



Centrum pro výzkum
toxických látek
v prostředí

Bi6420

Ekotoxikologie mikroorganismů

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jaro 2014



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Inovace tohoto předmětu byla spolufinancována Evropským sociálním fondem a státním rozpočtem České republiky



Centrum pro výzkum
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Bi6420: Ekotoxikologie mikroorganismů

Část 2: MO a toxické látky

Biodostupnost toxických látek pro MO v prostředí
Vstup toxických látek do MO a povrchové bariéry
Interakce toxikantů s mikrobiálními buňkami, obrana
MO proti toxikantům, toxicita, biodegradace polutantů



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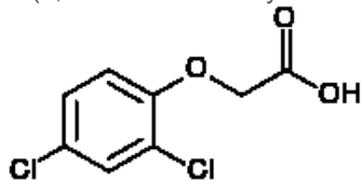
Organické kontaminanty a MO

Interakce organických polutantů s MO

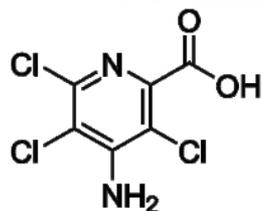
- **organické polutanty** – velice široká skupina látek různých vlastností
- jaké jsou jejich zdroje?
- persistentní organické polutanty (**POPs**): perzistence v prostředí, bioakumulace, toxicita (**PBTs**) – často lipofilní, hydrofóbní látky (**HOCs**)
- znát různé zkratky a základní organické názvosloví ...

Příklady struktur organických polutantů

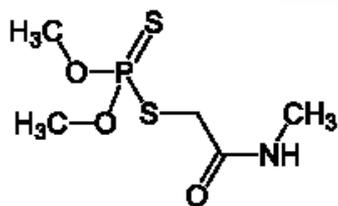
2,4 – D (2,4 – dichlorofenoxyoctová kyselina)



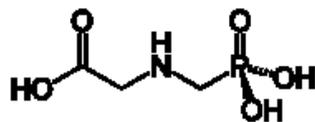
Picloram



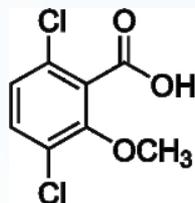
Dimethoate



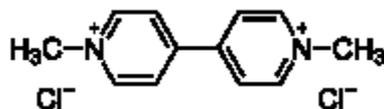
Glyphosate



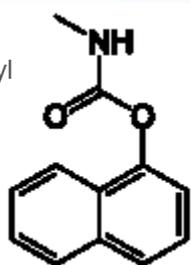
Dicamba



Paraquat



Carbaryl



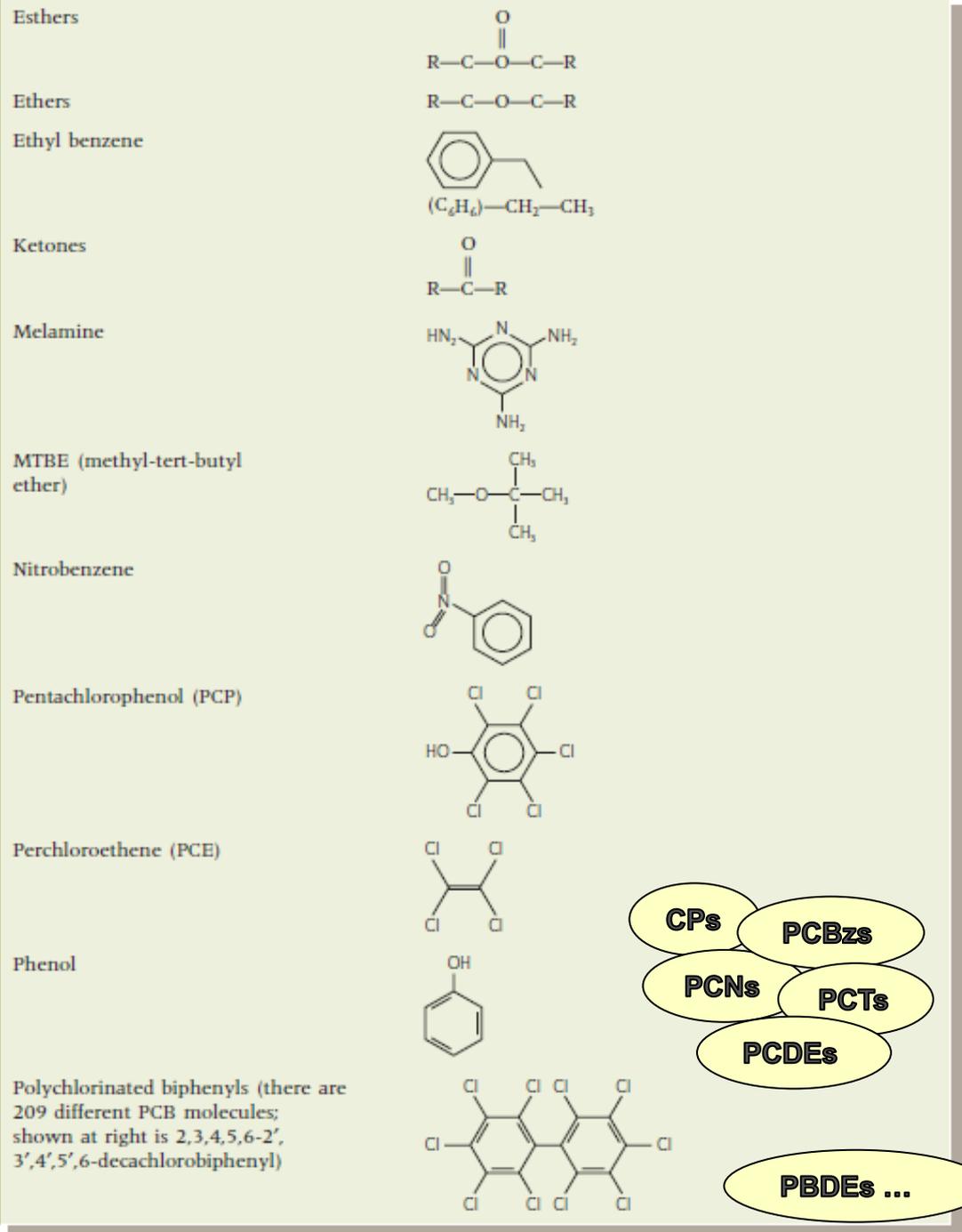
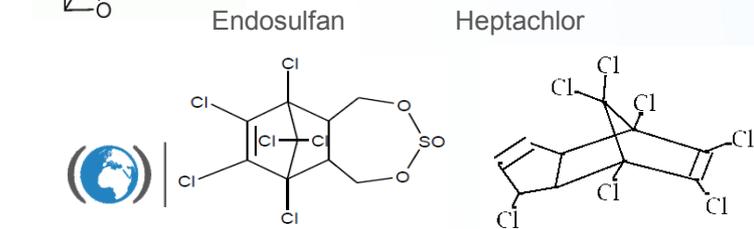
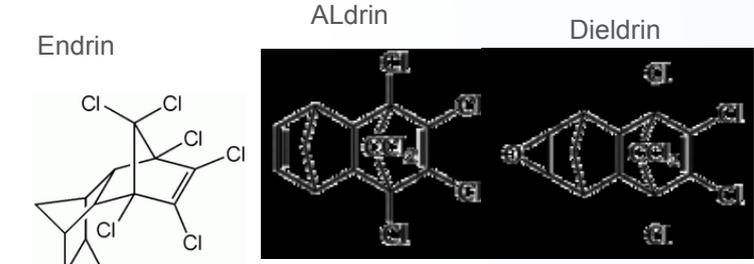
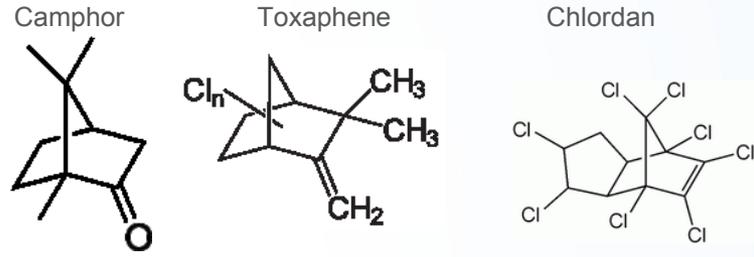
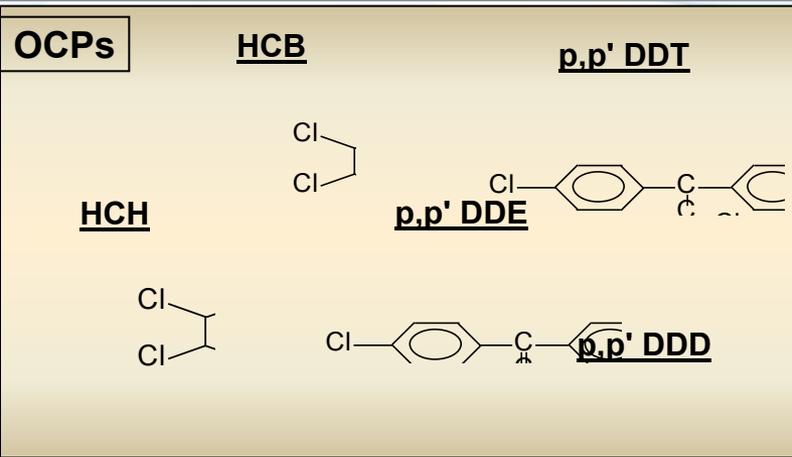
Box 8.7

A selection of widespread organic environmental contaminants and their chemical structures

Compound	Chemical structure
Alcohols	R—OH Hydroxyl group Methanol H ₃ C—OH
Alkanes	Saturated with H atoms, no double bonds Octane H ₃ C—(CH ₂) ₆ —CH ₃
Alkenes	Sites of unsaturation, at least one C double bond Ethene 2HC=CH ₂
Alkynes	Triple-bonded C R—C≡C—R Acetylene HC≡CH
Aromatic hydrocarbons	Hydrocarbons containing benzene rings
Atrazine	
Benzene	 Aromatic ring C ₆ H ₆
Chlorobenzene	
Creosote	Oily yellow-to-black substance rich in phenols, PAHs, and cresols. Used as a wood preservative
Cresol(s) (three different isomers)	
2,4-Dinitrotoluene	
Dioxins (e.g., TCDD)	 2,3,7,8-Tetrachloro-dibenzo- <i>p</i> -dioxin (TCDD)



Příklady struktur organických polutantů

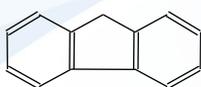


Příklady struktur organických polutantů

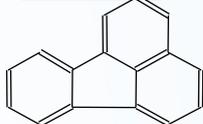
Naftalen



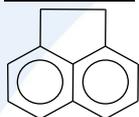
Fluoren



Fluoranten



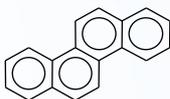
Acenaften



Antracen



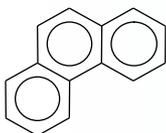
Chrysen



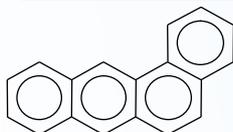
Acenaftylen



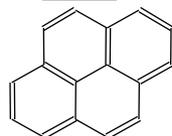
Fenantren



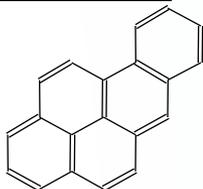
Benzo(a)antracen



Pyren



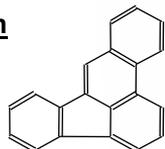
Benzo(a)pyren



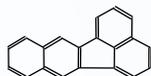
Benzo(ghi)perylene



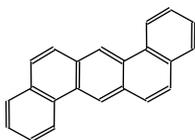
Benzo(b)fluoranten



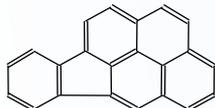
Benzo(k)fluoranten



Dibenzo(ah)antracen

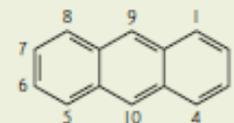


Indeno(123cd)pyren



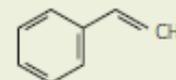
Box 8.7 Continued

Polycyclic aromatic hydrocarbons (PAHs)

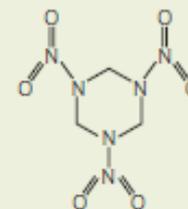


Anthracene, a three-ring PAH

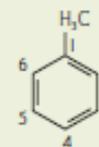
Styrene



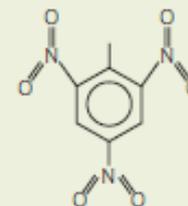
RDX (Royal Dutch Explosive)



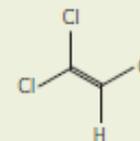
Toluene



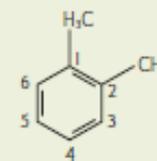
TNT (trinitrotoluene)



Trichloroethene



Xylene(s)
(three different isomers)

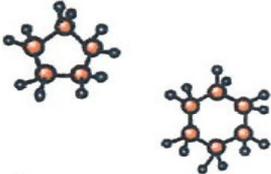
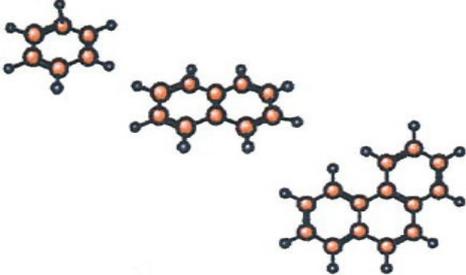
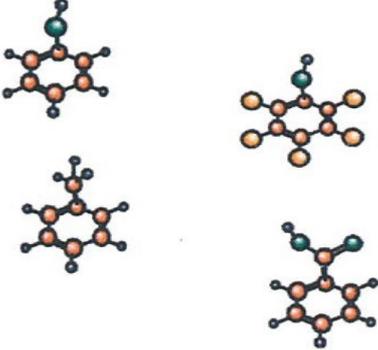


Xylene (o-shown)

Nomenclature: R, organic moiety; —, terminal methyl group (e.g., —CH₃).



Příklady organických polutantů - Meier et al. (2000)

Hydrocarbon Type	Structure	Name	Physical state at room temp.	Source and uses
Aliphatics		propane n=1 hexane n=4 hexatriacontane n=34	gas liquid solid	Petroleum contains both linear and branched aliphatics. The gasoline fraction of crude oil is 30 - 70% aliphatic depending on the source of the crude oil.
Alicyclics		cyclopentane cyclohexane	liquid liquid	Petroleum contains both unsubstituted and alkyl substituted alicyclics. The gasoline fraction of crude oil is 20-70% alicyclic depending on the source of the crude oil
Aromatics		benzene naphthalene phenanthrene	liquid solid solid	Petroleum contains both unsubstituted and alkyl substituted aromatics. The gasoline fraction of crude oil is 10-15% depending on the source of the crude oil.
Substituted aliphatics		chloroform trichloroethylene (TCE)	liquid liquid	Anthropogenically manufactured and used as solvents, degreasing agents, and in organic syntheses.
Substituted aromatics		phenol pentachlorophenol toluene benzoate	liquid liquid liquid liquid	Found in coal tar or manufactured and used as a disinfectant, and in manufacture of resins, dyes and industrial chemicals. Manufactured and used as an insecticide, defoliant, and wood preservative. Found in tar oil, used in manufacture of organics, explosives, and dyes. Also used as a solvent. Found in plants and animals and manufactured for use as a food preservative, dye component, and in curing tobacco.

Příklady organických polutantů - Meier et al. (2000)

Biaryl hydrocarbons		biphenyl polychlorinated biphenyls (PCBs)	solid liquid	Biphenyl is the parent compound of variously chlorinated biphenyl mixtures known as the PCBs. PCBs are used as transformer oils and plasticizers.
Heterocyclics		dibenzodioxin chlorinated dioxins pyridine thiophene	solid solid liquid liquid	Dioxins are created during incineration processes and are contaminants associated with the manufacture of herbicides including 2,4-D and 2,4,5-T. Found in coal tar. Used as a solvent and synthetic intermediate. Found in coal tar, coal gas and crude oil. Used as a solvent and in manufacture of resins, dyes, and pharmaceuticals.
Pesticides Organic acids Organophosphates Triazines Carbamates Chlorinated hydrocarbons		2,4-dichlorophenoxy acetic acid chlorpyrifos atrazine carbaryl 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane (DDT) methyl bromide	solid solid solid solid solid gas	Broadleaf herbicide Used as an insecticide and an acaricide Selective herbicide Contact insecticide Contact insecticide Used to degrease wool, extract oil from nuts, seeds and flowers, used as an insect and soil fumigant.

LEGEND

- Carbon
- Hydrogen
- Oxygen
- Chlorine
- Nitrogen
- Sulfur
- Phosphorus
- Bromine

Příklady organických polutantů - pesticidy

Organochlororové	aldrin, dieldrin, endosulfan, DDT, dicofol, chlordane, endrin, HCH, heptachlor, lindan, methoxychlor, toxaphene
Nesystémové	
Organofosfáty	acephate, azinphos methyl, diazinon, dichlorvos, ethion, fenitrothion, fomofos, chlorfenvinphos, chlorpyrifos, chlorpyrifos-methyl, malathion, mecarbam, mevinphos, methidathion, parathion ethyl, parathion methyl, phosalone, pirimiphos-methyl, quinalphos, sulfotep, terbufos, tetrachlorvinphos, tolclofos-methyl, triazophos,
Karbamáty	carbaryl, fenoxycarb, formethanate, methiocarb, methomyl, propoxur
Amidiny	amitraz, pymetrozine
Systémové	
Organofosfáty	acephate, dimethoate, disulfoton, formothion, heptenophos, methamidophos, mevinphos, phorate, phosphamidon, thiometon, trichlorphon, vamidothion
Karbamáty	aldicarb, bendiocarb, benfuracarb, carbofuran, carbosulfan, ethiofencarb, furathiocarb, pirimicarb, pyrethrins, methomyl, oxamyl
Syntetické pyretroidy	acrinathrin, allethrin, bifenthrin, bioresmethrin, cyfluthrin, lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, etofenprox, fenpropathrin, flucythrinate, fluvalinate, permethrin, piperonyl butoxid, tau-fluvalinate,



Interakce organických polutantů s MO

- specifikum ekotoxikologie mikroorganismů: evoluční strategie MO v kontaminovaném prostředí → interakce a transformace chemikálie s „cílem“ energetického profitu, růstu či snížení toxicity resistance → celá řada kontaminantů je transformována MO

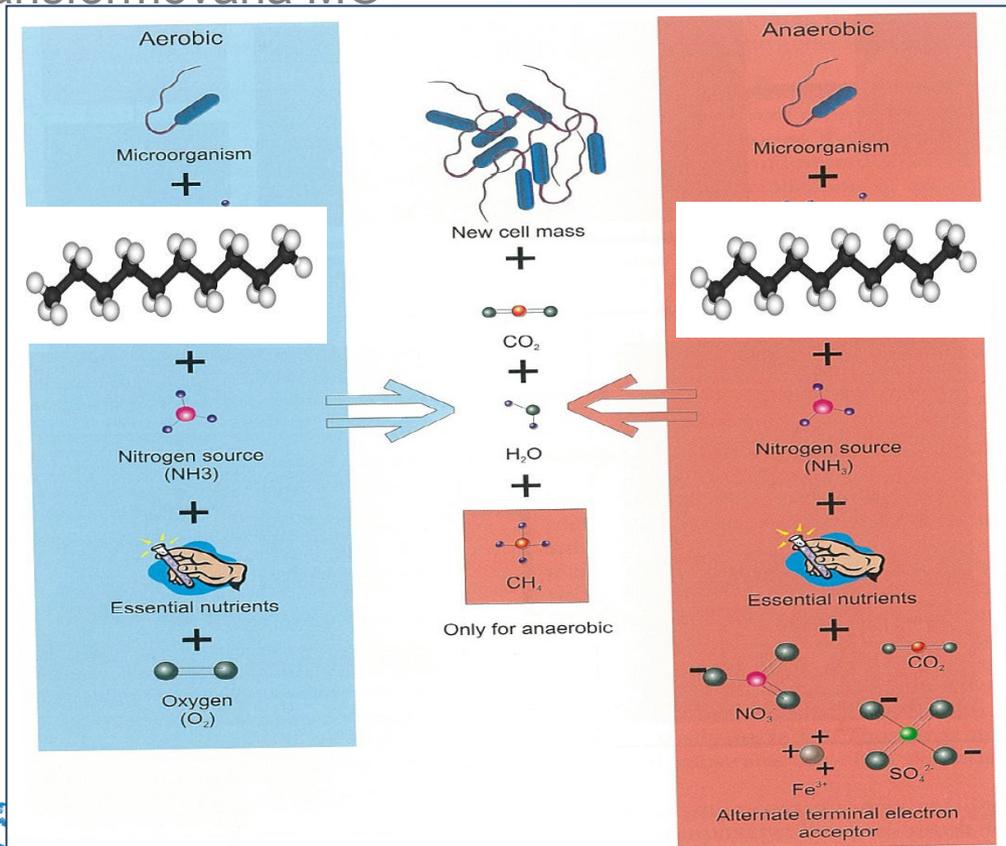
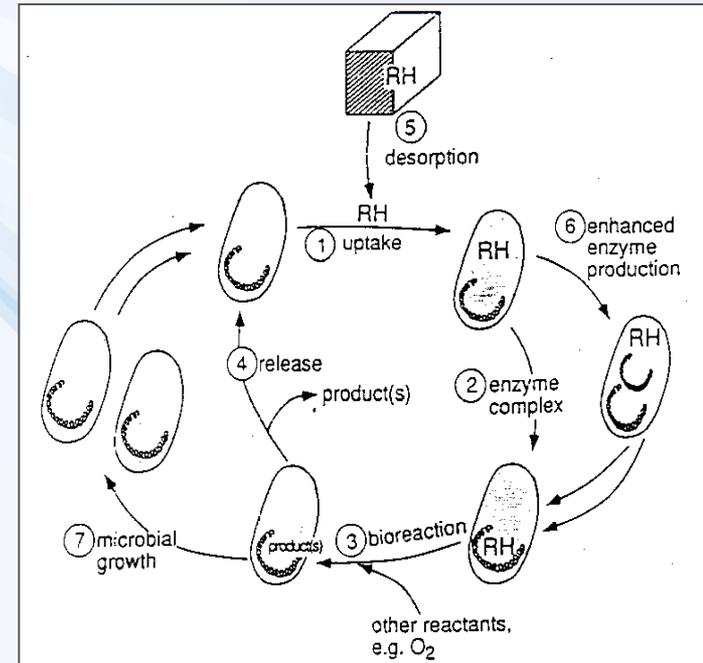
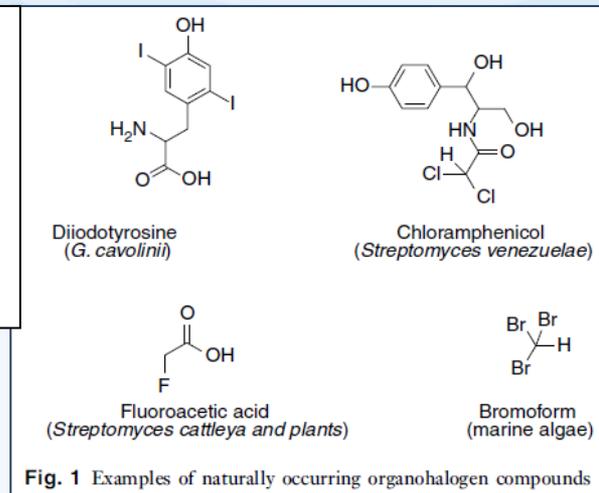
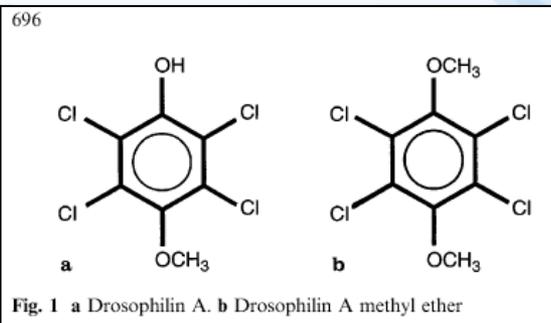
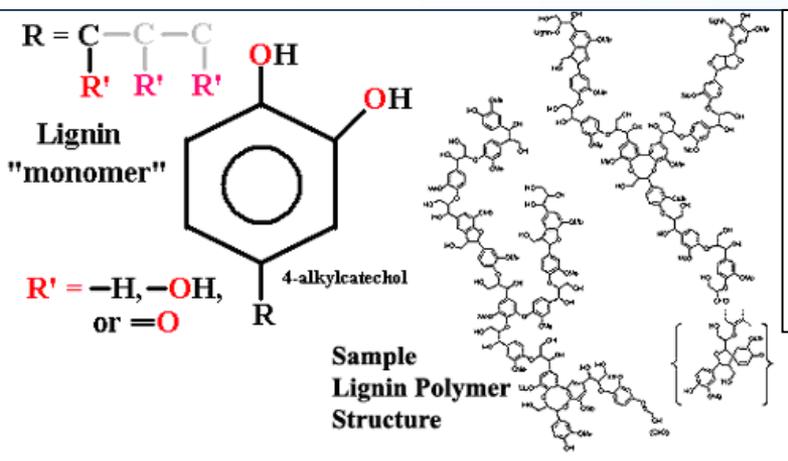


FIGURE 16.3 Aerobic (blue) or anaerobic (red) mineralization of an organic compound.

Interakce organických polutantů s MO

- díky dlouhé evoluci mají MO vyvinuté katabolické procesy na přírodní látky často podobné POPs (např. lignin a další biopolymery)
- řada POPs v prostředí odpradávná, přirozený původ:
 - např. PAHs, dioxiny – hoření
 - chlorované uhlovodíky – biologická halogenace houbami, řasami - 3000 látek



- **Carrying capacity** - i když dané polutanty umí MO degradovat (viz výše), je jich tolik, že tato degradace nestačí

Interakce organických polutantů s MO

• další přírodní látky podobné organickým polutantům

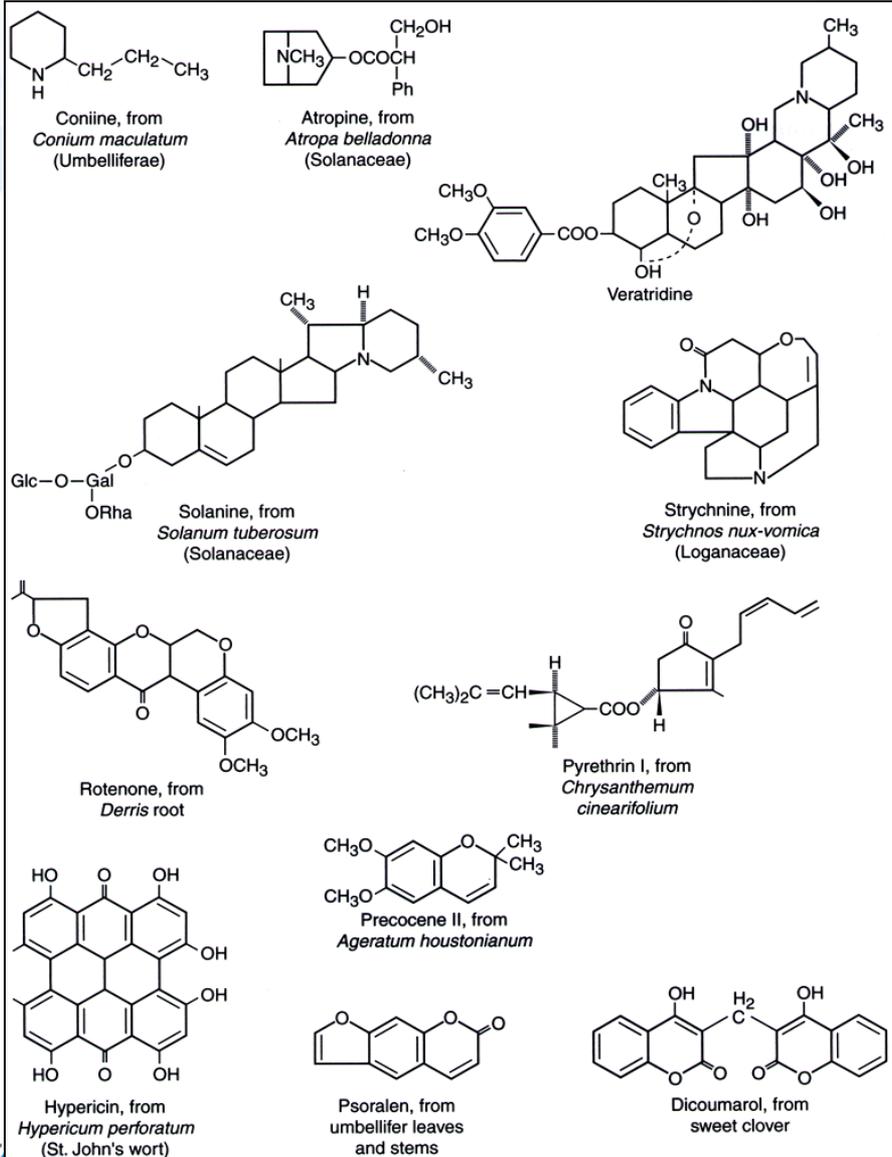


FIGURE 1.1 Some toxins produced by plants.

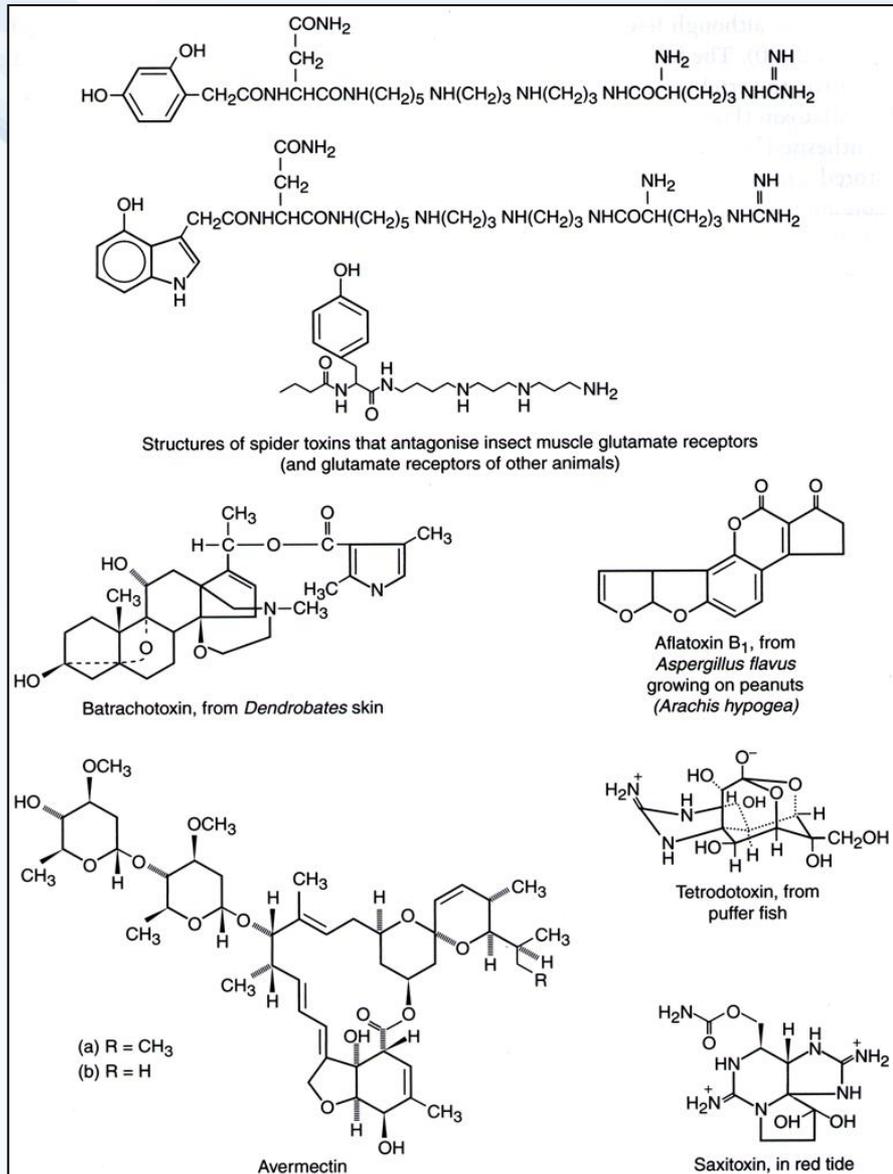


FIGURE 1.3 Some toxins from animals and microorganisms.

Interakce organických polutantů s MO

Dvě základní možnosti interakce:

1. biodegradace org. polutantů:

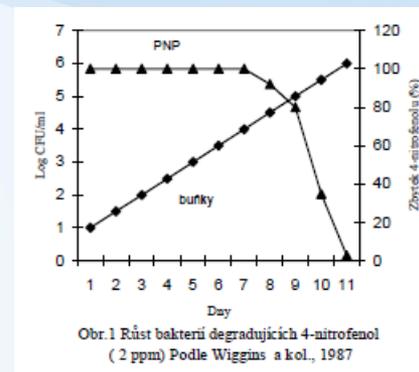
- MO transformují molekulu org. polutantu, částečně až zcela (zmineralizují)
- proces umožněný enzymy
- kromě kometabolismu platí, že MO má z biodegradace benefit:

- růst, biosyntéza - **asimilace**
- energie - **mineralizace**
- živiny (N z nitrofenolů, P z insekticidů)
- snížení toxicity - detoxifikace, metabolismus
- polutant je TEA
- atd.

$$C_{\text{substrát}} = C_{\text{asimilovaný}} + C_{\text{mineralizovaný}}$$

↑
↓
růstový výtěžek

- **využití v biotechnologiích**
- dominantní poznatky



Interakce organických polutantů s MO

Dvě základní možnosti interakce:

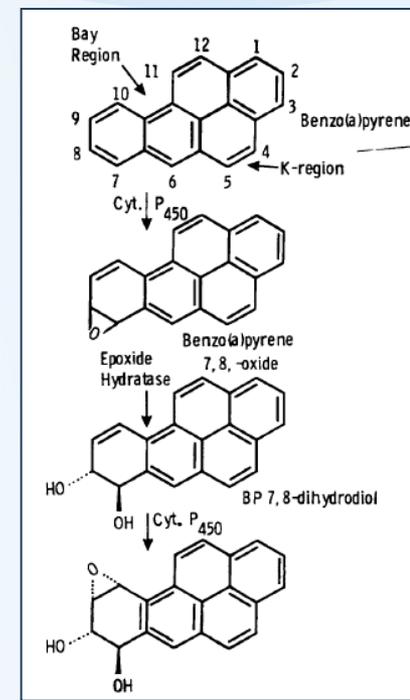
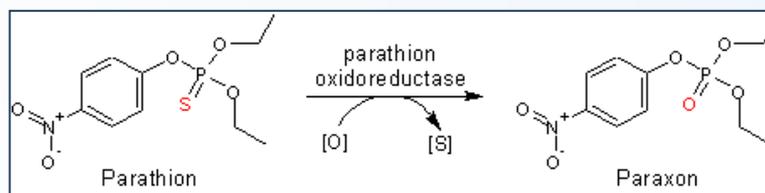
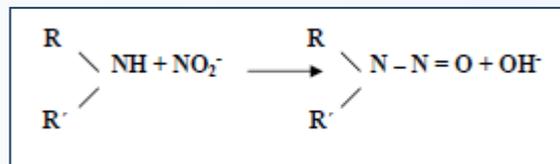
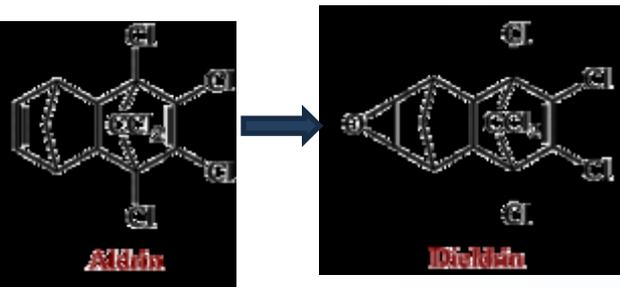
2. toxicita pro MO:

- obecně mají organické polutanty tendenci působit na biologické membrány (interakce s membr. fosfolipidy / proteiny) - nespecifická toxicita - narkóza (porušení integrity membrány, zvýšení její permeability)
- často však **také více či méně specifické reakce** s dalšími receptory (genotoxicita, ox. stres ...)
- spíše méně poznatků, zejména chybí pro „emerging“ POPs



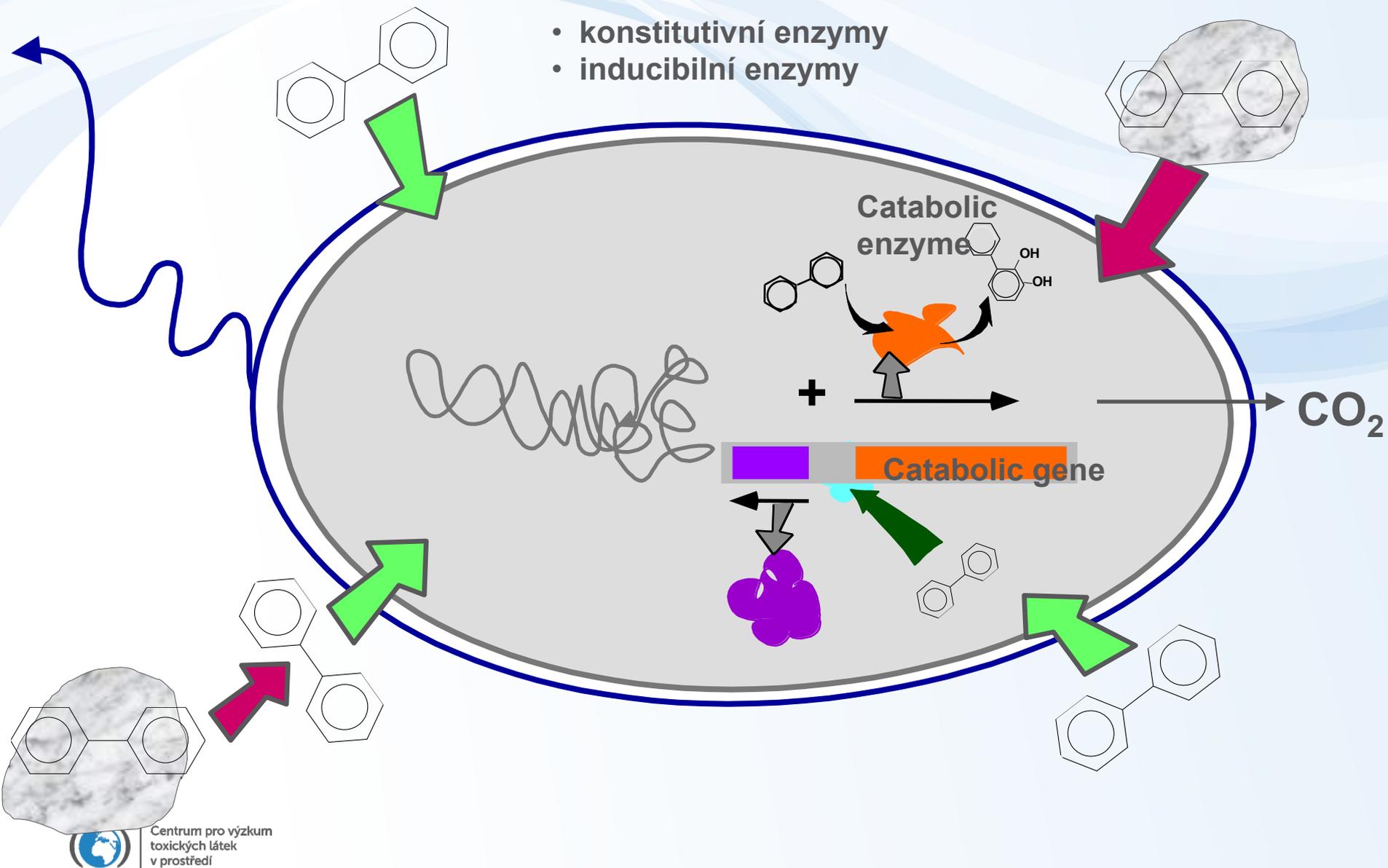
Interakce organických polutantů s MO

- Problematika interakcí MO a organických polutantů je úzce spjata s problematikou biodegradací a tyto dva obory (ekotoxikologie MO a biodegradace) nelze často zřetelně oddělit
- další možné typy interakce obou disciplín:
 - toxická látka snižuje biodegradční schopnosti MO → negativní dopad na čištění prostředí
 - degradovatelný polutant je přítomen v takové koncentraci, která je toxická 
 - při metabolizaci vzniká bioakumulativnější či toxicitější produkt → toxicita pro ostatní MO
 - TCE → vinylchlorid
 - fenol → PCP
 - aldrin → dieldrin
 - parathion → paraoxon
 - sekundární aminy → nitrosaminy

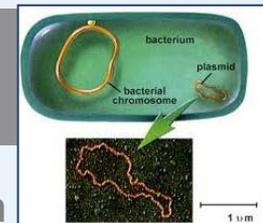


Biotransformace organických polutantů MO

- konstitutivní enzymy
- indukibilní enzymy



Biotransformace organických polutantů MO



- geny či operony kódující degradační enzymy jsou často kódovány na **plasmidech**
- to umožňuje genetické inženýrství nových degradátorů („superbiodegradátorů“) nebo snahy urychlit kinetiku (přirozené degradace jsou pomalé) nebo vývoj biosenzorů
- např. *Pseudomonas* sp. schopné degradovat více než 100 polutantů
- je také možné vymizení schopnosti (udržování selekčního tlaku při řízených bioremediacích)

Table 10.1 Typical *Pseudomonas* plasmids, their degradative pathways, and sizes.

Name of plasmid ^a	Compound degraded	Plasmid size (kb)
SAL	Salicylate	60
SAL	Salicylate	72
SAL	Salicylate	83
TOL	Xylene/toluene	113
pJP1	2,4-D	87
pJP2	2,4-D	54
pJP3	2,4-D	78
CAM	Camphor	225
XYL	Xylene	15
pAC31	3,5-Dichlorobenzoate	108
pAC25	3-Chlorobenzoate	102
pWWO	Xylene/toluene	176
NAH	Naphthalene	69
XYL-K	Xylene/toluene	135

^aPlasmids with the same name encode a similar degradative pathway despite the fact that they have different sizes and were described in different laboratories.

Adapted from Cork and Krueger, 1991, *Adv. Appl. Microbiol.* **36**:1–66.

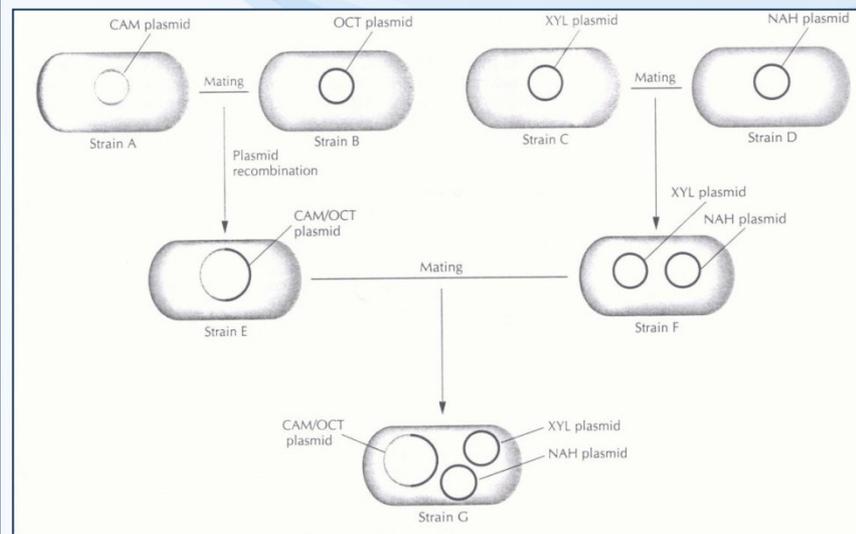


Figure 10.5 Schematic representation of the development of a bacterial strain that can degrade camphor, octane, xylene, and naphthalene. Strain A, which contains a CAM (camphor-degrading) plasmid, is mated with strain B, which carries an OCT (octane-degrading) plasmid. Following plasmid transfer and homologous recombination between the two plasmids, strain E carries a CAM and OCT biodegradative fusion plasmid. Strain C, which contains a XYL (xylene-degrading) plasmid, is mated with strain D, which contains a NAH (naphthalene-degrading) plasmid, to form strain F, which carries both of these plasmids. Finally, strain E and strain F are mated to yield strain G, which carries the CAM/OCT fusion plasmid, the XYL plasmid, and the NAH plasmid.



Biotransformace organických polutantů MO

- Stejný princip jako u organických látek (celulóza, lignin ...)
- série degradačních kroků podmíněných enzymy:
- **Extracelulární** - pokud je potřeba rozštěpit makromolekuly na menší látky, které mohou projít do buňky
- **Intracelulární** - další degradace
- Pokud nějaký enzym chybí - **konec degradace u meziproduktu**

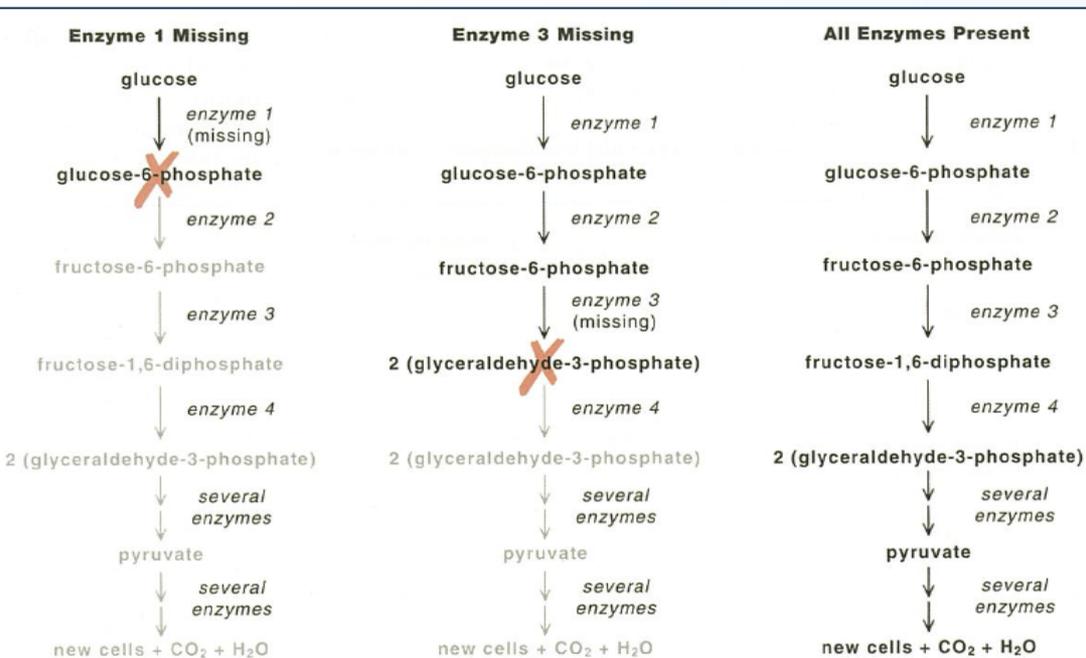
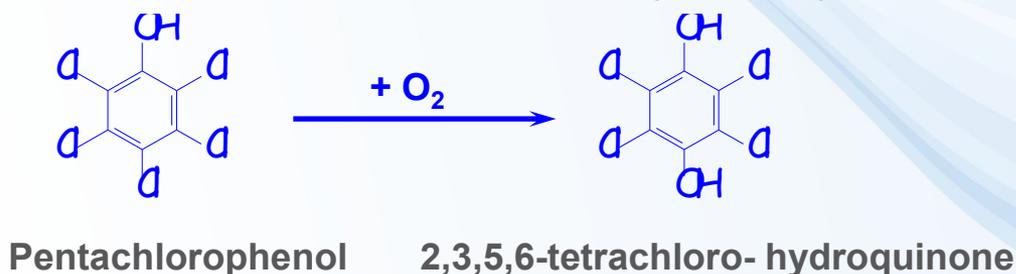


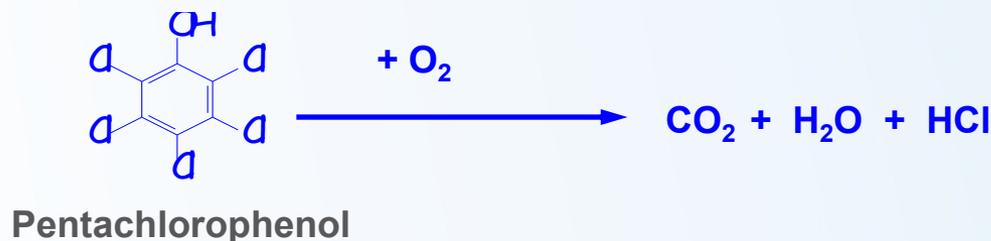
FIGURE 16.4 Stepwise degradation of organic compounds. A different enzyme catalyzes each step of the biodegradation pathway. (From *Pollution Science* © 1996, Academic Press, San Diego, CA.)

Biotransformace organických polutantů MO

- a) Částečná změna (metabolizace, částečná oxidace, hydroxylace, dehalogenace ...) s cílem zisku energie či obrany před toxikantem = **primární biodegradace**; často v anaerobním prostředí; řada enzymatických kroků



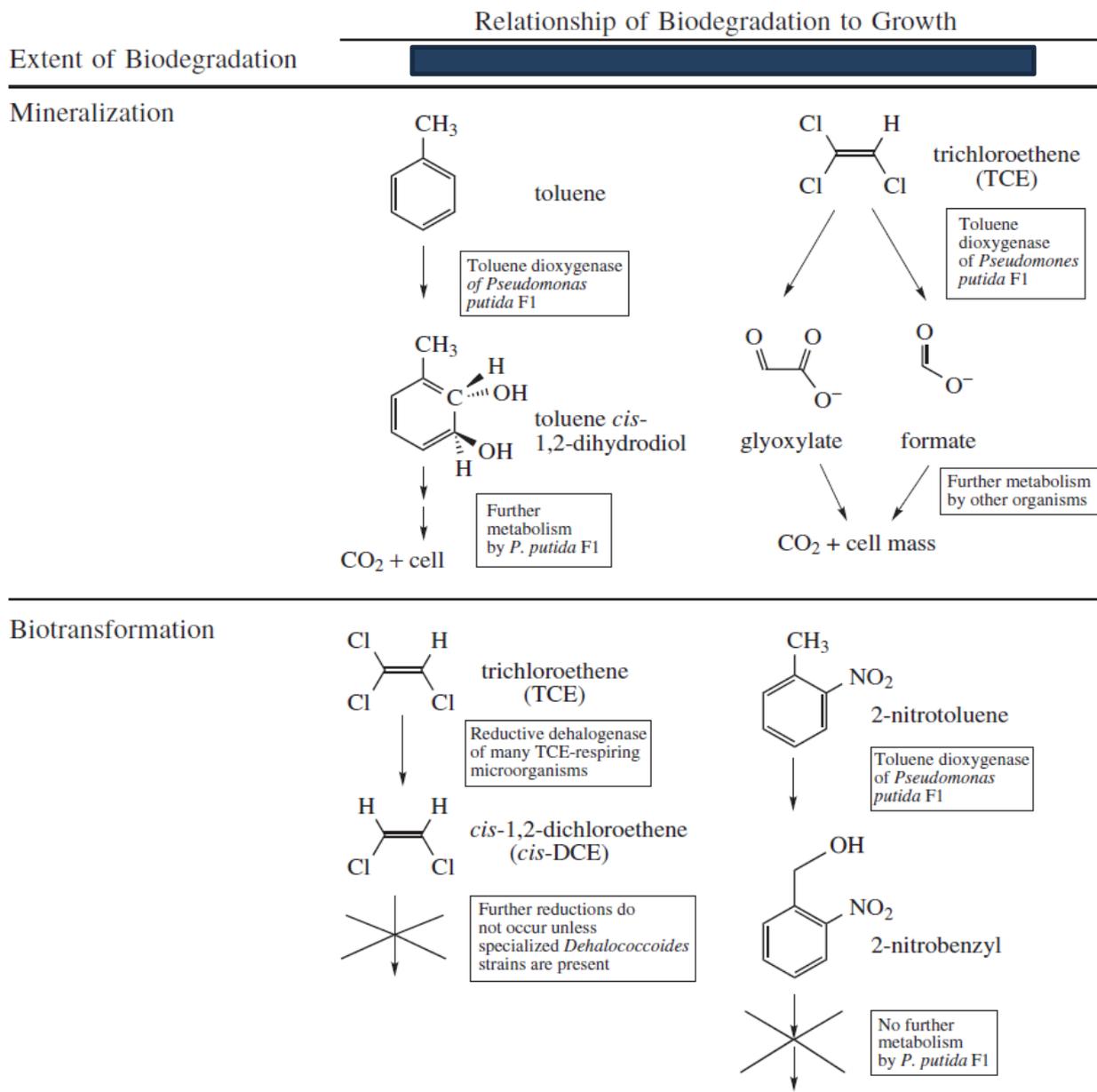
- b) **Mineralizace** – na CO_2 + energie = **ultimativní degradace**; vede k zisku energie a/nebo živin a/nebo růstu; může v mikrobiální buňce navazovat na předchozí nebo ...



- c) ... kooperace mnoha MO v konsorciu (substrátová symbióza)
d) další možnosti: polymerizace, kometabolismus

Biotransformace organických polutantů MO

Table 8.1 Classification of Example Biodegradation Reactions in a Bioremediation Matrix^a

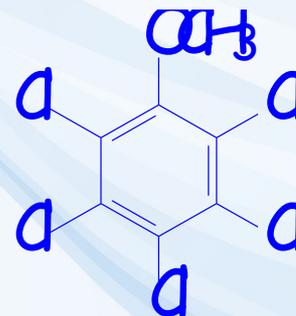
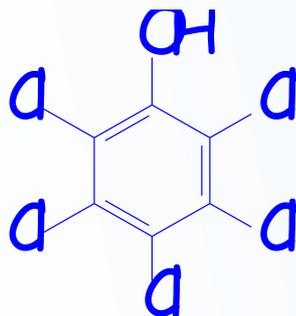


^aThe potential for successful bioremediation is highest for metabolic reactions that lead to mineralization and lowest for co-metabolic substrates that undergo minor biotransformations.

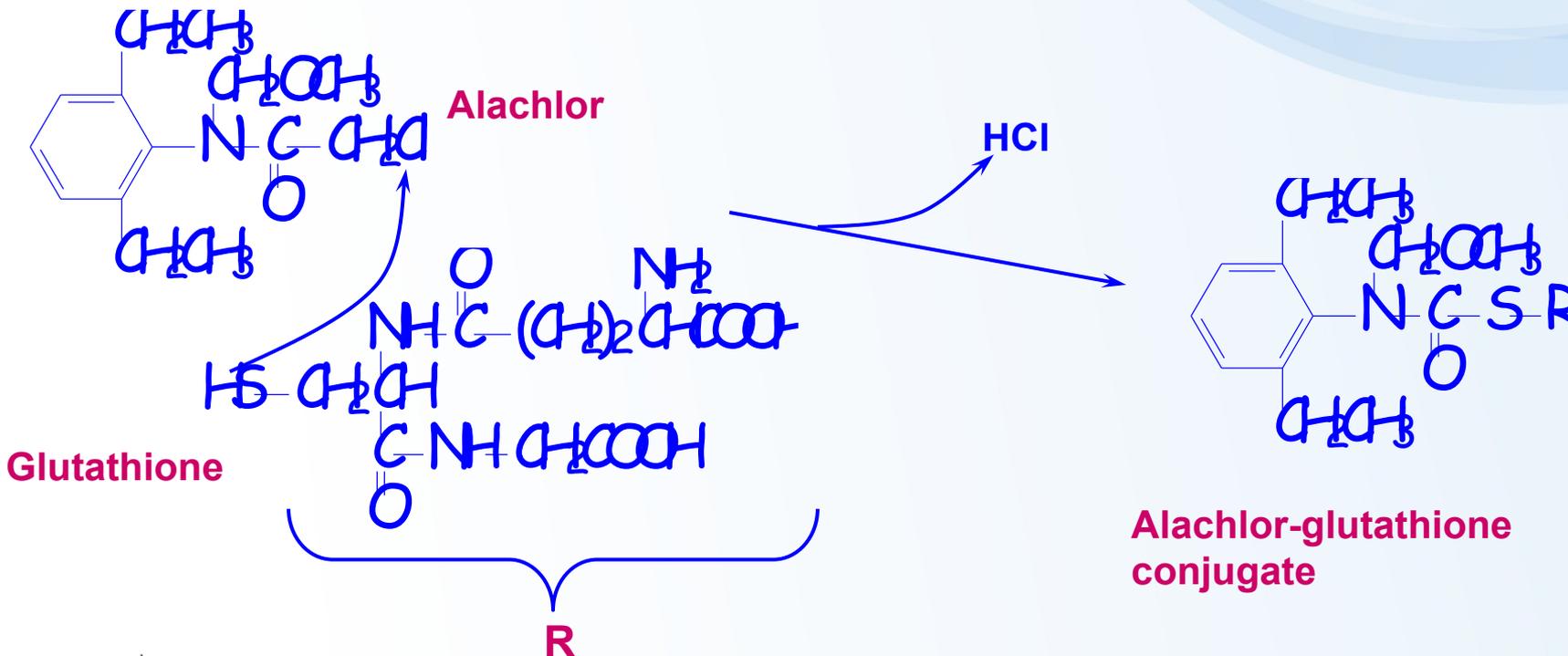
Biotransformace organických polutantů MO

Příklady biotransformace - metabolizace detoxifikace

Pentachloro-phenol

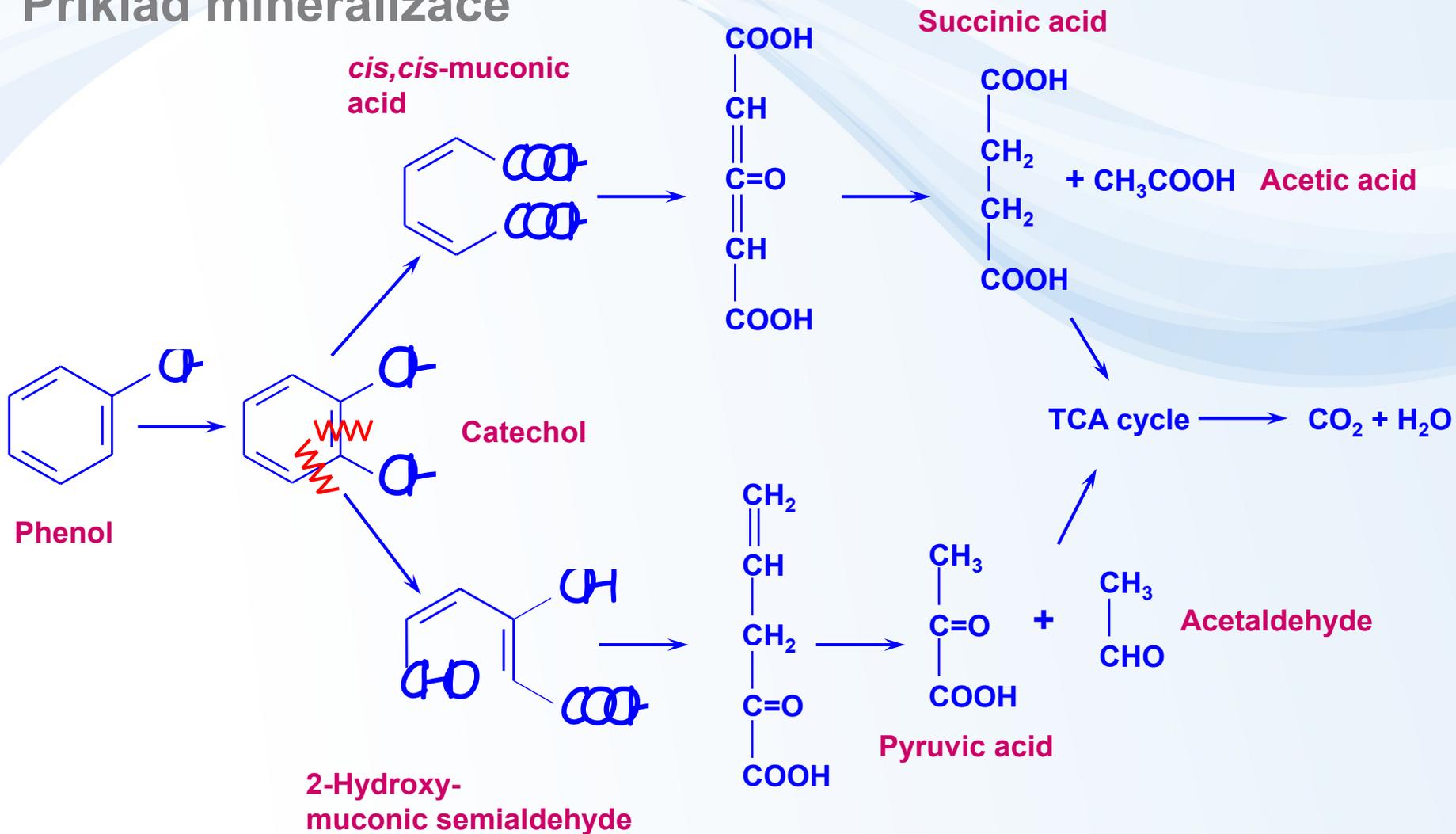


Pentachloro-anisole



Biotransformace organických polutantů MO

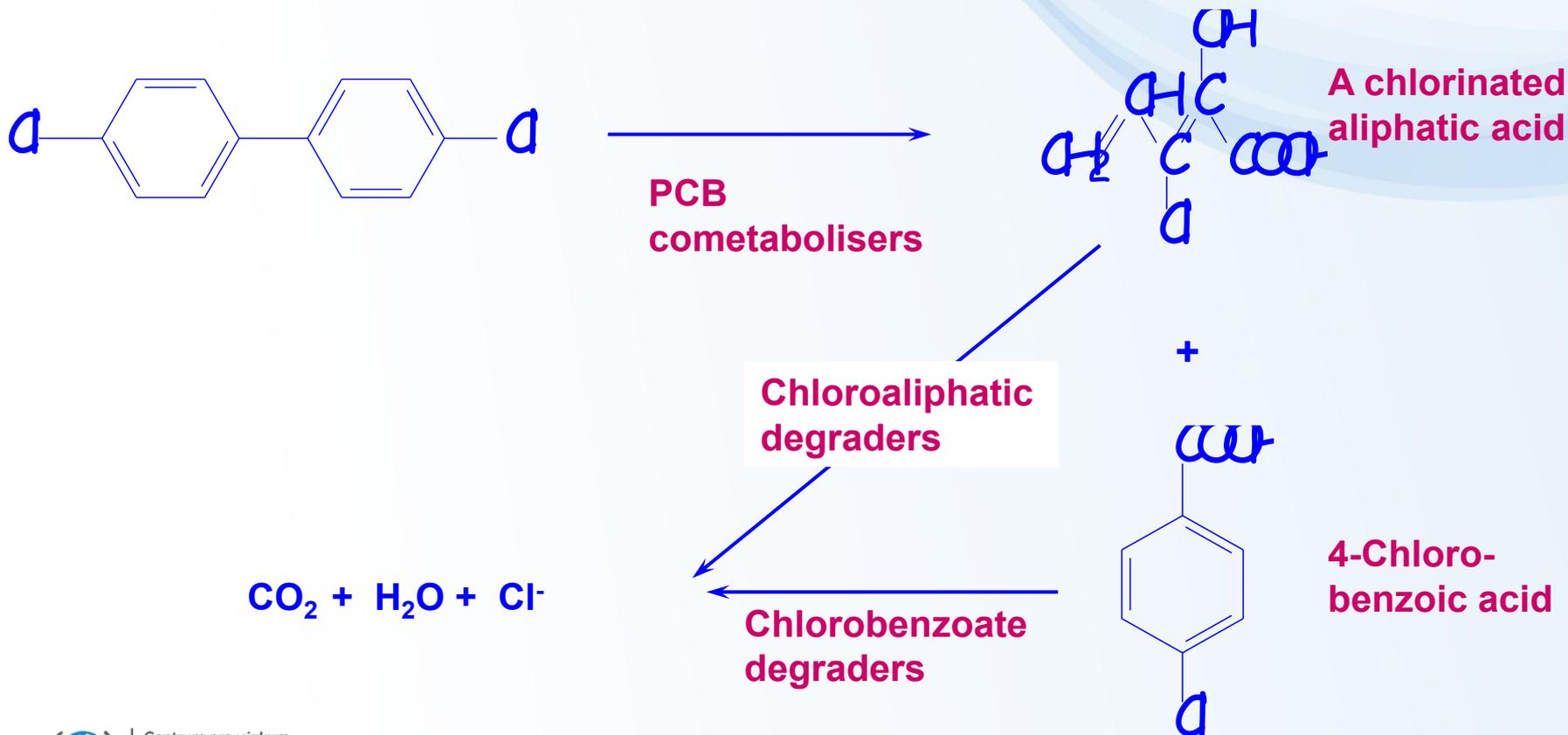
Příklad mineralizace



Biotransformace organických polutantů MO

Příklad práce MO v konsorciu

Aerobic:



Biotransformace organických polutantů MO

Příklad práce MO v konsorciu

Anaerobic:

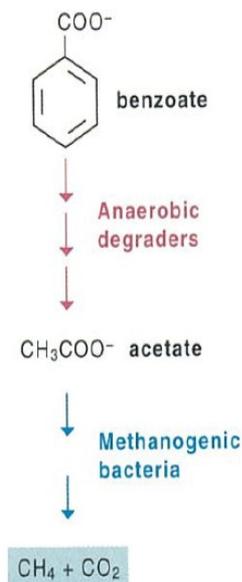
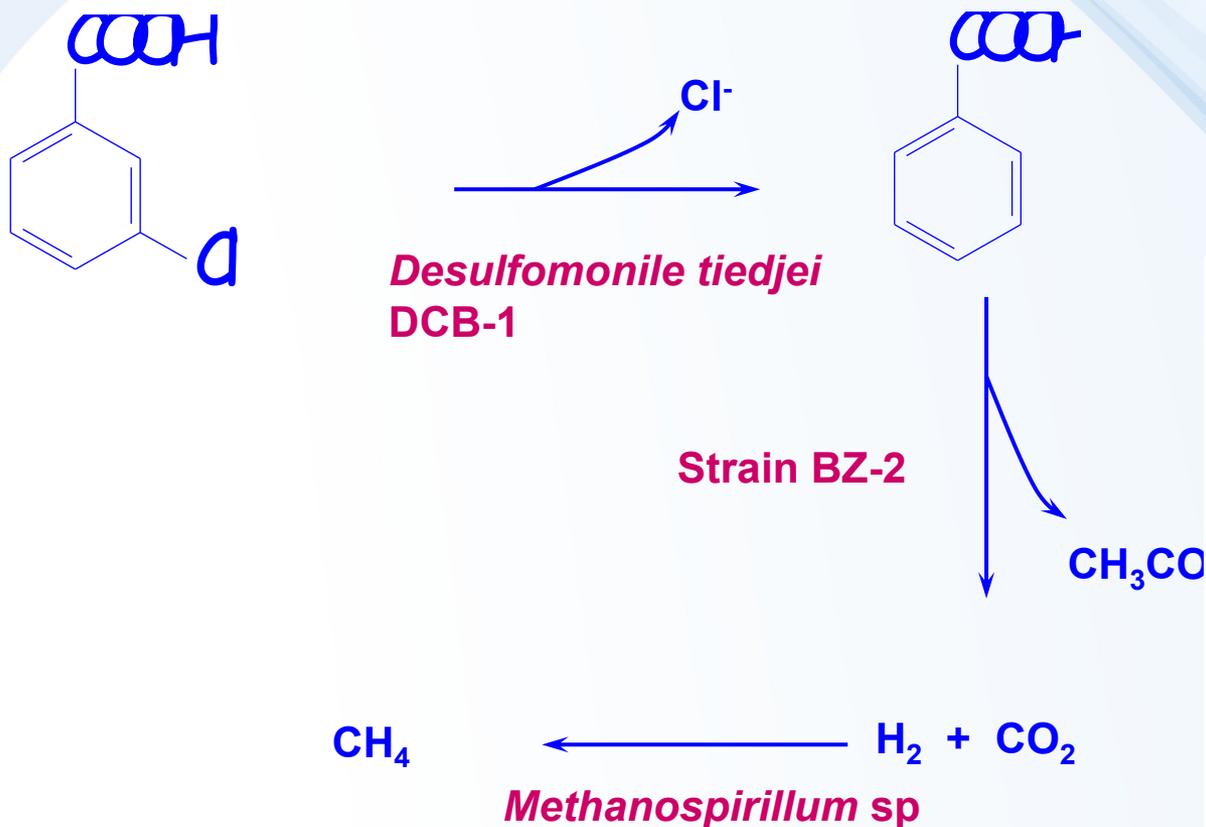


FIGURE 16.27 Anaerobic biodegradation of aromatic compounds by a consortium of anaerobic bacteria. (From *Pollution Science*, © 1996, Academic Press, San Diego, CA.)



Příklad práce MO

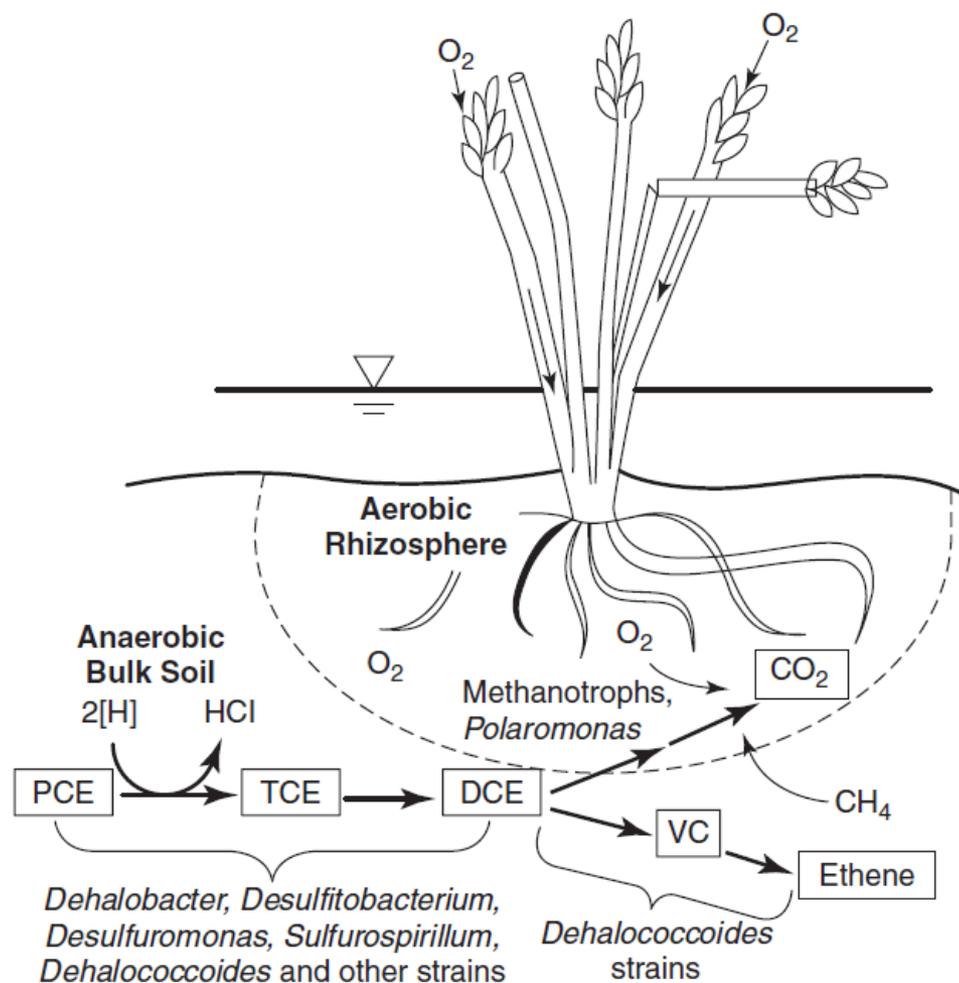


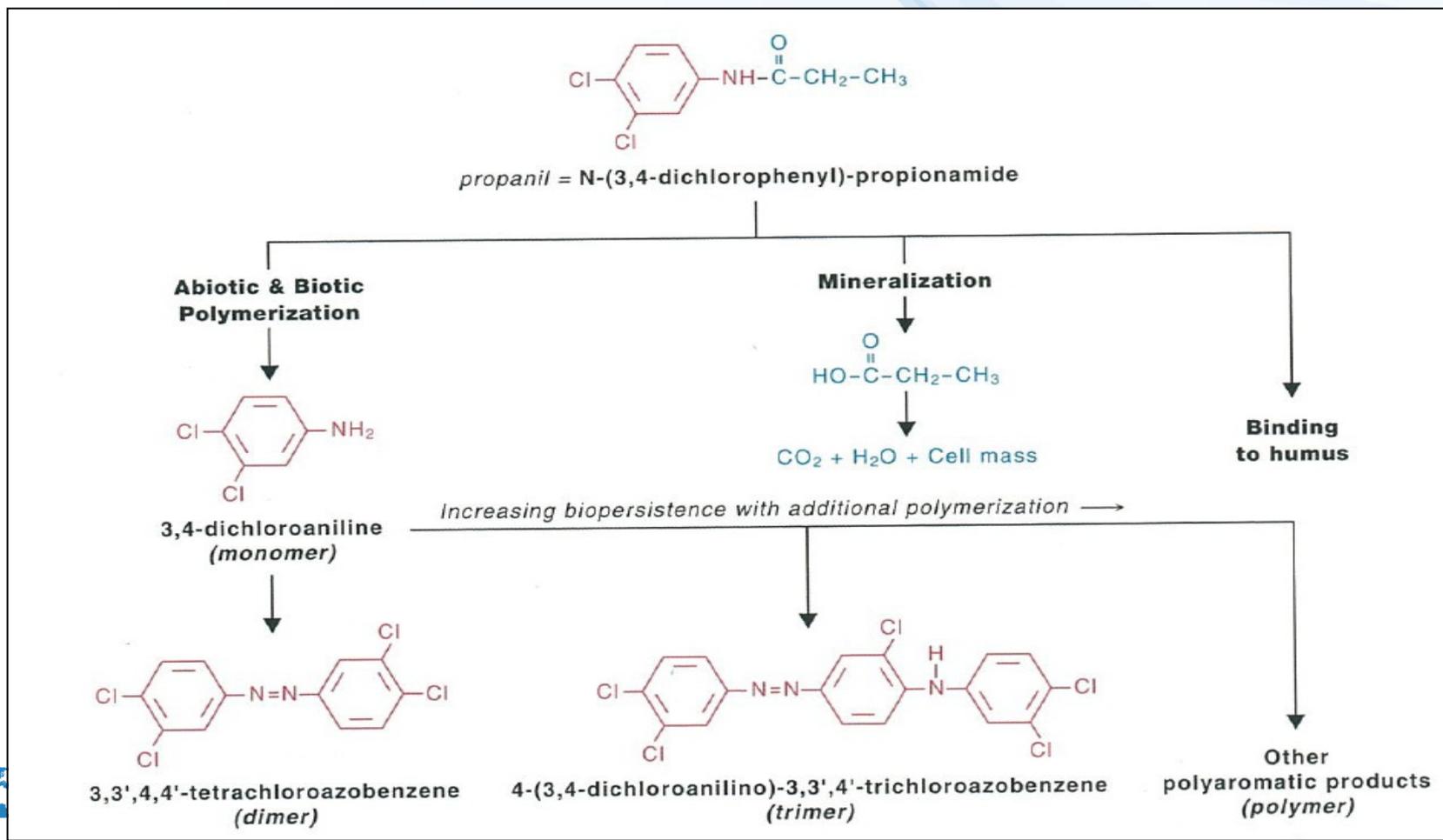
Figure 8.1 Potential pathways leading to complete detoxification of PCE in wetland environments, which typically contain anaerobic bulk soils and aerated zones within the rhizosphere. Certain strains within the genera listed can transform individual chlorinated ethenes metabolically via dehalorespiration or, in the case of *Polaromonas*, via oxidation. Some methanotrophs can oxidize lesser chlorinated ethenes co-metabolically.



Biotransformace organických polutantů MO

Příklad polymerizace

- Nastává často tam, kde extracelulární enzymy vytvoří reaktivní intermediáty, které následně zpolymerizují
- Důsledkem jsou nedegradovatelné látky (nerozpustné, sorbované, nejsou enzymy)

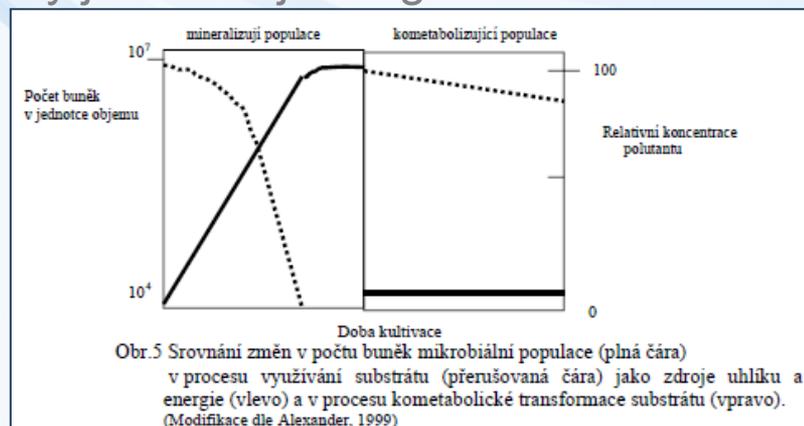


Biotransformace organických polutantů MO

• Velice často se vyskytuje tzv. kometabolismus

– přestože daný polutant je pro MO nevyužitelný jako zdroj energie nebo živin (ani C), probíhá ko-oxidace jeho molekuly

1. paralelně s jiným metabolickým dějem, nebo
2. v MO je enzym, který reakci „nechtěně“ provede, nebo
3. daná molekula je potřeba pro spřažení s další reakcí např. jako TEA (potřeba další substrát – např. e⁻ donor)

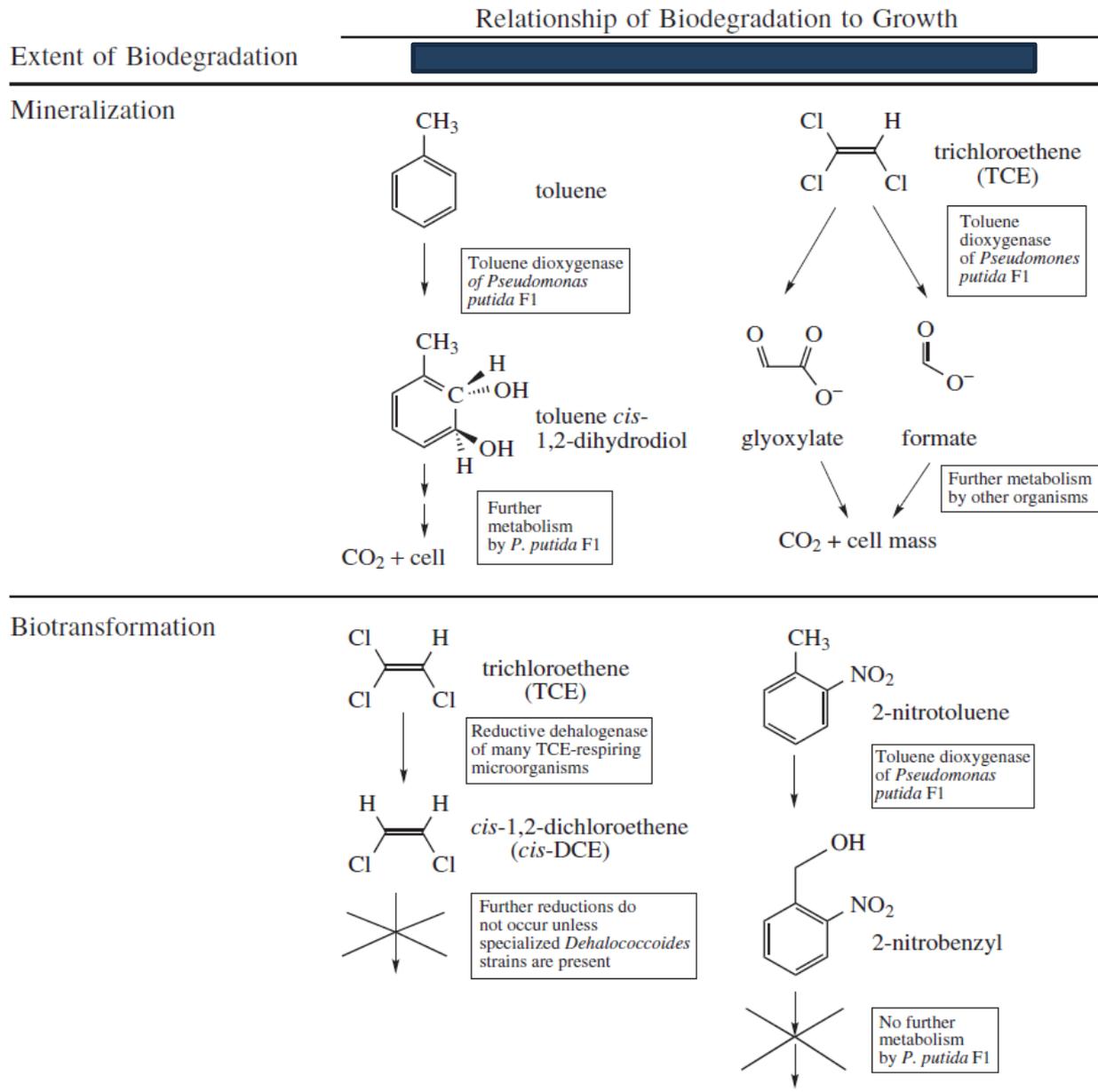


- enzymy s **nízkou substrátovou specifitou**: oxygenázy, dehalogenázy ...
- často končí dead-end produktem, nebo produktem bez dalšího využití - kumulace, nebo dokonce produktem, který je toxický a inhibuje růst a aktivitu daného MO či okolních MO
- příklady: cyklohexan → cyklohexanol, PCBs, chlorfenoly, 1,3,5-trinitrobenzen, alachlor, chlorbenzen → 3-chlorcatechol, parathion → 4-nitrofenol, DDT → DDE (DDD), propan → propionát (aceton), metylflorid → formaldehyd
- využití při bioremediacích: přídavek bifenyly spouští degradaci PCBs

Table 8.1 Classification of Example Biodegradation Reactions in a Bioremediation Matrix^a

Biotransformace organických polutantů MO

Kometabolismus



^aThe potential for successful bioremediation is highest for metabolic reactions that lead to mineralization and lowest for co-metabolic substrates that undergo minor biotransformations.

Biotransformace organických polutantů MO

Kometabolismus - příklad

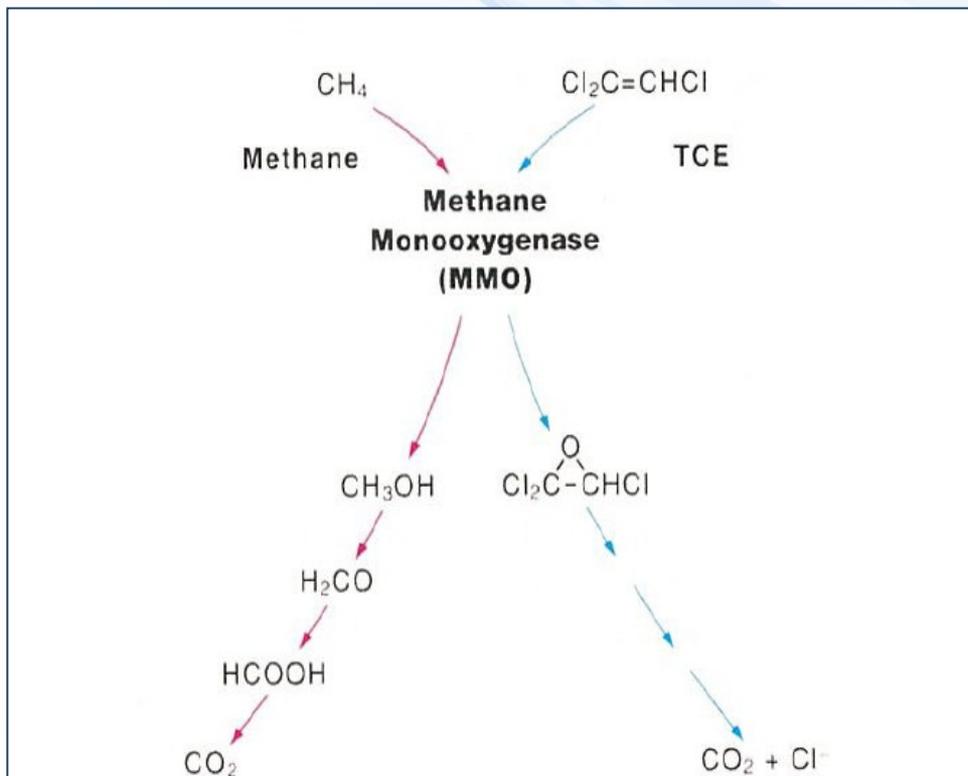


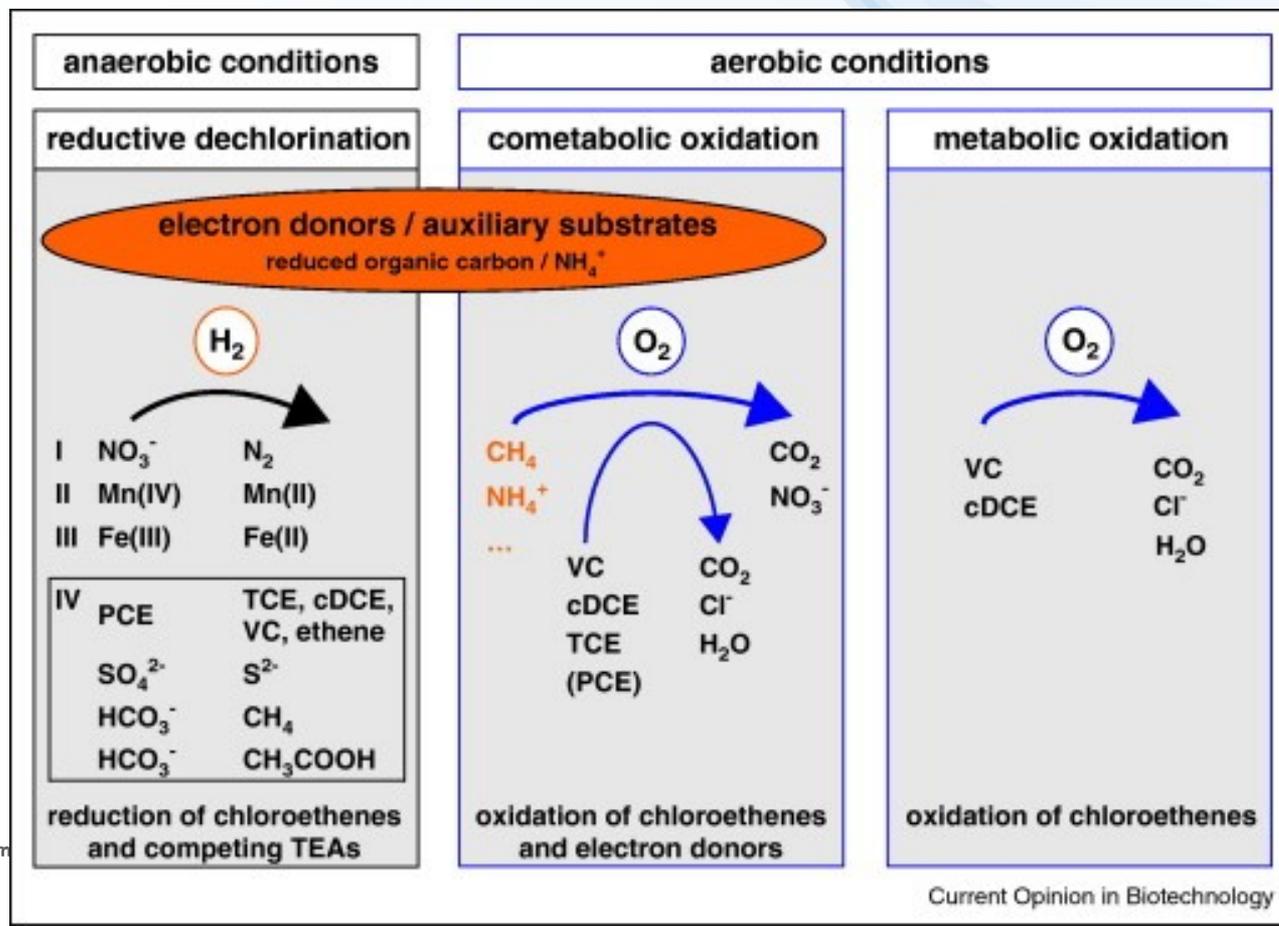
FIGURE 16.5 The oxidation of methane by methanotrophic bacteria is catalyzed by the enzyme methane monooxygenase. Subsequent degradation steps may be catalyzed spontaneously, by other bacteria, or in some cases by the methanotroph. (From *Pollution Science* © 1996, Academic Press, San Diego, CA.)



Biotransformace organických polutantů MO

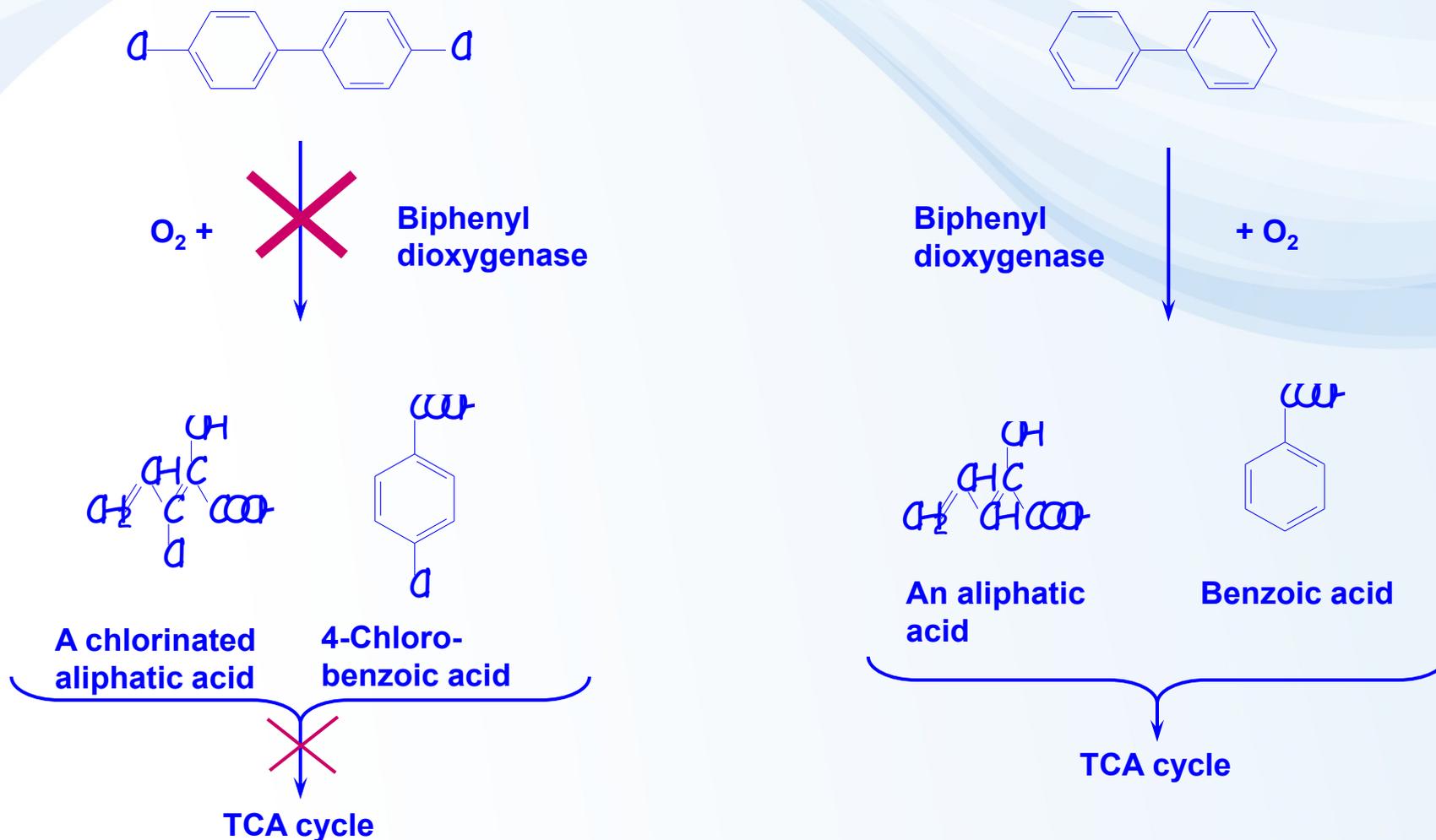
Kometabolismus - příklad

Comparison of reductive dechlorination with cometabolic and metabolic oxidation in terms of need for auxiliary substrates as electron donors and competing reactions (PCE: perchloroethene; TCE: trichloroethene; cDCE: *cis*-1,2-dichloroethene; VC: vinyl chloride; and TEA: terminal electron acceptor). The numbers indicate the order of the different respiration processes



Biotransformace organických polutantů MO

Kometabolismus - příklad



Faktory biotransformace organických polutantů MO

- **vlastnosti polutantu**, zejména:

- biodostupnost (ovlivněna též následujícími faktory + vlastnostmi prostředí)
- molekulová hmotnost
- struktura - stérické efekty, elektronové efekty
- hydrofobicita, polarita
- reaktivita
- toxicita polutantu pro MO
- tenze par, Henryho konstanta
- rozpustnost ve vodě, rozpustnost v tucích
- adsorptivita
- K_{OW} , K_{OC} , K_A
- koncentraci polutantů - nízká i vysoká je špatně

ovlivňují např. energetickou náročnost prvního ataku molekuly (E se spotřebovává)



Faktory biotransformace organických polutantů MO

Tabulka 8 Základní charakteristika některých organických polutantů (podle Montgomery, 1991)

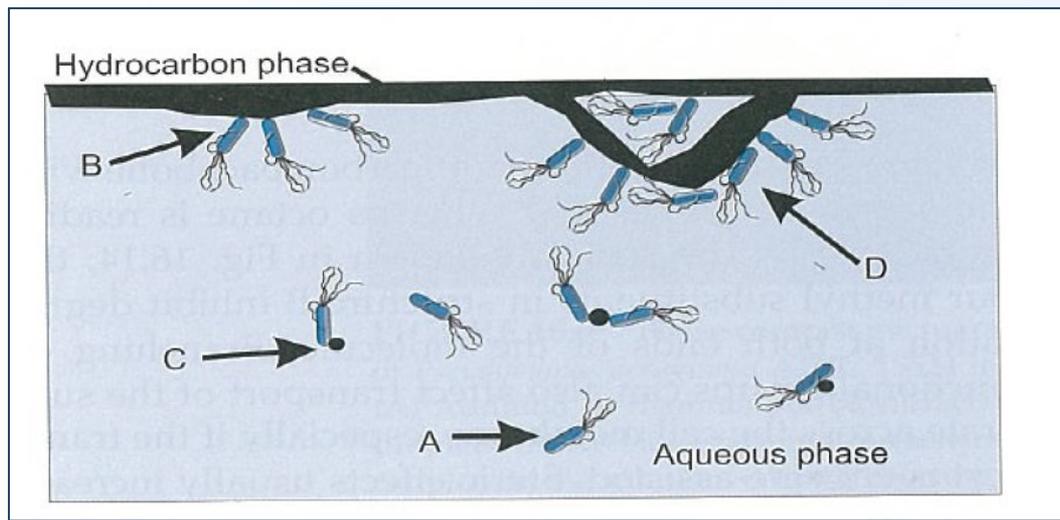
Polutant	Hustota	Bod varu (°C)	Rozpustnost ve vodě (mg/l)	tlak par (mm Hg)	Henryho konstanta při 25°C (atm:m ³ /mol)
Benzen	0,877	80,1	1780	76	5,48.10 ⁻³
Toluen	0,867	110,6	515	22	6,74.10 ⁻³
o-xylen	0,88	144,4	152	6,6	5,35.10 ⁻³
n pentan	0,626	36,1	39,5	426	1,255
1,1-DCA	1,176	57,3	5500	234	5,45.10 ⁻³
1,2-DCA	1,235	83,5	8690	64	1,31.10 ⁻³
1,2-DCE	1,257	47,5	6260	265	5,32.10 ⁻³
PCE	1,623	121,2	150	14	1,16.10 ⁻²
TCE	1,464	87,2	1100	57,8	9,9.10 ⁻³
Dodekan	0,749	216,3	3,4.10 ⁻³	0,3	2,4.10 ⁻²
naftalen	1,162	217,9	30	5,4.10 ⁻²	7,34.10 ⁻⁴
B(a)P	1,351	495	3,8.10 ⁻³	2,4-5,6.10 ⁻⁹	<2,4.10 ⁻⁶
fenol	1,058	181,7	93000	0,2	3,97.10 ⁻⁷
PCB 1221	1,15	275-320	0,59	6,7.10 ⁻³	3,24.10 ⁻⁴



Faktory biotransformace organických polutantů MO

biodostupnost - příklad

- ve vodním prostředí hraje roli také hustota polutantu:
 - lehčí než voda - klesne ke dnu (nádrž, jezero, moře, ale i zvodeň) - PCBs, TCE
 - těžší než voda - na povrchu - ropné látky, benzen
- organické látky ve vodě - několik možností příjmu MO:
 - A. příjem rozpuštěné frakce
 - B. přímý kontakt buněk (\uparrow hydrofobní povrch buněk)
 - C. dispergované kapičky polutantu $< \mu\text{m}$ (voceán vlny, vítr x půda)
 - D. MO produkují surfaktanty a ty zvýší rozpustnost, ale i hydrofobicitu buněk



Faktory biotransformace organických polutantů MO

- vlastnosti polutantu - příklady: vliv struktury - sterické efekty
- větvení, funkční skupiny - čím více a větší tím pomalejší degradace - vliv na vstup do reakčního místa či přestup přes membránu

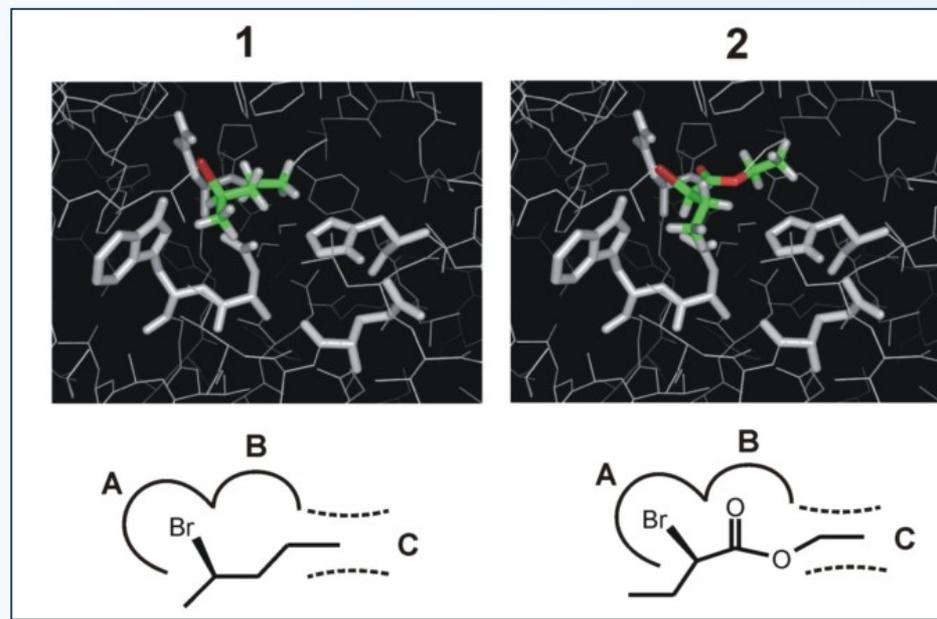
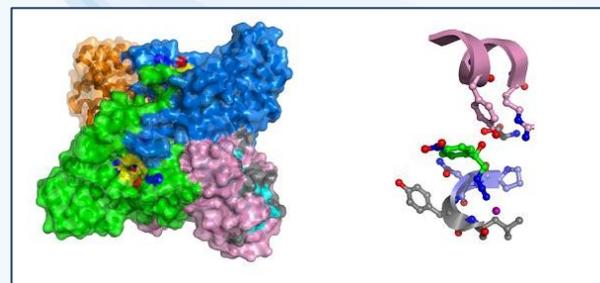
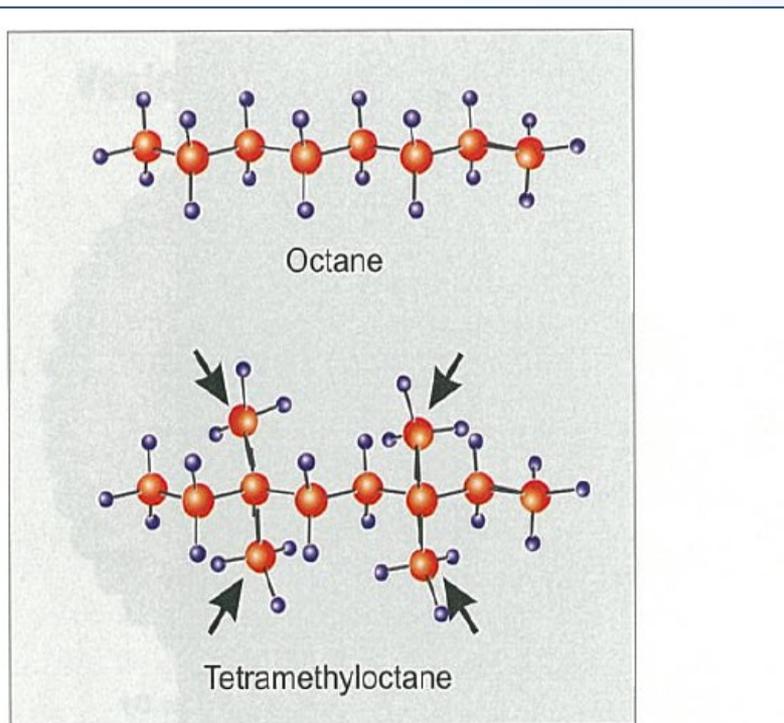


FIGURE 16.10 The structure of (A) octane, which is readily degradable, and (B) a tetramethyl-substituted octane that is not degraded because the methyl groups block the enzyme-substrate catalysis site.

Faktory biotransformace organických polutantů MO

- vlastnosti polutantu - příklady: vliv struktury - elektronové efekty
- substituenty mohou být e- donory (CH_3) či akceptory (Cl)
- změny elektronové hustoty v reakčním místě: zvyšující e- hustotu zvýší degradaci, snižující e- hustotu sníží degradaci

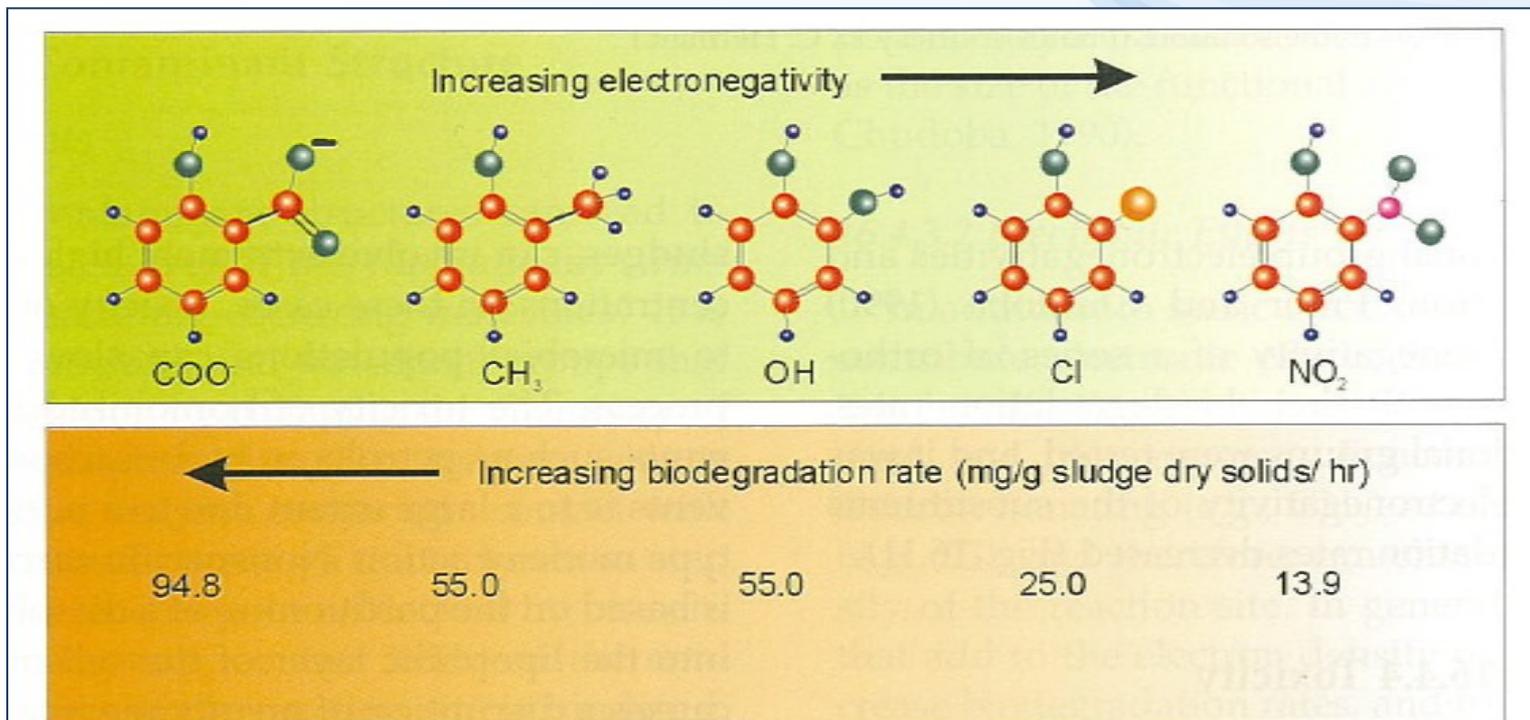
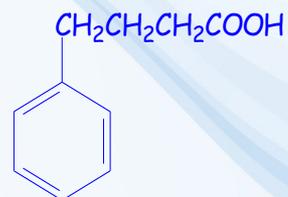
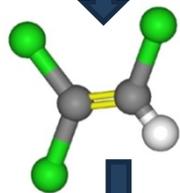
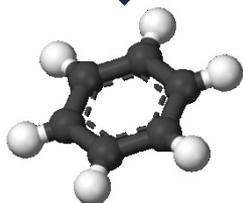
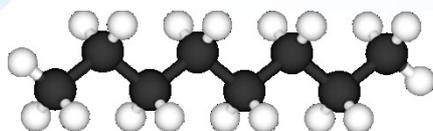


FIGURE 16.11 Various ortho-substituted phenols and their respective biodegradation rates. (Adapted from Pitter and Chudoba, 1990.)



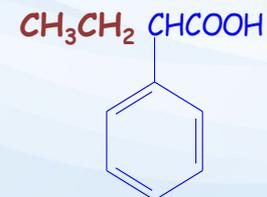
Faktory biotransformace organických polutantů MO

- vlastnosti polutantu - příklady: vliv struktury



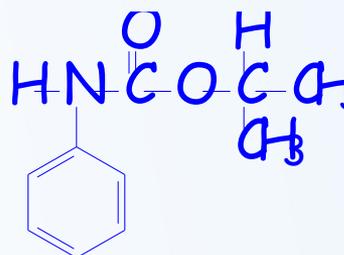
4-phenylbutyric acid

Rapidly degraded

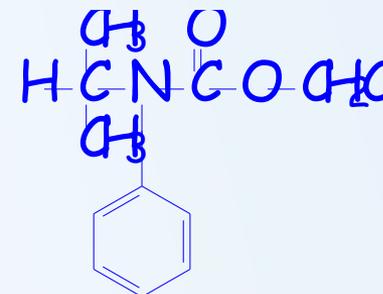


2-phenylbutyric acid

Slowly degraded



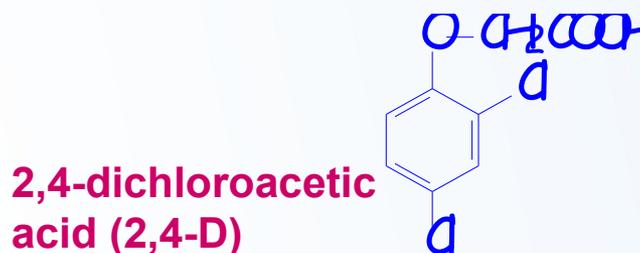
Propham



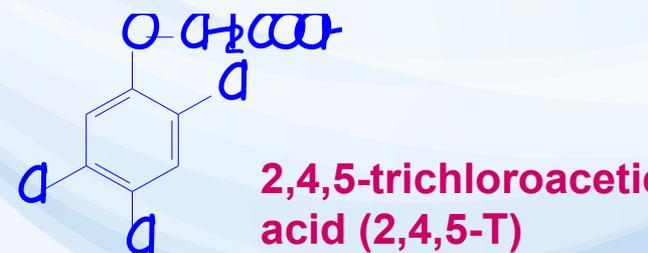
Propachlor

Faktory biotransformace organických polutantů MO

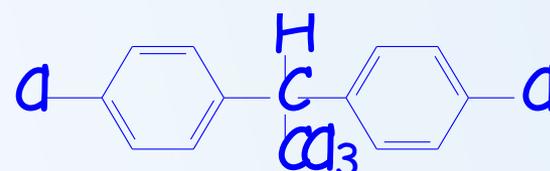
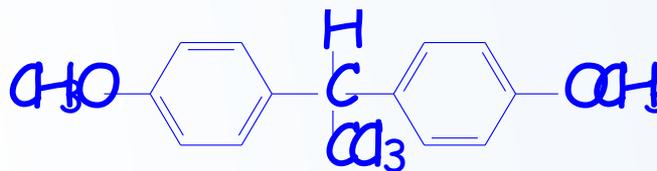
- vlastnosti polutantu - příklady: vliv pozice substituentů



Rapidly degraded



Slowly degraded

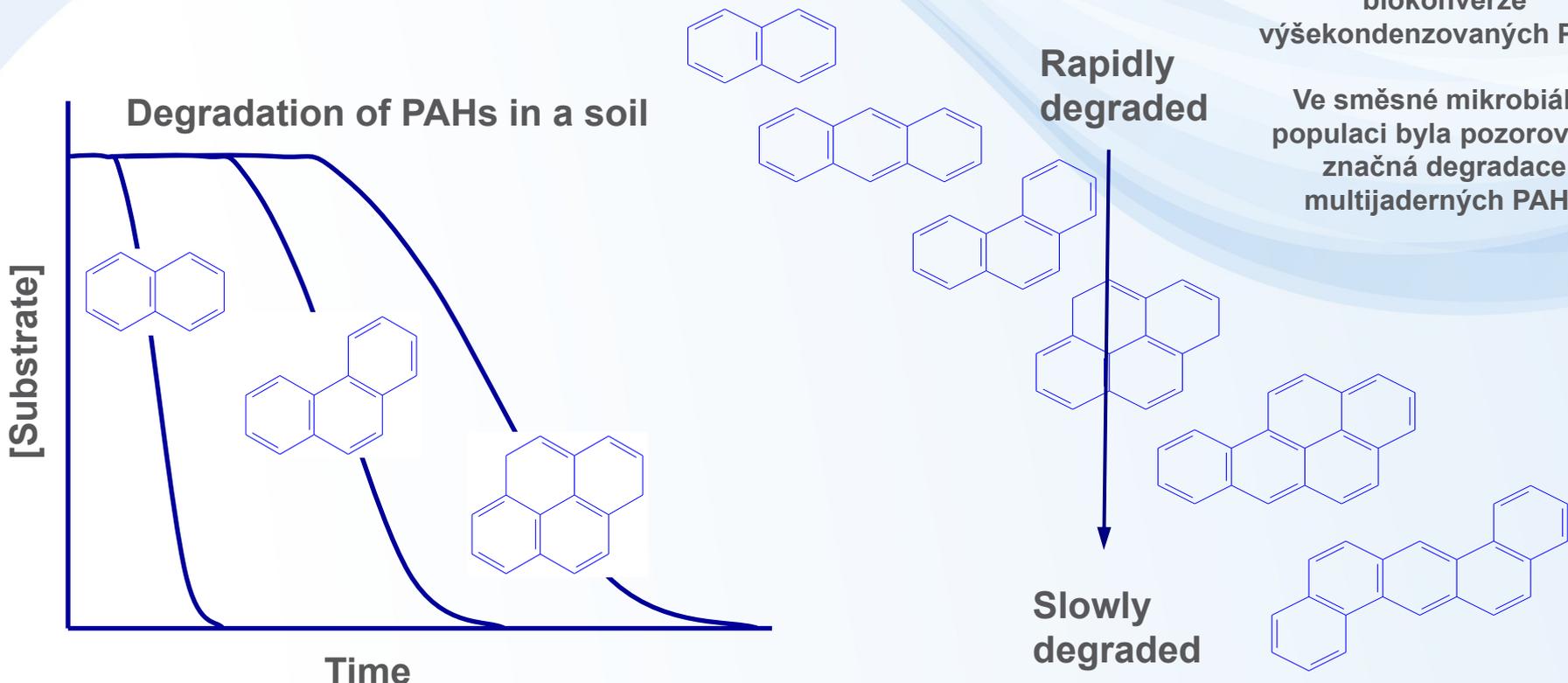


- Biodegradabilita chlorovaných fenolů: 2,4 > 4 > 3,5 > 2,6 > 3 nebo 5 nebo 2
- trichlorfenoly 2,3,6-, 2,4,5-, 3,4,5- biodegradabilní pouze za aerobních podmínek



Faktory biotransformace organických polutantů MO

- vlastnosti polutantu - příklady: vliv MW



Bakterie jsou schopny za přítomnosti jiného, využitelného zdroje uhlíku biokonverze výšekondenzovaných PAHs

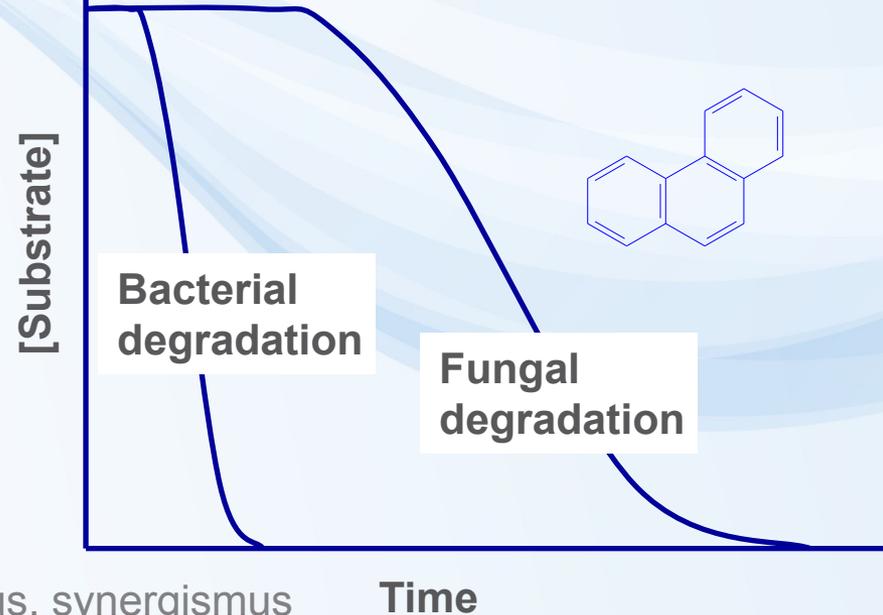
Ve směsné mikrobiální populaci byla pozorována značná degradace multijaderných PAHs

Pozn.: Ačkoliv rychlost degradace v sedimentech klesá s rostoucí molekulovou hmotností, rostoucí tendence vysokomolekulárních PAHs k adsorpci vyrovnává tento děj

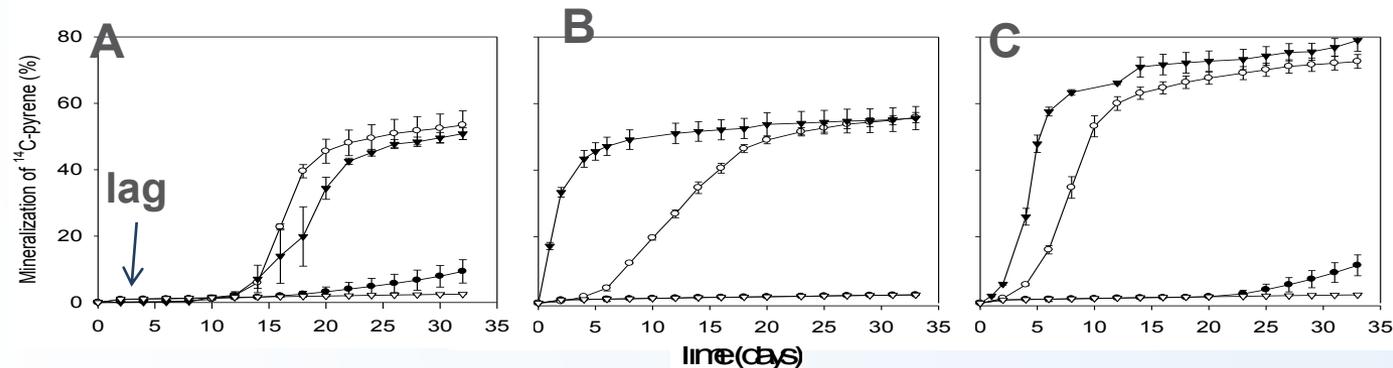
Faktory biotransformace organických polutantů MO

- schopnosti a stav MO, společenstva
- enzymatická výbava, genetická výbava
- typ metabolismu
- schopnosti přijmout polutant do buňky
- stavu buňky
- „aklimatizace“ (lag fáze)
 - dřívější „zkušenost“ s danou látkou či jejím přírodním analogem
 - schopnost adaptace, mutace, genový transfer
 - čas nutný k indukci biosyntézi enzymů
- vlastnostech celého konsorcia
 - návaznost metabolických drah - komenzalismus, synergismus

Degradation of phenanthrene in a soil by bacteria and fungi



Evolution of pyrene catabolism for 50 mg pyrene kg⁻¹ (A), 2 x 50 mg pyrene kg⁻¹ (B) and 4 x 50 mg pyrene kg⁻¹ (C) amendments, over 0 (▽), 4 (●), 8 (○) and 12 (▼) weeks soil-pyrene contact time. Error bars are the SEM of n=3.



Faktory biotransformace organických polutantů MO

- faktory prostředí
- vše, co ovlivní biodostupnost/osud látky
- pH, T, kyslík, substrát (např. dostupný zdroj C), živiny (C/N/P - 100/10/1)
- přítomnost dalších látek – kosubstráty, TEA apod.
- potřeby MO - zejména dostatek vody ($a_w > 0,96$ pro bct a $> 0,7$ pro houby)

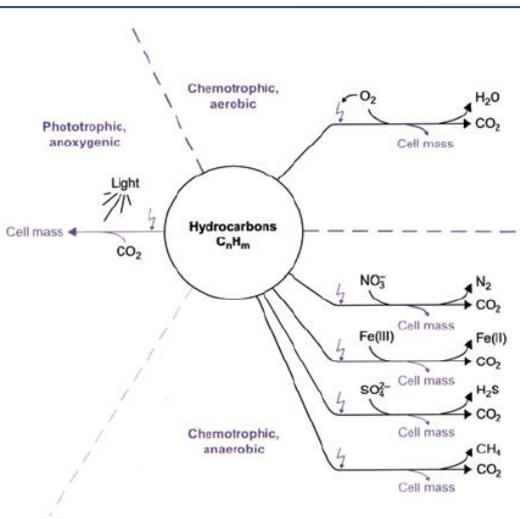


Figure 7.9 Experimentally verified possibilities for the microbial utilization of hydrocarbons. In all chemotrophic reactions, a part of the hydrocarbon is oxidized for energy conservation (catabolism) and another part is assimilated into cell mass. In the long-established aerobic oxidation of hydrocarbons (upper right), oxygen is not only the terminal electron acceptor, but is also needed for substrate activation (oxygenase reactions). The anaerobic pathways involve novel hydrocarbon activation mechanisms that differ completely from the aerobic mechanisms. Jagged arrows indicate hydrocarbon activation. (Reprinted from Widdel, F. and R. Rabus. 2001. Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr. Opin. Biotechnol.* 12:259–276. Copyright 2001, with permission from Elsevier.)

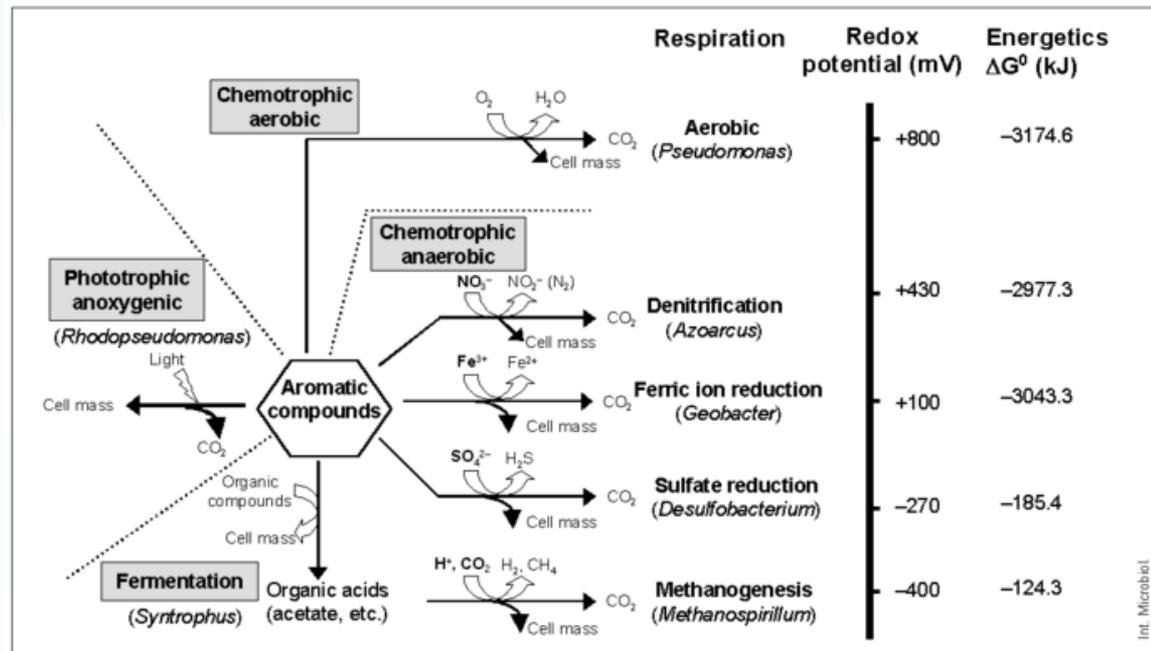


Fig. 2. Microbial utilization of aromatic compounds. The different terminal electron acceptors in respiration are indicated in bold and they are aligned with the redox potential bar. The energetics (free-energy changes) of the aerobic and anaerobic degradation of a model aromatic compound, benzoate, are indicated on the right. Methanogenesis needs to be coupled to fermentation reactions. Bacterial genera representative of each type of metabolism are shown in parentheses. Modified from [35].

Faktory biotransformace organických polutantů MO

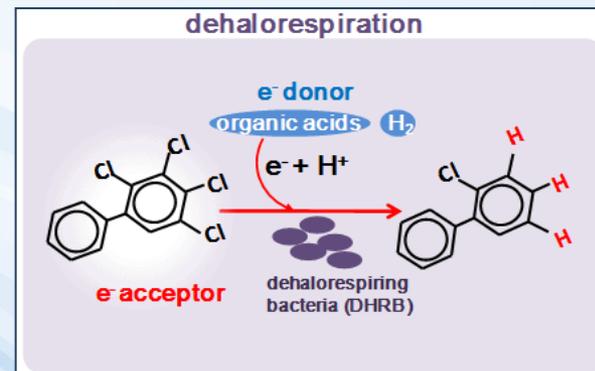
zásadní rozdíl mezi

• anaerobní

- podmínky bez kyslíku (např. sedimenty)
- pomalá, delší aklimatizace
- menší spektrum metabolických cest
- v ideálním případě produkce CH_4 a CO_2 (jako v ČOV)
- často ve spojitosti s redukčními pochody - reduktivní dehalogenace; např. propojení s denitrifikací, redukcí železa, desulfurikací

• aerobní

- prostředí s kyslíkem
- rychlejší, nutný např. pro vysoce redukované polutanty (alkany $> \text{C}_{16}$)
- vznik kyslíkatých meziproduktů
- nutná určitá struktura POPs - např. halogenace brání v přístupu enzymů štěpících aromatický kruh - kombinace aero- a anaero- v bioremediaci



Faktory biotransformace organických polutantů MO

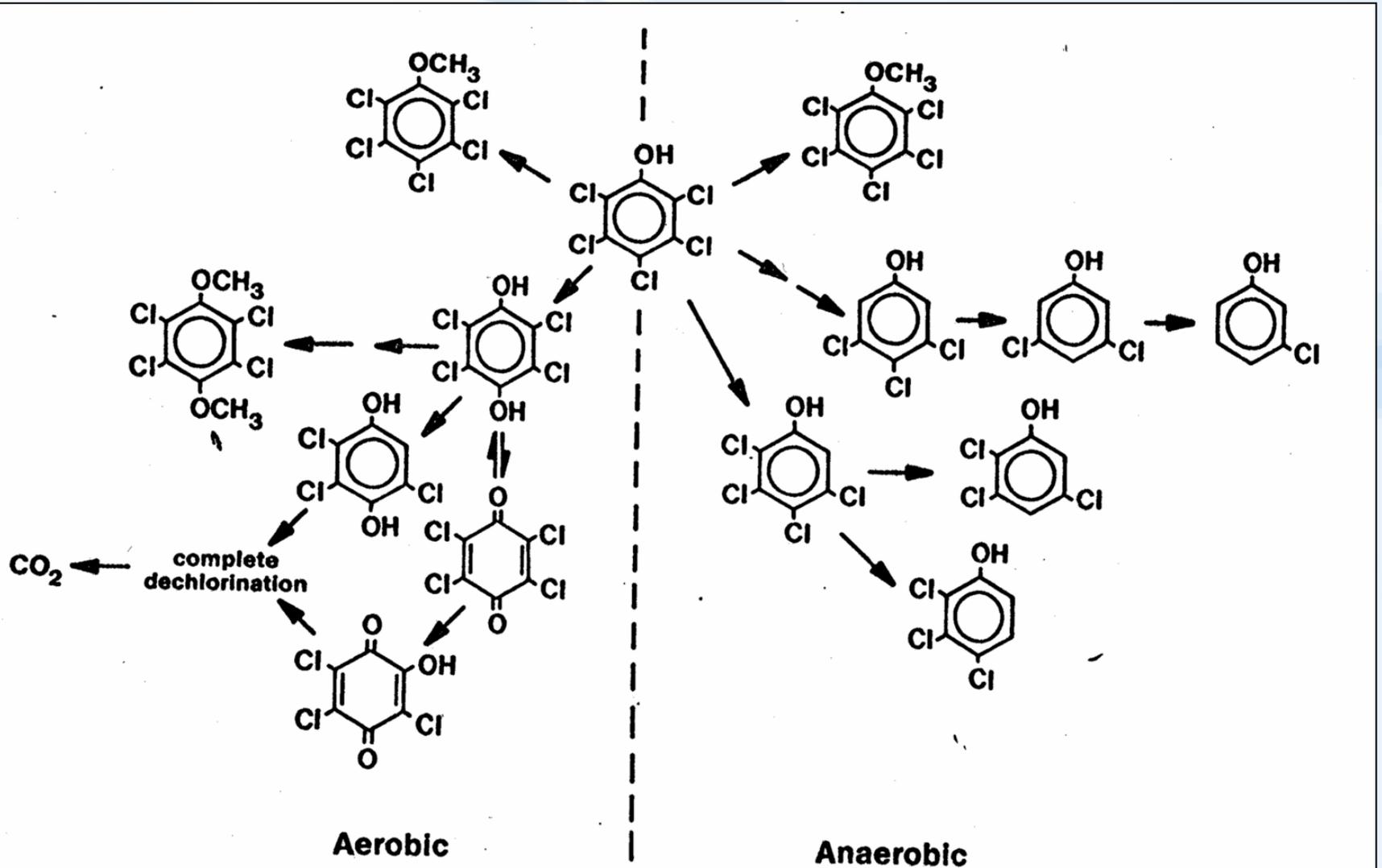


Fig. 8-4. Comparison of typical degradation pathways of pentachlorophenol in aerobic and anaerobic environments.

Faktory biotransformace organických polutantů MO

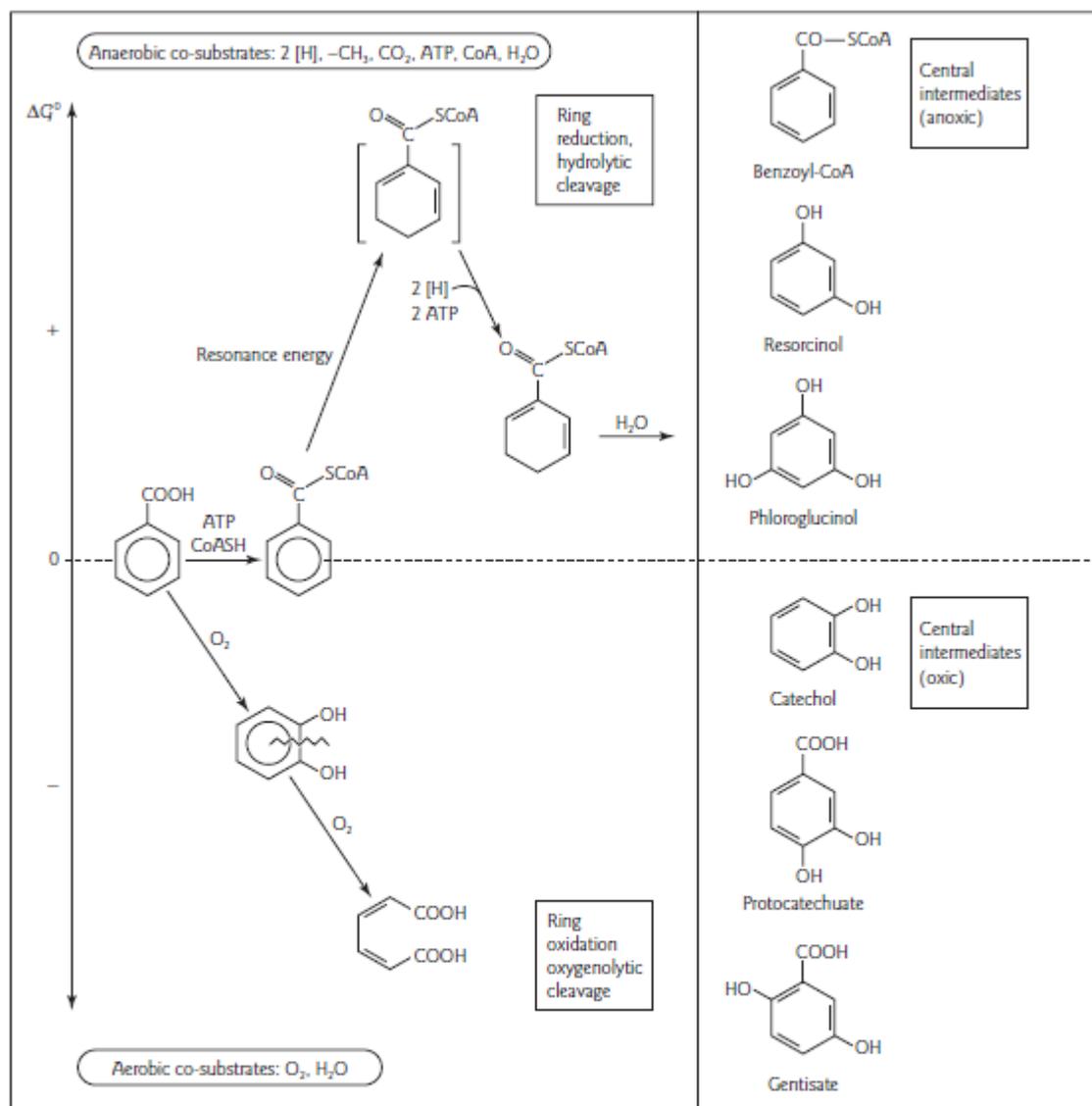


Figure 7.11 Comparison of aerobic (oxygen-dependent) and anaerobic (anoxic) metabolism of aromatic compounds. Shown are the co-substrates used and several key intermediary metabolites. Under oxic conditions, benzoate is converted, for example, to catechol, and the aromatic ring is oxygenolytically cleaved. Under anoxic conditions, the high resonance energy does not allow direct reduction of the aromatic ring. Rather, ring destabilization is achieved by forming the coenzyme A (CoA) thioester, which requires ATP. Then, the ring is reduced at the cost of another ATP, and finally the ring is opened hydrolytically. See text for additional details. (From Lengeler, J.W., G. Drews, and H.G. Schlegel. 1999. *Biology of Prokaryotes*, fig. 9.29. Blackwell Science, Stuttgart. With permission from Blackwell Science. Stuttgart.)



Faktory biotransformace organických polutantů MO

- často zapojeno více kroků
- kombinace aero-/anaero-bních podmínek (i záměrně)

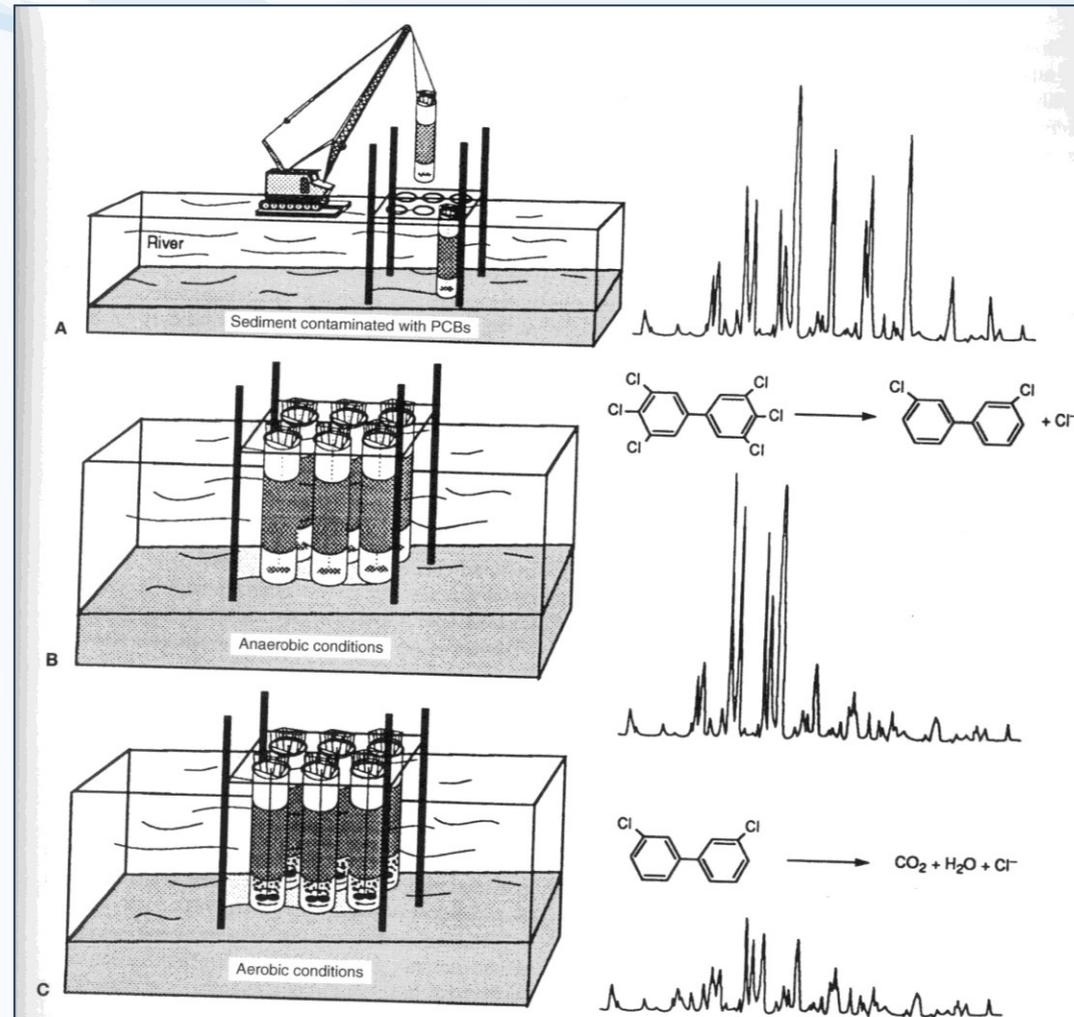


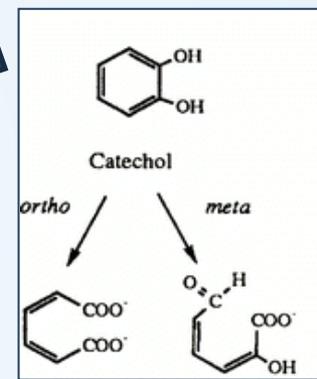
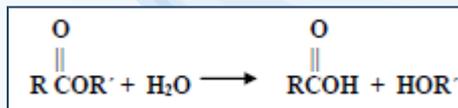
Figure 14.4

Bioremediation of PCB-contaminated river sediments. (A) Placement of steel caissons into sediments; chromatographic tracing showing full range of contaminating PCB congeners. (B) Nutrients added to sealed caissons lead to creation of anaerobic conditions: anaerobic dehalogenation converts higher-molecular-weight congeners to ones with fewer chlorines; chromatographic tracing shows disappearance of higher-molecular-weight congeners with 4-6 chlorines and increased concentrations of lower-molecular-weight PCBs with 2-3 chlorines. (C) Forced aeration and stirring create aerobic conditions; biodegradation of lower-molecular-weight congeners leads to cleaner sediments.



Základní mechanismy biotransformace org. polutantů MO

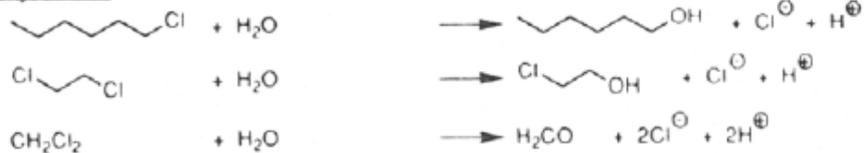
- dealkylace: odštěpení alkyly
- dealkoxylace: odštěpení alkoxy skupiny
- dekarboxylace: $-\text{COOH} \rightarrow -\text{COH}$ nebo $-\text{COOH} \rightarrow -\text{CH}$
- štěpení etherů
- hydrolýza: adice vody $\rightarrow -\text{OH}$ či $-\text{COOH}$
- oxidace: $-\text{OH} \rightarrow -\text{COH}$ či $-\text{COOH}$ nebo epoxid
- hydroxylace: připojení $-\text{OH}$
- epoxidace
- methylace: vznik methyletherů
- vznik nebo štěpení aromatického kruhu (např. aromatická oxidace)
- redukce: např. $-\text{NO}_2 \rightarrow -\text{NH}_2$
- dehalogenace: $-\text{X} \rightarrow -\text{H}$ (hydrolitická, reduktivní, dehydrodehalogenace)



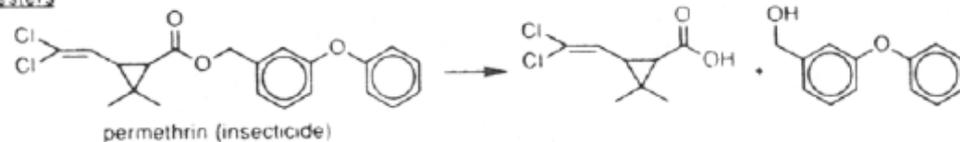
Základní mechanismy biotransformace org. polutantů MO

Hydrolýza

alkyl halides



esters



amides



carbamates



ureas



(thio)phosphates



Základní mechanismy biotransformace org. polutantů MO

Štěpení benzenového jádra

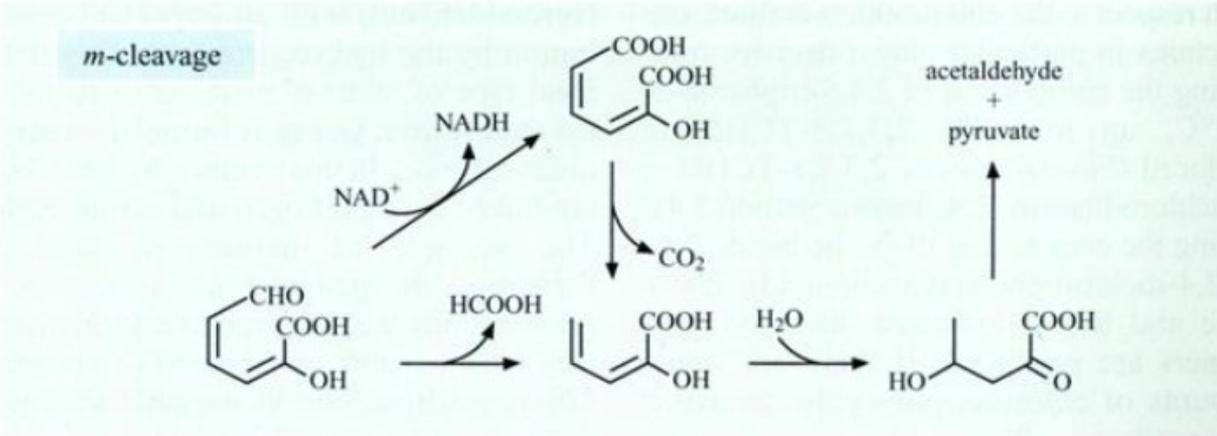
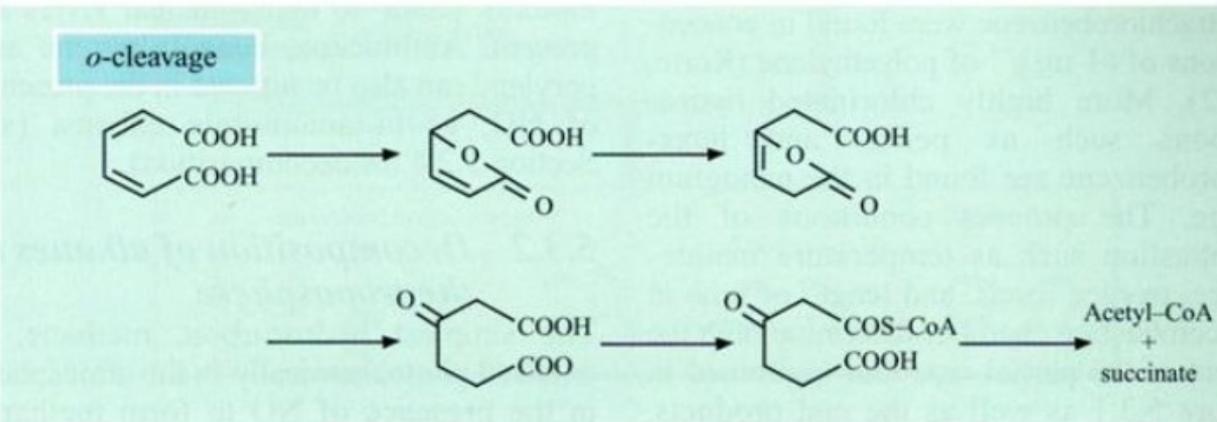
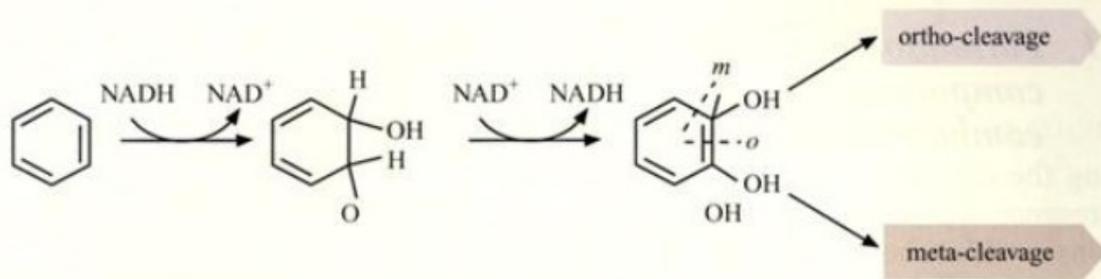
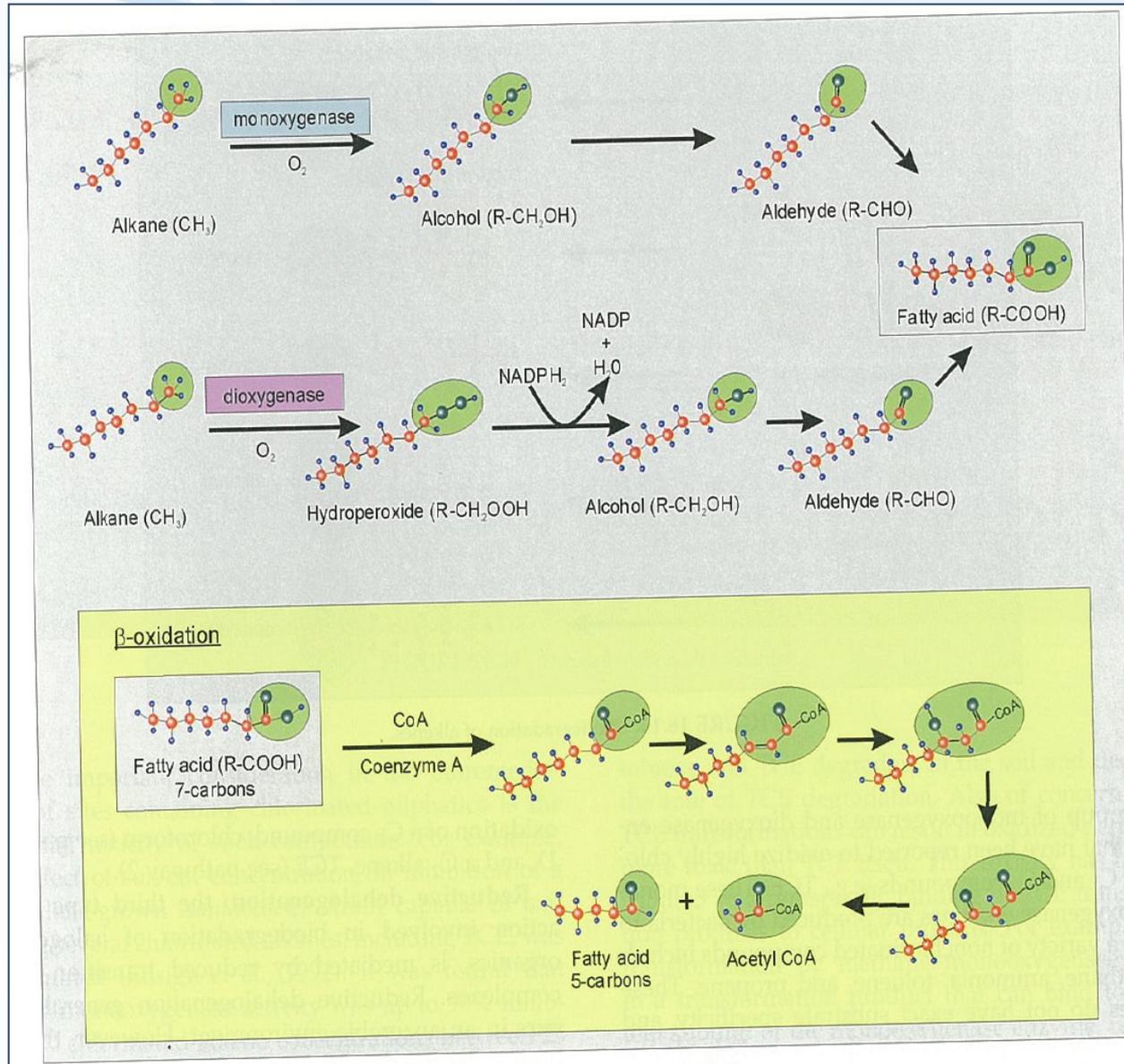


Figure 5.2.3 Bacterial decomposition of aromatic compounds (after Korte, 1992)

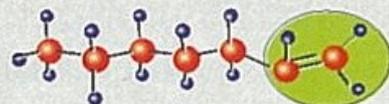
Příklad - mikrobiální degradace alkanů

- podoba s metabolismem mastných kyselin – β -oxidace
- optimální degradace je u n-alkanů C10-C18 (vyšší malá rozpustnost ve vodě, nižší možná toxicita – butan)
- proces vysoké BOD
- větvené – horší degradace
- metylace obecně snižuje degradabilitu
- v anaerobních podmínkách velice těžko odbouratelné (ropa před vytěžením nedegraduje i když v ní žijí MO ...)

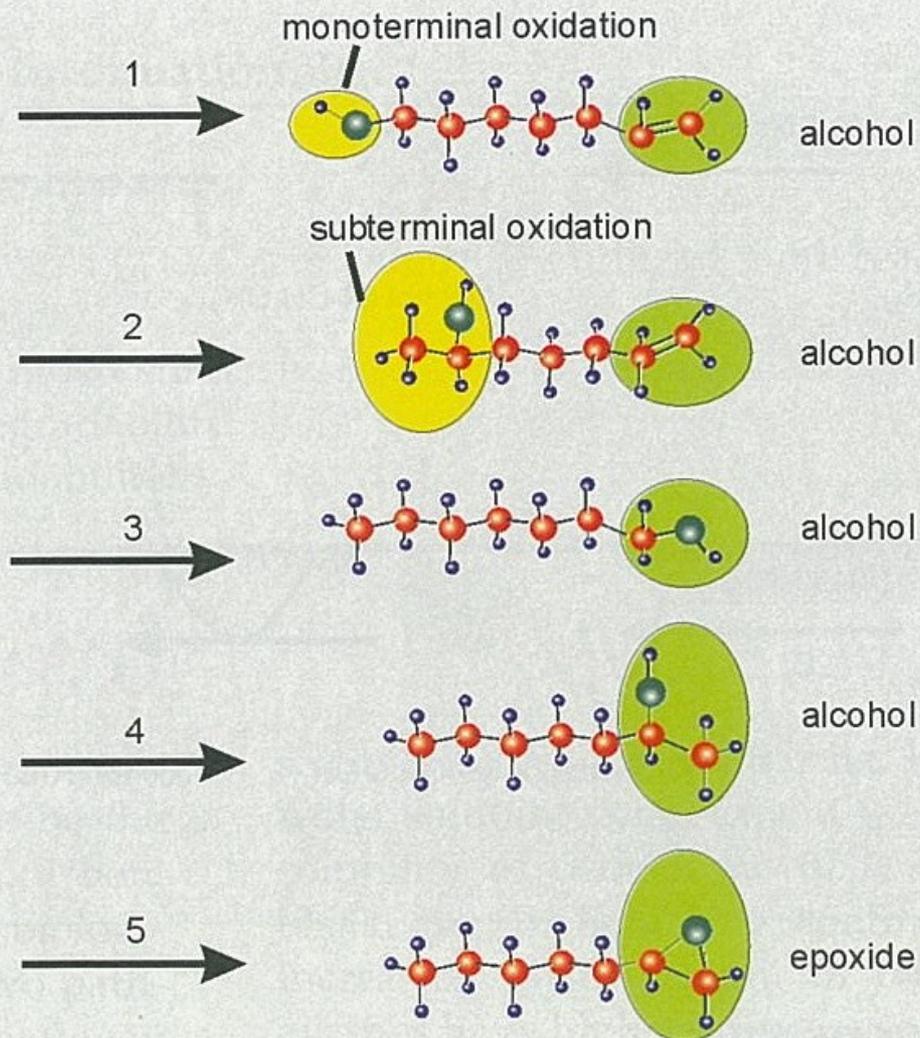


Příklad - mikrobiální degradace alkenů

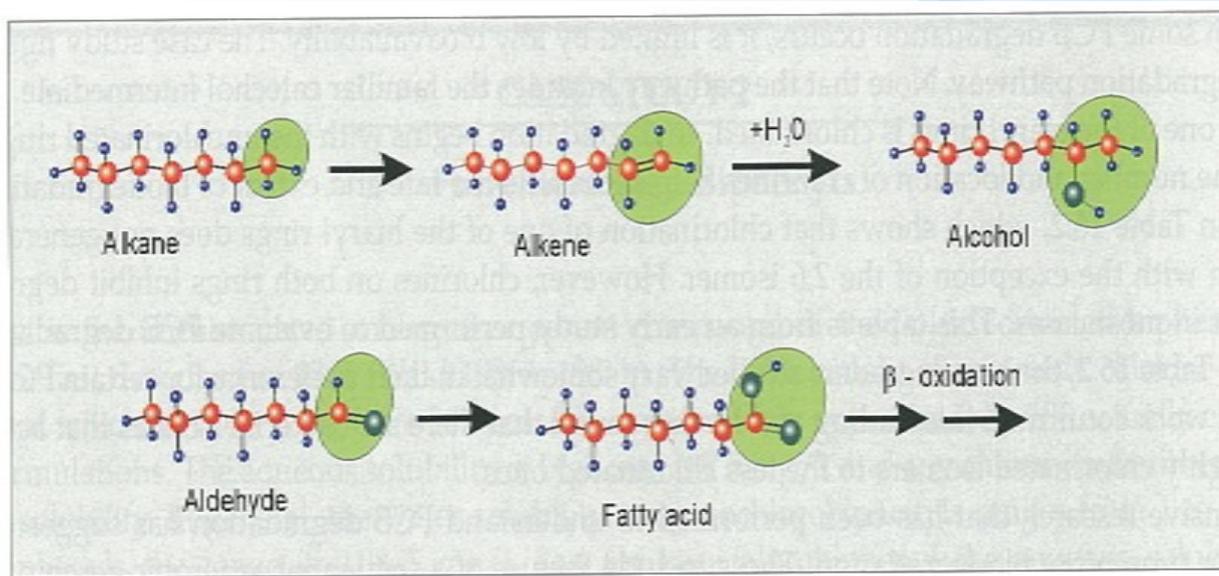
- podobná degradovatelnost jako alkanů
- atak koncové nebo n-1 metylové skupiny, nebo atak dvojné vazby vedoucí k alkoholu či epoxidu



Alkene ($R-CH=CH_2$)

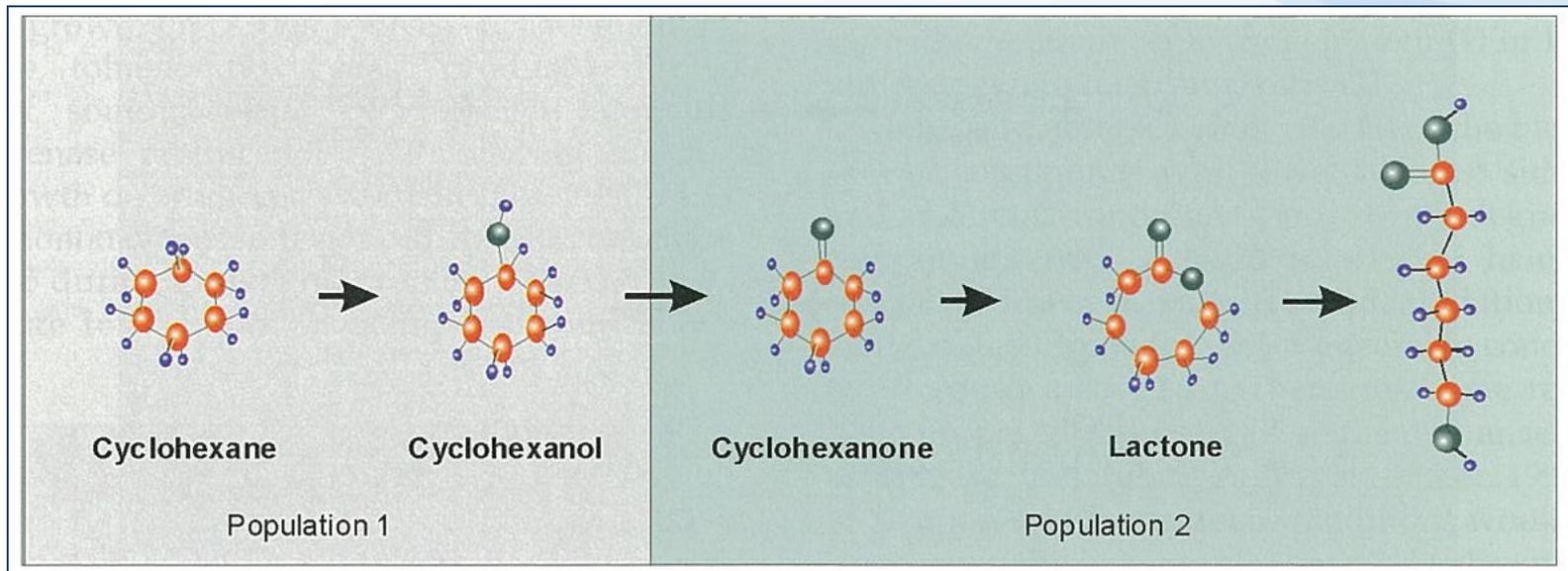


Příklad - mikrobiální degradace alifátů v anaer. podmínkách



Příklad - mikrobiální degradace cykloalkanů

- není korelace mezi biodegradací n-alkanů a n-cykloalkanů
- dosti často kometabolická degradace a komezalismus
- OH, C=O, COOH substituce zvyšují rychlost, alkyl- skupiny snižují



Příklad - mikrobiální degradace BTEX

Figure 10.6 The meta-cleavage pathway and the *xyl* operon of the toluene/xylene-degrading plasmid pWVO. Transcription of the *xyl* operon is controlled by the P_m promoter, which is regulated by the XylS gene product that must be activated by one of the initial pathway substrates. The genes from *xylX* to *xylH* are under the control of the P_m promoter. The *xylS* gene, which is not part of this operon, is constitutively expressed. Some of the primary substrates are benzoate where R and R' = H; 3-methylbenzoate where R = H and R' = CH₃; 3-ethylbenzoate where R = H and R' = CH₂CH₃; and 4-methylbenzoate where R = CH₃ and R' = H. The genes *xylXYZ* encode toluene dioxygenase; *xylL* encodes dihydroxycyclohexadiene carboxylate dehydrogenase; *xylE* encodes catechol 2,3-dioxygenase; *xylF* encodes hydroxymuconic semialdehyde hydrolase; *xylG* encodes hydroxymuconic semialdehyde dehydrogenase; *xylH* encodes 4-oxalocrotonate tautomerase; *xylI* encodes 4-oxalocrotonate decarboxylase; *xylJ* encodes 2-oxopent-4-enoate hydratase; and *xylK* encodes 2-oxo-4-hydroxypentonate aldolase.

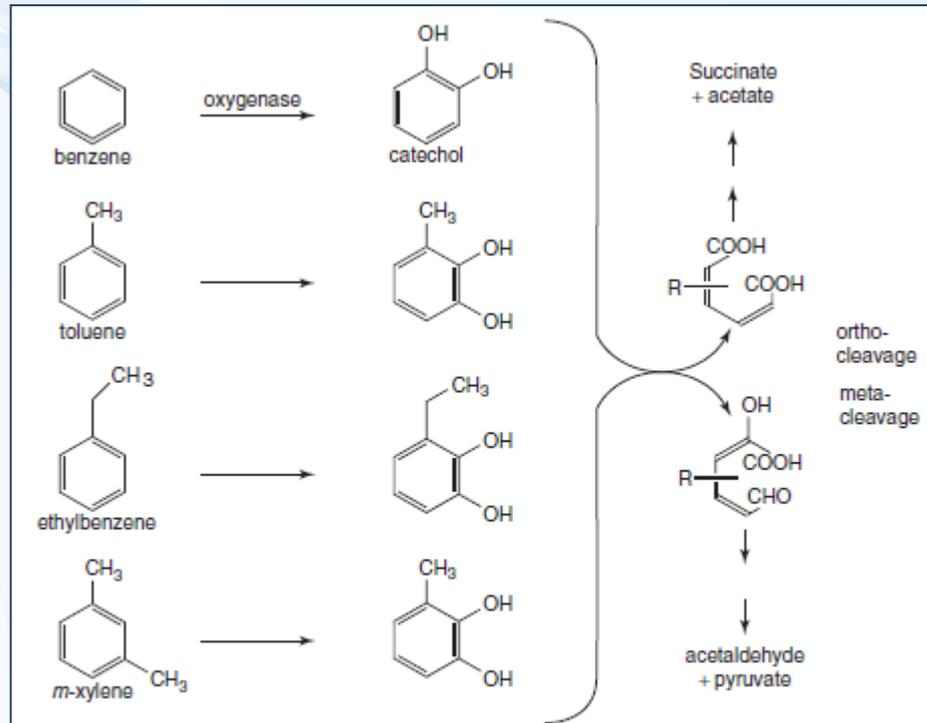
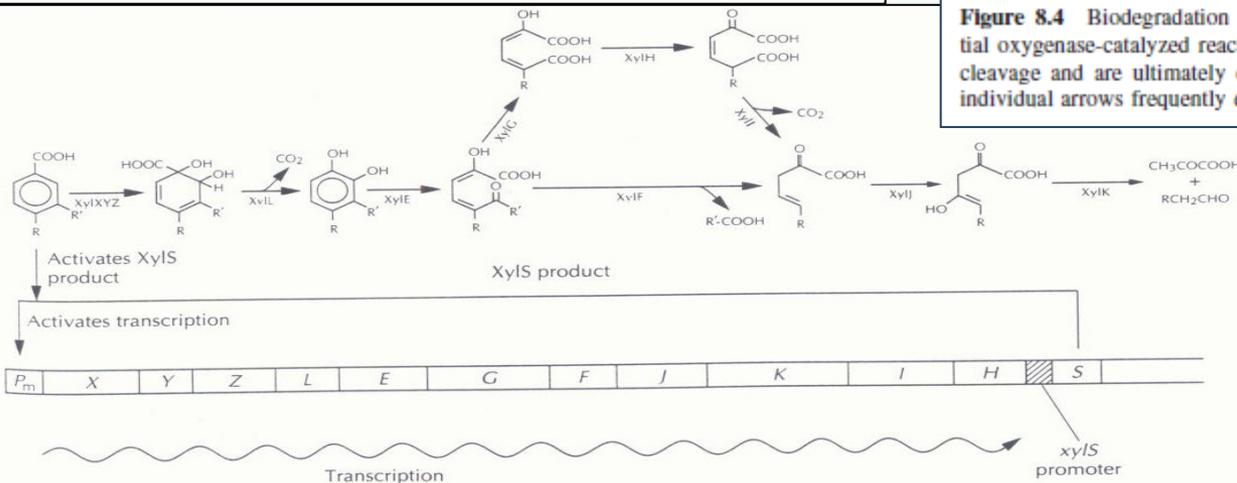


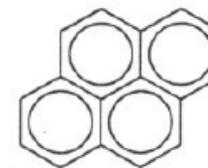
Figure 8.4 Biodegradation of benzene, toluene, ethylbenzene, and xylene (BTEX) via initial oxygenase-catalyzed reactions that lead to the formation of catechols, which undergo ring cleavage and are ultimately converted to central metabolic pathway intermediates. Note that individual arrows frequently encompass multiple reactions.



Příklad - mikrobiální degradace PAHs

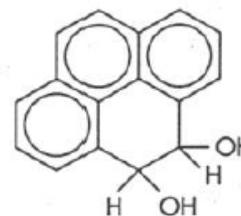
bakterie

příklady látek identifikovaných po metabolizaci pyrenu *Mycobacterium* sp.

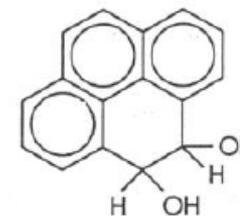


Pyrene

Ring-oxidation products:



cis-4,5-Pyrenediol

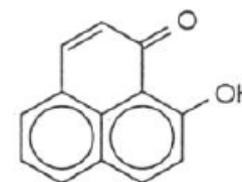


trans-4,5-Pyrenediol

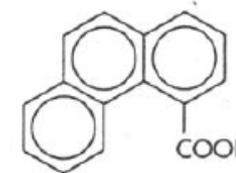


Pyrenol

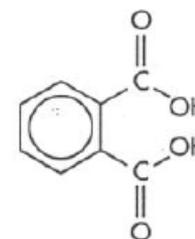
Ring-fission products:



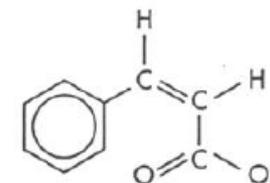
4-Hydroxyperinaphthenone



4-Phenanthroic acid



Phthalic acid



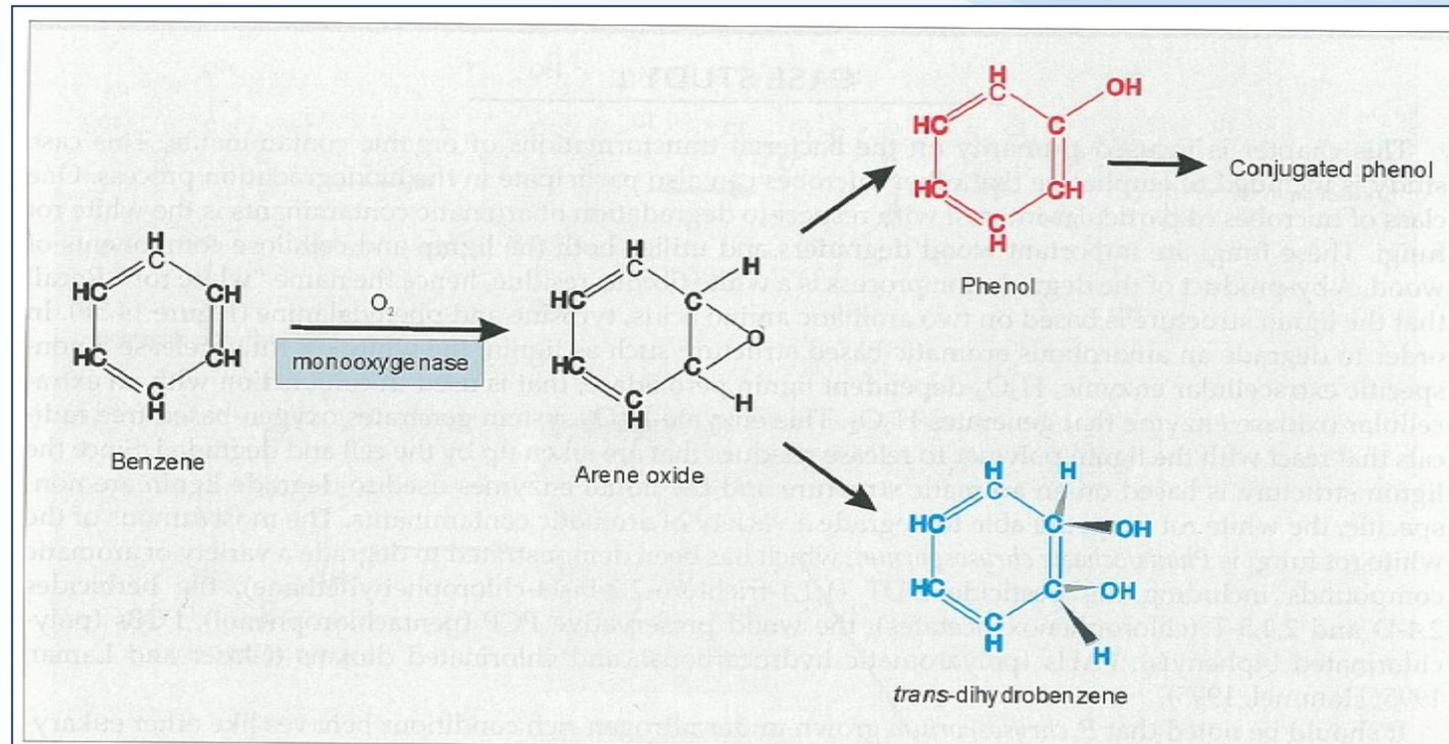
Cinnamic acid



Příklad - mikrobiální degradace PAHs

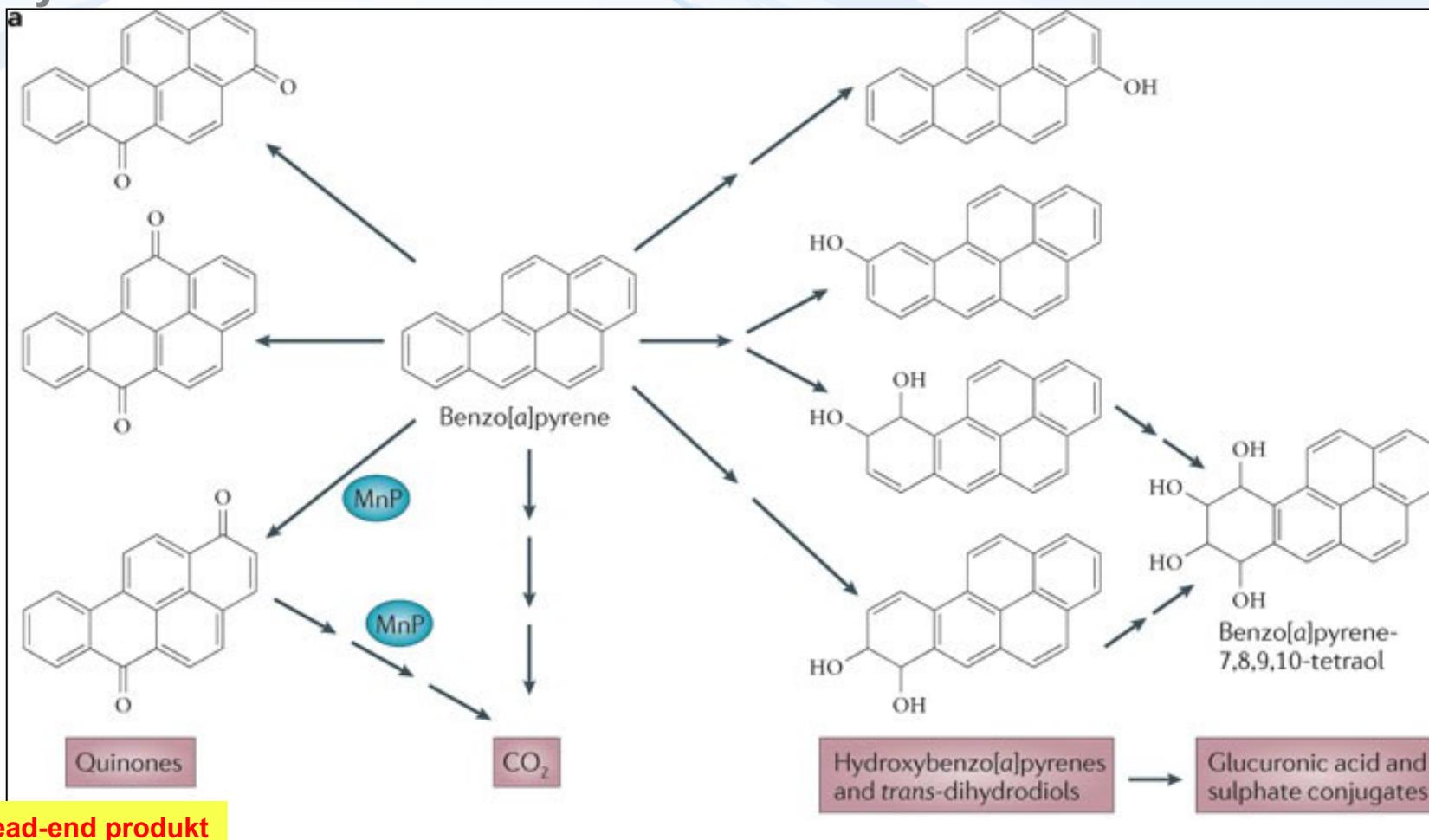
houby

- enzymatický systém cytochromu P-450 monoxygenázy → trans-dihydrodiol cestou arenoxidových meziproduktů
- případně vzniká fenol, který je konjugován (s sulfáty, glukuronovou kyselinou, GSH) a vyloučen či podléhá za účasti enzymatického systému cytochromu P450 epoxidaci
- white-rot fungi - kompletní mineralizace na CO₂ a vodu
- Epoxid - elektrofilní molekula schopná vazby na nukleofilní DNA a RNA → genotoxicita



Příklad - mikrobiální degradace PAHs

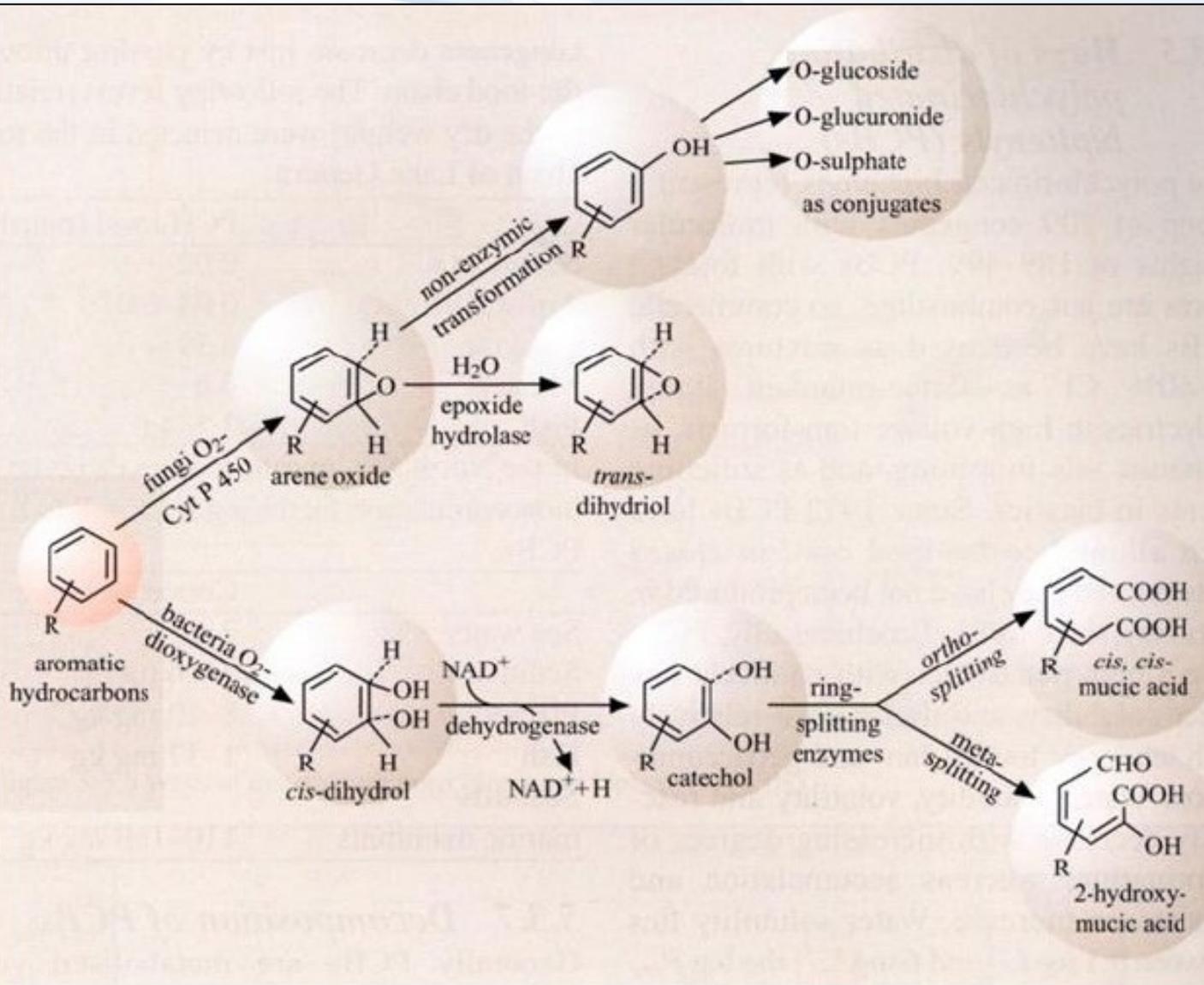
houby



An example of fungal polycyclic aromatic hydrocarbon biodegradation: the biochemical pathway and products reported for the co-metabolic fungal degradation of benzo[a]pyrene. Note that the epoxide intermediates that arise from initial intracellular attack by cytochrome P450s and precede the formation of hydroxylated products are not shown



Příklad - mikrobiální degradace PAHs



Příklad - mikrobiální degradace PAHs

Faktory ovlivňující mikrobiální degradaci PAHs:

- Předcházející expozice - delší expozice MO PAHs → adaptace; větší míra degradace v kontaminovaných prostředích
- Chemická struktura - PAHs s nižší Mr degradovány rychleji ; alkylace PAHs inhibuje degradaci
- Dostupný kyslík - molekulární kyslík nezbytný pro degradaci PAHs; za anaerobních podmínek probíhá degradace velmi pomalu, pokud vůbec
- Dostupné živiny - limitující v prostředí, kde je hodně organického odpadu (N,P); jiné zdroje C → degradace PAHs až po spotřebování C
- Teplota, pH, salinita - slabě probíhá degradace v zimě; rychleji při pH = 8 než pro pH = 5



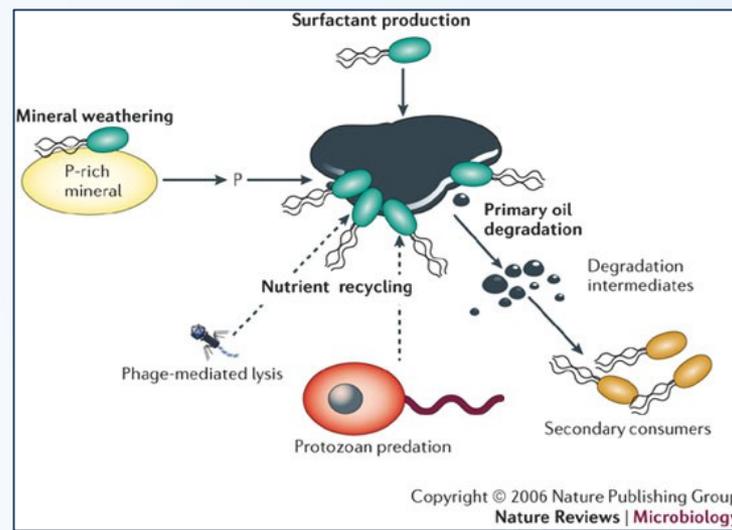
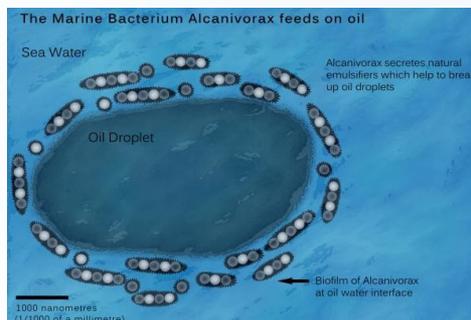
Příklad - mikrobiální degradace ropných látek

- **surová ropa** - komplexní směs uhlovodíků (alifatické, aromatické, asfalt)
- n-alkany, rozvětvené alkany, cykloalkany, aromáty - degradovatelnost viz výše
- asfalt, kerogen - sloučeniny nedegradovatelné nebo velmi pomalu
- ropné pryskyřice - omezená mikrobiální degradace
- **lehká ropa**
 - 10-40% degradovatelných alkanů
 - 2-20% lehkých aromatických uhlovodíků
 - 2-10% vysokomolekulárních těžce degradovatelných polyaromátů
 - 1-5% asfaltických látek a ropných pryskyřic
- **těžká ropa**
 - < 1% odbouratelných alifátů
 - < 2% lehkých aromatických uhlovodíků
 - až 35% vysokomolekulárních těžce degradovatelných polyaromátů
 - až 25% asfaltických látek a 20% ropných pryskyřic

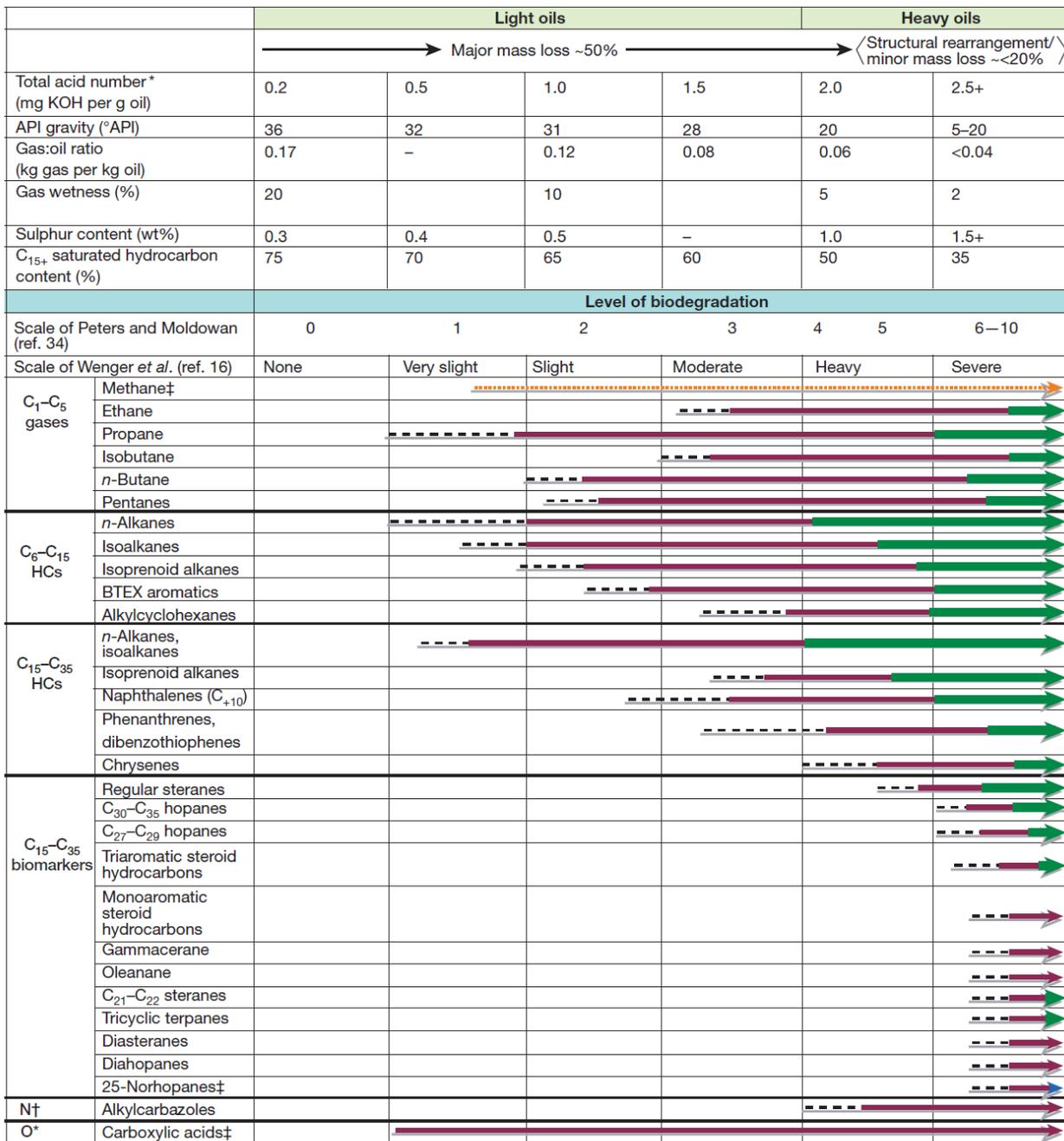


Příklad - mikrobiální degradace ropných látek

- žádná surová ropa není zcela degradována
- na procesech biologické degradace ropných látek se podílí více než 70 rodů MO
- většina těchto rodů je všeobecně rozšířena, ale platí: ekosystémy nekontaminované ropnými uhlovodíky - málo degradátorů ropných uhlovodíků \times prostředí ropnými uhlovodíky kontaminované - vysoké počty
- nejvýznamnější degradátoři ropy: rody *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Acinetobacter*, *Flavobacterium*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Nocardia*, *Candida*, *Rhodotorula* a *Sporobolomyces*
- ve vodě bakterie a kvasinky, v půdách rovněž houby (např. *Penicillium*)
- MO degradující ropné uhlovodíky produkují často emulzifikační látky



Příklad - mikrobiální degradace ropných látek



* Ref. 14

† Ref. 21

--- Minor removal

— Major alteration

→ Removal

--- Methane generation and possible destruction

‡ Produced and destroyed during biodegradation

insight review articles

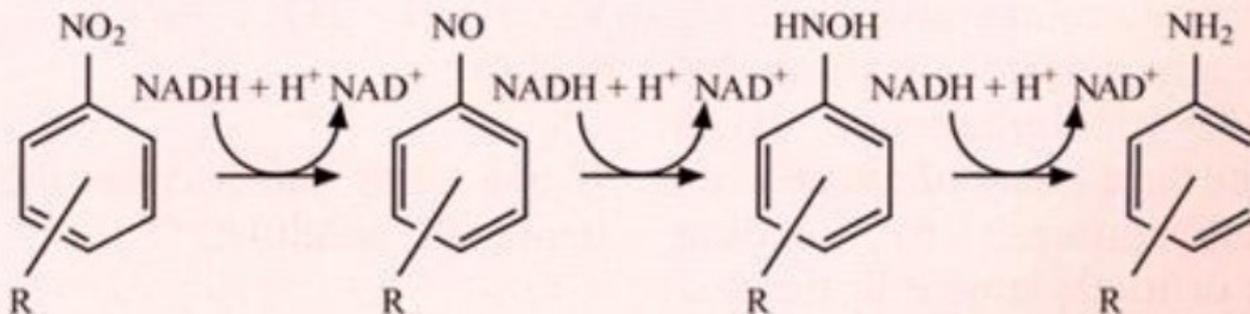
Biological activity in the deep subsurface and the origin of heavy oil

Ian M. Head, D. Martin Jones & Steve R. Larter

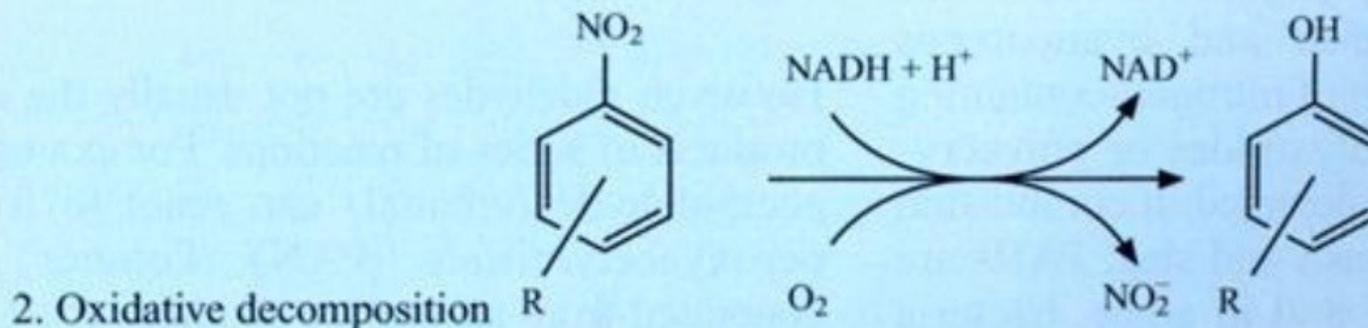
Figure 1 Schematic diagram of physical and chemical changes occurring during crude oil and natural gas biodegradation. The general sequence of removal of saturated hydrocarbon types during biodegradation is *n*-alkanes, alkylcyclohexanes, acyclic isoprenoid alkanes, bicyclic alkanes–steranes–hopanes⁷, with some production of new hydrocarbons, such as 17 α ,25-norhopanes from demethylation of hopanes at advanced levels of degradation^{32,33}. Similarly, for aromatic hydrocarbons, alkylbenzenes are removed before diaromatic and triaromatic hydrocarbons⁸, with aromatic steroid hydrocarbons being resistant until very severe levels of biodegradation are achieved³². With both alkanes and aromatic hydrocarbons, stereochemistry and the position of alkyl substituents confound such simplistic scenarios, and there is frequently much overlap and synchronous removal

of the various compound types. Removal of gas chromatography (GC)-resolvable components during degradation contributes to the production of the 'humps' of GC-unresolvable hydrocarbon mixtures that are typically found in degraded oils⁹¹. However, even when biodegradation levels are slight, mass balance calculations suggest that non-GC-resolvable components are also degraded. The indicative compositional and physical property changes with biodegradation assume that it starts with a light, low-sulphur undegraded oil. The lower diagram of compositional change is modified from Wenger *et al.* (ref. 13). Pervasive oil mixing and synchronous removal of several compound types result in the degradation scales being indicative rather than being a precise consistent form.

Příklad - mikrobiální degradace nitroláték



1. Reductive decomposition



2. Oxidative decomposition

Figure 5.2.4 Decomposition of aromatic nitro compounds

Příklad - mikrobiální degradace halogenovaných OPs

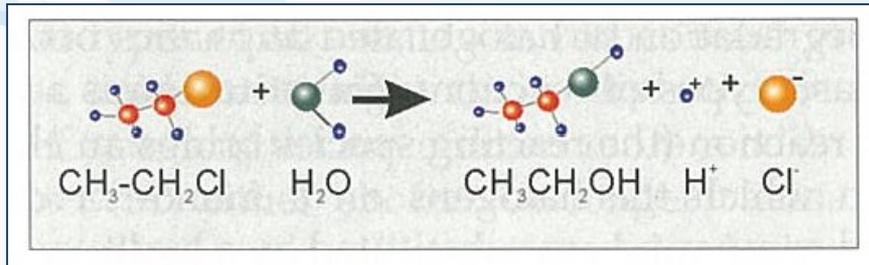
- přítomnost 2, 3 a více Cl zpomaluje aerobní degradaci
- problém nastává např. při degradaci aromatických látek, kdy adice hydroxyly na katechol či dihydroxybenzen vyžaduje nesubstituované uhlíky na štěpené vazbě (halogen je ale blokuje)
- méně-chlorované jsou lépe degradovány v aerobních podmínkách a více-chlorované v anaerobních



Příklad - mikrobiální degradace halogenovaných OPs

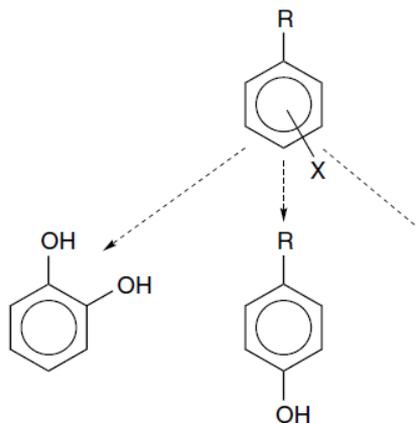
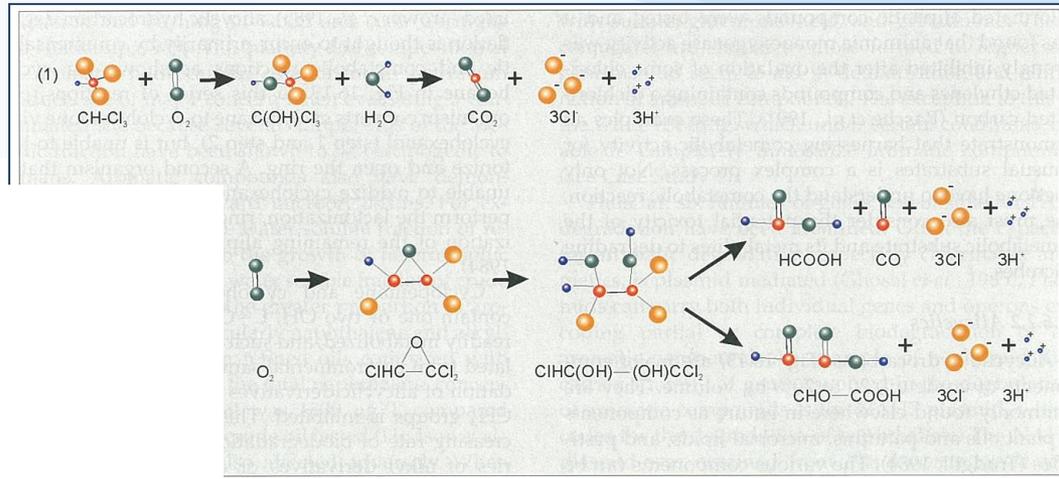
1. dehalogenace substitucí

– hydrolytická



2. dehalogenace oxidací

monooxygenázy či dioxygenázy oxidují vysocechlorované látky typu TCE – kometabolismus (enzymy mají širokou substrátovou specifitu, typicky oxidují metan, amoniak, toluen, propan)



Oxidative (dioxygenase) R = COOH, H, NH₂
X (ortho) = F, Cl, Br, I

Hydrolytic (dehalogenase) R = COOH, OH, NH₂
X (para) = F, Cl, Br, I

Příklad - mikrobiální degradace halogenovaných OPs

3. Reduktivní dehalogenace

- v anaerobním prostředí, ale také v aerobním, pokud má chlorovaná látka větší redukční potenciál než O₂ (hexachlorethan, dibromoetan)
- první krok: e⁻ z redukovaného kovu se přesune na alifát → alkyl-radikál a odštěpí se volný halogen; alkyl-radikál buď naváže vodík, nebo ztrácí další chlor a vzniká dvojná vazba

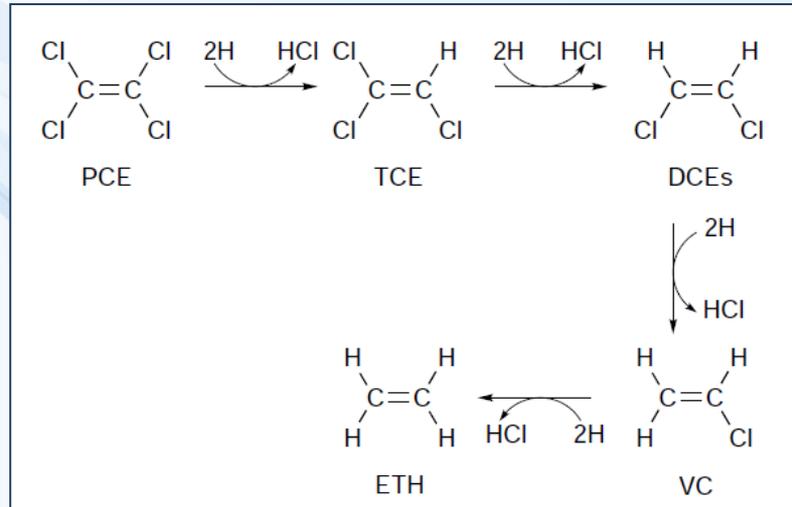
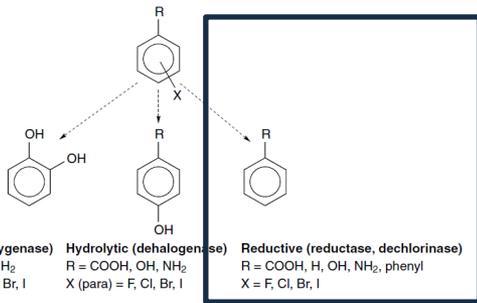
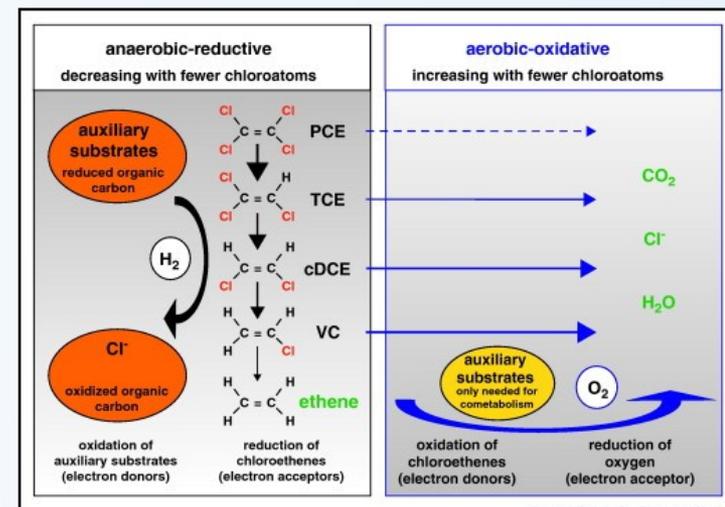
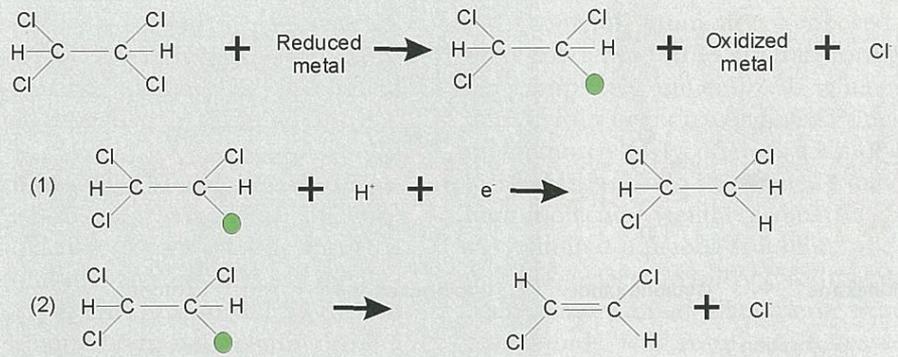


Figure 4. Reduction of tetrachloroethene (PCE) to ethene (ETH). Intermediates include trichloroethene (TCE), dichloroethene isomers (represented by the *cis* isomer), and vinyl chloride (VC).



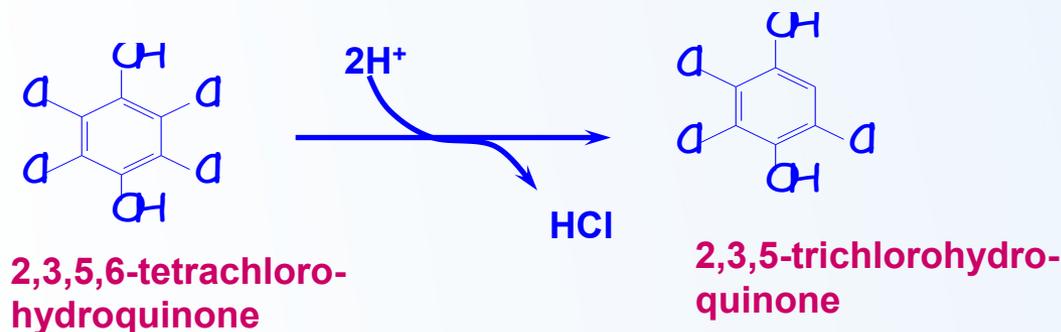
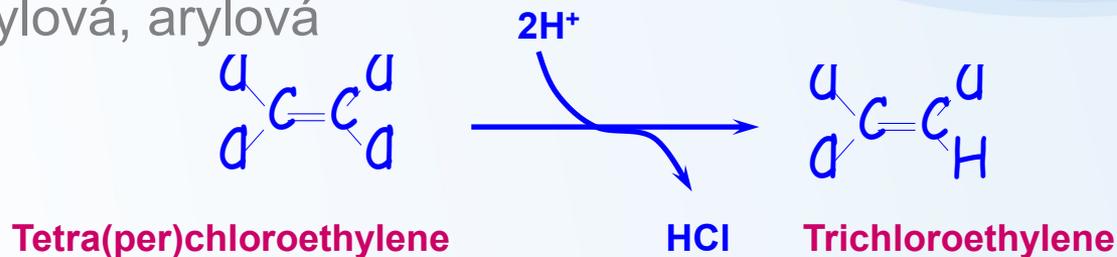
Příklad - mikrobiální degradace halogenovaných OPs

Reduktivní dehalogenace - 2 základní varianty

1. Eliminace dvou halogenů za vzniku = vazby (dihaloeliminace)



2. Hydrogenolysis - alkylová, arylová



Příklad - mikrobiální degradace halogenovaných OPs

Table 1. Representative Organisms Capable of Respiratory Reductive Dehalogenation

Organism	Representative Dehalogenations	Electron Donors	Other Electron Acceptors	Phylogenetic Position
<i>Desulfomonile tiedjei</i> (12)	3-chlorobenzoate to benzoate	H ₂ , formate, pyruvate	Sulfate, thiosulfate	Delta <i>Proteobacteria</i>
Isolate 2CP-1 (50)	2 chlorophenol to phenol	Acetate	Oxygen	Delta <i>Proteobacteria</i>
<i>Desulfitobacterium dehalogenans</i> (27)	2, 4, 6-trichlorophenol to 2,4-dichlorophenol	H ₂ , butyrate, pyruvate	Sulfite, fumarate, nitrate,	<i>Firmacutes</i>
<i>Dehalospirillum multivorans</i> (74)	PCE to <i>cis</i> -DCE	H ₂ , formate, pyruvate	Fumarate, nitrate	Epsilon <i>Proteobacteria</i>
<i>Desulfitobacterium frappieri</i> (26)	Pentachlorophenol to 3-chlorophenol, PCE to TCE/ <i>cis</i> -DCE	Pyruvate,	Sulfite, thiosulfate, nitrate	<i>Firmacutes</i>
<i>Dehalobacter restrictus</i> (25)	PCE to <i>cis</i> -DCE	H ₂	None	<i>Firmacutes</i>
<i>Enterobacter</i> strain MS-1 (30)	PCE to <i>cis</i> -DCE	Formate, pyruvate, acetate	Oxygen, nitrate	Gamma <i>Proteobacteria</i>
<i>Desulfuramonas chloro ethenica</i> (29)	PCE to <i>cis</i> -DCE	Acetate, pyruvate	Polysulfide, fumarate, Fe(III)	Delta <i>Proteobacteria</i>
<i>Dehalococcoides ethenogenes</i> (31)	PCE to ETH 1,2-DCA to ETH(VC)	H ₂	None	<i>Chloroflexi</i>
<i>Desulfovibrio</i> strain TBP-1 (53)	2,4,6-tribromophenol to phenol	H ₂ , formate, lactate	Sulfate, thiosulfate, S ⁰	Delta <i>Proteobacteria</i>
<i>Trichlorobacter thiogenes</i> (47)	Trichloroacetate to dichloroacetate	H ₂ S (acetate)	S ⁰ , fumarate	Delta <i>Proteobacteria</i>
<i>Dehalococcoides</i> strain CBDB1 (56)	Tetra- and trichlorobenzenes to tri- and dichlorobenzenes	H ₂	None	<i>Chloroflexi</i>

Příklad - mikrobiální degradace halogenovaných OPs

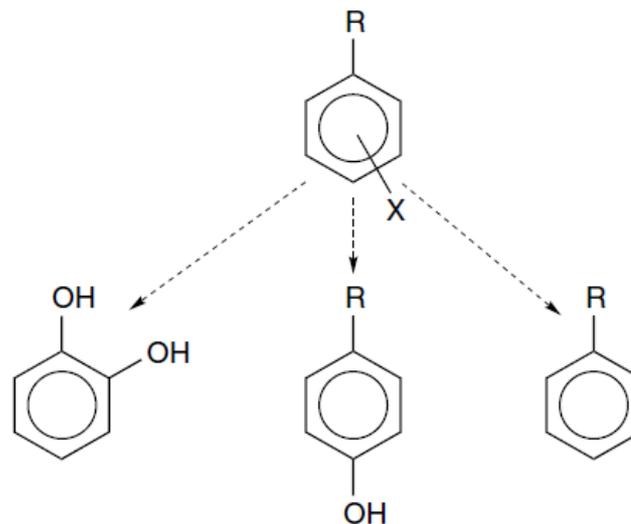


Figure 4. Dechlorination mechanisms exhibited by aerobic and anaerobic bacteria.

Oxidative (dioxygenase)

R = COOH, H, NH₂

X (ortho) = F, Cl, Br, I

Hydrolytic (dehalogenase)

R = COOH, OH, NH₂

X (para) = F, Cl, Br, I

Reductive (reductase, dechlorinase)

R = COOH, H, OH, NH₂, phenyl

X = F, Cl, Br, I



Příklad - mikrobiální degradace chlorfenolů

- dominují aerobní a anaerobní biodegradační procesy: oxidační procesy působením monooxygenáz a hydroxyláz; redukční dehalogenace působením dehalogenáz
- biodegradabilita klesá v tomto pořadí:
- 2,4 > 4 > 3,5 > 2,6 > 3 nebo 5 nebo 2
- trichlorfenoly 2,3,6-, 2,4,5-, 3,4,5- jsou biodegradabilní pouze za aerobních podmínek
- méně chlorované fenoly, včetně monochlorovaných jsou více rezistentní než pentachlorfenol (PeCP) vůči biodegradačnímu potenciálu aklimatizované PeCP degradující bakteriální kultury
- v tomto případě zvýšení stupně chlorace nevede ke zvýšení perzistence
- obecně ale lze potvrdit, že chlorace v pozicích 3 a 5 zvyšuje perzistenci

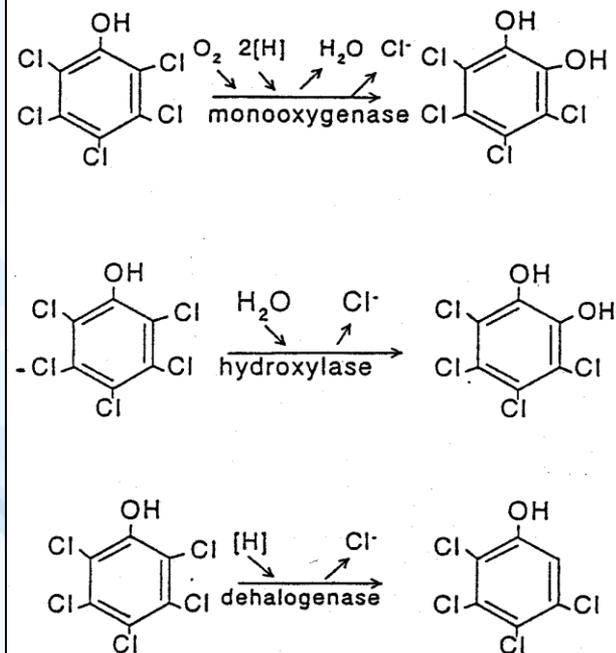


Fig. 1. Pathways of PCP degradation.

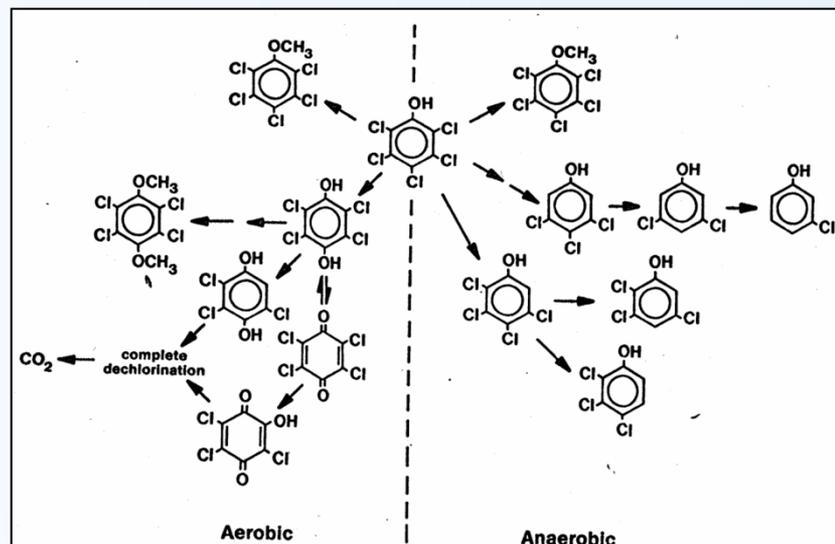
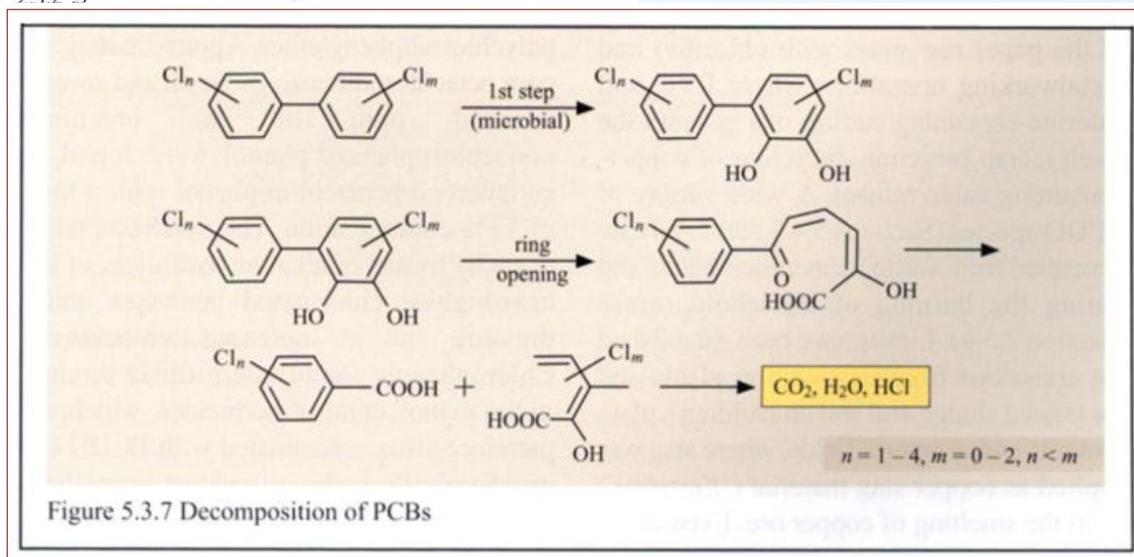


Fig. 8-4. Comparison of typical degradation pathways of pentachlorophenol in aerobic and anaerobic environments.



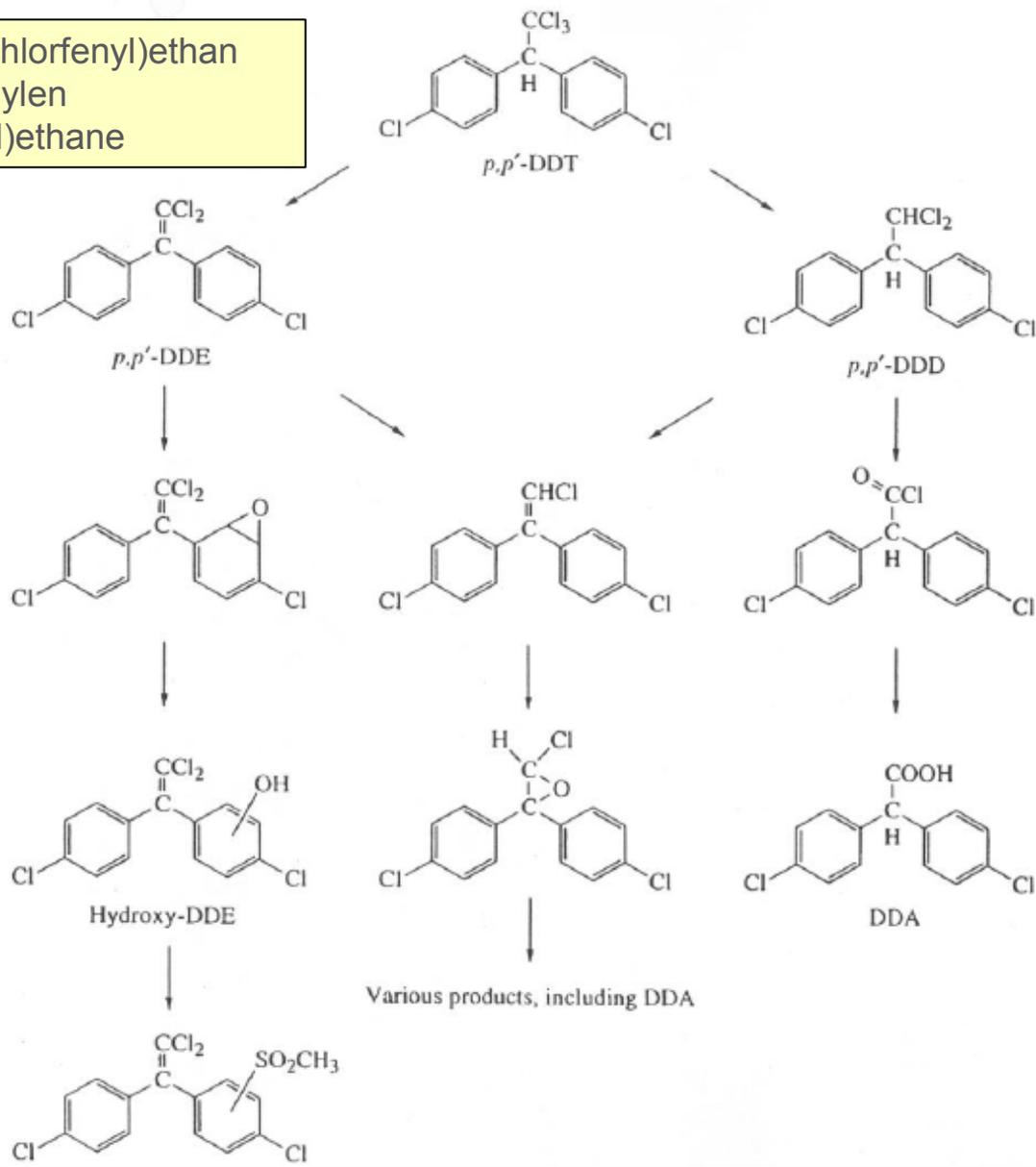
Příklad - mikrobiální degradace PCBs

PCB chlorine position	Degradation rate (nmol/ml/hr)		PCB chlorine position	Degradation rate (nmol/ml/hr)	
	<i>Alcaligenes sp.</i>	<i>Acinetobacter sp.</i>		<i>Alcaligenes sp.</i>	<i>Acinetobacter sp.</i>
2	>50	>50	2,4,6	3.1	46.0
3	>50	>50	2,5,2'	1.6	5.1
4	>50	>50	2,5,3'	42.1	41.3
2,3	>50	46.4	2,5,4'	21.8	30.4
2,4	>50	>50	2,4,4'	41.3	40.2
2,5	>50	>50	3,4,2'	15.6	38.6
2,6	0	4.1	2,3,4,5	25.8	19.1
3,4	>50	>50	2,3,5,6	0	0
3,5	>50	>50	2,3,2',3'	8.7	7.3
2,2'	6.3	14.0	2,4,2',4'	0	0
2,4'	48.2	49.1	2,4,3',4'	0	0
3,3'	—	18.5	2,5,2',5'	0	3.5
4,4'	16.2	25.2			
2,3,4	35.1	32.0			
2,3,6	0	0			
2,4,5	46.0	32.4			



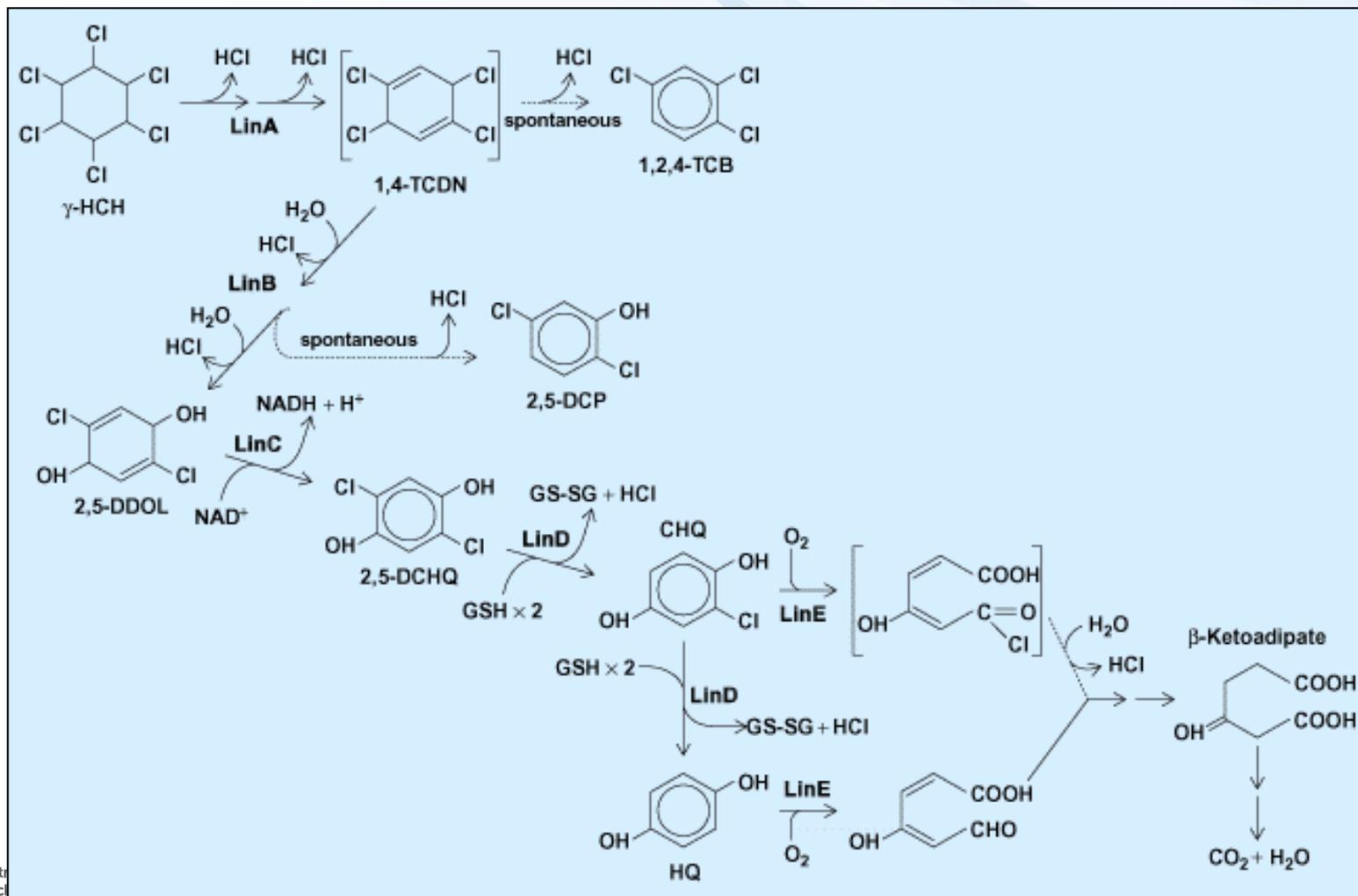
Příklad - mikrobiální degradace DDT

p, p' -DDT (4,4'-DDT) = 1,1,1-trichlor-2,2-bis(4-chlorofenyl)ethan
 p, p' -DDE = 1,1-dichlor-2,2-bis(4-chlorofenyl)ethylen
 p, p' -DDD = 1,1-dichloro-2,2-bis(4-chlorophe-nyl)ethane



Příklad - mikrobiální degradace HCHs

- Izomery HCH jsou relativně chemicky stálé látky lipofilní povahy.
- Postupnou mikrobiální dechlorací jsou převáděny na trichlorbenzeny a tetrachlorbenzeny



Příklad - mikrobiální degradace CB

Chlorobenzene: solvent in pesticide formulations, also used as a degreasing agent; produced by chlorination of benzene in the presence of a catalyst; first synthesized in 1851; used in high volume during World War I

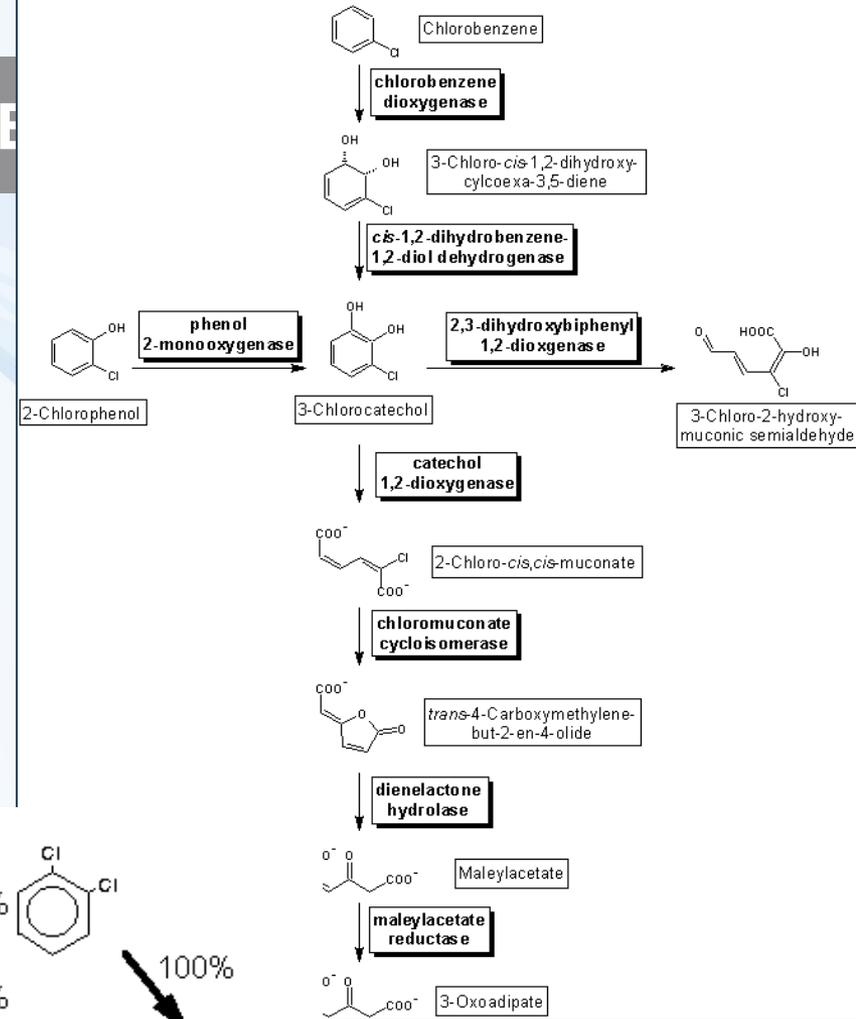
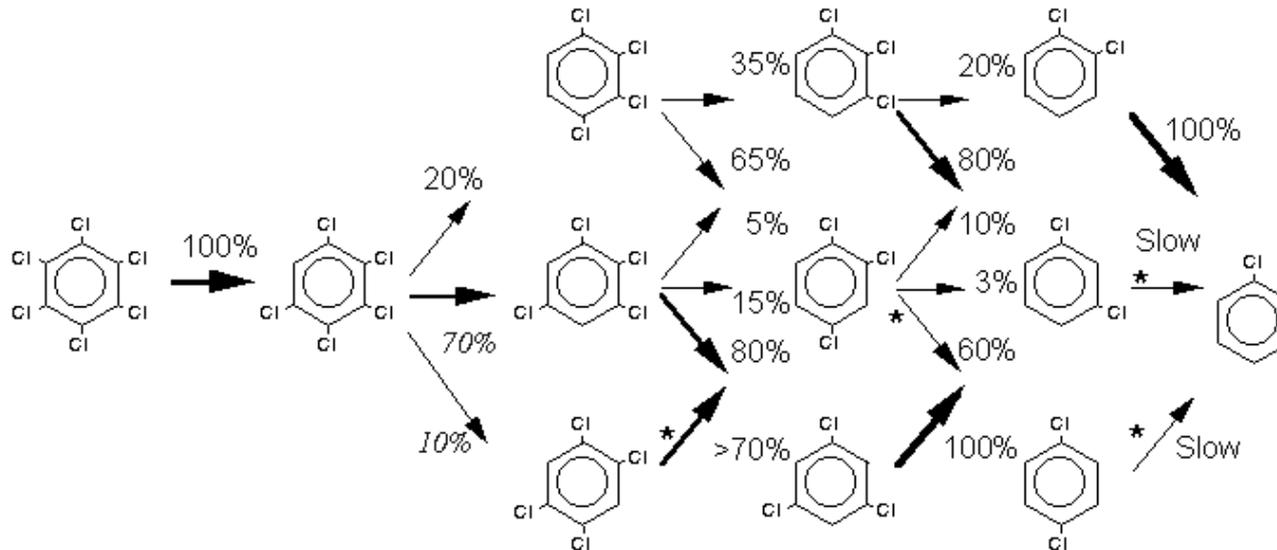
Chlorobenzene contamination persisted in a shallow aquifer at Kelly Air Force base for ~30 years. In the 1990s the levels of contamination began to decline

Chlorobenzene-degrading bacteria were not in the contaminated zone prior to the chlorobenzene spill, nor could they be isolated outside the contaminated zone. However, bacteria able to grow on toluene and chlorocatechol were present in the indigenous microflora across the site

A bacterium able to grow on chlorobenzene, *Ralstonia* sp. JS705, was isolated from the contaminated aquifer and was characterized biochemically and genetically

The chlorobenzene degradation pathway and the gene fragments encoding the enzymes represent a merger of two biodegradation pathways: toluene (*todCBA*) and chlorocatechol (*clcAB*). Thus, the chlorobenzene genes in the strain JS705 were assembled in situ within the contaminated aquifer

Analysis of the DNA sequence of the new combination of genes allowed the investigators to reconstruct the genetic history of strain JS705. It was originally a toluene-degrading bacterium and received the chlorocatechol gene on a self-transmissible mobile genetic element, termed a "genomic island" (see Science and the citizen box, Chapter 5). Sequence analysis also implicated involvement of insertion (IS) elements, hence transposons (other agents of gene rearrangement), in achieving the final configuration of genes conferring chlorobenzene metabolism to strain JS705



Příklad - mikrobiální degradace dioxinů

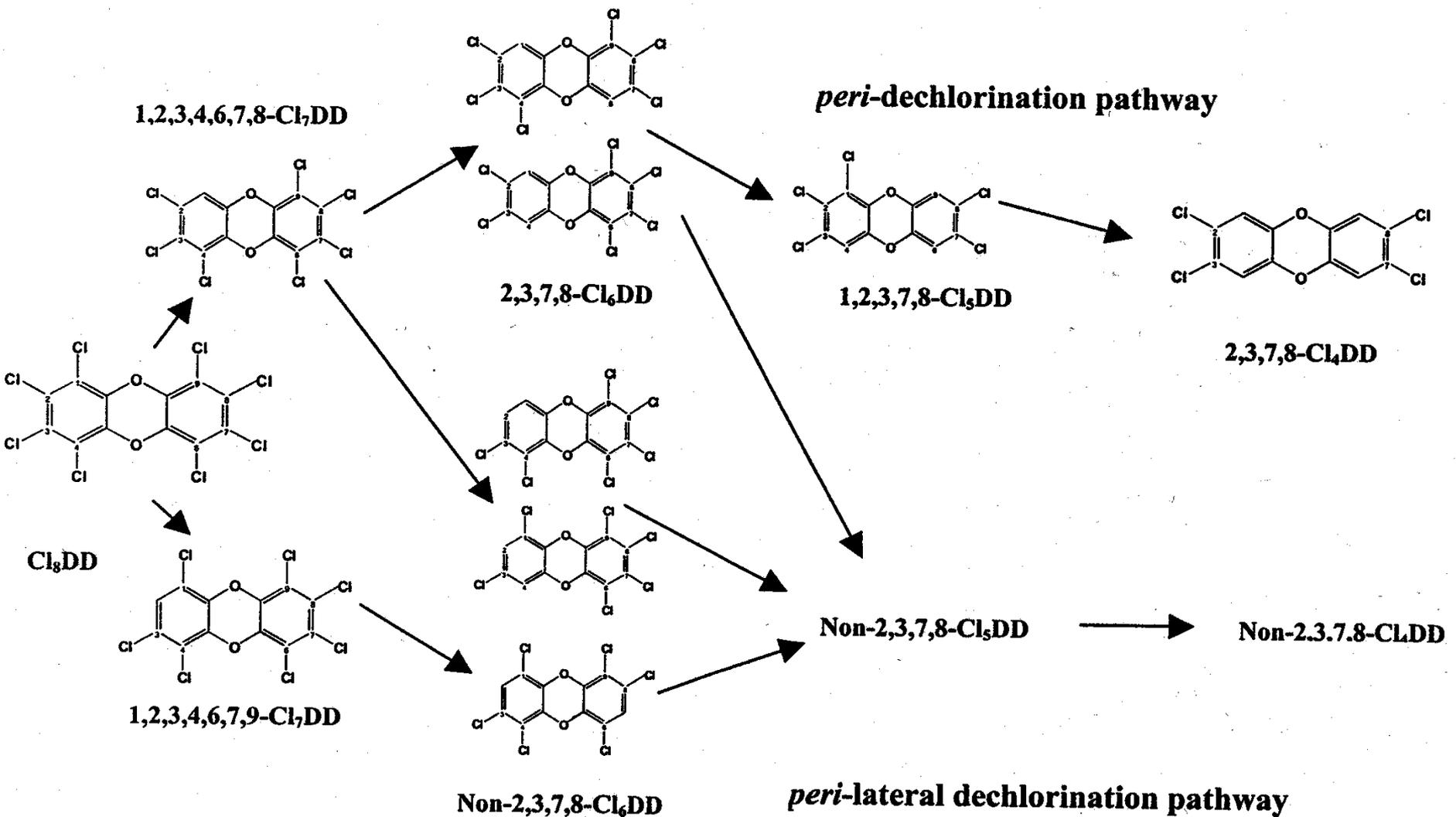
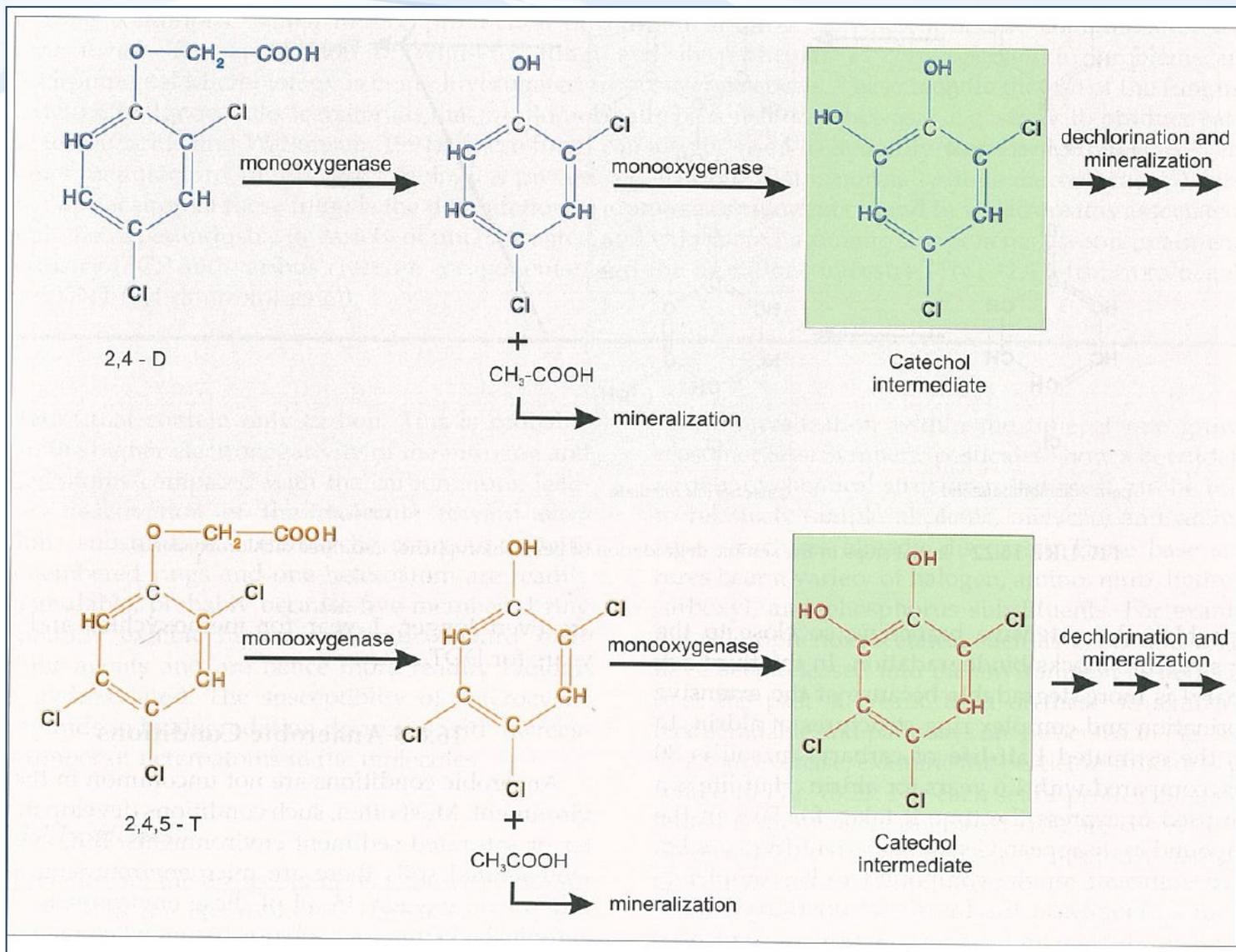


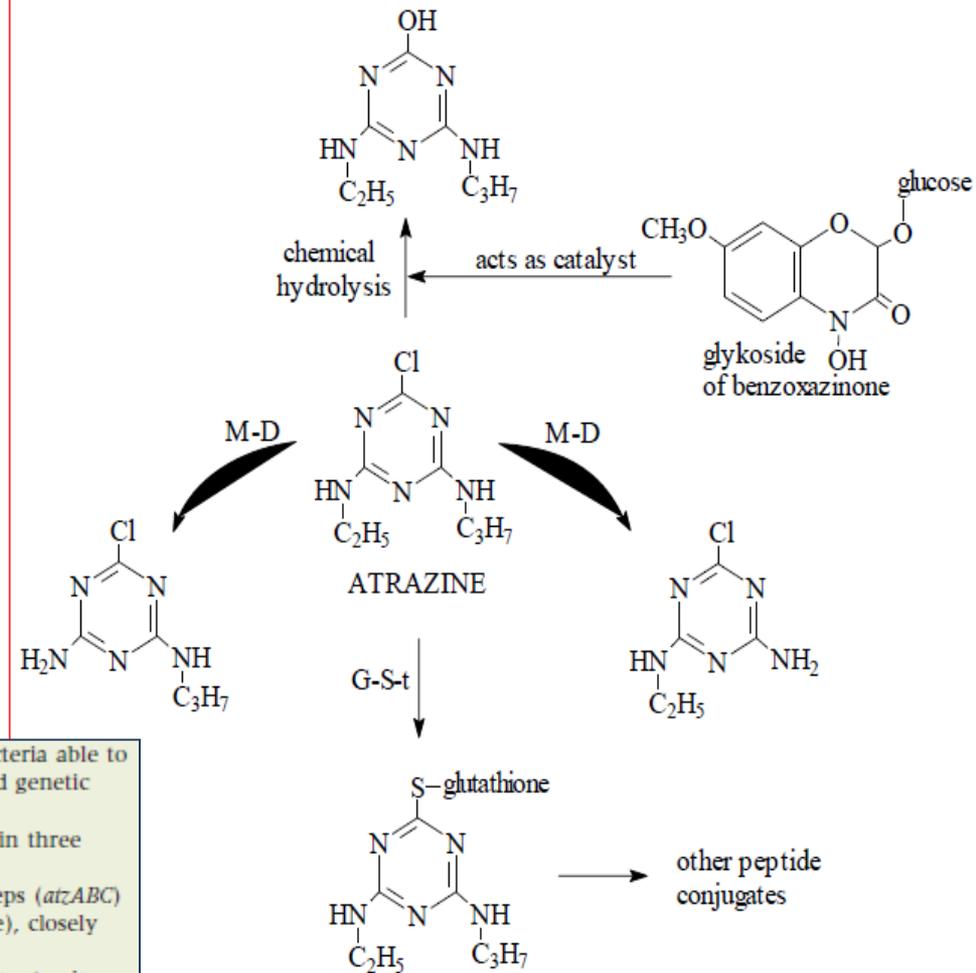
Figure 13.4. Differentiation of microbial *peri*-dechlorination (1,4,6,9) and *peri*-lateral dechlorination (2,3,7,8) pathways.

Příklad - mikrobiální degradace 2,4-D a 2,4,5-T



Příklad - mikrobiální degradace atrazinu

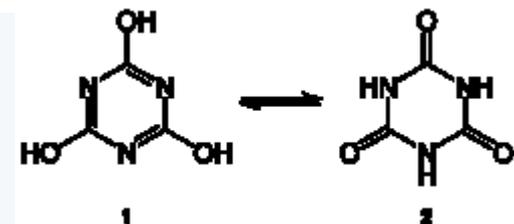
- heterocykly obecně:
- hůře degradovatelné než analogické látky pouze s C
- degradovatelnost klesá s počtem heteroatomů



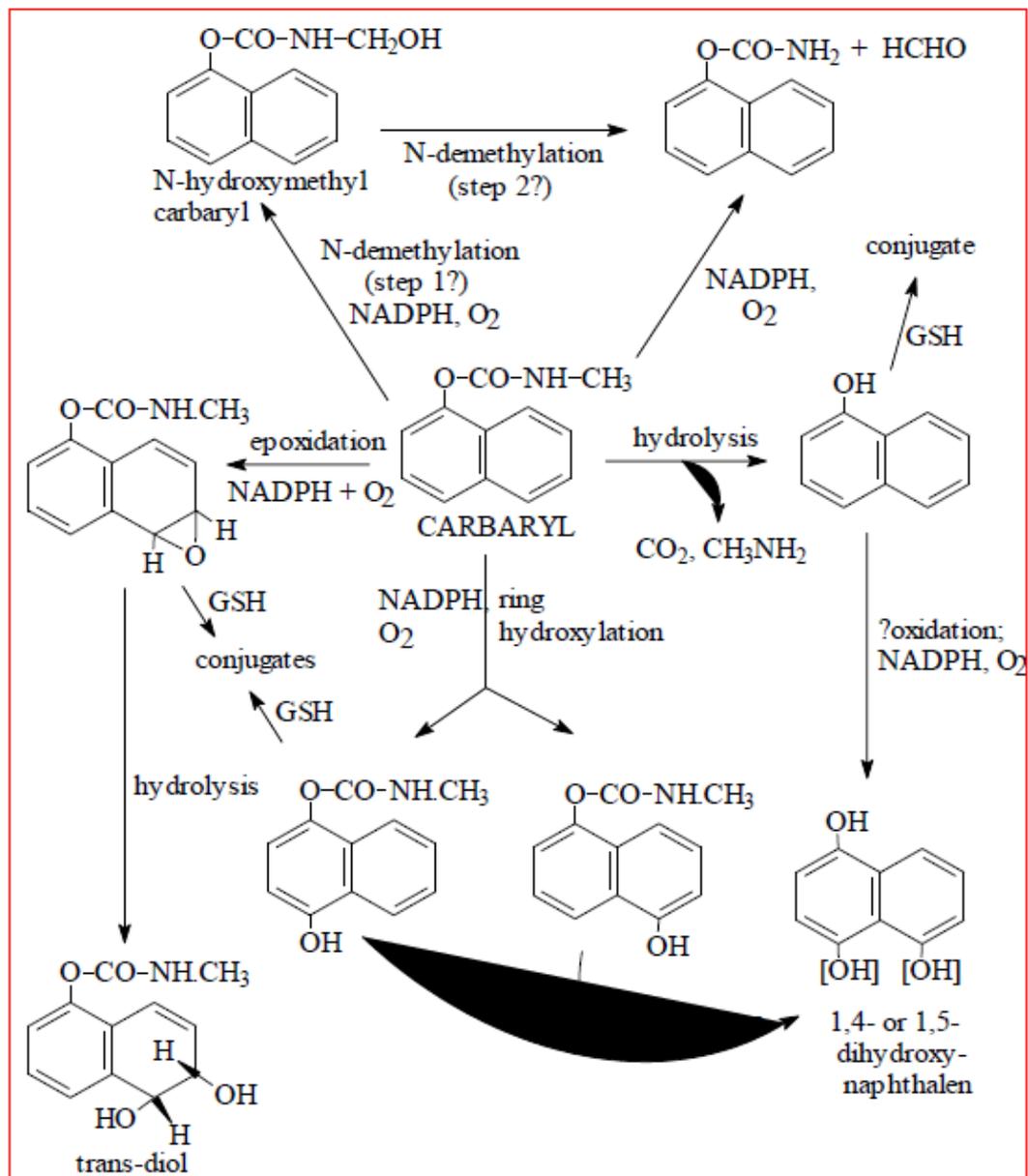
Atrazine: major triazine agricultural herbicide used to protect corn; introduced in the 1960s

Considered nonbiodegradable until the mid-1990s when bacteria able to grow on atrazine were isolated. Extensive biochemical and genetic characterization of atrazine biodegradation show that:

- the metabolic pathway converts atrazine to cyanuric acid in three steps
- the genes coding for enzymes responsible for the three steps (*atzABC*) all belong to the same family of enzymes (amidohydrolase), closely related to *trzA* (for melamine)
- nearly identical *atzABC* gene sequences have been found in atrazine-metabolizing bacteria in North America, Europe, Australia, and Asia
- although the genes have been found on plasmids (extrachromosomal, often self-transmissible, independently replicating, genetic elements), no single type of plasmid is common to all atrazine-degrading bacteria
- sequence analysis of DNA flanking the *atz* genes has discovered evidence for transposons. Thus transposons may be involved in horizontal gene transfer of atrazine biodegradation genes between different hosts



Příklad - mikrobiální degradace carbarylu



Degradace organických polutantů houbami

The classification of Fungi, the major organic chemicals that are degraded by various fungal phyla and subphyla, and the important ecological characteristics of these fungi.

Taxa that are relevant for the bioremediation of organic chemicals are bold. The less relevant ascomycete and basidiomycete subphyla *Taphrinomycotina* and *Ustilaginomycotina*, respectively, are omitted. The number of genera in each taxonomic group with alkane, biphenyl, coal tar, coal tar oil, crude oil, dibenzothiophene, diesel, polycyclic aromatic hydrocarbon (PAH) and toluene degraders are given in parentheses.

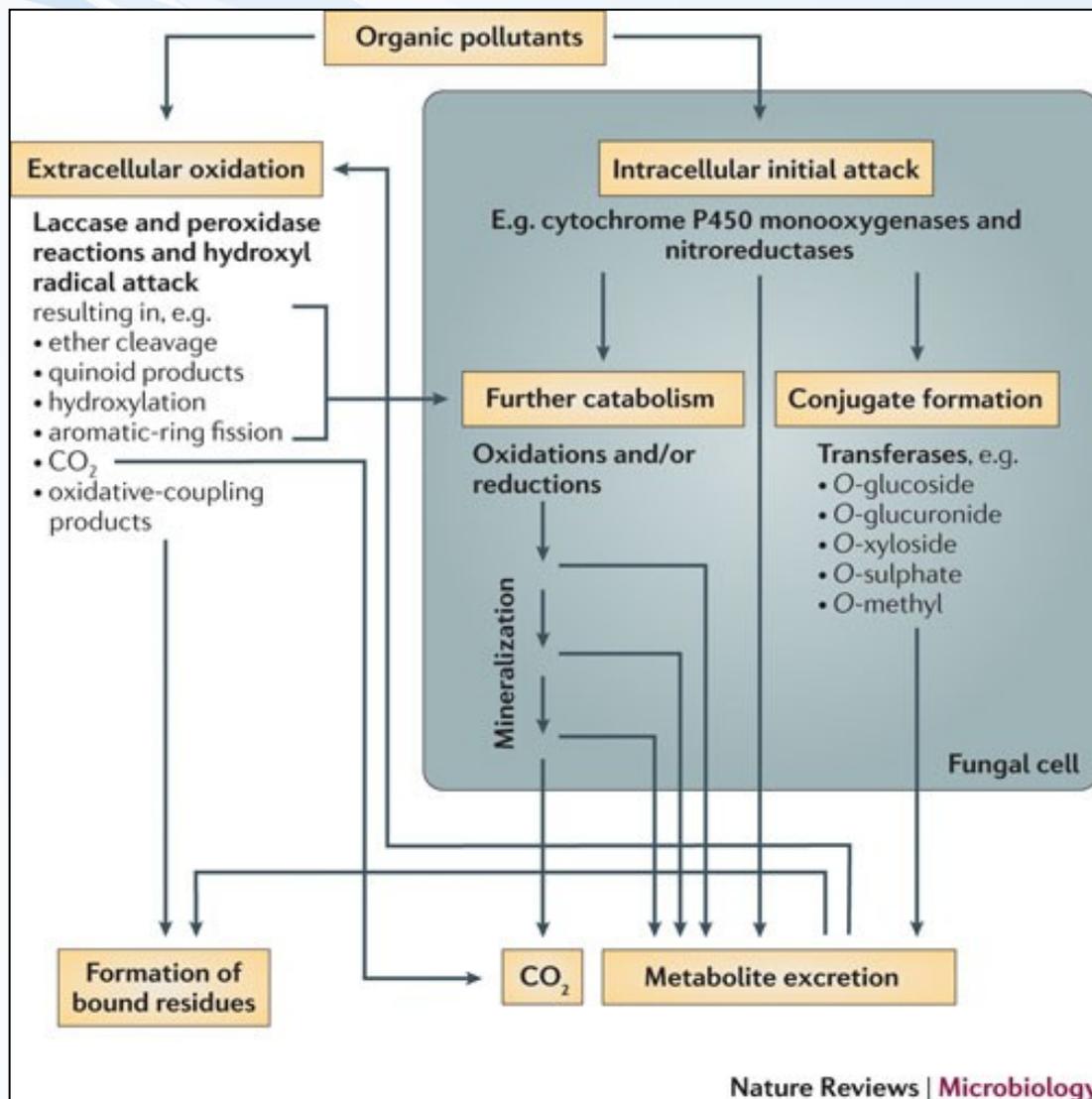
BTEX, benzene, toluene, ethylbenzene and xylenes; EDCs, endocrine disrupting chemicals; MTBE, methyl-*tert*-butylether; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; RDX, Royal Demolition Explosive; TNT, 2,4,6-trinitrotoluene.

	Phylum or subphylum	Organic chemicals degraded	Major ecological characteristics
Basal fungal lineages	Microsporidia		Obligate parasites of animals
	Kickxellomycotina (2)	PAHs	Saprobies, and parasites of animals and fungi
	Zoopagomycotina		Parasites of nematodes, protozoa and fungi
	Entomophthoromycotina (2)	PAHs	Parasites of insects
	Blastocladiomycota		Saprobies, and parasites of plants and animals; aquatic and terrestrial
	Mucoromycotina (16)	Benzoquinoline, biphenyl, PAHs, pesticides, synthetic dyes and TNT	Saprobies, parasites or ectomycorrhizal symbionts
	Neocallimastigomycota		Gut symbionts of ruminant herbivores
	Chytridiomycota (2)	PAHs	Saprobies, and parasites of plants and animals; fresh water and wet soil
	Glomeromycota	PAHs and pesticides	Arbuscular mycorrhizal symbionts
Dikarya	Ascomycota (88) Pezizomycotina (57)	Alkanes, alkylbenzenes, biphenyl, chlorophenols, coal tar oil, crude oil, diesel, EDCs, fragrances, PAHs, PCDDs, pesticides, synthetic dyes, TNT and toluene	Saprobies, pathogens of plants and animals, and symbiotes of algae (lichens), plants (ectomycorrhizae, ercoid mycorrhizae and endophytes) and insects; terrestrial and aquatic
	Saccharomycotina (9)	Alkanes, alkylbenzenes, biphenyl, crude oil, EDCs, PAHs and TNT	
	Other ascomycetes (22)	Alkanes, diesel, coal tar oil, crude oil, MTBE, PAHs, pesticides, RDX, toluene and synthetic dyes	
	Basidiomycota (53) Agaricomycotina (50)	Alkanes, BTEX compounds, chloroaliphatics, lignols and phenols, crude oil, coal tar, EDCs, PAHs, PCBs, PCDDs, PCDFs, personal care product ingredients, pesticides, pharmaceutical drugs, RDX, synthetic dyes, synthetic polymers, TNT and other nitroaromatics	Saprobies, ectomycorrhizal symbionts, pathogens of plants and animals, and parasites of other fungi; terrestrial and aquatic
	Pucciniomycotina (3)	Cresols, crude oil, dibenzothiophene, PAHs and RDX	

Degradace organických polutantů houbami

Although fungi primarily co-metabolize organic pollutants, they do grow on some aliphatic or aromatic compounds, including volatile organics.

Initial pollutant attack may occur extracellularly or intracellularly. Metabolites generated during extracellular pollutant oxidation may be subject to intracellular catabolism or may form bound residues of soil constituents. Metabolites arising from intracellular initial attack may be excreted and can then either undergo further extracellular enzymatic reactions or form bound residues through abiotic oxidative coupling. They may also be secreted in the form of conjugates (which usually persist) or may undergo further intracellular catabolism. This may result in mineralization or, again, in metabolite excretion at various oxidation stages if subsequent oxidation is impeded.



Degradace organických polutantů houbami

- pomáhají extracelulární nescifické enzymy z řady peroxidáz a laccáz, které houby produkují při rozkladu ligninu
- biodegradace vzrůstá při nedostatku N
- white rot fungi (např. *Phanerochaete chrysosporium*)
- = degradace řady polutantů: BTEX, DDT, PCBs, CPs, dioxinů, PAHs, nitroPAHs, 2,4-D, 2,4,5-T, TNT, RDX, pesticidy,

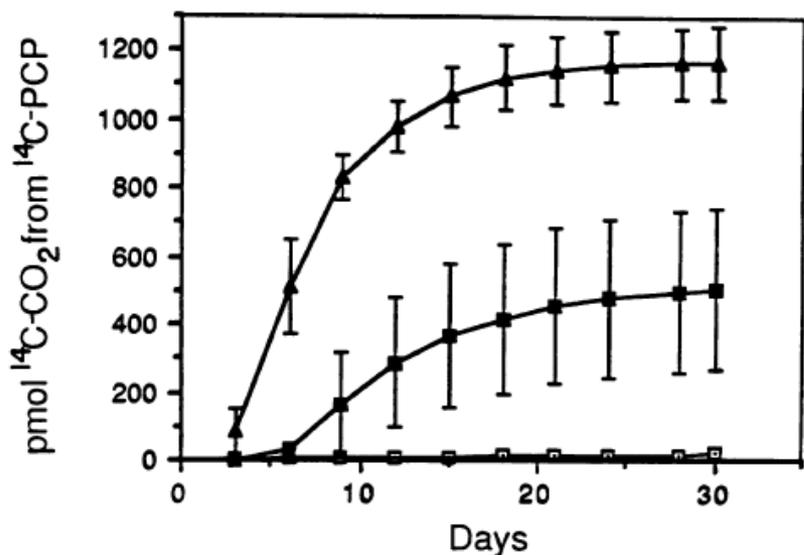


FIG. 2. Effect of nutrient nitrogen on PCP mineralization by *P. chrysosporium*. Cultures containing either 12 mM ammonium tartrate (■; nitrogen sufficient) or 1.2 mM ammonium tartrate (▲; nutrient nitrogen limited) were incubated with 5.0 nmol of [¹⁴C]PCP. Values are the means ± standard deviations for triplicate cultures. A single uninoculated sterile control (□) was incubated with 5.0 nmol of [¹⁴C]PCP.

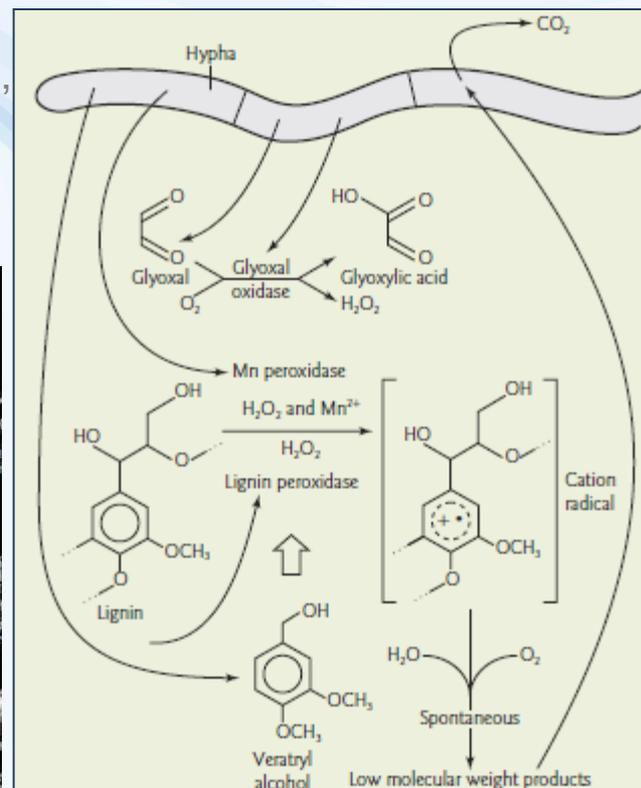
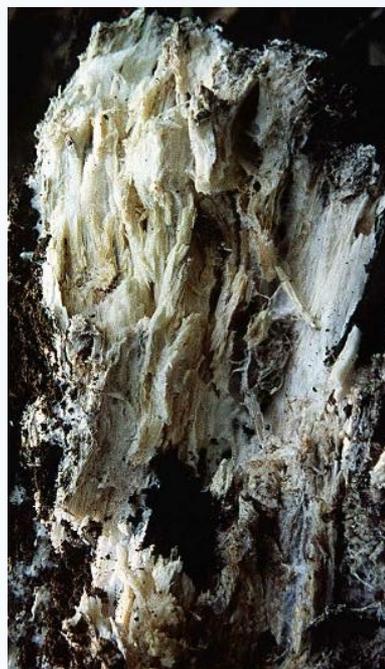


Figure 3 The ligninolytic system of the white rot fungus, *Phanerochaete chrysosporium*. See above box text for details. (From Lengeler, J.W., G. Drews, and H.G. Schlegel. 1999. *Biology of Prokaryotes*, fig. 9.10. Blackwell Science, Stuttgart. With permission from Blackwell Science, Stuttgart.)

Degradace organických polutantů houbami

Table 1 | Major classes of enzymes involved in the fungal catabolism of organic pollutants*

Enzymes (ExPASy ENZYME accession [†])	Fungal taxa	Localization and occurrence [‡]	Reaction mechanism	Comments
Laccases (EC 1.10.3.2) [¶]	Ascomycota and Basidiomycota	Extracellular	• O ₂ -dependent one-electron oxidation of organic compounds	• Redox potential of around 0.4–0.8 V • Direct oxidation of various phenols, aromatic amines and anthraquinone dyes • A wide range of pollutants oxidized in the presence of natural and synthetic redox mediators • Activity mostly in the acidic and rarely in the neutral or alkaline pH range
Tyrosinases (EC 1.14.18.1)	Ascomycota, Basidiomycota and Mucoromycotina	Sometimes extracellular but mainly intracellular	• O ₂ -dependent hydroxylation of monophenols to <i>o</i> -diphenols (resolase activity) • Oxidation of <i>o</i> -diphenols to catechols (catecholase activity)	• Oxidation of various phenols, including those that are highly chlorinated • Activity from the acidic to the alkaline pH range
Lignin peroxidases (EC 1.11.1.14) [¶]	Basidiomycota	Extracellular	• H ₂ O ₂ -dependent one-electron oxidation of aromatic compounds	• Redox potential of 1.4–1.5 V • Direct oxidation of various aromatics with high redox potentials, but rapid inactivation during oxidation of phenols • Direct oxidation of PAHs with an ionization potential of ≤7.55 eV • Extended substrate range (including dyes with high redox potentials and phenols) in the presence of the redox mediator veratryl alcohol • Activity in the acidic pH range
Manganese peroxidases (EC 1.11.1.13) [¶]	Basidiomycota	Extracellular	• H ₂ O ₂ -dependent one-electron oxidation of Mn ²⁺ to Mn ³⁺ , which subsequently oxidizes organic compounds	• Redox potential of 1.0–1.2 V • Mn ³⁺ -mediated oxidation of various phenols and aromatic amines • Extended substrate range in the presence of co-oxidants (organic SH-containing compounds, unsaturated fatty acids and their derivatives) • Activity in the acidic pH range
Versatile peroxidases (EC 1.11.1.16) [¶]	Basidiomycota	Extracellular	• H ₂ O ₂ -dependent direct one-electron oxidation of aromatic compounds • H ₂ O ₂ -dependent one-electron oxidation of Mn ²⁺ to Mn ³⁺ , which subsequently oxidizes organic compounds	• Redox potential of around 1.4–1.5 V • Direct oxidation of phenols and aromatics with high redox potentials, including dyes • Mn ³⁺ -dependent reactions as for manganese peroxidase • Activity in the acidic pH range
Coprinosin cinerea peroxidase (EC 1.11.1.7) [¶]	Basidiomycota	Extracellular	• H ₂ O ₂ -dependent one-electron oxidation of aromatic compounds	• Redox potential of around 0.9–1.1 V • Direct oxidation of phenols and dyes with low redox potentials • Activity from the acidic to the alkaline pH range
Dye-decolorizing peroxidases (EC 1.11.1.x) [‡]	Basidiomycota	Extracellular	• H ₂ O ₂ -dependent one-electron oxidation of organic compounds • Additional hydrolysing activity	• Redox potential of around 1.2–1.5 V • Oxidation of anthraquinone dyes with high redox potentials (only rarely oxidized by other peroxidases) • Highly stable at high pressure, high temperature and very low pH • Activity in the acidic pH range
<i>Caldariomyces fumago</i> haem-thiolate chloroperoxidase (EC 1.11.1.10) [¶]	Ascomycota	Extracellular	• H ₂ O ₂ -dependent halogenation of organic compounds in the presence of halides (one-electron transfer) • H ₂ O ₂ -dependent one-electron oxidations of phenols and anilines in the absence of halides • H ₂ O ₂ -dependent peroxygenation (two-electron oxidation), leading to epoxidation of (cyclo)alkenes, hydroxylation of benzylic carbon and sulphoxidation of S-containing organic compounds	• Redox potential not known • No activity on non-substituted aromatic rings and <i>n</i> -alkanes • Activity in the acidic pH range

Table 1 (cont.) | Major classes of enzymes involved in the fungal catabolism of organic pollutants*

Enzymes (ExPASy ENZYME accession [†])	Fungal taxa	Localization and occurrence [‡]	Reaction mechanism	Comments	Refs
Haem-thiolate peroxygenases [¶]	Basidiomycota	Extracellular	• H ₂ O ₂ -dependent peroxygenation of aromatic, aliphatic and heterocyclic compounds, leading to aromatic and alkylic carbon hydroxylation, double-bond epoxidation, ether cleavage, sulphoxidation or <i>N</i> -oxidation reactions (depending on the substrate); • H ₂ O ₂ -dependent one-electron abstractions from phenols; • H ₂ O ₂ -dependent bromination of organic substrates	• Redox potential not known • Peroxygenation of various monoaromatic to polyaromatic pollutants, including PAHs, dibenzofuran, and monohydroxylated and polyhydroxylated products • Ether bond cleavage between aromatic and aliphatic parts of molecules and in alicyclic and aliphatic ethers (for example, MTBE) • Activity from the acidic to the alkaline pH range	70
Cytochrome P450 monooxygenases [¶]	Ascomycota, Basidiomycota, Mucoromycotina and Chytridiomycota	Cell bound	• Incorporation of a single atom from O ₂ into a substrate molecule, with concomitant reduction of the other atom to H ₂ O	• Epoxidation and hydroxylation of aromatic or aliphatic structures of many pollutants, including PAHs, PCDDs, alkanes and alkyl-substituted aromatics	3, 80–82, 87
Phenol 2-monooxygenases (EC 1.14.13.7)	Ascomycota and Basidiomycota	Cell bound	• Incorporation of a single atom from O ₂ into a substrate molecule, with concomitant reduction of the other atom to H ₂ O	• <i>Ortho</i> -hydroxylation of various (halo) phenols to the corresponding catechols	33, 87
Nitroreductases [¶]	Ascomycota and Basidiomycota and Mucoromycotina	Cell bound	• NAD(P)H-dependent reduction of nitroaromatics to hydroxylamino and amino(nitro) compounds, and of nitro functional groups of N-containing heterocycles	• Reduction of TNT to hydroxylamino-dinitrotoluene and amino-dinitrotoluenes • Formation of mononitroso derivatives and ring cleavage products from cyclic nitramine explosives • Widespread among fungi	34, 90–94
Quinone reductases [¶]	Basidiomycota	Cell bound	• NAD(P)H-dependent reduction of quinones	• Functions in quinone detoxification, in the conversion of quinones arising from extracellular pollutant oxidation into substrates for extracellular and intracellular oxidoreductases, and in pollutant attack by hydroxyl radicals arising from quinone redox cycling • Occurrence in white-rot and brown-rot basidiomycetes	95–98, 102
Reductive dehalogenases [¶]	Basidiomycota and perhaps Ascomycota	Cell bound	• Two-component system comprising a membrane-bound glutathione <i>S</i> -transferase that produces glutathionyl conjugates with concomitant chlorine removal, and a soluble glutathione conjugate reductase that releases reductively dechlorinated compounds	• Reductive dechlorination of chlorohydroquinones arising from chlorophenol degradation and of diphenyl ether herbicides (basidiomycetes) • Perhaps responsible for reductive dechlorination of chlorocatechols arising from PCDD degradation (ascomycetes)	58, 86, 102, 104
Miscellaneous transferases	Ascomycota, Basidiomycota and Mucoromycotina	Cell bound	• Formation of glucoside, glucuronide, xyloside, sulphate or methyl conjugates from hydroxylated compounds	• Phase II enzymes are prominent in fungal PAH metabolism but also act on other pollutants • Widespread among fungi	3, 89

MTBE, methyl-*tert*-butylether; PAH, polycyclic aromatic hydrocarbon; PCDD, polychlorinated dibenzo-*p*-dioxin; TNT, 2,4,6-trinitrotoluene. *See Supplementary information_S1 (table) for an extended version of this table, additionally exemplifying known enzyme genes and providing information about the regulation of catabolic-enzyme production in fungi. [†]See the ExPASy ENZYME database. [‡]Further sequence information related to extracellular enzymes can be found in the Fungal Oxidative Lignin Enzymes (EOLs) database (laccases, and lignin, manganese, versatile and generic peroxidases) and the *ParaoxiBase* database (peroxidases). [¶]Particularly promising for the bioremediation of organic chemicals. ^{¶¶}Commercialized for textile dye-bleaching in finishing dyed cotton fabric (DenLite, a laccase, and Baylase, which uses *C. cinerea* peroxidase), for preparing cork stoppers for wine bottles (Suberase, a laccase) and for diagnostic and research applications (lignin and manganese peroxidases, and *C. fumago* haem-thiolate chloroperoxidase)^{¶¶¶}. ^{¶¶¶}Further sequence information about fungal cytochrome P450s is available at the *Fungal P450* page maintained by D. R. Nelson.

Degradace organických polutantů houbami vs. bct

Box 1 | **Bacterial approaches to pollutant degradation**

Bacterial degradation of pollutants differs in two aspects from most cases of fungal degradation:

- Bacteria typically use the pollutants as growth substrates. The efficiency of bacterial degradation thus relies on a positive feedback loop between pollutant degradation and the formation of more bacteria. However, unlike chemical catalysts, organisms require minimum substrate fluxes to persist. Below a crucial 'per cell' flux of maintenance energy, the biocatalyst concentration and its catabolic capacity decrease. This occurs when pollutant concentrations are very low (some trace chemicals in wastewater treatment) or when pollutants are poorly bioavailable (high-molecular-mass hydrocarbons) or contain very little energy (highly oxidized chemicals, such as chlorinated or nitrated compounds). Under such conditions, an organism persists only if it succeeds in reducing its maintenance requirements — usually coupled with a loss in activity (for example, entering dormancy or undergoing sporulation) — or in using other substrates along with the pollutant.
- Bacteria use specific biochemical pathways. Degradation pathways for new pollutants are mostly modifications or extensions of existing pathways. Alternatively, they may be newly assembled but based on existing enzymes gained, for example, by genetic transfer between species. However, degradative pathways will only evolve and radiate when there is a selective benefit for their encoding bacterium. Specific pathways are thus unlikely to exist for environmental chemicals that always occur at concentrations below those required for multiplication or for chemicals containing rare or novel structural elements. The difficulty in finding bacteria that productively degrade drugs, agricultural chemicals or ingredients of consumer products (cosmetics, dyes, detergents, and so on) can be explained in this way. However, bacteria are particularly successful degraders of structurally simple mass chemicals (for example, those that are aliphatic or aromatic with low numbers of functional groups).



Závěrečný přehled látek a jejich biodegradovatelnosti MO

Table 15–2 An Overview of Relationships between Chemicals, Their Properties, and Bioremediation Prospects

Chemical Classes ^a	Biodegradability ^b (A, N, AN)	Mobility ^c	Frequency of Occurrence ^d	Partitioning Reactions ^e	Prospects for Bioremediation ^f
Hydrocarbons					
BTEX	A1, N2, AN2	H	F	M	Es
Low MW, gasoline, #2 fuel oil	A1, N3, AN2	M	F	M	Es
High MW. oil, PAH	A2, N4, AN4	L	C	S	Em
Creosote	A1, N2, AN4	L	I	S	Em
Oxygenated hydrocarbons					
Low MW alcohols, ketones, esters, ethers	A1, N5, AN3	H	C	W	Es
Halogenated aliphatics					
Highly chlorinated	A4, A3, N5, AN2	M	F	M	Em
Less chlorinated	A2, A3, N5, AN2	H	F	M	Em
Halogenated aromatics					
Highly chlorinated	A4, A2, N5, AN2	L	C	S	Em
Less chlorinated	A2, A3, N2, AN2	M	C	M	Em
PCBs					
Highly chlorinated	A4, N5, AN2	L	I	S	Em
Less chlorinated	A2, A1, N5, AN4	L	I	S	Em
Nitroaromatics					
	A2, N5, AN2	M	C	M	Em

^aBTEX = benzene, toluene, ethylbenzene, xylenes; MW = molecular weight; PAH = polycyclic aromatic hydrocarbon; PCBs = polychlorinated biphenyls.

^bThe three alphanumeric entries for each compound provide a biodegradability rating (1–5) under aerobic (A), nitrate-reducing (N), and other anaerobic (AN) conditions. 1 = readily mineralizable as growth substrate; 2 = biodegradable under narrow range of conditions; 3 = metabolized partially when second substrate is present (co-metabolized); 4 = resistant; 5 = insufficient information.

^cH = highly mobile; M = moderately mobile; L = least mobile.

^dBased on survey of groundwater contaminants. F = very frequent; C = common; I = Infrequent.

^eS = strong sorptive characteristics; M = moderate characteristics; W = weak characteristics.

^fEs = established; Em = emerging.

Závěrečný přehled látek a jejich biodegradovatelnosti MO

TABLE 10.5 Summary

Chemical class	Frequency of occurrence	Status of bioremediation	Evidence of future success	Limitations
Hydrocarbons and derivatives				
Gasoline, fuel oil	Very frequent	Established	Aerobically biodegradable under a narrow range of conditions	Forms nonaqueous phase liquid Sorbs strongly to subsurface solids
PAHs	Common	Emerging	Readily biodegradable under aerobic conditions	Sorbs strongly to subsurface solids; forms nonaqueous phase liquid
Creosote	Infrequent	Emerging		
Alcohols, ketones, esters	Common	Established		
Ethers	Common	Emerging	Biodegradable under a narrow range of conditions using aerobic or nitrate-reducing microbes	
Halogenated aliphatics				
Highly chlorinated	Very frequent	Emerging	Cometabolized by anaerobic microbes; cometabolized by aerobes in special cases	Forms nonaqueous phase liquid
Less chlorinated	Very frequent	Emerging	Aerobically biodegradable under a narrow range of conditions; cometabolized by anaerobic microbes	Forms nonaqueous phase liquid
Halogenated aromatics				
Highly chlorinated	Common	Emerging	Aerobically biodegradable under a narrow range of conditions; cometabolized by anaerobic microbes	Sorbs strongly to subsurface solids; forms nonaqueous phase either liquid or solid
Less chlorinated	Common	Emerging	Readily biodegradable under aerobic conditions	Forms nonaqueous phase either liquid or solid
Polychlorinated biphenyls				
Highly chlorinated	Infrequent	Emerging	Cometabolized by anaerobic microbes	Sorbs strongly to subsurface solids
Less chlorinated	Infrequent	Emerging	Aerobically biodegradable under a narrow range of conditions	Sorbs strongly to subsurface solids
Nitroaromatics	Common	Emerging	Aerobically biodegradable; converted to innocuous volatile organic acids under anaerobic conditions	
Metals (Cr, Cu, Ni, Pb, Hg, Cd, Zn, etc.)	Common	Possible (see Chapter 17)	Solubility and reactivity can be changed by a variety of microbial processes	Availability highly variable and controlled by solution and solid-phase chemistry

Závěrečný přehled látek a jejich biodegradovatelnosti MO

Table 8.6

Overview of biodegradation and bioremediation potential for particular classes of environmental contaminants (modified from National Research Council. 2000. *Natural Attenuation for Groundwater Remediation*. National Academies Press, Washington, DC. Reprinted with permission from the National Academies Press. Copyright 2000, National Academy of Sciences)

Chemical class	Mechanisms of microbe–contaminant interactions	Type(s) of contaminant alteration	Susceptibility to microbiological transformation	
			Aerobic	Anaerobic
Organic				
Petroleum hydrocarbons				
Low molecular weight				
BTEX	Carbon and electron-donor source	Mineralized to CO ₂	1	2
Gasoline, fuel oil	Carbon and electron-donor source	Mineralized to CO ₂	1	2
High molecular weight				
Oils, PAHs	Carbon and electron-donor source	Mineralized to CO ₂ or partially degraded	1, 2	2, 4
Creosote	Carbon and electron-donor source	Mineralized to CO ₂ or partially degraded	1, 2	2, 4
Oxygenated hydrocarbons				
Low molecular weight				
Alcohols, ketones, esters, ethers	Carbon and electron-donor source	Mineralized to CO ₂	1, 2	2
MTBE	Cometabolized; occasionally used as carbon and electron-donor source	Partially degraded, sometimes mineralized to CO ₂	2–5	4, 5
Halogenated aliphatics				
Highly chlorinated	Electron acceptor under anaerobic conditions; cometabolized	Partially degraded, dechlorinated	2–5	2–5
Less chlorinated	Electron acceptor under anaerobic conditions; carbon and electron-donor source; cometabolized	Partially degraded, dechlorinated	2–5	2–5

Závěrečný přehled látek a jejich biodegradovatelnosti MO

Table 8.6 Continued

Chemical class	Mechanisms of microbe–contaminant interactions	Type(s) of contaminant alteration	Susceptibility to microbiological transformation*	
			Aerobic	Anaerobic
Halogenated aromatics				
Highly chlorinated	Electron acceptor under anaerobic conditions; carbon and electron-donor source; cometabolized	Partially degraded, dechlorinated	2–5	2, 3
Less chlorinated	Electron acceptor under anaerobic conditions; carbon and electron-donor source; cometabolized	Partially degraded, mineralized to CO ₂	1, 2	2
PCBs				
Highly chlorinated	Electron acceptor under anaerobic conditions	Partially degraded, dechlorinated	4	2, 3
Less chlorinated	Electron acceptor under anaerobic conditions; carbon and electron-donor source	Partially degraded or fully mineralized to CO ₂	1, 2	2, 4
Dioxins	Electron acceptor under anaerobic conditions	Partially degraded	4	4
Nitrogen-containing explosives (TNT, RDX)	Cometabolized	Partially degraded; immobilized by precipitation or polymerization	2	2

Biodegrace org. polutantů – neustálý rozvoj poznatků

Table 8.8

Selected examples of organic environmental pollutants that were initially considered nonbiodegradable and later reclassified as biodegradable (from J. Spain, personal communication; Seffernick and Wackett, 2001; Wackett et al., 2002; Johnson and Spain, 2003)

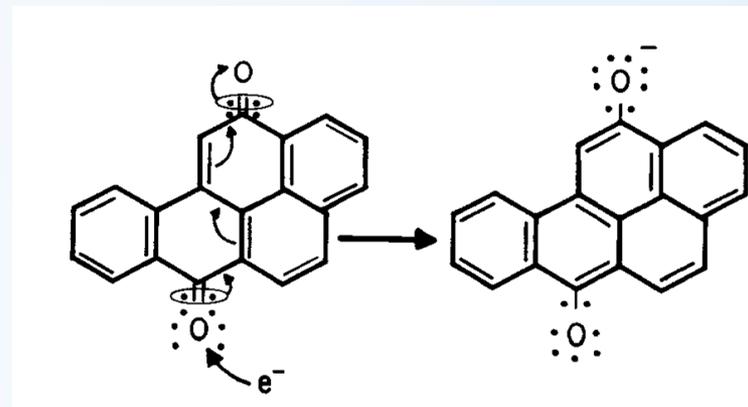
Compound	Use	Physiological role of compound	Genetic change conferring biodegradability
Melamine	Building block for early industrial polymers	Electron donor and carbon source	New enzymatic activity, melamine deaminase, in new combination with other genes
Atrazine	Chlorinated triazine herbicide	Electron donor and carbon source	New enzymatic activity, especially atrazine chlorohydrolase, in new combination with other genes
Styrene	Monomer for industrial polymer, polystyrene	Electron donor and carbon source	New enzymatic activity, styrene epoxide isomerase, in new combination with other genes
2,4-dinitrotoluene (2,4-DNT)	Manufacture of polyurethane foam; production of explosives	Electron donor and carbon source	New enzymatic activity, 2,4-DNT dioxygenase, in new combination with other genes
Chlorobenzene	Solvent for pesticide formulations; degreasing agent	Electron donor and carbon source	New enzymatic activity, chlorobenzene dioxygenase, in new combination with other genes
Nitrobenzene	Production of the industrial compound, aniline; ingredient in shoe and floor polishes	Electron donor and carbon source	New enzymatic activity, nitrobenzene nitroreductase, in new combination with other genes
Pentachlorophenol (PCP)	Antifungal wood preservative	Electron donor and carbon source	New enzymatic activity, PCP 4-monooxygenase, in new combination with other genes
Polychlorinated biphenyls (PCBs)	Dielectric fluid used in electrical transformers	If highly chlorinated, final electron acceptor If lightly chlorinated, electron donor and carbon source	[There are 209 different forms (congeners) of PCB molecules; explanations for metabolism resist generalization]
Tetrachloroethene and trichloroethene	Industrial solvents and degreasing agents	Final electron acceptors	New enzymatic activity, tetrachloroethene reductive dehalogenase

Several of the listed compounds (especially styrene) may be naturally occurring. Thus, some metabolic pathways may have evolved prior to widespread manufacture and environmental release of the compounds.



Efekty organických polutantů na buňky MO

- narušení integrity a fluidity membrány (důsledky: ztráta důležitých látek z buňky, vstup dalších polutantů ...)
- interakce s procesy a gradienty na membráně (např. chinony – přenos e- při respiraci)
- interakce s biologickými molekulami (většinou po aktivaci) – DNA, proteiny
- interakce s receptory (membránovými či jadernými)
- oxidativní stres (např. chinony) – oxidace makromolekul (NA, proteiny), peroxidace membránových lipidů



Efekty organických polutantů na buňky MO - příklad

• Aromatické látky toxické pro MO

- ve vodě rozpustná frakce rafinovaných ropných produktů (více toxické než tato frakce ze surové ropy - méně NAP a alkyl-NAP) - toxicita pro heterotrofické i autotrofické MO

• Zejména po metabolizaci (aktivaci) jsou vysoce reaktivní:

- mohou se vázat a vytvářet kovalentní adukty s životně důležitými makromolekulami, jako jsou celulární proteiny, DNA apod.
- interkalace v DNA
- podobně působí chinony, aza-PAHs, nitro-PAHs

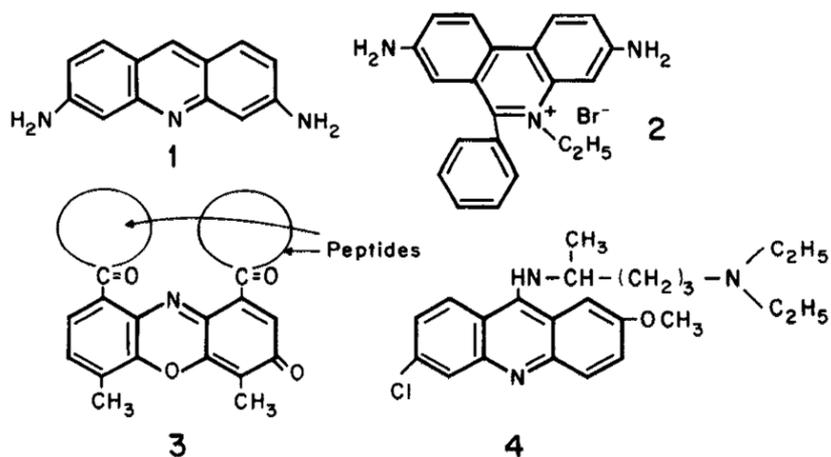
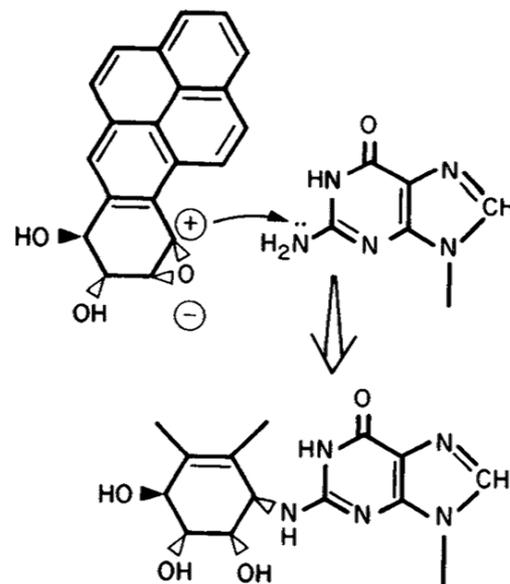


Chart 5.8. Examples of intercalating agents. Key: 1, acriflavine; 2, ethidium bromide; 3, actinomycin; 4, quinacrine.



Scheme 5.5. Interaction of 7,8-dihydrodiol-9,10-epoxide of benzo[a]pyrene with the amino group of guanine.

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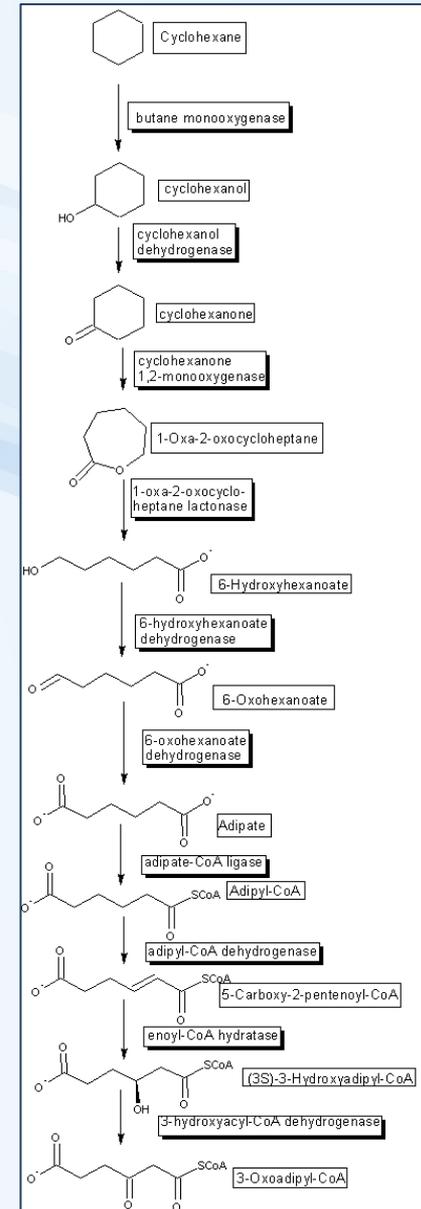
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Gao J, Ellis LBM, Wackett LP (2010) "The University of Minnesota Biocatalysis/Biodegradation Database: improving public access" *Nucleic Acids Research* 38: D488-D491

Další toxikanty a MO

Další toxikanty a MO

- halogeny
 - silná oxidovadla (H_2O_2 , KMnO_4)
 - barviva
 - syntetické tenzidy
 - některé plyny
- využívání pro desinfekci, ale úniky do prostředí poškozují přírodní populace MO



Povrchově aktivní látky

- hydrofilní a hydrofobní část molekuly – snižují povrchové napětí – tenzidy

- anionaktivní (mýdla)
- kationaktivní
- neionogenní

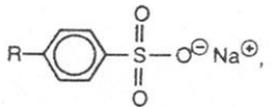
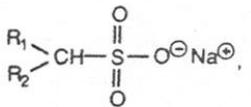
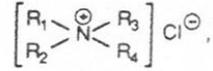
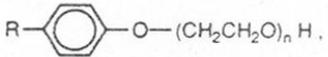
Anionaktivní

- ve vyšších koncentracích poškozují cytoplazmatickou membránu, způsobují denaturaci bílkovin
- při nižších koncentracích pronikají do buňky a ovlivňují metabolismus

Kvarterní amoniové soli

- působí baktericidně (především na G+, méně na G-), fungicidně, amoebicidně, virucidně

TABLE 2.6 Examples of Commercially Important Surfactants^a

Common Name of Surfactant Class (Acronym)	General Structure
<i>Anionic Surfactants</i>	
Soaps	$R-CH_2-COO^{\ominus}Na^{\oplus}$, R = C ₁₀₋₁₆
Linear alkylbenzene sulfonates (LAS)	 , R = C ₁₀₋₁₃
Secondary alkyl sulfonates (SAS)	 , R ₁ , R ₂ = C ₁₁₋₁₇
Fatty alcohol sulfates (Alkyl sulfates, FAS)	$R-CH_2-O-SO_3^{\ominus}Na^{\oplus}$, R = C ₁₁₋₁₇
<i>Cationic Surfactants</i>	
Quaternary ammonium chloride (QAC)	 , R ₁ = R ₂ = C ₁ , R ₃ = R ₄ = C ₁₆₋₁₈
<i>Nonionic Surfactants</i>	
Alkylphenol polyethyleneglycol ethers (APEO)	 , R = C ₈₋₁₂ , n = 5-10
Fatty alcohol polyethyleneglycol ethers (AEO)	$R-CH_2-O-(CH_2CH_2O)_nH$, R = C ₇₋₁₇ , n = 3-15

^aFrom Piorr (1987).



Halogeny

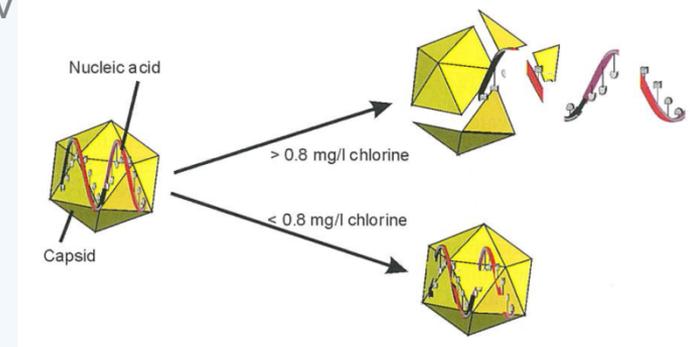
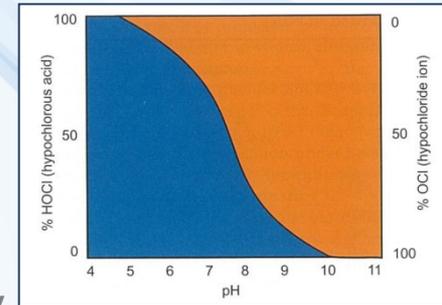
- zejména chlór - chlornany (-OCl) a chloraminy (Cl-NH₂)
- nejpoužívanější technologie čištění pitné a odpadní vody
- silné oxidační činidlo
- Cl₂ tvoří ve vodě:



- v prostředí se váže na amoniak či organické látky – vázány chlór
- chloraminy jsou méně efektivní než volně dostupný chlór
- působí na vegetativní i klidová stadia bakterií, virů i prvoků
 - mění permeabilitu membrány – únik důležitých látek v buněžt
 - interferuje s ději na membráně – fosforylace
 - nevratné vazby na SH skupiny enzymů a proteinů
 - denaturace NA



- Br, I – substituce tyrosylu a histidylu, oxidace SH



Halogeny

TABLE 23.3 C · t Values for Chlorine Inactivation of Microorganisms in Water (99% Inactivation)^a

Organism	°C	pH	C · t
Bacteria			
<i>E. coli</i>	5	6.0	0.04
<i>E. coli</i>	23	10.0	0.6
<i>L. pneumophila</i>	20	7.7	1.1
Viruses			
Polio 1	5	6.0	1.7
Protozoa			
<i>G. lamblia</i> cysts	5	6.0	54–87
<i>G. lamblia</i> cysts	5	7.0	83–133
<i>G. lamblia</i> cysts	5	8.0	119–192
<i>G. muris</i> cysts	5	6.0	250
<i>Cryptosporidium</i> oocysts	25	7.0	>7200

From Sobsey (1989); Rose *et al.* (1997).

^a In buffered distilled water.

TABLE 23.5 C · t Values for Chloramines in Water (99% Inactivation)^a

Microbe	°C	pH	C · t
Bacteria			
<i>E. coli</i>	5	9.0	113
<i>M. fortuitum</i>	20	7.0	2667
<i>M. avium</i>	17	7.0	ND**
<i>M. intracellulare</i>	17	7.0	ND
Viruses			
Polio 1	5	9.0	1420
Hepatitis A	5	8.0	592
Coliphage MS2	5	8.0	2100
Rotavirus SA11			
Dispersed	5	8.0	4034
Cell-associated	5	8.0	6124
Protozoa			
<i>G. muris</i>	3	6.5–7.5	430–580
<i>G. muris</i>	5	7.0	1400
<i>Cryptosporidium</i>	25	7.0	>7200

Adapted from Sobsey (1989); Rose *et al.* (1997).

^a In buffered distilled water.

** ND = no data.

TABLE 23.4 C · t Values for Chlorine Dioxide in Water

Microbe	ClO ₂ residual (mg/l)	Temperature (°C)	pH	% reduction	C · t
Bacteria					
<i>E. coli</i>	0.3–0.8	5	7.0	99	0.48
<i>L. pneumophila</i>	0.5–0.35	23		99.9–99.99	ND
Viruses					
Polio 1	0.4–14.3	5	7.0	99	0.2–6.7
Rotavirus SA11					
Dispersed	0.5–1.0	5	6.0	99	0.2–0.3
Cell-associated	0.45–1.0	5	6.0	99	1.0–2.1
Hepatitis A	0.14–0.23	5	6.0	99	1.7
Coliphage MS-2	0.15	5	6.0	99	5.1
Protozoa					
<i>G. muris</i>	0.1–5.55	5	7.0	99	10.7
<i>G. muris</i>	0.26–1.2	25	5.0	99	5.8
<i>G. muris</i>	0.21–1.12	25	7.0	99	5.1
<i>G. muris</i>	0.15–0.81	25	9.0	99	2.7
<i>Cryptosporidium</i>	4.03	10	7.0	95.8	6.0

Adapted from Sobsey (1989); Rose *et al.* (1997).

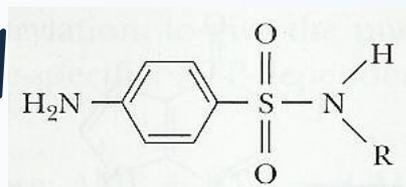
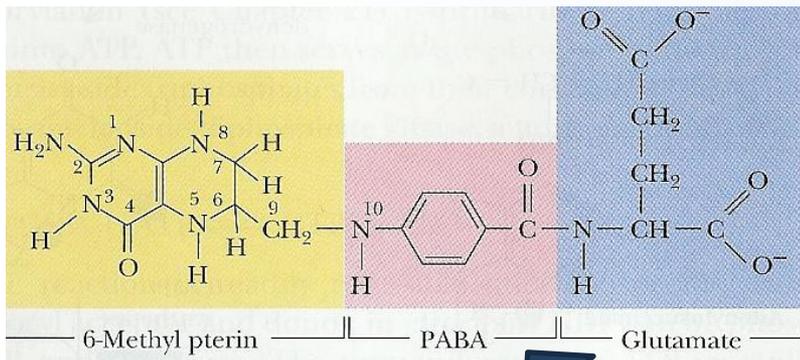
TABLE 23.6 C · t Values for Ozone Inactivation of Microorganisms in Water (99% Inactivation)

Organism	°C	pH	C · t
Bacteria			
<i>E. coli</i>	1	7.2	0.006–0.02
Viruses			
Polio 1	5	7.2	0.2
Polio 2	25	7.2	0.72
Rota SA11	4	6.0–8.0	0.019–0.064
Protozoa			
<i>G. muris</i>	5	7.0	1.94
<i>G. lamblia</i>	5	7.0	0.53
<i>Cryptosporidium</i>	7	—	7.0
<i>Cryptosporidium</i>	22	—	3.5

From Sobsey (1989); Rose *et al.* (1997).

Chemoterapeutika

- selektivní účinek
- inhibice procesů biosyntézy koenzymů, bílkovin a NA
- strukturálními analogy esenciálních látek s větší afinitou k daným enzymům
- isonikotinylhydrazid → pyridoxin
- kyselina p-aminosalicylová → kyselina salicylová
- sulfonamidy → kyselina p-aminobenzoová (PABA)



Antibiotika

- neřízené používání v živočišné výrobě + vstupy z komunálních odpadních vod a jejich kalů (spolu s dalšími farmaceutiky)
- negativní efekty na přirozené populace MO

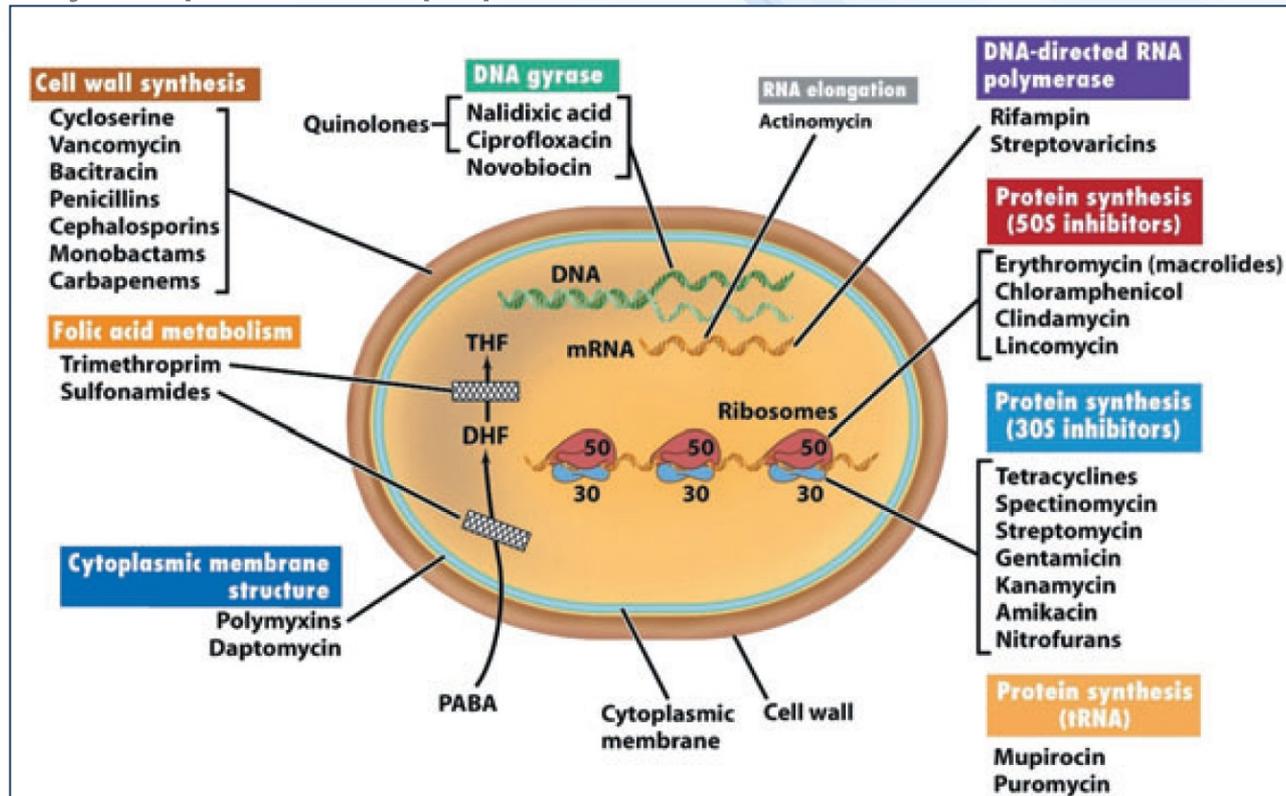


Figure 8.27 Mode of action of major antimicrobial agents. DHF, dihydrofolate; DNA, deoxyribonucleic acid; mRNA, messenger RNA; PABA, para-amino benzoic acid; THF, tetrahydrofolate; tRNA, transfer RNA. (From MADIGAN, M. and J. MARTINKO. 2006. *Brock Biology of Microorganisms*, 11th edn, p. 683. Prentice Hall, Upper Saddle River, NJ. Copyright 2006. Reprinted by permission of Pearson Education, Inc., Upper Saddle River, NJ.)

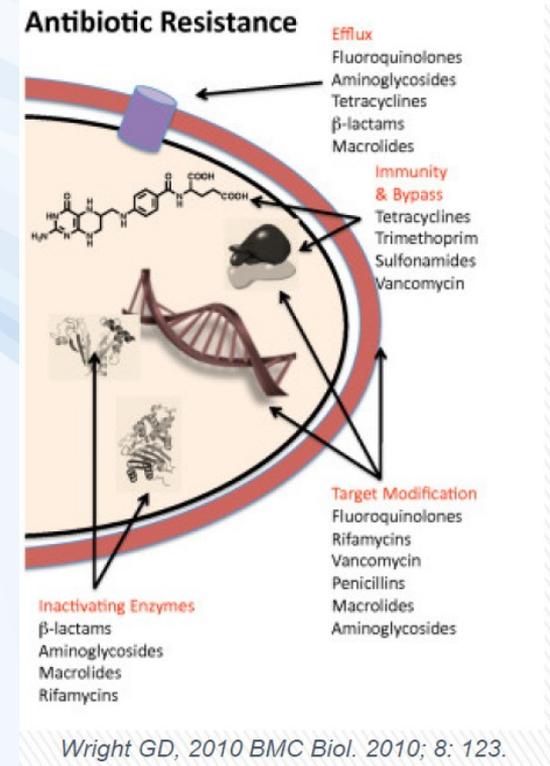


Antibiotika

Table 8.12

Mechanisms of antibiotic resistance in bacteria. The genes for each of these resistance traits can be transferred between bacteria (from Davies, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science* **264**:375–382. Reprinted with permission from AAAS)

Mechanism	Antibiotic
Reduced uptake into cell	Chloramphenicol
Active efflux from cell	Tetracycline
Modification of target to eliminate or reduce binding of antibiotic	β -lactams (e.g., penicillin G, amoxicillin) Erythromycin, lincomycin
Inactivation of antibiotic by enzymic modification:	
Hydrolysis	β -lactams Erythromycin
Derivatization	Aminoglycosides Chloramphenicol Fosfomicin Lincomycin
Sequestration of antibiotic by protein binding	β -lactams
Metabolic bypass of inhibited reaction	Fusidic acid
Binding of specific immunity protein to antibiotic	Sulfonamides Trimethoprim Bleomycin
Overproduction of antibiotic target (titration)	Sulfonamides Trimethoprim



Použitá literatura – MO a další toxikanty

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