Biologically weighted UV dosimetry by use of the biofilm technique

G. Horneck, P. Rettberg and W. Strauch, DLR, Institut für Luft- und Raumfahrtmedizin, Abteilung Strahlenbiologie, Linder Höhe, 51147 Köln, Germany,

G. Seckmeyer and B. Mayer, Fraunhofer Institut für Atmosphärische Umweltforschung, Kreuzeckbahnstraße 19, 82467 Garmisch-Partenkirchen, Germany,
and
P. C. Simon, D. Gillotay and D. Bolsée, Institut d' Aeronomie Spatiale de Belgique, Ave. Circulaire 3, 1180 Brussels, Belgium

INTRODUCTION

To determine the impact of environmental UV-radiation on critical processes of our biosphere demands accurate and reliable monitoring systems that weight the spectral irradiance according to the biological responses under consideration. The need for a biological weighting of solar UV-irradiance derives from the highly wavelength-specific absorption characteristics of atmospheric ozone and the wavelength specificity of the biological action spectra in the UV-B-range. Approaches to quantify the biologically effective solar irradiance are: (1) weighted spectroradiometry where the biologically weighted radiometric quantities are derived from spectral data by multiplication with an action spectrum of a relevant photobiological reaction, (2) wavelength integrating chemical-based or physical dosimetric systems with spectral sensitivities similar to a biological response curve, and (3) biological dosimeters that directly weight the incident UV components of sunlight in relation to the effectiveness of the different wavelengths and to interactions between them (Horneck 1995). In most cases, simple biological dosimeters are applied, such as bacteria, bacteriophages, or biomolecules. Induction rates for lethality, mutagenesis or photoproduct formation are used, which directly reflect the UV-sensitivity of DNA (reviewed by Rontó et al. 1994). In this project, a biofilm which comprises immobilised bacterial spores as a UV-sensitive target has been used as biological UV dosimeter. The biofilm has been systematically characterised and its reliability as biological and field dosimeter has been tested by intercomparison with weighted spectroradiometry.

METHOD

The biofilm consists of a monolayer of dried spores of *Bacillus subtilis* immobilised on a transparent polyester sheet (Quintern et al. 1992). After UV exposure, the biofilm is incubated in nutrient broth and the protein, synthesised by the immobilised microorganisms is stained with Coomassie dye. The processed biofilm is a transparent blue foil, where the intensity of the blue colour is a direct measure of the biological activity. Biofilms are divided in calibration fields (where they are calibrated at defined fluences of monochromatic UV radiation at 254 nm) and exposure fields (where they are exposed to environmental UV radiation for defined periods of time). Biofilm evaluation is done by image analysis. The biologically effective irradiance E_{eff} in W/m^2 is determined from the equivalent dose F at 254 nm producing the same response of the biofilm (Fig. 1):

$$E_{eff} = \frac{F}{t} \qquad (1)$$

with E_{eff} = biologically effective irradiance (W/m^2)
F = equivalent dose at 254 nm producing the same biofilm response (J/m^2)
t = time of exposure (s)

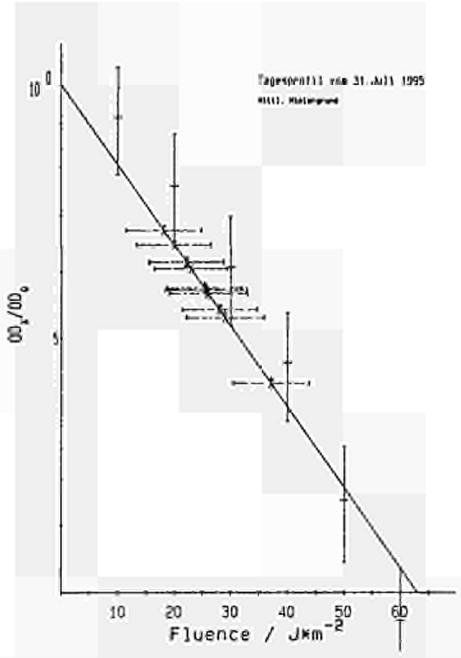

Fig. 1. Biofilm calibration curve, obtained by irradiation with defined fluences at 254 nm and corresponding relative optical densities of exposure fields.

For the full radiometric characterisation of the biofilm a calibration system has been constructed, which consists of a UV-light source (1 kW Xe arc lamp) controlled by a photo-feedback system with sufficiently high spectral irradiance at 300 nm, a filtering system of combinations of WG and UG Schott filters, and a calibration box with 8 times 4 exposure fields and a slide shutter mechanism. A calibration protocol has been developed which allows a systematic characterisation of the biofilm.

An intercomparison campaign was held at the Fraunhofer Institute for Atmospheric Environmental Research at Garmisch-Partenkirchen, Germany, in September 1994. Simultaneous measurements were performed during different atmospheric conditions with biofilms and the IFU spectroradiometer, which was in close agreement with other high accuracy spectroradiometers at an international intercomparison some weeks before (Seckmeyer et al. 1995). 62 data points were evaluated from biofilm dosimetry, the exposure times varying between 20 min and 3 h, corresponding to an erythemal dose of approximately 1 MED (MED = minimal erythemal dose, 0.21 kJ/m^2 at 297 nm), measured by a Robertson Berger meter (Solar Light). For comparison with the biofilm data, the spectral data were weighted with the biofilm action spectrum (Quintern et al 1994), integrated over wavelength and over biofilm exposure time (Fig. 2). The biologically effective irradiance E_{eff} (W/m^2) is determined according to (Horneck et al 1995).

$$E_{eff} = \int E_\lambda(\lambda) \bullet S_\lambda(\lambda) d\lambda \qquad (2)$$

with $E_\lambda(\lambda)$ = the solar irradiance (W/m^2 nm)
$S_\lambda(\lambda)$ = the biofilm action spectrum, normalised at $\lambda = 254$ nm

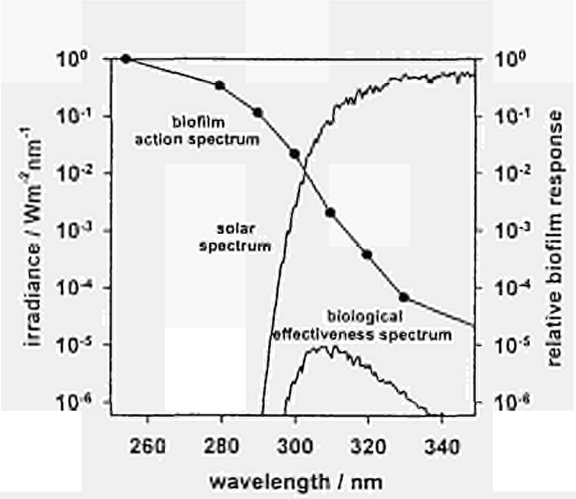

Fig. 2. Solar spectral irradiance at the Earth's surface, biofilm action spectrum, normalised at $\lambda = 254$ nm, and biological effectiveness spectrum, obtained as product of the irradiance times the action spectrum. The area under the curve is the biologically effective irradiance E_{eff}.

For continuous UV monitoring by use of the biofilm technique, a Monitoring System for Biofilm MOSYS B has been developed, which is a weather proof exposure device for automated measurements of diurnal profiles of biologically effective solar UV irradiance over one week (Fig. 3).

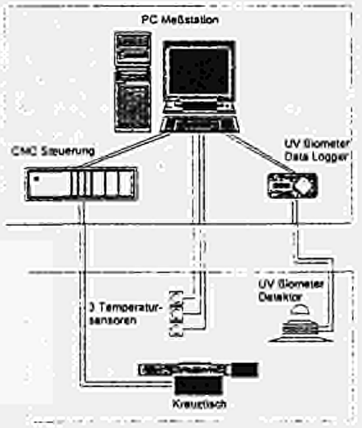

Fig. 3. Monitoring System for Biofilm, MOSYS B, for automated continuous measurements of diurnal profiles of biologically effective solar UV irradiance over one week.

RESULTS

The first intercomparison campaign in August/September 1994 (Fig. 4) gave the following results:

- *Precision*. The ratio between biofilm signal and weighted spectral data was found to have a standard deviation of 17 % from the average.
- *Absolute accuracy*. The data were found to differ by a constant factor of 3.6.
- No systematic diurnal variation of the ratio biofilm to weighted spectral data was found.

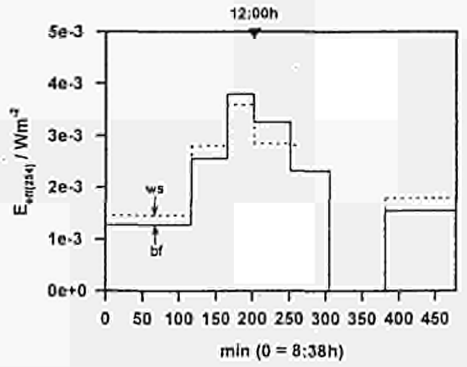

Fig. 4. Comparison of biofilm data (bf) and weighted spectral data (ws), where the biofilm data are scaled by a factor 1/3.6; results from the first intercomparison campaign August/September 1994 in Garmisch-Partenkirchen

CONCLUSIONS

The biofilm has proven to have the potential of a suitable biological dosimeter for monitoring the biological effectiveness of environmental UV radiation. Further studies are required to increase the precision between biofilm data and weighted spectral data. It is especially important to improve the reproducibility of the biofilm background (non-irradiated areas). Furthermore, the reason for the systematic deviation of the biofilm and weighted spectral data by a constant factor of 3.6 need further investigations. Possible sources of error could be: the absolute calibration of the biofilm, non-linearities of the biofilm, deviation of response between monochromatic (calibration) and polychromatic (sunlight) radiation, accuracy of the biofilm action spectrum. The results are expected from the on-going characterisation of the biofilm.

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