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In-situ formation and immobilization of biogenic nanopalladium into anaerobic granular sludge enhances azo dyes degradation

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ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form
22 March 2015
Accepted 24 March 2015
Available online 14 April 2015

Keywords:
Azo dye
Biogenic nanopalladium
Anaerobic granular sludge
Reduction
Decolorization

ABSTRACT

Azo dyes are toxic and recalcitrant wastewater pollutants. An innovative technology based on biogenic nanopalladium (Bio-Pd) supported anaerobic granular sludge (AGS) was developed for azo dyes reduction. In-situ formation of Bio-Pd in the AGS was observed by Scanning Electron Microscopy coupled with Energy Dispersive Spectrometer (SEM-EDS). The Pd associated AGS (Pd-AGS) showed enhanced decolorization rates to the three azo dyes of Congo Red, Evans Blue and Orange II, with the degradation kinetic constants increased by 2.3–10 fold compared to the control AGS in the presence of electron donor formate. Impacts of different electron donors on Orange II decolorization were further investigated. Results showed that formic acid, formate, acetate, glucose, ethanol and lactate could serve as electron and hydrogen donors to stimulate Orange II decolorization by the Pd-AGS, and their activities followed the order: formic acid > formate > ethanol > glucose > lactate > acetate. Most of the Bio-Pd was bound with microbes in the AGS with a small fraction in the extracellular polymer substances (EPS). Transmission Electronic Microscopy analysis revealed that the Bio-Pd formed in the periplasmic space, cytoplasm and on the cell walls of bacteria. This study provides a new concept for azo dye reduction, which couples sludge microbial degradation ability with Bio-Pd catalytic ability via in-situ formation and immobilization of Bio-Pd into AGS, and offers an alternative for the current azo dye treatment technology.

1. Introduction

Azo dyes, which constitute about 70% of the synthetic chemical dyes, are widely used in the textile, cosmetic, paper, drug and food processing industries (Huang et al., 2014). Most azo dyes are carcinogenic, harmful, and hard to be biodegraded due to their complex chemical structures and toxic breakdown products (Dos Santos et al., 2007). The discharge of azo dyes containing wastewater into water bodies can cause the reduction in water transparence and oxygen transfer rate into water, adversely influence photosynthesis by plants in the aquatic systems (Corso and De Almeida, 2009). Therefore, it is of great significance to find

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http://dx.doi.org/10.1016/j.watres.2015.03.024
0043-1354/

an effective way to remove these compounds from wastewater before discharging into environment.

In the last few decades, several physicochemical methods have been developed for azo dyes removal from wastewater, but they are not widely used due to large cost, less effectiveness, and production of secondary waste products and toxic sludge (Alhassani et al., 2007). Biological technology is an attractive method for azo dye removal because of eco-friendly nature, production of less toxic compounds and low cost (Wang et al., 2008). Anaerobic granular sludge (AGS) is a special type of microbial aggregates formed in a granular shape (Pol et al., 2004). As an advanced anaerobic treatment technology, AGS holds a great promise for recalcitrant organic compounds degradation due to the compact structure, large microbial density, complex microbial culture, and strong resistance to toxic compounds (Lim and Kim, 2014). AGS has a multi-layered architecture with a complex microbial community: the outer layer is dominated by acidogens; syntrophic microcolonies are in the middle layer; and the center core is aceticlastic methanogens (Pol et al., 2004). Microbial extracellular polymeric substances (EPS) are also the major components of AGS, which act as the gel-like matrix binding cells together (Pol et al., 2004). AGS has been reported to show a certain degradation ability to a variety of azo dyes (Dos Santos et al., 2007). However, AGS based reactor generally requires a long hydraulic retention time (HRT) to achieve a satisfactory decolorization level due to the relatively low azo dye degradation rate (Silva et al., 2012). Therefore, further enhancing the degradation rate of azo dyes by AGS is of great significance for the application of this technology in dye wastewater treatment.

Palladium (Pd)-based catalysis has emerged as a promising strategy for the reductive transform of lots of recalcitrant environmental contaminants, such as polychlorobifenyls (De Windt et al., 2005), trichloroethylene (Hennebel et al., 2009b) and azo dyes (Johnson et al., 2013), in the presence of hydrogen donors. Fabrication of Pd nanoparticles with chemical methods is generally conducted under harsh conditions using toxic chemical agents and solutions (Shen et al., 2003). Recently, a variety of microorganisms have been reported to produce biogenic nanopalladium (Bio-Pd) from soluble Pd (II) solution, such as *Shewanella oneidensis* (*S. oneidensis*) (Hennebel et al., 2009a), *Desulfovibrio* (Baxter-Plant et al., 2003), *Clostridium butyricum* (Hennebel et al., 2011), etc. The microbial method for Pd nanoparticle fabrication has been regarded as a promising alternative because it requires less chemical agents and reacts under benign and gentle conditions.

Although Bio-Pd can be produced by some pure strains, for a practical application, these nanoparticles are generally required to be immobilized into suitable matrixes in order to reduce catalyst leaching and facilitate their separation and recycling. For example, Hennebel et al. (2009b) immobilized Bio-Pd produced by *S. oneidensis* into polyurethane, polyacrylamide, alginate, silica and zeolites. However, the immobilization of Bio-Pd could lead to the loss of Pd catalytic activity (Hennebel et al., 2009b). This problem may be circumvented by in-situ formation and self-immobilization of the Bio-Pd into a mixed microbial culture such as biofilms or biogranules. Anaerobic granule sludge is special type of microbial aggregates, involving a variety of microbial species such as fermentative bacteria, hydrogen generating bacteria, homoacetogens, methanogen, etc. The problems whether the microbes in AGS could produce Bio-Pd and self-immobilize them into the structure of granular sludge, and whether the Bio-Pd hosting AGS (Pd-AGS) can promote azo dyes degradation by the catalytic activity of Pd and the microbial activity of AGS, remain open and deserve further study.

In this study, the possibility of the Bio-Pd formation by AGS was explored, and the degradation of three azo dyes of Orange II, Evans Blue and Congo Red by a Pd-hosting AGS (Pd-AGS) was investigated in the presence of different electron donors. The morphology and size distribution of the Bio-Pd in the AGS were characterized using Transmission Electronic Microscopy (TEM) and Scanning Electron Microscopy (SEM). This study will provide a novel strategy to treat azo dye containing wastewater based on in-situ formation and immobilization of the Bio-Pd into AGS.

2. Materials and methods

2.1. Chemicals and materials

Three azo dyes of Evans Blue, Congo Red and Orange II were purchased from Sigma–Aldrich (purity > 95%). Their chemical structures are presented in the Supporting Information (SI) (Fig. S1).

The AGS used for Bio-Pd formation and azo dye degradation was collected from a full-scale upflow anaerobic sludge bed (UASB) reactor treating beer production wastewater (Beijing, China). The sludge was acclimated to a synthetic wastewater with glucose as the carbon source (1500 mg/L COD) in a lab-scale UASB reactor (3 L) for more than three months until achieving a stable status. The stabilized sludge was sieved and washed with tap water before Pd (II) reduction experiments.

2.2. Pd (II) reduction and self-immobilization by the AGS

The AGS collected was first washed with phosphate buffer solution (PBS) three times and re-suspended in M9 medium. Serum bottles were added with the AGS suspension (final biomass concentration of 2 g VSS/L), Pd (II) solution (50 mg/L or 100 mg/L in Na_2PdCl_4), and 25 mM sodium formate (electron donor). The serum bottles were then flushed with N_2 gas for 10 min to exclude oxygen and then capped with inert viton stoppers and incubated on a shaker (180 rpm) at 35 ± 1 °C for 4 h. The medium turned black within one minute due to the reduction of Pd (II) to Pd (0). After Pd (II) reduction, the Bio-Pd supported AGS was collected through centrifugation at 5000 g for 10 min. The morphology and spatial distribution of the Bio-Pd in the AGS was investigated using a TEM and a SEM.

2.3. Degradation of azo dyes by the Pd-AGS in the presence of different electron donors

The degradation of three azo dyes of Evans Blue, Congo Red and Orange II using the Pd supported AGS and the control AGS were conducted with formate as the electron donor. Degradation experiments were conducted in 50 mL serum bottles