Biological Approaches for Low-Temperature Oxidation of Methane

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ARPA-E Workshop: Sensing and Mitigation Strategies for Systematic Improvements in the Efficiency of Natural Gas Production

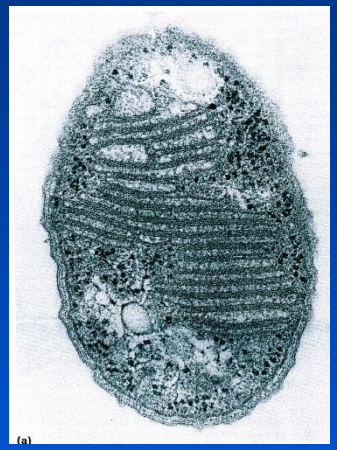
Biological Oxidation of Methane

- Methanotrophs grow on CH_4 as sole carbon and energy source and have a unique physiology
- Typically obligate aerobes, Gram-negative in the α and γ-Proteobacteria
  - Recent identification of methanotrophic Verrucomicrobia
  - Anaerobic methane oxidizers also discovered with methane oxidation coupled to sulfate or nitrite reduction
- Present in many environments, e.g., forest and agricultural soils, landfills, sediments, lakes, marine systems, hot springs, etc.

Proteobacteria Methanotrophs

γ-Proteobacteria Methanotrophs

- RuMP pathway of formaldehyde assimilation
- Intracytoplasmic membranes arranged in bundles



*Methlobacter**
*Methylococcus**
*Methylocaldum**
Methylohalobius
Methylomicrobium
Methylomonas
Methylospheara
*Methylosarcina**
*Methylosoma**
Methylothermus
Methylovulum

α-Proteobacteria Methanotrophs

- Serine pathway of formaldehyde assimilation
- Intracytoplasmic membranes arranged along cell periphery

Methylocapsa
*Methylocella***
*Methylocystis**
Methyloferula
*Methylosinus***

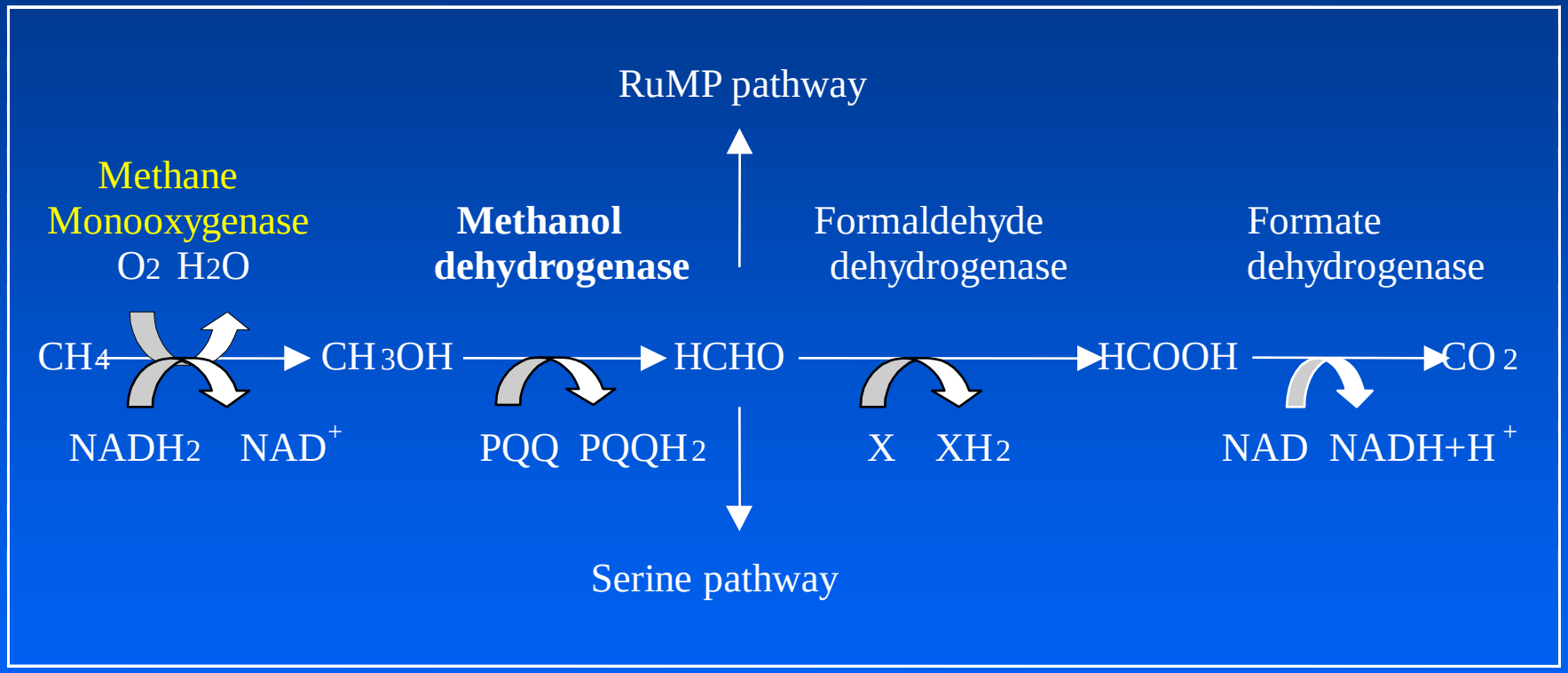
**cysts; **exospores*

Aerobic Methanotrophs Can Survive a Wide Range of Environmental Conditions

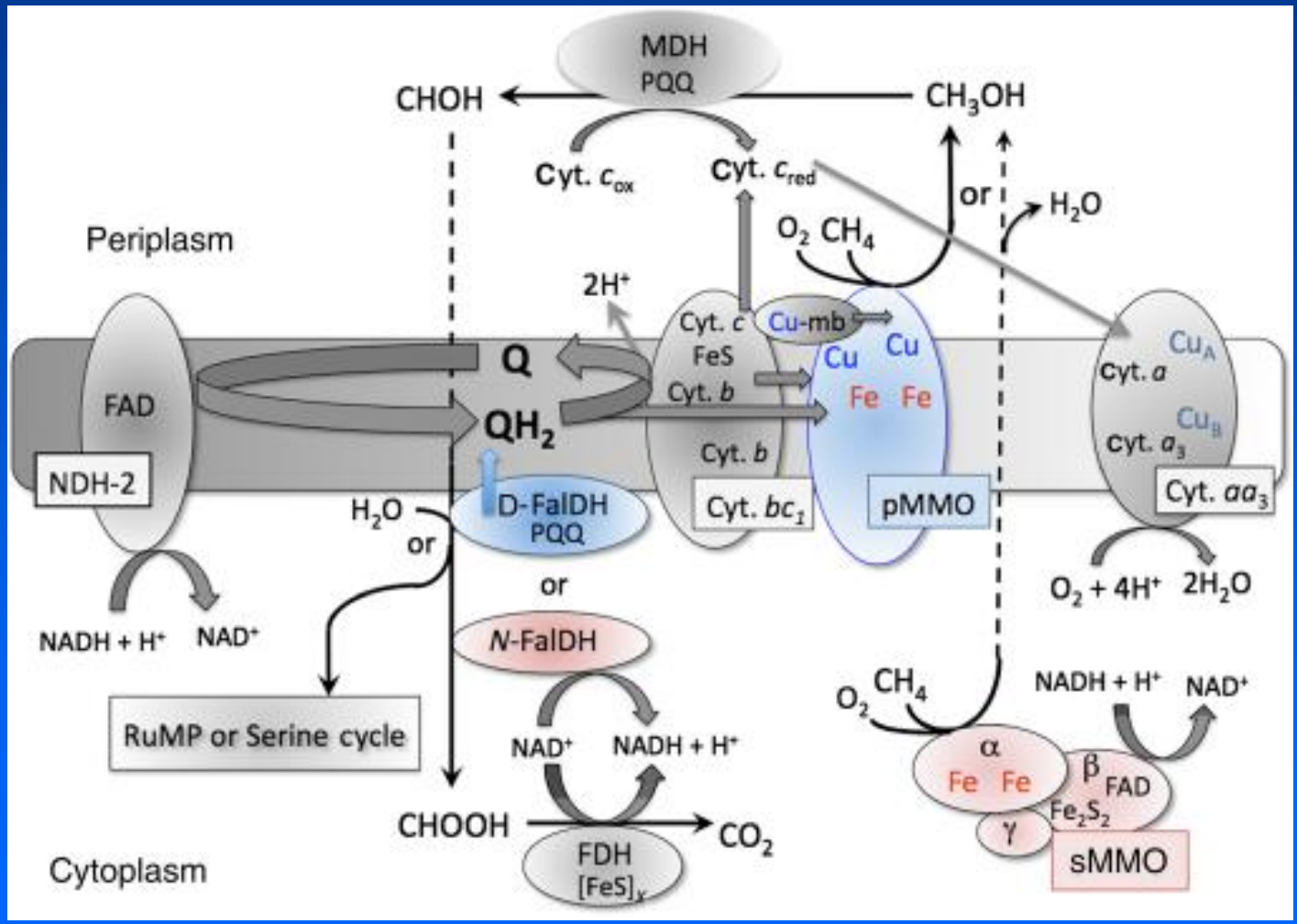
- pH: 4 - 9.5
- Temperature: 0 - 72°C
- Salt : 0.1 - 12% (w/v)
- Many form resting stages such as cysts or exospores
- Some can fix nitrogen
- Broad range of methane affinity:
  - 0.11 - 90 µM (~100 – 90,000 ppmv)

NOTE: Verrucomicrobia methanotrophs are acidophilic, and can grow at a pH of 0.8 (but are not known to form resting stages)

Pathway of Methane Oxidation in Methanotrophs

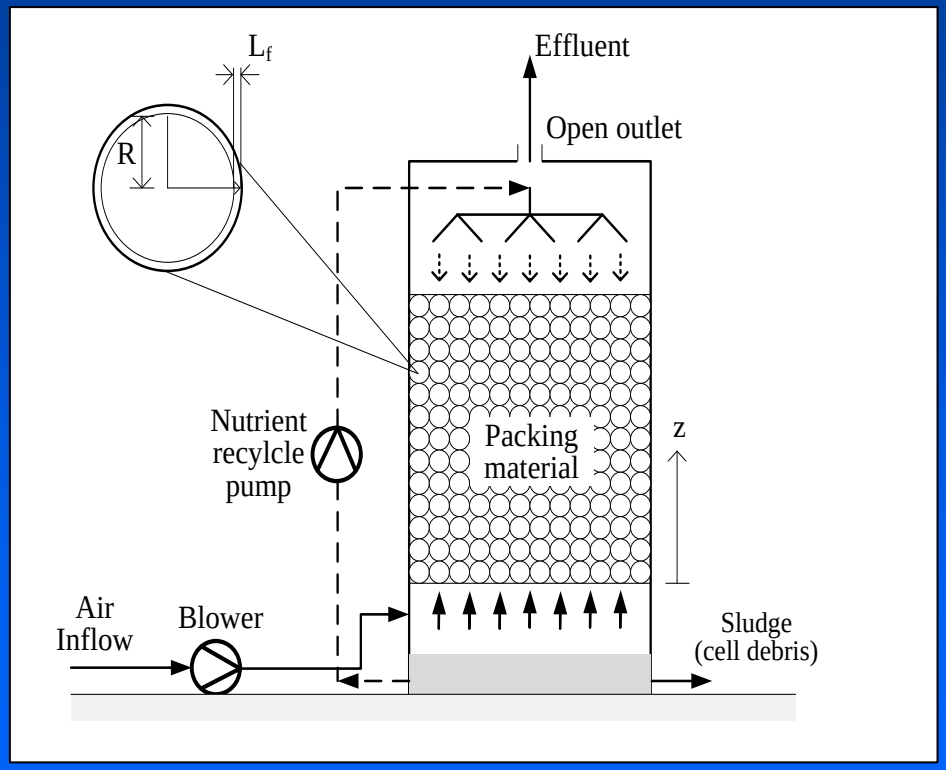


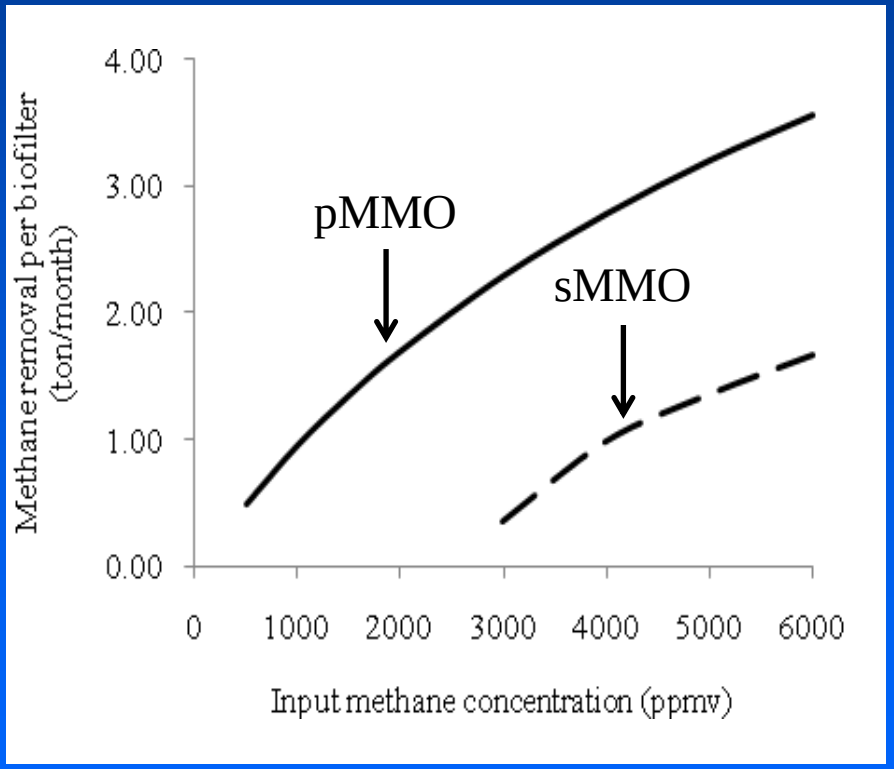
Two forms of MMO exist are known to exist



Semrau, et al., 2010

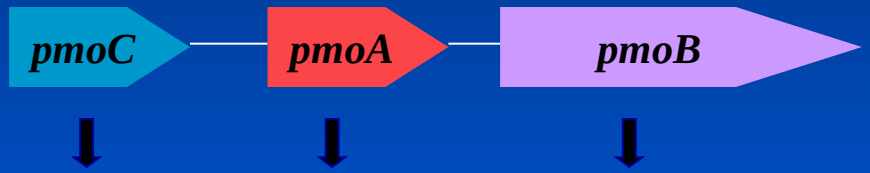
Mitigation of Methane Emissions via Biofiltration





Methane Monooxygenases (MMO): Gene Organization and Expression

pMMO

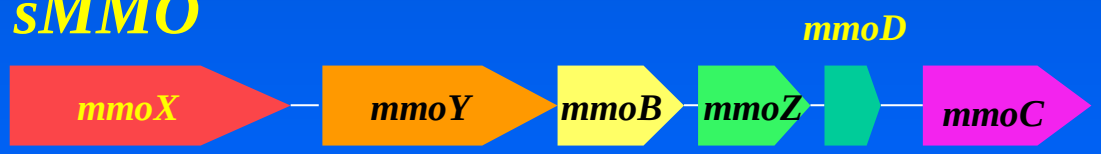


PmoC *23 kDa*

PmoA *27 kDa*

PmoB *45 kDa*

sMMO



α *60 kDa*

β *45 kDa*

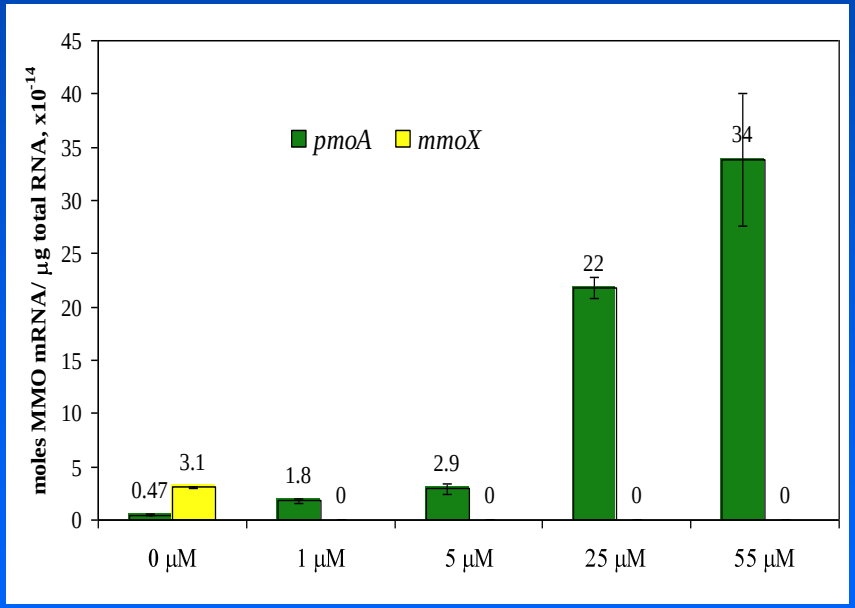
B *16 kDa*

γ *20 kDa*

?

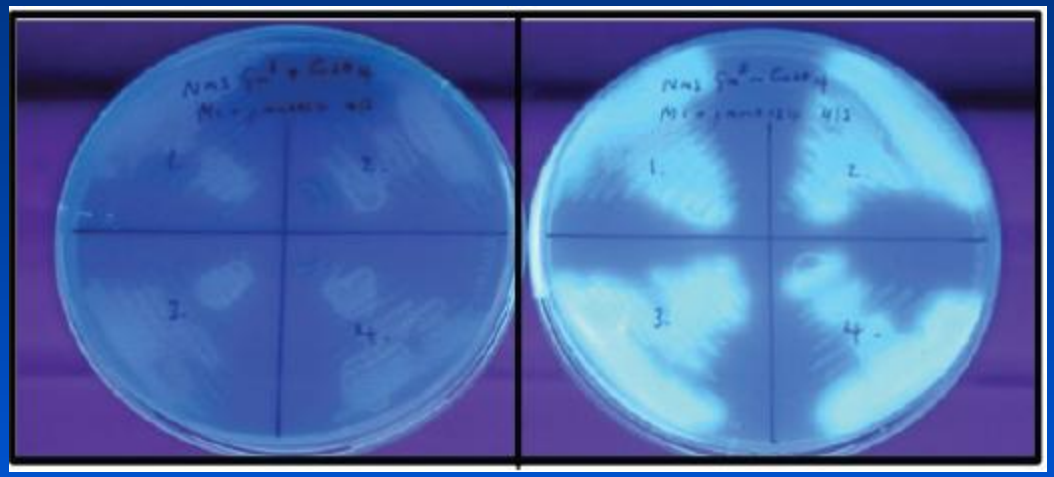
Reductase *39 kDa*

Gene expression as a function of copper



Choi, et al., (2003) J. Bacteriology 185: 5755-5764.

Insertion of Reporter Genes into Methanotrophs



Ali & Murrell, 2009

← *lacZ* fused to σ^{54} promoter in *Methylococcus capsulatus* Bath

Theisen, et al., 2005

← *gfp* fused to σ^{54} promoter in *Methylocella silvestris* BL2

Conclusions and Challenges

- Methanotrophs have been extensively studied and these microbes are remarkably versatile in the range of environmental conditions they can survive (if not thrive)

- Methanotrophy has significant environmental and industrial applications, e.g., prevention of methane emissions from various sources

- To utilize methanotrophy for monitoring fugitive methane emissions, one key issue is to insert a reporter system into an appropriate strain that is easily monitored and reliable.