THE TRANSFORMERS

FROM CARBON DIOXIDE TO BIOFUEL

Annika Fust[1], Birte Hollmann[1], Boas Pucker[1], David Wollborn[1], Janina Tiemann[1], Julian Droste[1], Sandra Brosda[1], Sebastian Blunk[1],Simon Riedl[1], Tore Bleckwehl[1]; [1]CeBiTec Bielefeld

iGEM BIELEFELD 2014

Policy and Practices

Beside public events we cooperated with SYNENERGENE.
According to the problem analysis we developed several application scenarios and technomoral vignettes during our project. This enabled us to have another view on our project and led to adjustments during the wet lab work.

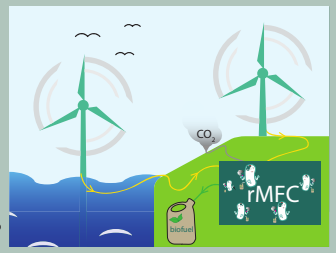
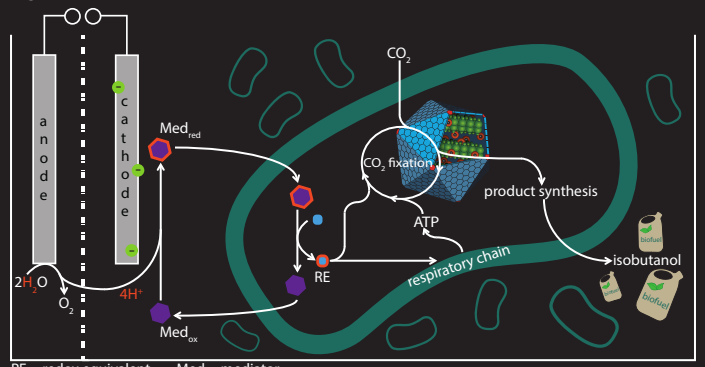


Figure 2: Application scenario for a wind power station.

Abstract

Within our project we aim to produce isobutanol by using electricity for the generation of redox and energy equivalents and carbon dioxide as a carbon source. In *Escherichia coli* this task is seperated into three parts shown below. In addition we developed an antiobiotic-free selection system shown on the right.



RE = redox equivalent Med = mediator

Figure 1: Schematic illustration of our project.

Antibiotic Free Selection

An antibiotic-free selection system was implemented by using the complementation of the D-alanine auxotrophy in the *E. coli* strain DH5α Δ*alr* Δ*dadX*. It turned out that the transformation efficiency is about three times higher compared to the classical selection with chloramphenicol (Cm).

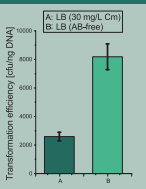
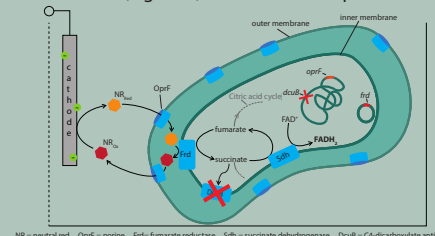


Figure 12: Comparision of the transformation efficiency.

reverse Microbial Fuel Cell

Our aim was to generate redox equivalents by supplying electrons from the cathode through mediators like neutral red or bromphenol blue. To engineer an electrophilic strain by enabling the cells for electron uptake, various genetic modifications (Figure 3) needed to be implemented.

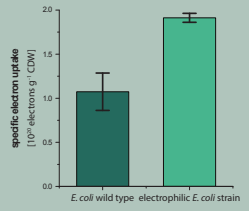


NR = neutral red OprF = porine Frd= fumarate reductase Sdh = succinate dehydrogenase DcuB = C4-dicarboxylate antiporter

Figure 3: Principle of electron transfer to *E. coli*. The mediator (neutral red) is reduced at the cathode and crosses the outer membrane through constitutive expressed outer membrane porine (OprF). It serves as an electron donor for the fumarate reductase (Frd). Fumarate is reduced into succinate, which would be reoxidized again by the succinate dehydrogenase (Sdh). In this step FADH$_2$ is restored. The antiporter *dcuB* is knocked out to avoid any succinate export.

To carry out our experiments we designed a reverse microbial fuel cell (rMFC). Such a system (Figure 4) is suitable for the investigation of mediator redox-characteristics and indirect electron transfer into electrotrophes.

Figure 4: Our electrobiochemical reactor system.



To evaluate the electron uptake we used a potentiostat for sensitive measurements.

Figure 5:
Constructed *E. coli* strain Δ*dcuB::oprF* with expressed fumarate reductase (Frd; BBa_K1465102) shows a higher electron uptake by cultivation in the electrobiochemical reactor compared to the *E. coli* wild type.

Fixation of Carbon Dioxide (CO_2)

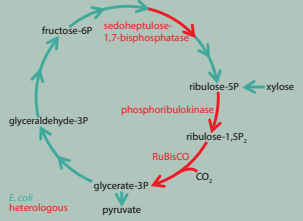


Figure 6: Reactions of the Calvin cycle.

Eight of the eleven enzymes involved in the **Calvin cycle** already exist in *E. coli* (Figure 6). For the CO_2 fixation the phosphoribulokinase (PrkA), the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and the sedoheptulose-1,7-bisphosphatase (SBPase) need to be expressed heterologous. The activity of the RuBisCO could be validated *in vitro* (Figure 7).

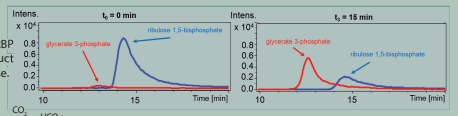


Figure 7: Decrease of the substrate RBP (blue) and an increase of the product 3-phosphoglycerate (red) over time course.

The correct carboxysome assembly was verified using a translational fusion of one shell protein coding sequence with *gfp* (Figure 9).

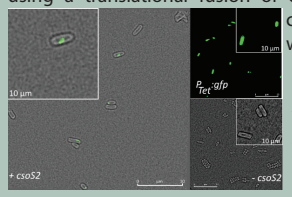


Figure 9: Fluorescence images taken through a structured illumination microscope. Expression of *E. coli* KRX with different plasmids, encoding the carboxysome, constitutive *gfp* expression and the carboxysome lacking the essential shell associated protein CsoS2.

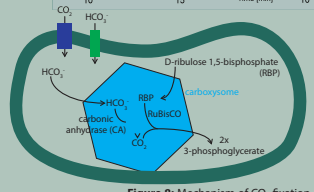


Figure 8: Mechanism of CO_2 fixation within the carboxysome in *E. coli*.

The **carboxysome** is a protein-enveloped microcompartment encapsulating the RuBisCO and the carbonic anhydrase. The advantage of the microcompartment is the concentration of carbon dioxide in its lumen, which allows efficient carbon dioxide fixation under aerobic growth conditions (Figure 8).

Isobutanol Production

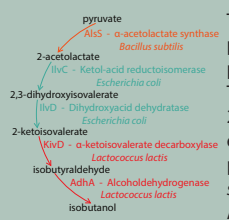


Figure 10: Isobutanol production pathway.

The aim was the production of an industrially relevant product. We decided to implement the isobutanol production pathway (Figure 10).
The steps in the conversion of pyruvate to 2-ketoisovalerate can be executed by proteins existing in *E. coli* (IlvIH, IlvC and IlvD). The native protein IlvIH is replaced by the AlsS from *Bacillus subtilis* to increase the isobutanol production and the AdhA from *Lactococcus lactis* is used. With our approach we achieved a production of about 56 mg isobutanol per liter medium.

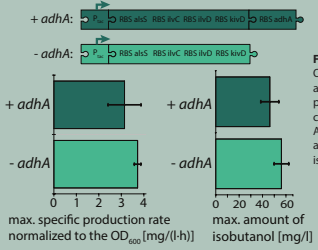


Figure 11:
Our designed constructs and the maximum specific production rate of cultures carrying them. Additionally the maximum amount of produced isobutanol is visualized.

The dynamic **modeling** approach containing ordinary differential equations indicated possible optimizations. Stronger expression of *kivD* and *adhA* could improve product synthesis (Figure 12).

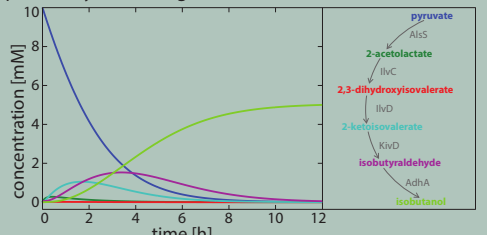


Figure 12: Predicted changes in the concentration of the metabolites over time.

References
Atsumi S *et al.*, Nature 451, pp. 86–89, 2008.
Bonacci *et al.*, Proceedings of the National Academy of Sciences, vol. 2, pp. 478 – 483, 2012.
Parikh *et al.*, Protein Engineering, Design & Selection, vol. 19, pp. 113-119, 2006.
Park *et al.*, Journal of Bacteriology, vol. 181, pp. 2403-2410, 1999.
Stolzenberger *et al.*, Journal of Bacteriology, vol. 195, pp. 5112 - 5122, 2013.

Acknowledgements
Prof. Dr. Jörn Kalinowski
Dr. Christian Rückert
Manuel Wittchen
Nils Lübke
Timo Wolf
Working groups
Fermentation Technology
Microbial Genomics and Biotechnology
Cellular and molecular Biotechnology