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Redox Regulation of Hydrogenase Activity and Polyhydroxyalkanoate Production in *Cupriavidus necator* H16 Cultivated on Dairy Industry Sidestreams

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ABSTRACT

Cupriavidus necator is a chemolithoautotrophic bacteria with high biotechnological relevance, particularly for bio-based production processes. Organic streams (whey) from the dairy industry, rich in proteins, sugars, and minerals, can serve as valuable sources of carbon and nitrogen for microbial growth [1]. This study evaluates the effect of redox reagent dithiothreitol (DTT) on bacterial growth, hydrogenase (Hyd) activity, and polyhydroxyalkanoate (PHA) accumulation in *C. necator* H16 cultured on acid whey (AW) and sweet whey (SW). Bacterial growth was monitored by measuring optical density at 600 nm (OD_{600}), and Hyd activity was quantified using hydrogen-dependent methylene blue reduction, while PHA accumulation was analyzed using high-performance liquid chromatography (HPLC) [2]. The results demonstrated enhanced bacterial growth in DTT-supplemented samples. The highest OD_{600} was observed on day 5 for the AW+DTT culture (OD_{600} = 5.1) and on 6th day for the SW+DTT culture (OD_{600} = 4.6), ~ 2 fold higher than their respective controls without DTT. Hyd activity was detected in all samples; however, its onset was earlier and more pronounced in DTT-treated cultures. The maximum Hyd activity reached 0.17 U/min/g CDW in the SW+DTT sample. PHA accumulation was observed during growth on both AW and SW. Interestingly, PHA levels decreased when Hyd activity was high, suggesting a possible inverse relationship between energy metabolism via hydrogen oxidation and carbon storage. In summary, supplementation with DTT enhances *C. necator* H16 growth on dairy side-streams, promotes earlier Hyd activation, and influences PHA accumulation dynamics. This approach highlights the potential of valorizing dairy industry by-products for sustainable microbial bioprocesses.

Keywords: dairy industry sidestreams, polyhydroxyalkanoate, hydrogenase enzymes, redox regulation

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