

Anaerobic Degradation of Aromatic Compounds — Ring Cleavage without Oxygen —

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Anaerobic Lifestyles — Electron acceptors

Metabolism in the absence of oxygen is dominated by the nature of the available electron acceptor or hydrogen sink.

Successively, nitrate, ferric iron, sulfate, and carbon dioxide serve as the preferred electron acceptor for the denitrifying, iron reducing, sulfate reducing, and methanogenic bacteria.

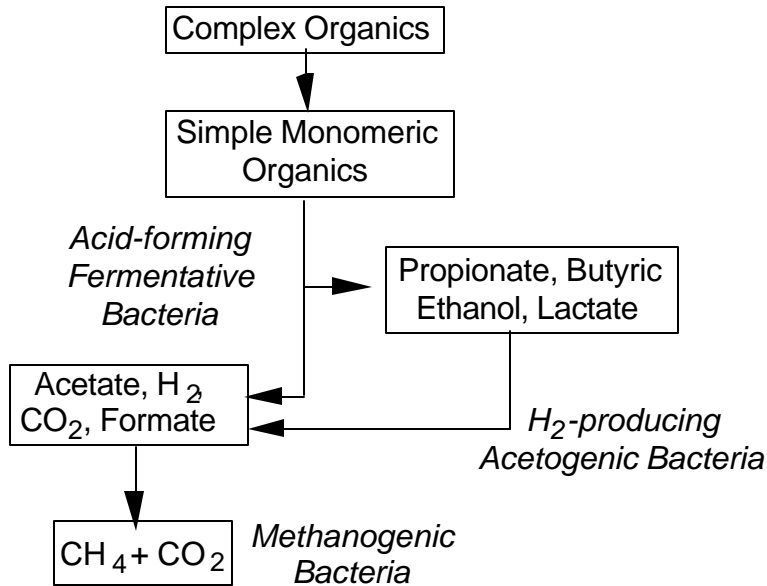
Environments of the terminal electron accepting processes (TEAPs):

Electron Acceptor	TEAP	Environment
O ₂	Aerobic	Sediment/GW
NO ₃ ⁻	Denitrifying	Nitrate-rich GW
Fe(III)	Iron reducing	GW
SO ₄ ²⁻	Sulfate reducing	Marine systems
CO ₂	Methanogenic	Organic rich systems

It also appears that oxidized groups on humic substances can serve as electron acceptors ¹.

Humic acids may act as redox shuttles between immobile ferric iron and bacteria in suspension.

In addition, acid-forming fermentative bacteria and H_2 -producing acetogenic bacteria are a part of many anaerobic communities:



In anaerobic environments oxygen is not available to promote activation of recalcitrant substrates, such as aromatics.

O_2 has two functions in metabolism: As electron acceptor and as a reactant in oxygenase reactions.

Until recently it was thought that only substituted benzenes were degradable without oxygen.

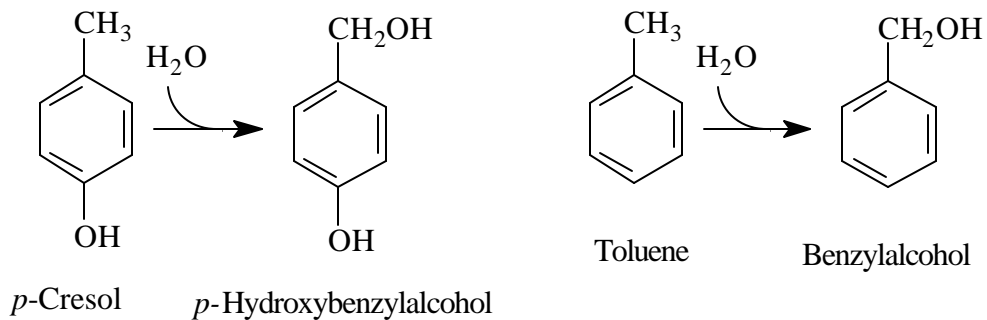
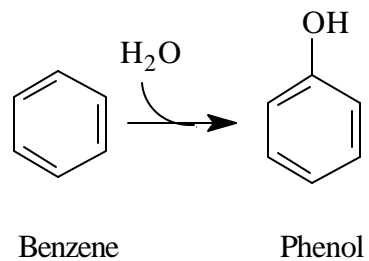
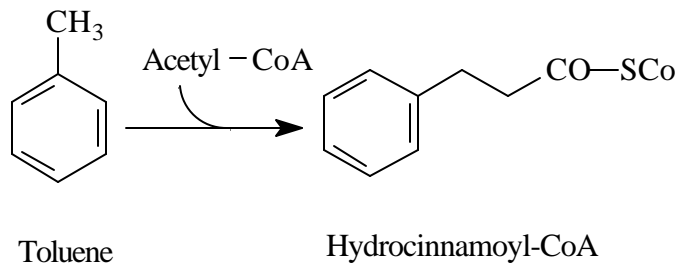
The substituents such as carboxyl, methyl, or hydroxyl decrease resonance energy by withdrawing electrons and destabilizing the ring and making ring cleavage easier.

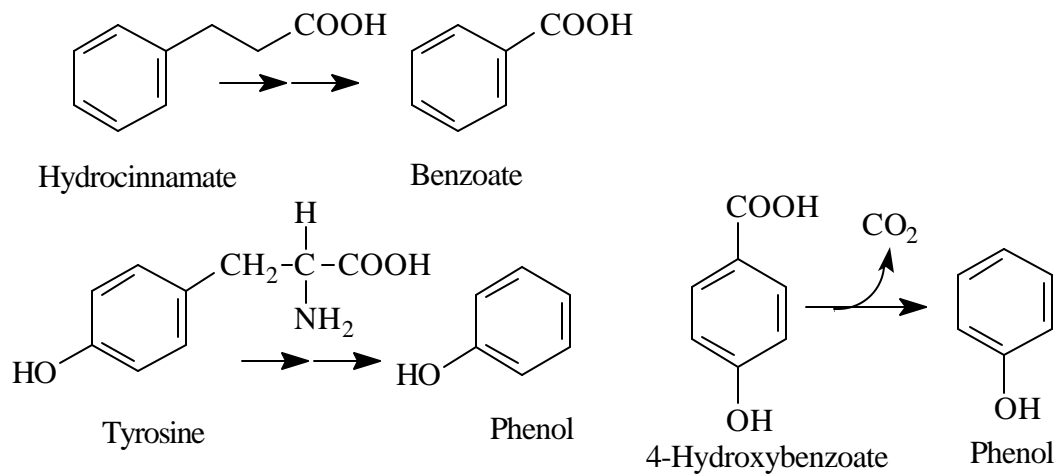
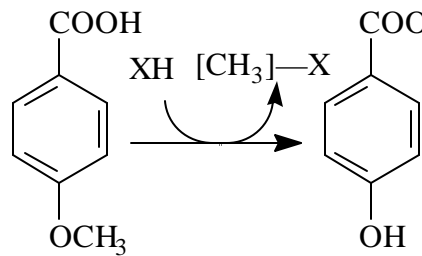
In the last few years toluene, xylenes, benzenes, and PAHs have been shown to be degradable.

Ring cleavage steps without oxygen

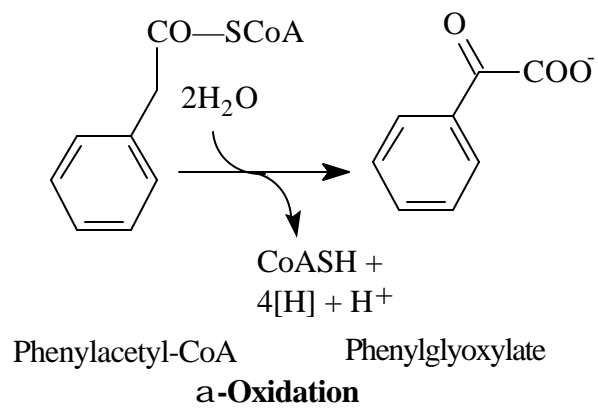
Three types of reactions:

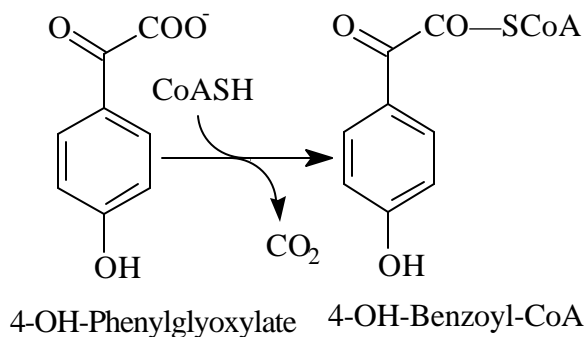
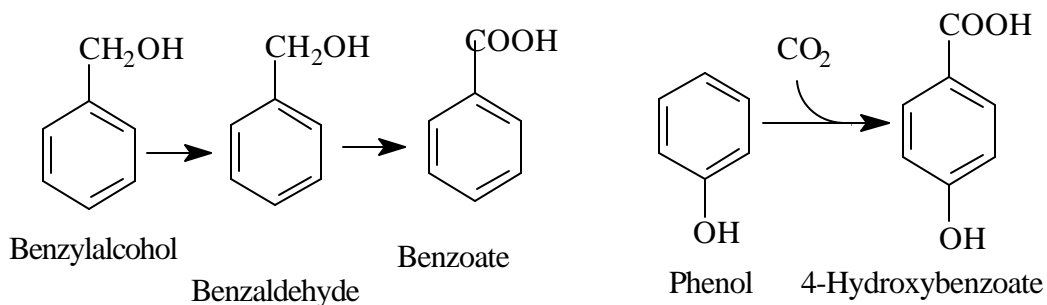
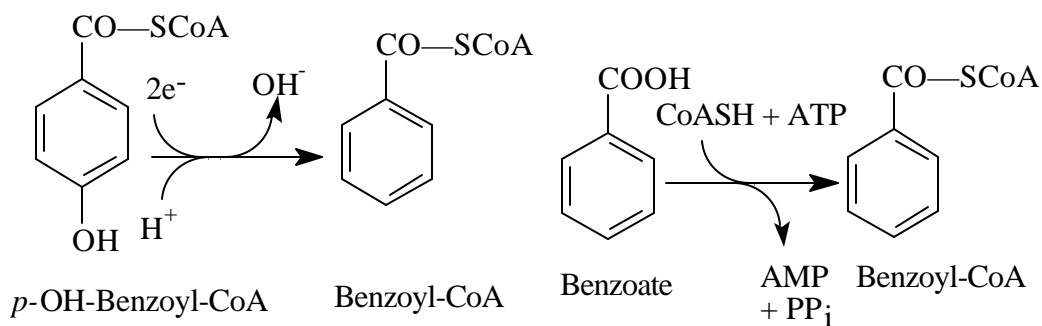
1. Activation reactions
2. Channeling reactions to central intermediates
3. Ring cleavage and β -oxidation reactions to form common intermediates for energy production and cell synthesis

Activation Reactions**Methyl Hydroxylation of *p*-Cresol Methyl Hydroxylation of Toluene****Hydroxylation of Benzene Ring****Methyl Group Oxidative Addition**

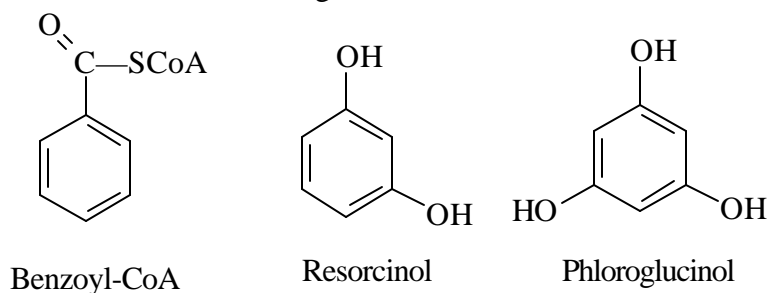
 **β -Oxidations and Lyase Reactions****Decarboxylation**

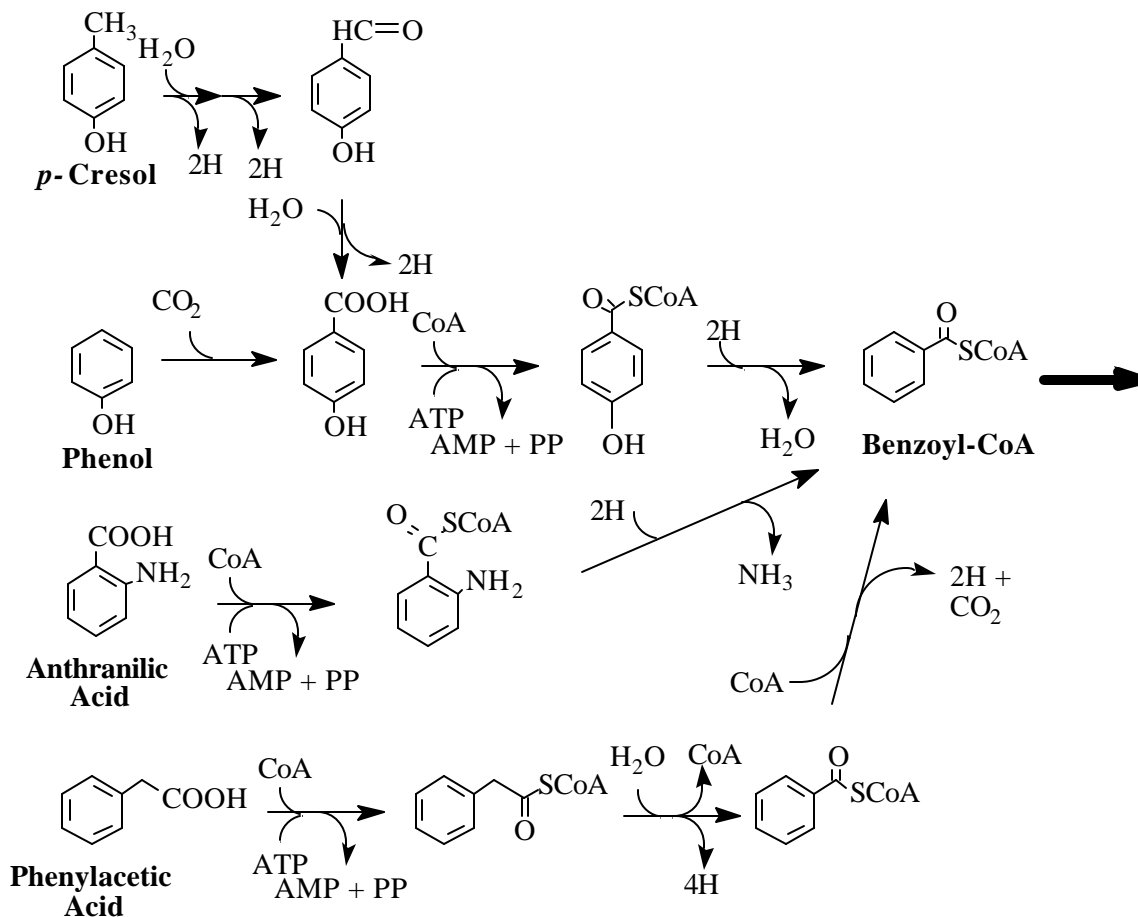
4-Methoxybenzoate 4-Hydroxybenzoate

O-Demethylation, Aryl Ether Cleavage

**Oxidative Decarboxylation****Aromatic Alcohol & Aldehyde Dehydrogenases****Carboxylation****Reductive Dehydroxylation****Coenzyme A Thioester Formation****Channeling Reactions**

Formation of central intermediates, thought to include

**Examples of Channeling to a Central Intermediate**



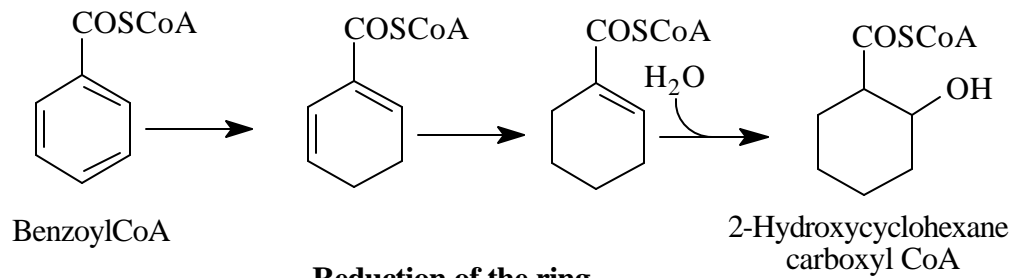
Ring Cleavage Reactions

Following production of central intermediates, the ring is cleaved to produce aliphatic acids, leading to common metabolic end products (CO_2 , CH_4 , and biomass)

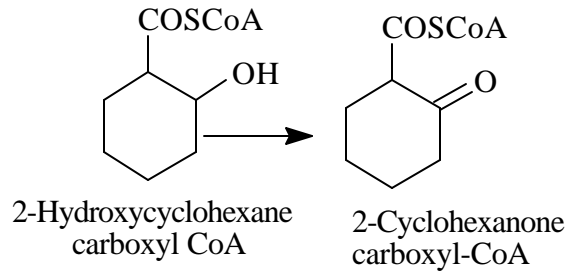
These generally involve three reactions:

1. Reduction of the aromatic ring to an alicyclic ring
2. Formation of cyclohexanone
3. Hydrolytic cleavage

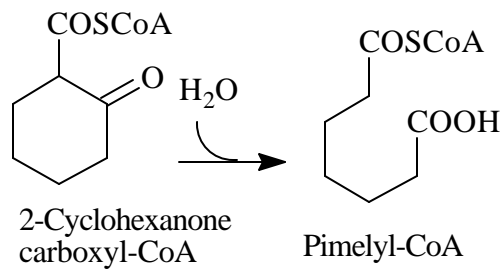
For example, this is how the benzoyl CoA ring is cleaved:



Reduction of the ring



Formation of hexanone



Hydrolytic Ring Cleavage

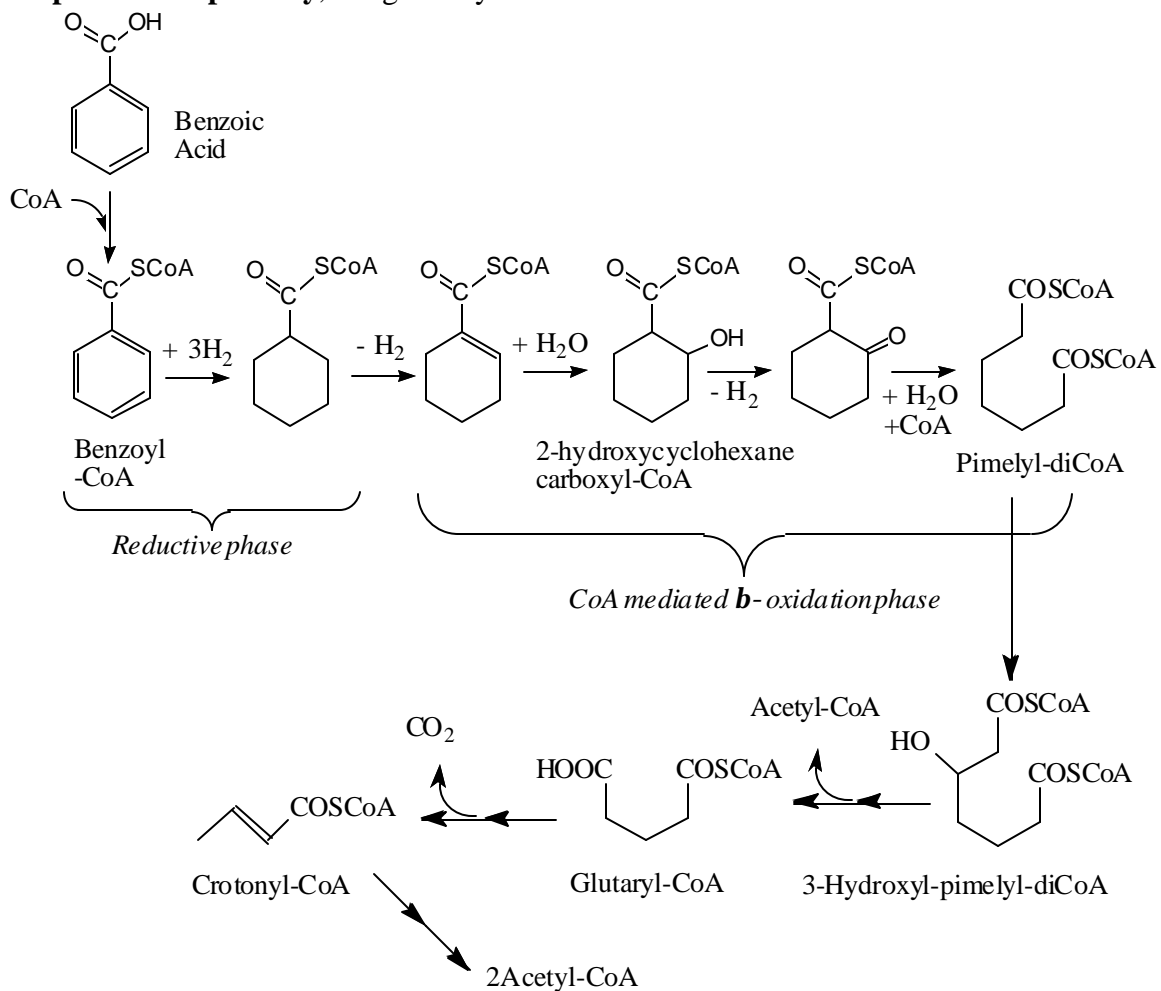
Examples of metabolic pathways*Anaerobic degradation of benzoic acid*²

Anaerobic degradation of benzoate proceeds by two pathways:

via pimelic acid and adipic acid

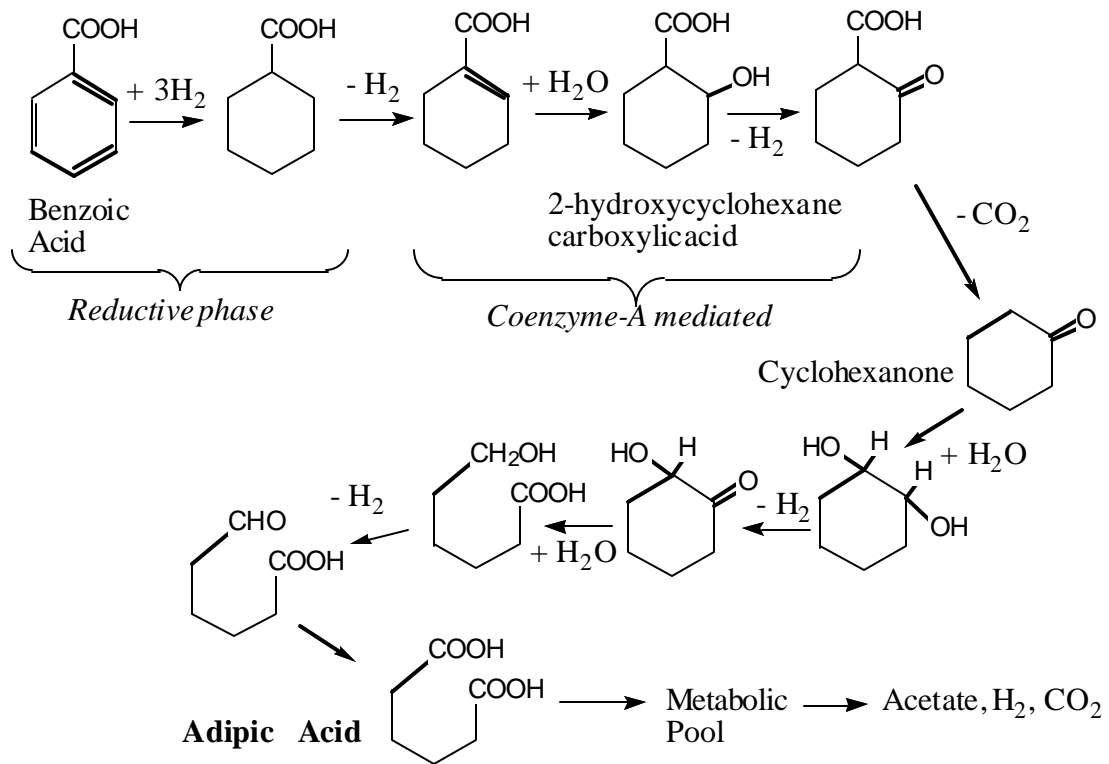
Both involve reduction (saturation) of the aromatic ring and incorporation of an oxygen from water leading to an enol prior to ring cleavage. By reduction of the aromatic ring the resonant electron distribution is destabilized.

The phototrophic purple nonsulfur bacterium *Rhodospseudomonas* attacks benzoic acid by the **pimelic acid pathway**, using coenzyme A as a cofactor:

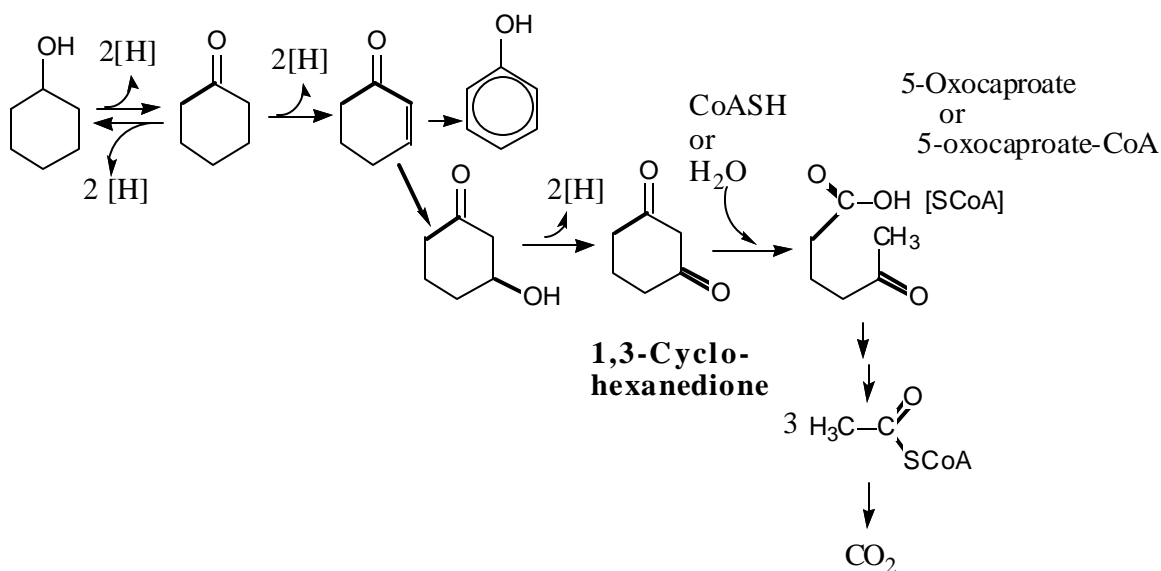


This pathway resembles the β -oxidation of fatty acids. Oxygen inhibits this degradation as it does other anaerobic ring cleavage pathways.

Denitrifiers, a *Moraxella* sp. and *Pseudomonas* PN1, degrades by reduction of the ring, followed by decarboxylation, as in the following pathway:



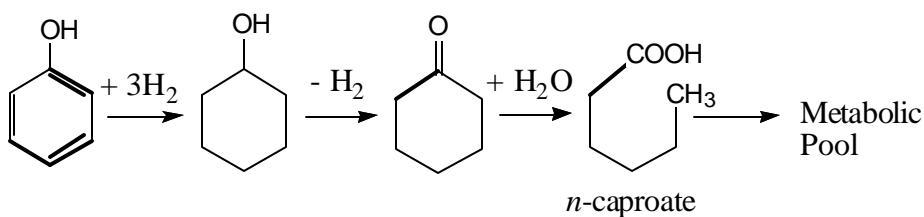
Recent work has provided details of the pathway used by a denitrifying *Pseudomonas* strain K601 to degrade cyclohexanol through 1,3-cyclohexadione, the substrate for ring opening, to 5-oxocaproate ³:



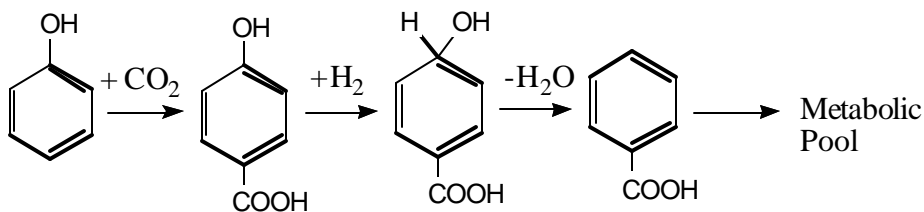
Sulfate reducers also able to degrade benzoates, but not through cyclohexanol or cyclohexanone. No pathways for aromatic metabolism by sulfate reducers yet published.

Anaerobic degradation of phenol

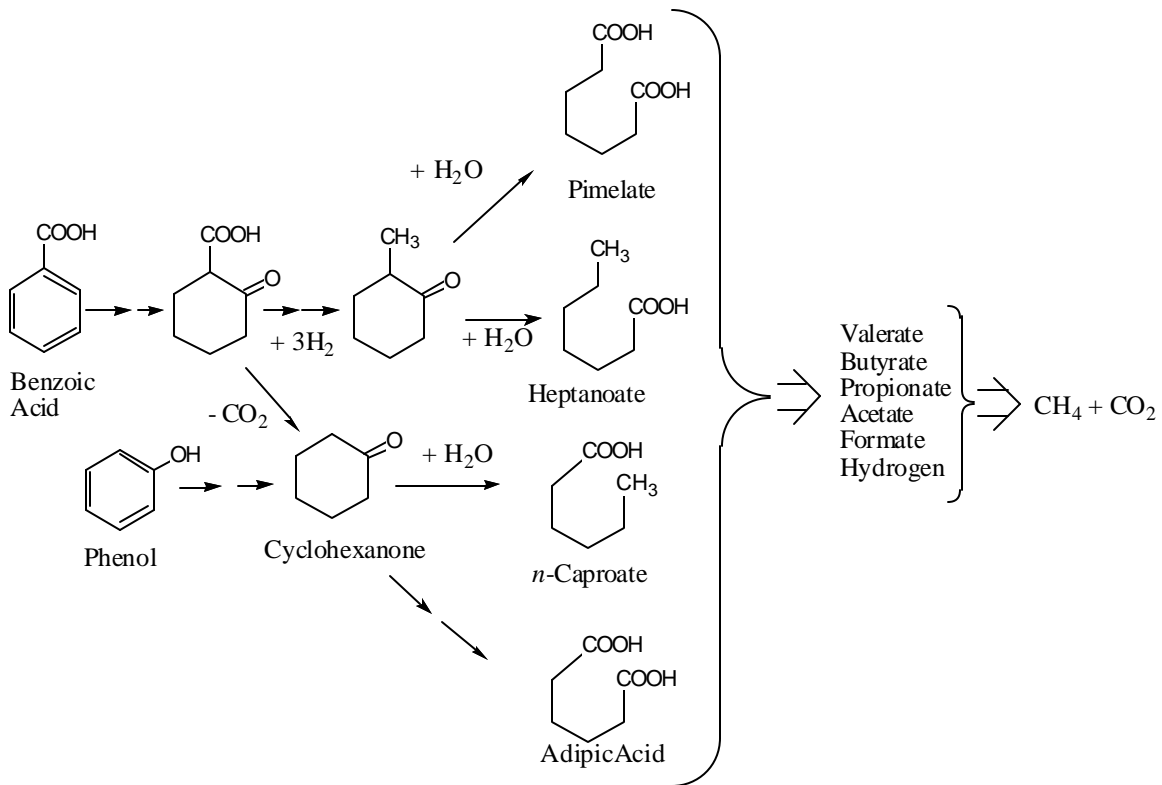
Phenols are degraded by denitrifiers via *n*-caproate:



or through benzoate:



Note that complete degradation of aromatics under methanogenic conditions requires interaction of a consortium:

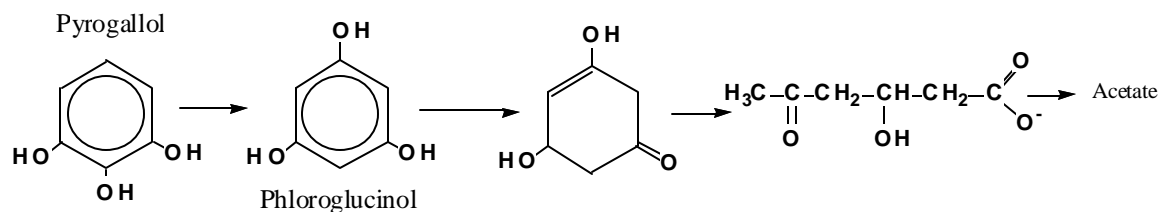


Sulfate reducers may take the place of methanogens as accepting hydrogen in consortia degrading aromatics.

All of these pathways involve

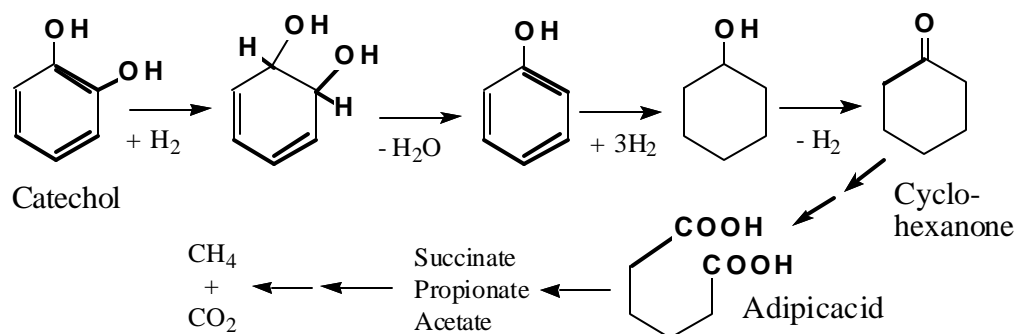
1. Reduction of the ring, often involving coenzyme A
2. Formation of a cyclohexanone or methylcyclohexanone
3. Reductive cleavage of the ring to aliphatic acids
4. Degradation of the cleavage products to compounds suitable for methanogenic use.

Other compounds, significant in degradation of lignin that occurs naturally and in pulp and paper making, can be anaerobically degraded: Catechol, vanillin, syringic acid, syringaldehyde, syringic acid, ferulic acid, cinnamic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and **pyrogallol**, a product of lignin degradation:



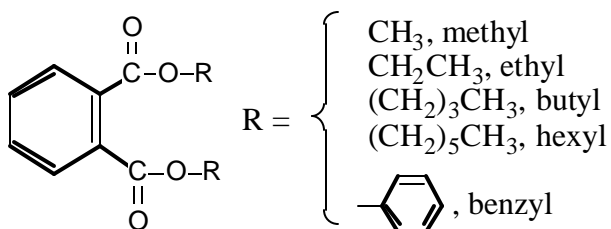
All require a reduction of the aromatic ring, formation of cyclohexanone, then a cleavage leading to a dicarboxylic acid, as for **catechol**

:



Anaerobic degradation of phthalates

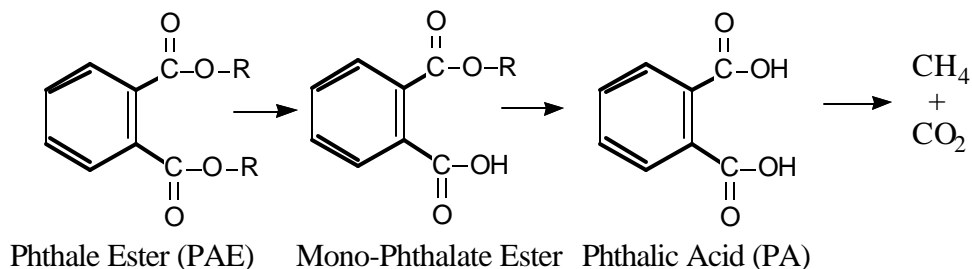
Phthalate acid esters are important as plasticizers. They leach out of plastics and are a major contaminant of municipal sludges.



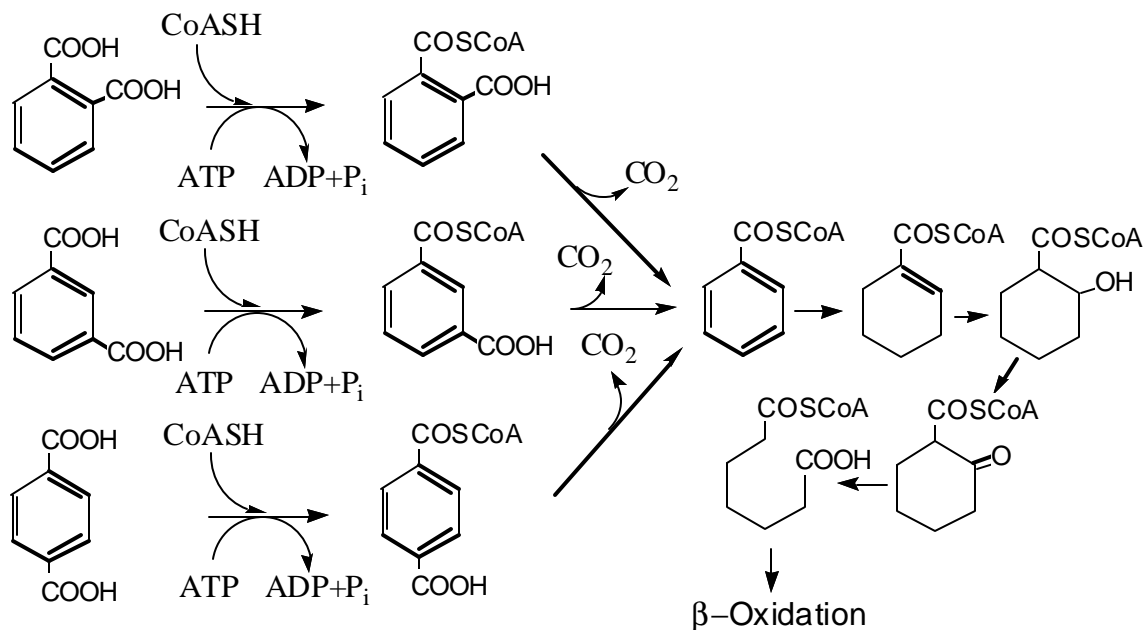
Bis(2-ethylhexyl) phthalate accounts for about 23% of phthalate production and it is a suspected carcinogen.

Phthalate esters are degraded slowly under anaerobic conditions with less degradation for longer side-chains: 100% loss in 7-42d for methyl, ethyl, butyl, and butylbenzylphthalates, but insignificant loss of octyl and ethylhexylphthalates ⁴.

The proposed pathway for the degradable phthalate esters is by cleavage of a side chains sequentially, forming *o*-phthalate, the ring cleavage by an unknown pathway:



Phthalic acids degraded by decarboxylation through benzoyl-CoA and pimelate as in the following degradation of phthalates by a denitrifying bacterium ⁵



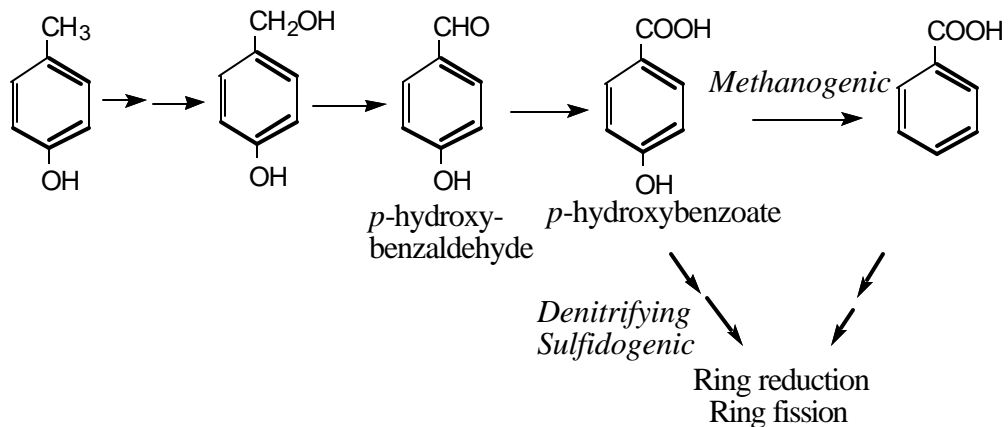
These anaerobic pathways leave hexylethylphthalate untransformed in digested municipal sludge.

Anaerobic degradation of cresols

Para-cresol was degraded rapidly in acclimated sediment systems under denitrifying, sulfate reducing, & methanogenic conditions, but took 3-4 weeks in unacclimated.

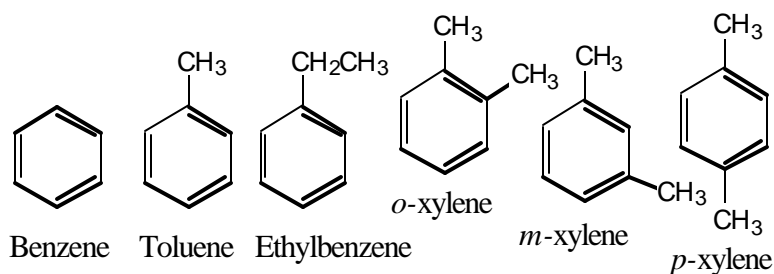
It was suggested that the same initial pathway was used for *p*-cresol metabolism: via oxidation of methyl substituent to *p*-hydroxybenzaldehyde and *p*-hydroxybenzoate, Both of these intermediates degraded rapidly in both acclimated and unacclimated sediments.

Benzoate appeared as an intermediate in methanogenic cultures, but not in denitrifying or sulfate-reducing. ⁶



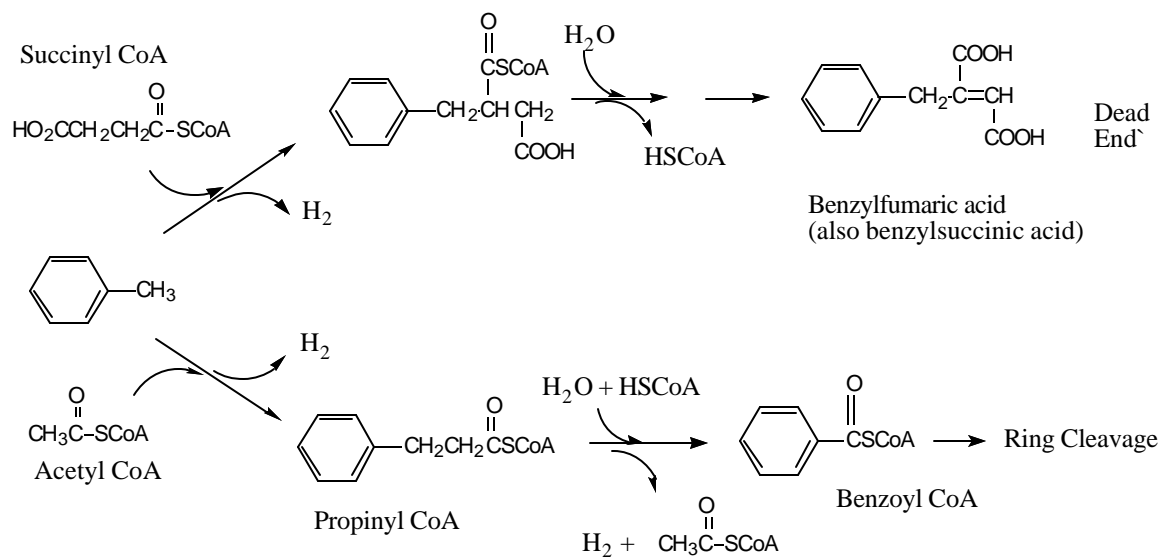
Anaerobic degradation of toluene and xylenes

The BTEX chemicals, benzene, toluene, ethylbenzene and *o*-, *m*-, and *p*-xylene are major contaminants of soils and groundwater, originating mainly from leaking underground gasoline tanks.



Some studies have found evidence for degradation of all of these compounds, but only toluene and *m*- and *o*-xylene have been studied with pure cultures. Both denitrifying (strain T1) and an iron reducing strain (strain GS-15) have been isolated that can grow on toluene and *o*-xylene and can transform *m*-xylene^{7, 8}

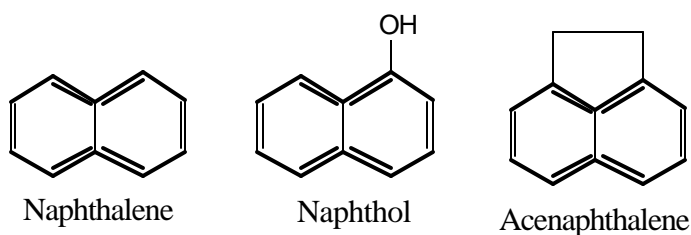
The proposed pathway is through oxidation of the methyl substituent to benzoyl coA through the involvement of acetyl coenzyme A, with concomitant formation of benzylfumaric and benzylsuccinic acid as deadend metabolites involving succinyl coenzyme A, as shown below⁹:



In mixed methanogenic cultures using ^{14}C -labeled toluene, labeled benzene, phenol, and *o*- and *p*-cresol have been observed as well as methylcyclohexane and benzoic acid. Labeled benzene led to recovery of cyclohexene, cyclohexanone and phenol ¹⁰

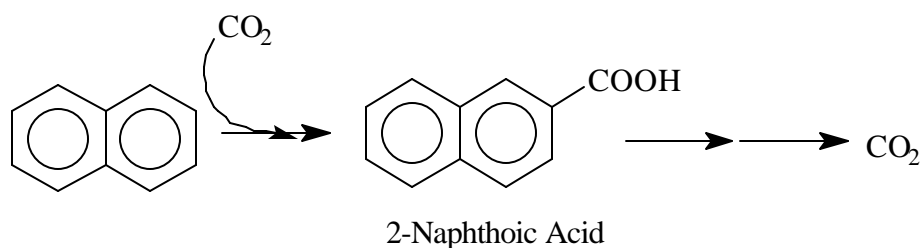
PAH degradation under anaerobic conditions

Degradation of naphthol, naphthalene, and acenaphthalene under denitrification conditions, but not sulfate-reducing conditions, has been observed in freshwater-soil slurries using radiolabeled PAHs and recovery of CO_2 ¹¹



Naphthalene, phenanthrene, and biphenyl were degraded under nitrate- and sulfate-reducing conditions in enrichments from marine sediments ^{12, 13}.

Carboxy-derivatives have been reported to be the first intermediate in the degradation of naphthalene and phenanthrene in a sulfate-reducing enrichment culture ¹⁴, but the activation mechanism is unknown.



Chloroethene mineralization under anaerobic conditions

Vinyl chloride and cis-1,2-dichloroethylene have been reported to be degradable to carbon dioxide under anaerobic conditions with ferric iron, humic acids, sulfate as electron acceptor and with methanogenesis¹⁵⁻²⁰.

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