Data Article

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

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**A R T I C L E   I N F O**

Article history:
Received 1 March 2019
Received in revised form 16 May 2019
Accepted 17 May 2019
Available online 25 May 2019

**Keywords:**
Biofilm monitoring
Biofilm cultivation
Confocal laser scanning microscopy
Cyclohexane conversion

**A B S T R A C T**

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 gCDW 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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DOI of original article: https://doi.org/10.1016/j.biortech.2019.02.093.
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https://doi.org/10.1016/j.dib.2019.104059
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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.
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.

![Flow cell setup](image)

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.

| Strain Description | Strain Description | Reference |
|--------------------|--------------------|-----------|
| E. coli DH5α       | F− λ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17 (rK-, mK+) phoA supE44 | [2] |
| Synchocystis sp. PCC 6803 | Geographical origin: California, USA; Received from Pasteur Culture Collection of Cyanobacteria (PCC, Paris, France) | [3] |
| Pseudomonas taiwanensis VLB120 | Wild-type Pseudomonas; styrene prototroph | [4] |
| Pseudomonas taiwanensis VLB120_eglfp | P. taiwanensis VLB120 harboring a chromosomally integrated egfp (enhanced green fluorescent protein) gene | This study |
| Plasmid Description | Plasmid Description | Reference |
| pAH032 | pPMQAK1 based, RSF ori, Kanamycin resistance, empty P_{trc} expression system | [5] |
| pAH050 | Based on pAH032; CYP, FnR, and Fn genes under control of P_{trc} promoter system, RBS* optimized for Synchocystis sp. PCC 6803 in front of CYP gene | [6] |
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 Na₂HPO₄, 0.0305 g L⁻¹ K₂HPO₄, 0.0305 g 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].

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].

| Inoculation                  | P. taiwanensis VLB120_egfp [µm³] | Synechocystis sp. PCC 6803 (pAH050) [µm³] | Volume ratio (Ps/Syn) |
|-----------------------------|----------------------------------|------------------------------------------|-----------------------|
| 1 day after medium flow     | 5.9 × 10⁴                         | 1.6 × 10⁴                                | 0.37                  |
| 3 days                      | 1.5 × 10⁴                         | 4.4 × 10⁴                                | 0.357                 |
| 11 days                     | 1.1 × 10⁴                         | 5.4 × 10⁴                                | 0.217                 |
| 25 days                     | 2.4 × 10⁴                         | 1.0 × 10⁵                                | 0.0239                |
| 11 days                     | 3.1 × 10⁴                         | 1.1 × 10⁵                                | 0.0284                |

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 µL min⁻¹. For segmented flow, a gaseous air phase was additionally fed at the same rate.

| Experimental setup | O₂ in aq. Phasea (µM) | Citrate Consumption (g L⁻¹) | Biofilm dry weightb (g L⁻¹) | Biofilm dry weightb (g L⁻¹) |
|--------------------|------------------------|----------------------------|----------------------------|----------------------------|
| i - Air            | 746                    | –                          | –                          | 1.5                        |
| ii + Air           | 284                    | –                          | –                          | 13.7                       |
| iii - Air          | 923                    | –                          | 0.1                        | 5.9                        |
| iv + Air           | 287                    | –                          | 0.2                        | 31.6                       |
| v - Air            | 0                      | 0.27                       | 7.2                        | 47.8                       |
| vi + Air           | 194                    | 0.39                       | 1.5                        | 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. 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 × 0.3 cm (C) with the light source on the top, (D) bubble traps for gas phase sampling and (E) waste bottles.
2.1. Monitoring biofilm growth in a flow-cell by confocal laser scanning microscopy

The development of a mixed trophees biofilm consisting of \textit{P. taiwanensis} VLB 120 (pAH050) and \textit{Synecocystis} sp. PCC 6803 (pAH050) was analyzed by confocal laser scanning microscopy (CLSM). The

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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 (g L$^{-1}$ 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).

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Fig. 3. The average cyclohexanol production rate in g$_{\text{CHXOL}}$ m$^{-2}$ d$^{-1}$ utilizing \textit{Synechocystis} sp. PCC 6803 (pAH050) and \textit{P. taiwanensis} VLB 120 (pAH050) as a dual-species mixed-trophies biofilm under light and dark conditions. Experiments were conducted at 50 \(\mu\)E m$^{-2}$ s$^{-1}$ providing organic carbon free YBG11 medium and air segments at a flow rate of 52 \(\mu\)L min$^{-1}$. Green and grey bars represent product formation under light and dark conditions, respectively. CHXOL = cyclohexanol.
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 auto-fluorescence 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. \( Ps = P. \text{taiwanensis} \ \text{VLB120_egfp} \), \( Syn = \text{Synechocystis} \ \text{sp. PCC 6803 (pAH050)} \). 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 \( P. \text{taiwanensis} \ \text{VLB120} \) 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 \( P. \text{taiwanensis} \ \text{VLB 120 (pAH050)} \) 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 \( g_{\text{CHXOH}} \ m^{-2} \ \text{day}^{-1} \) 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 1.0–1.3 \( g_{\text{CHXOH}} \ m^{-2} \ \text{day}^{-1} \) [1]. The average volumetric productivities during the light and dark conditions are 3.71 \( g_{\text{CHXOH}} \ m^{-2} \ \text{day}^{-1} \) and 1.35 \( g_{\text{CHXOH}} \ m^{-2} \ \text{day}^{-1} \), respectively (Fig. 3).

2.4. Benchmarking

The here presented biotransformation using a mixed-trophies biofilm consisting of a phototrophic 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.

Acknowledgments

We acknowledge the use of the facilities of the Centre for Biocatalysis (MiKat) at the Helmholtz Centre for Environmental Research, which is supported by European Regional Development Funds (EFRE, Europe funds Saxony) and the Helmholtz Association. IH was funded from the ERA-IB- Project PolyBugs ID:16–006 and the Sächsisches Ministerium für Wissenschaft und Kunst (SMWK) Project ID: 100318259.

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.
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