Ecotoxicology and Environmental Safety 156 (2018) 287–293

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effect of graphene oxide on the bioactivities of nitrifying and denitrifying bacteria in aerobic granular sludge

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ARTICLE INFO

Keywords:
Aerobic granular sludge (AGS)
Graphene oxide (GO)
Ammonium oxidizing bacteria (AOB)
Nitrite oxidizing bacteria (NOB)
Denitrifying bacteria
Extracellular polymeric substances (EPS)

ABSTRACT

With the widespread application of graphene oxide (GO), it would be inevitably released into wastewater treatment plants (WWTPs) and get involved in the biochemical process. So far, there are controversies on the effects of low GO concentration (0.05–0.1 g/L) on the nitrogen removal process. Therefore, this study essentially investigates any potential effects of GO on wastewater microbial communities functions. In present study, the nitrifying and denitrifying batch tests were introduced to investigate the influence of 0.06 g/L of GO on bacteria. The results showed that GO could be easily combined with the aerobic granular sludge (AGS), and NH_4^+-N was sharply absorbed, which directly promoted the bioactivities of ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) and extracellular polymeric substances (EPS) production. The influence of GO on the denitrifying bacteria was negligible, which resulted in the stable EPS production. Furthermore, as inferred from the near maximum chemical reaction rates, there were no obvious changes on the microbial community functions during nitrogen removal process.

1. Introduction

Today, graphene and its derivatives have been intensively applied in high-speed electronics, high-frequency transistor, energy generation and storage, supercapacitors, biosensors, environment, and other fields (Morales-Narváez et al., 2017; Novoselov et al., 2012; Suárez-Iglesias et al., 2017). As previous reported, graphene products market is expected to reach $195 million in 2018 (Mcwilliams, 2013). The current mass-production of graphene-based materials (GBM) inevitably causes graphene to be released into wastewater treatment plants (WWTPs) (Combarros et al., 2016; Suárez-Iglesias et al., 2017). To date, a few researchers have paid attention to the potential health and ecological risks of these nanomaterials (Jastrzębska and Olszyna, 2015; Nowack, 2017; Smith and Rodrigues, 2015). Furthermore, they have addressed the environmental behavior and eco-toxicity of these materials (Ioniţă et al., 2017; Liu et al., 2012; Marković et al., 2017). However, not sufficient information about their effect on biological treatment was provided in these reports.

Graphene oxide (GO) is an oxidized graphene derivative, in which epoxide groups and phenol hydroxyl are on basal plane, and carboxylic groups are at edges (Lalwani et al., 2016; Lee et al., 2016; Liu et al., 2012; Sharma et al., 2017; Suárez-Iglesias et al., 2017). Because of the high dispersibility and processibility of these groups in aqueous solvents, GO is used as an active alternative or precursor for graphene materials, which would accompany wastewater and enter the WWTPs (Li et al., 2008; Morales-Narváez et al., 2017; Suárez-Iglesias et al., 2017). Therefore, GO might have influence on the biological process of microorganisms. When bacteria are cultured with 0.025 g/L of GO, the optical density and bacteria growth rate are increased, resulting in the improvement of the cell attachment and proliferation (Ruiz et al., 2011). It was concluded that with 0.1 g/L of GO, the removal efficiency of NH_4^+-N is improved and the amount of extracellular polymeric substances (EPS) production is increased (Wang et al., 2013). Furthermore, when the anammox sludge is stored with 0.1 g/L of GO at 4 °C, the reaction of anammox is obviously promoted (Wang et al., 2014a). These studies show that GO at the different concentration levels have positive effect on different microorganisms. However, when GO concentrations changed from 0.05 to 0.3 g/L, the microbial communities functions and nitrogen removal efficiency are gradually impacted, especially at higher concentration (≥ 0.1 g/L) the sludge microbial community functions are remarkably inhibited (Ahmed and Rodrigues, 2013). Furthermore, previous findings suggest that 0.1 g/L of GO would

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https://doi.org/10.1016/j.ecoenv.2018.03.036
Received 7 December 2017; Received in revised form 8 March 2018; Accepted 9 March 2018

stimulate microorganisms to produce more reactive oxygen species (ROS) and damage the cell membrane (Ahmed and Rodrigues, 2013; Chen et al., 2016). Previous studies suggested that low GO concentration (< 0.05 g/L) has positive influence on the microbial community functions, while at higher concentration (> 0.1 g/L), the effects is significantly negative. However, the reports about the influence of GO concentration (between 0.05 and 0.1 g/L) are controversial, which would attract researchers to perform in-depth investigation.

Nitrogen is a key pollutant in sewage and some industrial wastewater. Nitrogen in water body would promote excessive growth of algae and aquatic plants, and result in dissolved oxygen (DO) decrease and water quality deterioration, which is potentially harmful to human health (Erisman et al., 2013; Nancharaiah et al., 2016). In China, nitrogen pollution of surface water is most widespread (Tong et al., 2017). It is highly essential to implement the removal of nitrogen compounds from wastewater. Conventional nitrogen removal methods include autotrophic nitrification and heterotrophic denitrification processes. In recent years, aerobic granular sludge (AGS) has been proposed as a promising strategy to nitrogen removal (Bassin et al., 2012; Caluwé et al., 2017; Dobbeleers et al., 2017; Henriet et al., 2016; Suja et al., 2015; Yan et al., 2016). Due to the decrease of oxygen gradient inside AGS, the aerobic and anoxic micro-zones are contributed to the simultaneous nitrification-denitrification, which is good for reducing energy input (Caluwé et al., 2017; Dobbeleers et al., 2017; Pronk et al., 2015, 2017). In order to ensure nitrogen removal efficiency under any possible conditions, the potential influence of some factors (such as GO) on the activities of microorganisms should be carefully evaluated. Thus, the investigation about the influence of low GO concentration on the autotrophic nitrobacteria and heterotrophic denitrifying bacteria in AGS is promising and would provide guidance for WWTPs operation.

As for AGS, EPS has to be introduced since EPS can protect bacteria against noxious shocks, enhance their aggregation and provide nutrition to microorganisms (More et al., 2014). EPS plays a crucial role in AGS formation and stability (Adav et al., 2008; Corsino et al., 2016, 2017). Most of the researchers think that EPS are composed of polysaccharides (PS), proteins (PN), phospholipids, nucleic acids, and humus (HA), and the former two are the major constituents (Adav and Lee, 2008; McSwain et al., 2005; Sarma et al., 2017). As a rich matrix of polymers, EPS can be used as an ion exchanger for cation (metal and ammonium ions) owing to its abundant oxygen-containing functional groups (e.g., -COOH, -NH, -OH, -CO-) (Bassin et al., 2011; Yan et al., 2016). With this regard, EPS has been believed to play an important role in NH_4^+-N adsorption in a pilot-scale AGS reactor (Bassin et al., 2011). Furthermore, Yan et al. (2016) pointed out that 32.94% of the total nitrogen (TN) removal is contributed to the adsorption of EPS to NH_4^+-N, NO_2^--N, and NO_3^--N. The functions of EPS in AGS have been reported in many studies. However, the effect of GO on EPS and nitrogen removal has been rarely emphasized, especially at low GO concentration level.

Therefore, since possible short-time exposure of WWTPs to the low GO concentration (between 0.05 and 0.1 g/L) could occur, the main objective of this study is to investigate the effect of low GO concentration on EPS and biological activities of autotrophic nitrobacteria and heterotrophic denitrifying bacteria. A 0.06 g/L of GO concentration was chosen. For this purpose, a series of denitrification and nitrification batch experiments of AGS were conducted with and without 0.06 g/L of GO. After the tests, AGS samples were taken out for EPS extraction and analysis was performed to determine the amount of PN, PS and HA. The outcomes from this research would help to better understand the complex relationships among GO, EPS, nitrogen removal efficiency, and microbial bioactivities.

2. Materials and methods

2.1. GO preparation and characterization

GO sheets were purchased from Xian Feng NANO Co., Ltd.. The product was prepared using the modified Hummer's method (Li et al., 2008; Papageorgiou et al., 2017), according to the manufactures' instructions. A stock solution of GO (6 g/L) was prepared in ultrapure water and then sonicated at 250 W (Sonics & Material, USA) for 2 h to disperse the natural materials. The obtained suspension was stored in the dark at 4 °C for future use.

In order to investigate GO characterization, some test techniques were applied. The GO powder was taken to observe the typical morphology by scanning electron microscopy (SEM, Zeiss Ultra Plus, Germany) and to identify the molecular structure and functional groups by Fourier Transform Infrared (FTIR) spectrometer (Bruker 27, Germany). The 0.06 g/L of GO solution was taken for UV–vis spectrum analysis (Lambda 750S, USA).

2.2. AGS preparation

AGS had been cultivated for 400 days with synthetic wastewater (see S1 of the Supplementary material) in a laboratory-scale sequencing batch reactor (SBR) (see Fig. S1 of the Supplementary material). The yellowish mature granules appeared in near round shape with compact structure and maintained a stable size distribution with a median value of 1 mm in accordance with the results from Corsino et al. (Corsino et al., 2016). The average settling velocity of individual granule was maintained at 0.9–1.1 cm/s with specific hydraulic selection pressure. The sludge volume index after 5 min settling (SVI_5) remained at around 22–25 mL/g SS. The mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were maintained at 10,000–11,000 mg/L and 7000–8000 mg/L, respectively. When the nitrogen removal efficiency of AGS in parent SBR were maintained at 92.8–96.3%, 0.2 L AGS mixture was taken out every day and washed with ultrapure water for 3 times (6000 rpm for 10 min, TGL-185, China) and the residuum was frozen for batch tests. Thus the sludge retention time (SRT) of AGS was approximately 20 days. SRT is calculated as follows:

$$\theta_c = \frac{CV}{X_e V_e + CQ_w} \quad (1)$$

where θ_c represents SRT (d); C represents MLSS (mg/L); V is the working volume of SBR (L); X_e is the concentration of suspended solids in the effluent (mg/L); V_e is the effluent volume for each day (L); and Q_w represents discharge volume (L) of the mixture.

The AGS, which would be used for the batch tests, was collected for 30 days. When the AGS mixture volume reached 2000 mL, the frozen AGS was taken out for thawing for batch tests.

2.3. Batch tests

The unfrozen AGS was rewashed by ultrapure water and re-suspended in ultrapure water to 2000 mL. Then the mixture was equally separated into four 1000 mL flat bottom conical flasks. In order to investigate the effects of 0.06 g/L of GO on the heterotrophic denitrifying bacteria and autotrophic nitrobacteria, two flasks, which was named flask 1# and 3#, were used for denitrification and nitrification tests without GO, the remaining two flasks, which was named flask 2# and 4#, were used for tests with GO.

2.3.1. Denitrification tests

A 10 mL 6 g/L GO stock suspension and 490 mL nutrient solution (containing 306 mg/L soluble chemical oxygen demand (COD_{Cr}) of glucose and 17.3 mg/L NO_3^--N) were simultaneously added into flask 2#, and then the reaction was started. After 10 s, 5 mL supernatant was