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The application of sonication for purifying soil contaminated with microorganism
超音波照射を用いた微生物汚染土壌の浄化処理

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1. Introduction

Cavitations formed with the ultrasonic irradiation create radicals and points of high temperature and pressure when they collapse. It has been reported that this property can be applied for the degradation of organic substances and the sterilization of microorganisms [1-3]. The present study aimed for the sterilization of soil and aquifer polluted with microorganisms, and investigated the influence of ultrasound treatment on soil particles. In addition, we examined the application of ultrasound [4-6] on the solid-liquid separation of suspended particles.

2. Experimental

The frequencies of ultrasonic generators were set to 200 kHz (TA-4021; KAIJO) and 28 kHz (C-6392; KAIJO). The outputs of these devices were adjusted to 200 W. The ultrasound powers applied to the solution were calculated by the calorimetry method as 11 W and 16 W. An submersible transducer was put on bottom of a tank filled with water, and a round bottom flask containing a solution was set right above the transducer. The temperature in the solution was controlled between 15 to 20 °C. The schematic design of the experimental apparatus is shown in **Fig.1**. Microorganisms used for this study belong to the genus of Bacillus that lives in underground aquifers. Green tuff (Akita, Japan) was used as soil particles. A "microbial solution" containing 2.5×10^{-5} CFU/ml microorganisms and a "microbial suspension" containing 2.5×10^{-5} CFU/ml microorganisms and 2×10^{-2} wt% green tuff were used as sample solutions. Ultrasound waves of 28 kHz or 200 kHz were irradiated to 50 ml of each sample for 3 hours, and the 100 µl of the irradiated samples were spread on agar medium every 30 minutes after the irradiation. The sterilization efficiency was evaluated by the survival rate after the culturing for 48 hours. The Zeta-potential of particles in the suspension of density 5×10^{-4} wt% was measured with a device (ELS-8000；OTSUKA ELECTRONICS) that used light scattering method. Measured solutions were adjusted for pH with nitric acid, and the ion strength was assumed to be 0.01.

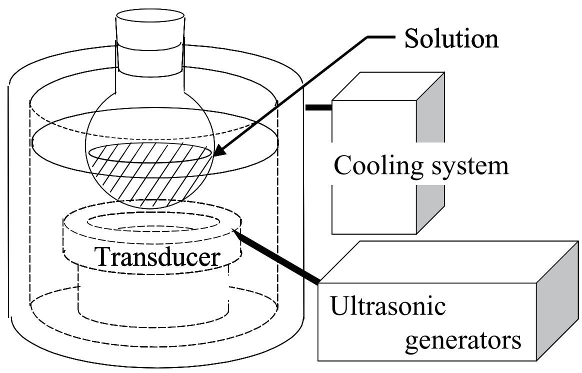

Fig.1 Schematic design of the experimental apparatus.

3. Results and Discussion

Fig.2 shows the survival rate of microorganisms after ultrasound irradiation. When the comparison is made for microbial solutions between 28 kHz and 200 kHz treatment, the microorganism survival rate was almost the same after 180 minutes of ultrasound irradiation. However, the survival rate of 28 kHz was lower than that of 200 kHz up to 120 minutes of ultrasound irradiation. This is presumably because the physical action (a shock) of the ultrasound is more effective at 28 kHz, which sterilize the solution with irradiation as short as around 60 minutes. On the other hand, the main mechanism of sterilization by 200 kHz irradiation seems to be the chemical action (i.e. the generation of radicals) of the ultrasound. The concentration of radicals and hydrogen peroxide generated by the short time of irradiation is not high enough for sterilization. It seems that a more than 120 minutes of irradiation is necessary to sterilize the solution since a change was hardly seen in a survival rate up to 60 minutes of irradiation. The same sterilization effect was observed for microbial suspensions when irradiated at 28 kHz. However, unlike the microbial solution, the irradiation of the microbial suspension at 200 kHz did not sterilize the microorganism up to 120 minutes. The reason of this deteriorated sterilization effect at 200 kHz is not clear, but likely that radicals

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and hydrogen peroxide reacted with the particles, thus reducing the rate of reaction for the sterilization.

Fig.3 shows the relationship between the Zeta-potential and the pH of green tuff suspension (5×10^{-4} wt%). The green tuff suspension had negative Zeta-potential at pH 9 when prepared, and the electric potential became smaller when the pH was lowered. The equivalent electric region was about pH 3.0. The particles precipitated by the adjustment of pH to 3.0 with chemicals and the standing for six hours (**Fig.4**). The turbidity was 2.74 NTU (the turbidity was 755 NTU before the pH adjustment). Because ultrasound irradiation can acidify solutions to pH 3.0, it is possible to collect particles by precipitating them with ultrasound irradiation.

4. Conclusions

We studied the effect of ultrasound on the purification of suspensions contaminated with microorganisms. Our studies suggest that ultrasound irradiation at 28 kHz can effectively sterilize suspensions in a short time, and that the sterilizing effect of 200 kHz ultrasound remains low unless the concentration of generated chemicals which react for sterilization exceeds a certain level. When a 2×10^{-2} wt% of particles were included in the solution, 28 kHz irradiation had the same sterilization effect as for the solution without particles, but the effect of 200 kHz irradiation was remarkably reduced.

The Zeta-potential measurements of the green tuff suspension revealed that the particles were able to precipitate at pH 3.0. Thus, the green tuff microbial suspension can be processed by sterilization with 28 kHz ultrasound irradiation followed by the precipitation of particles with 200 kHz ultrasound irradiation.

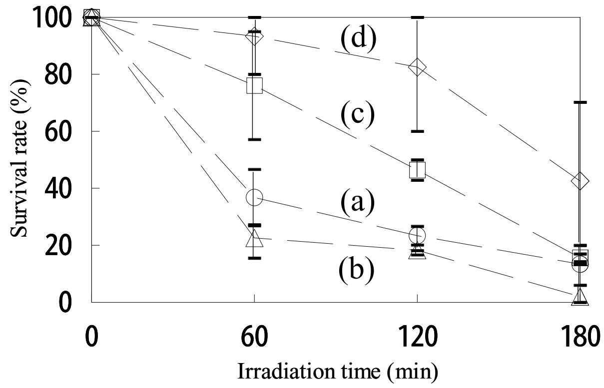

Fig.2 Survival rate of microorganism in the solution and the suspension treated by 28 kHz or 200 kHz ultrasound. (a) the solution treated by 28 kHz, (b) the suspension treated by 28 kHz, (c) the solution treated by 200 kHz, (d) the suspension treated by 200 kHz.

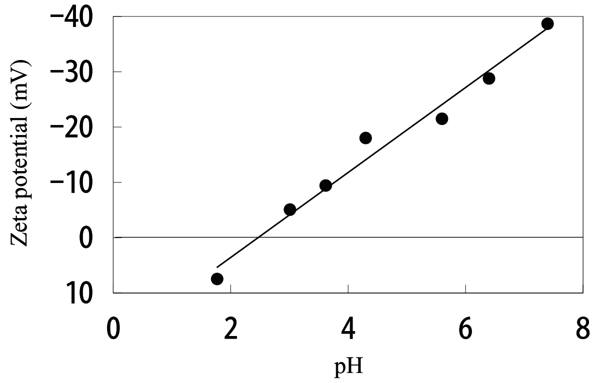

Fig.3 Zeta potential of Green tuff as a function of pH. (concentration 5×10^{-4} wt %).

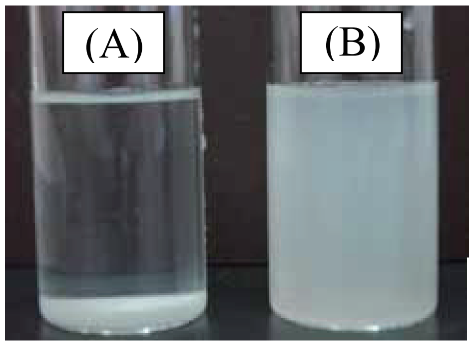

Fig.4 Transparent appearance of Green tuff suspension stood for 6h following the adjustment of pH to 3.0 with HNO_3. (A) pH 3.0, (B) pH 8.0.

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