Study of the factors influencing the robustness of genetically modified strains of *C. necator* for the production of hydrocarbons from CO_2 in a bioreactor

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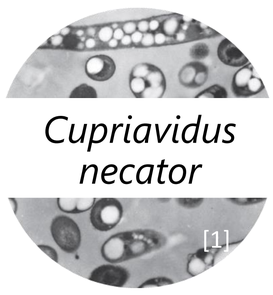
Background



Cupriavidus necator

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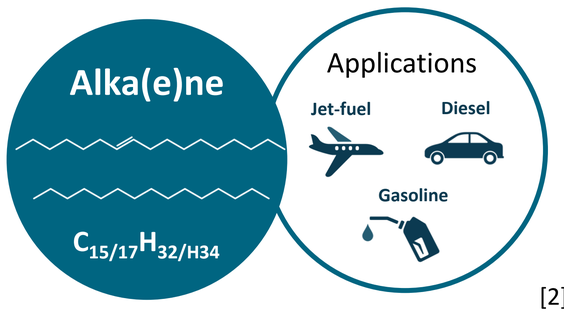
- Chemolithoautotroph strain.
- Bioproduction strain for multiple molecules of interest.
- Heterogeneity leading to reduced production yields.



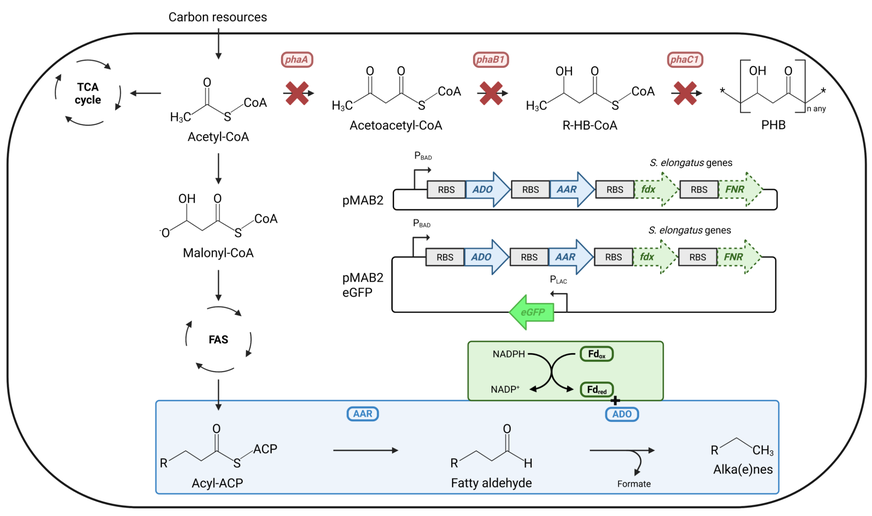
[2]

Key points

- Highest alka(e)ne yield never obtained in *C. necator* under autotrophic condition.
- Lack of plasmid stability throughout the cultivation and production phases in gas-fed cultures under tested conditions.

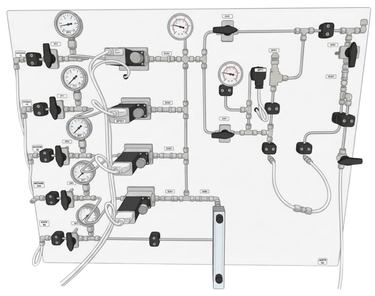
Methods

Genetically engineered strain

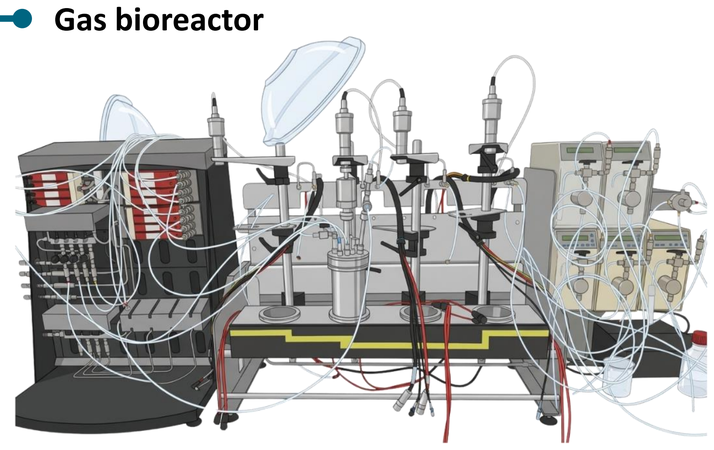


Gas cultivation under safety condition

- **Gas bottles**
- Potential explosion risk (H_2 + O_2).

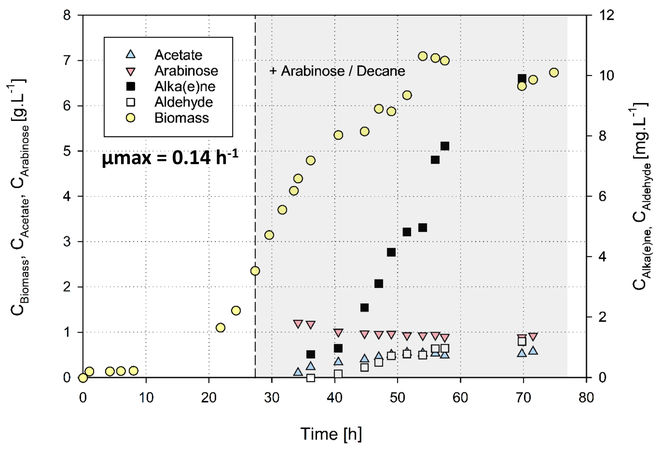


- Configurable gas feed system.
- **Gas bioreactor**

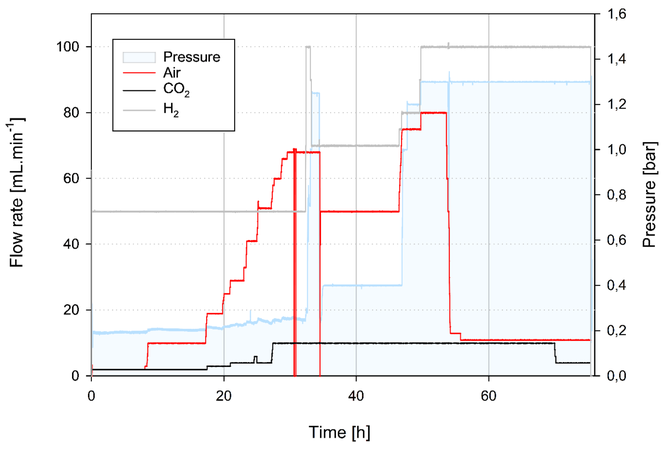


Results

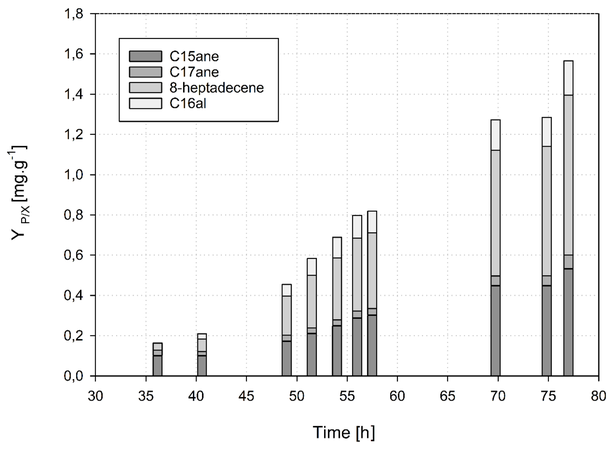
Alka(e)ne formation in 1L gas bioreactor by Re2061 pMAB2



Kinetics of Re2061 pMAB2 in 1L gas bioreactor. Production phase always induced by the adition of arabinose / decane.

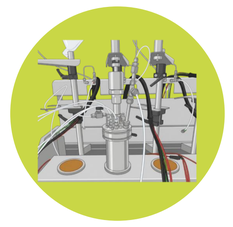


Gas composition in the bioreactor during the culture.

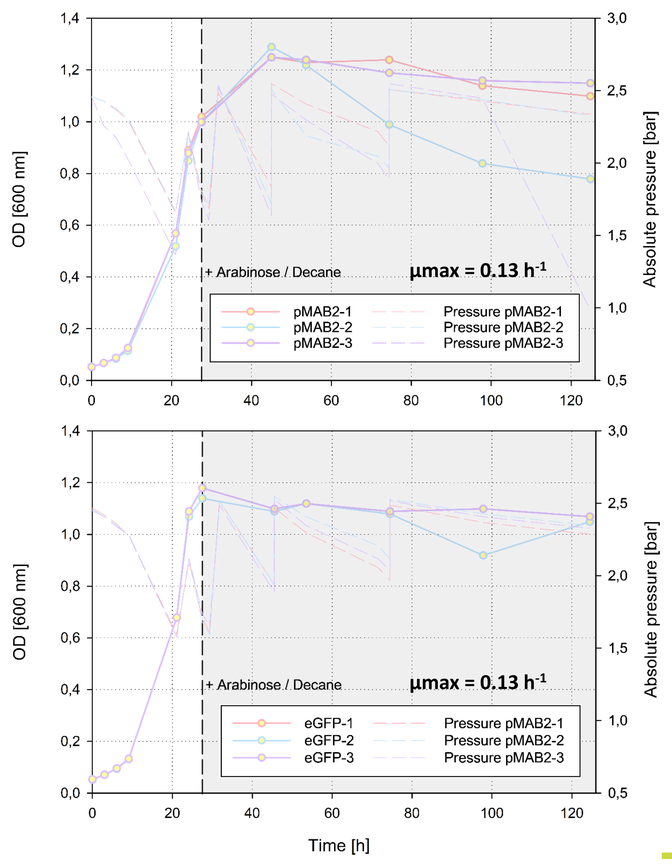


An alka(e)ne yield of 1,4 mg·g(X)$^{-1}$ was achieved. This represents the highest yield reported for *C. necator* on gas. C16al accumulation points to a bottleneck in the biosynthetic pathway.

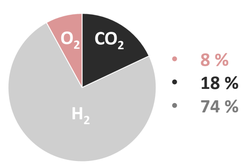
[3]



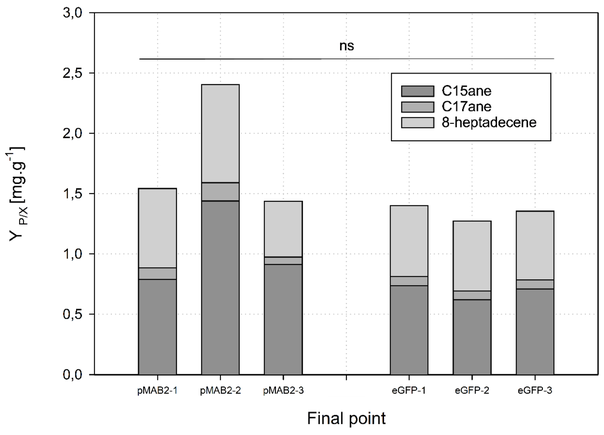
Alka(e)ne formation and plasmid stability in gas bottles with pMAB2 /pMAB2 eGFP



Kinetics of Re2061 pMAB2 (n=3) and Re2061 pMAB2 eGFP (n=3) in gas bottles.



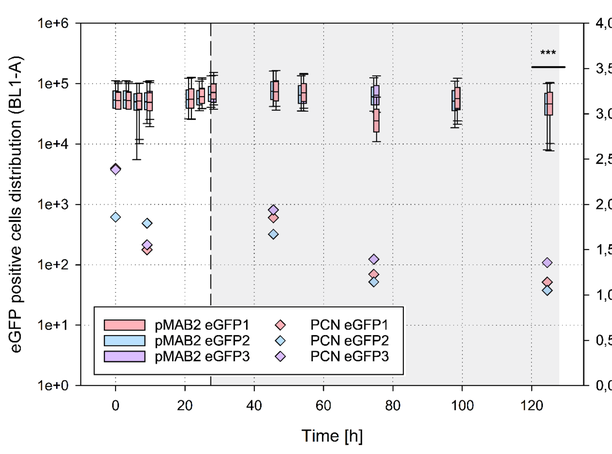
Production

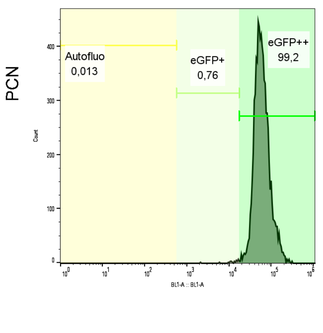


Alka(e)ne yield for Re2061 pMAB2/ pMAB2 eGFP. eGFP does not significantly affect yield.

ns (non significative), (Welch's t-test, P = 0,277).

Stability





Plasmid stability was assessed through flow cytometry and Plasmid Copy Number (PCN) quantification (determined by multiplex digital PCR). In all three bottles, the fluorescence intensity of the eGFP-positive population progressively decreased over time, with a significant difference between the first and last sampling points in each culture. This decrease in reporter fluorescence was correlated to a reduction in PCN, suggesting that plasmid loss may contribute to the observed decline in expression. However, the population underwent approximately six generations during the initial growth phase (5.8–24 h) and remained essentially stable during the production phase (0,1–100 h). Therefore, cell division alone cannot fully explain the observed plasmid loss, indicating that additional mechanisms may be involved.

***P < 0.001 (Mann–Whitney rank-sum test, n = 10,000).

[1] Panich, J., B. Fong, and S. W. Singer (2021). "Metabolic Engineering of *Cupriavidus necator* H16 for Sustainable Biofuels from CO_2". *Trends in Biotechnology* 39 **[2]** Peralta-Yahya, P.P., and Keasling, J.D. (2010) Advanced biofuel production in microbes. Biotechnol J 5: 147–162 **[3]** Crépin, L., Lombard, E., and Guillouet, S.E. (2016) Metabolic engineering of Cupriavidus necator for heterotrophic and autotrophic alka(e)ne production. Metab Eng 37: 92–101.

This project has received funding from the PEPR B BEST – OptiSfuel (ANR-22-PEBB-0011).
This work received government funding administered by the French National Research Agency under the Investments for the Future programme, reference number ANR-18-EURE-0021 .