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Lab-Scale Cultivation of *Cupriavidus necator* on Explosive Gas Mixtures: Carbon Dioxide Fixation into Polyhydroxybutyrate

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Abstract:

Aerobic, hydrogen oxidizing bacteria are capable of efficient, non-phototrophic CO₂ assimilation, using H₂ as a reducing agent. The presence of explosive gas mixtures requires strict safety measures for bioreactor and process design. Here, we report a simplified, reproducible, and safe cultivation method to produce *Cupriavidus necator* H16 on a gram scale. Conditions for long-term strain maintenance and mineral media composition were optimized. Cultivations on the gaseous substrates H₂, O₂, and CO₂ were accomplished in an explosion-proof bioreactor situated in a strong, grounded fume hood. Cells grew under O₂ control and H₂ and CO₂ excess. The starting gas mixture was H₂:CO₂:O₂ in a ratio of 85:10:2 (partial pressure of O₂ 0.02 atm). Dissolved oxygen was measured online and was kept below 1.6 mg/L by a stepwise increase of the O₂ supply. Use of gas compositions within the explosion limits of oxyhydrogen facilitated production of 13.1±0.4 g/L total biomass (gram cell dry mass) with a content of 79±2% poly-(R)-3-hydroxybutyrate in a simple cultivation set-up with dissolved oxygen as the single controlled parameter. Approximately 98% of the obtained PHB was formed from CO₂.

Keywords: non-phototrophic CO₂ assimilation, Knallgas cultivation, Chemolithotrophs, ATEX compliant bioreactor, dissolved oxygen control