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Short Communication

Enhanced reduction of an azo dye using henna plant biomass as a solid-phase electron donor, carbon source, and redox mediator

Jingang Huang [a,*], Shushan Chu [a], Jianjun Chen [a], Yi Chen [b], Zhengmiao Xie [a]

[a] Institute of Environmental Science and Engineering, Hangzhou Dianzi University, Hangzhou 310018, PR China
[b] Department of Applied Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, 16521 Prague 6, Czech Republic

HIGHLIGHTS

- Henna plant is a source of electron donor and redox mediator (RM) for AO7 reduction.
- Associated lawsone in henna powder can act as a fixed RM to shuttle electrons.
- Deficiency of electron donor along with the retention of henna weakens AO7 reduction.

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ABSTRACT

The multiple effects of henna plant biomass as a source of carbon, electron donor, and redox mediator (RM) on the enhanced bio-reduction of Orange II (AO7) were investigated. The results indicated that the maximum AO7 reduction rate in the culture with henna powder was ~6-fold that in the sludge control culture lacking henna. On the one hand, AO7 reduction can be advantageously enhanced by the release of available electron donors; on the other hand, the associated lawsone can act as a fixed RM and play a potential role in shuttling electrons from the released electron donors to the final electron acceptor, AO7. The soluble chemical oxygen demand (SCOD) during each experiment and the FTIR spectra suggested that the weakened AO7 reduction along with the retention of henna powder might not be attributed to the lack of fixed lawsone but rather to the insufficiency of electron donors.

1. Introduction

Azo dyes, which constitute about 70% of the synthetic chemical dyes, are widely used in many industrial applications such as in pharmaceuticals, textile dying, cosmetics, food, and so on. Although the dyes provide an esthetic function, their release from wastewater effluents poses a threat to the environment and, further, to human health (Saratale et al., 2011). Due to the strong electron affinity of the —N=N— bond, azo dyes are bio-persistent under aerobic conditions. Thus, a combined process incorporating anaerobic reduction and subsequent aerobic oxidation is usually applied during biological treatment, with the former reaction being the rate-limiting step. Anaerobic reduction of azo dyes is mainly dependent on the availability of electron donors, such as H_2, volatile fatty acids (VFAs) and carbohydrates (Hong et al., 2007); and the electron shuttling system of redox mediators (RMs) such as anthraquinone-2,6-disulfonate, humic acids, and riboflavin (van der Zee and Cervantes, 2009). Therefore, to overcome the low anaerobic reduction rate of the azo dyes, researchers have been trying to find effective and eco-friendly electron donors and RMs.

Recent years, with the pursuit of green industry and safety life, natural dyes have been popularly employed. These dyes come mainly from the leaves, flowers, or stems of plant biomass such as fabaceae (*Lonchocarpus cyanescens*), henna (*Lawsonia inermis*) and marigold (*Tagetes erecta*) (Oduro and Addo-Yobo, 2013; Sivakumar et al., 2011). However, the process of extracting natural dyes from plants produces large amount of biomass wastes, which add pollution to the environment. On the other hand, plant biomass such as cane molasses and cellulosic materials can also serve as solid electron donor sources for the bio-reduction of oxidative contaminants, e.g., nitrate and halogen derivatives (Brennan et al., 2006; Wen et al., 2010), and can further encourage the reductase activities of these reducing processes (Chen et al., 2014). Henna powder is a popular raw material of natural dye all over the world. After the utilization in dyeing hair or textile, it can then become biomass wastes. Apart from providing potential electron

* Corresponding author. Address: Room S104, The 6th Building, Hangzhou Dianzi University, Hangzhou 310018, PR China. Tel./fax: +86 571 86919158.
E-mail address: hjg@hdu.edu.cn (J. Huang).

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donors under anaerobic conditions, henna plant biomass also contains abundant lawsone, up to 1%, which is an effective RM and can promote the reduction of azo dyes (Rau et al., 2002). Therefore, henna plant biomass, comparing with other reported ones, can be advantageously used for the anaerobic reduction of azo dyes due to the simultaneous supply of electron donors and RM. In this way the accumulated biomass wastes would be adequately resolved as well.

The aim of this study was to investigate the combined multiple effects of henna plant biomass as a source of carbon, electron donor, and RM on the enhanced bio-reduction of azo dyes. Specifically, henna powder was subsequently tested on the reductive decolorization of synthetic azo dyes by anaerobic sludge.

2. Methods

2.1. Chemicals

Orange II, also known as Acid Orange 7 (AO7) which was purchased from Sigma–Aldrich, was used as a model azo dye. Commercial henna powder (leaves) was purchased from a company in Shanghai, China. Henna powder was dried at 45 °C for at least 8 h before usage. The inoculated sludge was obtained from a parent sequential batch reactor which had been operated for ~150 d and achieved stable biological AO7 removals (above 90%) with glucose as co-substrate. The sludge was washed for three times with de-ionized water before adding to the serum bottles.

2.2. Experimental set-up

Three 1000-mL serum bottles were set up as batch test (B1), sludge control test (B2), and adsorption control test (B3), respectively. Dried henna powder (4.0 g L^{-1}) and anaerobic sludge (3.5 g VSS L^{-1}) were added to B1; however, B2 was cultured without henna powder; and the sludge that added to B3 had been previously autoclaved (121 °C, 0.12 MPa). The initial AO7 concentrations in each bottle were ~1.2 mM. The serum bottles were magnetically stirred at a temperature of 25 ± 1 °C. The pH in each system was adjusted to 7.0 ± 0.2 using $NaHCO_3$ buffer solution. B1 was operated for three different continuous batches (Batch I, II and III), while B2 and B3 were operated for only one batch. Between different batch tests within B1, the mixed liquid was allowed to settle for 1.5 h, and then the supernatant was decanted. This procedure was repeated for three times, and then the sludge was re-suspended with AO7 solution to recover the initial concentration of 1.2 mM for the next batch test. NH_4Cl (80 mg L^{-1}) and KH_2PO_4 (20 mg L^{-1}) were added as nutrients; macro and trace metals and vitamins were supplied as described previously (Huang et al., 2012). All substances were prepared in N_2-flushed de-ionized water throughout all the experiments.

2.3. Chemical analysis

Three 2-mL mixture from each batch test, withdrawn at appropriate time intervals, were firstly centrifuged at 4000*g*, and then be filtered through 0.22 μm membrane filters (Anpel Co., Shanghai, China). pH was measured by a portable pH/mV/temperature meter (HACH, sensION1, USA) with a gel-filled pH electrode and a combination Ag/AgCl ORP electrode. For AO7 analysis, the filtered samples were first diluted with freshly prepared phosphate buffer (50 mM, pH 7.0) containing ascorbic acid (500 mg L^{-1}), and then were measured spectrophoto-metrically at the wavelength of 484 nm. Reducing sugar was measured by the DNS method with glucose as standard. Protein was determined according to the Bradford method with bovine serum albumin as the standard. Sulfanilic acid (SA), which is the reductive product of AO7, and volatile fatty acids (VFAs) were measured by high performance liquid chromatography unit (HPLC, Agilent 1200, Agilent Technologies, CA, USA) equipped with an UV detector. SA and VFAs were separated with a Shodex RSpak KC-811 analytical column following a Shodex RSpak KC-G guard column (Showa Denko, Japan) at 50 °C. The mobile phase was phosphoric acid solution (0.1%) at a flow rate of 0.7 mL min^{-1}, and the wave length was 210 nm. Soluble chemical oxygen demand (SCOD), volatile suspended solid (VSS) were determined according to Standard Methods. To analyze the functional groups in the liquid phase and in the solid phase of mixture (microorganisms plus henna powder), the collected samples were centrifuged at 12000*g* at 4 °C for 10 min. Afterwards, the liquid and the solid phases were freeze-dried for 48 h in a freeze dryer (LGJ-10, Xinzhi, China), and then a Bruker Alpha FTIR spectrometer was used for functional group chemical analysis.

3. Results and discussion

3.1. AO7 reduction and SA formation

To evaluate the enhanced bio-reduction of AO7 using henna powder, three continuous batch tests with a single addition of henna powder before Batch I in B1 were performed in this study. The results (Fig. 1A) indicate that the decrease of AO7 in the cultures with henna powder (Batch I, II and III) was much faster than those in their control cultures (adsorption and henna-free controls). The adsorption control test only showed a very limited decline in the AO7 concentration. Therefore, the adsorption of AO7 in these systems could be neglected. Under anaerobic conditions, AO7 can be biologically or chemically reduced to SA and 1-amino-2-naphtol (1A2N). The results from HPLC analysis (Fig. 1B) show that SA was simultaneously formed during the above tests. Furthermore, the AO7 removal rates and SA formation rates in the biological systems, i.e., Batch I, II and III in B1 and the henna-free sludge control in B2, can be balanced (Table S1). This suggests that most of the AO7 in the above systems was removed by reduction.

The henna-free sludge control test displayed a 33% reduction in the initial AO7 concentration, and 0.345 mM of SA was simultaneously formed over the course of 87 h. In this situation, AO7 reduction was driven by endogenous substrates from the decaying biomass. Batch I, II and III were conducted sequentially in B1 to evaluate the effect of retention time of henna powder on the ability to reduce AO7. It can be found from Fig. 1 that AO7 reduction and SA formation were much faster than those in the control tests. In addition, the AO7 reduction behaviors were quite different in these three batch tests in B1. The decrease of AO7 and the increase of SA in Batch I were much more rapid than those in the other two batches. AO7 was completely reduced to SA and 1A2N within 45 h and 87 h in Batch I and II, respectively. Nevertheless, by the end of Batch III, only 70% of that reduction had been achieved. The calculated average AO7 reduction rates (Table S1) of the above three batches in B1 are about 6-, 3- and 2-fold that of the sludge control in B2, respectively. These results indicate that AO7 reduction will be weakened along with the retention of henna powder.

Furthermore, it was found that AO7 reduction and SA formation were very slow during the initial several hours and present a lag phase. This lag phase was prolonged with the ongoing batches in B1 (Fig. 1). These could be explained in the following way. Microorganisms must take up the available carbon, nutrients and electrons to replenish their intracellular pools prior to initiating bio-reduction of AO7 (Hsueh and Chen, 2008). In these biological cases in B1, the available carbon sources and electron donors are dependent on the fermentation process of the henna powder.