Bioconversion of anaerobic digestion products to biomass and polyhydroxyalkanoates with *Cupriavidus necator*

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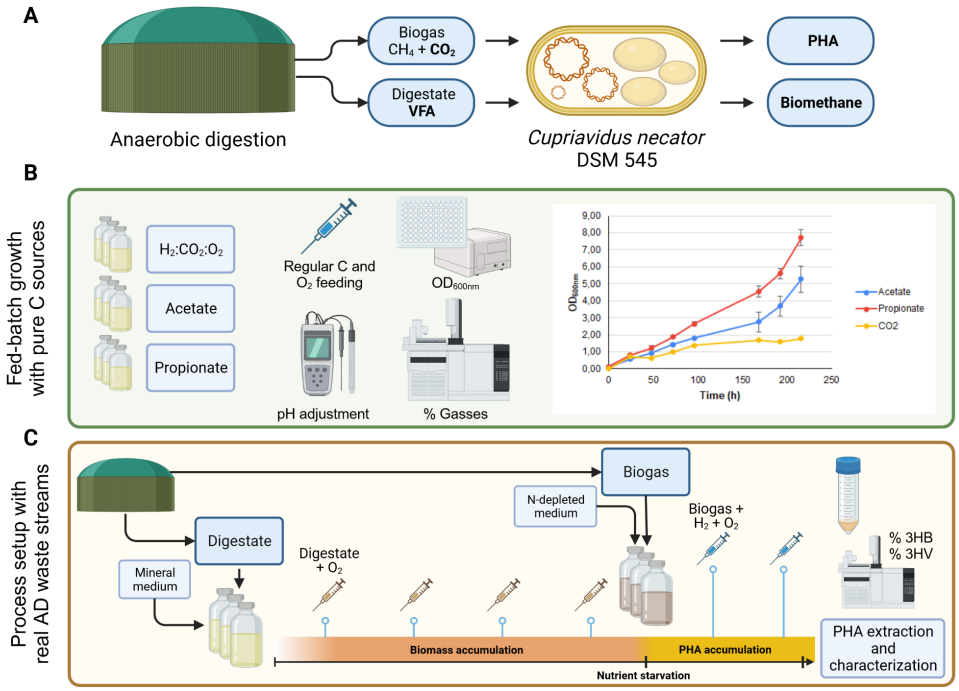

Figure 1. **A.** General concept for bioconversion of AD waste streams into PHA. **B.** Experimental setup and growth curves of fed-batch cultures with different carbon sources present in AD byproducts. **C.** Experimental setup for bioconversion of real AD waste streams into PHA.

Background

Anaerobic digestion (AD) is a widely employed process in industrial-scale valorization of organic waste, involving its conversion into biogas (50-70% CH_4, 30-50% CO_2) and digestate. Biogas, while suitable for heat and energy production, has a limited combustion efficiency due to its high CO_2 content. Biogas upgrading allows the removal of CO_2 to yield biomethane (>95% CH_4), which can be used as a natural gas substitute. Digestate, rich in volatile fatty acids (VFA) acetate and propionate, is another untapped carbon-rich AD byproduct (Kougias and Angelidaki, 2018).

The facultative chemolithotroph *Cupriavidus necator* is a promising platform for polyhydroxyalkanoate (PHA) production from CO_2 and VFA alike. PHAs, biodegradable polymers with mechanical properties comparable with traditional plastics, are difficult to produce competitively, due to the costly compounds (usually sugars) used for baterial growth. Hence, carbon-rich waste streams can be employed as carbon sources by taking advantage of the flexible metabolic properties of *C. necator* (Crutchik et al., 2020). *C. necator* growth and PHA production from CO_2 and VFA have been demonstrated at lab scale (Morlino et al., 2023), but few to no data are available on biogas and digestate utilization. Furthermore, no critical comparisons are available in terms of

growth rates obtained with the two substrates mentioned above, and there is a urgent need to establish a reliable strategy for biomass and PHA accumulation.

This research aims to set the groundwork for large-scale PHA production using biogas and digestate (Figure 1A). Preliminary experiments compared fed-batch growth with CO_2 (autotrophic) and VFA (heterotrophic). Further experiments involve: (i) replicating results with real biogas and digestate streams; (ii) fine-tune a two-step process to grow biomass and then accumulate PHA, maximizing CO_2 capture; (iii) assess the substrate effects on PHA monomeric composition.

Methods

C. necator DSM 545 (DSMZ, Germany) was cultured in DSMZ M81 medium. Fed-batch cultures were set up in gas-tight glass serum bottles, sealed with rubber stoppers and aluminum crimps. Daily monitoring included measuring optical density (OD_{600nm}) via spectrophotometry and gas concentrations in the headspace via chromatography. Acetic and propionic acid (Sigma-Aldrich, St. Louis, MO) were supplied to cultures at appropriate concentrations, adjusting pH with sodium hydroxide (Sigma-Aldrich). Autotrophic cultures received a daily supply of an H_2:O_2:CO_2 gas mixture (12:3:5, individual gases obtained from Sapio S.r.l., Milan, IT) at 2 atm pressure. PHA was extracted from lyophilized cells as described in Torri et al., (2014) and analyzed via gas chromatography using a flame ionization detector (FID) and AT-WAX column (30 m, 0.25 mm, 0.25 μm). Figures were created with Biorender.

Results and perspectives

C. necator DSM 545 was grown in fed-batch mode in three conditions: autotrophic growth with H_2:CO_2:O_2 gas mixture, and heterotrophic growth with acetate or propionate. To avoid VFA-induced growth inhibition, minimal amounts (0.6 g/L) of acetate and propionate were provided bi-daily, resulting in ODs of 5 and 8 after 9 days, respectively (Figure 1B). In contrast, autotrophic cultures supplied daily with the H_2:CO_2:O_2 mixture reached a final OD_{600nm} of 2 (Figure 1B), highlighting the effectiveness of an appropriate VFA feeding regime in accelerating biomass accumulation. Further experiments will aim to reproduce these outcomes using real biogas and digestate from the Mirandola plant in Bovolone, managed by BTS Biogas S.r.l. (Verona, IT). The final objective is to grow *C. necator* on digestate and, once sufficient biomass is reached, to shift the culture to autotrophic growth on biogas-derived CO_2, supplementing H_2 and O_2 to allow biogas upgrading. Simultaneously, the medium will be changed to introduce a nutrient depletion, triggering PHA accumulation. Overall, this work envisions lab-scale harnessing of AD byproducts, striding towards maximizing efficiency at closing loops in the short carbon cycle.

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