

Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents

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Received 22 December 2010; Accepted 31 March 2011

Abstract

We review the principles of ultraviolet (UV) irradiation, the inactivation of infectious agents by UV, and current applications for the control of microorganisms. In particular, wavelengths between 200 and 280 nm (germicidal UV) affect the double-bond stability of adjacent carbon atoms in molecules including pyrimidines, purines and flavin. Thus, UV inactivation of microorganisms results from the formation of dimers in RNA (uracil and cytosine) and DNA (thymine and cytosine). The classic application of UV irradiation is the inactivation of microorganisms in biological safety cabinets. In the food-processing industry, germicidal UV irradiation has shown potential for the surface disinfection of fresh-cut fruit and vegetables. UV treatment of water (potable and wastewater) is increasingly common because the process is effective against a wide range of microorganisms, overdose is not possible, chemical residues or by-products are avoided, and water quality is unaffected. UV has been used to reduce the concentration of airborne microorganisms in limited studies, but the technology will require further development if it is to gain wider application. For bioaerosols, the primary technical challenge is delivery of sufficient UV irradiation to large volumes of air, but the absence of UV inactivation constants for airborne pathogens under a range of environmental conditions (temperature, relative humidity) further compounds the problem.

Keywords: Ultraviolet, UV, UV irradiation, UVC, germicidal UV, Microbial Inactivation, Inactivation

Introduction

The purpose of this review is to provide an overview of ultraviolet (UV) irradiation and its application for the control of microorganisms. The first work on the inactivation of microorganisms by UV light was published in 1892 (Ward, 1892), with the 'germicidal' effects of UV wavelengths reported in 1903 (Barnard and Morgan, 1903). In 1927, Rivers and Gates used UV light to inactivate virus in suspension and proved the efficacy of the method through subcutaneous inoculation of rabbits (Rivers and Gates, 1927). The use of UV light to inactivate microorganisms in the environment began when Wells and Wells (1938) described the use of UV to inactivate microorganisms in hospital operating rooms. Wheeler *et al.* (1945) used UV light to 'disinfect' Army and Navy

barracks for the control of airborne rubella virus and *Streptococcus pyogenes* and in 1947, the use of UV irradiation reportedly reduced the spread of airborne viral pathogens ('measles') in classrooms (Perkins *et al.*, 1947). In 1961, Riley demonstrated the efficacy of UV light for the control of airborne tuberculosis by showing that untreated ventilated air from wards housing infectious TB patients produced infection in guinea pigs whereas air irradiated with UV light did not (Riley, 1961). Thereafter, the research moved from qualitative to quantitative measures of the effect of UV light on microorganisms.

In one of the first papers to quantify the genetic damage induced by UV, Miller and Plagemann (1974) calculated that a dose of 7 mJ cm⁻² induced the formation of 1.7 uracil dimers per mengovirus plaque-forming unit and that dimer formation increased the production of empty viral capsids, altered protein structures and increased RNase activity. Four years later, in 1978, Sarasin and Hanawalt reported that a 10 mJ cm⁻² dose resulted in

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seven pyrimidine dimers per simian virus 40 (SV40) genome (Kowalski, 2009). With the exception of water treatment applications, relatively little research on the effect of UV light on microorganisms was published from the mid-1970s to 2000. However, concerns about bioterrorism, antibiotic-resistant bacteria and airborne spread of emerging and reemerging pathogens (e.g., pandemic influenza and the severe acute respiratory syndrome (SARS) corona virus) have stimulated renewed interest in the use of UV light as a microbial inactivant (Walker and Ko, 2007).

The UV spectrum

The electromagnetic spectrum includes energies possessing both electrical and magnetic properties. Classified according to wavelength and photonic interaction with matter (ionizing or non-ionizing), these wavelengths vary from very high vibrational energy (1×10^{-7} nm) to infinitely long wavelengths and encompass all known forms of energy, from high-energy gamma rays (3×10^{-3} nm), microwaves to lower-energy radio waves (3×10^{13} nm) (Van Heuvelen, 1982). UV wavelengths lie between the high-energy X-rays (≤ 100 nm) and the lower-energy visual spectrum (>400 nm). Wavelengths longer than 300 nm begin the visible region of the electromagnetic spectrum, followed by the infrared spectrum beginning at 700 nm, microwave frequencies (starting at 3×10^6 nm) and the radar/radio frequencies (3×10^8 nm and beyond). There is no clear delineation between X-rays and the UV spectrum; rather it is the nature of their interaction with matter that best defines the end of X-rays and the beginning of the UV spectrum. The energy-matter interactions of wavelengths less than 100 nm result in ionization (a change in atomic charge) of the exposed matter. As the wavelengths increase, the energy-matter interaction results in less ionization and more electron excitation (electrons jumping to higher-energy levels) as the energy is absorbed by molecules.

Although there are several classification schemes (Jagger, 1967), the UV spectrum may be most simply divided into four general classifications based on the wavelength's interaction with molecules: (1) 'vacuum ultraviolet' (VUV), (2) ultraviolet 'C' (UVC), (3) ultraviolet 'B' (UVB) and (4) ultraviolet 'A' (UVA). The VUV spectrum includes wavelengths <200 nm. The most energetic wavelengths within the UV spectrum, VUV readily interacts with oxygen atoms and their interaction with organic molecules is detrimental even at low doses; however, these wavelengths exist only in a vacuum due to the high energy. UVC encompasses wavelengths between 200 and 280 nm. This spectrum is also called the 'germicidal' spectrum because of its biocidal effects on bacteria (Jagger, 1967). UVB ranges from 280 to 315 nm and is the wavelength responsible for 'sun burning' the skin and the synthesis of Vitamin D (Goodsell, 2001). UVA

ranges from 315 to 400 nm and is the primary light produced by black light fixtures (Stowe, 2005). Both UVA and UVB are used in industry, e.g., to activate organic polymers used in laminates and to produce medical devices (Stowe, 2005). UVA and UVB, but not UVC, wavelengths, are long enough to pass through the earth's atmosphere to the earth's crust and can penetrate a short distance into the world's oceans (Jagger, 1967). The focus of this review is UVC and its effect on microorganisms. UVC is sometimes termed 'ultraviolet germicidal irradiation' (UVGI) to distinguish it from the non-germicidal wavelengths, UVA and UVB (Kowalski, 2009).

UVC is biologically important because unsaturated organic compounds, i.e., compounds that are not fully saturated with hydrogen atoms or are composed of conjugated bonds, efficiently absorb wavelengths between 200 and 280 nm. Conjugated bonds hold two electron pairs, with each electron in the pair possessing an independent and opposite spin of equal energy. When a photon of UV radiation energy strikes an electron, it is induced to rise to an excited (higher-energy) level. This disruption of stable electrons can travel the entire organic structure, raising a bonded electron out of a bonding pair, and resulting in an unstable conformation. In conjugated bond-containing structures, the entire structure acts as a chromophore, i.e., the π orbitals are shared throughout the ringed structure (Jagger, 1967). Because of this, the entire structure absorbs the UV energy (photon) and this extra energy is drained off into the weakest bond, thereby causing conformational changes to occur (Jagger, 1967).

Conjugated organic structures include nitrogen-containing ring structures such as pyridines, pyrimidines, flavins and the aromatic amino acids (Jagger, 1967). Because these structures act as chromophores, these wavelengths can also be used for nucleic acid analysis. This process is based on the difference in absorption between nucleic acids and proteins. Essentially, this methodology relies on that fact that peptide bonds exhibit double-bond characteristics, whereas aromatic amino acids absorb UV (Jagger, 1967).

Measuring UV radiation: radiometry and actinometry

Measurement is the heart of science and the basis on which effects are evaluated. There are two basic ways to measure the intensity and duration of UV radiation: radiometry and actinometry.

Radiometry measures irradiance, the UV energy striking a surface from all forward angles at a point in time expressed as energy (watts) per unit area (Bolton and Linden, 2003). A radiometer is a sensor with an electronic readout device that displays the sensor readings. Sensors are wavelength specific and have a cosine response that accounts for the incident angle of the light source as

it strikes the sensor's surface. (Note: to ensure accurate UV measurements, radiometers need to be calibrated annually.) Since UV sensors typically have a measurement area of 1 cm², the amount of UV energy arriving at the surface is commonly measured in watts per m² or mW per cm². In biological experiments, it is necessary to account for both the intensity of the UV light energy (irradiance) and the length of exposure (time). Therefore, radiometers are often equipped to measure cumulative exposure over time (mW per cm²).

Actinometry is a second method of measuring UV light energy. Actinometry is based on chemical systems that undergo light-induced reactions at specific wavelengths for which the quantum yield is accurately known (Kuhn *et al.*, 2004). The quantum yield is a measure of molecular UV-absorption efficiency of a chemical and is described as the ratio of the number of chemical changes per unit time to the number of photons absorbed per unit time (Kuhn *et al.*, 2004):

$$\Phi = Nc/Np, \quad (1)$$

where Φ is the quantum yield, Nc is the number of molecules chemically reacting and Np , is the number of photons absorbed.

For example, iodide/iodate solution is an actinometer commonly used in the UVC spectrum because it absorbs energy between 200 and 300 nm. The concentration of the product (triiodide) is directly proportional to the intensity, i.e., the number of photons absorbed, as measured on a spectrophotometer at 352 nm (Rahn *et al.*, 2005). This is not to be confused with the radiometric UV dose that is independent of photon absorbance.

There are important differences between the two methods and it should be recognized that each provides results based on different parameters. Actinometry is the preferred method of measuring UV exposure in the field of photochemistry and photochemists express UV exposure in units of quantum yield. Actinometry is neither convenient nor practical in photobiology and photobiologists measure UV using radiometry and express exposure in joules per cm². This difference in the expression of UV exposure units presents a fundamental problem in communicating results across disciplines.

UV radiation is also sometimes described in terms of fluence rate, the energy (mW) passing through a cross-sectional area (cm²), with UV dose defined as the fluence rate per unit time in seconds (s), i.e., mW per cm² (Bolton, 2000). A joule is expressed as energy×time, and therefore UV dose is expressed as joules per cm². Because radiometers are in common use and more readily implemented than chemical actinometry, the term 'ultraviolet dose' is sometimes used interchangeably with 'fluence rate', regardless of the object's ability to absorb UV radiation. Therefore, depending on the field or discipline, the terminology used may not strictly conform to the definitions of the International Union of Pure and Applied

Chemistry Working Party on Ultraviolet Disinfection (Bolton and Linden, 2003).

Considerations in experimental UV₂₅₄ photobiology

Photobiology describes the interaction between light and living matter. Bolton and Linden (2003) outlined the basic requirements in the design of bench scale UV inactivation apparatus for wastewater experimentation, but these standards also apply to other types of UV light experimentation involving inactivation of microorganisms. Specifically, bench-scale UV light inactivation experiments should be reproducible across laboratories and contain the basic elements of good experimental design. Results that provide a foundation for forward progress will also use accepted terminology to describe the experimental design and results. Thus, the terminology presented in this review is based on the recommendations of the International Union of Pure and Applied Chemistry Working Party on Ultraviolet Disinfection (Bolton and Linden, 2003).

The single most significant physical factor in UV₂₅₄ inactivation is lamp design and performance (VanOsdell and Foarde, 2002). In microbiology, the majority of work involving photobiology involves 'germicidal' UVC, with UV₂₅₄ being the wavelength considered to possess the strongest inactivating effect. Importantly, VanOsdell and Foarde (2002) noted that for a UV₂₅₄ system to efficiently inactivate pathogens, the bulb design (type of quartz, internal gas composition, operating temperature and ballast type) must be matched with the environmental conditions (temperature and relative humidity).

The first mercury vapor arc lamp was developed by Wheatstone in 1835 (cited by Kowalski, 2009). Other metallic gases (zinc, iron or xenon) can be energized into UV emission, but excited mercury gas is the most efficient UV emitter and is used extensively in UV₂₅₄ bulbs. Mercury gas UV emitters are produced as low-, medium- and high-pressure bulbs. Low- and medium-pressure mercury lamps consist of electrodes that produce electrons that collide with mercury atoms causing them to emit photons, predominately at 253.7 nm (Jagger, 1967). High-pressure mercury lamps, while similar in design, use electrodes capable of high voltage, thus allowing for an increase in the efficiency of emitted photons (Jagger, 1967).

Low-pressure bulbs (internal pressure of less than 1 bar) operate at a low surface temperature and emit monochromatic (UV₂₅₄) wavelengths (Fig. 1). Medium-pressure bulbs (internal pressure slightly higher than 1 bar) operate at higher surface temperature and emit polychromatic light (Fig. 1). To eliminate undesired UV wavelengths, the synthetic quartz containing the vaporized mercury atoms can be treated with wavelength-dependent UV-absorbing components that block specific

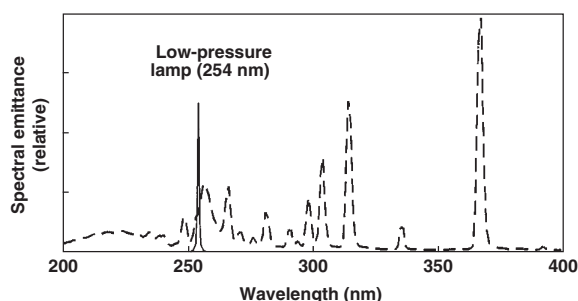


Fig. 1. Spectral output of low- and medium-pressure UV lamps. Low-pressure bulbs (solid line) are monochromatic (UV₂₅₄), whereas medium-pressure lamps (dashed line) are polychromatic (200–400 nm). Figure adapted from Bolton and Linden (2003). Reprinted by permission of the publisher.

wavelengths from exiting the bulb. Proper temperature control must be maintained when using medium-pressure vapor lamps because the lamps produce heat and the output (irradiance) of these lamps is temperature sensitive. As a result, these lamps require additional power to increase the internal temperature and pressure of the lamp. This increased temperature allows for an increased UV light spectrum, but until the bulbs reach operating temperature, their output fluctuates. For this reason, under experimental conditions, a shutter system is needed so that light-emitting bulbs reach peak performance prior to target exposure (Bolton and Linden, 2003).

Under experimental conditions, the structure of the UV-emitting apparatus must be designed to provide a spatially homogeneous field of irradiation. This can be accomplished through the use of a collimating tube (Bolton and Linden, 2003; Thurston-Enriquez *et al.*, 2003; Shen *et al.*, 2005). However, depending on the experimental design, a collimating tube may not be required. That is, in the absence of reflected UV energies and with irradiance from one plane only, the cosine sensors will account for any incident irradiation. Sensors for measuring UV and holding treatment samples should be secured to a thermally and physically stable exposure stage. For accurate measurements of UV exposure, sensors must be placed at the same distance from the energy source as the irradiated sample and absorbance of UV by the sample matrix must be taken into account. Bolton and Linden (2003) consider it necessary to stir the solution to ensure uniform UV dose, but small volumes or a matrix that does not readily absorb UV need not be stirred.

Lambert–Beer law

In brief, this law states that some of the UV₂₅₄ energy to which the target is exposed will be absorbed by the surrounding environment and this absorption must be taken into account. For microorganisms in suspension (liquid or aerosol), the average UV₂₅₄ intensity

may be calculated as follows (Thurston-Enriquez *et al.*, 2003):

$$I_{\text{average}} = I_0(1 - e^{-a_e L})/a_e L, \quad (2)$$

where I_{average} is the average UV₂₅₄ intensity (milliwatts per square centimeter), a_e is the absorbance of the virus suspension to the base e ,

I_0 is the UV₂₅₄ intensity after passing through solution and

L is the depth (cm) of the solution irradiated by the UV₂₅₄ energy.

For liquid media, the amount of UV energy absorbed by the solution can be determined by measuring the amount of UV light that passes through the matrix using a quartz cuvette equal to the depth of the sample (Lambert–Beer's law). Jagger (1967) provides information on the absorption coefficients for various solutions, but only empirical data will provide the researcher the information needed to determine if stirring is necessary.

Mechanism of inactivation by UV radiation

Inactivation of microorganisms by UV light is initiated at the quantum level. The quantum yield is the number of photons or the photon density, impacting a surface area. Each photon carries an amount of energy called a quantum (ϵ) determined from quantum mechanics (Jagger, 1967; Kowalski, 2009):

$$\epsilon = h(\nu), \quad (3)$$

where ϵ is the energy in one photon, h is the Planck's constant, 6.626×10^{-34} joules (J) and ν is the frequency in hertz (Hz).

Much of the quantal information was determined in the 1960s, when researchers on UV radiation focused on the mechanism of inactivation. During this time, researchers estimated the quantum yields required for dimerization of nucleic acids (Shore & Pardee 1956; Kleczkowski, 1963). Thus, Kowalski (2009) calculated that UV light at a wavelength of 253.7 nm has a frequency of 1.18×10^{15} Hz and 7.819×10^{-19} J of energy per photon. It follows that each joule contains 1.279×10^{18} photons and a UV light dose of 1 mJ cm^{-2} will produce 1.279×10^{15} photons cm^{-2} . Thus, a microorganism with a diameter of $0.1 \text{ }\mu\text{m}$, i.e., a cross-sectional area of $3.14 \times 10^{-14} \text{ m}^2$ ($3.14 \times 10^{-12} \text{ cm}^2$), will be subjected to the passage of about 401,000 photons per second (Kowalski, 2009).

UV energy at 254 nm readily affects the double-bond stability between adjacent carbons. There are two types of molecular bonds occurring in conjugated organic structures, the sigma (σ) orbitals and the Pi (π) orbitals. The higher-energy sigma orbitals (shorter wave function) are located closer to the nucleus of the two bonded atoms. The Pi orbitals are of lower energy and are non-localized

about the bonded pair. The lower-energy π orbitals are more stable and, therefore, have longer wave function (Kowalski, 2009). Conjugated ring structures, like the pyrimidines, purines and the aromatic amino acids have large, non-localized π orbitals (Smith and Hanawalt, 1969). When an incoming UV_{254} photon strikes a π orbital, the photon's energy is converted to vibrational energy (Kowalski, 2009). If this vibrational energy is sufficient, the π orbital is pushed into a transient unstable state that exists for a femtosecond (10^{-15} s). This unstable state must return to the ground state either by dissipation of the energy or through modification of the bond by rotation (Kowalski, 2009).

Unsaturated organic compounds are essential to cell reproduction and cell metabolism. Unsaturated organic compounds vulnerable to UV_{254} inactivation include pyrimidines, purines and flavin. Pyrimidines provide the basic structure for nucleobases uracil (a component of tRNA), thymine (a component of DNA and tRNA) and cytosine (a component of DNA and RNA). Purines provide the basic structure for nucleobases adenine and guanine (in DNA and RNA) and the aromatic amino acids, phenylalanine and tyrosine. Flavin is an unsaturated organic compound found in flavin adenine dinucleotide (FAD), a molecule that is necessary for metabolic redox reactions. FAD is an important hydrogen acceptor in the cell and ATP is generated as a result of the oxidation of FADH.

Nucleic acids are composed of bases, sugars and phosphates. Photons affect DNA and RNA by inducing molecular transformation, i.e., photoproducts, of the genetic material. The sugars and phosphate groups do not absorb wavelengths above 210 nm, but conjugated bases have peak absorption of UV light energy at 260 nm, with pyrimidines being 10 times more sensitive to UV_{254} than purines (Jagger, 1967). It follows that uracil and cytosine in RNA and thymine and cytosine in DNA are the targets of UV_{254} inactivation. Whether the target is RNA or DNA, the mechanism of UV_{254} inactivation is hydration of the base or base dimerization (Jagger, 1967). There are six possible photoproducts induced by UV light: (1) thymine–thymine dimer, (2) cytosine–cytosine dimer, (3) cytosine–thymine dimer, (4) uracil–uracil dimer, (5) uracil–thymine dimer and (6) uracil–cytosine dimer (Kowalski, 2009). The photoproducts requiring the least energy are the thymine–complex dimer and the uracil–complex dimer (Jagger, 1967; Kowalski, 2009). Cytosine hydrate, another UV_{254} -induced photoproduct, occurs in RNA and single-stranded DNA (Smith and Hanawalt, 1969). This structure requires more energy, but is formed when UV_{254} irradiation of cytosine yields 6-hydroxy-5,6-dihydrocytosine (O'Donnell *et al.*, 1994). In DNA, the thymine dimer is the photoproduct with the highest quantum yield (Kowalski, 2009). These dimers occur when the hydrogen bonds linking the thymine bases are lost and the respective 5 and 6 carbon atoms are cross-linked.

The biological effects of UV_{254} exposure are reversible. In bacterial cells, dimer formation is reversible via absorption of wavelengths between 300 and 500 nm (photo reactivation) or by photolyase enzymes that split the dimers. DNA viruses utilize host cellular polymerase enzymes to excise dimers and replace the damaged DNA (Kowalski, 2009). Generally, viruses do not produce their own photolyases. One exception is the fowlpox virus, the only virus known to code for its own photolyase enzyme production (Srinivasan *et al.*, 2001).

The UV inactivation of microorganisms can be achieved with either monochromatic or polychromatic emitters. Monochromatic lamps producing primarily UV_{254} are routinely used to inactivate microorganisms. Compared to monochromatic lamps, polychromatic lamps may possess greater efficiency (Linden *et al.*, 2007). For example, Eischeid *et al.* (2009) reported low-pressure monochromatic UV lamp doses of 30, 50 and 80 $mJ\ cm^{-2}$ resulted in (2 log), (3 log) and (4 log) reduction of adenovirus type 2 virus, respectively. In contrast, doses of 10 and 25 $mJ\ cm^{-2}$ from medium-pressure polychromatic UV lamps resulted in (2.5 log) and (4.5 log) adenovirus reductions, respectively. Presumably these differences in inactivation reflect the fact that monochromatic UV wavelengths only caused genetic damage, whereas polychromatic wavelengths also affect aromatic proteins. That is, the structure and function of microbial proteins depend on their primary, secondary and tertiary structures, which reflect their constituent amino acids. It is estimated that one in 10 amino acids is susceptible to photochemical processes and, hypothetically, the photochemical alternation of any of these amino acids could affect protein structure and function (Jagger, 1967). Therefore, the efficiency of UV light inactivation could be increased through the use of medium- or high-pressure polychromatic bulbs, but this gain is generally offset by the additional expense of operating this equipment.

Principles of inactivation by UV radiation

The inactivation kinetics of UV radiation can be described as a first-order chemical reaction. That is, the amount of reagent (UV irradiance) will equal the amount of product (modified conjugated bonds) in a given time period. The Stark–Einstein law states that if a photon is absorbed, then only one photon should be required for the formation of one photoproduct. This law is the foundation of the 'one-hit' (first-order) kinetics that have historically been used to describe UV_{254} inactivation (Hiatt, 1964; Qualls and Johnson, 1983; Thurston-Enriquez *et al.*, 2003; Kowalski, 2009).

Grotthus–Draper law

The Grotthus–Draper law states that photons must be absorbed for a photochemical reaction to occur

(Kowalski, 2009). Following this line of thought, Bolton and Linden (2003) suggest that the term 'ultraviolet dose' should be used to describe the total energy absorbed by the target. Problematically, energy striking an object is not necessarily absorbed by the object (Jagger, 1967) and absorbed photons may not produce a photochemical reaction (Kowalski, 2009). Currently, the only method to measure the absorptive efficiency of a conjugated organic molecule is actinometry.

Bunsen–Roscoe reciprocity law

The Bunsen–Roscoe reciprocity law states that microbial inactivation is dependent on dose and dose is the product of UV intensity expressed in mW per cm² and exposure time expressed in seconds (Riley and Kaufman, 1972).

$$D = I \times T, \quad (4)$$

where D is the UV dose, I is the irradiance (intensity) in mW per cm² and T is the exposure time in seconds.

The Bunsen–Roscoe law is important and relevant to microbial inactivation because it shows that, although the UV irradiance drops as the target moves further away from the source (except in a vacuum), the desired UV dose can be achieved by increasing the exposure time. This law is fundamental because it allows for the comparison of results from experiments using different UV equipment types, wattages and conditions, when the exposure time is known.

Chick's law

Chick's law states that as disinfectant contact time (t) increases, the ratio of viable microorganisms (N_t) to the total (N_0) microbes at time zero decreases. Chick's law was originally used to describe the relationship between chlorine and the inactivation of microorganisms in wastewater (Rubin and Elmaraghy, 1977). Figure 2 illustrates first-order inactivation kinetics where absorbance of one photon results in inactivation.

Since the 1950s, inactivation constants (k) derived using Chick's law have been used to measure the sensitivity of microorganisms to UV inactivation, with larger inactivation constant values (larger slope) indicative of greater susceptibility to UV inactivation. The following equation is a modification of the formula used by Tseng and Li (2005) to solve for the inactivation constant (k):

$$k = [\log_{10}(N_t / N_0)] / \text{Dose}, \quad (5)$$

where k is the inactivation constant, N_0 is the quantity of microbes at time zero, N_t is the quantity of microbes at UV₂₅₄ exposure time ' t ' and Dose is the ultraviolet light dose.

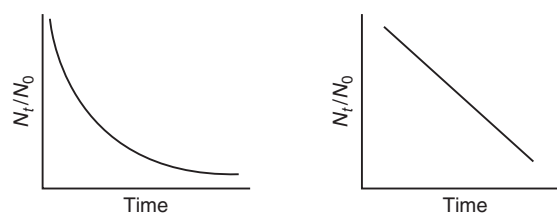


Fig. 2. Effect of disinfectant contact time on the proportion of remaining viable microorganisms (Chick's law). Non-transformed data are shown on the left; log₁₀-transformed data on the right.

One-stage versus two-stage inactivation

Hiatt (1964) published the first work detailing the kinetics of microbial activation and noted that the 'first-order' inactivation model, i.e., the single-hit hypothesis proposed by the Stark–Einstein law and described by a single inactivation constant (k), was accurate only if the exposed viral population was homogenous and inactivation did not require cumulative damage. Hiatt (1964) also described the classical inactivation curve dynamics of 'shouldering' and 'tailing'. 'Shouldering' refers to an increase of UV₂₅₄ dose with no corresponding increase in microbial inactivation, whereas 'tailing' is a decrease in UV₂₅₄ dose with no corresponding decrease in microbial inactivation (Hiatt, 1964). In contrast to the one-stage inactivation model, the two-stage model proposes that exposure of microbial populations to an inactivating agent may reveal two subpopulations: one subpopulation (f) more susceptible to inactivation and a second subpopulation ($1-f$) more resistant (Hiatt, 1964; Cox, 1976; Kowalski *et al.*, 2002).

$$\log_{10} N_t = \log_{10} N_0 + \log_{10} [(1-f) \cdot 10^{-k_1 \cdot \text{Dose}_t} + f \cdot 10^{-k_2 \cdot \text{Dose}_t}], \quad (6)$$

where N_t is the quantity of virus in the test sample after treatment with Dose _{t} , N_0 is the quantity of virus in the unexposed control sample, f is the resistant fraction of the total initial virus population with inactivation rate K_2 , $(1-f)$ is the susceptible virus population fraction with inactivation rate K_1 , k_1 is the inactivation rate of the inactivation curve for the 'fast decay population', k_2 is the inactivation rate of the inactivation curve for the 'resistant population' and Dose _{t} is the UV₂₅₄ intensity × time.

In practice, the two-stage inactivation analysis should be utilized when the statistical analysis indicates that the data are better described by two-stage versus one-stage inactivation kinetics.

Applications of UV light to the inactivation of microorganisms

Laboratory use

The classic application of UV is the inactivation of microorganisms in biological safety cabinets. In many

laboratories, turning on the UV lamp after using the cabinet is standard operating procedure. The current version of the National Science Foundation (NSF) International Standard 49 (Section 5.25.2) does not mandate the use of UV light in biosafety cabinets (Meechan and Wilson, 2006). Further, the Centers for Disease Control and Prevention states that the use of UV light in biosafety cabinets is neither recommended nor necessary (CDC, 2009). UV inactivation of microorganisms on surfaces is ancillary to standard chemical disinfection and should not be relied upon as the sole method of disinfection.

Food processing

In the food-processing industry, germicidal UV light has shown potential for the surface disinfection of fresh-cut fruit and vegetables. In studies on carrots (Mercier *et al.*, 1993), grapes (Nigro *et al.*, 1998), sweet potatoes (Stevens *et al.*, 1999) and spinach leaves (Artés-Hernández *et al.*, 2009), treatment with UVC was shown to reduce product deterioration and prolong storage life. Alone or in combination with ozone, UV light-reduced microorganisms in water used to wash fresh-cut onion, escarole, carrot and spinach (Selma *et al.*, 2008). UV light may provide a viable alternative to chemical sanitizers, e.g., titanium dioxide (TiO₂) or chlorine. Currently, there is interest in developing non-thermal methods for the sterilization of juices, an objective in which UVC may play a role (Guerrero-Beltrán and Barbosa-Cánovas, 2005).

Water treatment

The application of UV light to food preservation is a relatively recent development, but the use of UV light for the treatment of water has an extensive history: the first system for UV treatment of potable water went into operation in Marseilles, France in 1910 (cited by Kowalski, 2009). UV treatment of water was not widely implemented at the beginning of the twentieth century for a variety of reasons, including high operating costs, issues with equipment reliability and maintenance, and the availability of cost-effective, chemical water treatment systems (Wolfe, 1990). UV light is regarded as broadly effective against all human pathogens (bacterial, viral and protozoal) transmitted in water (Hijnen *et al.*, 2006) and guidelines for the treatment of waste water and potable water with UV light have been established (Environmental Protection Agency, 2003). UV treatment of water (potable and wastewater) is increasingly common because the technology is readily available, the process is effective against a wide range of microorganisms, over-dose is not possible, chemical residues or by-products are

avoided and water quality is unaffected (Wolfe, 1990; Hijnen *et al.*, 2006).

Bioaerosols

A wide variety of fungal, bacterial and viral pathogens may be transmitted by airborne droplets or droplet nuclei (Tang *et al.*, 2006; Blachere *et al.*, 2007). Airborne pathogens of humans include major emergent and re-emergent agents, e.g., *Mycobacterium tuberculosis*, influenza viruses, SARS corona virus, *Aspergillus* spp., *Legionella* spp. (Douwes *et al.*, 2003; Wong and Yuen, 2006; Escombe *et al.*, 2007). Likewise, some of the most economically significant pathogens of animals are transmitted via aerosols, e.g., foot and mouth disease and porcine reproductive and respiratory syndrome virus (Alexanderson *et al.* 2002; Hermann *et al.*, 2009). Regardless of the actual level of risk bioaerosols present to the public, events of recent history have raised society's awareness and concern. In the market place, UV emitters designed for residential application, e.g., portable units or those for ductwork insertion, and commercial application, e.g., upper room air, ceiling mount or cooling-coil disinfection are available, despite the fact that data on the efficacy of these systems are sparse (Environmental Protection Agency 2006a, b, c, d).

UV light has successfully reduced the concentration of airborne microorganisms in targeted applications. Berg *et al.* (1991) found that UV irradiation of air in operating rooms during surgery significantly reduced the number of viable airborne bacteria collected at the edge of the surgical site. Likewise, installation of UV light in air-handling units and ventilation systems reduced the concentration of airborne bacteria and fungi in indoor air (Menzies *et al.*, 1999; Levetin *et al.*, 2001; Menetrez *et al.*, 2010).

Moving beyond narrowly focused applications, the use of UV light for the routine inactivation of airborne microorganisms faces severe technical challenges. In the first place, the inactivation kinetics of most airborne pathogens is not known for the range of environmental conditions (temperature and relative humidity) under which such a system would need to function. This is a significant deficit because environmental conditions are known to affect UV light, e.g., as relative humidity increases, UV light becomes less efficient (Ko *et al.*, 2000; Peccia *et al.*, 2001; VanOsdell and Foarde, 2002; Lai *et al.*, 2004; Tseng and Li, 2005; Walker and Ko, 2007). Thus, acquiring baseline exposure doses to target UV exposure levels is the first priority. Beyond this, delivery of the inactivating dose uniformly and consistently to large volumes of air is a significant challenge given the current state of the technology. At present, UV inactivation of bioaerosols can only be considered one part of an overall biocontainment plan,

rather than a stand-alone solution (Memarzadeh *et al.*, 2010).

Conclusions

The use of UV light for the inactivation of microorganisms is appealing because it is a familiar, commercially available technology that does not involve the use of chemicals. Some applications are well developed, e.g., water treatment. Others show future promise, such as applications in food processing. One highly desirable application, the routine use of UV for the inactivation of microorganisms in aerosols, will require extensive development and ultimately may only function effectively in tandem with other technologies, e.g., with photocatalysis, where UV energies are used to induce the formation of metallic oxides and superoxides, rather than serving as the primary source of inactivation or with filtration where the UV energy is provided post-filtration.

References

- Alexanderson S, Brotherhood I and Donaldson AI (2002). Natural aerosol transmission of foot-and-mouth disease virus to pigs: minimal infectious dose for strain O1 Luasanne. *Epidemiology and Infection* **128**: 301–312.
- Artés-Hernández F, Escalona VH, Robles PA, Martínez-Hernández GB and Artés F (2009). Effect of UV-C radiations on quality of minimally processed spinach leaves. *Journal of the Science of Food and Agriculture* **89**: 414–421.
- Barnard J and Morgan H (1903). The physical factors in phototherapy. *British Medical Journal* **2**: 1269–1271.
- Berg M, Bergman BR and Hoborn J (1991). Ultraviolet radiation compared to an ultra-clean air enclosure. *Journal of Bone and Joint Surgery (British)* **73**: 811–815.
- Blachere FM, Lindsley WG, Slaven JE, Green BJ, Anderson SE, Chen BT and Beezhold DH (2007). Bioaerosol sampling for the detection of aerosolized influenza virus. *Influenza and Other Respiratory Viruses* **1**: 113–120.
- Bolton JR (2000). Calculation of ultraviolet fluence rate distributions in an annular reactor: significance of refraction and reflection. *Water Research* **34**: 3315–3324.
- Bolton JR and Linden KG (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering* **129**: 209–213.
- Centers for Disease Control and Prevention, (CDC) (2009). Appendix A – Primary containment for biohazards: selection, installation and use of biological safety cabinets. In: Chosewood L Casey and Deborah E (eds) *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. Wilson: HHS Publication No. (CDC), pp. 21–112.
- Cox CS (1976). Inactivation kinetics of some microorganisms subjected to a variety of stresses. *Applied and Environmental Microbiology* **31**: 836–846.
- Douwes J, Thorne P, Pearce N and Heederik D (2003). Bioaerosol health effects and exposure assessment: progress and prospects. *Annals of Occupational Hygiene* **47**: 187–200.
- Eischeid AC, Meyer JN and Linden KG (2009). UV disinfection of adenoviruses: molecular indications of DNA damage efficiency. *Applied and Environmental Microbiology* **75**: 23–28.
- Environmental Protection Agency (2003). *Ultraviolet Disinfection Guidance Manual*. EPA 815-D-03-007, June 2003. Washington, DC: EPA.
- Environmental Protection Agency (2006a). Biological inactivation efficiency by HVAC in-duct ultraviolet light systems. Novatron, Inc. BioProtector BP114i. September 2006. EPA 600/R-06/084.
- Environmental Protection Agency (2006b). Biological inactivation efficiency by HVAC in-duct ultraviolet light systems. American Ultraviolet Corporation ACP-24/HO-4. May 2006. EPA 600/R-06/054.
- Environmental Protection Agency (2006c). Biological inactivation efficiency by HVAC in-duct ultraviolet light systems. Lumalier. June 2006. EPA 600/R-06/055.
- Environmental Protection Agency (2006d). Biological inactivation efficiency by HVAC in-duct ultraviolet light systems. UltraViolet Devices, Inc. Altru-V V-Flex. May 2006. EPA 600/R-06/049.
- Escombe AR, Oeser CC, Gilman RH, Navincopa M, Ticona E, Pan W, Martínez C, Chacaltana J, Rodríguez R, Moore DA, Friedland JS and Evans CA (2007). Natural ventilation for the prevention of airborne contagion. *PLoS Medicine* **4**: e68.
- Goodsell DS (2001). The molecular perspective: ultraviolet light and pyrimidine dimers. *The Oncologist* **6**: 298–299.
- Guerrero-Beltrán JA and Barbosa-Cánovas BV (2005). Reduction of *Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. *Journal of Food Processing and Engineering* **28**: 437–452.
- Hermann JR, Muñoz-Zanzi CA and Zimmerman JJ (2009). A method to provide improved dose-response estimates for airborne pathogens in animals: an example using porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology* **133**: 297–302.
- Hiatt CW (1964). Kinetics of the inactivation of viruses. *Bacteriological Reviews* **28**: 150–163.
- Hijnen WAM, Beerendonk EF and Medema GJ (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research* **40**: 3–22.
- Jagger J (1967). *Introduction to Research in Ultraviolet Photo-biology*. Englewood Cliffs, NJ: Prentice-Hall, Inc.
- Kleczkowski A (1963). The inactivation of ribonucleic acid from tobacco mosaic virus by ultraviolet radiation at different wavelengths. *Photochemistry and Photobiology* **2**: 497–503.
- Ko G, First MW and Burge HA (2000). Influence of relative humidity on particle size and UV sensitivity of *Serratia marcescens* and *Mycobacterium bovis* BCG aerosols. *Tubercle and Lung Disease* **80**: 217–228.
- Kowalski W (2009). *Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection*. New York: Springer.
- Kowalski WJ, Bahnfleth WP and Carey DD (2002). Engineering control of airborne disease transmission in animal laboratories. *Contemporary Topics in Laboratory Animal Science* **41**: 9–17.
- Kuhn HJ, Braslavsky SE and Schmidt R (2004). Chemical actinometry. *Pure and Applied Chemistry* **76**: 2105–2146.
- Lai KM, Burge HA and First MW (2004). Size and UV germicidal irradiation susceptibility of *Serratia marcescens* when aerosolized from different suspending media. *Applied and Environmental Microbiology* **70**: 2021–2027.
- Levetin E, Shaughnessy R, Rogers CA and Scheir R (2001). Effectiveness of germicidal UV radiation for reducing fungal contamination within air-handling units. *Applied and Environmental Microbiology* **67**: 3712–3715.

- Linden KG, Thurston J, Schaefer R and Malley JP (2007). Enhanced UV inactivation of adenoviruses under polychromatic UV lamps. *Applied and Environmental Microbiology* **73**: 7571–7574.
- Meehan PJ and Wilson C (2006). Use of ultraviolet lights in biological safety cabinets: a contrarian view. *Applied Biosafety* **11**: 222–227.
- Memarzadeh F, Olmsted RN and Bartley JM (2010). Applications of ultraviolet germicidal irradiation disinfection in health care facilities: effective adjunct, but not stand-alone technology. *American Journal of Infection Control* **38**: 13–24.
- Menetrez MY, Foarde KK, Dean TR and Betancourt DA (2010). The effectiveness of UV irradiation on vegetative bacteria and fungi surface contamination. *Chemical Engineering Journal* **157**: 443–450.
- Menzies D, Pasztor J, Rand T and Bourbeau J (1999). Germicidal ultraviolet irradiation in air conditioning systems: effect on office worker health and wellbeing – a pilot study. *Occupational and Environmental Medicine* **56**: 397–402.
- Mercier J, Arul J, Ponnampalamm R and Boulet M (1993). Induction of 6-methoxymellein and resistance to storage pathogens in carrot slices by UV-C. *Journal of Phytopathology* **137**: 44–54.
- Miller RL and Plegemann PGW (1974). Effect of ultraviolet light on mengovirus: formation of uracil dimers, instability and degradation of capsid and covalent linkage of protein to viral RNA. *Journal of Virology* **13**: 729–739.
- Nigro F, Ippolito A and Lima G (1998). Use of UV-C light to reduce Botrytis storage rot of table grapes. *Postharvest Biology and Technology* **13**: 171–181.
- O'Donnell R, Boorstein ERJ, Cunningham RP and Teebor GW (1994). Effect of pH and temperature on the stability of UV-induced repairable pyrimidine hydrates in DNA. *Biochemistry* **33**: 9875–9880.
- Peccia J, Werth HM, Miller S and Hernandez M (2001). Effect of relative humidity on the ultraviolet-induced inactivation of airborne bacteria. *Aerosol Science and Technology* **35**: 728–740.
- Perkins JE, Bahlke AM and Silverman HF (1947). Effect of ultraviolet irradiation of classroom on the spread of measles in large rural central schools. *American Journal of Public Health* **37**: 529–537.
- Qualls RG and Johnson JD (1983). Bioassay and dose measurement in UV disinfection. *Applied and Environmental Microbiology* **45**: 872–877.
- Rahn RO, Bolton R and Stefan MI (2005). The iodide/iodate actinometer in UV disinfection: determination of the fluence rate distribution in UV reactors. *Photochemistry and Photobiology* **82**: 611–615.
- Riley RL (1961). Airborne pulmonary tuberculosis. *Bacteriological Reviews* **25**: 243–248.
- Riley RL and Kaufman JE (1972). Effect of relative humidity on the inactivation of *Serratia marcescens* by ultraviolet radiation. *Applied Microbiology* **23**: 1113–1120.
- Rivers T and Gates F (1927). Ultra-violet light and vaccine virus. II. The effect of monochromatic ultra-violet light upon vaccine virus. *Journal of Experimental Medicine* **47**: 45–49.
- Rubin A and Elmaraghy G (1977). Studies on the toxicity of ammonia, nitrate and their mixture to guppy fry. *Water Research* **11**: 927–935.
- Sarasin AR and Hanawalt PC (1978). Carcinogens enhance survival of UV-irradiated simian virus 40 in treated monkey kidney cells: induction of a recovery pathway? *Proceedings of the National Academy of Sciences of the United States of America* **75**: 346–350.
- Selma MV, Allende A, López-Gálvez F, Conesa MA and Gil MI (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology* **25**: 809–814.
- Shen C, Fang S, Bergstrom D and Blatchley E (2005). (E)-5-[2-(Methoxycarbonyl)ethenyl]-cytidine as a chemical actinometer for germicidal UV radiation. *Environmental Science and Technology* **39**: 3826–3832.
- Shore VG and Pardee A (1956). Energy transfer in conjugated proteins and nucleic acids. *Archives of Biochemistry and Biophysics* **62**: 355–368.
- Smith D and Hanawalt P (1969). Repair replications of DNA in ultraviolet irradiated *Mycoplasma laidlawii*. *Journal of Molecular Biology* **46**: 57–72.
- Srinivasan V, Schnitzlein W and Tripathy D (2001). Fowlpox virus encodes a novel DNA repair enzyme, CPD-Photolyase, that restores infectivity of UV light-damaged virus. *Journal of Virology* **75**: 1681–1688.
- Stevens C, Khan VA, Lu JY, Wilson CL, Chalutz E, Droby S, Kabwe MK, Haung Z, Adeyeye O, Pusey LP and Tang AYA (1999). Induced resistance of sweet potato to Fusarium root rot by UV-C hormesis. *Crop Protection* **18**: 463–470.
- Stowe R (2005). Radiometric methods for ultraviolet-process design and process monitoring-advanced radiometry. *Radtech Report* **4**: 12–20.
- Tang J, Eames I, Chan P and Ridgway G (2006). Factors involved in the aerosol transmission of infection and control of ventilation in healthcare premises. *Journal of Hospital Infection* **64**: 100–114.
- Thurston-Enriquez J, Haas C, Jacangelo J, Riley K and Gerba C (2003). Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied and Environmental Microbiology* **69**: 577–582.
- Tseng C and Li C (2005). Inactivation of virus-containing aerosols by ultraviolet irradiation. *Aerosol Science and Technology* **39**: 1136–1142.
- Van Heuvelen A (1982). *Physics: A General Introduction*. Boston, MA: Little, Brown & Co.
- VanOsdell D and Foarde K (2002). Defining the effectiveness of UV lamps in circulating air ductwork. ARTI 21-CR/610-40030-01.
- Walker C and Ko G (2007). Effect of ultraviolet germicidal irradiation on viral aerosols. *Environmental Science and Technology* **41**: 5460–5465.
- Ward M (1892). Experiments of the action of light on *Bacillus anthracis*. *Proceedings of the Royal Society of London* **52**: 393–403.
- Wells W and Wells M (1938). Measurement of sanitary ventilation. *American Journal of Public Health* **28**: 343–350.
- Wheeler SM, Ingraham HS, Hollaneder A, Lill ND, Gershon-Cohen Jr J and Brown EW (1945). Ultraviolet light control of airborne infections in a naval training center. *American Journal of Medicine* **35**: 457–468.
- Wolfe R (1990). Ultraviolet disinfection of potable water: current technology and research needs. *Environmental Science and Technology* **24**: 768–773.
- Wong S and Yuen K (2006). Avian influenza infections in humans. *Chest* **129**: 156–168.