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Effect of pre- and post-UV disinfection conditions on photoreactivation of fecal coliforms in wastewater effluents

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ABSTRACT

Photoreactivation of microorganisms following UV disinfection can represent a disadvantage to using UV technology for wastewater treatment since recovery may, in some cases, reach several logs. Thus, decreasing photoreactivation can lead to considerable savings in capital and operating costs. Objectives of this study were to determine pre- and post-UV irradiation conditions which could decrease fecal coliform (FC) photoreactivation in wastewater effluents. Results indicated that delaying exposure to photoreactivating light for 3 h suppressed photoreactivation after relatively low UV doses of 10 and 20 mJ/cm^2. Moreover, at least 440 lux (0.065 mW/cm^2) of visible light was needed to initiate photoreactivation. Additionally, photoreactivation decreased significantly when samples were exposed to visible light simultaneously or prior to UV irradiation. This was more significantly observed for winter samples, where photoreactivation decreased by nearly 50%. Finally, summer FC populations were more sensitive to inactivation and less able to photoreactivate than winter populations. The effect of visible light on photoreactivation levels may be explained by several photo-mechanisms of FC photolyase, such as photodecomposition of the MTHF co-factor and reduction of FAD.

1. Introduction

The increasing recognition of ultraviolet (UV) light as an effective water and wastewater disinfection technology and an alternative to chemical disinfection, has led to an increased number of UV treatment facilities throughout the world. UV light, emitted by mercury arc-lamps (low or medium pressure), is effective against a variety of pathogenic microorganisms including viruses, bacteria and protozoan cysts (Harris et al., 1987; Oguma et al., 2002; Hijnen et al., 2006). UV-induced DNA damage, namely *cis-syn* cyclobutane pyrimidine dimers (CPDs), can however be repaired following UV disinfection by light-dependent (photoreactivation) and light-independent (dark repair) mechanisms found in many organisms, such as fecal coliforms (FC), which include *Escherichia coli*. The count of viable organisms can increase by several log values due to photoreactivation, thus representing an obstacle to reaching safe disinfection levels and a potential disadvantage for application of UV disinfection. For example, *E. coli* can photoreactivate with maximum log repair values reaching 3–4 log (Knudson, 1985; Harris et al., 1987). These species are common biological indicators for disinfection efficiency monitoring in water and wastewater systems. The Quebec Ministère du Développement Durable, de l'Environnement et des Parcs (Ministry of Sustainable Development, Environment and Parks) typically assumes 1 log photoreactivation for UV-treated wastewater discharged into the Saint-Lawrence River and other waterways, and has used this value for specifying UV doses for wastewater treatment (Government of Quebec, 2005). However, 1-log photorepair

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Nomenclature

μS/cm	microsiemens per centimetre
μW/cm^2	microwatt per centimetre squared
CFU	colony forming unit
COD	chemical oxygen demand
CPD	*cis-syn* cyclobutane pyrimidine dimer
d	depth
DNA	deoxyribonucleic acid
FAD	flavin adenine dinucleotide
$FADH^-$	double electron reduced form of FAD
$FADH^o$	oxidized form of FAD
FC	fecal coliform
HDF	7,8-didemethyl-8-hydroxy-5-deazariboflavin
h	hour
I_{avg}	average irradiance
I_o	incident irradiance
LP	low pressure mercury arc (UV lamp)
min	minute
mJ	millijoule
MP	medium pressure mercury arc (UV lamp)
MTHF	5,10-methenyltetrahydrofolate
MWTP	Montreal Wastewater Treatment Plant
mW	milliwatt
N	number of bacteria which survived UV irradiation
N_d	total number of bacteria after 3 h in the dark
N_o	initial number of bacteria
N_p	total number of bacteria after 3 h of exposure to photoreactivation light
NTU	nephelometric turbidity unit
s	second
SS	suspended solids
T	transmittance
UVA	320–400 nm region of the light spectrum
UVB	280–320 nm region of the light spectrum
UVC	100–280 nm region of the light spectrum
UVT	UV transmittance

may be overly conservative under various treatment conditions and further research into the extent of photoreactivation likely to occur under realistic situations is necessary.

The aim of this study is therefore to evaluate pre- and post-UV irradiation conditions that can decrease photoreactivation of FC at those typically low UV doses (10 and 20 mJ/cm^2) used by wastewater treatment plants, and which may also occur in plants whose UV doses are below design levels due to factors related to maintenance or operation, such as lamp sleeve fouling.

The light-sensitive enzyme responsible for CPD repair is called photolyase. When light is absorbed in the 310–480 nm range, CPDs are uncoupled at a rate that is dependent upon temperature, light intensity, pH and ionic strength (Jagger et al., 1967; Chan and Killick, 1995). Of the two co-factors contained in FC photolyase, 5,10-methenyltetrahydrofolate (MTHF) absorbs over 90% of visible light, while the double electron reduced form of flavin adenine dinucleotide ($FADH^-$) catalytically reverses DNA damage (Sancar, 2003; Losi, 2007). Studies have shown that when reduced photolyase absorbs blue light (440–490 nm) in the absence of CPD, MTHF is photodecomposed in *E. coli*. Moreover, photodamaged MTHF was found to decrease photoreactivation by 30% in *E. coli* (Xu et al., 2006). The binding affinity of MTHF was also observed to decrease under low ionic strength conditions, which in turn decreases photoreactivation (Xu et al., 2006).

The effect of visible light alone on bacterial disinfection and photolyase, or of visible light with UV or prior to UV irradiation, is poorly understood. While some studies have shown that illuminating cells at wavelengths in the visible range (>400 nm) prior to UV irradiation increased bacterial resistance in *E. coli* (Tyrrell and Peak, 1978; Lage et al., 2000; Kohli and Gupta, 2003), others also found that irradiation with visible light alone could actually induce bacterial damage (Vermeulen et al., 2008). When visible light is combined with UV before or during disinfection, various photo-mechanisms act upon photolyase for which, depending on various illumination conditions, these mechanisms can take place simultaneously and at different speeds. A list of potential reactions and their quantum yields (for *E. coli*) is shown in Table 1.

Although most researchers agree that the simultaneous exposure to a broad range of wavelengths (such as those emitted by medium-pressure UV lamps) may be involved in decreasing cell survival, no study has identified specific wavelengths contributing to decreased photoreactivation. Studies have found that photoreactivation of *E. coli* following UV disinfection with MP lamps was lower than that with LP lamps at UV doses up to 10 mJ/cm^2 (Oguma et al., 2002; Zimmer and Slawson, 2002). However, at a UV dose of 40 mJ/cm^2, photoreactivation of *E. coli* and total coliforms, both *in vitro* and *in vivo*, was shown to be similar for both MP and LP lamps (Zimmer-Thomas et al., 2007; Quek and Hu, 2008; Guo et al., 2009).

Table 1 – Reactions occurring during UV disinfection under various illumination scenarios. Quantum yields (Φ) are given for *E. coli*.

UV disinfection	
Intact DNA → CPD	$\Phi \approx 24 \times 10^{-4}$ (Görner, 1994)
Pre-UV illumination with visible light	
$FADH^o \rightarrow FADH^-$	$\Phi = 0.05–0.1$ (Sancar, 2003)
$FADH^- - MTHF \rightarrow FADH^-$ –photodecomposed MTHF	$\Phi \approx 0.01$ (Heelis et al., 1987; Schuman Jorns et al., 1990)
Simultaneous illumination (UV + visible light)	
$FADH^o \rightarrow FADH^-$	$\Phi = 0.05–0.1$ (Sancar, 2003)
$FADH^- - MTHF \rightarrow FADH^-$ –photodecomposed MTHF	$\Phi \approx 0.01$ (Heelis et al., 1987; Schuman Jorns et al., 1990)
Intact DNA → CPD	$\Phi \approx 24 \times 10^{-4}$ (Görner, 1994)
Post-UV illumination with visible light	
CPD → Intact DNA	$\Phi = 0.7$ (Sancar, 2003)
$FADH^o \rightarrow FADH^-$	$\Phi = 0.05–0.1$ (Sancar, 2003)