FINAL REPORT – PHASE I

Proof-of-Concept Study: Novel Microbially-Driven Fenton Reaction for In Situ Remediation of Groundwater Contaminated with 1,4-Dioxane, Tetrachloroethene (PCE) and Trichloroethene (TCE)

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14. ABSTRACT A microbially-driven Fenton reaction was designed to autocatalytically generate hydroxyl ($HO^{\bullet}$) radicals that degrade 1,4-dioxane, TCE, and PCE. In comparison to conventional (purely abiotic) Fenton reactions, the microbially-driven Fenton reaction operates at circumneutral pH and does not require addition of exogenous H_2O_2 or UV irradiation to regenerate Fe(II). The 1,4-dioxane, TCE, and PCE degradation process was driven by the Fe(III)-reducing facultative anaerobe *Shewanella oneidensis. S. oneidensis* batch cultures were provided with lactate, Fe(III), and either 1,4-dioxane, TCE or PCE and exposed to alternating aerobic and anaerobic conditions. During the aerobic phase, *S. oneidensis* reduced O_2 to H_2O_2, while during the anaerobic phase, *S. oneidensis* reduced Fe(III) to Fe(II). During the aerobic-to-anaerobic transition period, the produced Fe(II) and H_2O_2 interacted chemically via the Fenton reaction to form Fe(III), OH^- ion, and $HO^{\bullet}$ radicals which oxidatively degraded 1,4-dioxane, TCE, and PCE at potential source zone concentrations. Under optimal conditions, the microbially-driven Fenton reaction completely degraded 1,4-dioxane, TCE, and PCE in 53 h with optimal aerobic-anaerobic cycling frequencies of 3 h. Acetate and oxalate were detected as transient intermediates during the microbially-driven Fenton degradation of 1,4-dioxane, an indication that conventional and microbially-driven Fenton degradation processes may follow similar reaction pathways.

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ABSTRACT

The carcinogenic cyclic ether compound 1,4-dioxane is used to stabilize chlorinated industrial solvents and is a widespread contaminant in surface groundwaters and subsurface aquifers. 1,4-dioxane is also often found in the presence of co-contaminants tetrachloroethene (PCE) and trichloroethene (TCE). Current 1,4-dioxane remediation technologies such as carbon absorption, air stripping, and distillation are limited by the physical properties of 1-4-dioxane. In the present study, a microbially-driven Fenton reaction was designed to autocatalytically generate hydroxyl ($HO^{\bullet}$) radicals that degrade 1,4-dioxane, TCE, and PCE. In comparison to conventional (purely abiotic) Fenton reactions, the microbially-driven Fenton reaction operates at circumneutral pH and does not require addition of exogenous H_2O_2 or UV irradiation to regenerate Fe(II). The 1,4-dioxane, TCE, and PCE degradation process was driven by the Fe(III)-reducing facultative anaerobe *Shewanella oneidensis*. *S. oneidensis* batch cultures were provided with lactate, Fe(III), and either 1,4-dioxane, TCE or PCE and exposed to alternating aerobic and anaerobic conditions. During the aerobic phase, *S. oneidensis* reduced O_2 to H_2O_2, while during the anaerobic phase, *S. oneidensis* reduced Fe(III) to Fe(II). During the aerobic-to-anaerobic transition period, the produced Fe(II) and H_2O_2 interacted chemically via the Fenton reaction to form Fe(III), OH^- ion, and $HO^{\bullet}$ radicals which oxidatively degraded 1,4-dioxane, TCE, and PCE at potential source zone concentrations. Under optimal conditions, the microbially-driven Fenton reaction completely degraded 1,4-dioxane, TCE, and PCE in 53 h with optimal aerobic-anaerobic cycling frequencies of 3 h. Acetate and oxalate were detected as transient intermediates during the microbially-driven Fenton degradation of 1,4-dioxane, an indication that conventional and microbially-driven Fenton degradation processes may follow similar reaction pathways. Current work is focused on applying the microbially-driven Fenton

degradation process to degrade various combinations of 1,4-dioxane, TCE, and PCE, and also to evaluate the ability of the novel degradation process to degrade perfluoroalkyl (PFAS) substances.

INTRODUCTION

1,4-dioxane is a cyclic ether compound detected in a variety of contaminated surface groundwaters and subsurface aquifers[1-7]. 1,4-dioxane is a probable human carcinogen since exposure is linked to tumor formation in rats and guinea pigs.[3, 8] 1,4-dioxane is often used as a stabilizing agent for the chlorinated solvents such as Trichloroethylene (TCE) and Tetrachloroethylene (PCE) in industrial processes, including the textile and paper industries.[9, 10] 1,4-dioxane is also a byproduct of surfactant and polyethylene terephthalate plastic manufacturing processes.[6, 11, 12] 1,4-dioxane is completely miscible in water, displays low volatility, and is thus highly mobile in the environment. Current 1,4-dioxane remediation technologies such as carbon absorption, air stripping, and distillation are limited by problems associated with 1,4-dioxane solubility, boiling point, and vapor pressure, respectively.[13] Alternate methods such as photo-remediation by UV light, ozone destruction in the presence of hydrogen peroxide (H_2O_2), and ultrasonic destruction are not cost effective situ remediation strategies.[14, 15]

Bioremediation is a promising alternative method for in situ remediation of 1,4-dioxane, TCE and PCE-contaminated waters. 1,4-dioxane, TCE and PCE may be degraded microbially via metabolic or cometabolic reactions. Microbial degradation is carried out aerobically by mixed microbial communities in industrial sludge,[16, 17] and aerobic 1,4-dioxane-degrading bacteria have been isolated.[4, 18, 19] Although a limited number of studies have examined microbial degradation of 1,4-dioxane under anaerobic conditions, the corresponding degradation rates under nitrate-, iron-, and sulfate-reducing conditions are exceedingly slow.[20] Ex situ treatment of 1,4-dioxane-contaminated ground water involves pumping with ex situ treatment via advanced oxidation processes (AOPs).[5, 21-23] Hydroxyl ($HO^{\cdot}$) radicals generated via

AOPs can oxidatively degrade a variety of organic contaminants due to their high oxidation potential, and a number of $HO^{\cdot}$ radical-generating processes have been developed including those based on the Fenton reaction.[24] In the Fenton reaction (equation 1), H_2O_2 reacts with ferrous iron (Fe(II)) to produce ferric iron (Fe(III)), hydroxyl ion (^{-}OH), and hydroxyl radical ($HO^{\cdot}$):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{-}OH + HO^{\cdot} \qquad \square(1)$$

Fenton reaction-generated $HO^{\cdot}$ radicals oxidatively degrade a wide variety of hazardous organic compounds, including landfill leachates,[25] chlorinated aliphatics and aromatics,[26] dry-cleaning solvents,[27] pentachlorophenol (PCP),[28, 29] , Tetrachloroethene (PCE),[23] Trichloroethene (TCE),[30] and 1,1,2-Trichloroethane (TCA).[31] Fenton reaction-driven AOPs have also recently attracted attention as an alternative means for degrading 1,4-dioxane.[1, 32-34] Fenton reaction-driven AOPs are expensive, however, since the Fenton reagents Fe(II) and H_2O_2 must be continuously supplied to drive the degradation reaction. UV irradiation is often employed to induce Fe(III) reduction and photolytic radical production in photo-Fenton systems. The UV irradiation systems, however, are limited by UV light penetration,[35] and H_2O_2 must still be continuously supplied to drive the degradation reaction.

PROJECT OBJECTIVES

The main objectives of the present study were to i) design a microbially-driven Fenton reaction (Figure 1) that autocatalytically generates $HO^{\cdot}$ radicals and degrades 1,4-dioxane, TCE and PCE at circumneutral pH without the need for continual addition of exogenous H_2O_2 or UV irradiation to regenerate Fe(II), ii) optimize the 1,4-dioxane degradation rates by varying the duration and frequency of the aerobic and anaerobic incubation periods, iii) determine the pathway for 1,4-dioxane degradation by identifying the transient intermediates

produced during the 1,4-dioxane degradation process, and iv) determine the ability of the microbially-driven Fenton reaction to degrade various combinations of 1,4-dioxane, TCE, and PCE.

MATERIALS AND METHODS

Culture Medium and Chemical Reagents. *S. oneidensis* was routinely cultured aerobically on LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl).[36] 1,4-dioxane, TCE and PCE degradation experiments were conducted in a lactate (10 mM)-supplemented minimal salt solution (LS; pH 7.0).[37] Fe(III) citrate was prepared by previously described methods[38] and added at a final concentration of 10 mM. 1,4-dioxane, TCE, PCE, acetonitrile, sodium glyoxylate, ferrozine, heptafluorobutyric acid, tetrabutylammonium hydroxide mannitol, sodium benzoate, and thiourea were obtained from Sigma-Aldrich. Sodium glycolate was obtained from Acros Organics. Ethanediol mono and diformate were obtained from Frinton Laboratories, Inc.

Design of a Microbially Driven Fenton Reaction for 1,4-Dioxane Degradation. The toxicity of 1,4-dioxane to *S. oneidensis* was tested by growing cell cultures in the presence of potential source zone levels (10 mM and 50 mM) of 1,4-dioxane in LS medium under aerobic conditions for 48 h. Periodic culture samples were withdrawn and the number of colony forming units (CFUs) were measured to monitor cell viability. In 1,4-dioxane degradation experiments, *S. oneidensis* was grown aerobically in LB on a rotary shaker (200 rpm, 30 °C) to early stationary phase (OD_{600} = 1.5), harvested by centrifugation at 6000 g, washed and resuspended in LS medium to a final cell density of 2 x 10^9 cells/ml. Anaerobic stock solutions of Fe(III) citrate

and 1,4-dioxane were added to final concentrations of 10 mM each. The cell culture was allowed to reduce Fe(III) citrate for pre-selected time periods (45 min, 1.5 h, and 3 h) under anaerobic conditions maintained by continuous sparging with compressed nitrogen. Reactor temperature (25°C) and pH (7.0) were held constant in all experiments. Aerobic conditions were initiated by sparging the culture with compressed air for pre-selected time periods (45 min, 1.5 h, and 3 h). Cell density was monitored by determining CFUs on LB agar plates incubated at 30 °C for 72 h.

Design of a Microbially Driven Fenton Reaction for TCE and PCE Degradation. An initial concentration of 500 μM was chosen to test the capability of *S. oneidensis*-based fenton degradation of TCE and PCE. *S. oneidensis* was grown under similar conditions as that of 1,4-dioxane. The reaction consists of cells at a final cell density of 2 x 10^9 cells/ml in a 60 ml sealed glass bottles with PTFE-lined stoppers. Anaerobic stock solutions of Fe(III) citrate, TCE (or PCE) were added to final concentrations of 10 mM and 500 μM respectively. The cell culture was allowed to reduce Fe(III) citrate by continuous sparging with compressed nitrogen for 12 h in the absence of TCE (or PCE) followed by sparging with compressed air for 12 h to initiate the generation of fenton radicals. 500 uM of TCE (or PCE) was added into the cell suspension between aerobic and anaerobic cycling during which the gas flow was stopped and the concentrations were monitored for 8 h. Reactor temperature (25°C) and pH (7.0) were held constant in all experiments.

Analytical Techniques. HCl-extracted Fe(II) concentrations were determined with a previously described Ferrozine-based detection technique.[39] H_2O_2 concentrations were determined in Fe(III)-free aerobic cultures and during abiotic Fe(II) oxidation under aerobic condition using a previously described spectrophotometric assay.[40] A solution consisting of 400 mM KI, 50 mM NaOH, and 170 μM of ammonium molybdate tetrahydrate was mixed with

equal volume of 100 mM potassium hydrogen phthalate. Cell-free supernatants of aerobically grown cells were added and absorbance of the resulting solution was measured at 350 nm to determine H_2O_2 concentration. H_2O_2 spectrophotometric measurements were not possible in Fe(III)-containing LS medium due to Fe(III) interference.

Chemical Analysis of 1,4-Dioxane, TCE, PCE and Transient Degradation Products. Samples were withdrawn and centrifuged at 6000 g for 10 min. 1,4-dioxane was analyzed using a ZORBAX SB-C18 column with 20% aqueous acetonitrile as the mobile phase and a constant flow rate of 1.0 ml/min.[41] Chromatograms were generated at 190 nm for 1,4-dioxane detected at a retention time of 2.4 min. Ethyl acetate extracted samples of TCE and PCE were analyzed using a ZORBAX SB-C18 column with 60% aqueous acetonitrile as the mobile phase and a constant flow rate of 1.2 ml/min. Chromatograms were generated at 214 nm for TCE and PCE detected at a retention time of 4.2 and 7.1 min respectively. Lactate, acetate, and oxalate were analyzed via an ion chromatograph (Dionex, DX-300 Series) equipped with a Dionex IonPac® ICE-AS6 chromatography column and AMMS® ICE 300 suppressor. The Dionex DX-300 uses a CDM II detector with suppressed conductivity detection. Anion analysis was performed with 0.4 mM heptafluorobutyric acid as the eluent and 5 mM tetrabutylammonium hydroxide as the regenerant. Chromatograms were generated for lactate, acetate, oxalate, glyoxalate, and glycolate at retention times of 10.0, 13.3, 4.3, 6.5, and 9.0 min, respectively. Calibration curves were generated from standards to determine the concentrations of each compound.

Inhibition of the Microbially-Driven Fenton Reaction. A series of four control experiments were carried out to confirm that 1,4-dioxane was degraded by $HO^{\cdot}$ radicals generated by the *S. oneidensis*-driven Fenton reaction. All four sets of control experiments were

carried out under incubation conditions identical to those described above for the microbially-driven 1,4-dioxane-degradation system. In the first set of control experiments, 1,4-dioxane degradation was monitored under identical alternating anaerobic and aerobic periods with 15 mM NO_3^- replacing Fe(III) as electron acceptor. In the second set of control experiments, the HO □radical scavengi mM) were added to reactors carrying out an otherwise identical 1,4-dioxane degradation process.[29] In the third set of control experiments, 1,4-dioxane degradation was monitored in the absence of either Fe(III) citrate, 1,4-dioxane, or cells (abiotic control). In the fourth set of control experiments, 1,4-dioxane concentration was monitored in sealed anaerobic bottles to examine the effects of compressed air and nitrogen gas flow on volatilization of 1,4-dioxane in LS medium.

RESULTS AND DISCUSSION

1,4-Dioxane Degradation by the Microbially Driven Fenton Reaction. To test for 1,4-dioxane toxicity, *S. oneidensis* was grown aerobically in LS growth medium supplemented with either 10 mM or 50 mM 1,4-dioxane. Compared to aerobic growth rates in the absence of 1,4-dioxane, aerobic growth rates decreased 10-16% in the presence of either 10 mM or 50 mM 1,4-dioxane, respectively (Figure 5). *S. oneidensis* produced approximately 8 μ M of extracellular H_2O_2 after 60 min of aerobic growth in the absence of Fe(III) (Figure 6). H_2O_2 was not detected during abiotic Fe(II) oxidation experiments (data not shown), an indication that H_2O_2 produced in the Fe(III)-containing aerobic *S. oneidensis* cultures was most likely a by-product of microbial aerobic respiration.

To initiate $HO^{\cdot}$ radical production by the *S. oneidensis*-driven Fenton reaction (Figure

1), Fe(III)-containing *S. oneidensis* cultures were exposed to alternating aerobic and anaerobic periods of 45 min, 1.5 h, and 3 h. 1,4-dioxane concentrations were monitored abiotically in the absence of nitrogen or compressed air flow to examine the effects of gas flow on 1,4-dioxane volatility. 1,4-dioxane volatilization in control reactions with continuous gas flow resulted depletion of approximately 50% (3-h cycling period), 37% (1.5-h cycling period), and 35% (45 min cycling period) of initial 1,4-dioxane levels (Figures 2B, 3B & 4B). Fe(II) concentrations oscillated between 1.3 mM and 6.3 mM during the alternating aerobic and anaerobic 3-h cycling periods, while rates of Fe(II) oxidation (0.89 mM/h) and Fe(III) reduction (0.99 mM/h) remained constant in each 3-h cycling period (Fig. 2A). 1,4-dioxane was degraded during the first 24 h at rates of approximately 0.26-0.42 mM/h. The 1,4-dioxane degradation rates were 5-fold higher during the second set of aerobic-anaerobic cycles (24-33 h). A similar observation was made under cycling frequency of 1.5 h (approximately 3-fold higher during the second and third cycling periods, respectively). Reasons for the increased rates of 1,4-dioxane degradation are unknown, but may reflect carry over of 1,4-dioxane-degrading $HO^{\cdot}$ radicals produced during the preceding aerobic period. 10 mM 1,4-dioxane was completely degraded in 53 h with 3-h aerobic-anaerobic cycling periods (Figure 2B). The rate of 1,4-dioxane degradation was 35% slower during the 1.5-h cycling experiment (Figure 7B) and 25% slower during the 45-min cycling experiment (Figure 8B). The highest 1,4-dioxane degradation rates detected during the 3-h cycling experiment may be due to accumulation of higher concentrations of $HO^{\cdot}$ radicals during the longer aerobic cycling periods. 1,4-dioxane was also degraded during anaerobic periods (Figure 2B, Figures 7B and 8B), again potentially due to carry over of $HO^{\cdot}$ radicals produced during the preceding aerobic period. Cell viability analyses indicated that cell numbers decreased approximately 70% during the anaerobic periods, yet rebounded to normal levels

during subsequent aerobic periods (Figure 2F, Figures 7F and 8F). In chemical (purely abiotic) Fenton reaction systems, 1,4-dioxane was degraded at rates (5.4-8 mm/h) approximately 20-fold greater than the microbially-driven system designed in the present study. Such differences may be due to the high concentration of exogenous H_2O_2 (15 mM) added to drive the chemical system.[1, 5]

Inhibition of the Microbially-Driven 1,4-Dioxane Degradation Process. A series of biotic and abiotic control experiments were carried out to confirm that the degradation of 1,4-dioxane was catalyzed by $HO^{\cdot}$ radicals generated by *S. oneidensis*-driven Fenton reaction. 1,4-dioxane was not degraded when the microbially-driven Fenton process was carried out in the presence of the $HO^{\cdot}$-radical scavenging compounds mannitol (120 mM), thiourea (40 mM), or benzoate (8 mM) (Figure 3B). Fe(II) concentrations oscillated between 0.8 mM and 4.8 mM during the alternating aerobic and anaerobic 3-h cycling periods, while rates of Fe(II) oxidation (1.14 mM/h) and Fe(III) reduction (0.52 mM/h) remained constant in each cycle (Fig. 3A). The ability of the $HO^{\cdot}$-radical scavenging compounds to inhibit 1,4-dioxane degradation indicates that $HO^{\cdot}$-radicals are required to degrade 1,4-dioxane. In the second set of control experiments, the requirement for microbial Fe(III) reduction was tested during 1,4-dioxane degradation. Fe(III) was replaced by NO_3^- and an otherwise identical *S. oneidensis* culture was subjected to aerobic and anaerobic cycling periods of 3-h. 1,4-dioxane was not degraded with NO_3^- as electron acceptor (Figure 3B), an indication that microbial Fe(III) reduction was required for 1,4-dioxane degradation. 1,4-dioxane was also not degraded in the absence of Fe(III) or *S. oneidensis* cells (Figure 2B). These results indicate that microbial Fe(III) reduction was required to drive the 1,4-dioxane degradation process.

Identification of Transient Degradation Products During the Microbially-Driven 1,4-Dioxane Degradation Process. The major transient degradation products detected during 1,4-dioxane degradation by conventional (purely abiotic) Fenton reactions include acetate and oxalate, which are produced by $HO^{\cdot}$ radical attack of 1,4-dioxane producing the α-oxy radical as a key intermediate.[1] Acetate and oxalate were also detected as transient degradation products during the microbially-driven 1,4-dioxane degradation process. Acetate and oxalate concentrations oscillated during the aerobic and anaerobic cycling periods with both intermediates highest during the 49-71 h period and lowest during the 6-24 h period (Figures 2D and 2E). The period of highest acetate and oxalate concentrations (49-71 h) corresponds to the period when 1,4-dioxane was completely degraded. Acetate and oxalate were not detected in abiotic control incubations. Lactate was also completely depleted in all biotic reactions, and may have thus potentially contributed to acetate production (Figure 2C, 3C & Table 1, 3). Total acetate and oxalate produced in the microbially-driven Fenton reaction were up to 4-fold greater than the acetate and oxalate concentrations detected in the negative control incubations (Table 1). This observation was supported by up to 4-fold greater amount of 1,4-dioxane degraded in the microbially-driven Fenton reaction compared to the negative controls (Table 1, Figures 2B, 3B). Similar results were obtained with cycling frequencies of 1.5 h and 45 min (Table 3). These findings suggest that acetate and oxalate were produced as intermediates of 1,4-dioxane degradation as opposed to lactate oxidation. Since acetate and oxalate are produced by $HO^{\cdot}$ radical attack of 1,4-dioxane with the α-oxy radical as the key intermediate,[1] the detection of higher concentrations of acetate and oxalate in the microbially-driven Fenton reaction also suggests that 1,4-dioxane is degraded by $HO^{\cdot}$ radical.

A carbon mass balance was carried out to account for total carbon input and output in the 1,4-dioxane degradation system. Carbon output (acetate plus oxalate) during 1,4-dioxane degradation were up to 4-fold greater than the negative controls (Table 2). Similar observations were made with cycling frequencies of 1.5 h and 45 min. Carbon output (acetate plus oxalate) during 1,4-dioxane degradation were up to 15-fold greater than the negative controls (Table 4). Total carbon input (lactate plus 1,4-dioxane) could not completely account for total carbon output (acetate plus oxalate), most likely due to further reaction of acetate and oxalate with $HO^{\cdot}$ radical and unaccounted carbon loss, potentially as CO_2 (Table 2).

Proposed Pathway for 1,4-Dioxane Degradation. 1,4-dioxane reacts with $HO^{\cdot}$ radicals to form α-oxy radicals, which are the main precursors for subsequent degradation reactions. The α-oxy radicals follow two separate degradation pathways depending on oxygen levels in the Fenton reaction system. In oxygen-limited systems, the α-oxy radicals are degraded to acetate, formate, formaldehyde ,and oxalate, while in oxygen-replete systems, the α-oxy radicals are degraded to mono and diformate esters of 1,2-ethanediol and formaldehyde.[1, 5] In the present study, 1,4-dioxane degradation by the microbially driven Fenton reaction resulted in production of acetate and oxalate as the main transformation intermediates, while 1,2-ethanediol formate esters were not detected. This finding indicates that the microbially-driven Fenton reaction degraded 1,4-dioxane under oxygen-limited conditions. Although the optimized microbially-driven Fenton reaction included 3-h aerobic cycling periods, high rates of Fe(II) oxidation and microbial O_2 consumption during the 3-h aerobic cycling periods may have effectively resulted in oxygen-limited reactor conditions. In addition, formate, glycolate, glyoxylate were not detected during the microbially-driven 1,4-dioxane degradation process,

potentially due to the high reactivity of these intermediates with $HO^{\cdot}$ and H_2O_2.[1]

The microbially-driven Fenton reaction designed in the present study autocatalytically generates $HO^{\cdot}$ radicals that degrade 1,4-dioxane at circumneutral pH, with optimal 1,4-dioxane degradation rates detected during 3-h aerobic and anaerobic cycling periods. Despite the 3-h aerobic cycling periods, transient intermediates detected during the 1,4-dioxane degradation process indicated that the microbially-driven Fenton reaction degraded 1,4-dioxane under oxygen-limited conditions. Furthermore, the 1,4-dioxane degradation process did not require continual addition of exogenous H_2O_2 or UV irradiation to regenerate Fe(II) as Fenton substrates. The microbially-driven Fenton reaction may thus provide a foundation for development of alternative in situ remediation technologies that degrade 1,4-dioxane or other contaminants (e.g., PCP[28, 29], PCE,[23] TCE,[30] and TCA [31]) susceptible to attack by $HO^{\cdot}$ radicals generated by the Fenton reaction.

TCE and PCE Degradation by the Microbially Driven Fenton Reaction.

To initiate $HO^{\cdot}$ radical production by the *S. oneidensis*-driven Fenton reaction, Fe(III)-containing *S. oneidensis* cultures were exposed to alternating aerobic and anaerobic periods for 8 h. TCE and PCE degradation rates in the reactor containing cells + Fe(III) + TCE (or PCE) were 6- and 3-fold higher than the control incubations (cells or Fe(III) omitted) respectively. However, TCE and PCE degradation was the most effective during the second phase of aerobic incubation resulting in 95% and 71% degradation in 8 h respectively (Figure 4A & B). Chemical fenton systems degrade TCE and PCE at rates 2- and 25-fold greater than the microbially-driven system developed in the present study. Such differences may be due to the high concentrations of exogenous H_2O_2 (170 μM[42] and 2 M[23] respectively) added the drive the chemical system.

Figure 1.

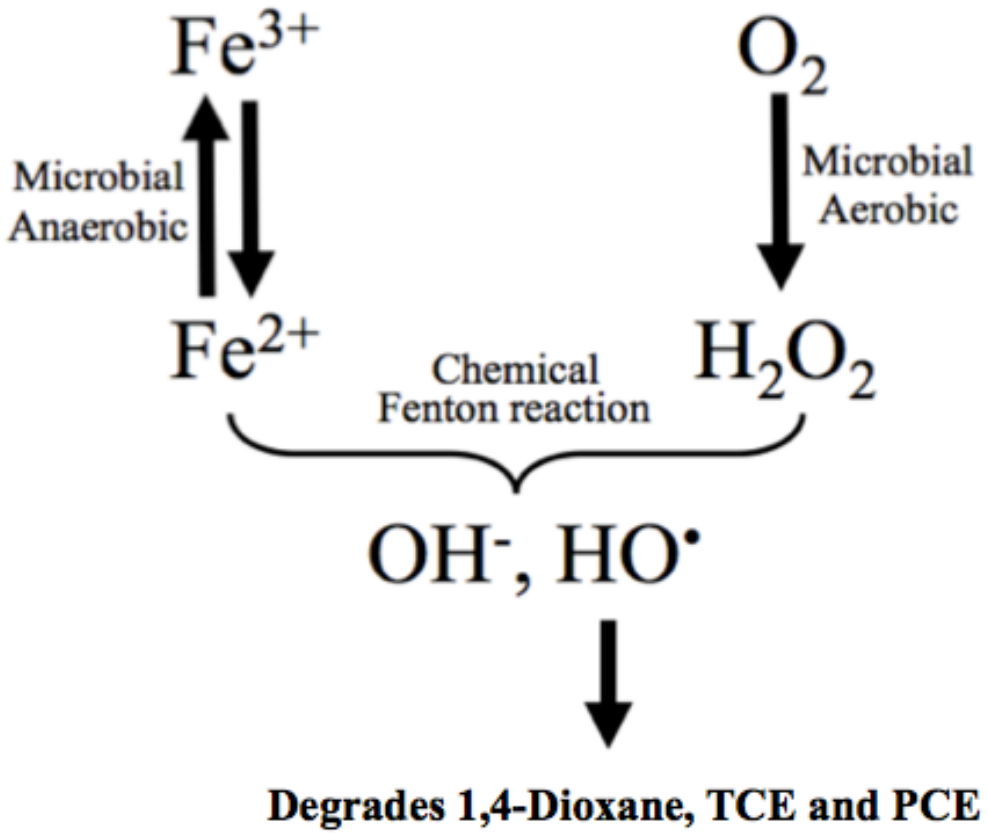
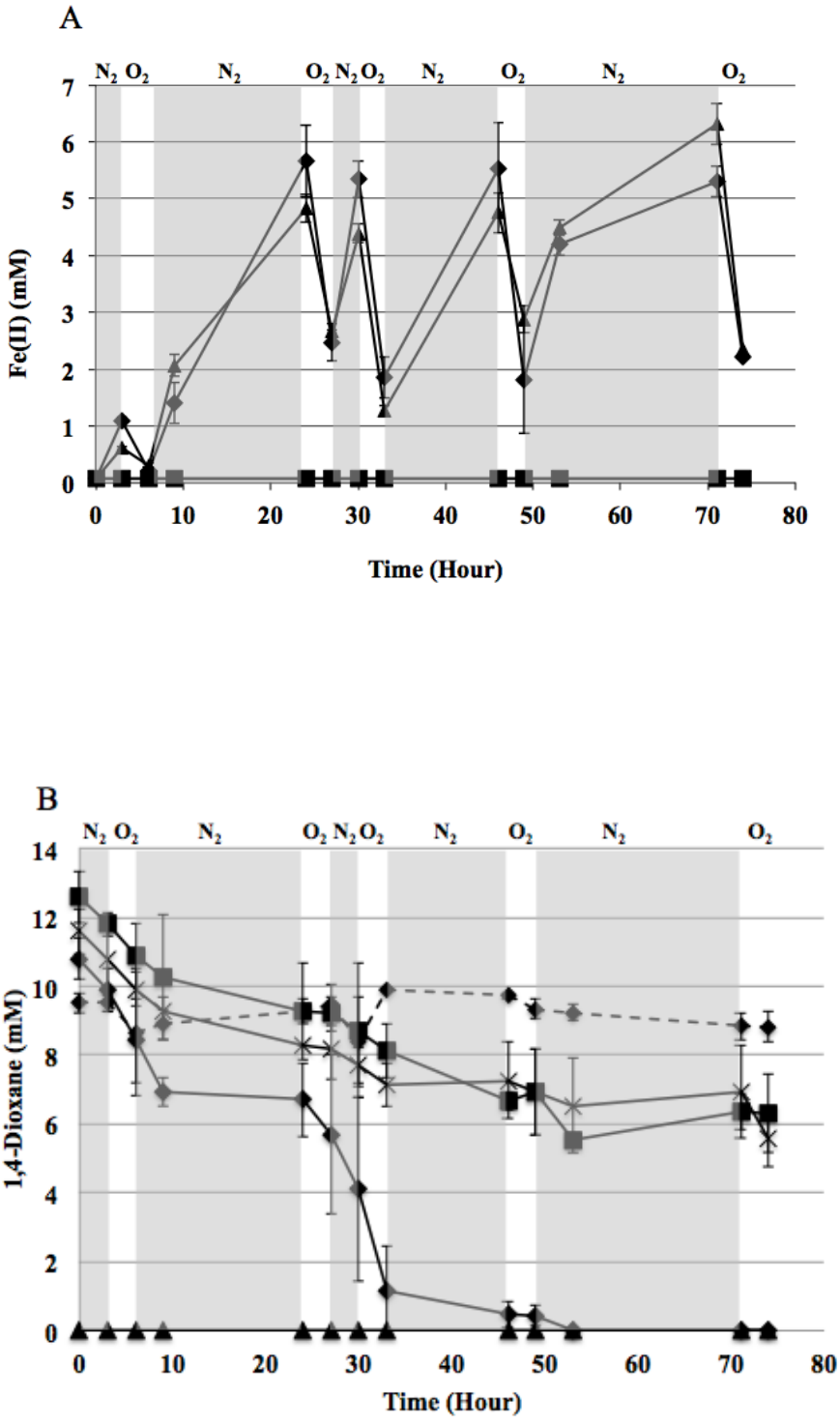
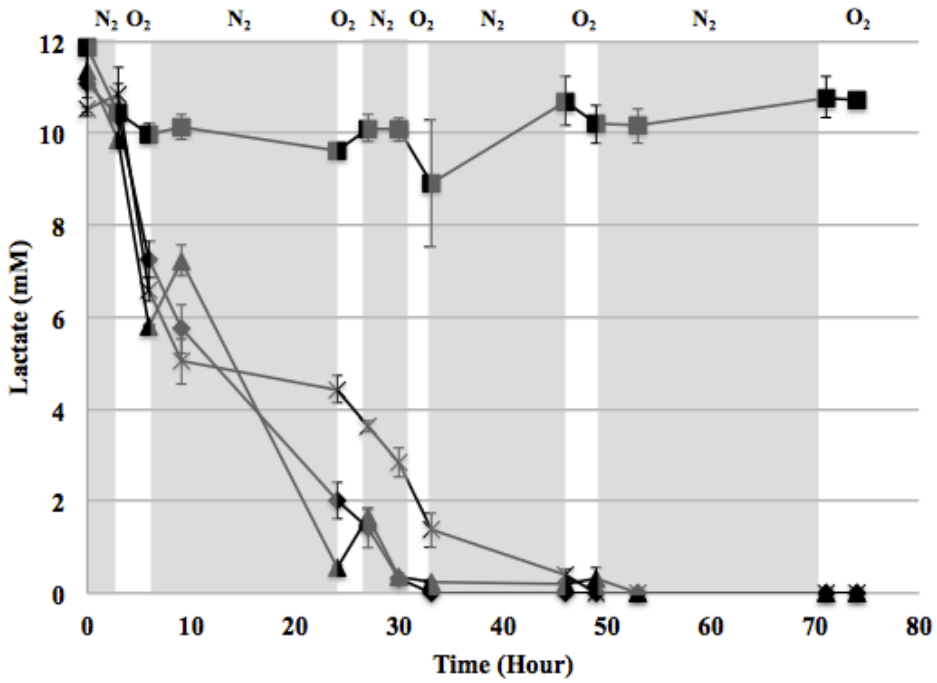


Figure 1. Overall strategy for generation of HO$^{\cdot}$ radicals by the *Shewanella oneidensis*-driven Fenton system. Fe(II) produced during anaerobic phases interacts chemically via the Fenton reaction with H_2O_2 produced during aerobic phases to yield HO$^{\cdot}$ radicals that oxidatively degrade 1,4-dioxane.

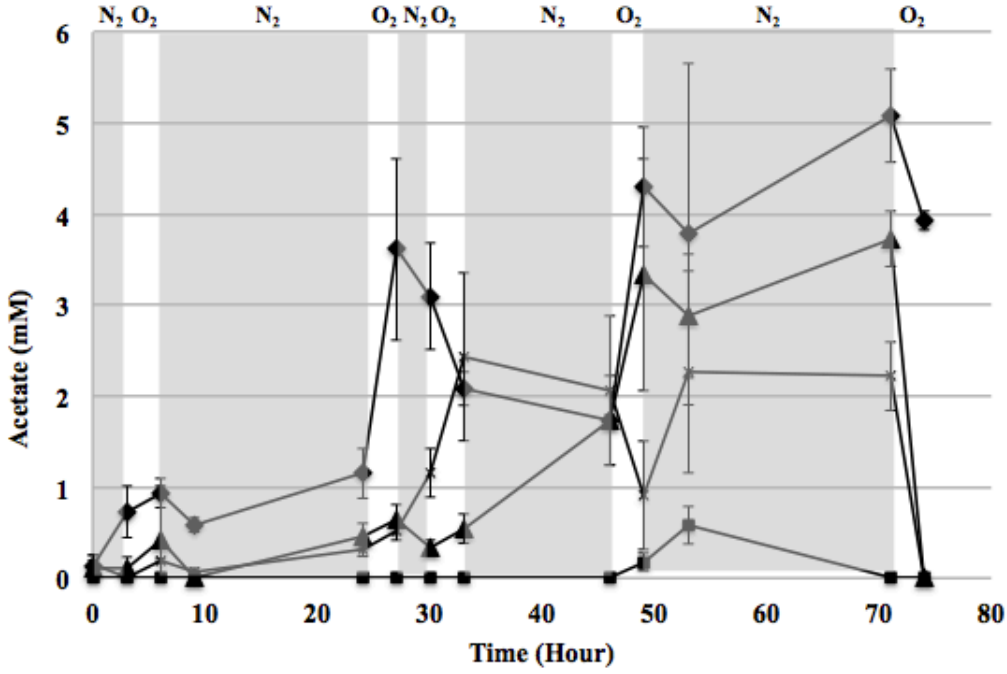
Figure 2.


A
N2 O2
N2
O2 N2 O2
N2
O2
N2
O2
Fe(II) (mM)
Time (Hour)
B
1,4-Dioxane (mM)
Time (Hour)

C


N2 O2 N2 O2 N2 O2 N2 O2 N2 O2
Lactate (mM)
Time (Hour)

D


N2 O2 N2 O2 N2 O2 N2 O2 N2 O2
Acetate (mM)
Time (Hour)

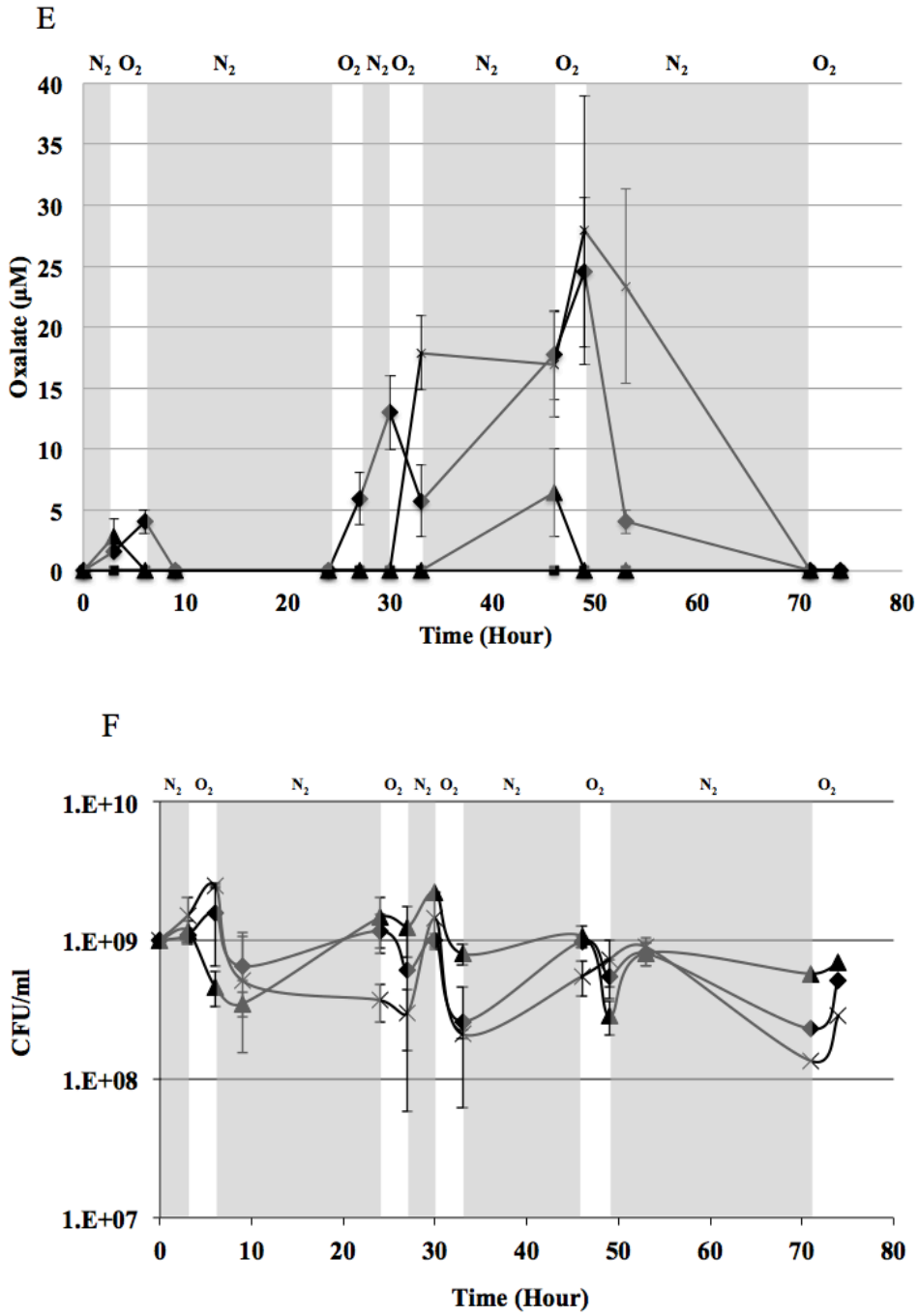
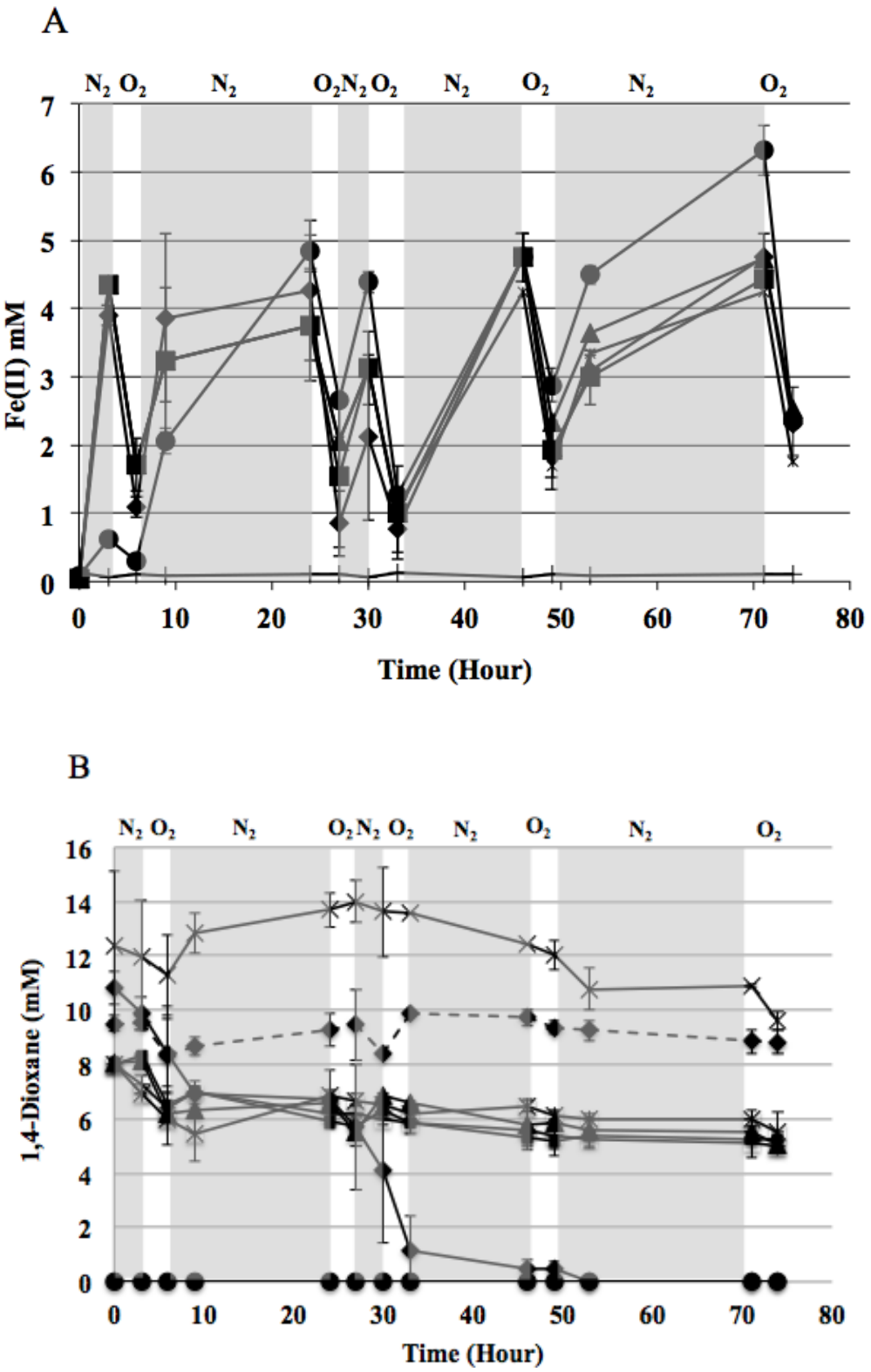
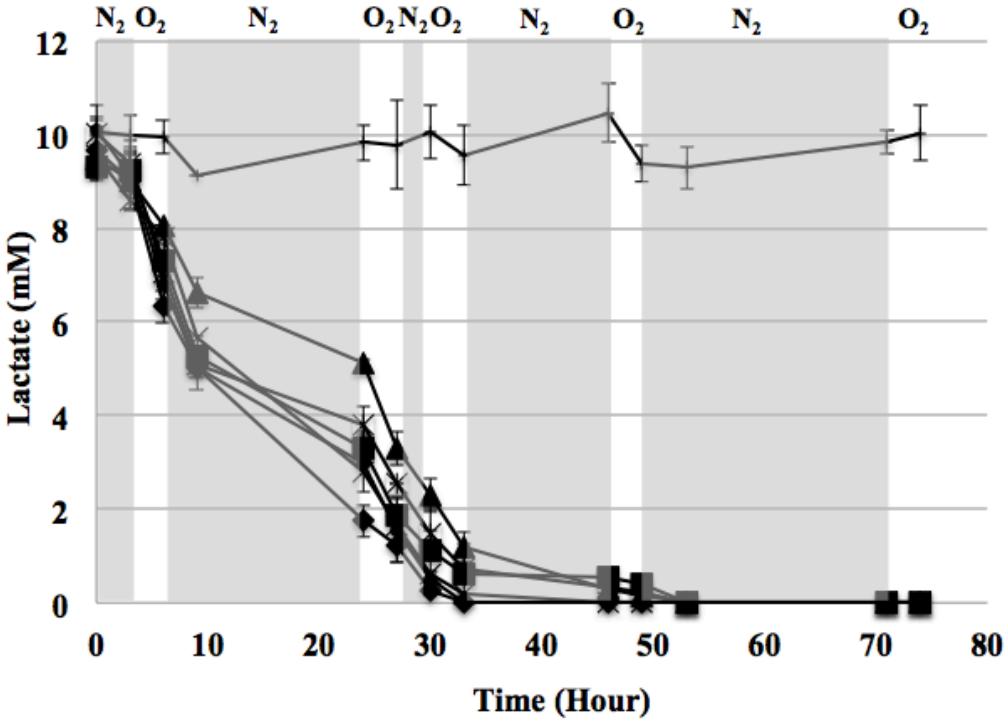


Figure 2. Microbially-driven Fenton degradation of 1,4-dioxane (10 mM) under aerobic and anaerobic cycling frequency of 3 h: (A) Fe(II); (B) 1,4-dioxane; (C) lactate; (D) acetate; (E) oxalate; (F) cell count. Symbols: ◆, cells + 1,4-dioxane + Fe(III); ■, cells omitted; ▲, 1,4-dioxane omitted; ×,Fe(III) omitted; dashed ◆, No Gas control**.** Incubations were carried in two parallel yet identical cultures and error bars indicate standard deviations between cultures.

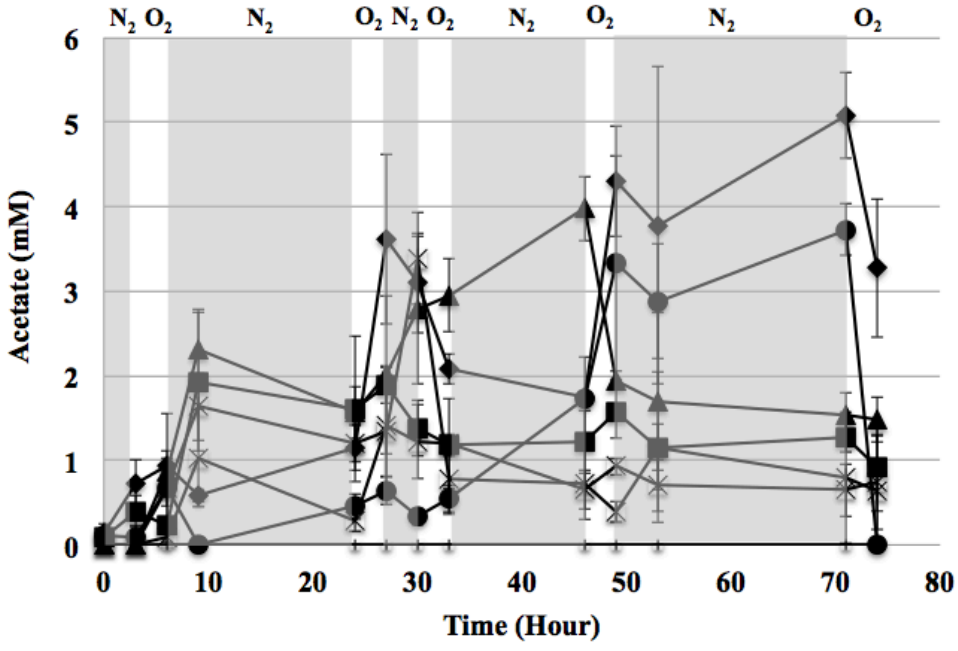
Figure 3.


A
N2 O2 N2 O2N2 O2 N2 O2 N2 O2
Fe(II) mM
Time (Hour)
B
N2 O2 N2 O2N2 O2 N2 O2 N2 O2
1,4-Dioxane (mM)
Time (Hour)

C


N2 O2 N2 O2 N2O2 N2 O2 N2 O2
12
10
8
6
4
2
0
Lactate (mM)
0 10 20 30 40 50 60 70 80
Time (Hour)

D


N2 O2 N2 O2 N2O2 N2 O2 N2 O2
6
5
4
3
2
1
0
Acetate (mM)
0 10 20 30 40 50 60 70 80
Time (Hour)

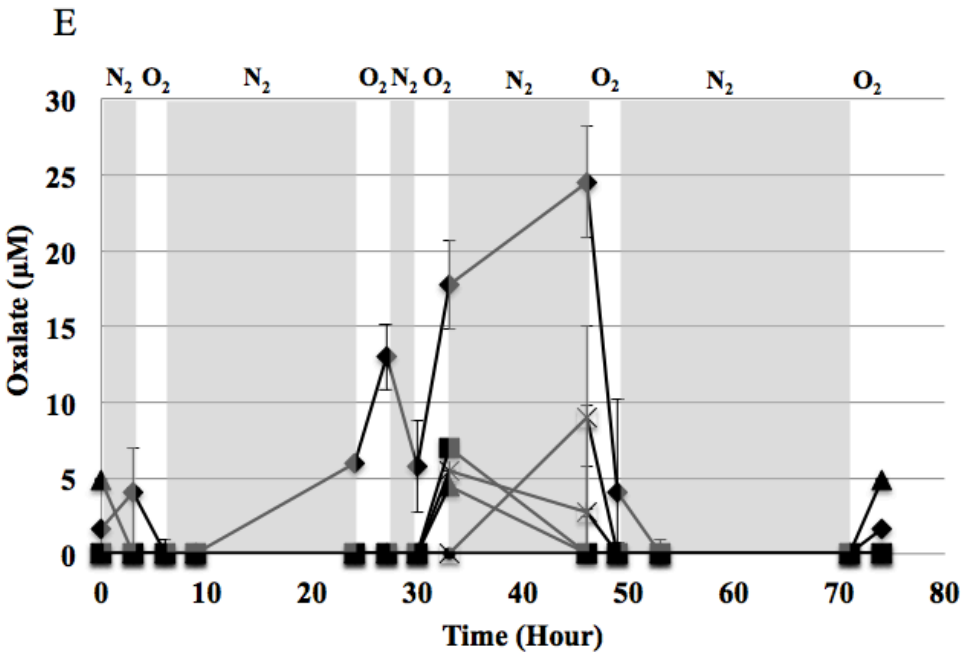
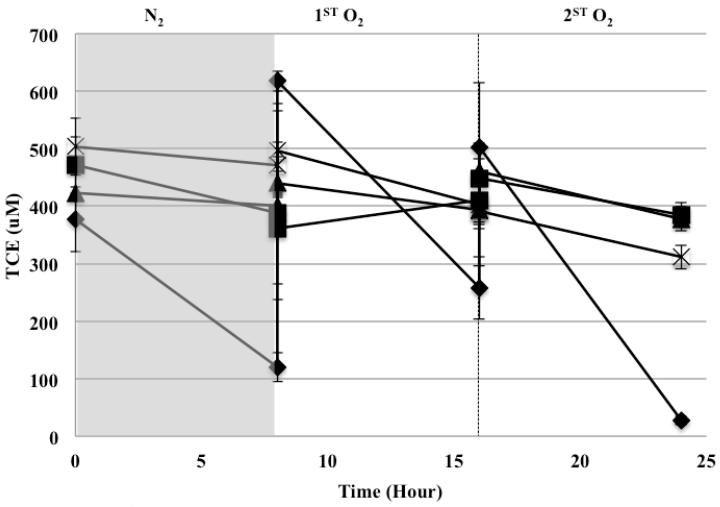


Figure 3. Microbially-driven Fenton degradation of 1,4-dioxane (10 mM) under aerobic and anaerobic cycling frequency of 3 h in the presence of inhibitors: (A) Fe(II); (B) 1,4-dioxane; (C) lactate; (D) acetate; (E) oxalate. Symbols: ◆, cells + 1,4-dioxane + Fe(III); •, mannitol; ▲, thiourea; *, Benzoate; ■| , cells omitted; □, 1,4-dioxane omitted; dashed ◆, No Gas control**.** Incubations were carried in two parallel yet identical cultures and error bars indicate standard deviations between cultures.

Figure 4 **A**



B

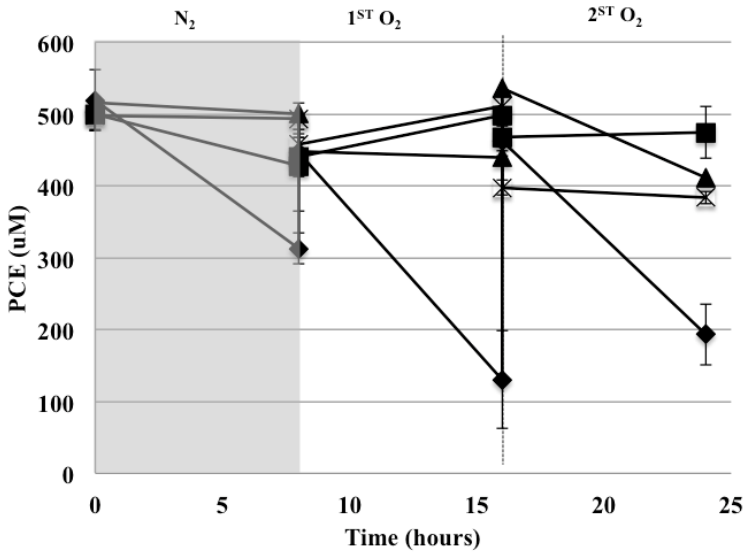


Figure 4. Microbially-driven Fenton degradation of TCE and PCE (500 μ M each) under aerobic and anaerobic cycling conditions: (A) Fenton degradation of TCE; (B) Fenton degradation of PCE. Symbols: ◆, cells + 1,4-dioxane + Fe(III); ■, cells omitted; ▲, 1,4-dioxane omitted; ×,Fe(III) omitted**.** Incubations were carried in two parallel yet identical cultures and error bars indicate standard deviations between cultures.

Figure 5.

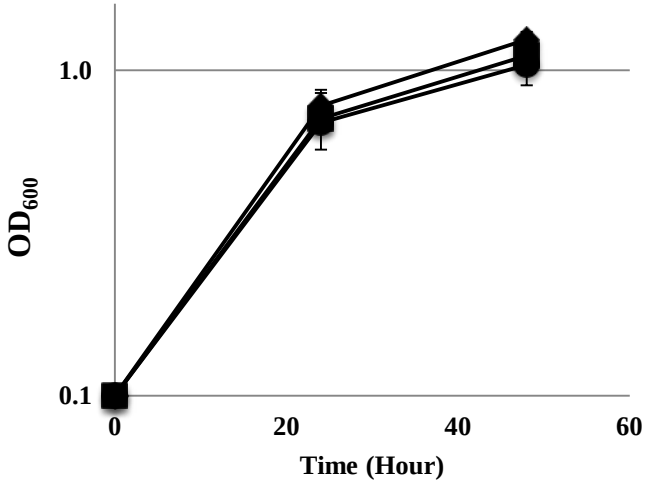


Figure 5. Growth profile of *Shewanella oneidensis* in the presence of 10 mM (◆), 50 mM (▲) and 0 mM (■) 1,4-dioxane. Cell cultures were grown in LS media under aerobic conditions in the presence of 10 mM lactate as electron donor. Experiments were performed in duplicates; error bars indicate standard deviations.

Figure 6.

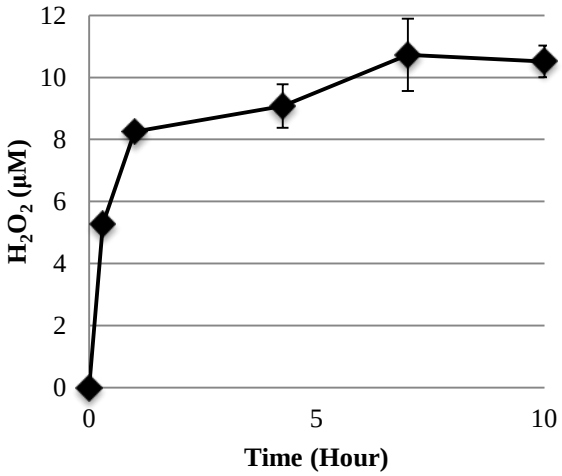
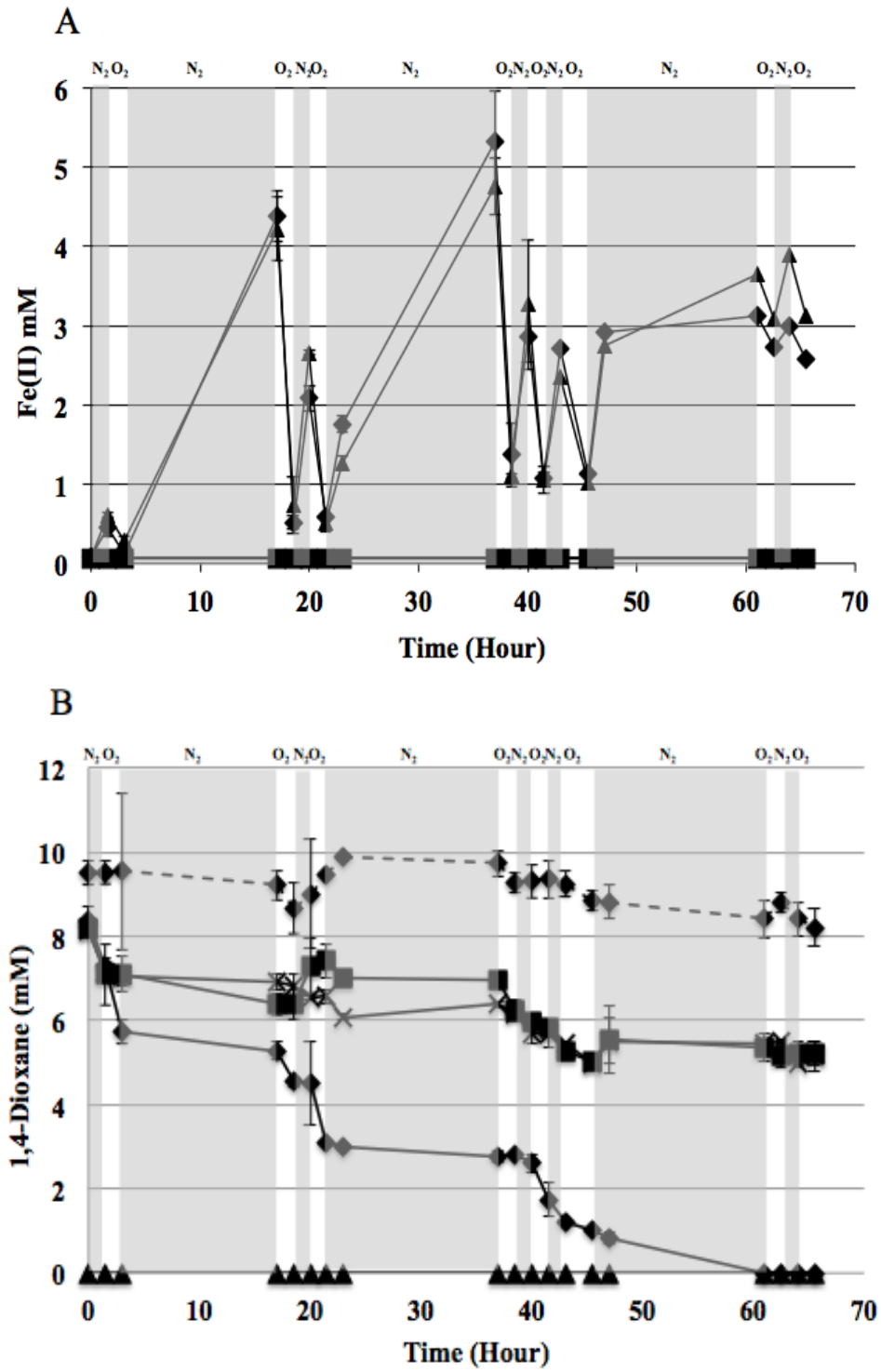
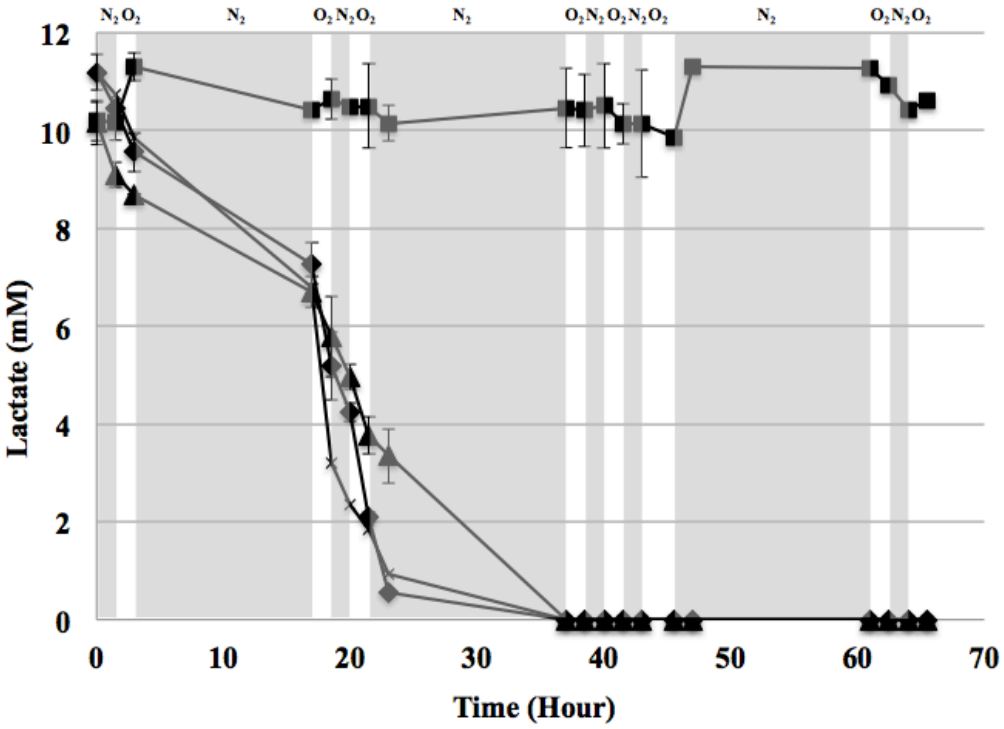


Figure 6. H_2O_2 production by *Shewanella oneidensis* exposed to aerobic conditions for 10 h in iron-free LS media. Experiments were performed in duplicates; error bars indicate standard deviations.

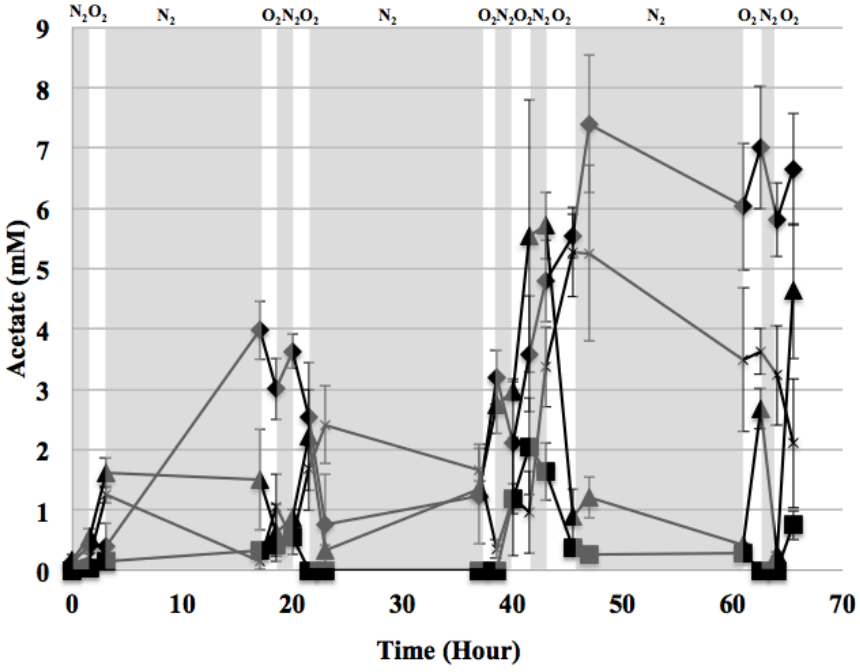
Figure 7.


A
N2 O2
N2
O2 N2 O2
N2
O2 N2 O2 N2 O2
N2
O2 N2 O2
Fe(II) mM
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
Time (Hour)
B
N2 O2
N2
O2 N2 O2
N2
O2 N2 O2 N2 O2
N2
O2 N2 O2
1,4-Dioxane (mM)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
Time (Hour)

C


N_2O_2
N_2
$O_2N_2O_2$
N_2
$O_2N_2O_2N_2O_2$
N_2
$O_2N_2O_2$
Lactate (mM)
Time (Hour)

D


N_2O_2
N_2
$O_2N_2O_2$
N_2
$O_2N_2O_2N_2O_2$
N_2
$O_2N_2O_2$
Acetate (mM)
Time (Hour)

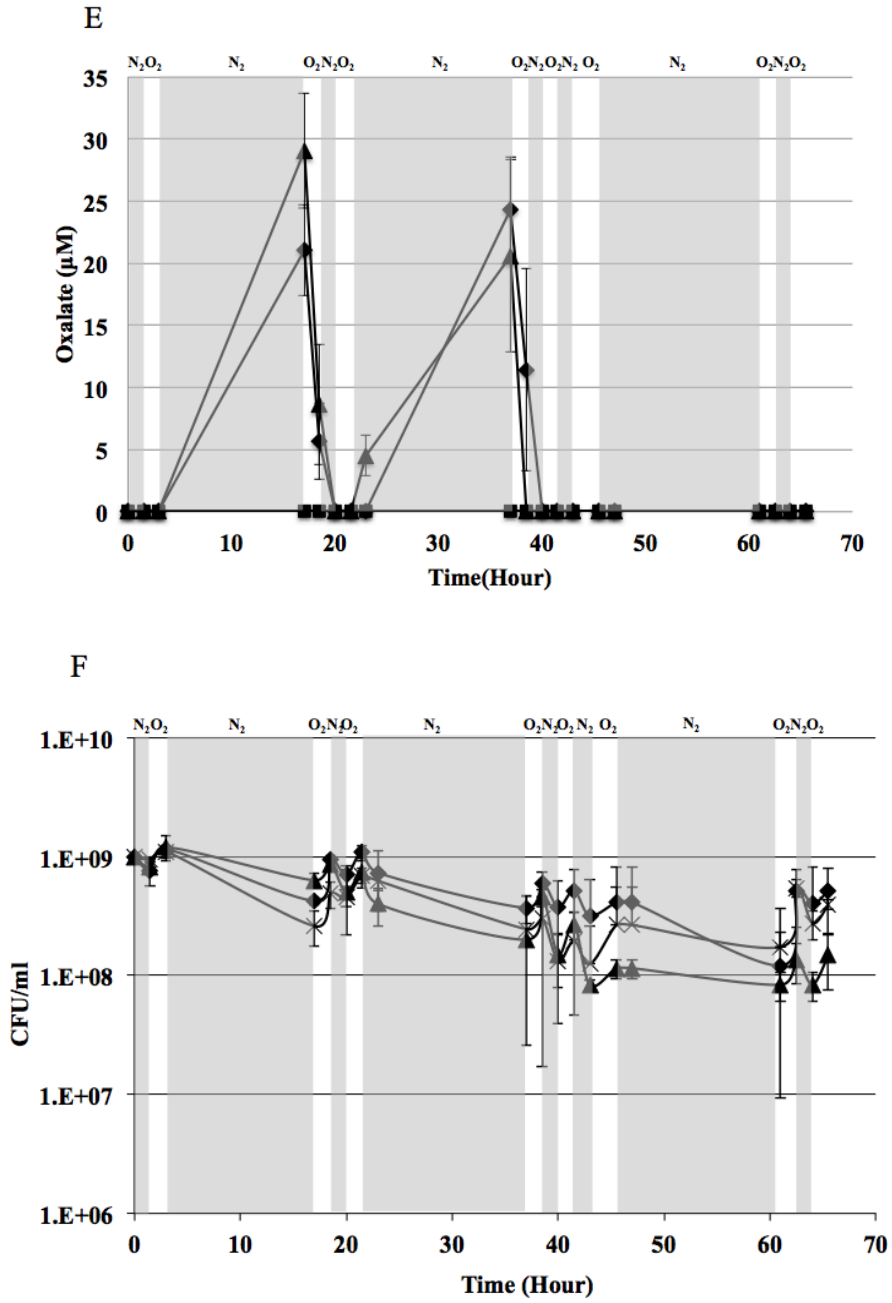
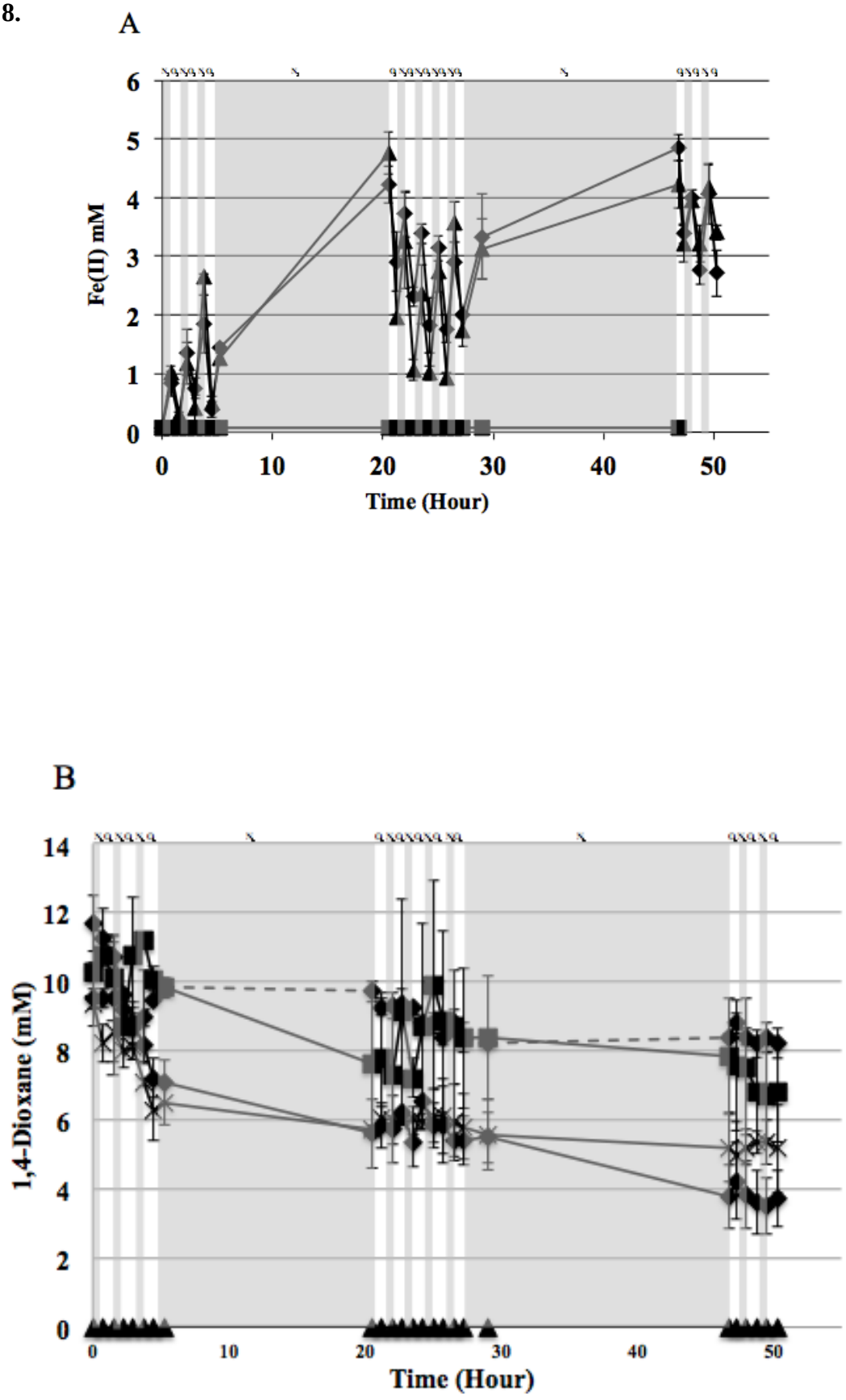
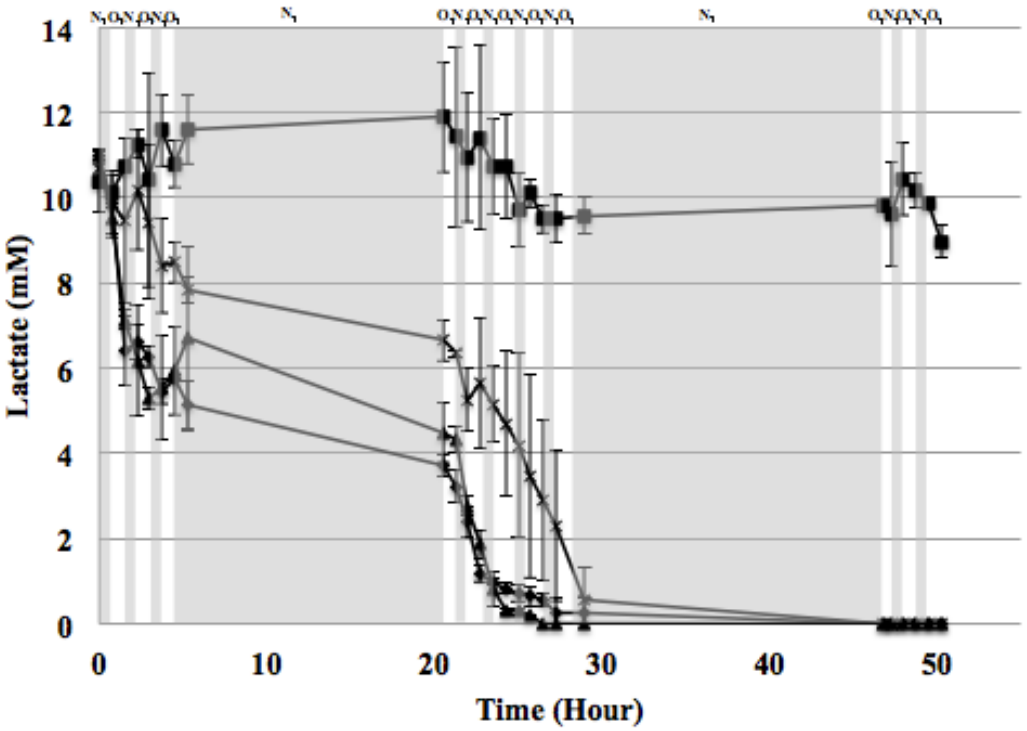


Figure 7. Microbially-driven Fenton degradation of 1,4-dioxane (10 mM) under aerobic and anaerobic cycling frequency of 1.5 h: (A) Fe(II); (B) 1,4-dioxane; (C) lactate; (D) acetate; (E) oxalate; (F) cell count. Symbols: ◆, cells + 1,4-dioxane + Fe(III); ■, cells omitted; ▲, 1,4-dioxane omitted; ×,Fe(III) omitted; dashed ◆, No Gas control**.** Incubations were carried in two parallel yet identical cultures and error bars indicate standard deviations between cultures.

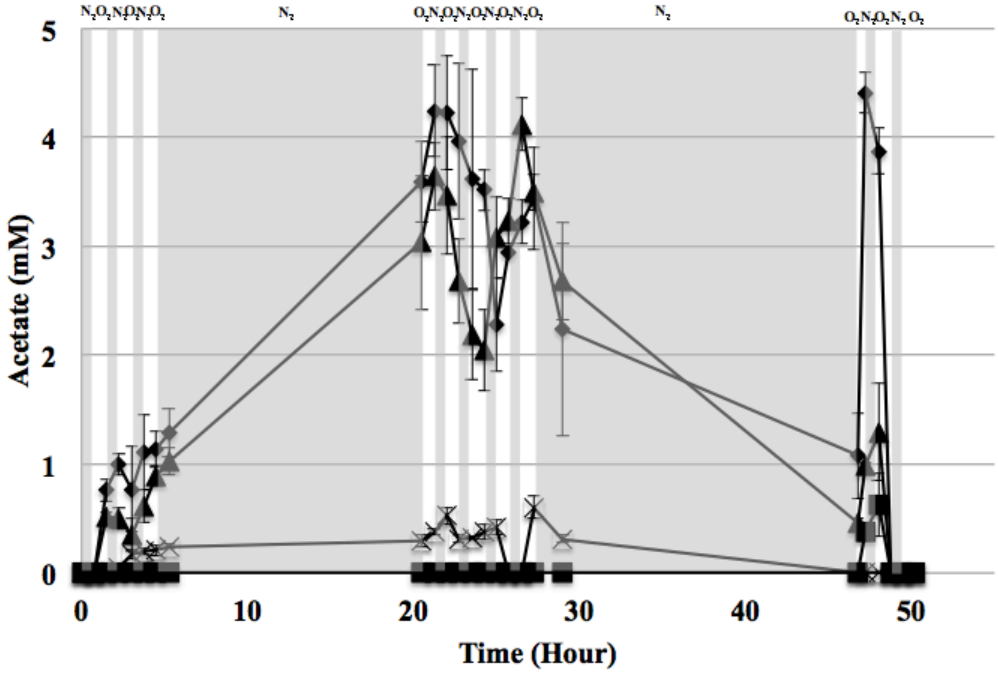
Figure 8.


A
Fe(II) mM
0
1
2
3
4
5
6
0
10
20
30
40
50
Time (Hour)
B
1,4-Dioxane (mM)
0
2
4
6
8
10
12
14
0
10
20
30
40
50
Time (Hour)

C


Lactate (mM)
Time (Hour)
0
2
4
6
8
10
12
14
0
10
20
30
40
50

D


Acetate (mM)
Time (Hour)
0
1
2
3
4
5
0
10
20
30
40
50

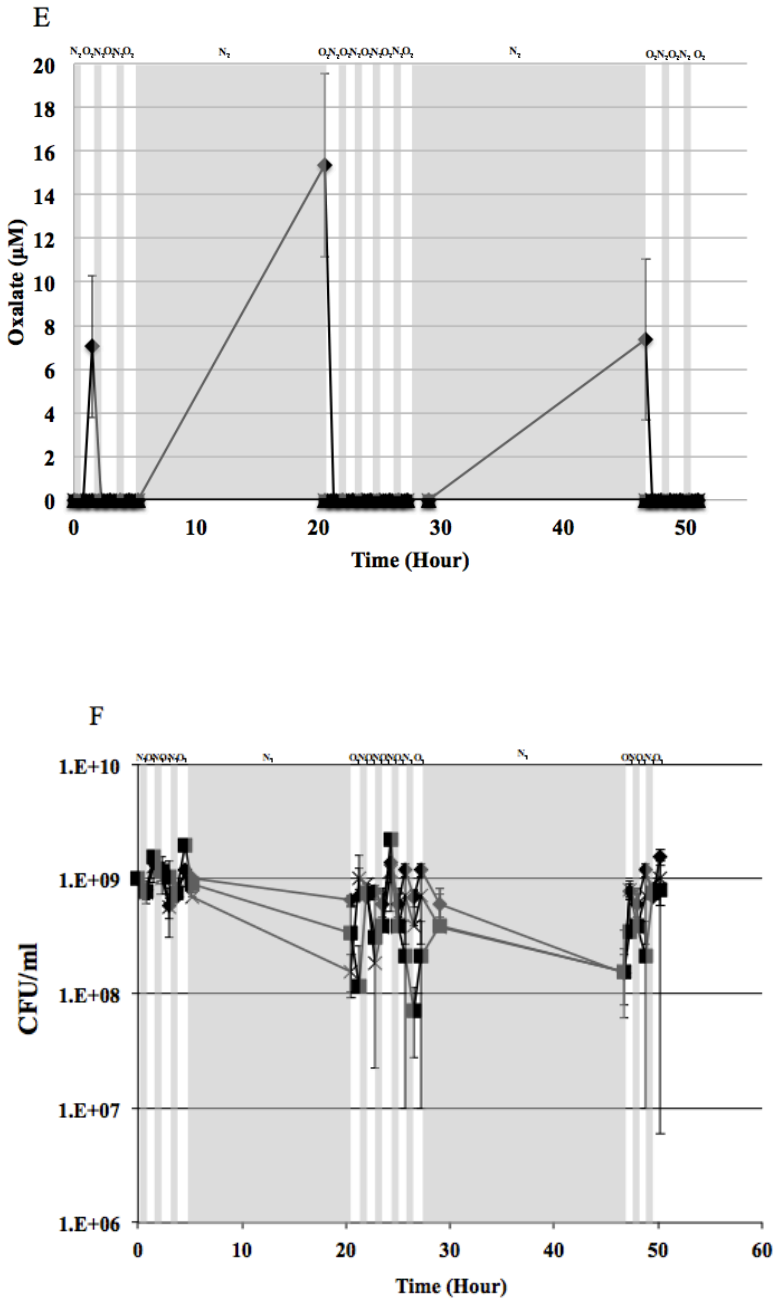


Figure 8. Microbially-driven Fenton degradation of 1,4-dioxane (10 mM) under aerobic and anaerobic cycling frequency of 45 min: (A) Fe(II); (B) 1,4-dioxane; (C) lactate; (D) acetate; (E) oxalate; (F) cell count. Symbols: ◆, cells + 1,4-dioxane + Fe(III); ■, cells omitted; ▲, 1,4-dioxane omitted; ×,Fe(III) omitted; dashed ◆, No Gas control. Incubations were carried in two parallel yet identical cultures and error bars indicate standard deviations between cultures.

Table 1. Substrate degradation and transient intermediate production during microbially-driven Fenton degradation of 1,4-dioxane*

	3 h cycling			
	Degraded (mM)		Produced (mM)	
	1,4-Dioxane	Lactate	Acetate	Oxalate
Cells + 1,4-dioxane+Fe(III)	10.8 ± 0.6	11.1 ± 0.6	7.7 ± 0.8	0.04 ± 0.002
Cells omitted	6.3 ± 0.4	1 ± 0.3	0	0
1,4-Dioxane omitted	0	11.4 ± 0.4	4.8 ± 0.4	0.01 ± 0.002
Fe(III) omitted	6 ± 0.2	10.5 ± 0.1	3.9 ± 0.4	0.03 ± 0.004
Mannitol	2.8 ± 0.5	9.3 ± 0.3	2.8 ± 0.3	0.01 ± 0.009
Thiourea	3 ± 0.01	9.5 ± 0.03	4.7 ± 0.6	0.01 ± 0.004
Benzoate	2.5 ± 0.6	10 ± 0.6	4.6 ± 0.5	0.01 ± 0.003
Nitrate	2.8 ± 2.4	9.5 ±0.1	2.5 ± 0.3	0.01 ± 0.006

*Experiments were performed in duplicates; errors indicate standard deviations.

Table 2. Total input and output carbon detected during microbially-driven Fenton degradation of 1,4-dioxane*

	3 h cycling			
	Input Carbon (mmol)		Output Carbon (mmol)	
	Lactate + 1,4-Dioxane	1,4-Dioxane degraded	Acetate	Oxalate
Cells+1,4-dioxane+Fe(III)	70	43.2 ± 2.5	15.4 ± 1.6	0.08 ± 0.004
Cells omitted	70	25.2 ± 1.6	0	0
1,4-Dioxane omitted	30	0	9.6 ± 0.8	0.02 ± 0.004
Fe(III) omitted	70	24 ± 0.7	7.8 ± 0.8	0.06 ± 0.008
Mannitol	70	11 ± 2	5.6 ± 0.6	0.02 ± 0.018
Thiourea	70	12 ± 0.04	9.4 ± 1.2	0.02 ± 0.008
Benzoate	70	10 ± 2.2	9.2 ± 1	0.02 ± 0.006
Nitrate	70	11.2 ± 9.6	5 ± 0.6	0.02 ± 0.012

*Experiments were performed in duplicates; errors indicate standard deviations.

*Carbon mmols per liter of reaction volume

Table 3. Total Substrate degradation and intermediate production during microbially-driven Fenton degradation of 1,4-dioxane*

	1.5 h cycling				45 min cycling			
	Degraded (mM)		Produced (mM)		Degraded (mM)		Produced (mM)	
	1,4-Dioxane	Lactate	Acetate	Oxalate	1,4-Dioxane	Lactate	Acetate	Oxalate
Cells + 1,4-dioxane+Fe(III)	8.4 ± 0.4	11.2 ± 0.4	14.1 ± 0.5	0.05 ± 0.003	8 ± 0.1	11 ± 0.1	9 ± 0.3	0.03 ± 0.003
Cells omitted	3 ± 0.2	0	3.4 ± 0.2	0	3.5 ± 1.7	1.4 ± 0.3	0.6 ± 0.2	0
1,4-Dioxane omitted	0	10.2 ± 0.4	10.6 ± 0.8	0.05 ± 0.005	0	10.4 ± 0.7	6.7 ± 0.3	0
Fe(III) omitted	3 ± 0.2	11.2 ± 0.4	9.3 ± 0.6	0	4.2 ± 1.9	10.7 ± 0.4	1.2 ± 0.04	0

*Experiments were performed in duplicates; errors indicate standard deviations.

Table 4. Total input and output carbon during microbially-driven Fenton degradation of 1,4-dioxane*

	1.5 h cycling				45 min cycling			
	Input Carbon (mmol)		Output Carbon (mmol)		Input Carbon (mmol)		Output Carbon (mmol)	
	Lactate + 1,4-Dioxane	1,4-Dioxane degraded	Acetate	Oxalate	Lactate + 1,4-Dioxane	1,4-Dioxane degraded	Acetate	Oxalate
Cells+1,4-dioxane+Fe(III)	70	33.5 ± 1.4	28.2 ± 1	0.1 ± 0.006	70	31.9 ± 0.3	18 ± 0.6	0.06 ± 0.006
Cells omitted	70	11.9 ± 0.8	6.8 ± 0.4	0	70	14 ± 6.9	1.2 ± 0.4	0

1,4-Dioxane omitted	30	0	21.2 ± 1.6	0.1 ± 0.01	30	0	13.4 ± 0.6	0
Fe(III) omitted	70	11.9 ± 0.9	18.6 ± 1.2	0	70	16.7 ± 7.7	2.4 ± 0.08	0

*Experiments were performed in duplicates; errors indicate standard deviations.

ACKNOWLEDGEMENT

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