Different strategies in anaerobic biodegradation of aromatic compounds: nitrate reducers versus strict anaerobes

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Summary

Mononuclear aromatic compounds are degraded anaerobically through pathways that are basically different from those used in the presence of oxygen. Whereas aerobic degradation destabilizes the aromatic π-electron system by oxidative steps through oxygenase reactions, anaerobic degradation is most often initiated by a reductive attack. The benzoyl-CoA pathway is the most important metabolic route in this context, and a broad variety of mononuclear aromatics, including phenol, cresols, toluene, xylenes and ethylbenzene, are channelled into this pathway through various modification reactions. Multifunctional phenolic compounds are metabolized via the reductive resorcinol pathway, the oxidative resorcinol pathway with hydroxyhydroquinone as key intermediate, and the phloroglucinol pathway. Comparison of the various pathways used for modification and degradation of aromatics in the absence of oxygen indicates that the strategies of breakdown of these compounds are largely determined by the redox potentials of the electron acceptors used, and by the overall reaction energetics. Consequently, nitrate reducers quite often use strategies for primary attack on aromatic compounds that differ from those used by sulfate-reducing, iron-reducing or fermenting bacteria.

Introduction

Aromatic compounds are widespread in nature as structural polymers (lignin), as functional units in biochemistry (amino acids, coenzymes), and as secondary metabolites of plants, fungi and bacteria. Aromatic compounds also make up a major part of petrol and are applied on a broad basis in chemical industry. Despite their often unusual structure, only few of these synthetic compounds appear to cause environmental problems due to incomplete degradation. Especially bacteria have developed an impressive adaptative capacity to successfully degrade synthetic compounds, even those which show only little resemblance to pre-existing templates among natural compounds.

Aerobic degradation of aromatic compounds employs molecular oxygen for substrate activation in oxygenase reactions, leading to catechol, protocatechuate and gentisate as key intermediates. These phenolic intermediates are subject to ring cleavage in a further oxygenase-dependent step, either between or vicinal to the hydroxy groups of the aromatic ring (Vaillancourt *et al.*, 2006) leading to an unsaturated, open-chain carboxylic acid which undergoes further degradation, typically to an acetyl and a succinyl derivative.

In anoxic environments, oxygenases cannot operate, and anaerobic degradation of aromatics, if possible at all, has to take alternative, oxygen-independent paths. Mainly through the 1970s and 1980s (Healy and Young, 1978; 1979), anaerobic breakdown of numerous mononuclear aromatics was documented in detail, leading to the identification of three different key intermediates through which mononuclear aromatic compounds are channelled, i.e. benzoyl-CoA, resorcinol, and phloroglucinol (Schink *et al.*, 1992; Boll *et al.*, 2002). The common feature of most of these pathways is that the aromatic nucleus is destabilized via a reductive rather than an oxidative attack. The benzoyl-CoA pathway appears to be the most important one because a broad variety of compounds enters this path, including phenol, various hydroxybenzoates, phenylacetate, aniline, cresols and alkylbenzenes (Schink *et al.*, 1992; Heider and Fuchs, 1997; Harwood *et al.*, 1999).

Nonetheless, the chemical constraints of microbial activities in anoxic environments differ with the respective

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electron acceptor system available. Reduction of nitrate (through nitrite, NO, N_2O) to N_2 operates at an average standard redox potential (E_0') of +750 mV (close to that of O_2/H_2O at +810 mV), reduction of $Fe(OH)_3$ to Fe^{2+} (pH 7.0) at *c.* +100 mV (Widdel *et al.*, 1993), sulfate reduction to sulfide at $E_0' = -218$ mV, and finally methanogenesis at −244 mV. Thus, it appears plausible that also the biochemical strategies taken in anaerobic degradation of aromatics should differ within anoxic environments, depending on the respective electron acceptor system in use.

The field of anaerobic degradation of aromatic compounds has been discussed in several other reviews in the recent past (Schink *et al.*, 2000; Boll *et al.*, 2002; Gibson and Harwood, 2002; Boll and Fuchs, 2005; Boll, 2005a,b; Heider, 2007; Fuchs, 2008; Carmona *et al.*, 2009). In the following overview, we want to focus on the different strategies taken in anaerobic microbial degradation of aromatic compounds by different metabolic types of bacteria, depending on the electron acceptor available for substrate oxidation. It turns out that, beyond the mere non-availability of oxygen, also the overall reaction energetics and the redox potential of the electron acceptor system in use have a profound influence on the biochemical strategies employed in degradation of aromatics.

Benzoate

Benzoate is degraded anaerobically by nitrate-reducing, sulfate-reducing, iron(III)-reducing and also by fermenting bacteria, which cooperate with methanogenic partners in a syntrophic manner. In all these cases, benzoate is first activated by a ligase enzyme to form benzoyl-CoA; this reaction consumes two ATP equivalents. In the nitrate reducer *Thauera aromatica*, the stability of the aromatic ring structure is overcome by a reduction reaction which forms cyclohexadiene carboxyl-CoA as the first identifiable product (class I benzoyl-CoA reductase, Fig. 1A; Koch and colleagues 1993; Boll and Fuchs, 1995) and consumes further two ATP equivalents. This energy is required to overcome the resonance energy of the benzene ring to form the cyclohexadiene derivative; the standard redox potential (E_0') of the benzoyl-CoA/cyclohexadiene carboxyl-CoA couple is at −620 mV (Schöcke and Schink, 1999) and the reaction is irreversible.

In the iron-reducing bacterium *Geobacter metallireducens* (Fig. 1B) the reduction of benzoyl-CoA involves an enzyme (class II benzoyl-CoA reductase), which contains tungsten, zinc (Wischgoll *et al.*, 2005; Boll, 2005b) and selenocysteine (Peters *et al.*, 2004). The primary reaction product is cyclohexadiene carboxyl-CoA as with the nitrate reducers (Peters *et al.*, 2007a; Kung *et al.*,

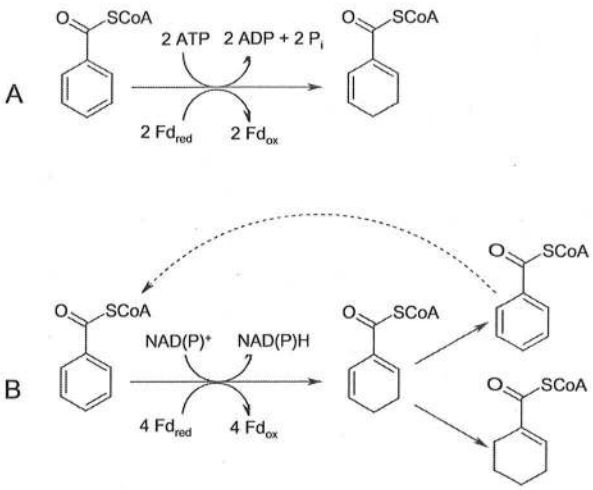

Fig. 1. Key steps in anaerobic degradation of benzoyl-CoA.
A. Benzoyl-CoA reduction by the nitrate-reducing bacterium *Thauera aromatica* strain K 172.
B. Benzoyl-CoA reduction by the iron-reducing bacterium *Geobacter metallireducens*.

2009), and the electrons for this reduction are supplied by a ferredoxin. The purified enzyme has no binding site for ATP, and ATP is not required (Fuchs, 2008; Kung *et al.*, 2009). The ferredoxin electrons are dismutated through a 'bifurcation' mechanism (Herrmann *et al.*, 2008; Thauer *et al.*, 2008) to the level of benzoyl-CoA reduction (−620 mV) and that of NAD(P)H (−320 mV); the NAD(P)H electrons have to be pushed again to the ferredoxin level, probably through an Rnf-like reversed electron transport system. The reaction equilibrium is shifted further by a subsequent highly exergonic dismutation of the dienoyl-CoA product to benzoyl-CoA and monoenoyl-CoA (A. Schmidt, pers. comm.). Thus, the overall reaction can be written as:

$$\text{Benzoyl-CoA} + 4e^- + 4H^+ \rightarrow \text{Cylohex-1-ene carboxyl CoA}$$

The redox potential of this reduction is at −350 mV (Schöcke and Schink, 1999). It was shown recently that the entire reaction chain is fully reversible (Kung *et al.*, 2010), thus demonstrating that it operates close to the thermodynamic equilibrium.

Iron reducers gain substantially less energy in the further oxidation of the carbon skeleton than nitrate reducers do. Thus, it appears plausible that they employ an enzyme system for ring de-aromatization that operates with far less ATP expenditure than nitrate reducers do. The energetic situation of benzoate-degrading sulfate reducers is similar to that of iron reducers, and it is even worse with syntrophically fermenting benzoate degraders. To balance the overall energetics of benzoate fermentation by the syntrophically fermenting *Syntrophus genti-*

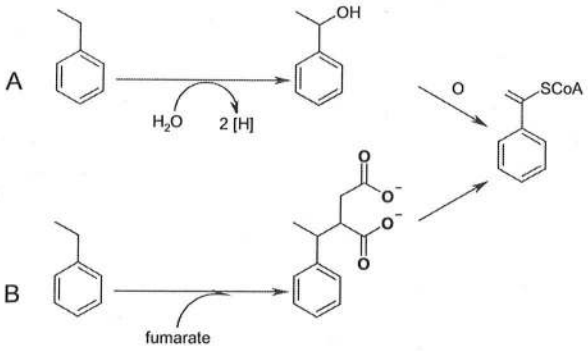

Fig. 2. Initial steps in anaerobic degradation of ethylbenzene.
A. Degradation by the nitrate-reducing bacterium '*Aromatoleum aromaticum*', leading to (*S*)-1-phenylethanol.
B. Degradation by the sulfate-reducing bacterium strain EbS7, forming phenylethyl succinate as intermediate.

anae it was suggested earlier that it reduces benzoyl-CoA in a four-electron transfer step to a cyclohex-1-ene derivative (Schöcke and Schink, 1999). Recent evidence (Peters *et al.*, 2004; 2007b; Löffler *et al.*, 2011) indicates that sulfate reducers and syntrophically fermenting benzoate degraders use an ATP-independent reductase system similar to that described above for *G. metallireducens*. This view is supported by the observation that with the syntrophically fermenting *Syntrophus aciditrophicus* the pathway from benzoyl-CoA to fatty acid derivatives appears to be fully reversible as well (Mouttaki *et al.*, 2007).

Ethylbenzene

For the initiating reaction in anaerobic degradation of ethylbenzene, two alternative strategies have been reported. The denitrifying bacteria '*Aromatoleum aromaticum*' strain EbN1 (Kniemeyer and Heider, 2001) and *Azoarcus* sp. strain EB1 (Johnson *et al.*, 2001) initiate degradation of ethylbenzene by hydroxylation to (*S*)-1-phenylethanol (Fig. 2A). Both enzymes contain molybdenum; the basic mechanism of oxygen-independent hydroxylation is probably analogous to that of other hydroxylating molybdenum enzymes (Hille *et al.*, 1999; Boll *et al.*, 2005). Further degradation leads via oxidation to acetophenone and carboxylation to benzoyl acetate (Heider, 2007). In contrast, the sulfate-reducing bacterium strain EbS7 initiates ethylbenzene degradation by fumarate addition, a reaction mechanism which was originally discovered in sulfate-dependent degradation of toluene (Biegert *et al.*, 1996; Beller and Spormann, 1997). Fumarate addition to ethylbenzene forms (1-phenylethyl) succinate (Fig. 2B), which is further degraded via rearrangement to 4-phenylpentanoyl-CoA (Kniemeyer *et al.*, 2003). Regardless of the different strategies for initiating the conversion, the ethylbenzene residue is channelled into the benzoyl-CoA pathway in both cases. Since the redox potential for the hydroxylation is rather positive (–12 mV, Johnson and Spormann, 1999; or +30 mV; Kniemeyer and Heider, 2001), this reaction would be difficult to perform by sulfate-reducing bacteria (Kniemeyer *et al.*, 2003).

Cresols

Cresols (methylphenols) are anaerobically degraded through different pathways, depending on the position of the hydroxy group. *p*-Cresol is hydroxylated by a nitrate reducer at the methyl group by an oxygen-independent reaction, possibly through a quinomethide intermediate as suggested earlier for an aerobic *Pseudomonas* strain (Fig. 3A; Hopper, 1978). The redox potential of this oxidation reaction is in the range of +100 mV (calculated after Thauer *et al.*, 1977) and the reaction is therefore easy for a nitrate-reducing bacterium that couples this oxidation, e.g., with the reduction of a *c*-type cytochrome at +232 mV (Hopper *et al.*, 1991). Recently, a *p*-cresol methylhydroxylase of *G. metallireducens* has been shown to be a periplasmic enzyme with two haem cofactors and one FAD cofactor (Peters *et al.*, 2007b; Johannes *et al.*, 2008). A sulfate-reducing bacterium uses an entirely different pathway for *p*-cresol degradation, which activates the substrate at the methyl group by fumarate addition (Müller *et al.*, 2001). *o*-Cresol can be carboxylated by fermenting and by nitrate-reducing bacteria to 3-methyl-4-hydroxybenzoate and degraded further analogous to phenol, producing 3-methyl benzoyl-CoA (Fig. 3B; Bisaillon *et al.*, 1991; Rudolphi *et al.*, 1991). In enrichment cultures with *m*-cresol plus sulfate, indications of a carboxylation as primary activation reaction were obtained (Roberts *et al.*, 1990; Ramanand and Suflita, 1991), but it was never proven that the 4-hydroxy-2-methyl benzoic acid detected was really a degradation intermediate rather than a side product. Degradation of *m*-cresol by the sulfate-reducing bacterium *Desulfobacterium cetonicum* follows a pathway similar to sulfate-dependent *p*-cresol oxidation, including fumarate addition to the methyl group (Müller *et al.*, 1999). Activation and β-oxidation lead to succinyl-CoA and 3-hydroxybenzoyl-CoA (Fig. 3C). Thus again, oxidative activation reactions are used by nitrate reducers whereas sulfate reducers prefer the addition of fumarate to the methyl group.

Resorcinol and resorcylic acids

There are two different pathways for anaerobic degradation of resorcinol (1,3-dihydroxybenzene), which can be attacked either reductively or oxidatively. Reduction of resorcinol was found with the fermenting *Clostridium*

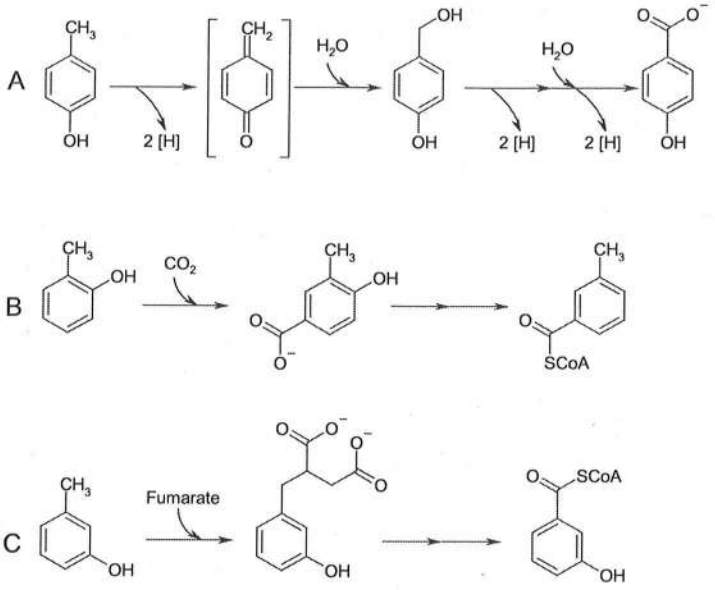

Fig. 3. Initial steps in anaerobic degradation of cresols.
A. Degradation of *p*-cresol.
B. Degradation of *o*-cresol.
C. Degradation of *m*-cresol.

co-culture KN 245 (Tschech and Schink, 1985), which harbours a resorcinol reductase catalysing the reduction of resorcinol to 1,3-cyclohexadione (Fig. 4A; Kluge *et al.*, 1990). This intermediate is hydrolytically cleaved to 5-oxocaproic acid which is further fermented to acetate and butyrate. The resorcinol reductase is a homo-multimeric enzyme consisting of 49.5 kDa subunits each with FAD as a co-factor (Schüler, 1997). The reduction of resorcinol resembles the analogous reactions with phloroglucinol (1,3,5-trihydroxybenzene; Haddock and Ferry, 1989) and hydroxyhydroquinone (1,2,4-trihydroxybenzene; Reichenbecher *et al.*, 2000; see below). In all these cases, the aromatic π-electron system is sufficiently destabilized to form 1,3-dioxo tautomers and thus to allow ring reduction without prior activation.

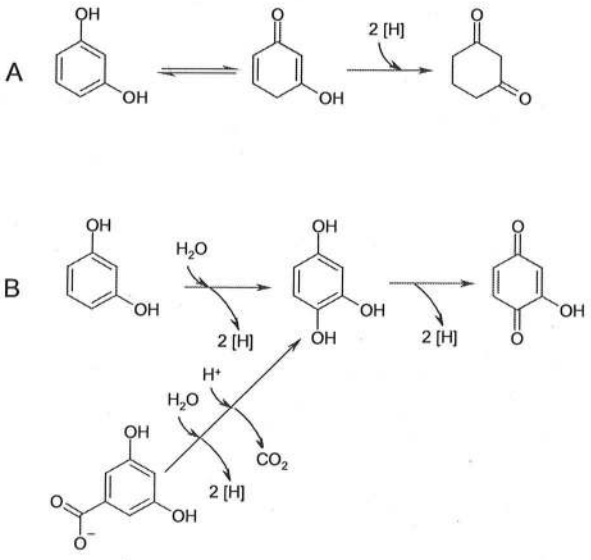

Fig. 4. Initial steps in anaerobic degradation of resorcinol.
A. Resorcinol degradation by a fermenting bacterium, *Clostridium* strain KN245.
B. Degradation of resorcinol and α-resorcylate by the denitrifying bacteria *Azoarcus anaerobius* and *Thauera aromatica* strain AR-1 respectively.

An entirely different pathway of resorcinol degradation was found with a nitrate reducing bacterium, *Azoarcus anaerobius* strain LuFRes1. This pathway proceeds via oxidative steps (Philipp and Schink, 1998), and the respective genes have been identified by heterologous expression in the *T. aromatica* strains AR-1 and K172 (Darley *et al.*, 2007). In the first step, resorcinol is hydroxylated by a membrane-bound enzyme to hydroxyhydroquinone (1,2,4-trihydroxybenzene; HHQ) (Fig. 4B). The resorcinol-hydroxylating enzyme is encoded by two genes named *rhLS*, which show high sequence identities to the subunits of pyrogallol-phloroglucinol transhydroxylase of *Pelobacter acidigallici*, a well-studied molybdoenzyme (Reichenbecher *et al.*, 1994; Messerschmidt *et al.*, 2004). Thus, resorcinol hydroxylation is likely catalysed by a molybdenum-containing enzyme. In the second step, HHQ is further oxidized to hydroxybenzoquinone by a membrane-bound HHQ-dehydrogenase.

Hydroxybenzoquinone, the product of the oxidative de-aromatization, is a very reactive compound which is further converted in cell-free extracts to acetate, malate, and succinate (Darley *et al.*, 2007). The first products of ring cleavage are probably aldehydes or ketones

since such compounds could be trapped with 2,4-dinitrophenylhydrazine (J. Hellstern, unpubl. results). This reaction sequence is most probably catalysed by enzymes which, according to the gene sequence information, show structural similarity to components of the multienzyme complex pyruvate dehydrogenase. Cleavage of the HBQ ring through an oxidative splitting between the two oxo carbon atoms would be compatible with an enzymatic system analogous to a pyruvate dehydrogenase complex.

Azoarcus anaerobius strain LuFRes1 can also grow with the resorcylic acids 2,4-dihydroxybenzoate and 2,6-dihydroxybenzoate, which both are prone to spontaneous decarboxylation and are degraded most likely via resorcinol (Gorny *et al.*, 1992). The third isomer 3,5-dihydroxybenzoate is chemically stable. *T. aromatica* strain AR-1 can grow with this compound (Gallus and Schink, 1998) and degrades it via hydroxylation to a trihydroxybenzoate, which is decarboxylated to HHQ (Fig. 4B). HHQ-dehydrogenating activity was detected in membrane fractions of this strain as well (Philipp and Schink, 2000).

The HHQ-pathway for degradation of aromatic compounds has so far been found only in nitrate-reducing bacteria, probably because the oxidation of resorcinol to HHQ requires electron acceptors of a positive redox potential (about +100 mV). Also the oxidation of HHQ to HBQ has a standard redox potential of +180 mV (Philipp and Schink, 1998), which could explain why this strategy is used only by nitrate-reducing bacteria but – as far as we know – not by sulfate-reducing or fermenting bacteria.

Trihydroxybenzenes

Among the three trihydroxybenzene isomers, pyrogallol and phloroglucinol are degraded quickly by fermenting bacteria, and were actually the first aromatic compounds degraded by fermentation in pure culture (Schink and Pfennig, 1982). Phloroglucinol (1,3,5-trihydroybenzene) degradation has been studied in detail with *Eubacterium oxidoreducens* and *P. acidigallici*. It is reduced by an NADPH-dependent reductase to dihydrophloroglucinol (Haddock and Ferry, 1989; Brune and Schink, 1992). Hydrolytic ring cleavage leads to 3-hydroxy-5-oxohexanoic acid, which is thiolytically cleaved and oxidized to three acetate residues (Brune and Schink, 1992). This pathway is easy to conceive because the 1,3,5-arrangement of the three hydroxyl groups on the aromatic ring allows tautomerization to 1,3,5-trioxocyclohexane to a certain degree which favours a nucleophilic attack on the oxo-carbon groups. The second trihydroxybenzene isomer, pyrogallol (1,2,3-trihydroxybenzene), cannot be hydrolysed or reduced directly but is isomerized to phloroglucinol through a transhydroxylation reaction

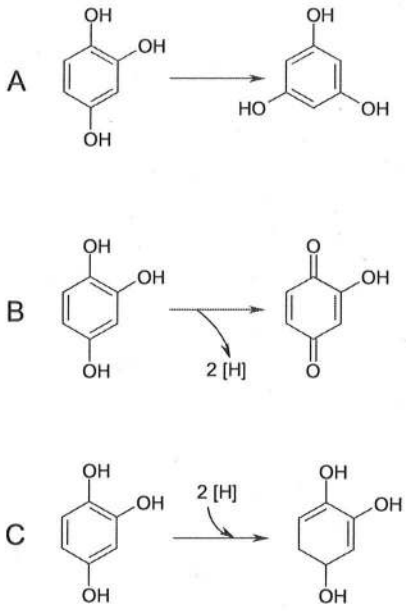

Fig. 5. Degradation of hydroxyhydroquinone.
A. Degradation by fermenting bacteria.
B. Degradation by nitrate-reducing bacteria.
C. Degradation by sulfate-reducing bacteria.

(Krumholz and Bryant, 1988; Brune and Schink, 1990). The reaction requires 1,2,3,5-tetrahydroxybenzene as a cosubstrate, and the enzyme transfers a hydroxyl group from the tetrahydroxybenzene to pyrogallol, thus releasing phloroglucinol as product and the tetrahydroxybenzene as a co-product (Brune and Schink, 1990; Messerschmidt *et al.*, 2004). The transhydroxylase enzyme contains an iron–sulfur centre and a molybdopterin cofactor (Reichenbecher *et al.*, 1996).

The third trihydroxybenzene isomer, hydroxyhydroquinone (HHQ; 1,2,4-trihydroxybenzene), is converted by the fermenting bacterium *Pelobacter massiliensis* to three acetate as well (Schnell *et al.*, 1991; Fig. 5A), indicating that also this pathway leads through phloroglucinol. The isomerization to phloroglucinol requires three subsequent transhydroxylation reactions analogous to the pyrogallol-phloroglucinol transhydroxylation (Brune *et al.*, 1992).

Alternative to the strategy of isomerization to phloroglucinol, we found different pathways of HHQ degradation with nitrate-reducing and sulfate-reducing bacteria. Oxidative degradation of HHQ by nitrate-reducing bacteria (Fig. 5B) was mentioned above in the context of nitrate-dependent degradation of resorcinol and resorcylic acids. A further alternative of HHQ degradation was found with the sulfate-reducing bacterium *Desulfovibrio inopinatus*. This bacterium metabolizes HHQ to two acetates and two CO_2 (Fig. 5C). In the first step, HHQ is destabilized by reduction to dihydro-HHQ, and later acetate and a so far

non-identified 4-carbon derivative are formed (Reichenbecher *et al.*, 2000). Since *D. inopinatus* cannot oxidize acetyl residues, the final products are two acetates and two CO_2, and 1 mol of sulfate is reduced concomitantly to sulfide. Thus, the strategies of substrate exploitation by the three different metabolic types of anaerobic bacteria are mirrored in their metabolic products: The fermenting bacterium releases all substrate carbon as acetate, and recovers a net amount of 2 ATP per mol HHQ via phosphotransacetylase and acetate kinase, following one hydrolytic and two thiolytic cleavage reactions. The sulfate reducer which cannot oxidize acetate produces two acetates and uses the additional electrons for sulfate reduction to gain some additional energy through sulfate respiration. The nitrate reducer gains most of its energy in the oxidation of acetyl residues and forms only CO_2 as terminal product.

Phenol, hydroquinone, catechol and aniline

Different from the cases treated so far, anaerobic degradation of other aromatics, such as phenol, hydroquinone and catechol, appears to take similar paths, no matter which electron acceptor is used.

Phenol degradation has been investigated into most detail with the denitrifying bacterium *T. aromatica* strain K172. It is carboxylated to 4-hydroxybenzoate (Tschech and Fuchs, 1989) via phenylphosphate (Lack and Fuchs, 1992), which is formed by an ATP-dependent reaction yielding phenylphosphate, AMP and inorganic phosphate (Lack and Fuchs, 1994; Schmeling *et al.*, 2004). The subsequent carboxylase forms 4-hydroxybenzoate plus phosphate (Schühle and Fuchs, 2004). Thus, the carboxylation of phenol to phenylphosphate is accomplished with the expenditure of two ATP equivalents. The further degradation of 4-hydroxybenzoate proceeds via activation with coenzyme A and reductive dehydroxylation to benzoyl-CoA; this compound is further degraded via reactions described before (Boll *et al.*, 2002). Also other nitrate reducers such as *Magnetospirillum* spp. and '*Aromatoleum aromaticum*' and even the iron-reducing strict anaerobe *G. metallireducens*, although energetically far less favoured, all carboxylate phenol via phenyl phosphate at the expense of two ATP equivalents (Schleinitz *et al.*, 2009; Schmeling and Fuchs, 2009). The same appears to be true for the sulfate-reducing *Desulfobacterium anilini* (Ahn *et al.*, 2009). So far, the biochemistry of phenol carboxylation in fermenting phenol degraders (Gallert *et al.*, 1991) has not been studied; the high energy expenditure of phenol carboxylation and hydroxybenzoic acid activation described above may ask for different biochemical strategies in these bacteria.

Hydroquinone is degraded by sulfate-reducing and fermenting bacteria via carboxylation to gentisate. This carboxylation could never be studied in cell-free extracts and the energetization of this reaction is unknown. Gentisate is activated to gentisyl-CoA through a CoA-ligase reaction (Gorny and Schink, 1994a,b). In *S. gentianae*, gentisyl-CoA is reductively dehydroxylated to benzoyl-CoA which enters a modified benzoyl-CoA pathway (Gorny and Schink, 1994b).

Degradation of catechol has been studied with a sulfate-reducing *Desulfobacterium* strain, which carboxylates catechol to protocatechuate (Gorny and Schink, 1994c). Protocatechuate is activated to form protocatechuyl-CoA, which is subsequently dehydroxylated to benzoyl-CoA. A similar pathway appears to be used by the nitrate reducer *T. aromatica* (Ding *et al.*, 2008). In this case, the initial carboxylation proceeds via activation of catechol to catechylphosphate.

Aniline is degraded anaerobically by the sulfate reducer *D. anilini* through a pathway analogous to phenol degradation. It is initially carboxylated to 4-aminobenzoate which is subsequently activated to 4-aminobenzoyl-CoA, with subsequent reductive deamination to benzoyl-CoA (Schnell and Schink, 1991). The carboxylation reaction has not been studied so far, and nothing is known about an activated intermediate to provide the necessary energy for the carboxylation reaction. A primary phosphorylation as in the case of phenol degradation (see above) appears unlikely, but no reliable biochemical studies have been performed on this system yet. Aniline degradation by nitrate reducers or other anaerobes has never been reported yet.

Conclusions

In this survey, we have shown that the degradation of aromatic compounds by anaerobic bacteria can follow rather different strategies with every substrate, indicating that anaerobic degradation of aromatic compounds is apparently more diverse with respect to the possible intermediates and reaction routes than the aerobic degradation of aromatics. Comparison of fermenting, sulfate-reducing, and nitrate-reducing bacteria exemplifies that the respective strategy applied in the absence of oxygen appears to be largely influenced by the energetic situation of the respective organism and the redox potentials of the electron acceptors it can use. In the benzoyl-CoA pathway, two different strategies are used to overcome the resonance energy barrier to destabilize the π-electron system: nitrate reducers invest a major amount of ATP into the reduction to the energy-rich cyclohexadiene derivative. Iron-reducing, sulfate-reducing and fermenting bacteria do the same without ATP, but shift and pull this reaction by bifurcation of ferredoxin electrons and subsequent dismutation to benzoyl-CoA and the cyclohexene derivative, which is

energetically more feasible. Thus, the net reduction of benzoyl-CoA to cyclohex-1-ene carboxyl-CoA with four electrons may require only fractions of an ATP equivalent which has to be invested into a reversed electron transport from NAD(P)H to ferredoxin.

In degradation of ethylbenzene and cresols, nitrate reducers start with oxidative steps with standard redox potentials around 0 to +100 mV. Electrons at these potentials are difficult to dispose of by sulfate reducers or other strict anaerobes; iron reducers are just able to handle such electrons as the case of *p*-cresol degradation shows. Sulfate reducers prefer the addition of fumarate to alkyl residues which is nearly an equilibrium reaction ($\Delta G_0' = -15$–25 kJ mol^{-1}). Of course, the problem of electron disposal follows then later, in the reoxidation of a succinyl to a fumaryl residue ($E_0' = +30$ mV) to complete the reaction cycle. Sulfur- and sulfate-reducing bacteria couple succinate oxidation with a reversed electron transport, which consumes a fraction of an ATP equivalent (Paulsen *et al.*, 1986; Thauer, 1988).

With resorcinol, two entirely different pathways were identified in fermenting versus nitrate-reducing bacteria, the one starting with a reductive, the other one with an oxidative strategy. The HHQ pathway may gain major importance in the transformation of phenolic compounds by nitrate-reducing bacteria, because they may prefer this pathway over the parallel pathways used by fermenting or sulfate-reducing bacteria. Degradation of HHQ takes even three different directions, isomerization to phloroglucinol in fermenting bacteria, primary reductive destabilization in sulfate reducers, and oxidative attack in nitrate reducers.

Nonetheless, some compounds are degraded through similar primary activation reactions, no matter which electron donor is used. This applies to phenol, hydroquinone and catechol, at least according to our present knowledge. All these substrates start (after phosphorylation of the respective substrate) with a carboxylation reaction, which is not directly coupled to redox processes and is therefore rather independent of the electron acceptor system.

Finally, some open questions remain with respect to the diversity of anaerobic pathways for degradation of aromatics. Whereas some aromatics, e.g., benzoate, can be degraded under any redox condition discussed, others are degraded only under certain conditions. It appears enigmatic why, e.g., aniline is degraded so far only by sulfate reducers although its degradation with nitrate should be much more feasible. The same applies to anaerobic naphthalene degradation which was shown reliably so far only with sulfate as electron acceptor (Galushko *et al.*, 1999; Musat *et al.*, 2009). Perhaps some key steps in the activation of these substrates interfere with the high reactivity of redox intermediates of nitrate reduction (nitrite, NO, N_2O), which preclude those reactions to proceed.

One may ask why there are different strategies taken for anaerobic degradation of aromatic compounds in the anoxic world. Since fermenting bacteria, sulfate reducers and iron reducers are supposed to have evolved their metabolic capacities far before aerobes and nitrate reducers entered the scene, one may argue that these bacteria could have used the same biochemical strategies as their strictly anaerobic predecessors. However, nitrate reduction was developed probably even after aerobic respiration as a secondary anaerobic lifestyle, and both oxygen and the various redox intermediates of denitrification (nitrite, NO, N_2O) are aggressive oxidants, which probably preclude the employment of highly oxygen-sensitive, often radical-catalysed reactions that the strict anaerobes handle with such impressive virtuosity. Therefore, nitrate reducers had to develop novel oxygen-independent strategies, at least in those cases in which rather delicate radical-catalysed reactions are used by the strict anaerobes.

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