Panel discussion

Strain development:

Messages from the full scale

J.J. (Sef) Heijnen
Department of Biotechnology
Delft University of Technology, The Netherlands

Technology challenges and opportunities in

Commercializing Industrial Biotechnology

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Cell Systems Engineering

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 (CO_2, 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 H_2, CO, (m)ethanol, biogas, glycerol offer design opportunities

↓

Microorganisms with broad substrate spectrum are needed

Full scale growth rate is always low 0.01 – 0.02h^{-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 form our immune system

Fundamental questions in strain development

- Inhibition (CO_2, 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