Growth suppression effect of high-frequency ultrasound on *Microcystis aeruginosa*

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1. Introduction

Due to excess nutrient loading in water bodies, harmful algal blooms have become an increasing issue globally, leading to negative effects in both wild and domestic animals, and in humans due to the release of cyanotoxins. Microsystin (MC) has been one of the most famouse cyanotoxins in Japan. MC, typically produced by *Microcystis*, is categorized into hepatotoxin. MCs exist intracellularly and are extracelluarly released due to the damage of cell membrane. Although there are several methods, such as activated carbon filtaeratoin, reverse osmosis process and electrochemical treatment[1], to remove extracelluar MC, they involve a significant cost. Thus, it is important to remove *Microcystis* while it prevents from damging them.

Among currently available treatment methods, ultrasonic treatment has received increasing attention for algal control because of its low impact on ecosystems and the environment although there are problems in field applicatoin. Gas vacuoles within the cells possibly play a important role in the mechanism of the mechanical and chemical interactions between ultrasound and *Microcystis* because the gas can work as cavitaton nuclei. The damage of cell membrane should become serious as the sound pressure becomes large. This report summarizes the growth suppression effects of ultrasound on *Microcystis* and its dependence on the sound pressures

2. Methods

2.1 Ultrasound exposure and observation system

Figure 1 shows the ultrasound exposure system. Two coaxial transducers, concave and annular transducers, were located in a bottom of water tank (150×150×150 mm^3). The resonant frequencies of annular and concave transducers were 2.8 and 4.8 MHz, respectively. In this experiment, the annular transducer was employed for emitting ultrasound. The concave transducer was used for observing signals for cavitation. A polymer cell (H 20 mm×W 10 mm×D 10 mm) filled with algal solution was located at the focal point of annular transducer. Continuous sinusoidal electrical signal with frequency of 2.8 MHz was

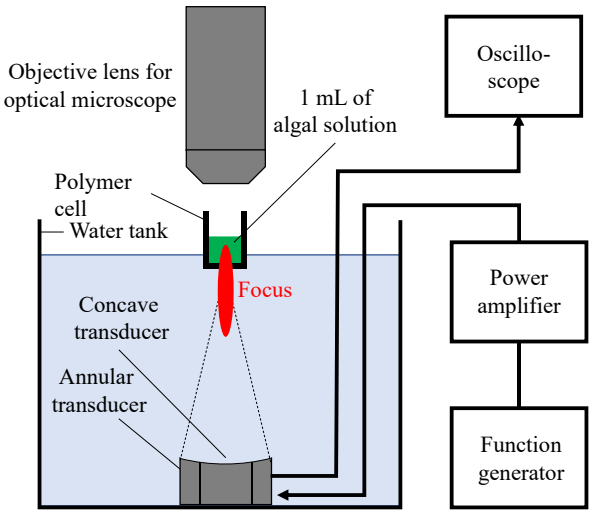

Fig. 1 Ultrasound exposure and optical observation system.

generated from a function generator (WF1968, NF). The voltage was 0 (control), 0.1, 1, 10, 50, 100, 150 and 200 mV$_{pp}$. The signal was amplified in a power amplifier (ZCA5252, RF AMP DESIGN) with amplification of 52 dB, and was input to the annular transducer. The ultrasound was irradiated during 2 min. The echo signal could be observed by the concave transducer and recorded by an oscilloscope (LeCroy, Wave Runner 6000). The behaviors of microcystis cells during ultrasound exposure were observed by using an optical microscope (KEYENCE, VHX-950F) with variable magnification lens (KEYENCE, VH-Z100T).

2.2 Culture of *Microcystis* cells

A unialgal culture of *Microcystis aeruginosa* strain NIES 3349 (National Institute for Environmental Studies, Japan) was grown in 10 L of MA medium at 23 °C under illumination at ca. 16 μmol m^{-2} s^{-1} and a 12:12-h light:dark cycle. Cultures were harvested during the exponential growth phase or the stationary growth phase. *M. aeruginosa* strain NIES 3349 produces microcystin-LR and -YR.

2.3 Evaluation of growth of *microcysitis aeruginosa*

The optical density of *microcysitis aeruginosa* at 405 nm (OD_{405}) was measured as an index of growth suppression effect of ultrasound. The measurement of OD405 was conducted every two days after ultrasound exposure.

3. Results

Optical observation showed that numerous microsystis cells aggregated due to the acoustic radiation force of standing wave formed in the polymer cell. **Fig. 2** shows the images of microsystis cells. Before ultrasound exposure, microsystis cells were homogeneously dispersed as shown in Fig. 2(a). Aggregates were formed during several ten seconds just after ultrasound exposure. The spatial interval between the aggregates was 500 μm~1 mm, which approximately corresponded to the wavelength of 2.8-MHz ultrasound. The size of an aggregate was 50~100 μm much larger than the diameter of a single cell. As soon as the ultrasound exposure was turned off, the aggregates were gradually separated. There is no difference in behaviors of microsystis cells between the stationary growth phase and late exponential growth phase.

Fig. 3 shows variation in OD_{405} (a) stationary growth phase and (b) late exponential growth phase. In case of exponential growth phase, there is no significant dependence of OD_{405} on the input voltage. In contrast, it seems that the ultrasound at input voltage of 100 and 200 mV_{pp} suppresses OD_{405} compared with control.

4. Summary

It was investigated how the ultrasound affect the growth of *Microcystis aeruginosa*. *Microcystis* in the stationary growth phase and late exponential growth phase were exposed to the continuous ultrasound with 2.8 MHz frequency. Optical observation showed that the acoustic radiation force induced the cell aggregation. The measurement of optical density suggested that there are the growth suppression effects of ultrasound only in case of stationary growth phase.

Acknowledgment
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References

1. B. Jeon, J. Han, K. Makino and H. Park: Environ. Eng. Sci., **31** (2014) 525.

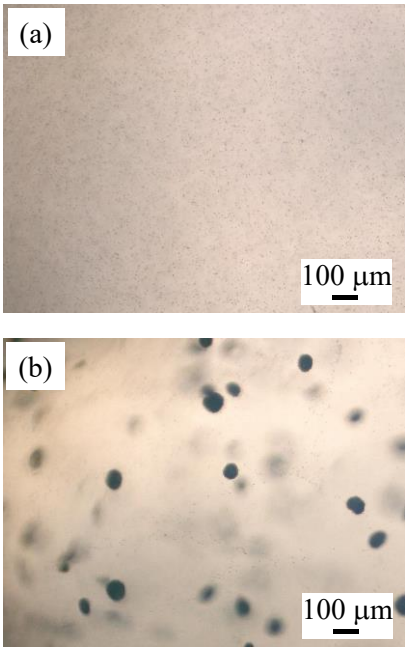

Fig. 2 Images of microcysitis cells (a) before and (b) during ultrasound exposure (Input voltage 200 mVpp).

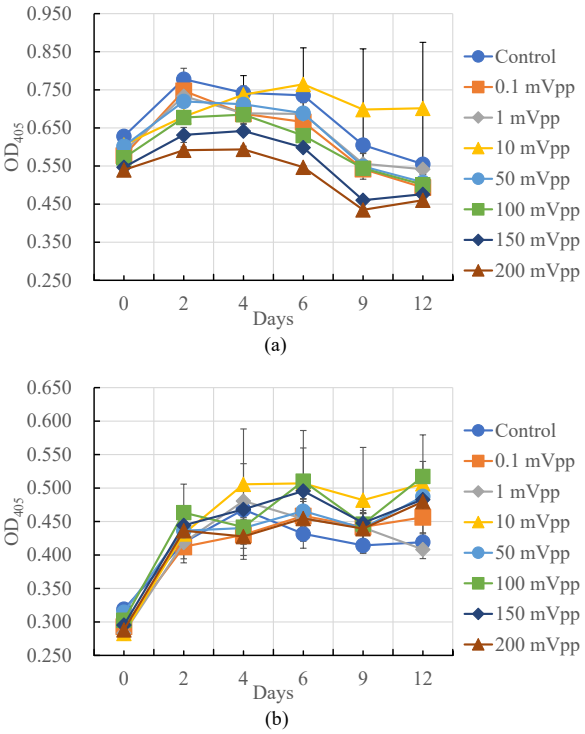

Fig. 3 Variation in optical density of *Microcystis* in medium.

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