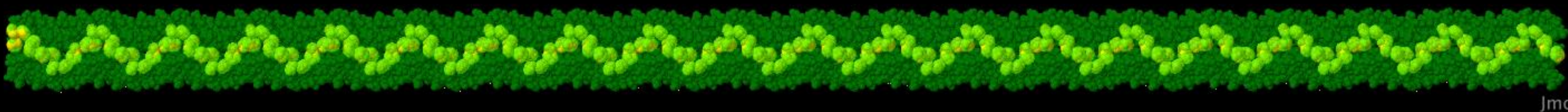
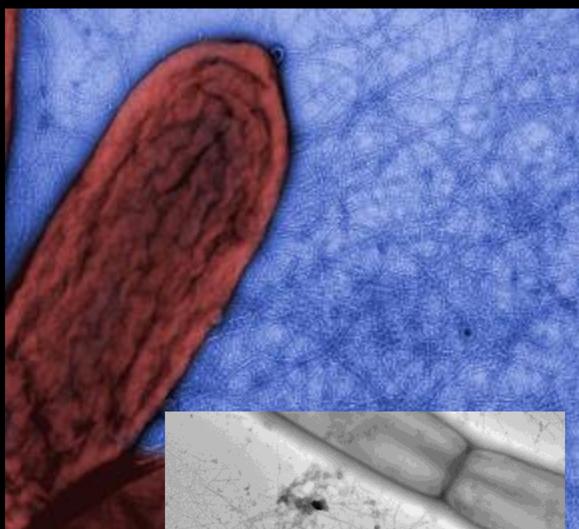
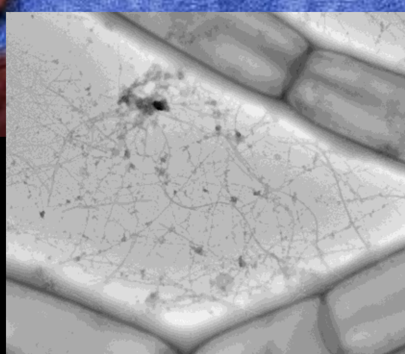
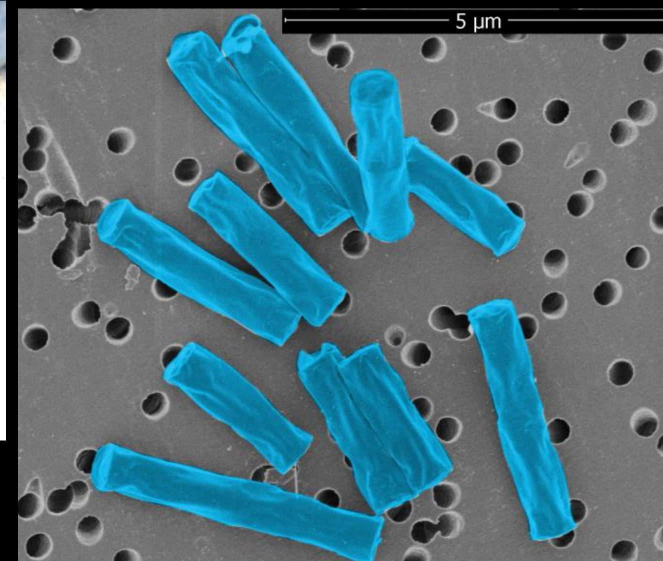
Electrobiocommodities from Carbon Dioxide: Enhancing Microbial Electrosynthesis with Synthetic Electromicrobiology and System Design









Derek Lovley

DOE Wastewater Workshop

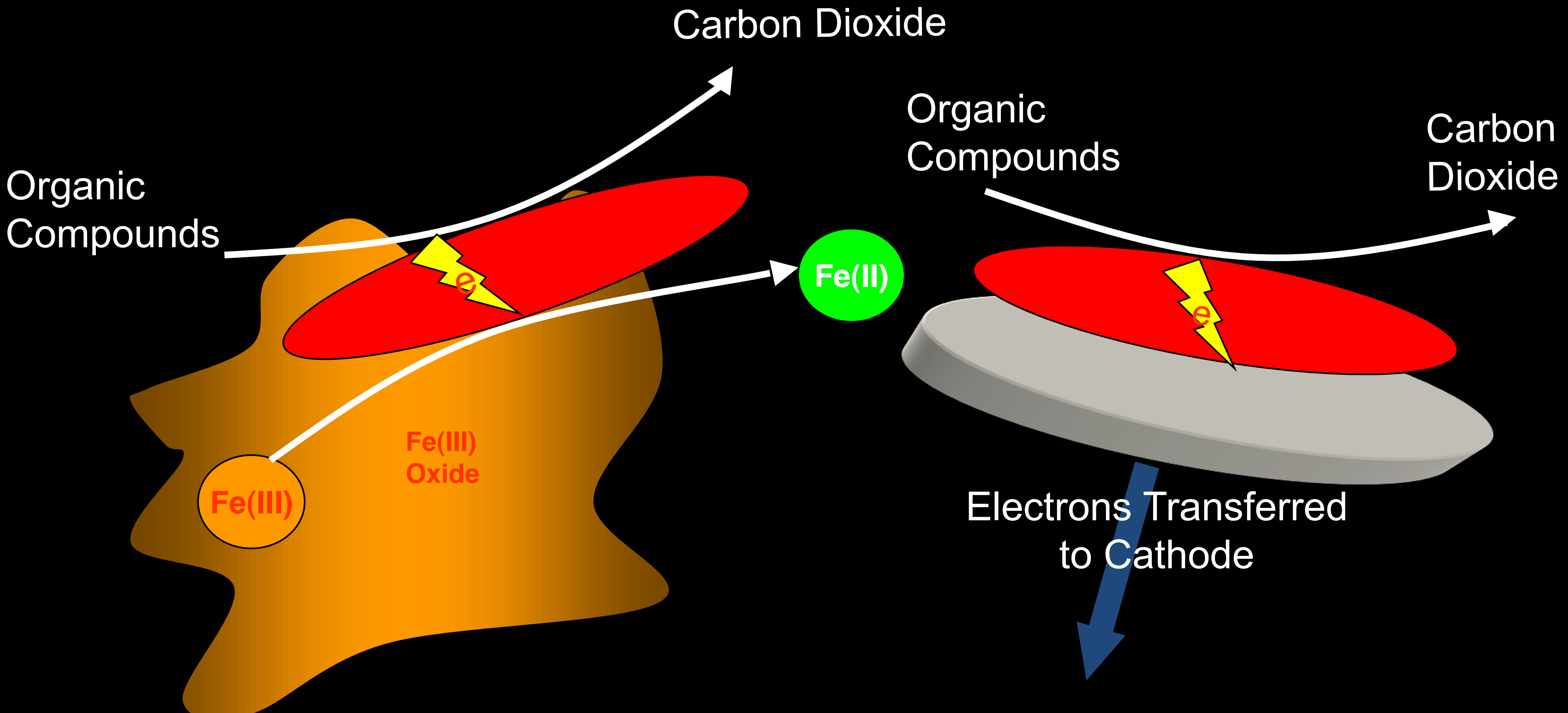
March 19, 2015

Geobacter Project

www.geobacter.org

ElectroFuels.org

Geobacter Species can Substitute Electrodes for Fe(III) as a Terminal Electron Acceptor

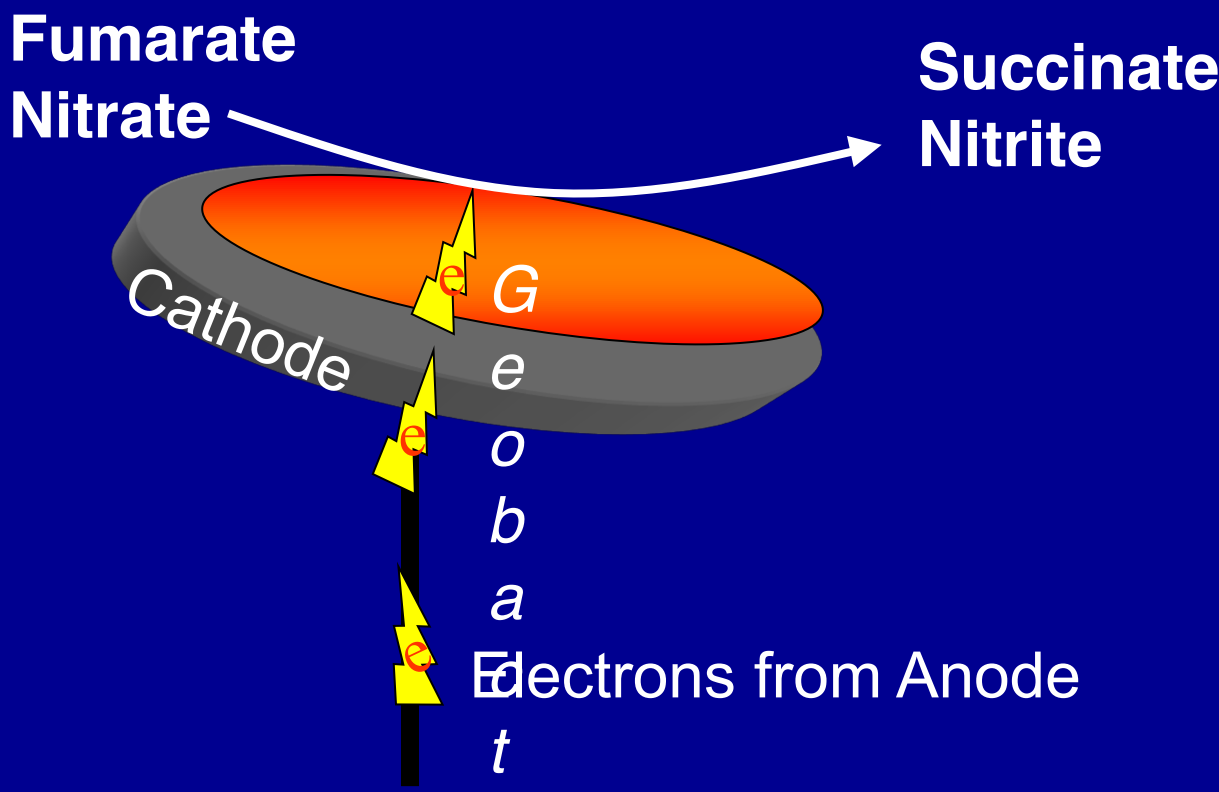


Lovley, D. R., J. F. Stolz, G. L. Nord, and E. J. P. Phillips. 1987. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. Nature 330:252-254.

Bond, D. R., D. E. Holmes, L. M. Tender, and D. R. Lovley. 2002. Electrode-reducing microorganisms harvesting energy from marine sediments. Science 295:483-485.

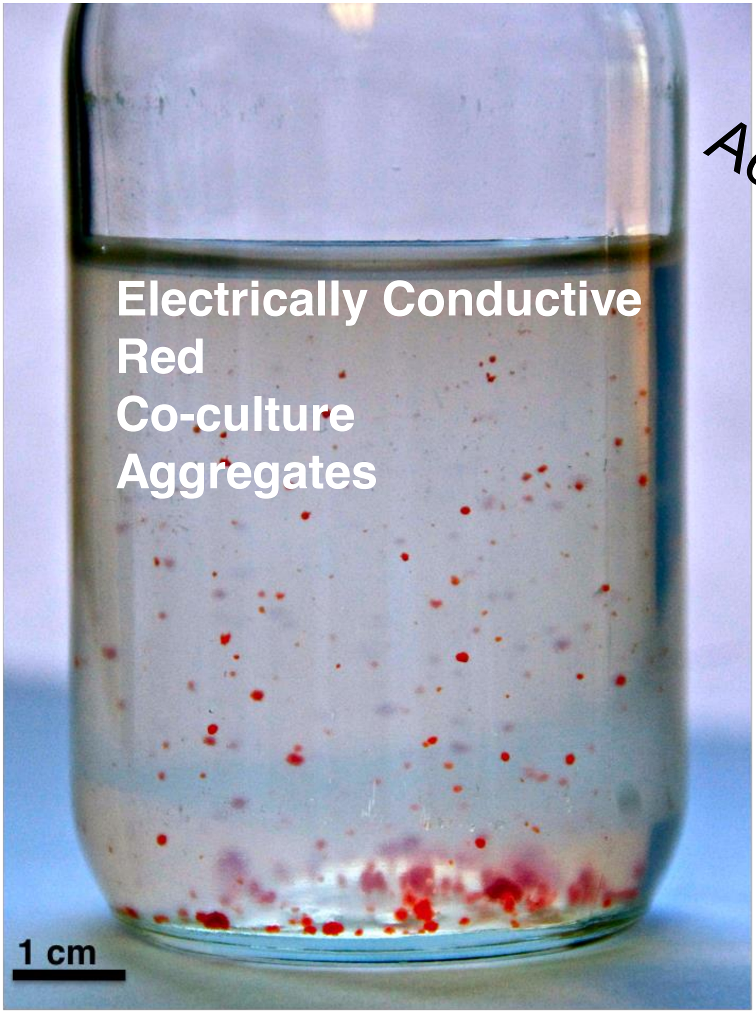
Feeding Microbes Electrons

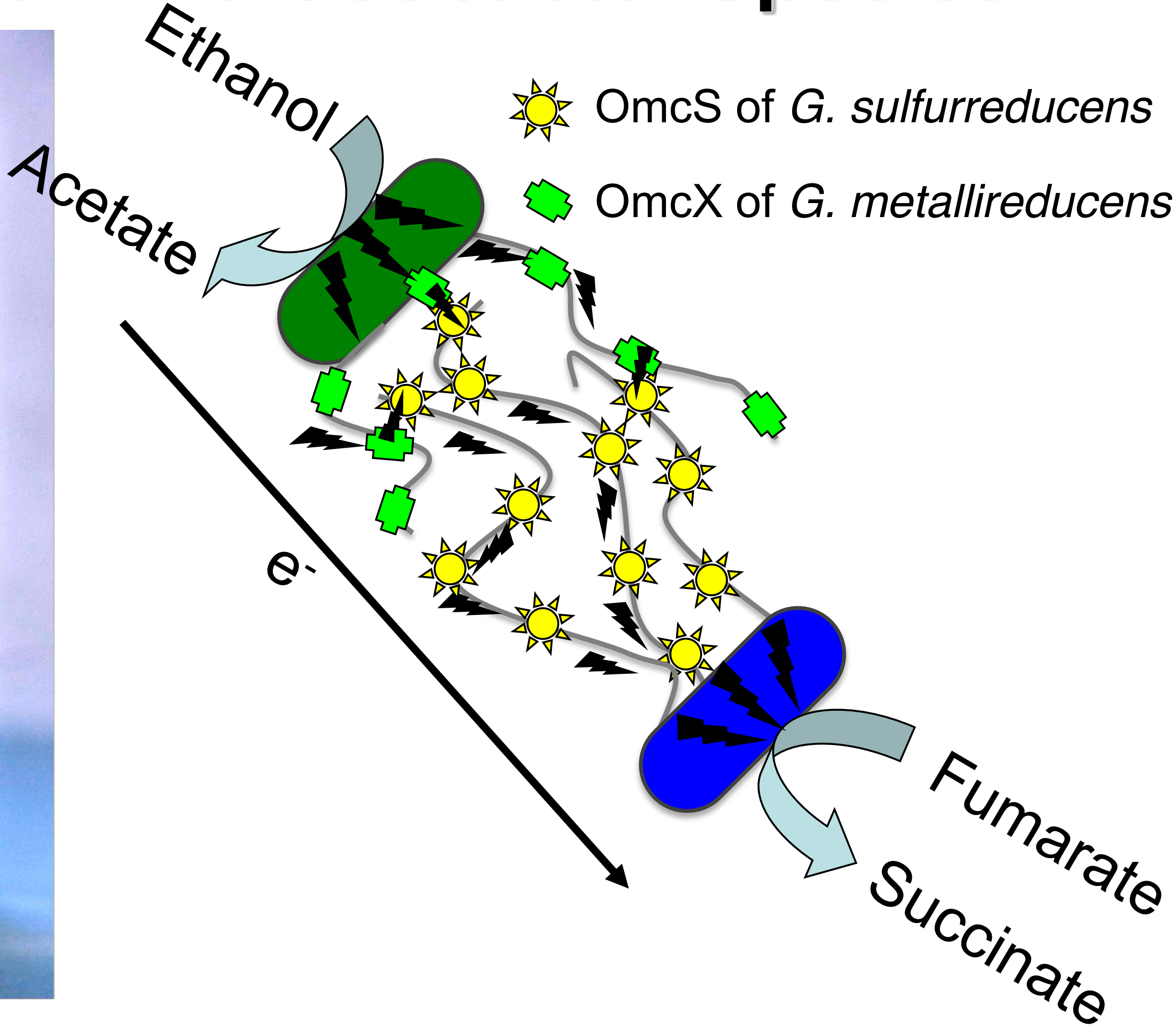
It has been known for over a decade that microorganisms can directly accept electrons to support anaerobic respiration



Gregory, K. B., D. R. Bond, and D. R. Lovley. 2004. Graphite electrodes as electron donors for anaerobic respiration. Environ. Microbiol. 6:596-604.

Direct Interspecies Electron Transfer (DIET) in Co-Cultures of Two Geobacter Species

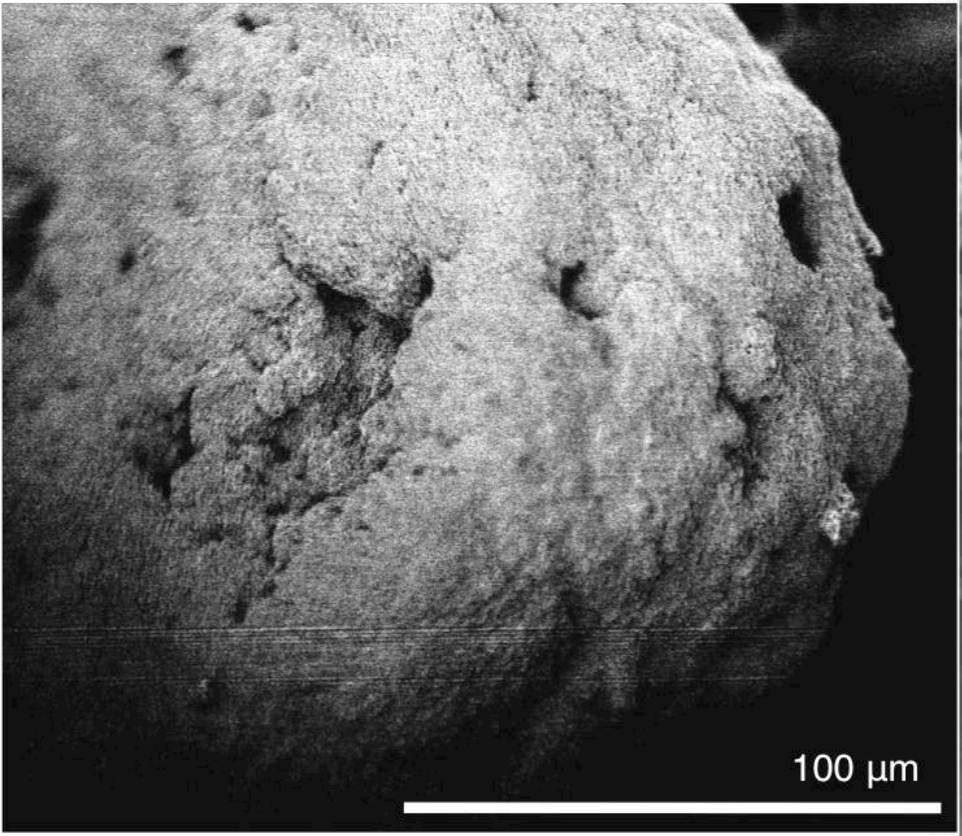




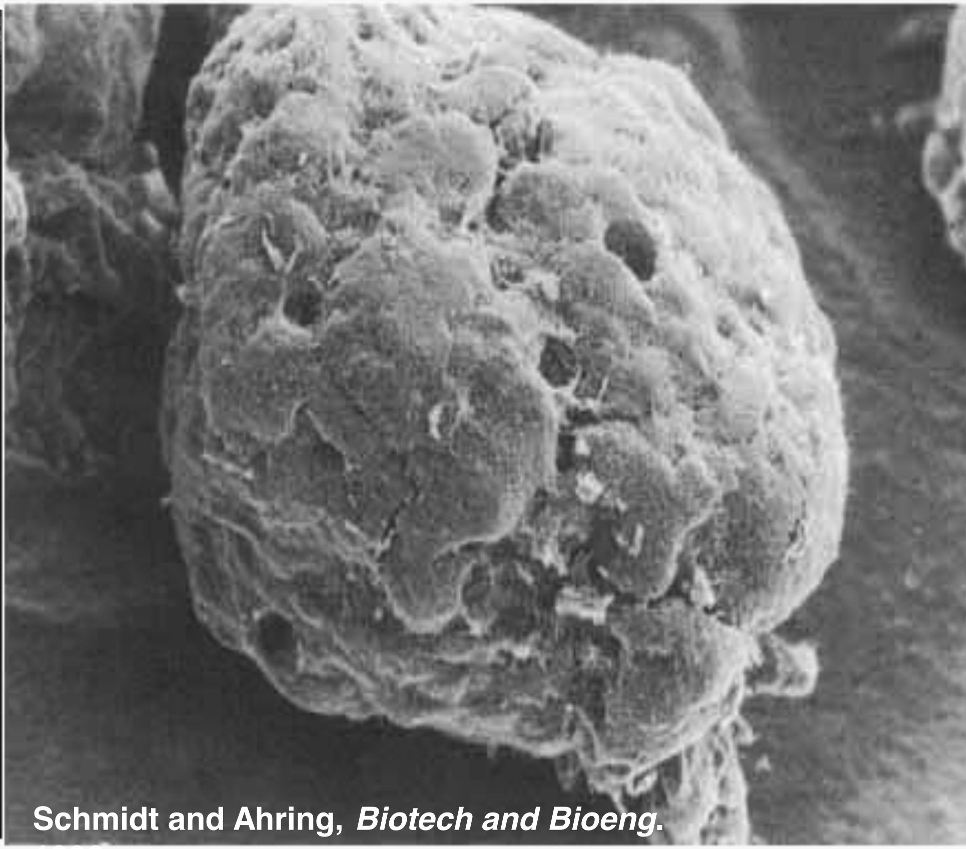
Summers, Z. M., H. Fogarty, C. Leang, A. E. Franks, N. S. Malvankar, and D. R. Lovley. 2010. Cooperative exchange of electrons within aggregates of an evolved syntrophic co-culture. Science 330:1413-1415.

Methanogenic Wastewater Aggregates Look Remarkably Similar *Geobacter* Co-Culture Aggregates that Exchange Electrons via Direct Interspecies Electron Transfer (DIET)

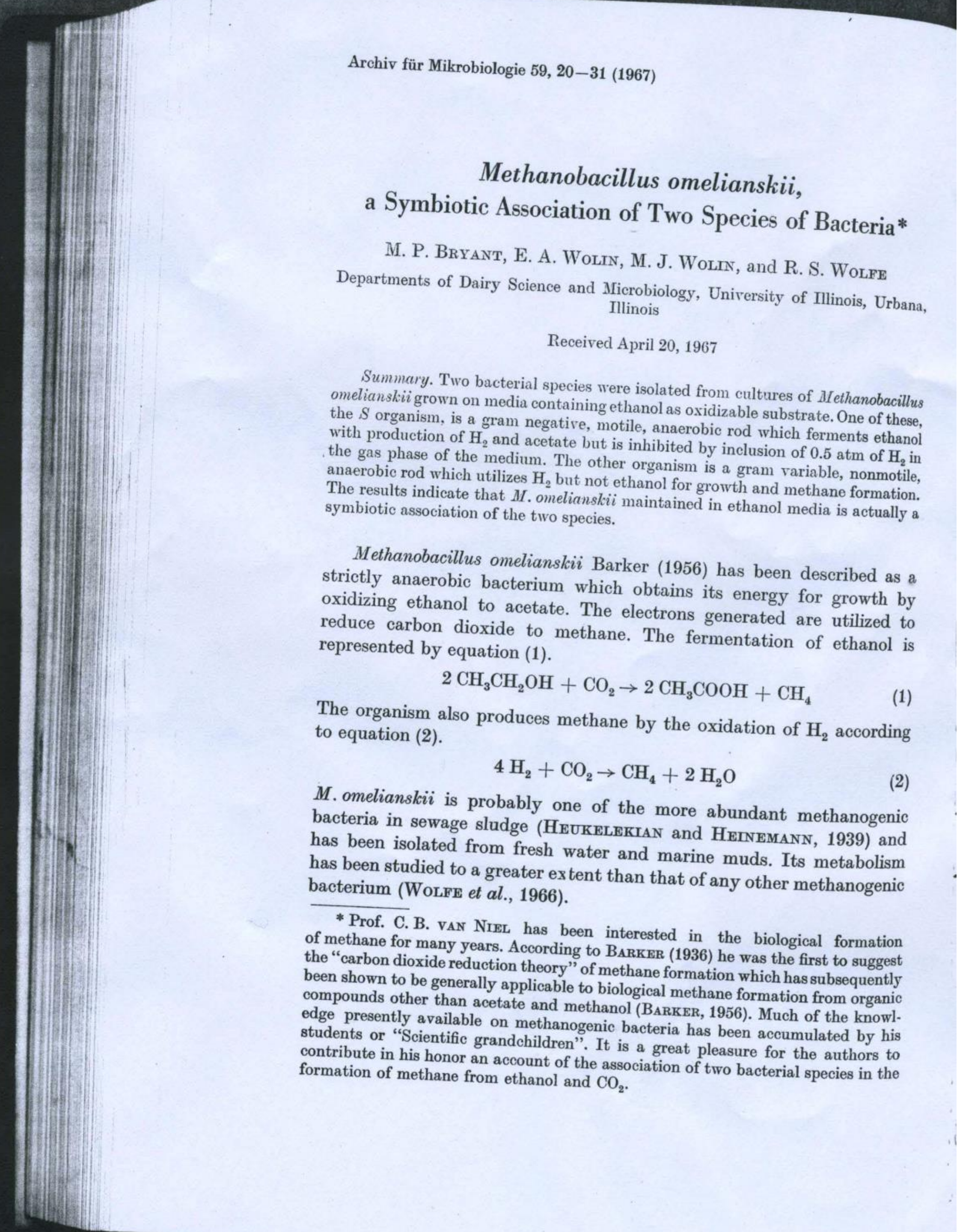
Geobacter Aggregate



Wastewater Aggregate



Schmidt and Ahring, *Biotech and Bioeng.* 1996



Archiv für Mikrobiologie 59, 20—31 (1967)

Methanobacillus omelianskii, a Symbiotic Association of Two Species of Bacteria*

M. P. Bryant, E. A. Wolin, M. J. Wolin, and R. S. Wolfe

Departments of Dairy Science and Microbiology, University of Illinois, Urbana, Illinois

Received April 20, 1967

Summary. Two bacterial species were isolated from cultures of *Methanobacillus omelianskii* grown on media containing ethanol as oxidizable substrate. One of these, the *S* organism, is a gram negative, motile, anaerobic rod which ferments ethanol with production of H_2 and acetate but is inhibited by inclusion of 0.5 atm of H_2 in the gas phase of the medium. The other organism is a gram variable, nonmotile, anaerobic rod which utilizes H_2 but not ethanol for growth and methane formation. The results indicate that *M. omelianskii* maintained in ethanol media is actually a symbiotic association of the two species.

Methanobacillus omelianskii Barker (1956) has been described as a strictly anaerobic bacterium which obtains its energy for growth by oxidizing ethanol to acetate. The electrons generated are utilized to reduce carbon dioxide to methane. The fermentation of ethanol is represented by equation (1).

$$2\ CH_3CH_2OH + CO_2 \rightarrow 2\ CH_3COOH + CH_4 \qquad (1)$$

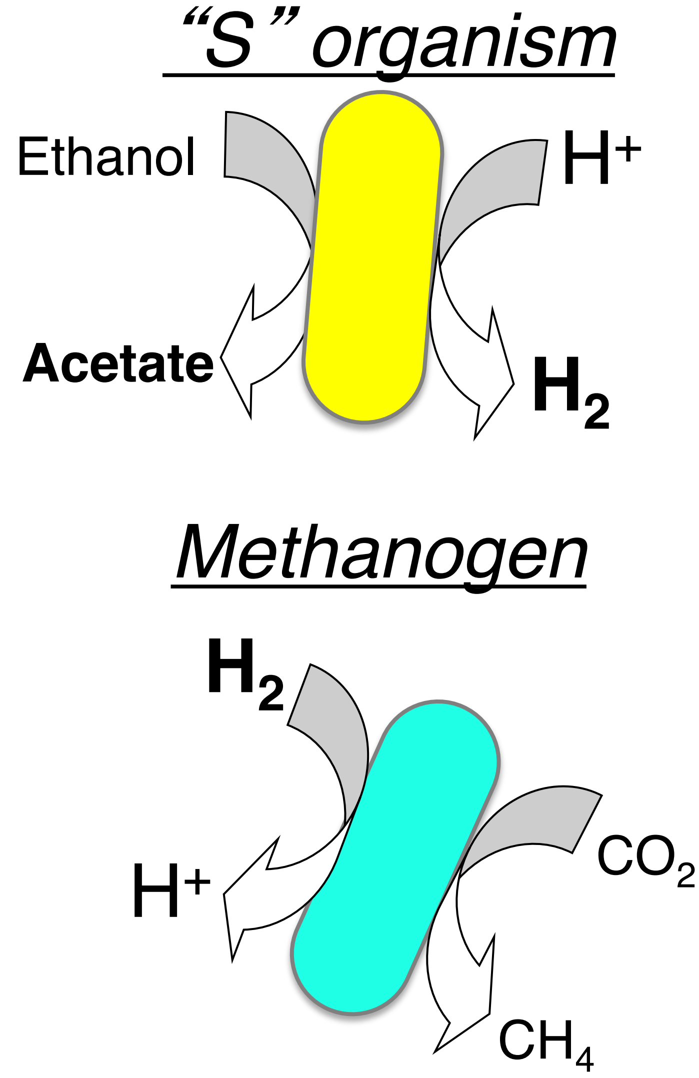
The organism also produces methane by the oxidation of H_2 according to equation (2).

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \qquad (2)$$

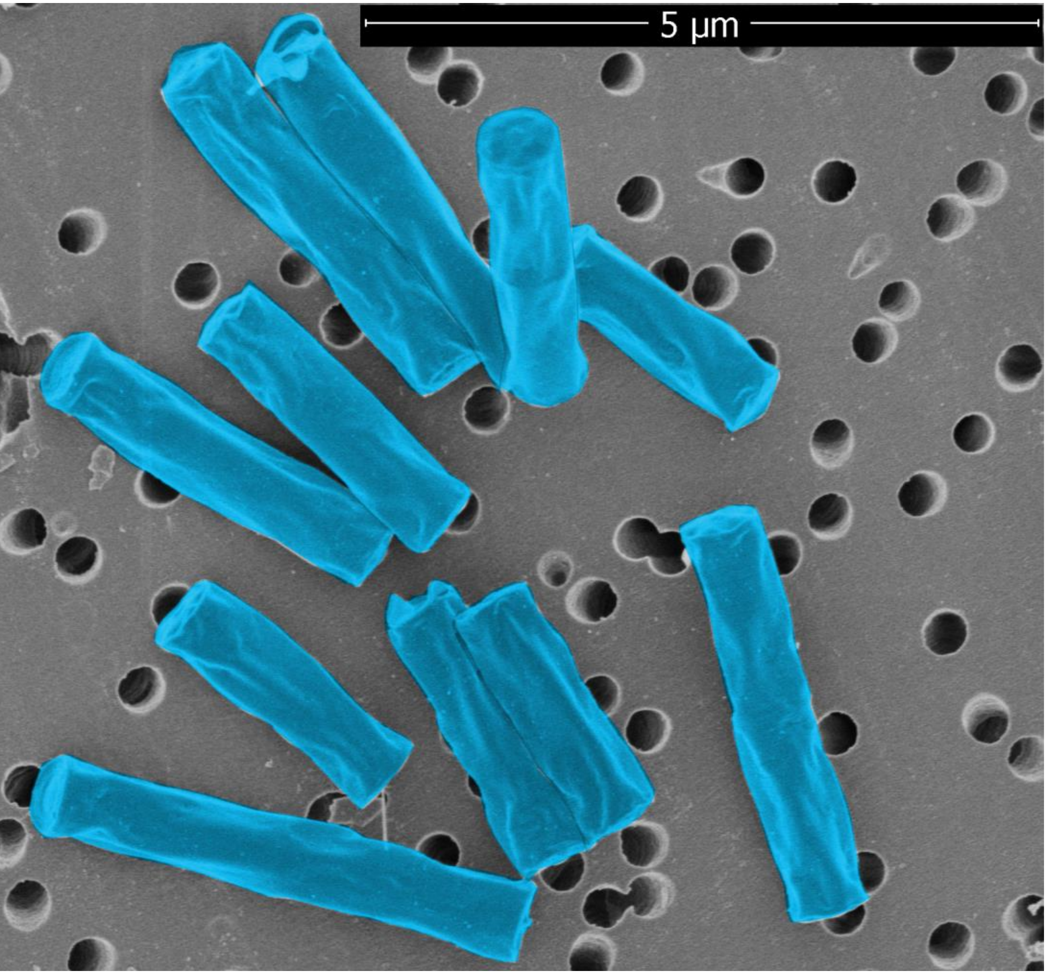
M. omelianskii is probably one of the more abundant methanogenic bacteria in sewage sludge (Heukelekian and Heinemann, 1939) and has been isolated from fresh water and marine muds. Its metabolism has been studied to a greater extent than that of any other methanogenic bacterium (Wolfe *et al.*, 1966).

* Prof. C. B. van Niel has been interested in the biological formation of methane for many years. According to Barker (1936) he was the first to suggest the "carbon dioxide reduction theory" of methane formation which has subsequently been shown to be generally applicable to biological methane formation from organic compounds other than acetate and methanol (Barker, 1956). Much of the knowledge presently available on methanogenic bacteria has been accumulated by his students or "Scientific grandchildren". It is a great pleasure for the authors to contribute in his honor an account of the association of two bacterial species in the formation of methane from ethanol and CO_2.

Interspecies Hydrogen Transfer



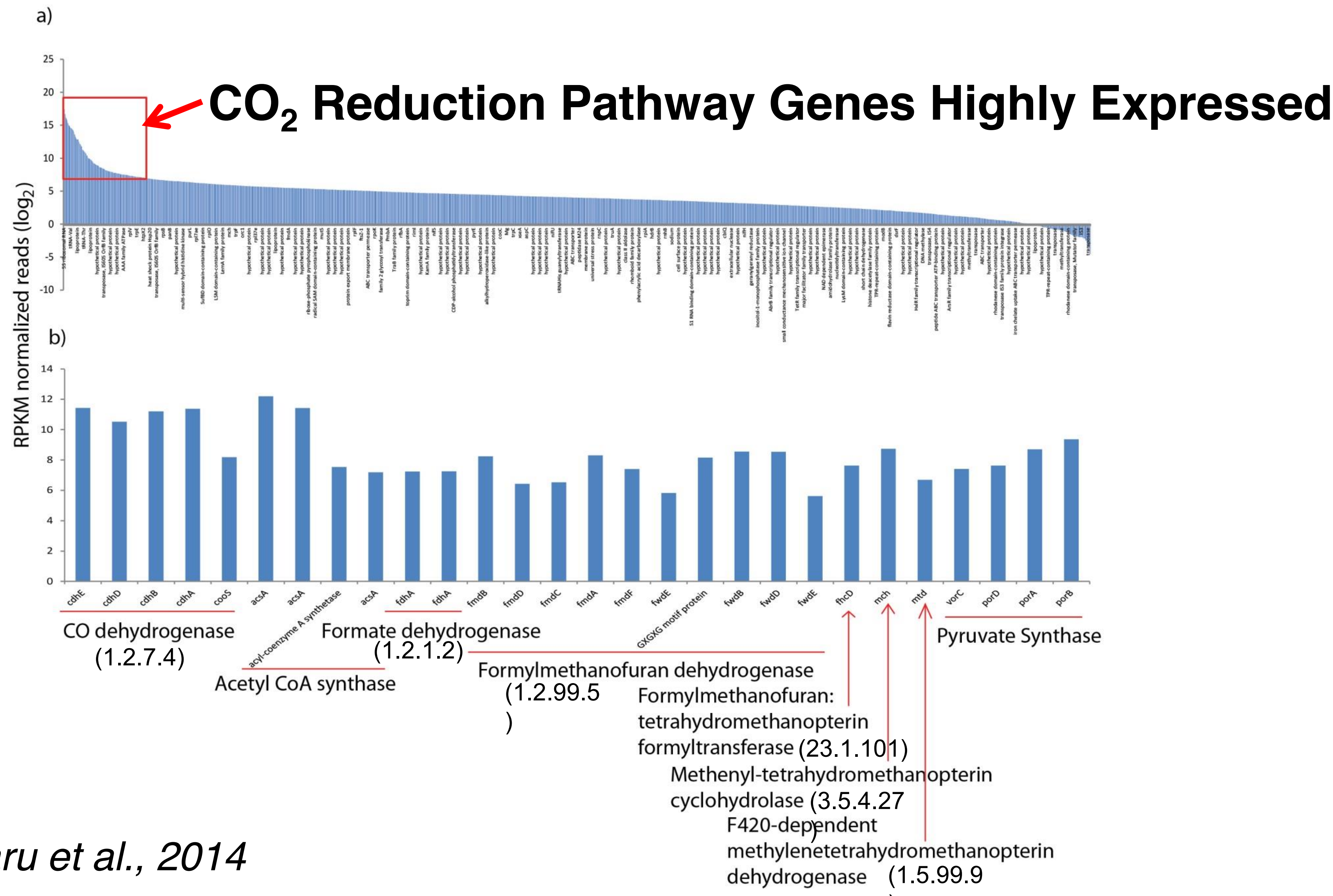
Methanosaeata the “Worlds Most Prodigious Methanogen” Participates in DIET



- It has been considered that acetate was the only substrate for methane production by *Methanosaeta*
- *Methanosaeta* can not use H_2 or formate as an electron donor
- However the genome contains a complete pathway for carbon dioxide reduction to methane

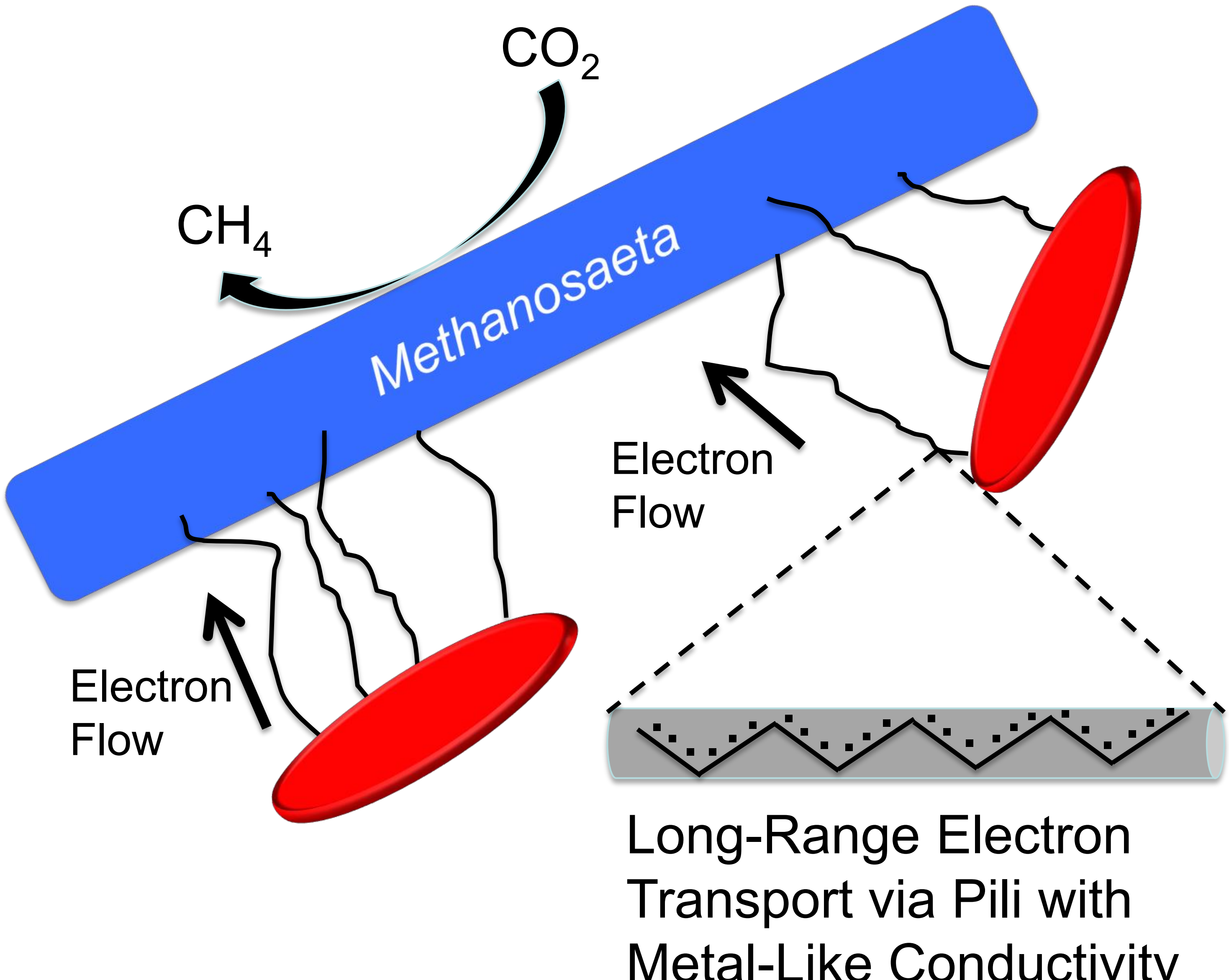
Rotaru A-E, Shrestha PM, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin KP, Lovley DR. 2014. A new model for electron fow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. Energy and Environmental Science 7:408-415.

Metatranscriptomic Analysis of UASB Digester Aggregates Revealed that Gene Expression Patterns Indicating that *Methanosaeta* species were Involved in DIET

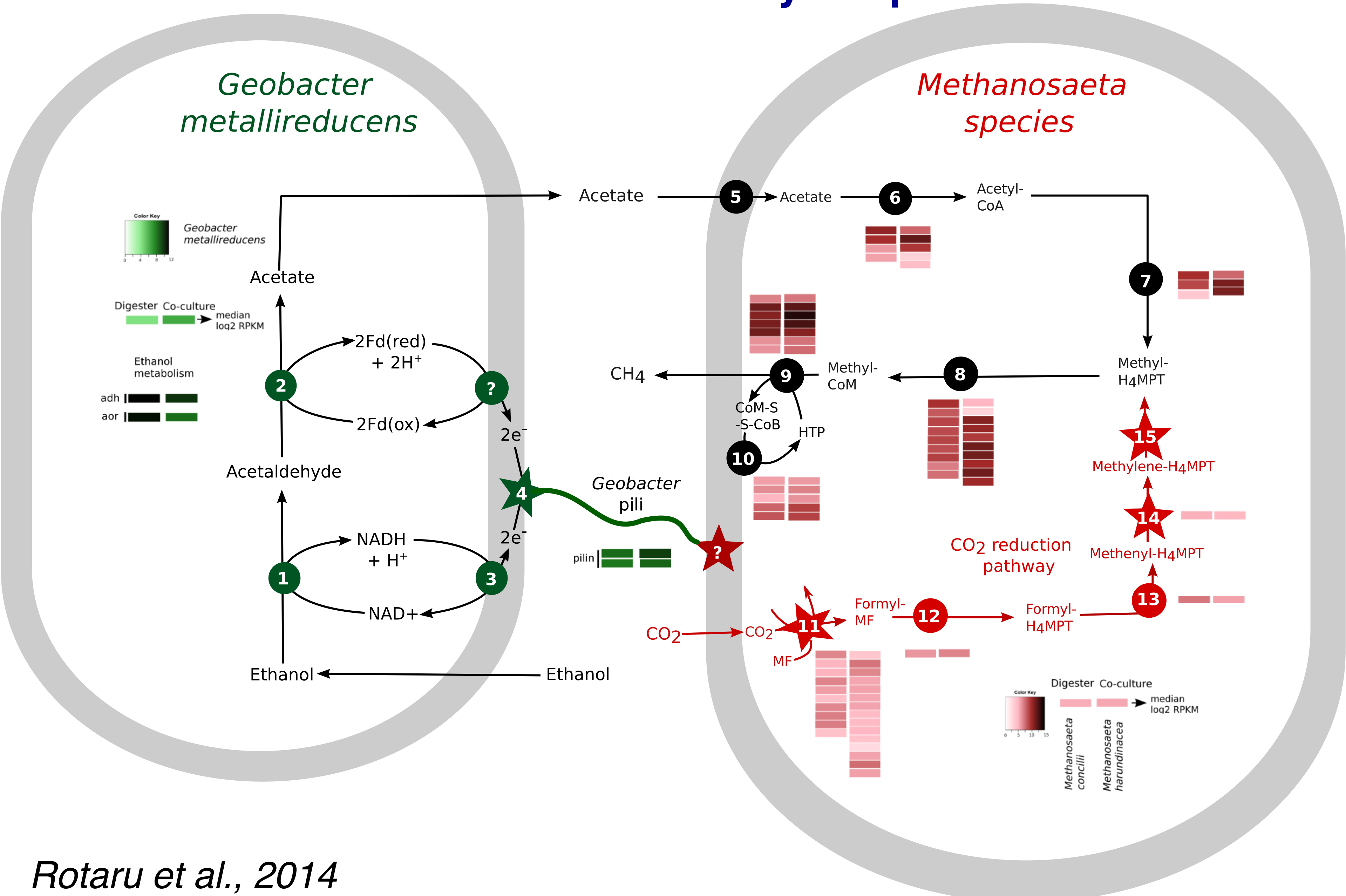


Rotaru et al., 2014

Metranscriptomic Data from Reactors and Genetic Analysis of Co-Cultures Demonstrated that *Geobacter* Species Make DIET Connections to *Methanosaeta* via Microbial Nanowires

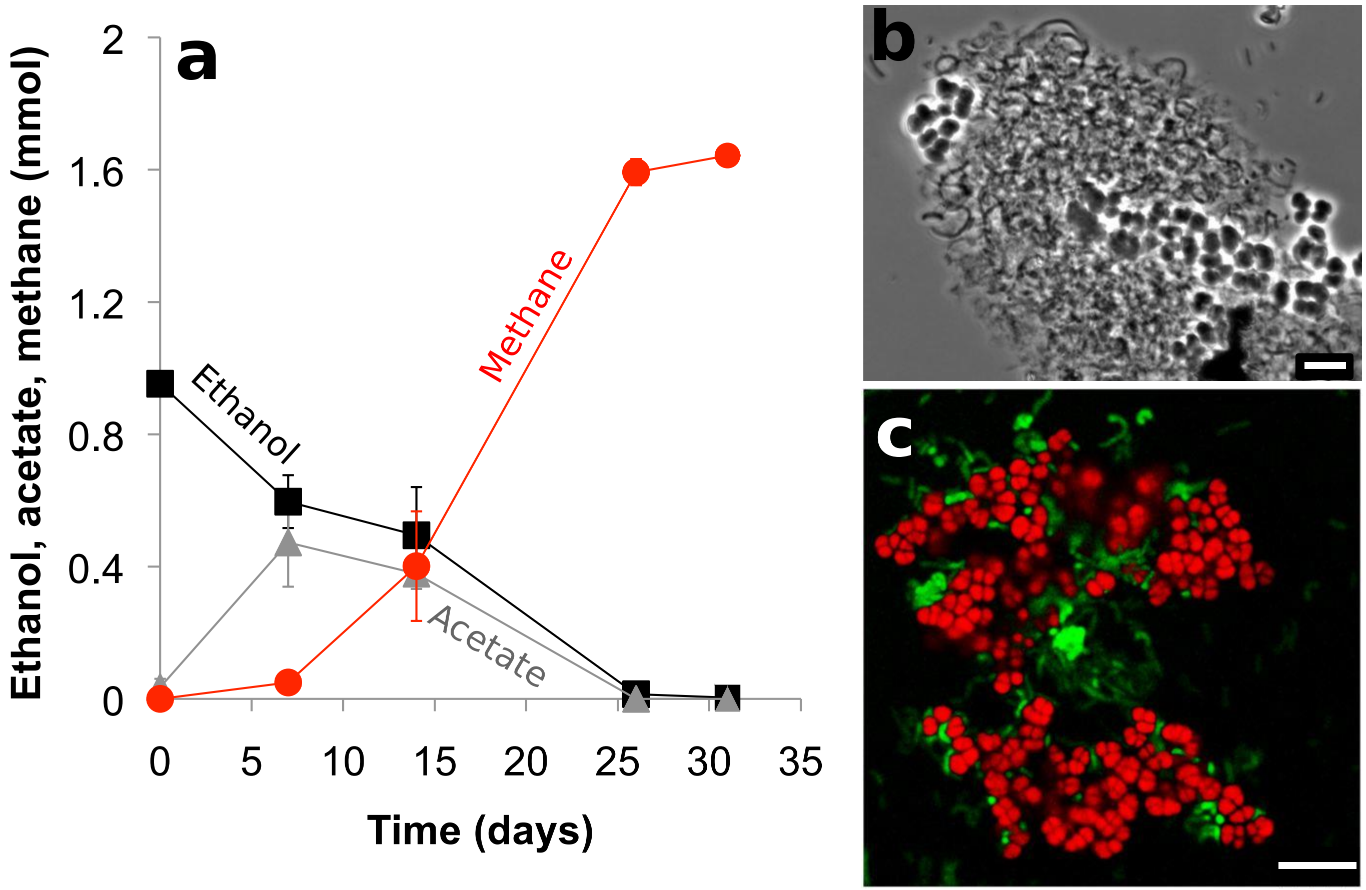


Transcriptomic Results are Consistent with the DIET Model for *Geobacter-Methanosaeta* Syntrophic Interaction



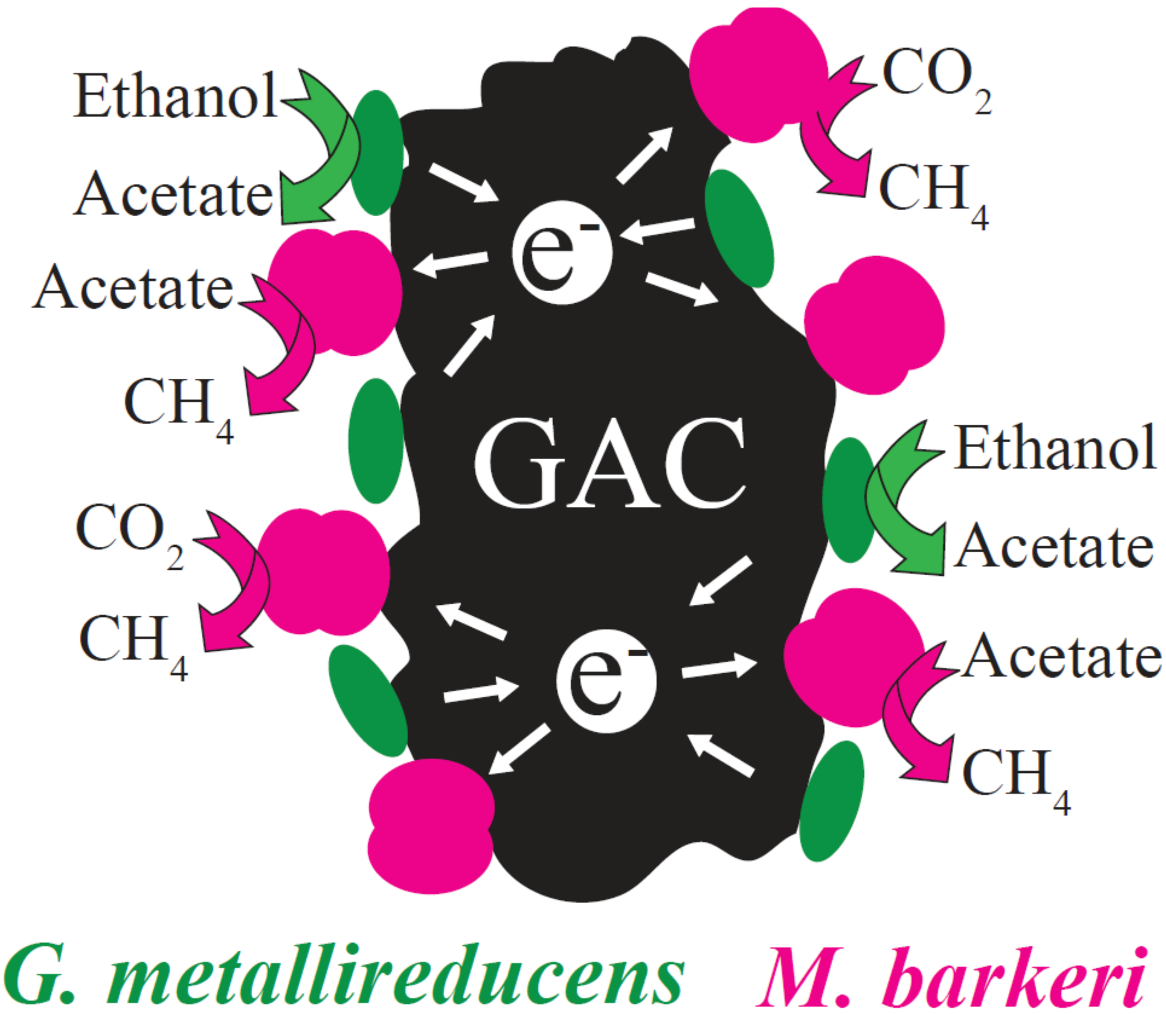
Rotaru et al., 2014

Methanosarcina barkeri Can Also Accept Electrons via DIET



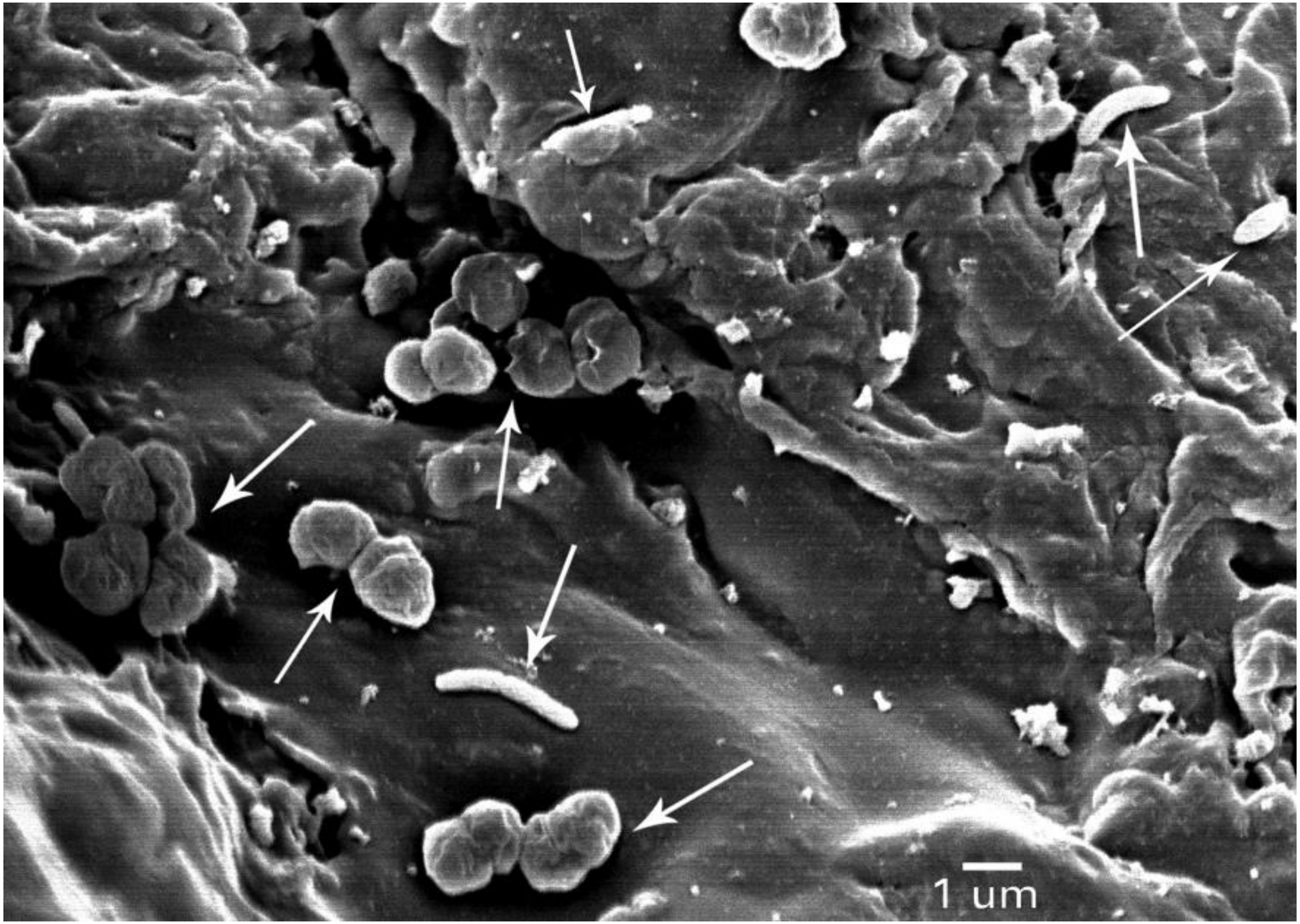
Rotaru A-E, Shrestha PM, Liu F, Markovaite B, Chen S, Nevin KP, Lovley DR. 2014. Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri Appl. Environ. Microbiol. 81:4599-4605.

Substituting Conductive Carbon Materials for Pili-Based Electrical Components Enhances Methanogenesis



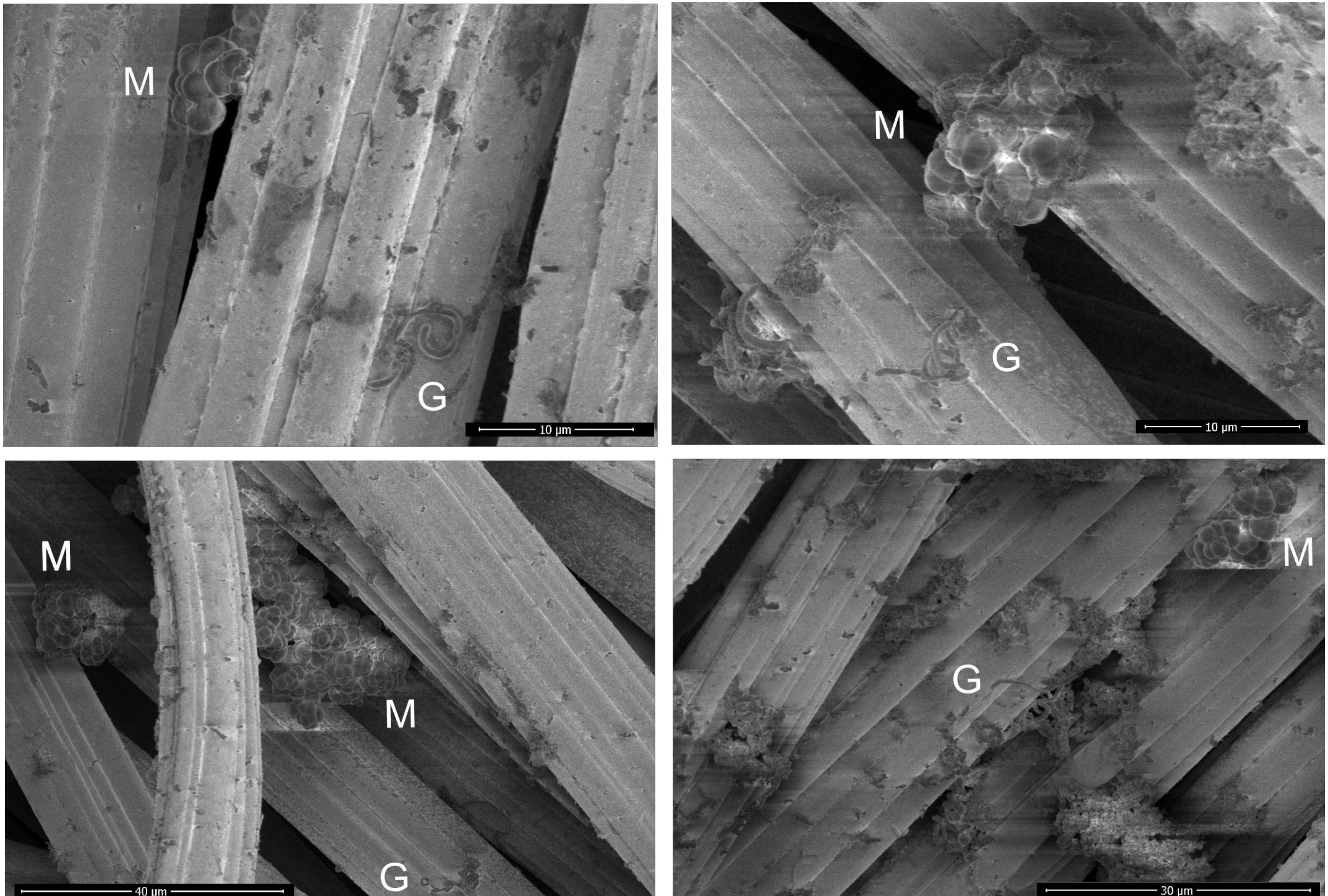
Liu, F. A-E. Rotaru, P. M. Shrestha, N. S. Malvankar, K. P. Nevin, and D. R. Lovley. 2012. Promoting direct interspecies electron transfer with activated carbon. *Energy & Environmental Science* 5: 8982-8989.

Biochar is 1000-fold Less Conductive than GAC but as Effective in Promoting DIET



Chen S, Rotaru A-E, Shrestha PM, Malvankar NS, Liu F, Fan W, Nevin KP, Lovley DR. 2014. Promoting interspecies electron transfer with biochar. Scientific Reports 4:5019.

Carbon Cloth is Also an Effective Conduit for DIET

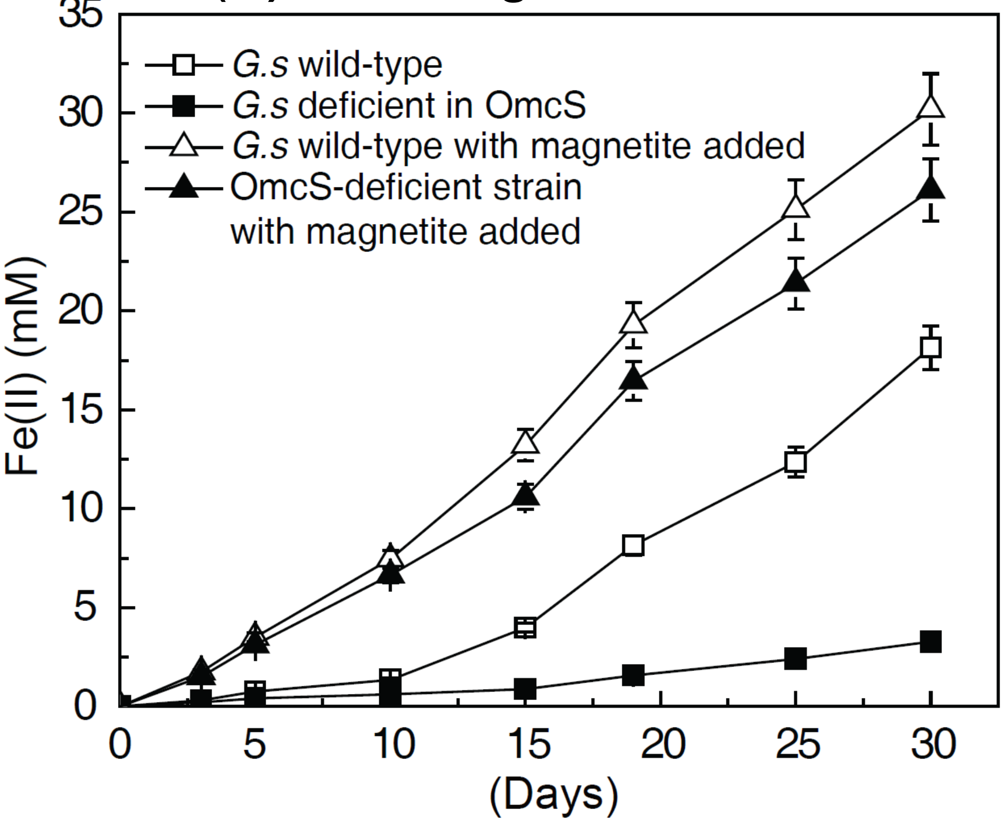


Chen, S. A-E. Rotaru, F. Liu, J. Phillips, T. Woodard, K.P. Nevin, and D.R. Lovley et al. 2014. Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures. Bioresource Technology 173:82-86.

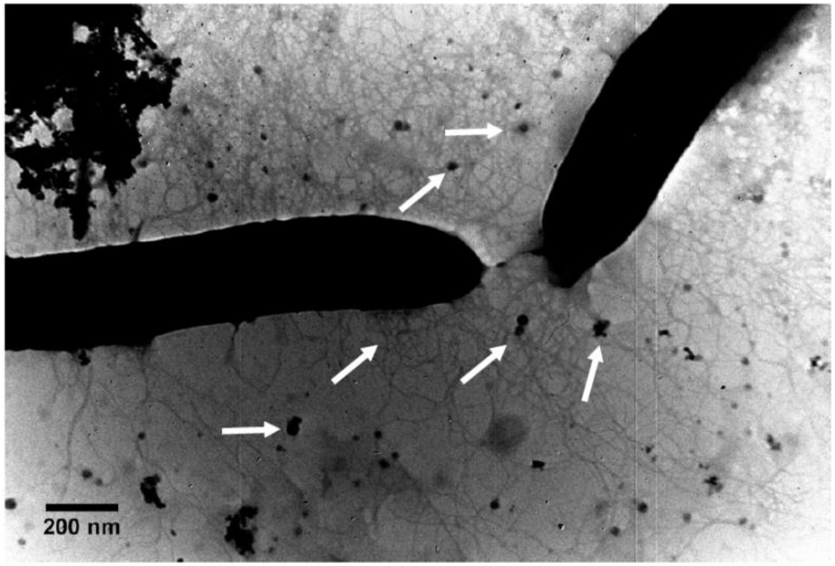
Minerals as Surrogate Electrobiological Components

Magnetite Substitutes for the Pili-Associated Cytochrome OmcS to Facilitate Extracelular Electron Transfer Between Cells

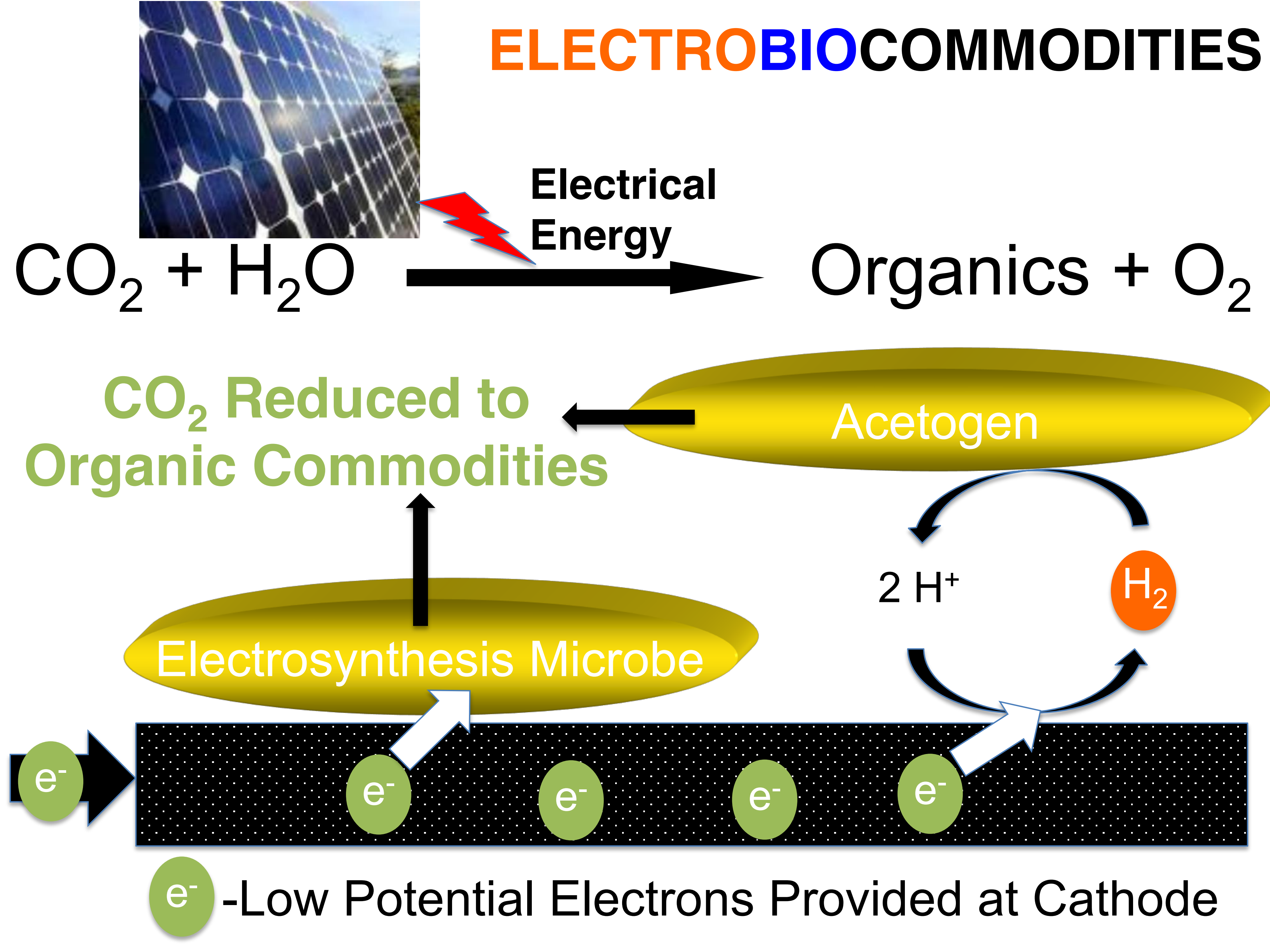
OmcS-Deficient Mutant Effectively Reduces Fe(III) when Magnetite is Present

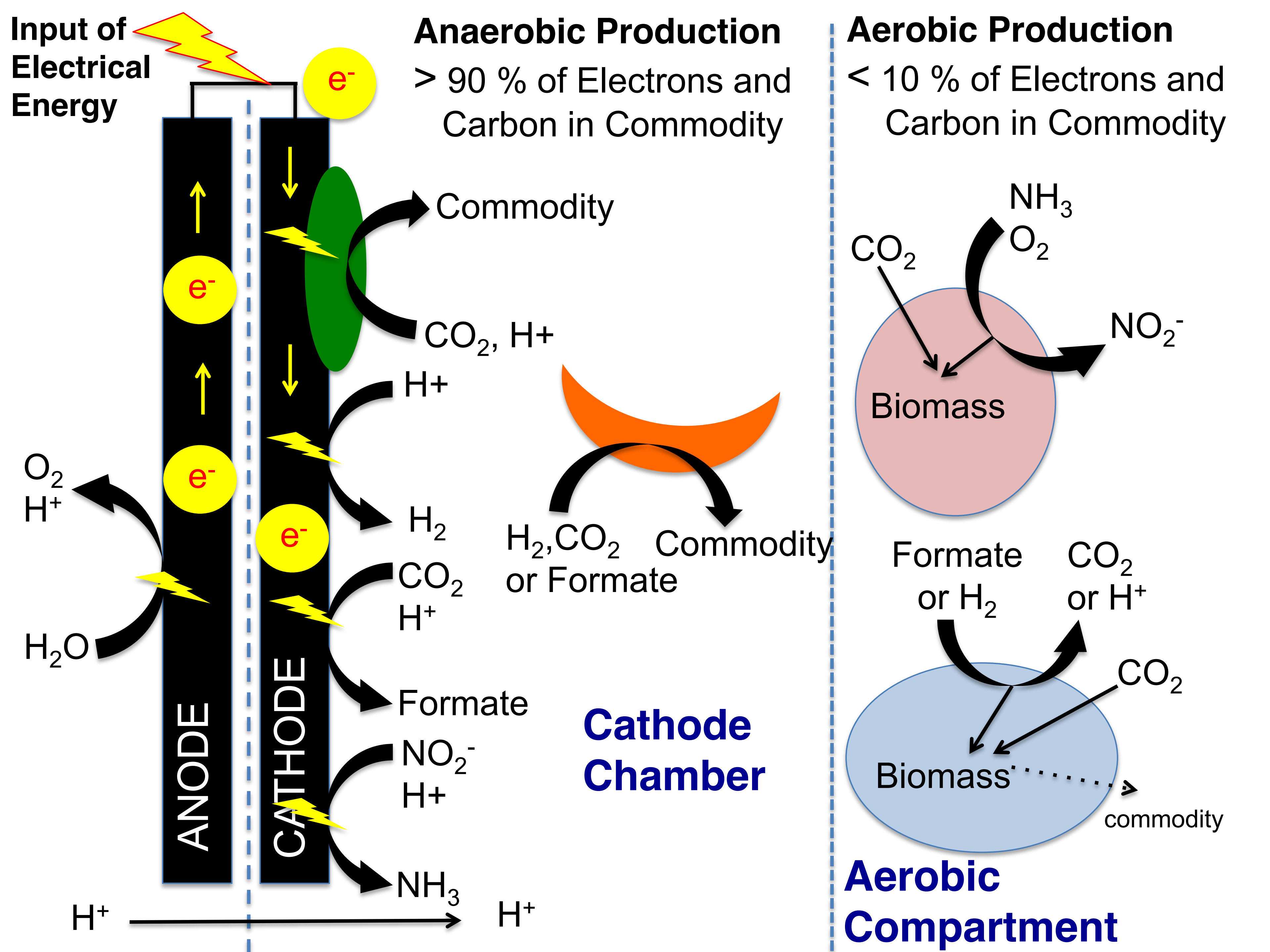


Magnetite Associated with Pili

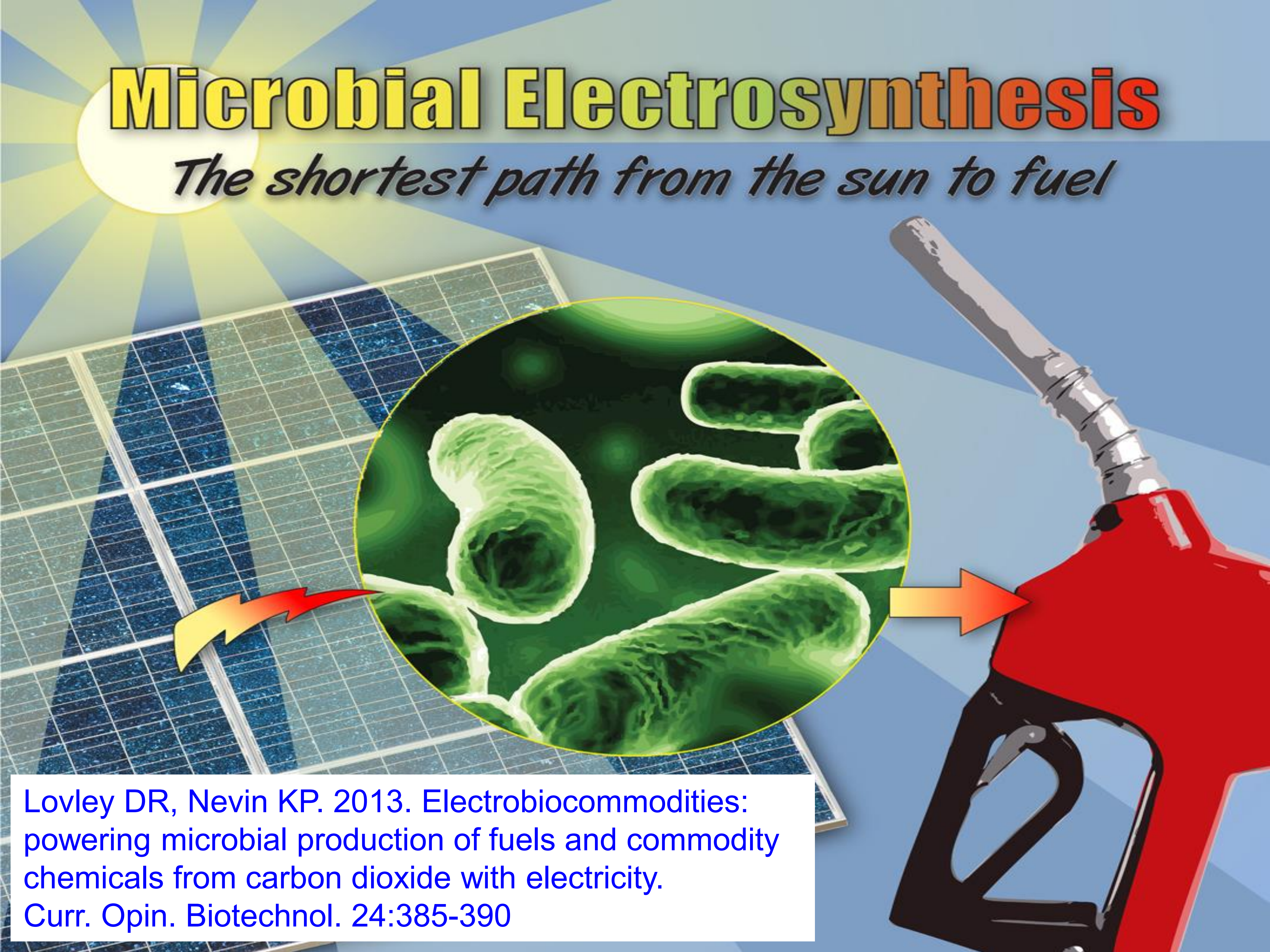


Liu F, Rotaru A-E, Shrestha PM, Malvankar NS, Nevin KP, Lovley DR. 2015. Magnetite compensates for the lack of a pilin-associated c-type cytochrome in extracellular electron exchange. Environ. Microbiol.:DOI: 10.1111/1462-2920.12485.


ELECTROBIOCOMMODITIES
Electrical Energy
$CO_2 + H_2O$
Organics + O_2
CO_2 Reduced to Organic Commodities
Acetogen
2 H^+
H_2
Electrosynthesis Microbe
e^-
e^- -Low Potential Electrons Provided at Cathode



Input of Electrical Energy
Anaerobic Production
> 90 % of Electrons and Carbon in Commodity
Aerobic Production
< 10 % of Electrons and Carbon in Commodity
e-
Commodity
CO2, H+
H+
H2
CO2 H+
Formate
NO2- H+
NH3
O2 H+
H2O
ANODE
CATHODE
H+
H+
H2,CO2 or Formate
Commodity
Cathode Chamber
NH3
O2
CO2
NO2-
Biomass
Formate or H2
CO2 or H+
CO2
Biomass
commodity
Aerobic Compartment



Lovley DR, Nevin KP. 2013. Electrobiocommodities: powering microbial production of fuels and commodity chemicals from carbon dioxide with electricity. Curr. Opin. Biotechnol. 24:385-390

Microbial Electrosynthesis and Biological Photosynthesis Have the Same Overall Reaction

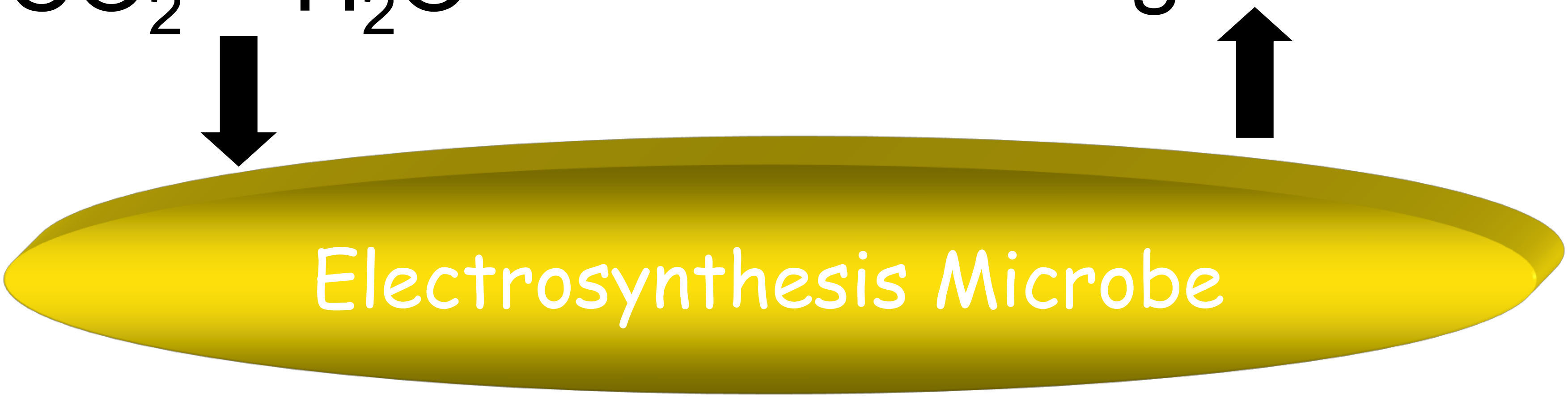
$$CO_2 + H_2O \xrightarrow{\text{Solar Energy}} \text{Organics} + O_2$$

The Difference is the Form of the Organic Product

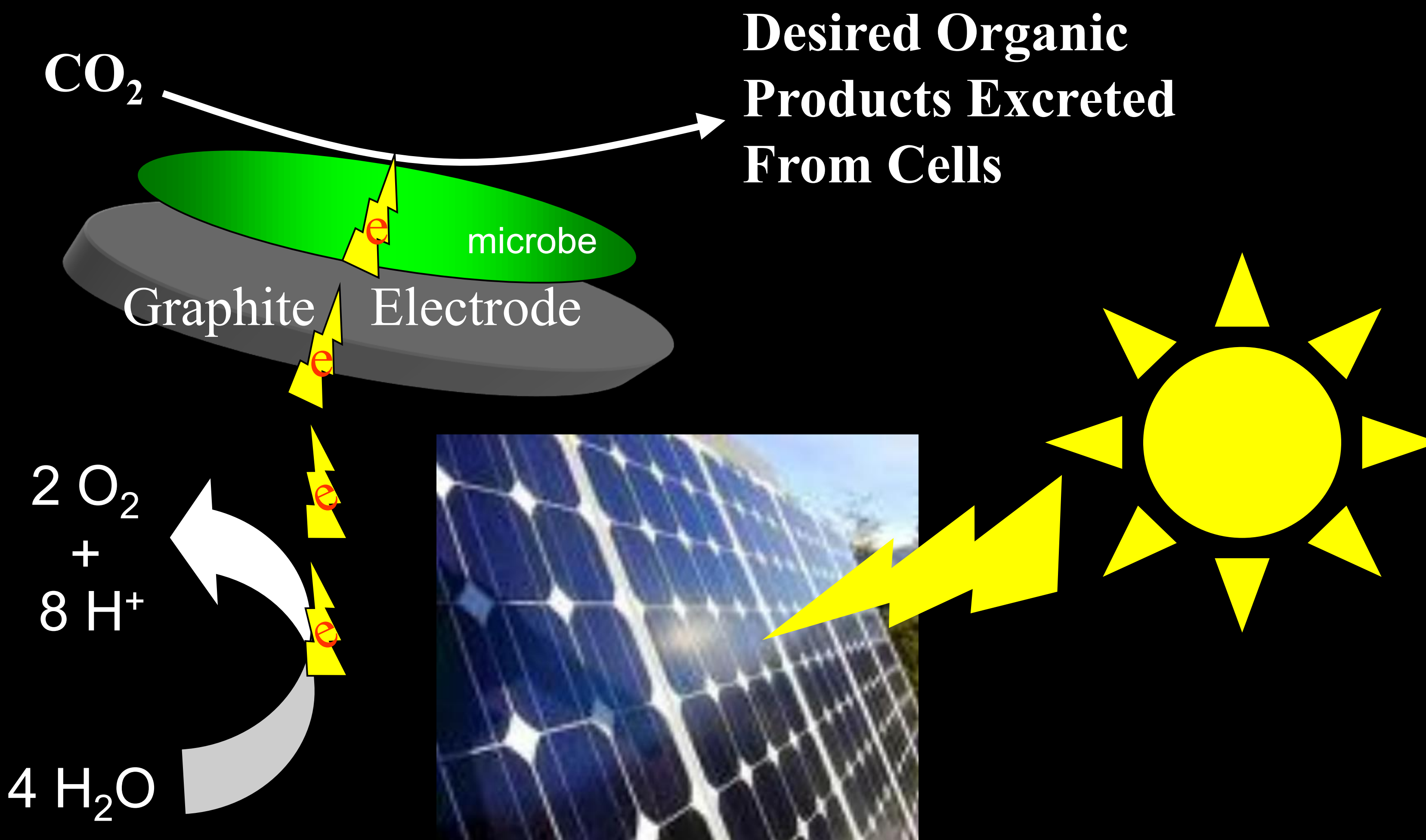
Microbial electrosynthesis - Desired commodity, released from cell

Biological photosynthesis – Biomass, requires further processing

$CO_2 + H_2O$ Excreted Organics + O_2



Microbial Electrosynthesis: Production of Fuels and Other Organic Chemicals from CO_2, Water, and Electricity



Nevin, K. P., et al. 2010. mBio 1: e00103-10

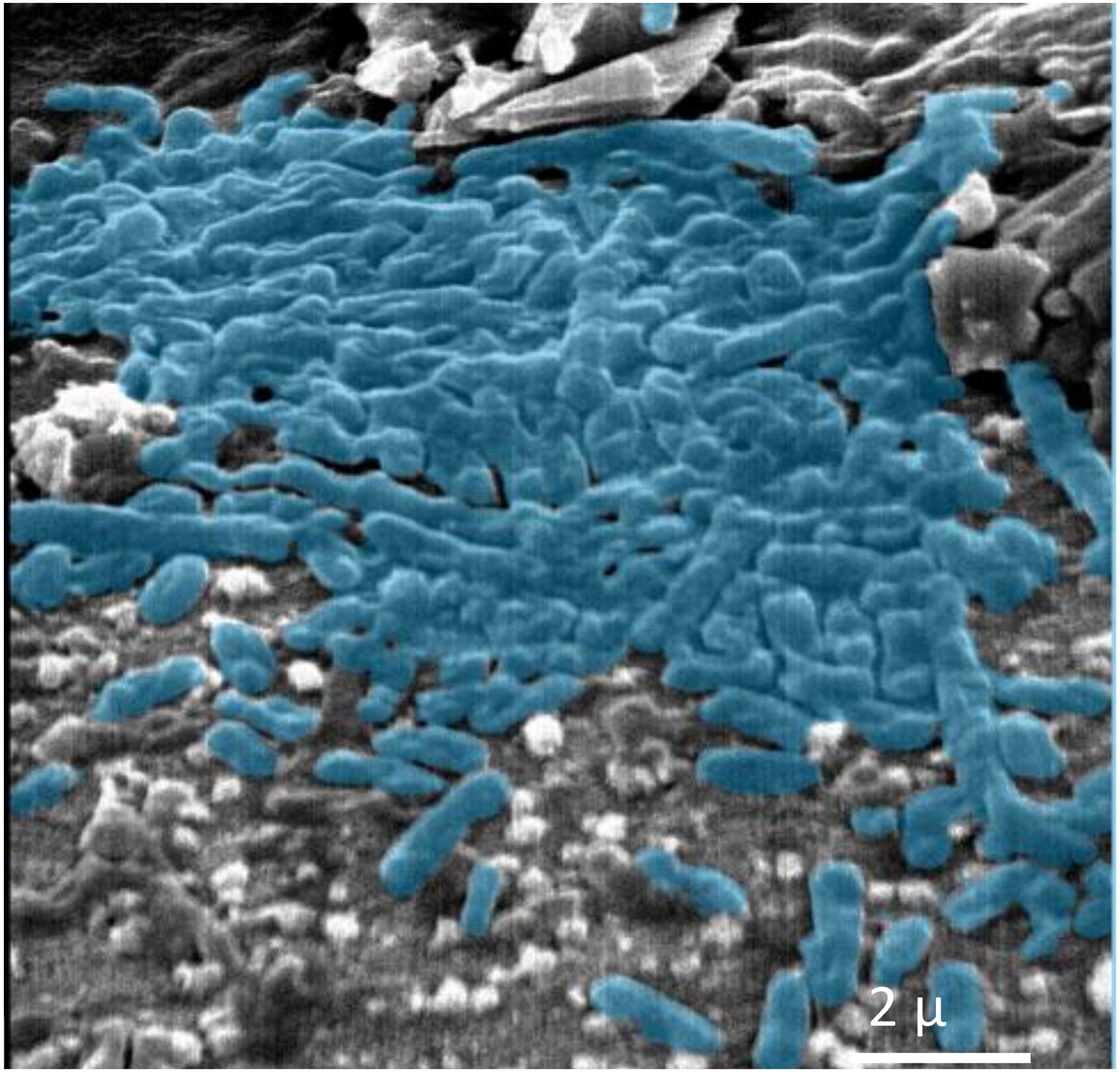
Patent Application PCT/US2010/061690

ElectroFuels.org

Advantages of Microbial Electrosynthesis Over Biomass-Based Strategies

- 100-fold more efficient in solar energy capture
- Directly produces fuel or other desired products rather than biomass that requires further processing
- Avoids water, additional energy consumption and waste generation associated with biomass processing
- Does not require arable land
- Avoids environmental degradation associated with intensive biomass production

Clostridium ljungdahlii is Highly Effective in Electrosynthesis



Initial Acetate Production Studies:

Columbic Efficiency 85%

Electrical Energy Input Recovered in Organic Products 70%

Nevin, K. P., S. A. Hensley, A. E. Franks, Z. M. Summers, J. Ou, T. L. Woodard, O. L. Snoeyenbo-West, and D. R. Lovley. 2011. Electrosynthesis of organic compounds from carbon dioxide catalyzed by a diversity of acetogenic microorganisms. Appl Environ Microbiol. 77: 2882-2886.

The Genetic Toolbox Newly Developed for *C. ljungdahlii* Makes It Feasible to Make New Products from Carbon Dioxide

Elimination of Unwanted Pathways for Carbon and Electron Flux

Leang, C., T. Ueki, K. P. Nevin, and D. R. Lovley. 2013. A genetic system for *Clostridium ljungdahlii*: a chassis for autotrophic production of biocommodities and a model homoacetogen. Appl Environ Microbiol 79:1102-1109.

Leang, C. R. Orellana, K.P. Nevin, and D.R. Lovley. 2015. Acetate kinase is essential for autotrophic growth of *Clostridium ljungdahlii* (manuscript to be submitted).

Elucidation of the Role of the Rnf Complex in Autotrophic Energy Conservation

Tremblay, P.-L., T. Zhang, S. A. Dar, C. Leang, and D. R. Lovley. 2013. The Rnf complex of Clostridium ljungdahlii is a proton translocating ferredoxin:NAD+ oxidoreductase essential for autotrophic growth. mBio 4: e00406-12.

Redirect Metabolic Flux for the Production of New Products from Carbon Dioxide

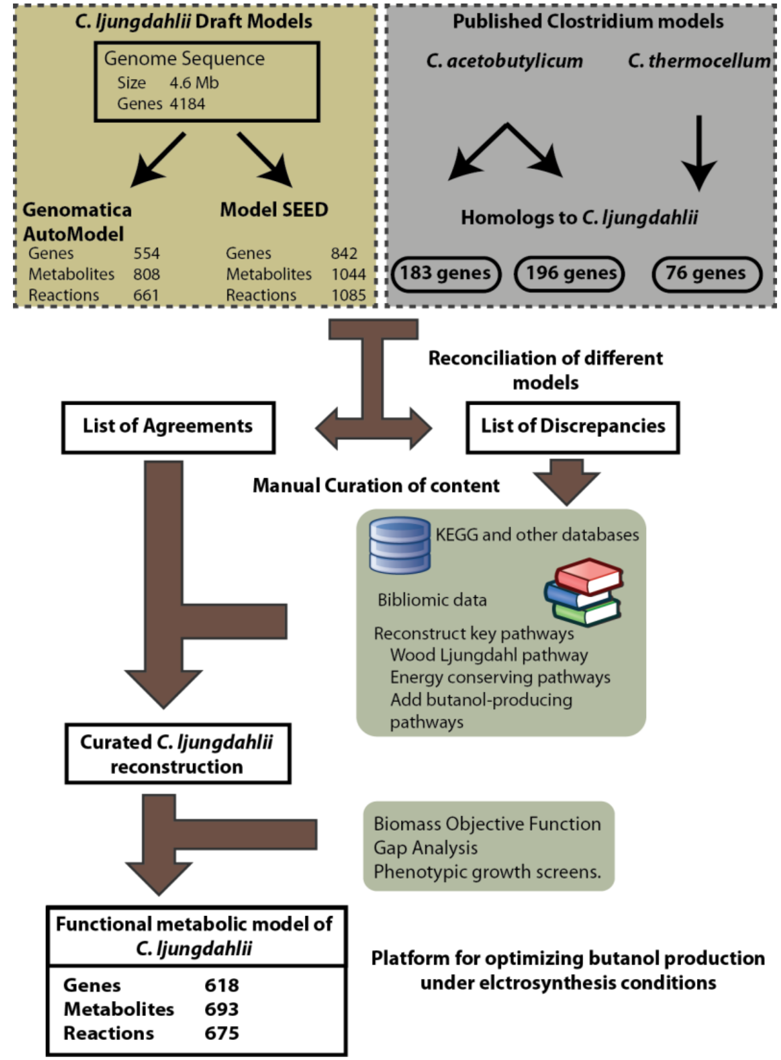
Banerjee A, Leang C, Ueki T, Nevin KP, Lovley DR. 2014. A lactose-inducible system for metabolic engineering of Clostridium ljungdahlii. Appl Environ Microbiol 80:2410-2416.

Ueki, T., K.P. Nevin, K.P., T.L. Woodard, and D.R. Lovley. 2014. Converting carbon dioxide to butyrate with an engineered strain of *Clostridium ljungdahlii*. mBio 5:e01636-14.

Genome-scale Model for *Clostridium ljungdahlii* Based on Revised Genome Annotation , RNA-Seq and Proteomics

RNA-Seq Analysis

Expression levels	*Cl. ljungdahlii* (H2/CO2)	*Cl. ljungdahlii* (Fructose)
High expression (≥9 RPKM values)	474	460
Intermediate expression (5 to <9 RPKM values)	1769	1569
Low expression (>2 to <5 RPKM values)	955	924
No expression (<2 RPKM values)	881	978

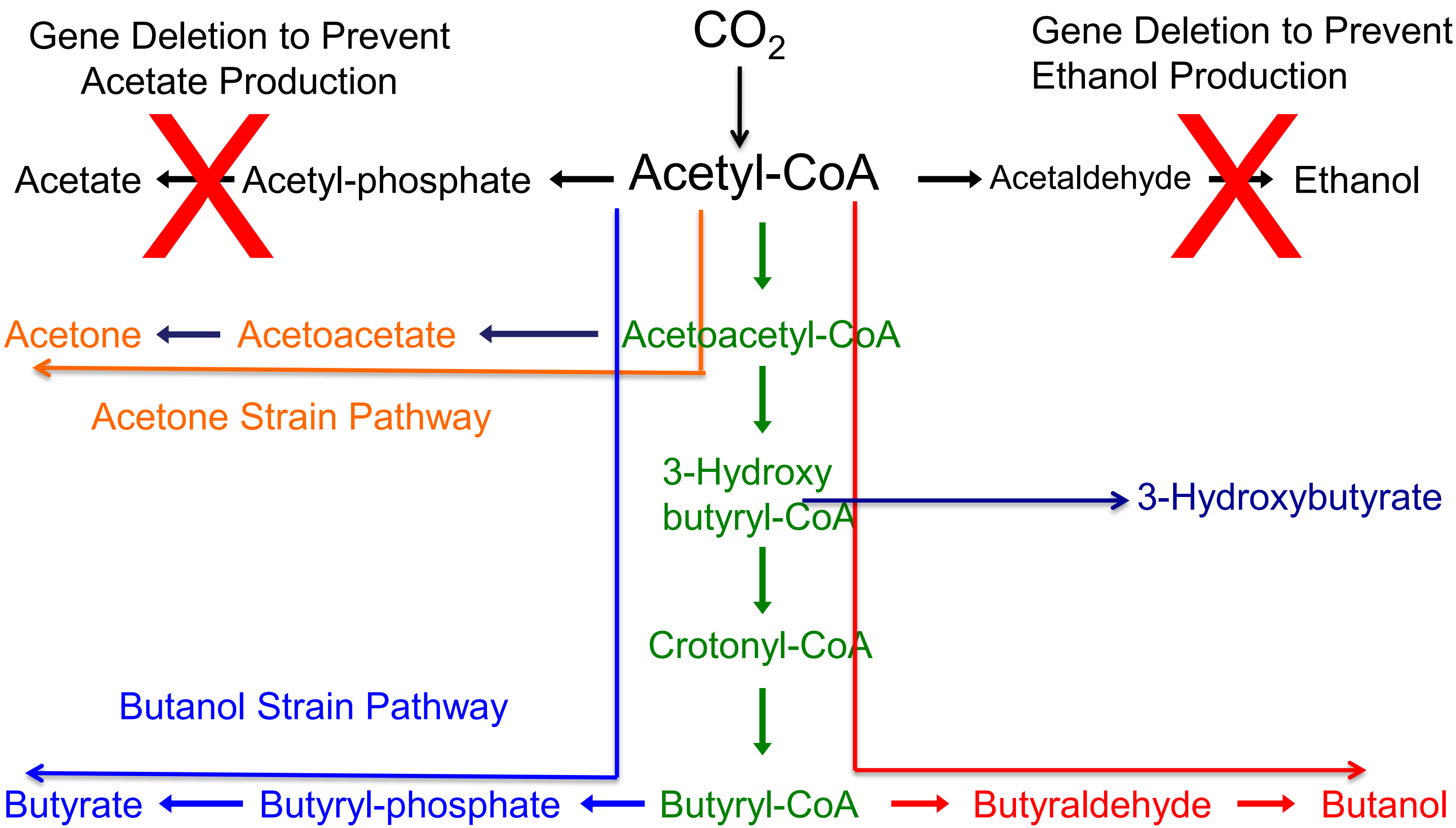


Model Statistics

Genes included	618
Total reactions	675
Total metabolites	693
External metabolites	90
Subsystems	61

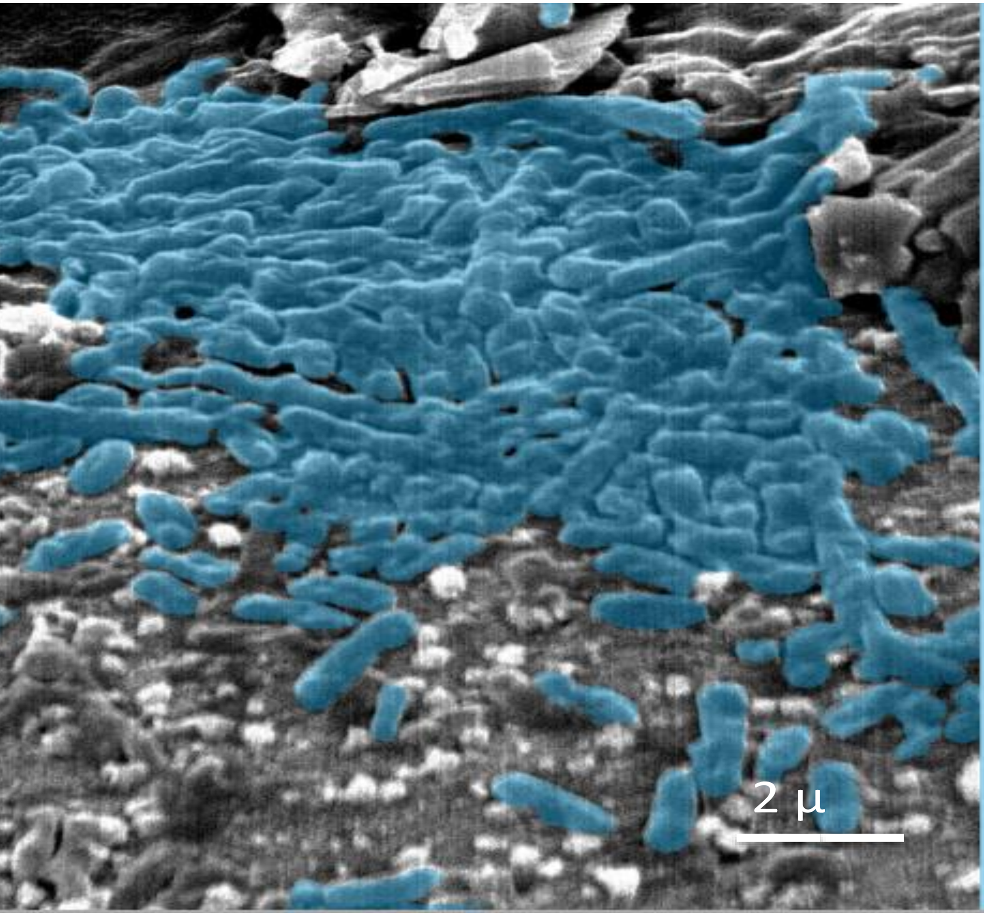
Nagarajan H, Sahin M, Nogales J, Latif H, Lovley DR, Ebrahim A, Zengler K. 2013. Characterizing acetogenic metabolism using a genome-scale metabolic reconstruction of *Clostridium ljungdahlii*. Microb Cell Fact 12:118.

New Strains that can Convert Carbon Dioxide to Acetone, 3-HydroxyButyrate, Butyrate or Butanol Rather than Natural Products Acetate or Ethanol Have been Engineered

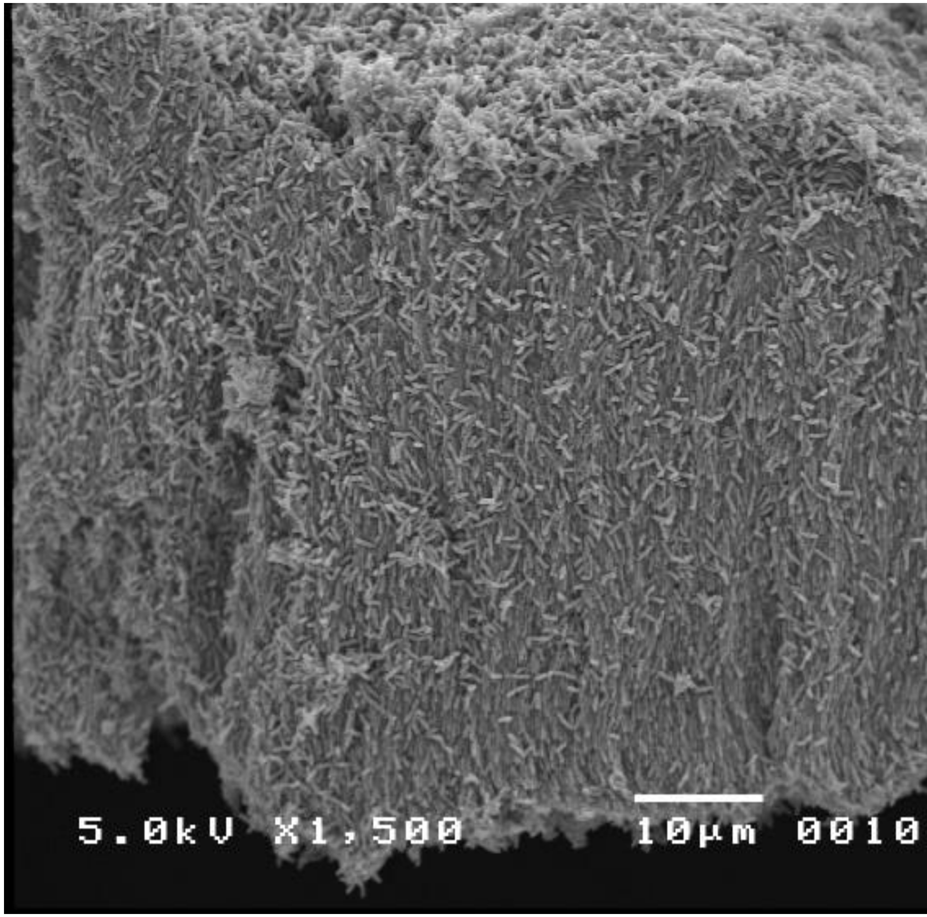


Biofilms of Acetogens are Very Sparse Compared to *Geobacter* Biofilms

Clostridium ljungdahlii



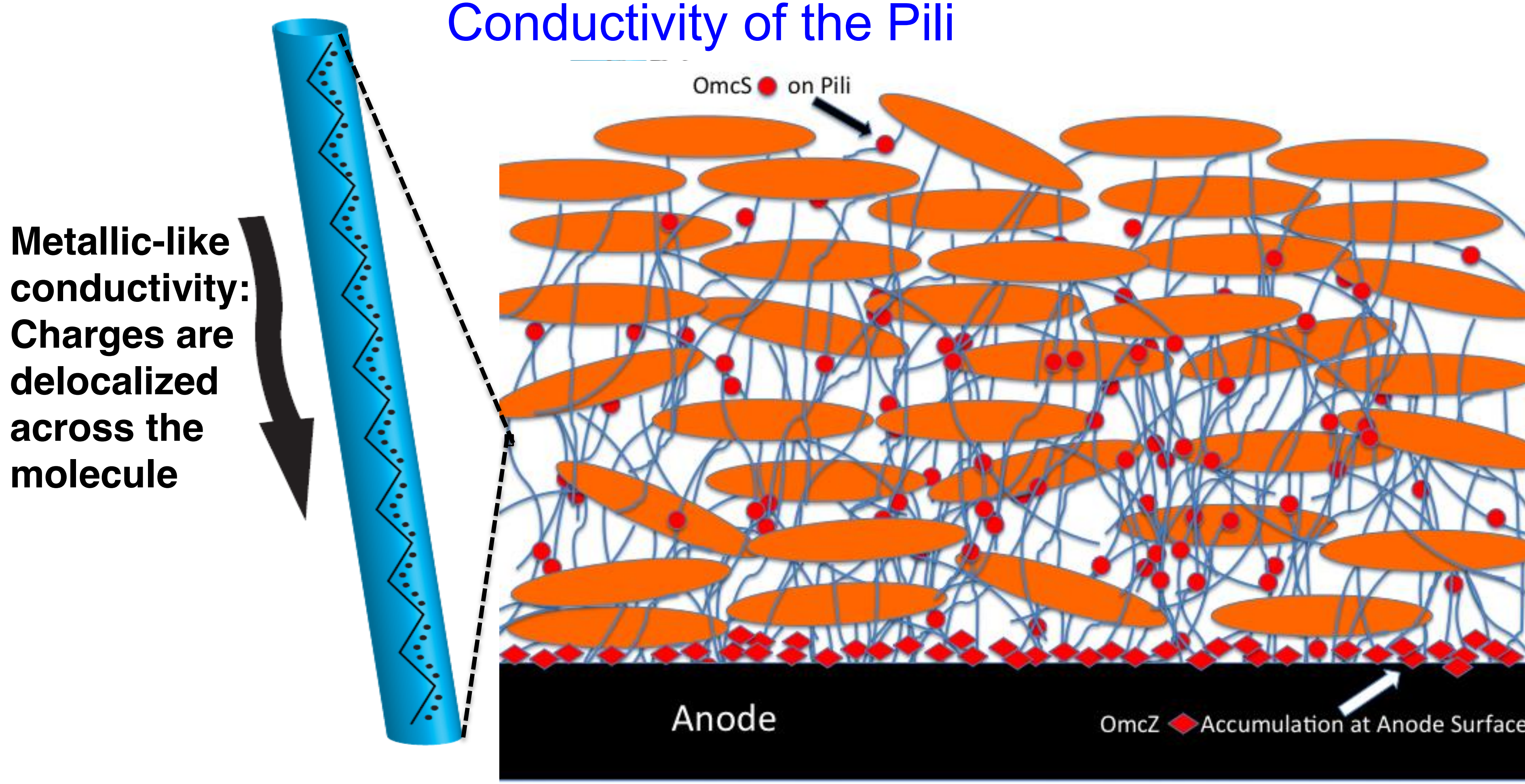
Geobacter sulfurreducens



Potential Explanations for the Difference:

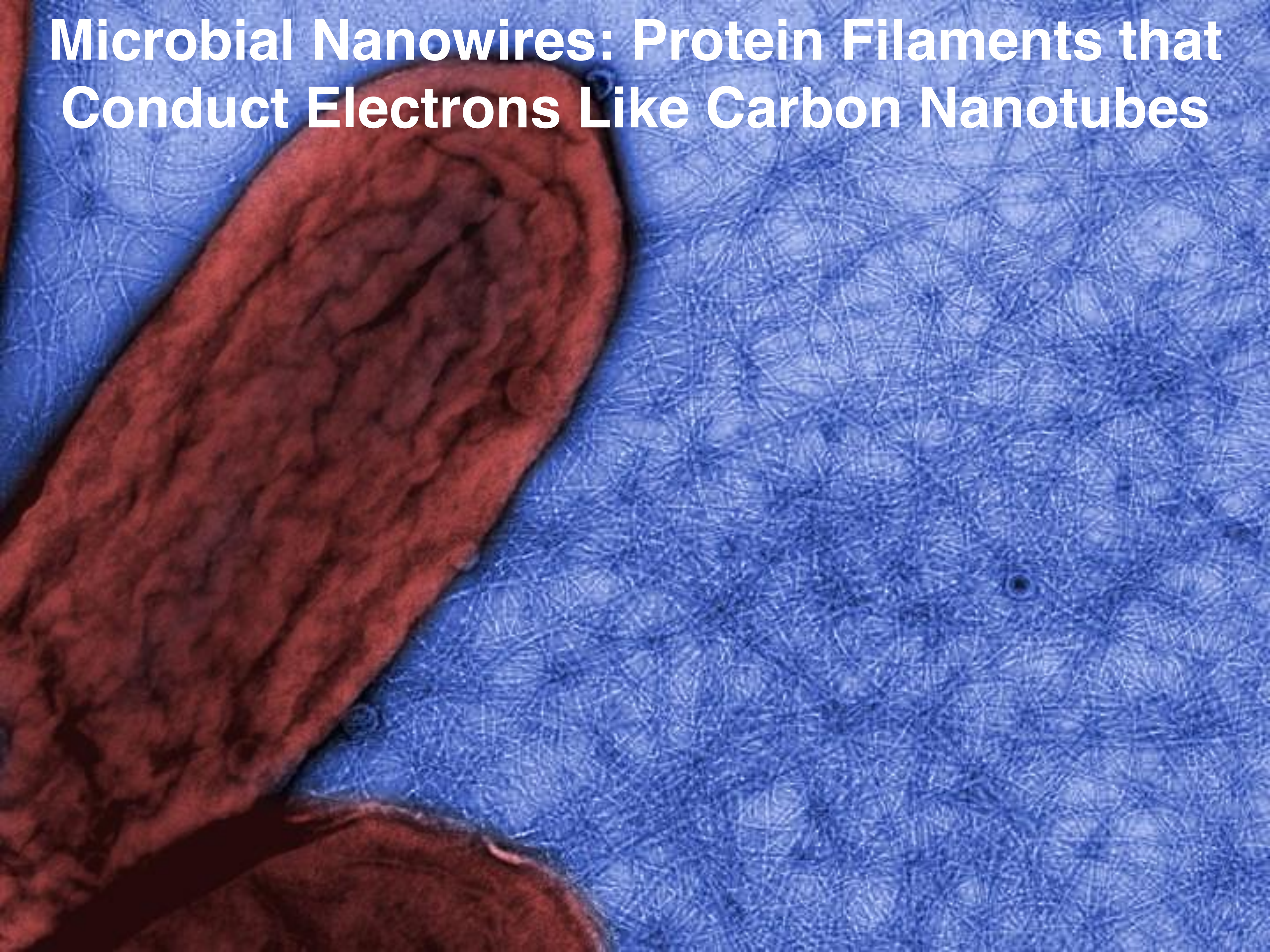
1. Poor ability of *C. ljungdahlii* to attach to surfaces
2. Inability of *C. ljungahlii* to produce conductive biofilms

Multiple Lines of Previously Published Evidence Suggest that Long-Range Electron Transfer Through Anode Biofilms of *Geobacter sulfurreducens* can be Attributed to the Metallic-Like Conductivity of the Pili

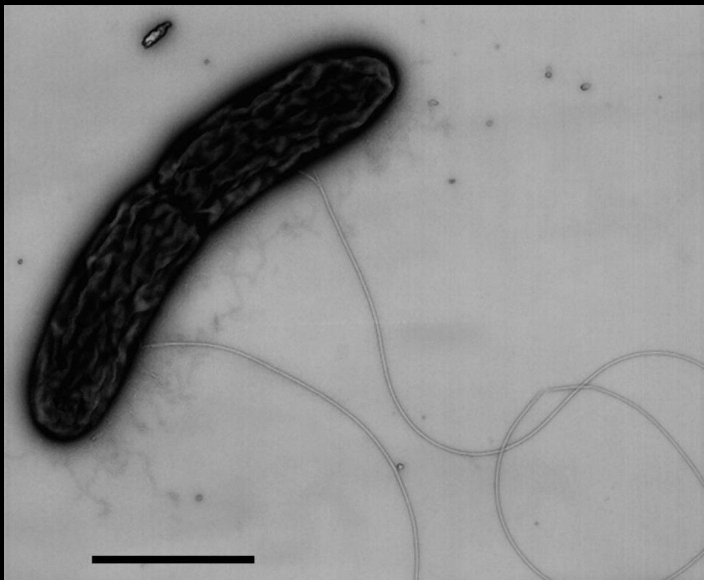


Reguera et al 2005. *Nature* 435:1098-1101
Malvankar et al. 2011. *Nature Nanotechnology* 6:573-579.
Vargas et al. 2013. mBio 4: 00105-13.

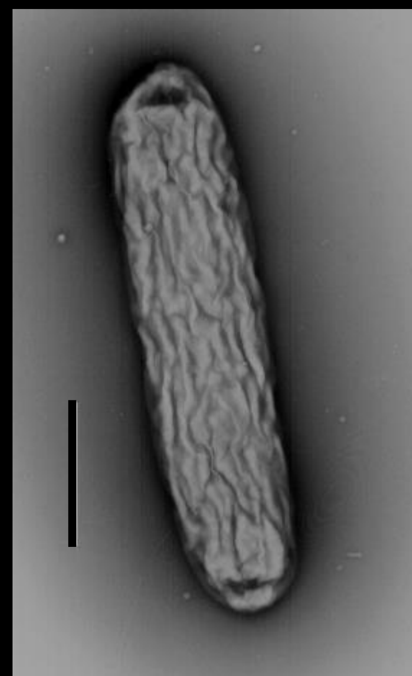
Microbial Nanowires: Protein Filaments that Conduct Electrons Like Carbon Nanotubes



Pili and Flagella are Specifically Produced When *Geobacter* are Grown on Insoluble Electron Acceptors



Mn(IV) Oxide



Fe(III) citrate

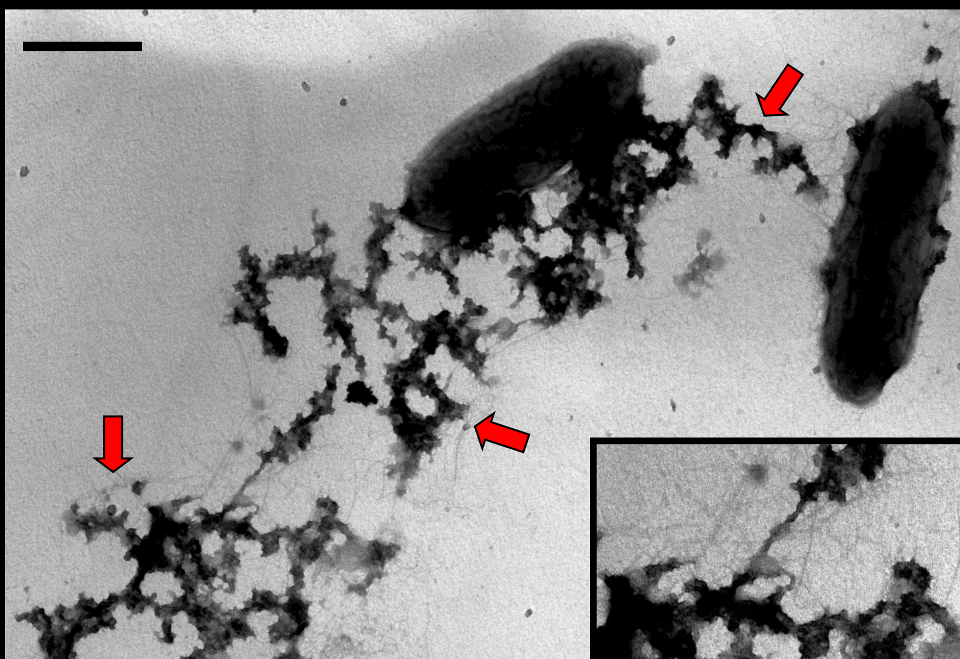
Fe(III) Oxide

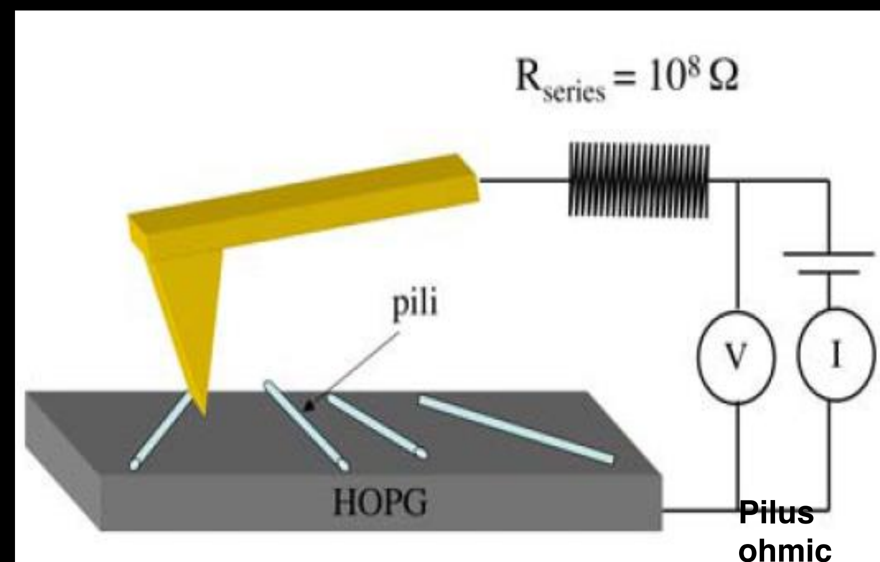
Bar=1um

Childers S.E., S. Ciufo, and D. R. Lovley. 2002. *Geobacter metallireducens* access Fe(III) oxide by chemotaxis. Nature 416:767-769.

Initial Evidence that *Geobacter* Pili are Conduits for Long-Range Electron Transport to Fe(III)

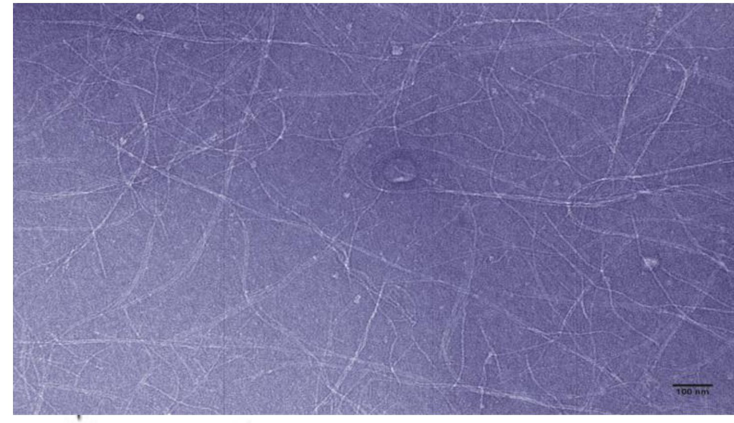
- Pilin genes specifically expressed during growth on Fe(III) oxide
- Knocking out pili gene eliminates Fe(III) oxide reduction
- Fe(III) oxide associated with pili
- Pili are electrically conductive across their width



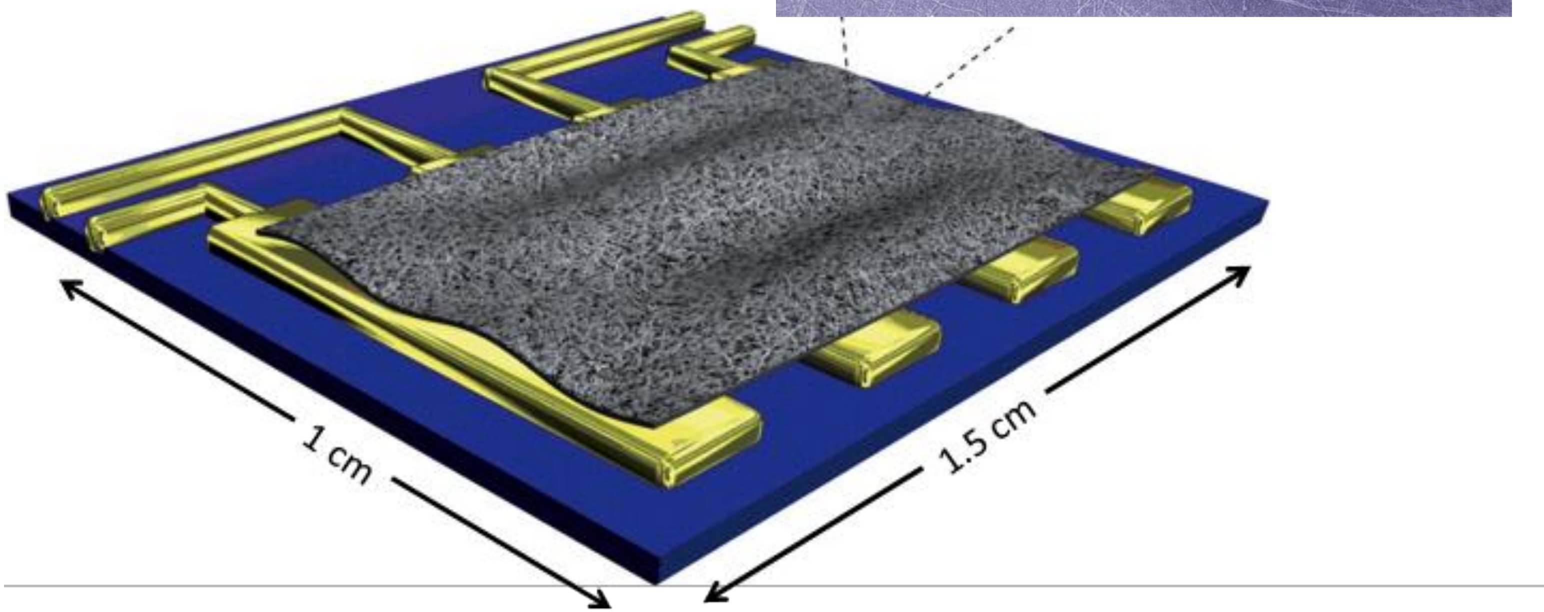


G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen, and D. R. Lovley 2005. Extracellular electron transfer via microbial nanowires. *Nature* 435:1098-1101.

Demonstration of Long-Range Conduction Through a Network of *Geobacter* Pili

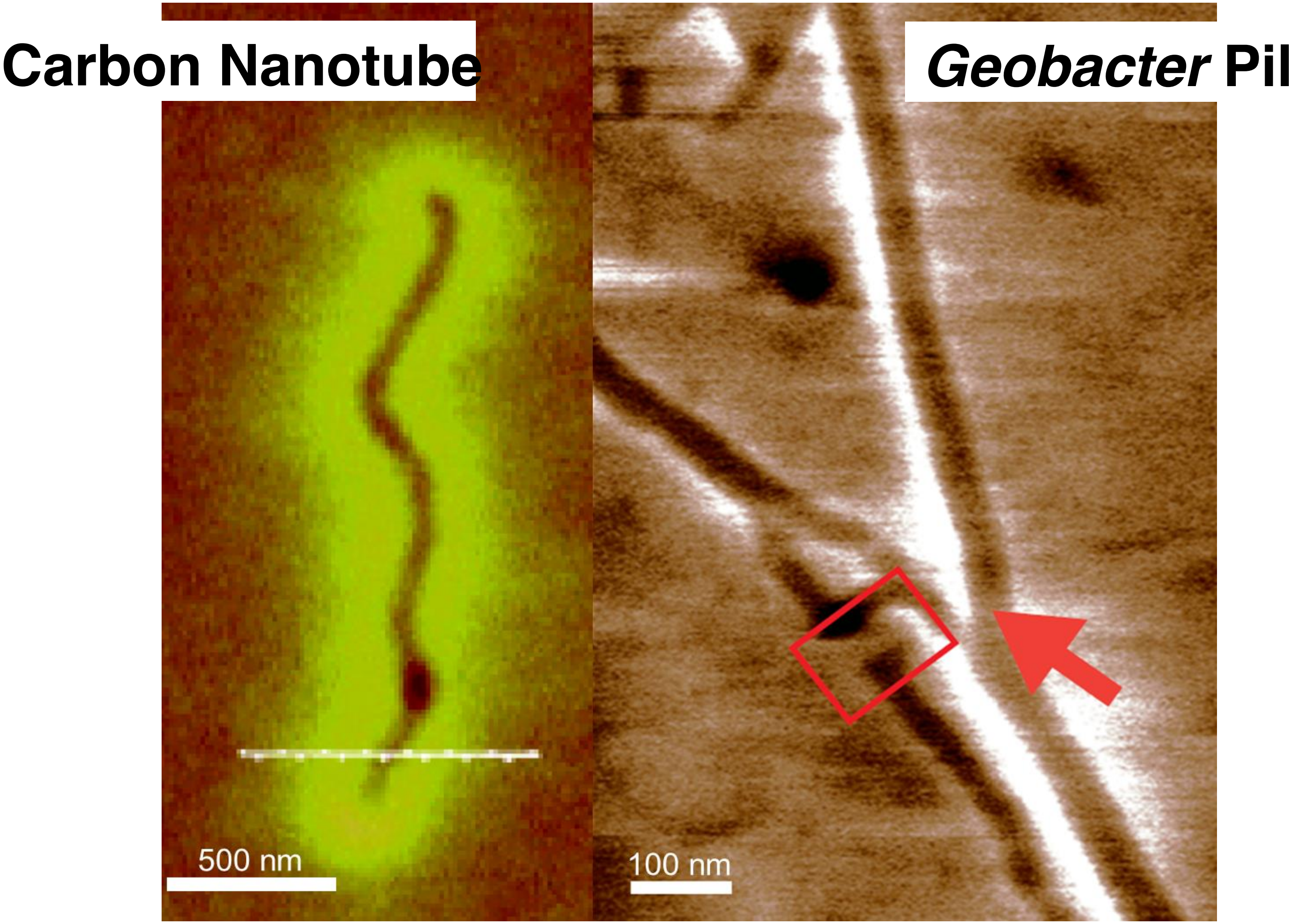


Physiologically Relevant Conditions:
No chemical fixative
Pili still hydrated



Malvankar, N., M. Vargas, K. P. Nevin, A. E. Franks, C. Leang, B.-C. Kim, K. Inoue, T. Mester, S. F. Covalla, J. P. Johnson, V. M. Rotello, M. T. Tuominen, and D. R. Lovley. 2011. Tunable metallic-like conductivity in nanostructured biofilms associated with microbial nanowires. Nature Nanotechnology 6:573-579.

Electrostatic Force Microscopy Revealed that Charges Propagate in *Geobacter* Pili Similar to Carbon Nanotubes and Consistent with Metallic-Like Conductivity



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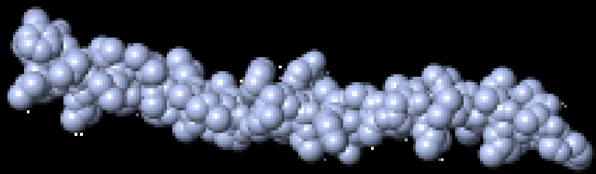
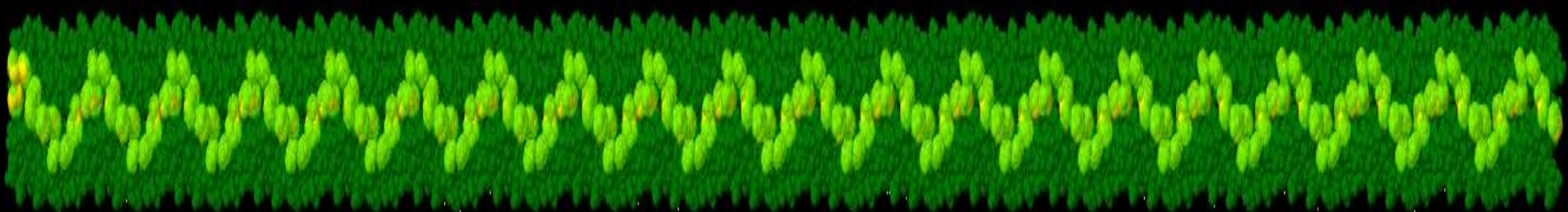
Modeling of the *G. sulfurreducens* Pilus Structure Predicts the Close (3-4 Å) Packing of Aromatics Packing Required for Metallic-Like Conductivity





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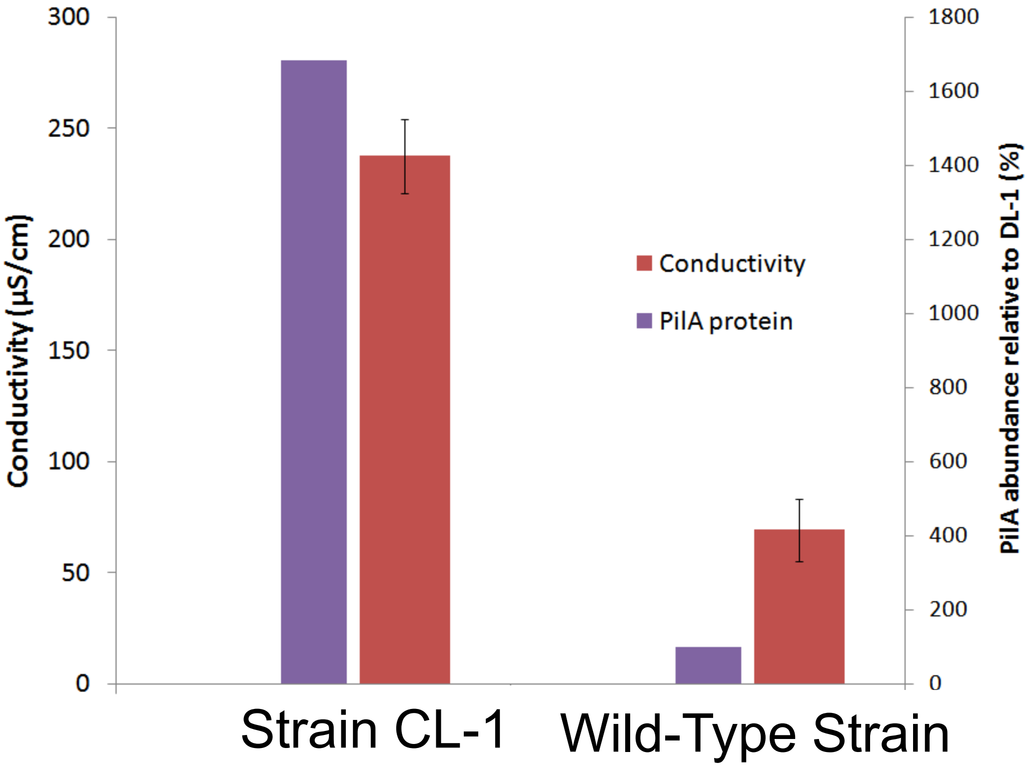
Genetically Modifying Gene Regulation Resulted in Strain CL-1 Which Over Expresses Nanowires

Strain CL-1 Produces Highly Cohesive Biofilms that are More Conductive and Generate Higher Power Densities



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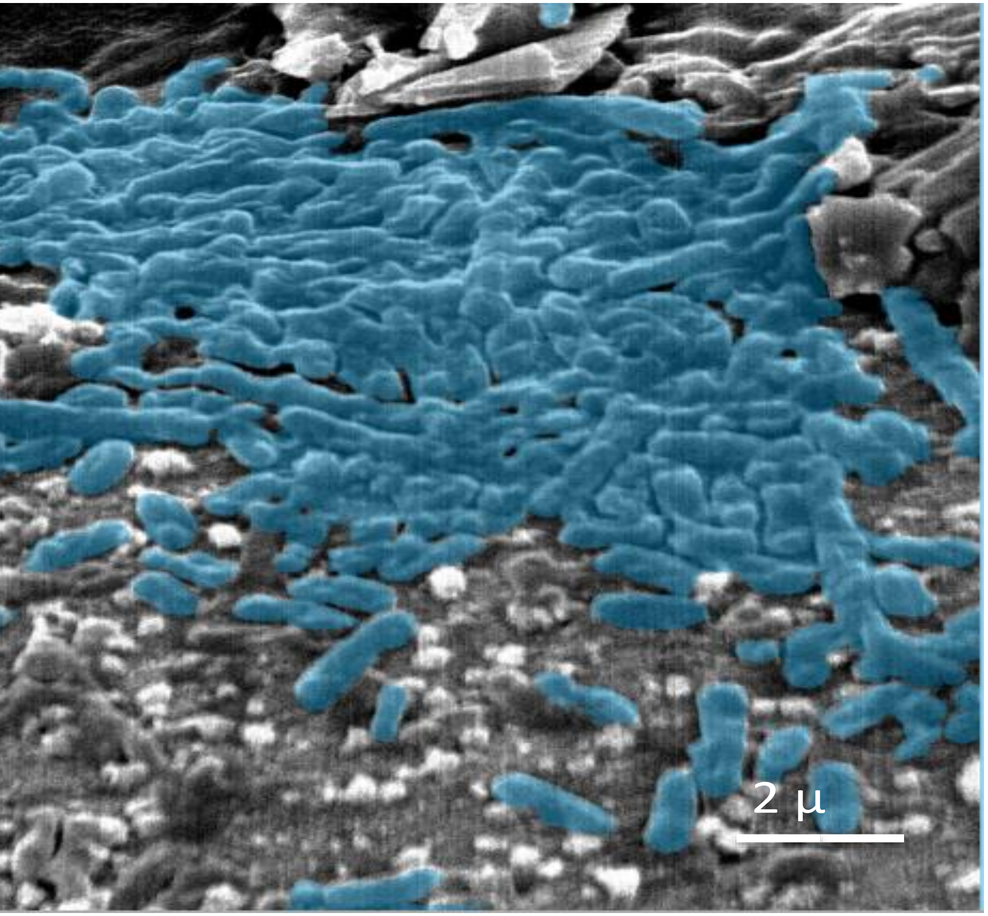
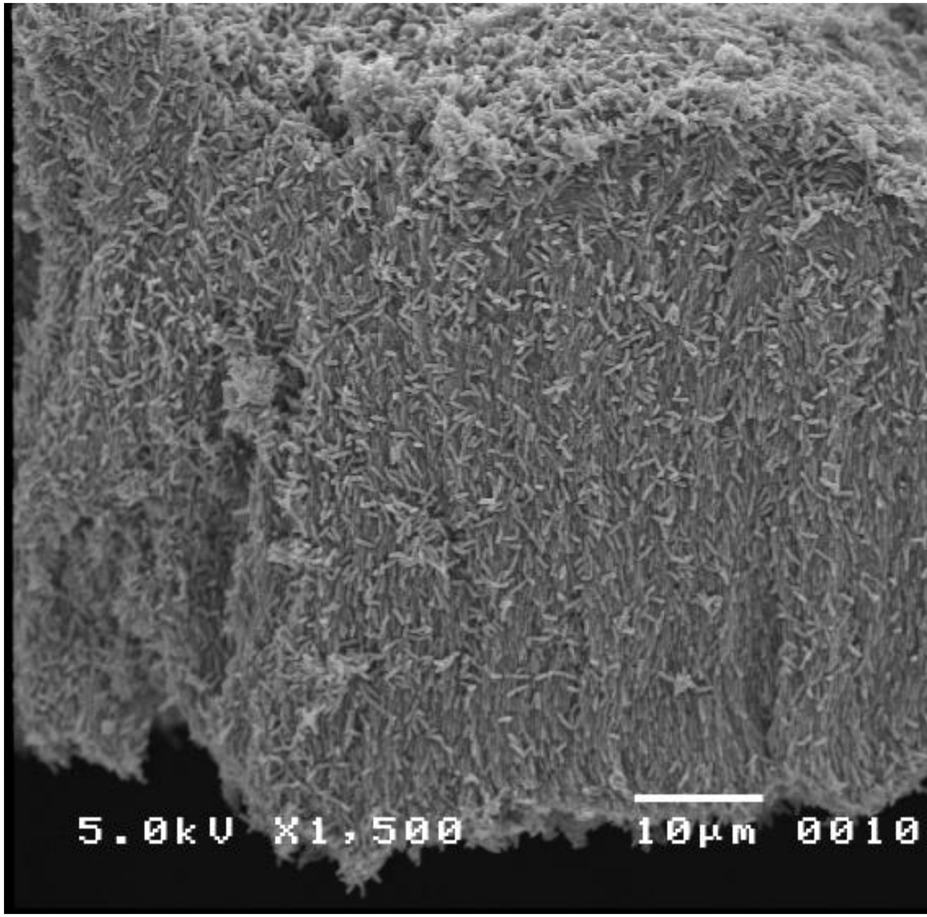
Will Installing Conductive Pili in *Clostridium ljungdahlii* Enable Production of Thick Electrically Conductive Biofilms?

Clostridium ljungdahlii



Geobacter sulfurreducens



Summary

- Promoting DIET with conductive materials may enhance methanogenic digestion rate and stability

- Microbial electrosyntheis shows promise for converting carbon dioxide produced during anaerobic digestion (or from other sources) can be converted to organic commodities with renewable electrical energy

- Production of commodities from carbon dioxide that are more valuable than methane will require synthetic biology approaches to engineer new pathways for carbon and electron flow and enhance electrosynthesis rates