PRODUCTION OF ISOBUTANOL FROM CO_2 IN A TWO-STEP PROCESS

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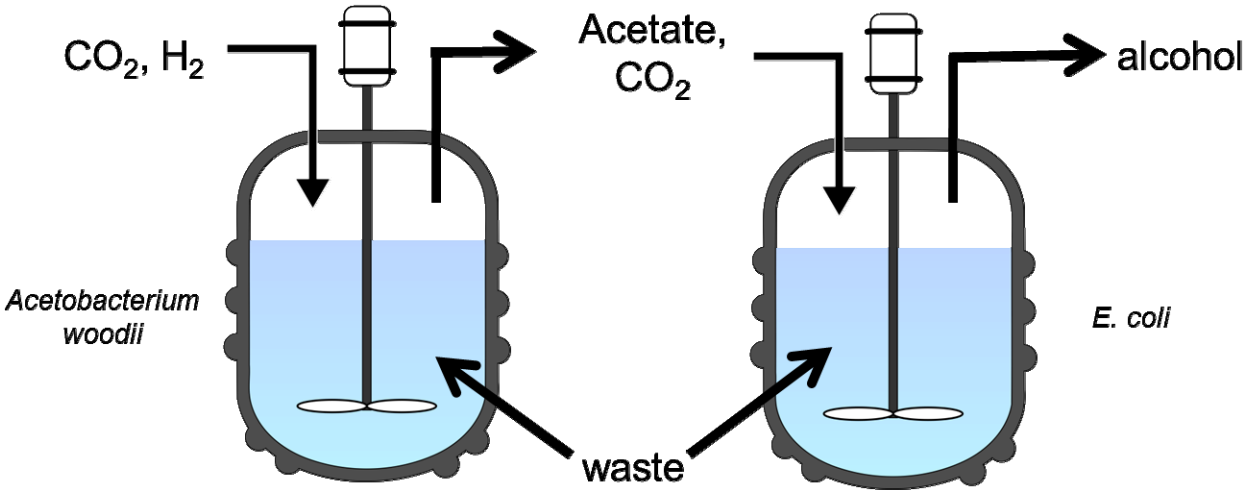
INTRODUCTION

The effects of climate change as a result of high greenhouse gas emissions force the industrial sector to reduce its carbon footprint, especially as it accounts for 44 % of the emissions in Austria[1]. Additionally, a high amount of energy is required for the transportation sector, mainly in the form of hydrocarbons, which offer a cheap energy source and are easily storable.

Finding a solution for these two challenges and concurrently investing into the transition towards a biobased future led to the design of a two-step process. In the first step, acetate is produced from CO_2 and H_2 by the acetogenic bacterium *Acetobacterium woodii*. The produced acetate is subsequently used in a second step for the conversion into isobutanol by genetically engineered *Escherichia coli*. Its property as a two-step process makes the system a highly flexible platform for the production of various fuel alcohols and chemicals.



Picture 1: Overviw of the two-step process for CO_2 fixation and alcohol prodcution

Biotechnological CO_2 fixation is challenging, because CO_2 is chemically inactive and it can be used as a carbon but not as an energy source. Hence, for biological utilization, an energy input is needed. This can be in the form of sunlight as for photoautrotrophic organisms, but also hydrogen gas from renewable resources and waste streams, with the last two being investigated in this work. Conventional cultivation media for the acetogen *Acetobacterium woodii* usually contain high amounts of expensive complex media components. For industrial applications, the omittance of this component is of great interest, which was shown to be possible in this work. Although the productivity was decreased in batch processes, good productivities of 11 g l^{-1} d^{-1} were shown in a chemostat operation.

Subsequently, acetate is taken up by *E. coli* in a second stage and converted into isobutanol. However, acetate utilization is challenging due to the low energy production from this carbon source in the bacterial cell. Genetic engineering of the acetate uptake system has been shown to

improve the utilization of acetate as a sole carbon source as well as in a co-utilization system with glucose[2].

For isobutanol production, a strain with high productivities was selected during a screening process from a genetic construct library. During bioreactor cultivations, important process parameters were optimized, resulting in the production of 9 g/l isobutanol, which could even be further increased in fed-batch cultivations. Out of four waste streams tested, two were successfully used for alcohol production, underlining the potential of the imagined process.

Until now, all parts of the imagined two-step process for CO_2 fixation and isobutanol production were investigated successfully. The next steps will be to improve the isobutanol production on acetate as a carbon source as well as to finally put all puzzle pieces together in order to investigate the whole process in its full complexity.

REFERENCES

[1] http://www.umweltbundesamt.at/umwelt/luft/treibhausgase/

[2] Novak K, Flöckner L, Erian AM et al. Characterizing the effect of expression of an acetyl-CoA synthetase insensitive to acetylation on co-utilization of glucose and acetate in batch and continuous cultures of E. coli W. Microbial Cell Factories 2018;17:109.