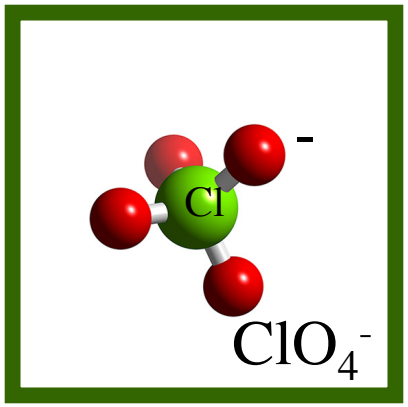
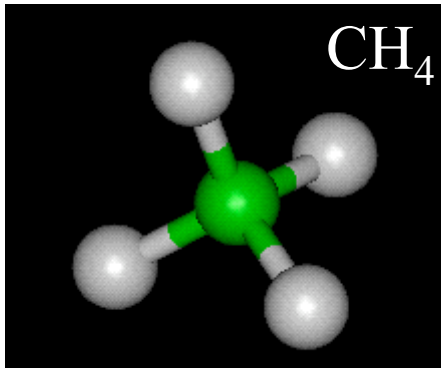


The possibility of methane oxidation coupled to microbial perchlorate metabolism



John D. Coates

Department of Plant and Microbial Biology

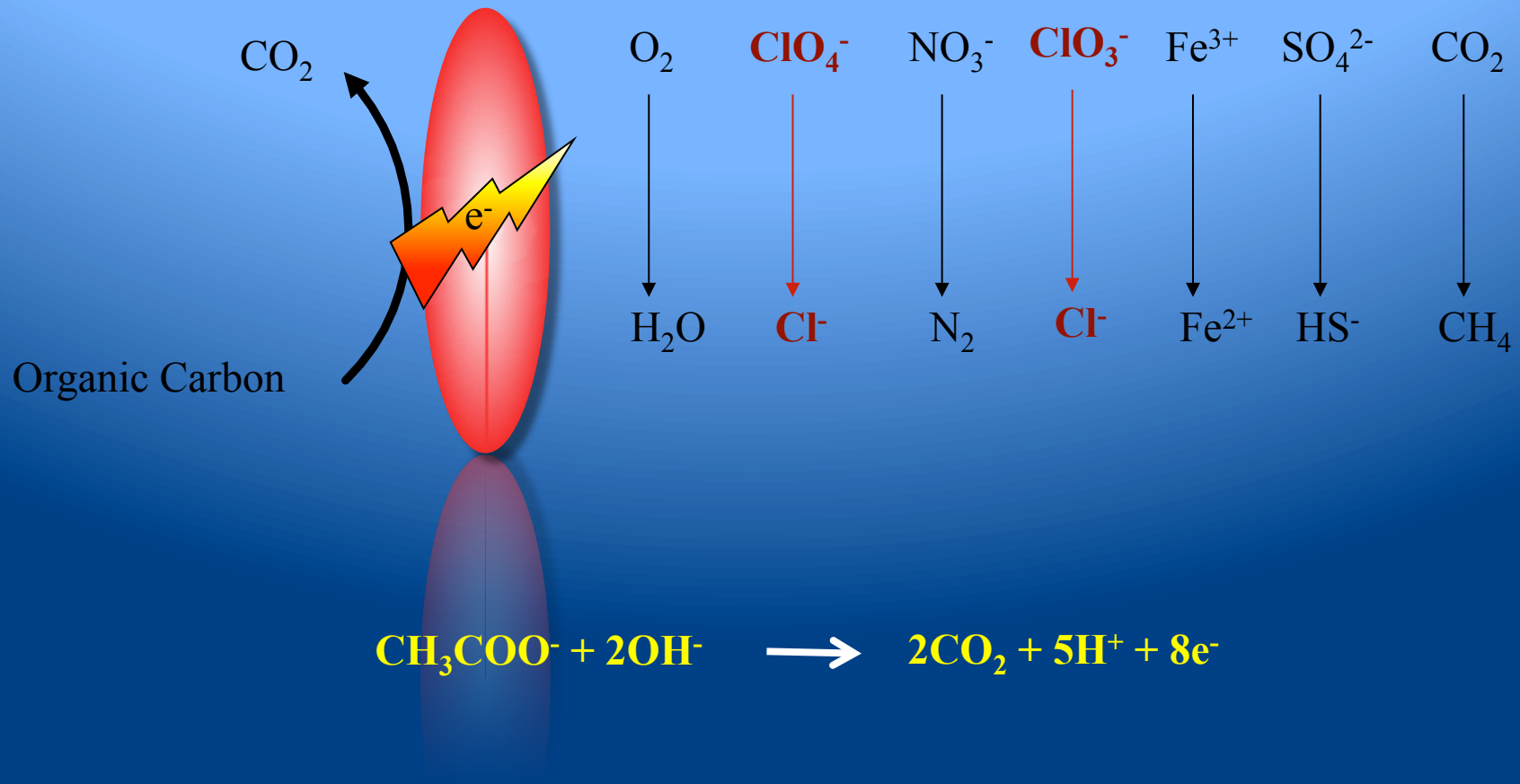
University of California, Berkeley

SERDP Strategic Environmental Research and Development Program

ESTCP Environmental Security Technology Certification Program

Environmental Remediation Sciences

Microbial Respiration

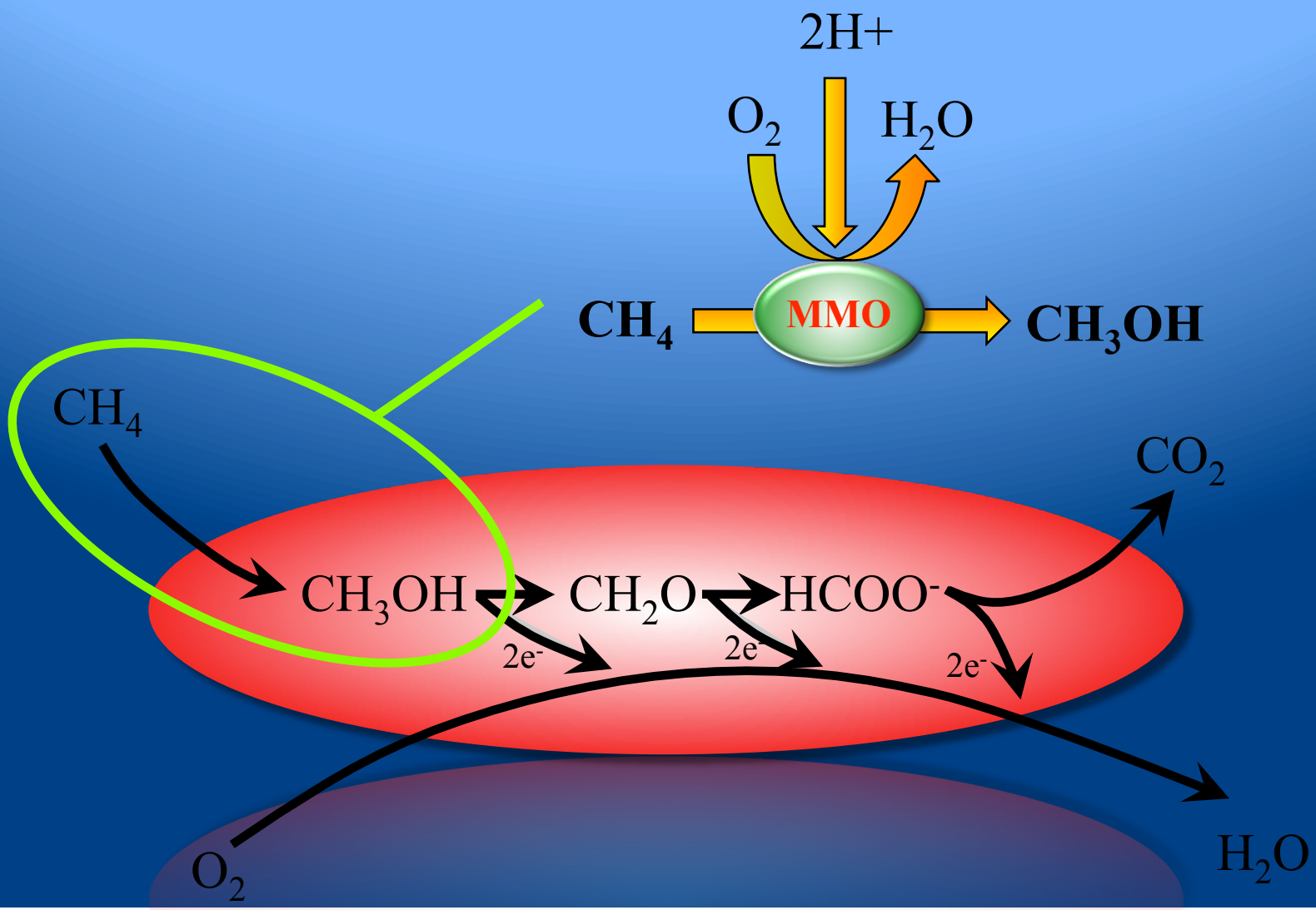


Microbial energetics are based on the transfer of electrons from reduced compounds to oxidizedr compounds with the conservation of energy in the form of ATP

Microbial Terrestrial Methane Oxidation

Aerobic

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ $\Delta G°'$ -818 kJ/mol



Microbial Terrestrial Methane Oxidation

Anaerobic

$\Delta G°'$ (kJ/mol)

$5CH_4 + 8NO_3^- + 8\ H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O$ -765

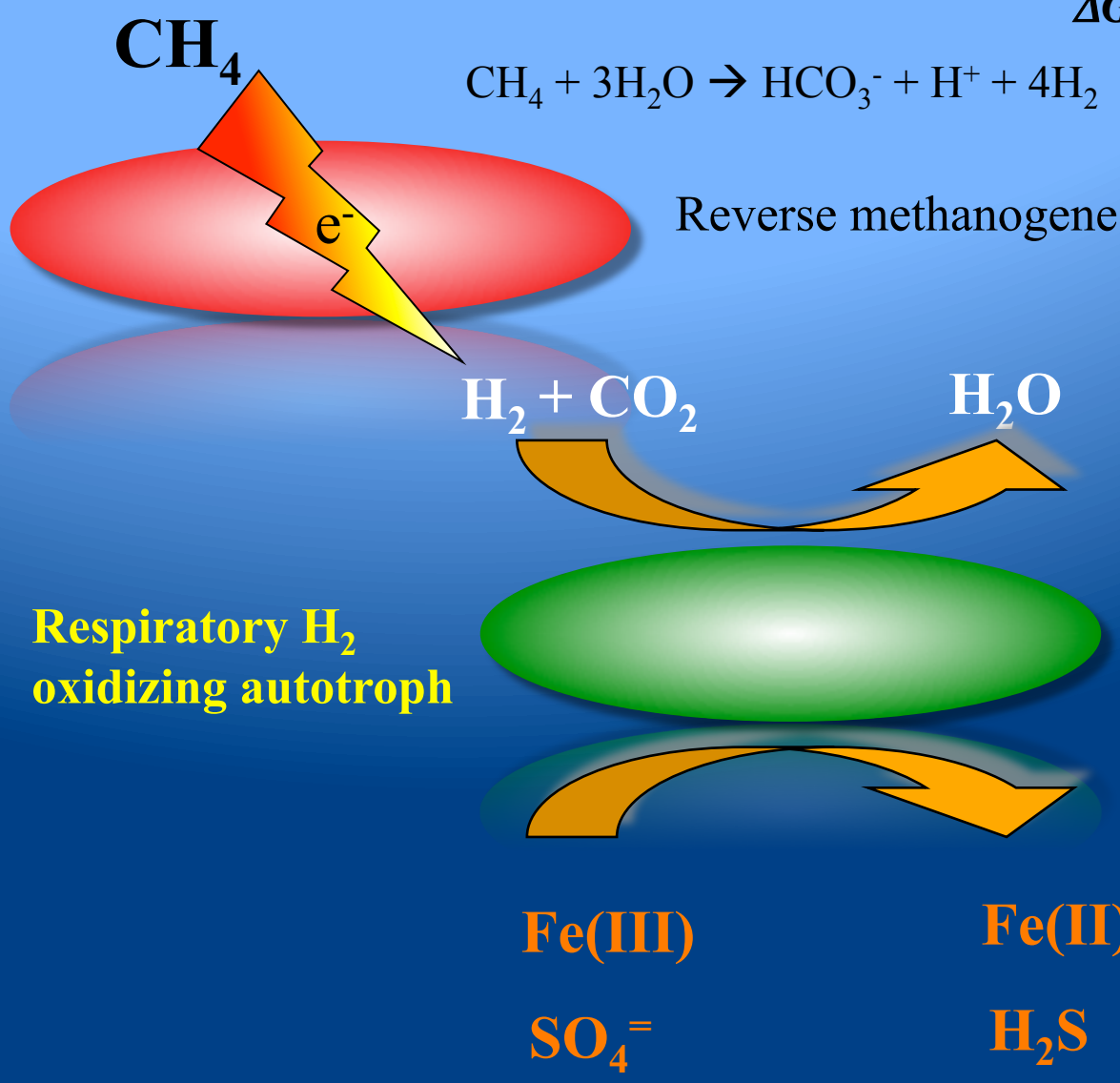
$CH_4 + 8Fe(OH)_3 + 16H^+ \rightarrow CO_2 + 8Fe^{2+} + 22H_2O$ -499

$CH_4 + SO_4^= \rightarrow HCO_3^- + HS^- + H_2O$ -16

No known anaerobic methane oxidizing respiratory organism

Microbial Terrestrial Methane Oxidation

Anaerobic



$\Delta G°'$ (kJ/mol)

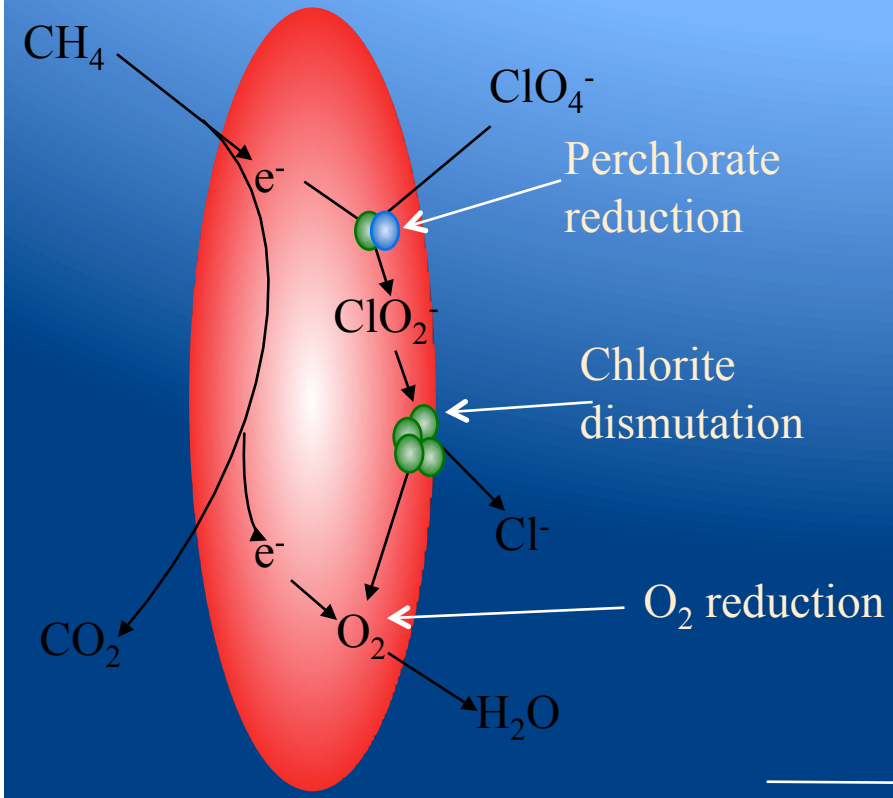
$CH_4 + 3H_2O \rightarrow HCO_3^- + H^+ + 4H_2$ +136

Reverse methanogenesis

Theoretical Anaerobic Methane Oxidation with perchlorate

$\Delta G°'$ (kJ/mol)

$CH_4 + ClO_4^- \rightarrow HCO_3^- + Cl^- + H_2O$ -1,165



BUT perchlorate reduction is a three step process

1. $CH_4 + 2ClO_4^- \rightarrow CO_2 + 2ClO_2^- + 2H_2O$

   $\Delta G°' = -764$ kJ/mol

2. $ClO_2^- \rightarrow O_2 + Cl^-$

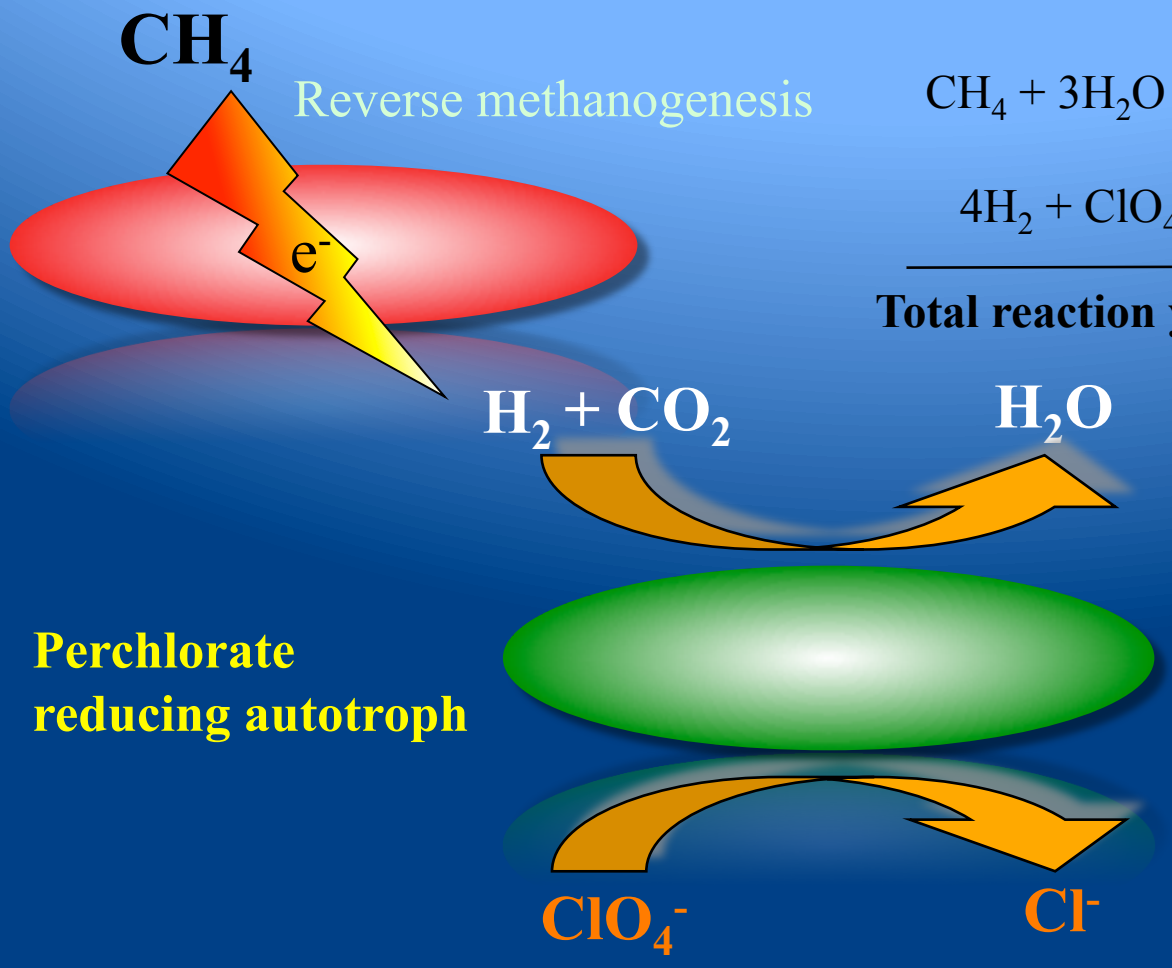
   $\Delta G°' = 0$ kJ/mol

3. $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$

   $\Delta G°' = -818$ kJ/mol

Total reaction yield: $\Delta G°' = -791$ kJ/mol CH_4

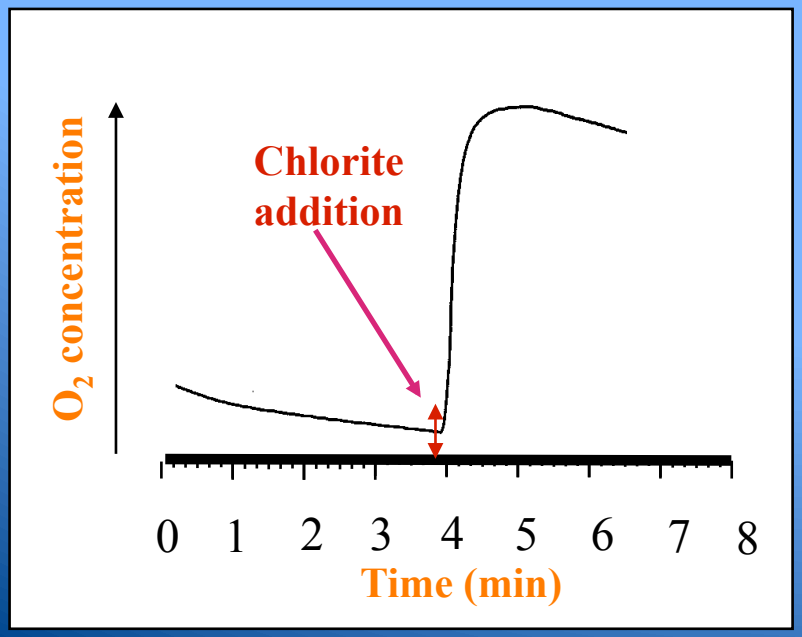
Alternatives for CH_4 oxidation with perchlorate

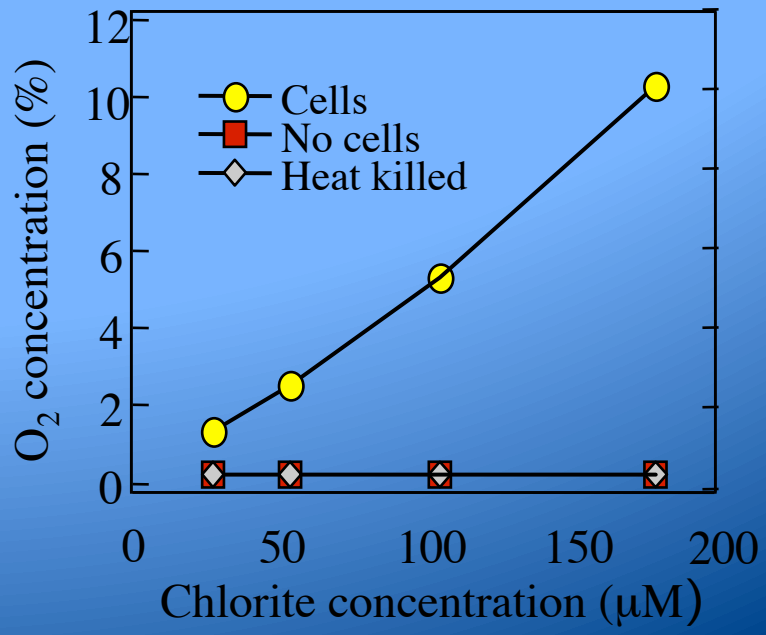


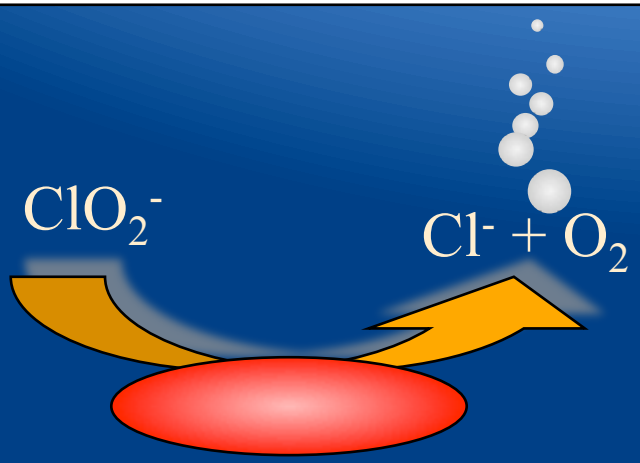
	$\Delta G°'$ (kJ/mol)
$CH_4 + 3H_2O \rightarrow HCO_3^- + H^+ + 4H_2$	+136
$4H_2 + ClO_4^- \rightarrow Cl^- + 4H_2O$	-928

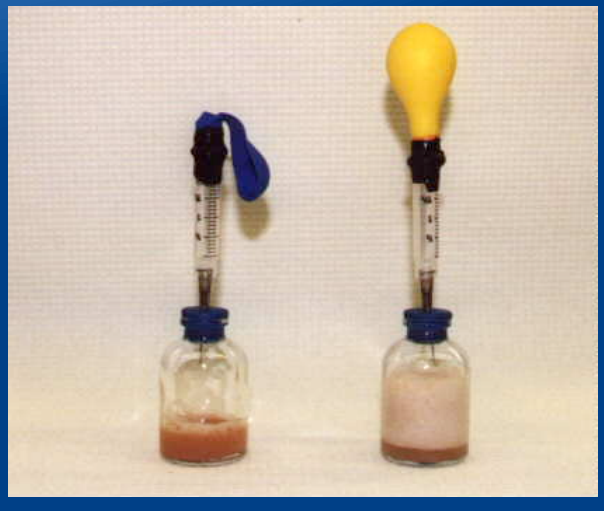
Total reaction yield: $\Delta G°'$= -792 kJ/mol CH_4

ClO_4^- respiration – a unique metabolism



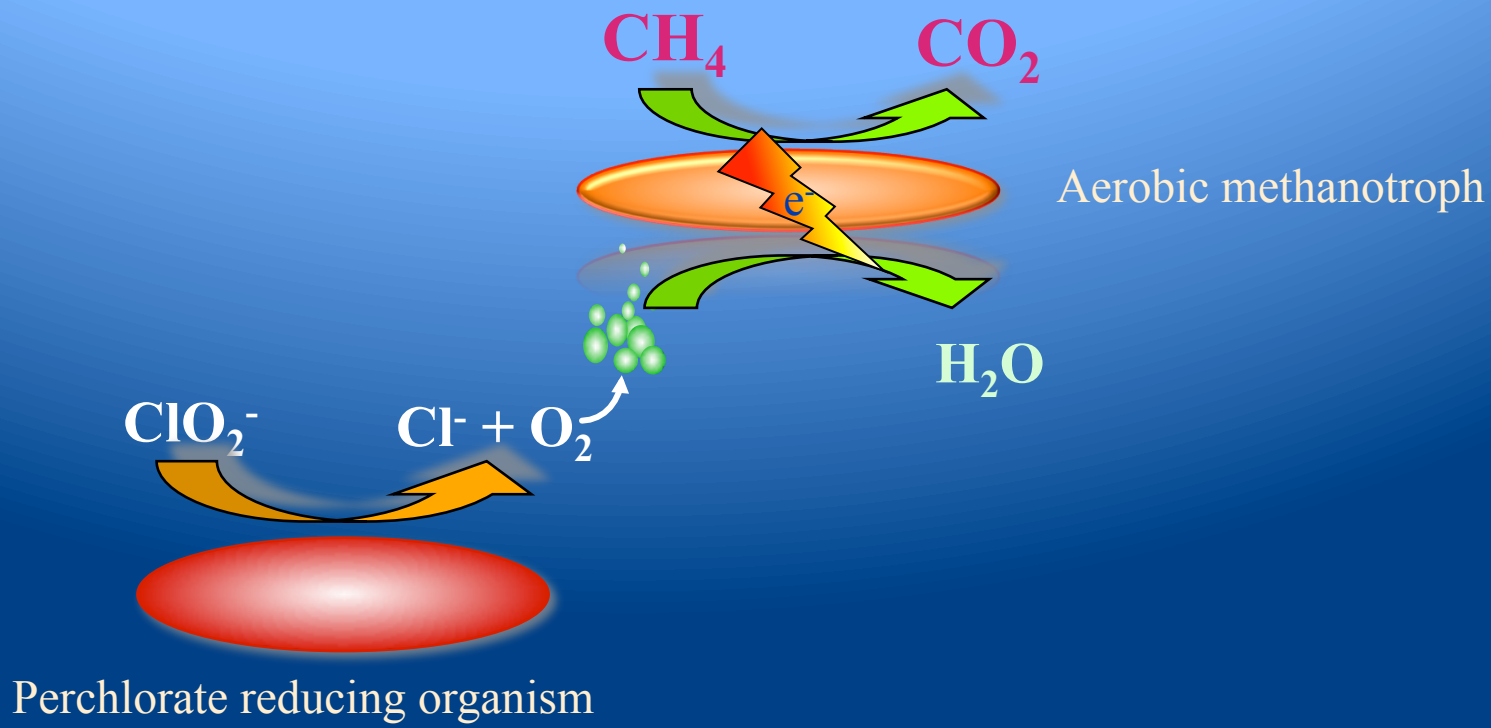




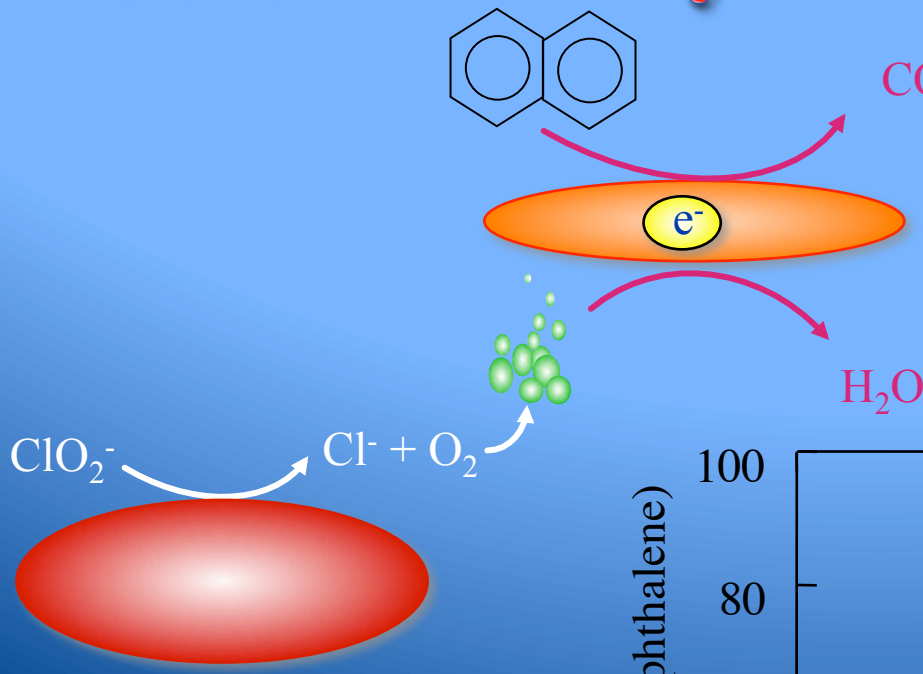


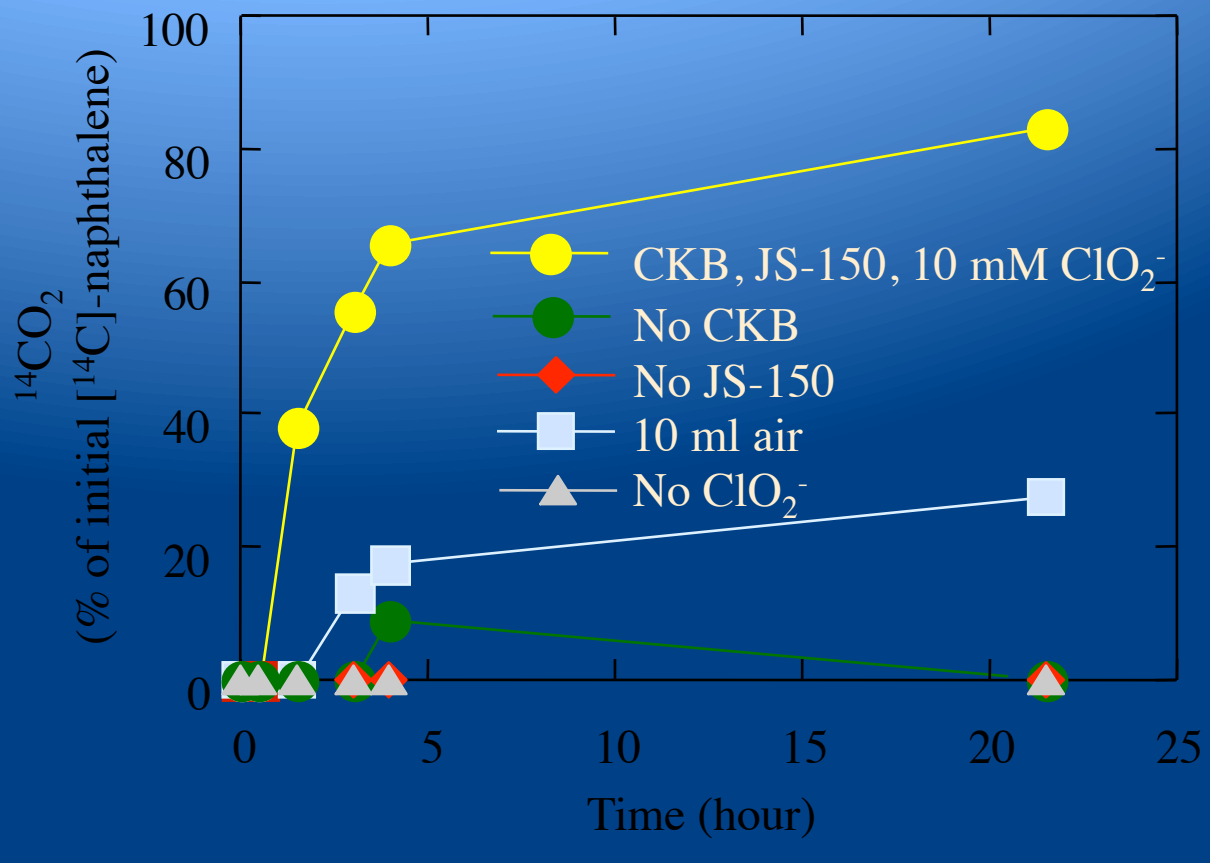
Cld is a highly active enzyme that is common to all perchlorate reducing bacteria.

Provision of extracellular O_2 to an aerobic methanotroph

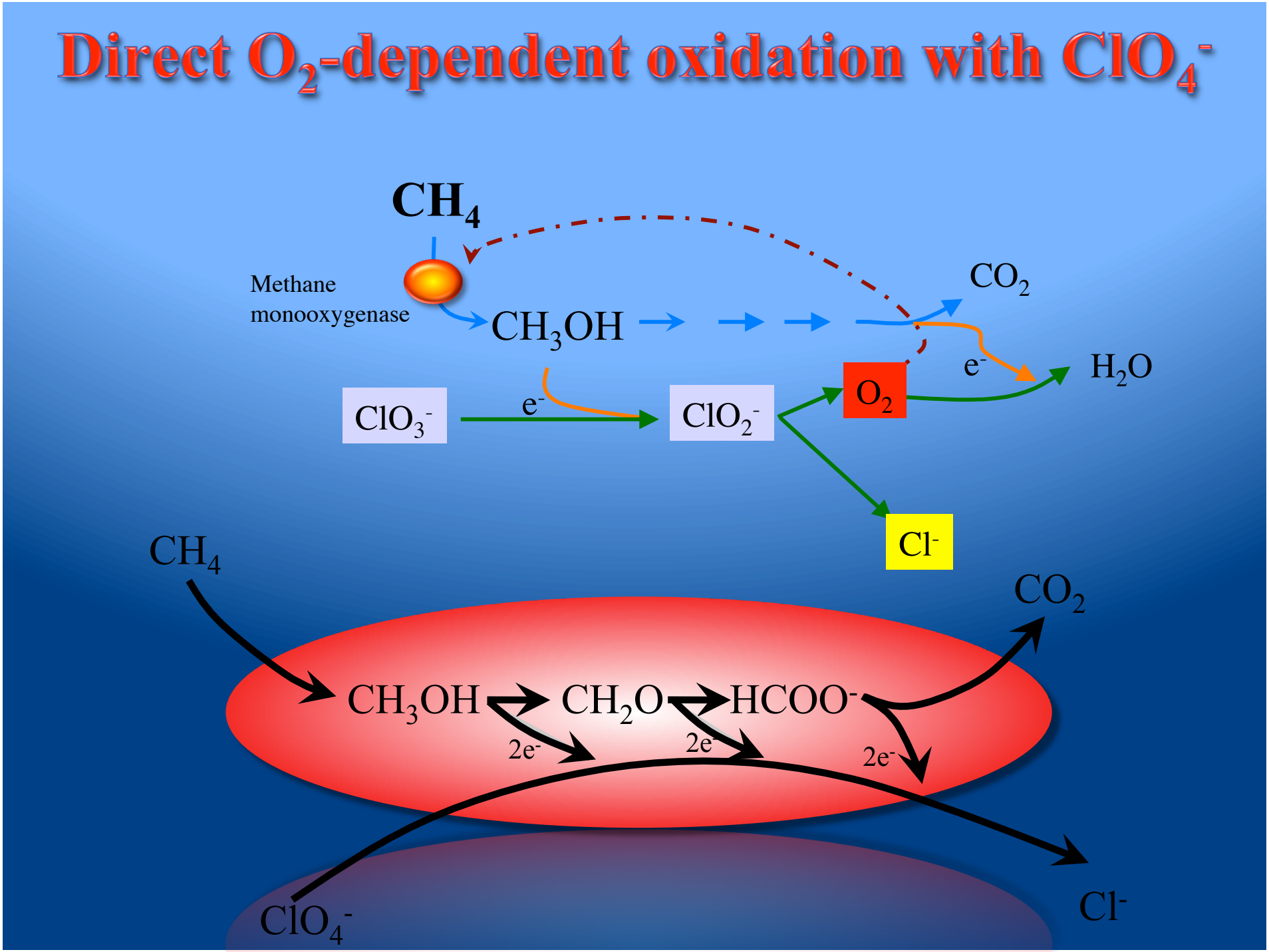


Co-culture Hydrocarbon Oxidation



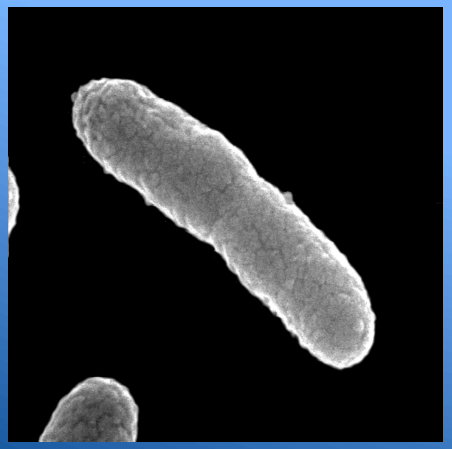


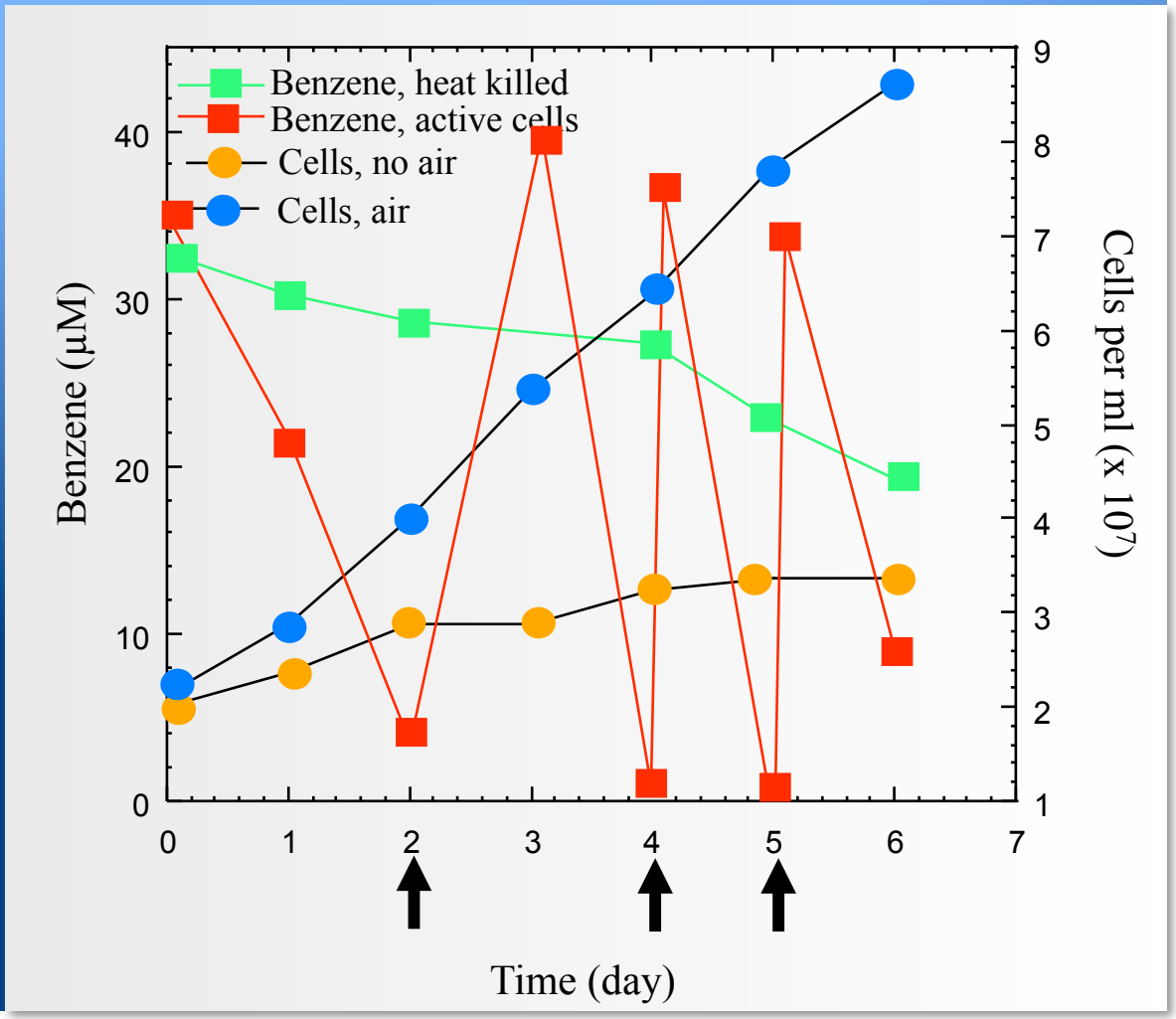
Direct O_2-dependent oxidation with ClO_4^-

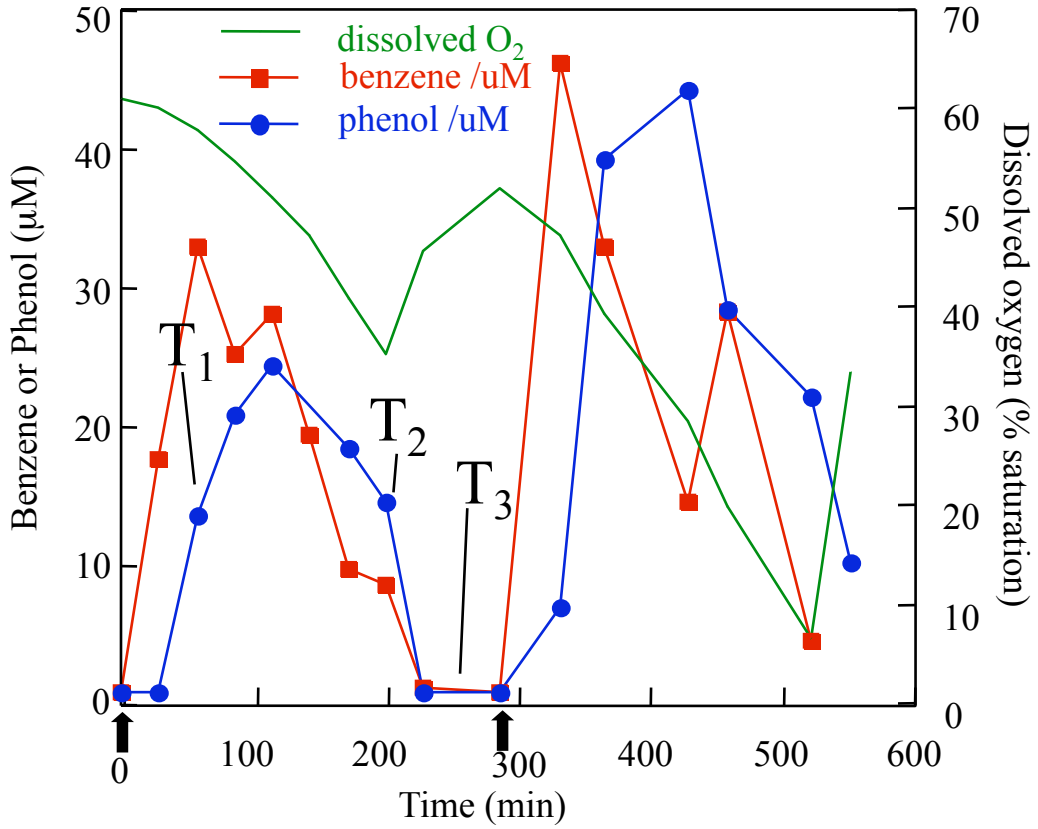


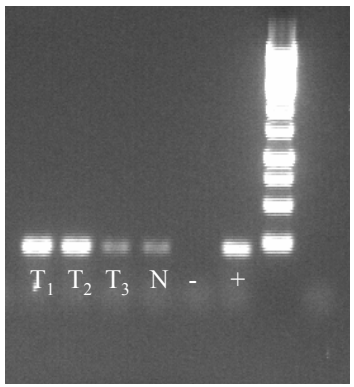
Evidence for internal oxygen cycling

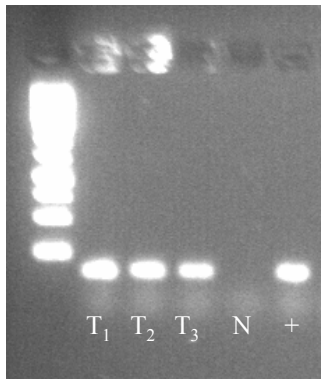
Dechloromonas aromatic strain RCB

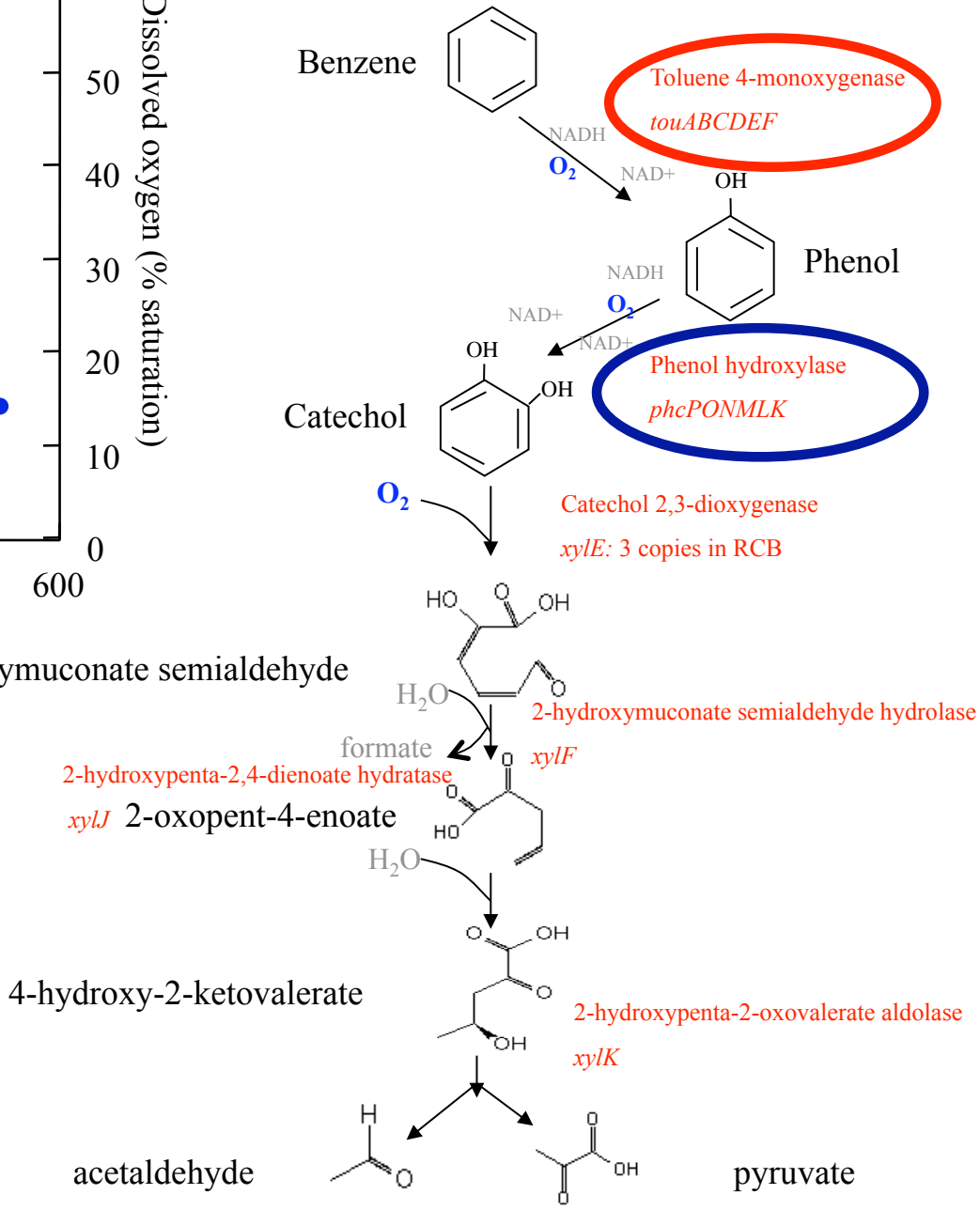


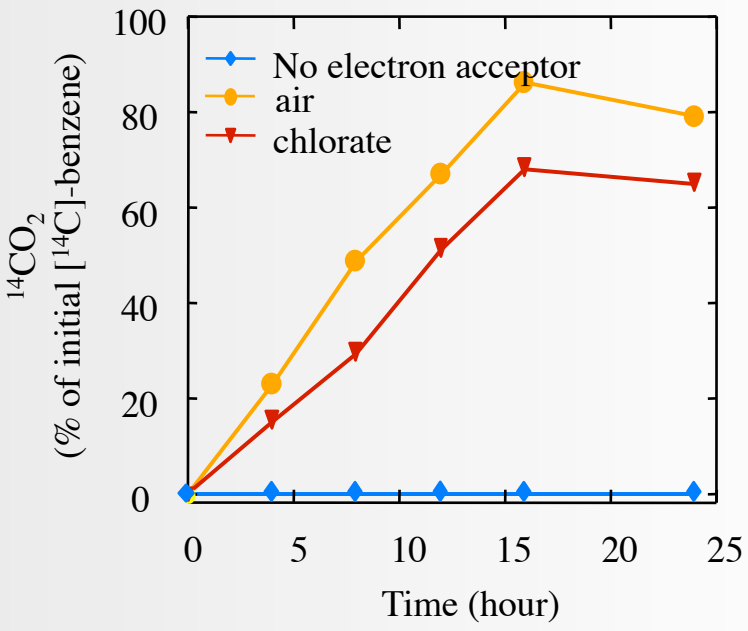



dissolved O_2
benzene /uM
phenol /uM
Benzene or Phenol (μM)
Dissolved oxygen (% saturation)
Time (min)
T_1
T_2
T_3


RT-PCR touA
T_1 T_2 T_3 N - +

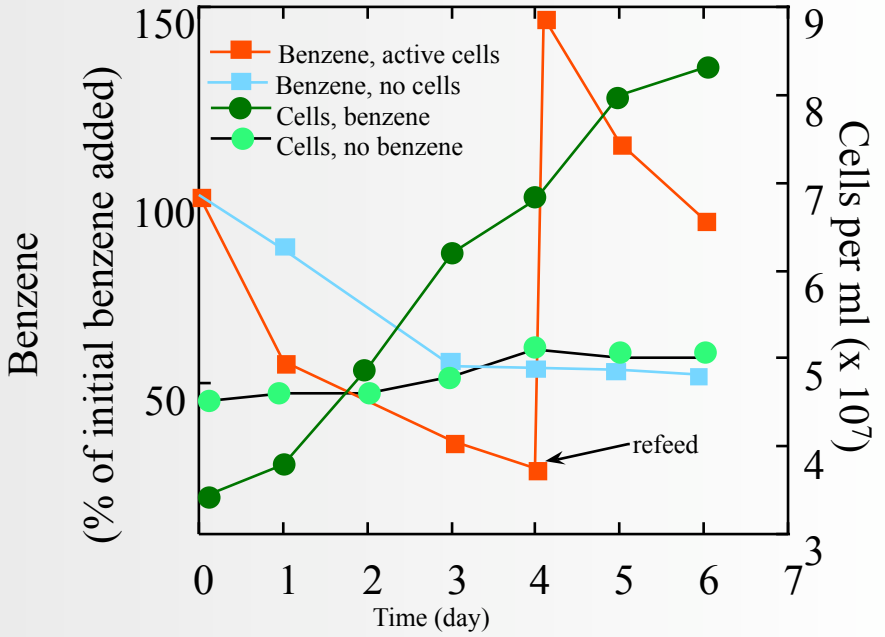

RT-PCR phcP
T_1 T_2 T_3 N +


Benzene
Toluene 4-monoxygenase
touABCDEF
NADH
O_2
NAD+
OH
Phenol
NADH
O_2
NAD+
NAD+
Phenol hydroxylase
phcPONMLK
OH
OH
Catechol
O_2
Catechol 2,3-dioxygenase
xylE: 3 copies in RCB
HO
O
OH
2-hydroxymuconate semialdehyde
H_2O
2-hydroxymuconate semialdehyde hydrolase
formate
xylF
2-hydroxypenta-2,4-dienoate hydratase
xylJ 2-oxopent-4-enoate
HO
H_2O
OH
4-hydroxy-2-ketovalerate
2-hydroxypenta-2-oxovalerate aldolase
xylK
H
acetaldehyde
OH
pyruvate

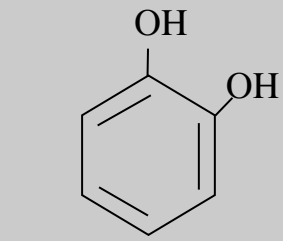


No O_2 present!

Benzene with chlorate (ClO_3^-)



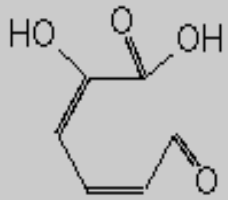
Catechol 2,3-dioxygenase activity in anaerobic benzene/chlorate cells

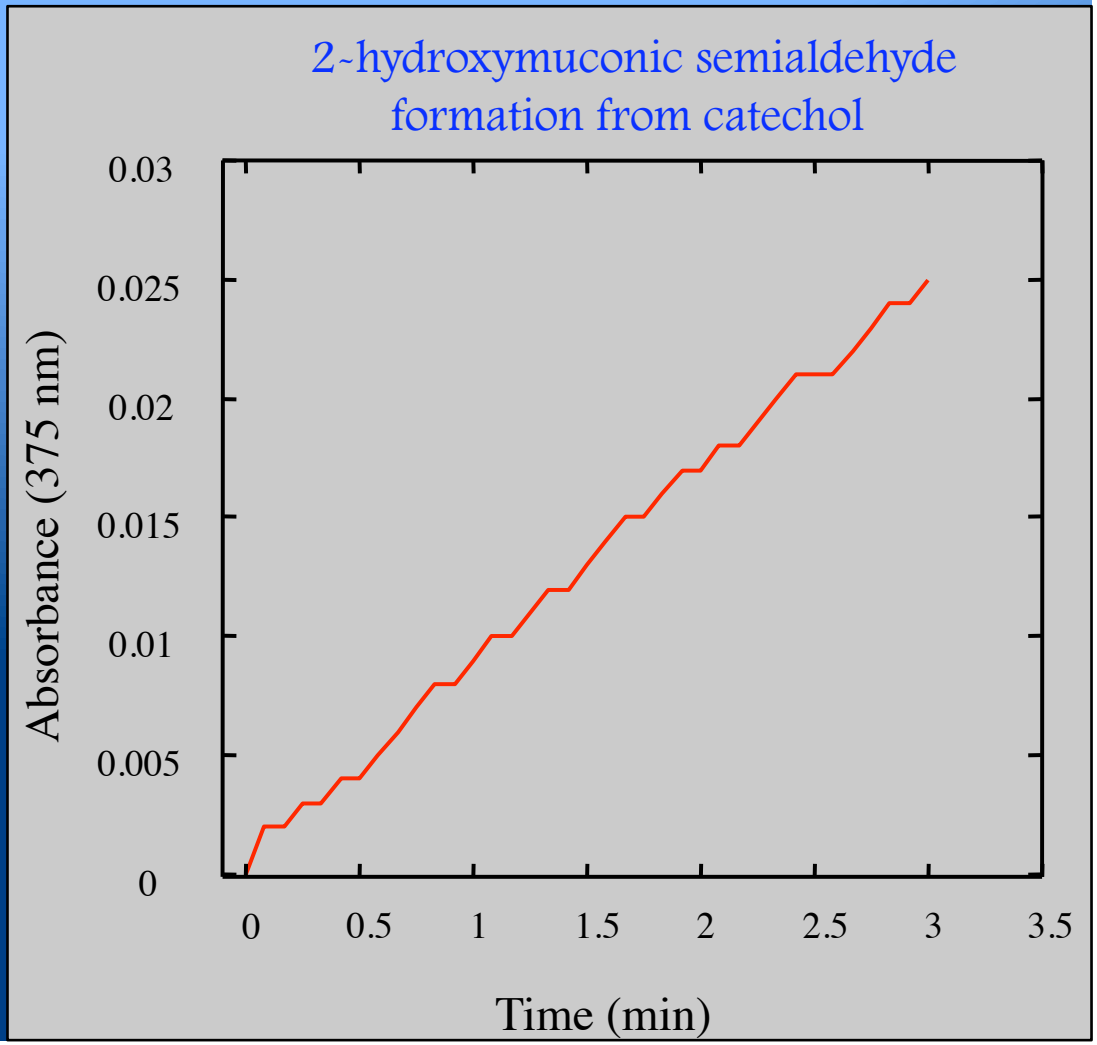


O_2

Catechol 2,3-dioxygenase

***xylE:* 3 copies in RCB**





Conclusions

1. CH_4 is oxidized on Earth both aerobically and anaerobically through syntrophic interaction.

2. The unique biochemistry of perchlorate reducing bacteria offers several alternatives of CH_4 oxidation.

    a) Direct anaerobic oxidation with perchlorate respiration

    b) Syntrophy interaction with reverse methanogens

    c) Provision of O_2 to aerobic methanotrophs

    d) Direct O_2-dependent CH_4 oxidation with perchlorate