

Catalytic role of Au@TiO₂ nanocomposite on enhanced degradation of an azo-dye by electrochemically active biofilms: a quantized charging effect

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Abstract A green and sustainable approach to azo dye degradation by an electrochemically active biofilm (EAB) with Au@TiO₂ nanocomposite assistance (average size of Au ~ 8 nm) has been developed with high efficiency and mineralization of toxic intermediates. The EAB-Au@TiO₂ system degraded the dye more rapidly than the EAB without the nanocomposite, which indicated the catalytic role of the Au@TiO₂ nanocomposite on the dye degradation. Toxicity measurements showed that the dye wastewater treated by the EAB-Au@TiO₂ system was almost non-toxic while the dye wastewater treated by the EAB without the nanocomposite showed a high toxicity compared to the parent dye. Quantized charging and Fermi level equilibration within the Au@TiO₂ nanocomposite may be attributed to the excellent catalytic activity of the nanocomposite on the dye degradation. A mechanism of the catalytic activity is also proposed. Redox behavior and quantized charging of the nanocomposite were confirmed by cyclic voltammetry (CV) and differential pulse voltammetry (DPV), respectively. The proposed protocol can be effectively utilized in wastewater treatment applications.

Keywords Electrochemically active biofilm · Dye degradation · Au@TiO₂ nanocomposite · Quantized charging · Fermi level equilibration

Introduction

Azo dyes are commonly used in textile industries. Treatment of dye wastewater prior to discharge is one of the daunting tasks as most of the dyes are recalcitrant molecules and highly toxic in nature (Husain 2010). There are many available methods for the degradation of azo dyes including chemical, physical, and biological methods; however, their performances are far from satisfactory (Robinson et al. 2001). Usually, intermediates formed after the degradation of dyes are highly toxic and sometimes even more toxic than the parent dye. Hence, a further treatment is needed for the degradation of the toxic intermediates. There are many metal nanoparticles such as Au, Ag, and Pt which were employed for the degradation of dyes by using various chemical reducing agents such as NaBH₄ (Gupta et al. 2011). The major disadvantage of these methods is the use of toxic chemicals which causes a secondary pollution.

In recent years, coupling of semiconductors with noble metals has been regarded as an interesting approach to enhance the performance of semiconductor-based nanostructures (Choi et al. 2012; Takai and Kamat 2011). For example, marrying TiO₂ nanoparticles with Au nanoparticles can enhance the

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electrochemical performance of the nanocomposite by promoting electron transfer to adsorbed species such as H^+ to make hydrogen molecule (Choi et al. 2012). It has been reported that Au@TiO₂ nanocomposite can boost photocurrent generation in dye-sensitized solar cells by capturing and discharging electrons (Choi et al. 2012). Deposition of Au nanoparticles on semiconductors enhances charge accumulation property of the nanocomposite and the metal nanoparticles facilitating electron discharge on demand (Choi et al. 2012).

Recently, bioelectrochemical systems (BESs) such as microbial fuel cells (MFCs) (Kalathil et al. 2012a; Kalathil et al. 2011a; Pant et al. 2010) and microbial electrolysis cells (Pant et al. 2010) have received great attraction for simultaneous treatment of dye wastewater and for bioelectricity generation. However, BESs are in early stages and still not practically established (Pant et al. 2010). Electrochemically active biofilms (EABs) developed by electrically active microorganisms have emerged as one of the promising tools for environmental remediation, chemical production, and bioelectricity generation (Borole et al. 2011; Erable et al. 2010). Electrically active microorganisms can be found in various natural sites such as sea water sediments, soils, and various wastewaters such as municipal, dye, and swine wastewaters (Erable et al. 2010). The real attraction of EABs is the bio-anode catalyst for the MFCs (Borole et al. 2011; Erable et al. 2010) where the EAB biologically oxidizes organic substrates to electrons and protons. Murray and co-workers have reported that noble metals especially Au nanoparticles possess the property of storing electrons in a quantized fashion (Chen et al. 1998; Murray 2008). In this study, methyl orange (MO) was degraded with mineralization of toxic intermediates by EABs using Au@TiO₂ nanocomposite as catalysts via quantized charging effect. To our best knowledge, it is the first time EAB with a nanocomposite is utilized for the rapid degradation of an azo dye.

Experimental methods

Au@TiO₂ nanocomposite synthesis

EABs were developed according to previous reports (Kalathil et al. 2011b; Kalathil et al. 2012b). The Au@TiO₂ nanocomposite was synthesized using an

EAB (Kalathil et al. 2012b). In brief, an as-prepared EAB was dipped in a solution (200 mL) containing sodium acetate (1 g/L) as electron donor, 4 mM Degussa-TiO₂, and 1 mM HAuCl₄ (gold precursor) under anaerobic condition by sparging N₂ gas for 5 min before the reaction. A color change after 1 h signaled the formation of the Au@TiO₂ nanocomposite. The as-synthesized nanocomposite solution was centrifuged and kept for drying in an oven (50 °C) overnight.

Characterizations of nanocomposite

Formation of Au@TiO₂ nanocomposite was monitored by UV–Vis absorption spectroscopy (UV–VIS–NIR, Cary 5000). The crystal structure of the nanocomposite was determined by X-ray diffractometry (PANalytical, X'Pert-PRO MPD). The morphology of the nanocomposite was determined by a high resolution transmission electron microscope (HRTEM; Tecnai G2F20, FEI, USA) operating at an accelerating voltage of 200 kV.

Dye degradation

The dye degradation was carried out by dipping an as-prepared EAB into a MO solution (20 mg/L) using the as-synthesized Au@TiO₂ nanocomposite (20 mg/L) as the catalyst containing sodium acetate (1 g/L) as electron donor under magnetic stirring. Also, the MO degradation was carried out by dipping an EAB without using the nanocomposite to investigate the role of the Au@TiO₂ nanocomposite on the dye degradation process. The reactions were carried out in dark to avoid photo effect on the dye degradation. The MO degradations were performed at pH 4 (30 °C) as electron transfer reaction, for the dye degradation is highly favorable at lower pH (Liu et al. 2009), and also methanogenic bacteria can be suppressed at lower pH, which inhibit electrically active bacteria (Borole et al. 2011).

Analyses

The dye degradation was monitored using a UV–Vis spectroscopy (UV–VIS–NIR, Cary 5000). Differential pulse voltammetry (DPV) analysis was carried out using a potentiostat (VersaSTAT 3, Princeton Research, USA) using standard three-electrode system. An Ag/AgCl (saturated with KCl) was used as the reference

electrode, a Pt foil as the counter electrode, and plain carbon paper as the working electrode with as-synthesized Au@TiO₂ nanocomposite colloidal solution as the electrolyte. Cyclic voltammetry (CV, multiple cycles) analysis was also performed by the potentiostat using standard three electrode system. An Ag/AgCl (saturated with KCl) was used as the reference electrode, a Pt foil as the counter electrode, and plain carbon paper as the working electrode with as-synthesized Au@TiO₂ nanocomposite colloidal solution as the electrolyte. The scan rate used was 50 mV/s. Toxicity analysis was performed by following the resazurin reduction method (Kalathil et al. 2012a; Kalathil et al. 2011a).

Results and discussion

The metal nanocomposites show characteristic colors due to excitation of surface plasmon vibrations. UV-Vis analysis showed a peak at around 550 nm (Fig. S1) which indicated the formation of the Au@TiO₂ nanocomposite (Dawson and Kamat 2001; Kalathil et al. 2012b). XRD measurement showed that sharp peaks correspond to both TiO₂ and Au (Fig. S2) indicating high crystallinity of the as-synthesized nanocomposite (Kalathil et al. 2012b). Transmission electron microscopic (TEM) image shows that the product is almost exclusively composed of discrete Au@TiO₂ nanoparticles (Fig. S3a). The HRTEM micrograph (Fig. S3b) indicates that average particle diameter of Au nanoparticles is around 8 nm. Selected area electron diffraction (SAED) pattern shows well-resolved (101) lattice fringes ($d_{101} = 0.35$ nm) and diffraction cycles indicative of a highly crystalline TiO₂ anatase framework (Fig. S3c).

MO reductions by the EAB in the presence of Au@TiO₂ and by the EAB without the nanocomposite were monitored using UV-Vis spectroscopy. The UV-Vis spectra (Fig. 1) showed that the MO reduction was completed within 2 h by the EAB-Au@TiO₂ nanocomposite system while only around 40 % degradation was observed for the EAB without the nanocomposite, which indicates the role of Au@TiO₂ on the degradation. To our pleasant surprise, the toxic intermediates (aromatic amines) formed by the MO degradation were also degraded after 2 h, which was confirmed by the disappearance of the peak around 270 nm by the EAB-Au@TiO₂ system (Fig. 1d). However, the MO degradation under strict anaerobic

condition by sparging N₂ gas continuously during degradation reaction into the EAB-Au@TiO₂ system increased the intensity (Fig. 1g) of the toxicity peak (more explanation in mechanism section, Fig. 2). The toxic intermediate peaks at 270 nm were enhanced after the MO degradation by the EAB only (without nanocomposite), which is anticipated in the anaerobic conditions without the catalyst (Fig. 1e–f).

Mechanism involved in the MO degradation by the EAB-Au@TiO₂ nanocomposite was depicted in Fig. 2. We used Au@TiO₂ nanocomposite instead of pure Au nanoparticles for the dye degradation as the Fermi level energy difference between the TiO₂ conduction band and the pure Au nanoparticles allows a much larger potential difference to be accommodated than is possible at the pure Au–H₂O interface (Oldfield et al. 2000). The TiO₂ can be an appropriate support to anchor the Au nanoparticles, and the anchoring of Au nanoparticles on the TiO₂ surfaces should avoid agglomeration and coalescence of Au nanoparticles during catalysis (Primo et al. 2011). The EAB biologically oxidized the acetate to electrons and protons. The electrons produced by the EAB were

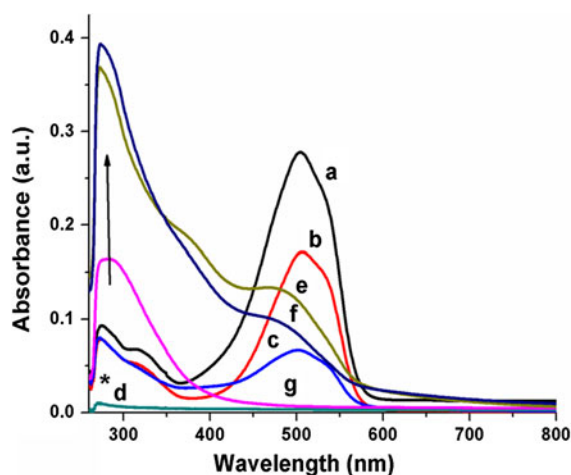


Fig. 1 UV-Vis spectra of MO degradation by the EAB-Au@TiO₂ nanocomposite and EAB only. **a** represents initial MO spectrum while **b**, **c**, and **d** represent MO degradation after 30 min, 1 h, and 2 h, respectively by the EAB-Au@TiO₂ nanocomposite system. **e** and **f** show the MO degradation after 2 and 3 h by the EAB only (without nanocomposite). **g** represents the MO degradation after 2 h by the EAB-Au@TiO₂ system under strict anaerobic condition by sparging N₂ gas continuously during the degradation reaction. The arrow indicates the increase of intermediates peaks by the EAB only, and * represents the reduction of intermediates peaks by the EAB-Au@TiO₂ nanocomposite system

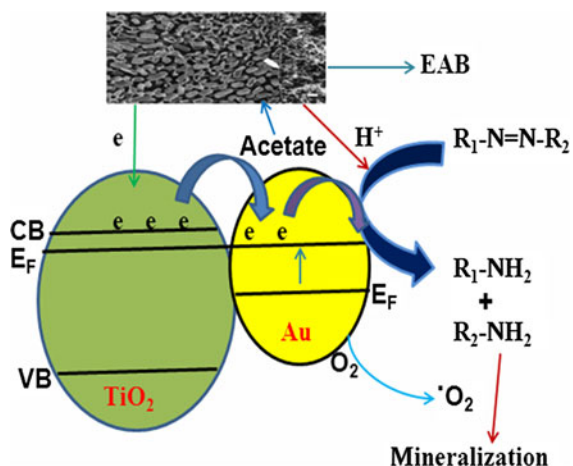


Fig. 2 A proposed mechanism for the degradation of MO by EAB-Au@TiO₂ nanocomposite system

injected into the conduction band (CB) of TiO₂ (Fig. 2), and the electron injection by the EAB to the CB of TiO₂ has already been documented (Kalathil et al. 2012b). Chemical injection of electrons to Au@SnO₂ nanocomposite by NaBH₄ has been already reported (Oldfield et al. 2000). Here, the EAB mimics NaBH₄ by giving electrons to the nanocomposite (Fig. 2), which offers a green and sustainable approach for the dye degradation. Spectroscopic studies prove that the electron transfer from a semiconductor to a metal nanoparticle is an ultrafast process (Harris and Kamat 2010). Since the Fermi level of Au ($E_F = +0.45$ V versus NHE) is more positive than the CB of TiO₂ ($E_{CB} = -0.5$ V versus NHE), the stored electrons in the CB of TiO₂ are easily transferred to Au nanoparticles until the Fermi level of the two systems attain equilibration (Choi et al. 2012; Kamat 2007). The electron accumulation increases the Fermi level of Au to more negative potentials, and hence the resultant Fermi level of the nanocomposite shifts closer to the CB of the TiO₂ (arrow in Fig. 2), which will impart excellent reductive power to the nanocomposite (Choi et al. 2012; Kamat 2007).

A convenient tool to investigate the charge storage within metal or metal nanocomposites is DPV analysis (Murray 2008). The DPV analysis showed a well-defined quantized charging peak at positive potential similar to many previous reports (Chen et al. 1998; Murray 2008) indicating excellent charge storage property of the nanocomposite (Fig. 3). CV analysis showed a well-pronounced reduction peak which

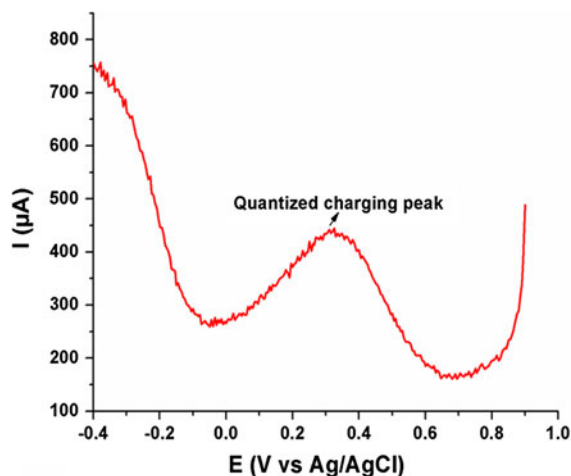


Fig. 3 DPV of as-synthesized Au@TiO₂ aqueous solution. DPV parameters: pulse amplitude 100 mV, pulse width 50 ms, step height 5 mV, and step width 100 ms

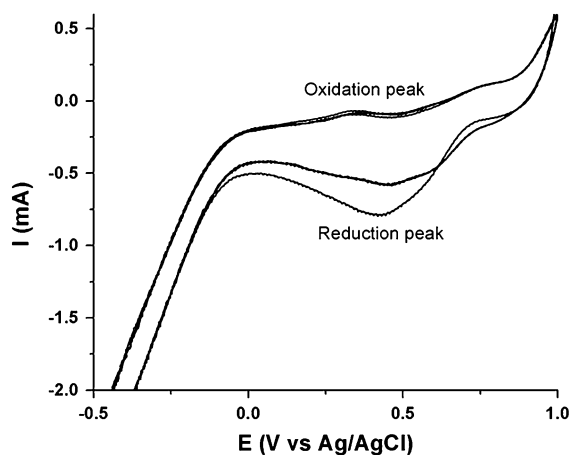


Fig. 4 CV of as-synthesized Au@TiO₂ aqueous solution (scan rate 50 mV/s)

further confirmed the excellent reduction capacity of the nanocomposite (Fig. 4). It can be seen that the second CV cycle is well-pronounced than the first CV cycle as the second cycle should be less influenced by ohmic drop compared to the first cycle (Lovric and Scholz 2003). The stored electrons within the nanocomposite were transferred to the azo bonds ($-N=N-$) of the MO which cleaved the bonds by producing intermediate aromatic compounds (Fig. 2). These toxic intermediates may be further mineralized by the EAB-Au@TiO₂ system which was confirmed by the UV analysis (Fig. 1d). Even though the system was kept as

Table 1 Advantages of the proposed degradation route over existing dye degradation methods

Proposed method	Biological method	Chemical method	Physical method
Rapid	Slow	Rapid	Rapid
High efficiency	Low efficiency	Low efficiency	Low efficiency
Green approach	Green approach	Use of toxic chemicals	Potentially dangerous or use of energy
Low cost	Moderate cost	High cost	High cost

anaerobic, we cannot neglect the possibility of some dissolved oxygen (DO) in the dye degradation system. The DO can accept the electrons stored in the nanocomposite to form superoxide ($\cdot\text{O}_2$) radicals (Fig. 2) which can destroy the toxic intermediates (Hoffmann et al. 1995). As observed in Fig. 1g, when the EAB-Au@TiO₂ system was kept strictly anaerobic by sparging N₂ gas continuously during the reaction time, peak corresponding to toxic intermediates increased. It should be noted that it is not possible to keep a strict anaerobic condition by sparging N₂ gas continuously in practical use as it consumes lots of energy, and it is also important to consider that biological systems in practical uses are often temporarily exposed to oxygen. Fortunately, EABs formed with mixed culture bacteria can usually survive at low DO concentration, and the presence of low DO can enhance substrate breakdown and enhances its utilization (Borole et al. 2011). This indicates the possible role of DO on the mineralization of toxic intermediates by forming $\cdot\text{O}_2$ radicals, and the $\cdot\text{O}_2$ radicals can be generated only in the presence of a catalyst (Tsunoyama et al. 2009).

Toxicity analysis (resazurin reduction method) was performed to investigate the toxicity of parent dye and intermediate products. The original MO showed $16 \pm 3\%$ inhibition on the resazurin reduction, which can be considered as toxic. The products formed by the degradation of MO by the EAB-Au@TiO₂ nanocomposite system after 2 h showed $6 \pm 2\%$ inhibition which can be considered as almost non-toxic concordant with disappearance of intermediates peak in the UV spectrum (Fig. 1d). On the other hand, the products formed after the MO degradation by the EAB (without nanocomposite) after 2 h showed $28 \pm 5\%$ inhibition which indicates the relatively higher toxicity of the intermediates compared with the parent dye which also satisfies the UV analysis (Fig. 1e).

The proposed EAB-Au@TiO₂ dye degradation route is far superior to other existing physical, chemical, and biological methods for the efficient degradation of dyes (Table 1). The dye degradation process was rapid

compared to other biological methods using fungi and bacteria which usually consume 24–96 h for the degradation of dyes (Chang et al. 2004; Lin et al. 2010; Saratale et al. 2011; Wang et al. 2009) while our approach degraded the dye within 2 h. Physical methods based on coagulation–flocculation suffer from lower color removal efficiency, use of copious amount of chemicals, and large amount of sludge production (Vandevivere et al. 1998). Adsorption method has been limited by problems associated with regeneration or disposal, high cost, and low effectiveness for the dye degradation (Anjaneyulu et al. 2005). Filtration methods using membranes have been severely hindered by high costs and potential membrane fouling (Robinson et al. 2001). Chemical oxidation methods such as ozonation limit practical applications due to their shorter life time and high cost of ozone (Anjaneyulu et al. 2005). Even though electrochemical oxidation is highly effective for the dye degradation, high cost of electricity limits its application (Morawski et al. 2000). Photo-degradation of dyes by UV light is potentially dangerous and consumes lots of energy (Primo et al. 2011). On the other hand, our proposed degradation protocol doesn't use any toxic chemicals or reagents which make the protocol eco-friendly. Also, there is no need of energy input as the EAB acts as an electron generator which makes the protocol highly sustainable for practical applications. The toxic intermediates formed during the dye degradation were also removed by the EAB-Au@TiO₂ system, and it avoids a secondary treatment of the treated dye wastewater.

Conclusions

A rapid and efficient degradation of MO with mineralization of toxic intermediates was achieved by EAB-Au@TiO₂ nanocomposite system. The quantized charging property of the nanocomposite may be responsible for the high catalytic activity of the nanocomposite. Also, Fermi level equilibration in the Au@TiO₂ nanocomposite plays a crucial role in

improving its catalytic performance. The extension of this study on other semiconductor–metal nanostructures for environmental remediation is on the anvil in our lab and will be reported elsewhere.

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