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Biohydrogen Production by Lactate-based Dark Fermentation from Cheese Whey in Continuous Mode

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Abstract

Cheese whey (CW) is the wastewater generated by the dairy industry during cheese making processing. CW is a yellow-green liquid and produced by the precipitation of casein from milk. Due to its relatively high organic load (40-60 g COD/L), it can be highly polluting if it is released into the environment without proper treatment (Ahmad et al., 2019). Additionally, this residue contains around 50% of milk nutrients, so it can be valorized and used in different fields such as the food industry (modification of physicochemical properties of food), energy (biofuel) and production of other value-added products (Ramos et al., 2021). Dark fermentation (DF) is a promising biological process to produce hydrogen as an energy vector or feedstock from organic wastes and wastewaters. CW is indeed considered a potential substrate for the production of hydrogen and other value-added products through DF. However, the DF of CW is commonly inhibited due to the overwhelming growth of lactic acid bacteria (LAB). Recently, it has been reported that lactate-based metabolic pathways are effective for hydrogen production, provided that lactate cross-feeding occurs between LAB and lactate-oxidizing-hydrogen-producing bacteria (LO-HPB). The objective of this study was to evaluate the continuous production of hydrogen from CW using a specialized biocatalyst able to biotransform lactate into hydrogen. This approach aimed to harness syntrophic interactions between LAB and LO-HPB (García-Depraect et al., 2021). To achieve the main aim, a laboratory scale stirred tank reactor (2.5 L) with a working volume of 1 L was used. The culture conditions were: 35 °C, 150 rpm, pH 5.8 ± 0.02, and hydraulic retention time (HRT) of 24 h and 18 h. CW (103 g COD/L, 13 g/L reducing sugars, 94.8 g TS/L, 82.6 g VS/L) and an inoculum mainly composed of LAB and LO-HPB (10% v/v) were used as substrate and biocatalyst, respectively. The removal of sugars, as well as the productivity of hydrogen (L H_2/L-d) were recorded. Regarding the results, a

productivity of 6.5 ± 0.5 L H_2/L-d was achieved at 24 h HRT, with an associated yield of 62.3 ± 5.4 mL H_2/g CODfed. A reducing sugars removal of 98.2 ± 0.1% was computed. Further, the change of HRT from 24 h to 18 h resulted in an increase in the productivity, reaching up to 7.8 L H_2/L-d (the highest so far reported for CW) with a corresponding hydrogen yield of 54.6 ± 2.7 mL H_2/g CODfed. No difference was observed in the removal efficiency of reducing sugars compared to that at 24 h HRT. As for the soluble metabolites, the major organic acid detected was butyrate, which peaked up to 33 g/L when hydrogen productivity peaked (7-9 L H_2/L-d). Hydrogen productivity correlated positively with butyrate but negatively with lactate, strongly suggesting the presence of lactate-driven DF. Altogether, it can be concluded that CW is a promising raw material to produce biogenic hydrogen via the lactate-driven DF. This non-conventional hydrogen-producing pathway is key to overcome issues related to the inhibition of hydrogen production by LAB. Here, marked fluctuations in hydrogen productivity were observed throughout the operation, likely due to the dynamic equilibrium between LAB and LO-HOB. Hence, further research is still needed to ensure long-term stability in hydrogen production via LD-DF.

Keywords: Cheese whey, Dark fermentation, Hydrogen

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