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Photoreactivation of *Escherichia coli* and *Yersinia enterolytica* after Irradiation with a 222 nm Excimer Lamp Compared to a 254 nm Low-pressure Mercury Lamp

Photoreactivation of *Escherichia coli* ATCC 11229 and *Yersinia enterolytica* ATCC 4780 after irradiation with a 222 nm krypton-chloride excimer lamp compared to a 254 nm mercury lamp was investigated under laboratory conditions. The bacteria samples were irradiated each with different doses of both wavelengths. After irradiation one sample of the bacteria was illuminated with fluorescent light, the other sample was stored in darkness to prevent photoreactivation. The inactivation curves were determined. Without photoreactivation, an irradiation of 69 J/m^2 at 254 nm was sufficient for a 4 log reduction for *E. coli*, and only 59 J/m^2 for *Y. enterolytica*. To get a 4 log reduction with following photoreactivation, 182 J/m^2 were necessary for *E. coli* and 180 J/m^2 for *Y. enterolytica*. After irradiation with the 222 nm excimer lamp the ratios were different. Without photoreactivation, an irradiation of 106 J/m^2 at 222 nm was sufficient for a 4 log reduction for *E. coli* and 88 J/m^2 for *Y. enterolytica*. With photoreactivation 161 J/m^2 were necessary for *E. coli* to get a 4 log reduction and 117 J/m^2 for *Y. enterolytica*.
When the photoreactivation after irradiation is excluded, the mercury lamp with 254 nm clearly shows better results regarding inactivation. Whereas, when included, the excimer lamp with 222 nm wavelength obviously shows better results.

Photoreaktivierung von *Escherichia coli* und *Yersinia enterolytica* nach Bestrahlung mit einem 222 nm-Excimerstrahler im Vergleich mit einem 254 nm-Niederdruck-Quecksilberstrahler

Die Photoreaktivierung von *Escherichia coli* ATCC 11229 und *Yersinia enterolytica* ATCC 4780 nach Bestrahlung mit einem 222 nm-Krypton-Chlorid-Excimerstrahler im Vergleich zu einem 254 nm-Niederdruck-Quecksilberstrahler wurde unter Laborbedingungen untersucht. Proben beider Bakterienarten wurden mit verschiedenen Dosen beider Wellenlängen bestrahlt. Danach wurde zur Photoreaktivierung eine Probe Fluoreszenzlicht ausgesetzt, die andere dunkel gehalten, um diese zu verhindern. Dann wurden die Inaktivierungskurven ermittelt. Bei der Bestrahlung mit dem 254 nm-Quecksilber-Niederdruckstrahler waren ohne anschließende Photoreaktivierung für eine Inaktivierung von 4 log-Stufen 69 J/m^2 für *E. coli* und 59 J/m^2 für *Y. enterolytica* nötig. Mit anschließender Photoreaktivierung waren es dagegen 182 J/m^2 für *E. coli* und 180 J/m^2 für *Y. enterolytica*. Bei Bestrahlung mit dem 222 nm-Excimerstrahler zeigen sich deutliche Unterschiede bei den Verhältnissen. Ohne anschließende Photoreaktivierung war hier für eine Reduktion von 4 log-Stufen eine Bestrahlung von 106 J/m^2 für *E. coli* und 88 J/m^2 für *Y. enterolytica* nötig. Mit Photoreaktivierung waren es 161 J/m^2 für *E. coli* und 117 J/m^2 für *Y. enterolytica*. Wird die Photoreaktivierung ausgeschlossen, zeigt der Quecksilberstrahler bessere Ergebnisse bei der Inaktivierung, mit anschließender Photoreaktivierung jedoch der Excimerstrahler.

Keywords: Ultraviolet Radiation, Water Disinfection, Photolyase, Proteins

Schlagwörter: Ultraviolette Strahlung, Wasserdesinfektion, Photolyase, Proteine

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Research Paper

1 Introduction

Low-pressure mercury lamps are traditionally used for water disinfection. Their nearly monochromatic emission of 254 nm almost corresponds with the maximum of DNA absorption at approx. 260 nm. This absorption causes damage to DNA by altering nucleotide base paring, especially 6−4 photoproducts and thymine dimers formation [1, 2]. If the damage remains unrepaired, DNA transcription and replication is blocked. This finally leads to cell death.

There are also other targets in the cell which are damaged by UV radiation by different wavelengths. Damage of membranes has been reported to occur in cells of *Escherichia coli* only after irradiation with UV radiation above 305 nm [3]. In contrast to this, membranes of *Saccharomyces cerevisiae* yeast cells were damaged by radiation at wavelengths less than 200 nm only [4]. This membrane damage is predominantly caused by UV radiation formed radicals which react to escharotic lipoperoxides in the unsaturated fatty acids of the membrane [5]. Much more important seems to be the damage done to amino acids, and thus also in proteins composed out of it. Out of the 20 most common amino acids only phenylalanin, tyrosin, tryptophan, cystein, cystin [6] and histidin [7] show a peak UV absorption in the area of 280 nm and a higher one at 220 nm. At wavelengths exceeding 200 nm the absorption spectra of proteins and those of the composition of their constituents are comparable [6, 8]. Thus, proteins also show absorption maxima at 220 nm and 280 nm.

Both prokaryotic and eukaryotic cells have special mechanisms to remove DNA defects [1]. Among the nucleotide excision repair (NER), also known as dark repair, one of the most important repair mechanisms is the photoreactivation [9]. This process has been well researched and uses a single enzyme called photolyase to repair UV-induced damage in the DNA [10−12]. The photolyase of *E. coli* is basically specific for repair of pyrimidine dimer. It catalyses the reaction from cis-syn pyrimidin dimers to the original pyrimidin monomers in DNA. It is a light-dependent process which requires specific wavelengths ranging from 300...500 nm and is much more effective and faster than the NER [9]. This leads to problems when treated water is exposed to light and microorganisms which obviously have already been inactivated begin to reactivate, for example in UV-treated wastewater after its discharge to runoff ditches. One possible solution being applied in practice is to increase the irradiance to such a high value that the DNA is extensively damaged and photoreactivation is no longer possible. However, the higher power consumption is still a disadvantage. Due to the fact that the photoreactivation only repairs DNA damage it would be interesting to investigate the photoreactivation of bacteria after irradiation with wavelengths in the range of the absorption maxima of proteins.

Several authors investigated the use of other types of UV lamps for water disinfection like medium-pressure mercury lamps with a broader emission spectrum from far UV to infrared [13−15] excimer lamps [16, 17] and excimer laser [18]. But special reactivation studies in the past only focused on DNA repair of microorganisms following UV-exposure from low-pressure [19, 20] and medium-pressure lamps [13−15]. The broad emission spectrum of a medium-pressure lamp indeed contains wavelengths also in the range of the absorption maxima of proteins, but it is rather unspecific. In contrast to this a krypton-chloride excimer lamp shows a relatively sharp emission spectrum with a peak at 222 nm (Fig. 1).

The intention of the following experiment was to compare the photoreactivation of *Escherichia coli* and *Yersinia enterolytica* after irradiation with a 222 nm (near protein absorption max.) excimer lamp with a 254 nm (near DNA absorption max.) low-pressure mercury lamp under laboratory conditions. The bacteria were irradiated with the UV radiation from the two lamps. Afterwards the suspension was illuminated with fluorescent light. Then the reduction of the colony forming units was investigated after different irradiation times with and without photoreactivation.

2 Materials and methods

UV source. A collimated beam device (WEDECO AG Water Technology) was used for irradiation corresponding to the details of the DVGW-guideline W 294 [21]. This device contains interchangeable lamp units. One lamp unit is equipped with four low-pressure mercury lamps, type NLR 2036; the other unit is equipped with two KrCl-excimer lamps. The distance between the probes and the UV lamps was 51 cm. For both units the emission spectrum and the irradiance were measured with a Bentham Spectrometer DM 150 Double Monochromator with a 200...450 nm standard sensing head. The real power consumption taken from the main supply was 185 W for the mercury lamp and 86 W for the excimer lamp.

Figure 1 shows the measured irradiance of both lamps plotted logarithmically against the wavelength. The irradiance of the mercury lamp in the whole UV region (200...380 nm) is 22.10 W/m^2 and therefore much higher than that of the excimer lamp with 3.55 W/m^2. In the UV-C region (200...280 nm) the irradiances are 20.95 W/m^2 and 3.38 W/m^2.

Photoreactivation. The inactivation was followed by exposure to fluorescent lamps. For the photoreactivation the probes were illuminated with four (7 cm horizontally apart) fluorescent tube lamps (Osram Biolux 18 W, 600 mm length; daylight spectrum from 360...700 nm). To ensure that the light intensity in the Petri dishes was even, the illuminations

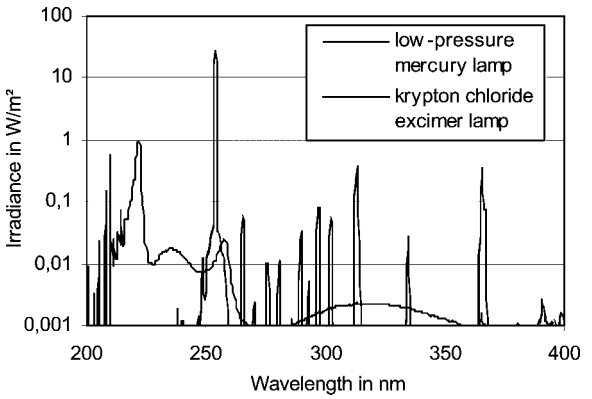

Fig. 1: Emission spectra of the 254 nm mercury lamp and the 222 nm KrCl-excimer lamp from 200...400 nm wavelength.

Emissionsspektren des 254 nm-Quecksilber-Niederdruckstrahlers und des 222 nm-Krypton-Chlorid-Excimerstrahlers im Wellenlängenbereich 200...400 nm.

were done in a box (62 cm l × 50 cm h × 32 cm w) lined with aluminium foil to reflect scattered light and to exclude light from outside.

Organisms and their cultivation. Culture: *Escherichia coli* ATCC 11229 and *Yersinia enterolytica* ATCC 4780 (American Type Culture Collection, Manassas, VA). Incubation of *E. coli* for 14 h in Endo's broth: Meat extract (Merck 103979) 3.0 g and tryptone (Oxoid LP0042) 2.5 g per litre aqua demin. Incubation of *Y. enterolytica* for 14 h in Caso's broth: Tryptone (Oxoid LP0042) 15.0 g, peptone from soymeal (Oxoid LP0044) 5.0 g, NaCl (Oxoid LP0005) 5.0 g per litre aqua demin. Bacteria were harvested by centrifugation (Biofuge 28 RS, company Heraeus) with 2600 upm for 10 min at 20 °C, the pellet was resuspended in 100 mL 0.65 % NaCl and filtrated through a 5.0 μm filter (Cellulose-Nitrate-Filter, Sartorius).

Standardization of bacteria titer. For irradiation the titer of the test suspension was standardized at $1 \cdot 10^6$ bacteria/mL as follows: The extinctions of the respective bacteria suspension were measured at 510 nm in a 5 cm quartz glass cuvette against a 0.65 % NaCl solution in a photometer (Hitachi U-1100 Spectrometer) and then further diluted with the solution until an extinction of 0.100 was reached. To this extinction the respective titer was determined. Then, the number of organisms/mL to $1 \cdot 10^6$ could be adjusted by diluting the corresponding stock suspension.

Irradiation and evaluation of the results. The irradiation and the consequent evaluation of the results were done according to the regulations of the DVGW [21] and the ÖNORM [22], which lay down the requirements and testing of plants for the disinfection of water using ultraviolet radiation. Each time 25 mL of test suspension were irradiated for different periods of times (depending on the lamps and the microorganisms to be irradiated) in 85 mm standard polystyrene Petri dishes (arithmetical thickness of 4.4 mm) without intermixing. In order to determine the exact inactivation kinetic, five duplicate samples of each of the microorganisms were irradiated. After irradiation one sample of the bacteria was illuminated with fluorescent light, the other sample was stored in darkness to prevent photoreactivation. To set a decimal dilution series after irradiation 1 mL of the test suspension was taken from the centre of the Petri dish each time. 100 μL of the dilutions were plated 3 x for the dilution steps 10^{-2} to 10^{-5} in pour-plate method with PC-Agar: Tryptone (Oxoid LP0042) 5.0 g, yeast extract (Oxoid LP0021) 2.5 g, glucose (Oxoid LP0071) 1.0 g and agar (Oxoid LP0011) 10.0 g in 1 L distilled water. For the dilution steps 10^0 and 10^{-1}, 1 mL and 100 μL of the test suspension were taken from the centre of the Petri dish and directly plated. The following incubation of the microbes was done at 37 °C in a darkened incubator for 24 hours. For the arithmetical evaluation of the results three agar plates each of this dilution step with 10...300 colonies were used. These were counted and the results arithmetically averaged. The mean was then divided by the corresponding dilution step and the common logarithm was calculated. From these five lg concentrations the average value was calculated. The corresponding reduction for the respective irradiation time is calculated by $\lg(N/N_0)$. This dose reduction factor was plotted logarithmically as function of the irradiance.

3 Results

The UV inactivation results for *E. coli* and *Y. enterolytica* are presented in Figures 2 and 3. They show only slight differences in the UV sensitivity of the two bacteria species. The UV irradiation/reduction response curves of *E. coli* and *Y. enterolytica* without photoreactivation developed in this study differ from the curves with photoreactivation.

Without photoreactivation, an irradiation of 69 J/m^2 at 254 nm was sufficient for a 4 log reduction for *E. coli*, and only 59 J/m^2 for *Y. enterolytica*. In contrast, the same irradiation with following photoreactivation showed an approx. 0.5 log reduction only for *E. coli* and 0.7 for *Y. enterolytica*. To get a 4 log reduction after irradiation and following photoreactivation, 182 J/m^2 were necessary for *E. coli* and 180 J/m^2 for *Y. enterolytica*.

After irradiation with the 222 nm excimer lamp the ratios were different compared to the 254 nm mercury lamp. Without photoreactivation, an irradiation of 106 J/m^2 at 222 nm

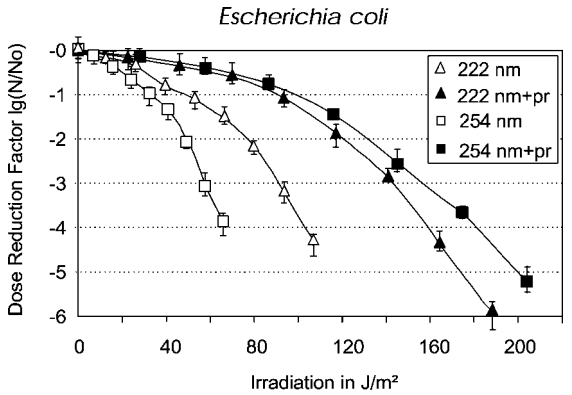

Fig. 2: Inactivation curves for *E. coli* ATCC 11229 after irradiation with a 222 nm KrCl excimer lamp and a 254 nm low-pressure mercury lamp with and without photoreactivation (pr) afterwards. All symbols indicate the mean of five independent series of experiments. Error bars denote the highest and lowest value.

Inaktivierungskurven für *E. coli* ATCC 11229 nach Bestrahlung mit einem 222 nm-Krypton-Chlorid-Excimerstrahler und einem 254 nm-Quecksilber-Niederdruckstrahler mit und ohne anschließende Photoreaktivierung (pr). Die Symbole stehen für den Mittelwert aus fünf unabhängigen Versuchsreihen. Die Fehlerbalken zeigen den höchsten und den niedrigsten Wert an.

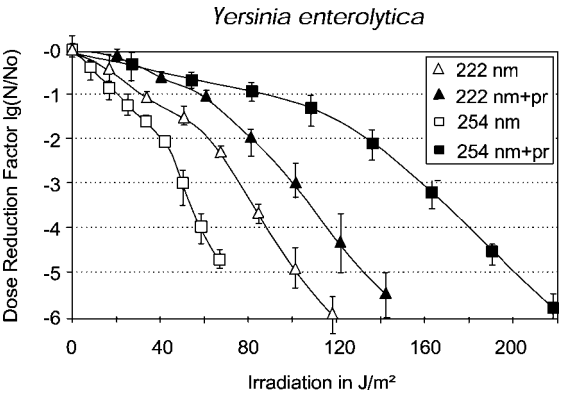

Fig. 3: Inactivation curves for *Y. enterolytica* ATCC 4780 after irradiation with a 222 nm KrCl excimer lamp and a 254 nm low-pressure mercury lamp with and without photoreactivation (pr) afterwards. All symbols indicate the results of five independent series of experiments. Error bars denote the highest and lowest value.

Inaktivierungskurven für *Y. enterolytica* ATCC 4780 nach Bestrahlung mit einem 222 nm-Krypton-Chlorid-Excimerstrahler und einem 254 nm-Quecksilber-Niederdruckstrahler mit und ohne anschließende Photoreaktivierung (pr). Die Symbole stehen für den Mittelwert aus fünf unabhängigen Versuchsreihen. Die Fehlerbalken zeigen den höchsten und den niedrigsten Wert an.

was sufficient for *E. coli* for a 4 log reduction. However, the same irradiation with following photoreactivation still showed a 1.4 log reduction. In this case only 161 J/m^2 were necessary for *E. coli* to get a 4 log reduction. Also *Y. enterolytica* showed similar results. Without photoreactivation, an irradiation of 88 J/m^2 at 222 nm was sufficient for a 4 log reduction. In contrast, the same irradiation with photoreactivation showed an approx. 2.3 log reduction. In this case 117 J/m^2 were necessary to get a 4 log reduction.

When the photoreactivation after irradiation is excluded, the mercury lamp with 254 nm clearly shows better results regarding inactivation. Whereas, on the other hand with photoreactivation afterwards the excimer lamp with 222 nm wavelength obviously shows better results.

4 Discussion

Photolyase activity and the resulting photoreactivation were found in many prokariotic and eukaryotic organisms [9]. *E. coli* was selected for this study because it is commonly used as a biological indicator of disinfection efficiency in water systems. Its dark- and photorepair processes following exposure to UV radiation are well known and have been extensively studied. This strain was specifically chosen because it is known to undergo photorepair following low-pressure UV exposure up to a dose of 280 J/m^2 [20]. *Y. enterolytica* is not a common test organism but similar to *E. coli*, it has a highly-efficient photorepair mechanism for UV radiation induced damage at 254 nm and is actually able to undergo photorepair up to 320 J/m^2 [20].

The bacterial low-pressure UV irradiation/survival response curves developed in this study are similar to other published curves for these bacteria [12, 17, 23, 24]. Also values for 3 or 4 log reductions are similar. After a 4 log reduction of the colony count (10^6/mL−10^2/mL) trough UV rays, the reduction rate could be decreased to only 1 log (10^5/mL) after UV disinfection and photoreactivation [19]. The same ratio was investigated in this study. But sometimes the values differ from some authors; however, the ratios are similar. For example, an irradiation for a 4 log reduction without photoreactivation was given for *E. coli* ATCC 11229 and *Y. enterolytica* (no strain was given), each with 100 J/m^2 [20] (in this study 69 J/m^2 for *E. coli* and 59 J/m^2 for *Y. enterolytica*). For the same reduction with photoreactivation 280 J/m^2 for *E. coli* and 320 J/m^2 for *Y. enterolytica* were necessary (182 J/m^2 and 180 J/m^2 in this study). It is commonly known that results regarding the inactivation of bacteria which can

be found in literature are contradictory, due to the different experimental conditions and the large variability of the bacteria.

No bacterial UV irradiation/survival response curve for the irradiation with a 222 nm excimer lamp could be found for these bacteria except for *E. coli* in preliminary investigations [17] and only some data in the literature [16]. In preliminary investigations it was found that under laboratory conditions the required irradiation for a 4 log reduction of *E. coli* ATCC 25922 is 60 J/m^2 with 254 nm UV radiation and 86 J/m^2 with 222 nm UV radiation [17]. At least the ratio is very similar to 69 J/m^2 for 254 nm and 106 J/m^2 for 222 nm found in this investigation. A 0.4 log reduction of *E. coli* as found in literature, irradiated with a 222 nm KrCl excimer lamp in a flowing system compared to a more than 4 log reduction under the same conditions with a 254 nm low-pressure UV lamp [16]. This reduction correlates with the one investigated in this study.

Also no data for photoreactivation at 222 nm could be found in literature. Nevertheless, clues are given in some papers that deal with the photoreactivation after irradiation with low- and medium-pressure UV sources [14, 15, 25]. The general conclusion in these papers is that the survival ratio of the bacteria after photoreactivation following medium-pressure lamps is smaller than that of low-pressure lamps. The emission spectrum of medium-pressure mercury lamps contains also wavelengths of around 222 nm. It could thus be assumed that this effect also occurs here.

In general, it is recognizable that without photoreactivation the inactivation with UV radiation with 254 nm wavelength near the absorption maxima of DNA is most effective, and DNA has always been regarded as the most important target molecule for UV radiation. To get the same inactivation results with 222 nm wavelength the necessary irradiation has to be 50 % higher. This is according to preliminary results for irradiated *E. coli*, *Enterococcus faecalis* and *Candida albicans,* which were inactivated 1.5 times better with 254 nm than with 222 nm [17]. But when the bacteria get the chance to photoreactivate, the ratios change. With photoreactivation and irradiation with 254 nm the bacteria has to be irradiated 300 % more to obtain the same reduction as without photoreactivation. At 222 nm a higher irradiation of only 25 % for *E. coli* and 50 % for *Y. enterolytica* are necessary to get the same inactivation as without photoreactivation.

In summary, the photoreactivation has a lower level after irradiation with 222 nm wavelength compared to 254 nm. These results indicate the damage of other molecules at 222 nm among the DNA, because photoreactivation is a DNA repair process only and not able to remove damage in other cell compartments like membranes or proteins. It is assumed that protein damage is most probable [6–8], whereas other authors suppose that this is due to the fact that repair of photodimers and 6-4 photoproducts is better than the one of damage induced due to photoionization at lower wavelength [18]. Another possibility is, that wavelength of 220...300 nm reduced the subsequent photorepair, possibly by causing a disorder in endogenous photolyase, the enzyme specific for photoreactivation [25].

With 3.38 W/m^2 measured in the UV-C region the excimer lamp has a much lower irradiance than the mercury lamp with 20.95 W/m^2 (Fig. 1), but the real power, actually taken from the main supply, is with 185 W for the mercury lamp 2.1 times higher than for the excimer lamp with 86.6 W. With regard to electric energy consumed and the resulting inactivation the mercury lamp is more efficient when photoreactivation is excluded. Whereas, when included, the excimer lamp with 222 nm wavelength is more efficient.

It should be pointed out that photons at the higher wavelength of 254 nm have a deeper penetration in water than 222 nm photons (for example 172 nm only approx. 30 μm, 222 nm approx. 3 cm [16]). If the transmission of 1 cm water at 254 nm is set as 100 %, the transmission at 222 nm is 97.3%, but the thickness of the suspensions which were irradiated in this study was less than 0.5 mm.

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