Implications for anaerobic methane oxidation: The Norman Landfill and beyond

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Global Impacts of Methane

- Over a 20 year period, one molecule of methane has a global warming potential 25 times that of one molecule of carbon dioxide
- Landfills are 3-7% of global methane emissions, and the largest anthropogenic source of methane in the United States
- The sources and sinks of methane cycling on the planet are not well understood, particularly in the absence of oxygen

(Thompson et al. 1992; Lelieveld et al. 1998; IPCC 2007)

Microbial Mediation of Methane in Landfills

- Microbial methane oxidation
  - Well defined process under aerobic conditions
    - Reports of methane oxidation in landfill cover soil
    - Genes involved in the process are characterized
  - Anaerobic oxidation of methane (AOM)
    - What role does it play in the terrestrial subsurface?
    - Mitigation of methane emissions from landfills?

Anaerobic Oxidation of Methane (AOM)

- **AOM has been heavily studied in marine environments**
- **Energetics:**
  - $CH_4 + SO_4^{2-} + H^+ \rightarrow CO_2 + HS^- + 2H_2O$

    $\Delta G°'$ -21 kJ mol^{-1} (Shima and Thauer, 2005)

    $\Delta G'$ -31.5 kJ mol^{-1} (Widdel et al., 2007)
- **Mechanisms?**
  - Reverse methanogenesis (Shima and Thauer, 2005)
  - Methyl sulfides (Moran et al., 2007)
- **Microbiology**?
  - Archaeal ANME-1,2,3 (*an*aerobic *me*thane oxidizers)
  - Sulfate-reducing bacterial partner *Desulfosarcina/ Desulfococcus*

Anaerobic Oxidation of Methane (AOM)

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  - CH_4 +
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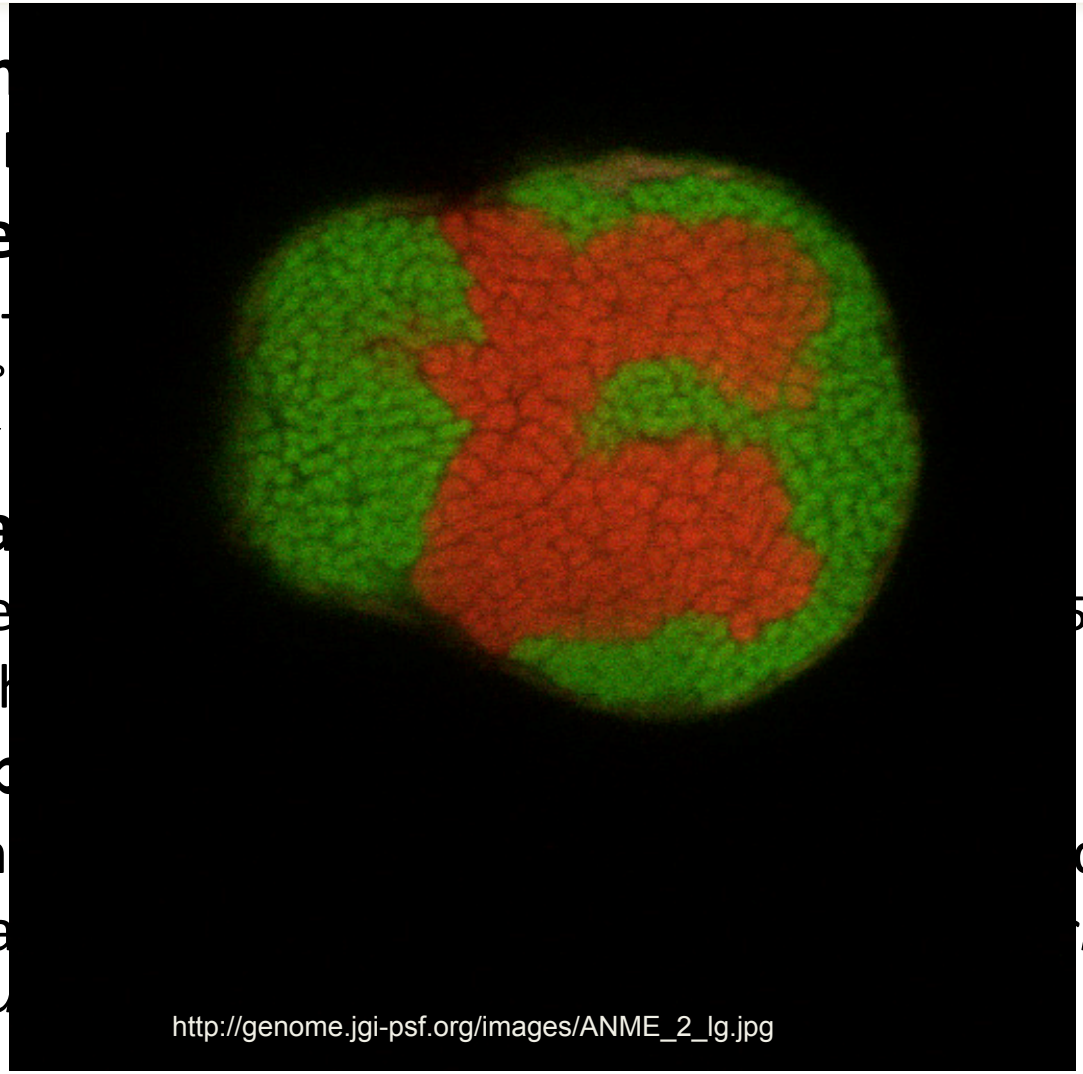

http://genome.jgi-psf.org/images/ANME_2_lg.jpg

Freshwater/terrestrial AOM

- AOM described in the terrestrial environment
  - $5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O$
    - $\Delta G°'$ -765 kJ mol^{-1} (Raghoebarsing et al., 2006 *Nature*)
  - Methane concentration 800 µM and 100 µM nitrate
- Incorporation of ^{13}C-labeled methane into bacterial/ Archaeal membrane
  - 16S rDNA data suggest a new subdivision for bacterial clone
  - Archaeal member only a distant relative of marine ANME
  - Following enrichment the bacterial member was dominant, suggesting Archaea may not be involved in AOM under denitrifying conditions (Ettwig et al., 2008)

AOM - what we don't know:

- The relationship between the archaeal and bacterial partner has not been elucidated.
  - There are no proven metabolic intermediates
  - What is the true **enzymatic mechanism?**
- To what extent are other terminal electron acceptors are involved in AOM?
  - Denitrifying consortia
  - Bemidji – hydrocarbon contaminated site, AOM via iron reduction? (Bekins et al., 2008 AGU)
- What are the microorganisms responsible for AOM?
  - There are no known isolates capable of AOM.

Fundamental Aspects of Anaerobic Hydrocarbon Metabolism

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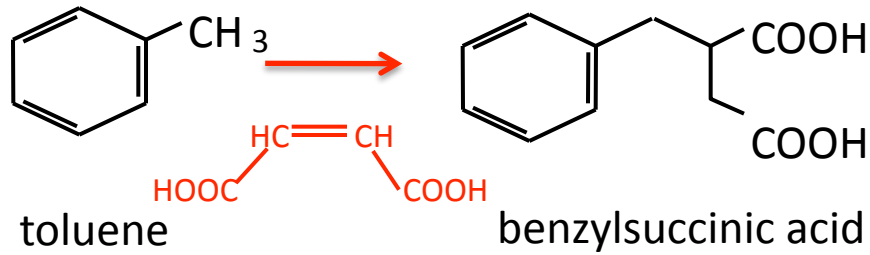
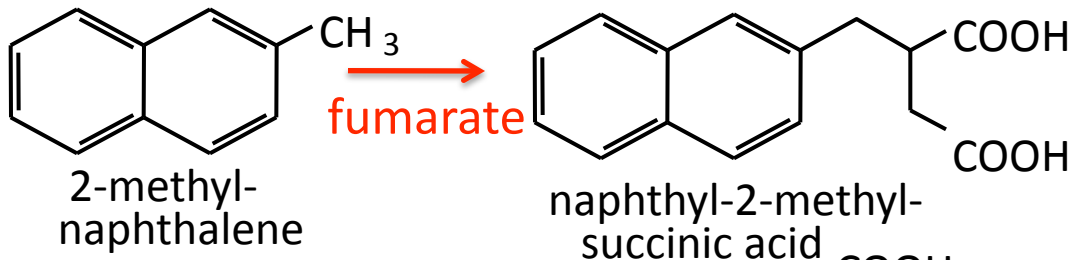
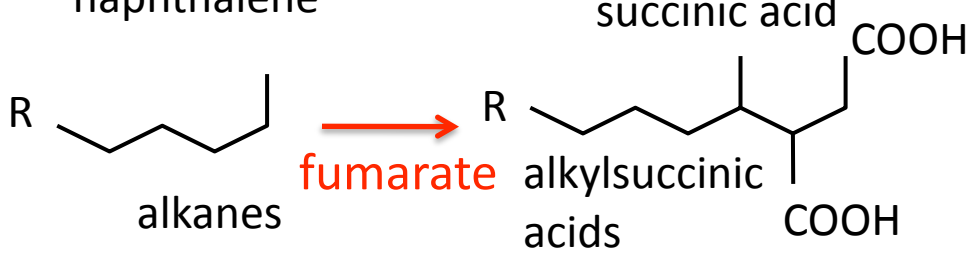


PAHs



Alkanes



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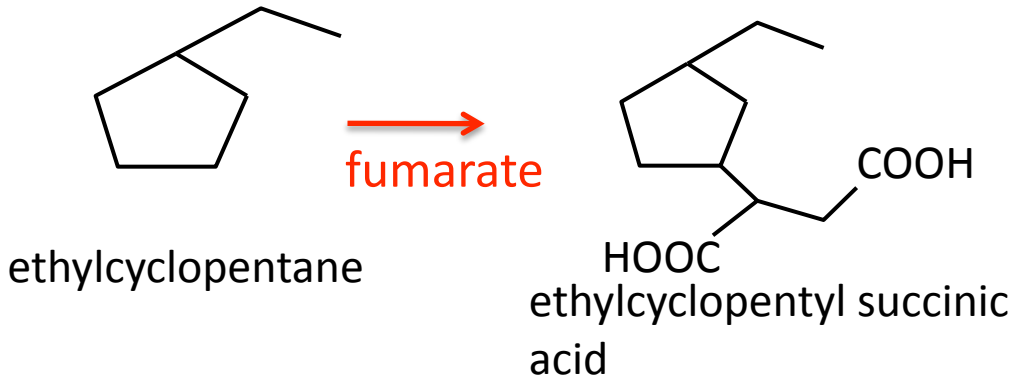
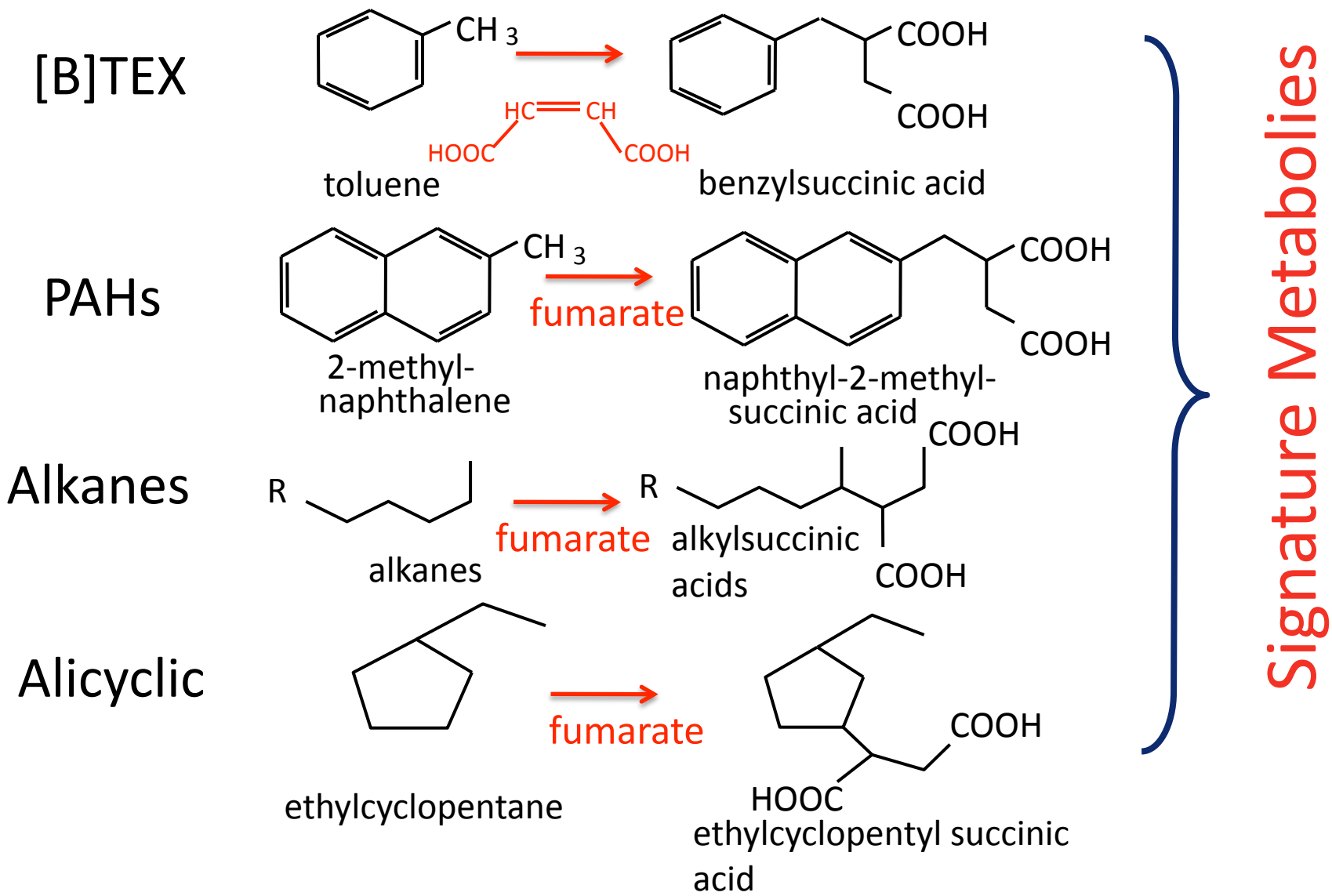
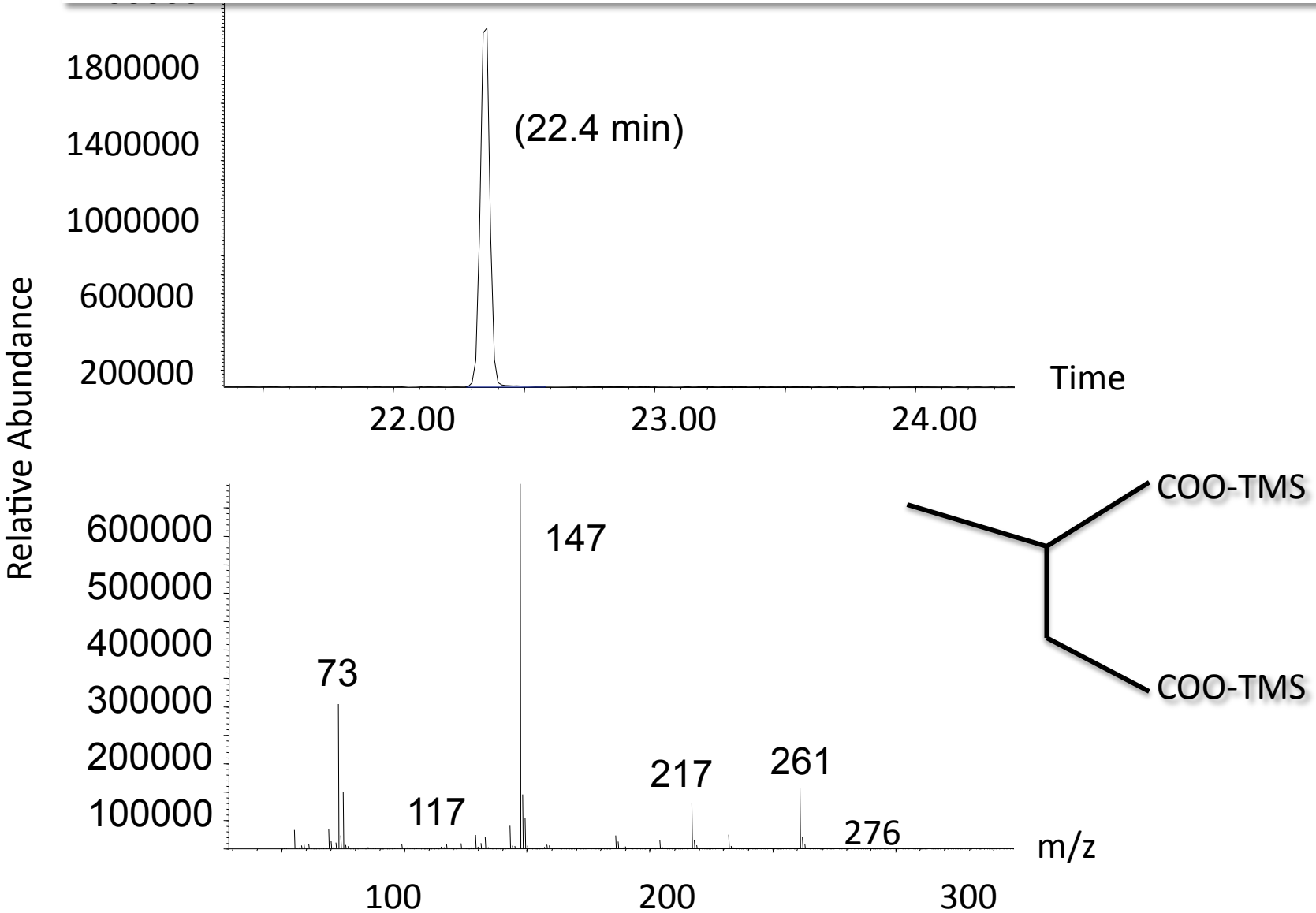


Fundamental Aspects of Anaerobic Hydrocarbon Metabolism



TIC: Methylsuccinate standard (1mM)



The Alaskan North Slope meets the Norman Landfill

- The metabolic profiling on the slope revealed the presence of a putative metabolite
  - Phylogenetic results (poster No. 13)
  - But no typical ANME organisms
- Where else?
  - Isotopic analyses revealed heavier methane at the margins of the plume at the Norman Landfill (Grossman et al., 2002)

Anaerobic Methane Oxidation at the Norman Landfill

Environ. Sci. Technol. **2002**, *36*, 2436–2442

Anaerobic Methane Oxidation in a Landfill-Leachate Plume

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to Norman Landfill in Oklahoma provides an excellent natural laboratory for the study of anaerobic methane oxidation in landfill-leachate plumes.

Several comprehensive studies have characterized the anaerobic environments of landfill-leachate plumes (e.g., refs 5–8). The abundance of electron donors in landfill-leachate plumes results in a paucity of electron acceptors. Ideally, methanogenesis dominates closest to the landfill source and is followed sequentially downgradient by sulfate reduction, iron reduction, nitrate reduction, and oxygen reduction. In reality, these processes can overlap and exhibit a complex distribution pattern reflecting the heterogeneity of the aquifer. The distribution of redox environments will have a profound

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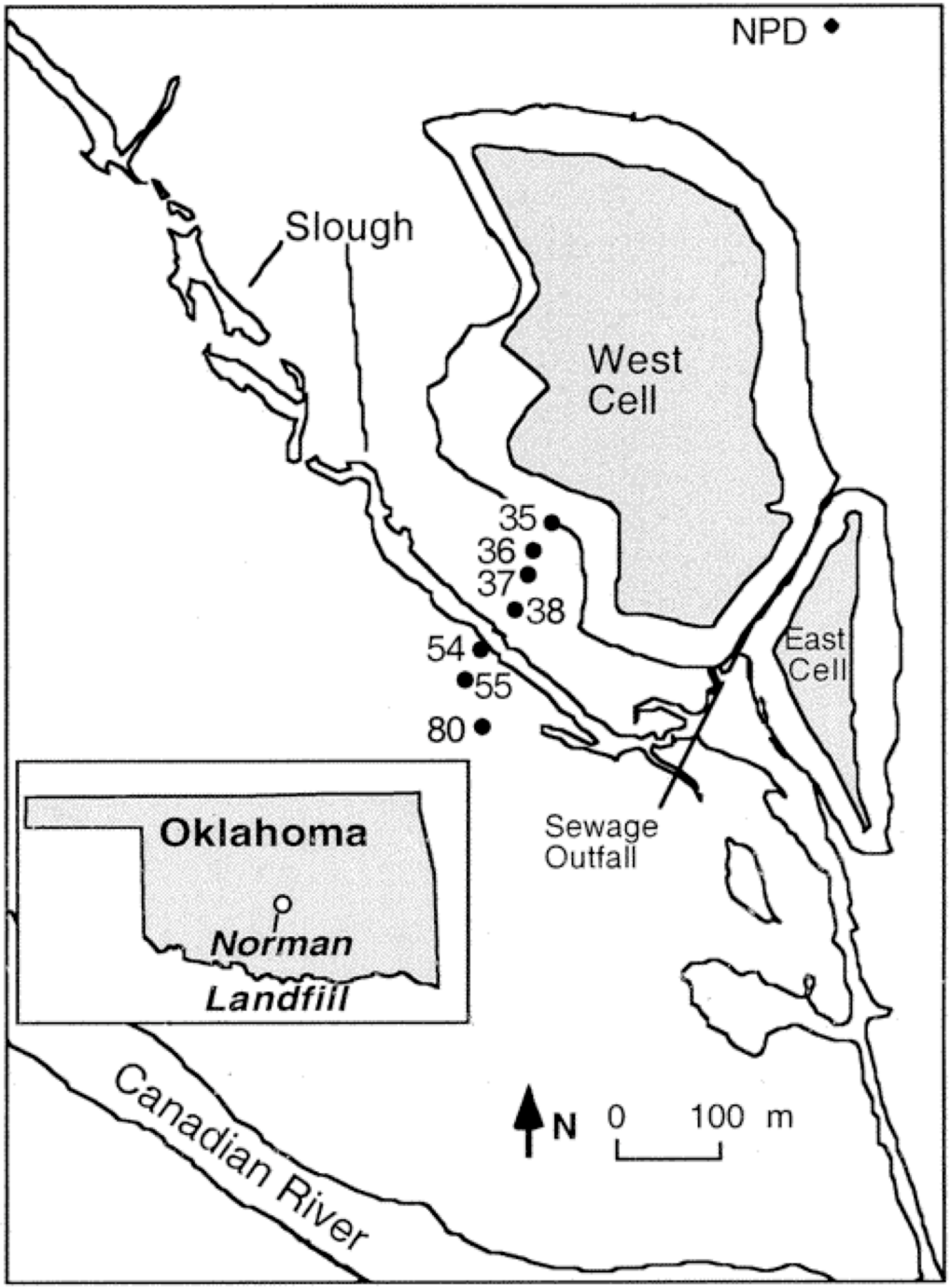
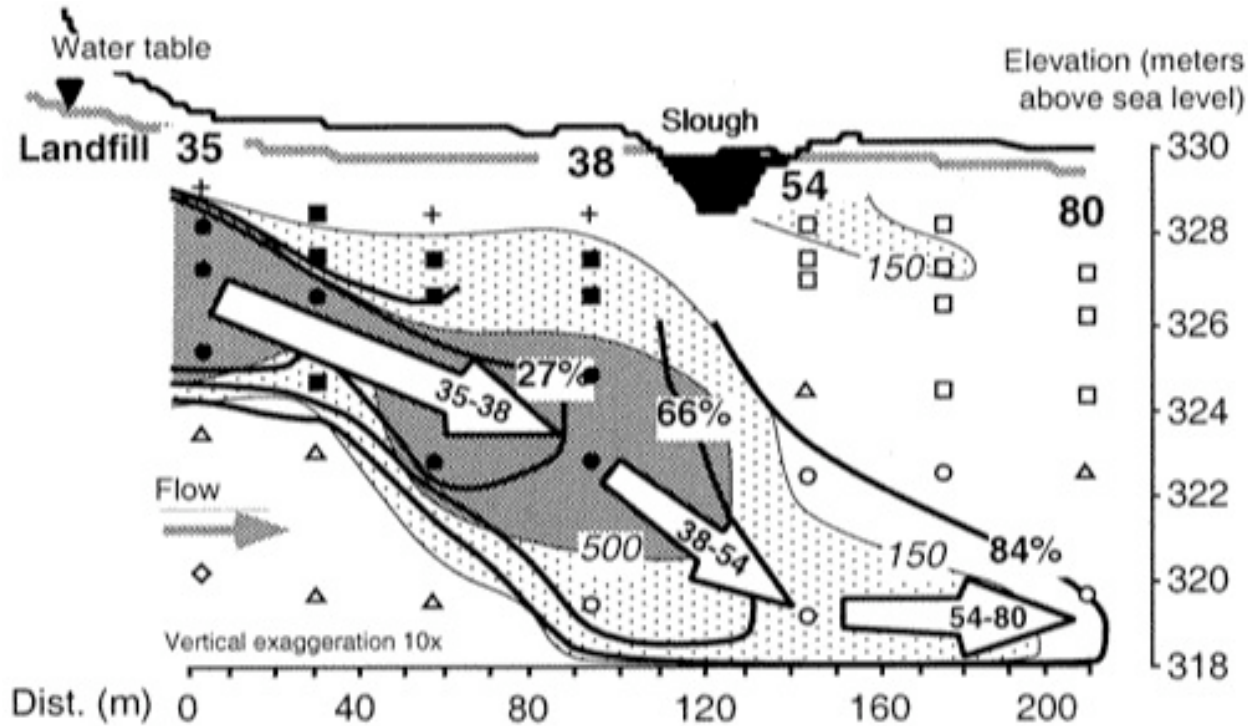


Suggested AOM at the Norman Landfill



Grossman et al. 2002 *Environ Sci Technol* ***36***, 2436-2442

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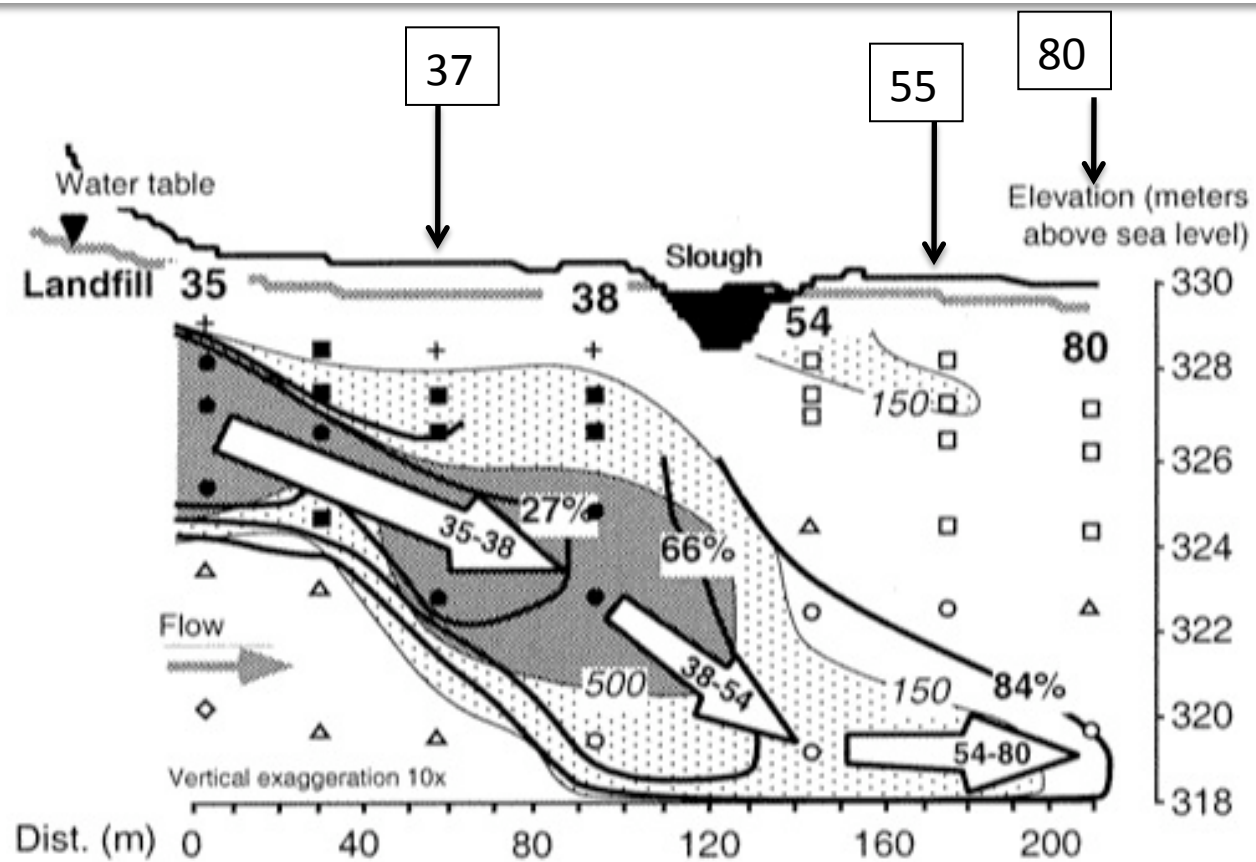

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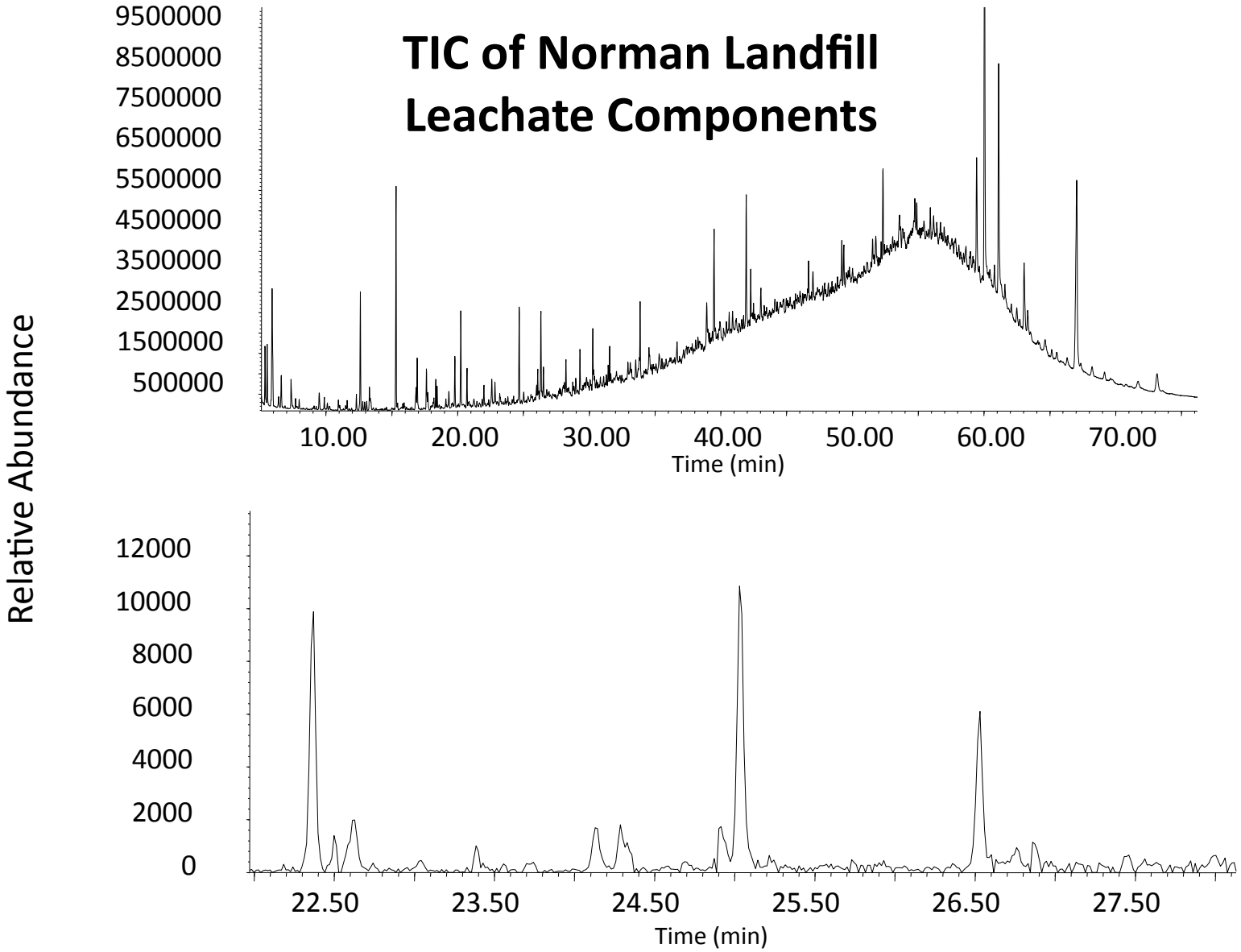
Interrogation of Landfill samples



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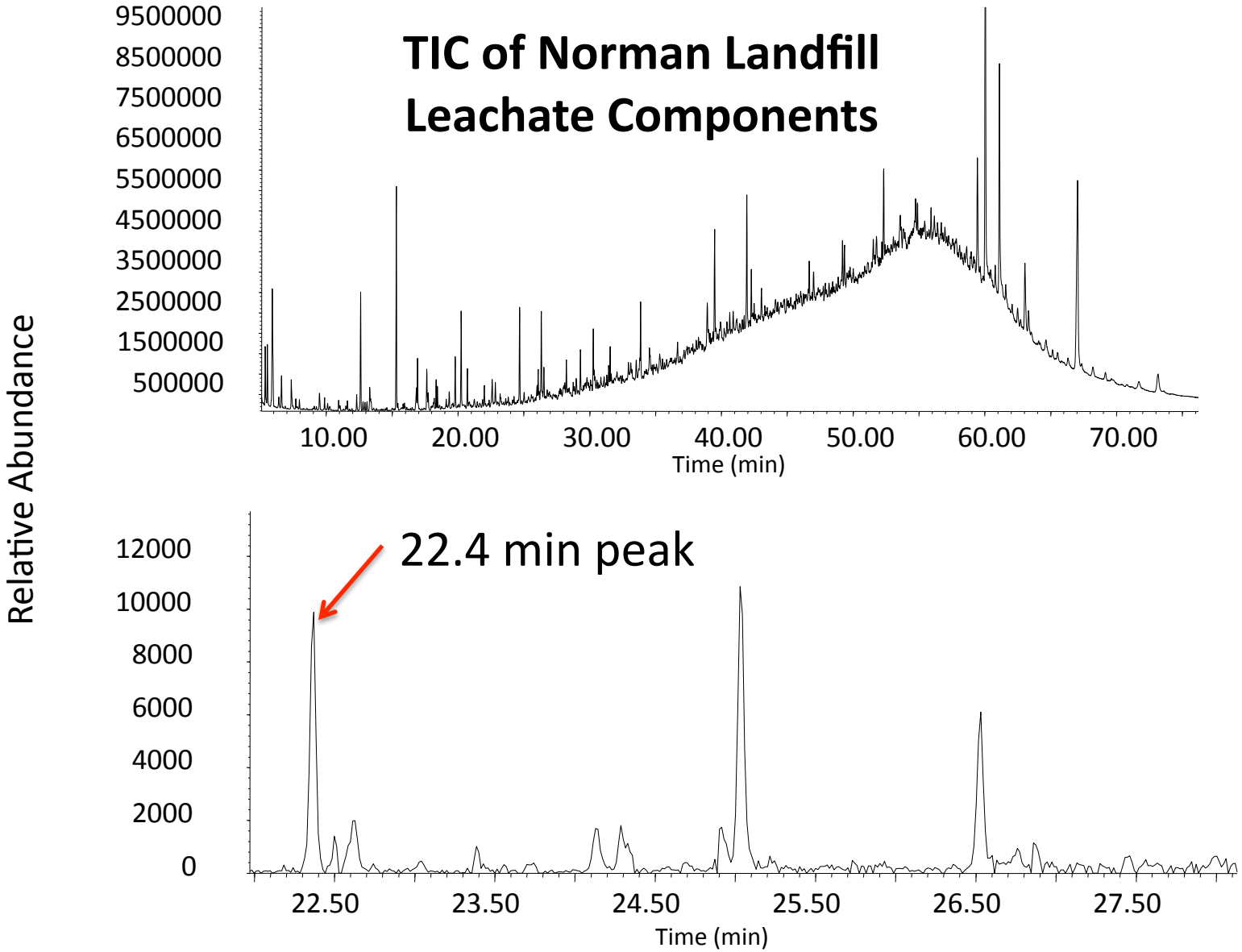

Expected mass spectral profile of methylsuccinate at 22.40 min

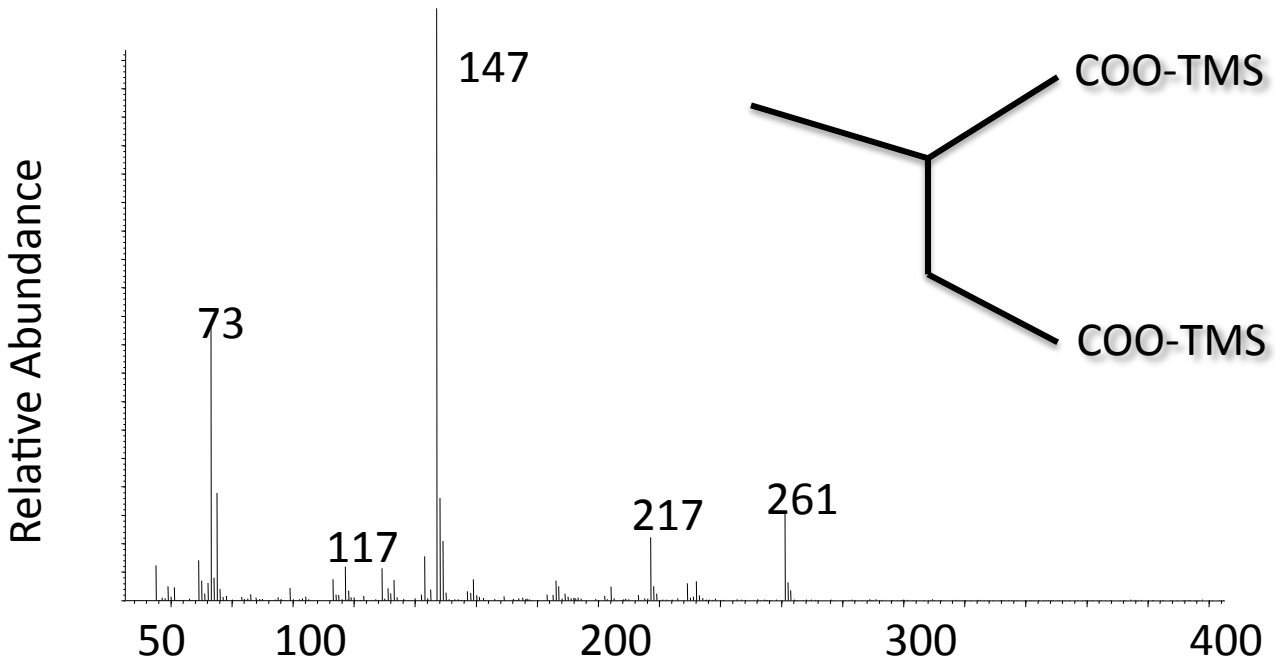

TABLE 1. Standard Free Energies of Reactions between Methane and Environmentally Relevant Electron Acceptors

reaction	ΔG_r^0 (kJ mol^{-1} CH_4) $CH_{4\,(g)}$	$CH_{4\,(aq)}$
$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$	−16.6	−33.0
$CH_4 + SO_4^{2-} + 2H^+ \rightarrow CO_2 + H_2S + 2H_2O$	−92.8	−109.2
$CH_4 + 2O_2 \rightarrow HCO_3^- + H_2O + H^+$	−806.0	−822.4
$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	−842.3	−858.7
$CH_4 + 4NO_3^- \rightarrow HCO_3^- + 4NO_2^- + H^+ + H_2O$	−467.0	−483.4
$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O$	−503.4	−519.8
$5CH_4 + 8MnO_4^- + 19H^+ \rightarrow 5HCO_3^- + 8Mn^{2+} + 17H_2O$	−991.7	−1008.1
$5CH_4 + 8MnO_4^- + 24H^+ \rightarrow 5CO_2 + 8Mn^{2+} + 22H_2O$	−1028.1	−1044.5
$CH_4 + 8Fe^{3+} + 3H_2O \rightarrow HCO_3^- + 8Fe^{2+} + 9H^+$	−418.3	−434.7
$CH_4 + 8Fe^{3+} + 2H_2O \rightarrow CO_2 + 8Fe^{2+} + 8H^+$	−454.6	−471.0
$CH_4 + ClO_4^- \rightarrow HCO_3^- + Cl^- + H^+ + H_2O$	−895.9	−912.3
$CH_4 + ClO_4^- \rightarrow CO_2 + Cl^- + 2H_2O$	−932.2	−948.6
$CH_4 + 4HAsO_4^{2-} + 3H^+ \rightarrow HCO_3^- + 4H_2AsO_3^- + H_2O$	−299.6	−316.0
$CH_4 + 4HAsO_4^{2-} + 4H^+ \rightarrow CO_2 + 4H_2AsO_3^- + 2H_2O$	−263.3	−279.7

Caldwell *et al.,* 2008 *EST*

Is fumarate addition thermodynamically favorable?

- Reverse methanogenesis
  CH_4 + CoM-S-S-CoB ⇒ CoM-S-CH_3 + HS-CoB
  ΔG°´ or ΔG´ = **+30 kJ mol^{-1}**
  (Widdel et al., 2007)

- Addition of fumarate (hypothetical)
  CH_4 + $^-$OOC-CH=CH-COO$^-$ ⇒ $^-$OOC-CH_2-[CH_3]CH-COO$^-$
  ΔG°´ or ΔG´ = **-27 to -31 kJ mol^{-1}** (Widdel et al., 2007)

Summary

- AOM has been shown to occur in terrestrial environments
- Alternate mechanism may be responsible for AOM
- The Norman Landfill has the potential to serve as a model site for elucidating AOM
  - Global biogeochemistry
  - Mitigation of anthropogenic methane releases