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Enhanced sulfate and metal removal by reduced graphene oxide self-assembled *Enterococcus avium* sulfate-reducing bacteria particles

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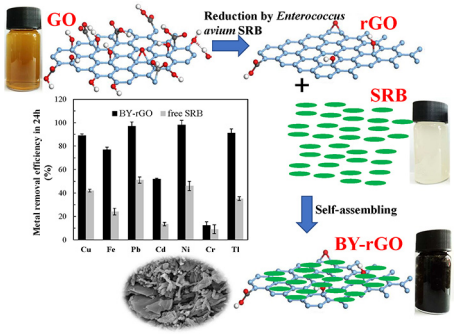
GRAPHICAL ABSTRACT



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ABSTRACT

Graphene oxide (GO) was introduced to *Enterococcus avium* strain BY7 sulfate-reducing bacteria culture as a carrier, GO was partially reduced by SRB to reduced graphene oxide (rGO). The rGO could further self-assemble *Enterococcus avium* strain BY7 sulfate-reducing bacteria to form BY-rGO particles. Growth and sulfate reduction activity of strain BY7 was promoted by rGO, which probably due to the protective effect of rGO, and enhanced electron transfer by rGO as electron shuttle. Effects of pH and temperature variance on strain BY-rGO were remarkably weakened, growth and sulfate reduction were observed from pH 2.0 to 12.0, and from 10 to 45 °C, respectively. Metal toxicity to BY7 strain SRB was sharply decreased in BY-rGO particles and heavy metal removal was remarkably accelerated (up to 50%). The immobilization methods established in this study might open a new way for the application of SRBs, especially under extreme environmental conditions.

1. Introduction

Heavy metal pollution is a serious problem, which was mainly attributed by anthropogenic activities, including mining, industrial production, agricultural drainage and atmospheric deposition (Kieu et al., 2011; Li et al., 2017). Heavy metal cannot be degraded but may be strongly accumulated in aquatic, terrestrial system and the food chain, which led to diseases and death for plants, animals and humans (Yi et al., 2011; Krishna and Mohan, 2016). Many methods have been developed for the treatment of heavy metal, for instance, soil washing,

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thermal extraction, ion exchange, electrolysis process, membrane technology, however, most of these methods are costly, complicated and inefficient for removal of heavy metal (Kurniawan et al., 2006).

Therefore, fixation of heavy metals through microbial-driven reduction and precipitation achieves considerable interest, which is environmental friendly and cost-effective. Microbial sulfate reduction under anaerobic condition is a promising method for treatment of sulfate- and metal-containing wastewater, since both sulfate and metal can be removed simultaneously (Zhang and Wang, 2016b; Yan et al., 2018). This method consists of two stages: First, production of sulfide by sulfate reducing microbes (SRM); Second, precipitation of metals by produced sulfide, a chemical reaction produces insoluble metal sulfides which can be easily removed from solution. However, heavy metals are commonly toxic to microorganisms (including SRM), because of their capacity to deactivate enzymes by reacting with their functional groups, denature proteins and compete with essential cations (Sani et al., 2001; Cabrera et al., 2006). The reported inhibitory concentrations of heavy metals to sulfate reducers range from a few ppm to 100 ppm, over different metals and microbes (Utgikar et al., 2002; Cabrera et al., 2006; Azabou et al., 2007).

Microbial immobilization, such as gel entrapment and biofilm formation, can be an effective solution to maintain high microbial activities, protect microbes from harmful compounds and unfavorable environments (Yan and Hu, 2009; Zhu et al., 2014; Li et al., 2017; Zhang and Wang, 2016b). Polyvinyl alcohol (PVA) and sodium alginate (SA) are most commonly used as an entrapment carrier for immobilization of SRB (Zhang and Wang, 2016b; Li et al., 2017). Moreover, polymer plate, tube, membrane and quartz sand have all been used as matrix for formatting SRB biofilm (Baskaran and Nemati, 2006; Cologgi et al., 2014). Enhanced tolerance to high concentration of heavy metal is achieved after immobilization, no matter which immobilization method and carrier is used (Cologgi et al., 2014; Li et al., 2018).

Recently, application of graphene-based nanomaterials, such as graphene oxide (GO) and reduced graphene oxide (rGO), has a great interest in many research areas, because of the mechanical strength, high surface area, electron transfer capacity, pollutant transformation and degradation (Holmes et al., 2004; Colunga et al., 2015; Chen et al., 2016, 2017). GO can be reduced to rGO through microbial reduction process, which is a green and cost-effective way to produce graphene, in comparison with the chemical and physical methods (Pei and Cheng, 2012; Gurunathan et al., 2013). Then, rGO can work as a matrix to self-assemble a large amount of cells to form complex (Yoshida et al., 2016; Chen et al., 2017). GO (rGO)-based materials remarkably promote bioelectricity generation, biotransformation and biodegradation of pollutants, due to the redox-mediating and electron transfer capacity of graphene (Zhang et al., 2014a; Yoshida et al., 2016; Toral-Sánchez et al., 2017). Besides, rGO-based materials are better supporters for immobilizing microbes than commonly used supports, such as activated carbon and carbon filter, because of the large surface area (Liu et al., 2015). However, the ability of GO-based materials for preventing harmful compounds and unfavorable conditions has not been studied yet.

Therefore, in this study, graphene oxide (GO) was introduced to a previously isolated sulfate-reducing bacteria culture (*Enterococcus avium* strain BY7) as a carrier. GO was partially reduced to reduced graphene oxide (rGO) by SRB. The rGO could further self-assemble *Enterococcus avium* strain BY7 sulfate-reducing bacteria to form BY-rGO particles. Effect of GO addition to the growth and sulfate reduction of SRB was investigated. Particle size distribution and electrochemical property of BY-rGO particles were compared. To verify the formation mechanism of rGO, compositions of GO and BY-rGO particles were compared by X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD). Morphology of BY-rGO particles was investigated by field emission scanning electron microscopy (FESEM). Then, growth and sulfate reduction activities of BY-rGO particles were studied under various pH values and temperatures, versus free SRB cells. Moreover, metal toxicity and removal efficiency of BY-rGO particles were evaluated with the presence of different heavy metals, including copper, lead, nickel, chromium, cadmium, thallium and ferrum.

2. Materials and methods

2.1. SRB culture and chemicals

Sulfate reducing bacteria used in this study was a previously isolated mesophilic *Enterococcus avium* strain BY7, which also available from Guangdong Microbial Culture Collection Center with the accession number of GDMCC1.1349. A modified Postgate growth medium was used as previously published (Yan et al., 2018). The pH value of culture medium was adjusted to 7.5 with addition of 0.1 mM NaOH and 0.1 mM HCl. Sodium lactate and sodium sulfate were used as carbon and sulfur source, with a COD concentration of about 12000 mg/L and sulfate concentration of 1000 mg/L, respectively. Copper(II) chloride, ferric (III) chloride, lead(II) nitrate, cadmium(II) nitrate, chromium(III) nitrate, thallium(I) nitrate, lactic nickel(II) were used to prepare heavy metal solution. All chemicals with 99% purity were used as received. Graphene oxide colloid was purchased from Aladdin (G139812), with viscosity of 1.038 mg/L.

2.2. Preparation of BY-rGO

Growth of BY7 strain was performed in a 100 ml serum bottle. About 50 ml medium was transferred into each serum bottle, and previously isolated strain BY7 was inoculated (0.5 ml, with OD600 of about 0.2), GO was introduced to serum bottle with required volume, as shown in Table 1. Serum bottle was capped with a butyl rubber stopper and aluminum crimp seal. To provide anaerobic condition, each serum bottle was flashed with di-nitrogen gas (99.99%) for 20 min. Incubation was performed in dark in a biology incubator (Fuma, Shanghai, China) without shaking.

2.3. Experimental procedure

Effect of GO addition, initial pH value, incubation temperature and metal addition on growth and activity of BY-rGO particles were investigated, a summary of the operating parameters for each test was given in Table 1. Three incubations were performed in parallel as replicates to investigate growth of strain BY7 bacteria. Aliquot samples were taken every 3 h for OD600 and sulfate analyses. Growth rate (μ) and doubling time (t_d) of strain BY-rGO was calculated as reported by Gu (2016). Concentration of soluble heavy metal in the medium was measured when metal was introduced. Precipitation obtained from

Table 1
Experimental settings in each test.

Variable parameter	Settings of each sample												Remark
GO addition (ml)	0		0.2		0.4		0.6		0.8		1.0		pH 7.5; Temperature 30 °C
pH	2	3	4	5	6	7	7.5	8	9	10	11	12	GO addition 0.4 ml; Temperature 30 °C
Temperature (°C)	10	15	20		25	30	35	40	45				GO addition 0.4 ml; pH 7.5
Heavy metal (mM)	No addition	Cu 1	Fe 1		Pb 1	Cd 1	Ni 1	Cr 1	Tl 0.25				GO addition 0.4 ml; Temperature 30 °C; pH 7.5