Productivity and stability of different methanogenesis routes in synthetic microbial communities

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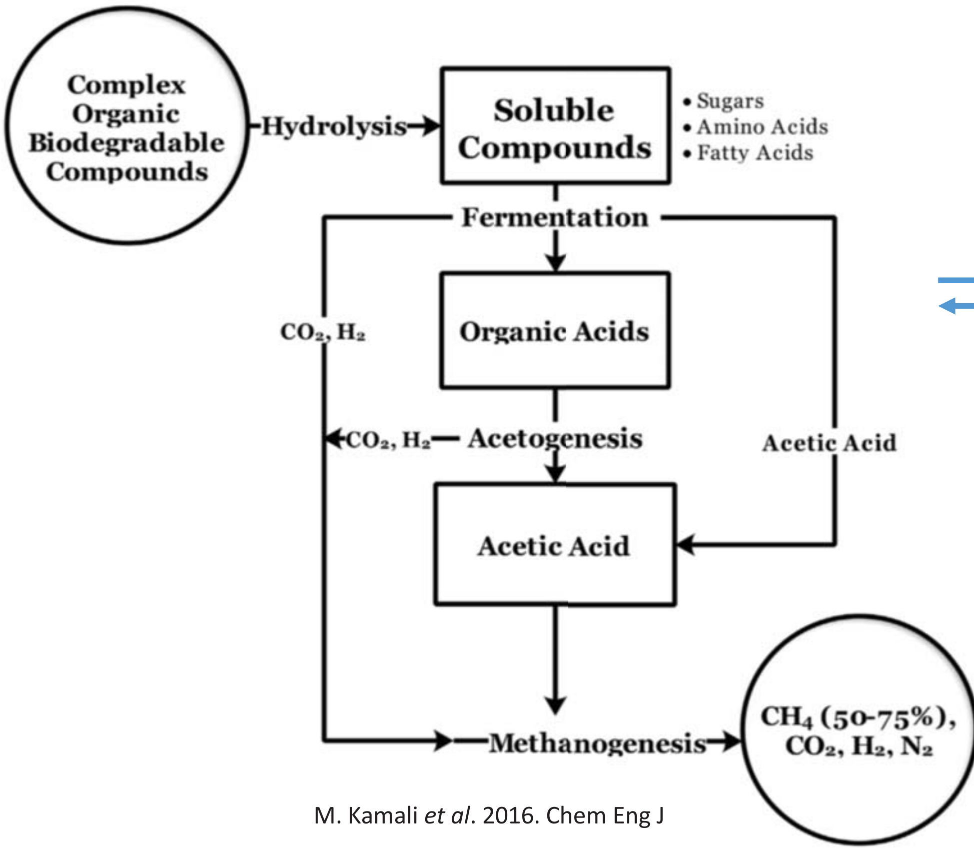
The University of Warwick

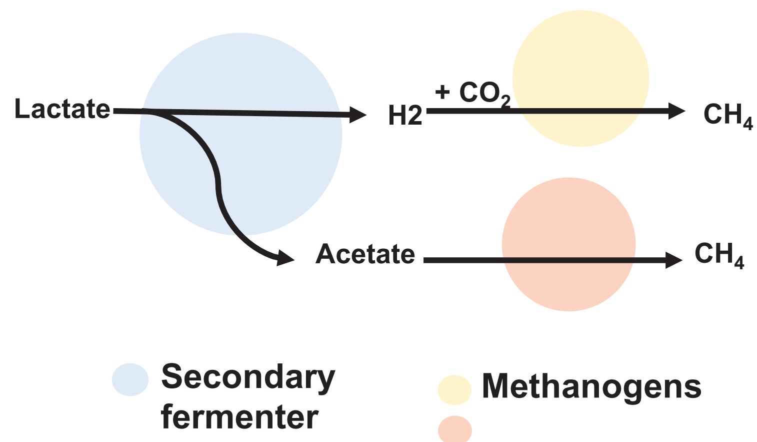
AgroSpace-MELiSSA workshop, 18-May-2018

Decomplexify natural system

Anaerobic Digestion (AD) system ⇄ Synthetic minimal community



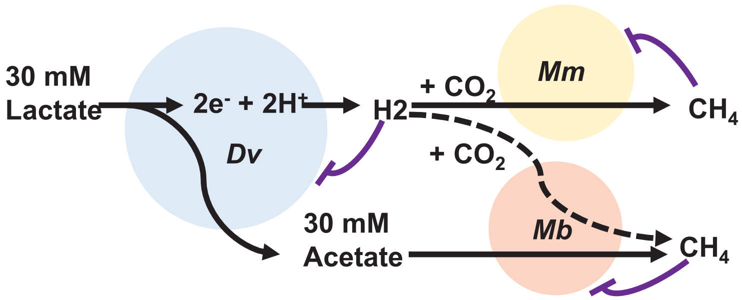
M. Kamali *et al*. 2016. Chem Eng J



Na-Lactate **$Na\text{-}C_3H_5O_3$** $\xrightarrow{?\ SO_4^{2-}}$ **CH_4**

- *Desulfovibrio vulgaris* **(Dv)** — **H_2** ↑ $\cancel{SO_4^{2-}}$ — $\cancel{H_2}$ **SO_4^{2-}** ↓
- *Methanococcus maripaludis* **(Mm)** — **CH_4** ↑ **Hydrogenotrophic methanogen**
- *Methanosarcina barkeri* **(Mb)** — **CH_4** ↑ **Acetotrophic methanogen**

(a)



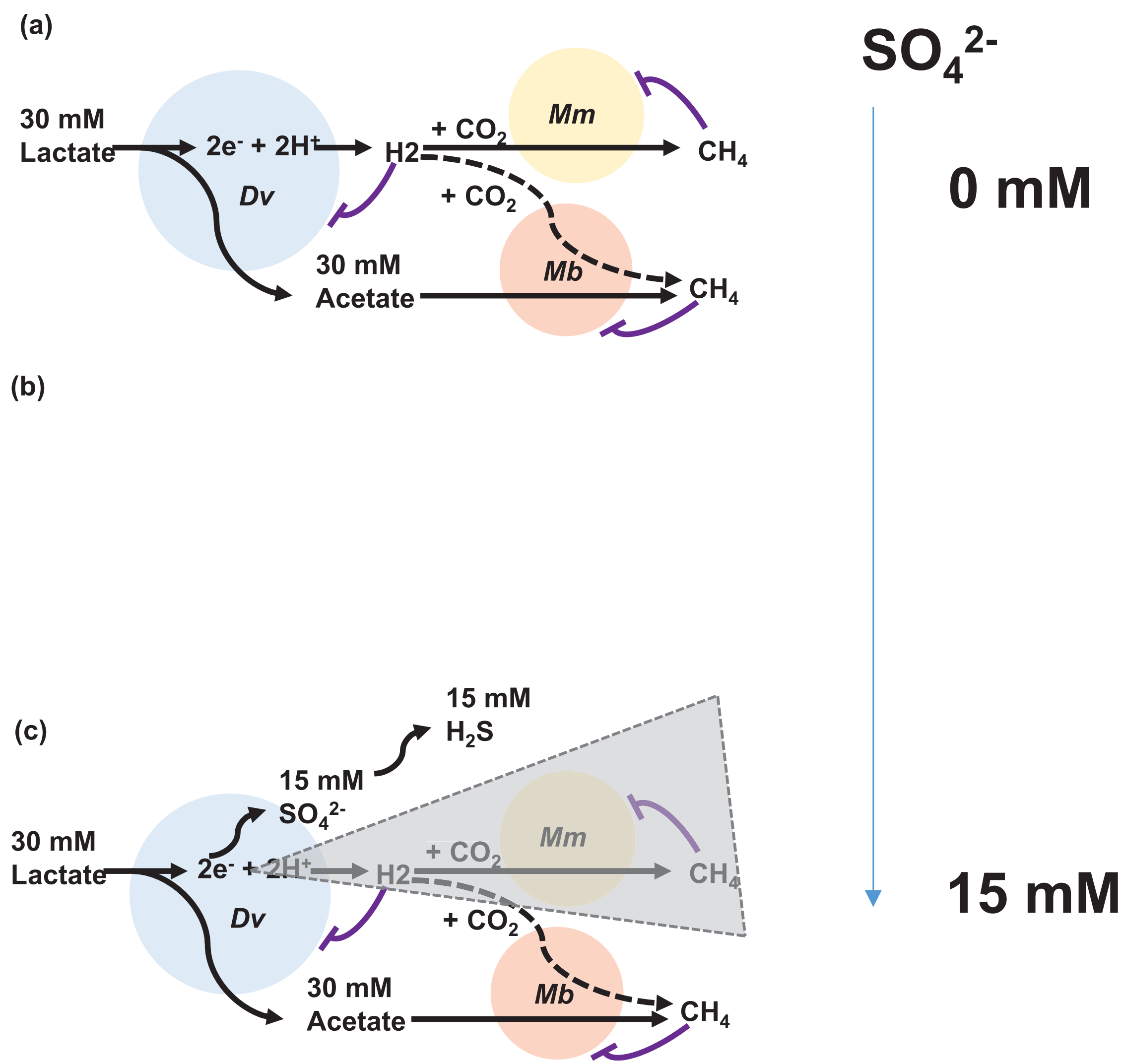
(b)

(c)

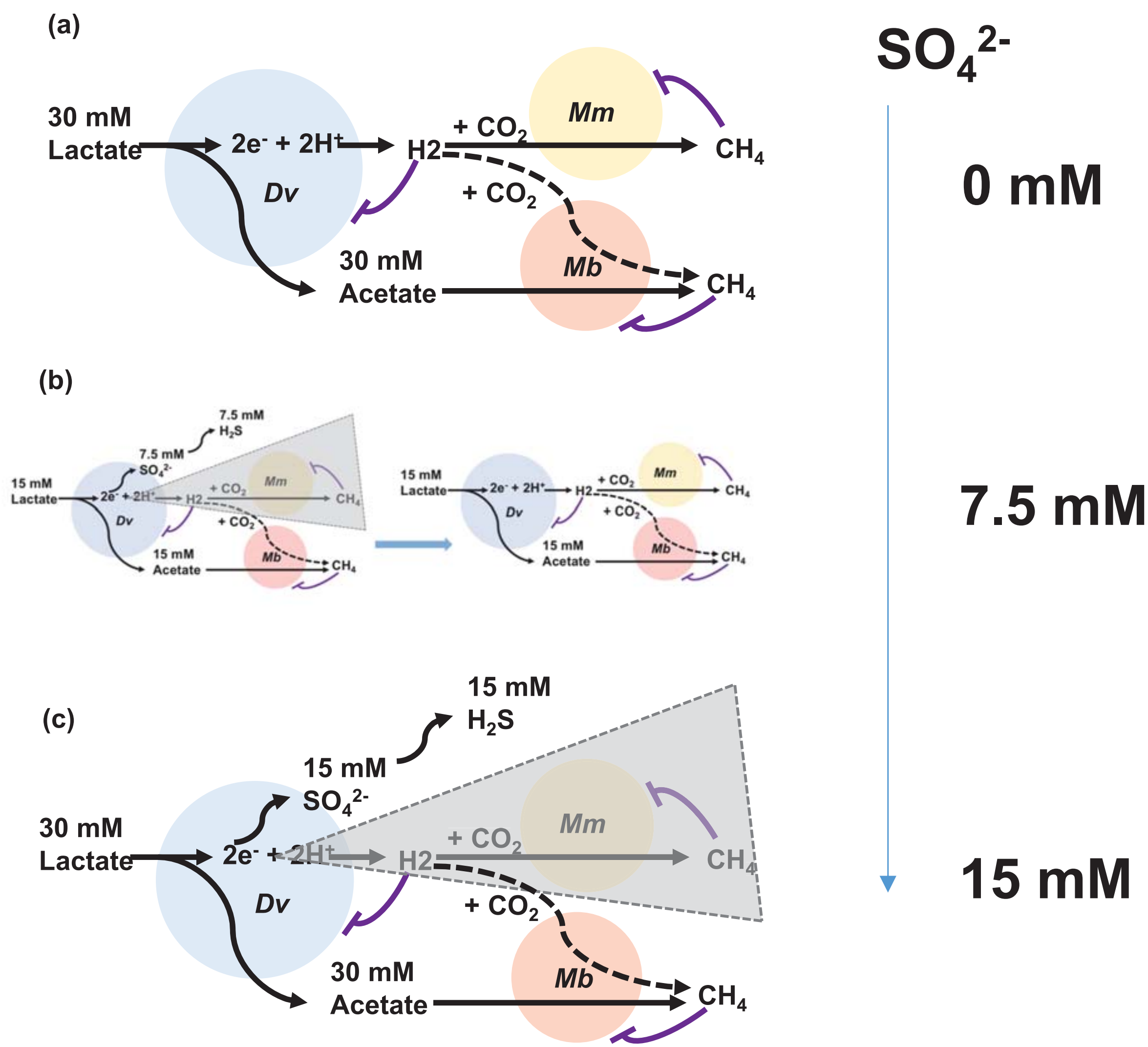
SO_4^{2-}

0 mM

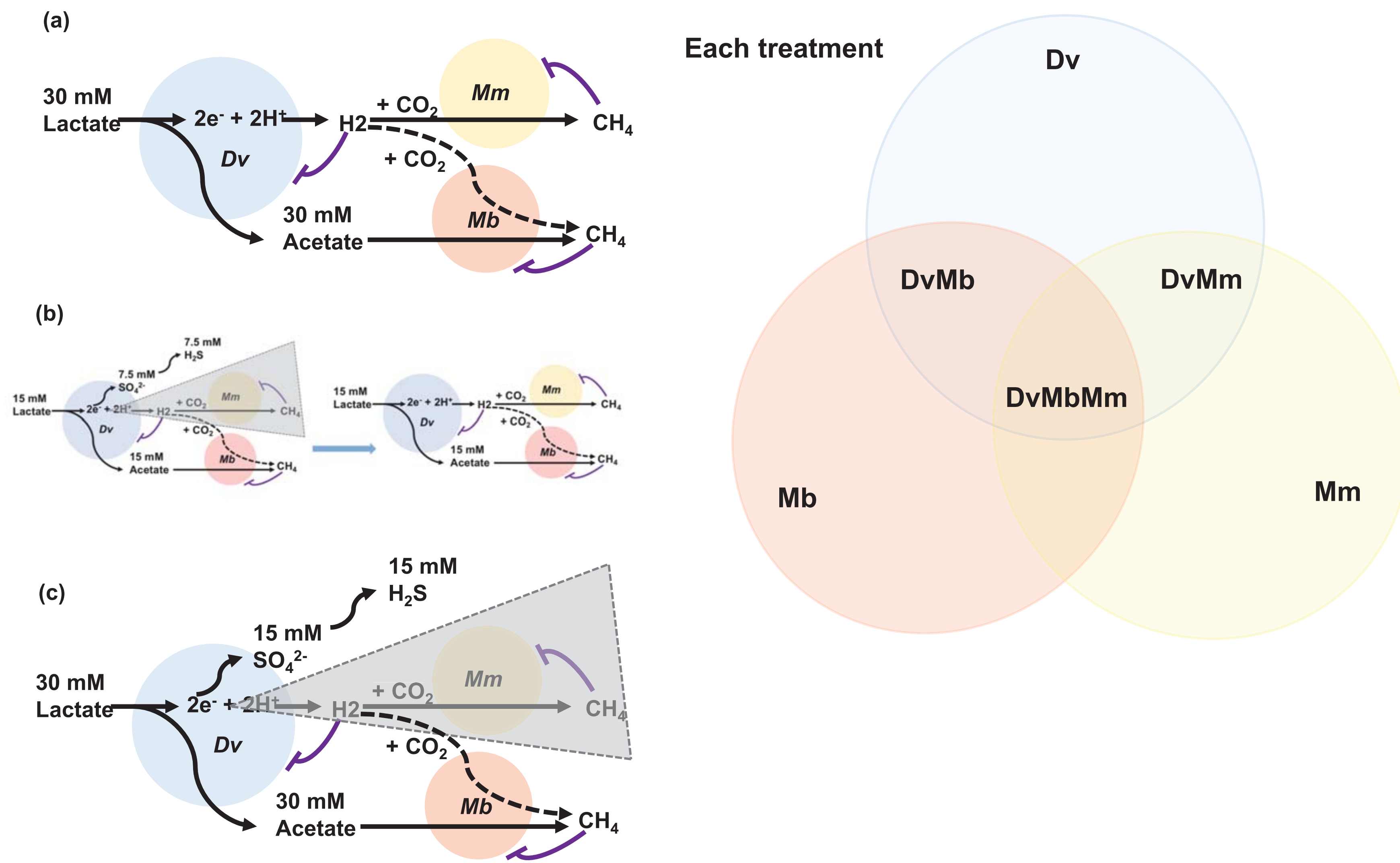
Three scenarios were designed



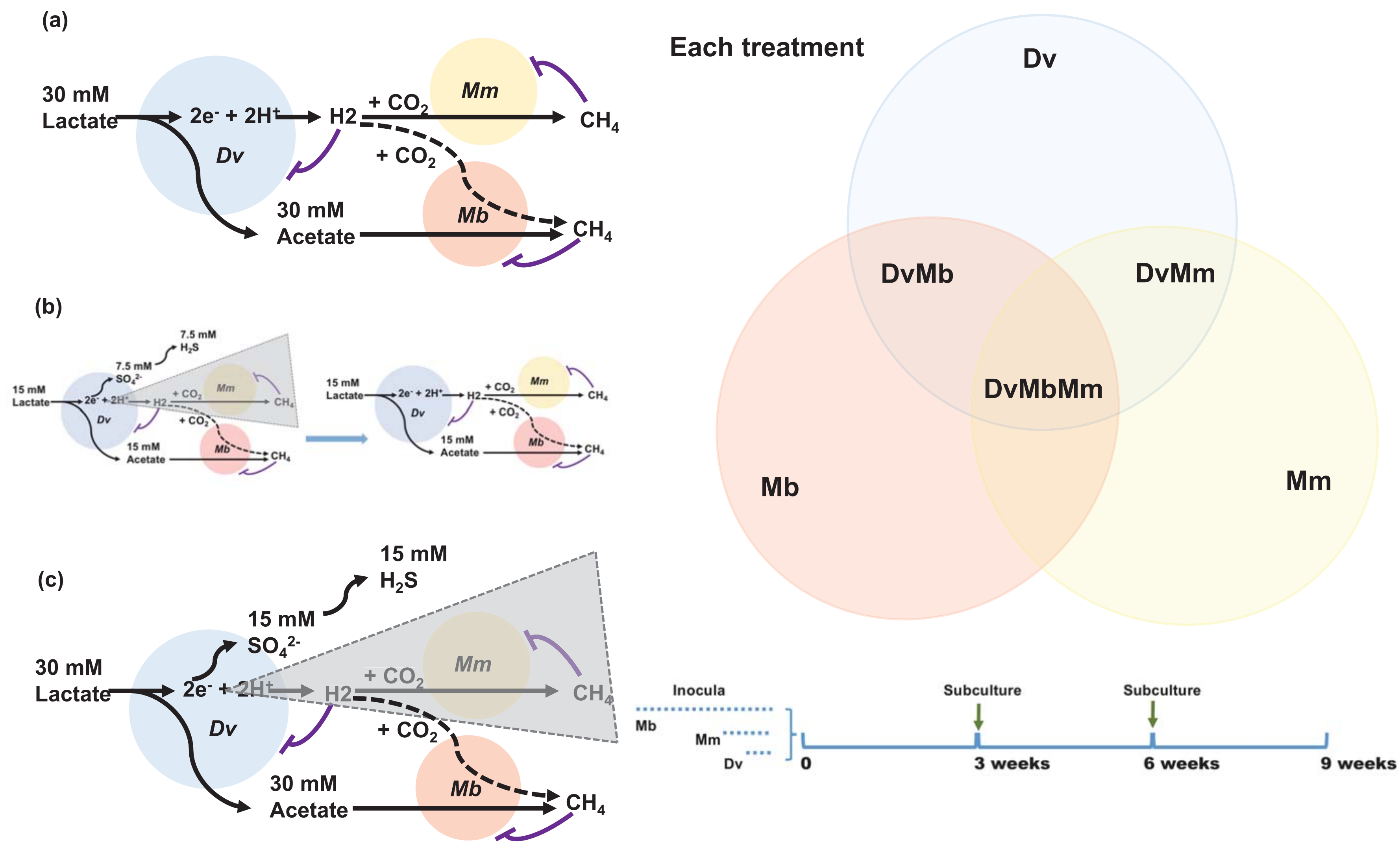
Three scenarios were designed



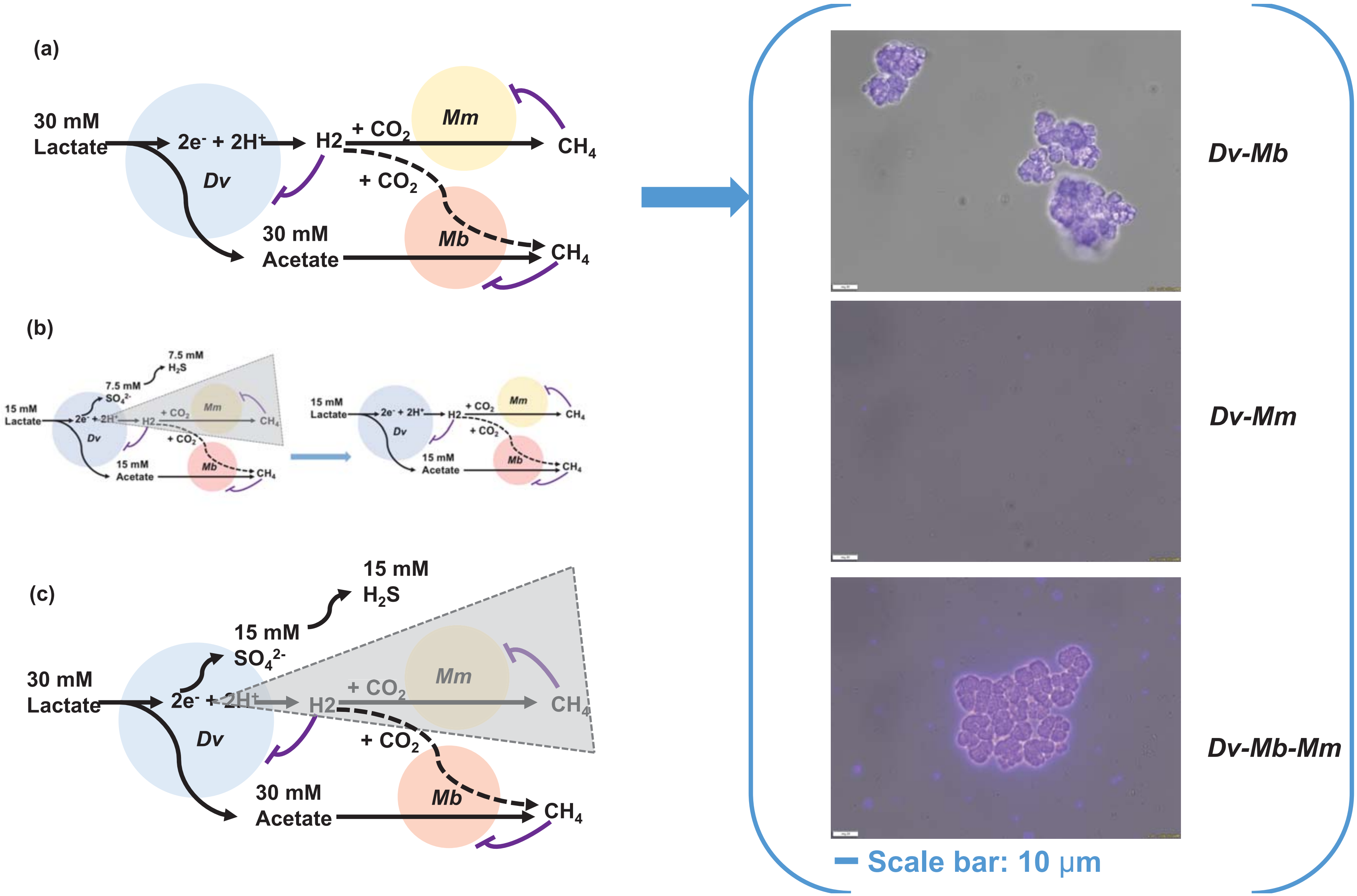
Three scenarios were designed



(a)
30 mM Lactate
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
+ CO2
30 mM Acetate
Mb
CH4
(b)
(c)
15 mM H2S
15 mM SO4 2-
30 mM Lactate
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
+ CO2
30 mM Acetate
Mb
CH4
Each treatment
Dv
DvMb
DvMm
DvMbMm
Mb
Mm

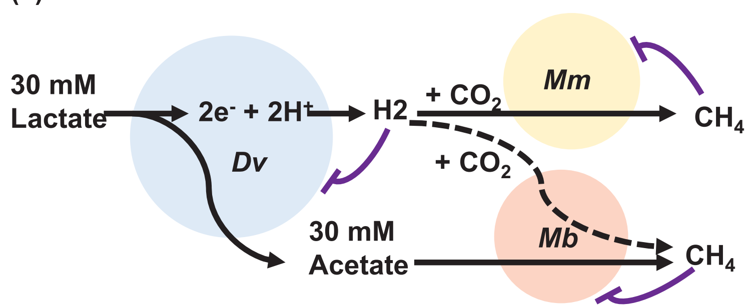


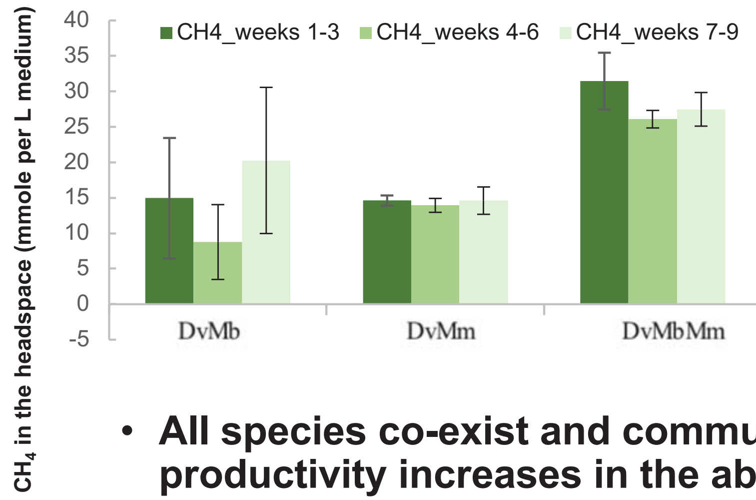
(a)
30 mM Lactate
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
+ CO2
30 mM Acetate
Mb
CH4
(b)
7.5 mM H2S
7.5 mM SO42-
15 mM Lactate
Dv
Mm
15 mM Acetate
Mb
CH4
15 mM Lactate
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
+ CO2
15 mM Acetate
Mb
CH4
(c)
15 mM H2S
15 mM SO42-
30 mM Lactate
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
+ CO2
30 mM Acetate
Mb
CH4
Each treatment
Dv
DvMb
DvMm
DvMbMm
Mb
Mm
Inocula
Mb
Mm
Dv
Subculture
Subculture
0
3 weeks
6 weeks
9 weeks



(a)
30 mM
Lactate
2e- + 2H+
Dv
H2
+ CO2
Mm
CH4
+ CO2
30 mM
Acetate
Mb
CH4
(b)
7.5 mM
H2S
7.5 mM
SO4 2-
15 mM
Lactate
Dv
Mm
Mb
15 mM
Acetate
CH4
(c)
15 mM
H2S
15 mM
SO4 2-
30 mM
Lactate
2e- + 2H+
Dv
H2
+ CO2
Mm
CH4
+ CO2
30 mM
Acetate
Mb
CH4
Dv-Mb
Dv-Mm
Dv-Mb-Mm
Scale bar: 10 μm

(a)



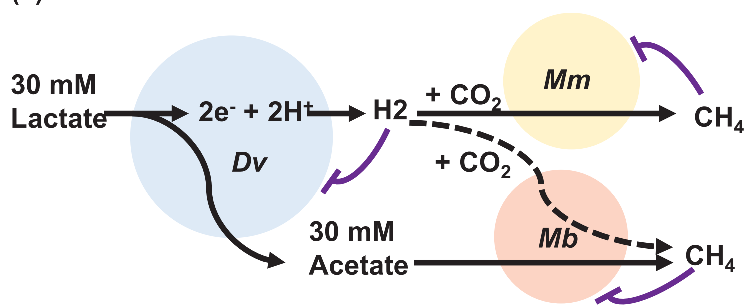


(b)

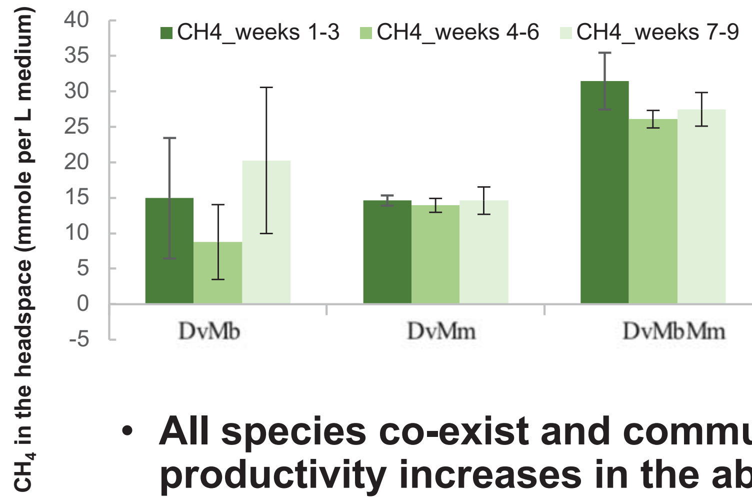
- **All species co-exist and community productivity increases in the absence of strong electron acceptors**

(c)

(a)



(b)



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

- ***Dv-Mm* followed 1 Lac ~ 0.5 CH_4 output; but not *Dv-Mb* (1 Lac ~1 CH_4)**

(c)

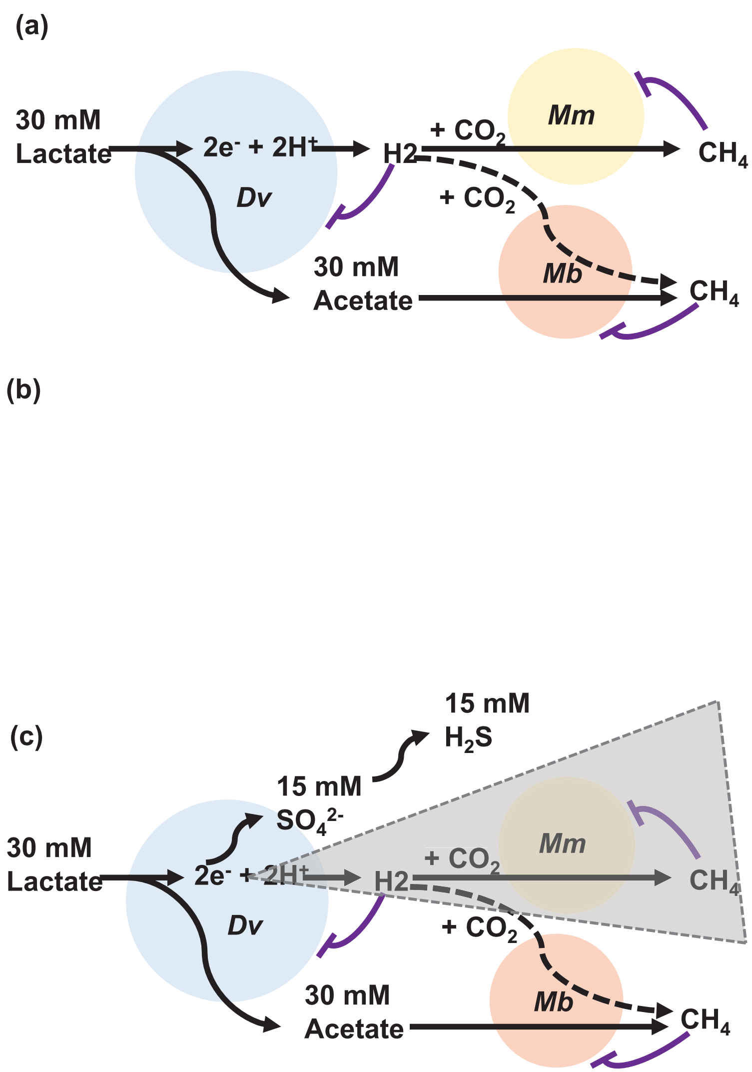
Dv-Mm

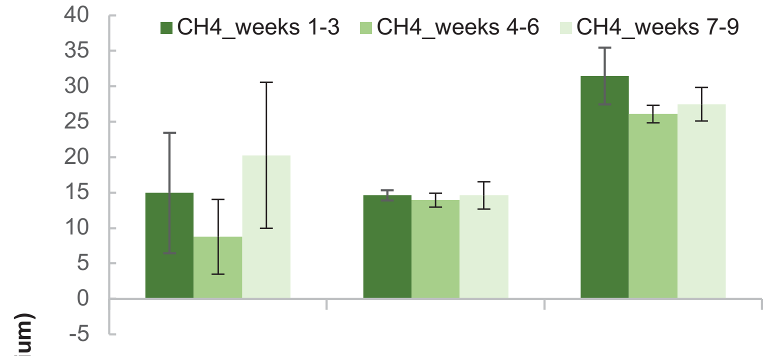
Dv-Mb

$$C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$$

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



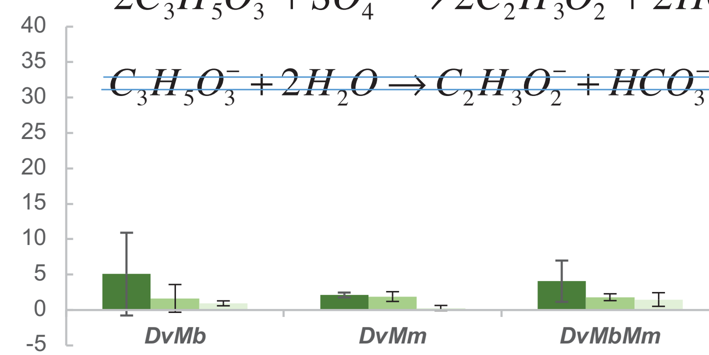


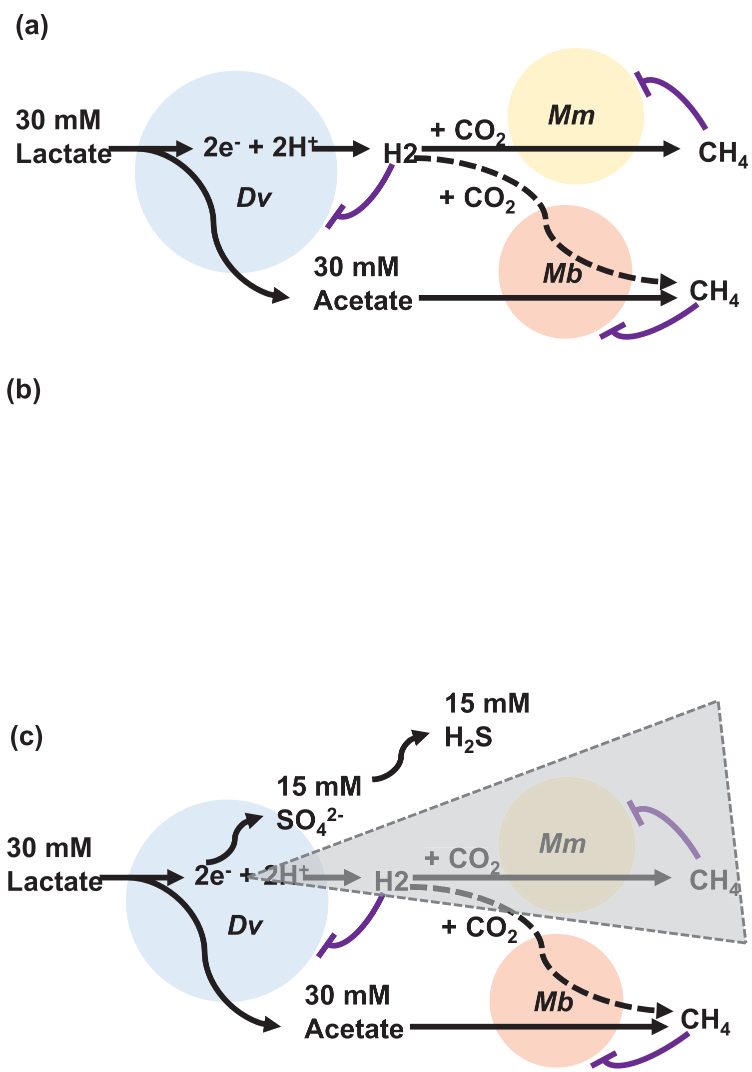
- **With sulfate, *Dv* scavenged H_2 instead of producing it. CH_4 production was inhibited**

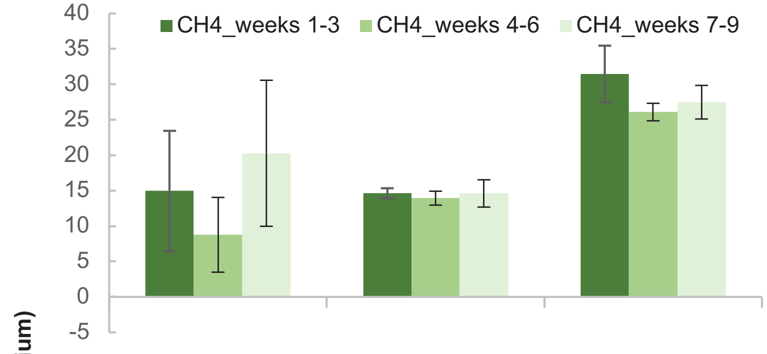
$$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$$

$$2C_3H_5O_3^- + SO_4^{2-} \rightarrow 2C_2H_3O_2^- + 2HCO_3^- + H_2S$$

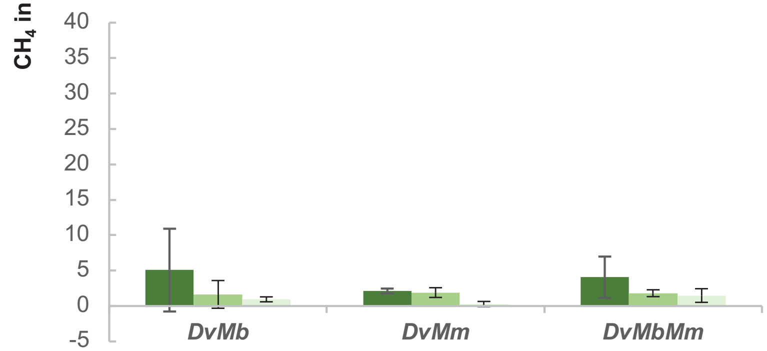
$$\cancel{C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+}$$

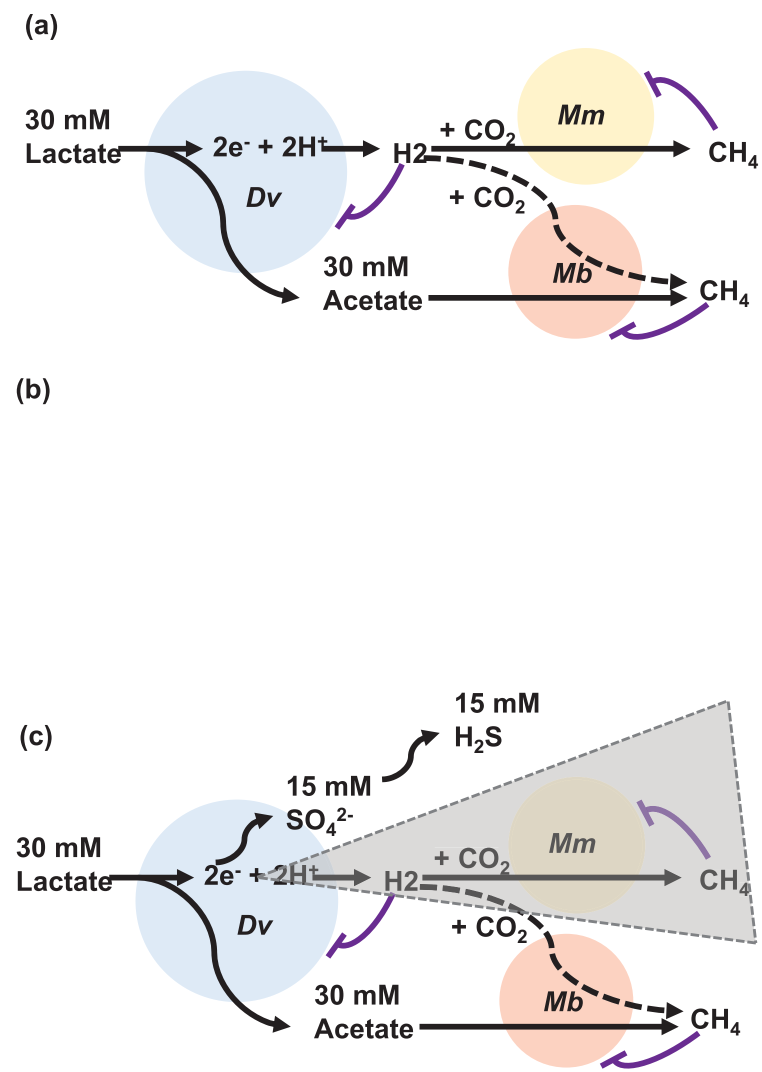


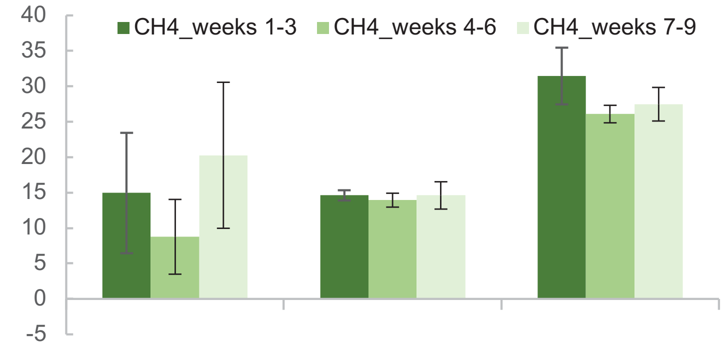




- **With sulfate, *Dv* scavenged H_2 instead of producing it. CH_4 production was inhibited**
- ***Dv-Mb* did not produce CH_4 as expected**

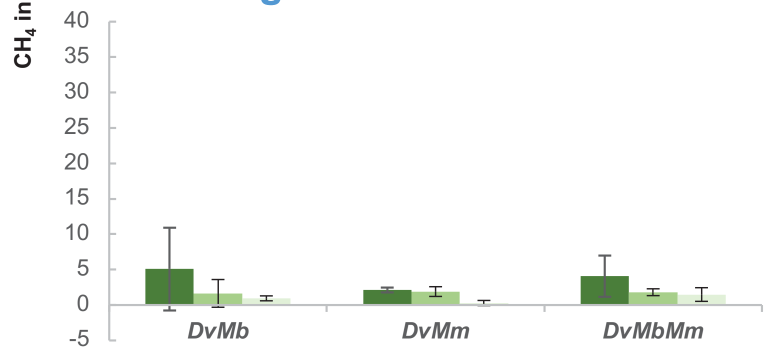


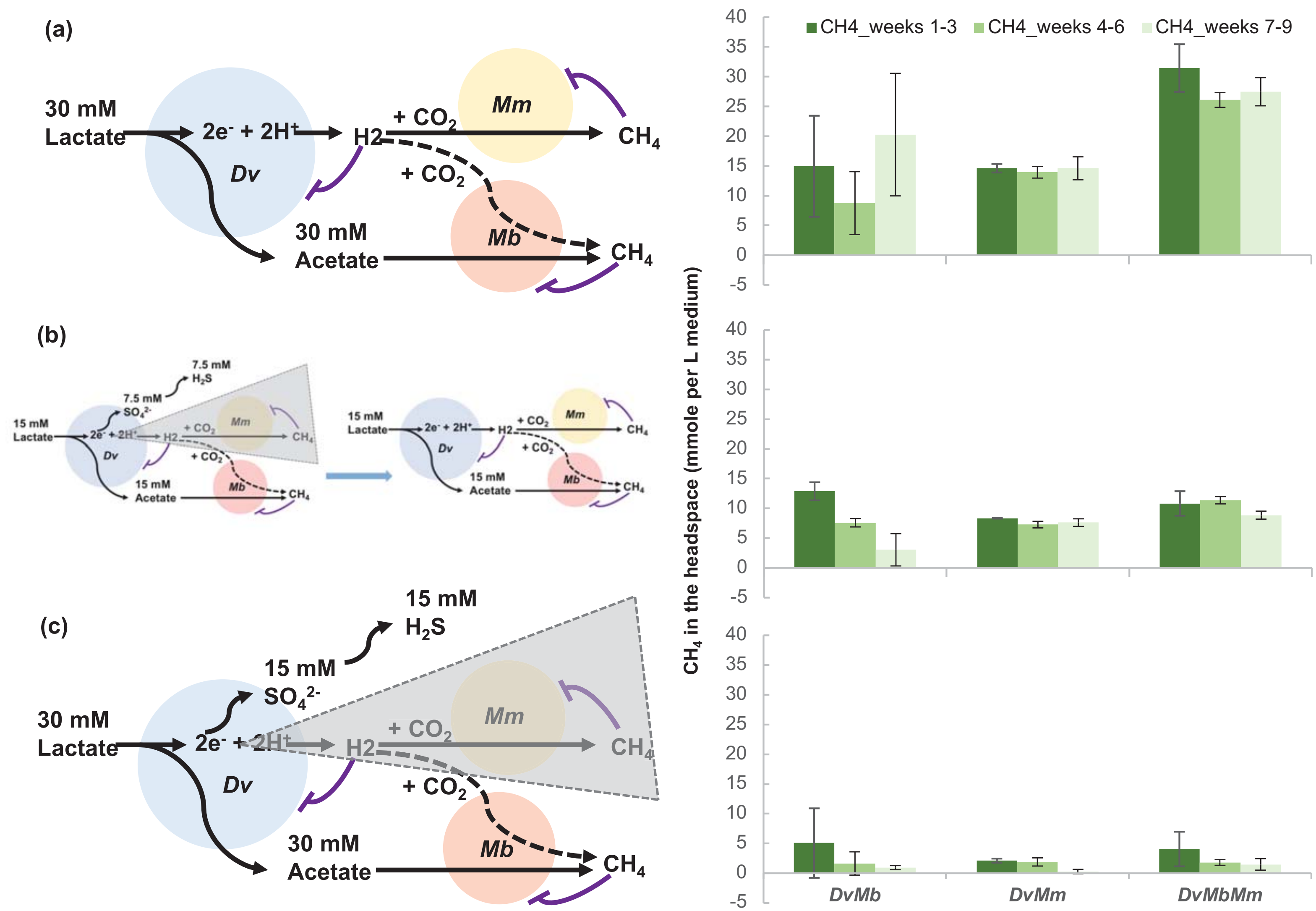




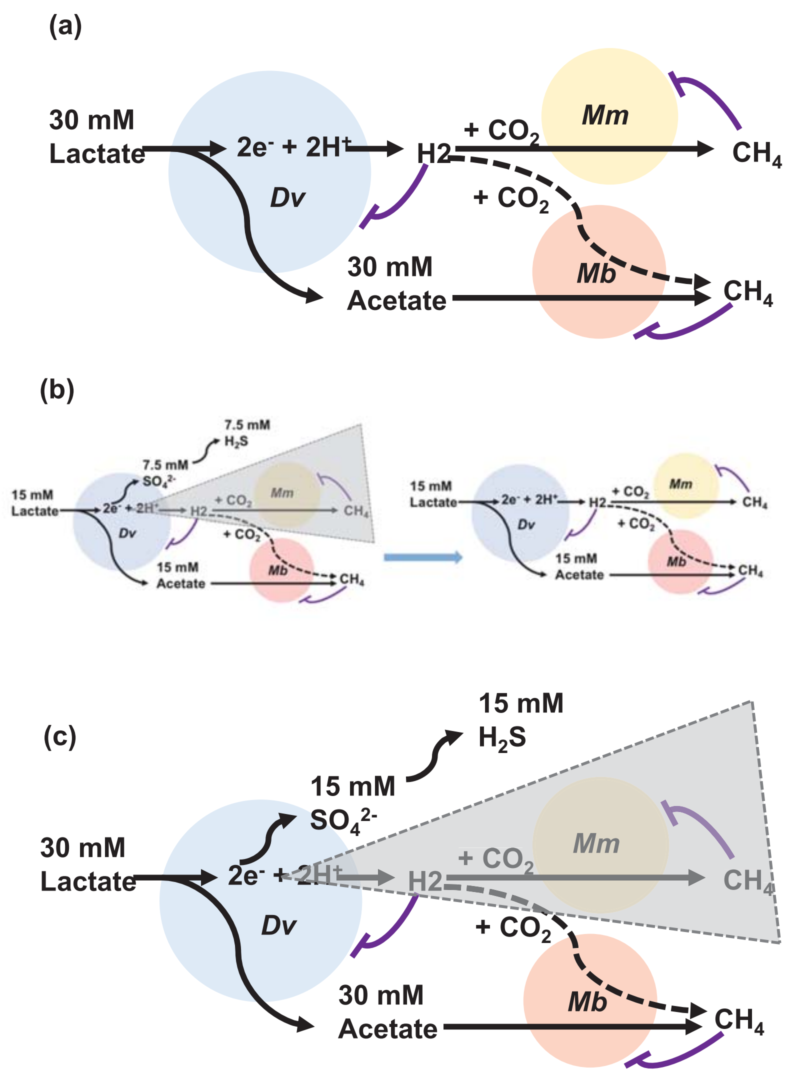
- **With sulfate, Dv scavenged H_2 instead of producing it. CH_4 production was inhibited**
- **Dv-Mb did not produce CH_4 as expected**

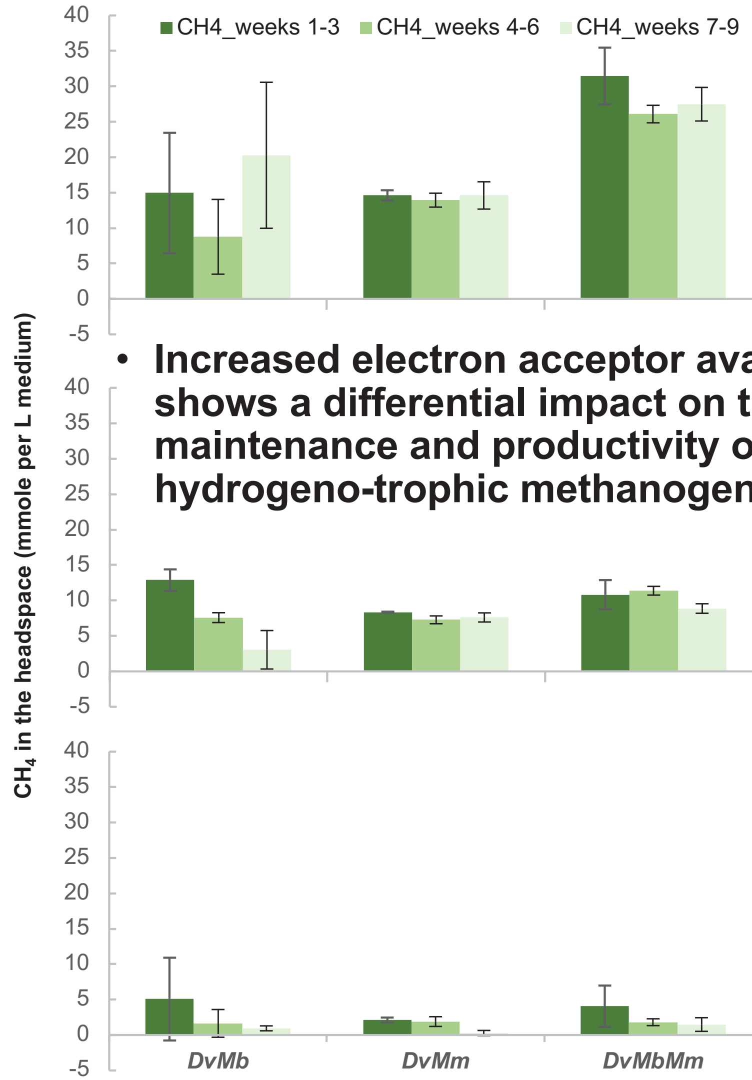
Coming back later for discussion





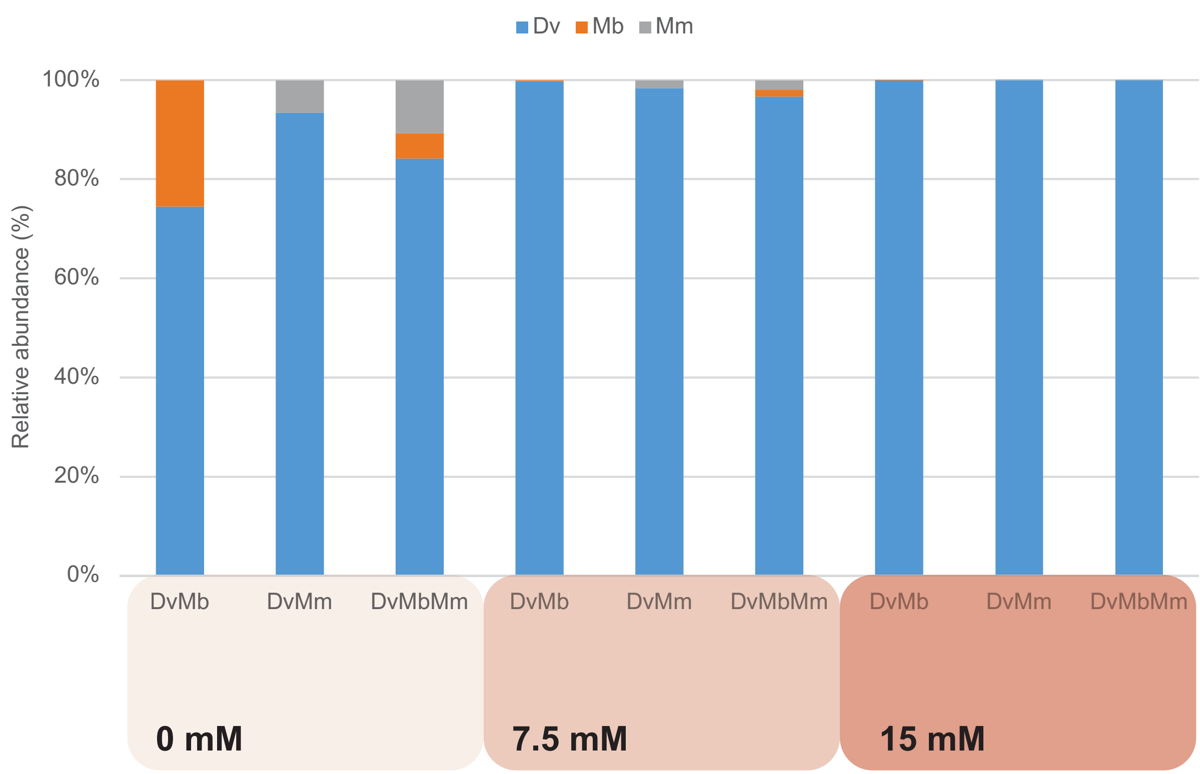
(a)
30 mM Lactate
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
+ CO2
Mb
30 mM Acetate
CH4
(b)
15 mM Lactate
7.5 mM SO4 2-
7.5 mM H2S
15 mM Acetate
(c)
30 mM Lactate
15 mM SO4 2-
15 mM H2S
2e- + 2H+
H2
+ CO2
Mm
CH4
Dv
Mb
30 mM Acetate
CH4
CH4_weeks 1-3
CH4_weeks 4-6
CH4_weeks 7-9
CH4 in the headspace (mmole per L medium)
DvMb
DvMm
DvMbMm



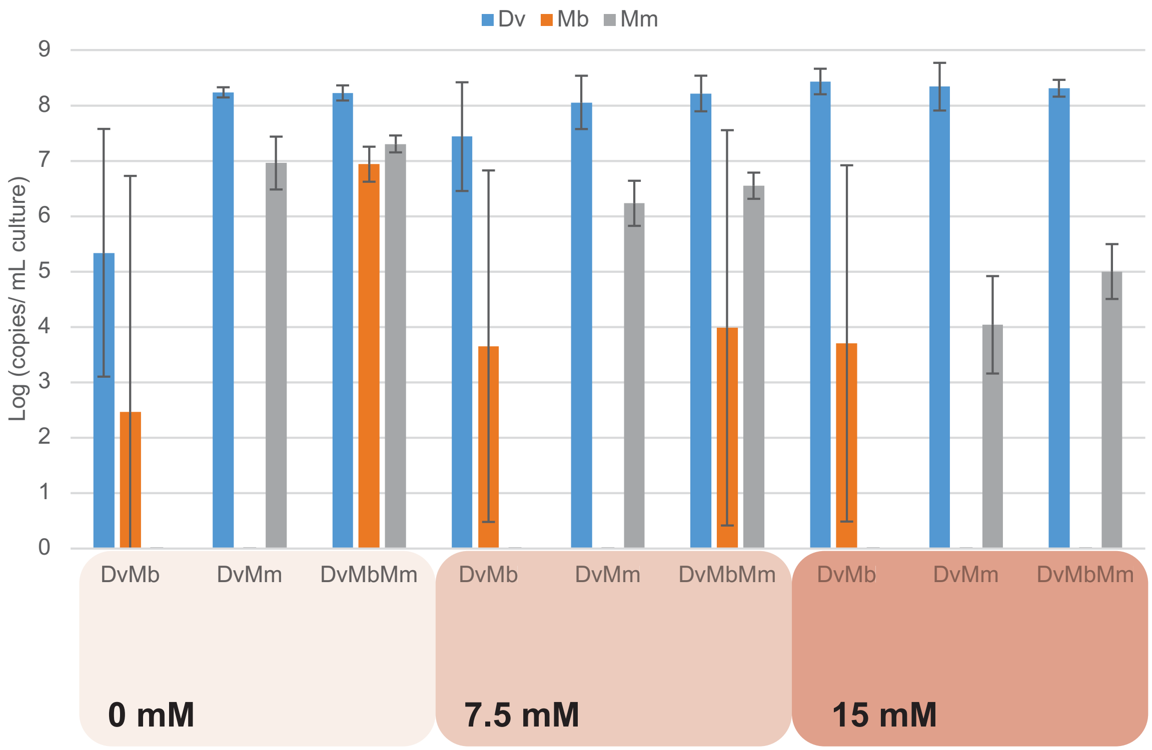


- **Increased electron acceptor availability shows a differential impact on the maintenance and productivity of aceto- and hydrogeno-trophic methanogens**

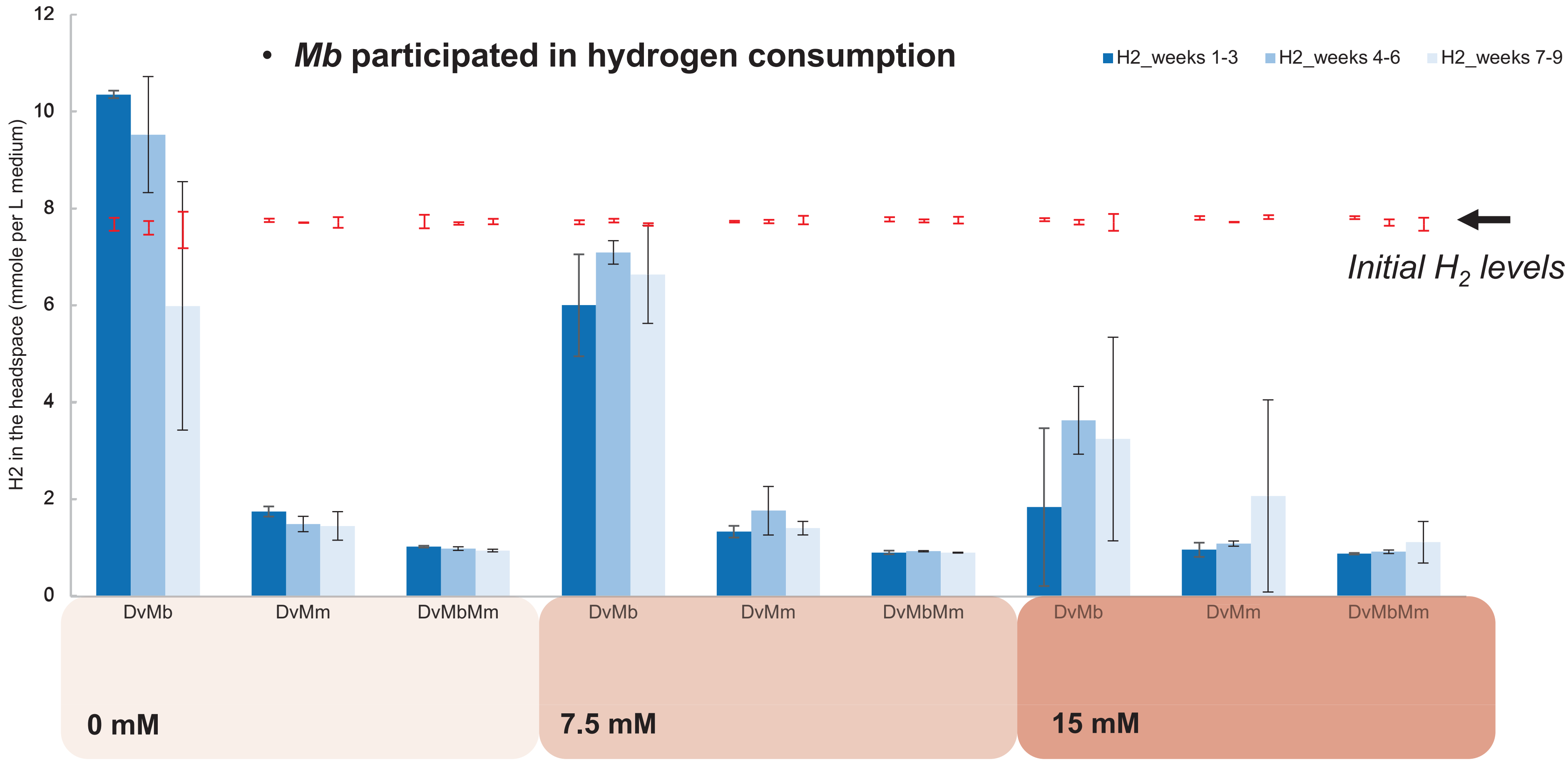
- *Dv* populations accounted for a large portion. No cross contamination

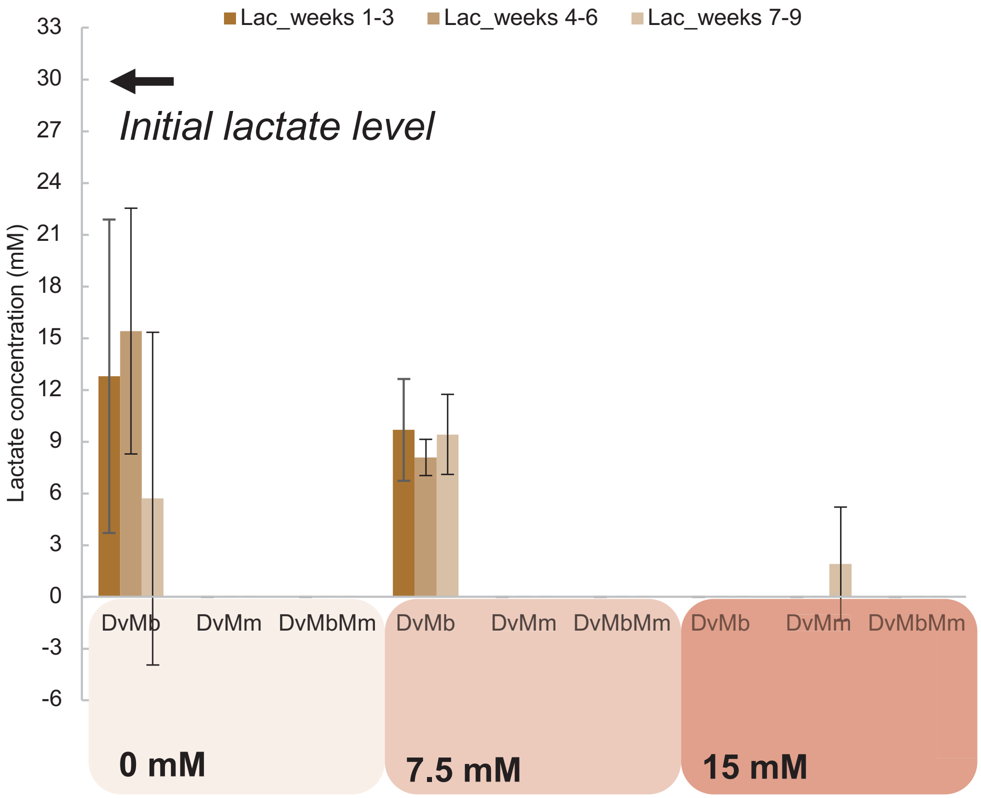


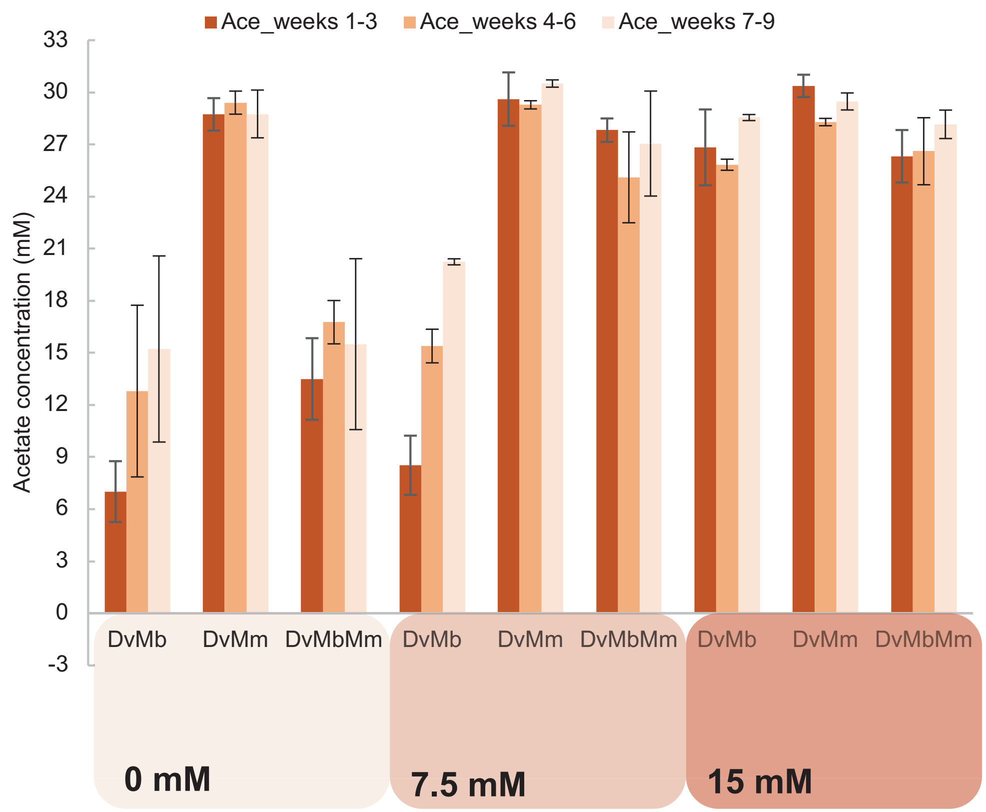
- *Dv* populations accounted for a large portion. No cross contamination
- *Mb* showed high variance, *Mm* were more stable
- An increased stability of methanogen populations with the increased community complexity (i.e. extended syntrophic interactions)



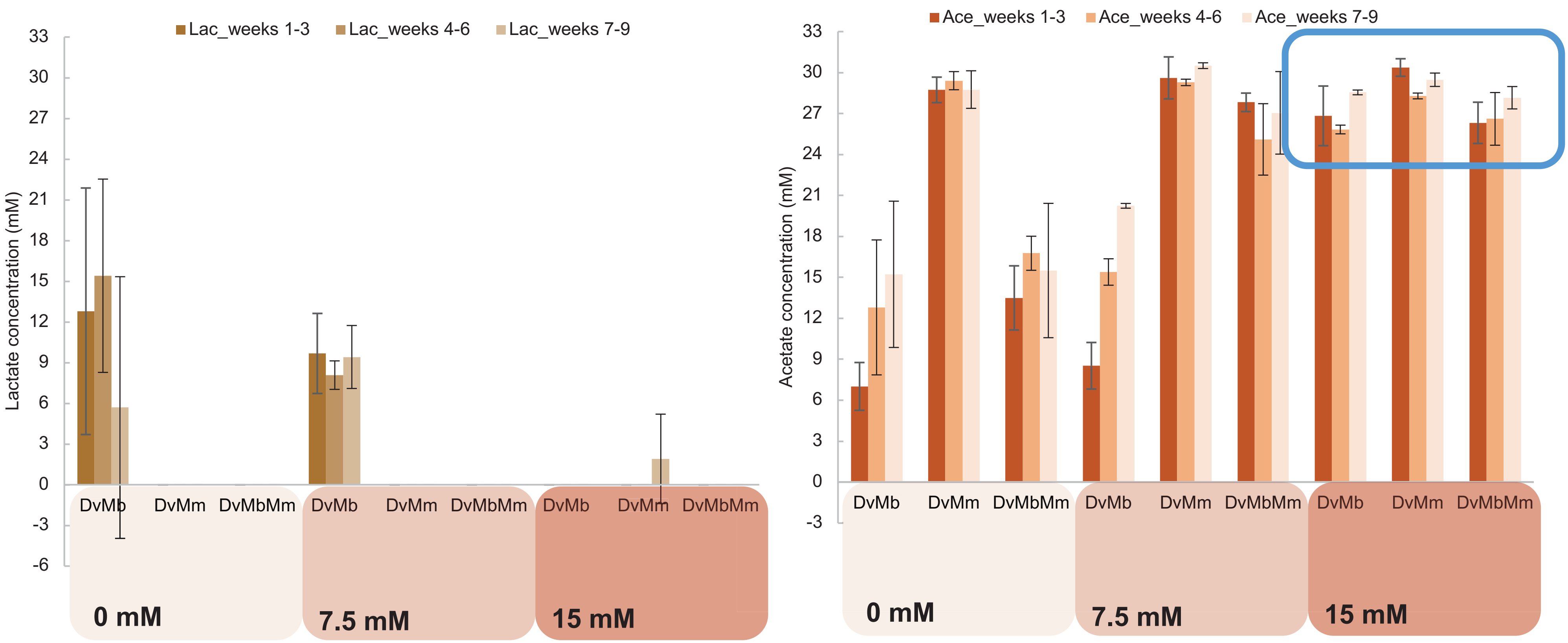
- **There were hydrogen decreases in all three scenarios, including *Dv-Mb* without sulfate addition**
- ***Mb* participated in hydrogen consumption**





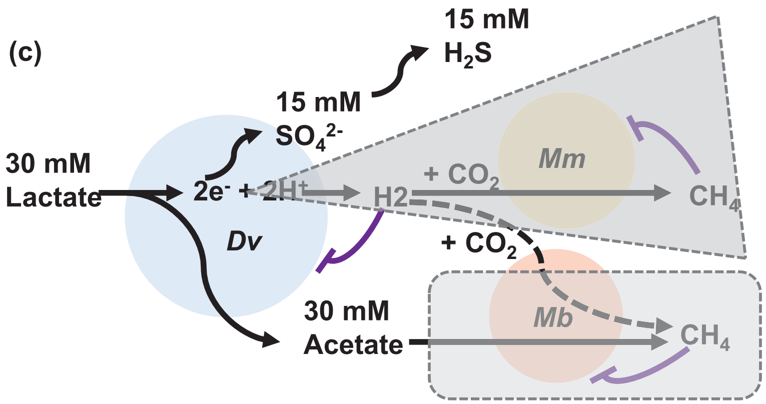


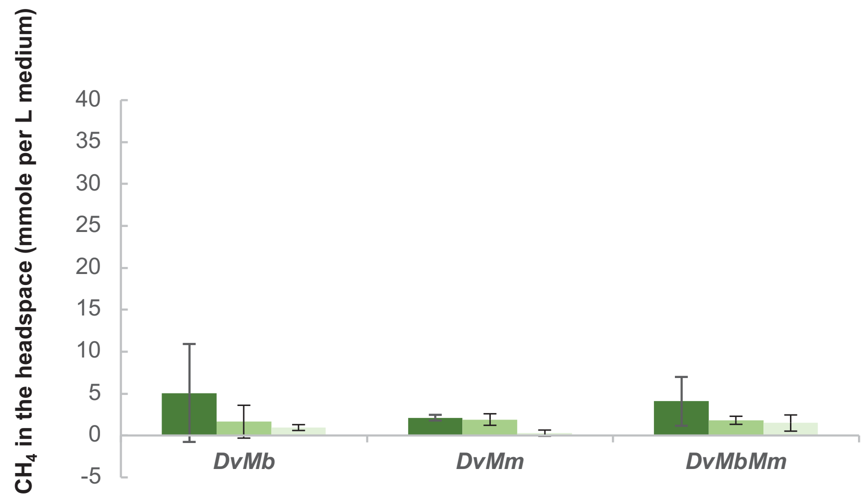
- ***Dv-Mm* consumed ~0 mM acetate**



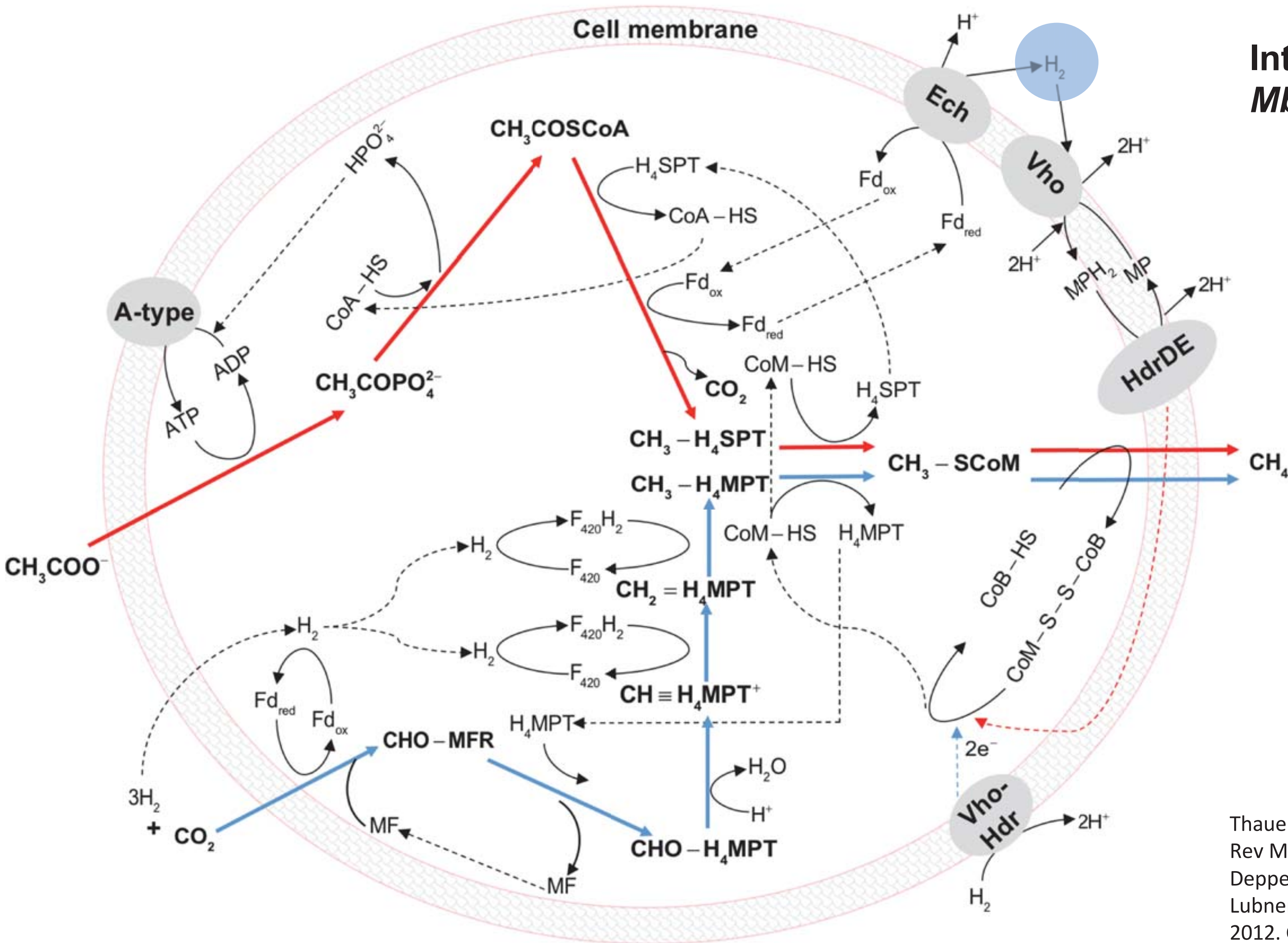
- ***Dv-Mm*** **consumed ~0 mM acetate**
- ***Dv-Mb*****+15 mM sulfate fully converted lactate to acetate, which Mb did not utilize for** CH_4 **production**

- ***Dv*** **might compete for Intermediate H_2 from *Mb* to block aceticlastic pathway**





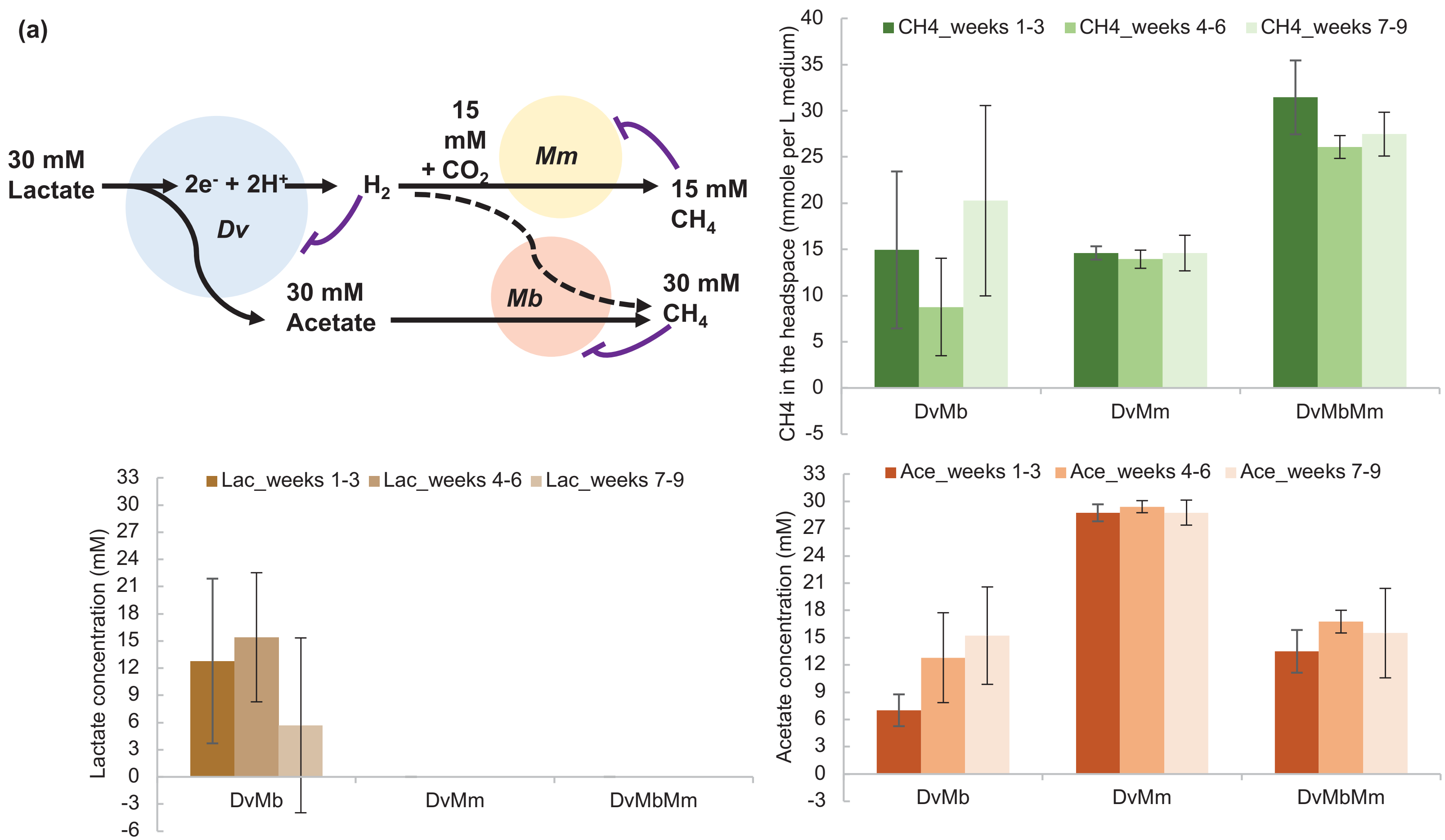
Intermediate H_2 makes *Mb* vulnerable



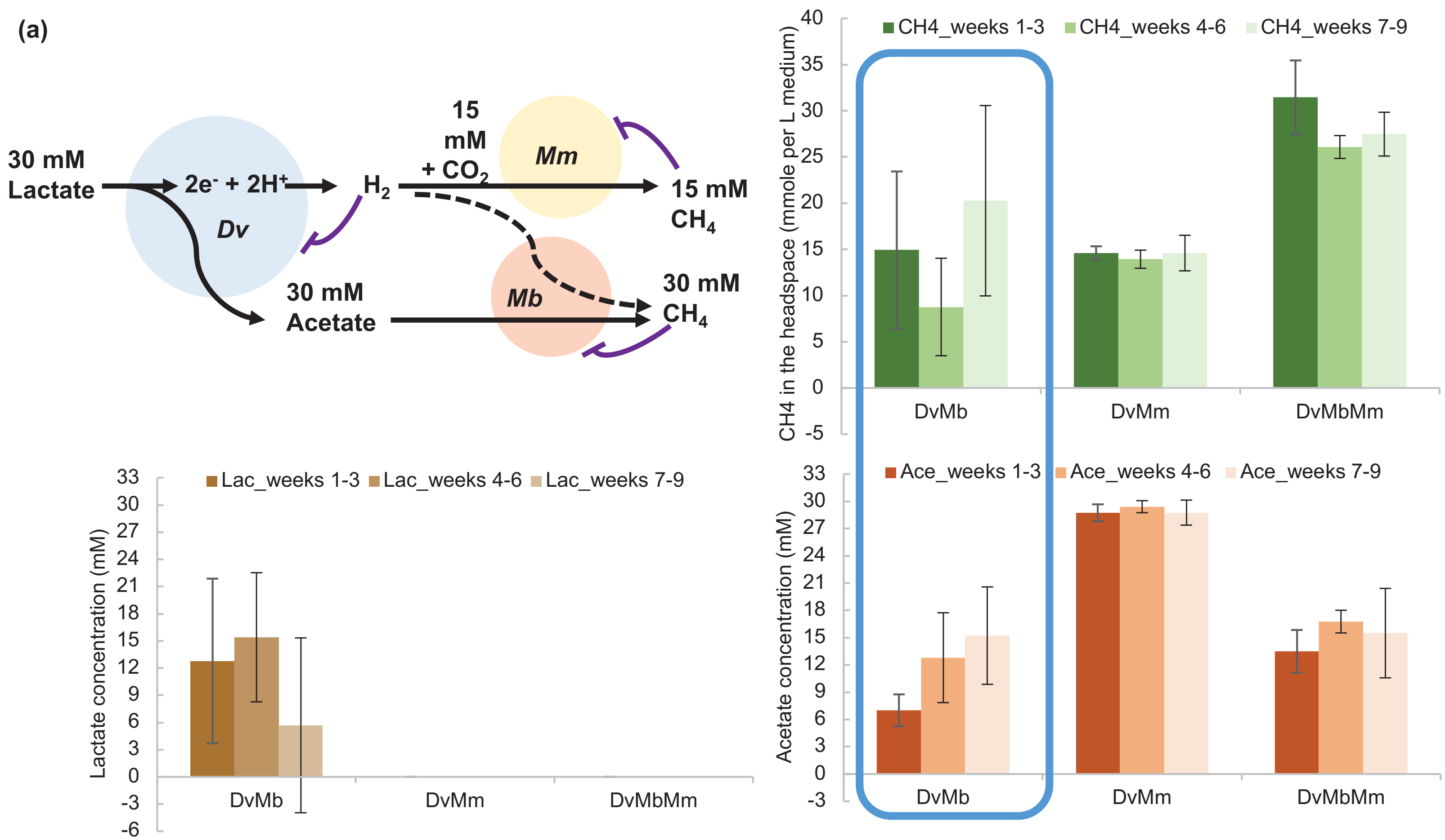
Acetate ->CH_4

CO_2 +H_2 ->CH_4

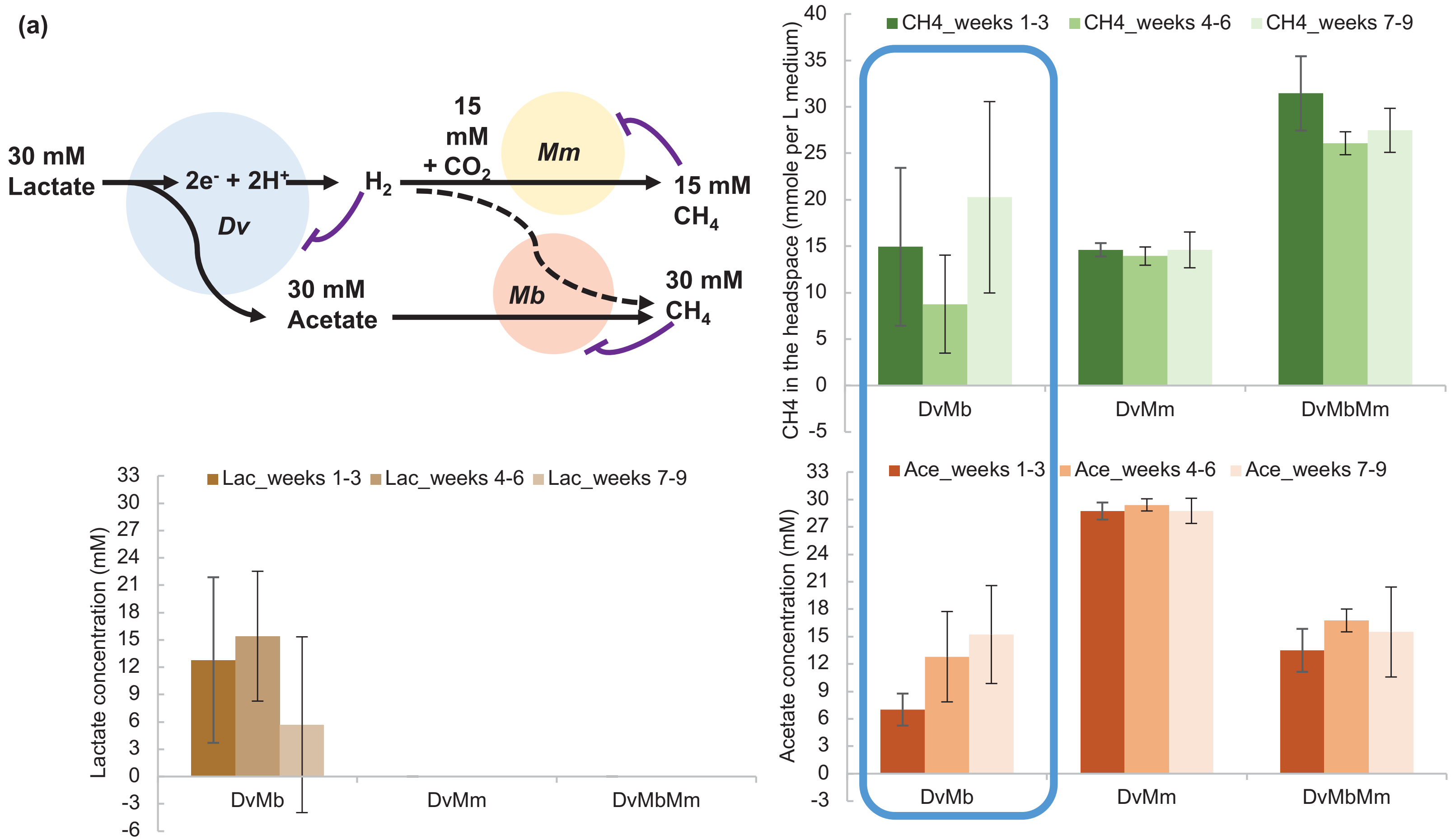
Thauer, *et al.* 2008. Nat Rev Microbiol; Ferry, 2010. Annu Rev Microbiol; Wang, *et al.* 2011. BMC Microbiol; Welte and Deppenmeier, 2014. Biochim Biophys Acta - Bioenerg; Lubner and Peters, 2017. ChemBioChem; Lane and Martin, 2012. Cell; Sousa, *et al.* 2013. Philos Trans R Soc B Biol Sci



- ***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.**

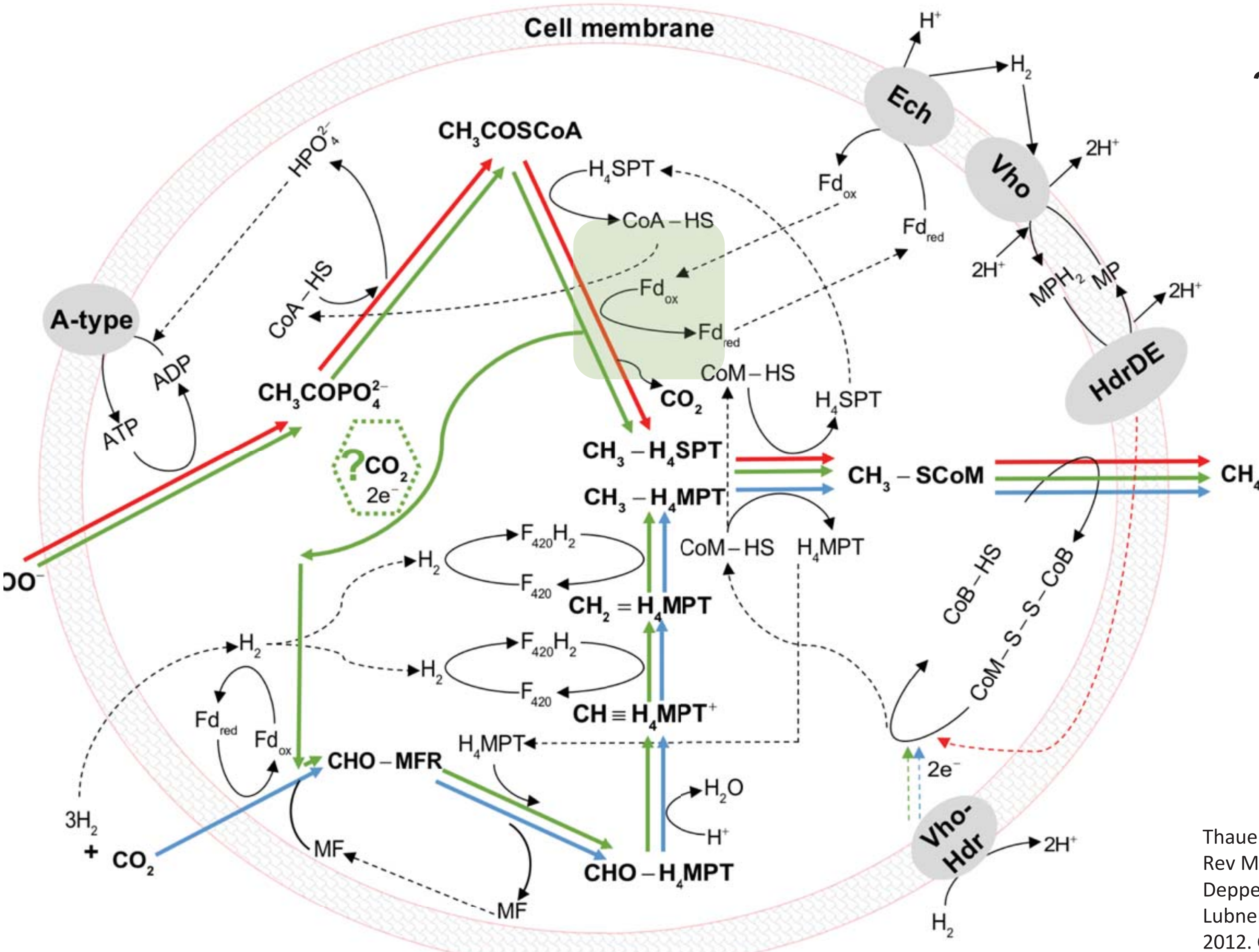


Dv $C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+$

? Mb $C_2H_3O_2^- + H^+ + 4H_2 \rightarrow 2CH_4 + 2H_2O$

H_2 limitation;

1 Lactate ~1 Acetate + $2H_2$; 0.5 Acetate + $2H_2$ ~ 1 CH_4



? Mb conserved 2 e^- and followed CO_2 reduction pathway with external H_2, instead of producing CO_2.

Acetate ->CH_4
CO_2 +H_2 ->CH_4
Acetate +H2 ->CH4

Thauer, *et al.* 2008. Nat Rev Microbiol; Ferry, 2010. Annu Rev Microbiol; Wang, *et al.* 2011. BMC Microbiol; Welte and Deppenmeier, 2014. Biochim Biophys Acta - Bioenerg; Lubner and Peters, 2017. ChemBioChem; Lane and Martin, 2012. Cell; Sousa, *et al.* 2013. Philos Trans R Soc B Biol Sci

Reaction number	Equation	ΔG°′ (KJ)
1	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-130.7
2	$C_2H_3O_2^- + H^+ \rightarrow CO_2 + CH_4$	-35.8
3	$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$	-157.8
4	$2C_3H_5O_3^- + SO_4^{2-} \rightarrow 2C_2H_3O_2^- + 2HCO_3^- + H_2S$	-165.8
5	$C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 2H_2 + H^+$	-4.0
6	$C_2H_3O_2^- + H^+ + 4H_2 \rightarrow 2CH_4 + 2H_2O$	-166.5

The standard free energy change at pH 7 (ΔG°′) was calculated from equilibrium constants (RK Thauer et al. 1977. Bacteriol Rev)

M. maripaludis **(Mm)**

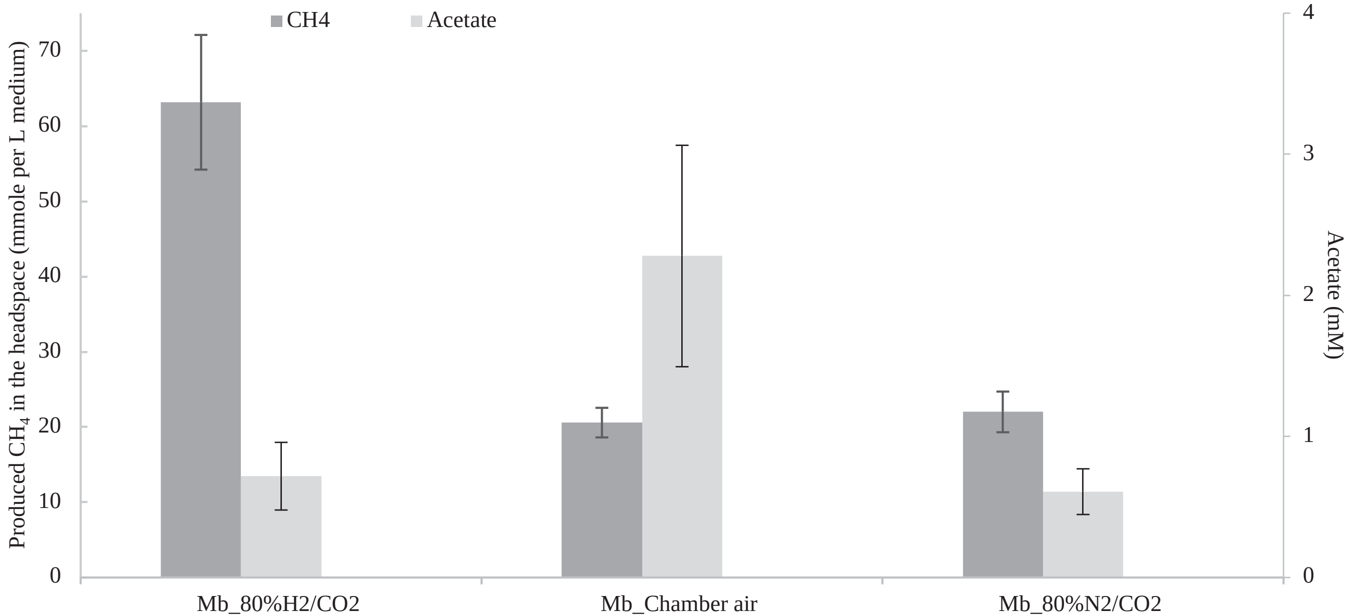
M. barkeri **(Mb)**

D. vulgaris **(Dv)**

? *M. barkeri* **(Mb)**

Needs further investigation

- **With acetate provided at 30 mM, increasing H_2 pressure in the headspace significantly increased *Mb* monoculture's methane production**



80% H_2	3.14% H_2	0% H_2
0% N_2	89.92% N_2	80% N_2
20% CO_2	5.32% CO_2	20% CO_2

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**
- **H_2 addition into AD system might benefit aceticlastic methanogenesis more**

THANK YOU !

Matthew J Wade (*Newcastle*)

Jan Dolfing *(Newcastle)*

Tobias Großkopf *(Warwick)*

Mary Coates *(Warwick)*

Fred Farrell *(Warwick)*

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