THE EFFECT OF GRAPHENE AND GRAPHENE-OXIDE NANOPARTICLES ON THE AEROBIC GRANULAR SLUDGE WASTEWATER TREATMENT PROCESS

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Abstract
The responses of aerobic granular sludge (AGS) to the presence of graphene and graphene-oxide nanoparticles (G and GO NPs) at various concentrations (1, 5 and 10 mg/L) during biological wastewater treatment processes were investigated. Bioreactor performance and extracellular polymeric substance (EPS) secretion were assessed. The results showed that the effects of G and GO NPs on bioreactor performances were dependent on the material and dose applied, especially in terms of chemical oxygen demand (COD) and phosphorus removal. Nitrite and nitrate removal rates were unchanged. The secretion of EPS, which could alleviate the toxicity of GO NPs, also changed. GO NPs were found to be more toxic than G NPs, indicating that structural changes may affect their activity.

Introduction
Carbon-based nanomaterials, such as fullerenes, carbon nanotubes, and graphene derivatives, have gained widespread use, and thus, their global production is continuously increasing [1]. The extremely interesting properties of graphene such as its great mechanical strength, high electrical, thermal and magnetic properties, and unique optical properties have made it a potential candidate to be used in several fields of engineering. They are widely used in industry as sensors and semiconductors as well as in energy storage and water purification [2]. Notably, its favorable biocompatibility, extensive surface area, impressive optical properties, and the ease with which it can be bio-functionalized, along with its derivatives like graphene oxide (GO) have gained attention in areas such as drug/gene delivery and tissue engineering. Due to their versatility, graphene and their derivatives are the most commonly produced carbon-based nanomaterial. However, graphene and graphene oxide NPs exhibit biotoxicity towards various microorganisms and can reduce soil enzyme activity. While the effects weaken with time and can be reversed after a single dosage, their increasing production raises concerns about environmental release and concentrations in wastewater [3]. Moreover, their long-term stability in water leads to their accumulation in wastewater treatment plants, potentially impacting biological wastewater processes. The conventional activated sludge (CAS) process, commonly used for sewage treatment, may be negatively affected by G and GO NPs, prompting the exploration of aerobic granular sludge (AGS) processes. AGS offers advantages, including tolerance to toxins, excellent settling, diverse microbial composition, and concurrent removal of organic matter, nitrogen, and phosphorus. Due to the need to understand the effects of G and GO NPs on AGS, we conducted experiments to assess the response of AGS towards these materials. We monitored reactor performance, including chemical oxygen demand, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, and phosphorus removal, and EPS production.

Experimental

Synthesis and characterization of G and GO NPs

A modified Hummer's method was used to prepare GO NPs [4]. All chemicals used were of reagent grade (Sigma-Aldrich). Firstly, 9 g of graphite powder and 9 g of sodium nitrate were mixed with 420 mL concentrated sulfuric acid solution at 0 °C in an ice bath for 30 min. This solution was oxidized by slowly adding 54 g of potassium permanganate and stirring at ambient temperature for 1 day. The mixture was then supplemented with 1000 mL deionized water and 20 mL 30 % (w/w) hydrogen peroxide and stirred at 0 °C for 1 h. Thereafter, the product was washed with an excess of distilled water, and the resulting brownish GO NPs were freeze-dried. G NPs were purchased purchased from Sigma Aldrich. To assess particle size and morphology of G and GO NPs, transmission electron microscopy (TEM) at 200 kV were used (FEI Tecnai G2 20 X-Twin microscope and Hitachi S-4700 Type II FE-SEM microscope). The crystalline structure of the particles was characterized via X-ray diffraction (XRD) using a Rigaku MiniFlex II powder X-ray diffractometer with a Cu Kα irradiation source. The scanning rate was $2°min^{-1}$ over a 2θ range of 5°–40°.

Configuration of AGS reactors

The control AGS sequencing batch reactor (SBR) was fed with synthetic wastewater (SWW), whereas other AGS reactor were fed with G and GO NP-contaminated SWW at different concentrations (1, 5 10 mg/L G and GO NPs, G1, G5, G10, GO1, GO5, GO10). The chemical oxygen demand (COD), NH_4-N, NO_2-N, NO_3-N, and PO_4-P concentrations were continuously measured, while the extracellular polymeric substance (EPS) content, were determined at the end of experiments.

Results and discussion

In case of control bioreactor, the effluent chemical oxygen demand, nitrate, nitrite, ammonia, phosphorus contents were stable during the whole experiment. In all experiments, the concentrations of nitrite and nitrate remained stable throughout the whole operation period, even in the control bioreactor (Figure 1). These observations correspond to the previous AGS studies wherein copper-oxide and silver nanoparticles did not influence the removal efficiency of these components [5,6].

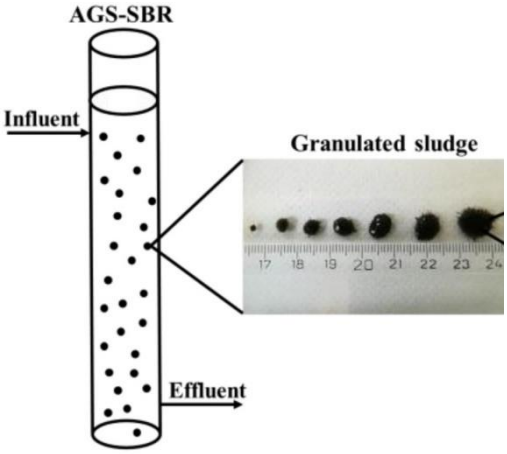

Figure 1. The schematic figure of the reactor [4]

The exposure to both graphene (G) and graphene oxide (GO) particles at various concentrations (1, 5 and 10 mg/L) did not have a detrimental impact on the reactor effluent's nitrate level, nitrate concentrations remained stable throughout the experiment. However, during this exposure, there was a notable and significant increase in ammonia concentration. At a low concentration of 1 mg/L, neither G nor GO nanoparticles affected COD removal even after 10 days. When exposed to 5 mg/L of GO nanoparticles, a slight increase in COD was noted after 6 days, while G nanoparticles exhibited stable COD removal, indicating their tolerance by the aerobic granular sludge. All the two nanomaterials significantly impacted COD removal when exposed to a higher concentration of 10 mg/L. GO NPs were more toxic with effluent COD exceeding limits, while G NPs had a milder effect, indicating granular sludge's ability to tolerate them.

Biological phosphorus removal in AGS changed in the same tendence as in case in COD removal. The phosphorus removal was adversely affected by graphene and graphene-oxide nanoparticles as well. G and GO at 1 mg/L did not significantly alter phosphorus removal. However, GO NPs at 5 mg/L slightly increased phosphate content in the effluent after 10 days. Moreover, GO NPs in higher concentrations strongly hindered phosphate removal by granular sludge. On the other hand, G NPs, even at the highest concentration, had a lesser impact, resulting in effluent phosphate contents. This suggests that microorganisms responsible for phosphorus removal tolerated G NPs better than GO NPs, likely due to the well-known high antimicrobial effects of GO NPs causing bacterial cell disruption. Similar observations were also reported, wherein ZnO and CuO NPs negatively influenced the phosphorus removal in case of AGS [5,6].

Extracellular polymeric substances (EPS), the primary components of granular sludge, are metabolic byproducts of various microorganisms and play a crucial role in protecting against toxic substances. Hence, it is essential to monitor EPS changes in granular sludge. In control AGS, PS levels did not change during nanoparticles exposure. This was in line with earlier studies, where AGS system was treated with different NPs (CeO_2, CuO, and ZnO) [5,6,7]. On the other hand, PN secretion increased significantly with nanomaterial introduction (Figure 2.).

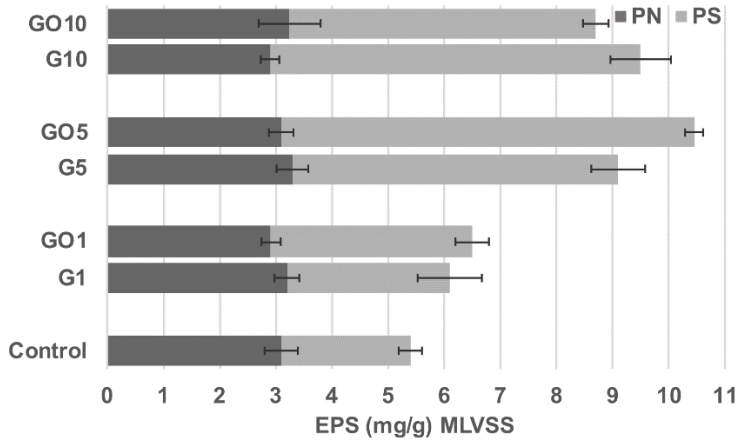

Figure 2. Effects of GO NPs on EPS contents of AGS

With G NPs addition, the PN and EPS levels slowly rose with increasing NP concentrations. This indicates that G NPs had low toxicity initially, allowing microorganisms to adapt and produce more extracellular polymeric substances.

The introduction of GO nanoparticles also enhanced the amount of EPS in sludge, although their utilization in high concentration (10 mg/L) caused high toxicity towards microorganisms which were not able to secrete the appropriate amount of EPS. The higher toxicity of GO NPs compared to G NPs may be due to structural alterations, as GO NPs tends to aggregate less than G nanoparticles.

Conclusion

In this study, the impact of graphene (G) and graphene oxide (GO) at various concentrations (1, 5, and 10 mg/L) on nutrient removal and extracellular polymeric substances secretion in aerobic granular sludge (AGS) SBRs was investigated. Overall, the findings highlight the complex and concentration-dependent effects of graphene and graphene oxide nanoparticles on AGS performance, especially in terms of COD and phosphorus removal. The research highlights AGS's ability to adapt to nanoparticle exposure, with G nanoparticles exhibiting lower toxicity and GO nanoparticles displaying higher toxicity, possibly due to structural variations. These observations emphasize the significance of carefully assessing nanoparticle effects in wastewater treatment and the need for further research to comprehend and mitigate their potential environmental consequences.

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