Functional Genomics of Metal-Reducing Microbes Degrading Hydrocarbons

11

Pier-Luc Tremblay and Tian Zhang

Contents

Abstract

The anaerobic oxidation of petroleum hydrocarbons can be coupled to the reduction of metals. At contaminated subsurface sites, this phenomenon will accelerate the removal of pollutants and will have an important influence on biogeochemical cycles. Due to its abundance, iron is the most prominent metallic terminal electron acceptor involved in hydrocarbon degradation followed by manganese. Dissimilatory metal-reducing microbes (DMRM) capable of oxidizing either monocyclic aromatic or polycyclic hydrocarbons are phylogenetically diverse with representatives from bacteria as well as from archaea. It has

P.-L. Tremblay (✉) · T. Zhang
School of Chemistry, Chemical Engineering and Life Science, Wuhan University of Technology, Wuhan, People's Republic of China

Bioelectrochemical Systems, The Novo Nordisk Foundation Center for Biosustainablity, Technical University of Denmark, Hørsholm, Denmark
e-mail: pierluct@whut.edu.cn; pitre@biosustain.dtu.dk; zhang@biosustain.dtu.dk

M. Boll (ed.), *Anaerobic Utilization of Hydrocarbons, Oils, and Lipids*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-319-50391-2_13

been shown that the monocyclic aromatic hydrocarbons benzene, toluene, ethylbenzene, and xylene and the polyaromatic hydrocarbons naphthalene, 1-methylnaphthalene, and 2-methylnaphtalene can be degraded by metal-reducing enrichment cultures or pure cultures. In recent years, significant breakthroughs have been made in the field of functional genomics for the characterization of the metabolic pathways, enzymes, and genes participating to hydrocarbons degradation by metal-reducing microbes. Here, we present an updated portrait of the monocyclic aromatic and polycyclic hydrocarbons metabolism of DMRM.

1 Introduction

Contamination of sediments, soil, aquifer, and groundwater with petroleum hydrocarbons is a widespread concern posing a threat to human health and ecosystems. Due to the fast depletion of oxygen, polluted sites often become anaerobic and under these circumstances, the degradation of hydrocarbons requires the metabolic activity of microbes using other terminal electron acceptors (TEA) (Aburto-Medina and Ball 2015). This includes metals abundant in soil and sediments such as iron and manganese. Dissimilatory metal-reducing microbes (DMRM) couple the oxidation of organic molecules such as hydrocarbons with the reduction of metals as their sole TEA for energy conservation (Foght 2008). Because metals in soil and sediments are usually in a solid state, DMRM have developed unique extracellular electron transfer mechanisms. This includes direct electron transfer *via* outer membrane c-type cytochromes, conductive nanowires and pili as well as the synthesis of soluble electron shuttles (Lovley 2012; White et al. 2016).

The anaerobic oxidation of monocyclic aromatic hydrocarbons including benzene, toluene, ethylbenzene, and xylene has been demonstrated with DMRM-containing consortia as well as with DMRM pure cultures either with Fe(III) or Mn(IV) as the TEA (Lovley et al. 2011; Meckenstock and Mouttaki 2011; Meckenstock et al. 2016; Lovley and Lonergan 1990; Heider et al. 1998; Chakraborty and Coates 2004; Botton and Parsons 2006; Rooney-Varga et al. 1999; Jahn et al. 2005; Langenhoff et al. 1997a, b; Villatoro-Monzón et al. 2003). Furthermore, several studies have reported the microbial degradation of polycyclic hydrocarbons such as naphthalene and its derivatives with Fe(III) as TEA (Anderson and Lovley 1999; Kleemann and Meckenstock 2011). Until now, the microbial degradation of aliphatic hydrocarbons including alkane, alkene, and cycloalkane coupled with metal reduction by DMRM has never been reported.

Metabolic pathways responsible for the anaerobic oxidation of aromatic hydrocarbons by DMRM are complex and comprise multiple reactions with unique chemistry. Currently, many of those reactions are still poorly understood. This chapter will focus on the ongoing research effort aiming at elucidating the anaerobic hydrocarbon oxidation metabolism of DMRM and at characterizing the genes and enzymes involved.

2 Dissimilatory Metal-Reducing Microbes Involved in Anaerobic Hydrocarbons Degradation

Key Fe(III)-reducing microorganisms involved in the complete oxidation of monocyclic aromatic hydrocarbons at anoxic contaminated environments are often members of the *Geobacteraceae* family (Rooney-Varga et al. 1999) (Table 1). From this group, only pure cultures *Geobacter metallireducens* and *Geobacter* strain Ben have been shown to degrade both benzene and toluene (Lovley and Lonergan 1990; Lovley et al. 1993; Zhang et al. 2012). Two other *Geobacter* species, *Geobacter grbiciae* and *Geobacter toluenoxydans*, only degrade toluene (Coates et al. 2001; Kunapuli et al. 2010). Other Fe(III)-reducing bacteria capable of oxidizing toluene in pure culture include *Georgfuchsia toluolica* and *Desulfitobacterium aromaticivorans*, which are members of the Betaproteobacteria and Clostridia, respectively (Kunapuli et al. 2010; Weelink et al. 2009). *D. aromaticivorans* can also oxidize *o*-xylene with Fe(III) as TEA. When manganese is the TEA, *G. toluolica* and an enrichment culture dominated by an *Azoarcus* species can

Table 1 DMRM coupling aromatic hydrocarbon oxidation with metal reduction

Species	Classification	Degraded aromatic hydrocarbons with reduced metals	Reference
Geobacter metallireducens	Gram - *Deltaproteobacteria Geobacteraceae*	Benzene-Fe(III) Toluene-Fe(III)	Lovley and Lonergan 1990; Lovley et al. 1993; Zhang et al. 2012
Geobacter strain Ben		Benzene-Fe(III) Toluene-Fe(III)	Zhang et al. 2012
Geobacter grbiciae		Toluene-Fe(III)	Coates et al. 2001
Geobacter toluenoxydans		Toluene-Fe(III)	Kunapuli et al. 2010
Georgfuchsia toluolica	Gram - *Betaproteobacteria Rhodocyclaceae*	Toluene-Fe(III) or Mn(IV) Ethylbenzene-Mn(IV)	Weelink et al. 2009; Dorer et al. 2016
Azoarcus sp.-dominated microbial consortium		Toluene-Mn(IV) Ethylbenzene-Mn(IV)	Dorer et al. 2016
Desulfitobacterium aromaticivorans	Gram + *Clostridiales Peptococcaceae*	Toluene-Fe(III) *o*-xylene-Fe(III)	Kunapuli et al. 2010
Culture N49[a]		Naphtalene-Fe(III) 1-methylnaphthalene-Fe(III) 2-methylnaphthalene-Fe(III)	Kleemann and Meckenstock 2011
Ferroglobus placidus	Archaea *Archaeoglobales Archaeoglobaceae*	Benzene-Fe(III)	Holmes et al. 2011

[a]Culture N49 is composed of 90% of a bacteria species from the *Peptococcaceae* family

degrade both toluene and ethylbenzene (Dorer et al. 2016). Besides mesophilic bacteria, the hyperthermophilic archaeon *Ferroglobus placidus* can also degrade benzene and transfer electrons to Fe(III) (Holmes et al. 2011).

Polycyclic aromatic hydrocarbons degradation by metal-reducing microbes was demonstrated initially *via* the anaerobic oxidation of naphthalene with Fe(III) as the TEA (Anderson and Lovley 1999). Community composition involved in polycyclic aromatic hydrocarbons degradation with metal as TEA has not been investigated as much as for BTEX degradation. The only study to our knowledge on bacterial population in Fe(III)-reducing enrichment degrading naphthalene identified a Gram-positive spore-forming bacterium of the *Peptococcaceae* family as the dominating species (Kleemann and Meckenstock 2011). This enrichment could also degrade 1-methylnaphthalene and 2-methylnaphthalene.

3 Degradation of Monocyclic Aromatic Compounds

Genes, enzymes, and metabolic pathways involved in monocyclic aromatic hydrocarbons degradation by metal-reducing bacteria have mainly been studied in *G. metallireducens*, which is genetically tractable and had its genome sequenced in 2005 (Tremblay et al. 2012; Oberender et al. 2012; Butler et al. 2007; Lovley and Lonergan 1990; Lovley et al. 1993; Zhang et al. 2012). Genome survey of *G. metallireducens* have led to the identification of genes participating in the anaerobic oxidation of monocyclic aromatic hydrocarbons and of other monocyclic aromatic compounds including phenol, *para*-cresol, benzyl alcohol, benzaldehyde, and 4-hydroxybenzoate (Butler et al. 2007; Heinnickel et al. 2010). Additionally, *G. metallireducens* has been used as a model bacterium to study extracellular electron transfer to insoluble TEA such as Fe(III) oxide or the electrode of a bioelectrochemical system for electricity production (Lovley 2012; Bond et al. 2002; Smith et al. 2013).

3.1 Degradation of Benzene to Benzoyl-CoA

Because of its structural stability and high solubility in water, benzene is the most persistent monocyclic aromatic hydrocarbon in the environment (Vogt et al. 2011). Benzene is harmful for humans and other animals as it can cause cancer and aplastic anemia and impairs the reproductive system (Aburto-Medina and Ball 2015). Anaerobic bacteria such as *G. metallireducens* convert monocyclic aromatic compounds including benzene into the central intermediate benzoyl-CoA before subsequent dearomatization (Heinnickel et al. 2010; Zhang et al. 2012) (Fig. 1). The metabolic pathway from benzene to benzoyl-CoA in *G. metallireducens* has not been completely characterized yet. Several activation mechanisms with specific thermodynamic constraints have been proposed as the first step of anaerobic benzene oxidation including hydroxylation to phenol, carboxylation to benzoate or methylation to toluene (Vogel and Grbìc-Galìc 1986; Grbić-Galić and Vogel 1987;

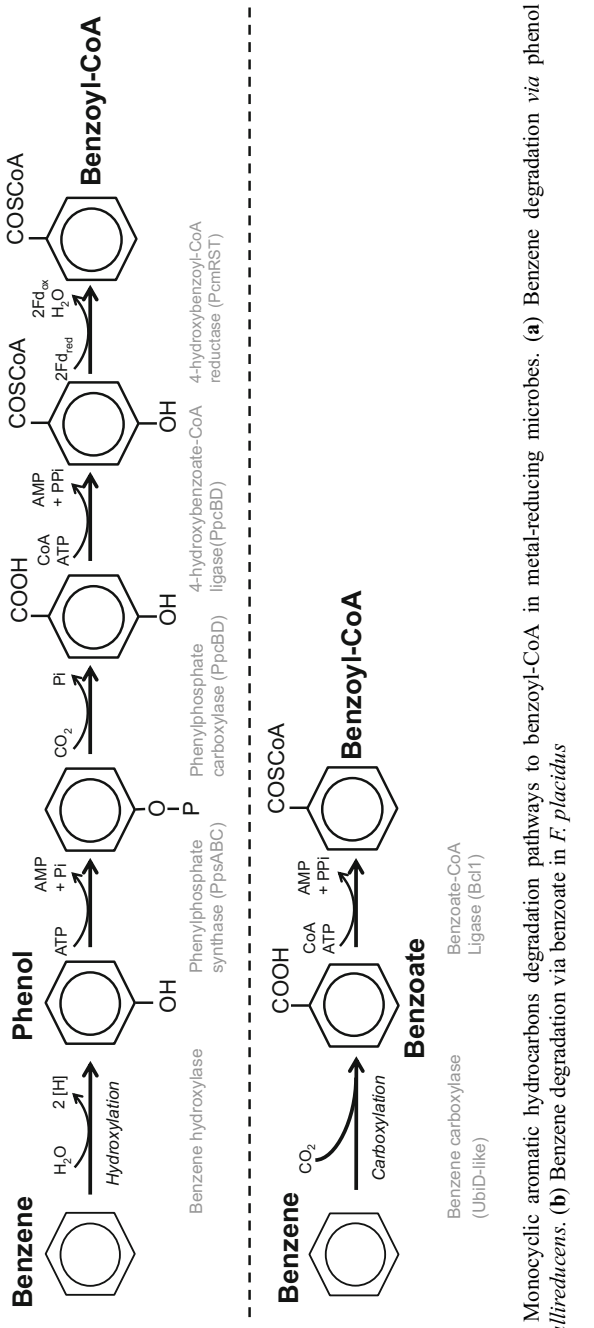

Fig. 1 Monocyclic aromatic hydrocarbons degradation pathways to benzoyl-CoA in metal-reducing microbes. (**a**) Benzene degradation *via* phenol in *G. metallireducens*. (**b**) Benzene degradation via benzoate in *F. placidus*

Caldwell and Suflita 2000; Chakraborty and Coates 2005; Abu Laban et al. 2009, 2010; Phelps et al. 2001; Kunapuli et al. 2008; Ulrich et al. 2005). In 2013, Zhang et al. presented evidences indicating that benzene is degraded *via* hydroxylation to phenol by *G. metallireducens*. In this study, phenol was labeled with ^{18}O when $H_2{}^{18}O$ was added to benzene-oxidizing cell suspension and complete benzene oxidation to CO_2 was inhibited when genes essential for the phenol metabolism were deleted (Zhang et al. 2013). A recent functional genetic study led to the identification of two adjacent genes (Gmet_0231 and Gmet_0232) coding for a zinc-containing oxidoreductase and a hypothetical protein required for benzene oxidation but not for phenol oxidation that may be involved in the first step of benzene degradation (Zhang et al. 2014). Still, the identity of the enzyme responsible for benzene hydroxylation and its mechanism of action are still unclear.

The suggested product of benzene hydroxylation, phenol, is degraded in *G. metallireducens via* a metabolic pathway analogous to the one found in the denitrifying bacterium *Thauera aromatica* (Butler et al. 2007; Breinig et al. 2000) (Fig. 1a). Phenol is first converted to phenylphosphate by an ATP-dependent phenylphosphate synthase composed of three different subunits (PpsABC) (Schmeling et al. 2004). Phenylphosphate is then carboxylated by a phenylphosphate carboxylase (PpcBD) to 4-hydroxybenzoate. In *T. aromatica*, the phenylphosphate carboxylase has four subunits. The δ-subunit is thought to catalyze the exergonic dephosphorylation of phenylphosphate resulting in a phenolate anion that will be carboxylated with CO_2 by the subunits α, β, and γ (Schühle and Fuchs 2004). Phenylphosphate carboxylase activity has been detected in *G. metallireducens* but the enzyme structure and mechanism of action may be different from *T. aromatica* since only two subunits are encoded on the genome (Schleinitz et al. 2009; Lovley et al. 2011). Subsequently, 4-hydroxybenzoate is converted to 4-hydroxybenzoyl-CoA by a 4-hydroxybenzoate-CoA ligase. Activity of this enzyme has been detected in *G. metallireducens* but its exact identity is still unknown (Peters et al. 2007; Wischgoll et al. 2005). Finally, 4-hydroxybenzoyl-CoA is reduced to benzoyl-CoA by a 4-hydroxybenzoyl-CoA reductase composed of three subunits ($\alpha_2\beta_2\gamma_2$) (PcmRST) (Butler et al. 2007; Breese and Fuchs 1998).

Other studies suggested that carboxylation is also an important benzene activation reaction in hydrocarbons contaminated environments. Proteome analysis of a benzene-degrading mixed community dominated by *Peptococcaceae*-related Gram-positive bacteria led to the identification of a putative anaerobic benzene carboxylase (Abu Laban et al. 2010). This enzyme is probably formed by at least two subunits (AbcDA) homologous to proteins comprised in two families of carboxylases: phenylphosphate carboxylase and 3-octaprenyl-4-hydroxybenzoate carboxy-lyase. Furthermore, benzoate is an important intermediate of benzene degradation in the Fe(III)-reducing archaeon *F. placidus* (Holmes et al. 2011) (Fig. 1b). Accumulation of trace amounts of benzoate as well as higher transcript abundance of genes thought to be involved in the conversion of benzene to benzoate during benzene metabolism are evidences supporting a carboxylation-dependent pathway in *F. placidus*. One upregulated gene in particular is thought to code for a putative benzene carboxylase homologous to proteins of the 3-octaprenyl-4-hydroxybenzoate carboxy-lyase (UbiD-like) family.

After benzene carboxylation, benzoate will be converted to benzoyl-CoA at the expense of ATP via a benzoate-CoA ligase (Bcl1/BamY) formed by a single protein (Holmes et al. 2011; Butler et al. 2007; Schühle et al. 2003).

3.2 Degradation of Toluene, Ethylbenzene, and Xylene

Degradation of toluene by DMRM such as *G. metallireducens* involves the addition of fumarate to toluene via glycyl radical mediated C-C bond formation by a benzylsuccinate synthase yielding benzylsuccinate (Butler et al. 2007; Rabus et al. 2005; Rabus 2005) (Fig. 2). The benzylsuccinate synthase comprises three subunits with a $(\alpha\beta\gamma)_2$ structure (BssABC) (Leuthner et al. 1998). The catalytic α-subunit harbors a glycyl radical enzyme fold surrounding the glycyl radical cofactor and the active site (Funk et al. 2014). The glycyl radical cofactor will be formed from a conserved glycine residue by an activating enzyme (BssD) of the S-adenosyl-L-methionine radical enzymes superfamily (Leuthner et al. 1998). The function of subunits β and γ that both possess a FeS cluster is still unclear (Li et al. 2009; Hilberg et al. 2012). Recently, it has been suggested that they may be involved in modulating the conformational dynamics of the benzylsuccinate synthase enzyme (Funk et al. 2014).

Subsequently, a succinyl-CoA:benzylsuccinate CoA transferase will transfer coenzyme A from succinyl-CoA to benzylsuccinate generating benzylsuccinyl-CoA and succinate (Heider et al. 1998). This enzyme comprises two subunits (BbsEF) arranged in a $\alpha_2\beta_2$ structure (Leutwein and Heider 2001). Benzylsuccinyl-CoA will then be oxidized to (E)-phenylitaconyl-CoA by a benzylsuccinyl-CoA dehydrogenase (BbsG) (Leutwein and Heider 2002). Deletion of the gene *bbsG* coding for this homotetrameric enzyme in *G. metallireducens* eliminates its capacity to degrade toluene further confirming the importance of this enzyme (Chaurasia et al. 2015). E-phenylitaconyl-CoA will then be converted by a (E)-phenylitaconyl-CoA hydratase (BbsH) into 2-carboxymethyl-3-hydroxy-phenylpropionyl-CoA which will be oxidized into benzoyl-succinyl-CoA by a 3-hydroxyacyl-CoA dehydrogenase (BbsCD). Finally, the succinyl-CoA moiety is replaced by CoA in a reaction catalyzed by a benzoylsuccinyl-CoA thiolase (BbsB) to generate the central metabolite benzoyl-CoA (Butler et al. 2007; Leuthner and Heider 2000).

Metabolic pathways involved in ethylbenzene and *o*-xylene oxidation by iron-reducing bacteria including *G. toluolica* and *D. aromaticivorans* are poorly characterized. A mixed culture dominated by an *Azoarcus* species has been shown to couple the degradation of ethylbenzene with manganese reduction (Dorer et al. 2016). In this study, it is not clear if the *Azoarcus* species is directly coupling ethylbenzene oxidation with manganese reduction or if a syntrophic interaction with another microbial species is required. In the closely related denitrifying bacterium *Azoarcus* sp. EbN1, degradation of ethylbenzene is initiated by the hydroxylation of the C1-methylene carbon of the sidechain yielding (*S*)-1-phenylethanol, which is catalyzed by an ethylbenzene dehydrogenase (EbdABC) (Fuchs et al. 2011; Ball et al. 1996; Kniemeyer and Heider 2001a) (Fig. 3). The ethylbenzene dehydrogenase is a heterotrimeric molybdoenzyme located in the periplasmic space of

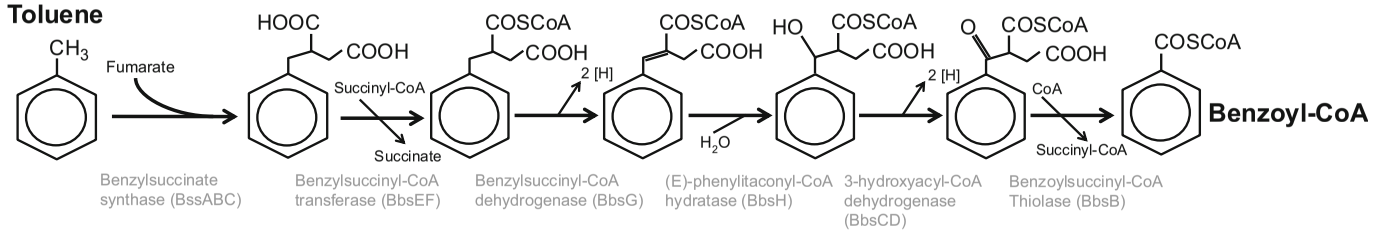

Fig. 2 Toluene degradation pathway in *G. metallireducens*

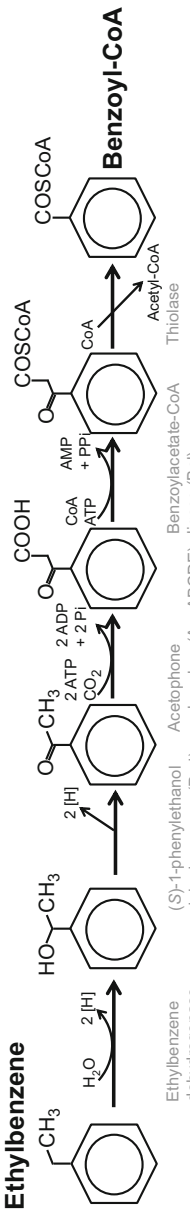

Fig. 3 Ethylbenzene degradation pathway in *Azoarcus* sp. EbN1

microbial cells (Heider et al. 2016; Kloer et al. 2006). (*S*)-1-phenylethanol is then oxidized to acetophenone by a (*S*)-1-phenylethanol dehydrogenase (Ped) (Kniemeyer and Heider 2001b). Later, acetophenone is carboxylated into benzoylacetate in an ATP-dependent manner by a complex acetophenone carboxylase comprising five different subunits (ApcABCDE) (Jobst et al. 2010). Subsequently, benzoylacetate is activated to benzoylacetate-CoA by a putative benzoylacetate-CoA ligase (Bal). In the last step, benzoylacetate-CoA is cleaved into benzoyl-CoA and acetyl-CoA by an unknown thiolase (Rabus 2005; Muhr et al. 2015). In the case of *o*-xylene, a lot less is known about its degradation pathway, but the initial reaction is possibly the addition of a fumarate molecule to the alkyl side-chain by a glycyl radical enzyme (Heider 2007).

3.3 Benzoyl-CoA Reduction

As described above, benzene, toluene, ethylbenzene, and *o*-xylene are converted to the central metabolite benzoyl-CoA in DMRM. The next step in the degradation of monocyclic aromatic hydrocarbons is the two electrons reduction of benzoyl-CoA to cyclohex-1,5-diene-1-carboxyl-CoA, a reaction comparable to the chemical Birch reduction catalyzed by the benzoyl-CoA reductases (Boll et al. 2014) (Fig. 4). Two different classes of benzoyl-CoA reductases are active in DMRM. In the strictly anaerobic archeon *F. placidus*, an ATP-consuming class I benzoyl-CoA reductase is responsible for the dearomatization of benzoyl-CoA during benzene metabolism (Holmes et al. 2011) (Fig. 4a). This is unusual since class I benzoyl-CoA reductases are normally found in facultative anaerobes, while strict anaerobes possess class II benzoyl-CoA reductases (Boll et al. 2014). The four genes (*bzdNOPQ*) coding for the class I benzoyl-CoA reductase found in the genome of *F. placidus* had higher transcript abundance in benzene-grown cells compared to acetate-grown cells. Recently, in vitro assays with purified BzdNOPQ showed that these proteins catalyze the ATP-dependent reduction of benzoyl-CoA with two electrons to cyclohex-1,5-diene-1-carboxyl-CoA (Schmid et al. 2015, 2016). The four subunits of the class I benzoyl-CoA reductases are organized in two different modules (Boll et al. 2014). The first module composed of subunits α (BzdQ) and δ (BzdP) harbors two ATP-binding sites and a single bridging [4Fe-4S] cluster. Its function is to transfer electrons from reduced ferredoxins in an ATP-dependent manner to the second modules catalyzing the benzoyl-CoA ring reduction (Boll 2005). The second module formed by subunits β (BzdO) and γ (BzdN) includes the benzoyl-CoA binding site as well as two [4Fe-4S] clusters involved in electron transport to the substrate.

In the model bacterium *G. metallireducens* that is a strict anaerobe, an ATP-independent class II benzoyl-CoA reductase is responsible for the dearomatization of benzoyl-CoA (Löffler et al. 2011; Boll et al. 2014) (Fig. 4a). Class II benzoyl-CoA reductases require significantly less energy than class I benzoyl-CoA reductases and this is probably the reason why class II enzymes are widespread in strict anaerobes. Class II benzoyl-CoA reductase of *G. metallireducens* is encoded by a cluster of eight genes, *bamBCDEFGHI* (Wischgoll et al. 2005).

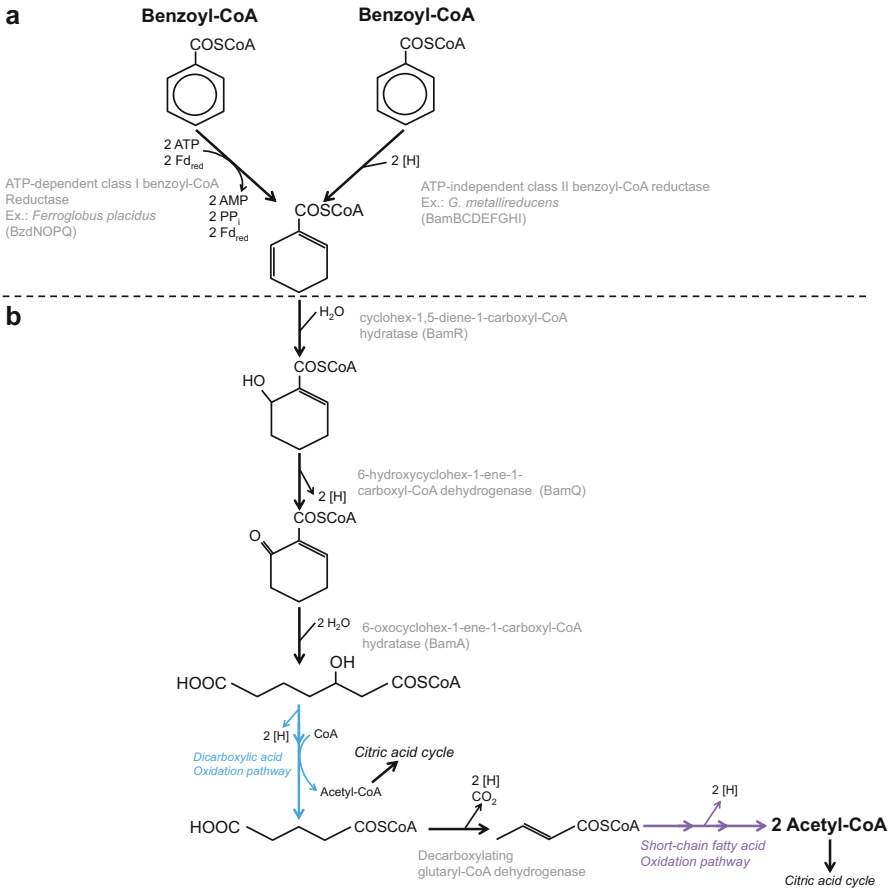

Fig. 4 Degradation pathways from benzoyl-CoA to acetyl-CoA. (**a**) Benzoyl-CoA reduction in *F. placidus* by a class I benzoyl-CoA reductase (BzdNOPQ) and in *G. metallireducens* by a class II benzoyl-CoA reductase (BamBCDEFGH). (**b**) Reduction of cyclohex-1,5-diene-1-carboxyl-CoA to acetyl-CoA. BamRQA enzymes are common to both *F. placidus* and *G. metallireducens*

A recent study showed that the enzyme is crystallized as a $Bam(BC)_2$ heterotetramer (Weinert et al. 2015). The substrate benzoyl-CoA binds to the subunit BamB (Kung et al. 2009). Reduction of the substrate occurs via electrons transferred from the tungsten-*bis*pyranopterin monophosphate cofactor of the BamB subunit. A single [4Fe-4S] cluster in BamB and three [4Fe-4S] clusters in BamC have been proposed to shuttle electrons from BamDEF to the tungsten-bispyranopterin monophosphate cofactor (Weinert et al. 2015). The exact function of the products of *bamDEFGHI* genes is still unknown, but the amino acids sequences and the presence of binding sites for flavin adenine dinucleotide cofactors suggest that they could participate in an energy conservation mechanism analogous to flavin-based electron bifurcation coupling endergonic and exergonic redox reactions (Boll et al. 2014; Buckel and Thauer

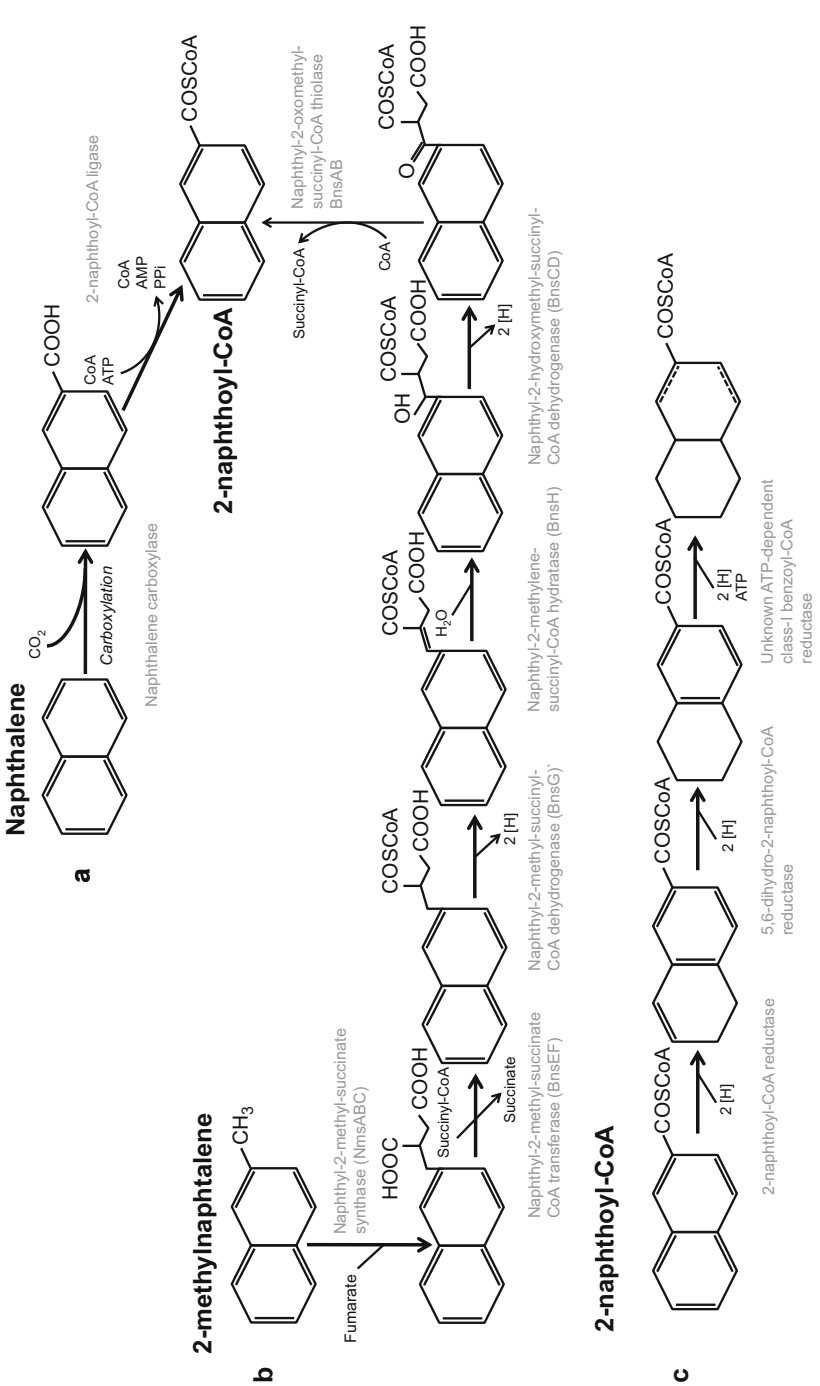

Fig. 5 (continued)

2013). It has been hypothesized that electrons from the oxidation of four reduced ferredoxins are bifurcated by the BamCDEF module for the endergonic reduction of benzoyl-CoA by BamB and the exergonic reduction of NAD^+, which has been predicted to bind to the BamGHI module (Boll et al. 2014; Fuchs et al. 2011).

3.4 Degradation of Cyclohex-1,5-Diene-1-Carboxyl-CoA to Central Carbon Metabolites

After benzoyl-CoA reduction, metal-reducing bacteria such as *G. metallireducens* as well as the archaeon *F. placidus* convert cyclohex-1,5-diene-1-carboxyl-CoA to 3-hydroxypimeloyl-CoA *via* a pathway widespread in microbes oxidizing monocyclic aromatic compounds that was initially characterized in *T. aromatica* (Butler et al. 2007) (Fig. 4b). This pathway comprises three reactions and starts with the addition of water to cyclohex-1,5-diene-1-carboxyl-CoA by a hydratase (BamR) yielding 6-hydroxycyclohex-1-ene-1-carboxyl-CoA (Peters et al. 2007; Laempe et al. 1998; Schmid et al. 2015). This product is then converted to 6-oxocyclohexa-1-ene-1-carboxyl-CoA by a NAD^+-dependent dehydrogenase (BamQ). The third reaction is catalyzed by a 6-oxocyclohex-1-ene-1-carboxyl-CoA hydratase (BamA) and consists in a hydration followed by ring cleavage to form 3-hydroxypimeloyl-CoA (Kuntze et al. 2008; Schmid et al. 2015; Laempe et al. 1998). This molecule will be further oxidized *via* three steps: (1) a dicarboxylic acid oxidation pathway, (2) followed by a decarboxylation and oxidation reaction catalyzed by a decarboxylating glutaryl-CoA dehydrogenase, and (3) a short-chain fatty acid oxidation pathway. In total, the oxidation of 3-hydroxypimeloyl-CoA requires six reactions and yields three acetyl-CoA molecules (Harwood and Gibson 1997; Carmona et al. 2009; Wischgoll et al. 2009).

4 Polycyclic Aromatic Hydrocarbons Degradation Pathway in Iron-Reducing Gram-Positive Bacteria

Two initial activation mechanisms have been proposed for the degradation of naphthalene by anaerobic microbes including DMRM (Fig. 5). The first mechanism proceeds *via* the carboxylation of naphthalene into 2-naphthoic acid. Experimental evidence supporting this pathway includes the accumulation of the metabolite 2-naphthoic acid during naphthalene degradation (Kleemann and Meckenstock 2011; Meckenstock et al.

Fig. 5 Naphthalene and 2-methylnaphthalene degradation pathway in anaerobic bacteria. (**a**) Carboxylation of naphthalene and degradation to 2-naphthoyl-CoA. (**b**) Addition of fumarate to 2-methylnaphthalene and degradation to 2-naphthoyl-CoA. (**c**) Reduction of 2-naphthoyl-CoA. 2-naphthoyl-CoA reductase and 5,6-dihydro-2-naphthoyl-CoA reductase may form an enzymatic complex in vivo. Identity of the enzyme responsible for the conversion of 5,6-dihydro-2-naphthoyl-CoA to hexahydro-naphthoyl-CoA is unknown. Downstream reactions are also poorly understood

2000; Zhang et al. 2000). Furthermore, growth with naphthalene in ^{13}C–bicarbonate-buffered medium resulted in the formation of ^{13}C–labelled 2-naphthoic acid (Zhang and Young 1997; Mouttaki et al. 2012). Alternatively, naphthalene degradation could be activated *via* a methylation reaction yielding 2-methyl-naphthalene (Safinowski and Meckenstock 2006). However, biochemical evidence supporting a naphthalene methylation pathway is lacking until now.

In recent years, the carboxylation pathway has mainly been investigated in naphthalene-degrading sulfate-reducing bacteria. The activation reaction in this pathway is mediated by an ATP-independent naphthalene carboxylase (Mouttaki et al. 2012) (Fig. 5a). Proteomic investigation and genome survey carried out in the sulfate-reducing enrichment culture N47 led to the identification of the α- and β-subunits of a putative carboxylase possibly catalyzing the conversion of naphthalene into 2-naphthoic acid (Bergmann et al. 2011). The genes coding for these two subunits are found in a cluster organized similarly in N47 as well as in the naphthalene-degrading sulfate-reducing Deltaproteobacterium NaphS2 (DiDonato et al. 2010).

Subsequently, 2-naphthoic acid is converted into 2-naphthoyl-CoA by an ATP-dependent 2-naphthoyl-CoA ligase (Eberlein et al. 2013b). 2-naphthoyl-CoA is then converted into 5,6-dihydro-2-naphthoyl-CoA *via* a two-electron reduction mediated by a 2-naphthoyl-CoA reductase, a member of the "old yellow enzyme family" of flavoproteins (Estelmann et al. 2015). The 5,6,-dihydro-2-naphthoyl-CoA formed is subsequently reduced with two electrons into 5,6,7,8-tetrahydro-2-naphthoyl-CoA by a 5,6-dihydro-2-naphthoyl-CoA reductase (Eberlein et al. 2013a; Estelmann et al. 2015). The 2-naphthoyl-CoA reductase and the 5,6-dihydro-2-naphthoyl-CoA reductase could not be completely separated *via* purification steps, and there is a significant possibility that both enzymes form a complex in vivo. In the next step, 5,6,7,8-tetrahydro-2-naphthoyl-CoA is possibly reduced to hexahydro-naphthoyl-CoA by an ATP-dependent class I benzoyl-CoA reductase with two electrons (Eberlein et al. 2013b; Estelmann et al. 2015). Until now, the natural electron donors of both the 2-naphtoyl-CoA reductase and the 5,6-dihydro-2-naphthoyl-CoA reductase are unknown, but genes located nearby as well as cofactors associated with the enzymes indicate that a flavin-based electron bifurcation energy conservation mechanism may be involved (Estelmann et al. 2015). After dearomatization, it has been suggested that the complete degradation of naphthalene to CO_2 is continued further *via* cyclohexane ring comprising CoA esters instead of monocyclic aromatic compounds (Annweiler et al. 2002).

Until now, the anaerobic degradation pathway of 1-methylnaphthalene remains unresolved (Kleemann and Meckenstock 2011). Like with naphthalene, the degradation pathway of 2-methyl-naphthalene has not been investigated in metal-reducing microbes but only in sulfate-reducing bacteria (Annweiler et al. 2000; Annweiler et al. 2002; Safinowski and Meckenstock 2004; Musat et al. 2009). Similar to anaerobic toluene oxidation, 2-methylnaphthalene is first converted to naphthyl-2-methyl-succinate by the addition of a fumarate molecule to the methyl group (Selesi et al. 2010) (Fig. 5b). This reaction is mediated by the glycyl radical enzyme naphthyl-2-methyl-succinate synthase (NmsABC). Subsequently, naphthyl-2-methyl-succinate is activated to yield naphthyl-2-methylene-succinyl-CoA by the naphthyl-2-methyl-succinate CoA transferase (BnsEF). The following four reactions catalyzed respectively by a naphthyl-2-methyl-succinyl-CoA dehydrogenase

(BnsG), a naphthyl-2-methylene-succinyl-CoA hydratase (BnsH), a naphthyl-2-hydroxymethyl-succinyl-CoA dehydrogenase (BnsCD), and a naphthyl-2-oxomethyl-succinyl-CoA thiolase (BnsAB) result in the β-oxidation of the side chain leading to the formation of 2-naphtoyl-CoA, which will then be dearomatized.

5 Research Needs

Exploring anaerobic hydrocarbon oxidation by DMRM could lead to several promising applications. For instance, many of the reactions involved in hydrocarbon oxidation have unusual chemistry that could be exploited for biotechnological purposes. Microbes capable of transferring electrons to solid metal oxides such as *Geobacter* spp. can also use a solid electrode as the TEA (Franks and Nevin 2010). This characteristic has conducted to the development of a bioremediation strategy where electrodes are buried in contaminated sediments to stimulate hydrocarbons degradation (Zhang et al. 2010; Morris and Jin 2012; Wang et al. 2012). Because electrodes are low-maintenance and can be installed permanently at polluted sites, electrobioremediation is a promising approach since it has the potential of being less expensive than competing technologies. Another possible application associated with DMRM, which usually generate magnetite from the Fe(III) oxide reduction, is the possibility of developing tools for the localization of petroleum deposit based on magnetic anomaly signals (Lovley et al. 2004).

Developing novel technologies related to hydrocarbons degradation with DMRM will require a better understanding of their metabolism. Although important progress has been made in the last 5 years, hydrocarbons degradation pathways in DMRM are still only partially characterized. Outstanding questions remaining include: (1) the identity of the enzymes responsible for benzene hydroxylation in *G. metallireducens* (Zhang et al. 2013) and benzene carboxylation in *F. placidus* (Holmes et al. 2011); (2) the nature of the electron transport route and energy conservation mechanism involved in benzoyl-CoA reductase, 2-naphthoyl-CoA reductase, and 5,6-dihydro-2-naphthoyl-CoA reductase activity (Boll et al. 2014; Estelmann et al. 2015); and (3) the nature in metal-reducing microbes of the polycyclic hydrocarbons degradation pathways described only in other phylogenetic classes of microbes until now. These examples are representing only a small fraction of the unexplored elements that will require extensive research work before achieving a complete portrait of the hydrocarbon degradation metabolism in metal-reducing microbes.

Acknowledgments This work is funded by the Novo Nordisk Foundation.

References

Abu Laban N, Selesi D, Jobelius C, Meckenstock RU (2009) Anaerobic benzene degradation by Gram-positive sulfate-reducing bacteria. FEMS Microbiol Ecol 68:300–311. https://doi.org/10.1111/j.1574-6941.2009.00672.x

Abu Laban N, Selesi D, Rattei T, Tischler P, Meckenstock RU (2010) Identification of enzymes involved in anaerobic benzene degradation by a strictly anaerobic iron-reducing enrichment culture. Environ Microbiol 12:2783–2796. https://doi.org/10.1111/j.1462-2920.2010.02248.x

Aburto-Medina A, Ball AS (2015) Microorganisms involved in anaerobic benzene degradation. Ann Microbiol 65:1201–1213. https://doi.org/10.1007/s13213-014-0926-8

Anderson RT, Lovley DR (1999) Naphthalene and benzene degradation under Fe(III)-reducing conditions in petroleum-contaminated aquifers. Biorem J 3:121–135. https://doi.org/10.1080/10889869991219271

Annweiler E, Richnow HH, Antranikian G, Hebenbrock S, Garms C, Franke S, Francke W, Michaelis W (2000) Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass by the thermophile *Bacillus thermoleovorans*. Appl Environ Microbiol 66:518–523

Annweiler E, Michaelis W, Meckenstock RU (2002) Identical ring cleavage products during anaerobic degradation of naphthalene, 2-methylnaphthalene, and tetralin indicate a new metabolic pathway. Appl Environ Microbiol 68:852–858

Ball HA, Johnson HA, Reinhard M, Spormann AM (1996) Initial reactions in anaerobic ethylbenzene oxidation by a denitrifying bacterium, strain EB1. J Bacteriol 178:5755–5761

Bergmann FD, Selesi D, Meckenstock RU (2011) Identification of new enzymes potentially involved in anaerobic naphthalene degradation by the sulfate-reducing enrichment culture N47. Arch Microbiol 193:241–250. https://doi.org/10.1007/s00203-010-0667-4

Boll M (2005) Dearomatizing benzene ring reductases. J Mol Microbiol Biotechnol 10:132–142. https://doi.org/10.1159/000091560

Boll M, Löffler C, Morris BEL, Kung JW (2014) Anaerobic degradation of homocyclic aromatic compounds via arylcarboxyl-coenzyme A esters: organisms, strategies and key enzymes. Environ Microbiol 16:612–627. https://doi.org/10.1111/1462-2920.12328

Bond DR, Holmes DE, Tender LM, Lovley DR (2002) Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295:483–485. https://doi.org/10.1126/science.1066771

Botton S, Parsons JR (2006) Degradation of btex compounds under iron-reducing conditions in contaminated aquifer microcosms. Environ Toxicol Chem 25:2630–2638

Breese K, Fuchs G (1998) 4-Hydroxybenzoyl-CoA reductase (dehydroxylating) from the denitrifying bacterium *Thauera aromatica*. Eur J Biochem 251:916–923. https://doi.org/10.1046/j.1432-1327.1998.2510916.x

Breinig S, Schiltz E, Fuchs G (2000) Genes involved in anaerobic metabolism of phenol in the bacterium *Thauera aromatica*. J Bacteriol 182:5849–5863

Buckel W, Thauer RK (2013) Energy conservation via electron bifurcating ferredoxin reduction and proton/Na(+) translocating ferredoxin oxidation. Biochim Biophys Acta 1827:94–113. https://doi.org/10.1016/j.bbabio.2012.07.002

Butler JE, He Q, Nevin KP, He Z, Zhou J, Lovley DR (2007) Genomic and microarray analysis of aromatics degradation in *Geobacter metallireducens* and comparison to a *Geobacter* isolate from a contaminated field site. BMC Genomics 8:180. https://doi.org/10.1186/1471-2164-8-180

Caldwell ME, Suflita JM (2000) Detection of phenol and benzoate as intermediates of anaerobic benzene biodegradation under different terminal electron-accepting conditions. Environ Sci Technol 34:1216–1220. https://doi.org/10.1021/es990849j

Carmona M, Zamarro MT, Blázquez B, Durante-Rodríguez G, Juárez JF, Valderrama JA, Barragán MJL, García JL, Díaz E (2009) Anaerobic catabolism of aromatic compounds: a genetic and genomic view. Microbiol Mol Biol Rev 73:71–133. https://doi.org/10.1128/MMBR.00021-08

Chakraborty R, Coates JD (2004) Anaerobic degradation of monoaromatic hydrocarbons. Appl Microbiol Biotechnol 64:437–446. https://doi.org/10.1007/s00253-003-1526-x

Chakraborty R, Coates JD (2005) Hydroxylation and carboxylation–two crucial steps of anaerobic benzene degradation by *Dechloromonas* strain RCB. Appl Environ Microbiol 71:5427–5432. https://doi.org/10.1128/AEM.71.9.5427-5432.2005

Chaurasia AK, Tremblay P-L, Holmes DE, Zhang T (2015) Genetic evidence that the degradation of para-cresol by *Geobacter metallireducens* is catalyzed by the periplasmic para-cresol methylhydroxylase. FEMS Microbiol Lett. https://doi.org/10.1093/femsle/fnv145

Coates JD, Bhupathiraju VK, Achenbach LA, Mclnerney MJ, Lovley DR (2001) *Geobacter hydrogenophilus*, *Geobacter chapellei* and *Geobacter grbiciae*, three new, strictly anaerobic, dissimilatory Fe(III)-reducers. Int J Syst Evol Microbiol 51:581–588. https://doi.org/10.1099/00207713-51-2-581

DiDonato RJ, Young ND, Butler JE, Chin K-J, Hixson KK, Mouser P, Lipton MS, DeBoy R, Methé BA (2010) Genome sequence of the deltaproteobacterial strain NaphS2 and analysis of differential gene expression during anaerobic growth on naphthalene. PLoS One 5:e14072. https://doi.org/10.1371/journal.pone.0014072

Dorer C, Vogt C, Neu TR, Stryhanyuk H, Richnow H-H (2016) Characterization of toluene and ethylbenzene biodegradation under nitrate-, iron(III)- and manganese(IV)-reducing conditions by compound-specific isotope analysis. Environ Pollut 211:271–281. https://doi.org/10.1016/j.envpol.2015.12.029

Eberlein C, Estelmann S, Seifert J, von Bergen M, Müller M, Meckenstock RU, Boll M (2013a) Identification and characterization of 2-naphthoyl-coenzyme A reductase, the prototype of a novel class of dearomatizing reductases. Mol Microbiol 88:1032–1039. https://doi.org/10.1111/mmi.12238

Eberlein C, Johannes J, Mouttaki H, Sadeghi M, Golding BT, Boll M, Meckenstock RU (2013b) ATP-dependent/−independent enzymatic ring reductions involved in the anaerobic catabolism of naphthalene. Environ Microbiol 15:1832–1841. https://doi.org/10.1111/1462-2920.12076

Estelmann S, Blank I, Feldmann A, Boll M (2015) Two distinct old yellow enzymes are involved in naphthyl ring reduction during anaerobic naphthalene degradation. Mol Microbiol 95:162–172. https://doi.org/10.1111/mmi.12875

Foght J (2008) Anaerobic biodegradation of aromatic hydrocarbons: pathways and prospects. J Mol Microbiol Biotechnol 15:93–120. https://doi.org/10.1159/000121324

Franks AE, Nevin KP (2010) Microbial Fuel Cells, A Current Review. Energies 3:899–919. https://doi.org/10.3390/en3050899

Fuchs G, Boll M, Heider J (2011) Microbial degradation of aromatic compounds – from one strategy to four. Nat Rev Microbiol 9:803–816. https://doi.org/10.1038/nrmicro2652

Funk MA, Judd ET, Marsh ENG, Elliott SJ, Drennan CL (2014) Structures of benzylsuccinate synthase elucidate roles of accessory subunits in glycyl radical enzyme activation and activity. Proc Natl Acad Sci U S A 111:10161–10166. https://doi.org/10.1073/pnas.1405983111

Grbić-Galić D, Vogel TM (1987) Transformation of toluene and benzene by mixed methanogenic cultures. Appl Environ Microbiol 53:254–260

Harwood CS, Gibson J (1997) Shedding light on anaerobic benzene ring degradation: a process unique to prokaryotes? J Bacteriol 179:301–309

Heider J (2007) Adding handles to unhandy substrates: anaerobic hydrocarbon activation mechanisms. Curr Opin Chem Biol 11:188–194. https://doi.org/10.1016/j.cbpa.2007.02.027

Heider J, Spormann AM, Beller HR, Widdel F (1998) Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiol Rev 22:459–473. https://doi.org/10.1111/j.1574-6976.1998.tb00381.x

Heider J, Szaleniec M, Sünwoldt K, Boll M (2016) Ethylbenzene dehydrogenase and related molybdenum enzymes involved in oxygen-independent alkyl chain hydroxylation. J Mol Microbiol Biotechnol 26:45–62. https://doi.org/10.1159/000441357

Heinnickel ML, Kaser FM, Coates JD (2010) Hydrocarbon degradation coupled to metal reduction. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 947–955

Hilberg M, Pierik AJ, Bill E, Friedrich T, Lippert M-L, Heider J (2012) Identification of FeS clusters in the glycyl-radical enzyme benzylsuccinate synthase via EPR and Mössbauer spectroscopy. J Biol Inorg Chem 17:49–56. https://doi.org/10.1007/s00775-011-0828-1

Holmes DE, Risso C, Smith JA, Lovley DR (2011) Anaerobic oxidation of benzene by the hyperthermophilic archaeon *Ferroglobus placidus*. Appl Environ Microbiol 77:5926–5933. https://doi.org/10.1128/AEM.05452-11

Jahn MK, Haderlein SB, Meckenstock RU (2005) Anaerobic degradation of benzene, toluene, ethylbenzene, and *o*-xylene in sediment-free iron-reducing enrichment cultures. Appl Environ Microbiol 71:3355–3358. https://doi.org/10.1128/AEM.71.6.3355-3358.2005

Jobst B, Schühle K, Linne U, Heider J (2010) ATP-dependent carboxylation of acetophenone by a novel type of carboxylase. J Bacteriol 192:1387–1394. https://doi.org/10.1128/JB.01423-09

Kleemann R, Meckenstock RU (2011) Anaerobic naphthalene degradation by Gram-positive, iron-reducing bacteria. FEMS Microbiol Ecol 78:488–496. https://doi.org/10.1111/j.1574-6941.2011.01193.x

Kloer DP, Hagel C, Heider J, Schulz GE (2006) Crystal structure of ethylbenzene dehydrogenase from *Aromatoleum aromaticum*. Structure 14:1377–1388. https://doi.org/10.1016/j.str.2006.07.001

Kniemeyer O, Heider J (2001a) Ethylbenzene dehydrogenase, a novel hydrocarbon-oxidizing molybdenum/iron-sulfur/heme enzyme. J Biol Chem 276:21381–21386. https://doi.org/10.1074/jbc.M101679200

Kniemeyer O, Heider J (2001b) (*S*)-1-phenylethanol dehydrogenase of *Azoarcus* sp. strain EbN1, an enzyme of anaerobic ethylbenzene catabolism. Arch Microbiol 176:129–135

Kunapuli U, Griebler C, Beller HR, Meckenstock RU (2008) Identification of intermediates formed during anaerobic benzene degradation by an iron-reducing enrichment culture. Environ Microbiol 10:1703–1712. https://doi.org/10.1111/j.1462-2920.2008.01588.x

Kunapuli U, Jahn MK, Lueders T, Geyer R, Heipieper HJ, Meckenstock RU (2010) *Desulfitobacterium aromaticivorans* sp. nov. and *Geobacter toluenoxydans* sp. nov., iron-reducing bacteria capable of anaerobic degradation of monoaromatic hydrocarbons. Int J Syst Evol Microbiol 60:686–695. https://doi.org/10.1099/ijs.0.003525-0

Kung JW, Löffler C, Dörner K, Heintz D, Gallien S, Van Dorsselaer A, Friedrich T, Boll M (2009) Identification and characterization of the tungsten-containing class of benzoyl-coenzyme A reductases. Proc Natl Acad Sci U S A 106:17687–17692. https://doi.org/10.1073/pnas.0905073106

Kuntze K, Shinoda Y, Moutakki H, McInerney MJ, Vogt C, Richnow H-H, Boll M (2008) 6-Oxocyclohex-1-ene-1-carbonyl-coenzyme A hydrolases from obligately anaerobic bacteria: characterization and identification of its gene as a functional marker for aromatic compounds degrading anaerobes. Environ Microbiol 10:1547–1556. https://doi.org/10.1111/j.1462-2920.2008.01570.x

Laempe D, Eisenreich W, Bacher A, Fuchs G (1998) Cyclohexa-1,5-diene-1-carbonyl-CoA hydratase [corrected], an enzyme involved in anaerobic metabolism of benzoyl-CoA in the denitrifying bacterium *Thauera aromatica*. Eur J Biochem 255:618–627

Langenhoff AA, Brouwers-Ceiler DL, Engelberting JH, Quist JJ, Wolkenfelt JGP, Zehnder AJ, Schraa G (1997a) Microbial reduction of manganese coupled to toluene oxidation. FEMS Microbiol Ecol 22:119–127. https://doi.org/10.1111/j.1574-6941.1997.tb00363.x

Langenhoff AA, Nijenhuis I, Tan NC, Briglia M, Zehnder AJ, Schraa G (1997b) Characterisation of a manganese-reducing, toluene-degrading enrichment culture. FEMS Microbiol Ecol 24:113–125. https://doi.org/10.1111/j.1574-6941.1997.tb00428.x

Leuthner B, Heider J (2000) Anaerobic toluene catabolism of *Thauera aromatica*: the *bbs* operon codes for enzymes of beta oxidation of the intermediate benzylsuccinate. J Bacteriol 182:272–277

Leuthner B, Leutwein C, Schulz H, Hörth P, Haehnel W, Schiltz E, Schägger H, Heider J (1998) Biochemical and genetic characterization of benzylsuccinate synthase from *Thauera aromatica*: a new glycyl radical enzyme catalysing the first step in anaerobic toluene metabolism. Mol Microbiol 28:615–628

Leutwein C, Heider J (2001) Succinyl-CoA:(R)-benzylsuccinate CoA-transferase: an enzyme of the anaerobic toluene catabolic pathway in denitrifying bacteria. J Bacteriol 183:4288–4295. https://doi.org/10.1128/JB.183.14.4288-4295.2001

Leutwein C, Heider J (2002) (R)-Benzylsuccinyl-CoA dehydrogenase of *Thauera aromatica*, an enzyme of the anaerobic toluene catabolic pathway. Arch Microbiol 178:517–524. https://doi.org/10.1007/s00203-002-0484-5

Li L, Patterson DP, Fox CC, Lin B, Coschigano PW, Marsh ENG (2009) Subunit structure of benzylsuccinate synthase. Biochemistry 48:1284–1292. https://doi.org/10.1021/bi801766g

Löffler C, Kuntze K, Vazquez JR, Rugor A, Kung JW, Böttcher A, Boll M (2011) Occurrence, genes and expression of the W/Se-containing class II benzoyl-coenzyme A reductases in anaerobic bacteria. Environ Microbiol 13:696–709. https://doi.org/10.1111/j.1462-2920.2010.02374.x

Lovley DR (2012) Electromicrobiology. Annu Rev Microbiol 66:391–409. https://doi.org/10.1146/annurev-micro-092611-150104

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

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

Lovley DR, Holmes DE, Nevin KP (2004) Dissimilatory Fe(III) and Mn(IV) reduction. Adv Microb Physiol 49:219–286. https://doi.org/10.1016/S0065-2911(04)49005-5

Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan KA, Aklujkar M, Butler JE, Giloteaux L, Rotaru A-E, Holmes DE, Franks AE, Orellana R, Risso C, Nevin KP (2011) *Geobacter*: the microbe electric's physiology, ecology, and practical applications. Adv Microb Physiol 59:1–100. https://doi.org/10.1016/B978-0-12-387661-4.00004-5

Meckenstock RU, Mouttaki H (2011) Anaerobic degradation of non-substituted aromatic hydrocarbons. Curr Opin Biotechnol 22:406–414. https://doi.org/10.1016/j.copbio.2011.02.009

Meckenstock RU, Annweiler E, Michaelis W, Richnow HH, Schink B (2000) Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. Appl Environ Microbiol 66:2743–2747

Meckenstock RU, Boll M, Mouttaki H, Koelschbach JS, Cunha Tarouco P, Weyrauch P, Dong X, Himmelberg AM (2016) Anaerobic degradation of benzene and polycyclic aromatic hydrocarbons. J Mol Microbiol Biotechnol 26:92–118. https://doi.org/10.1159/000441358

Morris JM, Jin S (2012) Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. J Hazard Mater 213–214:474–477. https://doi.org/10.1016/j.jhazmat.2012.02.029

Mouttaki H, Johannes J, Meckenstock RU (2012) Identification of naphthalene carboxylase as a prototype for the anaerobic activation of non-substituted aromatic hydrocarbons. Environ Microbiol 14:2770–2774. https://doi.org/10.1111/j.1462-2920.2012.02768.x

Muhr E, Leicht O, González Sierra S, Thanbichler M, Heider J (2015) A fluorescent bioreporter for acetophenone and 1-phenylethanol derived from a specifically induced catabolic operon. Front Microbiol. https://doi.org/10.3389/fmicb.2015.01561

Musat F, Galushko A, Jacob J, Widdel F, Kube M, Reinhardt R, Wilkes H, Schink B, Rabus R (2009) Anaerobic degradation of naphthalene and 2-methylnaphthalene by strains of marine sulfate-reducing bacteria. Environ Microbiol 11:209–219. https://doi.org/10.1111/j.1462-2920.2008.01756.x

Oberender J, Kung JW, Seifert J, von Bergen M, Boll M (2012) Identification and characterization of a succinyl-coenzyme A (CoA):benzoate CoA transferase in *Geobacter metallireducens*. J Bacteriol 194:2501–2508. https://doi.org/10.1128/JB.00306-12

Peters F, Shinoda Y, McInerney MJ, Boll M (2007) Cyclohexa-1,5-diene-1-carbonyl-coenzyme A (CoA) hydratases of *Geobacter metallireducens* and *Syntrophus aciditrophicus*: evidence for a common benzoyl-CoA degradation pathway in facultative and strict anaerobes. J Bacteriol 189:1055–1060. https://doi.org/10.1128/JB.01467-06

Phelps CD, Zhang X, Young LY (2001) Use of stable isotopes to identify benzoate as a metabolite of benzene degradation in a sulphidogenic consortium. Environ Microbiol 3:600–603

Rabus R (2005) Functional genomics of an anaerobic aromatic-degrading denitrifying bacterium, strain EbN1. Appl Microbiol Biotechnol 68:580–587. https://doi.org/10.1007/s00253-005-0030-x

Rabus R, Kube M, Heider J, Beck A, Heitmann K, Widdel F, Reinhardt R (2005) The genome sequence of an anaerobic aromatic-degrading denitrifying bacterium, strain EbN1. Arch Microbiol 183:27–36. https://doi.org/10.1007/s00203-004-0742-9

Rooney-Varga JN, Anderson RT, Fraga JL, Ringelberg D, Lovley DR (1999) Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. Appl Environ Microbiol 65:3056–3063

Safinowski M, Meckenstock RU (2004) Enzymatic reactions in anaerobic 2-methylnaphthalene degradation by the sulphate-reducing enrichment culture N 47. FEMS Microbiol Lett 240:99–104. https://doi.org/10.1016/j.femsle.2004.09.014

Safinowski M, Meckenstock RU (2006) Methylation is the initial reaction in anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. Environ Microbiol 8:347–352. https://doi.org/10.1111/j.1462-2920.2005.00900.x

Schleinitz KM, Schmeling S, Jehmlich N, von Bergen M, Harms H, Kleinsteuber S, Vogt C, Fuchs G (2009) Phenol degradation in the strictly anaerobic iron-reducing bacterium *Geobacter metallireducens* GS-15. Appl Environ Microbiol 75:3912–3919. https://doi.org/10.1128/AEM.01525-08

Schmeling S, Narmandakh A, Schmitt O, Gad'on N, Schühle K, Fuchs G (2004) Phenylphosphate synthase: a new phosphotransferase catalyzing the first step in anaerobic phenol metabolism in *Thauera aromatica*. J Bacteriol 186:8044–8057. https://doi.org/10.1128/JB.186.23.8044-8057.2004

Schmid G, René SB, Boll M (2015) Enzymes of the benzoyl-coenzyme A degradation pathway in the hyperthermophilic archaeon *Ferroglobus placidus*. Environ Microbiol 17:3289–3300. https://doi.org/10.1111/1462-2920.12785

Schmid G, Auerbach H, Pierik AJ, Schünemann V, Boll M (2016) ATP-dependent electron activation module of benzoyl-coenzyme A reductase from the hyperthermophilic archaeon *Ferroglobus placidus*. Biochemistry 55:5578–5586

Schühle K, Fuchs G (2004) Phenylphosphate carboxylase: a new C-C lyase involved in anaerobic phenol metabolism in *Thauera aromatica*. J Bacteriol 186:4556–4567. https://doi.org/10.1128/JB.186.14.4556-4567.2004

Schühle K, Gescher J, Feil U, Paul M, Jahn M, Schägger H, Fuchs G (2003) Benzoate-coenzyme A ligase from *Thauera aromatica*: an enzyme acting in anaerobic and aerobic pathways. J Bacteriol 185:4920–4929

Selesi D, Jehmlich N, von Bergen M, Schmidt F, Rattei T, Tischler P, Lueders T, Meckenstock RU (2010) Combined genomic and proteomic approaches identify gene clusters involved in anaerobic 2-methylnaphthalene degradation in the sulfate-reducing enrichment culture N47. J Bacteriol 192:295–306. https://doi.org/10.1128/JB.00874-09

Smith JA, Lovley DR, Tremblay P-L (2013) Outer cell surface components essential for Fe(III) oxide reduction by *Geobacter metallireducens*. Appl Environ Microbiol 79:901–907. https://doi.org/10.1128/AEM.02954-12

Tremblay P-L, Aklujkar M, Leang C, Nevin KP, Lovley D (2012) A genetic system for *Geobacter metallireducens*: role of the flagellin and pilin in the reduction of Fe(III) oxide. Environ Microbiol Rep 4:82–88. https://doi.org/10.1111/j.1758-2229.2011.00305.x

Ulrich AC, Beller HR, Edwards EA (2005) Metabolites detected during biodegradation of 13C6-benzene in nitrate-reducing and methanogenic enrichment cultures. Environ Sci Technol 39:6681–6691

Villatoro-Monzón WR, Mesta-Howard AM, Razo-Flores E (2003) Anaerobic biodegradation of BTEX using Mn(IV) and Fe(III) as alternative electron acceptors. Water Sci Technol 48:125–131

Vogel TM, Grbìc-Galìc D (1986) Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. Appl Environ Microbiol 52:200–202

Vogt C, Kleinsteuber S, Richnow H-H (2011) Anaerobic benzene degradation by bacteria. Microb Biotechnol 4:710–724. https://doi.org/10.1111/j.1751-7915.2011.00260.x

Wang X, Cai Z, Zhou Q, Zhang Z, Chen C (2012) Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. Biotechnol Bioeng 109:426–433. https://doi.org/10.1002/bit.23351

Weelink SAB, van Doesburg W, Saia FT, Rijpstra WIC, Röling WFM, Smidt H, Stams AJM (2009) A strictly anaerobic betaproteobacterium *Georgfuchsia toluolica* gen. nov., sp. nov. degrades aromatic compounds with Fe(III), Mn(IV) or nitrate as an electron acceptor. FEMS Microbiol Ecol 70:575–585. https://doi.org/10.1111/j.1574-6941.2009.00778.x

Weinert T, Huwiler SG, Kung JW, Weidenweber S, Hellwig P, Stärk H-J, Biskup T, Weber S, Cotelesage JJH, George GN, Ermler U, Boll M (2015) Structural basis of enzymatic benzene ring reduction. Nat Chem Biol 11:586–591. https://doi.org/10.1038/nchembio.1849

White GF, Edwards MJ, Gomez-Perez L, Richardson DL, Butt JN, Clarke TA (2016) Mechanisms of bacterial extracellular electron exchange. Adv Microb Physiol 68:87–138. https://doi.org/10.1016/bs.ampbs.2016.02.002

Wischgoll S, Heintz D, Peters F, Erxleben A, Sarnighausen E, Reski R, Van Dorsselaer A, Boll M (2005) Gene clusters involved in anaerobic benzoate degradation of *Geobacter metallireducens*. Mol Microbiol 58:1238–1252. https://doi.org/10.1111/j.1365-2958.2005.04909.x

Wischgoll S, Taubert M, Peters F, Jehmlich N, von Bergen M, Boll M (2009) Decarboxylating and nondecarboxylating glutaryl-coenzyme A dehydrogenases in the aromatic metabolism of obligately anaerobic bacteria. J Bacteriol 191:4401–4409. https://doi.org/10.1128/JB.00205-09

Zhang X, Young LY (1997) Carboxylation as an initial reaction in the anaerobic metabolism of naphthalene and phenanthrene by sulfidogenic consortia. Appl Environ Microbiol 63:4759–4764

Zhang X, Sullivan ER, Young LY (2000) Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulfate reducing consortium. Biodegradation 11:117–124

Zhang T, Gannon SM, Nevin KP, Franks AE, Lovley DR (2010) Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. Environ Microbiol 12:1011–1020. https://doi.org/10.1111/j.1462-2920.2009.02145.x

Zhang T, Bain TS, Nevin KP, Barlett MA, Lovley DR (2012) Anaerobic benzene oxidation by *Geobacter* species. Appl Environ Microbiol 78:8304–8310. https://doi.org/10.1128/AEM.02469-12

Zhang T, Tremblay P-L, Chaurasia AK, Smith JA, Bain TS, Lovley DR (2013) Anaerobic benzene oxidation via phenol in *Geobacter metallireducens*. Appl Environ Microbiol 79:7800–7806. https://doi.org/10.1128/AEM.03134-13

Zhang T, Tremblay P-L, Chaurasia AK, Smith JA, Bain TS, Lovley DR (2014) Identification of genes specifically required for the anaerobic metabolism of benzene in *Geobacter metallireducens*. Front Microbiol 5:245. https://doi.org/10.3389/fmicb.2014.00245