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Effect of tryptophan and phthalate on the germicidal activity of ultraviolet-A radiation in solar water disinfection

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A major contributor to disease in developing countries is the lack of clean drinking water. Solar water disinfection is a popular method used in such countries and involves elimination of pathogenic bacteria from contaminated water. SODIS requires the storage of contaminated water in clear plastic bottles and exposing the bottles to sunlight for several days. Sunlight contains mainly ultraviolet-A radiation, which has long been known to damage deoxyribonucleic acid and generate formation of reactive oxygen species, making it a popular tool for killing bacteria. Storage in clear plastic bottles ensures the contaminated water is not continuously exposed to bacteria and that sunlight can pass through and kill the bacteria contained within. It has been observed that trace amounts of aromatic compounds, polyethlylene terephthalate, are released from plastic bottles into the water. This suggests that aromatic compounds could play a role in either assisting or interfering with the efficiency of SODIS. In our study, we performed SODIS on contaminated water within PET bottles, with and without the addition of aromatic compounds, tryptophan and phthalate. Due to their ability to react with ROS, aromatic compounds have been speculated to induce quenching of these ROS, leading to a decrease in SODIS efficiency. Surprisingly, the addition of aromatic compounds resulted in a more rapid decline in bacterial survival. Our results suggested that aromatic compounds could enhance the germicidal effects of ROS.

Diarrhea is the second most frequent illness in the world (19). Acute infectious diarrhea and resulting diseases account for an estimated 12,600 deaths each day in children in developing countries, and the primary diarrhea inducers in these areas are enterotoxigenic *Escherichia coli* (ETEC) and rotaviruses (19). As diarrhea caused by ETEC generally transmits via the fecal-oral route and requires a relatively high infectious dose, environmental contamination in endemic areas is exceptionally prevalent (20). The most common vehicles of infection for these environmental ETEC are food and drinking water (20); therefore, food and water sterilization is essential in controlling the transmittance of infectious diarrhea. Of the various mechanisms of sterilization being utilized to de-contaminate drinking water in developing countries, solar water disinfection (SODIS) is one of the most accessible and affordable. Research on solar water disinfection was initiated in 1970s and matured into a reliable and effective water treatment method in the beginning of 1980s (1). Since high-intensity solar radiation is available in most developing countries, especially those around the equator, this method aims to utilize this solar energy to inactivate fecal coliform bacteria present in water (3). Of the arrays of solar radiation reaching the earth, the ultraviolet (UV)-A spectrum is the most crucial for SODIS (3). Due to its practicality and low cost, SODIS has been increasingly accepted as a feasible water disinfection method in Africa, Asia, and South America (3).

Polyethlylene terephthalate (PET) plastic bottles have been recommended as the best water containers for performing SODIS (3). However, it has been reported that small amounts of aromatic compounds, such as phthalates and adipates, are released into the water from PET bottles that have undergone prolonged SODIS usage (1). Since the amount released measured was similar to that present in regular tap water, it has been speculated that water treated by SODIS presents approximately the same level of safety as tap water with respect to phthalates and adipates (3). Interestingly, previous studies have shown a decrease of bacterial killing when the microorganisms being UV-irradiated were in spread plates instead of PET bottles (2). Furthermore, it has been suggested that aromatic compounds found in laboratory media used to culture bacteria, like tryptophan and tyrosine, were quenching the effects of reactive oxygen species (ROS) mediated killing. Quenching of ROS by aromatic compounds is possible because singlet oxygen is known to react with electron-rich double bonds without the formation of free radical intermediates (11). It has been shown that amino acids such as histidine and tyrosine eliminate ROS in the same manner as the quenching of singlet oxygen (11, 12). Moreover, Matysik *et al.* also demonstrated the ability of proline to eliminate singlet oxygen and hydroxy radicals (14).

On the other hand, aromatic compounds have also been indicated to promote the formation of ROS depending on the reaction solvent (13). Therefore, the aromatic compounds released from PET bottles may either decrease or increase the effectiveness of SODIS.

The goal of our study was to determine whether aromatic compounds inhibit bacterial killing via quenching of ROS or whether they aid in bacterial killing by increasing the levels of ROS. To answer this question, we compared the rate of killing of *Escherichia coli* B23 by applying UV-A irradiation on water contained in new PET bottles, aged PET bottles, and bottles artificially supplied with the aromatic compounds, tryptophan and phthalate. No viable bacteria were detected at the end of the irradiation course in any of the four PET bottle samples and no quenching of ROS was observed. Interestingly, a small amount of viable *E. coli* were discovered after a recovery period from the UV irradiation, suggesting incomplete bacterial killing either due to entry of the bacteria into a viable but non-culturable state (VBNC), a programmed decrease in metabolic activity induced by environmental stress, or recovery of cells transiently injured by UV radiation (4, 5). We determined that the amount of aromatic compounds released when bottles are exposed to UV-A radiation for prolonged periods promotes ROS-mediated killing.

MATERIALS AND METHODS

Bacterial strains and media *E. coli* B23 was used to inoculate Luria Broth (for 1 L: Trypticase peptone 10 g; Yeast Extract 5 g; Sodium Chloride 5 g). To test the viability of the bacteria they were plated on LB agar that was prepared based on Luria broth with addition of 1.6% agar. As additives 0.4 µg/L of phthalic acid or 0.4 µg/L of D/L-tryptophan, both dissolved in 100% ethanol, was added to the bottles filled with sterilized tap water.

Equipment The Nestle™ Pure Life 500 mL bottles were used to test the effect of reactive oxygen species on *E. coli* B23 killing. For aging, the bottles were placed into a 37ºC water bath for 18 hr. Sylvania 20 W Blacklight-blue with a peak wavelength at 366 nm and an intensity of 1.2 mW/cm^2 was used to test the ROS-mediated killing.

Incubation conditions The overnight cultures were inoculated from a single colony of *E. coli* B23 into 10 mL of Luria-Bertani (LB) broth in non-baffled 125 mL flask and incubated in 37ºC shaking water bath at 50 rpm for 20 hr. The overnight culture was then inoculated into 500 mL of sterilized tap water in new bottle and in aged bottle, as well as in new bottle with 0.4 µg/L phthalate or with 0.4 µg/L of D/L-tryptophan. The amount of the additives was calculated based on the amount of aromatic compounds leaching into water from aged PET bottles (3).

Bacterial killing measurement The water in the PET bottles was inoculated at a concentration of 1 x 10^5 cfu/mL and were placed 5 cm away from UV-blacklight to aid in killing of bacteria. Bacterial killing was measured by observing bacterial survival using spread plating of UV-treated *E. coli* B23 in each bottle type in the final dilutions of 10^{-2} and 10^{-3} at 0, 10, 30, 60, 120 and 180 min. post UV treatment. The bacteria were allowed to resuscitate after UV treatment by placing them in the dark at room temperature. See the results section for more detailed descriptions of each trial.

Confirmation of complete bacterial killing after UV irradiation The PET bottles that had been previously UV irradiated were filtered through 0.22 µm pore size (Millipore) Whatman filters using a standard vacuum pump. The subsequent retentate was scraped from filters onto LB agar and spread plated with 100 µL of sterile tap water. The scraped filters were then placed directly on LB agar plates.

Determination of viability after UV irradiation The PET bottles that had been previously UV irradiated were stored overnight at room temperature in the dark. The number of bacteria was enumerated the next day by plating on LB agar.

RESULTS

Aromatic compounds enhance UV-A induced killing of *E. coli* B23 in PET bottles. In the first trial, *E. coli* B23 was inoculated into sterile water contained in PET bottles and exposed to UV light for 90 min. Colony counts at 90 min. showed that there were substantial amounts of growth for all samples. Although there was not a complete cessation of growth in any of the samples by the last time-point, there was a decline in the number of colony forming units to a stable level for the aged and new bottles (Table I). It is possible that the lack of a significant decline in viable cells in the other two samples was a result of sub-maximal exposure of these samples to UV light. For this reason we decided to discard this trial from our analysis of the effect that aromatic compounds have on SODIS. However, the results from this trial highlight the importance of having stringently controlled conditions so that each sample receives equal amounts of maximal UV-A light exposure.

Table 1. Trial 1 results for *E. coli* B23 survival in PET bottles after UV-A irradiation with and without the addition of aromatic compounds.

	Concentration of Bacteria (1 x 10^5 cfu/mL) in PET Bottles			
Time (min.)	New Bottle	Aged Bottle	New Bottle + phthalate	New Bottle + tryptophan
0	84	91	46	59
10	88	75	61	57
20	66	88	30	79
30	67	76	53	71
60	69	74	44	76
90	64	69	26	73

For the later two trials testing the effect of aromatics on SODIS, we exposed *E. coli* B23 to UV-A light for 180 min., rather than 90 min., to ensure enough time for the UV-A light to induce generation of ROS. We also ensured the bottles were receiving identical amounts of maximal UV-A light exposure by stacking the bottles two per row, 5 cm in front of the UV-A light source. From colony counts taken at various time points it was evident that the aromatic compounds were not supporting growth of the bacteria

(FIG. 1). All samples had a cessation of bacterial growth after 120 min. of UV irradiation (FIG. 1A & B). However, this trend could not be confirmed for the tryptophan-treated samples in trial 2 because the 30 and 60-min. time points were contaminated. Despite this contamination, since the tryptophan-treated sample did not have any bacterial growth at 120 min., it is reasonable to assume cessation of bacterial growth occurred at 120 min. for the tryptophan-treated sample as well. To confirm this assumption we exposed the samples in trial 2 to UV-A light overnight (~17 hr) and then plated them on LB agar to determine whether there was any survival. We found that there was no bacterial survival in any of the samples after overnight irradiation (data not shown).

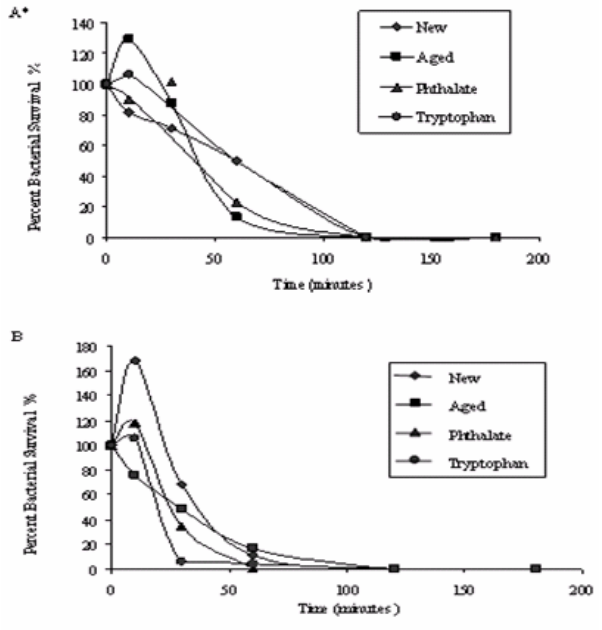

FIG. 1. Comparison of Percent survival of *E. coli* B23 in UV irradiated PET water bottles with and without the addition of aromatic compounds. Sterile water in PET bottles was inoculated with $1x10^5$ cfu/ml *E. coli* B23, UV irradiated at 355nm and colonies were counted after overnight incubation at 37°C. Four types of PET bottles used: new, aged, new with addition of phthalate, new with addition of tryptophan.
A. Trial 2: After 180 min UV irradiation, the aged sample, followed by the Pht-treated sample, shows the most rapid drop in survival. There is complete killing after 180 min irradiation for all samples.
B. Trial 3: After 180min UV irradiation, Trp-treated sample, followed by the Pht-treated sample, shows the most rapid drop in survival. There is complete killing after 120min irradiation for all samples.

*Data for Trp at 30, 60 and 180 min. is unavailable, as the plates were contaminated.

When the percent bacterial survival for both trials was averaged (FIG. 2), it appeared that the non-aromatic containing sample had the largest spike in growth after 10 min. of UV-A irradiation. This indicates that there was a lag period in which the damaging effects of UV radiation were not yet visible and the bacteria continued to proliferate. However, when trial 2 is observed on its own, (FIG. 1A) it appears that the bacteria within the aged bottle, that presumably contained trace aromatic compounds, had the largest spike in growth 10 min. post-irradiation. When trial 3 is observed on its own (FIG. 1B), it appears that the bacteria in the new bottle, without presence of aromatics, had the largest spike in growth 10 min. post-irradiation. The phthalate and tryptophan-treated samples also show a spike in growth but it is much lower. This suggests that the presence of aromatics are somehow dampening the effects of the UV lag period described. In terms of the rate of decline in percent bacterial survival, it can be seen from both trials that the rate was fastest for the samples in the aged bottles and new bottles containing aromatic compounds and slowest for the samples in the new bottles without aromatics (FIG. 1 & 2). Since the aged bottle seems to behave more similarly to the bottles containing the added aromatic compounds, it is likely that the spike in growth at 10 min. post-irradiation for the sample in the aged bottle (FIG. 1A) is an outlier.

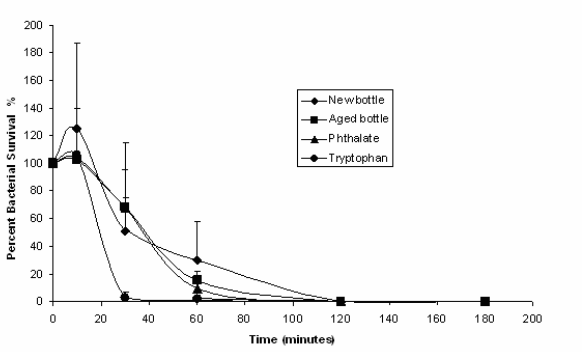

FIG. 2. Comparison of averaged percent survival of *E. coli* B23 in UV irradiated PET water bottles with and without the addition of aromatic compounds. After 180 min., the tryptophan-treated sample showed the most rapid decrease in bacterial survival. The new bottle showed a very rapid decrease in survival initially, which slowed after 30 min. of irradiation. For the phthalate-treated sample and the aged bottle, the decline in survival was not as rapid, but was more constant than the new bottle. However, there is no survival after 120 min. of UV irradiation for all samples.

It is also important to look at the trials individually to notice the inconsistency between the trials. For instance, in trial 3, the tryptophan-treated sample showed the most rapid drop in survival (FIG. 1B). This result is different from trial 2, where the aged sample showed the most rapid drop in survival (FIG. 1A). The fluctuating results between trials show the variant nature of bacterial survival measurements. It was crucial to only look for statistically significant trends found in the averaged results because of this variant

nature (FIG. 2). One such trend was that the samples in the new bottles that did not contain any aromatics had the most gradual decline in bacterial survival as compared with bottles that contained aromatics (FIG. 2).

Another inconsistency that is evident when comparing the trials individually is that overall, the bacterial survival decreased to lower numbers much sooner in trial 2 versus trial 3 (FIG. 1A & B). For instance, the tryptophan-treated sample dropped to 0% survival by 60 min. post-irradiation for trial 3 (FIG. 1B) whereas it was at ~25% survival at this same time-point for trial 2 (FIG. 1A). Hence, complete SODIS took 60 min. in trial 3 and 120 min. in trial 2. This is a surprisingly large difference in time for complete SODIS to occur between the two trials. The only factor that could have decreased the time for complete SODIS by half for all samples is the UV light. It would have to have been stronger in trial 3 than in trial 2. This is not possible, since the same UV light source was used for both trials. Despite the lack of an explanation for this unexpected result, it was helpful to average the result from these two trials (FIG. 2) and use the averaged result in our final analysis of the data.

In summary, the bacteria survived for a longer amount of time in the new bottle that had no additional aromatics compared to the aged bottle and the aromatic-treated bottles. Also, since the results for the aged bottle were more similar to the aromatic-treated bottles than the non-aromatic treated bottle, leakage of aromatic compounds from the aged plastic PET bottles into the water most likely occurred. The presence of aromatic compounds was found to induce killing via UV-A radiation and not dampen it.

UV-irradiation may induce VBNC phenomenon in *E. coli* B23. The decline in growth observed may have been due to actual UV killing of the bacteria or a temporary cessation in active cell growth. To test whether it was due to the latter case, the bottles were removed from the UV light after the final 180 min. time-point and left in the dark at room temperature. A sample from each bottle was then spread plated and incubated overnight. Furthermore, to confirm the lack of *E. coli* survival after UV irradiation was not due to failure to pick up the bacteria because of the decrease of bacterial population, 200mL of each PET bottle sample was plated by scraping cells from the filter onto LB plates and placing the remaining filter with any residual cells on separate LB plates. All plates were allowed to incubate for a period up to 42 hr.s at 37°C.

As seen from table II B, after 42 hr.s of incubation in the dark, growth began to occur on the plates with the non-aromatic containing (new bottle) and phthalate containing samples. This suggests that the cells had been too dilute to pick up while spread plating and that there was not complete bacterial killing by 180 min. post-irradiation. However, it can be seen in table III that a few colonies also appeared from the new and aged bottle samples after the period of overnight recovery. This data suggested that *E. coli* had survived the damage induced by 180 min. of UV irradiation but had entered a VBNC state.

Table 2. Appearance of growth of filtered material from 200 mL of water in each PET bottle on LB agar plates by *A.* placing the filter papers on the LB agar plates after *B.* scraping the majority of the material on the filter paper onto LB agar plates and directly spread plating the material.

A.

	Appearance of bacterial growth on filter paper placed on LB agar plates			
Incubation Period (hr.s)	New Bottle	Aged Bottle	New Bottle + phthalate	New Bottle + tryptophan
18	none	none	none	none
42	none	none	none	none

B.

	Appearance of bacterial growth on direct plating of material on filter paper on LB agar plates			
Incubation Period (hr.s)	New Bottle	Aged Bottle	New Bottle + phthalate	New Bottle + tryptophan
18	none	none	none	none
42	9 colonies	none	4 colonies	none

Table 3. Overnight spread plate count of UV irradiated *E. coli*-inoculated sterile water in PET bottles after 18h recovery in the dark.

Concentration of Bacteria (x 10^1 cfu/mL)			
New Bottle	Aged Bottle	New Bottle + phthalate	New Bottle + tryptophan
1	2	0	0

DISCUSSION

Studies conducted by the developers of SODIS, showed that trace amounts of aromatic compounds such as phthalates are released from used plastic PET bottles (3). In our study, bacterial survival in bottles that we had aged was distinctly lower than that in new bottles. This result indicates that the aging procedure used for our study was sufficient to cause aromatic compound release from plastic PET bottles. Previously, studies have shown that exposure of PET bottles under natural sunlight for a time period of months yielded distinctive shedding of photoproducts on the surface of the bottles (ref 1). Furthermore, analyses of the chemicals released inside PET bottles after a similar aging process indicated that trace amounts of aromatic

compounds were detected, including di(2-ethylhexyl)adipate (DEHA) and di(2-ethylhexyl)phthalate (DEHP), the plasticizers used to produce PET bottles (1). Considering the fact that we aged our bottles via heating in a hot water bath and had them exposed to natural daylight, the process seemed to be intensive and lengthy enough to yield a sufficient amount of aromatic compounds that were capable of changing bacterial survival to SODIS. Hence, the results observed from our aged PET bottles were more indicative of new PET bottles that had aromatic compounds added to them, than new PET bottles that did not have any aromatics added.

A recent study done on SODIS showed that when SODIS was performed on standard laboratory spread plates, the rate of bacterial killing decreased relative to when SODIS was performed in clear plastic PET bottles (2). The authors of that study explained their results by proposing that aromatic compounds present in the laboratory media were inhibiting the germicidal effects of UV-A radiation via quenching of ROS (2). However, another possible explanation for the inhibition of killing seen on the laboratory media could be due to the differences in the composition of media used. They compared bacterial killing in a rich media, LB agar, to a nutrient-deficient media, sterile water. Moreover, the results reported here indicate that trace amounts of aromatic compounds actually enhanced bacterial killing via UV-A irradiation, which further disproves the previous study's hypothesis (FIG. 3). Percent bacterial survival in the new bottles, which contained no added aromatic compounds, had a more gradual decline than percent bacterial survival in new bottles that had trace aromatic compounds present (FIG. 1). We proposed two different explanations for why the presence of aromatic compounds caused a faster decline in percent bacterial survival. One reason could be that the aromatic compounds were being oxidized by UV-A irradiation, thus adding more ROS to the water. The second reason could be that the aromatic compounds were inhibiting activity of *E. coli* catalase, leading to an accumulation of ROS.

If the reason for aromatic induced bacterial killing is due to oxidation of aromatics by UV irradiation, then this process would need to occur at a fast enough rate to generate enough ROS that could lead to an observed difference between aromatic and non-aromatic treated SODIS. When aromatic compounds get oxidized via UV light, reactive oxygen species can be generated by an as yet unknown mechanism. Although the mechanism is unknown, it has been described that photodegradation of aromatic pollutants, such as pesticides, occurs at a slow rate (8). However, one study showed that ROS can be produced during the oxidation of polycyclic aromatic hydrocarbon (PAH) *trans*-dihydrodiol and that this reaction is sped up by homogeneous dihydrodiol dehydrogenase that acts as a catalyst (6). Although this reaction involves a catalyst to aid in rapid ROS formation and our study did not have any catalysts present, it is possible that the low oxygen conditions acted as a catalyst for the oxidation of tryptophan and phthalate via UV-A irradiation. The reason for this, is that oxidation of aromatic compounds has been described as occurring ideally in low oxygen conditions (8). This is seen as well in our study since storage of the contaminated water in closed plastic bottles means the levels of available oxygen were low. The oxidation of tryptophan and phthalate would generate ROS, contributing to the existing pool of ROS and enhance ROS-mediated killing. This is opposite to the previous hypothesis that suggested oxidation of the aromatic compounds would lead to loss of ROS via quenching.

In our second proposed explanation, enhanced bacterial killing in the presence of aromatic compounds would be dependent on bacterial catalase. Catalase is an enzyme that normally breaks down the ROS, hydrogen peroxide, to non-reactive oxygen. Because *E. coli* is catalase positive, it should be able to stall ROS-mediated killing. However, since the samples containing aromatic compounds had a faster decline in bacterial survival (FIG. 2), it is possible that the aromatic compounds were inhibiting catalase activity in *E. coli*. This is a viable explanation since it has been observed in another study that 3,3'-diaminobenzidine, an aromatic compound, strongly inhibits bovine liver catalase (7). Furthermore, a much earlier study showed that simple aromatic compounds could also inhibit catalase isolated from beef liver (15). In both of these examples, aromatic compounds were inhibiting the activity of eukaryotic catalase. Since catalase enzymes are not entirely conserved between prokaryotes and eukaryotes (16), it is not safe to assume that aromatic compounds could also inhibit *E. coli* catalase, however it is still a possibility. Nonetheless, this explanation is not as favorable as the first aforementioned one. The reason for this is because there was a spike of growth after 10 min. of UV irradiation for the bottles not containing aromatic compounds (FIG. 3). This indicates there was a lag before UV damage to the cells occurred. This lag was not seen for the samples containing aromatic compounds, suggesting there was some factor speeding up their damage. The inhibition of catalase by aromatic compounds would take a longer time to take effect than the generation of more ROS via aromatic compound oxidation. To inhibit catalase activity, the aromatic compounds would first need to be taken up by the bacterial cells where they could still be used as a carbon source (17), further slowing the process of catalase inhibition.

Although there were differences in bacterial survival between the different samples, the percent of

bacterial survival dropped to zero percent for all samples by approximately 120 min. (FIG. 3). This made us wonder whether there was complete killing of the bacteria at this time or whether the bacteria had entered a viable but non-culturable (VBNC) state. Some strains of *E. coli* are capable of entering a VBNC state due to stresses such as starvation, elevated osmotic concentration and exposure to white light (4). Our study showed a decline in bacterial growth after UV irradiation. We wanted to test whether the decline was due to complete killing by UV or a temporary cessation in active cell growth because of the stressful conditions. In the latter case, *E. coli* may recover after overnight incubation without UV irradiation. Another possibility for the apparent loss of survival could be attributed to the fact that the bacteria was at such a low concentration, that none were being picked up for spread plating. To ensure that this was not the reason we observed zero percent survival, we filtered a portion of the UV irradiated sample, at its last time point of UV treatment, through a suction filter to concentrate any surviving cells and spread this onto LB agar plates.

None of the filtered material from the water samples showed growth overnight (Table II). However, when allowed to incubate for one more day, the samples from the new bottle and the phthalate-treated bottle showed some appearance of growth on the plates (Table II*B*). Moreover, after an 18-hr. recovery period in the dark, two samples showed some bacterial growth when incubated overnight on LB plates (Table III). These results suggest that although there is a drastic decrease in the bacterial population due to UV irradiation, as the concentrated bacterial samples showed no growth after incubation overnight, there is still recovery after incubation in the dark. Since some growth appeared after the filtered material was allowed one more day of incubation, and growth also was present from two water samples after recovery in the dark, it is still uncertain whether the decrease in cells observed was due to a decrease in number of cells or a decrease in culturable cells. It is possible that either the *E. coli* cells underwent transformation to a VBNC state and would slowly resuscitate when provided rich growth media or when the source causing VBNC (ie. UV irradiation) was removed. However, it is also possible that the *E. coli* cells did not exhibit VBNC, but some of the remaining cells, non-fatally injured by the UV light, were able to recover and regain the ability to divide after provision of LB agar and removal from UV light. The distinction between the VBNC phase and injured cells may be difficult, even though the two are very different physiological phenomenon, as cell injury is transient while VBNC is a specific cellular program designed for long term survival (5); they are not easily distinguished from our results and observations.

Previous studies have shown that *E. coli* develops resistance to UV light after cyclical exposure to UV radiation (18). This resistance was suggested to be mainly the result of SOS mutagenesis (18). The fact that we allowed for recovery from UV radiation means that there was also a cyclical treatment of the cells and thus it is possible that the cells also entered SOS response and underwent mutagenesis events that set them up for long-term survival. This means that UV resistant mutants may have been generated in the SODIS process of our study. This has dire implications for SODIS as poor compliance could lead to a rise in UV resistant *E. coli* mutants.

We made sure to mimic the natural circumstances under which SODIS normally takes place. We used UV-A radiation to induce bacterial killing because this is the most prevalent form of UV radiation that reaches the Earth. We also only observed the effects of trace amounts of aromatic compounds, the amount that would normally be released from PET bottles, on the SODIS process. For these reasons, our findings have direct implications for the SODIS protocol. In summary our study demonstrated that aromatic compounds, instead of quenching the ROS and thus decreasing its efficiency, may in fact assist in the process of SODIS. Furthermore, due to possible VBNC phenomenon, the germicidal effect of UV-A irradiation alone may not be sufficient in eliminating all bacteria from drinking water.

FUTURE EXPERIMENTS

In our study, we measured the percent bacterial survival after SODIS in the presence and absence of aromatic compounds. Although our data indicated that aromatic compounds enhance the germicidal effects of UV radiation, it did not indicate directly the cause of this phenomenon. We postulate that the aromatic compounds are being oxidized by UV light and by an as yet unknown mechanism, are generating more ROS. Due to limitations in supplies, we were unable to prove our hypothesis by directly measuring the levels of ROS. It would be interesting to have this measurement correlated to the presence of aromatic compounds in the water, thus determining whether aromatic compounds lead to an increase in the amount of ROS. Our study was also limited to measurement of gram-negative bacteria, *E. coli*, survival even though contaminated water normally contains a diverse plethora of microorganisms. To further confirm that aromatic compounds can enhance SODIS, it would be important to test whether this phenomenon holds true for viruses and gram-positive bacteria. Our experiments were also only performed in anaerobic conditions, where the water bottles were closed tight with a cap and let to stand while being irradiated with UV light.

Since our results showed a significant difference between SODIS in the presence versus absence of aromatic compounds, it was postulated that maybe the anaerobic conditions were acting as a catalyst for aromatic compound oxidation via UV irradiation. To determine whether this hypothesis is true, our study should be repeated in aerobic conditions. A simple strategy for inducing aerobic conditions would be to shake the bottles periodically while they are being irradiated. Research published on SODIS suggests that UV-A irradiation should last at least 12 hr.s to kill coliform bacteria (10); therefore it was not surprising to observe resuscitation of *E. coli* B23 in our experiments. UV light damages DNA, and it would be interesting to test if the bacteria that survived UV-A treatment developed mutations that made them more resistant to UV-A irradiation

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