Ghana J. Sci. **57** (2017), 57-64

PHOTOREACTIVATION OF TOTAL HETEROTROPHIC BACTERIA IN BOTTLED DRINKING WATER AFTER INACTIVATION WITH PULSED ULTRA-VIOLET LIGHT

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Abstract

Bacteria which cause opportunistic infections such as *Pseudomonas* can self resuscitate in circumstances where effective UV disinfection is compromised and is exposed to sunlight. The study investigated the effect of sub-lethal doses of pulsed ultra-violet (PUV) light on total heterotrophic bacteria (THB) in three brands of bottled water packed in glass and plastic bottles and how photoreactivation and dark repair occurred. The effect of exposure time on photoreactivation of *Escherichia coli* and *Pseudomonas aeruginosa* after inactivation with PUV was also investigated. THB in brands 1, 2 and 3 were completely inactivated by 7, 3 and 5 pulses of UV light respectively. Light repair of THB varied in the three brands of bottled water due perhaps to differences in the ionic composition of the three brands. Brands 1, 2 and 3 having 0.4, 0.7 and 1.7 log units of repair. respectively. Evidence of dark repair was not significant. Photo-repair in *E. coli* and *Pseudomonas aeruginosa* increased gradually with continual exposure to irradiating light for a period after which there was a decrease, suggesting that for a particular bacterium and illuminating source, an optimum time of exposure exist during which maximum photo-repair occur.

Introduction

In most developing countries, water resources are poorly managed resulting in increased eutrophication of water reservoirs. The use of eutrophic waters as sources of drinking water, results in use of high dosage of chlorine, as well as high water treatment costs. The possible long term adverse impact of such high dosages of chlorine has led to a search for alternative methods of water disinfection that is effective and affordable. The use of ultra-violet (UV) radiation in the disinfection of drinking water for example is widely practiced currently (Bohrerova et al., 2008). UV disinfection is achieved when DNA molecules absorb UV photons creating damage in the DNA by altering pyrimidine nucleotide base pairing, resulting in new linkages between adjacent nucleotides on the same DNA strand (Belov, krasavin & Parkhomenko, 2009). If the damage goes unrepaired, DNA replication is blocked, and this ultimately results in cell death (Zimmer & Slawson, 2002; Belov, Krasavind & Parkho-menko, 2009).

In recent times the use of pulsed ultra-violet light (PUV), as a variant of UV technology, is also gaining ground due to its potency and shorter duration of disinfection. PUV is a high 'blast' of UV energy generated from Xenon flash lamps and is usually accompanied by the generation of ions and heat (Wang *et al*., 2005) which is capable of damaging DNA of bacteria by altering its base pairings. The relative efficiency of PUV compared to the traditional continuous wave of UV disinfection is still under debate but some have come strongly in favour of PUV radiation (Bohrerova *et al.,* 2008; Luo et al, 2014; Uslua Demira & Regan, 2016). There is, however, limited data on the effectiveness of PUV on total heterotrophic bacteria and a wide range of pathogens in drinking water (Garvey

and Rowan, 2015), particularly packaged (bottled and sachet) drinking water. High counts of total heterotrophic bacteria (THB) had been found in bottled water in Ghana (Obiri-Danso *et al*., 2003; Osei et al, 2013), and other parts of the world (Nzanze *et al.,*1999; Islam et al, 2010; Semerjian, 2011;Majumder *et al.,* 2011; Pant Poudyal & Bhattacharya., 2016), including Europe and the bacteria are predominantly *Pseudomonas spp.* (Amas & Sutherland, 1999). One of the objectives of this study, therefore, would be to investigate the effect of PUV on THB in bottled drinking water.

The public health implications of high counts of THB in bottled water for vulnerable individuals such as infants, children, pregnant women and immuno-compromised persons, however, is not well understood (WHO, 2003). Indeed the presence of high numbers of *Pseudomonas aeruginosa* in potable water, notably packaged water, can be associated with complaints about taste, odour and turbidity (WHO, 2004). The presence of biofilm forming bacteria such as *Pseudomonas* in bottled water is a concern considering the ability of biofilms to enable the survival of bacteria under low nutrient conditions and to shield it from the effect of disinfection processes (Grobe *et al*., 2001). Infection with *Pseudomonas aeruginosa* is an opportunistic one that exploits the weakness of the host's defences, causing infections such as urinary tract and respiratory tract infections, dermatitis, gastrointestinal infections and immuno-compromised AIDS patients.

Limitations in the effectiveness of UV radiation disinfection due to turbidity and other issues provide the opportunity for bacteria to resuscitate itself. Many microorganisms have developed mechanisms to compensate for the damaging effects of UV radiation through multiple pathways to repair UV-induced DNA damage such as nucleotide excision repair (often referred to as dark repair) and photoreactivation (Zimmer & Slawson, 2002). Several bacterial species have been demonstrated to exhibit effective photorepair, including *E. coli* and *Pseudomonas* (Xu *et al*., 2015) but photoreactivation of *E. coli* and *Pseudomonas aeruginosa* after inactivation with PUV light and the effect of exposure time is rare to find in literature. The survival of bacteria stored in glass bottles is also different from that stored in plastic bottles due to substances released by plastic bottles that may serve as carbon and energy sources for survival and growth, including other characteristics such as the degree of carbonation and *p*H (Rosenberg, 2003).

The study investigated the effect of sub-lethal doses of PUV on THB in three brands of bottled water packed in glass and plastic bottles and how photoreactivation and dark repair occurred. The effect of exposure time on photoreactivation of *E. coli* and *Pseudomonas aeruginosa* after inactivation with PUV was also investigated with the overall aim of shedding some light on the effectiveness of PUV light technology in drinking water treatment.

Experimental

Preliminary screening of bottled water sold on the open market showed that at least three brands had total heterotrophic bacteria (THB) in concentrations of 10^3cfu/mL to 10^4cfu/mL. The three brands of this bottled water (10^3 - 10^4cfu/mL of THB) were named brand 1, 2 and 3 in this study for obvious reasons. Brand 1 and 2 had water bottles made of polyethylene terephthalate (PET) while brand 3 had glass water bottle. To understand how sunlight may affect bottled water on shelves, the absorbance and transmittance of light through the PET and glass bottle was determined. This was done using a spectrophotometer and determining the light passing through thin strips of these materials. Storage of bottled water before experiment was at room temperature.

To determine the concentrations of THB in this bottled water, dilutions were done using 25% ringer solution and yeast extract agar (prepared to manufacturer's specification, Oxoid Ltd, UK). Inoculated plates were incubated at 22 ºC for 72 hours for THB count and at 37 ºC for 24h to determine the presence of potentially pathogenic bacteria. These brands of bottled

water were used in subsequent experiments outlined below.

Effect of pulsed ultra-violet (PUV) light

Three brands 1, 2 and 3 of bottled water with THB concentration of 10^3 - 10^4cfu/mL were used for the experiment to investigate the effect of PUV on THB in bottled water.The pulsed UV-rich light system used in the study was made up of a pulsed power generator (PUV-1, Samtech Ltd, Glasgow), driving a low pressure (60kPa), xenon–filled flash lamp emitting 200-280 nm wavelength radiation and connected to a monochromator (Wang *et al.*, 2005; Excelitas Technologies Corporation, 2015). The setup was operated at 1kV, the energy per pulse being 20J. Water samples obtained from the three brands of bottled water were poured into petri dishes inside the Xenon flash lamp chamber and exposing the dishes to short duration pulses of UV rich light in the wavelength range of 200-280nm at 1 pulse/s. The bottled water samples were exposed to 1, 2, 3 and 5 pulses of ultra-violet light. THB count in sample after treatment with the PUV light was determined as outlined in Standard Methods (APHA, 2012).

Photoreactivation of bottled water flora

Twenty millilitre portions of brands 1, 2 and 3 bottled water in sterile petri dishes were treated with 2 pulses of UV (a sub-lethal dose). Conditions of darkness was created by wrapping replicate samples of brands 1,2 and 3 in petri dishes wrapped in aluminium foil. Wrapped and unwrapped samples were placed at the high intensity light region of the light cabinet for 2 hours to determine whether the bacteria in these waters show dark or light repair mechanisms. The light cabinet had 12 fluorescent lamps at the top of the upper shelf and light intensity ranged from 13,600 – 14,000 lux. During the experimental procedure, the lights in the laboratory were dimmed to minimize any effects of stray lights. Plating was done by the pour plate method in a dark room illuminated by a red light to avoid uncontrolled photoreactivation in between experimental analysis.

Effect of period of exposure on photoreactivation

Pure culture of the test bacteria was centrifuged at 4300 rpm for 20 min at 20 ºC and the pellet re-suspended in 100 mL sterile PBS. This was then serially diluted to a concentration of 10^8cfu/mL. Twenty millilitre portions of this sample were transferred unto sterile petri dishes for inactivation to 10^2 /cfu/mL. Preliminary experiments showed that 5 pulses of PUV light exposure reduced *E. coli* from a concentration of 10^8 /mL to 10^2/mL. The *E. coli* suspension (10^2 cfu/mL) was exposed to light and darkness for 30, 60, 120, 180 and 240 minutes in the light cabinet as described above. After this period, *E. coli* counts were determined as outlined in Ansa et al (2012).

Statistical analysis

Bacteria count before and after inactivation with PUV light was compared using student's t-test at a p-value of 0.05. T-test was also used to compare bacteria count before and after photoreactivation or dark repair. A correlational analysis between number of pulses of PUV light used and concentration of ions in the drinking water samples was also run. The relationship between bacteria count after photoreactivation or dark repair and exposure time was also determined using correlational analysis.

Results

TABLE 1

Table 1. Characteristics of bottled mineral water brands

Parameters	*Brand 1 (mg/L)*	*Brand 2 (mg/L)*	*Brand 3 (mg/L)*
pH	7.2	6.1	6.2
Magnesium	8.0	3.0	5.1
Calcium	32.0	4.0	14.0
Sodium	4.5	6.0	13.0
Nitrate	2.0	3.0	13.0
Sulphate	7.0	6.0	24.5
Chloride	5.0	9.0	22.7
Bicarbonate	133.0	26.0	40.0
Potassium	0.5	2.0	1.4

Table 1 shows the chemical composition of the various brands of bottled water used for the experiment. Brand 1 particularly had high content of bicarbonates. The glass bottle of brand 1 can transmit light of wavelengths above 336nm with 50% of transmittance occurring at 346nm (Fig. 1a). The PET bottle can transmit light of wavelengths above 321nm with 50% of transmittance occurring at 326nm (Fig.1b).

All bacteria in the various brands showed a decrease in bacterial count with increasing number of pulses of UV light. This Indicates that PUV light can be effective in inactivating heterotrophic bacteria in bottled water. THB in brands 1, 2 and 3 were completely inactivated by 7, 3 and 5 pulses of UV light respectively (Fig. 2). The amount of pulsed UV light required to completely inactivate all the THB in the different brands of bottled water correlated well with the concentration of bicarbonates present in the water ($R^2 = 0.85$).

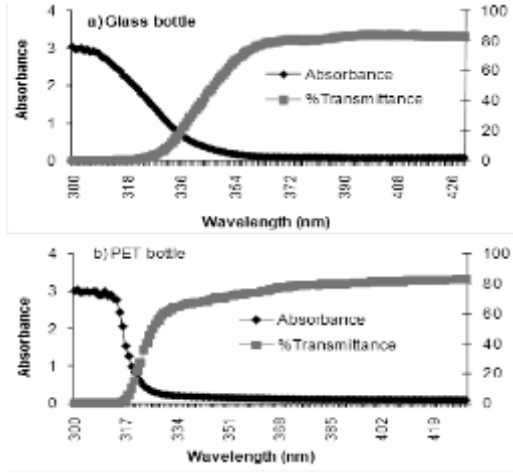

Fig. 1. Absorbance and transmittance through glass bottle and polyethylene terephthalate (PET)

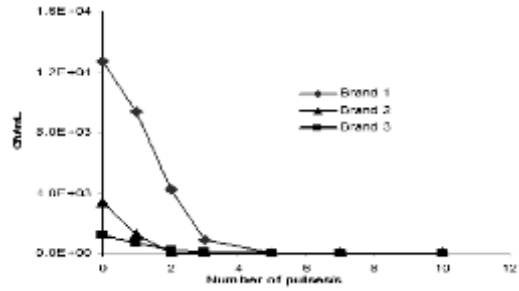

Fig. 2. Effect of PUV treatment on total heterotrophic bacteria present in selected brands of bottled water

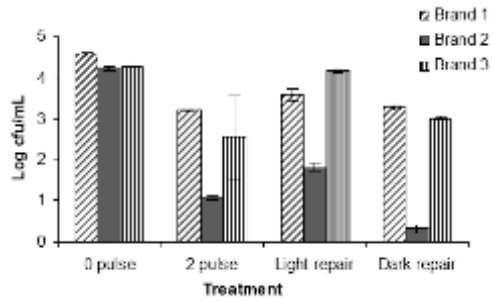

Fig. 3. Effect of sub-lethal PUV treatment and photoreactivation of total heterotrophic bacteria in three different brands of bottled water

Brand 1, after 2 pulses of UV treatment reduced by 1.3 log units. For light repair, exposure to 2h/ of light, led to an increase in 0.37 log units (Fig. 3). Differences in counts for dark repair were not significant. There was a 3.1 log unit decrease in bacterial count for brand 2 after 2 pulses of UV treatment. Light repair in brand 2 was a 0.74 log increase after 2h of exposure to continuous light following inactivation. No dark repair was however observed in brand 2.

After 2 pulses of UV treatment, there was a 1.7 log decrease in bacterial counts in brand 3. Brand 3 showed a log increase of 1.7 log units in bacteria count after 2 h of exposure to light and a 0.4 log increase following dark repair. Dark repair increases however were not signifi-cant. No pathogenic bacteria were present in the brands analysed after incubation at 37 °C for 24h/. After 5 pulses of UV treatment, *E. coli* population was reduced from 3.4 x 10^8 to 6.7 x 10^2 cfu/ml before photoreactivation study was carried out. *E. coli* NCTC 9001 showed a light repair of 2.1 log increase after 4h/ of continuous exposure to irradiation from 12 fluorescent lamps (intensity of 13,600 – 14, 000 lux). The exposure period correlated with the log count of *E. coli* /ml repair in light ($R^2 = 0.87$) and darkness ($R^2 = 0.91$) in an exponential relationship (Figure 4).

Using 8 pulses of UV treatment, *Pseudomonas aeruginosa* population was reduced from 4.4 x10^8 to 2.4 x10^3 cfu/ml. After exposure to 3h/ of light, a light repair of 1.2 log increase in bacteria count was observed. The exposure period correlated with the log count of *P. aeruginosa* /ml repair in light ($R^2 = 0.73$) and darkness ($R^2 = 0.96$) in an exponential relationship (Figure 5).

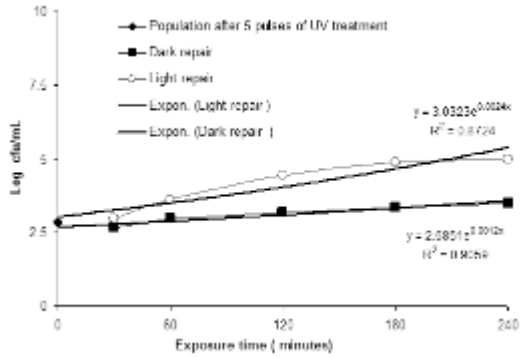

Fig. 4. Influence of period of exposure on E. coli photoreactivation

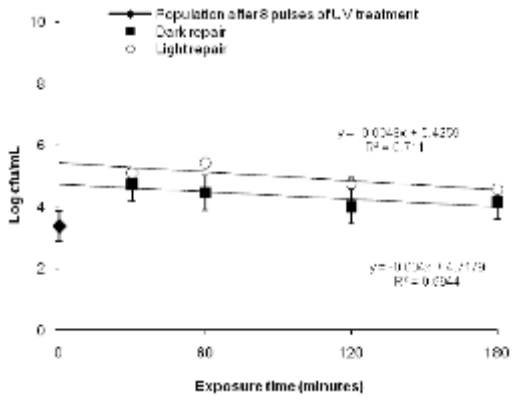

Fig. 5. Influence of period of exposure on Pseudomonas aeruginosa photoreactivation

Discussion

PUV inactivation of Total Heterotrophic Bacteria in bottled water

Generally, the levels of contamination of bottled water with THB varied from brand to brand and from batch to batch. The wavelengths of light inactivating THB in the glass and PET bottles were above 336nm and 321nm, respectively. The glass bottle containing brand 3 transmitted 50% of its light at a wavelength of 346nm (Fig. 1a). The PET bottle containing brand 1 and 2, transmitted 50% of its light at a wavelength of 326nm (Fig. 1b). This shows the ability of the glass and the PET bottles to transmit light of

longer wavelengths. THB in the various brands showed a decrease in bacterial count with increasing number of pulses of UV light. This Indicates that PUV light can be effective in inactivating heterotrophic bacteria in glass and PET bottled water.

THB in brands 1, 2 and 3 were completely inactivated by 7, 3 and 5 pulses of UV light, respectively, (Fig. 2). Differences in the requirement of pulses of UV for the inactivation of THB in the different brands could be attribu-ted to the chemical compositions of the different brands of the bottled water as transmittance of radiation in both bottle types were similar. Uptake of bicarbonates by some heterotrophic bacteria is an important source of carbon (Hongo et al, 2016) and brand 1 had as high as 133mg/l of bicarbonates (Table 1). The amount of pulsed UV light required to completely inactivate all the THB in the different brands of bottled water correlated well with the concen-tration of bicarbonates present in the water ($R^2 = 0.85$).

Photoreactivation of Total Heterotrophic bacteria in bottled water

Inactivation by 2 pulses of UV light is considered sub-lethal as not all THB are inactivated. With sub-lethal PUV treatment, light repair was evident in all the 3 brands of bottled water. Light repair in brand 1, 2 and 3 were 0.4, 0.7 and 1.7 log units, respectively, (Fig. 3). Dark repair increases in all the three brands were not significant. Differences in the degree of photoreactivation of THB in the three brands can be attributed to differences in the ionic composition of the bottled water (Table 1). Photoreactivation is a two-step process which involves first, the formation of a complex of the dimer to be repaired and the photoreactivation enzyme (PE). This step is dependent on temperature, pH and ionic strength of the water (Velez-Colmenares et al, 2012). All brands had similar temperatures (room temperature) and *p*H of brand 2 (6.1) and brand 3 (6.2), were similar (Table 1). The second step in the photoreactivation process is dependent on the intensity of the illumination source (Velez-Colmenares *et al*., 2012) which was also the same in all brands.

Effect of exposure time on photoreactivation

Photorepair seems to increase gradually with continual exposure to irradiating light. For both *E. coli* NCTC 9001 and *P. aeruginosa,* continuous exposure to irradiation from 12 fluorescent lamps with light intensity of 13,600 – 14,000 lux showed increasing photo-repair for a time after which photo-repair decreased. The exposure period correlated with the log count of *E. coli* repair in light ($R^2 = 0.87$) (Fig. 4) and with the log count of *P. aeruginosa* repair in light ($R^2 = 0.73$) (Fig. 5) in an exponential relationship. The exponential relationship of log increase in bacteria count till a certain optimum after which there is a decrease can be explained by the activities occurring in the second step of the photoreactivation process. During the second step, an extended period of exposure to light enables the release of more photoreactivation enzymes (PE), making them available to form new complexes (Nebot et al, 2007; Velez-Colmenares et al, 2012). The decreasing part of the curve can be explained by the fact that with time, formation of new complexes may decrease due to decreasing amounts of limiting substrate of enzyme.

Conclusion

THB in brands 1, 2 and 3 were completely inactivated by 7, 3 and 5 pulses of UV light respectively. This inactivation was achieved by wavelengths above 336nm and 321nm in glass bottles, and PET bottles respectively, 50% of transmittance occurring at 346nm and 326 respectively for glass and PET bottles. Light repair of THB varied in the three brands of bottled water due perhaps to differences in the ionic composition of the three brands. Brands 1, 2 and 3 having 0.4, 0.7 and 1.7 log units of repair, respectively. Evidence of dark repair was not significant. Photo-repair in *E. coli* and *Pseudomonas aeruginosa* increased gradually with

continual exposure to irradiating light for a period after which there was a decrease, suggesting that for a particular bacteria and illuminating source, an optimum time of exposure exist during which maximum photo-repair occur.

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Received 23 Mar 17; revised 11 May 17