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Preliminary data 1: 10-day proof-of-concept semi-sterile continuous cultivation of *Caldimonas thermodepolymerans* in turbidostat

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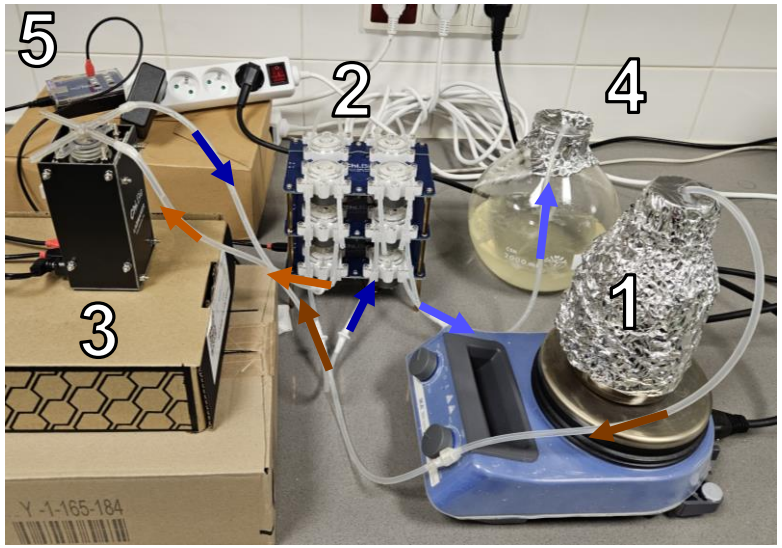
MOTIVATION

- The purpose of this 10-day experiment was to verify whether *C. thermodepolymerans* can be cultured in continuous mode (turbidostat) at 50°C in semi-sterile conditions without contamination of the culture with another organism.

- The minimal medium containing 5 g/L xylose **was not sterilised** prior to cultivation, the glass bioreactor vessel and connecting tubes were briefly rinsed with 70% ethanol prior to cultivation, the Chi.Bio reactor vessel containing the *C. thermodepolymerans* culture was connected to the outside environment via two outlets **without** air filters for the whole course of the experiment.

BIOREACTOR SETUP



Chi.bio minibioreactor set-up for open continuous *Caldimonas* culture at 50°C.

1 – Heating plate keeps the minimal medium with 5 g/L D-xylose at desired temperature (50 °C).

2 – Peristaltic pump for turbidostatic mode to regulate OD. Flows the medium from the medium bottle (1) to the reactor (3) and takes excess medium to waste (4). Flow represented by arrows: brown → orange → dark blue → light blue.

3 – Chi.bio minireactor that contains heating plate, magnetic stirrer and LED for OD measurement

4 – Waste bottle

5 – Chi.bio microcontroller controls the reactor and pumps. The microcontroller is connected to a computer with a graphical interface (6).

6 – Graphical interface allows to readily set cultivation parameters and monitor them in real-time.

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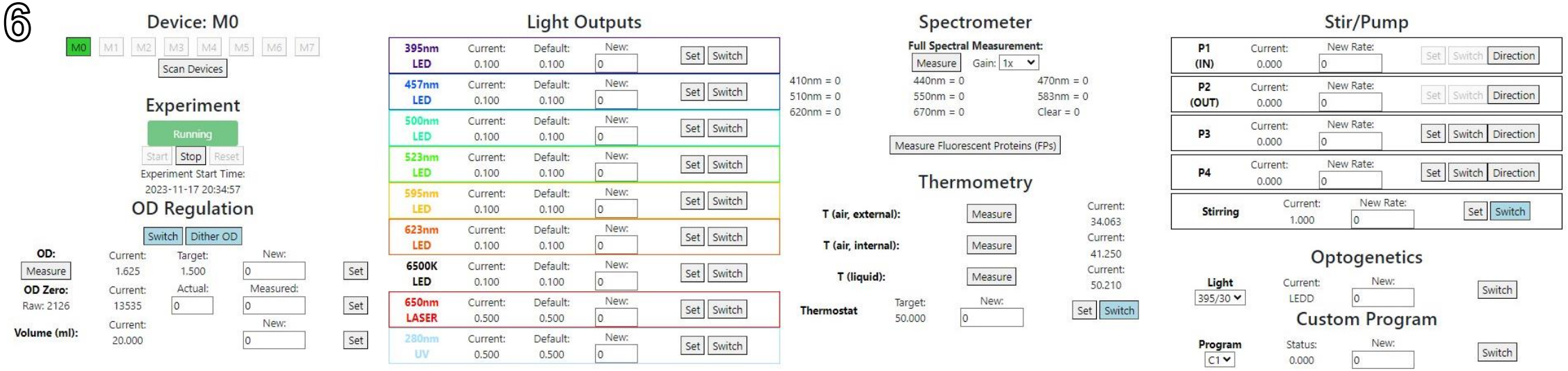

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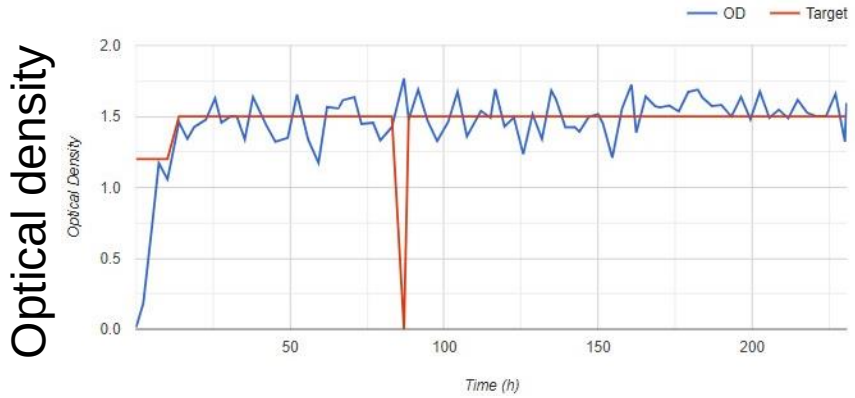
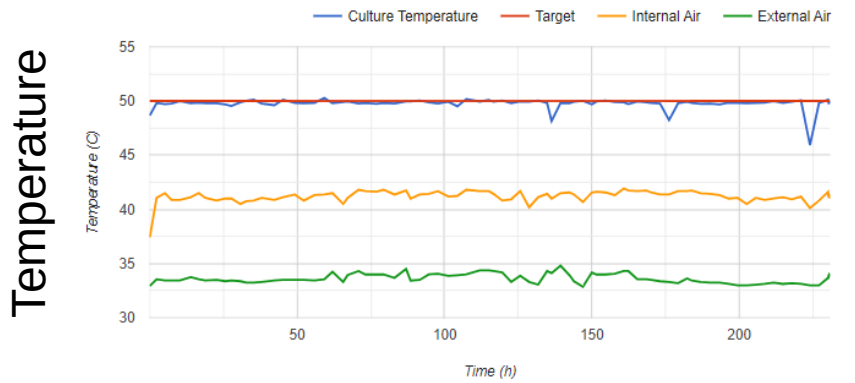
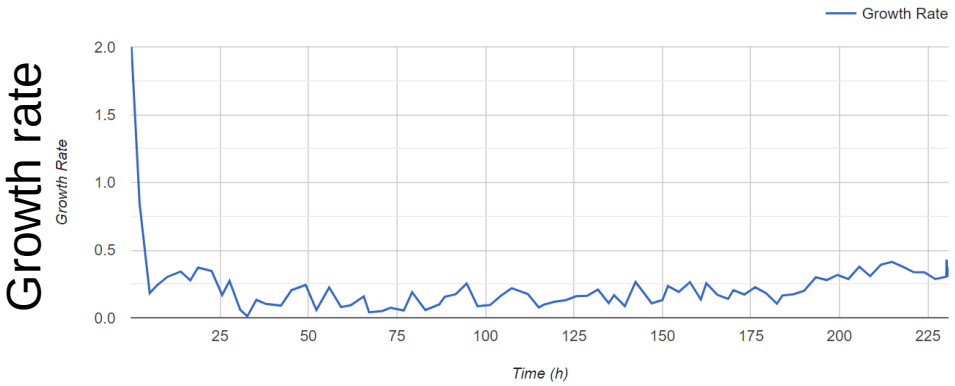
RESULTS

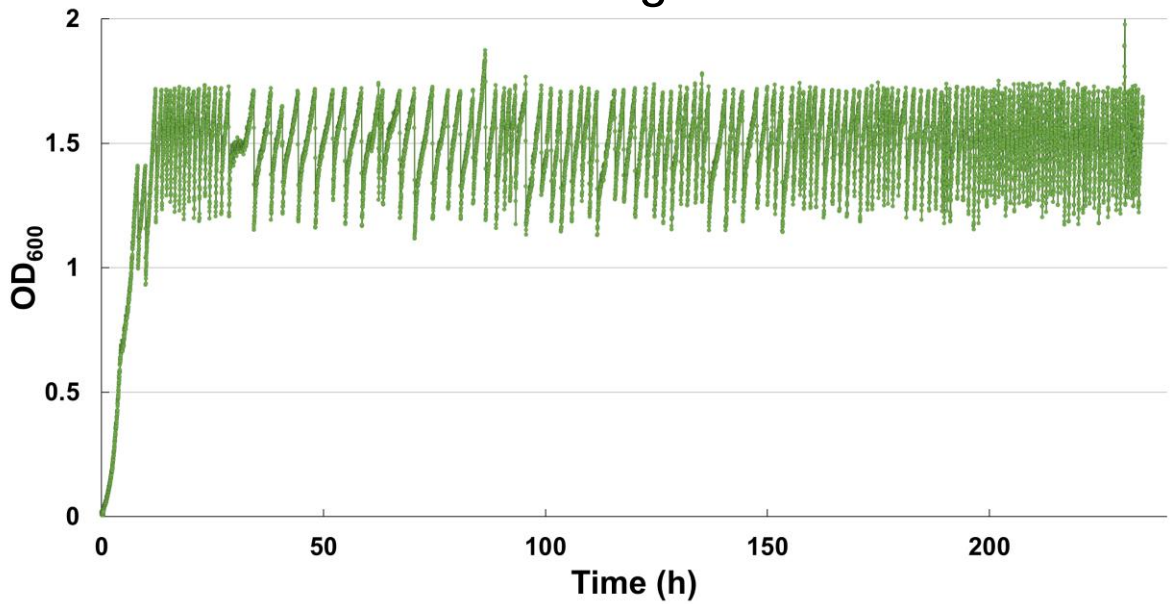
Real time monitoring of the continuous culture





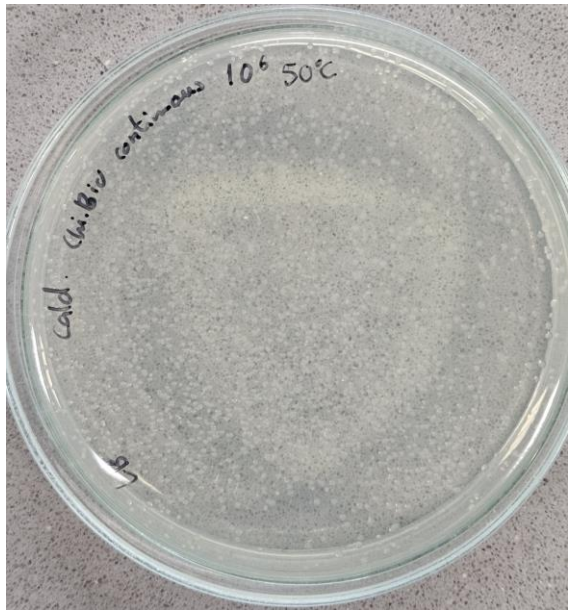


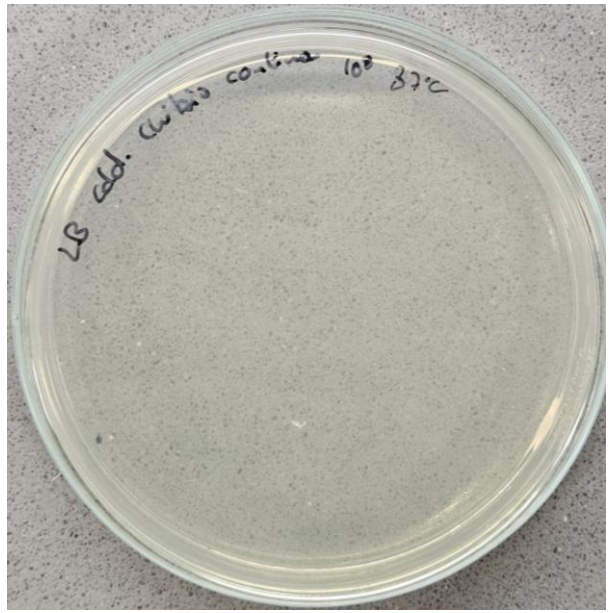
Post cultivation growth data



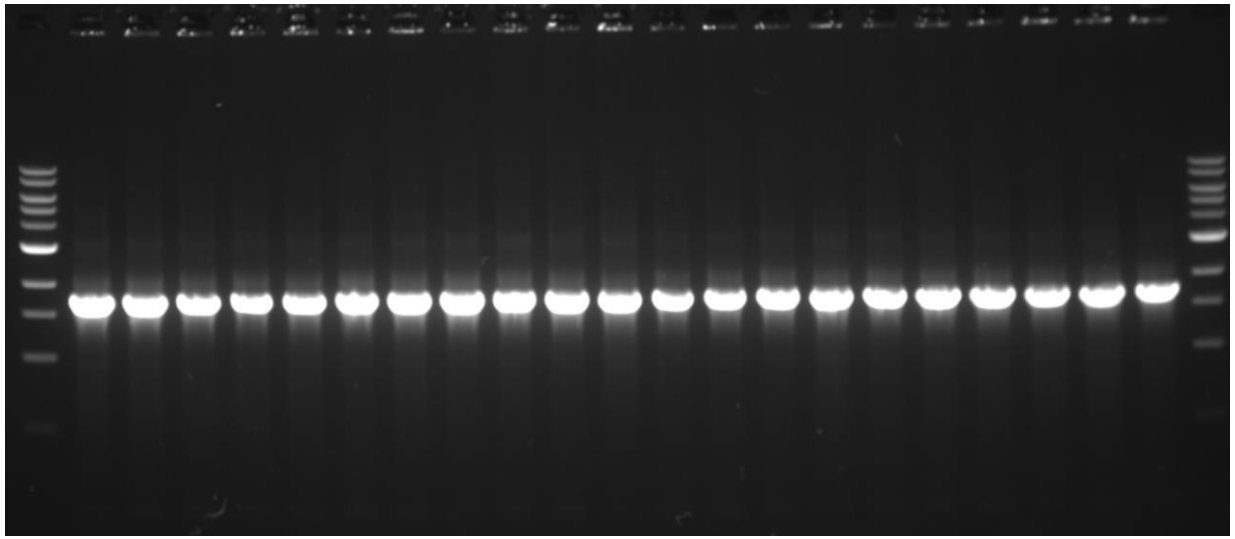
- Stable uninterrupted growth for 10 days.
- Graphical interface allows to spot deviations/errors that can be readily fixed without cancelling the cultivation.
- All data is collected, saved for posterior data analysis.

RESULTS: NO CONTAMINATION OBSERVED AFTER 10 DAYS OF THE CULTURE





- Cells were plated on LB agar after 10 days of continuous cultivation and incubated at 50 °C or 37 °C for 24 h.
  - Numerous colonies were obtained at 50 °C, while there were no visible colonies on plate incubated 24 h at 37 °C (slower growing *Caldimonas* colonies became visible later after 48 h).
  - This result indicates no contamination with mesophilic organisms.



- Colonies from 50°C plate were genotyped using *phaC* gene *Caldimonas thermodepolymerans* specific primers
  - Primer forward: ATGACACAACGCTCTGCACCCC.
  - Primer reverse: TCAGGCCCGTTCCTTGACGTAG
  - Annealing: 60 °C
  - Extension: 1 min/kb
  - Expected amplicon size: 1.7 kb
- 21 randomly picked colonies were positive, which indicates that the turbidostat culture contained only *C. thermodepolymerans* at the end of the experiment.

CONCLUSION

No contamination with mesophilic or thermophilic microflora was observed at the end of the 10-day proof-of-concept continuous culture with *C. thermodepolymerans* grown on D-xylose in non-sterile minimal medium at 50°C.