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Comparison of dark- and lightrepair tendencies between low and medium pressure UV lamps

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Abstract
Ultraviolet (UV) light has become widely accepted as an alternative to chlorination for the disinfection of potable water, process water and wastewater. To avoid the failure of UV disinfection systems due to the recovery of micro-organisms, certain additional wavelengths in the UV area are emitted by medium pressure UV lamps. To reduce the chance of microbial recovery after ultraviolet irradiation, damage must be inflicted in as many areas of the micro-organism as possible. The effective destruction of micro-organisms by polychromatic medium pressure UV lamps is due to the lamps' exceptionally high UV energy output at specific wavelengths across a broad section of the UV spectrum. The combination of properties results in a number of lethal effects in both small and large micro-organisms. Important biological molecules inside the microbial cell, in addition to DNA, are also likely to be damaged, helping to prevent the recovery of the irradiated micro-organisms. The absorption line spectra of absorbing nucleotide bases, DNA and other biological molecules such as proteins and enzymes show how effective UV light is in stopping the metabolism. Recent findings on the biological effects of short UV wavelengths on *Bacillus subtilis, Cryptosporidium parvum and Escherichia coli* confirm the destructive effects of wavelengths both shorter and longer wavelenghts than 254 nm. Practical comparisons with conventional low pressure UV lamps, at equal UV doses, show killing rates from medium pressure UV lamps to be more effective, without the formation of disinfection-by-products (DBPs). The results from a number of studies have shown that micro-organisms such as *E. Coli* underwent photorepair following exposure to the low pressure UV lamps (254nm), but no repair was detected following exposure to medium pressure lamps (200-400nm).

Keywords
low pressure UV lamps, medium pressure UV lamps, E. coli, Cryptosporidium parvum, biological molecules, DNA, enzymes, proteins, recovery, repair, UV disinfection

INTRODUCTION

Since the end of the nineteenth century, monochromatic UV light from low pressure (LP) mercury (Hg) vapour lamps (254 nm) have been known to be effective in killing micro-organisms. It is also known that enzymatic repair processes enable micro-organisms to recover from damage caused by low pressure Hg lamps.

By the end of the twenteeth century medium pressure (MP) UV lamps were introduced for disinfection purposes. Medium pressure UV lamps produce a wider range of UV wavelengths (200 - 400 nm) than their low pressure counterparts, allowing them to affect many other biomolecules in addition to DNA. Today, medium pressure UV lamps are used for a wide range of disinfection applications including potable water, waste water and industrial process water. Nowadays MP lamps combine a higher UV efficiency with the multiple germicidal effects of the wide-band output, to create multiple effects inside micro-organisms.

UV DISINFECTION

One of the earliest reports outlining the germicidal effects of UV was by Downes and Blount (1877). They described the lethal effects of sunlight on a mixed microbiological population and assigned the cause of these effects to UV radiation.

Earlier interest in the application of UV for disinfection was originally centred on potable water. Today, many different liquids are disinfected by UV light, including waste water, process water, ballast water, irrigation water, drainwater and ultrapure water.

UV light has proved to be a very “clean” alternative to those disinfection methods which use chemical agents such as chlorine, chlorine dioxide or ozone. Unlike these methods, UV does not produce any disinfection-by-products (DBPs). Even the formation of trihalomethanes (THMs), AOC/DOC, which is the result of pre- and or post-chlorination, will be reduced if chlorine is replaced by UV treatment. In addition, UV light normally has no effect on the taste, smell or colour of the liquid.

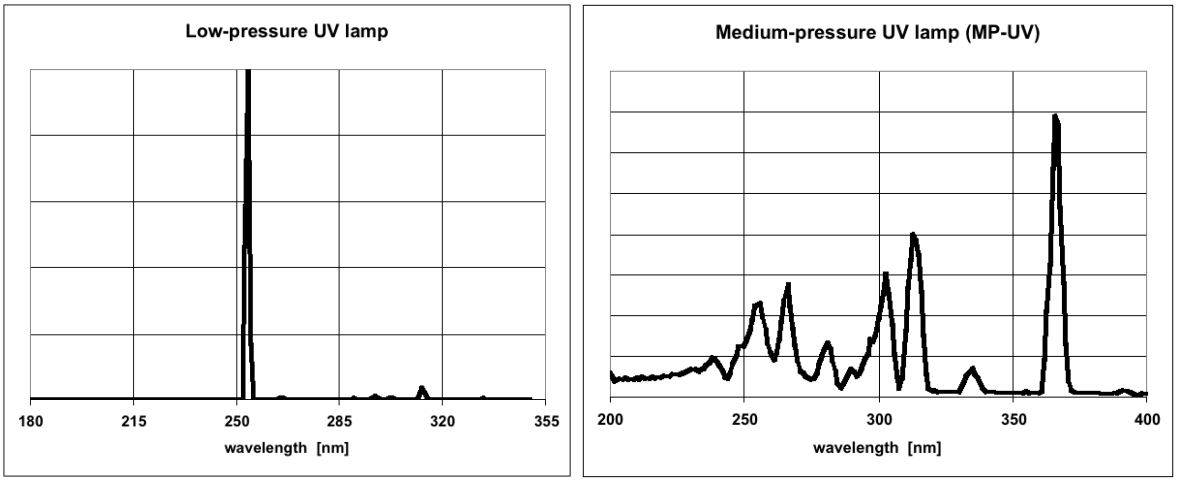
Emission spectrum of UV lamps

As UV emission spectrum and UV intensity both play an important role in killing micro-organisms, medium pressure lamps were developed to combine these elements. The improved performance of these type of MP lamps is achieved by:

- a broad emission spectrum in the UV area (wavelenghts, nm)
- high UV intensity (fluence rate, mW/cm2)
- improved UV efficiency.

A combination of above cause multiple germicidal effects inside micro-organisms.

The emission spectra and intensities of low and medium pressure UV lamps are significantly different. Low pressure lamps emit one single wavelenght (254nm), whereas medium pressure lamps emit a broad band of wavelengths all over the UV areas.



Emission spectrum of a low pressure and medium pressure UV lamp

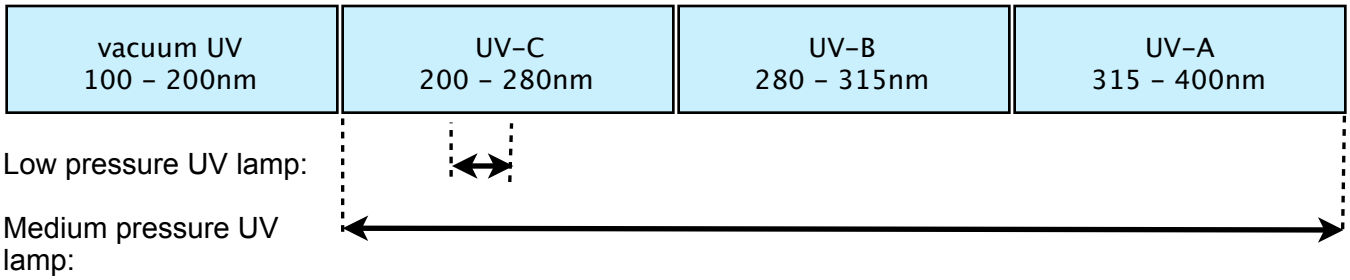
Electromagnetic spectrum

In the electromagnetic spectrum, the wavelengths between 100 and 400 nm are known as the ultraviolet (UV) region. This region can be roughly divided into three areas (Jagger, 1967):

- extreme, or vacuum, UV : 100-190 nm
- far-UV (UV-C and UV-B) : 190-300 nm
- near-UV (UV-A) : 300-400 nm

Because water and air absorb all wavelengths below 190 nm (extreme or vacuum UV), only the wavelengths between 190 and 380 nm (far-UV and near-UV) can be used for biological effect (Harm, 1980).

The International Commission of Light (CIE), subdivides the UV region into four areas: vacuum UV (100 - 200 nm), UV-C (200 - 280 nm), UV-B (280 - 315 nm) and UV-A (315 - 400 nm)



ABSORPTION OF UV LIGHT

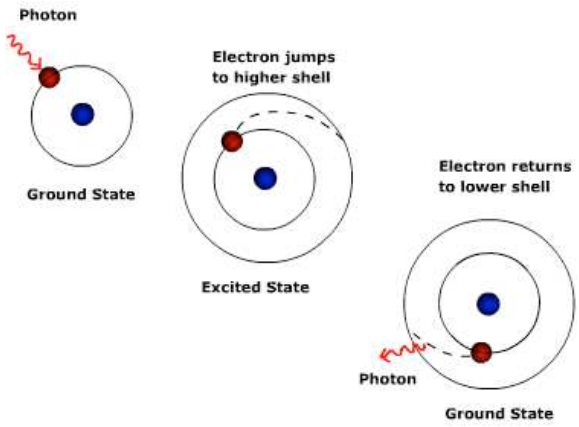
UV lamps emit light in the UV region at the following wavelengths:

- monochromatic low pressure UV lamps : (185) 254 nm
- polychromatic medium pressure UV lamps : (185) 200 - 400 nm

Photons of light are produced at each specific wavelength. Each photon has its own energy content, which depends upon the specific wavelength. When the photon is absorbed by a material, such as a biomolecule within a micro-organism, electrons in the atoms or molecules making up the material are excited.

The velocity of the photons is equal to the velocity of visible light (3.10^8 m/s), while the time needed for their absorption by atoms or molecules is about 10^{-15} seconds. The absorption of specific wavelengths is shown in a so-called 'absorption line spectrum'.

The larger and more complex the molecule (for example DNA or protein), the wider the range of wavelengths absorbed and therefore the wider the absorption line spectrum. Molecules – like atoms – are held together by bonds in the form of electrons shared between them. When the energy absorbed from the photons reaches a threshold known as ionisation energy (E_{ion}) , a bond can be broken, splitting the molecule ("reactant") into two or more parts ("products"). This photochemical reaction is called 'dissociation'. Obviously only the photons or radiation energy absorbed by the material can be photochemically effective (Draper-Grotthus principle). The dissociation or ionisation energies for several organic bonds are given in various literature (Meltzer, 1987).

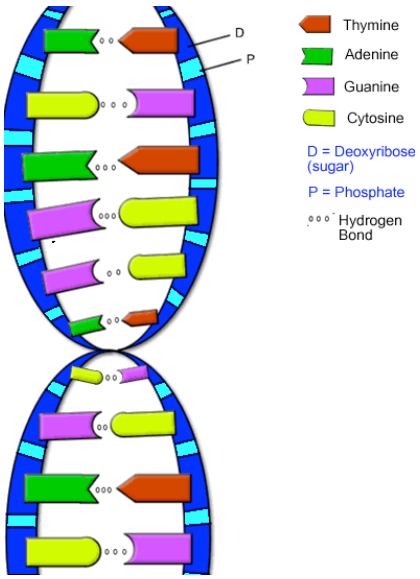


Photon attack to an atom. As sufficient photon energy, the right wavelength, hits the atom the electron jumps to a higher shell to cause dissociation.

Absorption of UV light by the major biomolecule, DNA

Harm (1980) considers that for UV radiation to achieve biological effects, the wavelength range 190 - 380 nm (far-UV and near-UV) is essential. The majority of biological effects, especially in very small micro-organisms, are due in the first place to photochemical reactions in the DNA.

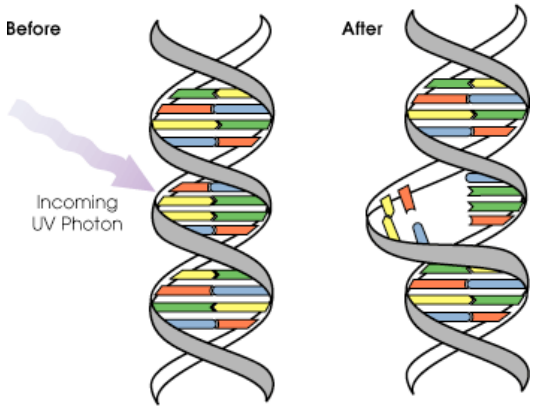
According to Von Sonntag (1986) the UV absorption curve shows maximum absorption at 200 nm. There is also an absorption peak at around 260-265 nm. The maximum absorption therefore does not occur at 254 nm, the wavelength often assumed to be the most effective for killing micro-organisms, this is a misunderstanding.



Linden (2005) states that the absorption curves of micro-organisms are different per type of micro-organism. The maximum absorption by *Cryptosporidium*, MS2, Herpes simples virus is at abt. 265 nm but they differ very strong in absorption below 240 nm.

In DNA the 'backbone' molecules of sugar (ribose) and phosphate do not absorb significantly above 210 nm. So, the absorption by the biomolecules DNA and RNA, at wavelengths above 200 nm, is due to absorption by the nucleotide bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). The absorption spectra of these nucleotide bases are found in the UV-C and UV-B regions (Jagger, 1967).

Absorption of UV light by the nucleotide bases



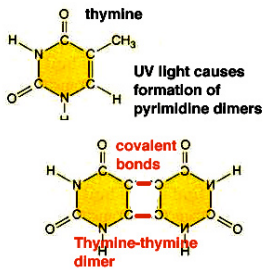
The absorption of photons by the nucleotide bases results in the formation of (photo-)products, the most common of which thymine dimers, formed when two adjacent thymine bases become covalently joined by cyclobutane (Adams, 1986). About 90% of the UV damages is dimer based (Kittler, 1976) When DNA is damaged in this way, the metabolism is stopped (Linden, 2005) it cannot replicate, so the bacterial cell is unable to multiply and is effectively dead (Lehninger, 1976).

Formation of dimers in DNA after exposure to UV light (photons)

Type of UV damages in DNA

Primarily 6 types of damages of pyrimidines (Linden, 2005)

- single and double strand breakage (requires high fluence)
- DNA-DNA cross-links (requires high fluence)
- Protein-DNA cross links (important for e.g. *Micrococcus*)
- Pyrimidine hydrates
- Pyrimidine (6-4) pyrimidine photoproducts
- Pyrimidine dimers (most common, abt. 90% of UV damages)



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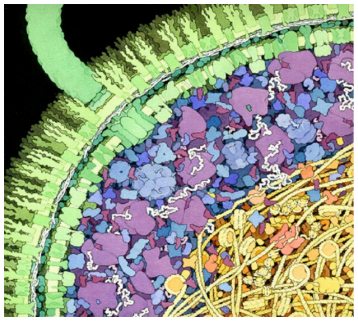
Absorption of UV light by proteins and enzymes

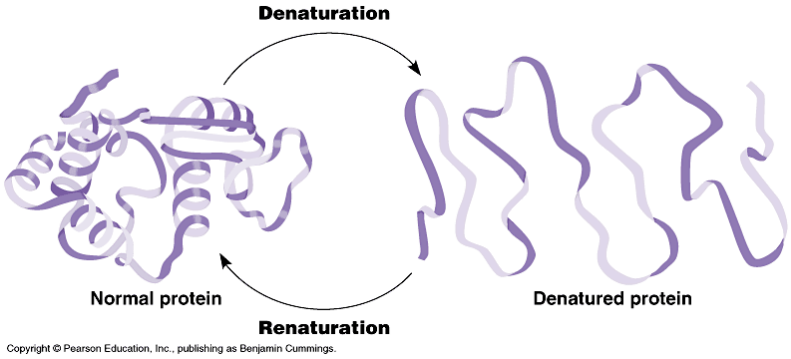
In addition to DNA and RNA, photochemical reactions in proteins, enzymes and other biomolecules are also important, particularly in the case of larger micro-organisms such as fungi, protozoa and algae, which have dimensions of tens or hundreds of microns. UV photons may be unable to penetrate far beneath the surfaces of these organisms, leaving the critical component, the DNA, scarcely affected.
Jagger (1967) suggests that it is probably no accident that, precisely at the point where solar radiation falls off below 300 nm, proteins and nucleic acids (DNA/RNA) – molecules which are of prime importance to life – begin to absorb and be damaged by UV radiation.



The absorption spectra of proteins show, in general, a maximum peak at around 280 nm. The peptide bond (-CONH-) in proteins displays some double bond characteristics and is relatively weak at absorbing UV, the only significant absorption occurring below 240 nm. However, as there is a peptide bond for every amino acid residue in a protein, UV absorption below 240 nm is nonetheless significant.

This *E.coli* cell, the proteins (shown in blue) crowd around ribosomes (purple). These regions have a high concentration of protein, typically greater than 30 percent,

Bensel (1977) claims that absorption of UV light by the amino acid cysteine makes proteins and enzymes unstable. If the dissociation energy for the disulfide (S-S) bond between cysteine molecules is reached, dissociation of the tertiary structure of the amino acid takes place, resulting in denaturation of the biomolecule and a loss of biological activity.



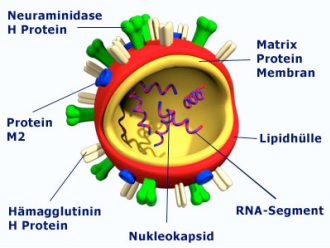
If, for example, denaturation of the enzyme polymerase takes place, the micro-organism loses its ability to multiply; while in the case of the enzyme photolyase, the micro-organism loses its ability to repair UV damages. Because of the high concentration of proteins in micro-organisms (about 50% of the dry weight), absorption of UV light can influence their role in nucleic acid synthesis and chromosome structure (Ingraham, 1994).

Absorption of UV light by other biomolecules

In addition to DNA, proteins and enzymes, other biomolecules with unsaturated bonds may be sensitive to the deactivation effects of UV radiation. Important examples include coenzymes, hormones and electron carriers.

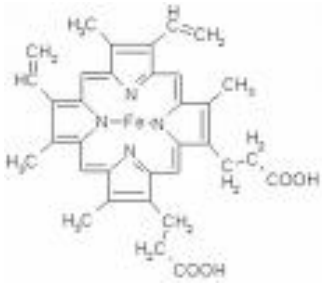
Biomolecules absorbing far-UV (below 300 nm)

Jagger (1967) claims that, generally, the most important UV absorbers are those with conjugated bonds (alternating single and double bonds). Structures containing conjugated rings are usually good absorbers of far-UV light (below 300 nm). Absorption of far-UV by aromatic acids may result in decarboxylation, deamination or breaking of ring structure.

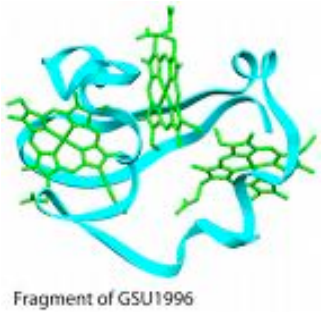


Some far-UV (< 300 nm) absorbing biomolecules are:
- six-membered carbon rings – benzene, toluene, phenol
- rings containing nitrogen – pyridine, imidazole, pyrimidine, cytosine, thymine, uracil
- double rings – naphtalene, purine, adenine, guanine
- triple rings – anthracene, riboflavin
- quadruple rings – steroids, porphyrins
- amino acids – tryptophan, tyrosine, phenylalanine, cystine, cysteine
- other biomolecules – nicotinamide adenine dinucleotide (NAD)

Biomolecules absorbing near-UV (above 300 nm)
Biomolecules absorbing UV above 300 nm include:
- triple rings – riboflavin
- four rings – porphyrins, steroids
- long-chain conjugated molecules – carotenoids
- other biomolecules – NADH2 (reduced form of NAD), isoprenoid quinones, vitamine K, phterins, vitamine A, flavins, cytochrome.



Cytochromes are, in general, membrane-bound hemoproteins that contain heme groups and carry out electron transport. They are found either as monomeric proteins (e.g., cytochrome c) or as subunits of bigger enzymatic complexes that catalyze redox reactions. They are found in the mitochondrial inner membrane and endoplasmic reticulum of eukaryotes, in the chloroplasts of plants, in photosynthetic microorganisms, and in bacteria.



GERMICIDAL EFFECTS OF VISIBLE LIGHT (above 400 nm)
The ability of wavelengths in the solar spectrum above 300 nm to kill small bacteria (< 10 microns) has been known for a long time (Ward, 1893). However, the affected biological molecules, or chromophores, have yet to be identified. Since proteins and nucleic acids show little or no absorption above 340 nm there must be other chromophores with sufficient absorbancy to result in the death of small micro-organisms.

UV-A 315 – 400nm	Visible light 400 – 700 nm

In 1952 it was discovered that wavelengths above 300 nm and in the adjacent visible spectrum destroy the capacity of micro-organisms to multiply. Radiation between 350 - 490 nm has been shown (Bruce, 1958), for example, to cause a leakage of ions.

It has often been assumed that the killing of micro-organisms above 300 nm occurs because the damage caused by these wavelengths is less effectively repaired than at 254 nm (UV-C). This suggests that, in addition to the formation of pyrimidine dimers, exposure to sunlight can result in the sort of poorly repairable – or even non-repairable – lethal damage which is rarely caused by low pressure lamps producing UV at 254 nm. Lethal damage of this sort, however, is well within the capacity of third generation, enhanced medium pressure lamps.

It has been shown experimentally that, by filtering out the shorter wavelengths below 360 nm, non-repairable, lethal damage can be inflicted. The germicidal effects of solar radiation is almost entirely due to the formation of oxygen radicals in the cytoplasm (Tortora, 1995).

Experiments with solar light indicate that effects on biomolecules other than DNA and proteins can also cause lethal damage to microbiological cells.

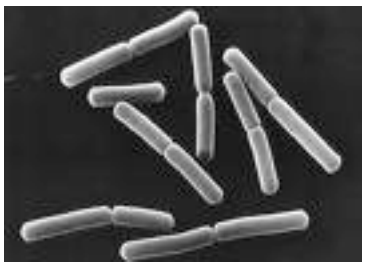
Wavelengths other than UV-C may become even more important in killing larger, more UV-resistant micro-organisms (e.g. *Cryptosporidium parvum* oocysts), as large cells tend to be less transparent to UV-C. The penetration of larger cells by wavelengths below 295 nm is a major problem which increases the importance of other wavelengths. It is assumed that absorption by organic molecules in the outer cell wall is a major contributor to the killing of larger micro-organisms.

Oguma (2002) observed that wavelengths between 300 and 580 nm play an important role in reducing the subsequent recovery of colony-forming ability by inducing damage other than damage to the pyrimidine dimers in the DNA. Therefore, it was found that inactivating light at a broad range of wavelengths effectively reduced the subsequent photoreactivation, which is an advantage that medium pressure lamps have over low-pressure UV lamps.

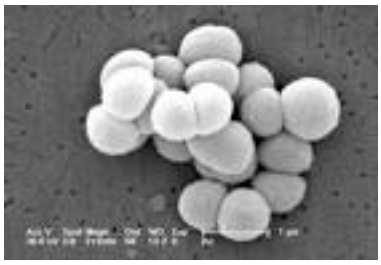
RECOVERY FROM UV DAMAGE

The need to recover from or repair UV damage is common to all organisms. Known as reactivation, the process can take place in both dark and light conditions and is consequently described as either dark repair or photoreactivation (Schlegel, 1992). The method of reactivation varies significantly according to the level of biological organisation and the kind of UV damage inflicted by UV.
The repair mechanism (EPA, 1986) in micro-organisms is not universal, and there is no clearly defined set of characteristics to determine which species have the ability to repair themselves and which do not.

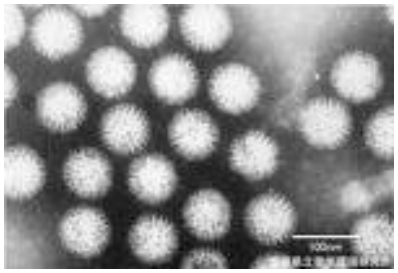
Micro-organisms shown to be unable to repair themselves include:
Heamophilus influenzae, Diplococcus pneumoniae, Bacillus subtilis, Micrococcus radiodurans, Viruses, Staphylococcus aureus phage A994, Rotavirus SA-11, Poliovirus, MS2 phage



Bacillus subtilis



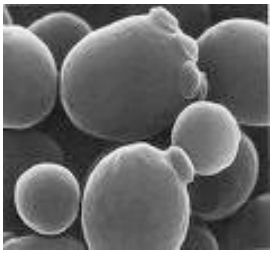
Micrococcus



Rotavirus

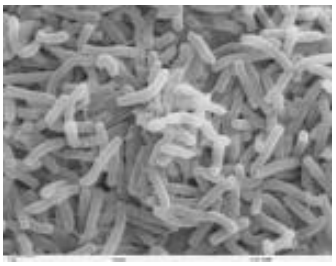
Organisms shown to be capable of photoreactivation include:
Streptomyces spp., Escherichia coli, Saccharomyces spp., Aerobacter spp., Micrococcus spp., Erwinia spp., Proteus spp., Penicillium spp., Neurospora spp., Enterobacter cloacae, Citrobacter freundii, Enterocolitica faecium, Klebsiella pneumoniae, Mycobacterium smegmatis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhimurium, Seratia marcescena, Vibrio cholerea, Yersinia enterocolitica, Cryptosporidium parvum.

Klebsiella pneumoniae



Saccharomyces cerevisiae

Neurospora spp.



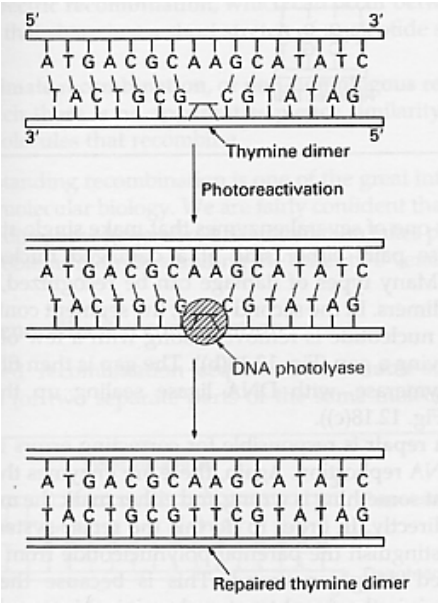
Aerobacter

In the microbial cell the most vulnerable component is the genetic material contained in DNA and RNA. This is due not only to its uniqueness, but also because of the molecule's complex structure and huge size. It is hardly surprising, therefore, that all known types of molecular repair processes have evolved to act upon the macromolecular nucleic acids, particularly the DNA. The effects of monochromatic UV light on the DNA molecule and the enzymatic repair processes are well described in literature (Ingraham, 1994).

Photoreactivation (EPA, 1986) is a phenomenon which can influence the performance and design of UV treatment systems – to prevent enzymatic repair, UV irradiation must damage a wide variety of molecules, including the DNA.

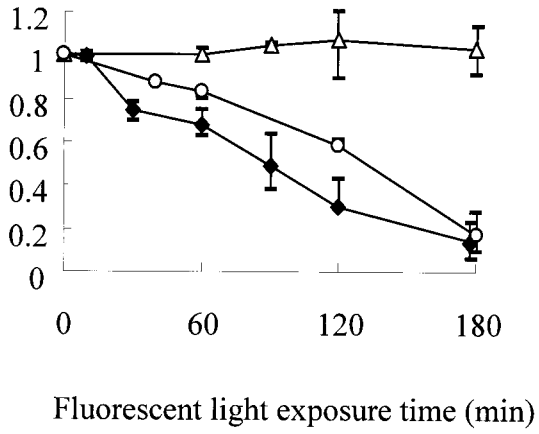
Exposure to light will result in repair of the damaged microbiological cells.

Damages caused by low pressure UV lamps, which produce monochromatic 254 nm UV light, can be repaired relatively easily using active enzymes like photolyase (Ingraham, 1995).



The enhanced recovery of UV irradiated micro-organisms, following exposure to visible light, is known to be due to the enzyme photolyase. This enzyme binds the pyrimidine dimers and uses the energy of visible light to split the dimers apart and repair the DNA string.

Oguma (2002) reported that wavelengths between 220 and 300 nm reduced the subsequent photorepair of endonuclease sensitivity site (ESS), by causing a disorder in endogenous photolyase.



ESS (quantity of UV damages) were determined after exposure to fluorescent light after exposure to LP (254nm), MPF filtered (300 - 580nm) and MP (220 - 580nm) UV lamps. The figure shows what happens with the quantity of UV damages after 3 hours of exposure to visible light. Conclusion: the MP UV lamp (triangle (top)) shows no reduction of the quantity of UV damages, meaning there is no photorepair. Both LP and MPF show reduction in UV damages after 3 hours exposure to light, meaning that UV damages caused by LP and MPF are repaired withing hours. (Oguma, 2002).

However, it has been demonstrated that UV damage caused by high intensity polychromatic lamps, such as enhanced medium pressure lamps, cannot be repaired (Oguma 2001, Oguma 2002, Zimmer 2002). The repair processes in biomolecules other than DNA have not been identified.

Differences between low and high fluence rate (UV intensities)
Micro-organisms have the capacity to recover using active enzymes such as photolyase, endonuclease, polymerase and ligase (Dressler, 1986).

Before the reactivation process starts and takes place, exposure times varying from a few minutes to several hours are needed, depending on the type of organism, with light between the wavelengths 310 to 480 nm. It is likely that the repair systems in micro-organisms are so efficient that, below the lethal UV dose, the potentially lethal effects of virtually all UV damage are avoided. As already mentioned, all known types of molecular repair process act upon the DNA because it is the most important molecule within micro-organisms. About 90% of the pyrimidine lesions (thymine dimers) caused by 254 nm UV irradiation can be repaired by active enzymes.

In studies of survival kinetics using biological materials, fluence rate – that is, the amount of UV energy (mW/cm2) absorbed – is often considered to be less important for disinfection than the UV fluence (UV dose) – the total number of photons (mJ/cm2) absorbed. The low fluence rate of low pressure lamps should therefore produce the same UV dose as the high fluence rate of medium pressure lamps, as long as the exposure time is increased by the appropriate factor. This is correct from a photochemical and UV dosecalculation point of view. The extent and nature of the damage caused, should depend only on the UV fluence (UV dose), which is a product of fluence rate and exposure time as stated by the Bunsen-Roscoe reciprocity law:

UV fluence (mWs/cm²) = UV fluence rate (mW/cm²) x time (sec) (1)

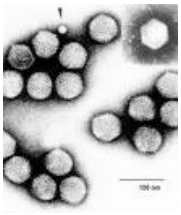
However, since the effectiveness of UV disinfection depends not only on photochemical reactions but also on biological processes (namely repair), it has been concluded (Harm, 1980) that the Bunsen-Roscoe law may not actually apply here. UV fluence rate (UV intensity) seems to play a very and/or more important role.

Harm (1980) shows that the most lethal effect can be achieved by combining high fluence rates with short exposure times. Harm concludes that at equal UV fluence (UV dose), significantly greater reductions in *Escherichia coli* are achieved using short-term, high fluence rate UV irradiation ('single exposure') rather than long-term, low fluence rate irradiation ('sector irradiation') respectively produced by MP and LP UV lamps.
Wayne (1999) states that studies of photochemical reactions involve multi-photon processes, in which a single particle absorbs more than one photon. Such absorption only occurs at sufficiently high fluence rates.

PRACTICAL EXPERIMENTS

Differences in inactivation and UV doses using low and medium pressure UV lamps

Adenovirus



Linden c.s. (2009) reported that bench-scale studies indicate that full-scale medium pressure UV systems may be capable of achieving significant inactivation of *Adenovirus* at lower UV doses than using low pressure lampssystems. The study demonstrated that medium pressure UV lamps can achieve >4-log reduction at economically viable UV doses lower than 100 mJ/cm2, where as low pressure requires for a 4-log reduction 186 mJ/cm2 acc to the reports of USEPA.

Escherichia coli

Results of collimated beam studies (Zimmer, 2002) show that *E.coli* underwent photorepair following exposure to low pressure UV lamps, but no repair was detectable following exposure to medium pressure lamps. The studies clearly indicate differences in repair potential under laboratory conditions between low pressure and medium pressure UV lamps.

Oguma (2001) investigated photoreactivation in *E.coli* and *Cryptosporidium parvum* using low pressure and enhanced medium pressure lamps. UV-induced pyrimidine dimers in DNA were continuously repaired using low pressure lamps, while none of these dimers were repaired when using enhanced medium pressure UV lamps.

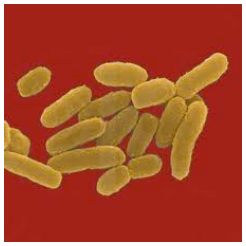
Cryptosporidium parvum

Linden (2001) describes the results of exposing *Cryptosporidium parvum* oocysts to distinct wavelengths of collimated beam ultraviolet radiation across the germicidal wavelengths 210 to 295 nm, using a medium pressure mercury vapour lamp. The highest reduction rates were reached at a wavelength of 271 nm, which was approximately 15% more effective than at 254 nm. Even 263 nm was shown to be more effective at inactivating *Cryptosporidium parvum* oocysts than 254 nm.

***Bacillus subtilis* spores**

Waites (1988) reports that the greatest kill of *Bacillus subtilis* is achieved using UV radiation around 270 nm. This suggests that the UV is not acting directly on the DNA of *Bacillus subtilis* spores, but rather on the dipicolinic acid (DPA), whose absorption peak is close to 270 nm. The absorption spectrum of DPA within spores of *Bacillus subtilis* shows that wavelengths at 270 nm are about 40% more effective compared to those at 254 nm.

Yersinia enterolytica



Claus (2005) reports that, using LP lamps, without exposure to light a fluence of 6,9 mWs/cm2 was sufficient for a 4 log (factor 10000) reduction for *E.coli* and 5,9 mWs/cm2 for *Y. enterolytica*. Whilst to get a 4 log reduction with exposure to light (photoreactivation can take place) a fluence of respectively 18,2 and 18,0 mWs/cm2 is required. The conclusion from this report is that abt. 3 times more UV fluence is required to prevent photoreactivation using low pressure UV lamps. The thesis from this report could be that a 3-fold less fluence and thus energy is required using MP lamps compared to LP lamps.

CONCLUSIONS

Medium pressure UV lamps combine a broad emission spectrum with high fluence rate, which appear to cause quick 'multi-photon photochemical damage' inside the microbiological cell.
In addition to far-UV (<300 nm), near-UV (>300 nm) and visible wavelengths may also be effective in killing micro-organisms, particularly the larger ones.
Most micro-organisms can repair UV-damaged DNA with enzymes in light or dark conditions. Repair processes of UV-damaged biomolecules other than DNA, however, have yet to be discovered.
The Bunsen-Roscoe reciprocity law may not be applicable for the destruction of micro-organisms, as biological processes also play an important role.
Wavelengths of polychromatic medium pressure UV lamps are more effective at killing larger and more UV-resistant micro-organisms than low pressure monochromatic UV lamps.
For the inactivation of *Cryptosporidium parvum* oocysts and *Bacillus subtilis* spores, wavelengths in the region of 270 nm appear to be more effective than those at 254 nm.
Differences in photoreactivation were observed between low pressure and medium pressure lamps. UV damage caused by low pressure UV lamps underwent photorepair, while no repair was detectable following exposure to medium pressure UV lamps.
It was found that inactivating light at a broad range of wavelengths, upto 580 nm, effectively reduced subsequent photoreactivation, which is an advantage that medium pressure lamps have over low-pressure UV lamps.
For inactivation of *Adenovirus* about factor 2 of UV dose is required for a 4-log reduction, using low pressure UV lamps (186 mJ/cm2) instead of medium pressure UV lamps (100 mJ/cm2).

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