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Enhance wastewater biological treatment through the bacteria induced graphene oxide hydrogel

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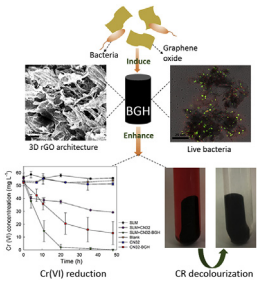
HIGHLIGHTS

- Some special bacteria can induce GO reduction and bio-rGO-hydrogel (BGH) formation.
- BGH was characterized as 3D porous structure containing live bacteria, EPS and rGO.
- Assemble mechanisms were proposed as stacking, bridging, rolling and cross-linking.
- BGH was proven superior to free bacteria when removing dye and Cr(VI).
- BGH suggests a new use of graphene material for biological wastewater treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction between bacteria and graphene-family materials like pristine graphene, graphene oxide (GO) and reduced graphene oxide (rGO) is such an elusive issue that its implication in environmental biotechnology is unclear. Herein, two kinds of self-assembled bio-rGO-hydrogels (BGHs) were prepared by cultivating specific *Shewanella* sp. strains with GO solution for the first time. The microscopic examination by SEM, TEM and CLSM indicated a porous 3D structure of BGHs, in which live bacteria firmly anchored and extracellular polymeric substances (EPS) abundantly distributed. Spectra of XRD, FTIR, XPS and Raman further proved that GO was reduced to rGO by bacteria along with the gelation process, which suggests a potential green technique to produce graphene. Based on the characterization results, four mechanisms for the BGH formation were proposed, i.e., stacking, bridging, rolling and cross-linking of rGO sheets, through the synergistic effect of activities and EPS from special bacteria. More importantly, the BGHs obtained in this study were found able to achieve unique cleanup performance that the counterpart free bacteria could not fulfill, as exemplified in Congo red decolorization and Cr(VI) bioreduction. These findings therefore enlighten a prospective application of graphene materials for the biological treatment of wastewaters in the future.

1. Introduction

Nowadays, graphene and its derivatives have attracted tremendous attention throughout the world, owing to their

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extraordinary physical, chemical and mechanical properties. Among them, graphene is the single atomic layer of sp^2 carbon atoms, whilst graphene oxide (GO) is the contiguous aromatic lattices of graphene grafted with epoxides, alcohols, ketone carbonyls, and carboxylic groups on the basal planes and their edges, which endows GO with more utilities as catalysts or supplements in special functional materials (Compton and Nguyen, 2010; Cai et al., 2016). Meanwhile, at present, the most important role of GO is still regarded as the precursor for large-scale graphene production through chemical reduction, since GO can be obtained from graphite powder at low cost. In this sense, the reduced GO (rGO), corresponding to C/O ratio of 4–246:1, is also a crucial intermediate within the transformation between GO and graphene (Pei and Cheng, 2012).

Though 2D sheet of graphene offers a unique delocalized network for electron conduction, it is the 3D assembled architecture that extends the applicability of the graphene-related materials from nano- to macro-scales (Jiang and Fan, 2014). These three-dimensional graphene-based macrostructures (3D GBMs) (Shen et al., 2015), typically in the form of foams, sponges, aerogels and hydrogels, can be made by either the direct synthesis through chemical vapor deposition or the assembly of 2D GO sheets through a series of reactions (Ma and Chen, 2015; Asim et al., 2017). Unfortunately, toxic reagents and all-time supervision are usually required in these chemical methods, hindering the bulk preparation and cost-effective utilization of 3D GBMs. Besides, so far the environmental applications of 3D GBMs are mainly restricted to membranes and adsorbents (Shen et al., 2015). The studies about 3D GBMs for biological wastewater treatment are still scarce, which can be ascribed to the controversial opinion on the biotoxicity of the graphene materials (Krishnamoorthy et al., 2012; Sanchez et al., 2012; Gurunathan et al., 2013a). The acute toxicity of graphene material led to significant reduction of the microbial community metabolic activity, which could affect wastewater treatment process (Nguyen et al., 2017). Zou et al. (2016) summarized the antimicrobial activities of graphene, GO and rGO for the selected microbes, and in turn systematically reviewed the proposed antimicrobial mechanisms (e.g., nanoknives, oxidative stress, and wrapping or trapping). Meanwhile, there is a bold disagreement among many other studies demonstrating a good compatibility of bacteria with graphene materials. A noticeable finding is that many bacteria can be used as reducing agents, implying that some bacteria can grow well with GO while reducing GO to rGO in solutions (Jiao et al., 2011; Wang et al., 2011; Gurunathan et al., 2013b; Chouhan et al., 2016). For the mixed bacteria, Tanizawa et al. (2012) developed a hybrid approach for the synthesis of rGO sheets from chemically derived GO using anaerobic microorganisms extracted from river sediments. Recently, there are a couple of attempts combining bacteria and graphene-related materials so as to construct 3D GBMs. The formation of some rGO/bacteria hybrid biofilm was firstly observed on the surface of the carbon cloth electrodes in microbial fuel cells (MFCs), by mixing GO solutions with either *Shewanella oneidensis* MR-1 in the anodic chamber (Yong et al., 2014) or anaerobic activated sludge in the cathodic chamber (Zhuang et al., 2012). Further, Yoshida et al. for the first time developed an rGO anode hydrogel directly mixing GO solution and the bacteria enriched by anaerobic cultivation of environmental samples (i.e., river water, sediment and soil) (Yoshida et al., 2016a) or anaerobic sludge (Yoshida et al., 2016b). All these special 3D BGMs, no matter the rGO/bacteria biofilm or the rGO/bacteria hydrogel, exhibited excellent electrical conductivity and enabled a remarkable improvement of the electricity generation. They also suggest that the bacteria induced assembly of rGO hydrogel, designated as bio-rGO-hydrogel (BGH) in this study, turn to be realistic for environment applications other than MFC.

However, the current knowledge about BGH remains very rare, demanding fundamental and critical investigations on many points. For instance, as the mixture of bacteria in the environmental samples and anaerobic sludge worked (Yoshida et al., 2016a, 2016b), does any bacterium have the ability to form BGH? Except for the electrochemical characteristics previously explored, how about other properties of the BGH? Especially, how about the biological traits of the BGH, or is it abiotic just like other chemical build-up hydrogel? What are the fundamental reasons to cause BGH formation? And for the ultimate concern, what is the distinctive goodness of the BGH in use?

The present paper therefore aimed to tackle the aforementioned problems. Seven pure bacterial strains were chosen as the reducing agents and the cross-linkers to induce BGH, in which three belonged to the well-known exoelectrogen *Shewanella* sp. that can transfer electrons directly to terminal electron accepters including GO (Shen et al., 2016). Besides, anaerobic conditions were maintained throughout the BGH formation process, to exclude the probable competition between oxygen and GO for the reducing agent. Once the BGHs assembled, a thorough characterization was carried out to verify their 3D structure and composition by different microscopic and spectroscopic methods, especially for the interior bacteria and rGO flakes. Then, the probable mechanism for the BGH formation was proposed and interpreted. In the end, the derived BGHs were employed to treat wastewater containing the toxic dye Congo Red (CR) or hexavalent chromium (Cr(VI)) to probe the prospective application of BGH for biological wastewater treatment.

2. Experimental

2.1. Materials and bacterial strains

Natural graphite powders were bought from Nanjing XFNANO Materials Tech Co., Ltd (Nanjing, China). CR (99%) was purchased from Sinopharm Chemical Reagents Co, Ltd. (Beijing, China). Fluorescein-isothiocyanate (abbreviated to FITC), Concanavalin A (abbreviated to Con A), Wheat Germ Agglutinin (abbreviated to WGA) and L13152 LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit were purchased from Thermo Fisher Scientific (Waltham, USA). All other reagents used in this study were of analytical grade. GO was prepared from natural graphite powder by a modified Hummers method, and the procedure for the synthesis of GO was explained elsewhere (Marcano et al., 2010). The prepared GO was dispersed in water and ultrasonicated for 4 h for the next BGH experiment.

In this study, seven wild-type facultative bacterial strains were used to fabricate BGH, as listed in Table S1. Specifically, they are *Shewanella xiamenensis* BC01 (abbreviated to BC01), *Shewanella putrefaciens* CN32 (abbreviated to CN32), *Shewanella oneidensis* MR-1 (abbreviated to MR-1), *Escherichia coli* BL21 (abbreviated to *E. coli*), *Bacillus subtilis* (abbreviated to *B. subtilis*), *Acetobacter tropicalis* CHM061701 (abbreviated to CHM061701) and *Weissella Cibaria* 27 (abbreviated to *W. Cibaria* 27). The enrichment conditions of each bacterium were illustrated in the Supplementary Material.

2.2. Preparation of BGH

The sodium acetate medium (SAM) used in this study was anaerobically prepared as described previously (per liter) (Yoshida et al., 2009): 0.5 g NH_4Cl; 1.0 g NaCl; 0.5 g KCl; 0.1 g $CaCl_2$; 0.1 g $MgCl_2 \cdot 6H_2O$; 0.2 g KH_2PO_4; 2.5 g $NaHCO_3$; 1 mL of trace element solution; 10 mL of vitamin solution; and 10 mM sodium acetate. The prepared basal medium was then supplemented GO of 0.17 g L^{-1} and adjusted pH to 6–6.5, 5.5–6.0 and 7 for *W. Cibaria* 27,