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Short Communication

Using graphene oxide to enhance the activity of anammox bacteria for nitrogen removal

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HIGHLIGHTS

- The activity of anammox bacteria can be enhanced in dose-dependent manner of GO.
- The maximum 10.26% increase in activity is obtained at a GO dose of 0.1 g L-1.
- The appropriate GO dose can efficiently stimulate the increase of EPS.
- GO can be used as a scaffold for anammox bacteria attachment.

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ABSTRACT

Graphene oxide (GO) was applied in this study to enhance the activity of anaerobic ammonium oxidation (anammox) bacteria for nitrogen removal. A GO dose-dependent effect on anammox bacteria was observed through batch tests. The results showed that the activity increased as the GO dose was varied within 0.05–0.1 g L^{-1}. A maximum 10.26% increase of anaerobic ammonium oxidizing activity was achieved at 0.1 g L^{-1} GO. Analysis of extracellular polymeric substances (EPS) indicated that the highest carbohydrate, protein, and total EPS contents (42.5, 125.7, and 168.2 mg (g volatile suspended solids)$^{-1}$, respectively) were obtained with 0.1 g L^{-1} GO. Appropriate GO dose stimulated EPS production to promote the activity of anammox bacteria. Transmission electron microscopy showed the large surface area of GO benefited cell attachment. These findings proved that the application of GO was an effective approach to enhancing the activity of anammox bacteria.

1. Introduction

The anaerobic ammonium oxidation (anammox) process is one of the most innovative technological advances for the removal of nitrogenous contaminants from wastewater. This process consists in a combination of ammonium and nitrite by Planctomycete-type bacteria under anoxic conditions to generate nitrogen gas. In contrast to conventional biological nitrogen removal processes, the anammox process does not require external electron donors (e.g. methanol) because of the chemolithoautotrophic lifestyle of anammox bacteria. In addition, 63% less oxygen is supplied to the system because only some ammonium ions need to be nitrified to nitrite (Fux et al., 2002). Surplus sludge production is also minimal because of the low cell production rate. Although the anammox process has many potential benefits, it is still difficult to be widely applied in practical wastewater treatment because the low activity and growth rate of anammox bacteria (estimated doubling time of 7–11 days) cause a long system start-up time.

Over the past years, numerous researchers have attempted to shorten the start-up period by various means. On one hand, the key point is aimed at increasing the volumetric loading rate of the reactor, e.g. studying the settling ability and community composition of anammox granules, using the sludge wash-out strategy (Kieling et al., 2007), and selecting suitable inoculation sludge or reactors. On the other hand, an interesting and promising alternative is to improve the volumetric loading rate by enhancing the anaerobic ammonium oxidizing activity of the anammox bacteria, e.g. applying the static magnetic field or the low intensity ultrasound to enhance the activity of anammox microbial consortium for nitrogen removal (Duan et al., 2011). By this way, the specific nitrogen removal performance can be increased and the system start-up time can be shortened, even though the concentration of anammox bacteria is low.

Graphene oxides (GOs), which are layered and oxygenated graphene sheets with epoxide, carboxyl, and hydroxyl groups on their basal planes and edges, are attracting extensive attention in

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microbiology. These materials have extraordinary characteristics, such as large surface area, good colloidal property, and low cytotoxicity (Akhavan and Ghaderi, 2009). Previous investigations have shown that GO is highly biocompatible in terms of allowing the effective proliferation of some bacteria. Moreover, the additional GO is also a promising approach for the enhancement of biological activity. For example, GOs coated on filters induce the faster growth of *Escherichia coli* bacteria (Ruiz et al., 2011). Another study has demonstrated that the activities of human fibroblast cells are enhanced by increasing the dose of GO. However, when the dose is more than 50 μg mL^{-1}, GO exhibits obvious cytotoxicity such as inducing cell apoptosis, decreasing cell adhesion, and entering lysosomes (Wang et al., 2011).

The successful application of GO in microbiology suggests that GO may be an alternative for shortening the start-up time of the anammox process by enhancing the activity of anammox bacteria. In this paper, we studied the possibility of enhancing biological activity of anammox bacteria using GO in batch experiments. Then, the optimum dose and specific effect of GO on anammox bacteria were investigated.

2. Methods

2.1. Synthesis and characterization of GO

GO was prepared from natural graphite powder (500 mesh, SCR-20019128; China) by the modified Hummers method (Kovtyukhova et al., 1999). The crystallographic structures of natural graphite powder and GO were characterized by a powder X-ray diffraction (XRD) system (D/max-2400) with Cu Kα radiation (λ = 1.451 Å) at a scanning speed of 8°min^{-1} and 2θ of 5°–50°. The micromorphology of GO was observed by transmission electron microscopy (TEM) under standard operating conditions, and the oxygen-containing functional groups of GO were characterized by Fourier transform infrared (FTIR) spectroscopy (Bruker, IFS 66 V/S, Germany).

2.2. Microorganisms and feeding medium

The activated anammox bacteria used for inoculation originated from a laboratory-scale anammox upflow column reactor. Anammox bacteria accounted for about 89% of Planctomycete-type bacteria and belonged to *Candidatus Brocadia anammoxidans*. The feeding medium used in this experiment mainly contained NH_4^+-N and NO_2^--N in the form of $(NH_4)_2SO_4$ and $NaNO_2$. The composition of the mineral medium was as described by Third et al. (2001). For batch tests, the medium and anammox bacteria were prepared, and each of the batch serum bottles contained 100 mL of medium and approximately 0.35 (g volatile suspended solids (VSS)) L^{-1} biomass concentration.

2.3. Batch tests

All serum bottles were tightly sealed with rubber caps to avoid any external O_2 influence. The serum bottles were incubated at 35 °C in a dark shaker at a speed of 150 r min^{-1} for anaerobic analysis. Liquid samples were collected using syringes with needles to monitor the nitrogen concentrations over time. The GO doses in the range of 0–0.15 g L^{-1} were added to the serum bottles. Each cycle conducted for the bacterial activity was lasted for 42 h, and after 7 repetitive cycles, the average changes in each of the batch serum bottles were calculated for analysis.

2.4. Chemical analysis

The nitrogen compound concentrations (NH_4^+-N,NO_2^--N, and NO_3^--N) were colorimetrically measured, and the VSS were determined to calculate the biomass concentration according to standard methods (APHA, 1998). Extracellular polymeric substances (EPS) of the activated sludge samples were extracted by the cation exchange method. The carbohydrate content of EPS was measured by the anthrone method using glucose as the standard. The protein content of EPS was measured by the Lowry method using bovine serum albumin as the standard (Frølund et al., 1996). Bacterial morphological observation was performed by TEM (JEM-1200EX).

3. Results and discussion

3.1. Characterization of GO

XRD experiments were carried out to determine the crystallographic structure of the as-synthesized samples (Supplementary Fig. 1a, supporting information). The XRD pattern of graphite powder exhibited a sharp diffraction peak at 26.4°, which corresponded to the graphite (002) plane (Li et al., 2011). This finding indicated that the interlayer distance, d_{002}, was approximately 0.32 nm as obtained by Bragg's equation. This d_{002} value for the graphite precursor gave an interlayer space close to highly oriented graphite carbons (Gong et al., 2007). When the natural graphite sample was oxidized to GO, the (002) peak, which became broad and relatively weak, obviously shifted to 11.4°, showing that the interlayer spacing increased to 0.73 nm. The *c*-axis spacing increased from 0.32 to 0.73 nm after graphite was modified to GO, which was attributed to the oxidization-induced expansion, thereby suggesting the creation of abundant oxygen-containing functional groups bonded on the surface of GO sheets (Hsieh and Chen, 2011). The oxygen functional groups, such as hydroxyl, epoxyl and carboxyl (Supplementary Fig. 2, supporting information), enabled GO to be readily dispersed in water, which is of the essence for GO in microbiology applications.

TEM analysis was conducted to further characterize the GO microstructure, and the result clearly showed that large GO nanofilms with wrinkled morphology consisted of many stacked GO layers and exhibited many interlaced edges (Supplementary Fig. 1b, supporting information). Corrugation and scrolling are parts of the intrinsic nature of GO sheets ascribed to the fact that the thermodynamic stability of the 2D membrane structure is achieved by bending (Wen et al., 1992).

3.2. Effect of GO on anammox bacteria for nitrogen removal

To investigate the effect of GO on the activity of anammox bacteria, batch tests were conducted to compare the nitrogen removal performance in the absence and presence of GO with various doses based on previous studies (Ruiz et al., 2011; Wang et al., 2011). The results are recorded and illustrated in Fig. 1a–c. In the control experiment without GO application, the NH_4^+-N and NO_2^--N concentrations decreased from 120 to 30.1 mg L^{-1} and 150 to 31.3 mg L^{-1}, respectively, after culturing for 42 h. Meanwhile, 23.4 mg L^{-1} NO_3^--N was also produced in this process. The change in the three kinds of nitrogen charge was approximately consistent with the anammox stoichiometry according to the approved N balances by Strous et al. (1998), demonstrating that the anammox reaction successfully occurred in the control experiment. The NH_4^+-N,NO_2^--N, andNO_3^--N concentrations in the serum bottle containing anammox bacteria with 0.05 g L^{-1} GO were 25.2, 24.8, and 24.6 mg L^{-1}, respectively, which also had the same molar rates as the anammox reaction. Nevertheless, no nitrogen loss occurred in the test with GO alone. These results indicated that GO alone did not affect nitrogen removal, although GO has some adsorption capacity, and that the only way to remove nitrogenous compounds was through the anammox process. Moreover, the