Nanocomposite Polymeric Membranes with Bio-Graphene and Enzymes for the Sustainable Mitigation of Biofouling

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Abstract In this study, nanocomposite polyethersulfone (PES) membranes were engineered to reduce biofouling during wastewater treatment. Green bio-graphene (bG) was synthesized via liquid-phase exfoliation of graphite in the presence of chitosan, enhancing its antimicrobial properties. Three antimicrobial agents—bG, α-amylase, and lysozyme—were incorporated into the PES casting solution, and the process was optimized using the phase inversion method to maximize antimicrobial efficacy.

The biocatalytic performance of the optimized membranes was then evaluated. Enzyme reusability tests showed that α-amylase retained approximately 42% of its activity after eight cycles, while lysozyme activity significantly declined. Storage stability tests revealed that the membranes maintained over 60% of their catalytic activity after three weeks and exhibited strong antibacterial activity for up to two weeks.

The biocatalytic nanocomposite membranes exhibited higher anti-biofouling and antimicrobial performance than the neat PES membrane, ensuring stable performance in challenging wastewater environments.

Keywords: Membranes, biofouling, wastewater, nanocomposite membranes,

1. Introduction

Ultrafiltration (UF) membranes play a vital role in wastewater treatment by efficiently removing bacteria and high-molecular-weight organic compounds, ensuring the continuous recycling and supply of fresh water (Wang et al., 2022). However, their hydrophobic nature constitutes a natural barrier that promotes organic fouling and biofilm formation, collectively known as biofouling. This often reduces ultrafiltration performance and shortens the operational life of the membranes (Kaneda et al., 2019)

In this work, PES neat and nanocomposite membranes with antibacterial activity were prepared. The amounts of three antibacterial agents, bG prepared with chitosan, lysozyme, and α-amylase (PES/bG/AL), were optimized to determine the ideal concentrations for the ultrafiltration membrane casting solution by evaluating their antimicrobial activity. The nanocomposite PES membranes were prepared by embedding the anti-foulants in the PES casting solution, followed by membrane formation using the phase inversion method. Various microscopic and spectroscopic techniques were used to characterize the resulting bG, as well as the neat and nanocomposite PES membranes. In the next step, the fabricated membranes were evaluated for their hydrophilicity, permeation flux, and antifouling performance. Finally, membrane operation was evaluated in the presence of synthetic wastewater containing E. coli to assess fouling behavior, treatment efficiency, and antibacterial activity under simulated operational conditions

2. Results and Discussion

2.1 Optimization of PES nanocomposite membranes fabrication

The membrane fabrication parameters were optimized to obtain the best antimicrobial performance of the PES nanocomposite membranes. Firstly, the antimicrobial experiments were conducted against C. glutamicum, which is a model Gram-positive bacterium. Its simplified cell structure allowed for a clearer evaluation of the antimicrobial efficacy of the membranes. Three critical parameters, including the concentration of bG, lysozyme, and α-amylase, were evaluated. The antibacterial activity of the membranes was also evaluated against E. coli cells to investigate their performance against Gram-negative bacteria. The neat PES membrane did not exhibit antibacterial activity, allowing bacterial growth on its surface.

2.2. Microscopic and spectroscopic characterization of membranes

The ATR-FTIR spectrum of PES membranes exhibits characteristic absorption bands that reflect their aromatic and sulfone-containing polymer structure. SEM and EDS were employed to study the morphology and chemical composition of the PES, PES/bG, and PES/bG/AL membranes. The porous network of the neat PES membrane, displaying a uniform pore size distribution. Upon incorporation of bG, amylase, and lysozyme, the nanocomposite membranes retained their porous structure. The bG layers were relatively homogeneously dispersed as exfoliated nanosheets on the membrane. The EDS analysis and mappings of the membranes revealed their chemical composition and element distribution.The addition of enzymes increased the N content of the nanocomposite PES/bG/AL membrane from 1.33% to 4.78%, confirming the successful incorporation of the enzymes into the membrane

2.3 Membrane organic fouling test

The normalized flux (J/J0) values decreased drastically after the first cycle of BSA filtration. It can be speculated that such a decline may be caused by the formation of a fouling layer or pore blockage due to protein deposition on membranes. After three fouling cycles, the pure PES membrane exhibited lower normalized permeate flux (J/J0) values than nanocomposite membranes ($p<0.01$, at 180 min). This improvement may be attributed to the incorporation of bG/AL into the polymer matrix, reinforcing the membrane's resistance to fouling.

Nanocomposite membranes present an elevated flux recovery rate compared to the neat PES membrane in all cycles, suggesting that fewer BSA molecules strongly adhered to the surface of these systems, allowing efficient permeability

2.4 Membrane bio-fouling test

A biofouling test was conducted by employing synthetic wastewater and E. coli as the feed solution. The permeate fluxes of both PES and PES/bG/AL membranes significantly decreased at the beginning of the wastewater effluent filtration and then gradually stabilized.

Additionally, live cell quantification on the membrane surfaces after filtration was conducted through the plate counting method. The PES/bG/AL membrane maintained high antimicrobial properties over the long-term filtration process, with 71.56% of E. coli bacteria surviving on its surface compared to the PES membrane, where no antimicrobial activity was recorded.

A static experiment was also conducted for a 24-hour incubation period for the two bacterial strains. In the case of PES/bG/AL, a severe decline in viability (~15% viability for E. coli and ~6% viability for C. glutamicum) is observed after long incubation time, while the neat PES membrane did not contribute to bacterial cell death. The prolonged exposure to antimicrobial agents enhances lethality due to possible cumulative damage and progressive cellular dysfunction, improving long-term membrane performance and reducing the risk of persistent fouling

3. Conclusions

A green-synthesized nanomaterial, bio-graphene combined with chitosan, was incorporated into the membrane casting solution alongside two hydrolytic enzymes, lysozyme and α-amylase, to develop biocatalytic membranes. Although the encapsulation of these agents decreased the pore size and pure water flux of the nanocomposite membranes, it significantly improved their hydrophilicity and storage stability, prioritizing long-term operational stability and water quality over maximal permeability. Despite their reduced initial permeability, the PES/bG/AL membranes maintain their flux over time, reflecting enhanced resistance to fouling and pore blockage compared to PES neat membranes. Finally, the membrane performance was evaluated by conducting filtration experiments using synthetic wastewater with E. coli designed to mimic the composition of real fouling conditions. The PES/bG/AL nanocomposite membrane's permeability and fouling resistance were superior to the pristine PES membranes. Moreover, the increased antibacterial efficiency after 24 h of incubation underscores its potential for self-cleaning and increased lifespan of the membrane. The developed nanocomposite membranes offer a sustainable alternative to conventional chemical cleaning methods, aligning with environmentally friendly regulations. Finally, these improvements highlight the effectiveness of ultrafiltration membrane surface modification in overcoming the limitations of the PES membrane, making them a more promising candidate for wastewater treatment applications.

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