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Upcycling Agro-Industrial Digestates into Polyhydroxyalkanoates with Cupriavidus Necator

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Abstract

The replacement of fossil-based plastics with biodegradable alternatives requires platforms that combine renewable feedstocks, economic viability and environmental benefits. Polyhydroxyalkanoates (PHAs) are bacterial polyesters with promising applications as fully biodegradable bioplastics; however, their application at the consumer stage remains limited by high production costs, particularly those associated with carbon substrates and downstream processing (Kourmentza et al., 2017; Mahato et al., 2023). Anaerobically digested agro-industrial wastewaters represent an attractive alternative feedstock, as anaerobic digestion converts complex organic substrates into more readily assimilable carbon sources; these include volatile fatty acids and lactic acid (Maragkaki et al., 2026), which can be readily converted to PHAs by bacteria (Al Battashi et al., 2021; Szacherska et al., 2021). By integrating anaerobic digestion with PHA production, enhanced waste valorization and remediation can be achieved, in addition to streamlining the production of biofuels and biopolymers (Martinez et al., 2016; Sekoai et al., 2022). This work presents a holistic evaluation of mesophilic and thermophilic anaerobically digested co-digestates as growth media for PHA production by *Cupriavidus necator*. A range of locally sourced agro-industrial waste streams and their mixtures, including cheese whey, food waste, olive mill wastewater, pig manure, sewage sludge, were characterized and screened for their suitability to support bacterial cultivation. The digestates were assessed through physicochemical analysis and organic carbon profiling, with emphasis on volatile fatty acids, lactic acid, sugars, chemical oxygen demand, total Kjeldahl nitrogen and other parameters relevant to microbial growth and process performance. *C. necator*'s growth performance was evaluated across the different waste media by monitoring growth kinetic parameters, as well as biopolymer accumulation and substrate consumption trends. Through this approach, feedstock composition and their nutrient profile parameters were connected to microbial growth and biopolymer productivity. Following cultivation, the accumulated PHA was extracted and analyzed quantitatively and qualitatively. Polymer production was evaluated in terms of intracellular PHA content, volumetric yield, monomeric composition, including 3-hydroxybutyrate and 3-hydroxyvalerate fractions and polymer purity. In parallel, pollutant removal was assessed by monitoring reductions in COD,

TKN, sugars, and organic acids post-cultivation, linking microbial valorization to wastewater treatment efficiency. Our results demonstrate that selected anaerobically digested agro-industrial co-digestates can effectively support C. necator growth and PHA accumulation, with substrate composition playing a decisive role in process efficiency. Digestates enriched in readily biodegradable carbon sources, particularly those derived from cheese whey and food waste, showed strong potential for integrated PHA production and pollutant reduction. Overall, this study supports the development of an AD–PHA bioprocess that combines waste treatment, resource recovery, and biodegradable polymer production, adhering to the principles of circular bioeconomy.

Keywords: agro-industrial waste, bioplastics, circular bioeconomy, Cupriavidus necator, Polyhydroxyalkanoates

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