Sunlight-mediated inactivation of health-relevant microorganisms in water: a review of mechanisms and modeling approaches


Health-relevant microorganisms present in natural surface waters and engineered treatment systems that are exposed to sunlight can be inactivated by a complex set of interacting mechanisms. The net impact of sunlight depends on the solar spectral irradiance, the susceptibility of the specific microorganism to each mechanism, and the water quality; inactivation rates can vary by orders of magnitude depending on the organism and environmental conditions. Natural organic matter (NOM) has a large influence, as it can attenuate radiation and thus decrease inactivation by endogenous mechanisms. Simultaneously NOM sensitizes the formation of reactive intermediates that can damage microorganisms via exogenous mechanisms. To accurately predict inactivation and design engineered systems that enhance solar inactivation, it is necessary to model these processes, although some details are not yet sufficiently well understood. In this critical review, we summarize the photo-physics, -chemistry, and -biology that underpin sunlight-mediated inactivation, as well as the targets of damage and cellular responses to sunlight exposure. Viruses that are not susceptible to exogenous inactivation are only inactivated if UVB wavelengths (280–320 nm) are present, such as in very clear, open waters or in containers that are transparent to UVB. Bacteria are susceptible to slightly longer wavelengths. Some viruses and bacteria (especially Gram-positive) are susceptible to exogenous inactivation, which can be initiated by visible as well as UV wavelengths. We review approaches to model sunlight-mediated inactivation and illustrate how the environmental conditions can dramatically shift the inactivation rate of organisms. The implications of this mechanistic understanding of solar inactivation are discussed for a range of applications, including recreational water quality, natural treatment systems, solar disinfection of drinking water (SODIS), and enhanced inactivation via the use of sensitizers and photocatalysts. Finally, priorities for future research are identified that will further our understanding of the key role that sunlight disinfection plays in natural systems and the potential to enhance this process in engineered systems.

Environmental significance
The manuscript provides a comprehensive synthesis of the current understanding of the mechanisms by which sunlight causes damage to microorganisms, ultimately leading to inactivation. This topic is important for understanding the fate and transport of microbiological contaminants in all sunlit surface waters, including fresh and marine ecosystems, as well as engineered treatment systems.
1. Introduction

Sunlight has long been recognized as a disinfectant. The sunlight-mediated inactivation of microorganisms is relevant in many types of applications and to many aquatic environments. In both fresh and marine surface waters, sunlight-mediated damage influences microbial ecology, with implications for microbial food webs and microbially mediated biogeochemical processes. It also strongly influences the persistence of human pathogens and indicator organisms in contaminated waters (e.g., sunlight is a major determinant of swimming beach water quality). Sunlight is the key factor contributing to inactivation of indicator organisms and pathogens in engineered natural systems like wastewater treatment ponds (WTP) and open-water wetlands for treatment of wastewater and stormwater.

Solar disinfection of drinking water (SODIS) is promoted around the world as a low-cost method for household water treatment. The goal of this paper is to review the tremendous progress that has been made in the last several decades in understanding the mechanisms by which sunlight damages health-relevant microorganisms in water. Based on this understanding, we present a mechanistic approach for modeling inactivation, discuss the implications of sunlight-mediated inactivation for common applications in the field of water quality, and identify knowledge gaps and research priorities. The review focuses on mechanisms that occur in both viruses and bacteria, including indicator organisms and human pathogens, because sunlight inactivation is most relevant and best understood for these two classes of microorganisms. Short sections review sunlight inactivation of protozoan cysts and antibiotic resistance genes.

2. Conceptual model of sunlight inactivation

Sunlight-mediated inactivation is a type of photoinactivation, a term that also includes disinfection by artificial radiation sources whose spectral irradiance typically differs appreciably from that of sunlight. Although the emphasis of this review is natural sunlight, the discussion of mechanisms is also relevant to artificial radiation sources. Indeed, much of the information on solar inactivation comes from experiments with artificial sources. A conceptual model of photoinactivation mechanisms of viruses and bacteria is shown in Fig. 1. This conceptual model provides a framework for discussing the underlying principles and mechanisms in more detail in subsequent sections. Direct photoinactivation occurs when a chromophore endogenous to the microorganism (e.g., nucleic acids, proteins, or other macromolecules that occur in microorganisms) absorbs a photon resulting in changes to the chemical structure of the chromophore. Indirect photoinactivation occurs when an endogenous (a constituent of the microorganism) or exogenous (not a constituent of the microorganism) chromophore absorbs a photon and sensitizes the production of photo-produced reactive intermediates (PPRI) that, in turn, damage virus or cell components. Chromophores that produce PPRI are called sensitzers. As indicated in Fig. 1, viruses are primarily damaged through endogenous direct and exogenous indirect mechanisms, whereas all three mechanisms may contribute to bacterial inactivation. Although the three mechanisms are described separately, they likely occur simultaneously and interact, especially in bacteria. For example, direct damage to a bacterial enzyme, such as catalase, could exacerbate indirect inactivation by causing higher levels of photo-chemically produced hydrogen peroxide to persist within a bacterial cell.

3. Solar irradiance and water optics

Different regions of the solar spectrum contribute to the three main mechanisms of damage, as shown in Fig. 1. Endogenous direct damage is primarily initiated by photons in the UVB range (280–320 nm) whereas endogenous indirect damage can involve photons in the UVB and UVA (320–400 nm) ranges. Photons in the UVB, UVA, and visible (400–700 nm) light regions can contribute to exogenous damage. The main reason for this dependence on wavelength is that different chromophores are involved, with different absorption spectra and quantum yields, as reviewed in Section 4. An implication of this dependence on wavelength is that different chromophores may contribute to exogenous damage. The main reason for this dependence on wavelength is that different chromophores are involved, with different absorption spectra and quantum yields, as reviewed in Section 4. An implication of this dependence on wavelength is that different chromophores may contribute to exogenous damage. The main reason for this dependence on wavelength is that different chromophores are involved, with different absorption spectra and quantum yields, as reviewed in Section 4. An implication of this dependence on wavelength is that different chromophores may contribute to exogenous damage. The main reason for this dependence on wavelength is that different chromophores are involved, with different absorption spectra and quantum yields, as reviewed in Section 4. An implication of this dependence on wavelength is that different chromophores may contribute to exogenous damage.

In Fig. 2, we provide examples of spectral irradiance of sunlight for different zenith angles, total atmospheric ozone concentrations, and for an overcast sky. The spectral quality of solar irradiance is fairly consistent throughout the visible range despite major changes in the magnitude of solar irradiance. UVB wavelengths, however, are preferentially absorbed by atmospheric ozone. Differential absorption of the sunlight spectrum is exacerbated when the sun is lower in the sky due to the longer path through the atmosphere (i.e., larger air mass). For example, while UVA and visible light vary seasonally in irradiance by about a factor of two between summer and winter at mid-latitudes, UVB varies by a factor of four (Table 1). A similar effect occurs over the course of a day. During the equinox, at mid-latitudes, the UVA and visible light intensities reach 50% of their maximum value about four hours before solar noon, while UVB reaches the 50% mark almost a full hour later (the UVB “sunrise” and “sunset” lag and precede visible sunrise and sunset). Due to these large differences in irradiance, we can expect the sunlight-mediated inactivation rate to vary by several orders of magnitude as a function of location, season, time of day, and weather conditions.

As solar radiation penetrates waters it undergoes further spectral shifts due to wavelength-dependent irradiance attenuation (spectral filtering) by water; as a result, the water quality and water depth also exert significant influence over the sunlight-mediated inactivation rates. The transmission of irradiance over a depth interval in the water column can be described as.
where \( E_d(z, \lambda) \) is the downwelling planar (spectral) irradiance (Watts m\(^{-2}\) nm\(^{-1}\)) at depth \( z \), \( E_d(0, \lambda) \) is just below the air–water interface, \( K_d(\lambda) \) is the diffuse downwelling attenuation coefficient, which varies with wavelength (m\(^{-1}\)), and \( z \) is depth (m) in the water column. Although \( K_d \) can be calculated based on first principles, it is typically treated as an empirical parameter that is measured for a particular water. \( K_d \) accounts for a range of factors that affect attenuation after reflection and refraction at the water–air interface, including absorption (by water, dissolved constituents, and particles), and scattering (primarily by particles) (see Kirk (2011)\(^{10} \) for more detail).

\[ E_d(z, \lambda) = E_d(0, \lambda)e^{-K_d(z)z} \]  

![Fig. 1](image) Conceptual model of sunlight inactivation mechanisms in viruses and bacteria. For direct mechanisms, the photon is absorbed by a chromophore at the site of damage (orange star). For indirect mechanisms, the photon is absorbed by a sensitizer (Sens), and damage (orange star) occurs at a different site. Green shapes represent proteins. PPRI = photo-produced reactive intermediates.

![Fig. 2](image) Spectral irradiance of sunlight under different conditions for (a) UV-visible range (300–700 nm) and (b) UV range (300–400 nm) shown using log scale. Sunlight spectra were generated with the RADTRANX\(^{\dagger} \) routine in Hydrolight5 (\(^{19} \)) for varying ozone concentration (300 ppb is approx. average in the stratosphere), solar altitudes (zenith angle), and overcast versus clear sun. The solar simulator spectrum is for a 1000 W Oriel simulator with airmass and atmospheric attenuation filters, measured using a Stellarnet spectroradiometer, as reported in Silverman and Nelson (2016).

### Table 1

<table>
<thead>
<tr>
<th>Radiation type</th>
<th>Wavelength range (nm)</th>
<th>Dec 21</th>
<th>Jun 21</th>
<th>( E_{summer}/E_{winter} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB</td>
<td>280–320</td>
<td>2.0</td>
<td>8.4</td>
<td>4.2</td>
</tr>
<tr>
<td>UVA</td>
<td>320–400</td>
<td>76.2</td>
<td>174</td>
<td>2.3</td>
</tr>
<tr>
<td>Visible</td>
<td>400–700</td>
<td>1010</td>
<td>2050</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\( a \) SMARTS was used for generating these values (reference atmosphere), because it can account for wavelengths down to 280 nm. A limitation of SMARTS is that it does not account for the impact of cloud cover. Ozone concentration = 300 Dobson units.

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1. Hydrolight5 was used for generating this figure, because the RADTRANX routine can account for overcast skies; however, a limitation of this model is that the lowest wavelength is 300 nm.
In very clear natural waters, the absorption spectrum (and attenuation spectrum) is dominated by water, such that spectral irradiance tends to be concentrated in the blue-visible window near the attenuation minimum (Fig. 3), giving such waters their blue color. However, most other constituents of natural waters result in preferential attenuation of shorter wavelengths. The main dissolved substance in natural waters that attenuates radiation is natural organic matter (NOM), specifically the colored dissolved organic matter (CDOM), whose absorbance increases exponentially with declining wavelength, resulting in yellow or orange (visible) light penetrating most strongly. Thus, UV wavelengths are strongly attenuated by CDOM in most natural waters. For example, irradiance attenuation in the solar UV is nearly 1000-fold higher in water from the humic-stained Lake Hochstetter, New Zealand, than in the very clearest natural waters (Fig. 3); this effect has been observed in many natural waters. Consequently, enhancements in CDOM concentrations caused by runoff tend to reduce inactivation by UVB whereas droughts that reduce runoff result in deeper UVB penetration that enhances inactivation.

Suspended particles exacerbate this bias against shorter wavelengths in colored waters, since short wavelengths are more efficiently scattered by particles, which increases their average pathlength and therefore their absorption. For example, suspended sediments in turbid waters such as the Mississippi River can dominate attenuation of solar UV radiation. In eutrophic waters, with high phytoplankton concentrations, irradiance attenuation has appreciable spectral structure owing to light absorption by chlorophyll-a (with two absorption peaks at about 440 nm and 676 nm) and accessory photosynthetic pigments, resulting in green (visible) light penetrating deepest. Solar UV is also strongly attenuated in these eutrophic waters. The spectral irradiance attenuation in a high rate algal pond (common wastewater treatment technology; Fig. 3) has broadly similar spectral shape to eutrophic waters generally, and with attenuation 3000-fold greater than in pure water in the solar UVB range.

Due to the greater attenuation of UVB, the relative importance of UVA and visible light compared to UVB increases with depth. A key implication for sunlight inactivation is that exogenous processes become relatively more important with increasing light attenuation (or depth in the water column). Because microorganisms have differing susceptibility to endogenous and exogenous mechanisms, this spectral filtering can lead to large shifts in the relative photoactivation rates between organisms with water depth (see Section 8).

4. Photochemistry and photobiology fundamentals

4.1. Chromophores and sensitizers

The first step in photoactivation is absorption of a photon by a chromophore (Fig. 1), but the chromophores involved in endogenous and exogenous processes are markedly different. In viruses, chromophores involved in the endogenous direct and indirect inactivation are limited to amino acids (tryptophan, tyrosine, cysteine disulfide) and nucleic acid bases that primarily absorb light in the UVB range. In bacteria, chromophores also include coenzymes, vitamins and metalloproteins (see Table 2); therefore the range of light absorption is wider and encompasses the UVB, UVA, and visible light ranges (Table 2). While there is documented evidence that some chromophores undergo direct damage (e.g., nucleotide bases) and others act as sensitizers (e.g., riboflavin), it is likely that most chromophores experience both direct damage and initiate sensitized reactions (i.e., most chromophores are also sensitizers).

Exogenous sensitizers are derived from the environment, with organic matter being the most important class. CDOM absorbs light over the UVB, UVA and visible range, though the absorption decreases exponentially with increasing wavelength (Table 2). This exponential decrease in absorption can be characterized as

\[ a_{CDOM,l} = a_{CDOM,0} e^{-S(l - l_0)} \]  

where \( a_0 \) is the Naperian absorption coefficient at wavelength \( l_0 \) and \( S \) (nm\(^{-1}\)) is the empirical spectral slope coefficient of the log (natural) absorbance curve; a reference wavelength (indicated by subscript 0) of 300 nm is typically used. The photoactivity of CDOM varies as a function of its chemical composition, which in turn depends on its origin and subsequent transformation by biological and chemical processes. Wastewater-derived CDOM exhibits higher production rates of PPRI compared to autochthonous CDOM; however, because it is also a more efficient quencher of PPRI, the higher production rates do not necessarily result in greater PPRI concentrations in wastewater-impacted waters. In addition to CDOM, nitrate and nitrite, as well as metal complexes can sensitize the formation of PPRI's in sunlit waters.
4.2. Photochemical reactions of chromophores (direct and indirect)

A chromophore (CHROM) that absorbs a photon is promoted to an excited singlet state (1CHROM*; Fig. 4). 1CHROM* are short-lived (nanosecond lifetimes) and generally return to their ground states, emitting heat or light (fluorescence), although some undergo intersystem crossing (ISC) to longer-lived (microsecond lifetimes) excited triplet states (3CHROM*). 3CHROM* or 3CHROM* may directly undergo photochemical transformation, resulting in endogenous direct inactivation. The best-studied chemical structures in biomolecules that promote direct photoreactions are adjacent pyrimidine nucleobases (C, T or U), which can dimerize upon irradiation; pyrimidine hydrates can also be formed. Double-stranded nucleic acids are generally less photoreactive than single-stranded nucleic acids. In RNA, uracil dimer reactions have lower quantum yields than the corresponding thymine dimer reactions in DNA. The extent of nucleic acid photoprocesses form to a greater extent in RNA than DNA due to the low quantum yields of the thymidine hydrate reactions compared to uracil hydrate reactions. The extent of nucleic acid photoprocesses is dependent on solution pH and ionic strength. Nucleic acids sequence and structure also have significant impacts on base photoreactivity. Although most research on the direct photolysis of nucleic acids have focused on UVC wavelengths, the pyrimidine products can also form by UVA and UVB.

Besides direct photoreactions, 3CHROM* can furthermore promote reactions of biomolecules through sensitized processes, resulting in endogenous or exogenous indirect inactivation. Sensitized photooxidations include 3CHROM* acting directly as an oxidant, or acting as a sensitizer and promoting the formation of PPRI, such as reactive oxygen species (ROS). ROS can further be categorized as: (1) singlet oxygen (1O2) formed by energy transfer to dissolved oxygen, (2) superoxide (O2•−) and hydrogen peroxide (H2O2) formed by electron and proton transfer to dissolved oxygen, and (3) hydroxyl radical (•OH) formed by processes involving 3CHROM*, but also including other processes, such as the photolysis of nitrate or nitrite and Fenton reactions involving dissolved iron and H2O2. In some waters, other intermediates such as carbonate radical (CO3•−)43 or reactive halogen species (RHS; X•, X2•−)44 might contribute to photoreactions; see Section 4.5.
The concentrations of individual PPRI can vary by orders of magnitude, depending on the water composition.\textsuperscript{42,47} Typical concentration ranges of some exogenous PPRI in sunlit surface waters are $10^{-17}$ to $10^{-15}$ M for hydroxyl radical, $10^{-14}$ to $10^{-12}$ M for singlet oxygen and carbonate radical, and $10^{-10}$ to $10^{-10}$ M for superoxide.\textsuperscript{46}

Most PPRI selectively react with electron-rich sites on biomolecules. In nucleic acids, PPRI most readily oxidize guanine (G), producing 7,8-dihydro-8-oxoguanine (8-oxo-G) and other products.\textsuperscript{47} In proteins, PPRI mostly target the electron-rich amino acid side chains of tryptophan, tyrosine, histidine, methionine, cysteine, and cystine.\textsuperscript{48–51} Hydroxyl radical, which is a highly reactive and nonselective oxidant, can, in principle react with all of the amino acid side chains and backbones. Nevertheless, ‘OH has been found to preferentially oxidize the so-called RKPT amino acids (arginine (R), lysine (K), proline (P), and threonine (T)), leading to formation of carbonyl-containing derivatives.\textsuperscript{52} In addition, ‘OH hydroxylates aromatic amino acids.\textsuperscript{56}

While the potential photochemical reactions of PPRI with individual biomolecules are fairly well understood, the reactions occurring with whole bacterial cells or virus particles are less well-understood, and may differ substantially. Due to the organisms’ higher order structure, additional damage may occur (\textit{e.g.}, via radical chain reactions to adjacent molecules), or damage may be mitigated or attenuated (\textit{e.g.}, due to poor accessibility of PPRI to reactive sites, or quenching of PPRI). Furthermore, modification of a site within an organism does not necessarily result in inactivation, due to repair mechanisms and to the high redundancy of protein and membrane components. Thus, the relevant sites of photochemical damage are difficult to predict based on the known photochemistry of free biomolecules alone. In Sections 5, 6, and 7, we review what is known about types of damage and causes of inactivation in microorganisms, as well as other pathways to damage in bacteria involving oxidative stress and internal Fenton chemistry.

### 4.3. Action spectra for endogenous inactivation

In the UVC range (100–280 nm), beyond the range of the solar spectrum at the Earth’s surface, action spectra (relative inactivation as a function of wavelength) for viruses and bacteria closely match the absorption spectra of nucleic acids (maxima around 260 nm), indicating that direct damage to nucleic acids is the primary mechanism of damage.\textsuperscript{54} As summarized in recent reviews, the wavelengths present in sunlight incident on the Earth’s surface (>280 nm) cause less inactivation of viruses and bacteria with increasing wavelength.\textsuperscript{54,55} In bacteria irradiated under aerobic conditions, the action spectrum deviates strongly from the absorption spectra of endogenous chromophores, due to the complex pathways involved in indirect endogenous damage.\textsuperscript{54} Thus, empirical relationships are needed to describe the wavelength-dependence of endogenous inactivation. Two main approaches have been used to develop quantitative relationships – either exposing microorganisms to narrow bands of radiation, or broadband exposure (polychromatic) modified with cutoff filters.\textsuperscript{7} Cullen proposed that only the former be called “action spectra” and that the latter be called “biological weighting functions”.\textsuperscript{28} Recent research on sunlight inactivation has not adhered to this distinction, but it is important to note that the former approach does not capture interactions between different wavelengths, nor photorepair, and these phenomena are believed to be particularly important for bacteria exposed to sunlight.\textsuperscript{57} A further disadvantage of using narrow bands is that to generate inactivation data in a reasonable timeframe, the irradiances are often much higher than in natural sunlight. An outstanding challenge with developing action spectra is the choice of a functional form (\textit{e.g.}, algebraic function or look-up table);\textsuperscript{57} to date, there is no consensus for the most useful functional form for waterborne indicator organisms and pathogens.\textsuperscript{58} Action spectra are discussed further in the mechanism and modeling sections.

### 4.4. Interaction of exogenous sensitizers with microorganisms

For exogenous sensitizers, the properties of the sensitizer itself, in particular its ability to associate with the organism, can affect the efficiency of exogenous inactivation. Natural organic matter exists as a mixture with components that are supramolecular, colloidal and particulate,\textsuperscript{59} and these assemblies may sorb to viruses and bacteria. Viruses and bacteria with sorbed DOM may experience enhanced photoreactivity, because they are bound to the sources of the PPRI. For example, singlet oxygen’s short lifetime (3.6 μs (ref. 60)) means that it can only take part in reactions within a small sphere of diffusion from where it was generated. This phenomenon is illustrated in Fig. 5, which shows that the probability of an encounter with photochemically produced $^{1}$O$_2$ decreases by 50% if the sensitizer is separated by a distance of 75 nm from the virus, compared to if the sensitizer is sorbed to the virus, due to quenching of $^3$O$_2$ as it diffuses away from the sensitizer.\textsuperscript{62} Higher rates of inactivation

![Fig. 5](image-url) Probability of a virus having an encounter with $^{1}$O$_2$ produced by sunlight irradiation of a sensitizer as a function of distance between the virus and sensitizer (based on values reported in Latch and McNeill 2006).
due to sorbed organic matter have been demonstrated for some viruses\textsuperscript{65,66} and \textit{Ent. faecalis}.\textsuperscript{84}

Relative to photoinactivation, there has been more work on the effects of sensitizer association on the photodegradation of small molecules. For example, studies have shown that the interaction with DOM enhanced the photodegradation of mirex,\textsuperscript{65,66} organic probe compounds of singlet oxygen,\textsuperscript{61,67} histidine,\textsuperscript{44} and also mercury(0).\textsuperscript{89} Compared to free molecules, it has proven difficult to experimentally demonstrate enhanced photoreactions for organic matter-bound microorganisms. Nevertheless, it is clear that viruses and bacteria will associate with organic matter-rich (micro)phases\textsuperscript{62-70} and the likelihood of enhanced exogenous photoinactivation in such cases deserves further study.

Association between organic matter and viruses is governed by interactions with the outer surface of the protein capsid, and is influenced by electrostatic, steric, and hydrophobic interactions, and cation bridging between carboxylate groups.\textsuperscript{71} Preferential adsorption of the hydrophobic, higher molecular weight fractions of an aquatic fulvic acid to \textit{Bacillus subtilis} was reported.\textsuperscript{79} The interaction of microorganisms with organic matter can be enhanced by ionic strength and divergent cations (see Sections 5 and 6). However, our current understanding is inadequate to predict the association between organic matter and microorganisms in real water matrices, and the subsequent influence on photoinactivation.

4.5. Photoinactivation in seawater

A number of studies have shown that photoinactivation occurs more quickly in marine versus fresh waters for both bacteria and viruses.\textsuperscript{72-79} To date, these studies have primarily been observational and a complete mechanistic understanding for the salinity and other water quality effects is lacking. Salinity can potentially influence both endogenous and exogenous mechanisms. In isolated DNA, ionic strength enhanced the quantum yield of pyrimidine dimer formation due to the impact ionic strength has on nucleic acid configuration;\textsuperscript{72} this effect could potentially be relevant for non-enveloped viruses, but has not been studied directly. In Gram-negative bacteria, enhanced inactivation in seawater was attributed to greater loss of internal cell integrity when cytoplasmic membranes were damaged by sunlight (through either endogenous or exogenous mechanisms).\textsuperscript{79,80}

With respect to exogenous mechanisms, the higher ionic strength in seawater may enhance organism–sensitizer interactions.\textsuperscript{72,81} In addition, high ionic strength can influence the concentration and relative distribution of PPRI by a variety of mechanisms. First, ionic strength has been shown to decrease the loss rate of excited triplet state chromophores (\textsuperscript{1}CHROM*) \emph{via} electron transfer interactions with solution constituents, including other DOM moieties.\textsuperscript{82} However, ionic strength did not affect \textsuperscript{1}CHROM* formation rates or loss rates by energy transfer to other solution components. The net result was a near doubling in the steady-state (ss) \textsuperscript{1}CHROM* concentration, \textsuperscript{1}CHROM*\textsubscript{ss}. \textsuperscript{1}CHROM* is the precursor for \textsuperscript{1}O\textsubscript{2} formation, and some studies report higher \textsuperscript{1}O\textsubscript{2}\textsubscript{ss} in seawater compared to freshwater.\textsuperscript{83} Overall, the skewing of \textsuperscript{1}CHROM* away from electron transfer processes may be expected to impact indirect exogenous inactivation processes. Furthermore, halides, particularly Br\textsuperscript{–}, are the predominant \textsuperscript{1}O\textsubscript{2} scavengers in seawater,\textsuperscript{40,84} leading to decreased \textsuperscript{1}O\textsubscript{2}\textsubscript{ss} in halide-rich waters. Halide scavenging of \textsuperscript{1}O\textsubscript{2} or halide oxidation by excited state ketones\textsuperscript{85} forms halogen radicals of the form X* or X\textsuperscript{–}.\textsuperscript{42,86} Modeling indicates that halogen radicals promote the formation of carbonate radical, and that concentrations of halogen and carbonate radicals exceed that of \textsuperscript{1}O\textsubscript{2} by several orders of magnitude in sunlit seawater.\textsuperscript{87,88} The conversion of \textsuperscript{1}O\textsubscript{2} to these more selective radical oxidants is anticipated to focus the oxidizing power of the system on electron-rich functional group targets.\textsuperscript{42,87,89}

5. Virus mechanisms

Sunlight disinfection mechanisms for viruses were first investigated by Davies-Colley \textit{et al.}\textsuperscript{90} DNA F+ coliphages were only susceptible to endogenous direct inactivation, whereas RNA F+ coliphages could also undergo exogenous indirect inactivation. Since this first study, sunlight disinfection rates have been assessed for various other human and bacterial viruses, and more insight has been gained into their susceptibility to the different inactivation mechanisms. The main findings are reviewed in this section. To assist with our discussion of mechanisms, the potential stages of the virus life cycle that could be disrupted due to endogenous or exogenous damage to viral nucleic acids or capsids are illustrated in Fig. 6. Many knowledge gaps remain in terms of identifying specific sites of damage and which life cycle stages are impacted.

5.1. Endogenous mechanisms

Under full-spectrum sunlight, all viruses investigated to date have been found to undergo endogenous inactivation.\textsuperscript{91-95} Among the viruses studied, human adenovirus (HAdV) and MS2 appear to be the most resistant whereas poliovirus and somatic phages are particularly sensitive.\textsuperscript{91,92,94} Even for the relatively resistant viruses, however, sunlight inactivation \textit{via} endogenous mechanisms was found to be the main inactivation process in clear natural waters.\textsuperscript{93}

It is likely that endogenous inactivation of viruses mainly occurs by the direct mechanism, though indirect processes have been documented. One example of endogenous indirect inactivation (photosensitization) was identified in MS2 illuminated with (UVC at 254 nm UV\textsubscript{254}), resulting in an RNA-sensitized cleavage of the capsid protein backbone.\textsuperscript{97} However, this mechanism was found to be of minor importance compared to overall inactivation.\textsuperscript{98} The negligible contribution of endogenous indirect inactivation can be explained by the simple structure of many viruses, which consist of a genome surrounded by a protein capsid, and lack intrinsic biochemistry. As a result of this simple structure, viruses contain few internal sensitizers that absorb light in the solar wavelength range; consequently, endogenous indirect inactivation is typically not an efficient inactivation mechanism, and occurs at a much
slower rate than endogenous direct inactivation (e.g., Love et al.93). However, experimentally, direct and indirect endogenous mechanisms are difficult to separate, and it is often more appropriate to group them together under the category “endogenous inactivation”.

Only one study to date has investigated the impact of endogenous sunlight damage on the virus life cycle. Sunlight was found to inhibit viral RNA synthesis (Fig. 6a(iii)) of rotavirus, which could explain about half of the inactivation that was observed; the remaining inactivation was attributed to post-translational steps. As discussed in Section 4, nucleic acid and protein monomers are susceptible to direct endogenous reactions. It is therefore likely that these reactions play a role in virus inactivation. At this time, no studies have identified the specific sites of virus genome and protein damage that are targeted in endogenous sunlight reactions. Qiao and Wigginton monitored the reactions in viral RNA oligomers exposed to simulated sunlight, but detected no decay with either mass spectrometry or RT-qPCR after 5100 J m⁻² UVB. Nonetheless, insight into the expected molecular-level modifications induced by sunlight can be obtained from laboratory studies using UVC radiation.

RNA coliphage inactivation studies using a low-pressure UVC lamp (emitting at 254 nm) showed that different regions in the RNA genome exhibited varied susceptibility to UVC irradiation,106,101 and that each genome lesion caused inactivation. Earlier work on ssRNA Tobacco Mosaic Virus found that under some conditions, not all RNA lesions caused inactivation. A complicating factor in studying viral genome reactivity is that commonly employed methods do not detect potentially important reactions in the nucleic acids. For example, reverse transcriptase-based methods (like RT-qPCR) do not detect the same UV-induced RNA reactions as mass spectrometry methods.11 In addition, UVC-induced protein damage, specifically cysteine oxidation followed by backbone cleavage, was reported for selected phages,97,100,102 and was associated with the coliphage’s inability to inject its genome into the host cell (Fig. 6b(ii)). Compared to low-pressure UV, the broad spectrum radiation of medium-pressure UV lamps (emitting down to 200 nm) led to a more significant contribution of protein damage in human adenovirus.103 It is therefore reasonable to expect that endogenous inactivation induced by sunlight causes damage to both genomes and protein capsids. The ability of some viruses, notably adenovirus104 and several bacteriophages,105 to hijack their host cells machinery and repair DNA damaged by UV254 has been reported. Similarly, repair of sunlight-induced damage has been reported.106

As might be expected based on viral chromophores, the action spectra of sunlight inactivation closely follow the absorption spectra of the nucleic acids and proteins, as shown by Lytle and Sagripanti, who developed a composite action spectrum for viruses by compiling inactivation data for both RNA and DNA viruses at different wavelengths in the solar spectrum.107 Although considerable work has been conducted to develop action spectra for several viruses in the UVC/low UVB range (e.g., 107–110) the only publications to date for the sunlight spectrum are for MS2 and PRD1.11
5.2. Exogenous mechanisms

In waters containing external sensitizers at concentrations that can occur in natural waters, inactivation rates by full spectrum sunlight of HAdV, human rotavirus, PRD1, and MS2 were faster than endogenous inactivation rates (after correcting for light attenuation), demonstrating that these viruses are susceptible to exogenous indirect inactivation. As discussed previously, exogenous inactivation of MS2 was greater with increasing association of the virus with the sensitizers. In contrast, inactivation of polio virus, porcine rotavirus as well as other F+ DNA coliphages did not increase markedly in the presence of exogenous sensitizers and at environmentally relevant temperatures, indicating that the rate of any exogenous inactivation is too low to detect in the presence of endogenous inactivation. Results for phiX174 have ranged from a small to a significant contribution from exogenous sensitizers.

Several studies have investigated sunlight-mediated inactivation in the absence of UVB, to study the contribution of different PPRI to exogenous inactivation without the confounding effects of endogenous inactivation (Table 2; light absorption by endogenous chromophores in viruses is limited to the UVB region). Results indicate that O2 is an important contributor to overall indirect inactivation of MS2, phiX174 and human adenovirus in natural waters. In contrast, O2 produced by NOM was not important for the inactivation of porcine rotavirus. Several other PPRI can inactivate viruses, including hydroxyl radicals, triplet state organic matter, and carbonate radicals. Although each of these species can inactivate viruses in isolation, their relative importance also depends on solution characteristics and the contribution of endogenous inactivation. In particular the concentration of NOM, which both produces and quenches reactive species and attenuates light, can be expected to play an important role, as explored in Section 8.

Damage induced by PPRI has been most thoroughly investigated for O2. Exposure to O2 inhibited MS2 genome replication and reduced the virus’s ability to bind to its E. coli host. The binding inhibition was due to chemical modifications in the virus assembly protein (Fig. 6b(i)). Specifically, damage to MS2 capsids as a result of O2 included oxidation of protein side chains, in particular of solvent-exposed methionine residues. RNA oligomers are reactive with O2, with purine bases being more reactive than pyrimidine bases; however the detected modifications in RNA oligomers have yet to be linked to inactivation of intact viruses. Protein damage (crosslinking) was also reported upon exposure to O2 produced by functionalized fullerenes. For adenovirus, both genome damage and significant protein damage by O2 was detected. Protein damage likely led to a loss in binding ability or a disruption of early infection processes within the host cell. Damage induced by environmentally relevant PPRI besides O2 have not been adequately examined.

5.3. Virus characteristics governing susceptibility to sunlight inactivation

If the factors that govern virus susceptibility to sunlight are understood, it may be possible to predict inactivation for viruses that are difficult to culture (and for which it is therefore difficult to quantify inactivation rates). For endogenous inactivation, some efforts have been made to establish generally applicable concepts of virus susceptibility. For example, Lytle and Sagripanti showed that endogenous inactivation by radiation in the UVC/B range depends on the size and type of the viral genome; when normalized by genome size, the inactivation of viruses of the same family, and to a lesser extent of the same genome type, can be estimated reasonably well. They generally found that double-stranded (ds) DNA viruses were the most resistant to UVC light, followed by dsRNA viruses, single-stranded (ss) RNA viruses, and finally ssDNA viruses; these findings are consistent with previous work by Rauth. The difference in the susceptibility of ds and ssDNA viruses was attributed to two main factors: the redundancy of the genetic information encoded in dsDNA, and their ability to undergo repair in the host cell. For viruses that are readily inactivated by sunlight, the relationship between genome length and inactivation is not linear. For example, the endogenous inactivation rate constant of polio virus was five times greater than that of MS2, whereas the length of the genome of polio is about twice that of MS2. Similarly, Reovirus was found to be more sensitive to UVC than expected based on its genome size. Two explanations were offered for the observed discrepancies: first, virus morphology affects genome packaging and may thereby influence its susceptibility to radiation damage; and second, the presence of light-sensitive proteins likely contributes to inactivation.

The characteristics that render a virus susceptible to exogenous indirect inactivation have proven difficult to assess. Generally, it appears that exogenous inactivation is only relevant for viruses that are relatively resistant to endogenous inactivation, and in waters that produce appreciable concentrations of PPRI. For viruses that are readily inactivated by endogenous inactivation, the exogenous contribution to inactivation may be too small to detect. In addition to the susceptibility of the protein capsid and genome to damage by PPRI, the association between viruses and sensitizers is expected to play a role, as discussed in Section 4.4 and Fig. 5. Recent advances have been made in understanding the interactions between viruses and DOM that could be insightful for explaining differential responses of viruses to sensitizers.

Few studies to date have attempted to pinpoint the virus characteristics that render a virus susceptible to inactivation by PPRI. The presence of oxidizable protein side chains has been suggested as a cause for a virus’ susceptibility to transient species. However, the presence of such side chains is not sufficient to explain inactivation: first, not all side chains are accessible to transient species, and second, protein oxidation is not always causal to inactivation. In fact, for MS2 as well
as for HAdV, the major contribution to inactivation by singlet oxygen was found to arise from genome damage rather than protein oxidation. Within a closely related group of F+ RNA coliphages, strong similarities in \(^3\)O\(_2\)-mediated inactivation kinetics were observed, and small differences could be explained based on the length and composition of the genome, in particular the number of the most easily oxidizable nucleobase guanine.\(^{106}\) Similar to endogenous inactivation, exogenous indirect inactivation may thus be governed by the genome composition, length and type. To further test this hypothesis, however, information is needed for a broad variety of viruses to assess the effects of PPRI on viral genome and proteins, and to determine the effect of this damage on virus infectivity.

6. Bacterial mechanisms

The ability of sunlight to inactivate bacteria has been recognized for a long time. Compared to viruses, bacterial cells are vastly more complex, with more potential targets of photochemical damage and molecules that can serve as sensitizers. Furthermore, bacteria have an adaptive regulatory response to sunlight, which induces several stress responses that help to protect against or repair damage.\(^{1,13,123,124}\) The general picture that emerges is that at some point during sunlight exposure, the protective and repair strategies become overwhelmed, leading to irreversible cell death (inactivation); for bacteria derived from batch cultures, these cellular processes are manifested as a lag phase that often precedes measurable inactivation. Thus, it is difficult to characterize the mechanisms that definitively lead to inactivation in bacteria, as many types of stress and damage may occur simultaneously, and it is challenging to identify which particular mechanism or combination of mechanisms leads to irreversible damage. Furthermore, the dominant mechanisms may be different for different environmental conditions, depending on changes in the solar spectrum, depth in the water column, and the water quality (type and concentration of sensitizers, oxygen, pH, salinity). The following sections summarize what is known about mechanisms, without attempting to rate their importance.

6.1. Endogenous mechanisms

Sunlight is known to cause direct damage to bacterial DNA, via similar mechanisms reviewed above for dsDNA viruses, resulting in various photoproducts including dimers and single-strand breaks.\(^{52}\) Studies of the wavelength effects on bacterial inactivation provided early evidence for the importance of the indirect endogenous mechanism in bacteria; inactivation of \(E. coli\) K12 by UV wavelengths up to 313 nm was independent of the oxygen concentration in solution (interpreted as direct endogenous inactivation), whereas inactivation above 313 nm was strongly dependent on oxygen (interpreted as indirect endogenous inactivation).\(^{123}\) Inactivation of \(Ent. faecalis\) and \(Staphylococcus aureus\) by natural sunlight was also shown to be much faster under oxic conditions than anoxic conditions.\(^{126,127}\) Most mechanistic studies have been carried out only under aerobic conditions, under which it is difficult to separate direct and indirect endogenous mechanisms. For this reason, and because the mechanisms clearly interact, they are discussed together.

To provide a framework for understanding many of the ways through which sunlight can cause cellular damage, we first review oxidative stress, summarizing from several recent reviews.\(^{128,129}\) Oxidative stress may affect any bacterial cell in an aerobic environment, and does not require exposure to radiation; these cells must constantly manage oxidative stress to survive. Dark oxidative processes are summarized in the left side of Fig. 7; we will return to a discussion of sunlight-mediated processes (right side of Fig. 7) in several paragraphs. The specific processes illustrated in Fig. 7 are referenced in the text with the corresponding number or letter. (1) Cytoplasmic \(O_2^{•−}\) is produced when dissolved oxygen oxidizes reduced enzyme moieties and electron shuttles, such as in flavoenzymes and quinones, either in the electron transport chain or the cytosol. \(H_2O_2\) is produced by a second electron transfer at the redox site of the enzyme, or by spontaneous or enzymatic dismutation in the cytoplasm. Neither \(H_2O_2\) nor \(O_2^{•−}\) is directly reactive with most organic biomolecules, including nucleotides, amino acids, and unsaturated lipids (unlike in eukaryotic cells, lipid peroxidation by \(O_2^{•−}\) is not believed to be significant, due to the lack of polyunsaturated lipids in bacterial membranes). (2) A more important pathway of damage is the production of \(OH/Fe(ν)\) by \(H_2O_2\) and ferrous iron \(via\) the Fenton reaction,\(^{130}\) making any biomolecule containing or associated with solvent-accessible reduced iron susceptible (with reactivity depending on the ligand). Because ‘OH is so reactive and non-specific, the reaction products are diverse. Iron has an affinity for nucleic acids and thus DNA is a key target of intracellular Fenton reactions, leading to strand cleavage and formation of adducts. A wide range of ferroenzymes can also be damaged, with ‘OH initiating a chain reaction in some cases, and resulting in a wide range of products, including carboxyls. (3) \(O_3\) can exacerbate Fenton damage by releasing iron from enzymes, and reducing ferric to ferrous iron.

Cellular defense mechanisms to cope with oxidative stress (even in the absence of radiation) include: (I) enzymes to reduce the intracellular concentrations of ROS (superoxide dismutases (SOD) for \(O_2^{•−}\), and catalases and peroxidases for \(H_2O_2\)), (II) extremely tight controls on the levels of intracellular iron (controlling import, and sequestering cytoplasmic iron in ferritins and other iron binding proteins like Dps), and (III) repair of damaged proteins and DNA. Some of these defense mechanisms are regulated by inducible stress responses that are activated by ROS (e.g., OxyR protein system, which among other things increases levels of alkylhydroperoxidase (Ahp) and catalase as well as iron sequestration by Dps, and SoxRS protein system, which increases levels of SOD).

We now return to the possible ways in which sunlight might contribute to endogenous damage, with reference to right side of Fig. 7. Sunlight wavelengths in the UVB range may cause direct damage to DNA and proteins (A);\(^{53}\) several specific examples are also mentioned below (D and H). There are multiple lines of evidence that sunlight increases ROS levels in cells: accumulation of ROS in \(E. coli\) exposed to sunlight as measured using a fluorescent probe;\(^{131}\) increased expression or levels of ROS scavenging enzymes in \(E. coli\) and \(Ent. faecalis\)
Exposed to sunlight wavelengths, the finding that *E. coli* mutants lacking genes that regulate production of ROS scavengers and iron levels are more sensitive to sunlight wavelengths, and increased survival of *E. coli* and *Ent. faecalis* under simulated sunlight by addition of histidine, a membrane-permeable O2 quencher. One source of ROS is photoproduction by endogenous sensitizers (B), which is typically described as the classic indirect endogenous mechanism of sunlight inactivation. Examples include the production of 1O2 by flavoenzymes and H2O2 by tryptophan. Porphyrins are also endogenous sensitizers in *E. coli*, but it is not clear whether the porphyrins themselves are damaged, disrupting the electron transport chain, or if they sensitize production of ROS. Others have suggested that once the respiratory chain is damaged by sunlight, ROS production by adventitious reduction of oxygen increases because the regular electron transfer pathway is disrupted (C). Another mechanism through which ROS levels may increase is via sunlight damage to scavenging enzymes (D), such as direct photolysis of catalase and Ahp.

Given the toxicity of iron in aerobic cells, reactive iron levels are tightly regulated in bacteria. There is evidence that sunlight increases the pool of reactive, reduced iron, for example via photoreduction (F) and release of iron from the siderophore enterobactin (E). The accumulation of reducing equivalents after the respiratory chain is damaged could also increase the rate at which Fe(III) is reduced to Fe(II), accelerating damage by the Fenton reaction (F). Another possibility is that DNA and protein repair processes that require energy are reduced, due to damage to the electron transport chain (G).

An intriguing possibility that is distinct from the mechanisms that exacerbate oxidative stress is that UVA wavelengths cause direct damage to transfer RNA, due to the chromophore 4-thiouridine, which causes a growth delay in *E. coli*, and has been suggested to offer a protective effect against UVA exposure by retarding protein expression (H).

Another line of research has been to characterize loss of function or activity in *E. coli* exposed to sunlight wavelengths. There is evidence that bacterial cell membranes are damaged.
force, which reduced membrane functions related to the electron transport chain were more potent than visible light.

Isolates of

not have been activated in this study when grown under anoxic although it possesses oxidative stress response genes, they may and Gram-negative bacteria under oxic and anoxic conditions; was inactivated faster than a suite of seven other Gram-positive and Gram-negative bacteria and diverse. The oxyR gene was identified in protecting

Also, it is likely that all bacteria have peroxidases or catalases to scavenge endogenous H₂O₂; Pseudomonas aeruginosa was found to be protected from UVA irradiation by catalase. However, there is evidence that oxidative stress responses are complex and diverse. The oxyR gene was identified in protecting Salmonella typhimurium, whereas the sodA gene was identified in Enterococcus faecalis, and the msrA gene in Staphylococcus aureus.

On the other hand, the obligate anaerobe Bacteroides thetaiotaomicron was inactivated faster than a suite of seven other Gram-positive and Gram-negative bacteria under oxic and anoxic conditions; although it possesses oxidative stress response genes, they may not have been activated in this study when grown under anoxic conditions. Evidence of damage to lipids and proteins was found in a study of Acinetobacter and Pseudomonas exposed to UVB and UVA wavelengths.

6.2. Exogenous mechanisms

Enterococci appear to be susceptible to exogenous inactivation, but E. coli are not noticeably susceptible except at high pH, which can occur (temporarily) in highly eutrophic waters such as wastewater treatment ponds or open water wetlands, due to the high photosynthetic rate of algae, at high salinity, such as in seawater. Evidence that enterococci are susceptible to inactivation by exogenous sensitizers was provided by experiments in WTP effluent using both simulated and natural sunlight; the inactivation rate of enterococci was higher in WTP effluent than in buffered, sensitizer-free water, indicating that the sensitizing effects of chromophores in the water outweighed light-attenuation in the shallow reactors used. A study of eight health-relevant bacteria (Bacteroides thetaiotaomicron, Campylobacter jejuni, Enterococcus faecalis, E. coli K12, E. coli O157:H7, Salmonella enterica serovar Typhimurium LT2, Staphylococcus aureus, and Streptococcus bovis) to exogenous inactivation by synthetic and natural sensitizers confirmed that the Gram-positive bacteria were more susceptible to exogenous inactivation than the Gram-negative bacteria. When UVB wavelengths were present, all of the Gram-positive bacteria experienced faster inactivation in the presence of at least one synthetic sensitizer. However, the natural sensitizers only increased the inactivation rate when the UVB wavelengths were not present. Interestingly, the natural sensitizers (Suwanee River NOM and DOM isolated from a constructed wetland) also increased inactivation rates (via the exogenous mechanism) of E. coli K12 and S. enterica when the UVB wavelengths were not present. Recent results indicate that DOM isolated from wastewater and constructed wetlands adsorbs to Enterococcus faecalis cells, and that sunlight inactivation rate increased with the mass of adsorbed DOM. Synthetic sensitizers are also known to be more effective when associated with, or taken up by bacteria.

The reactive species responsible for exogenous mechanisms have not been well characterized. Kadir and Nelson (2014) found that polyhistidine, which is too large to be transported across the cell wall, decreased the inactivation rate of Enterococcus faecalis in WTP water, implicating 1O₂ produced exogenously; consistent with this interpretation, quenchers of O₂⁻, and H₂O₂ did not reduce the inactivation rate. Singlet oxygen is also known to be an effective reactive species for photodynamic therapy, with Gram-positive bacteria being more susceptible than Gram-negative bacteria; one possible explanation is that Gram-negative bacteria are protected by their outer membrane, whereas O₂ can diffuse through the peptidoglycan layer of Gram-positive bacteria and damage the cytoplasmic membrane. Under conditions that compromise the outer membrane of Gram-negative bacteria, however, they appear to become more susceptible.

Overall, there are a complex set of factors that influence whether exogenous mechanisms are relevant under specific conditions. These factors include: bacterial species and physiological state, the wavelengths of light, the characteristics of the sensitizer and its association with the bacterium.

6.3. Interactions between mechanisms

The three mechanisms of sunlight damage may interact for bacteria. For example, catalase may be damaged directly, which then increases indirect endogenous and exogenous
damage. Similarly, a DNA repair enzyme may be damaged by an indirect endogenous mechanism, which then increases net direct DNA damage. As a final example, hydrogen peroxide produced exogenously may diffuse across cell membranes to cause indirect endogenous damage.\textsuperscript{162}

6.4. Pigmentation
Enterococci that contain carotenoids are less susceptible to both endogenous and exogenous sunlight inactivation, presumably due to the ability of the pigments to scavenge singlet oxygen and other reactive intermediates.\textsuperscript{161,163,164} As a result, pigmented strains become dominant with prolonged sunlight exposure.\textsuperscript{164,165} This difference in susceptibility complicates the use of enterococci as fecal indicator bacteria, as the fraction of pigmented and non-pigmented strains may vary with time and in different waters. Pigmentation may also protect some pathogenic bacteria from sunlight inactivation, such as \textit{Staphylococcus aureus}.\textsuperscript{161,166} Fortunately, the pigmented \textit{S. aureus} was found to be inactivated at higher rates than the non-pigmented \textit{Ent. faecalis},\textsuperscript{128} suggesting that non-pigmented enterococci may still be a conservative indicator of pathogenic bacteria.

6.5. Damage versus inactivation
Because bacteria have multiple strategies to repair sunlight damage, there is a possibility that sub-lethal injury could lead to recovery and regrowth. Nonetheless, multiple studies have demonstrated that regrowth is uncommon for a range of conditions. In laboratory experiments with \textit{E. coli} simulating disinfection with SODIS and photo-Fenton (see Section 10.3), no recovery or regrowth was observed, although cells retained culturability longer on less selective media.\textsuperscript{167} Using microcosm experiments, \textit{Ent. faecalis} appeared to become permanently inactivated by sunlight in clear seawater and not to experience repairable injuries within 48 h,\textsuperscript{127} similar to findings of others on \textit{Salmonella} and \textit{Shigella}.\textsuperscript{168} Davies-Colley et al. held pond samples in the dark after they were exposed to sunlight, and found that enterococci counts continued to decrease over the 24 h holding period, although \textit{E. coli} showed some increase – presumably due to repair mechanisms.\textsuperscript{158}

6.6. Effects of bacterial physiology
The susceptibility of bacteria to sunlight has been shown to be affected by the prior growth conditions,\textsuperscript{2} which has implications for the design of laboratory experiments, comparing results for different conditions, and relating experiments with lab cultures to environmentally acclimated bacteria. With respect to the latter, several studies have found bacteria sourced from wastewater to be less susceptible to sunlight than laboratory-grown organisms,\textsuperscript{158,159,164} although another study found that differences depended on the water quality.\textsuperscript{169} A faster growth rate during culturing was reported to increase the susceptibility of both \textit{E. coli}\textsuperscript{170} and \textit{Ent. faecalis} cells to sunlight wavelengths, and stationary phase \textit{Ent. faecalis} were more resistant than cells harvested in exponential phase.\textsuperscript{171} \textit{E. coli} grown under aerobic conditions were more susceptible to sunlight than cells grown under anaerobic conditions; furthermore, after sunlight exposure, cell counts were higher when plated in the presence of ROS scavengers (pyruvate or catalase).\textsuperscript{167,172} \textit{E. coli} grown in a low-iron media were inactivated more slowly by sunlight than cells grown on iron-rich media.\textsuperscript{112} Finaly, prior exposure of \textit{E. coli} cells to a sub-lethal UVA fluence rate increased survival to a lethal fluence rate of UVA.\textsuperscript{123} Thus, the life history of bacteria may also affect their susceptibility to sunlight. Based on the current understanding of sunlight inactivation mechanisms, it is likely that physiological differences influence susceptibility to sunlight because of varying sources of, or responses to, oxidative stress.

7. Other organisms and biomolecules
7.1. Sunlight-mediated inactivation of protozoan cysts
The sunlight-mediated inactivation mechanisms of \textit{Cryptosporidium parvum} oocysts have been explored by Liu et al. (2015).\textsuperscript{173} Inactivation rates (as determined in \textit{in vitro} cell culture) were faster in the presence of UVB light compared to when the UBV wavelengths were blocked. Direct damage to DNA was implicated as the dominant mechanism by UVB, whereas indirect endogenous mechanisms were implicated when only UVA and visible wavelengths were present. Inactivation by UVA-induced endogenous radical damage was higher at 40 °C than 25 °C, whereas inactivation by UVA-induced genome damage was not sensitive to temperature. Natural organic matter (Suwannee River NOM and wastewater effluent NOM) did not enhance inactivation, likely due to a thick oocyst wall, which renders oocysts resistant to exogenous inactivation. Studies focusing on mechanisms of damage have illustrated damage to the oocyst wall after 10 h of exposure to UVA/visible light,\textsuperscript{174} and interference with sporozoite exocytosis, which is a fundamental cellular process required for sporozoites to attach to and invade host cells.\textsuperscript{175}

Most other research investigating the effects of sunlight on waterborne protozoan pathogens has been conducted in the context of solar disinfection of drinking water (SODIS; see Section 9.3), and has focused on the effects of reactor geometry, water turbidity, and temperature. A number of studies on container effects have found that containers that transmit more or shorter-wavelength UV light achieved faster inactivation rates of \textit{C. parvum} oocysts,\textsuperscript{174,176–181} consistent with the findings on mechanisms above. In general, other protozoan cysts, including \textit{Entamoeba histolytica}/\textit{dispar}, \textit{Naegleria gruberi}, and \textit{Giardia lamblia/muris/duodenalis} have been found to be susceptible to photoinactivation.\textsuperscript{5,174,182–185} The cysts of \textit{Acanthamoeba polyphaga/castellanii} appear to be an exception,\textsuperscript{186} and were only detectably inactivated by sunlight at elevated temperatures (>45 °C)\textsuperscript{181} or in the presence of riboflavin.\textsuperscript{186} No studies have directly compared the inactivation rates of protozoan cysts to those of bacteria or viruses (with the exception of \textit{Acanthamoeba}). Although it is difficult to compare rates from different studies given the differences in light spectra and irradiance, results to date suggest that protozoan cysts may be generally more resistant to sunlight than viruses and bacteria. This trend is different from that for inactivation by UV,\textsuperscript{254,187} to which protozoan cysts have similar susceptibility as bacteria, and are more susceptible...
than most viruses, and underscores that there are differences in the principal inactivation mechanisms of UV$_{254}$ and sunlight.

A particular challenge with interpreting some of the published research on protozoan cysts is the use of different assays to measure inactivation. Dye permeability and in vitro excystation were found to underestimate oocyst inactivation compared to animal infectivity tests (Swiss CD-1 suckling mice).

Another challenge is different sources of oocysts; because oocysts cannot be propagated in vitro, propagation through animals such as calves and mice is required. Also, oocysts for experiments are usually purified from feces of infected animals; however, purified oocysts have been shown to lose infectivity within 24 weeks during storage at 4°C in autoclaved water. We recommend that future studies of oocyst inactivation should document the source of oocysts, the storage conditions of oocysts, and should quantify response by either in vitro cell culture or animal infectivity.

7.2. Sunlight-mediated degradation of antibiotic resistance genes

An emerging concern that is relevant to the transmission of bacterial pathogens via sunlit waters is the fate of antibiotic resistance genes (ARGs). ARGs are now recognized as widespread contaminants of aquatic systems, leading to concerns that their presence may contribute to the dissemination of antibiotic resistance traits amongst bacterial populations via horizontal gene transfer (HGT) processes (including conjugation, transduction, and natural transformation). ARGs are present as intracellular genomic and plasmid DNA in viable antibiotic resistant bacteria (ARB), and as extracellular (i.e., free) DNA protected within cell debris, phage capsids, extracellular polymeric substances, or on clay mineral surfaces. Even extracellular ARGs may be capable of transferring their encoded resistance traits to non-resistant bacterial populations by means of transduction or natural transformation. Thus, it is desirable to examine not only if solar radiation will yield inactivation of viable ARB cells, but also whether or not it is likely to render ARGs incapable of conferring resistance traits by any of the three means of HGT.

Although very little information is currently available regarding the effects of UVB, UVA, or broadband sunlight in ARGs, substantial past work illustrates that monochromatic UVC radiation (UV$_{254}$) can eliminate the ability of various ARGs to transform competent non-resistant recipient bacteria to the corresponding resistance phenotypes, whether such ARGs are contained in intracellular or extracellular DNA. Studies in which qPCR was utilized to quantify residual copy number of ARGs contained in extracellular and intracellular DNA from several genera of ARB during UVC irradiation are generally consistent with these findings. However, ~2- to 10-fold higher fluences are required to achieve >2-log degradation of ARGs than to yield comparable ARB cell inactivation (i.e., ARGs are degraded more slowly than ARB cells are inactivated).

One complicating factor of ARG fate is that regions in the DNA outside of the ARG sequence are necessary for transformation; thus measuring the decrease in portions or all of the resistance gene with qPCR following UV treatment is a conservative measurement of transformation potential. For extracellular plasmids carrying ARGs, plasmid nicking was not a major reaction pathway at UV$_{254}$ fluences used for water treatment.

In light of the above, it is also reasonable to anticipate some degradation of ARGs (intracellular or extracellular) during solar irradiation, given the susceptibility of DNA to direct and indirect damage by sunlight, as described in previous sections. In general, it can also be expected that extracellular ARGs will undergo more rapid sunlight-driven degradation than intracellular ARGs, as many bacterial species are capable of DNA photorepair under solar illumination, as well as dark repair. Furthermore, extracellular DNA is likely to be susceptible to both exogenous direct and indirect mechanisms of damage.

In one series of studies, accelerated decay of several intracellular resistance genes (as monitored by qPCR) was observed in micro- and mesocosms that were seeded with wastewater and irradiated with simulated and/or natural sunlight, relative to dark controls. However, considering that wastewater matrices were used to seed the meso-/microcosms – it remains unclear whether these observations were due specifically to sunlight-mediated DNA damage or to unidentified alterations in the microbial ecology of the investigated systems upon exposure to sunlight.

It has also been reported that the ability of a plasmid-borne cat gene to transform recipient bacterial cells to chloramphenicol resistance can be gradually eliminated during exposure of extracellular preparations of the host plasmid in TE buffer (pH 8) to artificial UVC, UVB, and UVA light, as well as natural sunlight. Fluence requirements to yield comparable levels of deactivation were ~10-fold higher for irradiation by UVA compared to UVB, and also ~10-fold higher for UVB compared to UVC. Loss of activity correlated well with induction of cyclobutane pyrimidine photodimers by artificial UV ranges and natural sunlight, suggesting that photodimer formation represents the primary mechanism of ARG deactivation. Although not specific to ARGs, several recent studies also illustrate that qPCR signals for the 23S rRNA gene contained within extracellular or intracellular genomic DNA of Enterococcus spp. can persist even at solar fluence values several times those sufficient to yield 5-log inactivation of the bacterial cells themselves.

Taken together, results to date suggest that sunlight-driven degradation of ARGs will likely proceed with markedly slower kinetics than ARB cell inactivation, in analogy with observations for UVC irradiation. Recent findings also suggest that ARB cells themselves may be more resistant to inactivation during solar irradiation than cells of non-resistant strains, possibly due to upregulation in expression of a wider array of stress response and repair genes in ARB than in non-resistant strains. Even if ARB cells are effectively inactivated by solar irradiation, their ARG-containing DNA may remain intact and capable of transferring resistance traits to non-resistant bacteria. A challenge with assessing this risk is that ARGs detected by qPCR may no longer be capable of transferring resistance via transformation. Considering the potential public health and ecological implications of ARGs persisting during transit through natural surface waters, further research on this topic is highly desirable.
8. Modeling of inactivation rates

Models of sunlight-mediated inactivation are needed for use in the design of treatment processes that rely on solar disinfection, to quantify the fate of pathogens and indicators in recreational waters, and more generally as components of ecological models for surface waters. Although the impact of sunlight has been incorporated into models for these different applications, in most cases inactivation is modeled as a simple first-order process with time. Reported sunlight inactivation rate constants vary over several orders of magnitude even for the same microorganism. One large source of variability is that these “overall” rate constants do not separate out the key parameters that are now known to influence inactivation rates based on the growing mechanistic understanding reviewed in the previous sections. To improve the predictive ability of models, and to better understand the sources of variability, in this section we review more detailed approaches that have been applied to model photoinactivation of viruses and bacteria. We do not consider the effects of other potential loss processes that may occur simultaneously with photoinactivation (e.g., physical removal, die-off due to unfavorable environmental conditions, predation) or transport processes. Thus, we analyze a volume with artifical or well-mixed. The approaches discussed here can be used to model laboratory experiments conducted with artificial light sources (e.g., UVB, UVA, visible light, or solar simulators) or natural sunlight, as well as to model surface water bodies exposed to natural sunlight. Of course, other die-off mechanisms and transport processes must also be accounted for in surface waters.

There are two main steps in modeling photo-inactivation (Fig. 8): (1) estimating the irradiance spectrum to which the organisms are exposed, and (2) predicting the inactivation that occurs as a result of the irradiance spectrum. The first step can be further broken down into: (1a) characterizing the radiation spectrum incident upon the water body of interest, and (1b) accounting for differential transmission across the UV-visible spectrum within the water column. The second step can be further broken down into: (2a) predicting inactivation due to endogenous inactivation and (2b) due to exogenous inactivation. Because exogenous inactivation results from absorption of photons by chromophores in the water, this step involves (2b1) predicting the concentration of reactive intermediates in the sunlit water, and (2b2) predicting the inactivation caused by the reactive intermediates.

8.1. Measuring or predicting the incident irradiance (step 1a)

Step 1a involves either empirical measurement of the irradiance spectrum for a specific radiation source or sunlight condition, or prediction of the solar irradiance based on (assumed or measured) meteorological and atmospheric conditions. Because of the marked wavelength-dependence of photoinactivation, it is necessary to characterize the irradiance spectrum over the entire (UV-visible) wavelength range that may contribute to inactivation (i.e., the curves shown in Fig. 2). The main option for empirical measurement of the irradiance incident upon a water body (e.g., an open reactor or a natural water body) is a spectroradiometer, which measures the wavelength-specific irradiance over the desired range. Additional discussion of approaches for measuring irradiance is provided in Section 9.

Alternatively, several models exist for predicting the incident irradiance from natural sunlight, which is necessary when modeling photoinactivation for light conditions that cannot be measured directly (e.g., different locations or times). To date, sunlight inactivation models have used the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS)\(^2\) and the Tropospheric Ultraviolet and Visible Radiation Model (TUV).\(^3\) However, a unique challenge with predicting photoinactivation compared to other sunlight processes is that rates are very sensitive to UVB wavelengths, which are highly variable and represent only a minor fraction of the total irradiance; neither measurement instruments nor atmospheric models have been tailored to provide accurate results in the UVB region.\(^21\) To improve the accuracy of photoinactivation models, it will be necessary to develop more accurate methods for both measuring and predicting sunlight in the UVB range. A further challenge with predictive modeling is the difficulty of accounting for the effects of cloud cover on the sunlight spectrum.

8.2. Accounting for spectral transmission into water (step 1b)

Models of sunlight inactivation have employed the same approaches developed for aquatic photochemistry to account for differing light transmission through water across the UV-visible solar spectrum. To determine the rate of photoinactivation at a given depth in the water column, the irradiance at that depth must be determined by correcting the incident irradiance (e.g., Fig. 2) for light attenuation within the water column (e.g., Fig. 3). Alternatively, the depth-average irradiance can be determined if the water body is well-mixed. For laboratory experiments with collimated light sources, it is common to
estimate sunlight attenuation due to absorption using Beer’s Law, assuming that reflection at the surface and scattering by particles suspended in the water are negligible. For natural water bodies exposed to sunlight, the diffuse downwelling attenuation coefficient is used (eqn (1)), which can either be measured directly or modeled using fundamental optical measurements (e.g. Kirk 2011, or using an optical model like Hydrolight*). In some strongly coloured waters, scattering effects are relatively minor and can be neglected (e.g., wastewater polishing wetland211) whereas in most waters scattering significantly increases attenuation above that due to absorption alone (e.g., high rate algal pond, Fig. 3 (ref. 212)). The models ideally should account for a weak influence of solar zenith angle. To date, more sophisticated radiative transfer models that account for sunlight penetration into water bodies, such as HydroLight,211 have not been used for modeling sunlight inactivation, and we emphasize that this is an important direction for future research.

8.3. Modeling inactivation rate constants (step 2)

Although synergies between sunlight inactivation mechanisms likely exist, the current understanding is insufficient to include such interactions in modeling, and so mechanisms are assumed to be independent and additive. Thus, the total inactivation rate constant ($k_{	ext{tot}}$) is represented as the sum of the rate constant for endogenous mechanisms ($k_{\text{endo}}$), exogenous mechanisms ($k_{\text{exo}}$), and any light-independent (dark) mechanisms ($k_{\text{dark}}$).

$$k_{\text{tot}} = k_{\text{endo}} + k_{\text{exo}} + k_{\text{dark}} \quad (3)$$

Current mechanistic models do not explicitly account for other factors that may influence sunlight inactivation rates, including dissolved oxygen, pH, the physiological state of bacteria, the extent of aggregation or particle-association, or repair processes (occurring at temperature-dependent rates). An approach to account for the synergistic effect of temperature (30–55 °C) on endogenous inactivation was recently developed and is discussed briefly in the next section.214

8.3.1. Inactivation due to endogenous mechanisms (step 2a). Currently, it is difficult to separate direct and indirect endogenous mechanisms, so they are lumped together for modeling purposes. Three different approaches have been used to model the endogenous contribution to inactivation. Each approach assumes that the inactivation rate constant ($k_{\text{endo}}$) is proportional to the sunlight irradiance incident upon the organism ($E$):

$$k_{\text{endo}} = \sum_{\lambda_2} \lambda_1 P(\lambda) E(\lambda) \quad (4)$$

where $P(\lambda)$ is the weighting factor. In the first approach, it is assumed that all wavelengths in a specific range contribute to endogenous inactivation, and all photons in this range contribute equally to inactivation (i.e., $P = \text{constant}$). For example, Silverman et al. assumed that only UVB wavelengths contributed to endogenous inactivation of the viruses MS2 and poliovirus;17 Nguyen et al. assumed that UVB and UVA wavelengths contributed to inactivation of E. coli and enterococci.44 Maracccini et al.18 assumed only UVB contributed to E. coli and enterococci across a range of water types.

In the second approach, Mattle et al. (2015) modeled $P(\lambda)$ for three viruses (MS2, PhiX174, and HAdV) as the product of a constant apparent quantum yield for the virus ($\Phi_1$; units = number of viruses inactivated per photon absorbed22) and the viral extinction coefficient ($l_{\text{virus}}(\lambda)$; virus $^{-1}$ cm$^{-1}$). This approach assumes that all photons absorbed by virus components have equal likelihood to cause inactivation, independent of their wavelength (i.e., the action spectrum is proportional to the absorption spectrum of the virus). The number of photons absorbed by virus components is a function of their nucleic acid and endogenous protein chromophores, is a small fraction of the photons incident upon a virus, and is restricted to UVB wavelengths (Section 5.1). To generate similar modeling values for viruses other than those studied by Mattle et al., two pieces of experimental information are needed: the absorption spectrum of the virus, and its inactivation rate under a known light spectrum. Alternatively, it has been proposed that the parameters for additional viruses can be estimated without further experimentation, if the genome size and type are known, by assuming that the quantum yield as well as the extinction coefficient scales with genome type and size.197 This approach is not applicable to bacteria, for which the absorption spectrum does not match the action spectrum.125 Instead, Zepp et al. proposed an approach for determining wavelength-specific quantum yields that could be applied to bacteria using cutoff filter experiments and a fitting function as described by Run- del,27 but this has yet to be applied in practice.

The third approach is similar to that proposed by Zepp (using experiments with cut-off filters), but directly yields values for $P(\lambda)$. Fisher et al. used an empirical approach to determine action spectra and values for $P(\lambda)$ based on cutoff filter experiments for the viruses MS2 and PRD1.215 This approach assumes that photons of different wavelengths may have different contributions to inactivation, but cannot differentiate whether the effect is due to differences in absorption by viral chromophores or differences in the damage caused by photons of different wavelength. Using this approach, Nguyen et al. captured seasonal effects (summer vs. winter sunlight) on the inactivation rate of MS2 in clear water, and the effect of light attenuation by strongly humic-coloured wetland water using simulated sunlight.214 However, successful prediction of inactivation rates was hampered by difficulty in accurately determining UVB irradiance, a problem noted above in Section 8.1. The third approach has also been applied to bacteria; Silverman et al. (2016) developed $P(\lambda)$ functions for E. coli and enterococci grown in the laboratory and concentrated from wastewater.19 The wastewater bacteria $P(\lambda)$ functions were used to predict inactivation rates in wetland water and clear seawater, and it was noted that further work is needed to account for the increased bacterial inactivation that occurs with high dissolved oxygen and pH in algal laden waters, which are important for E. coli.178,216 Mostafa et al. (2016) used an action spectrum for Ent. faecalis to predict the effect of light attenuation on its
endogenous inactivation rate in water containing different types of organic matter. Finally, Roser et al. (2016) recently published action spectra for E. coli and enterococci based on experiments with monochromatic LEDs that cover the sunlight range; the results appear roughly consistent with other work conducted with polychromatic light.

The main difference in these three approaches is their response to changes in the spectral irradiance. If the shape of the light spectrum does not change (i.e., all wavelengths increase or decrease directly proportional to total irradiance), the three approaches predict the same relative changes in \( k_{\text{endo}} \). However, as discussed in Section 3, the relative proportion of shorter (i.e., UVB) to longer wavelengths changes due to zenith angle and atmospheric conditions (Fig. 2), as well as wavelength-dependent attenuation in the water column (Fig. 3).

Another approach has been developed for modeling the indirect endogenous inactivation of E. coli that explicitly accounts for damage by ROS (e.g., superoxide, hydroxyl radical). Steady-state concentrations of intracellular ROS species are determined as a function of their production and loss processes (Fig. 7), and inactivation results from second-order reactions between ROS and bacteria. A strength of this approach is that it provides more insight into the specific endogenous reactions, which allows for the manipulation of a wide range of factors that influence \( k_{\text{endo}} \). For example, the authors expanded the model to account for the impact of temperature on the individual reactions, as well as the independent and synergistic effects of temperature in the range 30–55 °C. The authors have also explored the effect of different catalase loss rates (due to photoinactivation and thermal degradation). A disadvantage of this modeling approach is that it does not account for direct endogenous inactivation, nor wavelength-specific effects.

### 8.3.2. Exogenous inactivation (step 2b)

Exogenous inactivation has been modeled as a series of parallel apparent second-order processes in which individual reactive intermediates inactivate the organism under consideration:

\[
 k_{\text{exo}} = \sum_{[\text{PPRI}]} k_{\text{PPRI}} [\text{PPRI}]_{ss} \tag{5}
\]

where \( k_{\text{PPRI}} \) is the apparent second-order photoinactivation rate constant for a specific reactive intermediate and organism and \([\text{PPRI}]_{ss}\) is the steady-state concentration of the PPRI, which is specific to the environmental system under consideration.

Measuring PPRI concentrations in situ is experimentally challenging. However, models have been developed that predict \([\text{PPRI}]_{ss}\) as a function of water depth (or depth-averaged values) based on easy-to-determine water composition (e.g., the APEX model, Bodrato and Vione 2014; GCSOLAR, U.S. EPA). Such models take into account the dominant formation and quenching processes of each reactive species, as well as the changes in irradiance spectrum throughout the water column.

Second-order rate constants have been measured in laboratory experiments for MS2, PhIX174, HAdV, and rotavirus. Values of \( k_{\text{PPRI}} \) for all four viruses are close to the diffusion limit for hydroxyl radical (\( \sim 10^{10} \text{ M}^{-1} \text{ s}^{-1} \)) and 1–2 orders of magnitude lower for singlet oxygen, \( ^1\text{DOM}^* \) or carbonate radicals. Furthermore, the measured \( k_{\text{PPRI}} \) for a given reactive species were typically within one order of magnitude for the different viruses (MS2, PhIX174, HAdV), except for \( ^1\text{DOM}^* \), for which slightly larger differences between viruses were observed. It should be noted that the rate constants for singlet oxygen and \( ^1\text{DOM}^* \) were determined using model sensitizers (Rose Bengal and anthraquinone-2-sulfonate, respectively), and that association between the sensitizers and viruses cannot be ruled out. Therefore, the apparent rate constants may vary depending on the degree of association.

While relatively straightforward in its application, this model of exogenous virus inactivation has several shortcomings. First, it does not take into consideration synergies between different reactive species or between reactive species and UVB light. Second, the model does not capture virus-sensitizer interactions, which may enhance inactivation, as discussed in Section 4.4.

The approach of Silverman et al. attempts to capture both of these aspects, but is more empirical. Recognizing that \( ^1\text{O}_2 \) was found to be the most important contributor to exogenous inactivation of MS2, E. faecalis, and a likely contributor to inactivation of Ent. faecalis, exogenous inactivation was modeled as an apparent second-order reaction with \( ^1\text{O}_2 \) as the only reactive species, with the assumption that other reactive species scale proportionally with \( ^1\text{O}_2 \), which is an oversimplification. This approach, in which the apparent \( k_{\text{O}_2} \) values for each organism were first determined experimentally in water from a constructed wetland, was used to model MS2 and Ent. faecalis in the same wetland. The value for \( k_{\text{MS2-}^1\text{O}_2} \) of \( 1.1 \times 10^{13} \text{ M}^{-1} \text{ h}^{-1} \), is about ten times greater than that measured by Mattle et al. using Rose Bengal (RB). This difference may be due to two factors: (1) the apparent \( k_{\text{MS2-}^1\text{O}_2} \) accounts for the contribution of other reactive species and possibly their interactions; and (2) the sensitizers in the wetland may have had greater association with MS2 than RB, such that MS2 was exposed to higher concentration of ROS than the measured bulk-phase concentration. A strength of this approach is that the apparent \( k_{\text{O}_2} \) values account for other reactive species as well as association between the sensitizer and organisms. However, this is also a drawback, because the apparent \( k_{\text{O}_2} \) must be measured specifically for each water of interest, as the value has been observed to vary among waters.

### 8.4. Putting it all together

Combining the approaches described above, the overall inactivation rate constant due to sunlight (eqn [3]) can be determined. If the interest is in inactivation at a specific depth, \( k_{\text{tot}}(z) \) is calculated using \( k_{\text{tot}}(z) = \sum_{\text{species}} k_{\text{adapted}}(z) \). If the interest is a well-mixed water column, a depth-averaged \( k_{\text{tot}} \) is calculated using depth-averaged irradiance \( \langle E(z) \rangle \) and \( ([\text{PPRI}]_{ss}) \). Applications include modeling the effects of changes in the irradiance spectrum and/or changes in water quality on the sunlight-mediated inactivation of viruses and bacteria.

In Fig. 9 and 10, we illustrate several insights from this modeling approach. The strong influence of spectral sunlight attenuation on the endogenous inactivation rate constant
(k_{endo}) is shown in Fig. 9, by comparing the different waters from Fig. 2. We used a water depth of 20 cm and assumed the water was well mixed. The first step was thus to calculate the average irradiance spectrum transmitted through each water (Fig 9, panel (b)), using an incident irradiance spectrum for June in Northern California, as reported in Silverman et al. 2015.

The second step was to incorporate the action spectra (i.e., P(\lambda)) to calculate values for k_{endo}, P(\lambda) spectra for E. coli, enterococci, and MS2 are shown in Fig. 9 panel (a). In panel (c), the product of the irradiance spectrum and the action spectrum – referred to as the photodamage spectrum for endogenous inactivation – is shown for laboratory strain E. coli for the different waters. The area under each curve is k_{endo}. The modeled endogenous inactivation rate is reduced from 5.2 h^{-1} in clear water to 0.12 h^{-1} in the algal pond water, due to strong attenuation of sunlight by chromophores in the water. Another insight provided by the photodamage spectra is the dominant wavelengths contributing to inactivation. The peak wavelengths contributing to E. coli (panel c) and enterococci (not shown) inactivation occur around 330 nm. Note that a similar approach was used by Mbonimpa et al. (2012) to model inactivation of E. coli by sunlight; however, a standard DNA action spectrum was used which does not account for other mechanisms of sunlight damage.

Note that the curves in Fig. 9 do not account for the contribution of exogenous mechanisms to inactivation, which can be initiated by longer wavelengths (Table 2). In Fig. 10 we account for both endogenous and exogenous inactivation rates, and

![Fig. 10](image-url)
illustrate how dramatically the rate constants can vary for MS2 and poliovirus due to their different susceptibilities to endogenous and exogenous mechanisms, using the model parameters from Silverman et al., also for the month of June in Northern California. In clear water (only endogenous inactivation, minimal light absorption by water column), \( k_{\text{tot}} \) for poliovirus is \( \sim 8 \) times greater than MS2. However, in wetland water (containing sensitizers), \( k_{\text{tot}} \) for poliovirus is only \( \sim 2.5 \) times greater at the water surface, because MS2 is much more susceptible to exogenous inactivation. Even at 5 cm depth in wetland water, \( k_{\text{tot}} \) for MS2 is still greater than it is in clear water because of the large contribution by exogenous inactivation. In contrast, \( k_{\text{tot}} \) for poliovirus is smaller at 5 cm depth in the wetland relative to clear water, because attenuation of sunlight by the organic matter decreases the endogenous rate more than the sensitizing effect contributes to exogenous inactivation. At depths greater than 11 cm, \( k_{\text{tot}} \) for MS2 is actually greater than \( k_{\text{tot}} \) for poliovirus, because \( k_{\text{ex}} \) decreases less rapidly with depth than \( k_{\text{endo}} \) due to the dependence of \( k_{\text{endo}} \) on shorter wavelengths, which are attenuated more efficiently by organic matter, as discussed in Section 3. If the water column is well-mixed, \( k_{\text{tot}} \) for MS2 and poliovirus are equal if the water column is about 50 cm deep, and the inactivation rate constant for MS2 is still greater in this case than it is in shallow clear water.

Next, we describe several applications of the mechanistic modeling approach. Silverman et al. (2015) and Nguyen et al. (2015) modeled the sunlight-mediated and dark inactivation of MS2 and fecal indicator bacteria (E. coli and enterococci), respectively, in a pilot-scale, open water unit process wetland operating for one year. The sunlight spectrum was predicted using SMARTS, \( \text{O}_3 \) was used as a surrogate for all PPRI, and \( \text{O}_3 \) concentrations were estimated as a function of the organic matter concentration. The wetland hydraulics were modeled using simple two-parameter model, with the dispersion coefficient determined from a tracer test. There was surprisingly good agreement between modeled MS2 and measured F+ coliphage removals throughout the year (MS2 is one member of the F+ coliphage family). The agreement was not as good for the indicator bacteria, as the monitoring data were highly variable. Exogenous inactivation mechanisms were predicted to dominate inactivation of MS2 and also contributed significantly to the inactivation of non-pigmented enterococci. One challenge is that E. coli and enterococci, concentrated from wastewater were more resistant to sunlight than lab strains and isolates cultured in the lab; therefore, a correction factor was developed to convert rates measured in the lab to those in field.

Kohn et al. modeled the inactivation rates of phages MS2 and phiX174 in two different water matrices, a WTP and a natural surface water, for different sunlight conditions (season and latitudes). The endogenous inactivation rate was determined using eqn (4), in which \( P(\lambda) \) was determined from each virus’s molar extinction coefficient and quantum yield. The exogenous inactivation rate constant was determined using eqn (5), and the second-order rate constants reported in Matte et al. and steady-state PPRI concentrations determined using the APEX model. As expected, the contribution of the exogenous mechanism in WTP water was significant for MS2 (>50% for water depths > 1 m), and dominated by \( \text{O}_3 \) with small contributions from “CDOM” and OH. Inactivation of phiX174 was dominated by endogenous mechanisms. Because longer wavelengths contribute to exogenous mechanisms, the inactivation rate of MS2 was less sensitive than that of phiX174 to changes in solar irradiance due to season and latitude. Despite some differences in the modeling approaches used by Kohn et al. and Silverman et al., the Kohn et al. model was able to predict with fairly good agreement the experimentally measured rate of inactivation of F+ coliphage reported by Silverman et al.

Other modeling efforts have incorporated some aspects of this mechanistic approach. Williamson et al. predicted that climate change is reducing solar disinfection of surface waters due to higher CDOM concentrations from runoff; the influence of wavelength on endogenous photoinactivation of Cryptosporidium oocysts was accounted for using a photoaction spectrum modified from the DNA absorption spectrum. Empirical approaches have also been used to account for the dual role of natural organic matter (attenuation vs. photosensitization) in the inactivation of the bacteriophage phiX174 and Ent. faecalis.

The results in this section illustrate how models can be used to capture the sensitivity of sunlight inactivation rate constants to the magnitude and spectral qualities of sunlight irradiance, as well as the impacts of water quality on attenuating sunlight and producing PPRI. These environmental factors result in inactivation rate constants that can vary over several orders of magnitude for the same organism. Importantly, it was also shown that specific viruses and bacteria respond differently to these environmental conditions, leading to large changes in their relative inactivation rate constants.

9. Standardizing methods for photoinactivation experiments

The previous sections illustrate that a large number of factors influence photoinactivation rates. Thus, experiments to investigate the mechanisms or kinetics of sunlight-mediated inactivation of microorganisms should be conducted and reported in a manner to facilitate inter-laboratory comparisons. The light source is one of the most difficult aspects to characterize with sufficient detail, and is therefore one of the greatest sources of variability between field sites and laboratories. Given the wavelength-specificity of inactivation, the full UV-visible irradiance spectrum should be reported for both artificial sources and natural sunlight. For example, a graph should be provided of the spectral irradiance (280–700 nm; Fig. 2b), complemented by values of the integrated spectral irradiance for UVB, UVA, and visible radiation bands. A logarithmic scale (e.g., Fig. 2) often clarifies differences in the UVB region where spectral irradiances are much lower than elsewhere in the solar spectrum and change rapidly with wavelength. Alternatively, an inset of the UVB region can be provided.

A spectroradiometer is the preferred physical instrument for measuring the spectrum of incident irradiance over the entire UV-visible range. However, spectroradiometers for the solar spectrum have not been designed to measure UVB with much
accuracy, particularly <300 nm. Because these wavelengths contribute proportionally to inactivation, especially for viruses, the difficulty in measuring these wavelengths accurately is a major source of uncertainty (see Nguyen et al. 2014 for further discussion). The sunlight fluence (the product of irradiance and time) can be calculated for the wavelength range(s) of interest; fluence is useful for normalizing results to allow comparison between results from different radiation exposures. If a spectroradiometer is not available, a radiometer can be used to measure irradiance over a defined spectrum, and the wavelength range should be reported along with the total irradiance. Any physical device should be recently calibrated to NIST or other comparable international standards across the wavelength range of interest.

Spectral filtering of incident light by the water column can be modeled based on laboratory measurements of the UV-visible attenuation spectrum (see Section 8.2). Alternatively, the downwelling irradiance can be measured in situ in the water body of interest, using submersible instruments. In either case, it is important to provide a clear description of any assumptions that are made. In the solar UV range, light scattering can sometimes be neglected such that the vertical attenuation coefficient, $K_d(\lambda)$, can be approximated with the absorption coefficient, $a(\lambda)$ – notably in strongly colored wetland waters of relatively low scattering. To determine whether scattering is significant, the absorption spectrum of a filtered and unfiltered sample should be compared. If necessary, spectral scattering coefficients can be measured directly using modified spectrophotometers (e.g., with integrating sphere) or field measurements with suitable instruments. If the experiments are conducted in closed containers (i.e., the light passes through the wall of the container to reach the sample), then absorption of light by the containers must be characterized.

Chemical actinometry complements spectroradiometry by providing a measure of the average fluence received by cells or virions in a closed reactor. In particular, actinometry is a useful tool for normalizing fluence between different experimental conditions (e.g., variable irradiance or attenuation). For closed experimental reactors (no open water surface, e.g., merry-go-round reactor), it is difficult to measure the irradiance spectrum in the reactor with physical instruments, so chemical actinometry is preferred. The main drawback to using actinometry for characterizing photoinactivation is that the absorption spectra of existing chemical actinometers differ from the action spectrum for photoinactivation, requiring the use of cut-off filters or bandpass filters to isolate spectral regions of interest. The development of new chemical actinometers and methods to facilitate wavelength-specific actinometry would be a useful contribution to photoinactivation research.

Other water quality parameters should be reported to characterize potential sources of PPRI and any impacts of water quality on inactivation. Both dissolved and particulate sensitizers are potentially relevant. Dissolved organic sensitizers can be approximated as CDOM, which is quantified as the absorption coefficient of a filtered sample at standard wavelengths such as 440 nm or 340 nm or other UV wavelengths (e.g. 280 nm, 254 nm; see eqn [2]). Characterizing absorption by particles (e.g., wastewater particles, algae) is more difficult because particles can both absorb and scatter light (see previous page). In addition to scattering coefficients, bulk parameters such as total suspended solids, volatile suspended solids, chlorophyll a, or particulate organic carbon should be reported for samples with high particulate matter. Other parameters that can influence PPRI include NO$_3^-$, NO$_2^-$, and iron (sources of OH). Also, the steady-state concentrations of key PPRI such as O$_2$, OH, and H$_2$O$_2$ can be measured. Other relevant parameters that help capture effects of water quality include pH, salinity or ionic strength, divalent cations, dissolved oxygen, and temperature.

Experiments using organisms cultured in the laboratory must explain in detail the preparation [source of stock culture, growth media, host cells (viruses and phage only), culture conditions, purification steps] and enumeration methods (e.g., selective vs. non-selective media, additives such as pyruvate or antibiotics, and incubation conditions, such as aerobic vs. anaerobic). The extent of the enrichment or purification of the tested stock organism should be clearly described, as residual material from the cultivation (e.g., bacterial debris from bacteriophage propagation) may attenuate light or act as sensitizers. Experiments with indigenous organisms from the environment, which respond to sunlight differently than lab-derived organisms, should provide comprehensive information on the sources of the organisms, concentration methods, and specificity of the enumeration methods used.

Microbial concentration data should be presented as either the log$_{10}$ or Naperian (natural) logarithm of normalized and unnormalized concentrations, with clear indication of which is used. The Naperian (natural) logarithm of concentration values should always be used for calculation of first-order decay rates. When a shoulder or tail is present, more complex decay models may be necessary. To compare rates between different light conditions (due to differences in irradiance, spectra, or light attenuation), it is necessary to normalize decay rates based on light incident on the target microorganisms (i.e., light transmitted through the water column), which can be accomplished using the fluence, employing a screening factor, or calculating rates as a function of the photon flux.

### 10. Applications of sunlight disinfection

The growing understanding of sunlight-mediated inactivation mechanisms, and knowledge about which actual pathogens are susceptible to which mechanisms, provides opportunities to improve our understanding of the fate of microorganisms in engineered systems and natural surface waters exposed to sunlight, as well as optimize the design of engineered systems to enhance disinfection. There are still significant challenges that need to be overcome, however, to translate the mechanistic modeling approaches described in Section 8 into useful models for real-world application. In addition, accounting for other loss processes (not related to sunlight) and accurately characterizing transport in real water bodies are major challenges to modeling natural systems.
10.1. Recreational waters and shellfish waters

Recreational waters are monitored for fecal indicator bacteria (FIB; total coliforms, fecal coliforms, E. coli, and enterococci) around the world to characterize risk for bathers.228 If local standards are exceeded, then bathing waters are considered unfit for swimming. Sunlight has been shown to affect concentrations of FIB in recreational waters, both seawater229,230 and freshwater.231,232 This means that water sampled in the afternoon, after several hours of sunlight exposure, may have lower FIB levels and comply with water quality standards, while water sampled at night or in the early morning may not. Understanding when sunlight will be important in reducing bacterial concentrations can help guide the design of water quality monitoring to protect public health. Models of natural surface water quality that account for sunlight effects often assume either a constant sunlight inactivation rate, or a rate that varies as a function of sunlight irradiance.231,232 To date, the more complete approach outlined in Section 8 to account for sunlight inactivation has not been integrated into models predicting the concentrations of indicator organisms in recreational waters.

Microbial water quality is also a concern in surface waters used for shellfish harvesting and bivalve aquaculture, as pathogens can be concentrated in the flesh of filter-feeding shellfish. Sunlight is recognized as a key variable that can affect the concentrations of microbial pollutants in shellfisheries.234 Norovirus is a particular concern, and its inactivation by simulated sunlight has been studied with the aim of informing shellfish protection.235

Microbial community analysis has also been proposed to identify sources of microbial pollution by matching microbial communities between surface water samples and potential sources.236,237 However, the differential effect of sunlight on microbial species in surface waters over time complicates this potential source tracking method.238 Recreational waters’ bacterial standards were set using epidemiology studies that relate indicator microorganism concentrations to health risk. It is presumed in these studies that an indicator-pathogen relationship exists that gives rise to an indicator-health relationship.239 Epidemiology studies have not specifically considered the effect of sunlight, although a recent study found that risks were reduced when swimming on very sunny versus less sunny days.240 If sunlight differentially affects indicator bacteria and the actual pathogens causing recreational waterborne illness, then the indicator-health relationship may be different under high sunlight versus low sunlight conditions. Thus, research that aims to understand the differential response of indicators and pathogens to sunlight is a priority for improving the management of recreational water quality.

10.2. Natural treatment systems for wastewater and stormwater

One of the oldest and most widespread applications of sunlight-mediated inactivation is the disinfection of wastewater in natural treatment systems, such as wastewater treatment ponds (WTP).241 A typical WTP system has an overall hydraulic retention time of weeks to months, and is comprised of a series of ponds: primary (anaerobic or facultative), secondary (facultative), and maturation. The conditions are particularly conducive to photoinactivation in maturation ponds, due to their shallow depths (typically 0.5 m) and high concentrations of planktonic algae, which give rise to supersaturated dissolved oxygen and elevated pH during sunlit hours when photosynthetic rates are high.2 In such ponds, the rate of inactivation of indicator bacteria and viruses by sunlight-mediated processes has been shown to be much greater than the rate of removal by dark processes (such as sedimentation of particle-associated organisms and predation).212,242

Because algal pond waters have very high attenuation in the UV range (Fig. 3), and this attenuation is strongly wavelength-dependent, longer wavelengths are relatively more important to overall photoinactivation. Thus, direct inactivation by UVB may be low or negligible, whereas exogenous processes may be very important for organisms that are susceptible. Both dissolved organic matter238,243 and particulate organic matter214,232,235 have been shown to contribute to inactivation. Because direct inactivation may be minimal, a potential concern is that viruses that are not susceptible to exogenous inactivation may be removed less efficiently.18 For E. coli, inactivation was shown to be enhanced by high dissolved oxygen and high pH in microcosm experiments with algal pond water,216 although it is unclear whether the increased inactivation is due to more efficient endogenous indirect mechanisms or that the cells become susceptible to exogenous mechanisms,235 and it is difficult to isolate the effect of these mechanisms in full-scale ponds.212,244

The open water unit-process wetland discussed in Section 8 appears to be a promising design for maximizing sunlight-mediated removal of indicator bacteria and viruses in a natural treatment system. In this pilot-scale system (20 cm water depth), the removals were estimated to be dominated by exogenous mechanisms for F+ RNA coliphage, endogenous mechanisms for E. coli and poliovirus, and Ent. faecalis was removed by both endogenous and exogenous mechanisms. Modeling suggested that wetland cells up to 40 cm may achieve even higher removals, unless deeper water causes a shift in the algal population from diatoms that accumulate in the benthic sediment layer to planktonic algae, in which case the light attenuation increase could bias model predictions.

High-rate ponds (HRPs), which employ a low-power paddle-wheel to provide mixing and circulate the water in a raceway configuration, are a proven design for achieving high levels of sunlight-mediated disinfection212 as well as improved nutrient and oxygen-demand removal. HRPs have the major advantage over conventional ponds of preventing short-circuiting,213 which can dramatically compromise overall disinfection efficiency.4 A 30 cm and 45 cm deep HRP with the same hydraulic retention time were found to provide very similar removal of E. coli.11 Operating ponds in semi-batch (draw and fill) mode is another option for improving hydraulic efficiency and increasing sunlight-mediated inactivation.245 A direct comparison of the disinfection efficiency of open water wetlands (algae
10.3. Solar disinfection of drinking water (SODIS)

Thorough reviews on the theory and practice of SODIS have been published by Reed (2004) and more recently updated by McGuigan et al. (2012). The most common approach involves filling plastic beverage bottles (e.g., 1.5 L polyethylene terephthalate (PET)) with water to be treated and exposing them to sunlight for one day; if the weather is cloudy the recommended exposure time is 2 d. The main photochemical mechanism through which bacteria are inactivated during conventional SODIS is via endogenous indirect inactivation; direct inactivation is likely minimal because PET bottles do not transmit UVB light, and exogenous inactivation is likely minimal in most waters used for drinking because few exogenous sensitizers are present. In the absence of high temperatures, inactivation of most viruses during conventional SODIS is likely to be poor, particularly in waters with low photoreactivity, given that sunlight-mediated inactivation of viruses occurs via direct and exogenous mechanisms. The use of container materials that are more transparent to sunlight, in particular UVB wavelengths, can increase photoinactivation of indicator bacteria and viruses by SODIS.

During SODIS there is the potential for the temperature of the water to increase significantly during sunlight exposure, which increases the effectiveness of photoinactivation. Synergistic temperature effects are notable above 30 °C; above ~70 °C thermal inactivation (pasteurization) becomes faster than photoinactivation. Various modifications to SODIS containers have been reported to enhance temperature effects, including painting the bottom of the container black, placing the bottles on a black surface, placing the bottles in a solar box oven, and concentrating the sunlight through the use of mirrors, in particular Compound Parabolic Collectors. Mechanistic modeling has provided valuable insights into the effects of key parameters, such as clear versus cloudy skies, turbidity, and container material on the inactivation of E. coli in SODIS bottles and parabolic reactors.

Considerable research efforts have been directed toward the development of practical approaches for enhancing sunlight-driven disinfection. One of the simplest such approaches involves the addition of H₂O₂ into solution during solar irradiation to accelerate inactivation of certain viruses, bacteria, and fungi relative to conventional SODIS processes. The observed benefits of H₂O₂ addition can be attributed to its participation in endogenous or exogenous photo-Fenton processes involving Fe(II) or Fe(III) associated with biomolecules present in the target microbial agent, or naturally-occurring Fe(III) associated with Fe(hydr)oxide complexes, Fe-organic ligand complexes (formed through interactions with acidic groups in NOM), and/or solid Fe oxides present in the water to be treated. Photo-Fenton processes are in each case expected to lead to production of such oxidants as 'OH or Fe(IV), depending on solution pH. Accordingly, inactivation appears to be particularly effective when waters dosed with H₂O₂ already also contain significant iron in the bulk solution, or are amended with copper in the presence of ascorbic acid (likely due to the ability of copper to participate in Fenton-like reactions).

For waters that are not naturally enriched in Fe, the effectiveness of SODIS processes can also be improved by addition of Fe (with or without addition of H₂O₂) to drive exogenous photo-Fenton processes. Fe addition may also yield the benefit of enhanced endogenous photo-Fenton chemistry within bacterial cells due to siderophore-mediated intracellular accumulation of added Fe. Amending waters with an organic acid such as citrate can serve to yield further improvements in oxidant yields, by improving Fe solubility through formation of stable metal–ligand complexes, which may themselves participate in ligand-to-metal charge transfer upon solar irradiation. The use of innocuous, naturally-occurring, and widely-accessible reagents (Fe, H₂O₂, and/or organic acids) is an added benefit of this approach to SODIS enhancement. Applications of photo-Fenton processes to enhance microbial inactivation kinetics have been successfully demonstrated in a variety of natural water matrices under field conditions at scales ranging from 1 L water bottles up to 50 L.

An alternative approach to SODIS enhancement is the solar “photoactivation” of free available chlorine (HOCl/OCl⁻) to yield 'OH, RHS, and O₃. The production of 'OH and O₃ during exposure of chlorine-containing solutions to natural sunlight under conditions typical of SODIS processes (e.g., pH 8, T = 33 °C) can accelerate inactivation of highly chlorine-resistant B. subtilis endospores and Cryptosporidium parvum oocysts by more than 200% compared to either chlorine-containing dark controls or light controls in the absence of chlorine. This approach could present interesting opportunities for improving the effectiveness of either chlorine-based disinfection or SODIS. It is important to note that photoactivation of free available chlorine by UVB and UVA wavelengths may lead to elevated levels of disinfection byproducts. Thus, the use of this approach would require careful selection of treatment conditions to minimize risks of byproduct exposure while maximizing inactivation of recalcitrant pathogens.

The effectiveness of solar disinfection may also be enhanced by the addition of exogenous photosensitizer compounds to increase PPRI, although the practicality of this approach has not been demonstrated. Organic sensitizers include flavins and psoralens (furocoumarins). Riboflavin has been shown to accelerate the rates with which various viral, bacterial, and protozoan pathogens can be inactivated during exposure to...
simulated sunlight, presumably due to interactions of the pathogens with either $\text{O}_2^-$ or the excited triplet-state of the riboflavin itself. Significant enhancements of viral and bacterial inactivation rates have also been observed in UVA or natural sunlight-irradiated solutions dosed with pure synthetic 5-methoxy-psoralen (MOP), as well as lime juice, likely due to attack of DNA in the target organisms by photoexcited MOP and psoralens in the lime fruit (which is higher than in lemons).

Alternatively, heterogeneous photocatalysts have been used for production of PPRI, such as TiO$_2$ mineral phases. Undoped TiO$_2$ phases (e.g., anatase, Degussa P25) may be excited by absorption of UVA light, resulting in the coupled reduction of $\text{O}_2$ and oxidation of H$_2$O at the photocatalyst surface, in turn leading to the formation of such ROS as $\text{O}_2^-$, H$_2$O$_2$, and $\text{HO}^\bullet$. Certain doped TiO$_2$ phases are also known to exhibit similar photoactivity over the visible region of the solar spectrum. TiO$_2$ photocatalysts are generally used either in suspension or in coatings applied to the inner surfaces of plastic or glass reactors. The use of these materials during SODIS processes has been shown to yield improved inactivation of a wide variety of microorganisms and has been demonstrated to function under both small- and large-scale conditions. A number of recent studies have also evaluated the use of C$_{60}$ fullerences functionalized with hydrophilic surface groups (e.g., -OH, -NH$_2$, etc.) as photocatalysts in solar disinfection. Such materials are reported to generate $\text{O}_2^-$ and/or $\text{OH}^\bullet$ upon photoexcitation with UVA radiation, and have been shown to accelerate inactivation of various viruses, bacteria, and fungi in aqueous solution. Other nanomaterial-based catalysts are being explored, such as vertically aligned MoS$_2$ nanofilms. However, the practicality of applying such approaches under field conditions may ultimately be limited, as heterogeneous photocatalysis approaches require the use of either suspensions of photocatalyst – which must be removed prior to water consumption, or coated reactor surfaces – which suffer from mass transfer constraints on exposure of organisms to ROS generated at the catalyst–water interface. Despite decades of intensive investigation in the laboratory, practical field designs employing photocatalysts have not emerged.

### 10.4 Solar radiation and microbial ecology

Sunlight strongly influences the bacterial and viral assemblages found in surface waters. Bacteria can be inhibited or stimulated by sunlight exposure. Research focused on UV radiation (UVA and UVB) shows an inhibitory effect on cellular activities due to the mechanisms described in this review article. The effect of UV radiation, however, varies by bacterial group. Differences in sensitivity to UV radiation between bacterial groups result in changes in bacterial community composition depending on UV dose. Researchers have also found that sunlight stimulates bacterial growth by the production of bioavailable organic matter and photorepair of sunlight induced damage. Sunlight acts as an energy source, both directly for photosynthetic organisms and indirectly through the photo-Transformation of organic matter into a biologically labile form. The bioavailable organic matter supports growth of certain bacterial groups and repair after sunlight exposure, leading to changes in bacterial community composition. Specific wavelengths of sunlight can stimulate photorepair or photoreactivation. Sunlight also influences bacterial community composition by controlling bacteriophage populations in surface waters via destruction of viral particles or reduction of viral infectivity during sunlight exposure. Thus, the overall effect of sunlight on bacterial communities in surface waters is the sum of detrimental and beneficial processes. Advances in next-generation sequencing (NGS) technologies are making it cheaper, easier and faster to investigate bacterial communities in surface waters. Understanding the effect of sunlight on bacterial communities will be especially important when analyzing experimental findings and building models describing photo-induced chemical and biological processes affecting bacterial communities in aquatic environments.

### 11. Research priorities

Based on our critical review of the current literature on sunlight inactivation of microorganisms, we have identified a number of research areas to prioritize for future work:

#### 11.1 UVB measurements and predictions

More accurate approaches for quantifying the UVB portion of natural and simulated sunlight are needed. Improved models for radiative transfer of sunlight into surface waters should be applied to model sunlight inactivation.

#### 11.2 Reactivity of microbial building blocks

To enable truly mechanistic descriptions of endogenous and exogenous inactivation, the reactions of biomolecules exposed to solar radiation and PPRI need to be more fully characterized. In addition, an understanding of how reactivity is influenced by the higher-order structure of microorganisms (compared to biomolecules) is needed.

#### 11.3 Expansion of microorganisms studied

To date, most of the research on sunlight inactivation has been on a limited number of microorganisms. Our understanding needs to be expanded to a broader suite of health-relevant viruses, bacteria, and protozoa with varied structures and biology, including how the inactivation rates of actual pathogens compare to commonly used indicator organisms for the range of sunlight and water quality conditions. As one example, very little information is available on endogenous and exogenous reaction rates of enveloped viruses.

#### 11.4 Endogenous inactivation by solar wavelengths

Additional studies are needed on the mechanisms of endogenous inactivation induced by solar wavelengths. Rate constants for inactivation by sunlight, rather than UVC, should be measured for a wider range of microorganisms. Furthermore,
action spectra should be obtained for different microorganisms in the solar spectrum range. The development of methods for wavelength-specific actinometry would be a useful contribution to facilitate comparison of experiments conducted with light sources with different irradiance spectra and waters with different absorption spectra.

11.5 PPRI responsible for bacterial inactivation

Whereas the PPRI involved in virus inactivation have been largely characterized, more research on the key exogenous ROS for bacteria is necessary.

11.6 Beyond monodispersed microorganisms

Future efforts should seek to understand how sensitizer characteristics and association with microorganisms impacts inactivation rates and mechanisms. Also needed is a better understanding of how aggregation of the microorganisms or sorption on surfaces affects inactivation kinetics in the field.

11.7 Particulate organic matter (POM)

To date, most of the focus on the role of organic matter in sunlight inactivation has been on DOM. Wastewater effluent and wetland-derived organic matter are of particular interest as sensitizers, and typically contain high concentrations of particulate organic matter (POM). Association between microorganisms and POM is likely. A further complication is that particulate organic matter scatters radiation, as well as absorbing it, affecting spectral irradiance in waters. Future research could compare photoinactivation in filtered versus unfiltered waters.

11.8 Laboratory microorganism effects

More research is needed to identify the factors that alter the susceptibility of laboratory cultures to sunlight, and that underlie differences between laboratory-grown and ‘native’ (environmentally-adapted) bacteria to sunlight. Also, more work is needed to explain why growth phase/physiology affects inactivation of bacterial species. This understanding is important for informing how to conduct experiments with laboratory organisms that can be accurately extrapolated to different field conditions.

11.9 Influence of inorganic constituents

Research is needed to characterize the influence of inorganic constituents of waters, notably salinity, halides, iron, dissolved oxygen, and pH, on exogenous inactivation under real-world conditions.

11.10 Omics tools

High throughput sequencing, metagenomics, proteomics, transcriptomics, and other omic tools can be further applied to understand pathways of damage that lead to inactivation by sunlight. Also, the ability to characterize changes in the composition of entire microbial communities should be applied to expand the focus beyond readily culturable pathogens and indicator organisms.

11.11 Interactions with other inactivation processes

Sunlight can influence other inactivation processes by, for example, increasing the water temperature, enabling photosynthesis, altering the activity of grazers and other predators. These effects need to be incorporated into models to accurately predict overall inactivation.

11.12 Validation and improvement of models

More work is needed that compares the results from predictive, mechanistic models with actual measurements of inactivation under a range of conditions in natural and engineered systems. A comparison of mechanistic and statistical modeling approaches would also be insightful. This work will provide insight into the level of detail that is required to accurately predict inactivation, given the variability inherent to real-world systems.

11.13 Applications of sunlight inactivation

The improving mechanistic understanding of sunlight inactivation should be exploited to further develop creative and practical disinfection strategies for drinking water, wastewater, and stormwater and better management of natural waters.

Conflicts of interest

There are no conflicts of interest to declare.

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