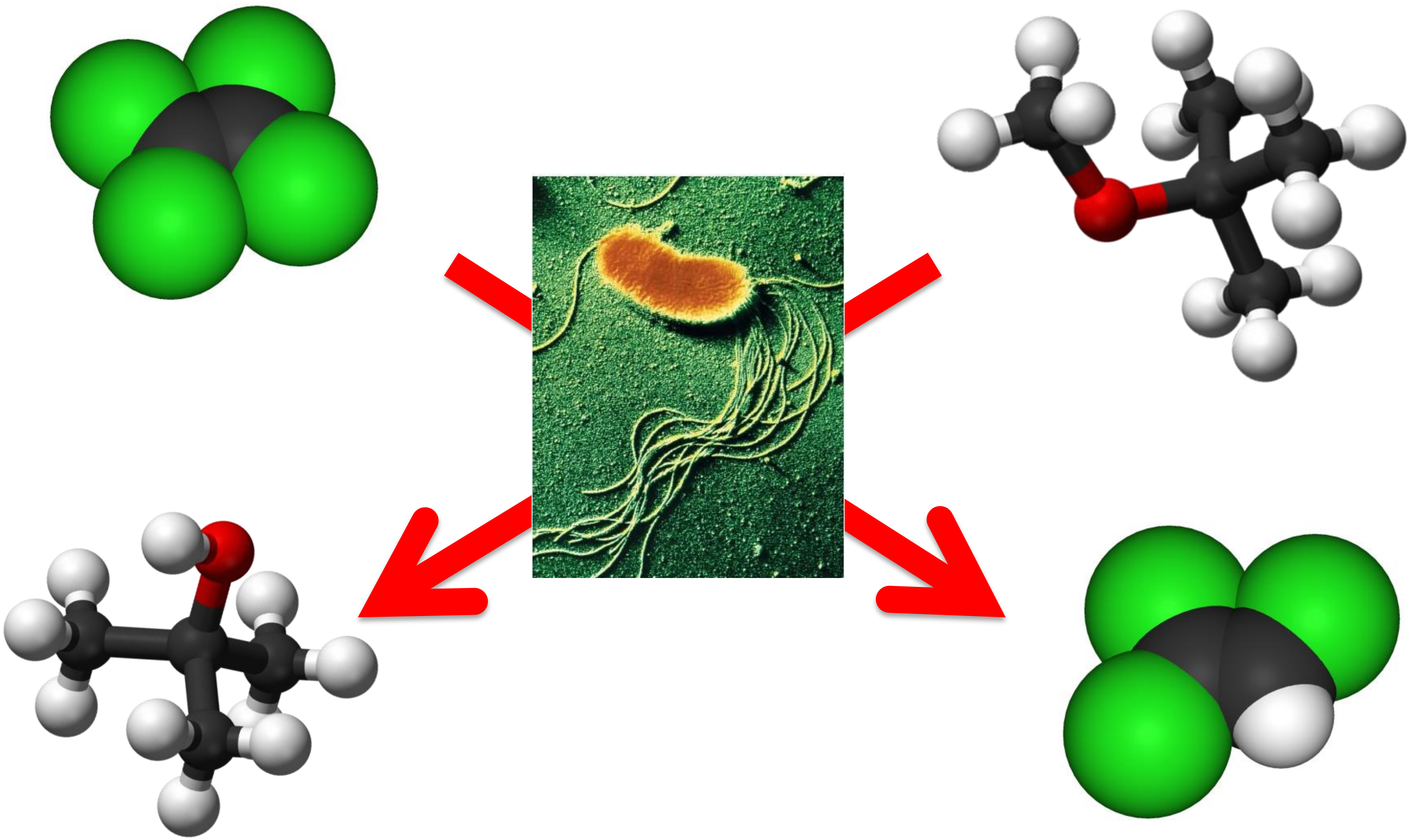
Biodegradation of chlorinated solvents and fuel oxygenates



Outline

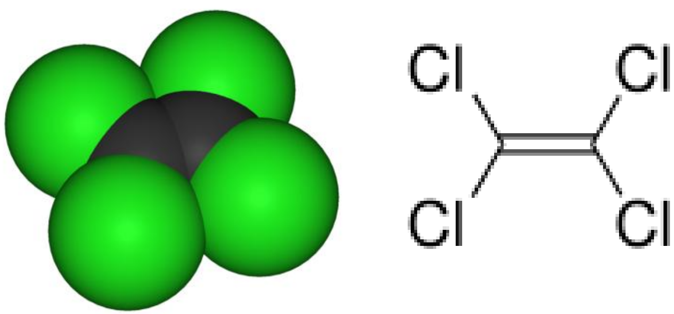
- **Chlorinated solvents--PCE and TCE**
  – Properties of pollutants and abiotic processes
  – Aerobic cometabolism and anaerobic metabolism
- **Fuel oxygenates--MTBE and TBA**
  – Properties of pollutants
  – Anaerobic and aerobic metabolism
  – Aerobic cometabolism
- **Fluorinated analogs as EAPS**

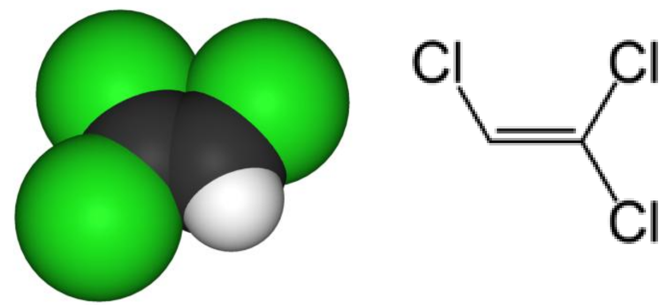

Chlorinated solvents
2 TON
COFFIN
PCE and TCE

Overview of PCE and TCE

- **PCE: (perchloroethylene, perc, tetrachloroethene)**
  - High volume chemical used as degreasing and dry cleaning solvent

- **TCE: (trichloroethylene, trike, 1,1,2-trichloroethene)**
  - Historically widely used as a solvent.



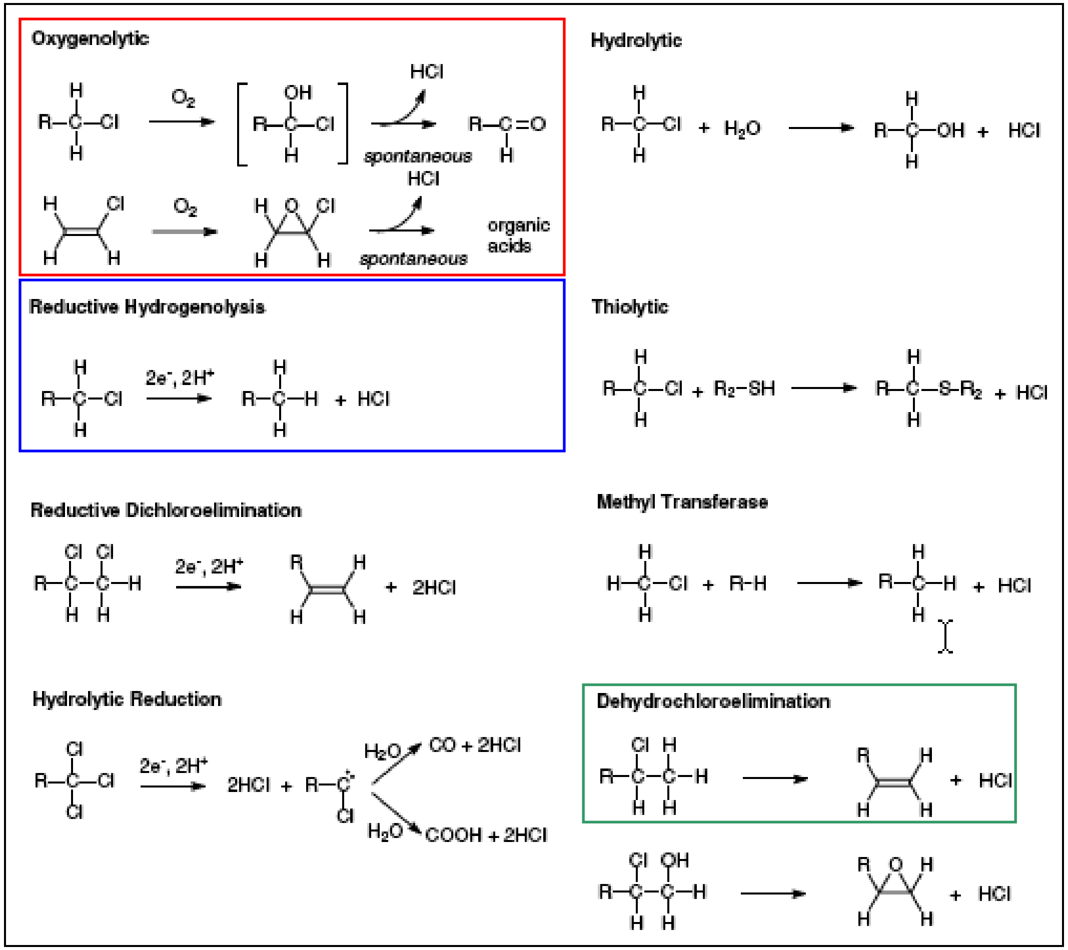


- Both common ground water pollutants and both are persistent under aerobic conditions

- No organisms know to use either compound as electron donors.

- PCE: undergoes limited abiotic hydrolysis; PCE and TCE undergo abiotic reduction

Dehalogenation mechanisms



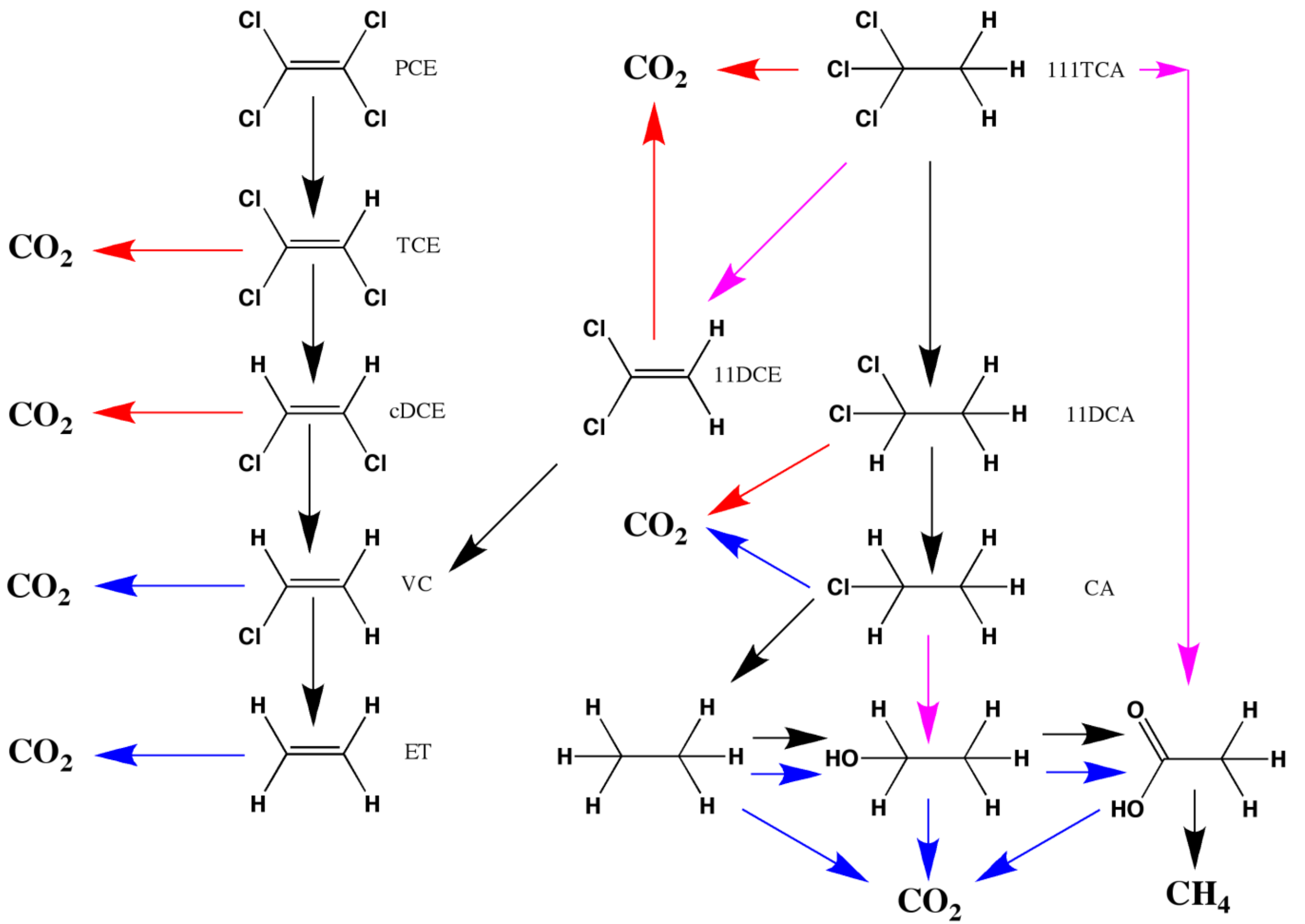
Many and varied. Some biotic, some abiotic, some both!

Oxygenolytic: Biodegradation process that requires O_2 for mono- or dioxygenases.

Reductive hydrogenolysis (*aka* reductive dehalogenation): Most common anaerobic biodegradation mechanism for PCE and TCE. Also occurs abiotically.

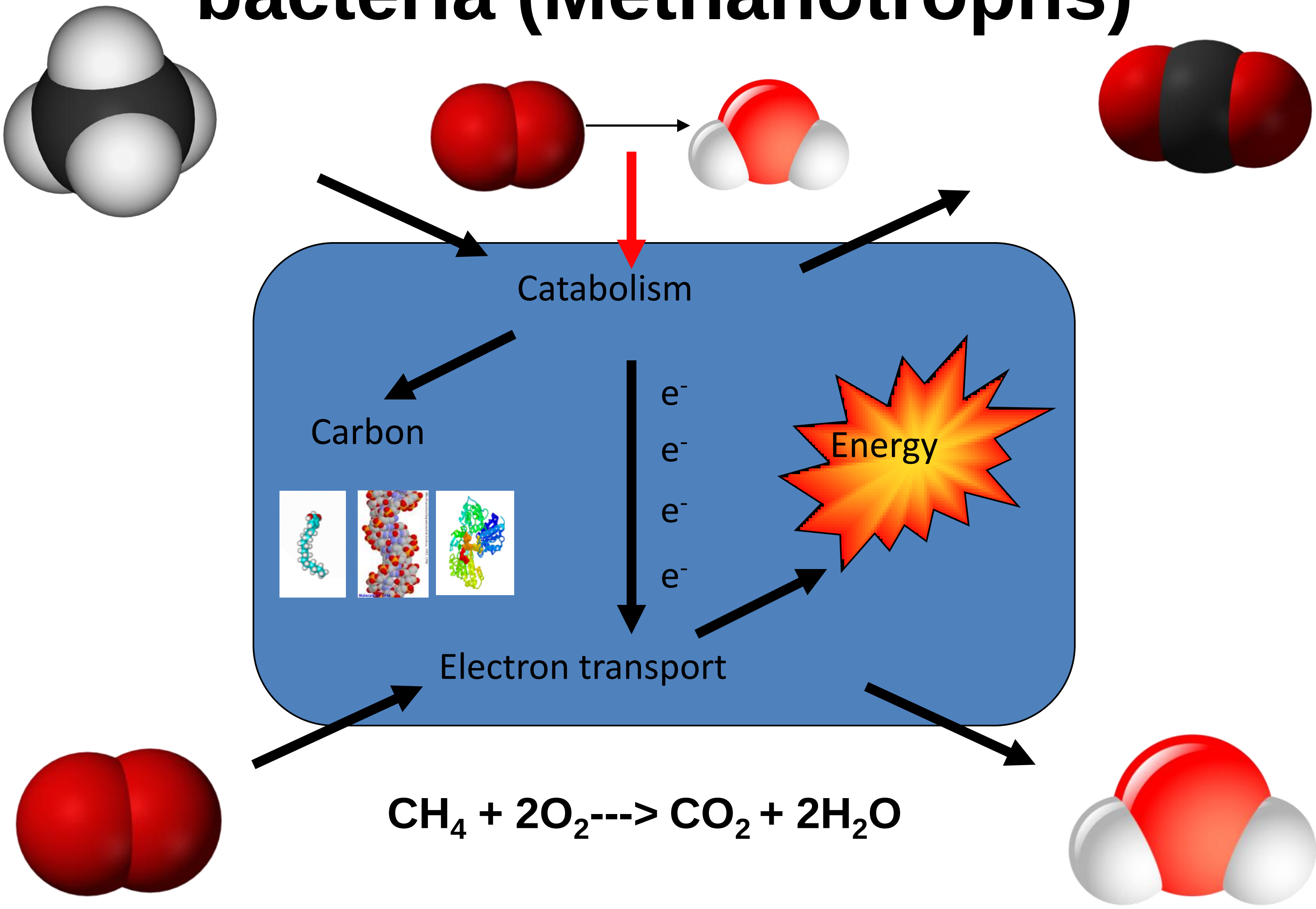
Dehydrochloroelimination: Common abiotic process that converts haloakanes to haloalkenes

Abiotic and Biotic Transformations



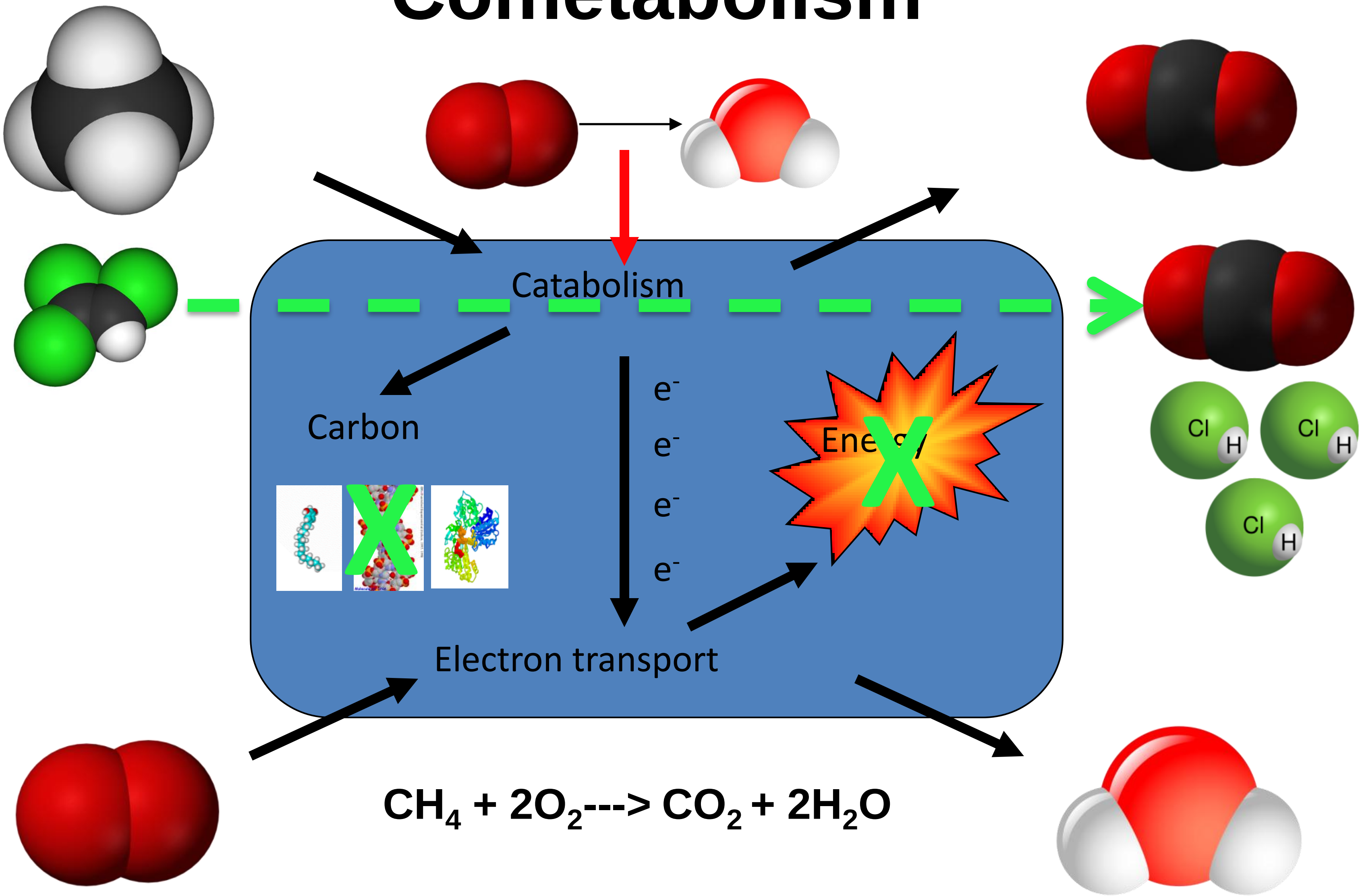
Aerobic Cometabolism	Aerobic Metabolism	Reductive Dehalogenation	Abiotic Transformations

Aerobic methane-oxidizing bacteria (Methanotrophs)



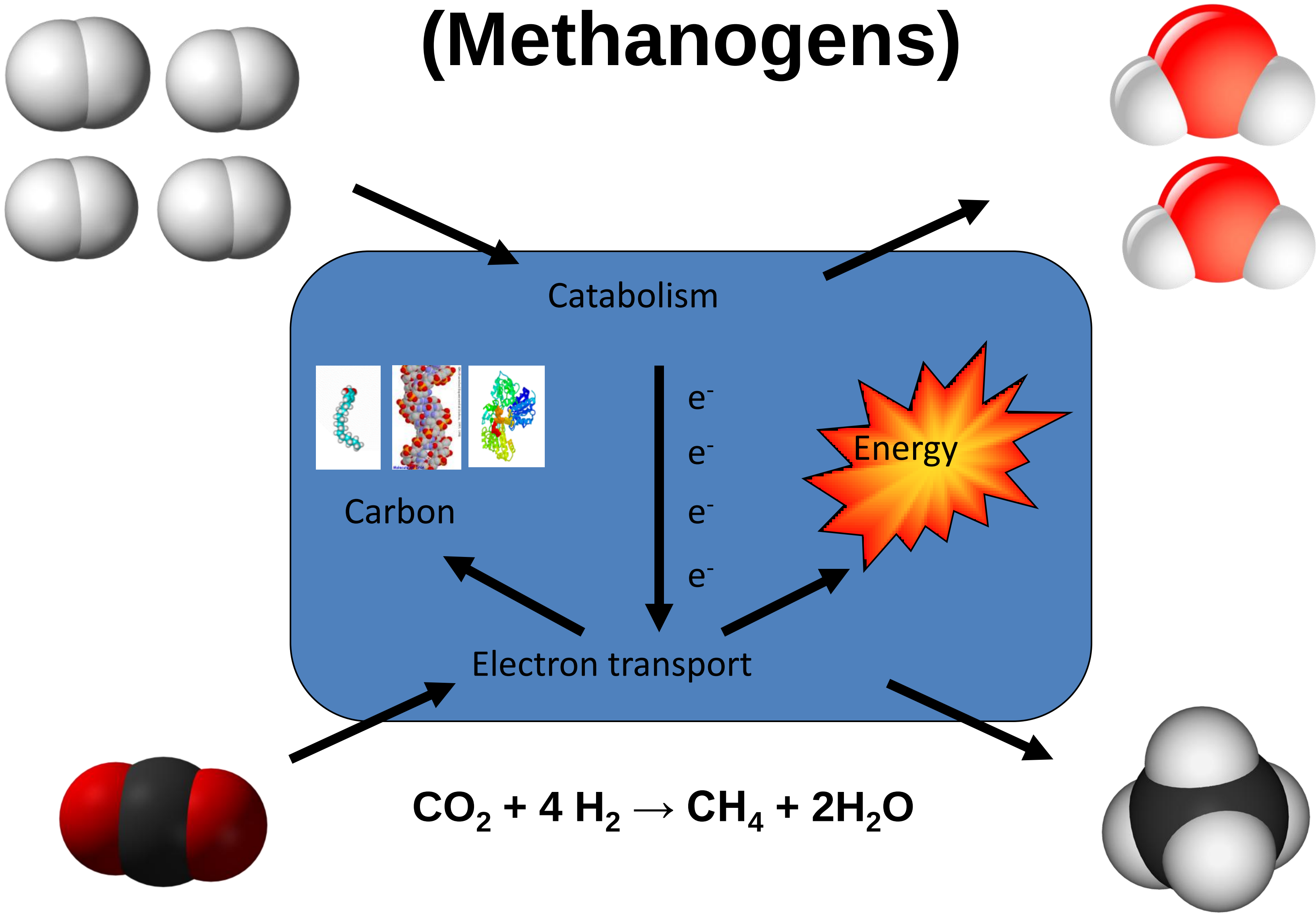
$$CH_4 + 2O_2 \text{--->} CO_2 + 2H_2O$$

Aerobic TCE Cometabolism



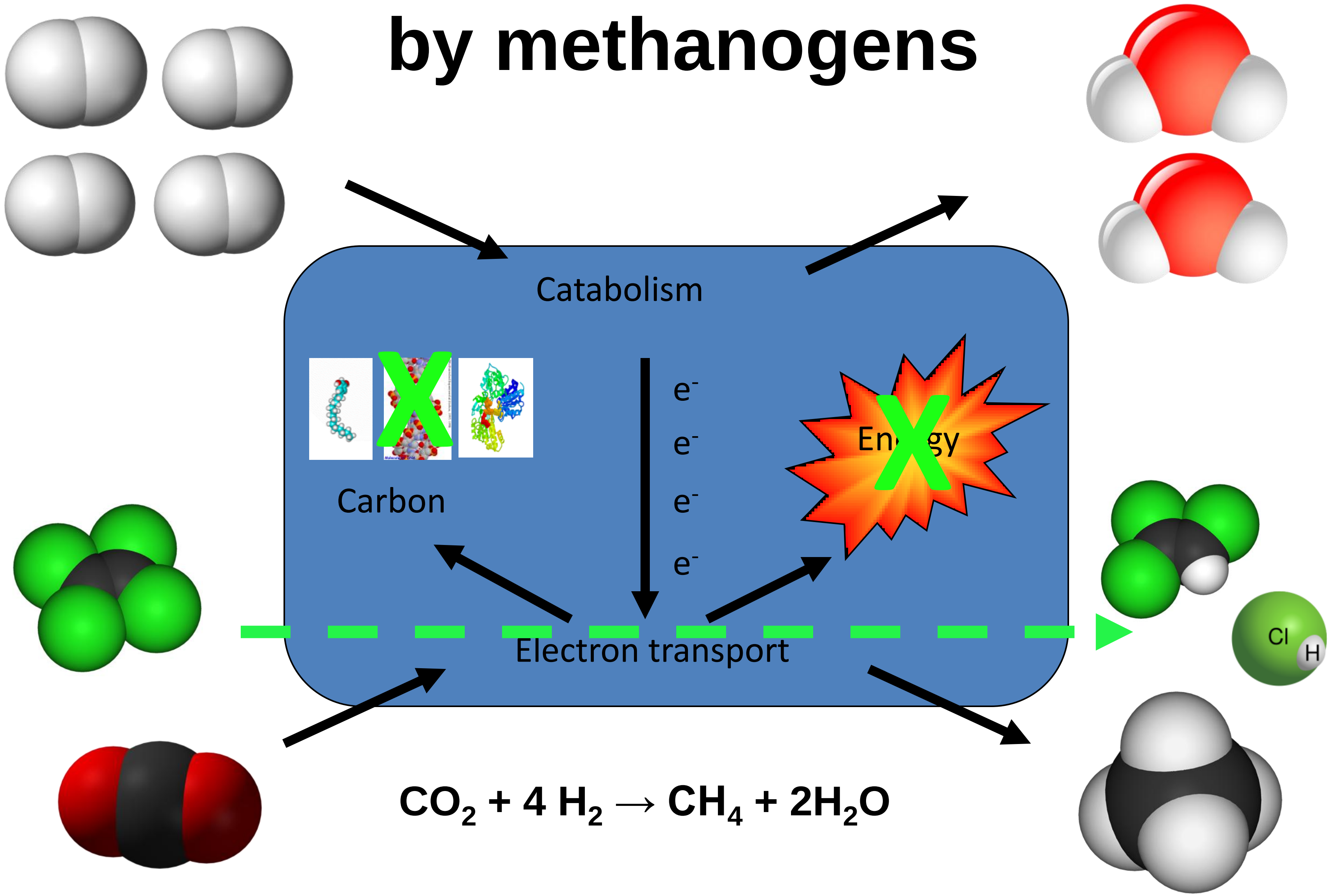
$$CH_4 + 2O_2 \text{--->} CO_2 + 2H_2O$$

Methane-generating anaerobes (Methanogens)



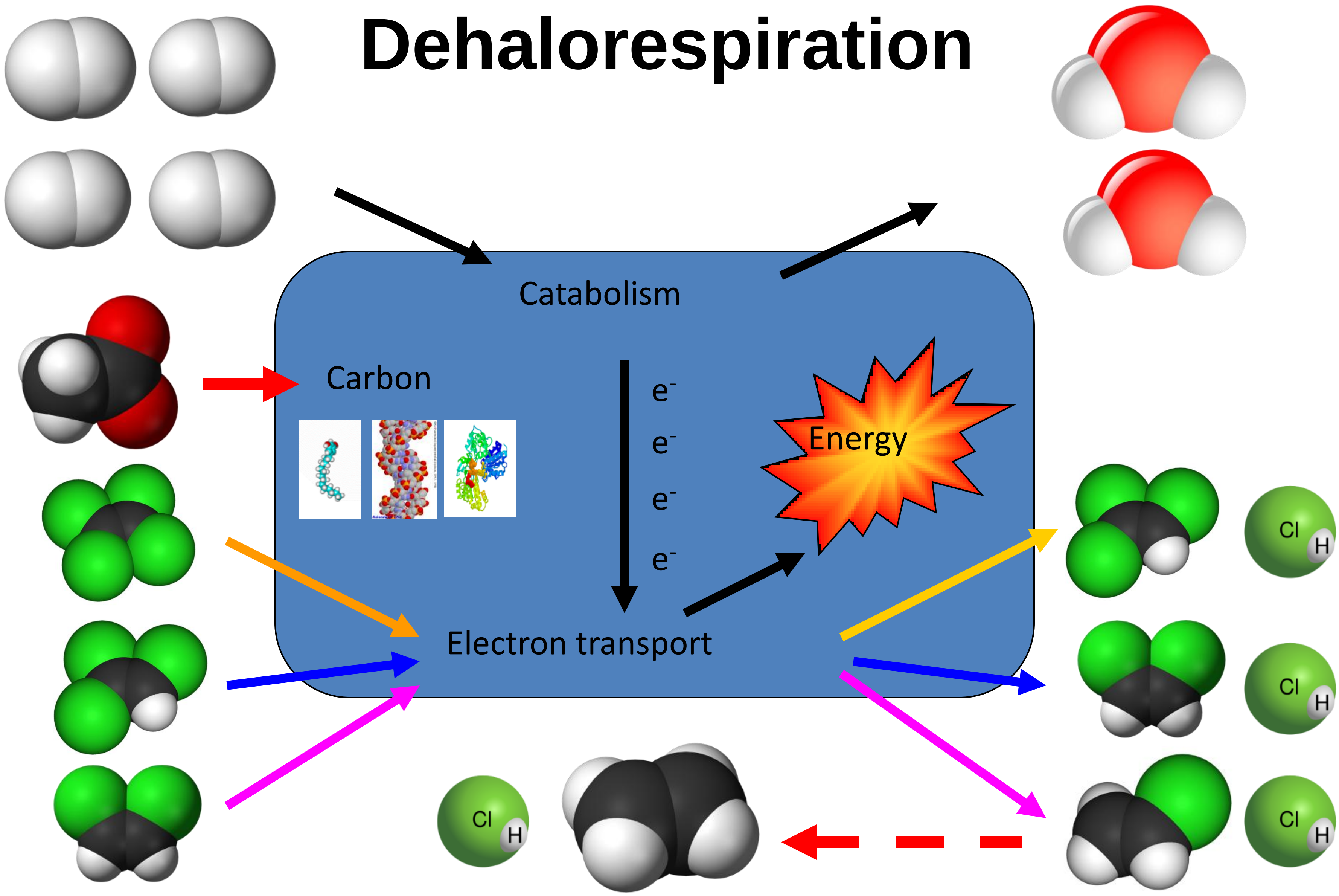
$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$$

Cometabolic dehalogenation of TCE by methanogens

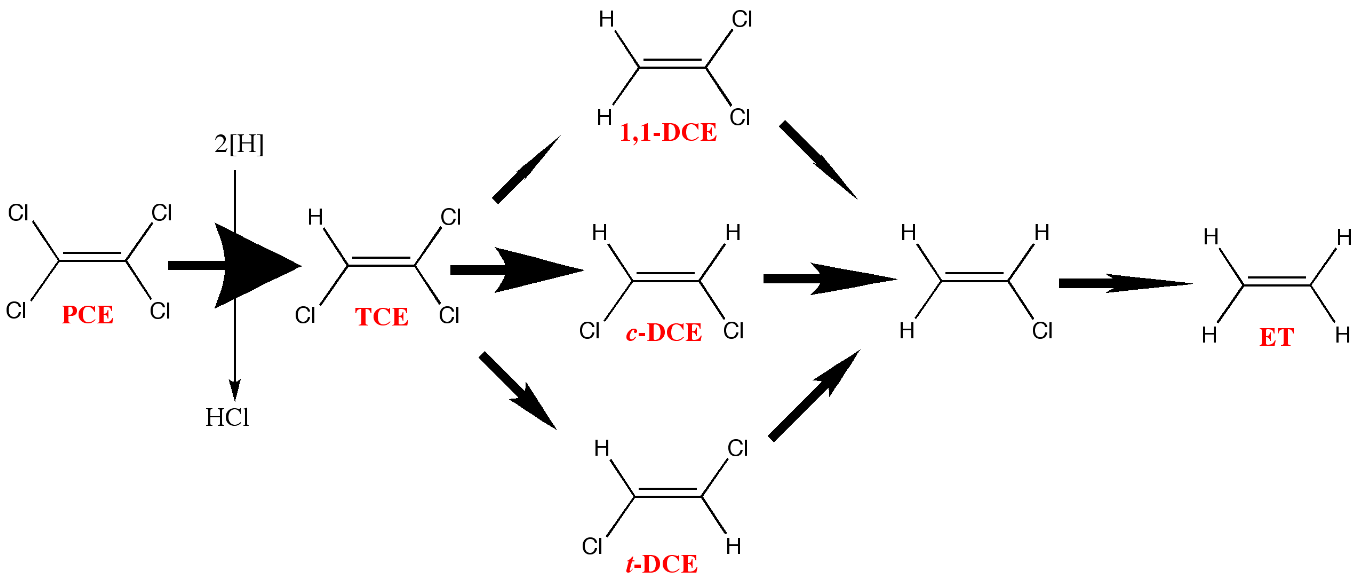


$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$$

Reductive dehalogenation: Dehalorespiration



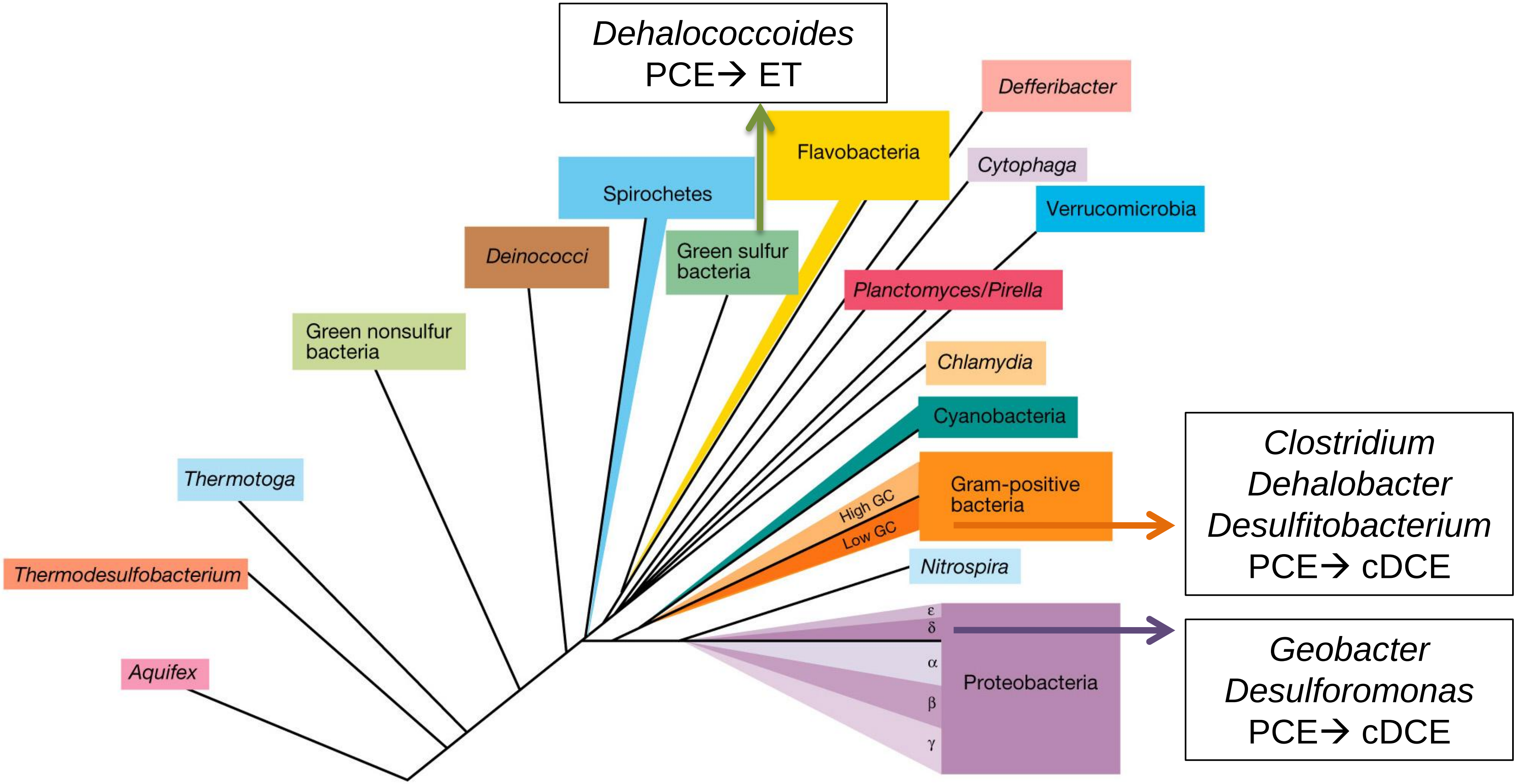
Pathway(s) of respiratory PCE reduction



- Respiratory process is much faster than anaerobic cometabolism
- Each reaction catalyzed by terminal reductase enzyme *(e.g. tceA)*.
- Main flux is through *c*-DCE
- Rates typically decrease as acceptors become more oxidized.
- Process can often stall at *c*-DCE in the field.
- While ethylene is innocuous, VC is more toxic than PCE and TCE

Organism	Strain	Phylogeny	PCE-TCE	TCE-cDCE	cDCE-VC	VC-ethene
Clostridium bifermentans	DPH-1	Low G+C G+	Y	Y	N	N
Dehalobacter restrictus	PER-K23, TEA	Low G+C G+	Y	Y	N	N
Desulfitobacterium frappieri	TCE1	Low G+C G+	Y	Y	N	N
Desulfitobacterium metallireducens	853-15A(T)	Low G+C G+	Y	Y	N	N
Desulfitobacterium sp.	Y51	Low G+C G+	Y	Y	N	N
Desulfitobacterium sp.	PCE1	Low G+C G+	Y	N	N	N
Desulfitobacterium sp.	PCE-S	Low G+C G+	Y	Y	N	N
Desulfitobacterium sp.	KBC1	Low G+C G+	Y	N	N	N
Enterobacter sp.	MS1	γ-proteobacteria	(Y)	(Y)	N	N
Desulfomonile tiedjei	DCB-1	δ-proteobacteria	Y	Y	N	N
Desulfuromonas michiganenis	BB1 & BRS2	δ-proteobacteria	Y	Y	N	N
Desulfuromonas chlorethenica	TT4B	δ-proteobacteria	Y	Y	N	N
Geobacter lovleyi	SZ	δ-proteobacteria	Y	Y	N	N
Sulfurospirillum multivorans		ε-proteobacteria	Y	Y	N	N
Sulfurospirillum halorespirans	PCE-M2	ε -proteobacteria	Y	Y	N	N
Dehalococcoides ethenogenes	195	Green non-sulfur	Y	Y	Y	(Y)
Dehalococcoides sp.	FL2	Green non-sulfur	(Y)	Y	Y	(Y)
Dehalococcoides sp.	CBDB1	Green non-sulfur	Y	N	N	N
Dehalococcoides sp.	GT	Green non-sulfur	N	Y	Y	Y
Dehalococcoides sp.	BAV1	Green non-sulfur	N	N	Y	Y
Dehalococcoides (enrichment)	VS	Green non-sulfur	N	N	Y	Y

Who are the PCE/TCE-respiring microorganisms?

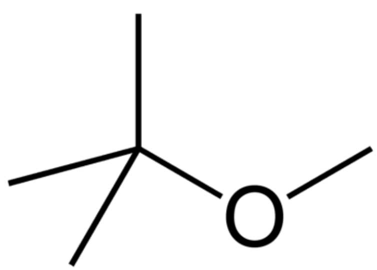


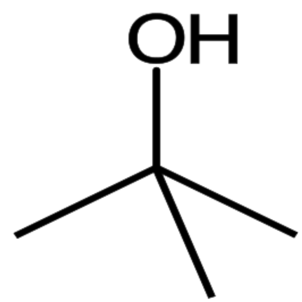
Fuel Oxygenates

MTBE and TBA

Overview of MTBE and TBA

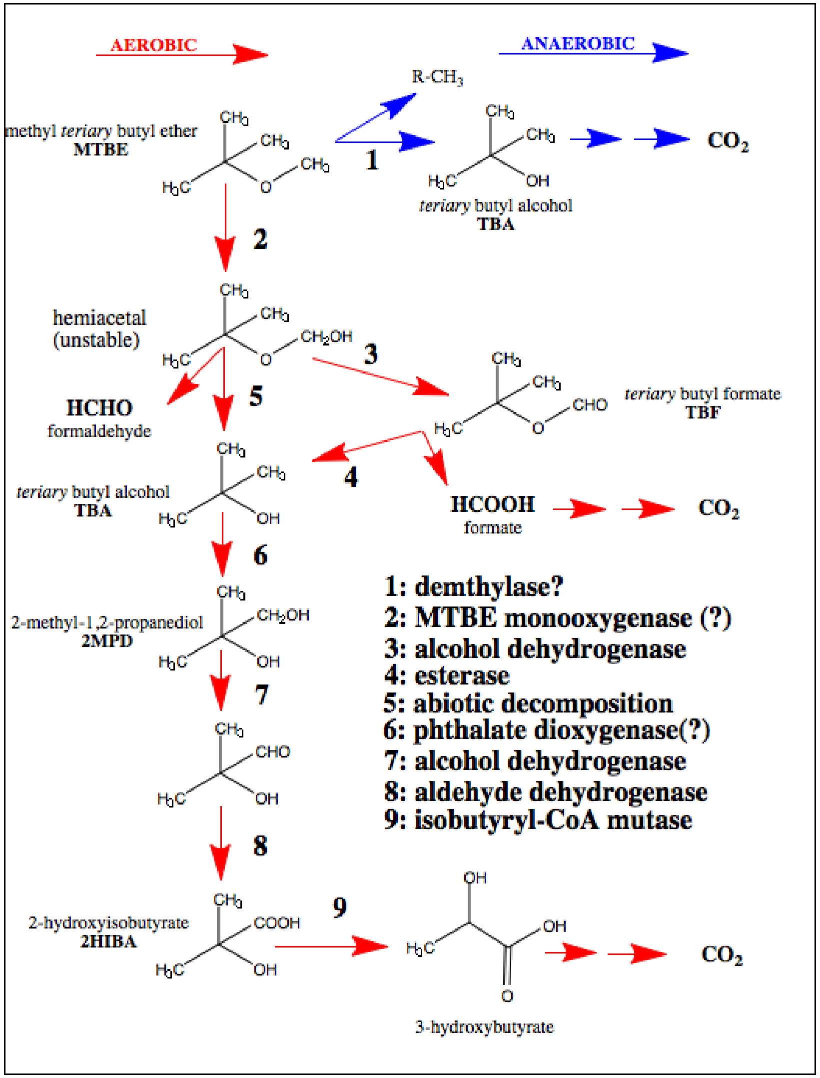
- **MTBE: (methyl *tertiary* butyl ether)**
  - High volume chemical used in US as octane enhancer and fuel oxygenate from 1980s to mid 2000's. Limited current use in US. Widespread current use outside US.
- **TBA: (*tertiary* butyl alcohol)**
  - Limited early use as fuel oxygenate. Major biodegradation metabolite of MTBE





  - Both common ground water pollutants, mainly from gasoline.
  - Biodegrades under both aerobic and anaerobic conditions but no organisms known that utilize either compound as an electron acceptor.
  - MTBE hydrolyzes to TBA at low pH. An analytical issue rather than environmental issue

MTBE biodegradation mechanisms

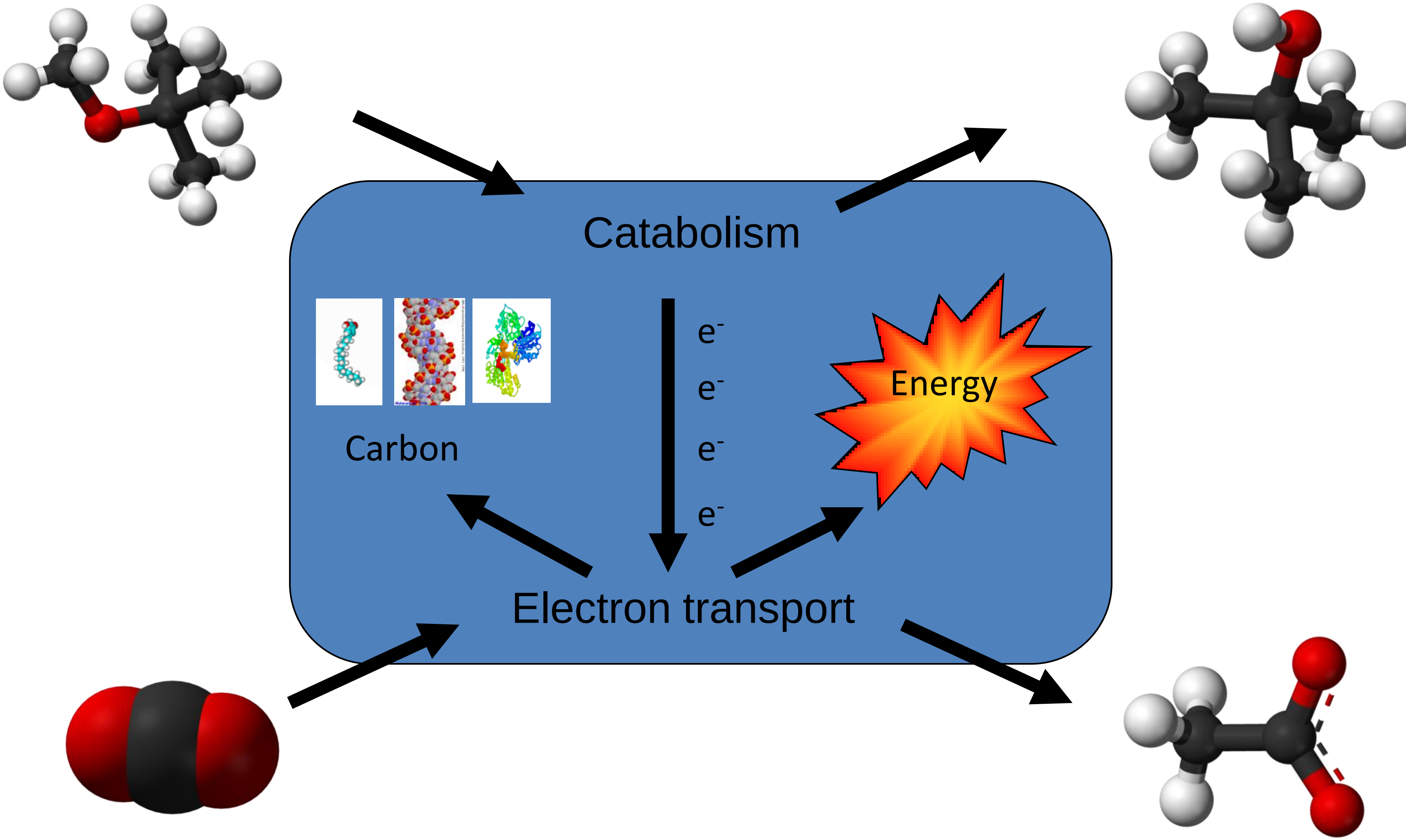


- **Anaerobic:**
  - No pure cultures available
  - Confirmed in microcosms and field
  - Possible role of acetogenic bacteria.
  - TBA metabolite can be longer-lived
- **Aerobic:**
  - MTBE and TBA metabolism occurs in several closely-related bacteria.
  - Initial oxidation by monooxygenases
  - C_1 metabolism is important.
  - Cometabolic processes occur with many alkane-oxidizing bacteria.
  - Cometabolic oxidation can stop at TBA.

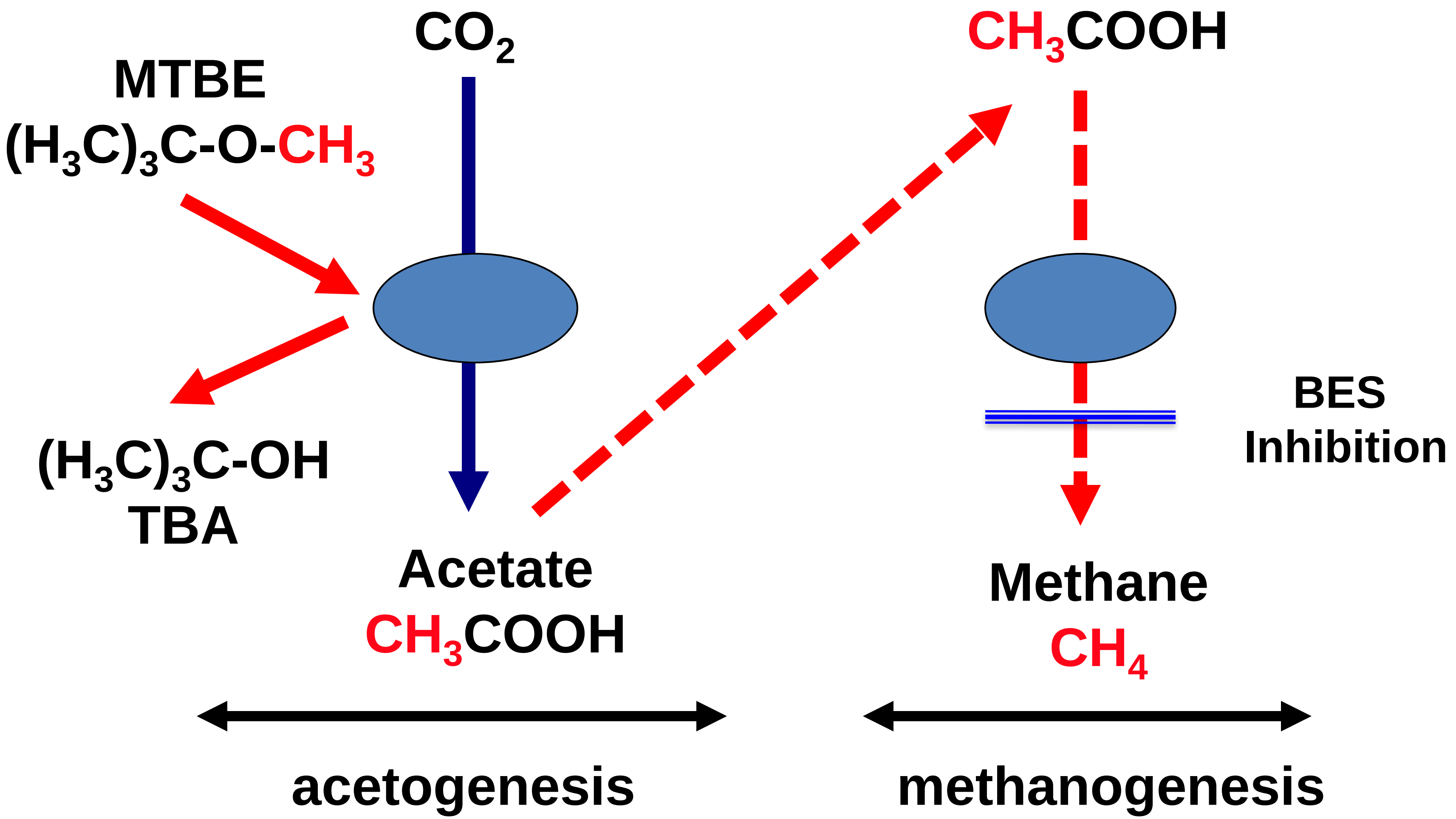
MTBE biodegradation summary

Electron Acceptor						
CO_2	CO_2	SO_4^{2-}	Fe^{3+}	Mn^{4+}	NO_3^-	O_2
CH_4	HAc	H_2S	Fe^{2+}	Mn^{2+}	N_2	H_2O
degradation rate, energy yield, biomass generation						
frequency of conditions in gasoline impacted sites						
MTBE	MTBE	MTBE	MTBE	MTBE	MTBE	MTBE
		TBA	TBA	TBA	TBA	TBA

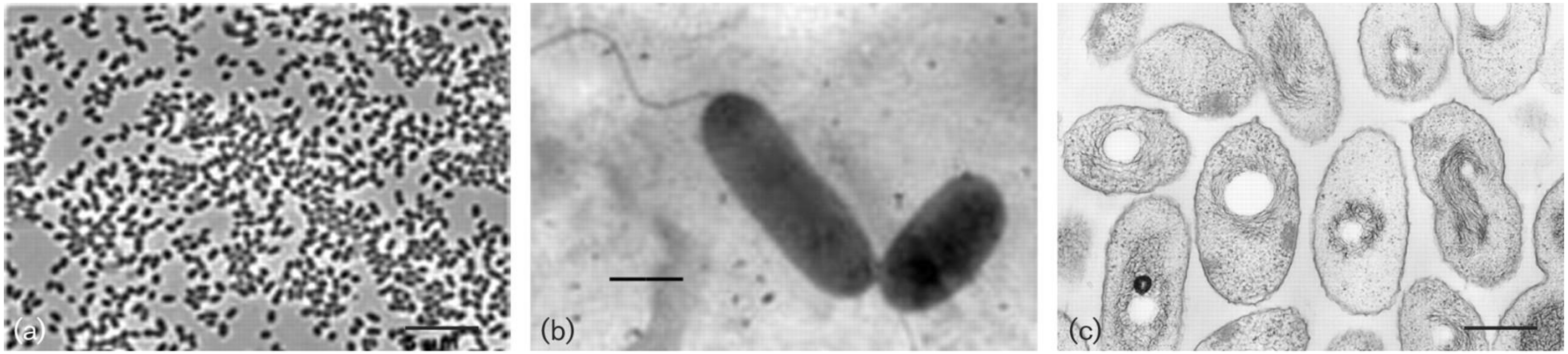
Acetate-generating anaerobes (Acetogens)



Acetogens, methanogens and anaerobic MTBE biodegradation?

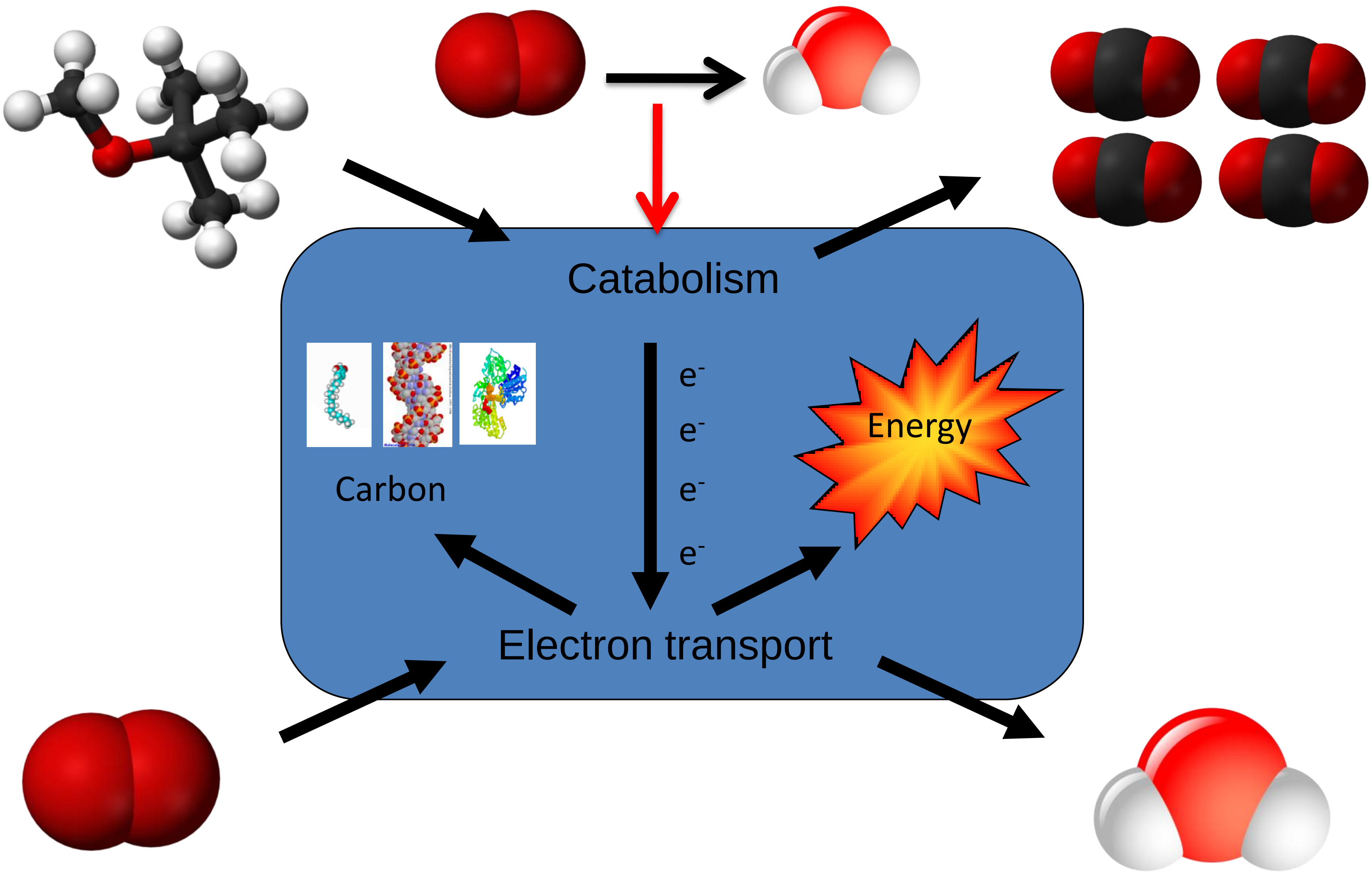


Aerobic MTBE/TBA metabolism

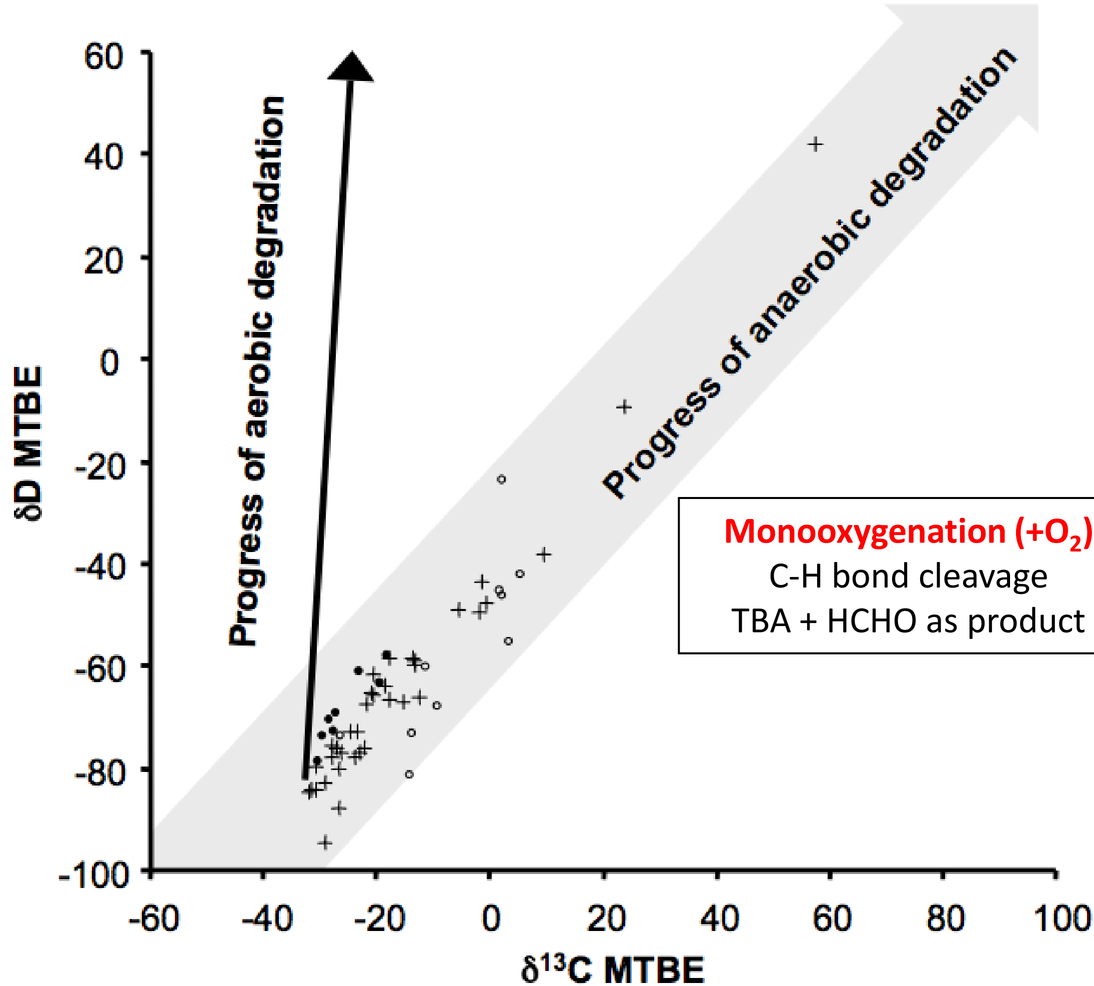


- ***Methylbium petroleiphilum* PM1**:
  - The DE 195 of the MTBE world!
  - Isolated from a peat moss biofilter.
  - Grows on MTBE, TBA, and several BTEX components.
  - Other MTBE-utilizing isolates are closely related (*Aquincola*, *Hydrogenophaga*)
  - Some closely-related strains lack the ability to oxidize MTBE but can grow on TBA.

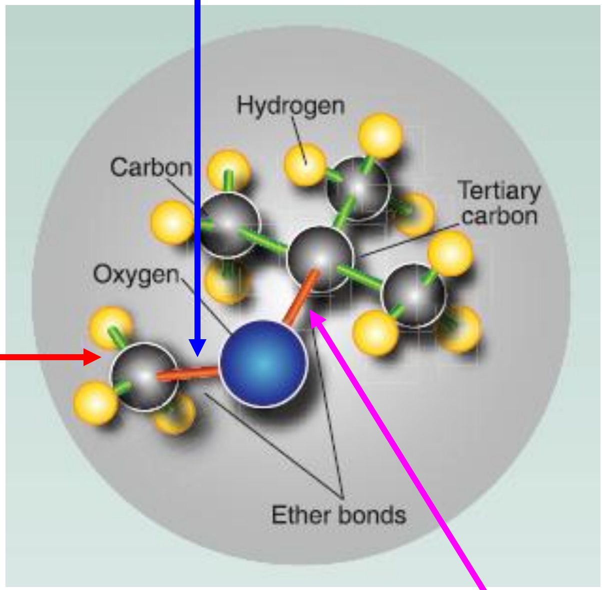
Methylibium petroleiphilum PM1



2D CSIA ($^{13}C/^{12}C$ & $^{2}H/^{1}H$) for MTBE



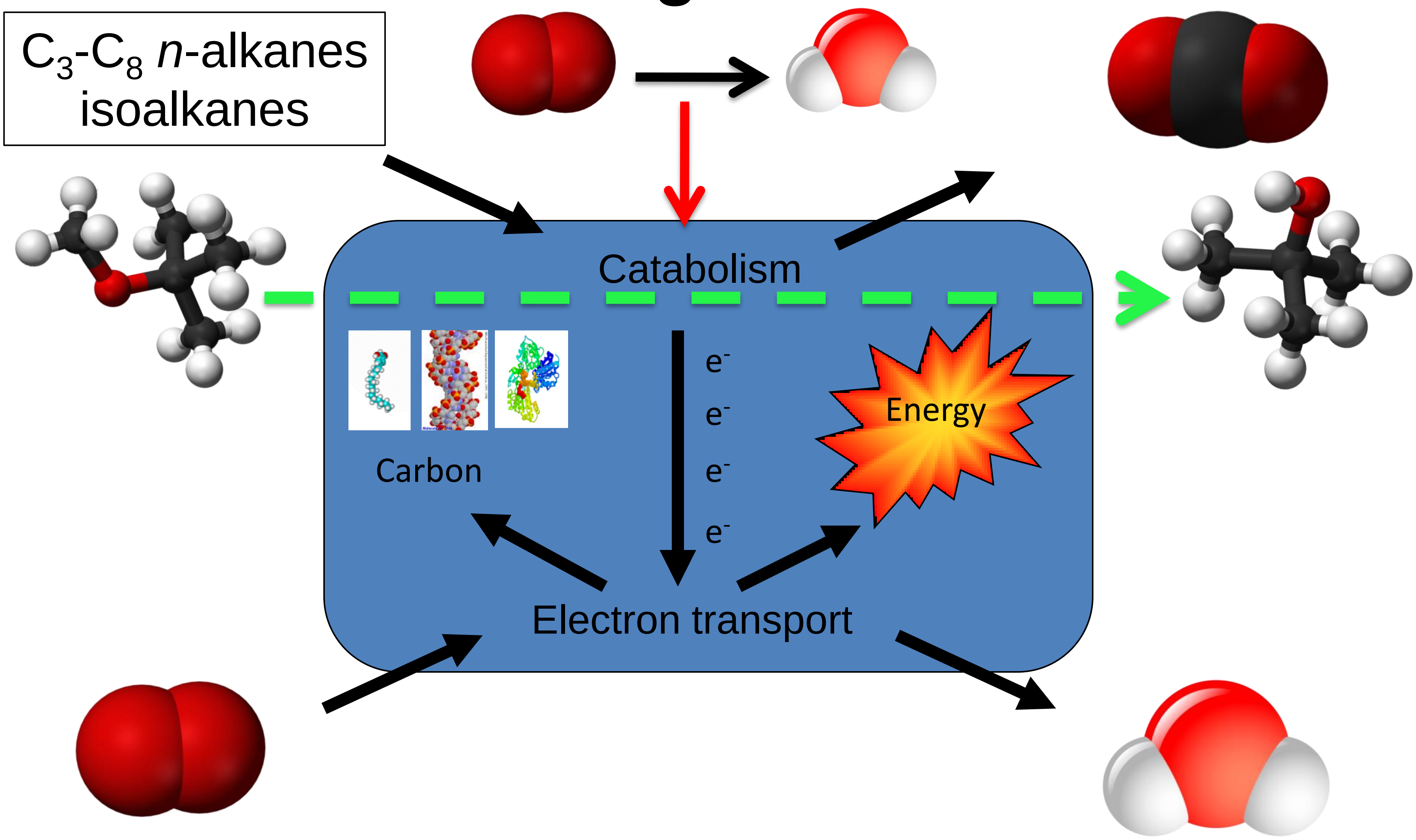
Demethylase (-O_2)
C-O bond cleavage
X-CH_3 + TBA as product



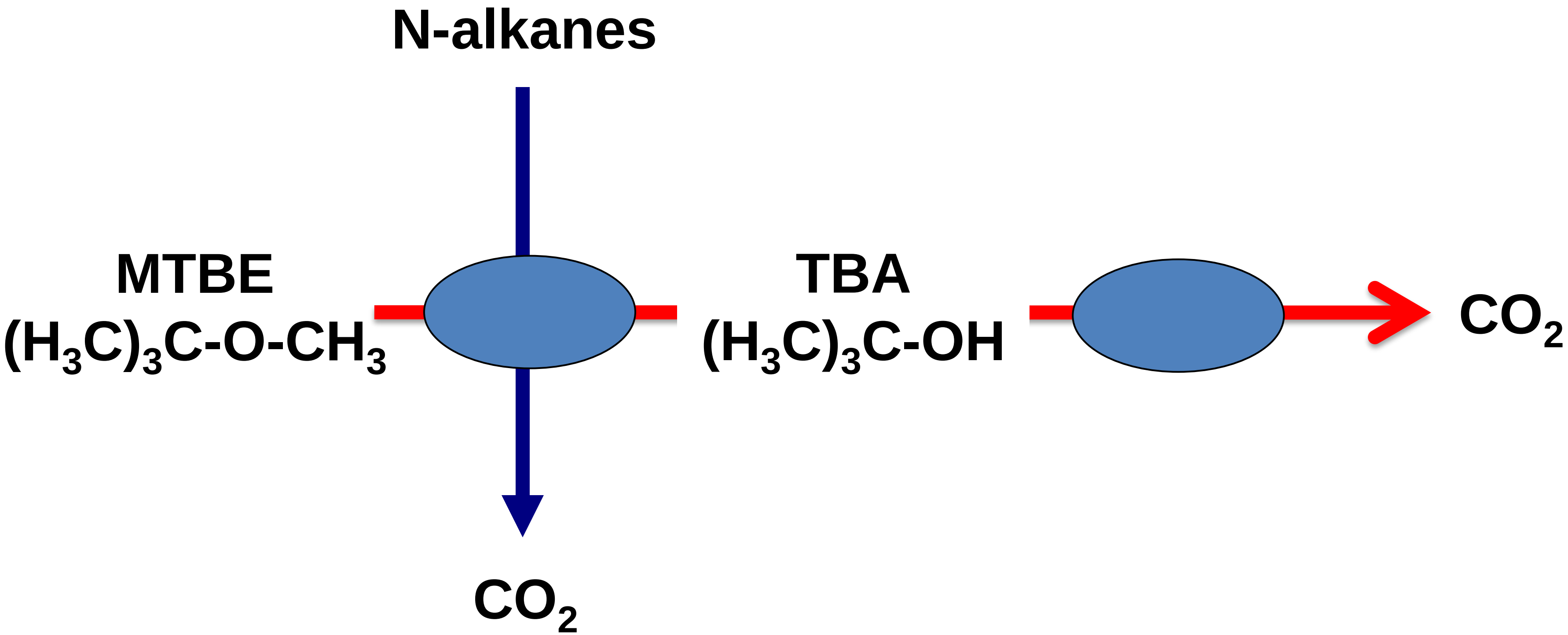
Monooxygenation (+O_2)
C-H bond cleavage
TBA + HCHO as product

Hydrolysis (+/- O_2)
C-O bond cleavage
CH_3OH +TBA as product

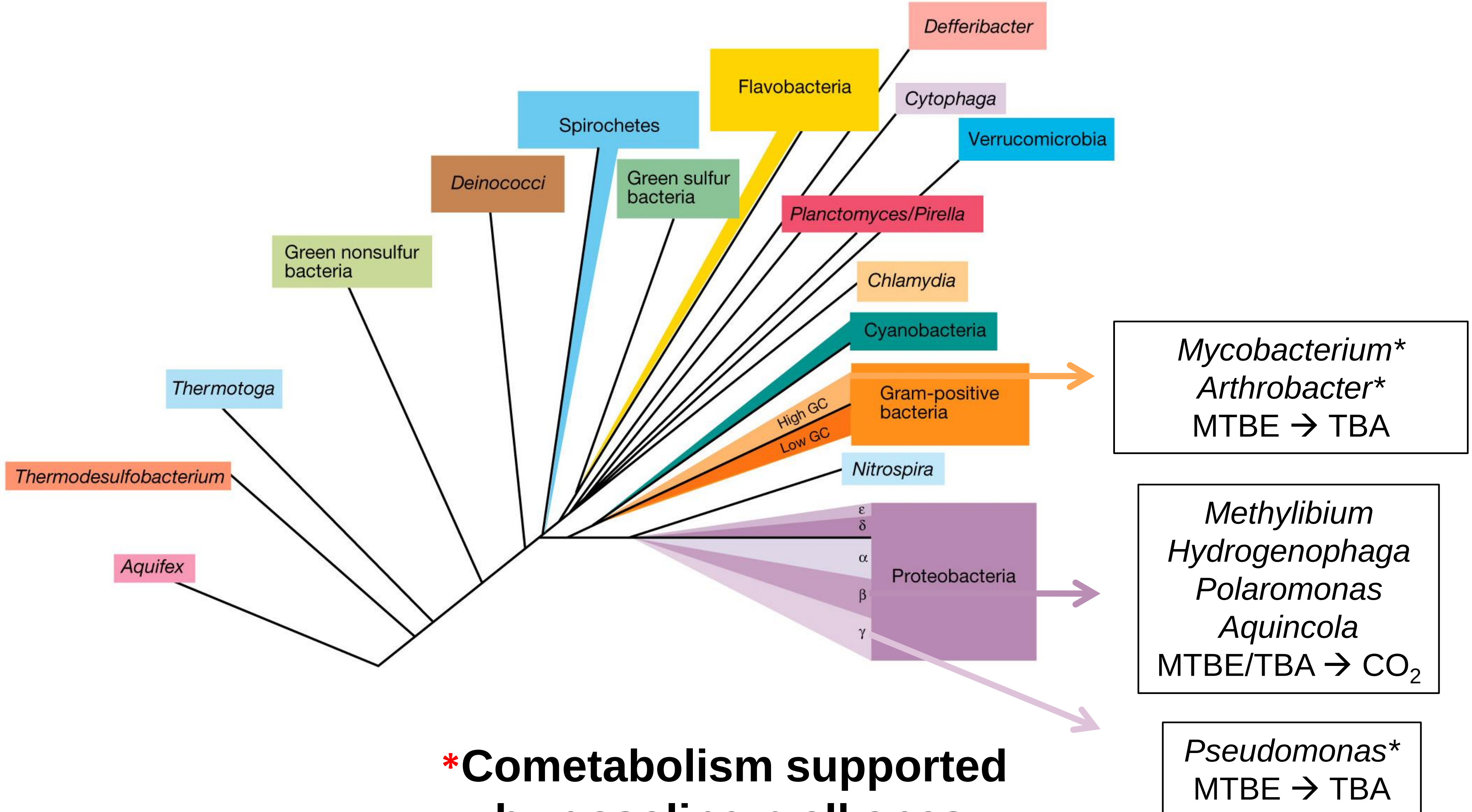
MTBE cometabolism by alkane-oxidizing bacteria



Commensalism and aerobic MTBE biodegradation?

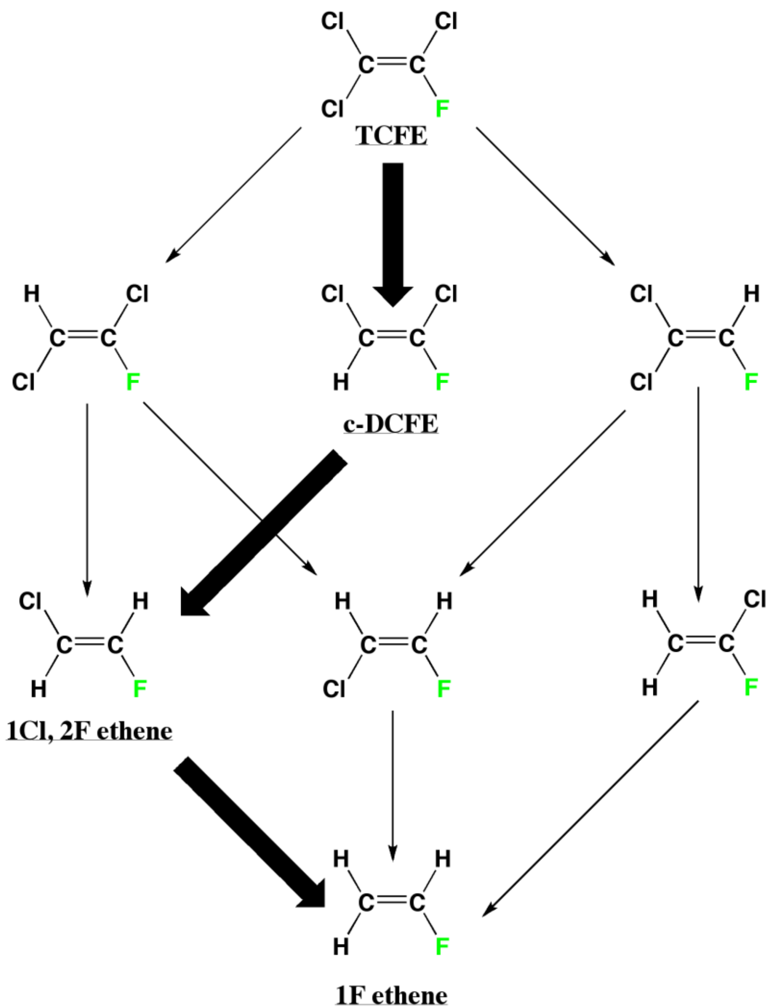


Who are the aerobic MTBE-oxidizing microorganisms?



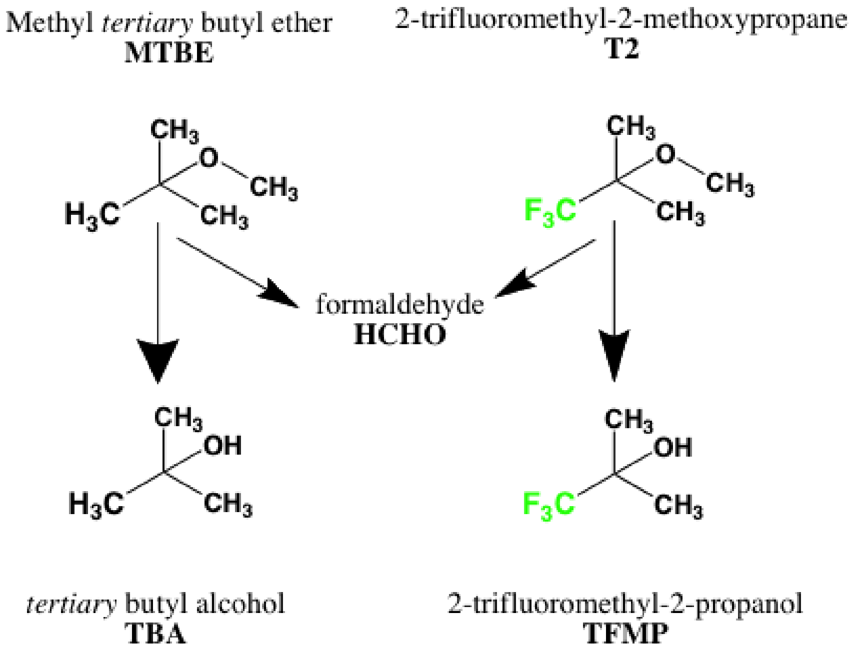
***Cometabolism supported by gasoline *n*-alkanes**

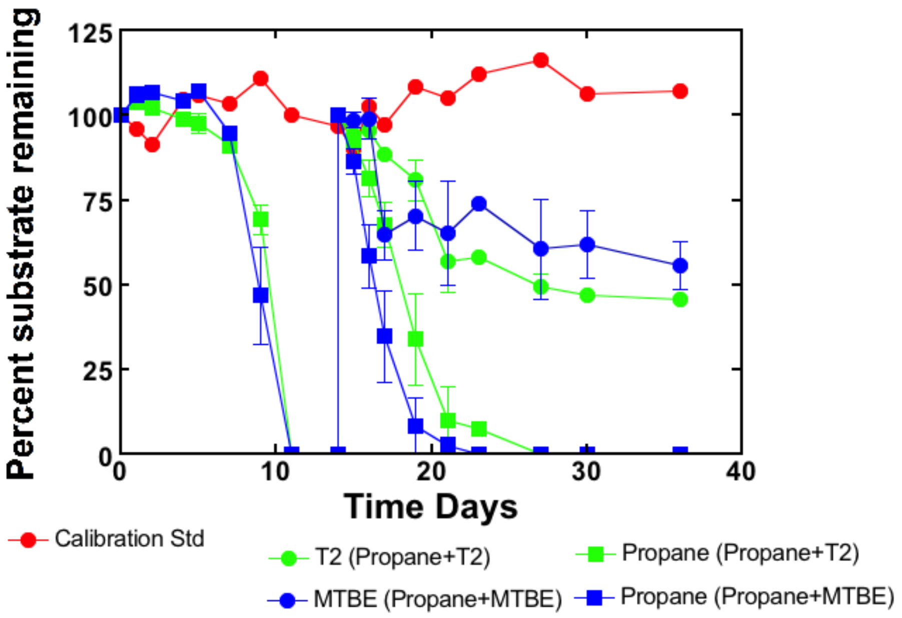
Fluorinated Enzyme Activity Probes



- Enzyme activity probes (EAPS) rely on lack of specificity of key enzymes in biodegradation pathways.
- Fluorinated analogs undergo same transformations as non-fluorinated compounds and at similar rates.
- Fluorine signature readily detectable, unique and retained during biodegradation.
- TCFE is analog for PCE/TCE
- VF also investigated for aerobic and anaerobic VC degradation.
- *In situ* applications using Push-Pull tests

Fluorinated MTBE analogs





- T2 serves as an accurate analog of MTBE in cometabolic micrcosms.
- TFMP used as a probe for pathway of MTBE/TBA oxidation.
- Both are useful as reactive probes for *in situ* determination of potential and rates of MTBE/TBA degradation.