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Overcoming propionic acid inhibition of hydrogen fermentation by temperature shift strategy

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ABSTRACT

A novel temperature shift strategy has been proposed to overcome an inhibition on hydrogen fermentation of beverage industry wastewater (BW) due to the accumulation of propionic acid (HPr) during continuous reactor operation. The continuous performance at constant pH 5.5, temperature 37 °C and hydraulic retention time (HRT) 8 h with BW concentration of 20 $g/L_{hexose\text{-}equivalent}$ in a stirred tank reactor (2 L) showed an accumulation of HPr to 2.36 g/L leading to a drop in hydrogen production rate (HPR) from 10 to 8.5 L L^{-1} d^{-1}. To overcome the HPr inhibition, a temperature shift (from 37 °C) to 45 °C for 8 h was applied. This significantly improved the inhibited HPR and HY to 13.6 L L^{-1} d^{-1} and 1.68 mol-H_2 mol^{-1} hexose, respectively, with a simultaneous reduction in the HPr concentration to 0.7 g/L. Microbial community analysis based on PCR-DGGE after temperature shift revealed the non-dominance of *Selenomonas lacticifex* and *Bifidobacterium catenulatum* (involved in HPr formation), and dominance of hydrogen producing bacteria namely *Clostridium butyricum*, *Clostridium perfringenes*, *Clostridium acetobutylicum*, and *Ethanoligenens harbinense*. This study demonstrated that temperature shift strategy could overcome the HPr inhibition and significantly improve the hydrogen fermentation of an industrial wastewater.

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Introduction

Biohydrogen production basically involves light-dependent process and the light-independent process, so called dark fermentation. Dark fermentation seems to be more advantageous owing to its low energy requirement for biohydrogen production and higher production rate than other biologically mediated hydrogen production systems [1]. Generally, under acidogenic phase, according to the main soluble metabolic end products three pathways has been observed and classified as butyric acid (HBu) type [2], ethanol (EtOH) type [3], and propionic acid (HPr) type [4]. The HBu and EtOH types have higher hydrogen generation rate. On the other hand, HPr type is associated with lower biohydrogen production rate [4]. Hence, HPr should be eliminated for an efficient hydrogen production in dark fermentation.

HPr production and accumulation in the acidogenic phase lead to a drop in hydrogen production activity [5]. The mechanism of the HPr accumulation in the acidogenic-reactor is

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not clearly understood. For example, it is indicated that the HPr accumulation is due to the shock loading or overloading, or in start-up stage [2,6]. Cohen et al. [7] demonstrated that the discontinuous feed supply enhanced the HPr production and reduced HBu and biogas productions. Some studies reported that higher hydrogen partial pressure is the main reason for HPr accumulation [8], because under the conditions of higher hydrogen production in acidogenic phase higher NADH yield is observed. HPr production produces more NAD^+ than HBu production and the HBu metabolic pathway is then replaced by HPr metabolic pathway to maintain a proper ratio of NADH/NAD^+ inside the cell. The hydrogen partial pressure is therefore, considered as the reason for HPr accumulation. However, Inanc et al. [9] reported that the accumulation of HPr seemed to be independent of hydrogen partial pressure, and demonstrated that the HPr production was a result of a shift in the dominant species of acidogenic populations.

The HPr inhibition seems to be a serious problem in a continuous hydrogen production system, in which HPr formation affects the volumetric hydrogen production rate (HPR) and hydrogen yield (HY). The inhibition levels as reported in several studies [10-12] vary from 0.2 to 12 g/L with the type of feedstock, inoculum source and reactor operational condition. As shown in Table 4, some strategies have been reported to overcoming the HPr accumulation by mixed cultures. However, few studies have been reported on the microbial communities responsible for HPr accumulation and their effects on hydrogen production [5,13]. Besides, the metabolic inter-relationship of different hydrogen-producing/consuming bacteria is not well elucidated. Therefore, in this study, we investigated the effect of the temperature shift on the HPr inhibition and the dynamic changes in the microbial community composition before and after the temperature shift in a continuously-stirred tank reactor (CSTR) fed on industrial wastewater.

Materials and methods

Microbial source and wastewater composition

Compost of food waste from central Taiwan (Kindly provided by Professor Dr. Kung-Yuh Chiang, Feng Chia University, Taiwan) was used to enrich the mesophilic hydrogen-producing bacteria. The pH of the compost was 6.8. The collected compost was stored at 4 °C. The compost was heated at 80 °C for 30 min to deactivate the hydrogenotrophic methanogens before it was used in the enrichment of hydrogen-producing bacteria using sucrose as the sole carbon source. The enrichment method was followed as described elsewhere [14]. Freshly grown (24 h) enriched mixed cultures was used as inoculum for CSTR experiments.

The wastewater feedstock was collected from a beverage industry located at central Taiwan. The characteristics of the beverage wastewater (BW) were pH 2.6–3.4, COD 760–900 g/L and total reducing-sugar 660–750 $g_{(glucose\ equivalent)}$/L. From this feedstock wastewater, 20 g total reducing-$sugar_{(hexose\ equivalent)}$/L substrate solution was made for the experiments. The wastewater substrate was stored at 4 °C.

Experimental setup for continuous operation

The schematic representation of the experimental setup is shown in Fig. 1. The working volume of the CSTR was 2.0 L. Initially, the reactor was seeded with 200 mL enriched mixed culture and 1800 mL substrate. The operation temperatures were 37 °C for hydrogen production and 45 °C during temperature shift for HPr reduction. The reactor was fed on the substrate wastewater with 20 g total reducing-sugar/L at an HRT of 8 h [15]. pH 5.5 was maintained using 3 M NaOH solution. The basal medium used was followed from the Endo formulation [16] and contained the following ingredients (g/L): 5.24, NH_4HCO_3; 6.72, $NaHCO_3$; 0.125, K_2HPO_4; 0.1, $MgCl_2 \cdot 6H_2O$; 0.015, $MnSO_4 \cdot 6H_2O$; 0.025, $FeSO_4 \cdot 7H_2O$; 0.005, $CuSO_4 \cdot 5H_2O$; 0.00012, $CoCl_2 \cdot 5H_2O$.

Analytical methods

The monitoring parameters were pH, ORP (oxidation–reduction potential), soluble metabolic products (SMPs) distribution and gas production. The biogas volume was measured using a wet-gas meter (Ritter, Bochum, Germany). The hydrogen production efficiency was evaluated using the hydrogen content in biogas, hydrogen yield (HY) and hydrogen production rate (HPR). Biogas composition (H_2 and CO_2) was analyzed with a gas chromatograph having a thermal conductivity detector (China Chromatograph 8700T). The volatile fatty acids and ethanol concentrations were detected by gas chromatography (Shimadzu GC-14A) using a flame ionization detector (FID). The temperatures at glass column and injection port were 145 and 175 °C, respectively. The

Table 1 – Hydrogen production during changes in reactor performance and process upset.

Day	Reactor performance/upset	Response of the reactor
0	Starting-up the reactor in a batch mode, pH 5.5, 37 °C	Hydrogen production started after a lag of 12 h with HPR 3.5 L L^{-1} d^{-1} and H_2 content 50%
1	Continuous flow started with HRT 8 h	Hydrogen production gradually increased to 13.65 L L^{-1} d^{-1} at day 7
8	pH increased to 10.0 due to feed pump failure	Hydrogen production significantly dropped to 7.8 L L^{-1} d^{-1}
12	Culture addition (200 mL)	Hydrogen production recovered quickly after the culture addition and stabilized to 10.5 L L^{-1} d^{-1}
36–49	Propionic acid accumulation in the reactor (HPr 2.36 g/L)	Hydrogen production dropped to 8.58 L L^{-1} d^{-1}
50	Reactor temperature was changed from 37 °C to 45 °C for one cycle of operation (8 h HRT)	Hydrogen production increased and stabilized to 13.58 L L^{-1} $d^{-1}$with significant reduction in propionic acid concentration (0.73 g/L)

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