StyGreen
Bioplastic from cellulolic waste through consolidated bioprocessing

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

Project
We equipped 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 FDC1 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 cellulobiose (a β-1,4 linked glucose dimer). Growth, monitored in a microtiter plate reader, was achieved on both cellulobiose 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 α-agglutinin adhesion receptor (Aga1) and (II) three enzymes: endoglucanase (EGE), cellobihaydrolyase (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.

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 cellulobiose. 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 cellulobiose in yeast. HPLC intensity at 254 nm is plotted against retention time.

Manufacturing

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

Team
Benno Diekmann, Bram Wiggers, Ingeborg Frenz
Jacques Hille, Jan Marten Wieleme, Jens Schepers, Matthijs Pals, Matthijs Tadmor, Noa Leijedalshoff, Owen Terstra, Phillip Vesley, Rianne Prins

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Faculty of Science and Engineering, University of Groningen
Prof. Dr. Jan Kok, Prof. Dr. Dirk Jan Scheffers, Dr. Andreas Millas Argeitis

Sources