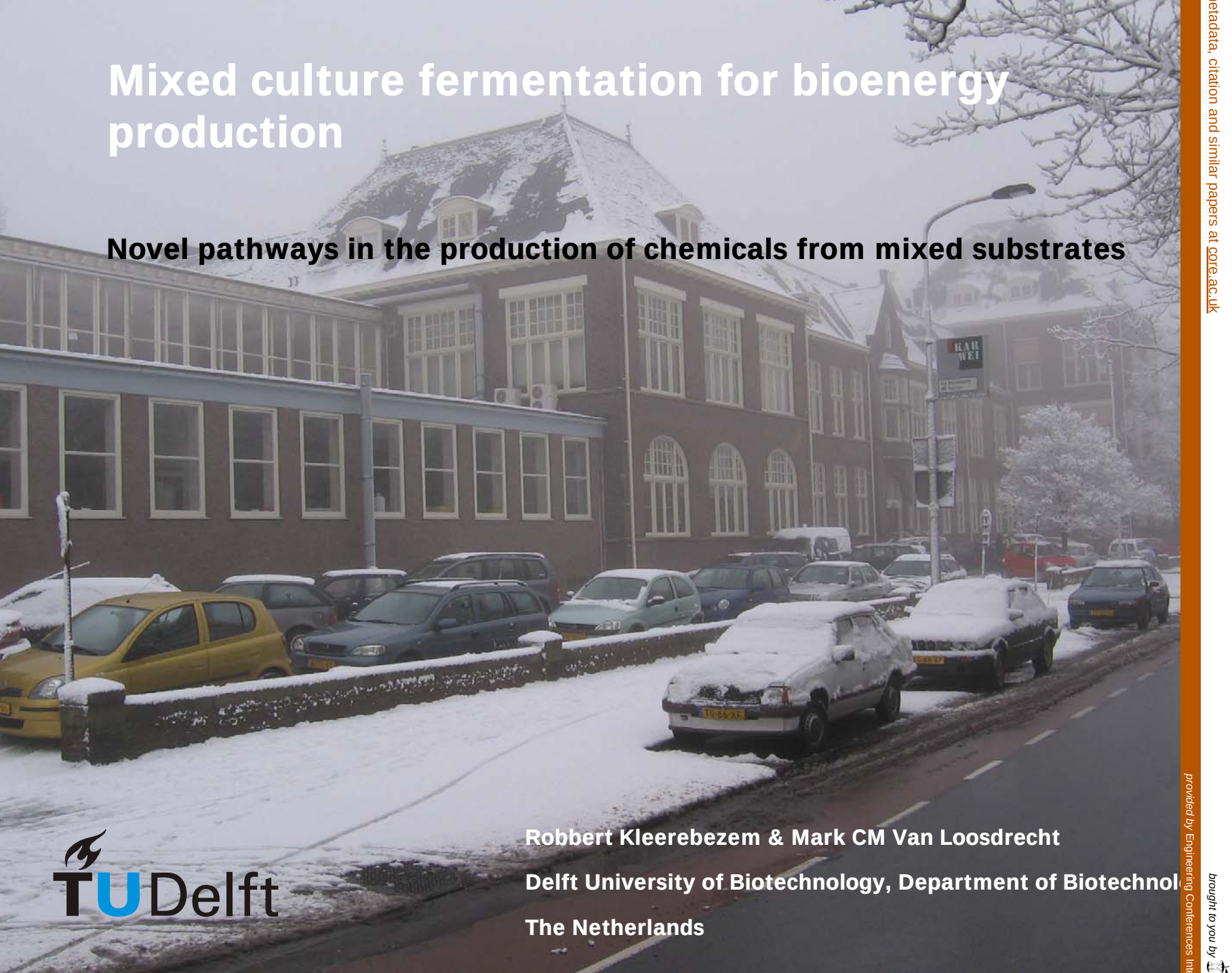
Mixed culture fermentation for bioenergy production

Novel pathways in the production of chemicals from mixed substrates



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Directed evolution in Mixed Culture Biotechnology (MCB)

Environmental Biotechnology

- Wastewater treatment,
- Mixed culture,
- Black b
- ...ration
- Mixed substrates
- Metabolic selection driven

Industrial Biotechnology

- (Anabolic) product f
- ...tabolic Information
- Maximize production
- Pure Substrates
- Specific organism and GE driven

Merge into Mixed Culture Biotechnology

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Advantages of MCB

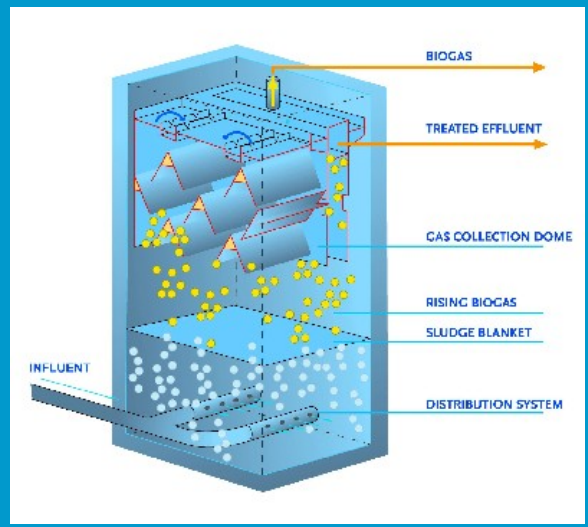
- Substrate mixtures e.g. agricultural waste,
- Mixed cultures can use complex waste streams,
- Adaptive capacity to changes in substrate/operation,
- No need for sterile operation,
- Lower energy demand,
- Simple process control.

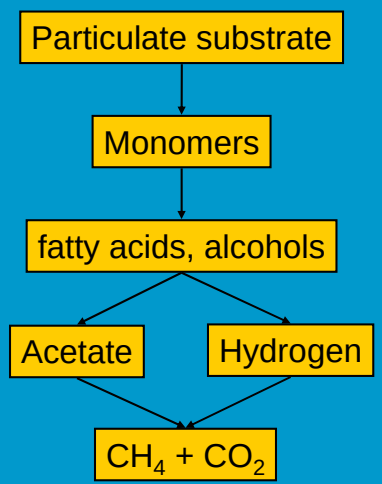
Disadvantages of MCB (?)

- Product purity,
- Product concentration (downstream processing),
- Only catabolic (lower value) products.

Classic example MCB: Anaerobic digestion

- Bioenergy production,
- Almost single product,
- No complex product recovery,
- Wide range of substrates,
- High volumetric productivity.





Hydrolysis/solubilization

Acidogenesis

Acetogenesis

Methanogenesis

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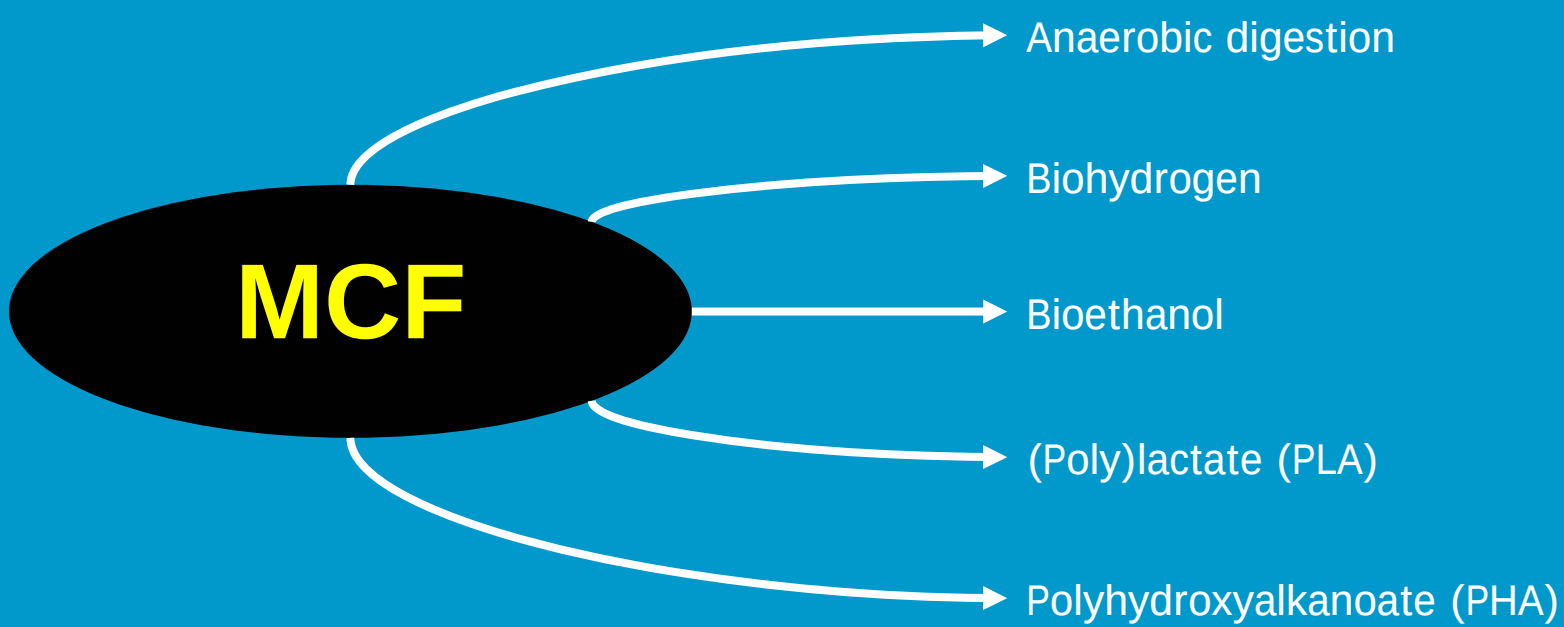
Basic research question

Can selective pressure in biological mixed culture processes be used to direct biological conversion of organic compounds towards useful products?

Process I: Mixed Culture Fermentation

Substrate: Carbohydrates, Glycerol...
Products: Acetate, Propionate, Butyrate, Ethanol, Lactate, Hydrogen...



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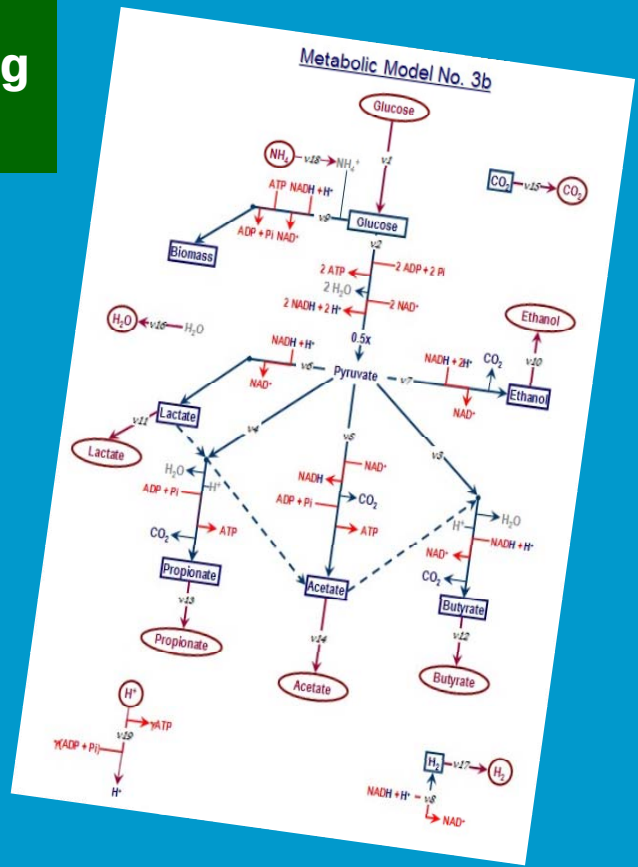
Basic research question

To which extent can we direct mixed culture fermentations to specific products by changing the process conditions?

Operational variables:

- Temperature
- Substrate composition and concentration
- pH
- Feed pattern (batch versus continuous)
- Nutrient (N, P...) availability
- Electron acceptor availability
- Seed material(?)



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Background

A lot of measurements are available, but there is no widely accepted selection mechanism that describes product formation in anaerobic Mixed Culture Fermentations.

But is there one dominant mechanism?

Influence of pH and Temperature

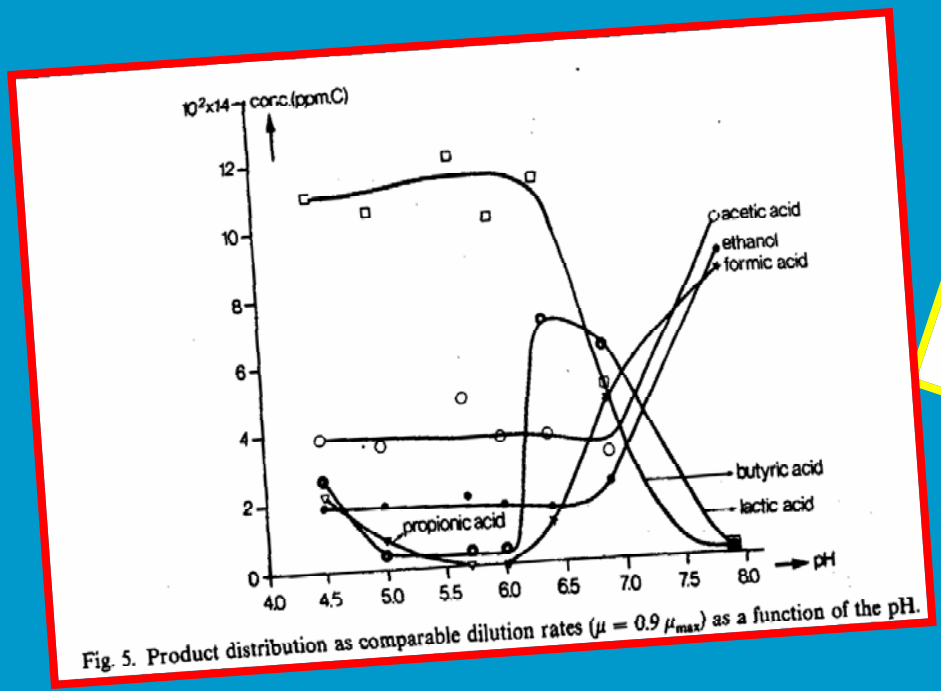

Fig. 5. Product distribution as comparable dilution rates ($\mu = 0.9\ \mu_{max}$) as a function of the pH.

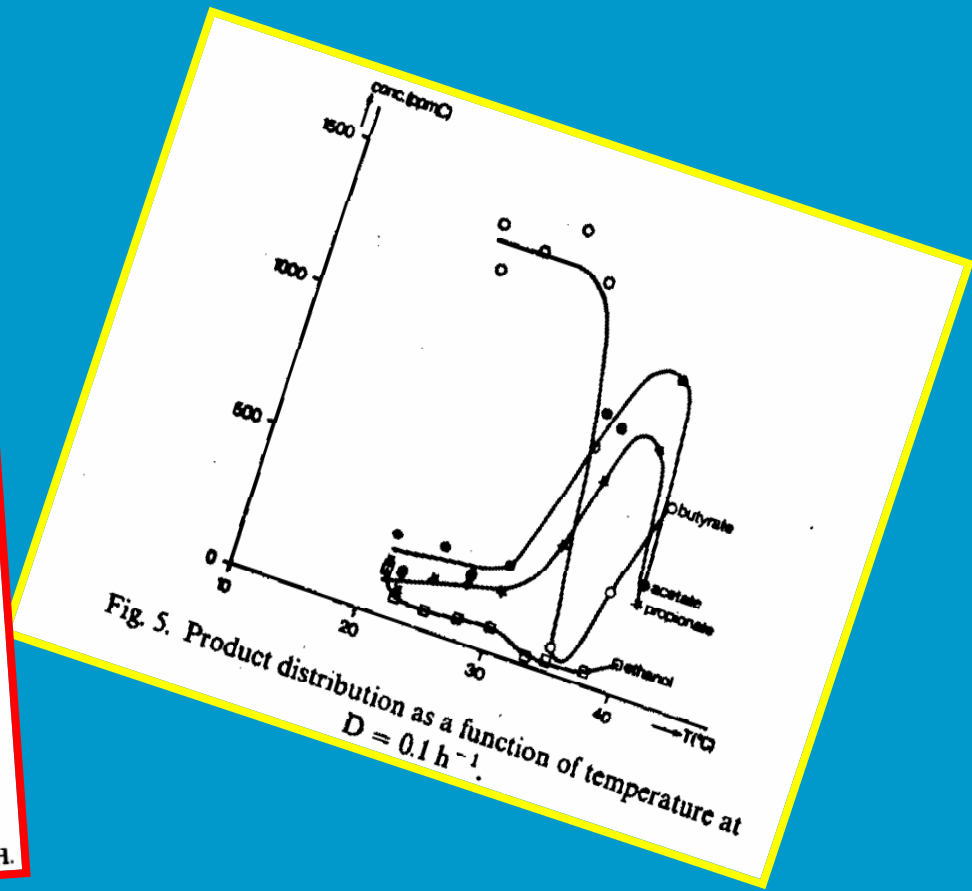

Fig. 5. Product distribution as a function of temperature at $D = 0.1\ h^{-1}$.

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Basic hypothesis

Environment selects for thermodynamically most favorable conversion, and environment selects organism that is capable of catalyzing this conversion most efficiently

And not the other way around!

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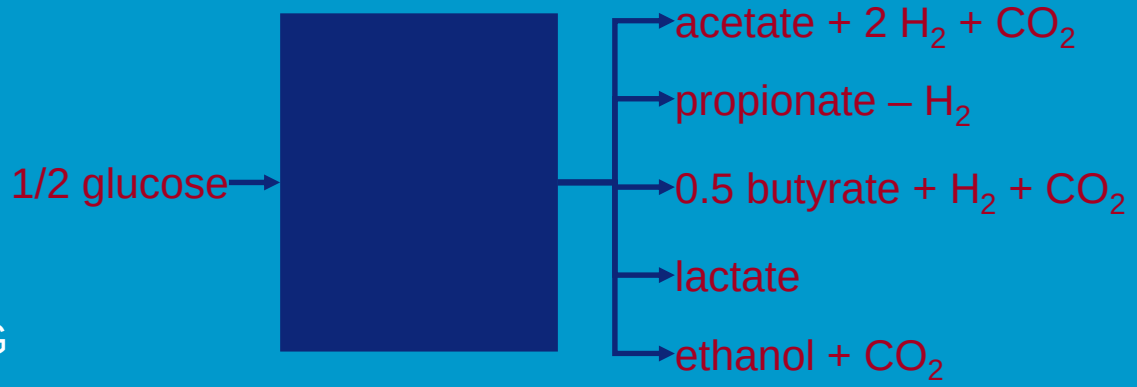
Modeling approach

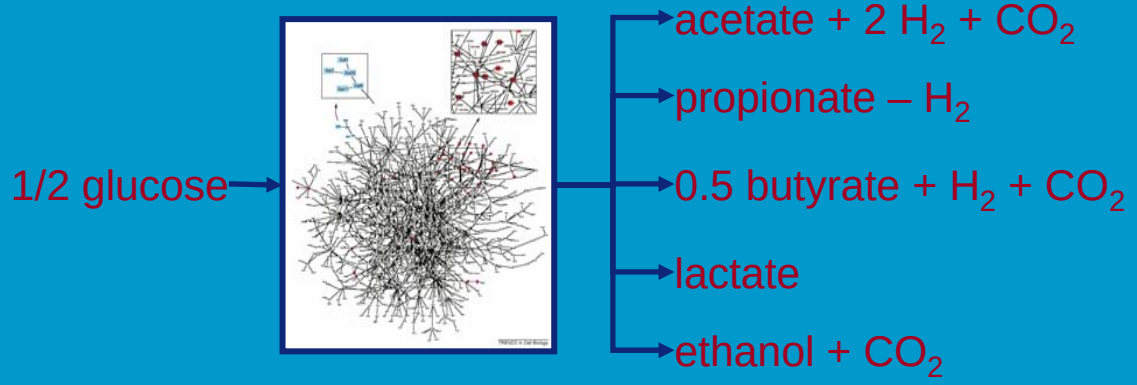
Black box approach

- Maximise Gibbs free energy production.
- Calculate free energy production of individual reactions and maximize ΔG of overall reaction.



White box approach

- Detailed biochemical network.
- Detailed kinetic and regulatory information.



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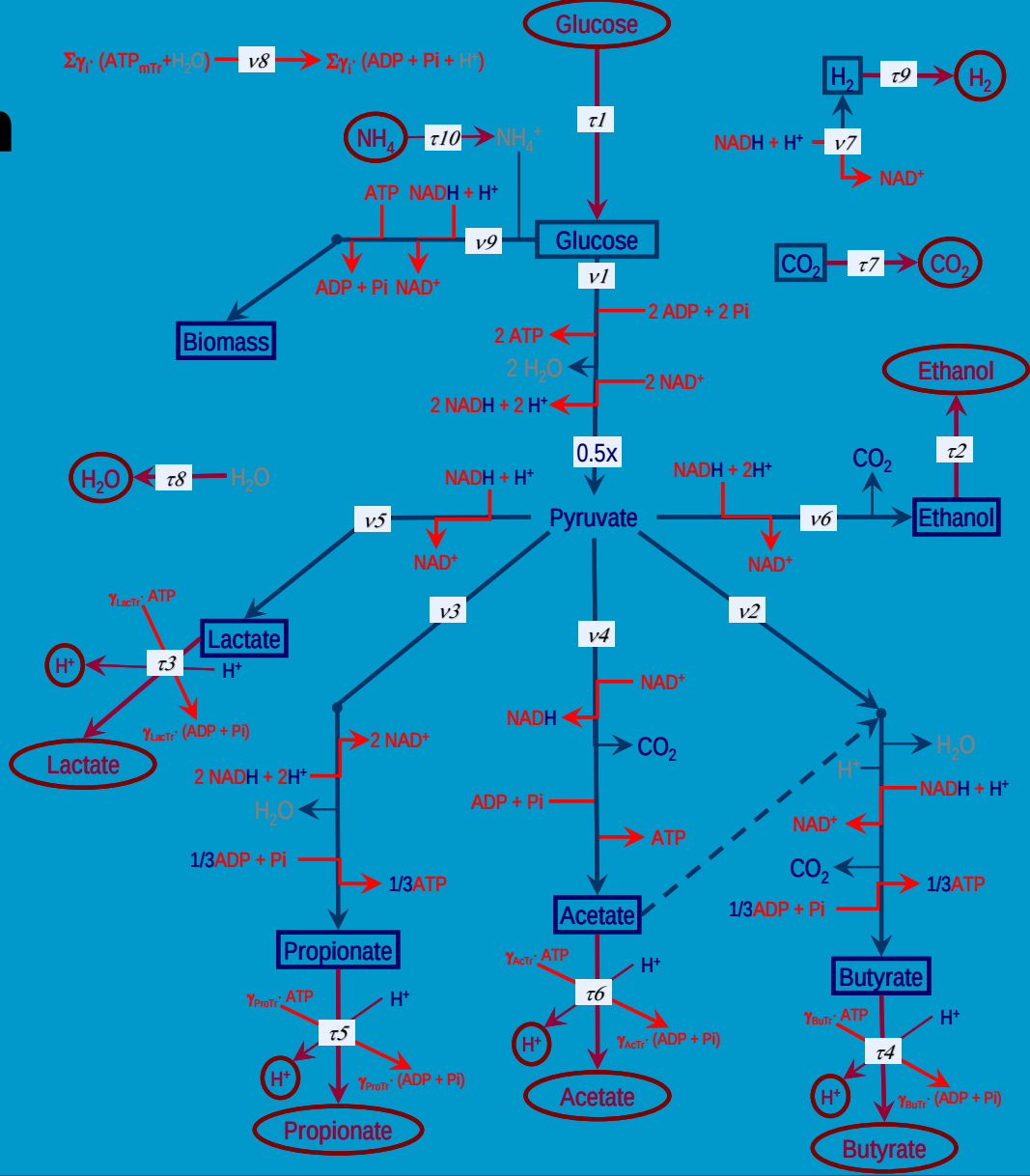
Grey box approach

Based on:

- Generalized biochemistry,
- Mass and Redox balances,
- ATP-yield



Reference:

Rodriguez, J, R Kleerebezem, JM Lema, and MCM van Loosdrecht (2006)
Biotechnology and Bioengineering **93:**592-606

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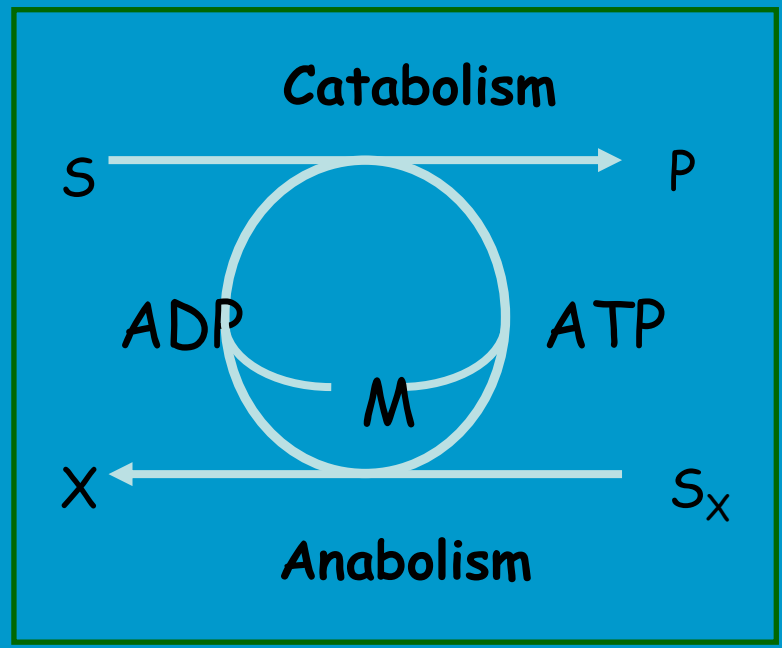
Assumptions and system solution

Flux optimization criterion:

- Maximisation of the free energy production and the biomass growth rate,
- Energy quantum ATP/ΔG



Boundary conditions:

- Thermodynamic feasibility,
- Mass and electron balancing
- Maximum intracellular product concentrations
- Lumped anabolic reaction
- Steady state, no competition

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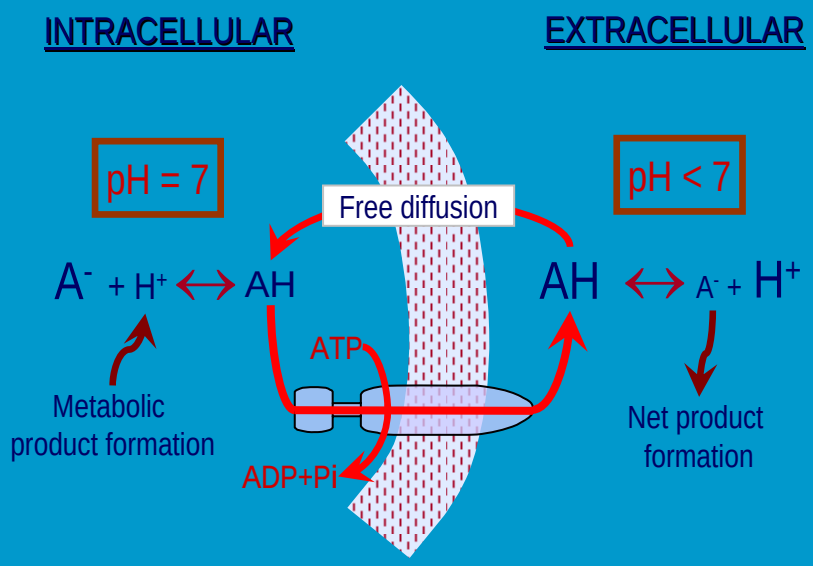
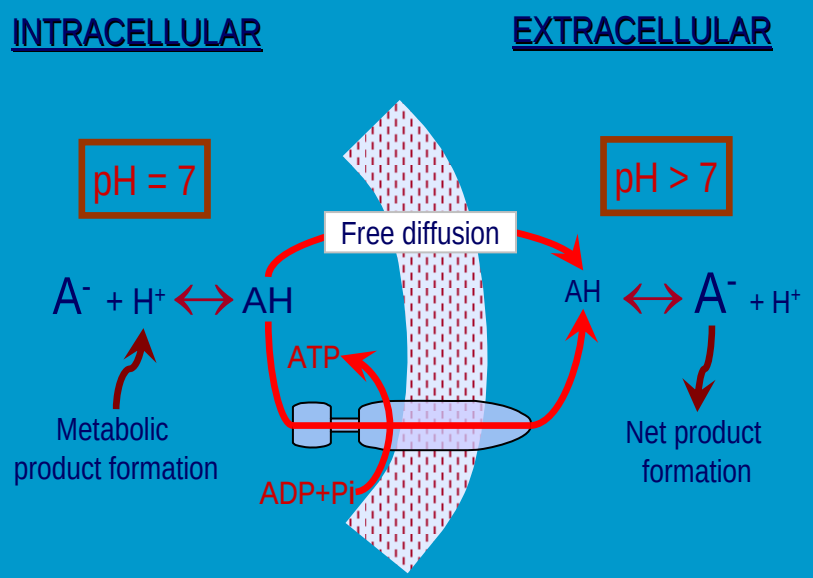
Membrane energy





The concentration gradients of chemical species over the cellular membranes can drive the generation or consumption of proton motive energy and metabolic energy is thus conserved.

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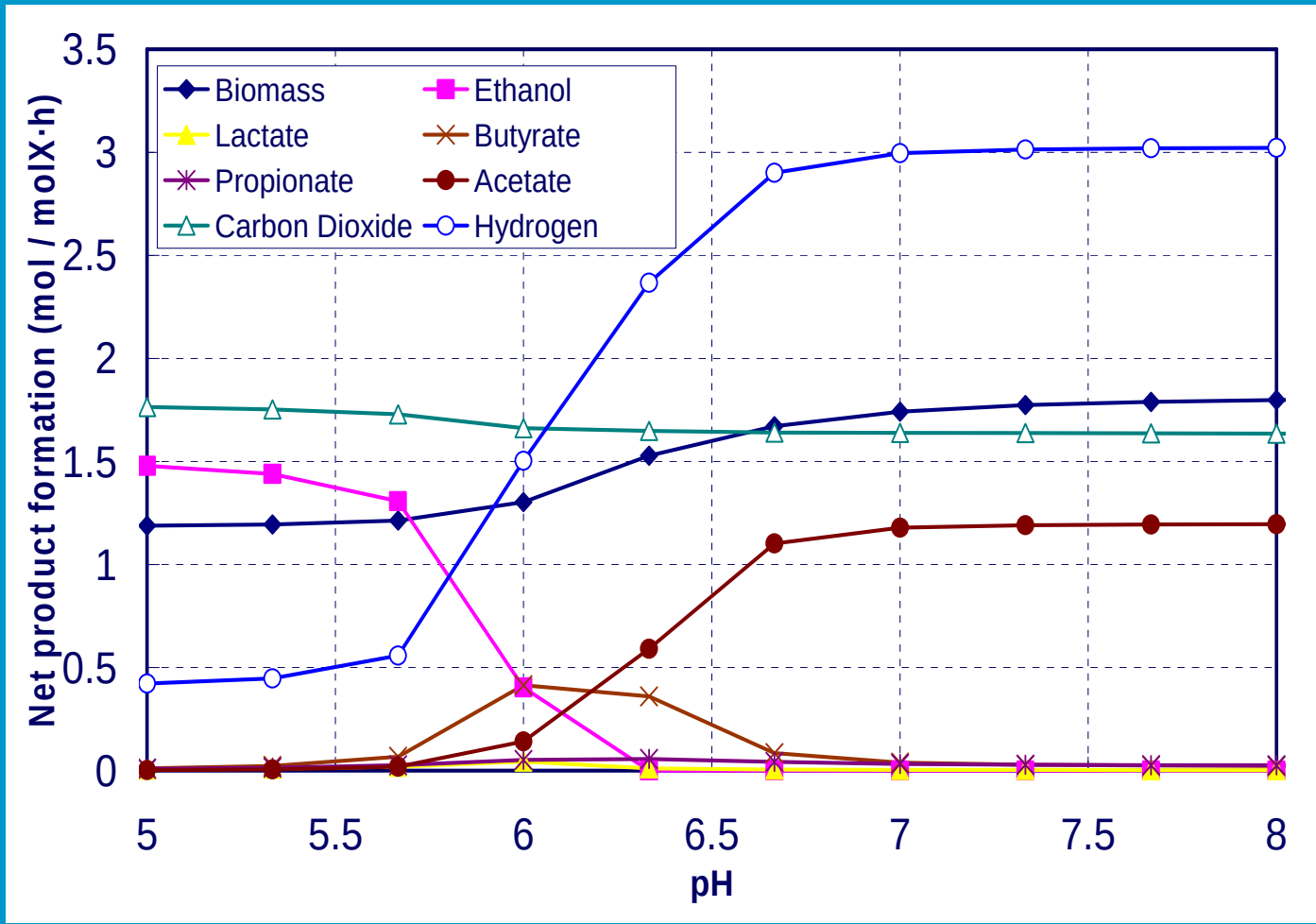
Results: pH



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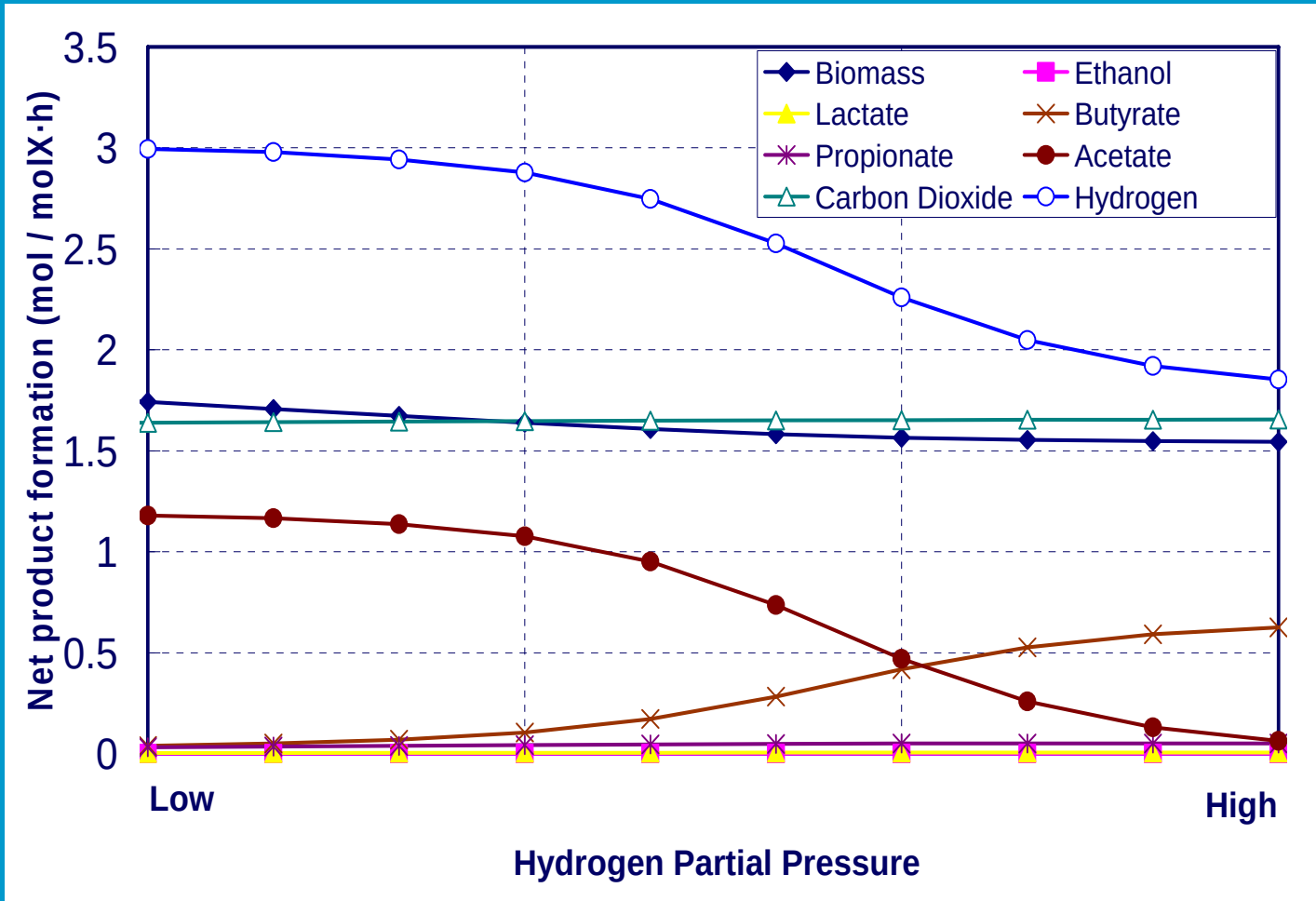
Results: H_2



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Model results

- **First** conceptual MCF model,
- Thermodynamics provides additional constraints,
- Introduction of biochemical generalizations increases information density of mixed culture models,
- High metabolic stability can be obtained with low population stability.

Preliminary experimental results

- Acetate and butyrate are the main products at around neutral pH,
- Fist lactate production,
- High lactate or ethanol production seem possible

But...

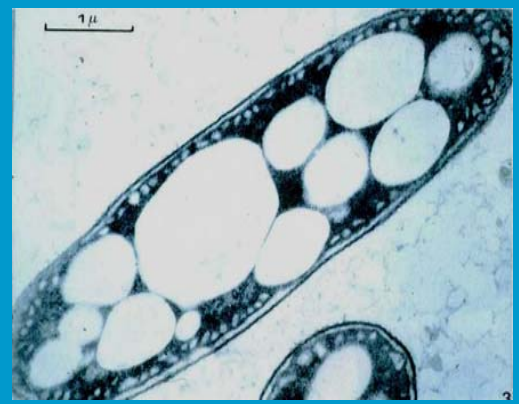
- Process generally unstable,
- Adaptation/selection periods can be very long.

Future...

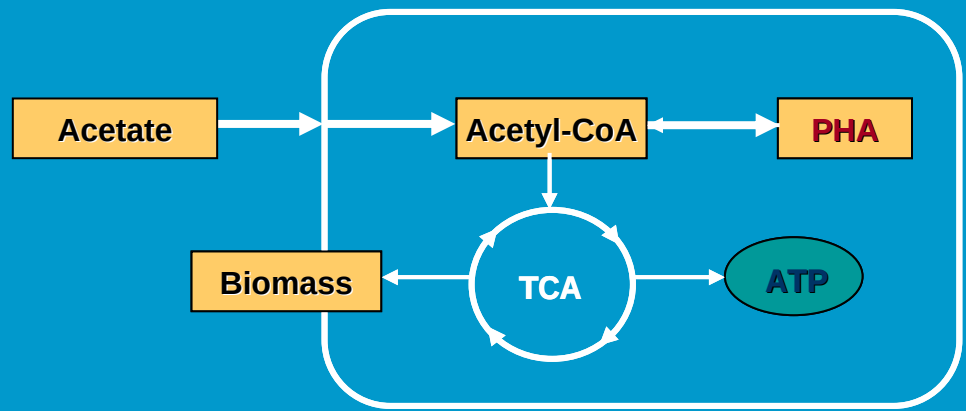
- More experimental data!
- Metabolite measurement / MCA
- Population dynamics
- Force / Flux
- Model extension

Example II: PHA production

- Polyhydroxyalkanoates, bioplastics,
- Up to 80% of cell dry material,
- Selective pressure: presence/absence of substrate,
- More balanced growth by storage
- Monomer building block organic synthesis



Feast:



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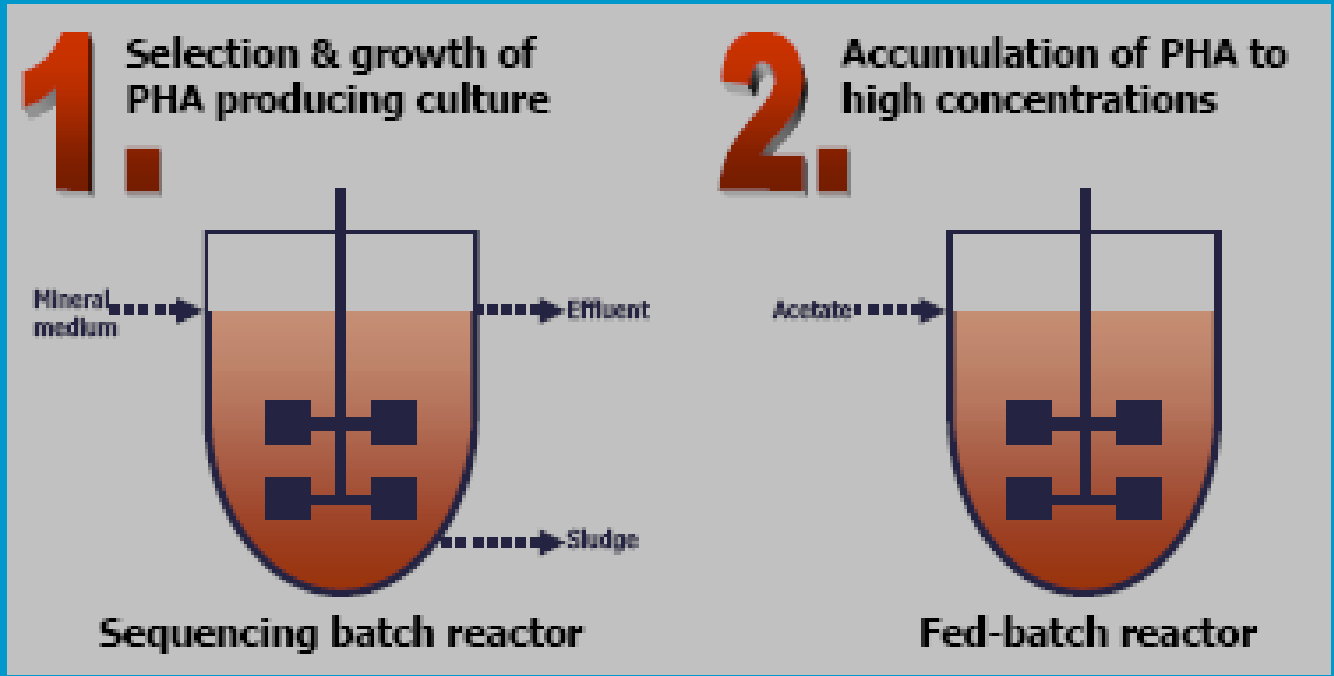
Production strategy



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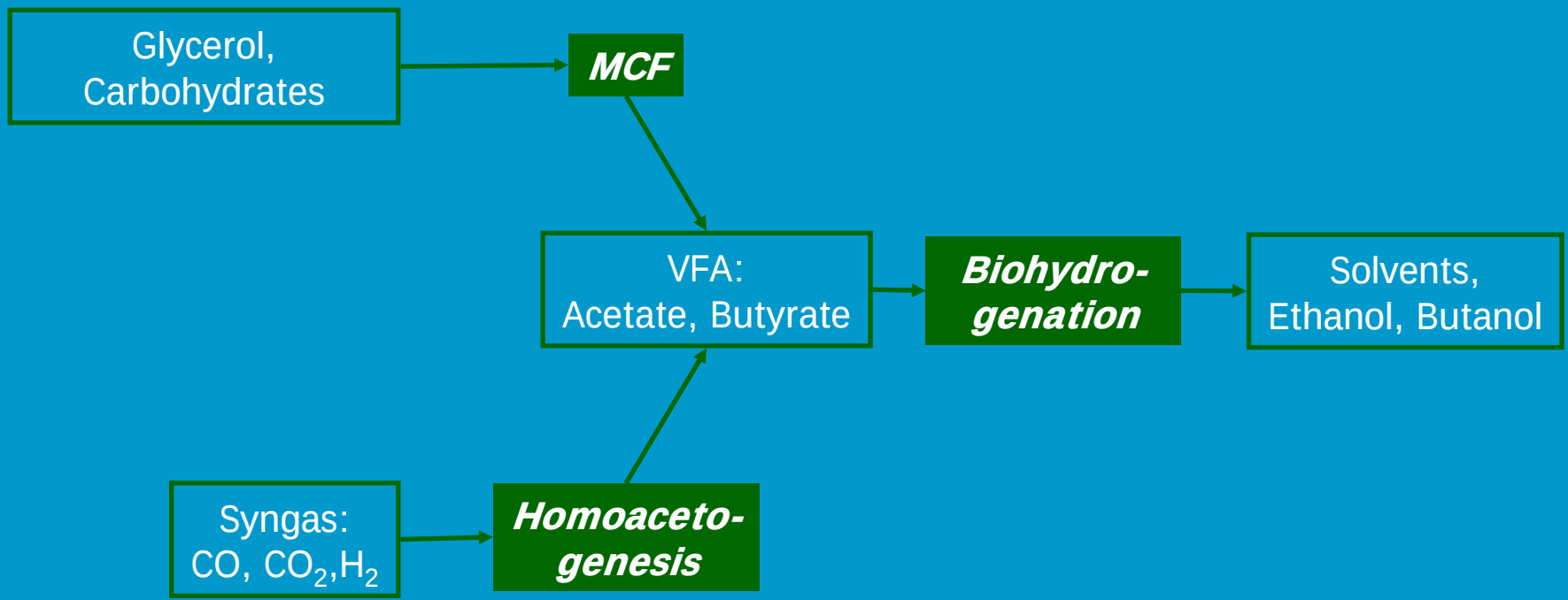
Example III: Biohydrogenation



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Workshop

- Mixed culture biotechnology for production of chemicals and bioenergy,
- Delft University of Technology
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- 12/13 June 2006
- www.bt.tudelft.nl

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Questions?