Al-Dalmaj Bulletin of Natural Science
Vol. 0, Issue 0, (2025), pp. 1-9
Available online at: https://dbns.qu.edu.iq/

Employing Biogenic Reduced Graphene Oxide as an Antibacterial Agent Against Some MDR Pathogenic Bacterial Isolates

Abtisam Jasim Abbas[1] Hazim Abed Walli[2] Ali A. Ghazzay[3]

Environment Department, College of Science, University of AL-Qadisiyah, Iraq[1,2]

Biology Department, College of Science, University of AL-Qadisiyah, Iraq[3]

Corresponding author: Abtisam Jasim Abbas

abtisam.jasim@qu.edui.iq

Abstract:

Antibiotic resistance in pathogenic microbes has become a severe health issue worldwide. Consequently, hard work has been made to progress novel materials with antimicrobial effectiveness. Current research accomplished to investigate the efficiency of biogenic reduced graphene oxide nanoparticles which were reduced for the first time by two bacterial strains *Heyndirckxia coagulans* (accession no. PP425868.1) and *Streptococcus thermophilus* (accession no. PP425862.1). The biosynthesized rGO NPs appeared to have antibacterial activity against (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia*, and *Pantoea agglomerans*), which were isolated from burns and wounds and urinary tract infections. Different concentrations of rGO NPs (0.05, 0.1, and 0.2 mg/ml) have been applied against the above MDR bacterial isolates. Results revealed that the development of inhibition areas augmented with the increase of rGO NPs concentration compared to the Ampicillin antibiotic. Using rGO NPs as antibacterial agents for curing infections associated with MDR bacteria is developing owing to exceptional physicochemical features such as high surface area, shape, and biocompatibility of rGO NPs.

Keywords: Biogenic reduced graphene oxide, *Heyndirckxia coagulans*, *Streptococcus thermophilus*, Antibacterial Activity, MDR bacteria.

1. Introduction

Recently, the random use of antibiotics has become a developing issue [1] and lead to the presence of multi-drug-resistance bacterial isolates [2]. When antibiotics discharge into the environment they destroy the bacteria realm, aquatic and soil organisms, as well as plants. As a result, bacteria particularly progress resistance or even MDR. These bacteria can produce deactivating enzymes and block drugs by changing the permeability of the cell membrane [3]. The noticeable development of antimicrobial resistance and the lack of new antimicrobial medicine improvement has gradually decreased the treatment elections for bacterial infection [4].

The current concept proposes that these bacteria develop resistance to numerous drugs by obtaining antimicrobial resistance genetic factors via horizontal transfer, facilitated by moveable genetic components like integrons, plasmids, and transposons among and across diverse bacterial strains is a significant factor that can be involved in the rise of multi-drug resistant bacteria [5]. Therefore, it is crucial to discover unconventional resources as an alternative of antibiotics to perform an antibacterial function [6]. One of the potential techniques used to overcome the great prevalence of MDR bacterial strains is Nanotechnology [7]. The Nano-scale materials that have showed antibacterial activity like Ag, AgO, Au, TiO2, Cu, Zn, ZnO and CuO [8,9] have shown noteworthy beneficial antimicrobial action.

Most recently, graphene-materials (GMs) have been widely studied for numerous applications for instance, environmental and medical fields [10,11]. Owing to the physicochemical features of GMs, researchers have studied their potential application in several biotechnologies [12-14]. Many prior studies have informed that rGO NPs has antimicrobial efficacy [15-17]. Altogether, the antibacterial efficacy of the graphene-based nanomaterials could be attributed to the synergy of oxidation stress and physical damages to the membrane [18]. The sharp edges of graphene oxide (GO) and rGO resulted in physical damages to the bacteria's cell wall and membrane. Moreover, graphene sheets could promote cellular oxidative stress, which contributed to the disruption of particular microbial processes [19,20].

The high NPs (SSA) allow particles to bind somewhat a great concentration of functional groups or serves as transporters for other active matters, improving their contact with bacteria [21]. In general, small size nanoparticles show better antibacterial activity, because decreasing of the volume will cause increasing of the surface area, and hence increase the antibacterial activity. Furthermore, the cell membrane has nanosized pores which allow the smaller nanoparticles easily to penetrate the cell and reach to the nuclear content of bacteria (reverse osmosis) [22]. Eventually, the antibacterial properties of NPs are difficult and include diverse routes. Consequently, the mechanism of antibacterial action is yet vague [23].

2. Experiment parts

2.1. Bacterial Isolates

Five bacterial isolates were used in the current research for the detection of rGO NPs biological activity, i.e., (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia*, and *Pantoea agglomerans*), which were isolated from different sources like burns, wounds, and urinary infections, diagnosed by VITEK-2 system.

2.2. Antibiotics sensitivity test of pathogenic isolates

The susceptibility of antibiotics test (AST) was conduct utilizing VITEK2 system.

2.3. Antibacterial Activity of rGO NPs

The antibacterial efficacy of rGO NPs were evaluated by using an agar-well diffusion technique against five bacterial pathogens i.e. (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Pantoea agglomerans*).

A free culture of pure five isolate colonies was suspended in brain heart infusion broth (5ml), to get an optical density equal to the 0.5 McFarland criteria (equivalent to 1.5x108 cells/ml). The mixture was incubated overnight at 37 °C. Moreover, the turbid growth culture was calibrated using sterile broth [24]. A sterilized cotton swab was dipped into the solution and used to streak it across the whole surface of a Mueller-Hinton agar plate. Stock concentrations of rGO nanoparticles and antibiotic standard drug ampicillin were prepared at three different concentrations (0.05, 0.1, and 0.2 mg/ml). After that, 4 wells were produced using a sterile cork porer in each plate and 100 µL of both rGO nanoparticles and ampicillin were poured into wells that had been prepared on agar plates. Distilled water served as the negative control. The pore diameter measure was 7 millimeters.

Finally, the Petri dishes were incubated at 37 °C overnight in an incubator to assess the antibacterial effect of rGO nanoparticles in comparison with antibiotic drug. The diameter of the growth inhibition zones was measured in millimeters. All the test plates were prepared in triplicate and incubated for 24 h at 37°C. After incubation, transparent ruler was used to measure the zone of inhibition (ZOI) diameter around the well [24,25]. Ampicillin represents the positive control, while distilled water acts as the negative control [26].

3. Results and Discussion

Antibacterial Activity of rGO NPs

The antibacterial activity of different concentrations (0.05, 0.1 and 0.2 mg/ml) of Bacillus coagulans-reduced graphene oxide (BA-rGO) and Streptococcus thermophilus-reduced graphene oxide (ST-rGO) nanoparticles against clinical pathogenic isolates Gram-positive isolate (*Staphylococcus aureus*) and Gram-negative isolates (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, and *Pantoea agglomerans*) were studied by agar well diffusion method. The inoculated plates were incubated at 37oC for overnight for detection of antibacterial activity.

The results showed that both nanoparticles had potent inhibitory effects against all the tested clinical pathogenic isolates, paradoxically ampicillin has slight inhibitory effects against all the tested clinical pathogenic isolates. The growth inhibition zones clearly revealed that both nanoparticles inhibited the growth of tested clinical pathogenic isolates where the highest concentration of both nanoparticles have the strongest antibacterial activity and their inhibited zone ranged from 10.0±1.73 to 27.0±1.73 mm, whereas ampicillin revealed inhibition zone ranged from 0 to 13.0± 1.0 mm.

The obtained results revealed a significantly high zone of inhibition against Gram-positive isolate and Gram negative isolates. BA-rGO revealed the highest zone of inhibition (27.0±1.73 mm) at concentration (0.2 mg/ml) against Escherichia coli and Pantoea agglomerans in comparison to positive control (ampicillin) with a zone of inhibition of (11.33±0.58 and 12.33±0.58), respectively ($p \leq 0.05$), while ST-rGO revealed the largest zone of inhibition (25.0±1.0 mm) at the same concentration against Escherichia coli in comparison to ampicillin with a zone of inhibition of (11.33±0.58) ($p \leq 0.05$). On the other hand, the negative control (distilled water) has showed no inhibition zone with 0.0 mm. As shown in Figure 1.

The obtained results are in agreement with those found by several investigators [27-29] who stated that the antibacterial activity of Bio-reduced graphene oxide (B-rGO) indeed demonstrated potent

growth inhibition zones was increased with increasing concentrations of B-rGO from Escherichia coli, Bacillus clausii and Lysinibacillus sphaericusm, respectively.

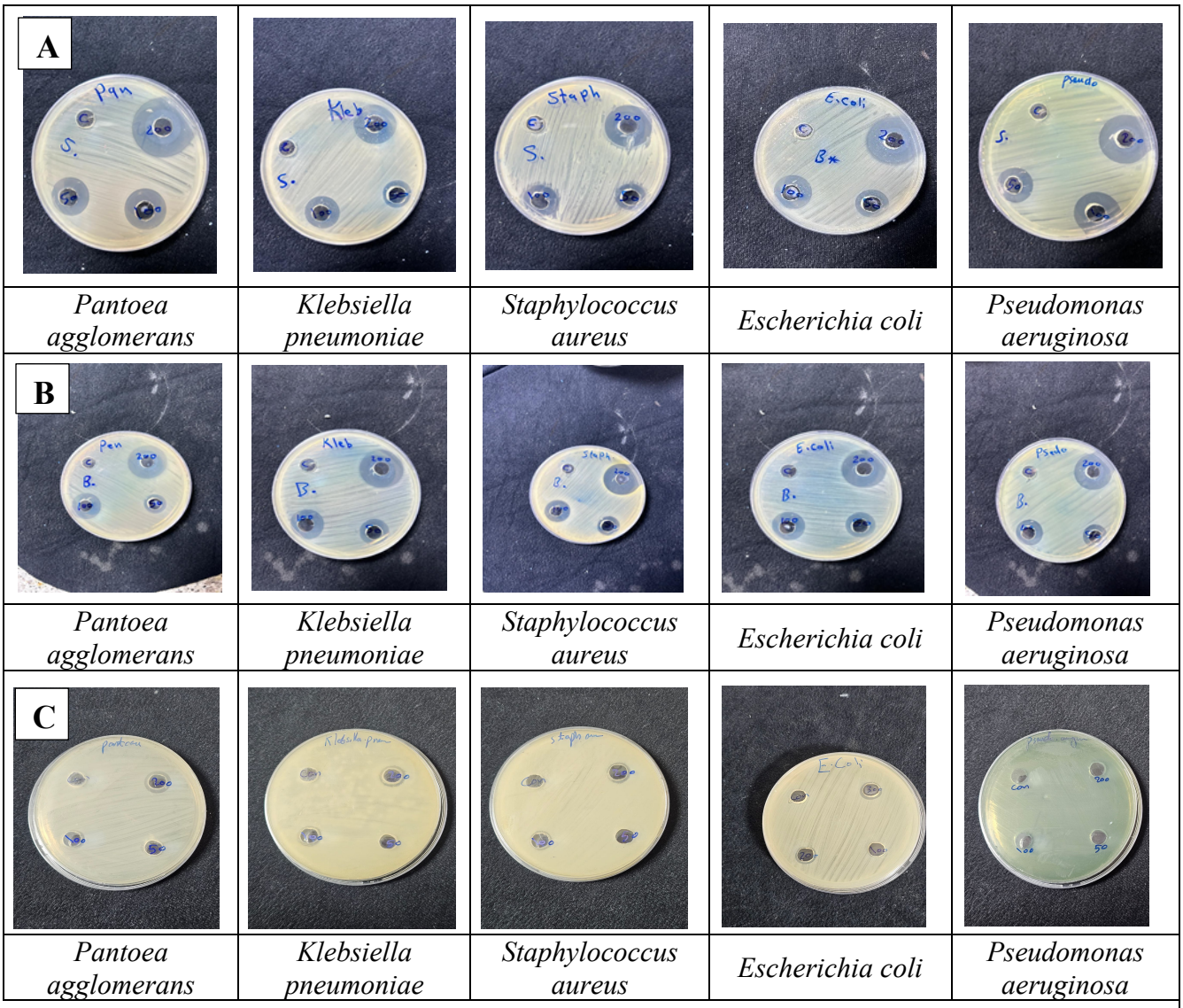

Figure 1: Illustrate the antibacterial activity of (A) ST-rGO, (B) BA-rGO Nanoparticles and (C) ampicillin against MDR bacterial isolates

Initially, B-rGO can provide a unique isolation barrier between two different regions and encapsulate microorganisms [30]. In addition, due to the lamellar structure of reduced graphene oxide (rGO), its sharp edges can cut and rupture cell membranes during contact with cells, thereby destroying the integrity of cell structure and being toxic to the cells [31,32], furthermore, B-rGO leads to oxidative stress by directly forming Reactive Oxygen Species (ROS) or consuming cellular antioxidants [33,34]. As a result, B-rGO shows steady biocompatibility compared to that commercial reduced graphene oxide (C-rGO) [35].

Table 1: Evaluation of antibacterial activity of BA-rGO nanoparticles against MDR pathogenic isolates as compared to ampicillin.

Microorganisms	Zone of inhibitions (mm), mean ± SD, n = 3					
	Concentration of Ampicillin (µg/ml)			Concentration of BA-rGO (µg/ml)		
	50	100	200	50	100	200
Pseudomonas aerugnosa	-	9.33±0.58	10.33±0.58	15.67±1.15	18.33±1.5	25.33±3.06
Staphylococcus aureus	-	9.67±0.58	11.0± 1.0	15.67±0.58	20.0±2.0	26.67±3.06
Pantoea agglomerans	-	10.67±0.58	12.33±0.58	16.67±1.53	22.0±0.0	27.0±1.73
Escherichia coli	-	9.33± 0.58	11.33±0.58	16.33±0.58	19.0±1.0	27.0±1.0
Klebsiella pneumoniae	-	10.33±0.58	13.0± 1.0	16.0±1.73	19.0±1.0	25.0±1.0

(-) indicates that the absence of inhibited zone as no ampicillin and antibacterial activities.

Values are means of 3 replicates ± standard deviations.

Table 2: Evaluation of antibacterial activity of ST-rGO nanoparticles against MDR pathogenic isolates as compared to ampicillin.

Microorganisms	Zone of inhibitions (mm), mean ± SD, n = 3					
	Concentration of Ampicillin (µg/ml)			Concentration of ST-rGO (µg/ml)		
	50	100	200	50	100	200
Pseudomonas aerugnosa	-	9.33±0.58	10.33±0.58	17.0±1.73	20.33±2.89	24.0±0.0
Staphylococcus aureus	-	9.67±0.58	11.0± 1.0	16.33±5.13	20.33±4.93	24.67±5.03
Pantoea agglomerans	-	10.67±0.58	12.33±0.58	14.67±3.06	18.67±1.15	24.67±4.62
Escherichia coli	-	9.33±0.58	11.33±0.58	10.0±1.73	18.0±1.0	25.0±1.0
Klebsiella pneumoniae	-	10.33±0.58	13.0± 1.0	12.0±2.0	19.0±1.0	23.0±1.73

(-) indicates that the absence of inhibited zone as no ampicillin and antibacterial activities.

Values are means of 3 replicates ± standard deviations.

Finally, from the current results above it can be concluded that rGO NPs demonstrated a significant antibacterial activity against the tested pathogenic MDR bacterial isolates, and the efficiency increased with the increasing of biogenic rGO NPs concentration.

4. References

[1] Muteeb, G., Rehman, M. T., Shahwan, M., & Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals, 16(11), 1615.

[2] Hirsch, E. B., Zucchi, P. C., Chen, A., Raux, B. R., Kirby, J. E., McCoy, C., & Eliopoulos, G. M. (2016). Susceptibility of multidrug-resistant gram-negative urine isolates to oral antibiotics. Antimicrobial agents and chemotherapy, 60(5), 3138-3140.

[3] Hu, Y., Zhang, T., Jiang, L., Yao, S., Ye, H., Lin, K., & Cui, C. (2019). Removal of sulfonamide antibiotic resistant bacterial and intracellular antibiotic resistance genes by UVC-activated peroxymonosulfate. Chemical Engineering Journal, 368, 888-895.

[4] Dhingra, S., Rahman, N. A. A., Peile, E., Rahman, M., Sartelli, M., Hassali, M. A., ... & Haque, M. (2020). Microbial resistance movements: an overview of global public health threats posed by antimicrobial resistance, and how best to counter. Frontiers in Public Health, 8, 535668.

[5] Yadegarynia, D., Karimi, J., Rahmati Roodsari, S., & Arab-Mazar, Z. (2017). Evaluation of the antimicrobial resistance of Klebsiella pneumoniae by E-Test method in Khatam_ol_Anbia Hospital, Tehran, Iran, during 2015. Infection Epidemiology and Microbiology, 3(1), 9-11.

[6] Gu, X., Xu, Z., Gu, L., Xu, H., Han, F., Chen, B., & Pan, X. (2021). Preparation and antibacterial properties of gold nanoparticles: A review. Environmental Chemistry Letters, 19, 167-187.

[7] Muthukrishnan, L. (2021). Multidrug resistant tuberculosis–Diagnostic challenges and its conquering by nanotechnology approach–An overview. Chemico-biological interactions, 337, 109397.

[8] Besinis, A., De Peralta, T., & Handy, R. D. (2014). The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays. Nanotoxicology, 8(1), 1-16.

[9] Zhao, X., Jia, Y., Li, J., Dong, R., Zhang, J., Ma, C., ... & Jiang, X. (2018). Indole derivative-capped gold nanoparticles as an effective bactericide in vivo. ACS applied materials & interfaces, 10(35), 29398-29406.

[10] Zou, X., Zhang, L., Wang, Z., & Luo, Y. (2016). Mechanisms of the antimicrobial activities of graphene materials. Journal of the american chemical society, 138(7), 2064-2077.

[11] ElMekawy, A., Hegab, H. M., Losic, D., Saint, C. P., & Pant, D. (2017). Applications of graphene in microbial fuel cells: the gap between promise and reality. Renewable and Sustainable Energy Reviews, 72, 1389-1403.

[12] Ji, H., Sun, H., & Qu, X. (2016). Antibacterial applications of graphene-based nanomaterials: recent achievements and challenges. Advanced drug delivery reviews, 105, 176-189.

[13] Tremblay, P. L., Li, Y., Xu, M., Yang, X., & Zhang, T. (2020). Graphene electrodes in bioelectrochemical systems. In Microbial Electrochemical Technologies (pp. 422-443). CRC Press.

[14] Mohammed, H., Kumar, A., Bekyarova, E., Al-Hadeethi, Y., Zhang, X., Chen, M., ... & Rimondini, L. (2020). Antimicrobial mechanisms and effectiveness of graphene and graphene-functionalized biomaterials. A scope review. Frontiers in bioengineering and biotechnology, 8, 465.

[15] Rani, M. N., Ananda, S., & Rangappa, D. (2017). Preparation of reduced graphene oxide and its antibacterial properties. Materials Today: Proceedings, 4(11), 12300-12305.

[16] Alayande, A. B., Obaid, M., & Kim, I. S. (2020). Antimicrobial mechanism of reduced graphene oxide-copper oxide (rGO-CuO) nanocomposite films: The case of Pseudomonas aeruginosa PAO1. Materials Science and Engineering: C, 109, 110596.

[17] Mann, R., Mitsidis, D., Xie, Z., McNeilly, O., Ng, Y. H., Amal, R., & Gunawan, C. (2021). Antibacterial activity of reduced graphene oxide. Journal of Nanomaterials, 2021(1), 9941577.

[18] Deng, C. H., Gong, J. L., Zeng, G. M., Niu, C. G., Niu, Q. Y., Zhang, W., & Liu, H. Y. (2014). Inactivation performance and mechanism of Escherichia coli in aqueous system exposed to iron oxide loaded graphene nanocomposites. Journal of hazardous materials, 276, 66-76.

[19] He, L., Dumée, L. F., Feng, C., Velleman, L., Reis, R., She, F., ... & Kong, L. (2015). Promoted water transport across graphene oxide–poly (amide) thin film composite membranes and their antibacterial activity. Desalination, 365, 126-135.

[20] Orooji, Y., Liang, F., Razmjou, A., Liu, G., & Jin, W. (2018). Preparation of anti-adhesion and bacterial destructive polymeric ultrafiltration membranes using modified mesoporous carbon. Separation and Purification Technology, 205, 273-283.

[21] Li, X., Robinson, S. M., Gupta, A., Saha, K., Jiang, Z., Moyano, D. F., ... & Rotello, V. M. (2014). Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. ACS nano, 8(10), 10682-10686.

[22] Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., ... & Mohamad, D. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-micro letters, 7, 219-242.

[23] Pandit, C., Roy, A., Ghotekar, S., Khusro, A., Islam, M. N., Emran, T. B., ... & Bradley, D. A. (2022). Biological agents for synthesis of nanoparticles and their applications. Journal of King Saud University-Science, 34(3), 101869.

[24] Ghanim, R. R., Mohammad, M. R., & Hussien, A. M. A. (2018). Antibacterial activity and morphological characterization of synthesis Graphene oxide Nanosheets by simplified Hummer's method. Biosciences Biotechnology Research Asia, 15(3), 627.

[25] Alangari, A., Mateen, A., Alqahtani, M. S., Shahid, M., Syed, R., Shaik, M. R., ... & Kuniyil, M. (2023). Antimicrobial, anticancer, and biofilm inhibition studies of highly reduced graphene oxide (HRG): In vitro and in silico analysis. Frontiers in Bioengineering and Biotechnology, 11, 1149588.

[26] Liaqat, N., Jahan, N., Anwar, T., & Qureshi, H. (2022). Green synthesized silver nanoparticles: Optimization, characterization, antimicrobial activity, and cytotoxicity study by hemolysis assay. Frontiers in Chemistry, 10, 952006.

[27] Al-Hilli, Z.B. and Aldujaili, N.H. (2020). Activity of Biosynthesized Reduced Graphene Oxide against Multidrug resistant Uropathogenic Bacteria. Indian Journal of Forensic Medicine and Toxicology, 14(2).

[28] Almamad, M.F. and Aldujaili, N.H. (2022). Antibacterial and antibiofilm activity of bacterially reduced graphene oxide against some MDR bacterial pathogens isolated from urinary tract infections. In AIP Conference Proceedings, 2386(1).

[29] Zhou, Y., Xue, C., Gan, L., Owens, G., & Chen, Z. (2023). Antibacterial activity of reduced graphene oxide prepared by microbe. Materials Today Sustainability, 22, 100341.

[30] Akhavan, O., Ghaderi, E., & Esfandiar, A. (2011). Wrapping bacteria by graphene nanosheets for isolation from environment, reactivation by sonication, and inactivation by near-infrared irradiation. The journal of physical chemistry B, 115(19), 6279-6288.

[31] Guo, S., Lin, J., Wang, Q., Megharaj, M., & Chen, Z. (2018). The toxicity of graphene and its impacting on bioleaching of metal ions from sewages sludge by Acidithiobacillus sp. Chemosphere, 195, 90-97.

[32] Zhou, X., Zhou, M., Ye, S., Xu, Y., Zhou, S., Cai, Q., ... & Li, Y. (2021). Antibacterial activity and mechanism of the graphene oxide (rGO)-modified TiO2 catalyst against Enterobacter hormaechei. International Biodeterioration & Biodegradation, 162, 105260.

[33] Gurunathan, S., Han, J. W., Dayem, A. A., Eppakayala, V., & Kim, J. H. (2012). Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in Pseudomonas aeruginosa. International journal of nanomedicine, 5901-5914.

[34] Dhakshinamoorthy, A., López-Francés, A., Navalon, S., & Garcia, H. (2022). Graphitic Carbon Nitride as Visible-Light Photocatalyst Boosting Ozonation in Wastewater Treatment. Nanomaterials, 12(19), 3494.

[35] Cheng, C., Nie, S., Li, S., Peng, H., Yang, H., Ma, L., ... & Zhao, C. (2013). Biopolymer functionalized reduced graphene oxide with enhanced biocompatibility via mussel inspired coatings/anchors. Journal of Materials Chemistry B, 1(3), 265-275.