WASTE AS A RENEWABLE ENERGY SOURCE
Anaerobic digesters can provide a balance in the electricity network

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PhD-candidate
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Principle of BioGas-Production

The four key stages of anaerobic digestion involve hydrolysis, acidogenesis, acetogenesis and methanogenesis.

Hydrolysis: A chemical reaction where particulates are solubilized and large polymers converted into simpler monomers;

Acidogenesis: A biological reaction where simple monomers are converted into volatile fatty acids;

Acetogenesis: A biological reaction where volatile fatty acids are converted into acetic acid, carbon dioxide, and hydrogen.

Methanogenesis: A biological reaction where acetates are converted into methane and carbon dioxide, while hydrogen is consumed.

The products produced by one group of bacteria serve as the substrates for the next group.
My research project is being performed to optimize the supply of hydrogen to an anaerobic digestion system.
Principle of BioP2G

Biogas (CH₄ + CO₂)

digestate

biomass

manure

Complex biopolymers (proteins, polysaccharides, fats/oils)

Broken down monomers and oligomers (Sugars, amino acids, peptides)

Propionate Butyrate, etc. (short-chain volatile organic acids)

Fermentative bacteria

Fermentative bacteria

Fermentative bacteria

H₂ + CO₂

Acetate

Acetogens (H₂ producing)

Acetogens (H₂ consuming)

Acetoclastic methanogens

CH₄ + CO₂

H₂O

O₂

H₂
Different methods of (renewable) hydrogen supply to a bioreactor
Diffuse rubber tubing:
Best way for the supply of hydrogen

Fisherbrand®
4,0 x 1,0 mm
Tube length calculator in Excel
[Andras PERL, 2017]

<table>
<thead>
<tr>
<th>Temperature in the reactor (K)</th>
<th>310</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal gas constant (/molK)</td>
<td>8.314</td>
</tr>
<tr>
<td>Compressibility factor of H2 1-3 bar:</td>
<td>1.0006</td>
</tr>
<tr>
<td>inner diameter of the pipe (mm):</td>
<td>4</td>
</tr>
<tr>
<td>outer diameter of the pipe (mm):</td>
<td>6</td>
</tr>
<tr>
<td>Diffusion coefficient of H2 (m²/s):</td>
<td>6.78E-10</td>
</tr>
<tr>
<td>Required total flow rate (m³/min):</td>
<td>12</td>
</tr>
<tr>
<td>Maximum H2 pressure in the tube (bar):</td>
<td>2</td>
</tr>
<tr>
<td>Calculated H₂ conc. (mol/m³):</td>
<td>77.5528537</td>
</tr>
<tr>
<td>Calculated molar flow (mol/s):</td>
<td>8.30637E-06</td>
</tr>
<tr>
<td>Calculated tube length:</td>
<td>10.81 m</td>
</tr>
</tbody>
</table>

The total quantity of hydrogen (Q_H₂) diffusing into the reactor through a unit length of the pipe in time t becomes:¹

\[ Q_{H₂} = \frac{2\pi D t (C_{out} - C_{in})}{\ln \left( \frac{D}{2} \right)} \]

Two reactor setups: *in situ* and *ex situ*

**in situ:**

- Biomass + $\text{H}_2$ → bioreactor *(in situ)* → biogas

**ex situ:**

- Biomass + $\text{H}_2$ → bioreactor → biogas → 2nd reactor *(ex situ)* → biogas

*share your talent. move the world.*
biogas \( \text{CH}_4/\text{CO}_2 \)

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \\
\text{CH}_4 + 2\text{H}_2\text{O} + \text{E}
\]

biochromatograph

- sugar beet pulp  100
- rabbit feed mix  23
- wheat flour  95

biomass

- sugar beet pulp  100
- rabbit feed mix  23
- wheat flour  95

biomass

control

in situ

share your talent. move the world.
biogas

\[ \text{CH}_4/\text{CO}_2 \]

\[ \text{bioreactor} \]

ex situ

\[ \text{sugar beet pulp} \quad 100 \]
\[ \text{rabbit feed mix} \quad 23 \]
\[ \text{wheat flour} \quad 95 \]

biomass

gas chromatograph

\[ 1\text{CO}_2 + 4\text{H}_2 \rightarrow \]
\[ 1\text{CH}_4 + 2\text{H}_2\text{O} + \text{E} \]

\[ \text{H}_2 \]

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Labscale 10 liter reactor set-up: *in situ*
The first thing that stands out is the color-change of the CO$_2$ fixation-unit:

\[3M \text{NaOH}\]
Relative vol.%-CH$_4^*$: control

* H$_2$, CH$_4$ and CO$_2$ = 100 vol.%
Relative vol.%-CH$_4^*$: *in situ*

- H$_2$, CH$_4$ and CO$_2$ = 100 vol.%
CH₄*- production: Control vs *in situ*

445 Nml \( \text{CH}_4/\text{gr biomass} \)

322 Nml \( \text{CH}_4/\text{gr biomass} \)

*\( \text{CO}_2\) is captured in 3M NaOH

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Relative vol.%-CH₄*: in situ dd 01-11-2017

* H₂, CH₄ and CO₂ = 100 vol.%
Conclusions

• Adding hydrogen *in situ* to an existing biogas plant results in more methane per unit biomass
• Use of silicone rubber tubing is attractive
Labscale 10 liter reactor set-up: *ex situ*

- **AMPTSII**
- **Fix-CO₂**
- **CH₄**
- **H₂**
- **LOW-ΔP-FLOW**

**biomass**

**bioreactor**

**ex situ**

---

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Relative vol.%-CH₄*: bioreactor

* H₂, CH₄ and CO₂ = 100 vol.%
Relative vol.%-CH$_4$*: ex situ

* H$_2$, CH$_4$ and CO$_2$ = 100 vol.%
Issue:
Has the addition of hydrogen effect on the methanogenic community?

Approach:
Development of a new Taqman® assay to study the effects of hydrogen supply on the microbial community.
Microbial-DNA isolation from bioreactor samples

- UltraClean microbial DNA isolation Kit (MP-biosystem, DNA kit for soil)
- Sampling at different time points
- Filter samples
- ‘Control’ vs *in situ* or *ex situ*
- Addition of Internal PCR Control (IPC) primers
- Dilution series (primer efficiency)
During the qPCR experiment, two separate dilution series are analyzed: one treatment sample ("2") consisting of target DNA (T2) as well as a known quantity of reference (R2) DNA, the other the control sample ("1") consisting of target DNA (T1) as well as reference DNA (R1). Both samples are analyzed at two different wavelength, yielding \( C_t \) values as a function of the dilution factor \( d \). Ordinary linear regression of \( C_t \) vs. the log of the dilution factor of the sample, \( 10\log(d)=x \), yields 4 different straight lines of the form \( y=a + bx \) for each of the signals T1, T2, R1 and R2.
Design and optimalization

- The primer $T_m$ should be the same for all primers used in the multiplex reaction.
- It is absolutely essential that results obtained from multiplex reactions are verified to confirm that the same results would be obtained if the reactions were performed individually.
- DNA + λ-DNA, both 100 ng/µl starting concentration, mix 1:1, 1:5, 1:10 and 1:100 F1 to λ, and dilute $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$.
- Since both assays are amplified in the same tube, they compete for the same reagents (dNTPs and polymerase). It is important that this competition be minimized. 500nM primer concentration will be used for ARC, MSL and MMB assays while 100nM primer concentration will be used for λ assays.
Experimental design: Taqman ®

**Internal Control**
- IPC-F taqman-primer
- IPC-Probe (6-VIC MGBNFQ)
- IPC-R

**Hydrogenotrophic**
- MC-274_F
- MC 361 Probe (6-FAM-MGBNFQ)
- MC-477_R

**Acetoclastic**
- MSL812F
- MSL860F-probe (6-FAM-MGBNFQ)
- MSL1159R

**Data Analysis**

<table>
<thead>
<tr>
<th>Amplicon size</th>
<th>204</th>
<th>224</th>
<th>354</th>
</tr>
</thead>
</table>

**Data Analysis: Ratio**

\[
\text{Ratio} = \frac{MC}{MSL}
\]

MC = Methanoculleus (H)
MSL = Methanosarcinales (A)
Method

**Duplex Taqman®-assay**

DNA templates were a mixture of 50 ng/μL gDNA (isolated from bioreactor community) and 50 ng/μL Internal Control DNA (Lambda DNA #SD0011 Thermo scientific) in a 99:1 target-to-IPC ratio. Real-time PCR was performed using a standard TaqMan® PCR kit protocol on an Biorad CFX96 system. The 20 μl PCR included 2,0 μl DNA-template, 1× TaqMan® Universal PCR Master Mix (4444557, TaqMan® Fast Advanced Master Mix), 0.2 μM TaqMan® probe, 0,5 μM forward primer and 0,5 μM reverse primer, and 0,1 μM IPC reverse primer and and 0,1 μM IPC forward primer. The reactions were incubated in a 96-well plate at 95°C for 8 min, followed by 40 cycles of 95°C for 15 s and 58°C for 45 sec. All reactions were run in triplicate. The threshold cycle (C_T) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. TaqMan® C_T values were converted into a relative to Archea amount with ‘R-studio’.
\[ LR = 10 \log \left( \frac{a_{T,2} / b_{T,2}}{a_{T,1} / b_{T,1}} - \frac{a_{R,2} / b_{R,2}}{a_{R,1} / b_{R,1}} \right) \]

<table>
<thead>
<tr>
<th></th>
<th>E</th>
<th>slope(b)=</th>
<th>intet(a)=</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL_BR</td>
<td>1.96</td>
<td>-3.42</td>
<td>19.79</td>
</tr>
<tr>
<td>MSL_BP2</td>
<td>1.86</td>
<td>-3.72</td>
<td>18.80</td>
</tr>
<tr>
<td>MC_BR</td>
<td>1.91</td>
<td>-3.55</td>
<td>19.11</td>
</tr>
<tr>
<td>MC_BP2</td>
<td>1.96</td>
<td>-3.44</td>
<td>18.96</td>
</tr>
<tr>
<td>LA_MSL_BR</td>
<td>1.94</td>
<td>-3.48</td>
<td>12.77</td>
</tr>
<tr>
<td>LA_MSL_BP2</td>
<td>1.95</td>
<td>-3.45</td>
<td>12.00</td>
</tr>
<tr>
<td>LA_MC_BR</td>
<td>2.01</td>
<td>-3.31</td>
<td>12.01</td>
</tr>
<tr>
<td>LA_MC_BP2</td>
<td>1.94</td>
<td>-3.48</td>
<td>11.77</td>
</tr>
</tbody>
</table>
1. Is the initial situation equal?
2. Does the community change in time?
3. Does the community change after $H_2$ addition?

**Ratio: MC/MSL**

<table>
<thead>
<tr>
<th>Day</th>
<th>MC (control)</th>
<th>MSL (in situ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day = 0</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Day = 21</td>
<td>1.4</td>
<td>40.1</td>
</tr>
<tr>
<td>Day = 28</td>
<td>3.5</td>
<td>48.7</td>
</tr>
</tbody>
</table>

MSL = Methanosarcinales (A)
MC = Methanoculleus (H)
Preliminary result:

Clear increase in the presence of hydrogenotrophic methanogens relative to the acetoclastic methanogens as a result of hydrogen supply
Conclusions

• Hydrogen can be supplied by using diffuse rubber piping in an existing biogas reactor
• Adding hydrogen this way results in more methane per unit biomass
• Hydrogen supply resulted in an increase in the numbers of hydrogenotrophic methanogens relative to acetoclastic methanogens.
Future plans

• Sequencing MinIon® (work in progress)
• Optimization and extending Taqman® assay*
• Based on lab scale experiments building a pilot reactor (start september 2017)
• Start PhD september 2018 ‘waste to Fuel‘

*A (high) abundance of microorganisms as deduced from metagenome analysis does not necessarily indicate high transcriptional or metabolic activity, and vice versa
We have already started with Chapter 4: Preparing community for [pilot]

H₂:CO₂ (80/20 mol/mol-%)
I have already started with collecting samples for [RNA-isolations]
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Thank you for your attention

Questions?

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