Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting information

Microbial factories: monitoring vitamin B_2 production by *Escherichia coli* in microfluidic cultivation chambers

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Supporting figures

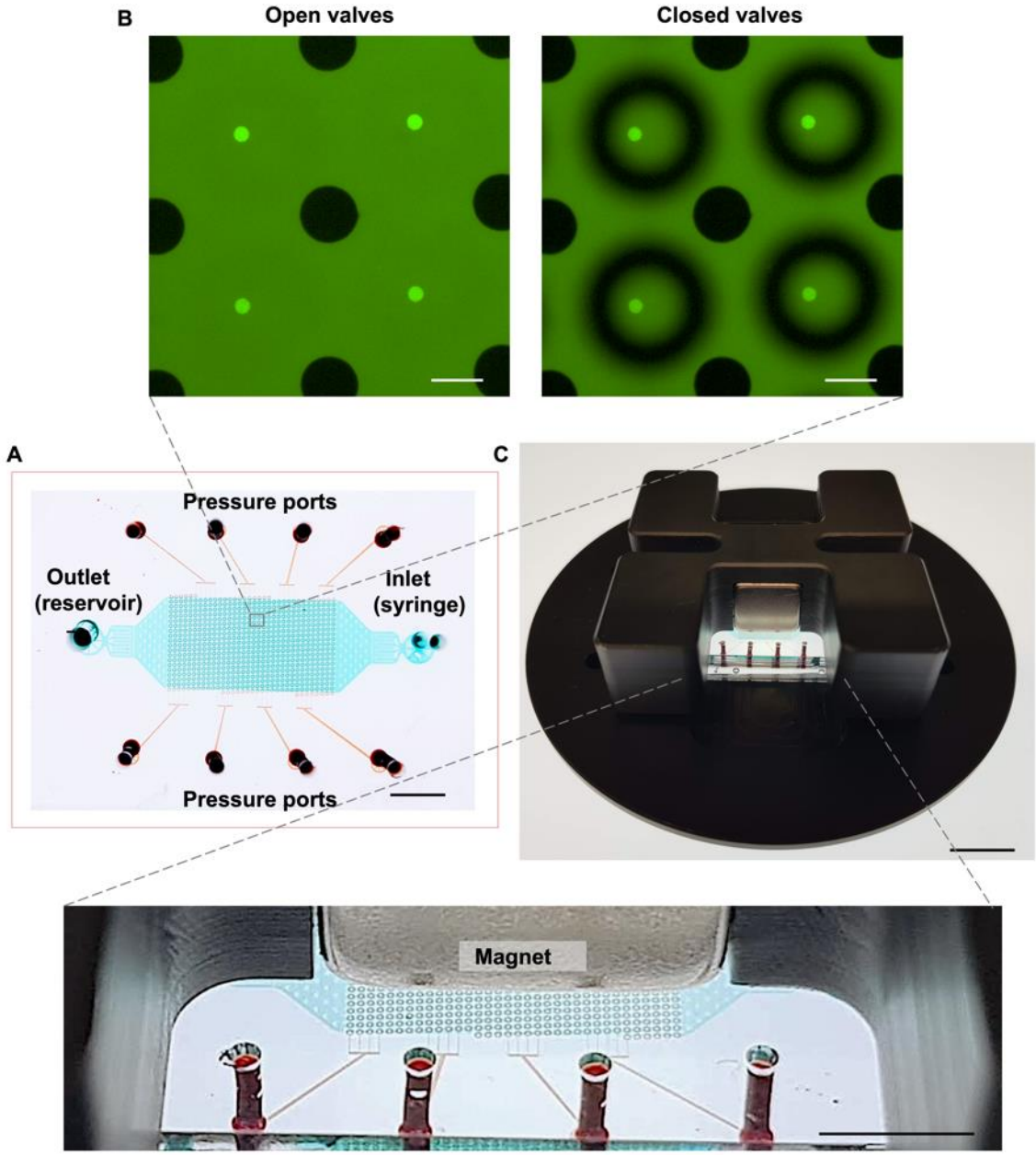

Figure S1. Microfluidic system, design and operation.
A: The microfluidic device is composed of two channel networks: the top part contains pressure actuated microfluidic valves (red) and the bottom part contains inlet and outlet for the bacteria and reagent supply (blue). Scale bar: 5 mm. B: The individual growth chambers are formed by application of pressure on the microfluidic valves. For better visualisation, the bottom channel contains auto-fluorescent riboflavin. Scale bar: 100 µm.
C: Magnetic holder to facilitate inoculation of the microfluidic system and microscopy. The holder allows to maintain the magnetic field above to growth chambers during the medium or reagent exchange.
Scale bar: 2.5 cm and 1 cm respectively.

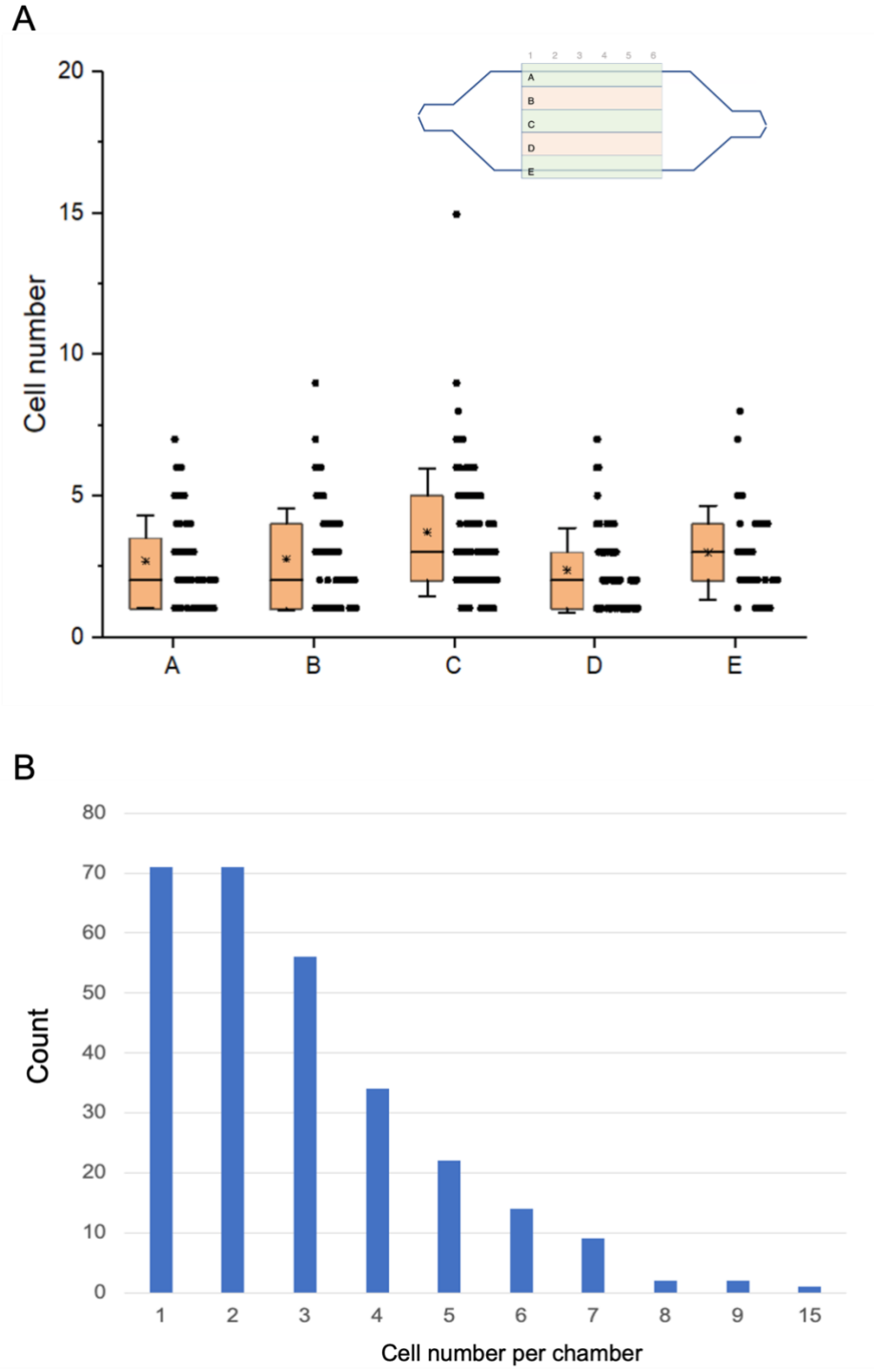

Figure S2. Distribution of the bacterial cells in the inoculated chambers. A: Distribution divided in the sectors based on the microscopic images. The box represents percentiles (25,75), whiskers: standard deviations, horizontal lines: medians and stars: the mean values. The mean cell number for all the sectors was 2.9 cells. Scheme in the corner shows distribution of the individual sectors over the chip area. B: Discrete frequency of the chamber inoculation by 1 to 15 cells (total number of chambers n=282). The data show that the relative frequency of the chamber inoculated by 1 to 5 cells is 90%, while only 1% of the chambers contains 9 or more cells.

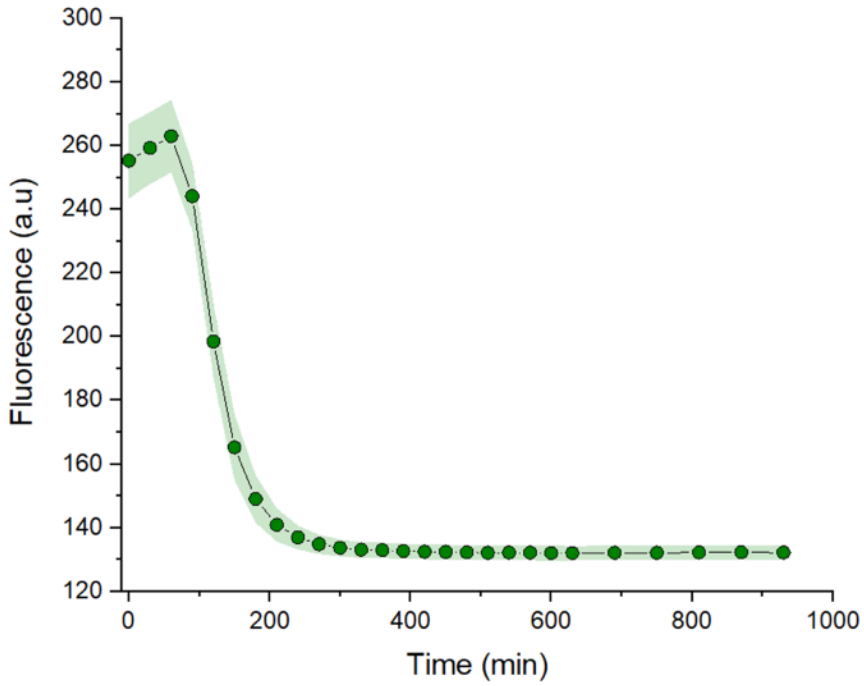

Figure S3. Exchange of fluids in the chamber demonstrated by replacing riboflavin (200 µM) with the non-fluorescent buffer. The pressure 2.2 bar is applied on the valves and buffer flow rate is 0.2 µL min^{-1}, identical to the experimental conditions. Initially, the chip is perfused with riboflavin. Here, exchange of the solutions starts after one hour. Most of the riboflavin is replaced within 2 hours, to achieve complete exchange, two additional hours are required. n = 64, plotted with the standard deviation.

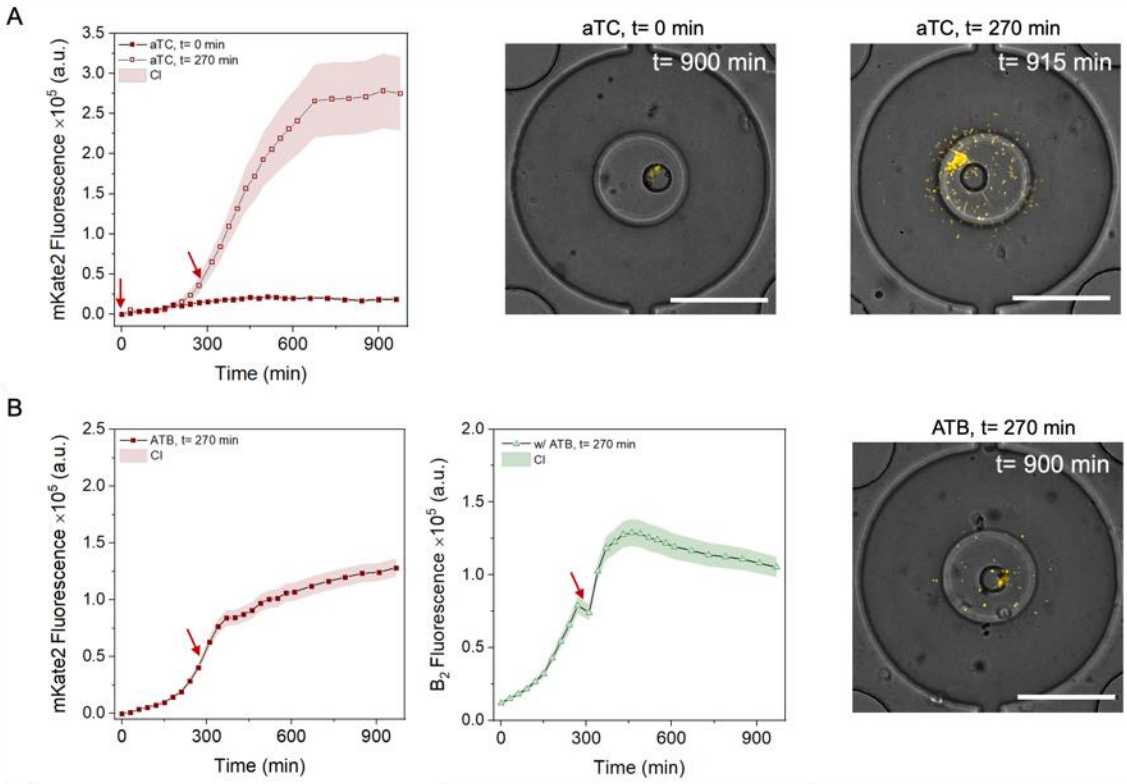

Figure S4. Production of mKate2 protein by *E. coli* BW23474 [pB2-*ribDBECA*-*mkate2*] under different conditions. A: Time-resolved changes of the bacterial growth estimated by the mKate2 production (right panel) with the inducer supplement at two different time points after inoculation (t= 0 min; n=131 and beginning of the exponential phase, after t=270 min; n=72 as indicated by the red arrows. The micrographs show bacterial culture for the two conditions at the end of the cultivation period with the decreased bacterial growth for the cultures where inducer was supplied directly after inoculation. Scale bar: 100 µm.
B: Time-resolved, mean mKate2 (left panel) and riboflavin (right panel) fluorescence measurements for bacterial cultures supplemented with the antibiotic at the time point 270 min (n=196). The growth (mKate2 production) is decreased approximately one hour after the addition of the antibiotic. This is also visible for the riboflavin biosynthesis; the production rate decreases after one hour and after two hours riboflavin starts to be slowly washed out of the chambers. The micrograph show the bacterial cells at the end of the cultivation period. Scale bar: 100 µm. CI: 95% confidence interval.

Table S1. Occupancy of chambers in %. The microfluidic device is separated in the 30 sectors, based on the positions where the microscopic images are taken. The table lists the occupancy (chambers containing bacteria as a percent of a total number of the chambers) of the individual sectors. The gradient fill shows that most of the cells are trapped in the entrance for the cell/beads suspension and the middle part of the device.

	1	2	3	4	5	6
A	38	25	58	88	50	92
B	69	67	36	69	75	100
C	75	75	64	94	100	83
D	50	56	75	88	94	92
E	75	58	38	50	38	100

Table S2. Details of the recombinant *E. coli* strain BW23474; Δ(*argF-lac*)169 Δ*uidA4*::pir-116 *recA1 rpoS396*(Am) *endA9*(del-ins)::FRT *rph-1 hsdR514 rob-1 creC510*; [1] with the details of the used plasmids.

Variant	Plasmid	Features	Reference
(i)	pB2-*ribDBECA-mkate2*	Expression of the genes for the riboflavin biosynthesis enzymes and the gene encoding for the red fluorescent protein mKate2 from the synthetic operon *ribDBECA-mKate2* under control of the aTC inducible P_{tet} promoter. Kanamycin resistance.	[2]
(ii)	pB2-*ribDBECA*	Expression of the genes for the riboflavin biosynthesis enzymes from the synthetic operon *ribDBECA* under control of the aTC inducible P_{tet} promoter." Kanamycin resistance.	[2]
	pKD13-*mkate2*	Constitutive expression of the gene for the red fluorescent protein mKate2 under control of the constitutive promoter BBa_J23100. Ampicillin resistance.	[3]
(iii)	pB2-*ribDBECA*$_{PL}$	As pB2-*ribDBECA*, but without the promoter. Decreased riboflavin production.	[2]
	pKD13-*mkate2*	As for variant (ii).	
(iv)	pB2-*ribDBECA*	As for the variant (ii)	
(v)	pB2-*ribDBECA*$_{PL}$	As for the variant (iii)	
(vi)	pB2-empty	No riboflavin overexpression. Kanamycin resistance.	[2]

REFERENCES

[1] Haldimann, A.; Prahalad, M. K.; Fisher, S. L.; Kim, S. K.; Walsh, C. T.; Wanner, B. L. Altered Recognition Mutants of the Response Regulator PhoB: A New Genetic Strategy for Studying Protein-Protein Interactions. *Proc. Natl. Acad. Sci. U. S. A.* 1996, 93 (25), 14361–14366.

[2] Schmitt, S.; Walser, M.; Rehmann, M.; Oesterle, S.; Panke, S.; Held, M. Archimedes' Principle for Characterisation of Recombinant Whole Cell Biocatalysts. *Sci. Rep.* 2018, 8 (1), 1–9.

[3] Anderson JC. Anderson promoter collection; Retrieved July 8, 2021; http://parts.igem.org/Promoters/Catalog/Anderson