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Immobilization of metal–humic acid complexes in anaerobic granular sludge for their application as solid-phase redox mediators in the biotransformation of iopromide in UASB reactors

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HIGHLIGHTS

- Metal–humic acid complexes were immobilized by a granulation process.
- Immobilized humic complexes enhanced the biotransformation of iopromide.
- Deiodination, N-dealkylation, decarboxylation and deacetylation occurred.
- First application of immobilized redox mediator without a supporting material.
- Redox catalyst suitable for enhancing degradation of recalcitrant pollutants.

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ABSTRACT

Metal–humic acid complexes were synthesized and immobilized by a granulation process in anaerobic sludge for their application as solid-phase redox mediators (RM) in the biotransformation of iopromide. Characterization of Ca- and Fe-humic acid complexes revealed electron accepting capacities of 0.472 and 0.556 milli-equivalents g^{-1}, respectively. Once immobilized, metal–humic acid complexes significantly increased the biotransformation of iopromide in upflow anaerobic sludge blanket (UASB) reactors. Control UASB reactor (without humic material) achieved 31.6% of iopromide removal, while 80% was removed in UASB reactors supplied with each metal–humic acid complex. Further analyses indicated multiple transformation reactions taking place in iopromide including deiodination, N-dealkylation, decarboxylation and deacetylation. This is the first successful application of immobilized RM, which does not require a supporting material to maintain the solid-phase RM in long term operation of bioreactors. The proposed redox catalyst could be suitable for enhancing the redox conversion of different recalcitrant pollutants present in industrial effluents.

1. Introduction

Humic substances (HS) constitute the major fraction of organic matter accumulating in terrestrial and aquatic environments originated from the decomposition of litter. HS are composed of heterogeneous organic compounds resulting from the biotransformation and (re)polymerization of phenolic and other aromatic components of litter such as lignin, tannins and secondary metabolites. HS are very persistent to biodegradation as evidenced by their millenary residence time in soil ranging up to a few thousand years. Nevertheless, HS do not remain as lethargic material in the environment as they play important roles in metals transport and soil fertility (Macalady and Walton-Day, 2011). Furthermore, HS have active roles in the redox (bio)transformation of azo dyes (Dos Santos et al., 2006; Kudlich et al., 1997; Martínez et al., 2013b), nitroaromatics (Borch et al., 2005), polyhalogenated compounds (Barkovskii and Adriaens, 1998; Cervantes et al., 2004; Martínez et al., 2012), among other electron-accepting contaminants (Martínez et al., 2013a; Van der Zee and Cervantes, 2009). The redox mediating properties of HS have mainly been attributed to quinone moieties (Martínez et al., 2013a), which are very

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abundant in humus, but recent studies suggest that non-quinone functional groups significantly contribute to the electron-transferring capacity of HS (Hernández-Montoya et al., 2012; Ratasuk and Nanny, 2007).

Several attempts to immobilize HS and other redox mediators (RM) have been explored lately to develop engineered systems integrating the redox mediating capacity of immobilized RM in order to promote enhanced conversion rates of contaminants susceptible to redox conversion. RM have been immobilized by entrapment in calcium alginate, polyvinyl alcohol-H_3BO_3 and agar (Guo et al., 2007), by covalent binding on ceramic material (Yuan et al., 2012), polyurethane foam (Lu et al., 2010) and activated carbon fibers (Amezquita-Garcia et al., 2015), by adsorption on metal oxides nanoparticles (Alvarez et al., 2010; Cervantes et al., 2015), by electrostatic attraction on anion exchange resins (Cervantes et al., 2010, 2011; Martínez et al., 2013b), and by electro-polymerization on activated carbon felt (Li et al., 2008).

Among the immobilizing techniques developed, several drawbacks have been identified in their application, such as mass transfer limitations (Guo et al., 2007; Lu et al., 2010), disruption of supporting material (Guo et al., 2007), desorption of immobilized RM (Cervantes et al., 2010), the possibility of washout of RM from bioreactors (Alvarez et al., 2010) and poor immobilizing capacity (Yuan et al., 2012). Furthermore, the vast majority of immobilizing techniques have been proposed and tested with expensive synthetic RM, and their applicability has mainly been demonstrated in batch experiments; although a few studies have also been performed in continuous bioreactors (Cervantes et al., 2015; Martínez et al., 2013b).

The aim of the present study was to synthesize and immobilize metal–humic acid complexes by a granulation process in anaerobic sludge. Immobilized HS were further tested as solid-phase RM for the microbial transformation of iopromide, an iodized contrast medium, which is usually persistent in conventional wastewater treatment systems, with a few exceptions (Casas et al., 2015), thus demanding a suitable technology to remove it from wastewaters originated from hospitals and pharmaceutical factories.

2. Methods

2.1. Synthesis of metal–humic acid complexes

Leonardite purchased from the International Humic Substances Society (Catalogue No. 1BS104L) was used as a bulk humic material for synthesizing metal–humic acid complexes. Production of Ca- and Fe-humic acid complexes was based on previously described protocols (Seki and Susuki, 1995; Zhang and Katayama, 2012). Briefly, a portion of 2.15 g of leonardite was firstly dissolved in one liter of deionized water under continuous stirring for 12 h and centrifuged (9900 rpm, 15 min) afterwards to remove insoluble particles. Solubilized HS were then amended with either $FeSO_4{\cdot}7H_2O$ or $CaCl_2{\cdot}2H_2O$ to obtain a final concentration of 5×10^{-3} M Fe^{+2} or 0.1 M Ca^{+2}, respectively, which were established based on a preliminary screening to optimize the yield. Both reaction systems were neutralized (pH 7.0) with NaOH 0.1 M and left under continuous stirring at 30 °C for one week. Precipitated metal–humic acid complexes obtained were finally collected and washed with deionized water by several centrifugation cycles (9900 rpm 20 min) and lyophilized for further characterization and used.

2.2. Characterization of metal–humic acid complexes

2.2.1. Electron accepting capacity (EAC)

EAC of parental leonardite and synthesized metal–humic acid complexes was measured by chemical and biological methods, using humic solutions of 2 g L^{-1}. The chemical method was based on a H_2/Pd reaction system as previously described (Hernández-Montoya et al., 2012). The biological method was based on the reduction by *Geobacter sulfurreducens*. An appropriate basal medium (pH 7.0) was prepared as previously reported (Caccavo et al., 1994) with acetate as electron donor (0.01 M), and 1.7 mg of protein (*G. sulfurreducens*) mL^{-1}. Incubations were placed in the dark at 30 °C. All EAC data were quantified by the ferrozine technique in a N_2/H_2 (95%/5%) anoxic chamber following the protocol described by Lovley et al. (1996). Briefly, Fe(III) citrate was mixed with samples to obtain a final concentration of 0.01 M, and allowed to react for 30 min. Then, equal volume of 0.5 M HCl was added to the sample, and an aliquot was mixed with the ferrozine solution for spectrophotometric (562 nm) determination of Fe(II). The Fe (II) concentration was used to calculate the electron transfer from humic samples to Fe(III) under all experimental conditions. All EAC measurements were corrected for intrinsic Fe(II) present in humic samples by using a control untreated with Fe(III) citrate. Thus, interference of this redox-active element on the EAC measurements can be dismissed.

2.2.2. FTIR spectra

Fourier transform-infrared (FTIR) spectra were recorded on a Thermo-Scientific FTIR (Nicolet 6700 model) spectrophotometer in transmission mode with a resolution of 4 cm^{-1} and 128 scans. For sample preparation, humic materials were mixed with KBr at a ratio of 1:99% (w/w) for subsequent drying at 60 °C for 48 h, and then compressed into a transparent pellet form for measurement.

2.2.3. Cyclic voltammetry (CV)

CV measurements were done using a multichannel potentiostat/galvanostat from VSP, BioLogic Science Instruments, equipped with a computer with the EC-Lab V10-10 software. The electro-chemical experiments were performed in a conventional three electrode cell. The cell contained the Ag/AgCl (3 M NaCl) system as a reference electrode, a platinum mesh (2.4 × 2.7 cm) as a counter electrode and a glassy carbon disc ($\phi = 0.25$ cm) as the working electrode. The working electrode was carefully polished with 1 μm alumina powder and ultrasonically rinsed with distilled water and ethanol before each run. All experiments were carried out in argon saturated solutions at room temperature. The voltammetric analysis was made after purging with argon for at least 15 min, by scanning from the potential of cero current $E_{i=0}$ in cathodic direction to $E_{\lambda} = -1.0$ V, then anodic direction to $E_{\lambda} = 1.0$ V, and finally cathodic direction to the initial potential, at a scan rate of 10 mV s^{-1}. The voltammetric responses were obtained following the protocol described by Zhang et al. (2014). Briefly: the insoluble metal–humic acid complexes were suspended (at a concentration of 2.5 g L^{-1}) in the mineral medium 0.022 M, which was used as supporting electrolyte containing NH_4Cl, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, K_2HPO_4 and $NaHCO_3$ at pH 7.2. A reduction potential of −0.5 V (*vs.* SHE) was applied for 2.5 h, followed by the recording of the cyclic voltammogram. As a control, the cyclic voltammetry measurements were done in the mineral medium in the absence of metal–humic acid complexes after the application of the same negative potential, and also with the mineral medium containing solubilized HS from leonardite.

2.3. Effect of metal–humic acid complexes on the microbial transformation of iopromide

2.3.1. Inoculum

Anaerobic granular sludge collected from a full-scale up-flow anaerobic sludge bed (UASB) reactor treating effluents from a brewery factory (Ciudad Obregón, Mexico) was used as inoculum