from 4000 to 600 cm^{-1} . Afterward, the adsorption performance of the BC-C gels for arsenic and methylene blue was evaluated.

Arsenic adsorption analysis

The BC-C gel samples were air-dried to reduce the moisture content to no more than 10%. The samples were then immersed in a 20 ppm arsenic trioxide solution (Loba Chemie PVT, LTD.) for 120 minutes, and then air-dried for another 30 minutes before digestion (Kroeksakul et al., 2023; Singhaboot et al., 2026). The samples (0.5 g) were digested in several 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 the 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 under standard conditions. The samples were compared against the ICP-OES multi-element standard solutions (AccuStandard, USA) (Singhaboot et al., 2026).

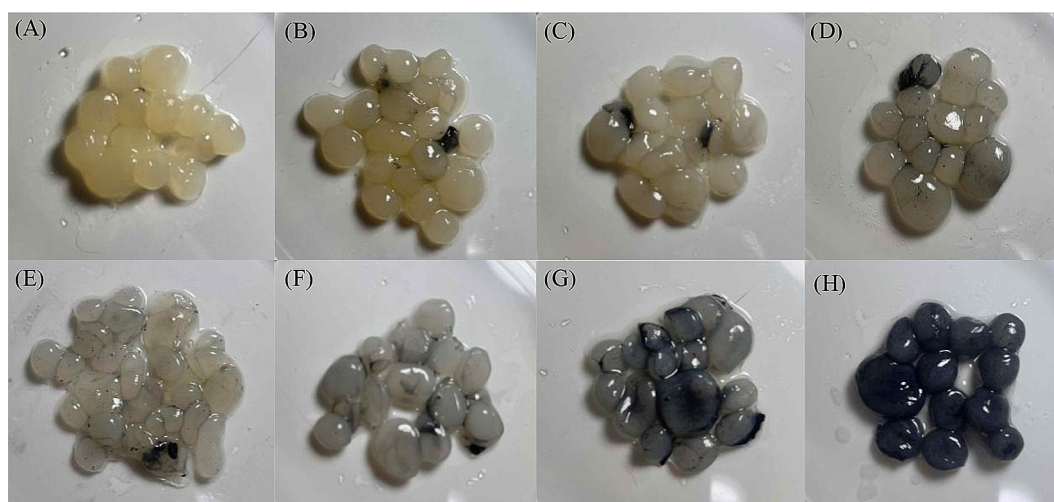


Figure 2. The BC-C gels with different charcoal concentrations of: (A) 0, (B) 0.005, (C) 0.01, (D) 0.03, (E) 0.05, (F) 0.07, (G) 0.10 and (H) 0.25% (w/v)

Methylene blue adsorption analysis

The efficiency of the BC-C gels in eliminating methylene blue from aqueous solutions was investigated using batch adsorption experiments. The methylene blue (Loba Chemie PVT, LTD) solution was prepared in different concentrations. The BC-C gels were allowed to air-dry to reduce moisture, then transferred to the methylene blue solution for two hours. After that, the absorbance at 660 nm was measured with an ultraviolet spectrophotometer (Unico (Shanghai) Instrument Co., Ltd.) to measure the residual methylene blue concentration in the solution. The amount of methylene blue adsorbed was used to calculate the adsorption efficiency in term of removal percentage according to Equation 1.

$$\begin{aligned} \text{Removal percentage (\%)} &= \\ &= ((C_0 - C_t) / C_0) \times 100 \end{aligned} \quad (1)$$

The amount adsorbed at time was calculated using Equation 2 (Kim, 2025) as follows:

$$q_t = (C_0 - C_t)(V) / m \quad (2)$$

where: C_0 is the initial methylene blue concentration (mg/L); C_t is the methylene blue concentration at time t ; V is the solution volume (L), and m is the weight of the BC-C gels (g).

RESULTS AND DISCUSSION

Condition of the BC-C gels

The results of the *in situ* preparation showed that the amount of charcoal incorporated into the BC-C gels increased significantly along with charcoal concentration (Table 2). The BC-C0

formula without added charcoal showed no charcoal content in the gel (0%), whereas in the BC-C0.005–BC-C0.25 formulas, the charcoal content increased, from 35.00 ± 7.07 to 78.12 ± 9.58 , respectively. This indicated that the charcoal could adhere well to the bacterial cellulose gel structure and that the initial concentration directly affected the amount of charcoal retained within the gel. The total gel weight (7.10–11.61 g) varied across formulations, but there was no clear linear relationship between gel weight and the percentage of charcoal in the gel. However, the charcoal content in gel weight (g) increased significantly, from 0.002 g in the BC-C0.005 formulation to 0.195 g in the BC-C0.25 formulation, reflecting the increased efficiency of the gel in retaining the carbon material. Increasing the concentration of activated charcoal in the range of 0.005–0.25% (w/v) resulted in an increase in the proportion of activated charcoal in the gel both in terms of weight and percentage; this is important for designing the adsorbent materials or composite materials that require the efficiency of additives within the bacterial cellulose gel structure.

Furthermore, the BC-C gels prepared with higher initial charcoal concentrations exhibited greater incorporation of charcoal within the gel network compared to those prepared at lower concentrations. This may be because, when the bacteria were cultured for gel production under shaking conditions and with high charcoal concentrations, the gels that had already formed were more likely to come into contact with charcoal particles in the culture medium. This was because the suspended amount was greater than at low concentrations, causing the bacterial cellulose fibers that were forming the gels to absorb more charcoal powder, resulting in the gels containing a higher amount

Table 2. Composition of charcoal in the BC-C gel prepared with different concentrations

Charcoal concentration (% w/v)	BC-C gel weight (g/100 mL culture medium)	Charcoal in BC-C gels (g)	Charcoal in the BC-C gels (%)
BC-C0	7.10 ± 2.67	0.000 ± 0.000	0.00 ± 0.00
BC-C0.005	8.92 ± 4.67	0.002 ± 0.001	35.00 ± 7.07
BC-C0.01	10.60 ± 4.60	0.004 ± 0.001	38.00 ± 8.49
BC-C0.03	7.95 ± 2.14	0.013 ± 0.016	42.33 ± 2.36
BC-C0.05	11.61 ± 2.85	0.024 ± 0.006	53.70 ± 4.67
BC-C0.07	8.73 ± 1.57	0.044 ± 0.000	63.21 ± 0.30
BC-C0.10	10.50 ± 2.02	0.074 ± 0.013	74.02 ± 1.34
BC-C0.25	7.73 ± 2.16	0.195 ± 0.010	78.12 ± 9.58

Note: BC-C gels denote bacterial cellulose-charcoal gels.

of charcoal powder. In addition, the bacterial cellulose gels from agitated or shaking cultures have a higher water holding capability (Krystynowicz et al., 2002), making this property suitable for adsorption application. Moreover, the results show that the BC-C gels can be produced using an *in situ* method. The *in situ* method is a technique to generate composite adsorbent materials by integrating additives directly into the bacterial cellulose network during fermentation. This finding is consistent with previous studies. For example, the bacterial cellulose aerogels prepared using the *in situ* biosynthesis method, in which covalent organic frameworks were directly incorporated during gel formation, exhibited significantly enhanced uranium adsorption (Li et al., 2025). Furthermore, *in situ* biosynthesis of a bacterial cellulose-based aerogel composite containing a yeast strain of *Aureobasidium pullulans* introduced abundant active sites for the removal of hexavalent chromium from wastewater (Dai et al., 2025).

The FTIR analysis

The FTIR spectral analysis of the BC-C gels in the concentration range of 0–0.25% (w/v) clearly showed changes in the infrared signal characteristics reflecting the interaction between the bacterial structure and charcoal (Figure 3). Considering the overall spectrum, the BC-C0 formulation without added charcoal exhibited the characteristics of pure bacterial cellulose, both in the O–H vibrational region around 3300–3400 cm^{-1} and the C–H group region around 2900–2850 cm^{-1} (Invernizzi et al., 2018; Hsiao et al., 2025). The signals were sharper and more intense compared to the formulations with different levels of added charcoal.

With increasing charcoal concentration, the O–H signal tended to decrease in intensity and slightly broaden. This may be due to the disruption of hydrogen bonds within the bacterial cellulose structure and to the interactions between –OH groups and the charcoal surface. In addition, the signal in the range of 2900–2850 cm^{-1} , is attributed to the vibration of methyl groups (Nandiyanto et al., 2019; Al-Kelani and Buthelezi, 2024), was less clear in the formulations containing high charcoal, especially the formulations BC-C0.10 and BC-C0.25, reflecting the infrared absorption property of charcoal materials that are more opaque to light than pure bacterial cellulose. The frequency region around 1640–1650 cm^{-1} , which

is related to the vibration of water trapped in the bacterial cellulose structure (Lucas-Aguirre et al., 2025), was altered by the addition of charcoal, with the formulations with high charcoal content tending to have a reduced sharpness that may be due to the reduction of water content in the structure resulting in a denser gel structure. The main signal band of glycosidic bonds (C–O–C) in the range around 1050–1030 cm^{-1} still appeared in all formulations (Tan et al., 2021), but its sharpness decreased with the increasing charcoal level, indicating the disturbance of the order of the bacterial cellulose structure when blended with charcoal materials.

However, the spectra of all charcoal formulations exhibited a flatter baseline and a lower percent transmittance, especially in the BC-C0.25 formulation, reflecting the higher infrared absorption of the charcoal. All these spectral changes were consistent with the amount of charcoal entrapped within the gel as determined by quantitative analysis, confirming that the charcoal could be effectively mixed and bound within the bacterial cellulose structure.

The impact of charcoal loading on arsenic adsorption by the BC-C gels

The results showed that the concentration of arsenic trapped in the BC-C gels varied significantly with charcoal addition level (Figure 4). The control (0%), which was bacterial cellulose gels without charcoal, had a relatively low arsenic concentration (approximately 300–400 mg/kg) and showed no significant difference compared to the levels of 0.005%, 0.03%, 0.07%, 0.10%, and 0.25% (w/v) ($p > 0.05$). The LSD test results confirmed that most of the mean differences between treatments were not statistically significant, indicating that the addition of charcoal in the very low to moderate concentration range (0.005–0.25% (w/v)) did not significantly enhance the arsenic adsorption efficiency. However, when charcoal was added at the level of 0.05% (w/v), there was a highly significant ($p < 0.001$) increase in arsenic adsorption capacity, with a high arsenic concentration of approximately 3.200 mg/kg, approximately 9–10 times higher than that of the control and all experimental treatments (Table 3).

A comparative analysis of pairs by LSD tests showed that the 0.05% (w/v) set differed significantly from all other experimental levels, with mean differences ranging from 2.844–3.095 mg/

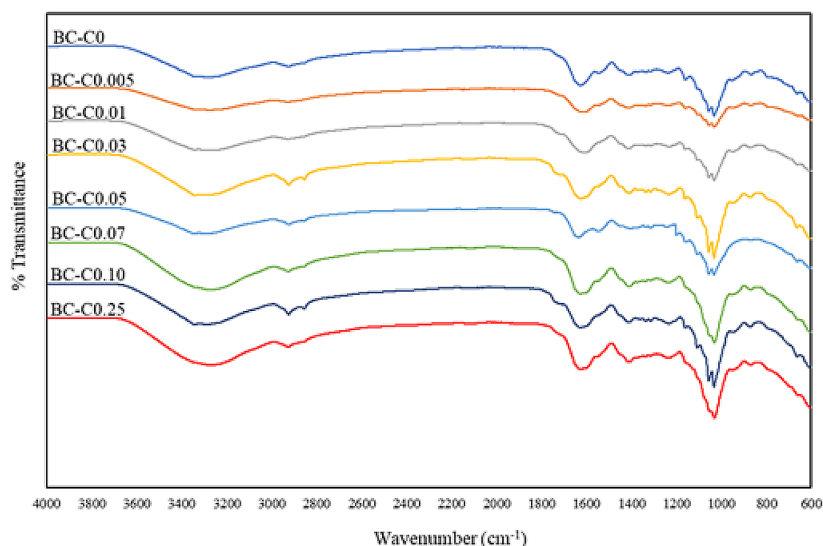


Figure 3. FTIR spectra of the BC-C gels with difference charcoal concentrations

kg, confirming that the 0.05% (w/v) level was the most effective condition for arsenic retention with the bacterial cellulose-charcoal gels.

The optimum charcoal level for arsenic adsorption efficiency indicated a non-linear relationship between the charcoal content and the arsenic adsorption efficiency, with very low and high concentrations (0.005–0.25% (w/v)) failing to significantly enhance the efficiency (Suresh et al., 2022; Sun et al., 2022; Hu et al., 2025). This may have been due to the insufficient number of positively charged functional groups or arsenic-binding groups, or the compaction of the BC-C gel structure, which reduced the usable surface area (Yang et al., 2019; Yang et al., 2024). As the 0.05% level resulted in the highest efficiency increase, the differences may have been due to (1) the distribution of charcoal functional groups on the BC-C gel structure, (2) the opening of the BC-C gel micropore structure, resulting in an increased surface area, or (3) the electrostatic interaction and complexation between arsenic and the charcoal-modified surface (Hossain et al., 2018; Khamkeaw et al., 2020; Lind et al., 2021). However, the result was a significant increase in adsorption capacity; at charcoal levels above 0.05%, there was no further increase in efficiency, possibly due to pore blocking or oversaturation of the functional groups, resulting in a decrease in the number of actual binding groups. This result is consistent with the concept of composite modification, meaning that there is an optimum loading level, and above this, the efficiency does not always increase with the loading amount.

Table 3. Quantity of arsenic adsorption of the BC-C gels

BC-C gel	Quantity of arsenic content (mg/kg of dry weight)
BC-C0	276 (\pm 2.80) ^a
BC-C0.005	111 (\pm 38.3) ^{ab}
BC-C0.01	299 (\pm 48.5) ^b
BC-C0.03	129 (\pm 28.3) ^{ab}
BC-C0.05	3206 (\pm 270) ^c
BC-C0.07	362 (\pm 15.7) ^{ab}
BC-C0.10	279 (\pm 11.1) ^{ab}
BC-C0.25	241 (\pm 6.45) ^{ab}
Average	613 (\pm 1007)

Note: ^{ab} Means that column differences are significant at $p < 0.05$ (LSD test).

Methylene blue adsorption by the BC-C gels

The results for methylene blue adsorption efficiency of the BC-C gels at dosing rates ranging from 0–0.25% (w/v), using gel weights of 0.1 and 1.0 g in 20 mL of solution, showed that all gel formulations were capable of removing methylene blue from the solution. However, significant differences in efficiency were observed among the formulations. The gel weight of 0.1 g yielded a removal percentage of 4.50–17.02%, and the BC-C0.07 formulation had the highest efficiency in terms of both removal percentage ($17.02 \pm 2.19\%$) and adsorption amount per gel weight ($q_t = 0.360 \pm 0.046$ mg/g). This indicated that the 0.07% dosage provided the best adsorption capacity per unit mass (Table 4).

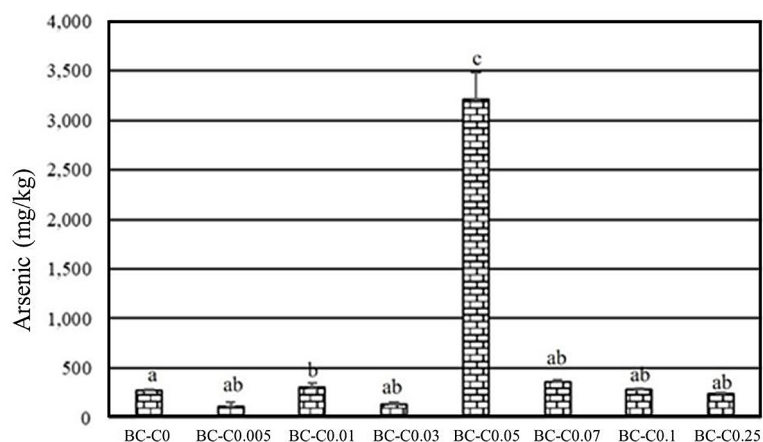


Figure 4. The concentration of arsenic retained in the BC-C gels under different charcoal concentrations

However, when the gel weight was increased to 1.0 g, the removal percentage increased significantly, ranging from 26.00% to 52.59%, with the BC-C0 formulation (without additive) exhibiting the highest efficiency at $52.59 \pm 10.92\%$. Although this value was higher than that of the gel with the additive, the q_t value decreased compared to using only 0.1 g of the gel, a typical characteristic of adsorption systems in which higher mass may not fully utilize the adsorption sites. The BC-C0.05, BC-C0.07, and BC-C0.25 formulations still exhibited higher q_t values than BC-C0, reflecting the role of the additive in increasing the surface area or adsorption sites of the gel (Figure 4). Thus, the results indicated that the addition level of 0.07%

(w/v) was optimal for increasing the adsorption efficiency per unit mass, while increasing the gel weight increased the total removal amount, but the q_t value decreased at the equilibrium of the adsorption system (Figure 5).

The results showed that the BC-C gels have the potential to adsorb methylene blue dye, but this capability was significantly dependent on both the gel content and the adsorbent loading rate (Hu et al., 2025; Wang et al., 2025). Using different gel weights yielded significantly different performance patterns. Increasing the gel weight from 0.1 to 1.0 g increased the removal percentage due to the greater number of adsorption sites, but the adsorption value per gram (q_t)

Table 4. The adsorption effect of methylene blue on the BC-C gels under different gel weights

BC-C gel	BC-C gel weight (g)	Methylene blue adsorption (mg/L)	Removal percentage (%)	q_t (mg/g)
BC-C0	0.1	2.14 ± 0.22	11.42 ± 1.19	0.306 ± 0.032
	1.0	9.76 ± 1.95	52.59 ± 10.92	0.164 ± 0.033
BC-C0.005	0.1	1.58 ± 0.45	8.45 ± 2.38	0.237 ± 0.067
	1.0	6.22 ± 1.86	33.41 ± 9.72	0.120 ± 0.036
BC-C0.01	0.1	1.14 ± 0.36	6.09 ± 1.90	0.201 ± 0.063
	1.0	4.83 ± 0.31	26.00 ± 1.64	0.096 ± 0.007
BC-C0.03	0.1	0.84 ± 0.36	4.50 ± 1.93	0.101 ± 0.043
	1.0	4.86 ± 0.34	26.14 ± 1.93	0.089 ± 0.003
BC-C0.05	0.1	1.95 ± 0.35	10.39 ± 1.87	0.215 ± 0.039
	1.0	7.26 ± 2.45	38.96 ± 12.85	0.184 ± 0.016
BC-C0.07	0.1	3.19 ± 0.41	17.02 ± 2.19	0.360 ± 0.046
	1.0	8.07 ± 0.14	43.44 ± 0.45	0.146 ± 0.004
BC-C0.10	0.1	2.68 ± 0.12	14.29 ± 0.65	0.313 ± 0.014
	1.0	6.92 ± 0.60	37.19 ± 2.91	0.135 ± 0.003
BC-C0.25	0.1	2.96 ± 0.26	15.82 ± 1.38	0.324 ± 0.028
	1.0	7.81 ± 2.04	42.08 ± 11.36	0.188 ± 0.005

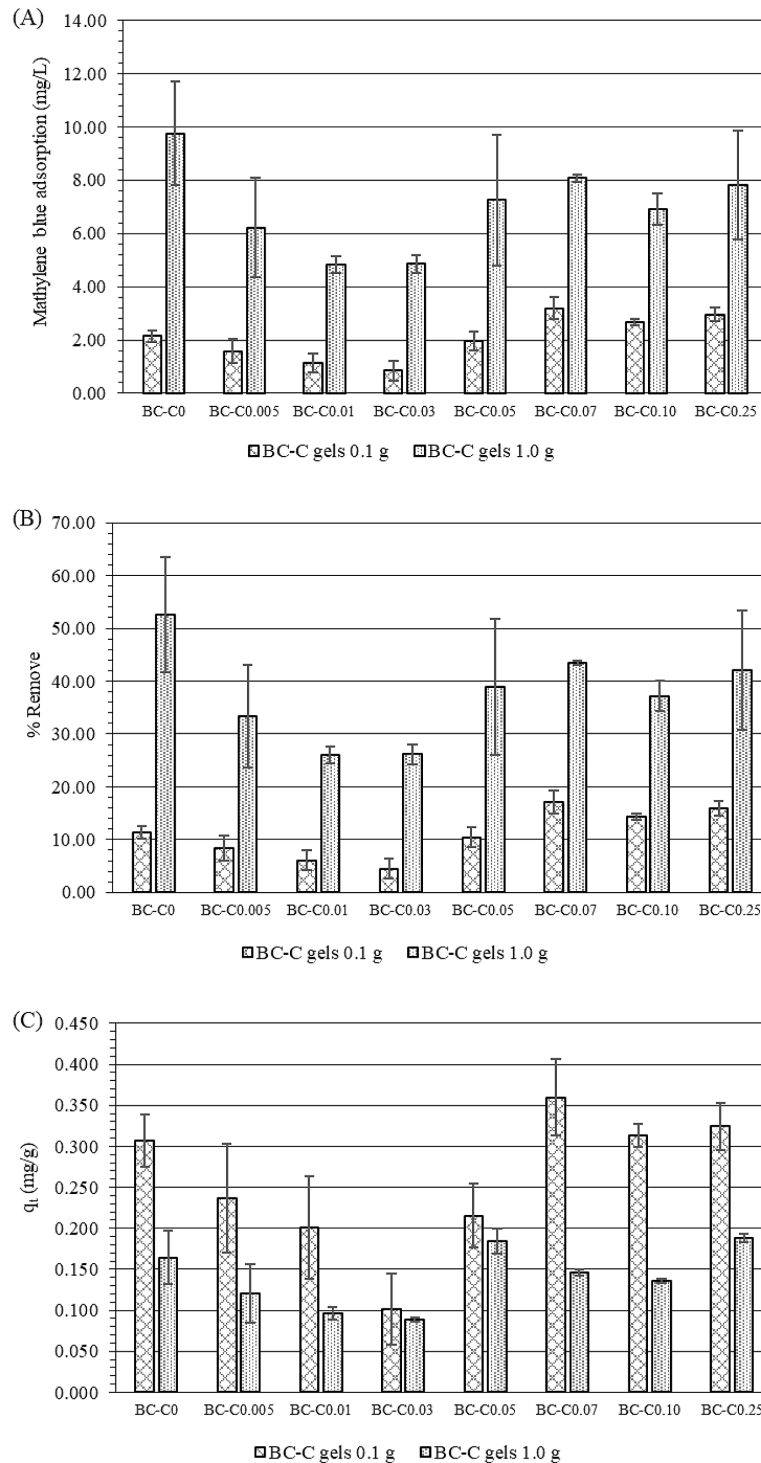


Figure 5. Methylene blue adsorption of the BC-C gels under different gel weights: (A) methylene blue adsorbed amount (mg/L), (B) removal percentage (% remove), and (C) adsorption value per gram (q_1 , mg/g)

decreased. This is a typical characteristic of an adsorption system with an excess of adsorbent: the dye concentration per unit mass of gel decreases, leaving some adsorption sites underutilized (Fadhillah et al., 2024).

Furthermore, the addition of different additives affected the internal gel structure, as

reflected in the adsorption values. The BC-C0.07 formulation showed higher removal and q_1 values than the other gels, especially at a gel weight of 0.1 g. This suggests that the addition of 0.07% (w/v) additive may increase the specific surface area, porosity, or the number of functional groups that can effectively interact with the methylene

blue molecules. In contrast, the addition of lower (0.005–0.03% (w/v)) and higher (0.25% (w/v)) percentages of additives did not result in a linear increase in efficiency, possibly due to the changes in the gel structure that caused pore clogging or the inhomogeneity of the polymer framework (Eliaz, 2019; Sugar et al., 2023).

However, compared to the control gel (BC-C0) without additives, the highest removal percentage was found when using 1.0 g of gel, but the q_t value was lower than BC-C0.07, indicating that the addition of additives does not always increase the total removal amount, but rather improves the adsorption efficiency per unit mass; this is useful for the design of adsorbent materials that require cost reduction or use of less adsorbent (Akhtar et al., 2025; Onyekachukwu et al., 2025). Therefore, the experimental results indicated that the optimal concentration was 0.07% (w/v), providing a balance between gel structure and adsorption efficiency in terms of removal concentration and efficiency per unit mass. The

results support the potential of the BC-C gels as adsorbents for wastewater treatment systems with low to moderate dye concentrations.

Figure 6 shows that the initial methylene blue concentration significantly affected the adsorption efficiency of the BC-C gels. As the methylene blue concentration increased from 1 to 20 mg/L in 10 mL of solution, the adsorption efficiency changed accordingly. The adsorption amount (mg/L) and the adsorption value per gram (q_t) were significantly increased for all gel formulations, reflecting the higher mass transfer driving force that enabled the methylene blue dye molecules to migrate into the adsorption sites of the gel more rapidly and efficiently (Li et al., 2024; Farasati Far et al., 2024; Carhuarupay-Molleda et al., 2025). This behavior is typical in physicochemical adsorption systems, where a high initial concentration accelerates the collisions and interactions between the methylene blue dye and functional groups on the gel surface (Zhang et al., 2023; Kuyucu et al., 2025).

Table 5. Methylene blue adsorption effect of the BC-C gels under different initial concentrations

Parameter	Methylene blue initial concentration (mg/L)	Methylene blue adsorption (mg/L)	% Remove	q_t (mg/g)
BC-C0	1.00	0.08 ± 0.04	6.54 ± 2.87	0.004 ± 0.002
	5.00	1.03 ± 0.04	19.11 ± 0.81	0.058 ± 0.002
	20.00	6.40 ± 0.16	35.00 ± 0.88	0.366 ± 0.009
BC-C0.005	1.00	0.09 ± 0.02	6.87 ± 1.36	0.004 ± 0.001
	5.00	2.10 ± 0.01	39.16 ± 0.10	0.121 ± 0.000
	20.00	2.22 ± 0.27	12.13 ± 1.45	0.130 ± 0.016
BC-C0.01	1.00	0.14 ± 0.06	11.67 ± 4.59	0.008 ± 0.003
	5.00	2.19 ± 0.09	40.87 ± 1.73	0.145 ± 0.006
	20.00	2.25 ± 0.36	12.29 ± 1.95	0.203 ± 0.032
BC-C0.03	1.00	0.17 ± 0.02	13.82 ± 1.42	0.009 ± 0.001
	5.00	1.96 ± 0.12	36.53 ± 2.18	0.105 ± 0.006
	20.00	2.30 ± 0.65	12.58 ± 3.54	0.143 ± 0.040
BC-C0.05	1.00	0.24 ± 0.05	19.35 ± 4.34	0.014 ± 0.003
	5.00	2.06 ± 0.03	38.41 ± 0.54	0.114 ± 0.002
	20.00	5.37 ± 0.58	29.37 ± 3.15	0.334 ± 0.036
BC-C0.07	1.00	0.25 ± 0.03	20.02 ± 2.28	0.012 ± 0.001
	5.00	2.08 ± 0.01	38.78 ± 0.27	0.117 ± 0.001
	20.00	4.04 ± 0.56	22.06 ± 3.09	0.235 ± 0.033
BC-C0.10	1.00	0.20 ± 0.08	16.37 ± 6.10	0.010 ± 0.004
	5.00	2.51 ± 0.34	46.70 ± 6.34	0.132 ± 0.018
	20.00	6.88 ± 0.64	37.59 ± 3.51	0.414 ± 0.039
BC-C0.25	1.00	0.19 ± 0.04	15.68 ± 3.27	0.010 ± 0.002
	5.00	2.32 ± 0.11	43.19 ± 2.13	0.120 ± 0.006
	20.00	3.97 ± 0.67	21.71 ± 3.67	0.223 ± 0.038

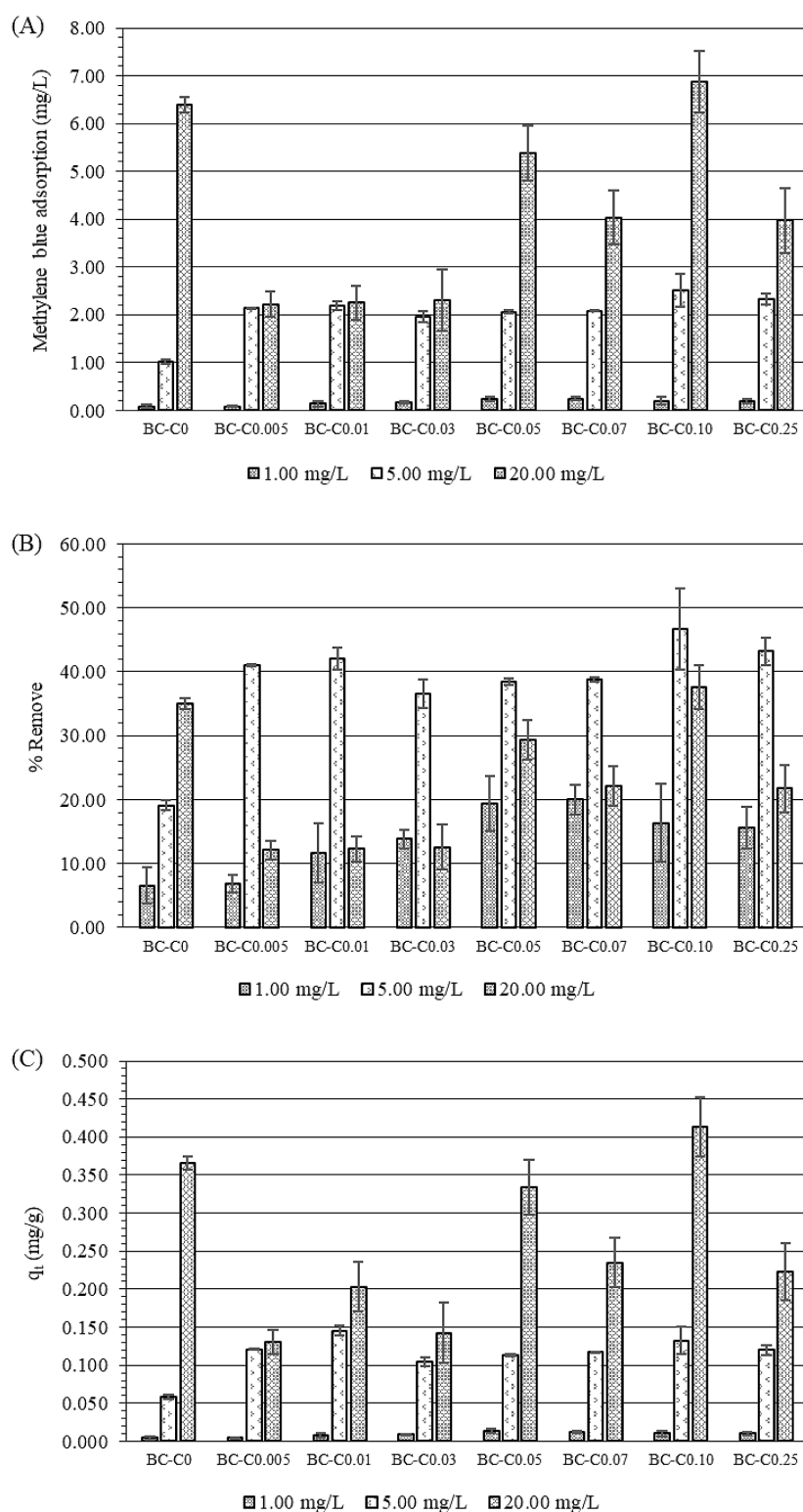


Figure 6. The adsorption of methylene blue by the BC-C gels under different initial concentrations

However, the comparison of results among gel formulations showed that BC-C0.10 and BC-C0.05 exhibited the best performance, especially at a concentration of 20 mg/L, yielding high q_t values of 0.414 and 0.334 mg/g, respectively (Table 5). This indicates that the addition of adjuvants could

increase specific surface area, porosity, and the number of anionic sites, thereby facilitating better binding of methylene blue, a cationic dye. In contrast, the formulations with low addition levels, such as BC-C0.005–BC0.03, showed consistently lower adsorption values, especially at 20 mg/L.

This may have been due to an insufficient number of adsorption sites or to the gel structure not being sufficiently developed for dye binding (Murcia-Salvador et al., 2024; Simion et al., 2025).

Additionally, at concentrations as low as 1 mg/L, all formulations gave very low q_t values (< 0.02 mg/g), consistent with the fact that in adsorption systems, if the number of contaminant molecules is too low, it will not be able to completely fill the adsorption sites (Satyam and Patra, 2024; Atangana et al., 2025). This results in low adsorption per unit mass, although the removal percentage value may increase in some formulations. In addition, the non-linear adsorption behavior with the level of supplement addition indicates that an excessive amount of the substance (Altwayti et al., 2022; Ogbeh et al., 2025; Salami et al., 2026), such as in BC-C0.25, may result in a denser gel structure, causing pore blocking and reducing the diffusion of dye molecules into the gel interior. The experiments indicated that the gel achieved its highest efficiency when auxiliary substances were added at 0.05–0.10%, as this provides a balance between gel structure and the number of adsorption sites. Especially when used in the systems with medium-to-high dye concentrations, the BC-C gels in this proportion range are suitable for the wastewater treatment processes involving residual dyes.

The relationship between the adsorption capacity of the BC-C gels for methylene blue and arsenic

A Pearson correlation analysis was performed for the amount of methylene blue adsorbed per

gram of adsorbent and the arsenic concentration (Charcoal; 0, 0.005, 0.01, 0.03, 0.05, 0.07, 0.1, and 0.25% (w/v)). The analysis showed that the adsorbed concentration of methylene blue had a significant positive relationship with the amount adsorbed ($r = 0.924–0.992$, $p < 0.01$), indicating that the absorbance values were in good agreement with the substance amounts (Table 5). In addition, the arsenic concentration showed a moderate positive correlation with the amount adsorbed ($r = 0.516$, $p < 0.01$) and methylene blue adsorption ($r = 0.418$, $p < 0.05$), indicating that arsenic concentrations affected the measurement method at low dilution levels. The final concentration showed a strong correlation with methylene blue concentration ($r = 0.470$, $p < 0.05$), indicating that the measurement level at dilution level 5 was most appropriate for estimating the true concentration. While some pairs of variables did not show a relationship, such as the concentrations of charcoal and arsenic or the amount adsorbed at level 5 and the amount adsorbed at level 20, this indicates different measurement characteristics and suggests different instrument sensitivity across measurement ranges (Table 6).

The results of this study reflect the consistency of the variables involved in the adsorption process, particularly the absorbance and the amount adsorbed per gram of adsorbent, which showed highly significant positive correlations ($r = 0.924–0.992$, $p < 0.01$) across all assay levels. This indicated that the spectrophotometric values could be used to accurately predict the true absorbance values and supports the effectiveness of both analytical methods in evaluating the amount

Table 6. Relationships between the adsorption capacity of the BC-C gels in different concentrations of charcoal and dye adsorption capacity

Parameter	Con	As Con	Con.Abs 1	Con.Abs 5	Con.Abs 20	QT1	QT5	QT20
Con	1	-0.057	0.398	.470*	0.163	0.379	0.231	0.087
As Con	-0.057	1	.418*	0.025	0.278	.516**	-0.002	0.325
Con.Abs 1	0.398	.418*	1	.527**	0.165	.992**	0.396	0.170
Con.Abs 5	.470*	0.025	.527**	1	-0.195	.492*	.924**	-0.140
Con.Abs 20	0.163	0.278	0.165	-0.195	1	0.181	-0.360	.973**
QT1	0.379	.516**	.992**	.492*	0.181	1	0.364	0.192
QT5	0.231	-0.002	0.396	.924**	-0.360	0.364	1	-0.240
QT20	0.087	0.325	0.170	-0.140	.973**	0.192	-0.240	1

Note: * The correlation is significant at the 0.05 level (two-tailed); ** The correlation is significant at the 0.01 level (two-tailed); Con: concentration of charcoal; As Con: Arsenic concentration; Con.Abs 1, 5, and 20: concentration of methylene blue 1, 5, and 20 (mg/L); and QT 1, 5, and 20: amount adsorbed at times 1, 5, and 20 (mg/g).

of dye or substance adsorbed in the same system (Khalifeh et al., 2025; Lessa et al., 2025). The relationship between arsenic concentration and the amount adsorbed and the concentration of methylene blue was moderate ($r = 0.516$ and $r = 0.418$, respectively), reflecting that at low dilutions, changes in arsenic concentration could still be detected by both methods, but the low correlation may have been due to matrix interference or reduced instrument sensitivity in the low concentration range (Kundu et al., 2002; Zhou et al., 2025). Furthermore, the significant positive correlation between charcoal concentration and methylene blue concentration ($r = 0.470$, $p < 0.05$) indicated that a dilution level of 5 mg/g was appropriate for reflecting the true concentration in the experimental system. However, some variable pairs, such as the concentrations of charcoal and arsenic or the amount adsorbed and the amount adsorbed, showed no clear correlation, which may indicate different response characteristics of the measurement methods or inconsistent sensitivity across measurement ranges. Therefore, selecting an appropriate concentration range and measurement method is a key factor in the accuracy of the evaluation in this type of research.

CONCLUSIONS

This study used an in-situ synthesis method with the microbe *Komagataeibacter intermedius* BE073 to successfully create bacterial cellulose-charcoal composite gels (BC-C gels). This eco-friendly method minimizes chemical and energy consumption in the manufacturing of adsorbent materials. The research established that bacterial cellulose effectively retains charcoal, and the incorporation of charcoal in suitable ratios markedly improves the adsorption characteristics of water pollutants. The adsorption results for methylene blue dye indicated that all BC-C gel formulations effectively removed the dye, with effectiveness contingent upon the gel mass and charcoal ratio. The formulations comprising 0.05–0.10% (w/v) of charcoal demonstrated elevated qt/unit mass values, appropriate for systems with medium to high dye concentrations. Although augmenting the gel mass enhanced the removal percentage, it diminished the efficiency per unit mass owing to adsorption equilibrium. A nonlinear association was seen between arsenic adsorption and the quantity of charcoal. The

formulation with 0.05% charcoal had the greatest efficacy (about 3.200 mg/kg), emphasizing the significance of the porous architecture and the surface-arsenic interaction. Excessive charcoal addition diminished effectiveness due to pore obstruction. This research corresponds with the United Nations Sustainable Development Goals, specifically SDG 6, by advancing efficient and cost-effective wastewater treatment technologies, alongside SDGs 3, 12, 13, and 14, which emphasize mitigating the health risks from toxic substances, employing eco-friendly biomaterials, and safeguarding aquatic ecosystems over the long term. Future research should examine the reusability of the bacterial cellulose-charcoal composite gel to evaluate its durability and cost-effectiveness in practical applications. Evaluating the substance using actual wastewater with diverse contaminants will validate its efficacy under authentic environmental conditions.

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