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Nonlinear dielectric properties and their relationship to the physiological state (resting or active) of *Saccharomyces cerevisiae*

by

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Synopsis

The construction of a nonlinear dielectric spectrometer is described. The system is applied to the study of cell suspensions of *Saccharomyces cerevisiae*. Substantial harmonics are generated by these cells when stimulated by a.c. exciting field. With resting suspensions of yeast, substantial third harmonics were generated with an optimum frequency (between 15 and 20 Hz) and optimum electric field (between 0.8 and 2.8). When D-glucose solution was added to the resting cell suspensions of *Saccharomyces cerevisiae,* glucose concentration was built up, then the third harmonic disappeared and second harmonic may be observed immediately. After two hours, glucose vanished and the nonlinear spectra returned to approximately their initial value.

Keywords : *Saccharomyces cerevisiae*, a.c.exciting field, substantial harmonics, nonlinear dielectric properties, resting cell, living cells,

1. Introduction

The nonlinear dielectric properties of biological systems was recently studied using resting cell suspensions of *Saccharomyces cerevisiae*[1]. Substantial 3rd harmonics were generated by these cells in an exciting a.c. electric field. The generation of these harmonics occurred only in living cells, with optimal field strength of 0.8-2.8 V/cm and optimal frequency of excitation of 15-20 Hz, respectively. It was suggested that it may be ascribed largely to the H^+-ATPase present in the plasma membranes of these cells, mainly on the basis of inhibitor studies, but latterly via mutant studies. Also, when stimulated by electrical fields containing more than one sinusoidal frequency, it was recognized that the response was characteristic for the nonlinear yeast ATPase system[2]. In experiments using the obligately aerobic soil bacterium *Micrococcus luteus*, it was found that the respiratory chain was the major source of nonlinear dielectricity in this organism, and that the type of harmonics observed depended upon whether or not the respiratory chain was active[3]. When studying the nonlinear dielectric properties of photosynthetic bacterium *Rhodobacter capasulatus*, it was shown that the photosynthetic electron transport chain was the major

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contributor to the nonlinear dielectric behaviour observed[4]. On the other hand, dormancy, or viable and vital states were used to refer to the physiological states of a cell, and were suggested as new problems in biological sciences[5]. It was therefore of interest to study whether the nonlinear dielectric method could be used to establish the physiological activity of a cell suspension.

The present approach illustrates how one can monitor the transition from a resting cell into an active state, by measuring the nonlinear dielectric properties of *Saccharomyces cerevisiae*. There is a good correlation between the glucose concentrations and the substantial harmonics.

2. Nonlinear dielectric measurements

Nonlinear dielectric measurements were carried out a computer system which was built around an IBM-PC-AT compatible microcomputer with a Cyrix 80C87 maths coprocessor. One of the expansion ports was fitted with a 16 bit A/D and D/A board. A sine wave was generated digitally from the PC, converted by the D/A converter, using a minimum of 20 steps/cycle, and applied across the outer current electrodes of an electrochemical cell. A block diagram of the nonlinear dielectric spectrometer is shown in Fig. 1. To minimize the contribution of electrodes was used[6]. Fig. 2 shows the dimensions and geometry of the

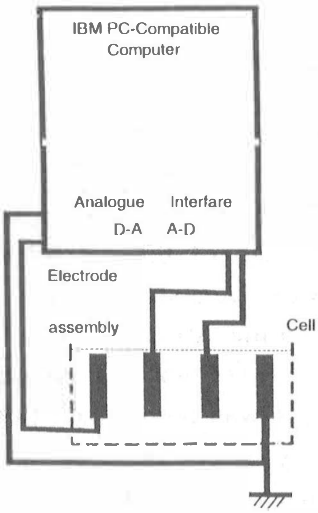

Fig. 1 A block diagram of the nonlinear dielectric spectrometer.

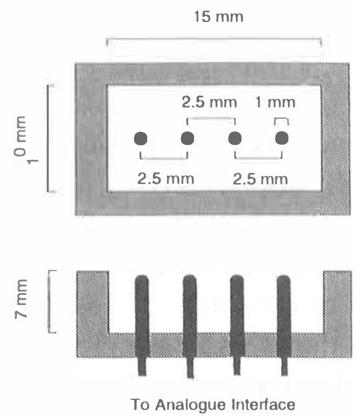

Fig. 2 Plan of the electrochemical cell, to show the dimensions and geometry. The electrodes were 24-carat gold pins, of length 6 mm. The volume of suspension used was 1 ml.

electrochemical cell. The data were logged from the inner electrodes at a sampling frequency which was typically at 20 times the fundamental frequency of exciting voltage and for a time that was specified in terms of a number of blocks, each block consisting of 512 samples. At the end of time specified, the data were Fourier transformed. These procedures were repeated 30 times for resting suspensions of *Saccharomyces cerevisiae* (concentration: 20 mg dw/ml), and the spectral data of averaging were stored on the computer's hard disk. The volume of suspension used was 1 ml. A reference spectrum was acquired using the supernatant of cell suspensions and the same procedures were carried out. Finally the nonlinear dielectric spectrum obtained was divided by the reference power spectrum, and also stored on the hard disk. A glucose concentration was measured by a Reflolux S (Boehringer Ltd.) at the same time. All measurements were done at room temperature.

3. Results

Fig. 3 shows a typical a.c. exciting voltage waveform with 10Hz (A) and a spectrum obtained from a supernatant of suspension of resting cells of *Saccharomyces cerevisiae* (B), with an exciting voltage measured between the outer electrodes of 1.5V (field strength of 2.0 V/cm) and the scanning frequencies between 10-50 Hz. A typical nonlinear dielectric spectrum obtained from a resting cell suspension of *Saccharomyces cerevisiae* is shown in

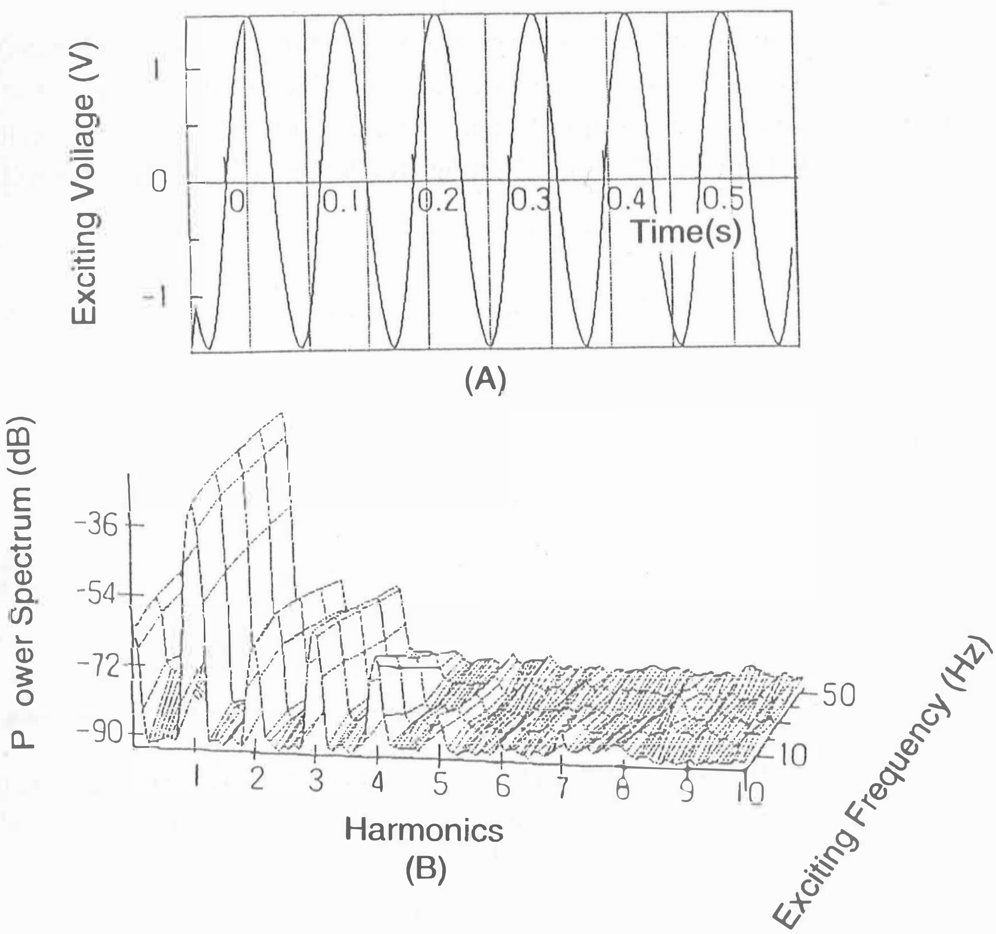

Fig. 3 Exciting Voltage and spectra.

(A) A typical exciting voltage waveform.

(B) The spectra obtained from a supernatant of a suspension of resting cells of *Saccharomyces cerevisiae*.

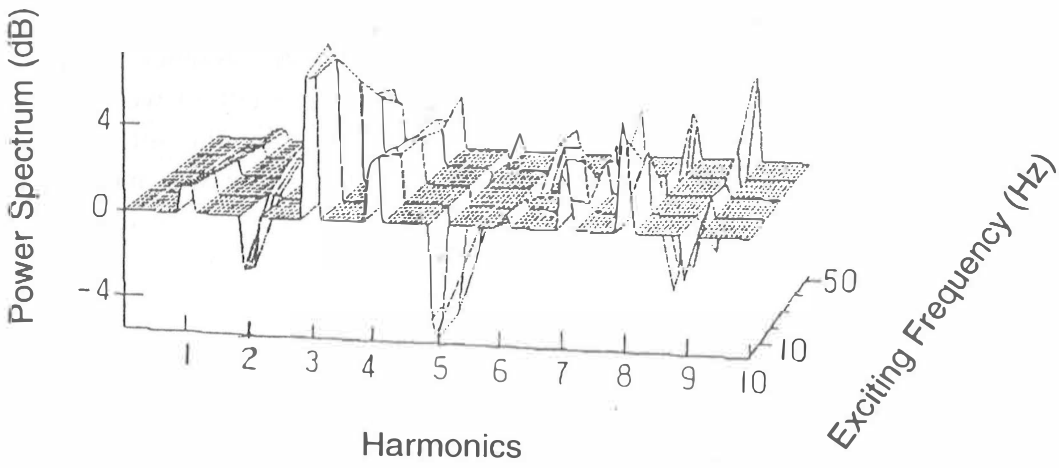

Fig. 4 The nonlinear dielectric spectra obtained from a resting cell suspension of *Saccharomyces cerevisiae*.

Fig. 4 (exciting voltage of 1.5V and scanning frequencies of 10-50 Hz).

A glucose solution of 1ml (the concentration of 100m mol/l after addition) was added to the resting cell suspension of *Saccharomyces cerevisiae* of 100ml. Fig. 5 shows the time variation of the glucose concentration (A) and second and third harmonic spectrums (2 and 1), with an exciting voltage of 1.5V and exciting frequency of 20 Hz (B), respectively.

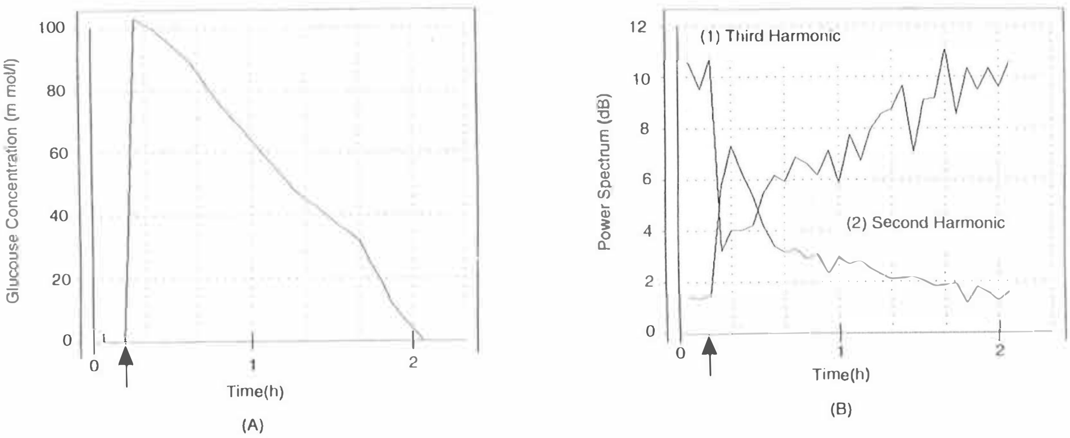

Fig. 5 Time variation of glucose concentrantion and substantial harmonics.
(A) Time variation of glucose concentration.
(B) The second (2) and third (1) nonlinear harmonics spectra at exciting frequency of 20 Hz, when the glucose solution was added in the cell suspensions of *Saccharomyces cerevisiae*.

4. Discussion

In the previous work[1)], and in the present experiments (Fig. 4), we have measured the 3rd harmonics from resting cell suspensions of *Saccharomyces cerevisiae*. The odd harmonics are the typical property obtained by the resting cell, but even harmonics are absent under the stated conditions. When the glucose solution was added to the suspensions of the resting cells (arrows in Fig. 5), the harmonics changed after a short time. It is considered that the time lag is due to activation of the yeast ATPase[2].

In these experiments, we have seen that the glucose concentration decreases at an essentially constant rate a few minutes after adding glucose, and odd harmonics disappear, whilst even harmonic appear immediately. After two hours, glucose vanishes and the nonlinear spectra return to their initial value. Although Fig. 5 (B) showed essentially the exciting frequency of 20Hz, the same phenomena were obtained with other frequencies. It is judged that the resting cells turn to a metabolically active state, and back to a resting state within a few minutes of the change of nutrient status.

5. Conclusion

Substantial harmonics are generated by the resting cell suspensions of *Saccharomyces cerevisiae*. The generation of these harmonics occurs only in living cells, and the 3rd harmonics disappear and 2nd harmonics appears when the cells transit from resting to active state by adding the glucose solution.

In these results, it is recognized that by measuring the nonlinear dielectric properties of cell suspensions, one can observe the transition from resting cell into the active state by the nature of the harmonics generated. Thus the nonlinear dielectric behaviour will be expected to monitor the physiological state of biological systems.

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