1P1-8

Effects of laser-induced stress wave irradiation on Saccharomyces cerevisiae

Kota Miyazaki[†1] and Koji Aizawa[1]
([1]Guraduate school of Engineering, Kanazawa Institute of Technology)

1. Introduction.

The laser-induced stress wave (LISW) becomes extremely strong when the ablation plasma and expansion generated at the medium surface are confined by the transparent medium [1-2]. In fact, it has been reported that LISW exceeding 100 MPa have been generated in solid media [3]. It has also been reported that irradiation of human fibroblasts with underwater spark discharge shock waves, which can generate equivalent pressure values, promotes cell proliferation [4]. We consider that LISW might have a similar effect on proliferation. For instance, the gene transfer efficiency of the electroporation method could be improved if the cell cycle could be artificially accelerated.

In this study, we investigate the effects of LISW on living organisms using Saccharomyces cerevisiae (yeast). The pressure values of the LISW generated in the cuvette were also examined to determine the actual effect on proliferation of yeast.

2. Pressure distribution inside the cuvette

2.1 Experimental Method

Figure 1 shows a photograph of experimental system using an Nd:YAG laser (Spectra-Physics, LAB-130-10) and a liquid target with a circulation device. The black ink (Kuretake, BA3-18) is circulating in a transparent resin tube (TYGON, R-3603, inner diameter: 4 mm, outer diameter: 6 mm) during experiment. The cuvette is then placed on a transparent resin tube circulating the black ink. A second harmonic pulsed light (wavelength: 532 nm, pulse width: 10 ns) was focused with a diameter of about 3 mm on the black ink inside the transparent resin tube fixed to the cuvette holder through a convex lens. The generated LISW propagates inside the cuvette through its bottom. Laser energy separated by a beam sampler (Sigma, BS4-30C03-10-550) was measured with an energy detector (Gentic, QE25LP-D-MB-QED-DO). The temporal pressure waveform of LISW were measured by a hydrophone sensor (MULLER instruments, 100-100-1) with pure water (500 μL) in the cuvette. The position of the hydrophone tip was manually adjusted, and the waveform measurements are taken several times at 1 mm, 4 mm, 6 mm, 11 mm, and 16.5 mm from the cuvette bottom where yeast is suspended. The pressure values were calculated from the voltage measured using a digital oscilloscope (Iwatsu, DS-5654A).

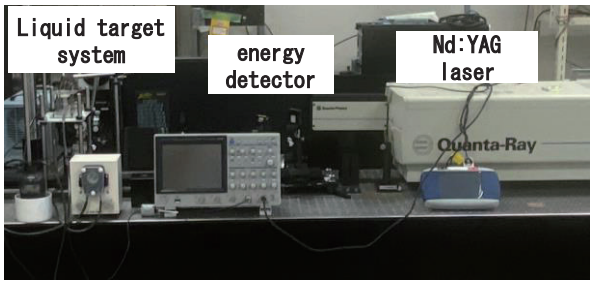

Fig. 1 Nd:YAG laser and experimental system

2.2 Experimental Results

Figure 2 shows the distribution of pressure values inside the cuvette. This experiment was performed with an average laser energy of 90.2 ± 1.7 mJ. The maximum pressure was 14.0±0.6 MP at 1 mm from cuvette bottom, on the other hand, the minimum pressure was 2.00 ± 0.1 MPa at 16.5 mm. The detailed results about pressure waveform have

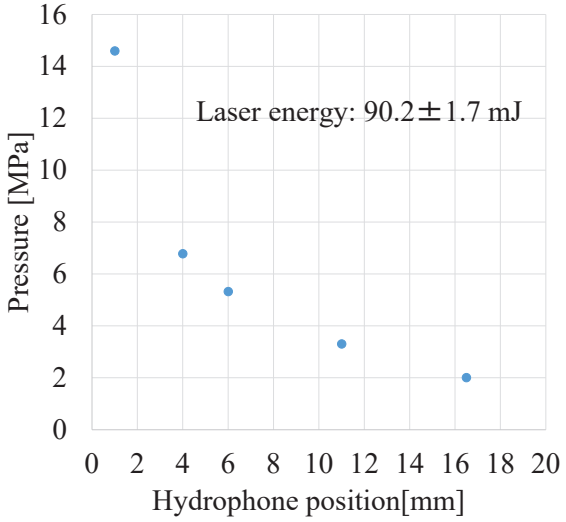

Fig. 2 Pressure distribution inside the cuvette

[†]6201733@st.kanazawa-it.ac.jp ,

reported in Ref. 5.

3. Effect of LISW irradiation on yeast

3.1 Experimental Method

From the pressure distribution experiment shown in Fig. 2, the pressure values at the cuvette bottom were 7 times larger than that at the top of the cuvette. In order to fully affect the yeast even at the top of the cuvette, therefore, the LISW was irradiated for 10, 50, and 100 cycles so that the cumulative pressure exceeded the pressure value at the bottom of the cuvette. The experimental procedure involved preparing two suspensions (500 μL) with yeast and irradiating to the other of two samples with LISW. After irradiation, the two samples were incubated in YPD liquid medium at the temperature of 34 ºC for 0 to 12 hours. Each medium taken every 2 hours from the sample was treated with trypan blue (BIORAD, trypan blue dye, 0.40% solution), and viability and concentration of yeast were measured in a cell counter (BIORAD, TC20) to determine the effect of LISW irradiation on yeast.

3.2 Experimental results

Figure 3 shows the concentration changes on time of yeast with and without 100 cycles LISW irradiations. From the results occurring between 0 and 8 hours, the concentration and specific growth rate showed 1.2-fold and 0.12 /h differences in maximum, respectively. The viability with and without LISW irradiations were almost the same ranged from 84% to 93% and from 77% to 94%, respectively. In the time elapsed between 8 and 10 hours, the experimental results showed little difference in concentration and specific growth rate. However, the concentration and specific growth rate observed from 10 to 12 hours showed 1.3-fold and 0.23 /h differences in maximum, respectively. As can be seen in the comparison of specific growth rate after 100 cycles irradiation, the growth rate decreased in the LISW-irradiated samples. In the case of 10- and 50- cycles irradiation, however, the irradiated samples showed high both concentration and specific growth rate.

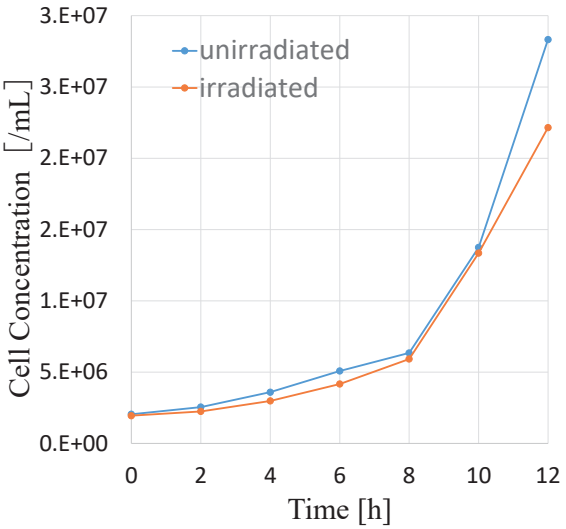

Fig. 3 Changes in cell concentration at elapsed time with and without 100 cycles LISW irradiations

4. Conclusion

In this study, we found that the growth tendency of the yeast was different depending on the number of irradiation cycle of LISW when the LISW irradiations were continuously applied between 2 and 20 MPa as pressure to the yeast in the cuvette.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (21K04202).

References

1) J. Nuckolls, L. Wood, A. Thiessen, and G. Zimmerman, Nature **239**, 139 (1972).

2) R. Fabbro, J. Fournier, P. Ballard, D. Devaux, and J. Virmont, J. Appl. Phys. **68**, 775 (1990).

3) T. Ando, S. Sato, S. Takano, H. Ashida, and M. Obara, Appl. Surf. Sci. **255**, 9898 (2009).

4) K. Miyazaki and K. Aizawa, Autumn Meeting Acoustical Society of Japan, 2022, 2-P-17 [in Japanese].

5) K. Miyazaki and K. Aizawa, to be submitted in 2023 Autumn Meeting Acoustical Society of Japan [in Japanese].