Take 1 colony and suspend in LB medium

Overnight culture

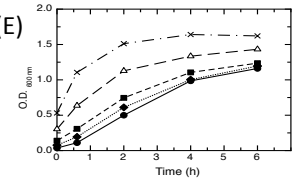
(A) Aerobic pre-culture

Centrifuge 1% v/v and suspend the pellet in 50 mL LB-glycerol

4 hours, 37 ºC

(B) Microaerobic inoculum

(E)



Centrifuge 4900 x g

Put the tubes inside the glove-box

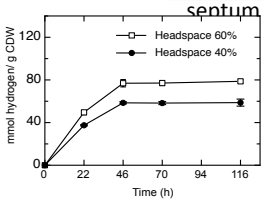
Sparged with argon up to <1 % oxygen

Decant the supernatant and suspend in glycerol based-medium

Inoculate 4.8 mL (60% headspace volume) in 6 mini reactors and seal with butyl rubber septum

(C) Anaerobic inoculum

(F)



Grow at 37 ºC and take samples at 22 and 46 h

(D) Anaerobic mini reactors

Measure H_2, ethanol and glycerol by using analytical techniques

HPLC
High **P**erformance
Liquid **C**hromatography

GC
Gas **C**hromatography