Biodegradation

- Why study?
  - One of few fate processes where material is gone from the environment
  - Change concentrations that are present to have effect
  - We can play with microbial communities to get them to do some things we want

Biodegradation

- **Three big categories- no one told bugs**
  - **Rapid breakdown- days to weeks**
  - **Slow breakdown- months to years**
  - **Almost no breakdown- many years**
- **Chemical structure important**
- **Biodegradation requires the presence of the appropriate organism, the chemical in an available form, and the right environmental conditions for organisms to function**

Molecular Recalcitrance and Microbial Fallibility

- **100 yrs of everything breaking down-**
- **In 50's and 60's synthetic organics appear that do not break down**
- **No prior exposure of microbes to chemicals**
- **Could not find bugs to grow on them**
- **Therefore, there are recalcitrant compounds**
- **Began a variety of studies on breakdown in the environment**
- **Can find degraders for many of these compounds now- evolution?**

Chemical Structure

- **Structure has to relate to degradability since it dictates what kind of enzyme is needed**
- **There have been few systematic studies**
- **Most with TOC in screening tests**
- **Many of the chemicals you need are not available.**
- **Generally the larger the molecule, the more substituents it has and less water soluble- the slower it degrades**

First substituent on ring

For others number, type and position all have large influence

Table II. Decomposition of Monosubstituted Benzenes by a Soil Microflora

Compound	Substituent	Decomposition Period, Days
Benzoate	COOH	1
Phenol	OH	1
Nitrobenzene	NO_2	>64
Aniline	NH_2	4
Anisole	OCH_3	8
Benzenesulfonate	SO_3H	16

Table III. Decomposition of Disubstituted Benzenes by a Soil Microflora

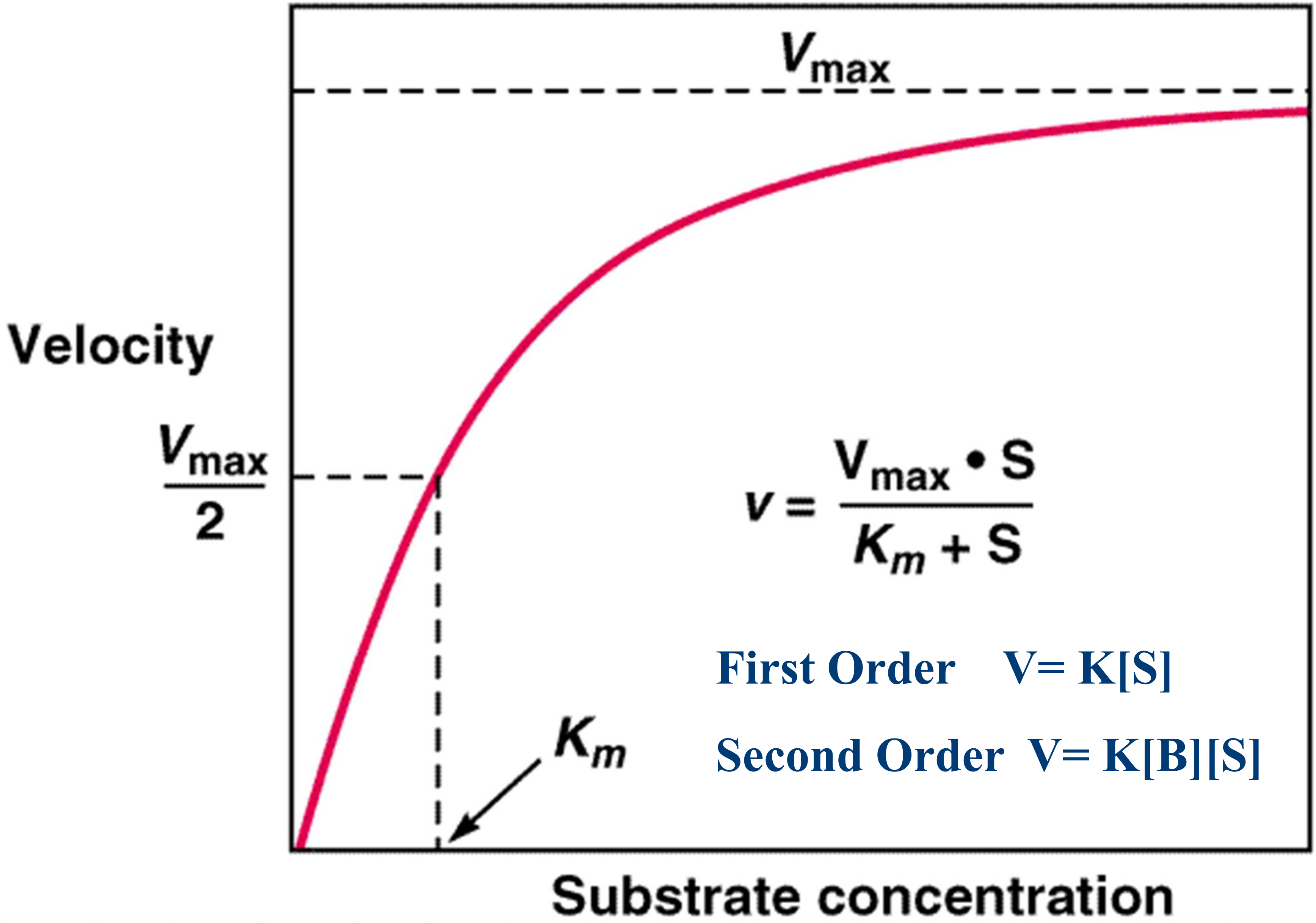
Second Substituent Type	Position	COOH	OH	NO_2	NH_2	OCH_3	SO_3H	Cl	CH_3
		First Substituent[a]							
COOH	o	2	2	8	2	4	>64	>64	16
	m	8	2	>64	>64	16	>64	32	2
	p	2	1	4	8	2	>64	64	8
OH	o		1	>64	4	4	...	>64	1
	m		8	4	>64	16	...	>64	1
	p		...	16	...	8	32	16	1
NO_2	o			>64	>64	>64	>64	>64	>64
	m			>64	>64	>64	>64	>64	>64
	p			>64	>64	>64	>64	>64	>64
NH_2	o				>64	>64	>64	>64	64
	m				>64	>64	>64	>64	8
	p				...	64	>64	>64	4
OCH_3	o					8	...	...	...
	m					>32	...	...	...
	p					8	...	...	...
SO_3H	m						>64	...	...
	p						...	16	>64

[a] Values reflect days for total loss of ultraviolet absorbancy at the designated wavelength.

Kinetics = How fast does it go

- **If something degrades the next question is how fast**
- **To predict how long it will persist need some idea of kinetics**
- **In most environments first order works for most chemicals**
- **More later on concentration effects**

Michaelis-Menton Kinetics



First Order V= K[S]

Second Order V= K[B][S]

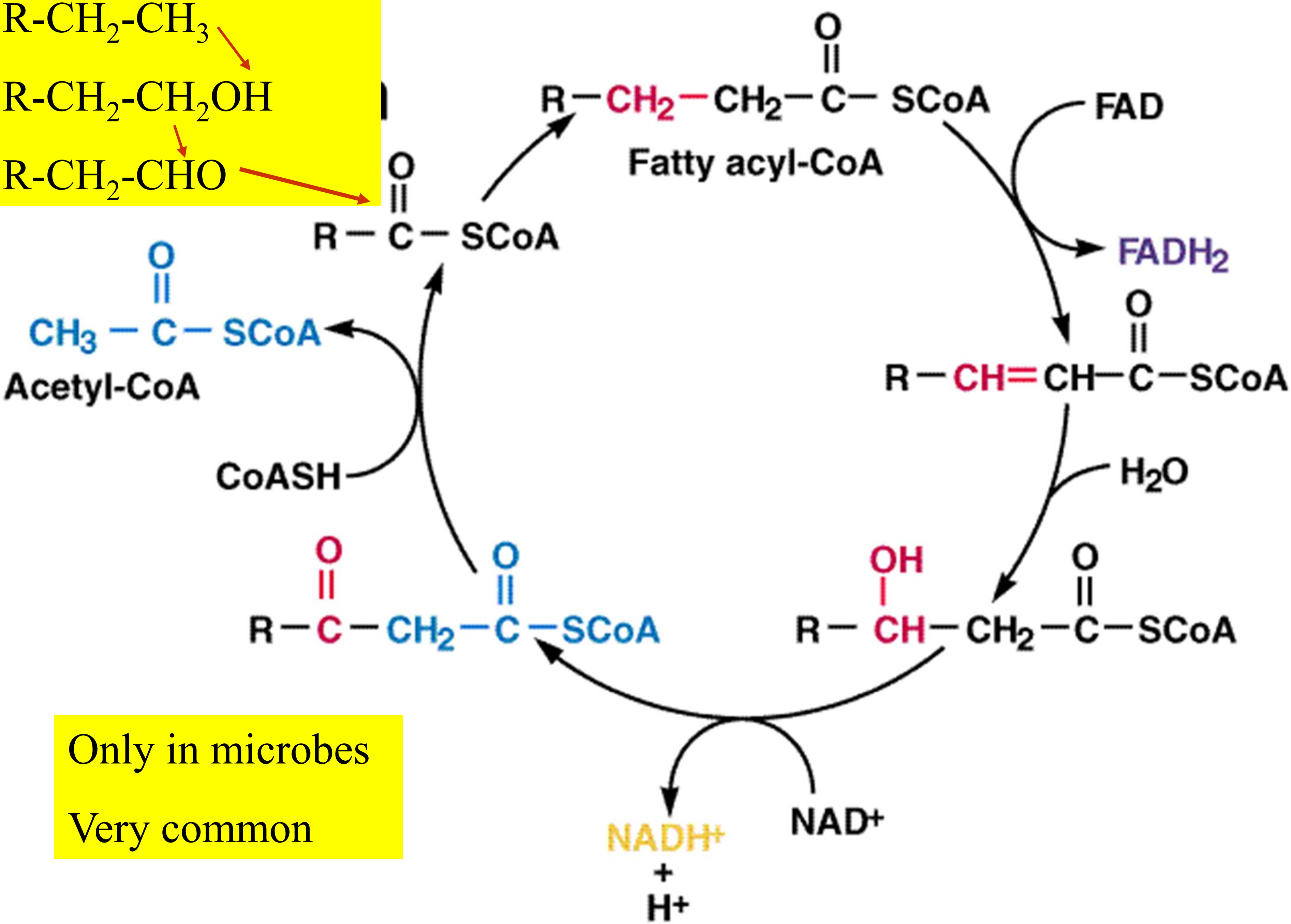
Rapid Biodegradation

- **Compounds that are identical or very similar to naturally occurring materials**
- **Use same or similar metabolic pathways as natural materials**
- **Usually support growth of some group of organisms**
- **Many examples- petroleum, pesticides, industrial chemicals**

$R-CH_2-CH_3$

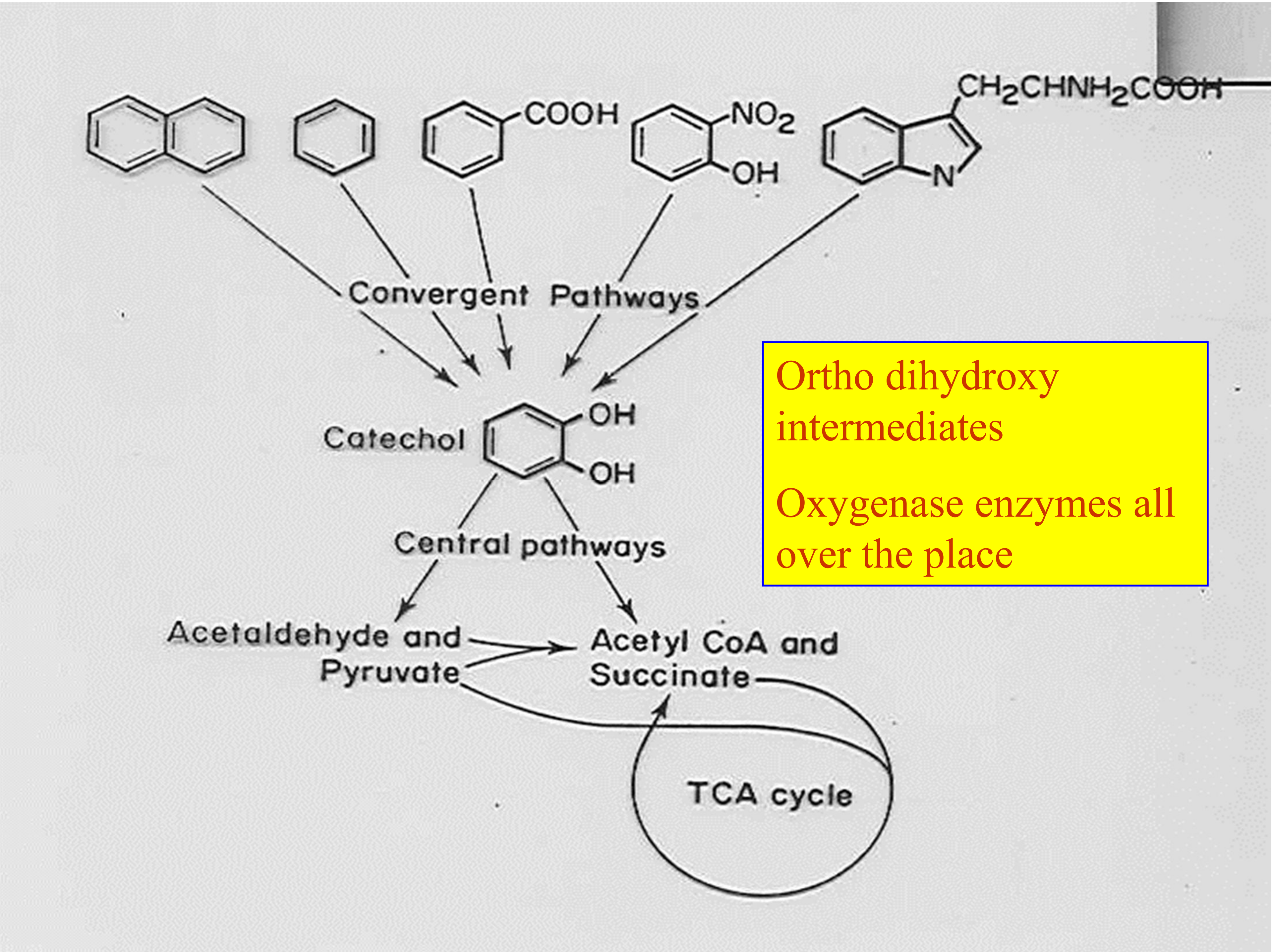
$R-CH_2-CH_2OH$

$R-CH_2-CHO$



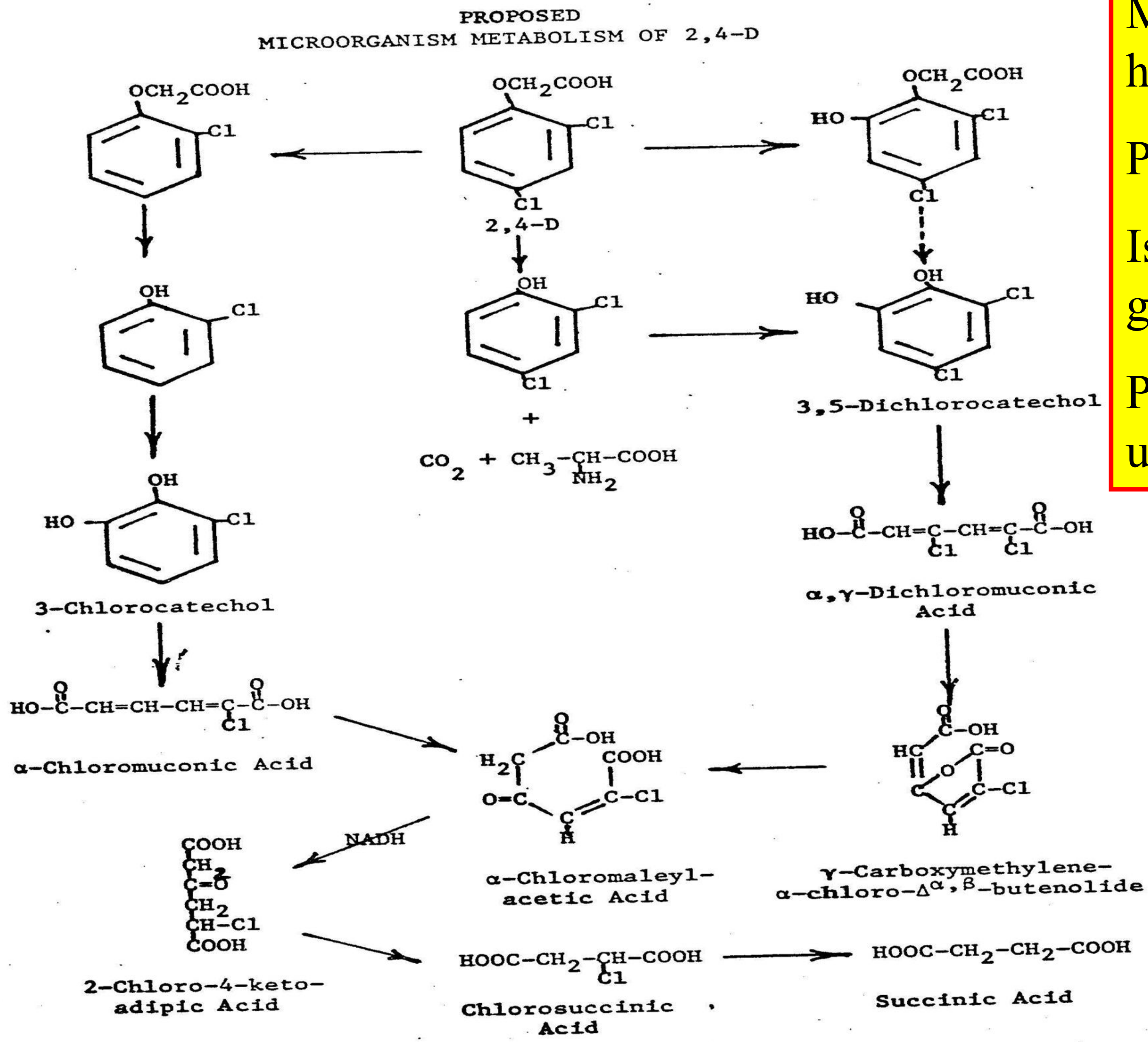
Only in microbes

Very common



Ortho dihydroxy intermediates

Oxygenase enzymes all over the place



Most Common herbicide

Persists 2 mo

Isolate bact that grow on it

Pathway well understoood

Slowly Degraded

- **Tends to be compounds with more than one substituent, or halogens**
- **Usually not present in high concentrations**
- **Generally do not support growth of degraders**
- **Partial degradation products common**
- **COMETABOLISM**

Cometabolism

TABLE 2. *Organic substances subject to co-metabolism and accumulated products*

Substrate	Product	Reference
Ethane	Acetic acid	37
Propane	Propionic acid, acetone	37
Butane	Butanoic acid, methyl ethyl ketone	37
m-Chlorobenzoate	4-chlorocatechol, 3 chlorocatechol	27, 33, 56
o-Fluorobenzoate	3-Fluorocatechol, fluoroacteate	15, [illegible]
2-Fluoro-4-nitrobenzoate	2-Fluoroprotocatechuic acid	54
4-Chlorocatechol	2-Hydroxy-4-chloro-muconic semialdehyde	23, 29
3,5-Dichlorocatechol	2-Hydroxy-3,5-dichloro-muconic semialdehyde	23, 29
3-Methylcatechol	2-Hydroxy-3-methyl-muconic semialdehyde	23, 29
o-Xylene	*o*-Toluic acid	44
p-Xylene	*p*-Toluic acid, 2,3-dihydroxy-*p*-toluic acid	44
Pyrrolidone	Glutamic acid	35
Cinerone	Cinerolone	53
n-Butylbenzene	Phenylacetic acid	[illegible]
Ethylbenzene	Phenylacetic acid	[illegible]
n-Phopylbenzene	Cynnamic acid	[illegible]
p-Isopropyltoluene	*p*-Isopropylbenzoate	[illegible]
n-Butyl-cyclohexane	Cyclohexaneacetic acid	[illegible]
2,3,6-Trichlorobenzoate	3,5-Dichlorocatechol	[illegible]
2,4,5-Trichlorophenoxy-acetic acid	3,5-Dichlorocatechol	24
p,p'-Dichlorodiphenyl methane	*p*-Chlorophenylacetate	17
1,1-Diphenyl-2,2,2-trichloroethane	2-Phenyl-3,3,3-trichloropropionic acid	17

They are small changes- one or two steps- then stops

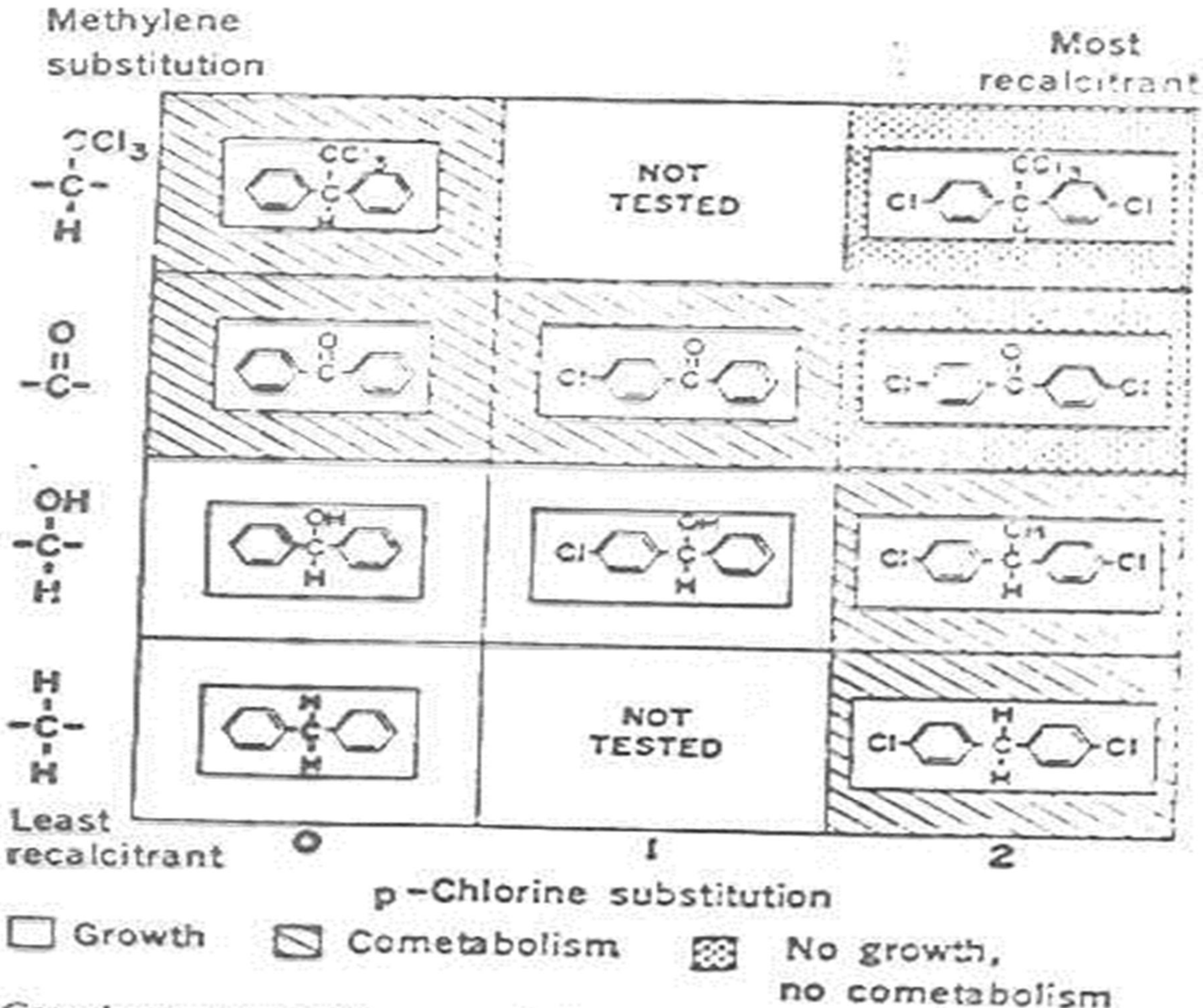
TABLE 1. *Metabolism of substituted benzoates by 20 bacterial isolates*

Substituted benzoate	No. of inactive cultures	Moles of O_2 consumed per mole of substrate metabolized[a]					
		0.5 ± 0.1	1.0 ± 0.1	1.5 ± 0.1	2.0 ± 0.1	2.5 ± 0.1	3.0 ± 0.1
o-Chloro	15	3	0	0	2	0	0
m-Chloro	9	6	0	2	3	0	0
p-Chloro	14	2	2	1	1	0	0
2,4-Dichloro	17	0	1	1	1	0	0
2,5-Dichloro	17	1	2	0	0	0	0
2,6-Dichloro	15	4	0	0	1	0	0
3,4-Dichloro	16	0	2	2	0	0	0
2,3,4-Trichloro	9	2	4	1	3	1	0
2,3,5-Trichloro	16	3	1	0	0	0	0
2,3,6-Trichloro	16	0	4	0	0	0	0
2,4,5-Trichloro	11	4	2	3	0	0	0
2,3,4,5-Tetrachloro	10	1	6	1	2	0	0
2,3,5,6-Tetrachloro	9	0	3	3	2	1	2
Pentachloro	19	0	1	0	0	0	0
m-Amino	18	1	1	0	0	0	0
p-Amino	18	1	1	0	0	0	0
3,5-Diamino	17	1	0	0	2	0	0
2-Chloro-4-amino	18	0	0	1	1	0	0
2-Chloro-5-amino	16	0	2	0	2	0	0
4-Chloro-2-amino	9	2	5	2	2	0	0
5-Chloro-2-amino	16	3	1	0	0	0	0
2,5-Dichloro-3-amino	17	1	0	0	2	0	0

[a] Figures in the columns represent the number of cultures metabolizing the compound with the consumption of the quantity of O_2 indicated.

Why partial metabolism?

- **Very common way for xenobiotic to degrade**
- **Either from unusual structure or very low concentration**
- **The enzymes early in a pathway are not very specific- so attack more than one thing**
- **As pathways proceed the enzymes become more specific- so it stops at some point**
- **Function of non-specific enzymes**



· 1. Growth on or aerobic cometabolism of diphenylmethanes and related com-
nds by *Hydrogenomonas* sp.

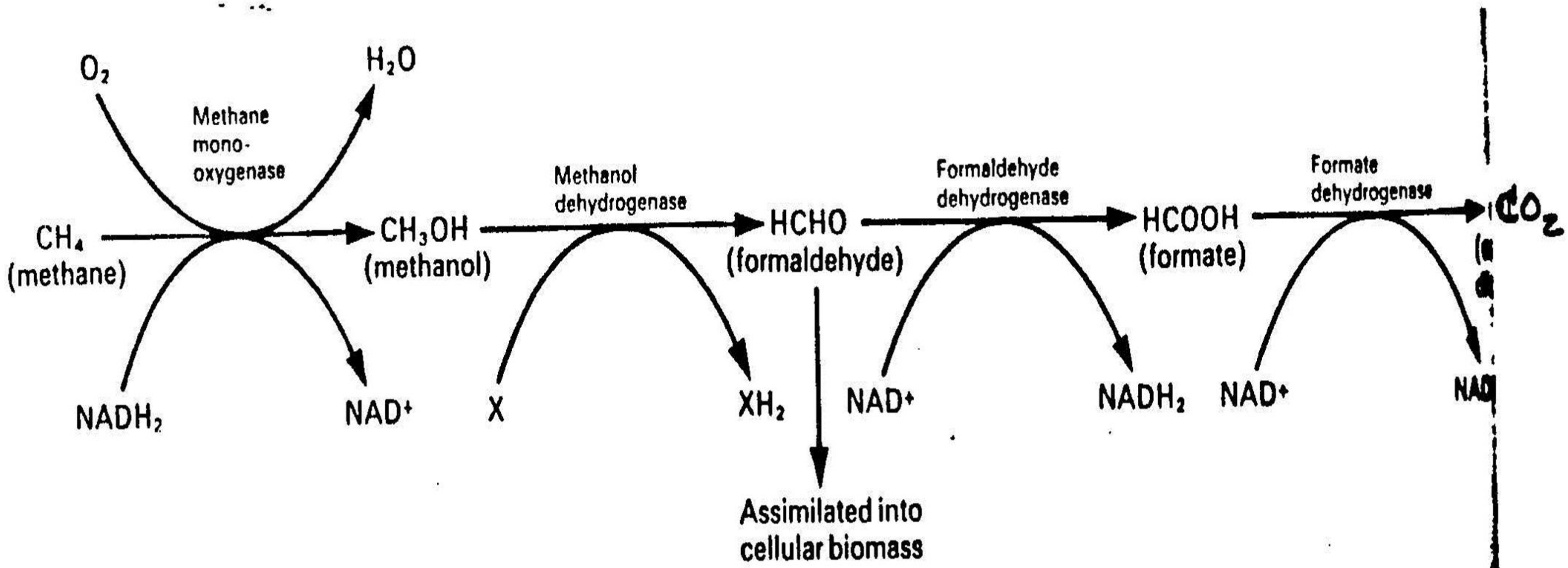
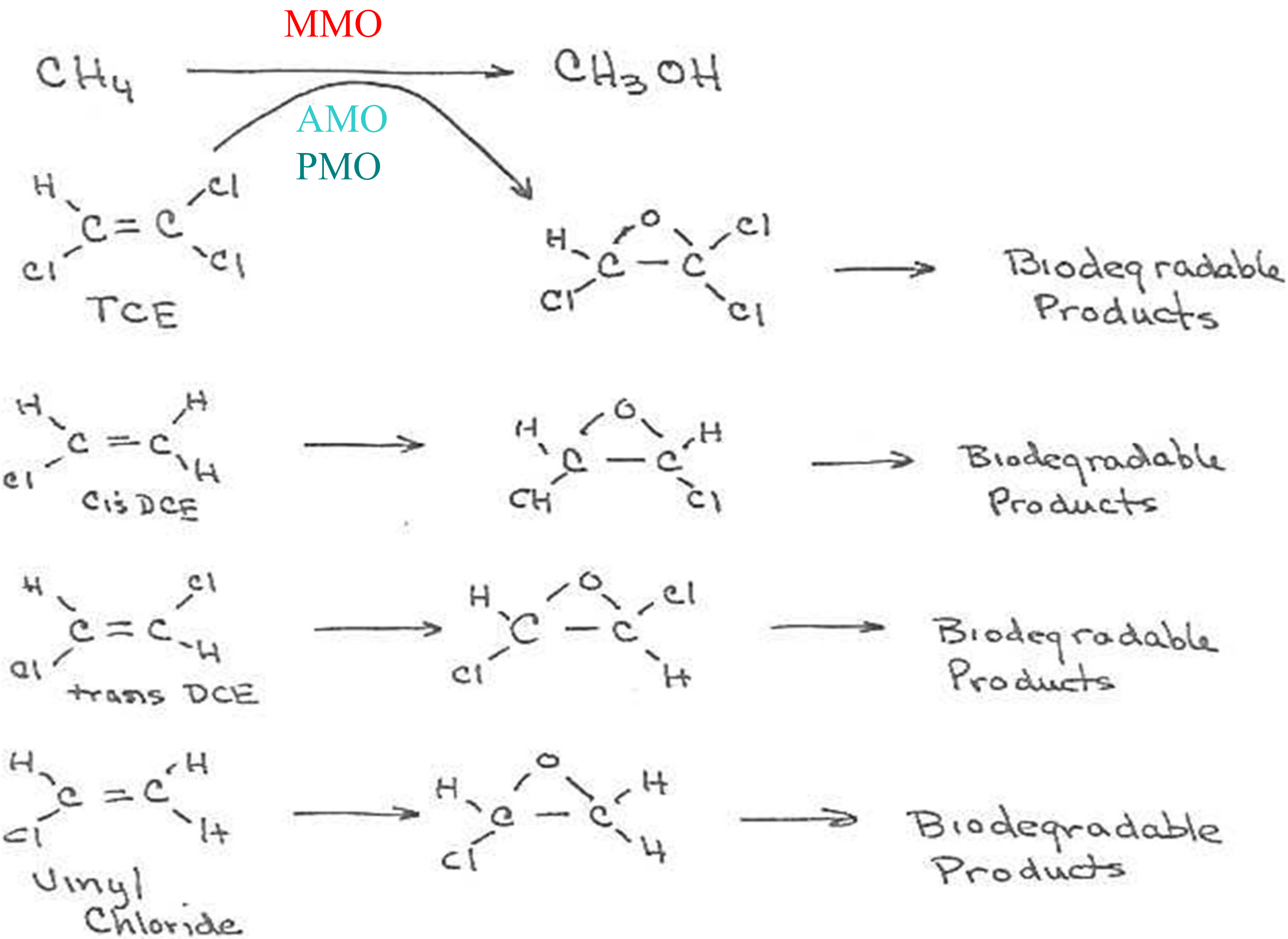


FIGURE 1. Microbial oxidation of methane.

TABLE 2. SUBSTRATES AND PRODUCTS OF METHANE MONOOXYGENASE

substrate	product(s)	substrate	product(s)
methane	methanol, formaldehyde	ethene	epoxyethane
ethane	ethanol, acetaldehyde	propene	epoxypropane
chloromethane	formaldehyde	benzene	phenol, hydroquinone
methanol	formaldehyde	toluene	benzyl alcohol, *p*-cresol
dimethyl ether	methanol, formaldehyde	pyridine	pyridine-*N*-oxide
carbon monoxide	carbon dioxide	ammonia	hydroxylamine, nitrite

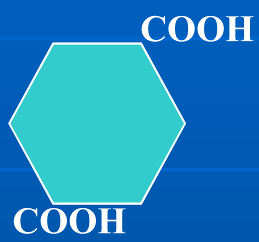
AEROBIC METABOLISM OF TRICHLOROETHYLENE (TCE)



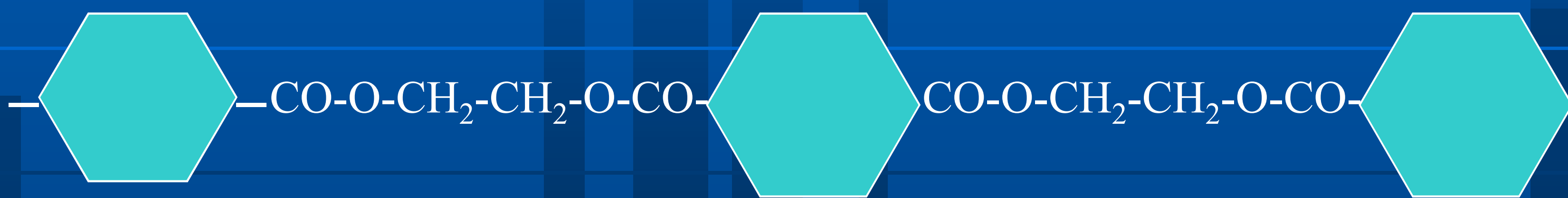
Almost Non-Degradable

- Mostly polymers – monomers often degradable
- Even natural polymers slowly degradable
- Large molecules cannot enter cells- need extracellular enzymes
- Surfaces often not wetable so water and enzyme does not see molecule
- Many examples

Simple Molecules linked



Terphthalic acid



DACRON

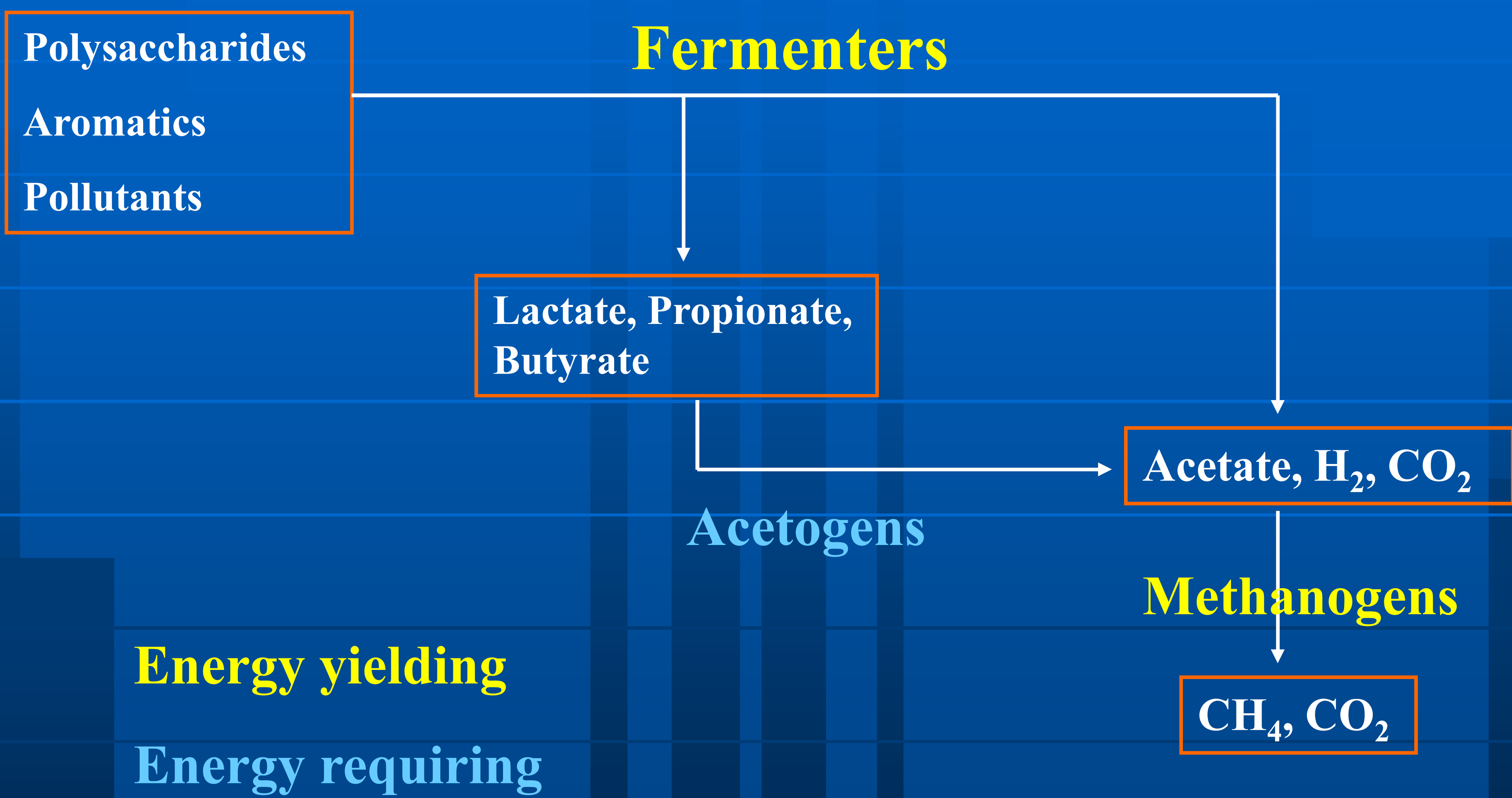
Environmental Factors

- **Presence of electron acceptors**
- **Concentration of the chemical**
- **Availability of nutrients**
- **Bioavailability of the chemical to the organisms**
- **Almost anything else you can think of can sometimes have an effect**

Characteristic of Anaerobic Processes

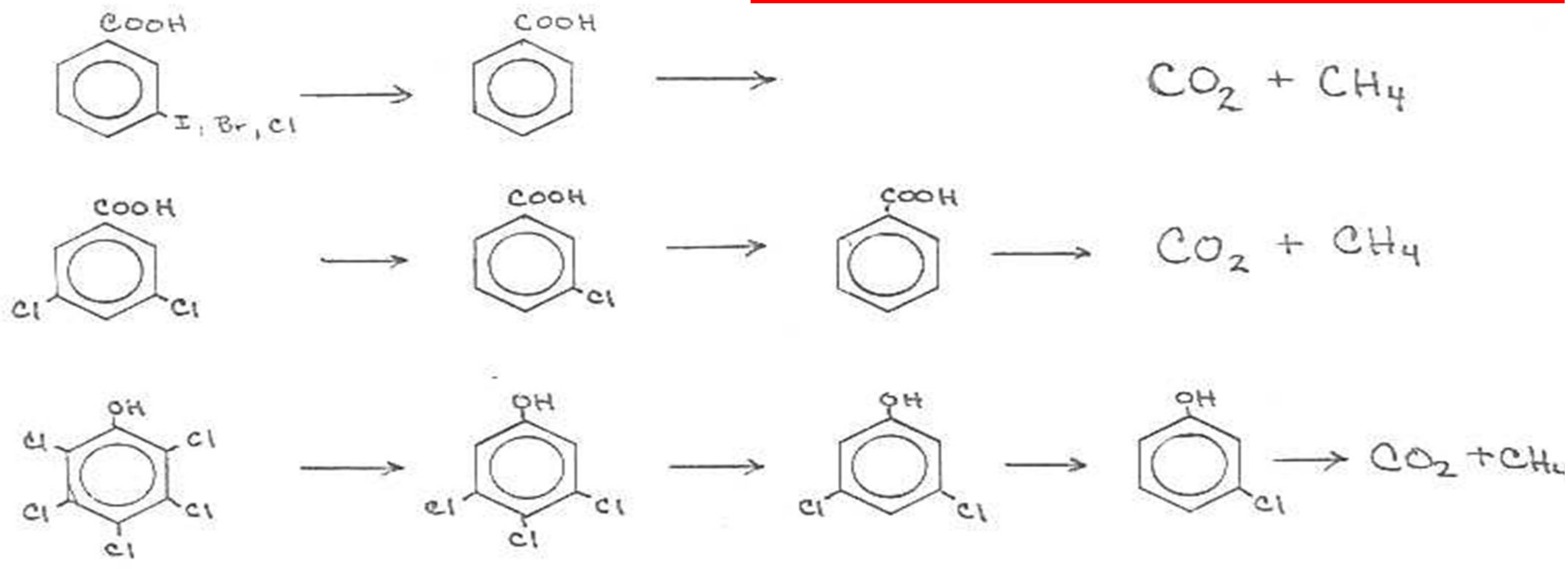
- **Slower than aerobic processes- 19X less energy**
- **Less oxidized processes**
- **Methane a common mineralization product**
- **Consortia of organisms almost always involved**
- **Use a variety of electron acceptors – not O_2**
- **Do many reactions not possible by aerobes**

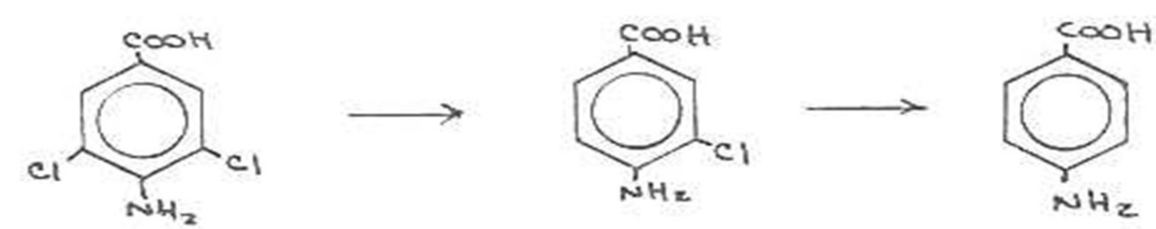
Anaerobic Consortia



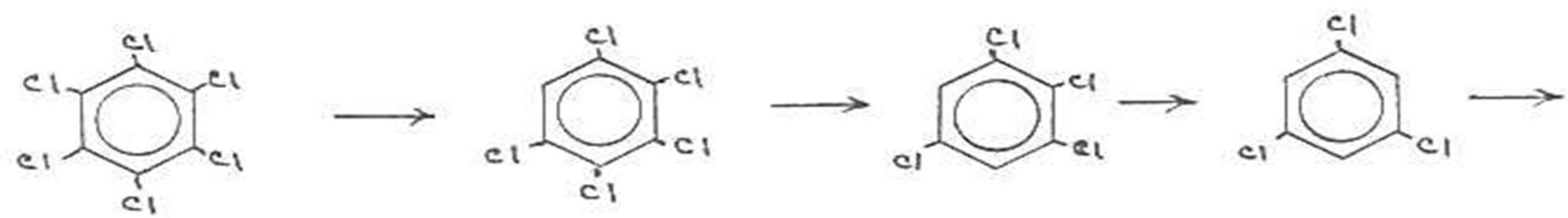
ANAEROBIC DEHALOGENATIONS

For at least some microbes the halogenated are electron acceptors

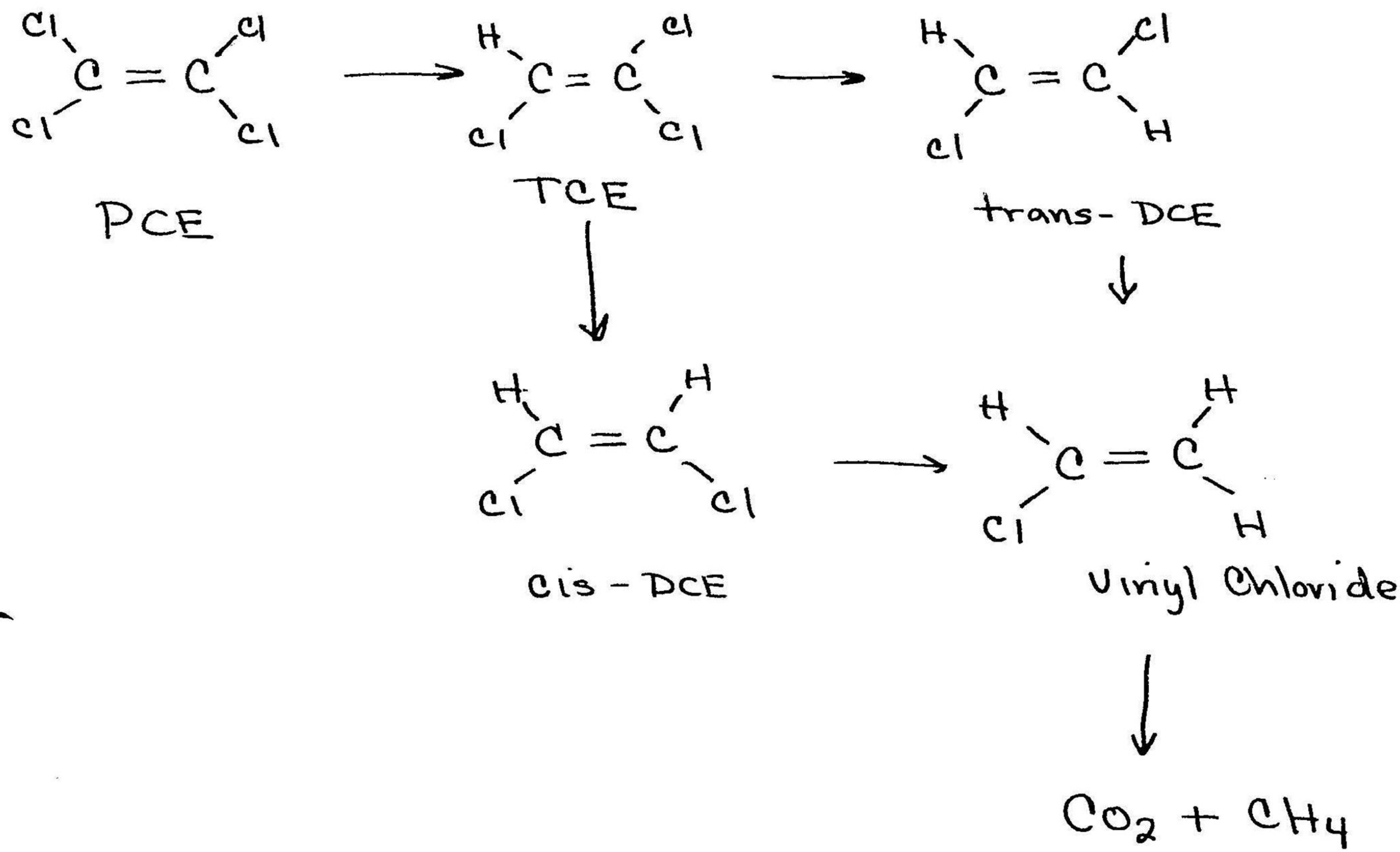




Anaerobically the more halogenated the better the e- acceptor



ANAEROBIC DEGRADATION OF CHLORINATED SOLVENTS



Concentration

- **Microbes have sophisticated ways to control enzyme production**
- **Thresholds do occur- some concentrations are too low to turn on enzyme synthesis**
- **Most Cpds do not have thresholds**
- **Toxicity often seen at high concentrations**
- **What may be too high or too low in one environment may be degraded in another**

Bioavailability of chemicals

- **Early observation that high organic contaminated sediments did not show toxicity to aquatic critters**
- **Toxicity related to pore water concentration**
- **Material sorbed to sediment was not biologically available to have a toxic effect**
- **At same time saw that microbes did not degrade material sorbed to soil or sediment**

Effect of nutrient availability

- **Most of the time there are adequate supplies of inorganic nutrients in most environments**
- **Where there are large amounts of organic materials need to add N and P**
- **Most often seen in oil spills where lots of carbon has been added**
- **Important consideration in remediation efforts**

Even though the compounds are degradable they do not unless N and P are added

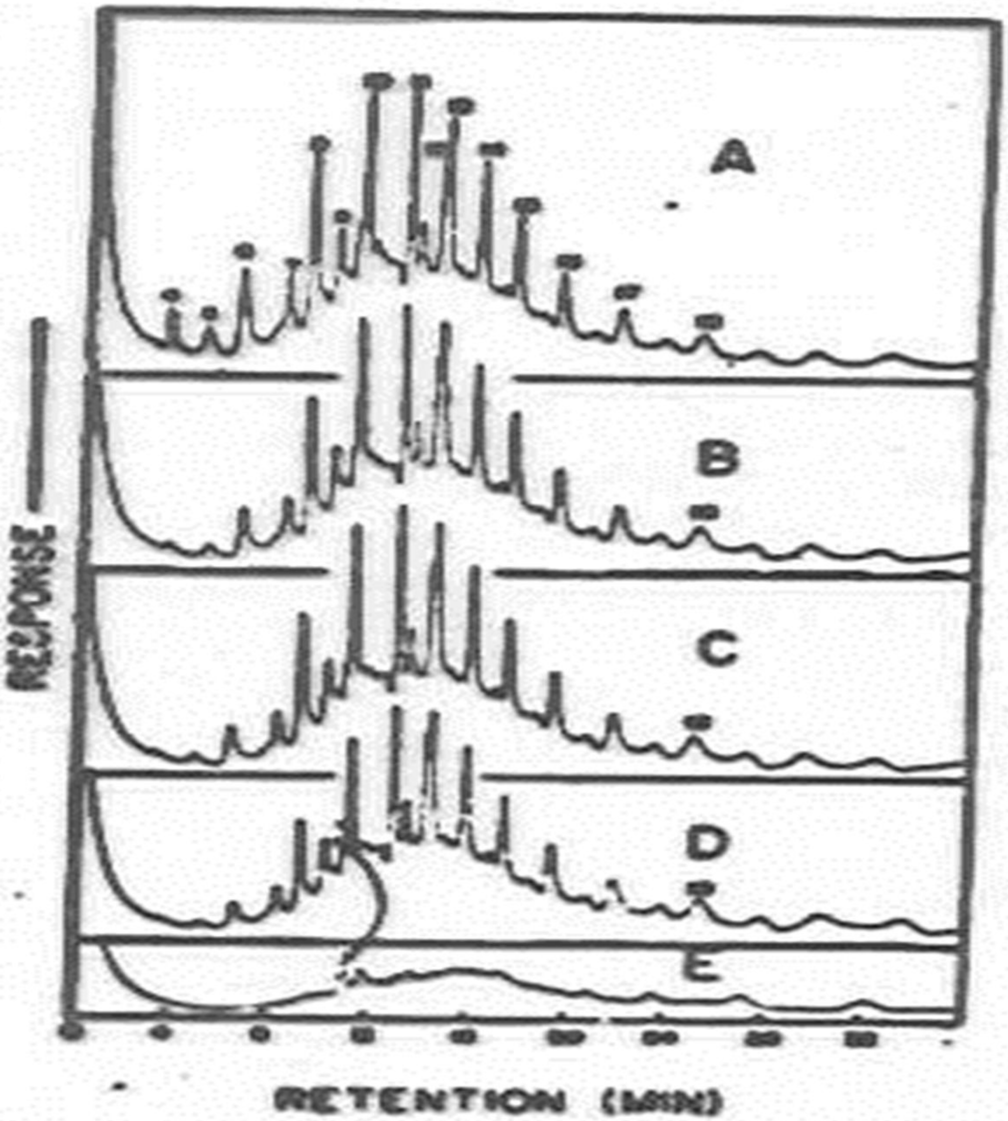


Fig. 4. Gas chromatograms of Sweden crude oil, incubated for 18 days under the following conditions: in filter-sterilized sea water (A), in natural sea water (B), in sea water supplemented with 7×10^{-4} M Na_2HPO_4 (C), 1×10^{-3} M KNO_3 (D), and both (E). For identity of the numbered peaks see Table I in ref. 3.

What can we use this for?

- **Bioremediation= lets use microbes to clean up our mess**
- **In US right now**
  - **100,000 petroleum leaks**
  - **15,000 VOC spills**
  - **8,000 wood treating sites**
  - **Many others**
- **Most can be bio-cleaned cheaper than other methods**

Bedtime Reading

- Books
  - Microbial Transformations and Degradation of Toxic Organic Chemicals- Young and Cerniglia- Wiley
  - Biology of Anaerobic Microorgansism- Zehnder- Wiley
  - Biodegradation and Bioremediation- Alexander- Wiley
- Journals
  - Applied and Environmental Microbiology
  - Environmental Science and Technology
  - Biodegradation
  - Environmental Toxicology and Chemistry
- Websites
  - ASMUSA.org- lots of good stuff
  - EPA.gov- search under biodegradation