Syngas fermentation: Toxicity analysis of potential products on *Clostridium ljungdahlii*

Ira Lauer, Gabriele Philipps and Stefan Jennewein

Fraunhofer Institute for Molecular Biology and Applied Ecology, 52074 Aachen, Germany

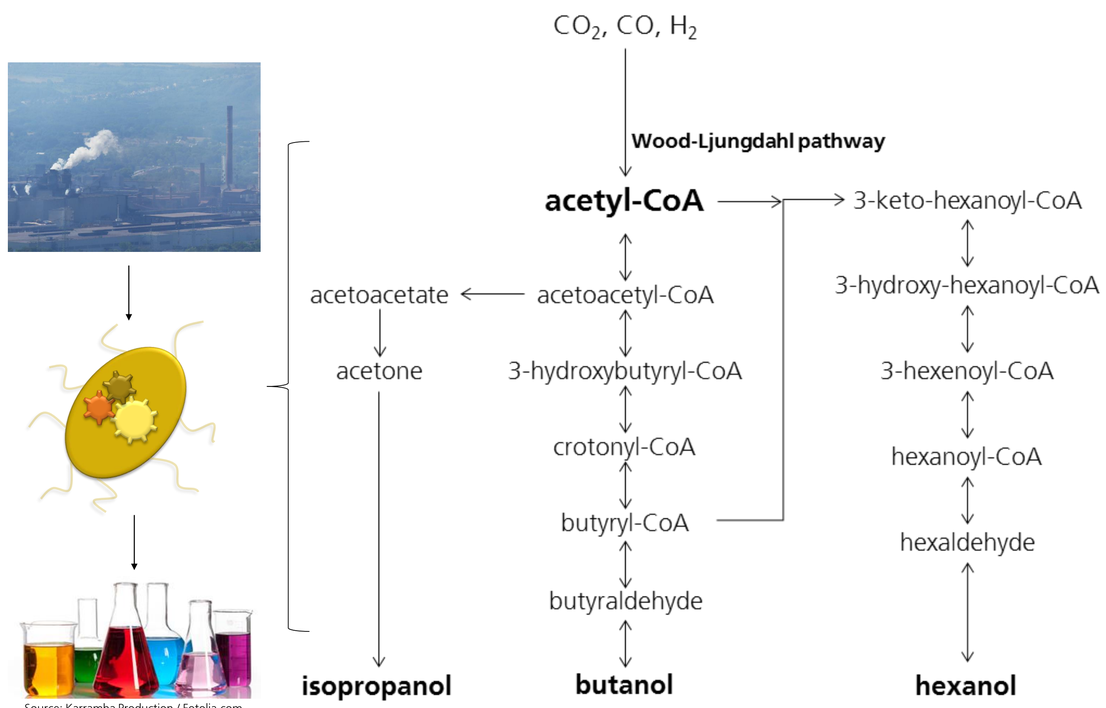
contact: stefan.jennewein@ime.fraunhofer.de

I Introduction

Syngas (a mixture of CO_2, CO and H_2) is a chemical feedstock which can be derived by e.g. the gasification of organic matter or from industrial production processes like steel manufacturing. Purified syngas is commonly used in the Fischer-Tropsch process for the synthesis of olefins and ethanol[1]. Acetogens like *Clostridium ljungdahlii* can use syngas as carbon and energy source via the Wood-Ljungdahl pathway to produce the key intermediate acetyl-CoA[2]. Based on this central intermediate a variety of valuable products can be built, either through endogenous or heterologous biosynthesis pathways[3]. Microorganisms of the genus Clostridia are known for their capability of synthesizing several industrially relevant organic solvents and potential biofuels, such as acetone, isopropanol, butanol and hexanol[4]. By genetic engineering we can transfer these pathways into *C. ljungdahlii* and use syngas as a carbon and energy source. Some of the desired products are known to possess certain toxic properties against microorganisms[5]. To evaluate the toxicity of our intended products (isopropanol, butanol and hexanol) on *C. ljungdahlii* we examined biomass formation by applying different concentrations of the targeted alcohols.

II Aim of the project

The project BIOCON-CO_2 (BIOtechnological processes based on microbial platforms for the CONversion of CO_2 from the iron and steel industry into commodities for chemicals and plastics) aims to reuse off-gases and thereby produce valuable products. Off-gases from steel industry pollute the environment and promote climate change. Therefore it is necessary to develop methods for recycling off-gases, especially CO_2, and establish alternative feedstocks to fossil resources for production of basic chemicals. *C. ljungdahlii* is able to metabolise these gases and should be tailored by metabolic engineering using heterologous biosynthetic pathways to produce valuable alcohols. In a first step, the toxicity of the targeted products on *C. ljungdahlii* wildtype was evaluated to get insights into tolerable product titers.



Source: Karramba Production / Fotolia.com

Figure 1: Scheme for using industrial off-gases as substrate to produce desired products using microbial cell factories and metabolic pathway to targeted alcohols in Clostridia [2, 6 (adapted)].

III Results

Product toxicity of the short chain alcohols isopropanol and butanol on *C. ljungdahlii* wildtype is not as high as of the longer chain alcohol hexanol. Half maximal inhibiting concentrations (IC_{50}) were estimated based on the biomass formation. For isopropanol and butanol IC_{50} values of about 500 mM and >100 mM were determined, respectively. Hexanol has a IC_{50} of between 10 and 50 mM, whereas at 50 mM, no biomass formation is detectable.

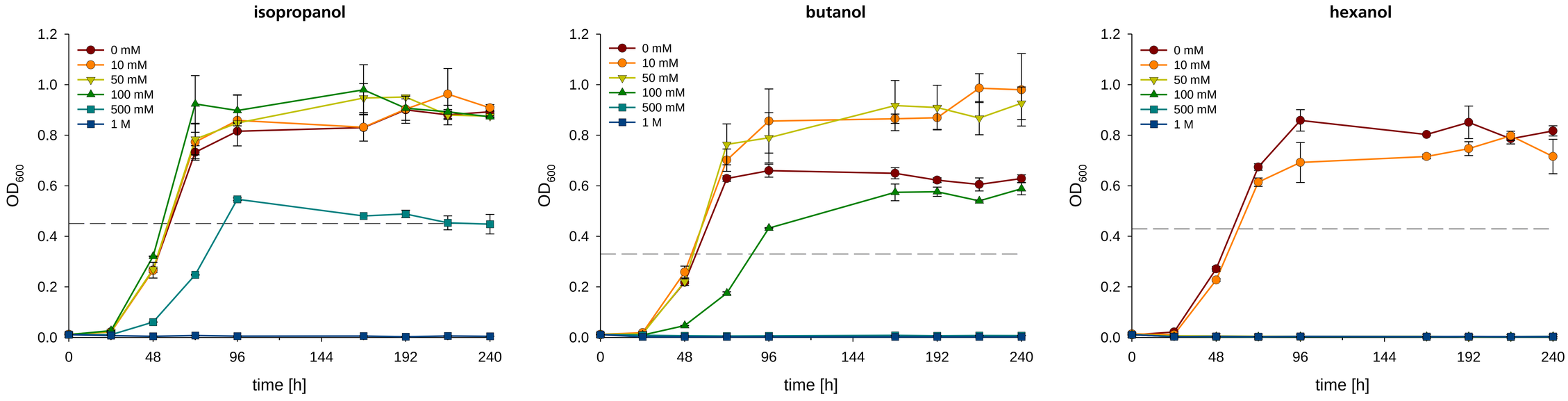


Figure 2: Biomass formation of *C. ljungdahlii* wildtype. Cultivations were performed in 25 mL anaerobic culture tubes with 5 mL modified ATCC1754 medium. Syngas in a composition of 33% CO_2, 33% CO and 33% H_2 with 1 bar overpressure was used as carbon and energy source for the preculture as well as for the main culture. Cultures were incubated horizontally at 37 °C and 150 rpm in a rotary shaker. The turbidity of the cultures was measured and converted into OD_{600}. The half maximal OD_{600} of the cultivations with 0 mM of supplement is marked as dashed line and the standard deviation of two technical replicates is given.

IV Conclusion and perspectives

Our findings show that as expected the longer chain alcohol hexanol is more toxic for *C. ljungdahlii* cell growth compared to the shorter chain alcohols. For isopropanol and butanol, the tolerable product titers seem not to be limiting for alcohol production. However, reasonable hexanol titers exceed the lethal concentration for *C. ljungdahlii* wildtype. For hexanol production with *C. ljungdahlii*, it is either necessary to develop more resistant producer strains or to establish a product recovery system. Knowing the influence of the products on *C. ljungdahlii*, the implementation, genomic integration and improvement of heterologous metabolic pathways into *C. ljungdahlii* can be started to enhance the product formation in stable producer strains.

V References

[1] **Dry, M.E.** (2002) The Fischer-Tropsch process: 1950-2000. *Catal Today* **71**, 227-241.
[2] **Bengelsdorf, F.R., Straub, M. and Dürre, P.** (2013) Bacterial synthesis gas (syngas) fermentation. *Environ Technol*, **34**, 1639-1651.
[3] **Köpke, M., Held, C., Hujer, S., Liesegang, H., Wiezer, A., Wollherr, A., Ehrenreich, A., Liebl, W., Gottschalk, G. and Dürre, P.** (2010) *Clostridium ljungdahlii* represents a microbial production platform based on syngas. *Proc Natl Acad Sci U S A*, **107**, 13087-13092.
[4] **Dürre, P.** (1998) New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. *Appl Microbiol Biotechnol*, **49**, 639-648.
[5] **Fernández-Naveira, Á., Veiga, M.C. and Kennes, C.** (2017) H-B-E (hexanol-butanol-ethanol) fermentation for the production of higher alcohols from syngas/waste gas. *J Chem Technol Biotechnol*, **92**, 712-731.
[6] **Dekishima, Y., Lan, E.I., Shen, C.R., Cho, K.M. and Liao, J.C.** (2011) Extending carbon chain length of 1-butanol pathway for 1-hexanol synthesis from glucose by engineered *Escherichia coli*. *J Am Chem Soc*, **133**, 11399-11401.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 761042 (BIOCON-CO2). This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.