Electrochemical Cultivation of Bacteria using a Quinone Compound as an Electron Carrier

Background

Understanding the behavior of microorganisms living underground is one of the important tasks to evaluate long term safety of underground facilities because these microorganisms will accelerate oxidation and reduction reactions of materials. However, the cultivation method for these microorganisms is almost still unknown. It has been suggested recently that quinone compounds, kinds of redox materials in soil, work as an electron carrier between environment and microorganisms and support the growth of these environmental microorganisms (Fig.1). Since the electrochemical cultivation technique developed can reproduce the behavior of quinone compounds in environment which works as electron carrier, the technique is expected to obtain previously uncultured environmental microorganisms related with formation of redox condition underground.

Objectives

To obtain quinone reducing bacteria that are useful to estimate safety of underground environment using the electrochemical cultivation technique.

Principal results

1. Acquisition of quinone reducing bacteria from soil samples

Bacteria with quinone reducing activity were obtained from environmental soil samples by 2-week cultivation under anaerobic condition with hydrogen gas and anthraquinone derivative, which is a kind of hydrophilic quinone compound.

2. Electrochemical cultivation of quinone reducing bacteria

Electrochemical cultivation was performed against quinone reducing bacteria obtained in the above condition to acquire a highly accumulated bacteria sample. Bacteria were inoculated into the electrochemical chamber described in Fig.2 and were cultivated with applying potential of 0.2 V vs. Ag/AgCl, at which quinone oxidation occurs, under anaerobic atmosphere created by hydrogen. After 5 days cultivation, a high density bacteria sample of 4 x 10^8 cells/mL was obtained that corresponded to 7 times higher density than the cultivation without an electrochemical technique (Fig.3). In addition, this was the first success of electrochemical cultivation using organic compound as the electron mediator.

3. Isolation of quinone reducing bacteria

Two different kinds of quinone reducing bacteria were isolated from electrochemically accumulated samples by colony isolation using an agar plate and named as G-1 and EMJ-1, respectively (Table 1). From DNA analysis, it was estimated that strain G-1 belonged to *Desulfitobacter* group, which mainly includes strictly anaerobes, and strain EMJ-1 belonged to *Enterobacter* group, which mainly includes facultative anaerobes. This result meant that the electrochemical cultivation with quinone compound was applicable to multiple types of bacteria with each different metabolic pathway.

Future Developments

Acquisition of bacteria from underground will be advanced using electrochemical cultivation to clarify the effect of bacteria on the safety evaluation of underground utilization.

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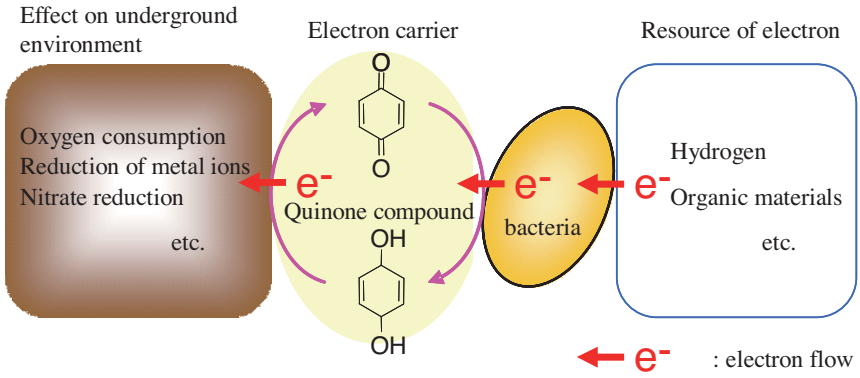
Reference

N. Matsumoto, et.al., 2006, “Electrochemical control of microorganisms (Part 9) -Electrochemical cultivation of bacteria using a quinone compound-” CRIEPI Report V05031 (in Japanese)



Microorganisms living underground are supposed to accelerate reduction and oxidation reactions of underground materials via quinone compound as an electron carrier.

Fig.1 Effect of microorganisms via a quinone compound on underground environment

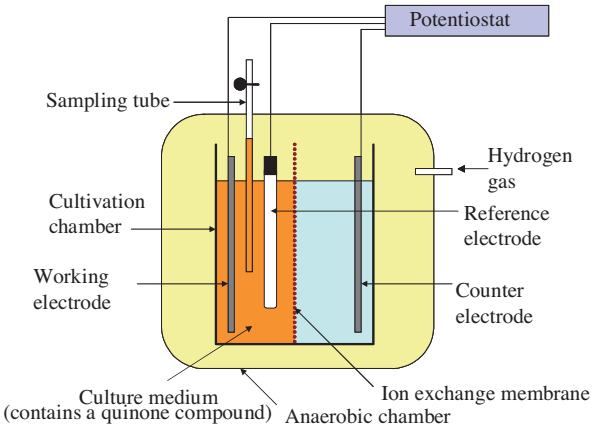


Fig.2 Schematic drawing of an electrochemical cultivation system

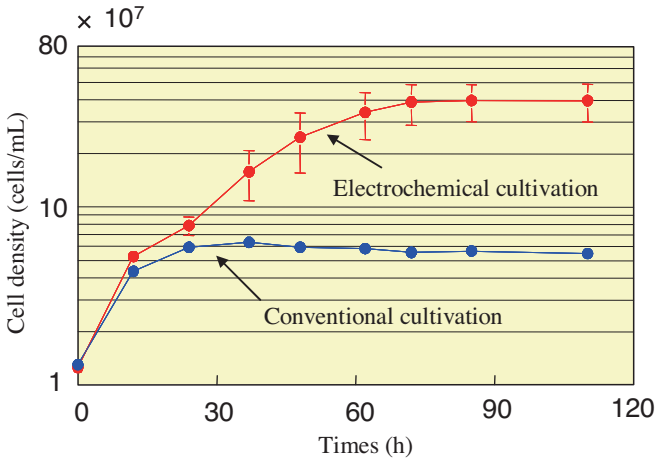


Fig.3 Comparison of growths of quinone reducing bacteria

7-fold high density growth of bacteria was observed in the electrochemical cultivation compared with the conventional cultivation system

Table 1 Property of quinone reducing bacteria isolated in this study

Two different kinds of quinone reducing bacteria were isolated

strain	EMJ-1	G-1
The nearest group from DNA analysis	*Enteorobacter*	*Desulfitobacter*
Typical growth condition of belonging group	facultative anaerobic	strictly anaerobic
Electron donor	Organic materials	Hydrogen
Electron acceptor	Organic materials	Quinone compound
Atomic force microscopy image (arrows indicate bacteria)