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Key Factors Regarding Decolorization of Synthetic Anthraquinone and Azo Dyes

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Abstract The factors affecting decolorization of anthraquinone dye represented by Reactive Blue 4 (RB4) and azo dye represented by Methyl Orange (MO) were studied in batch experiments under mesophilic (35 °C) and thermophilic (55 °C) anaerobic conditions. The results indicated differences in decolorization properties of the dyes with different chromophore structures. In abiotic conditions, MO could be decolorized by a physicochemical reaction when it was sterilized at 121 °C together with sludge cells or glucose. RB4 only showed absorption onto the cell mass. The presence of a redox mediator accelerated the decolorizing reaction when supplied together with glucose in the presence of sterilized sludge cells. In biotic conditions, the results indicated that the biological activity of microorganisms was an important factor in decolorization. The main factor involved in decolorization was the conversion of cosubstrate as electron donor, which reacted with dye as an electron acceptor in electron transfer. Redox mediators, anthraquinone-2-sulfonic acid, and anthraquinone could accelerate decolorization even if a small amount (0.2 mM) was applied. On the other hand, a high concentration of redox mediator (1.0 mM) had an inhibitory effect on decolorization especially under thermophilic conditions. In addition, the decolorization of dye was accelerated by increasing treatment temperature, as shown in biotic treatments. Based on these results, increasing the treatment temperature could be used to improve the decolorizing process of textile dye wastewater treatment, especially for recalcitrant dyes such as anthraquinone.

Keywords Factors of decolorization · Synthetic dye · Abiotic · Biotic · Anaerobic treatment · Redox mediator

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Introduction

Azo dye, which is widely used in the textile industry, represents the largest and most versatile group whose share in industrial application amounts to some 70% of all dyestuff consumed. Anthraquinone dyes are used for coloration of cotton and cellulose fibers as well as of hydrophobic, synthetic materials [1]. During dye processing, as much as 2–50% of dyestuffs applied may be discharged to wastewater and ultimately released into the environment [2]. Dye decolorization can be readily achieved under anaerobic conditions with either chemical or biological treatment. Biological dye decolorization involves unspecific enzymes usually found in a wide diversity of microorganisms. The exact mechanism of dye decolorization, whether occurring intracellularly or extracellularly, is still being investigated. It is hypothesized that dye reduction occurs by an extracellular or membrane-bond enzymatic reaction associated with biogenic water-soluble electron carriers, which act as electron shuttles to dye as the electron acceptor [3]. The transfer of reducing equivalent from cosubstrate (electron donor) to dye (electron acceptor) is the rate-limiting step in anaerobic decolorization [4]. Where membrane permeation is limited, use of cell-free extracts increases color removal rate of sulfonated azo dye. Quinone reductase activity located in the cell membrane also enhances sulfonated azo dye decolorization, and the transport of dye across the membrane is not needed [5].

The addition of redox mediator to the decolorizing process has been shown to accelerate the decolorizing potential and decolorizing rate [6, 7]. Quinone compounds acting as redox mediators were shown to accelerate chemical azo dye reduction by sulfide in abiotic systems [8]. These compounds, the electron accepting moieties of humic substances, facilitate electron transfer from an electron donor to an electron acceptor. The mechanism involves nonspecific enzymatic reduction of quinine to hydroquinone and then the chemical reoxidation of hydroquinone by azo dye [9]. In general, the chemical reaction follows the Arrthenius equation, indicating that an increase in temperature will proportionally increase the collision frequency of the reactant. Thus, the advantage of dye decolorization under thermophilic conditions might be not only the expected faster enzymatic reduction of quinine to hydroquinone compared to mesophilic conditions but also the faster regeneration of hydroquinone by the chemical reaction with dyes [7].

Furthermore, the rates of decolorization are dependent on the type of dye, the azo dyes generally presenting the highest rate of decolorization, with anthraquinone dyes being more recalcitrant. However, the mechanism of the reductive decolorization of azo and anthraquinone dyes is not yet known. In this study, the objective was to investigate the decolorizing factors for two kinds of dye viz., anthraquinone type represented by Reactive Blue 4 (RB4) and azo type represented by Methyl Orange (MO), under mesophilic (35 °C) and thermophilic (55 °C) conditions in batch experiments. To achieve this objective, abiotic and biotic decolorizations were investigated to assay the importance of the biological activity of decolorizing microorganisms. In addition, the effect of quinone compounds on acceleration of dye decolorization was evaluated.

Materials and Methods

Chemicals

MO (CI 13025) and RB4 (IC 61205) were selected as azo and anthraquinone dye classes, respectively (Fig. 1). Anthraquinone-2-sulfonic acid (AQDS), anthraquinone (ATQ), and general chemicals used were purchased from Wako and Sigma–Aldrich, Japan.

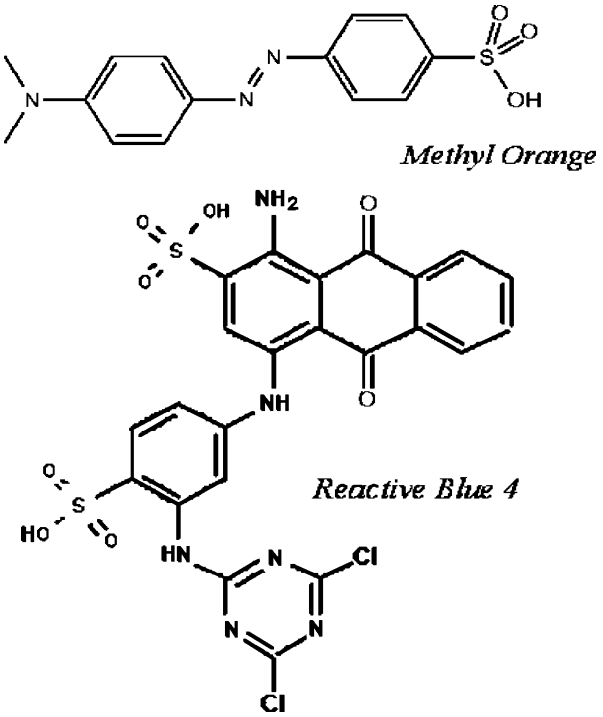

Fig. 1 Chemical structure of MO and RB4

Decolorizing Microorganisms

Digested sludge used as decolorizing microorganisms was obtained from an anaerobic digestion plant, Eastern Wastewater Treatment Centre, Ube City, Yamaguchi Prefecture, Japan. The initial suspended solid concentration of used sludge was approximately 13,000 mg SSL^{-1}. The batch experiment was conducted in serum bottles sealed with rubber, covered with aluminum caps and then purged with Ar gas. The initial pH was 7.5 without addition of buffering chemicals. The treatment temperatures were 35 and 55 °C in temperature controlled incubators without shaking.

Abiotic and Biotic Decolorizations

Abiotic and biotic decolorization was conducted as shown in Fig. 2. The sludge cell mass concentration was 0%, 10%, 20%, 30%, 40%, and 50% (*v*/*v*) after centrifuging at 6,000 rpm for 15 min. For 0% of sludge the supernatant was used, following sonication at 60% pulse for 3 min using an ultrasonic processor USP-300 (Shimadzu, Tokyo, Japan). The dye decolorization was studied by measuring the decolorizing efficiency of 200 mg L^{-1} dye as a percentage of decolorization compared with control using distilled water (DW). Abiotic conditions were established by sterilizing digested sludge at 121 °C for 15 min before adding to the culture bottle, with sterilization repeated after all mixture was in the serum bottle. For biotic conditions, sludge was directly used without sterilization. In all treatment of abiotic and biotic decolorization, the mixture was contained only sludge cell and dye without other supplementary. Decolorizing efficiency was measured at 0 and 48 h of incubating time under mesophilic and thermophilic conditions.

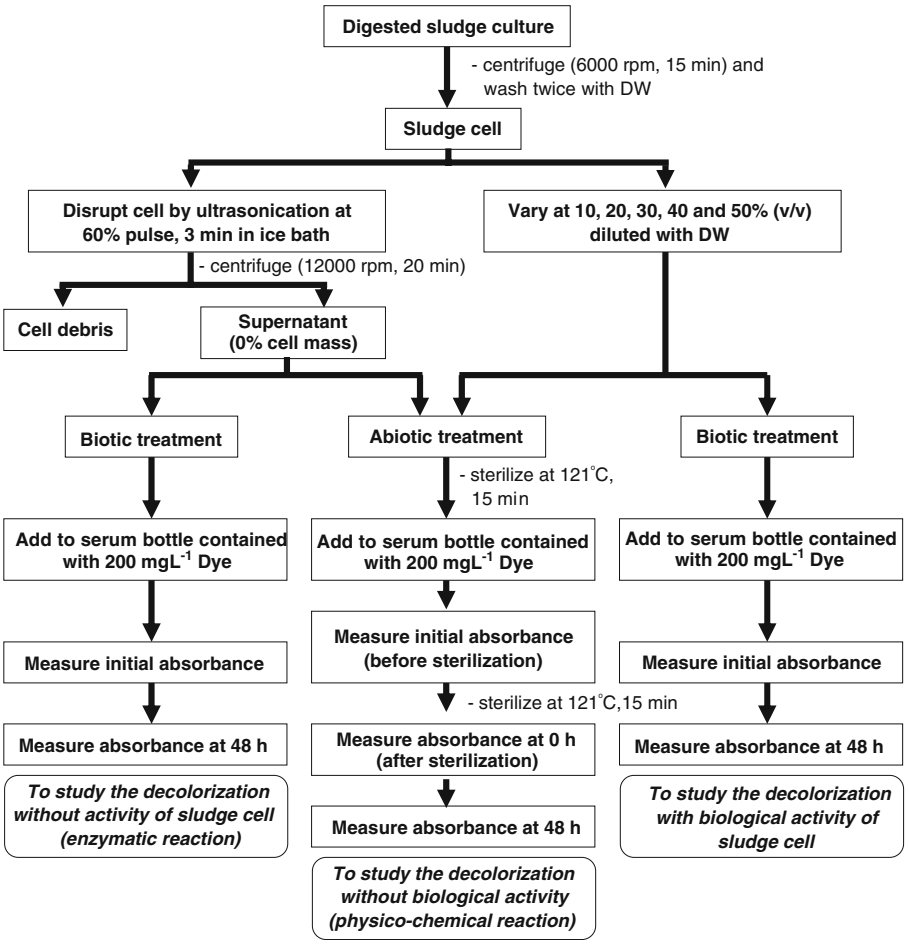

Fig. 2 The flowchart of experimental steps in abiotic and biotic decolorizations

Effect of Redox Mediator on Abiotic Decolorization

The effect of redox mediators on abiotic dye decolorization was studied by two experiments. The first experiment was conducted by using DW as the control and DW supplemented with glucose as organic material and/or redox mediator, 0.2 Mm AQDS or ATQ as treatment. The sterilized sludge cells, which were washed twice with DW before use, were used instead of DW in control and treatment bottles. The experiment was conducted by using 10% (*v*/*v*) of sludge cells sterilized at 121 °C for 15 min and mixed with glucose and/or redox mediator incubated under mesophilic and thermophilic anaerobic conditions. The dye concentration used in experiment was 200 mg L^{-1} and decolorizing efficiency was measured at 0 and 48 h of incubation.

Effect of Redox Mediators on Biotic Decolorization

The biological dye decolorization was conducted in 70-ml serum bottles containing 1,300 mg L^{-1} SS digested sludge, preincubated overnight without supplements, sealed with

rubber, and covered with aluminum caps and purged with Ar gas. Dye solution was added at concentrations of 50 and 200 mg L^{-1} for RB4 and MO, respectively, supplemented with 1 g L^{-1} of glucose as cosubstrate and 0.2 mM of AQDS or ATQ as redox mediator. Initial pH was adjusted to 7.5 before treatment and samples were then incubated under mesophilic and thermophilic anaerobic conditions.

Effect of Redox Mediator Concentration on Biotic Decolorization

Redox mediators, AQDS and ATQ, were added at concentrations of 0.2, 0.6, and 1.0 mM to study the effect of redox mediator on dye decolorization. Decolorizing conditions, sludge cultivation, and dyes were as described above.

Analysis

Dye reduction was determined photometrically by spectrophotometer (Hitachi U-2001). The appropriate dilution was made in distilled water. The absorbance was read at the maximum absorbance wavelength of 598 nm. The decolorizing efficiency was defined as a percentage of differentiation of initial (at 0 h) and final absorbance calculated as:

$$\text{Decolorization}(\%) = \frac{(\text{initial absorbance} - \text{final absorbance})}{\text{initial absorbance}} \times 100$$

Results

Abiotic and Biotic Decolorizations

Table 1 shows the comparison of decolorizing efficiency of model dyes with abiotic and biotic treatment under mesophilic and thermophilic conditions. The results indicate that RB4 decolorization in abiotic and biotic treatments occurred through dye precipitation and dye absorption onto cell mass, with no difference between decolorizing efficiency in any

Table 1 Decolorizing efficiencies of abiotic and biotic treatments of RB4 and MO with different masses of sterilized sludge cells under mesophilic and thermophilic conditions, measured at the 48th hour of the experiment.

	DW (control)	Cell mass (% *v*/*v*)					
		0	10	20	30	40	50
Reactive Blue 4							
Abiotic (mesophilic)	6.42	7.40	13.2	12.0	35.7	49.5	48.6
Biotic (mesophilic)		9.88	18.9	23.8	30.6	35.2	41.5
Abiotic (thermophilic)	7.78	9.50	9.47	11.6	36.9	48.4	49.8
Biotic (thermophilic)		10.4	14.6	29.9	29.1	44.4	38.3
Methyl Orange							
Abiotic (mesophilic)	0	95.9	93.1	93.1	91.2	97.8	97.3
Biotic (mesophilic)		2.83	91.9	91.9	90.8	88.2	87.1
Abiotic (thermophilic)	0	96.8	93.8	93.8	94.0	97.8	96.4
Biotic (thermophilic)		3.35	92.5	92.5	88.3	85.7	83.4

treatment in which the same concentration of cell mass was used. The precipitation of dye also occurred in the control (DW) and in the treatment using 0% (*v*/*v*) cell mass. In addition, the absorption of RB4 dye on cell mass was confirmed by the increase in decolorizing efficiency with increase of cell mass concentration. On the other hand, the decolorization of MO in both abiotic and biotic treatments did not occur in the control (DW), which indicated that MO was not precipitated as RB4. However, in the abiotic treatment of MO at all concentrations of cell mass 0–50% (*v*/*v*), the decolorization was more than 90%. This indicated that in the presence of organic matter such as protein from dead cell treated under sterilization, the MO could be decolorized by the reaction between dye and organic mater. The biotic treatment also showed high decolorizing efficiency with cell mass over 20% (*v*/*v*), which confirmed that the decolorization of MO was occurred by the reaction between dye and organic matter contained in treatment and also easy to decolorize compared with RB4 dye. Furthermore, MO did not show the absorption on cell mass which monitor from the color of cell mass after centrifugation.

Effect of Redox Mediator on Abiotic Dye Decolorization

Table 2 shows the results of the effect of redox mediators (AQDS and ATQ) on abiotic dye decolorization without sludge cells or supernatant. Redox mediator did not cause any reduction of dye in any treatments which are without glucose addition. For RB4 decolorization, the decolorization did not occur even when glucose was added together with redox mediator. On the other hand, MO was decolorized in the presence of glucose as organic matter in the high temperature treatment (121 °C during sterilization); 30% decolorization was occurred. And, in the absence of organic matter, the decolorization of MO did not increase even when redox mediator was added. With the presence of glucose and glucose + redox mediator in RB4 and MO decolorization, the decolorizing efficiency was increased in all treatment especially in MO decolorization. This indicated that the presence of glucose accelerate the decolorization more than redox mediator; furthermore, the decolorization could be improved by the supplementation of cosubstrate together with redox mediator.

Table 3 showed the result of treatment contained with sterilized cell mass instead of DW, which confirmed that the presence of only redox mediators did not enhance the decolorization. In contrast, treatment added with glucose + AQDS caused higher decolorization than treatments with glucose + ATQ and glucose only, respectively. This indicates that AQDS could be used as a redox mediator to enhance or accelerate the

Table 2 Effect of redox mediators on abiotic decolorization of RB4 and MO as percentage decolorization (%) under mesophilic and thermophilic conditions, measured at the 48th hour of the experiment.

	DW+dye (control)	DW + dye supplemented with				
		Glucose	AQDS	ATQ	Glucose + AQDS	Glucose + ATQ
Reactive Blue 4						
Mesophilic	6.35	7.03	6.75	4.76	9.38	4.38
Thermophilic	6.72	2.63	8.17	5.84	6.25	6.27
Methyl Orange						
Mesophilic	0	27.1	0	0	93.9	25.8
Thermophilic	0	38.1	0	0	97.0	35.8

Table 3 Effect of redox mediators on abiotic decolorization of RB4 and MO as percentage decolorization (%) with sterilized cell mass under mesophilic and thermophilic conditions, measured at the 48th hour of the experiment.

	10% (*v*/*v*) sterilized cell mass + dye (control)	10% (*v*/*v*) sterilized cell mass + dye supplemented with				
		Glucose	AQDS	ATQ	Glucose + AQDS	Glucose + ATQ
Reactive Blue 4						
Mesophilic condition (35°C)						
0 h	5.42	8.37	3.41	3.44	16.1	9.80
48th hour	10.8	17.2	8.78	11.3	20.5	15.7
Thermophilic condition (55°C)						
0 h	4.43	8.65	2.49	3.00	16.43	8.33
48th hour	9.85	19.7	8.46	1.50	6.28	6.86
Methyl Orange						
Mesophilic condition (35°C)						
0 h	38.3	70.0	30.5	32.5	98.0	75.2
48th hour	34.9	78.2	39.5	36.0	97.7	81.8
Thermophilic condition (55°C)						
0 h	33.4	65.7	35.2	37.7	98.2	76.7
48th hour	36.6	75.7	45.5	45.4	93.2	89.0

decolorization in both dyes. However, RB4 decolorization also needed the biological activity of microorganisms.

Effect of Redox Mediators on Biotic Dye Decolorization

Figure 3 shows the acceleration produced by redox mediators on RB4 decolorization under mesophilic and thermophilic conditions. Under both treatment temperatures, ATQ showed higher accelerating rate of decolorization than AQDS with clear acceleration under thermophilic conditions. However, under thermophilic conditions, AQDS caused lower decolorization than the control but still gave higher than treatments under mesophilic conditions. In addition, the results also showed that the increasing of treatment temperature was another factor in RB4 decolorizing acceleration. Under thermophilic condition, the decolorizing rate was higher than under mesophilic condition in which RB4 was completely

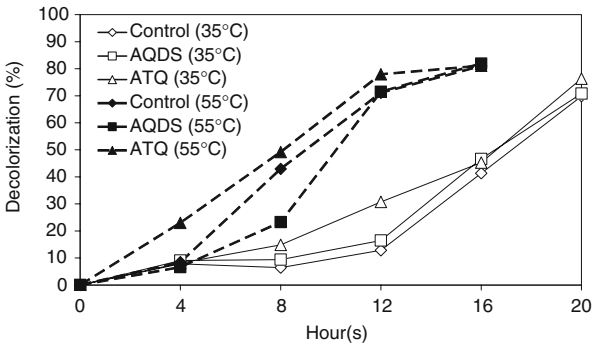

Fig. 3 Decolorizing efficiency of RB4 biotic decolorization using AQDS and ATQ as redox mediator under mesophilic (*blank symbol*) and thermophilic conditions (*solid symbol*)

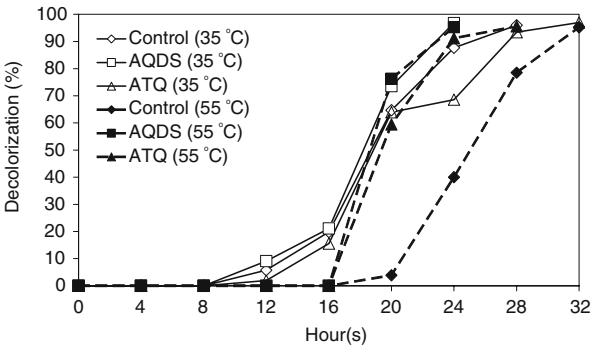

Fig. 4 Decolorizing efficiency of MO biotic decolorization using AQDS and ATQ as redox mediator under mesophilic (*blank symbol*) and thermophilic conditions (*solid symbol*)

decolorized within 16 h while the decolorization under mesophilic was completed with 20 h and showed lower decolorization at final time of experiment.

In contrast with MO decolorization, under thermophilic conditions, there was no decolorizing acceleration as shown in Fig. 4, while the presence of redox mediators clearly accelerated the rate of decolorization. Under mesophilic conditions, the presence of redox mediators showed no difference compared with the control (without redox mediator). These results indicate the differentiation of abiotic and biotic decolorization of both dyes. For RB4 decolorization, the presence of redox mediator could accelerate the decolorizing rate. But the important factors involved in decolorization of this dye are the biological activity of dye-reducing bacteria, the presence of co-substrate (glucose), and treatment temperature. Without the activity of dye-reducing bacteria, decolorization occurred by dye absorption as surface adsorption. However, for MO decolorization, the presence of redox mediator could enhance the rate of decolorization especially when treatment is conducted under high temperature. In addition, the presence of organic matter, both in abiotic and biotic treatments, was an important factor in dye decolorization. In Figs. 3 and 4, even all treatments showed the similar decolorizing efficiency at the end of experiment, but the rate of decolorization was difference. These mean that the decolorization supplemented with redox mediator in continuous treatment will give higher potential than without redox mediator.

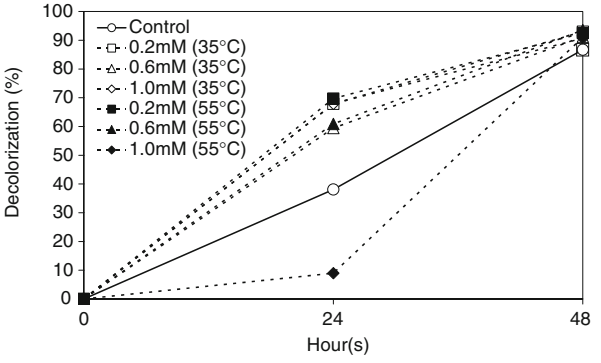

Fig. 5 Decolorizing efficiency of RB4 decolorizations with different AQDS concentrations under mesophilic (*blank symbol*) and thermophilic conditions (*solid symbol*)

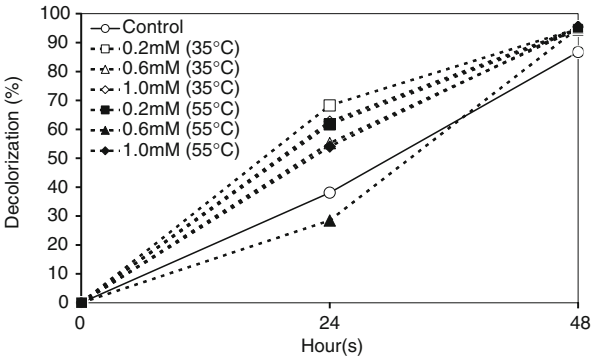

Fig. 6 Decolorizing efficiency of RB4 decolorizations with different ATQ concentrations under mesophilic (*blank symbol*) and thermophilic conditions (*solid symbol*)

Effect of Concentration of Redox Mediators on Biotic Dye Decolorization

The experiments with different concentrations of redox mediator were optimized to reveal the optimum dose for decolorization. Results shown in Figs. 5 and 6 indicate that the redox mediators AQDS and ATQ could accelerate the rate of RB4 decolorization at the initial time of decolorization. The addition of redox mediator could increase the reducing rate by about twofold compared with the control. In addition, the decolorizing efficiency could be increased from 80% to 95% at all doses of redox mediators. Increasing the concentration of redox mediator did not affect acceleration but under thermophilic conditions with 1.0 mM redox mediator, acceleration was inhibited in the initial period of RB4 decolorization. For MO, the presence of redox mediator caused similar acceleration to RB4 decolorization, but the decolorizing efficiency was no different from the control (Figs. 7 and 8). From the results, using redox mediator did not show effective on MO decolorization as the results showed in RB4 decolorization.

Discussion

In this study, the factors involved in the decolorizing process of two kinds of dye viz., azo type represented by MO and anthraquinone type represented by RB4 were investigated in batch experiments under mesophilic (35 °C) and thermophilic (55 °C) conditions. With the

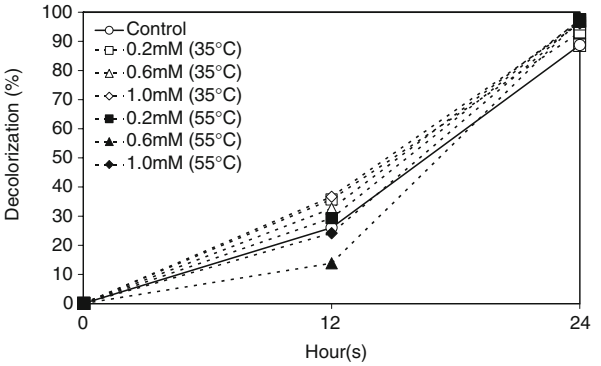

Fig. 7 Decolorizing efficiency of MO decolorizations with different AQDS concentrations under mesophilic (*blank symbol*) and thermophilic conditions (*solid symbol*)

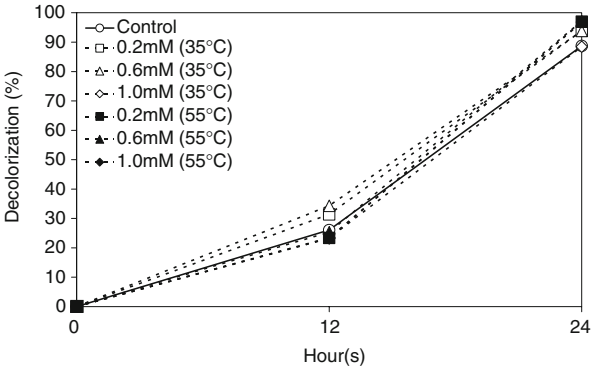

Fig. 8 Decolorizing efficiency of MO decolorizations with different ATQ concentrations under mesophilic (*blank symbol*) and thermophilic conditions (*solid symbol*)

different chromophore structures of azo dye (N=N) and anthraquinone dye (C=O), different results were obtained. For MO, decolorization could occur without biological activity, with only the physicochemical reaction between organic matter from cell debris or glucose as cosubstrate and dye under high temperature during the sterilization process being required. This could occur as some organic matter from cell extracts could act as electron donors to react with the dye. In addition, the addition of glucose increased decolorization. Kudlich et al. [5] have reported an increase in color removal rate of sulfonated azo dyes by free-cell extract due to azo reductase activity, which has generally been related to soluble cytoplasmic enzymes. This was based mainly on the observation that (i) cell extracts of various bacteria show azo reductase activity and (ii) cell extracts show higher azo reductase activity than intact cells do. On the other hand, RB4 did not show the same phenomena as in MO decolorization. The decolorization of RB4 occurred by absorption of dye onto the cell mass and increased with the increase in cell mass [5].

Redox mediators are known to mediate the reductive transfer of dye by decolorizing microorganisms [10]. The results obtained from this study showed that the presence of only redox mediator in treatment without organic matter such as sludge cells or glucose did not accelerate decolorization in either model dye. However, in the presence of organic matter, especially glucose, the addition of redox mediator increased decolorization especially of the azo dye. The acceleration by redox mediator was clearly seen when there was biological activity of microorganisms in the biotic treatment. Furthermore, at high concentrations of redox mediator, there was an inhibitory effect on decolorization under thermophilic conditions. This high concentration of redox mediation caused an inhibitory effect on acetate and propionate conversion and methanogenesis. The enzymatic generation of

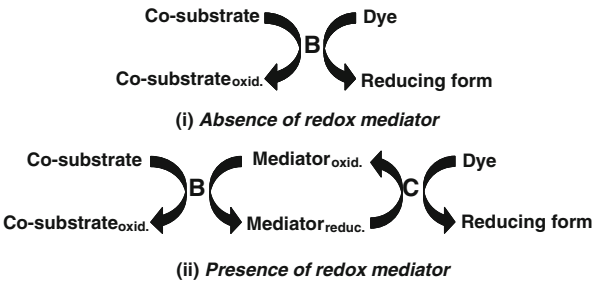

Fig. 9 Scheme of the biological and chemical steps involved in dye decolorization in absence (*i*) and presence (*ii*) of redox mediator. *RM*: redox mediator, *B*: biological enzymatic reaction and *C*: chemical reaction

hydroquinone was probably the rate-limiting step [10], and there are only a few microorganisms that can couple the conversion of acetate or propionate to quinone reduction under thermophilic or hyperthermophilic conditions [11]. This indicates that at low concentrations redox mediation may be added to accelerate the rate of decolorization. A schematic of biological and chemical steps involved in dye decolorization in the absence and presence of redox mediator is shown in Fig. 9. The mechanism of dye decolorization with redox mediator involved the organic matter or bacteria reducing the quinone group in AQDS and ATQ to hydroquinone and then hydroquinone reduced the dye structure to an aromatic compound.

The results also showed that temperature was an important factor in decolorization. Increasing treatment temperature during sterilization (121 °C) or treatment under thermophilic conditions (55 °C) accelerated the decolorizing rate. There was a suggestion that under thermophilic conditions, there was fast initial generation of reducing compounds via electron donor conversion, which was verified by comparing the k values. In addition, the more negative redox potential values obtained at 55 °C, compared to 30 °C, confirmed a better reduction capacity at 55 °C [10].

Conclusions

Microbial decolorization of two textile dyes (MO and RB4) showed the difference mechanism of decolorization in which MO could be decolorized under abiotic treatment with the reaction of organic matter from cell lysis and glucose, especially when treated under high temperature. RB4 decolorization occurred only by absorption onto cell mass and/or precipitation. In decolorizing reaction, biological activity was an important factor for dye decolorization and was needed for the cosubstrate conversion and induced the electron transfer between cosubstrate (electron donor) and dye (electron acceptor). Moreover, the addition of redox mediator in treatment could accelerate the decolorizing rate in both of abiotic and biotic treatments. And, treatment temperature was another important factor in decolorization. The increasing of treatment temperature resulted in a high rate of decolorization. In spite of the high rate and extent of decolorization observed in the batch assay conducted in present study, the need of glucose as cosubstrate inputted in treatment of thermophilic condition is lower than in mesophilic condition which could decolorize the high concentration of dye.

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