CO₂ as feedstock in microbial cultivations: Gas fermentations matched to microbial requirements and technical feasibility

Halima Aliyu Alhafiz^{1,2,3*}, Lars Lauterbach³, Regina Kratzer^{1,2}

¹Austrian Centre of Industrial Biotechnology (ACIB), Petersgasse 14, 8010 Graz, Austria. ²Institute for Biotechnology and Biochemical Engineering, TU Graz, NAWI Graz, Petersgasse 12, 8010 Graz, Austria. ³Institute of Applied Microbiology, RWTH Aachen University, Worringer Weg 1 D-52074 Aachen, Germany. * presenter/corresponding author.

Introduction

1).

Utilizing CO₂ as a feedstock for microbial cultivation offers a sustainable approach that reduces greenhouse gas emissions while producing valuable bio-based compounds (1). Cupriavidus necator, a hydrogen-oxidizing bacterium, uses the Calvin-Benson-Bassham (CBB) cycle to fix CO₂ into organic molecules, with H_2 as an electron donor and O_2 as the terminal electron acceptor (2). However, the combination of H_2 and O_2 brings with it the risk of explosive gas mixtures. Gas fermentation must therefore be optimized to match microbial requirements and technical capabilities (Table

Microbial requirements

Table 1. Gas composition ranges for cultivating *C. necator*.

H ₂ limitation (non-explosive)	$H_2 \le 4\% + atm. O_2 (21\%)$
O ₂ limitation (non-explosive)	$H_2 > 4\% + LEL of O_2 (4.8\%)$

H₂: O₂: CO₂ = 70%: 20%: 10% Optimum (Explosive)



Technical feasibilities

Under non-explosive O_2 levels (4%), biomass concentration (cell dry weight, CDW) can be increased by increasing the system's pressure and k₁ a, based on models (Equation 1, 2).

 $X = \frac{OUR}{}$ $OTR = k_L a \left(C_{O2}^* - C_{O2} \right)$ (1) q_{02} Cell Dry Weight (CDW) at Different $k_L a$ and Pressures 70 Pressure 1 bars 2 bars 60 -5 bars Cell Dry Weight (g/L) 10 -

PHB accumulation over time

In the presence of nitrogen, biomass was formed (CDW), after nitrogen depletion PHB (poly-3-hydroxybutyrate) was accumulated, the CDW was enhanced by increasing the k_1 a of the fermentation.



Figure 2. Figure 1. 20 300 600 k_La (per hour) Conclusions **PHB** production CDW: 13.5g/L PHB: ~70% CDW: 42.5g/L PHB: ~70% Achieved biomass concentration of up to 13.5g/L in a flask-scale Ο cultivation with a k_1 a of 20h⁻¹. Starting cond. Starting cond. • Reached biomass concentration of up to 42.5g/L in a lab-scale bioreactor $H_{2} 93\%$ cultivation with a k_1 a of 110h⁻¹. $H_2 40\%$ $O_2 2\%$ O₂ 2% 70% PHB accumulation of the total biomass, resulting in the production of Ο $CO_{2} 5\%$ $CO_{2} 5\%$ 29.8 g of PHB

1.2L stired tank reactor ($k_1 a = 110h^{-1}$)

References

(1) Lambauer, V., & Kratzer, R. (2022). Lab-scale cultivation of *Cupriavidus necator* on explosive gas mixtures: carbon dioxide fixation into polyhydroxybutyrate. Bioengineering 9(5), 204.

(2) Lauterbach, L., & Lenz, O. (2019). How to make the reducing power of H₂ available for *in vivo* biosyntheses and biotransformations. Current Opinion in Chemical Biology, 49, 91-96.

 $1L flask (k_1 a = 20h^{-1})$

PROJECT: ConCO₂rde

E-mail: halimaaliyualhafiz@acib.at

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 955740. This presentation reflects only the author's view, the Agency

is not responsible for any use that may be made of the information it contains.

