Anaerobic cultivation of *Escherichia coli*

- Hole anaerobic work takes place in a two-hand Glove Bag.
- All media and buffer has to be degased with nitrogen (N_2) gas via a sparger befor starting cultivation.
- Grow preculture under aerobic condition at 37°C.
- Cells were cultivated in a gas-tight 15 ml tube additional sealed with parafilm.
- Cultivation volume is about 10 mL. Fill cultivation tube with 8.5 ml steril degased cultivation medium.
- When preculture reaches OD_{600} of 0.6-0.8, take 1.5 ml into a steril tube.
- Centrifugate 1 min at 5,000 rpm and discard complete supernatant.
- resuspend pellet in 1.5 ml steril degased PBS buffer.
- Repeat previous step but resuspend in steril, degased cultivation medium. This two steps avoid residual oxygen in the inoculum.
- Inoculate culture with 1.5 ml washed and resuspended cells at OD_{600} of 0.1.
- Tubes are filled and opened for sampling only under nitrogen atmosphere.
- Tubes are incubated in a shaker at 37°C.
- At the beginning of anaerobic cultivation residual oxygen from preculture would be consumed. Therefore faster growth could be observed in the first period of cultivation until cells shift to anaerobic metabolism.
- There should be more frequent sampling at the beginning about 4 to 6-hour intervalls (two times) after inoculation.
- Regular sampling takes place at 1-day intervalls.
- Sampling volume is about 1 mL, using 500 µl for OD_{600} measurement and 500 µl for HPLC analysis.
- After sampling process tubes are overflowed with nitrogen gas, tightly closed and sealed with parafilm again.
- Tubes are further incubated in a shaker at 37°C.

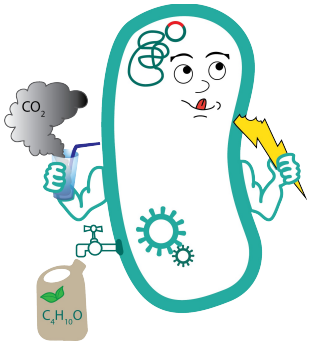

foto: sebastian kaulitzki