Strategies to increase CO_2 solubility

BioCON-CO_2 – Final Symposium

13.06.2022

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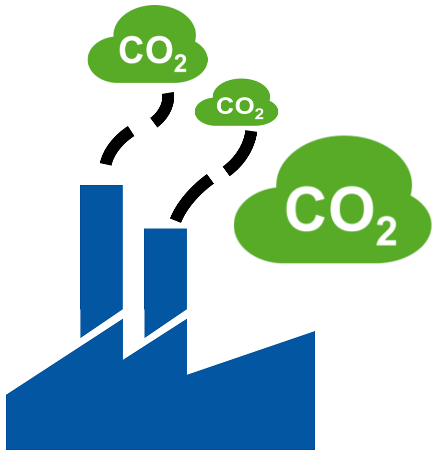
Project Overview

STAGE 1 CO_2 solubilisation strategies	STAGE 2 Bioprocess development	STAGE 3 Downstream technologies
	Anaerobic fermentation *Clostridium sp.* (acetogenic, solventogenic)	**C3-C6 alcohols**
	Aerobic fermentation *Oligotropha carboxivorans*	**3- Hydroxypropionic -acid**
	One-pot biocatalytic systems multi-enzymatic reaction (PDH)	**Lactic acid**
	FDH *(E. Coli)*	**Formic acid**



CO_2 solubilisation strategies

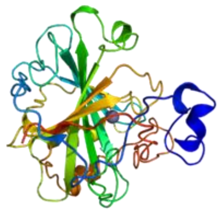
I. Carbonic Anhydrase

II. Trickle bed reactor

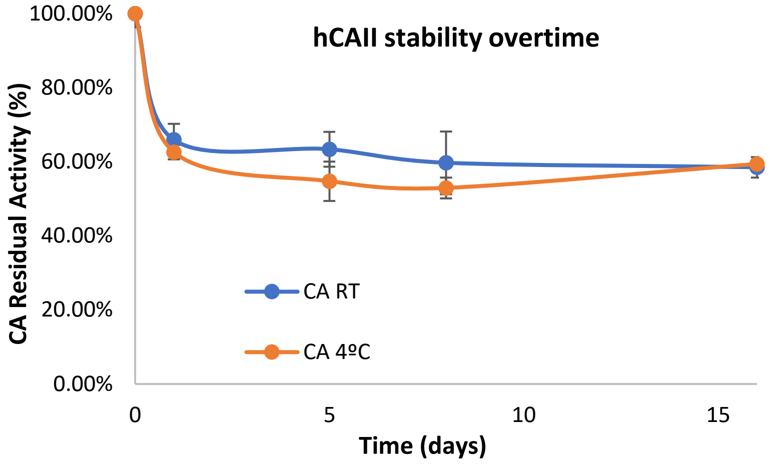
III. Process optimization (SynRAMOS)

I. Carbonic Anhydrase: Benefits and Bottlenecks

Human Carbonic Anhydrase II (hCAII)

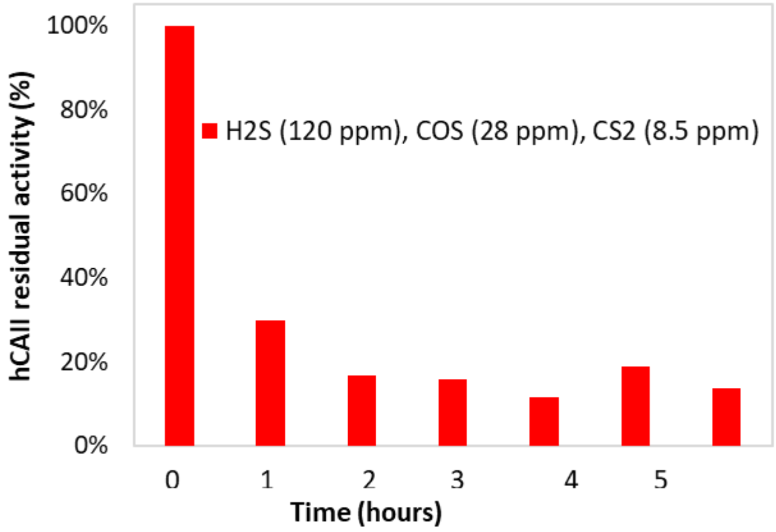


$$CO_2 + H_2O \xrightleftharpoons{CA} HCO_3^{\ominus} + H^{\oplus}$$



Advantages

- ✓ High turnover rate (k cat = 10^7 s^{-1})
- ✓ Mild operative conditions



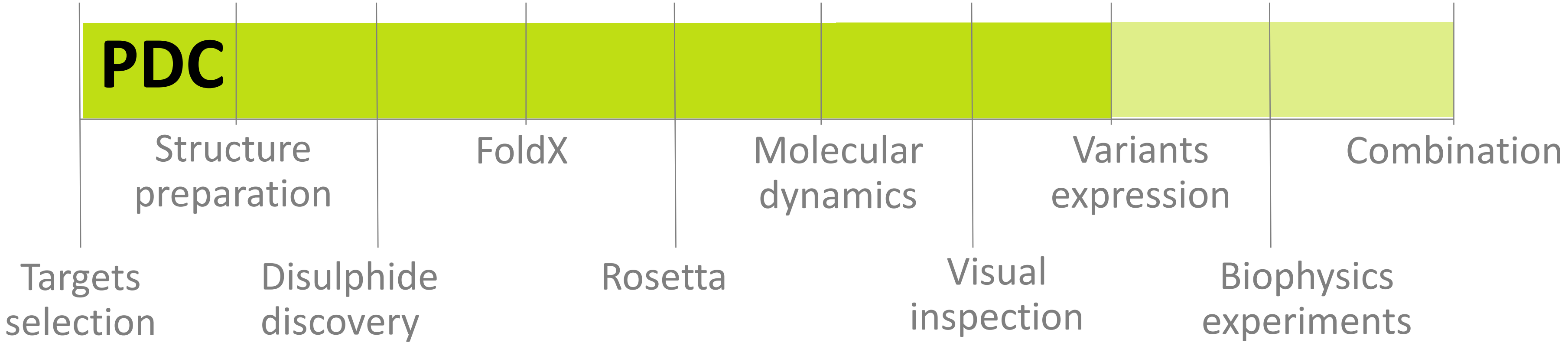
Disadvantages

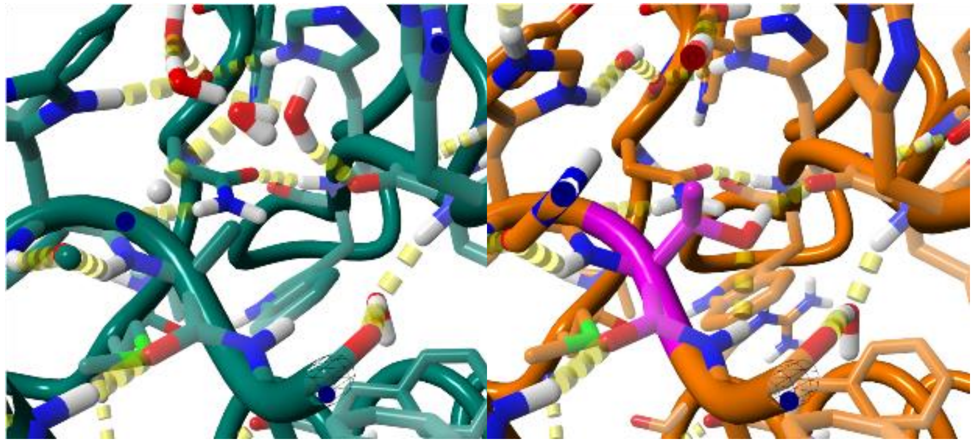
- ❑ Inhibition by sulphur-containing species
- ❑ Low Stability overtime

Computational Strategy for Increased Stability

BIOCON-CO2

Directed Evolution





755 screen mutations

49 selected mutations

21 primer sets designed for Leitat

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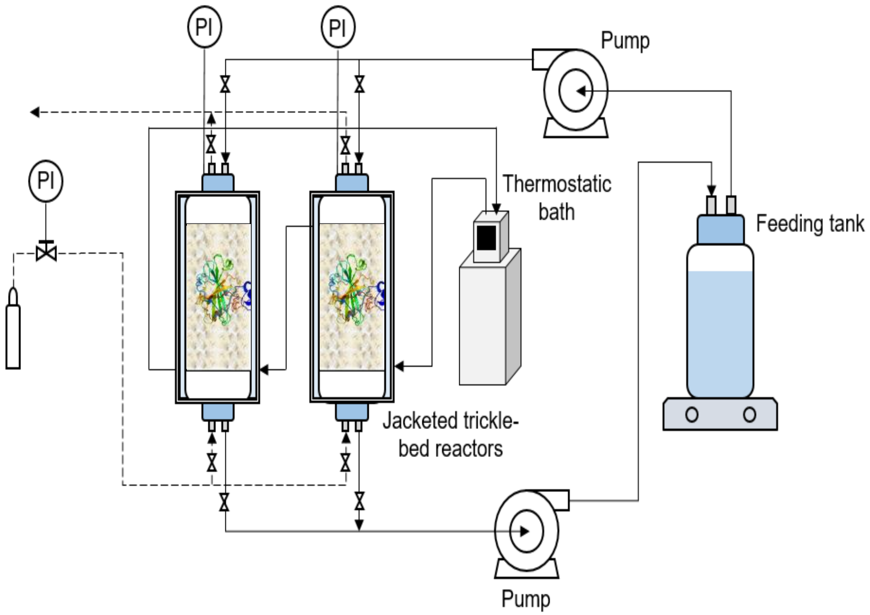
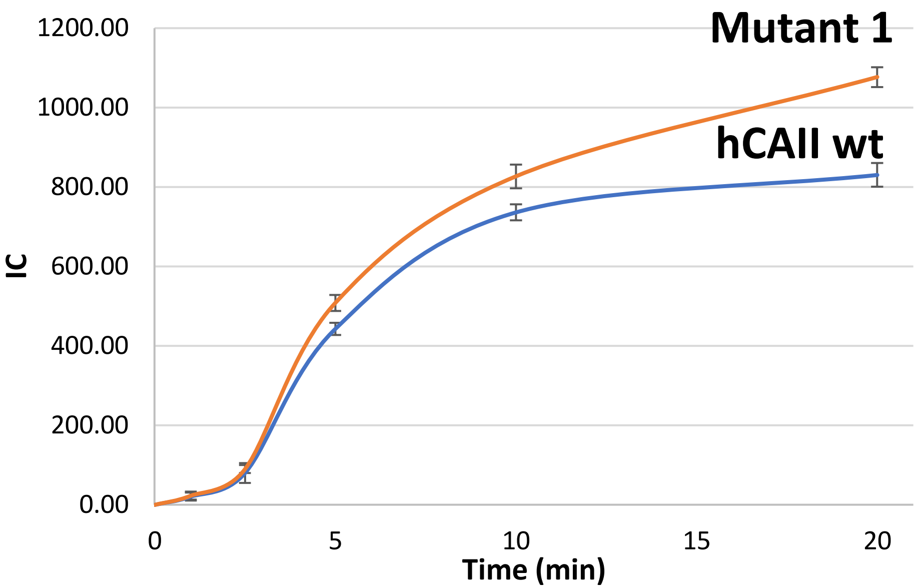
CO_2 Solubilisation with Carbonic Anhydrases



Experimental set-up for CA solubilisation



→ One of the mutants displays a higher activity (~1.5 fold) compared to hCAII wt but the same TM

→ It shows **higher CO_2 solubilisation** with a maximum of 30% after 20 minutes

II. Trickle-Bed Reactor

Definition:

The Trickle-Bed Reactor (TBR) is a chemical reactor that uses the downward movement of a liquid and the downward (co-current) or upward (counter-current) movement of gas over a packed bed of (catalyst) particles.

Critical parameters for the use in aerobic gas fermentation:

- Adhesion of bacteria *Cupriavidus necator* and biofilm formation
- Adaptability of the packing material inside the reactor
- Degradation suffered by the packing materials over time

Advanced packing materials

The aim of this research is to study innovative, efficient, environmentally friendly and low-cost packing materials, by analyzing their characteristics, bio-adhesion properties and growth of bacteria

POLYURETHANE FOAMS

Hard PU foam

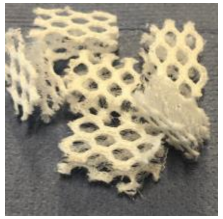
Soft PU foam

Polyisocyanurate (PIR) foam

FIBROUS MATERIALS (PES TEXTILES)

Woven

Non-woven



3D

HARDWOOD CHIPS

Beech wood

Eucalyptus wood

POLYPROPYLENE PELLETS

REUSE AND WASTE MANAGEMENT: Materials from industrial pre-consumer waste.

TBR Conclusion

- BIOCON-CO_2 project has demonstrate the viability of TBR uses in CO_2 capture
- 10 different packing materials have been evaluated by means of surface characterization, behaviour inside the reactor and biofilm adhesion and growth
- It was determined the capability of packing materials to create biofilms by *C. necator*
- Plasma treatments on selected textiles:
  - Atmospheric plasma using gases to increase the roughness of the surface
  - PECVD plasma to functionalise the surface with permanent functional groups
  - Surface properties and biofilm formation can be tuned changing plasma conditions
- It is expected to have a publication soon with more results

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III. Process optimization: Motivation

Characteristics of syngas fermentation for CO_2 utilization

- Low substrate solubility for carbon monoxide and hydrogen

- CO_2 conversion depends on additional energy supply

$$2\ CO_2 + 4\ H_2 \rightarrow CH_3COOH\ (Acetat) + 2\ H_2O$$
$$2\ CO_2 + 6\ H_2 \rightarrow CH_3CH_2OH\ (Ethanol) + 3\ H_2O$$

- CO conversion is energetically preferred and results in CO_2 production

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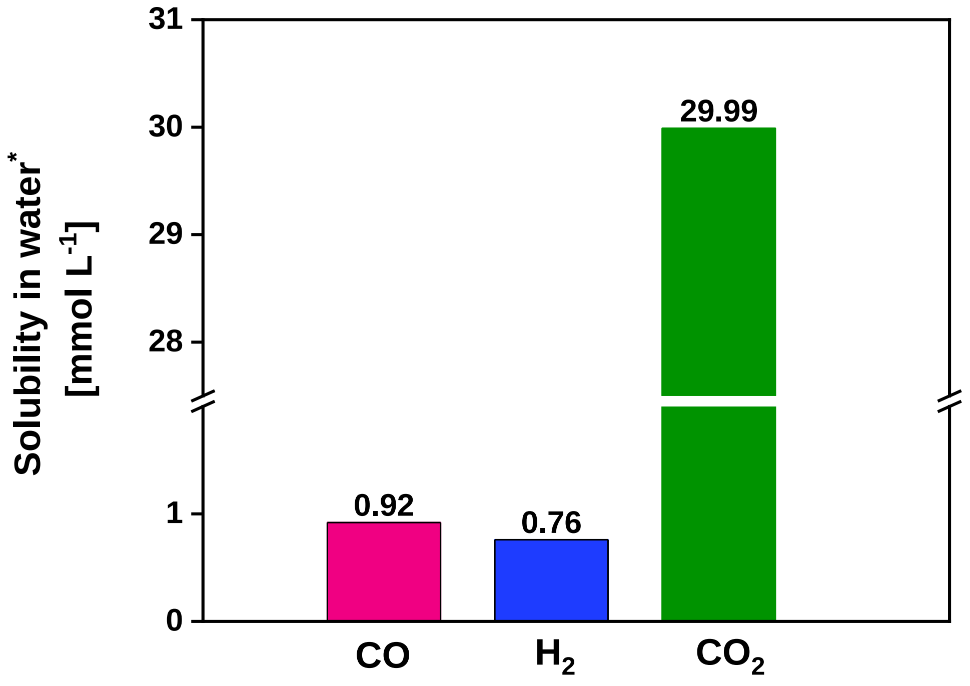

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BIOCON-CO_2

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- **Continuous gas supply** is necessary for sufficient growth
- **Monitoring CO_2 consumption/ production** is highly important for process development

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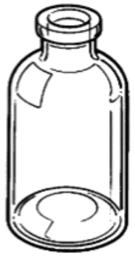
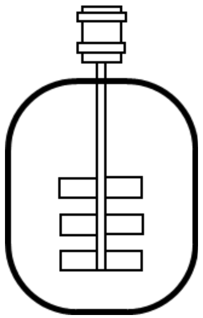
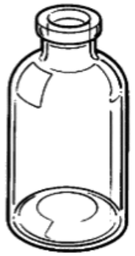
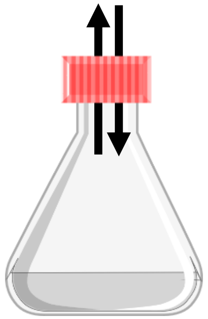
State of the art process development for syngas fermentation

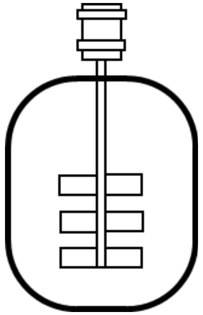
Serum bottle		Fermenter
	Is there something to fill the gap?	
No online monitoring		Online monitoring
Limited gas supply		Continuous gas supply
High throughput		Limited throughput

State of the art process development for syngas fermentation

Serum bottle	Gas shaker	Fermenter







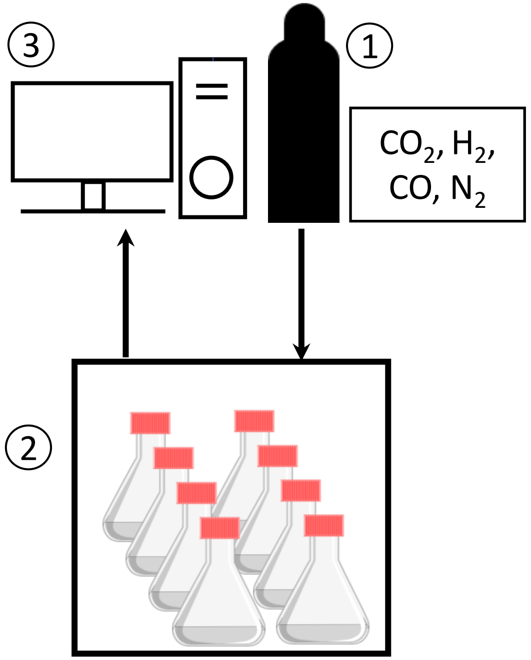
Serum bottle	Gas shaker	Fermenter
No online monitoring	Online monitoring	Online monitoring
Limited gas supply	Continuous gas supply	Continuous gas supply
High throughput	High throughput	Limited throughput

SynRAMOS - A device for gas fermentation in shake flasks

- Cultivation with gaseous carbon sources in up to 8 shake flasks
- Individually adjustable gas composition
- Safe to use with toxic and explosive gases (e.g. CO & H_2)

Measurement principle

- Measurement of the headspace pressure in each flask
- Measurement of the CO_2 partial pressure via high range gas sensors

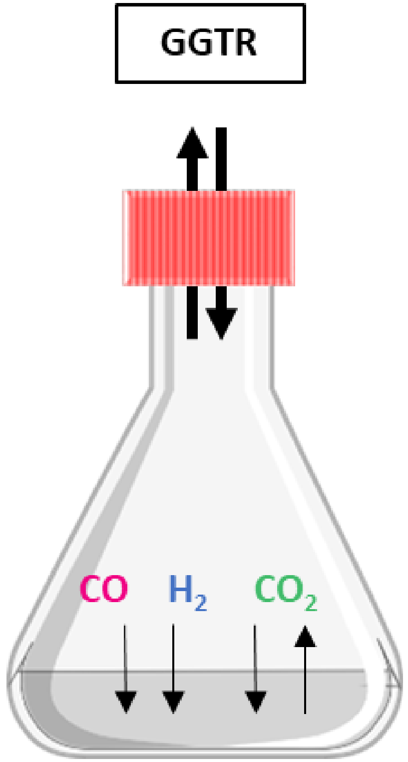


(1) Gas supply
(2) Shaking tablar with gasshaker setup
(3) Computer

[1] Mann, 2021, Online monitoring of gas transfer rates during CO and CO/H_2 gas fermentation in quasi-continuously ventilated shake flasks

Online Measurement of Gas Transfer Rates

- Gross Gas Transfer Rate (**GGTR)** calculation
  - Determined via a pressure sensor
  - Represents the total gas transfer into and out of the liquid phase
- Carbon dioxide transfer rate (**CO_2TR**) calculation
  - Measured via a CO_2-Sensor
  - Represents the transfer of consumed or produced carbon dioxide



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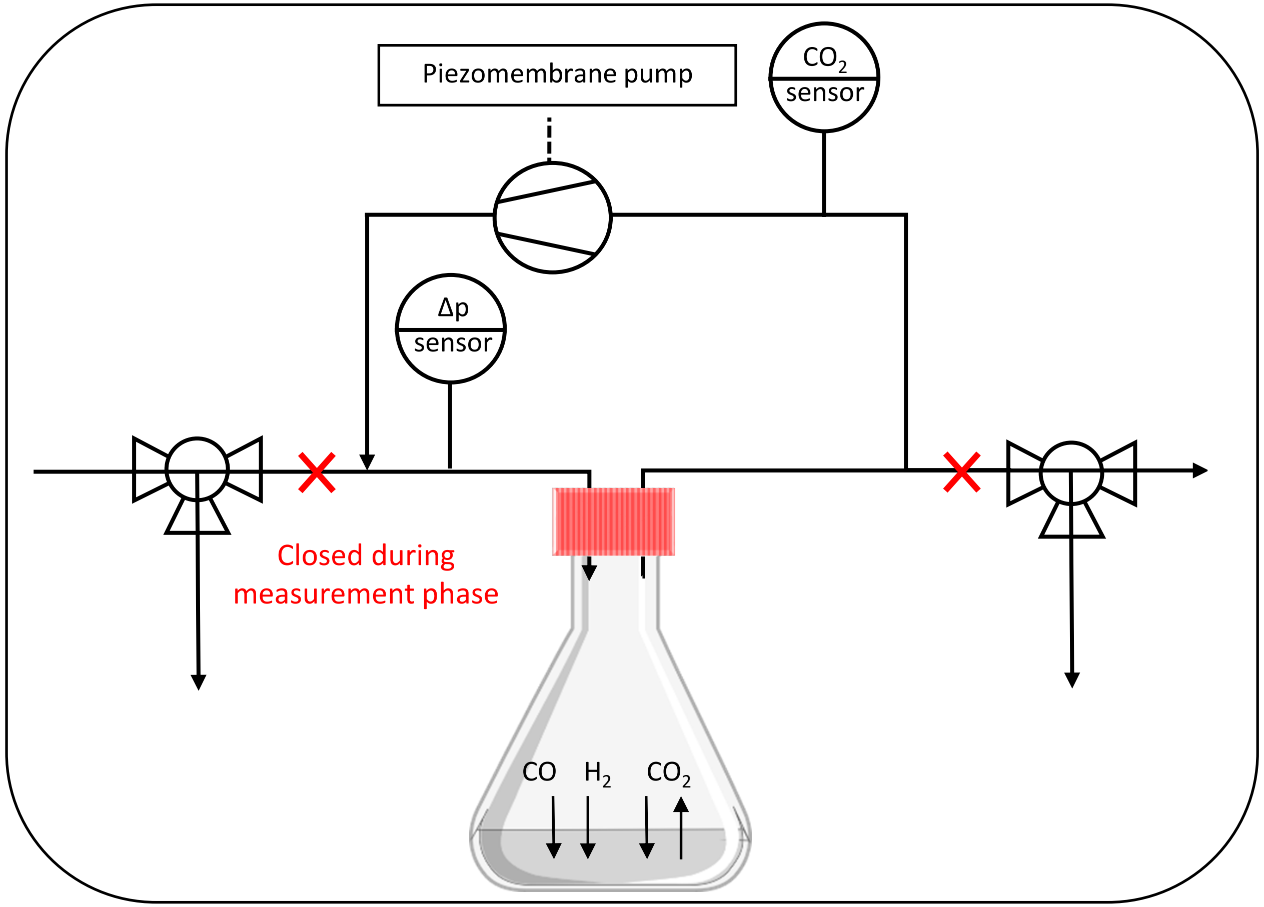
Setup for the gas shaker

- Gross Gas Transfer Rate (GGTR) calculation

$$GGTR = \frac{dn_{total}}{dt} = \frac{\sum p_i \cdot V_{gas}}{V_{liquid} \cdot \Delta t \cdot R \cdot T}$$



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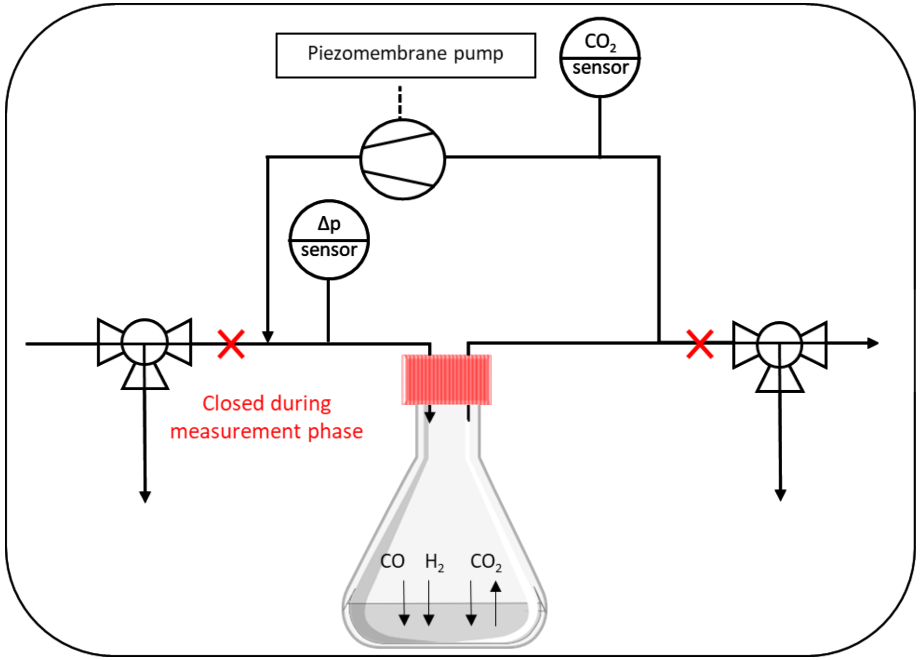

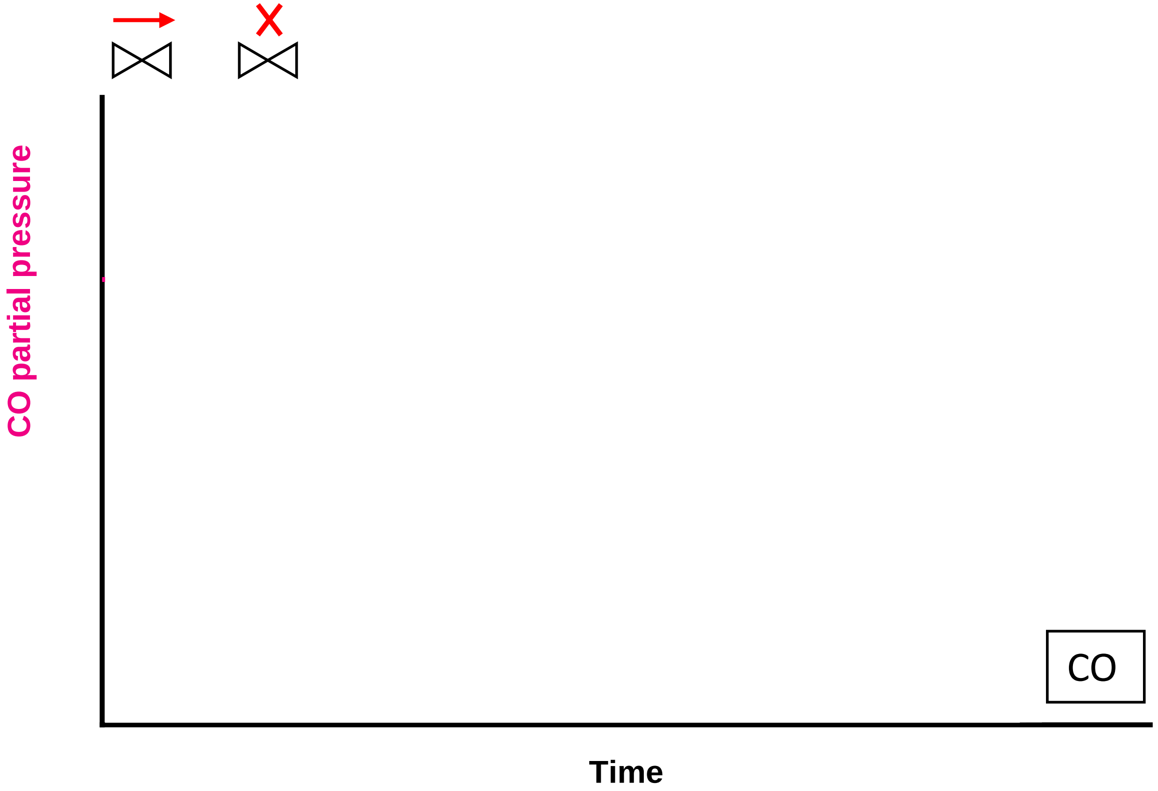

I : Flush phase
II: Measurement phase

Measurement principle

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CO as sole carbon source

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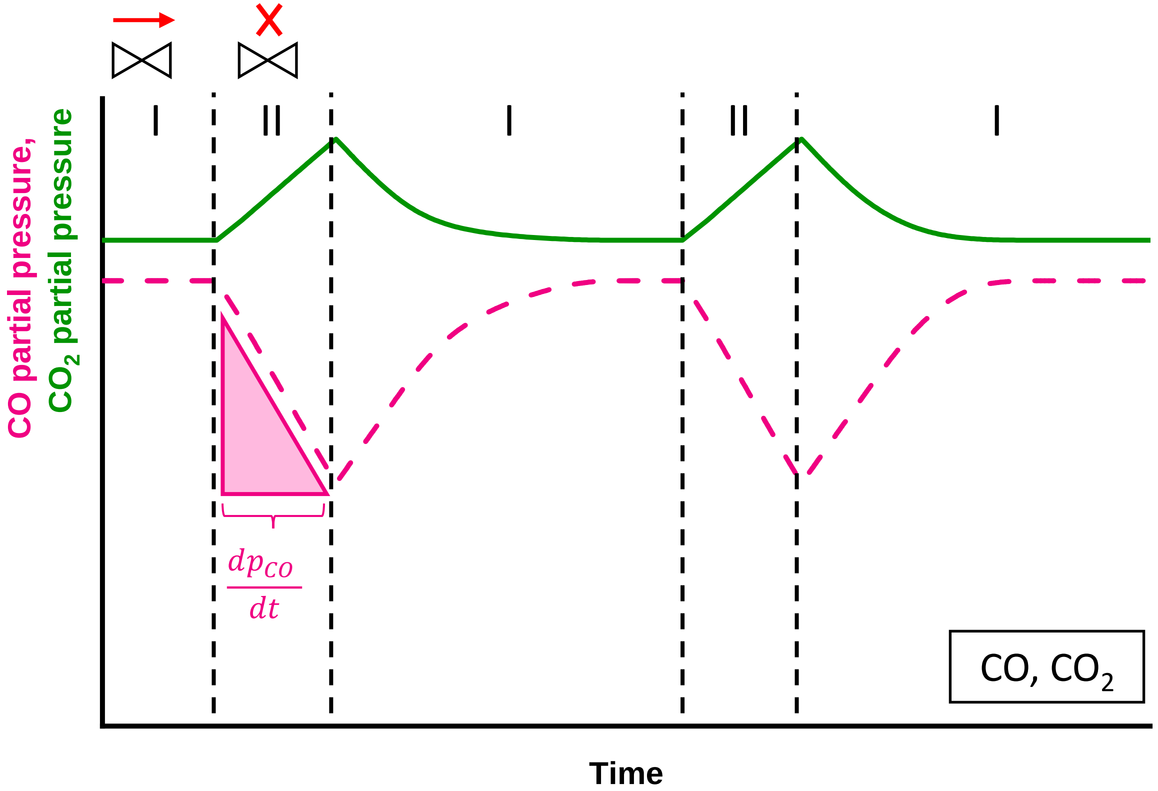

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Measurement principle

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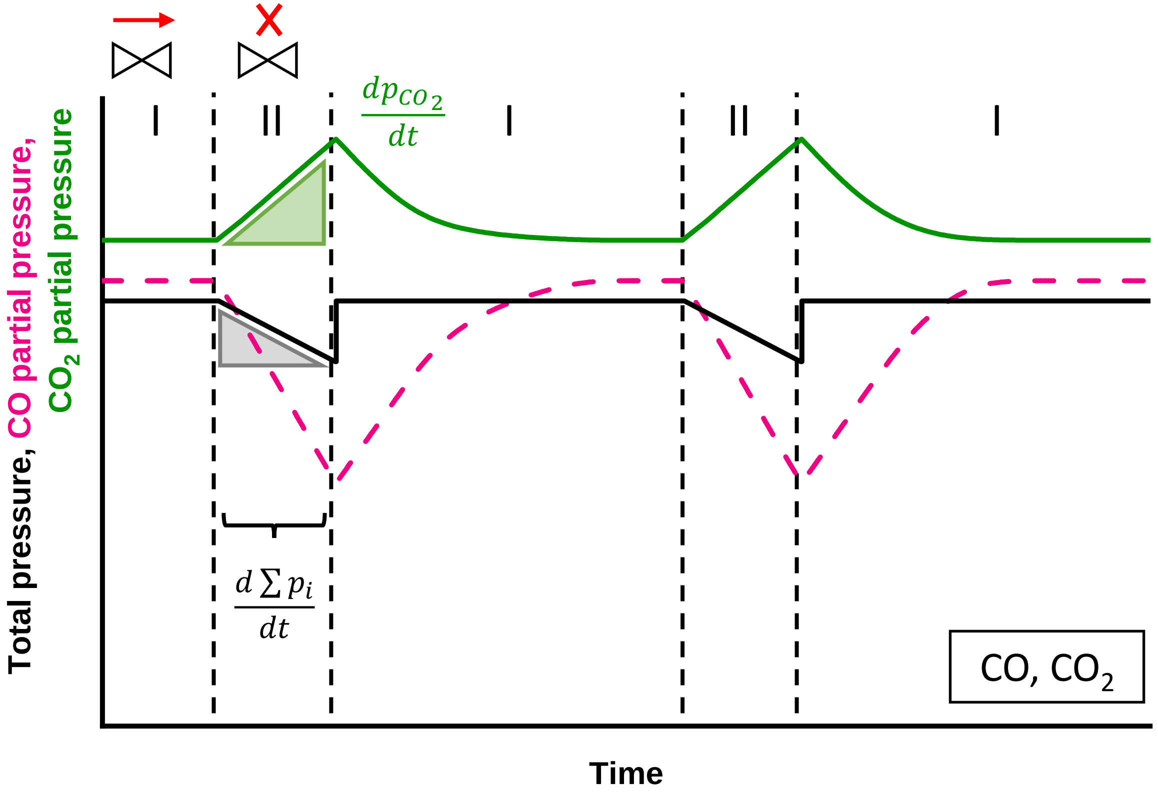
- Carbon Dioxide Transfer Rate (CO_2TR) calculation

$$CO_2TR$$

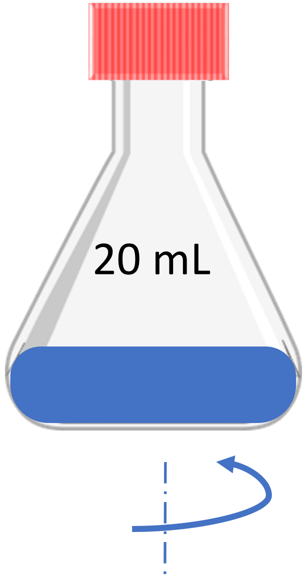


I : Flush phase
II: Measurement phase

Case study - Gas fermentation using *C. ljungdahlii*

- Investigation of different gas transfer rates
  - Effect on gas consumption and **carbon dioxide conversion**
  - Enhanced product formation
- Stepwise increase of shaking frequency
  - 100 rpm
  - 200 rpm
  - 300 rpm

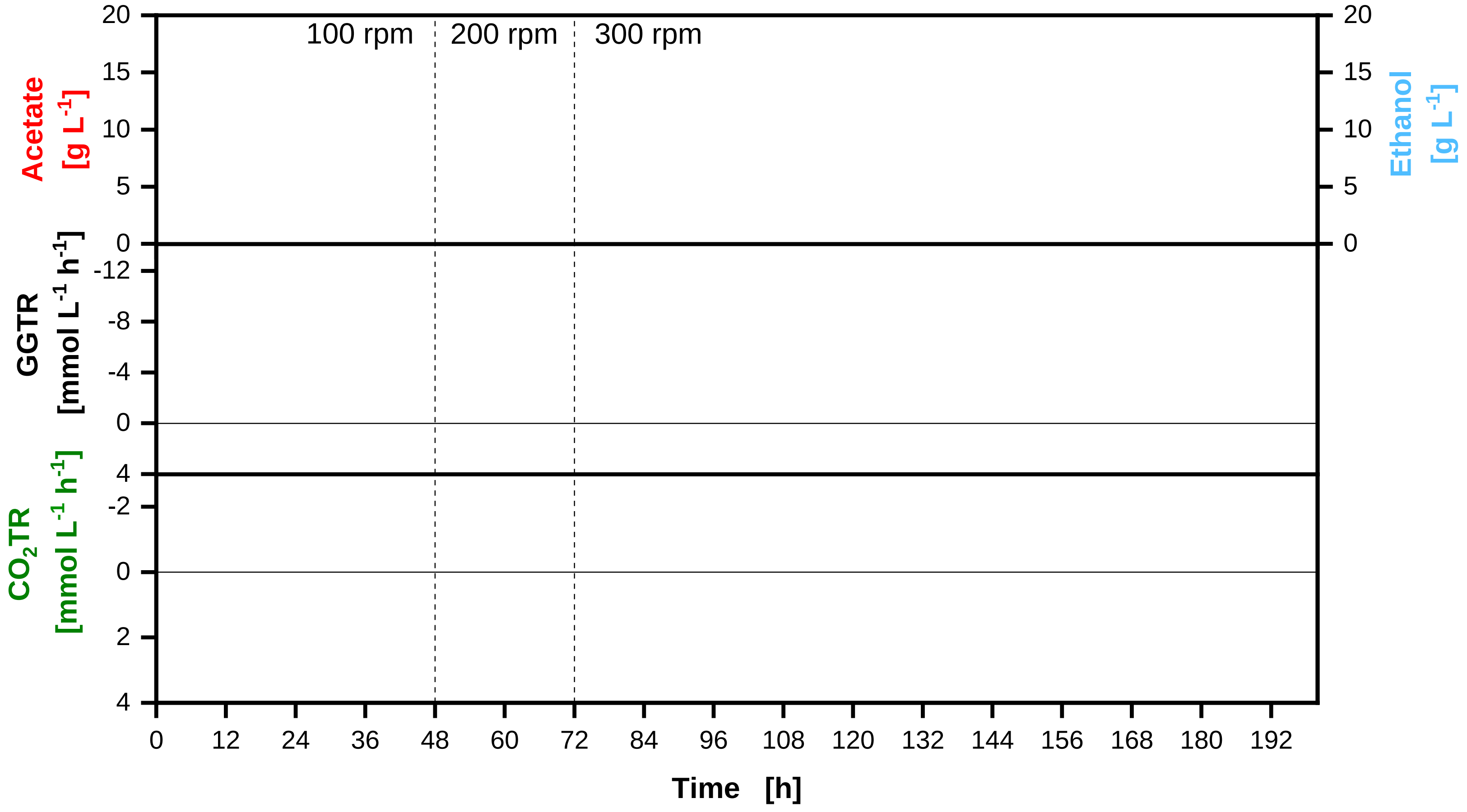
→ Higher shaking frequencies result in higher gas transfer rates



Effect of increased gas transfer rates

C. ljungdahlii wildtype, ATCC media, T = 37°C, pH 7, 100 mmol BisTris, n = 100 - 300 rpm,

d_0 = 50 mm, $\dot{V}_{Gas}$ = 5 mL min^{-1}, 10% CO / 20% CO_2 / 50% H_2 / 20% N_2



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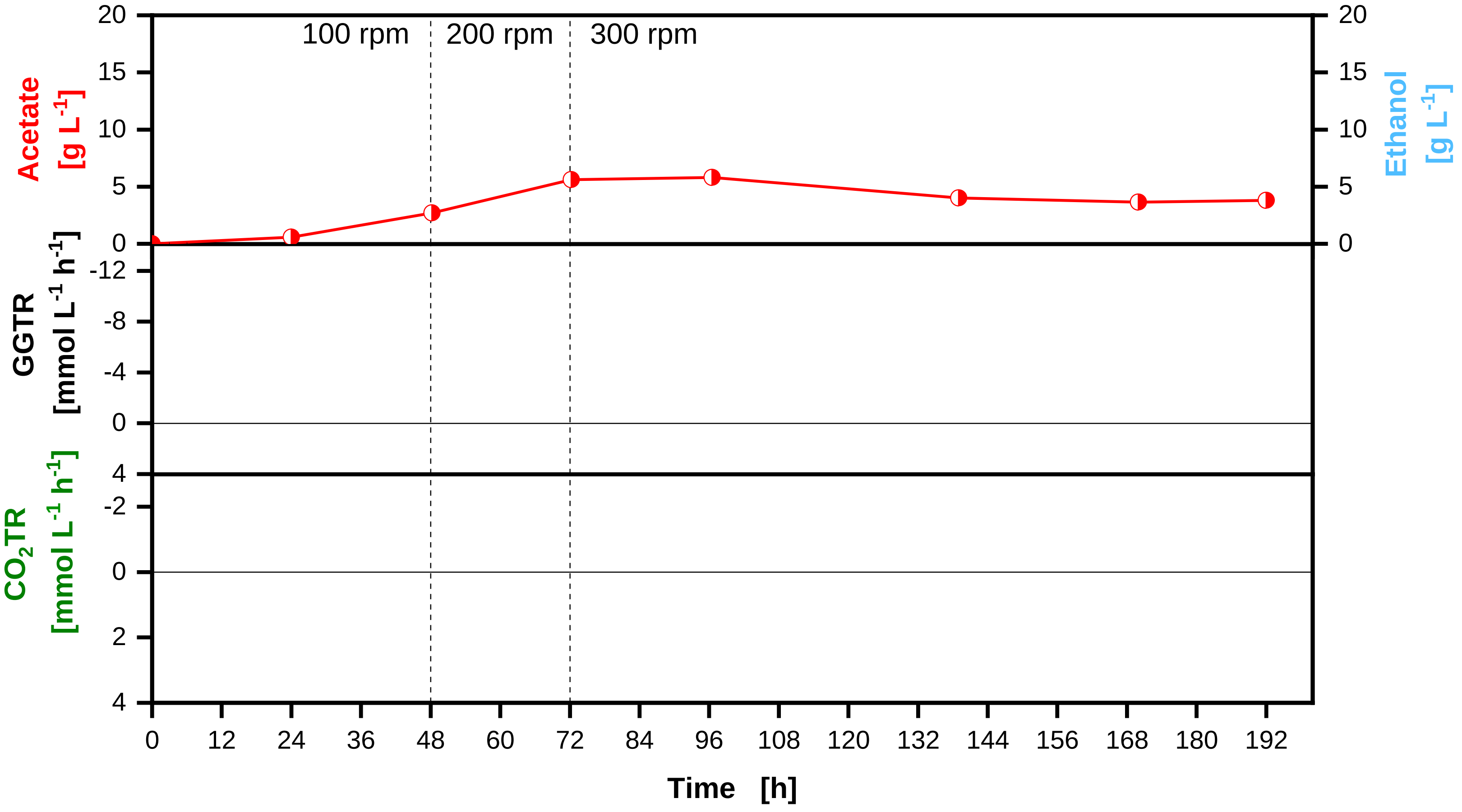

- Metabolic shift at 300 rpm

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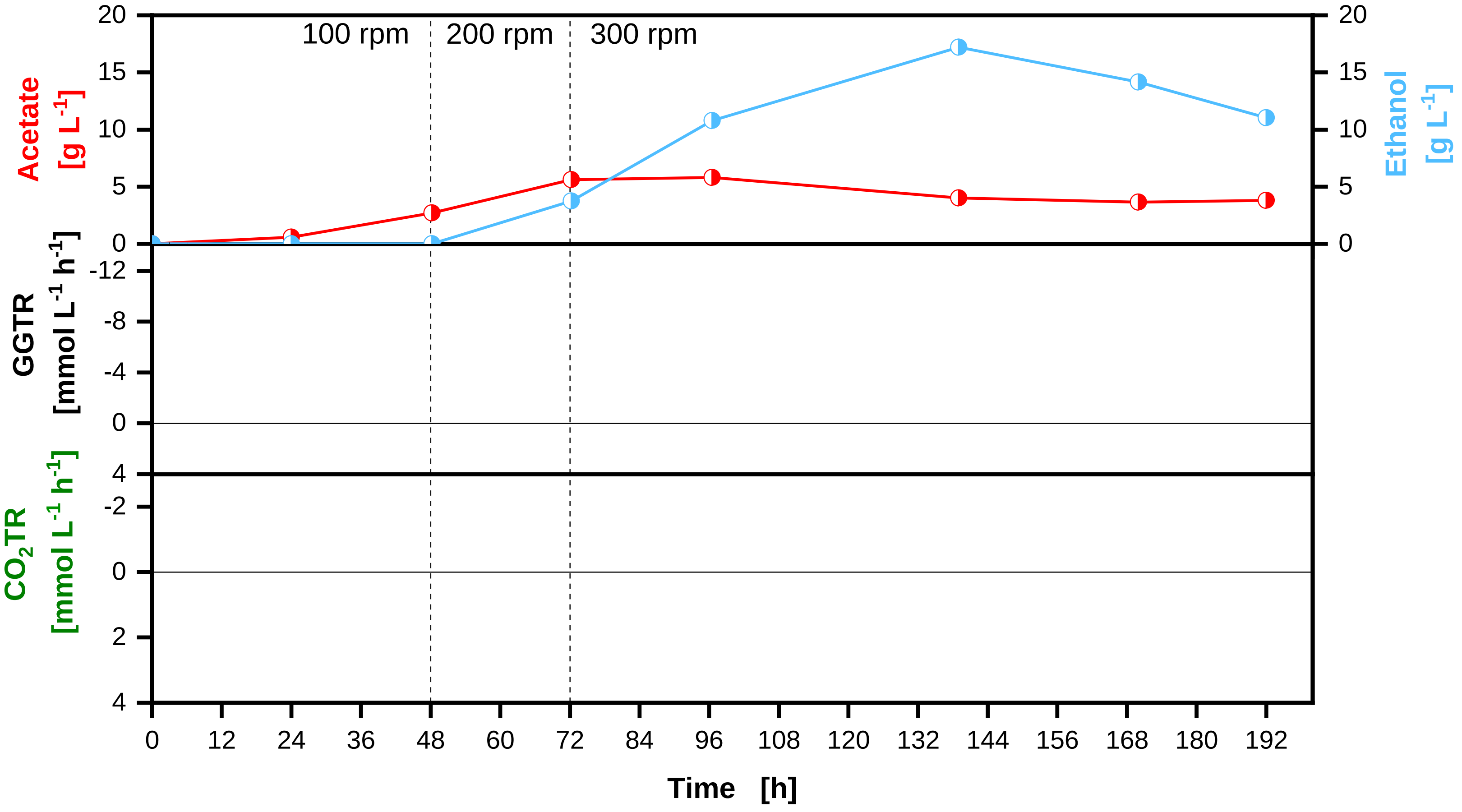

- Metabolic shift at 300 rpm
- Ethanol concentration of 15 g L^{-1}

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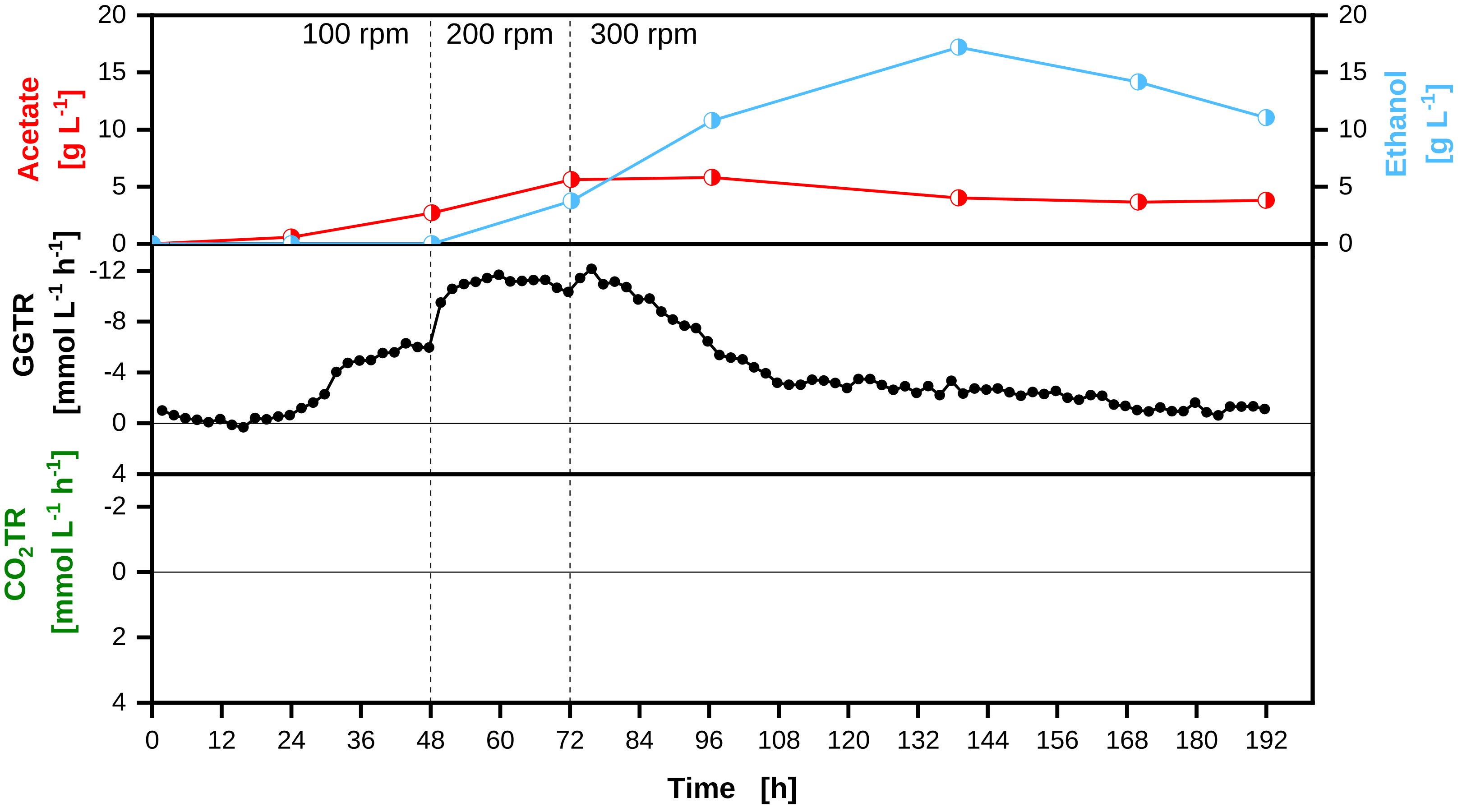

- Metabolic shift at 300 rpm
- Ethanol concentration of 15 g L^{-1}
- Gas consumption increases with increasing shaking frequency
- GGTR drops after increase to 300 rpm

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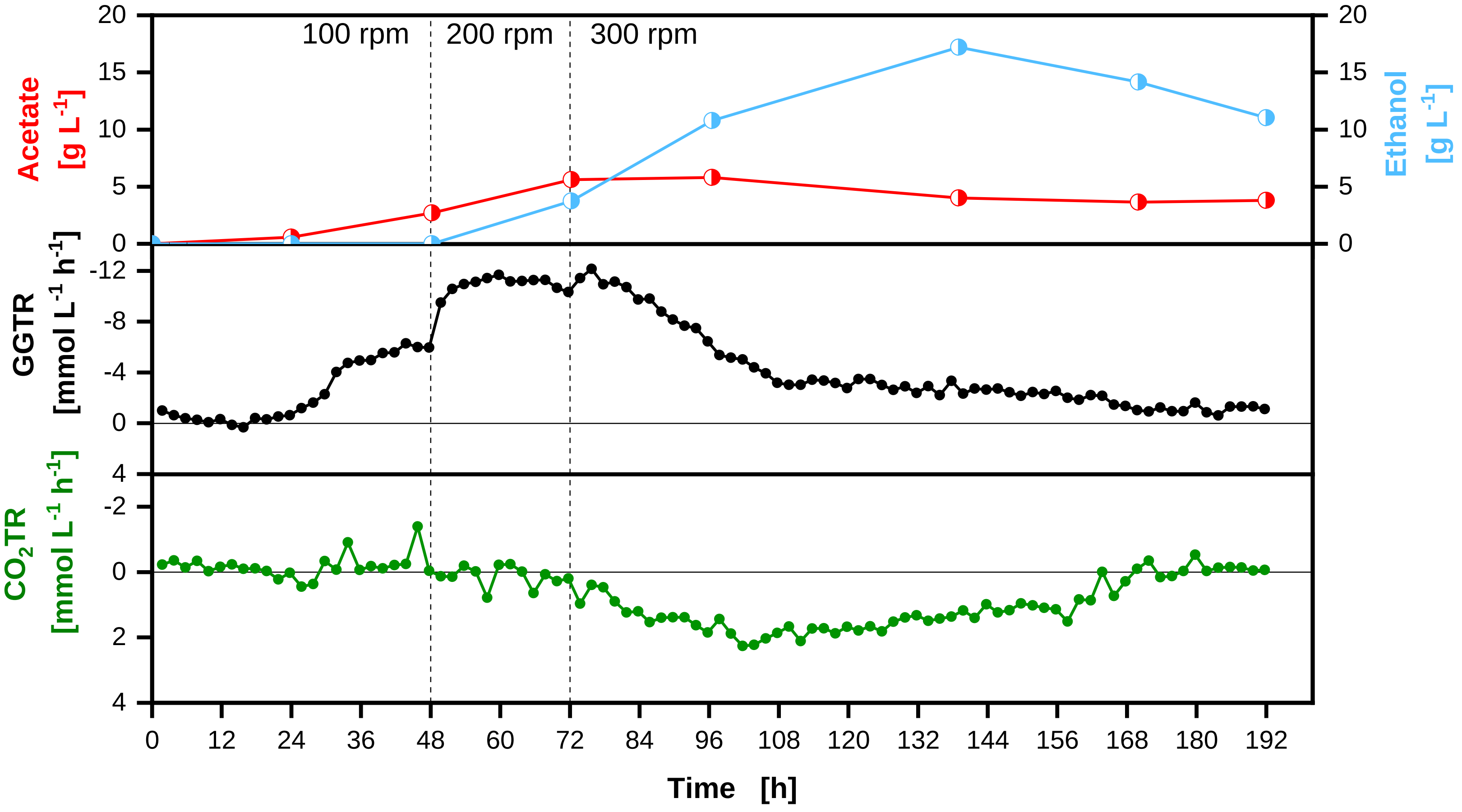
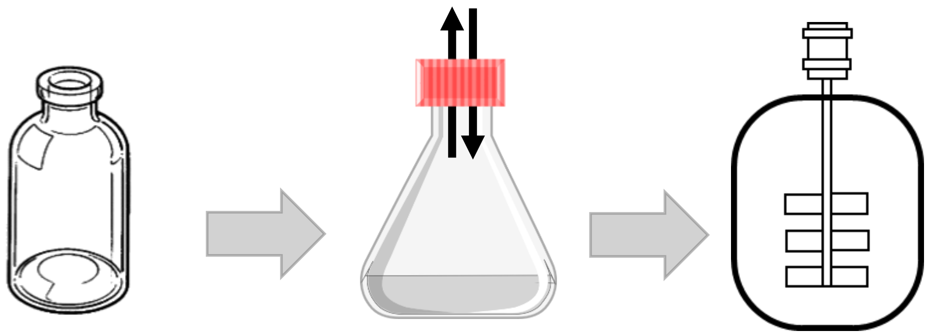


- Metabolic shift at 300 rpm
- Ethanol concentration of 15 g L^{-1}
- Gas consumption increases with increasing shaking frequency
- GGTR drops after increase to 300 rpm
- CO_2TR indicates excess CO_2 production after increase to 300 rpm

→ Increasing gas transfer leads to hydrogenase inhibition

III. Summary

Gas shaker



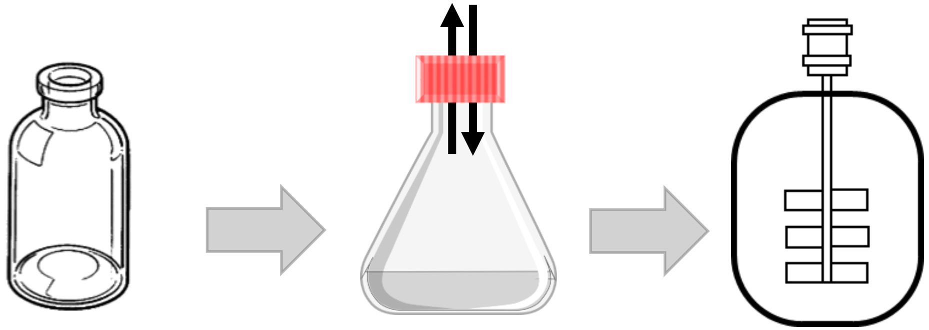
Novel shake flask system

Online monitoring of gas transfer rates

Applicable for process development

III. Summary

Gas shaker

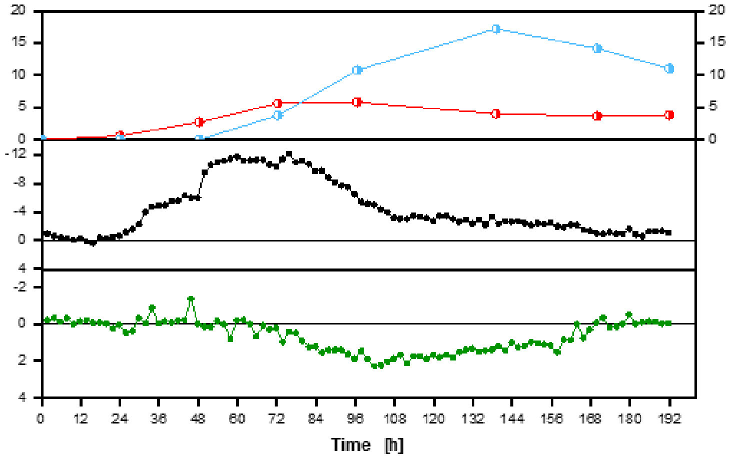


Novel shake flask system

Online monitoring of gas transfer rates

Applicable for process development

Analyzing gas transfer rates



Online measurement of GGTR and CO_2TR

enables deeper insights into small scale

gas fermentation processes.

Thank you

Aline Hüser, Marcel Mann, Prof. Dr. Jochen Büchs,
Montse Bosch, Aroa Rey Campa, Rubén Rodriguez Alegre,
Mari Carmen Royo Reverter

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