Review

Anaerobic degradation of xenobiotic organic contaminants (XOCs): The role of electron flow and potential enhancing strategies

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Abstract

In groundwater, deep soil layer, sediment, the widespread of xenobiotic organic contaminants (XOCs) have been leading to the concern of human health and eco-environment safety, which calls for a better understanding on the fate and remediation of XOCs in anoxic matrices. In the absence of oxygen, bacteria utilize various oxidized substances, e.g. nitrate, sulphate, metallic (hydr)oxides, humic substance, as terminal electron acceptors (TEAs) to fuel anaerobic XOCs degradation. Although there have been increasing anaerobic biodegradation studies focusing on species identification, degrading pathways, community dynamics, systematic reviews on the underlying mechanism of anaerobic contaminants removal from the perspective of electron flow are limited. In this review, we provide the insight on anaerobic biodegradation from electrons aspect — electron production, transport, and consumption. The mechanism of the coupling between TEAs reduction and pollutants degradation is deconstructed in the level of community, pure culture, and cellular biochemistry. Hereby, relevant strategies to promote anaerobic biodegradation are proposed for guiding to an efficient XOCs bioremediation.

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Introduction

In many aspects of modern anthropogenic activities — industrial manufactures, household lives, agriculture production, various xenobiotic organic contaminants (XOCs) are synthesized, applied into use, and released. Accordingly, occurrences of these organic contaminants in soil and aquatic system have been globally reported (Sousa et al., 2018; Sun et al., 2018), which becomes an increasingly important issue on human health as well as eco-environment safety, due to their proved and potential toxicity (Gogoi et al., 2018).

XOCs with molecular intrinsic resistance to sorption and chemical transformation can be highly mobile in soil environment, they can enter deep layer soil and groundwater...
through downward immigration under the effect of leaching (Nakada et al., 2008). Also, non-polar hydrophobic organic matter with relatively high octane-water distribution coefficient, represented by PAHs, tends to accumulate into soil particles or sediment rather than being transported by water flow (Gocht et al., 2007; Li et al., 2009). Both two conditions above are typical anoxic environment, which can be an important reservoir for XOCs.

When sufficient oxygen is available, aerobic biodegradation happens as a primary process for natural attenuation, where oxygen would serve as activated agent and terminal electron acceptor (TEA) for the breakdown of substrate as well as energy production. In anoxic condition, various inorganic/organic matter ubiquitous in environment could substitute the role of oxygen and function as non-O₂ TEAs, e.g. nitrate, sulphate, Fe(III) and Mn(IV) (hydr)oxides, allowing the occurrences of XOCs biodegradation in anaerobic pattern (Schmidt et al., 2017). For example, under limited oxygen condition, available oxygen can be used only for aromatic ring activation to catechol by oxygenase reaction, then the depletion of oxygen makes catechol a dead-end product, leading to the shutdown of aerobic biodegradation. But the presence of non-O₂ TEAs allows its subsequent anaerobic biotransformation via O₂ and oxygenate independent pathway, in which catechol is carboxylated, dehydroxylated, and ligated with coenzyme A to form benzoyl-CoA, the central intermediate of anaerobic aromatic compound biodegradation. Then benzoyl-CoA is cleaved to short-chain hydrocarbons followed by step-wise mineralization to CO₂ (Fuchs et al., 2011; Zheng et al., 2019).

There have been lots of researches illustrating aerobic XOCs biodegradation, but XOCs anaerobic degradation studies remain limited, despite that many anoxic niches such as flooding soil, groundwater, aquatic sediment, are important ecosystems calling for better exploration. It is accepted that, in general, aerobic and suboxic metabolism support more energy conservation and biomass assimilation, resulting in more rapid degradation than strict anaerobic metabolism (Philipp and Schink, 2012; Schaper et al., 2018). However, in recent decades, increasing reports have indicated that XOCs biodegradation can be redox-dependent (Fang et al., 2020), i.e. certain XOCs are less recalcitrant and more susceptible to degradation under anoxic condition (Boopathy, 2001; Rathour et al., 2019; Schmidt et al., 2017).

In brief, anoxic environment medium, low microbial activity and lack of oxygen can be the main reasons for the retard of XOCs degradation in many cases, whereas specific reactions prone to happen under anaerobic condition makes anaerobic biodegradation process irreplaceable. Furthermore, in comparison to traditional aerobic bioremediation strategy, anaerobic metabolism attracts public attention due to its inexpensive advantages (Lovley, 2001). Hence, the identification of anaerobic degraders, understanding of genetic and biochemical mechanism, ecological knowledge about cooperation among community members, and practice of bioremediation require more endeavors. In this review, we summarize typical terminal electron accepting processes (TEAPs) involved in anaerobic biodegradation, with a focus on electron production, transportation, and consumption. Specific anaerobic degradation pathways for different types of XOCs are not included here, readers are recommended to the review of Chattas et al. (2017). Furthermore, the mechanisms of anaerobic XOCs biodegradation in the level of both pure culture and microbial community are reviewed, potential methods to improve the efficiency of anaerobic biodegradation are proposed. Finally, current challenges in TEAP-based bioremediation practices and future research directions are suggested.

### 1. Anaerobic biodegradation coupled with various non-O₂ TEAs

Nitrate is an energetically favorable electron acceptor, its energy production from denitrification is close to oxygen reduction. Iron(III) reduction also has relatively high energy yields, followed by sulphate reduction, and methanogenesis (Rittmann and McCarty, 2005). According to the sequence of redox ladder, the low energy yield TEA respiratory guilds only outcompete when highly energetic TEAs are depleted, however, this traditional concept has been challenged by many studies revealing the inverse redox zonation or the simultaneous occurrences of multiple electron accepting processes (Meckenstock et al., 2015).

Oxygen reduction:

\[
0.25O₂ + H^+ + e^- = 0.5H₂O, \Delta G^0 = -78.72\text{kJ/e}_-\text{eq}
\]

Dissimilatory nitrate reduction (DNR):

\[
0.2NO₃⁻ +1.2H^+ + e^- = 0.1N₂+0.6H₂O, \Delta G^0 = -72.20\text{kJ/e}_-\text{eq}
\]

Dissimilatory metal reduction (DMR):

\[
Fe^{3+} + e^- = Fe^{2+}, \Delta G^0 = -74.27\text{kJ/e}_-\text{eq}
\]

Dissimilatory sulphate reduction (DSR):

\[
0.125SO₄²⁻ + 1.1875H^+ + e^- = 0.0625H₂S + 0.0625HS⁻ + 0.5H₂O, \Delta G^0 = +20.85\text{kJ/e}_-\text{eq}
\]

Methanogenesis:

\[
0.125CO₂ + H^+ + e^- = 0.125CH₄ + 0.25H₂O, \Delta G^0 = +23.53\text{kJ/e}_-\text{eq}
\]

The processes of non-O₂ TEAs reduction contribute numerously to the global biogeochemical cycle of various elements, resulting the couple between the cycle of organic carbon with the elemental dynamics of N, S, Fe, Mn, etc. Microbial driven denitrification flux contributes to nitrate loss six fold higher than leaching in forest ecosystem (Fang et al., 2015); DSR process accounts for over 50% organic matter mineralization, which plays a major role in anaerobic turnover of organic carbon in marine sediment (Jorgensen, 1982); DMR has been estimated bringing about from 10% to essentially all of the organic matter oxidation in aquatic sediments or submerged soils (Lovley, 2013). In anoxic environment like wetlands, methanogenic degradation contributes about 164 Tg CH₄ production per year (Bridgeham et al., 2013).

Beyond the ecological functions of non-O₂ TEAs respiring bacteria, the ability of proceeding anaerobic biodegradation through TEA reduction also makes them promising in XOCs.
removal. The decomposition of XOCs releases reductive equivalents, electrons are transported through respective electron transport chains (ETCs), finally consumed by TEA reducing enzymes. These three components make up the whole process of anaerobic XOCs biodegradation fueled by various TEAs reduction. Electron accepting processes, related electron transport system in different TEA reducers, as well as the examples of XOCs degradation of typical TEAPs are listed in Table 1.

Complete denitrification process involves a series of nitrous oxide reductase, i.e. nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR), and nitrous oxide reductase (NOS). Some corresponding genes such as narG, nirK, norB, also present in bacteria performing incomplete denitriﬁcation and dissimilatory nitrate reduction to ammonia (DNRA) (Lu et al., 2014). The reducing equivalents consumed by denitrifying enzymes are derived from carbon metabolism and transported via electron transfer system including complex I (NADH-ubiquinone oxidoreductase and ubiquinone pool) and complex III (ubiquinol-cytochrome c oxidoreductase) (Li et al., 2016). Many organic compounds less degradable under aerobic condition can be effectively utilized as carbon source by nitrate reducers under anoxic condition (Shen et al., 2015).

In dissimilatory sulphate reduction process, sulphate is initially reduced to sulfite via ATP sulfurylase and APS reductase, with adenosine phosphosulfate (APS) serving as an activated intermediate product, followed by the reduction of sulfite to sulfide via six electron transfer (Qian et al., 2019). Organic carbon metabolism releasing reductive equivalents is necessary to meet the demands of sulfide reduction. Protons and electrons produced from substrate fermentation would be converted into H2 in cytoplasm by membrane-bound hydrogenase. Hydrogen diffusing into cell periplasm space would be oxidized to protons and electrons again by dehydrogenase. Transmembrane protons gradient facilitates ATP synthesis, while electrons are passed into cytoplasmic DSR system by c-type cytochromes network (Li et al., 2018). Typically, the link between electron donor oxidation and TEA reduction in SRB can be explained by classic “hydrogen cycling” model.

Among all the dissimilatory metal reducing bacteria, Shenanelia and Geobacter spp. are two best studied genera for their ability of extracellular electron transfer (EET). In Shenanelia spp., the metal reducing (Mtr) pathway consists of a series of redox proteins, i.e. quinol dehydrogenase CymA, periplasmic STC and Fcc3, trans-outier-membrane protein complex MtrABC, and OmcA. In this way, quinol is oxidized on cytoplasmic membrane and then the released electrons can be transferred to extracellular minerals through periplasm. In Geobacter spp., porin-cytochrome (Pcc) pathway involved in EET includes quinol dehydrogenases CbcL and ImcH, periplasmic PpcA and PpcD, trans-outier-membrane protein complexes containing ExtA, ExtD, ExtC, OmaB, OmaC, OmbB, OmbC, OmcB and OmcC (Jiang et al., 2020b). Through such outward electron transport system, released electrons from oxidation of organic matter can be directly transferred to external solid metal (hydro)xides or electrodes (Chan et al., 2017). EET-capable microorganisms can also transfer electrons to other active cells to establish direct interspecies electron transfer (DIET) (Rotaru et al., 2014b), which is of great importance to the enhancement of microbial syntrophic interactions.

Unlike other TEAPs mentioned above, the proceeding of methanogenic degradation of organic matter are mainly through syntrophic metabolism between fermenting bacteria and methanogens, although some methylotrophic methanogens have been reported for the direct methanogenesis degradation of methyl compounds (Hu et al., 2018; Ticak et al., 2015). Through serial hydrolysis, acidogenesis, acetogenesis reactions, the complex organic matter is gradually broken into small molecules. Hydrogen gas is formed for indirect interspecies electron transfer (IIHT), finally consumed by hydrogenotrophic methanogens performing the reaction $\text{CO}_2 + 4\text{H}_2 = \text{CH}_4 + 2\text{H}_2\text{O}$ where $\text{CO}_2$ are used as TEA to reduce hydrogen. Although methanogens do not utilize organic substrate directly, they consume electron carrier intermediates to facilitate fermentation by decreasing the partial pressure of hydrogen (Stams and Plugge, 2009; Thauer et al., 2008).

Currently, direct interspecies electron transfer (DIET) among the members of methanogenic consortia via conductive material amendment and the formation of cell-to-cell contacts has attracted wide scientific concern. DIET-based anaerobic digestion performs more effective methanogenesis and more stable operation than IIHT does, which is promising to be the mainstream technology in anaerobic digestion (Rotaru et al., 2014; Zhao et al., 2017).

In addition to four typical non-O2 TEAs mentioned above, many other substances have been proved capable of receiving electrons from microorganisms and participating in anaerobic XOCs oxidation, here we give the examples of perchlorate, quinone-rich substance, and halogenated organic compounds.

Perchlorate is a highly stable oxide, its high reducing potential makes it a possible electron acceptor under anoxic conditions. The catalytic enzymes responsible for perchlorate reduction are perchlorate reductase ($\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^-$) and chlorite dismutase ($\text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2$). Most perchlorate respiring bacteria (PRB) are facultative anaerobes, nitrate, oxygen, chlorate are preferred by them (Liebensteiner et al., 2014). Through (per)chlorate respiration, PRB can degrade a variety of organic compounds, such as benzene and hydrocarbon (Mehboob et al., 2009, 2016; Weelink et al., 2008), showing a great potential in the simultaneous remediation for co-contamination of XOCs and perchlorate. Especially, the biogenic oxygen during (per)chlorate reduction enables oxygenease dependent XOCs degradation in PRB, even supports the activity of other aerobic degraders in co-cultures (Wang and Coates, 2017). The characteristic of oxygen production in perchlorate respiration could allow the occurrence of aerobic degradation under anoxic environment, which provides another unique choice for XOCs bioremediation.

Both humic substance (HS) and other carbon-based materials like biochar and activated carbon are rich in various chemical moieties, such as quinone/hydroquinone, alcoholic, phenolic, ketone, which endows them with the ability of giving or receiving electrons (Scott et al., 1998). Among all these moieties, quinone is considered to be the major redox-active group contributing to electron transferring capacity (Hernandez-Montoya et al., 2012). In 1996, Lovley et al. (1996) pioneeringly reported that Geobacter metalloreduccens and Shenanelia alga could anaerobically oxidized organic matter or hydrogen with HS as electron acceptor. Since
Table 1 – Electron production, electron transport and consumption in typical electron acceptor processes.

<table>
<thead>
<tr>
<th>TEAP</th>
<th>TEAs reductive process</th>
<th>Electron transport process</th>
<th>Example of XOCs degradation</th>
</tr>
</thead>
</table>
| DNR  | NO₃⁻ → NO₂⁻ → NO → N₂O → N₂ | N₂ | - Benzene (*Bacillus cereus*) ([Dou et al., 2010](#));  
|      | NO₂⁻ → NO₂⁻ → NO → N₂O → NH₄⁺  | N₂O | - Sodium dodecyl sulfate (SDS) (*Pseudomonas nitroreducens*) ([Paulo et al., 2013](#));  
|      |                                   |     | - Benzo(a)pyrene (*Microbacterium sp.*) ([Qin et al., 2017](#));  
|      |                                   |     | - Biphenyl, fluorene, phenanthrene and pyrene (consortia obtained from paddy soil) ([Ambrosoli et al., 2005](#));  
|      |                                   |     | - Naphthalene (enriched mix bacteria community from contaminated groundwater) ([Zhang et al., 2019b](#));  
|      |                                   |     | - Heterocyclic compound pyrrolidine and piperidine (enriched consortia from environment samples and activated sludge) ([Ba et al., 2002a](#));  
|      |                                   |     | - 2-Chlorophenol (enriched culture from activated sludge) ([Ba et al., 2002b](#));  
|      |                                   |     | - P-xylene (highly enriched Betaproteobacteria) ([Rotaru et al., 2010](#));  
|      |                                   |     | - Naphthalene and fluoranthene (sediment from highly industrialized river estuary) ([Ribeiro et al., 2018](#));  
|      |                                   |     | - Tetramobisphenol A (surface river sediment) ([Wang et al., 2019b](#));  
|      |                                   |     | - Alkane and alkene (*Desulfatibacillum aliphtaticivorans*) ([Grossi et al., 2007](#));  
|      |                                   |     | - P-xylene (*Desulfosarcina sp.*) ([Higashioka et al., 2012](#));  
|      |                                   |     | - Naphthalene (enrichment culture from contaminated aquifer) ([Kuemmel et al., 2015](#));  
|      |                                   |     | - Glycerol (consortium composed of *Desulfobulbus baarsii*, *Desulfomicrobium sp.*, and *Desulfuromonas sp.* and *Desulfobulbus baarsii*) ([Dinkel et al., 2010](#));  
|      |                                   |     | - Sulfonated diazo dye (phylogenetically diverse SRB culture which includes Desulfobulbus, Desulfokalibas, *Desulfuromonas*, and *Desulfovibrio*) ([Rasool et al., 2016](#));  
|      |                                   |     | - Antibiotics (SRB sludge system) ([Jia et al., 2018, 2017](#));  

- DSR | SO₄²⁻ → APS → SO₃²⁻ → S²⁻ | S²⁻ | - Alkane and alkene (*Desulfatibacillum aliphaticivorans*) ([Grossi et al., 2007](#));  
|      |                            |     | - P-xylene (*Desulfosarcina sp.*) ([Higashioka et al., 2012](#));  
|      |                            |     | - Naphthalene (enrichment culture from contaminated aquifer) ([Kuemmel et al., 2015](#));  
|      |                            |     | - Glycerol (consortium composed of *Desulfobulbus baarsii*, *Desulfomicrobium sp.*, and *Desulfuromonas sp.* and *Desulfobulbus baarsii*) ([Dinkel et al., 2010](#));  
|      |                            |     | - Sulfonated diazo dye (phylogenetically diverse SRB culture which includes Desulfobulbus, Desulfokalibas, *Desulfuromonas*, and *Desulfovibrio*) ([Rasool et al., 2016](#));  
|      |                            |     | - Antibiotics (SRB sludge system) ([Jia et al., 2018, 2017](#));  

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Table 1 (continued)

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<th>Electron transport process</th>
<th>Example of XOCs degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMR</td>
<td>Fe(III) → Fe(II)</td>
<td></td>
<td>- Monoaromatic hydrocarbons (Geobacter toluenoxydans) (Kunapuli et al., 2010);</td>
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<tr>
<td></td>
<td>(extracellular)</td>
<td></td>
<td>- Benzene (Geobacter metallireducens) (Zhang et al., 2013);</td>
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<td></td>
<td>- Estrogens (iron reducing bacteria affiliated to Shewanella baltica) (Ivanov et al., 2010);</td>
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<td></td>
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<td></td>
<td>- Simultaneous Cr(VI) removal and 2,2',4,4'-tetrabromodiphenyl ether biodegradation (Pseudomonas aeruginosa) (Tang et al., 2016);</td>
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<td></td>
<td>- Aromatic compounds biodegradation coupled with arsenate respiration (Desulsulporosinus sp.) (Liu et al., 2004);</td>
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<td>- Pharmaceuticals (mixture of anaerobic sediment in the effluent channel of WWTPs) (Liu et al., 2020a);</td>
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<td>- Quinoline (consortium from coking WWTPs) (Huang et al., 2020);</td>
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<td></td>
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<td></td>
<td>- Biodegradation of phenol with Cr(VI) reduction in an anaerobic fixed-biofilm process (Lin et al., 2009).</td>
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Table 1 (continued)

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</tr>
</thead>
<tbody>
<tr>
<td>Methanogenesis</td>
<td>CO₂ → CH₄</td>
<td></td>
<td>- Mineralization of benzene and phenol into CH₄ and CO₂ (Masumoto et al., 2012);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Syntrophic conversion of n-alkanes to methane (Wang et al., 2011);</td>
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<td></td>
<td></td>
<td></td>
<td>- Herbicides atrazine, cyanazine, and dicamba by methanogenic enrichment cultures from submerged soils (Gu et al., 2003);</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Mineralization of bensulfuron methyl by enrichment from anaerobic granular sludge collected from UASB (Zhu et al., 2018);</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Methanogenic biodegradation of PAHs by crude oil-degrading enrichment (Berdugo-Clavijio et al., 2012);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Direct methanogenic biodegradation of tetramethylammonium by Methanococcoides strain (Ticak et al., 2015).</td>
</tr>
</tbody>
</table>

(Baek et al., 2018)
then, increasing studies have confirmed that HS respiration is involved in anaerobic degradation of various XOCs, for example, benzene (Cervantes et al., 2011), toluene (Cervantes et al., 2001), 17α-ethinylestradiol (Huang et al., 2019), 17β-estradiol (Dai et al., 2019). Similarly, biochar has been proved to function as electron acceptor to support microbial extracellular respiration and cell growth (Yu et al., 2015), activated carbon could serve as TEA during the anaerobic biotransformation of azo dyes (Van der Zee et al., 2003). Based on their intrinsic redox properties, HS and carbon-based materials do not only act as TEAs but also serve as electron shuttles or redox mediators to facilitate the transport of electrons (Section 4.2).

Halogenated organic compounds like chlorinated hydrocarbons could act as TEA, leading to the generation of energy from electron flow along respiration chain as well as reduction of compounds, R-Cl+2[H]→R-H+H+Cl− (Hong and Gu, 2009). The application of halo-respiring bacteria (HRB) has achieved the dechlorination of hexachlorobenzene and pentachlorobenzene (Jayachandran et al., 2003), polychlorinated dibenzo-p-dioxins and dibenzofurans (Bunge et al., 2003), polychlorinated biphenyl (Payne et al., 2011), etc. Since halogenated XOCs act as electron acceptors as well as contaminants simultaneously, thereby the electron competition between them and other ubiquitously present TEAs like nitrate can be a prominent problem in practice. Inhibiting other TEAs reduction, for example, inhibit DSR by MoO42− addition, has been reported as a possible solution (Cheng et al., 2019). Exploring the role of other TEAs on target halogenated XOCs qualitatively and quantitively is essential for further design of halogenated XOCs bioremediation (Wang et al., 2020).

Fig. 1 – Mechanism of anaerobic XOCs biodegradation in pure culture in terms of electron production, transport and consumption, example adopted from Wang et al. (2019). The part to be regulated (inhibited or promoted) are labelled by flash symbols.

2. Insight into the coupling between TEAs reduction and XOCs degradation

2.1. Pure culture level

In anaerobic cellular respiration, the reduction of TEAs requires reductive equivalents (electrons) released from the oxidation of electron donors, i.e. the catabolism of organic substrates. The electrons released can be transferred by electron transport chains, and finally consumed by TEAs reducing enzyme systems. Accordingly, organic matter oxidation together with TEAs reduction form a chain of electron flow, where the electron pool and sink are linked by ETCs and driven by electron balance (Fig. 1).

In denitrifying bacteria, a series of denitrifying enzymes utilize NADH generated from carbon metabolism (e.g. EMP pathway and TCA cycle) as reducing powers to reduce nitrate (Wan et al., 2019). In Paracoccus pantotrophus, compared to control group without TEA, the supplementation of nitrate enhanced N-methylpyrrolidone biodegradation under anoxic condition, where a continuous “reception sink” for carbon metabolism provided by the sequential reduction of nitrate to dinitrogen was responsible (Wang et al., 2019).

In addition to the electron sink provided by TEAs, ETCs is an essential link between TEAs reduction and upstream XOCs biodegradation, whose activity can be further improved in order to accelerate degradation. Fulvic acid, a major component of HS, has been proved for the ability of enhancing ETCs activity, by facilitating electron transport from NADH to denitrifying enzymes via complex I and complex III (Li et al., 2016). Shewanella oneidensis MR-1 co-cultured with Paracoccus deni-
ficans also promoted the electrons transfer activity via the formation of nanotube between cells (Jiang et al., 2020a).

On the other hand, it is worth noting that, electron production, transport and consumption interacting with each other jointly constitute the whole process of microbial anaerobic metabolism, inhibition on one process would impact others in turn. For example, in Paracoccus denitrificans, the disturbance on carbon metabolism led to the decrease of NADH generation and deterioration of nitrate reduction (Luo et al., 2018; Wan et al., 2019). Similarly, studies focus on DSR inhibition have identified that, certain substances could serve as inhibitors for the central sulphate reducing pathway and carbon oxidation of SRB (Carlson et al., 2015; Gao et al., 2019), the reduced DSR rate can be attributed to down regulation of genes involved in electron transfer and respiration (Chen et al., 2019).

2.2. Microbial community level

Regarding in situ environment, the introduction of exogenous TEAs has been proved to be effective on improving XOCs removal, which involves complex interactions and successions among microbial populations (Wang et al., 2019b; Yan et al., 2018; Zheng et al., 2020). The elevation of TEAs concentration can be overwhelming selective pressure on indigenous microbial community, driving the shift of community composition and structure (Wang et al., 2015). When certain environmental samples are as inoculum to conduct the enrichment of functional consortia, microbial community become distinct under different TEAs conditions (Zhang et al., 2019c).

Based on the differentiated community, metabolism functions and degradation patterns of XOCs may be largely dependent on TEAPs correspondingly. Although a few studies suggested that TEAs did not affect anaerobic degradation products of XOCs (Lu et al., 2008), the fate and persistence of XOCs in situ are influenced by the prevailing TEAPs (Fang et al., 2020; Schmidt et al., 2017). For instance, petroleum hydrocarbons biodegradation patterns varied under different reducing conditions, carboxylation was the principal mechanism for n-alkane degradation coupled to denitrification, whereas addition of fumarate was the dominant activation mechanism under sulphate reducing conditions (Zhang et al., 2019). Besides, the source of environmental sample and incubation time in lab are important factors determining community composition and structure (Ribeiro et al., 2018).

The ecological mechanism behinds how TEAs enhance XOCs biodegradation can be explained from several aspects. First, the addition of certain TEAs enriches populations who possess both related TEAs respiration as well as XOCs degrading abilities. In aromatic contaminated sediment amended with elevated nitrate, genes involved in the degradation of aromatic compounds were significantly enriched, and most of the enriched genes were derived from nitrate reducing microorganisms (Xu et al., 2015) (Fig. 2a). Another study indicated that, after the addition of nitrate into complexly contaminated sediment, key genes involved in nitrogen cycling increased, showing significant positive correlations with genes related to labile organic carbon degradation, and all those key genes were largely derived from microorganisms with diverse physiological and metabolic properties (Xu et al., 2014) (Fig. 2b). However, there are also some reports showing that microbial community did not respond to elevated TEAs significantly. One possible explanation may be that many bacteria harboring versatile metabolisms can use various TEAs for XOCs degradation regardless of TEAs changes (Xu et al., 2014), thereby the respiratory pathway of these versatile metabolizers are adjusted but the overall community structure remains unchanged (Wang et al., 2018) (Fig. 2c). Also, the dosage of TEAs influences the interactions between different functional assemblages, i.e. XOCs degrading assemblages and TEA reducing assemblages. In a recent network analysis, it was found that, compared to sulphate, the favorable TEA nitrate could facilitate to establish significant and direct connectivity between potential denitrifiers and PAHs degraders (Yang et al., 2020) (Fig. 2d). However, there is still very limited research on the role of various assemblages

Fig. 2 – Community succeeding patterns in biostimulation by the addition of TEAs.
interactions underlying the mechanism of exogenous TEA stimulation.

2.3. XOCs degradation among multiple TEAs and functional groups

In nature, the co-existence of multiple TEAs can be the real situation, basically due to the spatial-temporal heterogeneity of environment medium (Bu et al., 2015), and the availability of various electron acceptors (Meckenstock et al., 2015). The degradation pathway or metabolites detected under different TEAPs are observed to be different in many researches (Zhang et al., 2019, 2020). Further, the strategy to break down XOCs taken by facultative nitrate reducers differs from those used by strict anaerobes, such as SRB, fermenting bacteria, which is largely determined by the redox potentials of TEAs they used and the overall reaction energetics (Philipp and Schink, 2012). Last but not least, even a certain bacterium cell responds differently to organic contaminants under the regulatory of fluctuating TEAs, based on the versatility of metabolism endowed by its genome diversity (Rabus et al., 2005). Hence, a mixture of multiple degradation pathways can be expected in realistic environmental medium, where different TEAs and TEA reducers are co-existed.

Currently, most studies about TEAP-mediated anaerobic biodegradation focus on using single TEA to enhance the activity of a certain group of microbes. Notably, a series of studies done by Boopathy have shown that XOCs always have higher degradation rates under mix electron acceptor condition than single TEAP condition, the reason was attributed to be that various groups of anaerobic degraders are encouraged to grow and compete for the degradation of contaminants. The metabolic potential of the diverse mix populations is over a selected group of enrichment cultures (Boopathy, 2001, 2002, 2003; Boopathy and Peters, 2001). The combination of different TEAs could further improve the diversity of microbial community (Zhao et al., 2018b), and the synergetic effect among different assemblages can significantly promote the utilization and transfer of electrons and enhance the biodegradation efficiency (Fig. 3a). Besides, many microorganism have been proved to be able to use a variety of electron acceptors for respiration (Xu et al., 2014; Zhao et al., 2013), thus we assume that the enhanced activity of versatile metabolizers by the augment of multiple TEAs could be another important reason.

When multiple TEAs are present, the competition for limited electron donor among different TEA reducers will occur, on the one hand, microorganisms with versatile metabolism or broader TEAP capabilities can be more competitive (Zhao et al., 2013). On the other hand, many studies have shown the consistent results on the dominance of nitrate reducers and/or nitrate reduction out-competing other TEAPs under mix TEAs condition (Cunningham et al., 2001; Song et al., 2019; Zheng et al., 2019), mainly because nitrate is a more energetic favorable electron acceptor than others. Nitrate addition could inhibit perchlorate reduction (Zhao et al., 2011), and can be used as a strategy for the inhibition on sulfdogenic in DSR (Carlson et al., 2015). However, it is worth noting that, as for the reduction of halogenated compounds who act as the electron acceptor, with electron naturally dis-
tributed towards multiple co-existed TEAs, the electron selectivity on the target contaminant can be adversely impacted, leading to the failure of XOCs removal (Cheng et al., 2019).

In addition to the simultaneous amendment of mix TEAs, integrated bioremediation approach with effective pollutants removal performance was developed in recent years, in which sequential TEAs reduction is coupled with XOCs degradation in two phases. It is believed that integrated remediation technology could lead to a higher XOC removal rate as well as effective control of secondary pollution via the complementary between each steps. For example, after the enhancement of sulphate reduction via co-substrate dosage in the first phase, nitrate addition could inhibit further DSR and oxidized the product sulfide by autotrophic denitrification in the second phase (Liu et al., 2017; Zhang et al., 2015). And sequential iron reduction-DSR was achieved in biodiesel blend contaminated groundwater by the amendment of goethite-like material, no obvious sulfide production was detected in the second phase, which was attributed to iron sulfide precipitate ( Muller et al., 2017) (Fig. 3b).

3. Strategies to enhance anaerobic XOCs biodegradation

3.1. The addition of appropriate TEAs

Oxygen is the best characteristic and energetically favorable electron acceptor in traditional degradation metabolism, while its disadvantages in bioremediation application are obvious, e.g. its solubility in water is low and easy to volatile; the energy consumption to distribute oxygen/air into subsurface can be a huge cost. By contrast, other TEAs mentioned above have several advantages in common: (1) many of them have higher electron acceptance than oxygen does, e.g. one molecular sulphate could accept eight electrons, which is twice of oxygen; (2) nitrate and sulphate are highly soluble in water and easy to be delivered; (3) wide and cheap sources, e.g. biochar is derived from organic waste (Fakayode et al., 2020), Fe(III)/Mn(IV) (hydr)oxides can be produced from drinking water treatment process (Liu et al., 2018); (4) it is easy to recover solid TEAs like metal (hydr)oxides; (5) re-oxidation of Fe(III)/Mn(IV) oxides is feasible (Zhou et al., 2018), and electron transfer with HS is fully reversible over successive redox cycle (Klüpfel et al., 2014).

As we stated above, TEAP is a determining factor for XOCs degradation pattern in addition to molecular characteristic. For example, the degradation rate of ethylthionocarbamate coupled with different TEAs follows the order: ferric > nitrate > sulfate (Chen et al., 2011). It can be seen that different predominant TEAs leads to specific biodegradability (Liu et al., 2011), catalytic effects of TEAs on the biodegradation of XOCs are not always in accordance with the order of redox potentials, i.e. oxygen > nitrate > Fe(III)/Mn(IV) > sulfate > CO2. Moreover, bioremediations via TEAs addition are not always satisfied with our expectation. In some cases, the addition of TEAs inhibits XOCs degradation instead (Li et al., 2010). For example, the amendant of Mn(IV) to PAHs contaminated mangrove sediment inhibited PAHs removal, which was attributed to the toxicity of produced Mn(II) (Li et al., 2011). Hence, the selection of appropriate TEAs is of vital importance to efficient bioremediation.

For practical bioremediation of XOCs contaminated sites, studies on indigenous community and the in situ dominant TEAPs are necessary (Zhao et al., 2015). Nieman et al. (2001) suggested that the intrinsically dominant TEAs are more important for biodegradation than the amended ones. Adding TEAs that indigenous microorganism has adapted could shorten the lag phase, otherwise, the community need longer time to adapt to the changed niche (Dini-Andreote et al., 2014; Yang et al., 2020). Thereby applying corresponding local electron acceptors could promote indigenous TEA reducers and XOCs degraders more efficiently. For example, Spersfeld et al. (2018) characterized microbial community in a BTEX polluted aquifer firstly, and predicted that nitrate reducing process was important for local aromatic degradation based on the dominance of nitrate reducers. This prejudgment was subsequently confirmed by the enrichment of nitrate reducing and pollutant degrading bacteria in nitrate amended enriching culture. In addition, the amendment of mix TEAs or the sequential addition of TEAs should be considered in a wide range, as we stated in Section 3.3, multiple TEAPs shows greater potential on biostimulation than single TEAP.

3.2. The enhancement of electrons transport

On the top of appropriate TEAPs, further enhancing approach is to promote electron transfer by the mediation of electron shuttles/redox mediators (RMs). Humic substance and biochar could facilitate the microbial electron shuttle process by serving as a redox pool, i.e. via their functional moieties that can be reversibly oxidized or reduced (Yuan et al., 2017). For example, the electrons derived from anaerobic oxidation of organic matter was first transferred to anthraquinone-2,6-disulphonate (AQDS), a model analogous of HS, then the reduced AH2-QDS transferred electrons to less accessible electron acceptor ferricydrite, meanwhile being oxidized back to the originated state (Bai et al., 2020; Nevin and Lovley, 2000).

The electron transfer between electron donors and electron acceptors via RMs in HS/biochar amended systems has been evidenced by many reports (Van der Zee and Cervantes, 2009), and the catalytic function of RMs could considerably improve the degradation of XOCs (Kwon and Finneran, 2010; Niedzwiecka et al., 2017; Zhao et al., 2018a). For example, RMs enhanced the degradation of sulfamethoxazole coupled with Fe(III) reduction in Sewardella oneidensis MR-1 (Zhou et al., 2018a). Recently, Liu et al. (2020b) isolated a quinone-respiring strain capable of degrading petroleum hydrocarbons under denitrifying condition. They found that AQDS can obtain electrons from respiration chains and be reduced to AH2-QDS. Then AH2-QDS passed electrons to nitrate, nitrite and nitrous oxide, which improved electron transfer efficiency, leading to the enhancement of upstream substrate biodegradation (Liu et al., 2020b).

In addition to the charging and discharging process of the surface functional groups, electron transfer efficiency can be improved by the conductivity of materials as well, where direct interspecies electron transfer is mediated by physical materials like iron oxides, activated carbon, rather than by biolog-
ical electrical connective structures, such as membrane associated cytochromes or pilis (Chen et al., 2014; Liu et al., 2012). Through the conductivity based-DIET among consortia members, a better interspecies electron connectivity can be built up, thus XOCs degradation could be stimulated as a consequence of higher syntrophic efficiency (Bonaglia et al., 2020).

4. Challenges and prospects

For bioremediation of anoxic contaminated medium, biostimulation via the amendment of various TEAs to stimulate anaerobic microbial respiration has been proved efficient in practical applications, e.g. cleanup of fuel blend contaminated groundwater/soil (Müller et al., 2017; Müller et al., 2020). In comparison to other physicochemical remediation measures, TEAP-based biostimulation strategy has the advantages of low side effects and low cost of operation and management. However, limitations from several aspects have been observed and some practical difficulties still need further study (Majone et al., 2015).

(1) The amended TEAs are not always consumed by microbial anaerobic respiration as our expectation. On the one hand, abiotic physicochemical reaction is a common process in complex subsurface systems, e.g. clay minerals in soil may partly remove nitrate via sorption or ion exchange (Lazaratou et al., 2020), leading to the loss of amended acceptors. On the other hand, TEAs utilized by bacteria are not always related to the purpose of contaminants degradation. For instance, nitrate could be used to drive inorganic sulfide oxidation by autotrophic denitrifiers (Yang et al., 2012), Fe(III) can be used as TEA in Feammox process (Wang et al., 2015). These reactions above may lead to a low utilizing efficiency of TEAs and impede bioremediation.

(2) Researchers should be careful when considering the amount and the form of TEAs intended to apply to the polluted sites. Aiming at achieving as much pollutant removal as possible, the amended TEAs are always excessive (Lu et al., 2011), which may increase the risk of secondary pollution and salinization. Also, the formation of metallic sulfide precipitate may result in the clog of soil voids. Thus, some researchers suggest that TEAs required in DMR can be applied in the form of solid Mn(IV)/Fe(III) (hydr)oxides to avoid these problems. Besides, solid TEAs also bring additional benefits, e.g. easy to amend and recover, removing other pollutants like ammonia or heavy metals simultaneously (Liu et al., 2020a; Park et al., 2009).

(3) Organic matter previously present in environment, or added growth-supporting additives may negatively affect the degradation of target pollutants sometimes (Ghosh and Philip, 2004). This seems to be reasonable, since microorganisms always preferentially utilize simple and energetically favorable substrates rather than recalcitrant compounds. Thus the amount of growth-supporting additives in biostimulation should be moderate to achieve the balance in co-metabolism (Qin et al., 2018). TEAs addition has been proved to be useful to eliminate the inhibition on XOCs degradation, through accelerating the removal of simple chemicals at the initial stage to mitigate the metabolic flux dilution (Corseuil et al., 2015).

(4) When we have everything amended and constructed in place, does it become one hundred percent certain that indigenous microbes could well behave as contaminants degraders? In many cases, an obvious lag phase in the startup of bioremediation can be observed (Yamazaki et al., 2020), because it takes time for local microorganisms to acclimate and experience successions to change into highly efficient degrading community. As for this problem, bioaugmentation strategy can be combined. By introducing microorganism possessing the ability of efficient degradation and already adapted to pollutants, the lag phase can be shortened to some extent (Siddique et al., 2020).

(5) In situ environment characteristic is one of the most important factors whether the bioremediation can be successful, meanwhile, it is also the most complicated and changeable variable. The investigation of in situ condition is necessary as the basis of bioremediation proposal (Pierro et al., 2017), factors need to be investigated including contaminants distribution profile, soil textures, pH, temperature, inorganic salt composition, soil organic matter, mineral composition, local microorganism community composition and activities, etc. And previous small scale experiments should be conducted to verify and optimize bioremediation schemes (Pierro et al., 2017), potential environmental impacts should be evaluated in advance, and whole process monitoring and management should be conducted (Majone et al., 2015).

Although considerable information about TEAP-based biostimulation has been summarized, it remains incompletely understood, and various restrictions are still present. To fill these knowledge gaps and obtain more theoretical basis, more relevant investigations are needed in future research.

(1) Most of TEAP-based anaerobic biodegradation studies are conducted in relatively simple systems with an interest towards single type contaminants or single TEA. However, the co-existence of multiple TEAs and XOCs in heterogeneous environment is the real case, where the interactions of various XOCs degradation and TEAs reduction among different functional groups are underestimated in current knowledge. Thus, future studies are urged to focus on the biodegradation of combined pollutants under multiple TEAP conditions, in order to simulate and approach to the degradation in real complex environment.

(2) Many studies done in this field mainly concentrate on conventional TEAPs, nitrate, iron and sulphate reducing condition. However, other TEAs, e.g. perchlorate, Cr(VI), oxidized organic contaminants, are less concerned, and relevant practical implications are scared even more. Also, available studies on the interactions among different TEAs reduction in degradation mainly focus on the electron competition between nitrate reduction and sulphate reduction, whereas the relationship between well studied TEAs and other less studied TEAs is poorly understood. More practice of TEAP-based bioremediation in large scale is in demand.

(3) Currently, the deduction of XOCs transformation is mostly based on the detection of metabolites intermediates in
medium, phylogenetic analysis of communities and literature investigations are combined to further explore related function microbial groups responsible for each degradation steps. However, the direct evidence linking bacteria community and metabolites formation out of degradation behaviors is lacking. In practice, the discovery of whole chains of degradation to obtain a precise picture of degradation pathways can be challenging based on this “black-box” pattern. In order to better answer the question of “who did what by how”, the integration of stable isotope probing, target metabolites identification as well as multiple metabolomics are the up-to-date technology to reveal the hidden connections between apparent overall community performance and the contributions and interactions of individual members (Kleinsteuber et al., 2012; Yu et al., 2019). With the aid of advanced methodology, the synergistic effects among different TEA respiring degraders, and/or the relay pattern of XOCs degradation by sequential TEA reduction in anoxic environment can be unraveled in depth, which provides the theoretical basis for further bioremediation design.

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REFERENCE


Chen, S., Gong, W., Mei, G., Han, W., 2011. Anaerobic biodegradation of ethylbenzocarbanate by the mixed bacteria under various electron acceptor conditions. Bioreourc. Technol. 102, 10772–10775.


