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Effect of Electrochemical Redox Reaction on Growth and Metabolism of *Saccharomyces cerevisiae* as an Environmental Factor

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Abstract The effect of an electrochemically generated oxidation-reduction potential and electric pulse on ethanol production and growth of *Saccharomyces cerevisiae* ATCC 26603 was experimented and compared with effects of electron mediators (neutral red, benzyl viologen, and thionine), chemical oxidants (hydrogen peroxide and hypochlorite), chemical reductants (sulfite and nitrite), oxygen, and hydrogen. The oxidation (anodic) and reduction (cathodic) potential and electric pulse activated ethanol production and growth, and changed the total soluble protein pattern of the test strain. Neutral red electrochemically reduced activated ethanol production and growth of the test strain, but benzyl viologen and thionine did not. Nitrite inhibited ethanol production but did not influence growth of the test strain. Hydrogen peroxide, hypochlorite, and sulfite did not influence ethanol production and growth of the test strain. Hydrogen and oxygen also did not influence the growth and ethanol production. It shows that the test strain may perceive electrochemically generated oxidation-reduction potential and electric pulse as an environmental factor.

Keywords: Electrochemical redox reaction, electron mediators, electric pulse, oxidation radical, reduction radical, *Saccharomyces cerevisiae*

Various environmental factors such as temperature, pH, ionic strength, osmotic pressure, and electric pulse affect the growth and metabolism of microorganisms [9, 21, 24, 27, 44]. Direct electric current between two electrodes installed in bacterial culture was reported to alter bacterial shape and increase cell surface hydrophobicity [22]. In particular, various researches were performed to prove the effects of electric field, electric pulse, or electric shock on bacterial physiology, morphology, and biofilm structure [15, 25, 35, 39]. However, few research related to growth and ethanol production of yeast has been reported. A direct electrochemical reaction between an electrode and bacterial cell was reported to induce electron transfer from the bacterial cell to the electrode through some specific coenzymes (electron carriers in respiratory metabolism) binding in the cytoplasmic membrane [2, 19]. However, the coenzymes for respiration are located in the mitochondria and NAD^+ is located in the cytoplasm of *Saccharomyces cerevisiae* [8, 13], which may be a barrier for electrochemical reactions between the electrode and yeast cells. The cell wall of *Saccharomyces* sp. is composed of β-glucans, chitin, and mannoproteins instead of peptidoglycan and the metabolism is different from bacterial cells [3, 11, 20]. We do not expect that the redox reaction of biochemical materials in a yeast cell may be electrochemically induced without electron mediators. To induce the electrochemical redox reaction, we applied neutral red, benzyl viologen, and thionine to a yeast culture. The benzyl viologen was reported to permeate into the cytoplasm of *E. coli* across the cytoplasmic membrane under a reduction environment [17]. Shin *et al.* [38] reported that the neutral red could mediate electron transfer from the electrode to *Trichospron capitatum* (yeast) and activate β-tetralol production. Hongo and Iwahara [14] reported that benzyl viologen electrochemically reduced activated growth and L-glutamic acid production of *Brevibacterium flavum* (bacterial cell), but natural benzyl viologen did not. Studies on benzyl viologen applied to yeast cells have not been reported and the researches about thionine are limited to bacterial fuel cell [37, 40]. Benzyl viologen and thionine were used to examine their effect on the growth and metabolism of yeast cells as the electron mediators not used before.

The objective of the present study was to understand the effect of electric pulse and electrochemically generated

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electric oxidation-reduction potential on ethanol production and growth of *Saccharomyces cerevisiae*. Our working hypothesis is that yeast cells may respond to the electric pulse and oxidation-reduction potential as an environmental factor, by which ethanol production and growth may be activated. The knowledge on the underlying mechanism responsible for the electrochemical oxidation-reduction potential affecting eukaryotic metabolism could be important in applying an electrochemical technique to the ethanol fermentation system and expanding the electrochemical bioreactor into the industrial system.

MATERIALS AND METHODS

Microorganism

Saccharomyces cerevisiae ATCC26603 was used as a test strain and cultivated in the medium containing 5 g/l yeast extract, 5 g/l peptone, and 1.0 M glucose (YPG) at 30°C. Seed culture was cultivated in the medium for 48 h.

Electron Mediators

Neutral red (E_o=−0.325 volt *vs.* NHE), benzyl viologen (E_o=−0.358 volt *vs.* NHE), and thionine (E_o=+0.06 volt *vs.* NHE) were selected based on the redox potential (E_o) differences. The redox potential of two mediators is lower and one mediator is higher than that of NAD^+/NADH (E_o=−0.32 volt *vs.* NHE). Theoretically, thionine cannot reduce NAD^+ to NADH because its redox potential is higher than NAD^+. The concentration of mediators added to the yeast culture was equally adjusted to 200 μM.

Chemical Oxidant and Reductant

Hydrogen peroxide and hypochlorite were used as chemical oxidants, and sulfite and nitrite were used as chemical reductants. These were added to yeast culture at the initial time when seed was inoculated after being sterilized with a membrane filter (pore size 0.22 μm).

Electrochemical Bioreactor

Two types of electrochemical bioreactors were designed to induce an electric pulse by equipped anode and cathode in a single reactor (Fig. 1A), and to generate a one-sided oxidation and reduction potential by separate installation of anode and cathode in a two-compartments reactor (Fig. 1B). The two-compartments bioreactor was compartmented by a porcelain membrane modified with cellulose acetate. The distance between the two electrodes was adjusted to 10 mm in reactor A and 200 mm in reactor B. The internal resistance between anode and cathode in the two-compartments reactor containing YPG medium was 4,500 Ω, and the internal resistance between the two electrodes in the single-compartment bioreactor containing the same

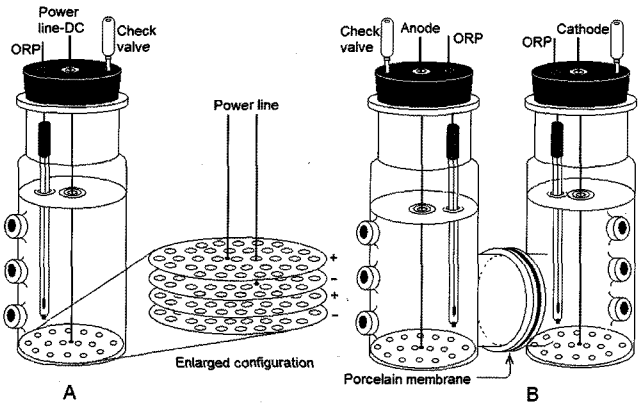

Fig. 1. Electrochemical bioreactors for yeast cultivation using electric pulse (**A**) and separated redox potential (**B**).
The redox potential of the yeast culture was measured with an ORP meter, and the anode and cathode of reactor A was regularly exchanged.

medium was 1,700 Ω. Titanium plates with holes were used as both anode and cathode. The diameters of the holes were 5 mm, and the space between holes was 5 mm. The bioreactor volume was 1,200 ml. The electrode diameter and thickness were adjusted to 100 mm and 2 mm, respectively. Yeast cells were cultivated in both parts of the two-compartments reactor containing 1.2 l of YPG medium.

Induction of One-Sided Redox Environment

To generate a one-sided oxidation and reduction environment, the anode (+) and cathode (−) were installed separately in two compartments, as shown in Fig. 1B [16, 30, 31]. In this system, the Ag/AgCl part of the ORP electrode can be a reference electrode for the anode or cathode located in the same partition, and the cathode can be a counter electrode for the anode when the anode is used as a working electrode; the anode can be a counter electrode for the cathode when the cathode is used as a working electrode.

When 2, 4, 6, 8, and 10 volts of DC electricity was charged between the anode and cathode in the two-compartments reactor containing YPG medium, the redox potential between anode and reference electrode was fixed to positive value in the anode compartment, and that between the cathode and reference electrode was fixed to a negative value in the cathode compartment. However, the ORP slightly little variable in both compartments, which indicates the oxidation-reduction tendency of the medium. Components of yeast extract and peptone were composed of free amino acids, vitamins, and oligopeptides [6]. Some components may be oxidized on the anode surface but others can be reduced on the cathode surface, by which oxidized and reduced compounds have to be unbalanced between the anode and cathode compartments. The imbalance of oxidized and reduced components between

Table 1. Effect of electron mediators on ethanol and biomass production of *S. cerevisiae* cultivated in the cathode compartment of an electrochemical bioreactor and a conventional bioreactor for 48 h.

Electron mediators	Ethanol production, M (Biomass, g/l)			
	Electrochemical reactor		Conventional reactor	
	24 h	48 h	24 h	48 h
None	0.427±0.03	0.935±0.04 (1.332)	0.357±0.03	0.782±0.06 (1.243)
Thionine	0.391±0.01	0.921±0.06 (1.284)	0.335±0.03	0.773±0.06 (0.966)
Benzyl Viologen	0.478±0.02	0.960±0.08 (1.196)	0.273±0.02	0.777±0.05 (0.894)
Neutral Red	0.469±0.04	1.163±0.05 (1.514)	0.445±0.05	0.759±0.06 (0.902)

anode and the cathode compartments induces the medium condition to be oxidized or reduced and the ORP of the medium to be variable.

Induction of Electric Pulse

Two to 10 volts of DC electricity was used as a source for formation of electric field between the electrodes parallel disposed (Fig. 1A). The electrode was disposed on the bottom because yeast cells are growing on the bottom without being suspended. To induce an electric pulse in the electric field, electrode poles (anode and cathode) were reciprocally exchanged at the intervals of 60 s. During the reciprocal exchange of anode and cathode, the redox potential between the reference electrode and an electrode at the top (Fig. 1A) was regularly exchanged from positive to negative values, by which an electric pulse can be induced.

Analysis

Ethanol and glucose were analyzed by HPLC (YoungLin, Korea) equipped with an Aminex HPX-87H ion-exchange column (Bio-Rad, CA, U.S.A.). Samples were prepared from yeast culture by filtration with a membrane filter (pore size 0.22 μM) and directly injected into the injector of the HPLC. The ethanol concentration was determined by comparison of the peak area obtained from the HPLC of standard material. Dry cell mass was determined with bacterial filtrate, dried at 110°C for 3 to 5 h until the filtrate weight was constant. The ORP (oxidation-reduction potential) of the yeast culture was continuously measured with an ORP electrode (Orion, U.S.A.) and potentiometer (Keithley, U.S.A.), which was communicated with a data acquisition system controlled with an IBM personal computer.

SDS-PAGE

The SDS-PAGE technique used in the present study was adapted from Laemmli [18]. Cell extract was prepared from a test strain cultivated in different growth conditions. Yeast cells were harvested, washed three times with 25 mM phosphate buffer (pH 7.0), and centrifuged at 4°C and 5,000 ×*g* for 30 min. The washed cells were disrupted by ultrasonic treatment (400 W) at 4°C for 120 min. Protein concentration was determined with Bradford reagent (BioRad) as a coloring agent and bovine serum albumin as a protein standard.

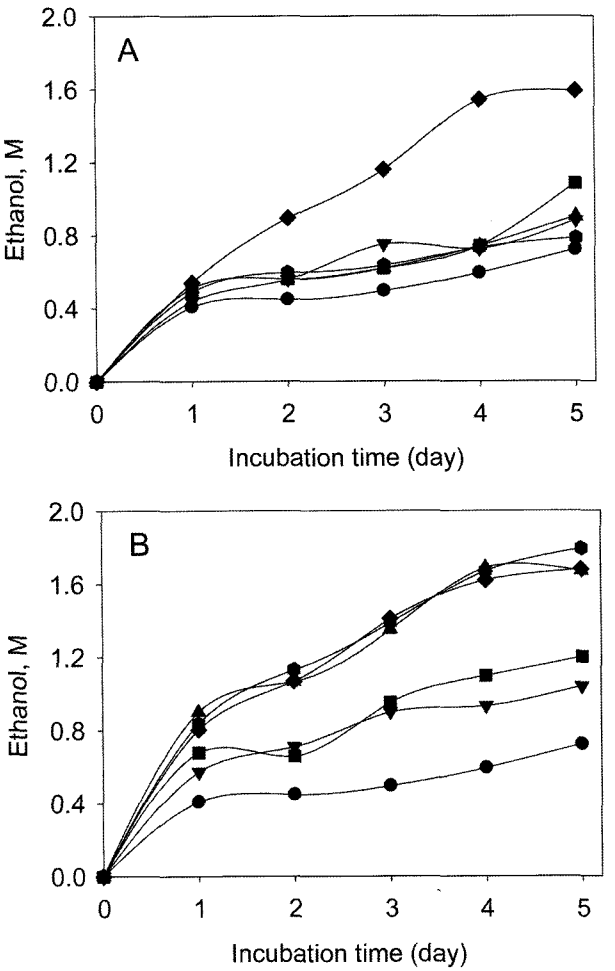

Fig. 2. Ethanol production by *S. cerevisiae* under reduction (**A**) and oxidation (**B**) environment.

An oxidation and reduction potential was electrochemically induced by separation of the anode and cathode. The electricity potential difference between the anode and cathode was adjusted to 0 volt (●), 2 volt (▼), 4 volt (■), 6 volt (▲), 8 volt (◆), and 10 volt (⬢). The ORP in the reduction and oxidation environment was proportional to the electric potential charged to the anode and cathode. A 5-times concentrated medium was added to the yeast culture at the intervals of 24 h.

RESULTS

Effect of Electron Mediators on Ethanol Production

Ethanol production was measured at 24 and 48 h of incubation time, and growth was measured by using dry cell mass at 48 h after cultivation was finished, because the yeast cells are clustered at the bottom of the bioreactor. The cluster is difficult to be homogeneously resuspended. The test strain was cultivated in the cathode compartment (reduction condition) of the electrochemical bioreactor (Fig. 1B), but not in the anode compartment because the electron mediators are oxidized forms in the natural condition and can be reduced only in the cathode compartment. The ethanol production was relatively higher in the electrochemical bioreactor than in the conventional bioreactor unrelated with the electron mediators, as shown in Table 1. The growth of the test strain was proportional to the ethanol production in both the conventional and electrochemical bioreactors. Natural forms of benzyl viologen and thionine inhibited the growth of the test strain, but those electrochemically reduced did not inhibit and activate the ethanol production and growth. Neutral red electrochemically reduced both activated growth and ethanol production, but the natural form of neutral red did not.

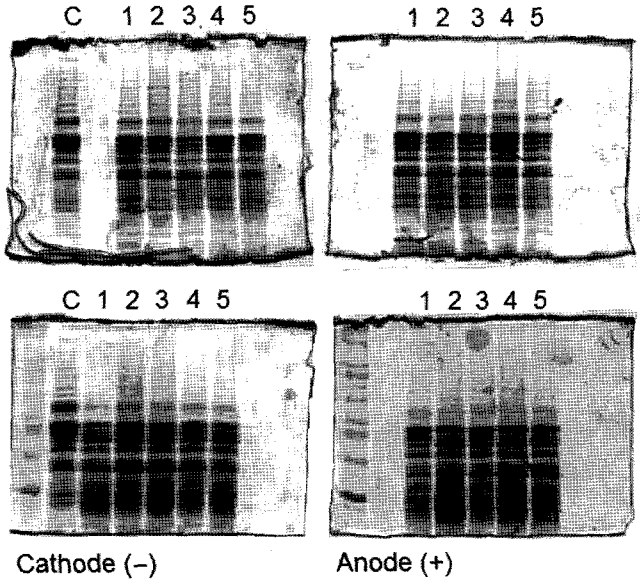

Fig. 3. SDS-PAGE profile of total soluble proteins of *Saccharomyces cerevisiae* cultivated in a cathode compartment (left) and anode compartment (right) for 24 h (upper) and 48 h (lower).

The electric potential charged to the anode and cathode was adjusted to 2 (lane 1), 4 (lane 2), 6 (lane 3), 8 (lane 4), and 10 volts (lane 5). Lane C indicates the soluble protein of *S. cerevisiae* cultivated in conventional condition.

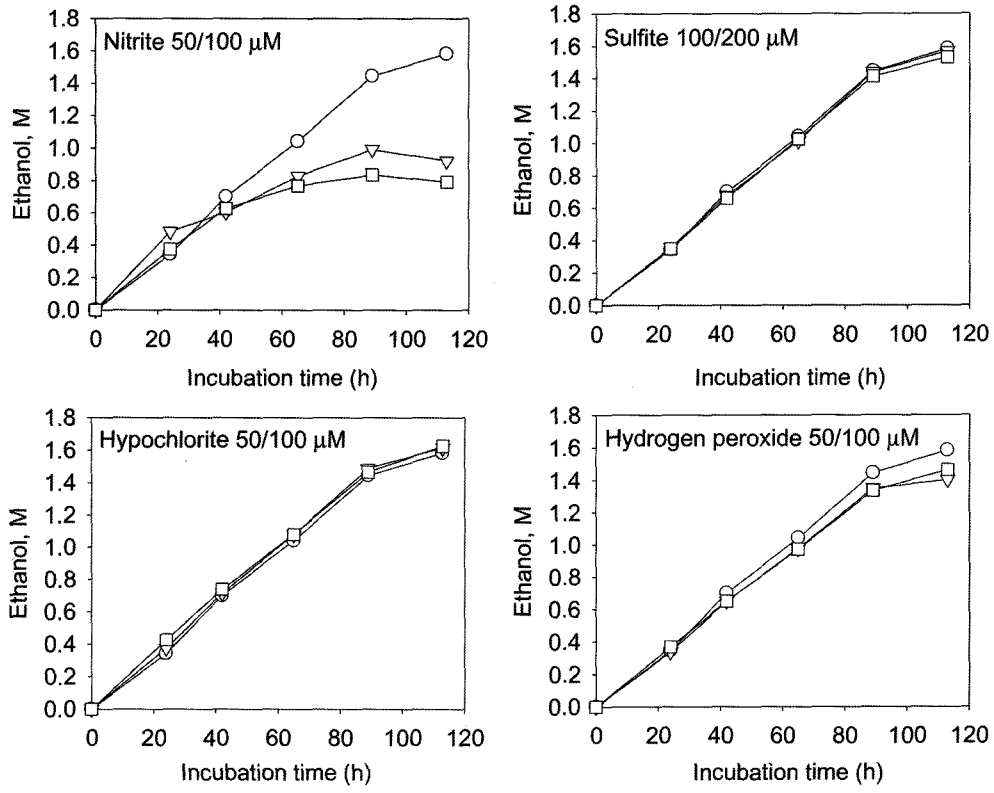

Fig. 4. Effect of oxidation and reduction radicals on ethanol production of *S. cerevisiae*.

The radical concentration was determined on the basis of MIC predetermined. The ethanol production was not influenced by low concentration (□) and high concentration (▽) of radical, which was nearly same as that produced in the condition without radical (○), except for nitrite.

Effect of Redox Potential on Ethanol Production

In this experiment, a five-times-concentrated medium was added to the yeast culture at intervals of 24 h when sampling, to exclude substrate limitation. The addition volume of the concentrated medium was adjusted to 0.1 volume of the culture, which was the same as the sampling volume. The ethanol production was more increased in the electrochemical bioreactor than in the conventional bioreactor (0 volt), and was higher in the oxidation environment (anode compartment) than in the reduction environment (cathode compartment) of the electrochemical bioreactor, as shown in Fig. 2. The ethanol production was maximal in the reduction environment generated at 6 volts electricity charge, and in the oxidation environment generated at 6, 8, and 10 volts electricity. It is a specific phenomenon that the ethanol production is maximal at a specific potential in both the oxidation and reduction environments. Totally, the ethanol production was higher in the oxidation environment than in reduction environment. The redox potential can be an indicator to monitor oxidation or reduction condition. The redox potentials (working electrode *vs.* Ag/AgCl) in the anode and cathode compartments were +300~+700 mV (*vs.* Ag/AgCl) and -200~-500 mV (*vs.* Ag/AgCl), respectively, during operation, which were fixed values in proportional to the electric potential charged to electrodes. The ethanol production differences according to the growth environments may be caused by gene expression differences, which were analyzed by comparison of total soluble proteins extracted from the test strain grown in the conventional, oxidation, and reduction environments. As shown in Fig. 3, the SDS-PAGE patterns of soluble proteins extracted from yeast cells grown in the conventional and electrochemical bioreactors were different from each other, and those extracted from the test strain grown in the anode and cathode compartments were also different from each other.

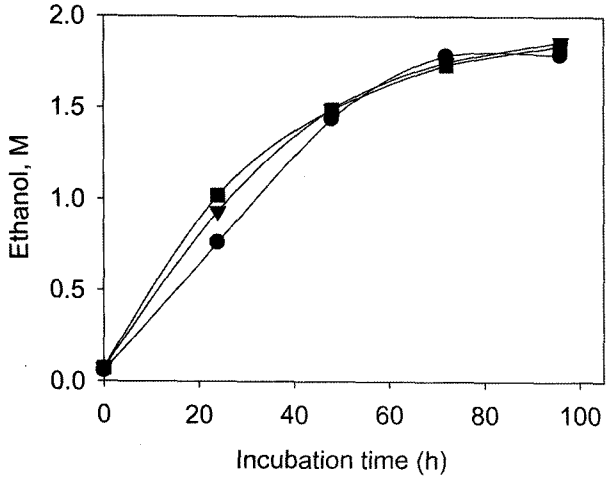

Fig. 5. Effect of hydrogen and oxygen on ethanol production of *S. cerevisiae*.

Yeast cells were cultivated in anaerobic serum vials of which the head space was filled with 99.99% of nitrogen (●), hydrogen (▼), and oxygen (■). Nitrogen was used to maintain an anoxic condition of the yeast culture as a control test.

Effect of Oxidant and Reductant on Ethanol Production

Two chemical reductants (nitrite and sulfite) and two chemical oxidants (hydrogen peroxide and hypochlorite), 99.99% hydrogen, and 99.99% oxygen were added to the yeast culture to compare ethanol production in a chemically induced oxidation or reduction environment. The concentration of chemical oxidants and reductants was determined on the basis of MIC previously measured. Concentration of nitrite, hydrogen peroxide, and hypochlorite was adjusted to 50 and 100 μM, and sulfite was adjusted to 100 and 200 μM. Hydrogen and oxygen (200 kp) was injected into the head space (115 ml) of an anaerobic serum vial (165 ml) containing 50 ml of medium after being completely vacuumed. As shown in Fig. 4, the ethanol production was not influenced by hydrogen peroxide, hypochlorite, and sulfite, but a little inhibited by nitrite. Hydrogen and oxygen also did not influence the ethanol production, as shown in Fig. 5. The ORP (Pt working electrode *vs.* Ag/

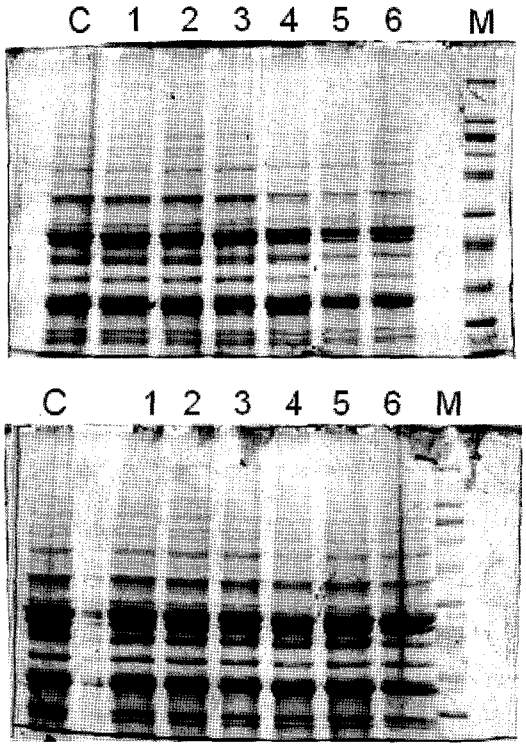

Fig. 6. SDS-PAGE profile of total soluble proteins of *S. cerevisiae* cultivated in different growth conditions for 24 h (upper) and 48 h (lower).

The yeast cells were cultivated in medium without radical (lane C), or in the medium containing hydrogen peroxide (lane 1), hypochlorite (lane 2), nitrite (lane 3), sulfite (lane 4), oxygen (lane 5), or hydrogen (lane 6). Lane M is the molecular weight marker.

AgCl electrode) of the medium containing hydrogen peroxide, hypochlorite, sulfite, nitrite, hydrogen, and oxygen was around +360 mV, +460 mV, -410 mV, -210 mV, -450 mV, and +550 mV, respectively. The redox potential chemically induced was in the range of the redox potential electrochemically generated. The chemical oxidants and reductants did not influence or had only a slight influence on the alcohol production, which was biochemically analyzed by comparison of the total soluble protein patterns of the test strain cultivated in the medium with and without chemical oxidants and reductants. As shown in Fig. 6, the total soluble protein patterns of the test strain grown in different growth conditions were the same as each other.

Effect of Electric Pulse on Ethanol Production

In the electric field between the anode and cathode, an electric pulse can be induced by reciprocal exchange of the anode and cathode. When 10 volts of electric potential between the anode and cathode was charged, the current between the anode and cathode was maximally increased to 5.5 mA at the second of exchange of the electrode poles, and minimally decreased to 0.1 mA right before the electrode poles were exchanged. The redox potential between the reference electrode (Ag/AgCl) and upper electrode was maximally increased to +4.5 volt at the second of exchange of the electrode poles, and minimally decreased to -0.3 volt right before the electrode poles were exchanged. This means that the oxidation reaction was more activated than the reduction reaction in the bioreactor. An electric pulse may induce a short-term redox reaction (for 60 s) around the electrode but cannot preserve continuous oxidation or reduction potential. The electric pulse cannot induce an oxidation or a reduction environment and can be generated only between two electrodes. The test strain cultivated in the pulsed electric field generated at the intervals of 60 sec produced more ethanol than in the conventional condition. The ethanol production was proportional to the electric potential charged to the electrodes, as shown in Fig. 7. The concentration of ethanol produced by the test strain grown in the electric pulse was very similar to that in the oxidation environment (anode compartment). However, the total soluble protein patterns of the test strain grown in the electric pulse were different from those in the oxidation environment but a little similar to those in the reduction environment.

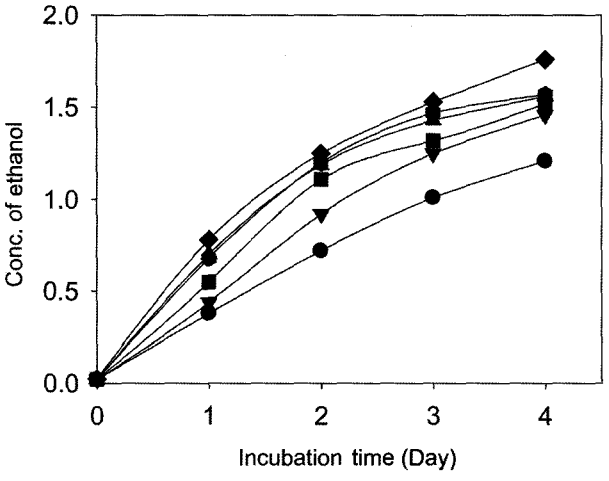

Fig. 7. Ethanol production by *Saccharomyces cerevisiae* growing in an electric field between the anode and cathode.
The electric potential between the anode and cathode was adjusted to 0 (●), 2 (▼), 4 (■), 6 (◆), 8 (▲), and 10 volts (⬢). The anode and cathode were reciprocally exchanged at the intervals of 60 sec, by which an electric pulse was generated in the electric field. The current was maximally increased to around 80 mA at the second of electric poles exchange, but was minimally decreased to 1-2 mA right before the electric poles were exchanged. However, the oxidation or reduction environment could not be preserved.

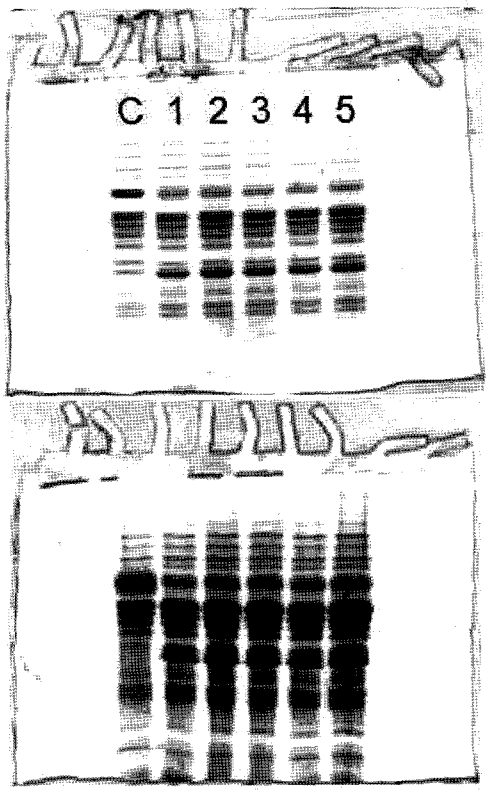

Fig. 8. SDS-PAGE profile of total soluble proteins of *S. cerevisiae* cultivated in the conventional condition (C) and on the electric pulse generated in an electric field, to which 2 volt (lane 1), 4 volt (lane 2), 6 volt (lane 3), 8 volt (lane 4), and 10 volt (lane 5) of electric potential was charged, for 24 h (upper) and 48 h (lower).

Effect of Redox Potential on Yeast Growth

Time-coursed biomass increase could not be measured because the yeast cells were grown on the bottom of the bioreactor but were not suspended during incubation. The

Table 2. Influence of oxidation and reduction potential, electrochemically and chemically induced, or electric pulse on biomass production of *S. cerevisiae* for 120 h. Each result was the mean value obtained from five repeat tests.

Dry cell mass (g/l) in the redox condition and electric pulse electrochemically induced				Dry cell mass (g/l) in the redox condition chemically induced	
Potential (volt)	Anodic reaction	Cathodic reaction	Electric pulse	Without radical	2.656
0	2.625		2.432	Hydrogen peroxide	1.652
2	3.336	3.792	3.089	Hypochlorite	1.756
4	3.614	3.179	3.553	Nitrite	2.445
6	3.832	3092	3.614	Sulfite	2.283
8	3.626	3.726	3.684	Pure oxygen	2.884
10	3.833	3.689	3.751	Pure hydrogen	2.632

biomass had to be measured after incubation was finished because shaking or agitation may disturb the experimental condition and yeast environment. Table 2 shows the dry cell mass obtained after cultivating for 120 h. The biomass of the test strain grown in the oxidation environment (anodic reaction), reduction environment (cathodic reaction), and in the electric pulse was proportion to the ethanol production and relatively higher than that in the conventional environment; however, oxidation and reduction radicals inhibited the biomass production of the test strain. Hydrogen and oxygen also did not influence the biomass production.

DISCUSSION

Electron mediators electrochemically reduced have been known to be able to catalyze the reduction reaction of specific coenzymes or enzymes [28, 36]. The reduced neutral red is known to catalyze NAD^+ reduction to NADH [33], but thionine (E_o=+0.06 V) cannot catalyze because its redox potential is much higher than that of NAD^+ [10, 29]. Thionine was used as a counterpart of benzyl viologen (E_o=−0.358 V) to compare the effect of electron mediators with higher and lower redox potential than NAD^+ (E_o= −0.320 V). Theoretically, the electron mediators with lower redox potential than NAD^+ can reduce NAD^+ to NADH, but those with higher redox potential cannot in a chemical reactor [41]. The metabolic pathway from pyruvate to ethanol may be more increased in a higher balance of $NADH/NAD^+$, because alcohol dehydrogenase is a NADH-dependent enzyme [1, 5, 42]. However, we obtained a different result from the expectation that benzyl viologen activated ethanol production of the test strain. The effect of neutral red on yeast metabolism was already proved by Park *et al.* [32] and Shin *et al.* [38], and we also had a positive result. In this experiment, the more significant observation than the neutral red effect was that the ethanol production was more increased in the electrochemical bioreactor than in the conventional bioreactor unrelated to the electron mediators. It means that the cathodic reaction (reduction reaction) can activate the yeast metabolism. On the basis of this result, we compared the effect of a cathodic reaction with an anodic reaction (oxidation reaction) on the ethanol production of the test strain. As mentioned above, an anoxic or a reduction environment may be more advantageous than an aerobic or oxidation environment for the fermentative metabolism of yeast [26]. However, the ethanol production was more increased in the anode compartment than in the cathode compartment. From these results, we supposed that a half-oxidation reaction in the anode compartment and a half-reduction reaction in the cathode compartment may induce generation of oxidant or reductant, which may cause yeast cells to induce consumption of extra free energy. To scavenge the oxidant or reductant, extra reducing power (NADH, NADPH) and free energy (ATP) have to be produced, by which the ethanol production may be increased because the reducing power and free energy production is balanced with metabolite production in the ethanol fermentation [7]. Oxygen and hydrogen production in the anode and cathode compartments has to be considered as well. Excessive oxygen can be a source for radical production [4] and hydrogen can be a reducing agent for the biochemical reaction [12]. No effectiveness of oxidant and reductant for the ethanol production, growth, and total soluble proteins pattern of the test strain presents another possibility that the electrochemical redox (oxidation-reduction) reaction itself, generated on the surface of the anode and cathode, may function as an environmental factor influencing the ethanol production of the test strain. To prove this possibility, the anode and cathode were disposed in a single bioreactor (Fig. 1A). To promote the electrochemical redox reaction, the anode and cathode were reciprocally exchanged, by which an electric pulse was generated. Wouters *et al.* [44] reported that *Listeria innocusa*, *Escherichia coli*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* were inactivated in the strong electric field of strength 2.7 volt/mm (27,000 volt/cm), which is much stronger than the electric field strength (maximal 10 volt/cm) used in this study. The report shows that the strong electric potential can damage or inactivate both bacteria

and yeast, but a weak electric pulse may not. It is very possible that the test strain may perceive the weak electrochemical redox reaction and electric pulse as an environmental factor and may respond to those. However, exactly how the weak electric pulse or electrode reaction activates ethanol production and changes the gene expression of the test strain is not known. We only show here that an electric pulse or electrode reaction may induce a test strain to produce more ethanol and more biomass, and to express specific or different genes. Gene expression has to be influenced by the various environmental factors, which can be shown with the total soluble protein [23, 34, 43]. SDS-PAGE is limited to showing of protein pattern, but useful to compare the expression intensity of proteins. In the SDS-PAGE, we found that some proteins were relatively increased but other proteins were relatively decreased, none of the proteins had disappeared on the gel. It means that electrochemical reaction can activate or partially inhibit a specific gene expression, but cannot block any gene expression. Accordingly, two-dimensional electrophoresis required for searching a specific protein not expressed is not useful in this research. In future, however, we will compare two-dimensional electrophoresis patterns of total soluble protein of the test strain grown in different environments.

Consequently, the electrochemical reactions such as an electric pulse, or anodic and cathodic reaction may be an environmental factor activating a test strain to produce more ethanol, because the environment around the electrode has to be continuously changed. Any changing around microorganisms can be an environmental factor. Accordingly, the electric pulse can be a new tool capable of applying to the industrial system for biological ethanol production. It is a more simple method than addition of electron mediators and genetic engineering technique for improvement of ethanol production because the electrode reaction generated at the level of voltage lower than 10 volts cannot be a mutagen and toxic for organisms. The electrochemical redox reaction may present a biologically safer environment than other chemical (biochemical) agents such as amino acids, vitamins, coenzymes, and antioxidants.

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References

1. Bakker, B. M., C. Bro, P. Kötter, M. A. H. Luttik, J. P. van Dijken, and J. T. Pronk. 2000. The mitochondrial alcohol dehydrogenase Adh3p is involved in a redox shuttle in *Saccharomyces cerevisiae*. *J. Bacteriol.* **182:** 4730–4737.
2. Bond, D. R. and D. R. Levley. 2005. Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. *Appl. Environ. Microbiol.* **71:** 2186–2189.
3. Bulik, D. A., M. Olczak, H. A. Lucero, B. C. Osmond, P. W. Robbins, and C. A. Specht. 2003. Chitin synthesis in *Saccharomyces cerevisiae* in response to supplementation of growth medium with glucosamine and cell wall stress. *Eukaryot. Cell* **2:** 886–900.
4. Carlsson, J., G. Nyberg, and J. Wrethén. 1978. Hydrogen peroxide and superoxide radical formation in anaerobic broth media exposed to atmospheric oxygen. *Appl. Environ. Microbiol.* **36:** 223–229.
5. Cho, J. Y. and T. W. Jeffries. 1998. *Pichia stipitis* genes for alcohol dehydrogenase with fermentative and respiratory functions. *Appl. Envir. Microbiol.* **64:** 1350–1358.
6. Crueger, W. and A. Crueger. 1989. *Biotechnology: A Textbook of Industrial Microbiology.* 2nd Ed. pp. 62. Science Tech Publisher, MA, U.S.A.
7. Dombek, K. M. and L. O. Ingram. 1987. Ethanol production during batch fermentation with *Saccharomyces cerevisiae*: Changes in glycolytic enzymes and internal pH. *Appl. Envir. Microbiol.* **53:** 1286–1291.
8. Dumont, M. E., J. B. Schlichter, T. S. Cardillo, J. K. Hayes, G. Bethlendy, and F. Sherman. 1993. CYC2 incodes a factor involved in mitochondrial import of yeast cytochromes C. *Mol. Cell. Biol.* **13:** 6442–6451.
9. Fey, A. and R. Conrad. 2000. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl. Environ. Microbiol.* **66:** 4790–4797.
10. Fultz, M. L. and R. A. Durst. 1982. Mediator compounds for the electrochemical study of biological redox system: A compilation. *Anal. Chim. Acta* **140:** 1–18.
11. Gombert, A. K., M. M. dos Santos, B. Christensen, and J. Nielsen. Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* under different conditions of glucose repression. *J. Bacteriol.* **183:** 1441–1451.
12. Gregory, E. M. and I. Fridovich. 1973. Oxygen toxicity and the superoxide dismutase. *J. Bacteriol.* **114:** 1193–1197.
13. Haucke, V., A. M. Dudley, and T. L. Mason. 1994. Analysis of the sorting signals directing NADH-cytochrome b5 reductase to two locations within yeast mitochondria. *Mol. Cell. Biol.* **17:** 4024–4032.
14. Hongo, M. and M. Iwahara. 1979. Application of electron-energizing method to L-glutamic acid fermentation. *Agric. Biol. Chem.* **43:** 2075–2081.
15. Iren, E. P., H. C. Mastwijk, P. V. Bartels, and E. J. Smid. 2000. Pulsed-electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*. *Appl. Environ. Microbiol.* **69:** 2405–2408.
16. Jeon, S. J., I. H. Shin, B. I. Sang, and D. H. Park. 2005. Electrochemical regeneration of FAD by catalytic electrode without electron mediator and biochemical reducing power. *J. Microbiol. Biotechnol.* **15:** 281–286.

17. Jones, R. W., T. A. Gray, and P. B. Garland. 1976. A study of the permeability of the cytoplasmic membrane of *Escherichia coli* to reduced and oxidized benzyl viologen and methyl viologen cations: Complications in the use of viologens as redox mediators for membrane-bound enzymes. *Biochemical Society Transaction*, 563rd Meeting, London.
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
19. Leagn, C., M. V. Coppi, and D. R. Levley. 2003. OmcB, a c-type polyheme cytochrome, involved in Fe(III)-reduction in *Geobacter sulfureducens*. *J. Bacteriol.* **185:** 2096–2103.
20. Lesage, G. and H. Bussey. 2006. Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **70:** 317–343.
21. Lloret, J., L. Bolanos, M. M. Lucas, J. M. Peart, N. J. Brewing, I. Bonilla, and R. Rivilla. 1995. Ionic strength and osmotic pressure induce different alterations in the lipopolysaccharide of a *Rhizobium meliloti* strain. *Appl. Environ. Microbiol.* **61:** 3701–3704.
22. Luo, Q., H. Wang, X. Zhang, and Y. Qian. 2005. Effect of direct electric current on the cell surface properties of phenol-degrading bacteria. *Appl. Environ. Microbiol.* **71:** 423–427.
23. Madsen, E. L., A. J. Francis, and J. M. Bollag. 1988. Environmental factors affecting indole metabolism under anaerobic conditions. *Appl. Envir. Microbiol.* **54:** 74–78.
24. McDermid, A. S., A. S. McKee, and P. D. Marsh. 1988. Effect of environmental pH on enzyme activity and growth of *Bacteroides ginivalis* W50. *Infect. Immun.* **56:** 1096–1100.
25. Na, B. K., B. I. Sang, D. W. Park, and D. H. Park. 2005. Influence of electric potential on structure and function of biofilm in wastewater treatment reactor: Bacterial oxidation of organic carbons coupled to bacterial denitrification. *J. Microbiol. Biotechnol.* **15:** 1221–1228
26. Nagodawithana, T. W., C. Castellano, and K. H. Steinkraus. 1974. Effect of dissolved oxygen, temperature, initial cell count, and sugar concentration on the viability of *Saccharomyces cerevisiae* in rapid fermentations. *Appl. Environ. Microbiol.* **28:** 383–391.
27. Otto, K., H. Elwing, and M. Hermansson. 1999. Effect of ionic strength on initial interactions of *Escherichia coli* with surfaces, studied on-line by a novel quartz crystal microbalance technique. *J. Bacteriol.* **181:** 5210–5218.
28. Park, D. H. and J. G. Zeikus. 1999. Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite producion. *Appl. Environ. Microbiol.* **65:** 2912–2917.
29. 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.
30. Park, D. H. and J. G. Zeikus. 2002. Impact of electrode composition on electricity generation in a single-compartment fuel cell using *Shewanella putrefaciens*. *Appl. Microbiol. Biotechnol.* **59:** 58–61.
31. Park, D. H. and J. G. Zeikus. 2003. Improved fuel cell and electrode designs for production of electricity from microbial degradation. *Biotechnol. Bioengin.* **81:** 348–355.
32. Park, S. M., B. I. Sang, D. W. Park, and D. H. Park. 2005. Electrochemical reduction of xylose to xylitol by whole cells or crude enzyme of *Candida peltata*. *J. Microbiol.* **43:** 451–455.
33. Park, S. M., H. S. Kang, D. W. Park, and D. H. Park. 2005. Electrochemical control of metabolic flux of *Weissella kimchii* sk10: Neutral red immobilized in cytoplasmic membrane as electron channel. *J. Microbiol. Biotechnol.* **15:** 80–85.
34. Patel, K. R., K. J. Mayberry-Carson, and P. F. Smith. 1978. Effect of external environmental factors on the morphology of *Spiroplasma citri*. *J. Bacteriol.* **133:** 925–931.
35. Rajnicek, A. M., C. D. McCaig, and N. A. Gow. 1994. Electric fields induce curved growth of *Enterobacter cloaceae*, *Escherichia coli*, and *Bacillus subtilus* cells: Implications for mechanisms of galvanotropism and bacterial growth. *J. Bacteriol.* **176:** 720–713.
36. Rao, G. and R. Mutharasan. 1987. Altered electron flow in continous cultures of *Clostridium acetobutylicum* induced by viologen dyes. *Appl. Environ. Microbiol.* **53:** 1232–1235.
37. Roller, S. D., H. P. Bennetto, G. M. Delaney, J. R. Mason, J. L. Stirling, and C. F. Thurston. 1984. Electron-transfer coupling in microbial fuel cells: 1. Comparison of redox-mediator reduction rates and respiratory rates of bacteria. *J. Chem. Tech. Biotechnol.* **34B:** 3–12.
38. Shin, H. S., M. K. Jain, and J. G. Zeikus. 2001. Evaluation of the electrochemical bioreactor system in biotransformation of β-tetralone to β-tetralol. *Appl. Microbiol. Biotechnol.* **57:** 506–510.
39. Stoodley, P., D. deBeer, and H. M. Pappin-Scott. 1997. Influcnce of electric fields and pH on biofilm structure as related to the bioelectric effect. *Antimicrob. Agents Chemother.* **41:** 1876–1879.
40. Thurston, C. F., H. P. Bennetto, G. M. Delaney, J. R. Mason, S. D. Roller, and J. L. Stirling. 1985. Glucose metabolism in a microbial fuel cell. Stoichiometry of product formation in a thionine-mediated *Proteus vulgaris* fuel cell and its relation to coulombic yields. *J. Gen. Microbiol.* **131:** 1391–1401.
41. Tinoco, I. Jr., K. Sauer, and J. C. Wang. 1985. *Physical Chemistry: Principles and Applications in Biological Sciences*. 2nd Edition. pp. 111–168. Prentice Hall. New York.
42. Verho, R., J. Londesborough, M. Penttilä, and P. Richard. 2003. Engineering redox cofactor regeneration for improved pentose fermentation in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **69:** 5892–5897.
43. Waligora, A.-J., M.-C. Barc, P. Bourlioux, A. Collignon, and T. Karjalainen. 1999. *Clostridium difficile* cell attachment is modified by environmental factors. *Appl. Envir. Microbiol.* **65:** 4234–4238.
44. Wouters, P. C., N. Dutreux, J. P. P. M. Smelt, and H. L. M. Lelieveld. 1999. Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *Appl. Environ. Microbiol.* **65:** 5346–5371.