Bioreactor Protocol

Materials

- Chloramphenicol (34 ug/ml final concentration)
- Kanamycin (25 ug/ml final concentration)
- LB medium
- 1 M IPTG

Protocol

Pre-culture 1

Strains seeded into the bioreactor are always seeded from glycerol stock into 5-20 ml of preculture 1 (PC1) consisting of LB with corresponding antibiotics. PC1 is left shaking aerobically at 37°C and 180 rpm in an incubator.

Pre-culture 2

PC 1 is transferred into 400ml of LB with corresponding antibiotics, and left shaking aerobically at 37°C and 180rpm overnight or is induced.

Induction

Some experiments require the cells to be induced before being seeded into the bioreactor which is conducted as following: PC 2 is left to grow until $0.4 < OD_{600} < 0.8$. IPTG from a stock solution (1M) is added to a final concentration of 0.8 mM, and this pre-culture 2 (PC2) is left shaking aerobically at 30°C and 180 rpm overnight.

Centrifugation, resuspension and seeding

If PC1/2 is of $OD_{600} > 0.8$ it is transferred to two plastic centrifugation tubes (300ml) which are centrifuged at 4500 rpm for 10 min at 4°C. The supernatant is discarded and the cells are resuspended in 30ml M4 minimal medium.

M4 medium consists of the following amounts of chemicals per L:

- 0.221 g K_2HPO_4
- 0.099 g KH_2PO_4
- 0.168 g $NaHCO_3$
- 1.189 g NH_4SO_4
- 7.305 g NaCl
- 1.192 g HEPES
- 10 mL $CaCl_2$ stock solution
- 10 mL trace element solution (for contents, look at the protocol of trace element solution)

The $CaCl_2$ stock consisted of 7.13 g $CaCl_2 \cdot 2H_2O$ dissolved in 1 L deionized water[1]; in addition corresponding antibiotics (AB) and 40mM of carbon source (C-source) is added, depending on the experiment. 30ml of M4 containing cells is seeded into a sterile bioreactor which is subsequently filled up to a total volume of 430ml with M4. N_2 gas is connected to the sterilization filter of the gas inlet, and the potentiostat (brand: BAS cv27) wires are attached to the corresponding electrodes with alligator clamps. The first OD_{600} is measured and the potentiostat is turned on with according potential. The OD_{600} is monitored when possible at approximately 1 h time intervals.

[1] C.P. Goldbeck et al., Tuning promoter strengths for improved synthesis and function of electron conduits in E. coli *ACS Synth. Biol.* 2 (3), pp 150–159 (2013)