A Novel *Cerrena caperata* Fungal-Derived Membrane: Antibacterial Effect of Deacetylation and Fatty Acid Coating

Retno Agnestisia[1*], Yuliana Yuliana[1], Erwin Prasetya Toepak[1], Remi Ayu Pratika[1], Muhammad Rumi Azhari Nur Irfani[2,4,5], Eka Jhonatan Krissilvio[1], Rendy Muhamad Iqbal[1,3,4]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya 73111, Central Kalimantan, Indonesia

[2]Department of Civil Engineering, Nagoya Institute of Technology (Nitech), Nagoya, Aichi 466-85555, Japan

[3]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia

[4]Advanced Membrane Technology Research Center (AMTEC), Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia

[5]Institute of Materials and Systems for Sustainability (IMaSS), Department of Civil Engineering, Graduate School of Engineering, Nagoya University, Aichi 464-8601, Japan

[*]Email: retno.agnestisia@mipa.upr.ac.id

Received 03 May 2025
Accepted 29 May 2025
Published 30 June 2025

Abstract

Biofouling commonly occurs due to bacterial accumulation that blocks membrane pores, especially in fungal-derived membrane technology. In this study, surface modification of *Cerrena caperata* fungal-derived membranes was conducted to enhance antibacterial activity, aiming to prevent biofouling on membrane surfaces. This modification is crucial to ensure the membrane's ability to inhibit or kill pathogenic bacteria during water purification, ultimately producing safer water. The membranes were synthesized from *C. caperata* mycelium cultured in potato dextrose broth for four weeks. Two treatments were applied: (1) deacetylation with 50% NaOH and (2) surface coating with 1% stearic acid. Both unmodified and modified membranes were characterized using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) to evaluate chemical and morphological changes. FTIR confirmed successful deacetylation through the appearance of hydroxyl (–OH) and amine (–NH) functional groups, while SEM revealed increased surface roughness following NaOH treatment. Stearic acid coating enhanced membrane hydrophobicity, as indicated by increased aliphatic C–H and carbonyl (C=O) peaks. Antibacterial activity was assessed via the disk diffusion method against *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*. Deacetylated membranes treated for 90 minutes exhibited the highest inhibition zones—9.88 mm (*B. cereus*), 9.82 mm (*E. coli*), and 9.24 mm (*S. aureus*)—demonstrating significant antibacterial efficacy due to chitosan formation. In contrast, stearic acid-modified membranes showed reduced activity. These findings highlight the potential of fungi as a sustainable raw material for producing filtration membranes that are effective, antibacterial, and environmentally friendly.

Keywords: *biofouling*, *Cerrena caperata, fungal-derived membrane*

How to cite

Agnestisia, R., Yuliana, Y., Toepak, E.P., Pratika, R.A., Irfani, M.R.A.N., Krissilvio, R.J., & Iqbal, R.M. (2025). A Novel *Cerrena caperata* Fungal-Derived Membrane: Antibacterial Effect of Deacetylation and Fatty Acid Coating. *Jurnal Kimia Riset*, *10*(1), 127–141.

Highlights

1. *Cerrena caperata* mycelium was successfully utilized to fabricate novel fungal-derived membranes for water purification, marking the first study to explore this native Indonesian fungus for such application.
2. Surface deacetylation using 50% NaOH for up to 90 minutes significantly enhanced the antibacterial activity of the membranes, with inhibition zones reaching up to 9.88 mm against *Bacillus cereus*.
3. FTIR and SEM analyses confirmed successful chemical and morphological transformations, including chitosan formation through deacetylation and increased hydrophobicity from stearic acid coating.
4. Stearic acid coating increased surface hydrophobicity but reduced antibacterial performance, highlighting a trade-off between anti-biofouling potential and antimicrobial efficacy.
5. The study demonstrates the viability of fungal-based membranes as eco-friendly and sustainable alternatives in membrane technology, offering promising potential for applications in biofouling-resistant water filtration systems.

Introduction

Recent advancements in water purification have increasingly focused on the development of fungal-derived membranes, an innovative and sustainable approach explored by numerous researchers (French et al., 2024; Isik et al., 2019). Fungal mycelium, characterized by its unique filamentous and porous structure, naturally forms highly efficient filtration materials capable of removing various contaminants from water (Isik et al., 2019). Beyond their physical filtration ability, the cell walls of fungal mycelium contain polysaccharides such as chitin and glucans, which possess functional groups able to adsorb hard-to-remove pollutants, including heavy metals and dyes metals (French et al., 2024). This dual functionality of filtration and adsorption positions fungal-based membranes as promising candidates for comprehensive water treatment solutions.

Previous research has demonstrated the successful fabrication of fungal-derived membranes from species such as *Aspergillus carbonarius* and *Pleurotus ostreatus*, targeting specific pollutants with considerable removal efficiencies. For instance, Isik et al. (2019) pioneered the fabrication of membranes from *A. carbonarius* M333, achieving dye decolorization efficiencies of up to 91% in wastewater treatment. Subsequently, French et al. (2024) expanded this work by synthesizing membranes from *P. ostreatus*, targeting inorganic pollutants such as copper, zinc, and cadmium, with removal efficiencies reaching 97%, 50%, and 68%, respectively. These studies underscore the effectiveness of fungal-derived membranes and their promise as eco-friendly alternatives to conventional filtration materials.

This study introduces a novel approach by utilizing the mycelium of *Cerrena caperata*, a native fungus of the Sebangau peat swamp forest in Central Kalimantan, Indonesia, for the fabrication of membranes. To the best of our knowledge, this is the first report that explores *C. caperata* as a membrane material for water purification. This species was selected based on its ecological abundance and the robust, fibrous nature of its mycelial network, which has not yet been exploited for membrane development. This macroscopic fungus, dominant in the Peat Swamp Forest at the Center for International Cooperation in Sustainable Management of Tropical Peatland (CIMTROP) in Sebangau, Central Kalimantan, has been successfully isolated and characterized in a previous study (Agnestisia et al., 2024). Its dense,

fibrous structure makes it a promising candidate for the development of bio-based water purification membranes. However, a significant limitation in membrane filtration systems is biofouling—the accumulation of bacteria on membrane surfaces that clogs pores and reduces filtration efficiency (Alnumani et al., 2024; Baker & Dudley, 1999; Iman et al., 2024). To overcome this issue, surface modification techniques such as deacetylation and fatty acid coating have been widely employed to improve membrane performance and resist bacterial attachment (Egorov et al., 2023; Elsoud & Kady, 2019; Islam et al., 2011; Prudnikov et al., 2023; Wu et al., 2021).

One of the key components of fungal-derived membranes is chitin, a natural polymer abundantly found in fungal cell walls. Chitin can be chemically converted into chitosan through deacetylation—a process commonly carried out using sodium hydroxide (NaOH). Chitosan has been extensively studied for its antimicrobial properties, biocompatibility, and film-forming capabilities, making it an effective additive for enhancing membrane function (Adhiksana et al., 2023; Egorov et al., 2023; Elsoud & Kady, 2019; Islam et al., 2011). The deacetylation process increases the concentration of amino groups, which are responsible for chitosan's antimicrobial activity and improved interaction with microbial cells (Egorov et al., 2023; Islam et al., 2011). Chitosan-modified membranes have shown increased resistance to biofouling, a key advantage in water treatment applications (Alnumani et al., 2024; Baker & Dudley, 1999; Iman et al., 2024). Meanwhile, surface modification using fatty acids, particularly stearic acid, has emerged as a complementary strategy to enhance membrane hydrophobicity and minimize bacterial adhesion (Manurung et al., 2024; Prudnikov et al., 2023; Wu et al., 2021). Fatty acid coatings form a hydrophobic layer on the membrane surface, reducing water retention and limiting microbial colonization. Therefore, this study aimed to investigate two distinct chemical modifications—deacetylation and stearic acid coating—to enhance the membrane's antibacterial activity. While these two methods have been explored separately in different materials, their impact has not been systematically studied on membranes derived from *C. caperata*. Importantly, our research does not combine the two modifications simultaneously but rather evaluates them as independent strategies, offering comparative insight into how each affects the antibacterial performance of fungal-derived membranes. The mycelium was cultured in potato dextrose broth for four weeks, then subjected to NaOH deacetylation and stearic acid coating for varying durations. Both unmodified and modified membranes were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) to assess chemical and structural changes. In addition, antibacterial activity was tested against *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* to evaluate the membranes' resistance to bacterial growth. By integrating insights from fungal-derived membrane technologies and antibacterial surface modifications, this study offers novel findings on the development of *C. caperata*-derived membranes. The results underscore their potential as effective, sustainable materials for enhanced water purification systems.

Research Methods

Materials

The *C. caperata* fungus was isolated from the Peat Swamp Forest at CIMTROP, Sebangau, Central Kalimantan, as reported in a previous study (Agnestisia et al., 2024). The fungal isolate was maintained on potato dextrose agar (PDA) and cultured in potato

dextrose broth (PDB), both of analytical grade and purchased from Merck Tbk., Bandung, Indonesia. Additional materials included Luria-Bertani agar (LB agar, analytical grade, Merck), sodium hydroxide (NaOH pellets, ≥98% purity, Merck), and stearic acid ($CH_3(CH_2)_{16}COOH$, ≥98% purity, Merck). All chemicals used were of analytical grade unless otherwise stated, and all solutions were prepared using distilled water. The bacterial strains used for antibacterial activity testing were *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*, obtained from the Institut Pertanian Bogor Culture Collection (IPBCC), Bogor, Indonesia.

Instrumentation

Membrane characterization was conducted using FTIR and SEM. FTIR spectra were recorded with a Shimadzu IRSpirit-T spectrometer (Shimadzu Corporation, Kyoto, Japan) using the KBr pellet technique over a range of 4000–400 cm^{-1} to identify functional groups. SEM analysis was carried out with an Advanced Microanalysis Solution SEM (TM4000Plus, Hitachi High-Technologies, Tokyo, Japan) to observe surface morphology at both micrometer and nanometer scales under low vacuum conditions with an accelerating voltage of 5–15 kV.

Procedure

1) Synthesis of fungal-derived membranes

   The fungal isolate was pre-cultured on PDA (39 g/L) in 9 cm Petri dishes at 27 ± 2°C under static and dark conditions. Ten agar discs (5 mm diameter) containing actively growing mycelium were transferred into 250 mL Erlenmeyer flasks containing 100 mL of PDB (24 g/L) and incubated statically in the dark at 27 ± 2°C for four weeks. The resulting mycelial mat was harvested, sterilized by UV exposure for 30 minutes, and immersed in 70% ethanol for 3 hours. It was then rinsed thoroughly with sterile distilled water. The prepared fungal mat was referred to as the fungal-derived membrane.

2) Modification of fungal-derived membranes

   The fungal-derived membranes (4 weeks old) were cut into 2 × 2 cm squares. For deacetylation, the samples were immersed in 50% (w/v) NaOH solution for 30, 60, or 90 minutes. For hydrophobic surface modification, the membranes were immersed in 1% (w/v) stearic acid solution (dissolved in ethanol) for 60, 120, or 180 minutes. After each treatment, the membranes were rinsed with sterile distilled water and oven-dried at 50°C for 60 minutes.

3) Antibacterial activity test

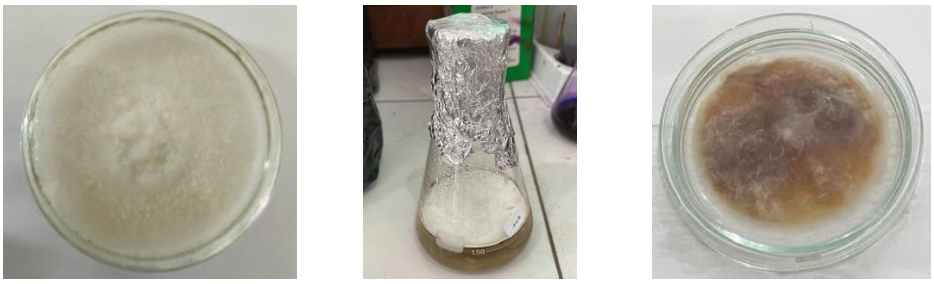
   The antibacterial activity of the membranes was evaluated using the disk diffusion method. LB agar plates were inoculated with *E. coli*, *B. cereus*, and *S. aureus* bacterial cultures. Membrane samples (both modified and unmodified) were placed on the inoculated plates and incubated at 37°C for 24 hours. Chloramphenicol (0.01%) was used as a positive control. Inhibition zones were measured in millimeters as an indicator of antibacterial efficacy. All tests were conducted in triplicate and independently repeated three times to ensure statistical reliability and reproducibility.

Results and Discussion

Synthesis of fungal-derived membranes

The synthesis of fungal-derived membranes began with the pre-culturing of *C. caperata* on PDA medium, which successfully supported the growth of the fungal mycelium (Figure 1a). The mycelium grew vigorously on the PDA surface under static and dark conditions, confirming the suitability of this medium and cultivation environment for *C. caperata* growth. After inoculating the pre-cultured fungal discs into the PDB

medium, the mycelium grew extensively in the liquid medium, forming a dense mat of mycelial biomass over the course of 4 weeks (Figure 1b). This is consistent with the optimal growth conditions reported for various fungi, where PDB serves as a nutrient-rich medium supporting robust fungal growth (French et al., 2024; Isik et al., 2019). The 4-week incubation period was also found to be ideal for generating sufficient biomass for membrane formation, with the fungal mat covering the entire surface area of the PDB liquid medium (Figure 1b). This resulted in a dense, flexible mycelial membrane that could be harvested. After harvesting, the fungal mycelium was subjected to sterilization processes to ensure the elimination of any microbial contaminants without compromising its structural integrity (Figure 1c).



(a) (b) (c)

Figure 1. Mycelium of *C. caperata* on PDA medium (a) and PBD medium (b), and a *C. caperata*-derived membrane after sterilization (c)

Modification of fungal-derived membranes

The fungal-derived membrane was then modified using two chemical treatments: 1) deacetylation with NaOH and 2) coating with stearic acid. These modifications aimed to enhance the membrane's antimicrobial properties and biofouling resistance. The fungal-derived membranes were soaked in a 50% (w/v) NaOH solution for varying durations (30, 60, and 90 minutes) to induce deacetylation. The NaOH treatment is known to convert chitin into chitosan by removing acetyl groups from the chitin polymer, a process that is expected to enhance the antimicrobial activity of the membrane (Egorov et al., 2023; Elsoud & Kady, 2019; Islam et al., 2011). The enhanced antimicrobial activity of deacetylated membranes is attributed to the formation of free amino groups ($-NH_2$) on the chitosan chains. These positively charged amino groups can interact electrostatically with the negatively charged components of bacterial cell membranes, leading to increased membrane permeability, disruption of cellular functions, and ultimately cell death (Ke et al., 2021; M. Wang et al., 2024). This mechanism is particularly effective against Gram-positive bacteria, which have a thicker peptidoglycan layer that is more susceptible to such interactions. As immersion time in NaOH increases, more amino groups become available on the membrane surface, enhancing these electrostatic interactions and thereby increasing the antibacterial efficacy of the fungal-derived membrane (Ke et al., 2021; S. Wang et al., 2024).

FTIR spectra were then used to provide insight into the potential transformation of chitin to chitosan in the fungal-derived membrane after hydroxylation using NaOH at varying immersion times (30, 60, and 90 minutes) (Figure 2). The neat membrane, which likely contains chitin as a structural component, exhibits characteristic peaks, including a strong band around 1600–1700 cm^{-1}, corresponding to the C=O stretching vibrations of the amide I group, and a peak near 1550 cm^{-1}, attributed to N–H

bending of the amide II group. These peaks are typical of the acetyl groups present in chitin (Nie et al., 2007; Raman & Ramasamy, 2017; Svecova et al., 2006). Following immersion in NaOH for 30, 60, and 90 minutes, notable changes occur in these regions. The gradual reduction in the intensity of the amide I and amide II peaks suggests the partial removal of acetyl groups, a key step in the deacetylation process that converts chitin to chitosan (Kumirska et al., 2010). Concurrently, the broadband observed around 3200–3400 cm^{-1}, associated with O–H and N–H stretching vibrations, becomes more pronounced after NaOH treatment (Boudouaia et al., 2019; Hassan et al., 2018). This indicates the generation of free amino groups (-NH_2) resulting from the replacement of acetyl groups during deacetylation (Hassan et al., 2018). The emergence or increase in peaks within this region further supports the hypothesis of chitosan formation. Additionally, changes in the C–O stretching vibrations between 1000 and 1200 cm^{-1} could signify alterations in the polysaccharide structure due to NaOH-induced hydrolysis (Hasan et al., 2022; Rehman et al., 2023).

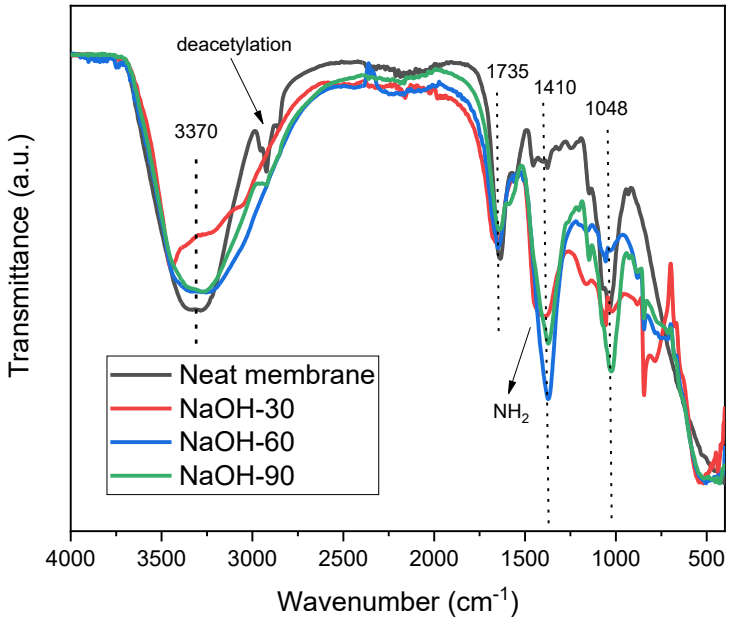


Figure 2. FTIR spectra of hydroxylated-fungal-derived membranes

The FTIR data also reveal a progressive trend with increased immersion time. Membranes treated for 90 minutes exhibit the most significant spectral changes, indicating a higher degree of deacetylation compared to shorter immersion times. This suggests that longer NaOH exposure enhances the conversion of chitin into chitosan. However, complete deacetylation is unlikely under these mild conditions, and the resulting material may still consist of a chitin-chitosan hybrid. While the FTIR spectra provide strong evidence of chemical transformation, quantitative analysis of the degree of deacetylation (DDA) is necessary to confirm the extent of chitosan formation. Complementary techniques, such as X-ray diffraction (XRD) or nuclear magnetic resonance (NMR), would provide more conclusive validation of the structural changes observed.

The fungal-derived membranes were then soaked in a 1% (w/v) stearic acid solution for varying durations (60, 120, and 180 minutes) to induce coating. Stearic acid treatment is known to

enhance the hydrophobicity of the membrane by depositing a fatty acid layer onto its surface, thereby modifying its surface energy and potentially improving antimicrobial resistance and anti-biofouling characteristics (Prudnikov et al., 2023; Wu et al., 2021). Fatty acids such as stearic acid are amphiphilic in nature and can disrupt bacterial membranes by inserting into the lipid bilayers, compromising membrane integrity and causing leakage of intracellular components (Casillas-Vargas et al., 2021). Additionally, the increased hydrophobicity of the stearic acid-coated membranes may reduce bacterial adhesion or limit bacterial access to active antimicrobial sites on the membrane surface (Prudnikov et al., 2023; Wu et al., 2021).

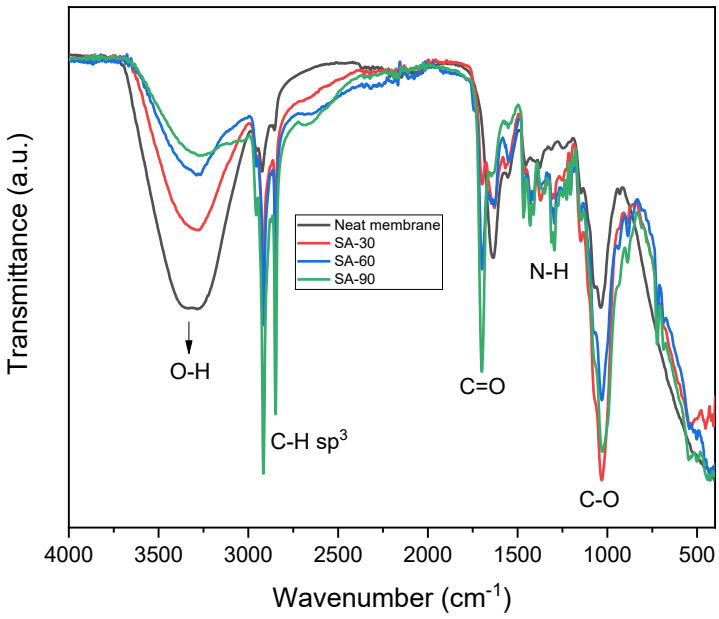


Figure 3. FTIR spectra of stearic acid-coated fungal-derived membranes

FTIR spectra were also used to confirm the chemical modification of membranes after immersion in stearic acid (SA) solution. The neat membrane, incubated for four weeks, serves as the control. Its FTIR spectrum exhibits key functional groups characteristic of fungal biomaterials, such as polysaccharides and proteins. The broad peak around 3200–3400 cm^{-1} corresponds to O–H stretching, indicative of hydroxyl groups in polysaccharides or adsorbed water (Bayramoğlu et al., 2005; Gupta, 2011). Peaks in the region of 1600–1700 cm^{-1} are attributed to C=O stretching, likely from carbonyl groups in polysaccharides or amide groups in proteins (Gupta, 2011). Additionally, the peaks between 1000 and 1200 cm^{-1} indicate C–O stretching vibrations, supporting the polysaccharide-rich nature of the fungal-derived membrane (Gupta, 2011). After modification with stearic acid, notable changes occur in the FTIR spectra, indicating successful chemical interactions between the fungal-derived membrane and stearic acid. The emergence of new peaks and changes in intensity confirm the grafting of stearic acid onto the membrane surface. A significant increase in intensity is observed in the region around 2800–3000 cm^{-1}, corresponding to C–H stretching vibrations from the aliphatic hydrocarbon chains of stearic acid (Lozano et al., 2024; S. Wang et al., 2024). The intensity of these peaks increases with longer immersion times, suggesting higher stearic acid deposition or more extensive surface coverage.

Furthermore, the peak around 1700 cm^{-1}, associated with C=O stretching of ester or carboxylic groups, becomes more pronounced after stearic acid modification (Larkin & Jackson, 2024; Sinha et al., 2017), especially for the 60- and 90-minute treatments. This indicates the presence of carboxylic groups from the stearic acid molecules on the membrane surface. The region between 1000 and 1200 cm^{-1} shows broadening, which might be linked to interactions between stearic acid and the hydroxyl or polysaccharide components of the membrane (Larkin & Jackson, 2024; Sinha et al., 2017). Among the modified samples, the membrane immersed for 90 minutes demonstrates the most pronounced spectral changes, indicating maximal modification. While longer immersion increases stearic acid deposition, excessive treatment could potentially compromise the uniformity or mechanical properties of the membrane. Overall, stearic acid modification enhances the hydrophobicity and functional properties of the fungal-derived membrane, making it suitable for applications requiring water-repellent or oil-selective materials.

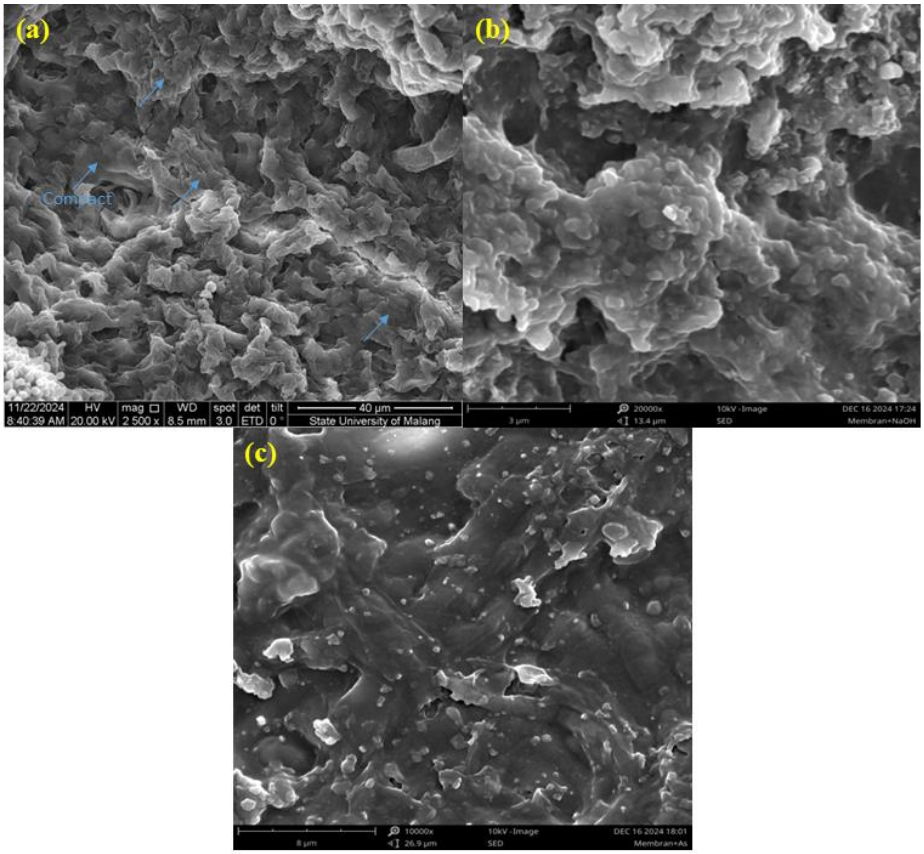


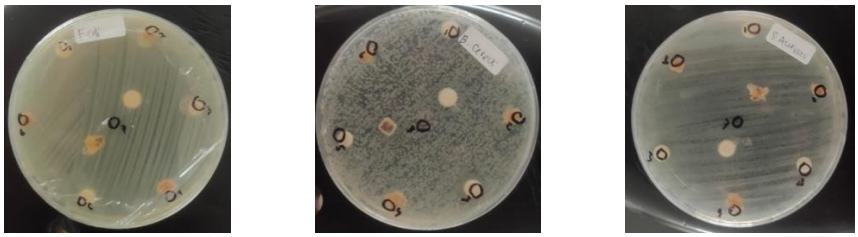
Figure 4. SEM photographs of the unmodified fungal-derived membrane (a), hydroxylated fungal-derived membrane (b), and stearic acid-coated fungal-derived membrane (c)

The structural and morphological changes in the fungal-derived membranes after chemical modification were further examined using Scanning Electron Microscopy (SEM) (Figure 4). SEM images of the unmodified membrane (Figure 4a) reveal a relatively smooth and compact surface, indicative of a dense network of fungal hyphae formed during the 4-week incubation period. The intact and interconnected mycelial structure suggests good membrane integrity and flexibility, with minimal porosity visible at the micrometer scale. After NaOH

treatment (Figure 4b), significant changes in surface texture are observed. The deacetylated membranes display a rougher and more fibrous morphology, with noticeable fragmentation and swelling of the hyphal walls. These morphological alterations are likely due to partial hydrolysis and disruption of the chitin structure during deacetylation, which leads to the formation of chitosan and a more open network. The increased surface roughness may enhance the membrane's surface reactivity and could contribute to improved antimicrobial activity, as previously reported for chitosan-rich materials (Goel & Bano, 2025; Ke et al., 2021). Further modification with stearic acid coating (Figure 4c) results in a distinct change in surface appearance. The stearic acid-treated membranes exhibit a more granular and uneven surface, consistent with the deposition of a hydrophobic fatty acid layer. The coated membrane appears to have a continuous, textured surface that covers the underlying mycelial structure. With increasing treatment time, more extensive surface coverage is evident, which correlates with FTIR findings of enhanced C–H and C=O vibrational peaks. This surface modification is expected to enhance water repellency and reduce microbial attachment by creating a low-energy surface barrier (Prudnikov et al., 2023; Wu et al., 2021). The combination of FTIR and SEM results confirms that the fungal-derived membranes underwent successful chemical and morphological transformations after NaOH and stearic acid treatments. These modifications not only alter the chemical composition but also significantly influence the surface architecture, which is critical for tailoring the membrane for specific applications such as antimicrobial filtration and or oil–water separation.

Antibacterial activity test

The antibacterial properties of the unmodified and modified membranes were evaluated in this study. The goal was to assess the effectiveness of the membrane modification in preventing bacterial contamination in water, which is prone to bacterial growth and can lead to biofouling or membrane surface clogging. This step was considered crucial to ensure that the fungal-derived membranes used in water purification processes could inhibit or even kill pathogenic bacteria, making the water produced safer for use. The bacterial species used in this study included *E. coli*, *B. cereus*, and *S. aureus*. The results of the antibacterial testing for unmodified membranes are shown in Figure 5.



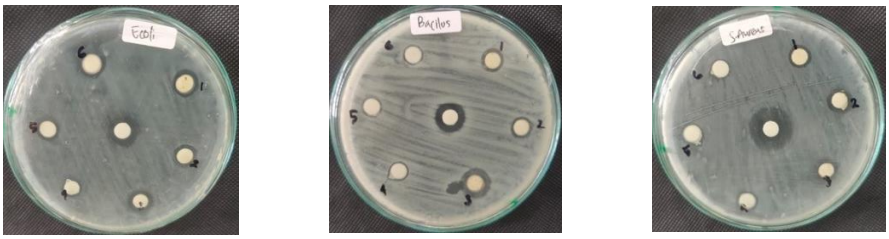
a B C

Figure 5. Antibacterial properties of fungal-derived membranes with *E. coli (a)*, *B. cereus (b)*, and *S. aureus* (c) bacteria

The results presented in Figure 5 show that no inhibition zones were formed around the membrane, indicating that the membrane derived from *C. caperata* mycelium did not exhibit antibacterial properties. Inhibition zones typically form when the material demonstrates antimicrobial activity, as bacterial growth around the membrane is suppressed. The absence of these zones suggests that the *C.*

caperata mycelium membrane was ineffective in preventing the growth of the tested bacteria, lacking significant antibacterial activity. In contrast, the modified membranes appeared to exhibit antibacterial properties that varied depending on the type of modification and the treatment duration (Figure 6).

The positive control (center of the plate) showed the highest inhibition zone for all three types of bacteria, indicating significant bacterial sensitivity to chloramphenicol (Figure 6). The inhibition zones for the control against *E. coli*, *B. cereus*, and *S. aureus* were 11.36, 9.44, and 14.84, respectively (Table 1).



a B C

Figure 6. Antibacterial properties of modified fungal-derived membranes with *E. coli (a)*, *B. cereus (b)*, and *S. aureus* (c) bacteria

Table 1. Diameter of the inhibition zone of the modified membrane against *E. coli*, *B. cereus*, and *S. aureus* bacteria

No. Sample (Figure 5)	Treatment	Inhibition Zone Diameter (mm)		
		E. coli	***B. cereus***	***S. aureus***
	Positive control	11.36	9.44	14.84
1	Deacetylation (30 min)	8.84	7.42	7
2	Deacetylation (60 min)	9.2	6.48	9.76
3	Deacetylation (90 min)	9.82	9.88	9.24
4	Coating (60 min)	6.24	5.68	5.88
5	Coating (120 min)	6.56	3.92	5
6	Coating (180 min)	6.82	3.92	2.32

As previously explained, deacetylation is a chemical modification that removes acetyl groups from chitin in fungal-derived membranes, converting it into chitosan, a well-known antibacterial agent. The results demonstrated a progressive increase in the inhibition zones as the deacetylation time was extended, indicating that the deacetylation method successfully enhanced the antibacterial activity of the membrane. For instance, after 90 minutes of deacetylation, the inhibition zones for *E. coli*, *B. cereus*, and *S. aureus* were 9.82, 9.88, and 9.24 mm, respectively, showing significant improvement compared to shorter treatment times. Specifically, for *B. cereus*, the inhibition zone exceeded that of the control after 90 minutes of deacetylation, suggesting that this bacterium was particularly responsive to prolonged deacetylation. Previous studies have also supported these findings, indicating that deacetylated fungal-derived membranes show enhanced antibacterial properties due to the increased exposure of chitosan on the membrane surface. Chitosan can disrupt bacterial cell membranes, leading to cell death, particularly in gram-positive bacteria such as *B. cereus* and *S. aureus*, which are more vulnerable to positively charged chitosan molecules. According to Aider et al. (2010), deacetylation to a certain degree optimizes antibacterial activity, which aligns with the observation

that 90 minutes of deacetylation resulted in optimal inhibition zones for the fungal-derived membrane s (Aider, 2010).

On the other hand, surface coating with stearic acid showed smaller inhibition zones, with a noticeable decrease as the coating duration increased. After coating for 180 minutes, the inhibition zones for *E. coli*, *B. cereus*, and *S. aureus* were 6.82, 3.92, and 2.32 mm, respectively. This trend suggests that prolonged exposure to stearic acid could inhibit antibacterial properties. This may be due to the hydrophobic nature of stearic acid, which creates a barrier that limits direct interaction between the membrane and bacterial cells. These findings are consistent with the previous research, which found that hydrophobic coatings on membrane surfaces reduce direct contact with bacteria, thereby decreasing their antibacterial effects (Rasitha. et al., 2022; Wu et al., 2021). The variation in bacterial response also highlights the importance of tailoring modification strategies to target specific microorganisms. *S. aureus* exhibited the most significant reduction in inhibition with increasing stearic acid coating time, from 2.94 mm at 60 minutes to 1.16 mm at 180 minutes. This is likely due to the structural sensitivity of *S. aureus* to the hydrophobic barrier, which restricts nutrient and ion exchange on the cell membrane. These findings underscore the importance of tailoring membrane modifications to optimize antibacterial performance. Deacetylation emerged as the most promising approach for enhancing fungal-derived membrane antimicrobial properties, while stearic acid coating may be better suited for applications where hydrophobicity is required, but antibacterial properties are less critical.

Conclusions

This study investigated the surface modification of *Cerrena caperata* fungal-derived membranes to enhance their antibacterial properties, a persistent challenge in membrane filtration systems. Two modification strategies were employed: deacetylation with 50% NaOH and coating with 1% stearic acid. Deacetylation for 90 minutes notably increased antibacterial activity, resulting in inhibition zones of up to 9.88 mm against *B. cereus*, 9.82 mm against *E. coli*, and 9.24 mm against *S. aureus*. This enhancement is attributed to the conversion of chitin to chitosan, which introduces amino groups known for their antimicrobial activity. In contrast, stearic acid coatings increased hydrophobicity but led to a decline in antibacterial performance with prolonged exposure, with inhibition zones decreasing to below 2.32 mm, indicating a trade-off between surface hydrophobicity and antimicrobial efficacy. The deacetylation approach demonstrated superior potential for applications in water filtration systems where biofouling resistance is critical. Meanwhile, stearic acid coating may be more suitable for systems prioritizing hydrophobic surfaces with moderate antimicrobial requirements. However, this study has certain limitations. The long-term stability of the modified membranes under operational conditions and their effectiveness against a broader spectrum of contaminants were not evaluated. Future research should explore these aspects, as well as the mechanical properties and filtration efficiency of the membranes under dynamic flow conditions. Additionally, scaling up the membrane fabrication process and assessing its economic viability would be valuable steps toward practical implementation in sustainable water treatment technologies.

Author Contributions

RA conceptualized the study, designed the experiments, conducted the experiments, and supervised the research. **YY**, **RMI**, **EPT**, and **RAP** assisted in experimental work, data interpretation, and contributed to manuscript drafting.

EJK collected data. **MRANI** provided technical support, contributed to data analysis, and revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript. The final version of the manuscript was read and approved by all authors.

Conflict of Interest

The authors have declared that there is no conflict of interest.

Acknowledgment

The authors would like to express their gratitude to the technical staff of the Biomedical Laboratory, Faculty of Medicine, Universitas Palangka Raya, for their valuable assistance during the research process, and to those who supported proofreading and data management.

Funding

This research was funded by the Institute for Research and Community Service of Universitas Palangka Raya, under grant number 7024/UN24/KP/2024.

References

Adhiksana, A., Wahyudi, W., Arifin, Z., & Irwan, M. (2023). Effect of Microwave Irradiation Time To Deacetylation Process of Chitin From Shrimp Shells. *Jurnal Kimia Riset*, *8*(1), 1–7. https://doi.org/10.20473/jkr.v8i1.40222

Agnestisia, R., Panjaitan, D., Erdene-ochir, T., & Nion, Y. A. (2024). Decolorization of Rhodamine B by the white rot fungus *Coriolopsis caperata* isolated from Central Kalimantan Forest. *BIO Web of Conferences*, *134*, 06011.

Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry: Review. *Lwt*, *43*(6), 837–842. https://doi.org/10.1016/j.lwt.2010.01.021

Alnumani, A., Abutaleb, A., Park, B., & Mubashir, M. (2024). Recent advancement on water filtration membranes: Navigating biofouling challenges. *Environmental Research*, *251*(1), 118615. https://doi.org/10.1016/j.envres.2024.118615

Baker, J. S., & Dudley, L. Y. (1999). Biofouling in membrane systems - a review. *Water Supply*, *17*(1), 173–181. https://doi.org/10.1016/S0011-9164(98)00091-5

Bayramoğlu, G., Çelik, G., Yalçin, E., Yilmaz, M., & Arica, M. Y. (2005). Modification of surface properties of Lentinus sajor-caju mycelia by physical and chemical methods: Evaluation of their Cr6+ removal efficiencies from aqueous medium. *Journal of Hazardous Materials*, *119*(1–3), 219–229. https://doi.org/10.1016/j.jhazmat.2004.12.022

Boudouaia, N., Bengharez, Z., & Jellali, S. (2019). Preparation and characterization of chitosan extracted from shrimp shells waste and chitosan film: application for Eriochrome black T removal from aqueous solutions. *Applied Water Science*, *9*(4), 1–12. https://doi.org/10.1007/s13201-019-0967-z

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., Del Valle, R. G., Carballeira, N. M., & Sanabria-Ríos, D. J. (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*, *82*, 101093. https://doi.org/10.1016/j.plipres.2021.101093

Egorov, A. R., Kirichuk, A. A., Rubanik, V. V., Rubanik, V. V., Tskhovrebov,

A. G., & Kritchenkov, A. S. (2023). Chitosan and Its Derivatives: Preparation and Antibacterial Properties. *Materials*, *16*, 6076. https://doi.org/https://doi.org/10.3390/ma16186076

Elsoud, M. M. A., & Kady, E. M. El. (2019). Current trends in fungal biosynthesis of chitin and chitosan. *Bulletin of the National Research Centre*, *43*(1), 59. https://doi.org/https://doi.org/10.1186/s42269-019-0105-y

French, V., Kleiman, M., Bhalla, K., Moreira, R., Jalaee, A., Kronstad, J. W., & Foster, E. J. (2024). Fungal-derived adsorption membrane to capture potentially toxic elements. *Chemical Engineering Journal*, *488*, 151028. https://doi.org/10.1016/j.cej.2024.151028

Goel, S., & Bano, Y. (2025). Chapter 13 - Chitosan-based nanofibrous membranes for antibacterial filter applications. In *Antimicrobial Materials and Coatings* (pp. 425–447). https://doi.org/https://doi.org/10.1016/B978-0-323-95460-0.00013-7

Gupta, B. S. (2011). Characterization of wood mould fungi by FTIR – A valuable step for prediction of initiation of decay. *12th DBMC International Conference on Durability of Building Materials and Components*, *3*, 1019–1027.

Hasan, S., Boddu, V. M., Viswanath, D. S., & Ghosh, T. K. (2022). *The Structural Difference Between Chitin and Chitosan* (pp. 79–102). Springer, Cham. https://doi.org/10.1007/978-3-031-01229-7_4

Hassan, M. A., Omer, A. M., Abbas, E., Baset, W. M. A., & Tamer, T. M. (2018). Preparation, physicochemical characterization and antimicrobial activities of novel two phenolic chitosan Schiff base derivatives. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-29650-w

Iman, H. N., Susilo, H., Satriyatama, A., Budi, I. D. M., Kurnia, K. A., Wenten, I. G., & Khoiruddin, K. (2024). Separation properties and fouling resistance of polyethersulfone membrane modified by fungal chitosan. *BMC Chemistry*, *18*(1). https://doi.org/10.1186/s13065-024-01341-w

Isik, Z., Arikan, E. B., Bouras, H. D., & Dizge, N. (2019). Bioactive ultrafiltration membrane manufactured from *Aspergillus carbonarius* M333 filamentous fungi for treatment of real textile wastewater. *Bioresource Technology Reports*, *5*, 212–219. https://doi.org/10.1016/j.biteb.2019.01.020

Islam, M., Masum, S., Rayhan, K., & Haque, Z. (2011). Antibacterial Activity of Crab-Chitosan against Staphylococcus aureus and *Escherichia coli*. *Journal of Advaced Scientific Research*, *2*(4), 63–66. doi: 10.4315/0362-028x-62.3.239

Ke, C. L., Deng, F. S., Chuang, C. Y., & Lin, C. H. (2021). Antimicrobial actions and applications of Chitosan. *Polymers*, *13*(6). https://doi.org/10.3390/polym13060904

Kumirska, J., Czerwicka, M., Kaczyński, Z., Bychowska, A., Brzozowski, K., Thöming, J., & Stepnowski, P. (2010). Application of spectroscopic methods for structural analysis of chitin and chitosan. *Marine Drugs*, *8*(5), 1567–1636. https://doi.org/10.3390/md8051567

Larkin, P. J., & Jackson, A. (2024). Interpretation of the Infrared Spectra of Metal-Stearate Salts. *Applied Spectroscopy Practica*, *2*(2), 1–10. https://doi.org/10.1177/2755185724 1253834

Lozano, K. P. C., Mendes, J. F., Ferreira,

L. F., Martins, M. A., Mendoza, J. S., & Mendes, R. F. (2024). Hydrophobic bio-composites of stearic acid starch esters and micro fibrillated cellulose processed by extrusion. *Industrial Crops and Products*, *221*, 119313. https://doi.org/10.1016/j.indcrop.2024.119313

Manurung, T. W., Beladona, S. U. M., Hakim, M. S., Tesalonika, L., Al-Hadi, R., & Iqba, R. M. (2024). Surcafe Modification Of Fly Ash From Asam-Asam Coal Power Plant Using Stearic Acid As Hydrophobic Inorganic Material. *Jurnal Kimia Riset*, *9*(1), 59–68. https://doi.org/10.20473/jkr.v9i1.57370

Nie, M., Luo, J., Xiao, M., Chen, J., Bao, K., Zhang, W., Chen, J., & Li, B. (2007). Structural differences between Fusarium strains investigated by FT-IR spectroscopy. *Biochemistry (Moscow)*, *72*(1), 61–67. https://doi.org/10.1134/S0006297907010075

Prudnikov, E., Polishchuk, I., Sand, A., Hamad, H. A., Massad-Ivanir, N., Segal, E., & Pokroy, B. (2023). Self-assembled fatty acid crystalline coatings display superhydrophobic antimicrobial properties. *Materials Today Bio*, *18*, 2024. https://doi.org/10.1016/j.mtbio.2022.100516

Raman, N. M., & Ramasamy, S. (2017). Genetic validation and spectroscopic detailing of DHN-melanin extracted from an environmental fungus. *Biochemistry and Biophysics Reports*, *12*, 98–107. https://doi.org/10.1016/j.bbrep.2017.08.008

Rasitha., T. P., Sofia., S., Anandkumar, B., & Philip, J. (2022). Long term antifouling performance of superhydrophobic surfaces in seawater environment: Effect of substrate material, hierarchical surface feature and surface chemistry. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *647*, 129194. https://doi.org/10.1016/j.colsurfa.2022.129194

Rehman, H. U., Cord-Landwehr, S., Shapaval, V., Dzurendova, S., Kohler, A., Moerschbacher, B. M., & Zimmermann, B. (2023). High-throughput vibrational spectroscopy methods for determination of degree of acetylation for chitin and chitosan. *Carbohydrate Polymers*, *302*, 2024. https://doi.org/10.1016/j.carbpol.2022.120428

Sinha, N., Singh, B. K., & Dutta, P. K. (2017). Research on antibacterial screening & drug delivery using chitosan-stearic acid derivative. *Journal of Polymer Materials*, *34*(1), 11–20.

Svecova, L., Spanelova, M., Kubal, M., & Guibal, E. (2006). Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies. *Separation and Purification Technology*, *52*(1), 142–153. https://doi.org/10.1016/j.seppur.2006.03.024

Wang, M., Wang, Y., Chen, G., Gao, H., & Peng, Q. (2024). Chitosan-Based Multifunctional Biomaterials as Active Agents or Delivery Systems for Antibacterial Therapy. *Bioengineering*, *11*(12). https://doi.org/10.3390/bioengineering11121278

Wang, S., Xu, J., Zhang, J., Lyu, Y., & Chen, S. (2024). Facile synthesis of natural stearic acid modified carbon adsorbent for toluene adsorption under high humidity conditions. *Separation and Purification Technology*, *344*, 127269. https://doi.org/10.1016/j.seppur.2024.127269

Wu, Y., Wu, X., Yang, F., Gan, J., & Jia,

H. (2021). The preparation of cotton fabric with super-hydrophobicity and antibacterial properties by the modification of the stearic acid. *Journal of Applied Polymer Science*, *138*(30), 1–9. https://doi.org/10.1002/app.50717