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University of technology

Building and construction engineering department

AEROBIC AND ANAEROBIC CHEMICAL DEGRADATION OF MICROORGANISMS

A project

submitted to the Department of Building and construction Engineering university of Technology in partial fulfillment of science in civil Engineering

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بسم الله الرحمن الرحيم

قالوا سبحانك لاعلم الا ماعلمتنا

انك انت العليم الحكيم

صدق الله العظيم

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الى من زرعوا على شفاهي البسمات

ورافقوني بأجمل اللحظات ... الى زملائي وزميلاتي

واخيرا... الى كل من علمني حرفا الى اساتذتي مع التقدير

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I want to express my deeb gratitude to my supervisor lecturer Dr. omar najdat for his big support , encouragement, invaluable suggestion, and guidance through this project

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Chapter one

introduction

Aerobic degradation

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Chapter one

1. introduction: Characteristics of Aerobic Microorganisms Capable of Degrading Organic Pollutants

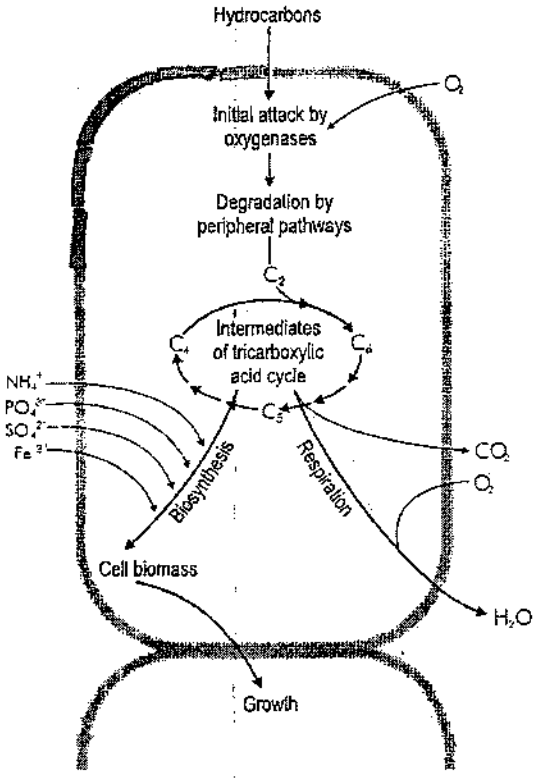
The most important classes of organic pollutants in the environment are mineral oil constituents and halogenated products of petrochemicals. The capacities of aerobic microorganisms are of particular relevance for the biodegradation of such compounds and are described as examples with reference to the degradation of aliphatic and aromatic hydrocarbons as well as their chlorinated derivatives. The most rapid and complete degradation of the majority of pollutants is brought about under aerobic condition.

The following are essential characteristics of aerobic microorganisms degrading organic pollutants (Fig 1.1)

- Metabolic processes for optimizing the contact between the microbial cells and the organic pollutants. The chemicals must be accessible the organisms having biodegradation activities. For example, hydrocarbons are water-insoluble and their degradation requires the production of biosurfactants.
- The initial intracellular attack on organic pollutants is an oxidative process; the activation and incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases.
- Peripheral degradation pathways convert the organic pollutants step by step into intermediates of the central intermediary metabolism, e.g., the tricarboxylic acid cycle.
- Biosynthesis of cell biomass from the central precursor metabolites, e.g., acetyl-coA, succinate, pyruvate. Sugars required for various biosyntheses and growth must be synthesized by gluconeogenesis.

A huge number of bacterial and fungal genera possess the ability to degrade organic pollutants. Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Alexander 1994). It is based on two processes: growth and cometabolism. In growth, an organic pollutants is used as sole source of

Fig 1.1 Main characteristics of aerobic degradation of hydrocarbons: processes associated with growth of microorganisms.



carbon and energy. This process results in a complete degradation (mineralization) of organic pollutants. Cometabolism is defined as the metabolism of an organic compound in the presence of a growth substrate that is used as the primary carbon and energy source.

Key enzymatic reactions of aerobic biodegradation are oxidations catalyzed by oxygenases and peroxidases. Oxygenases are oxidoreductases that use O_2 to incorporate oxygen into the substrate. Degradative organisms need oxygen at two metabolic sites the initial attack on the substrate and the end of the respiratory chain (Fig 1.1). Certain higher fungi have developed a unique oxidative system for the degradation of lignin based on extracellular ligninolytic peroxidases and laccases. This enzymatic system possesses increasing significance for the cometabolic degradation of persistent organopollutants. Thus, the basidiomycetous fungi require deeper insights and extensive consideration. Therefor, this chapter is divided into two sections: bacterial and fungal degradation.

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Chapter two

Principles of bacterial degradation

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Chapter 2

2. Principles of Bacterial Degradation

2.1
Typical Aerobic Degrading Bacteria

The predominant degraders of organopollutants in the oxic of contaminated areas are chemo-rganotrophic species, which are able to use a huge number of natural and xenobiotic compounds as carbon sources and as electron donors for the generation of energy. Although many bacteria are able to metabolize organic pollutants, a single bacteria species does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil. Mixed microbial communities have the most powerful biodegradative potential, because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas. The genetic potential and certain environmental factors such as temperature, pH, and available nitrogen and phosphorous sources, therefor, seem to determine the rate and the extend of degradation.

The predominant bacteria in polluted soils belong to a spectrum of genera and species (Table 2.1).The table lists only bacteria that can be cultured on nutrient-rich media. We have to consider that the majority of bacteria present in soils cannot yet be cultivated in the laboratory.

The pseudomonads, aerobic gram-negative rods that never show fermentative activity, seem to have the highest degradative potential, e.g., pseudomonas putida and p.fluorescens. further important degraders of organic pollutants are found within the genera Comamonas, Burkholderia, and Xanthomonas. Some species utilize > 100 different organic compounds as carbon source. The immense potential of the pseudomonads dose not solely depend on the catabolic enzymes, but also on their capability for metabolic regulation (Houghton and shanley 1994).

A second important group of degrading bacteria are the gram-positive rhodococci and corynefrom bacteria. Many species, now classified as Rhodococcus spp., were originally described as Nocardia spp., Mycobacterium spp., and Corynebacterium spp.. Rhodococci are aerobic

Table 2.1 Predominant bacteria in soil samples polluted with aliphatic and aromatic hydrocarbons, polycyclic aromatic hydrocarbons snd chlorinated compounds.[a]

Gram-negative bacteria	Gram-positive bacteria
Pseudomonas spp.	Nocardia spp.
Acinetobacter spp.	Mycobacterium spp.
Alcaligenes sp.	Corynebacterium spp.
Flavobacterium/Cytophaga group	Arthrobacter spp.
Xanthomonas spp.	Bacillus spp.

bic actinomycetes that show considerable morphological diversity. A certain group of these bacteria possesses mycolic acids on the external surface of the cell. These compounds are unusual long-chain alcohols and fatty acids, esterified to the pepti- doglycan of the cell wall. These lipophilic cell structures probably are important for the affinity of rhodococci to lipophilic pollutants. In general, rhodococci have high and diverse metabolic activities and can synthesize biosurfactants.

2.2
Growth-associated Degradation of Aliphatics

The aerobic initial attack on aliphatic and cycloaliphatic hydrocarbons requires molecular oxygen. Two types of enzymatic reactions are involved in these processes (Fig 2.1); whether a monooxygenase or dioxygenase reaction occurs depends on the nature of the substrate and the enzymes possessed by the microorganisms. The n-alkanes are the main constituents of mineral oil contaminations. Long-chain n-al-kanes (C_{10}-C_{24}) are degraded most rapidly by the pathways shown in figure 2.2 short-chain alkanes (less than C_9) are toxic to many microorganisms, but they evap-

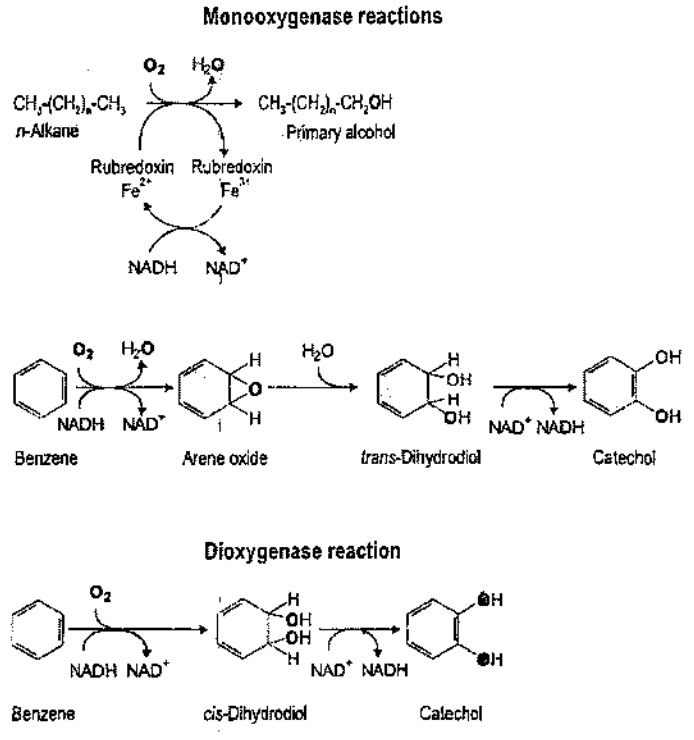


Fig. 2.1 initial attack on xenobiotics by oxygenases. Monooxygenases incorporate one atom of dioxygen (O_2) into the substrate, the second oxygen atom is reduced to H_2O by means of reduction equivalents. Dioxygenases incorporate both atoms of O_2 into the substrate.

branched chains, e.g., the tertiary butyl group, hinder the action of the degradative enzymes.

Cyclic alknes represent minor components of mineral oil and are relatively resistant to microbial attack. The absence of an exposed terminal methyl group complicates the primary attack. A few species can use cyclohexane as a sole carbon source, but it is more commonly cometabolized by mixed cultures. The pathway of cyclohexane degradation is shown in Figure 2.3. In general, the presence of alkyl sidechains on cycloalkanes facilitates their degradation.

Aliphatic hydrocarbons become less water soluble with increasing chain length; those with a chain length of C_{12} or more are virtually water-insoluble.Two mechanisms are involved in microbial uptake of these lipophilic substrates: attachment of microbial cells to oil droplets and production of biosurfactants. The uptake mechanism linked to attachment of the cells is still unknown, but the effect of biosurfac-

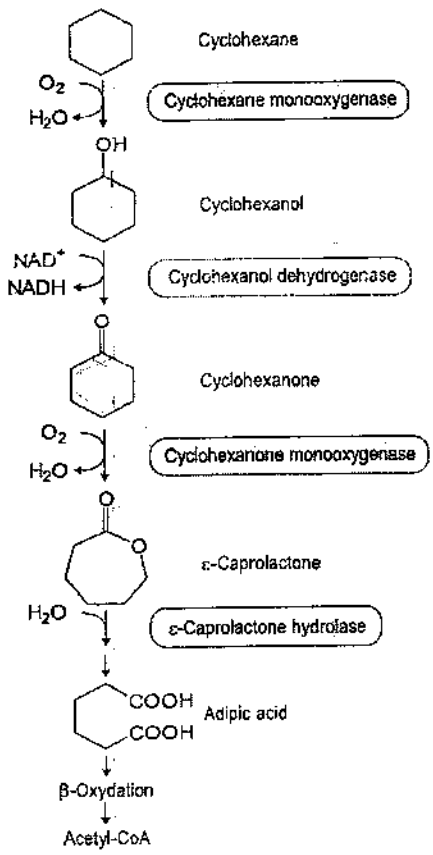
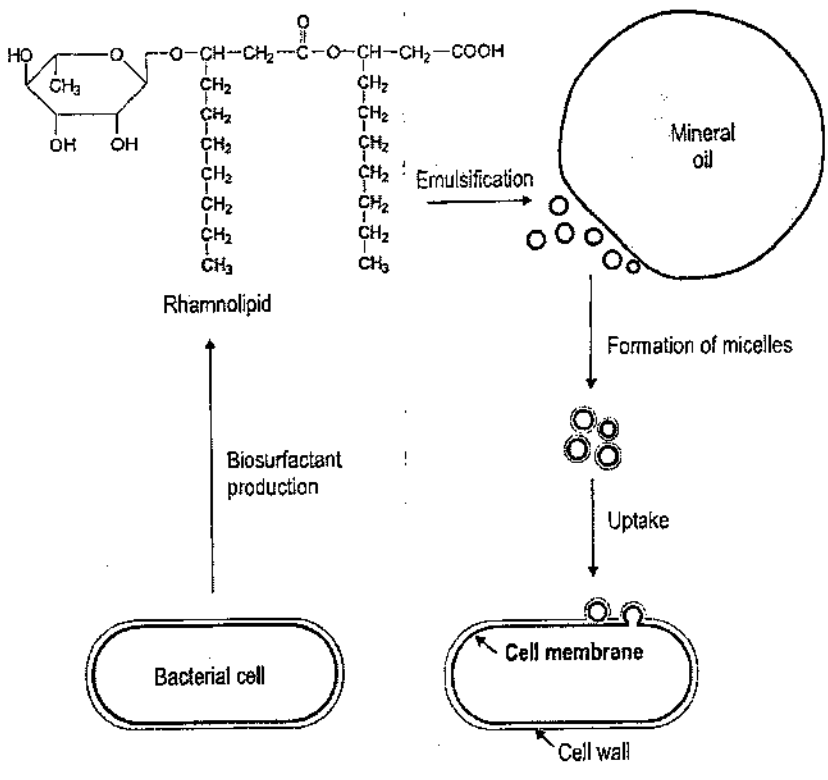


Fig.2.3 Metabolic pathways for degradation of cycloaliphatic compounds (cyclopar affins).

tants has been studied well (Fig2.4). Biosurfactants are molecules consisting of a hydrophilic and a lipohilic moiety. They act as emulsifying agents by decreasing the surface tension and by forming micelles. The latter can be encapsulated by the hydrophobcic microbial cell surface.

The products of hydrocarbon degradation that are fed into the central tricarboxylic acid cycle have a dual function: as substrates of energy metabolism and building blocks for the biosynthesis of cell biomass (Fig1.1). Synthesis of amino acids and proteins requires nitrogen and sulfur sources, that of nucleotides and nucleic acids a phosphorous source. Biosynthesis of the bacterial cell wall requires activated sugars synthesized by gluconeogenesis. The cells act as complex biocatalysts of degradation.

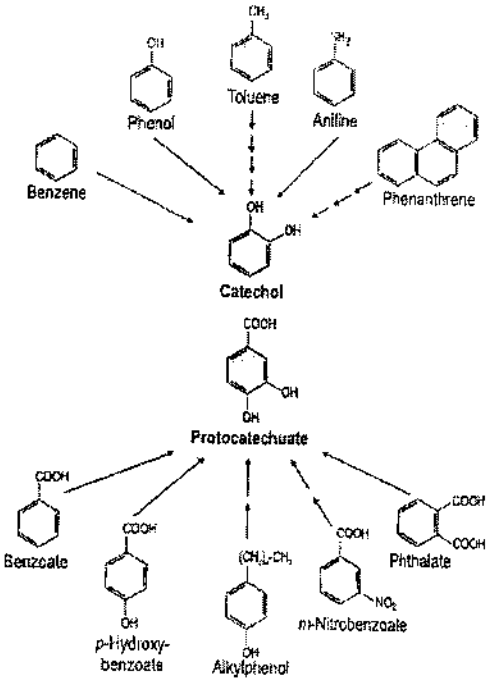
Products of growth-associated degradation are CO_2, H_2O, and cell biomass. The cell biomass can be mineralized after exhaustion of degradable pollutants in a contaminated site.



2.4 Involement of biosurfactants in the uptake of hydrocarbons. The emulsifying effect of a rhamnolipid produced by pseudomonas spp. Within the oil-water interphase and the formation of micelles are shown. Lipid phases are printed in bold.

2.3
Diversity of Aromatic Compounds: Unity of Catabolic processes

Aromatic hydrocarbons, e.g., benzene, toluene, ethylbenzene and xylenes (BTEX compounds), and naphthalene, belong to the large-volume petrochemicals, which are widely used as fuels and industrial solvents. Phenols and chlorophenols are released into the environment as products and waste metrials from industry. Aromatic compounds are formed in large amounts by all organisms, e.g., as aromatic amino acids, phenols, or hydroquinones / quinines. Thus, it is not surprising that many microorganisms have evolved catabolic pathways to degrade aromitc compounds, in general, man-made chemicals (xenobiotics) can be degraded by microorganisms when they are similar to natural compounds. The range of man-made aro-matic shown in Figure 2.5 can be converted enzymatically to the natural intermediates of degradation: catechol and protecatecatechuate. In general, benzene related compounds are characterized by greater thermodynamic stability than aliphatics are. The step in benzene oxidation is a hydroxylation that is catalyzed by a dioxygenase (Fig. 2.1). the product, a diol, is then converted to catechol by dehdrogenase. These initial reaction, hydroxylation and dehydrobenation, are also common



2.5 Degradation of a broad spectrum of natural snd xenobiotic aromatic compounds into two central intermediates: catechol and protocatechuate.

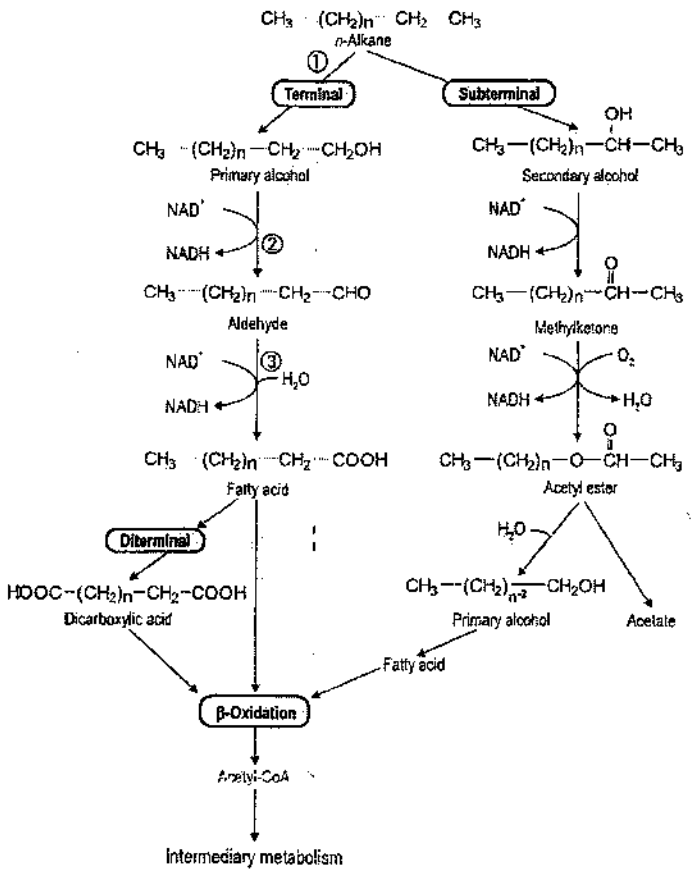
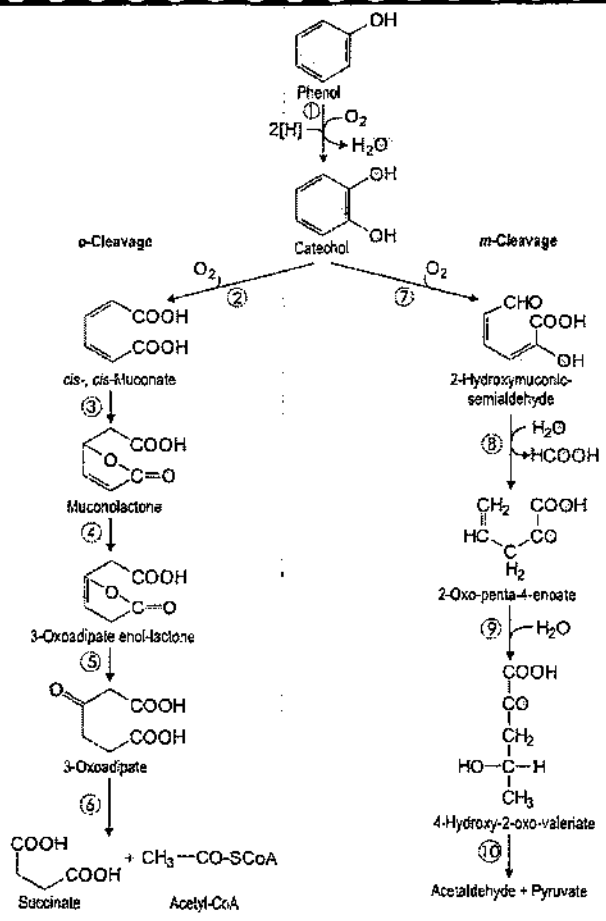


Fig. 2.2 pathways of alkane degradation. The main pathway is their terminal oxidation to fatty acids catalyzed by (1)n-alkane monooxygenase, (2) alcohol dehydrogenase, and (3) aldehyde dehydrogenase.

Orate rapidly from petroleum-contaminated sites. Oxidation of alkanes is classified as terminal or diterminal. Monoterminal oxidation is the main pathway and proceeds by formation of the corresponding alcohol, aldehyde, and fatty acid. Beta oxidation of fatty acids results in the formation of acetyl-CoA. n-alkanes having an odd number of carbon atoms are degraded to propionyl-CoA, which is in turn carboxylated to methylmalonyl-CoA and further converted to succinyl-CoA. Fatty acids having a physiological chain length may be directly incorporated in to membrane lipids, but most degradation products are fed in to the tricarboxylic acid cycle. Subterminal oxidation occurs with lower (C_3-C_6) and longer alkanes, with formation of a secondary alcohol and subsequently of a ketone. Unsaturated 1-alkenes are oxidized at the saturated end of the chains. A minor pathway proceeds via an epoxide, which is converted to a fatty acid. Branching generally reduces the rate of biodegradation . methyl side groups do not noticeably decrease the biodegradability, where complex



2.6 The two alternative pathways for aerobic degradation of aromatic compounds: ortho and meta cleavage. (1) phenol monooxygenase, (2) catechol 1,2-dioxygenase, (3) muconate-lactonizing enzyme, (4) muconolactone isomerase, (5) oxoadipate enol-lactone hydrolase, (6) oxoadipate succinyl-coa transferase, (7) catechol 2,3-dioxygenase, (8) hydroxymuconic semialdehyde hydrolase, (9) 2-oxopent-4-enoic acid hydrolase, (10) 4-hydroxy-2-oxovalerate aldolase.

Rhodococci, to adapt their metabolism to the effective utilization of substrate mixtures in polluted soils and to grow at a high rate.

2.4

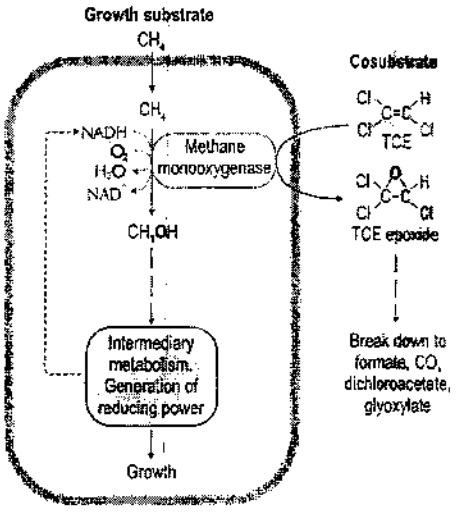
Extension of Degradative Capacities

2.4.1 Cometabolic Degradation of Organopollutants

Cometabolism, the transformtiom of a substance without nutritional benefit in the presence of a growth substrate, is a common phenomenon in microorganisms. It is the basis of biotransformtions (bioconversions) used in biotechnology to convert a

substance to a chemically modified form. Microorganisms growing on a particular substrate can gratuitously oxidize a second substrate (cosubstrate). The cosubstrate is not assimilated, but the product can be available as a substrate for other organisms in a mixed culture.

The prerequisites for cometabolic transformations are the enzymes of the growing cells and the synthesis of cofactors necessary for enzymatic reactions, e.g., of hydrogen donors (reducing equivalents, NAD(P)H) for oxygenases. For example, methanotrophic bacteria can use methane or other C1 compounds as a sole source of carbon and energy. They oxidize methane to CO2 via methanol, formaldehyde, and formate. The assimilation requires special pathways, and formaldehyde is the intermediate that is assimilated. The first step in methane oxidation is catalyzed by methane monooxygenase, which attacks the inert CH4 (Fig.2.7). Methane monooxygenase is unspecific and also oxidizes various other compounds, e.g., alkanes, aromatic compounds, and trichloroethylene (TCE). The proposed mechanism of TCE transformation according to Henry and Grbic-Gallic (1994) is shown in Figure2.7. TCE is oxidized to an epoxide that is excreted from the cell. The unstable oxidation product breaks down to compounds that can be used by other microorganisms. Methanotrophic bacteria are indigenous aerobic bacteria in soils and aquifers, but methane has to be added as growth substrate and inducer for the development of methanotrophic biomass, which limits their usefulness in bioremediation. Cometabolism of chloroaromatics is a widespread activity of bacteria in mixtures of industrial pollutants. Knackmuss (1997) demonstrated that the cometabolic transformation of 2-chlorophenol gives rise to dead-end metabolites, e.g., 3-chlorocatechol. This reaction product can be autoxidized or polymerized in soils to humic-like



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Chapter three

Principle of an aeropic degradation

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Chapter three

3. principles of Anaerobic Degradation of Organic Compounds

Bernhard Schink

3.1

General Aspects of Anaerobic Degradation Processes

The vast majority of organic compounds produced in nature or through human manufacture is degraded aerobically,with molecular oxygen as terminal electron acceptor.As long as oxygen is available, it is the preferred electron acceptor for microbial degradation processes in nature.

Anaerobic degradation processes have always been considered inferior to aerobic degradation in their kinetics and capacities.They are thought to be slow and inefficient, especially with certain comparably stable types of substrates. Nonetheless, in certain anoxic environments, such as the cow`s rumen, the turnover of, e.g.,cellulose is much faster than in the presence of oxygen, with average half-life times in the range of one day. Fermentative degradation of fibers in the rumen reaches its limit with plant tissues rich in lignin which largely withstands degradation in the absence of oxygen.

Also in waste treatment, especially with high loads of easy-to-deganic materil , anaerobic processes have proved to be efficient and far less expensive than aerobic treatment: they require only small amounts of energy input, in contrast to treatment in aeration basins, and can produce a mixture and co_2 (bio-gas`), which can be used efficiently for energy generation. This holds true for most waste materials that are easily accessible to degradation without the participation of oxygen, such as polysaccharides, proteins, fats, nucleic acids, etc. These polymers are hydrolyzed through specific extracellular enzymes, and the oligo- and monomers can be degraded inside the cell through enzyme reactions similar to those known in aerobic metabolism. The specific activities of such enzymes in anaerobic cultures are in the same range

(0.1-1 umol substrate per min and mg cell protein) as those of aerobic bacteria, and thus the transformation rates per unit biomass should be equivalent. Nonetheless, anaerobic bacteria obtain far less energy from substrate turnover than their aerobic counterparts. Whereas aerobic oxidation of hexose to six CO_2 yields 2870 kj per mol, dismutation of hexose to three CH_4 and three CO_2 yields

only 390 KJ per mol, about 15% of the aerobic process, and this small amount of energy has to be shared by at least three different metabolic groups of bacteria (see Schink, 1997). As a consequence, they can produce far less biomass per substrate molecule than aerobes can. Their growth yields are low, and most often growth is slower than that of aerobes. Maintaining the biomass inside specifically designed reastors (fixed bed, fluidized bed reactors, Upflow Anaerobic Sludge Blanket reactors) helps to overcome the problem of low and slow biomass production in anaerobic degradation and largely uncouples substrate turnover from biomass growth . These systems allow anaerobic wastewater treatment to be nearly as efficient as and less expensive than the aerobic process, with methane as a useful product; but the microbial communities in these advanced an-aerobic reactors still are compaeably sluggish in reacting to changes in substrate composition or in their reestablishment after accidental population losses due to toxic ingredients in the feeding waste.

Degradation of organic matter in the absence of oxygen can be coupled to the reduction of alternative electron acceptors following a certain sequence that appears to be determined by the respective redox potentials. Molecular oxygen (O_2/H_2O E_h= +810 mv; E_h values calculated for pH 7.0) is followed by nitrate (NO_3^-/NO_2^- E_h=+430 mV), Manganese (**IV**) oxide (MnO_2/Mn^{2+} E_h= +400mv), iron(**III**) hydroxides ($FeOOH/Fe^{2+}$ E_h= +150 mv), sulfate (SO_4^{2-}/HS^- E_h = -218 mv), and finally CO_2 (CO_2/CH_4 E_h = -244 mv), with the release of nitrite, ammonia, dinitrogen, manganese(II) and iron (II) carbonates, sulphides, and finally methane as products. Reduction of these acceptors with electrons from organic matter (average redox potential for glucose → 6 CO_2 is -0.434 v; calculated after data of thauer et al..1977) provides metabolic energy in the mentioned sequence. Thus, the energy yields of the various anaerobes mentioned
Differ, and the availability of either high- or low- potential electron acceptors may also influence the biochemistry of an-aerobic degradation processes.

Limits of anaerobic degradation become obvious with those organic compounds that accumulate in anoxic sediments or that persist in anoxic soil compartments contaminated with mineral oil or other rather recalcitrant compounds. Mineral oil consists mainly of aliphatic and aromatic hydrocarbons which, in the presence of molecular oxygen, are attacked biochemically through oxygenise reactions, which introduce molecular oxygen into the respective molecule (Lengeler et al.. 1999; see also Chapter 2). Oxygenise reactions

cannot be employed in the absence of oxygen, and, in particular, compounds that require oxygenases for aerobic breakdown might resist degradation under anoxic conditions. Alternatives usually exist in the anoxic world that also allow oxygen-independent degradation of such compounds.

Oxygen is not always advantageous in degradation processes. Oxygenises introduce hydroxyl groups into aromatics, and further oxygen may cause formation of of phenol radical that initiate un controlled polymerization and condensation to polymeric derivative , similar to humic compounds in soil, which are very difficult to degrade further , whether anaerobically or aerobically . therefore , anaerobic degradation processes may be used for treatment of specific wastewater rich in phenolic compounds ,e.g. , from the chemical industry , to avoid formation of unwanted side products such as condensed polyphenols . In other situations , aerobic treatment may cause technical problems, e.g., by extensive foam formation during aerobic treatment of surface-active compounds such as ten sides. Thus , knowledge of limitsand principles of anaerobic degradation processes under the various condition prevailing in natural habitats might help to design suitable alternative techniques for cleanup of contaminated soils or for treatment of specific wastewater that have so far been applied only insufficiently .

The following survey given an overview of our present knowledge of the limits and principles of anaerobic degradation of organic compounds . The focus is on those compounds that were for long times considered to be stable in the absence of oxygen.

3.2

Key Reactions in Anaerobic Degradation of Certain Organic compounds

3.2.1

Degradation of Hydrocarbons

Saturated aliphatic hydrocarbons are attacked only slowly in the absence of oxygen and the 1st reliable proof of such a process was provided only about eight years ago for a culture of sulfate-reducing bacteria . Growth of this culture with hexadence was very slow , with doubling times of more than one week under optimal conditions . In the mean time several strains of alkane-oxidizing anaerobic were isolated (Aeckersberg at al.,1998) which are specialized for either long - chain ($C_{12} - C_{20}$) or medium – chain ($C_6 - _{16}$) alkanes and use either sulfate or nitrate as electron acceptor.

Insight into the biochemistry of alkanes activation in the absence of oxygen has been obtained only recently . The initial Oxidation is basically similar to the

corresponding reaction involved in anaerobic oxidation of toluene (see Section 8.2.6.8): the hydrocarbon is added with its subterminal carbon atom to fumarate through a radical reaction , to form an alkyl succinate derivative . This strategy is used in a basically similar manner by nitrate – redusing and sulfate – redusing bactiria . in either In either case , anaerobic hydrocarbon degradation is very slow , but may play a role in e.g, natural attenuation of soil sites polluted with petroleum or diesel fuel.

A special example, although not of technical interest, is the anaerobic degradation of methane , e.g., with sulfate as electron acceptor, a process that is of major importance in global carbon transformations . No bacterium that catalyzes this reaction has been isolated so far , although it is thermodynamically feasible (see Schink, 1997). Recent evidence has shown that this reaction is most probably carried out by archaea similar to methanogens, which operate methane formation in the back-wards reaction, most often in syntrophic association with sulfate-reducing partner organisms. Although this concept was suggested many years a go, experimental evidence has been obtained only recently with samples from deep-sea sources, and efficient methane oxidation requires enhanced methane pressure (Nauhaus et al., 2002) .

Unsaturated long-chain hydrocarbons with terminal bonds can be hydrated to the corresponding primary alcohols (although against the Markownikoff rule) and completely degraded . A branched-chain unsaturated hydrocarbon sush as squalene was degraded in methanogenic enrichment cultures however, degradation was incomplete, probably due to the formation of Saturated branched derivatives . Other unsaturated isoprene derivatives such as terpenes have been shown recently to be completely degraded, with nitrate as electron acceptor. Al-though the structures of terpenes differ substantially with respect to the way of possible attack, an amazingly broad variety of terpenes was completely degraded. Some concepts of the biochemistry of degradation of these compounds have been developed, but experimental evidence is still lacking. Acetylene, a highly unsaturated hydrocarbon, is fermented comparably rapidly to acetate and ethanol through acetaldehyde, which is formed by a hydratase enzyme. Acetylene hydratase is an iron-sulfur protein containing a tungsten cofactor (Meckenstock et al., 1999); it is active only in the reduced state, but the reaction mechanism is still unknown. No anaerobic degradation has been documented so far for ethylene, propylene, propane, and higher homologs having up to six carbon atoms.

3.2.2
Degradation of Ether Compounds and Nonionic Surfactants

Ether linkages are rather stable, and their chemical cleavage requires severe conditions, e.g., boiling at strongly alkaline or acidic ph. Biological ether cleavage in the presence of oxygen employs oxygen as cosubstrate in an oxygenase reaction, which transforms the ether into an unstable hemiacetal. Thus, methyl groups of lignin monomers are released as formaldehyde, not as methanol.

Anaerobic demethylation of lignin monomers by the homoacetogen Acetobacterium woodii was first described by Bache and pfennig(1981) and later was repeatedly observed with several other homoacetogens. The mechanism of this phenyl methyl ether cleavage other were only recently elucidated. Studies with the homoacetogen Holophaga foetida showed that the methyl group is first transferred as a methyl cation to a fully reduced cod(I) alamin carrier, which later methylates the coenzyme Tetrahy-drofolate (Fig.3). Similar studies with Acetobacterium woodii, Sporomusa ovate, or Acetobacterium dehalogenans revealed that also in these species, the methyl group is transferred as a methyl cation, but that the details of further methyl transfer to coenzymes may differ with the strain studied.

A different type of anaerobic ether clravage was observed polyether polyether polyethylene glycol (PEG). Formation of acetaldehyde as the first cleavage product, extreme oxygen sensitivity of the ether-cleaving enzyme in cell-free extracts, and interference with cobalamins strongly

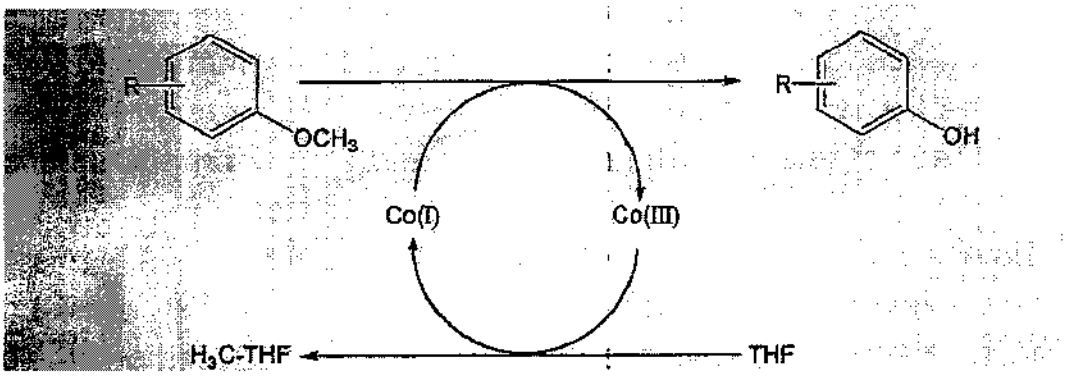

Fig 3.1 anaerobic demythlation of phenyl methy ether. Co(I), Co(III), cobalamin in different redox states; THF, tetrahydrofolate

Suggest that the first step in this degradation is a cobalamin-dependent shift of the terminal hydroxyl group to the sub terminal c atom analogous to a diol dhydratase reaction this reaction again transforms the ether into a hemiacetal derivative that decomposes spontaneously. Since the ether cleaving enzyme is located in the cytoplasmic space, the polymer PEG(which has molecular mass up to 40 kDa) has to cross the cell memberane(s) before its cleaved inside, and the same is true for PEG-containing non-ionic surfactants. Since the bacterial strains studied so far are specialized only for the degradation of the PEG chain, the lipophilic residues of the surfactants have to cross the membrane(s) again on their way back

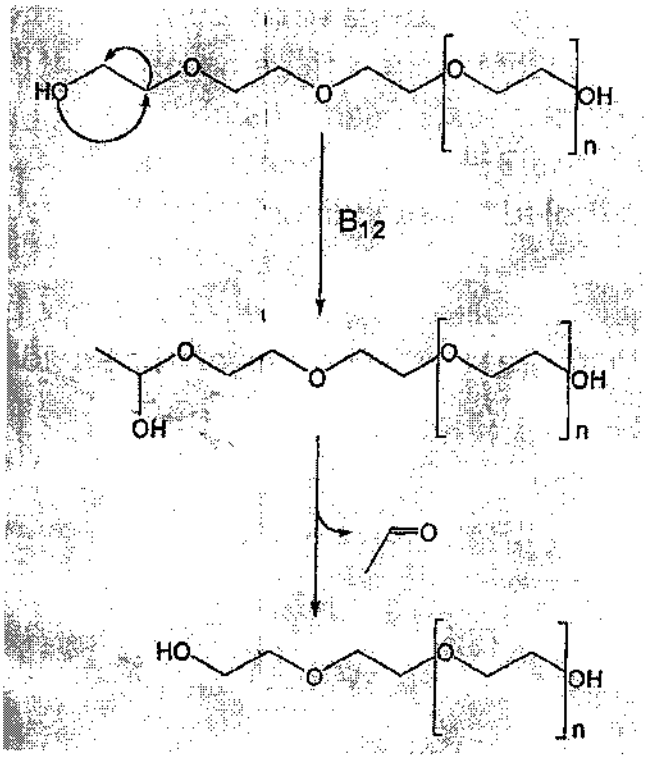

Fig 3.2 Anaerobic degradation of polyethylene glycol by fermemting bacteria.B_{12}, coenzyme B_{12}

Out.It is obvious that these transport steps considerably limit the applicability of such degradation capacities to treatment of,e.g., wastewaters rich in such surface-tants .Nonetheiess, the applicability of anaerobic fixed-bed reactors for treatment of nonionic surfactants of various types to methane, CO_2 , and mixtures of fatty acids has been demonstrated (Wagner and Schink, 1987).

The anaerobic ether cleavage reactions described here proceed only inside the bacterial cell. In this, they differ from the corresponding reactions reported for certain basidiomycetes, which use these cleavage capacities, e.g., in lignin degradation. It is not surprising therefore, that highly polymeric, condensed ether compounds such as lignin are not degraded to any significant extent in the absence of molecular oxygen.

3.2.3
Degradation of N-Alkyl Compounds and Nitrilotriacetate

Among the natural N-alkyl compounds are, in addition to the amino acids, several mentholated amines such as trimethylamine, which is formed during the initial decay of fish tissue through reduction of trimethylamine-N-oxide by several enterobacteria and others. Under strictly anoxic conditions, methanogenic archaea have been found to efficiently demethylate trimethylamine via dimethylamine to monomethylamine and to ferment the methyl moieties by dismutation to methane and CO_2. The cleavge between the nitrogen and the neighboring carbon atom is accomplished by a nucleophilic attack by a cob(I) alamin , analogous to the demethylation of phenyl methyl ethers by homoacetogenic bacteria (see above). So far, only methanogens have been found to demethylate methylamines, whereas ho_moacetogens appear to be specialists for demethylation of phenyl methl ethers which, in turn,are not attacked by methanogens . It should be emphasized at this point that the strategy of methyl cation removal by cob(I)alamin derivatives is applicable only to these one-carbon compounds. Ethyl or higher homologs cannot be cleaved this way.

N-alkyl compounds of technical and environmental concern include ethylenedi-aminetetraacetate (EDTA) and nitrilotriacetate (NTA); the latter has largely replaced polyphosphates as a calcium chelator in most Commercial washing detergents. The main problem in degrading EDTA is the formation of strong complexes of EDTA with metal ions, which make this

substrate very difficult to attack. Nonetheless, microbial EDTA degradation in the presence of oxygen has been documented (-Nortemamnn, 1992), but no reports exist on possible anaerobic degradation of EDTA. NTA is degraded aerobically through oxygenase-dependenthydroxylation of one methylene carbon. The resulting hydroxyl compound is unstable and releases glyoxylic acid. Removal of an additional carboxmethylene residue produces glycine as coproduct. Anaerobic degradation of NTA is oossible with nitrate as electron acceptor. The first degradation step is dehydrogenation to an unsaturated iminium derivative which, upon hydration , can again release glyoxylic acid to formthe iminodiacetate derivative (Egli et al.,1990). Since oxidation of NTA to the unsaturated derivative has a rather high redox potential (>+100 m V), it is not surprising that so far , only nitrate reducers have been found to be able to degrade NTA. We have to assume that , under strictly reducing condition , i.e., in deeper sediment layers, NTA is stable to microbial attack , because neither sulfate-reducing nor fermenting bacteria can release electrons arising at this high redox potential.

3.2.4

Degradation of S- Alkyl Compounds

Dimethlysulfoniopropionate is in osmoprotecant found in several green algae and seaweeds. Its cleavage by anaerobic bacteria leads to acrylate and dimethylsulfide, which can escape into the atmosphere. The thioether dimethylsulfide can also be degraded anaerobically by methanogenic archaea. The carbon – sulfur linkage is cleaved by methyl cation removal, analogous to the demethylation reactions described above.

3.2.5

Degradation of Ketones

Aerobic degradation of ketones appeared to be well established. Indications of an oxygenase – catalyzed hydroxylation of acetone to acetol by aerobic bacteria were provided early, but this type of reaction was never confirmed unequivocally. Anaerobic degradation of acetone by nitrate –reducing, sulfate – reducing, or fermenting bacteria cooperating with methanogenic partners uses a carboxylation reaction as primary step of activation , leading to an acetoacetyl derivative that undergoes subsequent cleavage to two acetate moieties. Unfortunately , the primary carboxylation reaction was never connivcingly proved with these cultures. Very little acetone caboxylation activity was observed in enzyme assays (platen and Schink,1991) or in radiotracer experiments using suspensions of intact cells. Acetone carboxylation activity in the phototrophic

anaerobe *Rhodobacter capsulatus* was also very weak in vitro. An acetone – carboxylating enzyme complex of high activity has been found in an aerobic *Xanthobacter* strain (sluis et al.,1996) and was purified and characterized. The reaction requires as energy source for the acetone carboxylation reaction one ATP, which is hydrolyzed to AMP plus

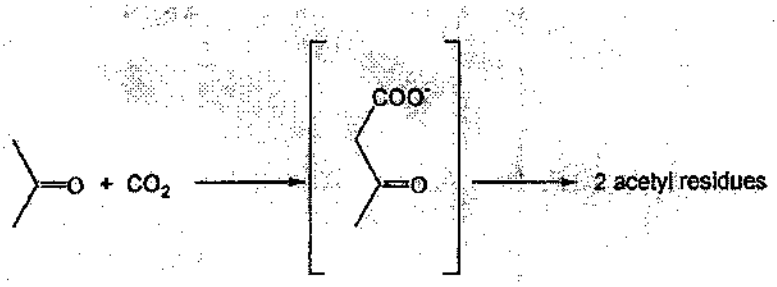

Fig 3.3 Anaerobic degradation of acetone through carboxylation.

two Pi. Whether sulfate-reducing or fermenting bacteria provide the necessary energy
for carboxylation in different ways still has to be examined. The fermenting bacteria
especially cannot afford to spend the equivalent of two ATP molecules on this
carboxylation reaction. To our surprise, all aerobic bacteria enriched with acetone also
used a carboxylation step rather than an oxygenase reaction in acetone activation,
even if CO2 was trapped during the enrichment process (Schink, unpublished data).
Thus, the original reports of oxygenase-dependent acetone activation may describe
an exceptional situation with one single strain, which is not representative of the
majority of aerobic acetone degraders.
Higher homologs of acetone appear to be attacked in a similar manner by carboxylations.

This applies also to acetophenone, the phenyl-substituted analog of acetone
(Schink, unpublished data).

3.2.6 Degradation of Aromatic Compounds

In aerobic degradation of aromatic compounds, oxygenases activate the comparably
stable oxygen molecule in such a way as to produce highly electrophilic species,
which add to a comparably inert aromatic compound to form hydroxylated products
such as catechol (). A further oxygenase-dependent step opens the aromatic ring, either between or vicinal to the two hydroxyl groups of catechol,
thus forming an unsaturated, open-chain carboxylic acid which undergoes further
degradation, typically to an acetyl and a succinyl derivative.
That aromatic compounds can also be degraded anaerobically was documented as
early as 1934: a broad variety of mononuclear aromatic compounds, such as benzoate,
phenols, and several lignin monomers, was converted stoichiometrically to
methane and CO2 (). Later these observations were forgotten,
and some textbooks maintained even into the 1980s the dogma that aromatics
can be attacked only with oxygen as cosubstrate. During the 1970s, Evans (1977) developed
the concept that destabilization of the aromatic nucleus in the absence of
oxygen could proceed through a reductive rather than an oxidative reaction. Today
we know at least three different pathways of anaerobic degradation of mononuclear

compounds, i.e., the benzoyl-CoA pathway, the resorcinol pathway, and the phloroglucinol pathway; Schink et al., 1992;). In all these pathways, a 1,3-dioxo structure is formed through a reduction step, either inside the ring itself or in combination with a carboxyl coenzyme A moiety. This structure allows a nucleophilic attack on one of the ring ketone carbon atoms and subsequent ring fission. Depending on the aromatic substrate, either a pimelic (C7-dicarboxylic) residue bound to coenzyme A or a partly oxidized caproic (C6-monocarboxylic) acid is formed, which undergoes subsequent beta oxidation to three acetyl moieties. Formation of other products in fermentative benzoate degradation, such as succinate or propionate as claimed in earlier papers, could never be reproduced with defined cultures and may have been due to uncontrolled side reactions or misinterpretations of insufficient chemical analyses.

3.2.6.1 Benzoate and the Benzoyl-CoA pathway

The benzoyl-CoA pathway appears to be the most important one in anaerobic degradation of aromatics, because a board varity of compounds enter this path, including phenol, various hydroxyl hydro benzoates, phenyl acetate, aniline, certain cresols, and even the hydrocarbon toluene(fig 3.4: Schink et al.). Once benzoyl-CoA is formed, the stability of aromatic ring structure is overcome by a reductive step that introduces two single electrons and protons, through radical intermediates, to form cyclohedroxyadiene carboxyl-CoA as the first identifiable product(fig 3.5: Koch et al., 1993). Becauce reduction of the benzene ring to a cyclohexadiene derivative, even with electrons at the ferredoxin level is

ender gonic, it requires the investment of energy in the form of two ATP molecules . Nitrate-reducing bacteria can recover this energy investment through the further breakdown of the C7-dicarboxylic acid derivative produced upon ring cleavage, via beta oxidation to three acetyle-CoA residues that are finally oxidized in the citric acid cycle. Fermenting bacteria and sulfate-reducing bacteria recover only a little energy in the further breakdown of the open-chain intermediate. They may use a different type of reaction for benzoyl-CoA dearomatization that leads to a cyclohexene carboxyl derivative or to a hydroxylated derivative through hydroxyl hydroxylation catalyzed by selenium- or molybdenum-containing enzymes. However, the biochemistry of these new reactions still needs to be elucidated.

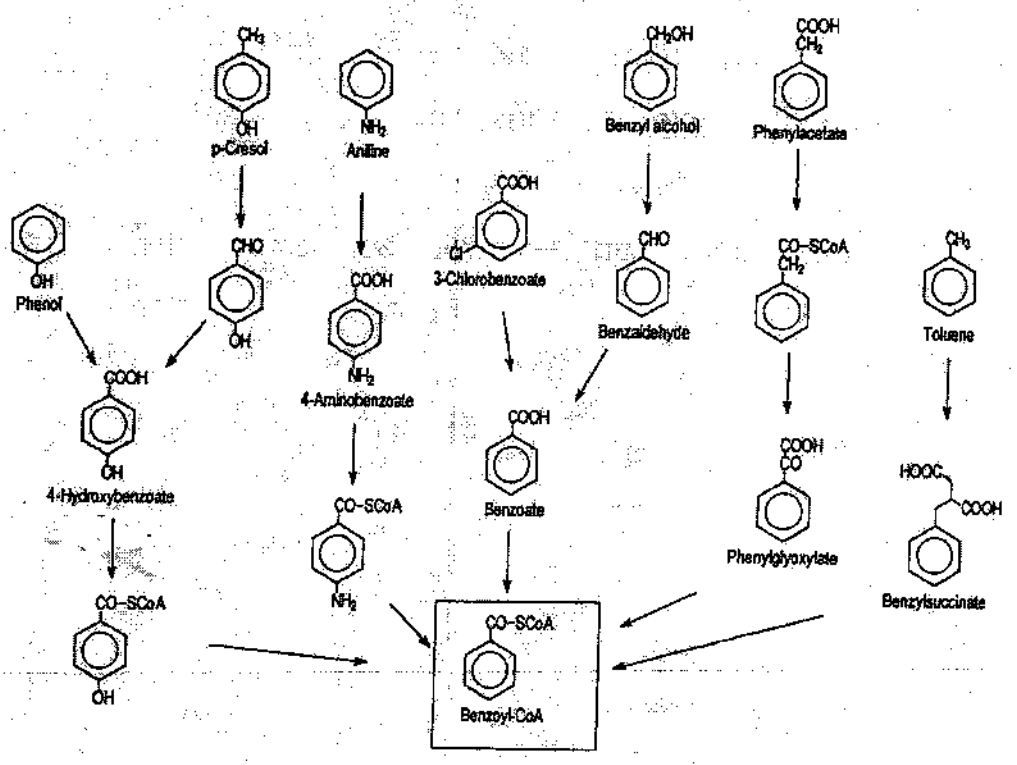

fig 3.4 Overview of mononuclear compounds entering the benzoyl-CoA pathway of anaerobic degradation(courtesy of prof. G, Fuchs, Freiburg).

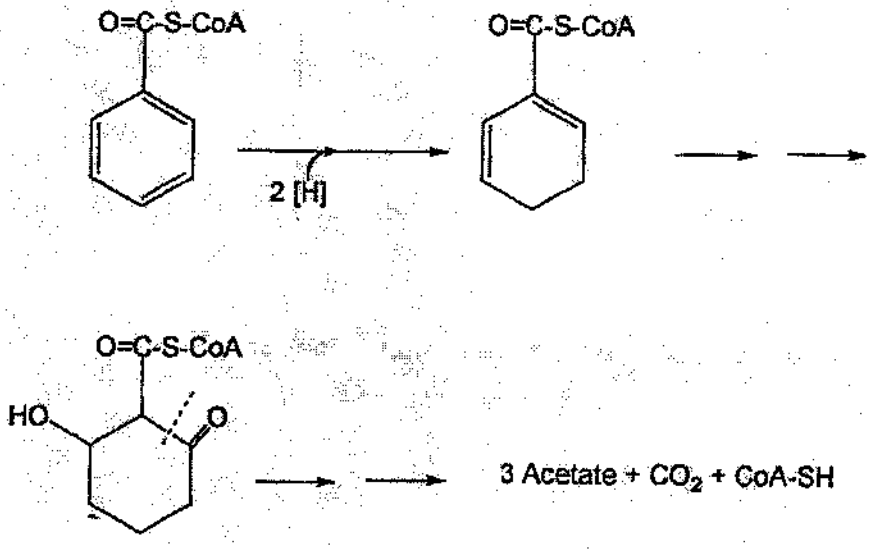

fig 3.5 Initial steps in anaerobic degradation of benzoate by the nitrate-reducing bacterium Thauera aromatica.

3.2.6.2 Phenol, Hydroxybenzoates, and Aniline

Aromatic compounds that do not carry a carboxyl group, such as phenol or aniline, are first carboxylated to a p-hydroxy or a p-amino benzoic acid residue, which is subsequently activated with coenzyme A(fig 3.6). The carboxylation of phenol by a nitrate-reducing Thauera aromatica strain can be followed in vitro with phenyl phosphate as substrate, which is carboxylated to 4-hydroxybenzoate and further degraded as such . The phosphate donor for phenol phosphorylation is still unknown (Berinig et al., 2000). Whether sulfate-reducing bacteria or fermenting bacteria cooperating with methanogentic partners use the same pathway for phenol degradation also remains to be examined. An H/D exchange

at carbon atom 4 of phenol by cell suspensions of a methanogenic phenol - degrading enrichment culture indicates that these cultures also activate phenol through carboxylation at the position. Whether the carboxylation reaction in these fermentative bacteria is also initiated by phenol phosphorylation is still an open question. The overall energy budget of fermentative phenol degradation is very tight and hardly allows spending a full ATP equivalent or even more on this carboxylation reaction. The biochemistry of phenol degradation by sulfate-reducing bacteria has not been studied so far, but it is likely to proceed basically through the same pathway.

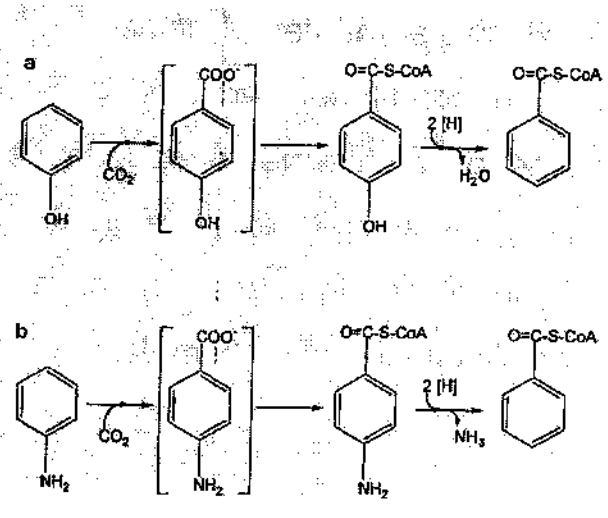

fig 3.6 anaerobic degradation of(a)phenol and (b) aniline by anaerobic bacteria. The compounds in brackets have beebn identified.

The 4-hydroxybenzoate formed is activated through a ligase reaction, analogous to benzoyl-CoA formation from benzoate, to form 4-hydroxybenzoyl-

CoA, which is subsequently reductively dehydroxylated to benzoyl-CoA, which is subsequently reductively dehydroxylated to benzoyl-CoA(fig 3.6a).

4-Hydroxybenzoate may be degraded anaerobically through the same pathway. However, in cultures 4-hydroxybenzoate is not stable but is slowly decarboxylated to phenol, perhaps by an enzyme activity related to phenyl phosphate carboxylase.

3-Hydroxybenzoate is comparably stable and does not decarboxylate spontaneously. Instead, the hydroxyl group is reductively eliminated by fermenting bacteria to allow further degradation through the benzoyl-CoA pathway, as shown with Sporotomaculum hydroxybenzoicum.

Aniline is degraded an aerobically through a pathway analogous to that for phenol degradation. The initial activation is accomplished through carboxylation to 4-aminobenzoate, which is subsequently activated to 4-aminobenzoyl-CoA and undergoes reductive deamination to benzoyl-CoA(fig 3.6b). The initial carboxylation reaction has not been studied in cell-free extracts so far, and nothing is known about an activated intermediate that can provide the carboxylation reaction with the necessary energy.

Amino benzoates, diamino benzenes, and amino hydroxyl benzenes are degraded very slowly in anaerobic enrichment cultures, and nothing is known about the degradation pathways (Schnell and Schink, unpublished data).

3.2.6.3 Cresols

Cresols (methyphenols)are an aerobically degraded through three different pathways, depending on the type of substitution. p-Cresol is hydroxylated at the methyl group by an oxygen-independent reaction, probably through a quinomethide intermediate as suggested earlier for an aerobic Pseudomonas strain(fig 3.7a). The redox potential of this oxidation reaction is in the range of +100 mV, and the reaction is therefore easy to do for a nitrate-reducing bacterium that couples this oxidation, e.g., with the reduction of a c-type cytochrome at+235 mV (Hopper et al., 1991). Sulfate-reducing or fermenting

bacteria, on the other hand, have difficulty in disposing of these electrons. o-Cresol can be carboxylated to 3-methyl-4-hydroxybenzoate and further degraded as such (fig 3.7b). And alternative pathway could lead through methyl ngroup hydroxylation, analogous to p-cresol, to form salicylic acid as intermediate (Schink et al., 1992), but this pathway has only been hypothesized so far. The pathway of anaerobic m-cresol degradation has been elucidated recently in the sulfate-reducing bacterium Desulfobacterium cetonicum. This degradation follows a strategy analogous to anaerobic toluene degradation by nitrate-reducing bacteria: the methyl group of m-cresol adds to fumarate to form 3-hydroxybenzylsuccinate. Activation and beta oxidation lead to

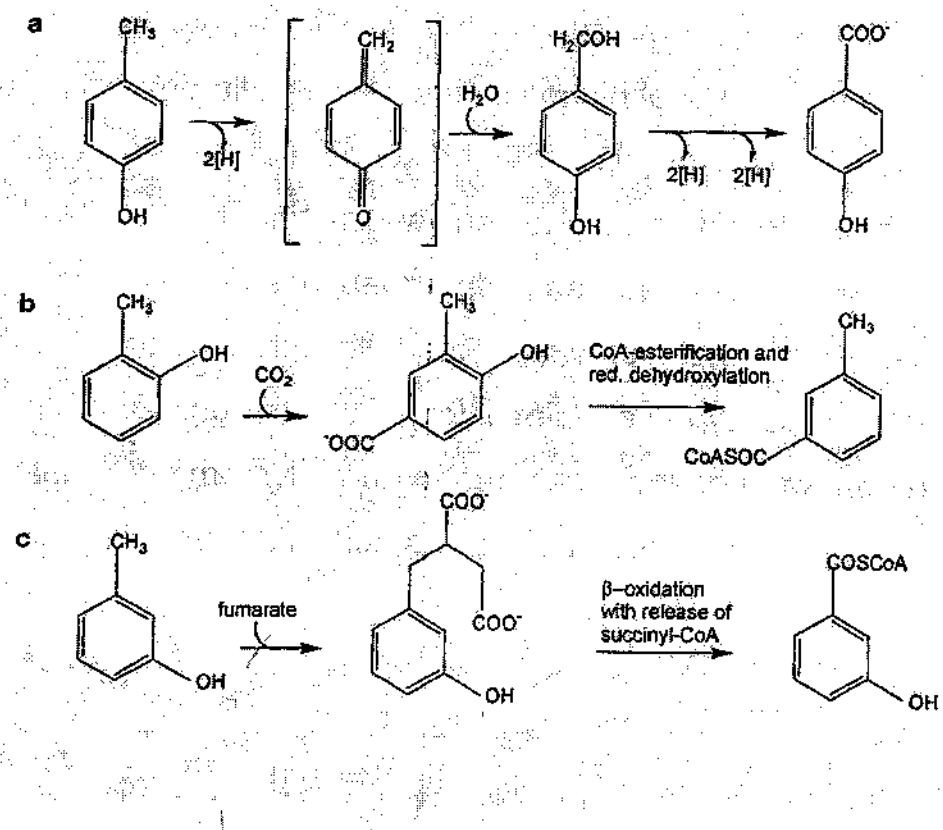

fig 3.7 Anaerobic degradation of cresols. (a)Degradation of p-cresol, (b) degradation of o-cresol,(c) degradation of m-cresol.

succinyl-CoA and benzoyl-CoA(fig 3.7c). Thus, the new type of methyl group activation by addition to fumarate appears not to be restricted to the activation of hydrocarbons (see below).

3.2.6.4 Hydroquinone and Catechol

Hydroquinone is degraded by sulfate-reducing and fermenting bacteria. The degradation pathway has been studied in a sulfate-reducing Desulfococcus strain and a fermenting bacterium that was later described as Syntrophus gentianae. In both species, hydroquinone is carboxylated to gentisic acid: again, this carboxylation could not be studied in cell-free extracts and the way of energization of this reaction is unknown. Gentisate is activated to gentisly-CoA through a CoA-ligase reaction and reductively dehydroxylated to benzoyl-CoA, which enters the modified benzoyl-CoA pathway. The dehydroxylation of both hydroxyl groups appears to proceed in a single step. Alternatively, gentosic acid is utilized by the fermenting bacterium.

Catechol, the key intermediate in aerobic breakdown of aromatic compounds, is by far the slowest phenolic compound to be degraded under anoxic conditions. The biochemistry of catechol degradation has been studied so far only in a sulfate-reducing Desulfobacterium strain, which carboxylates catechol to protocatechuate. Protocatechuate is activated to form protocatechuyl-CoA, which is subsequently dehydroxylated to benzoyl-CoA. Efforts to isolate nitrate-reducing or fermenting bacteria with hydroquinone or catechol as substrate have failed so far.

3.2.6.5 Resorcinol

An entirely different strategy is used in the anaerobic degradation of resorcinol and its derivatives. The two hydroxyl groups in resorcinol are in positions relative to each other that allow tautomerization to a cyclohexene dione derivative having three isolated double bonds(fig 3.8a). Cell-free extracts of a fermenting Clostridium strain convert resorcinol to dihydroresorcinol, which is further hydrolyzed to 5-oxohexanoate, probably through a nucleophilic attack on one of the carbonyl carbon atoms(fig 3.8 a).

The resorcinol carboxylates β- and Y-resorcylate are degraded by the same fermenting bacterium after decarboxylation to resorcinol. These decarboxylations are chemically easy, because in these compounds the carboxylic group is located ortho or para to electron-withdrawing hydroxyl groups.

In cultures of nitrate-reducing bacteria growing with resorcinol as the sole substrate, on resorcinol-reducing activity could be identified(Gorny et al., 1992). The nitrate reducer Azoarus anaerobius destabilizes the ring by introducing an additional hydroxyl group to form hydroxyhydroquinone(fig 3.8b). The enzyme involved is membrane-bound, and the hydroxylation is coupled to reduction of nitrate to nitrite. In a later oxidation step, hydroxyhydroquinone is oxidized to hydroxybenzoquinone. The ring fission reaction has not been resolved yet.

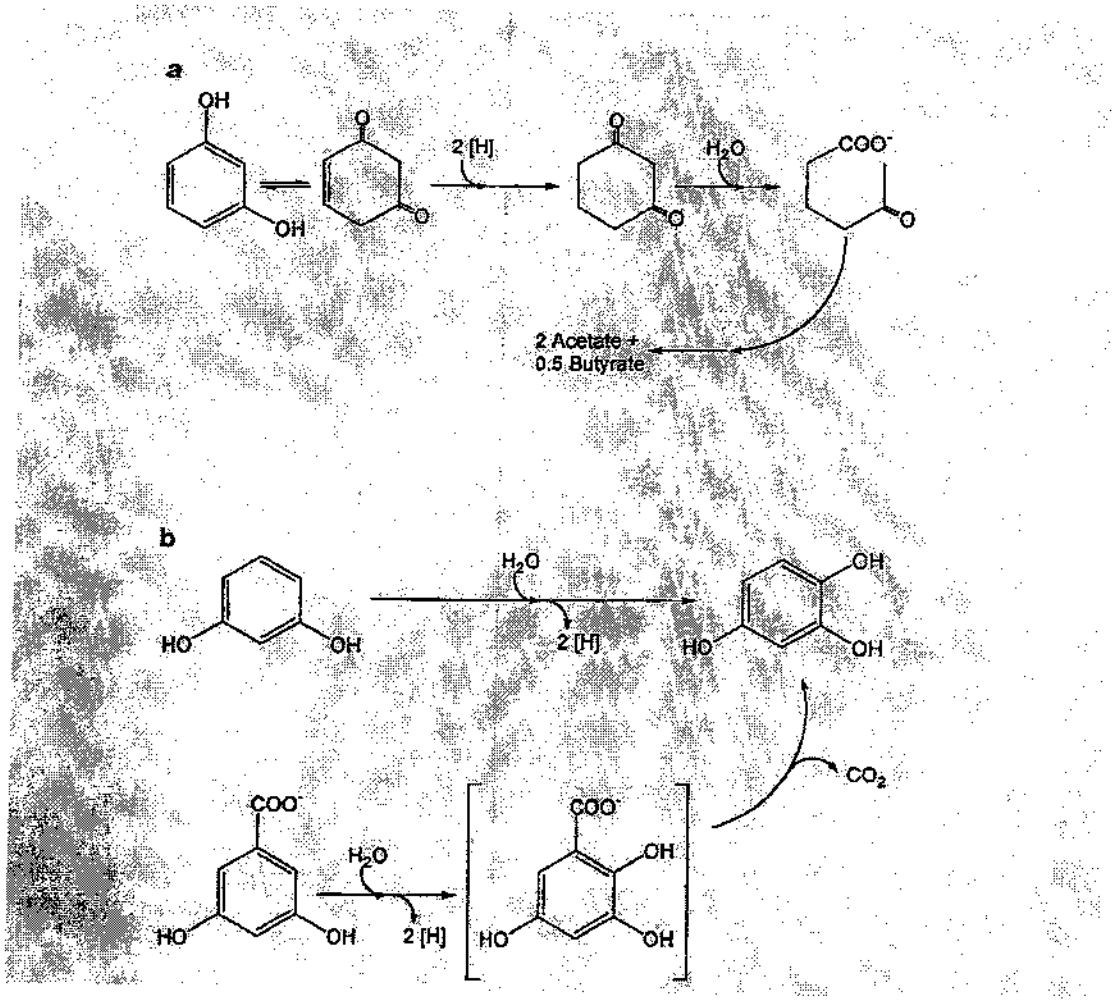

fig 3.8 Anaerobic degradation of resorcinol and α-resorcylate. (a) Resorcinol degradation by a fermentating bacterium,Clostridium strain KN245, (b)degradation of resorcinol and α-resorcylate by nitrate-reducing bacteria.

3.2.6.6 Trihydroxybenzenes and Trihydroxybenzoates

Among the three trihydroxybenzene isomers, pyurogallol and phloroglucinol are degraded quickly by fermenting bacteria. Phloroglucinol degradation has been studied in detail with Eubacterium oxidoreducens and Pelobacter acidigallici. Phloroglucinol is reduced by an NADPH-dependent reductase to dihydrophloroglucinol, and the same strategy is followed by Holophaga foetida strain TMBS4. Hydrolytic ring cleavage leads to 3-hydroxy-5-oxohexanoic acid, which is thiolyticallycleaved to three acetate residues (Brune and Schink, 1992).

This pathway is easy to conceive, because the 1,3,5 arrangement of three hydroxyl groups on

The aromatic ring allows tautomerization to 1,3,5-trioxocyclohexane to an extent that makes the molecule susceptible to a nucleophilic attack on the oxocarbon groups. The second trihydroxybenzene isomer, pyrogallol, cannot be hydrolyzeddirectly but is isomerized to phloroglucinol through a transhydroxylation reaction(fig 3.9). The reaction requires 1,3,4,5-tetrahydroxybenzenes as a co substrate, and the enzyme transfers a hydroxyl group from the tetrahydroxybenzene as co product.

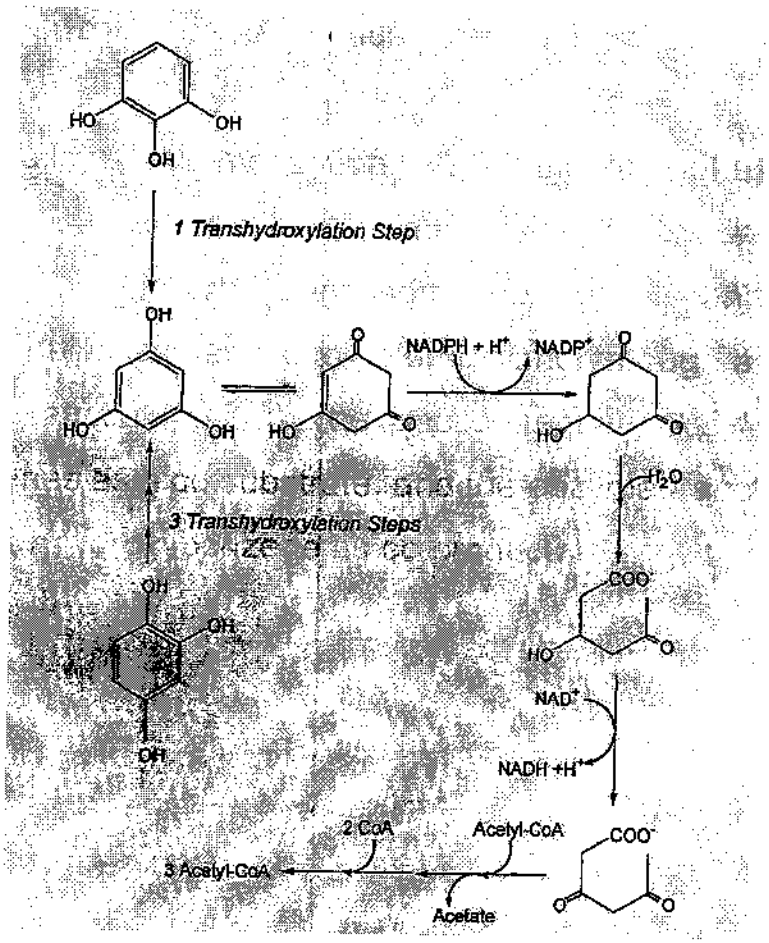

fig 3.9 Degradation of trihydroxybezenes by fermenting bacteria.

3.2.6.7 Hydroxyhydroquinone, a New Important Intermediate

The third trihydroxybenzene isomer, hyroxyhydroquinone, is converted by the fermenting bacterium Pelobacter massiliensis to three acetates via three subsequent transhydroxylation reaction, analogous to the pyrogallol-phloroglucinol transhydroxylation (Brune et al., 1992). Alternative pathways of hydroxyhydroquinone degradation were found in nitrate-reducing and sulfate-reducing bacteria. Hydroxyhydroquinone degradation by nitrate-reducing bacteria was mentioned above in the context of nitrate-dependent resorcinol degradation . The reaction sequence leads to an acetate and a succinate residue, suggesting that the hydroxyhydroquinone intermediate is cleaved between carbon atoms 1 and 2 and between 3 and 4(fig 3.10). Another alternative pathway of hydroxyhydroquinone degradation was found in the sulfate-reducing bacterium Desulfovibrio inopinatus. This bacterium destabilizes hydroxyhydroquinone by reduction to dihydrohydroxyhydroquinone, to form acetate and an as-yet unidentified 4-carbon derivative (Reichenbecher et al., unpublished data). Since D. inopinatus is unable to

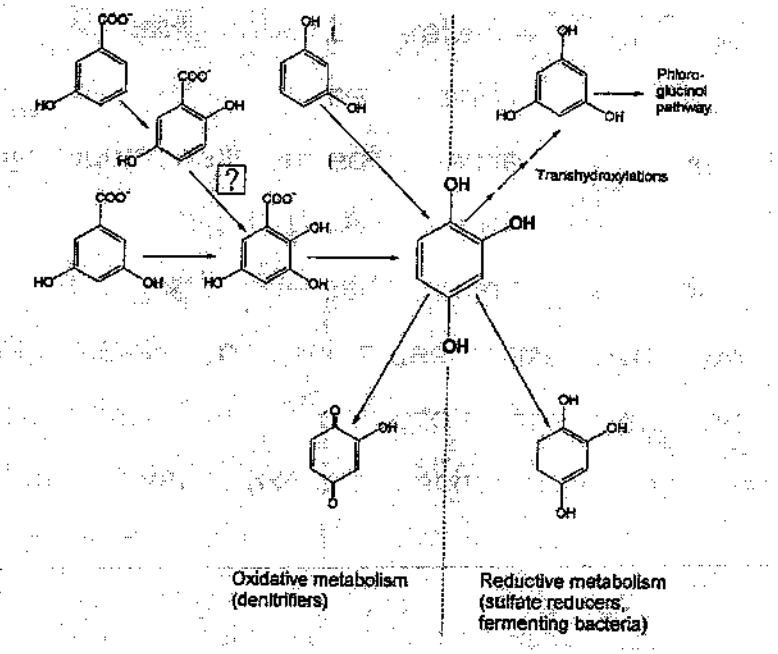

fig 3.10 Hydroxyhydroquinone as a new intermediate in anaerobic degradation of various aromatic compounds.

Oxidize acetate, the final products are two acetate and two CO2, and one mole of sulfate is reduced concomitantly to sulfide.

Hydroxyhydroquinone has gained additional interest recently because it was found to be an intermediate in the nitrate-dependent degradation of resorcinol, 3-hydroxybenzoate, 3,5-dihydroxybenzoate, perhaps also gentisate. the strategy of oxidative destabilization of aromatic compounds through hydroxylation, which these nitrate-reducing bacteria use, resembles to some extent the strategy of aerobic bacteria, and nitrate-dependent degradation of phenolic compounds thus follows a strategy that is somewhat of a mix between the oxidative aerobic strategy and the typical reductive strategy followed by strictly anaerobic bacteria.

3.2.6.8 Aromatic Hydrocarbons

Anaerobic degradation of aromatic hydrocarbons has been a matter of dispute for several years until reliable conversation balances with fast growing enrichment cultures or pure cultures were provided. Today, several pure cultures of nitrate-reducing or sulfate -reducing bacteria are available which oxidize toluene(methylbenzene) and have been characterized in detail.(Spormann and Widdle,2000). Initial experiments indicated that toluene degradation proceeded through oxidation of the methyl group ,with benzoyl-CoA as central intermediate. nonetheless, the anticipated methyl hydroxylation reaction transforming toluene to benzylalcoholcould never be observed in vitro. Labeling experiments with intact cells provided evidence that toluene was activated by addition of fumarate, through a radical intermediate, to form benzylsuccinate(Fig3.11a), and this mechanism was confirmed in nitrate-reducing and sulfate-reducing bacteria. Benzylsuccinate releases succinyl-CoA through beta oxidation, leading again to benzoyl-CoA as key intermediate. This new type of methyl group activation is also employed in anaerobic degradation of alkanes and of m-cresol.

Anaerobic degradation of o-, m-, and p-xylene has been documented mostly by tracer experiments wi8th sediment samples or in enrichment cultures. pure cultures of sulfate-reducing xylene degraders are now available, and evidence is available that at least m-xylene is activated by addition to fumarate.

Ethylbenzene is oxidized by denitrifying and sulfate-reducing bacteria. Here, the side chain is hydroxylated to form 1-phenylethanol as the first oxidation product(fig 3.11b)the oxidizing enzyme is a novel molybdenum enzyme. The subsequent pathway leads via oxidation to acetophenone and carboxylation to benzoylacetate and then throughthiolytic cleavage to an acetyl residue plus benzoyl-CoA.

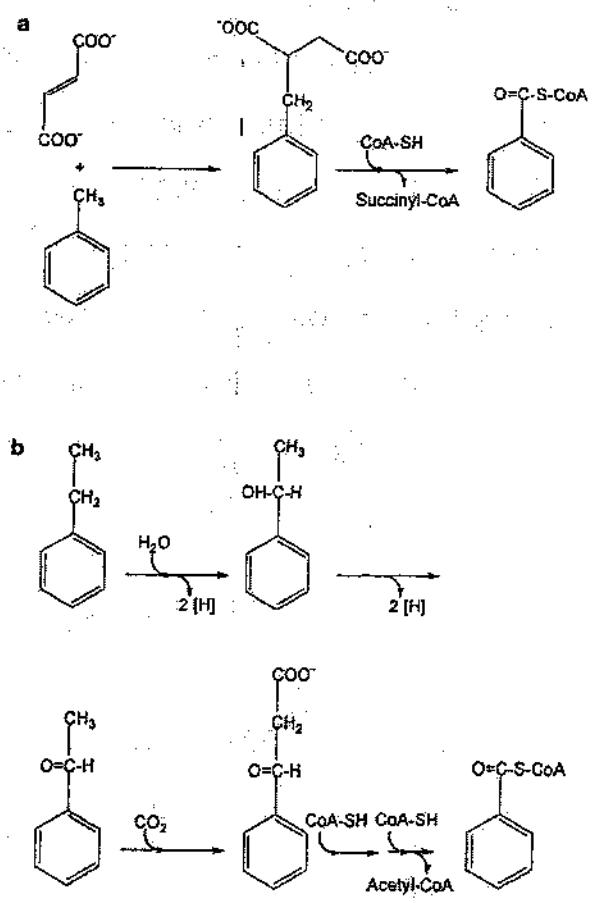

fig 3.11 anaerobic degradation of(a) toluene and(b) ethylbenzene by nitrate-reducing or sulfate-reducing bacteria.

Much less is known about anaerobic benzene oxidation. Oxygen-independent hydroxylation and degradation of benzene was observed in methanogenic enrichment cultures and in sulfate-reducing(lovely et al.,1995), nitrate-reducing, or iron(III)-reducing enrichments(lovely et al., 1996). Isolation of a pure culture of nitrate-reducing benzene degraders has been reported recently, but the biochemistry of benzene activation still remains an open question.

Naphthalene is degraded an aerobically by various enrichments and also by pure cultures of sulfate-reducing bacteria. Its degradation is probably initiated by a carboxylation reaction and subsequent reductive dearomatization, but the details of this pathway have still to be worked out.

3.2.7degradation of halogenated organics

Halogenated organics are widespread in nature and are formed especially as secondary metabolites by plants, marine algae, fungi, and certain bacteria at low rates. It is not surprising, therefore, that a broad variety of bacteria and fungi can degrade such compounds and that this capacity has grown in the past to include also the majority of synthetic halogenated compounds. Dehalogenation can proceed basically through an oxidative, a hydrolytic, or a reductive reaction. Among anaerobic bacteria, the reductive elimination of halogen substituents is the most common type of reaction and was first observed in enrichment cultures with 3,5-dihydroxybenzoate. Later, several other aliphatic and aromatic compounds were found to be dehalogenated(mainly dechlorinated) in anoxic incubation experiments (bouwer and McCarty, 1983), and today nearly all chlorinated organics can be dehalogenated and further degraded in strictly anaerobic microbial cultures. As a rule, reductive dechlorination is the preferred process for degradation of compounds with higher degrees of halogenation. The reaction is an excellent electron sink(Eh+250 to +580 mV), and highly halogenated compounds are much more amenable to a nucleophilic attack on the respective carbon atom than to an oxidative reaction.

The overall reaction of reductive dehalogenation can be diagrammed as in figure 3.12 for a chlorinated compound: electrons derived from molecular hydrogen, for-mate, or more complex organic compounds are transferred to the halogenated substrate to release the organic residue in a reduced form, together with chloride. It has been shown in several instances that this redox process can yield metabolic energy through a respiratory mechanism, which implies that the process may establish a net translocation of protons across the cytoplasmic membrane and that the proton gradient drives ATP synthesis through a membrane-bound ATP synthase complex. The biochemistry of the dechlorination reaction has been studied best with bacteria converting tetrachloroethene via trichloroethene to dichloroethene. The dechlorinatin reaction employs a corrinoid as cofactor, which attacks the carbon-chlorine linkage in its reduced Co(I) form and converts it to the Co(III) form, probably through two subsequent one-electron transfer reactions. whether this reaction mechanism is used also in the dehalogenation of aromatic compounds has still to be elucidated. There appear to be numerous differences with respect to the electron carriers involved and the spatial arrangement of the enzyme components in the cytoplasmic membrane.

fig 3.12 reductive dehalogenation of a chloro-rganic compound.

Halo-organics can also be reductively dehalogenated by reduced Fe(II) phases. that are formed on the surface of Fe(III) minerals.

3.2.8 degradation of sulfonates

Sulfonated organics are rare in nature: only taurine, coenzyme M, cysteate, and a few secondary metabolites are known to contain sulfonate substituents. Serobic degradation of such compounds typically requires an oxygenase

reaction, which hydroxylates the neighboring carbon atom and releases sulfite (Cook et al., 1999).

Some sulfonates can also be partly degraded in the absence of molecular oxygen and can serve as sulfur sources under conditions of sulfur limitation .(Cook et al., 1999). The biochemistry of anaerobic sulfur release from sulfonates has been studied extensively with taurine and related compounds, which are metabolized through sulfoacetadehyde as keyintermediate. The sulfono group is released from sulfoacetaldehyde probably through a thiamine pyrophosphate-dependent reaction that forms sulfite and an acetyl residue, analogous to the reaction elucidated earlier with an aerobic bacterium. Since this desulfonation reaction requires an oxo group positioned B{beta}to the sulfur atom for linkage to the coenzyme, it remains questionable whether this concept can also be applied to desulfonation of commercial sulfonates, such as alkyl sulfonates or alkylbenzene sufonates, which so far resist anaerobic degradation.

3.2.9 Degradation of Nitroorganics

Among the nitro aromatic compounds, trinitrotoluene (TNT) is of major importance as a soil pollutant because it has accumulated at old ammunition factory sites over several decades. The election-withdrawing effect of nitro substituents makes an oxidative attack on nitro armatics rather difficult. Indeed, trinitrotluene is attacked by aerobic bacteria primarily through a reductive reaction that transforms nitro aromatics to the corresponding amino derivatives or reductively eliminates the nitro groups via so-called Meisenheimer complex(Vorbeck et al.,1994). In the presence of oxygen, the partly reduced derivatives can react with each other to form a rather inert polymer. A reactive approach is taken also by strictly anaerobic bacteria, e.g., sulfate-reducing bacteria: trinitrotoluene is converted via diaminonitrotoluene to triaminotoluene.

Whereas the first step can also be catalyzed purely chemically without the participation of microbial cells or enzymes, reduction of diaminonitrotoluene to triaminotoluene requires the participation of microbial cells or enzyme fractions(Preuss et al.,1993).The further fate of triaminotoluene is unclear. It is partly utilized as a nitrogen source by a sulfite-reducing bacterium; the remnant product possibly polymerizes, especially in the presence of traces of oxygen(preuss et al., 1993). The same is likely to happen in contaminated soils; so far, there is no reliable proof of complete degradation of TNT by anaerobic bacteria or by anaerobic and aerobic bacteria cooperating in a two-step process.

University of technology

Chapter four

Conclosion remark

The end

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CIVIL ENGINEERING

CHAPTER FOUR

Conclution Remark

Anaerobic degradation can be applied in technical devices for treatment of waste material, often leading to CH4 and CO2 as products, which can be exploited as energy source or as a basis for biosynthetic processes. Moreover, anaerobic degradation proceeds in many anoxic habitats such as the intestinal tracts of humans and animals, sediments, and oxygen-deprived microenvironments in soil, sewage sludge, etc. Knowledge of the capacities, strategies, and limits of anaerobic degradation processes is therefore needed to assess the potential risk of synthetic compounds to health or to the environment, no matter whether such synthetics are released intentionally(as with plant protuction agents), inadvertently through wastewater treatment, or accidentally through spills.

This overview shows that the degradative potential of anaerobic microbial communities is much greater than assumed only a few years ago: a broad variety of compounds can be subject to anaerobic degradation, most often down to methane and carbon dioxide as final products. Aliphatic hydrocarbons are degraded if they contain unsaturated bonds, preferentially if these are located terminally, but saturated long-chain aliphatic hydroicarbons are also an aerobically degradable. These processes are slow and can be applied only in long-term incubations, if at all. Ether compounds are degraded an aerobically if they are methyl ethers or if they can be transformed into hemiacetals through, e.g., hydroxyl shift reactions. In the anaerobic degradation of ketones, the primary activation reaction is a carboxylation rather than an oxidation step.

Mononuclear aromatic compounds can be degraded an aerobically rather efficiently if they carry at least one carboxy, hydroxy,methoxy, amino, or methyl substituent, and four major degradation pathways have been elucidated in the

recent past, which differ basically from the well known aerobic oxygenase-dependent pathways. The degradation kinetics differ considerably, depending on the sites of substitution.

Halogenated aliphatics and aromatics are reductively dehalogenated, more efficiently and better than by aerobes, the higher the degree of halogenation. Anaerobic degradation of sulfonates appears to be restricted to only a few compounds, whereas the majority of synthetic sulfonates(detergents) are degraded efficiently only in the presence of oxygen. Nitro-substituted compounds are preferentially attacked through reduction, and anaerobic process therefore appear to be advantageous over aerobic ones. The same applies to azo compounds, which are not discussed here.

Several types of reactions were identified which activate or destabilize comparably inert substrates in the absence of oxygen. Among these are carboxylations,addition to furmarate, reductions and reductive eliminations, rearrangements of aliphgatic carbon skeletons, cobalamin-dependent nucleophilic substitutions, and oxygen-independent hydroxylations. Numerous reactions proceed through radical mechanisms, and the diversity of radical chemistry in the absence of oxygen appears to be considerably greater than in its presence.

Transformation of polymertic compounds is restricted in the anaerobic world to extracellular hydrolysis reactions unless the polymer can be taken up into the cell, as occurs with polyethylene glycol. there is no equivalent in the anoxic world to the fungal lignin-degrading enzyme apparatus. Therefore, polynuclear aromatics (lignin, other polyphenols) remain comparably recalcitrant in anoxic environments and represent barriers to microbial attack in the absence of molecular oxygen.

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