Water Science and Technology: Water Supply **Vol 2 No 3 pp 175–181** © IWA Publishing 2002

The spectral UV sensitivity of microorganisms used in biodosimetry

A. Cabaj*, R. Sommer, W. Pribil** and T. Haider*****

* Institut für Medizinische Physik und Biostatistik, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A 1210 Wien, Austria

** Klinisches Institut für Hygiene, Universität Wien, Kinderspitalgasse 15, A 1090 Wien, Austria

*** Institut für Umwelthygiene, Universität Wien, Kinderspitalgasse 15, A 1090 Wien, Austria

Abstract Biodosimetry, that is the application of microorganisms for the measurement of radiation, is already in wide use in the field of UV disinfection. The measurement of the mean fluence in flow-through systems with a biodosimeter (microorganisms with calibrated UV sensitivity) results in the Reduction Equivalent Fluence (REF). In the case of quasi monochromatic radiation (mercury low pressure lamp, 253.7 nm), the flow pattern of the water through the inhomogeneous radiation field, together with some other parameters, strongly influences the REF but the spectral sensitivity of the biodosimeter plays no role. If microorganisms with shouldered survival curves are used as biodosimeters two parameters (k and d) are necessary to describe their survival curves. In general, both parameters are wavelength dependent and the functions $k(\lambda)$ and $d(\lambda)$ must be known if medium pressure mercury lamps are used, which emit polychromatic UV radiation. The knowledge of $k(\lambda)$ also is necessary for selecting an appropriate UV sensor which controls the function of the UV lamps during the operation of the disinfection plant. In literature many different spectral sensitivity curves were published but they all differ somehow. The functions $k(\lambda)$ and $d(\lambda)$ were measured for spores of *Bacillus subtilis* ATCC 6633 in using quasi-monochromatic UV radiation and the results were tested in using polychromatic UV radiation.

Keywords *Bacillus subtilis*; biodosimetry; medium pressure lamps; REF; spectral sensitivity; UV-radiation

Introduction

In biodosimetry (Cabaj and Sommer, 2000) of UV disinfection plants for drinking water the biodosimeter to be used is well defined (spores of *Bacillus subtilis* ATCC 6633) in the Austrian standard (ÖNORM M 5873-1, 2001a) and the spectral sensitivity of the biodosimeter is not important. For plants with medium pressure lamps the spectral sensitivity of this biodosimeter must be known.

The spectral sensitivity of different microorganisms was measured at least since the 1930s when Gates (1930a,b,c) made his well known experiments. He was followed by many other authors like Luckiesh (1946), Rauth (1965), Hunter *et al*. (1982), Meulemans (1987), Requart (1984), Munakata *et al*. (1986, 1996). Meulemans and also some institutions like the Illuminating Engineering Society of North America IESNA (see Rea, 1995) and DIN 5031 (2000) published data whose origin was not clear.

In general, all experiments showed a higher sensitivity of microorganisms between 200 nm and about 290 nm, where a strong decline of the sensitivity starts, than from about 300 nm until 400 nm. In the visible region the sensitivity nearly is zero. Factors which make it difficult to compare the results of different authors are different experimental conditions like keeping the microorganisms in aqueous suspension or dried on a flat surface, or even the definition of the spectral sensitivity was not consistent. Many authors use the inverse of the fluence which causes a reduction of one power of ten, others, as we did in this work, use the slope of the linear part of the survival function and other parameters like the use of the cross section also exist. As Cerf (1977) pointed out survival curves of microorganisms may

have quite different shapes, in semilogarithmic representation they may be straight lines or shouldered or may have even other shapes. Functional representations of a linear and a shouldered survival curve are given in Eqs (1) and (2).

$$\frac{N}{N_0} = 10^{-kH_0} \tag{1}$$

$$\frac{N}{N_0} = 1-(1-10^{-kH_0})^{10^d} \tag{2}$$

where N_0 is the number of microorganisms before irradiation, N is the number of surviving microorganisms after irradiation, k (m^2/J) is the slope of the linear part of the survival function, H_0 (J/m^2) is the fluence and d is the intercept of the linear part of the survival function with the ordinate.

A graphical representation of these functions and the influence of approximations on the result of biodosimetry is shown in Figure 1.

Materials and methods

For our experiments we used spores of *Bacillus subtilis* ATCC 6633 whose cultivation method was described by Sommer and Cabaj (1993). The permanently stirred suspensions of the spores were irradiated with parallel and quasi-monochromatic UV radiation with six different wavelengths in the region from 214–352 nm. The radiation source was a 400W Cermax Xenon lamp together with a single monochromator (Jobin-Yvon HL) and collimating optics. In the longer wavelength region possible short wavelength components were filtered out by cut-off filters. Above 15 ml of the spore suspension was filled into 25 ml beaker glasses. The spectral irradiance at the surface of the suspension was measured with a spectro radiometer (Bentham DTM300) equipped with a quartz light guide and a teflon diffuser as entrance optics. The calibration of the spectro radiometer was traceable to PTB (Physikalisch Technische Bundesanstalt, Braunschweig, Germany). The bandwidth of the monochromatic irradiations was 20 nm. These measurements were done before and after each irradiation and the mean of both spectra was used for further calculations. The

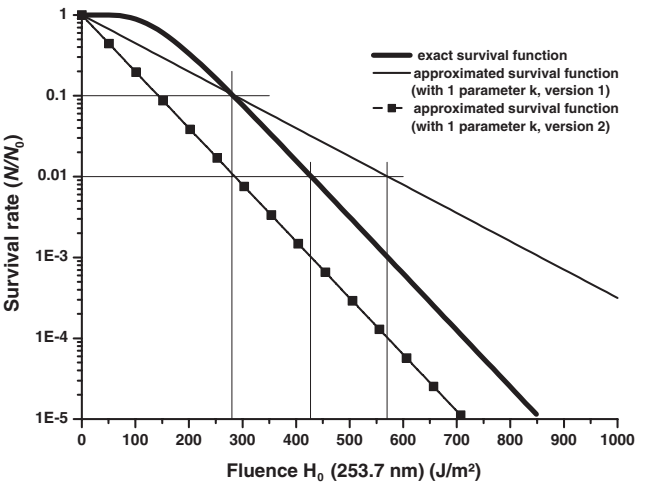

Figure 1 Approximation of shouldered survival curves (monochromatic radiation) by linear functions. Two methods are possible: to fit the linear curve through the point with survival rate 0.1 (version 1) or to use the slope k of the shouldered survival curve (version 2). Both methods result in deviations from the correct line. For instance, if used for biodosimetry, a survival rate of 0.001 would give a REF (Cabaj *et al.*, 1996) of about 570 J/m^2 (version 1), 430 J/m^2 (correct function) or 280 J/m^2 (version 2)

resulting fluence for each experiment was calculated from the spectral irradiance in taking into account the spectral reflection of the radiation at the surface of the suspension and the spectral absorption of the radiation in the suspension together with irradiation time. The spectral absorbance of the suspension was measured in using a spectro photometer (Hitachi U-3000) in 1 cm cells. In order to measure a survival curve the suspension was irradiated for a defined time and then 1 ml was taken out with a pipette. The change in depth of the suspension was also taken into account.

Experiments with polychromatic radiation were performed in using a medium pressure mercury lamp PHILIPS HPK125W. In one experiment its radiation was used unfiltered and in a second experiment an optical filter SCHOTT WG280 (2 mm) was put into the light path. The spectra for these two experiments are shown in Figure 2.

Results

The survival curves for quasi-monochromatic and for polychromatic radiation are shown in Figure 3.

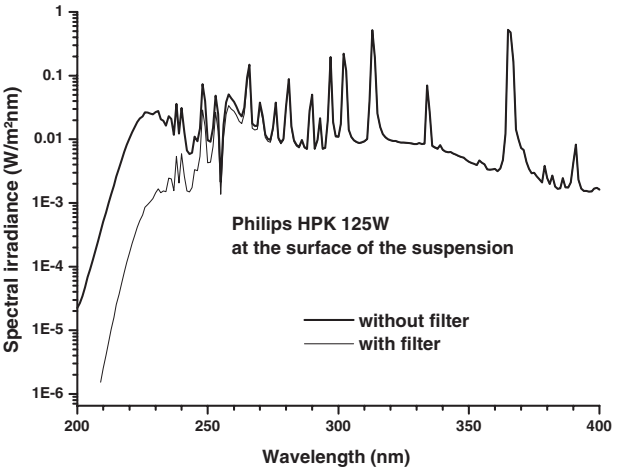

Figure 2 Spectral irradiance from the medium pressure lamp, unfiltered and filtered with Schott WG280 (2 mm) filter

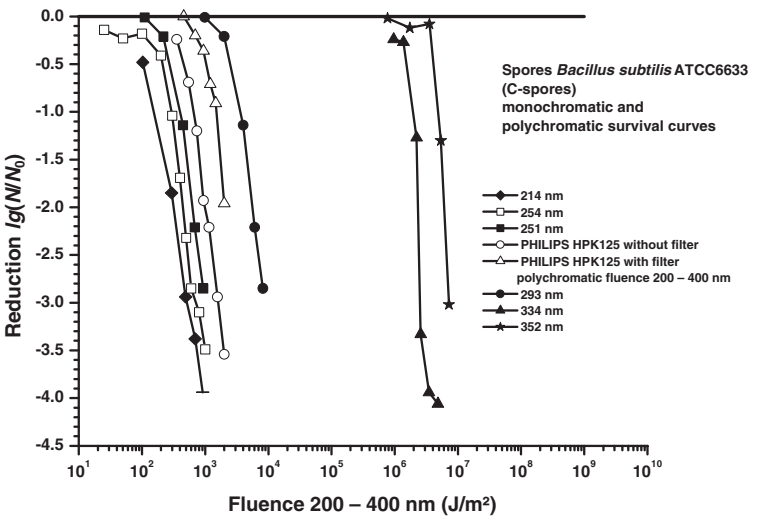

Figure 3 Survival curves of spores of *Bacillus subtilis* ATCC 6633 irradiated with quasi monochromatic and polychromatic radiation. Both the abscissa and the ordinate have logarithmic scale

Through the measured points of each curve (in semilogarithmic representation) functions as defined in Eq. (2) were fitted and the parameters k and d were determined. After normalizing these values to the values at 253.7 nm the relative functions $k_{rel}(\lambda)$ and $d_{rel}(\lambda)$ were received. Missing values were added in accordance to the literature. The resulting functions are shown in Figure 4. The function $k_{rel}(\lambda)$ has already been adopted in a draft of an Austrian standard for UV disinfection plants with medium pressure lamps ÖNORM M 5873-2 (2001b draft). Numerical values of $k_{rel}(\lambda)$ and $d_{rel}(\lambda)$ are given in Table 1.

These two functions were tested in using the two measured survival curves for polychromatic radiation. It should be possible to calculate the survival curves for polychromatic radiation if the monochromatic functions and the spectrum of the radiation are known. That means we calculated the parameters k_{res} and d_{res} of the following survival function:

$$\frac{N}{N_0} = 1 - (1 - 10^{-k_{res} H_0})^{10^{d_{res}}}$$

with

$$k_{abs}(\lambda) = k_{rel}(\lambda)\, k(253.7)$$

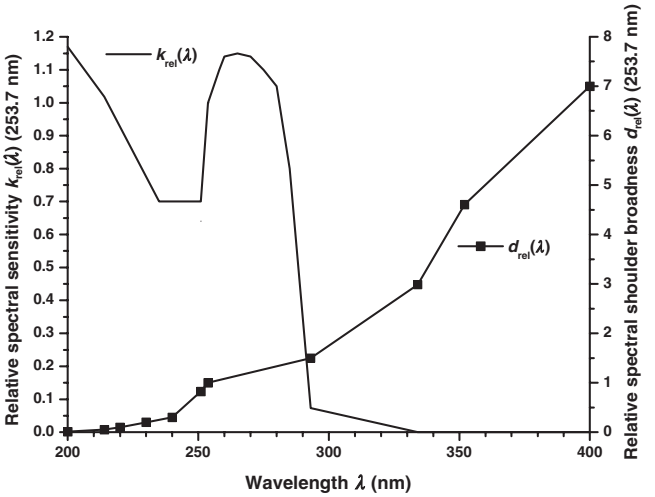

Figure 4 Relative spectral sensitivity functions $k_{rel}(\lambda)$ and $d_{rel}(\lambda)$ for spores of *Bacillus subtilis* ATCC 6633 in aqueous suspension. The values were normalized to 253.7 nm in using the following absolute values $k(253.7) = 0.00612\ m^2/J$ and $d(253.7) = 0.79$

Table 1 Numerical values of $k_{rel}(\lambda)$ and $d_{rel}(\lambda)$

Wavelength (nm)	$k_{rel,254}$	$d_{rel,254}$	Wavelength (nm)	$k_{rel,254}$	$d_{rel,254}$
200	1.17	0.01	265	1.15	
214	1.018	0.05	270	1.14	
220		0.1	275	1.1	
230		0.2	280	1.05	
235	0.7		285	0.8	
240		0.3	293	0.073	1.5
251	0.7	0.823	334	3.07E-4	2.99
253.7	1	1	352	1.5E-4	4.6
258	1.1		400	8.5E-6	7
260	1.14				

$$k_{res} = \int_{200}^{400} k_{abs}(\lambda) H_{0,\lambda}(\lambda)\, d\lambda \qquad H_0 = \int_{200}^{400} H_{0,\lambda}(\lambda)\, d\lambda$$

and

$$d_{res} = \frac{\int_{200}^{400} d_{abs}(\lambda) k_{abs}(\lambda) H_{0,\lambda}(\lambda)\, d\lambda}{\int_{200}^{400} k_{abs}(\lambda) H_{0,\lambda}(\lambda)\, d\lambda}$$

where $H_{0,l}(\lambda)$ is the absolute spectral fluence (J/m^{2}nm) of the UV radiation. A comparison of calculated with measured survival functions is shown in Figure 5.

Another important point was to test our function $k(\lambda)$ against some other sensitivity functions from the literature. We calculated the k_{res} in using the different sensitivity functions we found in literature together with the spectra from the medium pressure lamp and compared these values with the measured ones. The results are given in Table 2.

A graphical representation of the sensitivity function $k_{rel}(\lambda)$ measured by us as compared with some other functions given in the literature, can be seen in Figure 6. The origin of the data is given in the graph.

Discussion

The measured spectral sensitivity function is well suited to explain the slope of the survival curves of our biodosimeter which were produced with polychromatic UV radiation. Therefore this function can be used for the design of a spectral sensitivity function of sensors for disinfection plants with medium pressure lamps and for the calculation of microbicidally weighted fluences. In the Austrian national standard ÖNORM M 5873-2 (2001b, draft), which deals with the disinfection of drinking water with plants equipped with mercury medium pressure lamps, the function given in Figure 4 has been already adopted.

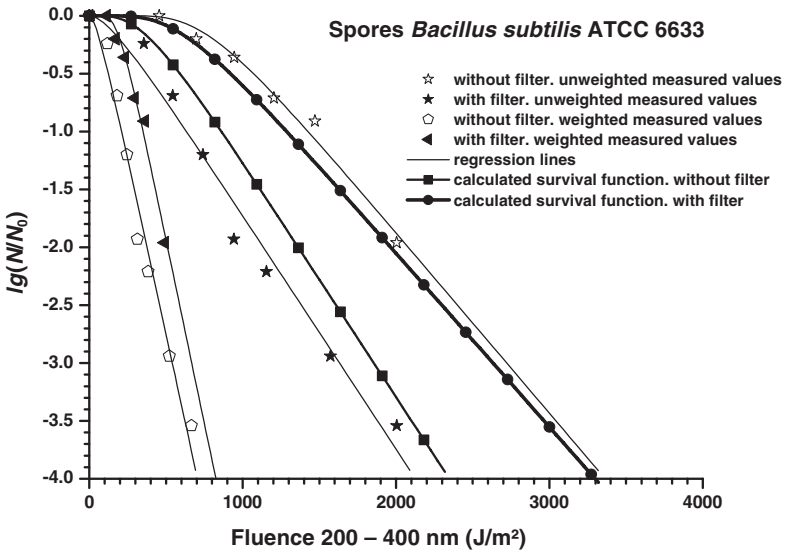

Figure 5 Comparison of calculated with measured survival curves for unfiltered and filtered radiation from medium pressure lamp. For filtered radiation, that means shortwave radiation had been cut off, the calculated and the measured curves show good agreement, *k* and *d* both could be sufficiently exact to be calculated. For unfiltered radiation, the calculated and the measured *k* show good agreement (the linear parts of the curves are parallel) but for *d* some discrepancy remains. The two curves at the left in the graph were calculated in using weighted fluences; they also are parallel but do not coincide completely

Table 2 Ratio of calculated values of k_{res} and of the measured values for polychromatic UV radiation. The calculation was performed by using the spectral sensitivity curve given in Figure 4. The radiation was from a medium pressure mercury lamp and the filtering was done with a cut off filter in the short wavelength region

	$k_{res,calculated}/k_{res,measured}$ (%)	
	unfiltered radiation	filtered radiation
DIN	+3.8	–5.7
Munakata	–18.6	+1.5
IESNA	–12.2	+0.7
Meulemans	+9.8	+22.3
IUPAC	–20.8	–4.7
ÖNORM M 5873-2 (draft)	+0.7	–3.6

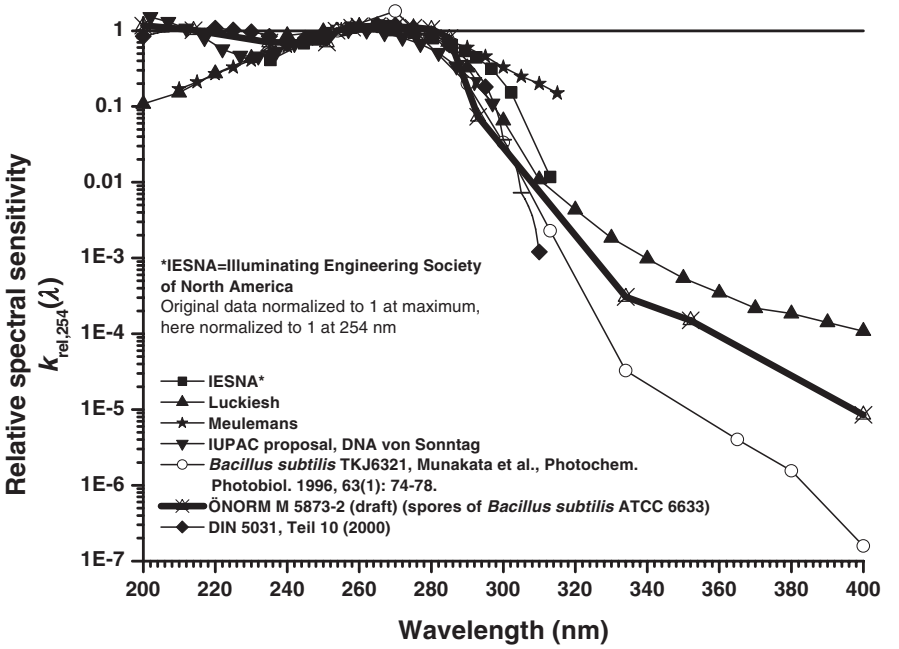

Figure 6 Several spectral sensitivity functions together with the function measured in this work (ÖNORM M 5873-2). The functions are normalized to 1 at 253.7 nm

The comparison of the different sensitivity functions $k(\lambda)$ from the literature showed certain deviations if they were applied to the spores of *Bacillus subtilis*, but the deviations were lower than we expected. In the literature we found no results for the wavelength dependence of the parameter d, therefore no possibility existed to test it against other authors.

The test of $k(\lambda)$ and $d(\lambda)$ against our measurements with polychromatic radiation gave good results for $k(\lambda)$ while the function $d(\lambda)$ resulted in somewhat greater deviations from our polychromatic results. A reason for this fact seems to be that the determination of k from the different survival curves by curve fitting is less uncertain than that of d.

Conclusions

As a conclusion from our experiments one can say that it is possible to calculate survival curves of microorganisms with survival curves without shoulder and also with shouldered

survival curves, for different polychromatic UV radiations if the proper functions $k(\lambda)$ and $d(\lambda)$ are known. Because these functions may be very different for different microorganisms it is not possible to speak of a general sensitivity function for microorganisms. Calkins and Barcelot (1979) gave an interesting collection of spectral sensitivity curves from the literature and it was shown that the spectral sensitivity functions of certain microorganisms may differ by orders of ten in some regions of UV wavelength. In our case we determined these functions for our biodosimeter, spores of *Bacillus subtilis* ATCC 6633, and the sensor of a UV disinfection plant would give reliable measurements of the k-weighted irradiance if its spectral sensitivity corresponds with $k(l)$.

References

Cabaj, A. and Sommer, R. (2000). Measurement of ultraviolet radiation with biological dosemeters. *Radiat. Prot. Dosim.*, **91**, 139–142.

Cabaj, A., Sommer, R. and Schoenen, D. (1996). Biodosimetry: model calculations for UV water disinfection devices with regard to dose distributions. *Wat. Res.*, **30**, 1003–1009.

Calkins, J. and Barcelot, J. (1979). Some further considerations on the use of repair-defective organisms as biological dosimeters for broad-band ultraviolet radiation sources. *Photochem. Photobiol.*, **30**, 733–737.

Cerf, O. (1977). Tailing of survival curves of bacterial spores. *J. Appl. Bacteriol.*, **42**, 1–19.

DIN 5031 and Teil 10 (2000). Strahlungsphysik im optischen Bereich und Lichttechnik. Teil 10: Photobiologisch wirksame Strahlung. Größen, Kurzzeichen und Wirkungsspektren. DIN Deutsches Institut für Normung e.V. Beuth Verlag GmbH, Berlin.

Gates, F. (1930a). A study of the bactericidal action of ultra violet light. I. The reaction to monochromatic radiations. *J. Gen. Physiol.*, **13**, 231–248.

Gates, F. (1930b). A study of the bactericidal action of ultra violet light. II. The effect of various environmental factors and conditions. *J. Gen. Physiol.*, **13**, 249–260.

Gates, F. (1930c). A study of the bactericidal action of ultra violet light. III. The absorption of ultra violet light by bacteria. *J. Gen. Physiol.*, **14**, 31–42.

Hunter, J., Kaupp, S. and Taylor, J. (1982). Assessment of effects of UV radiation on marine fish larvae. In: *Proceedings of a NATO Conference on The Role of Solar Ultraviolet Radiation in Marine Ecosystems*, J. Calkins (ed.) 28–31 July, Copenhagen, Denmark, Plenum Press, 459–497.

Luckiesh, M. (1946). *Applications of Germicidal, Erythemal and Infrared Energy* D. Van Nostrand Company, New York.

Meulemans, C. (1987). The basic principles of UV-disinfection of water, Ozone-Sci. Eng., **9**, 299–314.

Rea, M. (1995). *Lighting Handbook*, Illuminating Engineering Society of North America, USA, 152 pp.

Munakata, N., Hieda, K., Kobayashi, K., Ito, A. and Ito, T. (1986). Action spectra in ultraviolet wavelengths (150–250 nm) for inactivation and mutagenesis of *Bacillus subtilis* spores obtained with synchrotron radiation. *Photochem. Photobiol.*, **44**(3), 385–390.

Munakata, N., Morohoshi, F., Hieda, K., Suzuki, K., Furusawa, Y., Shimura, H. and Ito, T. (1996). Experimental correspondence between spore dosimetry and spectral photometry of solar ultraviolet radiation. *Photochem. Photobiol.*, **63**, 74–78.

ÖNORM M 5873-1 (2001a). Austrian National Standard, Plants for the disinfection of water using ultraviolet radiation. Requirements and testing. Part 1. Low pressure mercury lamp plants.

ÖNORM M 5873-2 (2001b draft). Austrian National Standard, Plants for the disinfection of water using ultraviolet radiation. Requirements and testing. Part 2. Medium pressure mercury lamp plants.

Rauth, A. (1965). The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. *Biophysical Journal*, **5**, 257–273.

Requart, H. (1984). *Aktionsspektroskopische Untersuchungen zur synergistischen Wirkung von UV-Strahlung und Vakuum auf Mikroorganismen*. PhD thesis, Naturwissenschaftliche Fakultät der Universität Frankfurt am Main, Germany.

Sommer, R. and Cabaj, A. (1993). Evaluation of the efficiency of a UV plant for drinking water disinfection. *Wat. Sci. Tech.*, **27**(3–4), 357–362.