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A quasi-homogeneous catalysis and electron transfer chain for biodecolorization of azo dye by immobilized phenazine redox mediator

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ABSTRACT

The anaerobic bio-reduction rate of many different contaminants is slow due to the limitations and efficiencies of electron transfer. In this study, the phenazine immobilized in hydrophilic material was tested as a redox mediator (RM) and quasi-homogeneous catalysis to reduce the mass transfer limitation and increase the decolorization efficiency. Neutral red (NR) was immobilized to polyacrylic acid (PAA) hydrogel by chemical reaction and obtained a content of NR as high as 3.58 mmol cm^{-3} of PAA. The novel functional material (PAA-NR), with three-dimensional polymeric network with high porosity and water contents (92–98%, w/v) achieved 13.5 fold higher in the anaerobic decolorization efficiency compared to the control. The optimal conditions for decolorization with PAA-NR hydrogel were 40 °C and pH 8.0. Additionally, the electron transfer mechanism of decolorization was investigated by testing with six inhibitors. NADH and FAD chains were involved in the decolorization process. PAA-NR acted as an insoluble redox mediator to accelerate Reactive Brilliant Red K-2BP (K-2BP) reduction through mediating electron transfer between the azoreductase and K-2BP, resulting in higher electron transfer and decolotization efficiency. This immobilizing technology extended the RM application and demonstrated high catalytic activity on pollutant anaerobic transformation.

1. Introduction

The anaerobic biological treatment, as a promising green technology for pollution control, has been evidenced to bio-reduce the environmental contaminants, including azo compounds, nitro-aromatics, and halogenated aromatics, etc. (Hong et al., 2008; Pandey et al., 2007; van Lier et al., 2015). However, the bio-reduction rate of many different contaminants seemed to be slow due to the electron transfer limitations. Redox mediators (RMs), which speed up bio-reduction rates by shuttling electrons from the primary electron donor to electron acceptors, can significantly increase the slow anaerobic bio-reduction rate (dos Santos et al., 2004; van der Zee et al., 2001).

In previous studies, quinone RMs have received most research attentions for the bio-reduction of contaminants, because of the high electron transfer efficiency (Guo et al., 2010). Quinones standard redox potentials are relatively intensive, although the redox activities are highly efficient, resulting in limited practical applications. Several studies have revealed that contaminants bio-reduction may involve a complex of either reductases, quinones, cytochromes, electron shuttles or phytochelatins (Ying, 2008), together playing important and complex roles in the microbial electron transport chain. Better understanding of non-quinone RM systems is significant. But, at present, little is known regarding the mechanisms of non-quinone mediators which play an important role in electron transfer chain (ETC) systems. Therefore, the use of non-quinone redox mediators for catalysis in anaerobic biological technology systems is of great importance.

Similar to the quinone RMs application, immobilized non-

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quinone RMs would be widely utilized to avoid secondary pollution which is caused by continuously dosing with dissolved RMs in water bodies. At the present stage, RMs have been immobilized by entrapment in a range of matrices, such as calcium alginate, polyvinyl alcohol-H_3BO_3 and agar (Guo et al., 2007), polyurethane foam and activated carbon fibers (Amezquita-Garcia et al., 2015), as well as by covalent binding to ceramic materials (Yuan et al., 2012), electrostatic attraction to anion exchange resins (Cervantes et al., 2011; Martinez et al., 2013), and adsorption to metal oxides nanoparticles (Cervantes et al., 2015). While these examples of immobilized quinone RMs show a certain degree of catalytic effect on the bio-reduction of contaminates, one notable disadvantage of these immobilized techniques in practical application is the gradual loss of redox mediating capacity due to either wash-out of the RMs from bioreactors, or due to disruption of immobilized material. Another notable disadvantage is limiting mass transfer due to the major fraction being entrapped within the immobilized material and the hydrophobicity of carrier material, forming heterogeneous catalysis systems (Alvarez et al., 2010). Therefore, good hydrophilicity and low mass transfer resistance are important indicators of materials that used for immobilizing RMs.

Hydrogels are three-dimensional polymeric networks of cross-linked polymers, which are generally considered to be biocompatible materials due to their high-water content, porosity, flexibility and biocompatibility (Drury and Mooney, 2003; Peppas et al., 2000). In general, hydrogels contain more than 90% water, which is propitious to construct homogeneous catalysis system and reduce mass transfer resistance during biological processes (Li et al., 2017; Lu et al., 2014). Moreover, the through pore structure of hydrogel will also be helpful for the mass transfer and to achieve high catalytic efficiency.

In the present study, the phenazine RM, neutral red (NR), was grafted to a polyacrylic acid (PAA) hydrogel via chemical reaction. The Reactive Brilliant Red K-2BP (K-2BP) decolorization efficiency with the immobilized functional material (PAA-NR) was investigated. Furthermore, electron transfer and accelerating mechanisms for PAA-NR were explored.

2. Materials and methods

2.1. Dyes and chemicals

Halomonas sp. GYW (EF188281), whose relative characteristic was studied in details in previous paper (Guo et al., 2009), isolate was obtained from the Hebei Key laboratory of Environmental Biotechnology (Shijiazhuang, China). Strain GYW was acclimatized in Luria-Bertani (LB) medium, containing: peptone (10 g L^{-1}); yeast extract (5 g L^{-1}); and NaCl (5 g L^{-1}). The K-2BP used in this study was reagent grade and purchased from Di Wei Ni Ao Pharmatech Ltd. (China, Tianjin). The chemical structures of both K-2BP and NR were provided in Fig. S1. Inhibitors were purchased from Sigma (St. Louis, MO). NR and all other reagents were of analytical grade and were purchased from Tianjin Heowns Biochemical Technology Co. Ltd (Tianjin, China).

2.2. Immobilization of NR on the PAA

2.2.1. The synthesis of PAA

PAAs were prepared by free radical polymerization of acrylic acid, in the presence of potassium peroxodisulfate (PPS) and N, N-methylenebis acrylamide (NMA). PPS and NMA were dissolved in a mixture of water and ethanol solution (v/v was 4:1 for water/ethanol), then acrylic acid (CA) was added and the solution was continually agitated by magnetic stirring at 65 °C until complete gelation had occurred, until no remaining flowing fluid could be observed. Different PAAs were synthesized by varying the reactant concentrations. Orthogonal experiments were designed according to different CA mass proportions of 5.8%, 6.8% and 7.8%, with different NMA/CA mole ratios of 2%, 3% and 4%. PAAs were then soaked in deionized water under normal condition and maintained until required for analysis.

Gelation time (T) was recorded from the point of addition of the mixed solution to the beaker. Unreacted hydrogel sections were washed three times prior to freeze-drying, while in addition PAAs were also freeze-dried following a reaction period of 24 h at 37 °C. The gel content (Ct) was established as defined by Eq. (1):

$$\text{Gel content}\ (\%) = \frac{W_1}{W_0} \times 100 \quad (1)$$

Where W_0 is the total weight of all three reactants; and W_1 represents the weight of the dry gel following freeze-drying.

Swelling ratios (SR) for PAAs were defined by comparing the change in weight of PAAs pre- and post-soaking in deionized water for 24 h, where W_1 represents the weight of the soaked PAA. PAAs were then freeze dried at −20 °C for 24 h and re-weighed, where W_2 represents the weight of the dry PAA. SR was calculated as outlined in Eq. (2) (Lu et al., 2014):

$$SR = \frac{(W_1 - W_2)}{W_2} \quad (2)$$

2.2.2. Immobilized NR on PAA

N-Hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodimide hydrochloride (EDC·HCl) and NR at 5:4:5 (*w*/*w*%) were dissolved in 100 ml deionized water. 1 cm diameter PAA cubes were then added to the above mixture, with reactions performed under static conditions for 24 h. The PAA-NR was washed several times with deionized water to remove excess NR remaining from the immobilizing procedure and then finally the PAA-NR was soaked in deionized water for 24 h. The full immobilization method is outlined in Fig. 1.

2.3. Decolorization experiments

Decolorization experiments on K-2BP, the selected model azo dye compound, were conducted in 250 ml serum bottles with the aforementioned base medium. The pH of the media was adjusted to 7.2 with 1 M HCl or 1 M NaOH. Following shaking at 35 °C and pH 7.0 for 12 h, the inoculated medium serum bottles had the rubber stoppers and aluminum covers sealed in order to maintain an anaerobic environment. All the inoculated bacteria in the experiments were at a logarithmic growth later period, which had the same growth state and maintained high biological activity (Guo et al., 2009). To verify the strength of both catalytic and adsorption properties of the PAA-NR, assays were performed comparing two control groups, namely control and sterile control. The control was with PAA and the sterile control PAA-NR but without bacteria. The experiment groups were designed with PAA-NR and bacteria. These initial concentrations of K-2BP in these groups were 160 mg L^{-1}. The decolorization experiments were performed in triplicate.

2.4. The effects of temperature and initial pH

Assays were performed to evaluate the impact of reaction temperature and initial pH on the PAA-NR catalytic decolorization performance. Different temperatures (25, 35, 40, and 45 °C) and