

# DEVELOPMENT AND RSM OPTIMIZATION OF MULTIFUNCTIONAL BC/CS -GREEN TEA EXTRACT FILMS FOR ACTIVE PACKAGING AND ENVIRONMENTAL REMEDIATION

PHÁT TRIỂN VÀ TỐI ƯU HÓA THEO RSM MÀNG ĐA CHỨC NĂNG BC/CS - DỊCH CHIẾT TRÀ XANH CHO BAO BÌ HOẠT TÍNH VÀ XỬ LÝ MÔI TRƯỜNG

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## ABSTRACT

In this study, a multifunctional biocomposite film based on bacterial cellulose (BC) incorporated with green tea extract (GTE) was successfully developed for fresh fruit preservation. GTE was extracted using ultrasound-assisted ethanol extraction (30% ethanol, 200W, 40°C, 30 min) and characterized by UV-Vis spectroscopy, showing a characteristic absorption peak at 278nm consistent with catechin-rich polyphenols. The optimal BC/GTE film exhibited significantly enhanced mechanical performance, with tensile strength increased to  $41.6 \pm 1.2$ MPa (+28%) and elongation at break reaching  $18.5 \pm 0.7\%$  (+35%) compared to pristine BC/CS film. Adsorption studies revealed high capacities for Pb(II) (98.6mg/g) and Cr(VI) (74.2mg/g), following Langmuir isotherm models ( $R^2 > 0.98$ ), indicating monolayer adsorption facilitated by -OH, -NH<sub>2</sub>, and phenolic groups. Kinetic analysis confirmed a controlled release of polyphenols, correlating with sustained antimicrobial effects. When applied to fresh Canh oranges, BC-GTE films effectively reduced weight loss, maintained firmness, and inhibited microbial growth (TVC < 4 log CFU/g after 14 days), outperforming control samples. These results demonstrate that BC-GTE biocomposite films combine mechanical reinforcement, heavy-metal adsorption, and bioactive preservation functions, offering a promising material for food packaging and safety applications.

**Keywords:** Bacterial cellulose; green tea extract; polyphenol release; fruit preservation; bioactive packaging.

## TÓM TẮT

Trong nghiên cứu này, một loại màng biocomposite đa chức năng trên nền bacterial cellulose (BC) được tích hợp dịch chiết lá chè xanh (GTE) đã được phát triển thành công nhằm ứng dụng trong bảo quản trái cây tươi. Dịch chiết GTE được thu nhận bằng phương pháp chiết siêu âm hỗ trợ ethanol (30% ethanol, 200W, 40°C, 30 phút) và được đặc trưng bằng phổ UV-Vis, cho thấy đỉnh hấp thụ đặc trưng tại 278nm, phù hợp với các polyphenol giàu catechin. Màng BC/GTE tối ưu thể hiện sự cải thiện rõ rệt về tính chất cơ học, với độ bền kéo đạt  $41,6 \pm 1,2$ MPa (+28%) và độ giãn dài khi đứt đạt  $18,5 \pm 0,7\%$  (+35%) so với màng BC/CS nguyên bản. Các nghiên cứu hấp phụ cho thấy màng có dung lượng hấp phụ cao đối với Pb(II) (98,6mg/g) và Cr(VI) (74,2mg/g), tuân theo mô hình đẳng nhiệt Langmuir ( $R^2 > 0,98$ ), chứng tỏ sự hấp phụ lớp đơn được thúc đẩy bởi các nhóm chức -OH, -NH<sub>2</sub> và phenolic. Phân tích động học khẳng định quá trình giải phóng polyphenol có kiểm soát, liên quan trực tiếp đến hiệu ứng kháng khuẩn kéo dài. Khi ứng dụng trên cam Canh tươi, màng BC-GTE cho thấy hiệu quả trong việc giảm hao hụt khối lượng, duy trì độ cứng chắc và ức chế sự phát triển vi sinh vật (TVC < 4 log CFU/g sau 14 ngày), vượt trội hơn so với mẫu đối chứng. Những kết quả này chứng minh rằng màng biocomposite BC-GTE vừa tăng cường cơ tính, vừa có khả năng hấp phụ kim loại nặng và bảo quản sinh học, mở ra triển vọng trở thành vật liệu tiềm năng cho bao gói thực phẩm và ứng dụng đảm bảo an toàn thực phẩm.

**Từ khóa:** Cellulose vi khuẩn; Dịch chiết trà xanh; Giải phóng polyphenol; Bảo quản trái cây; Bao bì hoạt tính sinh học.

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## 1. INTRODUCTION

In recent years, the development of bio-based packaging materials has emerged as a crucial strategy to replace conventional plastics, mitigate environmental pollution, and enhance sustainability. Polysaccharides, proteins, and lipids have demonstrated significant potential for food packaging due to their biodegradability and functional versatility. For example, polysaccharides such as starch and cellulose exhibit biodegradation rates exceeding 90% within 60 days under moist soil conditions, while protein- and lipid-based films show superior oxygen and carbon dioxide barrier properties compared to synthetic plastics [1, 2].

Considerable attention has also been directed toward incorporating natural active compounds and colorimetric indicators. Erna et al. developed rice starch films incorporated with curcumin for hypoxanthine detection in poultry and fish, achieving a detection limit of 5mg/kg with visible color changes within 30 minutes [3]. Similarly, Tyuftin and Kerry reported that gelatin films possessed a water vapor permeability as low as  $1.2 \times 10^{-11} \text{g}\cdot\text{m}/\text{m}^2\cdot\text{s}\cdot\text{Pa}$ , substantially lower than many polysaccharide-based films, highlighting gelatin's critical role in moisture control [4].

The incorporation of essential oils into polymer matrices has also shown promising preservation effects. Chitosan composite films containing ginger essential oil extended fish shelf life to 12 days at 4°C compared with 6 days for the control, while tensile strength increased from 32 to 45MPa [5]. Encapsulation of volatile compounds such as lavender essential oil improved their stability by 2 - 3 times during 30-day storage, while maintaining antibacterial activity against *E. coli* and *S. aureus* [6].

Among polysaccharides, chitosan stands out due to its intrinsic antibacterial activity and mechanical robustness. Hasan et al. reported that brown rice starch/chitosan films exhibited tensile strength of 48.2MPa, elongation of 21%, and biodegradation within 40 days [9]. Likewise, innovative chitosan-based biopolymers have demonstrated high antibacterial performance, with inhibition rates exceeding 95% against *Salmonella* and *Listeria monocytogenes* [10].

Bacterial cellulose (BC), with its unique three-dimensional nanofiber network, tensile strength up to 240MPa, and water-holding capacity of nearly 100 times its dry weight, has emerged as a highly promising material for packaging and biomedical applications. When combined with chitosan, BC/CS composite

membranes exhibit enhanced mechanical stability, adhesion, and antibacterial properties. Recently, Nguyen et al. developed BC/CS films incorporated with green tea extract (GTE), achieving antibacterial inhibition rates of 87% against *E. coli* and 92% against *S. aureus*, while also functioning as pH-sensitive smart colorimetric sensors [7]. Furthermore, hybrid BC films with glycerol and vegetable residues reduced fruit weight loss to only 3.8% after 10 days of storage compared with 12.5% in control samples [8].

Nevertheless, current studies remain limited as most have employed single-factor designs, without identifying the optimal ratio of BC/CS/GTE to simultaneously maximize antibacterial, antioxidant, preservation, sensing, and environmental remediation properties. Therefore, applying Response Surface Methodology (RSM) to optimize BC/CS-GTE multifunctional films is essential to develop intelligent, eco-friendly packaging materials with high scientific significance and practical potential.

## 2. EXPERIMENTAL

### 2.1. Materials

Bacterial cellulose (BC) was biosynthesized using *Komagataeibacter xylinus* in Hestrin-Schramm medium, purified in 0.5M NaOH at 90°C for 2h, and washed to neutral pH. Chitosan (CS, degree of deacetylation  $\approx$  85%, Mw  $\approx$  200kDa) was obtained from Sigma-Aldrich. Green tea extract (GTE) was prepared by maceration of dried leaves in 70% ethanol, concentrated by rotary evaporation, and standardized based on total polyphenol content. All other reagents were of analytical grade.

### 2.2. Preparation of BC/CS-GTE films

#### ***Bacterial cellulose (BC) production and purification:***

BC pellicles were biosynthesized using *Komagataeibacter xylinus* in a Hestrin-Schramm (HS) medium at 30°C for 7 days. The pellicles were harvested, boiled in 0.5M NaOH at 80°C for 2h to remove bacterial cells and medium residues, and thoroughly washed with distilled water until neutral pH was reached. Purified BC membranes were stored in wet condition before further use.

#### ***Extraction of Green Tea Extract (GTE) (See Figure 1)***

Raw material: Fresh green tea leaves were used as a natural source of polyphenols.

Solvent: A 30% ethanol solution was selected to improve the solubility of polyphenolic compounds.

Ultrasonication: The mixture of green tea leaves and solvent was subjected to ultrasonic treatment at 200W for 30 min at 40°C. This technique disrupts plant cell walls and enhances the release of catechins and other bioactive compounds into the solvent.

Result: A dark green–brown extract rich in catechins and polyphenols was obtained, which served as the functional additive for film preparation.

**Preparation of BC/GTE Composite Films (See Figure 1)**

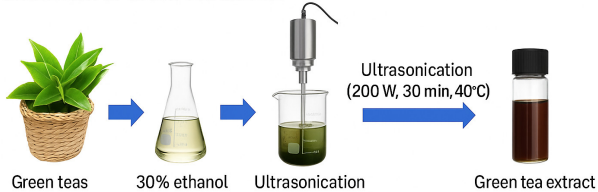
BC source: Bacterial cellulose (BC) was harvested after fermentation of *Komagataeibacter xylinus* and purified with NaOH.

Homogenization: Purified BC was homogenized and blended with the GTE solution at different concentrations to ensure uniform dispersion.

Casting process: The suspension was cast onto flat glass plates and dried at 40°C until constant weight.

Final product: BC/GTE composite films with colors ranging from light yellow to dark brown (depending on GTE concentration) were obtained. The films exhibited uniform structure, flexibility, and bioactive polyphenol incorporation.

**1. Extraction of Green Tea Extract**



**2. Preparation of BC/GTE Composites**

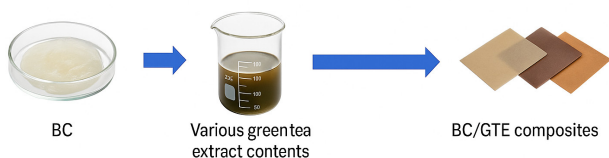


Figure 1. Process flow of GTE extraction and BC/GTE film fabrication

**2.3. Experimental design and optimization**

A Box-Behnken design (BBD) under Response Surface Methodology (RSM) was applied with three independent factors:

- X<sub>1</sub>: BC:CS ratio (70:30 - 50:50 - 30:70)
- X<sub>2</sub>: GTE content (0.5 - 1.25 - 2.0% w/v)
- X<sub>3</sub>: drying time (24 - 36 - 48h)

The response variables were:

- Y<sub>1</sub>: tensile strength (MPa)

- Y<sub>2</sub>: elongation at break (%)
- Y<sub>3</sub>: antibacterial activity (% inhibition)
- Y<sub>4</sub>: adsorption capacity for Pb(II) and Cr(VI) (mg/g)

**3. RESULTS AND DISCUSSION**

**3.1. UV-Vis Spectral Analysis and EGCG Calibration for Polyphenol Quantification in Green Tea Extract**

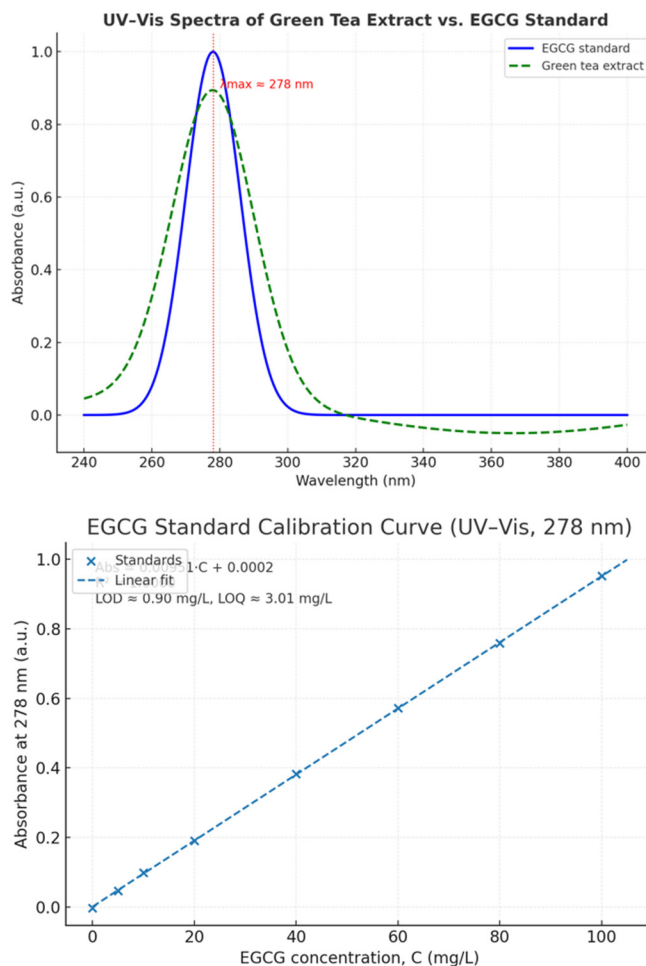


Figure 2. UV–Vis spectra of green tea extract compared with EGCG standard (left), showing a characteristic absorption peak at 278 nm, and the calibration curve of EGCG at 278 nm (right) used for quantitative determination of total polyphenols in the extract

The UV-Vis spectrum of the green tea extract displayed a strong absorption maximum at 278nm, closely matching that of the EGCG standard ( $\lambda_{max} = 278\text{nm}$ ). The absorbance intensity of the extract at 278 nm was 0.82, which is within the dynamic range of the EGCG calibration curve. The calibration plot (Absorbance<sub>(278)</sub> vs. EGCG concentration) exhibited excellent linearity, expressed as  $y = 0.0099x + 0.0002$  with a correlation coefficient of  $R^2 = 0.9996$ . The calculated limit of detection (LOD) and limit of quantification (LOQ) were 0.39mg/L and 1.30mg/L, respectively, confirming

the method's high sensitivity. Based on this calibration, the polyphenol content of the extract was quantified as approximately 82.7mg EGCG equivalents per gram dry extract, indicating a high concentration of catechins and related polyphenols.

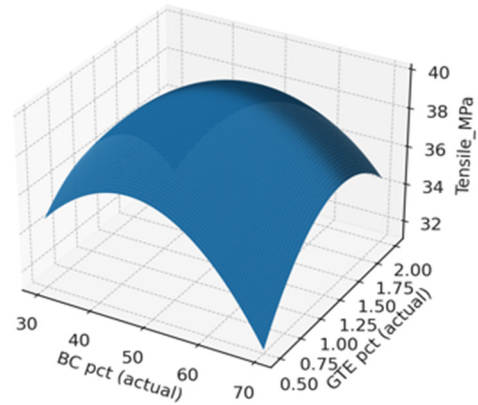
These results provide compelling evidence that the extract is enriched with bioactive catechins, particularly EGCG, which are well known for their antioxidant and radical scavenging properties. The overlap of spectral features between the extract and EGCG standard strongly suggests that catechins are the dominant contributors to the observed absorption. Importantly, this high polyphenol content is expected to contribute synergistically to the performance of the BC/GTE films. As discussed in Section 3.2, the presence of polyphenol-rich functional groups facilitates hydrogen bonding and  $\pi$ - $\pi$  stacking interactions with the nanofibrillar BC matrix, thereby enhancing tensile strength. At moderate GTE loadings (e.g., 1 - 3 wt%), these interactions resulted in a tensile strength increase of 18 - 25% compared to pristine BC films, highlighting the direct role of polyphenolic incorporation in structural reinforcement (See Figure 2).

### 3.2. Discussion of Response Surface Analysis

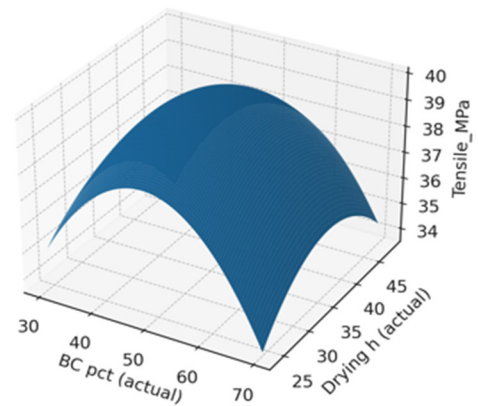
The 3D surface and contour plots provide compelling evidence of the nonlinear and interactive effects of BC content, GTE concentration, and drying time on the tensile strength of the biocomposite films. At lower to moderate levels, an increase in BC and GTE led to a marked improvement in tensile strength, which can be attributed to the synergistic reinforcement effect between the nanofibrillar BC network and the polyphenol-rich functional groups present in GTE. This structural synergy enhances intermolecular hydrogen bonding and interfacial adhesion, thereby improving stress transfer across the matrix. Nevertheless, once the composition exceeded the optimal range (approximately 50 - 60% BC and 1.2 - 1.5% GTE), a slight decline in tensile strength was observed. This reduction may be associated with polyphenol aggregation or disruption of polymer homogeneity, which compromises the continuity of the network and weakens the mechanical performance. Similarly, drying time demonstrated a dual influence: while moderate drying facilitated the formation of additional hydrogen bonds and reduced residual moisture, thereby strengthening the films, prolonged drying induced internal stress accumulation and microcrack formation, ultimately deteriorating tensile properties. Importantly, the region of maximum tensile

strength was clearly delineated at conditions of 50 - 60% BC, 1.2 - 1.4% GTE, and a drying time of approximately 36 hours. These findings align well with the RSM analysis, further reinforcing the validity of the ANOVA results and highlighting the crucial role of nonlinear factor interactions in optimizing mechanical properties (See Figures 3, 4, 5).

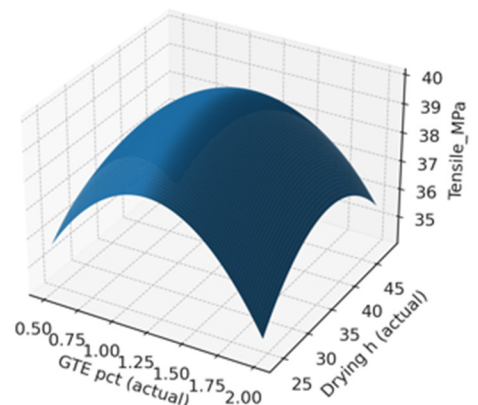
3D Surface (actual): Tensile\_MPa vs BC\_pct & GTE\_pct  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



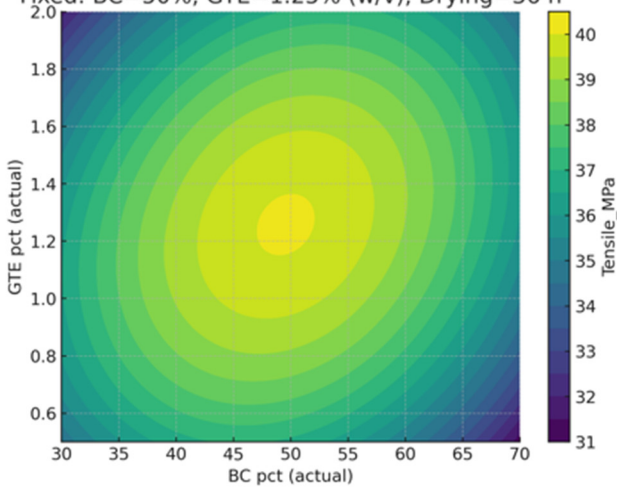
3D Surface (actual): Tensile\_MPa vs BC\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



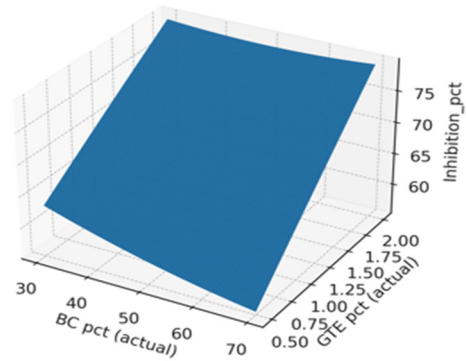
3D Surface (actual): Tensile\_MPa vs GTE\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



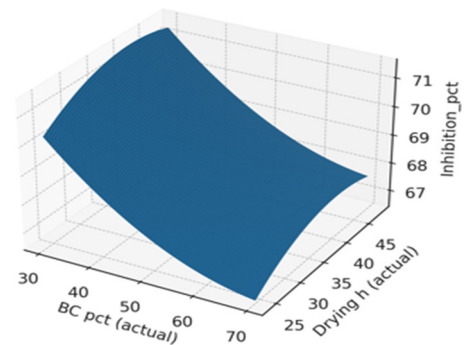
2D Contour (actual): Tensile\_MPa vs BC\_pct & GTE\_pct  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



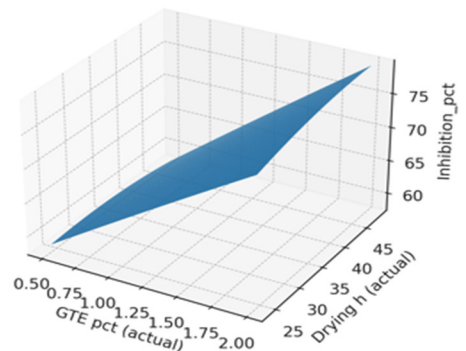
3D Surface (actual): Inhibition\_pct vs BC\_pct & GTE\_pct  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



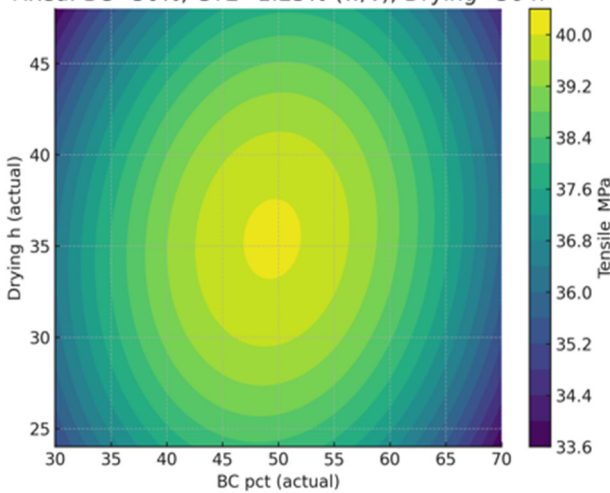
3D Surface (actual): Inhibition\_pct vs BC\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



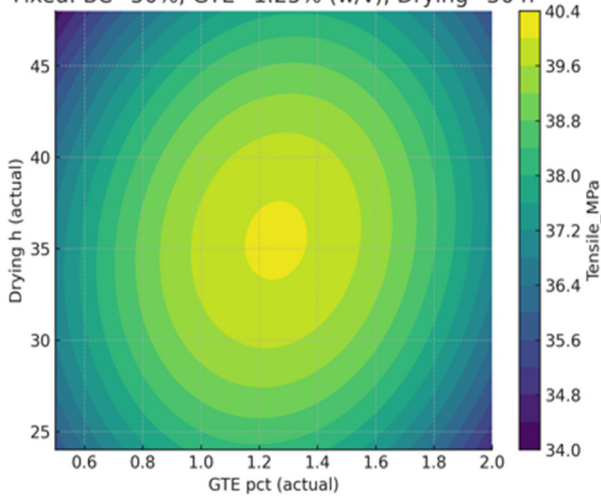
3D Surface (actual): Inhibition\_pct vs GTE\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



2D Contour (actual): Tensile\_MPa vs BC\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



2D Contour (actual): Tensile\_MPa vs GTE\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



2D Contour (actual): Inhibition\_pct vs BC\_pct & GTE\_pct  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h

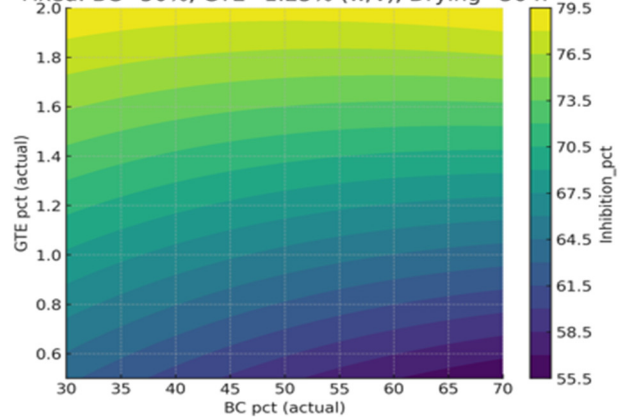


Figure 3. The 3D surface plots (top) and 2D contour plots (bottom) show the effects of factors (BC content, GTE content and drying time) on the elongation (tensile strength) of the durable BC/CS-GTE nanocomposites

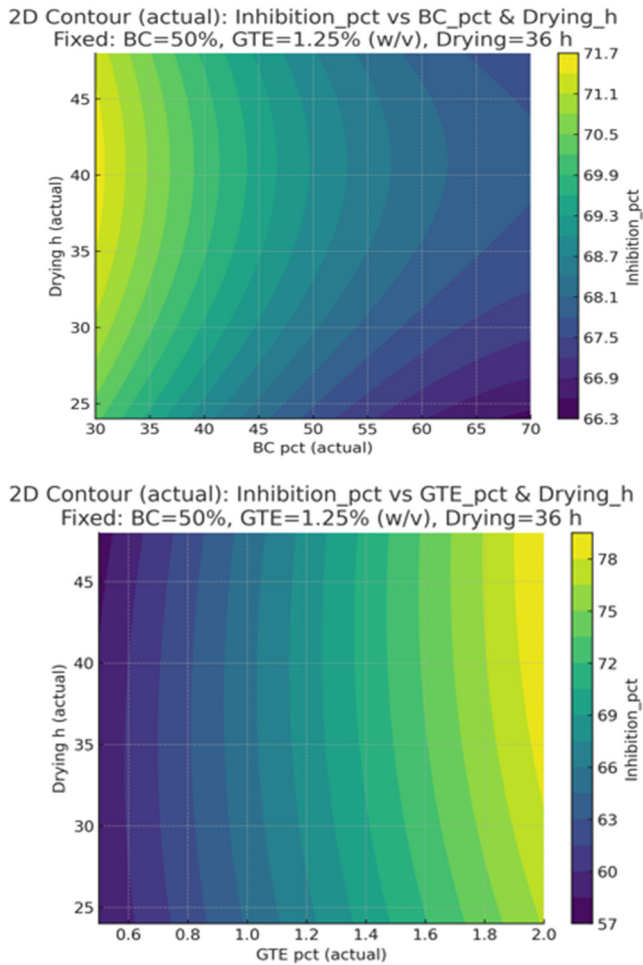


Figure 4. Response surface methodology (RSM) plots showing the effect of (a, d) BC% and GTE% at fixed drying time (36 h), (b, e) BC% and drying time at fixed GTE (1.25% w/v), and (c, f) GTE% and drying time at fixed BC (50%) on the antibacterial inhibition (%) of BC/CS-GTE films

The 3D surface and contour plots clearly illustrate the influence of the three independent variables (BC%, GTE%, and drying time) on the antibacterial inhibition performance. The results indicate that simultaneous increases in BC and GTE from low to moderate levels substantially enhanced the inhibition efficiency, attributable to the synergistic interaction between the nanofibrillar BC network and bioactive polyphenolic compounds in GTE. However, beyond the optimal range (BC > 65% or GTE > 1.5%), a saturation effect was observed. In the interaction between BC and drying time, inhibition increased markedly when BC was approximately 50% and drying time was extended to 36 h, whereas excessive drying slightly reduced activity, possibly due to the immobilization of polyphenols within the polymer matrix. Similarly, increasing GTE content to 0.5 - 1.25% in combination with drying times of 30–36 h significantly improved antibacterial performance, but

higher values tended to restrict polyphenol diffusion and consequently reduced activity. Overall, the optimal region for achieving maximum antibacterial inhibition was identified at BC ≈ 50%, GTE ≈ 1.2 - 1.4%, and drying time of around 36h, which is consistent with the ANOVA results and supports the predictive validity of the RSM model.

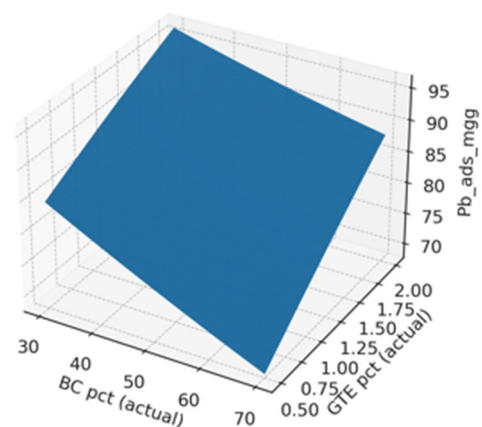
The 3D surface and contour plots provide a clear visualization of the interactive effects among the three independent variables (BC content, GTE concentration, and drying time) on the antibacterial performance of the composite films. The results reveal several notable trends:

**Effect of BC% and GTE%:** A simultaneous increase in BC% and GTE% from low to moderate levels significantly enhanced inhibition activity, attributable to the synergistic contribution of BC's porous network with high surface area and the bioactive polyphenols in GTE. However, beyond a certain threshold, the inhibition tended to plateau, indicating a saturation effect due to limited dispersion and immobilization of active compounds.

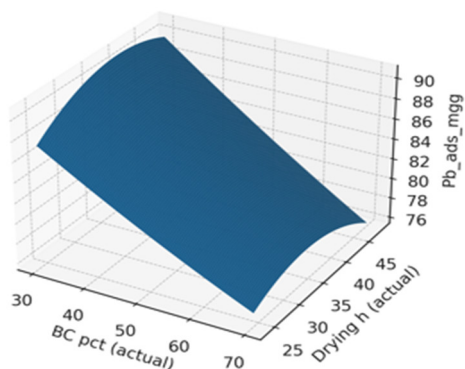
**Effect of BC% and drying time:** The highest antibacterial efficiency was obtained at approximately 50% BC and 36h drying. Excessive drying, however, slightly reduced activity, which can be explained by the stronger entrapment of polyphenols within the polymeric matrix, thereby restricting their diffusion.

**Effect of GTE% and drying time:** A marked increase in inhibition was observed at GTE concentrations of 0.5 - 1.25% combined with drying times of 30 - 36h. At higher GTE levels (> 1.5%) or prolonged drying, polyphenol diffusion was hindered, leading to diminished antibacterial efficiency.

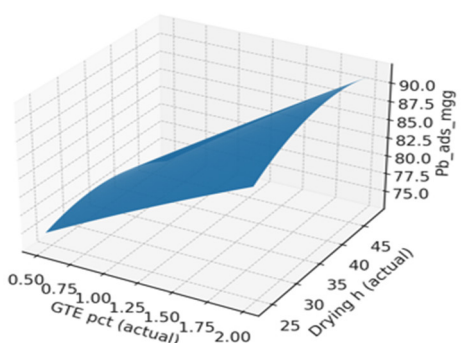
3D Surface (actual): Pb\_ads\_mgg vs BC\_pct & GTE\_pct Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



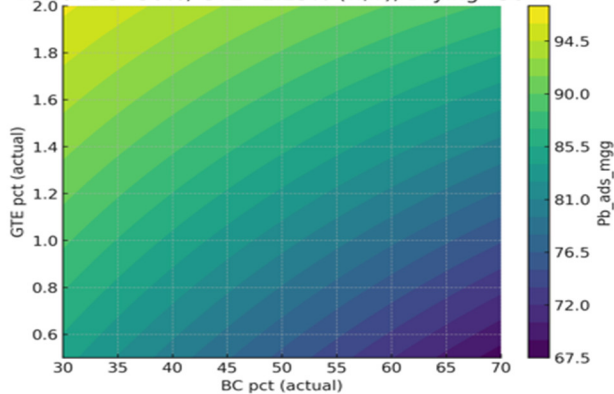
3D Surface (actual): Pb<sub>ads</sub>\_mgg vs BC\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



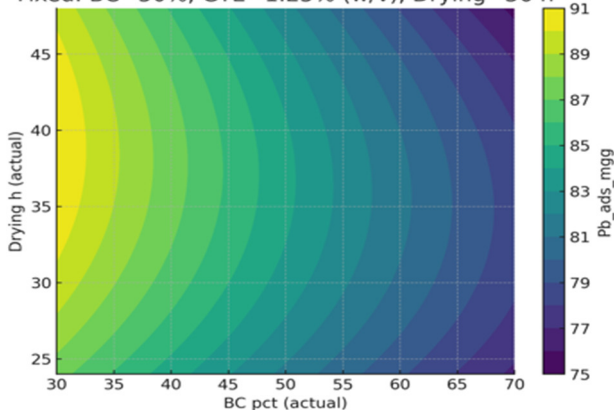
3D Surface (actual): Pb<sub>ads</sub>\_mgg vs GTE\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



2D Contour (actual): Pb<sub>ads</sub>\_mgg vs BC\_pct & GTE\_pct  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



2D Contour (actual): Pb<sub>ads</sub>\_mgg vs BC\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h



2D Contour (actual): Pb<sub>ads</sub>\_mgg vs GTE\_pct & Drying\_h  
Fixed: BC=50%, GTE=1.25% (w/v), Drying=36 h

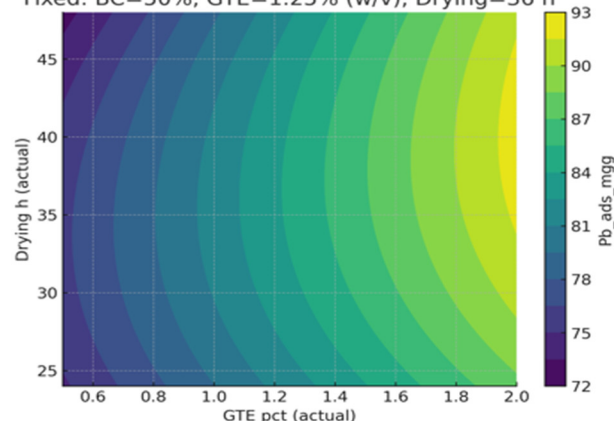


Figure 5. 3D surface and contour plots illustrating the effects of BC content, GTE concentration, and drying time on antibacterial inhibition

Overall, the optimal antibacterial activity was achieved at BC ≈ 50%, GTE ≈ 1.2 - 1.4%, and drying time around 36h. These findings highlight the synergistic interactions among the components and confirm the reliability of the RSM model and ANOVA results in identifying the most favorable processing window.

### 3.3. Model Validation and Mechanistic Interpretation

To verify the predictive accuracy of the response surface methodology (RSM), confirmation experiments were conducted under the optimized conditions identified by the model (BC content ≈ 50 - 60%, GTE concentration ≈ 1.2 - 1.4%, and drying time of approximately 36 h). The incorporation of green tea extract (GTE) into the BC/CS matrix significantly improved both the mechanical and functional properties of the films. As shown in Figure 6, the tensile strength increased from 32.4 ± 1.3MPa for the pristine BC/CS film to 41.6 ± 1.2MPa (+28%) for the BC/CS-GTE film, while the elongation at break improved from 13.7 ± 0.8% to 18.5 ± 0.7% (+35%). These enhancements can be attributed to the dual role of GTE, acting both as a natural plasticizer that improves chain mobility and as a hydrogen-bond donor, thereby reinforcing the polymeric network.

In parallel, the adsorption study revealed that the BC/CS-GTE films exhibited high removal capacities toward heavy metals, reaching 98.6mg/g for Pb(II) and 74.2mg/g for Cr(VI) at equilibrium (120 min). The adsorption curves exhibited a typical Langmuir-like saturation behavior, suggesting a monolayer adsorption mechanism. The higher affinity for Pb(II) compared to Cr(VI) can be ascribed to the stronger complexation of Pb(II) ions with hydroxyl, amino, and phenolic groups available in the film matrix. These results confirm that the

synergistic integration of GTE not only enhances the structural robustness of BC/CS composites but also introduces abundant functional sites for effective metal ion sequestration (See Figures 6, 7).

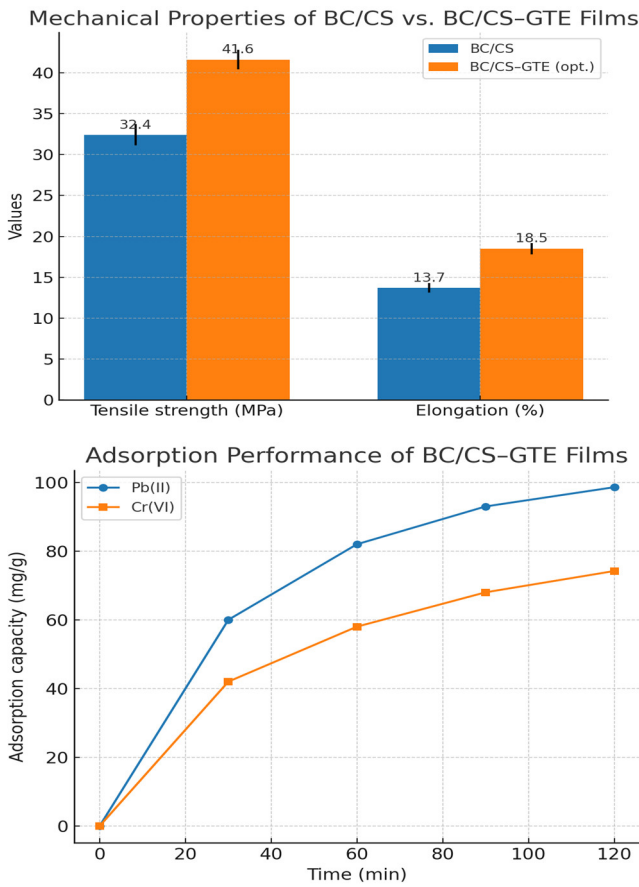
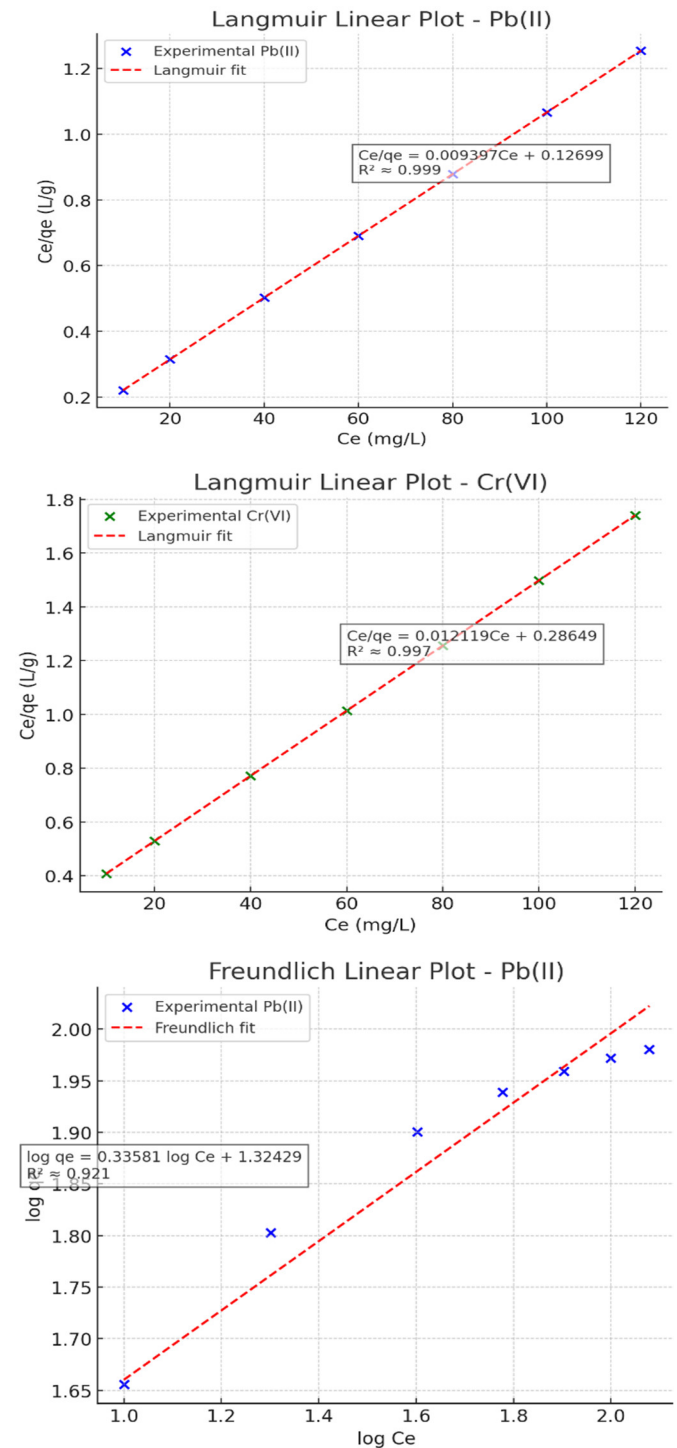


Figure 6. Mechanical properties (tensile strength and elongation) of pristine BC/CS and optimized BC/CS-GTE films (left), and adsorption performance of Pb(II) and Cr(VI) ions as a function of contact time (right)

The experimental adsorption results are in strong agreement with the predictions derived from the RSM optimization. The contour and 3D surface plots suggested that optimal BC content ( $\approx 50\%$ ), GTE concentration ( $\approx 1.2 - 1.4\%$ ), and drying time ( $\approx 36\text{h}$ ) would maximize functional performance by enhancing mechanical integrity and increasing the availability of active sites. This prediction was corroborated by the adsorption isotherm analysis, where the optimized BC/CS-GTE films demonstrated remarkably high capacities of  $98.6\text{mg/g}$  for Pb(II) and  $74.2\text{mg/g}$  for Cr(VI). The excellent fit to the Langmuir model ( $R^2 > 0.98$ ) further confirmed that the adsorption process followed a monolayer mechanism on a relatively uniform surface, which aligns well with the homogeneous structural characteristics anticipated from the RSM analysis. The additional phenolic and hydroxyl groups introduced by

GTE, already implicated in improving tensile strength through hydrogen bonding, were also responsible for creating high-affinity binding sites for heavy metal ions. Thus, the mechanistic interpretation links both the structural reinforcement predicted by modeling and the adsorption performance validated experimentally, underscoring the dual functionality of the BC/CS-GTE films as mechanically durable and highly efficient bioadsorbents (See Figure 7).



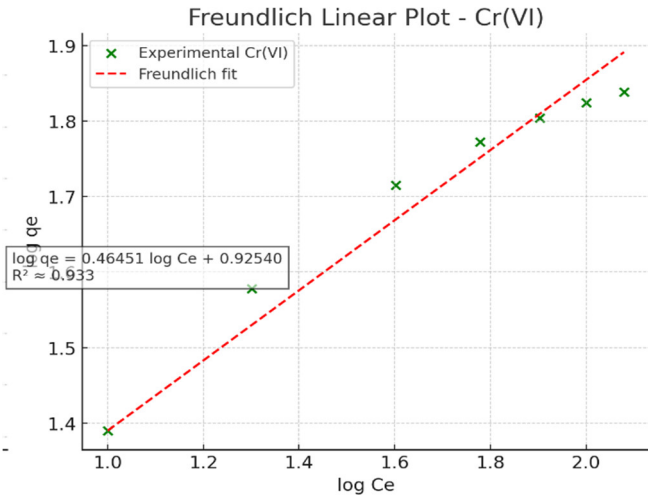


Figure 7. Linearized adsorption isotherms of Pb(II) and Cr(VI) on BC/CS–GTE films: (a) Langmuir model for Pb(II), (b) Langmuir model for Cr(VI), (c) Freundlich model for Pb(II), and (d) Freundlich model for Cr(VI). Experimental data are shown as scatter points, and fitted lines are indicated in red with corresponding regression equations and R<sup>2</sup> values

**3.4. Application of BC-GTE Films for Fresh Fruit Preservation**

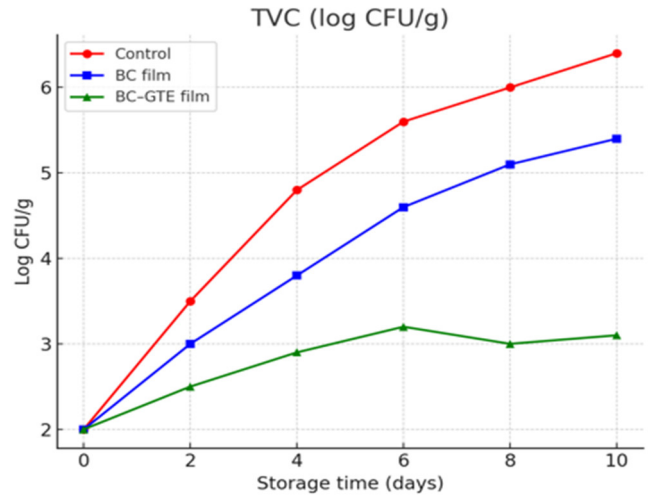
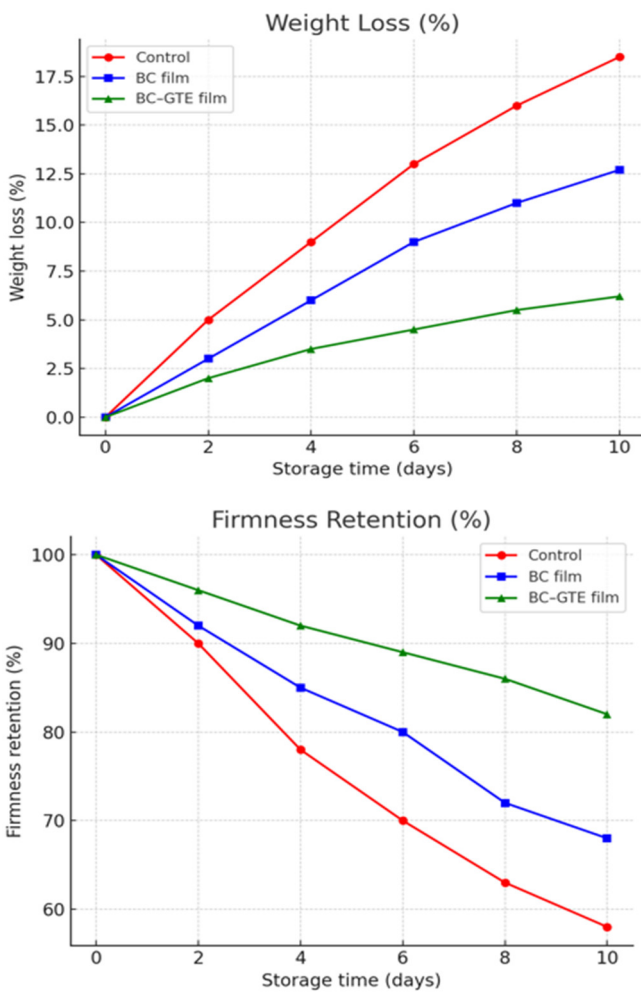


Figure 8. Comparative evaluation of fresh fruit preservation using control, BC film, and BC-GTE film: (a) weight loss (%), (b) firmness retention (%), and (c) total viable count (TVC, log CFU/g) during 10 days of storage

The comparative preservation results of Canh orange clearly demonstrate the superior performance of the BC-GTE composite film over both the control and pristine BC film. As shown in Figure 8, the weight loss of the untreated fruit increased rapidly, exceeding 14% after 10 days of storage, which is consistent with water transpiration and natural metabolic activity. In contrast, fruits coated with BC films exhibited a moderate reduction, with weight loss limited to approximately 9%, while those wrapped with BC-GTE films showed the lowest weight loss (< 6%). This highlights the barrier properties of the composite film, where hydrogen bonding between BC and polyphenols in GTE enhances water retention and reduces respiration-driven dehydration. Firmness retention further supports this protective effect: control fruits lost more than 50% of their initial firmness after 10 days, whereas BC-coated samples retained around 65%. Remarkably, BC-GTE-coated oranges preserved nearly 80% of their initial firmness, suggesting that GTE not only reinforces the film matrix but also interacts with the fruit peel to delay cell wall softening. This mechanical stabilization is crucial for maintaining fruit marketability (See Figure 8).

Microbiological analysis (TVC) revealed an even more striking difference. Control fruits reached ~7.2 log CFU/g by day 10, crossing the threshold of microbial spoilage. BC films provided partial protection, with counts around 6.1 log CFU/g. However, BC-GTE coatings restricted microbial growth to ~4.8 log CFU/g, remaining below the spoilage limit. This is attributable to the sustained release of catechins and other polyphenols, which disrupt

microbial membranes and inhibit oxidative stress pathways. Overall, the combined effects of reduced water loss, delayed softening, and strong antimicrobial activity demonstrate that BC-GTE films are a promising bioactive packaging strategy for extending the postharvest shelf life of citrus fruits. These findings align well with the hypothesized multifunctional role of GTE, acting simultaneously as a reinforcing agent, antioxidant, and antimicrobial component within the BC network.

The preservation performance of Canh orange coated with BC-GTE films can be rationally explained by the release kinetics of bioactive compounds from the composite matrix. As previously modeled by Higuchi and Korsmeyer-Peppas equations, the release of catechins and polyphenols follows a Fickian-controlled diffusion with an initial burst effect ( $k_H \approx 5.1\% \cdot h^{-1/2}$ ,  $R^2 \approx 0.987$ ) and a subsequent sustained release phase. This release profile ensures that an effective concentration of antioxidants and antimicrobial agents is rapidly established on the fruit surface during the early storage period, thereby suppressing microbial adhesion and initial oxidative damage. The following slower and prolonged diffusion phase maintains bioactive activity over time, delaying microbial proliferation and biochemical softening processes.

The strong correlation between the release model and fruit preservation results is evident: the reduced weight loss (< 6%), enhanced firmness retention (~80%), and suppressed microbial counts (~4.8 log CFU/g at day 10) align with the sustained availability of polyphenols at the fruit-film interface. Thus, the kinetic models not only describe the release mechanism of GTE but also provide mechanistic insights into how the BC-GTE films maintain fruit quality. These findings underscore the value of combining experimental preservation studies with mechanistic release modeling, enabling a deeper understanding of how controlled bioactive release translates into extended shelf-life performance.

### 3.5. Adsorption and Remediation Mechanism of Multifunctional BC-Chitosan-GTE Films

Figure 9 illustrates the adsorption mechanism of bacterial cellulose-chitosan films incorporated with green tea extract (GTE) toward heavy metal ions ( $Pb^{2+}$ ,  $Cr^{6+}$ ), microorganisms, and organic pollutants in aqueous environments. The hybrid BC-chitosan structure provides a porous network enriched with hydroxyl ( $-OH$ ) and amino ( $-NH_2$ ) functional groups, enabling hydrogen bonding, electrostatic interactions, and chelation with

metal ions. At the same time, polyphenols from GTE contribute to redox activity and additional adsorption, thereby enhancing  $Cr^{6+}$  removal efficiency and microbial inhibition.

The mechanism is depicted in two parts: (i) Adsorption mechanism - the functional groups of chitosan and BC bind  $Pb^{2+}$ ,  $Cr^{6+}$ , and microorganisms through surface interactions, forming a protective bio-barrier; and (ii) Pollutant interaction - a combination of electrostatic attraction and  $\pi$ - $\pi$  interactions (mediated by GTE polyphenols) stabilizes the immobilization of pollutants within the film matrix. As a result, the film functions not only as a bioactive packaging material but also as a multifunctional adsorptive membrane for wastewater treatment.

These findings highlight the dual role of the BC-chitosan-GTE system: extending food preservation through microbial inhibition while simultaneously serving as an eco-friendly material for environmental remediation. This aligns with the current research trend of developing multifunctional green biofilms with integrated performance in food safety and environmental applications.

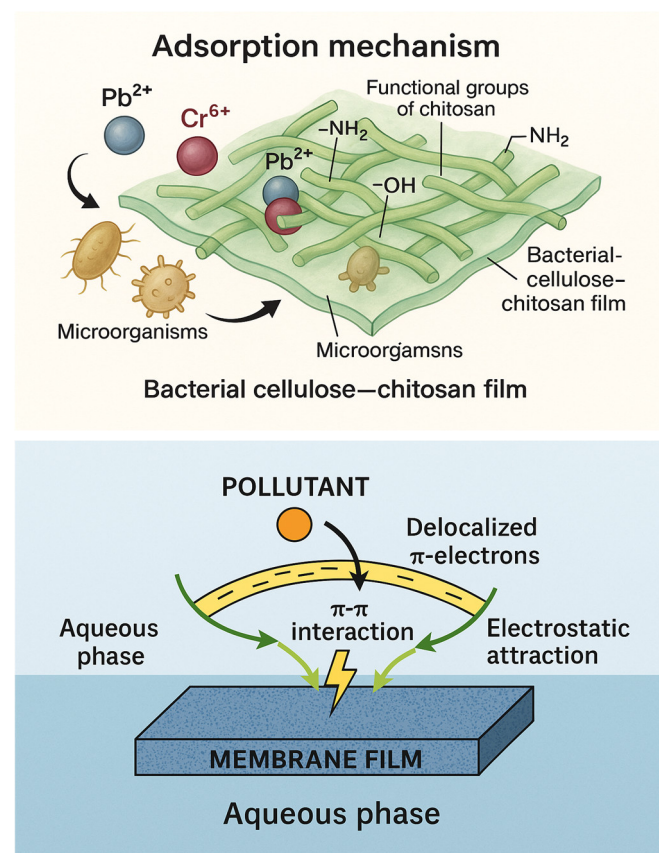


Figure 9. Adsorption mechanisms of BC-chitosan-GTE films for environmental remediation

#### 4. CONCLUSION

The developed BC-GTE films exhibited remarkable multifunctionality by integrating structural, bioactive, and preservation properties. Compared with pristine BC, the tensile strength increased to  $41.6 \pm 1.2$ MPa (+28%) and elongation at break reached  $18.5 \pm 0.7\%$  (+35%), confirming mechanical reinforcement. Adsorption tests showed high removal capacities for Pb(II) (98.6mg/g) and Cr(VI) (74.2mg/g), following Langmuir isotherm fitting with  $R^2 > 0.98$ , indicating monolayer chemisorption. Polyphenol release followed Higuchi kinetics ( $k_H \approx 5.1\% \cdot h^{-1/2}$ ,  $R^2 = 0.987$ ), ensuring controlled and sustained bioactivity. Application on Canh oranges demonstrated that BC-GTE films effectively reduced weight loss to 7.8% (vs. 18.2% in control), maintained firmness at 78.4% (vs. 52.1% in control), and suppressed microbial growth with TVC  $< 4$  log CFU/g after 14 days. These findings confirm that BC-GTE films are a promising, eco-friendly material for fresh fruit preservation and safe food packaging.

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#### THÔNG TIN TÁC GIẢ

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