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Bacteria attenuation by iron electrocoagulation governed by interactions between bacterial phosphate groups and Fe(III) precipitates

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ABSTRACT

Iron electrocoagulation (Fe-EC) is a low-cost process in which Fe(II) generated from an Fe(0) anode reacts with dissolved O_2 to form (1) Fe(III) precipitates with an affinity for bacterial cell walls and (2) bactericidal reactive oxidants. Previous work suggests that Fe-EC is a promising treatment option for groundwater containing arsenic and bacterial contamination. However, the mechanisms of bacteria attenuation and the impact of major groundwater ions are not well understood. In this work, using the model indicator *Escherichia coli* (*E. coli*), we show that physical removal via enmeshment in EC precipitate flocs is the primary process of bacteria attenuation in the presence of HCO_3^-, which significantly inhibits inactivation, possibly due to a reduction in the lifetime of reactive oxidants. We demonstrate that the adhesion of EC precipitates to cell walls, which results in bacteria encapsulation in flocs, is driven primarily by interactions between EC precipitates and phosphate functional groups on bacteria surfaces. In single solute electrolytes, both P (0.4 mM) and Ca/Mg (1–13 mM) inhibited the adhesion of EC precipitates to bacterial cell walls, whereas Si (0.4 mM) and ionic strength (2–200 mM) did not impact *E. coli* attenuation. Interestingly, P (0.4 mM) did not affect *E. coli* attenuation in electrolytes containing Ca/Mg, consistent with bivalent cation bridging between bacterial phosphate groups and inorganic P sorbed to EC precipitates. Finally, we found that EC precipitate adhesion is largely independent of cell wall composition, consistent with comparable densities of phosphate functional groups on Gram-positive and Gram-negative cells. Our results are critical to predict the performance of Fe-EC to eliminate bacterial contaminants from waters with diverse chemical compositions.

1. Introduction

Iron electrocoagulation (Fe-EC) is a process relying on the electrolytic dissolution of an Fe(0) anode to generate Fe(II), which is oxidized by dissolved O_2 to produce Fe(III) (oxyhydr)oxide precipitates with an affinity for microbial and chemical contaminants (Delaire et al., 2015; Tanneru and Chellam, 2012; van Genuchten et al., 2012). Fe-EC can efficiently remove arsenic from contaminated groundwater (Amrose et al., 2014; Li et al., 2012), and has also been shown to attenuate bacteria in a range of water matrices (Barrera-Díaz et al., 2003; Delaire et al., 2015; Ghernaout et al., 2008). In a recent study, we demonstrated that Fe-EC can attenuate *Escherichia coli* (*E. coli*) from synthetic Bengal groundwater (SBGW) without detriment to arsenic removal (Delaire et al., 2015), confirming that Fe-EC has promising applications for low-cost groundwater remediation (Amrose et al., 2014). Two processes contributed to bacteria attenuation in Fe-EC: (1) physical removal, caused by the adhesion of EC precipitates to cell walls, which results in bacteria enmeshment in Fe(III) flocs and subsequent settling, and (2) inactivation by reactive species produced upon Fe(II) oxidation by O_2. Fundamental aspects of the mechanisms underlying these two processes remain unknown. For example, the bacterial functional groups and the type of chemical interactions (electrostatic versus specific bonding) governing bacteria enmeshment in flocs are not well understood. In addition, the effect of major groundwater components, such as HCO_3^-, Ca, Mg, Si, and P, which can interfere with both inactivation and removal, has

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not been investigated. Finally, the impact of bacteria surface structure (Gram-positive versus Gram-negative, smooth versus rough Gram-negative) on attenuation has not been elucidated. By addressing these knowledge gaps, this study can improve considerably our predictions of Fe-EC performance in various water matrices containing different types of bacterial contamination.

Four types of surface functional groups are present on bacterial cell walls at comparable densities: hydroxyl (pK_a ~9.0), amine (pK_a ~9.0), carboxyl (pK_a ~4.7), and phosphate groups (pK_{a1} ~3.1, pK_{a2} ~6.6) (Borrok et al., 2005; Ngwenya et al., 2003). Hydroxyl and amine moieties do not have a strong affinity for Fe(III) oxides (McBride and Kung, 1991; Norén et al., 2008) and therefore they are not expected to strongly interact with EC precipitates. By contrast, carboxyl and phosphate moieties have strong affinities for Fe(III) oxides (Arai and Sparks, 2001; Chassé et al., 2015; Filius et al., 2000; van Genuchten et al., 2014a) and studies using Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (ATR-FTIR) have shown direct bonding of bacterial phosphate and carboxyl groups to hematite and goethite (Elzinga et al., 2012; Parikh and Chorover, 2006; Parikh et al., 2014). However, these studies were performed in controlled laboratory systems and simple water matrices, and they cannot be directly extrapolated to Fe-EC in groundwater, where precipitates and bacteria interact in an agitated suspension and in the presence of bivalent cations (Ca and Mg) and oxyanions (P and Si), which can sorb to bonding sites on bacteria (Beveridge and Koval, 1981; Johnson et al., 2007) and precipitates (van Genuchten et al., 2014b), respectively, and may therefore interfere with adhesion.

In addition to electrolyte composition, a number of studies have shown that the biomolecular structure of bacterial cell walls can affect their interactions with mineral surfaces through changes in surface charge, hydrophobicity, and steric hindrance (Chen and Walker, 2012; Jacobson et al., 2015; Walker et al., 2004). Because waterborne pathogenic bacteria and indicator organisms span the range of Gram-positive, smooth and rough (with and without O-antigen) Gram-negative strains (WHO, 2011), understanding the impact of cell wall structure on bacteria attenuation with Fe-EC is essential to generalize our findings to all bacterial species relevant to water quality.

Spectroscopic techniques such as ATR-FTIR, X-ray fluorescence (XRF) and X-ray absorption spectroscopy (XAS) have been used to study bacteria-Fe systems (Chan et al., 2009; Elzinga et al., 2012; Miot et al., 2009; Yan et al., 2016). However, these techniques cannot adequately determine bacteria-Fe(III) interactions in systems where Fe(III) is co-precipitated with bacteria in complex electrolytes similar to groundwater. For example, P–Fe bonds from bacteria-precipitate interactions and from aqueous P sorption to precipitates look very similar using ATR-FTIR (Elzinga et al., 2012) and would not be distinguishable with P K-edge XAS (Kelly et al., 2008). Additionally, ATR-FTIR is not suited to investigate interactions taking place inside large flocs due to the low penetration length of infrared beams in aqueous medium (~1 μm). To circumvent these limitations, the present study proposes an innovative approach, where macroscopic data of bacteria attenuation in systematically varied electrolytes are combined with ζ-potential measurements to elucidate the molecular interactions between bacteria and EC precipitates. Although this approach can only provide indirect evidence for specific interactions between bacteria and precipitates, it builds upon previous spectroscopic studies, which have identified bacteria-Fe oxide bonding processes in simple controlled systems (Elzinga et al., 2012; Parikh and Chorover, 2006; Parikh et al., 2014) and structures of Fe-EC precipitates in complex water matrices (van Genuchten et al., 2014a, 2014b), to gain information about bacteria removal mechanisms in groundwater-like electrolytes.

The goals of this study are to: (1) determine the impact of HCO_3^-, Ca, Mg, P, and Si on bacteria attenuation with Fe-EC, (2) identify the bacterial functional groups involved in the adhesion of EC precipitates to cell walls and investigate the type of interaction (electrostatic versus specific), and (3) test the generalizability of these conclusions to various bacteria types. To achieve these objectives, we first compared Fe-EC with $FeCl_3$ coagulation to distinguish the contributions of inactivation and removal via enmeshment in flocs to overall bacteria attenuation in Fe-EC as a function of the HCO_3^- concentration. Inactivation results were confirmed using live-dead staining. Second, we systematically investigated the effect of ionic strength, Ca/Mg, and P/Si on *E. coli* attenuation, both in single and in multiple solute electrolytes, to constrain the bacterial functional groups involved in precipitate adhesion to cell walls. ζ-potential, a proxy for surface charge, was used to assess the interaction of major groundwater ions with the surface of EC precipitates or *E. coli* cells. Third, we validated our proposed mechanism with 3 bacteria strains bearing different surface structures (smooth and rough Gram-negative, and Gram-positive). Our results strongly suggest that Fe-EC can be used to remove various types of bacteria from a wide range of water matrices representative of regions affected by arsenic and microbial contamination of groundwater. More generally, this study can help predict the performance of Fe-EC, and other Fe-based coagulation processes, to reduce bacterial contaminants from drinking water and wastewater.

2. Methods

2.1. Bacteria preparation and enumeration

One Gram-positive and two Gram-negative bacterial strains were used: *Enterococcus faecalis* (ATCC 19433, no antibiotic resistance), *Escherichia coli* K12 (NCM 4236, kanamycin-resistant), and *Escherichia coli* ECOR 10 (from STEC center, ampicillin-resistant (Mazel et al., 2000)). K12 is a rough strain (no O-antigen) (Stevenson et al., 1994) whereas ECOR 10 is a smooth strain (O-antigen present, serotype O6) (STEC center, 2016). After three propagations in growth media amended with appropriate antibiotics, stationary-phase bacteria were rinsed 3 times and resuspended in 100 mM NaCl as detailed in the Supporting Information. Bacteria were spiked in Fe-EC electrolytes to achieve initial concentrations of $10^{6.1-6.7}$ CFU/mL ($10^{5.0-5.8}$ CFU/mL for *E. faecalis*). Bacteria concentrations were enumerated in duplicate in 0.1 mL aliquots as colony forming units (CFU) using the spread plate technique on agar amended with appropriate antibiotics (detection limit of 10 CFU/mL), as described in the Supporting Information.

2.2. Electrolytes

The list of electrolytes used in bacteria attenuation experiments is specified in Table S1. In summary, we first varied the concentration of HCO_3^- (0.1–8.0 mM) to examine its impact on bacteria inactivation. Second, a range of ionic strengths was investigated by varying NaCl (in deionized water and in SBGW) or $NaClO_4$ (in 1 mM $CaCl_2$). Then, concentrations of bivalent cations (Ca: 0–13.5 mM and Mg: 0–10.6 mM) and oxyanions (P: 0–0.4 mM and Si: 0–0.4 mM) were systematically varied, in single and composite electrolytes, to elucidate their effect on bacteria removal. Finally, SBGW containing 8.2 mM HCO_3^-, 2.7 mM Ca, 2.0 mM Mg, 1.3 mM Si, 0.15 mM P, and 6.3 μM As(III), was prepared as described elsewhere (Delaire et al., 2015) and used as the electrolyte in some experiments. All experiments were conducted at pH 7.0 ± 0.3, except for the comparisons between the three bacterial strains, which were conducted at pH 7.5 ± 0.2. The pH was held constant throughout experiments by adding HCl, NaOH or $NaHCO_3$ as needed.