

Ph.D thesis: Valorization of CO₂ rich off gas through gas fermentation

Ph.D candidate: Giacomo Antonicelli

Summary

The transition from a fossil-based economy to a circular bioeconomy has accelerated the need for innovative and sustainable biotechnological processes. In recent years, the valorization of C₁-rich waste streams into liquid products through microbial-driven gas fermentation technology has gained significant attention. Notably, numerous studies have focused on the development of bioprocesses with acetogenic microbes to valorize industrial CO-rich off-gases. However, CO₂ emission sources are equally abundant and diverse in terms of purity, scale, and distribution. Among the high-value compounds produced by acetogens, hexanol is particularly appealing due to its wide range of industrial applications (e.g. food&beverage flavors, fragrances, cosmetics, chemicals and pharmaceuticals). The acetogen *Clostridium carboxidivorans* P7 is renowned for its natural ability to produce hexanol from CO-rich substrate. This thesis aims to develop a gas fermentation process with *C. carboxidivorans* P7 for bio-hexanol production using CO₂ and H₂ as feedstocks.

The growth and production performance of *C. carboxidivorans* P7 using CO₂ as carbon and H₂ as electron sources were enhanced via Adaptive Laboratory Evolution (ALE). The improved strain obtained named *C. carboxidivorans*_hex21 harbors a conserved frameshift mutation in the catalytic subunit of its hexameric hydrogenase, implicating alternative hydrogenases in more efficient H₂ utilization. Phenotypical analysis revealed a shortened lag phase and increased biomass growth relative to the wild type. Physiological characterization confirmed hexanol as the predominant fermentation product, achieving 45% carbon selectivity. This strain achieved the highest reported cell specific hexanol productivity of 0.74 g_{HexOH} g_{CDW}⁻¹ day⁻¹ directly from H₂ and CO₂.

The adapted strain was used for laboratory-scale process development in elevated pressure stirred tank batch reactors. The CO₂ and H₂ partial pressures, gas feeding strategies, and gas composition were systematically varied to evaluate effects on metabolic pathways. Continuous gas feeding promoted acetogenic and solventogenic activities, whereas gas limited conditions enhanced chain elongation to caproate, the hexanol precursor. Maximizing caproate accumulation in the first stage was critical for improving subsequent hexanol yields. An optimized two-stage configuration (initial gas limitation followed by continuous feeding) enhanced cell specific hexanol productivity to 0.9 g_{HexOH} g_{CDW}⁻¹ day⁻¹. In situ extraction further increased the hexanol carbon selectivity to 60%. These results demonstrate scalable H₂:CO₂-fed gas fermentation with enhanced growth and production.

The characterization of *C. carboxidivorans_hex21*'s metabolic response to process parameters enabled to transfer its performance to a different bioreactor system operating at atmospheric pressure. During a research period at Technical University of Wien (Prof. Stefan Pflügl's group), a cascade two-stage chemostat was designed to spatially separate caproate and hexanol production into sequential, distinct atmospheric pressure reactors. Operational variables such as pH, H₂:CO₂ ratios, gas flow rates, and agitation, were systematically optimized for each productive phase. Adjusting dilution rates within the cascade configuration achieved stable, continuous caproate synthesis in the first reactor and sustained hexanol formation in the second one. These results demonstrate the potential of reactor cascade design for process intensification and scalability for industrial-scale applications.

The energy and mass balance derived from laboratory-scale experiments was crucial for assessing scale-up feasibility. Using stoichiometric bioprocess reaction, commercial-scale energy demands were estimated, highlighting green hydrogen supply costs as the primary economic constraint. However, projected 2030 H₂ prices suggest competitive viability across industries. Mass balance calculations indicate consumption of 4.7 kg CO₂ per kg bio hexanol produced. A preliminary global warming potential assessment, encompassing mid and downstream stages, predicts net CO₂ equivalent savings of 3.5 and 5.4 kg of CO₂ per kg hexanol when powered by photovoltaic or wind energy, respectively. These findings confirm the process's carbon-negative footprint, further supporting sustainable deployment

This research successfully demonstrated the feasibility of a carbon-negative gas fermentation process for 1-hexanol production using green H₂ and CO₂ as feedstocks. The bioprocess was effectively optimized at laboratory scale, achieving stable 1-hexanol production performances. The newly microbial strain, obtained through laboratory evolution, exhibited outstanding production performance in the selected gas mixture. These findings lay a solid foundation for advancing H₂ and CO₂-based gas fermentation with acetogens toward industrial-scale bio-hexanol production, offering a sustainable and competitive alternative to conventional fossil- and palm oil-based methods.