Policy and Practices

Beside public events we cooperated with SYNERGENE. 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.

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 separated into three parts shown below. In addition we developed an antibiotic-free selection system shown on the right.

Fixation of Carbon Dioxide (CO₂)

Eight of the eleven enzymes involved in the Calvin cycle already exist in E. coli (Figure 6). For the CO₂ 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).

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

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).

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 ΔmalD ΔadiA. It turned out that the transformation efficiency is about three times higher compared to the classical selection with chloramphenicol (Cm).

Isobutanol Production

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 (IlvH, IlvC and IlvD). The native protein IlvH is replaced by the Alr5 from B. 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.

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

Reversal 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.

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.

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

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

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).

Reference


Acknowledgements

Prof. Dr. Jörn Kalinowski
Dr. Christian Ruckert
Marian Wilchen
Kati Lohke
Thomas Wolf
Working groups:
Fermentation Technology
Microbial Genomics and Biotechnology
Cellular and molecular Biotechnology

The TRANSFORMERS
FROM Carbon Dioxide TO Biofuel