Rev Environ Sci Biotechnol (2017) 16:273–287
DOI 10.1007/s11157-017-9424-3

REVIEW PAPER

Ferric reduction in organic matter oxidation and its applicability for anaerobic wastewater treatment: a review and future aspects

Musfique Ahmed · Lian-Shin Lin

Abstract Anaerobic treatment processes have the advantages of cost-effectiveness, energy efficiency, low sludge yield and potential of resource recovery over conventional aerobic treatment methods and have been gaining increasing attention as an approach for future wastewater management. An important feature of anaerobic processes is the use of alternative electron acceptors to oxygen, which renders treatment flexibility in using redox active elements such as iron and sulfate from other waste materials. Co-treatment of acid mine drainage and municipal wastewater, as an example, has been shown to be an effective method for removing organic materials, metals, and phosphate from the both wastes. It also suggested the applicability of ferric reduction process in wastewater treatment. Most of the previous studies on ferric reduction process and iron reducers were conducted in natural systems such as sediments, soils and groundwater. This paper reviews the significance and fundamentals of the ferric reduction process, its utility for organics oxidation, controlling factors, reaction kinetics, microbial processes of iron reduction and its ecology. The paper also evaluates the suitability and discusses future aspects of using iron reduction for wastewater treatment. Knowledge gaps are identified in this paper for developing such innovative wastewater technology and process optimization.

Keywords Anaerobic wastewater treatment · Ferric reduction · Iron reducers · Organic oxidation

1 Introduction

A wide range of treatment technologies have been developed and explored to dwindle the impacts of wastewater generated from household and industries on aquatic ecosystems. Although aerobic treatment process has been used worldwide to achieve high degree of treatment efficiency, anaerobic wastewater treatment has been gaining increasing attention because of its simplicity, energy efficiency, low sludge production and little or no use of chemicals (van Lier 2008; van Lier et al. 2015). Anaerobic treatment has distinct advantages over aerobic treatment when treating high concentrated organic wastewater including emission of significantly less amounts of greenhouse gas (Chan et al. 2009). Additional benefits such as lower capital, operational and maintenance costs and technological advancement of resource recovery have helped to justify the use of anaerobic treatment over aerobic treatment (Manariotis and Grigoropoulos 2002; Chan et al. 2009). Currently, anaerobic treatment systems have not been efficaciously in practice around the world because of inaptness of their process and inadequate

---

M. Ahmed · L.-S. Lin (✉)
Department of Civil and Environmental Engineering,
West Virginia University, Morgantown, WV 26506-6103,
USA
e-mail: lianshin.lin@mail.wvu.edu

capacity of the reactors to treat great amount of water (Saravanane and Murthy 2000). But, with recent scientific advancements in microbiological processes and environmental biotechnologies, these shortcomings are readily to be overcome if have not already been. In particular, continuous development in anaerobic treatment has led to the applications of different high-rate reactor systems. Among those, anaerobic fluidized bed reactor (AFBR), fixed film reactor, anaerobic baffled reactor (ABR) and upflow anaerobic sludge blanket (UASB) have demonstrated impressive organic removal rates (Saravanane and Murthy 2000; Chan et al. 2009).

Given the reducing conditions, opportunities exist for innovative approaches of incorporating natural redox active elements in anaerobic treatment systems. In this context, co-treatment of acid mine drainage (AMD) and municipal wastewater (MWW) is an example of an innovative treatment approach. It was first explored in the early twentieth century to reduce pathogens by low pHs and elevated metal concentrations in AMD (Roetman 1932). Recent developments of this co-treatment approach have produced impressive results of mitigating AMD and reduction of organic materials from wastewater (Strosnider et al. 2011; Deng and Lin 2013; Hughes and Gray 2013). Strosnider et al. (2011) studied a co-treatment of synthetic AMD and municipal wastewater to reduce the biological oxygen demand (BOD), nutrients and metals in a four stage passive process. A two stage co-treatment consisting of aerobic mixing and anaerobic biological treatment was recently tested and has shown significant chemical oxygen demand (COD) removal and sulfate reduction (>80%) under COD/sulfate ratios ranging from 0.6 to 5.4 in anaerobic biological reactors (Deng and Lin 2013). The two stage treatment also resulted in an average 75% reduction of a range of metals and excellent phosphorus removal. The kinetics and microbial ecology of the biological system were later quantified and characterized, with both affected by COD/sulfate ratio and iron concentrations (Deng et al. 2016). Hughes and Gray (2013) examined a co-treatment process by injecting AMD in different forms of organic wastes (i.e., raw AMD, pre-treated AMD and pre-treated AMD with screened MWW) and reported substantial removal of COD, BOD, total organic carbon (around 90%) with continuous loading of AMD into an activated sludge reactor.

Given high levels of sulfate in AMD, treatment of AMD has mainly focused on using sulfate reducing bacteria (SRB) to reduce sulfate to (bi)sulfide and metal sulfide precipitation by providing organics as electron donors. Iron is also a prevalent element in AMD as well as in a wide range of iron containing wastes. While numerous studies have been conducted to evaluate the performance of sulfidogenic bioreactors for treating sulfate-rich wastewater (Widdel 1988; Stefanie et al. 1994; Dar et al. 2007), only limited studies on iron amendment and its effects on sulfidogenic treatment can be found in literature. The studies examining iron reduction processes and iron reducers in the literature mostly focused on sediment and soil systems (Urrutia et al. 1998; Zachara et al. 2001) and studies related to wastewater is extremely scarce.

This review focuses on fundamentals of iron reduction process in organics oxidation and potential utilities of iron reducing bacteria (IRB) for removing organic matters from wastewater. Specifically, significance of ferric reduction process and its applicability in oxidizing organic matters are reviewed. This review also covers applications of iron reduction process in environmental remediation and microbial ecology of IRB and their interactions with other microorganisms such as SRB and methanogens. Knowledge gaps in applying iron reduction in wastewater treatment are identified and its future research scope is discussed.

2 Significance and fundamentals of iron reduction process

A main feature of anaerobic/anoxic wastewater treatment is the use of alternative electron acceptors other than oxygen for organics oxidation. In such environments, metabolic energy is supplied by oxidation of organic carbons and reduction of ions like sulfate, nitrate, and ferric ions. Microbial reducers utilizing these alternative electron acceptors such as SRB, IRB, denitrifying bacteria can provide diverse metabolic pathways for oxidation of organic and inorganic wastes. Given its abundance in the environment, reduction of ferric iron has long been recognized as an important biologically-mediated process with significant influence on the fate of organic and inorganic pollutants.

The fundamental iron reduction process involves Fe^{3+}/Fe^{2+} redox pair in which ferric ion is reduced to ferrous ion by receiving an electron from an electron donor. This reduction half reaction (i.e., $Fe^{3+} + e^- \rightarrow Fe^{2+}$) has a standard redox potential of +0.77 V at pH 2 and +0.2 V at pH 7 (Madigan et al. 2015). However, this potential can vary from −1 to +1 V due to the instability of complex compounds and dissimilar stability constants (Pierre et al. 2002). As Fe^{3+}/Fe^{2+} reduction potential is comparatively higher than other electron acceptors, ferric reducers can use this energy to respire a wide range of organic compounds. As a result, large number of organic materials can be used as a substrate by IRB as a mechanism of decomposing the organic matters. In the case of sulfate rich wastewater, ferric reduction and sulfate reduction can occur concurrently (Eqs. 1 and 2) and form ferrous sulfide (FeS) precipitation (Eq. 3).

$$Fe^{3+} + \text{Organic compound} \rightarrow Fe^{2+} + HCO_3^- + CO_2 + H^+ \quad (1)$$

$$SO_4^{2-} + \text{Organic compound} \rightarrow H_2S + HCO_3^- + CO_2 + H^+ \quad (2)$$

$$Fe^{2+} + H_2S/HS^- \rightarrow FeS + H^+ \quad (3)$$

Previous studies have shown various Fe^{3+} compounds as electron acceptors are effective for organic matter mineralization in groundwater, soils and sediments (Lovley 1987, 1995). This ferric to ferrous transformation can be an effective mechanism for organic oxidation in addition to its contribution to iron cycle in aquatic environments. For example, sediments found with comparatively higher concentration of Fe^{3+} than the other electron acceptors have the potential to use iron as oxidant to mineralize organic matters with releasing nutrients such as phosphate and trace metals adsorbed on ferric oxyhydroxides. In such sediment systems, most of the organic carbon has been found to be retained in the fermentation products (Lovley and Phillips 1986). With amorphous ferric as terminal electron acceptor, major fermentation products of acetate could be oxidized (Kamura et al. 1963).

Another oxidation pathway involving ferric reduction is known as ferric ammonium oxidation (Feammox). In strict anaerobic condition, ferric reduction has been found to be an effective process to oxidize ammonium (Clement et al. 2005; Sawayama 2006; Yang et al. 2012). Sawayama (2006) reported the evidence of ammonium oxidation to nitrate by IRB with Fe^{3+} EDTA as an oxidizing agent. This process was also observed to be thermodynamically feasible by Clement et al. (2005) by using goethite as the iron source for ferric reduction in wetland soils. All these examples substantiated the significance of ferric reduction process in remedying environmental pollutants.

3 Organic oxidation by ferric reduction

3.1 Factors effecting the ferric reduction

A number of factors have been reported to affect organics oxidation in iron reduction process, including the types of the ferric compound, microorganisms, and availability of substrate (Lovley 1987). In general, increasing degree of crystallinity of the iron source materials results in lower iron reduction. Less crystalline or amorphous compounds have larger surface areas and higher solubility rate compared to highly crystalline ferric materials (Lovley 1987). A sequence of preference had been established on different ferric compounds as $FePO_4{\cdot}4H_2O > Fe(OH)_3 > \gamma\text{-FeOH}>$, $\alpha\text{-FeOH} > Fe_2O_3$ with respect to their microbial reduction rate (Munch and Ottow 1983). This sequence corroborated on the hypothesis of decrease in microbial reduction rate with high crystallinity. Solubility of ferric compounds is another major factor to be considered in ferric reduction process. Most of the ferric compounds are highly insoluble and found as solid forms in nature. Due to this reason, ferric reduction may be hindered in sediments by other reducers such as denitrifiers, sulfate reducers, and methanogens that use highly soluble substrates (i.e., nitrate, sulfate, and hydrogen). Because of the low solubility of Fe^{3+} compounds, IRB may require direct contact with the surface of compounds to achieve the reduction process (Lovley 1987). In this regard, addition of Fe^{3+} chelating agents (e.g. Fe^{3+} NTA, Fe^{3+} citrate) can increase bacterial iron reduction (Lovley and Phillips 1988) and enhance ferric reduction rate of the cultures (Jones et al. 1983; Arnold et al. 1986).

Ferric reduction process has also been found to be pH dependent. Percentage of iron reduction was decreased when the seawater-nutrient medium was acidified and pH was decreased (Castro and Ehrlich 1970). With soil humic acid as a substrate, Chen et al. (2003) reported slower and lower ferric reduction capacity under a low pH condition (<4), and an improved reduction capacity with a higher pH (>4).

Different pure ferric reducing cultures have been utilized in carbon oxidation studies (Arnold et al. 1988; Lovley 1995; Lonergan et al. 1996; Hyun et al. 1999). *Geobacter metallireducens,* also known as GS-15 was first isolated and used as the model ferric reducing microorganism in several studies (Lovley and Phillips 1988; Lovley and Lonergan 1990; Lovley 1995). This iron reducer was found to generate different biogenic iron products with different ferric compounds. For example, with acetate as the electron donor, amorphous ferric oxide was reduced to magnetite (Fe_2O_3), and ferric citrate was reduced to ferrous compound. *Shewanella* sp. has also been identified as an important and effective ferric reducer (Nealson and Myers 1990; Lovley 1993; Hyun et al. 1999). The direct contact between the cells of microorganism and solid surface might be required for the ferric oxyhydroxide reduction by *Shewanella putrefaciens*, which reveals the occurrence of ferric reduction at membrane-bound sites (Arnold et al. 1988; Beliaev and Saffarini 1998). A detail section has been presented on the microbial iron reduction process and ecological diversity of these iron reducers in later part of this paper.

The effectiveness of Fe^{3+} reduction process in organic matter decomposition also depends on the competition of IRB with SRB and methanogens for electron donors. With amorphic ferric oxide added to sediments, Fe^{3+} reduction process was observed to inhibit sulfate reduction by 90% and methane production by 50–90% where sulfate reduction and methane production was the major terminal electron acceptors respectively (Lovley and Phillips 1987). In sediments, IRB can divert the electron flow away from SRB and methanogens and maintain low concentration of major substrates. As a result, SRB and methanogens do not get the enough substrate for metabolizing. Thermodynamically, ferric iron is a more favorable electron acceptor than sulfate and CO_2 (Madigan et al. 2015), causing this electron flow diversion. In some cases, however, ferric reduction was not found to hinder the sulfate reduction and methane production with sufficient substrate available in the environment (Lovley and Phillips 1987) and co-existence of ferric reduction and sulfate reduction were observed (Achtnich et al. 1995; Thomsen et al. 2004). Distinct to sediments, wastewater contains high amount of organic materials and availability of substrate may not be a limiting factor for sulfate reduction and methane production. However, evidences have been found where ferric reduction process inhibited the sulfate reduction and methane production in wastewater (Zhang et al. 2009). Utgikar et al. (2002) suggested that precipitation of metal sulfide on the surface of sulfate reducers and methanogens might be a possible reason behind such inhibition. But, the authors recommended further research at cellular and enzymatic levels to validate this hypothesis.

3.2 Kinetics of ferric reduction in organics oxidation

Most of the studies examining the kinetics of ferric reduction in organic matter oxidation focused on aquatic and freshwater sediment systems (Thamdrup 2000; Roden and Wetzel 2002; Jensen et al. 2003; Bonneville et al. 2004). Bioavailability of amorphous or poorly crystalline Fe^{3+} is an important factor and their concentration has been used as a variable for quantifying the ferric reduction rate as well as a predictor of the contribution of Fe^{3+} reduction to carbon mineralization (Lovley and Phillips 1987, 1988). Jensen et al. (2003) quantified the ferric reduction rate in a marine sediment system and found that the organics mineralization was highly correlated with the concentration of poorly crystalline Fe^{3+}. The authors then justified the use of Fe^{3+} concentration for estimating the ferric reduction rate. The study assumed only iron and sulfate reductions contributed to anaerobic carbon oxidation and the individual reduction rates were combined to derive a ferric reduction rate expression.

The Monod model has been used to characterize microbial ferric reduction by several iron reducers. Liu et al. (2001) studied goethite (α-FeOOH) reduction rate with lactate as the electron donor using a *S. putrefaciens* culture. The ferric bioreduction rate and extent were quantified with respect to the electron donor and electron acceptor. The initial ferric reduction rate was found to increase with increasing goethite and lactate concentrations. The authors used FeOOH sorption capacity for Fe(II) as a surrogate measure of the electron acceptor concentration to normalize the Fe(III) bioreduction rate. The normalized bioreduction rate was fairly constant (~0.027 ± 0.0023 h^{-1}) over varied FeOOH concentrations, indicating a first-order relationship of the ferric bioreduction with the goethite surface area for the concentration range examined. Whereas, the normalized bioreduction rate was well characterized by the Monod rate expression as a function of the lactate concentration with the maximum rate 0.029 ± 0.002 h^{-1} and half-saturation constant 0.52 ± 0.1 mM. In a study on nitric oxide removal from flue gas, Fe(III) EDTA-reduction rate was observed to vary with different electron donors in a mixed liquor bioreactor (BioDeNOx) with glucose giving the highest rate (13.9 mM h^{-1}) followed by ethanol (8.2 mM h^{-1}), acetate (5.1 mM h^{-1}), hydroge (5.1 mM h^{-1}) and methanol (4.1 mM h^{-1}) (Van der Maas 2005).

Bonneville et al. (2004) investigated the changes in ferric reduction rate with different forms of ferric oxyhydroxide with lactate as an electron donor. Using the Michaelis–Menten kinetics with Fe^{3+} concentration as the substrate for the rate expression, the maximum reduction rate (V_{max}) varied from 0.2×10^{-11} to 399×10^{-11} μmol h^{-1} $cell^{-1}$. The highest rates were observed with ferric citrate as the reductant and the lowest rate was obtained with hematite. A positive correlation was observed between the solubility of the ferric hydroxides with the maximum ferric reduction rate. Postma (1993) showed that the kinetic reactivity of iron oxides can be distinctive from each other by comparing the reduction rate of ferrihydrate with reductive dissolution of hematite and goethite. At same pH, the ferric reduction rate of ferrihydrate had been observed much higher than hematite and goethite. At pH 3, the initial reduction rate of ferrihydrate was 1.2×10^{-8} mol s^{-1} m^{-2} whereas the initial reduction rate was 6.1×10^{-11} mol s^{-1} m^{-2} for hematite and 1.8×10^{-11} mol s^{-1} m^{-2} for goethite (Zinder et al. 1986; Banwart et al. 1989). It was concluded that ferrihydrate dissolved much faster than hematite and goethite, resulting in 180 times faster reaction rate than hematite and 100 times faster than goethite.

Roden and Wetzel (2002) used a first-order rate expression to model Fe^{3+} oxide concentration as a function of time in sediments from a freshwater wetland system. The rate expression contained a fraction of ferric oxide as non-reacting. The study found that the exponential decrease in ferric reduction with time was most likely due to limited availability of Fe^{3+} oxide rather than organic matters. This study also provided an evidence to support the hypothesis that in wetland sediments, ferric reduction rate followed a first order relationship with amorphous Fe^{3+} oxide concentration.

3.3 Iron reduction process in environmental remediation

The potential of IRB to be employed in environmental remediation can be measured by the degree to which the IRB can utilize ferric compounds in successful organic oxidization and removal of organic pollutants from environment. Several aliphatic and aromatic organic compounds have been reported to be successfully oxidized by ferric reduction (Lovley and Phillips 1988; Lovley and Lonergan 1990; Azam and Finneran 2013). In this section, evidences of aliphatic and aromatic organic matter oxidation reported in the literature are summarized.

3.3.1 Fe^{3+} reduction in aliphatic compound oxidation

Azam and Finneran (2013) successfully demonstrated that various ferric amendments increased the rates and extent of mineralization of carbon compounds to different degrees in a septic tank system. Lepidocrocite and Fe^{3+} EDTA were found as the most effectual ferric compounds with high mineralization rate compared to Fe^{3+} citrate, ferrihydrate, Fe^{3+} NTA and Fe^{3+} pyrophosphate. Lepidocrocite achieved maximum mineralization rate for acetate (92%), lipid (98%) and lactate (82%) and Fe^{3+} EDTA attained the highest mineralization of glucose (74%), starch (93%) and butyrate (88%). The ferric amendment increased the generation of carbon dioxide and reduced the rate of greenhouse gas methane (CH_4) production. While most of the ferric compounds showed better results in carbon mineralization, there were exceptions such as ferric citrate which actually had a decreased mineralization rate of acetate. Fermentation of citrate to acetate was probably the reason of this anomaly (Azam and Finneran 2013). Finneran and Lovley (2001) studied anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in aquatic sediments amended with poorly crystalline ferric oxide. The study found that approximately 30 mol of Fe^{3+} compound was required to completely oxidize MTBE, and addition of humic substances stimulated the degradation. In their study, rapid degradation of TBA was observed in a strictly anaerobic condition which differed the observation of slow degradation of TBA in previous studies.

3.3.2 Fe^{3+} reduction in aromatic compound oxidation

Groundwater contaminated by organic materials develops an anaerobic environment in the aquifer and aromatic hydrocarbons present in this anaerobic condition can be oxidized by reducers using nitrate, sulfate and iron as electron acceptor (Reinhard et al. 1984; Kuhn et al. 1985; Major et al. 1987; Lovley et al. 1989). Geochemical evidences of accretion of Fe^{2+} precipitation in groundwater systems and depletion of Fe^{3+} from the aquifer sediments have suggested the applicability of ferric reduction as a remedial mechanism for groundwater systems contaminated by aromatic hydrocarbons (Reinhard et al. 1984; Wilson et al. 1986; Major et al. 1987). Previous studies have shown the effective decomposition of aromatic carbons by injecting ferric compounds (Lovley and Phillips 1988; Lovley and Lonergan 1990; Lovley 1995).

Various monoaromatic compounds (benzene, xylene, toluene) have been found to be oxidized by microorganisms such as GS-15 using Fe^{3+} as the major electron acceptor. In one of the preliminary experiments by Lovley et al. (1989), toluene was successfully oxidized with poorly crystalline ferric oxide to carbon dioxide. More different aromatic compounds (toluene, phenol and p-cresol) were later shown oxidized by an enriched culture of GS-15 in the ferric reduction process (Lovley and Lonergan 1990). Thirty six (36) moles of ferric oxide was required to completely oxidize 1 mol of toluene to carbon dioxide where ferric oxide was the sole oxidant. Likewise, 28 and 34 mol of ferric compound were required for oxidation of phenol and p-cresol respectively. The oxidation rates were highly varied with the type of ferric compounds that were used in the experiment. In contrast to the toluene oxidation with ferric oxide, 108 mol of ferric hydroxide, $Fe(OH)_3$ were required to oxidize 1 mol of toluene. However, these stoichiometric ratios do not represent the actual amounts as these metabolic reactions are much more complicated. Various forms of Fe^{2+} and Fe^{3+} and incorporation of some organic compounds on the cells of reducing microorganisms may be the reason for this complexity. Furthermore, these reactions are endothermic as good amount of energy is produced for the cell growth (Lovley and Lonergan 1990).

4 Microbial processes of iron reduction and ecology

Iron reducing bacteria gain their energy by the oxidation of organic materials or other available substrates via extracellular iron reduction, a process known as ‘extracellular respiration’ (Esther et al. 2015). Different mechanisms of microbial iron reduction have been proposed which include direct and indirect contact of IRB with the iron minerals (Weber et al. 2006; Esther et al. 2015). Also, a wide range of microorganisms belong to different taxa have been identified as iron reducers which can adapt to different chemical environments.

4.1 Microbial processes of iron reduction

The microbial iron reduction is mainly transpired by extracellular electron transfer from iron reducers to the solid surface of the Fe^{3+} compounds, typically insoluble Fe^{3+} oxide minerals. Several strategies of electron transference had been investigated and reported in the literature (Weber et al. 2006; Esther et al. 2015), and the strategies are dependent on the type of microorganism and the surrounding environment. In microbial ferric reduction, interaction of iron reducers with ferric mineral surface is often interceded by the formation of biofilm that is composed of extra polymeric substance (EPS) matrix (Thormann et al. 2004; Franks et al. 2010). Electron transfer is then mediated by the EPS as it creates support for electron transport proteins to attach with the microbes and improves the conductivity of the biofilm. Four approaches have been hypothesized by researchers on the interactions of microbial cells and Fe^{3+} surface which are illustrated in Fig. 1:

1. Direct contact between the bacteria surface and Fe^{3+} surface

2. Contact through pili, which is also known as 'protein nanowires'
3. Contact by using complex ligands which solubilize the Fe^{3+} compound
4. Electron shuttle which facilitates the electron transfer from and to solid Fe^{3+} surface

Understanding the complex interactions between iron surface and microorganism is a convoluted task. Most of the research on microbial iron reduction has been progressed around two model iron reducers: *Geobacter* and *Shewanella* (Nealson and Myers 1990; Lovley 1993; Fredrickson and Gorby 1996; Weber et al. 2006; Esther et al. 2015). The interactions of these two reducers with the ferric surface are quite dissimilar from each other. *Shewanella* has been found to have direct and indirect electron transfer to insoluble Fe^{3+} surface, including protein nanowires. In contrast, *Geobacter* species are strictly anaerobe and do not contain enough electron shuttling or chelating compounds to solubilize Fe^{3+} and hence have been found to mostly rely on pilin filaments (Esther et al. 2015). Direct electron transfer by *Shewanella oneidensis* occurs by following the 'porin-cytochorme model' via a Mtr pathway. This Mtr pathway was formed by four multi-heme cytochromes (MtraA, MtrB, CymA and OmcA) and one non-heme protein MtrB (Myers and Myers 1997, 2001; Beliaev et al. 2001; Pitts et al. 2003) that help in transferring electrons to the cell surface (Esther et al. 2015). The development of nanowire like pili was assumed as a requirement for the attachment of bacteria with ferric surface. But, later evidences showed, it works more likely as an electron conduit rather than an attachment medium (Weber et al. 2006). These conductive nanowires help to increase the spatial area outside the cell membrane and improve the cell to cell communications. Eventually, it improves the electron transfer to insoluble ferric oxide surface and other potential electron acceptors. Soluble redox-active compounds can serve as exogenous and endogenous electron shuttles mediating indirect electron transfer between iron reducers and the ferric mineral surfaces (Weber et al. 2006). Exogenous electron shuttles (e.g., humic substances and sulfur compounds) are compounds present in the surrounding environment and endogenous electron shuttles are mainly secreted from the microorganism itself (Esther et al. 2015). These compounds are reduced by the ferric reducers upon oxidization of an electron donor

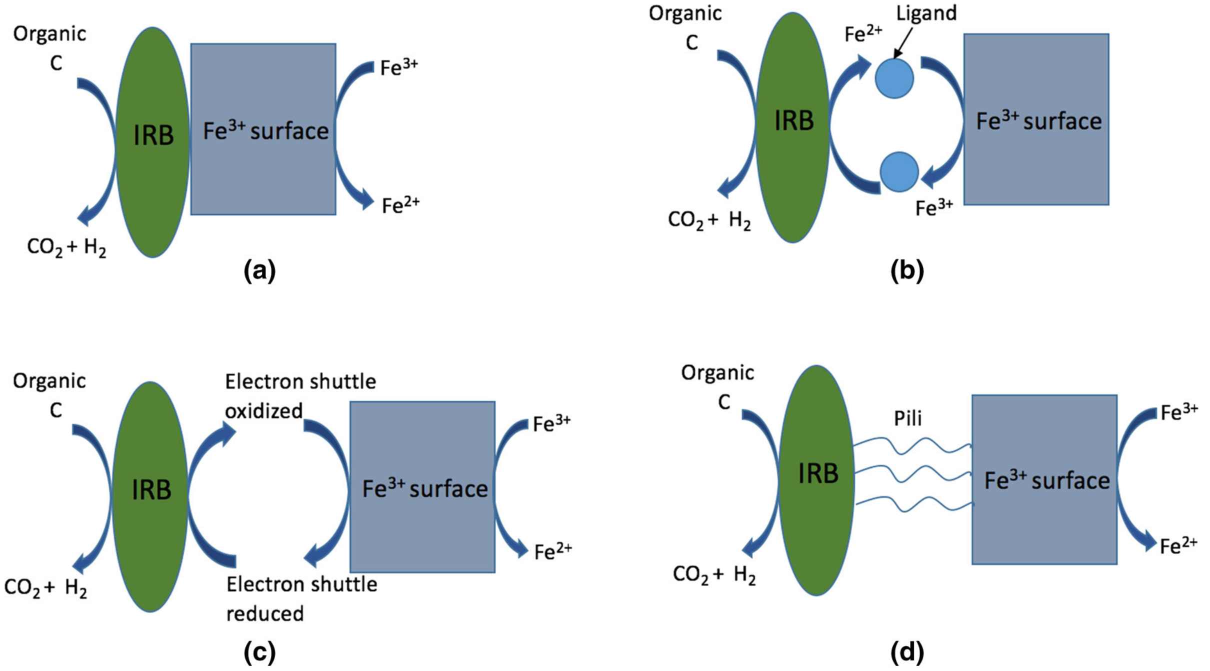

Fig. 1 Different strategies of microbial and ferric surface interaction **a** direct contact, **b** contact with ligand, **c** contact with electron shuttle, **d** contact with nanowires; adapted from Esther et al. (2015)

and that reduced electron shuttle subsequently diffuses and donates electrons to ferric oxide abiotically. These strategies are mainly investigated in sediments, submerged soils and aquifers, and are conceptually applicable in new applications of ferric reduction such as wastewater treatment.

4.2 Roles of iron reducing bacteria

Various bacterial strains have shown effectiveness to catalyze organics oxidation reactions coupled with ferric reduction. Dissimilatory ferric reducers have been shown to facilitate oxidation of glucose, amino acid, aromatics, long and short chain fatty acids (Lovley and Phillips 1989; Lovley 1993). Fermented sugar and amino acid could be metabolized by Fe^{3+} reducers to produce two major fermentative products: acetate and hydrogen (H_2). Other fermentative products include propionate and formate which could subsequently be transformed into CO_2 with the help of *Geobacter Desulfuromonas* and *Shewanella desulfovibrio*, respectively (Lovley 1993). Glucose can be oxidized directly to CO_2 with Fe^{3+} as the electron acceptor but the microorganisms that conduct this transformation are still unknown. However, these bacterial strains would be at detriments compared to the fermentative bacterial strain as glucose is always found to be fermented rather than directly oxidized to CO_2 in Fe^{3+} reducing sediments (Lovley and Phillips 1989). Figure 2 illustrates different pathways of organic matter oxidation coupled with ferric reduction and various microorganisms that have been found to catalyze the reactions. Although, most of the attempts to isolate these unknown species were unsuccessful, thermodynamic consideration has supported the assumption. Strains that help oxidize long chain fatty acids are still unknown whereas the aromatics are generally oxidized by *Geobacter* species with Fe^{3+} as the solitary electron acceptor (Lovley 1993).

Apart from IRB, the iron reduction competency of anaerobic ammonium oxidation (Anammox) bacteria in wastewater has been investigated in several studies (Park et al. 2009; Zhao, et al. 2014). The iron reduction ability of anammox bacteria showed impressive results when organic matters were used as electron donors and can be an impending way of removing ammonium from wastewater treatment. The heterotrophic IRB and autotrophic anammox bacteria compete against each other for utilizing ferric, but with the rising of nitrate production, the anammox bacteria could outcompete the heterotroph (Park et al. 2009). However, the iron reduction activity of anammox bacteria was inhibited to around 93% when nitrite was coinciding with ferric compound. The performance of anammox bacteria in iron reduction varied with respect to the electron donors and acceptors (Zhao et al. 2014). Among formate, acetate and propionate, formate had been found as the most effective electron donor with the highest production of Fe^{2+} concentration (~ 179.64 mol L^{-1}). Between Fe^{3+} NTA and Fe^{3+} EDTA, first complex had better results as an electron donor at pH 7.

4.3 Diversity of iron reducing microorganisms

A wide variety of bacteria and archaea belonging to diverse taxa have been found to perform the ferric reduction in different physical and chemical conditions (Fredrickson and Gorby 1996; Weber et al. 2006; Esther et al. 2015), suggesting prevalence of these ferric reducers in the environment. Most of the bacterial iron reducers belong to the Firmicutes, Proteobacteria, Deferibacteres and Actinobacteria taxa. The proteobacteria are commonly categorized in different classes as α-Proteobacteria, β Proteobacteria, Δ Proteobacteria and γ Proteobacteria. They can also be categorized as thermophilic, mesophilic, alkaliphilic, alkali-thermophilic, acido-thermophilic according to different adaptable conditions (Weber et al. 2006; Esther et al. 2015).

Model iron reducers *Geobacter* sp. and *Shewanella* sp. both belong to the group of Proteobacteria with *Geobacter* sp. residing in delta sub division and *Shewanella* sp. in gamma sub division. *Shewanella* sp. generally use lactate as carbon source and oxidize it to acetate, and *Geobacter* sp. use acetate and completely oxidize it to CO_2 (Fredrickson and Gorby 1996; Esther et al. 2015). *Geobacter metallireducens*, also known as GS-15 is one of the oldest and common ferric reducers which showed the capacity to reduce not only iron oxides but also Mn^{4+} and NO_3^-. GS-15 has been found to oxidize several carbon compounds to CO_2 by using Fe^{3+} (Nealson and Myers 1990). Some of the ion reducers have been found to be closely linked with sulfur reducers belonging to the same taxa and using same electron acceptor for growth. For example, *Geobacter acetoxidans*, another member of delta Proteobacteria have shown similarity with

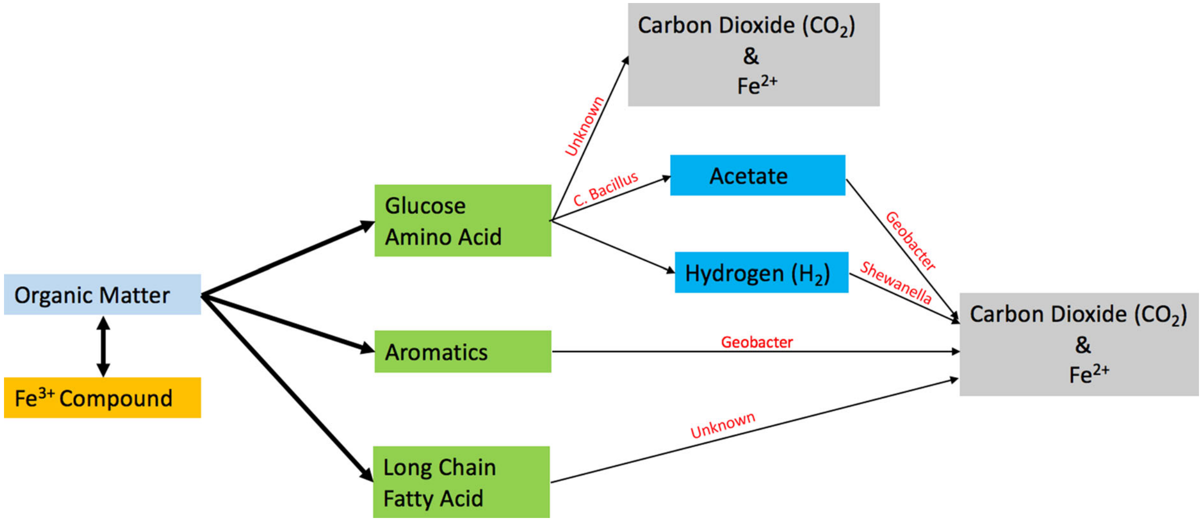

Fig. 2 Oxidation of organic matters with ferric reduction adapted from Lovley (1993)

Desulfuromonas acetoxidans, a sulfur reducing bacteria (Fredrickson and Gorby 1996) as both can use Fe^{3+} as electron acceptor for growth. *Pelobacter carbinolicus* has also shown ability to couple Fe^{3+} with oxidation of fermentation products and has an analogous phylogenetic relationship with *Geobacter* and *Desulfuromonas*. *Pelobacter* sp. generally uses formate, ethanol and H_2 as electron donors with Fe^{3+} as the electron acceptor. These bacterial strains were mainly isolated from iron-rich sediments. Apart from these, *G. sulfurreducens* and *D. palmitatis* which were isolated from submerged soils of a ditch and marine sediments, respectively, have been discovered to use Fe^{3+} as sole electron acceptor. *Desulfuromusa kysingii* and *Desulfuromusa bakaii* have also found to utilize Fe^{3+} as electron acceptor but were isolated as sulfur reducers. These findings supported the hypothesis that there are some sulfate reducers which can reduce Fe^{3+} as electron acceptor. Nevertheless, sulfate reducers with only iron as sole electron acceptor has not been found in any literature (Lonergan et al. 1996).

Shewanella putrefaciens, also known as MR-1, is a facultative anaerobe, in contrast to the *Geobacter* sp. which is a strict anaerobe. This bacterial strain was isolated from a culture that used non-fermentable carbon as electron donor (Nealson and Myers 1990). But, it has the capacity to use a wide range of electron acceptors including iron, manganese etc. *Shewanella* sp. is most effective in ferric reduction with lactate as the electron donor and the least effective with succinate. Although, these bacteria are proficient to preserve energy by ferric reduction, but possess extremely limited capacity to utilize organic matters as electron donors. Incomplete oxidation of lactate and pyruvate to acetate is commonly found redox reaction facilitated by these bacterial species (Lonergan et al. 1996). Similar to *S. putrefaciens,* BrY and *Desulfovibrio species* have also been noticed for their ability to oxidize lactate to acetate and CO_2 coupling with Fe^{3+} reduction (Lovley 1993). *Shewanella* sp. is also capable of reducing more crystalline form of ferric oxides compared to other iron reducers which reduce only amorphous or poorly crystalline ferric oxides. This revelation has helped to support the supposition that structure of ferric compound can also control the diversity of ferric reducing bacteria (Fredrickson and Gorby 1996). A thermodynamically unfavorable magnetite reduction by *S. putrefaciens* has also been discovered, which has made them a bacterial strain of significant importance. In this case, formate or lactate could have been used by the *S. putrefaciens* to reduce magnetite. These finding can ultimately help to understand the ecological diversity of iron reducers in the wastewater with different compositions. A list of major iron reducers that can reduce Fe^{3+} compounds is provided below with their major characteristics (Table 1):

5 Iron reduction in wastewater treatment and future prospect

Given that typical untreated municipal wastewater has COD 339–1016 mg L^{-1} and sulfate 24–72 mg L^{-1},

Table 1 Major iron reducing bacteria and their primary electron donors

Sl no.	Bacterial strain	Adaptable condition	Electron donors	References
1	*Geobacter fertireducens*	Mesophilic	Acetate, volatile fatty acid, alcohol	Lovley et al. (1993)
2	*Shewanella putrefaciens*	Mesophilic	Formate, lactate, pyruvate	Hyun et al. (1999)
3	*Aquaspirillum magnetotacticum*	Microaerophilic	Succinate	Guerin and Blakemore (1992)
4	*G. metallireducens*	Mesophilic	Mono aromatic compounds like toluene, p-cresol, and phenol	Lovley et al. (1989)
5	*G. chapelleii*	Mesophilic	Acetate, Hydrogen	Lonergan et al. (1996)
6	*G. hydrogenophilus*	Mesophilic	Acetate, Hydrogen	Lonergan et al. (1996)
7	*S. frigidimarina*	Psychrotropic	Sodium lactate, sodium acetate	Bowman et al. (1997)
8	*Geogemma pacifica* sp.	Hyper thermophilic	Formate, acetate,	Esther et al. (2015)
9	*Desulfovibrio* sp.	Mesophilic	Hydrogen	Lovley (1993)
10	*Shewanellasp. HN-41*	Mesophilic	Formate, lactate, pyruvate	Esther et al. (2015)
11	*Pelobacter carbinolicus*	Mesophilic	Fermentable substrate, formate, ethanol and H_2	Lonergan et al. (1996)

free ammonia 14–41 mg L^{-1}, insignificant nitrate and nitrite levels, and phosphate 3.7–11 mg L^{-1} (Tables 3–18, (Metcalf & Eddy, et al. 2013), using ferric Fe(III) in wastewater treatment has the multiple advantages. First, low solubility of iron phosphate can be an effective mechanism for retaining phosphorus from wastewater and reducing P loads to receiving waters. This could be achieved by mixing wastewater and iron to allow formation and settling of the chemical precipitation prior to the anaerobic treatment (Berner 1973; Ivanov et al. 2005). This is an important treatment aspect that nitrate and sulfate can not provide. Second, given the significant levels of sulfate in wastewater, sulfate reduction is expected to co-exist with iron reduction in the proposed iron-dosed anaerobic treatment. Precipitation of iron sulfide due to its low solubility (amorphous ferrous sulfide Ksp $\approx 10^{-3.05}$) can limit potential sulfide toxicity on iron reducers and sulfate reducers. Lastly, with abundance and widespread presence of iron (in particular, various forms of Fe-containing wastes such as chemical sludge from acid mine drainage treatment and wastes from steel industry), tremendous opportunities exist for incorporating iron as a green agent in innovative wastewater treatment technologies.

In light of the literature findings that showed effectiveness of IRB for organic matter oxidation in sediments, groundwater, soil systems, applicability of ferric reduction for organic matter removal from wastewater is explored in this section. Although the composition of wastewater is distinctively different from the other environmental sources, the mechanisms of the reduction process in organic oxidation in the above-mentioned studies are beneficial for the applications of iron reduction process in wastewater treatment. A few studies that evaluated the utilization of ferric reduction in wastewater treatment have been reported and future scope of this research is discussed here to identify knowledge gaps.

5.1 Organics mineralization by ferric reducers

Previous studies on co-treatment of AMD and municipal wastewater discussed in the introduction part of this paper have shown that substantial amount of COD, BOD, TOC, sulfate and other nutrients can be reduced from the wastewater through the co-treatment process. These results have suggested the potential benefits of using iron in anaerobic wastewater treatment. The study of Azam and Finneran (2013) has explored the pertinence of ferric amendments in mineralization of various carbon compounds in onsite anaerobic wastewater treatment. The mineralization of wide range of carbon molecules by different ferric compounds was one of the major illustrations of the study. As the composition of wastewater can vary significantly depending on the source of origin, there is a possibility that different ferric compounds will differ in performance of removing organic matter from wastewater of different composition. Other reduction processes such as fermentation and methanogens have limited substrate range and can not oxidize carbon molecules with high molecular mass, whereas ferric reduction process can be effective for mineralizing a broad array of carbon molecules (Lowe and Siegrist 2008; McKinley and Siegrist 2010; Chang et al. 2011). Further studies are required to examine biodegradability of different categories of organic matters typically found in wastewater.

5.2 Ferric iron bioavailability and bioreduction kinetics

Previous studies have indicated ferric bioavailability for IRB as a critical factor affecting ferric bioreduction kinetics and consequently carbon oxidation rate (Liu et al. 2001; Bonneville et al. 2004; Roden and Wetzel 2002). For iron-dosed wastewater treatment, identification of the chemical morphology and structure of ferric compounds which support and disfavor the ferric bioreduction can be used as a baseline for selecting iron source materials. To promote bioavailability and reduce environmental footprint of iron-dosed wastewater treatment processes, iron-containing materials with large surface areas and fast ferric dissolution rates are required. In this regard, chemical sludge from alkaline treatment of AMD can be suitable materials for such applications due to their amorphous nature and large surface areas. Use of AMD sludge would also reduce the environmental burdens and cost of disposing the sludge materials. However, presence of toxic heavy metals in the AMD sludge need to be examined and addressed. Formation of metal sulfides (e.g., PbS and CuS) with biogenic sulfide in the bioreactor can potentially be a mechanism used to remove heavy metals and address the concern. Alternatively, selective precipitation in AMD treatment to obtain high-purity iron hydroxide and exclude heavy metals from the chemical sludge (Wei et al. 2005) can be used to prevent introducing AMD heavy metals to the wastewater.

Another critical factor for ferric bioavailability is pH as ferric compounds are highly insoluble at circumneutral pH and most of the bacteria need an adaptable pH level of 5–8 (Straub et al. 2001). Soluble and insoluble ferric compounds can differ greatly in oxidizing the organic matters in wastewater. Hence, there is scope of future study to investigate optimal pH range for the biological treatment and variability in organics removal efficiency and kinetics in relation to pH.

Kinetic models that characterize ferric bioreduction and/or carbon oxidation are needed for designing the iron-dosed treatment process. The kinetic models can then be used to estimate hydraulic and biomass retention times in the design of the treatment process. Both retention times are critical design parameters to achieve sufficient wastewater treatment.

5.3 Microbial ecology

In wastewater treatment applications, organic substrate availability is typically not a limiting factor as wastewaters have relatively higher levels of organic matters available compared to electron acceptors (e.g., nitrate and sulfate). In the iron-dosed anaerobic treatment, IRB and SRB are expected to be the dominant bacterial species in the bioreactors. Iron/sulfate ratio can be a suitable parameter for gauging microbial activities of iron and sulfate reducers, and for developing kinetic models. Shift in microbial species distributions in relation to iron/sulfate ratio, and how variations in the microbial ecology affect organics removal are important knowledge gaps for developing such treatment technology. In particular, there is a need to understand the nature of the relationships between IRB and SRB (i.e., symbiotic or competitive) to identify optimal operating conditions for the treatment.

Studies aim to identify predominant microbial strains that truly responsible for ferric bioreduction and carbon oxidation are needed. The understanding of the microbial species and their ecology would help identify their mechanisms for utilizing ferric iron and carbon compounds. Although common strategies of IRB for utilizing ferric ion from iron material surfaces can be found in the literature, it is likely that new strategies and mechanisms can also be learned in wastewater treatment applications. Therefore, advancement in microbial studies is required to identify effective ferric reducers and their ecology, and their relationship with wastewater composition to better employ iron reduction in wastewater treatment.

5.4 Sample treatment process

A possible treatment train of the proposed iron-dosed wastewater treatment may consist of primary,

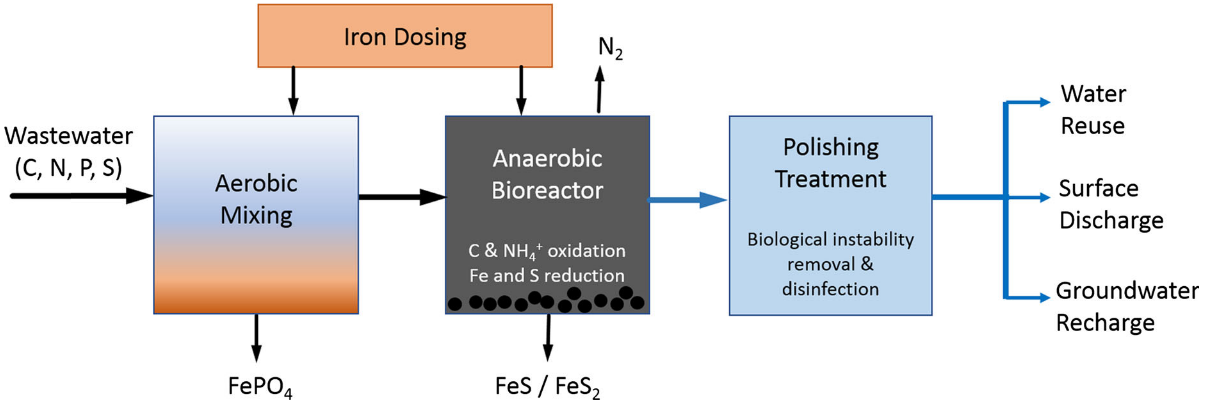

Fig. 3 Schematic diagram of the possible treatment process

secondary, and polishing treatment units (Fig. 3). The primary unit is a clarifier where the iron is mixed with raw wastewater to form iron phosphate precipitation and settle chemical sludge. The secondary treatment is an anaerobic bioreactor in which organic matters are oxidized by IRB and SRB. The polishing unit is to remove the remaining biological instability (e.g., ferrous Fe, sulfide, etc.) of the effluent from the biological system. Chlorine can be used as an oxidant that achieve both removal of biological instability and control of pathogens. The treated effluent can possibly be reused for a range of different purposes, which may or may not require additional treatment.

6 Conclusion

Microbial reduction processes are drawing increasing attention because of their benefits over conventional aerobic processes. Ferric reduction process has shown astonishing results in organic degradation in natural systems, and has a great potential to be used in novel wastewater treatment applications that provide multiple energy and environmental benefits. Majority of the studies on ferric reducers have focused on their fundamental biochemical mechanisms in soil and groundwater systems. Better understanding on ferric reduction process in engineering systems can broaden its applications to wastewater treatment using engineering systems. Future research in the areas identified in this paper is required to further develop this treatment concept and capitalize the benefits that are made available through adopting the biochemical reactions of IRB in wastewater treatment.

Acknowledgements This research is partly funded by a National Science Foundation Experimental Program to Stimulate Competitive Research program (NSF/EPSCoR, Award Number 1458952). The authors are thankful for the support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

References

Achtnich C, Bak F, Conrad R (1995) Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. Biol Fertil Soils 19:65–72

Arnold RG, DiChristina TJ, Hoffmann MR (1986) Inhibitor studies of dissimilative Fe(III) reduction by *Pseudomonas* sp. strain 200 ("*Pseudomonas ferrireductans*"). Appl Environ Microbiol 52:281–289

Arnold R, DiChristina T, Hoffmann M (1988) Reductive dissolution of iron(III) oxides by *Pseudomonas* sp. Biotechnol Bioeng 32:1081–1096

Azam HM, Finneran KT (2013) Ferric iron amendment increases Fe(III)-reducing microbial diversity and carbon oxidation in on-site wastewater systems. Chemosphere 90:1435–1443

Banwart SA, Davies S, Stumm W (1989) The role of oxalate in accelerating the reductive dissolution of hematite (α-Fe_2O_3) by ascorbate. Colloids Surf 39:303–309

Beliaev A, Saffarini D (1998) Shewanella putrefaciens mtrB encodes an outer membrane protein required for Fe(III) and Mn(IV) reduction. J Bacteriol 180:6292–6297

Beliaev A, Saffarini D, McLaughlin J, Hunnicutt D (2001) MtrC, an outer membrane decahaem c cytochrome required for metal reduction in Shewanella putrefaciens MR-1. Mol Microbiol 39:722–730

Berner RA (1973) Phosphate removal from sea water by adsorption on volcanogenic ferric oxides. Earth Planet Sci Lett 18:77–86

Bonneville S, Cappellen PV, Behrends T (2004) Microbial reduction of iron(III) oxyhydroxides: effects of mineral solubility and availability. Chem Geol 212:255–268

Bowman JP, Mccammon SA, Nichols DS, Skerratt JH, Rea SM, Nichols PD, Mcmeekin TA (1997) *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5 omega 3) and grow anaerobically by dissimilatory Fe(III) reduction. Int J Syst Bacteriol 47:1040–1047

Castro AFD, Ehrlich HL (1970) Reduction of iron oxide minerals by a marine Bacillus. Antonie Van Leeuwenhoek 36:317–327

Chan YJ, Chong MF, Law CL, Hassell D (2009) A review on anaerobic–aerobic treatment of industrial and municipal wastewater. Chem Eng J 155:1–18

Chang N, Xuan Z, Daranpob A, Wanielista M (2011) A subsurface upflow wetland system for removal of nutrients and pathogens in on-site sewage treatment and disposal systems. Environ Eng Sci 28:11–24

Chen J, Gu BA, Royer R, Burgos WD (2003) The roles of natural organic matter in chemical and microbial reduction of ferric iron. Sci Total Environ 307:167–178

Clement J-C, Shrestha J, Ehrenfeld JG, Jaffe PR (2005) Ammonium oxidation coupled to dissimilatory reduction of iron under anaerobic conditions in wetland soils. Soil Biol Biochem 37:2323–2328

Dar SA et al (2007) Analysis of diversity and activity of sulfate-reducing bacterial communities in sulfidogenic bioreactors using 16S rRNA and dsrB genes as molecular markers. Appl Environ Microbiol 73:594–604

Deng D, Lin L-S (2013) Two-stage combined treatment of acid mine drainage and municipal wastewater. Water Sci Technol 67(5):1000–1007

Deng D, Weidhaas JL, Lin L-S (2016) Kinetics and microbial ecology of batch sulfidogenic bioreactors for co-treatment of municipal wastewater and acid mine drainage. J Hazard Mater 305:200–208

Esther J, Sukla LB, Pradhan N, Panda S (2015) Fe(III) reduction strategies of dissimilatory iron reducing bacteria. Korean J Chem Eng 32:1–14

Finneran KT, Lovley DR (2001) Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA). Environ Sci Technol 35:1785–1790

Franks AE, Malvankar N, Nevin KP (2010) Bacterial biofilms: the powerhouse of a microbial fuel cell. Biofuels 1:589–604

Fredrickson JK, Gorby YA (1996) Environmental processes mediated by iron-reducing bacteria. Curr Opin Biotechnol 7:287–294

Guerin WF, Blakemore RP (1992) Redox cycling of iron supports growth and magnetite synthesis by *Aquaspirillum magnetotacticum*. Appl Environ Microbiol 58:1102–1109

Hughes TA, Gray NF (2013) Co-treatment of acid mine drainage with municipal wastewater: performance evaluation. Environ Sci Pollut Res 20:7863–7877

Hyun MS, Kim BH, Chang IS, Park HS, Kim HJ, Kim GT, Kim MA, Park DH (1999) Isolation and identification of metal-reducing bacterium, *Shewanella putrefaciens* IR-1. Korean J Microbiol 37:206–212

Ivanov V, Stabnikov V, Zhuang WQ, Tay JH, Tay STL (2005) Phosphate removal from the returned liquor of municipal wastewater treatment plant using iron-reducing bacteria. J Appl Microbiol 98:1152–1161

Jensen MM, Thamdrup B, Rysgaard S, Holmer M, Fossing H (2003) Rates and regulation of microbial iron reduction in sediments of the Baltic-North Sea transition. Biogeochemistry 65:295–317

Jones JG, Gardener S, Simon BM (1983) Bacterial reduction of ferrric iron in a stratified eutrophic lake. J Gen Microbiol 129:131–139

Kamura T, Takai Y, Ishikawa K (1963) Microbial reduction mechanism of ferric iron in paddy soils (Part I). Soil Sci Plant Nutr 9:5–9

Kuhn EP, Colberg PJ, Schnoor JL, Wanner O, Zehnder AJB, Schwarzenbach RP (1985) Microbial transformations of selected alkylbenzenes and halogenated aliphatic hydrocarbons in methanogenic aquifer material: a microcosm study. Environ Sci Technol 19:961–968

Liu C, Kota S, Zachara JM, Fredrickson JK, Brinkman CK (2001) Kinetic analysis of the bacterial reduction of goethite. Environ Sci Technol 35:2482–2490

Lonergan DJ, Jenter HL, Coates JD, Phillips EJP, Schimidt TM, Lovley DR (1996) Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J Bacteriol 178:2402–2408

Lovley DR, Phillips EJP (1986) Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl Environ Microbiol 51:683–689

Lovley DR, Stolz JF, Nord GL, Phillips EJP (1987) Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. Nature 330:252–254

Lovley DR (1987) Organic matter mineralization with the reduction of ferric iron: a review. Geomicrobiol J 5:375–399

Lovley DR (1993) Dissimilatory metal reduction. Annu Rev Microbiol 47:263–290

Lovley DR (1995) Bioremediation of organic and metal contaminants with dissimilatory metal reduction. J Ind Microbiol 14:85–93

Lovley DR, Lonergan DJ (1990) Anaerobic oxidation of toluene, phenol, and p-cresol by the disssimilatory iron-reducing organism, GS-15. Appl Environ Microbiol 56:1858–1864

Lovley DR, Phillips EJ (1987) Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl Environ Microbiol 53:2636–2641

Lovley DR, Phillips EJ (1988) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl Environ Microbiol 54:1472–1480

Lovley DR, Phillips EJP (1989) Requirement for a microbial consortium to completely oxidize glucose in Fe(III)-reducing sediments. Appl Environ Microbiol 55:3234–3236

Lovley DR, Baedecker MJ, Lonergan DJ, Cozzarelli IM, Phillips EJP, Siegel DI (1989) Oxidation of aromatic contaminants coupled to microbial iron reduction. Nature 339:297–299

Lovley DR, Giovannoni SJ, White DC, Champine JE, Phillips EJ, Gorby YA, Goodwin S (1993) *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch Microbiol 159:336–344

Lowe K, Siegrist R (2008) Controlled field experiment for performance evaluation of septic tank effluent treatment during soil infiltration. J Environ Eng 134:93–101

Madigan MT, Martinko JM, Bender KS, Buckley DH, Stahl DA (2015) Brock biology of microorganism, 14th edn. Pearson Education, IL

Major DW, Mayfield CI, Barker JF (1987) Biotrans-formation of benzene by denitrification in aquifer sand. Ground Water 26:8–14

Manariotis ID, Grigoropoulos SG (2002) Low-strength wastewater treatment using an anaerobic baffled reactor. Water Environ Res 74:170–176

McKinley J, Siegrist R (2010) Accumulation of organic matter components in soil under conditions imposed by wastewater infiltration. Soil Sci Soc Am J 74:1690–1700

Metcalf & Eddy Inc., Tchobanoglous G, Stensel HD, Tsuchihashi R, Burton F (2013) Wastewater engineering: treatment and resource recovery. McGraw-Hill, New York

Munch JC, Ottow JCG (1983) Reductive transformation mechanism of ferric oxides in hydromorphic soils. Environ Biogeochem 35:383–394

Myers CR, Myers JM (1997) Cloning and sequence of cymA, a gene encoding a tetraheme cytochrome c required for reduction of iron(III), fumarate, and nitrate by *Shewanella putrefaciens* MR-1. J Bacteriol 179:1143–1152

Myers JM, Myers CR (2001) Role for outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. Appl Environ Microbiol 67:260–269

Nealson KH, Myers CR (1990) Iron reduction by bacteria: a potential role in the genesis of banded iron formations. Am J Sci 290-A:35–45

Park W, Nam YK, Lee MJ, Kim TH (2009) Anaerobic ammonia-oxidation coupled with Fe^{3+} reduction by an anaerobic culture from a piggery wastewater acclimated to NH^{4+}/Fe^{3+} medium. Biotechnol Bioprocess Eng 14:680–685

Pierre J, Fontecave M, Crichton R (2002) Chemistry for an essential biological process: the reduction of ferric iron. Biometals 15:341–346

Pitts KE, Dobbin PS, Reyes-Ramirez F, Thomson AJ, Richardson DJ, Seward HE (2003) Characterization of the *Shewanella oneidensis* MR-1 decaheme cytochrome MtrA: expression in *Escherichia coli* confers the ability to reduce soluble Fe(III) chelates. J Biol Chem 278:27758–27765

Postma D (1993) The reactivity of iron oxides in sediments: a kinetic approach. Geochim Cosmochim Acta 57:5027–5034

Reinhard M, Goodman NL, Barker JF (1984) Occurrence and distribution of organic chemicals in two landfill leachate plumes. Environ Sci Technol 18:953–961

Roden EE, Wetzel RG (2002) Kinetics of microbial Fe(III) oxide reduction in freshwater wetland sediments. Limnol Oceanogr 47:198–211

Roetman E (1932) The sterilization of sewage by acid mine water. Dissertation, West Virginia University

Saravanane R, Murthy D (2000) Application of anaerobic fluidized bed reactors in wastewater treatment: a review. Environ Manag Health 11:97–117

Sawayama S (2006) Possibility of anoxic ferric ammonium oxidation. J Biosci Bioeng 101:70–72

Stefanie JWH, Elferink O, Visser A, Pol LWH, Stams AJM (1994) Sulfate reduction in methanogenic bioreactors. FEMS Microbiol Rev 15:119–136

Straub KL, Benz M, Schink B (2001) Iron metabolism in anoxic environments at near neutral pH. FEMS Microbiol Ecol 34:181–186

Strosnider W, Winfrey B, Nairn R (2011) Biochemical oxygen demand and nutrient processing in a novel multi-stage raw municipal wastewater and acid mine drainage passive co-treatment system. Water Res 45:1079–1086

Thamdrup B (2000) Bacterial manganese and iron reduction in aquatic sediments. Adv Microb Ecol 16:41–84

Thomsen U, Thamdrup B, Stahl DA, Canfield DE (2004) Pathways of organic carbon oxidation in a deep lacustrine sediment, Lake Michigan. Limnol Oceanogr 49:2046–2057

Thormann KM, Saville RM, Shukla S, Pelletier DA, Spormann AM (2004) Initial phases of biofilm formation in *Shewanella oneidensis* MR-1. J Bacteriol 186:8096–8104

Urrutia M, Roden E, Fredrickson J, Zachara J (1998) Microbial and surface chemistry controls on reduction of synthetic Fe(III) oxide minerals by the dissimilatory iron-reducing bacterium *Shewanella alga*. Geomicrobiol J 15:269–291

Utgikar VP, Harmon SM, Chaudhary N, Tabak HH, Govind R, Haines JR (2002) Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. Environ Toxicol 17:40–48

Van der Maas P (2005) Fe(III) EDTA-reduction in BioDeNOx reactors: kinetics and mechanism. In: Chemically enhanced biological NOx removal from flue gases. Wageningen, pp 109–124

Van Lier JB (2008) High-rate anaerobic wastewater treatment: diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. Water Sci Technol 57(8):1137–1148

Van Lier JB, van der Zee FP, Frijters CTMJ, Ersahin ME (2015) Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. Rev Environ Sci Biotechnol 14:681–702

Weber KA, Achenbach LA, Coates JD (2006) Microorganisms pumping iron:anaerobic microbial iron oxidation and reduction. Nature 4:752–764

Wei X, Viadero RC III, Buzby KM (2005) Recovery of iron and aluminum from acid mine drainage by selective precipitation. Environ Eng Sci 22(6):745–755

Widdel F (1988) Microbiology and ecology of sulfate and sulfur-reducing bacteria. In: Zehnder A (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 469–586

Wilson BH, Smith GB, Rees JF (1986) Biotiotransformations of selected alkylbenzenes and halogenated aliphatic hydrocarbons in methanogenic aquifer material: a microcosm study. Environ Sci Technol 20:997–1002

Yang WH, Weber KA, Silver WL (2012) Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. Nat Geosci 5:538–541

Zachara J, Fredrickson J, Smith S, Gassman P (2001) Solubilization of Fe(III) oxide-bound trace metals by a dissimilatory Fe(III) reducing bacterium. Geochim Cosmochim Acta 65:75–93

Zhang L, Keller J, Yuan Z (2009) Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. Water Res 43:4123–4132

Zhao R, Zhang H, Li Y, Jiang T, Yang F (2014) Research of iron reduction and the iron reductase localization of anammox bacteria. Curr Microbiol 69:880–887

Zinder B, Furrer G, Stumm W (1986) The coordination chemistry of weathering: II. Dissolution of Fe(III) oxides. Geochim Cosmochim Acta 50:1861–1869