StyGreen

Bioplastic from cellulosic waste through consolidated bioprocessing

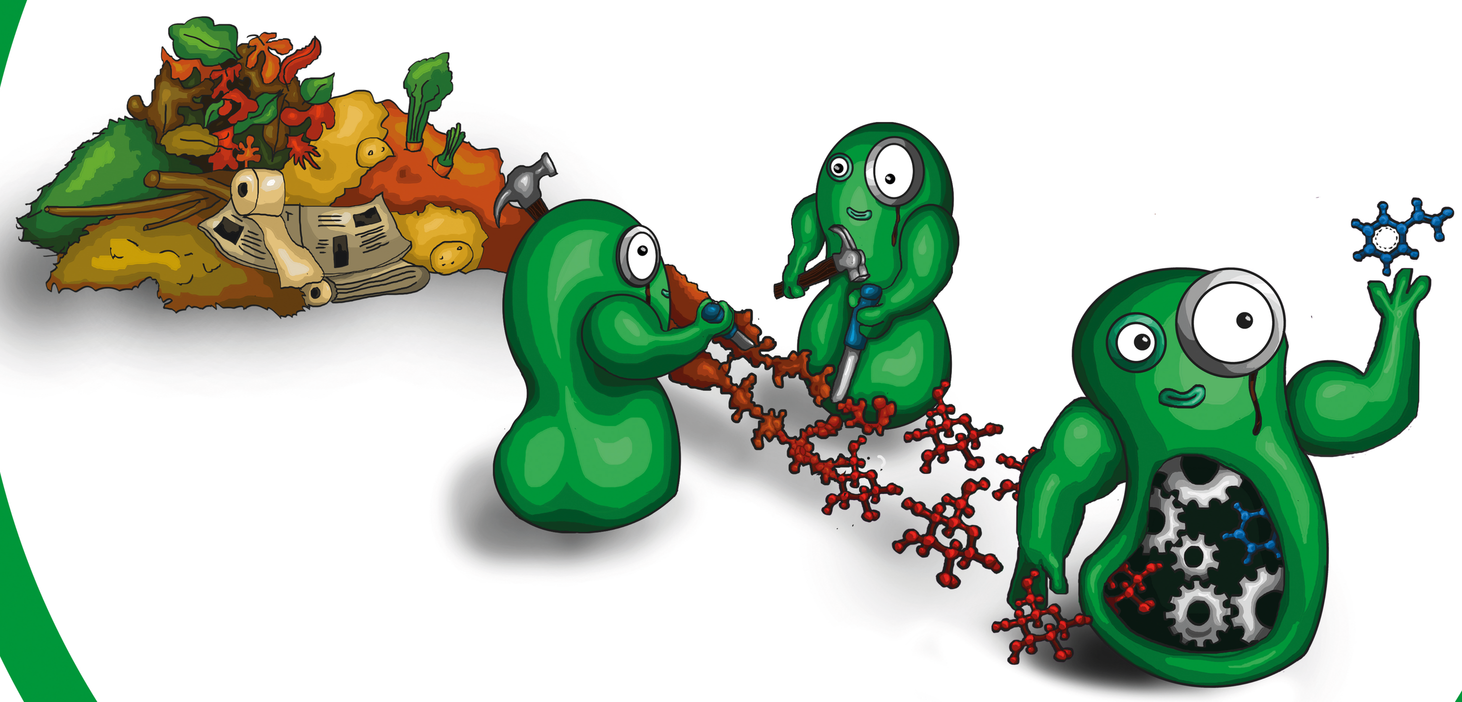
Our sponsors

Problem

Vast amounts of styrene are produced petrochemically. This is not sustainable as fossil fuels are running out.

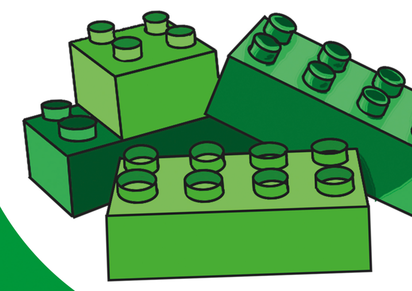
Project

We equiped *S. cerevisiae* with a mini-cellulosome containing a scaffold with 3 cellulases and a cellulose binding domain allowing it to degrade cellulose containing waste into glucose [1].



Furthermore we introduced the enzyme PAL2 which together with native FDCI allows for the conversion of phenylalanine to styrene [2].

Product



StyGreen, bioplastic made in an eco-friendly way, can be used for many great products such as truly green Lego bricks.

1. From waste

Growth on cellulose

Expression of the galactose regulated mini- cellulosome was induced in *S. cerevisiae*, after which the cells were switched to a medium with either phosphorylated cellulose or cellobiose (a β-1,4 linked glucose dimer).

Growth, monitored in a microtiter plate reader, was achieved on both cellobiose and phosphorylated cellulose.

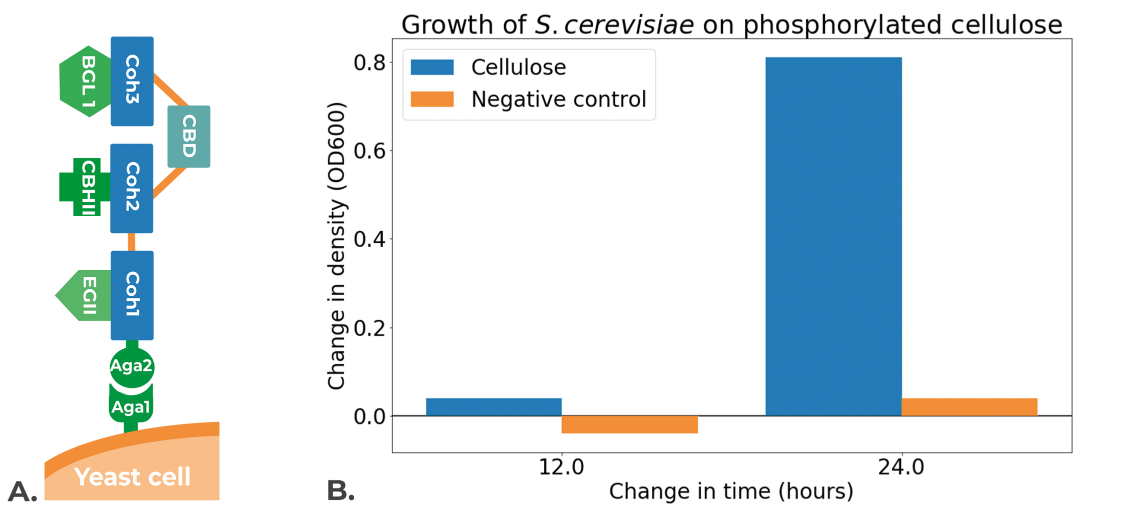


Figure 1. A: Mini-cellulosome consisting of (i) a miniscaffoldin containing a cellulose binding domain (CBD) and three cohesins tethered to the cell surface through the a-agglutinin adhesion receptor (Aga1) and (ii) three enzymes: endoglucanase (EGII), cellobiohydrolase (CBH), and a β-glucosidase (BGL). B: Growth of *S. cerevisiae* strains containing the artificial cellulosome.

Modeling the cellulosome

Coarse-grained molecular dynamics showed that a scaffold with a cellulose binding domain has an affinity for cellulose several orders of magnitude higher than the enzymes separately, while a mathematical model showed no significant decrease in enzyme performance upon scaffolding.

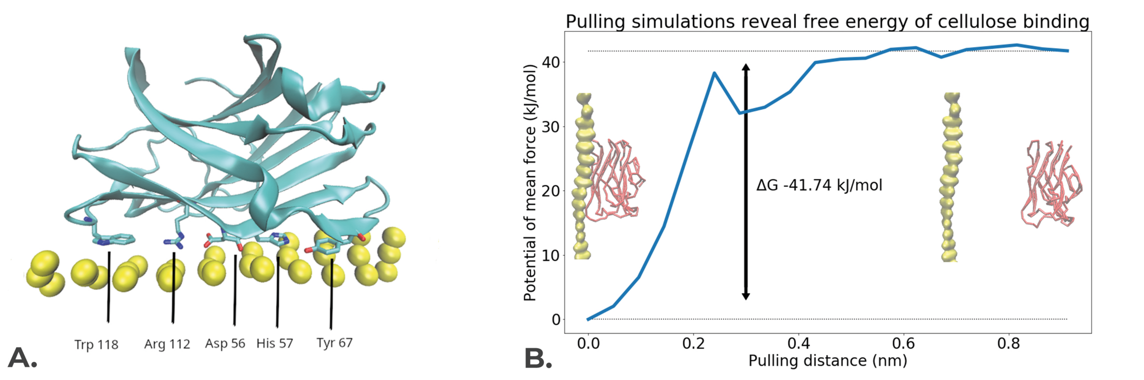
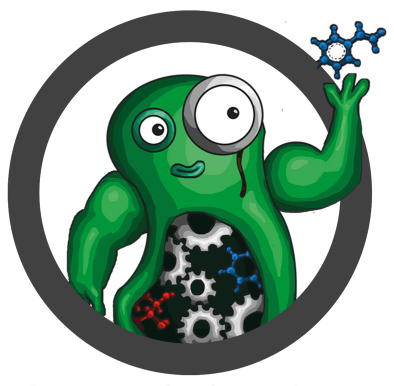


Figure 2. A: Coarse grained molecular dynamics confirms hypothesized binding interactions. B: Advanced sampling techniques uncover the underlying free-energy landscape along the reaction coordinate.

2. To bioplastic



Styrene production

S. cerevisiae expressing heterologous PAL2 (phenylalanine ammonia lyase) was grown on glucose and phenylalanine. **Trans-cinnamate (tCA) and styrene were detected in the supernatant using HPLC.**

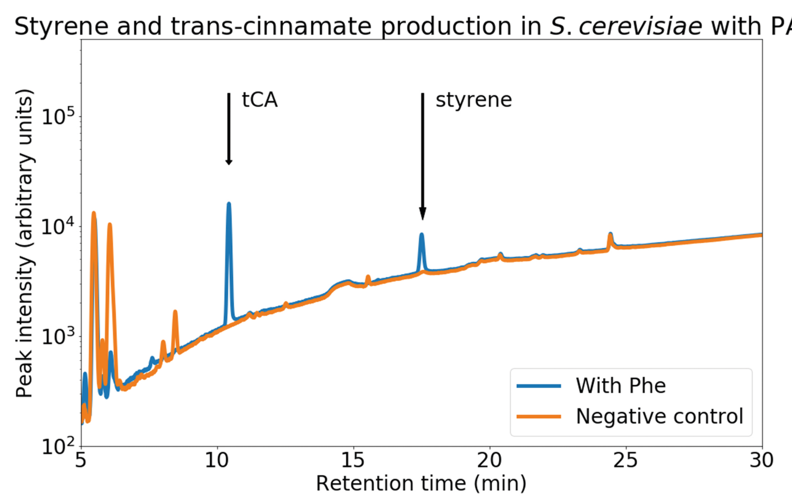


Figure 3. HPLC 254 nm intensity plotted against retention time.

Optimizing flux

Flux Based Analysis showed that our cells can simultaneously grow and produce styrene, while the OptForce algorithm was run to find interventions that would lead to StyGreen overproduction [3].

Human practices

- Use Recell®: recycled toilet paper
- Life-Cycle Analysis: 'cradle to gate'
  - Carbon emissions:
    - Petroleum-based styrene: 7.8 CO2-eq /kg
    - StyGreen, theoretical maximum yield: 2.1 CO2-eq/kg
  - Room for optimization
- Interest from industry

3. To a green world

Consolidated bioprocessing

S. cerevisiae expressing both the mini-cellulosome and PAL2 was grown on cellobiose. After 36 hours the styrene could be measured in the supernatant by HPLC. In other words: **we have successfully achieved consolidated bioprocessing.**

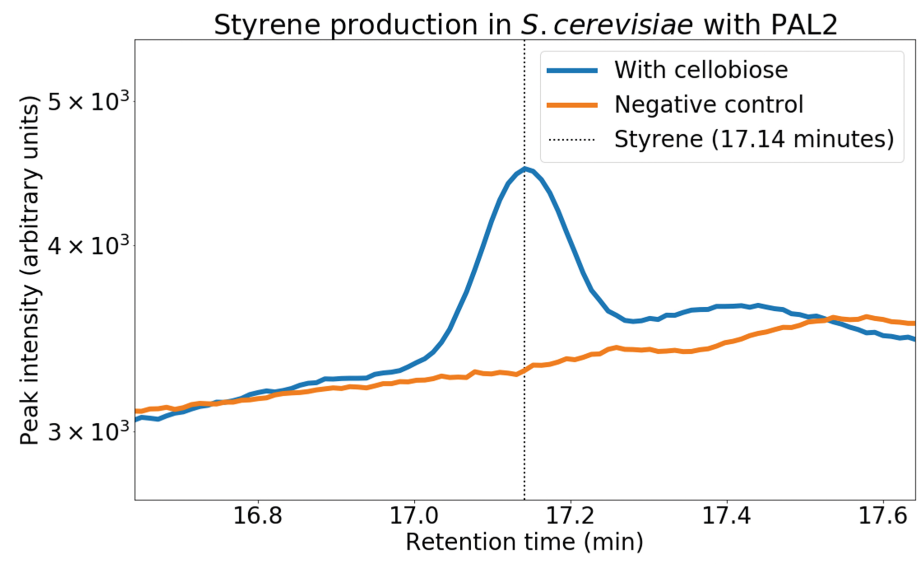


Figure 4. Styrene production from cellobiose in yeast. HPLC intensity at 254 nm is plotted against retention time.

Manufacturing

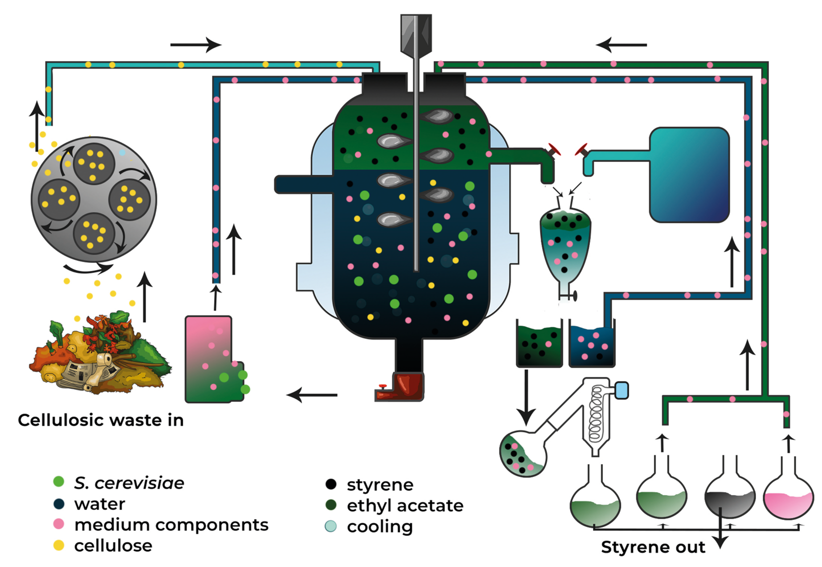


Figure 5: Scaling up our process in a bioreactor

Achievements

- Engineered a yeast strain able to produce styrene from cellobiose.
- Constructed three models that influenced and improved our project.
- Designed a way to upscale our process
- Succesfully tackled a relevant problem, making headway for a biobased economy.

Team

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Affiliations and Acknowledgements

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Sources

1. Wen, F., Sun, J., & Zhao, H. (2010). Applied and Environmental Microbiology, 76(4), 1251–60
2. Liu, C., Men, X., Chen, H., Li, M., Ding, Z., Chen, G., ... Xian, M. (2018). Biotechnology for Biofuels, 11.
3. Ranganathan, S., Suthers, P. F., & Maranas, C. D. (2010). PLoS Computational Biology, 6(4),