Productivity and stability of different methanogenesis routes in synthetic microbial communities

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Decomplexify natural system

Anaerobic Digestion (AD) system ↔ Synthetic minimal community

Complex Organic Biodegradable Compounds → Soluble Compounds
- Sugars
- Amino Acids
- Fatty Acids

Fermentation → Organic Acids

Acetogenesis → Acetic Acid

Acetic Acid → Methanogenesis
- CH₄ (50-75%), CO₂, H₂, N₂

Lactate → H₂ + CO₂ → CH₄

Acetate → CH₄

Secondary fermenter

Methanogens

M. Kamali et al. 2016. Chem Eng J
Na-Lactate $\text{Na-C}_3\text{H}_5\text{O}_3$ → $\text{CH}_4$

- *Desulfovibrio vulgaris* (Dv)
- *Methanococcus maripaludis* (Mm)
- *Methanosarcina barkeri* (Mb)

$\text{H}_2$ → $\text{SO}_4^{2-}$$\rightarrow$$\text{H}_2$ $\text{SO}_4^{2-}$

$\uparrow$ Hydrogenotrophic methanogen

$\uparrow$ Acetotrophic methanogen
Three scenarios were designed.
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SO$_4^{2-}$

0 mM

15 mM
Three scenarios were designed

- **SO_4^{2-}**
  - 0 mM
  - 7.5 mM
  - 15 mM
(a) 30 mM Lactate → 2e⁻ + 2H⁺ → H₂ + CO₂ → Mm → CH₄

(b) 15 mM Lactate + 7.5 mM H₂S → CO₂ → Mm → CH₄

(c) 30 mM Lactate + 15 mM H₂S → 2e⁻ + 2H⁺ → H₂ + CO₂ → Mm → CH₄

Dv-Mb

Dv-Mm

Dv-Mb-Mm

Scale bar: 10 μm
• All species co-exist and community productivity increases in the absence of strong electron acceptors.
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- *Dv-Mm* followed 1 Lac ~ 0.5 CH₄ output; but not *Dv-Mb* (1 Lac ~1 CH₄)

**Chemical Equations**

- \[ C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+ \] (Dv-Mm)
- \[ 4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \] (Dv-Mb)
- \[ C_2H_3O_2^- + H^+ \rightarrow CO_2 + CH_4 \] (Dv-Mb)
With sulfate, Dv scavenged H₂ instead of producing it. CH₄ production was inhibited

\[ 4H₂ + SO₄^{2−} + 2H⁺ \rightarrow H₂S + 4H₂O \]

\[ 2C₃H₅O₃^{-} + SO₄^{2−} \rightarrow 2C₂H₃O₂^{-} + 2HCO₃^{-} + H₂S \]

\[ C₃H₅O₃^{-} + 2H₂O \rightarrow C₂H₃O₂^{-} + HCO₃^{-} + 2H₂ + H⁺ \]
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Dv-Mb did not produce CH₄ as expected.
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- Dv-Mb did not produce CH₄ as expected.

Coming back later for discussion.
• Increased electron acceptor availability shows a differential impact on the maintenance and productivity of aceto- and hydrogeno- trophic methanogens.
- *Dv* populations accounted for a large portion. No cross contamination
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• *Mb* showed high variance, *Mm* were more stable

• An increased stability of methanogen populations with the increased community complexity (i.e. extended syntrophic interactions)
• There were hydrogen decreases in all three scenarios, including *Dv-Mb* without sulfate addition

• *Mb* participated in hydrogen consumption
• *Dv-Mm* consumed ~0 mM acetate
- *Dv-Mm* consumed \(~0\) mM acetate

- *Dv-Mb+15 mM* sulfate fully converted lactate to acetate, which *Mb* did not utilize for CH\(_4\) production
• *Dv* might compete for Intermediate H₂ from *Mb* to block aceticlastic pathway

CH₄ in the headspace (mmole per L medium)
Intermediate H₂ makes Mb vulnerable

- *Dv-Mb* had high variance among replicates, did not fully convert lactate, and accumulated acetate after sub-culturing
• *Dv-Mb* used less acetate, produced more CH$_4$. 
\[ \text{Dv} \quad C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+ \]

\[ \text{Mb} \quad C_2H_3O_2^- + H^+ + 4H_2 \rightarrow 2CH_4 + 2H_2O \]

\[ \text{H}_2 \text{~limitation;} \]

1 Lactate \~ 1 Acetate + 2H\_2; 0.5 Acetate + 2H\_2 \~ 1 CH\_4
Mb conserved 2 e⁻ and followed CO₂ reduction pathway with external H₂, instead of producing CO₂⁻.

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Equation</th>
<th>$\Delta G^\circ$ (KJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$</td>
<td>-130.7</td>
</tr>
<tr>
<td>2</td>
<td>$C_2H_3O_2^- + H^+ \rightarrow CO_2 + CH_4$</td>
<td>-35.8</td>
</tr>
<tr>
<td>3</td>
<td>$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$</td>
<td>-157.8</td>
</tr>
<tr>
<td>4</td>
<td>$2C_3H_5O_3^- + SO_4^{2-} \rightarrow 2C_2H_3O_2^- + 2HCO_3^- + H_2S$</td>
<td>-165.8</td>
</tr>
<tr>
<td>5</td>
<td>$C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+$</td>
<td>-4.0</td>
</tr>
<tr>
<td>6</td>
<td>$C_2H_3O_2^- + H^+ + 4H_2 \rightarrow 2CH_4 + 2H_2O$</td>
<td>-166.5</td>
</tr>
</tbody>
</table>

The standard free energy change at pH 7 ($\Delta G^\circ$) was calculated from equilibrium constants (RK Thauer et al. 1977. Bacteriol Rev)

- $M. maripaludis$ (Mm)
- $M. barkeri$ (Mb)
- $D. vulgaris$ (Dv)
- Needs further investigation
• With acetate provided at 30 mM, increasing H₂ pressure in the headspace significantly increased *Mb* monoculture’s methane production

![Graph showing produced CH₄ and acetate concentrations for different MB conditions](chart.png)
Summary

• All species co-exist and community productivity increases in the absence of strong electron acceptors

• Acetotrophic methanogen was more vulnerable by increased electron acceptor availability

• \( \text{H}_2 \) addition into AD system might benefit aceticlastic methanogenesis more
THANK YOU!

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