

Original Research Article

Preparation and Characterization of Dialdehyde Cellulose (DAC) with Enhanced Antibacterial Activity

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Abstract: Using cellulose as raw material, dialdehyde cellulose (DAC) was prepared via selective oxidation with sodium periodate. Single-factor and orthogonal experiments were conducted to optimize the oxidation conditions (sodium periodate concentration, suspension pH, and reaction time) and to evaluate their effects on the aldehyde content and sample retention rate of DAC. The structural characteristics and antibacterial performance of DAC films were also investigated. The results showed that the optimal preparation conditions were sodium periodate concentration of 7%, suspension pH of 3.0, and reaction time of 4.0 h. Under these conditions, the aldehyde content of DAC reached 83%, and the sample retention rate was 80.33%. Meanwhile, The order of influence of each factor on the aldehyde group content is as follows: sodium periodate concentration > reaction time > suspension pH. Fourier transform infrared spectroscopy (FTIR) revealed a new characteristic peak at 1712 cm^{-1} corresponding to aldehyde groups, confirming successful oxidation. Scanning electron microscopy (SEM) showed increased surface roughness, etching marks, and local damage after oxidation. The DAC film exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus anthracis*, with inhibition zone diameters of 1.50 mm, 8.33 mm, and 6.90 mm, respectively ($P < 0.05$), whereas the unmodified cellulose film showed no inhibitory effect. These results indicate that oxidation treatment endows DAC with significant antibacterial properties, providing a theoretical basis for the development of food preservative and active packaging materials.

Keyword: Dialdehyde cellulose (DAC), sodium periodate oxidation, process optimization, antibacterial activity, food packaging.

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INTRODUCTION

As a natural polymer, cellulose occupies an important position among renewable biomass resources due to its unique molecular structure and excellent comprehensive properties. Cellulose not only possesses relatively high mechanical strength and good thermal stability but also exhibits remarkable biocompatibility, biodegradability, wide availability, and low cost. These advantages have laid a solid foundation for its extensive applications in fields such as food packaging, biomedicine, water treatment, and the textile industry. However, the strong hydrogen bonding interactions between cellulose molecular chains lead to its poor solubility in conventional solvent systems. In addition, its inherent high crystallinity limits the accessibility of chemical reagents. Furthermore, drawbacks such as the lack of thermoplastic processability and poor wrinkle

resistance of cellulosic fibers severely restrict the high-value utilization of cellulose. Therefore, the chemical modification of cellulose to improve its processability and impart new functionalities has become a research hotspot in the field of cellulose science (Sun *et al.*, 2024).

Various strategies exist for the chemical modification of cellulose, including oxidation, etherification, esterification, amination, and graft copolymerization (Madhushree *et al.*, 2025). Among these, oxidation modification has attracted considerable attention due to its ability to selectively introduce specific functional groups, enabling precise regulation of cellulose structure and properties. Numerous studies have shown that oxidation treatment not only significantly improves the physical properties of cellulose, such as its dispersibility in aqueous media, water-holding capacity, and swelling performance, but

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also introduces reactive functional groups (e.g., aldehyde and carboxyl groups) at specific positions of the cellulose glucose units (e.g., C₂, C₃, or C₆), making it an important precursor for the preparation of novel functional materials. The introduction of these reactive sites provides possibilities for subsequent functionalization of cellulose, such as Schiff base reactions, metal ion coordination, and drug molecule conjugation.

Among the various cellulose oxidation methods, the sodium periodate (NaIO₄) system is particularly favored due to its unique regioselectivity. This system specifically cleaves the C₂–C₃ bond of the glucose units in cellulose, generating dialdehyde cellulose (DAC) containing two aldehyde groups. The reaction is characterized by mild conditions and high selectivity. The resulting aldehyde groups can be further coupled with amino compounds via Schiff base reactions, or reduced to hydroxyl groups or oxidized to carboxyl groups, demonstrating great potential in the preparation of high-value functional materials.

In this study, a sodium periodate system was selected for the is kept as oxidation modification of cellulose. Using aldehyde group yield and sample retention rate as evaluation indicators, the process parameters of the oxidation reaction were systematically optimized through single-factor experiments and orthogonal tests. Modern analytical techniques including FTIR, SEM, XRD, and TGA were employed to systematically characterize the chemical structure, micro morphology, crystalline characteristics, and thermal stability of cellulose before and after modification, and its antibacterial performance was evaluated. The purpose of this study is to elucidate the optimal process conditions for the oxidative modification of cellulose and to obtain dialdehyde cellulose (DAC) with a higher aldehyde group content, thereby opening up new avenues for enhancing the high-value utilization of cellulose resources.

1. MATERIALS AND METHODS

1.1 Materials and Reagents

The tested strains included *Colletotrichum asianum*, *Staphylococcus aureus*, and *Escherichia coli*. Among them, *Colletotrichum asianum* was isolated from mango fruit and identified by molecular biology techniques, while *Staphylococcus aureus* and *Escherichia coli* were both preserved in our laboratory.

The reagents details are as follows: Natural cellulose (cotton linter, α -cellulose > 95%, Mw = 1.07×10^5 g/mol) was purchased from Hubei Chemical Fiber Co., Ltd.; sodium hydroxide and urea were purchased from Shandong Keyuan Biochemical Co., Ltd.; sulfuric acid, hydrochloric acid, hydroxylamine hydrochloride, and sodium periodate were purchased from Chongqing Chuandong Chemical Co., Ltd.

1.2 Instrumentation

An adjustable high-speed homogenizer was supplied by Hangzhou Jingfei Instrument Technology Co., Ltd.; a heating magnetic stirrer was supplied by Shenzhen Dingxinyi Laboratory Equipment Co., Ltd.; an electronic balance was purchased from Shanghai Youke Instrument Co., Ltd.; a laboratory pH meter was supplied by Shanghai Youke Instrument Co., Ltd.; a medical low-temperature freezer was purchased from Qingdao Haier Co., Ltd.; a benchtop bell-type freeze dryer was supplied by Ningbo Haizi Biotechnology Co., Ltd.; an electric thermostatic drying oven was purchased from Zhejiang Lichen Instrument Technology Co., Ltd..

1.3 Preparation of cellulose membrane

Cellulose was dissolved using a NaOH/urea system (Chen *et al.*, 2023). Specifically, 8 g of sodium hydroxide and 12 g of urea were accurately weighed into a 250 mL beaker, and 100 mL of deionized water was added with stirring to dissolve. To ensure effective dissolution, the solvent system was pre-cooled, and then 4.2 g of cellulose was accurately weighed and added under ice-bath conditions with continuous stirring until dissolved. The resulting transparent composite liquid was transferred to a refrigerator and frozen at -18 °C for 12 h. After freezing, 15 mL of the thawed solution was accurately measured and poured into a glass Petri dish for gel sheet preparation. The Petri dish was then placed in a pre-heated forced-air drying oven and allowed to stand for 6 h for drying. After film formation, the membrane was treated in a 5 wt% sulfuric acid coagulation bath for 10 min to regenerate the cellulose (Fernandez-santos *et al.*, 2025). The obtained gel product was rinsed three times with deionized water to obtain the cellulose membrane. The prepared cellulose membrane was subsequently used for single-factor and orthogonal optimization experiments of sodium periodate oxidation.

1.4 Single-factor experiment for DAC preparation

The main influencing factors for cellulose oxidation using the sodium periodate method include sodium periodate concentration, suspension pH, and oxidation time. Single-factor experiments were conducted using the aldehyde content and retention rate of DAC as evaluation indicators to investigate the effects of the above factors on cellulose oxidation.

The cellulose membrane suspension prepared in step 1.3.1 was placed in an Erlenmeyer flask, and a certain amount of sodium periodate was added. The pH of the suspension was adjusted using hydrochloric acid solution. The flask was then placed in a constant-temperature magnetic stirrer and stirred for a certain period under light-proof conditions. The oxidized suspension was washed multiple times with water until the pH of the suspension reached neutrality. Finally, the DAC sample was freeze-dried.

- (1) Determination of the effect of sodium periodate concentration on the aldehyde content and

retention rate of DAC samples : Using the homogenized cellulose membrane suspension as the reaction system, sodium periodate was added at concentrations of 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%, respectively. The pH of the system was precisely adjusted to 4.0 using acid–base regulators, and the reaction was carried out in a magnetic stirrer under light-proof conditions for 3.0 h.

- (2) Determination of the effect of oxidation time on the aldehyde content and retention rate of DAC samples: Sodium periodate at a mass fraction of 6% was added to the homogenized cellulose membrane suspension to establish the reaction system. The oxidation time was set to 1, 2, 3, 4, 5, 6, 7, and 8 h, respectively. The pH of the reaction system was controlled at 4.0, and the reaction was performed using a magnetic stirrer under light-proof conditions to investigate the effect of reaction duration on the aldehyde content and retention rate of DAC samples.
- (3) Determination of the effect of suspension pH on the aldehyde content and retention rate of DAC samples: Sodium periodate (6%) was added to the homogenized cellulose membrane suspension, and the pH of the suspension was adjusted to 2.0, 3.0, 4.0, 5.0, and 6.0, respectively. The reaction was carried out under light-proof conditions using a magnetic stirrer for 3.0 h to analyze the influence of pH changes in the system on the aldehyde content and retention rate of DAC samples.

1.5 Determination of Aldehyde Content and Retention Rate

The hydroxylamine hydrochloride method is a common approach for determining aldehyde group content, which effectively reflects the degree of cellulose oxidation. The determination principle is based on the nucleophilic addition reaction between the amino groups of hydroxylamine hydrochloride molecules and the aldehyde groups present in the oxidized cellulose product, forming oxime compounds and releasing an equivalent amount of hydrochloric acid. The hydrochloric acid generated from the reaction is titrated with a standard sodium hydroxide solution. Based on the

stoichiometric relationship of the acid–base neutralization reaction, the amount of aldehyde groups in the sample can be calculated from the titration volume and solution concentration, thereby achieving quantitative determination of aldehyde group content. Specifically, 6 g of DAC was accurately weighed and added to 30 mL of distilled water. The pH of the solution was adjusted to 5.0 with dilute hydrochloric acid. Separately, 0.72 mol/L hydroxylamine hydrochloride solution was prepared and adjusted to pH 5.0 with dilute sodium hydroxide solution. The hydroxylamine hydrochloride solution was then added to the DAC solution, and the mixture was sealed and allowed to stand at room temperature for 24 h. Subsequently, the mixture was titrated with 0.01 mol/L sodium hydroxide solution. An unoxidized cellulose film solution at pH 5.0 was used as a blank control, with methyl red solution as the indicator. The aldehyde group content was calculated using Formula (1):

$$CHO/\% = \frac{(V_2 - V_1) \times C \times 162}{0.1 \times 1000} \times 100\% \quad (1)$$

Where V_2 and V_1 are the volumes of sodium hydroxide solution consumed by the sample and the blank control, respectively (L); C is the concentration of the standard sodium hydroxide solution (M); and 162 is the molar mass of the repeating unit of oxidized cellulose (g/mol).

Determination of Retention Rate: The cellulose samples before and after oxidation were weighed, and the retention rate was calculated using Formula (2):

$$\text{retention rate}/\% = \frac{m_1}{m_2} \times 100\% \quad (2)$$

Where: m_1 is the mass of cellulose before oxidation; m_2 is the mass of cellulose after oxidation.

1.6 Orthogonal Array Experiment of DAC

Based on the single-factor experiments, sodium periodate concentration, oxidation time, and suspension pH were selected as factors, and aldehyde group content and sample retention rate were used as evaluation criteria (with aldehyde group content as the primary criterion). A three-factor, three-level orthogonal experiment (see Table 1) was designed for the preliminary optimization of DAC.

Table 1: Single factor test factor level table

level.	Factors		
	A (Sodium periodate concentration) /%	B (Oxidation time) / h	C (Suspension pH)
1	5	2	3
2	6	3	4
3	7	4	5

1.7 SEM Analysis

The dried cellulose samples before and after oxidation were separately fixed onto conductive adhesive tape and sputter-coated with gold to provide good conductivity. The prepared samples were placed

into the sample chamber of a scanning electron microscope. After the vacuum system reached the required level, the surface morphology of the samples was observed at set magnifications, and corresponding

scanning electron microscopy (SEM) images were acquired.

1.8 FTIR Analysis

Approximately 3 mg of each dried cellulose sample (before and after oxidation) was accurately weighed, mixed uniformly with potassium bromide (KBr) in an agate mortar, and ground thoroughly. The mixture was then compressed into a pellet to prepare the test sample. The samples were analyzed using a Fourier transform infrared (FTIR) spectrometer, with KBr used as the background for baseline correction. The instrument parameters were set as follows: scanning wavenumber range of 4000–400 cm^{-1} , 32 scans, and a resolution of 4 cm^{-1} .

1.9 XRD Analysis

Approximately 1 g of each cellulose sample (before and after oxidation) was placed on the dedicated sample holder of an X-ray diffractometer, securely fixed, and then measured. The test conditions were as follows: tube voltage of 40 kV, tube current of 40 mA, scanning range of $2\theta = 5^\circ\text{--}40^\circ$, and step size of 0.02°.

1.10 TG Analysis

Cellulose samples (5–8 mg) before and after oxidation were analyzed using a thermogravimetric analyzer under a nitrogen atmosphere. The samples were heated from 0 °C to 500 °C at a rate of 10 °C/min, and the mass loss curves were recorded.

1.11 Antibacterial Activity Assay

The antimicrobial activity of different cellulose samples against *Staphylococcus aureus*, *Escherichia coli*, and *Colletotrichum asianum* was evaluated using the agar plate diffusion method (Kaczmarek *et al.*, 2024). *S. aureus* and *E. coli* were respectively inoculated into LB liquid medium and cultured at 37 °C with shaking until the logarithmic growth phase. *C. asianum* was inoculated into PDB liquid medium and cultured at 28 °C with shaking. An appropriate amount of the bacterial culture was added to melted LB solid medium that had been cooled to approximately 45 °C, and mixed thoroughly to achieve a final bacterial concentration of approximately 10^6 CFU/mL in the medium. The mixture was immediately poured into Petri dishes and allowed to cool and solidify. For *C. asianum*, PDA solid medium was used. The fungal culture was mixed with the medium to achieve a final spore concentration of approximately 10^5 CFU/mL, and the plates were poured.

A test DAC disk with a diameter of 10 mm was placed at the center of the solidified medium surface. Unoxidized cellulose was used as a control. The bacterial plates were incubated at 37 °C for 48 h, and the fungal plates were incubated at 28 °C for 72 h. After incubation, the diameters of the inhibition zones were measured using the cross method with a vernier caliper. Three parallel samples were prepared for each sample, and the

experiment was repeated three times. Statistical analysis was then performed.

2. RESULTS AND DISCUSSION

2.1 Analysis of Single-Factor Experiments

2.1.1 Effect of Sodium Periodate Concentration on Aldehyde Group Content and Retention Rate of DAC

As shown in Figure 1A, when the sodium periodate concentration increased from 3% to 6%, the aldehyde group content rose significantly from 27.27% to 73.71%, indicating that increasing the oxidant concentration facilitates the oxidation reaction and continuously improves the aldehyde group content. This trend is consistent with literature reports. This result may be attributed to the gradual saturation of reactive hydroxyl sites on the cellulose surface, or the condensation of generated aldehyde groups with adjacent hydroxyl groups to form hemiacetal structures, leading to the consumption of some aldehyde groups (Sultana *et al.*, 2024). Brault *et al.*, (2025) recently confirmed that non-oxidized aldehyde groups can be sterically hindered by the formation of hemiacetal bonds, resulting in an underestimation of titration results.

Considering both aldehyde group content and sample retention rate, a sodium periodate concentration of 6% was selected as appropriate. Under this condition, the aldehyde group content of cellulose was 73.71%, and the retention rate was 86.7%.

2.1.2 Effect of Oxidation Time on Aldehyde Group Content and Retention Rate of DAC

As shown in Figure 1B, within the range of 1.0–3.0 h, the aldehyde group content increased rapidly with increasing oxidation time, as sodium periodate reacted with the hydroxyl groups of cellulose to generate aldehyde groups. This rapid accumulation of aldehyde groups is closely related to the penetration efficiency of sodium periodate and the associated reaction kinetics. Chursin *et al.*, (2007) pointed out that oxidation time has a significant effect on the properties of the product during the oxidative degradation of hydroxyethyl cellulose.

The retention rate showed a monotonic decreasing trend with prolonged oxidation time, indicating an increasing degree of cellulose degradation. Yang *et al.*, (2007) also reported that as the aldehyde group content increased, the degree of polymerization of cellulose decreased. In a recent comprehensive review, Sun and Feng (2024) systematically described the preparation and functionalization of periodate-oxidized nanocellulose, highlighting that oxidation time, periodate concentration, and suspension pH significantly influence the degree of oxidation and morphology of the product. They noted that extending the reaction time can increase the aldehyde group content but leads to degradation of the cellulose chains. Based on the quality of the oxidized samples, a reaction time of 3.0h was selected. Under this condition, the aldehyde group

content was 71.01%, and the retention rate was 88.54%. Considering both aldehyde group content and sample retention rate, an oxidation time of 3.0 h was considered appropriate, under which the aldehyde group content of cellulose was 73.01%, and the retention rate was 88.54%.

2.1.3 Effect of Suspension pH on Aldehyde Group Content and Retention Rate of DAC

As shown in Figure 1 C, when the pH was below 4.00, the aldehyde group content decreased. This is attributed to the accelerated hydrolytic cleavage of β -1,4-glycosidic bonds of the cellulose chains under strongly acidic conditions, leading to further oxidation of aldehyde groups into carboxylic acids or dissolution

losses. When the pH was 4.00, the aldehyde group content reached its peak value of 64.80%, while the cellulose retention rate remained high at 87.94%. Furthermore, pH showed a positive correlation with the retention rate, which increased as the pH rose. This is because under acidic conditions ($\text{pH} < 4.00$), cellulose molecular chains are prone to degradation into small molecules and dissolution, resulting in a decreased retention rate. In summary, pH 4.00 was selected as the optimal condition to balance the dual requirements of aldehyde group content and cellulose retention rate. Under this condition, the aldehyde group content was 64.80%, and the retention rate was 89.94%.

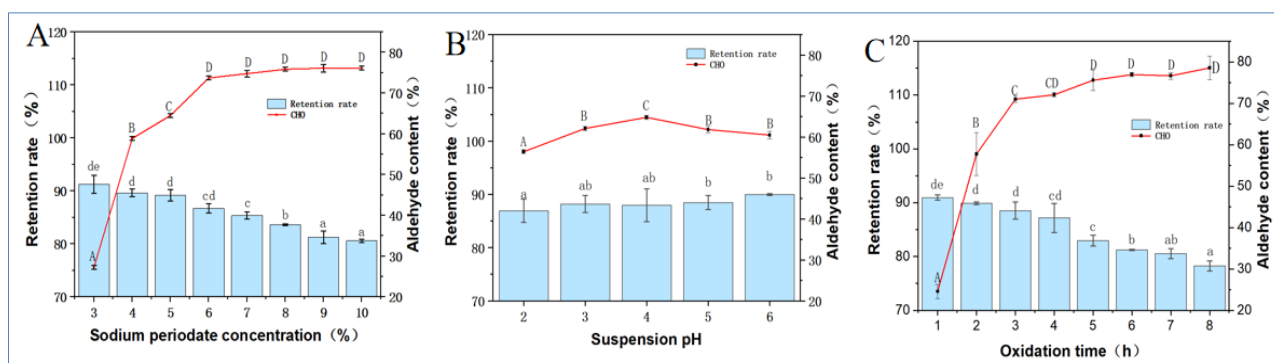


Fig. 1: The influence of different factors on DAC aldehyde group content and retention rate
 Note: A, Sodium periodate concentration (%); B, Suspension pH; C, Oxidation Time

2.2 Analysis of Orthogonal Experiment Optimization Results

Based on the single-factor experimental results, an L9 (3^4) orthogonal array was designed. As shown in Table 2, among the factors affecting the aldehyde group content (i.e., the degree of oxidation) of the samples, the order of priority was sodium periodate concentration > oxidation time > suspension pH. The same order of priority was observed for the factors affecting the retention rate of dialdehyde cellulose. Considering all indicators, the optimal combination of conditions for preparing dialdehyde cellulose was $A_3B_2C_2$, corresponding to factor A at level 3, factor B at level 2,

and factor C at level 2. Further variance analysis was conducted on the key evaluation indicator, aldehyde group content. The results showed that factor A had an F-value of 34.54, which is significantly higher than the critical value of 9, indicating that it has a significant effect on the experimental results. Based on this finding, it is inferred that under an appropriate concentration of sodium periodate, the carbon-carbon bonds at specific positions of the glucose units in cellulose are cleaved, thereby effectively releasing hydroxyl groups and achieving a high degree of cellulose oxidation.

Table 2: Orthogonal test results of DAC

Test number	Factors				Aldehyde content (%)	Sample retention rate (%)
	A Sodium periodate concentration (%)	B Oxidation time (h)	C Suspension pH	blank column		
1	1(5)	2(3)	3(5)	1	55	90
2	1	3(4)	2(4)	2	58	89
3	1	1(2)	1(3)	3	50	91
4	2(6)	3	3	3	72	84
5	2	2	1	1	78	83
6	2	1	2	2	70	85
7	3(7)	3	1	2	85	79
8	3	1	3	3	80	78
9	3	2	2	1	83	80
k_1	54.33	66.67	71	72		
k_2	73.33	72	70.33	68.67		

k ₃	82.67	71.67	69	69.67	Analysis of variance (ANOVA) based on aldehyde content
Combination	A ₃	B ₂	C ₂		
R	28.34	5.33	2	3.33	
S	1250.89	53.56	6.22	11.11	
df	2	2	2	2	
F-ratio	34.54	1.48	0.17		
F critical value	9	9	9		
Significance	Significant	Not significant	Not significant		
k ₁	90	84.67	84.33		Calculate the average value and range based on the sample retention rate
k ₂	84	84.33	84.67		
k ₃	79	84	84		
R	11	0.67	0.67		

2.3 Validation of the Optimal Process for Sodium Periodate Oxidation of Cellulose

Based on the optimal combination of conditions obtained from the orthogonal experiment, replicate validation experiments were conducted in this study to ensure the reliability and accuracy of the experimental results. The relevant experimental data are presented in Table 3. It can be clearly observed from the data that under the optimal experimental conditions, the aldehyde group content of the prepared DAC reached 83%, and the

sample retention rate was 80.33%. Compared with the data before optimization, the aldehyde group content was significantly improved. These results fully demonstrate that the conditions established by the orthogonal experiment are reasonable and appropriate, and that the process optimization achieved remarkable effectiveness. Furthermore, this finding further confirms that the orthogonal experimental method possesses high validity and practicality in the optimization of cellulose oxidation processes.

Table 3 Verification of the Optimal Preparation Process for DCA

Evaluation index	1	2	3	Mean
Aldehyde content (%)	83	82	84	83
Retention rate	80	80	81	80.33

2.4 SEM analysis

The SEM images of cellulose before and after sodium periodate oxidation are shown in Figure 2. Unoxidized cellulose (Figure 2A, B) exhibited a typical fibrous structure with a relatively smooth and flat surface, clear fiber contours, and no obvious damage or defects. At high magnification (Figure 2A, ×1000), the fiber surface appeared relatively dense and structurally intact, indicating an orderly arrangement of crystalline and amorphous regions in the pristine cellulose. After oxidation, the morphology of cellulose (Figure 2C, D) changed significantly. The fiber surface became rough, with varying degrees of etching and peeling. Longitudinal cracking and transverse breakage of the fibers were observed in some areas. At high magnification (Figure 2C, ×200), the fiber surface exhibited an uneven morphology with localized pores and fragments, indicating that the oxidation reaction caused erosion of the cellulose structure. These morphological changes are consistent with the appearance of the aldehyde characteristic peak (1712 cm⁻¹) and the weakening of the hydroxyl absorption peak observed in FTIR analysis, confirming that sodium periodate selectively oxidizes the adjacent hydroxyl groups at the C₂-C₃ positions of cellulose while also causing partial degradation of the cellulose molecular chains. Nevertheless, the overall contour and basic morphology of the fibers remained intact, suggesting that the oxidation reaction occurred primarily in the amorphous regions and on the surfaces of the crystalline

regions, without completely destroying the skeletal structure of the cellulose fibers (Firdiana *et al.*, 2025).

2.5 FTIR analysis

Figure 3 shows the FTIR spectra of cellulose before and after sodium periodate oxidation. The spectrum of unoxidized cellulose exhibits the characteristic absorption bands typical of cellulose (Li *et al.*, 2023): The broad and intense absorption band located near 3340 cm⁻¹ is attributed to O–H stretching vibrations, while the absorption band near 2900 cm⁻¹ corresponds to C–H stretching vibrations. The strong absorption band in the region of 1050–1160 cm⁻¹ is assigned to C–O–C stretching vibrations of the glucopyranose ring, which is characteristic of the cellulose skeleton. After oxidation, the spectrum of cellulose changed significantly, with a new absorption band appearing at 1712 cm⁻¹. This band is attributed to C=O stretching vibrations, confirming the successful introduction of aldehyde groups (–CHO) (Madhushree *et al.*, 2025). This change indicates that sodium periodate selectively oxidized the adjacent hydroxyl groups at the C₂ and C₃ positions of the glucose units in cellulose, cleaving them to form dialdehyde structures (Tian *et al.*, 2022). After oxidation, the intensity of the O–H absorption band at 3340 cm⁻¹ decreased, which is attributed to the oxidation of some hydroxyl groups into aldehyde groups, resulting in a reduction in the number of hydroxyl groups. In addition, the intensity of the cellulose skeleton absorption band in the region of 1050–

1160 cm^{-1} decreased slightly, indicating that the oxidation process had a certain impact on the main chain structure of cellulose, which is consistent with the observed decrease in sample retention rate (Pellegrino *et al.*, 2025). A study by Feng *et al.*, (2026) showed that the aldehyde groups of dialdehyde cellulose (DAC) prepared

by sodium periodate oxidation can react with samples containing functional groups such as alcohols, amino groups, and acids, enabling DAC to serve as an important intermediate for expanding the application range of cellulose.

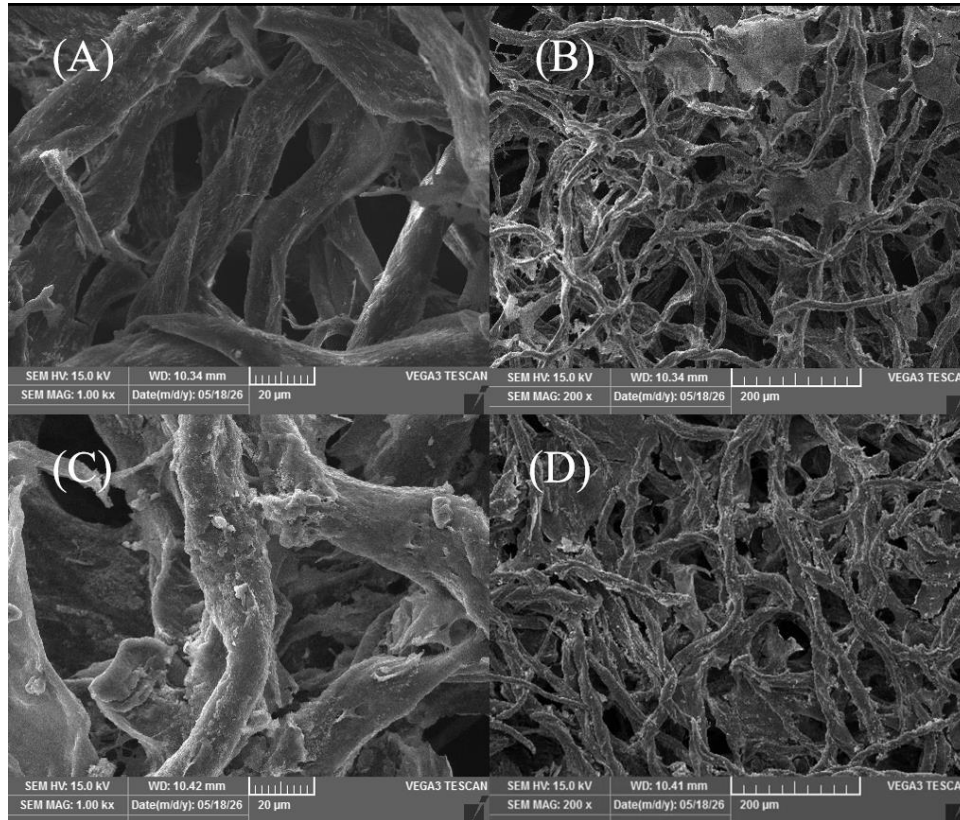


Fig. 2 SEM images

- A- SEM image of cellulose at a magnification of $\times 1000$**
- B- SEM image of cellulose at a magnification of $\times 200$**
- C- SEM image of DAC at a magnification of $\times 1000$**
- D- SEM image of DAC at a magnification of $\times 200$**

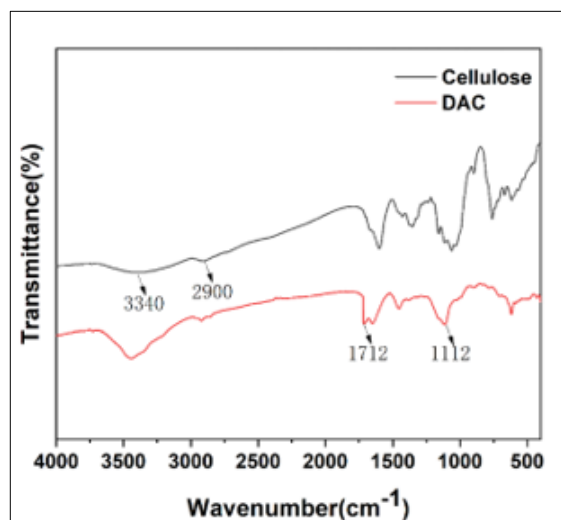


Fig. 3: FTIR spectra of cellulose and DAC

2.6 XRD analysis

Figure 4 presents a comparison of the X-ray diffraction patterns of cellulose before and after sodium

periodate oxidation. Unoxidized cellulose exhibited a typical cellulose I crystalline structure, with a sharp and strong diffraction peak corresponding to the (002) crystal plane at approximately $2\theta = 22.5^\circ$, overlapping doublet peaks corresponding to the (101) and (10 $\bar{1}$) crystal planes in the region of approximately $2\theta = 15^\circ$ – 17° , and a weak diffraction peak corresponding to the (040) crystal plane at approximately $2\theta = 34.5^\circ$. This pattern is highly consistent with the XRD patterns of natural cellulose reported in the literature (French *et al.*, 2014). After sodium periodate oxidation, the diffraction pattern changed significantly: the intensities of all diffraction peaks decreased substantially, the main peak at

approximately $2\theta = 22.5^\circ$ became broad and flattened, the doublet peak features in the 15° – 17° region nearly disappeared and transformed into a diffuse halo, and the overall pattern exhibited typical characteristics of an amorphous material. This change indicates that the oxidation process disrupted the crystalline structure of cellulose. The underlying mechanism is that sodium periodate selectively oxidizes the hydroxyl groups at the C₂-C₃ positions of the glucose units, opening the glucopyranose rings to form dialdehyde structures, thereby disrupting the ordered arrangement of the molecular chains (Varma *et al.*, 2002). According to Kim *et al.*, (2026).

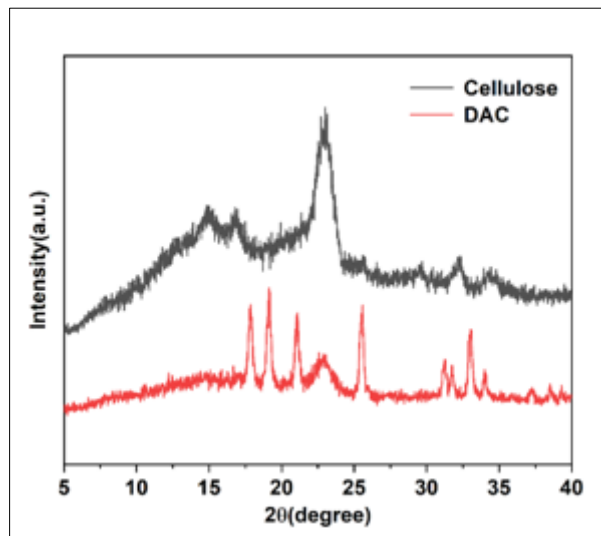
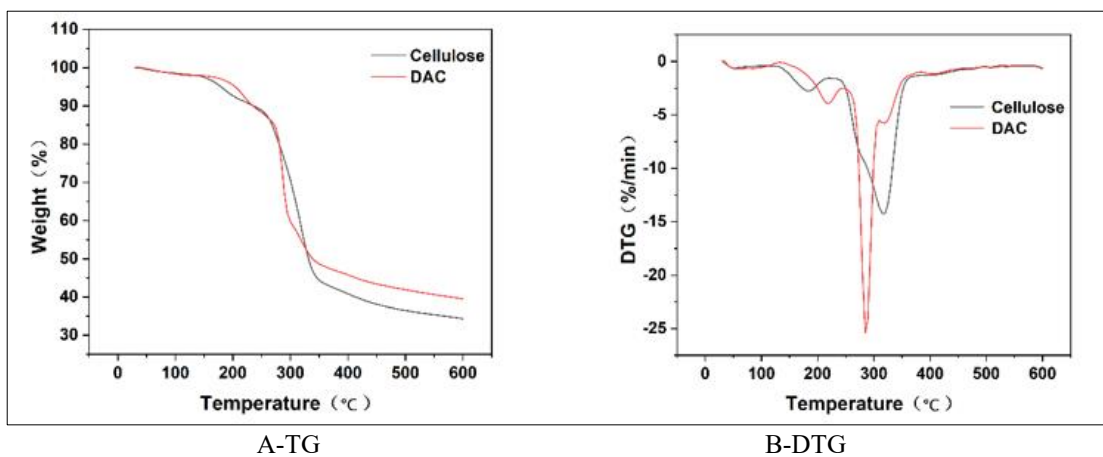


Fig. 4: XRD patterns of cellulose and DAC

2.7 TG analysis

Figure 5 presents the TGA curves of cellulose before and after sodium periodate oxidation. Unoxidized cellulose exhibited typical thermal degradation behavior, with a slight weight loss of approximately 3% in the range of 30–250 °C, corresponding to the removal of adsorbed water. Subsequently, a rapid weight loss occurred between 250 and 350 °C, which is attributed to the rapid thermal degradation resulting from glycosidic bond cleavage, dehydration, and depolymerization

reactions of the cellulose chains. The final residue was approximately 76%. After sodium periodate oxidation, the thermal stability of cellulose changed significantly. The initial degradation temperature shifted from approximately 250 °C to approximately 150–200 °C. The thermal degradation rate decreased, and the degradation interval became considerably broader. Moreover, the final residue increased compared with that of unoxidized cellulose.



A-TG B-DTG
Fig. 5: TGA thermograms of cellulose and DAC

2.8 Antibacterial performance analysis

The antibacterial activity of DAC films against *Staphylococcus aureus*, *Escherichia coli*, and *Colletotrichum asianum* was evaluated using the agar plate diffusion method, and the results are shown in Figure 6. As presented in Table 4, unoxidized cellulose films produced no obvious inhibition zones against any of the three tested strains, indicating that unoxidized cellulose itself lacks antibacterial activity. In contrast, DAC films exhibited inhibitory effects against all three strains, with mean inhibition zone diameters of 1.50 mm for *S. aureus*, 8.33 mm for *E. coli*, and 6.90 mm for *C. asianum*. Statistical analysis revealed that the differences in inhibition zone diameters between DAC films and unoxidized cellulose films were significant for all tested strains ($P < 0.05$), confirming that the oxidation treatment endows DAC films with antibacterial properties.

The antibacterial activity of DAC originates from the aldehyde groups introduced during the oxidation process. These aldehyde groups can disrupt microbial structures and inhibit growth through multiple mechanisms. These include reacting with amino groups in polysaccharides of the bacterial cell wall or membrane, thereby disrupting the cross-linked structure, increasing permeability, and causing leakage of intracellular contents, ultimately leading to cell death (Tian *et al.*, 2023). Furthermore, they can bind to intracellular active enzymes such as dehydrogenases and ATPases, occupying their active sites, which inhibits normal enzymatic function, blocks energy metabolism and biosynthesis, and results in growth arrest. Additionally, the aldehyde groups promote the accumulation of reactive oxygen species within bacterial cells, disturbing the redox balance and inducing oxidative stress, which triggers apoptosis (Song *et al.*, 2024).

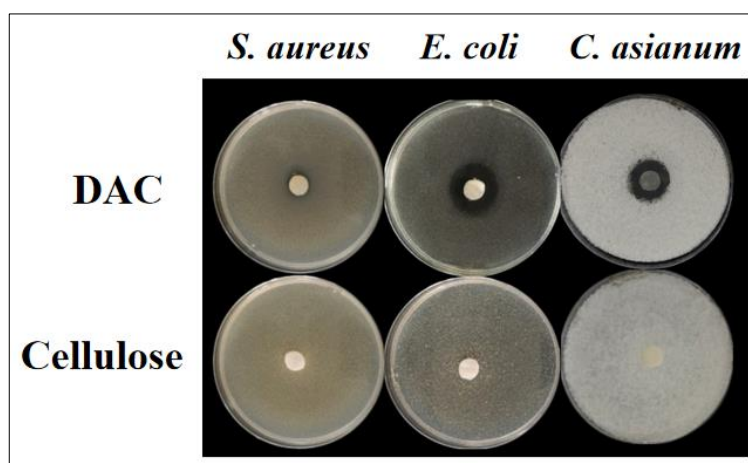


Fig. 6: Photographs of inhibition zone tests of cellulose and DAC against *S. aureus*, *E. coli*, and *C. asianum*

Table 4: Inhibition zone diameters (mm) of cellulose and DAC against *S.aureus*, *E. coli*, and *C. asianum*

Indicator strains	Inhibition zone diameters /mm	
	Cellulose	DAC
<i>S.aureus</i>	0.00 ^a	1.50 ± 0.52 ^b
<i>E.coli</i>	0.00 ^a	8.33 ± 0.22 ^b
<i>C. asianum</i>	0.00 ^a	6.90 ± 0.14 ^b

Note: Different lowercase letters indicate significant differences among different groups ($P < 0.05$)

3. CONCLUSIONS

In this study, the optimal preparation conditions for DAC were determined via single-factor experiments and orthogonal tests as follows: sodium periodate concentration of 7%, suspension pH of 3.0, and reaction time of 4.0 h. Under these conditions, the aldehyde group content reached 83%, and the sample retention rate was 80.33%. After oxidation, the DAC samples exhibited characteristic absorption peaks corresponding to carbonyl and hemiacetal stretching vibrations, along with obvious morphological evolution at the microscopic level, confirming the successful introduction of aldehyde groups. In contrast to unoxidized cellulose films, which

showed no antibacterial activity, DAC films exhibited significant inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Colletotrichum* species, demonstrating that oxidation treatment can effectively endow cellulose materials with good antibacterial performance. Overall, this study indicates that sodium periodate oxidation is an effective method for preparing aldehyde-functionalized cellulose while maintaining a high sample retention rate, and the resulting DAC films possess both well-defined structural characteristics and antibacterial functionality, providing theoretical support and technical reference for the development of antimicrobial materials for food preservation applications.

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