Photoinactivation of virus on iron-oxide coated sand:
Enhancing inactivation in sunlit waters

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Article history:
Received 7 July 2011
Received in revised form
23 December 2011
Accepted 24 December 2011
Available online 4 January 2012

Keywords:
Wetlands
Virus inactivation
Iron-oxide coated sand
Photocatalysis
Adsorption
Sunlight

Abstract
Adsorption onto iron oxides can enhance the removal of waterborne viruses in constructed wetlands and soils. If reversible adsorption is not coupled with inactivation, however, infective viruses may be released when changes in solution conditions cause desorption. The goals of this study were to investigate the release of infective bacteriophages MS2 and φX174 (two human viral indicators) after adsorption onto an iron oxide coated sand (IOCS), and to promote viral inactivation by exploiting the photoreactive properties of the IOCS. The iron oxide coating greatly enhanced viral adsorption (adsorption densities up to \(10^9\) infective viruses/g IOCS) onto the sand, but had no affect on infectivity. Viruses that were adsorbed onto IOCS under control conditions (pH 7.5, 10 mM Tris, 1250 \(\mu\)S/cm) were released into solution in an infective state with increases in pH and humic acid concentrations. The exposure of IOCS-adsorbed MS2 to sunlight irradiation caused significant inactivation via a photocatalytic mechanism in both buffered solutions and in wastewater samples (4.9 \(\log_{10}\) and 3.3 \(\log_{10}\) inactivation after 24-h exposure, respectively). Unlike MS2, φX174 inactivation was not enhanced by photocatalysis. In summary, IOCS enhanced the separation of viruses from the water column, and additionally provided a photocatalytic mechanism to promote inactivation of one of the surrogates studied. These qualities make it an attractive option for improving viral control strategies in constructed wetlands.

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1. Introduction
Controlling the transmission of waterborne viruses is made difficult by the fact that viruses are incompletely removed through conventional treatment processes. Their small size helps them to bypass physical removal treatments such as sedimentation and conventional filtration (Symonds et al., 2005; Thompson et al., 2003). Even when associated with particles, viruses tend to remain in suspension and pass through granular media filters (Rao et al., 1984; Templeton et al., 2005). They also exhibit higher resistance to many disinfectants compared to other pathogens, including chlorine and UV (LeChevallier, 2004; Thompson et al., 2003). Thus, alternative processes are needed to supplement conventional removal and inactivation strategies.

Natural treatment systems, such as constructed wetlands, have shown potential for virus removal. Viruses tend to adsorb poorly onto sand (Bales et al., 1991, 1993), which is commonly used as wetland matrix (Brix and Arias, 2005; Hoffmann et al., 2011). The presence of metal oxides can greatly enhance adsorption. The positively-charged metal oxide surfaces naturally present on sands and soils offer favorable sites for the adsorption of negatively charged viruses and other pathogens. Similarly, pure metal oxides or
metal oxide-coated sands have also been studied to enhance the removal of viruses, bacteria, and protozoa (Abudalo et al., 2005; Atherton and Bell, 1983; Gutierrez et al., 2009; Mills et al., 1994; Ryan et al., 2002).

While metal oxides enhance adsorption, the impact of this interaction on viral infectivity is unclear. Adsorption has been shown to produce the full range of outcomes, from increasing inactivation, to having no effect, to protecting viruses from inactivation (Grant et al., 1993). Viruses were not inactivated by adsorption onto Fe₂O₃ and silicon dioxide (Bales et al., 1991, 1993; Gutierrez et al., 2009; Murray and Laband, 1979), though they were inactivated by iron oxide-coated sand and aluminum (Ryan et al., 2002; Thurman and Gerba, 1988) and CuO and MnO₂ (Murray and Laband, 1979). If adsorption alone does not inactivate viruses, they may still pose a public health risk if they are released (desorbed) back into solution.

The photoreactive nature of the iron-oxide surface provides a unique opportunity for enhancing the inactivation of adsorbed viruses. Iron oxides are semiconductors capable of producing oxidants when irradiated with sunlight (Andreozzi et al., 2003; Du et al., 2008). They can also participate in photo-Fenton reactions leading to the production of hydroxyl radicals (He et al., 2002), which cause virus inactivation (Nieto-Juarez et al., 2010). Furthermore, the direct contact of the adsorbed viruses with the iron-oxide surface places them directly adjacent to the source of the inactivating oxidant. This physical proximity would be expected to greatly increase inactivation (Kohn et al., 2007; Koizumi and Taya, 2002; Nieto-Juarez et al., 2010).

To ensure effective wetland treatment, viruses must either be inactivated or irreversibly adsorbed across the water quality conditions that might be encountered in the wetland. The ideal wetland matrix aimed at reducing viral load would have several characteristics: it would 1) strongly adsorb viruses with a high adsorption density, 2) adsorb viruses over an environmental range of water quality conditions, and 3) pose no threat of releasing infective viruses because adsorption is irreversible and the particulate matter is immobile or 4) because the viruses are inactivated on the matrix. The latter two criteria are the focus of this study (adsorption of the phages onto iron-oxide coated sand was also characterized; data are presented in Supplemental Information). Specifically, the goals of this study were to determine if adsorbed bacteriophages MS2 and φX174 – two indicators of human viruses (Leclerc et al., 2000) – are released from iron-oxide coated sand in an infective state, and if inactivation of adsorbed viruses can be enhanced by exploiting the photoreactive properties of the iron-oxide coating.

2. Methods

Two sets of experiments were designed to answer the principal research questions: 1) are adsorbed bacteriophages MS2 and φX174 released from iron-oxide coated sand (IOCS) in an infective state, and 2) can the photoreactive properties of IOCS be used to enhance phage inactivation. The goal of the first group of experiments was to characterize the infectivity of viruses desorbed from IOCS. For these experiments, the basic adsorption characteristics of the phages onto IOCS were first determined under “control” water quality conditions meant to simulate a typical wastewater. Solution conditions were subsequently altered to span an environmental range of water qualities (conductivity, pH, and humic acid concentrations), and the desorption and infectivity of the viruses were measured.

In the second set of experiments, we tested if virus inactivation could be enhanced by exploiting the photoreactive properties of the IOCS. The importance of the physical proximity of the virus to the IOCS surface was studied by comparing inactivation of adsorbed and unadsorbed viruses. Virus inactivation was enumerated with two methods: plating and quantitative PCR (qPCR). These methods were chosen due to the complementarity of the information provided. Plating (virus culture) provides information on virus infectivity, but cannot enumerate inactivated viruses. In experiments involving viral adsorption, culturing must be preceded by a desorption step to release the viruses into solution. If the initial number of viruses is not recovered after desorption, at least two phenomena could be at play: 1) the viruses may remain adsorbed to the IOCS, or 2) the viruses could have been effectively desorbed, but in an inactivated and non-culturable state. qPCR served to expose this second possibility by quantifying the presence of total desorbed viruses, both infective and inactivated.

Batch tests were selected over column studies to facilitate experimental control and reproduction, and to more easily test a range of solution conditions.

2.1. Microorganisms

Bacteriophages MS2 and φX174 and their E. coli hosts were prepared as previously described (Pecson et al., 2009). Virus concentrations are reported as plaque forming units (pfu) per mL. Basic characteristics of the two phages are presented in Table 1.

2.2. Buffers

The control buffer for these experiments was 10 mM Tris (Ultrapure >99.9%, AppliChem, Darmstadt, Germany), pH 7.5, with a conductivity of 1250 µS/cm (Cond315i conductivity meter with TetraCon 325 probe, WTW, Weilheim, Germany). This concentration of Tris was necessary to prevent pH changes of greater than 0.1 pH units when 0.5 ml of the buffer was mixed with 1 g of the acidic IOCS. The buffer was adjusted with HCl or NaOH to obtain four different pH levels (6.5 – 9.5). The conductivity of all the buffers was adjusted to

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MS2</th>
<th>φX174</th>
</tr>
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<tbody>
<tr>
<td>Isoelectric point</td>
<td>3.9</td>
<td>6.6</td>
</tr>
<tr>
<td>Diameter (nm)</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Genome type</td>
<td>Single-stranded RNA</td>
<td>Single-stranded DNA</td>
</tr>
</tbody>
</table>

a (Michen and Graule, 2010).
b (Kutter and Sulakvelidze, 2005).
the highest conductivity buffer (1250 μS/cm at pH 6.5) using a saturated NaCl solution. This conductivity falls within the range of domestic wastewaters, but is at or above the level typically accepted for drinking waters (Metcalfe & Eddy. et al., 2003; WHO, 2008). For the experiments testing varying conductivities, the control buffer was adjusted from 1,250 μS/cm to 6,250 or 12,500 μS/cm with saturated NaCl. These higher conductivities fall into the range of brackish and tidal waters.

2.3. Humic acids

Aldrich humic acid was chosen as a representative of natural organic matter (NOM), as humic substances are arguably the most important dissolved/suspended organic matter in water (Schijven and Hassanizadeh, 2000). A 500 mg/l stock of humic acid was made by adding 25 mg of humic acid to 50 ml of milliQ. To promote dissolution, the pH of the water was adjusted to pH 10 and sonicated.

2.4. Acid-washed sand (AWS)

Ottawa sand (Fisher Scientific, Leicestershire, UK) with particle size of 20-30 mesh was acid washed to remove metals according to previously described methods (Chu et al., 2001). In a plastic bottle, 300 g of Ottawa sand were mixed with 500 ml of 0.2 M citrate buffer containing 42.82 g/l of sodium citrate (Na4C6H5O7·2H2O) and 10.5 g/l of citric acid (H2C6H7O7), with 15 g of sodium dithionite (Na2S2O4). The mixture was heated to 80°C in a water bath for 10 min, then shaken for 20 min on a rotating homogenizer. The reducing buffer was removed, and the sand was washed with deionized water until the conductivity dropped below 10 μS/cm. The sand was then dried in a 105°C oven for 2 days.

2.5. Iron-oxide coated sand

Acid-washed sand was coated with iron oxides following previously described procedures (Schwertmann and Cornell, 2000). One liter of distilled water was added to a 2 l glass bottle and sparged with nitrogen gas to remove the oxygen, after which 13.9 g of FeSO4·7H2O crystals were added. 300 g of acid-washed sand and 110 ml of 1 M NaHCO3 solution were added to the bottle, and the bottle was aerated while spinning axially on a rotating plate. After 48 h, the sand was rinsed with distilled water until the water used for rinsing obtained the same pH and conductivity as the distilled water. After coating, the mass of iron on the sand was determined in triplicate samples by inductively coupled plasma-atomic emission spectroscopy after I WCS digestion according to previously described method (McGrath and Cunliffe, 1985), and determined to be 1.70 ± 0.04 mg Fe/g sand (average ± 95% confidence interval).

2.6. Desorbing solution (beef extract)

To release adsorbed viruses, a 2× beef extract solution was diluted 1:1 into virus-sand mixtures and mixed for 1 h. The 2× solution contained 6% beef extract and 0.1 M glycine at pH 8. Control experiments verified that a 1-h desorption period provided for maximal desorption — no further desorption occurred after this time period.

2.7. Desorption of adsorbed viruses due to changing solution conditions

The viral desorption experiments consisted of two steps. In the first, viruses at 107 pfu/ml were pre-incubated in the control buffer with IOCS and AWS (0.5 ml of virus sample plus 1 g of sand matrix; see Supplemental Information for details). After 1 h, half of the supernatant was removed (0.25 ml) for plating, and replaced to achieve the desired pH, electrical conductivity, and humic acid concentrations. The samples were then again rotated for 1 h, after which 0.25 ml were removed for the quantification of unadsorbed viruses. The remaining adsorbed viruses were desorbed with beef extract (after 1-h rotation) and quantified by culturing. All experiments were conducted in triplicate.

2.7.1. pH

To achieve final pH levels of 8.5 and 9.5, it was necessary to add 0.5 ml of a 3.6 mM and 6.4 mM NaOH solution, respectively, to the 0.25 ml of virus-sand mixture.

2.7.2. Electrical conductivity and humic acids

To achieve the desired concentrations after 2× dilution, 2× stocks were made for each electrical conductivity (2,500, 12,500, and 25,000 μS/cm) and humic acid concentration (20, 60, 100, 200, and 500 mg/l).

2.8. Solar irradiation of virus/sand mixtures

Virus inactivation was tested after exposure to solar irradiation in the presence of the different sand matrices. The goal was to test how virus inactivation was influenced by adsorption onto the IOCS. For these experiments, three different conditions were tested both under solar irradiation and in the dark: IOCS with adsorbed viruses, IOCS with unadsorbed viruses, and AWS with unadsorbed viruses. To test the IOCS with adsorbed viruses, viruses were first pre-adsorbed under control conditions (0.5 ml of 107 pfu/ml virus, 1 g of IOCS, rotated for 1 h at pH 7.5, 1250 μS/cm). The whole contents of two of these tubes were transferred to a 20-ml glass beaker (total of 1.0 ml of 108 pfu/ml virus, 2 g of IOCS) which was then diluted 10× by the addition of 9 ml of the control buffer. For the IOCS and AWS with unadsorbed virus, 2 g of either IOCS or AWS was placed in a 20-ml beaker with 9 ml of control buffer and 1 ml of 106 pfu/ml virus. All samples were tested in triplicate. The beakers were gently stirred to ensure a uniform monolayer of sand grains on the bottom, and a 100-μl sample was taken to measure the concentration of free viruses before sunlight exposure. Samples were weighed, then placed in a plastic, water-filled tray that was cooled with a recirculating cooler to maintain the temperature in the beakers at 20°C (F240 Recirculating Cooler, Julabo, Sealbch, Germany). The entire cooling/stirring system was placed under a Sun 2000 Solar Simulator (ABET Technologies, Milford, Connecticut) equipped with 1000 W Xe lamp. An AM1.5 and a UVB cut-off filter. The cut-off filter was used to eliminate the possibility of direct genome damage by UV light. The total irradiance up
to 800 nm for this setup was determined spectroradiometrically (Model ILT-900-R, International Light) and was 300 W/m². After an 8–12 h exposure, the beakers were re-weighed and milliQ was added to account for any evaporation that occurred during the sunlight exposure. Half of the volume of sample was replaced by a 2 M beef extract solution. Samples were placed on an orbital shaker at 140 rpm (PSU-10i shaker, BIOSAN, Latvia) to promote virus desorption. After 1 h, samples were taken to measure virus desorption by plating and by qPCR.

2.9. qPCR analysis

qPCR analysis was conducted to measure the total concentration of viruses (infective and inactivated) in solution upon desorption from the IOCS. RNA extraction and whole genome standards were prepared as previously described (Pecson et al., 2009). PCR primers were designed with the Primer3, free, online software (http://primer3.sourceforge.net/) and synthesized by Microsynth (Balgach, Switzerland). Primer set 3 from an earlier experiment was utilized to detect MS2 in this study (Pecson et al., 2009). The complete genome of MS2 was taken from the NCBI GenBank database (www.ncbi.nlm.nih.gov; accession number NC_001417).

Experimental samples and RNA standards were reverse transcribed and amplified in parallel using the RotorGene 3000 quantitative PCR platform (Corbett Life Science, Sydney, Australia). Each RT-qPCR sample was run in 15 μl total volume comprising 7.5 μl of 2× One Step SYBR RT-PCR buffer III, 0.3 μl of TaKaRa ExTaq HS (5 U/μl), 0.3 μl of Prime Script RT enzyme Mix II, 0.3 μl of 10 μM forward and reverse primers, 3.3 μl of water, and 3 μl of RNA sample (Takara Bio, Shiga, Japan). The following thermocycling conditions were used: 10 min at 42 °C, 20 s at 95 °C, 45 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s, followed by a melting ramp from 72 to 95 °C, holding for 45 s on the first step (72 °C) followed by 5 s holds on all subsequent temperatures. The genome copies/ml in the original sample were calculated by dividing the qPCR results (in total genome copies) by the volume of the extracted sample used in each qPCR reaction (total genome copies per 3 μl of extraction) and accounting for the four-fold concentration during the RNA extraction (from an initial 200-μl sample to 50 μl). The detection limit for a given primer set was determined according to a modified version of the method of Hubaux and Vos, with a 95% confidence level (Hubaux and Vos, 1970). In brief, the detection limits were determined by relating the qPCR output, the cycle threshold (Ct) number, to the known amount of standard. Because the Ct values were inversely related to standard levels (i.e., lower quantities of standards corresponded to higher Cq values), the only modification for determining the detection limit consisted in subtracting all Ct values from a maximum Cq value of 40, the maximum number of amplification cycles used for the qPCR.

2.10. Zeta potential

Zeta potential were determined from streaming potential measurements performed by Anton Par using a SurPASS electrokinetic analyzer (Graz, Austria). The streaming potentials of both acid-washed sand and IOCS were tested in the presence of a background electrolyte (0.001 N KCl), and with the control 10 mM Tris buffer (described above) at pH 7.5, 8.5, and 9.5. The effect of a 50 mM solution of Aldrich humic acid was also tested.

3. Results and discussion

Adsorption isotherms were determined for both viruses onto IOCS and a control matrix, an acid-washed sand devoid of metal-oxide coating (see Supplemental Information, Fig. S1). While the AWS caused no discernible adsorption, the iron-oxide coating transformed the sand into a matrix capable of adsorbing large quantities of both viruses (>10⁹ pfu/g). Adsorption alone, however, had no impact on viral infectivity. Consequently, the release of adsorbed viruses reinserted infective viruses back into solution. These findings are consistent with a number of studies that found no change in infectivity with adsorption onto iron oxides (Gutierrez et al., 2009; Murray and Laband, 1979). Notably, these findings diverge from those of Ryan et al. (2002), who found that adsorption onto iron oxide surfaces provoked viral inactivation. Given the diversity of iron oxides and the unconfirmed identity of the iron oxide used in either study, these disparate findings may simply be attributed to differences in virus interactions between minerals.

3.1. Adsorbed viruses are released in an infective state in response to changes in solution pH and NOM content

The first major research focus sought to determine if changes in solution conditions could provoke the release of adsorbed viruses back into solution. While the adsorption of microorganisms onto metal-oxide surfaces has been well documented, the potential for the release (desorption) of infective viruses with changing solution conditions is not well known. The consequences of this situation have important and obvious implications for treatment design. This situation could arise if viruses adsorbed onto an IOCS were suddenly exposed to a pulse of water with different solution conditions, such as higher pH, electrical conductivity, or organic matter content. Ultimately, the goal was to determine if the viruses could be desorbed in a state that would continue to impact water quality.

Both viruses were released from the IOCS when the pH of the solution was elevated above 7.5 (Fig. 1A and B). Desorption was likely caused by electrostatic changes altering the surface of the IOCS at higher pH. MS2, with an isoelectric point (pI) of 8.5, could be desorbed in a state that would continue to impact water quality. The exact composition of the iron oxide on the sand was not determined; however, it was created following a protocol for the production of hematite (Schwertmann and Cornell, 2000). Assuming the iron oxide coating is hematite, and that its surface charge is not influenced by the underlying silica sand, it would have a pI in the range of 8–10 (Schwertmann and Cornell, 2000). Thus, the viruses should experience electrostatic attraction toward the IOCS whenever their net charges
are opposite. Electrostatic attraction occurs between pH 3.9 to 8–10 for MS2, and pH 6.6 to 8–10 for F/X174. At more elevated pH, the hematite surface becomes negatively charged leading to electrostatic repulsion between the virus and the IOCS.

This assumption was supported by zeta potential of the IOCS. When the pH was increased from 7.5 to 9.5, there was a significant decrease in the zeta potential which is consistent with the positive surface charge diminishing. Consequently, we would expect the increase in pH to weaken the electrostatic attraction between the IOCS and the viruses, causing partial desorption of the viruses.

The introduction of humic acid into the solution also dislodged the previously adsorbed viruses (Fig. 1B). Desorption was significant, but not complete: additions of 250 mg/l humic acid (the highest concentration tested) still left approximately 90% of all viruses remaining adsorbed. Increasing the electrical conductivity from 1250 to 6250 or 12,500 μS/cm did not release adsorbed viruses into solution. It should be noted, however, that the electrical conductivity was set by the combination of Tris buffer and monovalent ions (Na⁺ and Cl⁻). It is possible that a buffer of the same conductivity arising from higher valent ions could result in virus release, as adsorption is strongly controlled by ion charge.

All MS2 that adsorbed to the IOCS matrix were desorbed in an infective state with the addition of the beef extract elution. Small losses in F/X174 infectivity were measured at the highest pH (0.31 log loss), conductivity (0.26 log loss), and humic acid concentration (0.14 log loss). At all other conditions, F/X174 infectivity was unaffected by adsorption.

3.2. Combination of adsorption and sunlight exposure enhances loss of viruses

The results discussed above demonstrated that adsorption did not cause virus inactivation, and that infective viruses could be released as a result of changes to the solution conditions (pH and humic acid). To improve the microbial quality of the water, it would be desirable to couple virus adsorption with inactivation. Several studies have shown that the irradiation of iron oxides with sunlight leads to the degradation of organic contaminants, in particular if the contaminants are adsorbed onto the iron oxide surface (Andreozzi et al., 2003; Du et al., 2008).

Photooxidative damage has been shown to be an important pathogen inactivation mechanism in both fresh- and seawater, presumably from indirect mechanisms producing reactive oxygen species via photosensitizers (Kohn and Nelson, 2007; Sinton et al., 1999, 2002; Suttle and Feng, 1992). Previous work in our group has demonstrated that the photo-Fenton process, promoted by the presence of iron colloids and H₂O₂, is also effective at inactivating MS2 (Nieto-Juarez et al., 2010). Our previous results, in conjunction with the degradability of iron oxide-adsorbed contaminants, led us to investigate if the combination of iron oxides and light could also promote the inactivation of IOCS-adsorbed viruses.

To test this hypothesis, viruses were adsorbed to the matrix under control conditions and exposed to simulated sunlight. The photoinactivation properties of the IOCS were measured by assaying MS2 and F/X174 infectivity, both adsorbed and unadsorbed, in the presence of simulated sunlight and in the dark (Fig. 2). MS2 that were not adsorbed onto the IOCS showed low levels of inactivation after 8 h of sunlight exposure (<1 log₁₀). Inactivation was much greater if the viruses were first adsorbed onto the IOCS before exposure to sunlight. Almost no inactivation of adsorbed MS2 occurred in the dark, in line with the previously described adsorption experiments, which were run exclusively in the dark. Thus, the key factors leading to enhanced MS2 inactivation were (1) adsorption onto IOCS and (2) exposure to sunlight.

Fig. 1 – Effect of changing solution conditions after pre-adsorption of viruses onto IOCS. Circles and squares represent MS2 and F/X174 samples, respectively. Open and filled symbols represent experimental samples (in presence of IOCS) and control samples (in absence of IOCS). The values are normalized to the concentration of viruses that remain suspended after equilibration with IOCS under control conditions (approximately 10⁴ pfu/mL). Data symbols are the average of triplicate experiments, and error bars represent the 95% confidence intervals.
After sunlight treatment, MS2 were desorbed from the matrix and qPCR was used to analyze the total number of desorbed genomes in solution. Not all of the initial MS2 genomes were detected after desorption (5.4 \times 10^6 vs. 1.1 \times 10^6 genome copies/ml). Given the full recovery of viruses in all of the previous experiments, it seems likely that the loss in qPCR signal was the result of genome damage incurred during exposure to light (Pecson et al., 2011, 2009), and not a failure to desorb the viruses from the matrix. The number of detected genomes, however, greatly exceeded the number of culturable (infective) viruses (1.5 \times 10^7 pfu/ml). This gave us additional information about the fate of the adsorbed viruses: they were not irreversibly attached to the IOCS, but were desorbed upon addition of beef extract at a ratio of roughly 100 inactivated viruses for every infective virus.

### 3.4. Photoinactivation mechanism

To further investigate the nature of this inactivation process, a second set of experiments was conducted to distinguish between photo-Fenton and photocatalytic processes. An enhancement of inactivation upon H$_2$O$_2$ addition would be indicative of a photo-Fenton process, as this mechanism has previously been shown to depend linearly on the H$_2$O$_2$ concentration (Nieto-Juarez et al., 2010). The addition of 50 \mu M H$_2$O$_2$ did not enhance inactivation compared to the H$_2$O$_2$-free solutions. We therefore attribute the observed photoinactivation to a photocatalytic effect exerted by the IOCS.

Earlier work by Love et al. showed that PhiX174 was more susceptible to solar disinfection in clear water than either MS2 or PRD1 (Love et al., 2010). Thus, at least two inactivating factors are at work in the IOCS system: solar disinfection in the absence of particle adsorption (Fig. 2) and photocatalysis. Our results support the findings of Love et al. in that unadsorbed PhiX174 was inactivated more readily than MS2 (Fig. 2). Our results also show a difference in response of the two viruses to photocatalysis: while PhiX174 was not affected, MS2 inactivation was greatly enhanced by photocatalysis.

### 3.5. Photoinactivation of adsorbed MS2 occurs in wastewater samples

The enhanced inactivation of adsorbed MS2 led us to test if this inactivation also occurred in more typical wetland waters. To simulate wetland water, we mixed 10 mM Tris buffer at 1:1 and 10:1 ratios with filtered, secondary wastewater obtained from the Lausanne treatment plant, containing 10.9 mg/l of non-purgeable organic carbon. Control experiments showed that the presence of wastewater did not affect virus adsorption onto IOCS, nor did it cause virus inactivation (data not shown). All irradiated MS2 samples showed a dose-dependent increase in inactivation, with higher inactivation occurring at higher ratios of buffer to wastewater (Fig. 3). Even in the presence of wastewater (1:1), however, the sunlight enhanced inactivation of MS2 led to roughly 1 log inactivation of MS2 after a 10-h exposure; the photocatalytic inactivation by IOCS can thus also occur in the presence of wastewater organic matter.
4. Conclusion

The threat of waterborne viruses can be reduced by either removing them from the water column (physical separation) or inactivating them. Adsorption of viruses onto media surfaces can separate viruses from the liquid stream, and numerous studies have characterized the optimal conditions for virus adsorption. In this study, we show that virus adsorption is not necessarily linked with virus inactivation. Our main findings are the following:

- Unlike previous studies, the adsorption of viruses onto iron oxide coated sand was reversible, whether the viruses were infective or inactivated.
- Changes in solution conditions led to the desorption and release of infective viruses into the water column.
- This threat could be mitigated by taking advantage of the photoreactivity of the iron oxide surface to enhance the inactivation of adsorbed viruses. Photoinactivation can contribute to virus inactivation, and adsorption enhances this effect because it maintains the viruses closer to the inactivating surface. Many studies have reported on the protective effect of virus association with particles, such as increasing virus capsid stability, preventing viral aggregation, protecting them from proteolytic enzymes, and shielding them from UV light. This study supports the growing body of evidence that particle association can also enhance inactivation.
- IOCS has a number of characteristics that make it interesting for use as a wetland matrix. It has a high capacity for viral adsorption and its photoreactivity contributes to the inactivation of bound viruses.

Overall, our results indicate that combining an IOCS matrix with sunlight exposure could improve wetland performance with respect to virus inactivation. To take advantage of this mechanism, wetlands could be designed to enhance sunlight exposure to the IOCS, e.g., constructing vertical flow wetlands with the IOCS in the top layer. Under such conditions, it may be possible to achieve the 4-log inactivation that is typically required for drinking water disinfection. While this mechanism contributed to MS2 inactivation, the somatic phage φX174 showed no enhancement in inactivation due to adsorption. Future studies should seek to identify matrices with the broadest inactivating capacities.

Acknowledgments

This research was supported by Swiss National Science Foundation (Project No. 200021_118077). Support for B.M.P. was provided in part by Marie Curie Fellowship grant no. 220706. We thank Thomas Luxbacher at Anton Paar for his assistance with SurPASS measurements, and Romuald Curdy and Jean-David Teuscher for method development and laboratory analyses.

Appendix. Supplementary material


REFERENCES


