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On the generating factor of nonlinear dielectric responses of *Saccharomyces cerevisiae*

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Synopsis

The harmonics of response wave based on the nonlinear dielectric properties of yeast cells have particular patterns for biological activity. The method is a novel technique for determining the activity of living cells. In this paper, we examined the generating factor of the nonlinear dielectric responses by comparing the results in the case where yeast cells exist near the electrodes of an electrochemical cell with those in the case where yeast cells do not. It was ascertained that nonlinear dielectric responses were induced according to biological activities.

KEYWARDS: Nonlinear dielectric property, Biological activity, Membrane filter, Electrode

1. Introduction

When an ac voltage is applied to cell suspensions and response wave (electric current wave from cell suspension) is analyzed using FFT, the harmonics of the response wave show peculiar patterns according to biological activities. It is considered that the electric current wave is effected by the biological activity of cell. This phenomenon is called nonlinear dielectric property[(1)]. It is thought that these differences in the responses according to cell activity are derived from the membrane proteins and are mainly ascribable to by the H^+-ATPase in the plasma membrane.

H. Yositake *et al* studied nonlinear dielectric properties of yeast cells using 2-electrodes system[(2)] in which the responses are easier to be influenced by adhesion of yeast cells on electrodes than 4-electrodes system. We examine generating factor of nonlinear dielectric responses by using 2-electrodes system. First we measured nonlinear dielectric responses in each growth phase (2h, 7h, 25h) by using 2-electrode system. Then, to examine the effect of yeast adhesion on electrode surface, we produced the electrochemical cell to which we can mount membrane filters. We discuss the generating factor of nonlinear dielectric responses by comparing the results in the case where yeast cells exist near the electrodes with those in the case where yeast cells do not.

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2. Experimental

2.1 System

The block diagram of the system in this study used for measuring nonlinear dielectric properties of yeast cells is shown in Fig.1. The sinusoidal waves which were 64 steps per wavelength were output from a D/A converter in a microcomputer with configured exciting frequency and field. These waves were applied (14Hz, 1.25V) to the electrochemical cell as the exciting waves after being smoothed by the analog 2-dimension low pass filter. The current waves of the response were logged into the microcomputer with a sampling frequency 32 times the exciting frequency. The sampled data were FFT analyzed for 512 points per block. As a result of the nonlinear dielectric properties, the power spectrum was obtained from an average of 60 blocks. The power spectrum P_i is defined as following equation.

$$P_i = 20 \times \log_{10}(I_{rms}/I_{0rms}) \quad [\mathrm{dB}] \tag{1}$$

Here I_{rms} is the measured current, $I_{0rms} = 1 \times 10^{-10}$ [A].

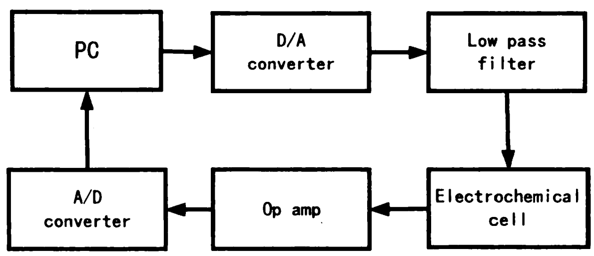

Fig.1. A block diagram of the measurement system

2.2 Electrochemical cell

We produced the electrochemical cell to which we can mount membrane filters as shown in Fig.2. We compared the nonlinear dielectric properties in the case where yeast suspensions were filled in all the domains A, B and C with those in the case where suspensions were filled in the domain B and their supernatant was filled in A and C. The hole size of a membrane filter is 0.45 μ m, and yeast (about 5 μ m) can not pass through membrane filters.

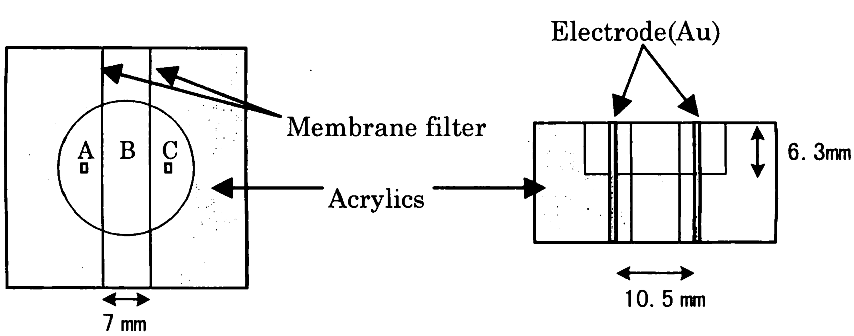

Fig.2. Electrochemical cell

2.3 Organisms

Yeast cells used in this study were *Saccharomyces cerevisiae* DKD - 5DH and were cultured (main-culture) in 30ml YEPD medium (0.3%yeast extract, 0.5%peptone, 1.0%dextrose, pH5.0) after pre-culture in order to keep the number of yeast always constant. The culture was maintained at a temperature of 25℃. Fig.3 shows the growth curve of the yeast cells under these conditions. The measurements were performed in the following growth phases: induction phase (Cultivation time 2h), logarithmic growth phase (Cultivation time 7h), and stationary phase (Cultivation time 25h). The number of cells in each experiment was adjusted to $10^7 ml^{-1}$ by centrifugation because the number of cells in suspensions affects to the characteristic responses[3].

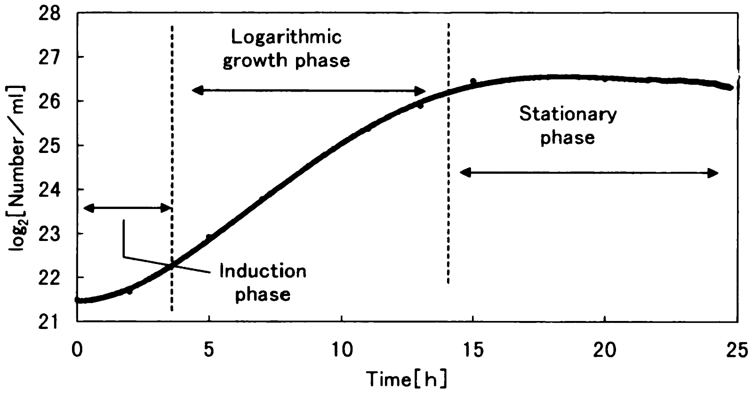

Fig.3. Growth curve of *Saccharomyces cerevisiae*

2.4 Process of measurement

We call the measurement result obtained from cell suspension Sample Data and the result obtained from its supernatant after centrifugation Control Data. Both are expressed the power spectrum values shown the equation (1). We regarded the value that was Sample Data minus Control Data as the result of the nonlinear dielectric properties.

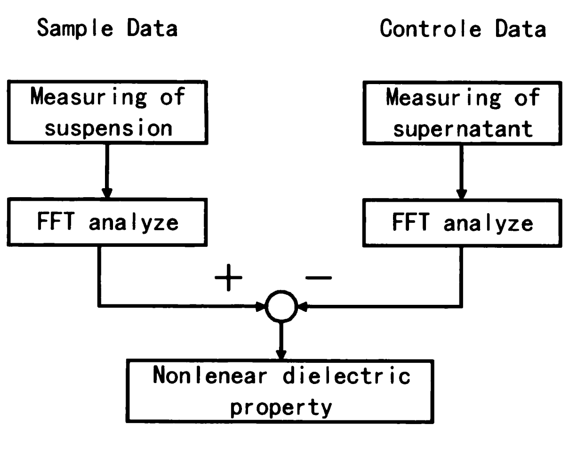

Fig.4. Process for measuring nonlinear dielectric properties

3. Result and discussion

Fig.5 shows the electrochemical cell (a) without membrane filter, (b) filled with yeast suspension in domain **A**, **B** and **C**, and (c) filled with yeast suspension in domain **B** and filled with its supernatant in domain **A** and **C**, and shows the obtained power spectra of the nonlinear dielectric properties under each condition. Shadow part in the figure indicates the domain of yeast suspensions.

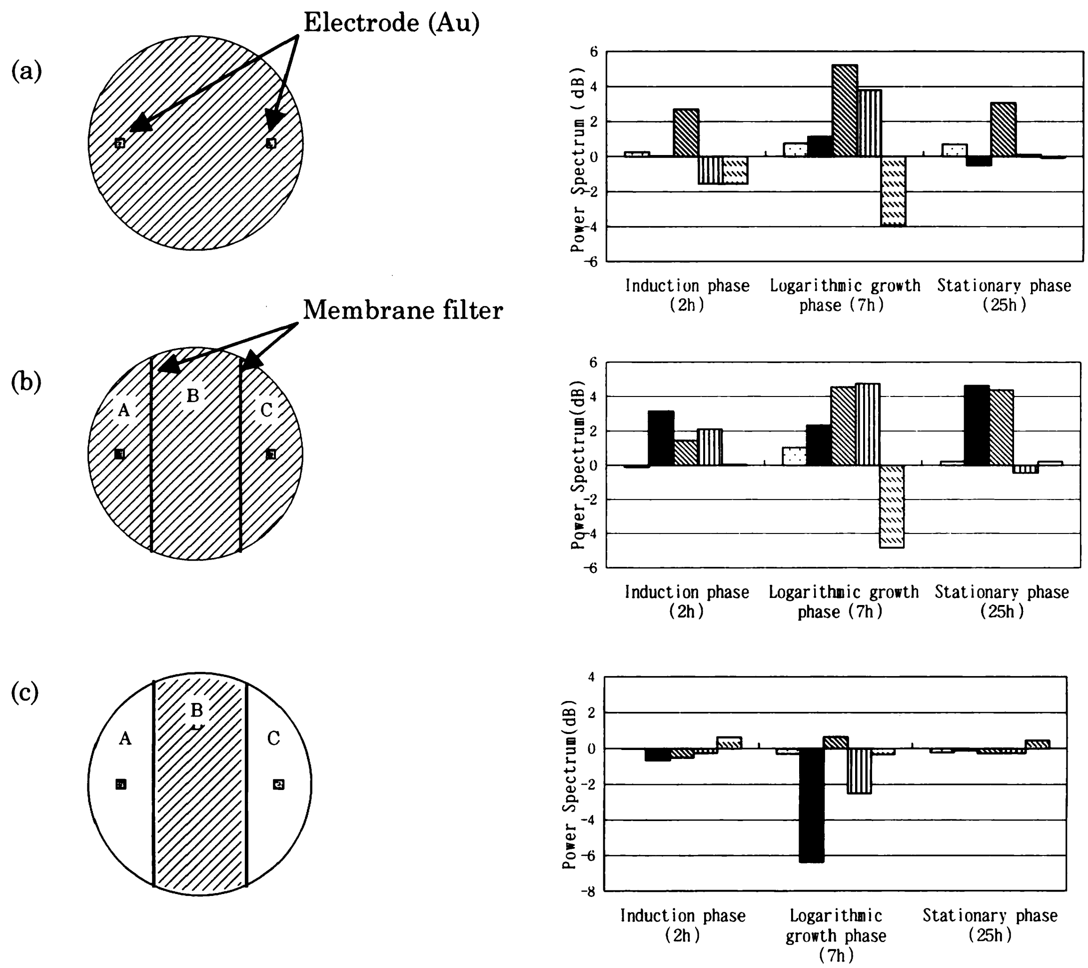

Fig.5. Result of the nonlinear dielectric properties under the condition
(a)Without membrane filter,
(b)Domain A, B, and C filled with yeast suspension,
(c) Domain B filled with yeast suspension and Domain A and C filled with its supernatant.

From Fig.5 (a) we can see that the power spectra show different pattern according to yeast activity based on each growth phase. The 3rd harmonic in the induction phase, the 3rd and the 4th harmonic in the logarithmic growth phase and the 3rd harmonic in the stationary phase appeared in the plus side. The 4th and the 5th harmonic in the induction phase and the 5th harmonic in the logarithmic growth phase, in the minus side. We made experiments to examine the effect of yeast adhesion on electrodes by using the electrochemical cell mounted with the membrane filter. We studied the effect of the membrane filter itself on the result of nonlinear dielectric responses. Cell suspension was put in the domain A, B and C. The graph in Fig.5 (b) showed that although the data at 2h and 25h had a large change, compared with those in Fig.5 (a), the data at 7h were relatively small. It follows from this that when yeast cells are relatively non-active in the induction phase and the stationary phase, the power spectra are influenced by the membrane filter as well as by biological activity, and when yeast cell is very active in the logarithmic growth phase, nonlinear responses by the biological activity of yeast cells are more dominant than by the membrane filter itself. In Fig.5 (c) the harmonics in the induction phase and the stationary phase hardly appeared. The 2nd and the 4th harmonics in the logarithmic growth phase appeared in the minus side greatly. Since the results of the nonlinear dielectric responses are Sample Data (power spectrum of yeast suspension) minus Control Data (power spectrum of its supernatant), it can be said that at 2h and 25h the power spectrum of Sample Data was almost the same as the power spectrum of the Control Data. Therefore we can say that, at 2h and 25h, the supernatant in domain **A** and **C** strongly reflected to response results and the effect of yeast suspensions in domain **B** are small. On the other hand, it is considered that yeast cells in the domain **B** at 7h which have strong multiplication capability and activity reflect to response results strongly rather than the effect of the supernatant near electrodes.

In concluding, we can say that nonlinear dielectric responses were induced according to biological activities based on each growth phase. Therefore this method can be useful to acquire the biological activities of yeast cells.

Reference

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