Growth Assay with E. coli

In a 96-well plate. As a benchmark for later co-culture experiments, the kinetics and toxicity of the cross-feeding metabolites on *E. coli* can be assessed.

Materials

- Overnight culture of *E. coli* in LB medium
- M9 minimal medium
- Desired additions to the medium (carbon source, toxic compound, etc.)
- PBS
- Mineral oil

Equipment

- Shaking incubator (37 °C)
- Plate reader with 37 °C incubation
- Spectrophotometer and cuvettes
- Microcentrifuge
- 96-well flat-bottom plate with lid (sterile)
- 1.5 mL Eppendorf tubes
- Single and multichannel pipettes and tips

Preparation

1. Dilute the overnight culture 1:100 into non-selective M9 medium.
2. Incubate overnight at 37 °C and 180 rpm, so the culture is fresh the next morning.
3. Transfer 1.5 mL of the culture to a sterile Eppendorf tube, spin for 3 min at 6800 × g, discard the supernatant and resuspend in 1.5 mL PBS. Repeat the wash three times.
4. Measure the OD_{600} of the washed cells and calculate the volume needed per well to reach the desired starting OD_{600} (typically 0.01–0.05).

Pipetting the 96-Well Plate

5. Pipette each condition to a total of 150 µL per well, at least in triplicate.
6. Cover each well with 50 µL mineral oil to prevent evaporation.
7. Note the plate layout before starting the run.

Plate Reader

8. Set the temperature to 37 °C.
9. Start a kinetic run of 24 h with readings every 15 min.
10. Set shaking to fast, double orbital, continuous.
11. Measure OD at 600 nm.