

Panel discussion

Strain development:

Messages from the full scale

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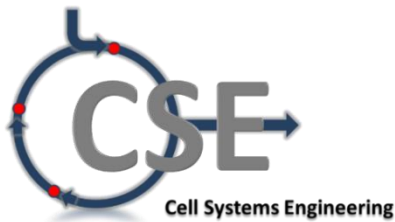
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Technology challenges and opportunities in

Commercializing Industrial Biotechnology

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Use more thermodynamics

- To calculate conditions in the best full scale in which the organism must function: need for **new lab evolution** approaches
- Future is **anaerobic**: hunt for robust anaerobic organisms
- **Energy design** of microorganisms: learn from anaerobic organisms in energy poor natural environments

Continuous process: culture degeneration problem

Approach:

Use stoichiometric (energy) coupling to growth with the product pathway as **only energy source**

- Anaerobic: coupling is realized
- Aerobic: strategies are needed
- Bonus of coupling: allows lab evolution to faster producers

Let the organism solve full scale problems

Cheap:

* subject organism to full scale conditions in lab evolution before using synthetic biology

Expensive:

* design the full scale to the properties of the organism

Combine



lab evolution protocols in scale down set-up

+

organisms selected from extreme environment
(CO₂, pH, osmo, temp, energy poor ...)

+

synthetic biology to guide direction of evolution

Feedstocks are a major cost factor

- There is more than (dilute) aqueous mixed carbohydrate solutions
- Try to avoid water addition, to lower DSP-cost
- Water free feedstocks H₂, CO, (m)ethanol, biogas, glycerol offer design opportunities



Microorganisms with broad substrate spectrum are needed

Full scale growth rate is always low $0.01 - 0.02\text{h}^{-1}$

Biomass retention to shrink fermentors

- Lab evolution to fast settling organism
- Synthetic biology targets
 - Flocculation
 - Modify cell size/cell density
 - Clumping by interfering with cell wall formation
- Reactor targets
 - Learn from biological waste water purification
 - Gravity based integrated biomass separation

← Separator

← Fermentor



Design the full scale as non-sterile selective environment

Industrial Biotechnology meets Environmental Biotechnology

- Continuous process
- Cheap, non-sterile, fermentors
- Stable product formation in presence of contaminating microorganisms

Challenge

How to design the full scale environment to maintain dominance of the producer organism

- **known:** acid treatment of yeast in ethanol fermentation
- ? learn from agricultural **Pest control**
- ? learn from our **immune system**

Fundamental questions in strain development

- **Inhibition** (CO₂, product, ...)
 - What are the mechanisms?
 - Role for product exporter?
- Which strategies do cells use to cope with full scale **feast/famine** dynamics (non-ideal mixing)
 - maintain stoichiometry using enormous flux flexibility
 - overflow metabolism
 - storage molecules
 - genetic control aspects
 - growth rate/cell cycle flexibility
- Culture **heterogeneity**
 - cell cycle
 - dead/living cells
 - producers/non-producers
 - substrate
- **Screening** under **full scale conditions**