Effects of destruction of *Euglena gracilis* by ultrasonic cavitation

Yoshihito Azuma[1‡], and Ken Yamamoto[1*]([1]Grad. School Eng., Kansai Univ.)

1. Introduction

Ultrasonic cavitation is a phenomenon in which microbubbles are generated when water is irradiated with ultrasonic waves. We have been studying the detailed mechanism underlying the physical and chemical effects of ultrasonic cavitation by using microscopic samples (e.g., microcapsules, algae, zooplankton, bacteria) as probes. It has been reported that ultrasonic irradiation can damage samples via physical effects such as mechanical resonance, shear stress, and microjets[1-3] and also by chemical effects such as OH radicals[4,5]. We have previously reported that small holes (pits) form on the surface of starch particles with diameter of 10–20 μm via microjets when the cavitation density is high and the bubble diameter is less than these values[6].

In this study, *Euglena gracilis* was used as a microscopic sample. Euglena are eukaryotic unicellular organisms that store various vitamins, amino acids, and paramylon (β-1,3-glucan). Paramylon is used as an ingredient in functional foods, and previous studies have suggested its potential use as a film or a filler to improve the functionality of resins[7,8]. In addition, paramylon nanofibers obtained by alkaline extraction of paramylon are attracting attention for their mechanical strength and environmental friendliness[9]. *E. gracilis* has a particularly high paramylon content[10,11], making it a potentially valuable resource. There are various methods for extracting paramylon from *E. gracilis*, including sodium dodecyl sulphate extraction[12], French press treatment[13], and sonication[14]. In this paper, we aim to destroy *E. gracilis* using ultrasonic cavitation to facilitate extraction of paramylon and to elucidate the mechanism. The particle size distribution was measured using a particle analyzer, and the number of undamaged cells was measured using optical microscopy after ultrasonic irradiation at various frequencies (26–3600 kHz). Furthermore, the influence of cell concentration on cell destruction was examined when 430 kHz ultrasonic waves, which displayed a high destruction rate, were used for irradiation.

2. Experimental

2.1 Sample

In this study, we used *E. gracilis* which has a spherical shape and an average diameter of about 20 μm, as shown in **Fig. 1**. For the ultrasonic irradiation experiments, the cultured *E. gracilis* suspension (5×10^7 cells/mL) was diluted in purified water by factors of 1, 2, 5, 10, and 30 in order to investigate the effects of concentration.

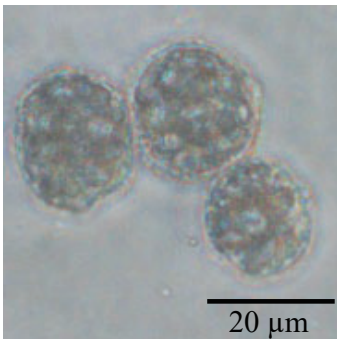

Fig. 1 Optical microscope image of *E. gracilis* before ultrasonic irradiation.

2.2 Sonication

The ultrasonic generator (QUAVA mini; Kaijo Corporation, Tokyo, Japan) comprised an oscillator unit and a transducer unit containing an ultrasonic transducer with a diameter of 30 mm, as shown in **Fig. 2**. A stainless-steel cylindrical sample tank with an inner diameter of 48 mm was installed at the top of the transducer unit. Ultrasonic frequencies of 26, 430, 1600, and 3600 kHz were applied and the acoustic power was measured using the calorimetric method and maintained at a constant power of 10±1 W. Cooling water inside the sample tank was used to maintain the sample temperature at 20±1°C, and 100 mL of sample was sonicated for up to 30 min.

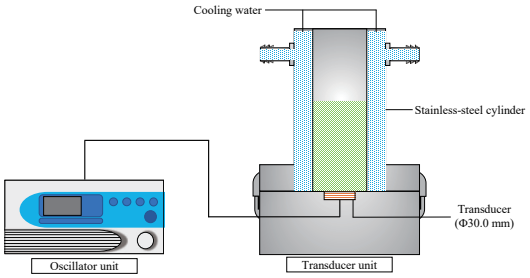

Fig. 2 Experimental apparatus for sonication.

2.3 Evaluation method

The particle size distribution was measured using a laser diffraction particle size analyzer (SALD-2300: Shimadzu Corporation, Kyoto, Japan).

The ultrasonic distribution rate was calculated using an optical microscope (IX73; Olympus, Tokyo, Japan) and a counting chamber (8100204; Hirschmann, Eberstadt, Germany). Optical micrographs of four sections were taken for each sample, and the undamaged cells were counted before and after ultrasonic irradiation. Then, the destruction rate was calculated from the number of

E-mail: †k313616@kansai-u.ac.jp, *ken@kansai-u.ac.jp

cells before sonication and the number of cells after ultrasonic irradiation for various lengths of time[3].

3. Results and Discussion

3.1 Influence of ultrasonic frequency

Fig. 3 shows a plot of the destruction rate at 5 min of irradiation according to frequency. The destruction rate increased with irradiation time for all frequencies. The destruction rate at 5 min of irradiation decreased in the order of 950 kHz ≥ 430 kHz > 200 kHz > 1600 kHz, and the destruction rate exceeded 90% at 430 and 950 kHz. It is thought that microjets form when the bubble diameter is smaller than that of the ultrasonicated object. Minnart's formula, which is commonly used to estimate the resonance diameter of cavitation bubbles[15], was calculated to be 15, 6.9, and 4.0 μm at 430, 950, and 1600 kHz, respectively. *E. gracilis* has a diameter of approximately 20 μm, and thus it is considered that the cell membrane was destruct by microjets because the bubble diameter was smaller than cell diameter. Moreover, the shear stress is proportional to the bubble wall velocity gradient, and the bubble wall velocity depends on the bubble diameter when the acoustic power is constant. Therefore, cell destruction due to shear stress is also possible at these frequencies[16]. However, the destruction rate was low at 26 kHz. At this frequency, the resonant bubble diameter was 250 μm. It is expected that a microjet did not form and shear stress did not occur because the bubble diameter was larger than the cell diameter. In addition, the destruction rate was significantly lower at 3600 kHz than at 1600 kHz. This is attributed to the decrease in microjet stress and shear stress resulting from the decrease in microjet velocity due to the increase in frequency[6].

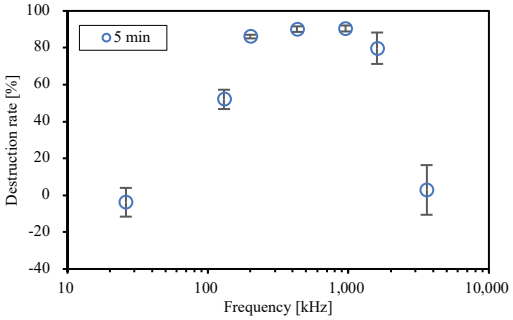

Fig. 3 Irradiation frequency dependence of the destruction rate of *E. gracilis* at 5 min.

3.2 Influence of concentration

Fig. 4 shows a plot of the cell destruction rate in *E. gracilis* according to dilution factor at 1 and 3 min of irradiation. The destruction rate increased with irradiation time for all dilution factors. When the dilution factor was 30, the destruction rate exceeded 90% at 1 min irradiation, but was about 40%–55% when the dilution factor was 1, 2, 5 and 10 ($1 \leq 2 < 5 \leq 10$). Such changes in the destruction rate are attributed to the effects of dilution of the suspension on viscosity. At a dilution factor of 1, the viscosity was 32.1 mPa·s (shear rate: 238 s^{-1}), while at a dilution factor of 30, the viscosity was 1.04 mPa·s (shear rate: 241 s^{-1}). It is considered that as the dilution factor decreases, the viscosity of the suspension increases, which in turn attenuates the sound waves propagating in the solution. These effects are expected to increase the time required for *E. gracilis* cell destruction. This result indicates that the size of bubbles and objects as well as the viscosity of the suspension are important for cell destruction of *E. gracilis* via ultrasonic irradiation.

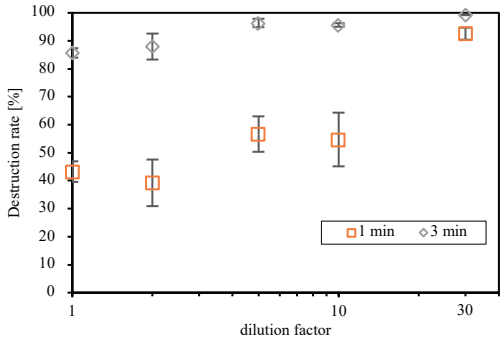

Fig. 4 Dilution factor dependence of the destruction rate of *E. gracilis* at 1 and 3 min.

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