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Ferrihydrite treatment to mitigate inhibition of RT-qPCR virus detection from large-volume environmental water samples

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ABSTRACT

Molecular assays are currently the most widely used method to quantify pathogenic viruses in water; however, their performance is often disrupted by matrices present in environmental samples. The present study used ferrihydrite (Fh) treatment to mitigate inhibition of RT-qPCR virus detection from environmental water concentrates. Fh treatment was performed to improve the detection of spiked Aichi virus 1 (AiV) and Qβ bacteriophage (Qβ) in commercial humic acids and seawater concentrates. The optimal Fh doses were found to be 200, 500, and 1000 mgFe/L for humic acid concentrates at UV_{254} of 1.5, 3.0, and 6.0 cm^{-1} respectively, whereas this value was 1000 mgFe/L for the seawater concentrates at UV_{254} of 1.5 cm^{-1}. At these optimal doses, the recoveries of spiked AiV and Qβ ranged from 2% to 12% in the humic acid and seawater concentrates, respectively. In addition, high levels of indigenous viruses (including AiV ≥ 2.81 log_{10} copies/mL and pepper mild mottle virus ≥ 2.77 log_{10} copies/mL) were detected from seawater concentrates after Fh treatment, while none were detected without Fh treatment. Fh treatment effectively mitigated the inhibitive effects from environmental water samples.

1. Introduction

Culture-independent molecular assays, such as quantitative real-time polymerase chain reaction (qPCR) or reverse transcription qPCR (RT-qPCR), are convenient tools commonly used to detect and quantify pathogenic viruses in water environments (Charles et al., 2009; Kluge et al., 2014) due to their high sensitivity, specificity, and rapidness. However, one major disadvantage of these assays is their susceptibility to inhibitive environmental matrices, such as humic substances, metal ions, and complex polysaccharides, that are frequently present in environmental waters (Schrader et al., 2012). These inhibitors can affect the performance of RT-qPCR/qPCR in several ways, including damaging the DNA template, inhibiting polymerase activity, and disturbing the florescence signal during PCR amplification (Schrader et al., 2012). As a result, the application of RT-qPCR/qPCR may underestimate or even lead to false-negative results. To detect viruses in environmental waters, large-volume water samples are concentrated to enrich the virus levels prior to RT-qPCR/qPCR detection; however, some environmental matrices, particularly humic acids, are often selectively coconcentrated/coextracted with target nucleic acids and subsequently inhibit RT-qPCR/qPCR (Hamza et al., 2009; Hata et al., 2017, 2015). Large-volume samples cause more inhibitive effects in RT-qPCR than small-volume samples (Hata et al., 2011). In addition, the presence of certain substances in the concentrated samples was found to disrupt the efficacy of the nucleic extraction step, which is conducted prior to RT-qPCR/qPCR (Hata et al., 2011; Schrader et al., 2012).

Process controls have been recommended to evaluate the extent of RT-qPCR/qPCR inhibition in environmental water samples (Haramoto et al., 2018; Hata et al., 2011; Hennechart-collette et al., 2015). This involves adding a known number of viral particles or DNA/RNA into the samples to evaluate the efficiency of nucleic acid extraction and/or PCR amplification. Nevertheless, the efficiency of the process controls is not sufficiently reliable to be used to back-calculate the actual number of target genomes in inhibited samples (Hennechart-collette et al., 2015). Therefore, removal of the inhibitor is important to improve the reliability of RT-qPCR/qPCR results. Dilution is one of the simplest approaches to reduce inhibitors in environmental samples (Gibson et al., 2012), yet this approach can give false-negative results if the target templates are diluted below their detection limit. Another approach is to spike additive compounds into the PCR reaction, such as skim milk (Arbeli and Fuentes, 2007), bovine serum albumin (Jiang et al., 2005; Wang et al., 2007), or T42 gene 32 protein (Jiang et al.,

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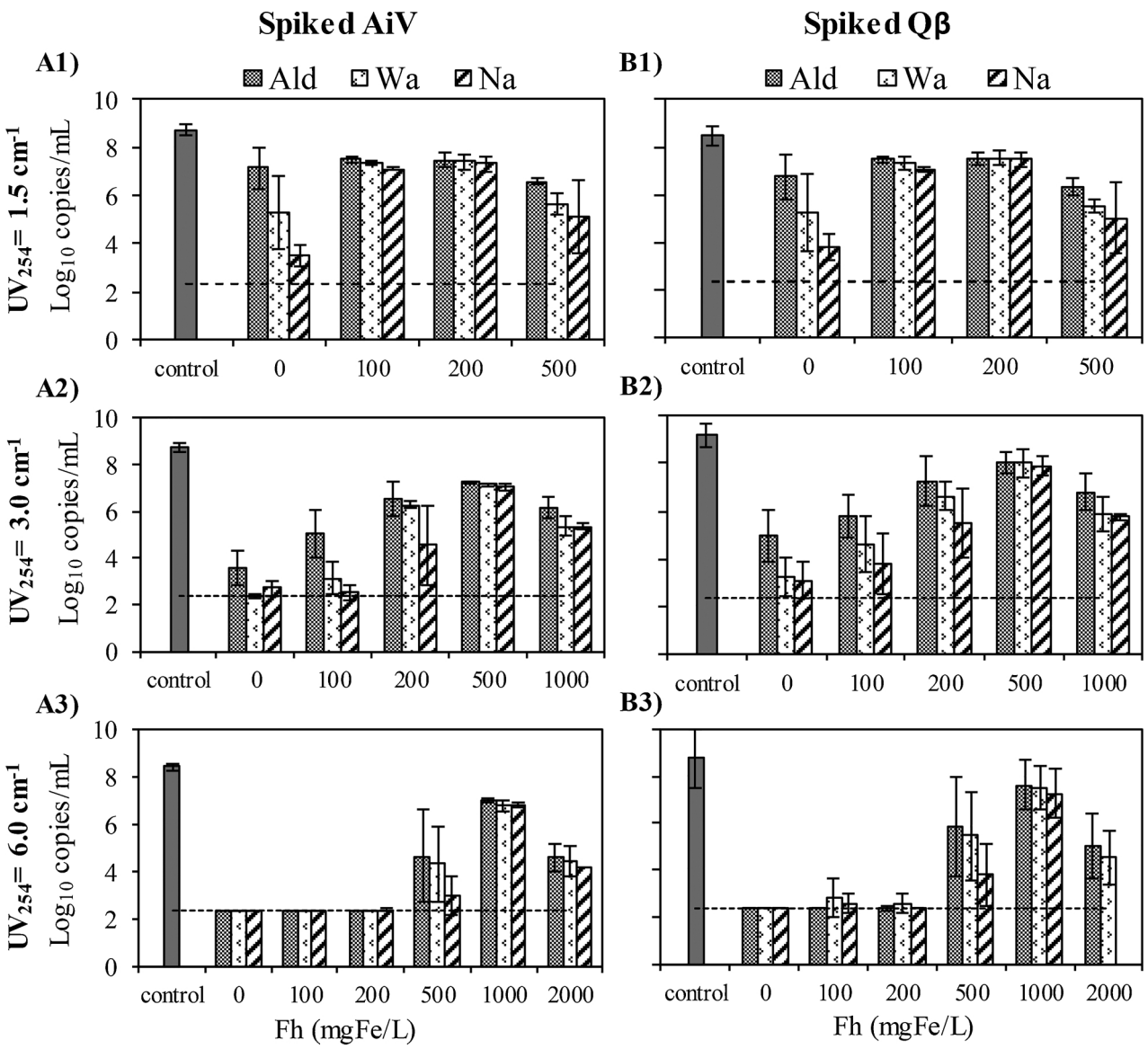

Fig. 1. RT-qPCR detection of spiked AiV and Qβ in humic acid concentrates (Ald, Wa, and Na) at an initial UV_{254} of 1.5 cm^{-1} (A1, B1), 3.0 cm^{-1} (A2, B2), and 6.0 cm^{-1} (A3, B3) after Fh treatment at doses ranging from 0 to 2000 mgFe/L. The positive control was prepared in Milli-Q. The dotted line represents the detection limit (2.33 log_{10} copies/mL), and error bars indicate standard deviation (n = 3).

2005). These compounds can interact with inhibitive substances and improve the annealing of primer, probe, and DNA polymerase during PCR amplification (Wang et al., 2007); however, this approach is not effective if the interference takes place during the nucleic acid extraction.

Other strategies to overcome PCR inhibition include purification techniques, such as the use of sepharose 4B-polyvinylpolypyrrolidone spin columns (Arbeli and Fuentes, 2007), and gel chromatography and chloroform extraction (Rodríguez et al., 2012). Recently, the polymeric adsorbent supelite DAX-8 was used to remove humic acids and other hydrophobic substances from environmental water samples (Asami et al., 2016; Kato et al., 2018; Schriewer et al., 2011). However, DAX-8 was employed during the nucleic acid extraction step and may have influenced the efficiency of the extraction. Besides, humic acids, which have been reported as a main cause of PCR inhibition in environmental samples (Hata et al., 2015; Kreader, 1996; Schrader et al., 2012), are particularly problematic for purification strategies since they are likely to be copurified with DNA (Steffan et al., 1988). Overall, none of these approaches has been fully effective; therefore, an alternative method is required to mitigate the inhibitive effects of environmental samples.

Ferrihydrite (Fh) is an iron oxide particle (IOP) with a large surface area and high adsorptive affinity. It has a higher capacity than activated carbon for adsorbing dissolved organic matter, especially those with a molecular weight > 1–3 kDa (Osawa et al., 2017; Yang et al., 2012). Fh has also been shown to greatly adsorb humic acids within a short contact time (Yang et al., 2014). More importantly, viruses are reported to be adsorbed by IOPs, but are released into the solution under the presence of humic acids (Pecson et al., 2012). Therefore, the application of Fh has a high potential to remove humic acids without the loss of viruses under optimized condition.

The present study aimed to develop a simple and effective method to mitigate inhibition of RT-qPCR virus detection from environmental sample concentrates. Humic acid and seawater concentrates spiked with Aichi virus (AiV) and bacteriophage (Qβ) were used to evaluate the ability of Fh treatment to improve RT-qPCR detection. The optimal Fh doses were adjusted based on the UV_{254} absorbance of the sample concentrates. Moreover, Fh treatment was tested to improve the detection of indigenous viruses in actual seawater samples.

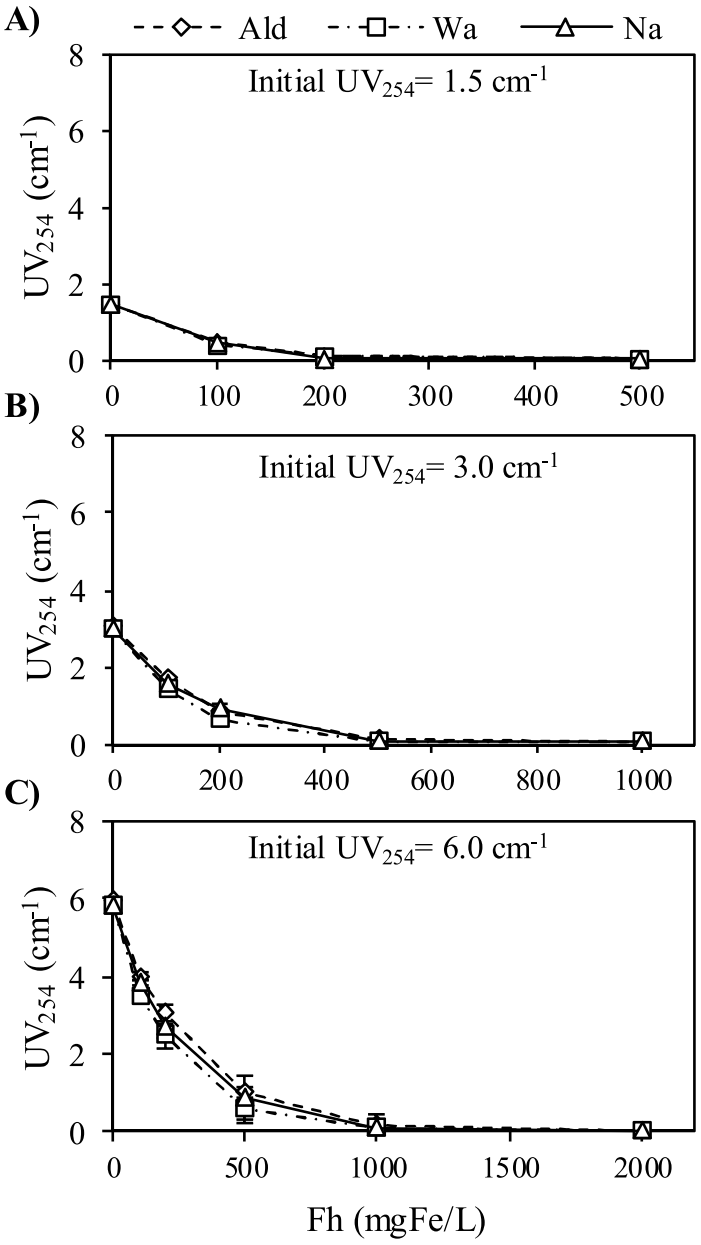

Fig. 2. UV_{254} of the humic acid concentrates (Ald, Wa, and Na) after Fh treatment at doses ranging from 0 to 2000 mgFe/L. Initial UV_{254} of around 1.5 cm^{-1} (A), 3.0 cm^{-1} (B), and 6.0 cm^{-1} (C). Error bars indicate standard deviation (n = 3).

2. Materials and methods

2.1. Water sample preparation

Humic acid stock solutions were prepared using three commercial reagents from Sigma-Aldrich, Tokyo, Japan (Ald), Wako Pure Chemical Industries, Osaka, Japan (Wa), and Nacalai Tesque, Kyoto, Japan (Na). Each humic acid reagent (300 mg) was dissolved in 300 mL of 1 M NaOH, neutralized using 5 M HCL, and then filtered through a membrane with a pore size of 0.45 µm (DISMIC-25HP, Advantec). Humic acid samples (1.0 L, UV_{254} = 0.5 cm^{-1}) were prepared by diluting a humic acid stock with Milli-Q water then subjecting the samples to the virus concentration processes using a negatively charged membrane, as described in detail elsewhere (Katayama et al., 2002). The humic acid concentrates were then adjusted using Milli-Q water to obtain final UV_{254} absorbances of 1.5, 3.0, and 6.0 cm^{-1}. The samples were then subjected to Fh treatment.

A total of six seawater samples (17–37 L) were collected on days 1, 2, and 3 after a rainfall event in June 2014 in Odaiba, Tokyo Bay, Japan. The surface water (SF) samples were collected at around 0.5 m under the surface, whereas the bottom (BT) samples were collected at around 5 m from the surface, close to the sandy sediment. These samples were concentrated by the virus concentration method using a negatively charged cartridge membrane for the large-volume samples, as described in detail elsewhere (Hata et al., 2015). The seawater concentrates were then diluted using Milli-Q water to obtain a final UV_{254} absorbance of approximately 1.5 cm^{-1}, then subjected to the Fh treatment.

2.2. Virus preparation

AiV and Qβ were used as model viruses to evaluate the performance of Fh treatment to mitigate the inhibition of RT-qPCR. AiV was selected as the representative of human enteric viruses in place of poliovirus, which was previously widely used but is currently not recommended, even in the laboratory, due to the World Health Organization polio

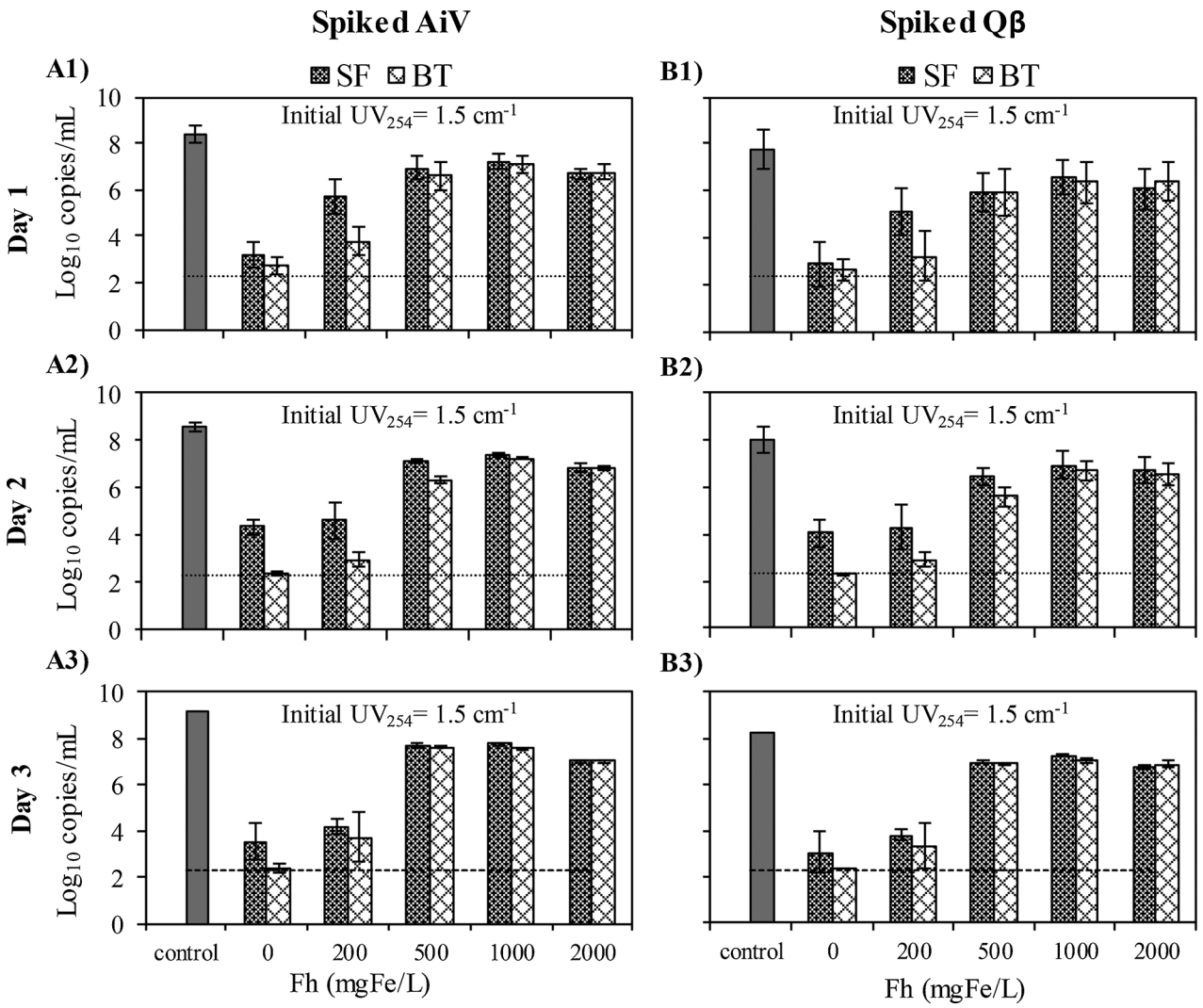

Fig. 3. RT-qPCR detection of spiked AiV and Qβ in the seawater concentrates (SF and BT) with an adjusted UV_{254} of around 1.5 cm^{-1} after Fh treatment at doses ranging from 0 to 2000 mgFe/L. The seawater concentrates were collected on days 1 (A1, B1), 2 (A2, B2), and 3 (A3, B3) after the rainfall event. The control was prepared in Milli-Q water. Dotted lines represent the detection limit (2.33 log_{10} copies/mL) and error bars indicate standard deviation (n = 3).

eradication program, while Qβ was selected as one of the most susceptible viruses to various environmental stresses (Schaper et al., 2002).

AiV (strain A846/88) was provided by Dr. Teruo Yamashita (Aichi Prefectural Institute of Public Health, Nagoya, Aichi, Japan) and propagated in Buffalo green monkey kidney cells (Yamashita et al., 1998). Qβ (ATCC 23631) was obtained from the American Type Culture Collection (Manassas, Virginia, USA) and was propagated in a culture of *Escherichia coli* K-12 F^+ A/ramda. Propagated AiV and Qβ were purified by membrane filtration using a cellulose acetate filter (0.2 µm, DISMIC-25CS, Advantec, Tokyo, Japan) and gel filtration with an Illustra Microspin S-300 HR column (GE Healthcare, Tokyo, Japan).

2.3. Fh preparation

Fh was synthesized according to the method described by Leone et al. (2001). Briefly, 2.5 g of $Fe(NO_3)_3.9H_2O$ (Wako Pure Chemical Industries, Tokyo, Japan) was dissolved in 31.5 mL of Milli-Q water and then neutralized using 1 M KOH. The Fh solution was then dialyzed to remove nitrate using a dialysis membrane (MWCO 100,000, Biotech cellulose Ester, Spectrum, Shiga, Japan) until the water conductivity was < 3 µS cm^{-1}. The concentration of the Fh stock solution (9 gFe/L) was determined by inductively coupled plasma mass spectrometry (Agilent Technologies, 7500 Series ICP-MS).

2.4. Fh treatment

A total volume of 500 µL was used for Fh treatment. AiV and Qβ were spiked into the humic acid or seawater concentrates to obtain a final concentration of approximately 10^9 copies/mL for each. Then, Fh was added to achieve a final concentration of 0, 100, 200, 500, 1000, and 2000 mgFe/L for the humic acid concentrates, and 0, 200, 500, 1000, and 2000 mgFe/L for the seawater concentrates. The samples were then vortexed for 5 min., then filtered using a 0.45-µm PVDF centrifugal filter (Ultrafree-MC-HV, Merck Millipore, Tokyo, Japan), with centrifugation at 12,000 rpm for 5 min to remove the Fh particles with adsorbed inhibitors. Next, 140 µL of filtrate was used for further steps, while the remainder was used for measuring UV_{254} absorbance using a UH5300 spectrophotometer (Hitachi, Japan) and pH using pH indicator strips (MColorpHas, Millipore). The pH of the Fh-treated samples was confirmed to be in the range of 5.0–6.0. A positive control was used in the absence of humic acids or environmental matrices. All treatments were performed in triplicate.

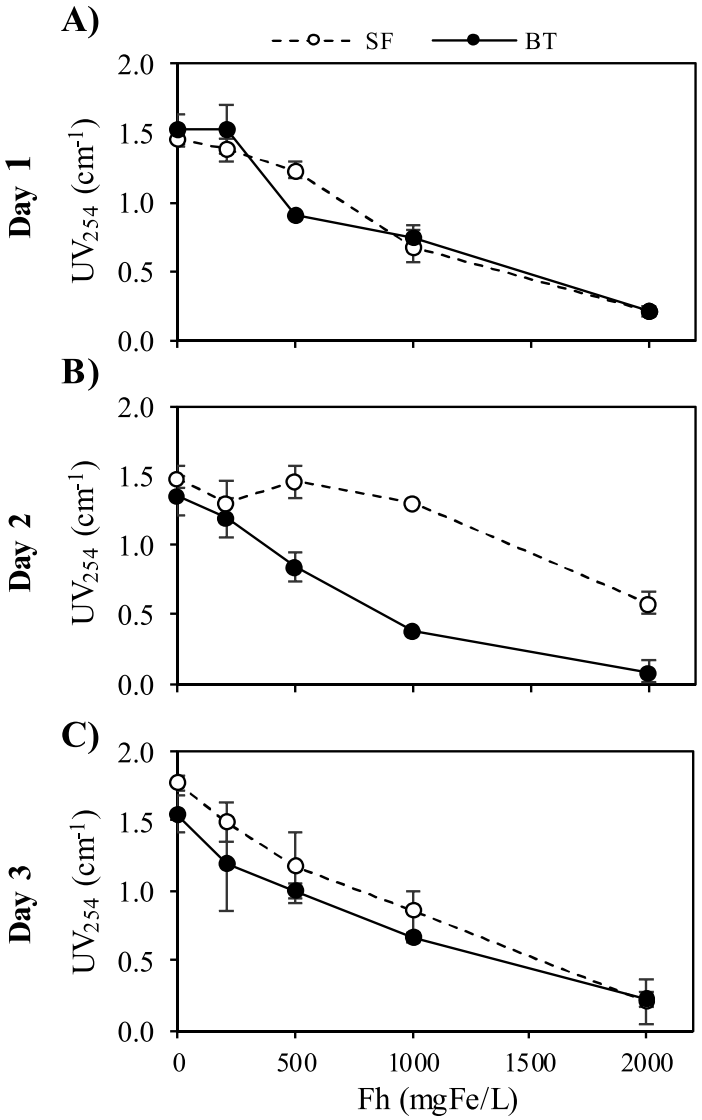

Fig. 4. UV_{254} of the SF and BT seawater concentrates after Fh treatment at doses ranging from 0 to 2000 mgFe/L. SF and BT samples were collected on days 1 (A), 2 (B), and 3 (C) after the rainfall event. The error bars indicate standard deviation (n = 3).

Table 1
Recovery efficiency of spiked AiV and Qβ from humic acid and seawater concentrates at optimal Fh doses.

Samples		Initial designed UV_{254} (cm^{-1})	Observed UV_{254} (cm^{-1})	Optimal Fh doses (mgFe/L)	Recovery efficiency (%)	
					AiV	Qβ
Humic acid concentrates	Ald		1.47	200	6	11
	Wa	1.5	1.47		5	12
	Na		1.48		4	11
	Ald		3.04	500	3	7
	Wa	3.0	2.95		2	6
	Na		2.97		2	5
	Ald		5.94	1000	4	8
	Wa	6.0	5.82		2	6
	Na		5.78		3	3
Seawater concentrates	SF (day 1)		1.46	1000	7	6
	BT (day 1)		1.53		5	4
	SF (day 2)	1.5	1.48	1000	6	8
	BT (day 2)		1.35		4	5
	SF (day 3)		1.77	1000	4	10
	BT (day 3)		1.55		2	6

Table 2
Detection of indigenous viruses (AiV and PMMoV) from seawater concentrates (adjusted UV_{254} of 1.5 cm^{-1}) with Fh treatment at an optimal dose of 1000 mgFe/L.

Samples		AiV (Log_{10} /mL)		PMMoV (Log_{10} /mL)	
		BF	AT	BF	AT
Seawater	SF (day 1)	n.d.	3.38	n.d.	3.90
	BT (day 1)	n.d.	2.81	n.d.	3.64
	SF (day 2)	n.d.	4.04	n.d.	4.28
	BT (day 2)	n.d.	3.04	n.d.	2.77
	SF (day 3)	n.d.	3.32	n.d.	4.09
	BT (day 3)	n.d.	3.22	n.d.	3.37

n.d., not detected.

2.5. Viral RNA extraction and reverse transcription

Viral RNA was extracted using a QIAamp viral RNA minikit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. RT was carried out using a High Capacity cDNA reverse transcription kit (Applied Biosystems, Tokyo, Japan) with the following thermal conditions: 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. The cDNA was utilized for qPCR analysis.

2.6. Virus quantification by qPCR

qPCR was performed using 20 µL of reaction mixture, containing 5 µL of cDNA, 10 µL of TaqMan Gene Expression Master Mix (Applied Biosystems), 1 µL each of 10 µM forward primer and reverse primers, 0.5 µL of 5 µM TaqMan probe, and 2.5 µL of nuclease-free water. The sequences of the primer pairs and TaqMan probes are described elsewhere for the detection of AiV (Kitajima et al., 2011), Qβ (Wolf et al., 2008), and pepper mild mottle virus (PMMoV) (Zhang et al., 2006). The StepOnePlus real-time PCR system (Applied Biosystems) was used for real-time PCR analysis with cycling conditions of 95 °C for 10 min, followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min. Ten-fold serial dilutions (ranging from $1.0 \times 10^{\circ}$ to 1.0×10^{4}) of plasmid DNA containing target sequences were amplified to obtain the calibration curve.

3. Results

3.1. Fh treatment for humic acid concentrates

To evaluate the ability of Fh treatment to mitigate the inhibitive effects of humic acid on RT-qPCR, Ald, Wa, and Na concentrates spiked with AiV and Qβ were treated with and without Fh, followed by RT-qPCR.

In the humic acid control (without humic acid), the detection of spiked AiV was nearly 8.6 log_{10} copies/mL, while the Ald, Wa, and Na concentrates at UV_{254} values of 1.5, 3.0, and 6.0 cm^{-1} showed a reduction of 1.5–5.1 log_{10}, nearly negative and completely negative, respectively (at Fh = 0 mgFe/L) (Fig. 1**A1–A3**). Similar tendencies were observed for the detection of spiked Qβ (Fig. 1**B1–B3**). In contrast, Fh treatment effectively improved the detection of spiked AiV and Qβ in the humic acid concentrates. The greatest improvement for the Ald, Wa, and Na concentrates at UV_{254} of 1.5, 3.0, and 6.0 cm^{-1} was observed at Fh doses of 200, 500, and 1000 mgFe/L, respectively, resulting in detection of spiked AiV and Qβ as high as 6.8–8.8 log_{10}/mL (Fig. 1). The improvement of inhibitory effects for all humic acid concentrates was confirmed (t-test, $p < 0.05$) except for the Ald and Wa concentrates at UV_{254} of 1.5 cm^{-1}. Therefore, Fh doses of 200, 500, and 1000 mgFe/L were considered optimum for minimizing the inhibitive effects of Ald, Wa, and Na concentrates at UV_{254} of 1.5, 3.0, and 6.0 cm^{-1}, respectively. On the other hand, there was a significant decrease in the detection of spiked AiV and Qβ in all humic acid concentrates when the Fh dose exceeded the optimal levels (t-test, $p < 0.05$), indicating that the spiked viruses were likely to be also adsorbed by Fh after the adsorption of humic acids.

Fig. 2 shows the UV_{254} of Ald, Na, and Wa concentrates were greatly reduced with increasing doses of Fh. Fh treatment at the optimal doses led to a reduction in the UV_{254} of all the humic acid concentrates (1.5, 3.0, and 6.0 cm^{-1}) to as low as approximately 0.1 cm^{-1} (Fig. 2).

3.2. Fh treatment for the concentrated seawater samples

The UV_{254} of the seawater concentrates (SF and BT) was adjusted to around 1.5 cm^{-1} by diluting with Milli-Q water, in which AiV and Qβ were spiked and detected by RT-qPCR, without and with the Fh treatment, at different doses (Fig. 3).

Using 8.7 log_{10} copies/mL of spiked AiV in the control, detection of spiked AiV was underestimated by approximately 4.0 log_{10} in the SF concentrates and was completely negative in the BT concentrates (at Fh = 0 mgFe/L) (Fig. 3**A1–A3**). Similar tendencies were observed for the detection of spiked Qβ (Fig. 3**B1–B3**). However, Fh treatment effectively mitigated the inhibitive effects of the seawater concentrates, and a dose of 1000 mgFe/L showed the highest detection of spiked AiV (6.7–7.8 log_{10} copies/mL) and Qβ (6.1–7.3 log_{10} copies/mL) in the SF and BT concentrates (Fig. 3), which was significantly higher than those without Fh treatment (t-test, $p < 0.05$). Therefore, 1000 mgFe/L was considered as the optimal dose to mitigate the inhibitive effects of seawater concentrates at UV_{254} of 1.5 cm^{-1}. Interestingly, detection of spiked AiV and Qβ at a dose of 2000 mgFe/L was not significantly different from those obtained at the optimal dose (t-test, $p > 0.05$) except for the detection of spiked AiV in the seawater concentrates collected on day 2 and 3 ($p < 0.05$), indicating that viruses in the seawater concentrates were not substantially adsorbed by Fh.

Fig. 4 shows that the UV_{254} of the SF and BT concentrates decreased as the Fh dose increased. At doses of 1000 and 2000 mgFe/L, the UV_{254} of all the SF and BT concentrates (1.5 cm^{-1}) was reduced to a range of 0.4–1.3 cm^{-1} and 0.1–0.6 cm^{-1}, respectively.

3.3. Recovery efficiency after the application of Fh treatment

The recovery efficiency for RT-qPCR of spiked AiV and Qβ in the humic acid and seawater concentrates after Fh treatment at the optimal doses are shown in Table 1. The recovery efficiency of spiked AiV and Qβ were in the range of 2%–6% and 3%–12%, respectively, in all the humic acid concentrates, whereas this was in the range of 2%–7% and 4%–10%, respectively, in all the seawater concentrates.

3.4. Detection of indigenous viruses in the seawater concentrates

Indigenous AiV and PMMoV in the seawater concentrates after adjustment of UV_{254} to around 1.5 cm^{-1} was determined by RT-qPCR without and with Fh treatment (Table 2), at an optimal dose of 1000 mgFe/L (Fig. 4).

Detection of AiV and PMMoV was completely inhibited by all the seawater concentrates without Fh treatment. However, concentrations ranging from 2.81 to 4.04 log_{10} copies/mL of AiV and 2.77–4.28 log_{10} copies/mL of PMMoV were detected after Fh treatment.

4. Discussion

Fh treatment was performed prior to nucleic acid extraction to mitigate the inhibitive effects of RT-qPCR caused by humic acid and seawater concentrates. Fh treatment greatly reduced the UV_{254} of all the humic acid and seawater concentrates with increasing Fh doses (Figs. 2 and 4). This clearly indicates that Fh treatment effectively removed humic acids and environmental organic compounds in the concentrates due to their rapid adsorption onto Fh (Yang et al., 2014,

2012). In principle, organic compounds can adsorb onto IOPs via anion exchange between the carboxyl and hydroxyl functional groups of organic compounds and the hydroxyl group of IOPs to form stable metal-bridged complexes (Batchelli et al., 2010; Gu et al., 1994; Sharma et al., 2010), while adsorption can be also promoted by hydrophobic interactions (Choo and Kang, 2003). Besides, Fh treatment decreased the UV_{254} of the humic acid concentrates more dramatically than that of the seawater concentrates (Figs. 2 and 4). This could be because the humic acid concentrates contained only hydrophobic organic compounds, while the seawater concentrates contained various environmental matrices, such as protein-like, humic acid-like, and fulvic acid-like compounds (Hata et al., 2017).

Fh treatment using doses of 200, 500, and 1000 mgFe/L were the most effective for all humic acid concentrates at UV_{254} of 1.5, 3.0, and 6.0 cm^{-1}, respectively (Fig. 1), while an optimal Fh dose of 1000 mgFe/L was observed for all the seawater concentrates at UV_{254} of 1.5 cm^{-1} (Fig. 3). In addition, comparing the humic acid with the seawater concentrates at a similar UV_{254} level of around 1.5 cm^{-1}, the optimal Fh dose for the humic acid concentrates (200 mgFe/L) was far less than that of the seawater concentrates (1000 mgFe/L) (Figs. 1 **A and 3**). This suggests that the optimal Fh dose was dependent on the level of inhibitors or the environmental matrix composition in the concentrates. This hypothesis was further confirmed since the UV_{254} of the humic acid concentrates was also lower than that of the seawater concentrates after Fh treatment at the optimal doses (Figs. 2 and 4).

At Fh doses exceeding the optimal doses for the humic acid concentrates, viruses (AiV and Qβ) were partially removed, whereas humic acids (Ald, Wa, and Na) were almost completely removed ($UV_{254} \leq 0.06$ cm^{-1}) (Figs. 2 and 4). This indicates that excess Fh doses greater than the humic acid adsorption were likely to adsorb viruses. In addition, testing the absorption capacity of Fh for viruses (AiV and Qβ) in the absence of humic acids (in Milli-Q water) showed a high number of AiV and Qβ (each, 10^9 copies/mL) were completely adsorbed by Fh at doses as low as 100 mgFe/L (data not shown). Virus absorption onto Fh may have been due to electrostatic interaction. Most viruses have a negative charge at neutral pH since their isoelectronic point (p*I*) is < 7.0 (Michen and Graule, 2010). To our knowledge, the p*I* of AiV has not been measured, whereas the p*I* of Qβ is in the range 1.9–2.7 (Langlet et al., 2008). In contrast, Fh has a positive charge below pH 7.0 (Choo and Kang, 2003; Gu et al., 1994). The pH was around 5.0–6.0 with Fh treatment. Under these conditions, AiV and Qβ were likely to have a negative charge, enabling them to adsorb on the positively charged Fh. This is consistent with previous studies indicating that rotavirus and bacteriophage MS2 and PhiX174 were adsorbed on IOPs since their net charges were opposite (Gutierrez et al., 2009; Pecson et al., 2012).

On the other hand, substantial absorption of AiV and Qβ onto Fh was not observed in the seawater concentrates, although the Fh doses (2000 mgFe/L) exceeded the optimal doses (1000 mgFe/L) (Fig. 3). Under these conditions, the relatively high levels of environmental organic compounds was still present in the Fh-treated samples (UV_{254} = 0.1–0.6 cm^{-1}) (Fig. 4). The remaining environmental matrices (after Fh treatment) possibly competed with the virus absorption. A previous study also found that bicarbonate irons and natural organic matter, commonly present in environmental waters, were strong competitors against viruses (rotavirus and MS2) for IOP adsorption sites (Gutierrez et al., 2009).

Although Fh treatment effectively mitigated RT-qPCR inhibition, complete mitigation was not achieved (Figs. 1 and 3). There appeared to be certain inhibitive substances that could not be removed by Fh treatment. In addition, RT-qPCR inhibition was still observed even if the UV_{254} absorbance was almost completely removed by Fh treatment (Figs. 2 and 4), suggesting that the UV_{254} absorbance might not fully represent all inhibitive substances in the concentrates. A previous study also demonstrated that the UV_{254} absorbance does not always correlate with the level of RT-qPCR inhibition (Gentry-Shields et al., 2013). However, it should be noted that the recovery efficiency of viruses at the optimal Fh doses was in the range of 2% to 12% (Table 1). Previous studies have shown that virus detection from environmental samples is considered valid when the recovery efficiency was greater than 1% (Randazzo et al., 2018, 2016). Therefore, the achieved efficiency in the present study would be valuable for virus detection in environmental samples.

Fh treatment represents a method to mitigate inhibition without compensating the concentration of target virus, where the optimal doses can be estimated from the reduction of UV_{254} absorbance in Fh-treated samples. In comparison with the dilution approach, which is commonly used to mitigate RT-qPCR inhibition, Fh treatment may have more advantages, since the determination of the optimal dilution ratio is problematic (Cao et al., 2012). Furthermore, dilution can also lower the target concentration, or even can cause a false-negative result.

5. Conclusions

Fh treatment was able to effectively mitigate inhibition of RT-qPCR virus detection from humic acid and seawater concentrates. The optimal Fh doses were 200, 500, and 1000 mgFe/L for concentrated humic acid samples at UV_{254} of 1.5, 3.0, and 6.0 cm^{-1}, whereas doses of 1000 mgFe/L were needed for both the SF and BT seawater concentrates at UV_{254} of 1.5 cm^{-1}. The recovery efficiency of viruses in the Fh-treated samples ranged from 2% to 12% for both humic acid and seawater concentrates. Therefore, this method may augment RT-qPCR virus detection in environmental samples.

Acknowledgments

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