TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

Bioprocess Technology

RESTRUCTURING RENEWABLE ENERGY SOURCES FOR MORE EFFICIENT BIOFUELS PRODUCTION WITH EXTREMOPHILIC MICROORGANISMS

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Agenda

Our mission and concept

I. Biological methanogenesis

II. Biohydrogen production

III. Biological conversion of waste streams to high value added products

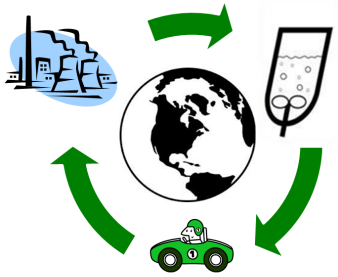
Conclusions

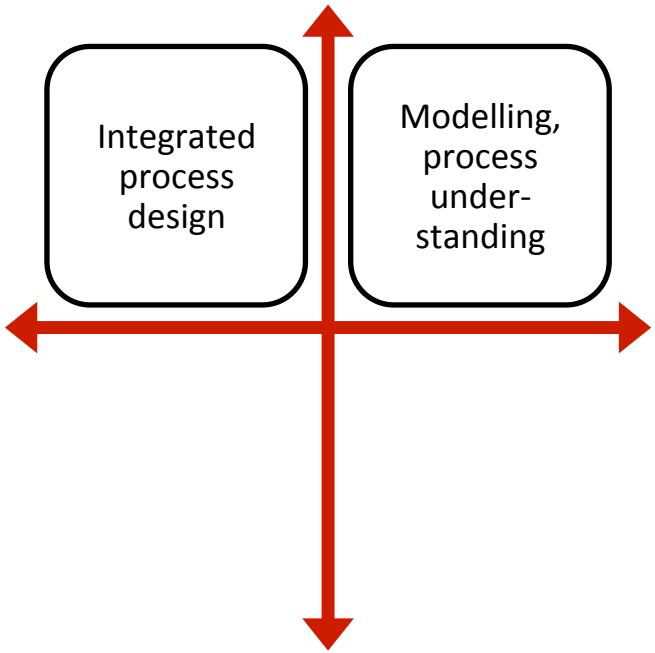
Outlook towards new biofuel generations & Waste to value

Mission

Contribution to new biofuel generations:

- integrated biological systems
- CO_2 neutrality
- process intensification by coupling of waste streams
- “Waste to value“ principles: bioproducts from waste streams





Sustainable bioprocessing

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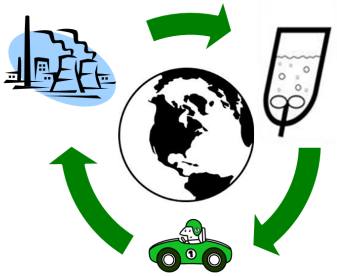
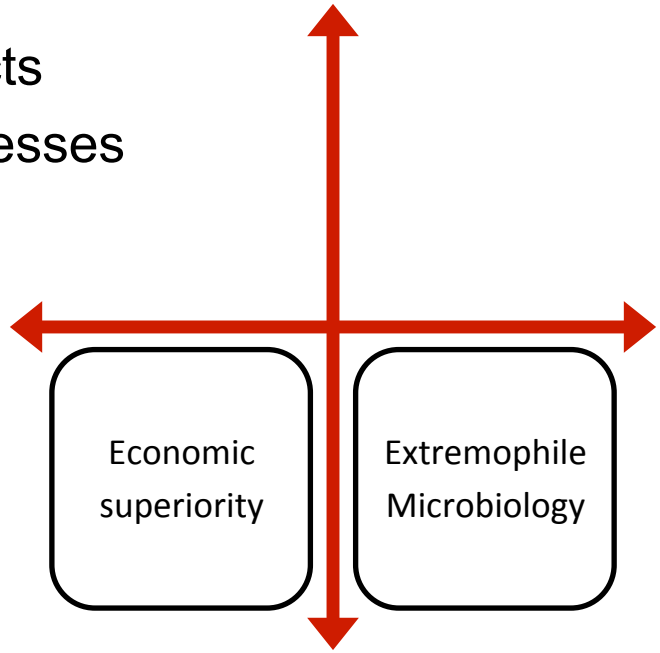
Extremophilic microoganisms

Living at extreme environmental conditions:

(e.g. high temperature, very alkaline conditions or high salt concentrations)

- special features and bioproducts
- cost-effective non-sterile processes





Coupling of effluent streams

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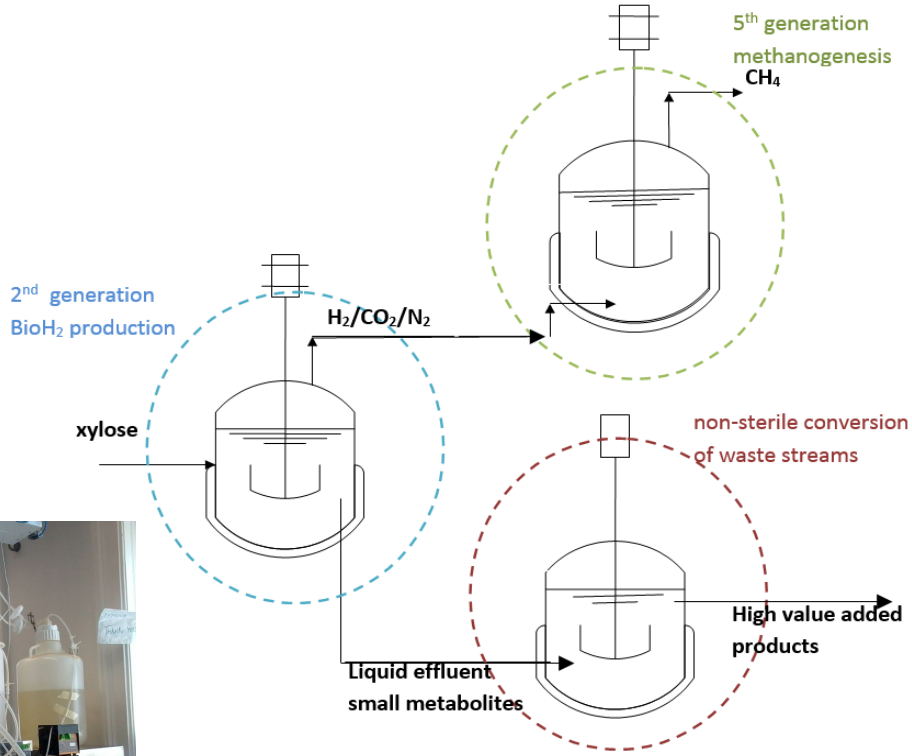
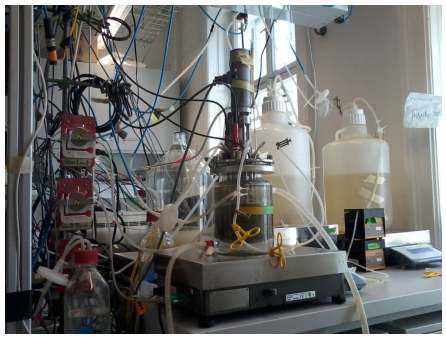
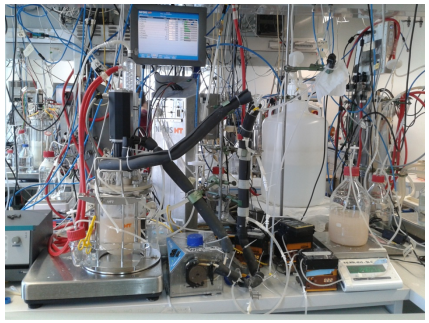
Process intensification







Sophisticated bioreactor setups

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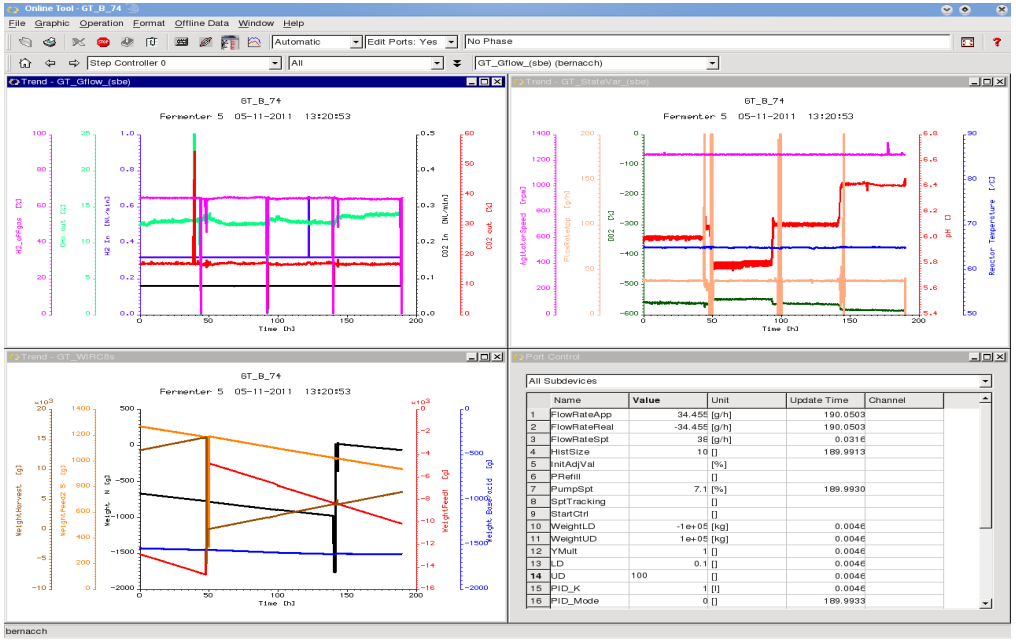
- Online process control with lucullus (PIMS)
- Online monitoring of process and signals
- Multi analytical methods for process quantification
  - GC, HPLC, ICP-OES/MS, Enzymatic/colorimetric methods on Cubian.XC and/or cedex bioHT and spectroscopic methods



Introducing biomethanogenesis

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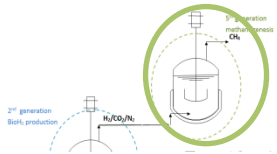


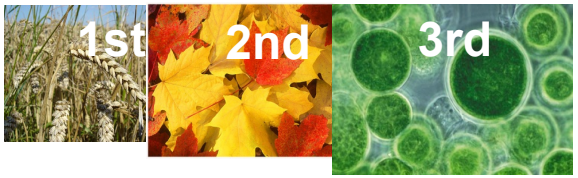
- Methanation reaction:

CO_2 + 4 H_2 → CH_4 + 2 H_2O - 130 kJ/mol

4 x 3 kWh 10 kWh efficiency, 83 % max.

Catalyst *"Archaea"*

Process *"Methanogenesis ("Biological Methanation")*

CO_2 / water —light→  —0.1 kg/m³ x h→ CH_4

+ H_2 reduction agent !

fast

out of "food for fuel"

"Photosynthetic Bypass"

4 th Generation Biofuels

(> 15 kg/m³ x h)

Why Biological and not Chemical?

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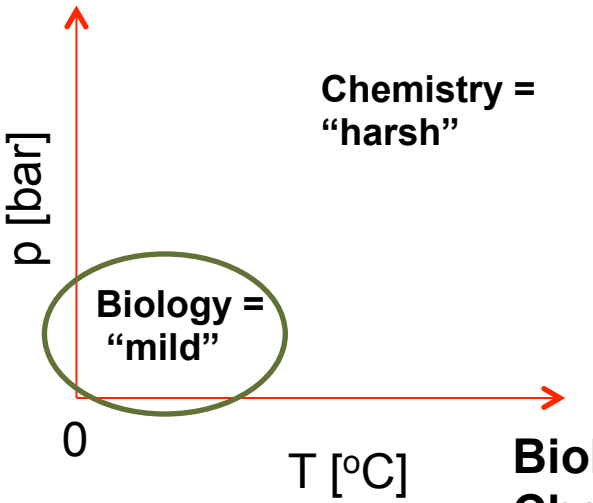
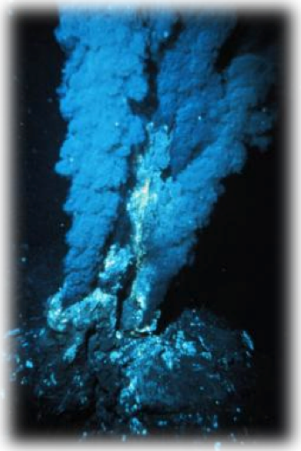
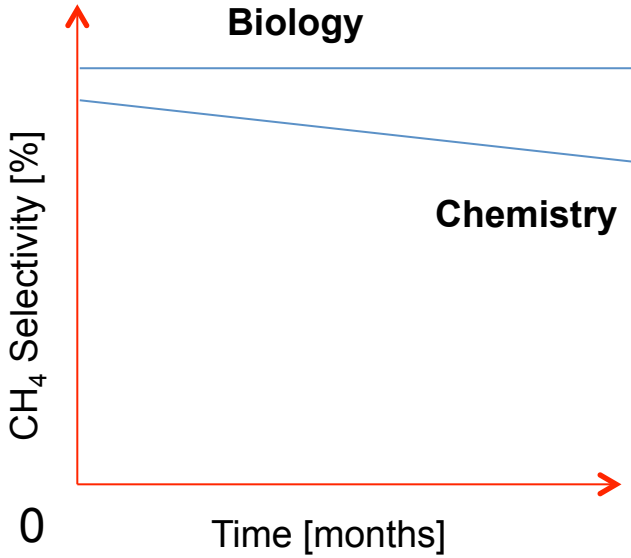
- Operating conditions
- Chemistry: T = 200 – 400 °C / P = 50 – 200 bar
- Biology: **T = 35 – 70 °C P = 1-10 bar**

- Chemical process form energy losses:
  - Heating
  - Compression





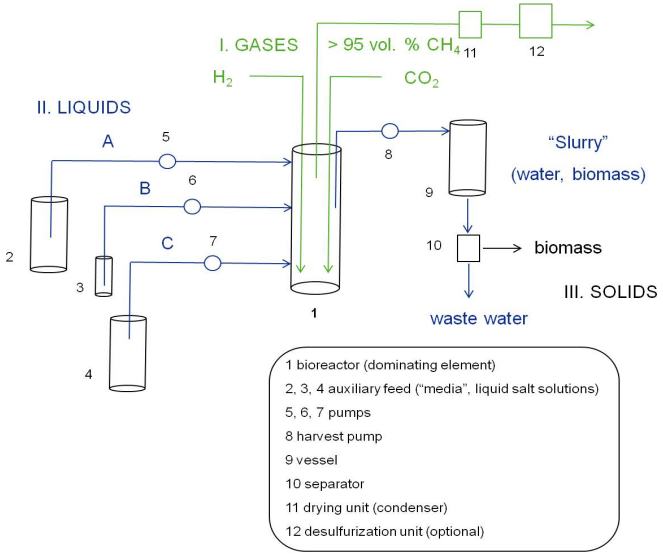
- Chemistry need high purity feed stocks
- Biology has strategies to **extract nutrients from gas mixtures**

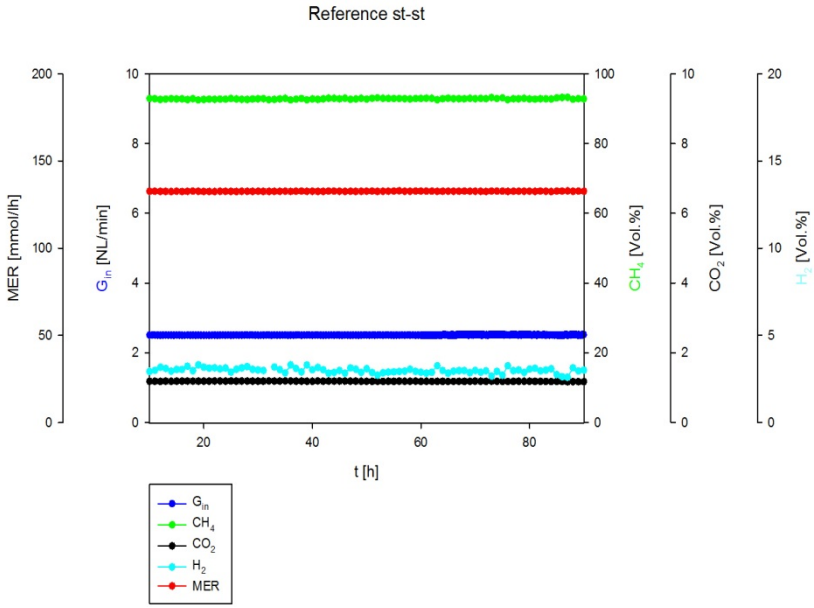


Biology makes from mixtures 1 product, Chemistry makes from pure components mixtures !

Biomethanogenesis

- Is a simple bioprocess running under gas transfer limitation
- Allows in a single step to produce from H_2/CO_2 mixtures an high quality CH_4 under mild conditions





Biomethanogenesis for “Power to gas”

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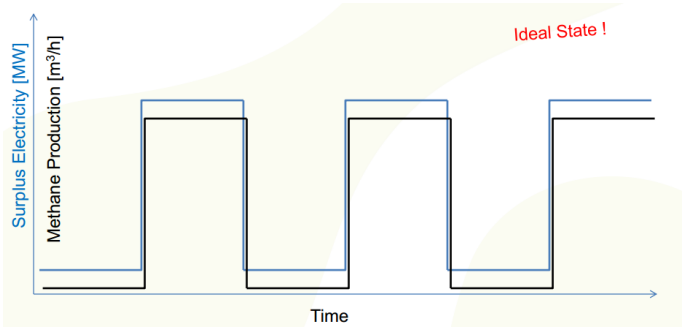
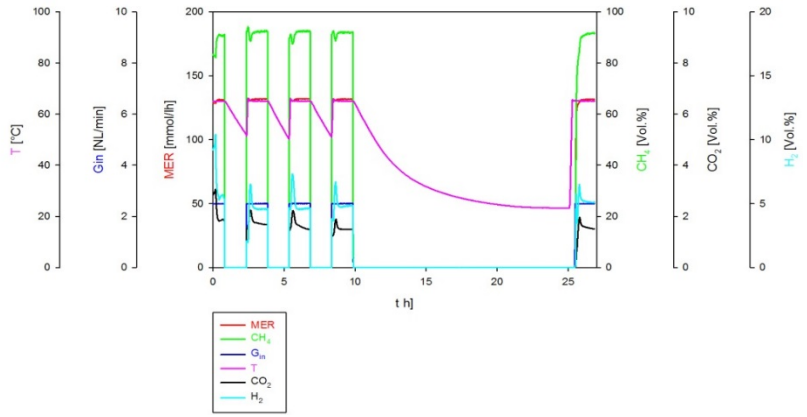
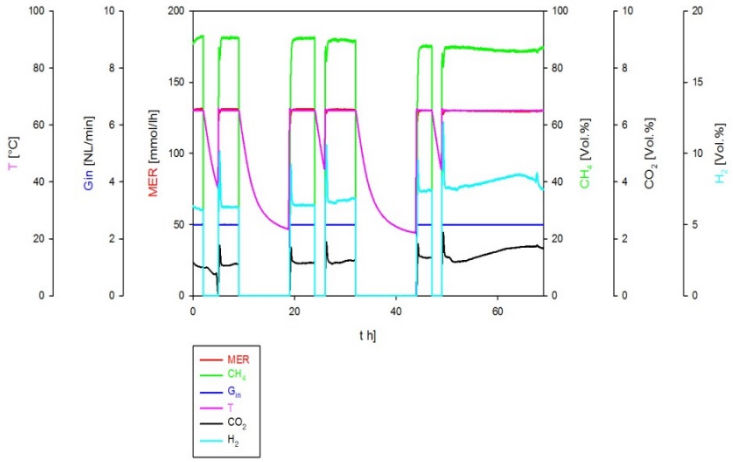
- The concept of power to gas originated from storing renewable electricity peaks into the form of natural gas
- How would an ideal response look?



Intermittent quadruplet



Intermittent profile 70h



Single step biogas upgrade to bioCH_4

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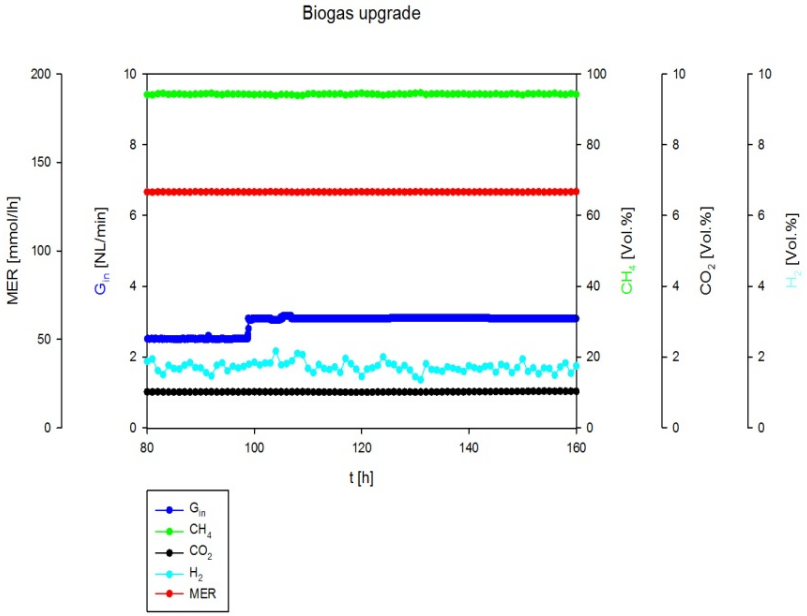
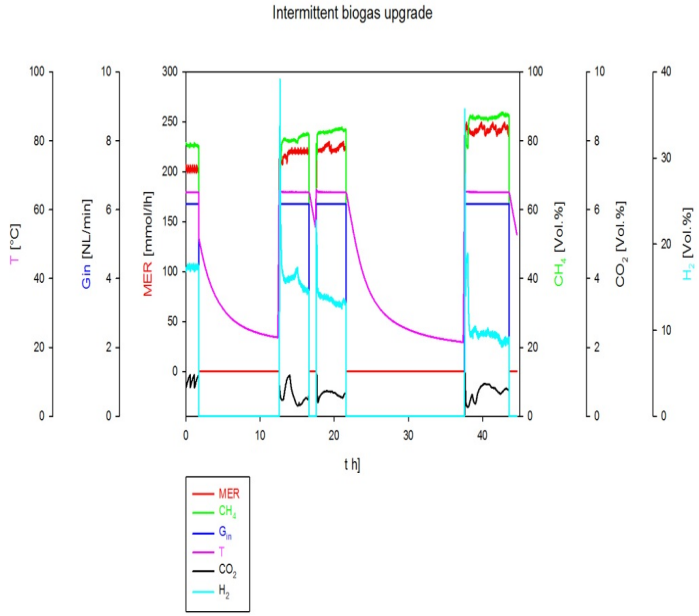
- Use of H_2S and CO_2 contained in biogas as raw materials for biomethanogenesis
- Efficient way of upgrading any kind of waste gas containing CO_2 (incineration gas, biogas etc.) and H_2 (syngas, waste streams etc.) into natural gas





Biohydrogen production

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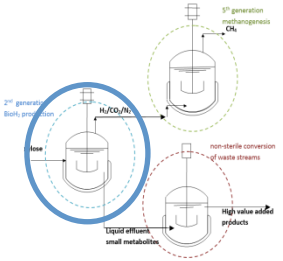
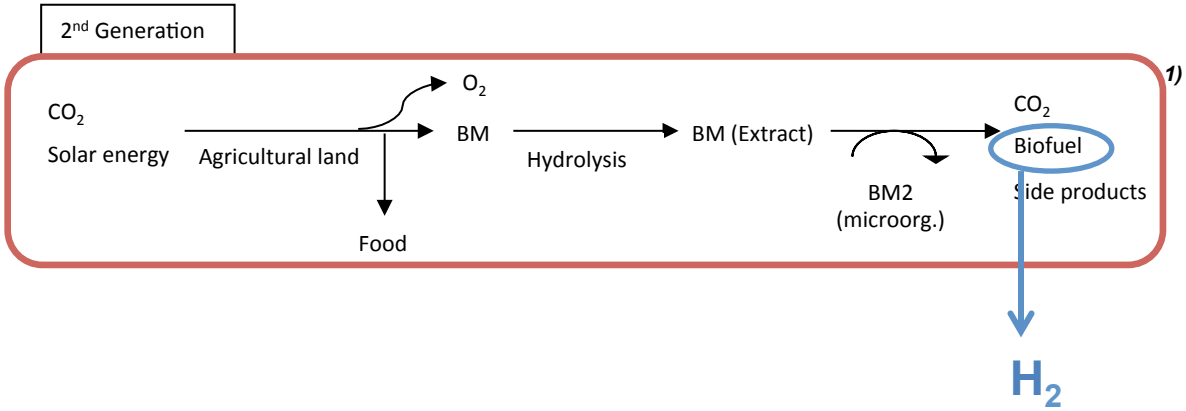
- H_2 production

Process	C-Source	Energy-Source
Dark Fermentation	Sugars	Sugars
Photofermentation	Organic acids	Light
Photosynthesis	CO_2	Light





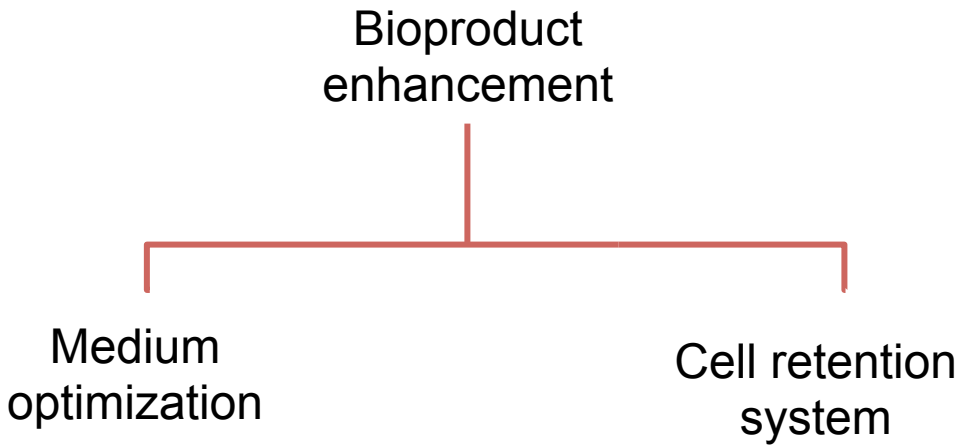
- BM (Extract): Xylose from the hemicellulose
- BM2: *Caldicellulosiruptor saccharolyticus* (strictly anaerobic asporogenous thermophilic Gram-positive bacterium)

(1) Martinez-Porqueras E, Rittmann S, Herwig C. Biofuels and CO2 neutrality: an opportunity. Biofuels. 2012;3(4):413-26

Goals

- Biohydrogen production

  To increase biohydrogen productivities and yields on xylose by the strain C. *saccharolyticus*



Technology

Bioprocess Technology

- Screening in serum flasks
- Batch and Continuous runs in a bioreactor
  - Verification of new medium components

		C. saccharolyticus (1)
Temperature	[°C]	72.5
pH	[-]	6.7
N_2 in	[L/h]	7.0
Agitator speed	[rpm]	150 / 350

Parameters:

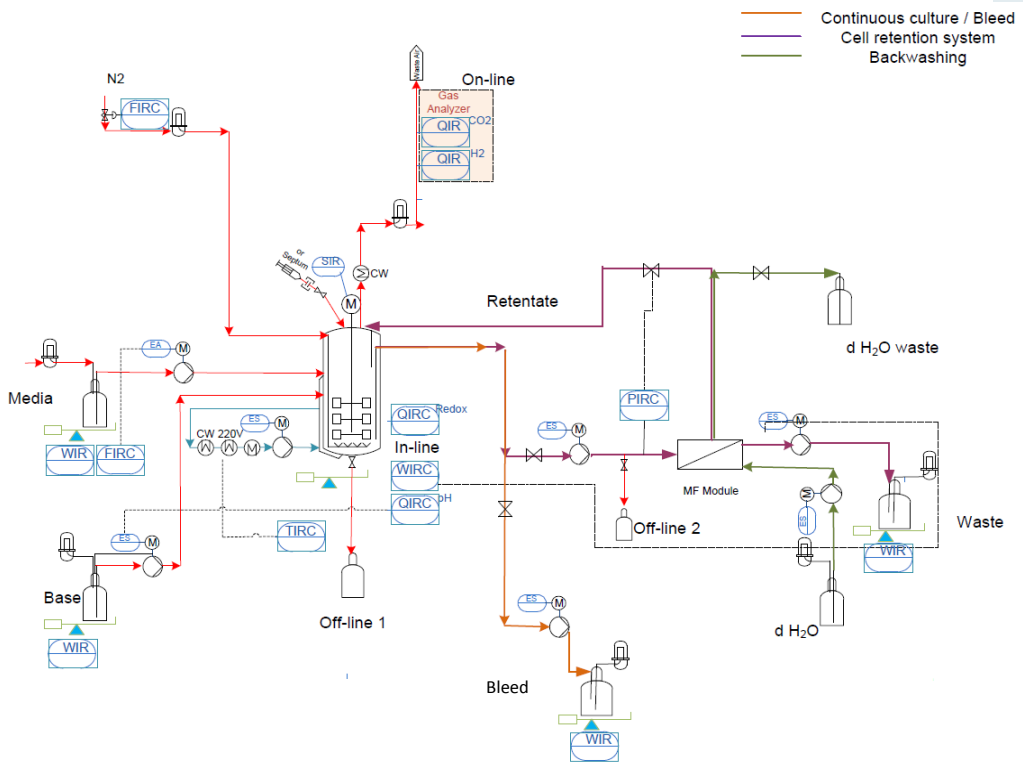
T= cte.

pH ≈ cte.

Agitator speed = 0 rpm

Tangential Cross Flow rate (TCFR)

X Cellular stress



(1) De Vrije T. et al. Glycolytic pathway and hydrogen yield studies of the extreme thermophile Caldicellulosiruptor saccharolyticus. Appl Microbiol Biotechnol. 2007;74(6):1358-67.

Medium optimization in biohydrogen production systems [1)]

Bioprocess Technology

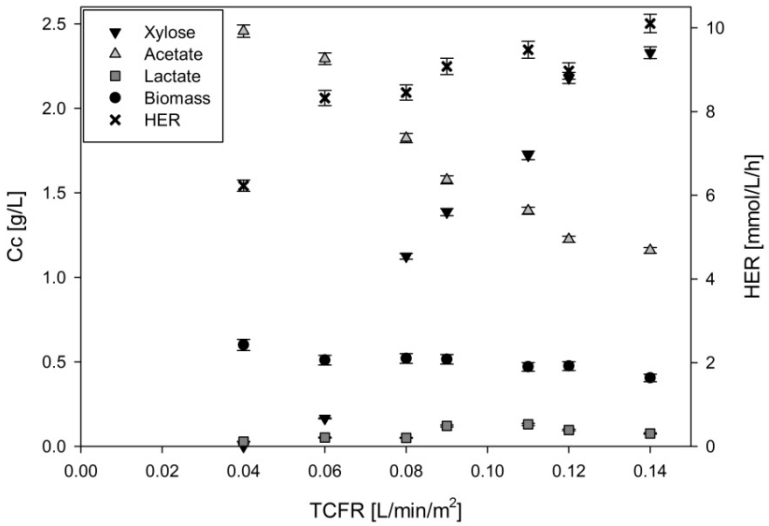
- Biohydrogen production on xylose by *C. saccharolyticus*:
  - Batch cultures:
    - HER, q_{H2} and $Y(H_2/s)$: Complex medium > Defined medium
    - $Y_{(H2/CO2)}$: Complex medium = Defined medium
  - Continuous culture:
    - C-limiting conditions at $D< 0.1\ h^{-1}$
      - HER_{max}, q_{H2max} => $D=0.1\ h^{-1}$
  - Double C-N-limitation on continuous mode:
    - $NH_4^+ < 1.5$ mM, if working with a defined medium in chemostat mode.
    - N-limitation => $q_{H2.}$

(1) Martinez-Porqueras E, Wechselberger P, Herwig C. Effect of medium composition on biohydrogen production by the extreme thermophilic bacterium Caldicellulosiruptor saccharolyticus. Int J Hydrogen Energy. 2013;38(27):11756-64.

Cell retention in biohydrogen production systems [1)]

Bioprocess Technology

- Start-up
  - Continuous culture with external loop (CCEL)
    - Adaptation of the cells to the new growing conditions
  - Total cell retention (TCR)
    - Increase of the biomass concentration



HER, xylose and metabolite concentrations of *C. saccharolyticus* cultivated on xylose (4.5 g/L) in a TCR system at different TCFRs

- C- limiting conditions at $D=0.1h^{-1}$ for $TCFR \leq 0.04$ L/min/m²
- HER increase at simultaneous increasing D and TCFR
- ✗ $HER_{TCR} \approx HER_{Conti}$
- ✗ No biomass increase
  - No cell lysis
  - Cellular stress

(1) Martinez-Porqueras E, Herwig C. Quantitative assessment of key physiological parameters of the extreme thermophilic bacterium Caldicellulosiruptor saccharolyticus grown in an external cell retention systems. Renewable Energy (under review).

“Waste to value” with Halophiles

Bioprocess Technology

Halophiles can generate extra values on diverse industrial waste streams

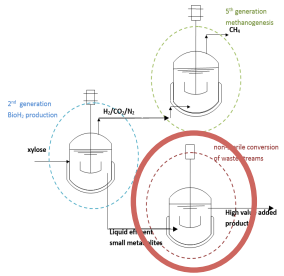
Use of wide variety of carbon sources: organic acids, diverse sugars, the sugar alcohol glycerol, aromatic compounds, etc.

Production of valuable bioproducts like carotenoids or biopolymers (PHA/ PHB), recombinant products

- quantitative bioprocessing technology
- on defined medium
- salt has to be added to the waste stream - in a corrosion resistant bioreactor
- the non-sterile process can be implemented in any industrial environment
- easy downstreaming of intracellular products, as disruption of cells can happen automatically in water due to osmotic shock
- scalability given through defined medium und the use of bioreactor with controlled and defined cultivation conditions

Technology - overview

Bioprocess Technology

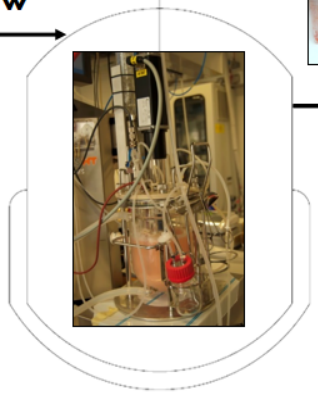


„Waste to value“ technology for hypersaline waste streams

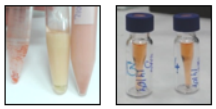
Waste streams
with organic matter
Non-sterile

Halophiles
NaCl > 20 %w/w

Non-sterile bioprocessing
in a corrosion resistant bioreactor

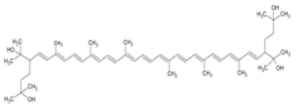


Downstream
processing

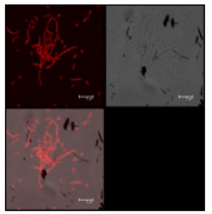


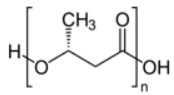
PHBs as
subcellular
granules

Carotenoids



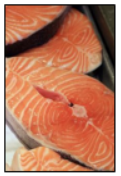
Bioproduct analysis





Biopolymers (PHB)

Use



Physiological characterization studies

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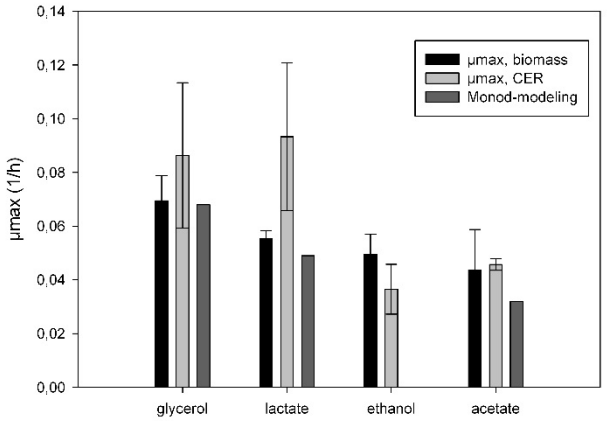
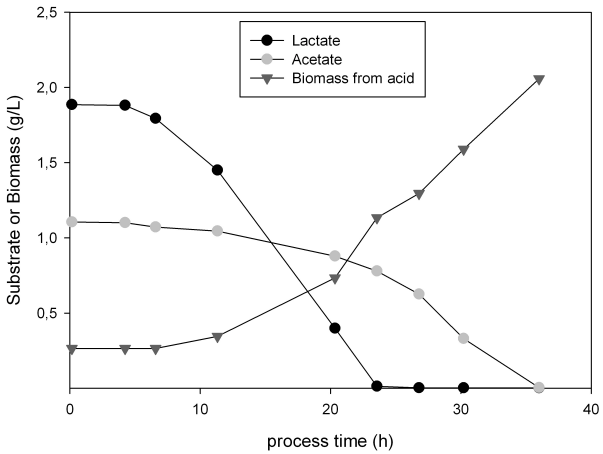
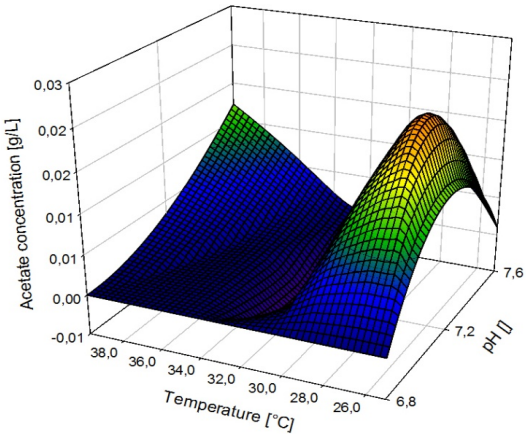
- corrosion resistant bioreactor setup for extreme halophiles
- quantitative data on stoichiometry and the kinetics on different carbon sources
- process parameter optimization
- used carbon sources: they are common residues in industrial waste streams







Halophilic bioproducts

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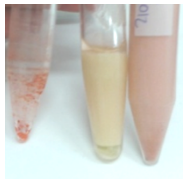
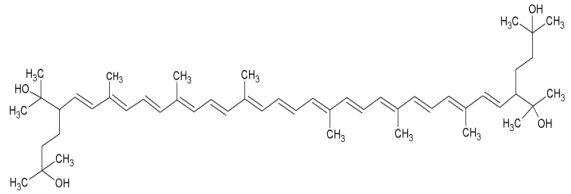
- Carotenoids

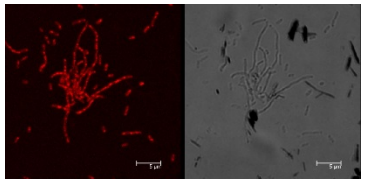
Potential applications: food colorant, dietary supplement, anticancer material

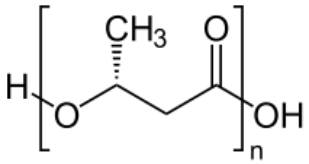




- Biopolymers: Poly-hydroxy-alkanoates

Potential applications: biodegradable thermopolyester





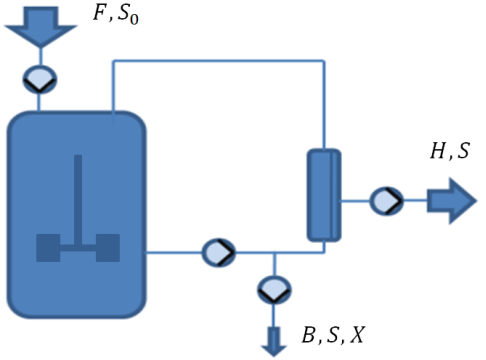
or

- Recombinant products

Potential applications: biopharmaceutical, therapeutic proteins, etc.

Productivity

- High biological activity and volumetric productivity
- the produced biomass is retained in a bioreactor
- Characterization of an external cell retention system
- 10-fold productivity increase



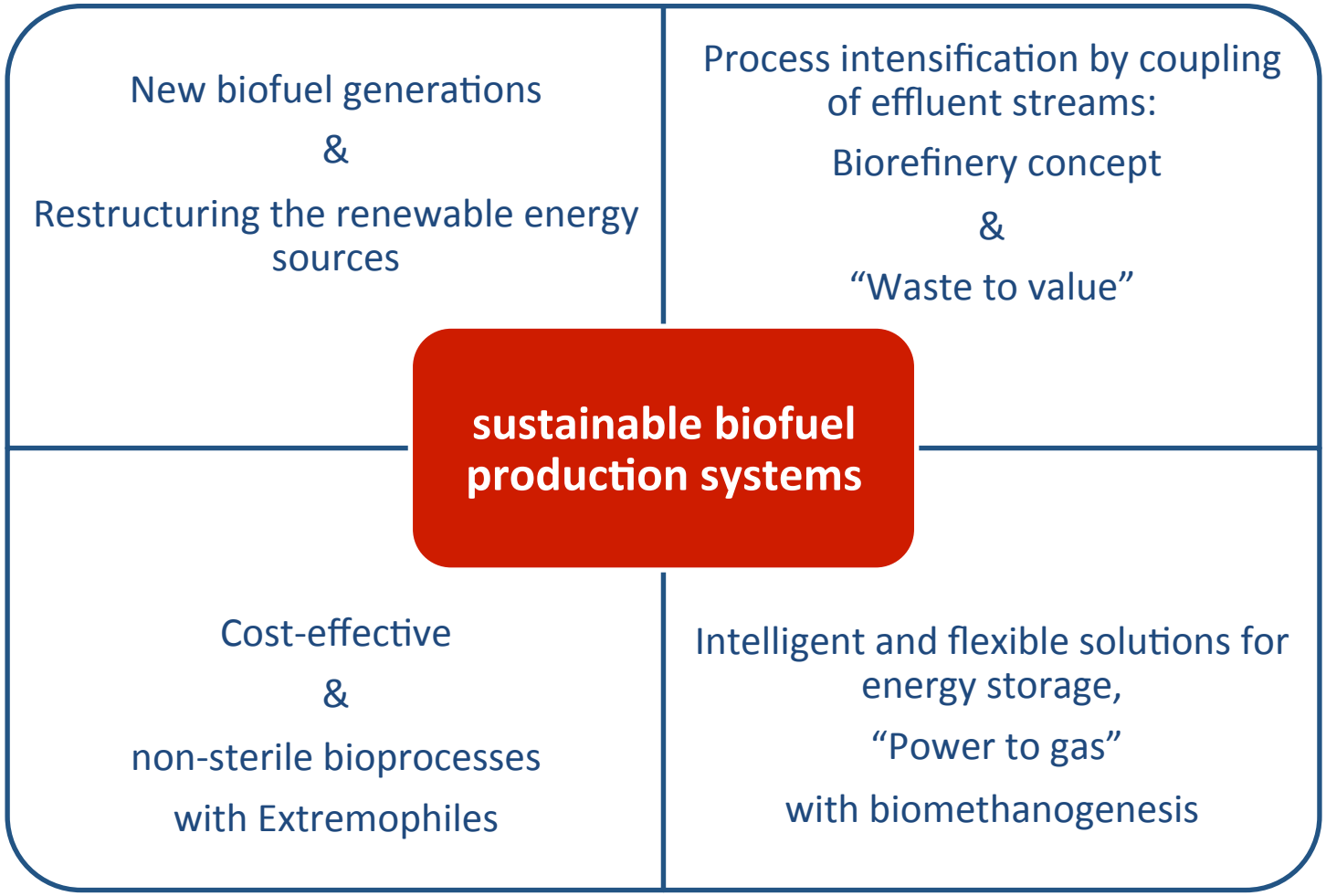
Real-medium applications

benefits of controlled bioprocessing with extreme Halophiles:

- pending patent application of TU Wien
  - the use of Halophiles
  - for biological conversion of the waste stream from biohydrogen production with small metabolites

- potential biotechnological applications with Halophiles
  - wide ranges of intelligent process intensification solutions “waste to value”
  - valuable bioproduct production with halophilic microorganisms
  - on any kinds of waste streams with organic content

Conclusions

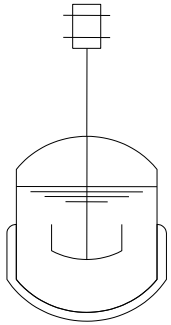


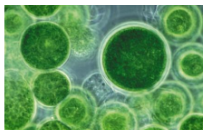
Outlook

microalgae from the 3rd biofuel generation

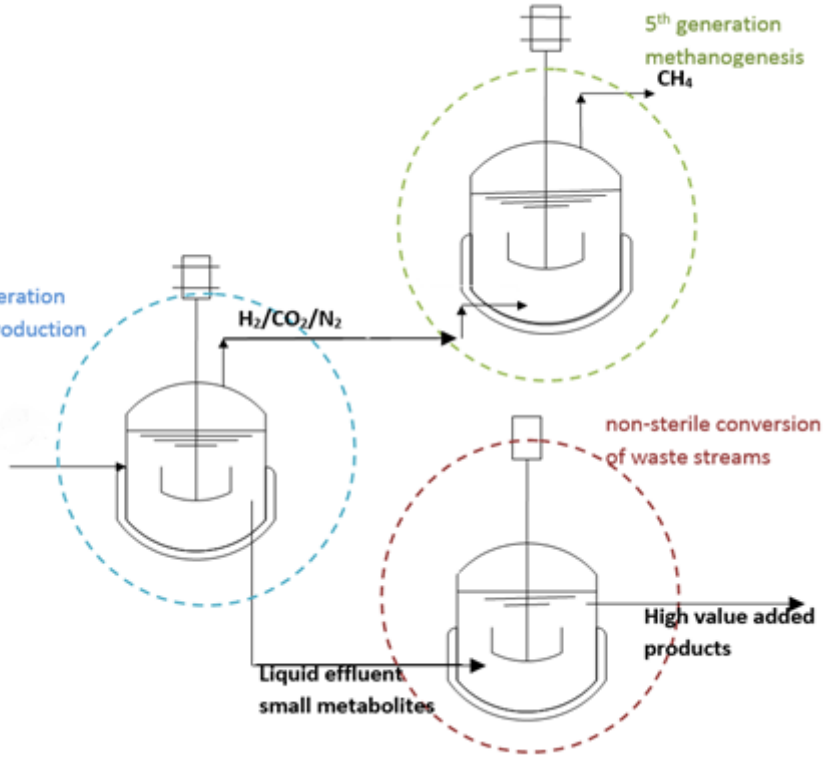
integrate as alternative renewable substrate source for biofuels production

Photobioreactor with algae for biodiesel production





Microalgal extracts



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Thank you for your attention!