

# Reduction of Bacterial Strains in Polluted Surface Water by Modified Chitosan

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**Abstract**— The purpose of this study was to investigate the efficacy of modified chitosan (CS) composites in inhibiting the proliferation of *Escherichia coli* (*E. coli*), and Fecal Coliforms in contaminated water. The antibacterial properties of the membranes were enhanced by progressively increasing the concentration of graphene oxide-silver nanoparticles (GO-AgNP) doped into the CS, which was achieved by preventing the growth and multiplication of the identified microorganisms. The modified CS exhibited enhanced antimicrobial activity as a result of the aggregated action of numerous antibacterial mechanisms demonstrated by each component in the CS-AgNP-GO composite. Moreover, the chemical structure and physical properties of chitosan were enhanced by a higher concentration of dimethyl acetamide (DMAc) in the membrane formulation, which was achieved by increasing the number of participating amino and hydroxyl chains responsible for hydrogen bonding within the CS-GO-AgNP composite. The surface morphology assessment indicated greater dispersion of AgNPs with an increase in the concentration of GO in the CS matrix. The highest membrane performance was attained at 78.5% CS, 21.5% DMAc, and 1.3 ml of GO/AgNP. This resulted in the inactivation of 96% of fecal and total coliforms, as well as full prevention of 99.9% of *E. coli* bacterial cells.

**Keywords**— Modified-Chitosan, DMAc, GO-AgNP, *E. coli*, Coliforms, filtration membranes

## I. INTRODUCTION

Access to pure drinkable water is essential for the well-being of individuals. The utilization of potable water of exceptional quality may provide substantial health benefits. However, microbial pollution and proliferation in surface water have been a significant social issue and a health hazard for a long time. The primary source of fecal bacteria, including pathogens, in surface water is the discharge of human and animal refuse. In turn, the development of numerous waterborne disorders has been facilitated by the presence of microorganisms. The digestive systems of warm-blooded animals are the source of bacteria in drinkable water. Pets, animals, and pollution from congested, malfunctioning, or poorly planned waste treatment facilities, such as private septic systems and sewage conduits, are among the causes. According to the World Health Organization, the preservation of public health necessitates the regular monitoring of fecal contamination in surface water [1]. Fecal indicator bacteria (FIB), including *E. coli*, Fecal

coliforms, and Total coliforms, have been employed for a long time to evaluate surface water fecal contamination.

In numerous industries, such as food, textiles, biomedicine, wastewater treatment, and cosmetics, chitosan (CS) is the most prevalent biopolymer. It is characterized by a strong antibacterial effect and a remarkable lack of toxicity [2]. However, its structural instability and low strength restrict its practical application as a membrane material. To overcome this limitation, people frequently modify the physicochemical properties of chitosan. A common substance employed to enhance the strength of chitosan (CS) is graphene oxide (GO). GO improves the mechanical strength, stability, and antibacterial properties of CS by providing a wide surface area, high adsorption capacity, improved mechanical strength, and antibacterial activity when used as a material for industrial polymer membranes [3].

Dimethylacetamide (DMAc), a polar aprotic solvent, is frequently employed in the synthesis of polymers to enhance their mechanical properties and permeability [4]. DMAc has been demonstrated to improve polymer membranes in numerous studies. For instance, it was examined the influence of DMF and DMAc solvents on the electrical and thermal properties of PVDF-CNF composites [5]. In terms of the dispersion of the polymer composite matrix, DMAc outperformed DMF, as indicated by the results.

This work delineates the utilization of customized 3D printed CS membranes to remove fecal coliforms, total coliforms, and *E. coli* bacterial strains from contaminated surface water. In order to enhance the mechanical and antibacterial properties of the modified Chitosan nanocomposites, AgNP-GO and DMAc were included, respectively.

## II. METHODS

### A. Preparation and printing of modified CS composites

Graphene oxide (GO) was synthesized from graphite particles using a modified Hummer and Offeman technique, while silver nitrate ( $\text{AgNO}_3$ ) was reduced with trisodium citrate ( $\text{C}_6\text{H}_8\text{O}_7\text{Na}$ ) in the presence of GO solution to produce AgNP-GO nanocomposites. Chitosan composite was synthesized using 1% aqueous solution of acetic acid (HAc). The mixture was agitated for 60 minutes. The mixture was treated with 0.5 mL of 2% (w/v) NaOH to neutralize the HAc. After an hour of agitation, 80 ml of CS solution was combined with 0.5

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milliliters of a 10 w/w% AgNP-GO aqueous solution. The modified CS composite was later printed in an A1630 UV-type flatbed inkjet printer (Colorsun China) equipped with an Epson L805 drop-on-demand piezoelectric inkjet print head, two UV LED lamps, and AcroRIP White ver9.0 printing software.

### B. Antibacterial analysis

The antibacterial performance of CS-GO-AgNP was accessed to ascertain its inhibitory capability against fecal coliforms according to established methods [6]. The Colilert-18 (QuantiTray/2000) approach was used to identify the total coliform and *E. coli* bacteria in accordance with the manufacturer's guidelines.

## III. RESULTS AND DISCUSSION

### A. SEM Analysis of the Modified CS Composite

Scanning electron microscopy (SEM) was employed to investigate the surface morphologies of modified chitosan membrane composite films, as illustrated in Figure 1.

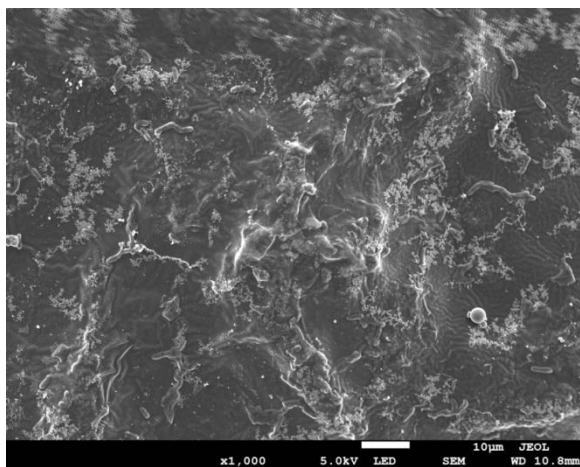


Fig. 1: SEM morphology of modified CS composite

The film's unique sandwich structural networks resulted in surface rippling as the concentrations of AgNP and GO increased. According to previous reports, this wrinkle characteristic prevents the membrane's structural network from disintegrating and maintains a substantial membrane surface area [49]. This result may be attributed to the reaction between OH- and amid groups from CS-DMAc, which has the possibility of forming H-bonds with the carboxyl, carbonyl, and hydroxyl functionalities in the AgNP-CS-GO composite sheets [7].

The modified CS inconsistent layer thickness in the sample's structural morphology suggests that it was not manufactured consistently. This could indicate the presence of numerous phases of particle generation that are the result of layer-by-layer interfacial polymerization during the 3D printing process. A study has demonstrated that 3D printed membranes have variable surface roughness, which enhances transport efficiency in contrast to ordinary membranes, which typically have smooth surface morphologies [8]. Additionally, the amide bonds that form between DMAc and CS molecules may

exacerbate intermolecular forces within the modified CS matrix, which could potentially alter its original structure as a result of the increased electrostatic interactions between the amide groups of the DMAc-CS configuration and the epoxy group of GO. AgNP's interaction with bacterial strains of *E. coli* and the coliforms may be enhanced by this interaction, which may also prevent AgNP from aggregating in the modified CS composite membranes.

### B. Quantification of bacterial analysis

The quantification of microbial analysis is summarized in Table 1 to display the quantity of bacterial cells identified during the microbiological examination of the modified CS membranes. The nanocomposite samples exhibited diminished levels of the investigated microorganisms, as indicated by the results. This trend implies that the inhibitory properties of the membrane samples were enhanced as the concentration of GO-AgNP-DMAc increased.

TABLE I: QUANTIFICATION OF BACTERIAL ANALYSIS

Sample	S1	S2	S3	S4	Saline	Posit. Contr.
<i>E. coli</i> conc. (MPN/100ml)	52.9±0.6	38.9±0.3	30.9±0.5	0.0±0.04	0.0 ±0.02	>2420 ±0.2
Total Coliform (MPN/100ml)	55.6±0.5	43.2±0.4	32.7±0.2	1±0.03	0.0 ±0.01	>2420 ±0.4
Fecal Coliform (CFU/100ml)	700±1.2	300 ±0.9	200 ±0.5	120 ±0.3	0 ±0.03	>1000 0 ±0.6

**Note:**S1(85%CS:15%DMAc/0.3mlGO/AgNP); S2(83%CS:17%DMAc/0.6mlGO/AgNP); S3(81%CS:19%DMAc/0.9mlGO/AgNP); S4(75%CS:21%DMAc/1.2mlGO/AgNP)

The microbial activity decreased between samples S1 and S4, with *E. coli* exhibiting the most suppression in the study. CS layer augmentation may serve as a deterrent to chitosan-microbial interactions. The antibacterial efficacy of samples S1 and S2, which contained a higher concentration of CS than composites S3 and S4, may have been diminished as a consequence.

Figure 2 shows the combination of the three bacterial strains that were investigated in this investigation. The CS nanocomposites' inadequate antibacterial activity against strains of *E. coli*, Fecal coliforms, and Total coliforms is demonstrated in sample S1, which follows samples S2 and S3. Sample S4 illustrates the most effective CS composites against the three bacterial species.

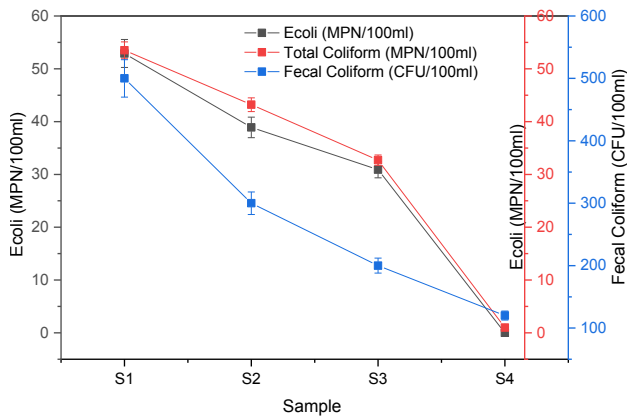


Fig. 2: Antibacterial analysis of modified CS composites

C. Comparative antibacterial analysis

A comparison study was conducted to assess the antibacterial efficacy of each composite material that was employed in the development of modified CS membranes. Figure 3 illustrates the average *E. coli* and Total coliform concentrations in the filtrate samples of CS, CS-GO, CS-AgNP, and CS-GO-AgNP membranes following a 24-hour incubation period.

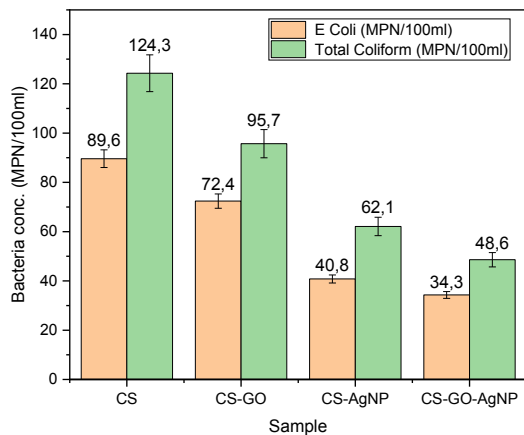


Fig. 3: Antibacterial statistical data of modified CS components

The CS composite exhibited the highest number of bacterial cells ( $89.6 \pm 0.32$  MPN/100 mL) during the incubation period in comparison to other membrane components, indicating that CS exhibited minimal inhibition of bacterial activity. This outcome is in accordance with prior research that demonstrated the minimal antibacterial activity of CS membranes [9]. The antibacterial activity of the CS matrix was enhanced by the addition of GO and AgNP, as evidenced by the decreased concentration levels of *E. coli* and Total coliforms.

Nevertheless, the CS-AgNP nanocomposite exhibited superior antibacterial activity in comparison to CS-GO. This implies that AgNP may enhance antibacterial efficacy more than GO when combined with a specific quantity of CS. This is due to the fact that the CS membrane network facilitates the

immobilization of Ag ions, which in turn enhances the contact killing of microorganisms, as evidenced by prior research [10]. Moreover, the antibacterial activity of the modified CS composite may have been improved by the interaction between AgNP and CS. The concentration of *E. coli* decreases swiftly in comparison to the concentration of Total coliform, as evidenced by the statistical data presented in Figure 3. The modified CS membrane network is more attractive to Gram-negative bacteria cells due to the increased concentration of Ag-GO, which results in their rapid inactivation. By reducing the quantities of *E. coli* and Total coliform strains from 40.8 to 34.3 MPN/100 mL and from 62.1 to 48.6 MPN/100 mL, respectively, the CS-GO-AgNP composites exhibited their potent antibacterial effect. Consequently, the comparative results corroborated the findings in the literature by demonstrating that the CS-GO-AgNP composite membrane exhibited the most significant synergistic effects when compared to its individual components [11]. Consequently, the integrated antibacterial activities of the membrane components enhanced the performance of the CS composite samples.

D. Mechanism of bacteria strains inactivation

In order to demonstrate the antimicrobial properties of each membrane component, the antibacterial mechanisms of the modified CS membranes are proposed as shown in Figure 4. Phase 1 involved exposing the CS compound to all viable bacterial cells in the contaminated water. However, the diminished antibacterial activity of CS allowed it to eliminate only a small number of the microorganisms' cell membranes that were attached to its surface. This could be due to the high porosity, limited mechanical strength, and instability of CS. As a result of exposure to the CS-GO surface, the inactivation of bacterial cells increased significantly during the subsequent phase (Phase 2). The potential for oxidative pressure to rupture cell membranes as a consequence of these reactions might have contributed to the increase in inactivation of bacterial strains between CS and GO bonding.

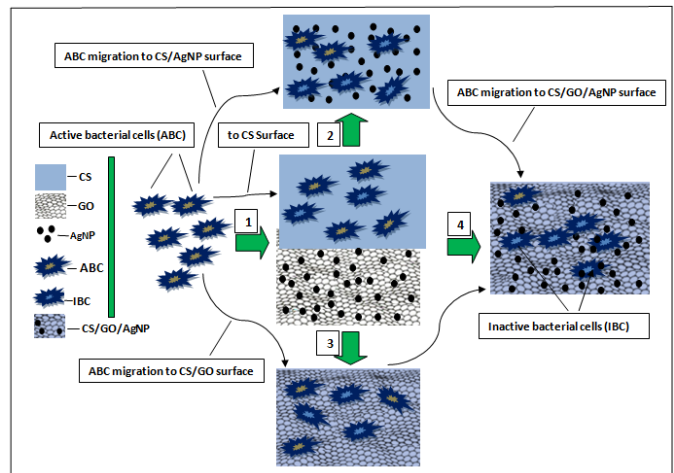


Fig. 4: Antibacterial mechanisms of the modified CS components

The incorporation of AgNP resulted to increased quantity of inactivated microorganisms, which had a more direct impact on the bacterial cells and expedited the penetration and disintegration of microbial cell walls, as demonstrated in the

Phase 3 mechanism (CS-AgNP). The antibacterial impact of the CS-AgNP-GO composite (Phase 4) was further enhanced against the microorganisms. This was due to the trapping of the negatively charged cell walls of *E. coli* and Coliforms bacteria on the surface of the more positively charged modified CS as a result of increased hydrogen bonding within the CS-GO-AgNP structure.

#### IV. CONCLUSION

The antifouling efficacy of CS/AgNP/GO membranes was dependent on the correct merging of membrane components and interaction with the BSA solute. Increased DMAc concentration and decreased CS mole ratio resulted in enhanced membrane wettability. Additionally, the membrane thermal stability was improved by the incorporation of DMAc and silver nanoparticles (AgNP) into the CS matrix, whereas the presence of more hydroxyl groups in the composite facilitated diffusion from the surface to the interior of the membrane composite via electrostatic and hydrogen bonding with water molecules.

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