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Enhancing Biohydrogen and VFA Production from Cheese Whey: Effects of Temperature and Co-digestion with Organic Waste

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Abstract

The growing generation of organic waste and continued reliance on fossil fuels have intensified the search for renewable energy carriers and sustainable chemicals, positioning the circular bioeconomy as a framework for valorizing waste into energy, fuels and platform chemicals (Chegini et al., 2026; Nemati et al., 2025). Dark fermentation, the light-independent anaerobic conversion of organic substrates into biohydrogen (H2) and volatile fatty acids (VFAs), offers a low-energy, scalable route to co-produce a clean energy carrier and valuable chemical precursors (Mokhtarani et al., 2025). Among suitable feedstocks, cheese whey (CW) is both a major environmental burden and an attractive substrate: global cheese production yields roughly 190 billion kg of whey annually, with a high organic load (COD 60-80 g/L) driven mainly by lactose (40-50 g/L) alongside proteins, lipids and minerals (Buchanan et al., 2023). While untreated discharge causes eutrophication and oxygen depletion, its readily hydrolysable lactose makes it an excellent substrate for H2 and VFA production; however, its high organic strength and low buffering capacity can trigger rapid acidification and process instability, motivating co-digestion with complementary substrates (Aranda-Jaramillo et al., 2023). This study examined the dark fermentation of cheese whey (CW) as the main substrate, both individually and co-digested with liquid pig manure (LPM), diluted food waste (FWdil), dried food waste (FWdry) and sewage sludge (SS), using continuous stirred tank reactors (CSTRs) operated under mesophilic (37 °C) and thermophilic (55 °C) conditions at a hydraulic retention time (HRT) of 24 hours. Eight experimental configurations were assessed to determine their influence on biohydrogen (H2) yield, volatile fatty acid composition and organic matter removal. Methane was absent under all conditions, confirming that fermentation proceeded exclusively via the acidogenic pathway. Thermophilic operation markedly improved H2 production across every co-digestion mixture, with the ternary blend (CW 60% & FWdry 20% & SS 20%) reaching the highest yield of 1.5 L H2/Lreactor/d at 55 °C roughly 3.8-fold greater than the corresponding mesophilic rate, alongside a total carbohydrate removal of 73.2%. In contrast, mesophilic conditions promoted greater VFA accumulation: the same mixture yielded 5573 ± 126 mg/L TVFAs at 37 oC, jointly dominated by acetic (2080 ± 365 mg/L) and butyric (2569 ± 356 mg/L) acids and exhibited the highest HBu/HAc ratio (1.23) among all conditions. Thermophilic operation consistently suppressed propionic acid, shifting the profiles toward acetate-butyrate dominance, which is advantageous for both H2 generation and downstream polyhydroxyalkanoate (PHA) synthesis. The CW & FWdry & SS effluent displayed one of the most favourable VFA profiles for subsequent PHA

production. Overall, these findings demonstrate that cheese whey dark fermentation can be directed toward either H2 production or VFA-rich effluents by adjusting temperature and co-substrate composition.

Keywords: dark fermentation, cheese whey, volatile fatty acids, biohydrogen, co-digestion, polyhydroxyalkanoates, mesophilic, thermophilic

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