The Influence of Relative Humidity on the UV Susceptibility of Airborne Gram Negative Bacteria

Louise A. Fletcher
Aerobiology Research Group, School of Civil Engineering,
University of Leeds, Leeds, LS2 9JT, UK L.A.Fletcher@leeds.ac.uk

INTRODUCTION

It is now widely accepted that ultraviolet germicidal irradiation at a wavelength close to 254 nm is an effective control measure for both waterborne and airborne microorganisms. Renewed interest in the disinfection properties of UV has been stimulated by the increased incidence of diseases, such as tuberculosis, increases in the occurrence of multi-drug resistant pathogens and also the increase in the number of nosocomial infections worldwide. UV disinfection systems are currently widely used in hospitals and other health care environments in the USA in order to protect patients and healthcare workers (Dumyahn and First 1999), although it is recognized that there are is a wide range of locational and operational parameters which will affect their efficacy (Lin and Li 2002).

In the UK as in many areas of the world nosocomial infections (i.e., those infections acquired in a hospital) are a major problem, with approximately 10% of the patients in the UK acquiring an infection during a hospital stay (Mertens 1996). Many of these infections are extremely difficult to treat, as the microorganisms responsible are multi-drug resistant (e.g., MRSA) and the resulting mortality rates can be relatively high. In addition to this, nosocomial infections have a large economic impact on the healthcare system. In the UK in 2000 this cost was estimated at approximately £1 billion per annum (NAO 2000).

Although it is accepted that many nosocomial infections are spread through direct person-to-person contact, there is increasing evidence to suggest that some are transmitted via the airborne route. However, the contribution made by airborne pathogens toward nosocomial infection is unclear and there is a great deal of skepticism regarding their importance (Beggs 2003). In the UK it is estimated that between 10 and 20% of endemic nosocomial infections may be transmitted by the airborne route at a cost of between £100-200 million annually.

Most airborne microorganisms found in hospitals are thought to originate from the staff, patients and visitors within the building rather than entering from outside. Microorganisms can enter the airborne state either as droplets/droplet nuclei dispersed through a person sneezing or coughing or on skin squamae shed naturally by all individuals. Larger droplets associated with sneezing and coughing will rapidly fall to the ground, whereas the smaller ones will evaporate and rapidly decrease in size to become droplet nuclei. An infectious person coughing or sneezing will produce thousands of droplet nuclei some of which will contain pathogenic microorganisms. These droplet nuclei are so small that they can remain suspended in the air for long periods of time. For example, 2 μm droplet nuclei in a calm room will take approximately 4.2 hours to fall a distance of 2 m (Beggs 2001). Since they remain suspended in the air for so long, they can travel long distances and have the potential to contaminate large areas of buildings.

Some microorganisms are designed to be transported in the airborne state (for example fungal spores which rely on air currents for their dispersal). However, in general, vegetative cells tend to suffer environmental stress resulting from desiccation, nutrient starvation and attack from free radicals while in the airborne state (Cox and Wathes 1995). It is generally accepted that gram positive microorganisms survive much longer in the airborne state than gram negative bacteria, making them much more amenable to long distance transportation. The reason for this is thought to be due to differences in the cell wall structure of gram positive and negative bacteria.

Gram positive bacteria possess a relatively thick (20-80 nm) continuous cell wall which is rich in peptidoglycan (~90%) and has a waxy mycolic acid rich layer on the surface, which is believed to make them more resistant to desiccation under low humidity conditions (Figure 1). Gram negative bacteria on the other hand have a lipid rich outer membrane (7-10 nm) and a thin peptidoglycan layer (5-10 nm) and are therefore, more likely to suffer moisture loss under low humidity conditions (Figure 2) (MicroBioNet).

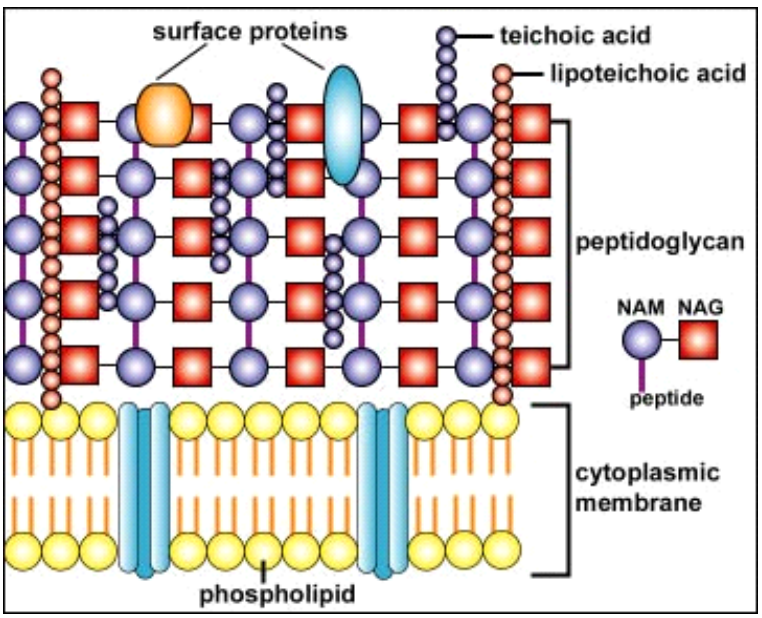

Figure 1. The structure of a gram positive cell wall (Courtesy of Dr. Kaiser, The Community College of Baltimore County).

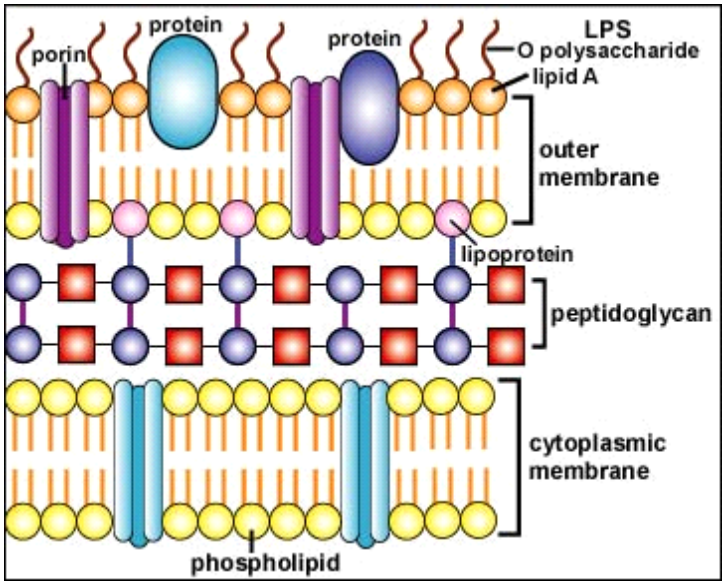

Figure 2. The structure of a gram- negative cell wall (Courtesy of Dr. Kaiser, The Community College of Baltimore County)

If this is the case, then it would suggest that under low humidity conditions gram negative bacteria will suffer more environmental stress than gram positive bacteria, and since they are already in a stressed state will be more susceptible to UV damage. When the humidity is increased gram negative bacteria will become less stressed, and this should be reflected in their increased resistance to the effects of UV at higher levels of humidity. This effect should be less apparent in gram positive bacteria as they are equally unstressed at both high and low levels of humidity.

Many authors have commented on the lack of information regarding the effect of relative humidity on the UV susceptibilities of microorganisms (Peccia et al. 2001, Ko et al. 2000, Lin and Li 2002) and also the contradictions within the data that does exist (Riley and Kaufman 1972). However, there appears to be little data regarding the effect of relative humidity on the UV susceptibilities of gram negative bacteria in particular, and the way in which their cellular structure may account for their behavior.

If relative humidity does affect the UV susceptibility of gram negative bacteria, then this will have implications for pathogen control in hospital buildings, since according to Greene *et al.* (1960, 1962a, 1962b) approximately one-third of the airborne microorganisms recovered from the air in hospitals were found to be gram negatives. This group includes pathogenic organisms such as *Pseudomonas aeruginosa*, *Bukrholderia cepacia*, *Serratia marsescens* and *Stenotrophomonas maltophilia*, which are important with regard to immuno-compromised individuals and in particular those suffering from cystic fibrosis.

It is clear therefore that there is some debate as to the importance of relative humidity and its effect on the UV susceptibility of aerosolized bacteria. With this in mind, a series of experiments were designed in order to quantify the UV inactivation of various aerosolized gram negative bacteria under controlled conditions at ambient and high relative humidities.

EXPERIMENTAL INVESTIGATION

All the experiments were performed in a specially designed airtight UV exposure chamber (Figure 3) into which an aerosolized bacterial suspension could be introduced. The aerosol is generated by a 6-jet Collison nebulizer mounted at the inlet end of the chamber (Plate 1). Aerosolized bacteria enter the chamber, are picked up into the air stream and pass through into the UV exposure section, the upper surface of which is constructed of UV transparent quartz glass. UV irradiation generated by the UV lamp assembly passes through the quartz glass and the aerosolized bacteria receive a dose of UV irradiation dependent on the air flow rate and the irradiance of the UV field. The UV irradiance can be measured through an access port located on the underside of the exposure section using a radiometer. The UV lamp apparatus consists of an enclosure containing four Philips TUV 15W lamps behind a fixed wire mesh screen. Further wire mesh screens can be added as necessary in order to adjust the UV intensity, and the whole enclosure is mounted on adjustable length legs. At the downstream end of the chamber there are two outlets, one of which is the bypass in which the exhaust air simply passes through a HEPA filter. The other outlet is the sample stream where the air passes through a 6-stage Andersen sampler, followed by a HEPA filter. Valves on both the bypass and sample streams allow air to be diverted between the two in order to allow accurate sampling times to be achieved.

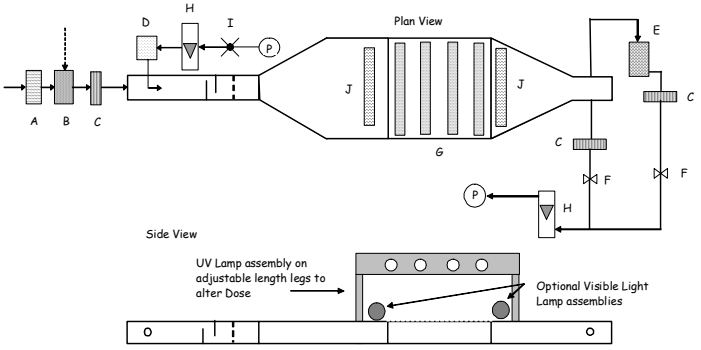

Figure 3 Schematic diagram of the laminar flow UV exposure apparatus (A: heater, B: Humidifier, C: HEPA Filter, D: 6-jet Collison Nebuliser, E: 6-stage Andersen sampler, F: air valve, G: UV exposure section, H: tube and float flow meter, I: pressure regulator and gauge, J: visible light lamps, P: air pump).

The experiments were performed using four gram negative bacteria: *Burkholderia cepacia*, *Serratia marcescens*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*. All of these microorganisms are known to be nosocomial pathogens and have been shown to have some degree of antibiotic resistance, making them extremely difficult to treat. Four separate experiments were carried out in which a suspension of the microorganism was nebulized into the test chamber for 5 minutes and the flow, temperature and relative

humidity allowed to stabilize. Five replicate three minute samples (a total of 84 liters per sample) were then taken with no UV irradiation and then at five different UV intensities. The samples were taken onto agar plates using stages 5 and 6 of the Andersen sampler (Plate 2). The plates were then incubated for 24 h at 37°C, after which the number of colonies were counted. The initial experiment was carried out at ambient temperature and relative humidity, and the whole procedure was then repeated under higher relative humidity conditions using humidified inlet air. The temperature and relative humidity of the exhaust air were measured using a portable thermohygrometer probe.

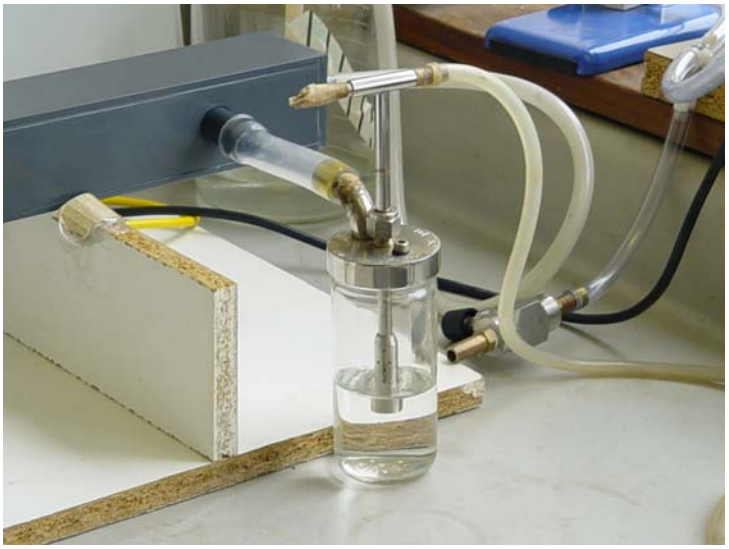

Plate 1. Six jet Collison nebulizer used to aerosolize the bacteria suspension

Plate 2. Six stage Andersen sampler

UV Susceptibility (Z value) calculation

After all the plates had been incubated for 24 hours, the colony counts on each of the plates were noted and corrected for multiple impaction (Macher 1989). For the controls (no UV) and each exposure, the number of colonies in each of the five replicates was used to calculate the average number of colonies. This was then used to calculate the survival fraction (SF) as follows:

$$SF = \frac{N_{UV}}{N_0}$$

N_{UV} = the average number of colonies on the plate after exposure to UV.

N_0 = the average number of colonies on the control plates.

In order to calculate the Z value, the natural log of the survival fraction is plotted against the UV dose (calculated by multiplying the UV average UV irradiance by the exposure time). A linear regression line is then fitted to the resulting line and the slope of the line represents the Z value of the microorganism.

$$Z = -\frac{\ln(SF)}{UVDose}$$

THE EFFFECT OF RELATIVE HUMIDITY ON THE UV SUSCEPTIBILITY

It is immediately clear from Figure 4 that increased relative humidity (from 58% up to 73%) has a marked effect on the survival of *Burkholderia cepacia* after UV exposure. At a UV dose of 5 J/m^2, only 9% of the microorganisms survived at the lower humidity compared with 50% at the higher humidity (Figure 4). At a higher UV dose of 20 J/m^2, the difference between the survival is less marked with survivals of only 2% at low humidity compared to 12% at high humidity. Figure 5 shows the plot of log survival from which the UV susceptibility is calculated and it is immediately clear from the difference in the slopes of the two regression lines that the relative humidity has a large effect on the susceptibility. At the lower relative humidity, the Z value was calculated to be 0.2115 m^2/J compared to 0.1052 m^2/J at the higher relative humidity. In effect, increasing the relative humidity by 15% produced a halving of the UV susceptibility of *Burkholderia cepacia*.

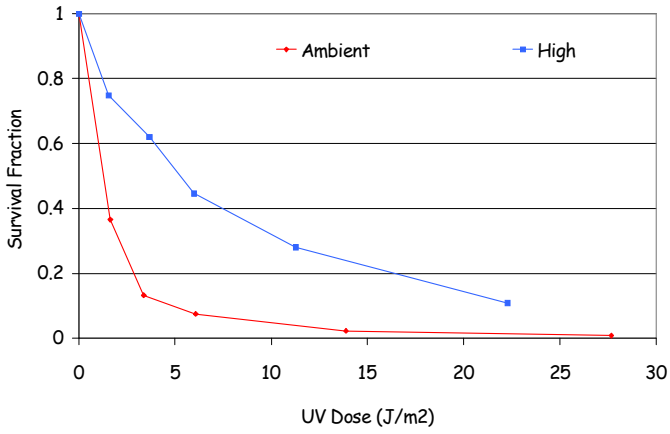

Figure 4. The effect of UV dose and RH on the survival of aerosolized *B. cepacia.*

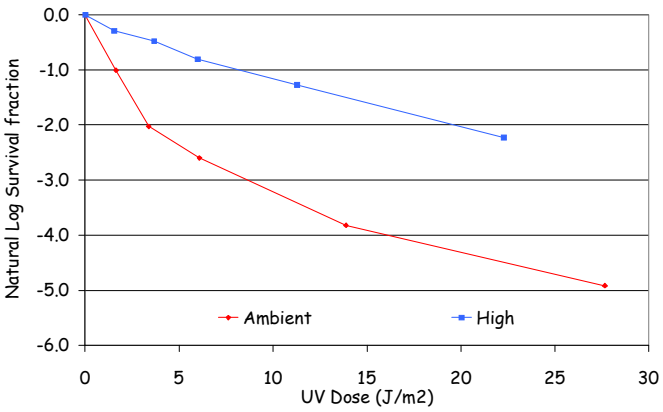

Figure 5. The effect of UV dose and RH on the UV susceptibility of aerosolized *B. cepacia*.

Similar trends were observed for the other microorganisms, although the degree to which the UV susceptibility was affected varied from as low as only 14% up to 74%. This has serious implications for the use of UV systems in tropical climates or situations where it is necessary to have increased relative humidity. In some cases, depending on the microorganism, the increased levels of relative humidity may result in more than 70% extra individual organisms surviving after UV irradiation.

The literature is somewhat divided as to the effect, if any, of relative humidity on the UV susceptibility of microorganisms. Many authors have suggested that UVGI is less effective at higher relative humidities (>60%). Peccia et al. (2001) found a significant decrease in inactivation rates at relative humidities in excess of 50% regardless of gram type, but also commented that the effect was species dependent, an effect which was also noted during this set of experiments. Gates (1929), Whistler (1940), Riley and Kaufman (1972) and Lin and Li (2002) also noted a decrease in UV susceptibility at increased levels of relative humidity. On the other hand, some authors, including Rentschler and Nagy (1942) and Ko et al. (2000) reportedly found no relationship between the two parameters.

WHY ARE GRAM NEGATIVE MICROORGANISMS MORE SUSCEPTIBLE TO UV DAMAGE AT LOWER RELATIVE HUMIDITY?

A number of theories have been put forward to account for the apparent reduction in the UV inactivation at increased relative humidity including the following:

Theory 1: Attenuation of the UV beam by additional moisture in the air;

Theory 2: Water sorption by cells provides protection against UV damage;

Theory 3: At lower relative humidity, the DNA inside the cell is in the A conformation, which is more susceptible to UV damage;

Theory 4: At lower humidity, gram negative bacterial cells are suffering environmental stress due to desiccation and are therefore more susceptible to UV damage.

The following sections discuss these theories in turn, and attempt to determine which, if any, may account for the reduced efficacy of the UV observed under controlled conditions in the laboratory experiments described.

Attenuation of the UV Beam

When designing UV systems for the treatment of water it is important to take into account the attenuation of the UV beam as it passes through the water. In air systems this is generally not thought to be an important parameter, particularly when the system is operating at low levels of relative humidity. However, if the relative humidity is increased, the increased amount of water vapor present in the air may reduce the intensity of the UV field and therefore reduce the UV dose received by the microorganisms. Peccia et al. (2001) reportedly measured UV spherical irradiance at a range of relative humidities and found that they did not change significantly in response to increased relative humidity. In the experiments described here the measured UV irradiance was found to be reduced by between 12.8 and 21.4% when the relative humidity was increased from approximately 50% up to 70%.

Although the beam was found to have been attenuated by the increased relative humidity during the experiments, this does not have an impact on the results, and cannot account for the reduced UV inactivation. The reason for this is that during the experiments the UV irradiance was recorded at each of the UV intensities used and also at both relative humidities, and is therefore incorporated into the UV dose used to calculate the UV susceptibility. UV beam attenuation would only become an issue here if it was assumed to be the same at the higher relative humidity and would result in higher UV doses being used to calculate the UV susceptibility than were actually experienced by the microorganisms.

Water Sorption

Most bacteria are composed of carbohydrate, proteins, lipid and nucleic acids which make them hygroscopic, and when in the airborne state the amount of water associated with them will be dependant on relative humidity (Cox 1987). As the relative humidity of the surrounding environment increases, then the amount of moisture attracted to the particle will increase thereby increasing the overall size of the particle. Ko et al. (2000) suggested that since germicidal ultraviolet radiation has limited penetration capabilities, then an increase in particle size may explain in part the decreased sensitivity of microorganisms at high humidity.

On the other hand Peccia et al. (2001) suggested that although bacteria can sorb up to 60% of their dry weight from water vapor at 95% relative humidity, the increased diameter associated with this (<0.5 μm) should not present a significant barrier to incident UV irradiation. They found that for *Serratia marsescens* there was no observable increase in aerodynamic

diameter with increasing relative humidity. Using a six stage Anderson impacter they found that the size of the bacterial aerosol did not change in response to increasing relative humidity.

Prior to the experiments described here, a number of experiments were carried out looking at the size distribution of the various microorganisms at ambient and high relative humidity. As reported by Peccia et al. (2001), no change in the aerodynamic diameter was observed when the relative humidity was increased. It would appear from the experimental results that increases in water sorption did not result in an increase in particle diameter, and are therefore unlikely to be responsible for the change in UV susceptibilities observed.

Changes in DNA Conformation

The hydration of intracellular DNA appears to be an important factor in understanding UV inactivation of biological cells (Peccia and Hernandez 2001). It is thought that in response to changes in relative humidity, DNA experiences various degrees of hydration, which in turn dictate the physical conformation of the genetic material contained within the cell. The two DNA conformations have different photochemical responses which will influence their response to UV irradiation at different relative humidities. At lower relative humidity, DNA is thought to undergo a reversible transition to an A-form, and at relative humidities in the range 50-65% then a transition to the B-form takes place and is completed above 75%. In addition to this, a change in the type of UV-induced DNA lesion from cyclobutane thymine dimers to spore photoproducts may be responsible for the greater susceptibility of bacterial cells at lower relative humidity.

DNA consists of two strands that coil around each other, usually in a clockwise or right-handed direction to make a double helix. Each strand is made up of four different nucleotides consisting of sugars, phosphates and a base. There are four different bases attached to the chain, and they form pairs that join the two chains together, the four bases being adenine, guanine, cytosine and thymine. Figure 6 shows the two different DNA conformations first observed by Rosaline Franklin during experiments in which she kept the DNA fiber dry (A-form) and wet (B-form) (Calladine and Drew 1997). The A-form was identified by X-ray diffraction at 75% RH, and it can be seen that the grooves are not as deep as in B-form and the base pairs are much more tilted. The B-form is the most common form of DNA and was originally deduced by X-ray diffraction at 92% RH. It contains two distinct grooves, a major and a minor groove, which provide distinct surfaces with which proteins can bind.

It is clear that there is ample evidence of conformation changes in the DNA strand in response to increases or decreases in the relative humidity, and that this may affect the UV susceptibility of biological cells. Falk *et al.* (1963) carried out a spectroscopic study of the effect of hydration on the structure of individual DNA films on plates and found that there was a decrease in the UV absorbance of the DNA at relative humidities in excess of 60%.

In reality, the DNA strand will be contained within a cell in a water filled structure and will be predominantly in the B-form. The question is therefore, whether changes in the relative humidity in the environment surrounding the cell will have an impact on the DNA within that cell. In a dry environment the cell may suffer desiccation/dehydration, but it is unclear whether this will seriously affect the water content surrounding the DNA strand. In the experiments described here, the residence time in the exposure chamber prior to UV irradiation is only approximately 10 s from the point of nebulization. It is unlikely that in such a short length of time, the change from 100% relative humidity inside the nebulizer to 50% in the test chamber would initiate a change in the conformation of the DNA strand. If this is in fact the case, then changing DNA conformation cannot account for the reduction in UV inactivation observed during the experiments.

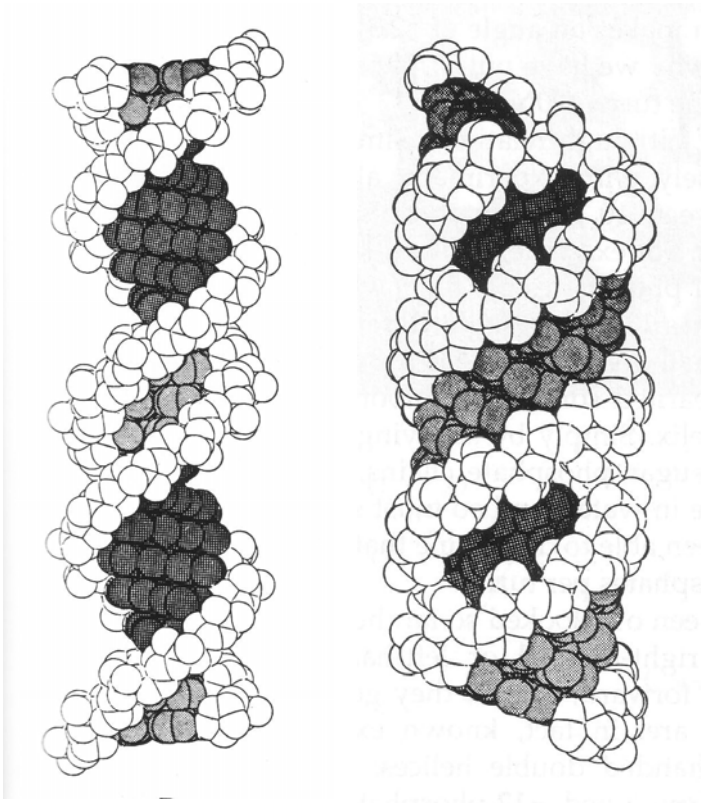

Figure 6. A form (left) and B Form (right) of DNA (Calladine and Drew 1997).

Environmental Stress

Vegetative cells are easily damaged by environmental stress when aerosolized, since they are used to moist environments and find the airborne environment extremely hostile and become subject to desiccation, nutrient starvation, and oxygen toxicity. The degree of desiccation required to put an organism under stress is far less than that required to cause serious changes to the cellular structure. The desiccation rate will

depend on the relative humidity and, depending on whether they are gram positive or negative, osmotic shock will have more or less of a lethal effect. As stated previously, it is generally accepted that gram positive bacteria survive longer in the aerosolized state since their peptidoglycan rich cell wall gives them resistance to desiccation. In addition to structural changes, damage due to dehydration can also manifest itself in terms of failure in various biochemical functions, such as protein synthesis, impaired active transport system and reduced oxygen consumption.

During the experiments, the microorganisms are initially suspended in water and are therefore in an environment at 100% relative humidity. After nebulization they are then subject to much lower relative humidities (around 50%) for a period of approximately 10 s before being exposed to the UV. Once again, the question is whether this is sufficient time for the microorganisms to experience desiccation/dehydration leading to environmental stress which in turn, will make them more prone to UV damage. Without more detailed information regarding cell physiology, it is not possible to determine if environmental stress could account for the reduction in UV inactivation at higher relative humidities.

The situation may be further complicated by the fact that the organisms may be under a certain amount of stress resulting from the nebulization process itself. Stone and Johnson (2002) found that the nebulization of *Bacillus* and *Pseudomonas* cause a decrease in the culturable concentrations of both species within the first five minutes of nebulization. They said that this was indicative of injury being caused to the organisms. It may be that the bacteria surviving the nebulization process, but under considerable stress from it are more prone to further stress as a result of the change in relative humidity. Together this may dramatically increase their susceptibility to damage through UV irradiation.

CONCLUSIONS

- The experiments have shown that increasing the relative humidity by approximately 20% leads to an observed decrease in the UV susceptibility of aerosolized gram negative microorganisms.
- The degree to which the UV susceptibility is affected by increased relative humidity varies from one species to another.
- Attenuation of the UV beam due to increased levels of water vapor in the air cannot account for the observed decrease in UV inactivation in this set of experiments.
- No increase in the aerodynamic diameter of the aerosols was observed, which would lead to the conclusion that increased water sorption by the microorganisms and the resultant protection this additional water layer may provide cannot adequately explain the reduced UV inactivation.
- Changes in DNA strand conformation has been suggested as a potential explanation for the variation in UV susceptibility. However, it is not clear whether, given the short length of time between nebulization and UV exposure, such changes will have taken place. Further studies will be required in order to determine if DNA changes can and are taking place in the exposure chamber.
- The most likely explanation for the variability in the UV susceptibility of the aerosolized microorganisms is that of environmental stress resulting from a combination of the nebulization process and desiccation/dehydration under lower relative humidity conditions. It has been stated that gram negatives are much more prone to desiccation/dehydration due to the structure of their cell walls, especially when aerosolized. Given that they are moving from 100% to 50% relative humidity and may already be in a stressed state, then they are likely to be suffering a degree of environmental stress at the lower relative humidities. If they are already stressed, they are then inherently weaker and will be more prone to UV damage at lower relative humidities.
- Since relative humidity has been shown to have an impact (in some cases a large impact) on the efficacy of UV system, this will have serious implications for the use of such systems in areas where the relative humidity is either naturally much higher or artificially controlled at higher levels. When considering the installation of UVGI systems or the preparation of guidelines for their installation, it will not be sufficient simply to include advice/suggestions on location and number of fittings. It will also be necessary to highlight the importance of looking at the whole environment into which they are being installed and looking at things such as potential climate control in order to enhance the efficacy of the UVGI system.

REFERENCES

Beggs, C.B. 2001. Ultraviolet sterilisation of air. Proceedings of the Conference of the International Society of the Built Environment, Dijon, 15 June 2001, International Society of the Built Environment, Switzerland.

Beggs, C.B. 2003. The Airborne Transmission of Infection in Hospital Buildings: Fact or Fiction. Indoor Built Environ. 12: 9-18.

Callandine, C.R. and Drew, H.R. 1997. Understanding DNA: The Molecule and how it Works, Academic, London.

Cox, C.S. 1987. The Aerobiological Pathways of Microorganisms, Wiley, UK.

Cox, C.S. and Wathes, C.M. 1995. Bioaerosols Handbook, Lewis, New York.

Dumyahn, T. and First, M. 1999. Characterization of Ultraviolet Upper room Air Disinfection Devices. Am. Indust. Hyg. Assoc. J. 60: 219-227.

Falk, M., Hartman, K.A. and Lord, R.C. 1963. Hydration of Deoxyribonucleic Acid III: A Spectroscopic study of the Effect of Hydration on the Structure of Deoxyribonucleic Acid. J. Am. Chem. Soc. 85: 391-394.

Gates, F.L. 1929. A Study of Bactericidal Action of Ultraviolet Light. I. The reaction of monochromatic radiations. J. Gen. Physiol. 13: 231-249.
Greene, V.W., Bond, R.G. and Michlaelsen, M.S. 1960. Air Handling Systems must be Planned to Reduce the Spread of Infection. Modern Hosp. August, 1960.
Greene, V.W., Vesley, D., Bond, R.G. and Michlaelsen, M.S. 1962a. Microbial Studies of Hospital Air I: Quantitative Studies. App. Microbiol. 10: 561-566.
Greene, V.W., Vesley, D., Bond, R.G. and Michlaelsen, M.S. 1962b. Microbial Studies of Hospital Air II: Quantitative Studies. Appl. Microbiol. 10: 567-571.
Kaiser, G., The Community College of Baltimore County, http://www.cat.cc.md.us.
Ko, G., First, M.W. and Burge, H.A. 2000. Influence of Relative Humidity on Particle Size and UV Sensitivity of *Serratia marsescens* and *Mycobacterium bovis* BCG Aerosols. Tubercle Lung Disease 80(4/5): 217-228.
Lin, C.Y. and Li, C.S. 2002. Control Effectiveness of Ultraviolet Germicidal Irradiation on Bioaerosols. Aerosol Sci. Technol. 36: 474-478.
Macher, J.M. 1989. Positive Hole Correction of Multiple Jet Impactors for Collecting Viable Organisms. Am. Indust. Hyg. Assoc. J. 50: 561-568.
Mertens, R.A.F. 1996. Methodologies and Results of National Surveillance. *Baillieres Clin.* Infect. Diseases 3: 159-178.
MicroBioNet. http://www.sciencenet.com.au
NAO 2000. The Management and Control of Hospital Acquired Infection in Acute NHS Trusts in England, National Audit Office Press Notice, ISBN: 0105566659
Peccia, J. and Hernandez, M. 2001. Photoreactivation in airborne *Mycobacterium parafortuitum*. Appl. Environ. Microbiol. 67(9): 4225-4232.
Peccia, J.L., Werth, H.M., Miller, S.M. and Hernandez, M.T. 2001. Effects of Relative Humidity on the Ultraviolet Induced Inactivation of Airborne Bacteria. Aerosol Sci. Technol. 35: 728-740.