Kinetics of anaerobic biodecolourisation of azo dyes

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Abstract Kinetics of anaerobic biodecolourisation (methanogenic environment) of four azo dyes (Acid Orange 6, Acid Orange 7, Methyl Orange and Methyl Red) was investigated with regard to their electrochemical properties as well as under variation of dye and sludge concentrations, pH and temperature. Cyclic voltammetry revealed a correlation between the potential of irreversible reduction peak of the dye and its first-order decolorisation constant. For each dye tested, this decolourisation constant was adversely proportional to dye concentration (0.086–1.7 mM) and had a saturation (hyperbolic) dependency on sludge concentration (0.04–1.1 g VSS/l), a bell-shape dependency on pH (4.0–9.0) and Arrhenius dependency on temperature (24–40 °C). Transfer from methanogenic to sulphate reducing environment led to an increase of decolorisation constant for all the dyes investigated due to the abundant presence of sulphide as a reducing agent in the reaction medium. Similar transfer to a denitrifying environment resulted in an almost complete decease of decolourisation because nitrate easily outcompetes azo dyes as an electron acceptor.
Keywords Anaerobic; azo dye; cyclic voltammetry; decolourisation; kinetics

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Introduction

The annual world market for dyes is estimated to be 0.7–1.1 mln. tonnes while the Russian one is estimated to be only 0.023 mln. tonnes (Karpov and Belov, 2002). 2% of dyes produced are discharged directly in aqueous effluent, and 10% are subsequently lost during the coloration process (Easton, 1995). A major class of all colorants used worldwide is represented by azo dyes – substituted aromatic moieties linked by azo groups (−N = N −). The presence of such compounds in the industrial wastewater may create serious environmental problems due to toxicity for aquatic life and mutagenecity to humans (Hildenbrand *et al.*, 1999). In spite of persistence to biodegradation under aerobic conditions (Pearce *et al.*, 2003; Yemashova and Kalyuzhnyi, 2005), azo dyes undergo reductive splitting of the azo bond relatively easily under anaerobic conditions (Kalyuzhnyi and Sklyar, 2000; Pearce *et al.*, 2003; Yemashova *et al.*, 2004) releasing corresponding aromatic amines. The extensive investigations with a wide range of azo dye structures revealed that anaerobic decolourisation is a microbiologically non-specific process (Pearce *et al.*, 2003; Yemashova and Kalyuzhnyi, 2006); moreover, it seems to be a common property of any anaerobic and even aerobic (Bromley-Challenor *et al.*, 2000) sludge. The mechanism currently accepted by the majority of specialists in the field assumes a chemical redox reaction of azo dye with extracellular reducing agents like sulphide, NADH and other reduced redox mediators produced and regenerated by bacteria (Pearce *et al.*, 2003). The ability of the sludges to degrade azo dyes depends on the redox potential of the dye and reaction medium, temperature and pH of treatment, presence of redox mediators and electron donors etc. (Pearce *et al.*, 2003). The objective of this paper was to assess the kinetics of anaerobic biodecolourisation of four selected azo dyes: Acid Orange 6 (AO6), Acid Orange 7 (AO7), Methyl Orange (MO) and Methyl Red (MR) under variation of the major factors mentioned above.

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Materials and methods

Azodyes, biomass and basal medium

Four azo dyes used and products of their reduction (4-aminoresorcinol, 1-amino-2-naphtol, N,N-dimethyl-1,4-phenylenediamine, anthranilic and sulphanilic acids) were of analytical grade (Acrus, USA) and were not further purified. Anaerobic granular sludge (10 g VSS/l, aceticlastic activity of 0.19 g CH_4-COD/g/VSS/l) from the EGSB-reactor treating brewery wastewater (Efes-Moscow) was used for experiments in the basal medium as described by Yemashova *et al.* (2004).

Electrochemical measurements

Cyclic voltammetry of the azo dyes and corresponding amines was performed at room temperature (18 ± 2 °C) using an IPC-200 potentiostat (IPC RAS, Russia) at 50 mV/s scan rate. The working, counter and reference electrodes were a glassy carbon, gold wire and standard Ag/AgCl electrodes, respectively. The experiments were performed in 0.01 M phosphate buffer (pH 7) supplemented with 0.1 M KCl at dye and amine concentrations of 0.5 and 0.25 g/l, respectively. Prior to analysis all solutions were purged with argon for 15 min. The redox potentials recorded versus Ag/AgCl reference electrode were corrected by 227 mV (18 °C) to the standard hydrogen electrode (SHE). Thus, all potentials given in this paper are versus SHE. All scannings were started at 227 mV in the negative direction. After reaching the potential of −673 mV, scannings were turned in the positive direction and continued until + 1 227 mV where the second turn of direction was performed. During each assay, 6 such cycles were recorded.

Decolourisation assays

Almost all the anaerobic biodecolorisation assays were performed under methanogenic conditions in 120 ml closed serum bottles filled with 50 ml of basal medium, granular sludge and corresponding substrate. Since some biodegradable organics were introduced with the sludge as well as due to anaerobic biodegradation of at least one biodecolourisation product formed (Yemashova and Kalyuzhnyi, 2006), no other primary electron donors were added to these assays. Moreover, in the preliminary experiments, it was shown that their addition (e.g., ethanol) did not significantly enhance decolourisation rates (data not shown). The headspace of the bottles was flushed with argon. The varying parameters were dye (0.086–1.7 mM) and sludge (0.04–1.1 g VSS/l) concentrations, pH (4.0–9.0), temperature (24–40 °C) and redox environment. The latter was realised via addition of sulphate and nitrate to the liquid phase creating (in addition to conventional methanogenic conditions) sulphate-reducing and denitrifying environments, respectively. All the assays were performed in duplicates.

Analysis

The azo dyes concentrations were measured spectrophotometrically with a UV-1202 spectrophotometer (Shimadzu, Japan) at maximum absorbance wavelength (pH 7.0) of 428, 431, 465 and 490 nm for AO6, MR, MO and AO7, respectively. Samples were centrifuged and then aliquots of 100 µl were diluted in a 2 ml 0.1 M phosphate buffer (pH 7.0) solution and measured in a 1.0 cm quartz cuvette.

Results and discussion

Cyclic voltammetry of azo dyes

All azo dyes tested showed similar cyclic voltammograms, data for Methyl Orange are presented as an example on Figure 1a. In the first scan of any dye, an irreversible reduction (II_r) and oxidation (II_o) peaks were observed in the potential range of −93 to

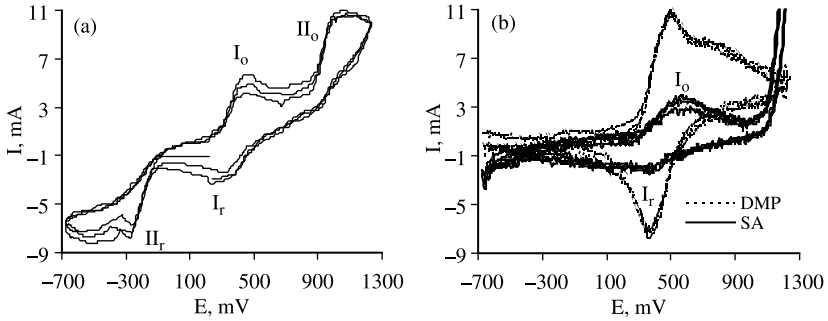

Figure 1 Cyclic voltammograms (3 cycles) of Methyl Orange (a) and its cleavage products – N,N-dimethyl-1,4-phenylenediamine (DMP) and sulphanilic acid (SA) (b)

−464 and +949 to +1,115 mV, respectively (Table 1). Both these peaks can be associated with irreversible redox reaction leading to cleavage of the azo dye. In the following scans, apparently semi-reversible redox couples (I_o, I_r) were detected. It should be noted that the reductive wave I_r did not appear in the first negative scan indicating that these redox couple peaks can be associated with the formation of unstable amine products which were oxidized and then reduced in the potential range of +260 to +465 and +64 to +240 mV, respectively (Table 1). To confirm this, the cyclic voltammograms of the pure amine product solutions were performed separately. The resulting peaks could be overlaid to the peaks of the azo dye voltammograms (compare Figures 1a and b). From Table 1, it is seen that there is a linear correlation between the potential of reductive peak II_r ($E_{II,r}$) and the first-order kinetic constant of anaerobic biodecolourisation (Figure 2). The results obtained are generally in agreement with the electrochemical data of other authors (Zille *et al.*, 2004).

Factors affecting biodecolourisation of azo dyes

Dye concentration. In the range of concentration tested, biodecolourisation of all four azo dyes proceeded without significant lag periods and usually followed the first-order kinetics (within experimental error) with respect to dye concentration independently on the initial concentration of the latter (data not shown). From Figure 3, it is seen that the calculated first-order kinetic constants generally decreased with increasing the dye concentration probably due to some inhibition of excess of substrate and/or product formed under elevated dye concentrations (Pearce *et al.*, 2003). Within an experimental error, the dependencies on Figure 3 can be described by a linear function:

$$k = k_{m,1} - \alpha \cdot C \quad (1)$$

where k is the observed first-order decolourisation constant; $k_{m,1(2,3\ldots)}$ is the "maximal" first-order decolourisation constant; α is the coefficient; C is the dye concentration.

Table 1 Oxidation and reduction peak potentials and decolourisation constants of tested azo dyes

Azo dye	II_r	mV	I_r	mV	I_o	mV	II_o	mV	First-order constant*, day^{-1}
Acid Orange 6	−425	−464	+64	+85	+260	+265	+979	+1038	0.24 ± 0.02
Acid Orange 7	−411	−428	+71	+82	+255	+273	+1080	+1115	0.46 ± 0.03
Methyl Orange	−250	−261	+222	+240	+459	+465	+1020	+1075	0.81 ± 0.04
Methyl Red	−93	−133	+76	+105	+270	+276	+949	+961	1.32 ± 0.14

*Initial azo dye concentrations 0.18–0.33 mM; ~0.4 g VSS/l of sludge; pH 7.0; 30 °C

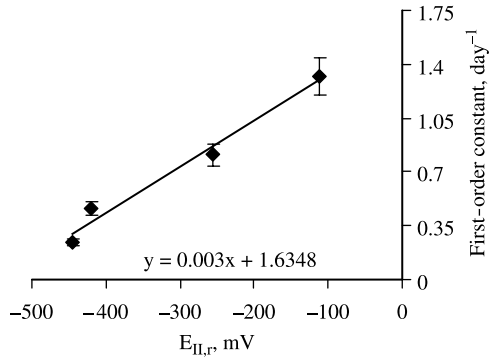

Figure 2 First-order decolourisation constants versus the potential of irreversible reductive peak ($E_{II,r}$, data of Table 1)

The results of determination of parameters $k_{m,1}$ and α from data of Figure 3 are presented in Table 2.

Sludge concentration. Though a linear correlation between activated sludge concentration (0–6 g/l) and decolourisation rate was found in previous studies (Bromley-Challenor *et al.*, 2000), in our case, the first-order decolourisation constants for all the dyes tested displayed a saturation type of dependency with regard to sludge concentration (Figure 4). This can be explained taking into account a regenerating function of sludge for reducing agents participating in azo dye splitting. Under increasing sludge concentration, hence, increasing metabolic activity per volume unit, the concentration and transport of reducing agents became less and less rate limiting for extracellular azo dye reduction. From a mathematical point of view, the curves of Figure 4 can be described by hyperbolic function similar to the Michaelis–Menten equation:

$$k = k_{m,2} \cdot VSS/(K_s + VSS) \quad (2)$$

where VSS is the volatile suspended solids concentration and K_s is the half-saturation constant. The results of determination of parameters $k_{m,2}$ and K_s from data of Figure 4 are presented in Table 2 (R^2 values varied between 0.94 and 0.98). Since not all the sludge VSS participate in regeneration of reducing agents, volumetric metabolic activity of anaerobic

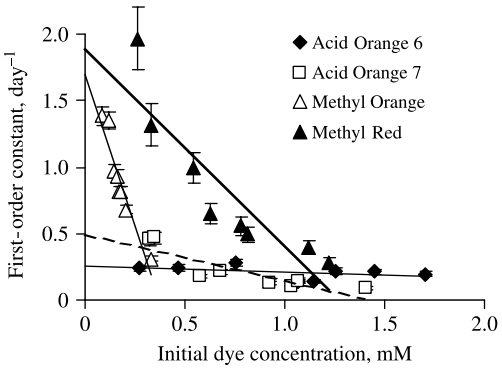

Figure 3 First-order decolourisation constants under varying initial dye concentrations (~0.4 g VSS/l of sludge; pH 7.0; 30 °C)

Table 2 Numerical values of parameters of Equations 1–4 for tested azo dyes

Parameter	Acid Orange 6	Acid Orange 7	Methyl Orange	Methyl Red
$k_{m,1}$, day^{-1}	0.26	0.5	1.69	1.82
α, $mM^{-1}.day^{-1}$	0.05	0.35	3.84	1.36
$k_{m,2}$, day^{-1}	0.25	0.34	0.79	1.81
K_s, g/l	0.06	0.02	0.05	0.52
$k_{m,3}$, day^{-1}	0.34	0.85	1.12	0.94
K_a	$8.0{\cdot}10^{-4}$	$4.5{\cdot}10^{-6}$	$5.5{\cdot}10^{-5}$	$4.0{\cdot}10^{-5}$
K_b	$1.0{\cdot}10^{-8}$	$8.6{\cdot}10^{-10}$	$7.0{\cdot}10^{-11}$	$2.1{\cdot}10^{-10}$
β	1	0.79	0.97	0.84
A, day^{-1}	$1.1{\cdot}10^{9}$	$8.5{\cdot}10^{6}$	$2.1{\cdot}10^{9}$	$1.2{\cdot}10^{6}$
E_a, kJ/mol	56.2	39.7	53.9	34.9

COD conversion (e.g., acidogenic, methanogenic, sulphate reducing or denitrifying activity) may be proposed as a more correct measure of sludge ability to regenerate reducing agents.

pH. From Figure 5, it is seen that the first-order decolourisation constants had a bell-shape dependency on pH, however, the shape of bell was not symmetrical, the acidic shoulder was generally less sloping compared to the alkaline one. From a mathematical point of view, the curves of Figure 4 can be described by the following function:

$$k = k_{m,3}/(1 + [H^+]^{\beta}/K_a + K_b/H^+) \quad (3)$$

where K_a and K_b are the dissociation constant and β is the coefficient. The results of determination of parameters $k_{m,3}$, K_a, K_b and β by the least square method from data of Figure 4 are also presented in Table 2.

Temperature. From Figure 6, it is seen that the first-order decolourisation constants increased with temperature increasing up to 40 °C. The curves of Figure 6, in general, followed the commonly used Arrhenius equation:

$$k = A \exp(-E_a/RT) \quad (4)$$

where A is the Arrhenius constant; E_a is the activation energy; R is the gas law constant; T is the absolute temperature. The results of determination of parameters A and E_a from data of Figure 6 are presented in Table 2.

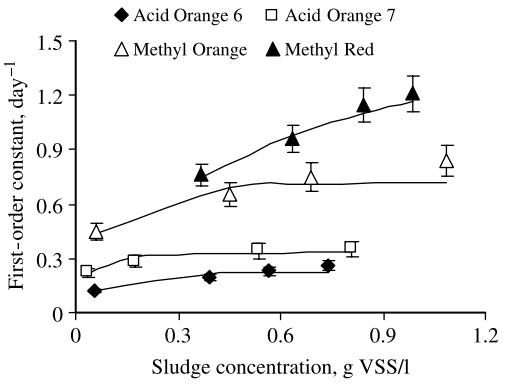

Figure 4 First-order decolourisation constants under varying initial sludge concentrations (AO6, AO7 and MR–0.6 mM; MO 0.16 mM; pH 7.0; 30 °C, points – experimental data; lines – theoretical curves obtained using Equation 2)

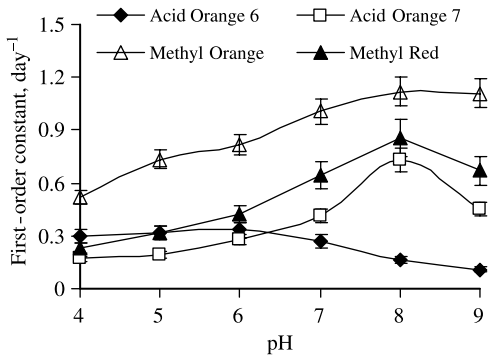

Figure 5 First-order decolourisation constants under varying pH (AO6 and AO7 – 0.6 mM; MO – 0.16 mM; MR – 0.8 mM; ~ 0.4 g VSS/l of sludge; 30 °C)

Redox environment. Since the rate limiting step of azo dye anaerobic reduction involves a redox equilibrium between the dye and the extracellular reducing agents (Pearce *et al.*, 2003), a decrease of decolourisation rate under increase of redox potential of reaction medium might be expected. This is indeed so for the transfer from methanogenic ($E_h = -420$ mV) to denitrifying ($E_h = +430$ mV) environments when an almost complete decease of decolourisation was observed (Figure 7) because nitrate easily outcompetes azo dyes as an electron acceptor. However, the transfer to sulphate reducing ($E_h = -200$ mV) environment led to an increase of decolorisation constant for all the

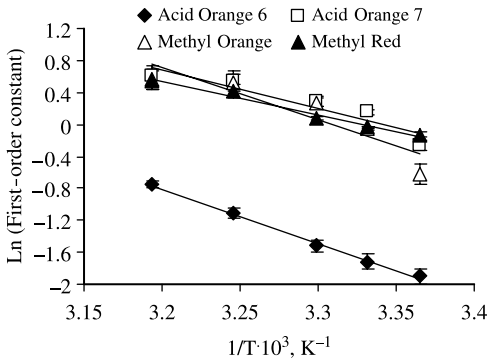

Figure 6 First-order decolourisation constants under varying temperature (AO6 and AO7 – 0.2 mM; MO and MR – 0.1 mM; ~0.4 g VSS/l of sludge; pH 7.0; 30 °C)

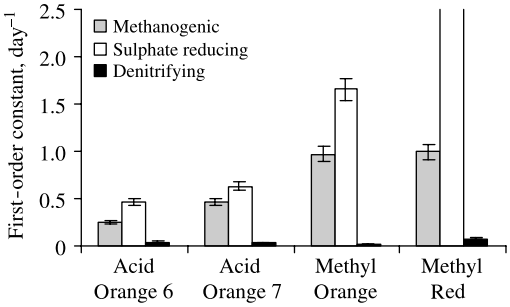

Figure 7 First-order decolourisation constants in methanogenic, sulphate reducing and denitrifying environments (AO6 and MR – 0.5 mM; AO7 – 0.3 mM; MO – 0.15 mM; ~0.4 g VSS/l of sludge)

dyes investigated due to an abundant presence of sulphide as a reducing agent and redox mediator in reaction medium (Figure 7).

Conclusions

We concluded that cyclic voltammetry of four tested azo dyes revealed a correlation between their potential of irreversible reduction peak and first-order decolorisation constant.

For each dye tested, the first-order decolourisation constant was adversely proportional to dye concentration (0.086–1.7 mM) and had a saturation (hyperbolic) type of dependency on sludge concentration (0.04–1.1 g VSS/l), a bell-shaped dependency on pH (4.0–9.0) and Arrhenius dependency on temperature (24–40 °C).

Transfer from methanogenic to sulphate reducing environment led to an increase of decolorisation constant for all the dyes investigated due to an abundant presence of sulphide as a reducing agent in the reaction medium. Similar transfer to a denitrifying environment resulted in an almost complete decease of decolourisation because nitrate easily outcompetes azo dyes as an electron acceptor**.**

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