

Sulfide-Induced Shifts in Biohydrogen Production and Microbial Dynamics during Dark Fermentation

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Most commercial hydrogen production has relied on fossil fuel-based reforming processes, which limits its sustainability (García *et al* 2024). In contrast, biohydrogen production using organic wastes has gained attention as an eco-friendly alternative that supports carbon neutrality (Sarkar *et al* 2021). Among various biological routes, dark fermentation (DF) has emerged as a promising process for hydrogen generation from organic wastes. DF offers notable economic and environmental merits, including low energy input, and rapid reaction rates (Fasheun *et al* 2024). However, the presence of sulfides is known to inhibit microbial activity and is considered a major limiting factor for hydrogen production in DF (Wang *et al* 2008). Despite its importance, limited studies have evaluated the impact of sulfide concentration on biohydrogen yields in DF. Therefore, this study aimed to investigate the effects of sulfide concentrations on hydrogen production and microbial community dynamics. Batch DF experiments were performed using sucrose as the substrate. Two experimental setups were prepared using inoculum with and without heat pretreatment (90 °C for 20 minutes). Each reactor had a working volume of 500 mL, with an initial total chemical oxygen demand (TCOD) of 40,000 mg/L. Anaerobic conditions were established by adjusting the initial pH to 8.0 ± 0.1 using 3 N KOH and purging the headspace with nitrogen gas. During the process, the operational pH was maintained at 5.5 ± 0.1 , and the temperature was controlled at 38 °C. In Experiment I, using non-pretreated inoculum, hydrogen production sharply decreased with increasing sulfide concentrations. As shown in Figure 1 (a), hydrogen production was 736.9 ± 12.0 mL at 200 mg S/L and 578.1 ± 16.6 mL at 400 mg S/L, corresponding to decreases of 55.4% and 64.9%, compared to the control ($1,649.3 \pm 34.3$ mL), respectively. At 800 mg S/L, hydrogen production was severely inhibited, indicating the toxic effects of high sulfide levels on hydrogen-producing bacteria. In contrast, Experiment II, which used heat-pretreated inoculum, showed an opposite trend. Sulfide addition positively affected hydrogen production, particularly at 200 mg S/L and 400 mg S/L. As shown in Figure 1 (b), the highest hydrogen production was observed at 200 mg S/L, reaching $4,627.5 \pm 16.9$ mL, which is 44.7% higher than the control ($3,199.2 \pm 36.2$ mL). These findings highlighted the beneficial impact of heat pretreatment in enhancing DF performance, likely by favoring the proliferation of hydrogen-producing bacteria. Analysis of organic acids further supported this trend; the proportion of butyrate increased to 56.0% at 200 mg S/L and 53.0% at 400 mg S/L, compared to 47.0% in the control. Moreover, microbial community analysis revealed that the relative abundance of *Clostridium*, a key genus involved in hydrogen production via the butyrate pathway, increased to 88.3% at 200 mg S/L and 95.4% at 400 mg S/L, compared to 78.6% in the control as shown in Figure 2 (Fritsch *et al* 1996). These results indicate that the combination of heat pretreatment and moderate sulfide addition promoted *Clostridium*-dominated pathways, particularly butyrate conversion, ultimately enhancing biohydrogen production.

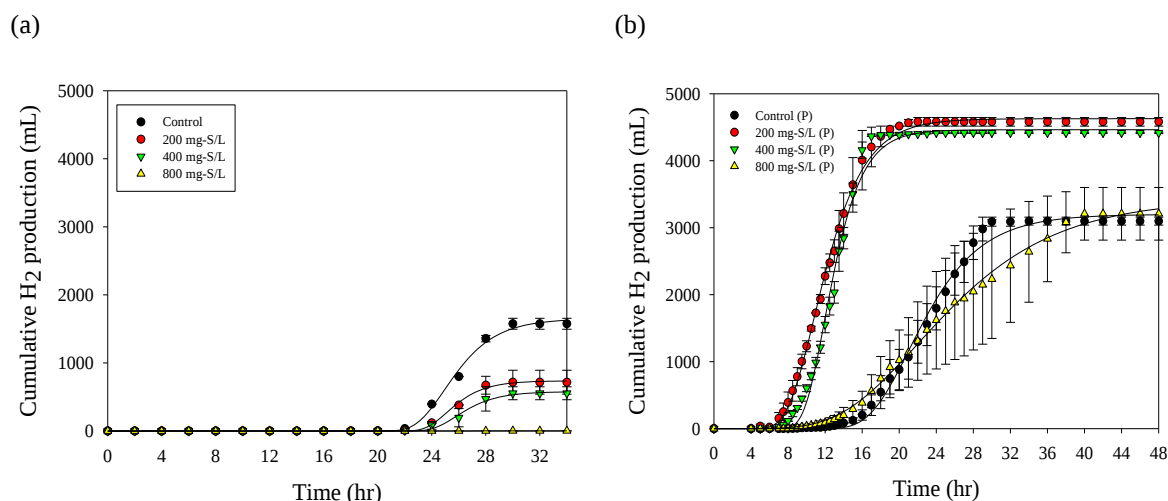


Figure 1. Variation of cumulative hydrogen production of (a) Experiment I and (b) Experiment II

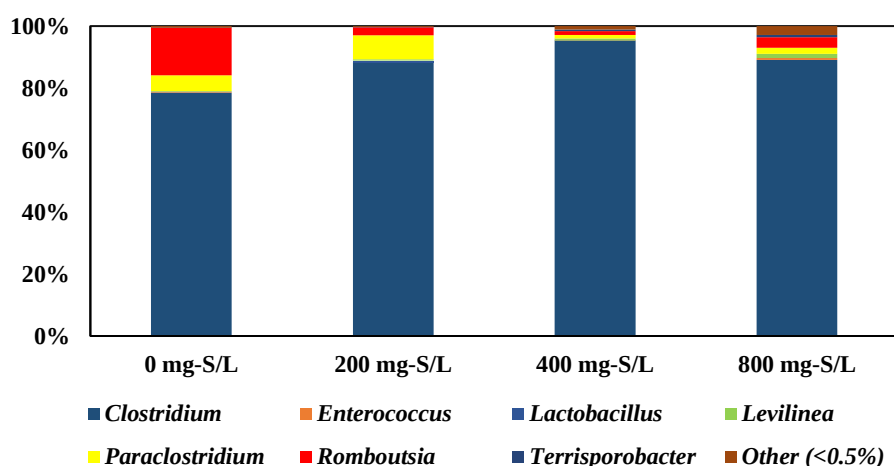


Figure 2. Comparison of microbial community analysis (genus) obtained from Experiment II

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