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Bacterial Assimilation of Carbon Dioxide by Electrochemical Bioreactor

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A two-compartmented electrochemical bioreactor was designed to induce regeneration of NADH and generation of hydrogen. Electric energy produced from solar cells charged to rechargeable battery. Anaerobic bacterial consortium was cultivated in cathode compartment to enrich bacterial cells capable of growing with carbon dioxide and hydrogen as a carbon and energy source. When bacterial cells were cultivated in the conventional bioreactor to which electricity was not charged, no bacterial growth was observed and no metabolite was produced. However, the bacterial cells cultivated in the electrochemical bioreactor produced acetate as a metabolite and accumulated PHB(poly-β-hydroxy butyrate) in cytoplasm. When glucose was added to the electrochemical bioreactor, butyrate concentration was greatly increased but PHB was not produced. In this study, we found that carbon dioxide may be assimilated into acetate and PHB, which is a new technology for biological fixation of carbon dioxide with natural energy.

Key words: Electrochemical bioreactor, NADH regeneration, solar energy, carbon dioxide fixation, anaerobic bacterial consortium

1. Introduction

Carbon dioxide is assimilated by Calvin cycle, Reversed TCA cycle or Wood pathway during the autotrophic bacteria growth.[1-3] In natural ecosystem, biological fixations of carbon dioxide are mainly performed by algae and plants but are partially by phototrophic and chemoautotrophic bacteria.[4-6] Theoretically, the carbon dioxide assimilated by the autotrophic organisms may be balanced to that generated by the heterotrophic organisms; however carbon dioxide is getting increased in global ecosystem by petroleum combustion that is getting increased too. Substantially, the carbon dioxide generated by petroleum combustion is difficult to be reduced but can be removed from atmosphere by chemical separation which has been utilized as industrial uses such as liquefied carbon dioxide and dry ice. To artificially assimilate carbon dioxide by using chemolithotrophic bacteria, the carbon dioxide has to be pure because the most chemolithotrophic bacteria are strict anaerobes, which can not grow in the condition contaminated with oxygen.[7-9] The photosynthetic bacteria are useful to assimilate carbon dioxide and may be economically profitable because of solar energy-dependent. However, the light energy is difficult to be equally reached to all photosynthetic bacterial cells growing in bioreactor because the light has to be dispersed by refraction, reflection and diffraction during transmitted through bacterial culture. The phototrophic bacteria do not produce specific metabolites but accumulate organic compounds as biomass however the chemolithotrophic bacteria produce specific metabolites such as acetic acid and butyric acid in addition to being biomass itself.[10-13] The chemolithotrophic bacteria can be easily isolated from anaerobic sediments of lakes or seashores, and anaerobic digestive reactor of wastewater treatment plants.[14-15)]

Anaerobic bacterial consortium is composed of sufidogens, methanogens, acetogens and anaerobic fermentation bacteria. Methanogens and acetogens can

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utilize hydrogen and carbon dioxide but sufidogens and fermentation bacteria require sugars or organic acids as energy and carbon source.[16-18] Methane is gaseous carbon compounds produced by only methanogens and the stronger agent for green house effect than carbon dioxide. Accordingly, acetogens are thought to be the most proper catalyst for biological carbon dioxide fixation because they produce organic compounds such as acetic acid and butyric acid from hydrogen and carbon dioxide.

In this study, we cultivated anaerobic bacterial consortium contained in anaerobic digestive sludge obtained from wastewater treatment plant by using an electrochemical bioreactor without selection of specific bacterial species. During cultivation of the anaerobic bacterial consortium in the electrochemical bioreactor for 3 months, specific bacterial cells capable of utilizing hydrogen, carbon dioxide or reducing power electrochemically generated were enriched. We expect that some bacterial cells may produce organic metabolites by assimilation of hydrogen and carbon dioxide, and others may accumulate some polymers from the organic metabolites in the enriched bacterial culture. The anaerobic bacterial consortium requires strict anaerobic condition at lower potential than -300 mV (vs. NHE), which is difficult to induce in the conventional bioreactor however may be easily induced in the electrochemical bioreactor because hydrogen and reducing power may effectively scavenge the oxidation potential or oxidation factors.

2. Materials and Methods

2.1. Organisms and Cultivation

Anaerobic bacterial consortium was isolated from anaerobic digestive reactor in Jungrang wastewater plant and cultivated in electrochemical bioreactor to enrich some bacterial cell capable of assimilating carbon dioxide and hydrogen for 3 months. The ingredients of making medium were 20 g $NaHCO_3$/L, 1 g yeast extract/L, 1 g K_2HPO_4/L, 1 g NH_4Cl/L and 2 ml trace mineral stock solution/L. The trace mineral stock solution contains 0.01 g/L $MnSO_4$, 0.01 g/L $MgSO_4$, 0.01 g/L $CaCl_2$, 0.002 g/L $NiCl_2$, 0.002 g/L $CoCl_2$, 0.002 g/L $SeSO_4$, 0.002 g/L WSO_4, 0.002 g/L $ZnSO_4$, 0.002 g/L $Al_2(SO_4)_3$, 0.0001 g/L $TiCl_3$, 0.002 g/L $MoSO_4$, and 10 mM EDTA. The glucose of 100 mM instead of $NaHCO_3$ was added to the medium to test glucose effect on bacterial growth and metabolites production.

2.2. Bioreactor

An electrochemical bioreactor was designed to induce hydrogen generation and maintain reduction potential as shown in Fig. 1. DC electricity was supplied to the bioreactor by rechargeable battery which was recharged by solar cells. Electric voltage and current were controlled by A/V (ampere/voltage) controller (DC Power Supply, DRP-303S, Digital Co.). Two electrodes were equipped in the cathode compartment. A graphite plate (100×200×10 mm) modified with neutral red as a catalyst for NAD^+ reduction to NADH in the cytoplasm of bacterial cells and a platinum wire (200×0.5 mm) as an agent for hydrogen generation by electrolysis of water.[19] DC electricity of 2 volts was charged to the graphite plate to induce NADH regeneration and current of 7.3 mA was charged to the platinum wire to induce hydrogen generation by which 1 ml hydrogen can be generated for 1 min. The same electrochemical bioreactor to which electricity was not charged was used as conventional bioreactor.

2.3. Membrane

A membrane was made from the mixture of 100% (w/w) white clay powder mainly composed of Kaolin whose mean particle size was 1-2 mm. Appropriate amount of distilled water was added to the white clay powder to make a clay paste, and the paste was configured to square-shaped plate (20 cm × 20 cm × 5 mm thickness) by pressing at 44 kg/cm^2, drying on air for two weeks at room temperature, and solidifying by baking at 1200°C for 8 hr in an electric Kiln (Red Corona Model 50 L, USA). After baking, the porcelain membrane was cut to adjust to bioreactor and modified with cellulose acetate to protect water transfer through the micro-pores. The porcelain membranes were immersed in 2 volumes of 3% cellulose acetate solution, which was dissolved in

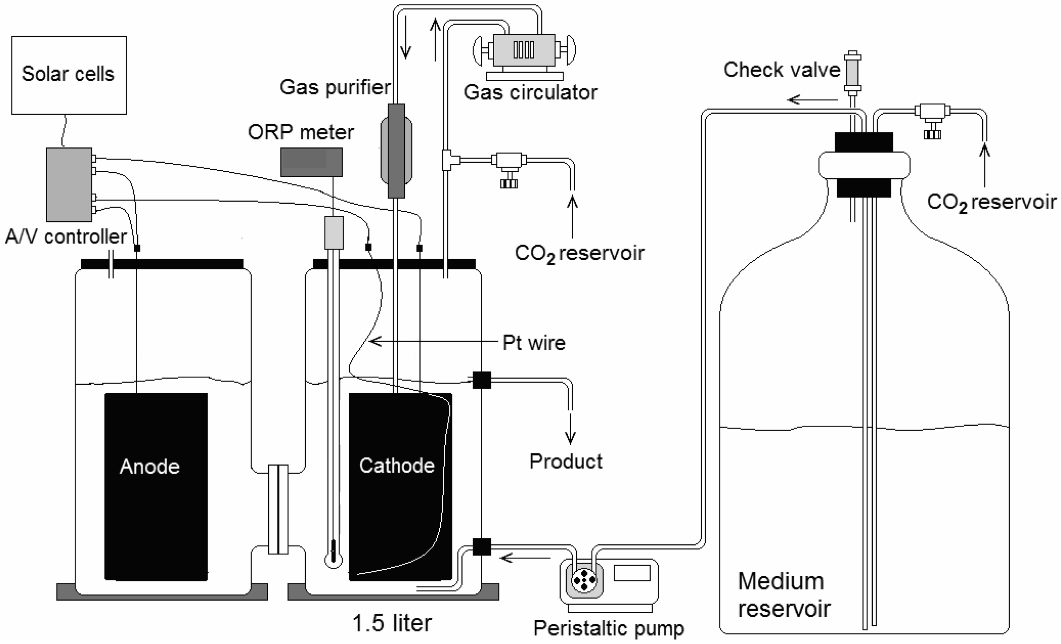

Fig. 1. Schematic structure of electrochemical bioreactor composed of anode compartment, cathode compartment and medium reservoir. Pure carbon dioxide was continuously recycled from medium to medium using gas circulator to saturate medium with carbon dioxide. The carbon dioxide consumed by bacterial cells was automatically refilled from carbon dioxide reservoir. Oxygen-free fresh medium sparged with carbon dioxide was continuously supplied from reservoir to reactor at the rates of 150 ml day^{-1} to supply organic N source and inorganic compounds. The medium in reservoir was sparged with carbon dioxide to remove dissolved oxygen and saturate with carbon dioxide.

100% acetone, and dried on air. Finally, the thickness and diameter of porcelain membrane used in the bioreactor was adjusted to 3 mm and 100 mm, respectively.

2.4. Analysis

Acetate and butyrate were analyzed with HPLC equipped with HPX-87H ion exchange column (BioRad, USA) whose temperature was adjusted to 35°C. H_2SO_4 of 0.08N was used as a mobile phase of which flow rate was adjusted to 0.6 ml/min. PHB was measured by Law and Slepeckys method.[20] The bacterial cells were centrifuged in polypropylene tubes, which had been previously washed thoroughly with ethanol and hot chloroform to remove plasticizers. The cell paste was resuspended in a volume of commercial sodium hypochlorite solution (Clorox) equal to the original volume of medium. After lipid granules were centrifuged for 1 hr at 37°C, it was washed with water, and then rewashed with acetone and alcohol. Finally, the polymer was dissolved by extraction with three small portions of boiling chloroform, the chloroform solution was filtered, and the filtrate was used for PHB assay. Chloroform was evaporated and 10 ml of concentrated H_2SO_4 are added, the tube is capped with a glass marble and heated for 10 min at 100°C in water bath. As shown in Fig. 2, PHB was hydrolyzed into crotonic acid. The crotonic acid was analyzed by same method with acetate and butyrate.

3. Results and Discussion

PHB (poly-β-hydroxy butyrate) is an inclusion body accumulated in bacterial cytoplasm, which is a storage nutrient like starch and glycogen.[21] Most of bacterial species have been known to accumulate PHB during growth in phosphorus-limiting condition. And, the PHB is secreted into medium as a metabolite in the condition with sufficient nutrient. Some chemolithotrophic bacteria assimilate carbon dioxide into acetate or methane

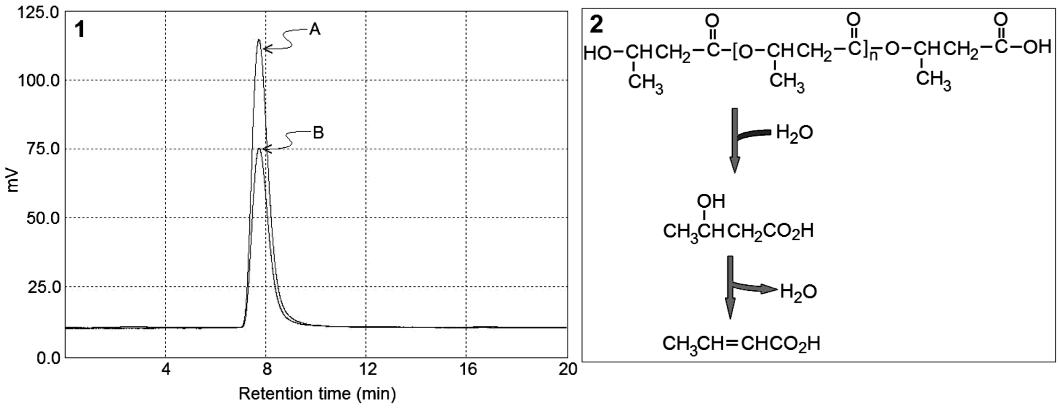

Fig. 2. Chromatograms of organic compound obtained by sulfuric hydrolysis of bacterial product (1-A) and standard PHB (1-B). Crotonic acid may be produced from PHB by hydrolysis of sulfuric acid. Concentration of standard PHB was adjusted to 10 mg ml^{-1} (2).

coupled to oxidation of hydrogen as a reducing power.[3,6) In the reduction condition or the circumstance with high $NADH/NAD^+$ balance, acetate can be reduced to butyrate coupled to oxidation of NADH and other reducing power as shown in Fig. 3. Accordingly, reducing power and hydrogen are absolutely required to assimilate carbon dioxide. Both reducing power and hydrogen can be electrochemically generated on the graphite electrode and platinum wire.[22,23) In the condition without hydrogen, yeast extract is a unique organic nutrient for bacterial cells growing in anaerobic condition. The bacterial cells are difficult to grow with yeast extract in the anaerobic condition because amino acids can not use as an electron donor or acceptor. As shown in Fig. 4, bacterial growth was not observed in the conventional bioreactor to which electricity did not charged. Meanwhile, bacterial growth, acetate, butyrate and PHB production were observed in the electrochemical bioreactor as shown in Fig. 5. ORP was stable from -0.42 to -0.47 (vs. NHE) which was electrochemically induced by the reducing power generated from cathodes. Acetate, butyrate and PHB production was proportional to the bacterial growth but butyrate

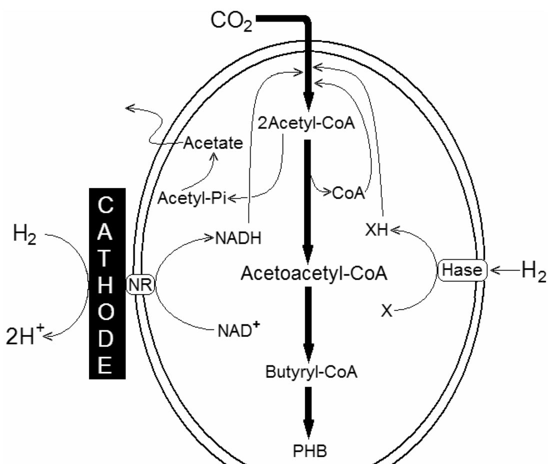

Fig. 3. Schematic structure for metabolic pathway from carbon dioxide to PHB, which was designed based on the chemoautotrophic bacterium such as homoacetogen and methanogen capable of growing with hydrogen and carbon dioxide as an energy and carbon source.

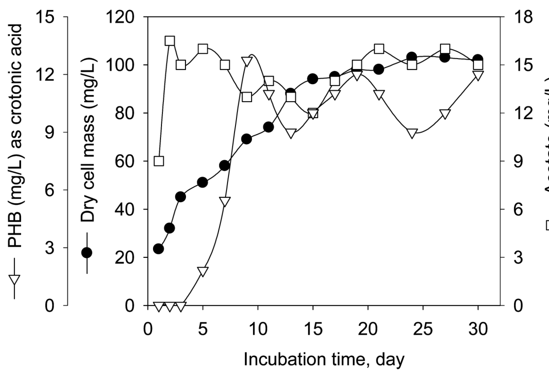

Fig. 4. Acetate and PHB produced from yeast extract by an anaerobic bacterial consortium grown in a conventional bioreactor, in which no hydrogen was generated.

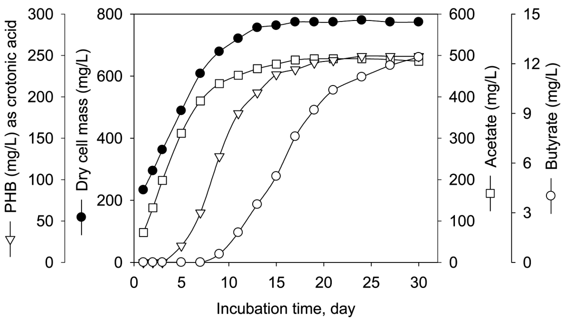

Fig. 5. Acetate and PHB produced from carbon dioxide and hydrogen by an anaerobic bacterial consortium grown in the cathode compartment of electrochemical bioreactor. PHB was determined as crotonic acid which was obtained from PHB by hydrolysis with sulfuric acid.

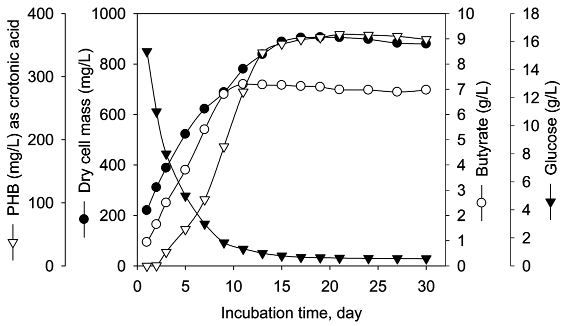

Fig. 6. Acetate and PHB produced from glucose and hydrogen by an anaerobic bacterial consortium grown in the cathode compartment of electrochemical bioreactor. PHB was determined as crotonic acid which was obtained from PHB by hydrolysis with sulfuric acid.

production was relatively lower than acetate. It shows that biochemical reduction reaction of acetate to butyrate is coupled to the oxidation of reducing powers and ATP consumption. When the bacterial cells were grown with hydrogen and carbon dioxide, one ATP is produced in the pathway from acetyl-phosphate to acetate. However four ATPs can be produced in the pathway from glyceraldehyde to pyruvate and two ATPs can be produced in the pathway from succinyl-CoA to succinate of TCA cycle when grown on glucose. In the condition with high ATP/ADP balance and sufficient nutrient, bacterial cells may produce relatively higher butyrate coupled to consumption of ATP and reducing power. As shown in Fig. 6, acetate was not produced but butyrate concentration showed about 600 times higher on glucose than on hydrogen and carbon dioxide. However, PHB production was not very higher in the growth condition with glucose than hydrogen and carbon dioxide. Bacterial cells can make easily inclusion body using glucose because it is an organic compound which can be converted into biochemical compounds by biosynthesis. But, hydrogen and carbon dioxide may be suppressed to accumulate organic compounds in the bacterial cells because those are inorganic compounds which are difficult to be converted into biochemical compounds.

Bacterial fixation of carbon dioxide by using electrochemical reaction and solar cells may be best choice to remove carbon dioxide from atmosphere. The catalytic electrode modified with neutral red can catalyze NAD^+ reduction to NADH without enzyme catalysis and platinum electrode can catalyze hydrogen generation from water by electrolysis. NADH and other reducing powers are absolutely required to reduce carbon dioxide to acetate and reduce acetate to butyrate. Lower voltage than 5.0 volt is enough to induce the electrochemical reaction coupled to biochemical redox reaction and can be generated from solar cells. This system can establish the carbon dioxide assimilation into PHB or butyrate without petroleum combustion, and PHB and butyrate is more stable than carbon dioxide for long-term storage and do not have green house effect.

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