The extracellular electron transfer capabilities of rumen microbes are stimulated by conductive nanomaterials

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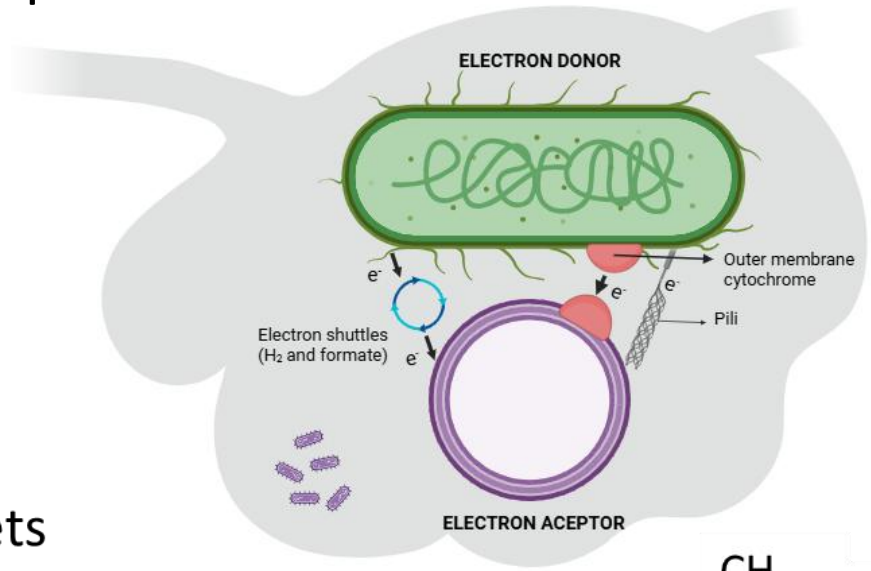
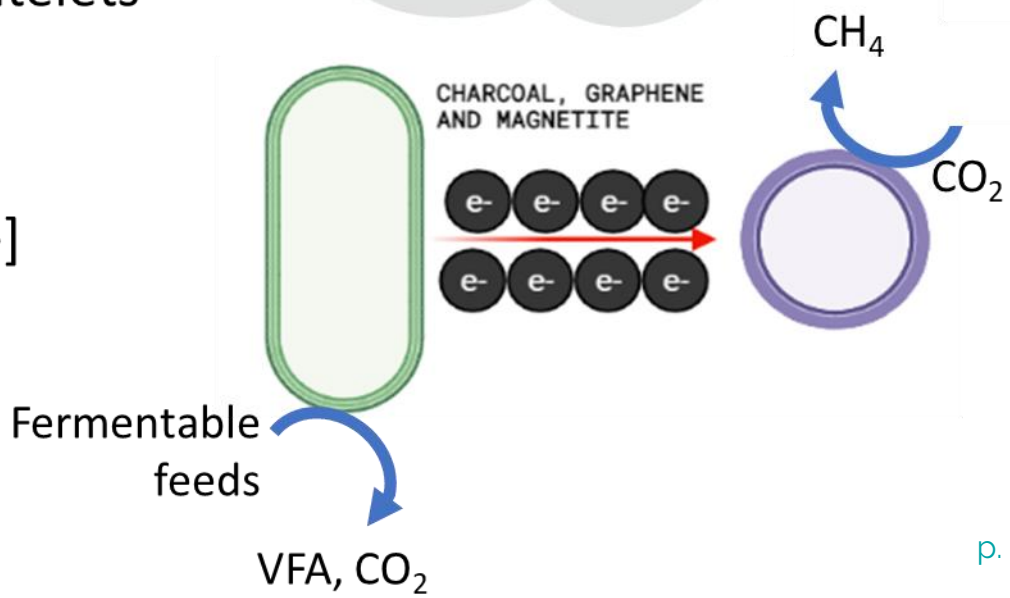
Strategies to explore Extracellular Electron Transfer (EET)

Rumen microbes capable to EET via DIET/redox protein remain undetected and underexplored

- Conductive materials (CMs) increase EET
  - Can promote biological processes
  - ↑ methane production in some ecosystems
- Carbon-based materials
  - Activated charcoal and graphene nanoplatelets
  - Provide an electron-conducting surface
- Mineral-based materials
  - Magnetite [$Fe^{2+}Fe_2^{3+}O_4$; Iron(II,III) oxide]
  - Acts as an electron conduit





Lovley, 2017. DOI 10.1016/j.coelec.2017.08.015
Lovley, 2017. DOI 10.1146/annurev-micro-030117-020420

Hypothesis

➢ Incubation of CMs, graphene and magnetite, in the rumen would enrich microbial communities associated with EET that induce shifts in fermentation parameters

- Does it change fermentation parameters? Methane?
- Which microbes could be associated with this presumed EET mechanism in the rumen?

Objective

➢ To test the effect of CMs on rumen fermentation and on microbial communities

METHODS

In vitro experiments

Rumen inoculum: Sheep

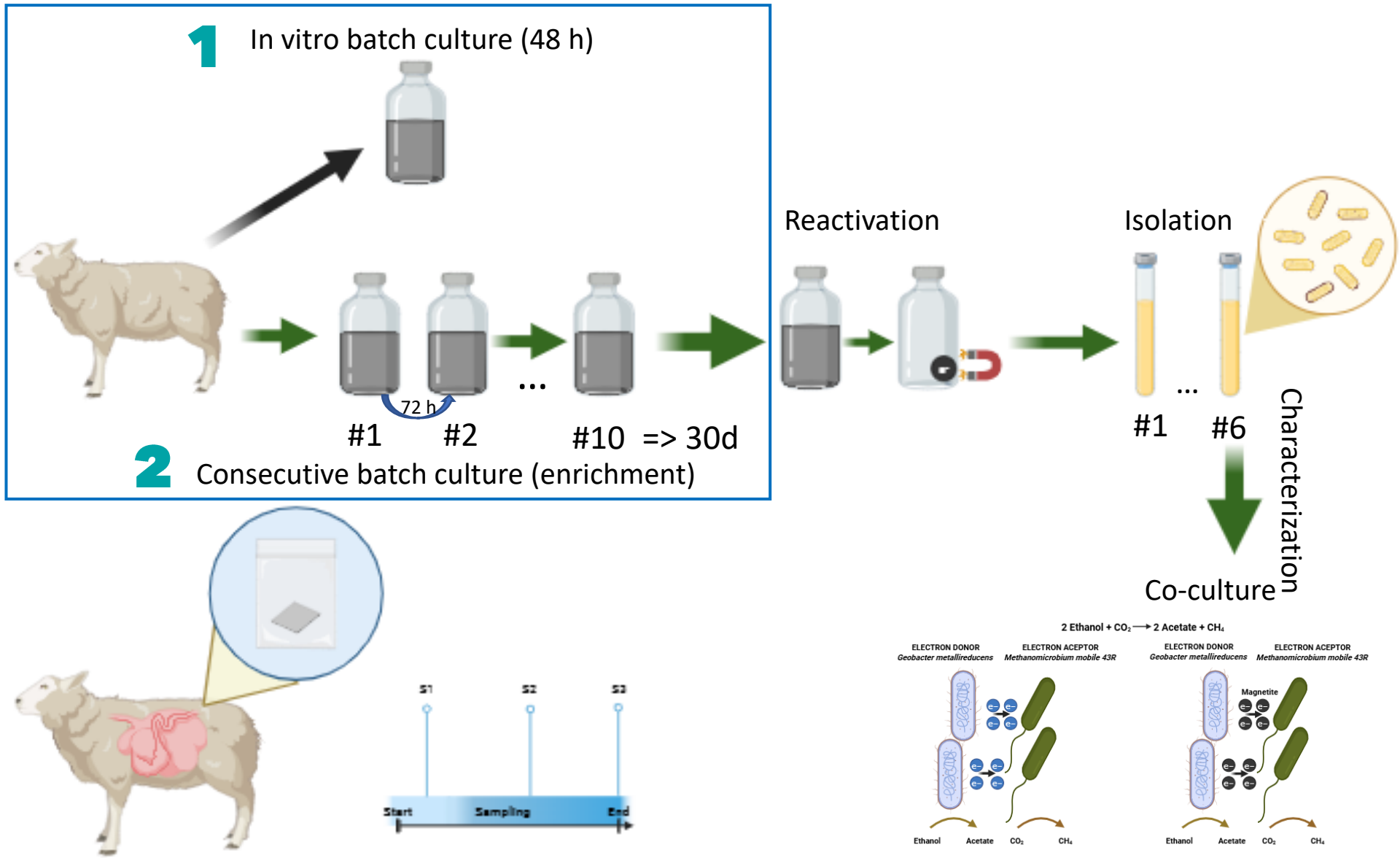
Treatments:

Control: Substrate alone

Graphene (GPH) 40 mg/bottle

Magnetite (MGN) 40 mg/bottle

Substrates: Solid or soluble



In sacco experiment

Rumen incubation: Sheep

Treatments:

Control: polymer PDMS alone

PDMS + Graphene (GPH)

PDMS + Magnetite (MGN)

Incubation time: 1d, 7d and 28d

3 In sacco experiment

RESULTS: Fermentation

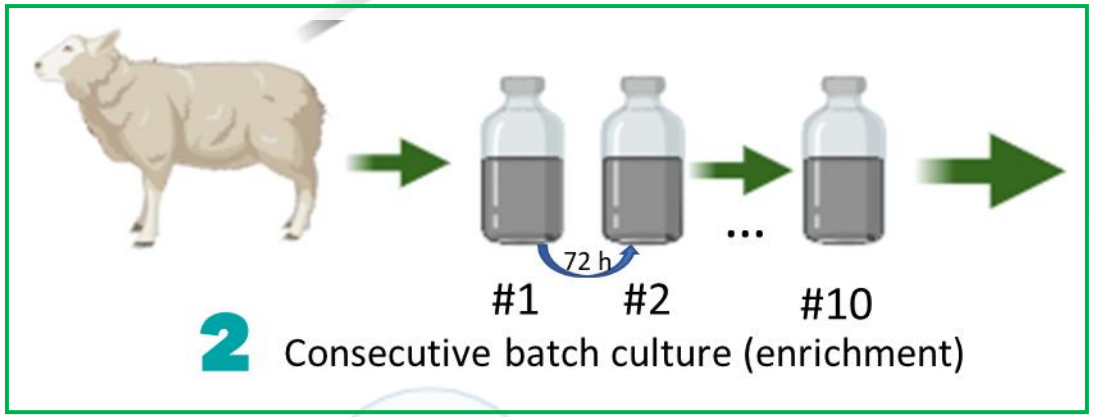
In vitro batch culture (48h)

Items	CTR	GPH 5%	GPH 10%	MGN 5%	MGN 10%	SEM	P-value
Gas production (mL)	156,0	154,0	157,0	158,0	155,0	2.24	0.542
Methane production (mL)	19.3	19.5	20.3	20.3	20.1	0.43	0.131
pH	6.84	6.81	6.81	6.78	6.77	0.03	0.321
Total VFA	107.1	100.3	96.7	98.7	96.6	3.42	0.053
Acetate	65.3	65.4	65,0	64.3	64,0	0.76	0.319
Propionate	18,0	17.7	18.6	18.5	18.2	0.48	0.342
Butyrate	7.7	7.8	7.3	7.7	8.1	0.35	0.271
Isobutyrate	2.1	2.1	2.1	2.1	2.1	0.02	0.268
Valerate	3.6	3.6	3.6	3.6	3.6	0.07	0.764
Isovalerate	3.1	3.2	3.1	3.4	3.7	0.21	0.072
Caproate	0.2	0.3	0.2	0.3	0.4	0.04	0.060
A/P	3.7	3.7	3.5	3.5	3.5	0.14	0.392

No major differences between treatments for any fermentative metabolites

CMs tend to increase methane production (~5%)

1 In vitro batch culture (48 h)



2 Consecutive batch culture (enrichment)

Reactivation

Isolation

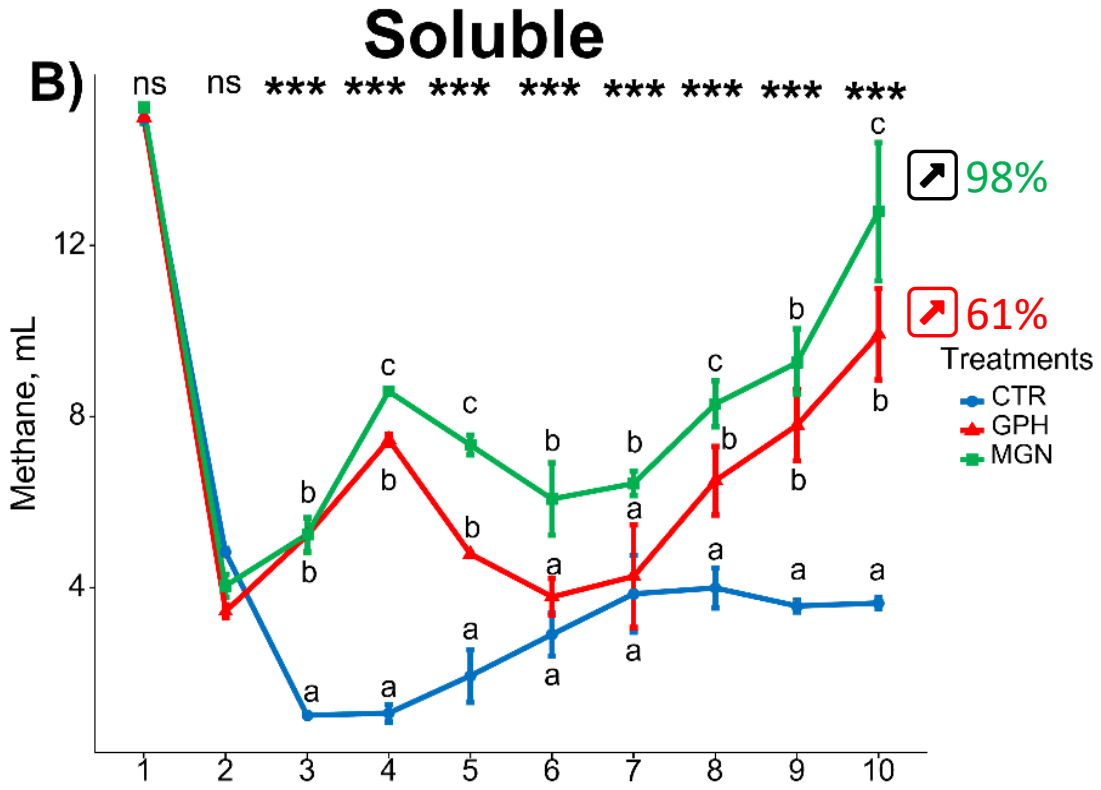
Characterization

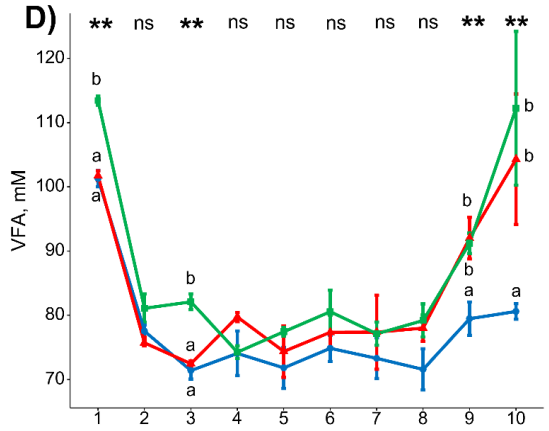
Co-culture

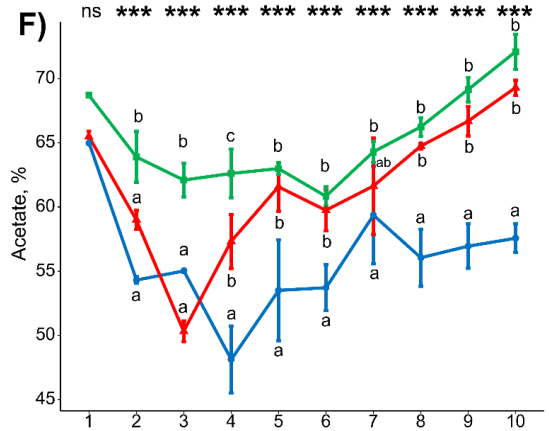
3 In sacco experiment

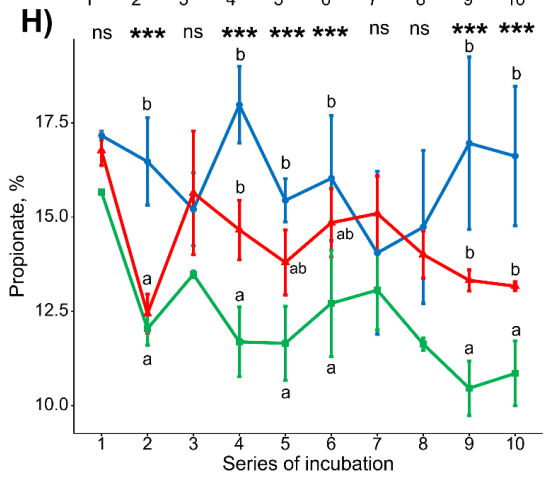
RESULTS: Fermentation

Consecutive batch culture









CMs increased methane production

↗ Total VFA and acetate

↘ propionate

RESULTS: Microbiota

Consecutive batch culture - Soluble substrate

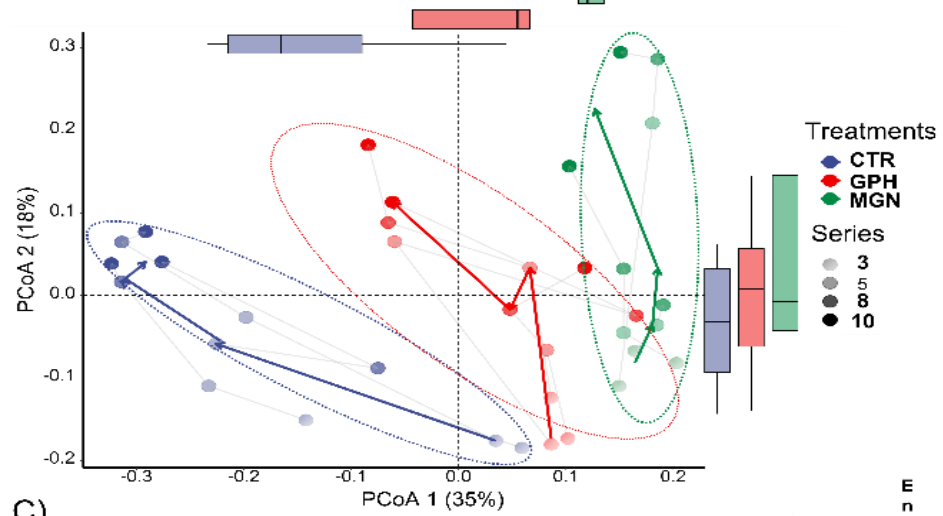
WUniFrac
S3 to S10

A) Mixed model

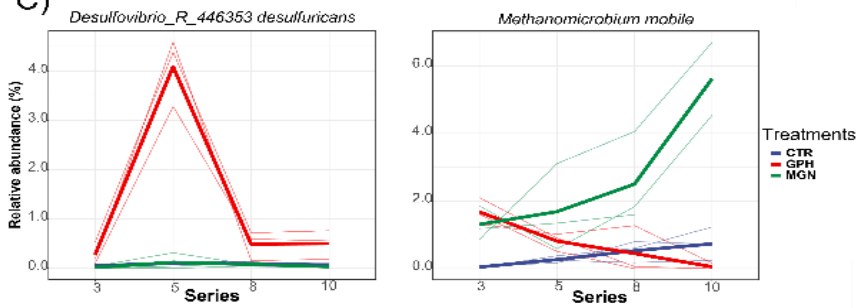
Term	Estimate	Std.Error	Statistic	P-value
Intercept	0.272	0.055	4.914	0.002
TreatmentsGPH	-0.009	0.078	-0.116	0.911
TreatmentsMGN	0.044	0.079	0.550	0.599
Series	0.018	0.005	3.405	0.004

PERMANOVA

Variable	R.Squared	P.Value
Series	0.212	0.001
Treatments	0.327	0.001

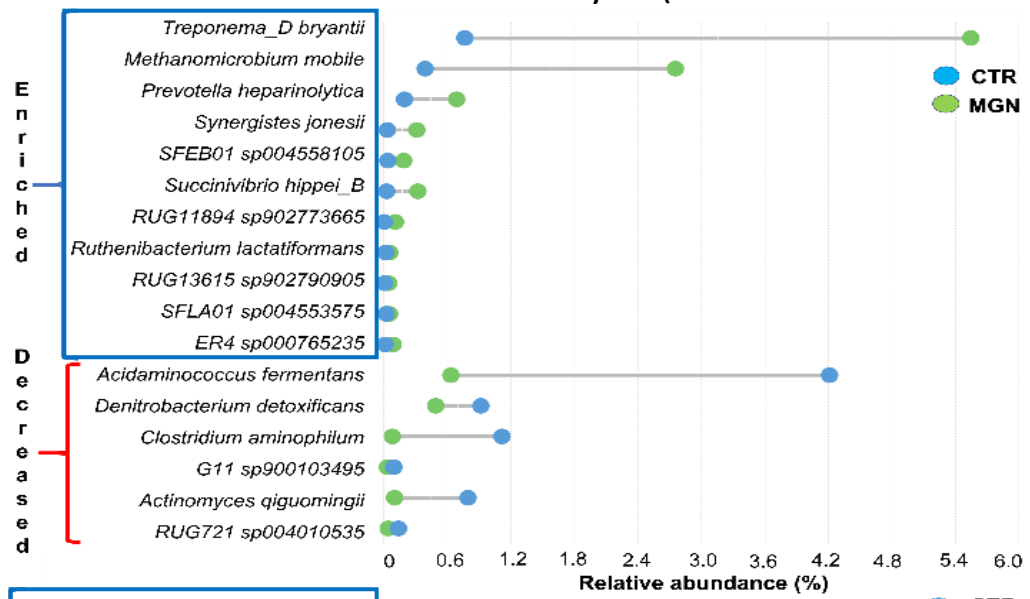


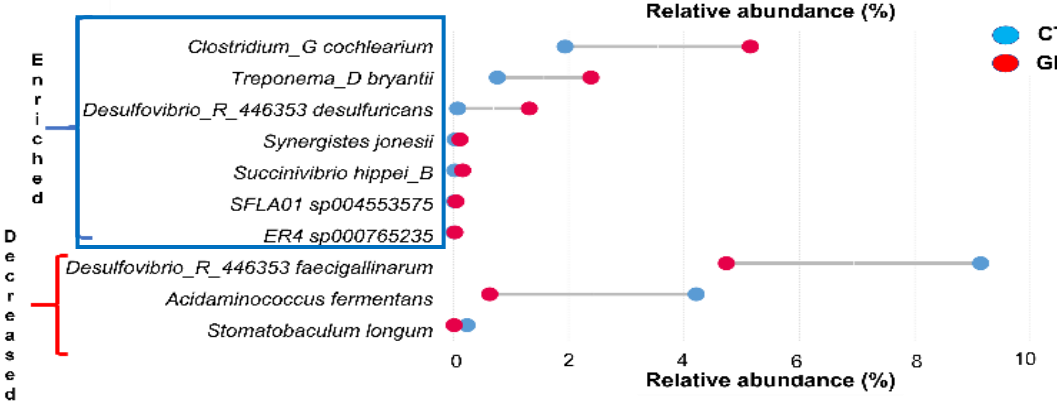
C)



B) Differential abundance analysis (consensus 3 stat tools)

MaAslin2
ANCOM BC2
LINDA





CMs enriched microbes that were poorly detected in the initial inoculum and CTR

Summary

- CMs Increased methane production

How? Enriched pathways/genes?

- Shift in microbes associated to conductive materials

suggests the presence of EET in the rumen

Correlation is not causation!

- EET and mechanisms involved should be validated with additional tools
- Its extent in the rumen should be assessed as it could be another mechanism to consider for modulating methanogenesis

Limitation and future directions

Next steps:

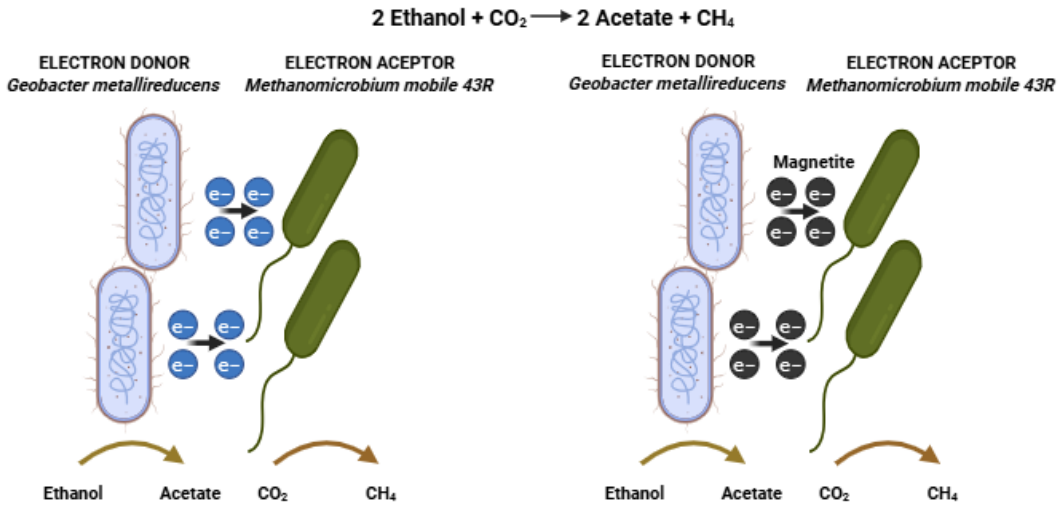
- **Functional analysis (genes/pathways)**
- **Isolation**

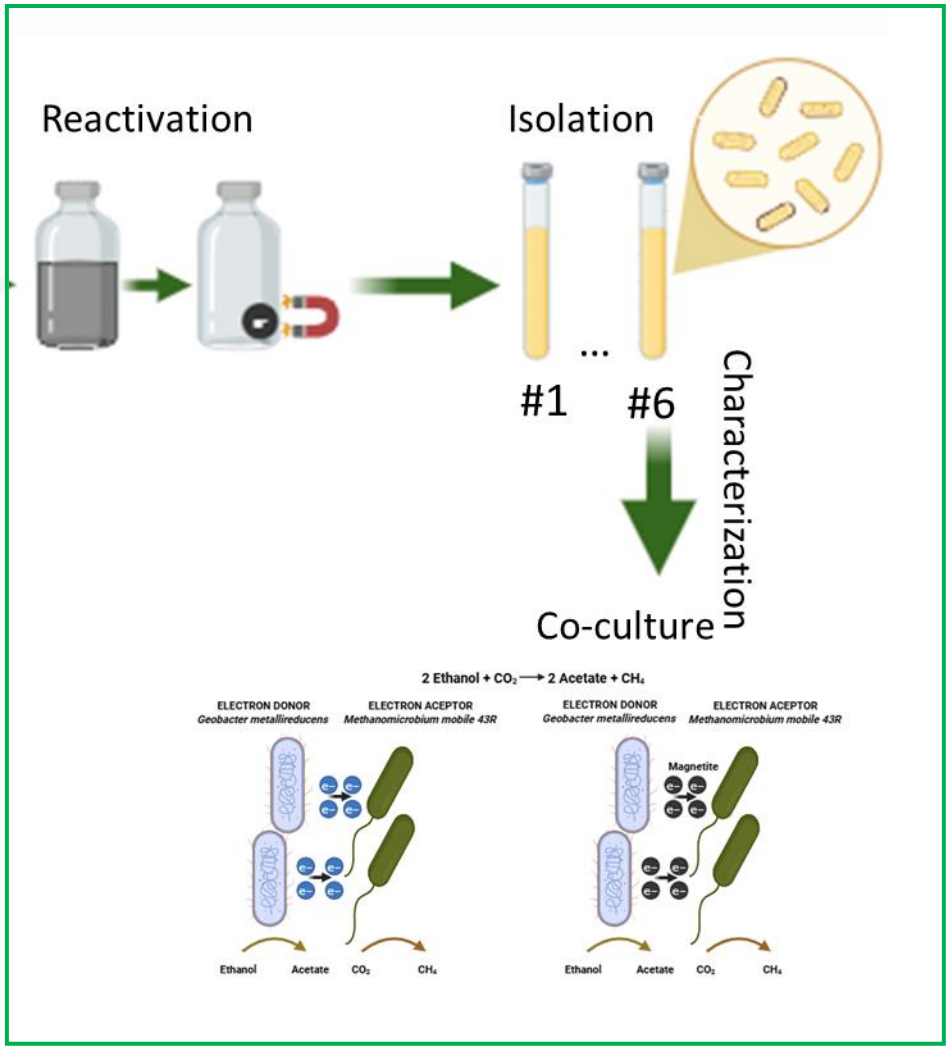
✓ A *Methanomicrobium mobile* strain was isolated

- **Co-culture**

Geobacter a well know DIET bacteria

G. Metallireducens <=> *M. mobile*?





Acknowledgments

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Thank you for your attention!