MICROORGANISMS AS CATALYSTS FOR THE DECONTAMINATION OF ECOSYSTEMS AND DETOXIFICATION OF ORGANIC CHEMICALS

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Keywords: aerobic and anaerobic degradation, auxiliary substrate, central precursor, cometabolism, convergent pathways, efficiency and velocity, fermentative conversion, indigent substrate, mixed culture, non-productive degradation, productive degradation, start assistance, xenobiotics

Contents

Summary

Living systems, cells and organisms, are geared toward survival. They are (biologically) stable by reproduction. For that purpose they need – above all – energy. Chemotrophic organisms win this energy from redox processes, chemo-organotrophs by degradation of organics. To survive they are not choosy, that means they catabolize not only bio-organics, but utilize also chemosynthetic, potentially hazardous compounds. Since microorganisms (can) colonize all ecosystems, they represent a self-defence power which, however, is too low. Starting from the modes of microbially mediated detoxification of organics, i.e. productive and non-productive degradation, the analysis of the reasons of the unsatisfactory situation and a physiological evaluation of the different organics, well-founded strategies and approaches for overcoming bottlenecks have been discussed and experimental examples presented.

1. Introduction

As a result of urbanization and industrialization, atmospheric, terrestrial and aqueous

environments, and their associated ecosystems have become more and more polluted, and contaminated with a range of substances, both organic and inorganic. A lot of these chemicals are problematic since they are potentially toxic, i.e. they can adversely affect all kinds of living systems. They become acutely toxic if their concentrations exceed certain thresholds and are bio-available. Hence, there are two fundamental approaches to decontamination: immobilization and removal, or conversion of the pollutants to harmless substances (Figure 1). All methods designed to decrease the (free) concentrations of hazardous chemicals use one or the other of these strategies.

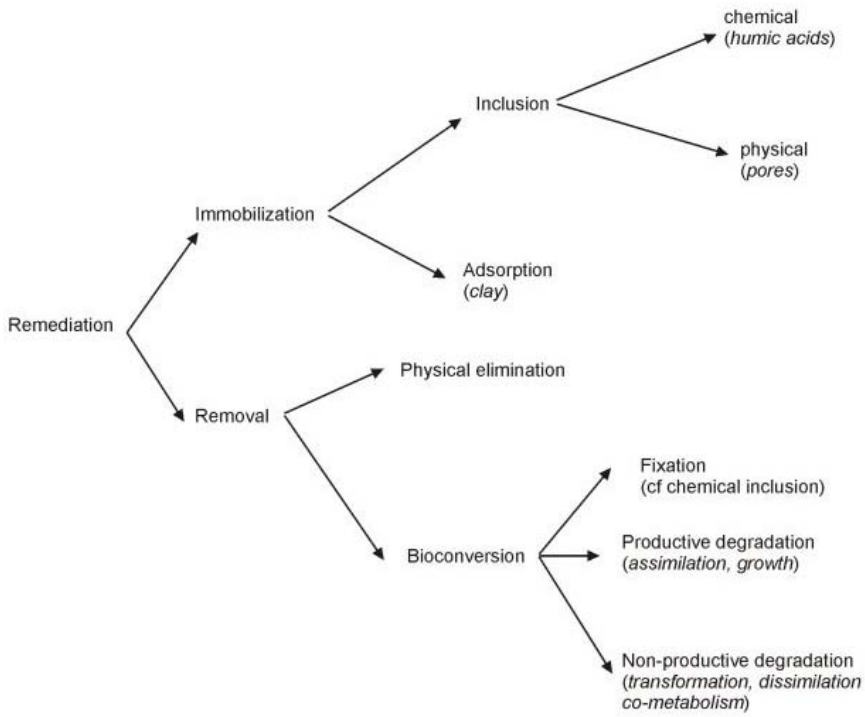

Figure 1: Survey of principles of physical, chemical and biological decontamination of ecosystems

Organics can be converted into a variety of other compounds, i.e. transformed and derivatized, partially degraded and even mineralized, either chemically or biochemically. Biochemical processes can be catalyzed by cell-free systems (via extracellular enzymes) or cellular systems (i.e. organisms). Microorganisms play an important role in these conversions. They are ubiquitous (MARTINUS BEIJERINCK: *Everything is everywhere, the environment selects*), and in nature, they metabolize bio-organics to utilize their carbon and energy for growth and multiplication (see also - *Microbial Cell Culture*) as a forward survival strategy. However, this ability is not restricted to bio-organics, since microorganisms can also attack and modify xenobiotics (see also - *Biodegradation of xenobiotics*). Furthermore, it appears that microorganisms are "teachable" (adaptable) since it is possible to broaden the spectrum of problematic compounds they can use as sources of carbon, nitrogen, hydrogen and as electron donors. Thus, in a sense, microorganisms have a predetermined capability for decontaminating polluted ecosystems (LOUIS PASTEUR: *Natura in minimis maxima*).

They represent the biologically based self-cleaning or defence potential of soil and water. This capability is an essential "chapter" of the phenomenon called *natural attenuation*. Interestingly enough, the number of terminal electron acceptors is comparatively small. There is neither an indication of, nor a good reason for, broadening the range of electron and hydrogen acceptors, for the well-known acceptors giving microorganisms great flexibility and versatility (see also - *Cell thermodynamic and energy metabolism*).

2. Key Reactions of Microbially Mediated Degradation of Organics

While fossil raw materials are being chemically processed, waste products enter the environment and pollute soil and ground-water. These waste products come from crude or mineral oil and contain many substances, especially aliphatic, alicyclic and aromatic hydrocarbons. Although these compounds are of biogenic origin and resemble natural materials, they are not readily biodegradable. Their microbiologically mediated degradation is influenced by various environmental factors and conditions, and their inherent biodegradability is determined by the composition, structure and thermodynamic stability of the compounds. Degradation of multi-carbon compounds in terms of catabolism and assimilation involves cleavage of covalent bonds, which must be preceded by an activation step. This may occur via either aerobic or anaerobic processes (see also - *Microbial physiology in anaerobic and aerobic atmospheres*). For aerobic activation, O_2 is required as an additional external co-substrate. In anaerobic activation, the activating agents required are generated internally within the cells (organisms): ATP, [2H] (= reducing equivalents, i.e. NADH and/or NADPH), CoASH, fumarate, CO_2 or H_2O act as cosubstrates. If they are not available endogenously, or cannot be acquired from the potential substrates themselves, an extra source of these agents is needed to provide start assistance.

Alkanes are readily biodegradable under aerobic conditions (see also - *Biodegradation of xenobiotics, – Basic Strategies of Cell Metabolism*). They can be activated terminally or sub-terminally. The incorporation of oxygen is catalyzed by a monooxygenase:

$$R\text{-}CH_2\text{-}CH_3 + O_2 + NAD(P)H + H^+ \longrightarrow R\text{-}CH_2\text{-}CH_2OH + NAD(P)^+ + H_2O$$

The resulting primary or secondary alcohols, respectively, are oxidized and are eventually converted to fatty acids, which can be converted into the well-known CoA-esters. Anaerobes are also able to utilize alkanes. In this case, the activation may take place by a carboxylation reaction:

$$CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}CH_3 + CO_2 \longrightarrow CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}COOH$$

The resulting elongated fatty acid can be channelled into the well-known intermediary or central metabolism, and assimilated and/or dissimilated (see also - *Cell thermodynamics and energy metabolism*).

The metabolism of alicyclic hydrocarbons has been less thoroughly studied because it is difficult to isolate pure cultures that are able to utilize these compounds as sole sources

of carbon and energy for growth. Productive degradation of alicyclic chemicals has only been reported for syntrophic cultures. Cooxidative reactions appear to be essential for the primary attack. Cyclohexane, for instance, is oxidized by a mixed function oxidase. The further metabolism of cyclohexanol leads, via several steps, to adipic acid and finally via β-oxidation to acetyl-CoA.

In order to utilize aromatic compounds it is necessary to open the ring structure. Ring structures are not used as prefabricated building blocks or precursors for growth and multiplication in microorganisms or in other living systems at all. Even in plants, the various ring structures that comprise the bulk of the structure-forming lignin**,** the main component of wood, are not early metabolites of assimilation. They are synthesized – like the so-called secondary metabolites – from chain-like small molecules (see also - *Secondary products in tissue culture*). They may be regarded as metabolic dead end products and are responsible for the mechanical stability of higher plants, as components of the lignin network that provides their scaffolding matrices. To fulfill this function, they must also be biochemically stable.

In contrast to lignin, function-bearing polymers like proteins and nucleic acids are not stable *per se*. They are stabilized and maintained by degradation and steady renewal, i.e. turnover. In these cases only their synthesis requires energy. The degradation is energetically neutral (or energy-generating). If the internal degradation of these polymers (which is a prerequisite for individual development, cell proliferation and evolution) is energy-consuming, the functional stability of these polymers, and thus homeostasis of the organisms, could only be maintained at great costs.

Ring cleavage must be prepared. Under aerobic conditions in the presence of molecular oxygen, aromatic rings are activated by monooxygenase or dioxygenase mechanisms. This step is referred to as peripheral, and involves considerable modifications of the ring and perhaps elimination of substituent groups. The modifications and conversions of the many different compounds result in convergence to a few metabolites (Figure 2). Catechol (1,2-dihydroxybenzene) and protocatechuate (3,4-dihydroxybenzoate) are the most common primary intermediates into which most of the aromatics are transformed (Table 1). Some aromatic compounds are degraded via gentisate (2,5-dihydroxybenzoate).

In Table 1 the stoichiometries of the formation of these peripheral metabolites are summarized. As can be seen, formation is accompanied by an increase in reduction equivalents in the case of phenolics, and they are generated expectedly in the case of methylated aromatics. Because formation of these metabolites initiates assimilation, the first steps influence the overall carbon conversion in productive degradation. Besides the primary intermediates already mentioned, further metabolites are formed simultaneously from “large” molecules, e.g. from polycyclic aromatic hydrocarbons (PAHs).

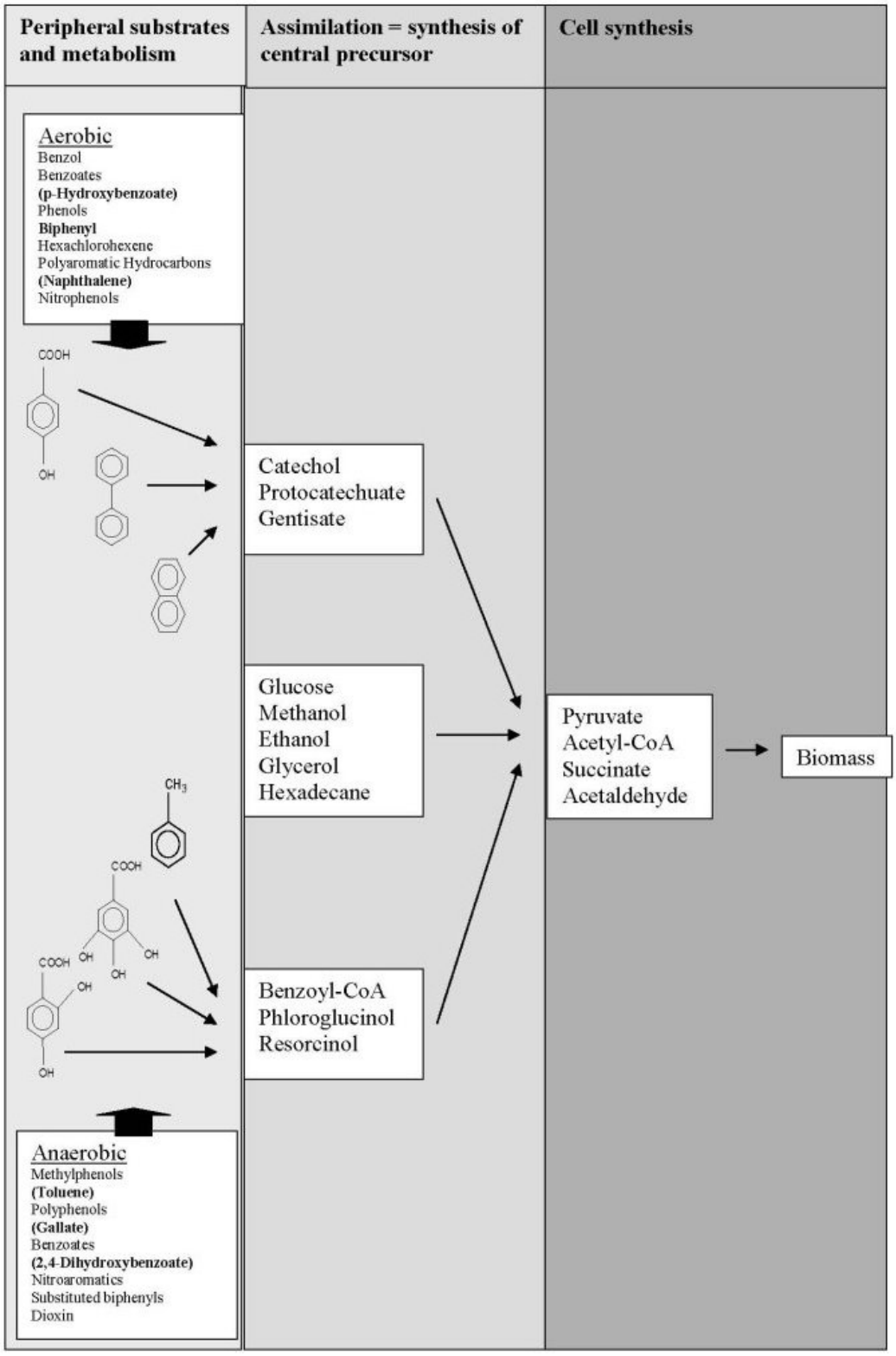

Figure 2: Metabolic funnel in aerobic and anaerobic preparation of aromatics for microbial assimilation and growth

Peripheral Substrate	O_2	[2H]	H_2O	Primary Intermediate	H_2O	CO_2	[2H]	Further Metabolites
				Catechol				
Benzene	1			1				
(Cl-)Benzoate	1			1 (chloro)		1		
Toluene	1			1		1	2	
Phenol	1	1		1	1			
DCP	1	1		1 (dichloro)	1			
MCP	1	1		1 (meth-chlor)	1			
Naphthalene	3		1	1		1		Pyr
Anthracene	6	2	1	1		2		Pyr + Ac + Ft
Biphenyl		3		1	1	1		2-Oxopentadienoate
				Gentisate				
Naphthalene	3		1	1				Pyr
Anthracene	6	2	1	1		1		Pyr + Ac + Ft
				Protocatechuate				
p-Hydroxy-benzoate	1	1		1	1			

Pyr, pyruvate; Ac, acetate; Ft, formate; DCP 2,4-dichlorophenol; MCP 4-chloro-2-methylphenol

Table 1: Balances of the aerobic formation of primary intermediates from peripheral substrates

The following oxygenolytic fission of the diols may be considered the first step of assimilation; the *ortho*-cleavage leads via *cis*,*cis*-muconic acid to succinate + acetyl-CoA and the *meta*-cleavage via 2-hydroxymuconic acid semialdehyde to pyruvate + acetaldehyde (from catechol; see Figure 3) or to only pyruvate (from protocatechuate). Gentisate and homogentisate are split into fumarate + pyruvate and fumarate + acetoacetate, respectively.

Anaerobes are also capable of activating aromatic rings. The initial reactions are independent of the presence of suitable electron acceptors such as NO_3^-, MnO_4^+, Fe^{3+} or SO_4^{2-}. The activation occurs via various mechanisms such as carboxylation of phenolic compounds, reductive removal of substituents, O-methyl ether cleavage,

transhydroxylation, and addition of fumarate (to methyl groups). The anoxic activations also result in just a few primary intermediates (Table 2), i.e. benzoyl-CoA, phloroglucinol (1,3,5-trihydroxybenzene) or resorcinol (1,3-dihydroxybenzene) and, possibly, some others (Figure 2). After reduction of the activated ring, the dearomatized ring is hydrolytically cleaved into a carboxylic acid which is finally converted via β-oxidation and decarboxylation into acetyl-CoA.

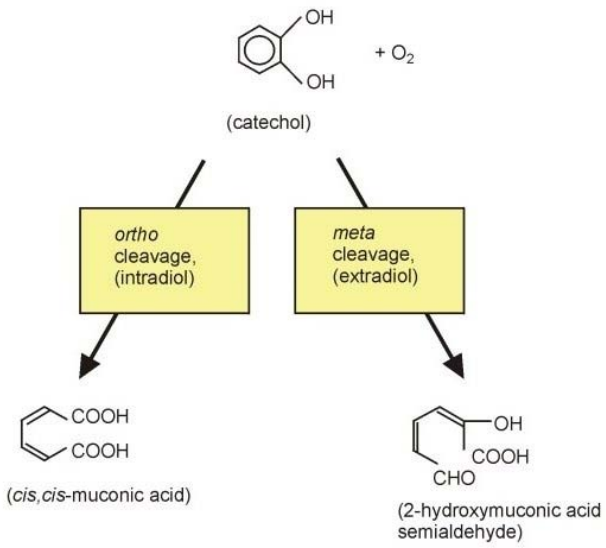

Figure 3: Model of cleavage of the aromatic ring

The stoichiometries of the anaerobic formation of these primary intermediates are shown in Table 2**.** The balances show a quite similar picture: claim or gain of reducing equivalents. As the reduction equivalents generated do not contribute to the energy budget of the cells, (they may even have to be disposed of), the ATP and/or [2H] required must be derived from an appropriate substrate.

Peripheral Substrate	**ATP**	**CoA**	**[2H]**	**H_2O**	**Metab.**	**Primary Intermediate**	**H_2O**	**CO_2**	**[2H]**	**Further Metab.**
						Benzoyl-CoA				
Benzoate	2	1				1				
Chloro-benzoate	2	1	1			1				HCl
Phenol	3	1	1			1	2	-1		
p-Cresol	2	1				1			2	
Toluene		1	1		Fum	1				Succ
Benzyl alcohol	2	1		1		1			2	

						Phloroglucinol				
Gallate						1		1		

Fum, fumarate; Succ, succinate

Table 2: Balances of the anaerobic formation of primary intermediates from peripheral substrates

Anthropogenic, chemically synthesized, man-made organics are very often harmful and foreign to living systems. Such chemicals, called xenobiotics, include pesticides, drugs, dyes, explosives etc. (see also - *Biodegradation of xenobiotics*). Their xenobiotic features are based on specific chemical bonds and structures (e.g. -N=N-), groups (e.g. -NO_2, -SO_3H) or elements (e.g. -F, -Cl, -Br) that are not involved in the steady turnover of biopolymers, or in the life cycle of organisms. Many of these compounds need to be reasonably chemically stable to fulfill their intended functions. This need not mean that they are biocatalytically inert, but as a rule they appear to be persistent and are not readily biodegradable or assimilable. The biodegradability of substituted chemicals is highly influenced by the kind, the number and the arrangement of the substituents: their resistance to degradation being increased by the electron-drawing property of the substituents. Therefore, an electrophilic attack by oxygenases becomes increasingly difficult the more substituents there are, and the greater the electron deficit at the carbon skeleton. Three mechanisms for eliminating xenobiotic groups, e.g. chlorine, are known:

(i) oxygenolytic, in which oxygen in the OH-group formed comes from O_2, for example,

$$\text{2-Chlorobenzoate} + [2H] + O_2 \longrightarrow \text{Brenzcatechol} + CO_2 + Cl^-$$

(ii) hydrogenolytic, i.e. reductive, for example,

$$\text{3-Chlorobenzoate} + [2H] \longrightarrow \text{Benzoate} + HCl$$

(iii) hydrolytic, whereby substituents are replaced by hydroxyl groups from water, for example,

$$\text{4-Chlorobenzoate} + H_2O \longrightarrow \text{4-Hydroxybenzoate} + HCl.$$

Depending on the resulting carbon skeletons and similarities to biogenic molecules, the products are further metabolized and may even be assimilated (e.g. 2,4-D derivatives), or remain intact as so-called dead end products. Many of the xenobiotics are not self-sufficient, i.e. they are not autarkically degradable. They are indigent, their utilization requires an external support. If the cometabolic activation of xenobiotics leads to dead end metabolites, a continuing source of such an aid is required. In co-oxidative degradation (very often initiated by a mixed function oxidase) and co-reductive processes (e.g. hydrogenolytic dehalogenation of trichloroethene, in which hydrogen is added to form dichloroethene and HCl), the external aid can be provided by typical growth substrates. They operate as cosubstrates.

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Biographical Sketches

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Roland H. Mueller studied Biochemistry at the University of Leipzig, where he also obtained his doctoral degree in 1979. He worked as scientific coworker at the Institute of Technical Chemistry and Institute of Biotechnology of the Academy of Sciences of the GDR, from 1980-1991. Since 1992, he has been a Senior Scientist at the UFZ Helmholtz Centre for Environmental Research Leipzig-Halle in the Department of Environmental Microbiology. His special interests and research fields are: physiology and biochemistry of microbial growth; production of metabolites and degradation of hazardous organics and xenobiotics; upper limits and bottlenecks of microbial performance.