

Article

Sodium Alginate-Encapsulated Oregano Essential Oil Microcapsules for the Conservation of Waterlogged Archaeological Wood

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Abstract

Waterlogged archaeological wood is highly vulnerable to degradation by wood-degrading microorganisms. Oregano essential oil (OEO) shows excellent antimicrobial activity against such microbes, but its high volatility and poor stability restrict direct application in cultural relic protection. This study aims to optimize the preparation of sodium alginate (SA)-based OEO microcapsules (OEO@SAM), characterize their structural and physicochemical properties, and evaluate their sustained-release antimicrobial performance for waterlogged archaeological wood conservation. OEO@SAM was fabricated via ionic crosslinking, with orthogonal experiments optimizing three key parameters: OEO:SA ratio, SA concentration, and CaCl_2 concentration. The microcapsules were characterized by morphological observation, Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), etc. Encapsulation efficiency (EE), in vitro sustained-release kinetics, and antimicrobial activity against dominant wood-degrading microorganisms (isolated from waterlogged archaeological sites) were tested. The OEO:SA ratio was the dominant factor regulating EE: EE decreased sharply as the OEO:SA ratio increased, with the highest EE (24.05%) achieved at OEO:SA = 0.5, SA = 2%, and CaCl_2 = 3%. Meanwhile, only 0.71 g/L of OEO@SAM is required to inhibit bacterial growth and achieve the conservation of waterlogged archaeological wood. OEO@SAM exhibited stable sustained release (fitting the zero-order kinetic model) and significant antimicrobial activity against target microorganisms. It provides a new type of antibacterial and antifungal material for the in situ conservation of waterlogged archaeological wood.

Keywords: oregano essential oil; encapsulation efficiency; sustained-release; antimicrobial; antifungal; waterlogged archaeological wood; cultural relic conservation



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1. Introduction

In 1973, archaeologists discovered the Hemudu culture, which dates back to 7000–5000 BP, in Hemudu Village, Yuyao City, Zhejiang Province, China. As an integral part of the Hemudu culture, the Tianluoshan site (7000–6300 cal BP) is famous for its remains of ancient wooden structural buildings [1,2], neolithic earthen settlement [3,4], and because it was an agricultural cultivation site [5–8]. It is the site with the most natural remains unearthed in the Hemudu culture, including 23,615 plant remains, 712 archaeological wood, and 8620 animal remains [9–11]. The archaeological wood in the Tianluoshan site investigated in this paper is the remains of ancient wooden structural buildings [12].

It serves as an important record of the evolution of the Hemudu culture's stilted wooden architecture, from its inception to full maturity through four sequential developmental stages [13–16], and holds exceptional historical, scientific, and artistic value. Most of the small archaeological wood pieces have been transferred to storage facilities for preservation. However, a great many other ancient wooden relics remain in situ at the Tianluoshan site. To preserve this important archaeological collection, staff from the Zhejiang Provincial Institute of Cultural Relics and Archaeology and the Hemudu Museum extracted large amounts of groundwater to submerge all large archaeological wood at the Tianluoshan site, aiming to create an oxygen-deprived environment for wood preservation similar to other sites [17–22]. Groundwater is extracted from near the venue and injected into the site through the site's southeast corner to preserve the archaeological wood. Excess water is pumped out from the northwest corner to achieve overall water circulation at the site. In this environment, archaeological wood is constantly exposed to multiphase environmental conditions, making it highly susceptible to microbial growth and posing a severe threat to its preservation [23–25]. Almost every archaeologist knows that soaking archaeological wood in water can provide temporary protection.

Essential oils are volatile oily liquids extracted from plants and exhibit inherent antimicrobial and insecticidal activities. They have a complex chemical composition, generally consisting of two to three major components along with a variety of trace constituents; the major components are predominantly terpenoids and phenylpropanoids, while the remaining ones include phenols, aldehydes, ketones, ethers, and other compounds [26]. Essential oils exert their antimicrobial effects through multiple mechanisms, such as disrupting cell membranes, inhibiting ATP synthesis, altering fatty acid ratios, impeding protein synthesis, and interfering with quorum sensing. Stupar et al. [27] investigated the antifungal efficacy of various essential oils against fungi colonizing stone and archaeological wood, and their findings revealed that oregano essential oil (OEO) exerted the strongest antifungal inhibitory effect. However, essential oils possess high volatility. Direct application of these oils may result in the massive loss of active ingredients, thereby compromising their antifungal efficacy and long-term durability [28]. However, simply submerging archaeological wood in water cannot achieve comprehensive protection of archaeological wood, as the erosion of archaeological wood by the large number of microorganisms present in the water must be taken into account. In recent years, with the growing demand for green and eco-friendly materials, the application of sodium alginate in essential oil encapsulation technology has attracted increasing research attention. This technology primarily leverages the crosslinking reaction between carboxyl groups on sodium alginate molecular chains and divalent metal ions (e.g., calcium ions) to form hydrogel microcapsules with a three-dimensional network structure under mild conditions, thereby achieving efficient encapsulation of essential oils [29]. This encapsulation approach not only protects the active components of essential oils from the adverse effects of external environmental factors (e.g., light, heat, oxygen, etc.), but also regulates their release rate and enhances the durability of antimicrobial efficacy, thus emerging as a key research focus in the field of microbial control [30]. Gholamian et al. [31] developed a novel encapsulation system based on calcium alginate hydrogels loaded with cumin essential oil, which enables the effective protection and sustained release of cumin essential oil. Yin et al. [32] investigated the performance of cinnamon essential oil-loaded microcapsules for the packaging of fresh mangoes, and the results demonstrated that the resultant composite film exhibited excellent fresh-keeping efficacy. Lertsutthiwong et al. [33] prepared sodium alginate microcapsules embedded with curcumin; these microcapsules possessed superior antioxidant activity, high water solubility, and targeted release properties, as well as favorable physical stability and a high recovery rate.

Previous studies have demonstrated the feasibility of encapsulating essential oils with sodium alginate. Therefore, this study develops a bacteriostatic agent suitable for the in situ submerged conservation conditions of archaeological wood. The development of this novel antibacterial and antifungal agent opens up new possibilities for the in situ conservation of archaeological wood at the Tianluoshan site.

2. Materials and Methods

2.1. Materials

Oregano essential oil (OEO, Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China); Sodium alginate (SA, analytical grade, Feijing Biotechnology Co., Ltd., Shanghai, China); Anhydrous calcium chloride (analytical grade, Fuyang Shengchang Desiccant Sales Co., Ltd., Fuyang, China); Tween-80 (TW-80, Langjiek Technology Co., Ltd., Beijing, China); Sodium citrate (analytical grade, Tianjin Zhonglian Chemical Reagent Co., Ltd., Tianjin, China); Anhydrous ethanol (analytical grade, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China).

Bacillus mycoides (M5), *Pseudomonas tianjinensis* (T6), *Trichoderma breve* (PM2), and *Fusarium foetens* (GM1) were isolated from waterlogged archaeological wood at the Tianluoshan site, China, and were preserved in our laboratory.

2.2. Methods

2.2.1. Preparation of OEO@SAM

SA solutions with mass-volume fractions of 1–5% were prepared by dissolving 10 g, 20 g, 30 g, 40 g, and 50 g of SA in 1 L deionized water, respectively. The mixtures were stirred at 50 °C for 4 h using a thermostatic heating magnetic stirrer (Model DF-101ST, Shenzhen Dingxinyi Laboratory Equipment Co., Ltd., Shenzhen, China) to ensure complete dissolution, followed by sterilization at 120 °C in an autoclave to further promote dissolution.

A total of 100 mL of SA aqueous solution was mixed with 1 mL of Tween-80 (TW-80) emulsifier under stirring. OEO was slowly added dropwise into the SA solution at OEO/SAM volume ratios of 1:2, 1:1, and 2:1. The mixture was sheared at 20,000 r/min for 3 min using a high-speed homogenizer (Model FSH-2A, Changzhou Yuexin Instrument Manufacturing Co., Ltd., Changzhou, China) to obtain an oil-in-water (O/W) OEO-SA emulsion.

Using a syringe with an outer diameter of 1.3 mm, the OEO-SA emulsion was added dropwise into CaCl₂ solutions of different concentrations. The weight of 100 drops of emulsion was recorded, and the mixture was stirred at 200 r/min for 45 min using a digital display magnetic stirrer to solidify the microcapsules. The obtained microcapsules were washed three times with deionized water by oscillation and dried under vacuum at 45 °C to constant weight, yielding OEO@SAM.

2.2.2. Orthogonal Experiments

(1) Preliminary orthogonal experiment

A preliminary orthogonal experiment with 3 factors (OEO/SAM ratio, SA concentration, and CaCl₂ concentration) and multiple levels was designed to optimize the preparation parameters. The levels of factors are shown in Table 1. A total of 15 groups of experiments were conducted, with the stability of the OEO-SA emulsion and the formation of microcapsules as the main evaluation indicators. The results were observed by ultra-depth-of-field microscopy to exclude the worst experimental conditions for the formal experiment.

Table 1. Factors and levels of the preliminary orthogonal experiment.

Factor	Level 1	Level 2	Level 3	Level 4	Level 5
OEO/SA ratio	1:2	1:1	2:1	-	-
SA concentration (%)	1%	2%	3%	4%	5%
CaCl ₂ concentration (%)	1%	3%	5%	7%	-

Note: The symbol “-” indicates that no corresponding level was set for the experimental factor.

Factor levels for the preliminary experiment were set based on the classical preparation process of SA microcapsules and the specific characteristics of OEO encapsulation, with a wide interval for primary screening. Partial levels (marked with “-”) were excluded due to the inherent factor properties or technological constraints, as detailed below:

OEO/SA mass ratio: Only 3 gradients were set according to the common range (1:2~2:1) for plant essential oil encapsulation. The 4th and 5th levels were excluded because an out-of-range ratio would cause low encapsulation efficiency or low drug loading, which is not experimentally valuable.

SA concentration (%): 5 gradients (1~5%) were set to investigate viscosity effects on microcapsule sphericity and crosslinking, covering low/medium/high viscosity ranges (conventional SA concentration: 0.5~5%).

CaCl₂ concentration (%): 4 gradients (1~7%) were set (conventional crosslinking concentration: 1~8%). The 5th level was excluded because CaCl₂ ≥ 8% caused excessive crosslinking, leading to microcapsule hardening and rupture in preliminary exploration.

(2) Formal orthogonal experiment

Based on the preliminary experiment, a 3-factor 3-level orthogonal experiment was designed with the encapsulation efficiency (EE) of OEO as the index. The factors and levels are shown in Table 2. The effects of OEO/SAM ratio (A), SA concentration (B), and CaCl₂ concentration (C) on the EE of OEO@SAM were investigated to determine the optimal preparation process.

Table 2. Factors and levels of the formal orthogonal experiment.

Factor	Level 1	Level 2	Level 3
OEO/SA ratio	1:2	1:1	2:1
SA concentration (%)	2%	3%	-
CaCl ₂ concentration (%)	3%	5%	7%

Note: The symbol “-” indicates that no corresponding level was set for the experimental factor.

For the formal experiment, factor levels were optimized based on the core property test results (encapsulation efficiency, sphericity, etc.) of microcapsules from the preliminary experiment, with ineffective levels eliminated and effective intervals focused on. The details are as follows:

OEO/SA mass ratio: This ratio had significant effects on encapsulation efficiency and drug loading within 1:2~2:1, and the three gradients showed distinct performances, so the original three levels were retained.

SA concentration (%): 1% SA caused poor sphericity and capsule wall rupture (low viscosity), and 5% SA led to agglomeration in emulsification and dropping (high viscosity). Only 2% and 3% SA showed favorable properties, so 1%, 4%, and 5% were eliminated with two effective gradients retained.

CaCl₂ concentration (%): 1% CaCl₂ resulted in low crosslinking degree and aqueous phase swelling/disintegration (no practical value), while 3%, 5%, and 7% CaCl₂ showed significant differences in crosslinking stability and mechanical strength. Thus, 1% was eliminated, and three gradients were retained for further study.

2.3. Characterization and Performance Testing

2.3.1. Morphological Characterization

The morphological characteristics of OEO@SAM were observed using an ultra-depth-of-field microscope (Model VHX-5000, Keyence Corporation, Osaka, Japan) at a magnification of 50–200 $\times$. The surface and cross-sectional morphologies, as well as element distribution, were analyzed by FE-SEM equipped with EDS.

2.3.2. Determination of Encapsulation Efficiency (EE)

(1) Establishment of OEO standard curve: A series of standard OEO solutions with concentrations of 2 $\mu\text{g/mL}$, 4 $\mu\text{g/mL}$, 6 $\mu\text{g/mL}$, 8 $\mu\text{g/mL}$, and 10 $\mu\text{g/mL}$ were prepared using anhydrous ethanol. A UV–Vis spectrophotometer (model UV-1800PC) was purchased from Shanghai Mapada Instruments Co., Ltd. (Shanghai, China). The UV–Vis spectrum was scanned over the range of 190–300 nm; 200 μL of the sample was pipetted, and the absorbance values at the maximum absorption wavelength were recorded to plot the standard curve.

(2) Calculation of theoretical OEO content: The total weight of 100 drops of OEO-SA emulsion was measured using an electronic balance. The theoretical total OEO content in 100 drops of emulsion was calculated by the formula: Theoretical total OEO content (g) = Weight of 100 drops of emulsion $\times$ Initial OEO concentration. The theoretical OEO content per microcapsule was obtained by dividing the theoretical total content by 100.

(3) Determination of actual OEO content: Ten intact OEO@SAM microcapsules were randomly selected and placed in a 2 mL centrifuge tube. In total, 1 mL of 0.2 mol/L sodium citrate aqueous solution was added to dissolve the SA wall material. The centrifuge tube was treated with ultrasound at 40 KHz for 30 min, oscillated for 30 s. This procedure was repeated until the microcapsules were completely disrupted. A total of 1 mL of anhydrous ethanol was added to the mixture, followed by oscillation to promote OEO dissolution. The mixture was centrifuged at 12,000 r/min for 5 min, and 400 μL of the supernatant was collected to measure the absorbance (A) using a UV–Vis spectrophotometer (Model V-1800, Aoe Instrument Co., Ltd., Shanghai, China). A mixture of 1 mL 0.2 mol/L sodium citrate aqueous solution and 1 mL anhydrous ethanol was used as the blank control to measure the background absorbance (A_0).

(4) Calculation of EE: The actual OEO concentration in the supernatant was calculated based on the standard curve, and the actual total OEO content in 10 microcapsules was derived. The EE was calculated by the formula: EE (%) = (Actual OEO content per microcapsule/Theoretical OEO content per microcapsule) $\times$ 100%.

2.3.3. Sustained-Release Performance Test

The sustained-release behavior of OEO@SAM was studied by the immersion method. Ten OEO@SAM microcapsules were immersed in 10 mL of 5% ethanol aqueous solution and gently oscillated at room temperature. At predetermined time intervals, 200 μL of the immersion solution was taken out, and an equal volume of fresh 5% ethanol aqueous solution was immediately added to maintain the total volume constant. The absorbance of the immersion solution at 274 nm was measured using a UV–Vis spectrophotometer, and the cumulative release rate (E_r) was calculated using the following formulas:

$$E_r = (M_t/M_0) \times 100\% \quad (1)$$

$$M_t = V_e \sum_{i=1}^{n-1} C_i + V_0 C_n \quad (2)$$

where M_t is the cumulative OEO release amount at time t , M_0 is the initial OEO loading amount in the microcapsules, V_e is the sampling volume, V_0 is the total volume of the

release medium, C_i is the OEO concentration in the release medium at the i -th sampling, and C_n is the OEO concentration in the release medium at the n -th sampling.

The release kinetics were analyzed by fitting the cumulative release data to the zero-order equation, first-order equation, Higuchi equation, and Ritger–Peppas equation using Origin 2021 software.

2.3.4. Physicochemical Characterization Test

A Fourier transform infrared spectrometer (FT-IR, Nicolet iS50) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). FT-IR spectra were recorded in the range of 500–4000 cm^{-1} via the KBr pellet method with a resolution of 4 cm^{-1} and 32 scans (standard test conditions for solid samples).

An X-ray diffractometer (XRD, D8 Advance) was purchased from the Bruker Corporation (Karlsruhe, Germany). XRD patterns were acquired with Cu $K\alpha$ radiation ($\lambda = 1.54056 \text{ \AA}$) at an operating voltage of 40 kV and a tube current of 30 mA, with a scanning range of 5° to 80° (2θ) and a scanning rate of $0.4^\circ \text{ min}^{-1}$.

A thermogravimetric analyzer (TGA/DTG, STA 449 F3) was purchased from Netzsch GmbH & Co. KG (Selb, Germany). TGA/DTG analyses were conducted under a continuous high-purity nitrogen atmosphere (flow rate: 20 mL min^{-1}) at a heating rate of $10^\circ \text{C min}^{-1}$ over the temperature range of 25°C to 600°C .

BET measurements were performed on an ASAP 2460 analyzer (Micromeritics, Norcross, GA, USA) at 77 K, with P/P_0 controlled at 0.05–0.35. N_2 adsorption capacity was recorded at adsorption equilibrium, and the sample specific surface area and pore structure parameters (pore volume and average pore size) were calculated by BET and BJH methods, respectively.

2.3.5. Minimum Inhibitory Concentration (MIC) Test

Bacterial strains (Table 3, M5 and T6) were inoculated into Erlenmeyer flasks containing 100 mL of liquid LB medium and incubated with shaking at 37°C and 180 r/min for 12–16 h until the OD_{600} value reached 1.0–1.2, after which the bacterial seed suspensions were prepared. Fungal strains (Table 3, PM2 and GM1) were inoculated into Erlenmeyer flasks with 100 mL of fungal fermentation medium and cultured in a constant temperature shaking incubator at 28°C and 180 r/min until the viable spore count reached $(0.8\text{--}1.2) \times 10^6$ spores/mL, to obtain the fungal seed suspensions. Molecular biological identification: Genomic DNA of the strains was extracted by the CTAB method; for bacterial strains (M5 and T6), 16S rDNA gene amplification and sequencing were performed with universal primers 27F/1492R; for fungal strains (PM2 and GM1), ITS rDNA gene amplification and sequencing were performed with universal primers ITS1/ITS4. The obtained sequences were compared against the NCBI GenBank database using the NCBI online BLAST tool (version 2.16.0, National Center for Biotechnology Information, Bethesda, MD, USA), and the taxonomic status of the strains was determined with a similarity threshold of $\geq 99\%$.

Table 3. Taxonomic identification and sequence information of isolated strains.

Strain No.	Phylum	Species	Similarity (%)	Accession No.
M5	Bacillota	<i>Bacillus mycoides</i>	100	ACMU01000002
T6	Pseudomonadota	<i>Pseudomonas tianjinensis</i>	99.47	MF083697
PM2	Ascomycota	<i>Trichoderma reesei</i>	99.71	NR_154574.1
GM1	Ascomycota	<i>Fusarium foetens</i>	100	NR_159865.1

2.3.6. Antimicrobial Activity Test

The antimicrobial activity of OEO@SAM was evaluated by the inhibition zone method against typical wood-degrading microorganisms isolated from the Tianluoshan site (M5, T6, PM2, and GM1). In total, 100 μ L of fungal spore suspension or bacterial suspension was uniformly spread on PDA agar plates or LB agar plates, respectively. A total of 150 μ g of OEO@SAM was placed in the center of the inoculated plates, and blank SA microcapsules (SAM) were used as the negative control. The plates were incubated at 28 °C for 2–3 days, and the diameter of the inhibition zone was measured by the cross method. Each experiment was repeated three times, and the average value was calculated.

3. Results

3.1. Formation of Microcapsules

Figure 1 illustrates the morphological outcomes of sodium alginate (SA) hydrogels fabricated via an orthogonal experimental design, which systematically evaluates the synergistic effects of three formulation variables: mass ratio of oregano essential oil (OEO) to sodium alginate (SA): 2:1, 1:1, and 1:2; sodium alginate (SA) concentration: 1%, 2%, 3%, 4%, and 5%; and calcium chloride (CaCl_2) concentration (as the crosslinking agent): 3%, 5%, and 7%.

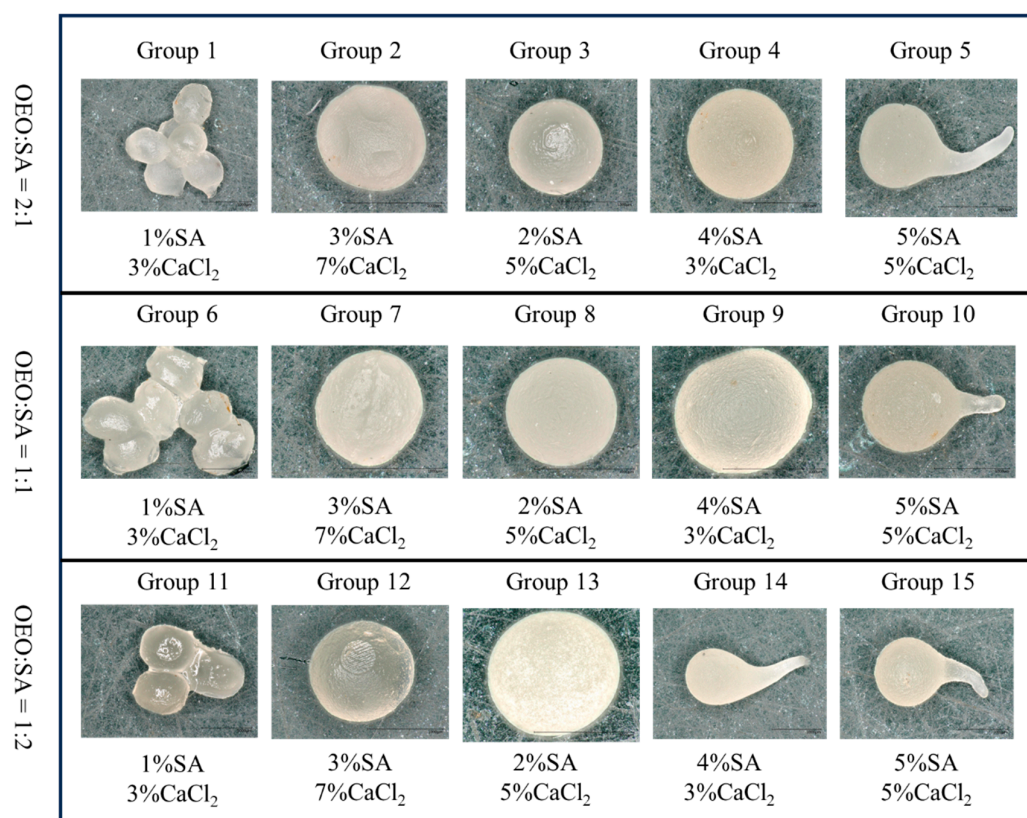


Figure 1. Ultra-depth-of-field microscopic images of OEO@SAM in orthogonal experiments.

(1) Top Row: OEO:SA = 2:1 (Groups 1–5)

Group 1 (1% SA, 3% CaCl_2): Exhibited irregular, fragmented gel aggregates with poor structural integrity, indicative of insufficient crosslinking.

Groups 2 (3% SA, 7% CaCl_2), 3 (2% SA, 5% CaCl_2), and 4 (4% SA, 3% CaCl_2): Formed well-defined, circular hydrogels with smooth, intact surfaces, demonstrating balanced crosslinking and uniform oil incorporation.

Group 5 (5% SA, 5% CaCl_2): Presented a teardrop-shaped morphology with noticeable deformation, suggesting excessive SA concentration disrupted gel uniformity.

(2) Middle Row: OEO:SA = 1:1 (Groups 6–10)

Group 6 (1% SA, 3% CaCl_2): Consisted of loose, clustered gel fragments lacking structural coherence, consistent with inadequate crosslinking density.

Groups 7 (3% SA, 7% CaCl_2), 8 (2% SA, 5% CaCl_2), and 9 (4% SA, 3% CaCl_2): Displayed uniform, circular hydrogels with homogeneous texture and smooth surfaces, reflecting optimal crosslinking under balanced OEO:SA ratios.

Group 10 (5% SA, 5% CaCl_2): Showed a teardrop-shaped gel with mild morphological distortion, similar to the trend observed in Group 5.

(3) Bottom Row: OEO:SA = 1:2 (Groups 11–15)

Group 11 (1% SA, 3% CaCl_2): Formed aggregated, irregular gel pieces with poor uniformity, attributable to low SA and CaCl_2 concentrations.

Groups 12 (3% SA, 7% CaCl_2), 13 (2% SA, 5% CaCl_2): Produced smooth, circular hydrogels.

Groups 14 (4% SA, 3% CaCl_2), 15 (5% SA, 5% CaCl_2): Developed teardrop-shaped, deformed gels with irregular contours, highlighting the sensitivity of gel morphology to high SA concentrations.

Accordingly, we conducted a full-factorial orthogonal experiment, in which the experimental variables were set as follows: sodium alginate (SA) concentrations of 2% and 3%, OEO:SA mass ratios of 0.5, 1, and 2, and calcium chloride (CaCl_2) concentrations of 3%, 5%, and 7%.

3.2. Encapsulation Efficiency (EE)

The OEO:SA ratio was identified as the most dominant factor affecting the encapsulation efficiency (EE), with EE decreasing sharply as the OEO:SA ratio increased (Figure 2). Specific data distribution under different experimental conditions is as follows:

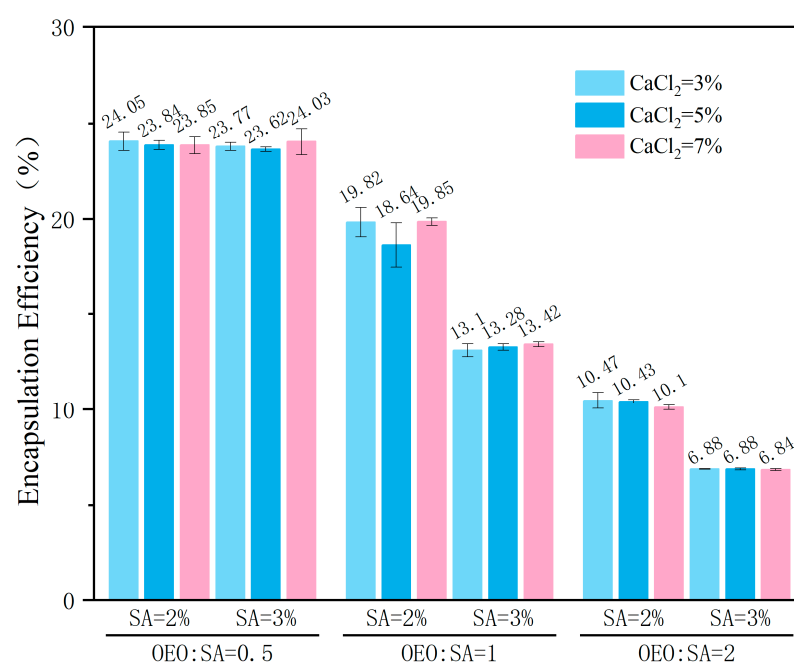


Figure 2. Encapsulation efficiency of OEO@SAM in orthogonal experiments.

When OEO:SA = 0.5 (low oil phase ratio), at SA = 2%, the EE corresponding to CaCl_2 concentrations of 3%, 5%, and 7% was 24.05%, 23.84%, and 23.85%, respectively.

At SA = 3%, the EE corresponding to CaCl₂ concentrations of 3%, 5%, and 7% was 23.77%, 23.62%, and 24.03%, respectively. The EE in this group fluctuated slightly (23.62–24.05%), showing no significant difference among different CaCl₂ concentrations.

When OEO:SA = 1 (medium oil phase ratio), EE decreased significantly compared with the OEO:SA = 0.5 group. At SA = 2%, EE was 19.82%, 18.61%, and 19.85% (corresponding to 3%, 5%, 7% CaCl₂). At SA = 3%, EE dropped to 13.28%, 13.28%, and 13.42% (corresponding to 3%, 5%, 7% CaCl₂). Notably, the EE of SA = 2% was significantly higher than that of SA = 3% in this group.

When OEO:SA = 2 (high oil phase ratio), EE decreased to the lowest level. At SA = 2%, EE was 10.47%, 10.43%, and 10.10% (corresponding to 3%, 5%, 7% CaCl₂). At SA = 3%, EE was only 6.88%, 6.88%, and 6.84% (corresponding to 3%, 5%, 7% CaCl₂), and SA = 2% still showed a higher EE.

3.3. Morphological and Physicochemical Characteristics

Based on the orthogonal experiments of the preliminary and formal tests, SA = 3%, OEO:SA = 0.5, and CaCl₂ = 3% were selected as the optimal final preparation parameters, and detailed physicochemical property characterization was subsequently conducted on the OEO@SAM microcapsules fabricated under these parameters.

The maximum absorption wavelength of OEO was 276 nm. The linear regression equation of the standard curve was $y = 0.03063 + 15.0605x$, with a correlation coefficient $R^2 = 0.99752$, indicating a good linear relationship between OEO concentration and absorbance.

3.3.1. Morphology and Element Distribution

SAM showed irregular bulk aggregates with rough surfaces, dense cross-sections, and no obvious pores. In contrast, OEO@SAM exhibited smaller and more uniform surface aggregates without obvious cracks, and the cross-section presented a porous/cavity structure (Figure 3). EDS analysis showed that the relative content of C element in OEO@SAM was higher than that in SAM, while the relative content of Ca element was lower, indicating the successful encapsulation of OEO.

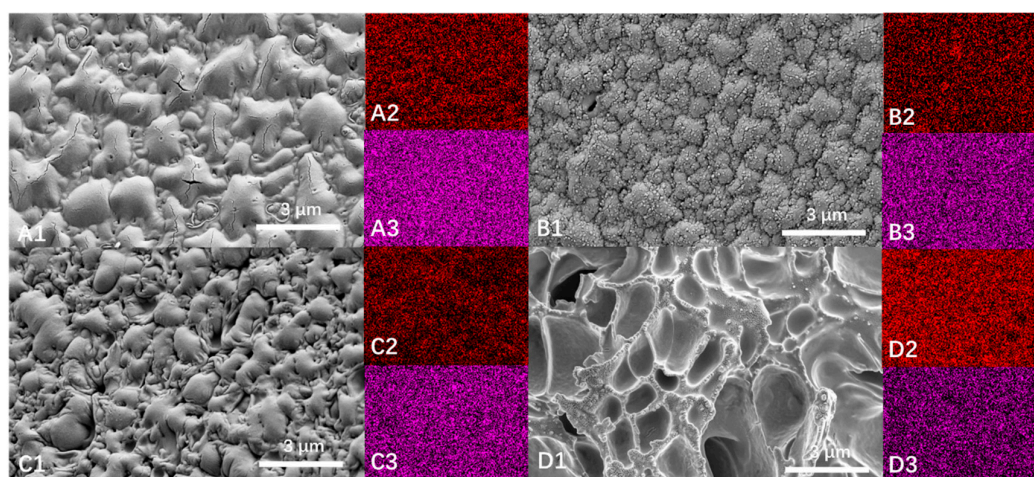


Figure 3. SEM and EDS images of SAM and OEO@SAM ((A1–A3): SAM surface, C and Ca element distribution; (B1–B3): SAM cross-section, C and Ca element distribution; (C1–C3): OEO@SAM surface, C and Ca element distribution; (D1–D3): OEO@SAM cross-section, C and Ca element distribution).

3.3.2. Characterization Result Analysis

FT-IR spectra confirmed the successful encapsulation of OEO and TW-80 in OEO@SAM (Figure 4A). The characteristic peaks of SA ($1500\text{--}1600\text{ cm}^{-1}$ and 1400 cm^{-1} for carboxylate

groups; 1000–1200 cm^{-1} for C-O bonds) were observed in both SAM and OEO@SAM. The characteristic peaks of TW-80 (1730 cm^{-1} for ester carbonyl; 1100 cm^{-1} for C-O-C) and OEO (2966 cm^{-1} for C-H stretching vibration of methyl groups) were detected in OEO@SAM, indicating the successful loading of OEO and TW-80. SA showed a strong diffraction peak at $2\theta = 20^\circ$, indicating a certain crystalline structure (Figure 4B). The diffraction peak intensity of SAM was weakened, and the peak shape was broadened, suggesting a decrease in crystallinity. The diffraction peak of OEO@SAM was further broadened with lower intensity, indicating that the introduction of OEO destroyed the crystalline order of the system, leading to an amorphous structure.

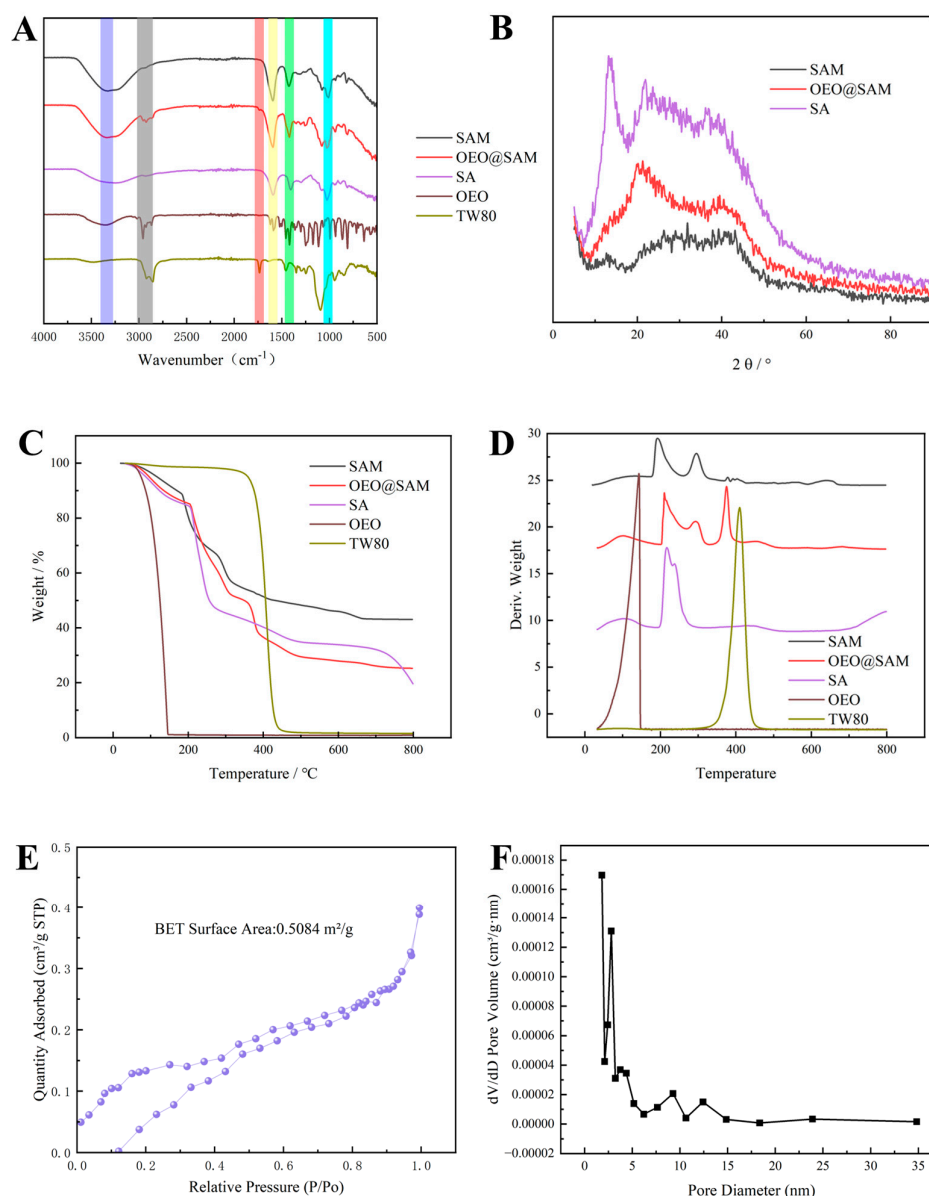


Figure 4. (A) FT-IR spectra of SAM, OEO@SAM, SA, OEO, and TW-80; (B) XRD patterns of SA, SAM, and OEO@SAM; (C) TGA curves of OEO, TW-80, SA, SAM, and OEO@SAM; (D) DTG curves of OEO, TW-80, SA, SAM, and OEO@SAM; (E,F) BET of OEO@SAM.

OEO showed rapid mass loss in the range of room temperature to 200 $^\circ\text{C}$, with almost no residue (Figure 4C). SA exhibited two stages of mass loss: slight water evaporation at 100 $^\circ\text{C}$ and main decomposition of the polysaccharide skeleton at 200–400 $^\circ\text{C}$. SAM showed similar thermal behavior to SA. OEO@SAM exhibited composite thermal behavior: additional mass loss in the low-temperature range (due to OEO volatilization), higher

mass loss rate at 200–400 °C (due to the SA, the final mass loss stage in the 350–400 °C range possibly associated with TW-80 decomposition), and lower residual mass compared to SAM. The DTG curve of OEO@SAM showed two peaks: the low-temperature peak corresponding to OEO volatilization and the high-temperature peak corresponding to the combined decomposition of TW-80 (350–400 °C) and SA (200–400 °C) (Figure 4D). BET analysis showed that OEO@SAM had a low specific surface area (0.5084 m²/g), low pore volume (0.000618 cm³/g), and average pore diameter of 4.4099 nm, indicating a dense structure (Figure 4E,F).

3.4. Sustained-Release Performance

The cumulative release rate of OEO from OEO@SAM increased gradually over time within 10 days, showing a stable and sustained release trend without obvious burst release. The release data were fitted to four kinetic models (Figure 5). The zero-order equation showed the highest fitting degree ($R^2 = 0.9959$), indicating a nearly constant release rate. The Ritger–Peppas equation fitting gave $n > 0.85$, suggesting that the release mechanism was dominated by SA skeleton erosion. The calculated release times were 13.9 days (zero-order equation), 14.3 days (Ritger–Peppas equation), and 18.3 days (Higuchi equation).

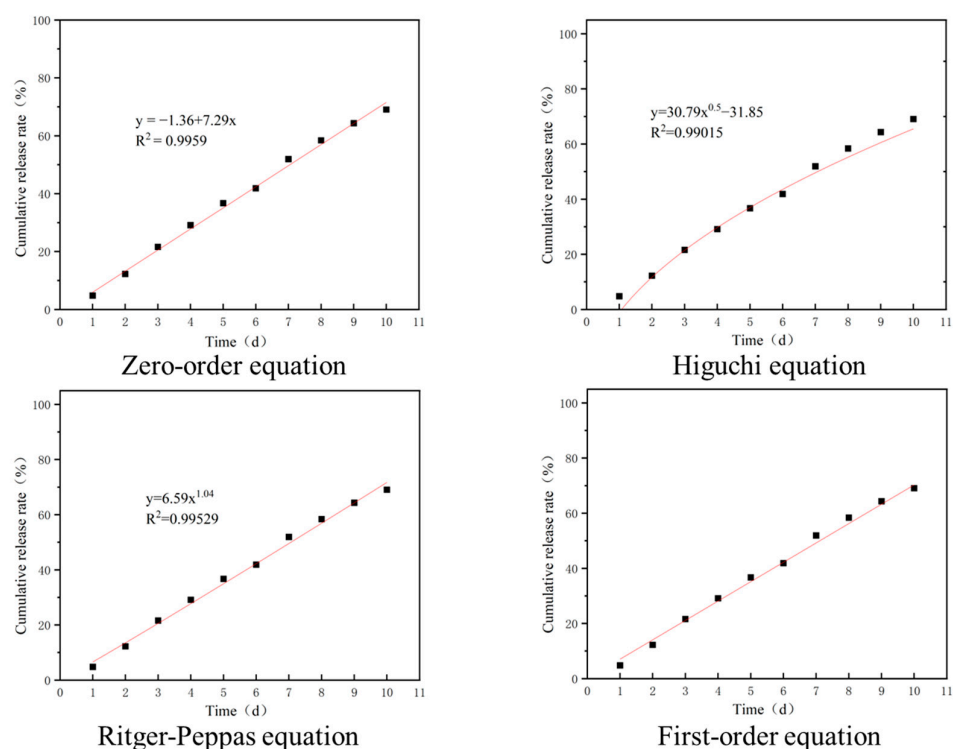


Figure 5. Fitting curves of OEO release from OEO@SAM using different kinetic models.

3.5. Antimicrobial Activity

OEO@SAM exhibited antimicrobial activity against all tested wood-degrading microorganisms (Figure 6). The largest inhibition zone diameter was observed against PM2 (24.67 ± 0.58 mm), followed by GM1 (12.67 ± 0.58 mm), T6 (9.33 ± 1.15 mm), and M5 (9.00 ± 1.00 mm), indicating effective inhibition of dominant wood-degrading microorganisms (Figure 6(A3,B3,C3,D3) and Table 4). The one-month simulation experiment verified the protective effect: visual observation showed reduced surface discoloration and softening of wood samples with OEO@SAM, while colorimeter data further quantified this difference—water chroma in the OEO@SAM group was only 30, compared to 300.01 in the control group without the antimicrobial agent (Figure 6E). The gradient dosing method in liquid medium was employed to determine the minimum inhibitory concentration (MIC)

of OEO@SAM microcapsules. The judgment criterion for MIC was defined as follows: the lowest dosage of microcapsules that could inhibit the visible growth of microorganisms (i.e., no turbidity observed by the naked eye, or a reduction of $\geq 90\%$ in OD value compared with the positive control group) was regarded as the MIC value of the microcapsules against the target microorganisms. In the experiment, the weight of 100 OEO@SAM microcapsules was 0.0714 g, which provided a fundamental basis for the subsequent mass concentration conversion. In the 5 mL liquid medium system, test tube groups (tubes 1–7, with tube 7 serving as the blank control) were set up with different microcapsule dosages, and the turbidity of the medium was used as the visual evaluation index. The results (Figure 6(A4,B4,C4,D4)) showed that OEO@SAM exhibited consistent inhibitory effects against bacteria and fungus PM2 in water: tubes 1–4 (supplemented with 0–3 microcapsules) all showed turbidity, while tubes 5–6 (supplemented with 4–5 microcapsules) were consistent with the blank control tube. The corresponding inhibitory concentration in terms of microcapsule count was 0.8 capsules/mL for both microorganisms. After conversion, the MIC of OEO@SAM in terms of mass concentration was 0.57 g/L against bacteria and 0.71 g/L against fungus PM2. In contrast, a higher dosage was required to inhibit fungus GM1: only tube 6 (supplemented with 5 microcapsules) was consistent with the blank control tube, corresponding to an inhibitory concentration of 1 capsule/mL in terms of microcapsule count and a MIC of 0.71 g/L in terms of mass concentration (Table 4).

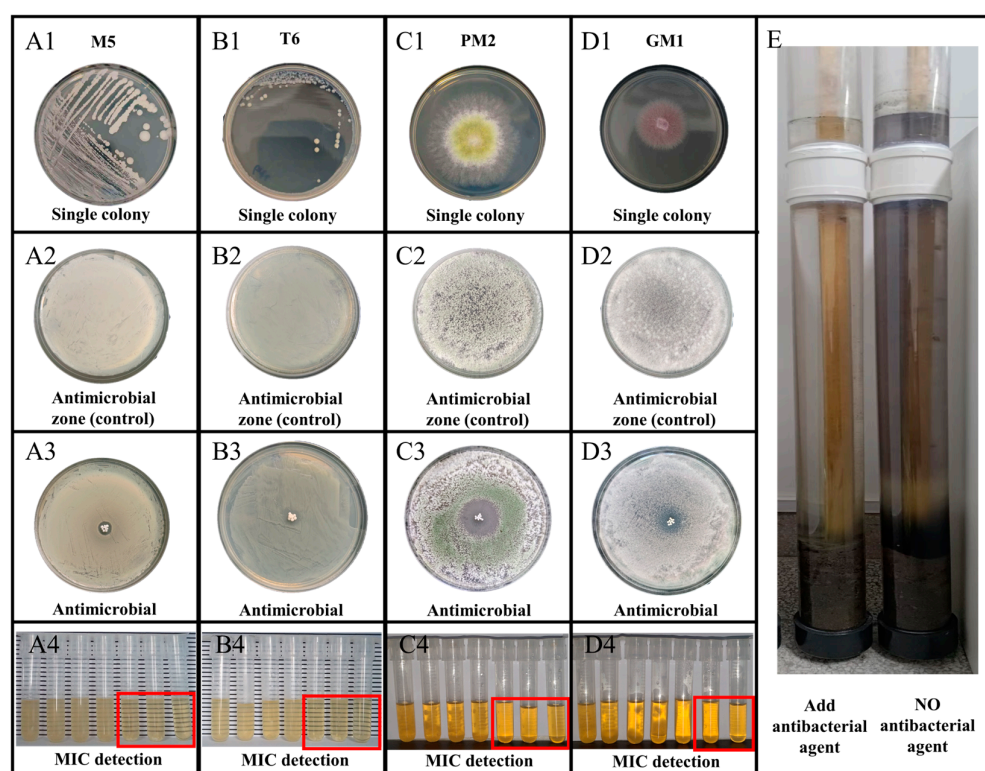


Figure 6. OEO@SAM antimicrobial experiment in the laboratory. (A) Tests for strain M5: (A1) purified single colony; (A2) antimicrobial zone blank control; (A3) antimicrobial zone test result; (A4) minimum inhibitory concentration (MIC) detection, with the red box indicating the MIC endpoint; (B) Tests for strain T6: (B1) purified single colony; (B2) antimicrobial zone blank control; (B3) antimicrobial zone test result; (B4) MIC detection, with the red box indicating the MIC endpoint; (C) Tests for strain PM2: (C1) purified single colony; (C2) antimicrobial zone blank control; (C3) antimicrobial zone test result; (C4) MIC detection, with the red box indicating the MIC endpoint; (D) Tests for strain GM1: (D1) purified single colony; (D2) antimicrobial zone blank control; (D3) antimicrobial zone test result; (D4) MIC detection, with the red box indicating the MIC endpoint; (E) Simulated protection experiment of waterlogged wooden relics: antibacterial agent-treated group (left) and untreated blank control group (right).

Table 4. MIC and inhibition zone diameter.

Factor	M5	T6	PM2	GM1
MIC (g/L)	0.57	0.57	0.57	0.71
Inhibition Zone Diameter (mm)	9.00 ± 1.00	9.33 ± 1.15	24.67 ± 0.58	12.67 ± 0.58

4. Discussion

The OEO/SAM ratio is the dominant factor affecting EE because the SA wall material has a limited loading capacity for OEO. The OEO:SA ratio dominates EE because the SA wall material has a limited loading capacity for OEO. As the OEO proportion increases, the theoretical OEO content exceeds the saturation load of SA, leading to a sharp decrease in EE. SA concentration affects EE by changing the viscosity of the emulsion: the higher viscosity of 3% SA may reduce the dispersion uniformity of OEO droplets, thus lowering the encapsulation effect (so SA = 2% shows higher EE). CaCl₂ concentration has a weak regulatory effect on EE in the experimental range: the crosslinking reaction can be sufficiently completed in 3–7%. With the increase in the OEO/SAM ratio, the theoretical OEO content per microcapsule increases significantly, but the actual loading capacity of SA cannot be improved correspondingly, resulting in a sharp decrease in EE. The slightly higher EE of 2% SA compared to 3% SA may be due to the higher viscosity of 3% SA, which reduces the dispersion uniformity of OEO droplets in the emulsion and the porosity of the crosslinked wall material, thereby decreasing the encapsulation efficiency. The effect of CaCl₂ concentration on EE is related to the size of OEO droplets. For small oil droplets (low OEO/SAM ratio), high CaCl₂ concentration (7%) accelerates the crosslinking reaction, forming a complete wall structure before OEO diffusion, thus improving EE. For large oil droplets (high OEO/SAM ratio), moderate CaCl₂ concentration (5%) balances the crosslinking rate and wrapping time, avoiding oil droplet rupture caused by slow crosslinking or wall material contraction caused by rapid crosslinking, thereby achieving optimal EE.

The porous cross-sectional structure of OEO@SAM is formed by the introduction of OEO, which provides channels for the sustained release of OEO. The successful encapsulation of OEO and TW-80 is confirmed by FT-IR and EDS analyses, and the crosslinking structure between Ca²⁺ and SA carboxyl groups remains intact, ensuring the stability of the microcapsule structure. The amorphous structure of OEO@SAM (XRD result) is beneficial to the uniform distribution of OEO and the stability of the release process. The thermal behavior of OEO@SAM reflects the composite properties of SA, OEO, and TW-80, with OEO volatilization in the low-temperature range and combined decomposition of SA and TW-80 in the high-temperature range, indicating good thermal stability of the microcapsule structure.

The release behavior of OEO@SAM conforms to the zero-order kinetic model, showing a constant release rate, which is attributed to the skeleton erosion mechanism (Ritger-Peppas model $n > 0.85$). SA, as a hydrophilic polymer, gradually swells and erodes in the aqueous medium, leading to the continuous and uniform release of encapsulated OEO. This sustained-release pattern effectively overcomes the defect of rapid OEO volatilization, ensuring long-term antimicrobial activity. The low specific surface area and dense structure of OEO@SAM (BET result) further contribute to the stable release by reducing the diffusion rate of OEO.

The antimicrobial activity of OEO@SAM is derived from the sustained release of OEO, which inhibits the growth of wood-degrading microorganisms. The strongest inhibition against PM2 (*Trichoderma breve*) is particularly important because *Trichoderma* spp. are key cellulose-degrading fungi in waterlogged archaeological wood, leading to wood softening and deterioration. The effective inhibition of PM2 and other dominant degrading microorganisms confirms the potential of OEO@SAM as an antimicrobial agent for waterlogged

archaeological wood protection. Compared with direct application of OEO, OEO@SAM reduces direct contact between OEO and wood, avoiding potential damage to the cultural relics, and meets the “safe and controllable” requirement of cultural relic protection. In field experiments on water-saturated archaeological wood, the minimum dosage of OEO@SAM microcapsules to be added per liter of water is at least 0.71 g. This conclusion has been verified by laboratory experiments.

5. Conclusions

In this study, OEO-loaded sodium alginate microcapsules (OEO@SAM) were successfully prepared via an ionic crosslinking method. The key preparation parameters were optimized through orthogonal experiments, and the optimal process was determined as OEO/SAM = 0.5, 2% SA, and 3% CaCl₂ (EE = 24.05%). For higher mechanical strength, the combination of OEO/SAM = 0.5, 3% SA, and 3% CaCl₂ is recommended. Morphological and physicochemical characterizations confirmed that OEO and TW-80 were successfully encapsulated in the SA-based microcapsules, and the Ca²⁺ crosslinking structure was stable. OEO@SAM exhibited a porous cross-sectional structure, amorphous crystal form, and good thermal stability. The sustained-release behavior of OEO from OEO@SAM followed the zero-order kinetic model, with a release mechanism dominated by SA skeleton erosion, achieving stable and long-term release of OEO. OEO@SAM showed effective antimicrobial activity against typical wood-degrading microorganisms isolated from the Tianluoshan site, especially against PM2 (inhibition zone diameter = 24.67 ± 0.58 mm). The minimum dosage of OEO@SAM microcapsules to be added per liter of water is at least 0.71 g. OEO@SAM is a green, eco-friendly, and pollution-free bacteriostatic agent. When deployed in the aqueous environment of waterlogged archaeological sites, it slowly releases oregano essential oil to inhibit microbial growth and is non-damaging to waterlogged archaeological wood. This study effectively solves the technical bottlenecks of natural essential oils in cultural relic protection and provides an environmentally friendly, safe, and efficient sustained-release antimicrobial material for the in situ protection of waterlogged archaeological wood.

The OEO@SAM antibacterial and antifungal agent developed in this study for the in situ conservation of waterlogged archaeological wood at the Tianluoshan site has successfully addressed the core technical challenges of oregano essential oil, OEO, namely its high volatility and poor stability. It has initially achieved controlled sustained release of OEO, and its excellent antibacterial activity and sustained-release performance have been verified under laboratory conditions. However, limitations still exist in its practical application. The sustained-release period of the OEO@SAM antibacterial and antifungal agent prepared in this study only lasts for approximately two weeks, which means that it is necessary to frequently supplement the microcapsule antibacterial and antifungal agent into the site water body, thereby increasing the workload of on-site maintenance. All performance tests conducted in this study were completed under controlled simulated laboratory conditions, while several issues may arise in actual on-site applications at the Tianluoshan site. Key parameters of the water at the Tianluoshan site, such as pH value, temperature, salinity, and organic matter content, undergo dynamic fluctuations with seasonal changes, climate variations, and surrounding environmental disturbances. These parameters directly affect the swelling properties and degradation rate of the sodium alginate wall material, making it difficult to ensure the stability of the antibacterial activity of OEO@SAM.

In summary, the combined effect of the aforementioned complex factors results in the antibacterial effect and sustained-release performance of OEO@SAM in actual applications at the Tianluoshan site being much lower than those in laboratory tests. This has become the core technical barrier preventing OEO@SAM from moving from the laboratory research and

development stage to on-site practical application, which requires focused breakthroughs in subsequent studies.

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