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Biological, Enzymatic, and Anaerobic Pretreatment of Cheese Whey for PHA Production by Cupriavidus Necator

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Abstract

Growing concerns over plastic pollution and agro-industrial waste accumulation have intensified the search for integrated and sustainable valorization strategies. Polyhydroxyalkanoates (PHAs) are microbial polyesters with potential as biodegradable plastics; however, their application remains limited by production costs, particularly those associated with carbon substrates (Kourmentza et al., 2017; Mahato et al., 2023). Cheese whey (CW) is a carbon-rich dairy by-product, but its direct valorization by *Cupriavidus necator* is limited by the strain's inability to assimilate lactose (Lohith Kumar et al., 2026). Pretreatments that convert lactose into readily metabolizable substrates could therefore enable whey-based PHA production (Basaglia et al., 2025; Zhang et al., 2022), while generating valuable coproducts.

In this work, CW was subjected to five pretreatment approaches prior to *C. necator* cultivation. A *Lactobacillus* strain was employed to produce lactic acid, while *Propionibacterium* was used for its ability to produce propionic acid. Treatment with *Scenedesmus obliquus* exploited extracellular lactase activity to hydrolyse lactose into glucose and galactose, generating microalgal biomass. Lactose was also hydrolysed using a commercial lactase. Finally, an anaerobic digestion (AD) approach was investigated under mesophilic and thermophilic conditions, yielding lactic acid, ethanol volatile fatty acids (VFAs) and hydrogen, while maintaining low or negligible methane formation. Untreated and pretreated whey substrates were assessed as growth media for *C. necator*, and growth, PHA accumulation, cell morphology and substrate consumption were evaluated.

Limited growth was observed in untreated and *Scenedesmus*-treated whey. In contrast, *Lactobacillus*-treated, enzymatically hydrolysed, and AD-whey supported growth comparable to, or higher than that in the control medium. PHA accumulation reached approximately 60% w/w of biomass. Nuclear magnetic resonance analysis demonstrated the production of poly(3-hydroxybutyrate - PHB) in the control, *Lactobacillus*-treated, and enzymatically hydrolysed whey. Both mesophilic and thermophilic digestates supported poly(3-hydroxybutyrate-co-3-hydroxyvalerate - PHBV) production, with a 3-hydroxyvalerate content of 5-6%. Microscopy

confirmed intracellular PHA granules and revealed elongated cells in whey-based media compared with the control, indicating a morphological stress response to the complex media. In samples with notable growth, quantitative NMR analysis showed that VFAs, monosaccharides, and ethanol were consumed.

Overall, appropriate pretreatment overcame the limitations of lactose utilization by *C. necator*. This integrated approach enabled the production of PHB or PHBV, depending on the pretreatment, together with coproducts such as hydrogen and microalgal biomass, supporting cheese whey valorization within a circular bioeconomy framework.

Keywords: Cheese whey, valorization, polyhydroxyalkanoates, bioplastics, Cupriavidus necator

References

Basaglia, M., Casella, S., Franzosi, G., Favaro, L. 2025. Sustainable Polyhydroxyalkanoates production by Cupriavidus necator DSM 545 from whey permeate. International Journal of Biological Macromolecules, 320, 146024.

Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H.N., Reis, M.A.M. 2017. Recent Advances and Challenges towards Sustainable Polyhydroxyalkanoate (PHA) Production. in: Bioengineering, Vol. 4, pp. 55.

Lohith Kumar, D.H., Sithole, S., Khumalo, G.A., Pillai, S., Kumari, S. 2026. Sequential hydrolysis and fermentation of polyethylene terephthalate and dairy wastewater for the production of polyhydroxyalkanoate and glycolic acid by Cupriavidus necator. International Journal of Biological Macromolecules, 368, 152705.

Mahato, R.P., Kumar, S., Singh, P. 2023. Production of polyhydroxyalkanoates from renewable resources: a review on prospects, challenges and applications. Archives of Microbiology, 205(5), 172.

Zhang, L., Jiang, Z., Tsui, T.H., Loh, K.C., Dai, Y., Tong, Y.W. 2022. A Review on Enhancing Cupriavidus necator Fermentation for Poly(3-hydroxybutyrate) (PHB) Production From Low-Cost Carbon Sources. Front Bioeng Biotechnol, 10, 946085.

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