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Removal of Maxilon Yellow GL in a mixed methanogenic anaerobic culture

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Abstract

Degradation of dye Maxilon Yellow GL (MY GL) (Basic Yellow 45) was investigated with anaerobic mixed culture using glucose (3000 mg l^{-1} COD) as carbon source and electron donor throughout batch experiments. 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 second-order reaction kinetics among the kinetic models studied. Decolorization process also approximates to second-order kinetics between 50 and 1000 mg l^{-1} of MY GL concentration. Substrate and color removal rates (mg l^{-1} h^{-1}) were found to be 6.38, 5.98, 4.6, 4.16, 3.64, 2.86, 2.34 and 0.075, 0.0149, 0.0265, 0.0303, 0.0426, 0.053, respectively, in all serum bottles throughout the incubation period. Color removal efficiencies decreased as the influent dye concentration increased. The highest removal efficiency (80%) was obtained with 50 and 100 mg l^{-1} of MY GL dye concentrations. However, the lowest removal efficiency (28%) was found with a 1000 mg l^{-1} of MY GL dye concentration. Complete dye reduction was not found for this basic dye. The results indicate that anaerobic mixed culture can decolorize low concentration of this basic dye.
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Keywords: Dye removal; Anaerobic mixed culture; Substrate removal; Kinetic constant

1. Introduction

Most of the industries, such as textiles, paper, plastics, leather, food, cosmetics, etc use dyes or pigments to color their final product. Effluents from these industries are generally highly colored. Therefore they are main sources of water pollution [1].

Dyes include a broad spectrum of different chemical structures, primarily based on substituted aromatic and heterocyclic groups such as aromatic amine (C_6H_5–NH_2) and phenyl (C_6H_5–CH_2) [2]. Azo dyes constitute the largest class (60–70%) of dyes used in industry [2,3]. They are characterized by their typical –N=N– nature [4]. Basic dyes are cationic compounds that are used for dyeing acid group-containing fibres, usually synthetic fibres like modified polyacryl. They bind to the acid groups of the fibres. Most basic dyes are diarylmethane, triarylmethane, anthraquinone and azo compounds. Basic dyes represent approximately 5% of all dyes listed in the Color Index [5]. The acute toxicity of dyestuffs is generally low. The most acutely toxic dyes for algae are – cationic – basic dyes. Many dyes and their breakdown products have toxic as well as carcinogenic and mutagenic effects on living organisms [1,6,7]. Therefore, decolorization of dyes is an essential aspect of wastewater treatment before discharge. Dyes are not easily degradable and are generally not removed from wastewater treatment systems [3]. Several physical, chemical and biological pretreatment, main treatment and post-treatment techniques can be employed to remove color from dye containing wastewaters [5]. Physico-chemical techniques include membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption, ion exchange, ion pair exctraction, ultrasonic mineralization,

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electrolysis, advanced oxidation (chlorination, bleaching, ozonation, Fenton oxidation and photocatalytic oxidation), and chemical reduction [1,5,8]. Biological techniques include bacterial and fungal biosorption and biodegradation in aerobic, anaerobic, anoxic or combined anaerobic/aerobic treatment processes. Biological processes provide a low cost and efficient means to treat the textile effluent [4,9,10]. Generally biological aerobic wastewater systems are not successful for decolorization of majority of dyes [1]. However, under strict anaerobic conditions, decolorization of dye can be achieved and is well documented [9,11]. Especially, anaerobic wastewater treatment is superior to aerobic treatment for azo dye removal [3,12,13]. Several researchers have reported that a low redox condition kept in the bioreactor by the methanogenic culture is responsible for color removal [9,11]. Decolorization of azo dye in the anaerobic batch culture has been studied by several researchers [3,9,11,13,14].

The objective of this study was to evaluate the use of anaerobic mesophilic culture for color removal from a basic dye (MY GL).

2. Kinetic models

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

Monod-type kinetics has been used for dye biodegradition and decolorization. However, some researchers showed that it was not successful to use these in their anaerobic systems. Hence, removal of co-substrate during decolorization of dyes can be expressed by zero-, first- and second-order reaction kinetics in an anaerobic batch reactor by the following equations [3,8]:

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

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

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

Similarly, zero-, first- and second-order reaction kinetics have been used to find out color removal rate constants by using the equations below:

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

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

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

3. Materials and methods

3.1. Batch experiments and experimental procedure

In the batch anaerobic experiments, 500 ml glass serum bottles sealed with rubber screw cap were used. Each of the serum bottles consisted of 18.5 ml anaerobic mixed culture to provide sludge concentration as 3000 mg MLVSS l^{-1} taken from UASB reactor treating the wastewaters of Pakmaya Yeast Factory in İzmit, Turkey, 3000 mg COD l^{-1} of glucose and the necessary Vanderbilt mineral medium for macro- and micronutrients. Table 1 gives the conditions of batch test.

This mineral medium was used in all batch experiments and contains the following inorganic composition (in mg l^{-1}): NH_4Cl, 400; $MgSO_4 \cdot 7H_2O$, 400; 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]. The alkalinity and neutral pH were kept constant by the addition of 5000 mg l^{-1} $NaHCO_3$. Temperature controlled incubator was used at 35 °C for all batch experiments. The serum bottles were shaken at 150 rpm during determined intervals. Syringe was used to take supernatant samples from bottles for analysis. λ_{max} of dye was found to be 430 nm (Fig. 1).

A control without dye and a seed blank sample were used to determine and compare COD measurements in all batch serum bottles. Substrate removal and decolorization experiments in all batch studies were performed in duplicates to control the accuracy of the experimental results. Experimental data were detected both from COD and dye measurements. The methane gas to COD conversion was considered as follows: 0.395 ml methane gas was produced from the removal of 1 mg of COD^{-1}.

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

Table 1
Experimental conditions of anaerobic batch study

Stock	Seed control (ml)	Abiotic control (ml)	Control (dye-free) (ml)	1 (50 mg l^{-1})	2 (100 mg l^{-1})	3 (250 mg l^{-1})	4 (500 mg l^{-1})	5 (750 mg l^{-1})	6 (1000 mg l^{-1})	Resulting control
Sludge, 32.5 g MLVSS l^{-1}, 40 g MLSS l^{-1}	18.5	–	18.5	18.5	18.5	18.5	18.5	18.5	18.5	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	20	20	20	20	20	20	20	20	20	Desired comp.
Dye, 10 g l^{-1}	–	1.0	–	1.0	2.0	5.0	10	15	20	Desired comp.
Total	200	200	200	200	200	200	200	200	200	1

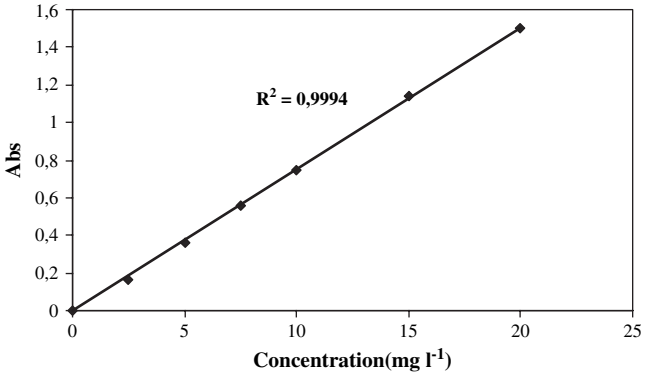

Fig. 1. Calibration curve of Maxilon Yellow GL (λ_{max}: 430 nm).

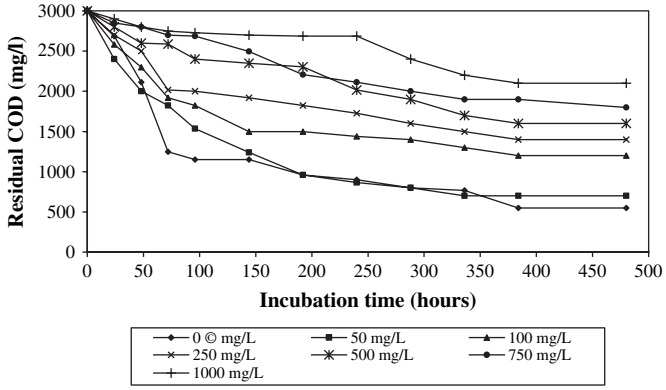

Fig. 2. The residual COD (Si) concentrations for 480 h incubation period for batch experiments.

3.2. Analytical procedure

TSS (total suspended solid) of anaerobic sludge was measured using 0.45 μm membrane filters by employing filtration technique [16]. Bicarbonate alkalinity and COD measurements were determined by titrimetric method [16]. Methane production was measured by using a sodium hydroxide solution (3%, w/v) displacement system. Color measurements were carried out in 5 ml samples removed from supernatants of serum bottles within 3 h. The Pharmacia Nova Model spectrophotometer was used at arranged wavelength of 430 nm for MY GL dye. The samples were centrifuged at 5000 rpm for 20 min and the absorbance values of supernatants were measured. Color removal ratio was determined using the following equation:

$$\text{Color removal}(\%) = \frac{(A_0 - A_t)}{A_0} \times 100$$

where A_0 is the absorbance at λ_{max} for the initial incubation time and A_t is the absorbance of dyes at λ_{max} for a determined time (t) of batch test.

4. Results and discussion

In this study effect of several MY GL concentrations on both degradation of co-substrate (glucose) and on decolorization of dyes was obtained by using anaerobic mixed culture.

4.1. Degradation kinetics of glucose (co-substrate)

In this study glucose was used as a co-substrate for carbon and energy source for microbial growth. The process of degradation and decolorization of non-growth substrate (MY GL) with a growth substrate (co-substrate) (3000 mg l^{-1} of COD) is termed as co-metabolism of azo dyes. It was considered that the dyes were not used as carbon source [17]. On the other hand, anaerobic bacteria do not take energy from dye.

Fig. 2 illustrates the residual COD (Si) concentrations during 480 h anaerobic incubation period of MY GL dye. Dye concentrations varied between 50 and 1000 mg l^{-1}. For low dye concentrations (50 and 100 mg l^{-1}), COD decreased linearly, however, for high dye concentrations (250, 500, 750, 1000 mg l^{-1}) degradation was not complete. Residual COD concentrations of 50 and 1000 mg l^{-1} of MY GL dye were 864 and 2688 mg l^{-1} at 240 h incubation time, respectively. However, residual COD concentrations of dye-free and 1000 mg l^{-1} of MY GL dye samples were 550 and 2100 mg l^{-1}, respectively, at the end of 480 h incubation period. Therefore it could be concluded that at low MY GL concentrations of sample, COD was biodegraded faster than high concentrations of MY GL dye samples. Cumulative methane gas productions in batch reactors were determined for each of the serum bottles (data not shown). Cumulative methane decreased as the dye concentrations increased. It could be considered that high concentrations of dye caused inhibition of anaerobic degradation of glucose. Therefore lower methane gas was produced at high dye concentrations. Higher dye

Table 2
Zero-, first- and second-order kinetic constants obtained in anaerobic batch tests during COD degradation

Kinetics	Constant	0 mg l^{-1}	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})	4.3697	4.3137	3.2287	2.9989	2.9665	2.6901	1.9099
	R^2	0.6762	0.7606	0.7352	0.7995	0.9436	0.9384	0.9138
First order	k_1 (h^{-1})	0.0033	0.0031	0.0018	0.0015	0.0014	0.0012	0.0008
	R^2	0.8362	0.8725	0.8165	0.8728	0.9607	0.9555	0.9076
Second order	k_2 (l mg^{-1} h^{-1})	0.000003	0.000003	0.000001	0.0000008	0.0000007	0.0000005	0.0000003
	R^2	0.9414	0.9424	0.8962	0.9288	0.9657	0.9676	0.9133

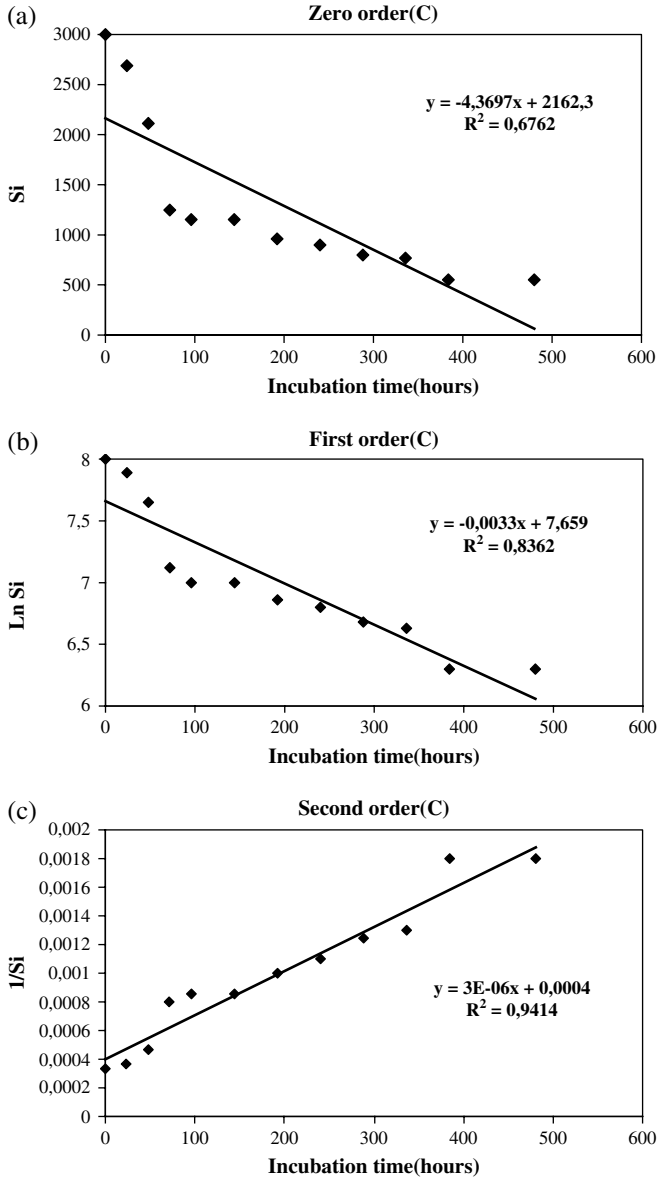

Fig. 3. (a) Zero-, (b) first-, and (c) second-order reaction kinetics for 0 mg l^{-1} of MY GL basic dye.

concentrations cause lower methane production. This is due to the accumulation of aromatic amines and volatile fatty acids, which are toxic to methanogenic bacteria [9].

Based on experimental data, kinetic constants of substrate (glucose) degradation were determined by using Eqs. (1)–(3).

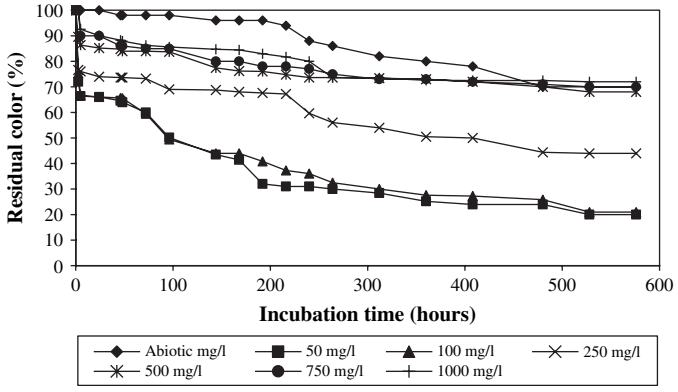

Fig. 4. Residual color (%) at different MY GL concentrations during the incubation period.

Si, ln Si and 1/Si versus time were plotted to find out 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 2. Considering regression coefficients (R^2), COD was removed according to second-order reaction kinetics. Second-order reaction kinetic constant (k_2) values decreased from 0.000003 to 0.0000003 l mg^{-1} h^{-1}, as the MY GL dye concentration increased from 0 to 1000 mg l^{-1} in the anaerobic serum bottle batch tests. Fig. 3 demonstrates kinetic constants of COD degradation for dye-free (control) samples. However, some researchers obtained that COD degradation occurred via first-order reaction kinetics [5,17]. Used basic dye (MY GL) in this study could inhibit the anaerobic metabolism more than the other used dyes in literature.

4.2. Decolorization kinetics of MY GL

The residual MY GL color percentages with constant initial substrate concentration (3000 mg l^{-1}) for different initial dye concentrations (50–1000 mg l^{-1} dye) throughout the 576 h of incubation period are demonstrated in Fig. 4. Abiotic tests performed with autoclaved anaerobic sludge showed that the microbial decolorization occurred primarily by biological degradation. As shown in Fig. 4, decolorization efficiency under abiotic conditions was only around 20–25%. This situation shows that physical adsorption of dye on anaerobic culture was not important. However, biological degradation was the main mechanism in color removal. As shown in the figure, percentages of color residue at the end of 576 h incubation were not zero for all dye concentrations. The highest removal

Table 3
The rate constants 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})	0.0522	0.1022	0.1773	0.1978	0.3331	0.4477
	R^2	0.755	0.783	0.847	0.778	0.794	0.823
First order	K_1 (h^{-1})	0.0025	0.0024	0.0012	0.0005	0.0005	0.0008
	R^2	0.893	0.924	0.913	0.827	0.8245	0.907
Second order	K_2 (l mg^{-1} h^{-1})	0.0001	0.00006	0.000008	0.000001	0.0000009	0.0000003
	R^2	0.967	0.982	0.945	0.861	0.873	0.913

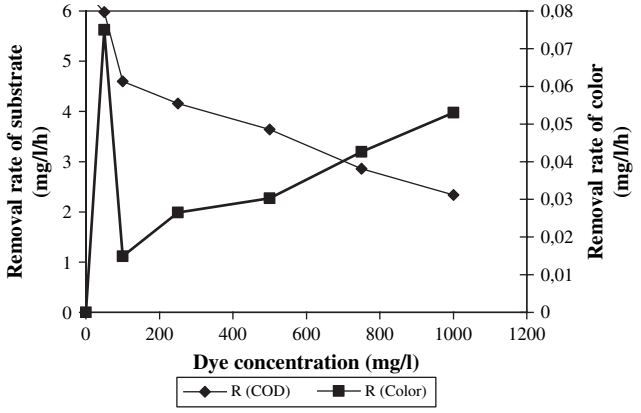

Fig. 5. Removal rates of COD and color throughout the incubation period.

efficiencies (80%) were obtained with 50 and 100 mg l^{-1} dye concentrations. However, the lowest removal efficiency (28%) was found with a 1000 mg l^{-1} of MY GL dye concentration during the incubation time. Dye removal was not completely found for this basic dye. The color removal results indicate that anaerobic mixed culture can decolorize low concentrations (50–100 mg l^{-1} dye) of MY GL basic dye.

Decolorization kinetic constants (K_0, K_1, K_2) are summarized in Table 3 based on experimental results. Zero-, first- and second-order reaction kinetics were used to determine kinetic constants of color removal. Therefore C, $\ln C$ and $\ln C/C_0$ versus time were plotted by using Eqs. (4)–(6). Based on R^2 values, the results showed that color was removed according to second-order kinetics in batch experiments. The rate constants from decolorization kinetic test showed that dye concentrations for MY GL varying between 50 and 1000 mg l^{-1} were appropriate for second-order rate. In literature, dye concentration removal was performed as first-order kinetics by several researchers [5,13,18,19,20]. However, some other researchers reported zero-order kinetics for decolorization experiments [21].

4.3. Removal rates of COD and color for MY GL dye

COD and color removal rates were calculated from experimental data throughout the anaerobic incubation time. As expected, substrate removal rates decreased from 6.38 to 2.34 mg l^{-1} h^{-1}, as the dye concentrations increased. As shown in Fig. 5, color removal rates first decreased from 0.075 to 0.0149 mg l^{-1} h^{-1} as the dye concentrations increased. Later, color removal rate started to increase slightly from 250 mg l^{-1} of MY GL concentration. This shows that highest color removal rate starts at 50 mg l^{-1} of MY GL concentration. Thus it could be considered that MY GL had slow rate of decolorization.

5. Conclusion

In this study, anaerobic mixed cultures with glucose as co-substrate and azo dye (MY GL) were used for decolorization batch experiments throughout the 576 h incubation period. A non-growth substrate (dye) cannot be utilized by microorganism for its growth for color removal experiments. However, glucose as a growth substrate is used as an electron donor to perform color removal by cleavage of the azo bond [22]. The results showed that color removals were not achieved at high dye concentrations. Abiotic test showed that chemical color removal was insignificant for this azo dye reduction.

Based on the experimental study, substrate degradation with MY GL dye was achieved according to second-order reaction kinetics. Substrate (COD) degradation kinetic rate constants varied between 0.000003 and 0.0000003 l mg^{-1} h^{-1}, as the MY GL dye concentration increased from 0 to 1000 mg l^{-1} in anaerobic batch tests.

Decolorization kinetic rate constants showed that dye concentration for MY GL varying between 50 and 1000 mg l^{-1} was of appropriate second-order rate. MY GL had a slow rate of decolorization, which was not complete yet after 576 h of incubation. Similar results were obtained by Van der Zee [5] by using Reactive Yellow 2 and Direct Yellow 12 azo dyes. Reactive Yellow 2 was not completely reduced within the 342 days of incubation.

Real textile wastewater generally contains only 100–500 mg l^{-1} of azo dyes. Therefore high dye concentrations are not important in practice.

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