Effect of the disinfection agents chlorine, UV irradiation, silver ions, and TiO_2 nanoparticles/near-UV on DNA molecules

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ABSTRACT

Extracellular DNA in municipal wastewater and effluents from hospitals and R&D laboratories contains antimicrobial resistance and recombinant genes that are today considered as a new class of emerging contaminants. The objective of this research was to investigate the effect of disinfection agents on the integrity of DNA molecules by using real-time PCR. *Escherichia coli* cell suspensions and genomic DNA in aqueous solution were exposed to increasing doses of disinfection systems, including chlorination, UV irradiation, silver ions, and TiO_2 nanoparticles/near-UV. The doses resulting in damage of DNA (16S rDNA) were determined using real-time PCR and compared with the doses resulting in the inactivation of bacterial cells. Our results showed that the disinfection agents chlorine, UV, and silver significantly inhibited the amplification of a fragment of 16S rDNA, but only when applied at doses much higher than the lethal doses for *E. coli* bacteria. The inactivation doses of TiO_2 nanoparticles/near-UV were of the same order of magnitude for both DNA and living cells. Our results raise questions about the efficacy of disinfection processes to destroy and prevent the dispersion of DNA pollutants into the environment. In addition, the damage of DNA by high levels of disinfectants may have implications for the utilization of PCR-based methods for bacterial detection.

Key words | Chlorine, disinfection, DNA damage, real-time PCR, silver, titanium dioxide, UV irradiation

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INTRODUCTION

Extracellular DNA in biological waste from municipalities, animal husbandries, and biomedical laboratories may contain antimicrobial resistance and recombinant genes that are increasingly considered as a new class of environmental pollutants.

Antimicrobial resistance is today a major concern for public health and it is widely accepted that wastewater treatment plants constitute a major route of entry of antimicrobial resistance genes into the environment (Pruden *et al.* 2006; Luczkiewicz *et al.* 2010). Recombinant DNA has been used extensively for the production of therapeutic molecules, vaccines and, more recently, bioethanol (Nielsen *et al.* 2007). Because of its potential impact on the environment, the release of recombinant DNA is currently regulated by the Toxic Substance Act (EPA) (Noordover *et al.* 2002). Genes on extracellular DNA can spread by horizontal gene transfer (HGT) involving mobile genetic elements, bacteriophages, or fragments of genomic DNA, potentially resulting in the acquisition of artificial traits by natural organisms and actual or perceived adverse effects on the environment (Stephenson & Warnes 1996).

The treatment of wastewater contaminated with biological wastes typically involves a disinfection process to prevent pathogens from being discharged into receiving waters. For more than a century, chlorination has been the primary disinfectant for the treatment of wastewater in the U.S. (Suquet *et al.* 2010). As the second most used wastewater disinfection process, UV irradiation has raised increasing interest due its high efficacy against the protozoae *Cryptosporidium* and *Giardia* and the detection of toxic chlorination byproducts (Hijnen *et al.* 2006; Wait *et al.* 2007). Silver is a disinfectant commonly applied for bacterial control in water supplies, cooling towers, swimming pools, and food, dietary and medical products (Silvestry-Rodriguez

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et al. 2007). While still largely experimental, titanium dioxide (TiO_2) irradiated with near-UV light generates hydroxyl free radicals that are potent disinfectant species (Maness *et al.* 1999). Although these disinfectants have been proven efficient in killing microorganisms, there is currently little information about their efficiency in destroying DNA molecules. Besides the environmental implications related to the dispersion of suspected harmful genes, potential DNA damage induced by disinfection agents may introduce a bias in the utilization of molecular methods for microbial detection and source tracking.

The objective of the present study was to characterize the effects of the disinfectants chlorine, UV irradiation, silver, and TiO_2 nanoparticles/near-UV on extracellular DNA molecules. Genomic DNA from *Escherichia coli* bacteria was exposed to increasing doses of disinfectants and DNA integrity was assessed by agarose gel electrophoresis (AGE) and the amplification of a marker gene, 16S rDNA, using real-time PCR. In parallel, *E. coli* cells were exposed to the same disinfectants and cell culturability was determined by counting the colony forming units (CFUs). Our results showed that the disinfection agents chlorine, UV, and silver significantly affected the DNA integrity, but only when applied at doses higher than lethal doses for *E. coli* bacteria.

METHODS

Bacterial cultures

E. coli DH5α was grown overnight in LB (Luria-Bertani) medium under agitation at 37 °C. Cells were harvested by centrifugation at 5,000 rpm for 15 min, washed three times with sterile phosphate buffer saline (PBS) and stored at 4 °C. For disinfection experiments, cells were diluted to an optical density at 600 nm (OD_{600}) ≈ 0.01 AU (based on plating results, final cell dilutions contained ~3×10^6 CFUs mL^{-1}). Except where stated otherwise, 20 mL of diluted suspension were used in each disinfection experiment.

DNA extraction

Total genomic DNA was extracted from overnight *E. coli* cell suspensions using DNeasy® Tissue Mini Kit (Qiagen, Carlsbad, CA). DNA extracts were quantified by the OD_{260} using a NanoDrop™ ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). The quality of the DNA was assessed by the ratio OD_{260}/OD_{280}. The DNA was stored at −80 °C. For disinfection experiments, the total genomic DNA (50–100 μg mL^{-1}) was diluted to 10 μg mL^{-1} in Tris buffer (2 mM). Except where stated otherwise, 5 mL of diluted solution were used in each disinfection experiment.

Chlorination

Diluted bacterial suspensions were exposed to increasing chlorine doses for 5 min under magnetic stirring. The initial chlorine demand of the bacterial suspension was determined using the *N,N′*-diethyl-*p*-phenylenediamine method. Chlorine was added as sodium hypochlorite (NaClO) solution (commercial bleach) at doses ranging from 0.0 to 2.0 mg L^{-1} min Cl for cell suspensions and from 0.0 to 4,000 mg L^{-1} min Cl for DNA (Freese & Nozaic 2004). After exposure, free chlorine was neutralized by the addition of an excess of sodium thiosulfate ($Na_2S_2O_3$). Control experiments consisted of non-treated samples with the addition of the neutralization agent $Na_2S_2O_3$.

UV irradiation

A UV collimated beam unit with a low-pressure UV mercury lamp (Atlantic Ultraviolet, Hauppauge, NY) that emits predominantly at 254 nm (UVC) was used. The UV intensity at the surface of the liquid was measured with a SEL240 Detector and IL1400A Radiometer (International Light, Peabody, MA) and was 0.1–0.2 mW cm^{-2}. Four mL of cell suspensions and DNA solutions in shallow quartz dishes (depth of the solution = 12.5 mm) were exposed to UV under magnetic stirring for increasing periods of time, resulting in doses ranging from 0.0 to 18 mJ cm^{-2} for cells and 0.0 to 540 mJ cm^{-2} for DNA (Freese & Nozaic 2004).

Silver ions

E. coli cell suspensions and DNA solutions were exposed to silver ions (Ag^+) by adding a silver nitrate ($AgNO_3$) solution to final concentrations ranging from 0.0 to 1.0 mg L^{-1} Ag for cells and 0.0 to 100 mg L^{-1} Ag for DNA (Butkus *et al.* 2003). Samples were incubated under magnetic stirring for 12 hours. In order to test the potential effect of silver ions carry-over on the PCR amplification, non-exposed DNA was applied on a silica gel cartridge and washed with 1 mL of PBS containing 100 mg L^{-1} Ag^+. DNA clean-up was then performed as for other silver-exposed samples.

TiO_2 nanoparticles/near-UV

E. coli cell suspensions and DNA solutions were exposed simultaneously to TiO_2 nanoparticles (500 μg L^{-1}) and

near-UV irradiation (365 nm) on a rotary shaker at 150 rpm for increasing periods of time (Maness *et al.* 1999). TiO_2 engineered nanoparticles were Degussa P25, 80% anatase and 20% rutile, with an average size of 21 nm and BET surface area of 50 $m^2\,g^{-1}$ (Degussa, Parsippany, NJ). A UV lamp (UVP, Upland, CA) that predominantly emits at 365 nm was used as a source of long-wavelength UV (UVA). The UV intensity was measured with a UV detector SEL005 and a radiometer IL1400A (International Light). Both cell and DNA samples were exposed for periods of time ranging from 0.0 to 12 hours, after which TiO_2 nanoparticles were removed by centrifugation at 1,000 g for 1 min. Two additional controls were exposed to UV only and TiO_2 only.

Characterization of the disinfection efficiency

After exposure to disinfection, serial dilutions of cell suspensions were plated on LB-agar and incubated at 37 °C for 18 to 24 hours. Cell culturability was determined by counting the colony forming units (CFUs). Exposed DNA was purified on silica columns, eluted in Tris buffer, and re-concentrated by ethanol precipitation. DNA was then washed two times with 70%-ethanol and eluted in Tris buffer to a final concentration of about 75 $\mu g\,mL^{-1}$. DNA damage was assessed by the level of amplification of a fragment of 16S ribosomal RNA gene (16S rDNA) by real-time PCR using the universal primers Eb787F: ATTAGATACCCTGGTA and E1115R: AGGGTTGCGCTCGTTG (Baker *et al.* 2003). Control reactions were run without DNA. PCR reactions were carried out on an ABI Prism® 7300 Sequence Detection System using SYBR® Green PCR Master Mix with passive reference ROX™ (Applied Biosystems, Foster City, CA). Cycling conditions were as prescribed by the manufacturer and involved an initial activation/denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and annealing-extension at 60 °C for 1 min. DNA amplification levels were calculated by the Comparative C_T (cycle threshold) method (or ΔC_T method) using ABI Prism 7300 Software v1.3.1 (Applied Biosystems). The amplification levels of exposed DNA were expressed by reference to non-exposed DNA and presented in the figures as relative amplification levels. The amplification efficiency was determined during all real-time PCR runs using 6 log dilutions of treated and non-treated DNA according to standard protocols (Applied Biosystems 2008).

Disinfection model

The effect of disinfection on *E. coli* cells and DNA molecules was calculated using the following variation of a first-order disinfection kinetics (Xiong *et al.* 1999):

$$\log\left(\frac{N}{N_0}\right) = \begin{cases} 0 & X < b \\ -k(X-b) & X \geq b \end{cases}$$

where X is the disinfection variable: the chemical dose (mg L^{-1} min Cl) for chlorination, physical dose (mJ cm^{-2}) for UV irradiation, concentration (mg L^{-1} Ag) for silver ions, and exposure time (min) for TiO_2/UVA. b is the *lag* coefficient and k is the coefficient of *specific lethality*. The model parameters were estimated by running piecewise regressions incrementing a trial breakpoint at dose = b. For each regression, a least square error algorithm was implemented in a macro to estimate the specific lethality coefficient and the sum of square errors (SSE) was calculated (Helsel & Hirsch 1993).

Statistical analyses

All experiments were conducted in triplicate and results are presented as means and standard deviations between replicates. The statistical significance of the differences between viabilities and amplification levels was assessed using t-tests at 95% confidence level.

RESULTS AND DISCUSSION

E. coli cell suspensions and genomic DNA solutions were exposed to increasing doses of the disinfectants sodium hypochlorite (NaClO), UV irradiation, silver ions (Ag^+), and TiO_2 nanoparticles/near-UV. Concentrations and doses of disinfectants were chosen based on typical bactericidal values used in wastewater treatment processes (for chlorine, UV, and Ag^+) and experimental values described in the literature (for TiO_2/near UV) (Maness *et al.* 1999; Butkus *et al.* 2003; Freese & Nozaic 2004). The inactivation dose for cells was defined as the dose resulting in a 2-log decrease of the viable bacterial counts. The damaging dose for DNA was defined as the dose resulting in 2-log reduction of the amplification levels of the gene coding for the ribosomal RNA, 16S rDNA, using real-time PCR. Real-time PCR has been used widely to detect DNA damage in specific gene segments (Ayala-Torres *et al.* 2000). Many DNA lesions, as induced by disinfection, are expected to block

the DNA polymerase resulting in a decrease of the amplification of damaged DNA as compared with intact DNA. The use of 16S rDNA as an indicator of DNA damage was motivated by the existence of multiple copies per cell (*E. coli* has 7 copies), making it a strong marker (Lee *et al.* 2009). The standard curves generated to assess the amplification efficiencies showed slopes ranging between −3.4 and −3.2 (corresponding to efficiencies of 96.8 to 105.4%) and r^2 higher than 0.98 for all non-treated DNA and treated DNA showing amplification levels at or above 10% of the amplification level of non-treated DNA. However, lower amplification levels were associated with a loss of linearity and more variable amplification efficiencies. Figures 1 to 4 present the dose-response data and regression trends for *E. coli* culturability (panel a) and DNA amplification (panel b) on both linear and logarithmic scales. Regression parameters and 2-log inactivation doses for cells and DNA are summarized in Table 1.

Figure 1 showed a significantly higher 2-log inactivation dose for DNA (1,980 mg L^{-1} min Cl) than for bacterial cells (0.52 mg L^{-1} min Cl). Consistently with these results, doses higher than 1,500 mg L^{-1} min Cl resulted in a complete disappearance of the DNA bands as shown by electrophoresis (data not presented). No effect of thiosulfate alone was recorded on cell growth and DNA amplification. Bactericidal doses of free chlorine reported in the literature (0.4 to 0.8 mg L^{-1} min) are consistent with our observations (Freese & Nozaic 2004). On the other hand, the inhibition of PCR amplification of DNA after exposure to high chlorine doses could be attributable to DNA base chlorination and subsequent impairment of *in vitro* replication by the DNA polymerase.

UV irradiation

As observed with chlorination, the UV dose resulting in 2-log DNA reduction (234 mJ cm^{-2}) was higher than the dose resulting in 2-log inactivation of cells (8.7 mJ cm^{-2}) (Figure 2). Exposure to 540 mJ cm^{-2} (1 hour) resulted in complete inhibition of DNA amplification. On the other hand, no observable effect of UV on DNA bands was recordable by electrophoresis (data not presented). Usually, 2- to 3-log reductions of indicator organisms are observed with UV doses ranging from 40 to 70 mJ cm^{-2}, which is

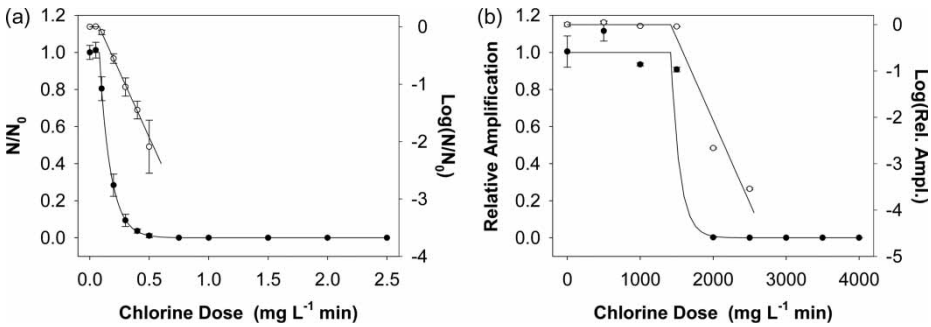

Figure 1 | Effect of chlorination (NaClO) on *E. coli* culturability (a) and PCR amplification of 16S rDNA (b). Cell inactivation is expressed as the ratio of viable cells in treated and non-treated samples (N/N_0) and DNA damage is expressed as the ratio of the amplification signal in treated and non-treated samples (Relative Amplification). These ratios were presented on linear (solid circles, left *Y*-axis) and logarithmic scales (open circles, right *Y*-axis).

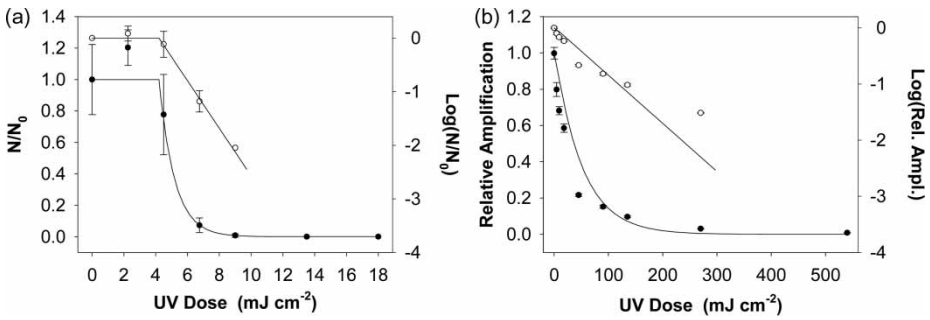

Figure 2 | Effect of UV irradiation (254 nm) on *E. coli* culturability (a) and PCR amplification of 16S rDNA (b). Cell inactivation is expressed as the ratio of viable cells in treated and non-treated samples (N/N_0) and DNA damage is expressed as the ratio of the amplification signal in treated and non-treated samples (Relative Amplification). These ratios were presented using linear (solid circles, left *Y*-axis) and logarithmic scales (open circles, right *Y*-axis).

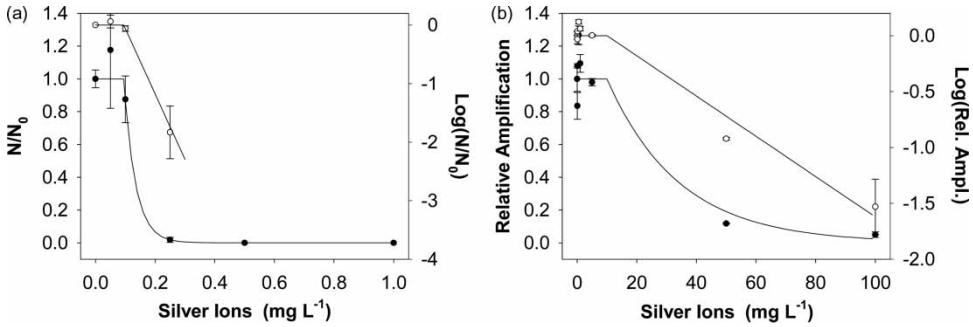

Figure 3 | Effect of silver ions (Ag^{+}) on *E. coli* culturability (a) and PCR amplification of 16S rDNA (b). Cell inactivation is expressed as the ratio of viable cells in treated and non-treated samples (N/N_0) and DNA damage is expressed as the ratio of the amplification signal in treated and non-treated samples (Relative Amplification). These ratios were presented using linear (solid circles, left *Y*-axis) and logarithmic scales (open circles, right *Y*-axis).

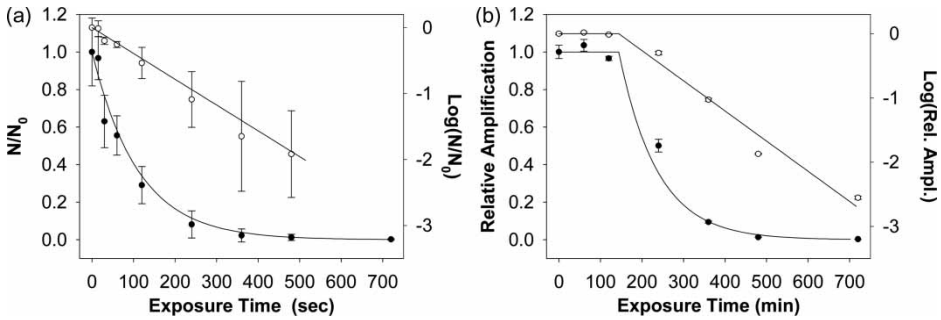

Figure 4 | Effect of exposure to TiO_2 nanoparticles (500 μg L^{-1})/near-UV (0.3 mW cm^{-2}) on *E. coli* culturability (a) and PCR amplification of 16S rDNA (b). Cell inactivation is expressed as the ratio of viable cells in treated and non-treated samples (N/N_0) and DNA damage is expressed as the ratio of the amplification signal in treated and non-treated samples (Relative Amplification). These ratios were presented using linear (solid circles, left *Y*-axis) and logarithmic scales (open circles, right *Y*-axis).

Table 1 | Estimated regression parameters of disinfection kinetics and 2-log inactivation doses of *E. coli* cells (cell culturability) and DNA molecules (real-time PCR) for the disinfection agents

Process	Lag		Specific lethality coefficient		2-Log inactivation dose		r^2 regression	
	Cells	DNA	Cells	DNA	Cells	DNA	Cells	DNA
Chlorination	0.08 mg L^{-1} min	1,417 mg L^{-1} min	−4.56	−0.0035	0.52 mg L^{-1} min	1.980 mg L^{-1} min	0.99	0.92
UV irradiation	4.2 mJ cm^{-2}	1.5 mJ cm^{-2}	−0.44	−0.0086	8.7 mJ cm^{-2}	234 mJ cm^{-2}	0.99	0.92
Silver ions	0.094 mg L^{-1}	10 mg L^{-1}	−11.16	−0.018	0.28 mg L^{-1}	121 mg L^{-1}	0.99	0.95
TiO_2/near-UV	0.0 min	144 min	−0.0039	−0.0047	514 min	571 min	0.99	0.97

higher than the 2-log inactivation dose that we observed for *E. coli* bacteria (Freese & Nozaic 2004). This is likely explained by the shallowness of the quartz dishes used for laboratory experiments (12.5 mm), allowing higher cell exposure than full size UV disinfection systems.

Silver ions

As observed previously, the 2-log reduction dose of DNA exposed to silver ions for 12 hours (121 mg L^{-1} Ag) was higher than the 2-log inactivation dose of *E. coli* cells (0.28 mg L^{-1} Ag) (Figure 3). The PCR control run with non-exposed DNA washed with 100 mg L^{-1} Ag^{+} resulted in the same amplification level as non-exposed DNA. As shown by electrophoresis, exposure to high concentration of Ag (50 and 100 mg L^{-1}) resulted in gradual disappearance of the DNA bands (data not presented). Reported minimum inhibitory concentrations of silver for *E. coli* cells for a period of 24 hours of exposure range from 0.1 to 0.4 mg L^{-1} Ag (Butkus *et al.* 2003), which is consistent with our results.

TiO_2 nanoparticles/near-UV

Exposure to TiO_2 nanoparticles (500 μg L^{-1}) and near-UV (3.0 mW cm^{-2}) resulted in similar 2-log inactivation times for *E. coli* cells and DNA (514 and 517 min, respectively) (Figure 4). The controls exposed respectively to UV only and TiO_2 only showed amplification levels comparable to non-exposed samples. As shown by electrophoresis, exposure times higher than 4 hours produced a significant decrease of the average size and quantity of DNA, suggesting DNA fragmentation (data not presented). Values obtained in the present study for *E. coli* cell inactivation are generally in accordance with the values reported in the literature (0.1 to 1.0 mg L^{-1} TiO_2) (Maness *et al.* 1999).

The disinfection systems used in this study are known to impact cells and DNA through different mechanisms. Although not fully understood, chlorine is believed to act as a strong oxidant, reacting nonspecifically with biological molecules, partially through the generation of reactive oxygen species (ROS). Chlorine-induced cellular damages include the disruption of cell permeability and unspecific denaturation of enzymes (Prütz 1996; Suquet *et al.* 2010). Although little information is available in the literature, a few reports provide evidence of the generation of chlorinated nucleotides by slow reaction with HOCl, leading to denaturation of DNA (Prütz 1996). Consistent with our experiments, damage to DNA was only observed in the presence of relatively high concentrations of chlorine. The higher susceptibility of living cells to chlorine, as compared with DNA, is likely due to the existence of multiple cellular targets more sensitive than DNA molecules. In addition, the disappearance of the DNA bands exposed to high levels of chlorine (electrophoresis) is consistent with a recent report that exposure of DNA to chlorine results in single and double strand breaks (Suquet *et al.* 2010). Short wave UV is known to react primarily with nucleic acids, leading to the formation of cyclobutane pyrimidine dimers and pyrimidine-pyrimidone photoproducts that interfere with DNA replication (Hijnen *et al.* 2006). The apparent higher potency of UV on bacterial cells by comparison to DNA molecules may be explained by the larger size of genomic DNA (i.e. containing more potential targets for UV irradiation) as compared to the fragment of 16S rDNA that we used as a marker of DNA integrity. Electrophoresis of UV-exposed DNA showed similar bands to non-exposed DNA, which suggests that the overall molecular structure and size of DNA remained intact, even when exposed to high UV doses. This observation, together with the dose-dependent reduction of the PCR amplification, is consistent with the major mechanism of action of UV known to damage DNA through intra-molecular alterations. Although its bactericidal effect is not well understood, silver is believed to act mainly by binding cysteine residues in proteins (Silvestry-Rodriguez *et al.* 2007). As for chlorine, the higher efficiency of silver ions on cell viability as compared to extracellular DNA integrity seems to be related to the multiplicity of potential cellular targets in the whole cells. Recently, silver ions have been shown to bind DNA, potentially impairing the replication process (Butkus *et al.* 2003; Silvestry-Rodriguez *et al.* 2007). This could explain the reduction of PCR amplification and the slower migration of DNA that was recorded by gel electrophoresis at high concentration (>50 mg L^{-1} Ag). Titanium dioxide (TiO_2) irradiated with near-UV light generates hydroxyl free radicals that can oxidize most biological molecules, including proteins and nucleic acids (Maness *et al.* 1999). DNA damages caused by ROS can potentially result in base lesions, rearrangements, deletions, and insertions (Coronado *et al.* 2005). However, extremely high doses of TiO_2 had to be used to produce a recordable effect on both cell viability and DNA integrity, which suggests that this disinfection system is rather inefficient for bacterial cell and extracellular DNA destruction.

It must be pointed out that drawing conclusions about the efficacy of disinfection systems on extracellular DNA and cell viability based solely on the respective DNA content is difficult because of the interference of many factors. The concentration of extracellular DNA exposed to disinfection systems (about 1.5×10^{-12} M) was significantly higher than the DNA content of the cell suspensions (about 5×10^{-15} M). On the other hand, the number of sites (e.g. nucleotides) that are potential targets for disinfectants is higher in genomic DNA than in the 16S rDNA fragment used as a marker of DNA integrity. In addition, many molecular targets other than DNA (e.g. proteins) can lead to cell inactivation without resulting in reduction of the real-time PCR signal. Finally, it is noteworthy that besides DNA integrity, disinfectants can also affect horizontal gene transfer by many other means, including the susceptibility of donor and recipient cells and the nature of the transfer vectors (e.g. bacteriophages).

CONCLUSION

The hypothesis behind this research was that various disinfection systems, which are used or can potentially be used for wastewater treatments, may not be efficient to destroy extracellular DNA molecules. The results presented above raise

the question whether chlorination, UV irradiation, and silver ions are sufficient to destroy extracellular resistance or recombinant genes when applied at lethal doses for bacteria. Our results also show that high concentrations of disinfectants could result in significant decrease of the PCR amplification of DNA, which may have critical implications for PCR-based methods of pathogen detection that are increasingly used for the assessment of water quality and food processing.

REFERENCES

Applied Biosystems 2008 Guide to Performing Relative Quantitation of Gene Expression Using Real-Time Quantitative PCR, Rev. A. Doc. 4371095 Rev B, Section VII. pp. 43–59, *Applied Biosystems*, http://docs.appliedbiosystems.com/.

Ayala-Torres, S., Chen, Y. M., Svoboda, T., Rosenblatt, J. & Van Houten, B. 2000 Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. *Methods* **22**, 135–147.

Baker, G. C., Smith, J. J. & Cowan, D. A. 2003 Review and re-analysis of domain-specific 16S primers. *Journal of Microbiology Methods* **55**, 541–555.

Butkus, M. A., Edling, L. & Labare, M. P. 2003 The efficacy of silver as a bactericidal agent: advantages, limitations and considerations for future use. *Journal of Water Supply Research and Technololgy-Aqua* **52**, 407–416.

Coronado, J. M., Soria, J., Conesa, J. C., Bellod, R., Adán, C., Yamaoka, H., Loddo, V. & Augugliaro, V. 2005 Photocatalytic inactivation of *Legionella pneumophila* and an aerobic bacteria consortium in water over TiO_2/SiO_2 fibres in a continuous reactor. *Topics in Catalysis* **35**, 279–286.

Freese, S. D. & Nozaic, D. J. 2004 Chlorine: is it really so bad and what are the alternatives? *Water SA* **30**, 566–572.

Helsel, D. R. & Hirsch, R. M. 1993 *Statistical Methods in Water Resources, Studies in Environmental Science*. Vol. 49. Elsevier Science, New York, pp. 226–227.

Hijnen, W. A. M., Beerendonk, E. F. & Medema, G. J. 2006 Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research* **40**, 3–22.

Lee, Z. M-P., Bussema III, C. & Schmidt, T. M. 2009 rrnDB: Documenting the number of rRNA and tRNA genes in Bacteria and Archaea. *Nucleic Acids Research* **37**, D489–D493.

Luczkiewicz, A., Fudala-Ksiazek, S., Jankowska, K., Quant, B. & Olaczuk-Neyman,, K. 2010 Diversity of fecal coliforms and their antimicrobial resistance patterns in wastewater treatment model plant. *Water Science and Technology* **61**, 1383–1392.

Maness, P. C., Smolinski, S., Blake, D. M., Huang, Z., Wolfrum, E. J. & Jacoby, W. A. 1999 Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. *Applied and Environmental Microbiology* **65**, 4094–4098.

Nielsen, K. M., Johnsen, P. J., Bensasson, D. & Daffonchio, D. 2007 Release and persistence of extracellular DNA in the environment. *Environmental Biosafety Research* **6**, 37–53.

Noordover, J. A. C., Hofmeester, J. J. M., van der Burg, J. P., de Leeuw, A., van Dijck, P. W. M., Luiten, R. G. M. & Groot, G. S. P. 2002 Containment in industrial biotechnology within wastewater treatment plants. *Journal of Industrial Microbiology and Biotechnology* **28**, 65–69.

Pruden, A., Pei, R. T., Storteboom, H. & Carlson, K. H. 2006 Antibiotic resistance genes as emerging contaminants: studies in Northern Colorado. *Environmental Science and Technology* **40**, 7445–7450.

Prütz, W. A. 1996 Hypochlorous acid interactions with thiols, nucleotides, DNA, and other biological substrates. *Archives of Biochemistry and Biophysics* **332**, 110–120.

Suquet, C., Warren, J. J., Seth, N. & Hurst, J. K. 2010 Comparative study of HOCl-inflicted damage to bacterial DNA ex vivo and within cells. *Archives of Biochemistry and Biophysics* **493**, 135–142.

Silvestry-Rodriguez, N., Sicairos-Ruelas, E. E., Gerba, C. P. & Bright, K. R. 2007 Silver as a disinfectant. *Reviews of Environmental Contamination and Toxicology* **191**, 23–45.

Stephenson, J. R. & Warnes, A. 1996 Release of genetically modified micro-organisms into the environment. *Journal of Chemical Technology and Biotechnology* **65**, 5–14.

Wait, I. W., Johnston, C. T. & Blatchley, E. R. 2007 The influence of oxidation reduction potential and water treatment processes on quartz lamp sleeve fouling in ultraviolet disinfection reactors. *Water Research* **41**, 2427–2436.

Xiong, R., Xie, G., Edmondson, A. E. & Sheard, M. A. 1999 A mathematical model for bacterial inactivation. *International Journal of Food Microbiology* **46**, 45–55.

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