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Enrichment of CO_2-Fixing Bacteria in Cylinder-Type Electrochemical Bioreactor with Built-In Anode Compartment

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Bacterial assimilation of CO_2 into stable biomolecules using electrochemical reducing power may be an effective method to reduce atmospheric CO_2 without fossil fuel combustion. For the enrichment of the CO_2-fixing bacteria using electrochemical reducing power as an energy source, a cylinder-type electrochemical bioreactor with a built-in anode compartment was developed. A graphite felt cathode modified with neutral red (NR–graphite cathode) was used as a solid electron mediator to induce bacterial cells to fix CO_2 using electrochemical reducing power. Bacterial CO_2 consumption was calculated based on the variation in the ratio of CO_2 to N_2 in the gas reservoir. CO_2 consumed by the bacteria grown in the electrochemical bioreactor (2,000 ml) reached a maximum of approximately 1,500 ml per week. Time-coursed variations in the bacterial community grown with the electrochemical reducing power and CO_2 in the mineral-based medium were analyzed *via* temperature gradient gel electrophoresis (TGGE) of the 16S rDNA variable region. Some of the bacterial community constituents noted at the initial time disappeared completely, but some of them observed as DNA signs at the initial time were clearly enriched in the electrochemical bioreactor during 24 weeks of incubation. Finally, *Alcaligenes* sp. and *Achromobacter* sp., which are capable of autotrophically fixing CO_2, were enriched to major constituents of the bacterial community in the electrochemical bioreactor.

Keywords: Electrochemical reducing power, CO_2-fixing bacteria, electrochemical bioreactor, built-in anode compartment, neutral red-modified cathode

Bacterial species capable of chemoautotrophically fixing CO_2 regenerate the biochemical reducing power (NADPH or NADH) with reduction energy generated by the metabolic oxidation of NH_4^+, S^o, or H_2, which functions as an electron donor for the respiration and reduction of CO_2 [10, 22, 26, 28]. In a variety of metabolic carboxylation reactions, CO_2 is assimilated into the biomolecules *via* the biochemical reducing power regenerated in tandem with the oxidation of organic compounds [29, 31, 32]. Meanwhile, the biochemical reducing power can be regenerated in combination with H_2O decomposition by the electromagnetic power in the photoautotrophic metabolism, along with O_2 generation [7].

Biochemical reducing power also can be electrochemically regenerated *via* the catalysis of neutral red, without resorting to enzyme catalysis [23, 25]. Neutral red can be electrochemically reduced by the cathodic reaction, which is coupled to the anodic reaction to generate O_2 from H_2O. Electrochemically reduced neutral red induces bacterial cells to regenerate biochemical reducing power; this is an effective reaction that is capable of inducing the autotrophic or mixotrophic CO_2-fixing bacteria to fix CO_2 with the electrochemical energy derived from solar energy. Experimentally, NR-graphite felt activated ethanol production in the fermentation metabolism of *Zymomonas mobilis*, denitrification reaction in *Ochromobactrum* sp., ammonium oxidation in *Nitrosomonas* sp., and the enrichment of hydrogenotrophic methanogens [1, 13–15, 17]. All of these reactions were activated by the electrochemically regenerated biochemical reducing power in the intact bacterial cells.

The balance between the CO_2 generated by heterotrophs and that fixed by autotrophs is unlikely to be profoundly altered in natural ecosystems; however, the concentration of CO_2 in the atmosphere has been increased continuously *via* fossil fuel combustion. Atmospheric CO_2 is naturally assimilated into biomolecules *via* autotrophic microorganisms and plants in the natural ecosystem; however, the extra CO_2 generated from the combustion of fossil fuels may not be biologically fixed. Accordingly, the bioreactor for

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bacterial CO_2 fixation using solar energy may constitute a method to reduce the atmospheric CO_2 generated from fossil fuel combustion. Practically, a very small percentage of solar energy is used for photosynthetic reactions; most solar energy is converted to radiant heat, which can be saved by converting it to electric energy with solar panels. The electricity obtained by the solar panels can be converted to electrochemical reducing power using neutral red. The bacterial cells cultivated using the electrochemical reducing power and atmospheric carbon dioxide may be not useful for industrial, nutritional, and pharmacological purpose, but may be effective for conversion of carbon dioxide to the chemically stable biopolymers in the conditions without combustion of fossil fuel and occupation of ecological habitats.

Cyanobacteria that perform a similar oxygenic photosynthesis to the higher plants [33] were studied with the object of production of valuable organic products that are foods and fodders [34]. Some cyanobacteria were reported to produce secondary metabolites that have therapeutic effects [2]. Deng and Coleman [8] transformed the cyanobacteria *Synechococcus* sp. in order to produce a novel photoautotroph capable of producing ethanol. Other useful products that are amino acids and PHB were reported to be produced by *Spirulina* sp. [5, 35]. According to the research data, the photoautotrophs may be the best organisms to fix the atmospheric carbon dioxide without combustion of fossil fuel but may occupy the ecological habitats for plants. Generally, cyanobacteria are cultivated in the water pool-type reactor under light of the sun like the agricultural system, by which the plants (trees and grasses) may be replaced by the cyanobacteria. The capacity of trees and grasses for carbon dioxide fixation is greatly higher than the cyanobacteria. The purpose of agriculture is not to fix atmospheric carbon dioxide but to produce crops that are oxidized to carbon dioxide by consumption as the nutrients. Meanwhile, high cost may be required to cultivate the cyanobacteria in the tank-type bioreactor owing to the employment of an awful lot of electric lamps.

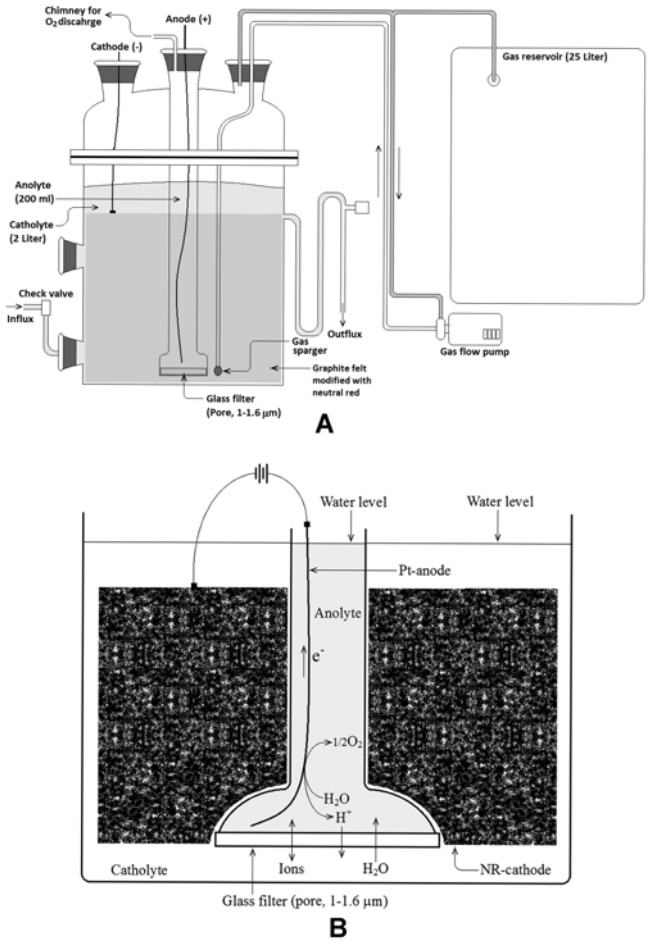

Fig. 1. Schematic structure of the electrochemical bioreactor with a built-in anode compartment for the enrichment of CO_2-fixing bacteria.

The glass filter septum was equipped at the bottom end of the anode compartment (**A**). Protons, electrons, and oxygen generated from water by the electrolysis may be transferred separately to the catholyte, the NR-cathode, and the atmosphere (**B**).

In this study, a cylinder-type electrochemical bioreactor with a built-in anode compartment was designed and employed to enrich the CO_2-fixing bacteria under CO_2–N_2 atmosphere. In order to convert electric energy to biochemical reducing power, neutral red was immobilized in the graphite felt electrode by a covalent bond. The time-coursed consumption of CO_2 by the bacterial community grown in the electrochemical bioreactor was analyzed at intervals of 1 week for more than 30 weeks. The time-coursed variation of the bacterial community that was enriched in the electrochemical bioreactor was analyzed on the basis of the 16S rDNA variable region *via* the TGGE technique during 24 weeks of cultivation.

Materials and Methods

Electrochemical Bioreactor

A cylinder-type electrochemical bioreactor (diameter, 120 mm; height, 200 mm; working volume, 2,000 ml; total volume, 3,000 ml; Pyrex, USA) with a built-in anode compartment was designed in the scale-up procedure for a pilot reactor or industrial reactor, as shown in Fig. 1A. A sintered glass filter (diameter, 50 mm; thickness, 5 mm; pore, 1~1.6 μm; Duran, Germany) was fixed at the bottom end of the anode compartment (diameter, 36 mm; height, 200 mm; working volume, 200 ml). The anolyte consumed by H_2O electrolysis was automatically refilled from the catholyte through the glass filter, as shown in Fig. 1B. O_2 generated by electrolysis in the anode compartment is removed through the chimney.

Operation of Electrochemical Bioreactor

The mixed culture of bacteria obtained from different sources was incubated in the electrochemical bioreactor under 50% (v/v) of CO_2

to N_2 without fresh medium feeding for 2 weeks. Approximate 3 V of electric power was charged directly to electrodes from a DC power supply to induce 1.5 mA of current. The electric voltage and current were measured precisely with a multimeter (Keithely 2700, USA). Approximate 50% (v/v) of CO_2 was sparged circularly into the medium at a rate of 200 ml/min to activate the dissolution of CO_2 into carbonic acid in the medium, by which carbon dioxide consumed in the electrochemical bioreactor may be replenished spontaneously. After the electrochemical bioreactor was operated at the initial time without feeding of fresh medium for 2 weeks, the fresh medium was continuously fed into the bacterial culture at a rate of 100 ml per day. The gas reservoir (Teflon gas bag; SKC, USA) was refilled freshly with 25,000 ml of 50±1% (v/v) of CO_2 to N_2 using the gas flow meter at intervals of 2 weeks. No electricity was charged to 3 of the electrochemical bioreactors for control test, but carbon dioxide was supplied by the same method to the test group.

Electrode

Neutral red was immobilized to 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 following procedures. The graphite felt was soaked into 1% (w/v) of polyvinyl alcohol solution (2,000 ml) at 60°C under vacuum for 3 h, and then dried for 48 h at 100°C. The completely dried graphite felt was then soaked into pure chloroform containing 10% thionylchloride and 0.1% neutral red to induce a covalent bond between the -OH of polyvinyl alcohol and the $-NH_2$ of neutral red by dehydration in a covered container for 12 h [19], after which the graphite felt was washed with methanol to remove the unbound neutral red. In this reaction, the water-soluble polyvinyl alcohol was converted to the water-insoluble polyvinyl neutral red and the graphite felt electrode was changed to a hardened one like a plastic sponge. The polyvinyl neutral red−graphite felt complex (NR−graphite felt) was very stable, and remained unchanged after more than 10 autoclavings. A platinum wire (thickness, 0.5 mm; length, 300 mm) was employed as an anode.

Medium

All of the ingredients for medium preparation were dissolved in double-distilled water. All of the chemicals were purchased from the Korean branch of Sigma-Aldrich (Yongin City, Korea). The mineral-based medium was composed of 2 g/l NH_4Cl, 2 g/l K_2HPO_4, 50 mM $NaHCO_3$, and 2 ml of trace mineral stock solution. The trace mineral stock solution was composed of 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 $Al_2(SO_4)_3$, 0.002 g/l $MoSO_4$, and 10 mM EDTA. The mineral-based medium was effectively exposed to CO_2 by continuous sparging using a cycloid gas flow pump, by which CO_2 was naturally dissolved into carbonic acid. The natural pH of the medium was 8.5~8.6, which was neutralized to 7.5~7.7 by carbonic acid.

Bacterial Source

About 300 ml of each bacterial culture obtained from the aerobic and anaerobic digestive reactor and anaerobic wastewater treatment reactor (Jungrang Wastewater Treatment Plant, Seoul, Korea) and forest soil (300 g) containing natural compost were mixed together with 1.5 l of fresh medium and strongly stirred under an aerobic atmosphere for 60 min, in order to deactivate strictly anaerobic bacteria. The mixture was filtered with filter paper (Whatman No.1) and the filtrate was used as the bacterial source for the enrichment of CO_2-fixing bacteria.

16S rDNA Amplification

The bacterial cells were collected from the electrochemical bioreactor and NR−graphite felt using a long-needled syringe immediately after inoculation, at the 2nd, 8th, 16th, and 24th weeks of incubation time during cultivation for more than 30 weeks. 16S Ribosomal DNA was amplified *via* direct PCR using the chromosomal DNA template and 16S rDNA specific universal primers as follows: forward 5'-GAGTTGGATCCTGGCTCAG-3' and reverse 5'-AAGGAGGGGATC-CAGCC-3'. The PCR reaction mixture (50 µl) consisted of 2.5 U *Taq* polymerase, 250 µM of each dNTP, 10 mM Tris-HCl (pH 9.0), 40 mM KCl, 100 ng template, 50 pM primer, and 1.5 mM $MgCl_2$ [36]. Amplification was conducted for 30 cycles of 1 min at 95°C, 1 min of annealing at 55°C, and 2 min of extension at 72°C, using a PCR machine (T Gradient model; Biometra, Germany).

TGGE

The 16S rDNA amplified from chromosomal DNA was employed as a template for TGGE sample preparation. A variable region of 16S rDNA was amplified with forward primer (eubacteria, V3 region) 341f 5'-CCTACGGGAGGCAGCAG-3' and reverse primer (universal, V3 region) 518r 5'-ATTACCGCGGCTGCTGG-3'. A GC clamp (5'-CGCCCGCCGCGCGC-GGCGGGCGGGGCGGGGGC ACGGGGGGCCTACGGGAGGCAGCAG-3') was attached to the 5'-end of the GC341f primer [30]. The procedures for PCR and DNA sequencing were the same as the 16S rDNA amplification conditions except for an annealing temperature of 53°C. The TGGE system (Bio-Rad, Dcode; Universal Mutation Detection System, USA) was operated in accordance with the manufacturer's instructions. Aliquots (45 µl) of the PCR products were electrophoresed in gels containing 8% acrylamide, 8 M urea, and 20% formamide with a 1.5×TAE buffer system at a constant voltage of 100 V for 12.5 h and then 40 V for 0.5 h, applying a thermal gradient of 39 to 52°C [9]. Prior to electrophoresis, the gels were equilibrated to the temperature gradient for 30 to 45 min.

Amplification of TGGE Band

DNA was extracted from the TGGE band and purified with a DNA gel purification kit (Accuprep; Bioneer, Korea). The purified DNA was then amplified with the same primers and procedures used for TGGE sample preparation, except that the GC clamp was not attached to the forward primer [6].

Analysis

The CO_2 and N_2 were analyzed with a gas chromatography (Acne 6000; YoungLin, Korea) equipped with a 45/60 Carboxen-1000 packed column (30ft length×2.1 mm ID; Supelco, USA) and a thermal conductive detector. The carrier gas used was 99.999% He, the flow rate of which was adjusted to 30 ml/min. Injector and detector temperatures were adjusted precisely to 50°C and 100°C, respectively. The initial column temperature was 35°C for 5 min, and was then increased gradually to 225°C at the rate of 20°C/min. The gas sample was directly injected from the CO_2 reservoir into the injector with an automatic sampler to precisely control the injection volume. CO_2 consumption was calculated on the basis of the variation of ratio (%) of CO_2 to N_2 as follows: 25,000×(50−CO_2 ratio)÷100 ml.

The ratio of CO_2 to N_2 in the gas reservoir was calibrated with the standard curve obtained from the results of analysis of the gas mixture (20–80 of CO_2–N_2, 50–50 of CO_2–N_2, and 80–20 of CO_2–N_2).

RESULTS

Initial CO_2 Consumption

CO_2 was continuously sparged into the bacterial culture prepared in the electrochemical bioreactor, but no fresh medium was fed to the culture for the initial incubation time of 2 weeks. The initial CO_2 consumption by the bacterial community originated from different sources may indicate that the CO_2-fixing bacteria can be successfully adapted to the electrochemical bioreactor. The initial CO_2 consumption by the bacterial community grown in the electrochemical bioreactor was not activated, but the ratio of CO_2 to N_2 in the gas reservoir was reduced in proportion to the incubation time, as shown in Fig. 2. The ratio (%, v/v) of CO_2 to N_2 in the gas reservoir was gradually reduced from 50–50 to 35–38 to 62–65 at the 28th day of incubation time. This is a useful result to verify CO_2 consumption by bacteria grown in the electrochemical bioreactor without the need for isotopic $^{14}CO_2$. The CO_2-fixing bacteria are unable to fix or consume N_2 as a nitrogen source, considering the NH_4^+ contained in the mineral-based medium. The practical volume of consumed CO_2 was calculated based on the CO_2 to N_2 ratio, which was approximate 1,500 {12,500×(0.5–0.38)} ml for 28 days. Initial carbon dioxide consumption per week was approximately 350 ml during enrichment for 4 weeks, and after that, the consumption was gradually increased in proportion to the incubation time.

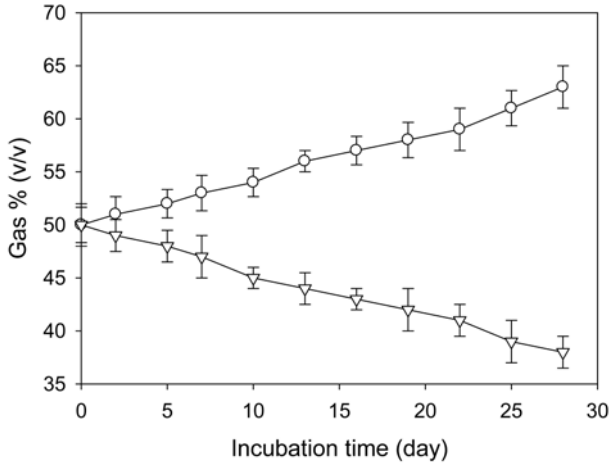

Fig. 2. Tendency of CO_2 consumption in the electrochemical bioreactor based on the variations in the ratio of CO_2 (▽) to N_2 (○) in the gas reservoir from initial time to the 28th day (4th week). The initial ratio of CO_2 to N_2 in the gas reservoir was adjusted to approximately 50±1% on a volume basis.

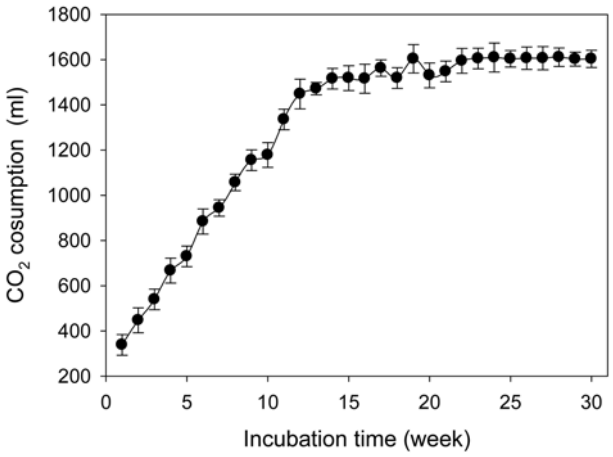

Fig. 3. Weekly consumption of CO_2 in the electrochemical bioreactor from the initial incubation time to 30 weeks.
CO_2 consumption was analyzed weekly and the gas reservoir was refilled with 50±1% of CO_2 to N_2 at 4-week intervals.

Long-Term CO_2 Consumption

The adaptation of CO_2-fixing bacteria to the electrochemical bioreactor can be predicted by initial CO_2 consumption; however, the enrichment of those bacteria cannot be predicted thusly. In order to identify the enrichment of the CO_2-fixing bacteria, the tendencies relevant to CO_2 consumption in the electrochemical bioreactor must be continuously analyzed until the consumption rate is stabilized. The CO_2 consumption was measured at intervals of 1 week and calculated based on the variations in the CO_2–N_2 ratio in the gas reservoir. The CO_2 consumption was proportional to the incubation time from the initial time to the 15th week, and then reached a maximum level of 1,600 ml per week afterward as shown in Fig. 3. This result is a clue that the CO_2-fixing bacteria may be continuously enriched and increased in the NR–graphite cathode in the electrochemical bioreactor for more than 15 weeks, after which time it stabilizes.

Variation of TGGE Pattern

The biomass of the CO_2-fixing bacteria grown in the electrochemical bioreactor was difficult to measure because the bacterial cells are selectively grown inside the NR–graphite cathode for the electrochemical redox reaction generated by contact with the electrode. However, the variations in the bacterial community growing inside the NR–graphite felt could be analyzed by TGGE of the 16S rDNA variable region. The genomic DNA was directly extracted from bacterial cells sampled from the NR–graphite cathode at the initial time, at the 2nd week when CO_2 consumption began, at the 8th week when CO_2 consumption steeply increased, at the 16th week when CO_2 consumption had reached a maximum, and at the 24th week when the CO_2 consumption tendency was stabilized. The

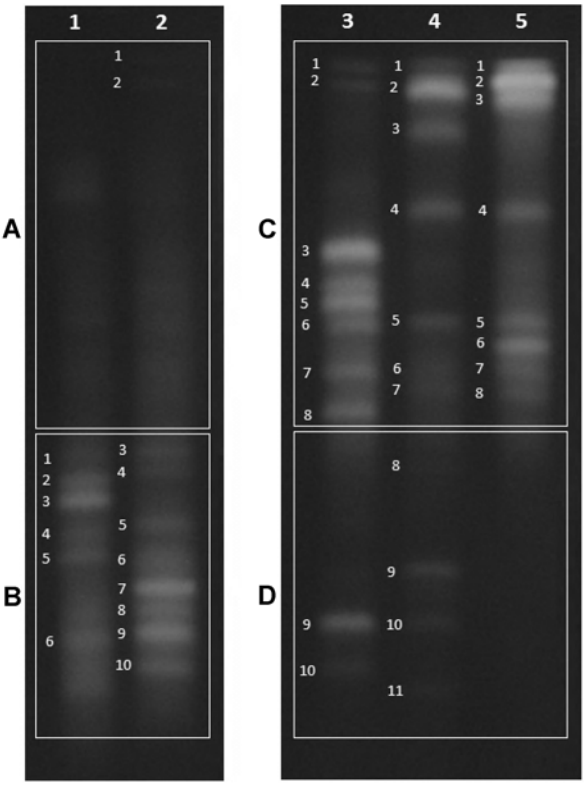

Fig. 4. TGGE patterns of 16S rDNA variable regions amplified with chromosomal DNA extracted from bacterial communities enriched in the electrochemical bioreactors.
Fifty ml of bacterial culture was isolated from the electrochemical bioreactor at the initial time immediately after inoculation (lane 1), 2nd week (lane 2), 8th week (lane 3), 16th week (lane 4), and 24th week of incubation time (lane 5).

pattern of DNA bands detected in the TGGE gel may be a clue that a bacterial community had survived or died out during enrichment but may not be an indicator that specific bacterial species predominated or were more relatively increased in the electrochemical bioreactor. DNA bands observed in the 2nd week were slightly increased in comparison with those at the initial time, as shown in box B of Fig. 4. Two very weak DNA bands were newly observed, and a slightly stronger DNA sign was observed in the 2nd week as opposed to the initial time, as shown in box A. These results demonstrated that it is possible for the bacterial community to adapt to the new environment developed by the electrochemical reduction reaction in 2 weeks, but this is not sufficient time for the bacterial community to adapt physiologically to the electrochemical reducing power. Most of the DNA bands observed at the initial time and in the 2nd week disappeared by the 8th and 16th weeks, and had completely disappeared by the 24th week as shown in boxes B and D; meanwhile, DNA observed as the sign in the 2nd week emerged as DNA bands in the 8th week as shown in boxes A and C. The DNA band pattern observed at the 24th week was more similar to that at week 16 than in the 8th week of incubation time, as shown in box C. These results demonstrate the possibility that organic nutrients contained in the initial bacterial culture may be consumed principally between the 2nd and 8th weeks of incubation time, and that the CO_2-fixing bacteria capable of converting the electrochemical reducing power to the biochemical reducing power may replace the organic nutrient-dependent heterotrophic bacteria.

Variation of Bacterial Diversity

The majority of heterotrophic bacterial species died out selectively and gradually in proportion to cultivation time in the electrochemical bioreactor using CO_2 as a sole carbon source, as shown by the TGGE patterns; however, the CO_2-fixing bacteria (*Alcaligenes* sp. and *Achromobacter* sp.) were successfully enriched, as is shown in Table 1. Information regarding the uncultured bacterial species (DNA bands 1 and 2 in lanes 2, 3, 4, and 5) that had begun to be enriched at the 2nd week of incubation time was not available from the GenBank database; however, the bacterial species were enriched and grew continuously from the 2nd week to the 24th week. Accordingly, the uncultured bacterial species are supposed to fix CO_2 in the electrochemical bioreactor. The other bacterial species, except for the CO_2-fixing bacteria observed at weeks 16 and 24, may have survived by syntrophism or may have been contaminated during bacterial sampling or DNA extraction. In particular, *Enterococcus* sp. observed abruptly at the 24th week could very easily have been the result of contamination during bacterial sampling for DNA extraction.

Bacterial Growth

The bacterial cells that have been cultivated in the NR-cathode for 7 months were grown continuously and released outside by accumulation of bacterial community inside the NR-cathode, as shown in Fig. 6. The bacterial community grown with electrochemical reducing power inside the NR-cathode may function as the producer of organic compounds; meanwhile, the bacterial cells released outside the NR-cathode may be regarded as the product that can be obtained from carbon dioxide.

DISCUSSION

Bacteria capable of growing in mineral- and carbonate-containing conditions have to physiologically regenerate biochemical reducing power from reduced inorganic compounds such as H_2, H_2S, S^o, and NH_4^+, or from solar energy [20]. Commonly, this biochemical reducing power (NADH or NADPH) is regenerated enzymatically with the electrons and protons generated by the oxidation of the reduced inorganic compounds or generated from H_2O by electromagnetic power (photosynthesis) [21]. Neutral red, whose redox

Table 1. The homologous bacterial species with the sequences of DNA extracted from TGGE bands (Fig. 3), which were identified based on the GenBank database.

Lane	Band	Genus or Species	Homology (%)	Accession No.
1	1	Uncultured *Burkholderia* sp.	98	FJ393136
	2	Groundwater biofilm bacterium	98	FJ204452
	3	*Hydrogenophaga* sp.	98	FM998722
	4	Uncultured bacterium sp.	97	HM481230
	5	*Aquamicrobium* sp.	98	GQ254286
	6	Uncultured *Actinobacterium* sp.	99	FM253013
2	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	Uncultured *Clostridim* sp.	99	FJ930072
	4	Uncultured *Polaromonas* sp.	99	HM486175
	5	Uncultured *Rhizobium* sp.	100	FM877981
	6	*Raoultella planticola*	98	EF551363
	7	Unidentified bacterium	98	AV669107
	8	Uncultured bacterium	99	HM920740
	9	Uncultured bacterium	97	GQ158957
	10	Uncultured *Klebsiella* sp.	98	GQ416299
3	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	*Enterococcus* sp.	98	DQ305313
	4	Uncultured bacterium sp.	98	HM820223
	5	*Aerosphaera taera*	99	EF111256
	6	*Alcaligenes* sp.	98	GQ383898
	7	Uncultured bacterium sp.	98	HM231340
	8	Uncultured bacterium sp.	97	FJ675330
	9	*Stenotrophomonas* sp.	98	EU635492
	10	Uncultured *Klebsiella* sp.	98	GQ416299
4	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	Uncultured bacterium sp.	98	HM575088
	4	*Alcaligenes* sp.	98	GQ200556
	5	*Alcaligenes* sp.	98	GQ383898
	6	Uncultured bacterium sp.	98	HM231340
	7	*Achromobacter* sp.	96	GQ214399
	8	Uncultured *Lactobacillales* sp.	96	HM231341
	9	Uncultured *Ochrombacterum* sp.	97	EU882419
	10	*Stenotrophomonas* sp.	98	EU635492
	11	*Tissierella* sp.	96	GQ461822
5	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	Uncultured bacterium sp.	98	HM820116
	4	*Alcaligenes* sp.	98	GQ200556
	5	*Alcaligenes* sp.	97	GQ383898
	6	*Enterococcus* sp.	99	FJ513901
	7	Uncultured bacterium sp.	98	HM231340
	8	*Achromobacter* sp.	96	GQ214399

potential is −0.325 volt *vs*. NHE, is an electron mediator capable of catalyzing the regeneration of biochemical reducing power, whose redox potential is −0.32 volt *vs*. NHE [23, 25]. The electrochemically reduced NR−graphite cathode can function as a solid electron donor, like the reduced inorganic compounds or the electromagnetic power [13, 15, 16]. Electrons and protons generated from H_2O by electrolysis on the anode surface are transferred to the

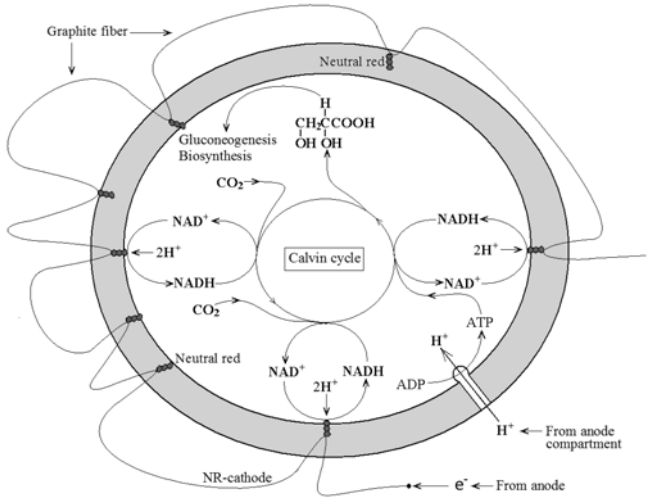

Fig. 5. Proposed mechanism of carbon dioxide fixation in bacteria capable of fixing carbon dioxide with electrochemical reducing power.

cathode through the electric circuit and cathode compartment through the membrane, respectively, and neutral red immobilized in the cathode catalyzes regeneration of NADH in the bacterial cell as shown in Fig. 5. The carbon dioxide may be assimilated into bacterial cell compounds by the Calvin cycle in the facultative anaerobic bacteria [3, 4].

Generally, an electrochemical bioreactor is composed of anode and cathode compartments separated by a heat- and pressure-labile ion-selective plastic membrane (Nafion), in which the volume and concentration of the anolyte must be balanced with the catholyte to adjust water pressure and osmotic pressure [24]. This may limit the ease with which such a two-compartmented electrochemical bioreactor can be scaled-up to a pilot- or industrial-sized reactor. The cylinder-type electrochemical bioreactor is composed of a built-in anode compartment and a heat- and pressure-resistant glass filter septum in water. In particular, the anolyte requires little special attention because it refills spontaneously from the cathode compartment through the glass filter septum (Fig. 1B) *via* the differential in water pressure between the anolyte (200 ml) and catholyte (2,000 ml). The oxygenic radical contained in the anolyte never flows backward to the cathode compartment because the anolyte has to be consumed *via* H_2O electrolysis, and the catholyte refills continuously with fresh medium. The oxidation–reduction potential of the NR–graphite cathode that was measured for the monitoring of the operation conditions was -0.45 ± 0.3 volt *vs*. Ag/AgCl, which reflects the normal generation and maintenance of the electrochemical reduction reaction.

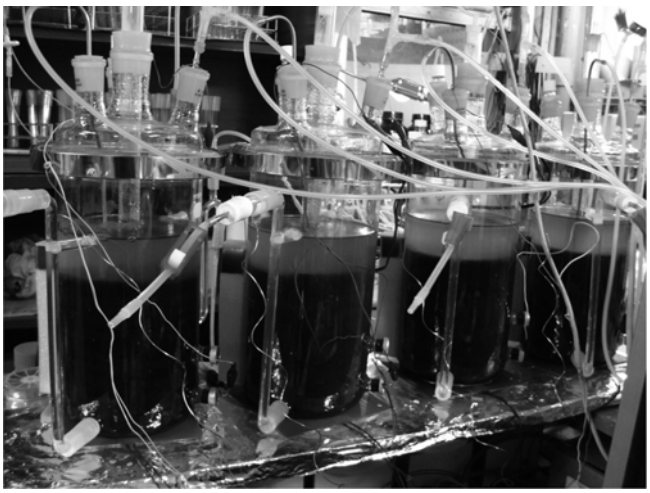

Fig. 6. The overgrown bacterial cells inside the NR-cathode was released outside of graphite fiber matrices of the NR-cathode.

The NR–graphite cathode that was electrochemically reduced by DC –3 volts of electricity catalyzed the regeneration of the biochemical reducing power required for CO_2 assimilation in the CO_2-fixing bacteria, by which *Achromobacter* sp. [12] and *Alcaligenes* sp. [10, 11, 18, 27], which are capable of autotrophically fixing CO_2, were enriched in tandem with CO_2 consumption for more than 30 weeks. The low voltage (3 volt) of DC electricity can be directly generated by the solar cells and supplied to the electrochemical bioreactor without an inverting system. The electrochemical bioreactor equipped with the NR–graphite cathode and glass filter septum can be employed in the bacterial fixation of CO_2 without limitations regarding scale-up, anolyte balance, and operational energy. The electrochemical bioreactor for bacterial CO_2-fixing can also be operated without limitations regarding installation location, seasonal variations, and energy supply; this is very unlike a phototrophic bacterial cultivation system, which absolutely requires open areas such as paddy fields, swimming pools, and salt farms for the solar light and a low latitude region for the proper temperature. The basement of a building or an underground area does not pose any problems for the installation of the electrochemical bioreactor, to which electric energy produced by solar cells can be supplied directly *via* the general technique, and may be minimally influenced by seasonal variations.

Bacterial cells grown in the electrochemical bioreactor with carbon dioxide as a sole carbon source may be the product obtained by carbon dioxide fixation. Bacterial cells are the smallest and the simplest in the organisms living in the Earth and stable structurally, which may be a useful character of the bacterial cells to be the carbon storage. The chemical or physical processes of bacterial cells are not required owing to the small-sized structure, which is a good character of a bacterial cell to be stored in a specific place, for example, the empty petroleum well. To

store the bacterial cells obtained from the electrochemical bioreactor in the empty petroleum well may be the best way to return the carbon to the original place.

In conclusion, the cultivation of CO_2-fixing bacteria in the cylinder-type electrochemical bioreactor may constitute a biotechnology that meets the requirements relevant to the reduction or removal of non-ecological atmospheric CO_2 generated from fossil fuel combustion. The cylinder-type electrochemical bioreactor, which was initially described herein, is not particularly limited in terms of scale-up, installation location, seasonal variations, and energy supply. In the future, we will attempt to develop a bioelectrochemical technology to generate useful organic compounds such as methane and ethanol from CO_2 by using the CO_2-fixing bacteria cultivated in the electrochemical reactor described herein, which again can operate without the need for electric energy generated by fossil fuel combustion.

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