Desalination and Water Treatment
www.deswater.com

23 (2010) 61–65
November

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doi: 10/5004/dwt.2010.1951

Decolorization of Basic Red 46 and Methylene Blue by anaerobic sludge: Biotic and abiotic processes

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Received 10 November 2009; Accepted 1 April 2010

ABSTRACT

Decolorization of Basic Red 46 (Maxilon Red GRL) (BR46; azo dye) and Methylene Blue (MB.; cationic dye) was studied by using anaerobic sludge taken from upflow anaerobic sludge blanket bed (UASB) reactor treating wastewaters of Pakmaya Yeast Factory in Izmit, Turkey. Experiments were carried out under abiotic and biotic conditions. Abiotic tests were carried out without living biomass in order to determine color removal by non-biological process. The biotic tests were achieved at two cases as dye main carbon source without co-substrate (glucose) and using different concentrations of glucose (500-1000-1500-2000-3000 mg/l). For first case; the concentration of dyes was 25(B1), 50(B2), 100(B3) mg/l. In the other case; dye concentration was kept constant as 100 mg/l for changing glucose concentrations. MB removal (40%) was better than BR46 removal (25%) physically as physically in abiotic conditions. In biotic tests without glucose, color removal for both dyes were achieved at almost within in the 120 h. The maximum dye removal rates were obtained at the starting of experiments. In biotic tests with co-substrate, decolorizations rate of dyes was obtained faster than first case experiments. Almost complete removal for BR46 and MB were found in 25 and 50 h, respectively. The highest removal rates for both dyes were determined by co-substrate with the highest concentration of glucose, 3000 mg/l in the biotic conditions. BR46 and MB can be biologically degraded by using anaerobic sludge with and without co substrate. Decolorization of BR46 and MB was achieved at short time with presence of co-substrate.

Keywords: Anaerobic sludge; Azo and Cationic dyes; Batch experiments; Color; COD

1. Introduction

Several industries, such as textiles, paper, plastics, leather, food, cosmetics, use dyes or pigments to color their final product. Especially, textile industry is responsible from highly colored wastewater given to aquatic environment. Therefore, they cause to water pollution problem [1,2]. Common group of dyes in textile industry, azo dyes (60–70%), are characterized by their typical –N = N– nature [3–5]. Basic dyes are cationic compounds that are used for dyeing acid group-containing fibres. Most of the basic dyes are diarylmethane, triarylmethane, anthraquinone and azo compounds. Basic dyes represent approximately 5% of all dyes listed in the color Index [6].

Some dyes and their breakdown products may be toxic towards living organisms [1,4]. Therefore decolorization of dyes by several physical, chemical and biological pretreatment, main treatment, post treatment methods are needed for dye containing wastewaters [1,2]. Biological aerobic wastewater treatment systems are generally not successful for color removal of some dyes [1,4]. However, under the anaerobic conditions, some dyes, such as azo dye, are degradated [7] and generation of aromatic amines from breakdown of azo dyes can be removed by using anaerobic-aerobic conditions [4,8,9]. Although some studies showed that many of the aromatic amines

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were removed from wastewater under aerobic conditions, not all aromatic amines could be mineralized [10]. Physico-chemical methods such as adsorption, coagulation-flocculation, membrane filtration and advanced oxidation, can be used effectively for color removal from wastewaters. However, they are expensive compare with biological processes [4,10].

In this study, decolorization of Basic Red 46 (BR46; azo dye) and Methylene Blue (MB; cationic dye) was investigated by using anaerobic sludge taken from upflow anaerobic sludge blanket bed (UASB) reactor treating wastewaters of Pakmaya Yeast Factory in Izmit Turkey. Experiments were carried out under anaerobic conditions with abiotic and biotic tests (with and without co-substrate (glucose)).

2. Materials and methods

2.1. Batch experiments and experimental procedure

A 500 ml glass serum bottles were used by sealed with a rubber screw cap. Experiments were performed with 200 ml of working volume by a batch test. Each of the serum bottles consisted of anaerobic sludge (as 3000 mg MLVSS/l) taken from UASB reactor treating the wastewaters of Pakmaya Yeast Factory, in Izmit Turkey, 3000 mg COD/l of glucose and necessary Vanderbilt mineral medium. This mineral medium contains the following inorganic composition (in mg/l): 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 [11]. Table 1 illustrates experimental conditions of anaerobic batch study. Substrate, metal ions, and distilled water were added into serum bottles. The alkalinity and neutral pH were kept constant by addition of 5000 mg/l $NaHCO_3$. The initial pH values were adjusted to 7.0 with NaOH (2M) and H_2SO_4 (2M) solutions. Temperature controlled incubator was used at 35 °C for all batch experiments. The serum bottles were shaken at 120 rpm. The syringe was used to take supernatant samples from the bottles. The mixed liquors samples were centrifuged for analysis of color and chemical oxygen demand (COD). Figs. 1 and 2 and Table 2 show the structures and properties of BR46 and MB, respectively. BR46 was

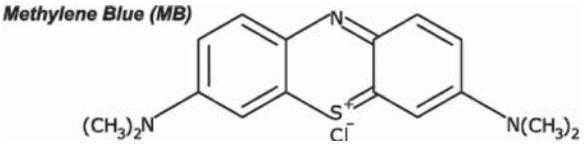

Fig. 1. Molecular structures of Methylene Blue (MB).

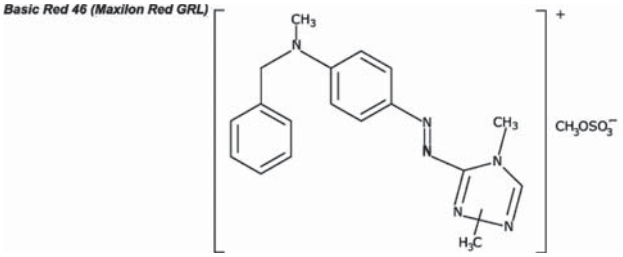

Fig. 2. Molecular structures of C.I. Basic Red 46 (BR46).

Table 1
Experimental conditions of anaerobic batch study

Stock	Seed cont. (ml).	Control (ml)	Abiotic control (ml)	A + B (ml)	Biotic 1 (ml)	Biotic 2 (ml)	Biotic 3 (ml)	*1 (ml)	*2 (ml)	*3 (ml)	*4 (ml)	*5 (ml)	Resulting conc.
Sludge, 40 g MLVSS/l, 48 g MLSS/l	15	15	–	15	15	15	15	15	15	15	15	15	3000 mg MLVSS/l
Glucose, 20 g COD/l	–	30	30	30	–	–	–	5.0	10	15	20	30	–
$NaHCO_3$, 50 g/l	20	20	20	20	20	20	20	20	20	20	20	20	5000 mg $NaHCO_3$/l
Vanderbilt	20	30	30	30	20	20	20	5.0	10	15	20	30	–
Dye, 10 g/l	–	–	2.0	2 + 2	0.5	1.0	2.0	2.0	2.0	2.0	2.0	2.0	–
Total Volume	200	200	200	200	200	200	200	200	200	200	200	200	–

*1 = 500 mg COD/l, *2 = 1000 mg COD/l, *3 = 1500 mg COD/l, *4 = 2000 mg COD/l, *5 = 3000 mg COD/l.
A + B = Mixture of BR46 and MB (Basic Red 46 + Methylene Blue).
Biotic 1 = 25 mg dye/l, Biotic 2 = 50 mg dye/l, Biotic 3 = 100 mg dye/l.
Biotic 1, 2, 3 = without co-substrate (glucose) and initial dye concentration was kept constant as 100 mg/l.
*1, *2, *3, *4, *5 = Biotic, with co-substrate (glucose) (500–1000–1500–2000–3000 mg/l COD).

Table 2
Properties of Basic Red 46 (BR46) and Methylene Blue (MB)

Color Index	Basic Red 46
Type	Cationic
Sulphonic group	–
Azo group	1
Λmax	530
pH range	(2–12)
*Molecular weight (g/mol)	322
*Width (nm)	1,3
*Depth (nm)	0.74
*Thickness (nm)	0.63
Color Index	Methylene Blue
Chemical formula	$C_{16}H_8N_3SCl \cdot 3H_2O$
Type	Basic, Cationic

Generally, it is known as nontoxic; but it is harmful for some cases.
*Associated counter ions are not included.

supplied from a textile factory in Bursa, Turkey and was of commercial quality. MB was purchased from chemical company in Turkey.

A control, without color, was used to determine COD measurements in all batch experiments. Abiotic tests were performed with autoclaved anaerobic sludge. COD and color removal experiments in all batch study were performed in duplicate to control the accuracy of the experimental results. Experimental data was detected both from COD and color measurements.

2.2. Analytical procedure

Total suspended solid (TSS) and total volatile solid (TVS) of anaerobic sludge was measured using the Standard Methods of American Public Health [12]. Bicarbonate alkalinity and COD measurements was determined by titrimetric method [12]. Color measurements were carried out in 5 ml samples removed from supernatants of serum bottles during the experimental study. Spectrophotometer was used for color measurements. The samples were centrifuged at 5000 rpm for 20 min. Absorbance of the supernatant was measured. λ_{max} of BR46 and MB. was determined in an aqueous medium by using a scanning UV-vis spectrophotometer. Pharmacia Nova Model spectrophotometer was used at 530 and 663 nm for BR46 and MB, respectively. 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.

3. Results and discussion

3.1. Abiotic study

Abiotic tests were carried out without living biomass and with glucose of 3000 mg/l. Initial concentration of BR46 and MB was kept constant as 100 mg/l. As shown in Figs. 3 and 4, approximately 25% BR46 and 40 % MB removal was achieved under abiotic tests, which was probably due to adsorption of dye molecules by the dead cells MB removal was better than BR46 removal by physically. As shown in Figs. 3 and 4, decolorization efficiency under abiotic conditions was only around 25% for BR46. This situation shows that physical adsorption of BR46 on anaerobic culture was not important. Similar results were found in other abiotic test studies [2,4,6].

3.2. Biotic studies

3.2.1. Biotic test without co-substrate

Decolorization of BR46 and MB was performed in biotic studies without co-substrate. As expected, it needs long time to achieve complete color removal

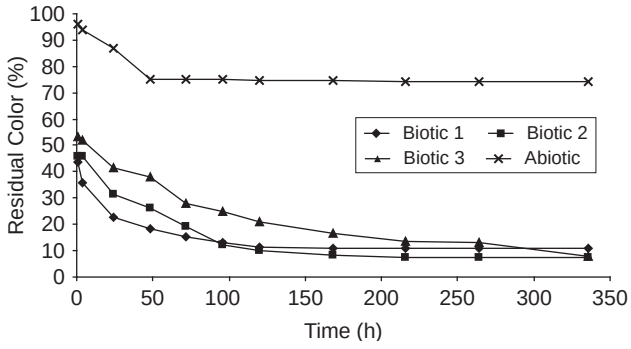

Fig. 3. Color removal of Basic Red 46(BR 46) for abiotic and B1, B2, B3 biotic tests.

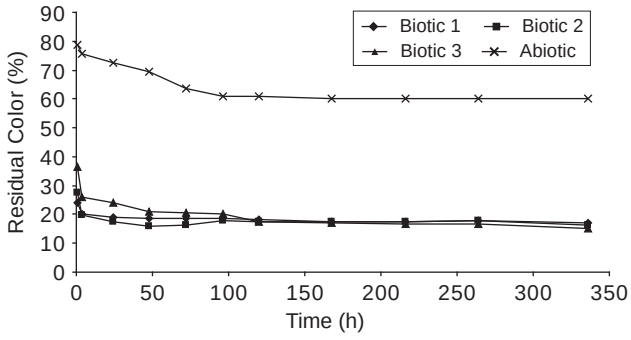

Fig. 4. Color removal of Methylene Blue (MB) for abiotic and B1, B2, B3 biotic tests.

due to absence of co-substrate (glucose). Decolorization was achieved at almost 120 h. Initial dye concentrations were 25–50–100 mg/l (B1, B2, B3) in the batch studies. As shown in Figs. 3 and 4, residual color as 10% (B1 and B2) and 20% (B3) for BR46 and approximately 20% (B1, B2 and B3) for MB after 120 h. Biological degradation of azo dye (Acid Orange 7) was investigated under the anaerobic batch conditions in presence and absence of co-substrate and it was shown that the dye needed longer times to be biologically degraded in the absence of co-substrate [4].

As shown in Figs. 3 and 4, the maximum BR46 and MB removal rates were observed at the beginning of experiment. MB was decreased faster than BR46 in biotic tests without co-substrate. But, residual color (%) was less for BR46 at the end of time. The decolorization efficiency for BR46 and MB was 80–90% and 80% after 120 h, respectively. Thus, biodegradation of BR46 was better than that of MB.

3.2.2. Biotic test for different co-substrate concentrations

A number of biotic tests were carried out in the presence of different concentrations of co-substrate (500-1000-1500-2000-3000 mg COD/L of glucose) and it was observed that the colorization rate of dyes was much faster in the presence of a co-substrate compared to those obtained in the absence of it.

As shown in Figs. 5 and 6, no significant change was observed in color removal when the COD was increased from 500 to 3000 mg/L. Almost complete removal of BR46 was achieved at 25th hour (Fig. 5). Residual color (%) was around 3% at the end of 25th h.Similar removal degree for MB was obtained at 50th hours. There is no change in color removal values after mentioned times given above (Figs. 5 and 6). Residual color (%) for MB changed between 3% and 7, 5% at the end of incubation

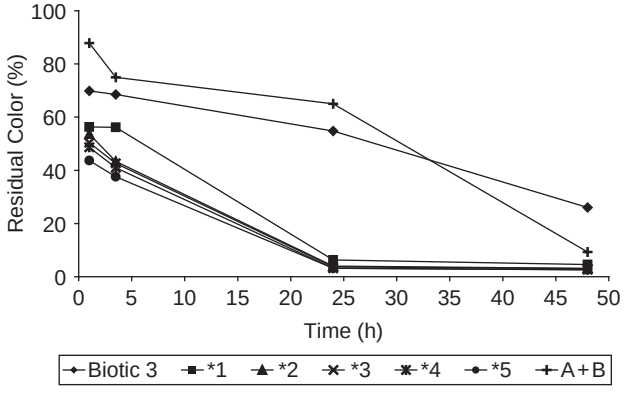

Fig. 5. Effect of COD concentrations onto removal of Basic Red 46(BR 46) and mixture of two dyes (A + B).

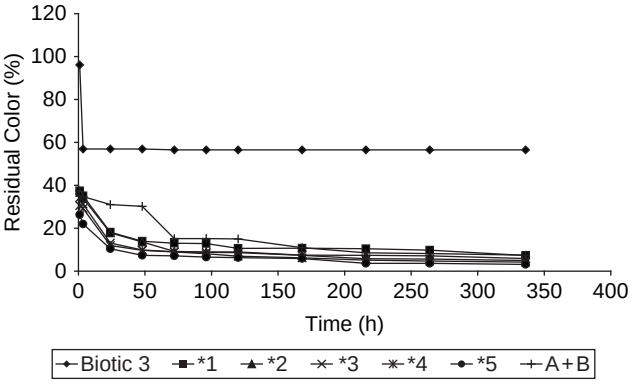

Fig. 6. Effect of COD concentrations onto Methylene Blue (MB) and mixture of two dyes (A + B).

time. Similar results were obtained by Van Der Zee [6] for Reactive Yellow 2 and Direct Yellow 12 azo dyes. Reactive Yellow 2 was not completely reduced within the 342 days of incubation. BR46 removal rate was higher than MB removal rate. In the literature, biodegradation of methylene blue by upflow anaerobic sludge blanket reactor was investigated. The presence of suitable amount of organic content (sucrose and peptone) as an electron donor played an important role for color removal (90%) of M.B [13]. Complete color removal was found for 20 selected azo dyes by anaerobic sludge [14].

Figs. 7 and 8 illustrate COD removal for both dyes during incubation time. Residual COD concentrations of 1000 mg/L initial COD concentration were 360 and 520 mg COD/L for BR46 and MB, respectively at the end of incubation time. Both Figs. 7 and 8 show that 100 mg/L dye concentration has a certain inhibition effect on COD degradation. When control and 5 were compared, the

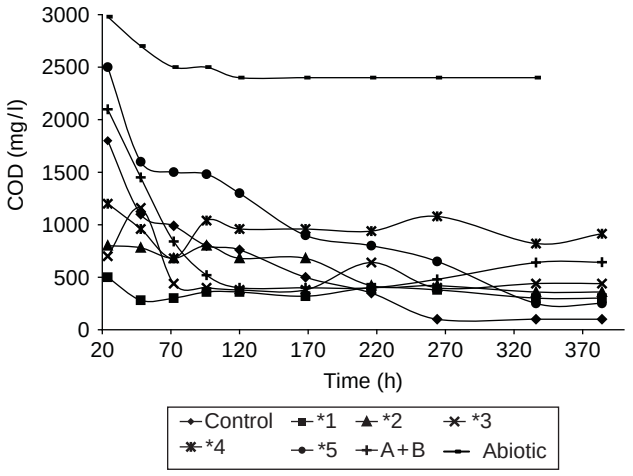

Fig. 7. COD Removal during Basic Red 46(BR 46)'s incubation time.

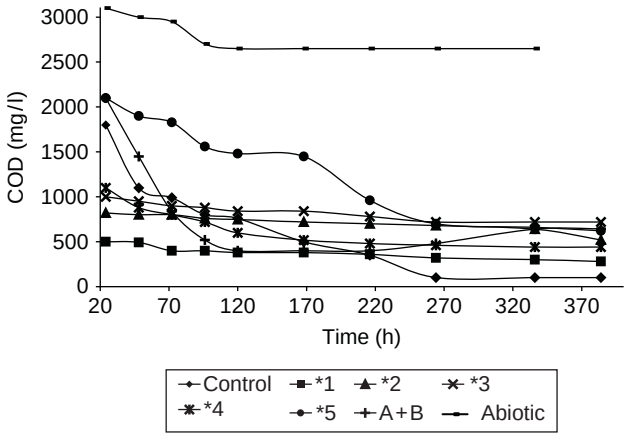

Fig. 8. COD removal during Methylene Blue (MB)'s incubation time.

residual COD for control and 5 at the end of incubation time were 100 and 250; 100 and 620 for BR46 and MB, respectively. As can be seen the results, the inhibition effect of MB was higher than that of BR46. Effect of dye concentrations on COD removal was studied in previous works [2,5,6]. It was mentioned that high concentrations of dye caused inhibition of anaerobic degradation of glucose.

Dyes used in this study was possible to biodegrade even at relatively low COD concentration. The statement mentioned is in good agreement with the results given in literature [2, 4, 5, and 6]. Color removal by anaerobic sludge as a pre-treatment for different industrial wastewaters seems to be an attractive technology with aerobic post treatment. Generally, COD removal can also be achieved during the anaerobic process [2,5,6]. Thus, anaerobic process is effective treatment of dye bearing wastewaters to remove COD and color

Decolorization of some dyes by using anaerobic granular sludge under mesophilic conditions was reviewed [15]. It was mentioned that comparison of investigations was extremely difficult because of the differences in type and concentrations of dyes (azo or others), sludge source and concentrations (from UASB reactor or others), electron donor (glucose or others) and so on. [15].

4. Conclusion

BR46 and MB can be removed in a combination of abiotic (non-biological) and biotic (biological) reaction processes in anaerobic experimental conditions. MB removal (40%) was better than BR46 removal (25%) as physically.

In biotic tests without co-substrate (glucose), color removal for dyes used this study were achieved at almost within the 120 hours, although the dye was the only carbon source in the medium. The maximum dye removal rates were obtained at the beginning of the experiments. It was observed that the colorization rate of dyes was much faster in the presence of a co-substrate compared to those obtained in the absence of it. Almost complete BR46 and MB removal were found at 25th and 50th hours, respectively. A significant color removal was observed for both R46 and MB in the absence of co-substrate after 120 hours of incuation in biotic tests. BR46 and MB can be biologically degraded by using anaerobic sludge with and without co-substrate.

Acknowledgement

This paper was presented at the 11th International Conference on Environmental Science and Technology, CEST 2009, Chania, Crete, Greece, 3–5 September 2009.

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