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Short communication

Inactivation of bacteria in batch suspension by fluidized ceramic tourmaline nanoparticles under oscillating radio frequency electric fields

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Abstract

Here, we investigated the synergistic effect of fluidized ceramic tourmaline nanoparticles (NPs) with oscillating electric fields to the inactivation of *Escherichia coli* in batch suspension. Different amounts of tourmaline NPs (5–100 mg) were dispersed in bacterial suspension (50 ml) and oscillating radio frequency (RF) electric field was applied at 3 V (equivalent of 1.5 V/cm electric field strength) and at varying frequencies (100 kHz and 1 MHz). The results showed high inactivation efficiency when combining fluidized tourmaline NPs and oscillating electric fields as compared to individual treatments of electric fields or tourmaline NPs only. The results showed 2.5–5-log reduction of viable *E. coli* concentration depending on the tourmaline concentration, electric field frequency and solution temperature. The present method shows a lot of promise as a novel and low-cost bacterial inactivation technique for water treatment applications.

Keywords: A. Suspensions; B. Interfaces; Oscillating electric fields; Tourmaline

1. Introduction

The presence of microorganisms in water is a major problem in drinking water, in industrial cooling water systems, and in desalination plants [1]. Various chemical and physical treatment techniques have been investigated to inactivate bacteria in water [2]. Due to increasing concern of the toxicity of chemical methods to human health and the environment as well as the increasing cost for disinfection, physical water treatment (PWT) techniques have gained vast interest as a "green" technology in the disinfection of water [3]. Among the PWT techniques, the use of electric fields has been found to have effective sterilization efficiency. When only electric fields are used, a high electric field density in the order of 100 kV/cm is needed to disinfect water [4]. So that, there is still a continuing research for enhancing the effectiveness of electric fields to inactivate bacteria.

Nanoscale materials, owing to their unique properties, have a wide range of applications in the field of water treatment, materials science, and energy sector. Several nanoparticles (NPs) have been utilized as photocatalysts and antibacterial agent such as Ag, Au, ZnO, TiO_2, MgO, carbon nanotubes, etc. [5–7]. A few studies report on the synergistic effect of electric field and nanoparticles in

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inactivating microorganisms [8,9]. Another potential nanomaterial that has not been explored extensively yet for antibacterial activity in water suspension is tourmaline NPs. Tourmaline is a complex borosilicate material with interesting and unique properties such as the possession of spontaneous surface electric fields, has ability to radiate far-infrared, and has antibacterial activities [10,11]. To the authors' best knowledge, based on literature review, the antibacterial activity of tourmaline NPs under oscillating electric fields has not been reported yet, and is ought to be recognized. Our previous study [11] showed good antibacterial activity by zone of inhibition test of electrospun polyurethane nanofibers decorated with tourmaline NPs against *Escherichia coli* and Entrococci bacteria. In this study, we investigated the synergistic effect of applied external oscillating electric fields at low power consumption and tourmaline NPs in suspension in the inactivation of bacteria in water. This method shows promise in the development of new water treatment technology without the use of chemicals that is safe, simple, robust, and low cost.

2. Experimental

A batch test set-up was used in the present study (Fig. 1). It was composed of two graphite plate electrodes kept at a distance of 20 mm, and placed inside a Teflon container. Alligator clips were attached on the tip of each electrode and the ends were connected to a power supply consisting of an amplifier (Biomechatron) and a function generator (WW1071, Tabor Electronics). The output amplitude and frequency were viewed through an oscilloscope (TDS 2002B, Tektronix). Bacterial suspensions with or without tourmaline NPs were placed inside the container and were exposed to oscillating electric fields. Tourmaline powder (TM, 325 mesh) was received from UBS Inc. Co., Ltd., Korea and was ball-milled for 12 h using 3 mm zirconia balls and sieved. After ball-milling, the TM NP size was in the range of 80–700 nm. In the present study, various tourmaline NP concentrations (i.e., 5, 20 and 100 mg) were dispersed in 50 ml bacterial solution and were exposed to oscillating electric fields at different square-wave frequencies (100 kHz, and 1 MHz, see Fig. 1b) and a peak-to-peak voltage (V_{pp}) of 3 V with an equivalent electric field strength of 1.5 V/cm.

E. coli Top 10 strain was used as the model microorganism, and was following our previous method [12]. The antibacterial experiments were carried out in a bench scale batch set-up containing 10^7 colony forming units (CFU)/ml of *E. coli* suspension (50 ml) with different amounts of tourmaline NPs, mixed by magnetic stirring. The tests were conducted at room temperature for a duration of 270 min. The baseline test was performed using a bacterial suspension with no tourmaline NPs and no oscillating electric field, and a reaction mixture with tourmaline NPs only was used as control. At given time intervals, 100 µl suspension was collected and diluted appropriately by serial dilution in distilled water. Sampling was done in triplicates. Ready-to-use petrifilm (3 M Petrifilm, USA) and prepared agar plates were used to plate the samples. After incubation for 24 h, the number of bacteria was manually counted using a colony counter.

Scanning electron microscopy (SEM, JSM-5900, JEOL Ltd., Japan) was utilized to observe the morphological changes of *E. coli* after treatment. At given time intervals, 100 µl of the cell suspension was placed onto a 0.2-µm-pore-size cellulose acetate membrane filter (Toyo Roshi Kaisha, Japan), were washed, fixed, and dehydrated. After which, the *E. coli* cells were subjected to SEM imaging.

3. Results and discussion

The effect of different concentrations of tourmaline NPs in suspension was observed at constant voltage and frequency of 3 V and 1 MHz, respectively (Fig. 2). The blank sample (Fig. 3a) exhibited almost constant bacterial count for the duration of the test, while treatment using only tourmaline NPs without electric field treatment showed a negligible decrease in bacterial count after 270 min of test. The application of electric field alone without tourmaline NPs in solution showed about 1.5 log decrease from the initial condition (Fig. 2d). Here, the electric field used was about 1.5 V/cm, which was quite low thus the electroporation mechanism of killing bacteria is ruled out since electroporation is reported to occur at around 1000 V/cm [13]. The present mechanism could be due to the effect of high-frequency oscillating electric fields (1 MHz) which induce particle and macromolecule distortion and movement, and affect the enzyme–substrate

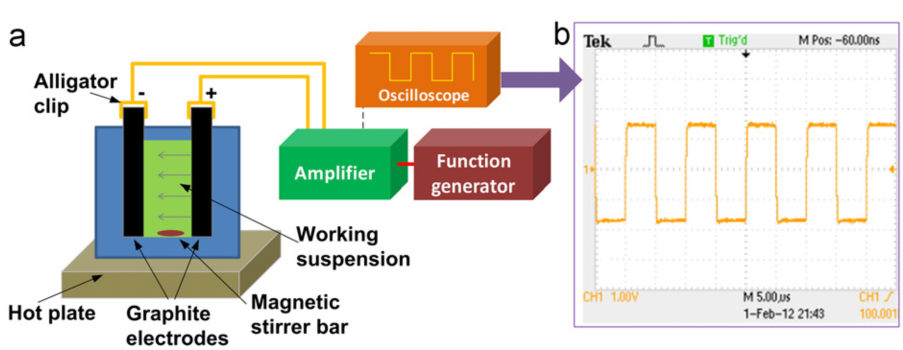

Fig. 1. The present (a) batch set-up for antibacterial activity and (b) sample oscilloscope output reading of square wave oscillating electric field.

reaction equilibrium [14]. When tourmaline NPs were added into the solution and was subjected to oscillating electric fields, the results showed 2.5–5 log reduction of bacteria count with the increase of tourmaline NP concentration from 5 mg to 100 mg. Here, the reduction of viable *E. coli* count with the use of tourmaline NPs under oscillating electric fields could be due to the following: (a) bacteria in solution adhere to the tourmaline NP surface, which could lead to disruption of cell membrane [15]. *E. coli* possess cell structures that make them possible to adhere to rough surface such as tourmaline NPs in the present study; (b) when the tourmaline NPs were exposed to external oscillating electric fields, it was possible to generate nonlinear and random electric field enhancements especially at the surface of tourmaline NPs as also observed in a simulation study of Yeckel et al. [16]. These enhanced surface electric fields could have helped in the breaking down of cell membrane in a process called electroporation [8,10]. The electroporation process opens up a tiny pore in the cell membrane, giving chance for ions released by tourmaline NPs surrounding the membrane to penetrate and be absorbed in the cell and cause cell damage [17].

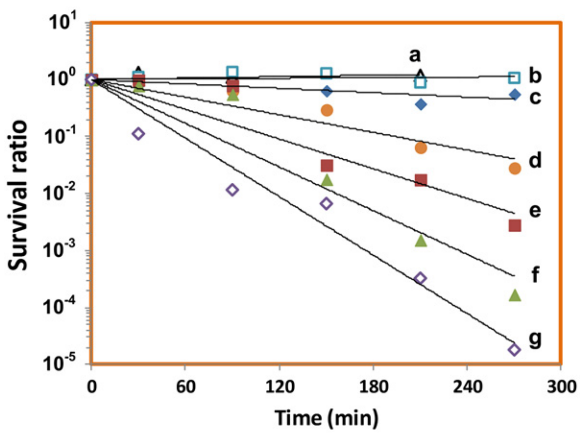

Fig. 2. The survival ratio of *E. coli* with respect to treatment time for (a) blank sample, and treatment at different tourmaline NP contents: (b) 5 mg+no electric field; (c) 20 mg+no electric field; (d) electric field only+no tourmaline NPs; (e) 5 mg+electric field; (f) 20 mg+electric field; and (g) 100 mg+electric field. Here, the voltage and frequency were maintained at 3 V and 1 MHz.

Representative SEM images of *E. coli* in contact with tourmaline NPs with electric field treatment (3 V, 1 MHz) are shown in Fig. 3. The control samples (Fig. 3a) showed intact cells with the expected morphology of *E. coli* cells. After exposure to electric field and tourmaline NPs for 30 min, *E. coli* cell showed a little distorted cell membrane with tourmaline NPs adhering on its surface (Fig. 3c). At longer treatment time (Fig. 3d and e), the majority of the cells were severely degraded and had lost cell membrane integrity. We found it hard to find *E. coli* cells trapped on cellulose acetate filter after a treatment time of 270 min (Fig. 3d), which suggests that *E. coli* were severely damaged and were drawn out of the filter since they decrease in size from cell dehydration.

The effect of electric field frequency at 3 V+20 mg TM NPs vs. time is shown in Fig. 4. The lower frequency at 100 kHz (Fig. 5c) showed much better inactivation efficiency (i.e., about 7 log decrease) compared to 1 MHz (only 4 log decrease) after 210 min. Studies show that different frequencies can lead to different phenomena such as surface polarization, increased cell membrane permeability and change in shape [18]. As reported by other groups, oscillating electric

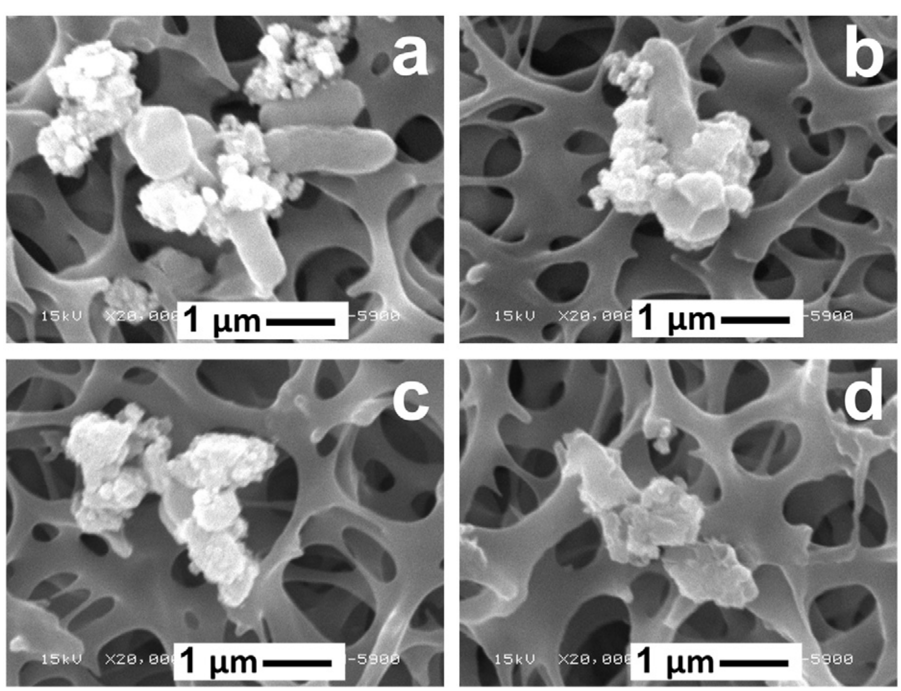

Fig. 3. Scanning electron micrographs of *E. coli* on a cellulose acetate filter membrane after subjecting to electric field treatment (3 V, 1 MHz) with tourmaline NPs at different treatment time: (a) 0 min; (b) 30 min; (c) 90 min; (d) 270 min.

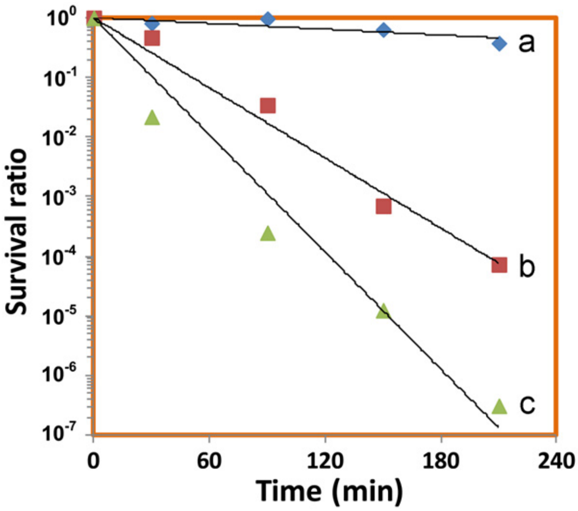

Fig. 4. Survival ratio of *E. coli* at (a) control and 20 mg tourmaline NPs subjected to constant voltage of 3 V and at frequencies of (b) 1 MHz and (c) 100 kHz.

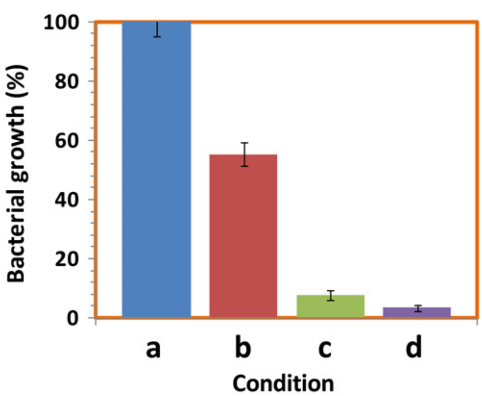

Fig. 5. Relative growth of *E. coli* after 90 min of electric field exposure (V=3 V) of 20 mg tourmaline NPs at different solution temperatures: (a) control; treatment at (b) 25 °C (room temperature), (c) 30 °C, and (d) 35 °C.

fields could cause the cells to rotate which could lead to cell fatigue, and for *E. coli*, a frequency range of 100–500 kHz is needed for rotational torque to arise [19]. This could be the reason why the lower frequency of 100 kHz had better performance than 1 MHz frequency in the presence of 20 mg TM NPs for both cases in the present study. Kotnik et al. [20] theorized that at higher frequencies such as in MHz region, the induced field strength across the cell membrane is substantially reduced, which consequently reduces the inactivation efficiency.

Fig. 5 shows the effect of solution temperature (i.e., 25, 30, and 35 °C) on the inactivation of bacteria by oscillating electric field (3 V, 1 MHz) and 20 mg tourmaline NPs after 90 min. It should be noted that the solution temperature was controlled and monitored during the whole test. Comparing from the control, 45% bacteria was inactivated at 25 °C, while about 93% and 97% were killed at temperatures of 30 and 35 °C, respectively. The present results agree well with other reports where higher inactivation was obtained when the solution temperature was increased at constant electric field strength [21]. It has been suggested that the solution temperature influences the phase transition of cytoplasmic membrane and phospholipids from gel to liquid crystalline structure, affecting the stability of the cell membrane [22]. Thus, when the cell membrane experiences thinning at higher temperatures, it renders itself susceptible to disruption by electric field by virtue of electric breakdown theory, and the surrounding ions released by tourmaline NPs, leading to cell death [17].

4. Conclusions

In summary, we have studied here the enhancement of bacterial inactivation of oscillating radio frequency (RF) electric fields when tourmaline nanoparticles were added into the solution. We found that the presence of tourmaline NPs could enhance the inactivation efficiency of oscillating RF electric fields depending on the frequency, tourmaline concentration and solution temperature. Here, the power consumption of the present method was very low, but was capable of 2.5–5 log reduction of *E. coli* concentration after 270 min of test. This method presents a promising alternative to chemical treatment and other high-power consumption methods of bacterial inactivation. The advantages of the present method include very low power consumption, low maintenance, environment-friendly as no chemicals are needed, and simple construction and its robustness. Based from the findings of this study, it is concluded that the present method of combining fluidized tourmaline NPs and oscillating radio frequency electric fields is a valid tool to inactivate bacteria in water.

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