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Research Letter

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Degradation of the recalcitrant oil spill components anthracene and pyrene by a microbially driven Fenton reaction

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†RS performed all experiments, developed part of the protocol and co-wrote the manuscript. TJD developed the concept and part of the protocol, co-analyzed all data and co-wrote the manuscript.
One sentence summary: This work demonstrates a newly developed microbially driven Fenton reaction (previously applied to degrade toxic chlorinated solvents and solvent stabilizers) that degrades the toxic oil spill components, pyrene and anthracene.
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ABSTRACT

Oil spill components include a range of toxic saturated, aromatic and polar hydrocarbons, including pyrene and anthracene. Such contaminants harm natural ecosystems, adversely affect human health and negatively impact tourism and the fishing industries. Current physical, chemical and biological remediation technologies are often unable to completely remove recalcitrant oil spill components, which accumulate at levels greater than regulatory limits set by the Environmental Protection Agency. In the present study, a microbially driven Fenton reaction, previously shown to produce hydroxyl ($HO^{\bullet}$) radicals that degrade chlorinated solvents and associated solvent stabilizers, was also found to degrade source zone concentrations of the oil spill components, pyrene (10 μM) and anthracene (1 μM), at initial rates of 0.82 and 0.20 μM h^{-1}, respectively. The pyrene- and anthracene-degrading Fenton reaction was driven by the metal-reducing facultative anaerobe *Shewanella oneidensis* exposed to alternating aerobic and anaerobic conditions in the presence of Fe(III). Similar to the chlorinated solvent degradation system, the pyrene and anthracene degradation systems required neither the continual supply of exogenous H_2O_2 nor UV-induced Fe(III) reduction to regenerate Fe(II). The microbially driven Fenton reaction provides the foundation for the development of alternate *ex situ* and *in situ* oil and gas spill remediation technologies.

Keywords: oil spill degradation; microbial Fenton reaction; anthracene; pyrene; *Shewanella oneidensis*

INTRODUCTION

Environmental catastrophes such as the *Deepwater Horizon* (DWH) oil spill resulted in an inadvertent release of a range of saturated, aromatic and polar hydrocarbons, including n-alkanes, branched alkanes, monochromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) (Reddy *et al.* 2012). In the DWH oil spill, for example, approximately 10^{11} g of net carbon were released in the form of C_1–C_5 hydrocarbon gases, including methane, ethane, propane and isobutene (Barron 2012) that adversely impacted marsh vegetation (Lin and Mendelssohn 2012), indigenous microbial and coral communities (White *et al.* 2012; Rodriguez *et al.* 2015) and aquatic and wildlife species (Barron 2012).

A variety of physical, chemical and biological methods are generally applied to contain oil spills and limit adverse effects on contaminated ecosystems. Physical methods include oil collection in containment booms with subsequent recovery by ships

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(Wang *et al.* 2014), while chemical methods include application of sorbents such as peat, sawdust and clays to adsorb lighter oils (Al-Majed, Adebayo and Hossain 2012; Owoseni *et al.* 2014), and wide-spread application of dispersants such as Corexit to accelerate the rate of oil dispersion. Chemical dispersants reduce the surface tension of the oil and the droplet sizes with subsequent increases in the available surface area for microbial growth and microbial oil degradation activity (Wang *et al.* 2016). Biological methods to remediate petroleum-contaminated sites include composting, bioventing and *ex situ* bioreactor treatment processes (Boopathy 2000).

Microbial hydrocarbon degradation is a major pathway for *in situ* removal of oil spill components from contaminated marine environments, particularly aerobic water columns and sediment surficial layers (Van Hamme, Singh and Ward 2003; Haritash and Kaushik 2009). Microbial oil degradation activity is affected by a variety of environmental factors, including the supply of carbon and energy sources such as acetic, benzoic and naphthenic acids, aliphatic and aromatic hydrocarbons, temperature, pH and dissolved oxygen concentration (Magot, Ollivier and Patel 2000). Intense aerobic microbial metabolic activity within oil plumes depletes oxygen to below detection levels in the plume interior (Hazen *et al.* 2010) and oil biodegradation rates are subsequently limited by oxygen supply (Lu, Zhang and Fang 2011). Anaerobic biodegradation is the primary pathway for hydrocarbon degradation in oxygen-depleted plumes and in deep petroleum reservoirs where oxygen is depleted at the oil–water interface (Roling, Head and Larter 2003).

Anaerobic, oil-degrading microorganisms (Rueter *et al.* 1994; Kodama and Watanabe 2003) activate hydrocarbons for use as an electron donor via hydroxylation, carboxylation, fumarate addition and methylation to yield more readily degradable intermediates (Ulrich, Beller and Edwards 2005). However, several oil and gas components such as the PAHs anthracene and pyrene are highly recalcitrant to microbial degradation pathways and thus may accumulate in contaminated sediments (Atlas and Bartha 1973; Chaillan *et al.* 2004; Anderson 2013; Hayworth *et al.* 2015; Zuijdgeest and Huettel 2012; Wang *et al.* 2014; Yin *et al.* 2015). Microbial PAH degradation rates are limited by PAH concentrations, temperature, pH and the composition of the indigenous microbial population (Smith, Schwab and Banks 2008; Horel, Mortazavi and Sobecky 2012).

Oil components such as pyrene and anthracene may also be oxidatively degraded by reactive oxygen species (ROS) such as hydroxyl ($HO^{\bullet}$) radicals with high oxidation potential (Andreozzi *et al.* 2004). Reactive oxygen species are produced during the oxidation of Fe(II) by dissolved oxygen in sediments not exposed to UV radiation (Murphy *et al.* 2016). These studies demonstrated that ROS may be generated with Fe(II) in pore waters or Fe(II) produced during the reduction of Fe(III) oxides by dissolved sulfides. Furthermore, hydrogen peroxide is not only detected in marine (Shaked, Harris and Klein-Kedem 2010), freshwater (Richard *et al.* 2007) and atmospheric environments (Lee, Heikes and O'Sullivan 2000), but has also recently been detected in subsurface aquifers (Yuan *et al.* 2017). $HO^{\bullet}$ radicals are produced by the conventional (purely abiotic) Fenton reaction in which H_2O_2 reacts with ferrous iron (Fe(II)) to produce ferric iron (Fe(III)), hydroxyl ion (OH^-), and hydroxyl radical ($HO^{\bullet}$) (Equation 1):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + HO^{\bullet} \quad (1)$$

In addition to pyrene and anthracene, conventional Fenton reaction-generated $HO^{\bullet}$ radicals oxidatively degrade a wide range of hazardous organic compounds, including polyethylene (Chow *et al.* 2016), chlorinated aliphatics and aromatics (Tyre, Watts and Miller 1991), dry-cleaning solvents (Topudurti *et al.* 1994), pentachlorophenol (PCP) (Barbeni *et al.* 1987), tetrachloroethylene (PCE) (Jho, Singhal and Turner 2010), trichloroethylene (TCE) (Tsai *et al.* 2010), 1,1,2-trichloroethane (TCA) (Pignatello, Liu and Huston 1999), 1,4-dioxane (Son, Im and Zoh 2009; Vescovi, Coleman and Amal 2010; Zeng *et al.* 2017), petroleum hydrocarbons and the oil dispersant Corexit (Millioli, Freire and Cammarota 2003; Glover *et al.* 2014; Ojinnaka, Osuji and Achugasim 2012; Zhang, Shao and Dong 2014). Petroleum hydrocarbon degradation is driven by the electrochemical production of $HO^{\bullet}$ radicals at optimal pH ranges of 2.5–3.5 (Zhang, Shao and Dong 2014). High concentrations of the reaction substrates Fe(II) and H_2O_2 must be continuously supplied in a conventional Fenton reaction system to drive the hydrocarbon degradation reaction at low pH (Lu *et al.* 2010; Ojinnaka, Osuji and Achugasim 2012; Wang *et al.* 2011). Continuous supply of H_2O_2 is often required in UV irradiation systems, often employed to drive photo-Fenton systems, since they are limited by UV light penetration (Kim and Vogelpohl 1998).

Microbially driven Fenton reactions that alternately produce the Fenton reagents H_2O_2 (via microbial O_2 respiration) and Fe(II) (via microbial Fe(III) reduction) do not require continual addition of Fenton substrates and UV-induced Fe(III) reduction to drive $HO^{\bullet}$ radical production (Sekar and DiChristina 2014; Sekar, Shin and DiChristina 2016; Sekar, Taillefert and DiChristina 2016b). The Fe(III)-reducing facultative anaerobe *Shewanella oneidensis* was recently employed to drive the Fenton reaction for oxidative degradation of PCP (McKinzi and DiChristina 1999), 1,4-dioxane (Sekar and DiChristina 2014), TCE and perchloroethylene (PCE) (Sekar, Taillefert and DiChristina 2016) and lignocellulose (Sekar, Shin and DiChristina 2016a). In the *S. oneidensis*-driven Fenton reaction, batch liquid cultures were amended with Fe(III) and contaminant and subsequently exposed to alternating anaerobic and aerobic conditions. *Shewanella oneidensis* produced Fe(II) via microbial Fe(III) reduction during the anaerobic period. During the aerobic period, *S. oneidensis* produced H_2O_2 via microbial O_2 respiration. During the transition from anaerobic-to-aerobic conditions, $HO^{\bullet}$ radicals were produced through the Fenton reaction between Fe(II) and H_2O_2 that completely degraded the contaminants PCP, 1,4-dioxane, TCE and PCE at source zone concentrations (McKinzi and DiChristina 1999; Sekar and DiChristina 2014; Sekar, Taillefert and DiChristina 2016b). The main objective of the present study was to determine the ability of the microbially driven Fenton reaction system to degrade the oil spill components, anthracene and pyrene, supplied at source zone concentrations.

MATERIALS AND METHODS

Culture medium and chemical reagents

Shewanella oneidensis overnight cultures were grown aerobically in Luria-Bertani medium (10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl) at 30°C (Sambrook, Fritsh and Maniatis 1989). Anthracene and pyrene degradation experiments were conducted in minimal salt solution (pH 7.0; 30°C, Supplementary Table S1). The limited salts (LS) medium for the microbially driven Fenton reaction contained 10 mM lactate. Unless otherwise specified, all chemicals were obtained from Sigma-Aldrich. In the microbially driven Fenton

degradation experiments, anthracene and pyrene were added to the batch reactor at source zone concentrations of 1 μM and 10 μM, respectively.

Design of the microbially driven Fenton reaction for degradation of anthracene and pyrene

The toxicities of anthracene and pyrene on *S. oneidensis* metabolic activity were tested in LS medium by comparing growth rates of *S. oneidensis* batch cultures in the presence and absence of the anthracene (1 μM) and pyrene (10 μM) under aerobic growth conditions. The experimental system for the microbially driven Fenton reaction for degradation of anthracene and pyrene was similar to the batch reactor system previously employed for degradation of 1,4-dioxane (Sekar and DiChristina 2014). A minimum cycling time of 3 h was chosen for the alternating aerobic and anaerobic periods. Based on previous studies, the 3-h cycling period was sufficient to achieve the maximum rate and extent of H_2O_2 production compared to other cycling times of 45 min, 1.5 h and 6 h (Sekar and DiChristina 2014). Time periods for the alternating aerobic and anaerobic cycles were similar to those previously employed for degradation of 1,4-dioxane with longer anaerobic periods (6–24 h, 33–47 h and 50–70 h) to ensure that Fe(III) was completely reduced prior to switching to aerobic conditions (Sekar and DiChristina 2014).

Cell densities of 10^9 cells per mL have been employed to degrade contaminants via the microbially driven Fenton reaction (Sekar and DiChristina 2014). This cell density was selected in anticipation of applying the microbially driven Fenton reaction to contaminant degradation processes carried out in *ex situ* reactors. Anaerobic stock solutions of anthracene and pyrene were added to 10^9 cells mL^{-1} in 60-mL glass serum bottles. Compressed nitrogen and air were sparged at 1 L/min gas flow rate. CO_2 was not scrubbed during the experiment and the pH of the medium was stable at 7.0±0.2 throughout the duration of the incubations (data not shown). The cell cultures were incubated under anaerobic conditions by injecting (hydrated) compressed nitrogen until 10 mM Fe(III) was reduced to 6.8–7.7 mM Fe(II) (3 h of anaerobic incubation; Fe(II) generating phase; Fig. 2). A (hydrated) compressed air line replaced the compressed nitrogen line to initiate aerobic conditions and 6.8–7.7 mM Fe(II) was oxidized to 1.6–3.0 mM Fe(III) (3 h of aerobic incubation; $HO^{\bullet}$ radical production phase; Fig. 2). $HO^{\bullet}$ radicals were generated under aerobic conditions by the chemical reaction between the products of microbial Fe(III) reduction (Fe(II)) and microbial aerobic respiration (H_2O_2). Anthracene and pyrene concentrations were monitored throughout the experiments via high-pressure liquid chromatography (HPLC; see below). The anaerobic and aerobic phases were alternated four additional times following the first aerobic phase during an overall 73-h reaction period similar to the batch reactor system previously employed for degradation of 1,4-dioxane (Sekar and DiChristina 2014). Three sets of control incubations were carried out to confirm that anthracene and pyrene were degraded by $HO^{\bullet}$ radicals generated by the *S. oneidensis*-driven Fenton reaction. The three control incubations were carried out in the batch reactor system described above with the following changes: In the first set of control incubations, anthracene and pyrene degradation were monitored in the absence of either Fe(III) or *S. oneidensis* cells (abiotic control). In the second set of control incubations, anthracene and pyrene concentrations were monitored in abiotic serum bottles maintained under strict anaerobic conditions with compressed nitrogen sparging for the entire 73-h reaction period. In the third set of control incubations, anthracene and pyrene concentrations were monitored in serum bottles maintained under strict aerobic conditions with compressed air sparging for the entire 73-h reaction period. Anthracene and pyrene concentrations were monitored throughout the experiments.

Analytical techniques

Samples were withdrawn and centrifuged at 6000 *g* for 10 min. HCl-extracted Fe(II) concentrations were determined with a previously described Ferrozine-based detection technique (Stookey 1970; Sorensen 1982). H_2O_2 concentrations were determined using a previously described spectrophotometric assay under similar conditions (Klassen, Marchington and McGowan 1994). H_2O_2 concentrations were monitored under (i) anthracene/pyrene degradation conditions with Fe(III) omitted and aerobic/anaerobic cycling periods of 3 h, and (ii) abiotic Fe(II) oxidation experiments in which 10 mM microbially produced Fe(II) (obtained by microbial Fe(III) reduction) was subjected to strictly aerobic conditions for 73 h.

To determine concentrations of anthracene and pyrene by HPLC, 1 mL of reactor contents was withdrawn and mixed with 700 μL of dichloromethane for 2 min. The mixture was centrifuged at 6000*g* for 10 min and the dichloromethane fraction was directly injected for analysis. Anthracene and pyrene were analyzed by HPLC (Waters 1525 binary pump system) with UV detection at 254 nm (Waters 2487 System) following separation using a Supelcosil LC-18 column (Supelco) with 80% aqueous acetonitrile as the mobile phase and a constant flow rate of 1.0 mL/min. Chromatograms were generated for anthracene and pyrene at retention times of 4.7 and 5.9 min, respectively. Calibration curves were generated from standards to determine the concentrations of each compound (Titato and Lancas 2006). The limits of detection for anthracene and pyrene were 0.02 μM and 0.23 μM, respectively, while the limits of quantitation of anthracene and pyrene were 0.06 μM and 0.69 μM, respectively.

Statistical analysis

For determination of concentrations of Fe^{2+}, H_2O_2, anthracene and pyrene, triplicate samples were taken at each time point and values reported as the mean with standard deviation. The statistically significant differences between the means were calculated using Student's *t*-test with $P < 0.05$.

RESULTS AND DISCUSSION

Microbially driven Fenton reaction for degradation of anthracene in liquid batch cultures

To test for pyrene and anthracene toxicity, *Shewanella oneidensis* was grown aerobically in LS growth medium supplemented with source zone concentrations of anthracene (1 μM) or pyrene (10 μM) (Reddy *et al.* 2012). Aerobic growth rates were not affected by the presence of either pyrene or anthracene, an indication that neither compound was toxic to *S. oneidensis* at source zone concentrations (Fig. 1). (Statistically significant differences were not found between treatments; $P > 0.05$.) Similar to previous findings (Sekar and DiChristina 2014), the results of cell viability experiments with anthracene and pyrene indicated that *S. oneidensis* cell concentrations were stable during the substrate switches between aerobic and anaerobic conditions (data not shown).

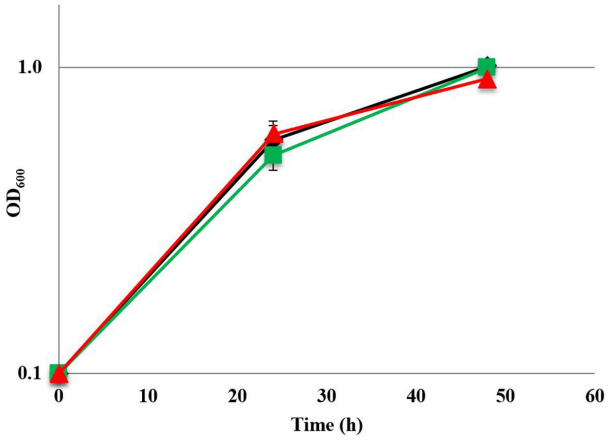

Figure 1. Aerobic growth of *S. oneidensis* in the presence of 1 μM anthracene; solid line, red (triangle), 10 μM pyrene; solid line, green (square) and no contaminants; solid line, black (diamond). Cell cultures were grown in LS media under aerobic conditions for 48 h with 10 mM lactate as an electron donor. Values represent means of triplicate samples; error bars represent one standard deviation.

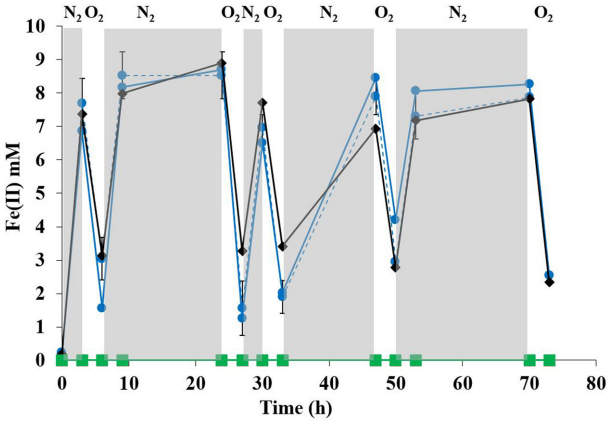

Figure 2. Fe(II) concentrations during microbially driven Fenton reaction for degradation of anthracene (1 μM) and pyrene (10 μM) in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III), 10 mM lactate and subjected to five alternate aerobic and anaerobic cycling periods; solid line, blue (circle), cells + Anthracene + Fe(III); solid line, green (square), cells + Anthracene (Fe(III) omitted); solid line, red (triangle), Anthracene + Fe(III) (cells omitted; abiotic control); solid line, black (diamond), no contaminant; dashed line, blue (circle), cells + Pyrene + Fe(III); dashed line, green (square), cells + Pyrene (Fe(III) omitted); dashed line, red (triangle), Pyrene + Fe(III) (cells omitted; abiotic control). Grey-shaded areas correspond to anaerobic phases, and unshaded areas correspond to aerobic phases. Values represent means of triplicate samples; error bars represent one standard deviation.

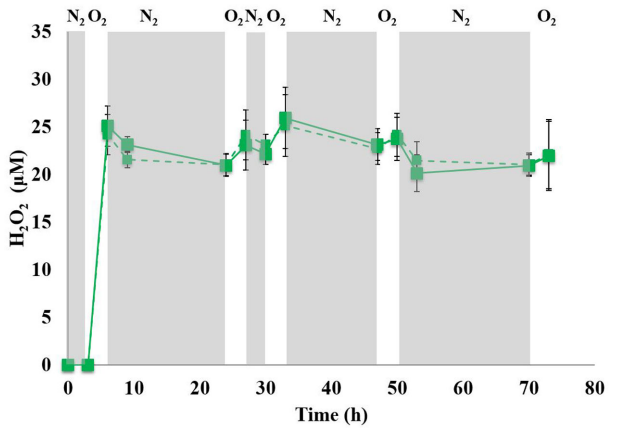

Figure 3. H_2O_2 concentrations during microbially driven Fenton reaction for degradation of anthracene (1 μM) and pyrene (10 μM) in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III), 10 mM lactate and subjected to five alternate aerobic and anaerobic cycling periods; solid line, green (square), cells + Anthracene (Fe(III) omitted); dashed line, green (square), cells + Pyrene (Fe(III) omitted). Grey-shaded areas correspond to anaerobic phases, and unshaded areas correspond to aerobic phases. Values represent means of triplicate samples; error bars represent one standard deviation.

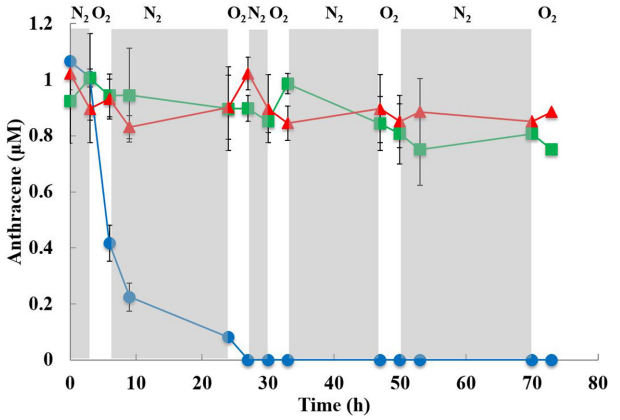

Figure 4. Anthracene concentrations during microbially driven Fenton reaction for degradation of anthracene (1 μM) in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III), 10 mM lactate and subjected to five alternate aerobic and anaerobic cycling periods; solid line, blue (circle), cells + Anthracene + Fe(III); solid line, green (square), cells + Anthracene (Fe(III) omitted); solid line, red (triangle), Anthracene + Fe(III) (cells omitted; abiotic control). Grey-shaded areas correspond to anaerobic phases, and unshaded areas correspond to aerobic phases. Values represent means of triplicate samples; error bars represent one standard deviation.

To initiate $HO^{\bullet}$ radical production by the *S. oneidensis*-driven Fenton reaction, Fe(III)-containing *S. oneidensis* cultures were incubated under anaerobic conditions for 3 h, during which the pool of 10 mM Fe(III) was microbially reduced at a rate of 2.3–2.6 mM h^{-1}, and Fe(II) concentrations increased to 6.8–7.7 mM (Fe(II)-generating phase; grey-shaded area in Fig. 2). At the 3-h time point, compressed air was injected for the next 3 h into all incubations, Fe(II) was air-oxidized at rates of 1.2–2.0 mM h^{-1}, and Fe(II) concentrations decreased from 6.8–7.7 mM to 1.6–3.1 mM (H_2O_2-generating phase; unshaded area in Fig. 2). At the end of the 3-h aerobic phase, we believe that our reaction system does not completely become anaerobic. Residual O_2 remaining in the system may still be microbially converted to H_2O_2 to react with Fe(II) and produce hydroxyl radicals that degrade anthracene and pyrene during the anaerobic phase. During the next four alternating anaerobic and aerobic phases, *S. oneidensis* reduced Fe(III) to approximately 7.7–8.9 mM Fe(II), while O_2 oxidized the produced Fe(II) to approximately 1.3–4.2 mM levels (Fig. 2). In control experiments with five alternating aerobic and anaerobic phases, *S. oneidensis* produced H_2O_2 to approximately 25 μM during the initial 3-h aerobic phase, and H_2O_2 subsequently oscillated between 20 and 25 μM levels throughout the remaining 73-h time period (Fig. 3). During the initial 3-h aerobic period (H_2O_2-generating phase; unshaded area in Fig. 4), anthracene was degraded from 1.0 μM to 0.41 μM at an initial rate of 0.2 μM h^{-1} (Fig. 4). During the ensuing 18-h anaerobic period (Fe(II)-generating phase; grey-shaded area in Fig. 4), anthracene was degraded from 0.41 to 0.08 μM (0.02 μM h^{-1}). During the second 3-h aerobic period (24-h time point; Fig. 4), anthracene was

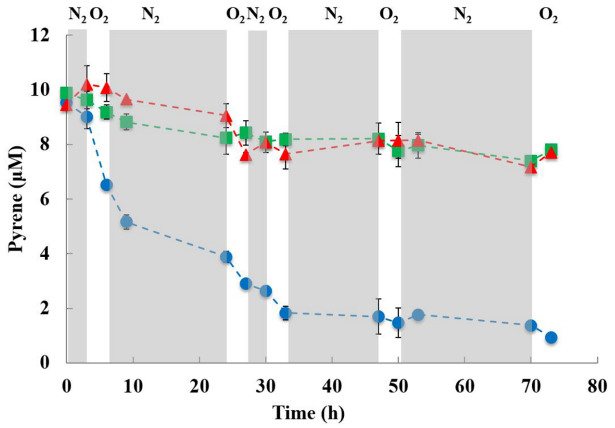

Figure 5. Pyrene concentrations during microbially driven Fenton reaction for degradation of pyrene (10 μM) in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III), 10 mM lactate and subjected to five alternate aerobic and anaerobic cycling periods; dashed line, blue (circle), cells + Pyrene + Fe(III); dashed line, green (square), cells + Pyrene (Fe(III) omitted); dashed line, red (triangle), Pyrene + Fe(III) (cells omitted; abiotic control). Grey-shaded areas correspond to anaerobic phases, and unshaded areas correspond to aerobic phases. Values represent means of triplicate samples; error bars represent one standard deviation.

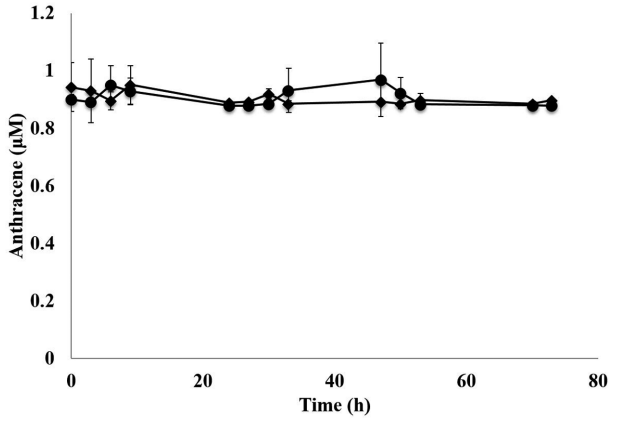

Figure 6. Anthracene profile (1 μM) under strict aerobic and anaerobic conditions; solid line, (circle), strictly aerobic; solid line, (diamond), strictly anaerobic. Values represent means of triplicate samples; error bars represent one standard deviation.

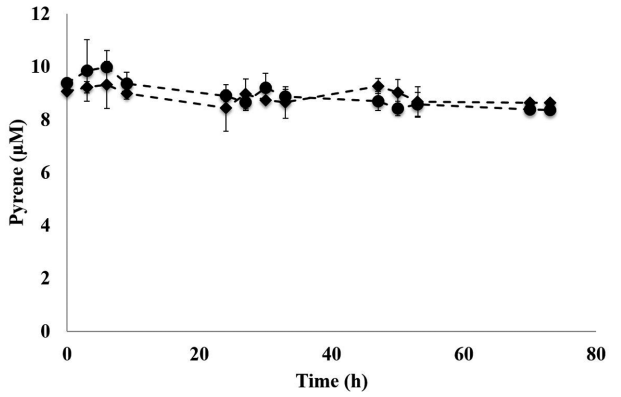

Figure 7. Pyrene profile (10 μM) under strict aerobic and anaerobic conditions; dashed line, (circle), strictly aerobic; dashed line, (diamond), strictly anaerobic. Values represent means of triplicate samples; error bars represent one standard deviation.

degraded to levels below detection limits (0.02 μM) and remained below detection levels throughout the remainder of the 73-h incubation.

Microbially driven Fenton reaction for degradation of pyrene in liquid batch cultures

Nearly identical patterns of microbial (*S. oneidensis*-catalyzed) Fe(III) reduction, chemical (O_2-catalyzed) Fe(II) oxidation and H_2O_2 production were observed in analogous pyrene degradation experiments (Figs 2 and 3). During the initial 3-h aerobic period (H_2O_2-generating phase; unshaded area in Fig. 5), pyrene was degraded from 9.0 μM to 6.5 μM (at an initial rate of 0.82 μM h^{-1}; Fig. 5). During the ensuing 18-h anaerobic period (Fe(II)-generating phase; grey-shaded area in Fig. 5), pyrene was degraded from 6.5 to 3.9 μM (0.15 μM h^{-1}). During the second and third 3-h degradation periods (24-h and 30-h time points respectively; Fig. 5), pyrene was degraded at rates of 0.32 and 0.27 μM h^{-1}, respectively. Pyrene concentrations remained constant at 1.4 μM throughout the remainder of the 73-h incubation (Fig. 5).

Anthracene and pyrene concentrations remained constant at 1.0 and 10.0 μM, respectively, in the first set of control incubations lacking Fe(III) or *S. oneidensis* cells (abiotic control; Figs 4 and 5) that also included five successive alternating anaerobic and aerobic cycles (statistically significant differences were found between Fenton reaction and control incubations; $P < 0.05$). In the second and third set of control incubations held under strict anaerobic and aerobic conditions respectively for 73 h, anthracene and pyrene concentrations remained constant at 1.0 and 10.0 μM, respectively (Figs 6 and 7). The absence of anthracene and pyrene degradation under strict aerobic or strict anaerobic Fe(III)-reducing conditions indicated that the contaminants were not degraded due to aerobic or anaerobic enzymatic degradation reactions catalyzed by *S. oneidensis*, or due to H_2O_2-catalyzed Fe(III) reduction and subsequent reaction of the produced Fe(II) with H_2O_2 (as in the conventional Fenton reaction) thus implicating $HO^{\bullet}$ radicals (formed by reaction of microbially produced Fe(II) and H_2O_2) as the major reactant of the 1,4-dioxane degradation process.

The anthracene and pyrene amendments did not affect *S. oneidensis* metabolic activity as the rates of microbial Fe(III) reduction and microbial aerobic respiration in the presence of anthracene and pyrene were identical to those rates measured in the absence of both contaminants (Fig. 2). (Statistically significant differences were not found between treatments; $P > 0.05$.)

The Environmental Protection Agency (EPA) has set the standard limit for anthracene and pyrene in the environment at 10^{-4} and 2×10^{-4} mg/L, respectively (Gehle 2009). The microbial Fe(III) reduction rates measured in the presence and absence of anthracene or pyrene are nearly identical (Fig. 2). Thus, the concentrations of anthracene (1 μM or 0.2 mg/L) and pyrene (10 μM or 2 mg/L) degraded in the present study indicate that concentrations 3- to 4-orders of magnitude higher than the EPA limits did not affect *S. oneidensis* metabolic activity. In addition, the predicted degradation products for anthracene are anthraquinone compounds (Gan *et al.* 2013) including palmitic acid and hexadecane (Choi *et al.* 2014), respectively. Although analyses for anthraquinone compounds were not carried out in the present study, neither of the predicted intermediates appear to affect *S. oneidensis* metabolic activity (Fig. 2).

Potential pathways for microbially driven Fenton degradation of recalcitrant oil spill components anthracene and pyrene in contaminated marine and freshwater environments

Degradation of oil spill components such as anthracene and pyrene by $HO^{\bullet}$ radicals may occur naturally at the sediment–water interface in contaminated marine and freshwater environments via the oxidation of Fe(II) by dissolved oxygen (Murphy *et al.* 2014, 2016). Unlike the chemical Fenton reaction, addition of exogenous H_2O_2 is not required for the microbially driven Fenton reaction to degrade anthracene and pyrene. The chemical Fenton reaction also requires re-reduction of Fe(III) produced during H_2O_2-catalyzed Fe(II) oxidation reactions. Fe(III) re-reduction processes such as those catalyzed by UV irradiation are possible during *ex situ* contaminant degradation processes, yet UV light penetration represents a formidable obstacle for *in situ* oil spill remediation technologies.

In the microbially driven Fenton reaction, *S. oneidensis* respiratory processes catalyze both H_2O_2 production and Fe(III) re-reduction. Since microbial Fe(III) reduction has been detected in a variety of environments, including anaerobic marine and freshwater environments, the microbially driven Fenton reaction may be induced by exposing Fe(III)-reducing facultative anaerobes in Fe(III)-containing contaminated environments to alternating aerobic and anaerobic conditions (via alternating injections of compressed air and nitrogen). Alternately, the microbially driven Fenton reaction may be stimulated by installing reactive iron barriers in the flow path of contaminated sediment pore waters and exposing Fe(III)-reducing bacteria attached to the iron barriers to alternating aerobic and anaerobic conditions. The microbially driven Fenton reaction may be especially useful for degradation of anthracene and pyrene, which are highly recalcitrant to microbial (enzymatic) degradation pathways and thus accumulate in oil spill-contaminated sediments (Ghosal *et al.* 2016; Bagby *et al.* 2017). The microbially driven Fenton reaction thus provides a foundation for development of more effective *in situ* and *ex situ* remediation technologies for degradation of anthracene, pyrene and other recalcitrant oil spill components in contaminated marine and freshwater environments.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

FUNDING

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Conflicts of interest. None declared.

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