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Bioelectrochemical Fixation of CO_2 using Autotrophic and Mixotrophic CO_2-Fixing Bacteria

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For the isolation of CO_2-fixing bacteria using electrochemical reducing power (ERP), a plate-type electrochemical bioreactor (PEB) was employed. Soluble neutral red (NR) was used as an electron mediator for carbonate-basal agar medium (CBAM) prepared in the PEB. For test of bacterial CO_2 fixation using the ERP, a single-compartmented electrochemical bioreactor (SCEB) was employed. NR immobilized in the graphite felt cathode (NR-cathode) was used as an electron mediator for carbonate-basal broth medium (CBBM) prepared in the SCEB. Two bacterial genera capable of electrochemically fixing CO_2 were isolated using the PEB and cultivated using the SCEB. The isolated bacterial species were 99% identified with *Achromobacter* sp. and *Alcaligenes* sp. Approximate 150 mL of CO_2 per day was consumed by 300 mL of the mixed culture of *Achromobacter* sp. and *Alcaligenes* sp. grown electrochemically in the SCEB.

Key words: CO_2-fixing bacteria, neutral red, electrochemical reducing power, *Alcaligenes* sp., *Achromobacter* sp.

Various bacteria are known to fix CO_2, which may function as a sole carbon source or as a co-carbon source. Chemoautotrophic bacteria can fix CO_2 as a sole carbon source with the biochemical reducing power (BRP: NADPH or NADH) regenerated in coupling with the oxidation of NH_4^+, S^o, or H_2[1-4]. Carboxylation is a metabolic reaction to fix CO_2 as a co-carbon source with the BRP regenerated coupled to the oxidation of organic or inorganic compounds[5,6]. The central reaction for metabolic carboxylation is regeneration of BRP, which functions as the sole driving-force for the assimilation of CO_2 into biomolecules in both autotrophic and mixotrophic organisms[7-9]. The regeneration of BRP can also be catalyzed by electrochemically reduced NR (neutral red) without resorting to enzymatic catalysis[10,11]. The electrochemically regenerated BRP can induce autotrophic or mixotrophic bacteria to assimilate CO_2 under conditions without chemical electron donors. The NR-cathode (surface area of graphite felt, 0.47 m^2, Electrosynthesis, USA) activated ethanol production in the fermentation metabolism of *Zymomonas mobilis*, the denitrification reaction in *Ochromobactrum* sp., and ammonium oxidation in *Nitrosomonas* sp.[12-14]. Goal of this research is to test the possibility that CO_2 may be fixed bioelectrochemically with the electrochemically enriched bacterial cells and the electricity obtained from the solar cell. In this research autotrophic and mixotrophic bacteria capable of growing with electrochemical reducing power and CO_2 were isolated using the PEB and an electrochemical bioreactor for cultivation of CO_2-fixing bacteria was designed and employed.

The CBAM (carbonate-basal agar medium) was composed of 2 g/L NH_4Cl, 2 g/L K_2HPO_4, 50 mM $NaHCO_3$, 200 μM NR, 2 mL trace mineral stock solution, and 20 g/L agar. The CBBM (carbonate-basal broth medium) was prepared by exclusion of agar. 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

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$ZnSO_4$, 0.002 g/L $Al_2(SO_4)_3$, 0.002 g/L $MoSO_4$, 10 mM EDTA. The CBAM was prepared in the PEB (plate-type electrochemical bioreactor) (Fig. 1A), of which anode compartment and anode function as the donor of proton and electron for the NR reduction (Fig. 1B). The PEB was incubated in the CO_2-incubator that was isolated completely from outside, to which 10,000 mL of gas reservoir filled with 99.99% CO_2 was connected, and the oxygen generated in the anode compartment was ventilated outside of the CO_2-incubator (Fig. 2). The CBBM was prepared in the SCEB (single-compartmented electrochemical bioreactor) (Fig. 3A) whose

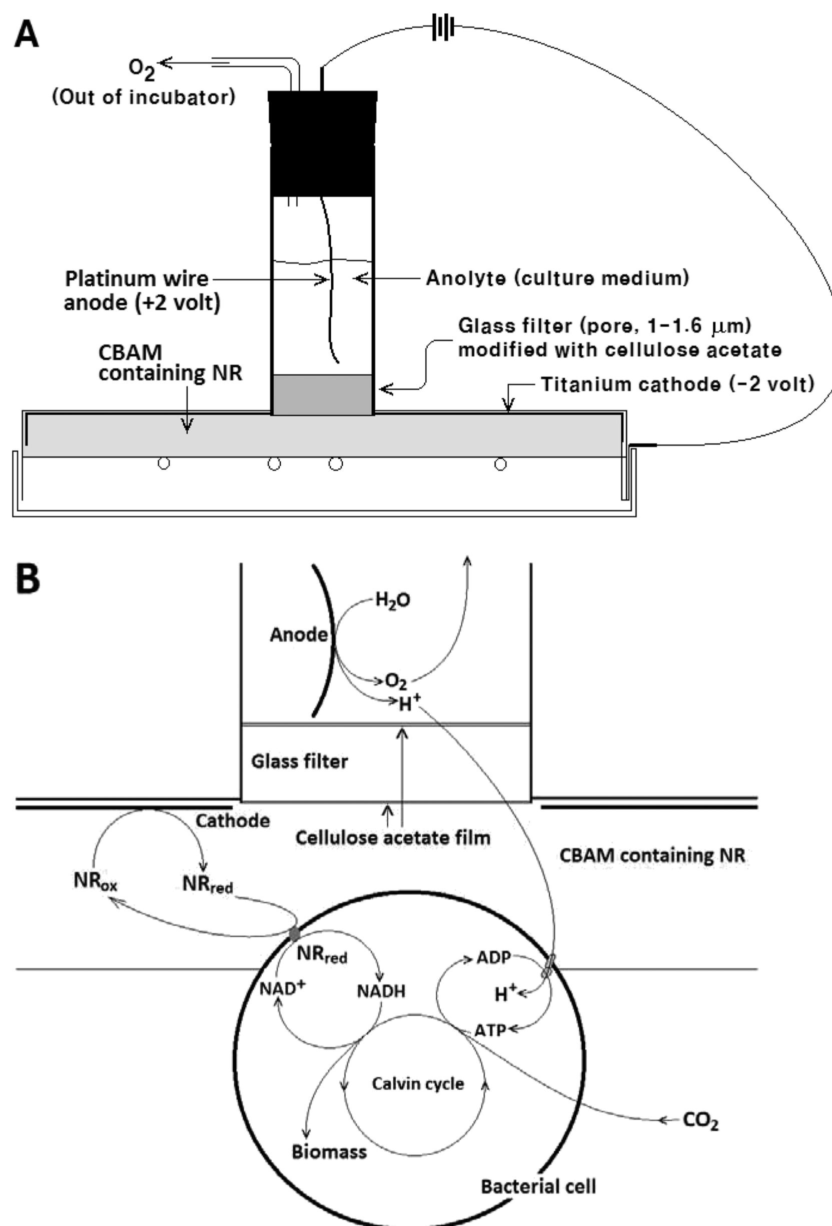

Fig. 1. Schematic structure of a plate-type electrochemical bioreactor for solid culture using CBAM (A) and proposed mechanism for explanation of bacterial growth with the biochemical reducing power (NADH) and free energy (ATP) generated from the electrochemically reduced NR (B). NR_{ox} and NR_{red} indicate oxidized NR in coupling with reduction of NADH and reduced NR by ERP, respectively.

anode was composed of cellulose acetate film, porous ceramic membrane, and porous carbon plate. The electrons and protons that were generated by electrolysis of water inside the porous carbon anode are the driving-force for regeneration of BRP by catalysis of NR-cathode (Fig. 3B).

50 mL of aerobic wastewater treatment reactant, 50 mL of anaerobic treatment reactant, and 10 g of forest soil were mixed thoroughly and then filtered with filter paper (Whatman No. 1) without vacuum inhalation under aerobic atmosphere. The filtered mixed bacterial community was 10 times diluted with the fresh saline and spread on CBAM prepared in the PEB, which was incubated in the CO_2-incubator at 25°C for 10 days. DC -2 volts of electricity were charged to the titanium cathode to induce electrochemical reduction of NR contained in the CBAM. The mixed bacterial community and the bacterial colonies grown electrochemically on the CBAM were analyzed separately by the pyrosequencing technique. The pyrosequencing was performed in Macrogen Bioinformatics Research Institute (Seoul, Korea) by Turnkey-base project. They provided us all of the sequencing, identification, and statistical data for community analysis. The classifiable sequences obtained by the pyrosequencing were identified based on the Ribosomal Database Project (RDP), and defined at the 100% sequence homologous level.

4,870 quality sequences with an average read length of 601 bp and a range of 150-800 bp were obtained from the mixed bacterial community. Of these sequences, 1,775 sequences were able to be classified at level of 100% homologous with the specific bacterial genus. The most abundant sequences (17.96%) obtained from the mixed bacterial community was identified as *Brevundimonas* sp., and the relative abundance of sequences identified with *Alcaligenes* sp. and *Achromobcter* sp. that are capable of fixing CO_2 was 0.98 and 0.12%, respectively, as shown in Table 1. Meanwhile, 5,129 quality sequences with an average read length of 599 bp and a range of 150-800 bp were obtained from the bacterial colonies grown on the CBAM cultivated in the PEB. Of these sequences, 4,986 sequences were able to be classified at the level of 100% homologous with the specific bacterial genus. The most abundant se-

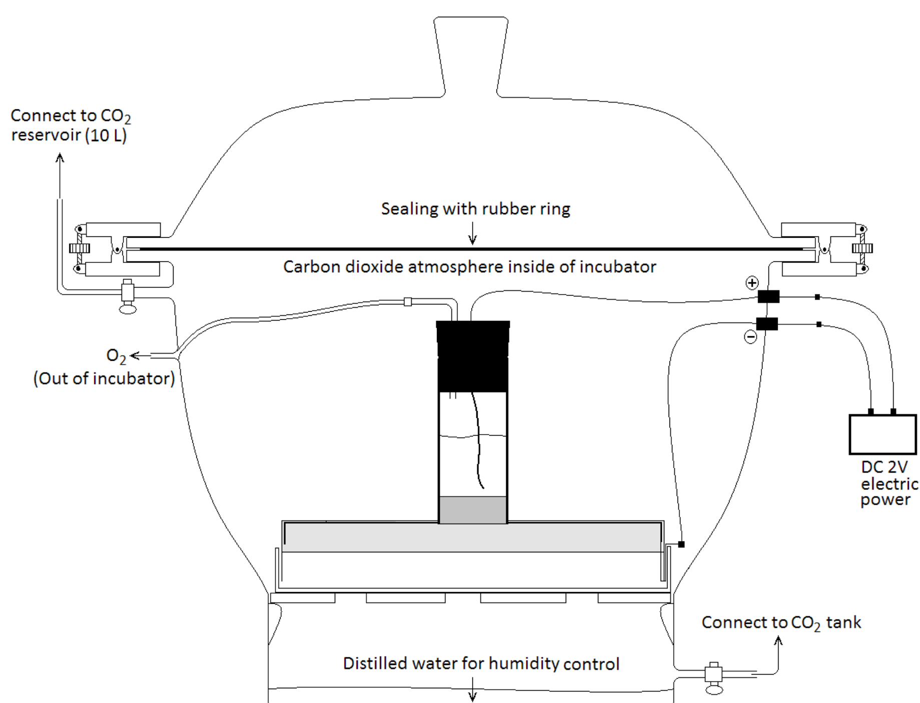

Fig. 2. Schematic structure of incubator for cultivation of CO_2-fixing bacteria with the electrochemical reducing power and CO_2.

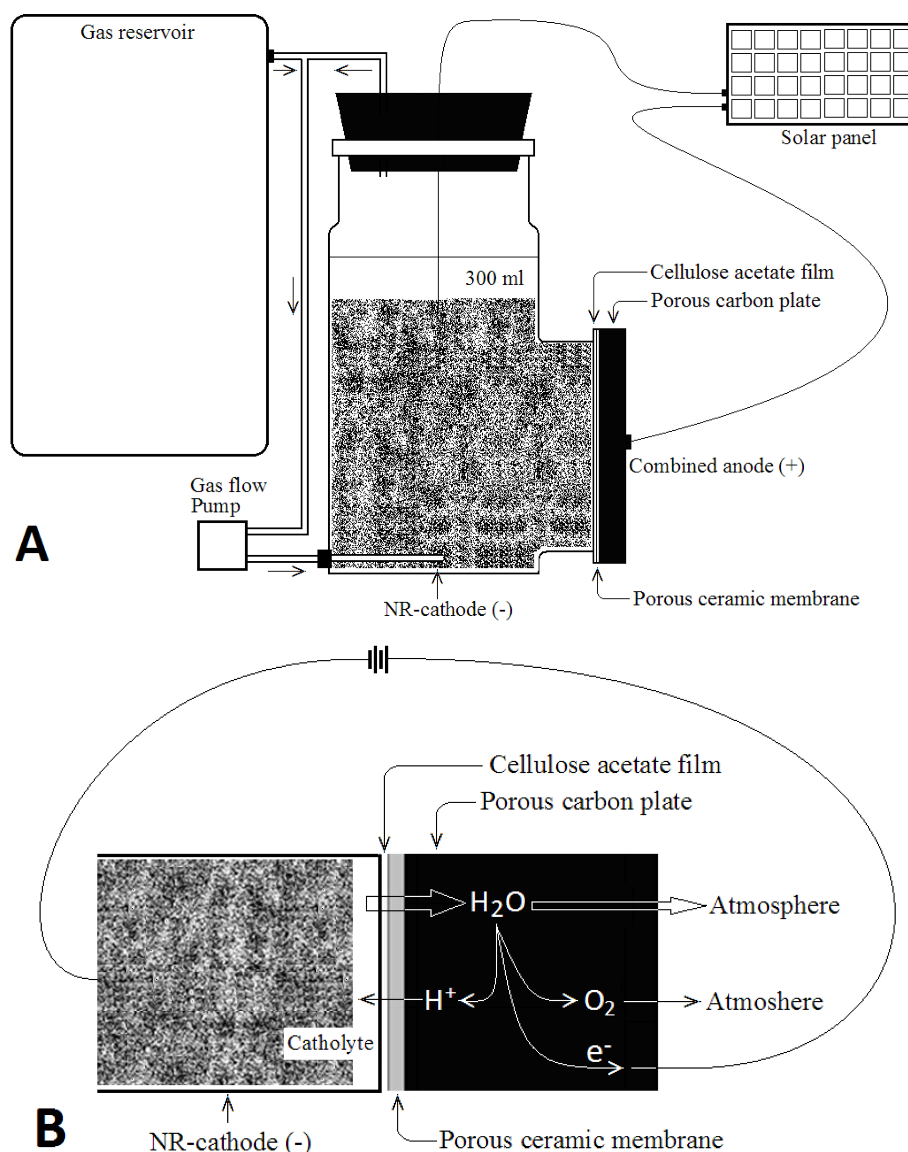

Fig. 3. Schematic structure of a single-compartmented electrochemical bioreactor (SCEB). The three-layered anode functions as a generator of electron and proton by electrolysis of H_2O. NR-cathode functions as both a catalyst and an electron donor for regeneration of biochemical reducing power.

quences (43.83%) obtained from the bacterial colonies grown on CBAM was identified as *Achromobacter* sp., and the most classifiable sequences were also identified as *Achromobacter* sp. and *Alcaligenes* sp. These results are clue that the heterotrophic bacteria originated from the mixed bacterial community were died out but CO_2-fixing bacterial community was proliferated selectively during cultivated electrochemically on CBAM. Five of colonies grown electrochemically on the CBAM was transferred separately on the fresh CBAM containing 0.1 g/L of yeast extract and cultivated electrochemically under CO_2 atmosphere for 10 days. The chromosomal DNA was extracted directly from the bacterial colonies electrochemically grown on the fresh CBAM. 16S ribosomal DNA was amplified via direct PCR using the chromosomal DNA template and 16S-rDNA specific universal primers as follows: forward 5'-GAGTTG-GATCCTGGCTCAG-3' and reverse 5'-AAGGAGGGG-

Table 1. Relative abundances of dominant bacterial taxa in the mixed bacterial community and the bacterial community grown electrochemically and selectively on the CBAM prepared in PEB. The relative abundances were estimated from the proportion of classifiable sequences that are 100% homology with the specific bacterial genus

Mixed bacterial community				Bacterial community grown on CBAM			
Classifiable sequences	Abundance (%)	Bacterial genus	Homology (%)	Classifiable sequences	Abundance (%)	Bacterial genus	Homology (%)
876	17.96	*Brevundimonas*	100	2248	43.83	*Achromobacter*	100
153	3.14	*Pseudomonas*	100	748	14.58	*Achromobacter*	100
111	2.28	*Hydrogenophaga*	100	595	5.87	*Stenotrophomonas*	100
99	2.03	*Delftia*	100	301	2.28	*Achromobacter*	100
86	1.76	*Stenotrophomonas*	100	263	1.77	*Achromobacter*	100
70	1.44	*Pseudomonas*	100	219	1.23	*Achromobacter*	100
53	1.09	*Parvibaculum*	100	117	0.90	*Achromobacter*	100
52	1.07	*Brevundimonas*	100	91	0.66	*Achromobacter*	100
48	0.98	*Alcaligenes*	100	63	0.57	*Alcaligenes*	100
32	0.66	*Comamonas*	100	46	0.53	*Achromobacter*	100
31	0.64	*Bacillus*	100	34	0.49	*Achromobacter*	100
26	0.53	*Bosea*	100	29	0.49	*Castellaniella*	100
21	0.43	*Devosia*	100	27	0.45	*Achromobacter*	100
17	0.35	*Acidovorax*	100	25	0.45	*Achromobacter*	100
12	0.25	*Brevundimonas*	100	25	0.39	*Stenotrophomonas*	100
12	0.25	*Sphaerobacter*	100	23	0.16	*Achromobacter*	100
11	0.23	*Brevundimonas*	100	23	0.14	*Alcaligenes*	100
9	0.18	*Acinetobacter*	100	20	0.12	*Achromobacter*	100
9	0.18	*Sphaerobacter*	100	14	0.10	*Alcaligenes*	100
8	0.16	*Brevundimonas*	100	14	0.10	*Pseudomonas*	100
7	0.14	*Hyphomicrobium*	100	11	0.08	*Achromobacter*	100
7	0.14	*Thermomonas*	100	10	0.08	*Achromobacter*	100
6	0.12	*Achromobacter*	100	8	0.06	*Achromobacter*	100
6	0.12	*Brevundimonas*	100	7	0.06	*Achromobacter*	100
4	0.10	*Devosia*	100	7	0.06	*Achromobacter*	100
3	0.08	*Pseudoxanthomonas*	100	6	0.04	*Alcaligenes*	100
3	0.06	*Castellaniella*	100	6	0.04	*Achromobacter*	100
3	0.06	*Gordonia*	100	6	0.04	*Achromobacter*	100

ATCCAGCC-3'. The PCR reaction mixture (50 μL) consisted of 2.5U Tag 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 $MgCl_2$. 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, Biometera, German). The amplified 16S-rDNA was sequenced by request to a professional sequencing system (Macrogen, Seoul, Korea). The species-specific identity of the amplified 16S-rDNA was determined on the basis of sequence homology, according to the information in the GenBank database system. *Alcaligenes* sp. and *Achromobcter* sp. that were isolated finally from single colony grown on the CBAM was cultivated mixedly in 300 mL of CBBM prepared in the SCEB (Fig. 2A), to which the 5,000 mL of gas reservoir filled with 50% N_2-CO_2 was connected. 100 mL of the gas mixture per min was sparged circularly into the CBBM to activate dissolution of CO_2. The CO_2 consumed by bacteria in the SCEB was supplied spontaneously from the gas reservoir. DC -2 V of electricity that was generated from the solar panel was charged to the NR-cathode to induce generation of ERP. Bacterial cells were grown inside of the NR-cathode from initial to 8th week of incubation time, after that, bacteria grown inside the NR-cathode were

begun to be released into medium. The bacterial cells released from NR-cathode were compared by optical density at 660 nm. The optical density of the released bacterial cells grown in CBBM without ERP, with ERP, and with both ERP and 0.1% yeast extract was 0.02, 0.07, and 0.19, respectively, at 16th week of incubation time, and 0.02, 0.12, and 0.34, respectively, at 24th week of incubation time. However, the bacterial cells grown inside of NR-cathode were impossible to be measured optically. CO_2 consumption was analyzed quantitatively based on the volume and N_2-CO_2 ratio of the residual gas in the gas reservoir with a gas flow meter (GMATE2000A, Lokas, Korea) and gas chromatography (Acme 6000, Young-Lin, Korea) at intervals of 1 week from 4th week of incubation time after the mixture of *Alcaligenes* sp. and *Achromobcter* sp. was inoculated into the SCEB. The N_2-CO_2 ratio of the residual gas was analysed qualitatively and quantitatively by gas chromatography equipped with 45/60 Carboxen-1000 packed column (30 ft length × 2.1 mm ID, Supelco, USA) and thermal conductive detector. A carrier gas was 99.999% He, of which flow rates were adjusted to 30 mL/min. Injector and detector temperatures were precisely adjusted to 50°C and 100°C, respectively. Initial column temperature was 35°C for 5 min then gradually increased to 225°C at the level of 20°C per min. Gas sample was injected directly from the gas reservoir into the injector by automatic sampler to control precisely the injection volume. CO_2 consumption by mixed culture of *Achromobacter* spp. and *Alcaligenes* spp. grown in the SCEB was maximal 950-1,050 mL per week when 0.1% yeast extract was added to the CBBM; meanwhile, maximal 400 mL per week in the condition without yeast extract shown as shown in Fig. 3. However, the CO_2 consumption was not observed in the SCEB without ERP.

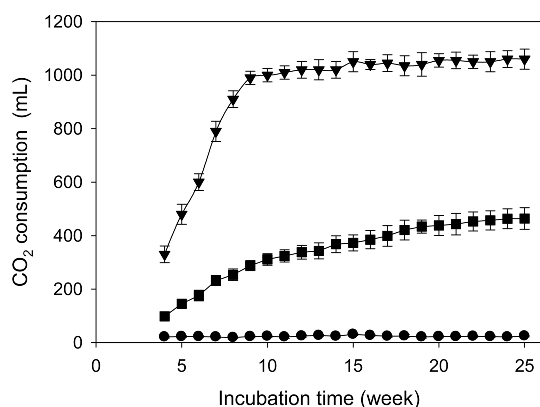

Fig. 4. Variations of CO_2 consumed by mixed culture of *Achromobacter* spp. and *Alkaligenes* sp. grown CBBM prepared in the SCEB without the ERP (●), with ERP (■), and with both the ERP and 0.1% (w/v) of yeast extract (▼). The error bars indicate the standard deviation that were calculated with data obtained from triple tests.

The majority of facultative chemoautotrophs are heterotrophs which preferentially oxidize organic substrates or grow mixotrophically with CO (or H_2) and organic compounds[15,16]. The ERP may function as the electron donor like CO or H_2 and CO_2 may function as the electron acceptor but yeast extract may function as the building block like organic compounds. The *Achromobacter* sp. described in a previous research was a facultative chemoautotroph[17]. According to the reports, *Alcaligenes* sp. is capable of growing autotrophically with a gas mixture of H_2, CO_2, and O_2, as well as heterotrophically under air on a broad variety of organic substrates[18-21]. *Alcaligenes* sp. was reported to oxidize metabolically H_2 to regenerate the BRP during autotrophic growth under an H_2-CO_2 atmosphere[22]. Meanwhile, both *Achromobacter* sp. and *Alcaligenes* sp. grew autotrophically with ERP and NH_4^+ or mixotrophically with ERP and yeast extract under a CO_2 atmosphere in this study.

Conclusively, the atmospheric CO_2 may be more effectively fixed by the mixotrophic growth than autotrophic growth of *Achromobacter* sp. and *Alcaligenes* sp. because bacterial growth rates are higher in the mixotrophic than autotrophic condition and the efficiency of CO_2 fixation is proportional to the bacterial growth rates. Sewage wastewater that is generated from human living activities contains various organic carbon and nitrogen, which may be a proper medium for mixotrophic growth of *Achromobacter* sp. and *Alcaligenes* sp.in the condition with ERP as the electron donor. Cultivation of *Achromobacter* sp. and *Alcaligenes* sp. using the sewage wastewater medium, ERP and CO_2 in the electrochemical bioreactor installed in a basement may

be the best way to produce stable organic carbons from wastewater and atmospheric CO_2 without combustion of fossil fuel. Cyanobacteria and green algae have been studied with the purpose of CO_2 fixation with the solar energy but are impossible to substitute for plants that are trees and grasses because they have to cultivated in the natural habitats for plants. Practically, ability of plants for CO_2 fixation per unit area is much higher than the cyanobacteria or green algae in the natural habitats.

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References

1. B. Alber, M. Olinger, A. Reider, D. Kockelkorn, B. Jobst, M. Hügler, and G. Fuchs, *J. Bacteriol*, **2006**, 188, 8551-8559.
2. U. Jahn, H. Huber, W. Eisenreich, M. Hügler, and G. Fuchs, *J. Bacteriol*. **2007**, 189, 4108-4119.
3. W.H. Ramos-Vera, I.A. Berg, and G. Fuchs,*J. Bacteriol,* **2009**, 191, 4286-4297.
4. S. Schouten, M. Strous, M.M.M. Kuypers, W.I.C. Rijpstra, M. Bass, C.J. Schubert, M.S.M. Jetten, and J.S.S. Damsté, *Appl. Environ. Microbiol*, **2004**, 70, 3785-3788.
5. F.J. Small and S.A. Ensign, *J. Bacteriol*, **1995**, 177, 6170-6175.
6. R.K. Thauer, K. Jungermann, and K. Decker, *Bacteriol. Rev,* **1977**, 41, 100-180.
7. R.R. Cogdell, N.W. Isaacs, T.D. Howard, K. McLuskey, N.J. Fraser, and S.M. Prince, *J. Bacteriol*, **1999**, 181, 3869-3879.
8. N. Ohmura, K. Sasaki, N. Matsumoto, and H. Saiki, *J. Bacteriol*, **2002**, 184, 2081-2087.
9. F.R. Tabita, *Microbiol. Rev,* **1988**, 52, 155-189.
10. D.H. Park, M. Laivenieks, M.V. Guettler, M.K. Jain, J.G. Zeikus, *Appl. Environ. Microbiol*, **1999**, 65, 2912-1917.
11. D.H. Park and J.G. Zeikus, *J. Bacteriol*, **1999**, 181, 2403-2410.
12. B.Y. Jeon, T.S. Hwang, and D.H. Park, *J. Microbiol. Biotechnol*. **2009**, 19, 666-674.
13. B.Y. Jeon, H.N. Seo, S.W. Kang, and D.H. Park, *J. Microbiol. Biotechnol*. **2010**, 20, 485-493.
14. W.J. Lee and D.H. Park, *J. Microbiol. Biotechnol.* **2009**, 19, 836-844.
15. I.R. Hamilton, R.J. Burris, P.W. Wilson, and C.H. Wang, *J. Bacteriol*, **1965**, 89, 647-653.
16. O. Meyer, K. Frunzke, D. Gadkari, S. Jacobitz, I. Hugendieck, and M. Karaut, *FEMS Microbiol. Rev,* **1990**, 87, 253-260.
17. A.K. Romanova, A.V. Nozhevnikova, J.G. Leonthev, and S.A. Alekseeva, *Microbiology,* **1977**, 46, 719-722.
18. C.M. Doyle, and D.J. Arp, *J. Bacteriol*, **1987**, 169, 4463-4468.
19. A. Freter and B. Bowien, *J. Bacteriol*, **1994**, 76, 5401-5408.
20. L. Leadbeater and B. Bowien, *J. Bacteriol*, **1984**, 157, 95-99.
21. I. Reutz, P. Schobert, and B. Bowien, *J. Bacteriol*, **1982**, 151, 8-14.
22. C. Hogrefe, D. Römermann, and B. Friedrich, *J. Bacteriol.* **1984**, 158, 43-48.