Biodegradation of anthraquinone dyes by *Shewanella* sp. NTOU1 under anaerobic conditions

Wei-Chiung Chi, Chih-Hung Chen and Shiu-Mei Liu

**ABSTRACT**

*Shewanella* sp. NTOU1 was able to decolorize a range of anthraquinone dyes [Reactive Blue 4 (RB4), Reactive Blue 19 (RB19), Mordant Red 11 (MR11), Disperse Red 15 (DR15), and Disperse Blue 3 (DB3)] under anaerobic conditions. By supplementing the medium with formate and ferric citrate as the electron donor and acceptor, respectively and cultivating it under the optimum pH (8–9) and temperature (45°C), this strain could decolorize these dyes (1,000 mg/L) at the initial color removal rates of 15–126 mg/L/h and the rates among them were RB19 > RB4 > DB3 > DR15 > MR11. The extent of color removal was in the range of 90–98% for RB19, 86–96% for RB4, 39–41% for MR11, 69–82% for DR15, and 89–91% for DB3. Based on the decolorization products detected by means of GC/MS analyses, probable pathways for the decolorization of these dyes by this strain were proposed.

**Key words** | decolorization pathways, disperse blue 3, disperse red 15, mordant red 11, reactive blue 4, reactive blue 19,

**INTRODUCTION**

Anthraquinone dyes constitute the second largest class of reactive dyes after azo dyes and were extensively used in the textile industry not only to color cotton, but also wool and polyamine fibers due to their wide variety of color shades and ease of application (Baughman & Weber 1994; Aspland 1997). A steady increase in reactive dye usage has been observed as a result of the increased cotton use worldwide.

Recent estimates indicate that as much as 2–50% of the dyes used each year are lost to waste streams as a result of manufacturing and processing operations. About 20% of these losses enter the environment through the effluent from wastewater treatment facilities. These effluents have resulted in some of the worst industrial pollution (O’Neill et al. 1999). Therefore, decolorization and detoxification of wastewaters containing these dyes prior to discharge is mandatory by environmental regulations in most countries.

Because of their complex fused aromatic structure (Itoh et al. 1996), anthraquinone-based dyes are highly resistant to decolorization (Pierce 1994; Seshadri et al. 1994; Vandevivere et al. 1998; Beydilli et al. 2000; Pearce et al. 2003). Although several physical and chemical methods, e.g. adsorption, chemical precipitation and flocculation, oxidation by chlorine, hydrogen peroxide and ozone, electrolysis, reduction, electrochemical treatment, and ion-pair extraction have been used to eliminate the color from these wastewaters (Reife 1993; Lin & Chen 1997; Azmi et al. 1998; Lall et al. 2003), these methods are generally costly and not very efficient. Thus, a viable alternative, biological process has received increasing interest owing to its cost effectiveness and environmental benignity (Banat et al. 1996). However, anthraquinone dyes are toxic to microorganisms (Cooling et al. 1996; Malpei et al. 1998; Lee & Pavlostathis 2004; Dos Santos et al. 2005), and few microorganisms have been reported that have the ability to decolorize them (Itoh et al. 1996; Chen et al. 2003; Lee & Pavlostathis 2004; Xu et al. 2005). Therefore, much work remains to be done to isolate more...
bacteria that are applicable for the biodegradation of anthraquinone dyes.

In this work, the ability of a new isolate, *Shewanella* sp. NTOU1 was tested to decolorize a range of anthraquinone dyes. Effects of temperature, pH, concentration, and different electron acceptors and carbon sources on the decolorization of anthraquinone dyes were determined. Meanwhile, degradation products of anthraquinone dyes by this strain were determined with GC/MS. Probable pathways for the degradation of these dyes by this *Shewanella* sp. NTOU1 was also proposed.

**MATERIALS AND METHODS**

**Chemicals**

Reactive Blue 4 (RB4, C.I. 61205) was obtained from Fluka, Germany.

Reactive Blue 19 (RB19, C.I. 61200), Disperse Blue 3 (DB3, C.I. 61505), Mordant Red 11 (MR11, C.I. 58000), Disperse Red 15 (DR15, C.I. 60710) were purchased from Sigma-Aldrich, and were used without any further purification. All other chemicals used were of analytical grade.

**Microbial strain and culture media**

*Shewanella* sp. NTOU1 was originally isolated from a cooling system in an oil refinery. The strain is Gram-negative, rod-shaped, pink to orange-pigmented and motile with a single polar flagellum. This strain can grow under aerobic conditions. It was not able to ferment glucose, but it was capable of anaerobic growth utilizing a variety of electron acceptors, including Fe(III), Mn(IV), iron oxide, sulfate, sulfite, thiosulfate, nitrate, nitrite, arsenate selenate, and selenite. Lactate, pyruvate, formate, and H₂ (It were used as electron donor only) were used as carbon and energy sources. The 16S rRNA gene (AB263191) sequence analysis indicated that this strain has 97% and 96% similarity with *Shewanella decolorationis* S12 and *Shewanella putrefaciens*, respectively (Chen et al. 2007). This strain has been deposited in the Bioresource Collection and Research Center in Taiwan as BCRC 910321 and in the Japan IAM Culture Collection as JCM 14211.

**Decolorization of anthraquinone dyes by strain NTOU1**

Decolorization of five anthraquinone dyes (RB4, RB19, MR11, DR15, and DB3) by strain NTOU1 was carried out in 50 mL or 117 mL serum bottles containing the anoxic phosphate-buffered basal medium (20 mL or 100 mL) (Saltikov et al. 2003) under an atmosphere of N₂. The phosphate-buffered basal medium contained (g/L of deionized water): K₂HPO₄, 0.225; KH₂PO₄, 0.225; NaCl, 0.46; (NH₄)₂SO₄, 0.225; MgSO₄·7H₂O, 0.117; trace element solution (Skerratt et al. 2002), 1 mL; and vitamin solution (Widdel & Bak 1992), 1 mL.

Thus, strain NTOU1 was first cultivated under aerobic conditions at 30°C overnight in Luria-Bertani medium (OD₆₀₀ was about 1.2) and then harvested by centrifugation (8,000 g, 10 min). The pellet was washed twice with 10 mL phosphate-buffered basal medium (pH 7.0) and then re-suspended in 10 mL of the same medium under a N₂ gas stream. Cell suspension was used to inoculate the serum bottles (50 mL) containing fresh anaerobic phosphate-buffered basal medium (20 mL) supplemented with formate (20 mM) as a carbon source and a dye (200 mg/L) to reach an initial cell mass of 0.4–0.5 g/L (wet weight) and incubated statically under anaerobic conditions at 45°C for the dye-decolorization test. Each experiment was conducted in duplicate. These serum bottles were capped with butyl rubber stoppers and were aluminum crimp sealed. A modified Hungate technique was used throughout the study for anaerobic cultivation (Ljungdahl & Wiegel 1986). Decolorization tests with heat-killed cell suspension or without cell suspension were served as controls. At times (every 1–2 h) during incubation, 1 mL sub-sample was taken and then centrifuged for monitoring color removal. The absorbance spectra of these samples were scanned by a UV-visible spectrophotometer. Spectrophotometric scanning of each dye solution (200 mg/L) was carried out at a wavelength range of 190 to 900 nm and absorbance maxima were identified. Concentration of each dye in the supernatant was determined based on sample absorbance at the maximum wavelength (i.e. 603 nm for RB4 and RB19; 524 nm for MR11; 529 nm for DR15, and 586 nm for DB3). The uninoculated medium supplemented with the respective dye was used as the reference.
Decolorization activity (%) was calculated according to the formula as follows.

\[
\text{Decolorization activity (\%)} = \left( \frac{A - B}{A} \right) \times 100
\]

\(A\)–initial absorbance; \(B\)–observed absorbance

Decolorization activity (%) was expressed as the average of the duplicate serum bottles.

To compare the decolorization rates between the reactivated and the un-reactivated dyes, reactivated dyes (RB4 and RB19) were prepared based on the typical textile dye-bath conditions as follows. Aliquots of 0.5 g each of dye were dissolved in 50 mL 50 mM NaOH solution (resulting pH 11.6 ± 0.1), heated to and kept at 85°C for 1 h and then diluted to 100 mL after cooling while adjusting the pH to 7.0 (Lee & Pavlostathis 2004). The wavelength of maximum absorbance of RB4 and RB19 (603 nm) did not shift after dye hydrolysis.

### Optimum temperature and pH value for the decolorization of anthraquinone dyes

With formate (20 mM) as the electron donor, the temperature range for decolorization of anthraquinone dyes (200 mg/L) was set at \(T = 20–50\)°C, respectively. The optimum pH value for decolorization of each anthraquinone dyes (200 mg/L) was determined with acetate buffer (50 mM) with a pH value of 5, 6, and 7, phosphate buffer (50 mM) with pH value of 7, 8, and 9, and Tris-HCl (50 mM) buffer with pH value of 9 and 10, respectively. Each experiment was performed in duplicate. Decolorization activity (%) was expressed as the average of the duplicate serum bottles.

### Effects of addition of different electron acceptors and electron donors on the decolorization of anthraquinone dyes

With formate (20 mM) as the electron donor, the effects of addition of different electron acceptors (20 mM), e.g. sulfate, thiosulfate, nitrate, ferric citrate, ferric oxide, manganese oxide, arsenate, and selenate on decolorization of each anthraquinone dye (200 mg/L) were determined at pH 9.0 and 45°C under anaerobic conditions. The culture medium without addition of extra electron acceptor was used as the reference. With ferric citrate (20 mM) as the electron acceptor, the effect of addition of different electron donor, e.g. \(H_2\), lactate, formate, glucose, arabinose, acetate or pyruvate (20 mM) on decolorization of each anthraquinone dye (200 mg/L) was determined at pH 9.0 and 45°C under anaerobic conditions. \(H_2\) was added to the culture medium by flushing a gas mixture of \(CO_2\) and \(H_2\) (80:20) to the headspace of the serum bottle. The culture medium without addition of an electron donor was used as the reference. Each experiment was performed in duplicate. Decolorization activity (%) was expressed as the average of the duplicate tubes.

### Effect of concentration of dyes on the rate and extent of decolorization

With formate (20 mM) as the electron donor and ferric citrate (20 mM) as the electron acceptor, the effect of concentration on the rate and extent of decolorization of these anthraquinone dyes was determined at 45°C with separate batches of medium (pH 9.0) containing from 100 to 1,000 mg/L of each anthraquinone dye. Each experiment was performed in duplicate. The initial color removal rate (mg/L/h) was determined within 4 h after incubation of the dye. The extent of the color removal (%) was determined after 57 h of incubation of the dye. Decolorization activity (%) was expressed as the average of the duplicate serum bottles.

### Determination of decolorization products of anthraquinone dyes

To study the degradation products of the dye (200 mg/L), cells (40 mL) were collected from each serum bottle, by centrifuged at 8,000 g for 10 min, and the supernatant collected was adjusted to pH 7.0 with 1 N NaOH. This supernatant was then extracted twice with chloroform (at the ratio of 1:1). The remaining aqueous layer was acidified with 1 N HCl to pH 2 and was extracted twice with diethyl ether (at the ration of 1:1). The diethyl ether extract was dried over anhydrous \(Na_2SO_4\) and evaporated under reduced pressure at 50°C. The residue was dissolved in a small amount of methanol. The resulting solution was used for the analysis by thin layer chromatography (TLC) and gas
chromatography-mass (GC/MS) spectrometry. TLC analysis was carried out on a silica gel 60 F254 (RP-18F254, 5 × 10 cm; E. Merck). Spots were detected under (UV) light (254 nm). The GC/MS system consisted of an Agilent 6890 GC equipped with an Agilent 5973N mass selective detector (MS). The mass spectrometer was operated in the electron impact mode with an electron current of 70 eV. Aliquots of 1 μL of a methanol extract of the major spot from the TLC plate were injected automatically with an auto sampler (7683 series) in splitless mode via a GC inlet (with injector temperature of 250°C). An HP-5MS capillary column, 30 m long, 0.25 mm ID, and with 0.25 μm film thickness was connected directly to the ion source of the mass spectrometer. The oven temperature was kept isothermal for 1 min at 50°C, was then increased to 200°C at the rate of 20°C min⁻¹, and then to 260°C at the rate of 3°C min⁻¹ and finally held constant at 260°C for 10 min. The GC/MS system was operated in full scan (m/z 50 to 500). Controls and samples were analyzed in duplicate.

RESULTS AND DISCUSSION

Decolorization of anthraquinone dyes by Shewanella sp. NTOU1

Shewanella sp. NTOU1 was capable of decolorizing these anthraquinone dyes from blue or red to pale yellow under static anaerobic conditions. Figure 1 displays the change of UV-visible spectra of these anthraquinone dyes in the culture medium. There was a significant decrease in the color intensity or in the peak absorbance of each dye during incubation. For RB4, RB19, MR11, DR15, and DB3, as the degree of dye decolorization increased with increasing incubation time, the visible absorbance maxima shifted from 603, 603, 524, 529, and 586 nm, respectively to 465, 485, 408, 459, and 470 nm, respectively attributed to the dye decolorization intermediate(s). No change occurred in the control bottles with heat-killed cell suspension or without cell suspension. We also noticed that after less than 2 h incubation with RB4, RB19, or DB3, the flocules with blue color were observed, while after less than 3 h incubation with MR11 or DR15, the flocules with red color were observed. However, the color of the supernatant of these cultures continued to decrease. At the end of the incubation period (24 h), the centrifuged biomass pellet had a pale yellow color. Thus, the initially aggregated/sorbed dye was reductively decolorized with the prolonged incubation time. No dye aggregation was found in cultures inoculated with heat-killed cell suspension or without cell suspension. Dye aggregation was also found in cultures supplemented with NaOH-hydrolyzed RB4 and RB19 (200 mg/L). Therefore, the changes of the absorption spectra of these anthraquinone dyes were due to the biological reaction through this strain, and were not due to adsorption to the cell membrane.

Dye aggregation has also been reported previously on the decolorization of RB4, RB19, and Reactive blue 5 (RB5) under methanogenic conditions (Panswad & Luangdilok 2000; Lee et al. 2006), and on the decolorization of Reactive Brilliant Blue K-GR (RB19) by Shewanella decolorationis S12 under anaerobic conditions (Xu et al. 2005). Although it has been reported that this aggregation phenomenon has been typically observed with anthraquinone dyes with a vinyl sulfone group (Lee et al. 2006), it has also occurred to anthraquinone dyes with other substituents.
**Optimum temperature and pH for decolorization of anthraquinone dyes**

RB19 was used as an example to show the optimum growth temperature and pH value for this strain to reach the highest decolorization rate for the dyes (200 mg/L). As shown in Figure 2a and b, the optimum growth temperature and pH value were 45°C and 8–9, respectively. At pH 9, medium with phosphate buffer had a higher decolorization rate than the medium with Tris-HCl buffer. In medium with phosphate buffer decolorization of these dyes reached a plateau when more than 90% of the residual color of RB19 was removed. The optimum growth temperature and pH value for this strain to decolorize the other anthraquinone dyes were also 45°C and 8–9, respectively (data not shown). In previous studies, the optimum temperature and pH for decolorization of RB19 by *S. decolorationis* S12 were around 30–37°C and pH 8.0 (*Xu et al. 2005*).

**Effects of different electron acceptors and electron donors on decolorization of anthraquinone dyes**

RB19 was used as an example to show the effect of different electron acceptors and electron donors on decolorization of anthraquinone dyes. As shown in Figure 2c, the decolorization rates of RB19 were different in the batch cultures supplemented with different electron acceptors. The addition of ferric citrate (20 mM) increased the decolorization rate of RB19. In this case the color of RB19 was decreased quickly, resulting in 90% color removal within 6 h of incubation. The addition of ferric oxide, thiosulfate, or selenate did not affect the decolorization, the addition of manganese oxide, sulfate or arsenate slightly inhibited decolorization, while the addition of nitrate almost completely inhibited decolorization of RB19. Thus decolorization of anthraquinone dyes by this strain was best under iron-reducing conditions. Similar results were also found in
decolorization of the other anthraquinone dyes by this strain (data not shown).

As shown in Figure 2d, decolorization rates of RB19 were different in batch cultures supplemented with a different carbon source. H2, pyruvate and formate were the better electron donors for this strain to decolorize RB19, and the maximum decolorization activity was obtained with formate. Similar results were also found in decolorization of the other anthraquinone dyes by this strain (data not shown).

The rate and extent of decolorization of anthraquinone dyes by *Shewanella* sp. NTOU1

The rate and extent of decolorization of anthraquinone dyes by this strain based on spectrophotometric analysis are shown in Table 1. Initial color removal rates (mg/L/h) of these dyes increased when the dye concentration increased from 100 to 1,000 mg L\(^{-1}\). This strain could decolorize 1,000 mg/L of these dyes at the initial color removal rates of 5.33–44.8 mg/L/h and the rates among them were ranked as follows: RB19 > RB4 > DB3 > DR15 > MR11. Lee & Pavlostathis (2004) also found that the initial color removal rates of RB4 and RB19 under methanogenic conditions increased when the dye concentration increased. However, in another report the authors found that when Reactive Brilliant Blue K-GR (RB19) at 50 mg/L, the dye was completely decolorized after 15 h cultivation with *S. decolorationis*, but the decolorization efficiency decreased with increasing dye concentration. When the dye concentration was as high as 1,200 mg/L, the dye could not be decolorized even after 300 h incubation (Xu et al. 2005).

Prolonged incubation period (57 h) the extent of decolorization was also different in these five dyes. The extent of color removal was in the range of 90–98% for RB19, 86–96% for RB4, 39–41% for MR11, 69–82% for DR15, and 89–91% for DB3. The extent of color removal among them were RB19 > RB4 > DB3 > DR15 > MR11. For RB4, RB19, and DB3 the extent of color removal increased as the dye concentration increased. For DR15 the extent of color removal decreased as the dye concentration increased. For MR11 the extent of color removal fluctuated but did not change much (39–41%) as the dye concentration increased from 100 to 1,000 mg/L.

Brown & Hamburger (1987) reported that more than 80% of acid blue (100 mg/L) was transformed to 1-amino-4-phenyl-aminoanthraquinone as a blue solid precipitate within 21 d after the start of the anaerobic sludge digestion process. Thus only sulfonate elimination occurred in this decolorization process. Itoh et al. (1993) reported that *Pigment Violet 12* (1,4-dihydroxyanthraquinone) was readily decolorized and degraded. However, anthraquinones bearing amino groups were hardly decolorized at all by *Bacillus subtilis* (Itoh et al. 1996). The rate and extent of decolorization of anthraquinone dyes by *B. subtilis* cultures followed a descending series relative to their substituents: dihydroxy > amino-hydroxy > amino-methyl > diamino (Itoh et al. 1993). Since the initial color removal rate and extent of decolorization of anthraquinone dyes by the strain NTOU1 was RB19 > RB4 > DR15 > DB3 > MR11, the decolorization of anthraquinones dyes by this strain followed a descending series relative to the substituents: amino-aromatic > amino-hydroxy > amino-aliphatic > dihydroxy, opposite to the results by the *B. subtilis* cultures. Cooling et al. (1996) reported that an increased degree of substitution of anthraquinone nucleus and addition of charged ring substituents resulted in an increased I\(_{50}\) value.

### Table 1

<table>
<thead>
<tr>
<th>Parameter/culture</th>
<th>Initial dye concentration (mg/l)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>100</td>
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<tr>
<td>Initial color removal rate†</td>
<td></td>
</tr>
<tr>
<td>RB4</td>
<td>4.63</td>
</tr>
<tr>
<td>RB19</td>
<td>6.76</td>
</tr>
<tr>
<td>MR11</td>
<td>1.78</td>
</tr>
<tr>
<td>DR15</td>
<td>1.42</td>
</tr>
<tr>
<td>DB3</td>
<td>2.13</td>
</tr>
<tr>
<td>Extent of color removal (100%)‡</td>
<td></td>
</tr>
<tr>
<td>RB4</td>
<td>86</td>
</tr>
<tr>
<td>RB19</td>
<td>90</td>
</tr>
<tr>
<td>MR11</td>
<td>39</td>
</tr>
<tr>
<td>DR15</td>
<td>82</td>
</tr>
<tr>
<td>DB3</td>
<td>89</td>
</tr>
</tbody>
</table>

†Within 4 h after the onset of incubation of the short-term decolorization assay.

‡Rate was calculated based on dye concentration (mg/l) h\(^{-1}\) protein concentration (mg/l)\(^{-1}\).

‡After 57 h of incubation.
i.e. a lower degree of inhibition of anthraquinone dyes on
pure cultures of sulfate-reducing bacteria as well as
sediment samples and waste water sludge. RB4 has two
amino groups and one dichlorotriazinyl reactive group,
RB19 has two amino groups and one vinyl sulfonil reactive
group, and DB3 has two amino groups and one aliphatic
reactive group in the anthraquinone nucleus. In addition,
RB4 and RB19 have an extra sulfonil group in the
2-positions of the anthraquinone nucleus. Thus RB4,
RB19, and DB3 have higher degree of substitution on the
anthraquinone nucleus than DR15 and MR11 have.
Toxicity of these three dyes might be lower than that of
DR15 and MR11 on Shewanella sp. NTOU1, and which
causes higher rate and extent of color removal.

Figure 3 shows that the reactivated RB 19 was
decolorized with a higher initial color removal rate than
that of the un-reactivated RB19. However, the extent of the
color removal of the reactivated and the un-reactivated
RB19 were similar. In contrast, the un-reactivated RB4 was
decolorized with a higher initial color removal rate than
that of the reactivated RB4. On the other hand, the
reactivated RB 4 was decolorized with a higher extent of
color removal than the un-reactivated RB4. Our results
were slightly different from the results of previous reports
that found that dyes reactivated under alkali and higher
temperatures could be decolorized at a higher rate and
extent of decolorization than dyes without reactivation
(Beydilli et al. 1998; Fontenot et al. 2002). At the present
time we are not quite clear about the effect of flocculation
on decolorization.

Figure 4 | GC/MS analysis of a methanol extract of the major spot ($R_f = 0.829$) from the
TLC plate containing the degradation products of RB19 by the
Shewanella sp. NTOU1 after color removal. (a) gas chromatogram; (b) mass
spectra for the peak with $R_t$ value of 16.55 min; (c) mass spectra for the
peak with $R_t$ value of 17.34 min; (d) mass spectra for the peak with $R_t$ value
of 17.66 min.
Degradation products of anthraquinone dyes

Except in the case of DR15 that two major spots ($R_f = 0.953$ and $R_f = 0.982$) were found, in the other cases one spot was found after TLC analyses of the extract from the culture medium after color removal. The major spot of RB4, RB19, MR11, and DB3 had $R_f$ value of 0.828, 0.828, 0.940, and 0.947, respectively.

With GC/MS, 1-aminoanthraquinone, 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione, and leuco-1,4-diaminoanthraquinone were detected from the methanol extract of the major spot of both RB4 and RB19 (Figure 4), while 1-hydroxy-9,10-anthracenedione was detected from that of the major spot of MR11, 1,4-dihydroxy-9,10-anthracenedione and 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione were detected from that of two major spots of DR15, respectively, and leuco-1,4-diaminoanthraquinone was detected from that of the major spot of DB3 (Figure 5).

These results showed that the anthraquinone nucleus could not further be degraded by this strain. In this study we also found that these dyes were decolorized by this strain from blue or red to pale yellow. Thus, the pale yellow color probably was from the unsubstituted anthraquinone, which is reported to have a weak band at ca. 405 nm (Zollinger 1987).

Since leuco-1,4-diaminoanthraquinone was detected as the intermediate compound after decolorization of RB4, RB19, and DB3, it is suggested that this strain could hydrolyze amino groups and eliminate sulfonyl group of these dyes to leuco-1,4-diaminoanthraquinone. In the past, Itoh et al. (1996) reported that decolorization of DR15 by a yeast strain Pichia anomala was involved in the displacement of hydroxyl groups resulting in the formation of 1,4-dihydroxyanthraquinone and...
2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione. In this study, 1,4-dihydroxy-9,10-anthracenedione and 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione were detected from DR15. Thus the decolorization pathway for DR15 by this strain was similar to that of the yeast. Revenga et al. (1994) reported that anaerobic decolorization of reactive anthraquinone dyes was achieved by the reductive transformation of the anthraquinone nucleus to
dihydroxyanthracene. Although DR15, 1,4-dihydroxy-9,10-anthracenedione and 1-hydroxy-9,10-anthracenedione were not detected from the major spot of RB4 and RB19, on the basis of our results of GC/MS analysis of MR11 and DR15 and other previous results (Itoh et al. 1996), plausible pathways for the decolorization of RB4, RB19, and DB3 by Shewanella sp. NTOU1 are shown in Figure 6.

As shown in Figure 6, the elimination of the sulfone group, the amino group, and the hydroxyl group, the elimination of substituents bound to the amino group, the displacement of the amino group with hydroxyl group, and the reductive transformation of the anthraquinone nucleus to anthracenedione were all involved in the decolorization of these dyes.

CONCLUSIONS

A facultative Shewanella sp. NTOU1 isolated from a cooling system of an oil refinery could decolorize a range of anthraquinone dyes (RB4, RB19, MR11, DR15, and DB3) under anaerobic conditions. The most suitable pH and temperature for decolorization of these dyes were pH 8.0–9.0 and 45°C, respectively. Decolorization rates were highest when formate was used as the electron donor and ferric citrate was used as the electron acceptor. For these dyes, as the initial dye concentration increased from 100 to 1,000 mg/L, the initial decolorization rates increased, and in most cases the extent of color removal increased as well.

1-aminoanthraquinone, 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione, and leuco-1,4-diaminoanthraquinone were detected with GC/MS after color removal from both RB4 and RB19, while 1-hydroxy-9,10-anthracenedione was detected from MR11, 1,4-dihydroxy-9,10-anthracenedione and 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione were detected from DR15, and leuco-1,4-diaminoanthraquinone was detected from DB3.

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