Supplementary Material (ESI) for Lab on a Chip

ON-LINE SUPPORTING INFORMATION

Continuous Two-Phase Flow Miniaturised Bioreactor For Monitoring Anaerobic Biocatalysis By Pentaerythritol Tetranitrate Reductase

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List of contents:

1. Phase separation

Here we present four videos showing the dispersion of aqueous droplets in an organic carrier fluid with subsequent phase separation. Droplets are charged at the aqueous inlet and collected in an aqueous column (earthed). The voltage at the inlet electrode is set between 200 and 400 V. The importance of equal rate of in- and outflow of the aqueous phase is shown.

Supporting Information video 1. (phase_sep1.mpg): Continuous dispersion and collection of an aqueous phase in a microchannel. The droplets have volumes of approximately 2 nL. The flows in and out of the chip are controlled by gravity by means of open syringes.

Supporting Information video 2. (phase_sep2.avi): Dispersed aqueous droplets are merging continuously into an aqueous column. The droplets have volumes of approximately 2 nL.

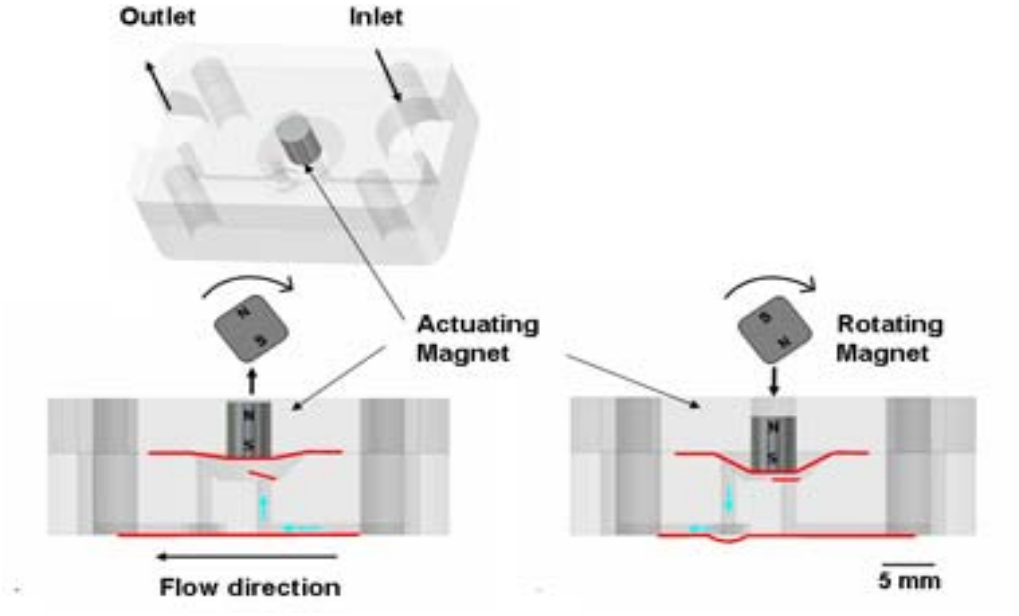
Supporting Information video 3. (selective_inject.avi): Selective collection of charged aqueous droplets by displacement of the aqueous column. The droplets have volumes of approximately 1 nL.

Supporting Information video 4. (phase_sep_pump.avi): Dispersed droplets merging back into the aqueous phase through electrostatic attraction using miniaturized membrane pumps. The volume of the droplets is approximately 20 nL. Videos are recorded with 25 frames per second.

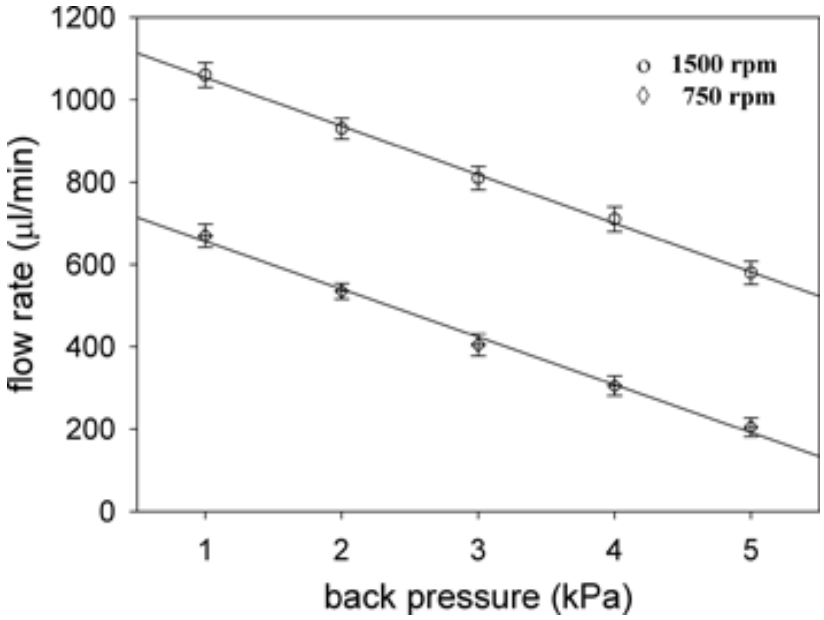
2. Micropumps for recirculation

The working membrane and the check valves are made of 25 μm thick fluorinated ethylene propylene (FEP) films with treated surfaces to improve bonding with conventional adhesives(1). We use UV curable epoxy (Norland 68, Norland Products Inc., New Brunswick, New Jersey, USA) to attach the membrane to a neodynium rod magnet and the check valves to the pump body.

The pump body is micro-CNC machined from polycarbonate. Work is in progress to manufacture the pump bodies by injection moulding.



Supporting Information Figure 1: Schematic view of the FEP membrane pumps comprising two Neodynium magnets for driving and actuation of the membrane. The FEP film is marked red and shown are the two pump operation (left: intake, right; outflow).



Supporting Information Figure 2: Membrane-pump characteristics for water for different rotational speeds of the driving magnet.

Error in plot: Changed to RPM

3. Details of microreactor fabrication

Details of chip fabrication: The microreactor was fabricated from 4 mm thick Polycarbonate sheet (RS Components Ltd, Corby, UK) cut into 75 by 75 mm square chips. 3D flow pattern were drawn in AUTODESK INVENTOR (Autodesk Inc., San Rafael, California, USA) and translated into CNC machine code by EdgeCam software (EdgeCam, Reading, UK). The chips were machined using a CAT3D M6 Computer Numerical Controlled (CNC) milling machine (Datron Technologies Ltd, Milton Keynes, UK) with 0.1 - 0.5 mm tungsten-carbide milling tools (Toolex Ltd, Trowbridge, UK).

FEP film bonding :

A good bond of the FEP film with the chip surface was crucial to the operation of the bioreactor. Firstly UV-curable epoxy (Norland 68, Norland Products Inc., New Brunswick, NJ, USA) was spread between two 100 μm thick acetate sheets with the help of a hot-roll laminator. After separating the sheets, a several micron thin layer of epoxy remained on the sheets which was subsequently transferred to the chip surface, this was followed by the attachment of the FEP film; both procedures were carried out with the aid of the laminator.

4. Calculation of pressure drop in the main channel

We have calculated the pressure drop in two-phase liquid-liquid flow in rectangular micro-channels using the formula developed by Jousse et al. (2):

In general, the pressure drop Δp can be described as:

$$\Delta p = rq$$

With r the hydrodynamic resistance and q the flow rate. In liquid-liquid flows of droplets larger than that of the channel and for complete dewetting of the dispersed phase from the channel walls the effective flow resistance can be calculated as:

$$r_{eff} = 12.8\frac{l}{b^3 w}\mu(\lambda\phi + 1 - \phi) + n_d \times 3.163 g(\lambda)\frac{\gamma}{b}\frac{Ca^{2/3}}{q}$$

With:

l: channel length: 0.12m
b: channel height: 0.0002m
w: channel width: 0.0003m
μ: viscosity continuous phase: 0.5mPas
Φ: volume fraction of dispersed phase: ~0.33
γ: surface tension: (isooctane/water) : 50.13 mN/m (3)
q: flow rate: 50 µL/min
λ: ratio of disperse to continues phase viscosity: 2
$g(\lambda)$: depends on viscosity ratio ~1 (ref)
n_d: number of droplets in channel: ~100
u: average speed of flow: ~0.008m/sec
$Ca = \mu u/\gamma$: capillary number: 0.00008
Prefactor 3.163 for rectangular channel with aspect ratio 1.5 (4)

We have calculated the pressure drop in the main channel for a typical experiment to be around **600** Pascal.

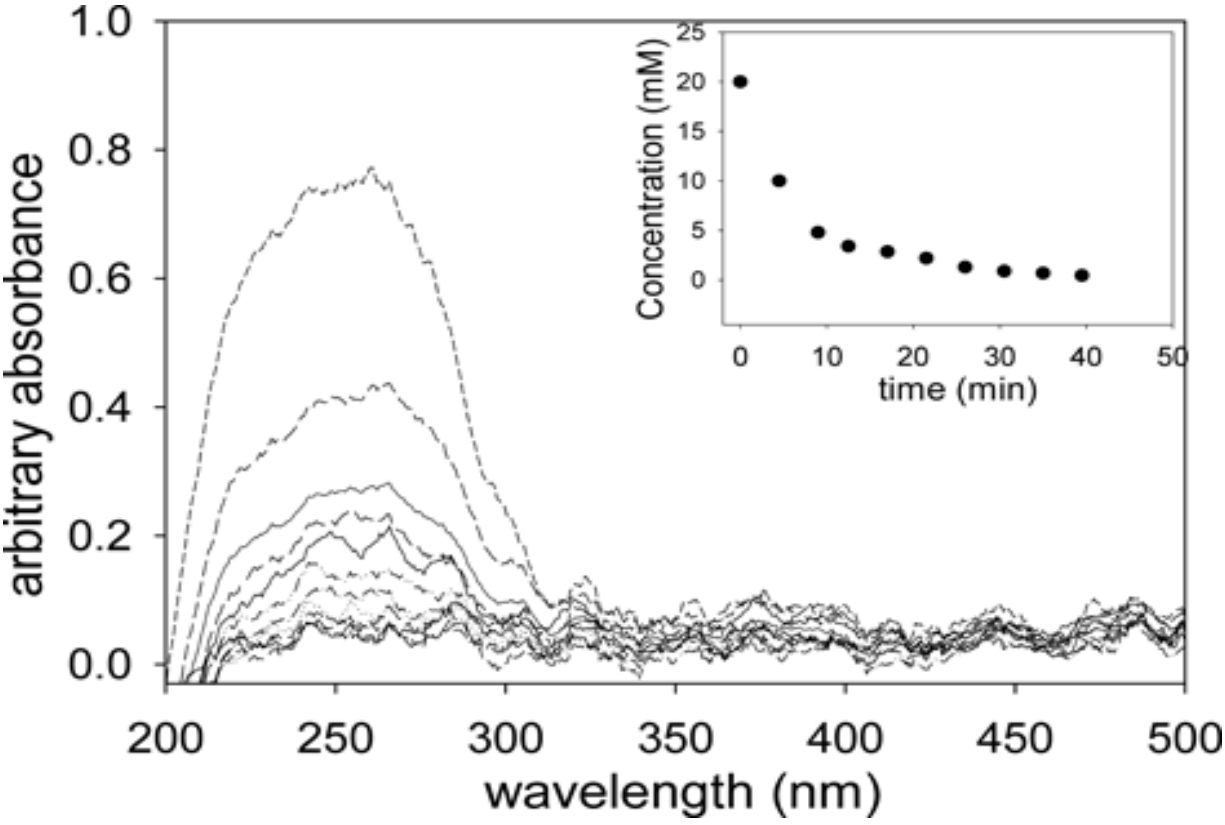
5. Surface to volume ratios in bioreactor

Figure SI 3. shows frames from videos taken of aquoues slugs in isooctane with different flow rates of the organic phase. Flow speeds and slug/droplets sizes were analysed from the videos using an image analysis program.
We have measured the relationship between the surface to volume ratio for varying flow rates of the organic phase with the flow rate of the aqueous phase kept constant. Two different flow rates for the aqueous phase have been chosen. We noticed that the most stable flow regime at a flow speed between 30 and 60 µL/min for the organic phase and between 10 and 20 µL/min for the aqueous flow rates.

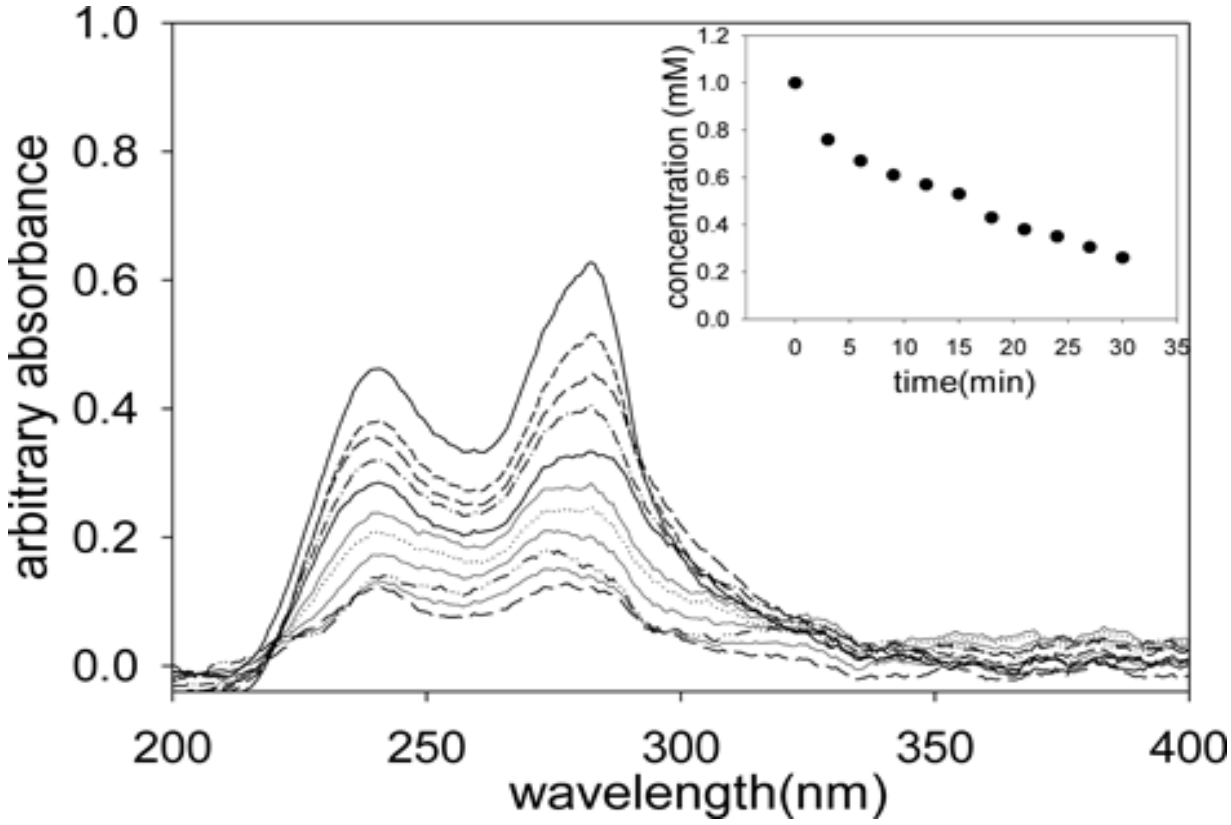
Supporting Information Figure 3: Frames taken from videos showing different flow rates of the organic phase with the aqueous flow rate kept at 18µL/min. The flow rates for the organic phase are: left: 27 µL/min, middle: 52 µL/min , right: 61 µL/min.

6. Spectral changes obtained during substrate reduction

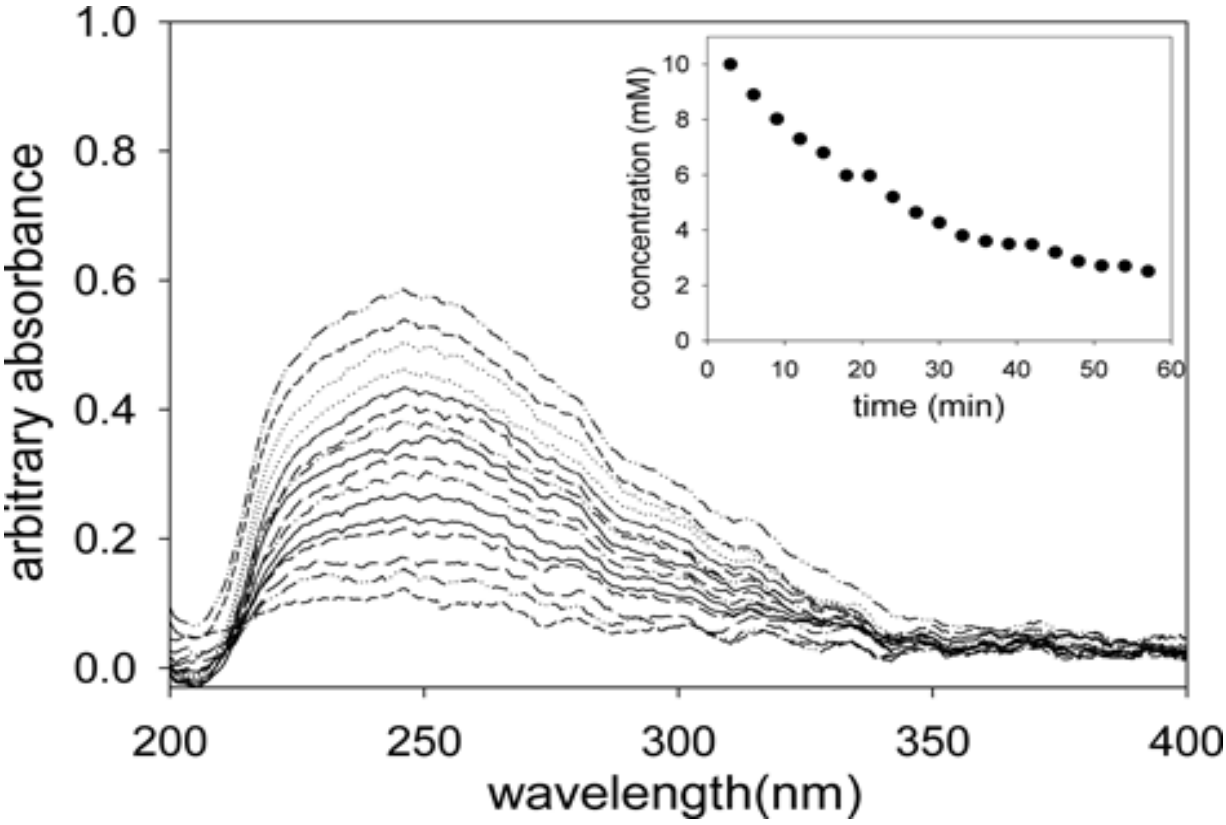
To highlight the applicability of PETN reductase as a generic biocatalyst in the recirculating bioreactor, we also tested the substrates ketioisophorone, trans-cinnamaldehyde and N-phenyl 2-methyl maleimide. These substrates were dissolved in anaerobic isooctane and the spectral changes observed during reduction are shown below.



Supporting Information Figure 4a: Spectral changes observed during the reduction of 20 mM ketoisophorone by 2 μM PETN reductase, measured with the on-chip spectroscopic cell for the organic phase. **Inset**: Concentration of ketoisophorone remaining as a function of time taken at λ=246nm.



Supporting Information Figure 4b: Spectral changes occurring during the reduction of 1 mM trans-cinnamaldehyde by 2 μM PETN reductase measured with the on-chip spectroscopic cell for the organic phase. **Inset:** Concentration of *trans*-cinnamaldehyde remaining at selected times during the reduction taken at λ=292nm.



Supporting Information Figure 4c: Changes in the spectrum of 10 mM N-phenyl 2-methyl maleimide during it's reduction by 2 μM PETN reductase, measured with the on-chip spectroscopic cell for the organic phase. Start concentration was 10mM. **Inset:** Time course of the reduction of N-phenyl 2-methyl maleimide by PETN reductase taken at λ=254nm.

References

1. Further information on FEP films: (http://www2.dupont.com/Teflon_Industrial/en_US/assets/downloads/h55007.pdf)
2. F. Jousse, R. Farr, D.R. Link, M.J. Fuerstman, P. Garstecki, Phys.Rev.E , 2006, **74**, 036311-1 – 036311-6
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