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Abstract:	Shewanella drives a variety of environmentally important processes, including the biogeochemical cycling of carbon, metals, metalloids, and radionuclides. The ability of Shewanella to deliver electrons extracellularly also renders this genus valuable for applications of contaminant remediation and energy generation in water treatment processes. Although the first Shewanella species was isolated and studied as a model metal-reducing microorganism over thirty years ago, only recently has research focused on employing Shewanella to drive water treatment processes. This chapter examines current and prospective applications of Shewanella to water treatment issues, highlighting biochemical details associated with each technology. The technologies include the remediation of metal-contaminated environments (Sections 1 and 2), the generation of electricity from wastewater streams (Section 3), the removal of hazardous contaminants under anaerobic conditions (Section 4), and precious metal recovery in combination with formation of novel biocatalysts (Section 5).

Application of *Shewanella to* Water Treatment Issues

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Anthropogenic activities have led to an increase of a wide range of organic and inorganic contaminants in aquatic environments. Biological water treatment relies on the activity of a wide variety of microorganisms to degrade the organic and inorganic compounds in waste waters. The genus *Shewanella* of the class Gammaproteobacteria represent a cosmopolitan group of facultatively anaerobic bacteria with high genetic diversity [1-3] and versatile respiratory [4-9] and central metabolic capabilities [4, 10]. Over 60 recognized species of *Shewanella* [9, 11, 12] have been isolated from aquatic and terrestrial environments over a wide range of salt concentrations, temperatures, and pressures [1, 8, 13, 14]. The distinctive trait of many *Shewanella* species is the ability to reductively transform a variety of extracellularly-localized organic and inorganic electron acceptors in both solid phase and water-soluble forms spanning a wide continuum of redox potentials [Fig. 1].

Shewanella drives a variety of environmentally important processes [1-9], including the biogeochemical cycling of carbon, metals, metalloids, and radionuclides. The ability of *Shewanella* to deliver electrons extracellularly also renders this genus valuable for applications of contaminant remediation and energy generation in water treatment processes. Although the first *Shewanella* species was isolated and studied as a model metal-reducing microorganism over thirty years ago [15], only recently has research focused on employing *Shewanella* to drive water treatment processes. This chapter examines current and prospective applications of *Shewanella* to water treatment issues, highlighting biochemical details associated with each technology. The technologies include the remediation of metal-contaminated environments (Sections 1 and 2), the generation of electricity from wastewater streams (Section 3), the removal of hazardous contaminants under anaerobic conditions (Section 4), and precious metal recovery in combination with formation of novel biocatalysts (Section 5).

1. *Extracellular electron transfer applied to water treatment technologies*

Biochemical pathway for extracellular electron transfer by Shewanella

Heavy metals, metalloids and radionuclides may be toxic and accumulate in living organisms [16]. Many *Shewanella* transform a wide variety of metals, metalloids, and radionuclides including toxic uranium and technetium [17]. The ability of *Shewanella* to reductively transform a diverse array of toxic compounds often relies on extracellular electron transfer (EET). EET is an anaerobic respiratory pathway to reduce electron acceptors unable to contact inner membrane (IM)-localized electron transport chains typical of aerobic respiration [18]. Transition metals such as Mn(IV) and Fe(III) oxides, for example, exist as sparingly soluble amorphous or crystalline (oxy)hydroxides at circumneutral pH and are unable to enter the cell to interact with IM-localized electron transport chains. To overcome this problem, *Shewanella* utilizes EET to transfer electrons from internal electron donors to external electron acceptors [19-21].

The biochemical machinery that transfers electrons from the electron donor to the cell surface is composed of dehydrogenases located at the head end of the electron transport chain to oxidize electron donors, pump protons to the periplasmic space, and transfer electrons to menaquinone [Fig. 2]. Reduced menaquinone transfers electrons to tetraheme *c*-type cytochrome CymA, which in turn transfers electrons to periplasmic decaheme *c*-type cytochrome MtrA. The outer-membrane (OM) β-barrel protein MtrB facilitates interaction and electron transfer between MtrA and OM decaheme *c*-type cytochrome MtrC. The type II protein secretion system secretes MtrC through the OM secretin protein GspD, and MtrC associates with the outside face of the OM with the aid of lipid tails. The MtrCAB complex functions as an extracellular electron conduit that

transfers electrons to extracellular solid iron[III] oxides and other electron acceptors [22]. Several novel strategies facilitate transfer to external Fe(III) oxides, including (a) direct reduction of external Fe(III) oxides with cell-surface localized *c*-type cytochromes [6, 23, 24]; (b) localization of *c*-type cytochromes along extracellular nanowires where they deliver electrons to external Fe(III) oxides; (c) delivery of electrons to external Fe(III) oxides via endogenous or exogenous electron shuttles [25-28] and (d) nonreductive dissolution of external Fe(III) oxides to form more readily reducible soluble organic-Fe(III) complexes [29-33].

Reductive precipitation as an alternative radionuclide remediation strategy

Subsurface contamination by the radionuclides uranium (U) and technetium (Tc) as byproducts of nuclear fuel processing is a global environmental problem. The mobility of radionuclides in groundwater largely depends on site-specific biogeochemical conditions. Under oxidizing conditions, both uranyl (U(VI)) and pertechnetate (Tc(VII)) form highly water soluble and mobile complexes with carbonate at pH >5. Under reducing conditions, U and Tc occur predominately as U(IV) and Tc(IV) oxides, respectively, which display much lower solubility and mobility than the oxidized forms [34]. Thus, dissimilatory metal- and radionuclide-reducing microorganisms may be employed in water treatment processes designed to selectively remove U- and Tc-contaminated plumes in subsurface aquifers [35, 36]. The *Shewanella* OM decaheme *c*-type cytochromes MtrC and OmcA (previously implicated in Mn(IV) and Fe(III) reduction) are also involved in electron transfer to U(VI) and Tc(VII). *mtrC* and *omcA* deletion mutants are severely impaired in U(VI) reduction activity and display decreased UO_2 associated with the outer membrane [37]. In the environment, association of UO_2 nanoparticles with biopolymers exerts a

strong influence on subsequent UO_2 reactivity, including susceptibility to oxidation by O_2 or NO_3^- and subsequent remobilization in U-contaminated soils and sediments [37]. The influence of U speciation on the kinetics and extent of microbial reductive precipitation of U(VI) may be modulated with multi-dentate organic acids. Strong complexing ligands such as citrate, NTA, and EDTA retard UO_2 precipitation in U(VI) bioreduction experiments by forming aqueous U(IV) complexes. The reduction rates of complexed U(VI) decrease with increasing stability constant values of the U:ligand complexes [38].

Oxidized	Reduced	mV
$S_4O_6^{2-}$	$S_2O_3^{2-}$	24
Fumarate	Succinate	33
TMAO	TMA	130
NO_2^-	NO	350
DMSO	DMS	160
$HCrO_2^{4-}$	Cr^{3+}	382
NO_3^-	NO_2^-	420
I3-	I3	570
Fe^{3+}	Fe^{2+}	760-200
MnO_2	Mn^{2+}	380
O_2	H_2O	820

Figure 1. E'_0 values of electron acceptors respired by *Shewanella oneidensis* span nearly the entire continuum of redox potentials encountered by bacteria in nature.

216. -linked Mn.

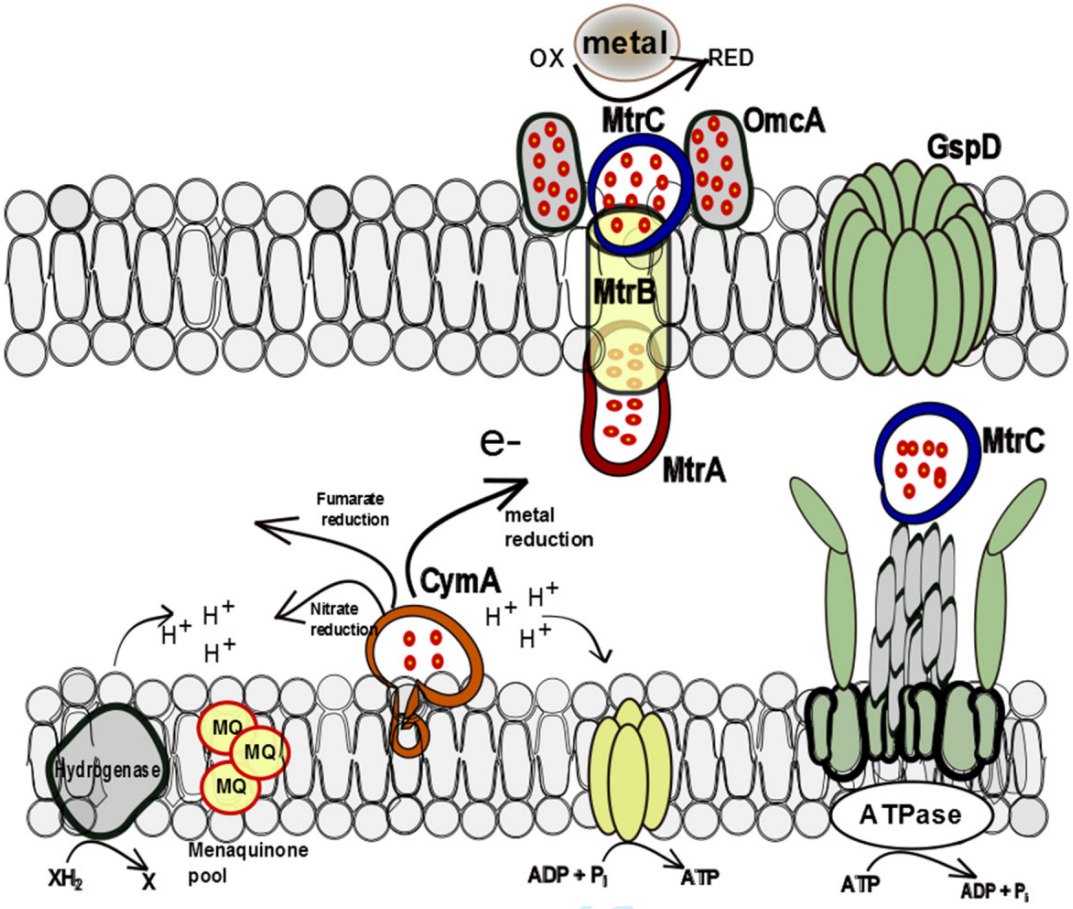

Figure 2. Working model of the *S. oneidensis* respiratory pathway for electron transfer to extracellular electron acceptors [22].

2. Anaerobic Reductive Biomethylation of Metals and Metalloids by *Shewanella*

Anaerobic reductive biomethylation of metals and metalloids is regarded as an alternative bioremediation strategy. Microorganisms biomethylate metals and metalloids under both aerobic and anaerobic conditions [39-48] in a variety of environments, including metal waste deposits, sewage sludge, and alluvial soils [45, 47, 48]. The resulting methylated metal compounds differ in solubility, volatility, and toxicity [49-51]. For example, volatile methylated species such as $(CH_3)_2Se$ and $(CH_3)_2Se_2$ may efflux

from aqueous to gas phases [48, 52]. Biomethylation reactions involve the enzymatic transfer of methyl groups via multiple stepwise methylation reactions resulting in both partially methylated nonvolatile species as well as fully methylated volatile metal and metalloid compounds. *Shewanella* species produce methylated derivatives of arsenic (As), selenium (Se), tellurium (Te), and iodine (I) via reduction of arsenate, selenite, tellurate, and iodate to reduced forms that are subsequently methylated under anaerobic conditions [53-60].

Reductive methylation of arsenic by Shewanella

Arsenic is introduced into the environment through the widespread use of organoarsenical herbicides and feed additives in agriculture. Arsenic primarily occurs in four oxidation states, arsenate (As(V)), arsenite (As(III)), elemental As (As(0)), and arsenide (As(-III)). As(V) and As(III) are highly water soluble with As(V) found mainly in aerobic environments and As(III) more common in anaerobic environments, while As(0) is rarely detected. *Shewanella* sp. strain ANA-3 contains As(V) reductases involved in both respiratory (encoded by the *arr* genes) and detoxification (encoded by the *ars* genes) processes [58]. The two As(V) reductases of strain ANA-3 respond to different amounts and types of inorganic As, which allow *Shewanella* sp. ANA-3 to tolerate high (μM) As levels. In *Shewanella* sp. ANA-3, the *ars* system is expressed under both aerobic and anaerobic conditions. *Shewanella* encoding the *ars* detoxification system typically tolerate higher As concentrations than *Shewanella* species that do not contain Ars. This feature indicates that the *ars* detoxification system is required for As(V)-respiring bacterial activity in environments where As concentrations are high [61]. Arr is a periplasmic heterodimer composed of ArrA and ArrB subunits [62]. Arr is only expressed anaerobically and is repressed by oxygen and nitrate. Both the *arr* and *ars* systems are induced by As(III), but

the *arr* system is activated by As(III) concentrations 3 orders of magnitude lower than that required for the ars system (≤100 nM versus ≤100 μM, respectively). As(V), on the other hand, induces the *arr* (but not the *ars*) system at low μM concentrations. The ArsR family protein ArsR2 is most likely the major As(III)-dependent regulator of *arr* and *ars* operons in *Shewanella* strain ANA-3. However, anaerobic growth with As(V) as electron acceptor requires co-regulation with global regulators such as the cyclic AMP-catabolite repressor complex (cAMP-CRP), which facilitates cross-talk between central metabolism and As toxicity responses [63].

Microbial As methylation involves As(V) reduction followed by oxidative addition of a methyl group [64], generating a growing series of methylated arsenic species of general structure $(CH_3)_nAsH_{3-n}$ with methyl arsenite (MMA), dimethyl arsenate (DMA-V), dimethyl arsenite (DMA-III), and trimethyl arsine oxide (TMAO) (n=1, 2, 3, respectively) as the major volatile As compounds [65]. Methylation occurs via the activity of As(III) methyltransferases (ArsM), a highly conserved set of proteins with three strictly conserved cysteine residues required for catalytic function [66]. *S. oneidensis* MR-1 transforms As through the partial methylation of inorganic arsenic species to less toxic methylated arsenic metabolites (e.g., DMA-III) by stepwise methylation with S-adenosyl methionine (SAM) as the methyl donor [60]. While the methyltransferases responsible for As biomethylation in *Shewanella* have yet to be identified, genomic analyses indicate potential pathways for As(III) methylation by a set of ArsM homologs (Fig. 1).

Reductive methylation of selenium (Se) and tellurium (Te)

Coal and phosphate mining are the main sources of Se- and Te-contaminated wastewaters. Se may enter the atmosphere via combustion of Se-bearing coals in power plants. Microbially-driven reductive methylation of Se and Te is widespread in aquatic and

terrestrial environments [47]. The most frequently produced methylated forms of Se and Te are volatile dimethyl selenide [$(CH_3)_2Se$; DMSe] and dimethyl telluride [$(CH_3)_2Te$; DMTe] (34-37). Bacterial thiopurine methyltransferase (bTPMT) is a methylating enzyme for transforming selenite and (methyl)selenocysteine to dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), with analogous transformations to tellurite [67]. Methylated Se and Te derivatives are volatile and less toxic than inorganic forms while reduction of selenite or tellurite to amorphous elemental Se or Te results in Se and Te immobilization and detoxification [48, 67, 68]. Therefore, subsequent multiple methylation of reduced Se or Te may be catalyzed by unidentified thiopurine methyltransferases (bTPMT) encoded in the *S. oneidensis* genome (Fig. 3).

Similar to U(VI) and Tc(VII) reductive precipitation reactions, the reductive precipitation of selenium and tellurium oxyanions may also be employed as Se and Te remediation technologies. While only selenate (SeO_4^{2-}) reduction supports anaerobic growth, selenite (SeO_3^{2-}) reduction is more widespread than SeO_4^{2-} reduction in the environment. SeO_4^{2-} reduction to Se^0 is a major sink for Se oxyanions in anoxic sediments [69, 70]. *Shewanella* species reduce SeO_4^{2-} and tellurate (TeO_4^{2-}) as well as SeO_3^{2-} and tellurite (TeO_3^{2-}) to Se^0 and Te^0 [59]. Recently, fumarate reductase (FccA) of *S. oneidensis* was identified as the terminal SeO_3^{2-} reductase in the periplasm, which also involves CymA, a *c*-type cytochrome central to anaerobic respiration in *Shewanella* (see Section 1) [71]. Se and Te reduction form Se(0) and Te(0) precipitates. Localization of extracellular Se precipitates and intracellular Te precipitates suggests that SeO_3^{2-} and TeO_3^{2-} are reduced by separate electron transport pathways [57, 72].

Reductive methylation of iodine (I).

Microbial iodate (IO_3^-) reduction is a major component of the biogeochemical cycling of iodine in marine and terrestrial environments [68, 73, 74] and the bioremediation of radioactive iodine in iodine-contaminated waters and sediments [75-77]. Radioactive isotope ^{129}I is produced during uranium and plutonium fission reactions with a half-life of 15.7 million years [77]. The iodine biogeochemical reaction network consists of a coupled abiotic (purely chemical) and biotic (enzymatic) reaction network [53]. In marine environments, for example, IO_3^- is reduced to I^- by IO_3^--reducing microorganisms [53, 56]. I^- is subsequently volatilized from marine surface waters by algae or bacteria by methylation to a variety of volatile organic iodine compounds, including methyl iodide (CH_3I), iodomethane (CH_2I_2), iodoethane (C_2H_5I), and iodopropane (C_3H_7I) [53, 78]. The iodine biogeochemical cycle is completed by I^- oxidation to IO_3^- as a step-wise via conversion of I^- to iodine (I_2) by I^--oxidizing microorganisms [79, 80] and subsequent hydrolysis of I_2 to HOI (+1 oxidation state), which subsequently disproportionates to IO_3^- to complete the iodine biogeochemical cycle [81, 82].

The molecular mechanism of microbial IO_3^- reductive methylation is poorly understood. SAM-dependent methyl halide transferases derived from plants, algae, fungi and bacteria catalyze methylation of I^- to methyl iodide (CH_3I) [78, 83]. Reductive methylation of IO_3^- has been reported for *S. putrefaciens* strain MR-4 [56] and methylation of I^- by *S. putrefaciens* IAM 12079 [78]. The majority of *Shewanella* strains reduce IO_3^- while *S. putrefaciens* 200, *S. algae* BrY and *S. oneidensis* display the highest IO_3^- reduction rates and extents of reaction [84]. IO_3^- reduction by *S. oneidensis* MR-1 involves metal reduction components MtrA and MtrB (but not MtrC), catabolite repressor protein

Crp, *c*-type cytochrome maturation protein CcmB, and the Type II protein secretin GspD. The *S. oneidensis* genome contains 14 putative SAM-dependent methyltransferases which are currently being examined for iodide methylation activity. A working model for iodine methylation based on the integration of phenotypic and genomic analysis of the *S. oneidensis* genome suggests that IO_3^- is reduced to I^- by an extracellular IO_3^- reductase complex composed of MtrA, MtrB and an unidentified IO_3^- terminal reductase. The produced I^- is subsequently converted to methyliodide (CH_3I) by an unidentified iodide-specific methylhalide transferase (IMHT)(Fig. 3).

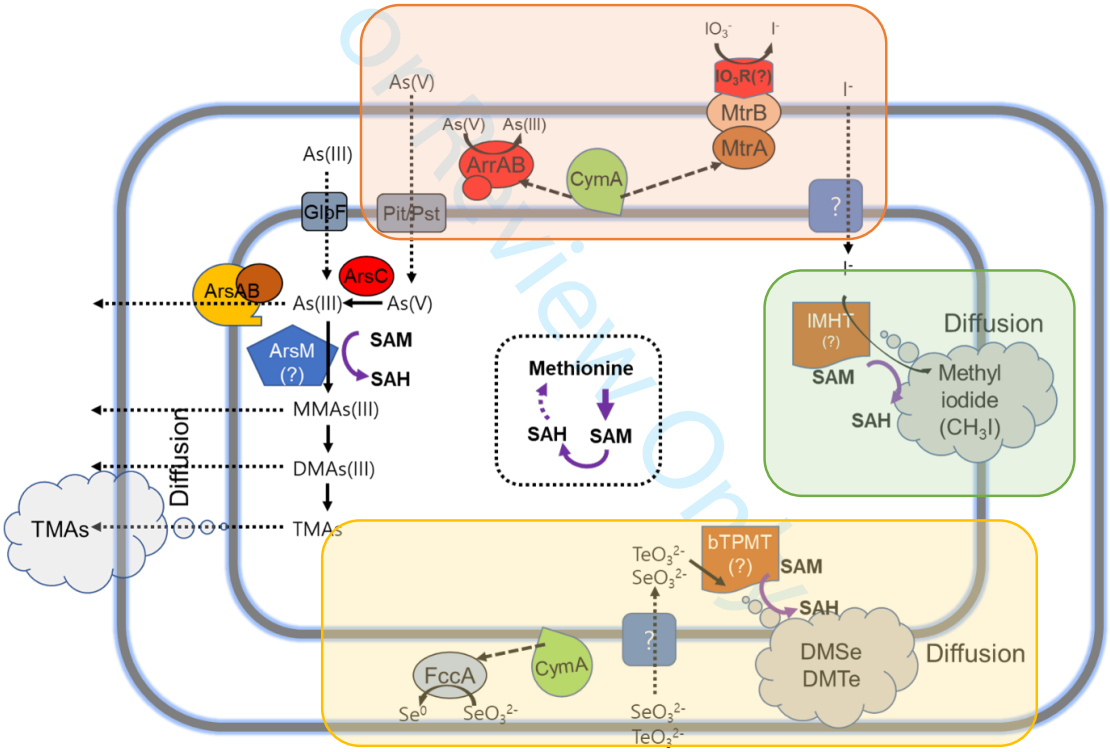

Figure 3. Reductive methylation pathways predicted from genomic analysis of the *S. oneidensis* genome. Top (orange box): Working model of reductive methylation of arsenate: periplasmic ArrAB reduces an extracellular As(V) to As(III) which is imported by Pit/Pst into the cytoplasm and cytoplasmic As(V) could be reduced to As(III) by ArsC.

Cytoplasmic As(III) is exported extracellularly by ArsAB or transformed to methylated arsenic compounds by sequential methylation of ArsM. Bottom (yellow box): Working model of reductive methylation of selenite or tellurite: periplasmic FccA reduces selenite (SeO_3^{2-}) to Se^0, however, the tellurite reduction mechanism is unknown. Selenite(SeO_3^{2-}) and tellurite(TeO_3^{2-}) are imported by unknown permeases and then transformed to gaseous methylated forms, DMSe or DmTe by bTPMT. Right (green box) working model of reductive methylation of iodate: IO_3^- is reduced to I^- by an extracellular IO_3^--reductase complex composed of MtrA, MtrB and an unidentified terminal IO_3^-reductase (IO_3R). Subsequently, the reduced I^- is converted to methyliodide (CH_3I) by an unidentified iodide-specific methylhalide transferase (IMHT) after cytoplasmic import of I^-. Abbreviations: ArrAB (Arsenite respiratory reductase), GlpF (Glycerol transporter), Pit/Pst (Phosphate transporter), CymA (Tetraheme c-type cytochrome), ArsAB (Arsenite detoxification efflux pump), ArsC (Arsenate detoxification reductase), FccA (fumarate reductase), MtrA, MtrB , IO_3R (Iodate reductase), IMHT (iodide-specific methylhalide transferase), bTPMT (bacterial thiopurine methyltransferase), DMSe (dimethylselenide), DMTe (dimethyltelluride), SAM (S-Adenosyl methionine), SAH (S-Adenosyl homocysteine).

2. *Shewanella*-driven bioelectrochemical water treatment systems

Bioelectrochemical systems such as microbial fuel cells (MFCs) consist of devices in which electroactive microbes (exoelectrogens) mediate electricity generation via EET from bacterial cells to an electrode and thus catalyze the transformation of chemical energy into electrical energy [85, 86]. MFCs generally contain a membrane that separates the anodic and cathodic compartments. The charge balance of the MFC system is compensated by ionic movement across an ionic membrane. MFCs utilize a variety of electron donors for electricity generation, including wastewater and

lignocellulosic waste. MFC technology is thus considered a seminal platform for coupling wastewater treatment to energy recovery from biodegradable compounds and sustainable remediation of contaminated water [87-89]. Low power density, however, is the current major obstacle for widespread application of MFC technology [90].

Microbial EET drives bioelectrochemical technologies for contaminant remediation, renewable energy recovery, and biofuel production coupled to water treatment. A wide range of renewable organic substrates drive MFCs, including acetate, glucose, lignocellulosic biomass, synthetic wastewater, brewery starch, dyes and land field wastewater, thus MFCs can be used as a wastewater treatment technology to decrease biological oxygen demand coupled to electricity generation which offsets operating costs [91, 92]. A variety of *Shewanella* strains display EET activity and dominate microbial populations enriched on MFC electrodes exposed to wastewater [93]. A comparison of *Shewanella* strains under identical BES configurations produce current values ranging from 0.7-3 uA/cm^2 with *S. putrefaciens* W3-18-1 generating the highest and *S. loihica* PV-4 generating the lowest current values [94]. *Shewanella* also reductively precipitate highly toxic metals, while simultaneously generating electricity. For example, *S. oneidensis*-driven MFCs with lactate as electron donor achieve a maximum current density of 32.5 mA/m^2 (1000 Ω external load) after receiving a 10 mg/L Cr(VI) addition to the cathode. Cathodic efficiency increased steadily over an 8-day operation period with successive Cr(VI) additions, thus demonstrating effective and continuous Cr(VI) reduction with associated current production [95]. In subsequent studies, *Shewanella* spp. W3-18-1, MR-4, and ANA-3 generated current during Cr(VI) reduction in BES, with values up to 5-fold higher than that of *S. oneidensis* MR-1 [96]. *S. oneidensis* MR-1 also reduced azo dye acid orange 7 (AO7) in a biocathode with lactate as electron donor and a

decolorization efficiency of over 96% [97].

Shewanella BES may also detect toxic compounds such as formaldehyde, in which current responses were detected over a concentration range from 0.01% to 0.10% in a single-chambered BES with 0 mV (versus saturated calomel electrode) applied on the anode [98] and fumarate. *S. oneidensis*–based fumarate-biosensing systems deliver a symmetric current peak directly proportional to increasing fumarate concentration in a linear rage between the values of 2 μM-10 mM [99]. *S. oneidensis* is also amenable to the development of genetically engineered biosensors such as arabinose- and arsenic-inducible *E. coli* promoters that produce current in BES reactors in response to arsenic. By placing the metal reduction (Mtr) pathway of *S. oneidensis* MR-1 under the control of an arsenic-sensitive promoter, the genetically engineered strain produces increased current output in response to arsenic. The BES-based biosensor displays a detection limit of 40 μM As(III) with a linear range up to 100 μM As(III). The transcriptional circuit relies on the activation of a single promoter, thus modular sensing systems may be developed to detect other analytes by the exchange of a single genetic component [100]. Another example of genetically-engineered BES systems involves trimethylamine-*N*-oxide (TMAO) to control EET rates via *mtrCAB*. The *torECAD* and *torF* promoters respond to TMAO concentrations and expression levels of TMAO-induced *S. oneidensis* cells to control *mtrCAB* expression with TMAO to induce Fe(III) reduction and current production [101]. Thus, *Shewanella*-based BES may simultaneously treat, monitor, and modify treatment by controlling the expression of genes encoding specific *Shewanella* biochemical pathways.

Mechanism of EET to MFC electrodes

MFCs may be driven by facultatively anaerobic bacteria such as *Shewanella* [102].

Two major factors affecting MFC efficiency are microbial attachment to the electrode surface and efficient electron transfer from the cells to the electrodes. *Shewanella* structural pilin genes (*mshA-D*, encoding extracellular mannose-sensitive hemagglutinin) structural proteins, involved in surface attachment and biofilm formation are involved in MFC current generation [102][103]. In addition, disruption of the putative cell surface polysaccharide biosynthesis gene SO_3177 in *S. oneidensis* MR-1 enhances adhesion to electrodes and current generation [104]. Conductivity in MFC-powering electroactive bacteria is dependent on EET (see Section 1) [105], and *Shewanella* transfer electrons directly or indirectly to electrode surfaces in either mediator- or mediator-free configurations [86]. The *Shewanella* Mtr electron transport machinery is involved in EET to electrodes [106]. For example, decahemes MtrA and MtrC and beta-barrel OM porin MtrB and homologs MtrDEF are required for EET and current production [106, 107]. *Shewanella* genomes encode other accessory proteins that impact MFC efficiency [108, 109]. In addition, different *Shewanella* strains contain different sets of EET genes that may impact MFC current production and coulombic efficiency [94]. Electron transfer to the electrode may be facilitated by mediators such as thionine, methyl viologen, methyl blue or humic acids [86]. Studies on the balance between the reduction of electrodes and competing acceptors are needed to determine the optimal combination of mediators. For example, the presence of O_2, while initially competing for electrons at the expense of cathode reduction, are used for riboflavin synthesis, which subsequently functions as a redox shuttle that increases electrical output [110].

Factors affecting bioelectrical output in Shewanella BES

The type of electron donors fed to MFC affects electrical performance, and thus a

fundamental mechanistic understanding of such effects is important to optimization of MFC performance. The effect of combined organic carbon compounds on the efficiency of contaminant degradation by *Shewanella* is of particular importance as wastewater composition is typically complex. For example, *Shewanella* readily oxidizes short and long chain carboxylic acids, including lactate or formate as electron donor. Formate and lactate, however, also act synergistically, and increase electrical output compared to formate or lactate as sole electron donor in *Shewanella* BES. During this synergy, lactate is metabolized as carbon source while formate is oxidized as electron donor [111]. The range and fate of organic substrates that *Shewanella* oxidizes to support current generation in MFCs is not well understood and a matter of ongoing research.

Shewanella form biofilms and produce electricity at circumneutral pH either as pure strains or as members of complex microbial consortia [86]. For example, *S. oneidens*is MR-1 clones overexpressing the c-di-GMP biosynthesis gene ydeH formed biofilms 3-fold thicker and generated electricity and current density 3-fold greater than wild-type *S. oneidensis* [112]. MFC driven by *S. loihica* PV-4 under aerobic or anaerobic conditions display different patterns of substrate utilization and biofilm formation. In turn, electrode composition also affects current output, possibly due to interactions with OM cytochrome lipoproteins. Surface wettability of electrodes, for example, alters EET activity of *S. loihica* PV-4. Current generated with superhydrophilic electrodes is substantially higher than normal hydrophilic and hydrophobic electrodes [113], while carbon-coated hematite electrodes are more efficient than bare carbon cloth electrodes for *S. oneidensis*-driven MFC, suggesting that semi-conductive properties of Fe(III) oxides play important roles for EET to electrode surfaces [114]. Increased electrical output is also achieved in higher ionic strength solutions, thus decreasing resistance and promoting

proton movement. Results are variable, however, since higher concentration of Ca^{2+} ions in the BES anodic electrolyte decreased current output by 72%, while addition of Ca^{2+} in a graphite felt MFC with *S. oneidensis* increased the current density by 80% [102].

Additional strategies to increase power density are based on isolation of synergistic microbial consortia. *Shewanella* EET is enhanced by co-culture with riboflavin-producing *Bacillus subtilis* RH33 which increases power densities. While *Shewanella* or *Bacillus* alone produce 56.9 mW/m2 and 6.9 mW/m2, respectively, a co-culture with both strains can reach a maximum power density of 277.4 mW/m2 [115]. Addition of mM levels of EDTA also increased EET activity 75-fold in BES cells driven by *S. loihica* PV-4 [90]. Electrode potentials also affect the physiological status of *Shewanella*. Mass spectrometric analysis of the *S. oneidensis* MR-1 proteome incubated in MFC at set anode potentials of + 0.71 V, + 0.21 V & − 0.19 V (versus SHE reference electrodes) displayed higher metabolic activity at higher electrode potentials, including a higher expression of riboflavin biosynthesis protein and EET and other energy-generating proteins [116]. At lower potentials (−0.19 V), cbb_3-type cytochrome c oxidase (CcoO), *c*-type cytochromes (SO3420 and CytB) and a number of chemotaxis and motility-related proteins were expressed at higher abundances, potentially indicating signs of oxidative stress at lower MFC potentials.

Shewanella-driven Fenton reaction drives hazardous contaminant degradation

Due to high oxidation potential, $OH^{\bullet}$ radicals generated from Fenton chemistry can oxidatively degrade a wide variety of recalcitrant hazardous contaminants found in

sediments, groundwater, and wastewater, including landfill leachates, chlorinated aliphatics and aromatics [117-120]. Advanced chemical oxidation processes such as the chemical Fenton reaction are often used as treatment strategies in such contaminated environments [118, 121]. In the chemical Fenton reaction, Fe(II) reacts with hydrogen peroxide (H_2O_2) under acidic conditions to form Fe(III), hydroxyl ion (OH^-), and $OH^\bullet$ radicals (Eq. 1) [122]:

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

In Fenton chemical treatments, however, the Fenton reagents Fe(II) and H_2O_2 must be continuously supplied to drive $OH^\bullet$ radical production via the chemical Fenton reaction, thus limiting *in situ* applications and increasing costs. Irradiation with UV light to induce Fe(III) reduction and photolytic radical production in photo-Fenton systems are also limited by UV light penetration and H_2O_2 must still be continuously supplied to drive the Fenton degradation reaction [123].

The microbially-driven Fenton reaction, on the other hand, is an emerging treatment strategy that continuously regenerates H_2O_2 and Fe(II), thus alleviating the need for continual addition of the Fenton reagents H_2O_2 and Fe(II). The microbially-driven Fenton reaction is carried out by alternating aerobic and anaerobic phases of bioreactors amended with Fe(III) and the Fe(III)-reducing facultative anaerobe *Shewanella*: H_2O_2 is produced via microbial O_2 respiration during the aerobic phase, while Fe(II) is produced via microbial Fe(III) reduction during the anaerobic phase (Fig. 4). In the transition between aerobic and anaerobic phases, the microbially-produced H_2O_2 and Fe(II) interact to produce $OH^\bullet$ radicals that degrade a variety of contaminants, including pentachlorophenol (PCP), trichloroethylene (TCE), tetrachloroethylene (PCE), 1,4-

dioxane, pyrene, and anthracene [123-125].

In the PCP case, the *S. putrefaciens*-driven Fenton reaction generates $OH^{\bullet}$ radicals in batch reactors to degrade PCP, a previously deployed pesticide and wood preservative. Approximately 60% of the PCP was degraded over the course of the experiments, and tetrachlorohydroquinone and tetrachlorocatechol were produced as daughter products accounting for 87% of PCP loss [126]. The *S. oneidensis*-driven Fenton reaction also generates $OH^{\bullet}$ radicals that degrade the chlorinated solvents TCE, PCE, and co-contaminant 1,4-dioxane. In bioreactors containing commingled contaminants, the ratio of the experimentally-derived rate of degradation of each contaminant was proportional to the corresponding $OH^{\bullet}$ radical reaction rate constant, thus indicating that the Fenton reagents generated by *S. oneidensis* subsequently resulted in $OH^{\bullet}$ radical production and contaminant degradation via fundamental Fenton reaction chemistry.

A similar *S. oneidensis*-driven Fenton reaction system was also recently employed to degrade the recalcitrant oil spill components pyrene and anthracene [127, 128] and to produce alternative energy biofuels with lignocellulosic materials from woody plants as starting substrate. Lignocellulose is primarily composed of the carbohydrate polymers cellulose and hemicellulose (consisting of sugar monomers glucose and xylose, respectively) tightly bound to lignin in a crystalline form highly resistant to enzymatic degradation reactions. The *S. oneidensis*-driven Fenton reaction system, however, degraded cellulose and hemicellulose to shorter oligosaccharides and monosaccharides that were subsequently fermented to the bioplastic polyhydroxybutyrate [129, 130].

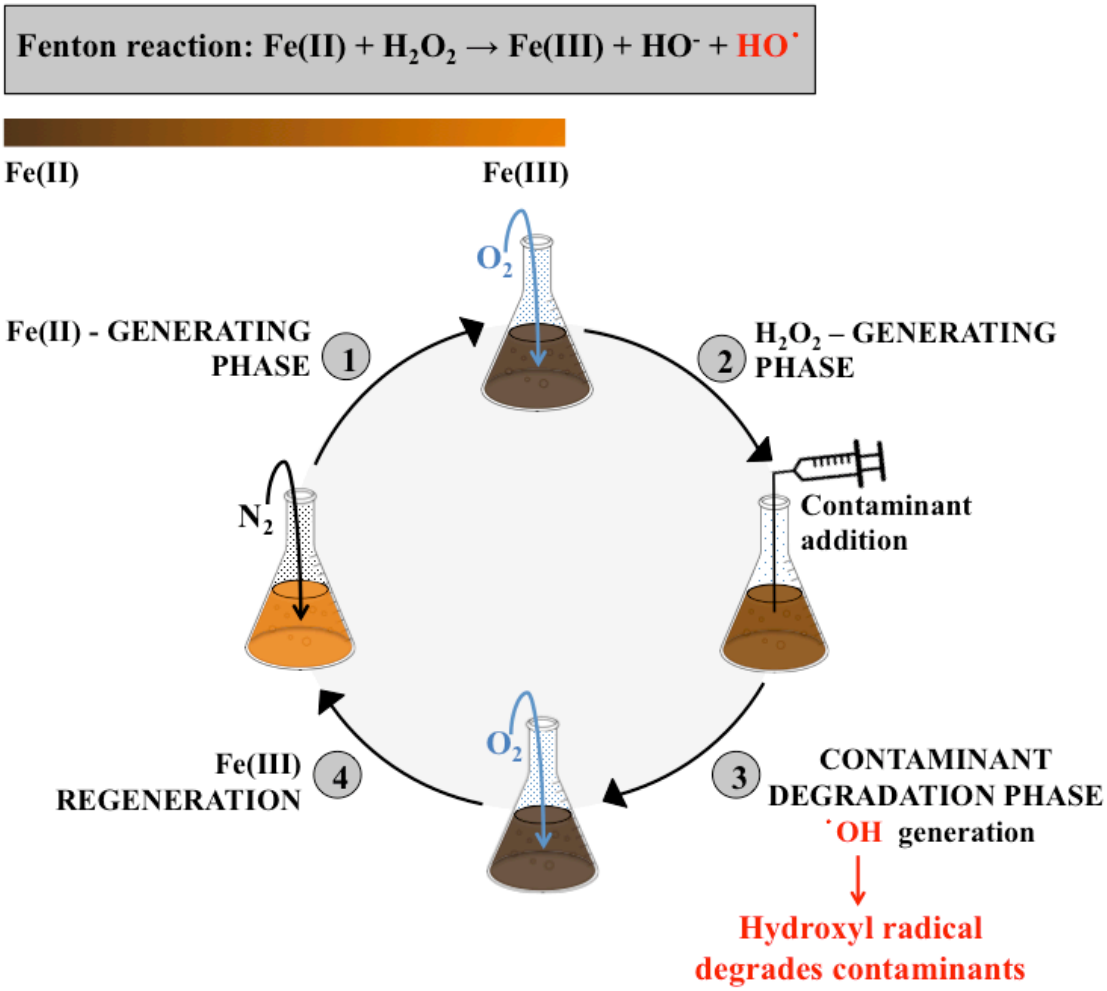

Figure 4. Microbially-driven Fenton reaction drives contaminant degradation in fed-batch reactor systems consisting of 4 main phases. Phase 1. Batch reactor containing Fe(III) and *S. oneidensis* cells is incubated under anaerobic conditions to initiate microbial Fe(III) reduction to Fe(II). **Phase 2.** Under aerobic conditions, *S. oneidensis* respires aerobically and reduces O_2 to H_2O_2, which reacts with Fe(II) produced in Phase 1 to generate $OH^{\bullet}$ radicals. **Phase 3.** The bioreactor is closed and contaminant (PCP, TCE, PCE, 1,4-dioxane, cellulose, hemicellulose, pyrene, or anthracene) is injected and degraded by the $OH^{\bullet}$ radicals generated in Phase 2. **Phase 4.** Fe(III) is regenerated via injection of compressed air and the 4-phase cycle is repeated. The

brown-colored bar in the upper left-hand corner indicates the relative reducing (brown-colored, fully Fe(II)) and oxidizing (orange-colored, fully Fe(III)) conditions in the bioreactor.

3. Application of *Shewanella* for resource recovery during water treatment: biogenic As and Se nanotubes

Selenium substitutes for sulfur in biological systems and is thus toxic at elevated concentrations [131]. Se is widely used in many industrial processes and products such as electronics, glass manufacturing, pigments, stainless steel, metallurgical additives, photoelectric cells, and pesticides. Se occurs predominately in four main oxidation states, selenate (Se(VI)), selenite (Se(IV)), elemental selenium (Se(0)), and selenide (Se(-II)). Soluble Se(VI) and Se(IV) are found in oxidizing environments, while insoluble Se(0) is more abundant in reducing environments. Chemical coprecipitation with Fe salts is often used to remove Se(VI) and Se(IV) from industrial wastewaters. However, this method generates Se sludge in a nonrecyclable form, which requires additional handling for disposal [132]. As occurs predominately in four main oxidation states (+5, +3, 0, and –3), with As(V) and As(III) the most common forms. Arsenic is a toxic heavy metal that occurs naturally in subsurface aquifers but its occurrence is magnified by anthropogenic emission as a byproduct of copper, lead, and zinc ore refining, and gold-producing industries [133], as well as in the pharmaceutical (pesticide), glass, timber, and leather industries [134].

The growing demand for electronic devices requires rare earth elements for materials fabrication, which leads to expansive volumes of toxic waste. While treatment is necessary to remove As and Se as a contaminant, recovery of As and Se as valuable

elements can partially offsets treatment costs. Due to high aspect ratios and unique size-dependent properties, As and Se nanotubes are valuable biotechnological building blocks for fabricating a variety of nanoscale electronic, optical, optoelectronic, electrochemical, and electromechanical devices. Microbially-produced electronic biological materials (e-biologics) represent potential green solutions for materials fabrication [135] since microorganisms are inexpensive catalysts sourced waste streams can be harvested for precursor As and Se [136].

Arsenic nanotube production by Shewanella

As(V) reduction is coupled to energy generation or As resistance via As(III) extracellular export and subsequent As detoxification. As(V)-respiring bacteria release As(III) from As(V)-containing minerals. Both respiratory As reductase (Arr) and resistance As reductase (Ars) are encoded in *Shewanella* genomes. The *arr* operon in *Shewanella* sp. strain ANA-3 lies immediately downstream of the ars operon and contains only two genes, *arrA* and *arrB*. ArrA contains motifs for binding an iron-sulfur cluster and molybdenum-containing pyranopterin cofactor. ArrB is predicted to contain three [4Fe-4S] and one [3Fe-4S] iron-sulfur clusters [137].

Shewanella sp. strain HN-41 produces an extracellular network of filamentous arsenic-sulfide (As-S) nanotubes under anaerobic conditions [138]. *Shewanella* As-S nanotubes (approximately 20 to 100 nm by 30 nm) form extracellular networks that upon aging continue to display electric conductivity, photoluminescence, photoactivity, and transistor-like properties [135]. Although not all *Shewanella* species produce As-S nanotubes, four *Shewanella* strains (*Shewanella* sp. strains HN-41, *S. alga* BrY, *S. oneidensis* MR-1 and *S. putrefaciens* CN-32) produce As-S nanotubes during incubation with lactate as carbon and energy source and As(V) and thiosulfate as electron acceptors

[139]. The main mineralogical components of the filamentous As-S nanotubes are comprised of a mixture of several As-S compounds. Biogenic As-S nanotubes consist primarily of amorphous As_2S_3 nanofibers with an indirect optical band gap of 2.37 eV but also contain crystalline As_8S_{9-x} minerals that were previously thought to form only at higher temperatures [140]. The ArrA and ArsC of *Shewanella*. sp. strain HN-41 and *S. putrefaciens* strain CN-32 display high amino acid sequence similarity to the corresponding proteins of *Shewanella* sp. strain ANA-3. In contrast, the *S. oneidensis* MR-1 genome harbors an ArsC (but not ArrA) homolog. Although the mechanism of (delayed) As-S nanotube formation by *S. oneidensis* MR-1 is not clearly understood, the (rapid) formation of As-S nanotubes by the other *Shewanella* strains may be due to the presence of two highly active ArrA and ArsC As(V) reductases [139].

Selenium nanotube production by Shewanella

As described above, dissimilatory Se(VI) and Se(IV) reduction by *Shewanella* reductively precipitates Se(0) nanoparticles. Se(IV) reduction by *Shewanella* species has drawn interest for nanoparticle synthesis. *Shewanella* sp. HN-41 synthesizes amorphous Se nanoparticles from aqueous Se(IV) compounds under anaerobic conditions (36). *S. putrefaciens* 200 produces reactive amorphous Se nanospheres and reduces Hg(II) rapidly. Biogenic Hg(0) reduced from Hg(II) by *S. putrefaciens* 200 was captured into extracellular amorphous Se nanospheres, resulting in the formation of stable HgSe nanoparticles. The new strategy lays the foundation for Hg removal strategies from aquatic environments without secondary pollution of Hg methylation or Hg(0) volatilization [141].

Se(0) nanospheres formed by Se-respiring bacteria are composed of Se with approximately the same uniform diameter (0.2–0.3 μm). Se(0) nanospheres are produced

outside the cell envelope and eventually slough off the cell surface [137]. Chemical synthesis of Se(0) nanospheres does not achieve the compacted nanostructural arrangement of Se atoms that results from microbial Se(VI) or Se(IV) reduction by *Shewanella* species. In addition to the compacted nanostructural arrangement, the structural features of extracellular Se(0) nanospheres produced by Se-respiring *Shewanella* include stable and uniform monoclinic crystalline nanospheres [142]. *Shewanella*-produced Se nanospheres, however, contain mixed Se redox species, heavy metals, and organics as impurities. Se recovery is technologically challenging since bacterially-produced Se(0) nanospheres exhibit colloidal properties that require development of novel methods for separation from treated wastewater [132].

Se(0) nanospheres may be stabilized in vitro against crystallization by inclusion of proteins or extracytoplasmic polymeric substances. Se(0) nanosphere-producing *Shewanella* sp. HN-41 generates 1D Se(0) nanostructures, nanowires, and nanoribbons at ambient conditions in dimethyl sulfoxide solutions. The crystallinity and shape of the Se(0) nanostructures are controlled by Se/DMSO ratios [143]. Although the average Se(0) particle size was unaffected by initial biomass concentration, the reduction rate and Se(0) particle size distribution were governed by biomass concentration. Se(0) nanoparticles of 1–20 nm were achievable at low initial biomass concentration and shorter reaction times. Initial Se(IV) concentrations did not have a significant effect on the average S(0) particle size, but affected the early-stage kinetics of Se(0) nanosphere production [144].

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