Valorization of carbon dioxide-rich gaseous waste through gas fermentative process: process development in lab scale bioreactors

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PhD aim

Hexanol is a C6 alcohol used in multiple market sectors. Currently, its production relies on fossil fuels or palm oils. However, C1-based gas fermentation with acetogens presents a promising and environmentally sustainable alternative. While gas fermentation with CO-rich waste is well-studied, hexanol production using CO_2 and green H_2 remains relatively unexplored. The aim of my PhD is to address this gap by developing a CO_2:H_2-based fermentation process for hexanol production. In the second year of my PhD, I focused on optimizing process parameters in stirred tank bioreactors. The adapted strain, *C. carboxidivorans*_hex21, which was developed in the first year of my PhD [1], serves as the biocatalyst due to its enhanced growth and its high hexanol selectivity.

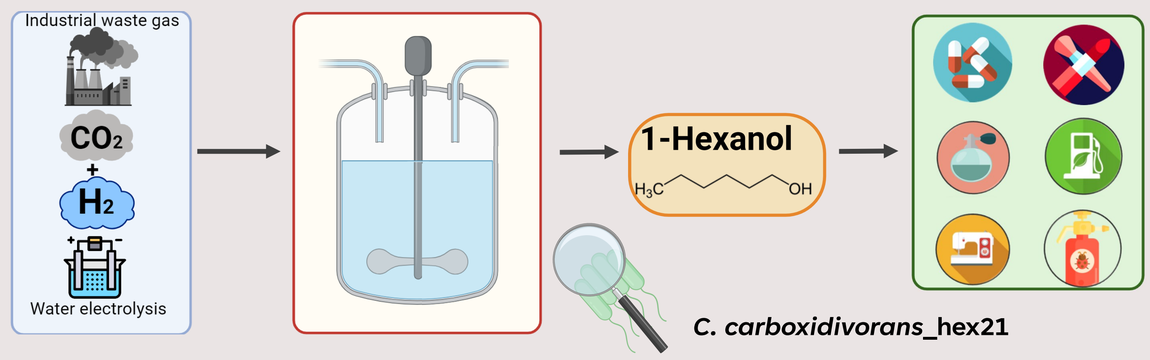
PhD activities

1°yr Biocatalyst development

2°yr **Bioprocess optimization in laboratory scale reactors**

- Process parameters investigation in reactor
- Hexanol toxicity mitigation

3°yr Continuous process and Scale-up feasibility evaluation



Methodology

Bioreactor process development

Experimental set-up

-500mL Automated CSTR

-300mL nominal volume

-Gas mix 80% H_2 20% CO_2

-200 rpm

Parameters investigated

Temperature and pH

H_2 and CO_2 partial pressures

Gas feeding mode

Hexanol toxicity mitigation

Experimental set-up

-250mL Serum bottles

-25mL nominal volume

-Gas mix 80% H_2 20% CO_2

-100 rpm

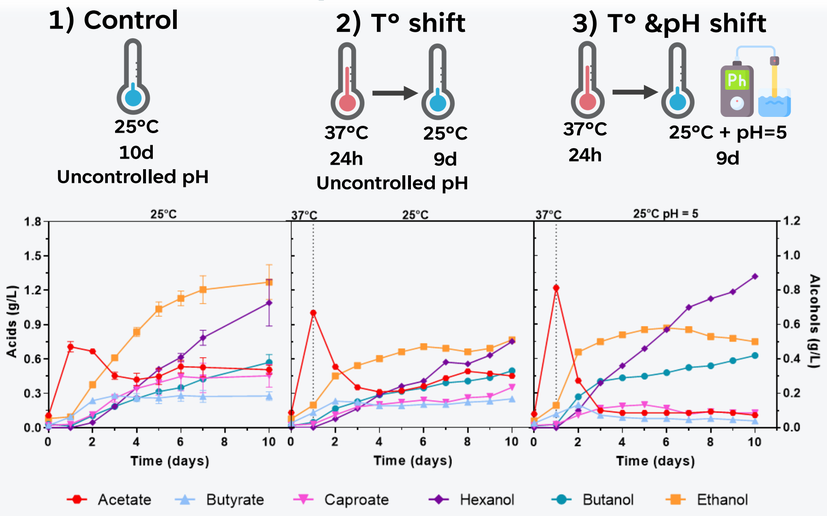
Test performed

-Adaptive Laboratory Evolution to improve hexanol tolerance

-Oleyl alcohol (OAL) as in situ hexanol extraction solvent

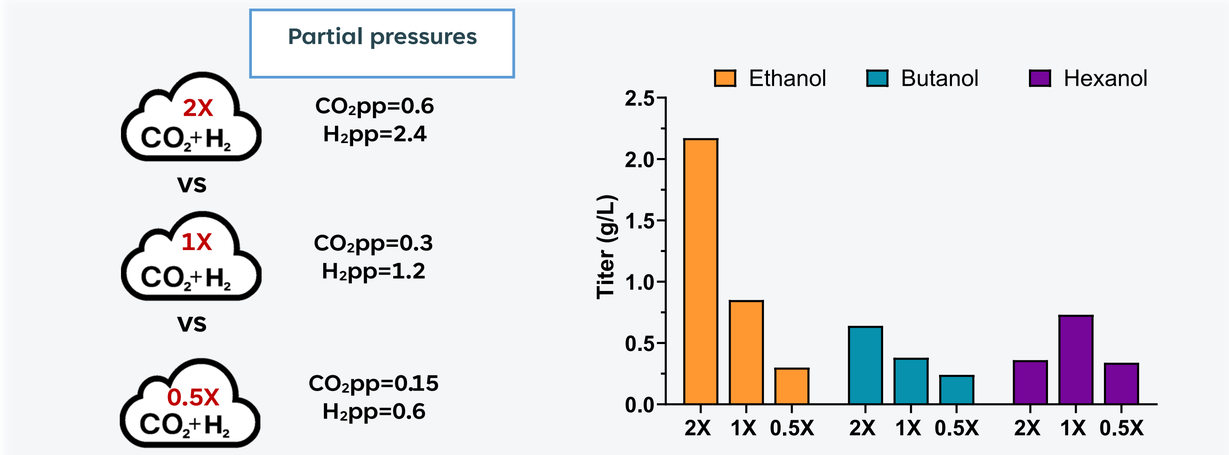
Results

1. Temperature shift and pH control effect



The temperature shift from 37°C to 25°C and the pH control to 5 increase acetate consumption and hexanol production

2. H_2 CO_2 partial pressures influence



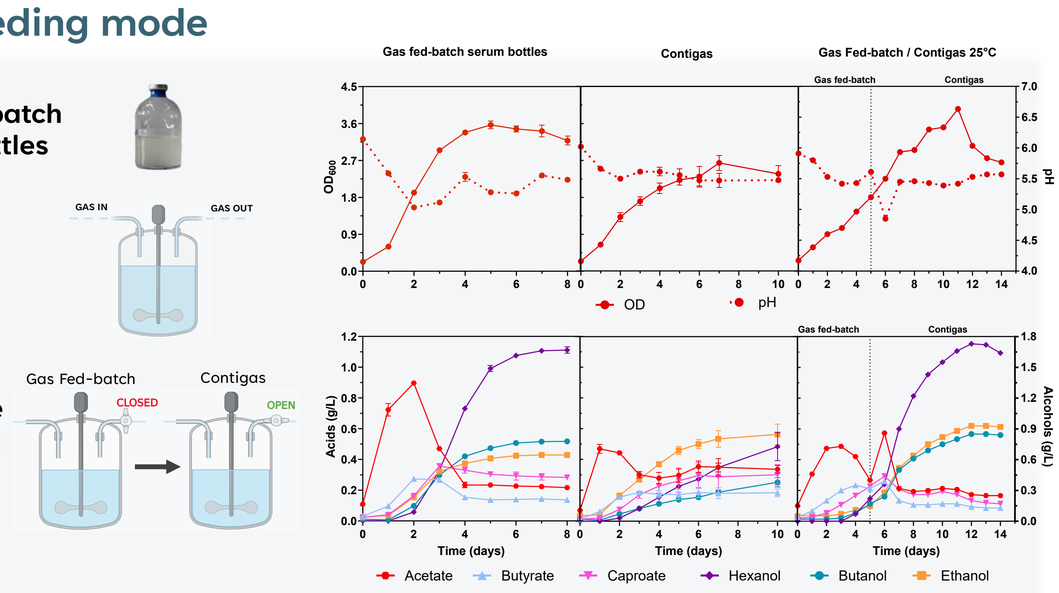
The increased gas partial pressures enhances alcohols production, however, ethanol is the primary product

3. Gas feeding mode

1) Gas Fed-batch Serum bottles

2) Contigas

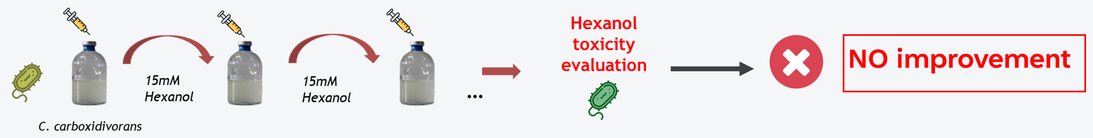
3) Two stage Gas feeding mode



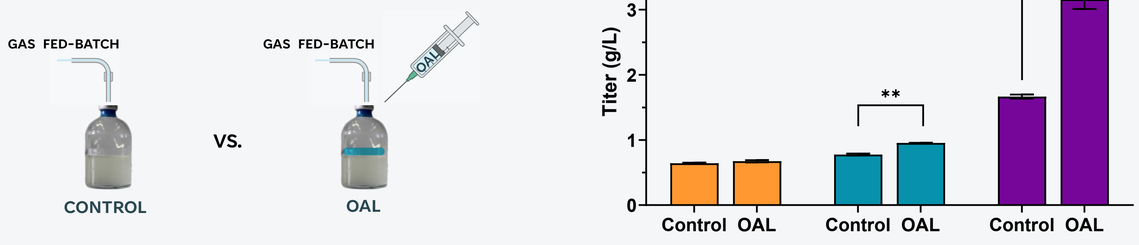
A two-stage gas feeding strategy enables the best hexanol production performances in bioreactor configuration

4. Hexanol toxicity mitigation

Aaptive Laboratory Evolution (15mM Hexanol)



In situ hexanol extraction with oleyl alcohol



The *in-vivo* use of oleyl alcohol doubles the hexanol titer compared to the control

Conclusion

- Lower hexanol production performances in conti-gas reactor compared to gas fed-batch experiment in serum bottles
- No enhancement of CO_2 conversion into hexanol by temperature shift, pH and partial pressures changes
- Hexanol tolerace improvement via ALE requires further culture transfers
- Hexanol carbon selectivity similar to serum bottles experiment (>40%) through two-stage gas feeding bioreactor strategy
- Doubling of hexanol titer and a selectivity >57% via in-situ extraction with oleyl alcohol

Summary of experiments	Hexanol ($g\ L^{-1}$)	HexOH Volumetric productivity ($g\ L^{-1}\ h^{-1}$)	Selectivity ($C_{mol\ hexanol}/C_{mol\ Tot}$)
(1) Control (25°C contigas 1Xpp)	0.73	0.005	0.247
(2) T° shift (37°C-25°C)	0.50	0.004	0.153
(3) T° shift + pH control	0.88	0.007	0.337
(4) 2X pp	0.36	0.004	0.107
(5) 0.5X pp	0.34	0.004	0.178
(6) Two stage	1.72	0.023	0.400
(7) Gas fed-batch (bottles)	1.67	0.031	0.458
(8) Gas fed-batch +OAL (bottles)	3.16	n.a.	0.576

Future activities

- Stabilization of the maximum hexanol productivity in chemostat reactor configuration
- Bioreactor in-situ hexanol extraction with oleyl alcohol
- Bioprocess simulation at pilot scale: mass and energy balance
- Environmental impact assessment: Life Cycle Assessment

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References

1. Antonicelli et al. "Expanding the product portfolio of carbon dioxide and hydrogen-based gas fermentation with an evolved strain of *Clostridium carboxidivorans*". Bioresource Technology 2023 DOI: 10.1016/j.biortech.2023.129689