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The removal of C.I. Basic Red 46 in a mixed methanogenic anaerobic culture

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Abstract

The removal of C.I. Basic Red 46 (BR 46) was investigated with anaerobic mixed culture using glucose (3000 mg l^{-1} COD) as carbon source and electron donor. Zero-, first- and second-order reaction kinetics were used to find out the suitable substrate removal and decolorization kinetics. The substrate removal (COD) process is suitable for first-order reaction kinetic among the kinetic models studied. Increases in dye concentrations from 0 to 1000 mg l^{-1} decrease the degradation rate constant (k_1) values from 0.0083 to 0.0032 h^{-1} in batch experiments carried out with BR 46. Decolorization process approximates to first-order kinetic at 50, 100 and 250 mg l^{-1} of BR 46, and to zero-order kinetic at 500, 750, 1000 mg l^{-1} of BR 46 concentration. Substrate and color removal rates (mg l^{-1} h^{-1}) were found to be 8.07, 7.78, 7.64, 7.23, 6.39, 5.98, 5.28 and 0.66, 1.37, 3.48, 4.95, 6.30, 5.99, respectively, in all serum bottles throughout incubation period.

Keywords: Dye removal; Anaerobic mixed culture; Substrate removal; Kinetic constant

1. Introduction

Effluents from the textile, leather, food processing, dyeing, cosmetics, paper, and dye manufacturing industries are major sources of dye pollution [1]. In general, dyes are not easily degradable and are not easily removed from wastewater by conventional wastewater treatment systems [2].

As some dyes and their breakdown products may be toxic towards living organisms [3], the decolorization of dyes is an essential aspect of wastewater treatment before discharge. Color removal has been extensively studied and various techniques have been explored such as physico-chemical methods, coagulation, ultra-filtration, electro-chemical, adsorption and photo-oxidation [3,4]. Generally biological aerobic wastewater treatment systems are not successful for the decolorization of many dyes [3], as azo dyes are readily decolorized by degradation under anaerobic conditions, anaerobic wastewater treatment systems are superior to aerobic methods for azo dye removal [2,5,6]. However, the degradation of azo dyes under anaerobic conditions results in the generation of aromatic amines that are not degraded in such anaerobic environments [2,7,8]. Anaerobic–aerobic conditions can be used to degrade both the azo dye and the corresponding aromatic derivatives because of the ultimate mineralization of azo dyes under aerobic conditions [6]. Hence, the decolorization of azo dyes in anaerobic batch cultures has been studied by several researchers [2,6,9,10,11]. Işık and Sponza [12] investigated the decolorization of Congo Red and C.I. Direct Black 38 using *Escherichia coli* and *Pseudomonas* sp. cultures under both anaerobic and aerobic conditions. These workers found that anaerobic conditions were more suitable for dye decolorization. Limited investigations have been carried out to determine the level of inhibition on anaerobic mixed cultures imparted by breakdown products such as aromatic amines. Wuhrmann et al. [13], Beydilli et al. [11], Razo-Flores et al. [10], Malpei et al. [14] studied the toxic effect of azo dyes on methane production and decolorization in anaerobic conditions. The

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Nomenclature

A_0	The absorbance at λ_{max} for the initial incubation time
A_t	The absorbance of dyes at λ_{max} at time t of batch test
C_0	Dye concentration at the beginning of the incubation during decolorization (mg l^{-1})
C_t	Residual dye concentration at time t of batch test (mg dye l^{-1})
i	Number of measured substrate concentrations remaining in the reactor at certain time intervals during the batch study
I_D	Inhibitor (dye) concentration (mg l^{-1})
k_0	Zero-order rate constant through co-substrate (COD) removal (mg l^{-1} h^{-1})
k_1	First-order rate constant through co-substrate (COD) removal (h^{-1})
k_2	Second-order rate constant through co-substrate (COD) removal (l mg^{-1} h^{-1})
k_{max}	Maximum specific substrate utilization rate (h^{-1})
K_0	Zero-order rate constant through decolorization (mg l^{-1} h^{-1})
K_1	First-order rate constant through decolorization (h^{-1})
K_2	Second-order rate constant through decolorization (l mg^{-1} h^{-1})
K_{ID}	Constant of inhibition (mg l^{-1})
K_S	Half saturation concentration (mg l^{-1})
n	Number of data obtained during the time course of the batch study
R	Substrate utilization rate (mg l^{-1} h^{-1})
R_i	Substrate removal rate at i steps (mg l^{-1} h^{-1})
R_{max}	Maximum substrate removal rate (mg l^{-1} h^{-1})
R^2	Regression analysis coefficient
S	Co-substrate concentration (COD) (mg l^{-1})
S_0	Glucose-COD concentration at the beginning of the batch incubation (mg l^{-1})
S_i	Substrate (COD) concentration at i steps (mg l^{-1})
$S_{(i-1)}$ and $S_{(i+1)}$	The substrate concentrations measured at time $t_{(i-1)}$ and $t_{(i+1)}$ (mg l^{-1})
t	Time (h)
X	Biomass concentration (mg l^{-1})

inhibition effects of C.I. Reactive Black 5 (RB 5), C.I. Direct Brown 2 (DB 2), C.I. Direct yellow 12 (DY 12) and C.I. Direct Black 38 (DB 38) azo dyes as well as the effects of the dyes on kinetic coefficients such as maximum substrate removal rate (R_{max}), half saturation constant (K_s) and dye inhibition constants (k_{ID}) during substrate (glucose) removal and decolorization were studied [2,5,6].

This paper concerns the kinetics of decolorization and substrate removal using the azo dye C.I. Basic Red 46 in an anaerobic mixed culture.

2. Kinetic models

2.1. Kinetic model for co-substrate (glucose) degradation and decolorization

Although Monod type kinetics have been applied to biodegradation and decolorization, some workers showed that they were applicable to their anaerobic systems. Hence, removal of the co-substrate during the decolorization of dyes can be expressed using zero-, first- and second-order reaction kinetics in an anaerobic batch reactor by means of Eqs. (1–3):

$$S_t = S_0 - k_0 t \tag{1}$$

$$S_t = S_0 e^{-k_1 t} \tag{2}$$

$$\frac{1}{S_t} = \frac{1}{S_0} + k_2 t \tag{3}$$

Similarly, zero-, first- and second-order reaction kinetics have been used to determine the color removal rate constants using Eqs. (4–6):

$$C_t = C_0 - K_0 t \tag{4}$$

$$C_t = C_0 e^{-K_1 t} \tag{5}$$

$$\frac{1}{C_t} = \frac{1}{C_0} + K_2 t \tag{6}$$

2.2. Kinetic coefficients

The substrate removal rate (R_i) was determined for different dye concentrations using a non-linear technique. The residual COD levels were measured at different substrate concentrations (S_i); ds/dt was calculated using Eq. (7):

$$\frac{ds}{dt} = R_i = \frac{S_{(i+1)} - S_{(i-1)}}{t_{(i+1)} - t_{(i-1)}} \tag{7}$$

Considering first-order substrate removal kinetic, K_S can be calculated from Eq. (8).

$$K_S = \frac{R_{max}}{k_1} - \frac{\sum_{i=0}^{n} S_i}{(n-1)} \tag{8}$$

3. Materials and methods

3.1. Batch experiments and experimental procedures

In the batch anaerobic experiments, 500-ml glass serum bottles were sealed with a rubber screw cap. Each of the serum bottles consisted of 15 ml anaerobic mixed culture to provide a sludge concentration of 3000 mg MLVSS l^{-1} taken from a UASB reactor that treated the wastewaters of the Pakmaya Yeast Factory in İzmit, Turkey, 3000 mg COD l^{-1} of glucose and the necessary Vanderbilt mineral medium for macro and micro nutrients. Table 1 gives the conditions of the batch test.

This mineral medium that was used in all batch experiments was of the following inorganic composition (in mg l^{-1}): NH_4Cl, 400; $MgSO_4 \cdot 7H_2O$, 400;

Table 1
Experimental conditions of anaerobic batch study

Stock	Seed control (ml)	Abiotic control (ml)	Control (dye-free) (ml)	1	2	3	4	5	6	Resulting concentrations
Sludge (40 g MLVSS l^{-1}, 78 g MLSS l^{-1})	15	–	15	15	15	15	15	15	15	3000 mg MLVSS l^{-1}
Glucose (30 g COD l^{-1})	–	20	20	20	20	20	20	20	20	3000 mg COD l^{-1}
$NaHCO_3$ (50 g l^{-1})	20	20	20	20	20	20	20	20	20	5000 mg l^{-1}
Vanderbilt mineral medium	20	20	20	20	20	20	20	20	20	Desired composition
Dye (10 g l^{-1})	–	1	–	1	2	5	10	15	20	Desired composition
Total volume (ml)	200	200	200	200	200	200	200	200	200	

1 = 50 mg dye l^{-1}, 2 = 100 mg dye l^{-1}, 3 = 250 mg dye l^{-1}, 4 = 500 mg dye l^{-1}, 5 = 750 mg dye l^{-1}, 6 = 1000 mg dye l^{-1}.

KCl, 400; $Na_2S \cdot 9H_2O$, 300; $(NH_4)_2HPO_4$, 80; $CaCl_2 \cdot 2H_2O$, 50; $FeCl_3 \cdot 4H_2O$, 40; $CoCl_2 \cdot 6H_2O$, 10; KI, 10; $(NaPO_3)_6$, 10; l-cysteine, 10; $AlCl_3 \cdot 6H_2O$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.5; $CuCl_2$, 0.5; $ZnCl_2$, 0.5; NH_4VO_3, 0.5; $NaMoO_4 \cdot 2H_2O$, 0.5; H_3BO_3, 0.5; $NiCl_2 \cdot 6H_2O$, 0.5; $NaWO_4 \cdot 2H_2O$, 0.5; Na_2SeO_3, 0.5 [15]. pH was kept constant by the addition of 5000 mg l^{-1} $NaHCO_3$. A temperature controlled incubator was used at 35 °C for all experiments. The serum bottles were shaken at 150 rpm at repeated intervals. A syringe was used to extract supernatant samples for analysis. Fig. 1 and Table 2 show the structures and properties of C.I. Basic Red 46 (Maxilon Red GRL). λ_{max} of C.I. Basic Red 46 was determined in an aqueous medium which contains only C.I. Basic Red 46 dye by using a scanning UV–vis spectrophotometer (Table 2). The dye was supplied from a textile factory in Bursa, Turkey and was of commercial quality.

A control without dye and a seed control (Table 1) were used to determine and compare COD measurements. Substrate removal and decolorization experiments were performed in duplicate. Experimental data were detected both from COD and dye measurements. Methane gas to COD convertion was considered as 395 ml methane gas produced from the removal of 1 g of COD^{-1}.

The microbial cultures were autoclaved at 121 °C for 15 min to measure the adsorption or abiotic removal of dyes.

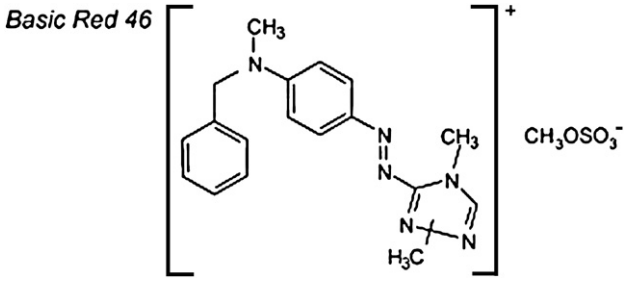

Fig. 1. Molecular structures of C.I. Basic Red 46 (Maxilon Red GRL).

Table 2
Properties of Basic Red 46 (Maxilon Red GRL)

Color index	Basic Red 46
Type	Cationic
Sulphonic group	None
Azo group	1
λ_{max}	530
pH range	2–12
Molecular weight[a] (g mol^{-1})	322
Width[a] (nm)	1.3
Depth[a] (nm)	0.74
Thickness[a] (nm)	0.63

[a] Associated counter ions are not included.

3.2. Analytical procedure

A filtration technique [16] was used to determine the TSS (total suspended solid) content of the anaerobic sludge using 0.45-μm membrane filters. Bicarbonate alkalinity and COD measurements were determined using titrimetric methods [16]. Methane production was measured by using a sodium hydroxide solution (3%, w/v) displacement system. Color measurements were carried out employing 5 ml samples removed from supernatants of serum bottles within 3 h. A Pharmacia Nova Model spectrophotometer was used at 530 nm (Fig. 2); dye samples were centrifuged at 5000 rpm for 20 min and the absorbance of the supernatant was measured. The color removal ratio was determined using the following equation:

$$\text{Color removal}(\%) = [(A_0 - A_t)/A_0]100$$

where A_0 is the absorbance at λ_{max} after the initial incubation time and A_t is the absorbance at λ_{max} after a predetermined time (t) of test.

4. Results and discussion

The effects of various dye concentrations on both the degradation of the co-substrate (glucose) and the dye decolorization were studied.

4.1. Degradation kinetics of the glucose co-substrate

In this study glucose was used as a co-substrate for carbon and as the energy source for microbial growth. Degradation and decolorization of the non-growth substrate (C.I. Basic Red 46) by a growth substrate (or co-substrate in this case) (3000 mg l^{-1} of COD) is termed the co-metabolism of the azo dye. It was considered that the dye was not a carbon source [17] as the anaerobic bacteria do not take energy from the dye.

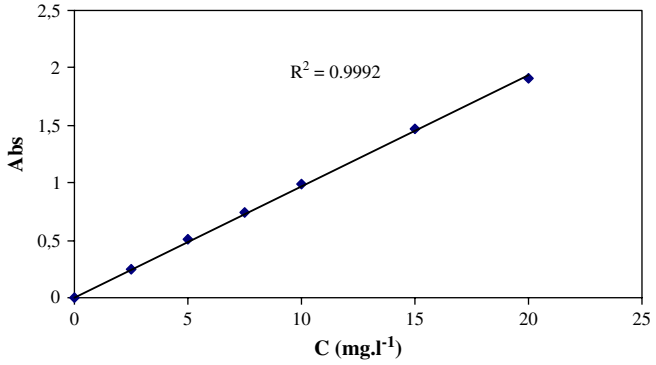

Fig. 2. Calibration curve of C.I. Basic Red 46 (λ_{max}: 530 nm).

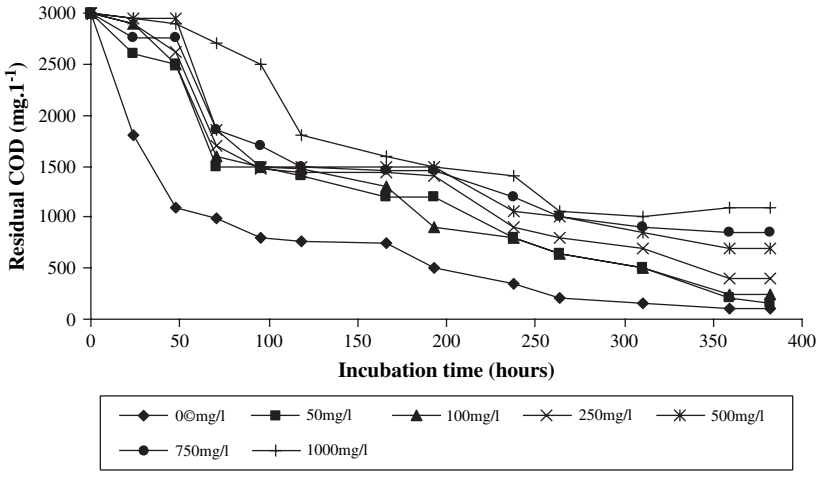

Fig. 3. The residual COD (S_i) concentrations for 382 h of incubation period batch experiments.

Fig. 3 shows the residual COD (Si) concentrations during 382 h of anaerobic incubation of C.I. Basic Red 46. Dye concentrations varied between 50 and 1000 mg l^{-1}. In the cases of low dye concentrations, COD decreased linearly, whereas for high dye concentrations (500, 750, 1000 mg l^{-1}) degradation was not complete. The residual COD concentrations of dye-free and 1000 mg l^{-1} of C.I. Basic Red 46 samples were 100 and 1000 mg l^{-1} at the end of the 382 h of incubation period. Thus, it could be concluded that at low dye concentrations, COD was biodegraded faster than in the case of high dye concentrations.

The kinetic constants for the substrate (glucose) degradation were determined using Eqs. (1–3). Plots of S_i, $\ln S_i$ and $1/S_i$ as functions of time were constructed to determine the kinetic data. Zero-, first- and second-orders were used to determine kinetic constants (k_0, $k_1$and k_2) as shown in Table 3. It is apparent that COD was removed according to first-order reaction kinetics. Values of the first-order reaction kinetic constant (k_1) decreased from 0.0083 to 0.0032 h^{-1}, as the dye concentration increased from 0 to 1000 mg l^{-1}. Fig. 4 shows the kinetic constants of COD degradation for dye-free (control) samples. Similarly, some researchers obtained that COD degradation occurred via first-order reaction kinetic [2,5,6,17].

4.2. Dye decolorization kinetics

The residual color achieved using a constant initial substrate concentration (3000 mg l^{-1}) for different initial dye concentrations (50–1000 mg l^{-1} dye) during 142.5 h of incubation is demonstrated in Fig. 5. Abiotic tests performed with autoclaved anaerobic sludge showed that the microbial decolorization occurred primarily by biological degradation. As shown in Fig. 5, compared to almost complete removal of color under biotic conditions (except 1000 mg l^{-1}), decolorization efficiency under abiotic conditions was only around 20–25% which was probably due to physical adsorption of dye molecules by the dead cells. It is clear from Fig. 5 that virtually no color remained at the end of 382 h of incubation.

Decolorization kinetic constants (K_0, K_1, K_2) are summarized in Table 4; zero-, first- and second-order reaction kinetics were used to determine the kinetic constants of color removal. Plots of C_t, $\ln C_t$ and $1/C_t$. as a function of time were constructed using Eqs. (4–6) from which it was evident that decolorization occurred according to zero and first-order kinetics. The rate constants showed that for dye concentrations of between 50 and 250 mg l^{-1}, first-order rates applied, whereas zero-order rate constants were most suitable for dye concentrations between 500 and 1000 mg l^{-1}. In the literature, dye removal has been described as following first-order kinetics [5,9,18,13,19,2,6]; however, other researchers have reported zero-order kinetics for decolorization experiments [5,20].

4.3. Bicarbonate alkalinity and pH

pH and bicarbonate alkalinity values were measured at different dye concentrations at the end of 382 h of incubation

Table 3
Zero-, first- and second-order kinetic constants obtained in anaerobic batch tests during COD degradation

Kinetics	Constant	0 mg l^{-1} (C)	50 mg l^{-1}	100 mg l^{-1}	250 mg l^{-1}	500 mg l^{-1}	750 mg l^{-1}	1000 mg l^{-1}
Zero order	k_0 (mg l^{-1} h^{-1})	5.2664	6.7235	6.9082	6.4355	5.9508	5.3721	5.8233
	R^2	0.683	0.896	0.882	0.870	0.818	0.832	0.877
First order	k_1 (h^{-1})	0.0083	0.007	0.0064	0.0051	0.0039	0.0034	0.0032
	R^2	0.966	0.932	0.973	0.954	0.931	0.931	0.907
Second order	k_2 (l mg^{-1} h^{-1})	0.00002	0.00001	0.000009	0.000005	0.000003	0.000002	0.000002
	R^2	0.864	0.682	0.799	0.848	0.953	0.971	0.902

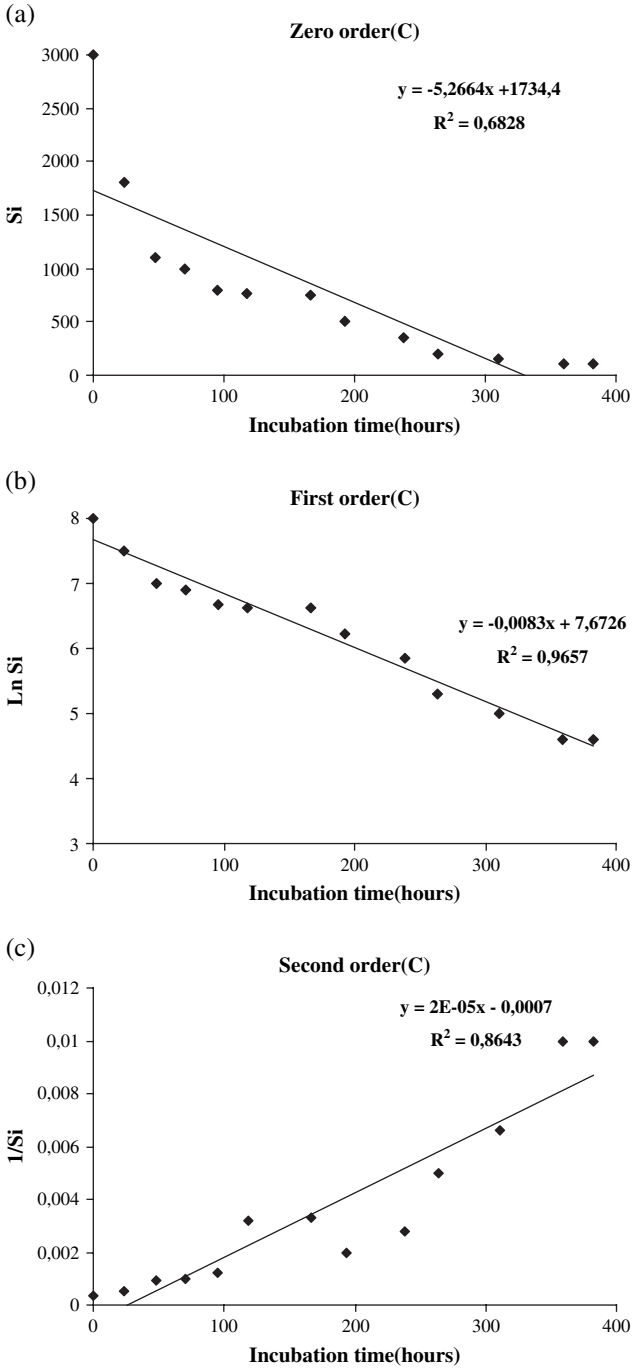

Fig. 4. Zero- (a), first- (b), and second-order (c) reaction kinetics for 0 mg l^{-1} of C.I. Basic Red 46 azo dye.

(Fig. 6) from which it is apparent that as dye concentration increased, both the pH and the alkalinity decreased. This can be attributed to the inhibition of methanogenesis and accumulation of intermetabolites through the simultaneous degradation and decolorization of the dye [4,11,17,20].

4.4. Determination of R_{max} and K_s

Substrate removal rates decreased from 8.07 to 5.28 mg l^{-1} h^{-1} as the dye concentrations increased. As shown in Fig. 7, the color removal rate first increased from 0.6 to 6.30 mg l^{-1} h^{-1} as dye concentration increased but later the color removal rate decreased at a dye concentration of 1000 mg l^{-1}. This shows that color removal decreased at high dye concentrations.

Substrate removal rates (R_i) and half saturation constants (K_s) were calculated in order to show inhibition effects. As shown in Table 3, the substrate was removed according to first-order kinetic reaction. Substrate removal rate (R_i) values were obtained for different dye concentrations using Eq. (7) and K_s values were calculated using Eq. (8); R_{max}, the maximum substrate removal rate, was calculated from the initial glucose-COD concentration

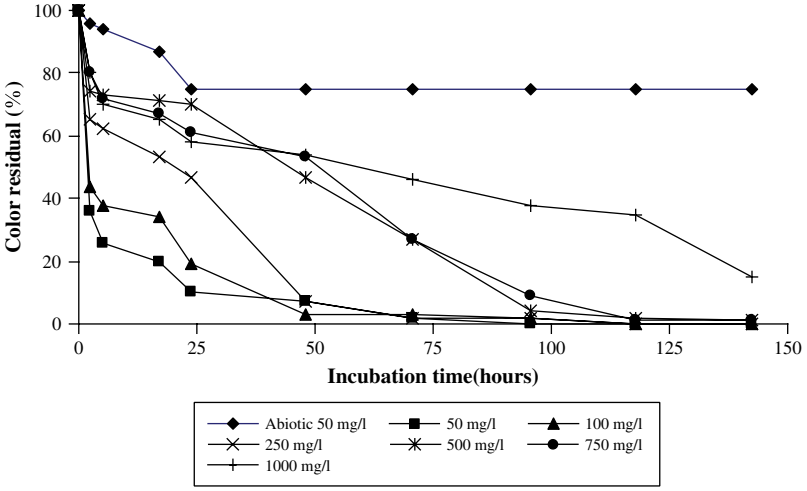

Fig. 5. Residual color (%) at different C.I. Basic Red 46 concentrations during the incubation period.

Table 4
The rate constants of observed from dye removal kinetic test

Kinetics	Constant	50 mg l^{-1}	100 mg l^{-1}	250 mg l^{-1}	500 mg l^{-1}	750 mg l^{-1}	1000 mg l^{-1}
Zero order	K_0 (mg l^{-1} h^{-1})	0.400	0.9633	3.0096	3.8481	5.6195	4.8513
	R^2	0.40	0.59	0.864	0.946	0.950	0.904
First order	K_1 (h^{-1})	0.0469	0.0618	0.0543	0.0345	0.0325	0.0255
	R^2	0.777	0.93	0.959	0.888	0.843	0.929
Second order	K_2 (l mg^{-1} h^{-1})	0.0056	0.0058	0.0026	0.0011	0.0008	0.0007
	R^2	0.939	0.828	0.798	0.634	0.496	0.5675

of 3000 mg l^{-1} to the first substrate removal interval. Maximum substrate removal rates (R_{max}; mg l^{-1} h^{-1}) were found to be 23.65, 21.50, 25.80, 23.65, 22.1 and 18.94 for between 50 and 1000 mg l^{-1} of dye, an average value of 25.02 was achieved. K_s values ranged from between 1561.9 and 4918 with an average value of 3444.36. Generally, the R_{max} values decreased, while the K_s values increased at high dye concentration indicating inhibition. The larger K_s values indicated low affinity of the dye for the substrate and anaerobic microorganisms in the batch reactor. Similar results have been observed by other workers [4,6].

5. Conclusions

In this study, anaerobic mixed cultures, using glucose as co-substrate and the azo C.I. Basic Red 46 were used to determine decolorization over a 382 h incubation period. Although a non-growth substrate (in this case the dye) cannot be utilized by microorganism to provide their growth for color removal, glucose as a growth substrate is used as an electron donor to perform color removal by cleavage of the azo group in the dye [21]. The results showed that color removal was achieved even at

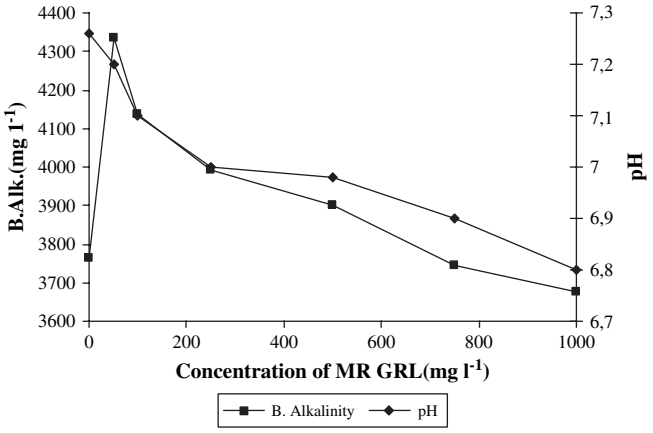

Fig. 6. Bicarbonate alkalinity (mg $CaCO_3$ l^{-1}) concentrations and pH values at the end of incubation period.

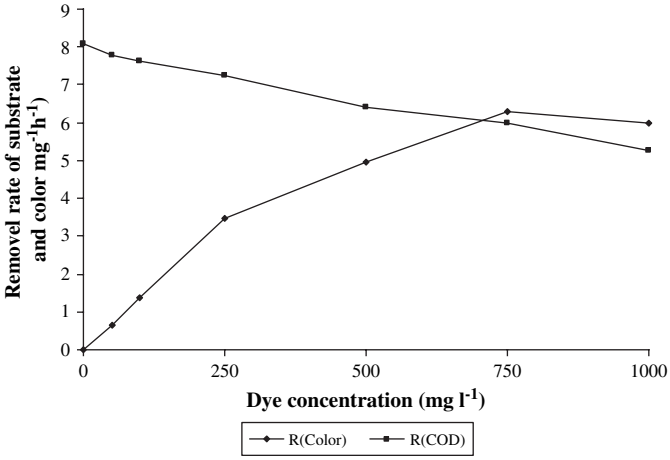

Fig. 7. Removal rates of COD and color throughout incubation period.

high dye concentrations. Decolorization efficiency under abiotic conditions was only around 20–25% which was probably due to physical adsorption of dye molecules by the dead cells.

Substrate degradation by the dye was achieved according to first-order kinetics; substrate (COD) degradation kinetic rate constants varied between 0.0083 and 0.0032 h^{-1}, as the dye concentration increased from 0 to 1000 mg l^{-1}.

Decolorization rate constants showed that for dye concentrations between 50and 250 mg l^{-1}, first-order rates applied, whereas zero-order kinetics were more applicable for dye concentrations between 500 and 1000 mg l^{-1} (i.e. at high dye concentration). As textile wastewater generally contains around 100–500 mg l^{-1} of azo dye, high dye concentrations are not important in practice.

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