Journal of Hazardous Materials B114 (2004) 29–39

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Monitoring of toxicity and intermediates of C.I. Direct Black 38 azo dye through decolorization in an anaerobic/aerobic sequential reactor system

Mustafa Işık, Delia Teresa Sponza*

Environmental Engineering Department, Engineering Faculty, Dokuz Eylul University, Buca Kaynaklar Campus, 35160 Izmir, Turkey

Received 8 April 2004; accepted 21 June 2004

Abstract

An Upflow Anaerobic Sludge Blanket Reactor/Continuous Stirred Tank Reactor was used sequentially to decolorize and mineralize C.I. Direct Black 38 azo dye (3200 mg/L) in a synthetic wastewater containing glucose as co-substrate. At the steady state conditions color was effectively removed under anaerobic condition, while the total aromatic amines and organic fraction could be mainly reduced under aerobic conditions. NO_3^-–N, COD, BOD_5, aromatic amine, HPLC and GC analyses showed that Direct Black 38 could be chiefly mineralized by the sequential system. The toxicity levels were determined using three different test organism (ATA-anaerobic toxicity, respiration/inhibition and *Daphnia magna* tests) through the continuous operation of anaerobic/aerobic sequential system treating Direct Black 38 dye containing synthetic wastewater. Feed and anaerobic effluent had greater toxicity than the aerobic effluent after mineralization of dye.

Keywords: Toxicity; Azo dyes; Direct Black 38; Benzidine; Microorganisms; Anaerobic/aerobic; Toxicity

1. Introduction

Azo dyes consist of a diazotized amine coupled to an amine or a phenol, and contain one ore more azo linkages. They are the largest class (60–70%) of dyes with the greatest variety of colors [1]. Approximately 10–15% of the dyes are released into the environment during dyeing of different substrates, such as synthetic and natural textile fibres, plastics, leather, paper, mineral oils, waxes and even (with selected types) foodstuffs and cosmetics [2]. Even at very low concentrations (10–50 mg/L) water soluble azo dyes can cause waste streams to become highly colored. Aside from their negative aesthetic effects certain azo dyes and biotransformation products have been shown to be toxic, and in some cases these compounds are carcinogenic and mutagenic [3]. Approximately, it was determined that 130 of 3200 azo dyes in use have produced carcinogenic aromatic amines because of reductive degradation of these [4]. In Turkey, the Turkish government declared that the azo dyes are banned dyes since 1 March 1995 due to the relevant aromatic amines. However, they have been still in use in textile dyeing processes, due to their efficiency of dyeing and cost, in Turkey and in some countries.

Azo dyes are reduced under anaerobic conditions to the corresponding aromatic amines [5,6] which though resisting further anaerobic degradation, are reported to be well amenable for aerobic degradation. During the last few years, several laboratory-scale continuous anaerobic/aerobic processes for the treatment of wastewater containing azo dyes have been described [7–11]. Aerobic stage of combined anaerobic/aerobic treatment of dye wastes eliminated the chemical oxygen demand (COD), attributed to removal of aromatic amines, which are anaerobically recalcitrant [12]. Aromatic amines are generally not degraded and accumulate under anaerobic conditions [13,14], with the exception of a few aromatic amines characterized by the presence of hydroxyl and/or carboxyl groups [15,16]. Mineralization

* Corresponding author. Tel.: +90 232 4531008/1119; fax: +90 232 4531153.
E-mail addresses: mustafa.isik@deu.edu.tr (M. Işık), delya.sponza@deu.edu.tr (D.T. Sponza).

0304-3894/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2004.06.011

of the aromatic amines by aerobic bacteria and aerobic sludge in treatment plants is more common and, therefore, aerobic conditions are prefered to degrade the accumulated aromatic amines [17,18]. The color was removed under anaerobic conditions while the complete mineralization of amines to CO_2, H_2O and NH_3 occurs under aerobic conditions. Therefore, anaerobic treatment followed by aerobic treatment can be used to decompose putatively toxic and recalcitrant compounds [6,15,16,19]. Mineralization of the aromatic amines by aerobic bacteria and aerobic sludge in treatment plants is more common and, therefore, aerobic conditions are prefered to degrade the COD remaining from the anaerobic stage [20,21].

The studies performed to decolorize the azo dyes using the anaerobic/aerobic sequential processes were with Anthraquinone and diazo dyes [22], Reactive azo dyes [23], Orange G and Mordan Green azo dyes [24], Acid Yellow and Basic Red dyes [25], Remazol Brilliant Orange 3R and Black 5 dyes [12], Remazol Brilliant Violet 5R dyes [26], Congo Red dyes [10] and dispersed blue 79 dyes [27].

The studies, in the past, including the decolorization of DB 38 are limited with the studies carried out by Işik and Sponza [11,28,29,31] and Bradford et al. [30]. The effect of increasing DB 38 dye loadings from 6 g/(m^3 h) (100 mg/L of DB 38 dye) to 192. 13 g/(m^3 h) (3200 mg/L of DB 38 dye) on decolorization and COD, volatile fatty acid (VFA), total aromatic amine removal efficiencies were investigated in sequential anaerobic upflow anaerobic sludge blanket reactor (UASB)/aerobic CSTR reactors [28,29]. For 3200 mg/L of DB 38 and 2000 mg/L glucose COD as co-substrate in the feed, 48.4% COD and 80% color removal efficiencies were observed at a maximum COD and dye loading rates of 5.37 kg COD/(m^3 d) and 213 g dye/(m^3 h), respectively, at a hydraulic retention time (HRT) of 15 h in the UASB reactor. The remaining COD (67%) was removed at an HRT of 2.3 days and a maximum organic loading rate of 0.77 kg COD/(m^3 d) in the aerobic stage. The TAA produced under anaerobic conditions was ultimately removed in the aerobic stage and aromatic amine recoveries such as 45–50% were obtained in the aerobic reactor. The anaerobic/aerobic sequential process provides simultaneous color, COD and carcinogenic amine removal [28,29]. The metabolism of DB 38 dye was investigated using human intestinal microbiota. Benzidine, 4-aminobiphenol, monoacetylebenzene and acetylamino-biphenyl (AABP) were isolated as metabolites in a semi-continuous reactor system [30]. The aromatic amines arising from the metabolites of anaerobic biodegradation was recorded under anaerobic conditions by *Escherichia coli* and *Pseudomonas* sp. High benzidine recoveries indicated the accumulation of aromatic amines under aerobic conditions. The color of the Congo Red and DB 38 dyes were removed up to 98 and 72%, respectively, by *E. coli* at the end of anaerobic incubation, while no decolorization occurred throughout the aerobic incubation [31].

Azo dyes were inhibitory to microbial oxidation processes in both activated sludge and stream water [32]. Dyes in aquatic environments were reported to affect the microbial population and their activities. Toxicity on microorganism in the environment could be eliminated through the mineralization of azo dyes by anaerobic/aerobic sequential process. Dyes in aquatic environments were reported to affect the microbial population and their activities. Azo dyes were inhibitory to microbial oxidation processes in both activated sludge and stream water [32]. Toxicity on microorganism in the environment could be eliminated through the mineralization of azo dyes by anaerobic/aerobic sequential process.

The novelty of this work is the utilization of UASB with partially granulated sludge as seed and the operation in continuous mode with a sequential aerobic completely stirred sludge reactor through decolorization of DB 38 azo dye. The anaerobic intermediates of the DB 38 dye through continuous operation was only determined in this study. Therefore, the main objectives of this study were to investigate the COD and color removal efficiencies of a sequential anaerobic/aerobic reactor system treating C.I. Direct Black 38, which is a banned azo dye and to determine the intermediate products produced through dye decolorization. The anaerobic intermediates of the DB 38 dye through continuous operation was only determined in this study. Furthermore, the toxic effect of C.I. Direct Black 38 was investigated using anaerobic, aerobic bacteria and *Daphnia magna* in influent, and effluent samples of anaerobic/aerobic system.

2. Material and methods

2.1. Synthetic wastewater

Synthetic wastewater containing 3200 mg/L of DB 38 azo dye, 3000 mg/L of glucose COD, 3000 mg $NaHCO_3$/L of alkalinity and Vanderbilt mineral medium [33] were treated using an anaerobic/aerobic sequential reactor system. Glucose is a good electron donor for cleavage of azo dyes under reducing equivalents. The characteristics of DB 38 azo dye is given in Fig. 1.

2.2. Configuration, seeding and operation of the reactor system

A continuously fed stainless steel anaerobic UASB and aerobic CSTR reactors were used in sequence for the experimentation. The UASB reactor had 1.8 L of effective volume with an internal diameter of 8 cm and a height of 40 cm. The

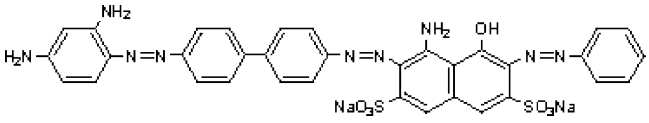

Fig. 1. Open formula and characteristics of C.I. Direct Black 38 used in this study (color index number: 30235; λ_{max}: 520 nm; COD of 1000 mg/L of Direct Black 38 solution: 370 mg/L; molecular formula: $C_{34}H_{28}N_9O_8S_2Na$; azo group: tri-azo; molecular weight: 781.7 g/mol and water solubility: 93 g/L (318 K)).

CSTR reactor consisted of an aeration tank (effective volume = 9 L) and a settling compartment (effective volume= 1.32 L). Partially granulated anaerobic sludge was used as seed in UASB reactor and was taken from the methanogenic reactor of Pakmaya Yeast Baker Factory in Izmir. CSTR inoculated with the activated sludge taken from the lab scale CSTR reactor treating molasses and operated with a MLSS concentration of 3000 mg/L. The UASB/CSTR reactor systems were initially started to operate after 15 days of adaptation period with free-azo dye. Then the azo dye concentrations increased stepwise from 100 to 3200 mg/L in order to minimize the adverse effect of increasing dye loadings rates. Once steady-state conditions were achieved at an organic loading rates of 1.14 kg/(m^3 d) in the UASB reactors and at a HRT 3.6 days and a sludge retention time (SRT) of 86 days for the treatment of DB 38 azo dye. HRTs of CSTR reactors were 18 days corresponding to a flowrate of 0.5 L/day. An UASB and CSTR reactor were operated without dye as control. These reactors were operated in continuous mode to compare the inhibitions in dye containing reactors.

2.3. Analytical procedure

The total and soluble COD was measured calorimetrically using closed reflux methods [34]. Inert COD content of the samples was determined with glucose comparison method developed by Germirli [35] measuring all the soluble form of COD under aerobic conditions. TOC was determined with DOHRMANN DC-190 Model High-Temperature TOC Analyzer. Bicarbonate alkalinity (B.Alk.) and total VFA concentrations were measured simultaneously by Anderson and Yang [36] using titrimetric method. Biomass measurements were performed according to Standard Methods for Examination of Water and Wastewater [34]. Color was measured by an UV–vis spectrophotometer (Pharmacia LKB-NovaPec II) at a wavelength of 520 nm in which maximum absorbance spectra was obtained for DB 38 azo dye. The samples were centrifuged at 7000 rpm for 10 min and the absorbance values of supernatants were recorded for color measurements. Since the effluents of the reactors contained not only the undergraded substances but also some of the colloidal organic matters such as metabolic excretions and dead cells interfered with color measurements. Therefore, in order to measure the real color removal the absorbance values of the reactor effluents at λ_{max} was calibrated with the control samples containing no dye, Vanderbilt mineral medium and anaerobic, aerobic reactor effluents. Therefore, an anaerobic and aerobic reactor were continuously operated without dye as control. The decolorization of azo dyes was measured as the decrease of light absorbance at their maximum levels as follows after the corrections mentioned above are carried out [37]:

$$\text{Color removal efficiency} = \frac{A_{\text{influent sample}} - A_{\text{effluent sample}}}{A_{\text{influent sample}}} \times 100$$

BOD_5 was measured using the WTW Oxi Top IS 12 system. Ammonium and nitrate nitrogen were quantified using specific chemical analysis kits numbered 14536 and 14598 (Merck-Spectroquant), using spectrometric methods. Oxidation reduction potential (ORP) was measured using WTW Sentix ORP meter (Germany) with a $Ag/AgCl_2$ reference electrode in saturated KCl solution and Pt electrode. Total aromatic amines were determined calorimetrically at 440 nm after reacting with 4-dimethylamino-benzaldehyde–HCl according to the method described by Oren et al. [37]. The chemical reduction of azo dyes was carried out with sodium dithionite. The reduction process was as follows: 0.06 g of dye sample was heated at boiling point with 1 M NaOH for 1 h and after 30 min 0.6 g sodium dithionite was added. Dye (1000 mg/L) was reduced according to the method described by Pielesz [38]. TAA content (benzidine) of DB 38 azo dyes in the feed (influent sample) was calculated from the calibration curves given in Fig. 2. The aromatic amines produced from the reduction of DB 38 in influent was calculated from these figures in order to determine the aromatic amines removed in anaerobic UASB reactor.

Abiotic tests performed with autoclaved non-living anaerobic partially granulated sludge and mineral medium showed that microbial decolorization was preceded primarily by biological degradation instead of abiotic removal.

2.4. Toxicity testes

2.4.1. Anaerobic toxicity assay (ATA) and specific methanogenic activity (SMA)

ATA test was performed at 37 °C using serum bottles with a capacity of 115 mL as described by Owen et al. [39] and

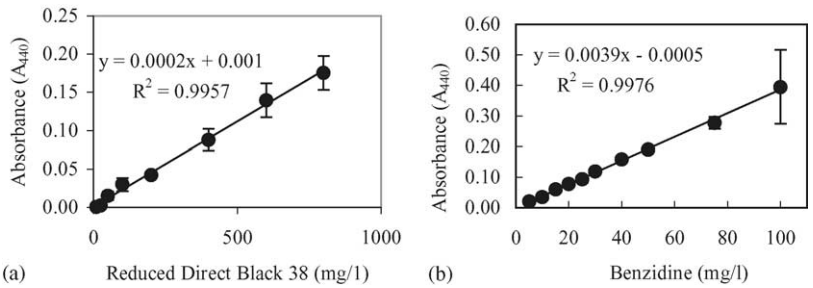

Fig. 2. The calibration curves of benzidine and chemically reduced DB 38.

Libra et al. [40]. Serum bottles were filled with stock solutions to give suitable Vanderbilt mineral medium, 3000 mg/L of glucose COD, 667 mg/L of sodium thioglycollate for providing the reductive conditions. Furthermore, 5000 mg/L of $NaHCO_3$ was used for maintaining the neutral pH, 4000 mg/L of MLSS for providing the anaerobic granules enriched in the anaerobic treatment plant of a yeast industry were used. The glucose COD in the serum bottles was stoichiometrically replenished to 3000 mg/L with stock solution after 3 days of exposure containing dye and its metabolites. The glucose COD level of 3000 mg/L ensured the presence of non-limiting substrate conditions in the serum bottles. Duplicate controls were performed on the assay containing no-dye while the analyses were carried out in triplicate samples. The results of ATA test given in Table 3 illustrates the mean ± standard deviation values. Methane production of each assay bottle was determined during subsequent 6 h of incubation period. Maximum specific methanogenic activity was calculated from the methane production through this period. Methanogenic activities of samples containing dye and its metabolites were compared to the control samples to determine the degree of inhibition effect on glucose utilization. Inhibition was defined as a decrease in cumulative methane compared to the control samples.

2.4.2. Respiration–inhibition test

This test was based on BOD measurements and the simulation to ATA test. Respiration–inhibition tests were carried out into bottles of WTW Oxi Top IS 12 system. Aerobic sludge from the lab scale CSTR reactor was fed with molasses, mineral medium and glucose COD of 1500 mg/L. Furthermore, wastewater samples in certain volumes were added to bottles. The final volume was adjusted to 97 mL with distilled water. The glucose COD in the bottles was stoichiometrically replenished to 1500 mg/L with a stock solution after 3–4 days of exposure to synthetic wastewater. Duplicate controls were performed in the bottles containing no DB 38 azo dye and its metabolites while the analyses were carried out in triplicate samples. The results given in Table 3 illustrates the mean ± standard deviation values. Oxygen consumption was monitored every day and compared to the control samples. Inhibition was defined as a decrease in oxygen consumption compared to the control samples.

2.4.3. Daphnia magna toxicity test

Toxicity was tested using 24 h *D. magna*. Test animals were obtained from the Zoology Department of the Engineering, Faculty at Aegean, University of Izmir. After a cloning periods in the laboratory, toxicity experiments were performed. Experiments were carried out using five daphnids in test beakers with 100 mL of effective volume at a pH 7–8 providing 6 mg/L of dissolved oxygen at the ambient temperature (20–30 °C) in five different concentrations as five series. A blank was also carried on. Results were expressed as mortality percentage of the daphnids after 24 h. The immobile animals which were not able to move were determined as death of daphnids.

2.5. High performance liquid chromatography–diode array detection (HPLC–DAD) and gas chromatography–mass spectra (GC–MS)

HPLC analyses were carried out using LC 10 model (Shimadzu) HPLC–DAD equipped with C 18 (Russia) chromatographic column (i.d. 4 mm, length 25 cm, stationary phase particle size 7 μm. In the operation mobile phase: methanol/water (55:45) was used at a flowrate of 1 mL/min. All samples were diluted at a ratio of 1:10 with distilled water.

For GC–MS analysis, the samples was extracted by liquid/liquid extraction as follows. The pH of the aqueous sample first adjusted to a pH greater than 10 with sodium hydroxide. Sodium chloride was added until it reached saturation. The sample was then extracted twice with 25 mL of methyl-*tert*-butyl-ether. The ether fraction was combined and evaporated to dryness. Finally, the extract was reconstituted in 2 mL of methanol for GC/MS analysis. The analyses were done using a Shimadzu QP5050A model GC–MS device. The mass spectrometer was operated in the electron impact mode with electron current of 70 eV. Aliquots of 1 μL were injected automatically by an auto sampler (AUC20i) in splitless mode via an GC inlet (injector temperature 250 °C). A Optima-5-MS capillary column, 30 m long, 0.25 mm i.d., 0.25 μm film thickness was connected directly to the ion source of the mass spectrometer. The oven temperature was kept isothermal for 10 min at 60 °C, then increased to 300 °C at a rate of 10 °C/min and finally, 10-min hold step was applied at 300 °C. GC–MS and HPLC–DAD analyses were performed in TÜBITAK Bursa Test and Analysis Laboratories.

3. Results and discussion

3.1. Removal of organics in the UASB/CSTR sequential system

The data collected from UASB/CSTR sequential system at a steady state conditions were summarized in Table 1 and the treatment performances based on COD, TOC, TAAs and color removals of UASB/CSTR reactor system are given in Table 2. Since the influent COD contained 3000 mg glucose COD/L, the remaining COD value was originated from the azo dyes. BOD_5 concentrations were 3018, 360 and 30 mg/L in the influent and effluent of the UASB reactor, and effluent of CSTR reactor, respectively. Inert COD measurements of samples corresponded to effluent CODs of CSTR showed that the inert COD levels are quite close to COD concentrations. Since the CSTR reactors were operated at long HRTs (18 days), probably the degradable fraction of the UASB effluent could be degraded by the aerobic microorganisms. Inert COD measurement showed that effluent COD of CSTR reactor constituted mainly from the inert COD produced under

Table 1
The influent and effluent parameters of anaerobic/aerobic system

Parameter	Sample		
	Feed	UASB effluent	CSTR effluent
COD (mg/L)	4100	928	335
BOD_5 (mg/L)	3018	360	30
COD/BOD_5 ratio	1.35	2.6	11.6
Inert COD (mg/L)	1440	381	335
TOC (mg/L)	1236	496	231
TAAs (mg benzidine/L)	ND[a]	121.4	23.7
Absorbance at λ_{max}	21.43	4.45	1.33
NH_3—N (mg/L)	1.15	1.6	2
NO_3—N (mg/L)	16	15	84
VFA (mg CH_3COOH/L)	ND[a]	503	ND[a]
T.Alk (mg $CaCO_3$/L)	2200	2400	1500
VFA/B.Alk ratio	ND[a]	0.23	ND[a]
pH	8.18	6.91	8.65
ORP (mV)	ND[a]	−339	−57

[a] ND, not detected.

aerobic conditions. Approximately 5% of inert COD in anaerobic effluent was degraded in aerobic CSTR reactor. Effluent COD of the system probably contained soluble microbial products produced from the microorganisms and not mineralized dye metabolites. The oxidation–reduction potential was measured as −339 mV in UASB reactor which indicates the strict anaerobic conditions. However, the ORP potential was −57 mV in aerobic reactor since the effluent of aerobic reactor was used as the influent of aerobic rector. Although high positive ORP values indicates the strict aerobic conditions, in this study lower ORP values did not negatively affect the COD and TAA removal efficiencies. It is important to note that although the ORP value is lower in aerobic reactor, the dissolved oxygen concentration varied between 2 and 3 mg/L (data not shown).

t-Test statistics for the parameters measured in influent, anaerobic and aerobic effluents showed that they are not significantly different (see Table 1; t_{critic} = 2.064, $t_{test\ statistic}$ = 0.10, α = 0.025).

Table 2 shows the treatment performances of the UASB/CSTR reactor system. COD removal efficiencies were found to be 77, 64 and 92% while the BOD removal efficiencies were 88, 92 and 99% for UASB, CSTR and UASB/CSTR reactors, respectively. As shown in this Table 64% of COD was removed under aerobic conditions while 77% of COD was removed under anaerobic conditions. Although, in literature it was reported that COD was removed mainly under aerobic conditions in this study significant amount of COD was removed under anaerobic conditions resulting in high methane gas productions (data not shown). The results obtained in this study exhibits similar results with the studies performed by Donlon et al. [41]. In this study it was reported that a small amount of removed COD (E = 36) was oxidized by aerobic bacteria in the second stage of anaerobic/aerobic treatment system treating Reactive Black 5 azo dye. However, the studies performed by, Field et al. [9], Supaka et al. [12] and Sponza and Işık [29] demonstrated that the great amount of COD removed under aerobic conditions while the COD ratio treated under anaerobic conditions also exhibits significant level. In these studies it was demonstrated that aerobic stage eliminated the additional COD and attributed to removal of aromatic amines. In the last study it was demonstrated that 25 and 45% of COD were removed in anaerobic UASB reactor at HRTs 5 and 15 h, respectively. The COD/BOD_5 ratio increased to 2.6 and 11.6 from 1.35 after the anaerobic and aerobic treatment indicating that the biodegradable material was removed as reported by O'Neill et al. [42]. In other words, this phenomenon was probably a result of metabolic activity due to the presence of the more easily degradable compounds which seem to be less toxic. Overall 81% reduction in TOC was achieved as a result of anaerobic/aerobic treatment of wastewater containing DB 38 at a concentration of 3200 mg/L.

Table 2
Treatment performances of the UASB, CSTR and UASB/CSTR sequential system

Parameter	Removal efficiencies (%)		
	UASB	CSTR	UASB/CSTR
COD	77	64	92
TOC	60	54	81
BOD_5	88	92	99
TAAs	26	81	86
Color	81	67	94

The concentration of TAA obtained from the degradation of DB 38 were 121 and 24 mg benzidine/L in the effluent of UASB and CSTR, respectively. The theoretical TAA concentration in the influent of anaerobic reactor was obtained from the benzidine levels produced from the chemical reduction of DB 38 azo dye as shown in Fig. 2. As shown in Table 2, TAA was mainly removed under aerobic conditions (E = 81) resulting in a TAA removal efficiency of 86% in the whole system. Most researches agree with these findings that the aromatic amines released from the cleavage of azo bond accumulated in the anaerobic conditions [5,6,43]. The TAA was partially removed (E = 26%) under anaerobic conditions. This results agree with the studies performed by Işık and Sponza [31], Razo-Flores et al. [43] and Donlon et al. [44], however, it contradicts with the studies carried out by Field et al. [9], Brown and Hamburger [45], Haug and Schmidt [46] and Seshadri et al. [47]. A study showed that the recovery percentage of total aromatic amines was about 100% as the DB 38 concentrations were increased to 3200 mg/L in anaerobic batch reactors [29].

t-Test statistics for the COD, TOC, BOD_5, TAA and color removal efficiencies measured in anaerobic and aerobic and UASB/CSTR reactor effluents showed that they are not significantly different (see Table 2; t_{critic} = 1.790, $t_{test\ statistic}$ = 0.275, α = 0.025).

3.2. Color removal in the UASB/CSTR sequential system

Microbial decolorization of DB 38 occurred anaerobically due to reduction and cleavage of the azo bonds, causing color,

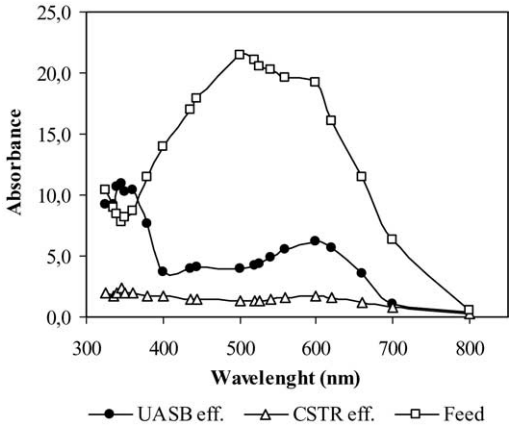

Fig. 3. Absorbance spectra of influent and effluent samples in the reactors.

which is associated with the production of the TAAs and was biodegradable in aerobic conditions. The peaks in visible region at 520 nm in the feed were removed by anaerobic treatment, and replaced by a small peak in UV region around 345 nm as shown in Fig. 3. The anaerobic and aerobic reactor effluents illustrated a peak at a wavelength of 350 nm. Color removals were 81, 67 and 94% in the UASB, CSTR and UASB/CSTR reactors, respectively, showing that both anaerobic and aerobic reactor could remove color. Probably, colorful intermediates occurred from the decolorization of DB 38 azo dye in the anaerobic stage, and then these intermediates degraded in the aerobic CSTR reactor. The effluent samples of UASB and CSTR reactors exhibited maximum absorbance spectra at low wavelengths (UV region) due to aromatic amines produced through dye degradation [43]. This shows that the parent dye converted to other intermediate organic forms. When COD, VFA, VFA/B.Alk, ORP and pH parameters were taken into consideration, inhibition was not observed in the UASB and CSTR reactors compared to the batch and continuous fed control reactors containing no dye. Color and organic matter could be effectively removed in the UASB/CSTR system.

3.3. Monitoring of intermediates through decolorization of DB 38 azo dye

To avoid the time-consuming and analytical complex procedures necessary for the identification of each derivative, simple HPLC–DAD and GC–MS analysis were carried out to determine qualitatively whether UV-absorbing amino derivatives were formed and/or degraded during each treatment step. Similar studies were performed by O'Neill et al. [42]. Fig. 4 shows the HPLC chromatograms of effluents of UASB and CSTR, respectively, relevant to determined aromatic amine "benzidine".

Fig. 5 shows the chromatograms of benzidine standard (20 mg/L) in HPLC analysis for comparison of the samples containing dye as a by-product. Since DB 38 is benzidine-based dyes it is assumed that if azo bond on these dyes is broken down, benzidine will be formed under anaerobic conditions. Fig. 4 clearly demonstrates that UV-absorbing by-product was degraded through aerobic treatment in the samples containing DB 38 by-products. The standard benzidine peaks in Fig. 5 was obtained at a retention time of 3.5 min. The similar peaks are shown on the chromatograms at the same retention times in the studied samples. Decreases in detectable area in the particularly benzidine peaks during aerobic treatment indicated the degradation of benzidine and other metabolites.

GC–MS analyses were also performed in order to determine the intermediates of DB 38 azo dye. In the samples containing only benzidine could be identified by comparison

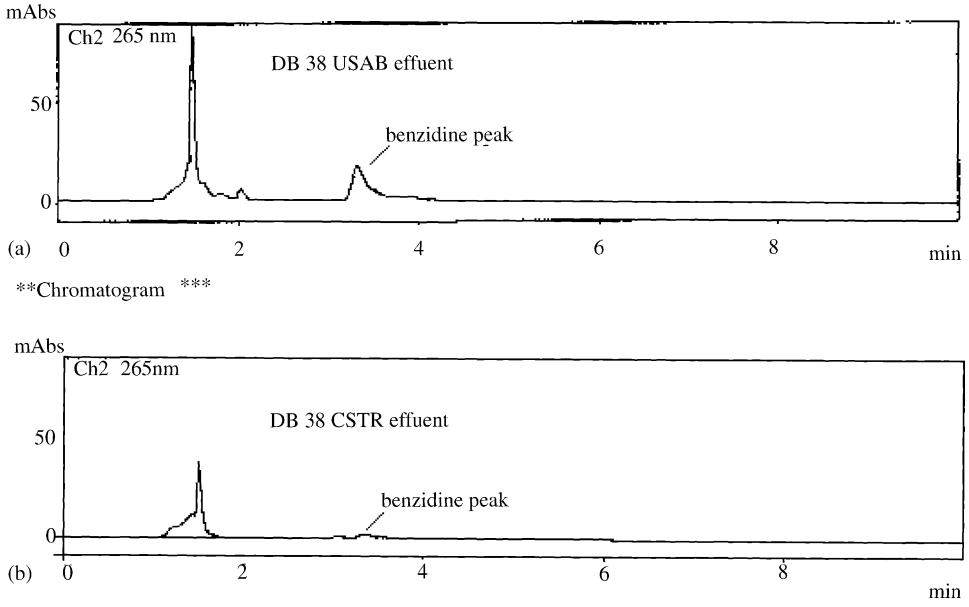

Fig. 4. HPLC chromatograms of effluent (a) UASB and (b) CSTR reactor.

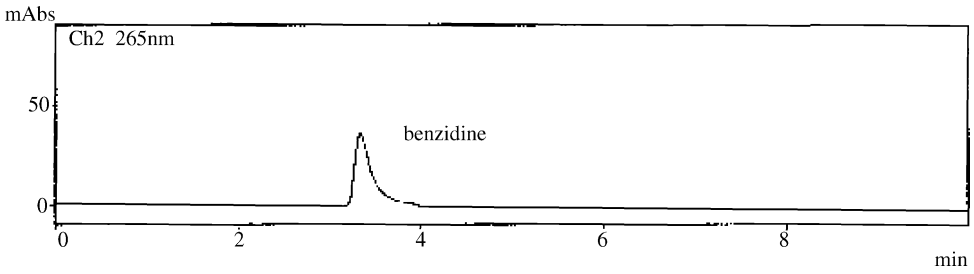

Fig. 5. HPLC chromatogram of benzidine (20 mg/L).

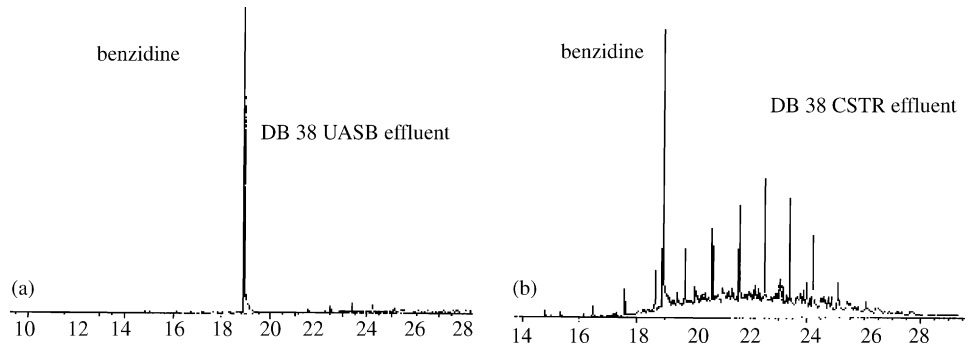

Fig. 6. Gas chromatograms of effluent in (a) UASB and (b) CSTR reactors.

of the retention times and mass spectra of the samples and benzidine standards. Fig. 6 shows the GC chromatograms of UASB and CSTR effluents samples. Azo dye metabolites were detected in the anaerobic and aerobic effluents. The benzidine gives a peak at 19 min in each one sample. No other organic inter-metabolites were determined both in GC and HPLC analysis. In this study the suggested stochiometric approaches including the benzidine formation through decolorization of DB 38 dye could be illustrated using Eqs. (1) and (2) for anaerobic and aerobic conditions, respectively:

$$C_{34}H_{28}N_9O_8S_2Na_2 + 25NH_3 + 68OH^- + 69H^+ \rightarrow 17C_2H_{10}N_2 + 2SO_3 + 2Na + 70OH^- \quad (1)$$

$$13C_2H_{10}N_2 + \tfrac{683}{2}O_2 \rightarrow C_2H_{10}N_2 + 65H_2O + 70NO_3^- + 144CO_2 + 60NO_2^- \quad (2)$$

According to the equations given above 60% of 3200 mg/L DB 38 was converted to benzidine (Eq. (1)) while approximately 45% of benzidine was mineralized to H_2O, NO_3^-, CO_2 and NO_2^- (Eq. (2)), under anaerobic and aerobic conditions, respectively.

These results and the mass spectra in aerobic effluents show that DB 38 azo dyes could not be completely mineralized by means of anaerobic/aerobic sequential process. The proposed intermediates produced from the sequential anaerobic/aerobic degradation of DB 38 are illustrated in Fig. 7 [48,49]. As shown in this figure 1,2,5-triaminobenzene, benzidine, 2,8,9-triamino-1-hydroxynaphthalene-3,7-disulphonic acid and aniline were produced under anaerobic conditions while SO_3^-, Na^+, NH_3, NO_2^-,NO_3^-, CO_2, H_2O and OH^- were released through aerobic stage. In a study performed by Bradford et al. [30] it was found that the anaerobic pathway of 500 mg/L DB 38 consist from the 4-aminobipheyl (4-ABP), AABP and monoacetylbenzidine (MABZ). In this study, the concentration of metabolites produced were 250 nmol/mL benzidine, 1 nmol/mL 4-ABP and 2 nmol AABP during 24 h in anaerobic stage. As shown, benzidine is the main metabolite product produced from the DB 38 dye via anaerobic pathway compared to the other organic metabolites. In our study the other organic metabolites mentioned in the literature did not produced. The main aromatic amine determined was benzidine. This could be attributed to the metabolism of partially granulated anaerobic sludge and to the continuous operation of the reactors since in the references mentioned above it was studied under batch conditions and suspended anaerobic sludge was used as seed. However, the concentration of other metabolites could be lower than the minimum detection limits of the GC and HPLC.

As shown in Table 1, the degradation of azo dyes and their metabolites were associated with increases in NO_3^-–N concentrations in the aerobic effluent indicating NH_3 released from the azo dyes and it was converted to NO_3^- by nitrification under aerobic conditions. These findings show that mineralization of azo dyes by anaerobic/aerobic processes occurred [50]. NH_4–N values were not meaningful in samples since it might be converted to nitrate at long HRTs in aer-

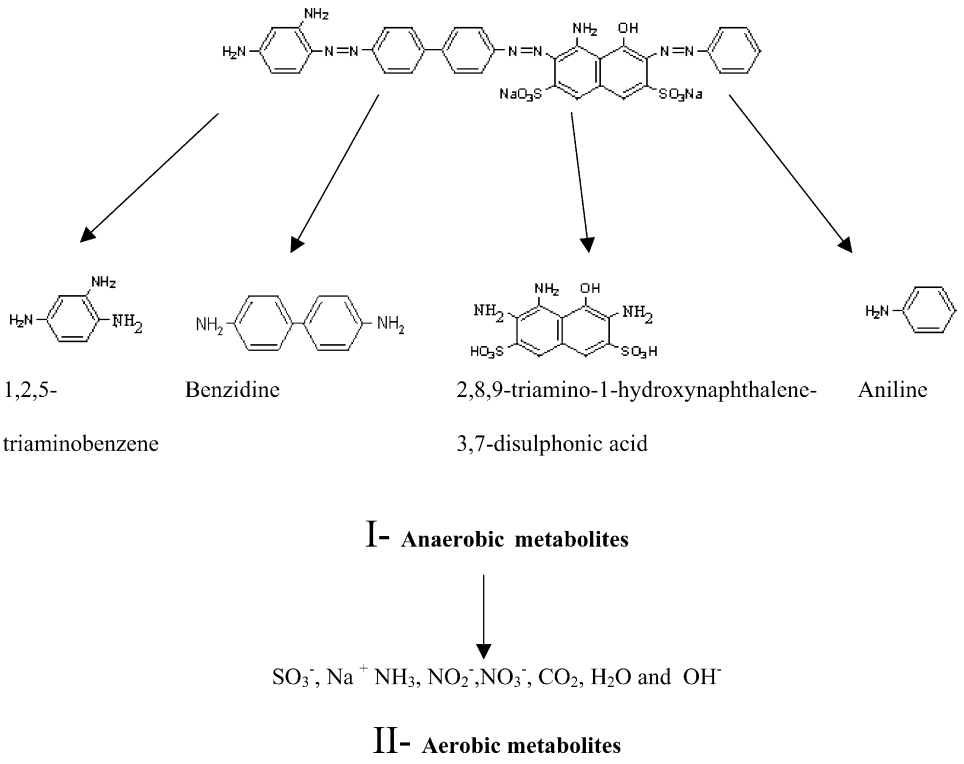

Fig. 7. Metabolites produced from the sequential anaerobic/aerobic degradation of DB 38 dye.

obic tank. Ammonia is the intermediate product for azo dye mineralization but nitrate is the final product for the anaerobic/aerobic system treating azo dyes.

3.4. Monitoring of toxicity through decolorization of DB 38 azo dye

Respiration/inhibition, *D. magna* and ATA toxicity assays were performed in order to determine the toxicity of azo dyes through anaerobic/aerobic treatment in the influent, and effluents of anaerobic/aerobic system. The results obtained from the toxicity tests were tabulated in Table 3. Toxicity test results are given as percent (%) inhibition obtained from the activity of the samples compared to control containing no dyes and toxic substances. Respiration–inhibition test showed that feed and anaerobic effluent had higher toxicity than effluent of the anaerobic/aerobic sequential system. ANOVA test statistics showed that the *F*-values between diluted samples and inhibitions in influent, anaerobic and aerobic effluent samples are lower than the critical *F*-value resulting in a strong statistical evidence in respiration inhibition tests, respectively (F_{critic} = 19.345, $F_{influent}$ = 2.350, α = 0.05; F_{critic} = 9.275, $F_{anaerobic\ effluent}$ = 0.61, α = 0.05; F_{critic} = 9.275, $F_{aerobic\ effluent}$ = 0.67, α = 0.05, respectively).

Table 3
Percent Inhibition (I%) results in different toxicity tests

Toxicity tests	Influent sample ratio (%)					Anaerobic effluent sample ratio (%)					Aerobic effluent sample ratio(%)				
Inhibition (%)	25	50	75		100[a]	25	50	75		100	25	50	75		100
Respiration/ inhibition (I%)	37 ± 2	58 ± 2	ND		75 ± 3	59 ± 3	65 ± 4	65 ± 4		53 ± 2	32 ± 2	NI	32 ± 2		47
Daphnia magna (M%)	100	100	100	100	100	100	100	100	100	100	NI	20	20	40	80
	100	99	100	99	99	100	98	99	99	100	NI	21	20	40	80
	99	100	99	99	99	100	100	100	99	99	NI	20	20	40	76
	100	99	99	100	100	99	100	100	100	100	NI	20	19	40	80
	99	100	100	100	100	99	99	100	100	100	NI	21	20	40	80
	12.5	25	50	75	100[b]	12.5	25	50	75	100	12.5	25	50	75	100
ATA (I%)	12 ± 1	NI	37 ± 2		29 ± 2	NI	NI	NI		NI	NI	NI	NI		NI

NI, no inhibition; ND, not determined; M, mortality.
[a] Dilution for respiration/inhibition and ATA tests.
[b] Dilutions for *Daphnia magna* mortality tests.

D. magna toxicity tests showed that the aerobic effluents did not exhibit mortality in samples containing 25% aliquot of effluents. This toxicity test showed that the anaerobic degradation of azo dyes generated metabolites which were toxic to *D. magna* test organisms. Toxicity was eliminated after aerobic treatment, indicating that the products causing toxicity to *D. magna* partially degraded under aerobic conditions. ANOVA test statistics showed that the *F*-values between diluted samples and mortalities in influent, anaerobic and aerobic effluent samples is higher than the critical *F*-value, respectively. Therefore, it can be concluded that no statistical evidence was observed in *D. magna* toxicity test ($F_{critic} = 2.365$, $F_{influent} = 4.710$, $\alpha = 0.05$; $F_{critic} = 2.365$, $F_{anaerobic\ effluent} = 7.38$, $\alpha = 0.05$; $F_{critic} = 2.365$, $F_{aerobic\ effluent} = 4.91$, $\alpha = 0.05$, respectively).

In a study performed by Moran et al. [51] *D. magna* toxicity test results showed that the aerobic treatment of textile wastewater did not remove the COD, and the non-degradable portion of the dye is responsible from the toxicity. On the other hand anaerobic decolorization also was not removed in the toxicity of dyes. For instance, in a study carried out by Chen, it was showed that biotoxicity of Reactive Red 22 dye is greater than Reactive Black B. High toxicity to *Pseudomonas luteola* was due to two azo bond present on Reactive Red 22 [52]. In addition intermediary products of Reactive Black B may be more toxic or chemically stable than the parent dye. A longer persistence of intermediary metabolites in anaerobic conditions may also augment toxicity to bacterial populations.

The data obtained with ATA test based on percent inhibitions (I%) showed that differences in toxicity were before and after the azo dye has been metabolized. Analysis revealed significant differences between the results obtained by the influent and those obtained by the anaerobic and aerobic effluents indicating that the toxicity of the metabolic products decreased after anaerobic and aerobic stage. This observation suggests that the toxicity of the parental compound decreased as the degradation proceeded, indicating that DB 38 azo dye were more toxic than its products. Both the parental dye compounds and the metabolic products of dyes were reported to affect the aquatic microbial population [3,53]. As seen in Table 3, the ATA tests results of parent dye, anaerobic and aerobic intermediates showed that reduction in toxicity as a result of the treatment of DB 38 azo dye. ANOVA test statistics showed that the *F*-values between diluted samples and inhibitions in influent, anaerobic and aerobic effluent samples are lower than the critical *F*-value resulting in a strong statistical evidence in ATA test for influent samples ($F_{critic} = 9.27$, $F_{influent} = 1.730$, $\alpha = 0.05$). However, no statistical evidence was observed between diluted samples and inhibitions in anaerobic and aerobic effluent samples since no *F*-values could be calculated according to ANOVA test statistics in ATA test.

The differences between ATA test and respiration/inhibition and *D. magna* toxicity tests could be explained by the resistance of anaerobic bacteria used in ATA test resulting in methane gas production of methanogenic bacteria in the anaerobic reactor effluents.

AOPs sometimes did not reduced the toxicity. For instance, the inhibitory effect on the bioluminescence bacteria is increasing as a function of ozonation time most probably due to the formation of first by products with high toxic potential. The dilution factor (G_L value) for bioluminescence bacteria increases to 64 from 16 after ozonation at ozone and Remazol Black concentrations of 20.5 mg/L and 2 g/L, respectively. However, with prolonged ozonation time and increased ozone concentration (60 mg/L) [54], Guyer and İnce [55] determined that 20 and 30 mg/L of Reactive Red 141 and Reactive Black 5, respectively, are toxic prior to sonolysis. After ultrasound at a power output of 40 W slower decreases of toxicity were accomplished along with partial color and aromatic carbon degradation.

The sequential anaerobic/aerobic biological processes are more successful to reduce the toxicity. Beside our study, Gottlieb et al. [32] reported that LUMIStox toxicity was not detectable when the Reactive Black 5 was decolorized in a baffled bioreactor with anaerobic and aerobic compartments.

Reactive azo dyes can be decolorized using either anaerobic/aerobic sequential treatment systems, microbial generation of oxygen radicals or expensive physicochemical treatment [23]. The structure of dye affects significantly the removal of recalcitrant COD and the toxicity. Many physical and chemical methods including adsorption, coagulation, precipitation, filtration, oxidation and advanced treatment processes have been used for the treatment of azo dye contaminated effluents [51]. These methods, however, may generate a significant amount of sludge or may easily cause secondary pollution due to excessive chemical usage. Moreover, their municipal treatment costs are high. Therefore, it may be economical to develop alternative means of dye decolorization, such as bioremediation due to its reputation as on environmentally friendly and publicly acceptable biological treatment technologies [56].

From AOPs the effect of ozonation (20.5 mg/L) on the degradation process of Remazol Black was studied. COD and TOC reductions were about 35 and 20%, respectively, while 89% color removal efficiency was obtained after 360 min ozonation time [54]. The degradation of Disperse Red 354 azo dye in water was investigated using four advanced oxidation processes (AOPs) ozonation, fenton, UV/H_2O_2 and photo-Fenton. A color removal of 85 and 90% removal were achieved for the photo-Fenton process while 60–70 color and 60% COD removals were obtained in the other AOPs [56]. Color (60%) in Acid Red 14, Acid Violet 12 and Acid Brown 14 in the presence of sodium sulfate and sodium chloride by ozonation [57]. Maximum COD and color removals of 75 and 70% were obtained through the decolorization of Acid Red 88 by ozone treatment [58]. In the study performed by Konstantinou and Triantafyllos it was showed that the degradation of dyes depend to pH, catalyst concentration (TiO_2) and the presence of electron acceptors such as H_2O_2 and $(NH_4)_2{\cdot}SO_4$(58). In this study the photo catalyzed azo

dyes does not mineralize to CO_2 and accumulation of organic metabolites was observed [59]. Abdelmalek et al. [60] used humic air plasma to decolorize the Yellow Supranol and Scarlet Red Nylosan azo dyes. Decolorization (90%) and COD removal efficiencies were obtained. Rehorek et al. [61] studied the decolorization of Acid Orange 5, Reactive Orange 10 and 17 dyes using power ultrasound of 850 kHz at 60, 90 and 120 W. This process provided 90% color removal efficiency. Okitsu et al. [62] studied the sonochemical decolorization of Reactive Red 22 and methyl orange with the addition butyl alcohol radical scavenger resulting in 87% color removal efficiency. Karkmaz et al. [63] studied the photocatalytic degradation of the alimentary azo dye amaranth using an irradiated titanium dioxide aqueous suspension. TiO_2/UV based photocatalysis provides 90% color and 85% COD removal efficiencies resulting in production of N_2 containing pollutants.

The AOPs summarized above showed that non-biological technologies have proven similar decolorization and sufficient depletion in organic matter compared to anaerobic/aerobic sequential treatment system. However, in some studies it was observed that the color and COD removal efficiencies were lower in AOPs and the aromatic amines were not ultimately mineralized. A single AOPs can not eliminate all contaminants in azo dye containing textile industry wastewater. In ozonation process, in order to obtain high color removal efficiency, longer ozonation periods with high ozone doses have to be applied. Otherwise partial reduction of COD and significant increases in toxicity appears. In other words, short-term ozonation results in a significant increase of toxicity caused by non-mineralized toxic intermediates [64]. They found that the COD and TOC reductions were about 40 and 25% for 6 h ozonation of 2 g/L Remazol Black 5 aqueous solution at an ozone dosage of 20.6 mg/L.

The COD removals observed in AOPs system varied between 70 and 90% indicating that the remaining COD is recalcitrant. In this study 99% BOD_5 removal indicates the success in the removal of biologically degradable organics while the COD removal was 92% in the whole system. Five percent of inert COD was removed under aerobic conditions. The removed recalcitrant COD in AOPs is similar or sometimes lower than the removal obtained in this study through anaerobic/aerobic degradation. It is important to note that the BD 38 concentration used in this study is as high as 3200 mg/L and the refractory content of removed COD depends to dye structure.

In combined anaerobic/aerobic sequential process the goal is to decolorize the dye through remove the COD in the anaerobic stage, as possible as, recovering energy as biogas or methane gas. The remaining COD can be mineralized by aerobic bacteria in second stage. This combination produces relatively little waste stage, since a part of COD is removed by methanogenic bacteria, which produce little biomass. In order to reduce the biomass production and aeration costs as well as gain energy transformation of co-substrate to biogas is desirable. Therefore, anaerobic/aerobic sequential treatment systems could be used to achieve the best economical and ecological results.

4. Conclusions

The detoxification of the anaerobic effluent was demonstrated by higher aromatic amine removals through aerobic stage. However, it was important to not that a significant amount of COD, TOC and BOD_5 were removed under anaerobic UASB reactor together with color removals. Meanwhile, in this study a great amount of COD and color were simultaneously removed under aerobic conditions. Therefore, coupled anaerobic–aerobic systems have proven to be successful in achieving the biodegradation of azo dyes. In such systems, azo dyes are reduced and decolorized anaerobically, followed by subsequent aerobic degradation of the aromatic amines produced although in this study a part of aromatic amines was removed under anaerobic conditions. HPLC–DAD and GC–MS analysis showed that dyes could be mineralized by a UASB/CSTR sequential reactor system although a small amount of benzidine was detected in the effluent of aerobic reactor. Furthermore, NO_3^-–N concentration of the effluent is an evidence of metabolizing of N in azo bond of DB 38 through aerobic conditions.

The findings obtained in this study demonstrated that DB 38 dyes could be reduced and decolorized in the anaerobic pre-treatment with glucose which is acting as electron donor for reducing equivalents. The enzyme cofactors transferring electrons for azo dye reduction by specific enzymes during the breakdown of azo dye and the reduction products were acceptable for aerobic mineralization. Toxicity values of aerobic effluents were significantly lower compared to anaerobic effluents exhibiting low toxicity resulting in complete mineralization of carcinogenic amines. Differences in toxicity results may arise from the different responses of test microorganisms and the structure of dye to toxicity in the samples. This finding has important implications for the environment since the benzidine is an important widespread contaminant of aqueous ecosystem; therefore an aerobic post-treatment step would be required for the mineralization of azo dyes containing textile and dye industry effluents.

Acknowledgments

This study was supported by the Turkish Scientific and Technical Research Council (TÜBİTAK) for the project numbered 199 Y 110, and fund of Niğde University with a project numbered 2002/03, TURKEY. The authors gratefully acknowledge the financial support from these organizations.

References

[1] C.M. Carliell, S.J. Barclay, N. Naidoo, C.A. Buckley, D.A. Mulholland, E. Senior, Water SA 21 (1995) 61.

[2] R. Anliker, Ecotoxicol. Environ. Saf. 1 (1979) 211.
[3] K.T. Chung, S.E. Stevens, Environ. Toxicol. Chem. 12 (1993) 2121.
[4] F. Şengül, A. Müezzinoğlu, The Strategies of Ecological Production. The Example of Eco-Textile Products in Cotton Textile Industry, TTGV 130/S Final Reports, Izmir, 1996.
[5] M. Haug, A. Schmiat, J.S. Nortman, D.C. Hempel, H.J. Stolz, Appl. Environ. Microbiol. 57 (1991) 3144.
[6] E.J. Weber, Environ. Toxicol. Chem. 9 (1991) 609.
[7] S. Seshadri, P.L. Bishop, A.M. Agha, Waste Manag. 14 (1994) 127.
[8] Y. Zaoyan, S. Ke, Y. Guangliang, D. Fan, Y. Jinshan, M. Hunonian, Water Sci. Tech. 26 (1992) 2093.
[9] J.A. Field, A.J.M. Stems, M. Kata, Antonie van Leeuwenhoek 67 (1995) 47.
[10] M. Işık, D.T. Sponza, J. Environ. Sci. Health A 38 (2003) 2301.
[11] D.T. Sponza, M. Işık, Process Biochem. (in press).
[12] N. Supaka, L. Juntagjin, S. Damronglerd, M.L. Delia, L. Strehaiano, Chem. Eng. J. 99 (2004) 169.
[13] D. Brown, P. Laboreur, Chemosphere 12 (1983) 397.
[14] J.A. Field, A.J.M. Stams, M. Kato, G. Schraa, Antonie van Leeuwenhoek 67 (1995) 47.
[15] E. Razo-Flores, M. Luijten, B.A. Donlon, G. Lettinga, J.A. Field, Environ. Sci. Technol. 31 (1997) 2098.
[16] S. Kalyuzhnyi, V. Sklyar, T. Mosolova, I. Kucherenko, J.A. Russkova, N. Degtyaryova, Water Sci. Technol. 42 (2000) 363.
[17] D. Brown, B. Hamburger, Chemosphere 16 (1987) 1539.
[18] P. Ekici, G. Leupold, H. Parlar, Chemosphere 44 (2001) 721.
[19] P. Nigam, I.M. Banat, R. Singh, S. Merachant, Process Biochem. 31 (1996) 435.
[20] H. An, Y. Qian, X. Gu, W.Z. Tang, Chemosphere 33 (1996) 2533.
[21] O.A. O'Connor, L.Y. Young, Environ. Toxicol. Chem. 8 (1989) 853.
[22] W. Luangdilok, T. Panswad, Water Sci. Technol. 42 (2000) 377.
[23] C. O'Neill, F.R. Hawkes, S.R.R. Esteves, D.L. Kawkes, J. Chem. Technol. Biotechnol. 74 (1999) 993.
[24] P. Rajaguru, M. Kalailselvi, M. Palanivel, V. Subburam, Appl. Microbiol. Biotechnol. 54 (2000) 268.
[25] S. Kalyuzhnyi, V. Sklyar, Water Sci. Technol. 41 (2000) 23.
[26] N.D. Lourenco, J.M. Novais, H.M. Pinheiro, Environ. Technol. 24 (2003) 679.
[27] A. Cruz, G. Buitron, Water Sci. Technol. 44 (2001) 159.
[28] D.T. Sponza, M. Işık, Water Sci. Technol. 34 (2001) 234.
[29] D.T. Sponza, M. Işık, Enzyme Microbiol. Technol. 32 (2002) 102.
[30] W. Bradford, M. Carl, E. Corniglia, J.M. Federle, Appl. Environ. Microbiol. 50 (1985) 10.
[31] M. Işık, D.T. Sponza, Process Biochem. 38 (2003) 1183.
[32] A. Gottlieb, C. Shaw, A. Smith, A. Weatley, S. Forsythe, J. Biotechnol. 101 (2003) 49.
[33] R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, Archae Press, Nashville Tennessee, USA, 1996 (p. 115).
[34] APHA-AWWA, Standard Methods for Water and Wastewater, 17th ed., American Public Health Association/American Water Works Association, Washington, DC, USA, 1992, pp. 1–860.
[35] F. Germirli, The Incremental and Comparison Methods for the Assessment of Initial Soluble Inert COD, Ph.D. Thesis, Istanbul Technical University, Istanbul, Turkey, 1990.
[36] G.K. Anderson, G. Yang, Water Environ. Res. 64 (1992) 53.
[37] A. Oren, P. Gurevich, Y. Henis, Appl. Environ. Microbiol. 57 (1991) 3367.
[38] A. Pielesz, J. Mol. Struct. 511–512 (1999) 337.
[39] W.F. Owen, D.C. Stuckey, J.B. Healy Jr., L.Y. Young, P.L. McCarty, Water Res. 13 (1979) 485.
[40] J.A. Libra, M. Borchert, L. Vigelahn, T. Storm, Chemosphere 56 (2004) 167.
[41] B.A. Donlon, E. Razo-Flores, J.A. Field, G. Lettinga, Appl. Environ. Microbiol. 61 (1995) 3889.
[42] C. O'Neill, F.R. Hawkes, S.R.R. Esteves, S.C. Wilcox, J. Chem. Technol. Biotechnol. 84 (2001) 673.
[43] E. Razo-Flores, M. Luijten, B.A. Donlon, G. Lettinga, J.A. Field, Water Sci. Technol. 36 (1997) 65.
[44] B. Donlon, E. Razo-Flores, M. Luijten, H. Smarts, G. lettinga, J. Field, Appl. Microbiol. Technol. 47 (1997) 83.
[45] D. Brown, B. Hamburger, Chemosphere 12 (1987) 397.
[46] W. Haug, A. Schmidt, Xenobiotica 1 (1971) 127.
[47] S. Seshadri, P.L. Bishop, A.M. Agha, Waste Manag. 14 (1994) 127.
[48] B. Manu, S. Chaudhari, Biores. Technol. 82 (2002) 225.
[49] C. O'Neill, A. Lopez, S. Esteves, F.R. Hawkes, D.L. Hawkes, S.J. Wilcox, Appl. Micobiol. Biotechnol. 53 (2000) 249.
[50] M. Işık, D.T. Sponza, Chemosphere 55 (2003) 119.
[51] C. Moran, M.E. Hall, R. Howell, J. Soc. Dyes Col. 113 (1997) 272.
[52] B.-Y. Chen, Process Biochem. 38 (2004) 437.
[53] E.J. Weber, N.L. Wolfe, Environ. Toxicol. Chem. 6 (1987) 911.
[54] C. Wang, A. Yediler, D. Lienert, Z. Wang, A. Kettrup, Chemosphere 52 (2003) 1225.
[55] G.T. Guyer, N.H. İnce, Ultrason. Sonochem. 10 (2003) 235.
[56] M. Neamtu, A. Yediler, I. Siminiceanu, M. Maceveanu, A. Kettrup, Dyes Pigments 60 (2004) 961.
[57] M. Muthukumar, S. Selvakumar, Dyes Pigments 62 (2004) 221.
[58] M. Muthukumar, D. Sargunamani, N. Selvakumar, J.V. Rao, Dyes Pigments 62 (2004) 221.
[59] I.K. Konstantinou, A.A. Triantafyllos, Appl. Catal. B: Environ. 20 (2004) 1.
[60] F. Abdelmalek, S. Gharbi, B. Benstali, A. Addou, J.L. Brisset, Water Res. 9 (2004) 2339.
[61] A. Rehorek, M. Tauber, G. Gübitz, Ultrason. Sonochem. 11 (2004) 177.
[62] K. Okitsu, K. Iwasaki, Y. Yobiko, H. Bandow, R. Nishimura, Y. Maeda, Ultrason. Sonochem. (in press).
[63] M. Karkmaz, E. Puzenat, C. Guillard, J.M. Herrmann, Appl. Catal. B: Environ. (in press).
[64] C.X. Wang, A. Yediler, D. Lienert, Z. Wang, A. Kettrup, Chemosphere 46 (2002) 339.