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Short Communication

The structure activity relationship of non-dissolved redox mediators during azo dye bio-decolorization processes

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Structure activity relationships were elucidated by applying chemical structure, electrochemistry and quantum chemical calculations for non-dissolved redox mediators (RM, quinones) with similar chemical structure. The decolorization efficiencies of acid red B by a *Halomonas* sp. GYW were enhanced 2.68, 2.58, 1.91 and 1.49 times with 1,5-dichloroanthraquinone, 1,8-dichloroanthraquinone, anthraquinone, and 1,4,5,8-tetrachloroanthraquinone, respectively. The order of oxidation reduction potential (ORP) during the decolorization process with four redox mediators agreed with their order of reduction potential (Ea) from cyclic voltammetry. The decolorization rate (k) with four redox mediators has a linear relation with their Ea values ($k = 269.05Ea + 85.782$, $R^2 = 0.9226$). The calculated $\rho(r_c)$ at the Ring Critical Point (RCP) based on Atoms in Molecules (AIM) and the inductive/resonance effects of the four redox mediators were also consistent with the accelerating effects on the decolorization. These established relationships might to be predictive models and mechanistic explanations for the accelerating decolorization with redox mediator.

1. Introduction

During the last two decades, the catalytic effects of different redox mediators (RM) on the bio-transformation processes of pollutants have been studied, which include azo dyes (Van der Zee and Cervantes, 2009; Guo et al., 2007), polyhalogenated compounds (Collins and Picardal, 1999; Kappler and Haderlein, 2003), nitro aromatics (Li et al., 2008), nitrate (Guo et al., 2010; Aranda-Tamaura et al., 2007), U(VI), Tc(VII), As(V), Cr(VI) and Se(IV) (Van der Zee and Cervantes, 2009; Field et al., 2004; Fredrickson et al., 2000; Zhang et al., 2007; Liu et al., 2010). Redox mediators accelerate reactions by lowering the activation energy of the total reaction, and can be reversibly oxidized and reduced serving as electron carriers in multiple redox reactions. Commonly studied redox mediators include non-dissolved and dissolved quinone compounds, such as anthraquinone, juglone, lawsone, anthraquinone-2-sulfonate (AQS) and anthraquinone-2,6-disulfonate (AQDS) (Rau et al., 2002; Kappler and Haderlein, 2003; Field et al., 2004; Zhang et al., 2007). Many researchers have focused on the characteristics of dissolved redox mediators and their ability to catalyze biogeochemical processes. The thermodynamics and kinetics of electron transfer reactions with both the electron donor and the terminal electron acceptor, and the stability towards irreversible side reactions are reported to be key factors in determining anaerobic biotransformation or biodegradation with redox mediator (Van der Zee and Cervantes, 2009; Uchimiya and Stone, 2009; Cape et al., 2006). Nurmi and Tratnyek used model quinone compounds to quantitate the electron-carrying capacity (ECC) of reversible redox sites present in humic substances (HS) (Nurmi and Tratnyek, 2002); Aeschbacher et al. explored two electrochemical methods to assess the redox properties of humic substances (Aeschbacher et al., 2010). In order to effectively illustrate the successful application of redox mediators, a key question is how to assess, predict and establish the relationship between characteristics of non-dissolved redox mediators and their catalytic ability in various reactions. However, most of the previously mentioned researches in this subject area have studied dissolved redox mediators and not given enough detailed information on the structure activity relationship of non-dissolved redox mediators.

In this work, four quinone compounds (1,4,5,8-tetrachloroanthraquinone, anthraquinone, 1,8-dichloroanthraquinone and 1,5-dichloroanthraquinone) with similar chemical structure were selected as non-dissolved redox mediators to enhance the bio-decolorization process. The structure activity relationship of non-dissolved redox mediators was elucidated applying knowledge of chemical structure, electrochemistry and quantum chemical calculations.

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2. Methods

2.1. Dyes and chemicals

The chemical structures of acid red B and four selected quinone compounds (1,4,5,8-tetrachloroanthraquinone, anthraquinone, 1,8-dichloroanthraquinone and 1,5-dichloroanthraquinone) are shown in Supporting information (SI) Fig. S1. All reagents were purchased from Xiandai Ltd. (Shijiazhuang, China).

2.2. Bacteria strain and growth medium

A *Halomonas* sp. GYW (EF188281) was obtained from "The Hebei key laboratory of Environmental Biotechnology". Strain GYW was incubated in a salt-tolerant medium (STM) at 30 °C and pH 7.0 on a rotary shaker at 150 rpm. The salt-tolerant medium contained yeast extract 5 g L^{-1}, peptone 10 g L^{-1} and NaCl 100 g L^{-1}, (pH 7.0).

2.3. Redox mediator immobilization method

Calcium alginate (CA) was selected for redox mediator immobilization in accordance with previous studies (Guo et al., 2007, 2010). For the purpose of comparison, CA bead was also prepared in the same method except for the addition of redox mediator.

2.4. Cyclic voltammetric experiments

Cyclic voltammetric experiments were carried out by employing the reported three-electrode configuration (Guo et al., 2007). The electrochemical measurements were performed using a Voltalab Powersuite/LK98B (Tianjin Lanlike Electronics Technology Ltd., China), controlled by power suite electrochemical software at 50 mV s^{-1} scan rate and 25 °C with a redox mediator concentration of 3.63 μM L^{-1} and 0.1 mol L^{-1} H_2SO_4. The glassy carbon and working electrodes were polished in succession with 5 mm, 1 mm, 0.3 mm and 0.05 mm diamond polishes and then rinsed with 8 M nitric acid and distilled water before use. Redox mediator solutions were prepared by adding stock ethanol solution in distilled water and subsequent evaporation of the solvent under a stream of sterile N_2. All solutions were purged with N_2 for 30 min prior to analysis.

2.5. Batch bio-decolorization with redox mediator immobilization beads

Adsorption experiments were conducted to estimate the adsorption ability of acid red B to CA beads. A desired amount of immobilization beads was added into 100 mL STM medium containing 200 mg L^{-1} acid red B. The residual dye concentration in the solution was detected as a function of time, until equilibrium was reached.

Redox mediator immobilization beads were placed in a 250 mL STM medium containing designated dye concentrations for decolorization. Decolorization with an identical amount of CA beads was performed as the blank control. The above experiments were performed at initial dye concentrations (200 mg L^{-1}), temperatures (30 °C), and pH 7.0.

2.6. Calculation methods

The B3LYP method was applied to optimize the structures of the four redox mediators and 6-311G (d) was chosen as the basis set in Gaussian 03'. Based on the theory of Atoms in Molecules (AIM) developed by Bader (1990), the topological analyses of electron density of the system were performed using AIM2000 (Biegler-Kònìng et al., 2000).

2.7. Analytical methods

The cell concentration was measured by optical density at 660 nm and converted to dry cell weight [OD_{660} of 0.720 = l g dry cell weight L^{-1}, ($R^2 = 0.997$)]. The measured method of ORP was conducted according to previous research (Guo et al., 2006) and measured by a digital pH Meter (Delta-320, China) with the ORP composited electrode (Leici-501, China). The pH was determined using the aforementioned digital pH Meter (Delta-320, China). Absorbance of the dye-containing solution was measured at its λ_{max} values using a UV–visible spectrophotometer (UV-2600, China), and absorbance was found to be proportional to concentration in the tested range.

To prevent oxygen contamination during sampling, bottles were opened only once, and as many sample bottles were incubated as measurements were planned. The assays for the experiment were performed in triplicate.

3. Results and discussion

3.1. Cyclic voltammetry characteristic of selected redox mediators

At the 50 mV s^{-1} scan rate and a redox mediator concentration of 3.6 mmol L^{-1}, Fig. 1 showed similar electrochemical characteristics, well-defined reduction peaks (*Ea*) and oxidation peaks (*Er*) for the four redox mediators. The four redox mediators displayed a reversible oxidation peak in the potential range of −0.13 to −0.23 V vs. Hg—Hg_2Cl_2(s) and a reversible reduction peak in the range of −0.25 to −0.28 V vs. Hg—Hg_2Cl_2(s). The reduction potential of 1,4,5,8-tetrachloroanthraquinone had the lowest *Ea* owing to its four chloro substituent derivatives. The electron-withdrawing properties of the chloro group played an important role in the electro-reduction of the four selected redox mediators.

3.2. The acid red B decolorization efficiency with different redox mediators

Without bacteria, the same sum of beads with and without redox mediator caused less than 2% decolorization efficiencies at a fixed initial dye concentration (200 mg L^{-1}) after 24 h. Therefore, the adsorption of acid red B by beads could be neglected in this study.

Fig. 2 showed that acid red B concentration was about 131 mg L^{-1}, 97 mg L^{-1}, 68 mg L^{-1}, 22 mg L^{-1} and 15 mg L^{-1} for the control, 1,4,5,8-tetrachloroanthraquinone, anthraquinone, 1,8-dichloroanthraquinone and 1,5-dichloroanthraquinone after 4 h of decolorization, respectively. Compared to the control, the decolorization efficiencies of acid red B were enhanced 2.68, 2.58, 1.91 and 1.49 times by 1,5-dichloroanthraquinone, 1,8-dichloroanthraquinone, anthraquinone, and 1,4,5,8-tetrachloroanthraquinone, respectively. The catalyzed decolorization process by different redox mediators followed a modified pseudo-zero order model with the reaction constant of k computed by multiple regression analysis, which was $k = dC_{dye}/dt$. The reaction constant of k (mg dye L^{-1} h^{-1}) was 24.87, 33.19, 46.43 and 50.30 for 1,4,5,8-tetrachloroanthraquinone, anthraquinone, 1,8-dichloroanthraquinone and 1,5-dichloroanthraquinone, respectively. The decolorization rate of acid red B could be correlated with the reduction potential (*Ea*) of each redox mediator. The accelerating effects of the redox mediators increased with higher reduction potential (*Ea*) values, corresponding to the linear equation $k = 269.05Ea + 85.782$ ($R^2 = 0.9226$) (Supporting information (SI) Fig. S2).