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Correlation of anaerobic biodegradability and the electrochemical characteristic of azo dyes

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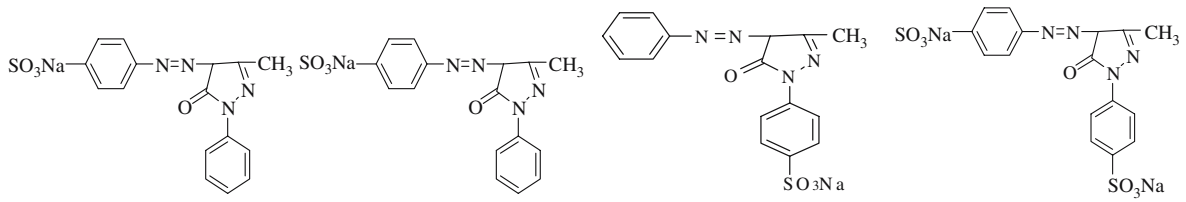
Abstract

Some experiments were conducted to study some electrochemical factors affecting the bacterial reduction (cleavage) of azo dyes, knowledge of which will be useful in the wastewater treatments of azo dyes. A common mixed culture was used as a test organism and the reductions of Acid Yellow 4, 11, 17 and Acid Yellow BIS were studied. It was found that the azo dyes were reduced at different rates, which could be correlated with the reduction potential of the azo compounds in cyclic voltammetric experiments. Acid Yellow BIS (E_r − 616.75 mV) was reduced at the highest rate of 0.0284 mol g dry cell weight^{-1} h^{-1}, Acid Yellow 11 (E_r − 593.25 mV) at 0.0245 mol g dry cell weight^{-1} h^{-1} and Acid Yellow 4 (E_r − 513 mV) at 0.0178 mol g dry cell weight^{-1} h^{-1}. At the same time, the decolourization rate of Acid Yellow 17 (E_r − 627.5 mV) was 0.0238 mol g dry cell weight^{-1} h^{-1}, which was affected by the nature of chlorine substituent. Reduction of these azo dyes did not occur under aeration conditions. These studies with a common mixed culture indicate that the reduction of azo dyes may be influenced by the chemical nature of the azo compound. The reduction potential is a preliminary tool to predict the decolourization capacity of oxidative and reductive biocatalysts.

Introduction

Aromatic azo dyes are the largest group of organic dyes for their widespread applications in many areas of textile and medicine (Schacht et al. 1996; Fu et al. 2001). They can be hardly biodegraded under an aerobic environment, but can be decolourized by anaerobic biological process (Knapp & Newby 1995; Supaka et al. 2004). It is well known that the bacterial cleavage of azo compounds is a reductive process (Mandić et al. 2003). Colour removal depends on the reduction potential of the electron donors and acceptors, because the rate-controlling step involves reduction equilibrium between the dye and the extracellular reducing agent. So the reduction potential is a measure of the ease of a molecule accepting electrons and being reduced, and electrochemistry provides convenient methods for studying mechanisms and kinetics of such reactions.

It is well known that the reduction of aromatic azo compounds depends on the type and position of substituents on aromatic rings and often is accompanied by fast homogenous chemical reactions that yield corresponding amines (Bourbonnais 1998; Eriksson & Nyholm 1999). But very few attempts have been made to study the correlation of anaerobic biodegradability and the electrochemical characteristic of azo dyes in details. This study discusses the biodegradation under anaerobic conditions of azo dyes by microorganism with reducing activity. The aim of this paper is to find out whether the reduction potential is a preliminary tool to predict the anaerobic biodegradability of azo dyes.



Acid Yellow 17 Acid Yellow-BIS Acid Yellow-11 Acid Yellow-4

Figure 1. The chemical structures of azo dyes used in this study.

Materials and methods

Dyes and reagents

The dyes used in this study were from Dye Synthesize Laboratory, Dalian University of Technology. The chemical structures of these dyes were shown in Figure 1. All other reagents were analytical grade and were purchased from Shenlian Ltd. (Dalian, China).

Organism and medium

The mixed bacterial culture was obtained from the aerobic part of the Sewage Treatment Plant of Chunliu (Dalian, China), and cultivated in the growth medium which contained: peptone 10,000 mg l^{-1}, NaCl 5000 mg l^{-1}, $(NH_4)_2SO_4$ 1000 mg l^{-1}, K_2HPO_4 1400 mg l^{-1}, KH_2PO_4 600 mg l^{-1}, mgSO_4 100 mg l^{-1}, $CaCl_2$ 100 mg l^{-1}, $FeCl_3{\cdot}6H_2O$ 0.005 mg l^{-1}, $CuSO_4{\cdot}5H_2O$ 0.0005 mg l^{-1}, H_3BO_4 1 mg l^{-1}, $MnCl_2{\cdot}4H_2O$ 0.0005 mg l^{-1}, $ZnSO_4{\cdot}7H_2O$ 0.001 mg l^{-1}, $NiSO_4$ 0.0008 mg l^{-1}, and the operation conditions of growth was pH 7.0, 30 °C on a rotary shaker at 150 r min^{-1}.

Decolourization of azo dyes by the mixed culture

The decolourization experiments were conducted in rubber-stoppered serum bottles, and acclimatization of dyes was not carried out. First, mixed culture was cultivated in the growth medium. Cells were harvested by centrifugation at 8000×*g* for 10 min after 24 h incubation and resuspended in the growth medium with different dyes to optical density 0.26–0.30 at 660 nm (OD_{660} = 0.26–0.30). Then, these cell suspensions were transferred into rubber-stoppered serum bottles (35 ml), containing 35 ml growth medium with 100 mg l^{-1} different dyes. The culture was cultivated under anaerobic condition, at 30 °C. The reduction of dyes by the mixed culture was monitored by the absorbance decrease at the maximum absorbance wavelength (λ_{max}) in the visible region for each dye (λ_{max} = 390 nm for Acid Yellow 4, λ_{max} = 393 nm for Acid Yellow 11, λ_{max} = 404 nm for Acid Yellow 17 and λ_{max} = 390 nm for Acid Yellow BIS), after centrifugation at 8000×*g* for 10 min. The decolourization rate was calculated at each $\lambda_{max.}$ The cell concentration was measured by optical density at 660 nm and converted to the dry cell weight [0.705 (OD_{660}) = 1 g l^{-1}, (R^2 = 0.995)]. To prevent possible contamination by oxygen during sampling, bottles were opened only once, and as many bottles as samples were incubated. The assays were performed in triplet. Both cell-free and sterilized controls of those mixed cultures were conducted.

The effect of Oxidation–Reduction Potential (ORP) on decolourization

All ORP measurements were performed using a Bench Digital pH/mV Meter (cyberscan pH1500, Singapore) and an ORP electrode (platinum electrode with a Ag/AgCl reference electrode in a 3.5 M KCl gel). The meter was calibrated prior to each use by short-circuiting the measuring and reference electrode input ports of the meter with a standard resistance cable (provided with the meter for this purpose), and zeroing the output reading. The meter and electrode output were periodically checked using a poised ORP reference solution containing 39.21 g ferrous ammonium sulfate, 48.22 g ferric ammonium sulfate, and 56.2 ml sulfuric acid. The meter reading was adjusted to 430 ± 10 mV which was the theoretical value of the reference solution at 25 °C. The ORP values

were reported directly as measured by the instrument and were not converted to E_h. To obtain ORP values with reference to the standard hydrogen electrode, a correction factor of +245.8 mV must be added to the reported ORP measurements. The measurement was recorded when the instrument reading was not changing more than 1 mV min^{-1}, typically within 30 min. And a cell-free control of those mixed cultures was conducted, which the ORP was obtained with nitrogen.

Electrochemical measurements

Cyclic voltammetric experiments were carried out employing a three-electrode configuration consisting of a glassy carbon (disk, 3 mm diameter) as a working electrode and ($Hg–Hg_2Cl_2(s)|KCl(s)$) electrode as the reference. All potentials given are related to this reference electrode. A platinum wire in an electrode bridge tube filled with ground electrolyte and separated from the sample by a porous frit served as counter electrode. The electrochemical measurements were performed using a Voltalab Potentiostai/Galvanostat Model 263A (Advanced Measurement Technology, USA), controlled by the Powersuite electrochemical software, at 20 mV s^{-1} scan rate. The glassy carbon electrode was successively polished with 5, 1, 0.3 and 0.05 μm alumina polish and then rinsed with 8 M nitric acid and distilled water before use. The experiments were performed at 30 °C, dye concentration 100 mg l^{-1}. Prior to analysis all solutions were purged with nitrogen for 15 min. the reduction potentials recorded vs. $Hg\text{-}Hg_2Cl_2(s)$ reference electrode were corrected by 0.2408 V to the standard hydrogen electrode (SHE). Nicotinamide adenine dinucleotide phosphate (NADH) was provided from the literature and is −320 mV vs. NHE (Bourbonnais 1998).

Analytical methods

Absorbance of the dye-containing solution was measured at their respective λ_{max} values using an UV–Visible spectrophotometer (JASCO, V−560, UV/VS spectrophotometer), and absorbance was proportional to concentration over the tested range. The relationship between absorbance and concentration was unaffected by pH in the range of 5–9.

Results and discussion

Cyclic voltammetry characteristic of azo dyes

Azo dyes showed similar electrochemical characteristics in the cyclic voltammograms. All compounds exhibited well-defined reduction peak, E_r, and oxidation peak, E_o. The azo dyes tested in this study presented similar cyclic voltammograms at 20 mV s^{-1} scan rate. The tested dyes displayed an irreversible oxidation peak in the potential range of +0.696 to 0.857 V vs. NHE and an irreversible reduction peak in the range of −1.094 to −0.74 V vs. NHE (Figures 2, 3, 4 and 5).

The effect of the sulfo and chlorine substituent on the E_r was showed in Table 1. The relative position of the sulfo group and chlorine to azo bridge has signification influence on the reduction potential. The reduction potential of Acid Yellow 4 was the greatest positive, which has two sulfo-substituent derivatives. Compared the reduction potentials of Acid Yellow 11 and Acid Yellow BIS, the electron-withdrawing properties of sulfo group does not play an important role in the electroreduction of sulfophenyl azo benzoic acids. Furthermore, the reduction potential of Acid Yellow BIS is even more negative and there is no correlation between reduction potentials and Hammet substitution constants, σ. These results indicate that the reduction potentials are solely governed by inductive or/and field effect of sulfo group. The chlorines cause the least negative potential that the electron donating chlorine substituent hinders the cleavage of azo bond.

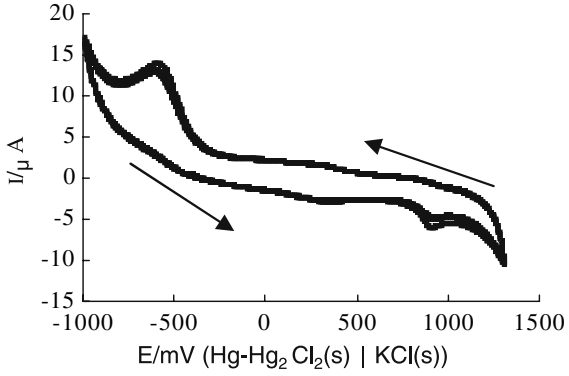


Figure 2. Cyclic voltammogram of dye AY-17: 3 cycles at 20 mV s^{-1} scan rate.

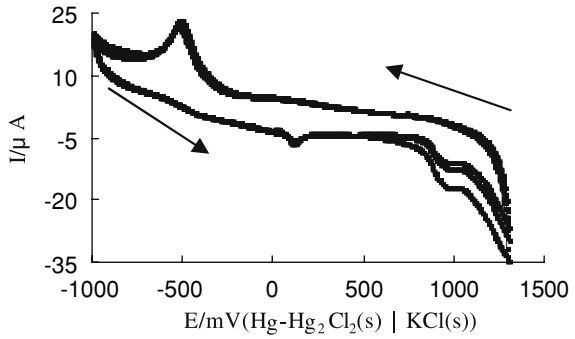


Figure 3. Cyclic voltammogram of dye AY-4: 3 cycles at 20 mV s^{-1} scan rate.

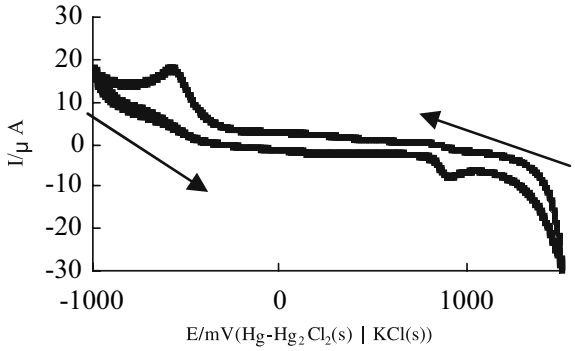


Figure 4. Cyclic voltammogram of dye AY-11: 3 cycles at 20 mV s^{-1} scan rate.

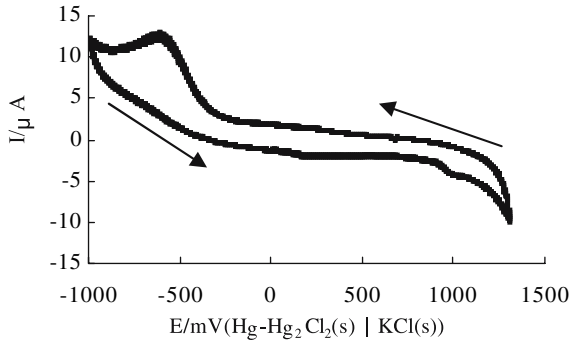


Figure 5. Cyclic voltammogram of dye AY-BIS: 3 cycles at 20 mV s^{-1} scan rate.

From the results of reduction potentials, it is possible to deduce the most important factors of carbonyl substituent, which is in agreement with the previous research results (Bourbonnais 1998; Eriksson & Nyholm 1999).

Table 1. The oxidation peak and reduction peak of Cyclic voltammetry of azo dyes (pH 5, 20 mV s^{-1})

Name of dye	Oxidation peak (V)	Reduction peak (V)
Acid Yellow 4	0.97075	−0.51300
Acid Yellow 11	0.90200	−0.59325
Acid Yellow 17	0.93625	−0.62750
Acid Yellow-BIS	0.97075	−0.61675

The effect of Oxidation–Reduction Potential (ORP) on decolourization

The objectives of these experiments were to determine the tested dyes decolourization potential of the aerobic culture under anoxic conditions gradually developed by the microbial activity. The oxidation–reduction potential (ORP) values of Acid Yellow BIS decreased from an initial value of 160–165 mV to around −250 mV and stabilized after 7 h incubation (Figure 6). During the first 2 days of incubation (i.e., the very 2 days after anoxic conditions were imposed), only 1% absorbance decline at 390 nm was observed. However, rapid decolourization was observed when the ORP values dropped below −50 mV. The extent of decolourization after 9 h of incubation was 97%. The similar changes of other dyes were shown in Figures 7, 8 and 9, and the similar results were reported (Bromley et al. 2000). And the cell-free control gave no significant reduction at high negative ORP by nitrogen, which suggested that the decolourization of the dye was caused by organism.

Effect of pH, temperature and dissolved oxygen (OD)

The optimum pH of the mixed culture was 6–9. Out of this range, the decolourization was much lower (data not shown). And 30–37 °C was the optimum temperature for growth in the mixed cultures (data not shown). The dyes were decolourizated only under anaerobic conditions.

Effect of different carbon sources

Addition of carbon sources (yeast extract and peptone) accelerated decolourization of the tested dyes; however, glucose and glycerol resulted in lower rates of decolourization of these dyes (data not shown). So peptone was selected as the carbon source in this study.

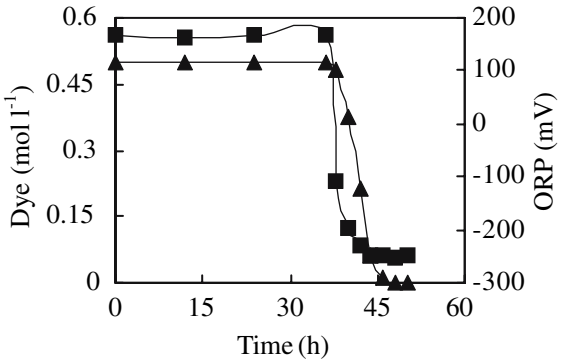


Figure 6. Acid Yellow BIS and ORP profiles in salt-tolerant culture under aerobic, anoxic and anaerobic conditions. (■: ORP; ▲: concentration of Acid Yellow BIS).

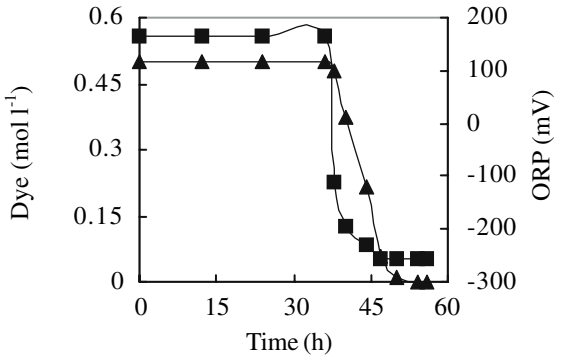


Figure 7. Acid Yellow 11 and ORP profiles in salt-tolerant culture under aerobic, anoxic and anaerobic conditions. (■: ORP; ▲: concentration of Acid Yellow 11).

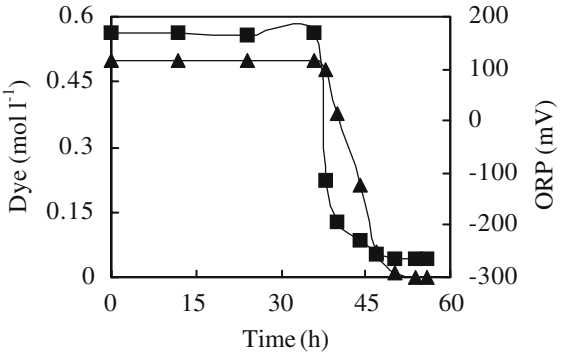


Figure 8. Acid Yellow 4 and ORP profiles in salt-tolerant culture under aerobic, anoxic and anaerobic conditions. (■: ORP; ▲: concentration of Acid Yellow 4:).

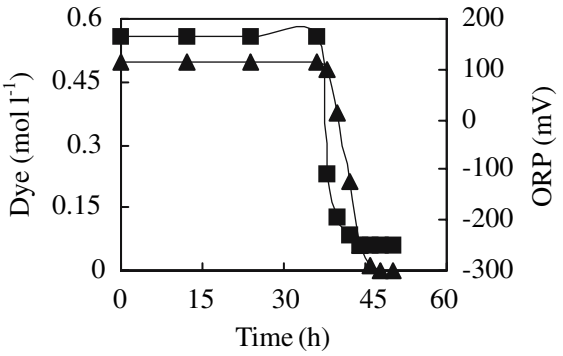


Figure 9. Acid Yellow 17 and ORP profiles in salt-tolerant culture under aerobic, anoxic and anaerobic conditions. (■: ORP; ▲: concentration of Acid Yellow 17).

Decolourization by the mixed culture

Four dyes tested in the experiment were decolourized by the mixed culture, and reduced at different rates. In contrast, less than 1% of dyes were reduced in the cell-free and sterilized controls.

The rate of reduction could be correlated with the reduction potential of the azo compounds (Figure 10), Acid Yellow BIS (E_r − 616.75 mV) was reduced at the highest rate of 0.0284 mol g dry cell weight^{-1} h^{-1}, Acid Yellow 11 (E_r − 593.25 mV) at 0.0245 mol g dry cell weight^{-1} h^{-1} and Acid Yellow 4 (E_r − 513 mV) at 0.0178 mol g dry cell weight^{-1} h^{-1}. These facts can be understood in the light of the Nernst facts equation. Any reduction depends on the formal reduction potential and on the concentrations of the reduced and oxidized species. The more negative the reduction potential, the more readily the molecule is reduced, which is not agreed with the previous results (Bragger et al.

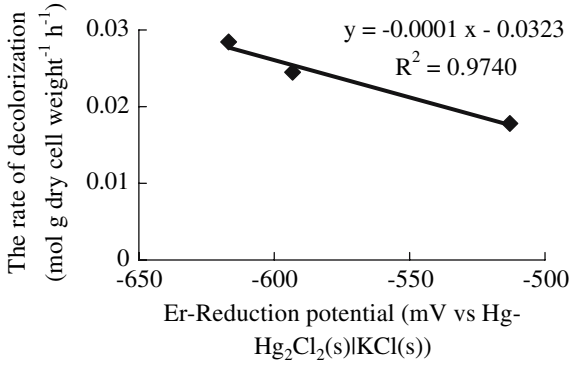


Figure 10. Correlation between reduction potentials (E_r) and the rate of dye decolourization (mol g^{-1} h^{-1}).

1997), and the rate of colour removal will increase with decreasing reduction potential of the azo substrate. The different microorganism and different mechanism of decolourization might explain the contradiction.

Concerning cell-mediated reductions, Nicotinamide adenine dinucleotide phosphate (NADH) is generally assumed to be primary electron donor and −320 mV vs. NHE (Bragger et al. 1997; Semde et al. 1998). The driving force for the reduction reactions promoted by NAD(P)H will therefore be proportional to the difference between the reduction potentials of the donor and acceptor species: the less negative the reduction potential of the azo dye, the more favorable (and faster) will be its reduction. Our observations confirmed this principle.

The inhibition of decolourization rate by chlorine

It had been reported that the chemical structures of the dyes greatly influenced their decolourization rates (Suzuki et al. 2001). Comparing the relationship of the reduction potential and the decolourization rate of four azo dyes, the inhibitory effect of chlorine was observed in the Acid Yellow 17, which was the least negative potential.

Conclusions

Reduction of these azo dyes did not occur under aeration conditions, and azo dyes were reduced by microorganisms only when the Oxidation–Reduction Potential (ORP) values decreased to the certain limits and stabled at the range of −250 to 300 mV during the decolourization process.

A linear relationship between the decolourization rates and the reduction potential of the tested azo dyes were found in this study. The results indicate that the reduction potential of the dyes is a preliminary tool to predict the anaerobic biodegradability of azo dyes.

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References

Bourbonnais R, Leech D & Paice MG (1998) Electrochemical analysis of the interaction of laccase with lignin model compounds. J. Biochim. Biophys. Acta. 1379: 381–390

Bragger JL, Lloyd AW, Soozandehfar SH, Bloomfield SF & Martin GP (1997) Investigation into the azo reducing activity of a common colonic microorganism. J. Int. J. Pharm. 157: 61–71

Bromley KCA., Knapp JS, Zhang Z, Gray NCC, Hetherdige MJ & Evans MR (2000) Decolourization of an azo dye by unacclimated activated sludge under anaerobic conditions. Water Res. 34: 4410–4418

Eriksson A & Nyholm L (1999) A comparison of the electrochemical properties of some azosalicylic acids at glassy carbon electrodes by cyclic and hydrodynamic voltammetry. J. Electrochem. Acta. 44: 4029–4040

Fu Y & Viraraghavan T (2001) Fungal decolorization of dye wastewaters: a review. Bioresour. Technol. 79: 251–262

Knapp JS & Newby PS (1995) The microbiological decolourization of an industrial effluent containing a diazo-linked chromophore. J. Water Res. 29: 1807–1809

Mandić Z, Nigović B & Simunić B (2003) The mechanism and kinetics of the electrochemical ć cleavage of azo bond of 2-hydroxy−5-sulfophenyl-azo-benzoic acids. J. Electrochem. Acta. 49: 607–615

Schacht E, Gevaert A, Kenawy ER, Molly K, Verstraete W, Adriaensens P, Carleer R & Gelan J (1996) Polymers for colon specific drug delivery. J. Control. Release. 39: 327–338

Semde R, Pierre D, Geuskens G, Devleeschouwer M & Moes AJ (1998) Study of some important factors involved in azo derivative reduction by *Clostridium perfringens*. J. Int. J.Pharm. 161: 45–54

Supaka N, Juntongjin K, Damronglerd S, Delia ML & Strehaiano P (2004) Microbial decolourization of reactive azo dyes in a sequential anaerobic-aerobic system. J. Chem. Eng. 99: 169–176

Suzuki T, Timofei S, Kurunczi L, Dietze U & Schüürmann G (2001) Correlation of aerobic biodegradability of sulfonated azo dyes with the chemical structure. J. Chemosphere. 45: 1–9