Electronic Supplementary Information

A Compact BrFAFC (Bio-reformed Formic Acid Fuel Cell) Converting Formate to Power

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1. Experimental

A seed was cultured in a 120-mL serum bottle (working volume 20 mL) at 37 °C and agitated in a shaking incubator at 220 rpm. After seed cultivation, the inoculum at the late exponential phase (OD_{680}=1.8) was transferred anaerobically into a 500-mL serum bottle (working volume 360 mL). All anaerobic procedures were carried out in an anaerobic chamber (SK-G002-A1, Dwyer Instruments Inc., USA). The serum bottles were sealed with a 12 mm-thick butyl rubber septum and an aluminum cap. For cell growth, cells were cultured at 37 °C in 360 mL of PYG (peptone, yeast extract, and glucose) medium for 6 hr. The PYG medium that was used for cell growth contained 10 g/L glucose, 15 g/L peptone, 3 g/L yeast extract, 0.5 g/L $MgSO_4 \cdot 7H_2O$, and 2 g/L KH_2PO_4. Following 6 hr of cultivation under anaerobic conditions, the cells were harvested by centrifugation at 11300 g for 20 min. The harvested cells, at the appropriate concentration, were transferred to a reactor (fermentor or bio-reformer) containing a formate solution for hydrogen production, which is separated from cell growth. Formate was used as a substrate in the hydrogen production stage, which is separated from the cell growth stage.

The cell concentration in the culture broth was determined by measuring the absorbance at 680 nm using a spectrophotometer (Genesys, Spectronic Instruments, USA). One unit of absorbance was equivalent to 0.42 g dry cell/L. The formate concentration in the culture broth was analyzed by high performance liquid chromatography (HPLC) (HP1100, Agilent Technologies, USA) with an Aminex HPX-87H packed column (ϕ300 x 7.8 mm, Bio-Rad, USA). The liquid samples were purified through a 0.2-μm disposable filter prior to injection. A refractive index detector (RID) was used to quantify the formate. A 0.01 N H_2SO_4 solution

at 0.6 mL/min was used as the mobile phase and the column temperature was maintained at 40 °C. The injection volume of the samples was 10 μL. The H_2 concentration in the gas phase was analyzed using a gas chromatograph (HP 5890 Series II, Hewlett-Packard, USA) equipped with a thermal conductivity detector and a Carboxen-1000 column (Supelco, USA). Argon (Ar) was used as the carrier gas at 58 mL/min. The oven, injector and detector were maintained at 30 °C, 120 °C, and 120 °C, respectively. The production of hydrogen gas was calculated from the hydrogen concentration in the outlet gas and the total volume of outlet gas produced, at each time interval. The hydrogen production rates were calculated by the amount of hydrogen produced as a function of time.

All the fuel cell experiments were carried out in the BrFAFC, which consists of bio-reformer and MEA. The bio-reformer was made of 1 L glass bottle. The CO_2 removal part containing 1 M NaOH solution was made in a glass bottle with 30 mL. The initial formate solution (0.35 M, 700 mL, pH 6) was used after neutralization with 10 M KOH. The pH-stat feeding strategy was used for continuous formate supply of 2 M formic acid stock solution (400 mL, pH 2) with pump and pH probe. A magnetic bar and portable stirrer were used for mixing. The biocatalyst was obtained as previously described. A membrane electrode assembly (MEA) was fabricated via catalyst-coated membrane (CCM) by spraying the platinum catalyst on both sides of the polymer membrane, Nafion 212 (Dupont). The amount of Pt catalyst used in the MEA was 0.2 mg cm^{-2} at the anode and cathode, respectively. The fabricated CCM with the active area of 5 cm^2 was then dried for approximately 1 hr. The CCM was placed with two gas diffusion layers (GDLs) between the graphite plates with a serpentine flow-field, and the whole unit was tightened between the metal plates with the proper pressure. The single cell performance tests were conducted to compare pure H_2 with

the bio-reformed H_2 produced from 0.35 M formate for the anode fuel. The humidified air was equally supplied to the cathode in all the fuel cell tests. The BrFAFC was maintained at 40 °C and ambient pressure during the single cell test. The polarization and power density curves were measured using an electronic loader (WFCTS, WonATech, Korea). The constant current experiment was conducted at 0.5 A and 40 °C.

2. Formate decomposition to hydrogen by the biocatalyst

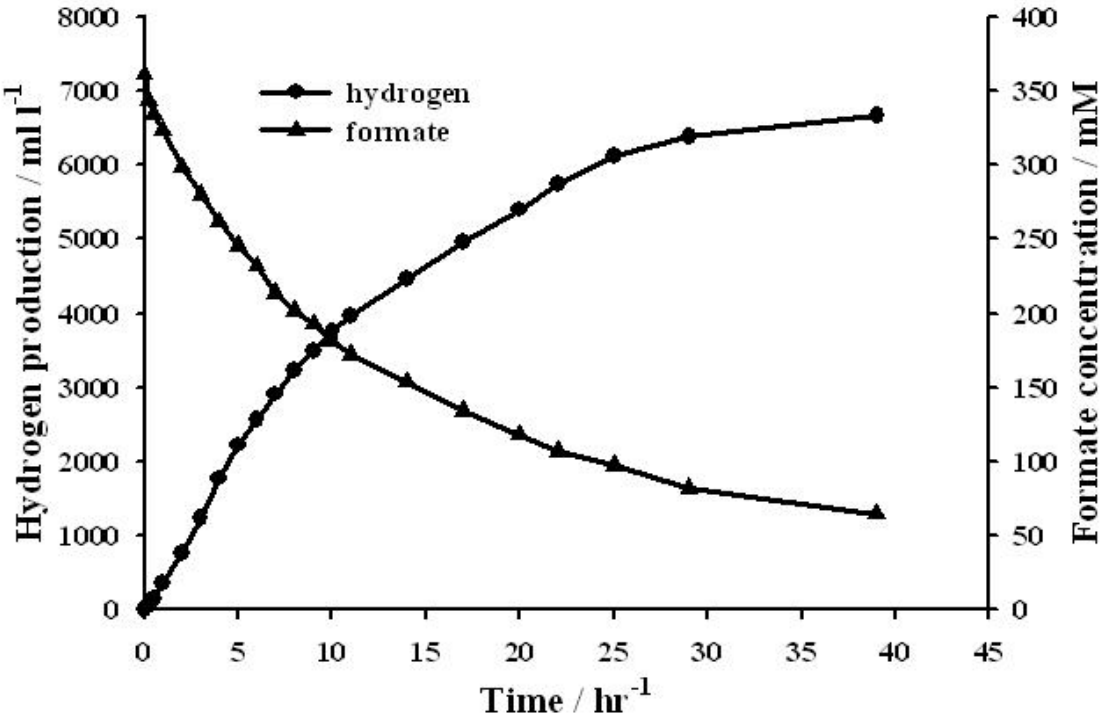

Fig. S1 Hydrogen production profile from formate under optimal conditions when *Enterobacter asburiae* SNU-1 was used as the biocatalyst.

Fig. S1 shows the profile of hydrogen production through the decomposition of formate using *Enterobacter asburiae* SNU-1 at 350 mM formate, 37 □, and pH 6. Under batch conditions, when the biocatalyst was added to the formate solution, hydrogen was immediately produced without a lag phase. Hydrogen production continued until 39 hr; however, the hydrogen production rate gradually decreased because of the decrease in the formate concentration and biocatalytic activity.

3. Bio-reformed hydrogen purity in BrFAFC

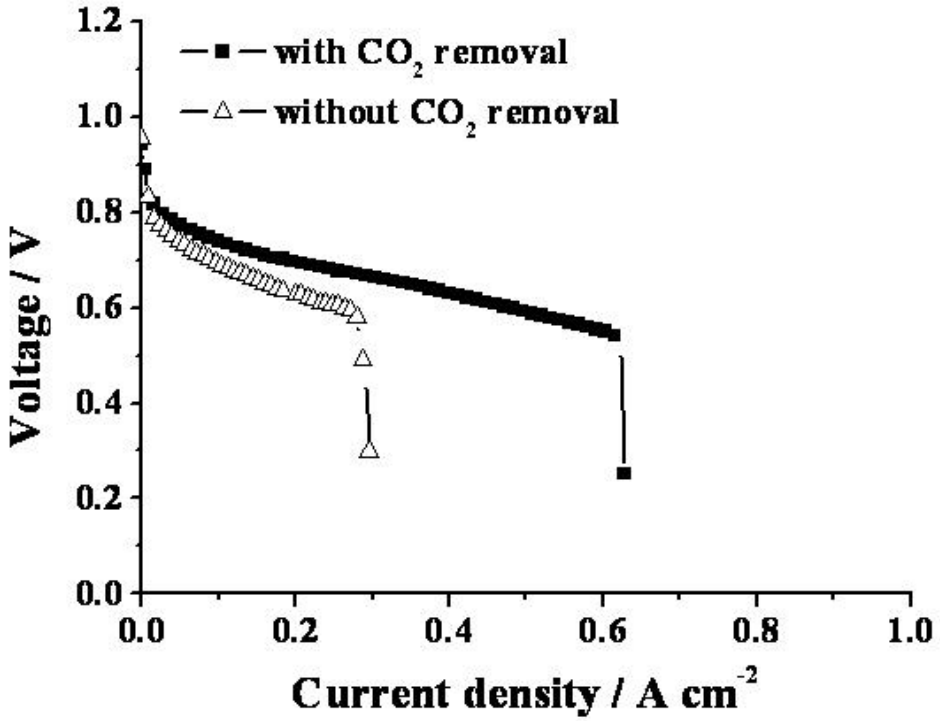

Fig. S2 Effect of carbon dioxide removal on the polarization curves. The bio-reformed H_2 with CO_2 removal or without CO_2 removal produced from 0.35 M formate was fed into the anode at 40 ℃. Humidified air was supplied to the cathode.

The hydrogen purity is crucial in a H_2/O_2 fuel cell system. Because the hydrogen content in the outlet gas from the bio-reformer was 50 %, we introduced a CO_2 removal step using the NaOH solution. The maximum power density of the BrFAFC using the bio-reformed H_2 with

CO_2 was 0.16 W cm^{-2}, which is much lower than that using bio-reformed H_2 gas without CO_2 (0.33 W cm^{-2}).

4. BrFAFC performance with various mass of biocatalyst

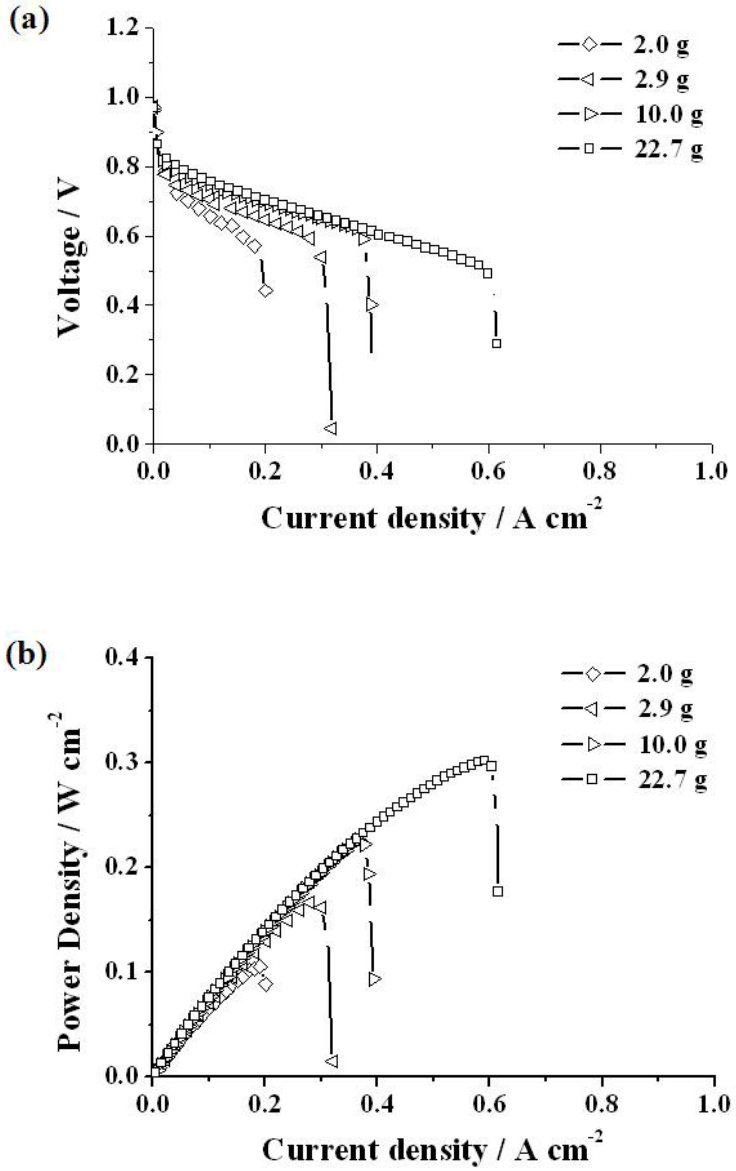

Fig. S3 Effect of the amount of biocatalyst on (a) polarization and (b) power density curves. The anode was fed with the bio-reformed H_2 produced from 0.35 M formate at 40 ℃ after the

removal of CO_2. Humidified air was supplied to the cathode.

5. Enhancement of hydrogen production rate by the addition of the biocatalyst

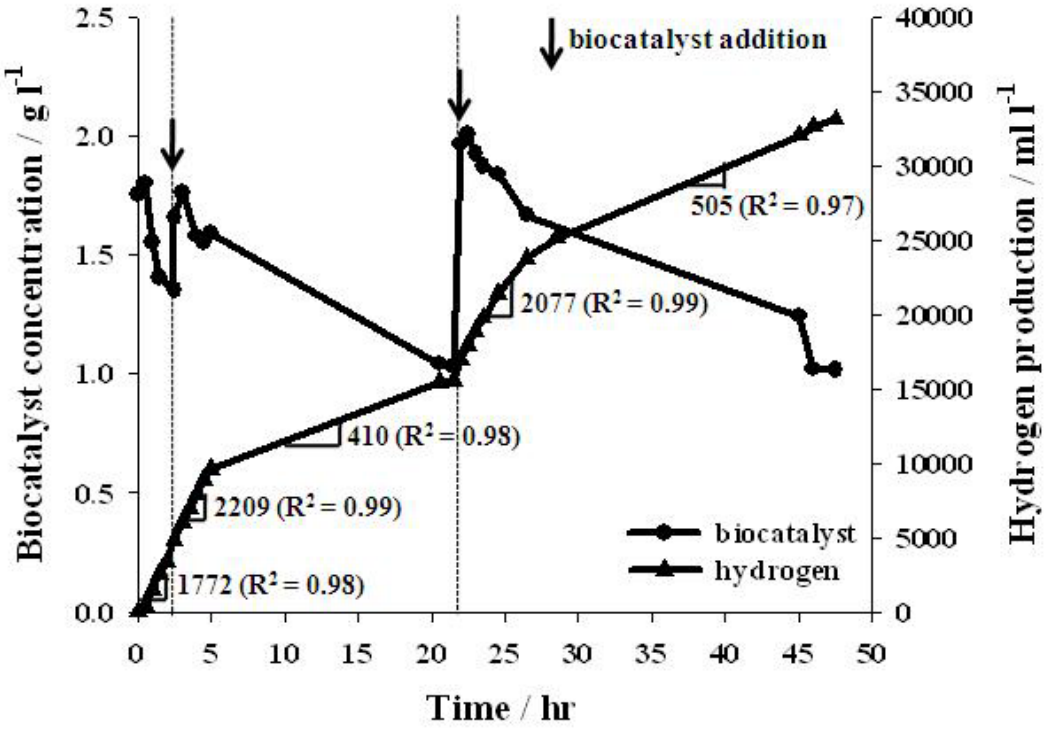

Fig. S4 Effect of the adding a new biocatalyst on the hydrogen production rate. The slope of hydrogen production represents the rate of hydrogen production.

Fig. S4 shows the enhancement of the hydrogen production rate when a new biocatalyst was added to the formate solution. The decrease in the hydrogen production rate was caused by the lysis of the biocatalyst and the addition of a new biocatalyst recovered the hydrogen production rate.

6. Re-activation of the biocatalyst by nutrient addition

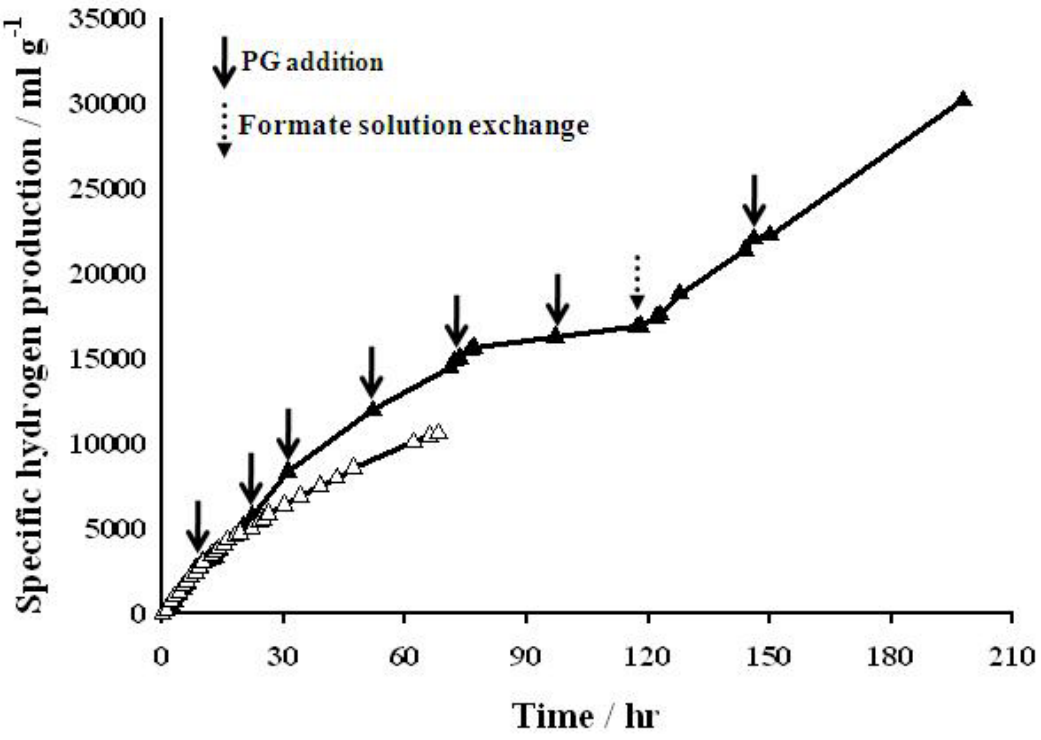

Fig. S5 Effect of nutrient addition on hydrogen production. 2 M formic acid solution was continuously supplied to the bio-reformer using the pH-stat feeding method. Mixture of peptone and glucose (PG) solutions were intermittently added to the bio-reformer (closed symbol) using a 25 g/L PG mixture solution so that the final concentration of each component was 2.5 g/L. After 118 hr in fed-batch system with formate solution exchange, 60 % of the solution in the bio-reformer was exchanged with a fresh formate solution

The hydrogen production rate gradually decreased because of the deactivation of the biocatalyst (open symbol). Therefore, re-activation of the biocatalyst was required to recycle

the biocatalyst. Various combinations of nutrients were tested to determine the optimal composition needed to recover the biocatalytic activity effectively, and a mixture of peptone and glucose was selected. Figure S5 shows the effect of adding this mixture on the recovery of hydrogen production. As shown in Figure S5, when the mixture of peptone and glucose solutions were intermittently added to the bio-reformer, the hydrogen production rate and stability were improved. When the solution in the bio-reformer was partially replaced with a fresh formate solution, the hydrogen production rate and stability were further improved, and the hydrogen production was maintained for more than 200 hr.