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Effect of Environmental Factors on Photoreactivation of Microorganisms under Indoor Conditions

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***Abstract*—**Ultraviolet (UV) disinfection causes damage to the DNA or RNA of microorganisms, but many microorganisms can repair this damage after exposure to near-UV or visible wavelengths (310–480 nm) by a mechanism called photoreactivation. Photoreactivation is gaining more attention because it can reduce the efficiency of UV disinfection of wastewater several hours after treatment. The focus of many photoreactivation research activities on the single species has caused a considerable lack in knowledge about complex natural communities of microorganisms and their response to UV treatment. In this research, photoreactivation experiments were carried out on the influent of the UV disinfection unit at a municipal wastewater treatment plant (WWTP) in Edmonton, Alberta after exposure to a Medium-Pressure (MP) UV lamp system to evaluate the effect of environmental factors on photoreactivation of microorganisms in the actual municipal wastewater. The effect of reactivation fluence, temperature, and river water on photoreactivation of total coliforms was examined under indoor conditions. The results showed that higher effective reactivation fluence values (up to 20 J/cm^2) and higher temperatures (up to 25 °C) increased the photoreactivation of total coliforms. However, increasing the percentage of river in the mixtures of the effluent and river water decreased the photoreactivation of the mixtures. The results of this research can help the municipal wastewater treatment industry to examine the environmental effects of discharging their effluents into receiving waters.

***Keywords*—**Photoreactivation, reactivation fluence, river water, temperature, ultraviolet disinfection, wastewater effluent.

I. Introduction

ULTRAVIOLET (UV) treatment has been used increasingly in recent years in many water and wastewater treatment plants as an alternative disinfection method to chlorination. Exposure to a sufficient inactivation fluence results in pathogen inactivation, which results in damage to the nucleic acids of the microorganism and stops pathogen reproduction [1]-[4]. However, microorganisms possess the ability to repair the DNA damage caused by UV exposure by two mechanisms including light-dependent (photoreactivation) and light-independent (dark repair) mechanisms [1]-[5].

Photoreactivation is a process by which light in the wavelength range of 310–480 nm is utilized by microorganisms to repair damaged DNA [2]-[5]. This issue has gained importance because the number of microorganisms can increase as a result of photoreactivation in a few hours after treatment, representing a potential disadvantage for the application of UV disinfection [1]-[2]. Photoreactivation has an important impact on wastewater disinfection, where the discharged water can potentially be exposed to sunlight immediately after the UV treatment. Therefore, the investigation of subsequent bacterial repair after UV treatment in wastewater treatment plants is necessary [5].

Irradiance and reactivation fluence have major effects on the photoreactivation process. When investigating the effect of irradiance on photoreactivation of *E. coli*, [6] reported an increase by 7 times in the number of bacteria at 5600 lx in 6 h compared to a dark control. The increase was about 4 times at 1600 lx. However, based on another research study, high average photoreactivation irradiance has lethal effects on bacteria [5].

Temperature is another important factor for photoreactivation. The temperature effect on photoreactivation has been investigated in some research studies [7]-[8]. It was concluded that higher temperatures increased the percent photoreactivation significantly. For example, [6] showed that photoreactivation in *E. coli* increased by 5 times at 25°C compared to 4°C over 6 h.

Water bodies and their nutrients also play an important role during photoreactivation process. Reference [9] added pure culture *E. coli* to the filtered river and found that the number of bacteria decreased after a long exposure (24 h) to a sunlight lamp. Reference [10] investigated the effect of a mixture of effluent and seawater on fecal coliforms. They showed that inactivation of bacteria occurred under both dark and sunlight conditions because of osmotic stress under saline conditions.

As the focus of many research activities was on the single species, this study focuses on an investigation of the potential photoreactivation of microorganisms in the wastewater effluent from a municipal plant in Alberta after MP ultraviolet disinfection. Also, this research will address the effect of environmental factors, such as reactivation fluence, temperature, and river water on photoreactivation. To make the results of photoreactivation experiments independent of light source, a concept, namely the 'effective reactivation fluence' (ERF) is introduced and all the photoreactivation results were evaluated based on it. Effective spectral irradiance (ESI) values were used to calculate ERF. To determine the ESI values, average spectral irradiance (SI) values were multiplied by the average action spectrum factor (AS) values in each band. The SI values were measured by a spectroradiometer (Fig. 1) and the AS values were estimated by the data presented in a research study for *E. coli* [11]. The ERF can be determined by integrating the ESI values in each band, summing over all the wavelengths, and multiplying by time in seconds.

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II. Materials and Methods

The wastewater samples were collected from influent and effluent of the UV disinfection unit at a municipal wastewater treatment plant (WWTP) in Edmonton, Alberta and stored in plastic containers at 4°C. River water samples from the North Saskatchewan River were stored in the same manner. Water dissolved organic carbon (DOC) was measured for effluent and river water samples according to Standard Methods [12]. The DOC was measured with a TOC analyzer (Apollo 9000 TOC Combustion Analyzer, FOLIO Instruments Inc.) and samples were filtered using a prerinsed filter (0.45 μm, Millipore, USA) prior to measurement.

A collimated beam apparatus (Calgon Carbon Corp., Pittsburgh, PA, USA. Model No. ps 1-1-120) equipped with a 1 kW medium pressure (MP) lamp (Calgon Carbon Corp.) was used for bacterial disinfection prior to the photoreactivation experiments according to the standard protocol described by [13]. The irradiance incident on the water surface for each sample was measured with a calibrated radiometer (International Light Inc. Model IL 1400A) along with a detector (International Light Inc. Model 18 SED240). A sun lamp (20 W, F20T12, Philips, USA) was used as the light source for the photoreactivation experiments. The absolute irradiance of sun lamp was measured with a spectroradiometer (JAZ-A, Ocean Optics Inc.) with the software program SpectraSuite. The spectral irradiance for the sun lamp is shown in Fig. 1.

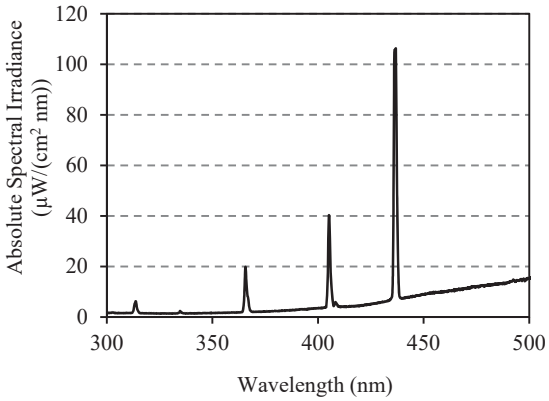

Fig. 1 Absolute irradiance of the sun lamp

Bacterial counts were recorded using the heterotrophic plate count standard. After light exposure, samples were diluted and filtered through a cellulose ester membrane (0.45 μm, Millipore, USA), and incubated at 37 ºC for 24 h on MF-Endo agar to culture total coliforms (used as representative bacteria for all experiments).

Photoreactivation experiments were carried out in Pyrex® dishes covered with Saran Wrap® to avoid sample evaporation. Controls ('dark control') were covered with black plastic and kept at 20 ± 1 °C in a water bath. The sun lamp was positioned 10 cm above the samples and sample collection was every hour for the first hour and every two hours for the next 7 hours to investigate the effect of temperature and river water on photoreactivation. To investigate the effect of the maximum reactivation fluence on photoreactivation, exposure time was extended to 10 h and the sun lamp was positioned 5 cm above the samples.

The temperature effect experiments were conducted using a water bath at temperatures of 5, 10, 15, 20 and 25 ºC following an application of 23 mJ/cm^2 inactivation fluence. The river water addition experiments were conducted following an application of 10 mJ/cm^2 inactivation fluence. Bacteria present in the effluent were separated by centrifugation (Eppendorf centrifuge 5810R, Brinkmann instruments Inc., USA) at 10,000 RPM for 45 min. The river water was filtered through a cellulose ester membrane (0.45 µm, Millipore, USA) to remove natural bacteria. Then, filtered river water samples were spiked with the separated cells to the final concentration as the effluent samples. After that, the mixture of river and effluent water with different ratios (0:100, 20:80, 40:60, 60:40, 80:20 and 100:0) were applied for the photoreactivation study.

III. Quantitative Evaluation of Photoreactivation

The effects of dark reactivation and photoreactivation were evaluated by computing the percent photoreactivation, as defined by [14] as:

$$\text{Percent photoreactivation } (\%) = \frac{N_p - N}{N_0 - N} \times 100 \quad (1)$$

where, N_p = cell number in the photoreactivated sample (CFU/mL), N = immediate survival cell count after UV disinfection (CFU/mL), and N_0 = cell number before UV disinfection (CFU/mL). Dark reactivation was determined in the same manner; the only difference is N_p, which is cell number in the dark reactivated sample.

After calculating the total percent reactivation, the percent dark reactivation was subtracted from the total percent reactivation for each sample to determine the net photoreactivation.

IV. Results and Discussion

A. Effect of Reactivation Fluence

The net percent photoreactivation of total coliforms versus effective reactivation fluence (ERF) is presented in Fig. 2. It can be seen that the percent photoreactivation increased with increased fluence under the sun lamp. During photoreactivation process an enzyme called *photolyase* absorbs near ultraviolet and visible light to initiate an enzymatic reaction for repairing the pyrimidine dimers in DNA by using light energy according to the classical Michaelis–Menten reaction [15]. The enzyme activation depends on wavelengths and light intensity for photoreactivation [15]-[17]. Small amounts of UV-B (280–315) can induce adverse impacts on living systems which reduces bacterial activity [18]-[19]. So, the lack of a UV–B portion in the spectrum of the sunlight lamp (Fig. 1) results in increased photoreactivation under the sun lamp. Another

reason is the low ERF of the sun lamp. Reference [20] showed that *E. coli* cells can be repaired or recovered after exposure to UV–A (320–400 nm) with fluences below 30 J/cm^2. As *photolyase* needs an adequate time to absorb light and initiate the repair reaction, the photoreactivation increased by increasing effective reactivation fluence up to 20 J/cm^2 as in Fig. 2.

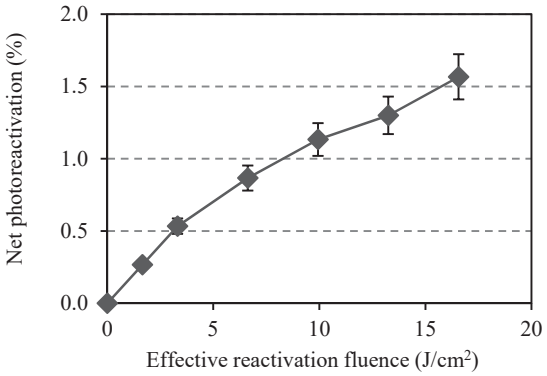

Fig. 2 Net photoreactivation of total coliforms based on the ERF under a sun lamp after applying a 23 mJ/cm^2 inactivation fluence as determined by the target wastewater plant

B. Effect of Temperature

The percent photoreactivation of total coliforms in the effluent of the WWTP increases with increasing temperature (5-25°C), reaching approximately 0.9% at 25°C (Fig. 3). This proves the results of other research studies which reported the same trend for the temperature effect on the photoreactivation of bacteria under indoor conditions [6]-[8].

C. Effect of River Water

The net percent photoreactivation of total coliforms versus the ERF in the mixtures of the effluent and spiked filtered river water after applying an inactivation fluence of 10 mJ/cm^2 under the sun lamp was investigated and is shown in Fig. 4. The final percent photoreactivation of the mixtures decreases from 11% to 4% by increasing the percentage of spiked filtered river water from 0 to 100 % under the sun lamp. The reason might be attributed to the lower nutrient content in the spiked filtered river water. Concentrations of dissolved organic carbon decreased with increasing ratio of spiked filtered river water in the mixtures as shown in Table I. This parameter gives an indication of the amount of nutrients available for bacterial growth, which is probably a reason for the decreasing percent photoreactivation of the samples with increasing filtered river water ratio in the mixtures.

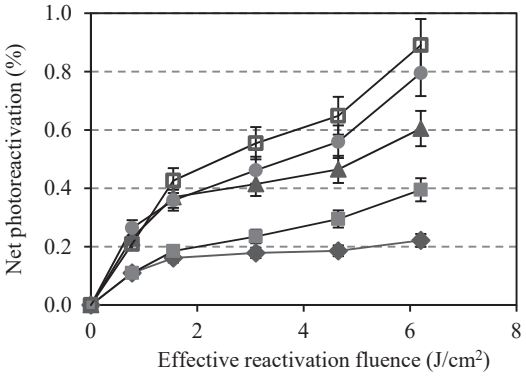

Fig. 3 Net photoreactivation of total coliforms at various temperatures under a sun lamp after applying a 23 mJ/cm^2 inactivation fluence by the target wastewater plant. ♦ 5 ºC; ■ 10 ºC; ▲15 ºC; • 20 ºC; □ 25 ºC

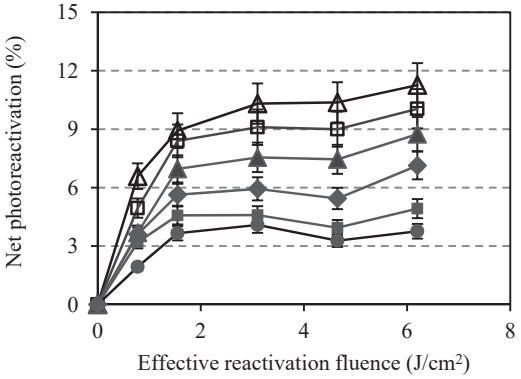

Fig. 4 Net photoreactivation of total coliforms under a sunlight lamp after applying a 10 mJ/cm^2 inactivation fluence. • 100% spiked filtered river water; ■ 80% spiked filtered river water + 20% effluent; ♦ 60% spiked filtered river water + 40% effluent; ▲ 40% spiked filtered river water + 60% effluent; □ 20% spiked filtered river water + 80% effluent; △ 100% effluent

TABLE I
CONCENTRATION OF DISSOLVED ORGANIC CARBON (DOC) OF WASTEWATER EFFLUENT, SPIKED FILTERED RIVER WATER, AND MIXTURES OF EFFLUENT WITH SPIKED FILTERED RIVER WATER

Sample	DOC (mg/L)
Effluent	17.7 ± 0.2
Spiked filtered river water	15.5 ± 0.2
80% Effluent + 20% Spiked filtered river water	17.2 ± 0.2
60% Effluent + 40% Spiked filtered river water	16.7 ± 0.2
40% Effluent + 60% Spiked filtered river water	16.3 ± 0.2
20% Effluent + 80% Spiked filtered river water	15.6 ± 0.2

V. CONCLUSIONS

In summary, the results showed that higher effective reactivation fluence values (up to 20 J/cm^2) and higher temperatures increased the photoreactivation of total coliforms by stimulating the reactivation process. In addition, the results suggested that the bacterial photoreactivation in the effluent

samples decreased by adding river water because of lower nutrient values.

This study demonstrated that the percent photoreactivation of the bacteria, after mixing natural river water with the wastewater effluent, was reduced to about 5%. Therefore, discharging wastewater effluent to river water may not have serious risk for the water body.

Acknowledgments

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