Molecular density regulating electron transfer efficiency of S. oneidensis MR-1 mediated roxarsone biotransformation

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1. Introduction

As an effective organoarsenical feed additive, 4-hydroxy-3-nitrobenzenearsonic acid (roxarsone) has been widely used in poultry industry to promote growth and/or prevent coccidial infections (Garbarino et al., 2003; Zhao et al., 2020). Although application of roxarsone has been banned in most developed countries, including China (MARA, 2018), long-term historical extensive application has resulted in a massive accumulation of roxarsone in the environment (Liu et al., 2013; Mangalgiri et al., 2015; Huang et al., 2019). Roxarsone is barely decomposed within animal body, and is usually excreted as its initial form along the manure (Makris et al., 2008; Jiang et al., 2013). Along with the storage or field application of animal waste or direct discharge into the environment, roxarsone can easily enter into soil, surface- or ground-water bodies due to its water-soluble capacity (Silbergeld and Nachman, 2008; Huang et al., 2019). Roxarsone itself is a moderately toxic compound, but it can be converted into higher toxic products upon exposure to the environment, such as trivalent inorganic arsenic or methylarsenicals, and eventually leads to severe environmental and public health risks (Cortinas et al., 2006; Stolz et al., 2007; Chen et al., 2018). In nature, roxarsone can be oxidized, reduced, methylated or dimethylated through numerous physical, chemical and biological interactions, and eventually produce a variety of arsenic compounds (Garbarino et al., 2003; Chen et al., 2016; Oyewumi and Schreiber, 2017). For example, nitro group of roxarsone was observed to be quickly reduced into an amino group in the absence of oxygen, yielding an intermediate product of 3-amino-4-hydroxybenzene arsonic acid (AHBAA) in anaerobic environment (Shi et al., 2014; Liu et al., 2017). Shewanella species are widely distributed in nature, including soil, sedimentary, freshwater and marine environments (Heidelberg et al., 2002; Harris et al., 2010), and they are capable of utilizing a variety of electron acceptors for anaerobic respiration and thus have attracted massive attention especially in biotechnology applications (Bretschger et al., 2007). Numerous respiration pathways of Shewanella oneidensis MR-1 and their underlying
mechanisms at the presence of various electron donors have been reported (Liang et al., 2014; Zhang et al., 2015). For example, S. oneidensis MR-1 was able to utilize roxarsone as a sole terminal electron acceptor during its anaerobic respiration, and enabled roxarsone transformation (Chen et al., 2016, 2018). Meanwhile, as a dissimilative iron-reducing bacterium, S. oneidensis MR-1 is able to reduce Fe(III) into Fe(II) in the presence of both Fe(III) and roxarsone, with the formed Fe(II) capable of transmitting electrons to roxarsone and thereby, conspire to stimulate roxarsone transformation (Chen et al., 2016). The yielded byproduct of roxarsone transformation (e.g., AHBAA) can be either adsorbed and immobilized or to be further biodegraded into inorganic arsenics (Liang et al., 2014; Liu et al., 2017), adding uncertainty to environmental- and public-health risks (Cortinas et al., 2006; Shi et al., 2014). During these transformation processes, one molecular roxarsone receives six electrons from bacterial cells or electron donors to form a primary byproduct of AHBAA (Stolz et al., 2007), whereby efficient extracellular electron transport is a key for sufficient reduction of roxarsone. Shewanella species have evolved various strategies of extracellular electron transport, including direct electron transfer using multiheme cytochromes (Breitschger et al., 2007; McLean et al. 2010) or via conductive nanowires (Reguera et al., 2005; Zacharoff and El-Naggar, 2017), as well as using soluble shuttle mediators that transfer electrons between cells and acceptors (Marsili et al., 2008; Wu et al., 2014). Harris et al. (2010) have shown that S. oneidensis MR-1 cells tended to stay on (or in close proximity to) a redox active surface (that served as electron acceptors) to facilitate extracellular electron transport. A recent study showed that nanowires existed as extensions of the outer membrane and periplasm of a S. oneidensis MR-1 cell, which associating with localized multiheme cytochromes MtrC and OmcA enhanced extracellular electron transport (Pirbadian et al., 2014). Notwithstanding the importance of the metal-reducing bacterium S. oneidensis MR-1 for the transformation and potentially bioremediation of the abandoned roxarsone in nature, the electron transfer characteristics between roxarsone and partnering bacterial cells are poorly understood, which are essential for full understanding the biotransformation process and environmental fate of roxarsone in nature or in waste treatment systems. In this study, we investigated the electron transfer performance of S. oneidensis MR-1 for the transformation and potentially bioremediation of the abandoned roxarsone in nature, the electron transfer characteristics between roxarsone and partnering bacterial cells are poorly understood, which are essential for full understanding the biotransformation process and environmental fate of roxarsone in nature or in waste treatment systems. In this study, we investigated the electron transfer performance of S. oneidensis MR-1 induced roxarsone transformation in a model aqueous system, and quantified the kinetics characteristics of both roxarsone transformation and the participating bacterial population growth.

2. Material and methods

The study is a reanalysis of raw data from an earlier research (Chen et al., 2016), focusing on the extracellular electron transfer characteristics between the target toxicant, roxarsone, and the bacterium, S. oneidensis MR-1, as well as bacterial growth kinetics, which are essential for full understanding of bacteria-mediated roxarsone (bio)transformation process and engineering practices. The experimental material and setup, as well as the analytical methods are identical to (and can be found in) Chen et al. (2016), with key information summarized in 2.1.

2.1. Experimental materials, setup, and analytical methods

The S. oneidensis MR-1 (MCCC 1A01706) culture was from the Marine Culture Collection of China (Xiamen, China). Roxarsone and AHBA are purchased from Sigma-Aldrich, Inc. (Shanghai, China) and International Laboratory (South San Francisco, U.S.A.), respectively. Batch experiments were conducted in 250.0 ml butyl-stopper glass bottles that contains 100.0 ml of basal medium with an initial bacterial cell density of 0.8 × 10⁷ cells/ml (Chen et al., 2016). Roxarsone concentration was initialized at 0, 0.10, 0.50, 1.00 and 2.50 mmol/l for five parallel experiments, respectively. 50 mmol/l of sodium lactate was supplemented as the sole source of carbon and energy. Fe(III) induced roxarsone biotransformation experiments were conducted in 250.0 ml butyl-stopper glass bottles under anaerobic condition, with an initial S. oneidensis MR-1 cell density of 0.8 × 10⁷ cells/ml. The initial concentration of roxarsone and Fe(III)-citrate were set as 1.0 and 10.0 mmol/l, respectively. Fe(II) induced abiotic roxarsone transformation experiments were conducted anaerobically in 250.0 ml butyl-stopper glass bottles, with initial roxarsone and Fe(II) concentration of 1.0 and 50.0 mmol/l, respectively. Aqueous sample was collected from each batch experiment for analysis of bacterial biomass, concentration of roxarsone, AHBA and ferrous. Detailed information can be found in Chen et al. (2016).

2.2. Kinetic models

We employed a modified logistic model according to Zwietering et al. (1990) to mimic the growth kinetics of S. oneidensis MR-1 population (X, expressed as relative cell density, unitless) during roxarsone transformation,

\[ X = \frac{X_{M}}{1 + \exp\left(4\mu_{M}/X_{M}(\lambda_{B} - t) + 2\right)} \]

where \( \mu_{M} \) (h⁻¹) is the maximum specific growth rate, \( X_{M} \) (unitless) is the maximum relative cell density, and \( \lambda_{B} \) (h) is the lag time, with its value calculated as \( \lambda_{B} = \frac{\ln(X_{M}/X_{0} - 1)}{X_{0}/X_{M}} \), with \( X_{0} \) (unitless) representing the initial relative bacterial cell density (value of 1). An estimation of detailed bacterial population growth kinetics is to calculate the inflection point (\( T_{i} \), h) of the population growth curve by setting the second derivative of Eq. (1) to zero,

\[ T_{i} = \frac{X_{M}\ln(X_{M}/X_{0} - 1)/(4\mu_{M})}{(\lambda_{B} - t) + 2} \]

The modified first-order kinetics model was applied in roxarsone reduction, which can be expressed as (Song et al., 2005),

\[ \frac{dS}{dt} = rS[1 - a(S/S_{0})^{m}] \]

where \( S \) (mmol/l) is roxarsone concentration, \( S_{0} \) (mmol/l) is initial roxarsone concentration, \( r \) (h⁻¹) is specific reduction rate, \( m \) (unitless) is the model parameter that represents the magnitude of joint biotic-abiotic environmental impact, and \( a \) (unitless) is the linear constraint of environmental impact describing the trend of curve (\( a < 0.5 \), from convex to concave) or not (\( a < 0.5 \), concave). Larger value of \( m \) means that the environmental conditions have significant impact on roxarsone transformation. An integration of Eq. (3) gives the expression of roxarsone concentration (S) as,

\[ S = S_{0}/[a + (1-a)\exp(mrt)]^{1/m} \]

The half-life time (\( T_{1/2} \), h⁻¹) of roxarsone transformation, according to the modified first-order model, can be expressed as,

\[ T_{1/2} = \frac{1}{(rm)\ln\left(\frac{2^{m} - a}{1 - a}\right)} \]

The modified Gompertz model was used to describe the kinetics of AHBA formation (Zwietering et al., 1990),

\[ S_{A} = S_{A_{max}}\exp\left\{-\exp\left[\frac{R_{A_{max}} \times e^{-t}}{5\times S_{A_{max}}} + 1\right]\right\} \]

where \( S_{A} \) (mmol/l) is AHBA concentration, \( S_{A_{max}} \) (mmol/l) is the maximum concentration of AHBA, and \( R_{A_{max}} \) (mmol/l/h) is the
maximum forming rate of AHBAAs. For experimental scenarios with the presence of Fe(III), there was a clear lag time ($\lambda$, h) for the formation of AHBAAs, where Eq. (6) can be expressed as,

$$S_A = S_{A\max} \exp \left\{ - \exp \left( \frac{R_{A\max} \times e}{S_{A\max}} (\lambda - t) + 1 \right) \right\}. \quad (7)$$

2.3. Product yield and bacterial apparent electron transfer performance

The relationship between roxarsone transformation and product formation could be described by the yield of AHBAAs ($Y_A$, unitless) during roxarsone transformation, as shown in Eq. (8),

$$\frac{dS_A}{dt} = -Y_A \frac{dS}{dt} \quad (8)$$

with its integration form as,

$$S_A = Y_A (S_0 - S). \quad (9)$$

According to the biochemical stoichiometry, it needs 6 mol of electrons for 1 mol of roxarsone to be entirely reduced into AHBAAs (Stolz et al., 2007; Chen et al., 2016). Therefore, the number of electrons acquired for the reduction of roxarsone within a unit volume of experimental medium can be expressed as,

$$B_{Rox} = 0.006 N_A (S_0 - S). \quad (10)$$

where $N_A$ is the Avogadro constant. During the anoxic transformation, roxarsone served as a sole electron receptor in the absence of oxygen and other electron receptors, receiving electrons from S. oneidensis MR-1 cells, and is eventually reduced into AHBAAs. For simplification, we assumed a constant bacterial apparent electron transfer rate ($Y_e$, electrons/cell/second, and thereafter simplified as electrons/cell/s) at a certain residual roxarsone concentration. Therefore, the number of electrons transferred by a number of bacteria with a certain time (e.g., incubation time) is proportional to the integration of bacterial population and incubation time, i.e.,

$$A_{MR-1} = \int_0^T Y_e X dt, \text{ which can be expressed according to Eq. (1) as,}$$

$$A_{MR-1} = 900 Y_e P X_M \mu M \ln(k_2 (k_1 + 1) / k_1 (k_2 + 1)), \quad (11)$$

where $k_1 = \exp(4 \mu M / \lambda - T) / X_M + 2$, $k_2 = \exp(4 \mu M / \lambda + 2)$, $P$ (cells/l) is the absolute inoculum bacterial cell density, and $T$ (h) is incubation time. Since roxarsone was the sole electron receptor during the biotransformation, $A_{MR-1}$ equals to $B_{Rox}$, and it enables an estimation of the apparent electron transfer rate ($Y_e$) of S. oneidensis MR-1 population according to Eqs. (10) and (11).

3. Results and discussion

3.1. Bacterial population growth kinetics at the presence of roxarsone

Simulation results revealed that the logistic model was capable of mimicking bacterial population growth kinetics for all scenarios (Fig. 1), evidenced by high values of the coefficient of determination ($R^2 > 0.9376$), as shown in Table 1. The maximum specific growth rate of S. oneidensis MR-1 under 0.10 mmol/l of roxarsone was estimated doubled (1.02 h$^{-1}$) as compared to that of none-roxarsone scenario (0.49 h$^{-1}$), associating with significantly amplified (nearly by half) maximum population. It is consistent with recently reported studies where the growth kinetics (i.e., the maximum populations and growth rate) of a bacterial consortium that capable of roxarsone degradation were clearly simulated at higher initial roxarsone concentration (Guzmán-Fierro et al., 2015). The results indicate that the presence of roxarsone would rather promote than suppress the population growth of S. oneidensis MR-1 strain, with roxarsone serving a terminal electron acceptor for anaerobic respiration of S. oneidensis MR-1. Similarly, recent studies reported that population growth of S. oneidensis MR-1 in a microbial fuel cells system was clearly stimulated upon supply of terminal electron acceptors, such as Fe(III) (Liu et al., 2011; Wu et al., 2013), Fe$_2$O$_3$ (Yang et al., 2014) or fumarate (Zhang et al., 2017). Surprisingly, the initial concentration of roxarsone tested in the study (of 0.10–2.50 mmol/l) had barely influence on the maximum specific growth rate of S. oneidensis MR-1, as evidenced by its nearly equal values ranging from 0.95 to 1.05 h$^{-1}$ for initial roxarsone concentration of 0.10–2.50 mmol/l. It was reported that the S. oneidensis MR-1 consists of an arsP gene (Heidelberg et al., 2002), which may encode the ArsP efflux permeases that likely confers resistance to trivalent roxarsone. Therefore, expression of the arsP gene does not guarantee S. oneidensis MR-1 to be resistant to roxarsone(V), and other unknown mechanisms may likely exist. For example, Han et al. (2017) reported that at the presence of S. putrefaciens CN32, roxarsone was rapidly degraded into AHBAAs(V) before further degradation into AHBAAs(III) and arsenite. Nevertheless, an earlier study showed that the accumulation of roxarsone degradation byproducts, such as inorganic arsenic, may exert moderate toxicity to the functioning (biodegradation) soil bacteria causing population lysis (Liang et al., 2014). On the other hand, the emergence of roxarsone did affect bacterial population growth kinetics, as shown in Table 1 of a gradually increased lag time from 1.3 to 2.9 h with increasing roxarsone concentration from 0.10 to 2.50 mmol/l, while zero for none roxarsone scenario. Considering the nearly equivalent specific growth rate, it seems possible of a decayed maximum population size over increased initial roxarsone concentration. However, simulations showed a significant increase in the inflection time from 7.4 to 10.8 h when the initial roxarsone concentration was amplified from 0.10 to 2.50 mmol/l, announcing an extended exponential population growth phase, which may overcompensate for the slightly enlarged lag time and was then responsible for the gradually amplified bacterial maximum population.

3.2. Kinetics of S. oneidensis MR-1 induced roxarsone biotransformation

Simulations applying the modified first-order model (Eq. (4)) achieved large $R^2$ values of higher than 0.9983 (Table 2), indicating a good fitness of the first-order model in predicting S. oneidensis MR-1 induced roxarsone biotransformation kinetics. Specifically, all the modelling fitting curves showed a sigmoid shape consisting of a first convex and a following concave patterns (evidenced by an a value of being globally larger than 0.5), the results revealed that roxarsone reduction process was gradually accelerated, and was in coincidence with amplified bacterial population growth in the presence of roxarsone. In addition, simulation results showed that the specific reduction rate ($r$) of roxarsone dropped from 0.59 to 0.032 h$^{-1}$ when the initial roxarsone concentration was amplified from 0.10 to 2.50 mmol/l, indicating that the apparent roxarsone concentration deemed affected S. oneidensis MR-1 induced roxarsone biotransformation process, with higher roxarsone concentrations clearly retarding the specific reduction rate. It was also evidenced by a rapidly increased $m$ value from 0.12 to 5.14 with
Fig. 1. Experimental (symbols) and modelling (lines) data of (A) relative bacterial cell density ($X$), (B) roxarsone concentration ($S$), and (C) AHBAA concentration ($S_A$) for *S. oneidensis* MR-1 induced transformation. Note that bacterial population growth modeling was limited to 65-h due to the nearly depletion of roxarsone at the 65th hour of incubation, when bacterial population size started to decay.
increasing roxarsone concentration from 0.10 to 2.50 mmol/l that reflected dramatically boosted joint biotic-abiotic environmental impacts on roxarsone transformation at higher initial roxarsone concentration. The results indicated that the S. oneidensis MR-1 induced early stage roxarsone biotransformation was likely roxarsone concentration dependent process.

Simulations applying a modified Gompertz model reviewed a good fitness to the AHBAA formation profiles as evidenced by high R² values (to be larger than 0.9884) as listed in Table 3. As modelling outputs, the maximum specific formation rate of AHBAA was found to drop from 0.033 to 0.016 h⁻¹ when the roxarsone concentration was increased from 0.10 to 2.50 mmol/l, and then decreased slightly with further increased roxarsone concentration until to 2.50 mmol/l, associated with its maximum concentration (SAmax) approaching to the initial roxarsone concentration (Fig. 1C and Table 3). AHBAA was formed upon the reception (by roxarsone molecule) of electrons transferred from the S. oneidensis MR-1 cells, and therefore, the AHBAA concentration profiles were simply a reflection of those of roxarsone reduction one. It suggested that AHBAA was the sole transformation product for Fe(III)-presence one (of 2.85), indicating that presence of Fe(III) likely played a more significant impact on S. oneidensis MR-1 induced early stage roxarsone biotransformation.

Simulations applying a modified Gompertz model also showed a good modeling fitness with the measured AHBAA concentration profiles, evidenced by a high R² value of 0.9998 and 0.9999 for Fe(III)-absence and presence scenarios, respectively, as shown in Fig. 3B and Table 5. Specifically, the simulated AHBAA maximum formation rate (Rₘₐₓₐₛ) was 0.015 mmol/l/h (or the specific rate of 0.014 h⁻¹) in the absence of Fe(III), with a lag time (λ) of 57.7 h. In comparison, the maximum formation rate of AHBAA jumped up to 0.133 mmol/l/h (or the specific rate of 0.125 h⁻¹) in the presence of Fe(III), associated with a largely reduced lag time of 18.3 h. There is barely difference in the simulated maximum concentration of AHBAA between the two scenarios, with its estimated value of 1.057 and 1.062 mmol/L for Fe(III)-absence and Fe(III)-presence scenarios, respectively. Indeed, Fe(III) cannot induce roxarsone reduction by itself, while the dissimilative iron-reducing bacteria S. oneidensis MR-1 can reduce Fe(III) to Fe(II) (Heidelberg et al., 2002), which may serve as an efficient reductant for direct roxarsone reduction (Chen et al., 2016), and eventually simulated roxarsone reduction. The AHBAA concentration gradually decayed upon its peak value at around 40-h of incubation, which was most likely contributed to the Fe(III)/Fe(II) mineral precipitates occurred following the biotic reduction of Fe(III) that created conditions for adsorption of AHBAA (Jackson and Miller, 2000; Chen et al., 2016).

Simulation employing the modified Gompertz model harvested a good modeling performance on mimicking Fe(II) formation kinetics (Fig. 3), with a high R² value of 0.9972 (Table 5). The simulated maximum Fe(II) formation rate (Rₘₐₓₐₛ) was 0.015 mmol/l/h (or the specific rate of 0.014 h⁻¹) in the absence of Fe(III), with a lag time (λ) of 57.7 h. In comparison, the maximum formation rate of AHBAA jumped up to 0.133 mmol/l/h (or the specific rate of 0.125 h⁻¹) in the presence of Fe(III), associated with a largely reduced lag time of 18.3 h. There is barely difference in the simulated maximum concentration of AHBAA between the two scenarios, with its estimated value of 1.057 and 1.062 mmol/L for Fe(III)-absence and Fe(III)-presence scenarios, respectively. The results further indicate that the presence of Fe(III) may largely stimulate S. oneidensis MR-1 induced early stage roxarsone biotransformation. In addition, Fe(III) did not alternate the maximum production yield of AHBAA as evidenced by its value of 105.7% and 106.2% for Fe(III)-absence and Fe(III)-presence scenarios, respectively. Indeed, Fe(III) cannot induce roxarsone reduction by itself, while the dissimilative iron-reducing bacteria S. oneidensis MR-1 can reduce Fe(III) to Fe(II) (Heidelberg et al., 2002), which may serve as an efficient reductant for direct roxarsone reduction (Chen et al., 2016), and eventually simulated roxarsone reduction. The AHBAA concentration gradually decayed upon its peak value at around 40-h of incubation, which was most likely contributed to the Fe(III)/Fe(II) mineral precipitates occurred following the biotic reduction of Fe(III) that created conditions for adsorption of AHBAA (Jackson and Miller, 2000; Chen et al., 2016).
transformation into AHBAA, associated with direct reduction of roxarsone by *S. oneidensis* MR-1, until complete reduction of roxarsone. The newly formed Fe(II) upon roxarsone completion, associating with the existing Fe(III) and bicarbonate, would likely form Fe(III)/Fe(II) precipitates, which provided a basis for the adsorption of AHBAA (Chen et al., 2016).

3.4. Bacterial apparent electron transfer performance

Implementing with measured bacterial cell density and residual roxarsone concentration values, bacterial apparent electron transfer rate was estimated ranging from $0.017 \times 10^6$ to $1.4 \times 10^6$ electrons/cell/s when the residual roxarsone concentration varied from 0 to 2.12 mmol/l for Fe(III)-absence scenarios (seeing symbols in Fig. 4A). It is in agreement with recently reported studies where *S. oneidensis* MR-1 was found to be able to transfer electron at a rate in order of $10^6$ electrons/cell/s (McLean et al., 2010). In addition, a linear regression showed a good correlation between the apparent roxarsone concentration and electron transfer rate as evidenced by high $R^2$ value of 0.9672 and Pearson’s $r$ value of 0.9845 (Fig. 4A), revealing a concentration-dependent effect of roxarsone on bacterial apparent electron transfer for the tested initial roxarsone concentration range. It implies that the apparent (or residual) roxarsone concentration is likely a determinant manipulating successful electron transfer from *S. oneidensis* MR-1 cells into roxarsone molecules. A further analysis of the relationship between the electron transfer performance and bacterial cell density exhibited a poor correlation with a relatively small Pearson’s $r$ value of $-0.3078$ and $R^2$ value of $0.0300$ (Fig. 4B). The results indicate that it is rather the apparent roxarsone molecules than the present cell density in the study (ranging from $0.80 \times 10^7$ to $13.18 \times 10^7$ cells/ml) that served the primary limiting factor regulating extracellular electron transfer and thereby roxarsone reduction process. Interestingly, an implementation of the kinetics models of roxarsone reduction and bacterial population growth for the calculation of electron transfer rate showed a dramatic rise of its value typically at the early stage of roxarsone reduction, i.e., relatively low inoculum cell density and high initial roxarsone concentration (seeing the solid curves in Fig. 4A). A simple calculation showed that one bacterial cell may occupy by average an aqueous volume of around $1.25 \times 10^{-7}$ ml at the initial cell density of $0.80 \times 10^7$ cells/ml, or equivalently $7.58 \times 10^{10}$ roxarsone molecules at a concentration of 1.00 mmol/l, which acquires $4.5 \times 10^{11}$ external electrons for complete reduction that is far beyond the electron transfer capacity (on the order of $10^6$ electrons per cell per second) of an active *S. oneidensis* MR-1 cell (El-Naggar et al., 2010). It implies that complete reduction of the presented roxarsone would require hours (e.g., 125 h) for 1.00 mmol/l roxarsone and initial bacterial cell density of $0.80 \times 10^7$ cells/ml neglecting population augmentation. Therefore, an alternation in the initial cell density at this level would barely affect electron transfer process, and it is deemed that roxarsone concentration (molecule density) in the experimental system manipulates the interacting probability between bacterial cells and roxarsone molecules, and for subsequent roxarsone reduction. In addition, it suggested that the roxarsone concentration range tested in the study was apparently over

![Fig. 2](image_url). The relationship (linear correlation) between the roxarsone transformation ($S_0 - S$) and AHBAA formation ($S_A$) at different initial roxarsone concentration of (A) 0.10 mM, (B) 0.50 mM, (C) 1.00 mM, and (D) 2.50 mM.
Fig. 3. Experimental [symbols] and modeling [lines] data of (A) roxarsone transformation ($S$), (B) AHBAA formation ($S_A$), and (C) Fe(II) concentration in S. oneidensis MR-1 induced roxarsone transformation with [marked as MR-1] and without [marked as MR-1+Fe(III)] soluble Fe(III).
saturated to immediate roxarsone reduction capacity of *S. oneidensis* MR-1, which may explain the dramatically declined specific reduction rate of roxarsone with increasing initial roxarsone concentration. On the other hand, considering an average cell size of one cubic micrometer (for simplification) of an active *S. oneidensis* MR-1 cell, it may continuously discover (relying on its self-motion, Alcalde et al., 2019) an aqueous volume equals to its body size. It implies that the amount of roxarsone molecules a *S. oneidensis* MR-1 cell may account at a time interval was proportional to the apparent roxarsone concentration, with the value being of $1.5 \times 10^7$ molecules at the highest tested initial roxarsone concentration of 2.50 mmol/l. This value is on the relevant magnitude of the electron transfer capacity of a single *S. oneidensis* MR-1 cell, i.e., a value on the order of $10^6$ electrons per second (EI-Naggar et al., 2010). It indicates that an initial roxarsone concentration of 2.50 mmol/l would likely support sufficient and rapid electron transfer between a *S. oneidensis* MR-1 cell and its closely-surrounded roxarsone molecules, yet for scenarios of lower concentrations that certainly requires longer interacting time (allowing cell movement or molecule diffusion) for timely electron transfer. EI-Naggar et al. (2010) have shown that the rapid supply of respiratory electrons (up to $2.6 \times 10^6$ electrons/cell/s) to a terminal acceptor was guaranteed by a single bacterial nanowire, which was otherwise hindered at high electric resistance circumstance (McLean et al., 2010), for instance, sparse electron acceptors environment (i.e., low roxarsone concentration in this study). In other words, the nanowires of a *S. oneidensis* MR-1 cell may offer an enhanced extracellular electron transport pathway for linking the respiratory chain of a bacterium to the external electron acceptors, as well as the nanowire-induced cell-cell communications that facilitated electron transfer (Reguera et al., 2005; Pirbadian et al., 2014; Zacharoff and EI-Naggar, 2017). In addition, studies have shown that electrons move through the outer membrane (MtrA and MtrB proteins) of an active *S. oneidensis* MR-1 cell to the extracellular surface (MtrC and OmcA), where electrons can be shuttled by intermediary flavins and thereby, enabling long-distance electron transport to terminal electron acceptors (Coursolle et al., 2010; White et al., 2013; Xu et al., 2018). Similarly, a recent study showed that MtrC and UndA were the key cytochromes involved in the extracellular reduction of roxarsone by *S. putrefaciens* CN32 (Han et al., 2017). Considering an initial cell density of $0.80 \times 10^7$ cells/ml, calculation showed that the distance for an electron to transport from an active cell to a roxarsone molecular may expand up to 50 μm (when the apparent roxarsone concentration approaching to zero) which is far beyond the physical size of a *S. oneidensis* MR-1 cell (Gorby et al., 2006). Therefore, an electron would experience dramatically amplified electric resistance while to be transferred into the external electron acceptors (roxarsone) when the residual roxarsone concentration was gradually approached zero, and thereby decayed apparent electron transfer performance.

At the presence of both *S. oneidensis* MR-1 and Fe(III), roxarsone transformation process was alternated because the Fe(II) yielded from bacterial induced Fe(III) reduction would actively contribute to roxarsone reduction (Cortinas et al., 2006). However, the yielded Fe(III)/Fe(II) mineral precipitates likely created conditions for adsorption of both *S. oneidensis* MR-1 cells and intermediate organoarsenic compounds (Chen et al., 2016; Zhang et al., 2018), which brought a barrier for successful quantifying the Fe(III) and *S. oneidensis* MR-1 induced roxarsone transformation kinetics due

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### Table 4
The modified first-order model simulated Fe(III)-mediated roxarsone transformation characteristics.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>$T_{1/2}$ (h)</th>
<th>$r$ (h⁻¹)</th>
<th>$a$</th>
<th>$m$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-1 + Rox</td>
<td>78.1</td>
<td>0.032</td>
<td>0.995</td>
<td>2.854</td>
<td>0.9990</td>
</tr>
<tr>
<td>MR-1 + Rox + Fe(III)</td>
<td>18.8</td>
<td>0.133</td>
<td>0.999</td>
<td>3.776</td>
<td>0.9964</td>
</tr>
</tbody>
</table>

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### Table 5
The modified Gompertz model simulated Fe(III)-mediated AHBA A and Fe(II) formation characteristics.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>$R_{max}$ (mmol/l/h)</th>
<th>$S_{max}$ (mmol/l)</th>
<th>$i$ (h⁻¹)</th>
<th>$R$ (h⁻¹)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-1 + Rox</td>
<td>0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.057&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9998&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MR-1 + Rox + Fe(III)</td>
<td>0.133&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.062&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.125&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9999&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.490&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.897&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9972&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> AHBA formation characteristics.

<sup>b</sup> Fe(II) formation characteristics.

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![Fig. 4](image-url). The relationship between bacterial apparent electron transfer rate and (A) residual roxarsone concentration, and (B) population density of *S. oneidensis* MR-1.
to inefficient accounting bacterial population and quantifying electron transfer process. Therefore, further study considering accurate measurements of both S. oneidensis MR-1 population and ferric/ferrous composition is needed for quantifying bacterial apparent electron transfer process at the presence of both S. oneidensis MR-1 and Fe(III).

4. Conclusions

The electron transfer characteristics between roxarsone and the closely associated partnering S. oneidensis MR-1 cells were investigated by linking the bioelectrochemistry and kinetics of roxarsone biotransformation and bacterial population growth in an aqueous system. The apparent electron transfer rate between a S. oneidensis MR-1 cell and the closely-surrounding roxarsone molecules was estimated up to $3.1 \times 10^6$ electrons/cell/s, which was in good agreement with recently reported literature data. The electron transfer performance was clearly associated with the apparent roxarsone molecular (that serve as electron receptor) density of the experimental system. Lowering molecular density would likely extend the average separation distance between bacterial cells and neighboring roxarsone molecules and thereby augmented electric resistance, which resulted in reduced electron transfer rate. Simulations results indicated that the tested models were capable of well mimicking the kinetics of bacterial population growth, roxarsone biotransformation and byproduct formation as evidenced by high correlation coefficient ($R^2$ value of >0.93). In addition, simulations revealed that the presence of roxarsone clearly stimulated the population growth of S. oneidensis MR-1 with its maximum specific growth rate being doubled, associating with significantly amplified (by at least 50%) maximum population, albeit with clearly increased lag time, as compared with that of none-roxarsone scenario. Such information is important for full understanding of roxarsone transformation process in bio-treatment systems that is necessary for waste-treatment engineering practice and/or environmental risks assessment. In addition, it has solid implications for understanding roxarsone transformation process and fate in nature, e.g., for bioremediation activity in soils where heterogeneous roxarsone spatial distribution as well as the tortuous pathways for roxarsone transport and bacterial cell movement through soil pore structures would likely confine sufficient bacteria-molecular (roxarsone) interactions, and thereby efficient bioremediation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Gang Wang: Conceptualization, Investigation, Methodology, Writing - review & editing. Neng Han: Investigation, Writing - original draft. Li Liu: Writing - review & editing. Zhongchen Ke: Investigation. Baoguo Li: Writing - review & editing. Guowei Chen: Conceptualization, Writing - review & editing.

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