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Acetylene as a low cost and effective inhibitor of methanogenesis in microbial electrolysis

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Roadmap

- Introducing Microbial Electrolysis
- Address Obstacles
- Formation of Study
- Materials and Methods
- Results
- Discussion and Future Work

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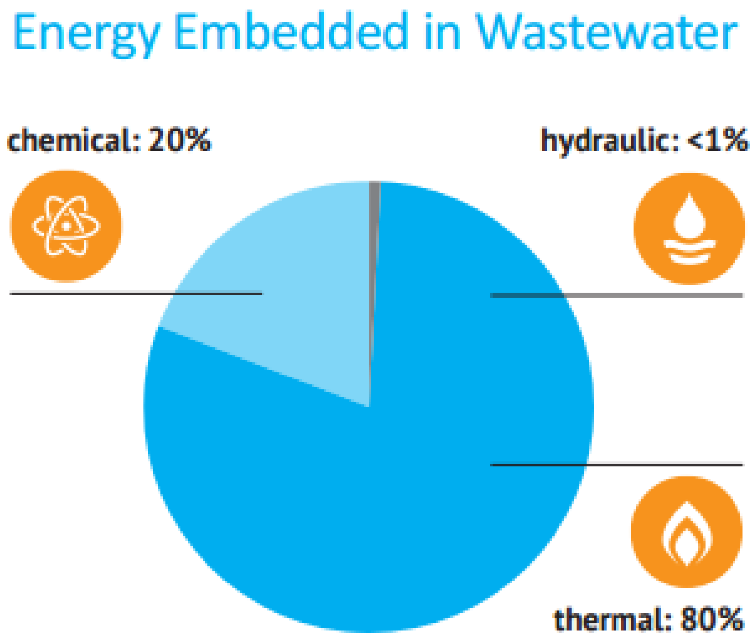
Wastewater

(Source: Sensorex)

- Wastewater treatment (WWT) 3% of electricity consumption in US (EPA 2006)
- Approximately 2 kJ electrical energy required to treat 1 L wastewater (McCarty 2011)

Energy positive wastewater treatment

- Estimated 8-17 kJ/L chemical energy (Heidrich 2011)
- Anaerobic digestion to produce methane
- Microbial fuel cells (MFCs) directly produce electricity
- Microbial electrolysis (MECs) use MFCs as basis for producing hydrogen



(Source: WERF)

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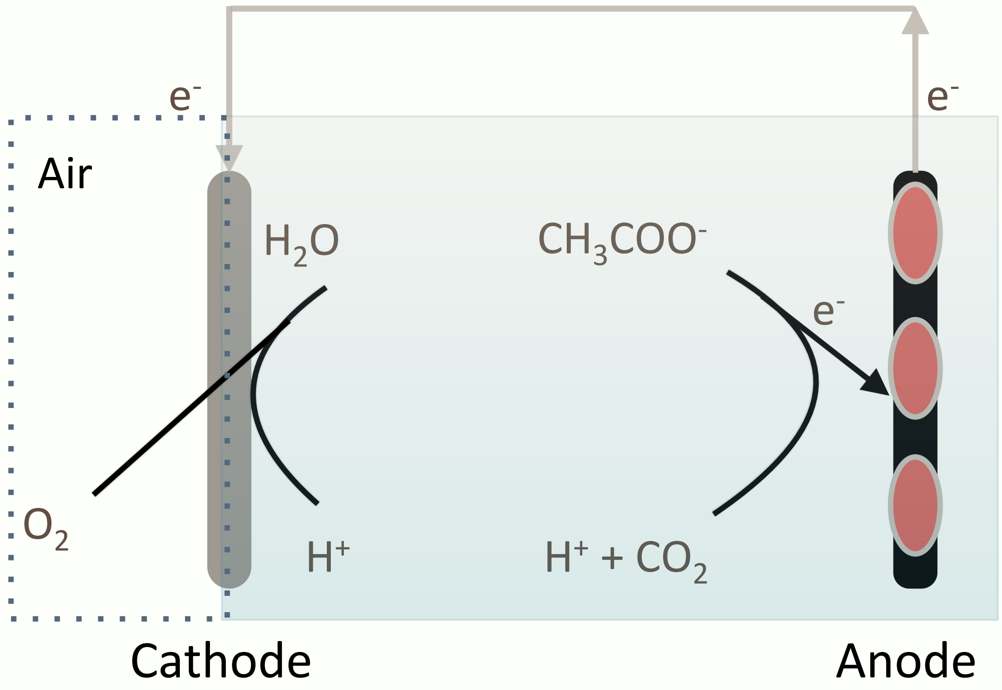
Introduction

Microbial Fuel Cells and Exoelectrogens



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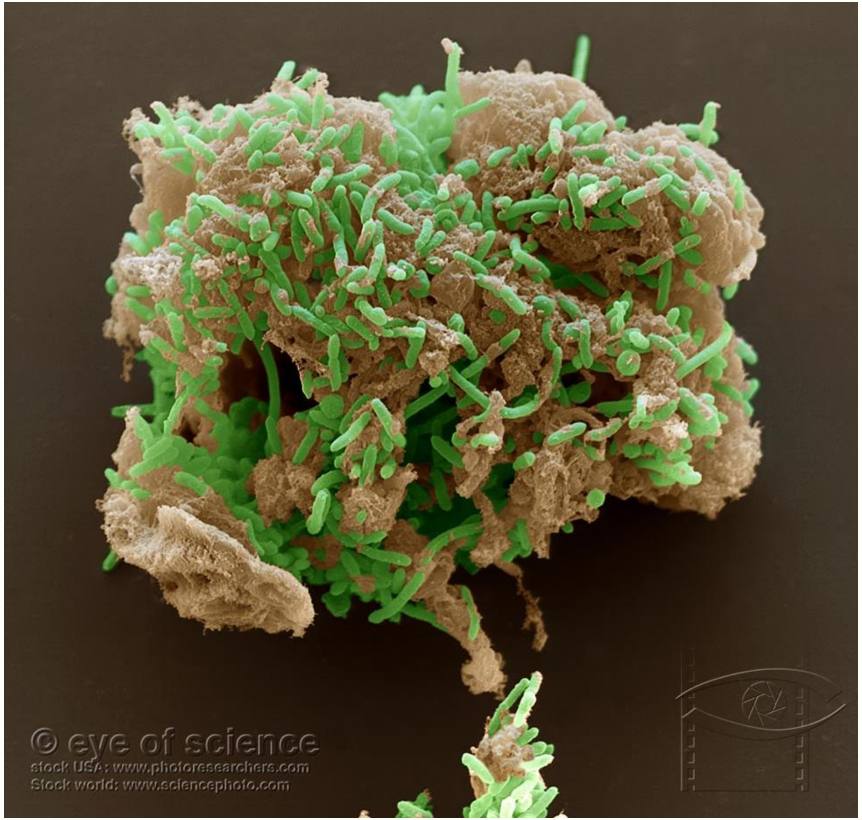
What is an exoelectrogen?



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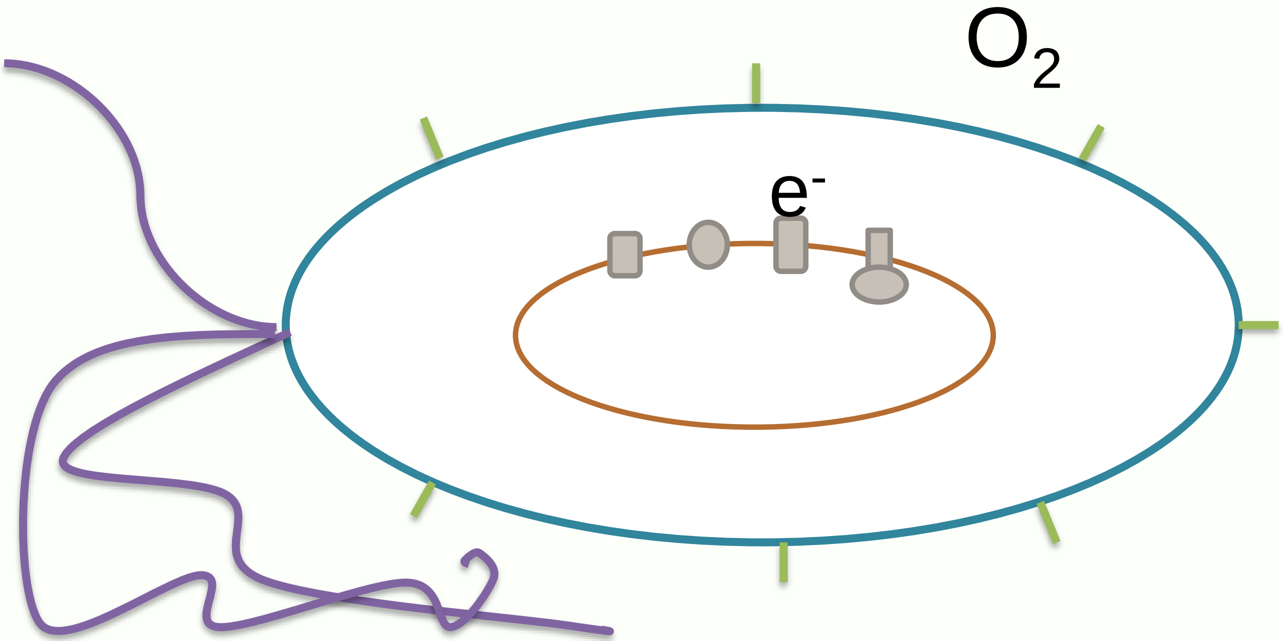
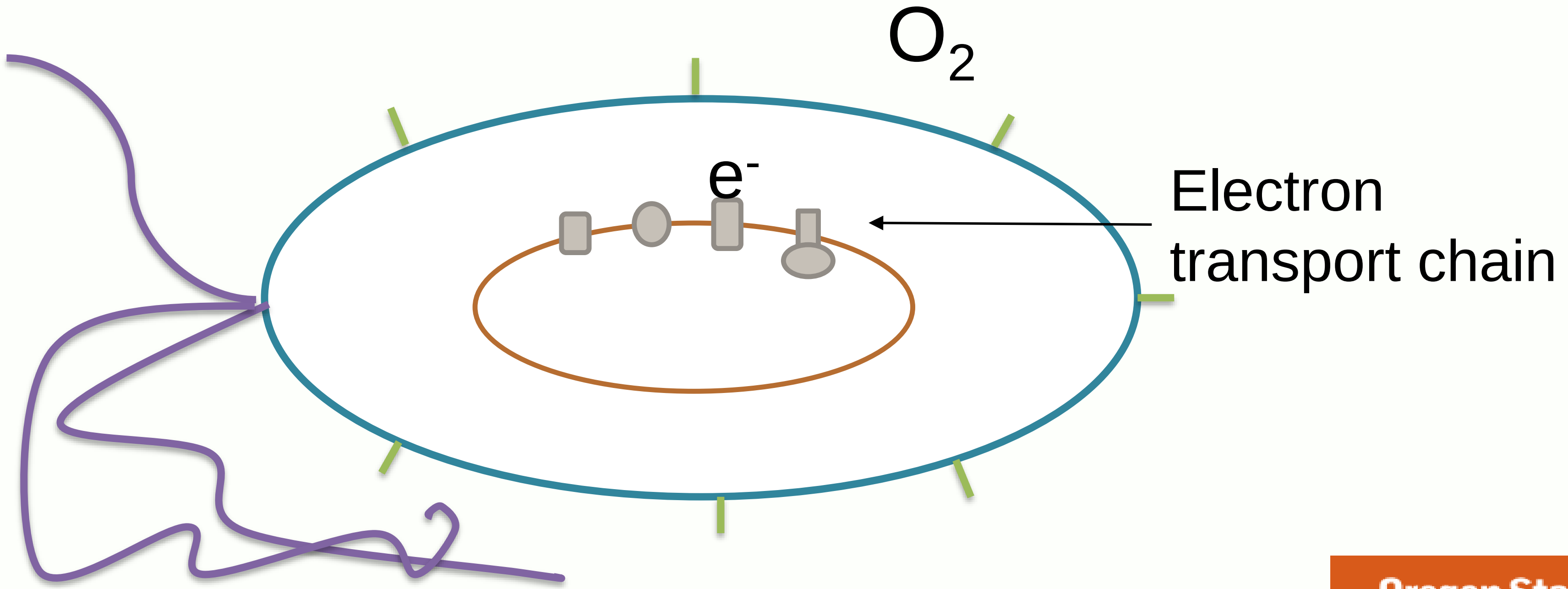
Aerobic metabolism



Aerobic metabolism



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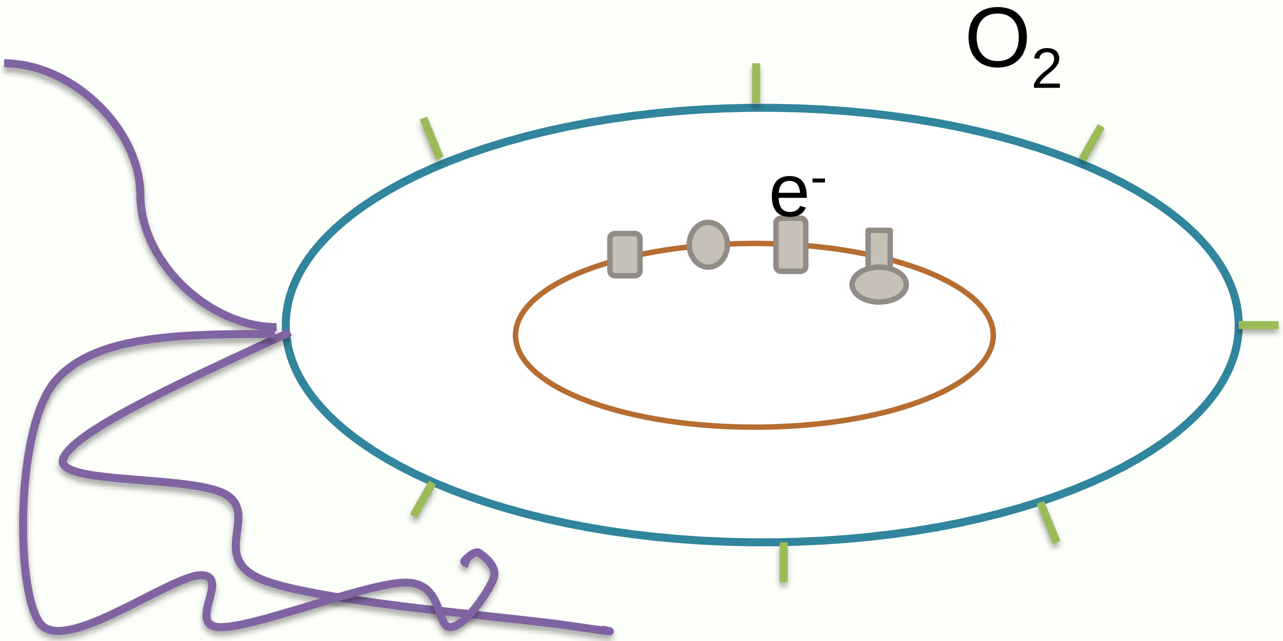
Aerobic metabolism



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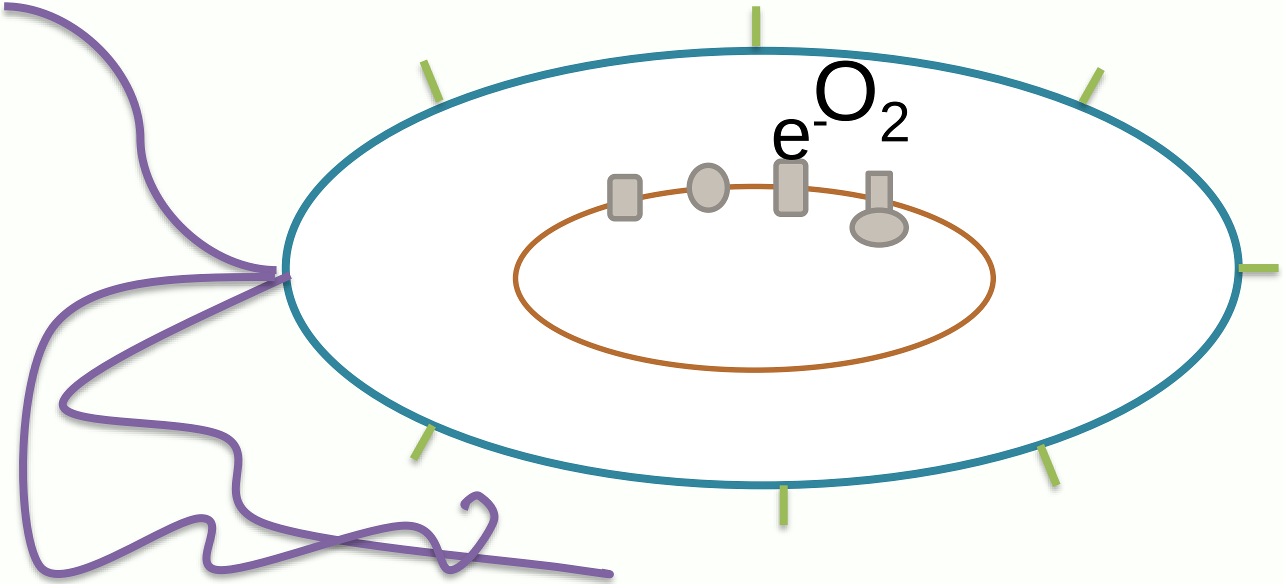
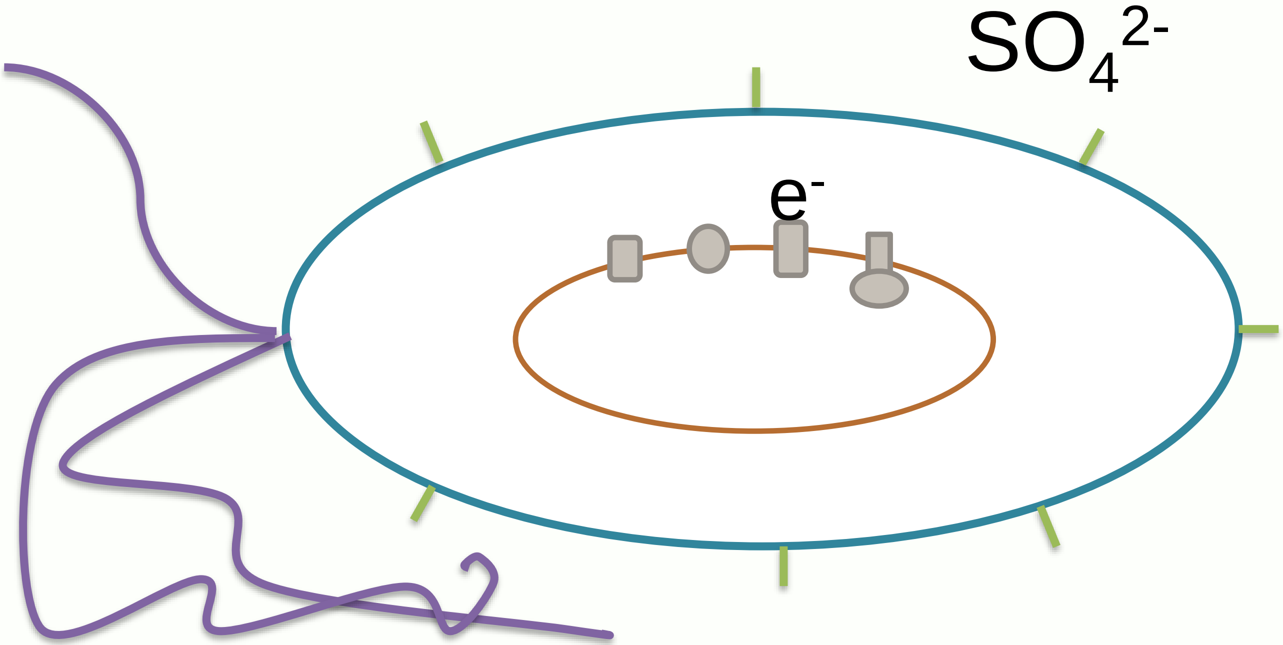
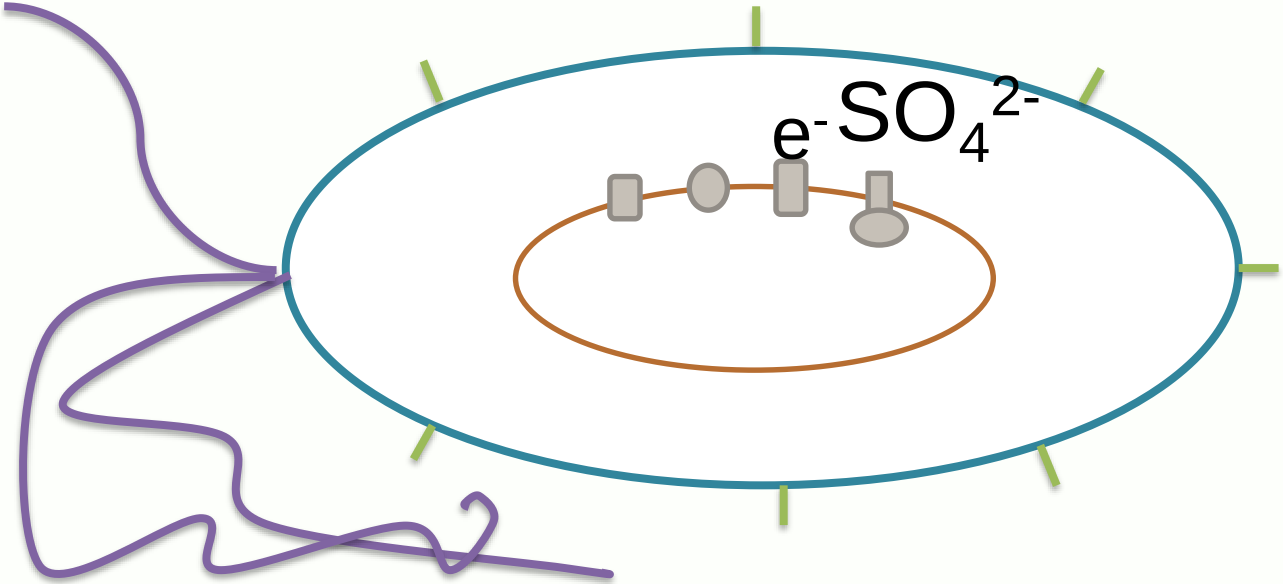
Aerobic metabolism



Anaerobic metabolism

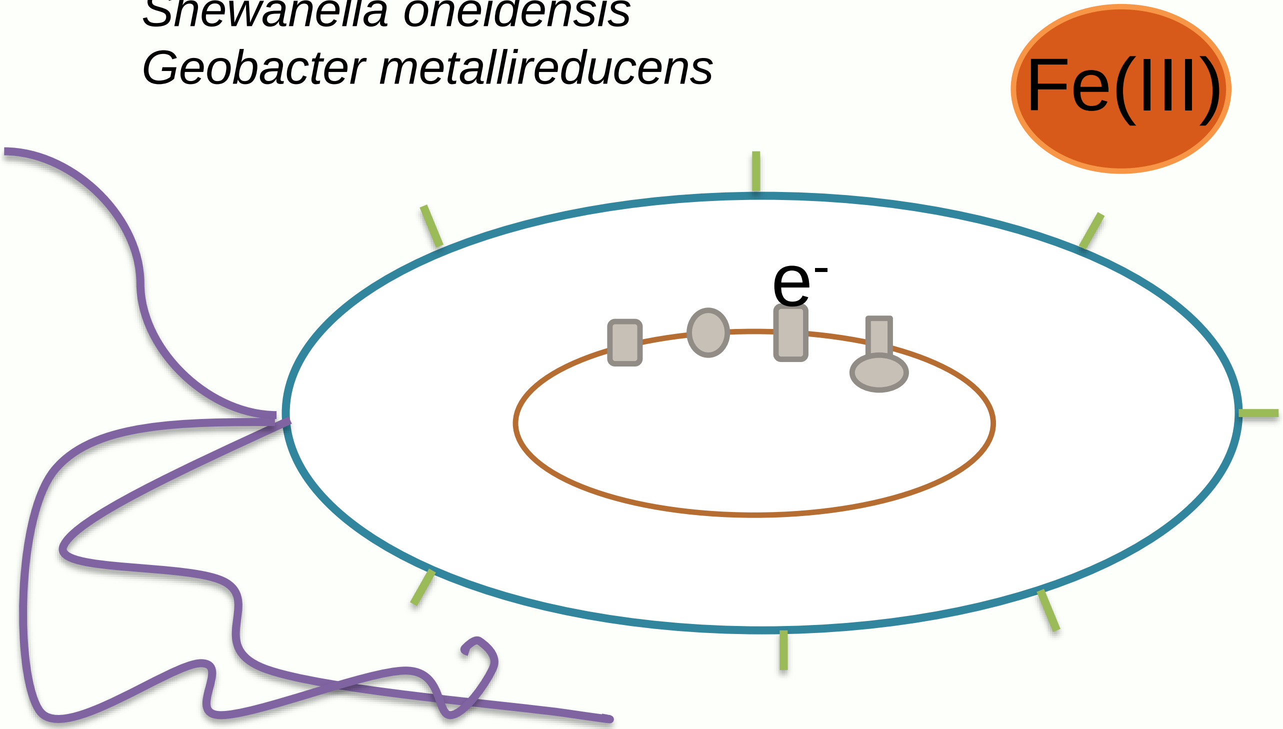


Anaerobic metabolism



Extracellular electron transport

Shewanella oneidensis
Geobacter metallireducens



Extracellular electron transport

Shewanella oneidensis
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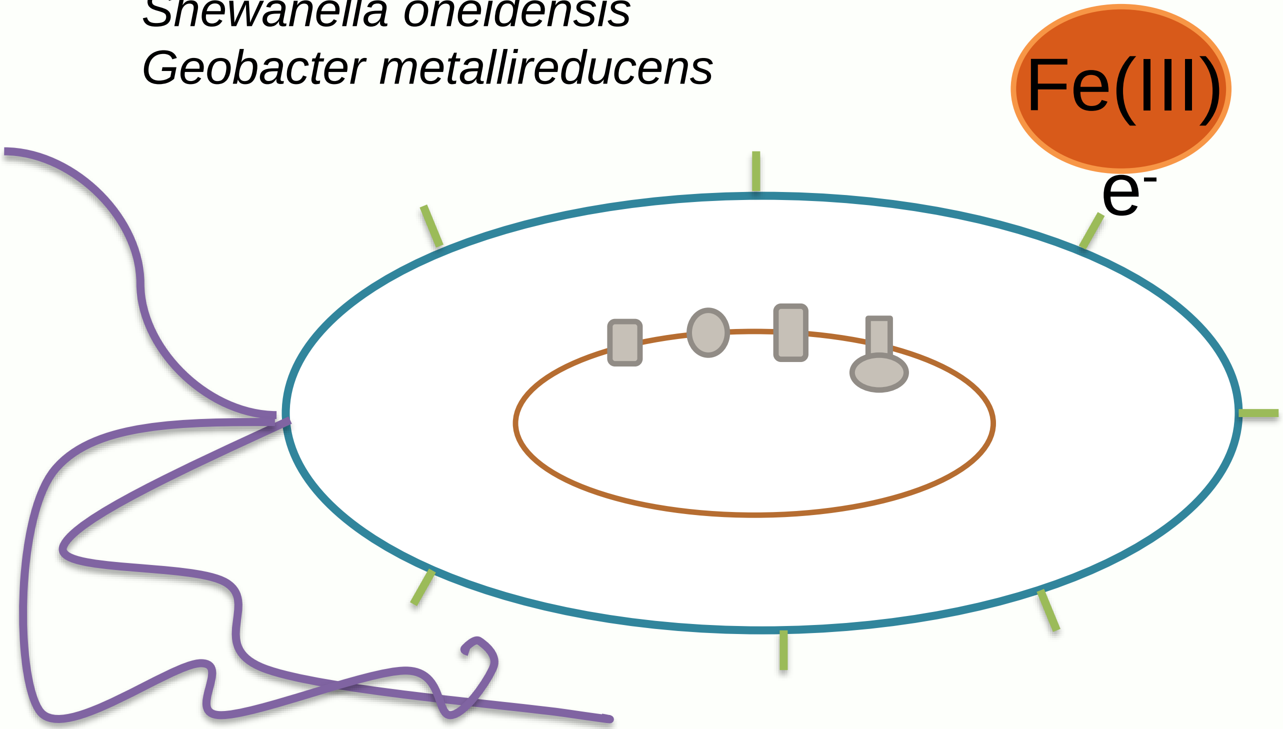
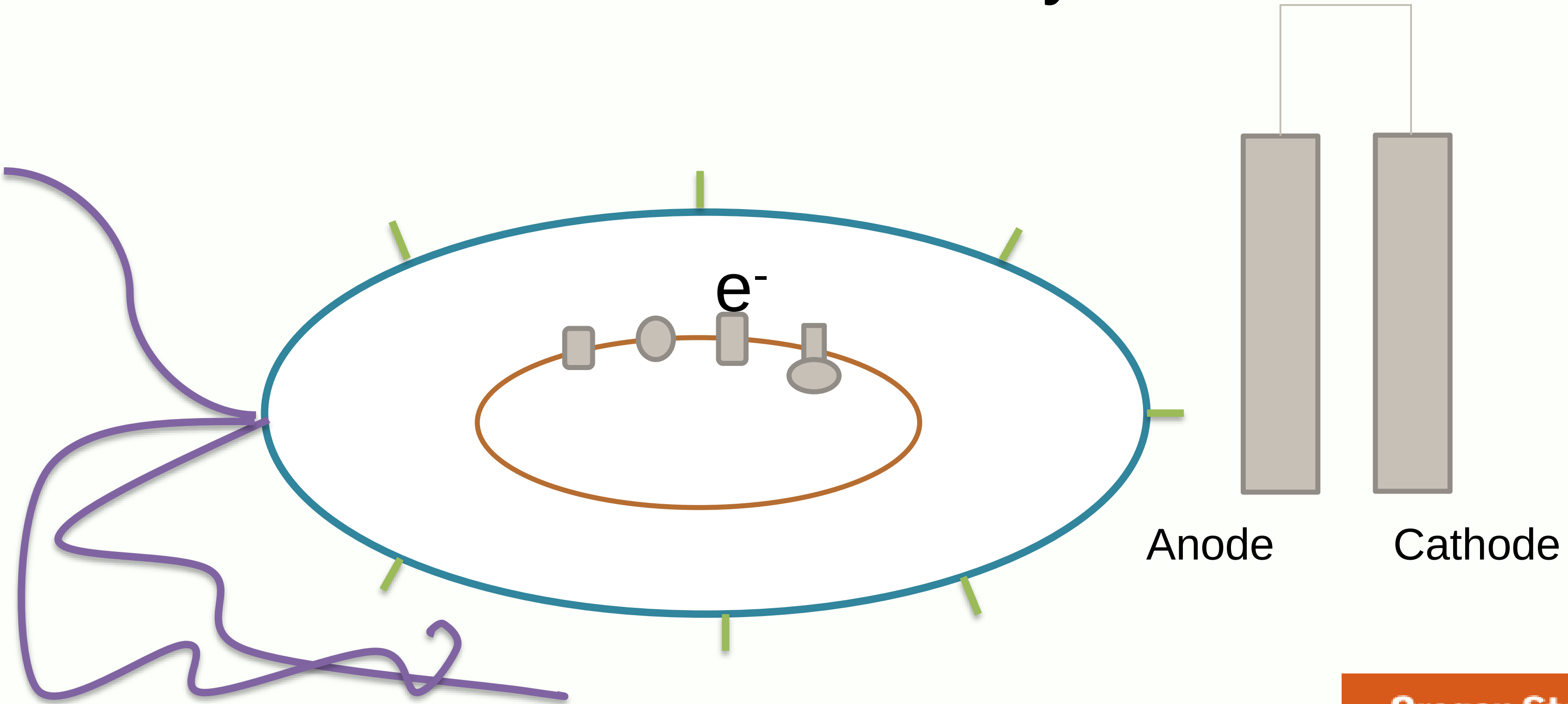
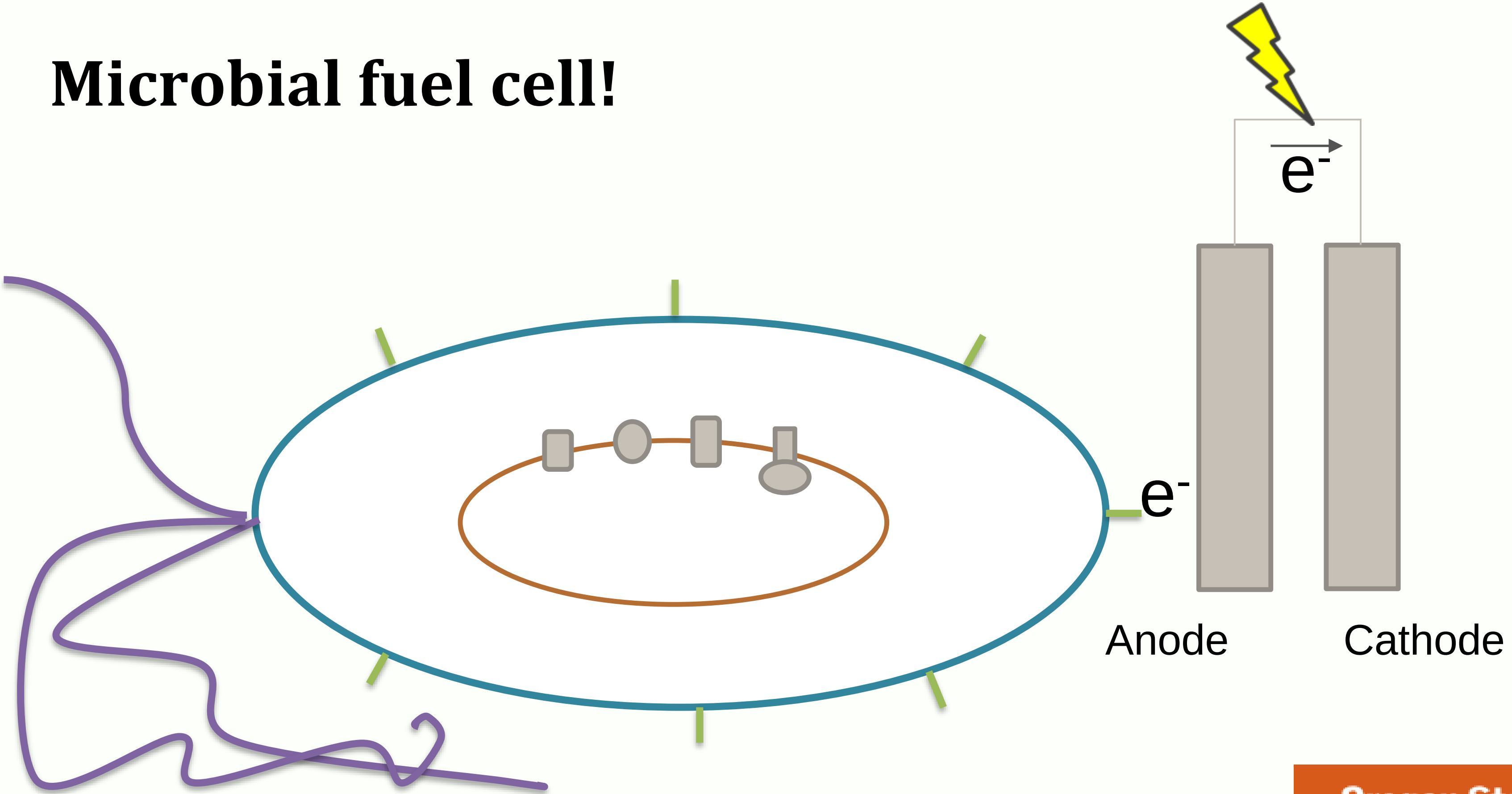


...addition of electrode assembly



Microbial fuel cell!



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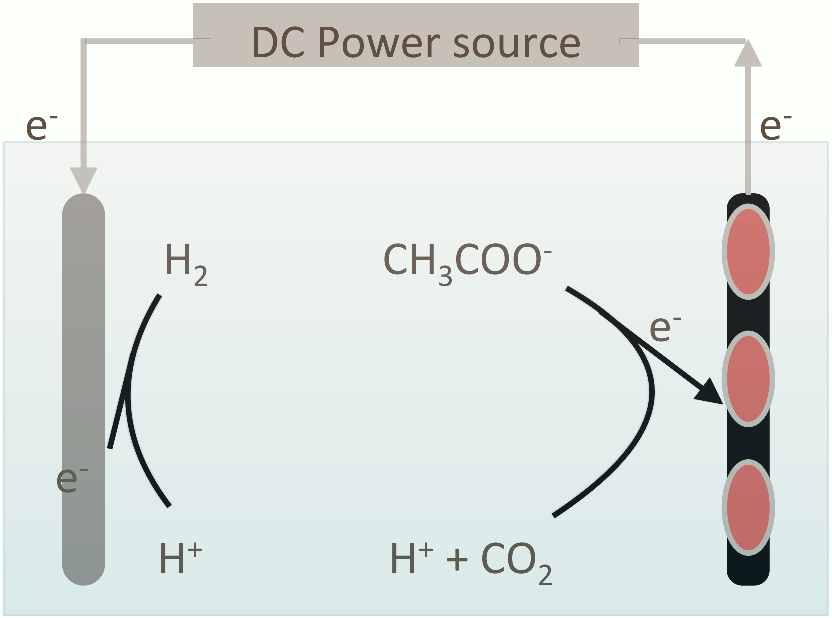
Hydrogen generation by Microbial Electrolysis Cell (MEC)



$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+ \quad \Delta G^\circ = +104\ kJ/mol$$

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Hydrogen is an attractive energy carrier

High in energy density

Higher (HHV) and lower (LHV) heating values of some common fuels[4]

Fuel	HHV MJ/kg	HHV BTU/lb	HHV kJ/mol	LHV MJ/kg
Hydrogen	141.80	61,000	286	119.96
Methane	55.50	23,900	889	50.00

Water is the only product of combustion

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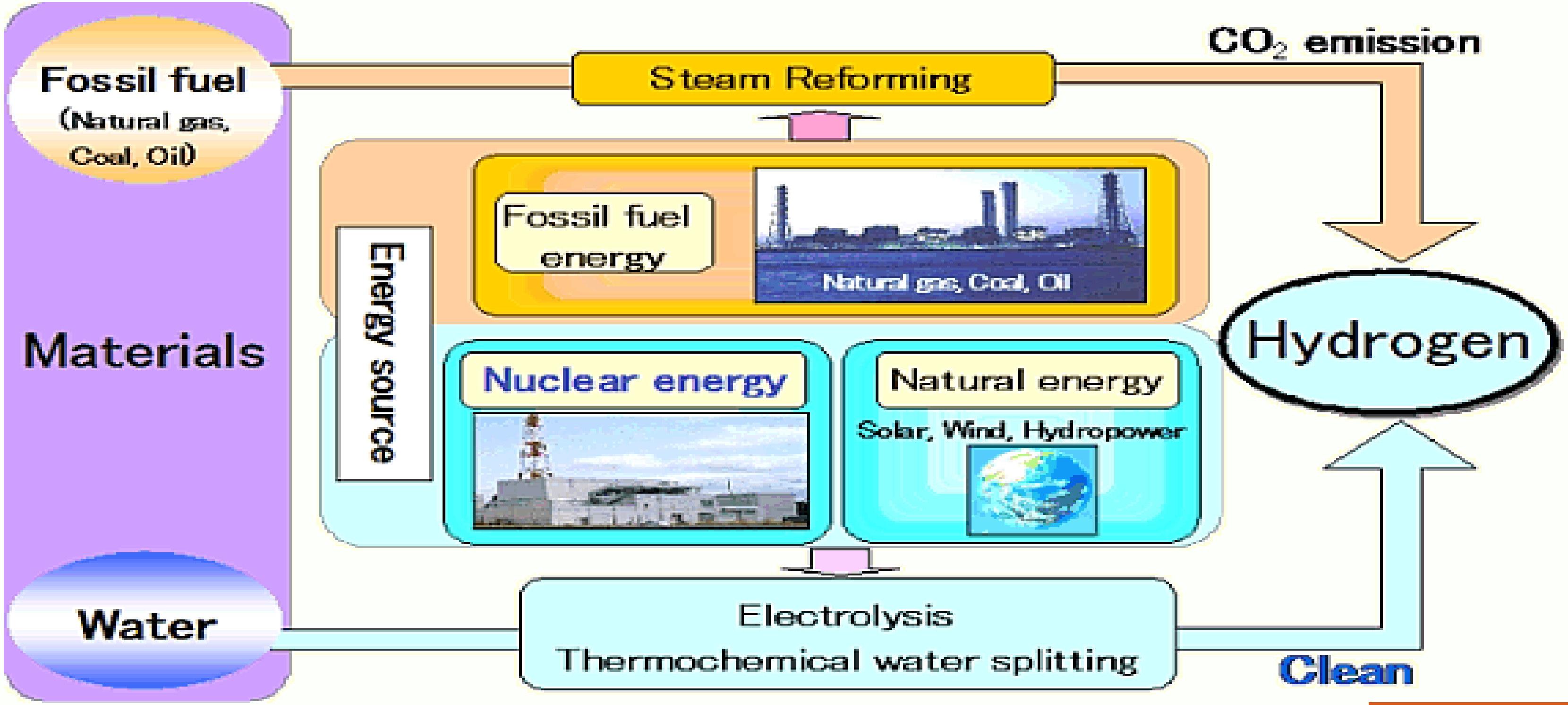
(Source: Wikipedia/NIST Chemistry Webbook)

Hydrogen Generation



Source: ZME Science

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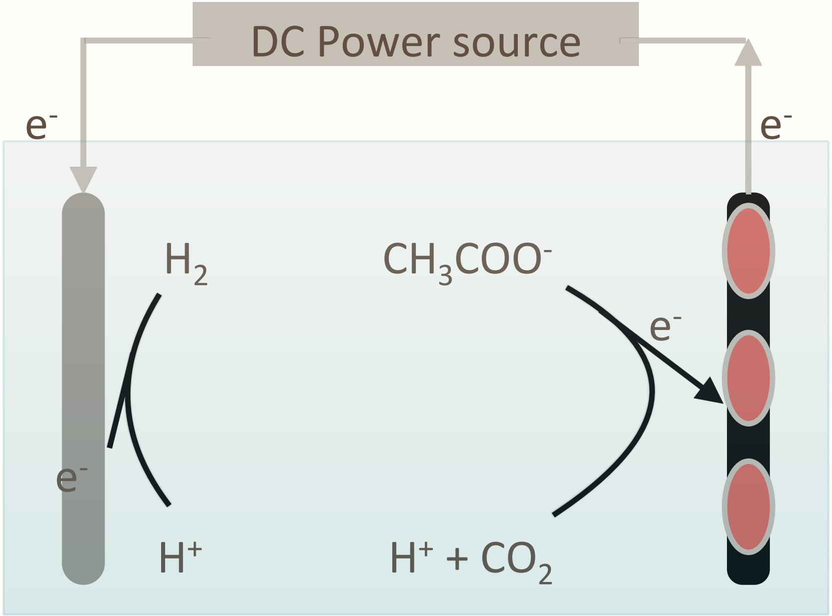
Hydrogen generation by Microbial Electrolysis Cell (MEC)



$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+ \quad \Delta G^\circ = +104\ kJ/mol$$

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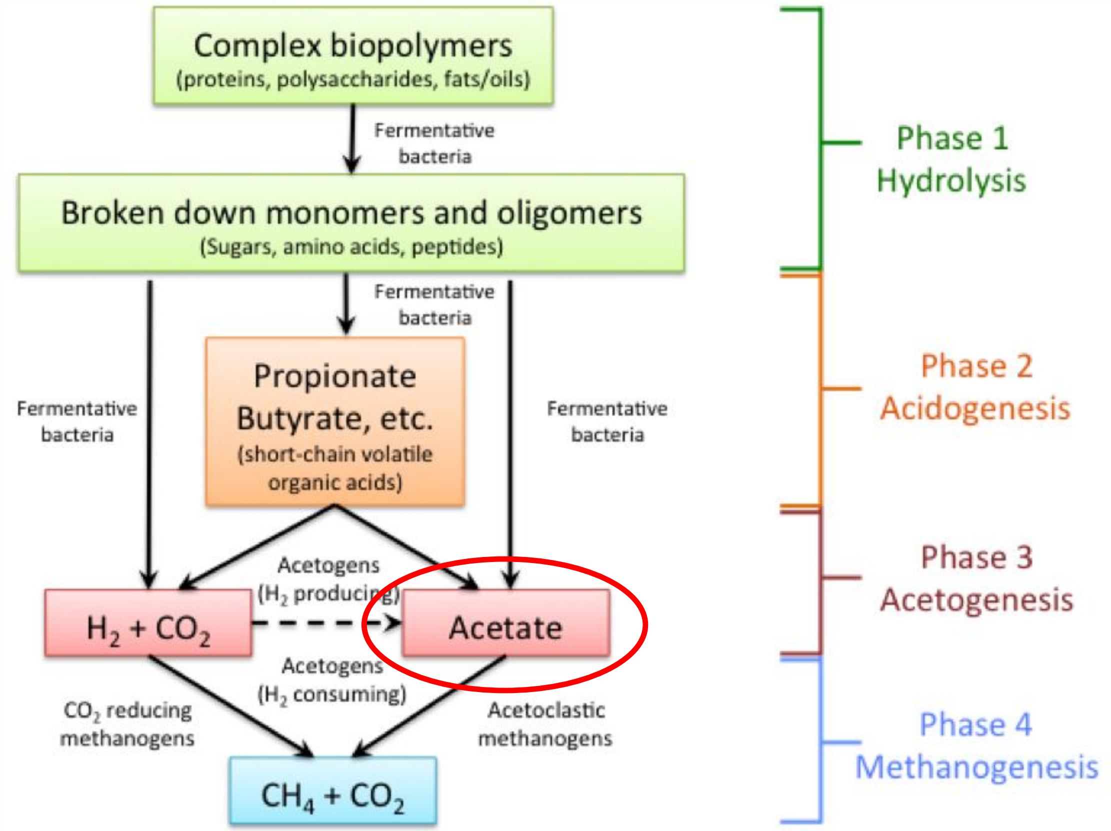
Building Better Biofilms

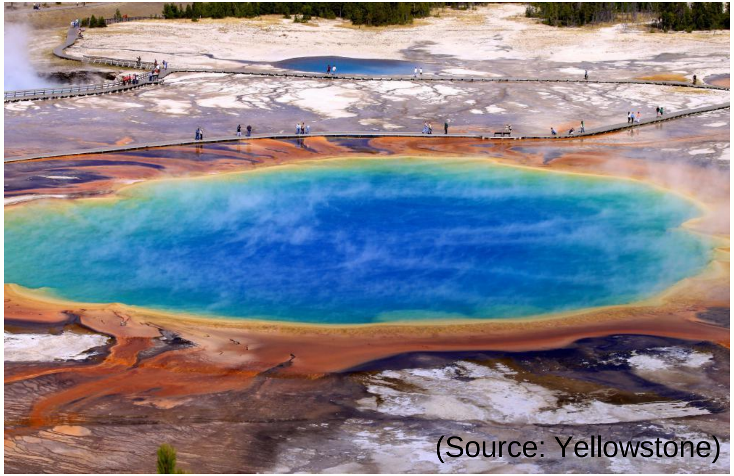


(Source: Pennsylvania State University)

- Mixed community to breakdown variety of substrates
- Anaerobic digestion

Methanogens

- Ubiquitous throughout the environment
- Sensitive to pH, temperature, oxygen
- Optimal pH 6.8 - 7.5
- Optimal temperature 25 - 30 °C
- Some are extremophiles


(Source: Yellowstone)


(Source: USDA)

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Methanogen Metabolism

$C_2H_3O_2^- + H^+ \rightarrow CH_4 + CO_2$ Acetotrophic methanogens

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ Hydrogenotrophic methanogens

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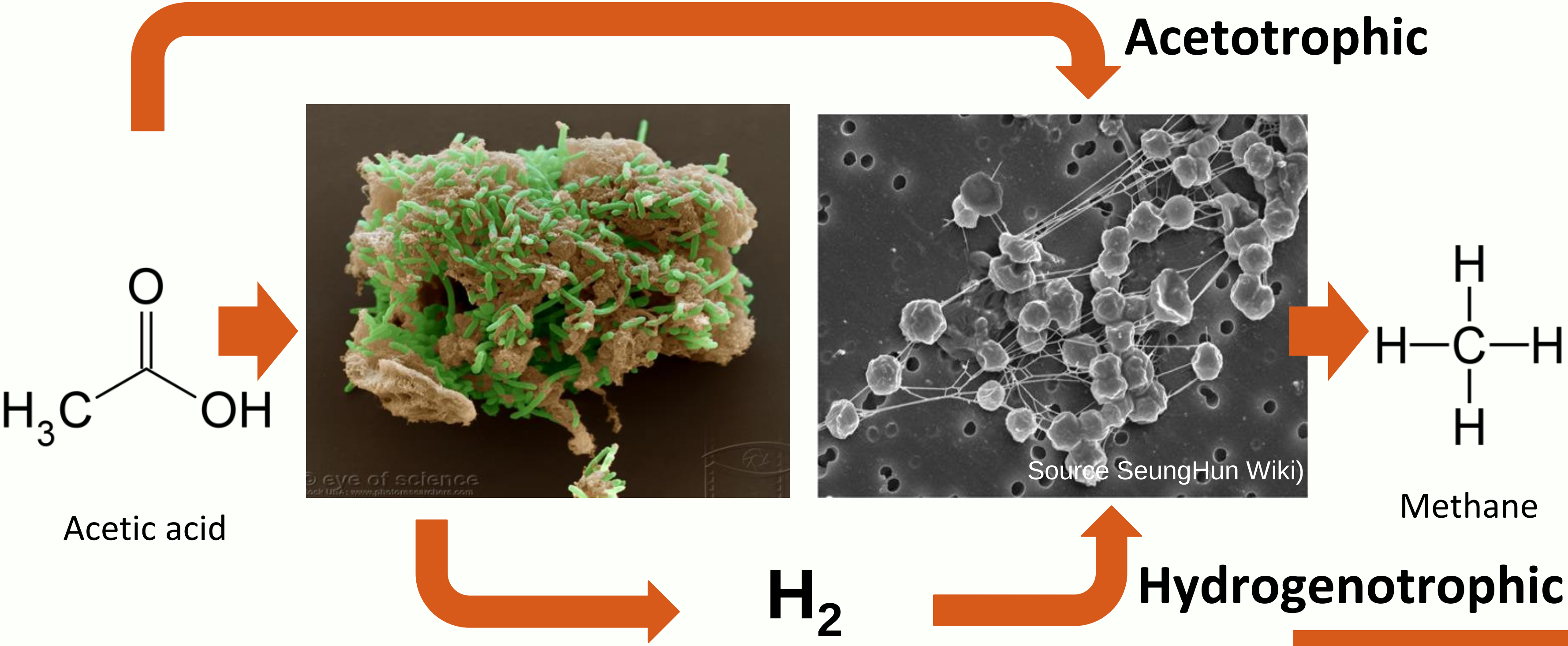
Scavenging by Methanogens in MECs



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Methanogens

252 Y. Gao et al. / Bioresource Technology 153 (2014) 245–253

Table 3
Kinetics parameters of ARB, methanogens, and homoacetogens from literature.

Substrate	Microorganisms	q_{max} (g COD/g VSS-d)	μ_{max} (d^{-1})	K_s (mg COD/L)	Threshold H_2 concentration (Pa)
Acetate	*Geobacter sulfurreducens*	22.7	2.4	0.64	–
	ARB	22.3	~3.2	119	–
	Acetoclastic methanogens	7.6	0.28	180–430	–
Hydrogen	ARB	–	–	–	–
	Hydrogenotrophic methanogens	4.7 g H_2/g VSS-d	0.77	0.006–1.4 (K_m)	2.3–9.3
	Homoacetogens	–	0.576	0.065–3.87 (K_m)	48.9–89.2

Assuming substrate-limiting conditions

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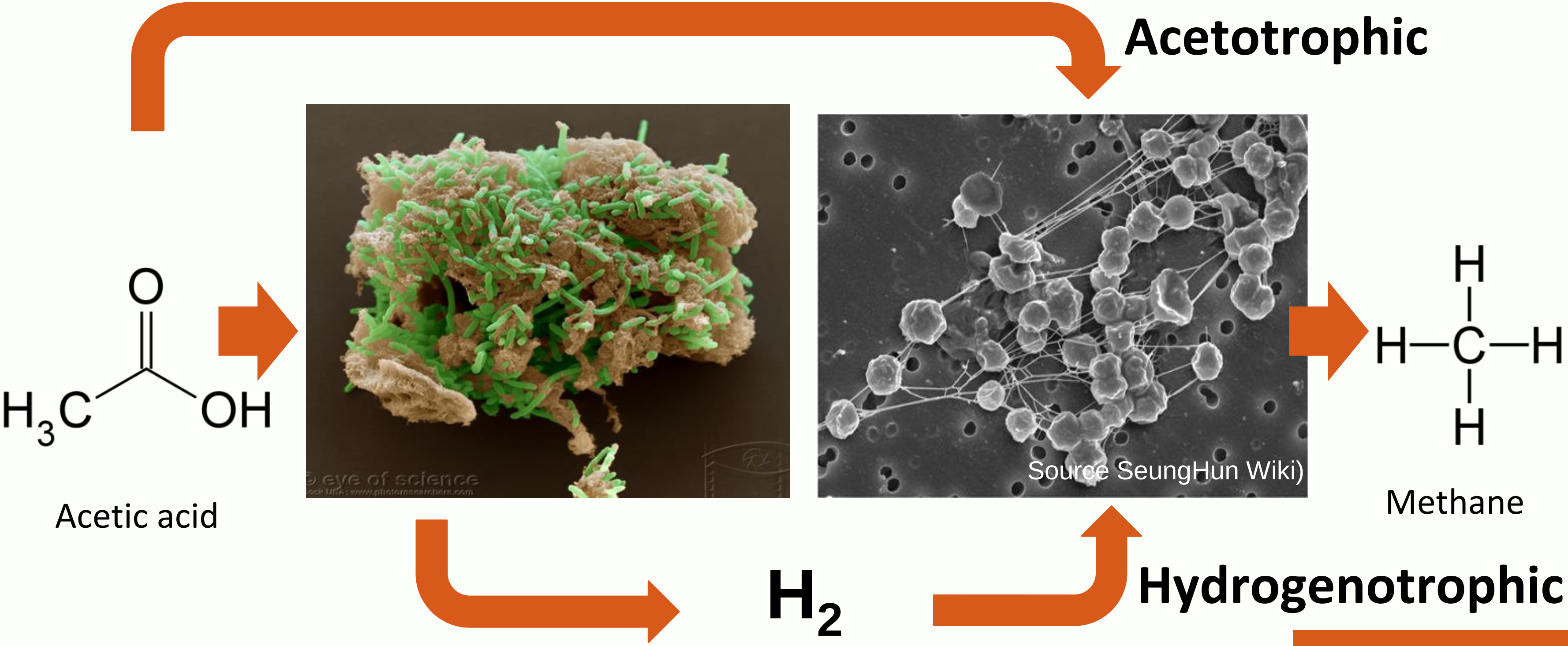
However,

Single chamber MECs, which decrease cost by requiring less materials, have had **diminished performance** due to **methanogen scavenging** of substrate and hydrogen (Call and Logan 2008; Hu et al 2008)

Scavenging by Methanogens in MECs



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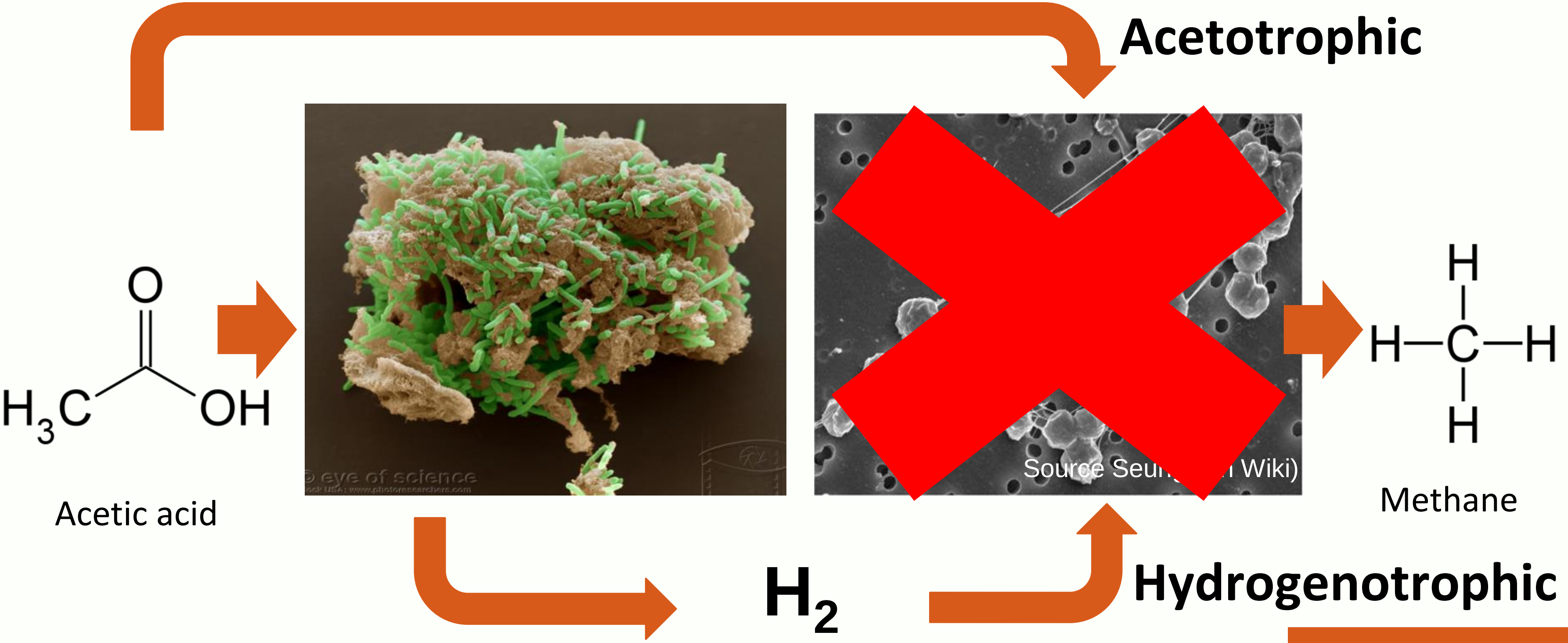
Scavenging by Methanogens in MECs



Methanogen Inhibition

- pH, temperature, and oxygen shock in MECs for inhibition of methanogenesis (Chae et al 2010)
- Methane generation recovered soon after dropping pH to 4.9 and temperature to 20 °C
- Oxygen caused damage to both methanogens and exoelectrogens

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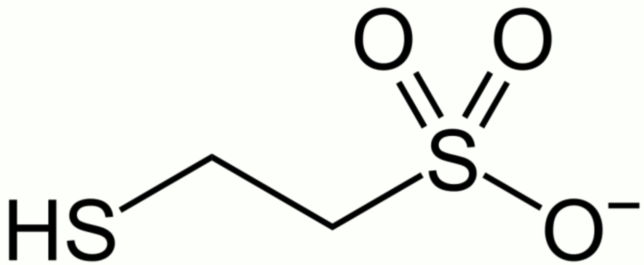
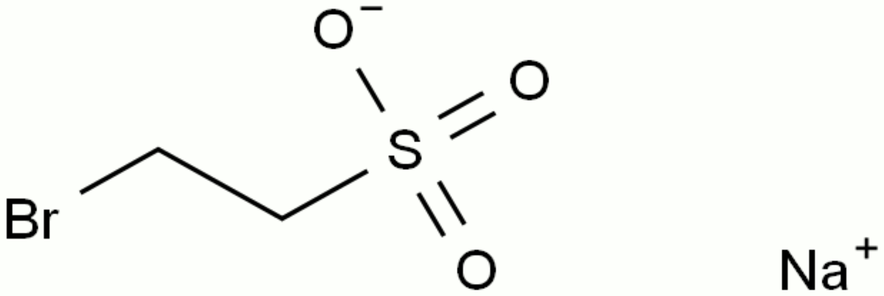
Methanogen Inhibition

- Coenzyme M precursor to methyl-coenzyme M
- 2-bromoethanesulfonate (BES) competitive inhibitor
  - Inactivates methyl-coenzyme M reductase reversibly and irreversibly (Konisky 1990; Boyd et al 2006)
  - Expensive

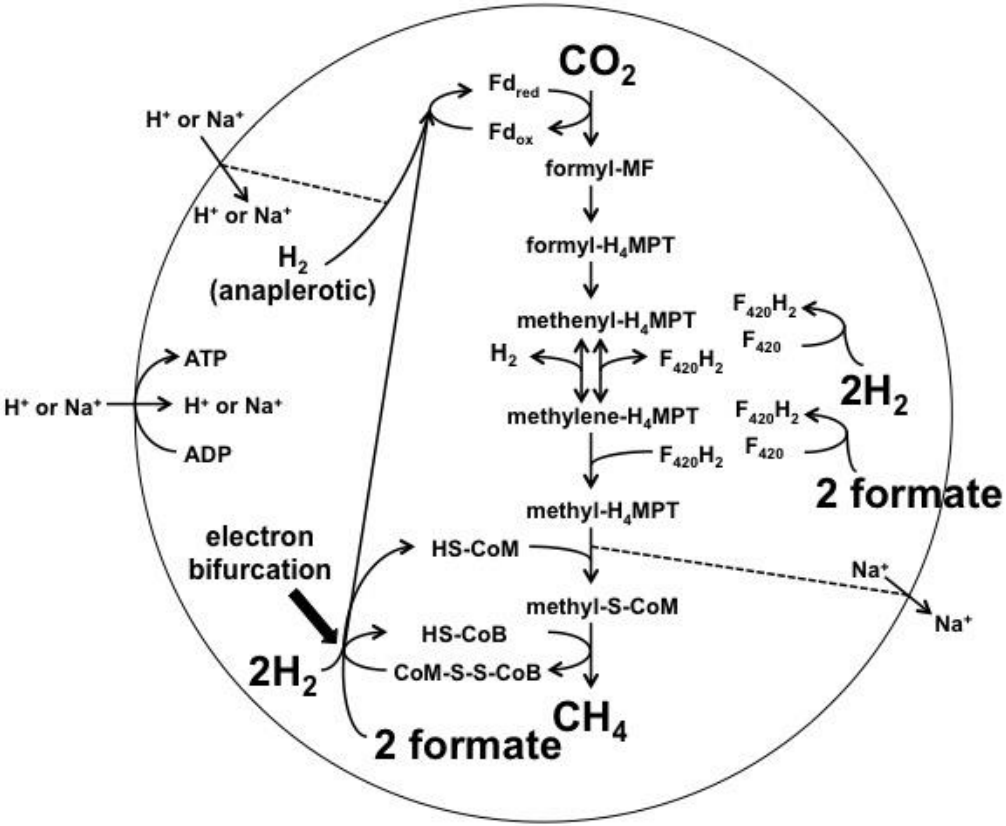
(Source: Organic Syntheses)



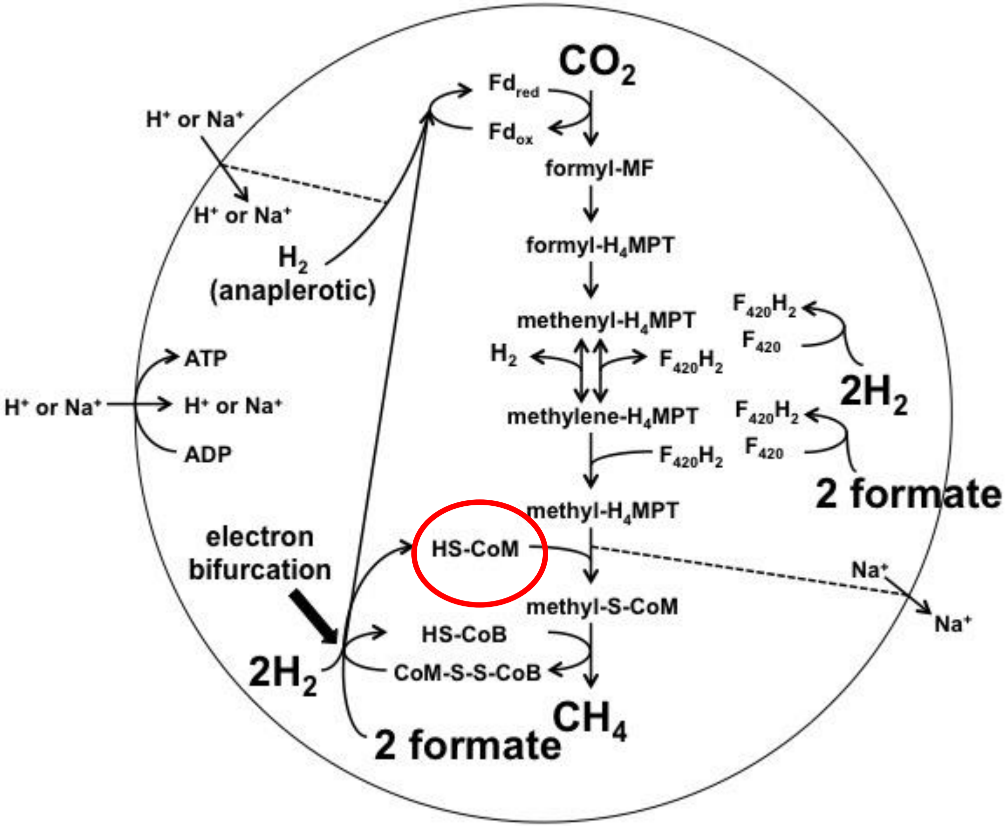
Coenzyme M



2-bromoethanesulfonate

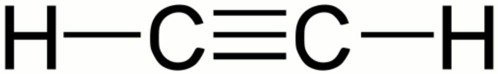


(Source: University of Washington)



(Source: University of Washington)

Acetylene: Classic methane inhibitor



(Source: BKChem)

- Hindered ability to maintain transmembrane proton gradient, decline in intracelllular ATP
- Did not cause physical damage to membrane (Sprott et al 1982)
- reduced activity of NiFe hydrogenase in *Methanosarcina*, which cleaves H_2 and reduces F_{420} coenzyme (He et al 1989)

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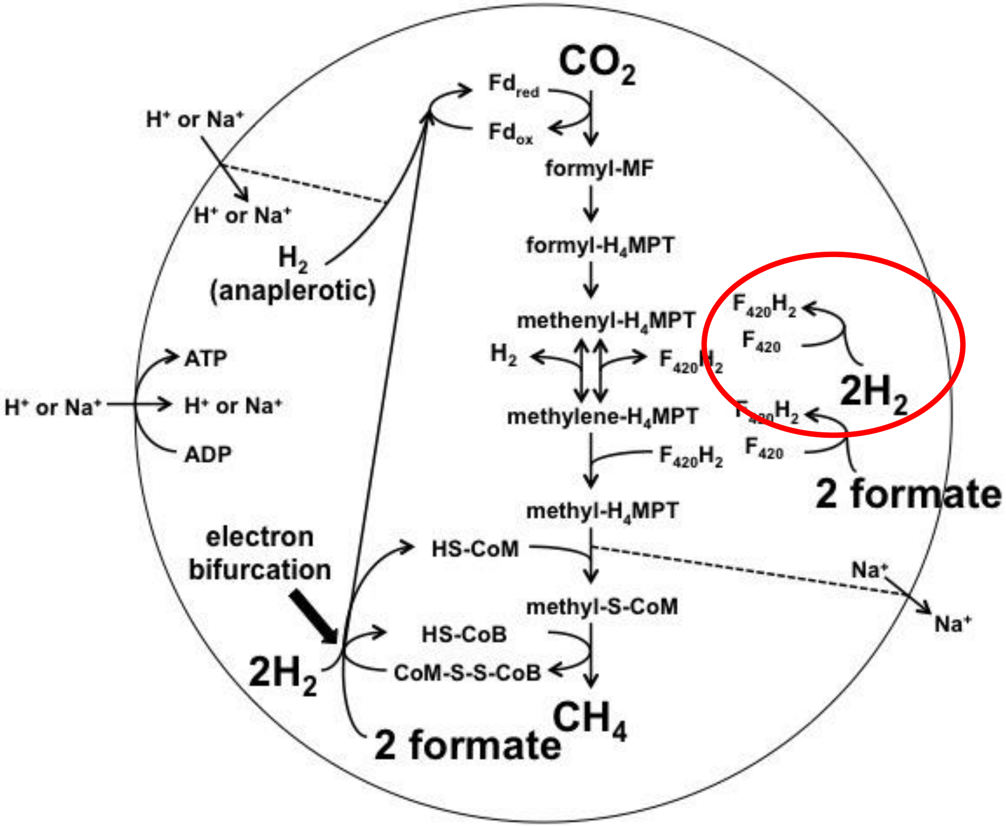

(Source: University of Washington)

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Acetylene Literature

- Acetylene used to inhibit methane in landfill cover soil
- 0.01% and up statistically significant from control
- 0.01% inhibited methanogenesis by 80% (Chan and Parkin 2000)
- Study only lasted up to 7 days
- 1% Acetylene as effective as 25 mM BES in anaerobic composters (Sparling et al 1997)

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Relevant questions

- How long is each inhibitor (BES and acetylene) effective?
- How often does each treatment need to be administered?
- What are the lower and upper limitations on concentration?
- Will acetylene have a negative impact on exoelectrogens?

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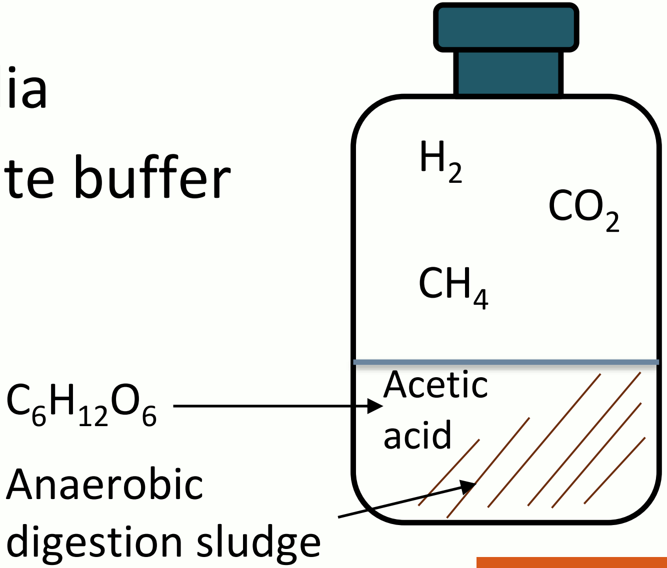
Fermentation Trials

- Effectiveness of methanogen inhibition methods in fermentation
- 120 mL serum bottles, 50 mL media
- 11 mM glucose, 100 mM phosphate buffer
- Control
- Heat shock
- 5 mM BES
- Acetylene at 0.1%, 1.0%, 5.0%



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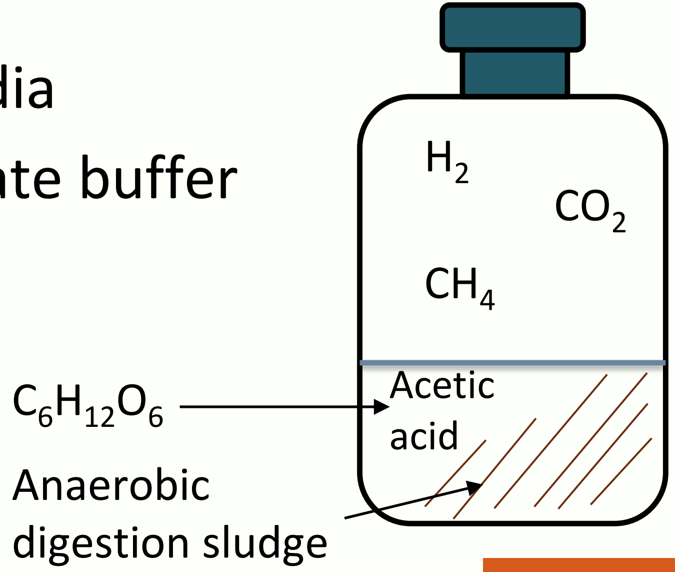
Fermentation Trials

- Effectiveness of methanogen inhibition methods in fermentation
- 120 mL serum bottles, 50 mL media
- 11 mM glucose, 100 mM phosphate buffer
- Control
- Heat shock (day 12)
- 5 mM BES (day 20)
- Acetylene at 0.1%, 1.0%, 5.0%



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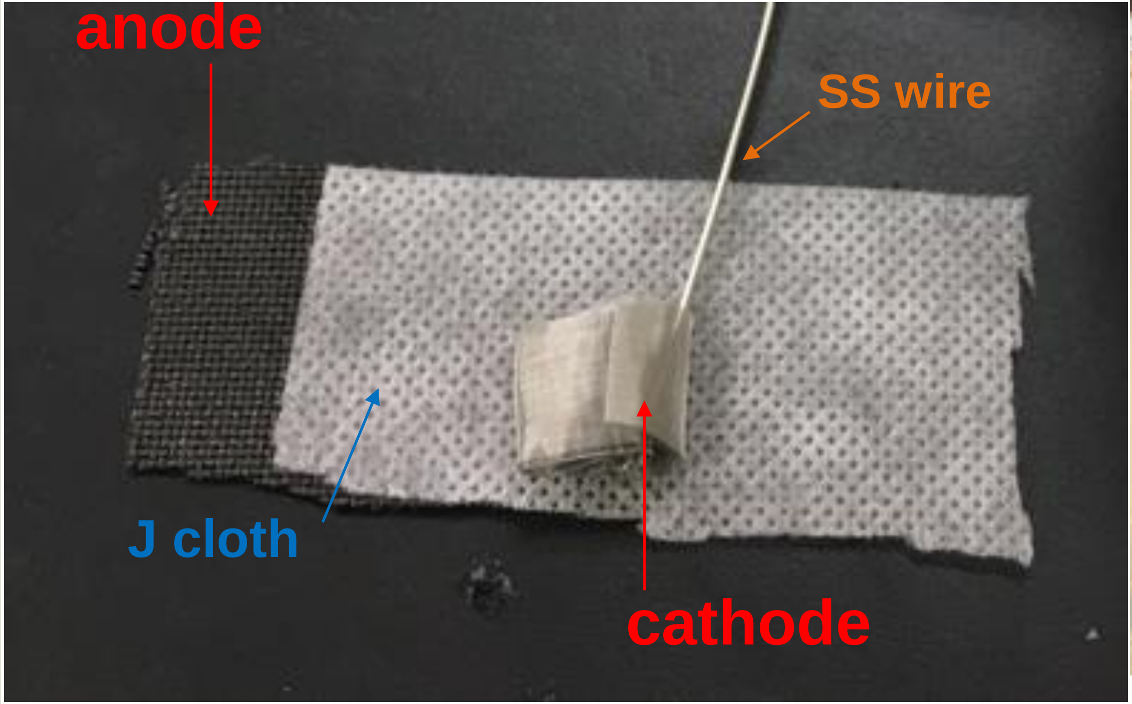
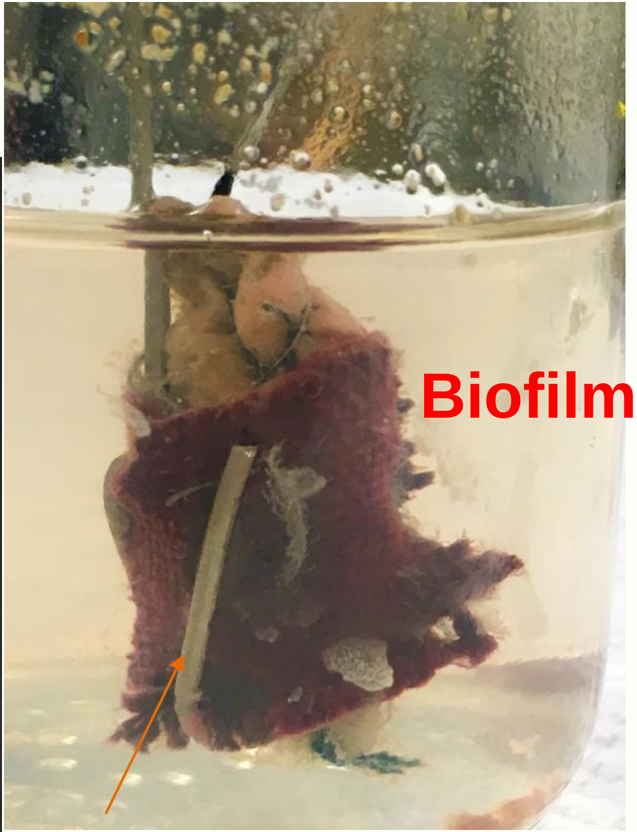
MEC Configuration and Set-up

- Single-chamber, bottle-type (320 mL)
- 10 cm^2 carbon cloth anode, 50 cm^2 stainless steel cathode
- Inoculated using biomass from previously operated MECs and anaerobic digestion sludge from WWT
- 100 mL media (100 mM phosphate buffer, vitamins/minerals)
- Sealed and purged with N_2 to achieve anaerobic conditions
- 1.0 V applied from power supply

MEC Assembly





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Materials and Methods

- 30 mM acetate
- Treatments
  - Control (no inhibition)
  - 5 mM BES
  - 1% Acetylene
  - 5% Acetylene
- Duplicates

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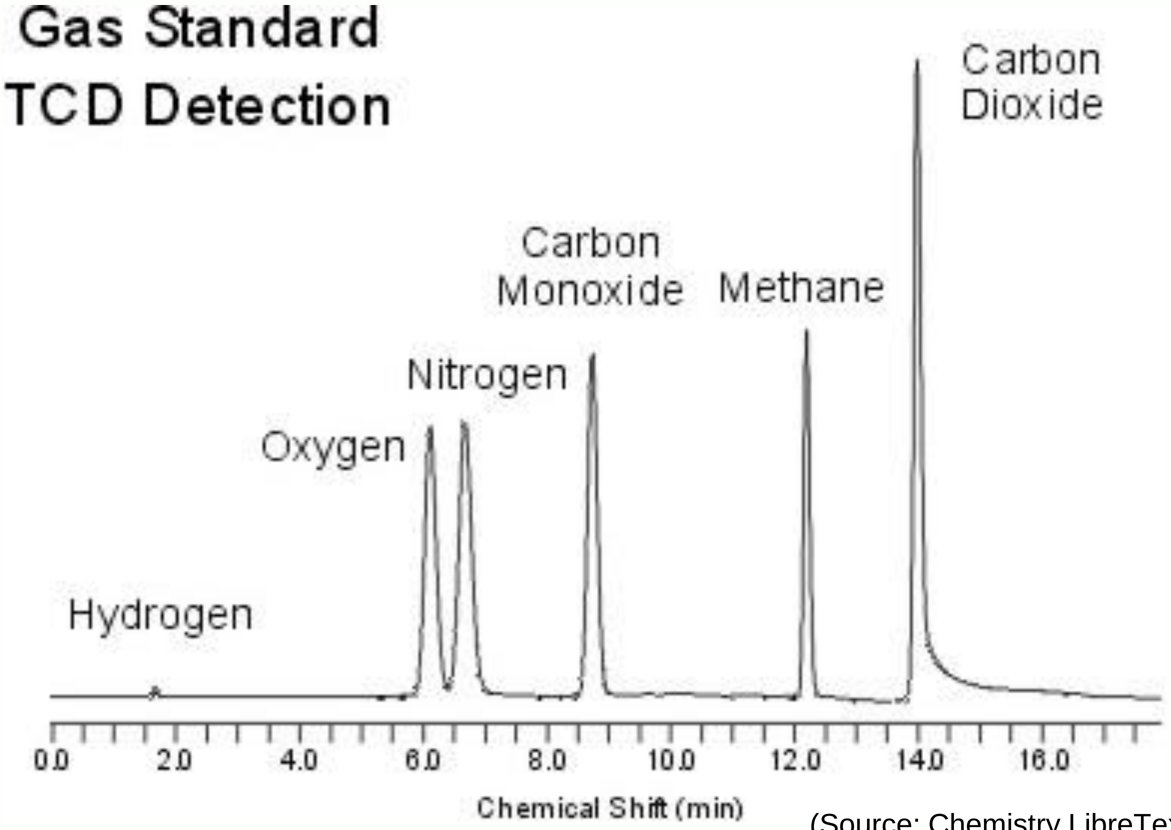
Materials and Methods

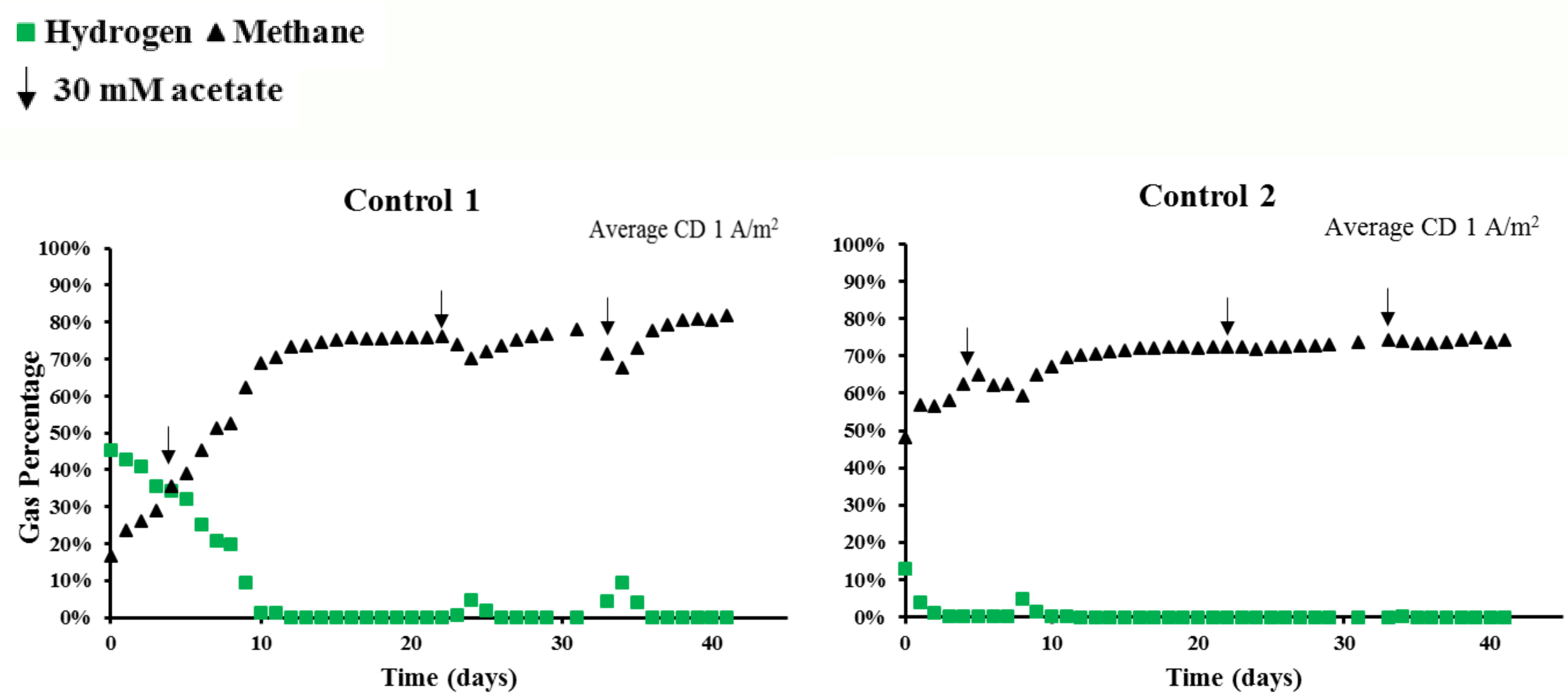
- Measured biogas and analyzed using gas chromatography every 24 hours
- Voltage monitored with a multimeter with data acquisition system (2700, Keithley)
- Current = Voltage/Resistance
- Current density (CD) = Current/Anode area

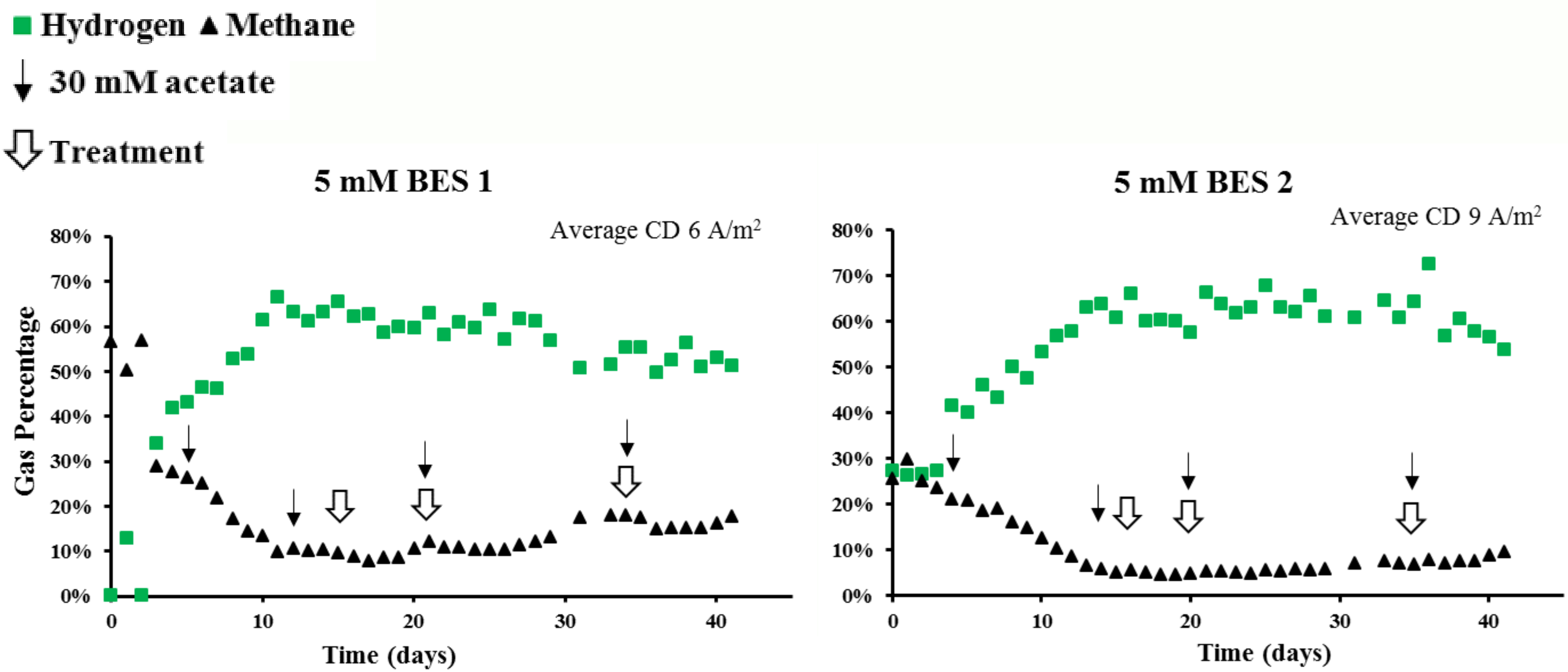


(Source: Chemistry LibreTexts)

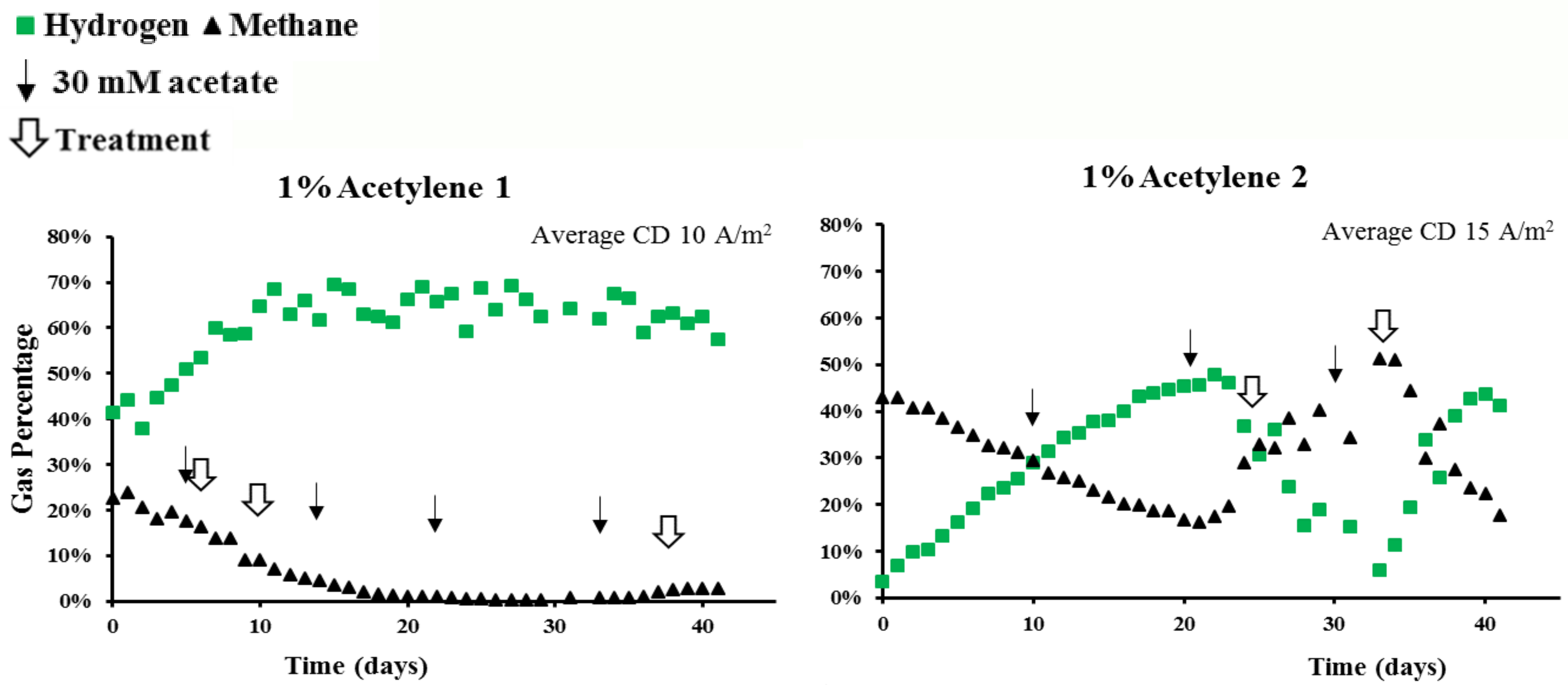
Administering treatment

- Treatment administered when methane recovery occurred
- Assumed conc. coming out = headspace
- Acetylene losses from biogas release approximated
- Administered volume required to reach desired concentration
- BES administered by concentrated liquid injection (500 mM)


Hydrogen
Methane
30 mM acetate
Control 1
Average CD 1 A/m²
Gas Percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
10
20
30
40
Time (days)
Control 2
Average CD 1 A/m²
Time (days)



Hydrogen
Methane
30 mM acetate
Treatment
5 mM BES 1
Average CD 6 A/m2
Gas Percentage
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
10
20
30
40
Time (days)
5 mM BES 2
Average CD 9 A/m2
Time (days)



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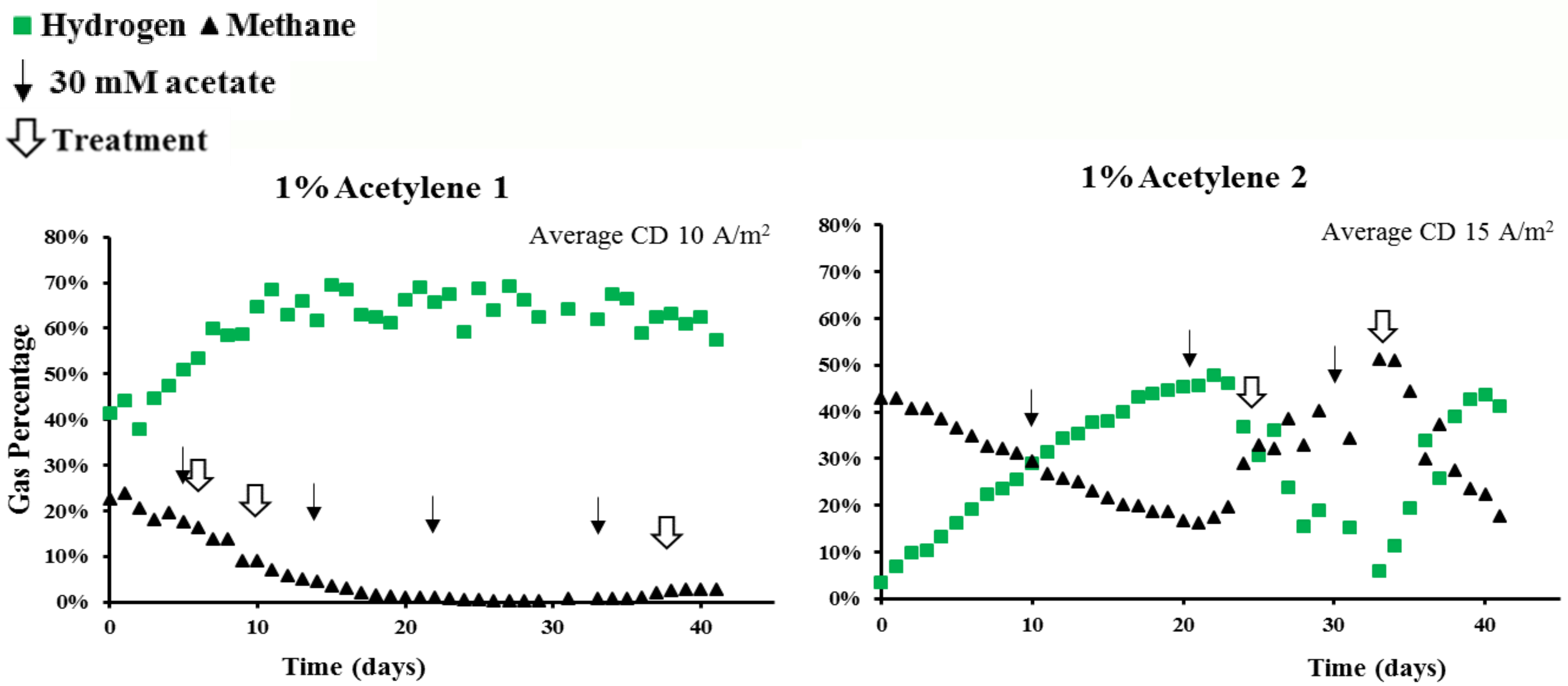
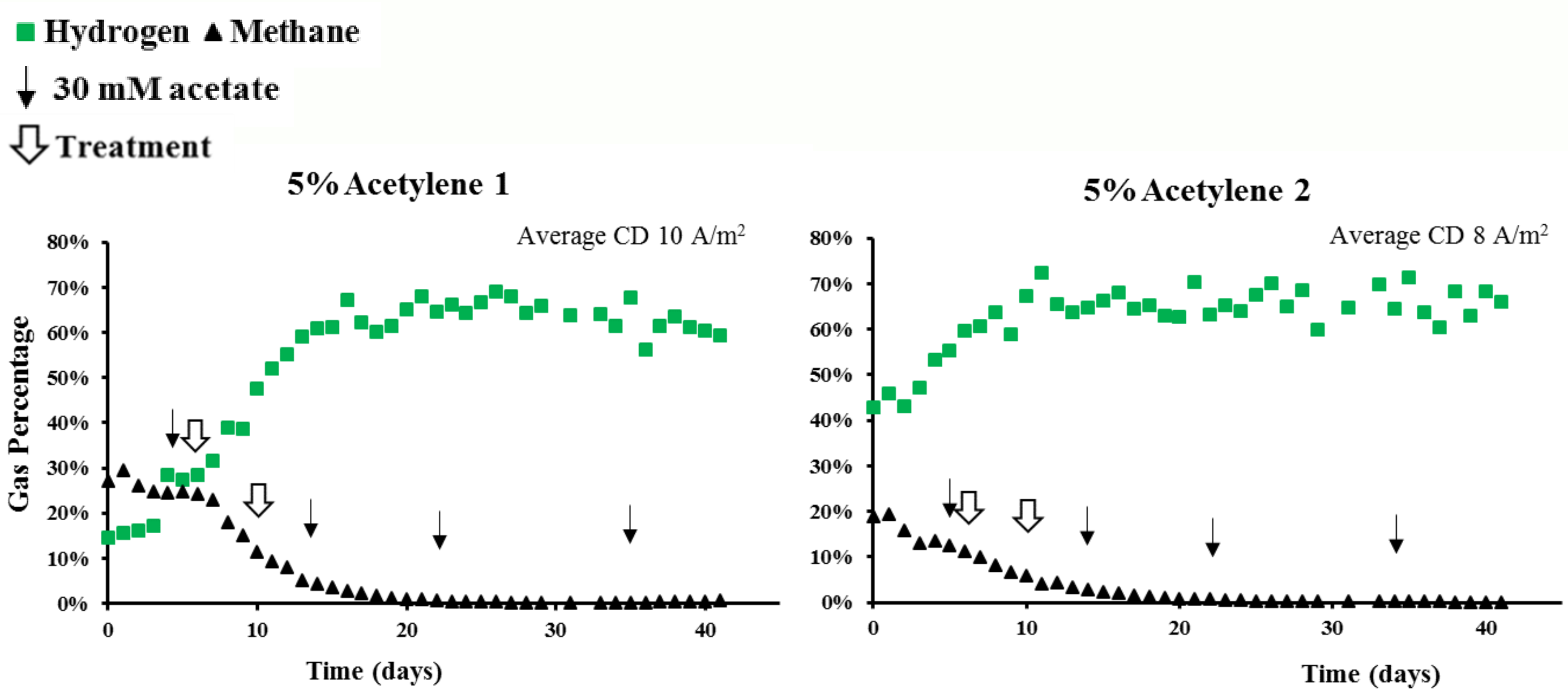


- Reactor 1 exhibited same behavior as reactor 2 later on, with continued methane generation even after re-injecting and maintaining acetylene at constant 1%



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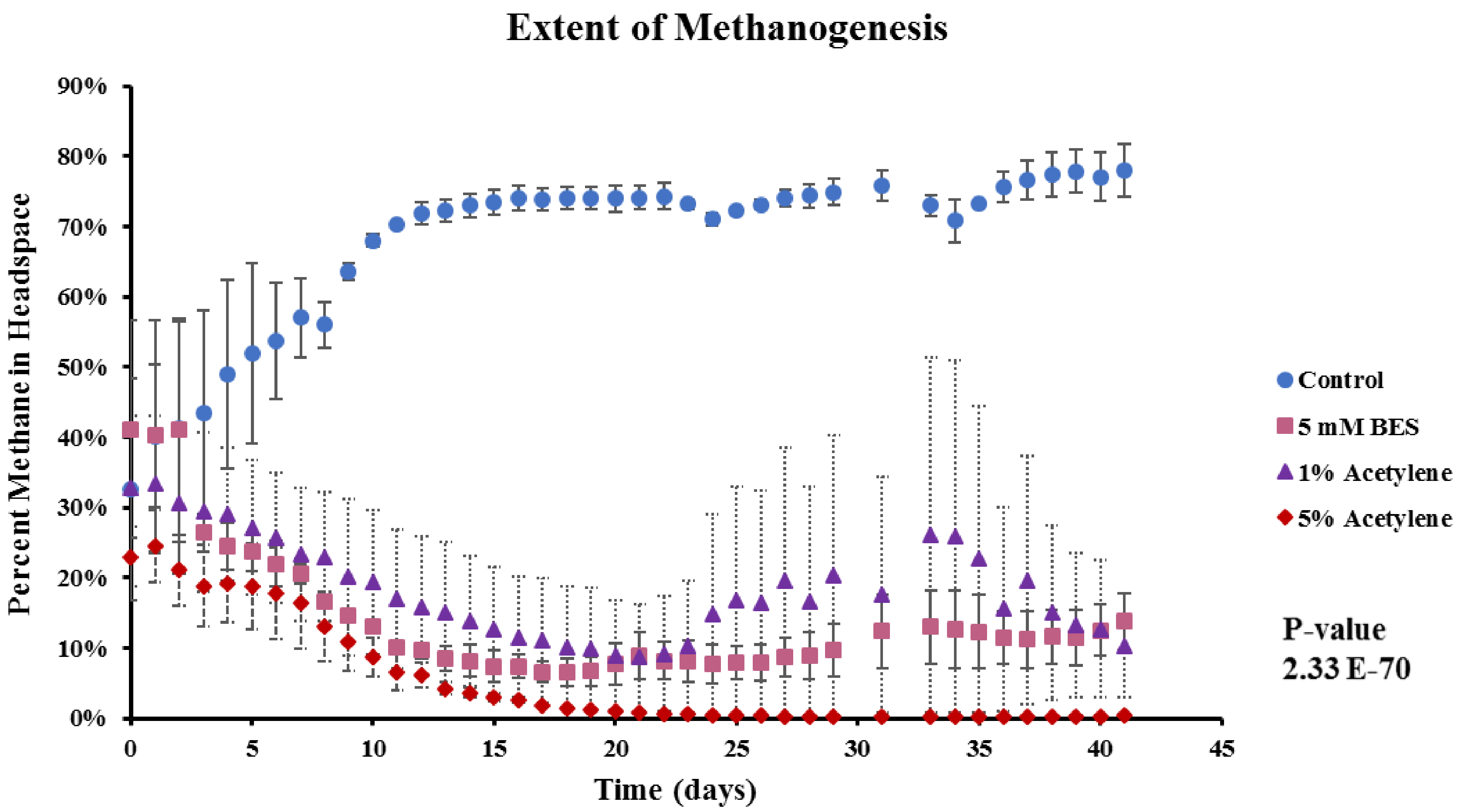
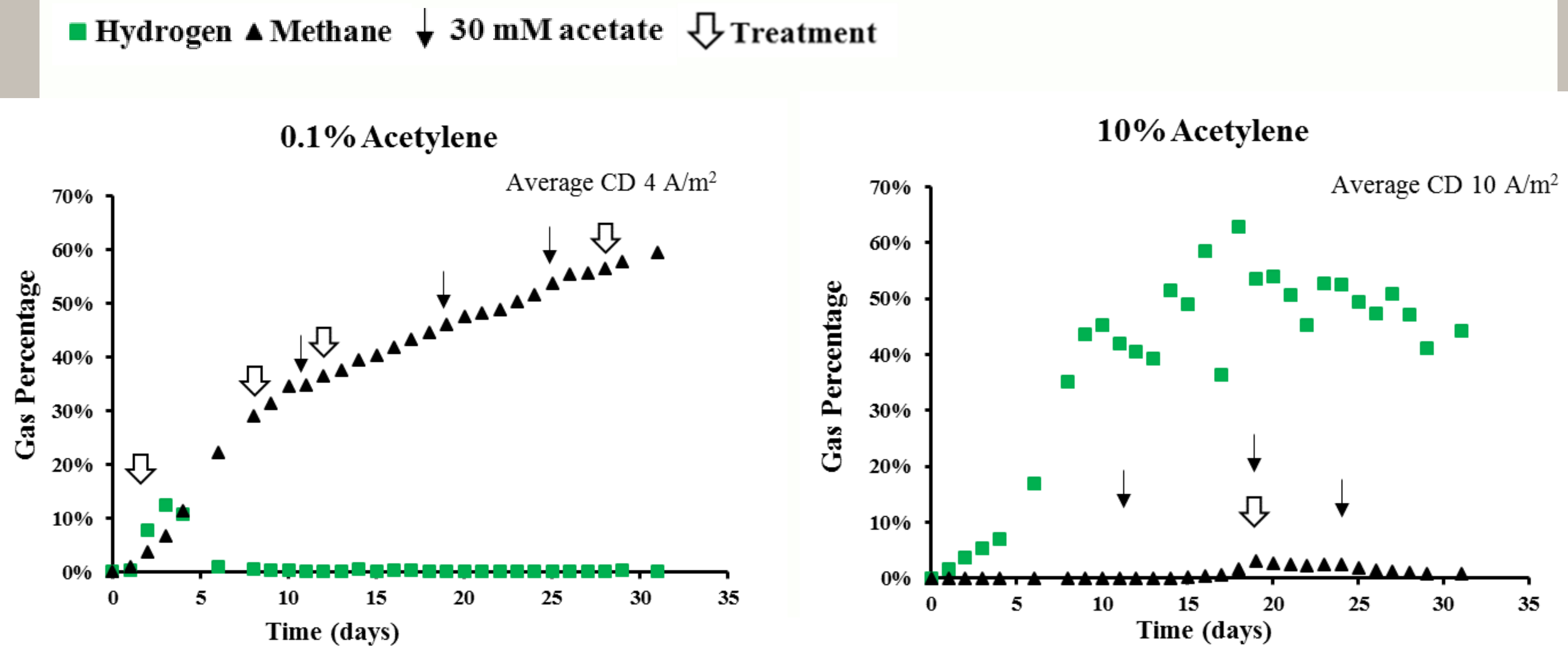


Extent of Methanogenesis
Percent Methane in Headspace
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
5
10
15
20
25
30
35
40
45
Time (days)
Control
5 mM BES
1% Acetylene
5% Acetylene
P-value
2.33 E-70



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Discussion of results

- 5% acetylene and up more effective than 5 mM BES
  - Complete inhibition
  - Less frequent application of treatment
- Decrease in effectiveness of 1% acetylene and 5 mM BES could be due to biofilm thickness
- Diffusion of compounds results in lower concentration

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Discussion of results

- Sources of error
  - MEC biological, performance/feeding not always the same
  - Connection issues to power supply
  - GC calibration
  - Leaks in rubber stopper
  - Hydrogen bubbles trapped in SS mesh

Wastewater Characterization and Trials

- Preliminary trials to evaluate performance of MECs operated with brewery wastewater
- Chemical Oxygen Demand (COD) determination
  - Digestion method following USEPA Method 410.4
- COD ~ 60,000 mg/L
- Diluted 1:10, 200 mM phosphate buffer
- MECs tested previously treated with 1% and 5% acetylene

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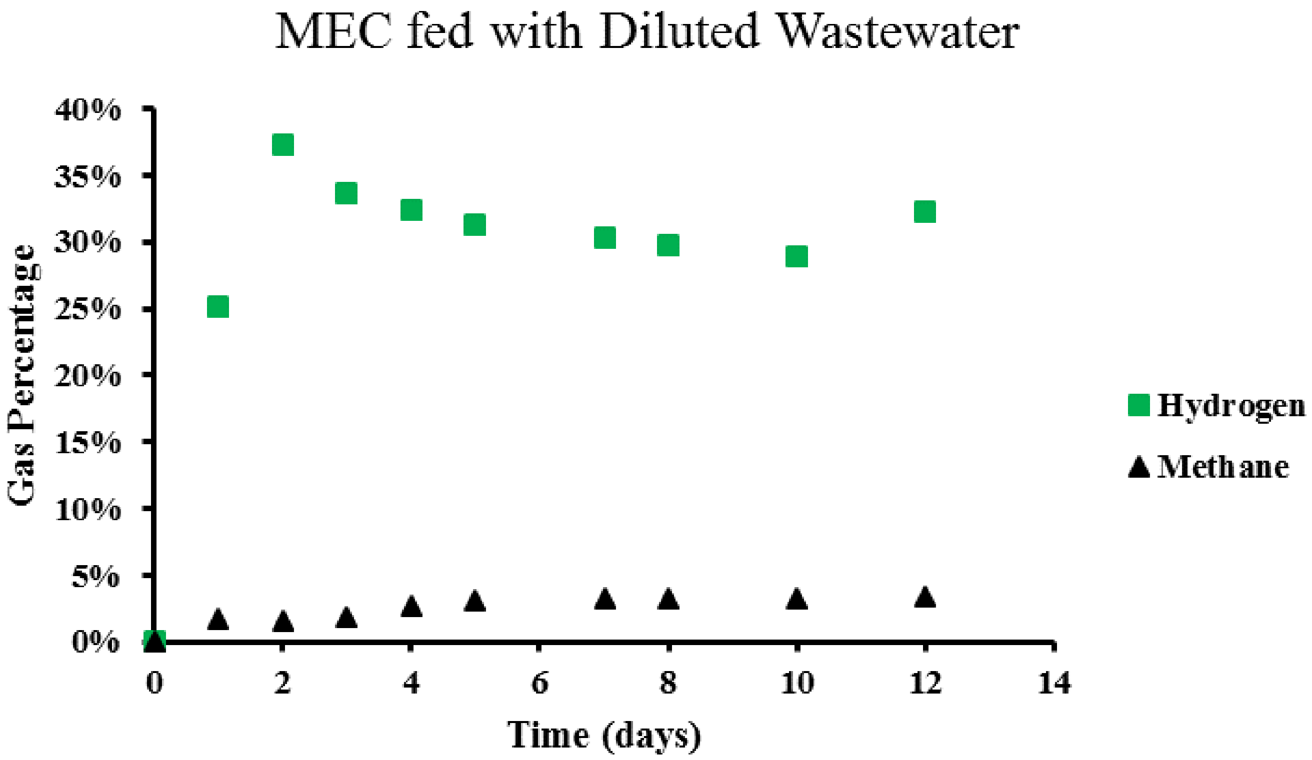

CD drop from 15 A/m^2 to 5 A/m^2

pH drop from 7 to 4

Conclusion and Future Work

- What we showed:
  - Acetylene at 5% and up keeps methanogenesis at bay over the long term
  - Does not cause harm to MEC performance
- Future work:
  - Longer study on fermentative conditions
  - Control pH under batch conditions to test acetylene's effectiveness for real wastewater treatment

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Acknowledgments

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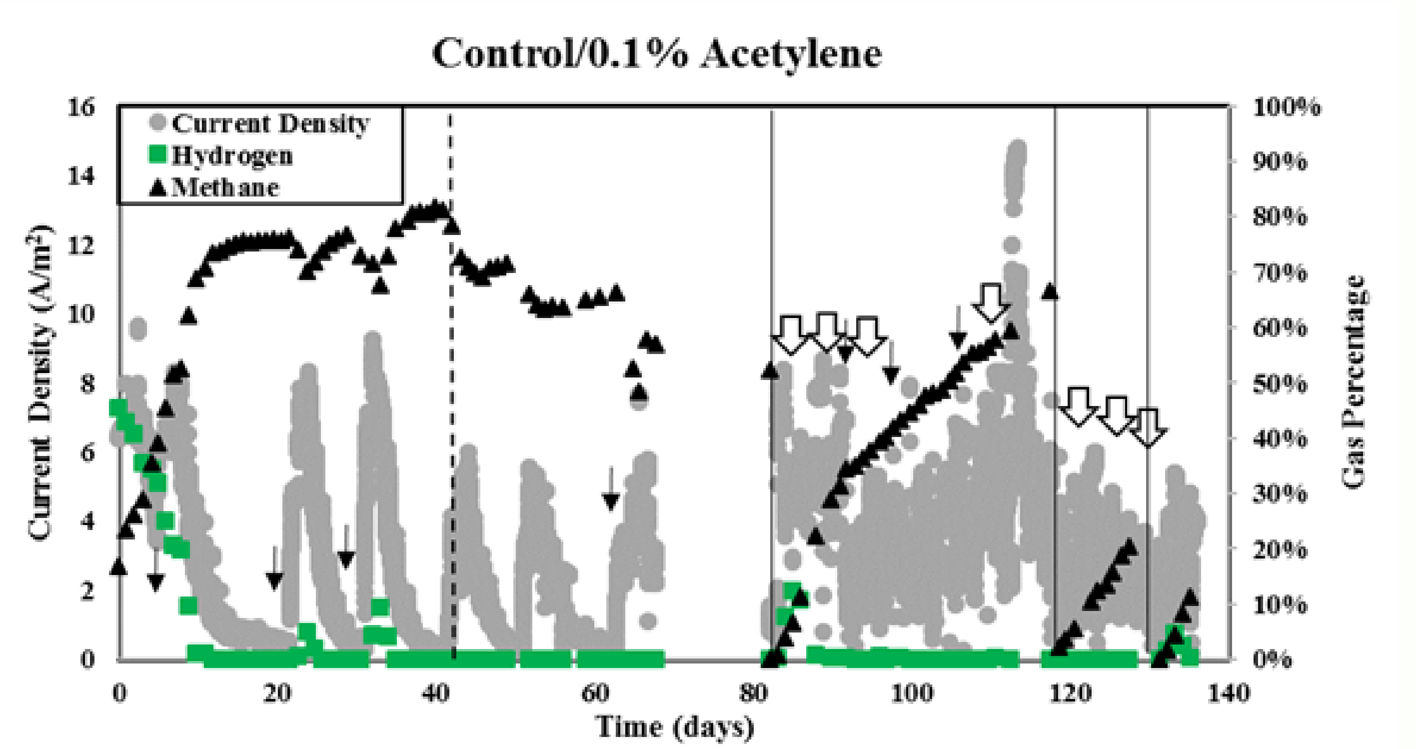
Thank you



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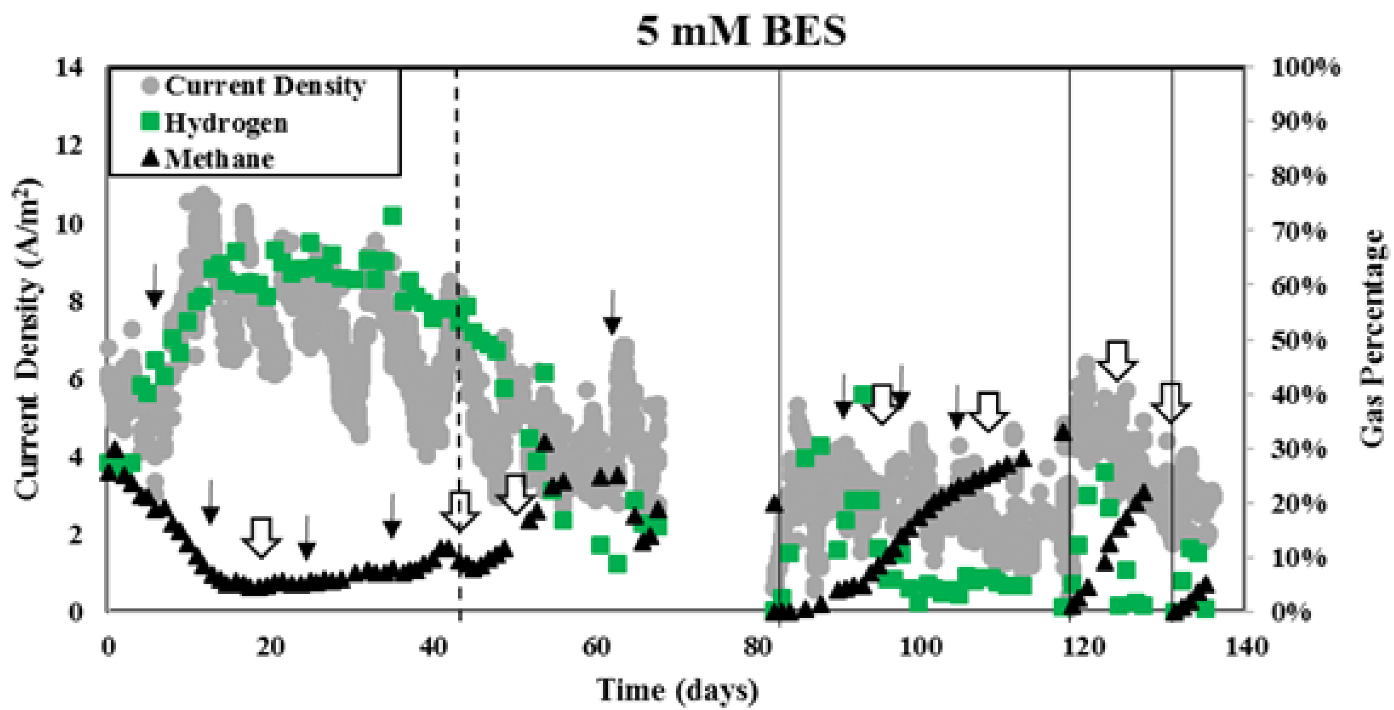
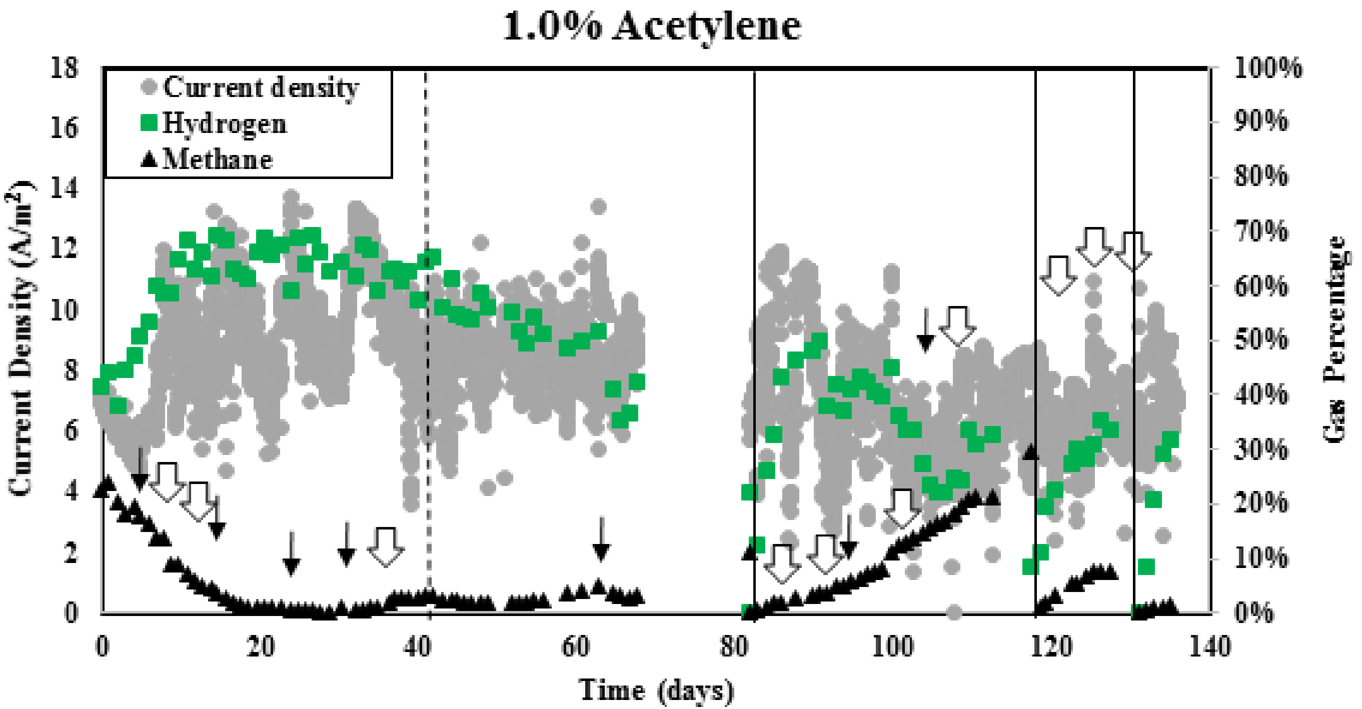
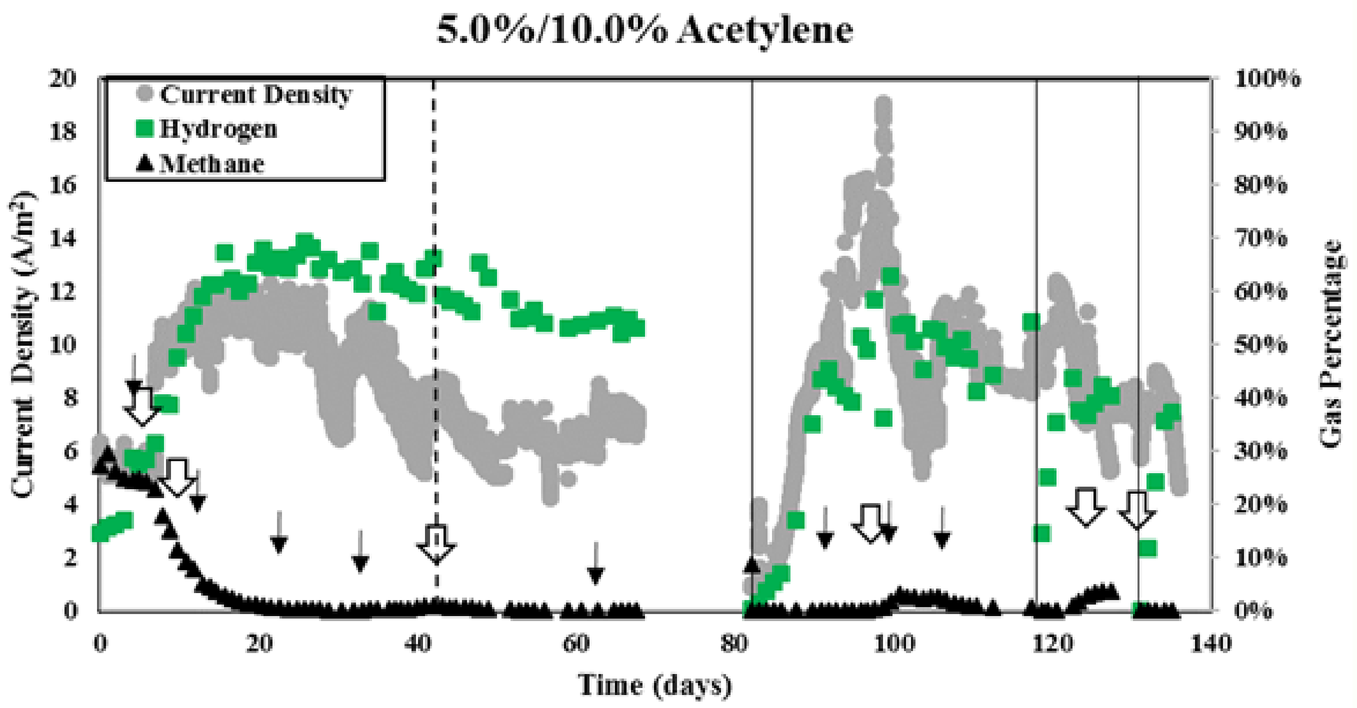


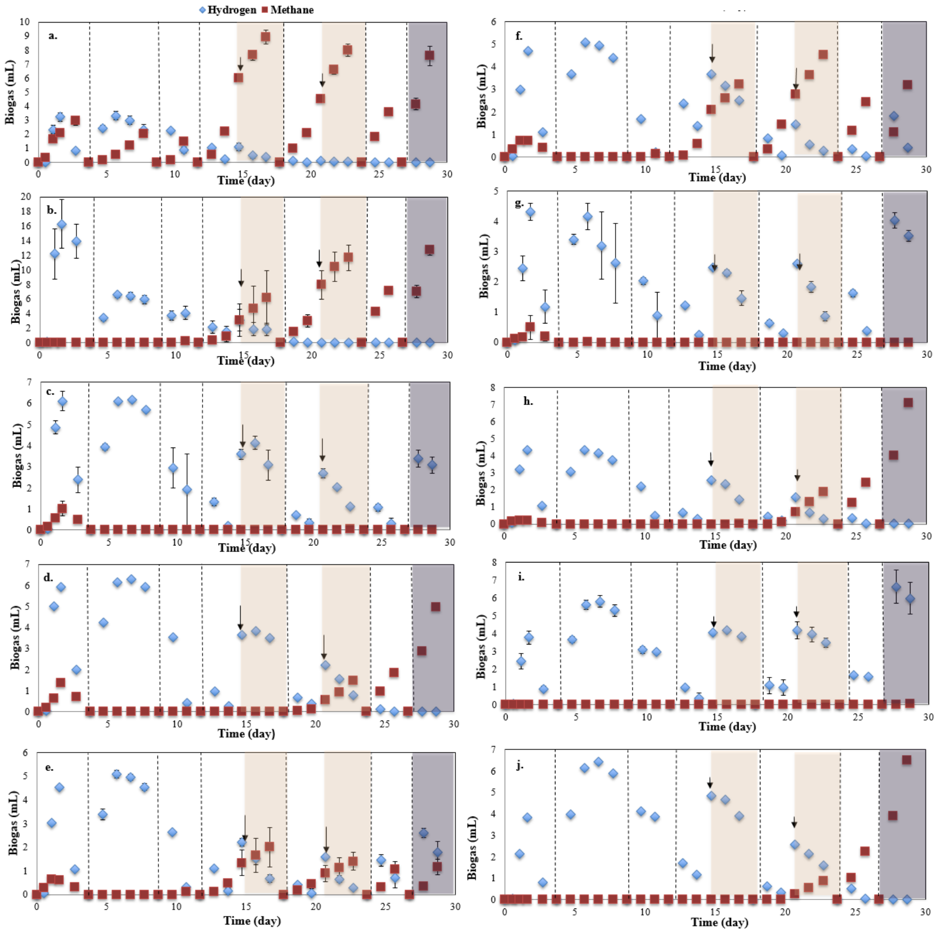
5 mM BES
Current Density
Hydrogen
Methane
Current Density (A/m²)
Gas Percentage
Time (days)
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%



1.0% Acetylene
Current density
Hydrogen
Methane
Current Density (A/m^2)
Gas Percentage
Time (days)
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
16
18
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

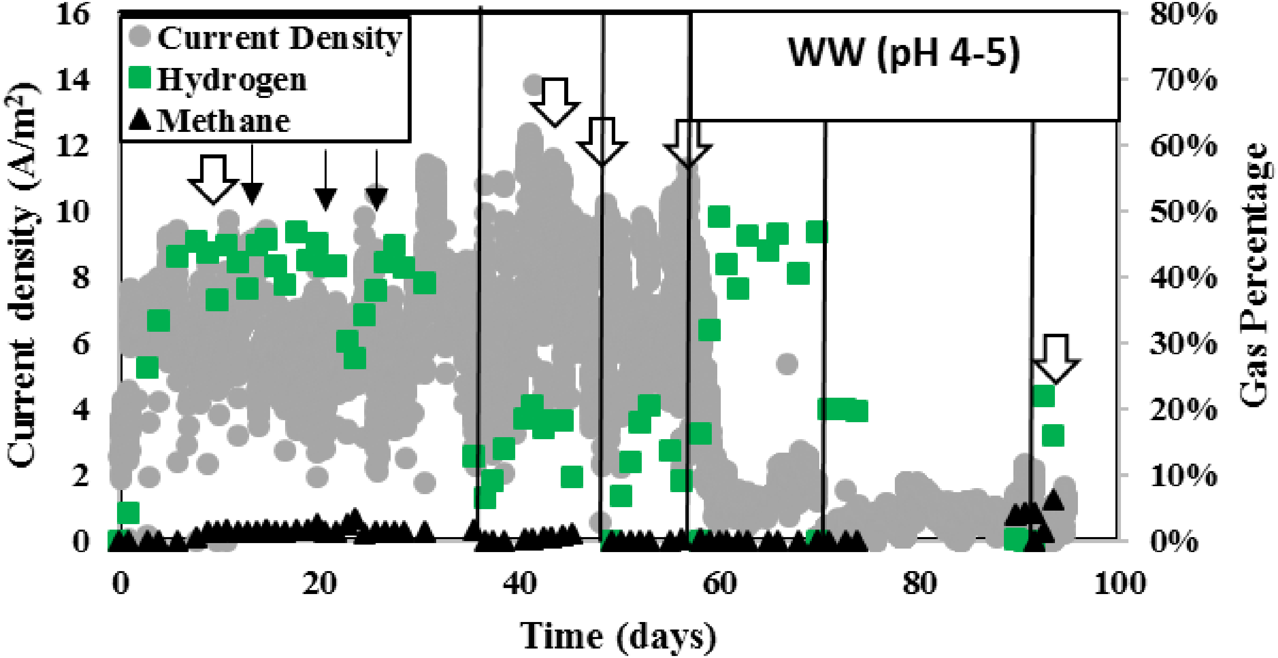


Oregon State University



Oregon State
UNIVERSITY

5% 2, Phase 2



Current Density
Hydrogen
Methane
WW (pH 4-5)
Current density (A/m²)
Gas Percentage
Time (days)
16
14
12
10
8
6
4
2
0
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
20
40
60
80
100