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# Immobilized redox mediator on metal-oxides nanoparticles and its catalytic effect in a reductive decolorization process

L.H. Alvarez [a], M.A. Perez-Cruz [b], J.R. Rangel-Mendez [a], F.J. Cervantes [a,*]

[a] División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica (IPICyT), Camino a la Presa San José 2055, Col. Lomas 4a. Sección, 78216 San Luis Potosí, SLP, Mexico
[b] Facultad de Ciencias Químicas, Universidad Autónoma de Puebla, 72571 Puebla, Mexico

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ABSTRACT

Different metal-oxides nanoparticles (MONP) including $\alpha$-$Al_2O_3$, ZnO and $Al(OH)_3$, were utilized as adsorbents to immobilize anthraquinone-2,6-disulfonate (AQDS). Immobilized AQDS was subsequently tested as a solid-phase redox mediator (RMs) for the reductive decolorization of the azo dye, reactive red 2 (RR2), by anaerobic sludge. The highest adsorption capacity of AQDS was achieved on $Al(OH)_3$ nanoparticles, which was ~0.16 mmol $g^{-1}$ at pH 4. Immobilized AQDS increased up to 7.5-fold the rate of decolorization of RR2 by anaerobic sludge as compared with sludge incubations lacking AQDS. Sterile controls including immobilized AQDS did not show significant (<3.5%) RR2 decolorization, suggesting that physical–chemical processes (*e.g.* adsorption or chemical reduction) were not responsible for the enhanced decolorization achieved. Immobilization of AQDS on MONP was very stable under the applied experimental conditions and spectrophotometric screening did not detect any detachment of AQDS during the reductive decolorization of RR2, confirming that immobilized AQDS served as an effective RMs. The present study constitutes the first demonstration that immobilized quinones on MONP can serve as effective RMs in the reductive decolorization of an azo dye. The immobilizing technique developed could be applied in anaerobic wastewater treatment systems to accelerate the redox biotransformation of recalcitrant pollutants.



## 1. Introduction

Several industrial sectors obtain great economical benefits due to the large and continuous production of different chemicals [1]. Nevertheless, linked to these economical benefits, several toxic and recalcitrant pollutants are discharged in large volumes of wastewater. Many of these contaminants are electron-accepting compounds, such as nitroaromatics, azo dyes, polyhalogenated compounds and metalloids, due to the presence of electrophilic functional groups in their structures, making difficult their treatment by convectional aerobic processes [2]. On the other hand, these pollutants can undergo reductive biotransformation under anaerobic conditions, producing compounds susceptible to aerobic biodegradation [3]. However, anaerobic reduction of recalcitrant pollutants occurs slowly as a result of toxicity effects on anaerobic consortia [4] or due to electron transfer limitations; consequently, anaerobic bioreactors could have deficient performance or can even collapse [5,6].

In the last years, humic substances (HS) and quinones (main redox reactive functional groups in HS) have been tested as redox mediators (RM) during the reductive biotransformation of electron-accepting priority pollutants [7–9]. RM decrease electron transfer limitations, so that biotransformation of these contaminants is accelerated, which minimizes the toxic effects in anaerobic microorganisms [4,6]. Nevertheless, water soluble RM, such as anthraquinone-2,6-disulfonate (AQDS), need to be continuously added to achieve increased reduction rates in anaerobic wastewater treatment processes; the continuous addition of RM increases the cost of treatment and generates contaminated effluents.

Few studies have shown the use of solid-phase RM (RMs) during the reductive biotransformation of priority pollutants. For instance, activated carbon was tested as a RMs due to the presence of quinone moieties in this material [10]. Moreover, quinoid RM has been immobilized in polymeric matrixes [11], on anion exchange resins [12], and on composites of polypyrrole [13]. In all these cases, the immobilized catalysts were shown to enhance the anaerobic biotransformation of azo dyes [10–12] or nitroaromatics [13].

Few years ago, with the emergence of nanotechnology, new materials have been designed and aimed to improve environmental quality through pollution prevention and treatment processes [14]. Mainly, nanoparticles are used as adsorbent materials of differ-

* Corresponding author. Tel.: +52 444 834 2041; fax: +52 444 834 2010.
*E-mail address:* fjcervantes@ipicyt.edu.mx (F.J. Cervantes).

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ent organic and inorganic compounds for water treatment [14,15], including HS [16] and natural organic matter. Additionally, the adsorption of AQDS and HS has been conducted on ferrihydrite nanoparticles to evaluate the effect of adsorbed quinones on ferrihydrite reduction [17]. Furthermore, AQDS has also been adsorbed on hematite in order to understand geochemical variables during the reduction of iron oxides [18]. Nevertheless, to our knowledge, RM adsorbed on metal-oxides nanoparticles (MONP) have never been tested as RMs for the reductive biotransformation of priority pollutants, such as azo dyes. In this work, the capacity of different MONP to adsorb AQDS was evaluated. The catalytic properties of immobilized AQDS were subsequently tested in the reductive biotransformation of the azo model compound, reactive red 2 (RR2). RR2 is a very recalcitrant azo compound commonly used to represent reductive decolorization processes for textile wastewater treatment [6,12,19].

## 2. Experimental

### 2.1. Reagents and nanoparticles

AQDS (98% purity, Sigma Aldrich) was selected as a model RM. RR2 was purchased from Sigma Aldrich (purity of 40%) and used without further purification. Nanoparticles used for the immobilization of AQDS were the following metal-oxides: α-$Al_2O_3$, ZnO and $Al(OH)_3$. All nanoparticles have a purity ≥99%, and were purchased from Nanostructured & Amorphous Materials Inc. (Houston, TX, USA).

### 2.2. Inoculum

Anaerobic granular sludge was used as inoculum, and was collected from a full-scale upflow anaerobic sludge bed (UASB) reactor treating effluents from a malt-processing factory (Lara-Grajales, Puebla, Mexico). The sludge was previously acclimated in a lab-scale UASB reactor (1.5 L) operated at a hydraulic residence time of 12 h. Glucose was used as a sole energy source for the UASB reactor, which showed stable efficiencies in terms of chemical oxygen demand (COD) removal (>90%) during steady state conditions. Prior to incubations, stabilized sludge was washed with distilled water and disintegrated with sterile needles (Microlance 3, 25G5/8, 0.5 mm × 16 mm); in the same step, the sludge was stored in a glass serum bottle containing basal medium as describe in Section 2.4, and anaerobic conditions were established by saturating the inoculation bottle with a gas mixture of $N_2/CO_2$ (80%/20%).

### 2.3. Characterization of nanoparticles

Nanoparticles were characterized by nitrogen adsorption at 77 K, using a Physisorption equipment (Micromeritics ASAP 2020, Norcross, GA, USA) to calculate surface area (SA), applying the BET method. Before adsorption, samples were degassed at 383 K for 3 h. Pore volume ($V_{P_0}$) was calculated from the maximum adsorption amount of nitrogen at $p/p_0 = 0.99$ applying the Harkins and Jura method. Additionally, batch experiments were used to determinate the surface charge of all nanoparticles at different pH values under a $CO_2$-free atmosphere. $N_2$ was bubbled for 15 min into the solutions and also in the headspace of vials before sealing. First, 30 mg of nanoparticles were dispensed in vials, and portions of 0.1 M NaCl were used to keep a constant ionic strength. Initial pH values (3–11) were obtained by adding NaOH or HCl 0.1 M, for a total volume of 15 mL. After 7 days in stirring, the final pH was measured and the surface charge (expressed as the amount of ions released) was obtained with a mass balance based on pH change.

### 2.4. Immobilization of AQDS on nanoparticles

The capacity of MONP to immobilize AQDS was conducted by adsorption isotherms using the batch equilibrium technique. Different concentrations (50, 100, 200, 300, 400, 500 and 600 mg $L^{-1}$) of AQDS were prepared and the pH was adjust to 4.0 using 0.1 M HCl. Then, 50 mg of nanoparticles and 10 mL of AQDS solution were mixed in vials. Vials were placed on a shaker (180 rpm at 25 °C) until the equilibrium was accomplished. After centrifugation (5000 rpm, 10 min) the supernatant was analyzed in order to determine the equilibrium concentration of AQDS and the adsorption capacity.

The saturated materials were exposed several times to basal medium (pH 7.2) in order to verify the adsorption strength of AQDS on MONP. The basal medium was prepared according to the typical nutritional requirements for anaerobic wastewater treatment systems [4,6,10,12], which composition was as follows (mg $L^{-1}$): $NaHCO_3$ (3000), $NH_4Cl$ (280), $K_2HPO_4$ (250), $MgSO_4.7H_2O$ (100), $CaCl_2.2H_2O$ (10), and 1 mL $L^{-1}$ of trace element solution, which composition was as follows (mg $L^{-1}$): $FeCl_2{\cdot}4H_2O$ (2000), $H_3BO_3$ (50), $ZnCl_2$ (50), $CuCl_2{\cdot}2H_2O$ (38), $MnCl_2{\cdot}4H_2O$ (500), $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$ (50), $AlCl_3{\cdot}6H_2O$ (90), $CoCl_2{\cdot}6H_2O$ (2000), $NiCl_2{\cdot}6H_2O$ (92), $Na_2SeO{\cdot}5H_2O$ (162), EDTA (1000), and 1 mL $L^{-1}$ of HCl (36%). The produced materials were characterized by Fourier transform infrared (FTIR) spectrometry and were compared with the FTIR spectra of AQDS and nanoparticles, as mentioned in Section 2.6. In addition, energy dispersive X ray (EDX) analysis was determined as previously established [12].

### 2.5. Decolorization assays of RR2

Decolorization assays were performed in 120 mL serum glass bottles with the basal medium described above; $NaHCO_3$ was change to 5000 mg $L^{-1}$ to create the proper buffer capacity (pH 7.2). Portions of basal medium, for a total volume of 50 mL, were dispensed in the bottles, which were then sealed with rubber stoppers and aluminum caps. The atmosphere in the headspace (70 mL) of the bottles was changed with a mixture of $N_2/CO_2$ (80%/20%) in order to create anaerobic conditions. Once the atmosphere was changed, the bottles were inoculated with anaerobic sludge (disintegrated) at 1 g of volatile suspended solids (VSS) per liter. All bottles were supplied with glucose (1 g COD $L^{-1}$) and pre-incubated during 12 h at 25 °C and 180 rpm. After the pre-incubation period, the bottles were flushed again with the same gas mixture and supplied with a second glucose pulse (2 g COD $L^{-1}$). RR2 was added from a stock solution prepared with sterile basal medium, and the initial concentration in the incubations was 0.3 mM. Different treatments and controls were tested in order to elucidate the catalytic effect of immobilized AQDS on nanoparticles. The treatments included three different concentrations (1.2, 2.4 and 4.8 mM) of AQDS in soluble and immobilized form. The controls were: first, a control without AQDS in any form, but including glucose, basal medium and sludge. Second, sterile controls with soluble or immobilized AQDS at 4.8 mM and basal medium. Finally, a control with nanoparticles, basal medium, sludge and glucose, but in the absence of AQDS. All conditions were carried out by triplicate and incubated at 25 °C and 180 rpm.

### 2.6. Analytical methods

AQDS concentration was spectrophotometrically measured at 328 nm. Liquid samples were firstly centrifuged (10 min at 10,000 rpm) and diluted in bicarbonate buffer (60 mM, pH 7.2). RR2 decolorization was also documented spectrophotometrically at the maximum wavelength of visible absorbance (539 nm). Samples (0.75 mL) were centrifuged and diluted in a 0.1 M phosphate buffer at pH 7.0. FTIR spectra were obtained at a resolution of