



Manganese ferrite graphene nanocomposite synthesis and the investigation of its antibacterial properties for water treatment purposes

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ABSTRACT

The main objective of this study was to synthesize a nanocomposite using graphene and manganese ferrite nanoparticles (MnFe₂O₄-G) and to evaluate its antibacterial activity for water treatment purposes. Its morphological characteristics were evaluated by instrumental techniques, such as scanning electron microscopy and transmission electron microscopy. The characterization results indicated that the nanocomposite presented nanoparticles of approximately 25 nm well dispersed in transparent and large (14 μm) graphene nanosheets. The antibacterial activity was evaluated in a batch experiment using a concentration of 40 μg mL⁻¹ of nanocomposite (MnFe₂O₄-G, bare MnFe₂O₄ nanoparticles or graphene oxide), 1x10⁵ CFU mL⁻¹ of *Escherichia coli*, and 8 h of contact time at room temperature. The highest antibacterial capacity was observed for the hybrid nanocomposite (91.91%), due to the synergic effect of graphene and MnFe₂O₄ nanoparticles. Various mechanisms were proposed to explain the effective antibacterial activity of MnFe₂O₄-G, such as wrapping, oxidative stress, sharp-edge cutting effect, among others. The results showed that MnFe₂O₄-G is a potential alternative in water treatment processes as an antibacterial agent.

Keywords: antimicrobial, magnetic, nanoparticle.



Síntese de nanocompósito de grafeno e ferrita de manganês e a investigação de suas propriedades antibacterianas para uso no tratamento de água

RESUMO

O principal objetivo do presente estudo foi sintetizar um nanocompósito usando grafeno e nanopartículas de ferrita de manganês ($\text{MnFe}_2\text{O}_4\text{-G}$) e avaliar sua atividade antibacteriana para aplicações em processos de tratamento de água. Suas características morfológicas foram avaliadas por técnicas instrumentais tais como microscopia eletrônica de varredura e microscopia eletrônica de transmissão. Os resultados de caracterização indicaram que o nanocompósito apresentou nanopartículas de aproximadamente 25 nm bem dispersas em nanofolhas de grafeno grandes (14 μm) e transparentes. A atividade antibacteriana foi avaliada em um experimento batelada usando uma concentração de 40 $\mu\text{g mL}^{-1}$ de nanocompósito ($\text{MnFe}_2\text{O}_4\text{-G}$, nanopartículas de MnFe_2O_4 ou óxido de grafeno), 1×10^5 CFU mL^{-1} de *Escherichia coli* e 8 h de tempo de contato à temperatura ambiente. A maior capacidade antibacteriana foi observada para o nanocompósito híbrido (91,91%), decorrente do efeito sinérgico do grafeno e as nanopartículas de ferrita de manganês. Vários mecanismos foram propostos para explicar atividade antibacteriana efetiva do $\text{MnFe}_2\text{O}_4\text{-G}$, tais como aprisionamento, stress oxidativo, efeito de corte afiado, entre outros. Portanto, os resultados mostraram que $\text{MnFe}_2\text{O}_4\text{-G}$ é uma alternativa em potencial para processos de tratamento de água como agente antibacteriano.

Palavras-chave: antimicrobiano, magnético, nanopartícula.

1. INTRODUCTION

Water is an essential resource for all beings. However, unsafe drinking water still constitutes a major burden on public health in developing countries. Diseases related to drinking water contamination lead to millions of deaths every year and diarrhea remains a major cause of child deaths. The main health risk is ingestion of water contaminated with feces that contains pathogens that cause infectious diseases such as cholera and other diarrheal diseases, dysenteries and enteric fevers (Liu *et al.*, 2012a). Therefore, the development of novel and efficient antibacterial agents to control and prevent contamination by pathogenic microorganisms in water is vital for human health and well-being.

Antibacterial nanomaterials provide great opportunities to develop next-generation sustainable water-disinfection technologies. Among all the antibacterial nanomaterials, graphene-based nanomaterials have emerged recently as a novel green broad-spectrum antibacterial material, with a severe cytotoxic effect on bacteria, fungi, and plant pathogens, with little resistance and tolerable cytotoxic effect on mammalian cells (Hegab *et al.*, 2016; Ji *et al.*, 2016).

Graphene is a two-dimensional monolayer of sp^2 -hybridized carbon atoms that form a honeycomb structure with unique properties. Because of its peculiar configuration, it has unique properties, such as high mechanical strength and elasticity, and excellent conduction of electric current and heat, in addition to having good dispersion and remarkable thermal stability (Syama *et al.*, 2016).

The degree of antibacterial effect of nanomaterials is determined by their shape, surface functionalization, size, stability and size distribution. Single-component graphene-based materials present slow antibacterial activity; generally, it takes several hours to totally inactivate bacterial cells in diluted suspensions. In practical applications, it is essential to optimize and accelerate the process minimizing the disinfection time (Zhou *et al.*, 2016b).

Another major problem related to the use of graphene in water treatment is that graphene nanosheets tend to aggregate and re-stack, forming graphite when used during process

operations and when used in larger quantities due to strong interplanar interactions (Cheng *et al.*, 2012). As for GO, they have poor affinity for binding with anionic compounds due to their strong electrostatic repulsion.

These disadvantages can be overcome by covalent or non-covalent functionalization of different molecules and other nanomaterials (Xu *et al.*, 2009). The surface functionalization of graphene materials with nanoparticles or other functional groups increases their sensitivity, selectivity and detection limit, and also may improve their antibacterial effect and stability (Hegab *et al.*, 2016; Tu *et al.*, 2016). The use of nanoparticles can be beneficial both to facilitate separation in the water treatment process and to confer antibacterial properties, which opens new opportunities to further explore their potential for water- and wastewater-treatment applications (Farghali *et al.*, 2013; Gutes *et al.*, 2012).

Thus, graphene derivatives are rapidly emerging as an extremely promising class of nanomaterials due to the combination of graphene derivatives and currently utilized antibacterial metal and metal-oxide nanostructures, in order to obtain the synergistic antibacterial effect, achieving exceptional bactericidal activity (Hegab *et al.*, 2016; Rojas-Andrade *et al.*, 2017).

Spinel ferrites represent an important family of iron-based heterostructured oxide materials and display great potential. Among these materials, MnFe_2O_4 has been considered a very attractive nanomaterial due to its high capacity, excellent chemical stability, easy fabrication, low cost and non-toxicity (Sakho *et al.*, 2019).

Few studies were found in the literature with research focusing on the use of MnFe_2O_4 or composites of graphene and MnFe_2O_4 for bactericidal activity in water treatment (Chella *et al.*, 2015). Most research focused on their antibacterial activity for the development of novel nanomaterials for biomedical applications (Esmaeili and Ghobadianpour, 2016; Sakho *et al.*, 2019), drug-delivery (Wang *et al.*, 2016) and photocatalytic activity (Zhou *et al.*, 2016a).

This paper therefore reports on the synthesis of a nanocomposite of manganese ferrite graphene ($\text{MnFe}_2\text{O}_4\text{-G}$), followed by an investigation of its antibacterial properties, evaluating its efficiency at removing *Escherichia coli*, to verify its potential use in water- and wastewater-treatment processes.

2. MATERIALS AND METHODS

2.1. Manganese ferrite graphene nanocomposite synthesis

GO was synthesized according to the modified Hummers method (Hummers and Offeman, 1958; Kovtyukhova *et al.*, 1999). The preparation of $\text{MnFe}_2\text{O}_4\text{-G}$ was based on a simple one-pot solvothermal method reported in our previous work (Yamaguchi *et al.*, 2016). In short, anhydrous ethylene glycol ($\text{HOCH}_2\text{CH}_2\text{OH}$, $\geq 99.8\%$), GO, ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\geq 97\%$), manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\geq 99\%$) were dispersed under ultrasonication. Later, anhydrous sodium acetate (CH_3COONa , $\geq 99\%$) was added and stirred for 30 min. The mixture was then autoclaved at 200°C for 10 h. The obtained mixture was then washed several times with deionized water and ethanol and dried in a hot air oven at 60°C . All chemicals were purchased from Sigma Aldrich. Bare MnFe_2O_4 nanoparticles were prepared using a similar approach, but in the absence of GO.

2.2. Nanocomposite characterization

The surface morphology of the as-synthesized nanocomposite was verified by scanning electron microscopy (SEM) under Shimadzu SS-550 - Scanning Electron Microscope and transmission electron microscopy (MET) under JEM-1400 - JEOL microscope. An extensive chemical characterization of the nanocomposites was performed in our previous work (YAMAGUCHI *et al.*, 2016) and will be used for further discussion of antibacterial results in Section 3.2.

2.3. Antibacterial properties evaluation

Assays for the evaluation of *E. coli* removal were based on the *Standard Methods for the Examination for Water and Wastewater* (APHA et al., 2017). A stationary phase culture of *E. coli* ATCC 11229 was incubated at 35°C for 24 h in trypticasein soy broth (TSB). From the culture obtained, a bacterial suspension of 1.5×10^8 CFU mL⁻¹ (colony forming unit) was prepared in a saline tube and determined by comparison with the turbidity of the McFarland scale # 0.5 tube.

To determine the antibacterial effect of the nanomaterials, a batch experiment was performed. Typically, 2 L of distilled water was contaminated with 1 mL of the previously prepared *E. coli* ATCC 11229 suspension to give a concentration of approximately 1×10^5 CFU mL⁻¹. Then, 100 mL of contaminated water was dispensed into 250 mL vials containing 10 mg of nanocomposite, resulting in $40 \mu\text{g mL}^{-1}$ nanocomposite concentration. After inoculation, they were shaken at 200 rpm and 35°C for 8 h. After 8 h, the antibacterial effect was evaluated using an adapted filter membrane technique of the *Standard Methods for the Examination for Water and Wastewater* (APHA et al., 2017), as illustrated in Figure 1.

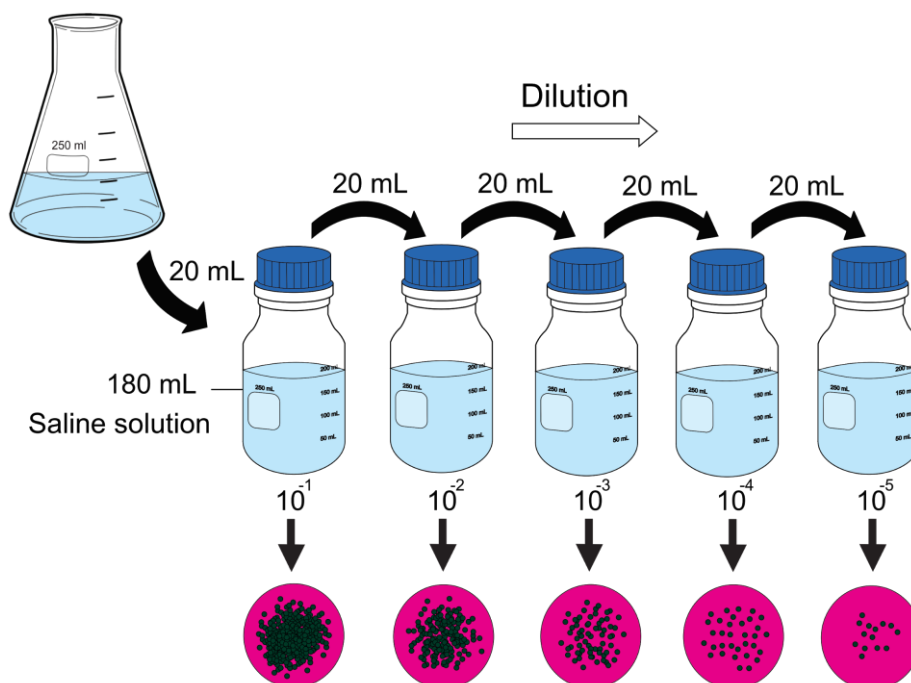


Figure 1. Scheme of filter membrane technique.

The filter membrane technique used can be summarized by: (1) First the sample was homogenized; then (2) 20 mL of the sample was diluted in 180 mL of 0.85% saline in a Schott® vial, resulting in a 10^{-1} dilution; (3) the vial was shaken and then 20 mL of the 10^{-1} dilution vial was collected and added to another vial containing 180 mL of 0.85% saline resulting in a 10^{-2} dilution; (4) this procedure was repeated until a 10^{-5} dilution was obtained, as shown in Figure 1; next, (5) in a laminar-flow chamber previously sterilized with ultraviolet radiation, 100 mL of each 10^{-5} dilution was vacuum filtered in a previously autoclaved Manifold Microfil® Millipore using a membrane of $0.45 \mu\text{m}$ pore and 47 mm diameter; (6) the membranes were placed in Petri dishes containing M-Endo LES agar and then placed in an oven at 35°C; (7) after 24 h, the Petri dish readings were taken by counting the number of CFU. The *E. coli* viability loss was calculated using Equation 1.

$$\varepsilon (\%) = ((N1 - N2) / N1) \times 100 \quad (1)$$

Where, N_1 is the number of colonies grown on the control Petri dish, N_2 is the number of colonies grown on the treated Petri dish and ϵ is the *E. coli* viability loss (%).

3. RESULTS AND DISCUSSION

3.1. Nanocomposite characterization

In the micrographs of the hybrid composite of MnFe_2O_4 -G shown in Figure 2, it is possible to observe the graphene nanosheets, which look like crumpled sheets. Similar structures were found in micrographs obtained in our previous work (Yamaguchi *et al.*, 2016) and by Yao *et al.* (2014), who used a similar methodology to that employed in this work.

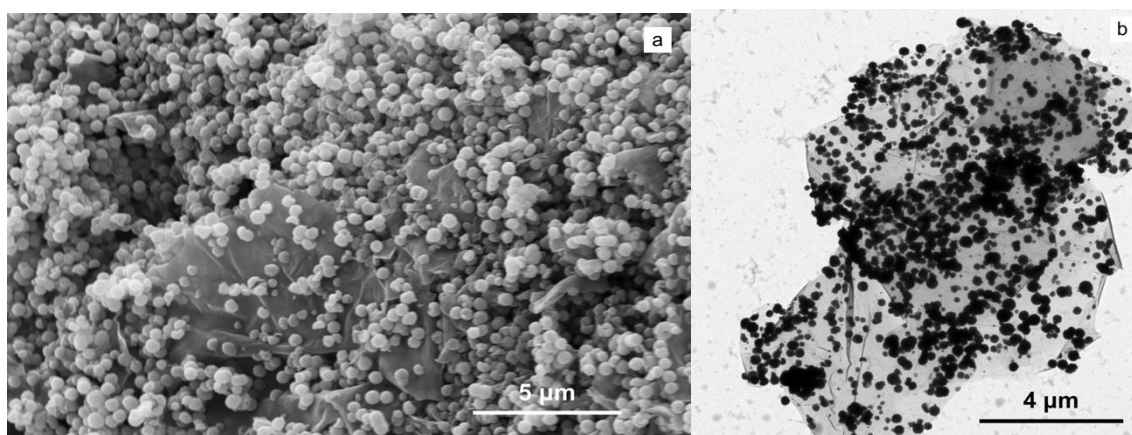


Figure 2. SEM (a) and TEM (b) micrographs.

It was also noted that MnFe_2O_4 nanoparticles were uniformly anchored on the transparent graphene nanosheets (Figure 2b). The size of MnFe_2O_4 nanoparticles can be confirmed by TEM and SEM micrographs (Figure 2), showing an average particle size of 25 nm. It is noteworthy that MnFe_2O_4 particles are still strongly anchored to the graphene surface even after sample preparation for MET analysis (agitation and sonication), suggesting that there is a strong interaction between MnFe_2O_4 nanoparticles and graphene nanosheets, also showing mechanical stability (Yao *et al.*, 2012).

In Figure 2b, the graphene nanosheet observed is approximately 14 nm and is much bigger than a bacteria cell ($\sim 1 \mu\text{m}$), indicating that the mechanism of cell wrapping (discussed in Section 3.2) is able to contribute to the antibacterial activity when using MnFe_2O_4 -G for antibacterial suspension tests. Also, large-size graphene nanosheets enhance the adhesion ability of bacteria, which means more chance to be in contact with and inactivate bacteria (Han *et al.*, 2019). Additionally, the transparent nanosheets presented in Figure 2b indicate a few-layer graphene. It is known that the number of layers that the graphene has significantly affects its antimicrobial activities, as graphene dispersibility in biological media displays a remarkable decrease with the increase in its thicknesses, resulting in agglomeration, which may affect the interactions between graphene and bacteria. Therefore, it was expected that our few-layer graphene nanocomposite would exhibit high antibacterial activity (Zheng *et al.*, 2018).

Hybrid nanomaterials with graphene structure are known to help promote a smaller agglomeration of nanoparticles, ensuring a large specific area due to the close interaction between the nanoparticles and graphene sheets (Liu *et al.*, 2013). Also in Figure 2, functionalized surfaces with considerable roughness and frequent ridges can be seen, which is also a favorable aspect for antibacterial activity, as it can cause cracking of bacterial cell walls during contact. The physical surface morphology resulting from graphene functionalization with MnFe_2O_4 is a crucial factor in affecting interaction with bacterial cells and can display a powerful antibacterial action by generating increased surface roughness, improving bacterial

cell adhesion. Higher surface area and deep terrains in the surface will result in more contact with bacteria cells, which can easily destroy them (Hegab *et al.*, 2016).

3.2. Antibacterial properties evaluation

The results of the evaluation of the antibacterial properties of the GO, MnFe₂O₄ and MnFe₂O₄-G are presented in Figure 3. Bacterial cell viability loss is the percentage of *E. coli* bacteria that were inhibited by the developed materials. GO exhibited 62.63% of cell inactivation presenting moderate cytotoxicity, while the nanohybrids showed 91.91% of cell inactivation. Bare MnFe₂O₄ nanoparticles showed lower removal in comparison to the nanohybrids, presenting 89.50% of bacterial viability loss.

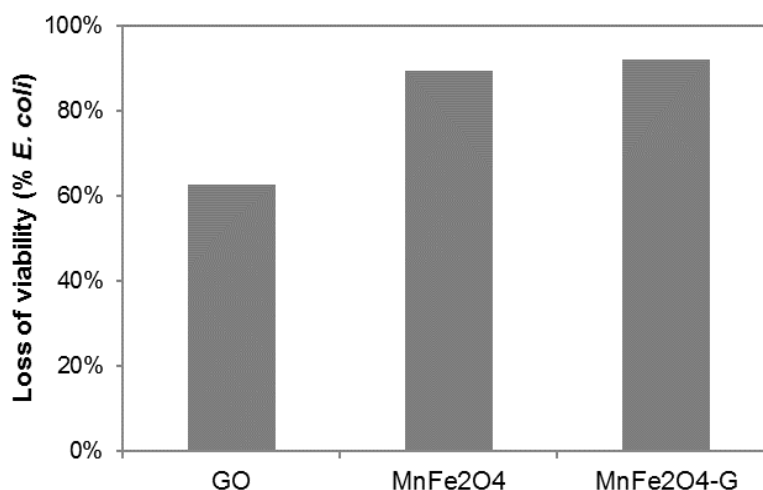


Figure 3. Loss of viability of *E. coli* (100 mL, 1x10⁵ CFU mL⁻¹, 10 mg of nanocomposite, 200 rpm, 35°C, 8 h).

Wide interest and several reports have been observed in the literature in developing GO-based antibacterial nanomaterials since its first observation in 2010 by Hu *et al.* Similar results were obtained by Liu *et al.* (2011) who evaluated the antibacterial GO efficiency for *E. coli* and reported ~69.3% performance using 85 µg mL⁻¹ for 2 h and they explained their results by the high density of oxygen-containing groups present in GO, which induce membrane stress leading to cell death. Similarly, experimental data from other groups obtained better results (> 80%) using higher material concentrations (Akhavan *et al.*, 2010; Begum *et al.*, 2020; Nine *et al.*, 2017).

The antibacterial activity of graphene has been confirmed to be dependent on its carbon radical density, as the oxygen content of the nanosheets plays an important role in bacterial killing through the induction of oxidative stress, which will be further explained below. Thus, a higher carbon-radical density implies a stronger antibacterial effect (Han *et al.*, 2019). GO is widely heterogeneous in their physicochemical properties resulting from its oxidation in the Hummers method. More oxidative content can generate more reactive oxygen species (ROS), which contribute to the higher bactericidal ability. GO possesses a high oxidation level, with oxidized groups such as C-OH, C=O and C-O-C, and can result in high oxidation performance for antibacterial capacity (Han *et al.*, 2019). In our previous work (Yamaguchi *et al.*, 2016), GO was characterized by FTIR analysis and the presence of these functional groups in GO surface was proved. Also, the ROS functional groups were observed in MnFe₂O₄-G nanohybrid samples when performing the XPS analysis (Yamaguchi *et al.*, 2016), indicating that the complete reduction of GO was not achieved, and that these functional groups may have contributed to the better efficiency of the nanohybrid nanomaterial compared to bare MnFe₂O₄.

However, the slight difference in antibacterial efficiency may be related to the charge of

the nanohybrids, which could have contributed to the improved adsorption of bare MnFe_2O_4 by the attraction of negatively charged bacteria cells through Coulombic interaction. The potential zeta characterization analysis (Yamaguchi *et al.*, 2016) showed that MnFe_2O_4 is more positively charged compared to GO and $\text{MnFe}_2\text{O}_4\text{-G}$. Thus, our nanohybrids have a negative global charge, specially graphene, while bare MnFe_2O_4 nanoparticles have a positive charge, which may have contributed to the electrostatic attraction and later ion release, and thus presented a higher *E. coli* removal (Hegab *et al.*, 2016; Ji *et al.*, 2016).

Chella *et al.* (2015) found a similar behavior when using $\text{MnFe}_2\text{O}_4\text{-G}$ nanocomposite for antibacterial investigations. They obtained lower cell inactivation percentages (82%) than those obtained in the present study using $100 \mu\text{g mL}^{-1}$ of $\text{MnFe}_2\text{O}_4\text{-G}$ for 2h of contact time. Another recent study, Sakho *et al.* (2019) reported 90% of *E. coli* death rate using $15 \mu\text{g mL}^{-1}$ of nanohybrid $\text{MnFe}_2\text{O}_4\text{-G}$ after 2 h of contact time. In both studies, they attributed the antibacterial activity to the oxidation stress and membrane interaction (Liu *et al.*, 2011). They explained that the antibacterial activity mechanism functioned by binding the hybrid composite with the *E. coli* cell wall by adsorption and electrostatic attraction and the cell wall rupture occurred by direct contact with the graphene nanosheets and also ROS generation, which induced oxidative stress to the membrane, disturbing the cell structure, leading to the death of bacterial cells. Graphene-based materials, which contain a great number of functional groups, are likely to interact with bacterial cell structures, resulting in damage and their death (Chella *et al.*, 2015; Sakho *et al.*, 2019). These mechanisms are further explained below, and presented in Figure 4, together with the major antibacterial mechanisms proposed for $\text{MnFe}_2\text{O}_4\text{-G}$ according to the several mechanisms reported in the literature for graphene and graphene metal-oxide based nanocomposites.

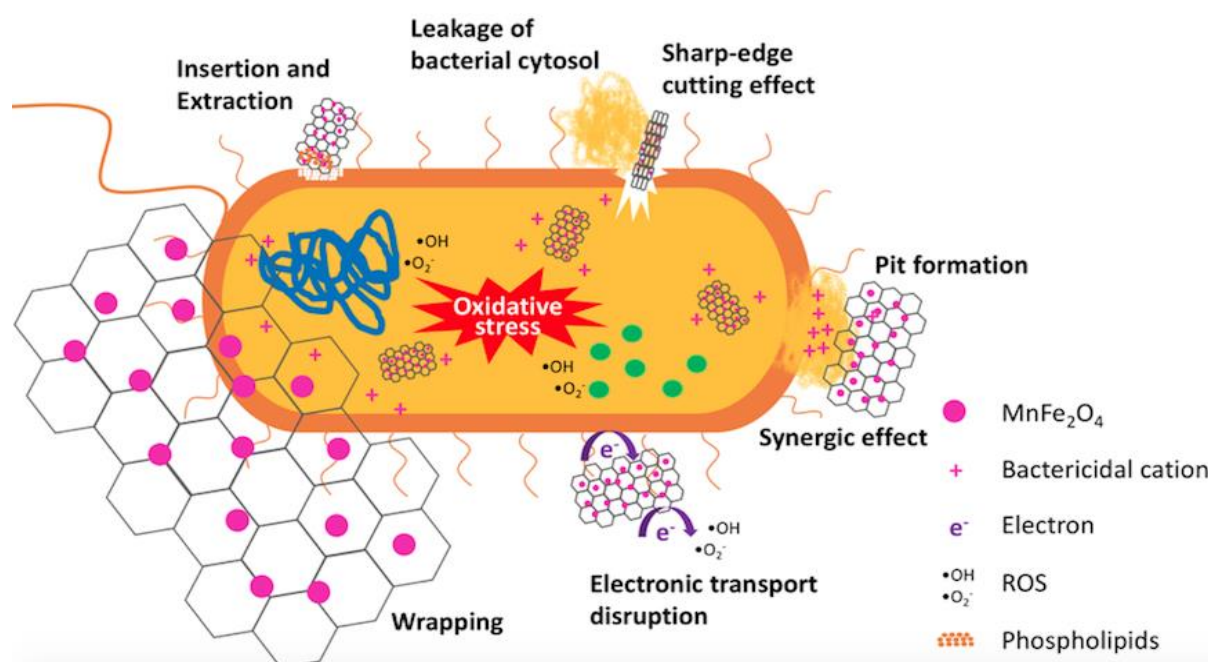


Figure 4. Major proposed mechanisms for antibacterial activity of $\text{MnFe}_2\text{O}_4\text{-G}$ nanocomposite.

Sharp-edge cutting effect: The physical damage in the bacteria membrane by graphene sharp edges is one of the main mechanisms of the antibacterial activity. The destruction of the integrity of the bacteria lipid bilayer upon direct contact with the atomically sharp edges of graphene nanosheets leads to bacterial inactivation, as they mediate the release of functional intracellular contents and form pores that could lead to osmotic imbalance, resulting in the reduction of the energy barrier required for membrane penetration (Hegab *et al.*, 2016; Hu *et al.*, 2010; Ji *et al.*, 2016).

Wrapping: The cell wrapping antibacterial physical activity mechanism is caused by the graphene sheet area, which hinders the bacterial growth in cell suspensions. Isolated from the external environment, the bactericidal activity of graphene is amplified as the sheet area increases. The bacteria is trapped in a large graphene nanosheet, becoming isolated and inactive, affecting their interaction with cells in the environment, which has the consequence of preventing nutrient acquisition and, subsequently, cellular growth and proliferation (Liu *et al.*, 2012b). This mechanism is more bacteriostatic, as it inhibits the growth of bacteria temporarily and normally does not kill them (Xia *et al.*, 2019).

Insertion and extraction: The destruction mechanisms of graphene insertion and lipid extraction can cause bacterial membrane stress leading to a reduction in cell viability. When graphene is in contact with the bacteria, the graphene nanosheet starts to vibrate around the bacteria cell (swing mode); then the insertion mode starts, when the sheet edge moves in and pierces the cell membranes, due to the strong van der Waals interactions. Finally, the extraction step is when the nanosheet extracts the phospholipids from the lipid bilayers of the bacteria cell membrane causing damage to the cell and cytoplasm leakage (Hegab *et al.*, 2016).

Oxidative stress: Oxidative stress leads to an imbalance between oxidation and antioxidation, which interferes with the bacterial metabolism, disrupts essential cellular functions, destroys cell structure, oxidized lipids, DNA and proteins that can ultimately lead to cell destruction and/or cellular growth inhibition. The large production of ROS, direct oxidation and charge transfer by MnFe₂O₄-G is believed to be the primary mechanism of cytotoxicity causing the oxidative stress of bacteria (Rojas-Andrade *et al.*, 2017). Also, when the ROS-mediated oxidation of lipid molecules takes place, the result is the formation of lipid peroxide radicals which initiate a chain reaction that leads to cell membrane oxidative destruction (Begum *et al.*, 2020; Hegab *et al.*, 2016).

Electronic transport disruption: The electron transfer from the bacterial membrane to the graphene surface is also a mechanism of the antibacterial activity of MnFe₂O₄-G. The negative charges of the bacterial cells interact with the graphene nanosheet edges which act as good electron acceptors and, therefore, contribute toward bacterial cell membrane damage. Respiratory chain electrons are extracted from the electron transport chain by graphene through a charge transfer mechanism, as graphene has high electronic conductivity. The electron transference can damage the membrane integrity, cause a depletion of the intracellular respiratory chain to extracellular molecular oxygen and provoke oxidative stress on bacteria metabolism, eventually causing its inactivation. Also, electron transfer from antioxidant biomolecules to graphene can also directly cause damage to bacterial antioxidant systems. In addition, the electron transfer occurring on MnFe₂O₄-G surface can generate free oxidative radicals, which are toxic to bacteria (Han *et al.*, 2019; Hegab *et al.*, 2016; Ji *et al.*, 2016; Rojas-Andrade *et al.*, 2017).

Synergic effect: The synergic effect is composed of the cytotoxicity effect of metal oxide nanoparticles enhanced by the graphene substrate, due to reduced agglomeration of metal oxide nanoparticles. The BET analysis performed previously (Yamaguchi *et al.*, 2016) for MnFe₂O₄-G and MnFe₂O₄ samples indicated that BET surface area presented a more than 4-fold increase. With this increase in surface area, the active surface area for adsorption properties towards bacteria cells is also increased, providing enhanced stability to immobilized metal oxide nanoparticles, and more effective release of cytotoxic nanoparticles in proximity to the bacterial cell, increasing bacterial inactivation (Begum *et al.*, 2020). Additionally, the graphene substrate affords additional membrane damaging effects, such as wrapping of bacteria and lipid extraction, which increase the overall antibacterial performance. Also, there is a close relationship between the cytotoxic effects of ROS generated by MnFe₂O₄-G and its destructive effects on bacterial membranes, which enables the metal cations (Mn²⁺ and Fe³⁺) to enter the bacteria more easily acting synergistically with ROS to destroy DNA and proteins (Begum *et*

al., 2020; Rojas-Andrade *et al.*, 2017). Furthermore, the positive ions released are easily absorbed by the bacterial membrane surface, with negative charge, leading to cell wall damage by pit formation. This electrostatic interaction between positive charge of metal ions and negative charge of membrane cells causes changes in the efflux and influx of biomaterials from bacteria. Moreover, in the surface or inner part of bacteria, small nanoparticles (~10 nm) can bind to sulfur-containing amino acids with thiol functional groups as they have more affinity to attach metal ions; this attachment may lead to the enzyme malfunction (Alavi and Rai, 2019).

Other mechanisms were also reported in the literature, such as photothermal reaction and photocatalytic activity for microorganism inactivation. However, our experiments were performed without light and/or infrared irradiation. An enhanced antibacterial activity can be achieved by sunlight irradiation provoked by high oxidative stress, as sunlight produces ROS, and accelerates the electron transfer from bacteria to graphene, by this means destroying bacterial antioxidant systems and causing great membrane disruption (Han *et al.*, 2019). Thus, a possibility to improve the antibacterial performance of MnFe₂O₄-G obtained in this work could be achieved using visible light irradiation in antibacterial assays.

4. CONCLUSIONS

The MnFe₂O₄-G nanohybrid was successfully synthesized in this research, as verified by SEM and TEM analysis. MnFe₂O₄ nanoparticles of an average particle size of 25 nm were anchored in crumpled large and transparent graphene nanosheets. The nanohybrid presented higher antibacterial activity when compared to GO and bare MnFe₂O₄ nanoparticles. The bactericidal activity of MnFe₂O₄-G was thoroughly discussed and it was concluded that graphene exerts its antibacterial action via physical damage such as direct contact of its sharp edges with bacterial membranes, destructive extraction of lipid molecules and wrapping mechanisms. Further, the chemical damage of bacteria is caused by oxidative stress with the generation of ROS and charge transfer. Furthermore, the synergic effect is observed when graphene and MnFe₂O₄ are together in the hybrid nanomaterial, obtaining improved antibacterial efficiency due to the reduced agglomeration of metal oxide nanoparticles, adsorption of bacterial cells, effective release of cytotoxic ions with greater proximity and the simultaneous damaging effects of graphene and metal cations on bacteria. Thus, due to the superior antibacterial properties obtained and to its good biocompatibility, MnFe₂O₄-G has been shown to have important potential for antibacterial purposes in water and wastewater treatment processes.

5. ACKNOWLEDGEMENTS

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