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Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions

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Abstract

In the present study mixed cultures that could grew in the molasses media were isolated from textile dye effluent and its decolorization activity was studied in a batch system under anaerobic conditions, in order to determine the optimal conditions required for the highest decolorization activity. The optimum pH value for decolorization was determined as 8 for all the dyes tested. In the experiment with pH 8 dye decolorizations by mixed cultures were investigated at about 96.2–1031.3 mg l^{-1} initial dye concentrations. The highest dye removal rates of mixed cultures were 94.9% for Reactive Red RB, 91.0% for Reactive Black B and 63.6% for Remazol Blue at 953.2, 864.9 and 1031.3 mg l^{-1} initial dye concentrations respectively within 24 h incubation period. When the Reactive Red RB was used, approximately 82–98% total color removal was obtained at between 96.2 and 953.2 mg l^{-1} initial dye concentrations after 12 h of incubation at 35 °C. These results show that our enriched mixed cultures have the potential to serve as an excellent biomass for the use in reactive dye removal from wastewaters under anaerobic conditions.

Keywords: Reactive dyes; Decolorization; Mixed culture; Anaerobic; Molasses

1. Introduction

Synthetic dyes including several structural varieties such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal–complex dyes are widely used as coloring agents in textile, food, paper and cosmetic industries. The effluents of these industries are highly colored and the disposal of these wastes into receiving waters causes damage to the environment as they may significantly affect photosynthetic activity in aquatic life due to reduced light penetration and may also be toxic to some aquatic organisms due to the presence of metals, chlorides, etc., in them and the breakdown products of dyes [1–3].

Many bacteria and fungi are used for the development of biologic processes for the treatment of textile effluents [4–7]. Recently, different kinds of dyes decolorizing bacterial cultures under anaerobic conditions were isolated from textile effluent in the media containing dyes [8,9]. Anaerobic reductive cleavage of the azo bond in dye molecular structure resulted in the formation of colorless aromatic amines that can be further degraded by aerobic microorganisms [10–15]. In the previous studies, the mixed bacterial cultures having the highest decolorization activity under anaerobic conditions were grown on rich media supplemented with yeast extract or glucose [16–18]. However, these complex laboratory substrates would not be suitable for in situ application.

The major objective of this study was to investigate the growth and decolorization properties of the mixed cultures isolated from textile effluents under anaerobic conditions in the media including molasses. Molasses was chosen in the present study due to its high sucrose and other nutrient contents, low cost, ready availability and easy of storage.

2. Materials and methods

2.1. Microorganism

The mixed cultures were obtained from water samples contaminated with dye effluents from textile industries at Adana, Turkey, and enriched by the following procedure consisting of periodic subculturing of samples taken from wastewater in molasses medium containing 50 mg l^{-1} dye. The composition of the molasses medium is as follows: beet molasses solution (approximately equivalent to 10 g l^{-1} sucrose), 1.0 g $(NH_4)_2SO_4$, 0.5 g KH_2PO_4 in 1 l [19]. Remazol Blue, Reactive Black B and Reactive Red RB dye stock solutions which are obtained from AYTEMİZLER Textile Co., Turkey in pure form were prepared by dissolving the powdered dyestuff in distilled water to a concentration of 2% (w/v). Appropriate volumes of the stock dye solution were added to media and

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the initial pH was adjusted to 6, 7 and 8 with 0.1 M NaOH and 0.1 M HCl. The cultures developing on three different dyes and pH values were kept at 4 °C and were transferred to molasses medium including dye every 3 months.

2.2. Culture conditions

The mixed cultures were transferred into 15 ml of the molasses medium including dye in a 15 ml glass screw tubes. Test tubes were filled up to top with medium and tightly closed. The cultivation was carried out at 35 °C for 48–76 h.

2.3. Decolorization experiments

To examine the effect of initial pH on the decolorization, molasses medium was prepared at pH 6, 7 and 8. After that, 100 mg l^{-1} of the selected dye was added to medium. To acclimatize the mixed cultures to various dye and pH values, repeated transfers of cultures were done into fresh molasses medium prepared at pH 6, 7 and 8. The tubes were inoculated with acclimatized cultures. At 0, 6, 12, 24, 30, 36 and 48th hours corresponding tube was opened and analysed.

In order to examine the effect of initial dye concentration on the decolorization, molasses medium was prepared at optimum pH value with 100, 200, 400, 600 and 1000 mg l^{-1} of the selected dye. To acclimatize the mixed cultures to increasing concentrations of textile dyes, the cultures were gradually exposed to increasing concentrations of dye. This was achieved through successive transfers of cultures into fresh molasses medium containing 100, 200, 400, 600, 1000 mg l^{-1} of the selected dye. The tubes were inoculated with acclimatized cultures. At 0, 6, 12, 24, 30, 36, 50, 56, 76th hours corresponding tube was opened and analysed.

The effect of different temperatures on the decolorization was investigated in another series of experiments. The mixed cultures that were grown on about 100 mg l^{-1} initial dye concentration and optimum pH value were inoculated with acclimatized cultures and incubated at 25, 35 and 45 °C. At 0, 24, 48, 72th hours corresponding tube was opened and analysed.

Each experiment was carried out in triplicate. In addition, control tubes containing both dye and molasses without inoculation of cultures were prepared to observe any reaction of molasses with dye.

2.4. Analytical methods

At the end of incubation period, tubes were opened and centrifuged to precipitate suspended biomass at 5000 rpm for 10 min. Cell growth was determined by measuring dry weight of washed biomass. The concentration of dye in the supernatant was determined by reading absorbance at 600 nm for Remazol Blue, at 590 nm for Reactive Black B and at 495 nm for Reactive Red RB. Cell free molasses medium was used as the blank. Absorbance measurements and centrifugation were done by using a Shimadzu UV 2001 model spectrophotometer and Hettich EBA12 model centrifuge, respectively.

3. Results

Dye decolorization properties by mixed cultures were investigated as a function of changes in the initial pH values, initial dye concentrations and temperature. The results are given as decolorized dye concentration at the end of growth ($C_{acc,m}$: mg l^{-1}) and the units of specific growth rate of mixed culture (μ: h^{-1}). Decolorization yield (decolorization: %) is defined as the ratio of decolorized concentration of dye at the end of microbial growth to the initial dye concentration (C_0: mg l^{-1}).

3.1. Effect of initial pH on decolorization

In order to find a suitable pH for the effective dye bioaccumulation by mixed cultures, experiments were performed at three different initial pH values. The effect of initial pH on the specific dye decolorization rate of mixed cultures is given in Table 1 at about 100 mg l^{-1} initial dye concentration. Decolorization yield of the mixed cultures exceeded 84.5–100% after 24–48 h at all tested pH and dyes. At Remazol Blue and Reactive Black B dyes, decolorization yield increased with increases in pH values to a certain level, the highest yield was obtained at pH 8. In the samples with Reactive Red RB there was no significant difference in the decolorization yield of dye between the pH values tested at the end of 30 h incubation period.

Table 1
The effect of initial pH on the maximum decolorized dye concentration ($C_{acc,m}$) and decolorization yield of mixed cultures at about 100 mg l^{-1} initial dye (C_0) concentration (T: 35 °C)

pH	C_0 (mg l^{-1})	$C_{acc,m}$ (mg l^{-1})	Decolorization (%)	Exposure time (h)
Remazol Blue				
6	108.9	96.8	88.9	48
7	102.0	100.7	98.7	48
8	125.6	125.6	100	48
Reactive Black B				
6	94.7	80.0	84.5	24
7	94.3	92.2	97.8	24
8	106.3	106.3	100	24
Reactive Red RB				
6	96.7	96.7	100	30
7	99.6	99.6	100	30
8	96.2	96.2	100	30

At the end of this series of experiments, the optimum pH values for dye decolorization were determined as pH 8. In the experiments with pH 8, the shortest incubation period for complete decolorization was obtained by Reactive Black B followed by Reactive Red RB and Remazol Blue.

3.2. Effect of initial dye concentration on decolorization

Decolorization percentages of the Remazol Blue dye by mixed cultures at different initial dye concentrations varied between 125.6 and 1031.3 mg l^{-1} after 76 h are shown in Fig. 1. At 125.6–206.3 mg l^{-1} dye concentrations 100–90% of the initial dye is decolorized at the end of 30 h incubation period. At

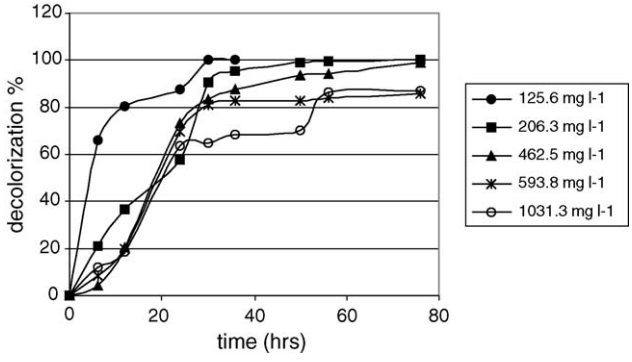

Fig. 1. Effect of initial dye concentration on decolorization yield of Remazol Blue by mixed cultures (T: 35 °C; pH 8).

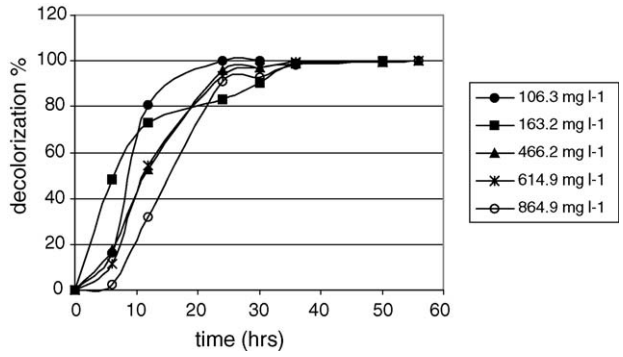

Fig. 2. Effect of initial dye concentration on decolorization yield of Reactive Black B by mixed cultures (T: 35 °C; pH 8).

462.5 mg l^{-1} initial dye concentration, 90% of dye is removed after 50 h incubation period. At high dye concentrations (593.8 and 1031.3 mg l^{-1}), in spite of longer incubation periods decolorization yield could only reach about 85%.

Reactive Black B dye decolorization by mixed cultures was also investigated at different initial dye concentrations (106.3–864.9 mg l^{-1}) during the 56 h incubation period (Fig. 2). After 24 h of incubation, about 90–100% dye removal was achieved for all dye concentrations of Reactive Black B. The mixed culture was found to decolorize more than 99% of the initial dye levels, which ranged from 466.2 to 864.9 mg l^{-1} within 36 h period and then the decolorization yield remained constant at the following 56 h during the cultivation in molasses medium under anaerobic conditions.

When the Reactive Red RB was used, approximately 82–98% total color removal was obtained at between 96.2 and 953.2 mg l^{-1} initial dye concentrations after 12 h of incubation (Fig. 3). For all tested dyes, this decolorization yield was the highest yield obtained at 12 h incubation period. In the experiment with Reactive Red RB decolorization began immediately and no lag period was observed. At all tested dye concentrations decolorization yield exceeded 98–100% after 30 h.

At the end of 24–36 h incubation period, the growth rates and decolorization yields obtained at different initial dye concentrations are compared in Table 2. As it can be seen from the table, at the increasing dye concentrations the growth rate

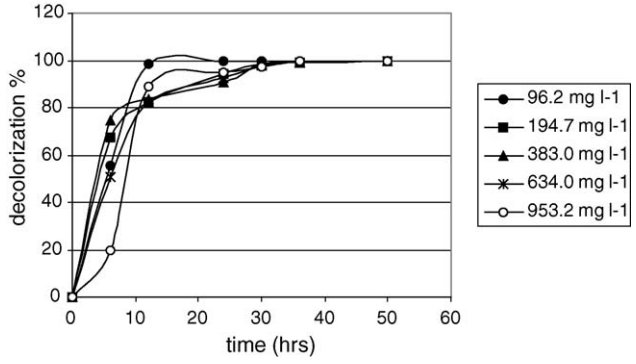

Fig. 3. Effect of initial dye concentration on decolorization yield of Reactive Red RB by mixed cultures (T: 35 °C; pH 8).

Table 2
The comparison of the maximum decolorized dye concentration ($C_{acc,m}$), decolorization yields and growth rates obtained at different initial dye (C_0) concentrations (T: 35 °C; pH 8)

C_0 (mg l^{-1})	$C_{acc,m}$ (mg l^{-1})	Decolorization (%)	μ (h^{-1})	Exposure time (h)
Remazol Blue				
125.6	125.6	100	0.12	36
206.3	196.4	95.2	0.12	36
462.5	406.1	87.8	0.10	36
593.8	490.5	82.6	0.09	36
1031.3	706.4	68.5	0.07	36
Reactive Black B				
106.3	106.3	100	0.16	30
163.2	147.7	90.5	0.17	30
466.2	452.7	97.1	0.11	30
614.9	595.8	96.9	0.10	30
864.9	805.2	93.1	0.10	30
Reactive Red RB				
96.2	96.2	100	0.23	24
197.4	186.3	94.4	0.23	24
383.0	348.1	90.9	0.16	24
634.0	587.1	92.6	0.15	24
953.2	904.6	94.9	0.11	24

of the mixed cultures decreased for all tested dyes. The maximum growth rate was obtained in the samples having lower initial dye concentrations. When the Reactive Red RB was used, the mixed cultures were shown to have the maximum growth rate and decolorization yield, which can be considered as satisfactory in terms of dye removal from subject wastewater effluents.

3.3. Effect of temperature on decolorization

The effect of different temperatures on the decolorization by mixed cultures has also been investigated under anaerobic conditions (Table 3). In the experiments, no growth occurred at 45 °C while growth observed at 25 and 35 °C incubation temperature for all tested dyes. Temperature variation had a significant effect on the decolorization of all tested dyes by mixed cultures within 24 h incubation period. The highest color removal was detected at 35 °C within 24 h. After 48 h incubation period, sim-

Table 3
The effect of temperature on the maximum decolorized dye concentration ($C_{acc,m}$) and decolorization yield of mixed cultures after 24 and 48 h incubation period (pH 8)

Dye C_0 (mg l^{-1})	Temperature (°C)	$C_{acc,m}$ (mg l^{-1})		Decolorization (%)	
		24 h	48 h	24 h	48 h
R. Blue 113.3	25	49.5	89.6	43.7	79.1
	35	80.9	86.9	71.4	76.7
R. Black B 169.2	25	94.6	161.4	55.9	95.4
	35	150.9	165.3	89.2	97.7
R. Red RB 134.7	25	56.7	126.1	42.1	93.6
	35	127.6	129.7	94.7	96.3

ilar decolorization yields was obtained by mixed cultures at both temperature used.

4. Discussion

Molasses is the residual syrup derived from the sugar beet pulp that strongly stimulates microbial growth. Therefore, molasses was used in the media with reactive dyes for all of the experiments described in this study. At the molasses media, high concentrations of dyes were decolorized at the conditions described above. The maximum yields obtained ranged from 90 to 100% within 12–36 h period, depending on the dye concentrations of the media.

Reactive dye decolorization under anaerobic conditions was also reported in the previous studies, but at relatively lower initial concentrations of dye in the media, which did not contain molasses. For example under anaerobic conditions, PDW-mixed culture which was found to be composed of at least two bacterial strains, *Alcaligenes faecalis* and *Comomonas acidovorans* removed 79–88% of color from effluent containing diazo-reactive dyes after 24 h at about 500 mg l^{-1} dye concentration in rich media supplemented with 5 g l^{-1} yeast extract [9,17,18]. In this study, it is readily apparent from Figs. 2 and 3 that reactive dye decolorization efficiency in the molasses media varied from 90 to 95% at high initial dye concentrations within 24 h incubation period. Data presented in figures show that decolorization yield was not highly affected by increasing dye concentrations at all tested dye types excepting Remazol Blue.

Although the literature contains many studies approaching to the topic [20], reactive azo dye decolorization by mixed cultures in the molasses media has not been extensively studied. Most of the researchers believe that anaerobic azo dye reduction is non-specific and extracellular process in which reducing equivalents from either biological or chemical source are transferred to the dye. It was stated that the reducing equivalents, which are formed during anaerobic oxidation of carbon sources, are used for the reduction of azo bond [14]. The substrates used for dye removal varied from simple substrates to ones that are more complex. Azo dye decolorization was achieved with all substrates like glucose, acetate, ethanol, starch, etc., however, some substrates may be more successful in delivering reducing equivalents to azo dyes either because of the substrate itself or the used microorganisms [15]. In this study, possibly because the reasons outlined above, dye removal rates obtained with enriched microbial culture in dye containing molasses medium is more successful than that of other reported studies.

Conventional treatment facilities may not be satisfactory for the treatment of textile dye effluents because of color, COD, N, other micro pollutants present in them [21]. Pretreatment of effluent with anaerobic bacteria will provide color removal, but anaerobic cleavage of azo dyes leads to formation of hazardous aromatic amines. Under anaerobic conditions, these amines are not degraded and accumulated. Some aerobic bacteria can degrade many aromatic amines. For this reason, it has been suggested to combine anaerobic treatment with aerobic treatment, sequentially or simultaneously to achieve complete mineralization of dyes. This study shows that isolated and enriched microbial cultures deserve attention as a new biomass media, which can be utilized with combined aerobic treatment in the decolorization of wastewater effluents containing dyes before the conventional treatment.

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