Chemostat no carbon sources and chain pilot

Date: 2025-08-12
Tags: ML CT OA chemostat community Carbon sources AT
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Goal :

This experiments contains four conditions:

- C1: No carbon source with Ct, Oa community for the Ct Oa coexistence paper
- C2: No carbon source with Ct, Oa, At, Ml for Snorre's transfer paper
- C3: Two chemostats connected in a chain with the four species on Glucose for a pilot

Condition	Reactor
C1	M0
C2	M1
C3	M2+M3

Procedure :

Required solutions:

- MM no CS with thiamine
- MM no CS no thiamine
- MM with 2.5 mM glucose
- 25 mM D-glucose

Solution	No CS thiamine	No CS no thiamine	Glucose
M9	50	50	50
HMB	10	10	10
10X glucose	0	0	50
10X thiamine	50	0	0
H20	390	440	390

Cell culture

- Inoculate 1 colony of each species (At, Ct, Oa, Ml) in 10 mL TSB for overnight culture

OD ON cultures:

At	Ct	Ml	Oa
5.7	4.8	5.8	6.1

- 2 day cultures per species in 10 mL TSB
- Merge two day cultures per species in 50 mL tube and centrifuge at 4000 rpm for 10 min
- Dissolve pellet in 1 mL No CS no thiamine, centrifuged at 8000 rpm for 6 min
- Dissolve pellet in 1 mL NO CS no thaimine, measure OD

OD washed cultures:

At	Ct	Ml	Oa
8.6	15.8	8.6	7.9

- Adjust OD to OD of species with lowest OD in 1 mL (7.9)
- For C1, Mix 330 uL Ct and 330 uL Oa
    - Adjusted OD to 0.1
- For C2 and C3, mix 660 of each species
    - For C2 adjusted OD to 0.03
    - For C3 adjusted OD to 0.1

Chained chemostat setup

- Connect M2 and M3 so that the waste flows into C3
- Only M2 has medium in vial, M3 No Cs no thiamine

Oxygen test

For a project idea, I turn off aeration and want to find out if oxygen is depleted in the chemostat.
I want to test Resazurin as an indicator.

- 1 mg/mL stock
- 1:1000 to test medium.
- It works as an indicator, because in a batch culture in a closed falcon the indicator turned from blue to purple
- When I turned off the oxygen supply in the second chemostat of the chain and added the dye, it was the first reactor that was more purple than the second one, even though both are still pretty blue (see picture)

Results :

I sample the reactore twice per day from Tuesday until (including) Friday. Below are the sampling times in experimental time. For every sample I did CFUs and measured the OD on a plate reader in the morning. The CFUs and ODs are in the attached sheets.

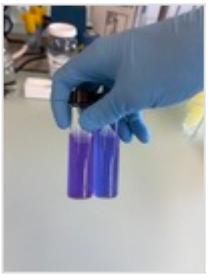
Name	Time	Measured OD

T1	18.8	YES
T2	24.4	NO
T3	43.4	YES
T4	48.5	NO
T5	67.5	YES
T6	71.3	NO

I attached the CFUs, the ODs and the plots below. Here is a table that maps experimental conditions to filenames:

Attached files

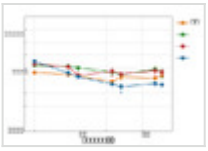
oxygen_dye.jpg
sha256: 26d4892a9a6547244db26b876e4cb3fb0badac74184e52ca6bbeffa6cda35026



od_commmunity_no_cs.csv
sha256: 12a9530acc1ca809e5e2d18c0276a8c774f39466db5069a280760f47e6297e6a

community_no_cs.csv
sha256: 2742fb037c70391bce80db80ba6ea593eae6c943f510fc85d4047d6f14d18f5d

community_no_cs.svg
sha256: c02efee1d3dc1f7792d01dc02fcb5dcaf27a93edd71f90523bce881943b4edb5



Unique eLabID: 20250812-9e1fffff033859e15dfe4d33ebb14d14be9eed66
Link: https://dmf-labbook.unil.ch/experiments.php?mode=view&id=871