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.

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 heterologously. 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 gfp (Figure 9).

The carboxysome is a protein-enveloped microcompartment encapsulating the Rubisco and the carboxic 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

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 IlvI is replaced by the AlsS from Bacillus. The steps in the conversion of pyruvate to isobutyraldehyde are complemented with SYNENERGEnE's AddA and AdhA from Lactococcus lactis. 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 `addA` could improve product synthesis (Figure 12).

References


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