NANO/MICROFLUIDIC DEVICES FOR WHOLE-CELL BIOCATALYST ANALYSIS BY UV/VIS SPECTROSCOPY

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ABSTRACT

Escherichia coli (*E. coli*) are widely used as whole-cell biocatalysts. Their advantage is they provide a stable environment with an enzyme stabilizing intra-cellular matrix and most of the necessary cofactors relevant for many industrially used enzymes, e.g. alcohol dehydrogenase or monooxygenase. As a consequence the biocatalysis becomes more stable and reliable. In this study, the impact of various external factors to the production and the whole-cell biocatalyst itself are investigated to gain a better understanding and the ability to enhance whole-cell biocatalytic reactions. For that purpose, the leaching of cofactors is studied for *E. coli* trapped in a nano/microfluidic confinement.

KEYWORDS: Whole-cell Biocatalyst, Analysis, UV/VIS Spectroscopy

INTRODUCTION

Manifold reasons for leaching of cofactors out of cells are related to the substrates of the biocatalytic reactions. Some of the substrates have a low solubility in aqueous solutions, and thus, are solved in organic solvents, which are assumed to impact the cell permeability. For some substrates the permeability of the whole-cell reactor is increased non-specifically either genetically or by freeze-thawing cycles of the cells to allow the passage into the cell. Additionally, to increase the specific permeability of whole-cell catalysts those are genetically adapted to form substrate channels, which should not be permeable for the cofactors. In this work, we study how the leaching of cofactors is affected by the performances mentioned above.

EXPERIMENTAL

A nano/microfluidic device suitable for UV/VIS-spectroscopy was designed and produced with poly(dimethylsiloxane) (PDMS) soft lithography. A black-PDMS spot at the detection point, figure 1, downstream of the trapping feature provided a significant reduction of the autofluorescence of the PDMS [1]. The native fluorescence of the cofactors NAD^+, NADH, $NADP^+$, and NADPH was exploited for their detection. Therefore, the technique was label-free.

RESULTS AND DISCUSSION

We tested the minimum concentration of cofactors that were detected in pinched injection mode, and detection limits were about 0.2 mM, table 1. The typical concentrations of NAD^+, NADH, $NADP^+$, and NADPH in *E. coli* are 2.49 mM, 1.07 mM, 0.59 mM, and 0.33 mM respectively [2]. The leached cofactors are diluted in the surrounding volume. But the detection limit is reached with five *E. coli* in a channel with dimensions 15 µm width and 5 µm height and a plug length of 5 µm.

Table 1. Peak heights for pinched injection of cofactors. The noise was 75 counts/100 ms.

	peak height [counts/100ms]			
cofactor	**1.6 mM**	**0.8 mM**	**0.4 mM**	**0.2 mM**
NAD+	1350	1196	775	307
NADH	1198	1107	512	246
NADP+	1795	1516	1425	282
NADPH	798	551	403	247

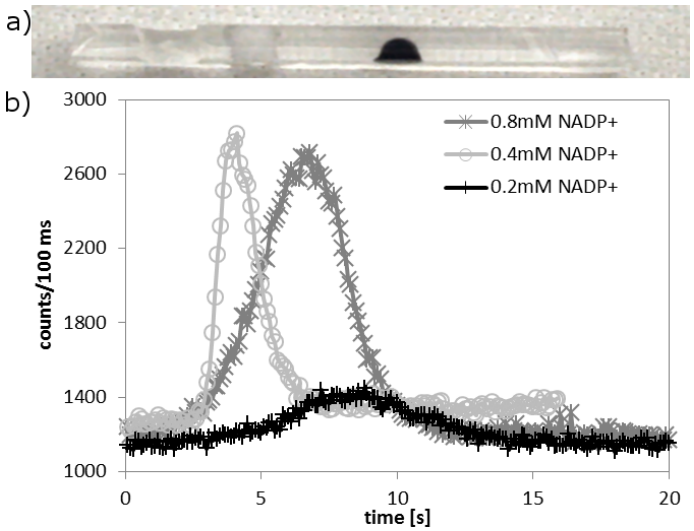

Figure 1: Cofactor detection by UV/VIS spectroscopy. a) Cross-section of chip with black-PDMS spot at detection point. b) Fluorescence intensity for pinched injection of $NADP^+$ at varying concentrations.

The nano/microfluidic trapping feature of the *E. coli* is shown in figure 2. A confined area was structured by ridges, with dimensions to host about 75 *E. coli* to study also the loneliness impact. The cells were trapped mechanically and flushed with the respective buffers or solutions. To ensure the cells were flushed properly the ridges did not reach from top to bottom of the channel but form a gap of about 600 nm height. Hence, the cells could not pass the ridged but the liquids.

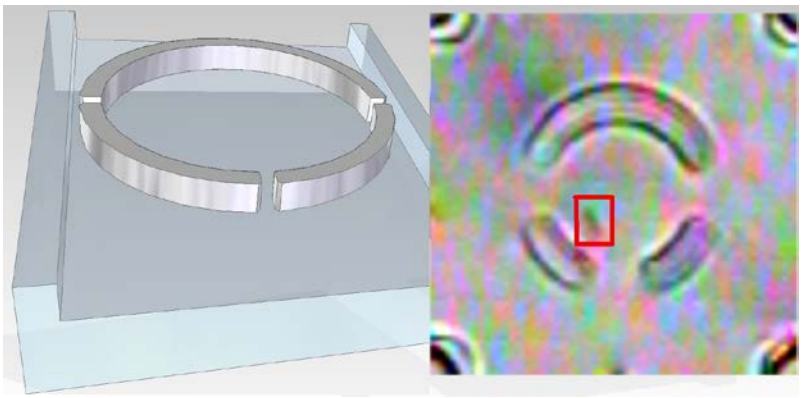

Figure 2: Trapping feature for the E. coli. Left) sketch of the feature. Right) microscopy image of a single E. coli, marked with the red square, in the trapping feature. In the corners of the picture the corners of the channel is visible to indicate the dimension; the channel width was 30 µm, the channel height was 6 µm.

CONCLUSION

We trapped *E. coli* in nano/microfluidic devices and cultured them. Additionally, we used UV/VIS spectroscopy to detect cofactors by native fluorescence. The amount of cofactors of five *E. coli* was calculated to be above the determined detection limit. Next, we will combine both experiments to detect cofactor leaching of *E. coli*.

REFERENCES

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