Microbes as Electrochemical CO₂ Conversion Catalysts

Jieun Song, Yousung Kim, Miran Lim, Hojun Lee, Jong In Lee, and Woonsup Shin[a]

Carbon dioxide conversion is not only important because it removes a greenhouse gas, but because it enables one to use the most-abundant carbon compound for producing useful organic compounds. Fundamentally, CO₂ activation is a process that requires energy, and the development of efficient catalysts is a key issue. In Nature, microbes, including acetogens and methanogens, exhibit C₁ chemistry capable of activating CO₂ to single- or multi-carbon compounds. Herein, we report that microbes are excellent electrocatalysts for the conversion of CO₂ to useful organic compounds. Among five microorganisms tested, two acetogenic bacteria, *Moorella thermoacetica* (MT, formerly *Clostridium thermoaceticum*, ATCC 35608) and *Clostridium formicoaceticum* (CF, DSM 92) efficiently converted CO₂ to formate. The current efficiency was 80% for MT and 100% for CF upon electrolysis in 1 atm CO₂-saturated 0.1 M phosphate buffer solution (pH 7.0) at −0.58 V vs. NHE, which is near the equilibrium potential of CO₂/formate.

Previously, we reported that CO dehydrogenase/acytob Co synthase (CODH/ACS) from MT could electrocatalytically convert CO₂ into CO at −0.57 V with 100% current efficiency,[1] and examined the optimum conversion conditions such as pH, temperature, electrode materials, and applied potential.[2] Acetogens can grow anaerobically under CO₂/H₂ or CO to produce acetate using a novel metabolic pathway, known as the Wood/Ljungdahl or acetyl-CoA pathway.[3] Formate dehydrogenase (FDH) is the first enzyme that fixes gaseous CO₂ to enter the pathway, and CODH/ACS is a key bifunctional enzyme for fixing CO₂ to CO and synthesizing acetyl coenzyme A. The catalytic system in the organism is based on reductive activation of CO₂, and can be applied to convert CO₂ into useful organic acids by electrochemical reduction. Although transition metal complexes are the most intensively investigated and developed catalysts,[4] enzymes such as CODH[5] and FDH[6] have started to receive a lot of attention owing to their catalytic units, developed naturally. The direct application of microbes to the electrochemical conversion of CO₂ could eliminate expensive enzyme purification steps and utilize fully not only the catalytic, but also transport and protection systems in the cell that nature has already optimized. Recently, a microbial film grown with *Sporomusa ovata* was shown to convert CO₂ to acetate in a growth medium.[7]

Bacterial cells in solution do not communicate with the electrode directly, and methyl viologen was used as an electron-shuttling mediator. Scheme 1 shows a schematic representation of the electrocatalytic reduction. The cyclic voltammo-

[1] J. Song, Dr. Y. Kim, M. Lim, H. Lee, J. I. Lee, Prof. Dr. W. Shin
Department of Chemistry and
Interdisciplinary Program of Integrated Biotechnology
Sogang University
Seoul 121-742 (Korea)
Fax: (+82)-2-3273-0993
E-mail: shinws@sogang.ac.kr

[a] Supporting Information for this article is available on the WWW under http://dx.doi.org/10.1002/cssc.201100107.
We also tested Clostridium formicoaceticum (Cf, DSM 92), Clostridium pasteurianum (Cp, DSM 525), Clostridium acetobutylicum (Ca, DSM 792), and Pseudomonas sp. (Ps, DSM 7220) for the electrocatalytic reduction of CO₂ under the same conditions as Mt except for the electrolysis temperature, which was varied to each organism’s optimal growth temperature: 37°C for Cf, Cp, and Ca, and 25°C for Ps (Figure S1). Among the tested bacteria, Cf, Cp, and Ca showed electrocatalytic activity and charge accumulation was observed, while Ps showed no electrochemical activity toward CO₂ reduction. Cf showed 100% current efficiency in formate conversion. On the other hand Cp showed only 3% formate conversion efficiency, and the major reaction was H₂ production. In case of Ca, H₂ was produced exclusively. Interestingly, the microorganisms showing electrocatalytic activity towards CO₂ reduction belong to acetogens, having an acetyl-CoA pathway. Mt is the best-characterized homoacetogen to have acetate as a sole metabolic product. Cf is a unique microorganism because of its ability to produce formate following the accumulation of acetate in growth conditions. Cp is known to have highly active hydrogenases, and the electrons are mostly used for reducing protons to hydrogen instead of activating CO₂. Ca has no acetyl-CoA pathway and is known to have active hydrogenases, and only hydrogen was produced. Ps showed no electrochemical reduction activity, although it is known to have active FDH. The FDH in the organism seems capable of only formate oxidation, and the reverse reaction does not occur.

A possible electrochemical activation pathway by acetogens is proposed in Scheme 2, and the product distribution can be explained by the relative enzymatic activities of FDH, CODH, ACS, and hydrogenase (Hase) in each organism. Notably, proton reduction (reaction 3) always competes with CO₂ reduction (reactions 1 and 2) because thermodynamically protons are more easily reduced than CO₂ in neutral solution (\(E^{0'}_{\text{H_2/H+}} = -0.42\, \text{V}, \quad E^{0'}_{\text{CO_2/CO}} = -0.52\, \text{V}, \quad E^{0'}_{\text{CO_2/formate}} = -0.61\, \text{V}, \quad \text{vs. NHE}\)). The high reductive activity of FDH in Mt and Cf results in electron transfer to mainly CO₂ kinetically, not to protons. The unsuccessful further reduction to acetate may result from either nutrient deficiency or from the use of the methyl viologen mediator, blocking the acetyl-CoA synthesis reaction pathway.

![Figure 1](image1.png) (a) Cyclic voltammograms of 1.0 mM MV²⁺ in 0.1 mM phosphate buffer (pH 7.0) with Mt (OD₆₆₀ = 1.0) under N₂ (solid line) and under CO₂ (dashed line) at 55°C on a glassy carbon disk (3 mm diameter) working electrode at a scan rate of 10 mV/s. (b) Electrolysis of CO₂ at −0.58 V vs. NHE in the presence of Mt (OD₆₆₀ = 1.0) and 1.0 mM MV²⁺ in 6.0 mL of 1 atm CO₂-saturated 0.1 mM phosphate buffer (pH 7.0) with glassy carbon rod electrode (7 mm diameter, 25 mm length) at 55°C.

![Figure 2](image2.png) (a) GC mass spectrometry data of (a) products formed by electrolysis under 1 atm of¹³CO₂, and (b) products formed by electrolysis under 1 atm of CO₂.

Scheme 2. Reaction pathways for electrochemical CO₂ reduction by microbes.
The effects of external variables, such as pH, electrolysis temperature, mediator concentration, and bacterial cell concentration, on formate conversion efficiency were examined for Mt and Cf. Mt showed only a slight variation of the electrocatalytic CO₂ conversion activity in the range of pH 5–8, while Cf showed a sharp pH optimum at pH 7 and the formate conversion activity dropped significantly, to ca. 60% at pH 5–6 (Figure S2). The addition of 0.5 mol NaCl did not affect the electrocatalytic capability of Mt, while Cf lost more than 20% of its activity. The thick cell walls of Mt (Gram-positive), compared to the thin cell walls Cf (Gram-negative), may be responsible for the less-sensitive response to changes in the solution conditions.[3] In the case of Mt and Cf, a maximum formate concentration reached 60 mm (Figure S3). These concentrations dropped to ca. 60% at pH 5–6 (Figure S3a). In the case of Mt, the maximum formate concentration was obtained at OD₆₆₀ nm = 5.0 and 5.0 mm MV²⁺ in 1 atm CO₂ saturated 0.1 mol/l phosphate buffer solution (pH 7.0) at 55 °C, and the maximum formate concentration reached 60 mm (Figure S3a). At OD₆₆₀ nm > 5, the catalytic reaction did not increase further due to the less-efficient electron transfer in the highly concentrated and viscous bacterial cell solution. The catalytic current increased with increasing mediator concentration, but the catalytic activity dropped quickly (within 1 h) at a methyl viologen concentration of 10–15 mm (Figure S3b). The high concentration of methyl viologen is toxic to the cells. The thermophile Mt showed the best catalytic performance at 55 °C (its optimum growth temperature), while the mesophile Cf showed similar catalytic behaviors in the range of 25–45 °C, although its optimum growth temperature is 37 °C.

In conclusion, the use of microbial cells as electrochemical CO₂ conversion catalysts is successfully demonstrated and it widens the choice of catalysts by including diverse microorganisms already developed and optimized in nature. The microorganisms are a potentially "green" alternative to the more traditional inorganic electrocatalysts. Engineering the bacterial activities is another important tool for developing the microbial catalysts.

Experimental Section

*Moorella thermoacetica* (Mt, formerly *Clostridium thermoacetemicum*, ATCC 35608) was purchased Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, German Collection of Microorganisms and Cell Cultures) and cultivated in CBBM (Carbonate-buffered basal medium) [13] in strictly anaerobic conditions (80% CO₂, 10% H₂ and 10% N₂) and cultivated in CBBM (Carbonate-buffered basal medium) and buffered with 0.1 mol/l phosphate buffer (pH 7.0) at 55 °C. The maximum formate concentration was obtained at OD₆₆₀ nm = 5.0 and 5.0 mm MV²⁺ in 1 atm CO₂ saturated 0.1 mol/l phosphate buffer solution (pH 7.0) at 55 °C, and the maximum formate concentration reached 60 mm (Figure S3a). At OD₆₆₀ nm > 5, the catalytic reaction did not increase further due to the less-efficient electron transfer in the highly concentrated and viscous bacterial cell solution. The catalytic current increased with increasing mediator concentration, but the catalytic activity dropped quickly (within 1 h) at a methyl viologen concentration of 10–15 mm (Figure S3b). The high concentration of methyl viologen is toxic to the cells. The thermophile Mt showed the best catalytic performance at 55 °C (its optimum growth temperature), while the mesophile Cf showed similar catalytic behaviors in the range of 25–45 °C, although its optimum growth temperature is 37 °C.

In conclusion, the use of microbial cells as electrochemical CO₂ conversion catalysts is successfully demonstrated and it widens the choice of catalysts by including diverse microorganisms already developed and optimized in nature. The microorganisms are a potentially "green" alternative to the more traditional inorganic electrocatalysts. Engineering the bacterial activities is another important tool for developing the microbial catalysts.

Cyclic voltammetry and bulk electrolysis were carried out with a BAS-50W electrochemical analyzer in a three-electrode cell system with glassy carbon, Ag/AgCl (3 mol KCl), and coiled platinum wire as working, reference, and counter electrode, respectively. All electrochemical experiments were performed in a glove-box (Vacuum Atmosphere Co, HE-243-2, MO-20-SSG purifier). A glassy carbon disk (3 mm diameter, 0.071 cm²) was used for cyclic voltammetry and a glassy carbon rod (7 mm diameter, 0.071 cm²) was used for electrolysis. The glassy carbon electrodes were polished with 1 mm alumina powder and sonicated in water for 5 min before use. All experiments were conducted in 0.1 mol/l phosphate buffer solution (pH 7.0), and the solutions were purged with high-purity CO₂ gas (99.999%) for 10 min before measurement. The redox potential was measured versus Ag/AgCl electrode, converted to the normal hydrogen electrode (NHE), and reported.

For the analysis of the liquid products, high-pressure liquid chromatography analyzer (Agilent 1100 series) was used with RSpak KC-811 column (Shodex, Japan) by using the settings: oven temperature 40 °C; eluent 0.1% phosphoric acid; flow rate 1 ml/min⁻¹; injection volume 20 µl. Gaseous products were analyzed by gas chromatography (HP 5890 Series II) using Porapak R80/100 packed column (Alltech) with a TCD detector. The operating conditions were: oven temperature 35 °C (isothermal); injection volume 100 µl; carrier gas N₂; flow rate 10 ml/min⁻¹. For the mass analysis GC-Mass (Agilent 5973N MSD) was used with carboxen/polydimethylsiloxane fiber using SPME (solid phase microextraction) technique.

**Acknowledgements**

This research was supported by the Korea Institute of Energy Technology Evaluation and Planning (2006CCD03P010000) and the Korea Center for Artificial Photosynthesis (KCAP) located in Sogang University funded by the Ministry of Education, Science, and Technology (MEST) through the National Research Foundation of Korea (NRF-2009-C1 AA A001–2009–0093879).

**Keywords:** carbon · bacteria · biotransformations · electrochemistry · renewable resources

---


Received: February 28, 2011
Published online on April 28, 2011