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Evaluation of DNA damage reversal during medium-pressure UV disinfection

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ABSTRACT

Ultraviolet (UV) disinfection relies on the principal that DNA exposure to UV irradiation leads to the formation of cytotoxic lesions resulting in the inactivation of microorganisms. Cyclobutane pyrimidine dimers (CPDs) account for the majority of DNA lesions upon UV exposure. Past research has demonstrated reversal of CPDs in extracted DNA formed at high UV-C wavelength irradiation (280 nm) upon subsequent irradiation at lower UVC wavelengths (230–240 nm). Medium-pressure (MP) UV lamps produce a polychromatic emission giving rise to the possibility that cellular DNA in a target pathogen may undergo simultaneous damage and repair when exposed to multiple wavelengths during the disinfection process, decreasing the efficiency of MP UV lamp disinfection. Culture techniques and a quantitative polymerase chain reaction (qPCR) assay were used to examine cell viability and DNA damage reversal. qPCR results indicated direct photoreversal of UV-induced DNA damage through sequential irradiations of 280 nm followed by 228 nm in *Escherichia coli* DNA. However, significant photoreversal was only observed after high initial doses and secondary doses of UV light. The doses where significant photoreversal took place were more than 10 times higher than those typically used in UV disinfection. Despite evidence of CPD photoreversal, bacterial growth assays showed no indication that sequential-wavelength irradiations result in higher survival rates than single-wavelength irradiations.

1. Introduction

Ultraviolet (UV) light is a proven and effective method for the inactivation of microorganisms. Consequently, it has become a widely used technology in water and wastewater treatment facilities. The benefits of UV are well known throughout the water industry; however, increased use of UV disinfection and technological advancement has spurred an interest for further research concerning process challenges and optimization. The foundation of UV disinfection lies in the ability of UV light to induce damage to DNA leading to inhibition of vital cellular processes such as transcription and replication and ultimately lead to the inactivation of the organism (Moné et al., 2011; Sinha and Häder, 2002; Rodríguez et al., 2013). DNA strongly absorbs UV-C (200–280 nm) with a relative peak at 260 nm and the absorption can lead to the formation of lesions between neighboring nucleobases, primarily pyrimidines (Douki, 2013; Eischeid and Linden, 2007; Rastogi et al., 2010). Two primary types of lesions may form:

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cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts. CPDs are characterized as four-membered ring structures formed between the C5 and C6 atoms on each pyrimidine base and 6–4 photoproducts are characterized by an interaction between one carbon on each base (Douki, 2013; Rastogi et al., 2010). CPDs represent the majority of lesions induced by UV-C light accounting for about 75% of lesions formed while the remaining 25% are 6–4 photoproducts (Sinha and Häder, 2002). Many microbes have evolved mechanisms to repair certain UV-induced lesions, including CPDs and 6-4 photoproducts, creating a potential concern for UV disinfection processes (Thoma, 1999). These mechanisms have been studied extensively and are well understood from a molecular point-of-view. Briefly, there are two principal categories of DNA damage repair: photoreactivation and excision repair. Photoreactivation is an enzyme-mediated mechanism stimulated by the exposure to visible, near-UV light. Excision repair is also an enzyme-mediated mechanism, but does not require visible light (Thoma, 1999; Sinha and Häder, 2002).

In addition to enzyme-mediated DNA repair mechanisms, there has been evidence that UV-induced lesions can be directly cleaved through subsequent UV-C exposure (Setlow and Setlow, 1962; Pan et al., 2012). Past studies utilizing isolated DNA observed that CPDs formed via irradiation at 280 nm can be directly cleaved, or split into their original components, with subsequent irradiation from lower wavelength UV-C irradiation near 230–240 nm. Setlow and Setlow (1962) used this concept to investigate the role of thymine dimers in the biological damage to microbes by UV light. Other studies used direct photocleavage to further investigate the prevalence of thymine dimers in UV-induced damage or reported on the phenomenon using absorbance measurements or paper chromatogaraphy (Johns et al., 1962; Setlow and Setlow, 1962; Setlow and Carrier, 1963; Johns et al., 1964). Recently, Pan et al. (2012) used the concept of direct photocleavage from sequential irradiations to investigate the effect of neighboring purines on CPD formation with the principal method of detection being absorption. It is important to note that direct photocleavage of CPDs is generally observed at higher than typical UV disinfection doses. For example, the minimum initial dose at 280 nm required to observe DNA damage reversal through subsequent irradiation at wavelengths below 240 nm is at least 100 mJ/cm^2 (Setlow and Setlow, 1962). Furthermore, the degree of photoreversal increases with increasing lower wavelength irradiation (Setlow and Setlow, 1962).

Medium-pressure (MP) UV lamps produce an emission spectrum spanning the 200–400 nm UV range and into the visible range, resulting in simultaneous exposure to multiple wavelengths. In contrast, low-pressure (LP) UV lamps have a monochromatic UV emission at 253.7 nm. Fig. 1 illustrates the polychromatic and monochromatic emission of MP and LP lamps, respectively. For UV systems utilizing LP UV lamps, direct photocleavage of CPDs would not be a concern; however, the polychromatic emission of MP lamps simultaneously exposes DNA to multiple wavelengths, including those below 240 nm. Therefore, there exists the opportunity for reversal of DNA damage through direct photocleavage of UV-induced CPDs during MP UV irradiation. MP UV lamps are becoming increasingly popular, as they exhibit increased effectiveness

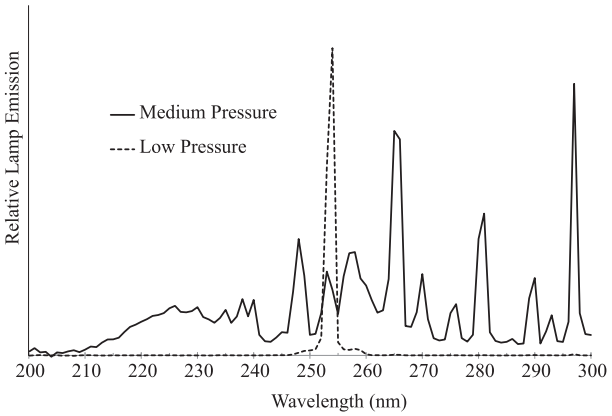

Fig. 1 – Emission spectra of LP and MP lamps.

for the inactivation of adenovirus (Linden et al., 2007). Furthermore, MP UV irradiation has been shown to limit the degree of photoreactivation seen after UV exposure in bacteria compared to LP UV irradiation (Zimmer and Slawson, 2002). The broad band emission characteristic of the MP UV lamp has been shown to cause damage to DNA other than CPDs and 6-4 photoproducts, including Dewar isomers and single-strand breaks (Cadet et al., 2005).

While there have been numerous studies examining the phenomenon of direct photocleavage of CPDs, there is a lack of research regarding this concept in the framework of UV disinfection which is founded on the capability of UV light to induce CPDs, primarily, in microbial DNA. Herein, direct photocleavage of UV-induced CPDs is examined in the context of the MP UV disinfection process. Specifically, the research aimed to 1) determine if direct photoreversal occurs in cellular DNA (previous research has used isolated DNA in the irradiations) and to 2) determine if the amount of high and low-wavelength UV-C typically present in MP UV doses results in direct photocleavage. These questions are addressed using conventional culture techniques to examine cell viability and quantitative polymerase chain reaction (qPCR) to quantify damage to the bacterial genome. Since cell viability can be affected by any UV-induced damage, this dual approach provides fundamental insight into whether or not direct CPD photocleavage can account for enough DNA damage reversal to return DNA to a viable state in *Escherichia coli*.

2. Materials and methods

2.1. UV Exposures and sample preparation

A bench-scale, collimated beam apparatus equipped with a 1-kW MP UV lamp (Calgon Carbon Corporation Inc., Pittsburgh, PA, USA) was used for the exposures. For the wavelength-specific exposures, the lamp was outfitted with 280 and 228 nm bandpass filters (Andover Corporation, Salem, NH, USA), which had a full width at half maximum (FWHM) of 10–12 nm and emission spectra as illustrated in Fig. 2. The emission spectra for the LP and MP sources (Fig. 1) and the bandpass filters (Fig. 2) were measured with an Ocean Optics