Characterisation of isopropanol-producing *Cupriavidus necator* strains under autotrophic conditions

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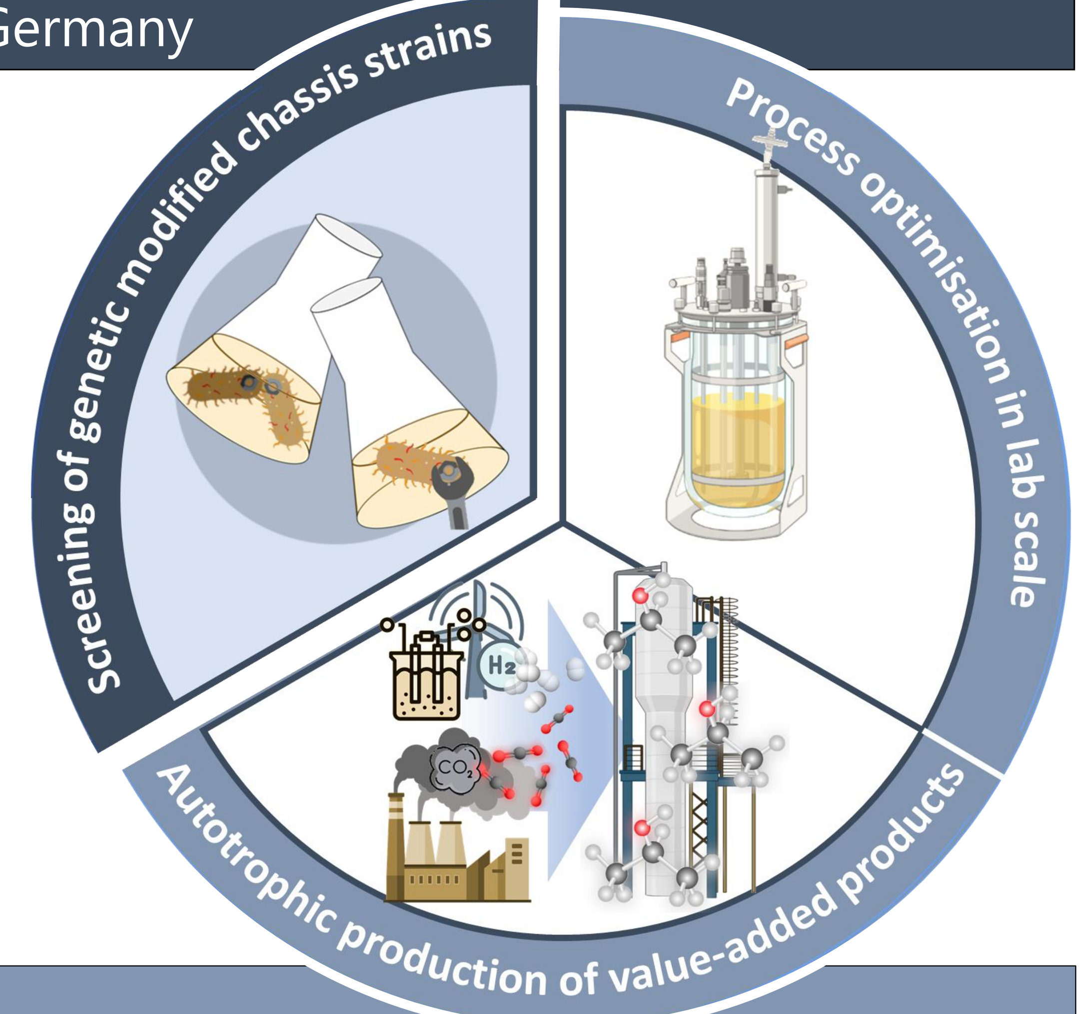

Background

Autotrophic fermentation is a promising approach to replace petroleum-based industrial processes while valorising CO_2 emissions as so far underexploited low-cost feedstocks.
Preceding a widespread industrial application, bottlenecks inherent to the process have to be overcome. Bioreactor fermentation is work-intensive, so here a cultivation system on a shake flask scale was set up to accelerate process optimisation. This facilitates preliminary strain screening and allows the determination of key process parameters as a basis for scale-up.
Here, isopropanol formation by the aerobic bacterium *C. necator* as an example of autotrophic fermentation was chosen. Isopropanol is a bulk chemical used as a solvent, disinfectant and platform chemical. Because traditional production based on fossil resources results in toxic waste and greenhouse gas emissions, alternative processes must be developed.
In this work, the suitability of the autotrophic shake flask system was demonstrated by applying it to the characterisation of several *C. necator* strains expressing the isopropanol pathway either constitutively or inducible.

Methods

Set-up of autotrophic shake flask cultivation

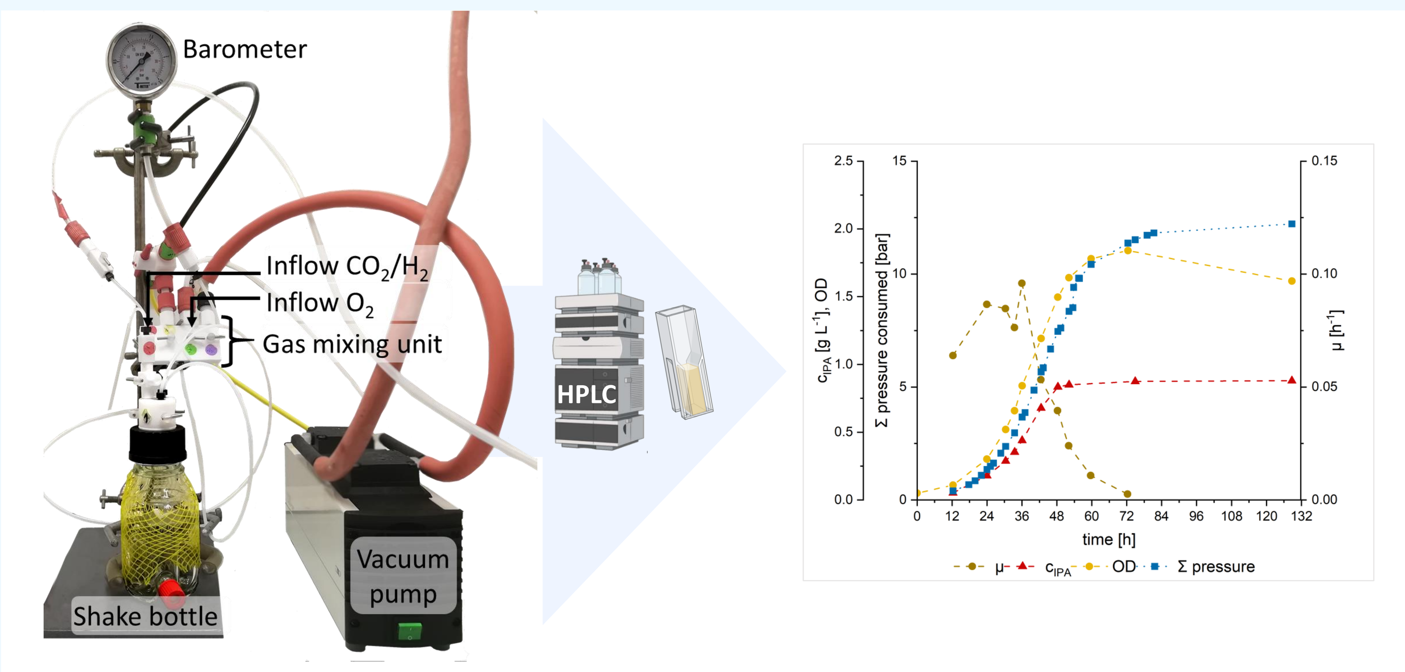

Fig. 1: Autotrophic shake flask during the gas refill process. The cultivation is followed by measuring the optical density (OD), product concentration and pressure loss.

- Hydrogen, oxygen and carbon dioxide are fed at 1.5 bar ($H_2/O_2/CO_2$ = 74/8/17)
- Pressure is controlled by barometer
- After consumption of 1 bar gas mixture the gas phase is replaced ($H_2/O_2/CO_2$ = 70/12/18)
- Metabolic activity is followed up simply by measuring decreased headspace pressure
- Allows parallel replicates
- Higher gas transfer than in serum bottles due to baffles and overpressure

Isopropanol producing *C. necator* strains

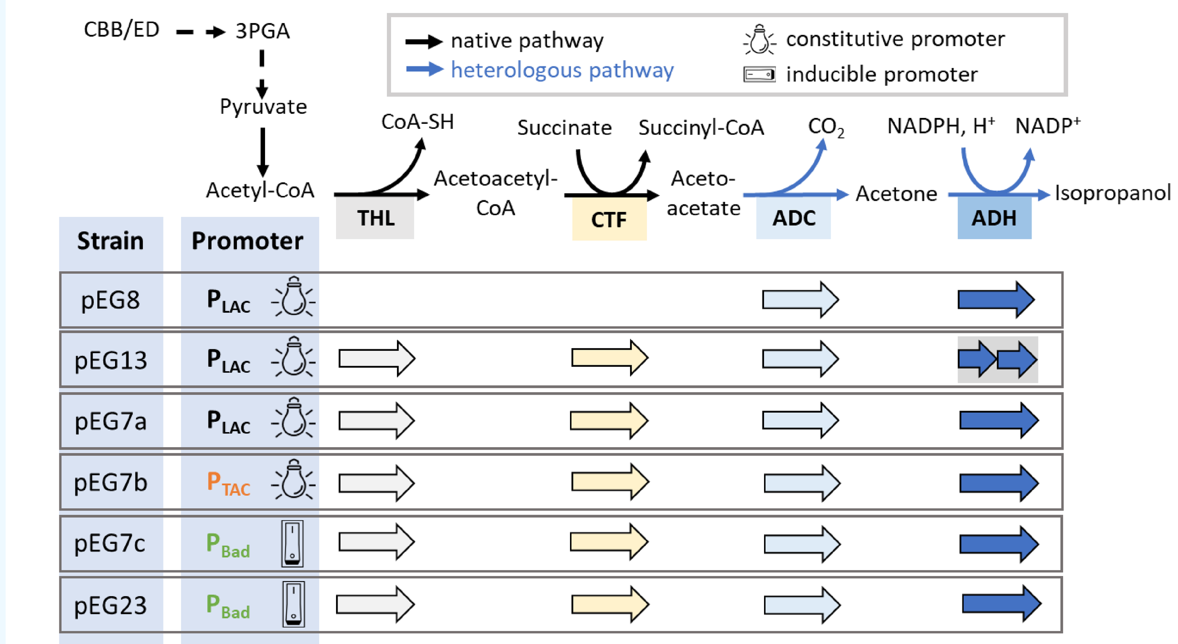

Fig. 2: Isopropanol-producing *C. necator* strains designed for heterotrophic fermentation by Grousseau et al. 2014 and Marc et al. 2017 which were evaluated here under autotrophic conditions.

Results

Link of growth, isopropanol formation and nickel availability

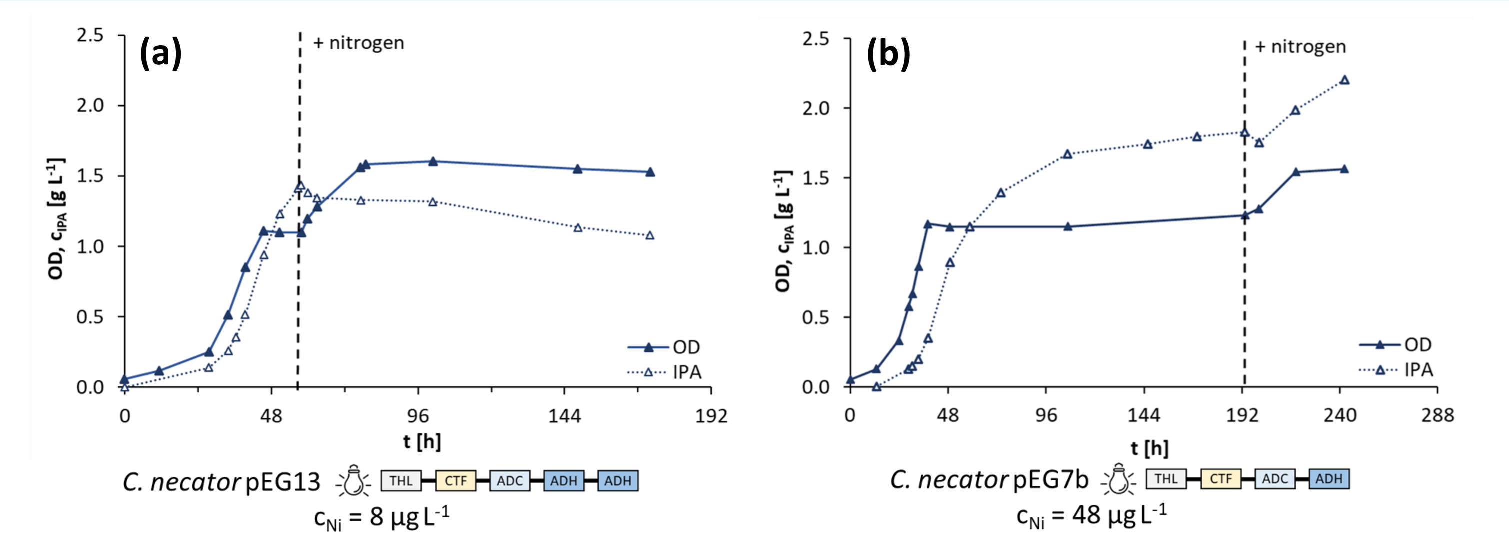

Fig. 3: (a) Nitrogen addition after presumed nickel limitation restarts growth (b) Higher nickel concentration prolongs isopropanol (IPA) formation, addition of nitrogen in presence of nickel restarts growth and product formation

Isopropanol formation stopped early due to a low concentration of nickel which is essential for hydrogenases and thus for CO_2 fixation (Burgdorf et al. 2005). Nevertheless, growth was still possible, showing the functionality of the hydrogenases. When product formation stopped while nickel was still available, it restarted with growth induced by nitrogen addition. Summarised, nickel availability and growth-coupled metabolic pathways supported a prolonged product formation.

High phenotypic variation of strains with constitutive promoters

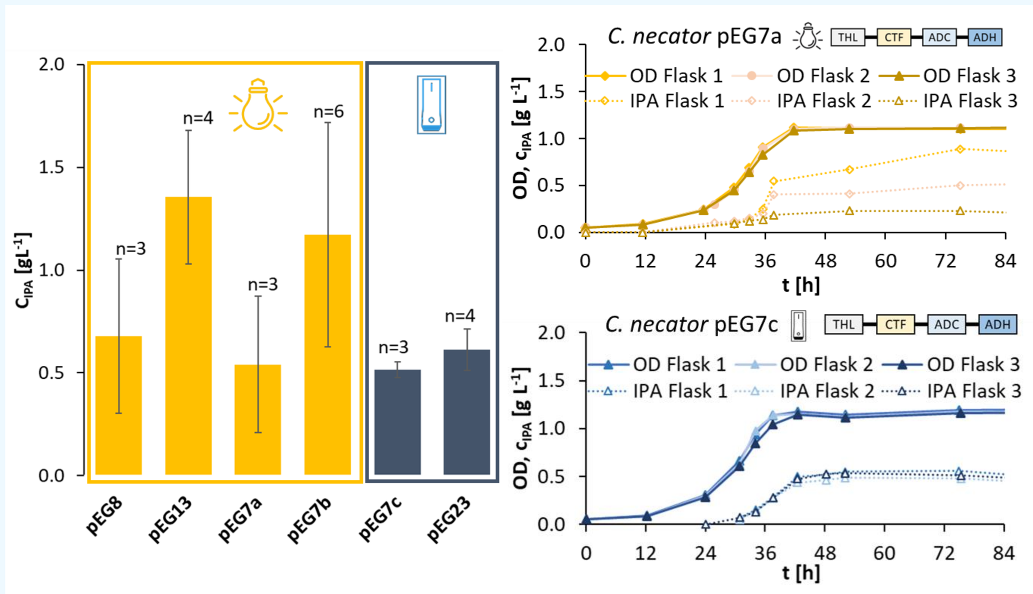

Fig. 4: Optical density (OD) and isopropanol (IPA) formation during cultivation of *C. necator* strains with inducible and constitutive promoters

While growth was reproducible for all strains, the isopropanol concentration showed a high standard deviation for the strains with constitutive promoter. In these strains the product formation started to differ between replicates after the exponential growth phase.

Key data of isopropanol formation by *C. necator*

Tab. 1: Comparison of autotrophically obtained isopropanol (IPA) concentration and yields with literature

C. necator strain	Fermentation system	c_{IPA} [$g\,L^{-1}$]	$Y_{P/X}$ [$g\,g^{-1}$]	r_{IPA} [$g\,L^{-1}h^{-1}$]	Source
pEG7b	Shake Flask	3.8	1.4	0.017	this work
pEG23	Bioreactor	0.3	0.3	0.009	[1]
pEG7b	Bioreactor	3.5	3.4	0.041	[2]
H16-CB-IPA-4	Bioreactor, continuous	7.7	5.4	0.016	[3]

[1] Marc et al. 2017, [2] Garrigues et al. 2020, [3] Bommareddy et al. 2020

The developed experimental setup allowed to obtain a similar product concentration as on bioreactor scale. Furthermore, a growth rate of 0.11 h^{-1} was obtained which is twice as high as the published 0.05 h^{-1} for the same strain (Garrigues et al. 2020).

Summary and outlook

A simple and straightforward cultivation method on shake flask scale under aerobic, autotrophic conditions was developed which will be useful for faster optimisation of fermentation processes using gaseous substrates.
For the here chosen model system – isopropanol production by *C. necator* – a high phenotypic variation of product formation was unveiled which has to be considered when interpreting the results of bioreactor fermentations, especially for strains with constitutive promoters. Furthermore, nickel limitation had a stronger impact on isopropanol production rather than growth and growth-associated processes prolonged product formation.
Among the screened strains *C. necator* Re2133/pEG7b achieved the highest isopropanol concentration of 3.8 $g\,L^{-1}$ with a specific yield of 1.4 $g\,g^{-1}$. This is promising for the now following process transfer into the bioreactor scale.

References

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