Take 1 colony and suspend in LB medium

Overnight culture

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

4 hours, 37 ºC

Centrifuge 4900 x g

Put the tubes inside the glove-box

Sparged with argon up to <1% oxygen

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

Decant the supernatant and suspend in glycerol based-medium

Measure \( \text{H}_2 \), ethanol and glycerol by using analytical techniques

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

(A) Aerobic pre-culture

(B) Microaerobic inoculum

(C) Anaerobic inoculum

(D) Anaerobic mini reactors

(E) O.D. 600 nm

(F) HPLC

High Performance Liquid Chromatography

GC

Gas Chromatography