

A disposable picoliter bioreactor for cultivation and investigation of industrially relevant bacteria on single cell level

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Supplementary Materials

Bacteria Strains

For proof of principle growth experiments, *E. coli* BL 21 and *C. glutamicum* ATCC130332 was used. For reactor comparison studies, *C. glutamicum* DM 1800 was used. For combined growth and L-arginine production studies, *C. glutamicum* wild type strain was transformed with plasmid pSenLysTKP-argB(fbr), containing a feedback resistant mutant of *argB* (coding for N-acetylglutamate kinase) and a metabolite sensor cassette, enabling EYFP expression in response to enhanced intracellular L-arginine concentration (Binder2012, in submission).

Media and Biological Sample Preparation

BHI medium (Becton-Dickinson/237500-Bacto Brain Heart Infusion) was used for *C. glutamicum* to prepare starter cultures for microfluidic experiments. *E. coli* BL 21 starter cultures were cultured in LB medium, containing 5 g yeast extract, 10 g peptone and 10 g NaCl per liter.

20 ml of sterile BHI medium (autoclaved and sterile filtered to prevent particles) were transferred into 100 ml culture flasks (Erlenmeyer shape, triple battled) and was inoculated with a single colony of *C. glutamicum* from BHI agar plates, containing no antibiotics. These cultures were incubated on an Incubator (Inforce) at 150 rpm at 30°C 10 hours. New culture using CGXII was started and cells were resuspended in new medium and transferred to the new culture starting optical density of 0.05 for adaption to the medium overnight. This step was done, that *C. glutamicum* could adapt to the new medium. The main culture was harvested in exponential phase (OD ~ 1-4) and washed with fresh sterile filtered medium prior to inoculation of the microfluidic system. For *E. coli* the same procedure was applied, using LB- medium for preculture. *E. coli* was directly transferred to chip after the first preculture.

Microfluidic experiments

For proof of principle experiments with *E. coli* BL 21 LB medium was used. The chip was flushed with Pluronic F-127 (Invitrogen) for 2 hours before culturing *E. coli*, to prevent undesired adhesion. No anti-adhesion additive was added to the medium. For microfluidic experiments with *C. glutamicum* ATCC130332 and DM 1800 CGXII medium³⁴ was used. The medium was adjusted to pH of 7.0 with sodium hydroxide. For artificial stationary phase CGXII medium without glucose was used. For microfluidic experiments with *C. glutamicum* the microfluidic chip was not coated before. Again, medium was not supplemented with any anti-adhesion detergence, to prevent unknown interaction between detergence and colony growth. No active biofilm control was necessary, since experiments

were finished before wall attachment and growth leads to formation of microcolonies that clog fluidic channels in average after 24-48 hours.

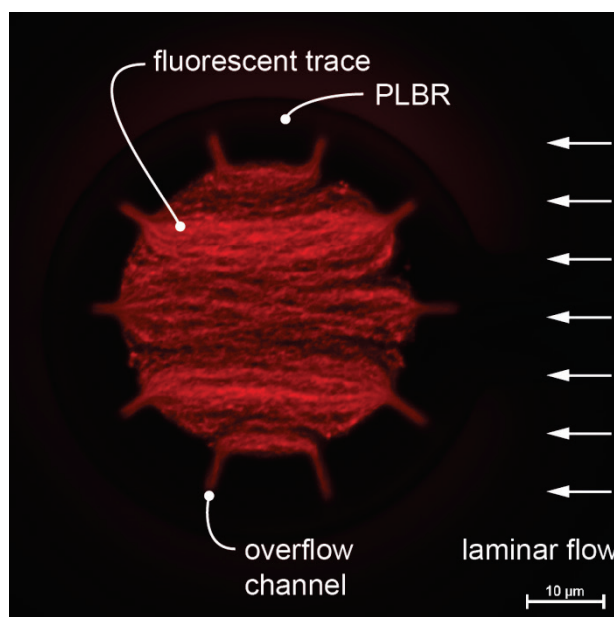


Figure S1 Image showing the flow pattern of fluorescently labeled latex beads with 200nm diameter through a single PLBR. The PLBR chip was coated with a 0.1% Pluoronic F68 solution for 60 minutes at a total flow-rate of 700 nl/min to minimize bead adhesion to the chip material. Red fluorescent latex beads (FluoSpheres® Carboxylate-Modified Microspheres, 0.2 μm, Red Fluorescent (580/605) 2% Solids, Molecular Probes) were diluted 100 times and flushed through the microfluidic device at 10 nl/min per PLBR channel each. Image exposure time was 10 s. Fluorescent bead trajectories clearly show flow through the PLBR device.

Lab-Scale Cultivation

Cultivation conditions

For cultivation of *C. glutamicum* DM 1800 the defined minimal medium CGXII³⁴ was used containing per liter of distilled water: 20 g (NH₄)₂SO₄, 1 g K₂HPO₄, 1 g KH₂PO₄, 5 g Urea, 10 g D-glucose, 13.25 mg CaCl₂*2H₂O, 0.25 g MgSO₄*7H₂O, 1 mg FeSO₄*7H₂O, 1 mg MnSO₄*H₂O, 0.02 mg NiCl₂*6H₂O, 0.313 mg CuSO₄*5H₂O, 1 mg ZnSO₄*7H₂O. The medium was adjusted to pH of 7.0 with sodium hydroxide. The medium contained additionally 3 ml of 10 % (v/v) AF 204 (Sigma) and 1 mL of a 0.2 g/L biotin stock solution per liter which were added after sterilization. Cryocultures of the two strains were stored at -80°C in CGXII medium containing 20 % (v/v) glycerol.

For batch-cultivation a 1.5 L bioreactor (DASGIP AG, Jülich) with a working volume of 1 L was prepared and inoculated directly with 2 mL of cryoculture. To increase reproducibility no pre-cultivation was performed. All cultivations were carried out at constant air flow (1 vvm) and 30°C. The pH was maintained at 7.0 by adding 4 M NaOH and 4 M HCl, respectively. Aerobic process conditions (dissolved oxygen > 30 %) were ensured via stirrer speed control (200-1200 rpm). During cultivation dissolved oxygen (Visiferm DO 225, Hamilton), pH (405-DPAS-SC-K80/225, Mettler Toledo) and exhaust gas concentrations of carbon dioxide and oxygen (GA4, DASGIP AG Jülich) were measured online.

Offline-Analysis

Cell number and size were monitored offline via coulter counter equipped with a 45 µm capillary (CASY® 1 Modell TT, Roche Diagnostics). For analysis a cell suspension volume of 2 ml was sucked into a 5 ml plastic syringe and dropped to withdrawal the dead volume of the sample port. After that 1 ml was sucked into a fresh 2 ml plastic syringe. The cell suspension was diluted with isotonic dilution liquid (CASY®ton, Roche Diagnostics) into the detection range of the coulter counter and measured as triplicates.

Supplemental Videos

- Video S1** Time lapse movie showing the cultivation of *E. coli* BL21 inside a PLBR. A single *E. coli* cell was seeded into the PLBR and complex LB growth medium was infused. After 2.5 hours of cultivation at T=37°C a microcolony of app. 30 cells was formed. After 3.5 hours of cultivation the overflow phase was reached. Cells were pushed continuously out of the PLBR maintaining a constant density. The overflow channels have different lengths due to a slight misalignment during the two layer photolithography process. The functionality was not affected by this misalignment.
- Video S2** Time-lapse movie showing the growth and production of *C. glutamicum* pSenLysTKP-argB(fbr) during PLBR cultivation. The strain contains a metabolite sensor enabling EYFP expression in response to enhanced intracellular L-arginine concentration. The seeded mother cell starts to emit fluorescence after 12 hours indicating production of L-arginine. While undergoing a change to maximum cell growth EYFP emission declined.