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Adenovirus inactivation by in situ photocatalytically and photoelectrocatalytically generated halogen viricides

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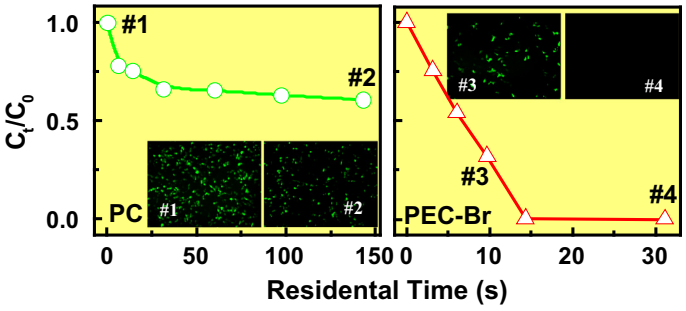
HIGHLIGHTS

- Adenovirus was inactivated by in situ PC and PEC generated halogen viricides.
- The PEC-Br shows the highest virucidal efficiency.
- The PEC-Br can completely inactivate ~1000 TCID50 RDRADS within 31.7 s.
- The superior performances is mainly due to the increased productions of AOSs.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigates and compares the virucidal performances of photocatalytic (PC) and photoelectrocatalytic (PEC) treatments in the presence and absence of halides, such as Br^- and Cl^-, under comparable experimental conditions. The results confirm that the PC virucidal efficiency can be enhanced in the presence of low halide concentrations (e.g., X = Br^- or Cl^-) and further enhanced by applying potential bias onto the photoanode in a PEC system. The PEC treatment in the presence of 1.0 mM Br (PEC-Br) shows the highest virucidal efficiency, enabling complete inactivation of a ~1000 TCID50 replication-deficient recombinant adenovirus (RDRADS) population within 31.7 s. The superior virucidal performances of PEC-X treatments can be attributed to the increased production of active oxygen species and additional viricides resulting from the PEC halide oxidation, as well as prolonged lifetime of photoholes (h^+) for direct inactivation. The findings of this work confirm that new forms of active species generated in situ via a PC or PEC process are effective for viruses.

1. Introduction

The outbreak of severe acute respiratory syndrome (SARS) in February 2003, caused by the deadly coronavirus, affected 3547 individuals, resulting in 182 deaths by April 2003 [1]. In recent history, numerous emerging and re-emerging viruses have caused widespread human disease and deaths, raising worldwide fears. The most well-known examples are the avian influenza H5N1 virus

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in 1997, the H7N9 virus in 2013, and the swine influenza H1N1 virus in 2009–2010. Although the transmission modes of viruses are diverse, wastewater is recognized as an important source of contaminated microbial aerosols and liquid. Given the importance of this factor in virus transmission [2], a number of research projects have focused on developing highly effective inactivation/separation water treatment techniques [3,4]. However, virus are submicroscopic particles. As such, the conventional filtration methods are generally less effective; furthermore, biofouling also leads to extra operational costs [5]. When considering UV and chlorination inactivation techniques, a number of pathogens, particularly some virus species, are naturally resistant to such traditional treatments [6,7]. In addition, the formations of toxic and potential carcinogenic by-products are also drawbacks of chlorine treatment [8–10]. Ozone disinfection has been considered as an effective alternative [11]; however, disadvantages, such as its toxic and corrosive characteristics, also hinder its practical application [12].

Recently developed TiO_2 photocatalysis (PC) based advanced oxidation processes for inactivation of virus have demonstrated promising outcomes [13,14]. These works have revealed the virucidal effect of PC processes at nanostructured TiO_2 on the inactivation of different viruses, including phage MS2, phage PL-1, and bacteriophage Qβ [3,15,16]. Nevertheless, PC inactivation of adenovirus has not been previously attempted, despite being indicated in the "Drinking Water Contaminant Candidate List" [17] because of their prevalent potential health risks and important role for water epidemiology [18–20]. Moreover, adenoviruses are highly resistant to common treatments when compared to other viral pathogens of concern in wastewater and drinking water [21,22], due to their DNA's double-stranded nature [23].

TiO_2 photoelectrocatalysis (PEC) has been proven to enhance the degradation efficiencies of organic pollutants in water [24], but are rarely used to improve the virus inactivation efficiency [25]. We have previously demonstrated the effectiveness of a PEC-based bactericidal technique in the presence of Br^- (PEC-Br) for instant inactivation and rapid decomposition of gram-negative bacteria [26,27]. In this study, we investigate the application of PC and PEC-based bactericidal techniques in the presence of halides to for inactivation of replication-deficient recombinant adenovirus (RDRADS). The specific study scope was to systematically evaluate and quantitatively compare the virucidal performance of PC and PEC treatment of RDRADS in the presence and absence of halides such as Cl^- and Br^-, using RDRADS encoding green fluorescent protein (GFP). The infectivity of the RDRADS can be accurately and conveniently determined using this method, because the virus encodes a GFP gene that can be expressed in host cells [28].

2. Materials and methods

2.1. Materials

Materials used for this study included indium tin oxide conducting glass slides (ITO, 8 Ω/square) from Delta Technologies Ltd. (USA), Titanium butoxide (97%), and other chemicals of analytical grade from Aldrich (unless otherwise stated). All solutions were prepared using high purity deionized water (Millipore Corp., 18 MΩ cm).

2.2. UV-LED array photoelectrochemical cell

All photocatalytic and photoelectrocatalytic disinfection experiments were performed in a UV-LED array thin layer electrochemical cell with a quartz window for illumination. The preparation of TiO_2 electrodes, cell configuration, and system set up were reported in detail, in our previous studies [26]. Briefly, the thickness of the reaction chamber and the illumination window area were 0.25 mm and 462 mm^2, respectively. A UV-LED array consisting of 4 pieces of UV-LED (NCCU033 (T), Nichia Corporation) was used as the light source. The emission electrum of the LED was centered at 365 nm with a spectrum half width of 8 nm. The UV intensity was adjusted by a power supply and measured with an UV-irradiance meter (UV-A, Beijing Normal University). A 0.1 M $NaNO_3$ was used as the supporting electrolyte. UV light intensity on the electrode surface was measured with an UV-irradiance meter (UV-A, Instruments of Beijing Normal University).

2.3. Viral strains, culture conditions and virus preparation

In this study, we used RDRADS encoding GFP as a model virus based on its sensitivity, specificity, and experimental safety. All experiments were performed in a biosafety level II laboratory and were conducted under appropriate conditions according to the Ref. [29]. A seed stock solution of the virus was obtained from the Experimental Medical Research Centre of Guangzhou Medical College. The viruses are replication deficient and can be propagated only in their packing cells. This feature enabled us to assay infected cells accurately, by counting cells with green fluorescence under a fluorescence microscope. The virus-containing cell was obtained by culturing with HEK293 (human embryonic kidney) cells and subsequent triple freezing of the infected cell culture in Dulbecco's-modified Eagle medium (DMEM) maintenance medium.

The viral suspension was purified using Sartobind Q 75 (Sartorius, Göttingen, Germany), according to the manufacturer menu. Prior to treatment, cell debris were removed by centrifugation at 3500 rpm for 15 min; the resulting supernatant was passed through a polyvinylidene fluoride (PVDF) MF membrane with nominal pore size of 0.22 μm. Then, 12.5 U/ml of nucleases (Takara Biotechnology (Dalian) Co., Ltd., China) were used to cleave the phosphodiester bonds between the nucleotide subunits of nucleic acids. The resulting viral suspension was passed through a centrifugal concentrator (100 000 MWCO) (Sartorius, Göttingen, Germany) to remove the salt. The virus concentrated with the centrifuge was finally diluted with sterilized H_2O to a desired concentration for the experiment. The initial population of RDRADS for each disinfection experiment was approximately 1000 Tissue Culture Infective Dose 50 (TCID50).

2.4. Inactivation of viruses and virological analytical procedure

Both PC and PEC disinfection experiments were performed under identical UV intensity using the same UV-LED array photoelectrochemical cell. For the PEC degradation experiments, a 0.1 M $NaNO_3$ solution was used as the supporting electrolyte, and a voltammograph (cv-27, BAS) was used for electrochemical control. Potential and current signals were monitored using a Macintosh computer (7220/200) coupled with a Maclab 400 interface (AD Instruments). A suspended solution containing a known number of RDRADS and 0.1 M of $NaNO_3$ prepared in sterilized tubes on a super clean bench was continuously injected into the cell via a precision pump during the inactivation process. The inactivation time of each sample was controlled by adjusting the flow rate. A sufficient volume of the inactivation sample was collected for further analysis after the system reached its steady-state. A 0.1 M $NaNO_3$ solution was used to clean the cell between the two sample injections. The samples collected after PEC treatment were analyzed the infectivity by green fluorescence of PK15 (pig kidney) cells. For assaying virus infectivity, 100 μl samples were added into 80% confluent PK15 cell monolayer (Fig. S1) in 48-well plates.

All of the disinfection experiments were repeated three times. The plates were rocked every 10–15 min during a 90 min cultured