Karlsruher Institut für Technologie

Microbe-Electrode-Interactions

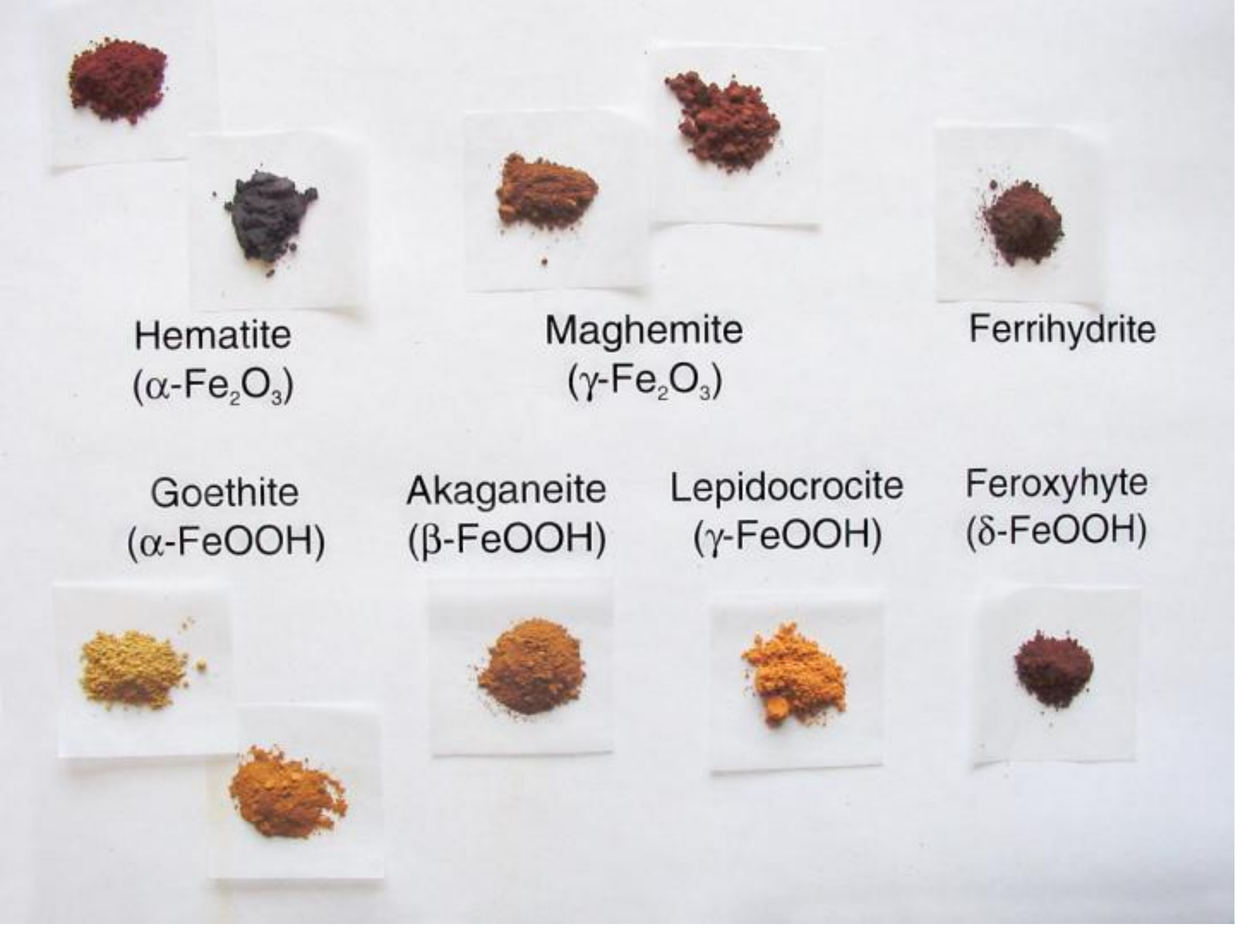
Johannes Gescher

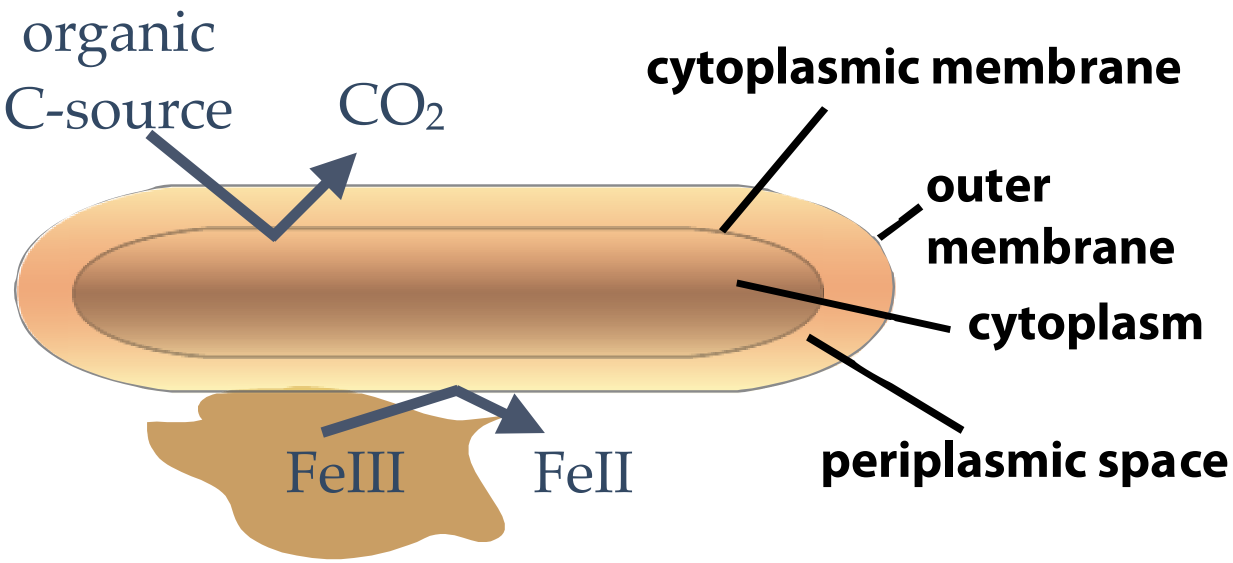
Institut for Applied Biosciences, Applied Biology

dissimilatory iron reduction - electrodes (Anodes) as electron acceptors

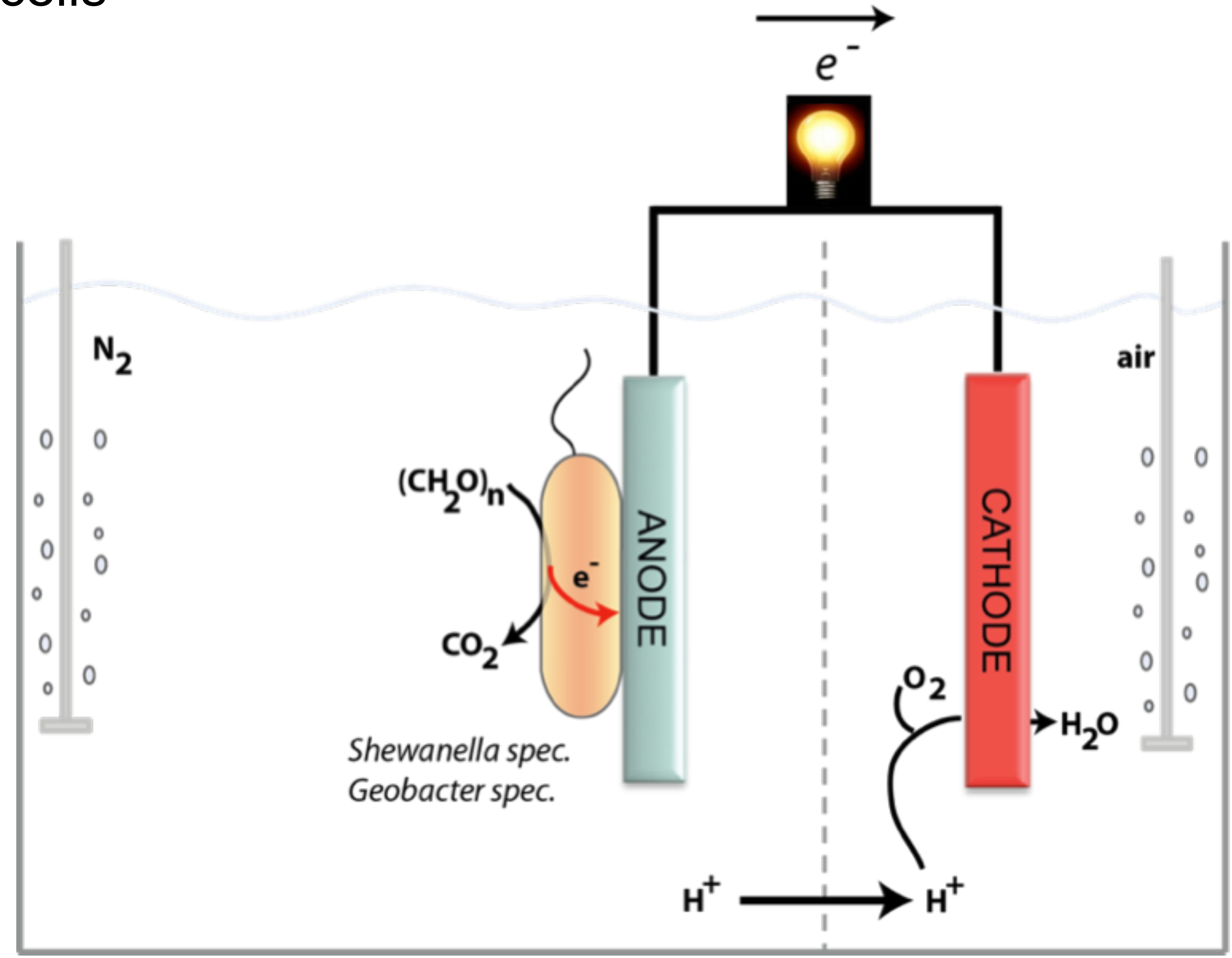
- at pH 7 and 25°C →
  ferrihydrite/Fe^{2+} $E_0{}' = +24$ mV
  hematite/Fe^{2+} $E_0{}' = -177$ mV

environmental iron forms are mostly insoluble minerals

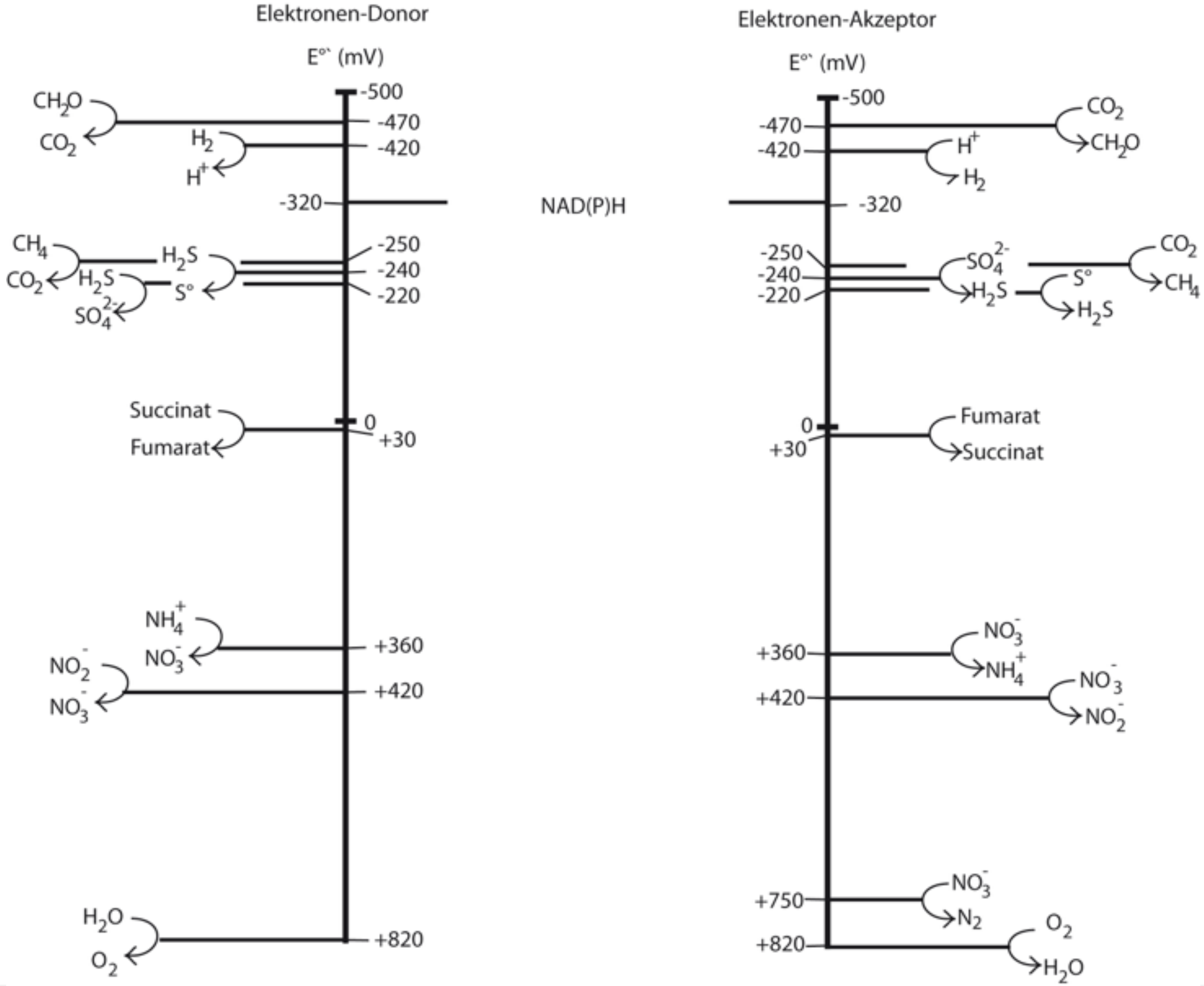




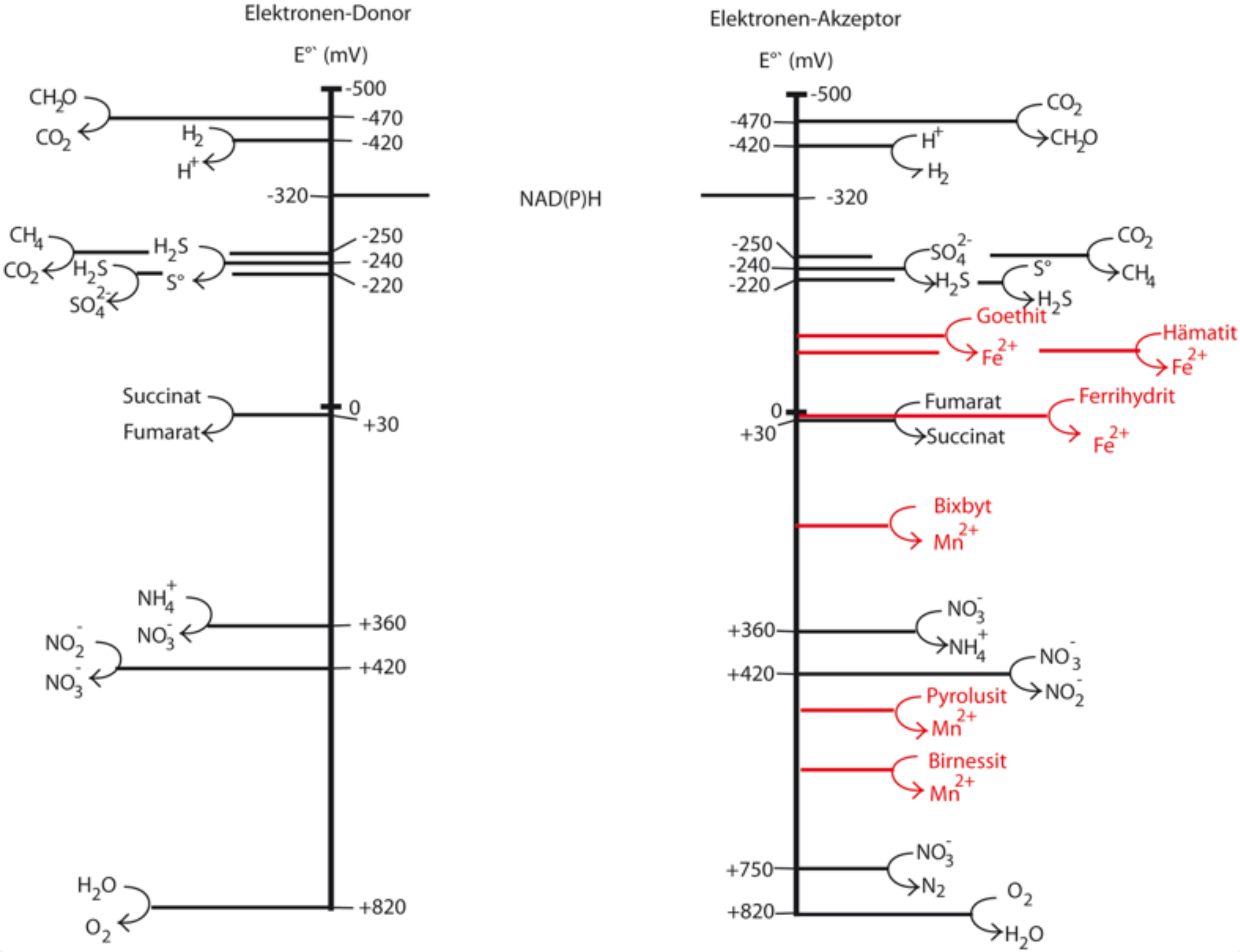
the concept of microbial fuel cells



redox-scales



redox-scales



redox-scales

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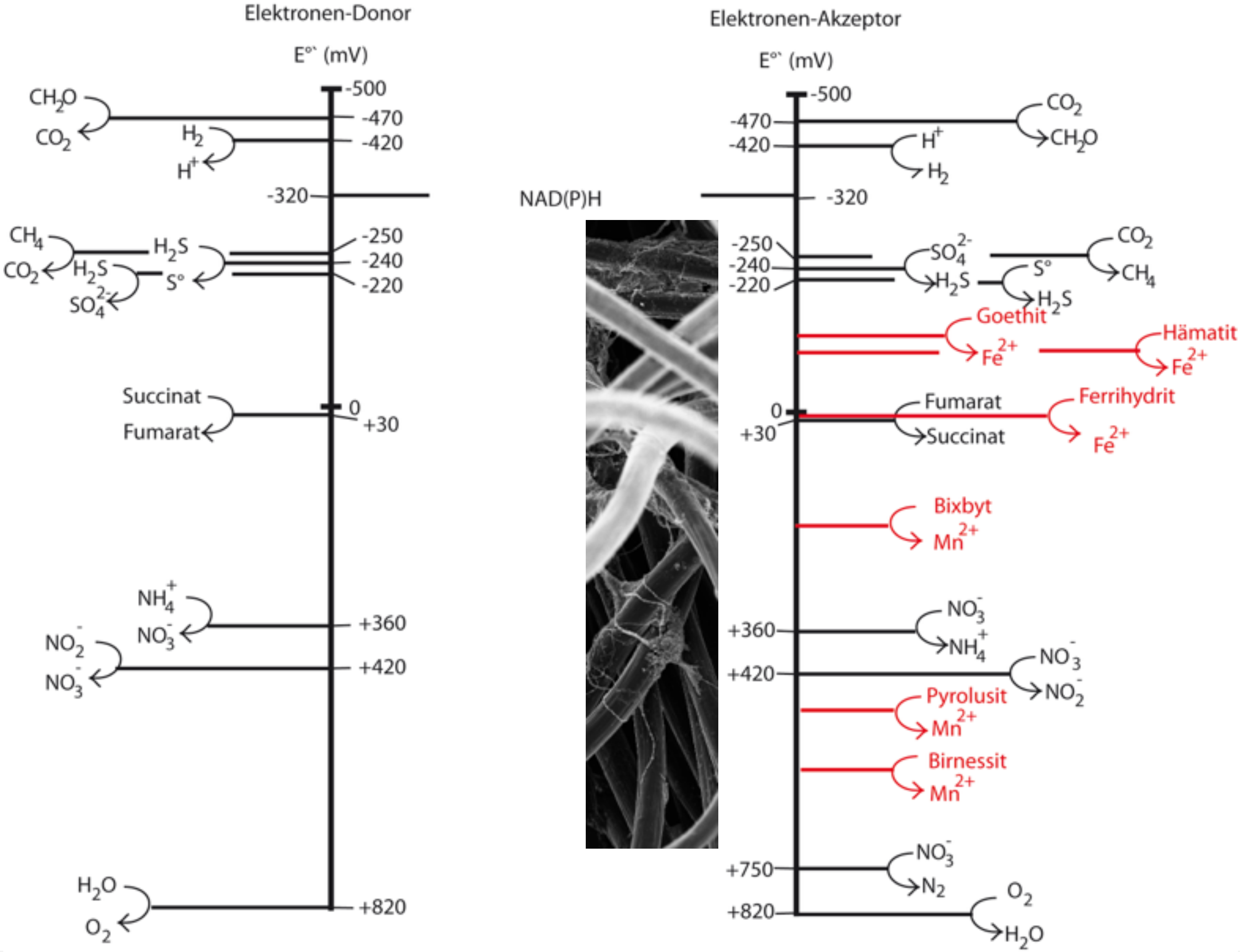

electron transfer rates can be regulated by the anode potential

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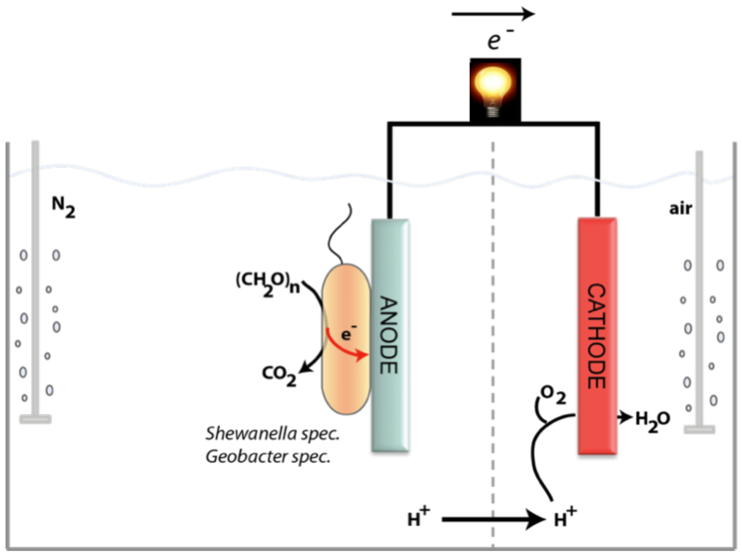
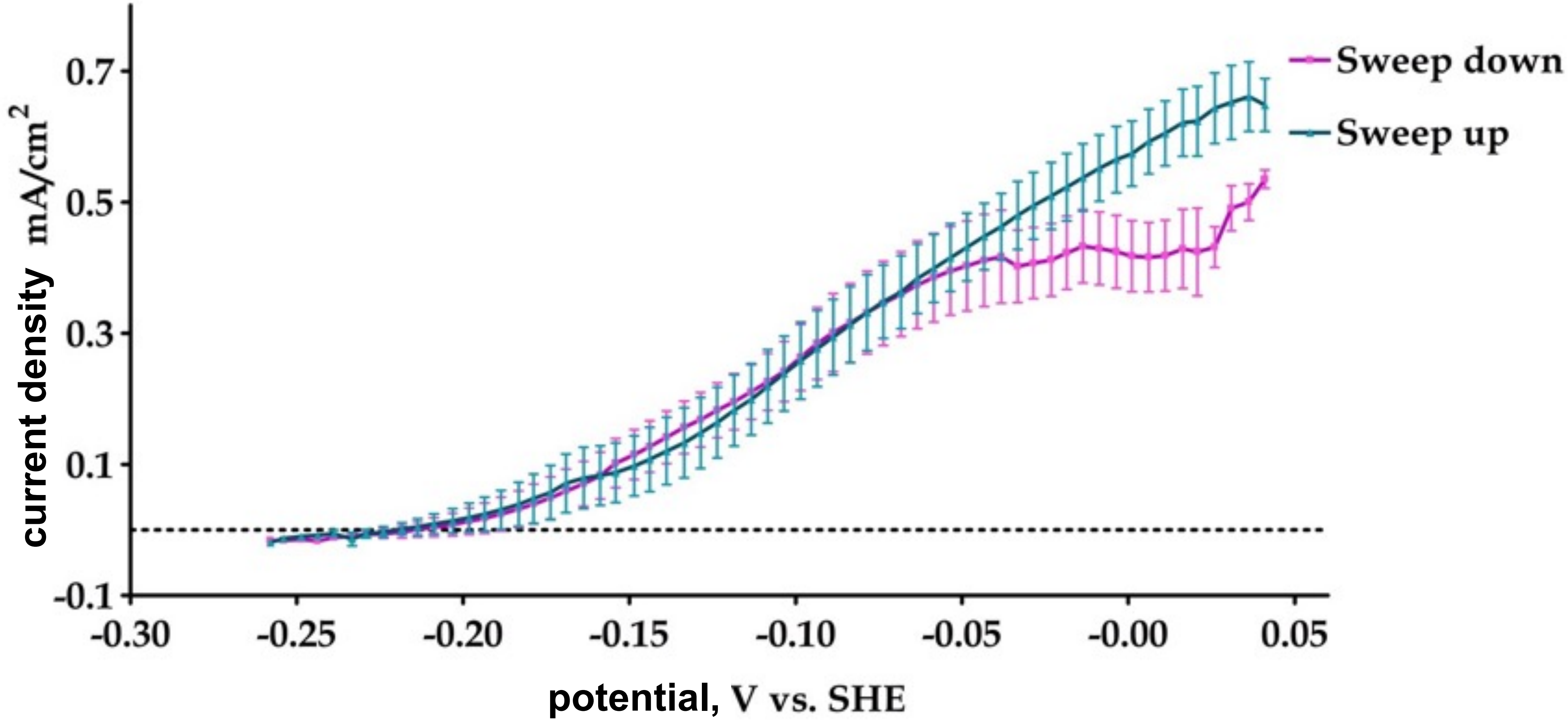
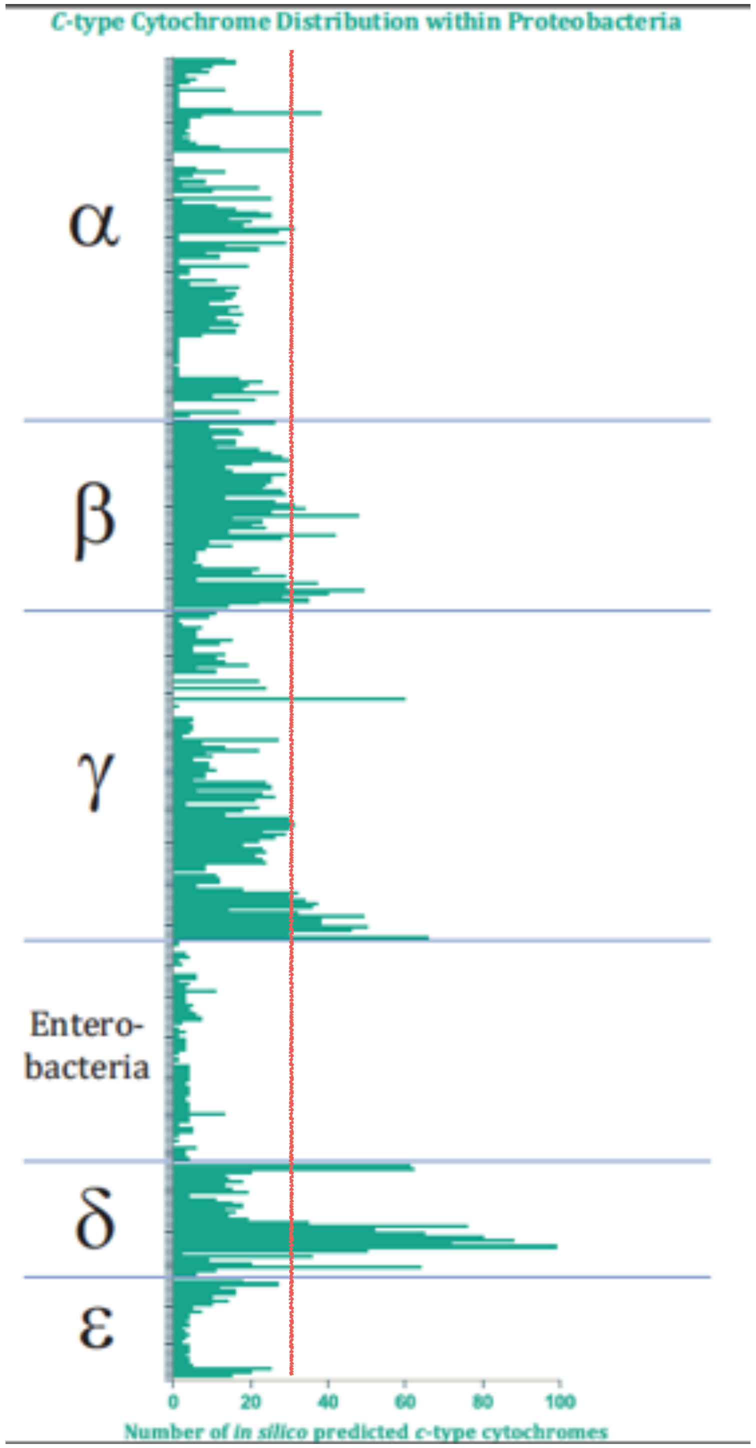
- ➢ sweep rate = 0,75 µV/s
- ➢ anode surface area = 2,25 cm^2
- ➢ working volume = 25 ml





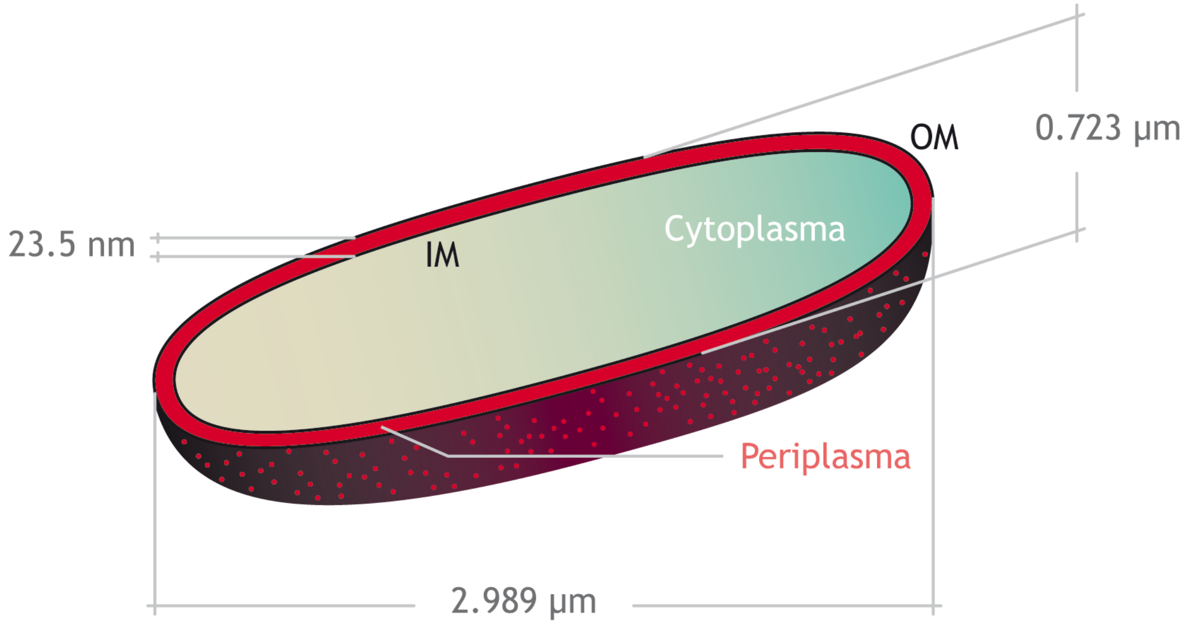
c-type cytochromes and iron/anode reducers



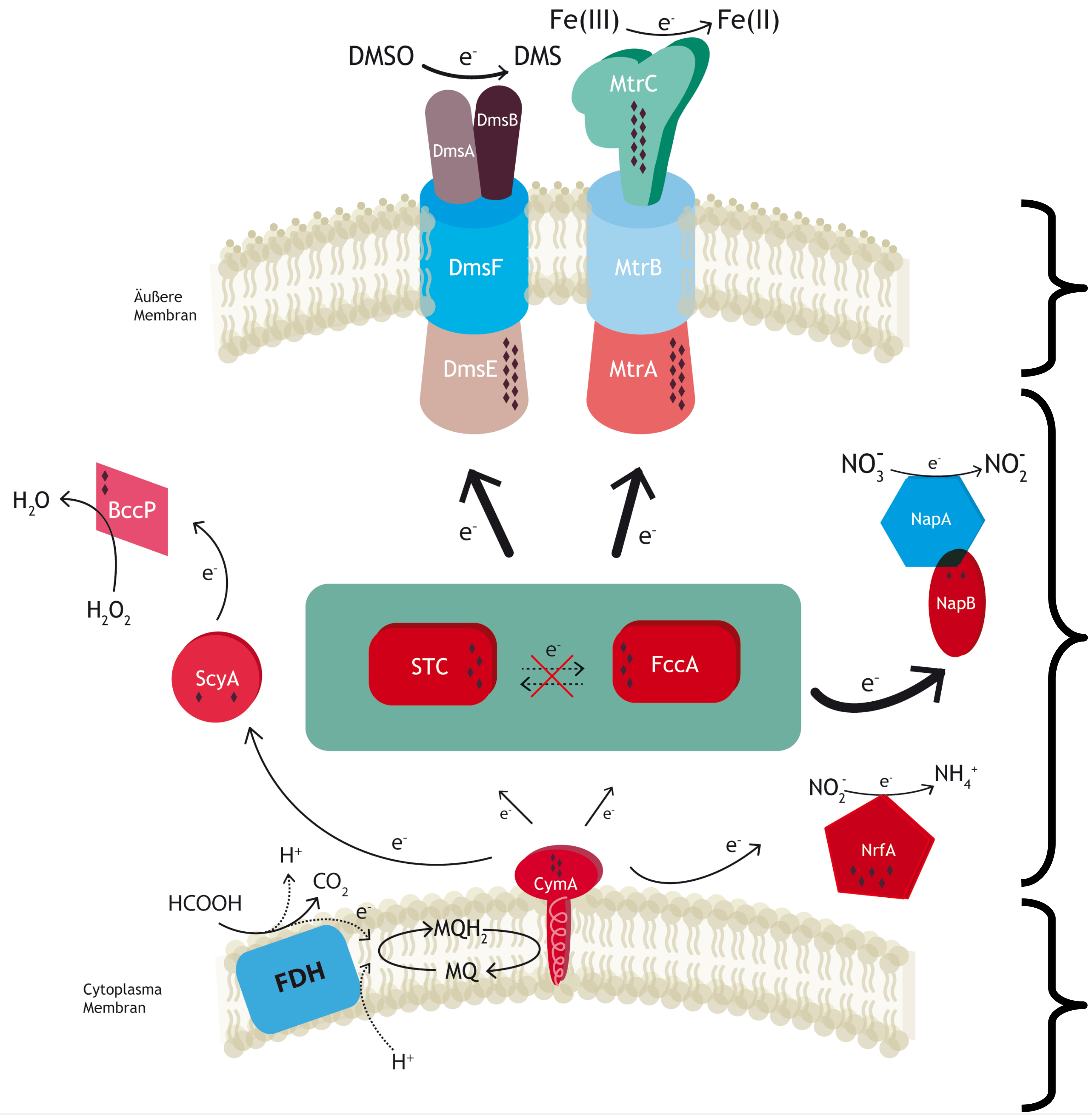
- average number of genes for c-Typ cytochromes in prokaryotes (n = 483 genomes): **13**
- *S. oneidensis:* **41**, *Geobacter sulfurreducens* **111**

structural preconditions for extracellular electron transfer in *S. oneidensis*

- periplasmic volume: **0.153 fl**
- **680.000** hemegroups per *S. oneidensis cell*
- periplasmic heme-concentration **3,8 mM**



a c-type cytochrome based electron transfer network

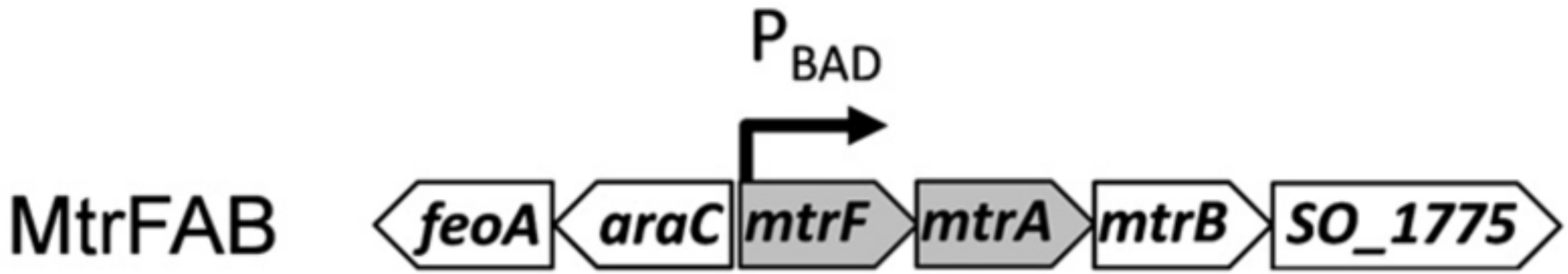


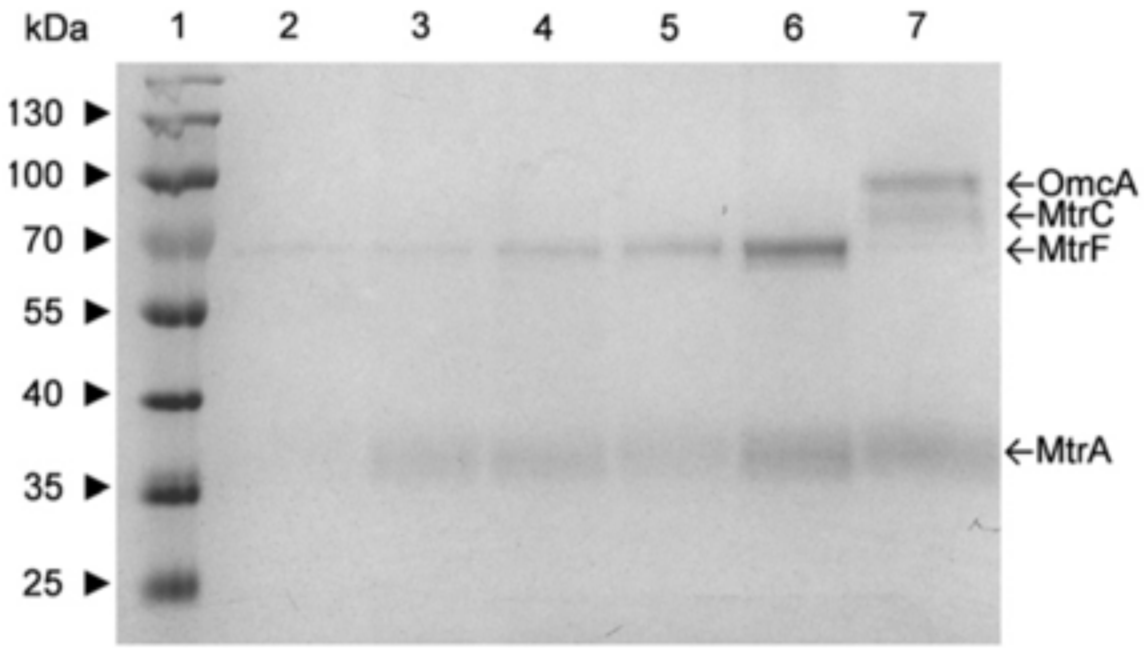
outer membrane

periplasm

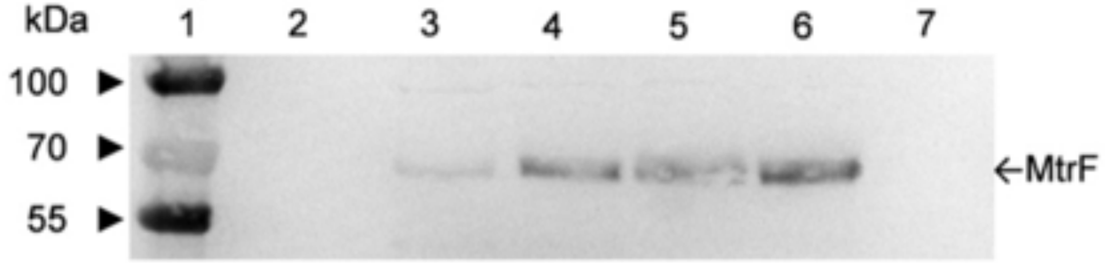
cytoplasmic membrane

using exoelectrogens as biosensors



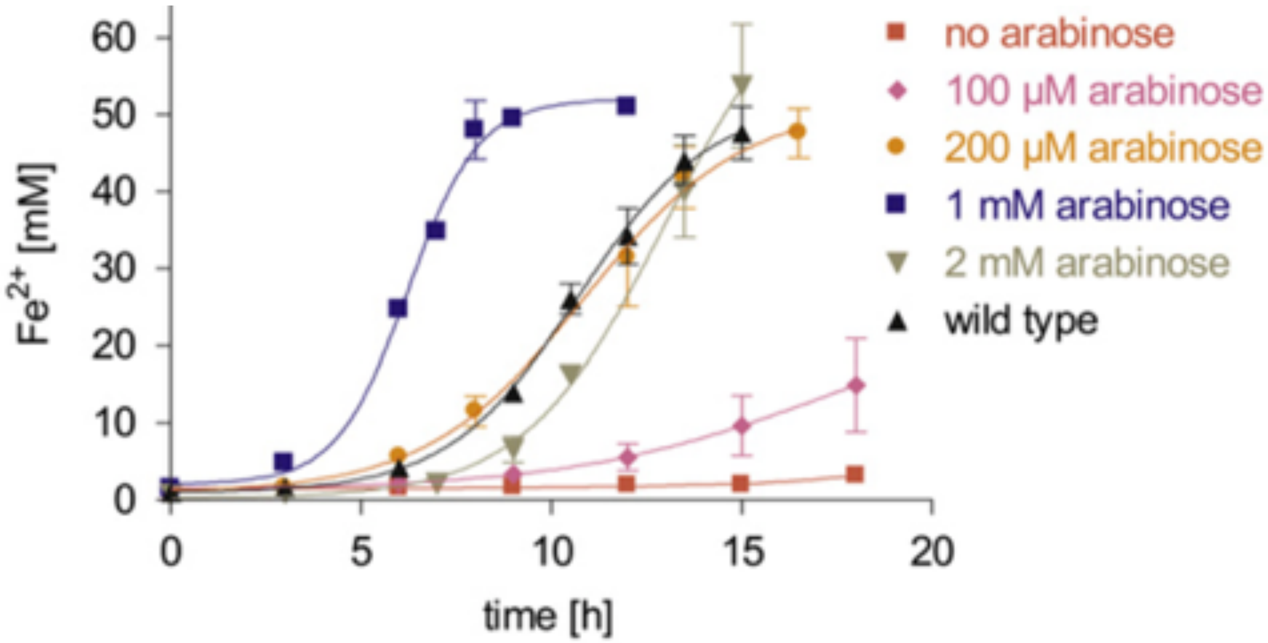


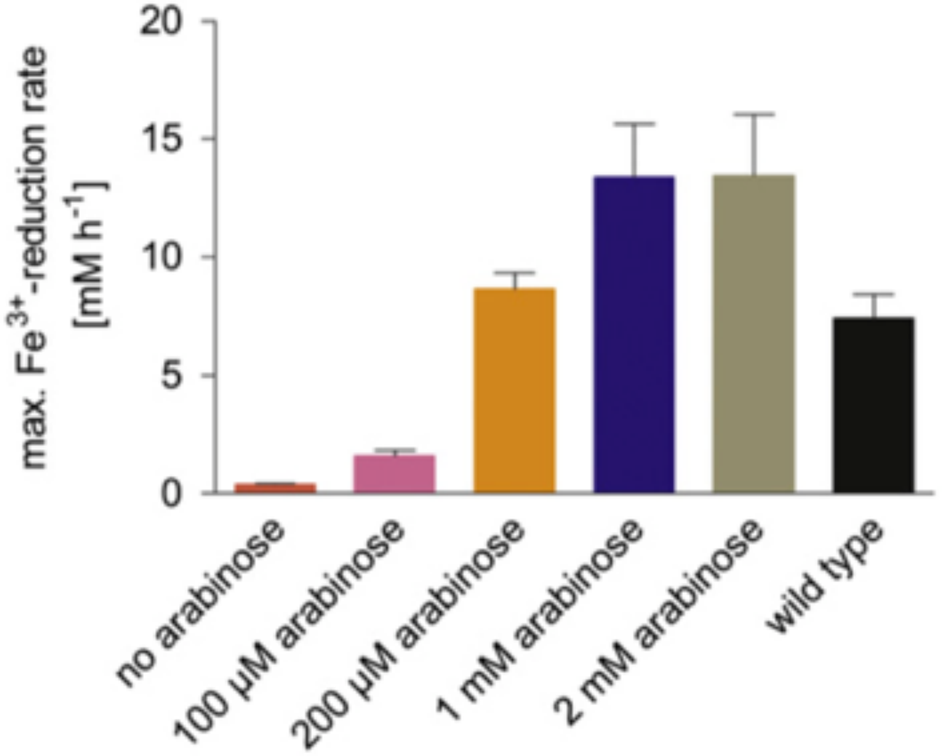
heme stain



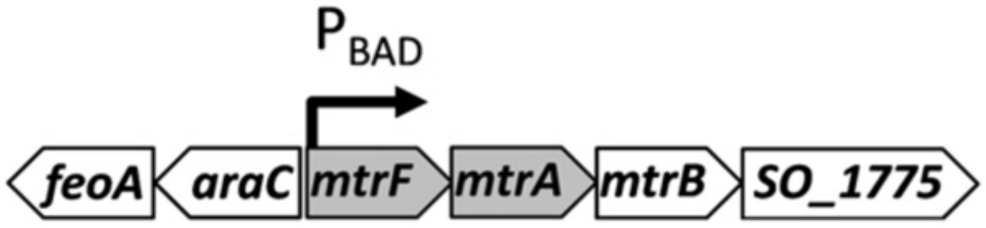
spec. MtrF-detection

arabinose dependent iron reduction

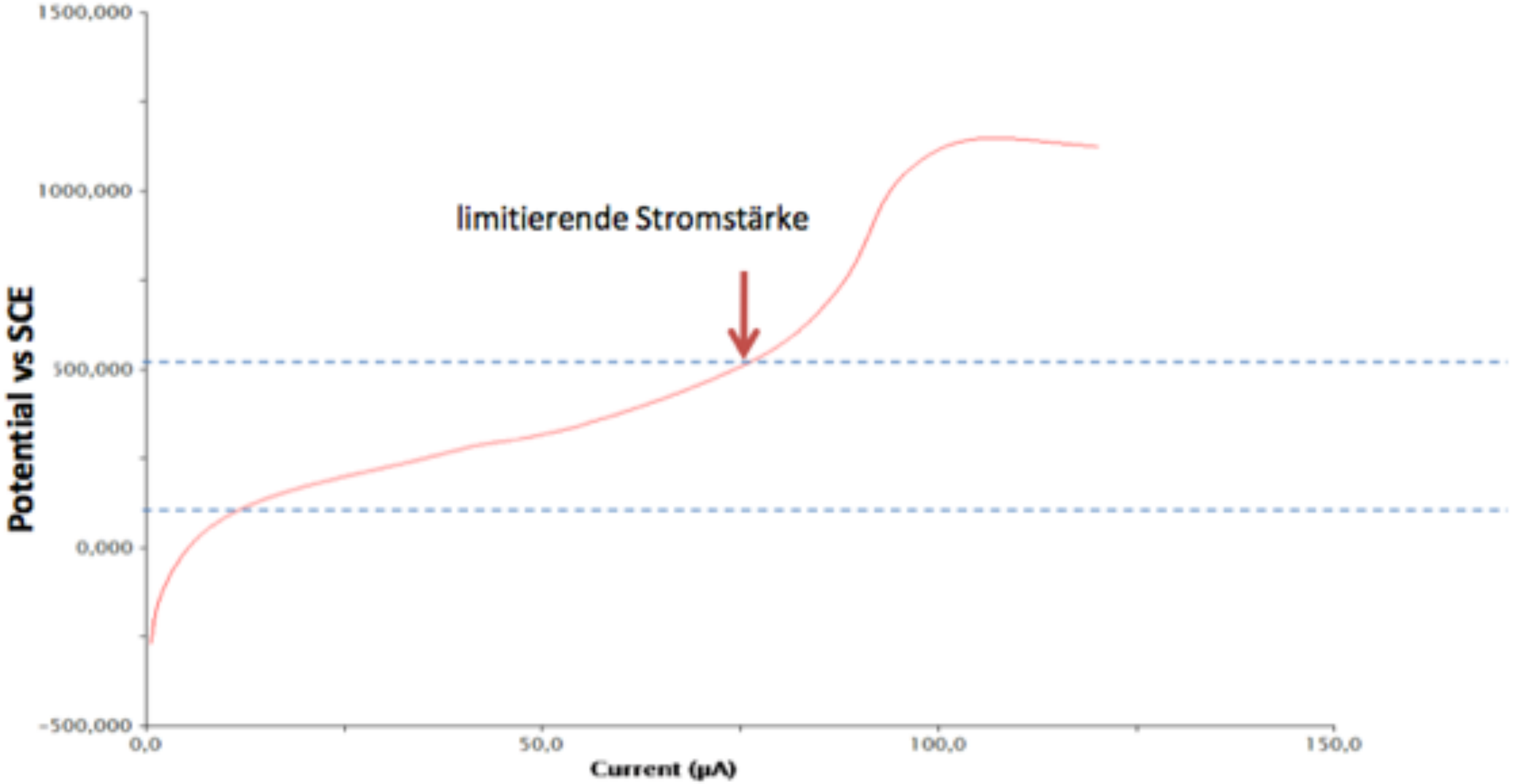




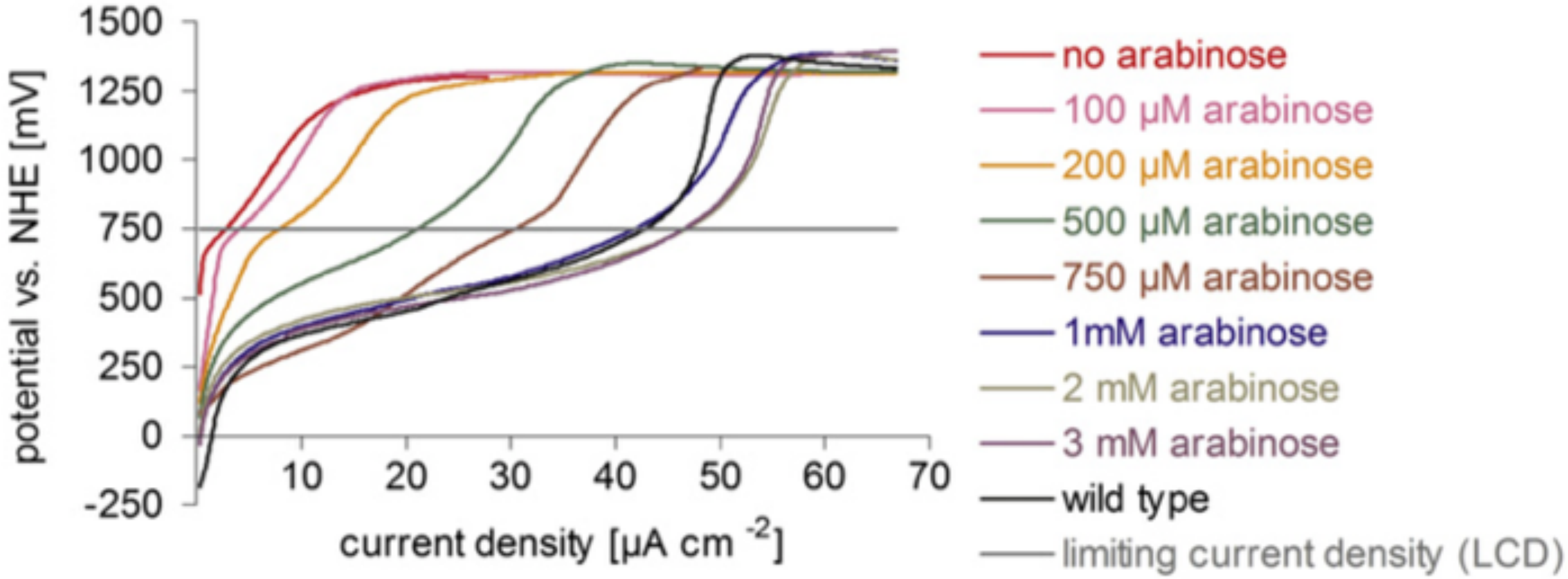
MtrFAB



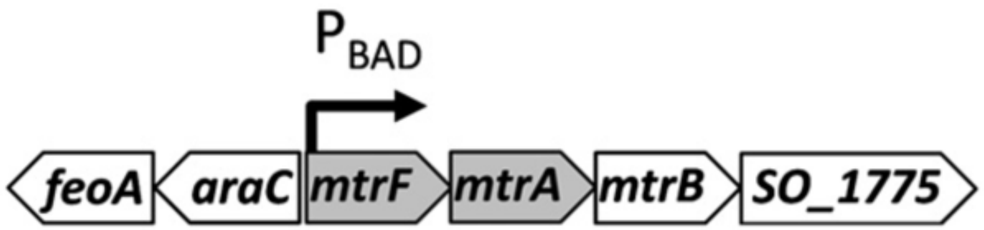
characterization of strains using polarization curves



arabinose biosensor

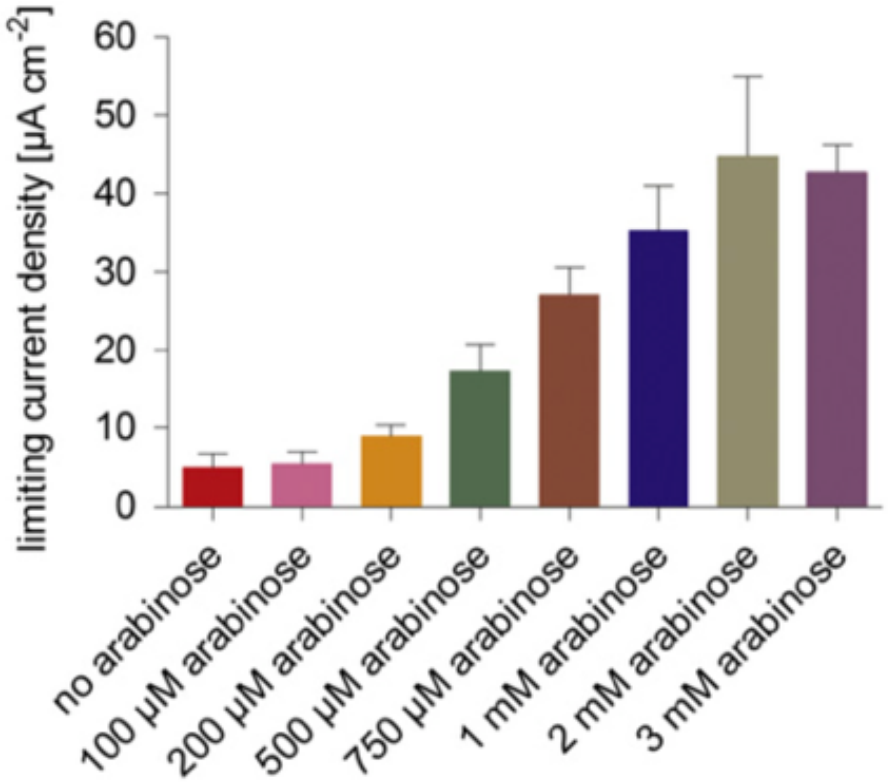


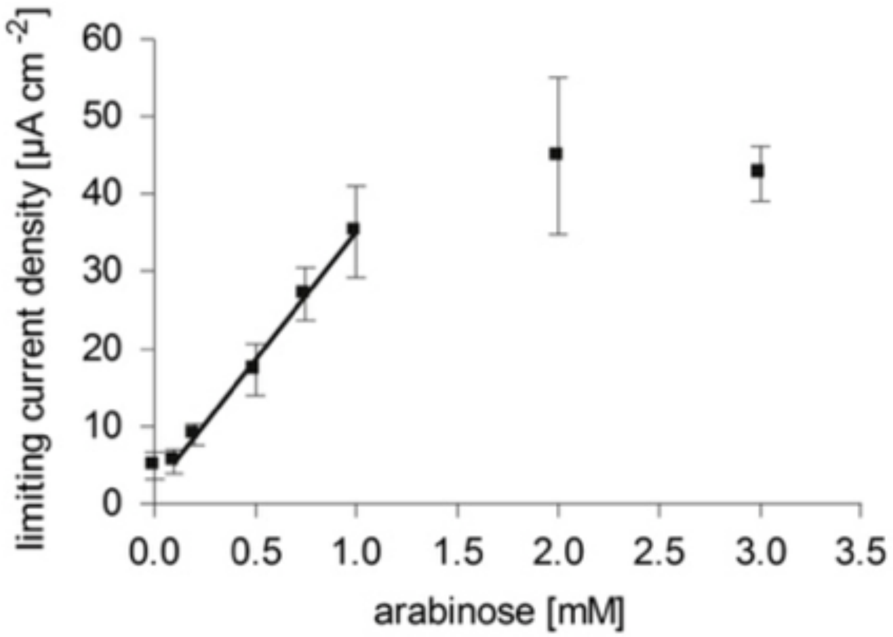
MtrFAB



Golitsch et al., Biosens. and Bioelectr. 2013
EP 12183209.1

arabinose biosensor





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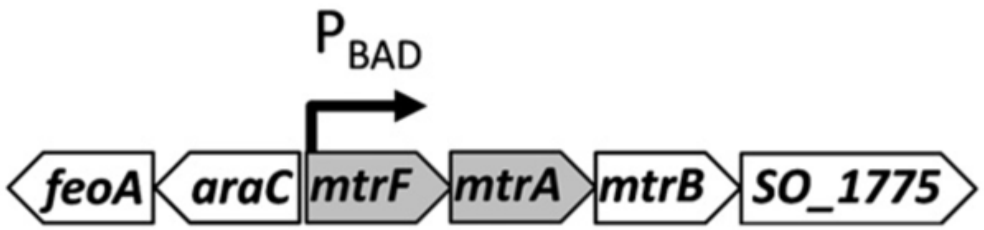

Golitsch et al., Biosens. and Bioelectr. 2013
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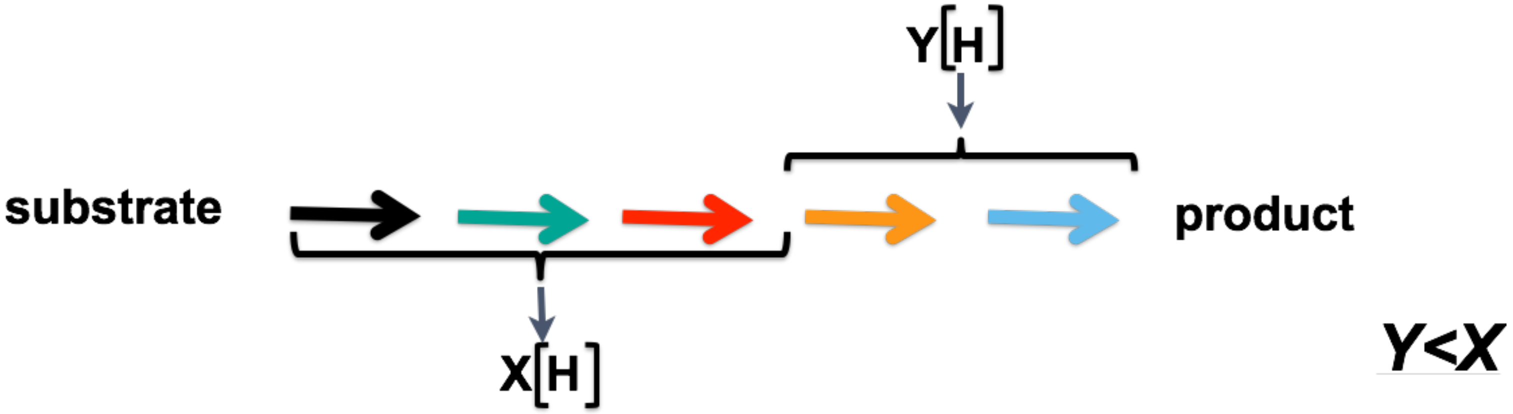
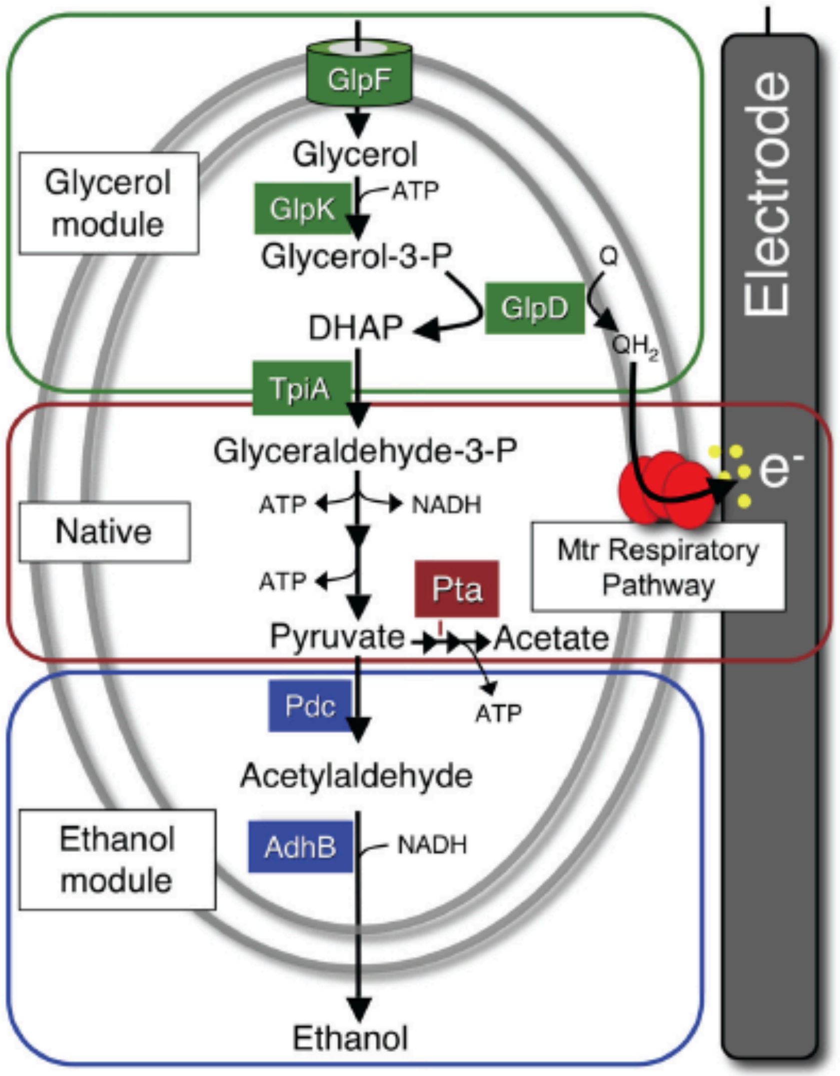
enabling unbalanced fermentations






RESEARCH ARTICLE

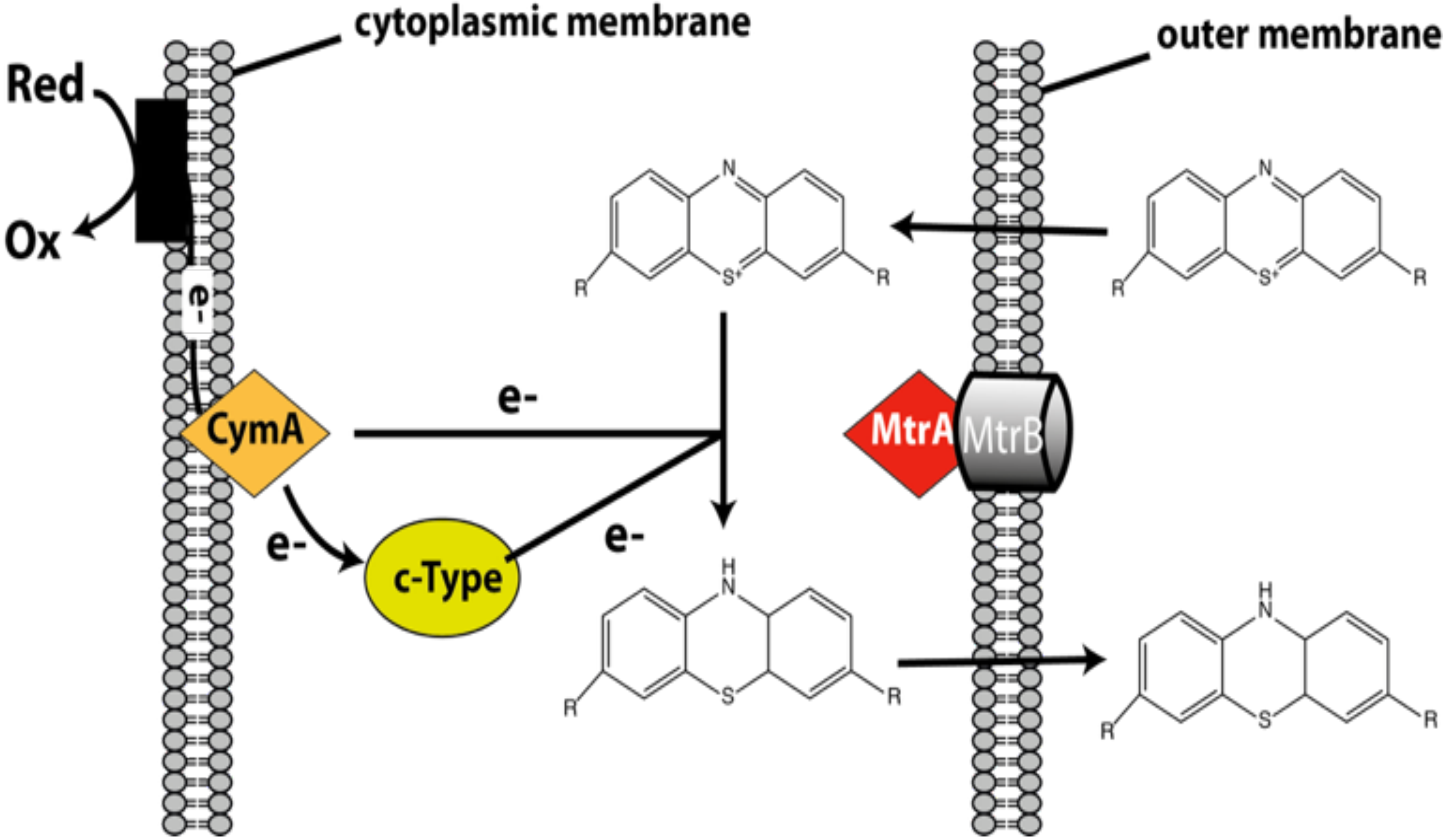
Enabling Unbalanced Fermentations by Using Engineered Electrode-Interfaced Bacteria

Jeffrey M. Flynn,[a] Daniel E. Ross,[a] Kristopher A. Hunt,[a] Daniel R. Bond,[a,b] and Jeffrey A. Gralnick[a,b]

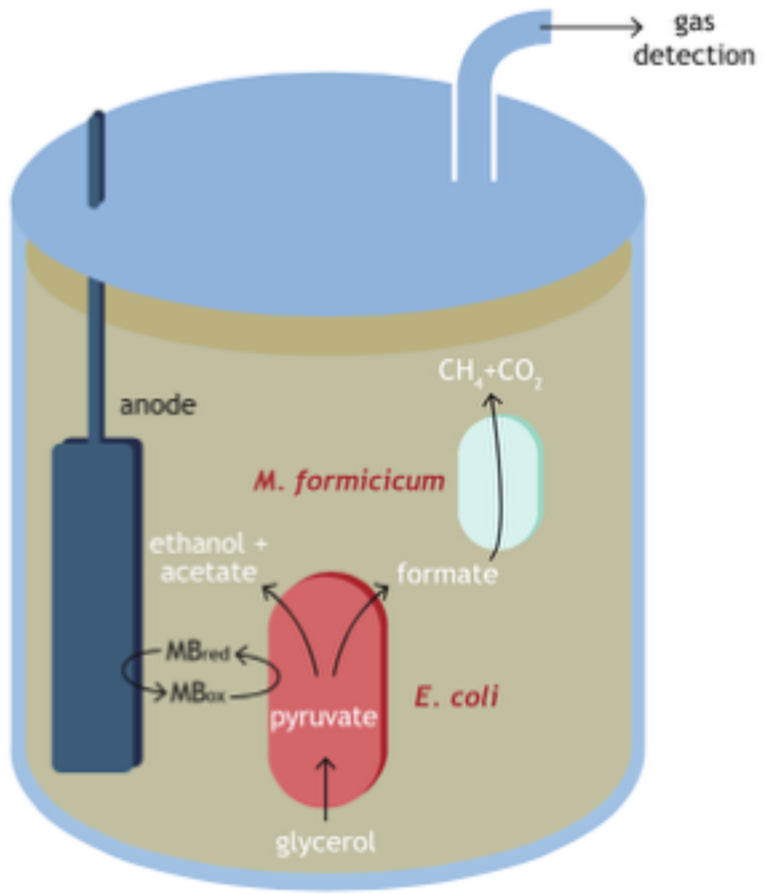
BioTechnology Institute[a] and Department of Microbiology,[b] University of Minnesota Twin Cities, St. Paul, Minnesota, USA

J.M.F. and D.E.R. contributed equally to this article.

enabling unbalanced fermentations in *E. coli*

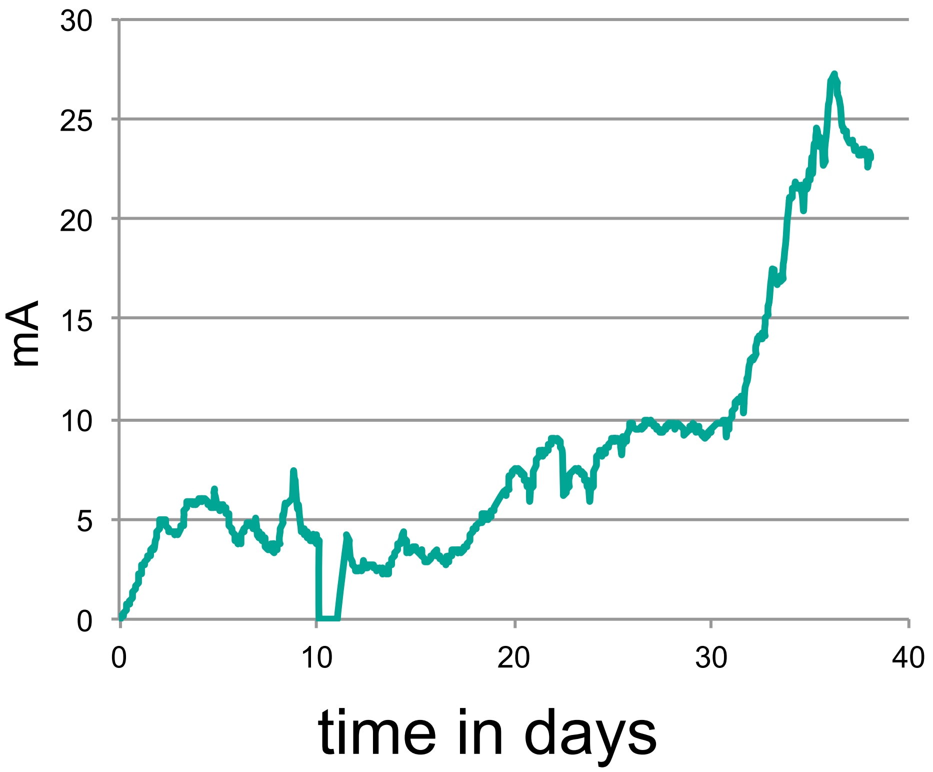


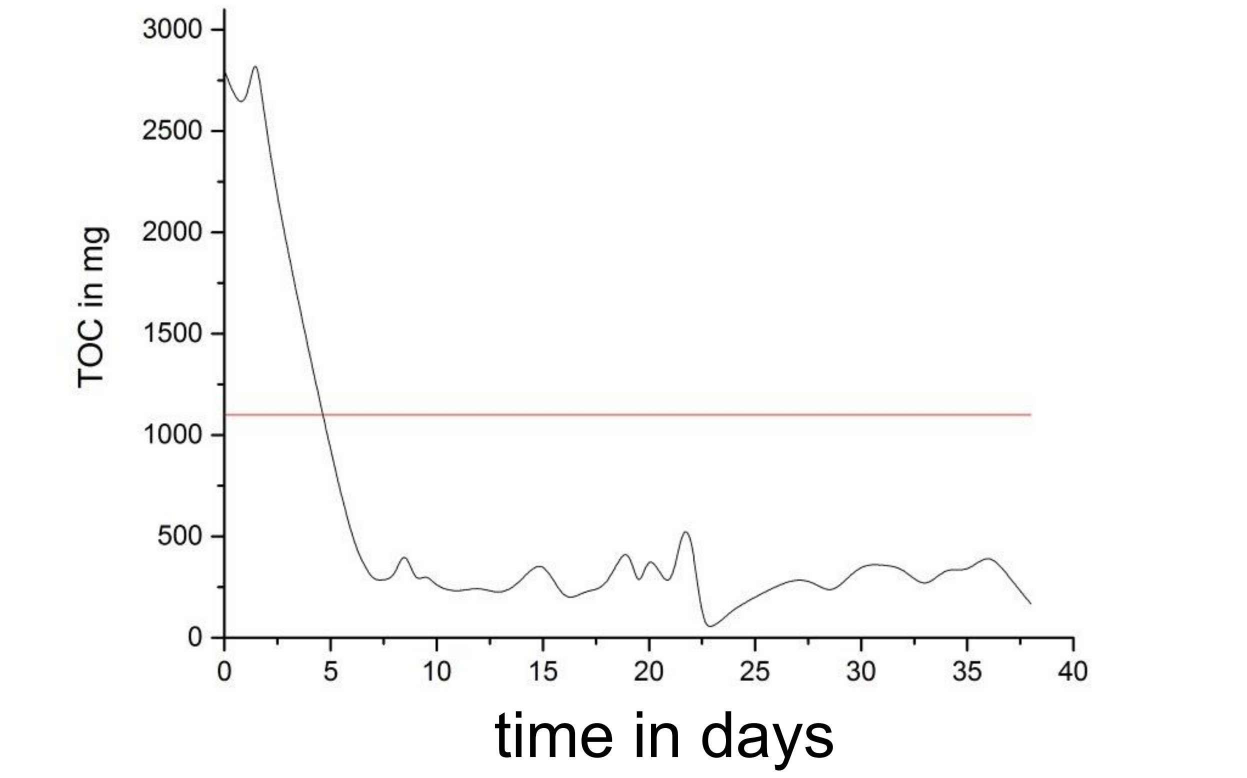
enabling unbalanced fermentations in *E. coli*

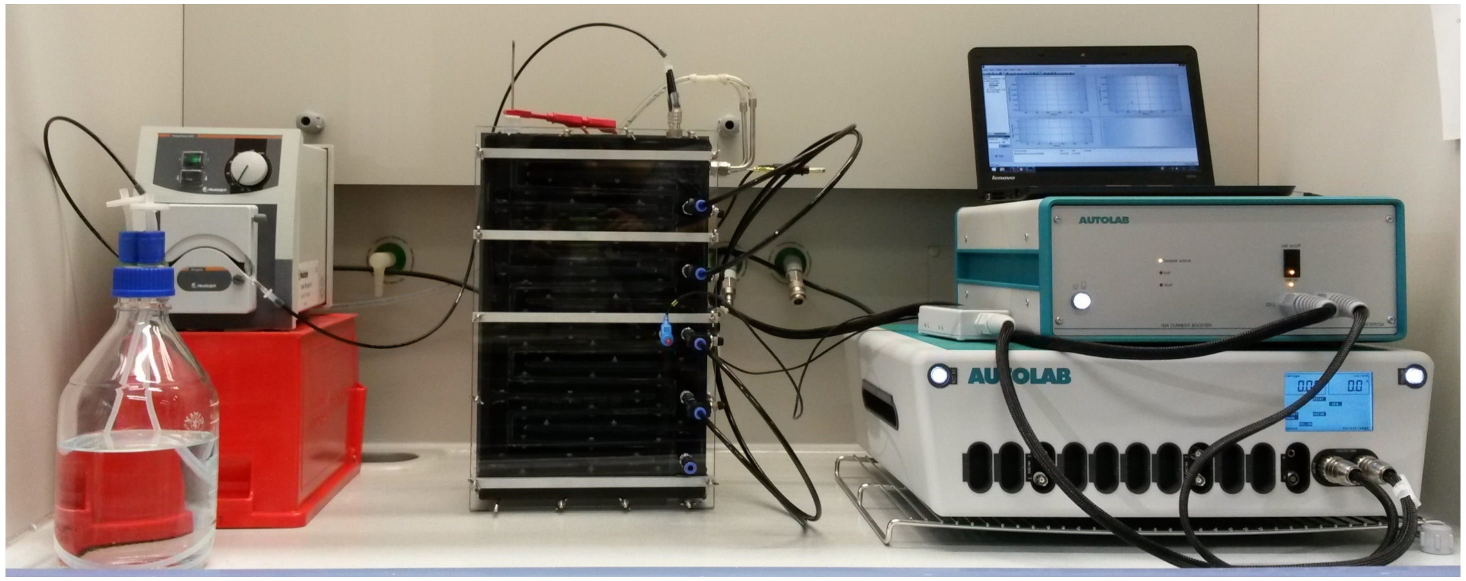


	mol product mol glycerol		oxidation state	redox balance	
	induced	not induced		induced	not induced
glycerol			-2		
acetate	0.30 ± 0.01	0.14 ± 0.04	0	0	0
ethanol	0.53 ± 0.07	0.90 ± 0.12	-4	-2.12	-3.60
formate*	0.83 ± 0.08	1.14 ± 0.18	2	1.66	2.28
total				-0.46	-1.32

microbial fuel cells and waste water treatment







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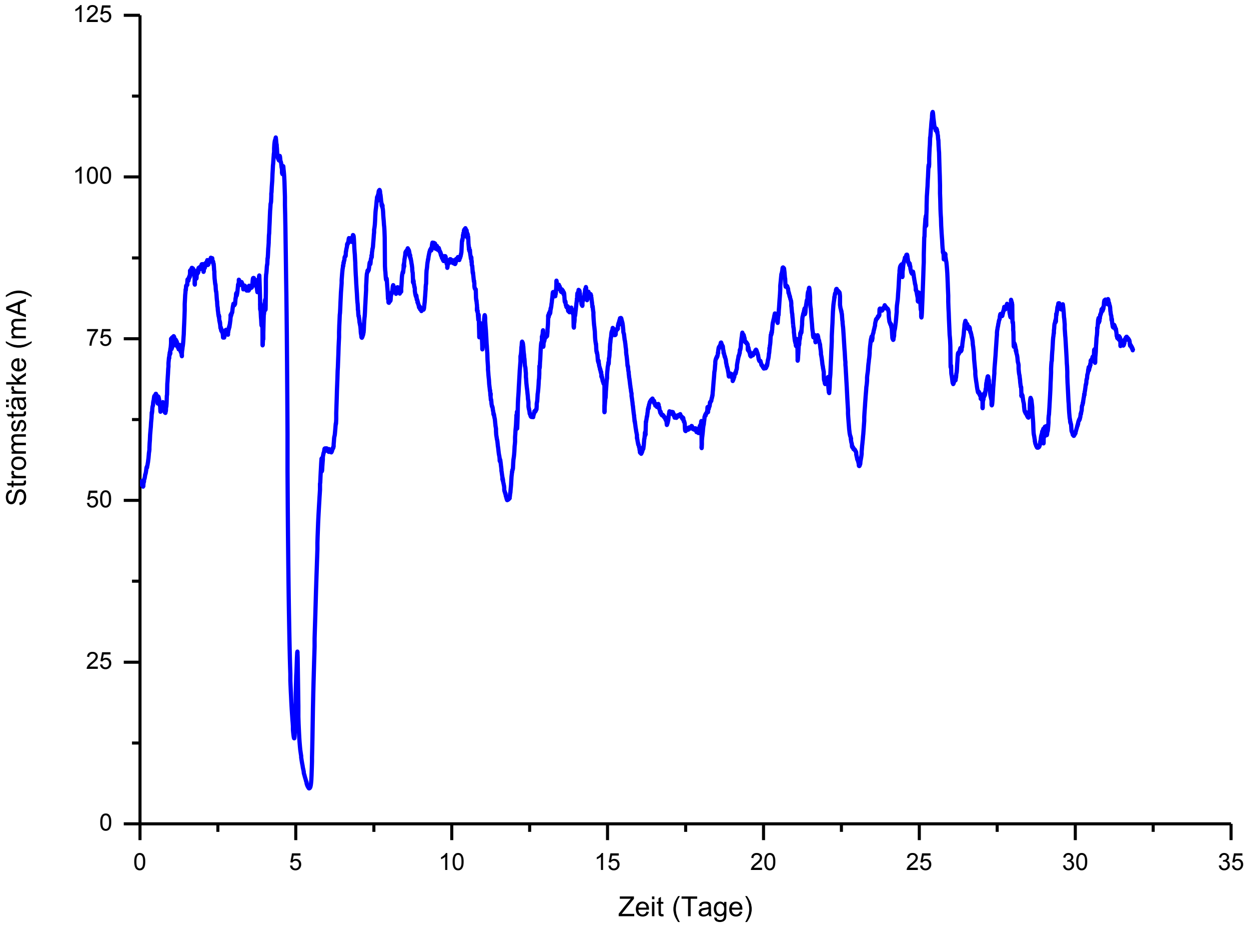
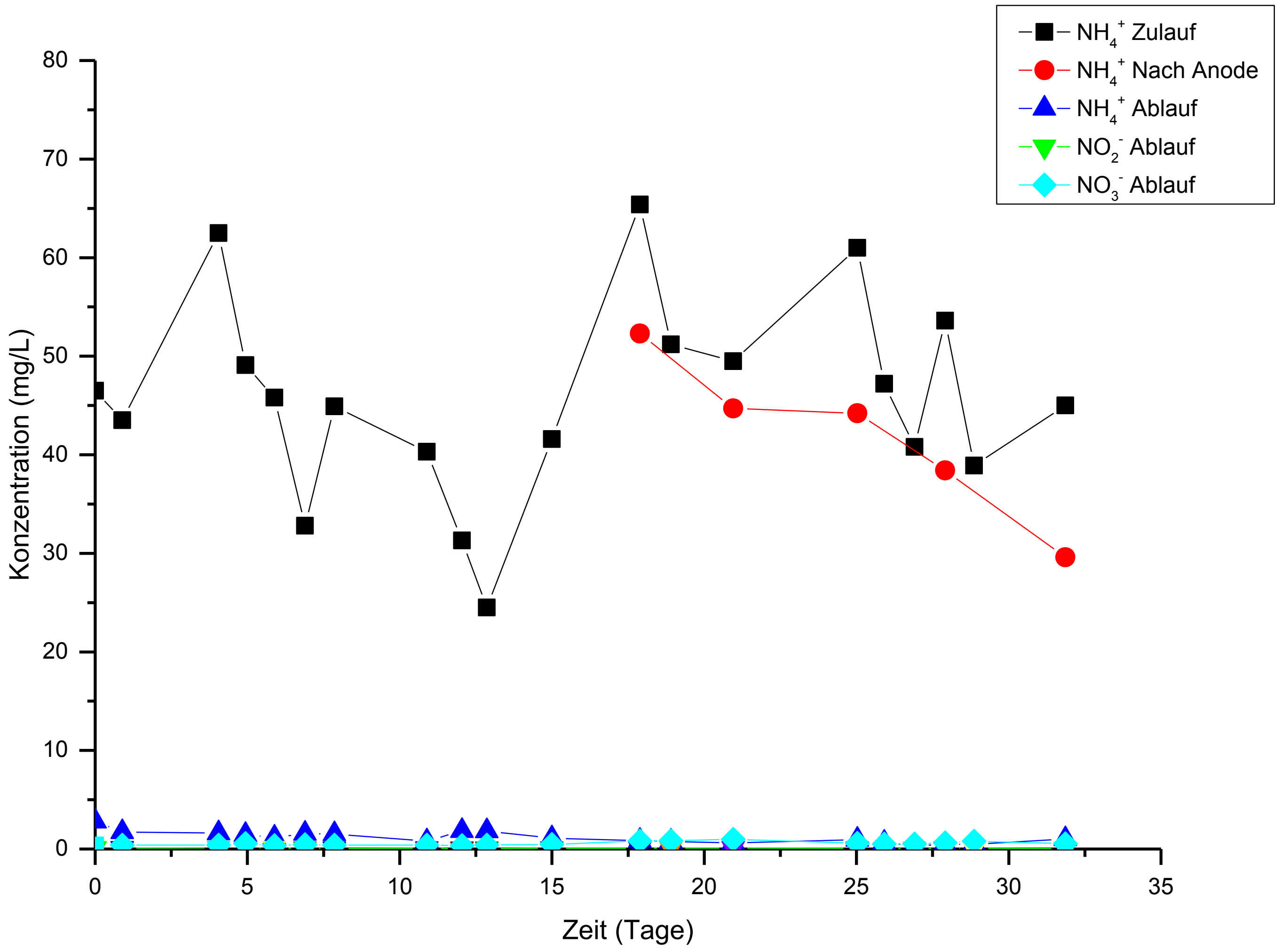
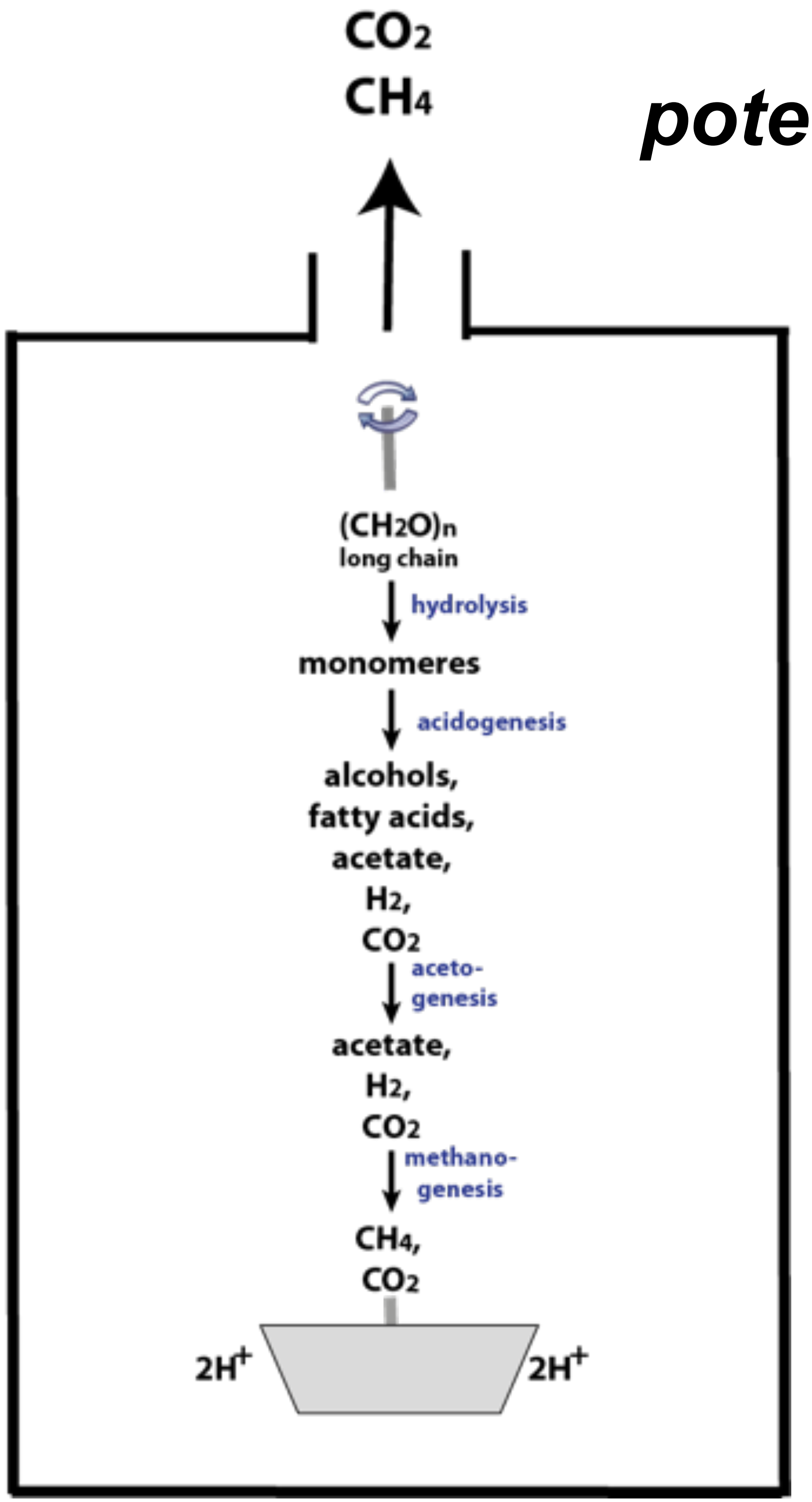




potential limitations in the biogas process



CO_2
CH_4

$(CH_2O)_n$
long chain

hydrolysis

monomeres

acidogenesis

alcohols,
fatty acids,
acetate,
H_2,
CO_2

aceto-genesis

acetate,
H_2,
CO_2

methano-genesis

CH_4,
CO_2

$2H^+$ $2H^+$

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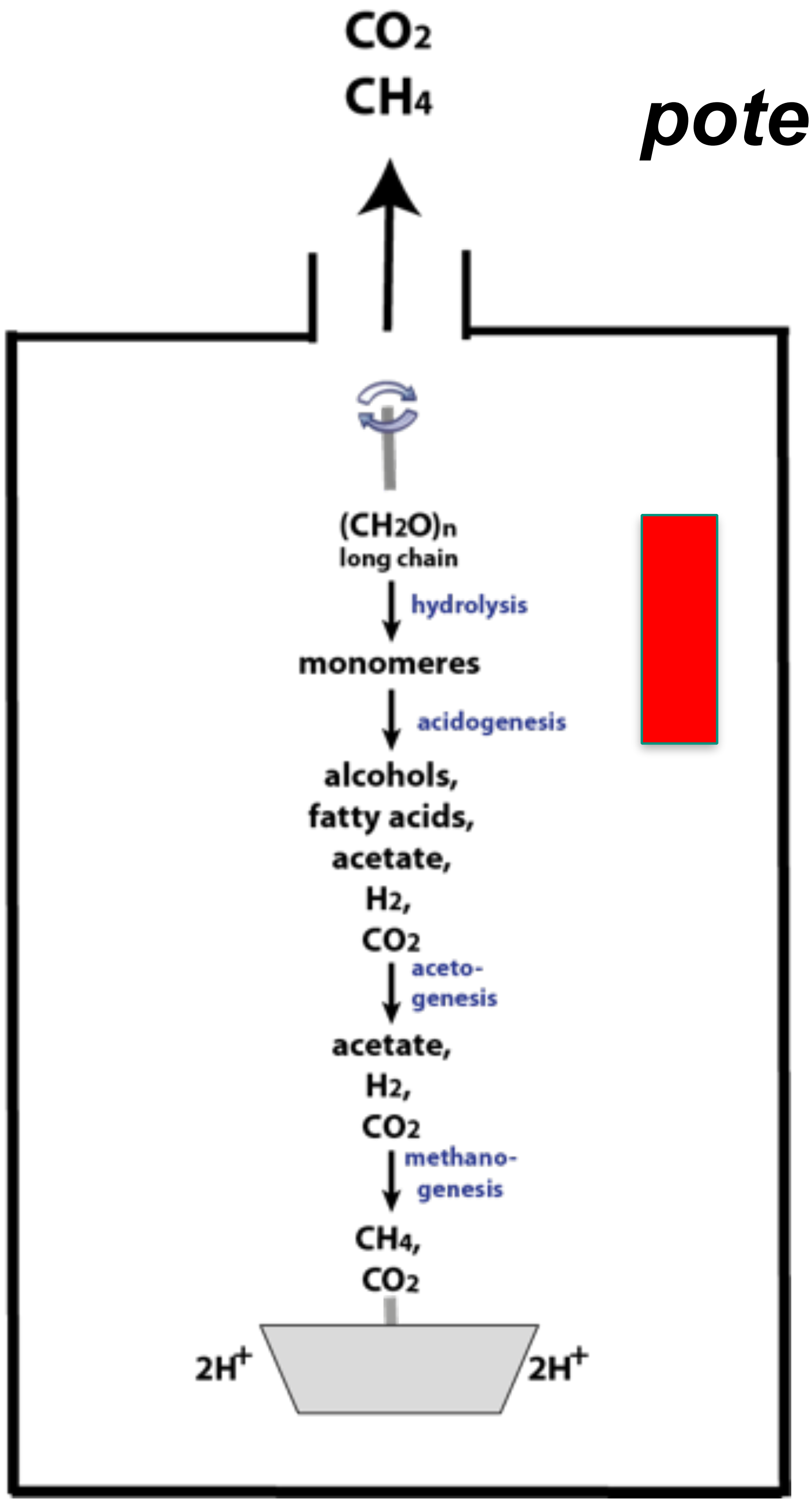

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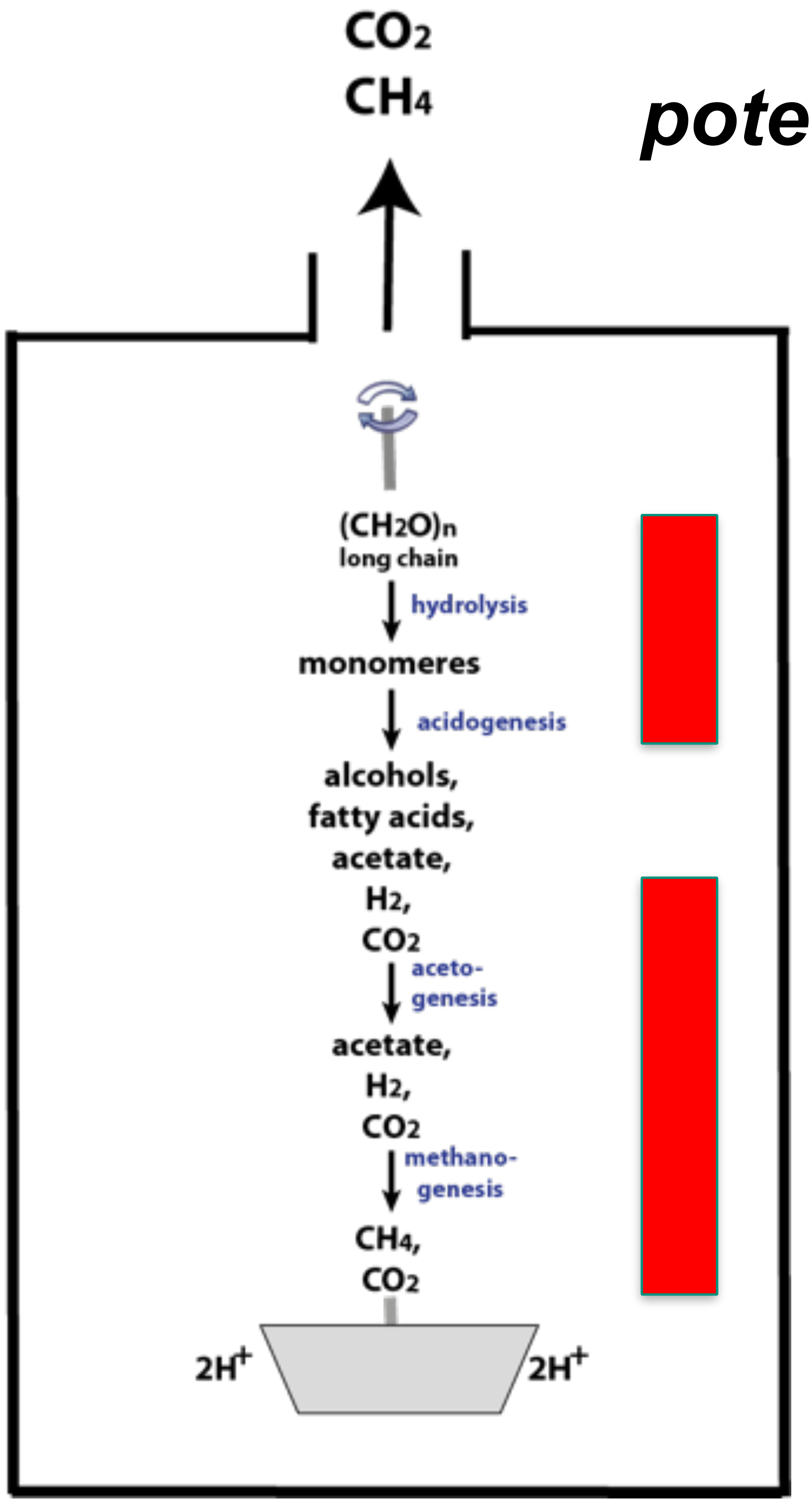
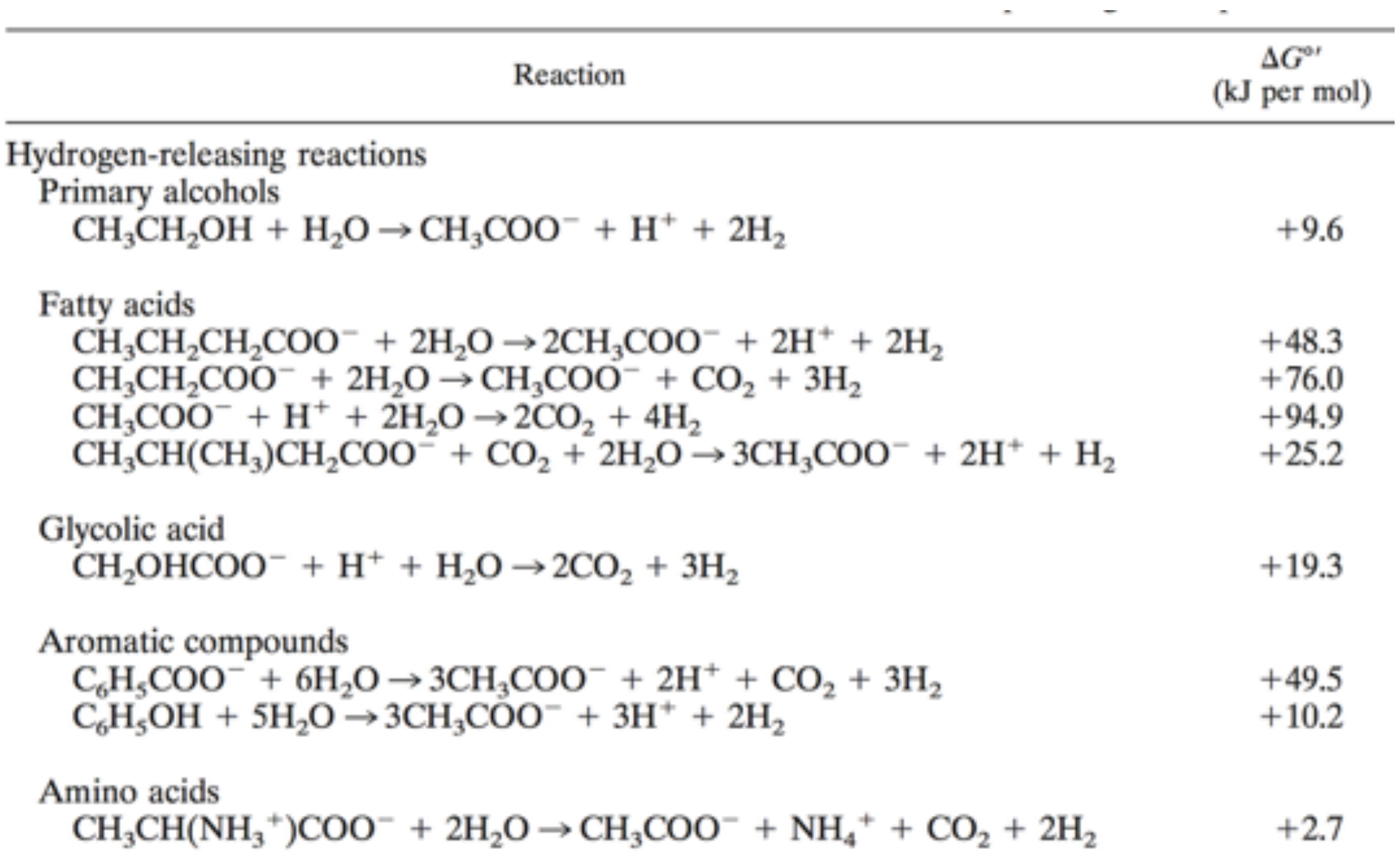
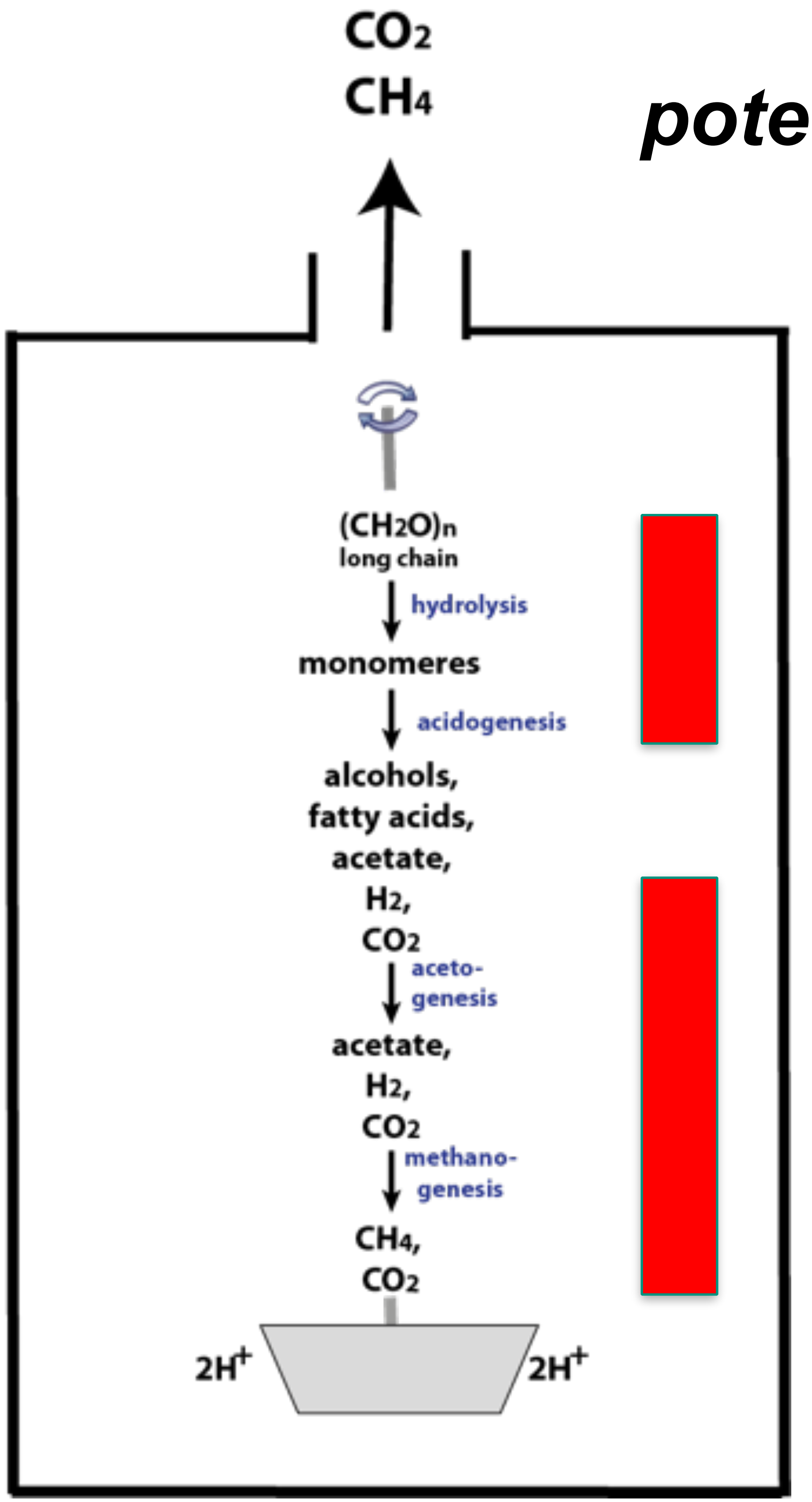




Reaction	$\Delta G^{\circ\prime}$ (kJ per mol)
Hydrogen-releasing reactions	
Primary alcohols	
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6
Fatty acids	
$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2H_2$	+48.3
$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2$	+76.0
$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2$	+94.9
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \rightarrow 3CH_3COO^- + 2H^+ + H_2$	+25.2
Glycolic acid	
$CH_2OHCOO^- + H^+ + H_2O \rightarrow 2CO_2 + 3H_2$	+19.3
Aromatic compounds	
$C_6H_5COO^- + 6H_2O \rightarrow 3CH_3COO^- + 2H^+ + CO_2 + 3H_2$	+49.5
$C_6H_5OH + 5H_2O \rightarrow 3CH_3COO^- + 3H^+ + 2H_2$	+10.2
Amino acids	
$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2$	+2.7

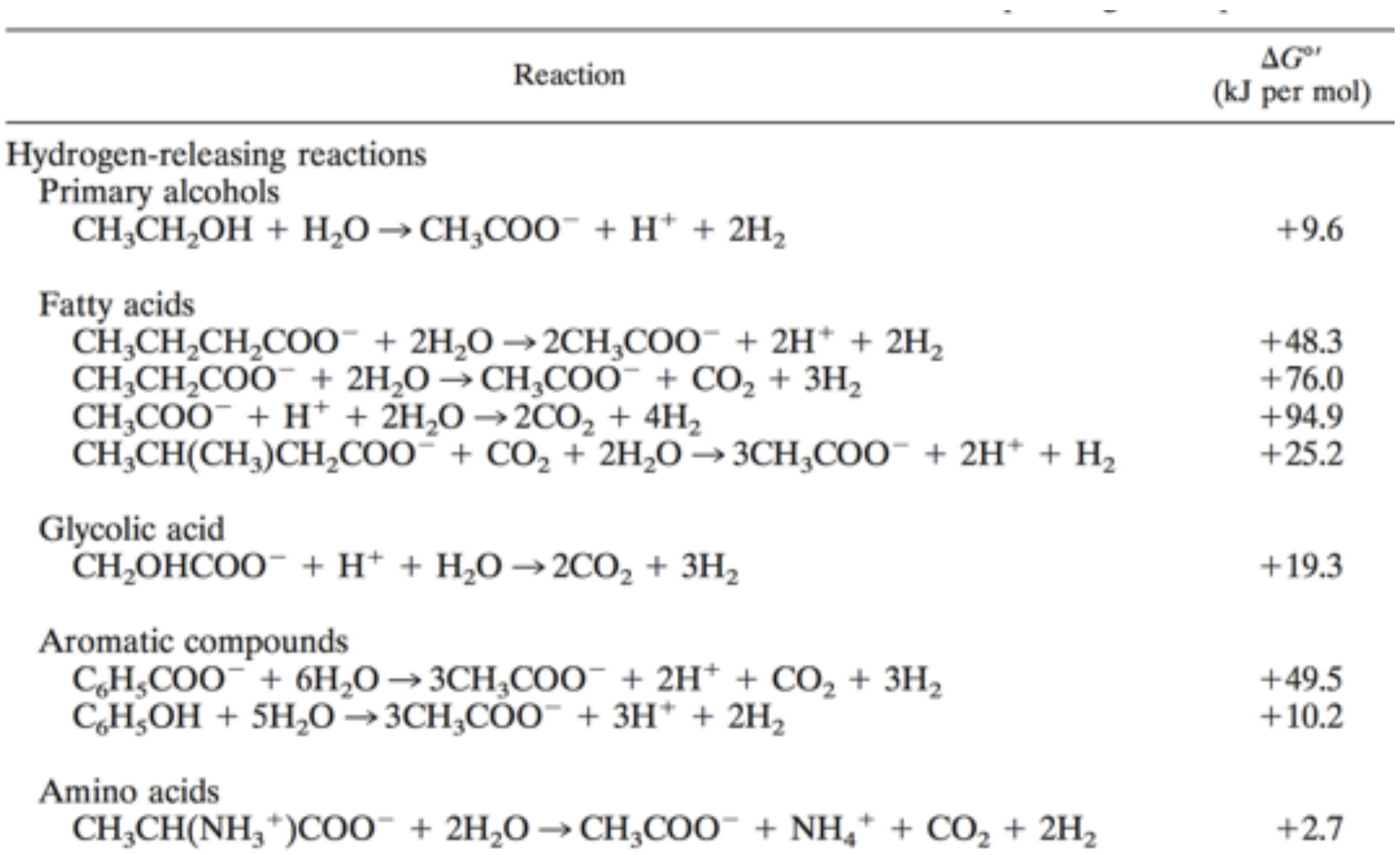
potential limitations in the biogas process



CO_2
CH_4

$(CH_2O)_n$
long chain
↓ hydrolysis
monomeres
↓ acidogenesis
alcohols,
fatty acids,
acetate,
H_2,
CO_2
↓ aceto-genesis
acetate,
H_2,
CO_2
↓ methano-genesis
CH_4,
CO_2

$2H^+$ $2H^+$



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$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2$	+76.0
$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2$	+94.9
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \rightarrow 3CH_3COO^- + 2H^+ + H_2$	+25.2
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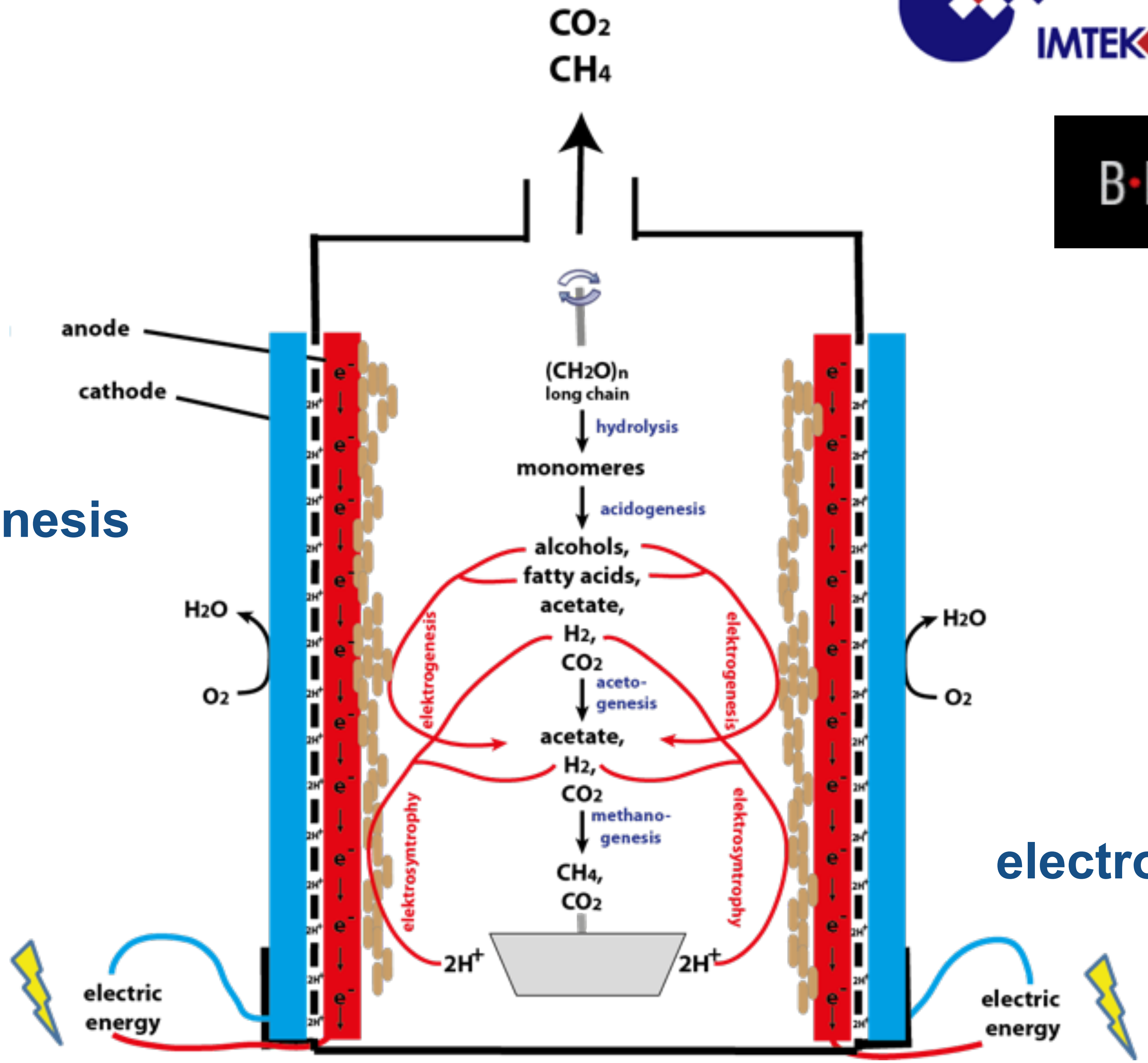
$C_6H_5OH + 5H_2O \rightarrow 3CH_3COO^- + 3H^+ + 2H_2$	+10.2
Amino acids	
$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2$	+2.7

- *missing monitoring*
- *limited possibilities for regulation*

microbial fuel cells in biogas production

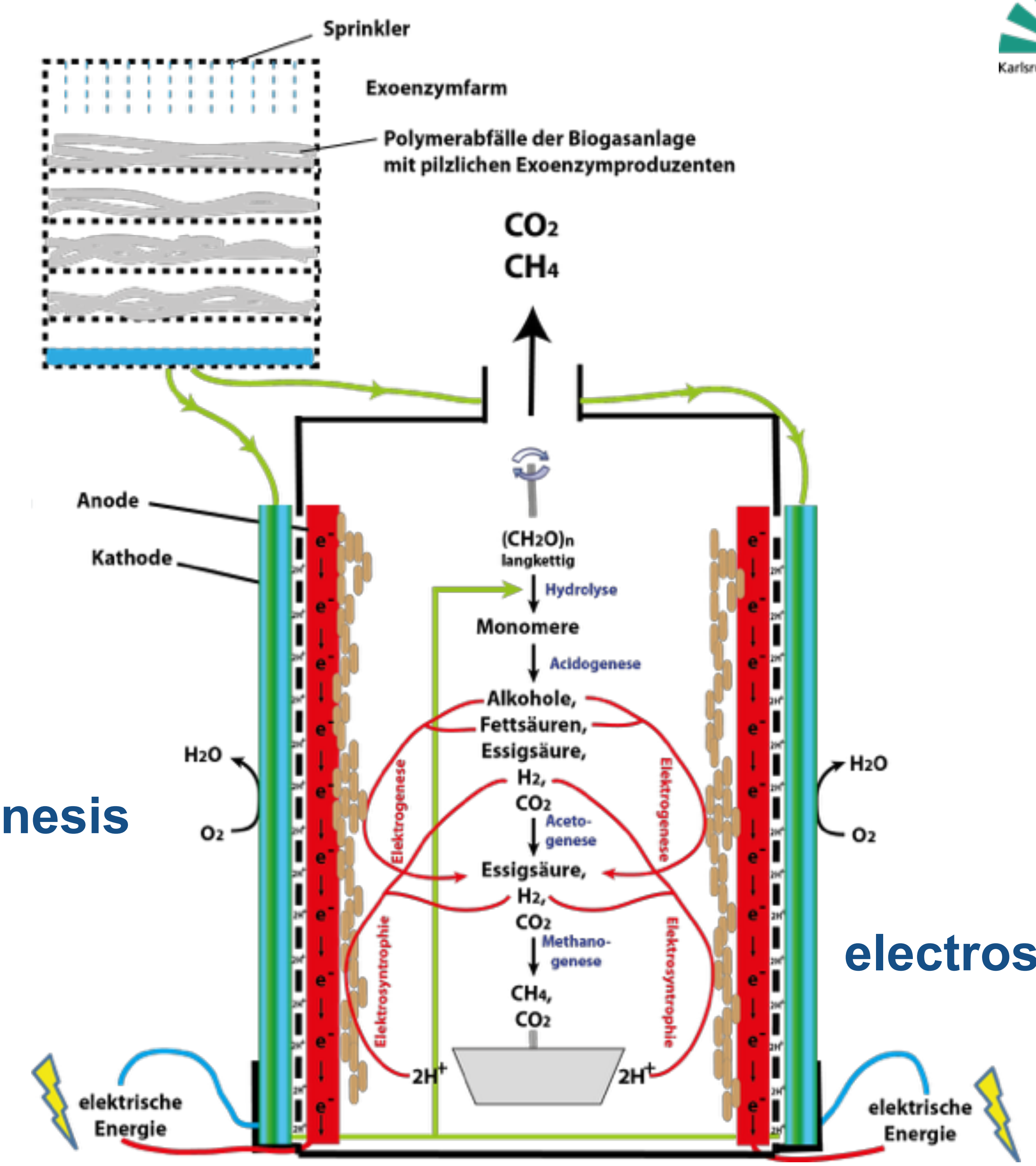


electrogenesis

electrosyntrophy

Die ESE-Biogas

Strategie II

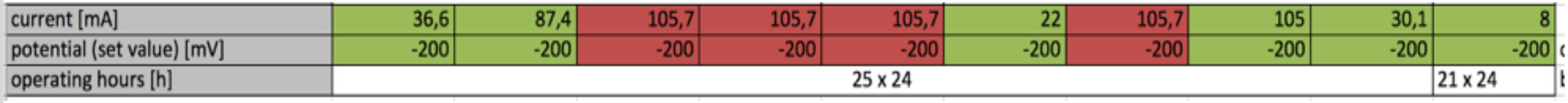


exoenzymproduction

electrogenesis

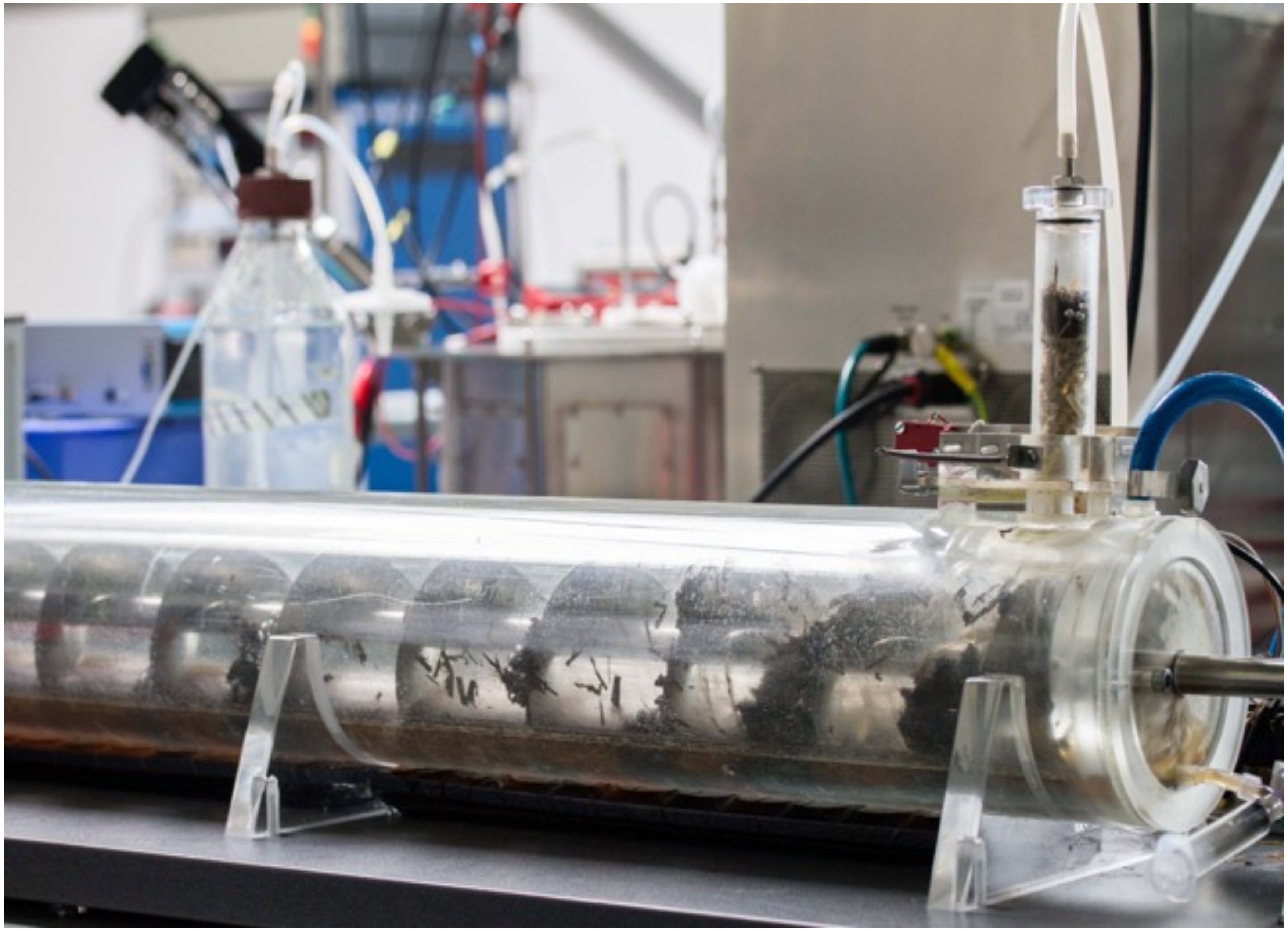
electrosyntrophy

demonstrator development

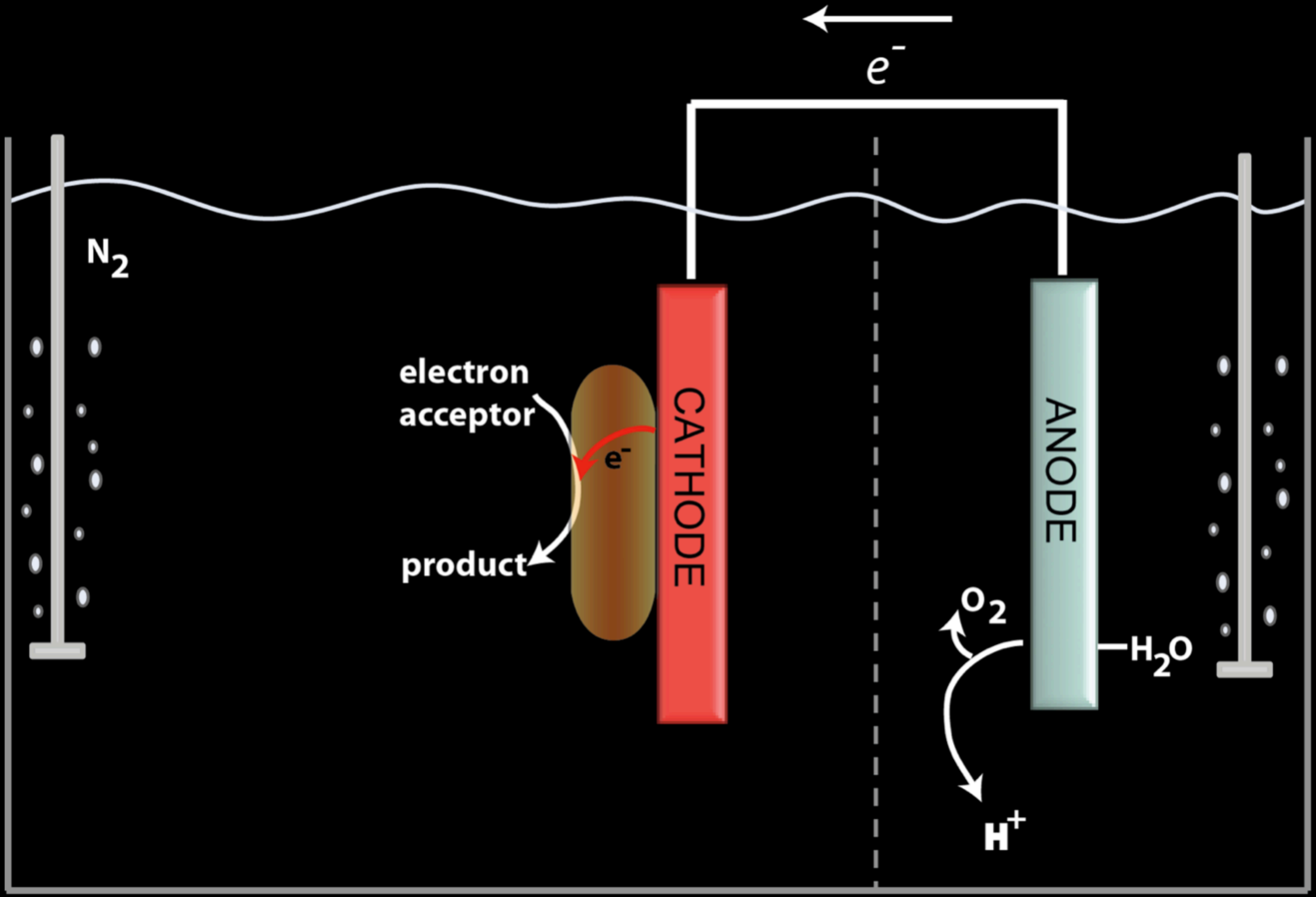


current [mA]	36,6	87,4	105,7	105,7	105,7	22	105,7	105	30,1	8
potential (set value) [mV]	-200	-200	-200	-200	-200	-200	-200	-200	-200	-200
operating hours [h]	25 x 24									21 x 24

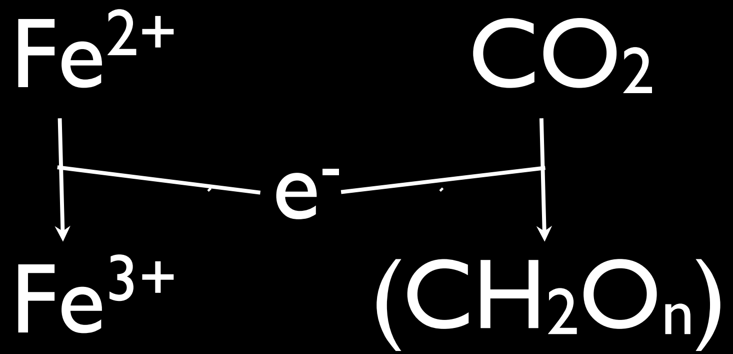
demonstrator development

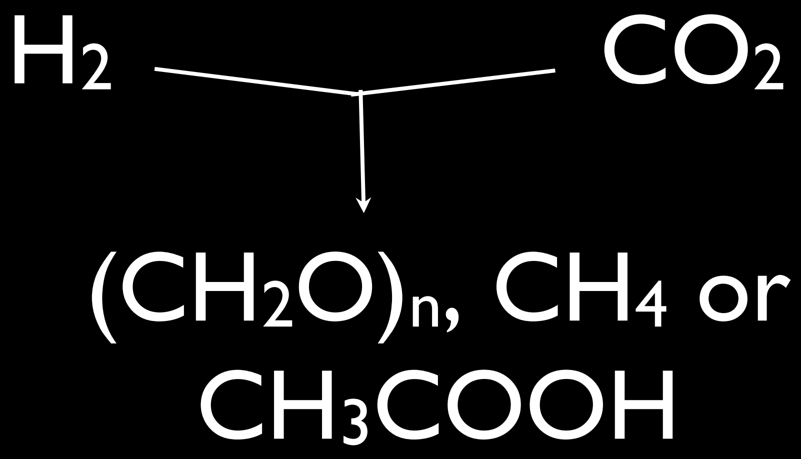


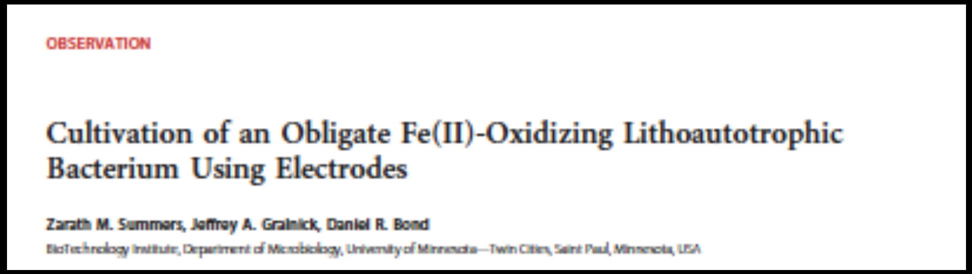
current as energy and electron donor



lithotrophic organisms
with
electrosynthetic activity

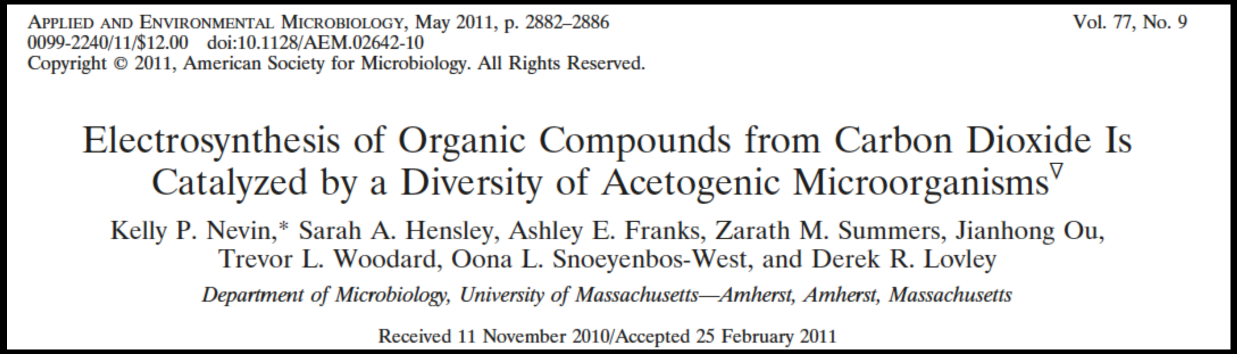





OBSERVATION

Cultivation of an Obligate Fe(II)-Oxidizing Lithoautotrophic Bacterium Using Electrodes

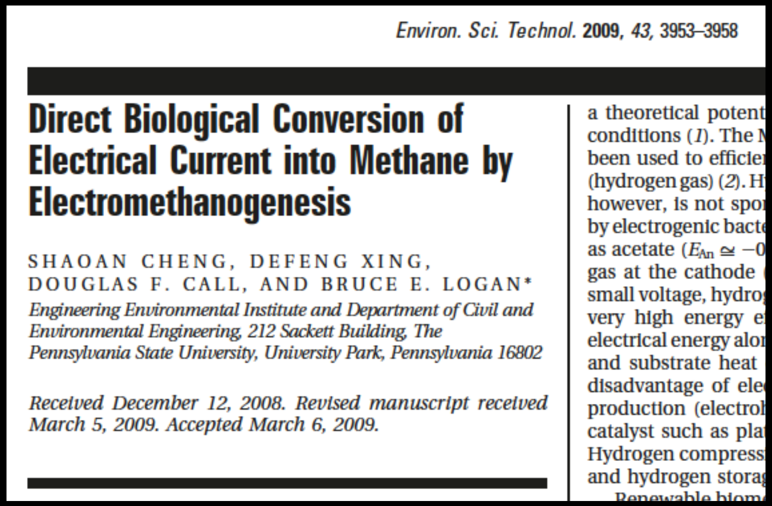
Zarath M. Summers, Jeffrey A. Gralnick, Daniel R. Bond
BioTechnology Institute, Department of Microbiology, University of Minnesota—Twin Cities, Saint Paul, Minnesota, USA


Applied and Environmental Microbiology, May 2011, p. 2882–2886 Vol. 77, No. 9
0099-2240/11/$12.00 doi:10.1128/AEM.02642-10

Electrosynthesis of Organic Compounds from Carbon Dioxide Is Catalyzed by a Diversity of Acetogenic Microorganisms[∇]

Kelly P. Nevin,* Sarah A. Hensley, Ashley E. Franks, Zarath M. Summers, Jianhong Ou, Trevor L. Woodard, Oona L. Snoeyenbos-West, and Derek R. Lovley
Department of Microbiology, University of Massachusetts—Amherst, Amherst, Massachusetts

Received 11 November 2010/Accepted 25 February 2011


Environ. Sci. Technol. **2009,** *43,* 3953–3958

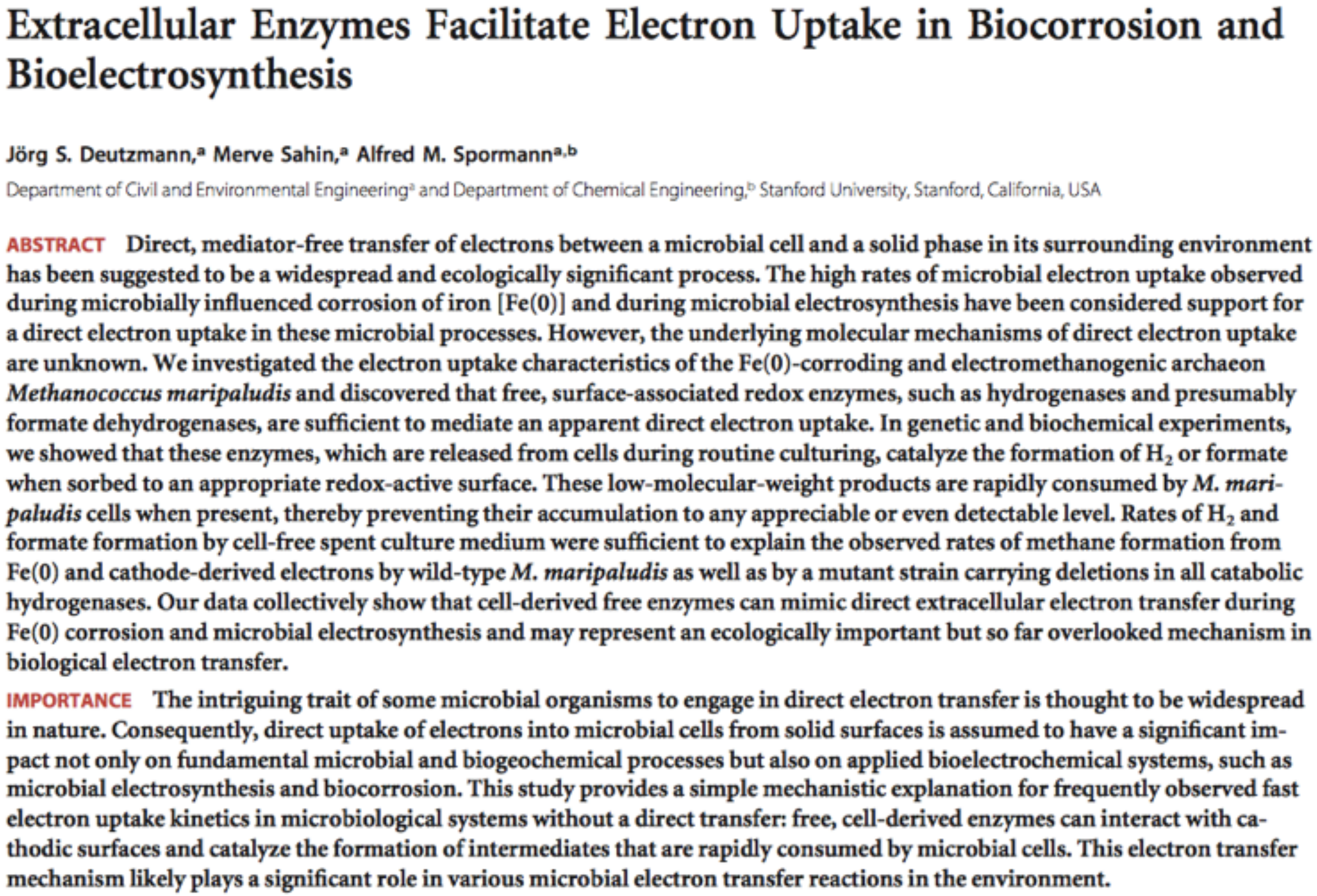
Direct Biological Conversion of Electrical Current into Methane by Electromethanogenesis

SHAOAN CHENG, DEFENG XING, DOUGLAS F. CALL, AND BRUCE E. LOGAN*
Engineering Environmental Institute and Department of Civil and Environmental Engineering, 212 Sackett Building, The Pennsylvania State University, University Park, Pennsylvania 16802

Received December 12, 2008. Revised manuscript received March 5, 2009. Accepted March 6, 2009.

a theoretical potent
conditions (*1*). The N
been used to efficien
(hydrogen gas) (*2*). H
however, is not spon
by electrogenic bacte
as acetate ($E_{An} \cong -0$
gas at the cathode
small voltage, hydrog
very high energy e
electrical energy alon
and substrate heat
disadvantage of ele
production (electrol
catalyst such as plat
Hydrogen compress
and hydrogen storag

enzyme enabled hydrogen and formate production



Extracellular Enzymes Facilitate Electron Uptake in Biocorrosion and Bioelectrosynthesis

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ABSTRACT Direct, mediator-free transfer of electrons between a microbial cell and a solid phase in its surrounding environment has been suggested to be a widespread and ecologically significant process. The high rates of microbial electron uptake observed during microbially influenced corrosion of iron [Fe(0)] and during microbial electrosynthesis have been considered support for a direct electron uptake in these microbial processes. However, the underlying molecular mechanisms of direct electron uptake are unknown. We investigated the electron uptake characteristics of the Fe(0)-corroding and electromethanogenic archaeon *Methanococcus maripaludis* and discovered that free, surface-associated redox enzymes, such as hydrogenases and presumably formate dehydrogenases, are sufficient to mediate an apparent direct electron uptake. In genetic and biochemical experiments, we showed that these enzymes, which are released from cells during routine culturing, catalyze the formation of H_2 or formate when sorbed to an appropriate redox-active surface. These low-molecular-weight products are rapidly consumed by *M. maripaludis* cells when present, thereby preventing their accumulation to any appreciable or even detectable level. Rates of H_2 and formate formation by cell-free spent culture medium were sufficient to explain the observed rates of methane formation from Fe(0) and cathode-derived electrons by wild-type *M. maripaludis* as well as by a mutant strain carrying deletions in all catabolic hydrogenases. Our data collectively show that cell-derived free enzymes can mimic direct extracellular electron transfer during Fe(0) corrosion and microbial electrosynthesis and may represent an ecologically important but so far overlooked mechanism in biological electron transfer.

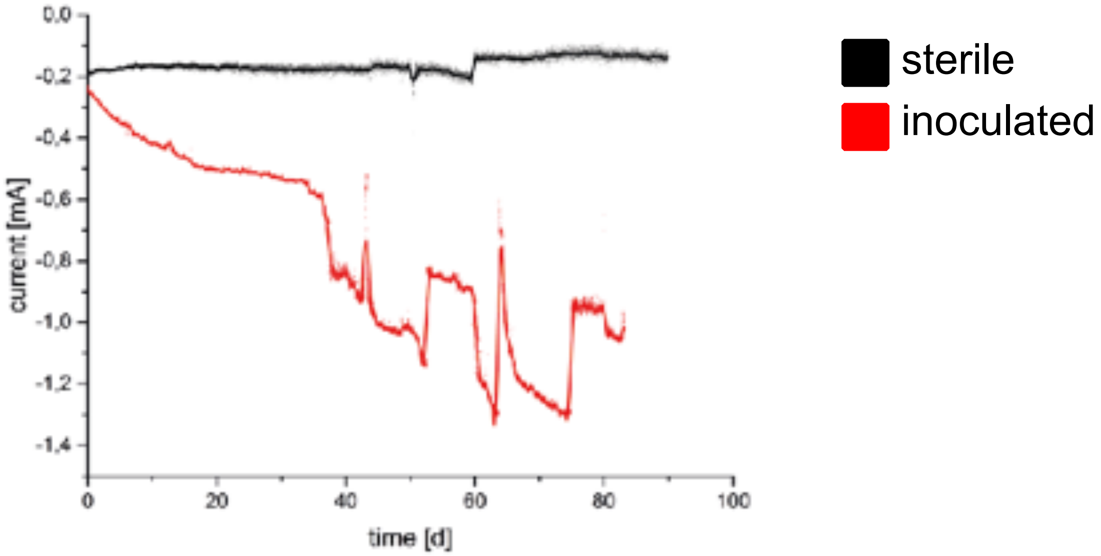
IMPORTANCE The intriguing trait of some microbial organisms to engage in direct electron transfer is thought to be widespread in nature. Consequently, direct uptake of electrons into microbial cells from solid surfaces is assumed to have a significant impact not only on fundamental microbial and biogeochemical processes but also on applied bioelectrochemical systems, such as microbial electrosynthesis and biocorrosion. This study provides a simple mechanistic explanation for frequently observed fast electron uptake kinetics in microbiological systems without a direct transfer: free, cell-derived enzymes can interact with cathodic surfaces and catalyze the formation of intermediates that are rapidly consumed by microbial cells. This electron transfer mechanism likely plays a significant role in various microbial electron transfer reactions in the environment.

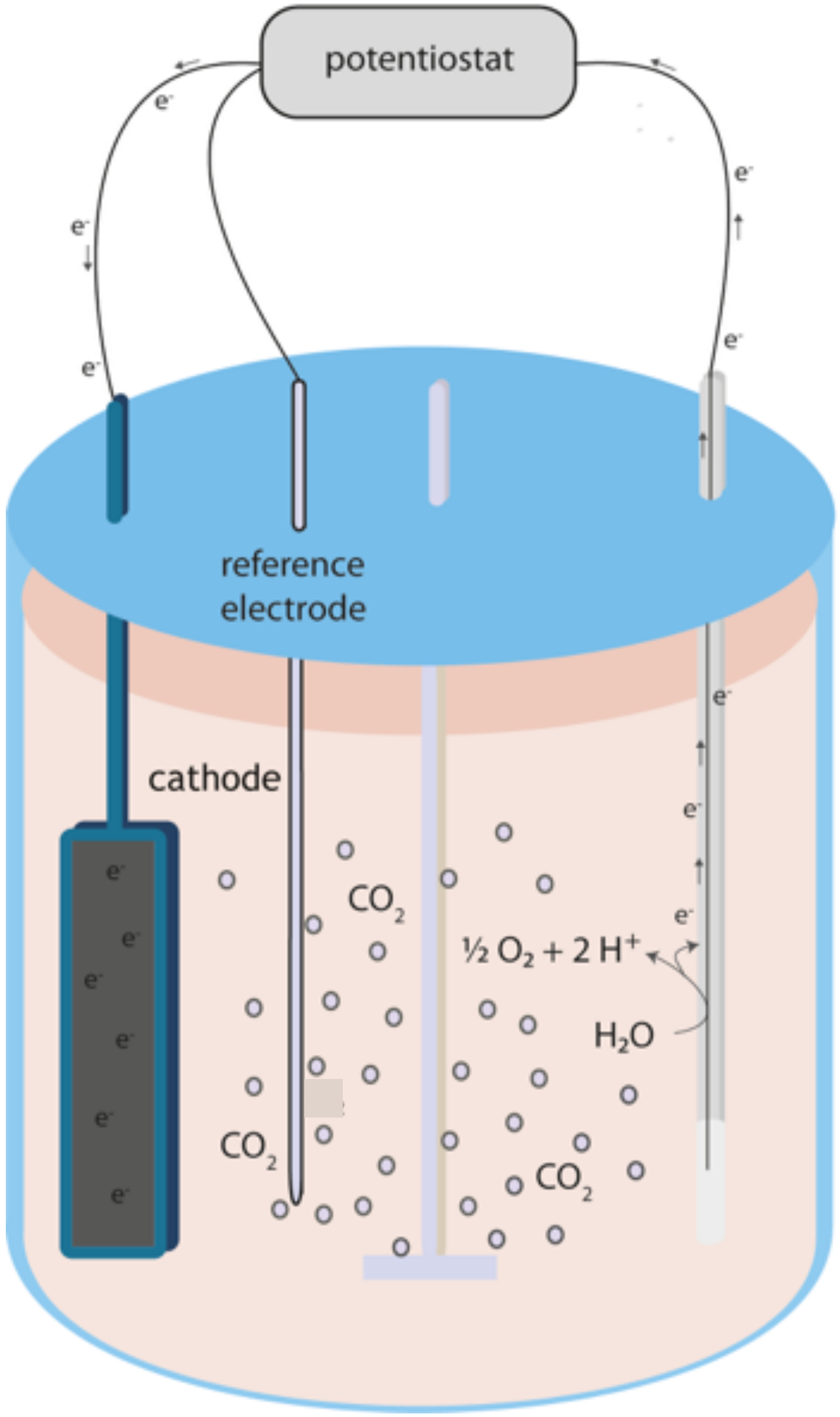
microbial electrosynthesis

Experimental setup

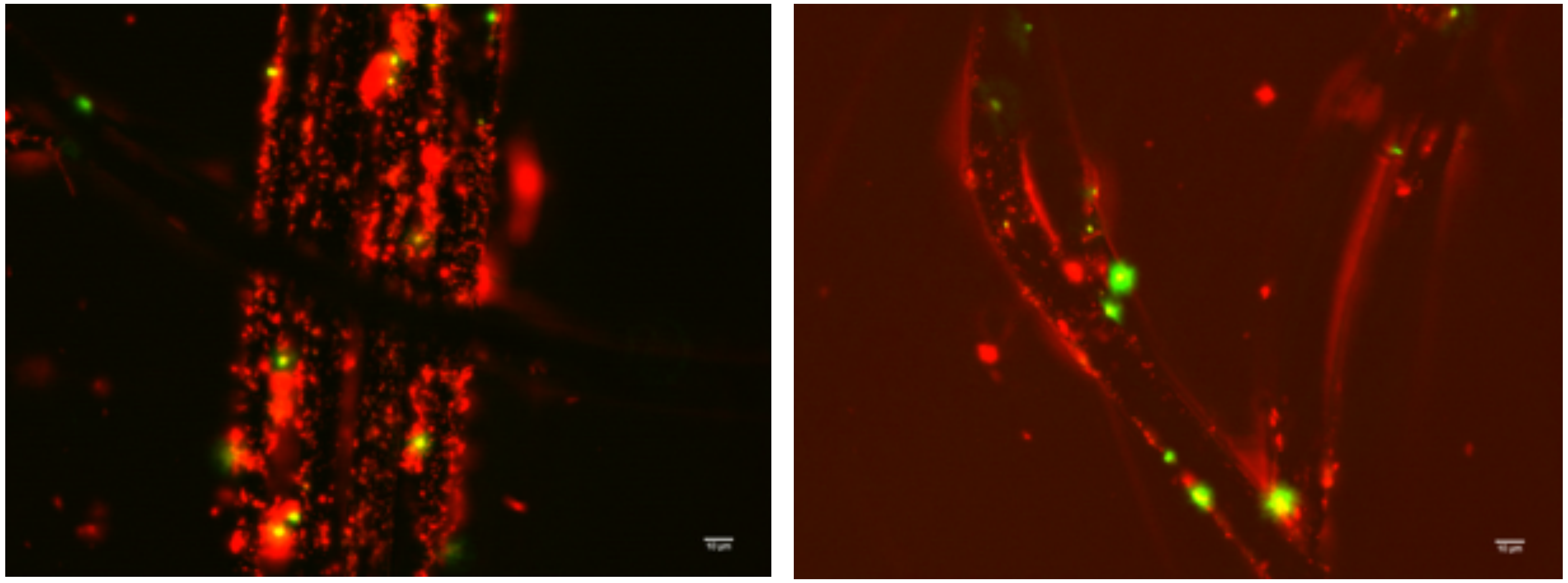
- -350 mV vs. SHE
- 60°C
- N_2/CO_2 (80/20)
- pH 3,5
- inoculated with a mixture of 24 samples from the azores

→monitoring of current





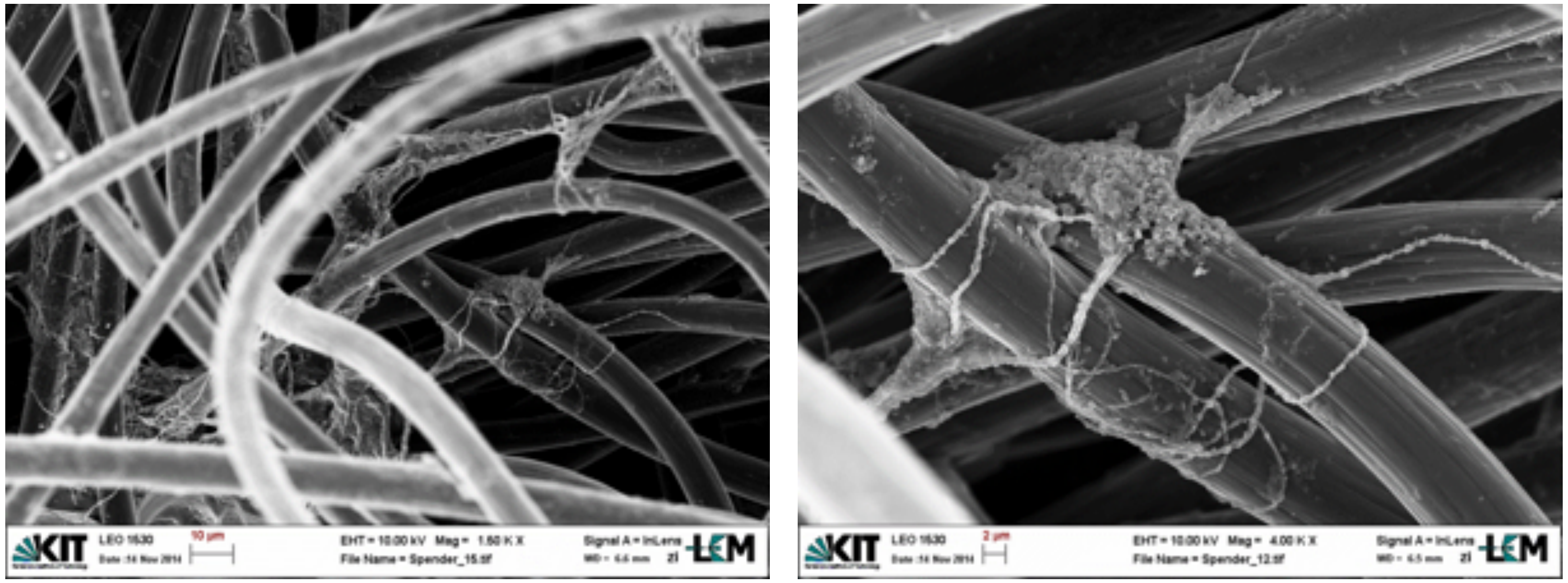
microscopic analysis (CARD-FISH and REM)



🟥 Bacteria (EUB338, Alexa546)

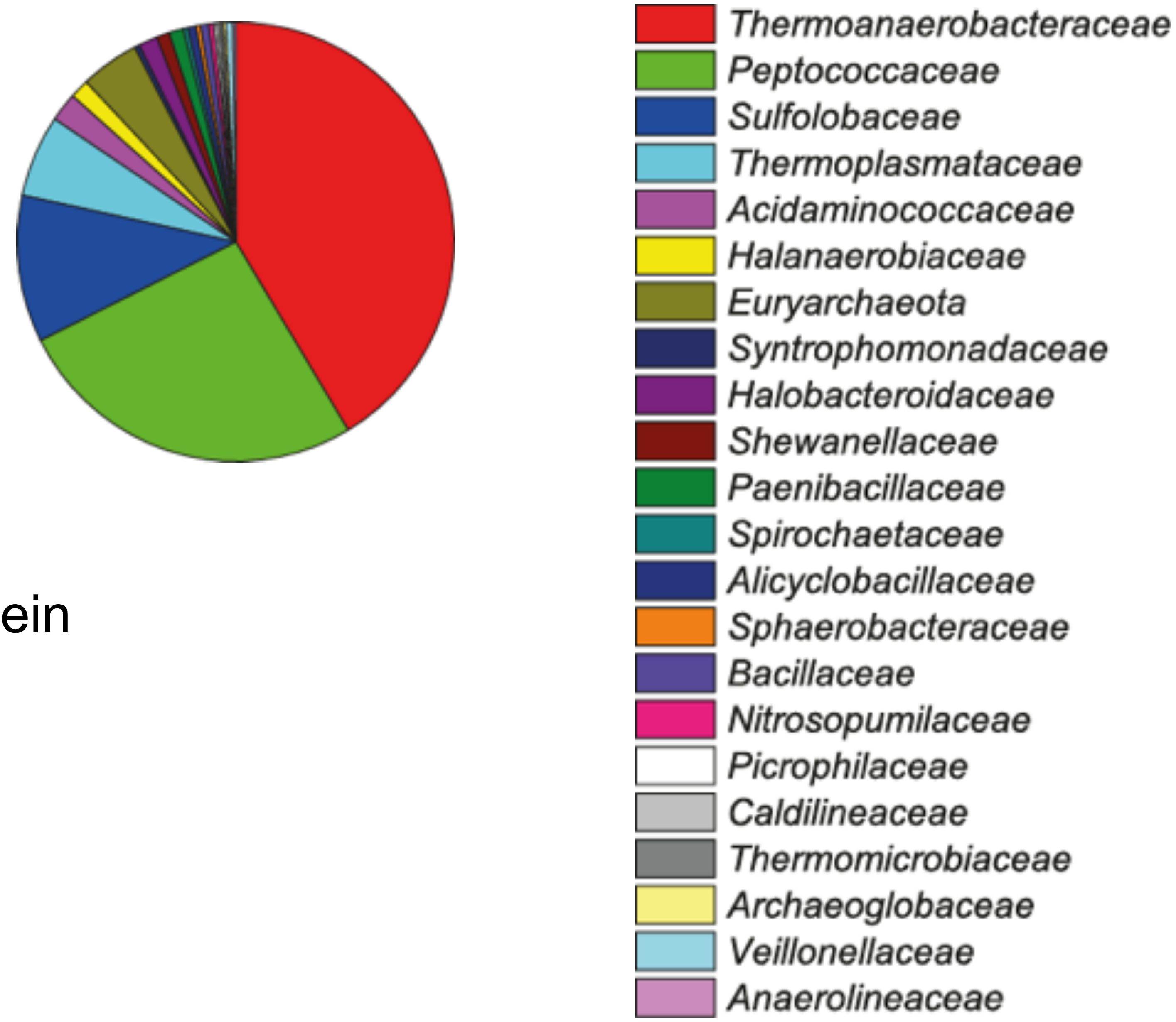
🟩 Archaea (Arch915, Alexa488)

→ CARD-FISH pictures show that bacteria comprise the majority of the sessile community



→ REM pictures show the organisms on the carbon fibres

metagenomic analysis (Illumina)



→ Taxa distribution calculated on the percentage of protein coding genes.

→ Moorella most abundant

→ reductive citric acid cycle,

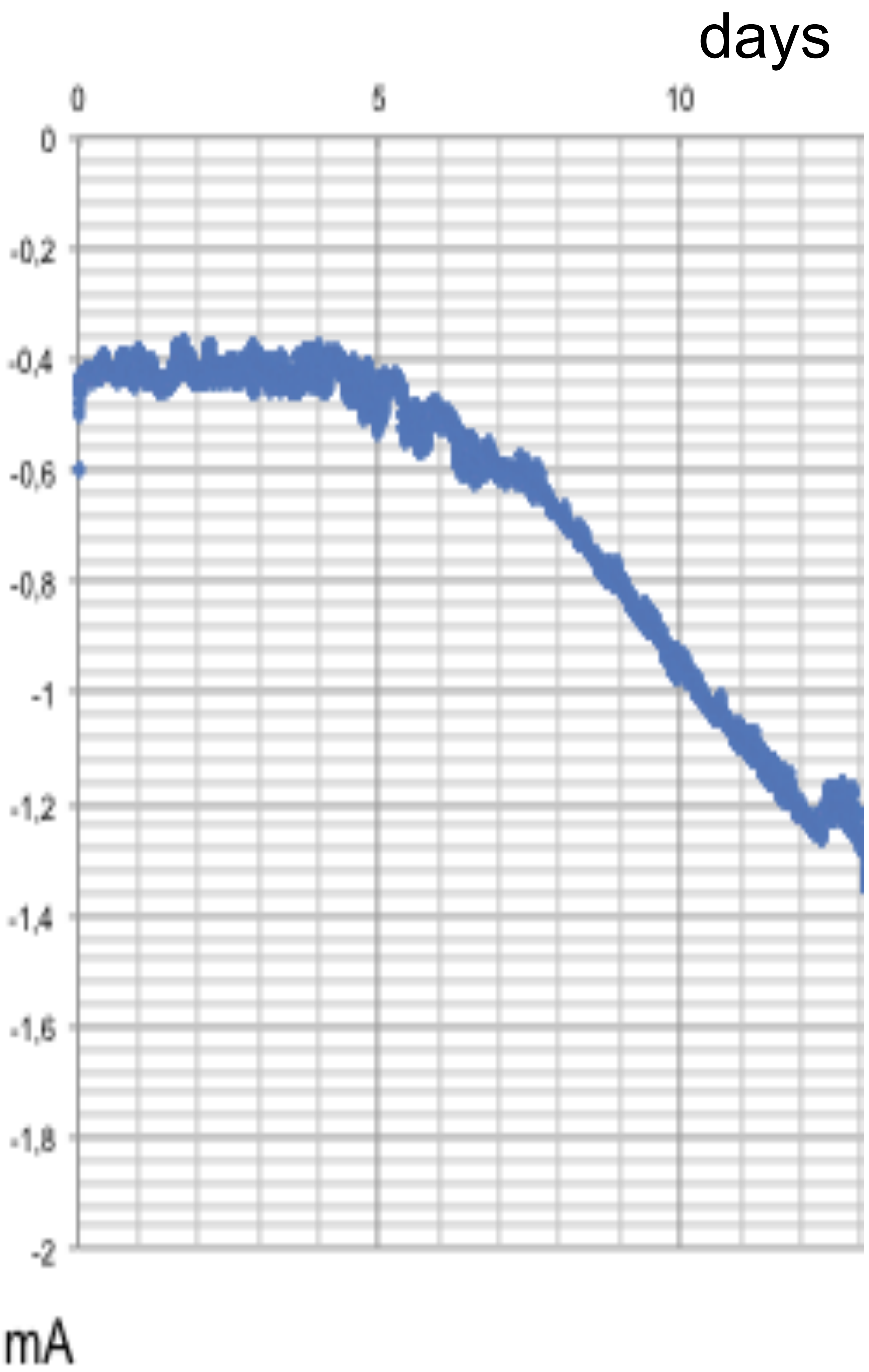
→ hydroxypropionate/hydroxybutyrate cycle

→ Calvin Cycle

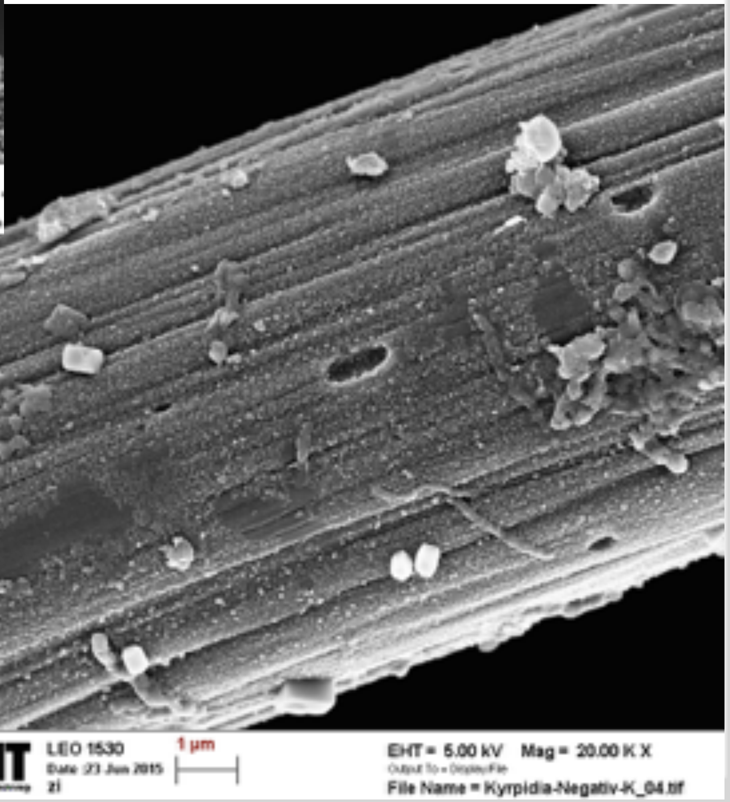
→ Wood-Ljungdahl pathway

Two step isolation strategy

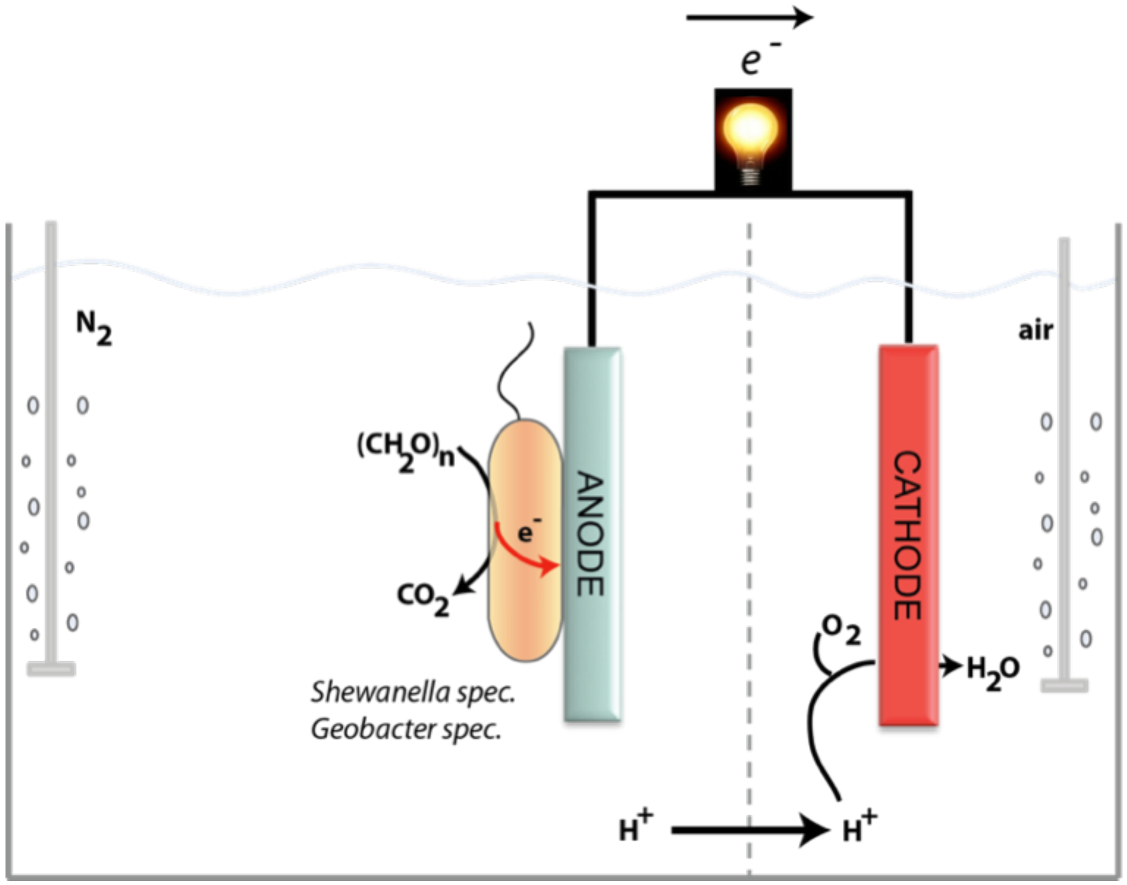
…for example O_2, CO_2, current



with…

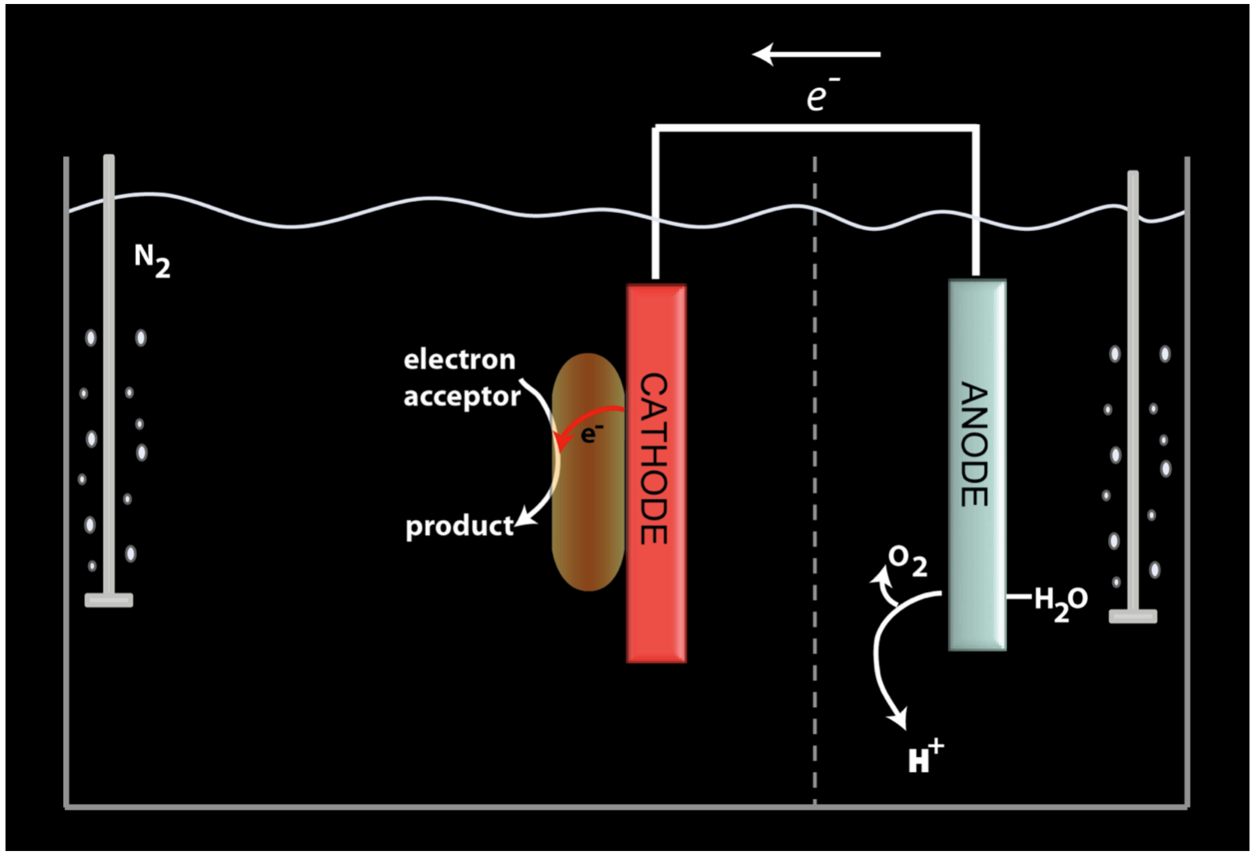


…and without potential



- carbon elimination
- regulation of biogas production
- unbalanced fermentations
- biosensor development

- electrical current as energy and electron source
- blackbox biochemistry
- future biotechnology applications using carbon dioxide as carbon source



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ZeroCarbFP

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