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Influence of solution chemistry on the inactivation of particle-associated viruses by UV irradiation

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ABSTRACT

MS2 inactivation by UV irradiance was investigated with the focus on how the disinfection efficacy is influenced by bacteriophage MS2 aggregation and adsorption to particles in solutions with different compositions. Kaolinite and *Microcystis aeruginosa* were used as model inorganic and organic particles, respectively. In the absence of model particles, MS2 aggregates formed in either 1 mM NaCl at pH = 3 or 50–200 mM ionic strength $CaCl_2$ solutions at pH = 7 led to a decrease in the MS2 inactivation efficacy because the virions located inside the aggregate were protected from the UV irradiation. In the presence of kaolinite and *Microcystis aeruginosa*, MS2 adsorbed onto the particles in either 1 mM NaCl at pH = 3 or 50–200 mM $CaCl_2$ solutions at pH = 7. In contrast to MS2 aggregates formed without the presence of particles, more MS2 virions adsorbed on these particles were exposed to UV irradiation to allow an increase in MS2 inactivation. In either 1 mM NaCl at pH from 4 to 8 or 2–200 mM NaCl solutions at pH = 7, the absence of MS2 aggregation and adsorption onto the model particles explained why MS2 inactivation was not influenced by pH, ionic strength, and the presence of model particles in these conditions. The influence of virus adsorption and aggregation on the UV disinfection efficiency found in this research suggests the necessity of accounting for particles and cation composition in virus inactivation for drinking water.

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1. Introduction

Virus disinfection can be a serious challenge in providing safe drinking water. However, not enough attention has been paid to virus disinfection compared to bacteria disinfection because of the lack of systematic monitoring of viruses in most countries. Many human enteric viruses, like rotavirus, norovirus, and enterovirus, could cause outbreaks of infectious diseases [1]. Severe diarrhea for children under the age of 5 due to rotavirus infection caused up to 591,000 deaths/year in 2000 and 233,000 deaths/year in 2013 [2]. Hepatitis A and hepatitis E viral infections could be related to untreated or improperly treated drinking water [3]. Hepatitis E infection is particularly severe for pregnant women, with the mortality rate as high as 25% [4]. Norovirus transmits through the fecal-oral route and causes acute gastroenteritis among people of all ages [5]. To prevent the transmission of human pathogenic viruses, special attention needs to be paid to virus disinfection in water treatment.

Most physical processes in water treatment, including coagulation, sedimentation, and granular filtration, are not capable of sufficiently removing viruses because of the nanometer size of the virions [6] and the unfavorable virus aggregation or adsorption to sand surfaces [7–9]. For these reasons, the control of virus contamination in drinking water relies on disinfection processes. Chlorination, ozonation, and UV irradiation are widely accepted disinfection processes. Compared to chlorination, UV irradiation has the advantage of producing less toxic disinfection byproducts (DBP) [10]. Though ozone is a strong disinfectant [11], the high cost of operation and maintenance and the production of toxic bromate DBP are of concern [12,13]. UV disinfection has some advantages over chlorination and ozonation, because of the lack of toxic DBP formation and the relatively low cost, respectively [14].

In this study, we investigated how virus disinfection by UV irradiation is influenced by virus aggregation and adsorption to suspended particles. While in physical treatment processes including coagulation and filtration, the occurrence of virus aggregation or adsorption to suspended particles has been reported to increase

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the virus removal [15–17], it is not understood yet how the virus aggregation or adsorption to suspended particles would influence the virus disinfection by UV irradiation. Bacteria disinfection efficacy by UV irradiation has been shown to decrease when bacteria cells adsorbed to suspended particles [18]. However, this observation may not be applicable for virus disinfection due to the different structures of virus and bacteria.

We hypothesize that virus aggregation and adsorption influence virus disinfection efficacy by UV irradiation due to the decrease in UV exposure. Algae bloom has become one of most serious water problem in south China [19,20]. Because algal particles are difficult to neutralize and destabilize under the conditions relevant for drinking water treatment in south China [21], the removal of algae is usually not satisfactory, allowing them to be present in the disinfection reactor [22]. Besides algae, inorganic particles could also be present, especially if the water source is turbid surface water. These inorganic particles could influence the virus disinfection efficacy by UV irradiation. For these reasons, the influence of *Microcystis aeruginosa*, a model algae, and kaolinite, a model inorganic particle, on virus disinfection efficacy by UV irradiation is investigated in this study. Bacteriophage MS2 is used as the surrogate for enteric viruses for their similarities in size and structure [23]. MS2 aggregation and adsorption to suspended particles are studied under relevant environmental factors including pH conditions, concentrations of suspended particles, and cation concentrations [7,24]. By understanding the influence of water source chemical composition on the relationship between the disinfection efficacy and virus adsorption/aggregation, virus disinfection by UV irradiation can be better controlled for safe water treatment.

2. Materials and methods

2.1. Material and solution preparation

Analytical grade NaOH, HCl, and $NaHCO_3$ were purchased from Sinopharm Chemical Reagent CO., Ltd (Shanghai, China). Kaolinite was purchased from Sigma-Aldrich (United States). Mediums for MS2 propagation (solid, semisolid, and liquid) were purchased from Qingdao Hope Bio-Technogy Co., Ltd (Qingdao, China). MS2 (ATCC 15597-B1) and its *E. coli* host (ATCC 15597) were purchased from American Type Culture Collection (ATCC, Manassas, USA). *Microcystis aeruginosa* was supplied by the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China).

2.2. MS2 propagation and purification

The method for MS2 propagation and purification was reported previously [17,25]. Briefly, the ATCC 15597 *E. coli* was used as the host bacteria for MS2. The *E. coli* was amplified in the tryptic soy broth liquid media at 37 °C and shaken at 200 rpm. The MS2 was inoculated into the *E. coli* suspension when the absorbance at 420 nm wavelength reached 0.8. The MS2 incubation lasted for 24 h at 37 °C with the suspension shaken at 200 rpm. After incubation, the bacteria debris were precipitated by centrifuge at 5000 rpm for 15 min, and were further removed by filtering the supernatant with a 0.2 μm Millipore hydrophilic membrane (Bedford, MA). Further purification of the MS2 stock was conducted in a Millipore stirred cell with a Koch 10 kDa MWCO membrane (Wilmington, MA). Each 200 mL MS2 suspension was washed constantly with 1 L of the 1 mM $NaHCO_3$ solution. The residue of the growth media was washed away, while the MS2 bacteriophage was retained in the stirred cell by the membrane. The retained MS2 suspension was used as the stock solution for the MS2 inactivation experiment. The MS2 concentration was determined with the plaque forming unit (PFU) method as previously described [26].

2.3. Hydrodynamic diameter and electrophoretic mobility (EPM) measurement for particles

The hydrodynamic diameters and the EPMs of MS2, kaolinite, and *Microcystis aerugunosa* were determined with a Malvern NanoZS90 Zetasizer (Southeborough, MA) at 25 °C. The concentration of MS2, kaolinite, and *Microcystis aerugunosa* in the measurement were 1.0×10^{11} pfu/mL, 100 mg/L, and 1.0×10^5 Cells/mL, respectively. There were 3 replicates in each measurement. The hydrodynamic diameters of MS2, kaolinite, and *Microcystis aerugunosa* were measured at pH = 7 in 1 mM NaCl solution. The EPMs were measured at pH from 3.0 to 8.0. The solution pH was adjusted by 1 mol/L NaOH and HCl. The EPMs were also determined in ionic strength from 2 mM to 200 mM for NaCl and $CaCl_2$ solutions at pH = 7.0.

2.4. MS2 adsorption onto kaolinite and Microcystis aeruginosa

100 mg/L kaolinite (or 10^5 Cells/mL *Microcystis aeruginosa*) was prepared in NaCl or ($CaCl_2$ solution) at a certain ionic strength. The solution pH was adjusted to the target value with 1 mol/L NaOH or HCl solution. 0.1 mL MS2 at a concentration of 1.0×10^8 pfu/mL was added to 100 mL of the particle suspension. The mixture was shaken at 200 rpm at 25 °C for 24 h. The sample was then centrifuged at 2000 *g* for 30 min to separate the suspended and the particle-associated MS2. The MS2 in the supernatant was quantified with the PFU method [27]. A control experiment in the absence of suspended particles was performed in DI water. Three replicates were conducted for each experiment.

2.5. MS2 inactivation by UV irradiation

The UV collimated beam apparatus (CBA) with only significant UV emission at 253.7 nm was constructed as recommended by the USEPA [28]. 20 mL of sample in a quartz dish was gently stirred and exposed to the UV irradiation. Prior to each experiment, the CBA was stabilized for 30 min to obtain a stable UV irradiation. Every inactivation experiment was replicated twice. A UV dosage of 40 mJ/cm^2 was chosen because this is the recommended UV dosage in water treatment [29]. The actual UV dosage was determined with the method described previously [30]. Briefly, the average germicidal fluence rate E'_{avg} (UV intensity) in the water was calculated as the product of the radiometer reading at the center of the dish at a vertical position (E_0), the ratio of the average of the incident irradiance over the area of the petri dish to the irradiance at the center of the dish ($P_f = 0.96$), the reflection factor ($R_f = 0.975$) the water absorption factor (W_f), and the divergent factor (D_f). W_f is a water factor, which accounts for the decrease in irradiance arising from absorption as the beam passed through the water, and it is defined as:

$$W_f = \frac{1 - 10^{-al}}{al\ln(10)}$$

where a is the absorbance for a 1 cm path length (cm^{-1}), and l is the vertical path length (cm) of the water in the petri dish. D_f is the divergence factor because the beam is not perfectly collimated and diverges significantly:

$$D_f = \frac{L}{l + L}$$

L is the distance from the UV lamp to the surface of the cell suspension. ($E'_{avg} \times t$) is a constant.

A control experiment to determine MS2 desorption from particles was conducted in the absence of UV irradiation. After adsorption, a sample was taken from the suspension solution and then was injected into elution solution to elute the adsorbed MS2 by

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