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Comparison of the action spectra and relative DNA absorbance spectra of microorganisms: Information important for the determination of germicidal fluence (UV dose) in an ultraviolet disinfection of water

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ABSTRACT

The action spectra of *Bacillus subtilis* spores (ATCC6633) and *Salmonella typhimurium* LT2 were characterized using physical radiometry for irradiance measurements and a multiple target model to interpret the inactivation kinetics. The observed action spectrum of *B. subtilis* spores deviated significantly from the relative absorbance spectrum of the DNA purified from the spores, but matched quite well with the relative absorbance spectrum of decoated spores. The action spectrum of *B. subtilis* spores determined in this study was statistically different from those reported in previous studies. On the other hand, the action spectrum of *S. typhimurium* bacteria matched quite well with the relative absorbance spectrum of DNA extracted from vegetative cells, except in the region below 240 nm. It is concluded that the common use of the relative DNA absorbance spectrum as a surrogate for the germicidal action spectrum can result in systematic errors when evaluating the performance of a polychromatic UV light reactors using bioassays. For example, if the weighted germicidal fluence (UV dose) calculated using the relative DNA absorbance spectrum as the germicidal weighting factor is found to be 40 mJ cm^{-2} for a medium pressure lamp UV reactor, that calculated using the relative action spectrum of *B. subtilis* spores, as determined in this study, would be 66 mJ cm^{-2}.

1. Introduction

Ultraviolet (UV) treatment is an increasingly popular technology for the disinfection of drinking water and secondary or tertiary wastewater effluents. Although low pressure (LP) UV lamps are commonly used for small UV disinfection plants, medium pressure (MP) mercury discharge lamps, which produce polychromatic germicidal UV light, are found in many large-scale UV disinfection facilities for drinking water and wastewater treatment. Many UV disinfection studies have been carried out using MP lamps in bench-scale batch UV reactors (Bolton et al., 1998; Craik et al., 2000; Mamane-Gravetz et al., 2005; Linden et al., 2005). The computation of the fluence (UV dose) in large continuous-flow UV reactors, used for the disinfection of drinking water, is complex. The fluence (UV dose) delivery for a given UV reactor design must be validated. Several approaches (biodosimetry, computational fluid dynamic (CFD) modeling and dyed microsphere

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Nomenclature

d	Shoulder inactivation coefficient
BPF	Band-Pass Filter
CFU	Colony Forming Units
DPA	Dipicolinic acid
F	Fluence (UV dose)
IL	International Light
LP	Low Pressure mercury discharge lamp
k	Inactivation rate constant ($cm^2\,mJ^{-1}$)
k_{rel}	Inactivation rate constant relative to 254 nm
MP	Medium Pressure mercury discharge lamp
n_c	Number of critical targets of multiple target model
N	Concentration of viable microorganisms after UV exposure
N_0	Concentration of viable microorganisms before UV exposure
N_i	Relative photon flux at wavelength λ_i
PBDI	Phosphate Buffered Deionized
REF	Reduction Equivalent Fluence
SASP	Small Acid Soluble Proteins

actinometry) are available or potentially available for the validation of UV reactors (USEPA, 2006). Biodosimetry, in which the inactivation of a surrogate microorganism added to the water is measured, has been accepted by regulatory agencies. For reactors equipped with MP lamps, the evaluation of the reactor performance using a bioassay is affected by the assumption made concerning the relative action spectra of the surrogate microorganism and target pathogen.

A standardized method, which uses a germicidal factor to calculate the germicidal fluence (UV dose) from a polychromatic source in bench-scale UV experiment, has been proposed (Bolton and Linden, 2003). When polychromatic lamps are used for UV disinfection studies and for interpreting the results of MP UV reactor bioassays, careful determination of the relative action spectrum (relative to a 'germicidal action' of 1.00 at 254 nm) of a microorganism is crucial for the accurate determination of the germicidal factors and hence the germicidal fluence (UV dose). The experimental methods to determine action spectra, however, are not standardized. Different methods have been reported in the literature for the determination of the germicidally weighted fluence (UV dose) for polychromatic UV sources. Moreover, studies have reported that the germicidal efficacy of UV light is highly dependent on the wavelength and might vary from microorganism to microorganism (Gates, 1929; Rauth, 1965; Linden et al., 2001; Linden et al., 2005).

The DNA absorbance spectrum reported by von Sonntag and Schuchmann (1992) has been conventionally used as a surrogate when the inactivation action spectrum of a microorganism is not available. However, it is not clear from their paper as to what the source of the DNA was. Since the nucleotide composition of DNA varies from one organism to another, the DNA absorbance spectrum might vary. In this study, action spectra are compared with the relative absorption spectra of DNA and other components from the same microorganism. Two microorganisms were chosen for the study: *Bacillus subtilis* spores, which are widely used as a non-pathogenic surrogate in biodosimetry testing of UV reactors and *Salmonella typhimurium* LT2, an attenuated strain of an important pathogenic bacterium.

B. subtilis spores have been used commonly as a surrogate microorganism to validate the performance of continuous-flow UV reactors used in drinking water treatment plants (USEPA, 2006; DVGW, 2006; ÖNORM, 2001, 2003) and for fundamental UV disinfection studies (Sommer and Cabaj, 1993; Uvbiama and Craik, 2005). The action spectra for *B. subtilis* have been determined for spores produced from liquid-cultivated media (Cabaj et al., 2002) and from surface-cultivated sporulation media (Mamane-Gravetz et al., 2005). Both studies reported similar first-order spore UV inactivation rate constants. However, the reported wavelength dependence of the 'shoulder' in the fluence (UV dose)–response curve for *B. subtilis* spores, differed between the two studies. The shoulder region at low fluence (UV dose), where there is little or no inactivation, must be considered carefully when evaluating UV reactors using bioassays (Cabaj et al., 2002).

Riesenman and Nicholson (2000) concluded that the spore coat does not contribute to the resistance of spores to 254 nm UV light. It has been reported that *B. subtilis* spores have their own unique photochemistry and repair mechanisms, and this may account for why spores are more resistant to UV light than are vegetative *B. subtilis* cells (Setlow, 2001).

Salmonella spp. is an important waterborne pathogen in drinking water. Most UV disinfection studies of *Salmonella* spp. have been carried out using monochromatic UV light (Chang et al., 1985; Tosa and Hirata, 1998; Koivunen and Heinonen-Tanski, 2005). However, the inactivation action spectrum of *Salmonella* has not yet been reported. Moreover, *Salmonella* DNA might have its own unique absorbance spectrum. The strain of *S. typhimurium* LT2 used in this study has a highly attenuated virulence and has been commonly used as a surrogate for virulent *Salmonella* spp. in laboratory studies.

In this paper not only are the relative action spectra of *B. subtilis* spores and *S. typhimurium* bacteria determined, but also, for the first time, the action spectra are compared to the relative DNA absorbance spectra extracted from the same microorganism. Also, in the case of *B. subtilis* spores, a comparison is made between the action spectrum and the relative absorbance spectrum of decoated spores. Where available, the relative action spectra are compared to previously published action spectra. Finally, the potential effect of different action spectra on the determination of the germicidal fluence (UV dose) in a MP UV reactor validation was evaluated.

2. Materials and methods

2.1. *B. subtilis* spore production, preparation of test suspensions, and enumeration

All sterile media, reagents and materials were pre-sterilized. Procedures for the preparation of *B. subtilis* spores (ATCC 6633)