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Microbial decolorization of reactive azo dyes in a sequential anaerobic–aerobic system

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Abstract

A sequential anaerobic–aerobic treatment process based on mixed culture of bacteria isolated from textile dye effluent-contaminated soil was used to degrade reactive azo dyes Remazol Brilliant Orange 3R. Remazol Black B and Remazol Brilliant Violet 5R. Treating synthetic dye wastewater with the combination anaerobic and aerobic process showed that the majority of colors were removed by the anaerobic process, on the other hand the majority of chemical oxygen demand (COD) was removed in the subsequent aerobic process. Samples from combined anaerobic–aerobic system at the beginning of anaerobic process, after anaerobic process and after subsequent aerobic process were analyzed by high performance liquid chromatography (HPLC). The results suggested that under anaerobic conditions, the azo dyes were reduced and the aromatic amines were generated by the bacterial biomass. After re-aeration of the synthetic dye wastewater, these amines were further degraded by the same isolates. Thus, total degradation of reactive azo dyes was achieved by using an anaerobic–aerobic treatment.
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Keywords: Reactive azo dyes; Mixed culture; Anaerobic–aerobic treatment; Aromatic amines

1. Introduction

Over 100,000 commercially available dyes exist and more than 7×10^5 metric tonnes of dyestuff are produced worldwide annually [1]. In textile and paper coloration industries synthetic dyes from residual dyebaths are released to waste streams. It is estimated that up to 50% of the applied dye, depending on the type, can be lost in effluents during textile dyeing processes [2]. Azo dyes, characterized by nitrogen to nitrogen double bonds (–N=N–), account for up to 70% of all textile dyestuffs produced, and are the most common chromophore in reactive dyes [3]. Reactive azo dyes are very soluble by design and, as a result, not all are exhausted by textile fibers during the dyeing process and therefore end up in the discharge from dyehouses. The reactive azo dyes-containing effluents from these industries have caused serious environment pollution because the presence of dyes in water is highly visible and affects their transparency and aesthetics even if the concentration of the dyes is low. Therefore, industrial effluents containing dyes must be treated before their discharge into the environment [4]. Many physical and chemical methods including adsorption, coagulation, precipitation, filtration, and oxidation have been used for the treatment of azo dye-contaminated effluents [4]. These methods, however, may generate a significant amount of sludge or may easily cause secondary pollution due to excessive chemical usage. Moreover, their municipal treatment costs are high. Therefore, it may be economical to develop alternative means of dye decolorization, such as bioremediation due to its reputation as on environmentally friendly and publicly acceptable treatment technology [4].

Reactive azo dyes are recalcitrant to microbial degradation because they have complex aromatic molecular structures and the strong electron-withdrawing property of the azo groups is thought to protect against attack by oxygenases so that the conventional aerobic wastewater treatment processes usually cannot efficiently decolorize azo dye-contaminated effluents [5]. The azo dyes, however, are reduced and hence, decolorized when acting as electron acceptors for the microbial electron transport chain, so a source of labile carbon is required [6]. Azo dyes

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are reduced under anaerobic conditions to the corresponding aromatic amines [7,8], which though resisting further anaerobic degradation, are reported to be well amenable for aerobic degradation [7]. Aromatic amines can be mineralized by means of aerobic treatment by non-specific enzymes through hydroxylation and ring-fission of aromatic compounds [6,9]. It has been suggested to combine the anaerobic cleavage of the azo dyes with an aerobic treatment system for the amines formed. The feasibility of this strategy was first demonstrated for the sulfonated azo dye Mordant Yellow 3 [7]. During the last few years, several laboratory-scale continuous anaerobic–aerobic processes for the treatments of wastewater containing azo dyes have been described [10–12]. Field et al. showed that the aerobic stage of combined anaerobic–aerobic treatment of dye wastes eliminated the additional chemical oxygen demand (COD), attributed to removal of aromatic amines, which are anaerobically recalcitrant [13].

Efficiency fluctuation in color removal by using anaerobic–aerobic process, however, was observed in an industrial facility for treatment of dyeing house effluent [14]. If azo dyes are not reduced and cleaved in anaerobic stage, they most probably leave the aerobic stage intact. Dye composition might be an important factor causing unstable decolorization because the textile effluent containing a wide range of structurally diverse dyes [4]. As would be expected, the degree of decolorization depends on the type of the dye, molecular weight, and substitution groups of the dye molecules—azo compounds with an hydroxyl or amino group being more likely to be degraded than those with methyl, methoxy, sulfo or nitro groups [5,15]. Further research is needed to establish the relationships between dye molecule structure and bacterial decolorization.

In order to develop an efficient decolorization process and to solve the problem of unstable decolorization performance, knowledge regarding the mechanism of bacterial azo reduction needs to be well identified. In this study, an anaerobic–aerobic sequencing batch system using a mixed bacterial culture was investigated for its performance with sulfonated monoazo, diazo and metal-containing reactive azo dyes. We also analyzed the degradation products of azo reduction (decolorization) of all dyes by mixed bacterial culture.

2. Experimental materials and methods

2.1. Chemicals

Commercially important and commonly used reactive azo dyes for cotton dyeing Remazol Brilliant Orange 3R (C.I. reactive orange 16, Fig. 1A), Remazol Black B (C.I. reactive black 5, Fig. 1B) and Remazol Brilliant Violet 5R (C.I. reactive violet 5, Fig. 1C) were obtained from Dystar Thai Ltd., Bangkok, Thailand. The dyes were used at a quality identical to that being used in the textile industry. Dye stock solutions were prepared and used in all experiments.

2.2. Synthetic wastewater

The basic composition of synthetic dye wastewater was ($g\,l^{-1}$): reactive azo dye 0.1, soluble starch 1.0, acetic acid 0.15, $(NH_4)_2SO_4$ 0.28, NH_4Cl 0.23, KH_2PO_4 0.067, $MgSO_4{\cdot}7H_2O$ 0.04, $CaCl_2{\cdot}2H_2O$ 0.022, $FeCl_3{\cdot}6H_2O$ 0.005, yeast extract 0.2, NaCl 0.15, $NaHCO_3$ 1.0, and $1\,ml\,l^{-1}$ of a trace element solution containing ($g\,l^{-1}$) $ZnSO_4{\cdot}7H_2O$ 0.01, $MnCl_2{\cdot}4H_2O$ 0.1, $CuSO_4{\cdot}5H_2O$ 0.392, $CoCl_2{\cdot}6H_2O$ 0.248, $NaB_4O_7{\cdot}10H_2O$ 0.177, and $NiCl_2{\cdot}6H_2O$ 0.02.

2.3. Bacterial isolation and cultivation

Wastewater and activated sludge samples were collected from the wastewater treatment facility of a local dyeing house and cultivated in a synthetic wastewater under static

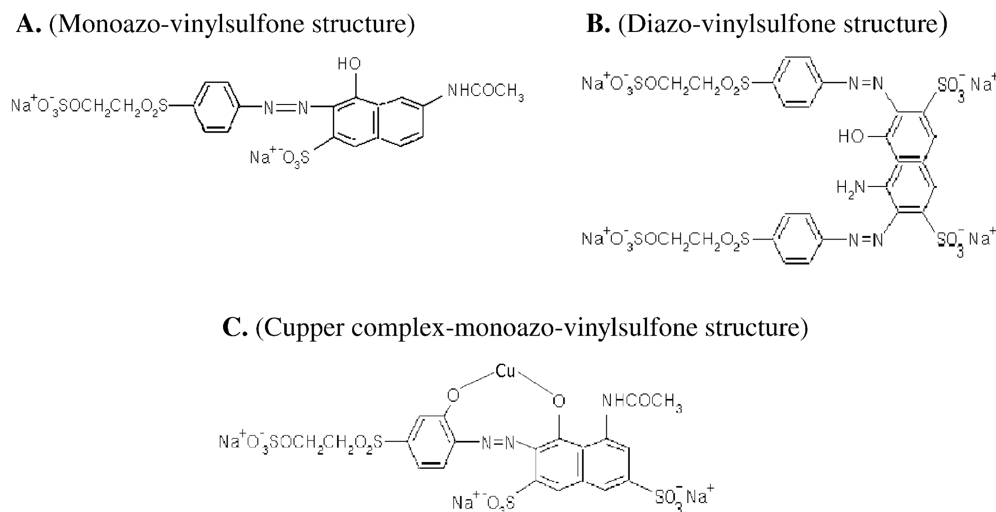


Fig. 1. Chemical structures of: (A) Remazol Brilliant Orange 3R; (B) Remazol Black B; and (C) Remazol Brilliant Violet 5R.

conditions. Three azo dyes were used as the indicators of microbial decolorization activity. A mixed culture that showed quick and stable decolorization activity was transferred to newly prepared synthetic wastewater. After five success transfers, it was plated on synthetic wastewater agar containing 100 mg l^{-1} of each dye. The plate was incubated at 30 °C in an anaerobic jar with Gaspak (CO_2–N_2–H_2, 80:15:5) sachets (Oxoid Ltd., Basingstoke, UK). Bacterial colonies around which clear zones expanded quickly were collected for further physiological identification. The single strains transferred from storage slants were cultivated in 500 ml flasks containing 100 ml of synthetic wastewater without dyes at 30 °C on a rotary shaker at 200 rpm. After 24 h of cultivation, the cells mass were harvested by centrifugation (10,000 rpm, 10 min) and resuspended in 0.85% NaCl solution. Equal volumes of each bacterial suspension were mixed together to make a defined mixed bacterial culture used as an inoculum in anaerobic–aerobic decolorization.

2.4. Anaerobic–aerobic batch decolorization operations

A mixed bacterial culture was aseptically transferred into dye-containing synthetic wastewaters to reach an initial dry weight cell concentration of 0.5–1.0 g l^{-1} for color removal experiments. The anaerobic batch decolorization experiments were performed in serum bottles (160 ml total volume), sealed with rubber septa, containing 150 ml synthetic wastewater under static-incubation conditions and incubated at 30 °C. At the end of the anaerobic phase (24 h), each incubated synthetic dye wastewater was aseptically transferred into 500 ml Erlenmeyer flasks. Then the flasks were incubated at 30 °C with rotary shaking at 200 rpm. The dye concentrations, COD values and decolorized by-products were monitored as a function of time during the batch decolorization runs. The assays were performed in duplicate.

2.5. Analytical methods

Color measurements in clarified samples from synthetic wastewaters were performed in a Shimadzu UV-160A spectrophotometer in the UV-Visible range against a baseline defined by clarified samples from dye-free synthetic wastewater. Calibration graphs of absorbance versus dye concentration were constructed from solutions of each azo dye in synthetic wastewater for the calculation of the individual dye concentrations. Samples were withdrawn from the serum bottles with a hypodermic needle and syringe. The samples were centrifuged at 8,000 rpm for 10 min and the absorbance values of supernatants were determined. Absorbance of the samples was measured at the maximum absorption wavelength (λ_{max}) in the visible region for each dye ($\lambda_{max} = 492$ nm for Remazol Brilliant Orange 3R, $\lambda_{max} = 595$ nm for Remazol Black B, and $\lambda_{max} = 557$ nm for Remazol Brilliant Violet 5R). COD was measured according to a standard procedure [16]. Decolorization metabolites formed during anaerobic and aerobic incubation were determined by reversed phase HPLC.

2.6. HPLC analysis of decolorization metabolites

The anaerobic and aerobic metabolites of three reactive azo dyes were extracted with equal volume of ethyl acetate after acidification to pH 2–3 with 6 N HCl. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary evaporator. The residue was dissolved in small volume of methanol. HPLC analysis was carried out on a Shimadzu model LC-3A chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with Shimadzu model SPD-2A detector and Pegasil ODS, (column with 4.6 mm × 150 mm inside diameter, Senshu Scientific Co. Ltd., Tokyo, Japan). A mobile phase composed of 50% methanol, 0.3% H_3PO_4, and 49.7% water was used with the flow rate of 0.5 ml min^{-1}. The eluates were monitored by UV absorption at 275 nm.

3. Results and discussions

3.1. Isolation of bacterial cultures

Few bacterial cultures were isolated from the prolonged anaerobic enrichment cultures in synthetic wastewater containing three reactive azo dyes. Three strains (A5, S1, and A6) were isolated as the most active azo dye-decolorizing bacteria. Identification of the bacterial strain was performed based on the methods of in Bergey's Manual of Systematic Bacteriology [17], using standard microbiological procedures. Strain A5 and S1 were gram-positive but gram-variable in old culture, rod shaped, endospore forming, aerobic or facultative anaerobic, catalase-positive, oxidase-positive and could reduce nitrate. These bacteria were assigned to the genus *Paenibacillus* based on biochemical and physiological properties and 16S rDNA analysis data [18]. However, these strains were different colony appearance and some physiological reactions. Strain A6 was gram-negative, rod shaped, aerobic or facultative anaerobic, catalase-positive, oxidase-positive, nitrate reduction and form acid from glucose by oxidation. These properties indicated that the strain was a member of the genus *Pseudomonas*.

3.2. Remazol Brilliant Orange 3R

The results obtained for the treatment of Remazol Brilliant Orange 3R are shown in Fig. 2 and Fig. 3. From Fig. 2, it can be seen that the color was completely removed in the anaerobic phase. A rapid decrease in dye concentration during first 10 min of incubation occurred as a result of abiotic decolorization. This resulted in an actual initial dye concentration of approximately 80–85 mg l^{-1} for bacterial decolorization. The majority of COD was removed in the aerobic phase (68.2% as opposed to 24.5% in the anaerobic phase).

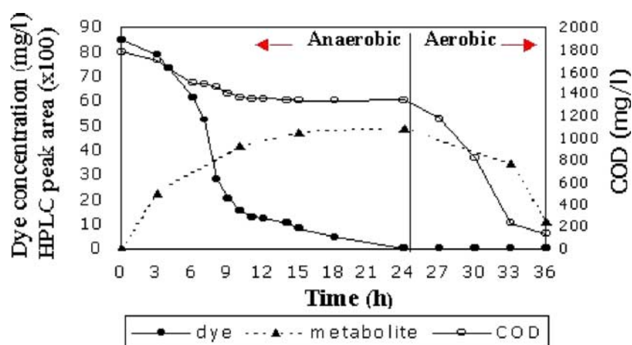


Fig. 2. Time-course profile of Remazol Brilliant Orange 3R, COD, and decolorized metabolite (retention time; 5.3 min) of synthetic wastewater under anaerobic and aerobic conditions.

The major COD in synthetic wastewater are due to soluble starch, acetic acid and yeast extract, all of which are known as anaerobically and aerobically biodegradable. In this study, bacteria aerobically oxidized most of the energy source available in synthetic wastewater for cellular growth and maintenance resulting in rapid COD reduction in the aerobic phase. The HPLC analysis performed to supernatants taken during the different duration of the anaerobic and aerobic phases showed the correspondence of the evolution of decolorization metabolite to the color removal. The chromatographic peak areas corresponding to the dye-degradation metabolite (retention time; 5.3 min) increased as the incubation time of anaerobic phase increased. The UV-absorbing area of the metabolite formed in anaerobic phase significantly decreased in the subsequent aerobic phase.

UV-Visible spectra (data not shown) obtained for the filtered samples showed marked alterations with reaction time. These changes could be explained by structural modifications of dye molecule due to azo bond reduction as under anaerobic conditions many types of bacteria perform this reaction [19], producing metabolites such as aromatic amines. In order to clarify this phenomenon, the ethyl acetate extracts taken during anaerobic and aerobic phases of decolorization operations of Remazol Brilliant Orange 3R were analyzed by HPLC.

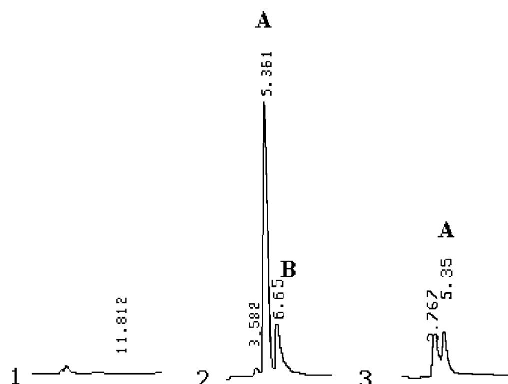


Fig. 3. HPLC analysis on metabolites resulting from decolorization of Remazol Brilliant Orange 3R under anaerobic–aerobic conditions: (1) at the beginning of the anaerobic incubation; (2) after anaerobic incubation for 24 h; and (3) after aerobic incubation for 12 h.

Fig. 3 shows the result of HPLC analysis on the metabolites of Remazol Brilliant Orange 3R decolorization by the mixed bacterial culture. At the beginning of the anaerobic incubation, the parent compound was not detected under these chromatographic conditions. After anaerobic incubation for 24 h, the intensity of peaks A and B (retention time; ca. 5.3 and 6.6 min, respectively) increased significantly. As the decolorization proceeded (Fig. 2), the area of peak A increased along with a decrease in the concentration of Remazol Brilliant Orange 3R. At this point, It is thus reasonable to assume that the mixed culture caused cleavage of the azo bond of Remazol Brilliant Orange 3R, which decomposed to form two aromatic amines represented by peaks A and B, respectively. It should be noted that due to the unavailability of authentic standards, the chromatographic peaks appearing in samples taken during the anaerobic phase could not be identified or quantified. Recently, it has suggested that both partial reduction and complete cleavage of the azo bond could contribute to decolorization of reactive red 22 by *Pseudomonas luteola* [20]. We also chemically reduced Remazol Brilliant Orange 3R by sodium by sodium dithionite to achieve complete cleavage of the azo bond [21]. The HPLC chromatogram of the chemically reduced products (data not shown) shows the same retention times of both peak A and B (5.3 and 6.6 min, respectively), which found in chromatogram of bacterial reduction. Therefore, it is reasonable to consider that peak A and B contain the aromatic amines originating from total reduction of the orange azo dye.

At the end of aerobic phase, the HPLC analyzes seem to indicate that the decolorization metabolites produced during the anaerobic phase were removed in the subsequent aerobic phase. Fig. 3.2 and 3.3 also shows that when such metabolites were metabolized aerobically they formed less aromatic, more polar compounds, since the metabolite peak area decreased and shifted towards a lower retention time (3.7 min). Degradation in the aerobic stage may result in the formation of oxidized and very polar derivatives (e.g., aldehydes, carboxylic acids) having a lower aromaticity, as suggested by Nortemann et al. [22] in a study of 6-aminonaphthalene-2-sulfonic acid degradation. No new UV absorbance was found in uninoculated controls nor inoculated synthetic wastewater without dye controls.

3.3. Remazol Black B

The typical profiles for Remazol Black B and COD removal and its decolorization metabolite evolution are represented in Fig. 4. Similar with what has been revealed for the decolorization of Remazol Brilliant Orange 3R, the majority of color was removed in the anaerobic phase and the majority of COD was removed in the aerobic phase. However, the decolorization rate of Remazol Black B was slower than the decolorization of Remazol Brilliant Orange 3R at the same incubation time. As shown in Fig. 4,

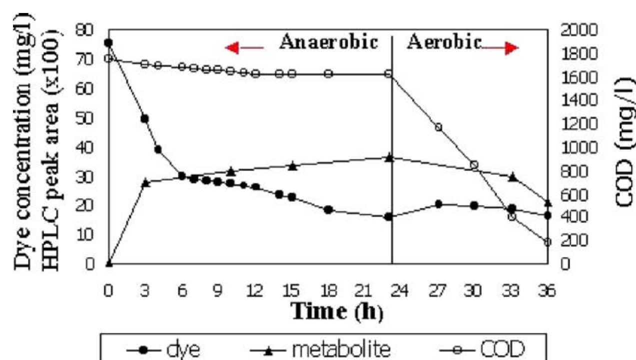
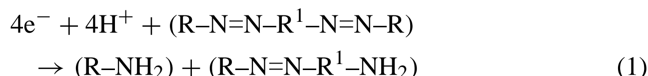


Fig. 4. Time-course profile of Remazol Black B, COD, and decolorized metabolite (retention time; 5.3 min) of synthetic wastewater under anaerobic and aerobic conditions.

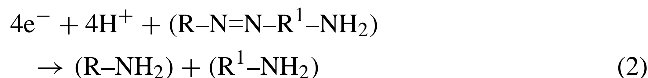
decolorization of Remazol Black B took place with anaerobic incubation and was repressed in aerobic culture. At the end of the anaerobic phase, 78.9% color and 14.8% COD removal efficiencies were obtained. The overall color removal was 78.2% and the COD removal was 90%. The studies concerning the treatability of Remazol Black B dye by bacteria are limited. Nigam et al. [23], achieved 67% color removal efficiency within 24 h by an anaerobic culture with an initial dye concentration of $500\,mg\,l^{-1}$ as a mixture of nine reactive dyes when yeast extract was used as carbon source. In a further investigation Oxspring et al. [24] developed an up-flow anaerobic filter operated under batch conditions for 96 h and 95% removal efficiency was obtained after 48 h of operation using an acclimated anaerobic consortium. Furthermore, in some previous batch studies the following decolorization efficiencies were obtained for Reactive Black B: 67 and 73% color removal in 10.5 and 79 h, respectively [25], 79% in 10 h [26] and 66% in 24 h [27].

From Fig. 4, the rate of decolorization of Remazol Black B was high for the early period of operation but it decreased with an increase in the operation time. The decolorization of Remazol Black B may be undertaken in two steps as follows: fast decolorization during which the concentration of this dye decreased rapidly within 6 h ($-(\mathrm{d}\ln[\text{dye}])/\mathrm{d}t \cong 0.154\,h^{-1}$, incubation time < 6 h) and slow decolorization during which concentration decreased to a smaller value for a longer period of time ($-(\mathrm{d}\ln[\text{dye}])/\mathrm{d}t \cong 0.083\,h^{-1}$, incubation time > 6 h). This two steps of decolorization of Remazol Black B have also been found by several researchers. Panswad and Luangdilok [28] reported that the decolorization rate of the black dye in an anaerobic–aerobic sequencing batch reactor (SBR) reactor was 11.9 space units (SU) h^{-1} in the first 2 h of the anaerobic stage and after that the decolorization rate dropped to 0.44 space units (SU) h^{-1}. In the study of Chen [29], the first order kinetic of decolorization of Remazol Black B was 0.413 and $0.0129\,h^{-1}$ within 2 and after 2 h of anaerobic incubation time, respectively.

A mechanism for Remazol Black B reduction was proposed by Chen [29] involving a two-stage reduction of the azo bond as given in reactions (1) and (2). One of decolorization products of reaction (1) still contains an azo bond in its molecule.



$$4e^- + 4H^+ + (R\text{–}N{=}N\text{–}R^1\text{–}N{=}N\text{–}R) \rightarrow (R\text{–}NH_2) + (R\text{–}N{=}N\text{–}R^1\text{–}NH_2) \quad (1)$$



$$4e^- + 4H^+ + (R\text{–}N{=}N\text{–}R^1\text{–}NH_2) \rightarrow (R\text{–}NH_2) + (R^1\text{–}NH_2) \quad (2)$$

where R and R^1 are variously substituted phenyl and naphthol residues.

This analysis also indicated that decolorizing-metabolites are likely to be more chemically stable than the parent compound, as metabolite ($R\text{–}N{=}N\text{–}R^{1-}NH_2$) is more persistent than Remazol Black B [30]. This two-step decolorization occurred due to the different reactivities of two azo-bonds existing in Remazol Black B. Such difference in azo-bond cleavage may be attributed to the reactivity difference between the of hydroxyl (–OH) and the amino ($-NH_2$) group which are the activating groups of aromatic electrophilic substitution, present on the naphthalene structure in Remazol Black B at the position *ortho* related to the azo bonds [29].

After change to aerobic phase, the initially clear cultured medium of the reduction products then turned quickly slightly deep blue. This behavior could be explained by the formation of autoxidation products formed from anaerobic reduction of parent azo dyes, which commonly occurred after exposed to air [31]. Moreover, Kudlich et al. has recently reported that disulfonated naphthalene derivative, 1,2,7-triamino-8-hydroxynaphthalene-3,6-disulfonate, which is formed during decolorization of Remazol Black B was rapidly oxidized even in the presence of trace amount of oxygen and could not be detected by HPLC [31]. The autoxidation of this compound led to the formation of rather stable products, 7-amino-8-hydroxy-1,2-naphthoquinone-3,6-disulfonate-1,2-diimine and dihydroxynaphthoquinone-3,6-disulfonatediimine, resulting to the appearance of deep blueish color.

Fig. 5 shows the result of HPLC analysis on the metabolites of Remazol Black B decolorization by the mixed bacterial culture. After anaerobic incubation for 24 h, the intensity of peaks A and B (retention time; ca. 5.3 and 6.6 min, respectively) increased significantly. It is thus reasonable to suggest that peaks A and B represent the decolorization metabolites confirming the formation of additional aromatic metabolites. The benzene-based amine is the common part in both Remazol Black B and Remazol Brilliant Orange 3R (Fig. 1A and B). Therefore, the reduction of Remazol Black B theoretically yielded benzene-based amine approximately two-times higher concentration than the reduction of Remazol Brilliant Orange 3R. To test this hypothesis, HPLC results from decolorization of Remazol Brilliant Orange 3R and Remazol Black B were compared. Based on the stoichiometric amount, the initial concentration ($100\,mg\,l^{-1}$) of Remazol Brilliant Orange 3R and Remazol Black B was

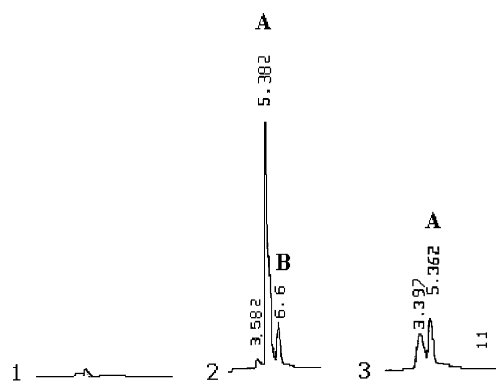


Fig. 5. HPLC analysis on metabolites resulting from decolorization of Remazol Black B under anaerobic–aerobic conditions: (1) at the beginning of the anaerobic incubation; (2) after anaerobic incubation for 24 h; and (3) after aerobic incubation for 12 h.

162 and 101 μM, respectively. In spite of the lower initial concentration of Remazol Black B, the area of peak A assumed as benzene-based amine formed from decolorization of the black dye, was significantly higher than the area of peak A obtained from decolorization of Remazol Brilliant Orange 3R at the third hour of anaerobic incubation. In the case of Remazol Black B, however, the area of peak A increased very slowly after 3 h of incubation, meanwhile, the same peak was observed in higher amount in the case of Remazol Brilliant Orange 3R decolorization. It is possible that two-stage reduction and incomplete cleavage of azo bonds caused the amount of benzene-based amines formed from Remazol Black B decolorization did not follow the theoretical stoichiometric amount.

Fig 5.2 and 5.3 also show that when such metabolites were metabolized aerobically, they formed less aromatic more polar compounds since the metabolite peak area decreased and shifted towards a lower retention time (3.3 min). There are several reports on the mineralization of sulfonated aminobenzenes and sulfonated aminonaphthalenes [22,32] by bacteria. Therefore, the decrease of peak area of decolorization metabolites may cause by the aerobic degradation processes of the bacterial isolates. Identification of metabolites of aerobic biodegradation should be the objective of further investigations in order to understand removal mechanisms of these metabolites under aerobic phase.

3.4. Remazol Brilliant Violet 5R

The results obtained for the treatment of Remazol Brilliant Violet 5R are shown in Fig. 6. The trend in terms of percentage removal of COD, dye and its decolorization metabolite are similar to that of Remazol Brilliant Orange 3R and Remazol Black B. Though Remazol Brilliant Violet 5R has a monoazo bond in complex with copper as its chromophore, this dye was decolorized completely within 24 h under anaerobic incubation. The COD removal were 17.8 and 74.1% in anaerobic and aerobic phase, respectively. The

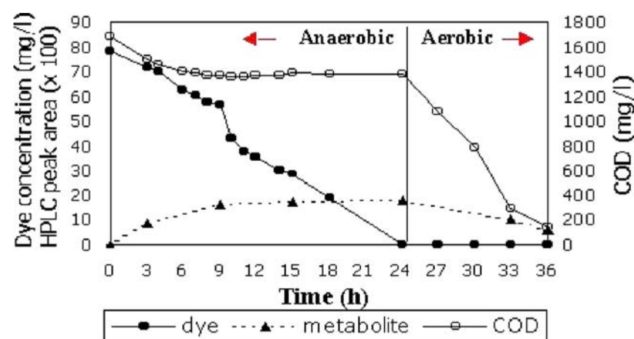


Fig. 6. Time-course profile of Remazol Brilliant Violet 5R, COD, and decolorized metabolite (retention time; 4.5 min) of synthetic wastewater under anaerobic and aerobic conditions.

studies concerning the treatability of Remazol Brilliant Violet 5R by bacteria are also limited. Lourenco et al. [33], achieved 90% violet dye removal efficiency within 24 h in the operation cycle of a SBR.

UV-Visible spectra (data not shown) obtained for the filtered samples showed marked alterations with reaction time. These changes could be explained by structural modifications of dye molecule due to azo-bond reduction. In order to clarify this phenomenon, the ethyl acetate extracts taken during the anaerobic and aerobic phases of decolorization operations of Remazol Brilliant Violet 5R were analyzed by HPLC.

The result of HPLC analysis on the metabolites of Remazol Brilliant Violet 5R decolorization by the mixed bacterial culture is shown in Fig. 7. After anaerobic incubation for 24 h, the area of appearing peak (retention time; ca. 4.5 min) increased significantly. It is thus reasonable to suggest that this peak represents the decolorization metabolite, confirming the formation of additional aromatic metabolites. The retention time of metabolite formed from decolorization of violet dye is different from the decolorizing-metabolites of orange and black dye. In this case, it may be due to the formation of the different decolorizing-metabolites resulting from decolorization of the different chemical structure of parent compounds.

It has been reported that decolorization of Remazol Brilliant Violet 5R can occur easily under anaerobic condi-

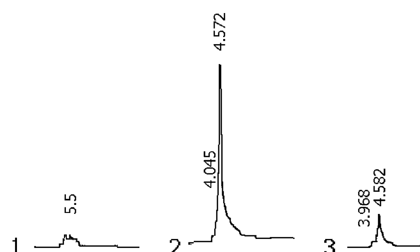


Fig. 7. HPLC analysis on metabolites resulting from decolorization of Remazol Brilliant Violet 5R under anaerobic–aerobic conditions: (1) at the beginning of the anaerobic incubation; (2) after anaerobic incubation for 24 h; and (3) after aerobic incubation for 12 h.

tion, however, certain of its decolorization metabolites are known to be resistant to degradation by aerobic bacteria [33,34]. Contrary to several reports, which report difficulties with the degradation of violet dye metabolites, the decolorizing-metabolites decreased aerobically, since the peak area of such metabolite decreased during the aerobic incubation as shown in this study.

4. Conclusions

Microbial decolorization of three reactive azo dyes in an anaerobic environment occurred as a result of reduction of azo bonds. This gave rise to the liberation of decolorization metabolites. No ready biodegradation of these metabolites was expected in the anaerobic system that gives rise to decolorization. However these metabolites could be further reduced by means of either biodegradation or autooxidation under subsequent aerobic treatment. As a characteristic of the textile-processing industry, a wide range of structurally diverse dyes is used within short periods in one and the same factory, and therefore, effluents from textile industry are extremely variable in composition. Results obtained from this work show that the mixed bacterial culture possesses high decolorization efficiency. The mixed bacterial cultures decolorized three structurally dissimilar azo dyes, suggesting that anaerobic decolorization was not a specific process. It was clear that the majority of the color removal occurred in the anaerobic stage. On the other hand, the anaerobic phase of decolorization operations gave low COD removal and most of the COD was removed in the subsequent aerobic phase, including the decolorized metabolites formed from anaerobic phases. It is possible that the mixed culture metabolized small amounts of COD under anaerobic conditions to the source of reduction equivalents (for example, NADH) to reduce the azo bonds present in reactive dyes. The color removal yield with the more complex diazo dye Remazol Black B was, however, much lower than that obtained with the monoazo dye Remazol Brilliant Orange 3R and Remazol Brilliant Violet 5R. The rate of decolorization of azo dyes is affected by their molecular weights, substitution groups of the dye molecules, and the intramolecular hydrogen bond between the azo and hydroxy groups. It is considered that the low decolorization of Remazol Black B is attributed to this factor. A longer retention time of anaerobic phase may be, therefore, required to enhance the rate of elimination of black dye. Hence, decolorization of Remazol Brilliant Orange 3R, Remazol Black B, and Remazol Brilliant Violet 5R by a mixed bacterial culture are most likely due to complete breakdown of the reactive azo dyes to form aromatic amines. Removal of azo dye metabolites after anaerobic reduction can be done in aerobic phases by the same mixed bacterial culture. However, further investigation should be clarified the removal mechanism of decolorization metabolites under aerobic phase.

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