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SURFICIAL DISINFECTION OF *ESCHERIACHIA COLI*-CONTAMINATED PLAYGROUND SOIL BY UV IRRADIATION

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Abstract : The necessity of disinfecting playground soil is an important issue, because pathogenic protozoa, bacteria, and parasite eggs remain viable for several months and can infect children. UV irradiation has been used to decontaminate water but its effectiveness on soil is unclear. We determined the efficacy of UV radiation for inactivation of an indicator bacteria, *E. coli* (strain ATCC 8739), on playground soil. While 99% inactivation of *E. coli* in the soil was readily achieved by UV radiation within 55 min at 0.4 mW cm^{-2}, complete inactivation was not achieved, even after prolonged treatment at 4 mW cm^{-2}. This was attributed to the irregular surface of the soil. A small number of *E. coli* escaped the UV radiation because they were situated in indentations or under small particles on the soil surface. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) confirmed that the surface characteristics of the soil is the major limiting factor in the inactivation of *E. coli* by UV radiation. Thus UV treatment may not be adequate for disinfecting some soils and should be carefully evaluated before being used on playground soils.

Key Words : Disinfection, *E. coli*, Inactivation, Playground Soil, UV

INTRODUCTION

Concern over the control of pathogens in public and industrial facilities has increased over the past few decades. Many studies have focused on public health issues, such as the disinfection of drinking water[1-3] and control of microbial growth in cooling towers.[4] However, few studies have been performed to control microorganisms in the soil environment, despite reports that some sandpits in the public parks and playgrounds of many countries are contaminated by bacterial pathogens and parasite eggs (e.g., Argentina,[5] Brazil,[6] Germany,[7] Italy,[8,9] Japan,[10] and Korea[11]). The presence of pathogenic microorganisms in playgrounds can be particularly dangerous to children, who

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have weaker immune systems than adults. Pathogens, such as *Escheriachia coli* O-157: H7, *Cryptosporidium parvum* oocyst, and parasite eggs can survive for months in untreated media.[12-15] Therefore, the importance of disinfecting playground soil should be emphasized.

Studies involving sterilization of soil by microwave radiation, γ-radiation, and chemical biocidal agents have been conducted. However, the purpose of these studies was not disinfection for public health, but sterilization as a pre-treatment in various experiments, such as the estimation of soil biomass, soil enzyme assays[16-18] or the study of soil ecology.[19-22] UV radiation, which is generally used for disinfecting drinking water and wastewater, is effective against numerous microorganisms, including bacteria,[1] protozoa,[23-25] and parasite eggs.[13] UV irradiation may be considered for treating infected soils, but to our knowledge its effectiveness has not been determined.

Therefore, in this study, the efficacy of UV irradiation for the disinfection of soil was evaluated using a well-known indicator microorganism (*E. coli*). The influence of soil surface characteristics on UV inactivation of *E. coli* was investigated by surface analyses using scanning electron microscopy (SEM) and atomic force microscopy (AFM).

MATERIALS AND METOHDS

Materials

Solutions and reagents were prepared using Milli-Q water (Millipore, Billerica, MA, USA), and analytical grade chemicals were used (Sigma-Aldrich, St. Louis, MO, USA). Glassware was rinsed with distilled water and autoclaved at 121°C for 15 min prior to use.

Soil Preparation

The soil was obtained from an apartment playground in Ansan City, Kyunggi-Do, Korea. Prior to the experiment and analysis, the playground soil was air-dried and sieved to obtain particles 180~335 μm in diameter. The physicochemical properties of the soil were determined by the Soil Testing Laboratory, Jeil Analysis Center Co. (Korea) and are shown in Table 1.

Table 1. Physicochemical characteristics of playground soil used in the experiments

Characteristics	
Sand (%)	87.12
Silt (%)	2.10
Clay (%)	10.78
Organic C (%)	0.37
CEC (me/100 g)	1.81
pH	8.4

Bacterial Culture and Analysis

E. coli, considered a representative indicator microorganism of vegetative bacteria, was selected for the purpose of estimating the microbial inactivation efficacy of UV irradiation in soil. *E. coli* strain (ATCC 8739) was inoculated into 50 mL of Tryptic Soy Broth medium in a 200 mL-flask, and grown at 37°C for 18 hr. The cells were harvested by centrifugation at 1,000 × g for 10 min and washed twice with phosphate buffered saline (PBS, pH 7) solution. *E. coli* stock solution was prepared by resuspending the cell pellets in 50 mL of PBS solution. The cell population was determined by plating on nutrient agar and counting the number of colonies. One mL aliquots of the solution were withdrawn and diluted to 10^0, 10^{-1}, and 10^{-2}. Finally, 0.1 mL samples of the diluted solutions were spread to count the number of colonies of *E. coli*. The number of colony forming units (CFUs) was determined after incubating for 24 hr at 37°C. The *E. coli* inoculum population was determined by enumerating the population of the heterotrophic plate count (HPC) of the bacteria in the natural playground soil. The HPC population was determined using the spread plate method.[26] A 10 g sample of playground soil was transferred to a 200-mL flask containing 20 mL of nonionic surfactant (Tween 20, 2% v/v; Aldrich Co.) in PBS solution and mixed thoroughly using a vortex mixer.[27,28] A 1 mL aliquot of the solution was withdrawn and serially diluted. One-tenth ml of the diluted solutions was spread on Tryptone Glucose Extract

Agar medium (Difco, BD, Franklin Lakes, NJ, USA). After incubating for 24 hr at 37°C, the population of the HPC was determined. The HPC population in natural playground soil was approximately 10^6~10^7 CFU g^{-1}. Thus, the soil samples were inoculated to obtain an *E. coli* population of 10^7 CFU g^{-1}.

Experimental Procedures

For the inactivation experiments, 10 g of the soil was transferred to a Falcon tube and autoclaved three times at 121°C for 30 min. To confirm the sterilization of the soil samples after autoclaving, one of them was incubated in 50 mL of Tryptic Soy Broth medium at 37°C for 5 days, and a one milliliter aliquot of the solution was diluted and spread on Tryptone Glucose Extract Agar medium. No microbial growth was observed in this soil sample. The initial population of *E. coli* (1.0×10^7 CFU g^{-1}) was obtained by inoculating 0.1 mL of stock solution (1.0×109 CFU mL^{-1}) into 10 g of sterilized soil and incubating at room temperature for 5 d. The soil samples were then spread on a sterilized Petri dish (9 cm diameter) and exposed to UV radiation for specific time periods (e.g., 5, 10, and 20 min) with hand-shaking every 1 min. In one experiment, in which irradiation exceeded 2 hr, the sample was shaken every 1 min during the first 2 hr and every 10 min after 2 hr. A collimated-beam UV apparatus was used, consisting of four, 4 W low-pressure mercury vapor UV lamps (253.7 nm, Philips, Amsterdam, Netherlands.). The UV light was directly irradiated through a circular collimating tube onto the surface of the soil samples contained in a Petri dish at room temperature. The light intensity was measured using a UVX radiometer (UVP Inc., Upland, CA, USA) and adjusted to 0.4 and 4 mW cm^{-2} by changing the distance between the UV lamp and the sample.[23] The *E. coli* remaining in the treated soil sample were extracted by adding 20 mL of Tween 20 (0.2%, v/v) in PBS solution. A 1 mL aliquot of the solution was diluted and spread on Tryptic Soy Broth agar medium, as previously described. The recovery efficiency of *E. coli* from the soil sample with phosphate buffer and surfactant was 90 ± 6%. The level of inactivation is expressed as the log reduction of the microbial survival ratio during the disinfection experiments.

After the initial UV treatment, a series of experiments were conducted with UV/Tween 20 (0.2%, v/v) in PBS solution. A 0.2% (v/v) Tween 20 in PBS solution was used as a surfactant to remove the small particles adhering to the soil surface. Soil treated with Tween 20 is referred to as surface-modified soil. A UV/distilled water experiment was also conducted to control the humidity of the soil. Tween 20 (0.2%, v/v) solution and distilled water were sprayed (0.1 mL g^{-1}) onto respective soil samples in the Petri dish, and re-exposed to UV radiation for 20 min with hand-shaking every 1 min.

A 'hard surface' test was conducted to determine the influence of surface roughness on the inactivation efficacy of UV irradiation. The surfaces included a Petri dish (hard surface 1) and the back side of slide glass (hard surface 2). A suspension of *E. coli* (1 mL, 1.0×10^7 CFU mL^{-1}) was placed on a 4 cm^2 area of each surface and air-dried for 2 h to allow attachment of the bacteria and drying of the medium. The contaminated surfaces were irradiated with UV (0.4 mW cm^{-2}) at room temperature for specific periods (e.g., 20, 40, and 60 s), then soaked in 20 mL of Tween 20 (0.2%, v/v) in PBS solution.[29,30] The *E. coli* present in the aqueous sample were enumerated by plating and counting. A control test was conducted by dipping a contaminated carrier in 20 mL of Tween 20 (0.2%, v/v) in PBS solution without the irradiation treatment.

In the suspension test, UV radiation at 253.7 nm was directly irradiated onto the surface of the suspension in a sterilized Petri dish for 1 min, as described above. The test suspension was prepared using 20 mL of phosphate buffer suspension (pH 7) containing *E. coli* at 1.0×10^6 CFU mL^{-1}. An aqueous sample was periodically withdrawn and spread on Tryptic Soy Broth agar medium to count the number of cells.

Surface Analyses

The soil samples were pretreated using the method of Foschino et al.[31] and observed under SEM. The procedure involved fixing with 2% paraformaldehyde in 50 mM sodium cacodylate buffer (pH 7.2) at 4°C for 4 hr, post-fixing with 1% osmium tetroxide in 50 mM sodium cacodylate buffer at 4°C for 2 hr, and dehydrating with a graded series of ethanol (30, 50, 70, 80, 90, and 100%). The specimens were dried with 100% hexamethlydisilazane, mounted on metal stubs, and sputter-coated with gold. The images were observed with a JSM-5410LV scanning electron microscope (JEOL Ltd., Tokyo, Japan).

The hard surface samples were observed with an AFM in the tapping-mode with a Multimode Nanoscope III a system (Digital Instruments Co., Santa Barbara, CA, USA) in air. Standard 125 um silicon microcantilevers (Digital Instruments Co.) were used. The cantilever oscillation frequency ranged from 200 to 300 kHz. The experimental conditions described by Wu et al.[32] were used for the AFM analysis.

RESULTS AND DISCUSSION

Inactivation of *E. coli* in Soil by UV Radiation

Figure 1 shows the inactivation efficacy for *E. coli* in soil at UV light intensities of 0.4 and 4 mW cm^{-2}. Irradiation periods of 30 and 55 min were necessary to achieve 99% (2-log) inactivation of *E. coli* in the soil for the UV doses of 4 and 0.4 mW cm^{-2}, respectively. During the initial phase of treatment, the inactivation efficacy rate increased with increasing irradiation time. However, once 99% (2-log) inactivation was achieved, no further inactivation of *E. coli* was observed, regardless of the duration or intensity of irradiation.

Images of the soil surface and *E. coli* adhered to the soil are shown in the electron micrographs (Figure 2). The *E. coli* were about 1 μm in length, and less than 0.5 μm in width. The bacteria can be seen adhering to the flat surface of the soil in Figure 2(a). In addition, surface indentations larger than the *E. coli*, are present

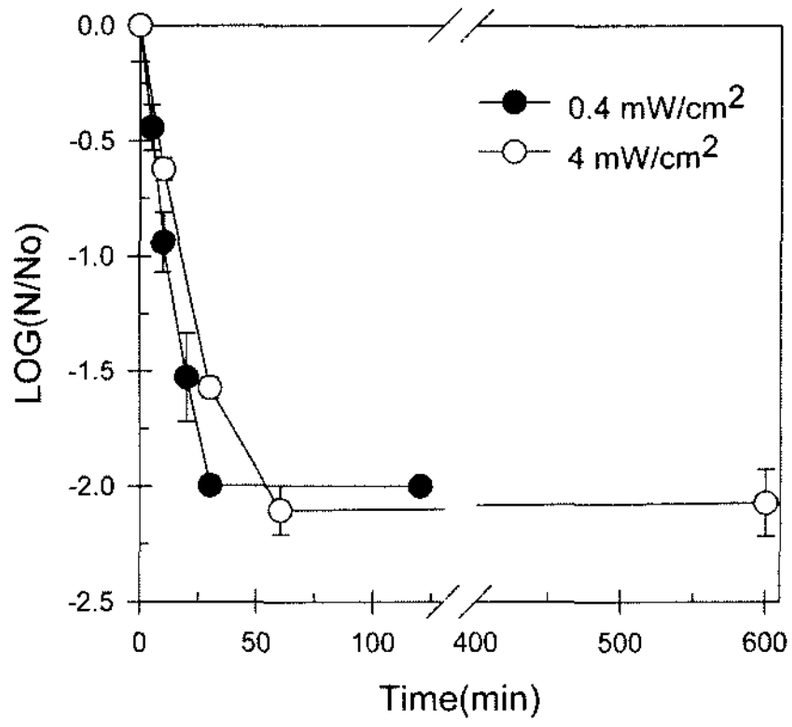


Figure 1. Inactivation efficacy of UV radiation (0.4 and 4 mW cm^{-2}) for *E. coli* in soil.

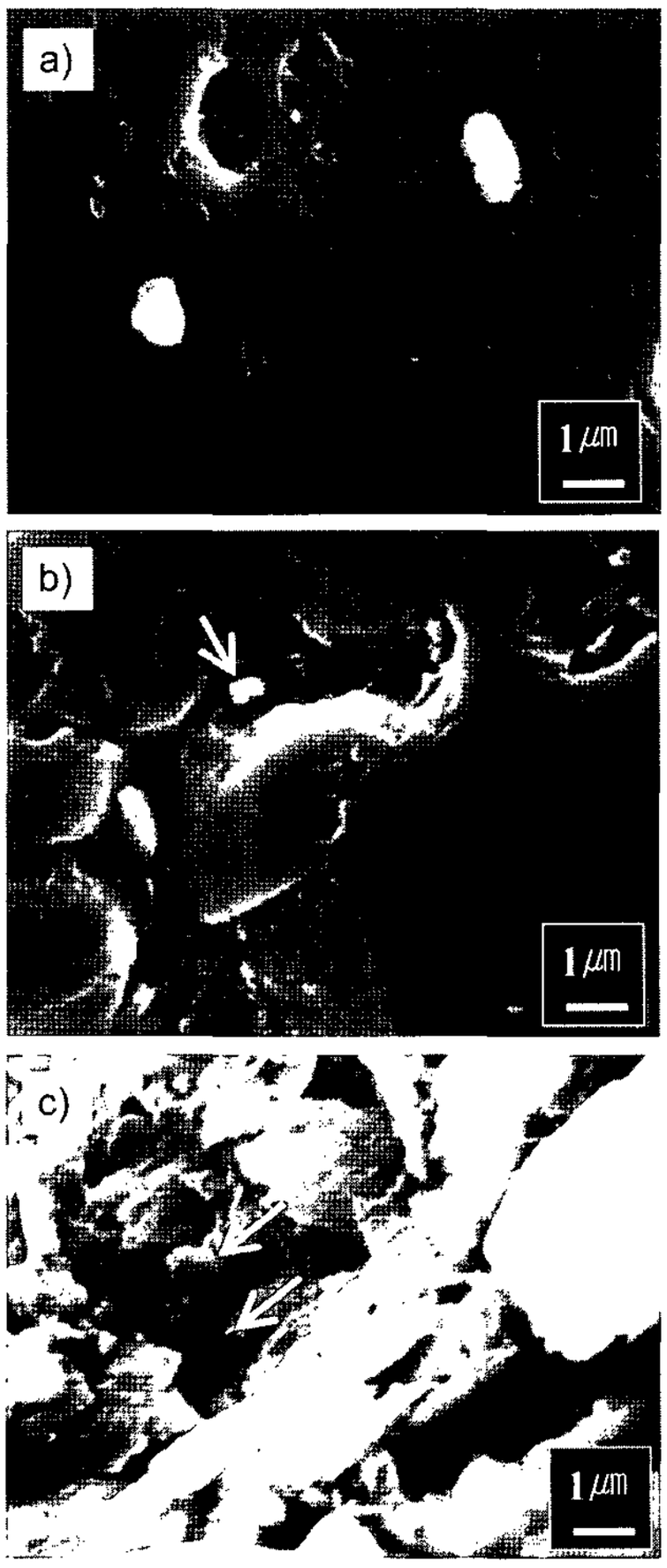


Figure 2. SEM images of soil samples. (a) *E. coli* adhered to the flat soil surface. (b) *E. coli* in indentations in the soil surface. (c) *E. coli* protected by small particles before treatment with Tween 20 solution. Arrows indicate *E. coli* on the soil surface.

in the soil. Figure 2(b) shows *E. coli* in these indentations and Figure 2(c) shows *E. coli* covered with many small particles on the surface of the soil.

Influence of Surfaces on UV Efficacy

To examine the effect of surface characteristics on the inactivation of *E. coli* by UV radiation, experiments were conducted with three sets of treatments (Figure 3). Inactivation of *E. coli* in the buffered solution and on the hard surfaces is illustrated in Figure 3(a). Two different hard surfaces were used for the experiments, and their surface characteristics were analyzed by AFM (Figure 3(b)). In this experiment, hard surface 1 (Petri dish) was very flat (depth < 21 nm), while hard surface 2 (back side of slide glass) had indentations approximately 150 times deeper (3.1 μm) than hard surface 1. Inactivation of *E. coli* in the suspension and on the Petri dish, which was scarcely indented, occurred rapidly (Figure 3(a)). Conversely, the slide glass, with its relatively large indentations exhibited a slow inactivation rate and a tailing effect, similar to that observed in the soil experiment (Figure 1).

To determine the influence of surface irregularities on UV efficacy, Tween 20 (0.2%, v/v) solution and distilled water were added after the initial 99% inactivation was achieved (Figure 4(a)). The addition of Tween 20 solution resulted in a further 1.5-log inactivation, for a total of 99.97% (3.5-log) inactivation, whereas the results for the control to which distilled water was added were similar to those of the initial 99% inactivation (Figure 1). The SEM image (Figure 4(b)) shows that the surfactant removed the small particles from the surface of the soil, accounting for the increased inactivation after treatment.

Limitations of UV Treatment

Most (99%) of the *E. coli* in the soil was readily inactivated by UV light of weak intensity (0.4 mW cm^{-2}). However, no further inactivation was observed once 99% (2-log) inactivation was achieved, regardless of the light intensity or duration of irradiation. This indicates that approximately 1% of the *E. coli* population present on the soil surface may not be exposed to the UV radiation. Unlike the aqueous phase, the disinfection efficacy in soil was not significantly influenced by the intensity of UV radiation. In solution, the microbial inactivation efficacy generally increases as light intensity and radiation time increase.[1] Therefore, the results obtained for irradiated soil

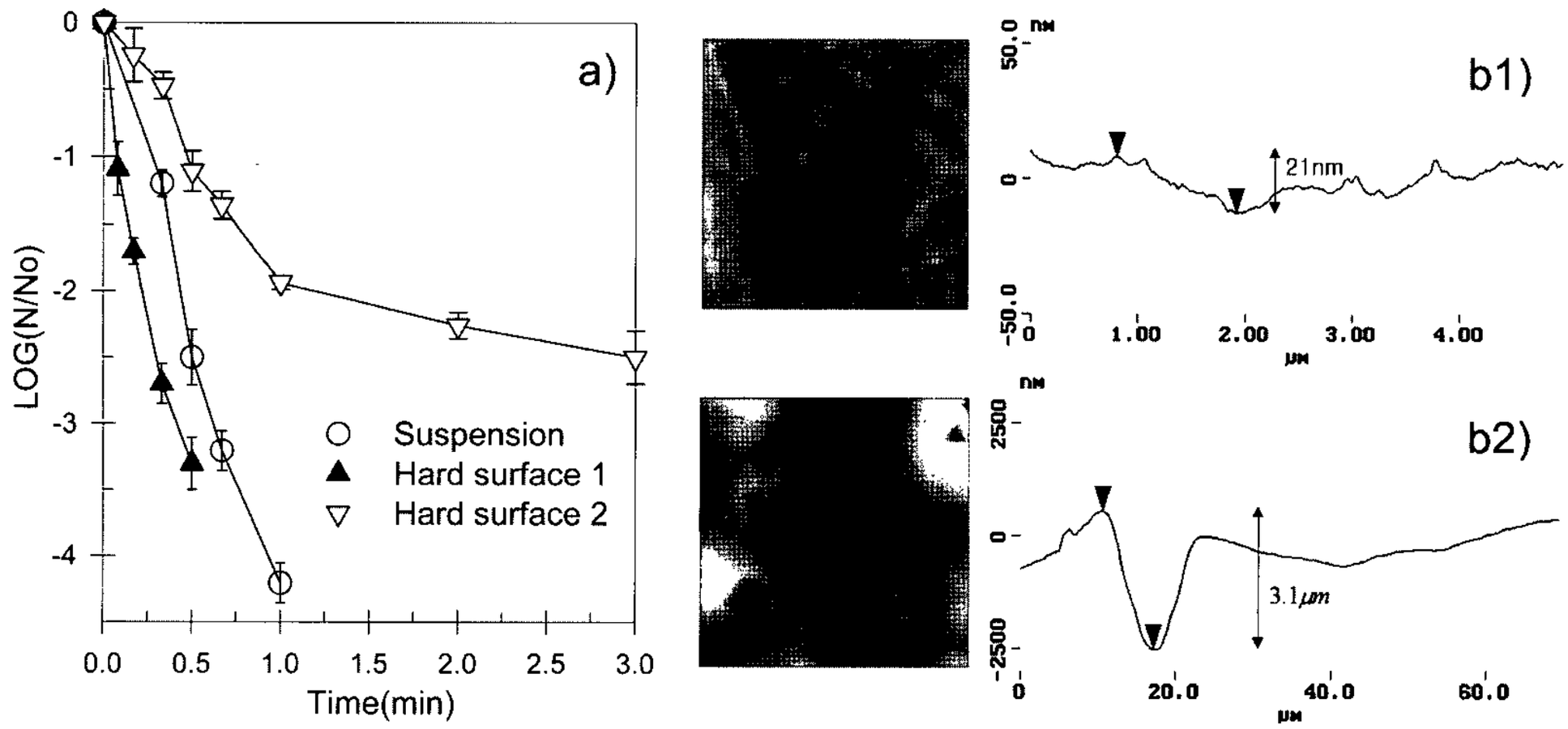
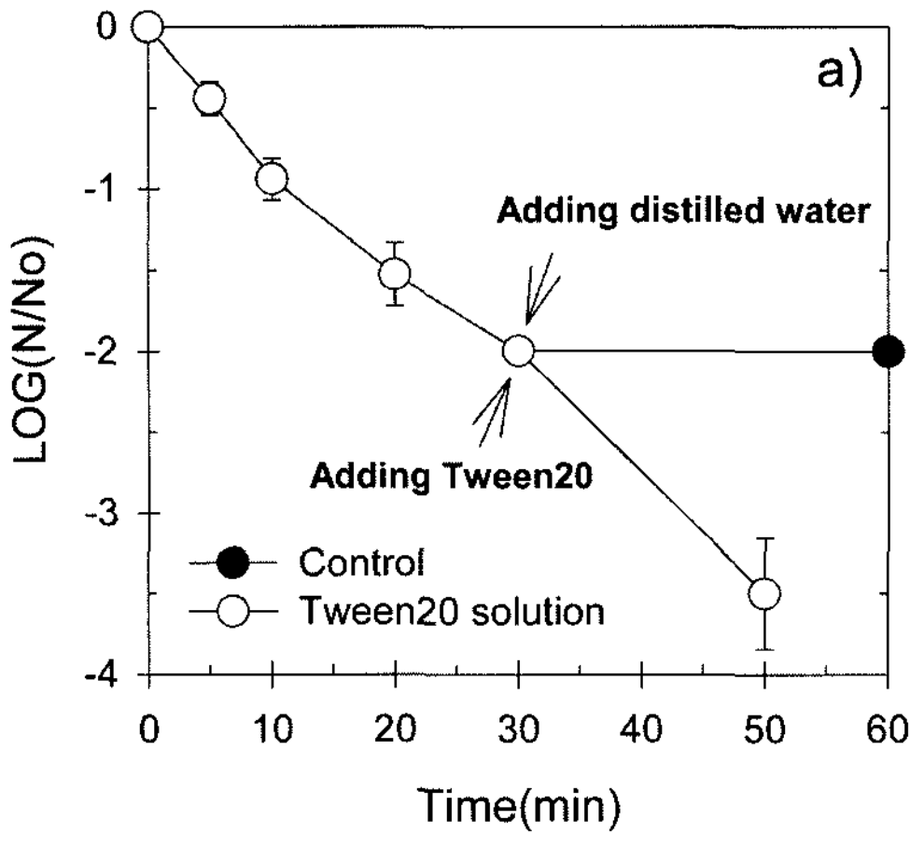


Figure 3. Influence of surface irregularities on UV disinfection efficacy. (a) Comparison of inactivation efficacy of UV radiation for *E. coli* on hard surfaces and in suspension (0.4 mW cm^{-2}). (b) AFM images of two different hard surfaces, (b1) hard surface 1 (Petri dish) and (b2) hard surface 2 (back side of slide glass).



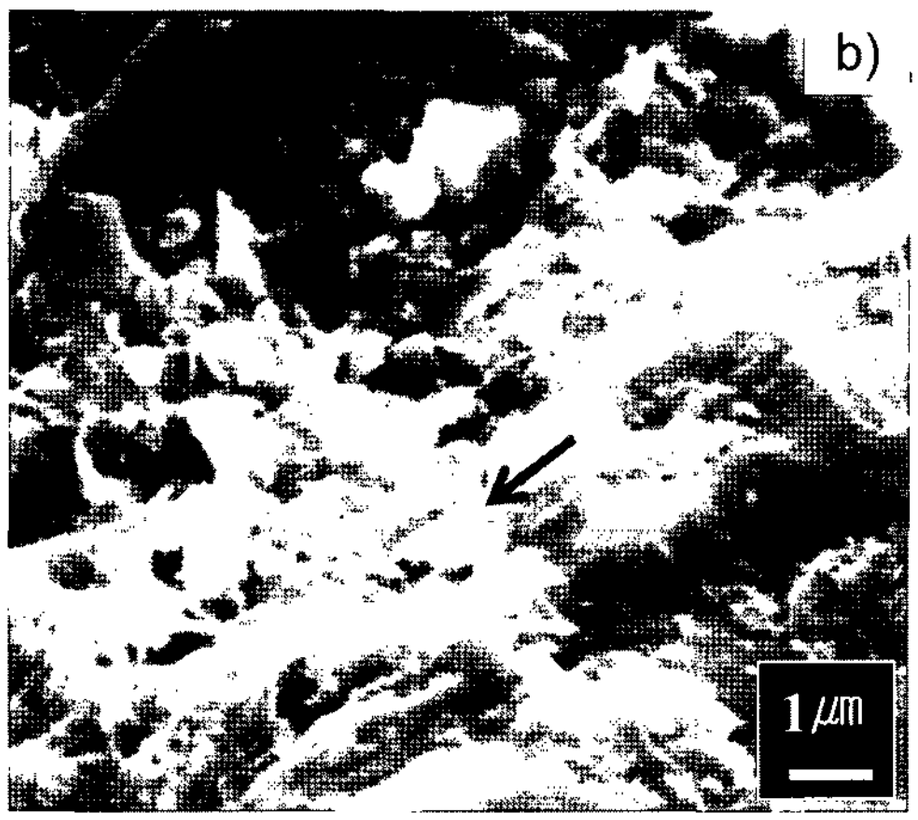


Figure 4. (a) Enhancement of the inactivation efficacy achieved by modifying the surface of the soil with Tween 20 (0.2%, v/v) solution. (b) SEM image of surface-modified soil after treatment with Tween 20 solution. *E. coli* were exposed after removal of the small protecting particles by surfactant treatment. Arrows indicate *E. coli* on the soil surface.

are due to the irregular surface of the soil, which provides partial protection of bacteria against exposure to the UV radiation. Large indentations and small particles were observed in SEM images of the soil surface. SEM analysis showed *E. coli* adhering to flat surfaces, within indentations, and under small particles. After the surface is treated with nonionic surfactant solution, the *E. coli* are directly exposed to the UV radiation, because the small particles are removed, leading to further inactivation. This is consistent with the work of Mancinelli and Klovstad,[22] who reported that an unprotected monolayer of *Bacillus* spores could be killed quickly when exposed to UV radiation, but an additional layer of spores or dust protected the *Bacillus* spores from the UV radiation.

Our experiments indicate that UV radiation can inactivate bacteria on soil surfaces that are directly exposed, but will not be effective on unexposed bacteria present in surface indentations and under small particles. While 99% inactivation of *E. coli* was achieved in the playground soil used in our tests, the remaining bacteria may still pose an unacceptable risk. The surface characteristics of soil, such as indentations and the amount of adhered particles are critical to the effectiveness of disinfection by UV radiation and will vary among soils. It is also important to recognize that the effectiveness of UV light for inactivation of some pathogens may differ from that observed for the indicator strain of *E. coli*. Thus UV treatment may not be adequate for disinfecting some contaminated soils and should be carefully evaluated before being used on playground soils.

CONCLUSIONS

The disinfection efficacy of UV radiation (by 99%) for *E. coli* in the soil was readily achieved. Complete inactivation, however, was not occurred due to the irregular surface of the soil. Indentations or under small particles on the soil surface provided shelter for a few *E. coli*. The surface characteristics of the soil can be the major factor in the inactivation of *E. coli* by UV radiation. It is advisable that UV treatment should be carefully evaluated for disinfecting soils, and that an alternative treatment should be investigated through further research.

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