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UV inactivation and characteristics after photoreactivation of *Escherichia coli* with plasmid: Health safety concern about UV disinfection

Meiting Guo [a,b,c], *Jingjing Huang* [c], *Hongying Hu* [c,d,*], *Wenjun Liu* [c], *Jian Yang* [a]

[a] *College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China*
[b] *State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai 200092, China*
[c] *School of Environment, Tsinghua University, Beijing 100084, China*
[d] *Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China*

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ABSTRACT

Occurrence and degree of photoreactivation after ultraviolet (UV) exposure have been widely studied. However, the characteristics of photoreactivated microorganisms were rarely investigated. Hence, in this study, *Escherichia coli* with plasmids of ampicillin (amp)-resistance or fluorescence was used as indicators to examine the UV inactivation efficiencies and variations of characteristics of *E. coli* after subsequent photoreactivation.

The experimental results indicate that the amp-resistant bacteria and the fluorescent bacteria used in this study had similar trends of UV dose–response curves. 3.5-$\log_{10}$ and 3-$\log_{10}$ reductions were achieved with a UV dose of 5 mJ/cm^2 for the amp-resistant and fluorescent *E. coli*, respectively. There was no significant difference in the UV inactivation behavior, as compared with common strains of *E. coli*.

For the amp-resistant *E. coli* and the fluorescent *E. coli*, after exposures with UV doses of 5, 15, 25, 40 and 80 mJ/cm^2, the corresponding percent photoreactivations after a 4 h exposure to photoreactivating light were 1% and 46% respectively for a UV dose of 5 mJ/cm^2, and essentially negligible for all other UV doses. Furthermore, the photoreactivated amp-resistant bacteria still have the ability of amp-resistance. And the revived fluorescent *E. coli* showed similar fluorescent behavior, compared with the untreated bacteria. The experimental results imply that after UV inactivation and subsequent photoreactivation, the bacteria retained the initial characteristics coded in the plasmid. This reveals a possibility that some characteristics of bacteria can retain or recover through photoreactivation, and a safety concern about pathogenicity revival might need to be considered with UV disinfection and photoreactivation.

* *Corresponding author*. School of Environment, Tsinghua University, Beijing 100084, China. Tel.: +86 10 6279 4005; fax: +86 10 6279 7265.
E-mail addresses: guomeiting@tongji.edu.cn (M. Guo), hjj03@tsinghua.edu.cn (J. Huang), hyhu@tsinghua.edu.cn (H. Hu), wjliu@tsinghua.edu.cn (W. Liu), yangjian@tongji.edu.cn (J. Yang).
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1. Introduction

Ultraviolet (UV) light disinfection is gaining more attention as an alternative to chlorine disinfection (Reed, 1998) because of its ability to inactivate a wide-range of pathogens, a lack of hazardous disinfection byproducts and safe and easy operation (Mechsner et al., 1991; Lazarova et al., 1999; Liberti et al., 2000; Martin and Gehr, 2007). However, certain UV irradiated bacteria are able to repair their UV damage under near UV or visible light, which weakens the disinfection efficiency and increases the microbial risk after UV disinfection (Linden et al., 2002). This repair mechanism is called photoreactivation, and it is a major disadvantage of UV technology (Hijnen et al., 2006; Guo et al., 2011).

The photoreactivation potential of bacteria has been widely studied in recent years (Harris et al., 1987; Oguma et al., 2001; Zimmer and Slawson, 2002; Hu et al., 2005). Hoyer (1998) investigated more than a dozen microorganisms as regards photoreactivation. He found that the minimum UV dose in order to achieve 4-$\log_{10}$ reduction of *Escherichia coli* ATCC 11229, considering possible photoreactivation, was 30 mJ/cm^2. In the absence of photoreactivation, he found that a UV dose of only about 10 mJ/cm^2 was sufficient. The results were similar with 16 other microorganisms in his studies. Kashimada et al. (1996) reported that, coliform group and fecal coliform from raw sewage recovered immediately after irradiation and saturated within 120 min. In their review, Hijnen et al. (2006) listed the types of bacteria that can photoreactivate and their reactivation mechanisms. Martin and Gehr (2007) investigated photoreactivation of fecal coliform in the effluent from one wastewater treatment plant in Canada. They found that, the average photoreactivation was 1.2 $\log_{10}$ after exposure under sunlight for 3 h. Since photoreactivation is such a common phenomenon when applying UV technology, many control measures have to be proposed, such as a combination of peracetic acid with UV (Martin and Gehr, 2007), or application of high enough UV dose (Sommer et al., 2000; Guo et al., 2009).

However, most of the studies have focused on the ability of microorganisms to photoreactivate, that is, whether or not photoreactivation can occur. Few studies have investigated the characteristics of the photoreactivated bacteria. This raises a question as to what happens to the photoreactivated microorganisms. It is still not yet known if these revived microorganisms are the same, less or more dangerous than the initial ones in terms of health safety. It is reported that *Cryptosporidium parvum* has the ability to repair, but its pathogenicity cannot recover (Oguma et al., 2001; Zimmer et al., 2003). This result indicated that, although photoreactivation did occur in *C. parvum*, the safety concern is not at the same level as compared to the case before UV treatment. Li et al. (2009) showed in her study not only a reduction in the number of *Giardia lamblia* cysts with secondary wastewater treatment, but also a reduction in the intensities of infection caused by those cysts. However, no results about photoreactivation were mentioned in her study. As regards disinfection, what really matters is reduction of pathogenicity instead of the concentration of microorganisms. It can be assumed that if the photoreactivated bacteria lose part of their functions, this will decrease the risk of photoreactivation to microbial safety. It is important to know whether or not the photoreactivated bacteria still hold their initial characteristics, such as pathogenicity, resistance to some chemicals or some special functions, etc. The answer to that question has a significant impact on microbial safety. So it is important to examine any possible changes in the characteristics of bacteria after photoreactivation. Thus, the significance of this study is to examine some essential factors in terms of photoreactivation and provide guidance regarding UV disinfection and relevant photoreactivation control. To the best of our knowledge, the characteristic variations of bacteria before and after photoreactivation have not been studied so far.

Hence, the objective of the research described herein was to investigate the UV inactivation efficiency, induce photoreactivation and then examine the characteristics of *E. coli* with plasmids after photoreactivation. Ampicillin (Amp)-resistant *E. coli* and green fluorescent *E. coli* were selected in this study as test microorganisms.

2. Materials and methods

2.1. Microorganisms and chemicals

Amp-resistant *E. coli* and fluorescent *E. coli* were used as test microorganisms because they have plasmids, which provide special characteristics. Amp-resistant *E. coli* (*E. coli* DH5α with a pUCm-T carrier) has the gene of amp-resistance. Fluorescent *E. coli* (*E. coli* JM109) has the plasmid pEGFP, which can produce a green fluorescence.

2.2. Water samples

50 μL of preserved cultures of amp-resistant *E. coli* or fluorescent *E. coli* were incubated separately in nutrient broth (beef extract 3 g/L, peptone 10 g/L and NaCl 5 g/L, pH: 7.2 ± 0.2) at 37 °C overnight until the stationary phase was reached. The cells were collected by centrifugation (10,000 rpm, 10 min, 4 °C), washed twice with a sterilized saline solution (0.9%), and subsequently suspended in the sterilized saline solution (0.9%), achieving a concentration of approximately 10^5 to 10^6 CFU/mL.

2.3. UV disinfection experiments

A bench-scale collimated beam apparatus, designed in the laboratory, was used for the UV exposure of samples. This apparatus contains a low-pressure (40 W, 30% UV-C, Cnlight, China) mercury UV lamp. The selected UV lamp is housed in a polyvinyl chloride collimating tube (33 cm) that aids in collimating the UV beam onto the sample to be exposed. The UV dose delivered from the low-pressure mercury lamp was determined according to the Bolton–Linden protocol (Bolton and Linden, 2003).

A 15 mL water sample contained in a Petri dish (60 mm diameter) was placed under the collimated tube and stirred gently during the UV exposure time. The irradiance values were fixed throughout the experiment, and the UV doses were