Conversion of Carbon Dioxide to Metabolites by
*Clostridium acetobutylicum* KCTC1037 Cultivated with
Electrochemical Reducing Power

Bo Young Jeon¹, Il Lae Jung², Doo Hyun Park¹*

¹Department of Biological Engineering, Seokyeong University, Seoul, South Korea
²Department of Radiation Biology, Environmental Radiation Research Group,
Korea Atomic Energy Research Institute, Daejeon, South Korea

Email:¹ baakdoo@skuniv.ac.kr

Received June 21, 2012; revised July 29, 2012; accepted August 6, 2012

ABSTRACT

In this research, metabolic fixation of CO₂ by growing cells of *C. acetobutylicum* cultivated with electrochemical reducing power was tested on the basis of the metabolites production and genes expression. In cyclic voltammetry, electrochemical oxidation and reduction reaction of neutral red (NR) immobilized in intact cells of *C. acetobutylicum* was stationarily repeated like the soluble one in the condition without CO₂ but the electrochemical reduction reaction was selectively increased by addition of CO₂. In electrochemical bioreactor, the modified graphite felt cathode with NR (NR-cathode) induced *C. acetobutylicum* to generate acetate, propionate, and butyrate from CO₂ in defined medium. When H₂ and CO₂ were used as an electron donor and an electron acceptor, respectively, *C. acetobutylicum* also produced the same metabolites in a defined medium. *C. acetobutylicum* was not grown in the defined medium without substituted electron donors (H₂ or electrochemical reducing power). *C. acetobutylicum* cultivated with electrochemical reducing power produced more butyrate than acetate in complex medium but produced more acetate than butyrate in defined medium. The genes of encoding the enzymes catalyzing acetyl-CoA in *C. acetobutylicum* electrochemically cultivated in defined medium than conventionally cultivated in complex medium. These results are a clue that *C. acetobutylicum* may metabolically convert CO₂ to metabolites and produce free energy from the electrochemical reducing power.

Keywords: *C. acetobutylicum*; CO₂-Assimilation; Electrochemical Reducing Power; Coupling Redox Reaction

1. Introduction

Chemoautotrophs that regenerate reducing power and produce free energy in coupling with oxidation of H₂ can more effectively fix CO₂ than ammonium, nitrite, and ferrous-oxidizing bacteria because redox potential of H₂/2H⁺ (−0.42 V vs. NHE) is lower than NAD⁺/NADH (−0.32 V vs. NHE) [1]. The reducing power generated in coupling with oxidation of ammonium, nitrite, and ferrous ion can’t induce regeneration of NAD(P)H without a reverse electron transport system coupled to consumption of external energy [2-5]. Experimentally measured redox potential of NR is −0.325 V (vs. NHE), which is theoretically enough to mediate generation of electron-driving force from electrode to NADH [6]. Practically, electrochemically reduced NR catalyzes NADH regeneration by non-enzymatic catalysis [7]. Theoretically, electrochemically reduced NR may be more effective in reducing power than H₂ on the basis of non-enzymatic catalysis of NADH regeneration.

*Corresponding author.*
been developed [13,14]. Electricity generated from the solar cell can be directly converted to biochemical reducing power in coupling with the redox reaction of NR immobilized in bacterial cell or graphite felt electrode [6]. Bacterial CO₂ fixation induced by biochemical reducing power electrochemically regenerated by the solar cell electricity may correspond to the photosynthesis because O₂ is generated from anode compartment and CO₂ is biochemically assimilated into biomass and converted to metabolites in cathode compartment [15]. Covalently immobilized NR in graphite felt electrode can function as a catalyst for NADH regeneration and a redox carrier for electron transfer from electrode to bacterial cell [16]. Redox potential of NR is –0.325 volt (vs. NHE), which is 0.05 volt lower than NAD⁺. The electrochemical redox reaction of NR can be coupled to biochemical redox reaction as follows: [NR₉ + 2e⁻ + H⁺ → NR₉⁻; NR₉⁻ + NAD⁺ → NR₉ + NADH]. Commonly, NR₉ and NAD⁺ are reduced to NR₉⁻ and NADH, respectively by accepting two electrons from electrode and NR₉⁻ (ox: oxidation; red: reduction).

In this study, electrochemical reducing power was charged to C. acetobutylicum culture using the NR-cathode to induce autotrophic production of acetate and butyrate from CO₂ in a defined medium and increase of butyrate production in a complex medium. The NR-cathode, to which –2 V of DC-electricity was charged, may be an optimized habitat for strict anaerobes because the lower oxidation-reduction potential than –300 mV (vs. NHE) is electrochemically generated and the electrochemically reduced NR may catalyze bacterial NADH regeneration.

2. Materials and Methods

2.1. Medium

Reinforced clostridial (RC) medium (Tryptone 10 g/L, Sodium chloride 5 g/L, Beef extract 10 g/L, Yeast extract 3 g/L, Glucose 20 g/L, Starch 1 g/L, L-cystein hydrochloride 0.5 g/L, Sodium acetate 3 g/L) was used as a complex medium and for successive cultivation of C. acetobutylicum. M9 mineral medium (Disodium phosphate 6.8 g/L, Monosodium phosphate 3 g/L, Ammonium chloride 5 g/L, Sodium chloride 0.5 g/L, Magnesium sulfate 0.246 g/L, Calcium chloride 0.0147 g/L) supplemented with sodium bicarbonate (25 mM) and yeast extract (3 g/L) was used as a defined medium. Fifty ml of RC or defined medium was prepared in anaerobic serum vials (total volume 165 ml) whose headspace was filled with 2 atmospheres of oxygen-free N₂ or H₂.

2.2. Electrochemical Bioreactor

An electrochemical bioreactor that was designed for continuous culture in previous research [16] was partially modified for cultivation of strict anaerobic bacterium in batch culture, as is shown in Figure 1. The electrochemical bioreactor (inner diameter, 80 mm; height, 200 mm; medium volume, 500 ml; electrode volume, 250 ml; total volume, 1000 ml; Pyrex, USA) with a built-in anode compartment was designed to equalize distance between anode and all round of cylindrical cathode. A sintered glass filter (diameter, 50 mm; thickness, 5 mm; pore, 1 - 1.6 μm, Duran, Germany) that was modified with cellulose acetate film (35 μm thickness, Electron Microscopy Sciences, USA) was fixed at the bottom end of the tube-type anode compartment (inner diameter 20 mm; height, 150 mm; working volume, 50 ml). Cellulose acetate film attached to the sintered glass functions as a semipermeable membrane capable of selectively transferring water, gas, and proton. Five hundred ml of the media was prepared in the electrochemical bioreactor (Figure 1) to which O₂-free CO₂ (50 ml·min⁻¹) was continuously supplied during cultivation. Inoculation ratio was adjusted to 5% (w/w) of medium volume. DC –2 V of electricity was charged to NR-graphite felt cathode to induce electrochemical reduction reaction of NR for C. acetobutylicum. The defined medium was used as anolyte to avoid generation of osmosis between anode and cathode compartment.

2.3. Electrode

Graphite felt (thickness, 10 mm; height, 200 mm; length,
500 mm; Electrosynthesis, USA) was rolled up to be a cylinder type (internal diameter, 40 mm; external diameter, 75 mm). Neutral red was immobilized to the graphite felt (10 × 200 × 500 mm, Electrosynthesis, USA) by the covalent bond between neutral red and polyvinyl alcohol (mean molecular weight, 80,000, Sigma, USA) according to the technique used in previous research [16]. The graphite felt modified with NR was used as a cathode and a platinum wire (thickness 0.5 mm, length 150 mm) was employed as an anode. Electric potential charged to NR-cathode was precisely adjusted to –2 V.

2.4. Analysis of Electrochemical Reaction of C. acetobutylicum

The cyclic voltammetry was employed in order to analyze electrochemical redox reactions between electrode and intact cell of C. acetobutylicum. The cyclic voltammetry was conducted using a voltammetric potentiostat (BAS model CV50W, USA) linked to a data acquisition system. Aglassy carbon electrode (5mm diameter, Electrosynthesis, USA), a platinum wire, and an Ag/AgCl electrode (redox potential, +0.2 V vs. NHE, Electrosynthesis, USA) were utilized as a working electrode, counter-electrode, and reference electrode, respectively. The reactant was composed of 25 mM Tris-HCl buffer (pH 7.5) containing 5 mM NaCl and 100 μM NR. C. acetobutylicum that was anaerobically cultivated in the modified M9 medium for 48 hr under H2 atmosphere was anaerobically centrifuged at 1500 × g and 4°C for 60 min. The precipitated bacterial cells were suspended in 0.05 volume of the oxygen-free reaction mixture, in which NR was spontaneously immobilized in bacterial cells. Prior to and during the cyclic voltammetry measurement, argon (99.9999%) was sparged into headspace of reaction beaker in order to protect contamination of the reaction mixture by oxygen. The scanning rate was 25 mV·s–1 over a range of 0 to –1200 mV. During cyclic voltammetry for NR dissolved in reactant or immobilized in C. acetobutylicum, the variations of upper voltammetric peaks (an indicator for electron transfer from electrode to bacterial cells through NR) and lower voltammetric peaks (an indicator for electron transfer from bacterial cells to electrode through NR) by addition of CO2 were recorded.

2.5. Analysis of Metabolites

Bacterial metabolites were analyzed using a Gas Chromatography/Mass Spectrometry (Clarus 600 series + TurboMatrix HSS Trap, PerkinElmer, USA) equipped with Elite-FFAP column (ID 0.25 μm, OD 0.32 μm, length 30 m) and electron ionization system. Bacterial culture was centrifugation at 10,000 × g and 4°C for 30 min and filtered with membrane filter (pore 0.22 μm), and then directly injected into the GC/MS injector. Concentration of metabolites was determined based on peak area of standard compounds and chemical species was determined based on mass profile database.

2.6. Microarray of mRNA

C. acetobutylicum was cultivated in the electrochemical bioreactor using the defined medium under strict anaerobic CO2 atmosphere and in the complex medium under strict anaerobic N2 atmosphere for 5 days. Total RNA was isolated and purified from harvested bacterial cells using a RNA purification kit (Total RNA, spin-column format, Oligotex mRNA mini kit, Qiagen Korea, Seoul). Microarray analysis of mRNA was conducted at Gnomictree (Daejeon, Korea) using the systems, kits, DNA chips, and analysis software offered by Agilent Technologies (Korea branch, Seoul) via a turnkey-based analyzing order. The significantly expressed genes that are concerned with CO2 fixation and energy metabolism were selectively analyzed to compare the relationship between metabolic pathway related with CO2 fixation and cultivation conditions.

3. Results

3.1. Electrochemical Redox Reaction in Coupling with CO2

Cyclic voltammetry is a useful technique to measure electrochemical coupling redox reaction of electron mediator immobilized in bacterial cells. In the cyclic voltammetry without bacterial cells, the electrochemical redox reaction of NR was measured to be –0.52 V (vs. Ag/AgCl), which is very similar to the experimental value –0.525 V (vs. Ag/AgCl) measured in standard condition. Both the upper and lower voltammetric peaks were not altered by addition of CO2 as expected; in contrast, the upper voltammetric peak generated by NR immobilized in C. acetobutylicum was shifted upward from 2.4 to 2.9 μA and rightward from –0.52 to –0.55 V by addition of CO2 as shown in Figure 2. Increase of 0.4 μA of current indicates that electrons are transferred from electrode to bacterial cells coupled to redox reaction of NR. Increase of –0.3 V of redox potential is a clue that electrons are transferred from electrode to NR by lower electrode (working electrode) potential than intrinsic redox potential of NR. Relatively higher electron-driving force (electrode potential) may be required for electrons to move through the electric resistance generated between electrode and NR immobilized in bacterial membrane. Meanwhile, other upper voltammetric peak (bold arrow mark) located at –0.9 V (vs. Ag/AgCl) was also shifted upward from 2.7 to 3.0 μA and rightward from –0.9 V to –0.93 V by addition of CO2. It seems possible that electrons are transferred from the electrode via one of the electron carriers located in the bacterial membrane,
allowing for current and potential increase by addition of CO$_2$. CO$_2$ could act as an electron acceptor to induce biochemical oxidation of NADH that may be electrochemically regenerated, by which electrons may be transferred from electrode to bacterial cells via the coupling redox reaction of NR and NAD$^+$.  

3.2. Growth and Metabolite Production of *C. acetobutylicum*

*C. acetobutylicum* did not grow and didn’t produce metabolites in the defined medium under N$_2$ atmosphere (DM-N$_2$) but grew and produced acetate, propionate, and butyrate under H$_2$ atmosphere (DM-H$_2$), as shown in Table 1. The metabolites detected in the chromatography for culture fluid of *C. acetobutylicum* cultivated in the DM-N$_2$ may have originated from the metabolites contained in the inoculum. The electrochemical reducing power generated from NR-cathode (reduced NR) is converted to the biochemical reducing power (NADH), which can be presumed on the basis of the growth and metabolite production of *C. acetobutylicum* cultivated in the DM-ER. *C. acetobutylicum* cultivated with electrochemical reducing power produced more acetate than butyrate in DM-ER but more butyrate than acetate in CM-ER. These are more clues that biochemical reducing power (NADH) may be regenerated by electrochemical reducing power generated from –2 V of NR-cathode and the high balance of NADH/NAD$^+$ may induce metabolic conversion of CO$_2$ to metabolites in coupling with free energy synthesis.

3.3. Quantitative and Qualitative Verification of Metabolites

Metabolites generated by *C. acetobutylicum* in different cultivation conditions were quantitatively and qualitatively analyzed by a specially trained expert, and found to be acetate, propionate, and butyrate as shown in Figure 3, as expected. This analytical process is absolutely

<table>
<thead>
<tr>
<th>Cultivation conditions</th>
<th>Growth at OD$_{660}$ (initial-final)</th>
<th>Acetic acid (mM)</th>
<th>Propionic acid (mM)</th>
<th>Butyric acid (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM-N$_2$</td>
<td>0.08 - 0.06</td>
<td>0.6 ± 0.04</td>
<td>0.1 ± 0.01</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>DM-ER</td>
<td>0.08 - 0.36</td>
<td>9.2 ± 0.2</td>
<td>0.8 ± 0.03</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>DM-H$_2$</td>
<td>0.08 - 0.38</td>
<td>9.8 ± 0.3</td>
<td>0.9 ± 0.02</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>CM-N$_2$</td>
<td>0.08 - 1.28</td>
<td>35.8 ± 1.4</td>
<td>2.8 ± 0.1</td>
<td>21.6 ± 1.1</td>
</tr>
<tr>
<td>CM-ER</td>
<td>0.08 - 1.06</td>
<td>19.6 ± 0.8</td>
<td>4.8 ± 0.2</td>
<td>38.4 ± 0.9</td>
</tr>
<tr>
<td>CM-H$_2$</td>
<td>0.08 - 1.21</td>
<td>24.4 ± 0.9</td>
<td>4.2 ± 0.2</td>
<td>36.6 ± 1.3</td>
</tr>
</tbody>
</table>
required because some metabolic intermediates derived from amino acids (yeast extract) may be produced by bacterial cells cultivated in the DM-H2 and DM-ER condition.

3.4. Analysis of Genes Induced by Electrochemical Reducing Power

Significant genes (higher than twice the signal intensity) commonly expressed in *C. acetobutylicum* that was electrochemically cultivated in the defined medium under CO2-atmosphere and in the complex medium under N2-atmosphere numbered in 318. All of the fundamental genes related to CO2-fixation were not detected; however, the genes of encoding the enzymes catalyzing acetyl-CoA synthesis from CO2, ATP synthesis, and butyrate production were quantitatively analyzed and compared as shown in Table 2. The genes encoding the enzymes catalyzing acetyl-CoA generation from CO2 and ATP synthesis in pathway from acetyl-CoA to acetate were more expressed but those catalyzing NADH regeneration coupled to oxidation of substrates (metabolic intermediates) and butyric acid production were less expressed in *C. acetobutylicum* cultivated in DM-ER condition than CM-N2 condition (Table 1).

4. Discussion

Electron transfer from electrode to bacterial cells can be generated by the simultaneous contact of an electron mediator with both electrode and bacterial cells. Contact of an electrode with the electron mediator (NR) immobilized in bacterial cell or contact of bacterial cells with the electron mediator immobilized in an electrode is a unique way to induce electron transfer between bacterial cells and electrode [17-19]. Patterns of cyclic voltammetry of NR immobilized in bacterial cells were identical to those of NR dissolved in reactant in the condition uncoupled to the external redox reaction, because the redox reaction is proportional to the concentration of NR contacted stably with the electrode (Figure 2). Some NRs immobilized in *C. acetobutylicum* are electrochemically reduced and biochemically oxidized coupled to NADH regeneration [6]. This electrochemical and biochemical coupling redox reaction of NR and NAD+ may be continuously repeated in this condition with both electron donor and acceptor. The electrode may be an electron donor and CO2 may be an electron acceptor in the cyclic voltammetry for the modified *C. acetobutylicum* with NR, considering that addition of CO2 induced electron transfer from electrode to bacterial cells via NR immobilized in bacterial cells or other bacterial electron carrier (upper voltammetric peaks in Figure 2) can be quantitatively analyzed based on the increase of current (electron number) and variation of redox potential (electron-driving force). The NR immobilized in bacterial cells can temporarily function in proportion to physiological activity.
Table 2. Comparison of genes expressed in *C. acetobutylicum* cultivated with electrochemical reducing power in the CO$_2$-saturated defined medium and cultivated with glucose in the complex medium.

<table>
<thead>
<tr>
<th>Ratio of A/B</th>
<th>Gene products (Functions)</th>
<th>Signal intensity for specific genes expressed in <em>C. acetobutylicum</em></th>
<th>Electrocromically cultivated (A)</th>
<th>Cultivated in complex medium (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.87</td>
<td>Carbon monoxide dehydrogenase (CO formation from CO$_2$)</td>
<td></td>
<td>7764</td>
<td>1595</td>
</tr>
<tr>
<td>4.71</td>
<td>Biotin-acetyl-CoA-carboxylase (Malonyl-CoA production)</td>
<td></td>
<td>2846</td>
<td>604</td>
</tr>
<tr>
<td>3.17</td>
<td>Formyl-H$_2$folate synthase (Methyl-formation from CO$_2$)</td>
<td></td>
<td>317</td>
<td>10</td>
</tr>
<tr>
<td>2.89</td>
<td>Formyl-H$_2$folate cyclohydrolase (Methyl-formation from CO$_2$)</td>
<td></td>
<td>772</td>
<td>267</td>
</tr>
<tr>
<td>3.24</td>
<td>Putative methyltransferase (Methyl-formation from CO$_2$)</td>
<td></td>
<td>13,908</td>
<td>4287</td>
</tr>
<tr>
<td>244.8</td>
<td>Phosphotransacetylase (Formation of acetyl-Pi from acetyl-CoA)</td>
<td></td>
<td>2448</td>
<td>10</td>
</tr>
<tr>
<td>212.4</td>
<td>Acetate kinase (Acetate production coupled to ATP synthesis)</td>
<td></td>
<td>2124</td>
<td>10</td>
</tr>
<tr>
<td>0.74</td>
<td>NAD-dependent dehydrogenase (NADH regeneration coupled to substrate oxidation)</td>
<td></td>
<td>1856</td>
<td>2524</td>
</tr>
<tr>
<td>0.21</td>
<td>Acetyl-CoA acetyltransferase (Butyric acid production)</td>
<td></td>
<td>4179</td>
<td>19,492</td>
</tr>
</tbody>
</table>

of the bacteria modified with NR; however, NR immobilized in the graphite felt cathode can semipermanently function as long as it is contacting with intact cells of bacteria.

In the electrochemical bioreactor equipped with the NR-cathode, the electron transfer from electrode to bacterial cells can’t be quantitatively analyzed because the number of electrons (current) transferred from power supply to NR-cathode is not proportional to the metabolic reduction reaction catalyzed by bacterial cells, and –2 V of electrode potential charged to the bioreactor was stronger than the redox potential of NR. This can generate electron-driving force to transfer electrons via NR immobilized in electrode to bacterial cells but may induce electrochemical reduction of medium ingredients and other organic compounds. The –2 V of electrode potential may be too strong to induce the electrochemical reduction of NR (–0.325 V vs. NHE) but is required to induce electron transfer from electrode to bacterial cells through the electron barrier (cytoplasmic membrane) because electric resistance may be generated by reactor membrane between analyte and catholyte, connecting error between NR and bacterial cells, and structural mismatch between NR and NAD$^+$ [20].

It is unquestionable that the NR immobilized in graphite felt electrode mediated electron transfer from electrode to bacterial cells and catalyzed regeneration of biochemical reducing power on the basis of acetic and butyric acid production from CO$_2$ and production of more butyric acid than acetic acid from glucose. This result is very similar to that obtained from *C. acetobutylicum* culture using H$_2$ and CO$_2$ as an electron donor and acceptor. Metabolic production of acetic acid is coupled to NADH regeneration but that of butyric acid is coupled to NADH oxidation in *C. acetobutylicum* grown in glucose. Accordingly, higher production of butyric acid than acetic acid by *C. acetobutylicum* cultivated in CM-ER and CM-H$_2$ is another clue that H$_2$ and electrochemically reduced NR may be an additional reducing power to increase ratio of NADH/NAD$^+$ [21].

Metabolic conversion of CO$_2$ to metabolites is coupled to oxidation of biochemical reducing power regenerated by the electrochemically reduced NR or H$_2$; however, the metabolic pathways related to the autotrophic CO$_2$ fixation can be assumed only by the metabolites produced by *C. acetobutylicum* cultivated in different media and under different conditions. Microarray analysis of mRNA is effective to analyze variations of metabolic pathway and free energy production related to autotrophic CO$_2$ fixation or heterotrophic growth. Practically, the specific genes related to the CO$_2$ fixation and energy metabolism expressed in *C. acetobutylicum* electrochemically or conventionally cultivated are a clue that the metabolic conversion of CO$_2$ to metabolites in coupling with the free energy production and redox reaction of reducing power may be generated by the electrochemical reducing power. Theoretically, –2 V of electricity charged to the NR-cathode located in culture medium may induce H$_2$ generation by electrolysis of H$_2$O. However, H$_2$ was not detected in the electrochemical bioreactor even by precision analysis. Accordingly, the NR-cathode may directly transfer electrons from electrode to intact cells of *C. acetobutylicum* and induce catalyzing of NADH regeneration in metabolism of *C. acetobutylicum*.

### 5. Conclusion

The electrochemical redox reaction of NR, the catalytic function of NR for NADH regeneration, and the immobi-
lization technique of NR in the electrode permit \textit{C. acetobutylicum} KCTC1037 to grow and produce metabolites using electrochemical reducing power. Mixed acid fermentation bacteria produced the relatively reduced metabolite (butyrate) or oxidized metabolite (acetate) depending on balance of NADH/NAD\textsuperscript{+}. In autotrophic microbes, CO\textsubscript{2} can be reduced to CO by catalysis of carbon monoxide dehydrogenase in coupling with oxidation of biochemical reducing power (NADH or NADPH). Practically, \textit{C. acetobutylicum} produced more butyrate than acetate from glucose and more acetate than butyrate from CO\textsubscript{2}, reasonable on the basis of metabolic pathway for ATP regenerations. \textit{C. acetobutylicum} cultivated heterotrophically with glucose synthesizes ATP by substrate-level phosphorylation and regenerates NADH in both trophically with glucose synthesizes ATP by substrate-level phosphorylation and regenerates NADH in both glycolysis and pathway from pyruvate to acetate but that cultivated autotrophically with electrochemical reducing power and CO\textsubscript{2} synthesizes ATP in the pathway from acetyl-CoA to acetate and regenerates NADH coupled to electrochemical redox reaction of NR.

6. Acknowledgements

This work was supported by the New \& Renewable Energy of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea governmental Ministry of Knowledge Economy (2012-T1001100334).

REFERENCES


Copyright © 2012 SciRes.

