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時空間制御された電場・プラズマ場での水・タンパク質・生体の状態変化
Property Change of Biological Components (Water, Protein and Living Organisms) in Temporally and Spatially Controlled Electric Field and Plasma

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1. Introduction

In bio-applications of pulsed power, the novel control of plasma and intense pulsed electric field (PEF) with spatially and temporally is important to adapt whole bio-scale as shown in **Fig. 1**. Especially, the nano- or sub-nanoseconds pulse is required to satisfy the limit of relaxation or charging time of water molecule and protein. The pulsed power generator is designed to match temporally changed impedance of bio-specimen by choosing optimum system (pulse forming line, H-bridge circuit, inductive and capacitive energy storage circuit) and semiconductor switches such as SiC-FET [1, 2]. In this article, the effect of applying voltage and plasma on property of biological components such as water, protein and living organisms is described.

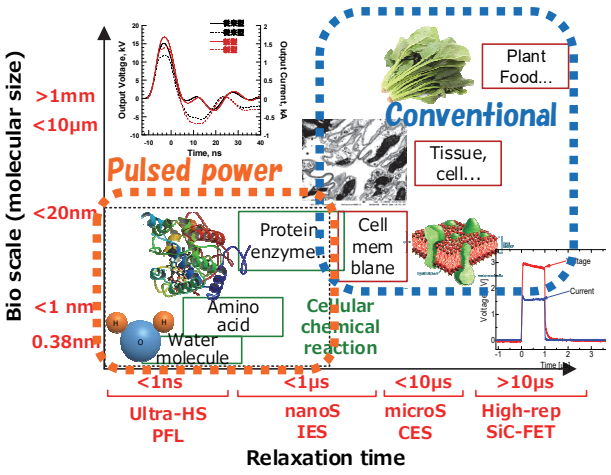

Fig. 1 Relation between bio-scale and relaxation (or charging) time required as pulsed power specification.

2. Water Nucleation in AC Electric Field

A water nucleation is the process in freezing water containing materials such as gel-type foods, vegetables, meets and fishes. Recently, the freezing process with applying an AC electric field (acEF) is trying to be utilized in some companies for controlling ice nucleation in the freezing process.

Figure 2 shows ice nucleation rate of distilled water for different frequencies of acEF [3]. The distilled water is used as samples, and is placed between the parallel plate electrodes in a freezer. The temperature inside the freezer is fixed at -15 ℃. The sinusoidal wave voltage is applied to the electrode to generate acEF. The acEF strength is fixed at 50 kV/m. A thermistor is used to measure the temperature. The ice nucleation rate R is obtained using the equation;

$$R(T_i) = \frac{r n_i}{\Delta T_i \left(\frac{n_i}{2} + \sum_{j>i} n_i\right)} \qquad (1),$$

where, r is the freezing speed [℃/s], n_i is the nucleation number for each temperature band, and ΔT_i is the band width of temperature (=1 K).

The nucleation rate increase with increasing acEF frequency. The nuclation rate R is expressed using Arrhenius equation as follows;

$$R = J_0 \exp\left(-\frac{\Delta G^*}{k_B T}\right) \qquad (2),$$

where, J_0 is the frequency factor, ΔG^* is the activation energy, and k_B is Boltzmann constant. Figure 2 shows that the slope of the lines is independent of acEF frequency and the nucleation rate shifts in parallel with increasing the frequency. The result indicates that the acEF mainly affects the nucleation frequency factor J_0.

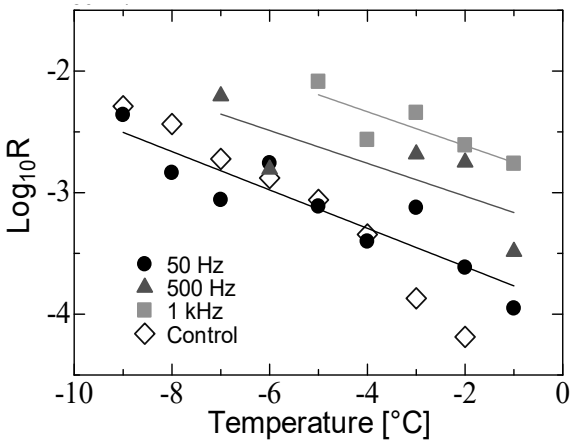

Fig. 2 Nucleation rate of distilled water for different frequencies at 50 kV/m applied electric field.

3. Conformational Change of Protein

Enzymes are large-size proteins which works as biological catalysts. The enzymes are frequently utilized in food industry such as fermentation in brewing process and making dairy products. Pulsed electric field (PEF) technology is a basically non-thermal food treatment method that involves the use of short electricity pulses for microbial and enzymatic inactivation through the protein conformational change.

Figure 3 shows the residual activity of α-amylase for various electric field strengths at same input energy of 720 J [4]. The residual activity of α-amylase decreases with increasing strength of electric field at same input energy. The strength of electric field is strongly related to efficiency of protein conformational change. Therefore, the strength of electric field is dominant parameter in PEF treatment for enzyme inactivation.

Figure 4 shows the tertiary structure change by PEF treatment [4]. The fluorescence spectra of α-amylase at 280 nm wavelength of excitation light is used to monitor the tertiary structure change for various strength of pulsed electric field at same input energy of 720 J. The tertiary structure (mainly tryptophan) of α-amylase also decreases with increasing strength of electric field at same input energy. This result indicates that the strength of electric field strongly affects the efficiency of protein conformational change, resulting in enzyme inactivation.

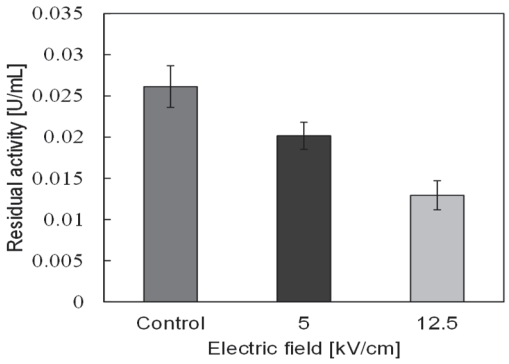

Fig. 3 Residual activity of α-amylase as a function of electric field strength at same input energy of 720 J.

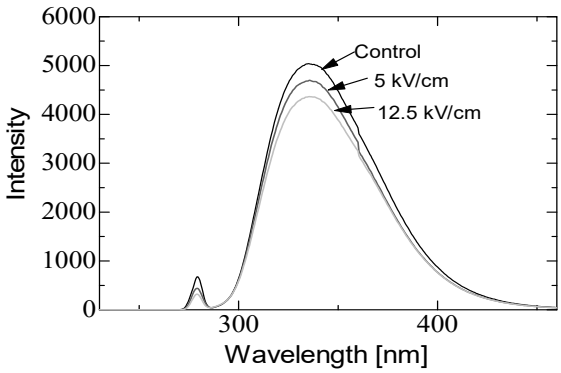

Fig. 4 Fluorescence spectra of α–amylase at 280 nm wavelength of excitation light emission after PEF treatment for various PEF strength at 720 J input energy.

4. Pulsed Electric Stimulation for Fruiting

The pulsed high-voltage can be used as stimulation for fruiting promotion. The mushroom fruiting bodies are also promoted by the pulsed voltage application as electrical stimulation. The basically process is as follows; the mushroom hyphae are accelerated with Coulomb force owing to the strong electrical field. As the result, some hyphae are scratched and cut by the movement. These hyphae cutting or scratching are worked as trigger to transfer from vegetative growth to reproduction growth. This process can be confirmed using metabolism analysis. **Figure 5** shows the hyphae activity analysis using hydrophobin release, which is mainly observed before the fruit body formation in nature [5]. The hydrophobin release decreases for three hours after stimulation. However, the hydrophobin release from the vegetative hyphae increases 2.3 times one day after the stimulation.

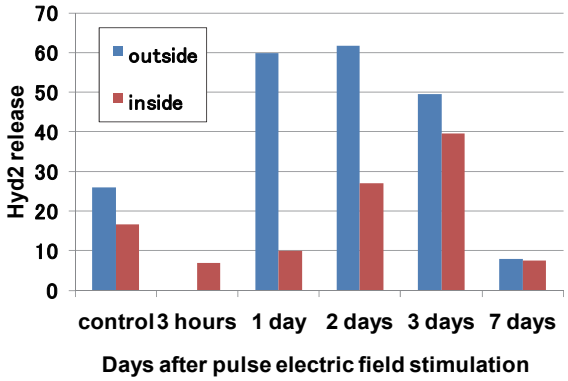

Fig. 5 Hydrophobin release for various periods after 100 kV pulsed high-voltage stimulation at two different parts of the hyphae. The label "outside" indicates actively cell separation (vegetative) hyphae and the "inside" is inactive hyphae.

Acknowledgments

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