Observation of *Zygosaccharomyces rouxii* cells after pulsed electric field

Yuta Nakada[1], Tomohiro Oku[1], Akinori Zukeran[1]
Risei Wada[2], Jun Sawai[3], Takashi Inui[4]

[1]Dept. of Electrical and Electronic Engineering, Kanagawa Institute of Technology, Kanagawa, 243-0292, Japan
[2]Dept. of Applied Bioscience, Kanagawa Institute of Technology, Kanagawa, 243-0292, Japan
[3]Dept. of Nutrition and Life Science, Kanagawa Institute of Technology, Kanagawa, 243-0292, Japan
[4]Facility Engineering Dept., Fuji Electric Co., Ltd., Tokyo, 191-8502, Japan
Keywords: inactivation, pulsed electric field, ballast water, scanning electron microscope,
Corresponding author email: zukeran-akinori@ele.kanagawa-it.ac.jp

Ballast water is used as weights for balancing ships. The ballast water is discharged at a distinction port where is different from a source port country, whereby that may cause problem in a marine ecosystem. The purpose of this study is to inactivate microorganisms in seawater using pulsed electric field.

The schematic diagram of the experimental system is shown in Fig. 1. Simulated seawater was poured into a beaker and installed a needle electrodes, which the electrode gap was between 1 and 10 mm. Microorganism concentration, which was *Zygosaccharomyces rouxii* (Z. *roxii)*, in simulated seawater was 10^4~10^5 CFU/ml. The pulsed electric field was generated in the seawater due to applying the pulse high voltage to between electrodes. The voltage and the current were measured using a high voltage probe and a current probe. The peak value, the rising time and the frequency of the pulse voltage were 10 kV, 75 ns and 50 Hz, respectively. Seawater of 1 mL was sampled periodically, and then incubated for 72 hours at 25 °C. The survival ratio η was calculated by equation (1):

$$\eta = \log (N/N_0) \qquad (1)$$

where N is the number of colony count after treatment, and N_0 is the initial number of colony count.

The survival ratio as a function of the number of pulses for various electrode gaps is shown in Fig. 2. Decreasing the survival ratio means improving the inactivation effect. The survival ratio decreased with increasing the number of pulses for any gaps. And the survival ratio decreased as the gap decreased.

The SEM images of *Z. rouxii* before and after applying the pulsed electric field were shown in Fig. 3. The microorganisms were washed three times with pure water by centrifugation to remove seawater before observation. *Z. rouxii* before treatment was a spherical shape as shown in Fig. 3 - a). However, it was clear that *Z. rouxii* after treatment was distorted as shown in Fig. 3 - b). This result shows that the survival ratio decreased as shown in Fig. 2 due to destroying cell membrane. It is considered that the survival ratio decreased with decreasing the gap because cell membrane tend to be destroyed due to increasing the electric field intensity.

These results indicated that the microorganism in simulated seawater was destroyed in the pulsed electric field due to electroporation.

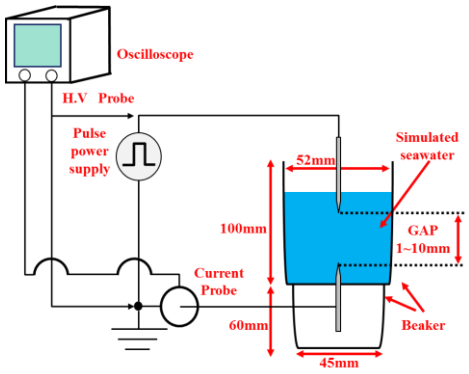


Fig. 1 Schematic diagram of experimental system.

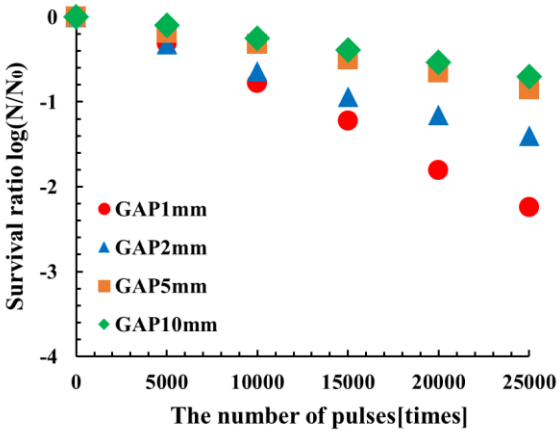
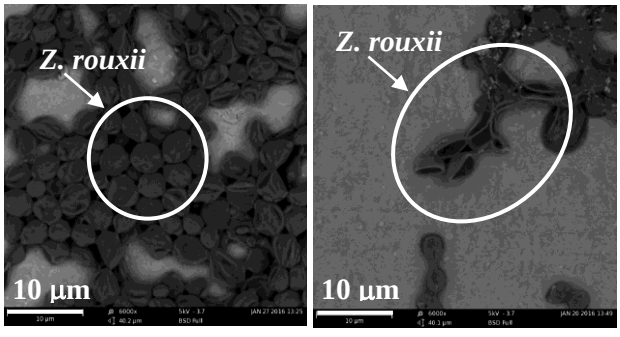


Fig. 2 The survival ratio as a function of the number of pulses for various electrode gaps.



a) before b) after

Fig. 3 SEM images of *Z. rouxii* before and after applying the pulsed electric field.