Construction of Microbial Fuel Cells Using Thermophilic Microorganisms, *Bacillus licheniformis* and *Bacillus thermoglucosidasius*

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A systematic study of microbial fuel cells comprised of thermophilic *Bacillus licheniformis* and *Bacillus thermoglucosidasius* has been carried out under various operating conditions. Substantial amount of electricity was generated when a redox mediator was used. Being affected by operation temperature, the maximum efficiency was obtained at 50 °C with an open circuit voltage of *ca*. 0.7 V. While a small change around the optimum temperature did not make much effect on the cell performance, the rapid decrease in performance was observed above 70 °C. It was noticeable that fuel cell efficiency and discharge pattern strongly depended on the kind of carbon sources used in the initial culture medium. In the case of *B. thermoglucosidasius*, glucose alone was utilized constitutively as a substrate in the microbial fuel cell irrespective of used carbons sources. When *B. licheniformis* was cultivated with lactose as a carbon source, best charging characteristics were recorded. Trehalose, in particular, showed 41.2% coulombic efficiency when *B. thermoglucosidasius* was cultured in a starch-containing medium. Relatively good repetitive operation was possible with *B. thermoglucosidasius* cells up to 12 cycles using glucose as a carbon source, when they were cultured with lactose as an initial carbon source. This study demonstrates that highly efficient thermophilic microbial fuel cells can be constructed by a pertinent modulation of the operating conditions and by carefully selecting carbon sources used in the initial culture medium.

Key Words : Coulombic efficiency, Microbial fuel cell, Thermophilic microorganism

Introduction

As a clean energy source and an alternative to the conventional energy production device, a microbial fuel cell has drawn much attention, in which intact microorganisms are utilized as catalysts for converting of chemical energy into electrical energy.[1-3] Electrons are initially trapped as a form of reduced intermediates following the degradation of substrate and transferred to the anode.[4-6] Microorganisms as well as redox mediators constitute an essential part of the microbial fuel cell, in that a large improvement in a fuel cell operation could be achieved by selecting suitable catalytic bacteria.[7-9] It is notable in the use of *Anabaena variabilis*[10] or *Synechococcus* sp.[11] in which even light energy can be utilized instead of carbon sources and *Shewanella putrefaciens*[12] where microbial fuel cells are constructed without electron transfer mediators. Among many kinds of microorganisms, *Proteus vulgaris* is one of the well known and intensively studied microbial catalysts.[13] Reduction rates of various organic molecules by *P. vulgaris* have been measured.[14]

This paper describes the preliminary study of the microbial fuel cells using thermophilic bacteria. Microorganisms capable of growing optimally at temperatures between 50 °C and 60 °C are designated as moderate thermophiles.[15] Most of these microorganisms belong to many different taxonomic groups of eukaryotic and prokaryotic microorganisms. While most studies have concentrated on room temperature conditions, operations under thermophilic condition may be advantageous over those under the "normal" temperature in view of anodic reaction kinetics, resulting in better fuel cell operation. Generally, thermophilic microorganisms are widely distributed around the high temperature ecosystem in nature so that they can develop their own physiology and characteristic membrane structures. For this reason, thermophiles have been applied to various industrial areas such as food and paper industry, detergents, drugs, toxic wastes removal and starch industry.[16] In this work, thermophilic bacteria have been used for construction of the effective microbial fuel cell. Various operative conditions related with redox mediators, temperature, pH, and carbon sources in the microbial fuel cell system were investigated with the thermophilic microorganisms, *B. licheniformis* and *B. thermoglucosidasius*.

Experimental Section

Preparation of microorganisms. *Bacillus licheniformis* (ATCC 27811) and *Bacillus thermoglucosidasius* (ATCC 43742) were obtained from the culture collection of KCTC (Korean Collection for Type Cultures) and grown aerobically at 50 °C in a nutrient broth medium (pH 7.0). To test initial carbon source effects on the fuel cell efficiency, the

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microorganism was cultured in a medium that contained 10 g of trypton, and 5 g of a carbon source per liter. Fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose, and trehalose were used as an initial carbon source and each was added to the medium after sterilization. Each culture containing a carbon source was daily sub-cultured with 5% inoculums for three days. Cells were harvested by centrifugation at 3,000 × g for 10 min at their early stationary phase and washed three times with 0.05 M of phosphate buffer of pH 7.0 at 4 °C. The washed microorganisms were resuspended in the buffer to give 20 mg (dry wt) per ml for the experiments.

Fuel cell assembly. Each cell unit is composed of anode and cathode compartments (internal dimensions 45 × 45 × 15 mm) and separated by a cation exchange membrane (Nafion, Aldrich, USA).[17] A reticulated vitreous carbon (RVC, 30 × 30 × 12 mm) plate was used as an anode. RVC has a physical structure that can allow easy access of organisms and mediators to the electrode surface through the open network and provide a high surface area for the reaction. Anolyte and catholyte were composed of 0.05 M phosphate buffer and 0.1 M ferricyanide solutions, respectively. Microorganism and mediators were added to the anodic compartment. A platinum plate (30 × 30 × 0.2 mm) was used as a cathode. Each compartment was sealed by 1.5 mm-thick silicon rubber gaskets, and held in a frame that was tightly bolted together. During the experiments, nitrogen flowed through the cell compartments to keep oxygen from entering the cell and for effective mixing of the solution. Operation temperature was maintained constant in a water bath. A principal feature of the microbial fuel cell is summarized in Figure 1.

Electrical measurements. The discharge curve was recorded only after the open circuit voltage was stabilized with nitrogen gas flowing through the cell. Discharging was done by connecting an external resistor of various values between the anode and the cathode to obtain a polarization curve. The cell voltage with time was then recorded with a personal computer equipped with an analog-to-digital board (Computer Boards, Mansfield, MA, USA). Current was calculated by using the Ohm's law, $I = V_{cell}/R_{load}$. When the cell voltage dropped to the background level, the cell was charged with a carbon source for another measurement. Generally, the cell voltage increases rapidly upon injection of carbohydrates and reaches a plateau level as long as there are enough carbohydrates to be consumed by microorganisms, and then the cell voltage begins to gradually decrease. Actually produced electricity can be calculated by integrating the discharge curve with time, $Q = \int Idt$. For the complete oxidation of glucose, the anodic reaction is given by

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

24 electrons should be generated from one molecule of glucose in an ideal condition. The coulombic efficiency was then calculated as the ratio of the output charge obtained from the fuel cell to the theoretical maximum coulombic equivalent of 24 × 96,485 C/mol of added monosaccharide.

Results and Discussion

The effects of operation temperature and buffer pH have been investigated for the optimal condition of the fuel cells. While the cell voltage from *B. licheniformis* decreased at 30 °C, *B. thermoglucosidasius* showed a better performance in the range of 30 to 60 °C. In view of operative temperature, the best efficiency was observed at 50-60 °C in both bacteria (Figure 2). However, above 70 °C, a rapid deterioration of

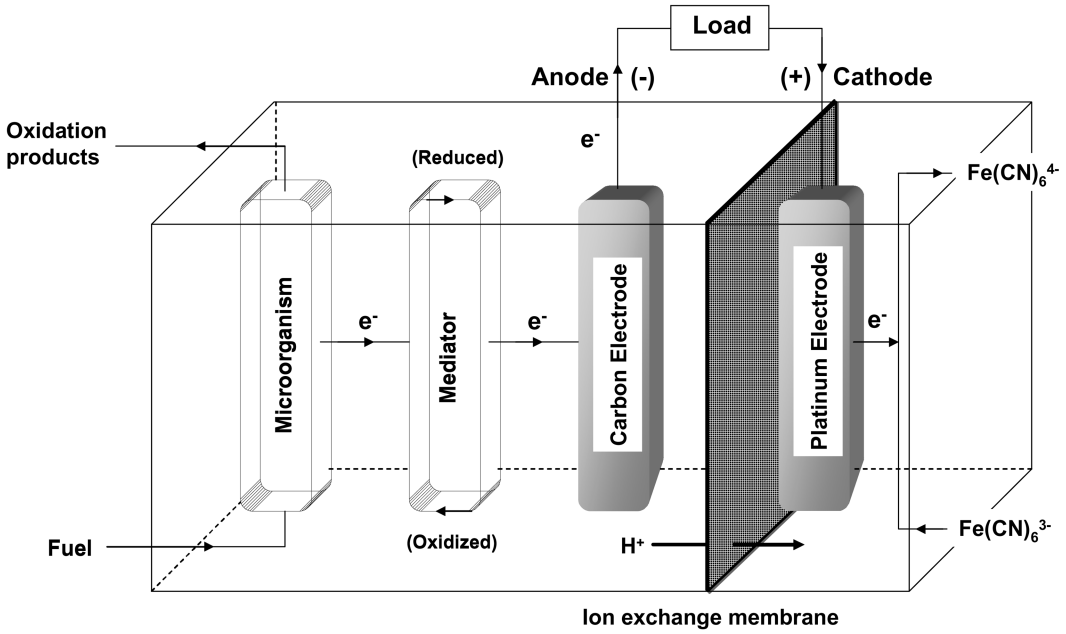

Figure 1. Schematic diagram showing the principal features of a microbial fuel cell.

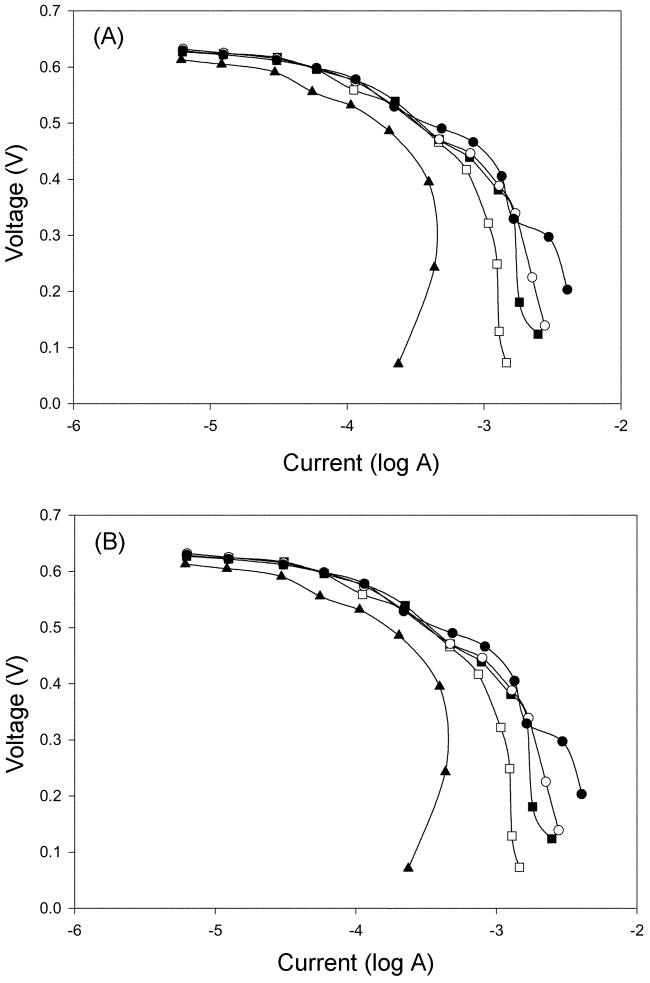

Figure 2. Current-voltage curves for fuel cells containing (A) *B. licheniformis* (B) *B. thermoglucosidasius* obtained at 30 °C (□), 40 °C (■), 50 °C (○), 60 °C (●), and 70 °C (▲) with azure A as a mediator. Organism concentration: 2 mg (dry wt) mL^{-1}; 4 μmol of mediator and 100 μmol of glucose added initially.

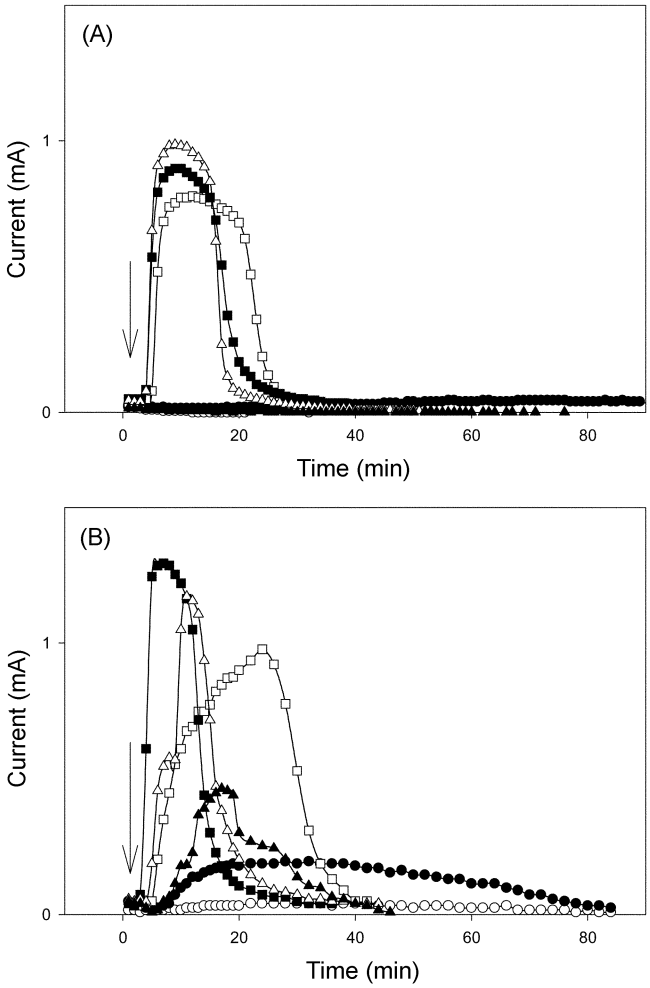

Figure 3. Variation of current output with time through the 300-Ω external load for fuel cells containing (A) *B. licheniformis* (B) *B. thermoglucosidasius* obtained at different buffer pH 5 (○), 6 (●), 7 (□), 8 (■), 9 (△), and 10 (▲) on the addition 1.0 μmol of glucose. Organism concentration: 1 mg (dry wt) mL^{-1}; azure A: 4 μmol.

the fuel cell performance was resulted. These results indicated that the rate of electron transfer of a mediator increased with temperature until the microorganisms ceased their metabolic activity. Figure 3 shows the dependence of a discharging pattern on pH. The maximum fuel cell efficiency was found at pH 7.0. As pH rises, the rapid current rising pattern was observed for both bacteria. These observed operative conditions were similar to those of typical bacterial cell culture. These results surely indicate that the fuel cell efficiency depends on the metabolic status within microorganisms.

Figure 4 shows current output characteristics with time through the external load (300-Ω) for the cells containing 4 μmol of azure A and 1 μmol of carbon sources when both *B. licheniformis* (Panel A) and *B. thermoglucosidasius* (Panel B) were initially grown with yeast extract. Only glucose, trehalose or maltose were utilized for *B. licheniformis* and glucose, trehalose or mannose were utilized for *B. thermoglucosidasius*, among tested 11 carbon sources (fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose, and trehalose). The characteristic slow and steady current output patterns from maltose or mannose indicated that both microorganisms were not metabolically pre-adapted to the maltose or mannose. These carbon sources might be utilized by the microorganisms after primary carbon sources such as glucose were consumed. Other initial carbon sources than yeast extract include fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose, and trehalose. The total 264 (11 × 12 × 2) different bacterial fuel cell systems, therefore, have been investigated for two thermophilic microorganisms. Tables 1 and 2 show the complete list of coulombic efficiencies for these systems. Throughout this investigation, we optimized the operative performance of these bacterial fuel cell systems by the regulation of metabolic adaptation of the bacteria. The coulombic efficiencies and charging characteristics were significantly altered according both to the initial carbon sources used for culture conditions and to the utilized carbon sources for the

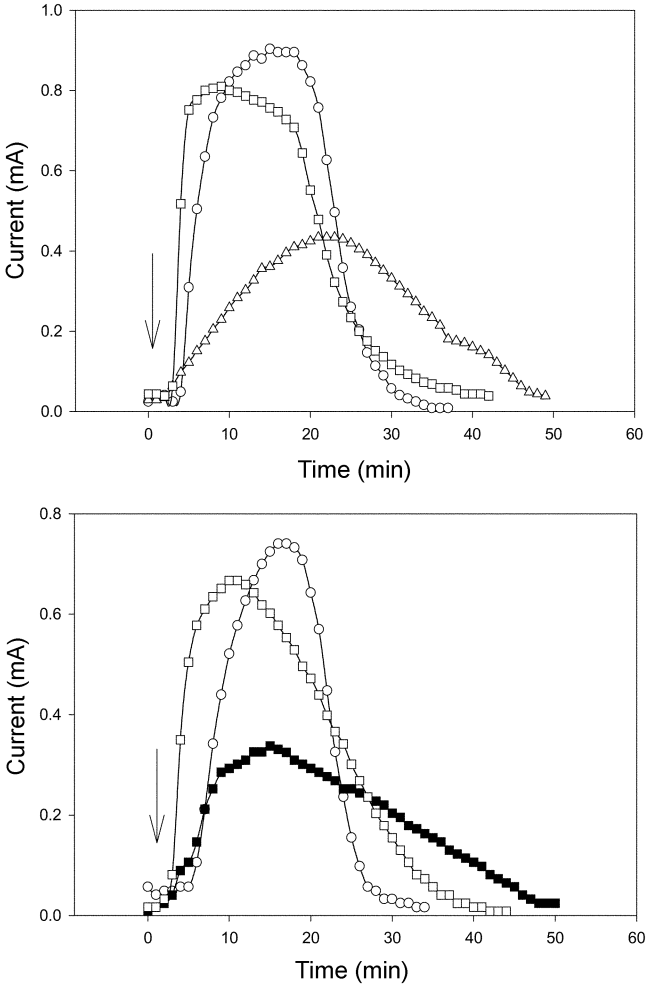

Figure 4. Variation of current outputs with time for bacterial fuel cells containing (A) *B. licheniformis* and (B) *B. thermoglucosidasius* (organism concentration: 1 mg (dry wt) mL^{-1}; 4.0 μmol of azure A and 1.0 μmol of 11 different utilized carbon sources such as fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose and trehalose; external resistive load: 300-Ω). Measurable current outputs were detected only four utilized carbon sources, glucose (○), maltose (△), mannose (■), and trehalose (□). Thermophilic microorganisms were initially grown in yeast extract, a complex medium before the fuel cell operation.

fuel cell operation. Coulombic efficiency was estimated to 100% when the theoretical value of 2.32 C was obtained from 1.0 μmol of monosaccharides or from 0.5 μmol of disaccharides with 24 or 48 electrons generated, respectively by the complete oxidation of saccharides. In this manner, the coulombic output directly reflects the coulombic efficiency. Current output did not drop to zero even after a long-time discharge, but rather reached a constant value of *ca*. 0.03 mA in all cases. Coulombic efficiency was calculated by measuring charge above this constant level, which was regarded as a background. *B. licheniformis* and *B. thermoglucosidasius* were grown at different initial culture conditions. Fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose, and trehalose were tested as an initial culture carbon source. We denoted Glu-ini, for example, to describe the condition where glucose was added initially at culture as a carbon source. Likewise, Fru-ini, Gal-ini, Lac-ini, Mal-ini, Mai-ini, Man-ini, Sor-ini, Sta-ini, Suc-ini and Tre-ini mean that fructose, galactose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose and trehalose were initially used as a carbon source, respectively.

The cell was fully discharged before each carbon source was added. It is noteworthy that glucose alone was significantly utilized regardless of initial culture conditions among carbon sources tested. In case of the *B. licheniformis*, current output was hardly observed when fructose, galactose, lactose, sorbitol, or starch was used as a substrate for fuel cell operation. Utilization of other carbon sources strongly

Table 1. Coulombic Efficiency (%) for Various Carbon Source Conditions in the Microbial Fuel Cell Containing *Bacillus licheniformis*

Initial Carbon Source Condition	Utilized Carbon Sources					
	glucose	maltose	mannitol	mannose	sucrose	trehalose
yeast extract	38.9	27.6	–	–	–	37.0
fructose	–	–	–	–	–	–
galactose	30.4	–	–	–	–	5.7
glucose	17.9	–	–	–	–	0.7
lactose	40.4	–	–	–	–	–
maltose	18.1	–	–	–	15.4	25.0
mannitol	–	–	2.0	–	–	–
mannose	6.8	–	–	4.2	–	–
sorbitol	–	–	–	–	–	–
starch	–	–	–	–	–	–
sucrose	–	–	–	–	–	–
trehalose	23.7	–	–	–	-	37.0

Current output was not observed when the bacterial fuel cells were operated with fructose, galactose, lactose, sorbitol or starch as utilized carbon sources

Table 2. Coulombic Efficiency (%) for Various Carbon Source Conditions in the Microbial Fuel Cell Containing *Bacillus thermoglucosidasius*

Initial Carbon Source Condition	Utilized Carbon Sources				
	glucose	maltose	mannitol	mannose	trehalose
yeast extract	22.9	–	–	22.5	33.0
fructose	4.0	–	–	–	–
galactose	38.3	–	21.2	8.2	–
glucose	12.6	1.2	-	-	6.5
lactose	30.0	–	–	–	–
maltose	19.5	18.8	–	–	–
mannitol	37.3	–	31.2	–	–
mannose	19.0	–	–	–	–
sorbitol	14.1	–	–	–	–
starch	35.7	–	–	21.2	41.2
sucrose	16.9	–	–	–	12.9
trehalose	15.8	–	–	–	10.1

Current output was not observed when the bacterial fuel cells were operated with fructose, galactose, lactose, sorbitol, starch, or sucrose as utilized carbon sources

depended on the initial culture conditions. Sucrose, for example, did not show any measurable current output except for the Mal-ini condition, but even in this case, only 15.4% of coulombic efficiency was obtained. None of the carbon sources were utilized under the Lac-ini condition except the glucose which gave the highest coulombic yield of 40.4%. Lac-ini cells may be metabolically adapted for the glucose utilization such as induction of more general carbohydrate transporters[18,19] in the cellular membrane of *B. licheniformis*. Although the glucose showed the best coulombic yield with the Lac-ini condition, it was hardly utilized under other initial conditions such as Fru-ini, Sor-ini, Sta-ini and Suc-ini. Trehalose as a substrate for fuel cell was the second candidate for the maximizing current output of the *B. licheniformis* fuel cell. It gave a current output of 0.86 C (37.0%) when the *B. licheniformis* was cultured under the Tre-ini condition. The best efficiency of 40.4% was obtained when glucose was used for the lactose-adapted cells of *B. licheniformis* (Table 1). These tendencies were almost similarly reflected in the features of a fuel cell containing *B. thermoglucosidasius*. Current output was hardly observed when fructose, galactose, lactose, sorbtiol, sucrose, or starch was used as a substrate for fuel cell operation. The best efficiency of 41.2% was obtained when trehalose was used for the starch-adapted cells of *B. thermoglucosidasius* (Table 2).

While trehalose was somewhat utilized regardless of the various culture conditions, maltose showed a negligible utilization except for the complex medium (yeast extract) condition. This strongly indicates that coulombic efficiency depends on the linkage pattern of two glucose units in disaccharides where trehalose is connected by an $\alpha 1 \rightarrow \alpha 1$ linkage and maltose by an $\alpha 1 \rightarrow \alpha 4$ linkage, suggesting that the linkage pattern is an important factor in a fuel cell operation. With the sucrose initial condition, sucrose itself showed non-current output in both bacterial fuel cells. Kim *et al.*[20] explained that these phenomena would be induced by different metabolic adaptation states resulting from different initial carbon sources used, which directly or indirectly, influences some of the redox reactions among the many metabolic reactions within the microorganism. Then, the induced redox reactions may change the coulombic efficiency via mediators, though its exact mechanism needs to be explored.

The fact that the current output pattern changed dramatically when their initial culture condition was altered by various carbon sources implies that the fuel cell performance could be easily manipulated by the simple change in culture conditions. We found that the maximum fuel cell efficiency was obtainable with trehalose as a substrate and starch as an initial carbon source with *B. thermoglucosidasius*. *B. thermoglucosidasius* showed also reasonable current outputs in the repetitive fuel cell operation only for two adaptive cell conditions, mannitol substrate at Mai-ini and glucose at Lac-ini (Figure 5). These results suggested that the coulombic efficiency in the *B. thermoglucosidasius* fuel cell could be enhanced or regulated by tuning the carbon source conditions (Figure 5). *B. thermoglucosidasius* cells were able to generate electricity repetitively up to 12 cycles using glucose as a carbon source (Figure 5A). This efficient repetitive operation would be a major advance in the field of microbial fuel cell containing unusual microorganisms.

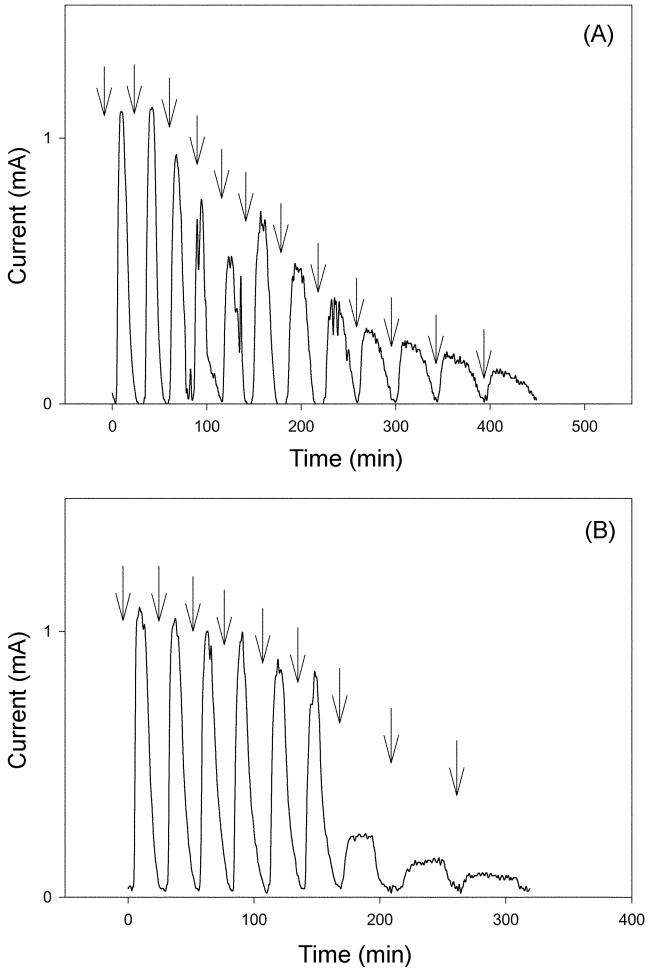

Figure 5. Variation of current output with time through the 300 Ω external load for bacterial fuel cells containing *B. thermoglucosidasius* (1 mg (dry wt) mL^{-1}) and the repetitive addition 1.0 μmol of carbon source in the presence of azure A (4 μmol); initial carbon source for bacterial culture: (A) lactose (B) mannitol, utilized carbon source for fuel cell operation: (A) glucose (B) mannitol. The constant amount of a carbon source was added after the current output reached a background level.

In summary, a fundamental study for the microbial fuel cells containing thermophilic bacteria was performed in order to achieve the optimal efficiency of the fuel cell operation. The current output was highly dependent on the operating conditions, especially initial culture medium. Coulombic efficiency and charging characteristics of the thermophilic bacterial fuel cells were enhanced or regulated by the modulation of carbon sources used for bacterial fuel cell system.

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