Respiration and Growth of Shewanella decolorationis S12 with an Azo Compound as the Sole Electron Acceptor†

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The ability of Shewanella decolorationis S12 to obtain energy for growth by coupling the oxidation of various electron donors to dissimilatory azoreduction was investigated. This microorganism can reduce a variety of azo dyes by use of formate, lactate, pyruvate, or H2 as the electron donor. Furthermore, strain S12 grew to a maximal density of 3.0 × 10⁷ cells per ml after compete reduction of 2.0 mM amaranth in a defined medium. This was accompanied by a stoichiometric consumption of 4.0 mM formate over time when amaranth and formate were supplied as the sole electron acceptor and donor, respectively, suggesting that microbial azoreduction is an electron transport process and that this electron transport can yield energy to support growth. Purified membranous, periplasmic, and cytoplasmic fractions from S12 were analyzed, but only the membranous fraction was capable of reducing azo dyes with formate, lactate, pyruvate, or H2 as the electron donor. The presence of 5 μM Cu²⁺ ions, 200 μM dicumarol, 100 μM stigmatellin, and 100 μM metyrapone inhibited anaerobic azoreduction activity by both whole cells and the purified membrane fraction, showing that dehydrogenases, cytochromes, and menaquinone are essential electron transfer components for azoreduction. These results provide evidence that the microbial anaerobic azoreduction is linked to the electron transport chain and suggest that the dissimilatory azoreduction is a form of microbial anaerobic respiration. These findings not only expand the number of potential electron acceptors known for microbial energy conservation but also elucidate the mechanisms of microbial anaerobic azoreduction.

Bacteria within the Shewanella genus are metabolically versatile and are able to use a diverse range of organic substrates and metals as terminal electron acceptors for growth and survival. Shewanella oneidensis MR-1, which can utilize a variety of compounds as terminal electron acceptors, including Fe(III), Mn(IV), U(VI), As(V), and humic substances, is arguably the most versatile bacterium studied to date (9, 24–28, 35). The diverse metabolic and physiological capabilities of Shewanella species make them useful for environmental cleanup and bioremediation (17, 40). Shewanella decolorationis S12, a new species of the genus Shewanella, was isolated from the activated sludge of a textile printing wastewater treatment plant in Guangzhou, China. Apart from oxygen, strain S12 can grow with various electron acceptors, including nitrate (NO³⁻), nitrite (NO₂⁻), ferric iron (Fe³⁺), sulfite (SO₄²⁻), and anthraquinone-2,6-disulfonate (AQDS), but not with sulfate (SO₄²⁻) (44), demonstrating that it has remarkable respiratory versatility, as do other members of Shewanella.

Azo dyes are almost all xenobiotic compounds, characterized by one or more azo groups (-N≡N-). They are toxic, highly persistent, and ubiquitously distributed in the environment, therefore resulting in serious environmental pollution.

MATERIALS AND METHODS

Azo dyes and chemical reagents. Azo dyes were purchased from Sigma-Aldrich, and their chemical structures are depicted in Table 1. Amaranth was used as a model azo dye in this study. The ion-pair agent tetrabutylammonium hydrogen-sulfate (TBAHS) was purchased (purity of >99%) from Fluka. 1-Naphthyl-
amine-4-sulfonic acid sodium and 1-naphthylamine-2-hydroxy-3,6-disulfonic acid sodium standards were obtained from Sigma-Aldrich. All other reagents are analytical grade.

**Media, strains, and cultivation.** *S. decolorationis* S12T (CCTCC M 203093, IAM 15094) was isolated from activated sludge from a textile printing wastewater treatment plant in Guangzhou, China (44). *S. decolorationis* was cultured aerobically at 32°C in LB medium or anaerobically in a defined medium (pH 7.4) (10 mM succinate, 5.7 mM Na2HPO4, 3.3 mM KH2PO4, 18.0 mM NH4Cl, 1.01 mM MgSO4, L-cysteine [20 μg/ml], vitamin solution, and mineral solution [43]). This defined medium, when supplemented with appropriate alternative electron donors and terminal electron acceptors, was able to support the growth of *S. decolorationis* S12.

Standard anaerobic techniques were used throughout this study as described by Vargas et al. (41). All gases were passed through a filter prior to use. The medium was prepared by adding concentrated stock solutions of all medium components into O2-free distilled water, and the solution was equilibrated with N2-CO2 (4:1). To study the coupling of azoreduction to the oxidation of electron donors, cells were grown in the defined medium (initial pH, 7.4) containing different electron donors with the azo compound amaranth serving as the electron acceptor. Cells were cultured in 50-ml serum bottles sealed with butyl rubber stoppers at a constant temperature (32°C) in an anaerobic station (RU SKINN C0105). The medium was bubbled with N2-CO2 (4:1) and filtered (0.2-μm filters) before incubation. H2 was provided at 96 kPa as the electron donor. The initial concentration of cells was 3.0 × 10^5 to 3.8 × 10^5 CFU/ml unless otherwise indicated.

**Membrane isolation and vesicle preparation.** Membrane isolation and vesicle preparation were performed using the method of Sapra et al. (36), with some modifications. Buffer A (50 mM Tris-HCl buffer [pH 8.0], containing 2 mM sodium dithionite) was used throughout. Cell extracts of *S. decolorationis* S12 were prepared by suspending 5 g (wet weight) of frozen cells in 50 ml of buffer A. The cell suspension was sonicated in an ice bath (3 s, 40% output, 80; SONICS VC-505). Cell breakage was monitored by examining the cells under a microscope. Unbroken cells were removed by centrifugation at 10,000 g for 15 min. The crude extract was then centrifuged at 150,000 g for 2.0 h. The resulting pellet contained the cell membrane whereas cytoplasmic proteins remained in the supernatant. The membrane fraction was resuspended in buffer A, which allowed vesicles to form spontaneously. The formation of vesicles was confirmed by transmission electron microscopy (Analysis and Test Centre, Zhongshan University). Protein concentrations were determined by the method of Bradford (5), using bovine serum albumin as the standard.

**Isolation of periplasmic proteins.** Cell cultures were harvested during late log phase, after approximately 15 h of growth, by centrifugation at 10,000 × g for 20 min (4°C) and washed once with 40 ml ice-cold 10 mM Tris-HCl buffer, pH 8.0. All isolation steps were carried out under aerobic conditions at 4°C with gentle stirring, unless otherwise indicated. The periplasmic fraction was prepared using a modification of the method of Osborn and Munson (30). Cells were resus-

### TABLE 1. Characteristics of azo dyes used in this study

<table>
<thead>
<tr>
<th>Azo dye</th>
<th>CAS no.</th>
<th>C.I. no.</th>
<th>Maximal absorbing wavelength (nm)</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranth</td>
<td>915-67-3</td>
<td>16185</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>Brilliant crocein MOO</td>
<td>5413-75-2</td>
<td>27290</td>
<td>510</td>
<td></td>
</tr>
<tr>
<td>Methyl red</td>
<td>845-10-3</td>
<td>13020</td>
<td>430</td>
<td></td>
</tr>
<tr>
<td>Orange G</td>
<td>1936-15-8</td>
<td>16230</td>
<td>483</td>
<td></td>
</tr>
<tr>
<td>Orange II</td>
<td>633-96-5</td>
<td>15510</td>
<td>487.5</td>
<td></td>
</tr>
<tr>
<td>Metanil yellow</td>
<td>587-98-4</td>
<td>13065</td>
<td>414</td>
<td></td>
</tr>
<tr>
<td>Acid red 13</td>
<td>6039-95-8</td>
<td>16045</td>
<td>507</td>
<td></td>
</tr>
<tr>
<td>Xylidine Ponceau</td>
<td>3761-53-3</td>
<td>16150</td>
<td>520</td>
<td></td>
</tr>
</tbody>
</table>

* CAS no., Chemical Abstracts Service registry number.  
  b C.I. no., Color Index number.
pended in ice-cold 750 mM sucrose, 30 mM Tris-HCl, pH 8.0, and incubated for 5 min. Lysozyme was added to a final concentration of 0.15 mg per ml and incubation was continued for another 2 min. Two volumes of 7.5 mM EDTA, pH 8.0, were added over a period of 10 min and then stirred for an additional 10 min. The suspension was then placed, without stirring, at room temperature for 15 min to permit the formation of spheroplasts. Spheroplasts were separated from the periplasm by centrifugation at 27,000 × g for 20 min. The resulting pellet contained the spheroplasts whereas the supernatant contained the periplasmic fraction.

Liquid chromatography. The azoreduction products were analyzed using a liquid chromatography system consisting of a 510 pump (Waters), a Waters 996 programmable photodiode array detector (Waters Chromatography Division, Milford, MA) operated at 254 nm, and a model 7125 valve injector (Rheodyne, Cotati, CA) with a 20-μl loop. The temperature was controlled by a Goldenfoil programmable oven (Milford, MA) operated at 254 nm, and a model 7125 valve injector (Rheodyne, Cotati, CA) with a 20-μl loop. The temperature was fixed at 20°C. The chromatographic separations were performed on Hypersil ODS columns (250 by 4.6 mm inside diameter). Acetonitrile-1 mM aqueous TBAHS (10:90, vol/vol) was used as eluent A against pure acetonitrile as eluent B. The velocity of flow remained 1 ml/min. From 0 to 5 min, the analysis ran isocratically, 100% for eluent A, and then a 25-min gradient was run with a

H2 98.5 68.4 ± 8.3 8.4 98.4 99.3 98.2 64.3 ± 4.3 97.5 ± 0.2 97.6 ± 0.6
Formate 99.6 75.3 ± 3.9 96.0 99.5 97.4 ± 0.2 58.5 ± 2.6 90.1 96.5 ± 0.3
Lactate 99.4 80.4 ± 4.6 97.3 ± 0.1 99.5 99.3 76.4 ± 1.8 99.9 99.7
Pyruvate 72.1 ± 5.8 52.2 ± 5.7 86.2 ± 2.2 79.3 ± 10.2 91.3 ± 0.7 58.3 ± 3.3 78.2 ± 4.2 82.2 ± 3.7

a Experiments were performed under anaerobic conditions at 32°C. Azo dye (1 mM) was used as the electron acceptor and H2 or 10 mM formate, lactate, or pyruvate was used as the electron donor. Data were obtained from separate triplicate experiments.

b Azoreduction rate was measured spectrophotometrically after a 3-h incubation and calculated as follows: percent azoreduction = [(A - B)/A] × 100, where A is initial absorbance and B is observed absorbance. The standard deviation is not shown if lower than 0.1. Abbreviations in column headings are defined as follows: A, amaranth; B, brilliant crocein MOC; C, methyl red; D, orange G; E, orange II; F, metanil yellow; G, acid red 13; H, xylidine Ponceau.

FIG. 1. Azoreduction coupled to the oxidation of electron donors by S. decolorationis S12 under anaerobic conditions. (A) Electron donors (ED) for anaerobic azoreduction by S. decolorationis S12. The initial concentration of formate, lactate, and pyruvate was 5 mM, and H2 was provided at 96 kPa. (B) Reduction of amaranth coupled to formate oxidation. All measurements are the averages of three independent experiments done using amaranth as the electron acceptor and different electron donors at 32°C under anaerobic conditions.

TABLE 2. Reduction of azo dyes by S. decolorationis S12 with H2 or an organic compound as the electron donora

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>No donor</td>
<td>2.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>4.8 ± 0.5</td>
<td>2.6 ± 0.2</td>
<td>3.5 ± 0.4</td>
<td>1.9 ± 0.1</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>H2</td>
<td>98.5</td>
<td>68.4 ± 8.3</td>
<td>98.4</td>
<td>99.3</td>
<td>98.2</td>
<td>64.3 ± 4.3</td>
<td>97.5 ± 0.2</td>
</tr>
<tr>
<td>Formate</td>
<td>99.6</td>
<td>75.3 ± 3.9</td>
<td>96.0</td>
<td>99.5</td>
<td>97.4 ± 0.2</td>
<td>58.5 ± 2.6</td>
<td>90.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>99.4</td>
<td>80.4 ± 4.6</td>
<td>97.3 ± 0.1</td>
<td>99.5</td>
<td>99.3</td>
<td>76.4 ± 1.8</td>
<td>99.9</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>72.1 ± 5.8</td>
<td>52.2 ± 5.7</td>
<td>86.2 ± 2.2</td>
<td>79.3 ± 10.2</td>
<td>91.3 ± 0.7</td>
<td>58.3 ± 3.3</td>
<td>78.2 ± 4.2</td>
</tr>
</tbody>
</table>

RESULTS

Azoreduction coupled to the oxidation of electron donors. To demonstrate the dependence of azoreduction in strain S12 on oxidation of primer electron donors, we inoculated S12 into a defined medium supplemented with different electron donors and azo compounds under anaerobic conditions. Experimental results showed that H2, formate, lactate, and pyruvate were able to serve as electron donors for reduction of amaranth (Fig. 1A) and other azo compounds (Table 2). However, S12 was not able to use acetate, propionate, salicylate, glycerin, glucose, carbinol, ethanol, sucrose, fructose, glucose, citrate, succinate, fumarate, or benzoate as electron donors for azoreduction. Azoreduction was an enzymatic process, as neither H2 nor any of the organic electron donors were able to reduce amaranth or other azo compounds in the absence of S12.
Azoreduction also did not occur when cells were killed by incubation at 95°C for 30 min.

To quantify the electron transfer from the electron donor to the azo dyes, the oxidization of formate and the reduction of amaranth were determined simultaneously in cultures containing 5 mM formate and 1 mM amaranth. Within 40 h, the complete disappearance of 1.0 mM amaranth was accompanied by a stoichiometric consumption of 2.0 mM formate over time (Fig. 1B). When 1.0 mM amaranth was completely reduced, the consumption of formate simultaneously ceased. The molar ratio of the formate oxidized to the amaranth reduced was 1.92. Because one molecule of formate provides two electrons and one molecule of amaranth (one azo bond) can accept four electrons, the ratio should theoretically be 2.0. The measured molar ratio suggests that almost all of the electrons accepted by amaranth were from formate. The amounts of formate consumed and amaranth reduced were consistent with the following reaction: 

\[
\text{Ar}_1\text{N} = \text{N-Ar}_2 + 2 \text{ formate} + 2 \text{ H}_2\text{O} \rightarrow \text{Ar}_1\text{NH}_2 + \text{H}_2\text{N-Ar}_2 + 2 \text{ HCO}_3^- + 2 \text{ H}^+
\]

**Analysis of azoreduction products.** A detection assay was designed based on the characteristics of azo compounds. The double bond of the azo linkage together with the conjugated-bond system of the aromatic components constitutes the chromophore of the azo dyes. The color of the azo compound is the result of the conjugated system, which facilitates electron delocalization, with the energy absorption in the visible region of the spectrum (45). If the double bond of the azo compounds is
strain, cysteine must be added to the medium in order for S. decolorationis to grow. When S. decolorationis was incubated for 7 days under anaerobic conditions, the concentration of the reduction products remained unchanged, indicating that the reduction products from amaranth were not formed concomitantly. After the reduced amaranth was incubated for 7 days under anaerobic conditions, the reduction products of peak 1 and peak 2 match with 1-naphthylamine-4-sulfonic acid sodium standards were 22.183, 14.524, and 19.201 min, respectively (Fig. 2). Once amaranth was reduced, there were two new peaks (Rt1 = 14.487 min, Rt2 = 18.924 min) in the chromatographs (Fig. 2B). Reduction products of peak 1 and peak 2 match with 1-naphthylamine-4-sulfonic acid sodium and 1-naphthylamine-2-hydroxy-3,6-disulfonic acid sodium standards were 22.183, 14.524, and 19.201 min, respectively (Fig. 2). Once amaranth was reduced, there were two new peaks (Rt1 = 14.487 min, Rt2 = 18.924 min) in the chromatographs (Fig. 2B). Reduction products of peak 1 and peak 2 match with 1-naphthylamine-4-sulfonic acid sodium and 1-naphthylamine-2-hydroxy-3,6-disulfonic acid sodium in both retention time and full wavelength spectrograms, confirming that when amaranth was reduced by cleavage of the azo bond, two reduction products, 1-naphthylamine-4-sulfonic acid sodium and 1-naphthylamine-2-hydroxy-3,6-disulfonic acid sodium, were formed concomitantly. After the reduced amaranth was incubated for 7 days under anaerobic conditions, the concentration of the reduction products remained unchanged, indicating that the reduction products from amaranth were not used further by strain S12.

**Growth of S. decolorationis S12 with amaranth as the sole electron acceptor.** As S. decolorationis S12 is a Cys-deficient strain, cysteine must be added to the medium in order for S. decolorationis S12 to grow. When S. decolorationis S12 was grown in defined medium with formate as the electron donor, amaranth was reduced to aromatic amines over time. Figure 3 shows the anaerobic growth of S. decolorationis S12 coupled to the reduction of amaranth. The biomass yield was directly proportional to the amount of amaranth reduced (Fig. 3A). Growth of S12 coincided with the reduction of amaranth and stopped as amaranth became depleted. No growth occurred when electron donors were absent from the medium or when the electron donors were provided but the azo compound was omitted.

S12 grew to a maximal density of 3.0 × 10^7 cells per ml at 32°C in the defined medium and completely reduced the 2.0 mM amaranth (Fig. 3B). These results indicate that with azo compounds as an electron acceptor, the presence of succinate and the use of formate or H2 as the electron donor are essential for the anaerobic growth of S. decolorationis S12. Succinate alone did not support growth or azoreduction, suggesting that it was used only as a carbon source and not as an electron donor for azoreduction. Strain S12 can obtain energy for anaerobic growth by coupling the oxidation of formate or H2 to the reduction of azo compounds. Energy conservation from the oxidation of formate or H2 was proportional to the azoreduction. This energy must be generated from electron transport and oxidative phosphorylation, because there is no known mechanism to generate ATP through substrate-level phosphorylation with formate or H2 as the substrate.

**Effect of electron acceptors on azoreduction.** Apart from oxygen, strain S12 can grow with several different electron acceptors, including nitrate (NO₃⁻), nitrite (NO₂⁻), ferric iron (Fe³⁺), sulfite (SO₃²⁻), anthraquinone-2-sulfonate, and AQDS, but not with sulfate (SO₄²⁻). Azoreduction of amaranth by S12 was fully inhibited by molecular oxygen and by several typical electron acceptors, including 0.9 mM NO₂⁻ and 6.0 mM NO₃⁻, but not by 10.0 mM Fe(III). This inhibition may be due to competition for electrons from the electron transport chain.

**Experiments with respiratory inhibitors.** The results of studies using respiratory inhibitors offer evidence for a chemiosmotic model of dissimilatory azoreduction in S. decolorationis S12. Azoreduction by S. decolorationis S12 with H2 or formate as the electron donor was almost completely inhibited by 5 μM Cu²⁺ ions (Fig. 4A), a membrane-impermeable dehydrogenase inhibitor (14, 20), indicating that hydrogenase and formate dehydrogenase are important components of electron transfer. Stigmatellin, a quinone analog able to bind to cyto-
chrome $b$ (15, 31), inhibited anaerobic azoreduction (Fig. 4B), suggesting that a low-potential cytochrome $b$ is involved in the electron transport from the electron donor to the azo dye. It is likely that cytochrome $b$ shuttles electrons between primer dehydrogenases and menaquinone (MK). Dicumarol, which is thought to inhibit electron transport of MK in bacteria (1, 16), also inhibited azoreduction (Fig. 4C). This observation supports MK being an essential compound of electron transport for azoreduction. Furthermore, anaerobic azoreduction was sensitive to metyrapone (Fig. 4D), a specific cytochrome P450 inhibitor (42), indicating that cytochrome P450 plays an important role in anaerobic azoreduction by strain S12. These results show that anaerobic azoreduction by S12 is catalyzed by a multicompound system including a dehydrogenase (hydrogenase), cytochrome $b$, MK, a P450-type cytochrome, and a deduced terminal azoreductase.

**Location of the azoreduction enzyme system.** To localize the anaerobic azoreduction enzyme system in *S. decolorationis* S12, the cytoplasmic, periplasmic, and membranous proteins were isolated from anaerobically grown cells of S12. Equal concentrations of each protein fraction were suspended in phosphate buffer (20 mM Na$_2$HPO$_4$ · 7H$_2$O, 20 mM K$_3$H$_2$PO$_4$, pH 8.0). The azoreduction activity of each cellular fraction was measured in defined medium under anaerobic conditions at 32°C, with lactate, H$_2$, or formate as the electron donor. The cytoplasmic and periplasmic fractions showed very little azoreduction. However, freshly prepared membrane vesicles of S12 did effectively reduce azo compounds with H$_2$, formate, or lactate as the electron donor (Fig. 5). No azoreduction was detected when the membranous protein was treated at 95°C for 10 min before amaranth was added. These results show that the membrane fraction contains all of the essential components required for electron transport from the electron donors to the azo compounds. The capacity for azoreduction was constitutive, as the vesicles could reduce azo compounds even though the organism had been grown with other electron acceptors.

**DISCUSSION**

Oxidation of electron donors coupled to azoreduction. Azoreduction is an important process for azo dye degradation. As mentioned in the introduction, many microorganisms are capable of decolorizing azo dyes anaerobically (6, 7, 12, 13, 18, 19, 32, 37). However, while the mechanism of microbial anaerobic azoreduction remains unclear, the redox mediator model of azoreduction is the currently accepted hypothesis. In this hypothesis, the reduction of the azo dye is catalyzed extracellularly by the action of redox mediator compounds, which are
either formed during the metabolism of certain substrates or added externally. These mediators enable the transfer of redox equivalents from the bacterial cell membrane to the azo dye. The action between the azo compounds and the redox mediator is a purely chemical redox reaction (19, 33, 38). A previous study has shown that anaerobic reduction of azo dyes by Sphingomonas sp. strain BN6 is linked to the bacterial electron transport chain (19). Results from the current study show that S. decolorationis S12 can reduce a variety of azo dyes by coupling the anaerobic oxidation of formate, lactate, pyruvate, or H2 to azoreduction. Furthermore, the electrons accepted by the azo bond are transferred from the electron donors. These findings suggest that azoreduction under anaerobic conditions is a dissimilatory process and that several dehydrogenases are involved. Moreover, azoreduction by strain S12 under physiological conditions does not depend on supplying any of the redox mediators mentioned above, but azoreduction can be improved by supplying AQDS. Thus, it can be concluded that an external redox mediator is not necessary for azoreduction by strain S12. However, we could not exclude the possibility that S12 may have produced a redox mediator which was used for azoreduction.

Energy conservation from dissimilatory azoreduction. Over more than 3.5 billion years of evolutionary history, prokaryotes have acquired the ability to use a broad range of electron-accepting substances. Shewanella species are highly advanced among microbes in their ability to exploit these various electron acceptors (40), and S12 has been shown to grow using many different electron acceptors (44). Generally, energy generation is a by-product of electron transfer. Thermodynamic calculations indicate that, per electron transferred, formate or H2 oxidation coupled to azoreduction of amaranth has the potential to yield enough energy to sustain microbial growth (see the supplemental material). The growth data provided here demonstrate that S12 is indeed capable of using the azo bond as a terminal electron acceptor for energy conservation under the growth condition used in this study. It is evident that microbial dissimilatory azoreduction is a respiratory process.

We propose that this biochemical reaction process be called azorespiration. This newly recognized form of anaerobic respiration expands the potential electron acceptors known for microbial energy conservation.

Location of dissimilatory azoreduction and electron transportation systems. To date, Sphingomonas sp. strain BN6 is the only reported organism with an enzymatic system of anaerobic azoreduction. For strain BN6, azo reductase activities were present in both the cytoplasmic and membrane fractions (19). In contrast, anaerobic azoreduction in S12 occurs almost exclusively in the membrane fraction. There was little azo reductase activity in the cytoplasmic and periplasmic fractions. These results provide strong evidence that dissimilatory azoreduction is a process of respiration in microorganisms. In addition, membrane vesicles were capable of azoreduction without an external redox mediator, suggesting that the azoreduction by S12 is a direct enzymatic process. It is possible that azoreduction was catalyzed by an unspecified azoreductase, which may be one of the components of the respiration chain. This is currently under investigation.

One of the basic methods of studying the activity of respiratory chain components is to use electron transport inhibitors. Experiments using specific inhibitors showed that anaerobic azoreduction by the bacterium was catalyzed by a multicomponent system including dehydrogenases, MK, cytochromes, and a deduced terminal azoreductase. A hypothetical chemiosmotic model of azorespiration is shown in Fig. 6.
model, the membrane-associated, cytoplasmic-oriented formate dehydrogenase or hydrogenase is the primary dehydrogenase. The membrane-bound, putative azoreductase functions as the terminal reductase. The electrons produced by the primary dehydrogenase are transported through the electron transport chain, causing an electrochemical protonotive force across the membrane, driving ATP synthesis. However, the precise mechanism of microbial anaerobic azorespiration remains to be elucidated.

**Environmental significance.** Anaerobic respiration by bacteria is an essential metabolic process. Microorganisms are often able to use a diverse range of electron acceptors, depending on the environmental conditions to which they are exposed (34). Under anaerobic conditions, bacteria can respire using diverse noxious substances as terminal electronic acceptors. Novel forms of anaerobic respiration have been and continue to be discovered (23). These new discoveries have environmental and biotechnological significance because these types of biochemical reactions impact the degradation of environmental contaminants and the cycling of organic carbon as well as many inorganic compounds. Furthermore, anaerobic respiration is increasingly recognized as a strategy for the remediation of environments contaminated by priority pollutants (21, 22). In this study, we illustrated that azoreduction by strain S12 is a process of respiration. This newly recognized microbial anaerobic respiration may have important environmental and biotechnological impact on the treatment of dye-containing wastewater and bioremediation of sites contaminated with azo dyes. Based on this study, anaerobic azoreduction by *Shewanella* strain S12 is a biochemical process coupling the oxidation of electron donors with azoreduction. As such, the addition of electron donors may stimulate the reduction of azo dyes. Usually, there is an abundance of organic substances in activated sludge which can be used as electron donors to support azoreduction; however, a lack of electron donors in the treatment reactor will decrease the rate of azoreduction. Our experiments show that tolune and aniline can also serve as electron donors for anaerobic azoreduction by S12 (data not shown), suggesting that bacteria capable of dissimilatory azoreduction might be able to couple the decomposition of toxic organic substances to the reduction of azo compounds. *S. decolorationis* S12 is able to grow both aerobically and anaerobically in many different environments and does not cause disease in humans or other organisms (44). These properties make it an ideal bacterium for bioremediation of environments contaminated with azo dyes and other toxic organic substances.

In summary, the experimental results presented in this study strongly suggest that azoreduction by S12 under anaerobic conditions is a new form of microbial respiration. Furthermore, these results demonstrate that rapid and extensive azoreduction can be accomplished by this respiration process and suggest that microbial azorespiration plays a great and direct role in azo dye degradation. Besides revealing a new form of microbial respiration, this discovery leads to a better understanding of the physiology and biochemistry of microbial azoreduction. However, the precise mechanism of microbial anaerobic azorespiration remains to be elucidated. Nonetheless, these findings suggest a strategy for bioremediation of soil and aquatic environments contaminated by azo dyes.
AUTHOR’S CORRECTION

Respiration and Growth of *Shewanella decolorationis* S12 with an Azo Compound as the Sole Electron Acceptor

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Volume 73, no. 1, p. 64–72, 2007. Table 2, Fig. 1B, and the corresponding description in Results (p. 66–67, with the subhead “Azoreduction coupled to the oxidation of electron donors”) were reprinted from “Effects of electron donors and acceptors on anaerobic reduction of azo dyes by *Shewanella decolorationis* S12” (Y. Hong, X. Chen, J. Guo, Z. Xu, M. Xu, and G. Sun, Appl. Microbiol. Biotechnol. 74:230–238, 2007) without appropriate credit and without permission. We apologize for this mistake.