30 Years of Anaerobic Benzene Biodegradation

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Benzene - C_6H_6 – and most petroleum hydrocarbons are in theory good electron donors for microbes

Electron Acceptor (ox/red)	Overall Energetic Equation	ΔG°' kJ/mol
O_2/H_2O	$C_6H_6 + 7.5\ O_2 + 3\ H_2O \rightarrow 6\ HCO_3^- + 6\ H^+$	-3180
NO_3^-/N_2	$C_6H_6 + 6\ NO_3^- \rightarrow 6\ HCO_3^- + 3\ N_2$	-2990
Fe^{3+}/Fe^{2+}	$C_6H_6 + 90\ Fe(OH)_{3\ (s)} \rightarrow 6\ HCO_3^- + 132\ H_2O + 30\ Fe_3O_{4\ (s)} + 6\ H^+$	-2660
SO_4^{2-}/H_2S	$C_6H_6 + 3\ H_2O + 3.75\ SO_4^{2-} \rightarrow$ $6\ HCO_3^- + 1.88\ H_2S + 1.88\ HS^- + 0.38\ H^+$	-200
CO_2/CH_4	Benzene: $C_6H_6 + 4.5\ H_2O \rightarrow 3.75\ CH_4 + 2.25\ CO_2$	-62

Microbial Biodegradation and Chemical Evolution of Oil from the Amoco Spill

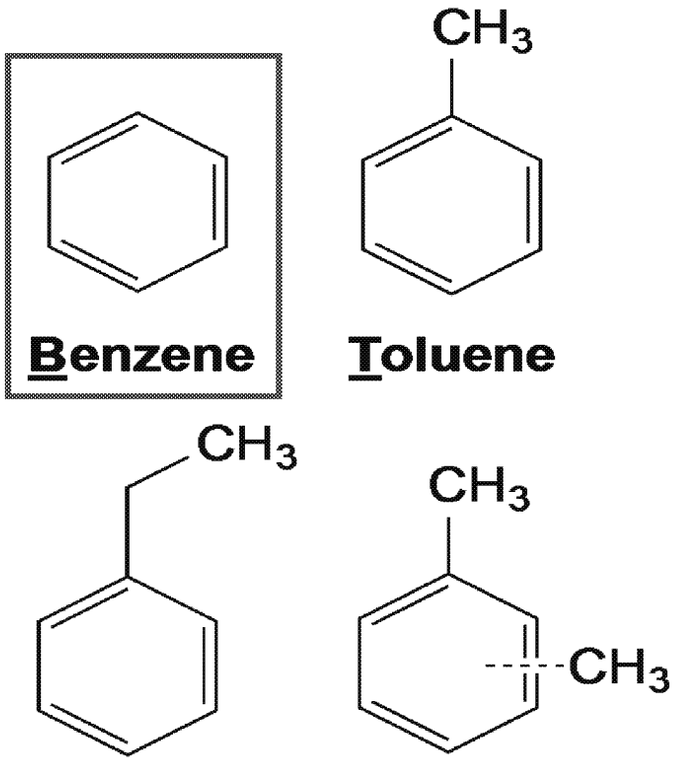
BY DAVID M WARD, RONALD M ATLAS, PAUL D BOEHM, JOHN A CALDER

1980

Ambio, 9(6), 277–283.

AMOCO CADIZ

Microorganisms played a role in the degradation or "weathering" of the oil from the Amoco Cadiz. After the spill, bacteria developed naturally to remove some but not all of the components of the oil. The effectiveness of microbial degradation, varies because of the complexity of the oil and the differing microbial responses in aerobic and anaerobic environments.



BTEX Compounds

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1986, p. 200–202 Vol. 52, No. 1
0099-2240/86/070200-03$02.00/0
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Incorporation of Oxygen from Water into Toluene and Benzene during Anaerobic Fermentative Transformation

1986

TIMOTHY M. VOGEL AND DUNJA GRBIĆ-GALIĆ*

Environmental Engineering and Science, Department of Civil Engineering, Stanford University, Stanford, California 94305

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1987, p. 254–260 Vol. 53, No. 2
0099-2240/87/020254-07$02.00/0
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Transformation of Toluene and Benzene by Mixed Methanogenic Cultures

1987

DUNJA GRBIĆ-GALIĆ* AND TIMOTHY M. VOGEL

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1992, p. 2663–2666 Vol. 58, No. 8
0099-2240/92/082663-04$02.00/0
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Complete Mineralization of Benzene by Aquifer Microorganisms under Strictly Anaerobic Conditions

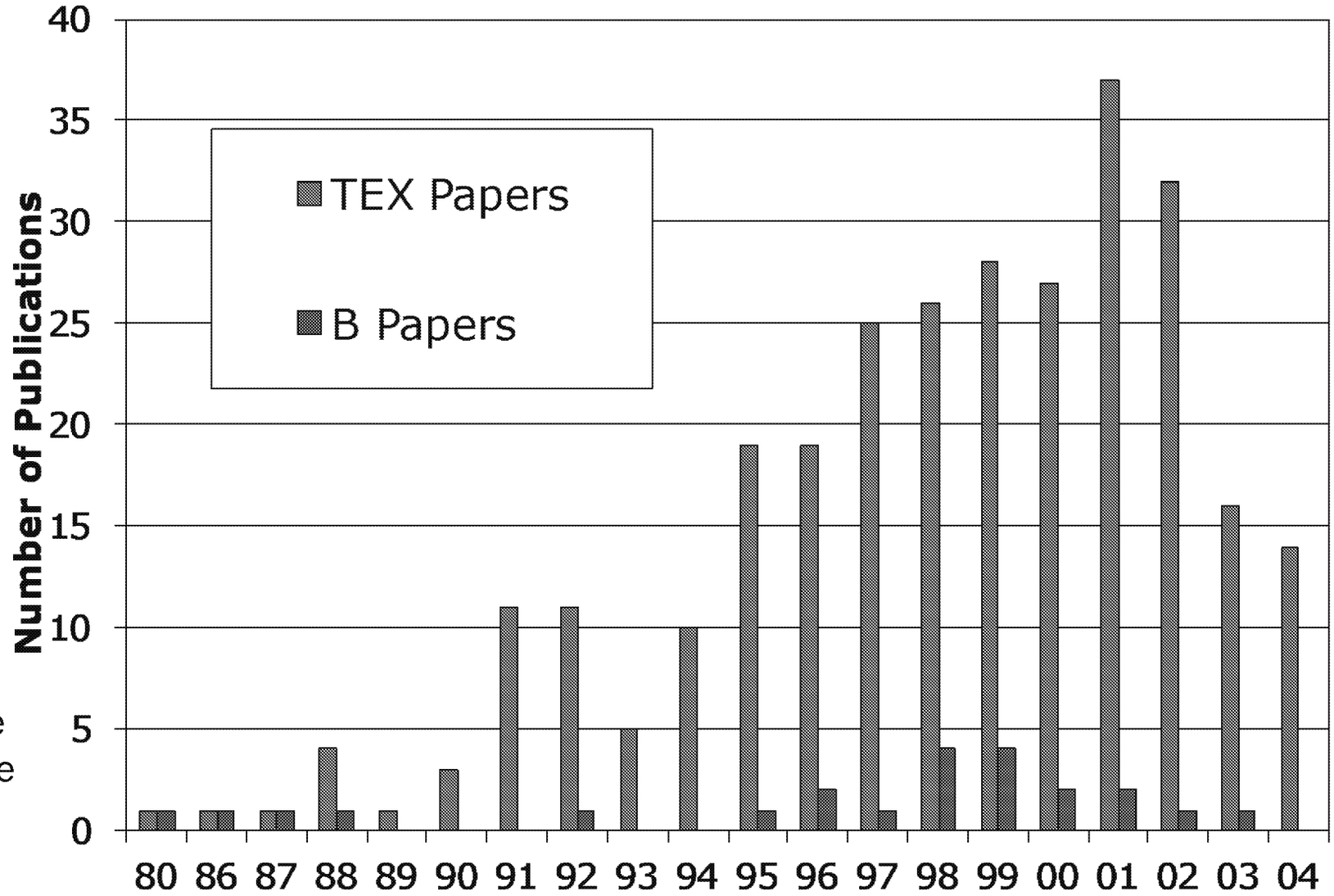
1992

ELIZABETH A. EDWARDS* AND DUNJA GRBIĆ-GALIĆ

Environmental Engineering and Science, Department of Civil Engineering, Stanford University, Stanford, California 94305-4020

ED_005025_00011617-00004

Anaerobic Publications



Presented at the ISME Conference 2004 in Cancun

microbial biotechnology

society for applied microbiology sfam

Microbial Biotechnology (2011) 4(6), 710–724 doi:10.1111/j.1751-7915.2011.00260.x

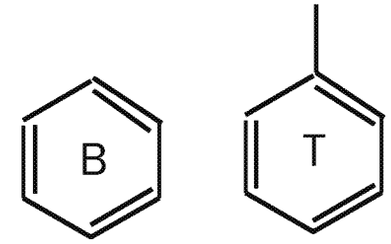
Minireview

Anaerobic benzene degradation by bacteria

Carsten Vogt,[1]* Sabine Kleinsteuber[2] and Hans-Hermann Richnow[1]
Departments of [1]Isotope Biogeochemistry and [2]Environmental Microbiology, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany.

As of 2011:
Identified 47 Anaerobic Benzene publications from 21 enrichments/cultures or sites... ever

Questions I had posed in 2004



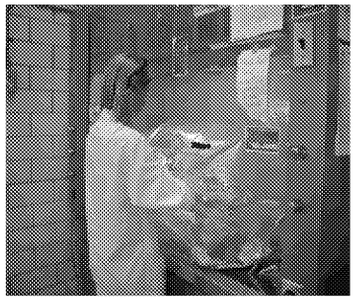
- Why is it so difficult to obtain cultures capable of anaerobic benzene degradation?

Benzene Thermodynamically ~equivalent to Toluene

  - Organisms/Processes don't exist?
  - Organisms uncommon? (kinetic barrier?)
  - Inappropriate laboratory enrichment and culturing technique?

Where we are now in 2018?

Benzene-Degrading Enrichment Cultures



Contaminated Site Material (1995)
(>20 Years of Enrichment in the Lab)

20+ years

Site 1

Swamp

Sw-NO_3

Site 2

Cartwright

Cart-NO_3

Cart-SO_4

Cart-CH_4

Site 3

Oil Refinery (OR)

OR-CH_4

Degrade: Benzene, Benzoate, Toluene, Phenol
Key Microbe: ***Peptococcaceae sp.***

Nitrate-reducing

Degrade: Benzene
Key Microbe**: δ-Proteobacterium "ORM2"**

Sulfate-reducing or methanogenic

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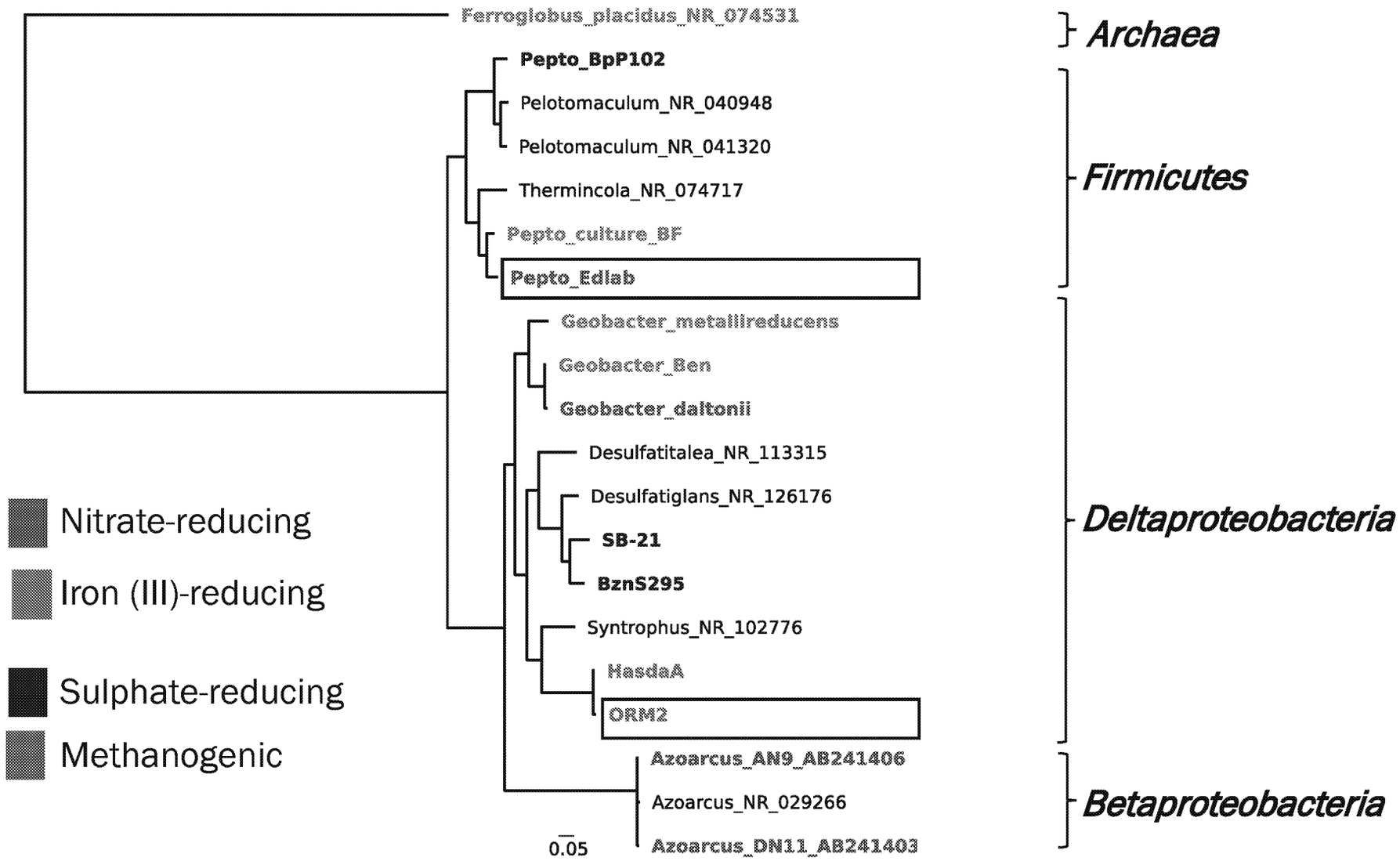
Known Anaerobic Benzene-Degraders



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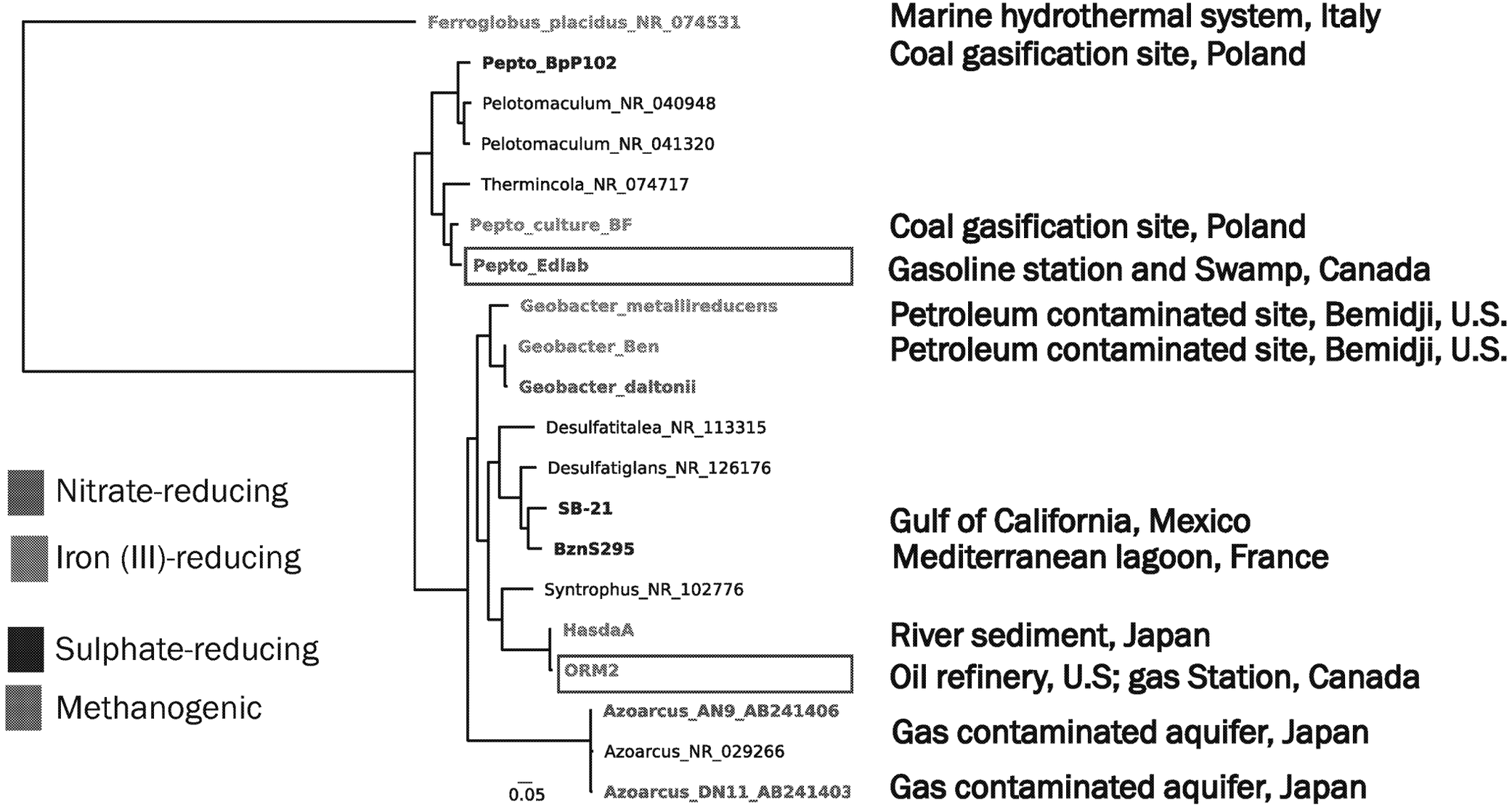
Known Anaerobic Benzene-Degraders



BTEX Degrading Enrichment Cultures: Rates

Enrichment Culture	Substrates	Main Organism	Rate (mg/L/day)	Doubling Time
OR-CH_4	Benzene/CO_2	***Deltaproteobacterium ORM2***	0.3-2.0	20-30 days
Cart-SO_4	Benzene/SO_4	***Deltaproteobacterium ORM2***	0.1-0.3	
Sw-NO_3	Benzene/NO_3	***Peptococcaceae sp.***	0.1-0.4	
Pen-CH_4	Toluene and o-Xylene/CO_2	***Pelotomaculum sp.***	>10	<10 days

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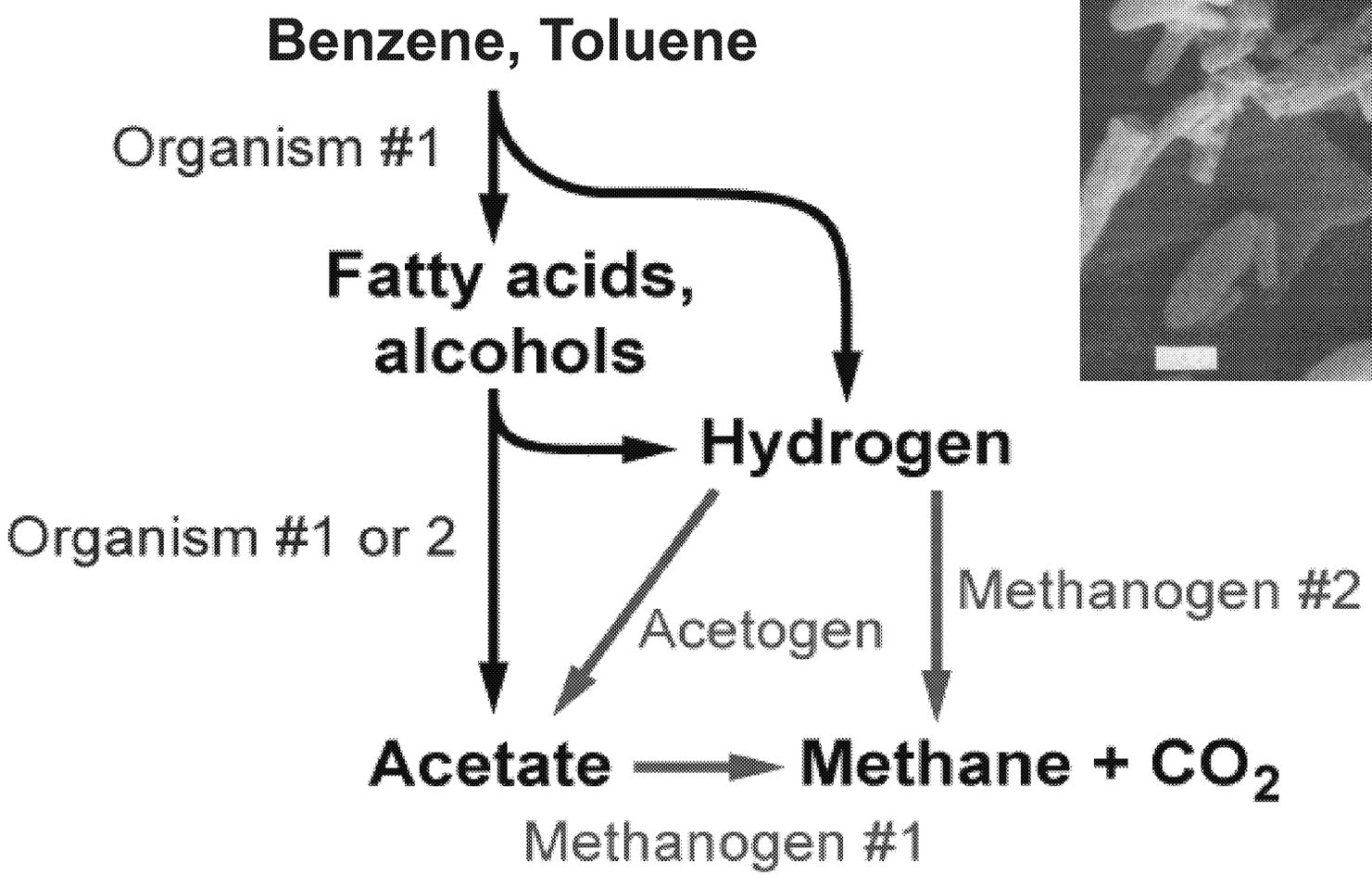
Concepts of *syntrophy* and *microbial consortium*

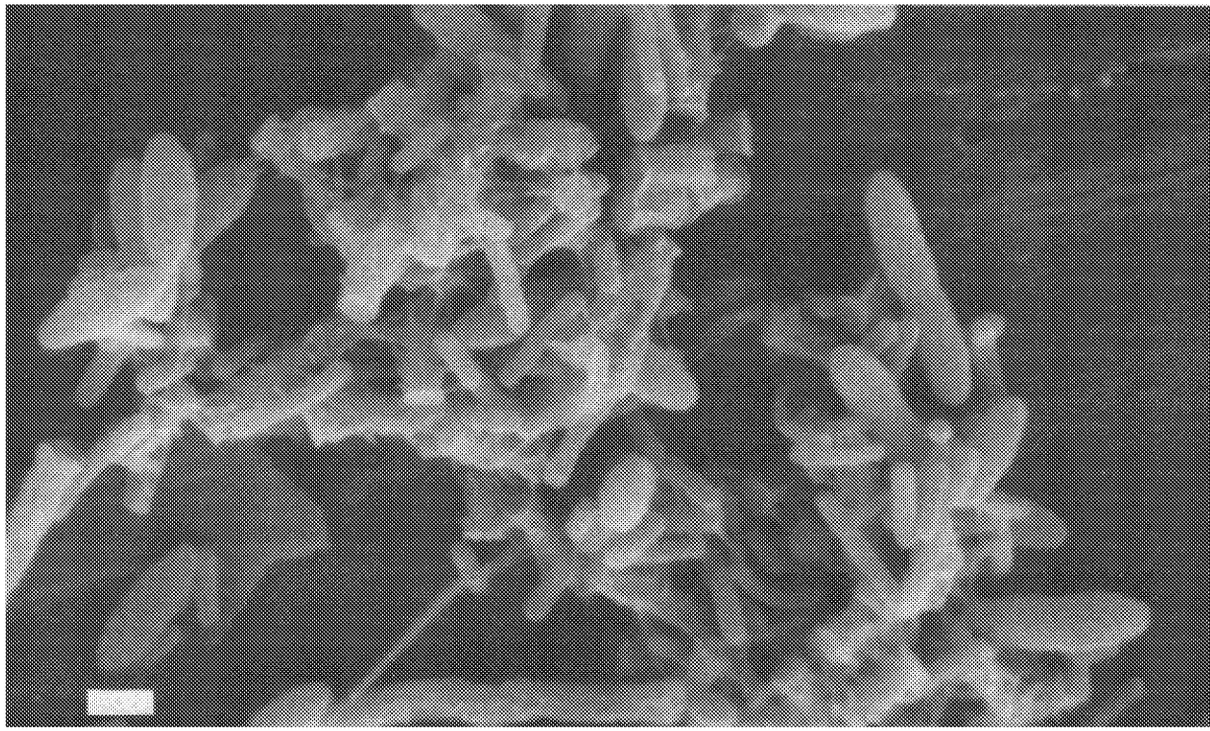
Example of benzene degradation under methanogenic conditions

	Reactants to Products		$\Delta G^{o\prime}$ Standard Conditions
	Fermentation reaction:		
Org. #1	C_6H_6 + 6 H_2O	--> 3 CH_3COOH + 3 H_2	153
	Benzene	Acetate Hydrogen	
	Methanogenic reactions:		
Org. #2	3 CH_3COOH	--> 3 CH_4 + 3 CO_2	-45
Org. #3	**3 H_2** + 0.75 CO_2	--> 0.75 CH_4 + 1.5 H_2O	-170
	Overall reaction		
Together	**C_6H_6 + 4.5 H_2O**	**--> 3.75 CH_4 + 2.25 CO_2**	**-62**

KJ per mole benzene

Microbial Consortia





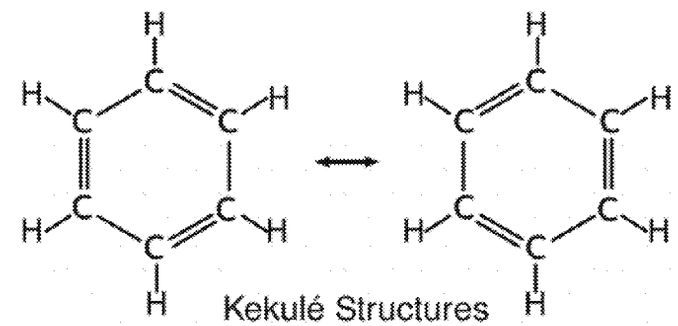
Hydrogen and Acetate concentrations must be kept low (by methanogens) to drive otherwise energetically unfavourable toluene fermentation reactions

Where we are now in 2018?

- Why is it so difficult to obtain cultures capable of anaerobic benzene degradation?

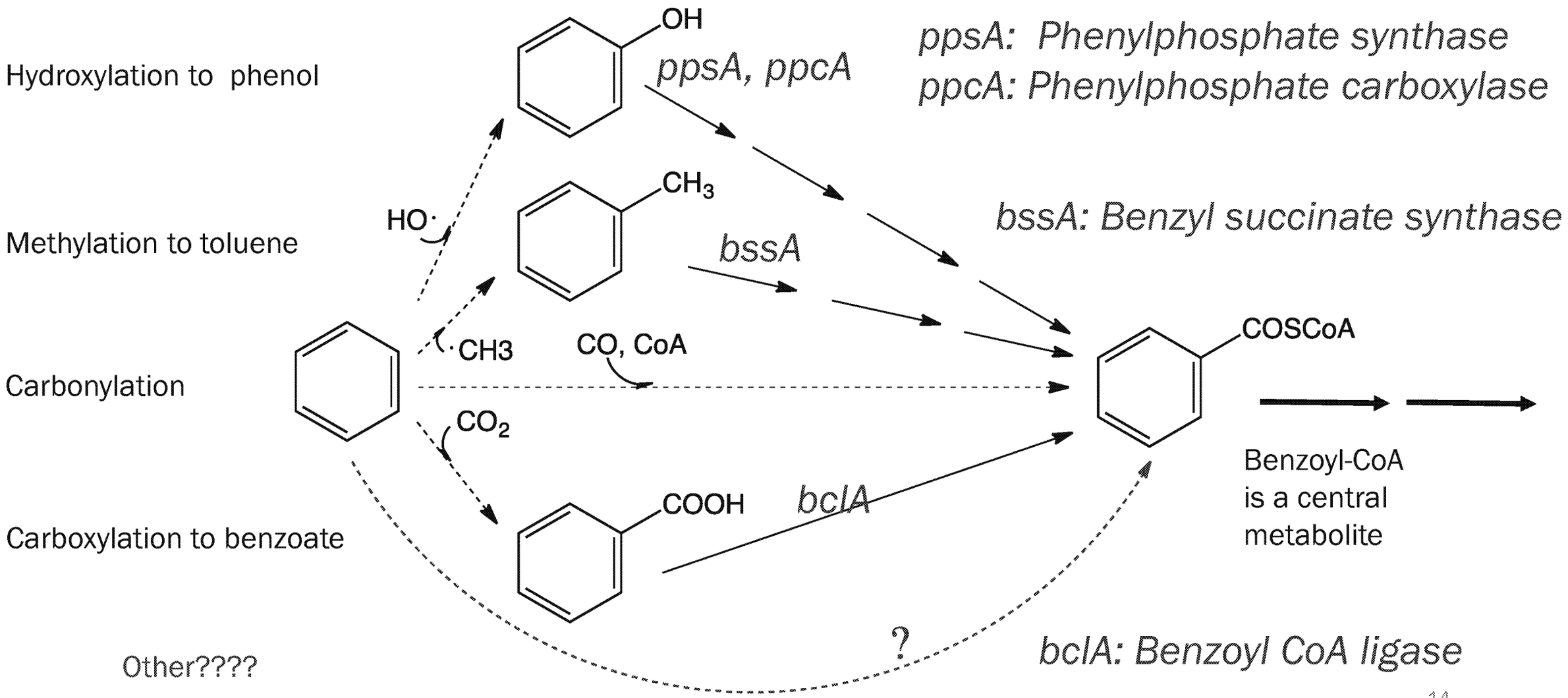
Benzene Thermodynamically ~equivalent to Toluene

- ~~Organisms/Processes don't exist?~~
- Organisms uncommon? (kinetic barrier?) **YES; low abundance**
- Inappropriate laboratory enrichment and culturing technique? **YES**



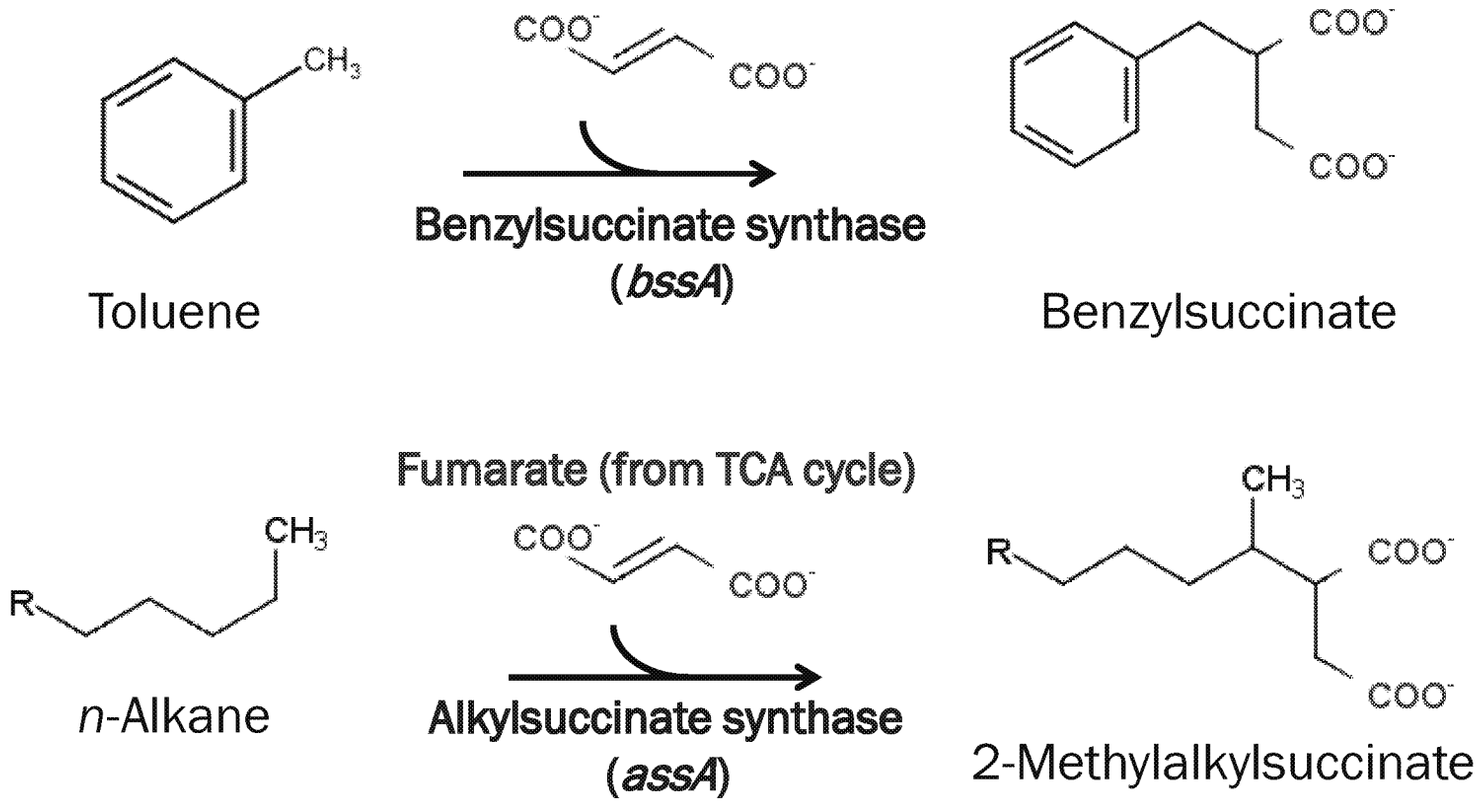
Kekulé Structures
(Isomers)

Elusive Anaerobic Benzene Activation Mechanism



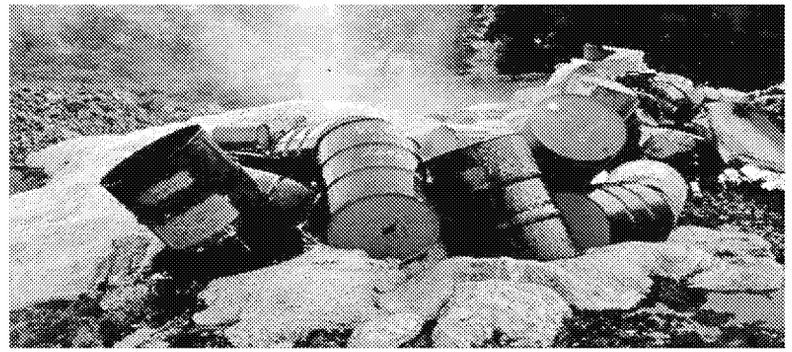
Introduction to Fumarate Addition

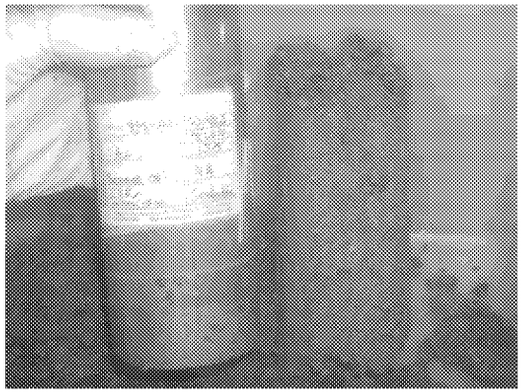
Alkylbenzenes (e.g., toluene) and saturates (e.g., alkanes, cycloalkanes) are activated via addition to fumarate under all anaerobic electron accepting conditions

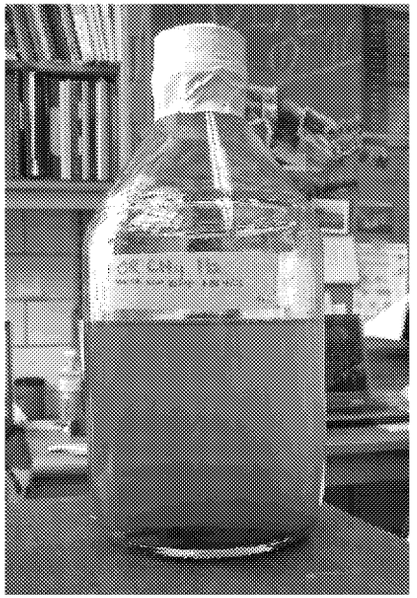


Slide courtesy of Courtney Toth

Let's look at our cultures more closely







1. Nitrate-Reducing Benzene degrading Culture

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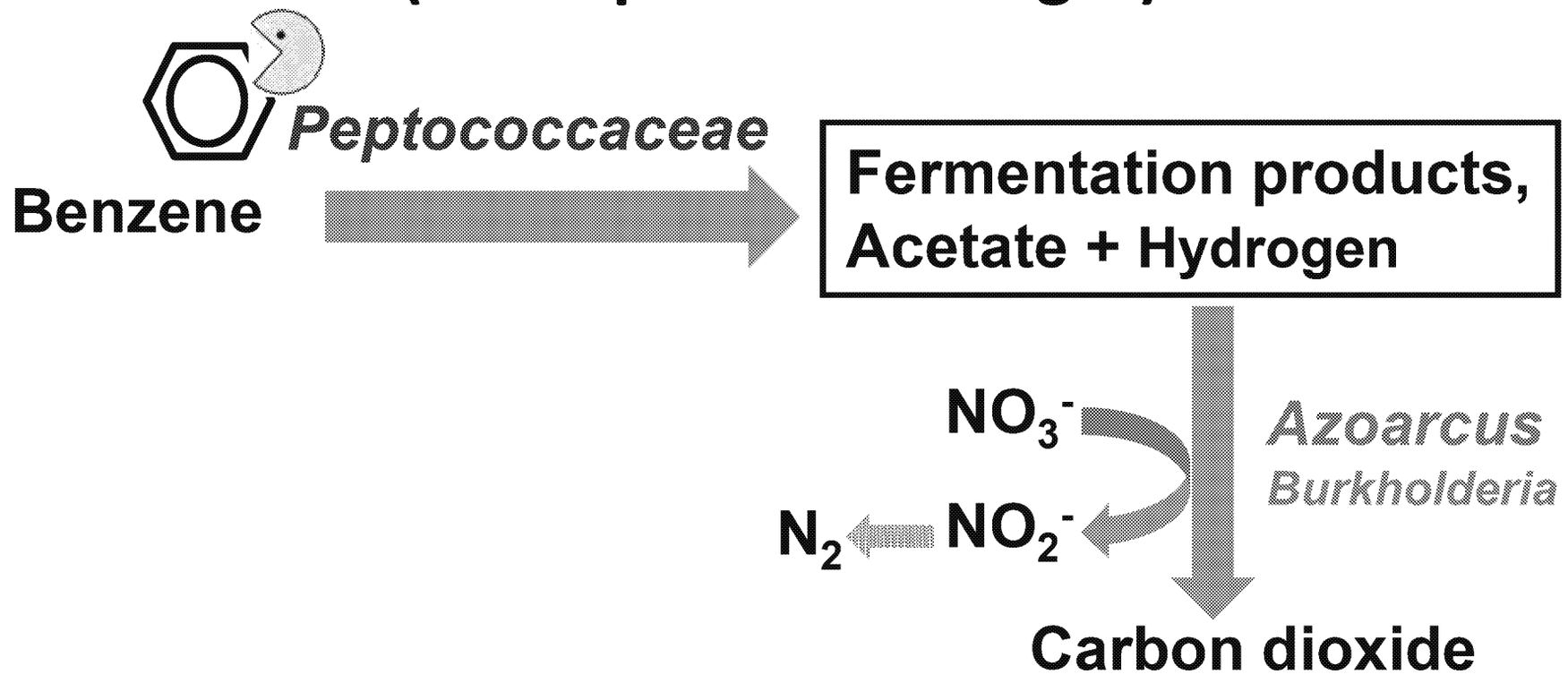
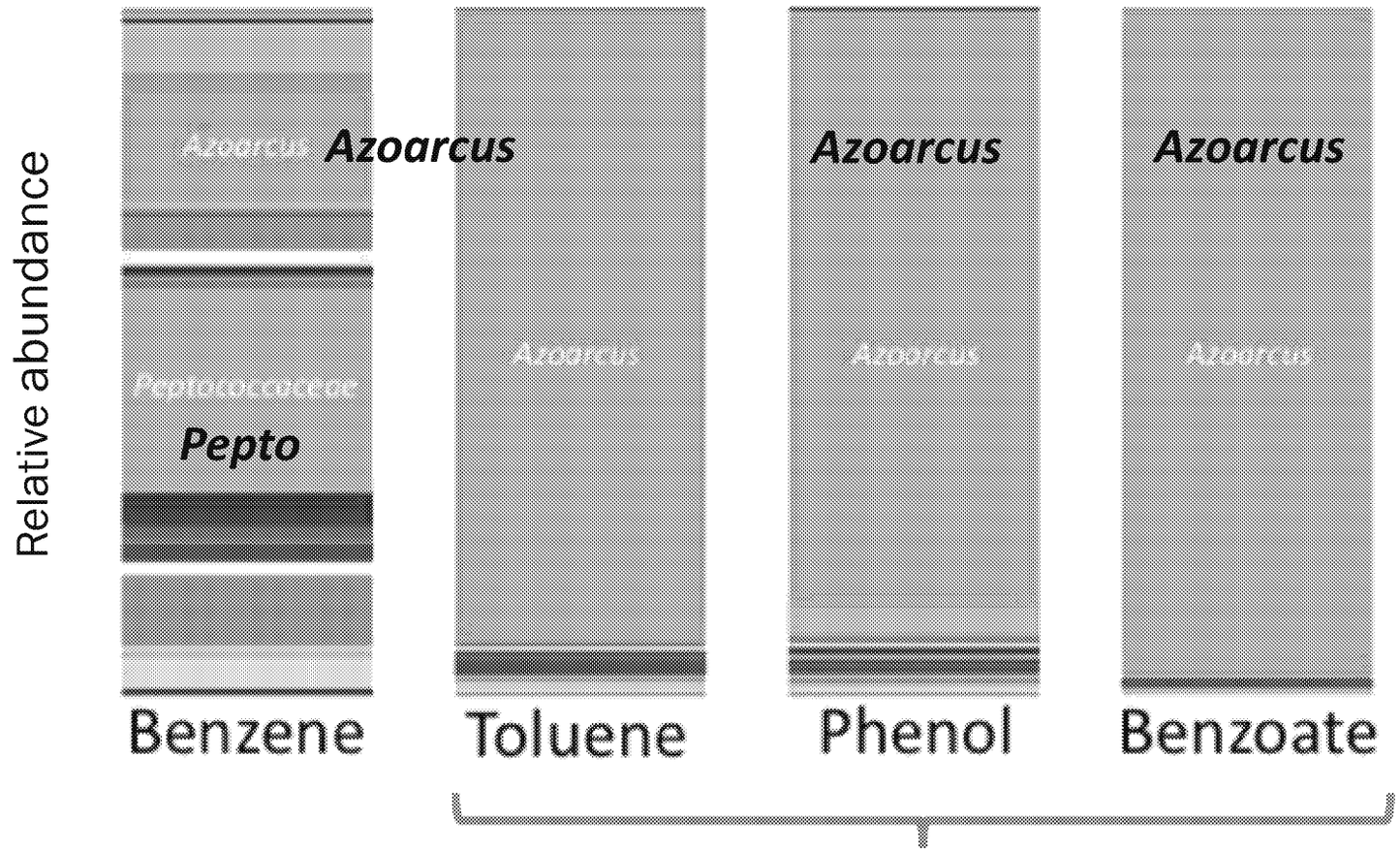
Syntrophy in nitrate-reducing enrichment cultures (Swamp and Cartwright)



Culture becomes dominated by *Azoarcus* if fed benzoate, toluene or phenol with nitrate



Based on Sequencing DNA

Anaerobic pathways clearly established in *Azoarcus*

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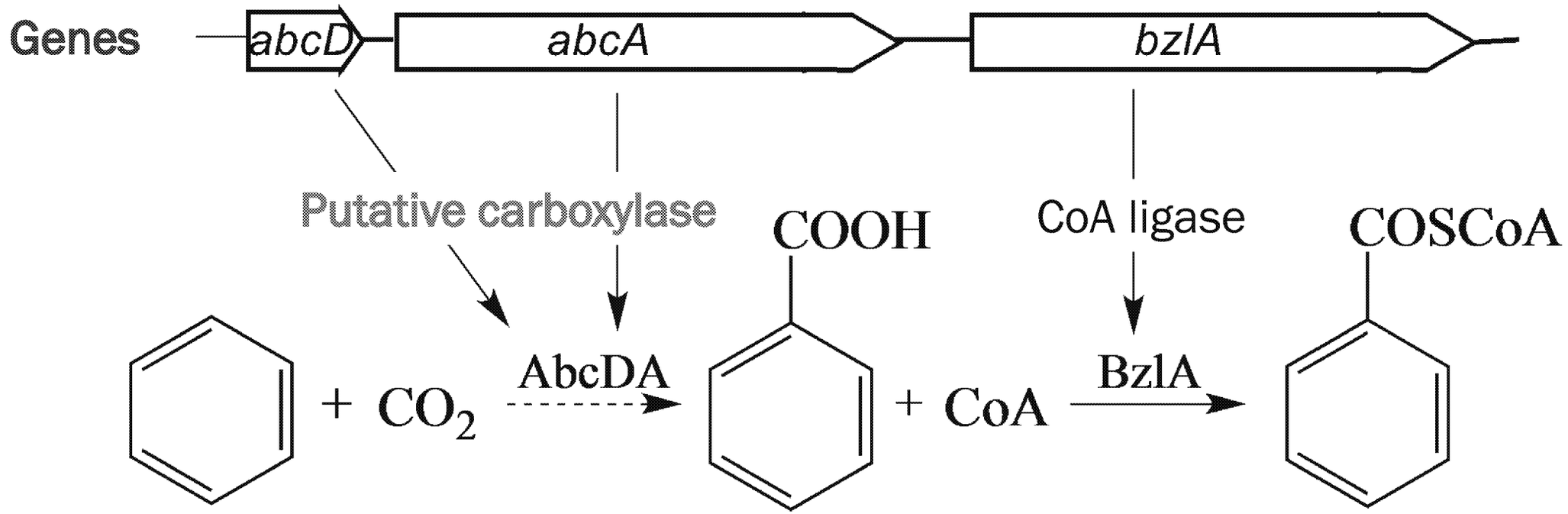
The anaerobic benzene carboxylase gene neighborhood in *Peptococcaceae*

DNA sequencing



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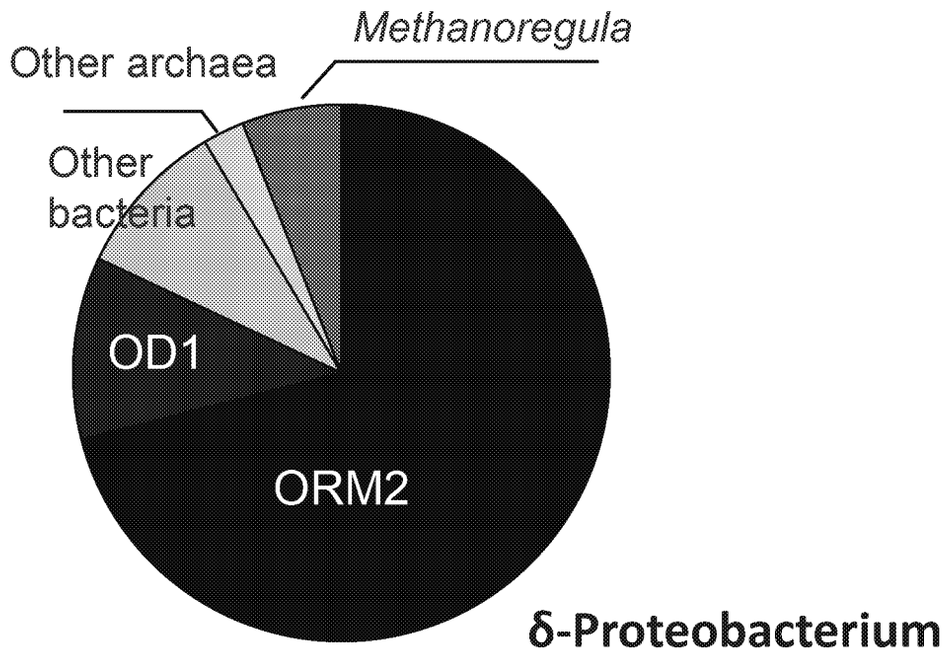
2. Methanogenic Benzene Degrading Culture

Microbial community composition quantified via metagenome analysis



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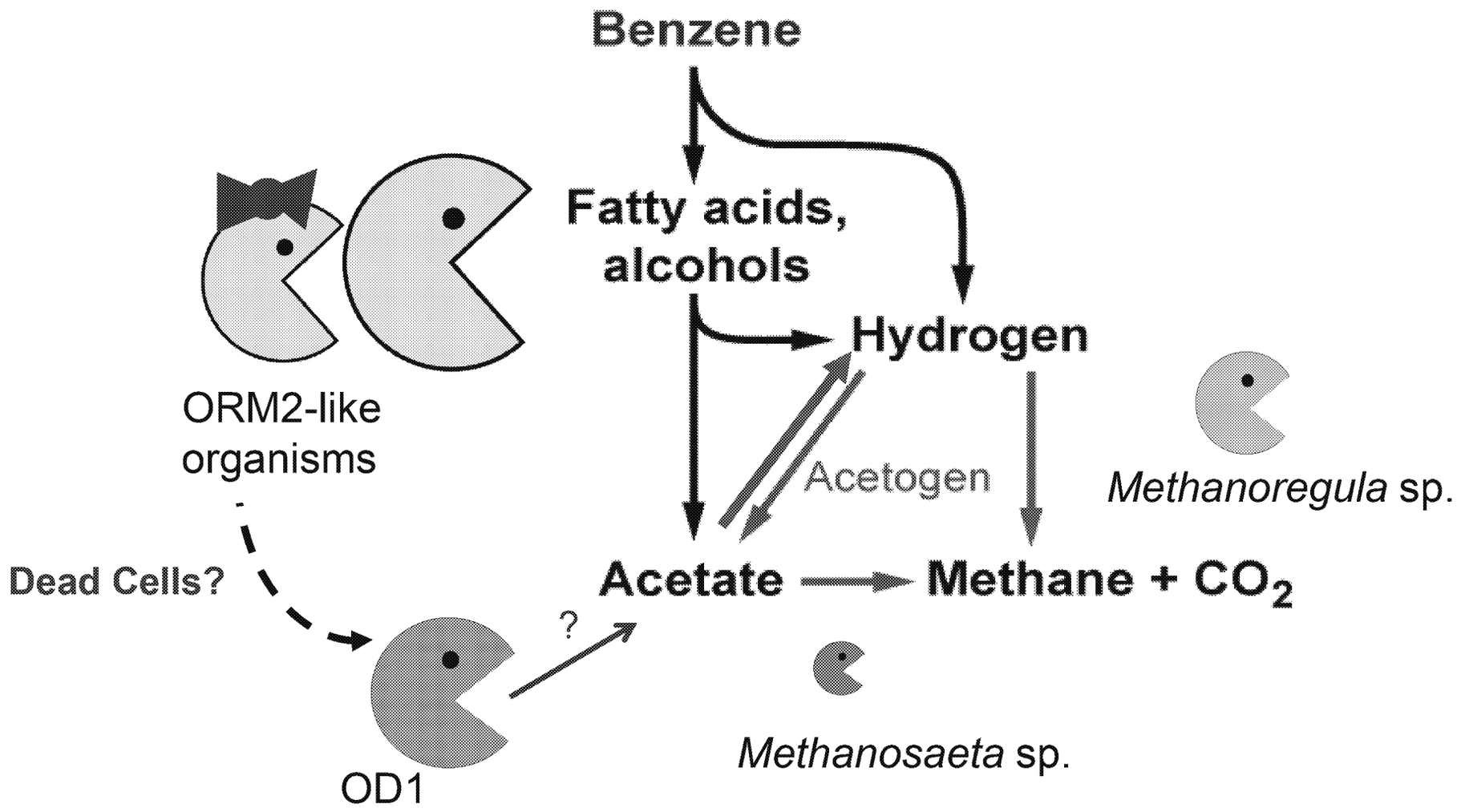
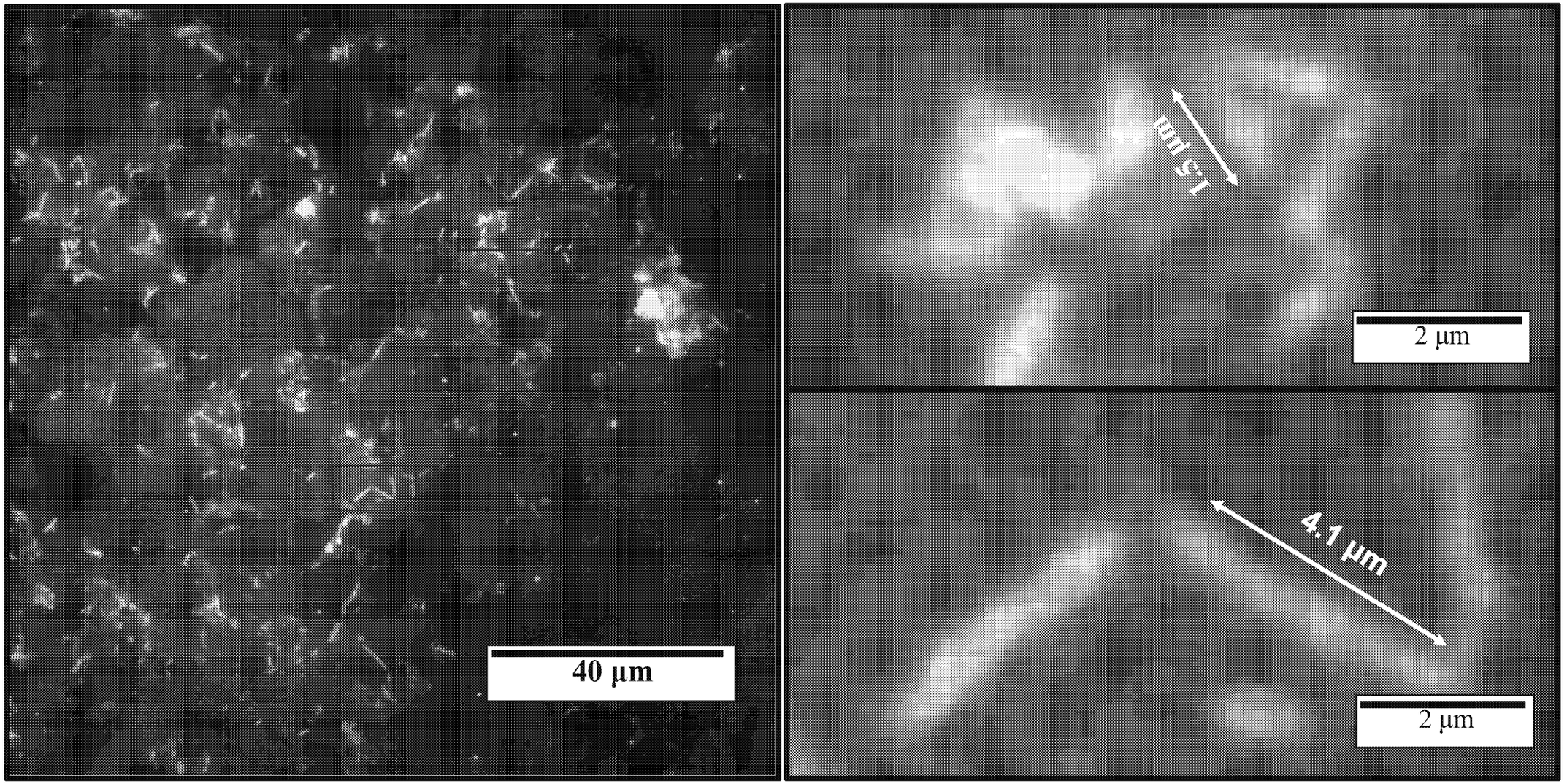
Syntrophy in methanogenic enrichment cultures



Fluorescent in situ Hybridization (FISH) Images of Deltaproteobacterium ORM2



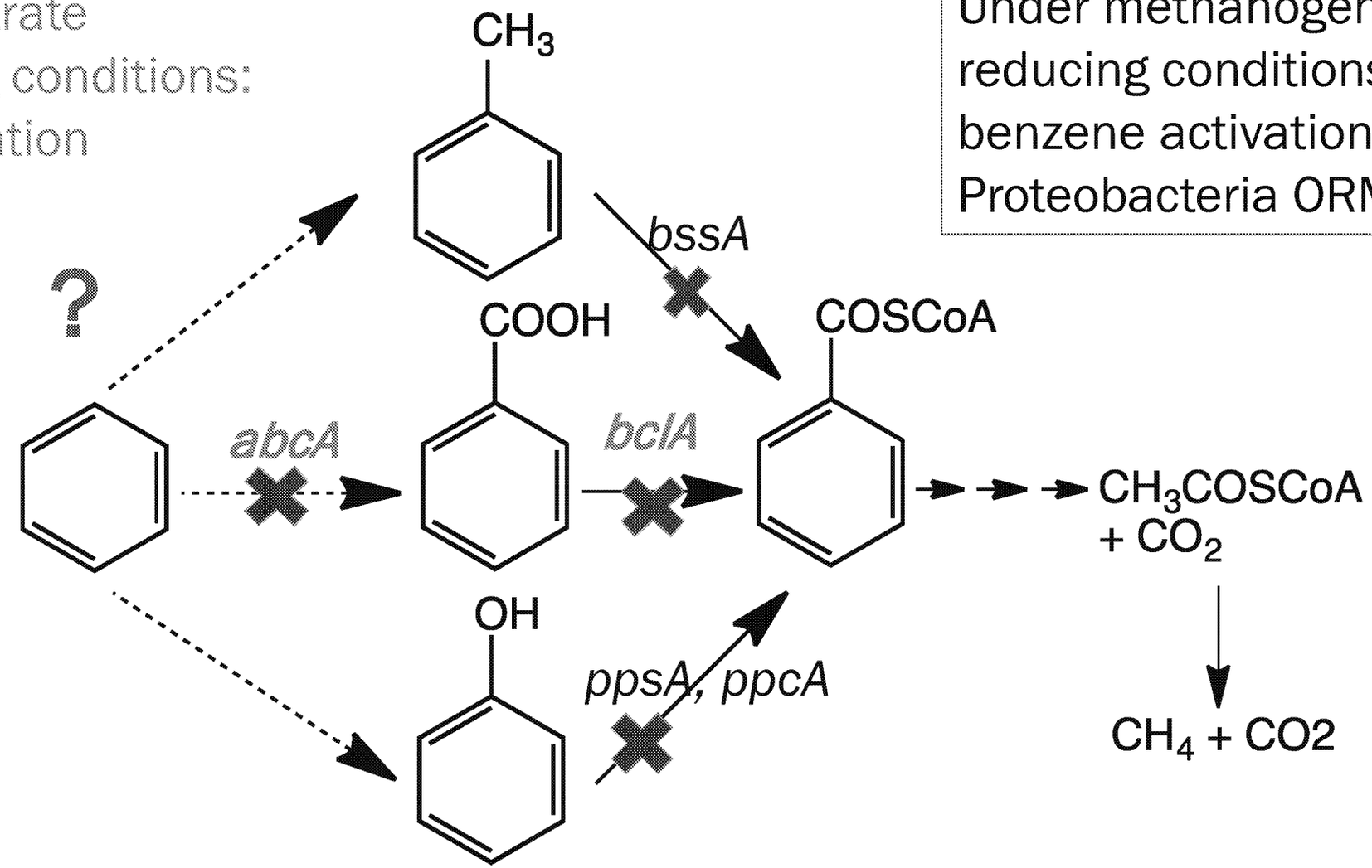
ORM2

ALL DNA

Anaerobic Benzene Activation Pathways

Under Nitrate Reducing conditions: Carboxylation

Under methanogenic & sulfate-reducing conditions: Mechanism for benzene activation by δ-Proteobacteria ORM2 is still unknown



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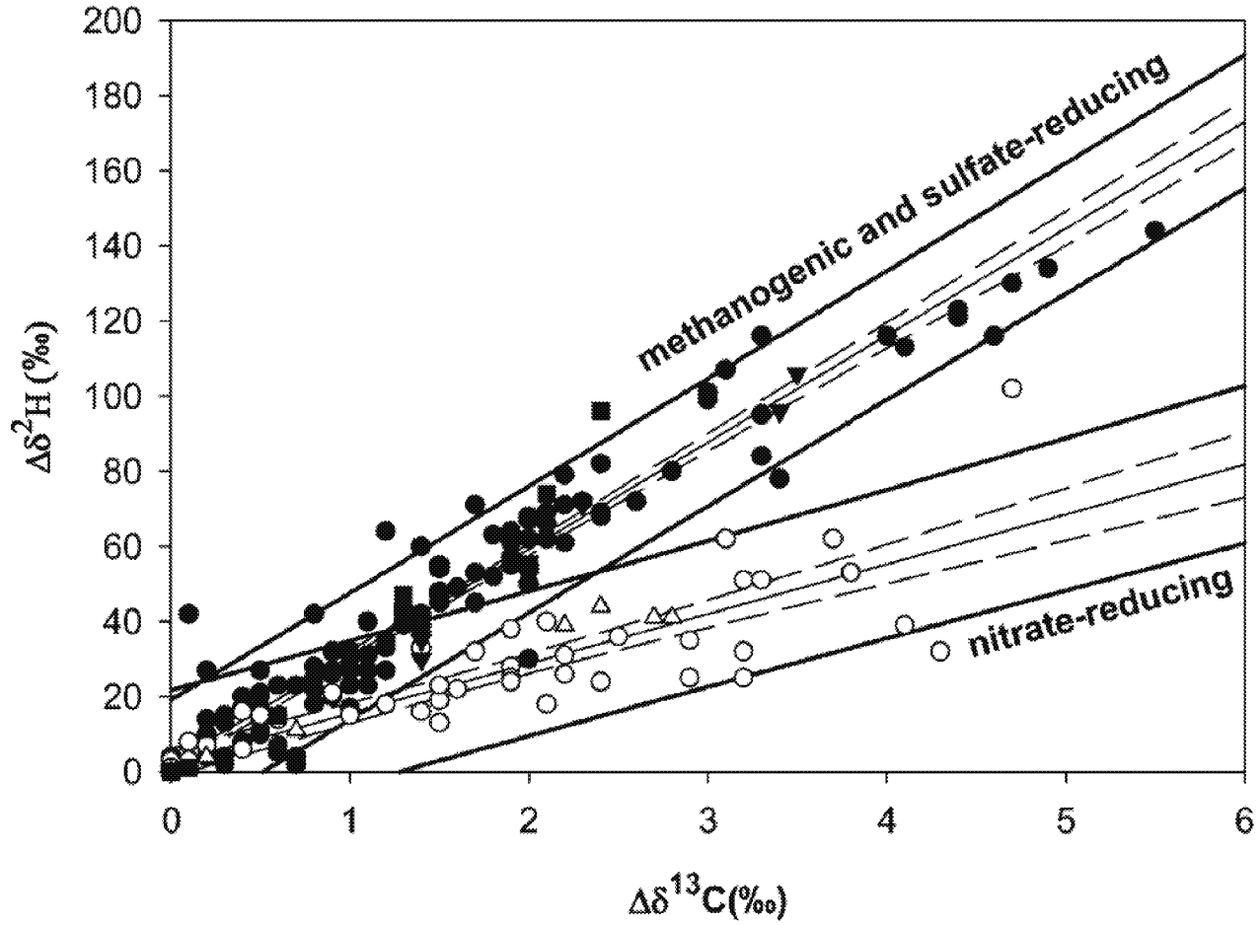
2008 - Dual Isotope Plot Suggested two Mechanisms



Mancini, S. A., Devine, C. E., Elsner, M., Nandi, M. E., Ulrich, A. C., Edwards, E. A., & Sherwood Lollar, B. (2008). Isotopic Evidence Suggests **Different Initial Reaction Mechanisms for Anaerobic Benzene Biodegradation**. *Environmental Science & Technology*, *42*(22), 8290–8296.

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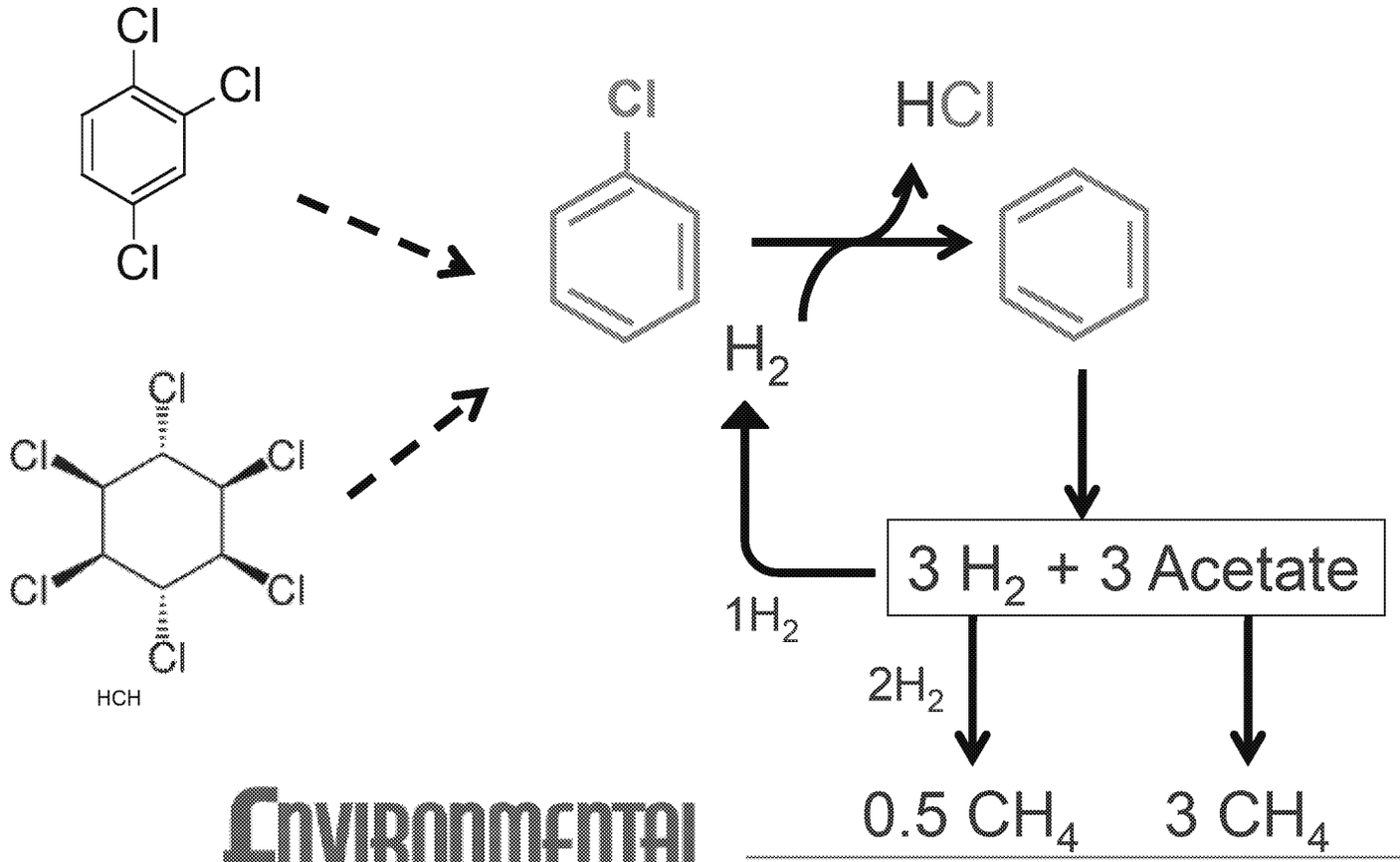
Monochlorobenzene to CH_4 and CO_2



Xiaoming Liang

Environmental Science & Technology

Article

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Anaerobic Conversion of Chlorobenzene and Benzene to CH_4 and CO_2 in Bioaugmented Microcosms

Xiaoming Liang,[†] Cheryl E. Devine,[†] Jennifer Nelson,[‡] Barbara Sherwood Lollar,[§] Stephen Zinder,[‡] and Elizabeth A. Edwards[†,*]

Conclusions

- **Anaerobic benzene** degradation appears limited to a few groups of organisms that we can now track. Could these organisms also be **specialists** (like *Dehalococcoides*) offering hope for bioaugmentation?
- *Peptococcaceae*-containing benzene-degrading cultures appear specific to slightly higher redox like nitrate-reducing & iron-reducing conditions. Initial step likely a carboxylation.
- *Deltaproteobacteria* (ORM2)-dominated cultures grow well under both under methanogenic and sulfate-reducing conditions, yet pathways for anaerobic benzene oxidation are still elusive.
- **Both groups** can be found in samples from field sites and are linked to increased rates of benzene transformation in the absence of oxygen.

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Benzene Research team (past and current):
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SiREM
Leading Science · Lasting Solutions

Sandra Dworatzek, Jennifer Webb, Jennifer Wilkinson, Jeff Roberts, Phil Dennis and others

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Thank you!

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