

Simultaneous removal of rotavirus and adenovirus from artificial ground water using hydrochar derived from swine feces

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ABSTRACT

Hydrothermal carbonization technology can convert fecal waste into a valuable carbonaceous product referred to as hydrochar. We investigated the potential of fecal waste-derived hydrochar as an adsorbent for virus removal in water treatment. Swine feces was hydrothermally treated under two conditions: at 180 °C for 2 h and 230 °C for 7 h. The resulting solid products (hydrochar) were evaluated as virus adsorbents in water treatment. Simultaneous removal of pathogenic rotavirus (RV) and human adenovirus (HAdV) was investigated using a sand column set-up of 10 cm bed height with and without hydrochar supplement (1.5%, w/w). The removal efficiency of both viruses in a hydrochar-amended column was >3 log (complete removal). The amount of virus released in deionized water when flushed into the virus-retaining columns indicated that the secondary energy minimum played a more important role in RV retention than that of HAdV. Zeta-potential and hydrophobicity measurements on hydrochar materials indicated that the improved virus removal performance of hydrochar-amended columns was induced by the provision of extra hydrophobic surfaces. This study provides evidence that fecal waste-derived hydrochar can be used as a competent virus adsorbent.

Key words | fecal waste, hydrothermal carbonization (HTC), polymerase chain reaction, sand filter, virus, water treatment

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INTRODUCTION

Provision of adequate sanitation and clean water is an important challenge for public health in many developing countries. In spite of international efforts and notable progress in the last decades, still over 700 million people are disconnected from improved drinking water sources, and 2.5 billion do not have access to proper sanitation (WHO & UNICEF 2014). In urban areas of developing countries, where sewer systems and sewer-based fecal waste treatment systems are not affordable, on-site sanitation technologies are predominantly used generating large quantities of untreated fecal waste which causes extensive environmental contamination (Koné 2010).

One of the main threats induced from improperly managed fecal waste is microbial contamination in surface water and groundwater of peri-urban areas (Katukiza *et al.* 2013). Large quantities of highly infective enteric viruses are shed in feces of infected persons: 10^5 – 10^{11} virions/g feces of infected person (Farthing 1989; Yezli & Otter 2011). They are considered to be responsible for outbreaks of water-borne diseases (Bosch 1998). Similar to the sanitation issue, conventional centralized water treatment systems are inappropriate in places in need due to technical and financial limitations (Bartram *et al.* 2005). Instead, simple and low-cost decentralized (point-of-use) technologies such

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as a biosand filter, a ceramic filter, solar disinfection and chlorination (combination with flocculation) have been recommended as affordable solutions for safe drinking water provision (Sobsey *et al.* 2008). These technologies have, however, relatively limited removal capacities for pathogenic viruses (Sobsey *et al.* 2008).

With proper measures, fecal waste can be utilized as a valuable resource. Recently, Diener *et al.* (2014) suggested the conversion of fecal sludge into market products: dry sludge as fuel for combustion, animal protein (food for larvae of black soldier fly), feed stock for biogas generation, soil conditioner and building materials. In this context, hydrothermal carbonization (HTC), also known as wet pyrolysis, of fecal waste can be an attractive technology providing not only a better hygienic environment, but also valuable carbonaceous materials (Katukiza *et al.* 2012; Breulmann *et al.* 2015). Fecal waste, the most important source of human pathogens, is totally sanitized during the HTC process under wet and pressurized (~20 bar) conditions at relatively low temperatures (~200 °C). The HTC product is a thick slurry consisting of solid particles (hydrochar) and a liquid (process water) phase. The hydrochar can be utilized as pathogen-free agricultural supplements (Berge *et al.* 2013), energy source (Mumme *et al.* 2011), phosphorus source (Heilmann *et al.* 2014) and adsorbent in water and wastewater treatment (Regmi *et al.* 2012; Minani *et al.* 2014; Sun *et al.* 2015).

Application of hydrochar as an adsorbent in water treatment for pathogen removal is still in its infancy. Chung *et al.* (2014) suggested a simple sand filtration unit supplemented with hydrochar derived from agricultural residues from maize for removal of *Escherichia coli*, a representative model for bacterial pathogens, from artificial groundwater (AGW). A more recent study carried out under similar experimental conditions showed considerable pathogenic virus removal capacity of hydrochar produced from stabilized sewage sludge (Chung *et al.* 2015). However, fecal waste-based hydrochar has not yet been investigated for its application in pathogen removal.

Since the retention of pathogens in the filtration unit does not guarantee their inactivation (Camper *et al.* 1985; Rollinger & Dott 1987), the detachment of pathogens from the filter media must be carefully considered in order to have better water quality surveillance. A large part of the interactions

between viruses and porous media can be explained by the classic Derjaguin-Landau-Verwey-Overbeek theory (Schijven & Hassanizadeh 2000). Under unfavourable (electrostatically negative) conditions, the secondary energy minimum was reported to play an important role in the colloidal deposition (Hahn & O'Melia 2004). Due to its reversibility, the viral attachment in the secondary energy minimum is important when evaluating filtration systems. Another important parameter in filtration systems is the flow rate because it not only determines the production rate, but also the attachment/detachment behavior of the pathogens (Tong & Johnson 2006; Su *et al.* 2009).

In this research, we investigated the use of fecal waste-derived hydrochar as an adsorbent for virus removal with potential application in water treatment. Fresh swine feces was hydrothermally converted into carbonaceous adsorbents. Simultaneous removal of infective pathogenic rotavirus and adenovirus, two of the most significant viral agents responsible for diarrheal diseases (Gomara *et al.* 2008; Parashar *et al.* 2009), was carried out by performing breakthrough analyses using a sand filtration set-up supplemented with a small amount of hydrochar. In order to have better understanding of the use of hydrochar in virus removal, the effect of flow rate and the secondary energy minimum was also investigated.

METHODS AND MATERIALS

Hydrochar

Fresh swine feces was collected at the research farm of the Faculty of Veterinary Medicine of Leipzig University (Germany). The feedstock for HTC was prepared by mixing fresh swine feces and deionized (DI) water at a dry matter content of 15.8%. The pH was adjusted to 4.6 by the addition of 0.5 M H₂SO₄. The hydrothermal conversion of swine feces was carried out in a high-pressure reactor (BR-500, Berghof, Eningen, Germany) equipped with a stirrer drive (BRM-1, Berghof, Eningen, Germany). Approximately, 55 g dry weight of swine feces was loaded in a 500 mL volume stainless steel vessel with polytetrafluoroethylene insert.

Two experimental conditions were employed for hydrochar production: at 180 °C for 2 h (180-HTC), and at 230 °C

for 7 h (230-HTC), representing two extremes of reaction severity (Funke & Ziegler 2010). The reactions were performed under a heating rate of 2 K/min with a stirring speed of 150 rpm. The pressure and temperature inside the reactor were monitored by a data logger (BTC-3000, Berghof, Eningen, Germany). Afterwards, the reactor was cooled down to room temperature. Then, the gaseous product was released into an inverse volumetric cylinder immersed in water in order to measure the volume of gaseous products. The hydrochar was separated from the resulting slurry by vacuum filtration using a ceramic funnel and filter paper. The hydrochar was dried at 70 °C overnight and stored at room temperature.

The weight of the resulting material at each step was measured. The moisture, volatile organic matter, fixed carbon and ash contents of oven-dried (at 70 °C) swine feces and both types of hydrochar were analyzed according to standard protocol (ASTM 2003).

Virus suspension

Active human pathogenic *Rotavirus WA* (RV) and *Human adenovirus C* type 2 (HAdV) were kindly provided by the Netherlands National Institute for Public Health and Environment (RIVM, Bilthoven, The Netherlands). Both are non-enveloped viruses and similar in their morphology (icosahedral shape) and size (80–100 nm) (Stewart *et al.* 1993; Parashar *et al.* 1998). Stocks of RV with a concentration of $10^{5.8}$ tissue culture infectious dose 50 (TCID₅₀)/mL and HAdV stocks with a concentration of 10^6 plaque-forming unit (PFU)/mL were aliquoted in Eppendorf vials and stored in a freezer (–80 °C).

The virus removal experiments were carried out using AGW, which was prepared by dissolving 526 mg CaCl₂·2H₂O, 184 mg MgSO₄·7H₂O, 8.5 mg KH₂PO₄, 21.8 mg K₂HPO₄ and 17.7 mg Na₂HPO₄ in 1 L DI water (Chung *et al.* 2014). The pH and electrical conductivity of the AGW were ~6.8 and 1,000 µS/cm, respectively. The influent was prepared by suspending RV and HAdV aliquots in AGW at a concentration of ~ 10^3 TCID₅₀ (RV) and PFU (HAdV)/mL. This influent was placed on a lab bench for 30 min prior to the virus removal experiments for stabilization of viruses in AGW. All operations with pathogenic viruses were carried out in a biosafety cabinet satisfying biosafety level 2.

Virus-contaminated materials were disinfected by autoclaving at 121 °C for 20 min.

Virus quantification

The nucleic acid of the virus contained in 100 µL of each sample was extracted by using chaotropic buffers and silica colloids as previously described (Boom *et al.* 1990, 1999). Established reverse transcription-polymerase chain reaction (RT-qPCR) protocols were used to determine the concentration of RV (Chung *et al.* 2013) and HAdV (Chung *et al.* 2015) with minor modifications. The lowest detection limits for the RV and the HAdV were $10^{-0.2}$ TCID₅₀/mL and 1 PFU/mL, respectively. Throughout the virus quantification process, the nucleic acid was used without any dilution. Briefly, denaturation and annealing of RV RNA with random primers were performed in a reaction mix consisting of 5 µL nucleic acid extract, 0.3 µL (0.06 µg) random hexamer (Fermentas) and 8.7 µL DEPC treated water at 70 °C for 5 min. Reverse transcription was carried out by the addition of 0.3 µL (60 unit) RevertAid reverse transcriptase (Fermentas), 1 µL dNTP mix (4 mM of each dNTP, GenScript), and 5 µL 5× RT buffer provided with the reverse transcriptase and 4 µL DEPC treated water into the product of the previous step. Then, the resulting reverse transcription mix with a final volume of 25 µL was subjected to a temperature of 25 °C for 10 min, 42 °C for 60 min, and at 70 °C for 10 min. All thermal treatments in this research were carried out in a thermal cycler BioRad MJMini™ (real time PCR system, MiniOpticon).

The real-time PCR assessments of RV cDNA or HAdV DNA were carried out using an identical protocol except for the use of primers and probes, both with FAM fluorophore and Black Hole Quencher, for RV (Pang *et al.* 2004) and HAdV (Hernroth *et al.* 2002). These probe and primers were synthesized by Biolegio (Nijmegen, The Netherlands). Briefly, 4 µL of template (RV cDNA or nucleic acid extract) was added to a qPCR mix containing 2.5 µL home-made qPCR buffer (150 mM Tris-HCl pH 8.2 at 25 °C, 300 mM KCl, 50 mM (NH₄)₂SO₄, 25 mM MgCl₂, and 0.2% BSA), 1 µL dNTP mix (4 mM of each dNTP), 1 µL (0.5 unit) Taq polymerase (GenScript), 15.4 µL DEPC treated water, 0.4 µL of forward and reverse primer (both at a 200 nM final concentration), and 0.3 µL probe (150 nM final

concentration). The (RT)-qPCR analysis for the samples from the column experiments was performed in duplicate. The thermal cycling consisted of an initial denaturation at 95 °C for 5 min, and 40 cycles of denaturation at 94 °C for 20 s followed by annealing/extension at 60 °C for 60 s. The fluorescence signal was monitored at the end of every cycle. The threshold cycle values (C_ts) obtained from qPCR of samples were converted into actual virus concentrations using regression curves derived from 10-fold dilution series of standard virus stocks (data not shown).

Material characterization

Zeta potential

The zeta potential values of the two types of hydrochar were determined in the pH range 4–10 using a Zetasizer Nano ZS (Malvern, UK) equipped with a MPT-2 pH auto-titration unit. The hydrochar sample was washed three times by being suspended in AGW followed by centrifugation (3 min at 2,700 g) prior to the zeta potential measurements. The concentration of hydrochar in the sample was controlled to have an adequate attenuator (6–8) selection of the instrument.

Hydrophobicity

To analyze hydrophobicity of two types of hydrochar samples, static contact angle analysis was performed using a Drop Shape Analyzer (DSA100, Krüss, Hamburg, Germany). A flat surface of hydrochar was prepared by pelletizing of powdered hydrochar (Jeong *et al.* 2009) using a manual hydraulic press (Atlas Manual Hydraulic Press, Kent, UK).

Column experiments

Column preparation

The virus removal efficiencies of both hydrochars (180-HTC and 230-HTC) were investigated by performing breakthrough analyses in a simple sand filtration set-up as described previously (Chung *et al.* 2014). Briefly, acid-washed quartz sand with 99.1% purity (Kristall quartz-sand, Dorsilit, Germany) was used as a supporting matrix for the hydrochar powders (<0.125 mm). A cumulative

mass distribution curve was plotted through sieve analysis of sand granules; a D₅₀ value of 0.79 mm and a U value (D₁₀/D₆₀) of 1.81 were obtained (Matthess *et al.* 1991). A borosilicate glass column with a 2.5 cm inner diameter was packed with either sand or with a sand-hydrochar mixture (at 1.5% dry weight hydrochar/sand) to have a 10 cm bed height (Omnifit, Cambridge, UK). In order to prevent air entrapment and channeling in the sand-hydrochar matrix, the column was regularly agitated and the packing materials were carefully compacted by using a glass rod during the packing process. Then, the column was connected to a peristaltic pump (MasterFlex model 77201-60, Vernon Hills, Illinois, USA) and washed with DI water overnight to remove residual fine particles and chemicals from the column matrix. Prior to the virus breakthrough analysis, the column was saturated with AGW.

Design of the virus removal experiments

To investigate the simultaneous removal of RV and HAdV, both types of viruses were seeded in the influent AGW. The breakthrough analysis was carried out by flushing of 50 mL virus-containing AGW (loading) followed by flushing 50 mL virus-free AGW (deloading), and finally 100 mL DI water was fed into the column. It was anticipated that the flushing with DI water can be an indicator for the role of the secondary energy minimum in the viral retention in the column. The low ionic strength of DI water (4–5 µS/cm) would have increased the repulsive electrostatic force between the virus and column media surface by expansion of the electrical double layer between the virus and the surface of the column media. As a result, a certain amount of viruses deposited in the secondary energy minimum will be released (Foppen *et al.* 2007; Chung *et al.* 2014).

The effect of the flow rate in virus retention and release behavior was examined by applying three flow rates in an upward direction: 1, 2.5 and 5 mL/min, which correspond to 0.12, 0.3, and 0.6 m/h, respectively. These can be considered as a high range of slow sand filtration rates. It was assumed that the lower flow rate induced a longer retention time and weaker shear stress, and the opposite conditions were true for the higher flow rates. In total, 36 breakthrough curves (BTC) were obtained from 18 duplicate experiments with two viruses (RV and HAdV), three packing materials

(sand, sand-180-HTC or sand-230-HTC) and three flow rates (1, 2.5 and 5 mL/min). The effluent was sampled at 5 min intervals, and stored in a freezer (-20°C). The concentration of RV and HAdV was measured by using the aforementioned (RT)-qPCR assays within 48 h from the column experiments. After each breakthrough analysis, column packing materials were excavated and autoclaved prior to disposal.

RESULTS

HTC of swine feces

Swine feces was hydrothermally converted under two reaction conditions: 180°C for 2 h (180-HTC, representing mild reaction) and 230°C for 7 h (230-HTC, representing severe reaction). The profiles of pressure and temperature development inside the reactor are given in Figure 1. The operating pressure during the HTC process maintained at 10–13 bar for the reaction at 180°C and 35–38 bar for the reaction at 230°C . These different hydrothermal conditions resulted

in different characteristics of the hydrochar materials. The solid product yield, gas production, and the constitution of materials are summarized in Table 1. The yield of solid product was 14.1% higher for 180-HTC than 230-HTC. In contrast, the volume of gaseous product was twice more with 230-HTC than with 180-HTC. Though the proportion of the carbonaceous contents in solid products increased from 76% (dry swine manure) to $\sim 80\%$ during both HTC processes, only 50–60% of the total carbon input was recovered in the hydrochars (Table 1). It was apparent that a certain amount of volatile organic matter in the feedstock was converted into different forms such as fixed carbon in hydrochar, soluble products in the process water or gaseous products. Only around a quarter of the volatile organic matter in the feedstock was recovered in both hydrochars.

Material characterization

Zeta potential

The zeta potential values of both 180-HTC and 230-HTC were all negative in the pH range 4–10 (Figure 2). This

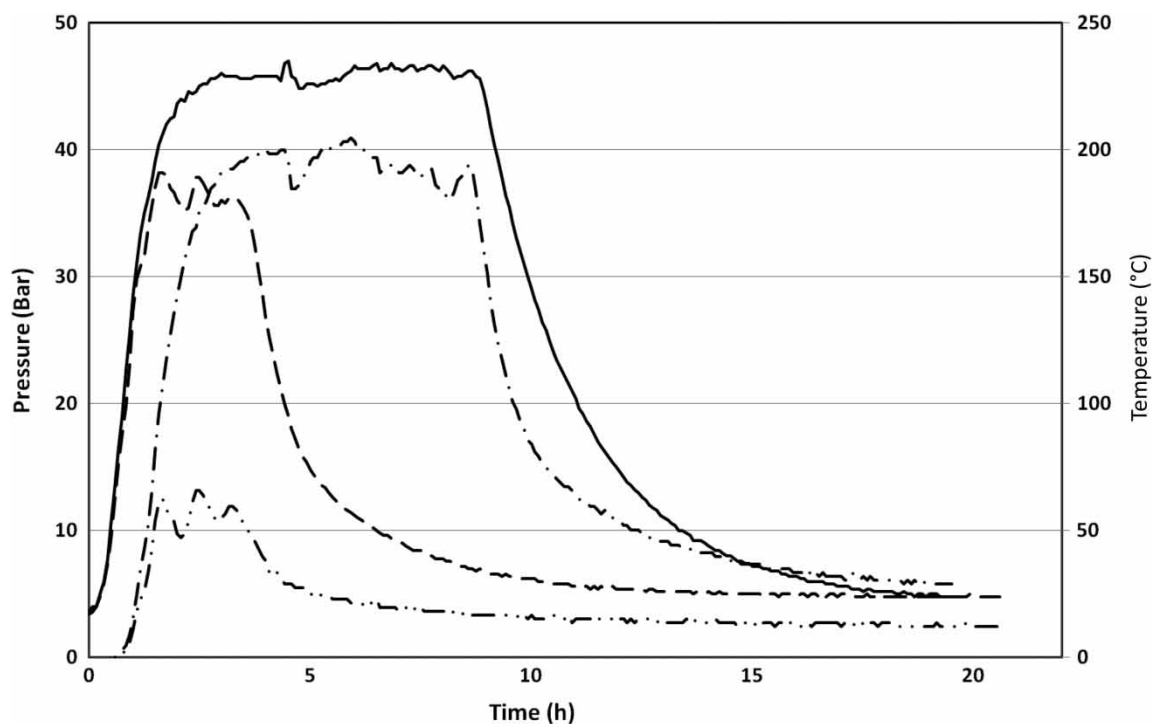
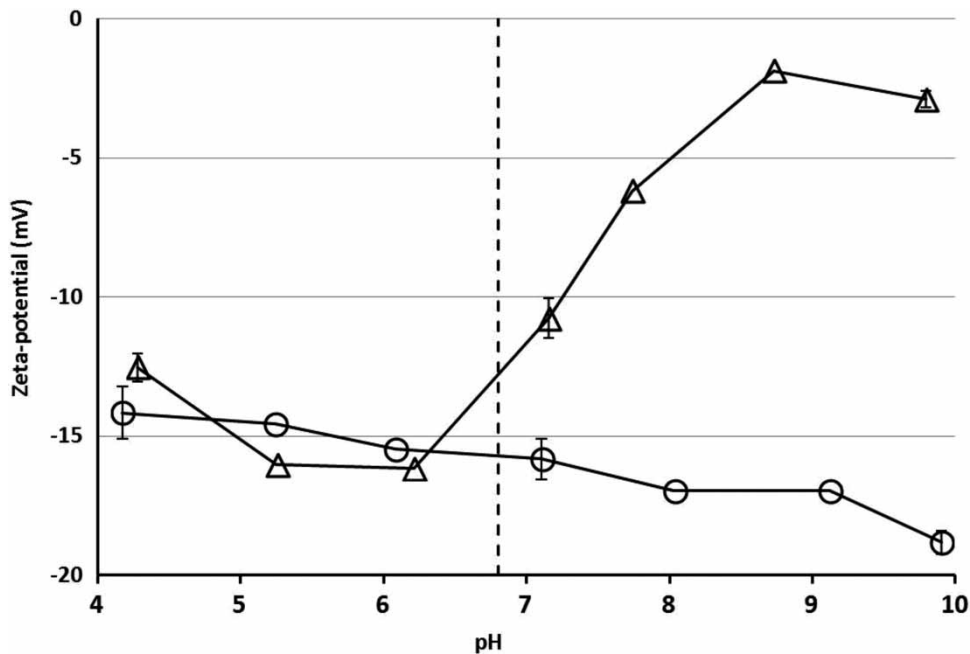


Figure 1 | Temperature and pressure profile during HTC of swine waste. Legends: (—) 230-HTC temperature, (---) 180-HTC temperature, (- -) 230-HTC pressure and (· · ·) 180-HTC pressure.

Table 1 | Composition of HTC materials, solid yield and gas production capacity derived from swine manure

Sample	Moisture (%)	Volatile organic matter (%)	Fixed carbon (%)	Total carbon (%)	Ash (%)	Solid yield (%)	Gas (mL/g dry manure)
Dry swine manure	8.4 ^a	62.6	13.5	76.1	15.5	n. a. ^b	n. a.
230-HTC	1.9 ^c (1.1)	44.2 (26.1)	38.2 (22.5)	82.4 (48.6)	15.7 (9.26)	59.0 ^d	46.0 ^e
180-HTC	1.0 (0.7)	32.4 (23.7)	50.9 (37.2)	83.3 (60.9)	15.6 (11.4)	73.1	22.1

^aAverage % of duplicate measurements.^bNot available.^cThe number in brackets indicates the mass conversion of each content (solid yield × average %).^dAverage % of triplicate measurements, dry weight hydrochar/dry weight feedstock.^eAverage of triplicate measurements, mL gaseous product/dry weight feedstock.**Figure 2** | Zeta potential of (○) 230-HTC and (Δ) 180-HTC as a function of pH. The solid lines represent the average zeta-potential value of triplicate assessments and error bars indicate the standard deviation. The dashed line indicates the pH value of AGW (6.8).

observation implied that slightly stronger electrostatic repulsion existed in the columns with 230-HTC (−16 mV) than the ones with 180-HTC (−13 mV) at the operational pH of AGW (pH 6.8).

Hydrophobicity

Comparable contact angle results were obtained from both hydrochar discs: 103.8 (±1.2)° for HTC-180 (average result from four replications ± standard deviation) compared to 101.8 (±2.6)° for HTC-230.

Column experiments

The BTCs of HAdV and RV are given in Figures 3 and 4, respectively. The virus concentration in the effluent was expressed as a log removal value (−log C/C₀, LRV). Virus removal efficiencies were calculated from the effluent samples collected in the loading (5–50 mL) and deloading (55–100 mL) phase. The amount of viruses observed in the DI water flushing (105–200 mL) stage was considered as an indicator for the role of the secondary energy minimum.

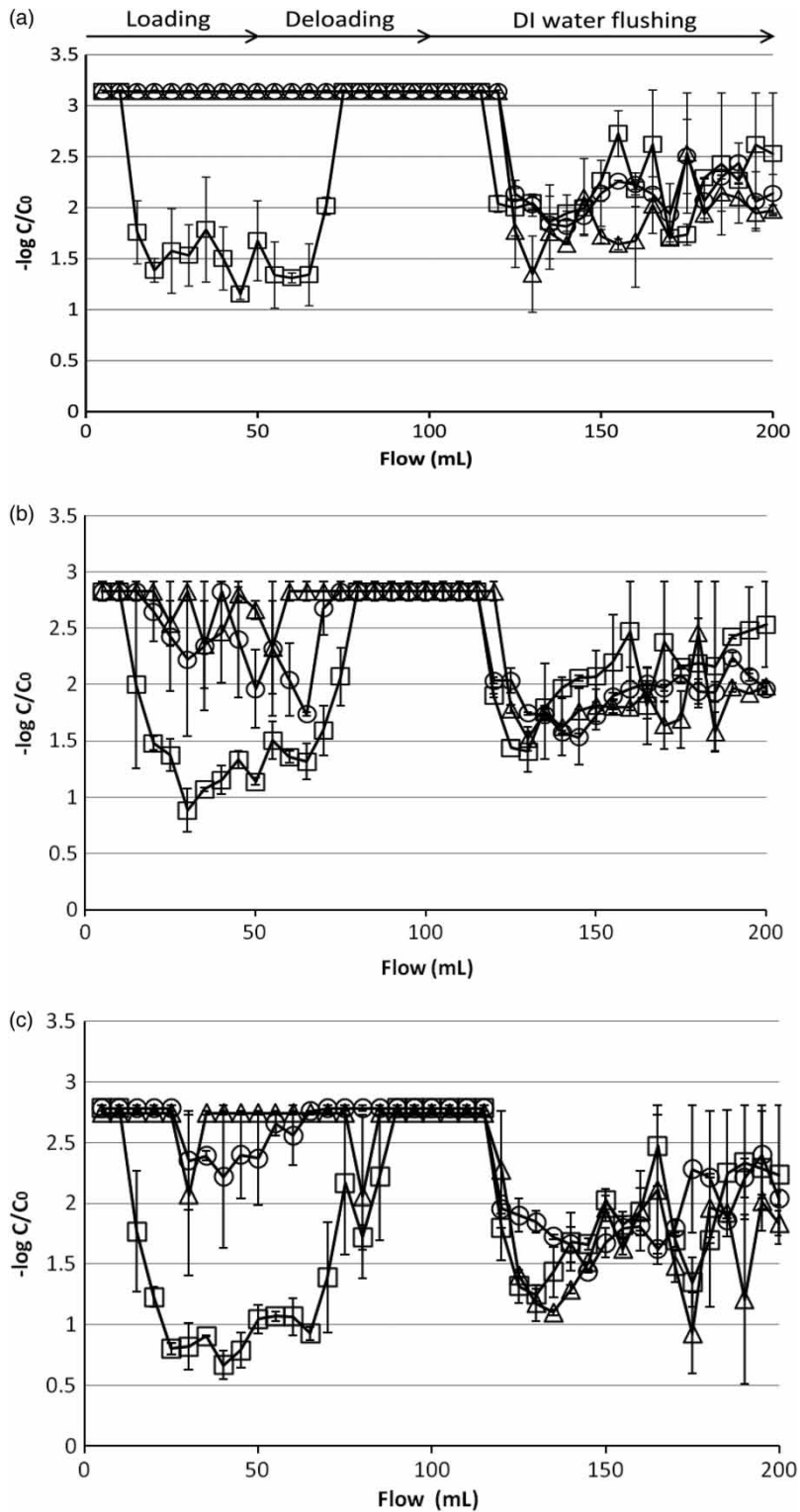


Figure 3 Adenovirus BTC at a flow rate of (a) 1 mL/min, (b) 2.5 mL/min and (c) 5 mL/min: Solid lines represent the mean log removal values ($-\log C/C_0$, LRV) of duplicated breakthrough experiments and error bars indicate the individual data points from each column. Virus concentrations were measured by using qPCR. Note that the DI water flushing started at 100 mL. The log removal values of samples under the detection limit were presented as C_0 . Columns: (□) Sand, (Δ) 180-HTC and (○) 230-HTC.

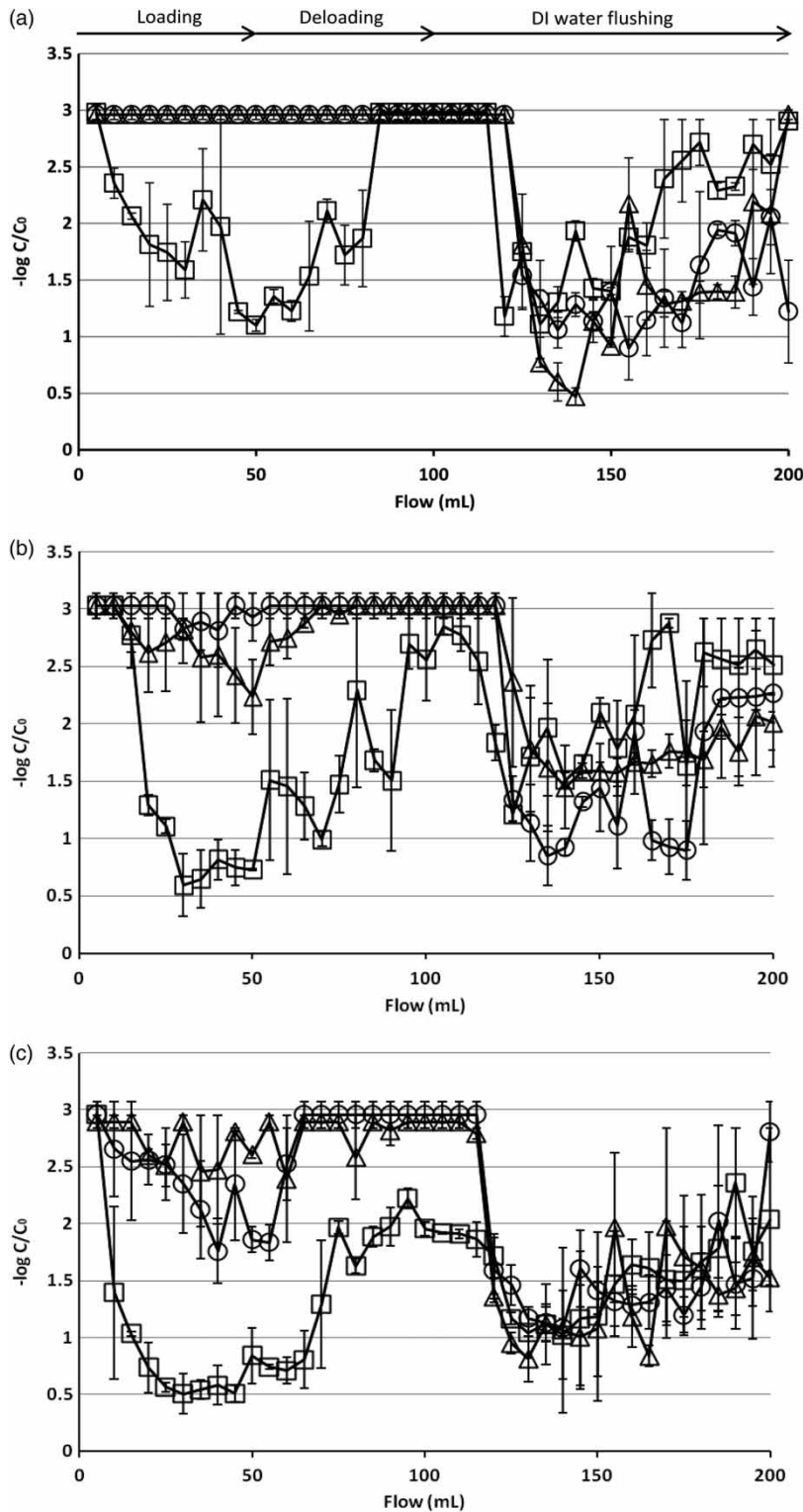


Figure 4 | Rotavirus BTC at a flow rate of (a) 1 mL/min, (b) 2.5 mL/min and (c) 5 mL/min: Solid lines represent the mean log removal values ($-\log C/C_0$, LRV) of duplicated breakthrough experiments and error bars indicate the individual data points from each column. Virus concentrations were measured by using RT-qPCR. Note that the DI water flushing started at 100 mL. The log removal values of samples under the detection limit were presented as C_0 . Columns: (□) Sand, (Δ) 180-HTC and (○) 230-HTC.

Regardless of the virus type or the flow rate, all experiments conducted in the sand-only columns produced BTCs with a typical pattern consisting of a falling limb, a plateau phase and a rising limb (Figures 3 and 4). It was common that the falling limb started from 10 mL, and arrived at the plateau phase at around 20 mL. Then, the rising limb appeared at around 65 mL, followed by a transition phase which clearly separated the virus release between the loading-deloaded and the DI water flushing. The depth and duration of the plateaus varied depending on the flow rates. In general, low flow rates resulted in shallower and shorter plateaus, or in higher removal efficiencies than when using higher flow rates (Table 2). The highest virus removal efficiency of sand-only columns was 1.3 log at 1 mL/min flow rate. At the higher flow rates, the virus removal efficiencies ranged from 0.6 to 1.2 log (70–93%).

Interestingly, at flow rates of 2.5 and 5 mL/min, RV was observed in the effluent samples from the sand-only columns throughout the breakthrough experiments. LRVs in the transition phase decreased from 2.7 to 2 as the flow rate increased from 2.5 to 5 mL/min (Figure 4(b) and 4(c)). It was apparent that a part of the RV deposited on the surface of the sand media had been continuously detached. This phenomenon was not observed in HAdV BTCs obtained from sand-only columns.

In contrast, the sand columns amended with either 180-HTC or 230-HTC did not release any viruses at 1 mL/min flow rate during either the loading or de-loading phase, resulting in complete removal (Figures 3(a)

and 4(a)). Different from the typical breakthrough pattern shown in sand-only columns, only irregular release of viruses at low concentrations (LRV >2) was observed at higher flow rates (Figures 3(b), 3(c) and 4(b), 4(c)). Hence, due to the amendment of the sand columns with hydrochar, total removal efficiencies improved (Table 2). In terms of virus removal, both hydrochar types did not have a significant difference. Due to the decrease in the virus removal efficiency of sand-only columns at higher flow rates, the effect of hydrochar amendments became more apparent at higher flow rates (2.5 and 5 mL/min): in those flow ranges, the virus removal efficiencies of hydrochar-amended columns ranged from 2.1 log to complete removal (99–100%).

In general, the viral detachment upon flushing with DI water was larger for RV than HAdV for all experimental conditions employed (Table 2). Under the employed experimental conditions, it was apparent that the increase in the flow rate does not have a clear impact on the amount of virus released. Though it could be speculated that the role of the secondary energy minima in the RV retention by the sand-only column was more significant at 5 mL/min than the lower flow rates, the result should be carefully interpreted because of the possible effect of increased physical stress that might have facilitated the viral detachment, shown as a decrease in LRV during the transition phase (Figure 4(c)). This could lead to over-estimating the mass of RV residing in the secondary energy minimum.

Table 2 | Removal and release of viruses in the breakthrough analysis with DI water flushing

Flow rate (mL/min)		Sand-only		180-HTC + Sand		230-HTC + Sand	
		RV	HAdV	RV	HAdV	RV	HAdV
1.0	LRV	1.3 ^a	1.3	>3.0	>3.1	>3.0	>3.1
	Release in DI water (%)	(4.0) ^b	(1.6)	(13.0)	(3.1)	(9.9)	(1.5)
2.5	LRV	0.8	1.2	2.5	2.6	>3.0	2.2
	Release in DI water (%)	(3.3)	(2.3)	(3.8)	(3.0)	(11.5)	(2.4)
5.0	LRV	0.6	0.9	2.5	2.4	2.1	2.5
	Release in DI water (%)	(11.4)	(3.9)	(14.6)	(7.1)	(8.3)	(2.6)

^aAverage log removal efficiency obtained from duplicated breakthrough analysis. The values with a sign of inequality represent C_0 , which indicates complete virus removal.

^bAverage percentage of virus release in DI water flushing stage.

DISCUSSION

Improved virus removal with hydrochar supplement in sand columns

This study showed that the virus removal efficiency of sand columns was significantly increased by supplementing hydrochar (1.5%, *w/w*) to the column media (Table 2). The adsorptive retention capacity of sand columns (with or without hydrochar supplement) can be proportional to the surface area and the favorable surface functional groups of the column media. The viral attachment on solid media is primarily controlled by van der Waals, electrostatic (electrical double-layer) and hydrophobic interactions that vary depending on surface characteristics of the virus and media, temperature and solution chemistry (Israelachvili & Wennerstrom 1996; John & Rose 2005). Since the net surface charge of non-enveloped viruses is determined by functional groups on the viral capsid and their protonation-deprotonation states, the solution pH plays a key role determining the electrostatic interaction (Michen & Graule 2010). Most frequently, viruses in polar media (such as water) have a negative surface charge at a neutral pH (Michen & Graule 2010). Because both hydrochar types (180-HTC and 230-HTC) in the AGW also showed a negative zeta-potential (Figure 2), electrostatic interactions between viruses and media surfaces in the column experiments are expected to be of a repulsive nature.

One of the possible virus removal mechanisms of the hydrochar amendment can be due to their strongly hydrophobic nature. Because the hydrochar powders (<0.125 mm) were much smaller than the sand granules (~0.8 mm), hydrochar supplements would have provided a meaningful extra surface area onto which the viruses can attach. Also, the results from hydrophobicity measurements on hydrochar samples suggested an increase in hydrophobic attraction between viruses and the hydrochar surface (Bales *et al.* 1991, 1993). The importance of hydrophobic interactions in virus retention was reported in a previous work that employed a membrane filtration unit (van Voorthuizen *et al.* 2001). In a direct comparison between a hydrophilic and a hydrophobic membrane, greater removal efficiencies of bacteriophage

MS-2 were obtained with the hydrophobic membrane in a wide range of pH and ionic strength of the suspension, and the hydrophobic interaction was pointed out as the main cause for the superior virus removal performance.

Another mechanism that likely contributed to the RV and HAdV removal is viral deposition in flow stagnation zones: viruses would be retained without direct contact to the hydrochar surface, but immobilized by the hydrochar surface owing to the secondary energy minimum (Table 2) (Redman *et al.* 2004; Brow *et al.* 2005). Other possible causes for virus deposition under unfavorable conditions include heterogeneous charge distribution, the presence of localized patches with positive charge that would have induced attractive electrostatic interactions despite a net negative charge (Elimelech & Omelia 1990; Elimelech *et al.* 2000).

Reversible attachment of virus particles is of major interest for effluent quality surveillance, because changes in influent ionic strength and/or pH can result in undesirable releases of infectious virus particles accumulated on the hydrochar surface. In our previous work carried out in a comparable experimental set-up using sand columns supplemented with a sewage sludge-derived hydrochar (1.5%, *w/w*), the release of HAdV in DI water flushing was comparable to the results of this research (~5%) (Chung *et al.* 2015). In contrast, the RV release from sewage sludge-amended sand columns was larger (24%, Chung *et al.* 2015) than the release from the swine manure derived 180-HTC (13%, Table 2) or 230-HTC (10%, Table 2). It can be speculated that the fecal waste-derived hydrochar retained a larger proportion of RV in the primary energy minimum, which is considered as irreversible attachment. Although the fecal waste-derived hydrochar seems a better adsorbent than the sewage sludge-derived hydrochar in this viewpoint, still a meaningful portion of RV was retained by reversible attachment. This has to be taken into consideration when large fluctuations of pH and/or the ionic strength are expected in the target water for virus removal.

The virus concentrations in the samples were measured by PCR-based methods. Due to their inability in assessing viral infectivity, the virus removal performance observed in this research must be interpreted as the worst-case scenario assuming all viruses in the samples are still infective (Lodder *et al.* 2013).

Effect of flow rate and secondary energy minimum

Colloidal deposition is affected by the flow rate (Li *et al.* 2005; Johnson *et al.* 2007). Depending on the presence or absence of an energy barrier to deposition, the flow rate affects colloidal deposition and release (re-entrainment) in opposite ways: in the presence of an energy barrier, colloidal deposition rates decrease and detachment rates increase with increasing flow rate (Tong & Johnson 2006; Johnson *et al.* 2007). Since both biological colloids and media surfaces (silicate mineral grains) carry an overall negative surface charge (unfavorable condition) under most environmental conditions (Davis 1982; Tipping & Cooke 1982), an increase in flow rate has a negative impact on pathogen removal in adsorption-based water treatment systems. Our observations agree well with these investigations: the removal efficiency decreased with increasing flow rate (Table 2).

The effect of increased flow rate (flow velocity) on the deposition and release behavior was attributed to an increase in fluid drag (Tong & Johnson 2006; Johnson *et al.* 2007). Li *et al.* (2005) suggested a close relation between the effects of the fluid drag and the role of the secondary energy minimum on colloidal deposition and re-entrainment. Briefly, the increased fluid drag may, to a certain extent, shift the deposition of colloids from the primary energy minimum (strong attachment) to the secondary energy minimum (weak attachment). Then, this increase in the role of the secondary energy minimum would have exposed more colloids to chances of detachments. Potential mechanisms for the effect of fluid drag on deposition and re-entrainment behavior of colloids can be summarized as follows (Li *et al.* 2005): (i) decrease in stagnation flow zones in which colloids can accommodate, (ii) increase in colloidal diffusion induced by enlarged concentration gradients between fluid and zones in which colloids accumulate, (iii) increased hydrodynamic collisions between mobile and deposited colloids, and (iv) increased hydrodynamic torque relative to the adhesive torque. The effect of increased flow rates was more prominent in sand-only columns than in hydrochar-amended columns (Table 2). We speculated that hydrophobic interactions between hydrochar surfaces and virus particles had provided an additional attractive force which compensated the negative

impact on the virus removal induced from increased fluid drag.

Despite the similarities of both RV and HAdV in their morphology (Friefeld *et al.* 1984; Stewart *et al.* 1993; Parashar *et al.* 1998), the difference in surface characteristics can explain the different response to the flow rate increase (Schijven & Hassanizadeh 2000). The results of this study suggest that the secondary energy minimum was more pronounced for RV than HAdV. For each experimental condition, regardless of the type of column packing material and flow rate, the RV release in DI water flushing was greater than the release of HAdV (Table 2). This observation corresponded well with our previous work (Chung *et al.* 2015). The higher variation of the RV removal efficiency depending on the flow rate in the sand media can be explained by the larger role of the secondary energy minimum (weak attachment), suggesting larger detachment rates throughout flushing experiments. The extended tailing of RV and elevated LRV level in the intermediate phase in the sand-only columns at 5 mL/min (Figure 3(c)) support this hypothesis (Li *et al.* 2005), and the response to the flow rate variation can be considered as virus-specific.

HTC for fecal waste treatment

The difference in reaction intensity during the hydrochar production resulted in a different hydrochar yield and composition (Figure 1 and Table 1). However, no clear difference in virus removal efficiency between 180-HTC and 230-HTC was observed for all experiments (Table 2). If we limit the potential use of hydrochar to a virus adsorbent in groundwater treatment, HTC produced at 180 °C could be a more attractive option due to the higher hydrochar yield (Table 1) and lower energy requirement during its production. Higher temperatures and extended reaction times in HTC production at 230 °C yield more operational costs. Also, the higher pressure induced in 230-HTC might necessitate a more pressure-resistant reactor, which will increase the capital costs.

Sustainable implementation of fecal waste treatment with HTC would be feasible in cases where the cost needed for HTC is lower than current treatment in specific cases (e.g. planted or unplanted sludge drying bed) or when the cost can be compensated by the benefits from HTC products (Koné 2010). Since the economic value of

the end-products varies significantly depending on implementation sites and their market demands (Diener *et al.* 2014), more research on fecal waste-HTC products is needed (e.g. calorific value, plant nutrient availability, biogas production capacity). Also, indirect benefits such as better hygienic surveillance (Hutton *et al.* 2007) or preventing deforestation (replacement of conventional plant-based energy source with HTC products) (Miles & Kapos 2008) need to be considered as an asset.

Since the characteristics of hydrochar are determined by the nature of the feed stock and several process parameters such as moisture conditions, temperature, residence time, pressure, solid load and pH (Libra *et al.* 2011), optimization of the HTC process (e.g. co-carbonization of fecal waste with locally available agricultural residues) pursuing maximum economic value of the HTC products is an important topic for further research.

CONCLUSIONS

This study showed the following:

- (i) Hydrochar amendments (1.5%) clearly enhanced the virus removal performance of sand columns for all flow rates employed. This can be mainly attributed to the provision of extra hydrophobic surfaces enforcing hydrophobic attraction between the viruses and column media.
- (ii) Though different HTC conditions resulted in different characteristics of 180-HTC and 230-HTC, no clear difference in virus removal performance was observed between both hydrochars.
- (iii) In sand-only columns, an increase in flow rate decreased the virus removal efficiency. Supplementing the columns with hydrochar (1.5%) mitigated the negative impacts from the increased flow rate in the virus retention.
- (iv) During DI water flushing, more release was observed for RV than HAdV despite the similar size and shape of both viruses. Apparently, the contribution of the secondary energy minimum in the virus retention was more determined by the surface characteristics of the viruses than their size and shape.

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