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Electrochemical Control of Metabolic Flux of *Weissella kimchii* sk10: Neutral Red Immobilized in Cytoplasmic Membrane as Electron Channel

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Abstract Electrochemical control of the metabolic flux of *W. kimchii* sk10 on glucose and pyruvate was studied. The growing cell of *W. kimchii* sk10 produced 87.4 mM lactate, 69.3 mM ethanol, and 4.9 mM lactate from 83.1 mM glucose under oxidation condition of the anode compartment, but 98.9 mM lactate, 84.3 mM ethanol, and 0.2 mM acetate were produced from 90.8 mM glucose under reduction condition of the cathode compartment for 24 h, respectively. The resting cell of *W. kimchii* sk10 produced 15.9 mM lactate and 15.2 mM acetate from 32.1 mM pyruvate under oxidation condition of the anode compartment, and 71.3 mM lactate and 3.8 mM acetate from 79.8 mM pyruvate under reduction condition of the cathode compartment. The redox balance (NADH/NAD^+) of metabolites electrochemically produced from pyruvate was 1.05 and 18.76 under oxidation and reduction conditions, respectively. On the basis of these results, we suggest that the neutral red (NR) immobilized in bacterial membrane can function as an electron channel for the electron transfer between electrode and cytoplasm without dissipation of membrane potential, and that the bacterial fermentation of *W. kimchii* sk10 can be shifted to oxidized or reduced pathways by the electrochemical oxidation or reduction, respectively.

Key words: *Weissella kimchii*, metabolic flux shift, electrochemical oxidation-reduction (redox), NR_{red} (reduced form of neutral red), NR_{ox} (oxidized form of neutral red)

The various energy sources such as light, reduced inorganic compounds, and organic carbons can be utilized by bacteria. The light energy acts as a source for the electron-driving force, which is converted to the proton motive force and the reducing powers NAD(P)H, and other energy sources are oxidized in coupling with reduction of the primary electron donor NAD^+ or $NADP^+$. In the microbial respiratory metabolism, the reducing power produced by the electron-driving force is directly proportional to the $\Delta E_o'$ value between the initial electron donor and the final electron acceptor [26] and, in the fermentative metabolism, the reducing power produced in coupling with oxidation of substrate is re-oxidized in coupling with reduction of the metabolic intermediate, such as pyruvate to lactate. The redox enzymes involved in the bacterial catabolism, such as hydrogenase, dehydrogenase, or reductase, can catalyze the oxidation-reduction of NAD^+ or quinone, and are in coupling with the oxidation-reduction of the artificial electron mediator (e.g., neutral red or benzyl viologen) [1, 3, 9, 18, 31]. This reaction is coupled to the generation of transmembrane proton gradient, which is used by the organism to support growth and metabolic function [13, 32]. Application of the oxidation-reduction characteristics of biological systems by electrochemical techniques is useful to control the metabolic flux. A useful electron mediator for the bioelectrochemical systems must easily react with all of the electrode, biological electron carriers, and electron mediator [34]. The various redox enzymes, including nitrite reductase [29], nitrate reductase [30], fumarate reductase [24], and hydrogenase [23], and various biological electron carriers such as NAD^+ [17, 25], quinone [22], and cytochromes [33], have been reported to electrochemically react with the electron mediator such as NR [4, 10, 18] *in vivo*. Ever since Park and Zeikus [18] found NR to be able to catalyze the redox reaction of NAD^+/NADH without enzymes and mediate electron transfer between electrode and bacterial cells, NR has been applied to the biofuel cell [19, 20], but not to metabolic flux control of bacterial cell. We have been interested in the electro-energization of living systems driven by the electrochemical energy. Therefore, we applied NR as an electron mediator to an electro-energization system to control electrochemical metabolic flux. Electrons are funneled from the cathode to a metabolic intermediate in the fermentative pathway of the bacterial cell through the oxidation-reduction of an electron mediator

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and an electron carrier. In this process, the electrochemically reduced electron mediator can be re-oxidized in coupling with reduction of NAD^+ to NADH, and the NADH is re-oxidized in coupling with reduction of pyruvate to lactate or fumarate to succinate [6, 7]. On the other hand, the electrons are also transferred from a metabolic intermediate to the electrode. In this process, NAD^+ can be reduced to NADH in coupling with oxidation of a metabolic intermediate and the NADH can be re-oxidized coupled to reduction of NR, which can be re-oxidized by transferring electrons to the electrode. In other words, some metabolic intermediates can be electrochemically reduced or oxidized [19]. This implies that it might be possible to control or alter metabolism by plugging biochemical processes into an external electrochemical system. Collins *et al.* [2] proposed the genus *Weissella*, on the basis of a 16s rRNA phylogenetic analysis, as a non-spore-forming heterofermentative bacterium. Park and Park [21] and Kang and Park [8] reported that the metabolic flux of *W. kimchii* could be shifted to oxidized pathway under aerobic, but reduced pathway under anaerobic, growth condition. In the present study, we attempted to electrochemically alter the metabolic flux of *W. kimchii* by using NR as an electron mediator. Thus, we estimated the growth and metabolites of *W. kimchii* sk10 in anode (oxidation reaction) and cathode compartments (reduction reaction) under anaerobic growth condition, respectively, and analyzed the metabolic flux by using stoichiometric balance of the metabolites produced and the substrate consumed [29].

MATERIALS AND METHODS

Chemicals and Reproducibility of Results

All chemicals were of reagent grade, and all the experiments were repeated two to three times with identical results.

Organism

The strain sk10 was isolated from kimchi and identified as *W. kimchii* on the basis of 16s-rDNA sequencing and sugar fermentation profile [1].

Cultivation and Growth

Strain sk10 was cultivated on modified MRS medium (proteose peptone 10 g/l, beef ext 10 g/l, yeast ext 5 g/l, sorbitan monooleate 1 g/l, ammonium citrate 2 g/l, magnesium sulfate 0.1 g/l, manganese sulfate 0.05 g/l, disodium phosphate 2 g/l). Glucose and pyruvate were added to the medium after autoclave, and final concentration was adjusted to 100 mM. The pH of the medium was adjusted to 8.0 before autoclave, and bacterium was cultivated at 30°C. Bacterial growth was monitored by optical density at 660 nm with a spectrophotometer (Jasco Model V-550, Tokyo, Japan). O_2-free N_2 was supplied to the bacterial culture and sparged in growth medium to maintain complete anaerobic condition [11, 12].

Cyclic Voltammetry of Neutral Red

Cyclic voltammograms of NR were obtained by using the glassy carbon (diameter 5 mm; Electrosynthesis, U.S.A.) as a working electrode, platinum wire as a counter electrode, and Ag/AgCl as a reference electrode in 200 μM NR solutions in 25 mM Tris-Cl buffer (pH 7.5). Cyclic voltammetry was performed using a cyclic voltammetric potential (model CV50W, BAS, U.S.A.) linked to an IBM personal computer data acquisition system. Prior to use, the electrodes were cleaned with ultrasonic cleaner. The scanning rate was adjusted to 25 mVs^{-1} over the range of 0.0 volt to - 1.0 volt. To test electron transfer between NAD^+ and NR, the NAD^+ was added to the NR solution during the cyclic voltammetry, and transition of the cyclic voltammogram was observed and compared before the addition of NAD^+.

Electrochemical Bioreactor

An electrochemical bioreactor (working volume: 50- 200 ml), specially designed for autoclaving, maintaining anaerobic conditions, and growing bacteria, was made of Pyrex glass. The bioreactor was separated into anode and cathode compartments by a porcelain membrane (diameter ϕ= 52 mm, thickness=3.2 mm, and resistance=15 Ω cm^{-2} in 0.25 N NaOH). A platinum wire (ϕ=0.5 mm; <1.0 Ω cm^{-2}) was connected to the graphite felt with graphite epoxy (<1.0 Ω cm^{-2}; Electrosynthesis, Amherst, New York, U.S.A.), and the theoretical surface area of graphite felt electrode was adjusted to 1.9 m^2. The electrical resistance between anode and cathode was <0.4 KΩ The current and voltage between anode and cathode were measured by a precision multimeter (model 45; Fluke, Everett, WA, U.S.A.) and adjusted to 1.0 to 10.0 mA and 2.0 volt, respectively. The growth medium, containing 50 mM phosphate buffer (pH 7.0), was used as the catholyte, and 200 mM phosphate buffer (pH 7.0) with 200 mM NaCl was used as the anolyte. The growing cells and resting cells were used for the test of the metabolic flux shift. Five % (v/v) pre-cultivated bacterial culture for 24 h was used as an inoculum for the growing cells and 200 μM NR was added to the bacterial culture after inoculation, which was spontaneously immobilized to the bacterial membrane [4]. The harvested bacterial cells were used as the resting cells. The resting cells were prepared as follows: a 18-h *W. kimchii* culture was aseptically harvested and washed twice with 50 mM phosphate buffer (pH 7.0) by centrifugation at 5,000 ×*g* and 4°C for 30 min. For immobilization of NR in the cytoplasmic membrane of the resting cells, 500 μM NR was added to the harvested bacterial cells and the mixture of NR and bacterial suspension was shaken at 80 rpm and 4°C for 30 min in a reciprocal shaker. The residual NR, which was not immobilized in the bacterial membrane, was removed by centrifugation. The modified resting cells with NR were resuspended in fresh medium, and bacterial density was adjusted to 4.0 on the basis of optical density at 660 nm.

Analysis

Glucose, pyruvate, lactate, acetate, and ethanol were analyzed by using HPLC (YoungLin system M925 pump, Seoul Korea) equipped with an RI detector (RI750F model) and Aminex HPX-87H ion-exchange column (Bio-Rad, Burlington, U.S.A.). The bacterial culture, which was periodically separated from bacterial-growing medium, was centrifuged at 10,000 ×*g* for 30 min and filtrated through a membrane filter (pore sized, 0.22 μM), and the filtrate was used for analysis. The concentration was calculated using a standard calibration curve that was previously made.

Calculation of Stoichiometric Balance

The bacterial growth was measured at 2-h intervals for 48 h, together with measurment of substrate consumption and metabolite production. Stoichiometric balance was calculated at mid-time of the stationary phase when all factors, biomass (dry cell weight), and substrate and metabolites concentrations were unchanged. Dry cell weight was determined by using a predetermined calibration curve as a function of absorbance at 660 nm [5].

$$\text{Dry cell weight (g/l)} = 0.329 \times A_{660}$$

Results and Discussion

The NR can be electrochemically oxidized and reduced [18], and also biochemically reduced by coupling to oxidation of NADH to NAD^+, and oxidized by coupling to reduction of NAD^+ to NADH [19]. The redox potential of NR_{red}/NR_{ox} and $NADH/NAD^+$ are -0.325 and -0.32 volt (vs. NHE), respectively, by which electrons can be transferred from NR_{red} to NAD^+ or NADH to NR_{ox} with low variation of entropy (-0.325+0.32=-0.005; -0.005 volt×96487 J/volt= 482.44 J/mol). The cyclic voltammetry is a useful tool for determination of experimental redox potential of the electrode-active compounds or materials and electron transfer between electron mediators and electron carriers. As shown in Fig. 1, the cyclic voltammogram shows the experimental value of the redox potential of NR. The theoretical redox potential of NR is -0.325 volt (vs. NHE), and experimentally -0.33 volt (vs. NHE) was obtained, which is very close to the theoretical value. The upper and lower peaks show the reduction and oxidation reaction of NR with electrode, respectively. Figure 2 shows the electron transfer from NR_{red} (upper peak), which was electrochemically reduced, to NAD^+ and NADH to NR_{ox} (lower peak), which was electrochemically oxidized. The increase of the upper and lower peak height indicates that the electrons can be transferred from electrode to NAD^+ and from NADH to electrode via oxidation-reduction of the NR, respectively. This is clear evidence that the NR_{red} can be oxidized to NR_{ox}, coupled to reduction of NAD^+ to NADH, but NADH

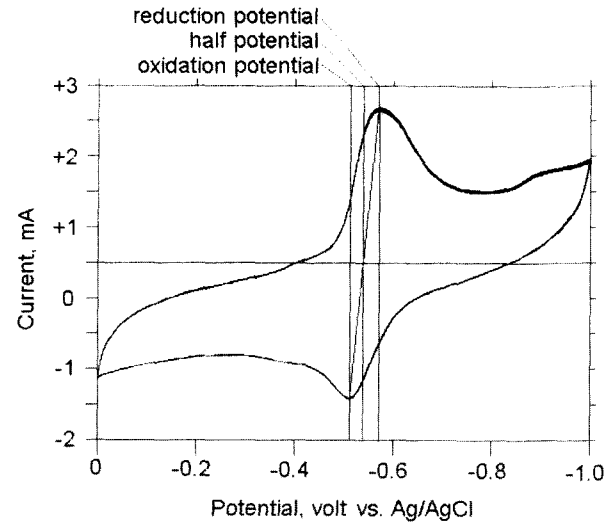

Fig. 1. Cyclic voltammogram of neutral red (100 μM in 50 mM KPi buffer, pH 7.0) for determination of the redox potential under test condition.

The half potential is a real redox potential of neutral red. The theoretical and experimental redox potentials of neutral red are -0.525 volt versus Ag/AgCl (-0.325 volt versus NHE) and -0.530 volt versus Ag/AgCl (-0.33 volt versus NHE), respectively.

can be re-oxidized to NAD^+, coupled to reduction of NR_{ox} to NR_{red} on the electrode surface. As shown in Fig. 3 and Fig. 4, this result led us to suggest that the NR can act as an electron channel across the bacterial membrane from inside to outside or in reversed direction. The electrons cannot be transferred across the bacterial membrane in the normal

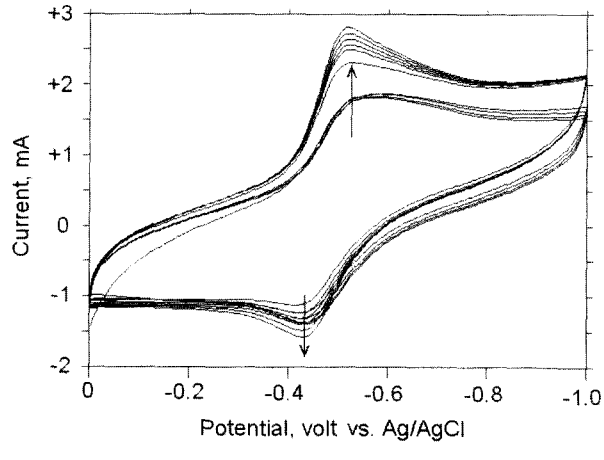

Fig. 2. Cyclic voltammogram of neutral red (100 μM in 50 mM KPi buffer, pH 7.0) for test of reaction with NAD^+ under test condition.

By the addition of NAD^+ (100 μM), the current was increased in both reduction reaction (upper peak) and oxidation reaction (lower peak). The increase of upper and lower current shows that electrons are transferred from electrode to NAD^+ and from NADH to electrode through oxidation-reduction of neutral red, respectively. The electrochemical oxidation-reduction of NAD^+-NADH, coupled to oxidation-reduction of neutral red, was reported by Park and Zeikus [2, 21].

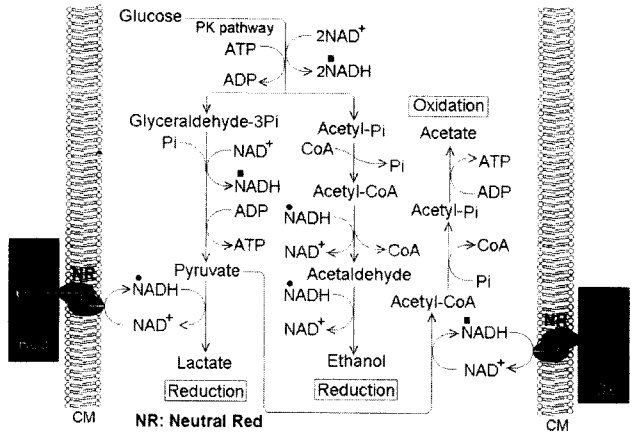

Fig. 3. Schematic mechanism for the oxidation or reduction reaction of the metabolic intermediates, coupling to oxidoreduction of NAD^+/NADH, which is electrochemically oxidized and reduced.
The neutral red immobilized in cytoplasmic membrane (CM) is to function as an electron mediator (channel) from cathode (-) to NAD^+ or NADH to anode (+). The symbol (■) indicates electrochemical oxidation of NADH, coupling to oxidation of the metabolic intermediate, but the symbol (●) indicates the electrochemical reduction of NAD^+, coupling to reduction of metabolic intermediate.

condition, because the bacterial membrane is an electron barrier composed of lipid, which is physiologically required for maintenance of membrane potential. Theoretically, the NR immobilized in bacterial membrane cannot transfer electrons across the membrane under the normal growth condition, because the NR needs the oxidation-reduction potential to be lower than -0.325 volt for the redox reaction.

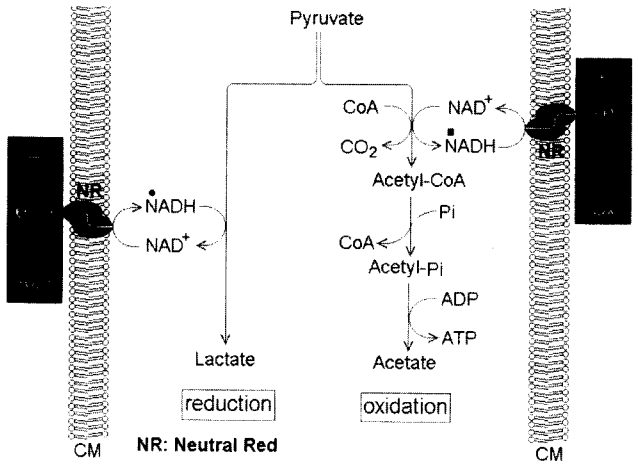

Fig. 4. Schematic mechanism for the oxidation or reduction reaction of pyruvate, coupling to the oxidoreduction of NAD^+/NADH, which is electrochemically oxidized and reduced.
The neutral red immobilized in cytoplasmic membrane (CM) is to function as an electron mediator (channel) from cathode (-) to NAD^+ or NADH to anode (+). The symbol (■) indicates electrochemical oxidation of NADH, coupling to oxidation of metabolic intermediate, but the symbol (●) indicates the electrochemical reduction of NAD^+, coupling to reduction of metabolic intermediate.

The NR can be chemically reduced by coupling to oxidation of sulfide (-0.52 volt) and thiosulfate (-0.45 volt), and chemically oxidized by coupling to reduction of NAD^+. However, the chemical oxidation-reduction of NR/sulfide is not useful for the bacterial reactor, because the chemicals are harmful for bacteria growth and they are continuously to be added to the reactor. On the other hand, the NR immobilized in the membrane can transfer electrons across the membrane by selective oxidation-reduction reaction in the electrochemical bioreactor capable of tuning the oxidation-reduction potential. The cathode was -2.0 volts and the anode was +2.0 volts, which are enough for reduction and re-oxidation of the NR immobilized in the membrane, however, H_2O cannot electrolyze without strong electrolytes such as NaOH or HCl. We hypothesize that electrochemically reduced and oxidized NR can reduce NAD^+ to NADH and oxidize NADH to NAD^+ in bacterial cytoplasm, respectively. This hypothesis implies that the redox balance (NADH/NAD^+) in cytoplasm can electrochemically be controlled by the electrode located outside of the bacterial cell. In high NADH/NAD^+ state, the reduced metabolic pathway may be activated, however the oxidized metabolic pathway may be activated in low NADH/NAD^+ state [14].

Bacterial growth was measured, coupled to substrate consumption and metabolite production, for 48 h at 2 h intervals. When bacterial growth is stopped at 12-14 h of incubation and then maintained at stationary phase for 14 h, the substrate consumption and metabolite production are not varied any longer after 24 h of incubation [21] (data not shown). Therefore, on the basis of these results, we used the biomass, substrate, and metabolite concentration measured at 24 h of incubation for calculation of stoichiometric balance [5]. As shown in Table 1, the growing cells of *W. kimchii* produced 98.9 mM lactate, 84.3 mM ethanol, and 0.2 mM acetate in the high NADH/NAD^+ state, but

Table 1. Fermentation parameters of growing *Weissella kimchii* cells on MRS with 100 μM neutral red, electrochemically oxidized or reduced under anaerobic condition for 24 h.

Fermentation parameters	Growth condition	
	Anode-Oxidized	Cathode-Reduced
Glucose consumption (mM)	83.1	90.8
Lactate production (mM)	87.4	98.9
Acetate production (mM)	4.9	0.2
Ethanol production (mM)	69.3	84.3
Total growth (g cell/l)	1.104	1.112
Carbon recovery (%)[a]	97.2	101.0
Y_{ATP} (mol ATP/mol substrate)[b]	1.16	1.18
Redox balance (NADH/NAD^+)[c]	0.810	0.927

[a]Carbon recovery: (sum of metabolites)÷(glucose consumption×2)×100.
[b]1ATP/1acetate production, 2ATP/1lactate production, -1ATP/1glucose consumption.
[c]Redox balance: (2NADH/1ethanol)÷(2 NAD^+/1glucose+1 NAD^+/1acetate).

87.4 mM lactate, 69.3 mM ethanol, and 4.9 mM acetate in the low NADH/NAD$^+$ balance state. Lactate and ethanol are reduced metabolites, and acetate is an oxidized product (shown in the pathway of Figs. 3 and 4). About 10% more lactate and ethanol were produced in the high balance of NADH/NAD$^+$, but about 240% more acetate was produced in the low balance of NADH/NAD$^+$. This result indicates that the metabolism of growing *W. kimchii* can be controlled by difference of electrochemical oxidation and reduction potentials, built outside of the bacterial cell. When the resting cell of *W. kimchii* was subjected to the electrochemical reactor, results very similar to those from the growing cell were obtained (Table 2). From the fermentation of glucose by *W. kimchii*, two reduced and one oxidized metabolites are produced, however, it is not enough in comparison with the dramatically different redox balance in oxidized and reduced metabolites [21]. As shown in Fig. 4.[8], we used in another test a simpler pathway, involving pyruvate to lactate and acetate: One reduced metabolite and one oxidized metabolite are easy to calculate the redox balance. The resting cells were used for this test, because *W. kimchii* cannot produce the reducing power to drive pyruvate reduction to lactate [27]. As shown in Table 3, the production of lactate and acetate were 15.9 mM and 15.2 mM, respectively, from 32.1 mM pyruvate consumed in the low balance of NADH/NAD$^+$. However, 71.3 mM lactate and 3.8 mM acetate were produced from 79.8 mM pyruvate consumed in the high balance of NADH/NAD$^+$. The redox balances calculated from these results were 1.05 and 18.76 in the low and high balance of NADH/NAD$^+$, respectively. Such definitely significant difference indicates that electrons can transfer from outside of the bacterial cell to electron carriers in the fermentation pathway and also in reversed direction [15]. The electrochemical control of bacterial metabolism is a useful tool to enhance production of specific metabolites such as succinate, malate, and xylitol. Succinate and malate have been produced from glucose by fermentation, and the productivity has been enhanced by various alterations of fermentation condition [22] or bacterial mutation [16].

Table 2. Fermentation parameters of resting *Weissella kimchii* cells on MRS with 100 μM of neutral red, electrochemically oxidized or reduced under anaerobic condition for 24 h.

Fermentation parameters	Growth condition	
	Anode-Oxidized	Cathode-Reduced
Glucose consumption (mM)	86.6	90.0
Lactate production (mM)	70.5	84.8
Acetate production (mM)	17.5	0.1
Ethanol production (mM)	76.8	94.3
Carbon recovery (%)[a]	95.2	99.5
Redox balance (NADH/NAD$^+$)[b]	0.805	1.05

[a]Carbon recovery: (sum of metabolites)÷(glucose consumption×2)×100.
[b]Redox balance: (2NADH/1ethanol)÷(2 NAD$^+$/1glucose+1 NAD$^+$/1acetate).

Table 3. Fermentation parameters of resting *Weissella kimchii* cells on modified MRS with pyruvate instead of glucose and 100 μM neutral red, electrochemically oxidized or reduced under anaerobic condition for 24 h.

Fermentation parameters	Growth condition	
	Anode-Oxidized	Cathode-Reduced
Pyruvate consumption (mM)	32.1	79.8
Lactate production (mM)	15.9	71.3
Acetate production (mM)	15.2	3.8
Ethanol production (mM)	Not detected	Not detected
Carbon recovery (%)[a]	96.8	94.1
Redox balance (NADH/NAD$^+$)[b]	1.05	18.76

[a]Carbon recovery: (sum of metabolites)÷(pyruvate consumption)×100.
[b]Redox balance: (1NADH/lactate)÷(1NAD$^+$/acetate).

Similarly, xylitol has been produced from xylose by yeast *Saccharomyces cerevisiae*, using glucose as a reducing power [28]. We are in a process to enhance succinate production from glucose fermentation, and electrochemical reduction of xylose to xylitol by electricity in place of glucose as a reducing power.

In the near future, we will confront dangerous environmental pollution with high concentration of carbon dioxide if we do not try to decrease carbon dioxide production. One of the solutions to decrease carbon dioxide is to develop a bioelectrochemical system capable of converting carbon dioxide to organic carbons such as PHB. To some extent, electricity has to be consumed during the night or holidays without any work, which constitutes a very useful energy source for operation of electrochemical or bioelectrochemical systems.

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REFERENCES

1. Cecchini, G., C. R. Thompson, B. A. Ackrell, D. J. Westenberg, N. Dean, and R. P. Gunsalus. 1986. Oxidation of reduced meanquinone by the fumarate reductase complex in *Escherichia coli* requires the hydrophobic FrdD peptide. *Proc. Natl. Acad. Sci. USA* **83:** 8898-8902.
2. Collins, M. D., J. Samelis, J. Metaxopoulos, and S. Wallbanks. 1993. Taxonomic studies on some *Leuconostoc*-like organisms from fermented sausages: Description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *J. Appl. Bacteriol.* **75:** 595-603.
3. Dickie, P. and J. Weiner. 1979. Purification and characterization of membrane-bound fumarate reductase from anaerobically grown *Escherichia coli*. *Can. J. Biochem.* **57:** 813-821.

4. Girbal, L., I. Vasconcelos, A. Silvie Saint, and P. Soucaille. 1995. How neutral red modified carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH. *FEMS Microbiol. Rev.* **16:** 151-162.
5. Hong, S. H., S. Y. Moon, and S. Y. Lee. 2003. Prediction of maximum yields of metabolites and optimal pathways for their production by metabolic flux analysis. *J. Microbiol. Biotechnol.* **13:** 571-577.
6. Hongo, M. and M. Iwahara. 1979. Determination of electro-energizing conditions for L-glutamic acid fermentation. *Agric. Biol. Chem.* **43B:** 2083-2086.
7. Hongo, M. and M. Iwahara. 1979. Application of electron-energizing method to L-glutamic acid fermentation. *Agric. Biol. Chem.* **43A:** 2075-2081.
8. Kang, H. S. and D. H. Park. 2004. Biocatalytic oxidation-reduction of pyruvate and ethanol by *Weissella kimchii* sk10 under aerobic and anaerobic condition. *J. Microbiol. Biotechnol.* **14:** 914-918.
9. Kemner, J. M. and J. G. Zeikus. 1992. Purification and characterization of membrane-bound hydrogenase from *Methanosarcina barkeri* MS. *Arch. Microbiol.* **161:** 47-54.
10. Kim, B. H. and J. G. Zeikus. 1992. Hydrogen metabolism in *Clostridium acetobutylicum* fermentation. *J. Microbiol. Biotechnol.* **2:** 2771-2776.
11. Kim, T. W., J. Y. Lee, S. H. Jung, Y. M. Kim, J. S. Jo, D. K. Chung, H. J. Lee, and H. Y. Kim. 2002. Identification and distribution of predominant lactic acid bacteria in kimchi, a Korean traditional fermented food. *J. Microbiol. Biotechnol.* **12:** 635-642.
12. Kim, T. W., S. H. Jung, J. Y. Lee, S. K. Choi, S. H. Park, J. S. Jo, and H. Y. Kim. 2003. Identification of lactic acid bacteria in kimchi using SDS-PAGE profiles of whole cell proteins. *J. Microbiol. Biotechnol.* **13:** 119-124.
13. Kötner, C., F. Lauterbach, D. Tripier, G. Unden, and A. Kröger. 1990. *Wolinella succinogenes* fumarate reductase contains a dihaem cytochrome b. *Mol. Microbiol.* **4:** 855-860.
14. Lee, J. W., A. Goel, M. M. Ataai, and M. M. Domach. 2002. Flux regulation patterns and energy audit of *E. coli* B/r and K-12. *J. Microbiol. Biotechnol.* **12:** 258-267.
15. Lee, Y. J., K. H. Cho, and Y. J. Kim. 2003. The membrane-bound NADH: Ubiquinone oxidoreductase in the aerobic respiratory chain of marine bacterium *Pseudomonas nautical. J. Microbiol. Biotechnol.* **13:** 225-229.
16. Millard, C. S., Y. P. Chao, J. C. Liao, and M. I. Donnelly. 1996. Enhanced production of succinic acid by overexpression of phosphoenolpyruvate carboxylase in *Escherichia coli. Appl. Environ. Microbiol.* **62:** 1808-1810.
17. Miyawaki, O. and T. Yano. 1992. Electrochemical bioreactor with regeneration of NAD^+ by rotating graphite disk electrode with PMS absorbed. *Enzyme Microb. Technol.* **14:** 474-478.
18. Park, D. H. and J. G. Zeikus. 1999. Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: Physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. *J. Bacteriol.* **181:** 2403-2410.
19. Park, D. H. and J. G. Zeikus. 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. *Appl. Environ. Microbiol.* **66:** 1292-1297.
20. Park, D. H. and J. G. Zeikus. 2003. Improved fuel cell and electrode designs for producing from microbial degradation. *Biotechnol. Bioeng.* **81:** 348-355.
21. Park, S. M. and D. H. Park. 2004. Metabolic flux shift of *Weissella kimchii* sk10 grown under aerobic conditions. *J. Microbiol. Biotechnol.* **14:** 919-923.
22. Samuelov, N. S., R. Lamed, S. Lowe, and J. G. Zeikus. 1991. Influence of CO_2-HCO_3^- levels and pH on growth, succinate production, and enzyme activities of *Anaerobiospirillum succinidiproducens. Appl. Environ. Microbiol.* **57:** 3013-3019.
23. Sánchez, S., A. Arratia, R. Córdova. H. Gómez, and R. Schrebler. 1995. Electron transport in biological processes. II. Electrochemical behavior of Q10 immersed in a phospholipid matrix added on a pyrolytic graphite electrode. *Bioelectrochem. Bioenerg.* **36:** 67-71.
24. Schlereth, D. D. and V. M. Fernández. 1992. Direct electron transfer between *Alcaligenes eutrophus* Z-1 hydrogenase and glassy carbon electrodes. *Bioelectrochem. Bioenerg.* **28:** 473-482.
25. Sucheta, A., R. Cammack, J. H. Weiner, and F. A. Armstrong. 1993. Reversible electrochemistry of fumarate reductase immobilized on an electrode surface. Direct voltammetric observation of redox centers and their participation in rapid catalytic electron transport. *Biochemistry* **32:** 5455-5465.
26. Surya, A., N. Murthy, and S. Anita. 1994. Tetracyanoquinodimethane (TCNQ) modified electrode for NADH oxidation. *Bioelectrochem. Bioenerg.* **33:** 71-73.
27. Thauer, R. K., K. Jungermann, and K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* **41:** 100-180.
28. Thestrup, H. N. and B. Hahn-Hagerdal. 1995. Xylitol formation and reduction equivalent generation during anaerobic xylose conversion with glucose as cosubstrate in recombinant *Saccharomycess cerevisiae* expressing the *xyl1* gene. *Appl. Environ. Microbiol.* **61:** 2043-2045.
29. Varma, A. and B. O. Palsson. 1994. Metabolic flux balancing: Basic concepts, scientific and practical use. Review. *Bio/Technology* **12:** 994-998.
30. White, H., H. Lebertz, I. Thanons, and H. Simon. 1987. *Clostridium thermoaceticum* production of methanol from carbon monoxide in the presence of viologen dyes. *FEMS Microbiol. Lett.* **43:** 173-176.
31. Willner, I., E. Katz, and N. Lapidot. 1992. Bioelectrocatalysed reduction of nitrate utilizing polythiophene bipyridium enzyme electrodes. *Bioelectrochem. Bioenerg.* **29:** 29-45.
32. Wissenbach, U., A. Kröger, and G. Unden. 1990. The specific functions of menaquinone and demethylmenaquinone in anaerobic respiration with fumarate, dimethylsulfoxide, trimethylamine N-oxide and nitrate by *Escherichia coli. Arch. Microbiol.* **154:** 60-66.
33. Wissenbach, U., D. Thernes, and G. Unden. 1992. An *Escherichia coli* mutant containing only demethylmenaquinone, but not menaquinone: Effects on fumarate, dimethylsulfoxide, trimethylamine N-oxide and nitrate respiration. *Arch. Microbiol.* **158:** 68-73.
34. Xie, Y. and S. Dong. 1992. Effects of pH on the electron transfer of cytochrome-c on a gold electrode modified with bis(4-pyridyl) disulphide. *Bioelectrochem. Bioenerg.* **29:** 71-79.