Science of the Total Environment 541 (2016) 1–7

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Sunlight inactivation of somatic coliphage in the presence of natural organic matter

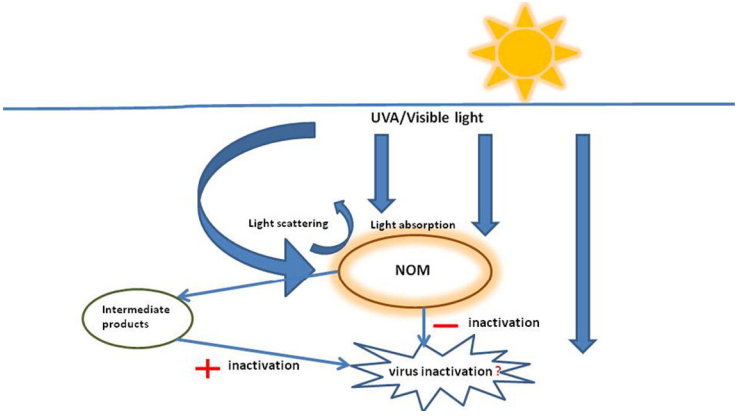
Chen-Xi Sun [a], Masaaki Kitajima [b], Karina Yew-Hoong Gin [a,c,*]

[a] Department of Civil and Environmental Engineering, National University of Singapore, Singapore
[b] Center for Environmental Sensing and Modeling, Singapore-MIT Alliance for Research and Technology, Singapore
[c] NUS Environmental Research Institute, National University of Singapore, Singapore

HIGHLIGHTS

- UVA and visible lightcan cause direct inactivation of DNA virus
- NOM can either enhance virus inactivation of reduce virus inactivation
- The effect of NOM on virus inactivation follows parabolic relationship

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 27 August 2015
Accepted 27 August 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
phiX174
Somatic coliphage
Sunlight inactivation
NOM
DNA virus

ABSTRACT

Long wavelengths of sunlight spectrum (UVA and visible light), as well as natural organic matter (NOM) are important environmental factors affecting survival of viruses in aquatic environment through direct and indirect inactivation. In order to understand the virus inactivation kinetics under such conditions, this study investigated the effects of Suwannee River natural organic matter (NOM) on the inactivation of a somatic coliphage, phiX174, by UVA and visible light. Experiments were carried out to examine the virucidal effects of UVA/visible light, assess the influence of SRNOM at different concentrations, and identify the effective ROS in virus inactivation. The results from this study showed that the presence of NOM could either enhance virus inactivation or reduce virus inactivation depending on the concentration, where the inactivation rate followed a parabolic relationship against NOM concentration. The results indicated that moderate levels of NOM (11 ppm) had the strongest antiviral activity, while very low or very high NOM concentrations prolonged virus survival. The results also showed that OH▪ was the primary ROS in causing phiX174 (ssDNA virus) inactivation, unlike previous findings where $^1O_2$ was the primary ROS causing MS2 (ssRNA virus) inactivation. The phiX174 inactivation by OH· could be described as $k = 3.7 \times 10^{13}[OH\cdot] + 1.404$ ($R^2 = 0.8527$).

* Corresponding author at: Department of Civil and Environmental Engineering, National University of Singapore, 1 Engineering Drive 2, E1A 07–03, 117576, Singapore.
E-mail address: ceeginyh@nus.edu.sg (K.Y.-H. Gin).

http://dx.doi.org/10.1016/j.scitotenv.2015.08.136
0048-9697/

1. Introduction

Human enteric viruses infect humans via the gastrointestinal tract and cause acute gastroenteritis, hepatitis, and/or other adverse health effects (Wyn-Jones and Sellwood, 2001). It has been found that enteric viruses occur widely in various environmental waters, including drinking water sources (e.g., river, reservoir, lake, and groundwater) and recreational water (Fong and Lipp, 2005). Recently, global attention has been drawn to better understand the survival of these viruses in environmental waters. However, enteric viruses are difficult to culture and in some cases, even impossible (Hamza et al., 2011). As a result, surrogate viruses, such as MS2 and phiX174, have been used as model viruses to study the behavior of enteric viruses (Gerhardts et al., 2009).

A number of studies have reported that the survival of these viruses in aquatic environments can be affected by various physical, chemical and biological factors, including sunlight (Watts et al., 1995) and natural organic matter (NOM) (Kohn et al., 2007). Sunlight-induced virus inactivation may involve three independent mechanisms: direct photobiological damage, exogenous photooxidation (capsid damage by exogenous chromophores) and endogenous photooxidation (Davies-Colley et al., 1999). The photobiological damage on the virus occurs when photons are absorbed by viral nucleic acid or proteins, leading to structural damage of the viral genome or protein (McLaren and Shugar, 1964). Photooxidation processes involve the absorbance of photons, transformation of energy and subsequent generation of reactive intermediates ($^1O_2$, ▪OH, *DOM, etc.) which can lead to virus inactivation (Kohn and Nelson, 2007). This process is catalyzed by photosensitizers (substances that initiate or catalyze photochemical reactions). NOM is a commonly found photosensitizer in surface waters with complex and undefined characteristics (Canonica et al., 1995). It has been found to either contribute to virus inactivation through generation of virucidal reactive intermediates or increase virus inactivation through association with virus particles (Kohn et al., 2007). In addition, NOM can act as a light scattering and absorbing substance, reducing the effective sunlight intensity penetrating into the water column for both the UV and visible wavelengths (Bricaud et al., 1981). This reduces the photobiological damage of the virus, leading to greater survival of the virus. Therefore, the overall effects of NOM on virus inactivation by sunlight are a combination of several processes and highly dependent on NOM composition and concentration.

Several studies have been performed to investigate the influence of NOM on virus survival. Under the full spectrum of sunlight exposure, the presence of Suwannee River Natural Organic Matter (SRNOM) was found to decrease the overall sunlight inactivation rates of porcine rotavirus and MS2 coliphage due to attenuation of lower wavelengths of light (Romero et al., 2011). A sunlight inactivation study on MS2 carried out using waste stabilization pond water with a concentration of 15 mg/L total organic carbon (TOC) showed that the indirect inactivation process mediated by the organic matter was more dominant than the direct photoinactivation process (Kohn and Nelson, 2007). However, most of these studies were carried out at particular values of NOM and did not cover a wide range of all the possible concentrations found in nature and their impact on virus survival in aquatic environments. In addition, most of these studies were performed with RNA viruses such as MS2 and rotavirus. However, a significant portion of viruses in surface waters are DNA viruses whose survival is different from that of RNA viruses (Sinton and Finlay, 1999), and relatively less efforts were made to understand their survival pattern.

For these above-mentioned reasons, the present study aimed to provide a more comprehensive understanding on the role of NOM on DNA virus inactivation by long wavelengths of sunlight in water. Specifically, we investigated the effects of NOM (ranging from 0 to 65 ppm TOC which covered typical surface water NOM concentrations) on the inactivation kinetics of DNA virus using the somatic coliphage phiX174 as a model virus. The results of this study provide information on virus survival influenced by NOM across a wide range of concentrations and answer the question of whether the presence of NOM increases or decreases the persistence of viruses in aquatic environments. The relationship between phiX174 inactivation and NOM concentration was described quantitatively by a mathematical equation, which can be used for predicting virus survival in NOM containing waters.

2. Materials and methods

Suwannee River NOM was obtained from the International Humic Substances Society (IHSS). All experiments were conducted in 1.0 mM $NaHCO_3$ (Sigma S6014) solution. A sunlight simulator (Atlas SUNTEST CPS+) was used to generate sunlight (UVA and visible light) with constant intensity (450 W/m^2). Temperature was maintained constant at 30 °C by circulating water from a chiller (Shelton SAE-AC1).

2.1. Coliphage and host bacteria preparation

The somatic coliphage, phiX174 (ATCC 13706-B1), and its host bacteria, *Escherichia coli* CN-13 (ATCC 700609), were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). PhiX174 was propagated with the *E. coli* host using the agar overlay method as described by ATCC for *E. coli* phage with slight modifications. Briefly, 0.7% tryptic soy agar (TSA; Sigma-Aldrich) mixed with log-phase *E. coli* and somatic coliphage phiX174 was overlaid on 1.5% TSA plates. Each plate contained 5 mL of 0.7% TSA with 200-μL *E. coli* and 500-μL phiX174 stock. The total number of plates was determined by the total volume of 0.7% TSA. The inoculated plates were then incubated for 16–24 h before the soft agar layers from different plates were scraped off the surfaces and centrifuged at 1000 ×*g* for 25 min to sediment the host cellular debris and agar. The supernatant was then collected and filtered through a 0.22-μm-pore-size membrane (Merck Millipore).

The coliphage phiX174 stock was then concentrated and purified with polyethylene glycol (PEG) precipitation, as described previously (Lewis and Metcalf, 1988) with modification. Briefly, PEG 8000 (Sigma P5413) and NaCl (Sigma S3014) were added to the phiX174 stock to form a final concentration of 8% (wt/vol) and 0.5 M, respectively. The resulting suspension was then stirred for 2 h at 4 °C and centrifuged at 12,000 ×*g* for 60 min. The PEG containing supernatant was discarded. The pellet was then resuspended in sterile 1.0 mM $NaHCO_3$ solution, sonicated for 30 s, shaken for 20 min at 250 rpm, and centrifuged at 10,000 ×*g* for 20 min. The supernatant was collected and the titer of phiX174 in the purified stock was determined to be 3×10^9 plaque forming unit (PFU)/mL. The purified stock was kept at 4 °C until the experiments were conducted.

2.2. Coliphage enumeration

The concentration of phiX174 was determined by the double-agar layer (DAL) plaque assay, as described in USEPA Method 1602 (Association APH, 2001). The samples were diluted as needed, and all dilutions were assayed in duplicate.

2.3. Sunlight inactivation experiment

The purified phiX174 stock was spiked into 10 mL of each water sample to a final coliphage concentration of approx. 1.0×10^6 PFU/mL. The samples were irradiated by UVA and visible light from a sunlight simulator (Atlas SUNTEST CPS+) at 450 W/m^2 for 2 h each. The irradiation spectrum is shown in Fig. 1. The temperature for the experiment was maintained by circulating water from a chiller at 30 °C. A 200-μL sample was taken from each reactor at 30 min intervals and kept at 4 °C in the dark until samples were subjected to coliphage titration.