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Data on mixed trophies biofilm for continuous cyclohexane oxidation to cyclohexanol using *Synechocystis* sp. PCC 6803



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ABSTRACT

Photosynthetic microorganisms offer promising perspectives for the sustainable production of value-added compounds. Nevertheless, the cultivation of phototrophic organisms to high cell densities (HCDs) is hampered by limited reactor concepts. Co-cultivation of the photoautotrophic *Synechocystis* sp. PCC 6803 and the chemoheterotrophic *P. taiwanensis* VLB 120 enabled HCDs up to 51.8 g_{CDW} L⁻¹. Respective biofilms have been grown as a biofilm in capillary flow-reactors, and oxygen evolution, total biomass, as well as the ratio of the two strains, have been followed under various cultivation conditions. Furthermore, biofilm formation on a microscopic level was analyzed via confocal laser scanning microscopy using a custom made flow-cell setup. The concept of mixed trophies co-cultivation was coupled to biotransformation, namely the oxyfunctionalization of cyclohexane to cyclohexanol. For benchmarking, the performance of the phototrophic reaction was compared to the chemical process, and to a biotechnological approach using a heterotrophic organism only. The data presented refer to our research paper "Mixed-species biofilms for high-cell-density application of *Synechocystis* sp. PCC 6803 in capillary reactors for continuous cyclohexane oxidation to cyclohexanol" Hoschek et al., 2019.

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Specifications table

Subject area	Biotechnology
More specific subject area	Phototrophic mixed trophies biofilm cultivation and biotransformation
Type of data	Table, text file, graph
How data was acquired	Gas chromatography, dissolved oxygen measurement via oxygen microsensor, surface calculation of 3D reconstructed images acquired by confocal laser scanning microscopy
Data format	<i>analyzed</i>
Experimental factors	The biofilm was cultivated in a custom made flow-cell developed for <i>in-vivo</i> confocal microscopy. For the dissolved oxygen determination in the liquid phase of the capillary reactors, microsensors were used. Whereas, oxygen in the gas phase was quantified via gas chromatography. For biomass analysis, the total biomass was mechanically removed from the capillary, re-suspended, and taken directly for coulter counter measurements and cell dry weight determination. To follow cyclohexane conversion, the substrate and the product were extracted from the aqueous phase with diethyl ether and quantified directly by gas chromatography.
Experimental features	The biofilm was either cultivated in the custom made flow-cell or in the capillary flow-reactor
Data source location	<i>Leipzig, Germany</i>
Data accessibility	All data are available in this document and in the Mendeley data sets (https://doi.org/10.17632/vgx2hgxsc4.1).
Related research article	"Mixed-species biofilms for high-cell-density application of <i>Synechocystis</i> sp. PCC 6803 in capillary reactors for continuous cyclohexane oxidation to cyclohexanol", <i>Bioresource Technology</i> , 282, (2019), 171–178 [1].

Value of the data

- The biofilm growth and development in a mixed-trophies format was investigated to gain insight on the role of individual species in biofilm formation.
- The recorded data of oxygen produced per biomass and the ratio between different species are valuable to understand and optimize high cell density culture of phototrophic organisms.
- The applicability of the bioreactor concept was demonstrated by continuous biotransformation of volatile and toxic substrates.
- For benchmarking, the performance of the mixed-trophies biofilm was compared to the chemical process, and to a biotechnological approach using a heterotrophic organism only

1. Data

This dataset contains information on strain development, biofilm cultivation devices, and imaging techniques, as well as analysis tools for characterizing productive biofilms converting cyclohexane to cyclohexanol. Bacterial strains and plasmids used for biocatalyst development are listed in Table 1, and their genetic features are briefly described. The schematic representation of the cultivation system developed for biofilm imaging using a confocal laser scanning microscope (CLSM) is given in Fig. 1. The central cultivation device is a flow cell made of stainless steel with the dimensions 65 mm × 4.5 mm fitting beneath the microscope. The respective volumina of the biological specimen recorded using CLSM were calculated after 3D reconstruction from the acquired images using Imaris 8.2.0 [1] and are presented in Table 2.

In Fig. 2, a schematic representation of the biofilm reactor system developed for the transformation of cyclohexane to cyclohexanol using mixed trophies biofilms comprising photoautotrophic and chemoheterotrophic organisms is shown. Biofilms are cultivated in capillaries with the dimensions 20 cm × 0.3 cm. Performance parameters like oxygen concentration, citrate consumption, and biofilm dry weight are summarized in Table 3, while average cyclohexanol production rates in light and dark conditions are given in Fig. 3. Different process concepts for cyclohexanol production are compared in Table 4.

Table 1

Strains, plasmids, and primers used in this study.

Strain	Description	Reference
<i>E. coli</i> DH5 α	F ⁻ Φ 80lacZ Δ M15 Δ (lacZYA-argF) U169 recA1 endA1 hsdR17 (rK ⁻ , mK ⁺) phoA supE 44 λ B ⁻ thi ⁻¹ gyrA96 relA1	[2]
<i>Synechocystis</i> sp. PCC 6803	Geographical origin: California, USA; Received from Pasteur Culture Collection of Cyanobacteria (PCC, Paris, France)	[3]
<i>Pseudomonas taiwanensis</i> VLB120	Wild-type <i>Pseudomonas</i> ; styrene prototroph	[4]
<i>Pseudomonas taiwanensis</i> VLB120_egfp	<i>P. taiwanensis</i> VLB120 harboring a chromosomally integrated <i>egfp</i> (enhanced green fluorescent protein) gene	This study
Plasmid	Description	Reference
pAH032	pPMQAK1 based, RSF ori, Kanamycin resistance, empty P _{trc10} expression system	[5]
pAH050	Based on pAH032; CYP, FnR, and Fn genes under control of P _{trc10} promoter system, RBS* optimized for <i>Synechocystis</i> sp. PCC 6803 in front of CYP gene	[6]

2. Experimental design, materials, and methods

Bacterial strains and plasmids used in this study are listed in Table 1. Additionally, the composition of YBG11 media used in this study is given.

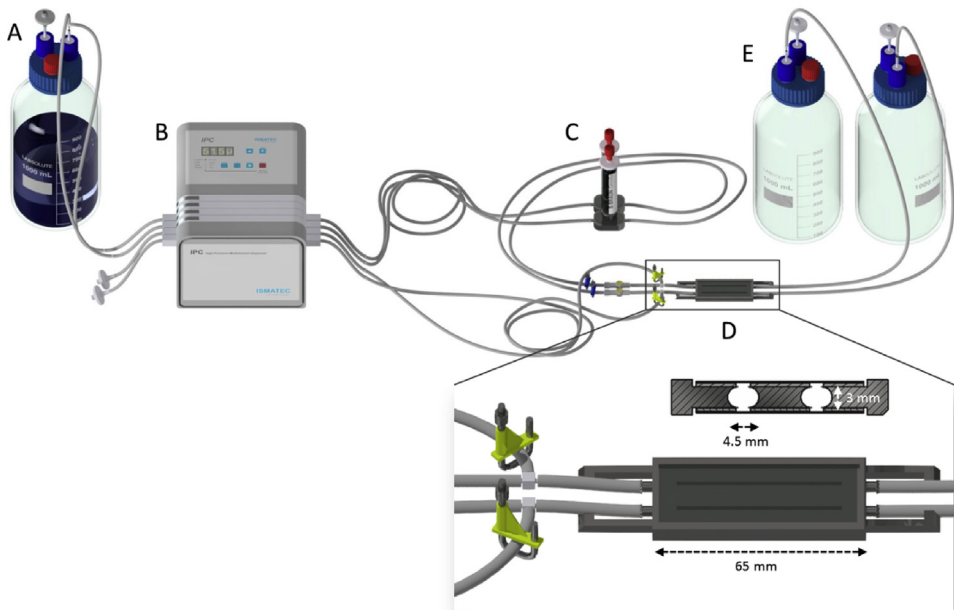


Fig. 1. Flow cell setup for biofilm analysis using CLSM. Medium reservoir (A), a multichannel peristaltic pump (B, IPC 4 peristaltic pump, Ismatec), bubble trap (C), flow-cell (D), and waste bottles (E) with the respective tubing. An enlarged view of the flow cell (D), as well as the cross-section, is shown. The body of the flow cell is made of stainless steel, whereas the top, and bottom parts are made of glass to enable microscopic images.

Table 2

Volumina of *P. taiwanensis* VLB 120_egfp and *Synechocystis* sp. PCC 6803 (pAH050) has been calculated from the CLSM images presented in [1].

	<i>P. taiwanensis</i> VLB120_egfp [μm^3]	<i>Synechocystis</i> sp. PCC 6803 (pAH050) [μm^3]	Volume ratio (<i>Ps</i> / <i>Syn</i>)
Inoculation	5.9×10^3	1.6×10^4	0.37
1 day after medium flow	1.5×10^4	4.4×10^4	0.357
3 days	1.1×10^4	5.4×10^4	0.217
11 days	2.4×10^3	1.0×10^5	0.0239
25 days	3.1×10^3	1.1×10^5	0.0284

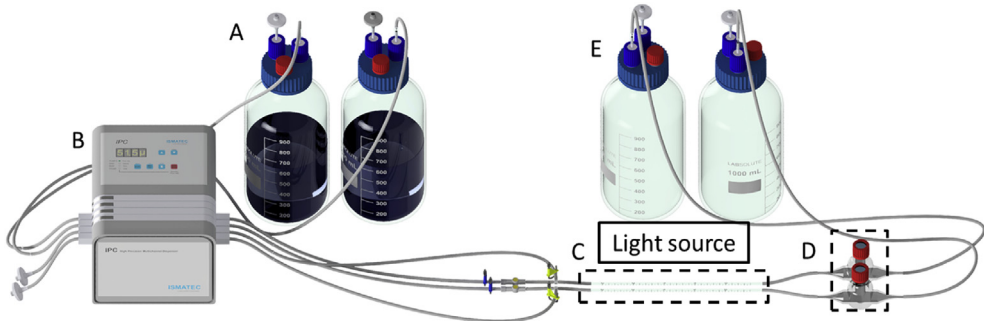


Fig. 2. Set-up of the biofilm capillary reactor system. The figure shows the medium reservoir (A), a multichannel peristaltic pump (B), peristaltic pump from Ismatec, capillary reactors, dimensions: 20 cm \times 0.3 cm (C) with the light source on the top, (D) bubble traps for gas phase sampling and (E) waste bottles.

Table 3

Biofilm cultivation parameters for single- and dual-species capillary reactors. i) and ii) depict monoseptic biofilm cultures of *Synechocystis* sp. PCC 6803 (pAH032) without (-Air) and with air segments (+Air), respectively. Dual species biofilm cultures of *Synechocystis* sp. PCC 6803 (pAH032) and *P. taiwanensis* VLB120 (pAH032) were inoculated at a ratio of 1:1 and operated iii) without and iv) with air segments. v) and vi) correspond to iii) and iv) with citrate in the aqueous medium feed. The aqueous medium was fed at a rate of 52 $\mu\text{L min}^{-1}$. For segmented flow, a gaseous air phase was additionally fed at the same rate.

Experimental setup	O ₂ in aq. Phase ^a (μM)	Citrate Consumption (g L^{-1})	Biofilm dry weight ^b (g L^{-1})		
			Ps	Syn	total
i - Air	746	—	—	1.5	1.5
ii + Air	284	—	—	13.7	13.7
iii - Air	923	—	0.1	5.8	5.9
iv + Air	287	—	0.2	31.4	31.6
v - Air	~0	0.27	7.2	40.6	47.8
vi + Air	194	0.39	1.5	17.3	18.8

^a Solubility of O₂ at 26 °C and a salinity of 3.5 g kg^{-1} : ~250 μM (21% O₂) and ~1190 μM (100% O₂); based on the respective partitioning, aqueous phase O₂ concentrations given for experiments performed with air segments are calculated from O₂ concentrations measured in the gas phase.

^b The biofilm dry weight is calculated based on 1.2 mL tube volume. *Synechocystis* sp. PCC 6803 (pAH032) and *P. taiwanensis* VLB120 (pAH032) specific biofilm dry weights are calculated based on cell numbers and cell volumes and the respective total biofilm dry weight, assuming that both strains constitute equal biovolume to biofilm dry weight ratio.

Composition of YBG11 (50 mM NaHCO₃, without citrate): 1.49 g L^{-1} NaNO₃, 0.074 g L^{-1} MgSO₄ 7H₂O, 0.0305 g L^{-1} K₂HPO₄, 10 mL L⁻¹ YBG11 trace elements (100x), 0.019 g L^{-1} Na₂CO₃, 50 mM HEPES (pH 7.2); YBG11 trace elements (100x): 3.6 g L^{-1} CaCl₂ 2H₂O, 0.28 g L^{-1} boric acid, 0.11 g L^{-1} MnCl₂ 4H₂O, 0.02 g L^{-1} ZnSO₄ 7H₂O, 0.039 g L^{-1} Na₂MoO₄ 2H₂O, 0.007 g L^{-1} CuSO₄ 5H₂O, 0.005 g L^{-1} Co(NO₃)₂ 6H₂O, 0.162 g L^{-1} FeCl₃ 6H₂O, 0.6 g L^{-1} Na₂EDTA 2H₂O, 4.2 g L^{-1} NaHCO₃ [7].

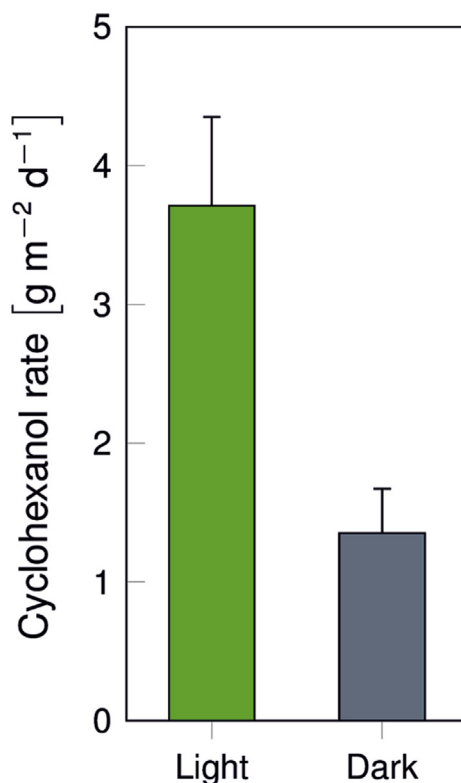


Fig. 3. The average cyclohexanol production rate in $\text{g}_{\text{CHXOL}} \text{m}^{-2} \text{d}^{-1}$ utilizing *Synechocystis* sp. PCC 6803 (pAH050) and *P. taiwanensis* VLB 120 (pAH050) as a dual-species mixed-trophies biofilm under light and dark conditions. Experiments were conducted at $50 \mu\text{E m}^{-2} \text{s}^{-1}$ providing organic carbon free YBG11 medium and air segments at a flow rate of $52 \mu\text{L min}^{-1}$. Green and grey bars represent product formation under light and dark conditions, respectively. CHXOL = cyclohexanol.

Table 4

Comparison of key parameters of different reaction concepts for cyclohexanol production.

Parameters	Conventional process ^a	Heterotrophic bioprocess ^b	Phototrophic bioprocess ^c
Reaction temperature (K)	413–453	303	295
Pressure (atm)	7–20	1	1
Residence time (min)	7–20	5–16	13
Cyclohexane conversion (%)	ca. 6	NA	98.9
Combinatorial selectivity (%)	80–90	NA	100
Space-time-yield ($\text{g L}^{-1} \text{h}^{-1}$)	ca. 25	ca. 0.4 ^d	ca. 0.2 ^e

NA: not available.

Combinatorial selectivity refers to the formation of cyclohexanol and cyclohexanone.

^a Data from reference [8].

^b Data from reference [9].

^c This study.

^d In complex medium (LB media).

^e In minimal medium (YBG11 media).

2.1. Monitoring biofilm growth in a flow-cell by confocal laser scanning microscopy

The development of a mixed trophies biofilm consisting of *P. taiwanensis* VLB 120_egfp and *Synechocystis* sp. PCC 6803 (pAH050) was analyzed by confocal laser scanning microscopy (CLSM). The

schematic representation of the experimental set-up is given in Fig. 1. The respective volumina of the biological specimen were calculated after 3D reconstruction from the acquired images using Imaris 8.2.0 [1] and are presented in Table 2. The eGFP signal of *Pseudomonas* sp. as well as the autofluorescence of *Synechocystis* sp. was recorded individually so that the volume could be calculated for each channel individually. The total volume of the biofilm equals the sum of the volume occupied by each species. *Ps* = *P. taiwanensis* VLB120_egfp, *Syn* = *Synechocystis* sp. PCC 6803 (pAH050). For the volume ratio of *Ps/Syn*, the calculated volume of *Pseudomonas* sp. was divided by the volume of *Synechocystis* sp. PCC 6803.

2.2. Biofilm cultivation in capillary reactors

Biofilms were cultivated as mixed and single species biofilms of *Synechocystis* sp. PCC 6803 and *P. taiwanensis* VLB120 both containing the plasmid pAH032. The schematic representation of the experimental set-up is given in Fig. 2.

The amount of oxygen produced as well as the biofilm dry weight and composition regarding bacterial species was determined for each cultivation condition (Table 3). For further cultivation details, please refer to [1].

2.3. Biotransformation of cyclohexane to cyclohexanol in capillary reactors

After 36 days of cultivation, the biotransformation was initiated for a mixed species biofilm of *Synechocystis* sp. PCC 6803 (pAH050) and *P. taiwanensis* VLB 120 (pAH050) by the addition of cyclohexane. The biotransformation substrate cyclohexane was supplied via saturation of the medium and air phase by a silicon membrane, before the reactor inlet. The productivity of $3.76 \text{ g}_{\text{CH}_2\text{OH}} \text{ m}^{-2} \text{ day}^{-1}$ was reached after 1 day of adaptation and was stable for 30 days. After 31 days, the setup was actively terminated [1]. The light was turned off during day 8 and 10 so that *Synechocystis* sp. PCC 6803 (pAH050) was no longer able to perform photosynthesis, and this resulted in to decrease of the productivity $1.0\text{--}1.3 \text{ g}_{\text{CH}_2\text{OH}} \text{ m}^{-2} \text{ day}^{-1}$ [1]. The average volumetric productivities during the light and dark conditions are $3.71 \text{ g}_{\text{CH}_2\text{OH}} \text{ m}^{-2} \text{ day}^{-1}$ and $1.35 \text{ g}_{\text{CH}_2\text{OH}} \text{ m}^{-2} \text{ day}^{-1}$, respectively (Fig. 3).

2.4. Benchmarking

The here presented biotransformation using a mixed-trophies biofilm consisting of a photoautotrophic and a chemoheterotrophic strain has been compared to the conventional chemical process and to a biotechnological approach using a heterotrophic organism only (Table 4). Thereby, the advantages and disadvantages of the different concepts become obvious, and new engineering targets may be identified to develop an economic and sustainable process.

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Transparency document

Transparency document associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2019.104059>.

References

- [1] A. Hoschek, I. Heuschkel, A. Schmid, B. Buehler, R. Karande, K. Buehler, Mixed-species biofilms for high-cell-density application of *Synechocystis* sp. PCC 6803 in capillary reactors for continuous cyclohexane oxidation to cyclohexanol, *Bioresour. Technol.* 282 (2019) 171–178, <https://doi.org/10.1016/j.biortech.2019.02.093>.
- [2] D. Hanahan, Studies on transformation of *Escherichia coli* with plasmids, *J. Mol. Biol.* 166 (4) (1983) 557–580, [https://doi.org/10.1016/S0022-2836\(83\)80284-8](https://doi.org/10.1016/S0022-2836(83)80284-8).
- [3] R. Stanier, R. Kunisawa, M. Mandel, G. Cohen-Bazire, Purification and properties of unicellular blue-green algae (order *Chroococcales*), *Bacteriol. Rev.* 35 (2) (1971) 171–205.
- [4] S. Panke, B. Witholt, A. Schmid, M. Wubbolts, Towards a biocatalyst for (S)-styrene oxide production: characterization of the styrene degradation pathway of *Pseudomonas* sp. strain VLB120, *Appl. Environ. Microbiol.* 64 (6) (1998) 2032–2043.
- [5] A. Hoschek, J. Toepel, A. Hochkeppel, R. Karande, B. Buehler, A. Schmid, Light-dependent and Aeration-independent Gram-Scale Hydroxylation of Cyclohexane to Cyclohexanol by CYP450 Harboring *Synechocystis* Sp. PCC 6803, *Biotechnol. J.* 2019, <https://doi.org/10.1002/biot.201800724>.
- [6] A. Hoschek, B. Bühler, A. Schmid, Overcoming the gas-liquid mass transfer of oxygen by coupling photosynthetic water oxidation with biocatalytic oxyfunctionalization, *Angew. Chem. Int. Ed.* 56 (47) (2017) 15146–15149, <https://doi.org/10.1002/anie.201706886>.
- [7] S. Shcolnick, Y. Shaked, N. Keren, A role for mrgA, a DPS family protein, in the internal transport of Fe in the cyanobacterium *Synechocystis* sp. PCC6803, *Biochim. Biophys. Acta Bioenerg.* 1767 (6) (2007) 814–819, <https://doi.org/10.1016/j.bbabi.2006.11.015>.
- [8] J. Fischer, T. Lange, R. Boehling, A. Rehfinger, E. Klemm, Uncatalyzed selective oxidation of liquid cyclohexane with air in a microcapillary, *Chem. Eng. Sci.* 65 (16) (2010) 4866–4872, <https://doi.org/10.1016/j.ces.2010.05.028>.
- [9] R. Karande, L. Debor, D. Salamanca, F. Bogdahn, K. Engesser, K. Buehler, A. Schmid, Continuous cyclohexane oxidation to cyclohexanol using a novel cytochrome P450 monooxygenase from *Acidovorax* sp. CHX100 in recombinant *P. taiwanensis* VLB120 biofilms, *Biotechnol. Bioeng.* 113 (1) (2016) 52–61, <https://doi.org/10.1002/bit.25696>.