Snorre Chemostat Experiments

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Goal :

Those set of experiments are chemostat experiments for Snorre's paper using the four species. We planned two set of experiments with four chemostats per round.
In experiment 1 we test if the four species At, Ct, Oa and Ml coexist on four different carbon sources. In experiment 1, we test the carbon sources Ribose, Acetate, Histidine and Gluterate.

Procedure :

Solution preparation 16.6.2025

- Weighted in mass of carbon source required for the corresponding molarity in 400 mL (see table below) and dissolved it in 30 mL H20
- Mixed solutions according to table below
- Adjusted pH to 7.4
- Filtered media

	Ribose	Sodium acetate	Histidine	Glutaric acid
Carbon-atoms	5	2	6	5
MW	150.13	82.0343	155.15	132.12
Concentration [mM]	18	45	15	18
Volume [mL]	400	400	400	400
Calculated weight in [mg]	1080	1476	930	951.2
Weight in [mg]	1079.4	1476.6	931.3	952.5

Solution	Volume
M9 10x	50
HMB	10
H20	310
Carbon sorce	30

Pre-culture and chemostat inoculation 16.6.2025

- Inoculated 1 colony of each species (At, Ct, Oa, Ml) in 10 mL TSB for overnight culture
- The next day, started 2 day cultures in 10 mL per species with OD 0.05 at 11:00, yielded cells at 16:00 (longer day culture to have enough cells)
- Merged two day cultures per species in 50 mL falcon tube and centrifuged at 4000 rpm for 10 min
- Dissolved pellet in 1 mL M9 + HMB, centrifuged at 8000 rpm for 6 min
- Dissolved pellet in 1 mL M9 + HMB and measured OD (based on 1:10 dilution, values in table below are OD of culture)

	At	Ct	Oa	Ml
OD	2.37	9.07	2.2	2.3

- Adjusted all cultures to OD 2.2 in 1 mL
- Pooled cultures (all at equal OD)
- Measured OD 3 for community (based on 1:10 dilution)
- Prepared 4 chemstat vials with media according to table below

Chemostat	C1	C2	C3	C4
Carbon source	Ribose	Acetate	Histidine	Glutaric Acid

- Inoculated chemsotat vials with community in order that OD is 0.03
- CFUs of community in chemostat vials

Chemostat sampling

Date	Time	Hours in experiment	Plate ID	Measured OD
17.6	06:30:00 PM	0	T0	No
18.6	10:00:00 AM	15.5	T1	Yes
18.6	06:30:00 PM	24	T2	No
19.6	10:00:00 AM	39.5	T3	Yes
19.6	05:00:00 PM	46.5	T4	No
20.6	10:00:00 AM	63.5	T5	Yes