Effective Disposal of Methylene Blue and Bactericidal Benefits of Using GO-Doped MnO$_2$ Nanorods Synthesized through One-Pot Synthesis

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ABSTRACT: Graphene oxide (GO)-doped MnO$_2$ nanorods loaded with 2, 4, and 6% GO were synthesized via the chemical precipitation route at room temperature. The aim of this work was to determine the catalytic and bactericidal activities of prepared nanocomposites. Structural, optical, and morphological properties as well as elemental composition of samples were investigated with advanced techniques such as X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, UV−visible (vis) spectroscopy, photoluminescence (PL), energy-dispersive spectrometry (EDS), and high-resolution transmission electron microscopy (HR-TEM). XRD measurements confirmed the monoclinic structure of MnO$_2$. Vibrational mode and rotational mode of functional groups (O−H, C=O, C−O, and Mn−O) were evaluated using FTIR results. Band gap energy and blueshift in the absorption spectra of MnO$_2$ and GO-doped MnO$_2$ were identified with UV−vis spectroscopy. Emission spectra were attained using PL spectroscopy, whereas elemental composition of prepared materials was recorded with scanning electron microscopy (SEM)-EDS. Moreover, HR-TEM micrographs of doped and undoped MnO$_2$ revealed elongated nanorod-like structure. Efficient degradation of methylene blue enhanced the catalytic activity in the presence of a reducing agent (NaBH$_4$); this was attributed to the implantation of GO on MnO$_2$ nanorods. Furthermore, substantial inhibition areas were measured for *Escherichia coli* (EC) ranging 2.10−2.85 mm and 2.50−3.15 mm at decreased and increased levels for doped MnO$_2$ nanorods and 3.05−4.25 mm and 4.20−5.15 mm for both attentions against SA, respectively. In silico molecular docking studies suggested the inhibition of FabH and DNA gyrase of *E. coli* and *Staphylococcus aureus* as a possible mechanism behind the bactericidal activity of MnO$_2$ and MnO$_2$-doped GO nanoparticles (NPs).

1. INTRODUCTION

Water is an indispensable ingredient of life on earth as well as overwhelmingly utilized in industrialization resulting in economic growth. However, industrial growth has caused water shortages while polluting and exposing it to hazardous industrial wastes (dyes, heavy metals, etc.), thus affecting its quality. The issue of water pollution is a grave one and has become one of the top-most public health concerns in the world. Harmful industrial wastes discharged from various industries mix into river streams, lakes, aquifers, oceans, and other water bodies to pollute them and make the water toxic not only for humans but for the whole biosphere.1−6 The grave damage caused to the environment by pollution is due to organic and inorganic pollutants, including long- and short-term contamination in ecosystem caused by rapid industrialization in developing countries.7 Researchers suggest that approximately 15% of dyes produced globally are directly and indirectly driven into the environment.8 Especially basic dyes such as methylene blue (MB) have more stable, non-biodegradable, aromatic molecular structures that pose a serious threat to the environment.9 To appease this grave situation, many scientists and researchers have embarked upon a journey to pursue solutions at multiple levels. In this regard, promising technological developments have occurred, including bio-based utilization of contaminants, development of different functionalized filtrations such as bio-filter, ultrafiltration, electrodialysis, aerobic treatment, adsorption,10 and as well as photocatalytic techniques to degrade the polluted materials.11−13 Adsorption technology is an efficient technique due to its use of carbon-containing materials, while it can also be used for incorporating inorganic materials as a framework. Metal nanoparticles (NPs) have been incorporated into
organic framework to breed the composite, which is then used for bactericidal and water treatment applications.\textsuperscript{9,14}

Pure MnO\textsubscript{2} has attracted a lot of interest as a host material in many oxide semiconductors because of its low cost, low toxicity, abundant availability, environmental compatibility, narrow band gap, tunnel structures, and high redox potential.\textsuperscript{15−19} Nanoparticles of MnO\textsubscript{2} are prone to agglomerate and are difficult to recycle due to their ease of suspension in water.\textsuperscript{20} The use of MnO\textsubscript{2} as a photocatalyst has become increasingly common in recent years.\textsuperscript{18} Recently, manganese-(IV) oxide has sparked a lot of interest in photocatalytic applications because of its crystallinity, large surface area, and ability to disintegrate water molecules into hydrogen and hydrogen ions.\textsuperscript{21−26} Furthermore, even at low temperatures, manganese dioxide has a high catalytic ability.\textsuperscript{27} It is reported that MnO\textsubscript{2} is one of the most feasible inorganic semiconductors for antibacterial actions due to physical and chemical properties. It has a wide range of applications in different fields such as energy storage, medicine, ion exchange, and imaging contrast.\textsuperscript{28} For the development of photoefficient composite catalysts, graphene and its derivatives such as graphene oxide (GO) are promising candidates.\textsuperscript{29} A single-atom-thick sheet of GO localizes sp\textsuperscript{3} defects within sp\textsuperscript{2}-bonded atoms of carbon in the hexagonal structure within planar two-dimensional (2D) sheets\textsuperscript{30} and is considered an effective material due to its hydroxyl and carboxyl groups. Among semiconductor materials, graphene hybridization has attained significance due to its electrical and optical properties along with a large surface area that can promote semiconductor photocatalysis\textsuperscript{31,32} and catalysis. Graphene oxide has many applications as it is used for biosensing, energy storage devices, biomedical (drug delivery, cancer therapy, and biological imaging), and water purification (filter) applications. Permeation of water through the membrane is attributed to swelling of GO structures, which enables a water penetration path between individual GO layers. It is also used in the fabrication of electronic devices.\textsuperscript{33,34} Graphene oxide has a unique structure such that it has biocompatible functional groups, i.e., hydroxyl (OH) and carboxylic (COOH), etc. Further functionalization and conjugation/immobilization of additional nanoparticles on its surface is enabled by the attached functional groups. GO plays a vital role due to its size (no. of layers and lateral dimension) and shape factor in deciding its properties that can be utilized in different fields. GO sheet thickness influences its tunable properties and different functionality.\textsuperscript{35} Due to its tunable properties, it has been used with metals and metal oxide nanoparticles to enhance the catalytic activity. Nevertheless, its exceptional chemical stability and ultrahigh thermal conductivity may be the cause of high catalytically active sites loading.\textsuperscript{36}

In this work, we perform a low-cost synthesis of MnO\textsubscript{2} and GO-doped MnO\textsubscript{2} nanorods (NRs) with various concentrations of GO (0, 2, 4, and 6%) for bactericidal and catalytic applications. We also discuss the effects of GO as a dopant on the structural, morphological, and optical properties of MnO\textsubscript{2}. Herein, the present work has proved helpful in evaluating the degradation ability of GO doped onto MnO\textsubscript{2} in the dark against a known pollutant methylene blue (MB). The novelty of this research work is that an affordable, low-temperature, sustainable, and multifunctional material (GO-MnO\textsubscript{2}) can be single-handedly used to maintain water quality by reducing/disintegrating organic toxins and preventing the bacterial cell growth. Furthermore, in silico molecular docking studies were performed against selected enzyme targets (i.e., FabH and DNA gyrase) to identify the possible mechanism behind its in vitro antibacterial activity.

2. RESULTS AND DISCUSSION

The crystallographic structures of all concerned samples were identified with X-ray diffraction (XRD), and the corresponding results are depicted in Figure 1a. The sharp diffraction peaks of MnO\textsubscript{2} NRs appeared at 2θ values of 12.8° (110), 18.2° (200), 28.8° (310), 37.6° (211), 42.1° (301), 49.8° (411), 56.3° (600), 60.33° (521), and 69.5° (541), which is in good

Figure 1. (a) XRD pattern, (b) Fourier transform infrared (FTIR) pattern, and (c–e) selected area electron diffraction (SAED) image of MnO\textsubscript{2} and various concentrations of GO-MnO\textsubscript{2}.
agreement with XRD standard card (JSPDF 44-0141) and suggested successful preparation of MnO$_2$ NRs.\textsuperscript{37,38} Compared with pristine MnO$_2$ NRs, an additional peak for all GO-doped samples was observed at $\sim$10.33$^\circ$ (002), which was attributed to the oxygen-containing groups of GO.\textsuperscript{39} The XRD patterns demonstrated that GO-MnO$_2$ NRs were successfully synthesized. The inset of Figure 1a reveals that the (211) peak position of MnO$_2$ NRs has been slightly shifted to lower 2$\theta$ values for (2, 4, and 6%) GO-doped MnO$_2$ NRs. The crystallite size of the pure sample was calculated as 36.4 nm using the Debye–Schererrer formula.

FTIR spectroscopy was used to analyze the presence of functional groups and MnO$_2$ as shown in the range of 450–400 cm$^{-1}$ in Figure 1b. Manifested peaks at 3417 and 1626 cm$^{-1}$ corresponded to O–H as shown in Figure 1b.\textsuperscript{40} Furthermore, FTIR spectra revealed peaks at 1053, 1238, and 1613 cm$^{-1}$ that were attributed to stretching vibrations of C–O (alkoxy), C–O (epoxy), and C=C, respectively.\textsuperscript{41} However, the peaks observed below 750 cm$^{-1}$ such as 705,
608, and 507 cm$^{-1}$ were attributed to stretching vibrations of Mn–O group. The peak located at 1238 cm$^{-1}$ was ascribed to the vibration of different oxygen functional groups present on GO NRs sheet.

Selected area electron diffraction (SAED) analysis indicates the crystalline nature of as-prepared nanocomposites as demonstrated in Figure 1c–e. Various planes of NRs are well related with XRD measurements and satisfied condition of Bragg’s diffraction.

The optical properties of MnO$_2$ and GO-doped MnO$_2$ NRs are illustrated in Figure 2. Curves of absorption spectra (prepared samples) were observed in the range of 225–700 nm (Figure 2a). The host sample showed a broad absorption peak at 300 nm, and doped MnO$_2$ curves occurred at $\sim$285 nm that indicated blueshift. The peak generated at 225–310 nm in the visible (vis) light region confirmed the presence of MnO$_2$ and was attributed to the d–d transition of Mn ions. The absorption peak of GO observed at 231 nm in 2, 4, and 6% GO-doped MnO$_2$ corresponded to $\pi-\pi^*$ transitions of aromatic C–C bonds. These peaks showed the same pattern. Band gap energies of dopant-free and doped MnO$_2$ were measured using Tauc’s plot equation. The $E_g$ value of MnO$_2$ was 4.1 eV, while it was measured between 4.2 and 4.25 eV for GO (0, 2, 4, and 6%)-doped MnO$_2$ (Figure 2b).

PL spectroscopy is a useful tool to examine the charge transfer, photogenerated carrier trapping, and optical properties of semiconductor material. A lower peak intensity for nanomaterial was caused by the lower recombination rate of electron–hole pairs, which showed a high photocatalytic activity (PCA) of nanocrystals. PL emission spectra of GO (0, 2, 4, and 6%)-doped MnO$_2$ were recorded at room temperature in the range of 375–610 nm as depicted in Figure 2c. Three emission bands of MnO$_2$ were located at 380 nm (ultraviolet region), 400–500 nm (blue region), and 509–554 nm (green region). The peak at 380 nm was attributed to band edge emission signals of MnO$_2$. The curve at 400–500 nm may correspond to oxygen vacancy-related defects, and the weak emission band at 500–555 nm was ascribed to surface...
dangling bonds. By sp² clusters, PL emission in amorphous carbon arises from electron−hole recombination pair in band-tails states. Substantially, PL emission originates from electronic-transitions between the region of nonoxidized carbon (−C=C−) and oxidized carbon atom (C−O, C==O, and O==C−OH) in the atomic structure.

Scanning electron microscopy (SEM)−energy-dispersive spectrometry (EDS) was used for the composition analysis of nanomaterials. EDS micrographs of the pristine sample and MnO₂ doped with GO (2, 4, and 6%) are depicted in Figure 3. These spectra revealed three evident Mn peaks, which confirmed the presence of Mn atoms, in MnO₂, indicating high purity. Peaks of potassium (K) and sulfur (S) were observed, which was caused by the precursor used in the experiment, while carbon (C) peak was attributed to nanosheets of GO.

Micrographs of high-resolution transmission electron microscopy (HR-TEM) revealed the morphological features of pure and doped MnO₂ (Figure 4a−d). HR-TEM results indicated the evolution of NRs of MnO₂ and (2, 4%) GO-MnO₂ as shown in Figure 4a−c. Agglomeration of NRs was observed with increasing amount of GO dopant (Figure 4d). Meanwhile, interlayer d-spacing can be calculated using high-resolution TEM images. d-Spacing values of MnO₂ and 2, 4, and 6% GO doped with MnO₂ were estimated as 0.30, 0.37, 0.25, and 0.38 nm, respectively, that are well matched with XRD measurements.

UV−vis absorption spectroscopy was used to investigate the catalytic activity of GO (2, 4, and 6%)-doped MnO₂ nanostructures in the presence of NaBH₄ for MB degradation in acidic, neutral, and basic media (Figure 5). Solutions of pH play an important role in the degree of degradation; dye effluents are often discharged at various values of pH. In the absence of catalysts, reducing capacity of NaBH₄ was also analyzed. The catalytic activity of the prepared nanocatalysts depends on pH condition. In acidic medium, 0.5 M solution of H₂SO₄ was added in MB dye while pH = 4 was maintained. After that, a diluted solution of samples (400 μL) and NaBH₄ (400 μL) was added in 3 mL of MB dye. Samples revealed non-negligible results; pure sample showed 53.5% within 30 min, while 2, 4, and 6% GO-doped samples showed 45.7, 55.7, and 94% degradation, respectively, as illustrated in Figure 5a. The efficiency of dye degradation depends upon the pH of dye solution. Catalytic activity increased in acidic medium, which is due to the excess of H⁺ ions available to be adsorbed on the nanostructure surface. For neutral pH (~7), the above-mentioned route was appropriated to investigate the degradation extent. Compared to acidic medium, catalytic activity enhanced for MB degradation at neutral pH. Undoped and doped MnO₂ indicated 60, 48, 52, and 95% degradation performance, respectively, within 30 min (Figure 5b). Generally, a large surface area of any catalyst showed that it has more active sites, and according to our results, catalytic efficiency enhanced. Also, the catalytic efficiency was diminished in the case of a large surface area, which may be due to microporosity that prevents the reactants from diffusing to active sites. Prior to the start of testing, NaOH (0.5 M) solution was used to maintain dye pH ~ 12 for basic medium. In basic medium, minimum degradation was observed compared to the acidic and neutral media. Pure sample showed a 44.8% degradation, while MnO₂ with doping of GO (2, 4, and 6%) showed 40.7, 35, and 31.8% degradations,
Figure 6. (a) Catalysis mechanism of GO-MnO$_2$ reproduced with permission from ref 52. Copyright 2018 American Chemical Society. (b) Absorbance effect of dye vs time.

Figure 7. In vitro antibacterial efficiency of doped MnO$_2$ NRs against EC (a) and SA (b), and the corresponding efficiency % of doped samples for EC (c) and SA (d).

Table 1. Antibacterial Action of Doped MnO$_2$ Nanorods

| sample         | inhibition areas$^d$ (mm) 500 µg/50 µL | 1000 µg/50 µL | inhibition areas$^d$ (mm) 500 µg/50 µL | 1000 µg/50 µL |
|----------------|----------------------------------------|--------------|----------------------------------------|--------------|
| MnO$_2$        | 1.85                                   | 2.35         | 2.30                                   | 3.65         |
| GO:MnO$_2$ 2%  | 2.10                                   | 2.50         | 3.05                                   | 4.20         |
| GO:MnO$_2$ 4%  | 2.45                                   | 2.85         | 3.65                                   | 4.60         |
| GO:MnO$_2$ 6%  | 2.85                                   | 3.15         | 4.25                                   | 5.15         |
| enrofloxacin   | 4.25                                   | 4.25         | 7.15                                   | 7.15         |
| DIW            | 0                                      | 0            | 0                                      | 0            |

$^d$Doped MnO$_2$ nanorods inhibition areas (mm) for EC. $^e$Areas of inhibition sizes of doped MnO$_2$ for SA.
respectively, which gradually decreased as shown in Figure 5c. In basic medium, the catalytic activity decrease can be attributed to the increasing number of hydroxyl groups (leading to reduced products oxidation) upon addition of sodium hydroxide (NaOH). Catalytic activity depends upon the crystallinity, morphology, and surface area of the particles. Figure 6b reveals that contact time increases gradually with an increase in absorbance of dye and the resultant equilibrium time was 30 min. These results showed that elimination of dye depends on its initial concentration. To evaluate the effect of the initial concentration of dye and contact time, a fixed amount of nanocatalyst was used as the dye concentration.

In the context of the EC and SA microorganisms, antibacterial behavior was investigated as presented in Figure 7a–d and Table 1, depicting an inhibition zone for the pristine and doped MnO2 samples. Substantial inhibition areas were measured for EC ranges of 2.10–2.85 and 2.50–3.15 mm at decreased and increased levels for doped MnO2 nanorods and 3.05–4.25 and 4.20–5.15 mm for both attentions against SA. The pristine sample showed 1.85 and 2.35 mm inhibition areas for EC and, similarly, 2.30 and 3.65 mm for SA at both concentrations, respectively. Broadly, the efficiency % age was increased from 49.4 to 67.1% and 58.8 to 74.1% for EC, and similarly, from 42.7 to 59.4% and 58.7 to 72.0% for SA at both attentions, respectively. Ciprofloxacin depicted 4.25 and 7.15 mm inhibition for EC and SA, respectively, in contrast to deionized water (DIW) (0 mm). GO-MnO2 with 6 wt % doping concentration presented a strong antibacterial efficiency for EC relative to SA. The apparent lack of peptidoglycan layer makes it possible to find optimum microbial suppression capacities in EC bacteria, independent of SA, which contains thick peptidoglycan and robust chitinoid membrane for defense.

Enriched permeability reactions, release of reactive oxygen species (ROS), and electrostatic attraction in positive nano-particles and negative microbial organisms eventually deteriorate key microbial proteins. The bacteria counter charge and Mn4+ ions released by MnO2 nanorods are thought to lead electrostatic bonding and bioactivity. Nanomaterial antibacterial activity may be attributed to Mn4+ ions responsible for membrane permeability and oxidative stress pathways important for cellular mortality. The potential of microbicidal is accomplished by the development of broad surface oxygen species by creating inhibition regions of NPs directly dependent on the concentration and size of NPs as the particle.

Table 2. Comparison of Doped MnO2 Nanorods with Previous Study

| material                  | catalysis (degradation (%)) | time | reference | antimicrobial activity | material                  | conc | S. aureus (mm) | E. coli (mm) | references |
|---------------------------|----------------------------|------|-----------|------------------------|---------------------------|------|----------------|--------------|------------|
| aFe3O4@GO                | 34                         | 59   |           |                        | AgNP@mesoMnOx             | 100 μg | 12             | 12           | 60         |
| NiCoMnO4                 | 75.9                       | 10 h | 61        |                        | Ag-doped MnO2 NPs        | 3.52 Ag doped in 2.48 μg of MnO2 | 14 mm | 12             | 62         |
| NiCoMnO4                 | 92.3                       | 40 h | 61        |                        | Ag-doped MnO2 NPs        | 60 μg  | 3.2 ± 3 cm     |             | 63         |
| PEDOT/GO/MnO2 hybrid     | 92.7                       | 7 h  | 64        |                        | GO-Ag NPs                | 100 μg/mL | 28 mm         | 18           | 65         |
| GO/MnO2 composite        | 79                         | 66   |           |                        | AgNP@GO                  | 5 mg/mL | 15.2 ± 1.6 mm  |            | 67         |
| GO/MnO2 composite        | 77                         | 68   |           |                        | CuONPs@GO                | 5 mg/mL | 11.2 ± 1.5     |            | 67         |
| RGO/MnO2 composite       | 66                         | 69   |           |                        | GO/CuONPs                | 5 mg/mL | 12.5 ± 2.1     |            | 70         |
| ZnG                      | 85.71                      | 2 h  | 71        |                        | GO/AgNP                  | 5 mg/mL | 17.3 ± 2.3 mm  |            | 70         |
| GO (6%)–doped MnO2       | 95                         | 30 min | present work |                        | GO (6%)–doped MnO2       | 1000 μg/mL | 5.15 mm       | 3.15         | present work |

Figure 8. Illustration of antimicrobial activity of prepared sample.
dimensions have the opposite relation to antibacterial ability (Figure 8 and Table 2).\textsuperscript{58}

2.1. Molecular Docking Studies. In silico approaches being an effective way for a clear understanding of the mechanism behind various biological activities have gained much attention in recent decades. The FabH enzyme belonging to type II (FASII) fatty acid synthesis is essential to bacterial cell viability, and inhibiting its activity has a huge significance in antibiotic discovery.\textsuperscript{72}

For FabH\textsubscript{E. coli}, the best docked conformation of MnO\textsubscript{2} showed involvement of three amino acid residues of active pocket showing H-bond interaction with overall binding score as $-7.835$ kcal/mol. The H-bond distances observed were Ala246 (1.7 Å), Asn247 (1.9 and 3.3 Å), and Gln245 (2.2 and 2.8 Å) alongside metal contact interaction with Ile250 as depicted in Figure 9a. Similarly, MnO\textsubscript{2}-doped GO also showed good binding tendency (binding score: $-5.995$ kcal/mol) with active site residues involving H-bond with Gln245 (1.6 and 2.4 Å) and Asn247 (1.9 and 3.2 Å) shown in Figure 9b.

Furthermore, we evaluated the binding interaction of MnO\textsubscript{2} and MnO\textsubscript{2}-GO against DNA gyrase (a well-known target for antibiotic discovery)\textsuperscript{73} from both Staphylococcus aureus (SA) and E. coli. The best docked conformation observed for MnO\textsubscript{2} binding into active site of DNA gyrase from E. coli showed H-
bonding with Ala47 (3.2 Å), Asp73 (2.2 Å), and Thr165 (1.6 and 3.5 Å) with an overall binding score of −8.729 kcal/mol, suggesting the inhibition potential of MnO2 against DNA gyrase. On the other hand, the MnO2-doped GO showed one H-bond with Asp73 (2.3 Å) alongside metal contact with Thr165 as depicted in Figure 10a,b.

Similarly, MnO2 interaction with the active site of DNA gyrase from S. aureus showed involvement of H-bond with Asp81 (2.6 and 3.2 Å) and Thr173 (1.5 and 3.6 Å) with a binding score of −4.628 kcal/mol. For MnO2-doped GO, the best docked conformation with a binding score of −5.619 kcal/mol was observed showing involvement of three amino acids having H-bond interaction, i.e., Gly85 (2.3 Å), Thr173 (1.6 and 3.5 Å), and Asp81 (2.4 and 2.8 Å) as depicted in Figure 11a,b.

In silico, molecular docking studies revealed that both MnO2 and MnO2-doped GO interacted with key residues of active pocket in a similar way to the reported inhibitors for both FabH and DNA gyrase and predicted them as possible inhibitors that are suggested to be further confirmed through enzyme inhibition studies.

3. CONCLUSIONS

Herein, we described the successful synthesis of dopant-free and GO-doped MnO2 nanorods with the chemical precipitation technique to evaluate the enhanced catalytic activity of MnO2 when doped with GO. The optical properties, morphology, structure, and elemental composition of pure and doped-MnO2 were studied. XRD and EDS confirmed the crystallite size (36.4 nm), monoclinic structure of MnO2, and implantation of GO on MnO2. Interplanar d-spacing and plane orientation were affirmed from XRD that was consistent with HR-TEM analysis. UV–vis spectra showed a 4.1 eV peak at 300 nm and exhibited a blueshift, which could be attributed to the doping effect of GO. In view of this blueshift band gap increase, peaks of emission spectra were attributed to defects related to oxygen vacancies. In the dark, 94% degradation of methylene blue in acidic medium and 95% in neutral medium revealed successful doping of GO (6%), which served to enhance the catalytic activity substantially. The doped samples’ synergistic antibacterial response against E. coli was compared with S. aureus. This study investigated the dopant-dependent properties of MnO2 nanorods, which could be used as antibiotic substitutes in antibacterial applications as suggested by in vitro antibacterial activities and in silico molecular docking studies.

4. MATERIALS AND METHODS

4.1. Materials. Manganese sulfate monohydrate (Mn-(SO4)·H2O, 99%), graphene oxide (GO), sodium hydroxide (NaOH, 98.0%), and sodium hydroxide (KMnO4, 98%) of analytical grade were purchased from Sigma-Aldrich Co., Germany. Sulfuric acid (H2SO4) was acquired from Analar, and all chemicals were used without any further purification.
4.2. Synthesis of GO-MnO₂. GO-doped MnO₂ was prepared using the chemical precipitation method. Initially, Mn(SO₄)_2·H₂O was dissolved in DI water under constant stirring to prepare 0.5 M solution while KMnO₄ was added and stirred for 2 h. Modified Hummers’ method was used to obtain GO from refined graphite powder as shown in Figure 12a. GO was dissolved in DI water in an ultrasonic bath for 2 h. GO-precipitated solution was added in MnO₂ solution and stirred for 24 h and then centrifuged at 4000 rpm. Finally, nanomaterials were dried at 100 °C overnight to obtain a GO-MnO₂ powder. The same method was repeated by adding various concentrations (4 and 6%) of GO to obtain GO-MnO₂ NRs (Figure 12b).

4.3. Catalysis. The catalytic activity of prepared samples was examined against MB at different pH values. In neutral medium, freshly prepared 400 μL NaBH₄ (used as a reducing agent) suspension was added in 3 mL of MB solution. Alongside, 400 μL of doped GO mixture was put in base solution. H₂SO₄ and NaOH were used to obtain acidic and basic solutions, respectively. Gradual removal of dye color shows degradation of the blue color of MB into leuco-MB. A UV−vis spectrophotometer is used to obtained results of collected samples at different intervals, and % degradation was calculated using eq 1

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\% \text{ degradation} = \frac{C_0 - C_f}{C_0} \times 100
\]

4.4. Antibacterial Evaluation. Antibacterial behavior of produced GO-MnO₂ nanorods (2, 4, and 6 wt %) was validated using pathogenic E. coli (EC) and S. aureus (SA) via a well diffusion test. Bacterial growth was achieved on manniot salt (MSA) and MacConkey agar (MCA). Wells of approximately 6 mm internal diameter with a sanitized borer were pierced on MSA and MCA plates swabbed by 0.5 McFarland of EC and SA. Afterward, plates were incubated at 37 °C overnight, and inhibited regions around wells were noticed. Quantifying diameters (mm) of inhibition areas around wells indicated antibacterial potential. The basic antibiotic used for comparison was ciprofloxacin (5 μg/50 μL), and DIW (50 μL) was used as negative regulation. The dishes were aerobically invaded overnight at 37 °C, and the inhibition areas measured with a Vernier caliper were used for the evaluation of antibacterial efficiency.

4.5. Materials Characterization. To analyze the crystallinity and phase constitution of samples through X-ray diffraction, PANalytical X Pert PRO was used with Cu Kα radiation (λ = 0.15418 Å) and 20° range of 5–80°. Fourier transform infrared (FTIR) spectroscopy (PerkinElmer spectrophotometer) was employed to carry out functional group analysis in the range of 4000–400 cm⁻¹. A scanning electron microscope (JEOL JSM 6460LV) and a high-resolution transmission electron microscope (JEOL JEM 2100F) were used to analyze the particle size, morphology, and interlayer d-spacing. The optical properties were obtained with a UV−vis spectrophotometer (Genesys 10S) in the range of 190–900 nm. The photoluminescence spectra (JASCO, FP-8300) of prepared samples were recorded.

4.6. Molecular Docking Studies. Molecular docking studies were performed against two enzymes, i.e., β-ketoacyl-carrier protein synthase III (FabH) and DNA gyrase from E. coli and S. aureus selected as targets, to evaluate the roles of MnO₂ and MnO₂-doped GO as their possible inhibitors. The selection of enzymes was based on the fact that their inhibition leads to disruption of proper cell processes and ultimate death of bacteria. The enzyme structures possessing better resolution were obtained from Protein Data Bank with IDs 4Z8D for FabHE.coli and 6KZX for DNA gyraseE.coli and 4URO for DNA gyraseS.aureus.

ICM Molsoft software was employed for molecular docking predictions, and enzyme structures were prepared using receptor preparation tool of ICM that involved removal of water molecules and native ligand followed by energy minimization with default parameters and force fields. Docking site was specified in close vicinity (within 10 Å) of co-crystallized ligand, and top-ranked docked conformations with the lowest binding score were selected in each case for further analysis. The analysis and interaction patterns of docked complexes were made using ICM Viewer and Pymol software to represent three-dimensional (3D) view. The ligand structure was built using LigEdit tool of ICM.

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**Notes**
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