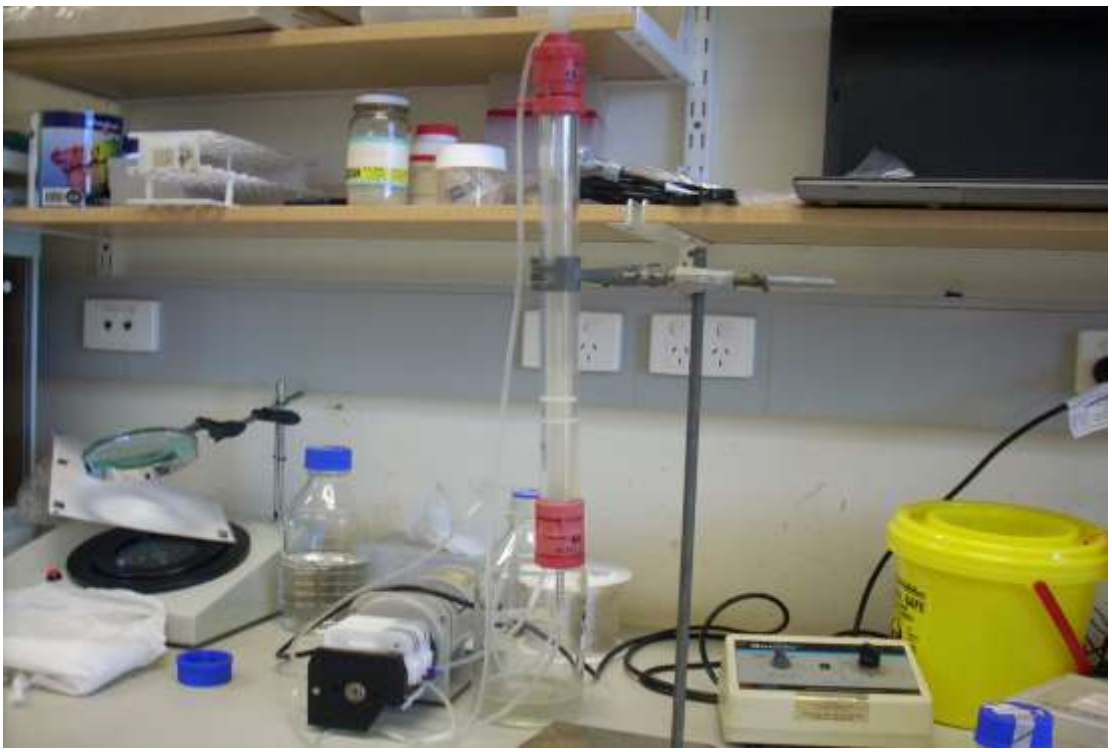
Concentration of Enteric Virus from Raw Wastewater and Treated Effluent with Silica Beads

Jatinder P.S. Sidhu and Simon Toze

November 2011



Urban Water Security Research Alliance
Technical Report No. 56

Queensland Government
National Research FLAGSHIPS Water for a Healthy Country
CSIRO
Griffith UNIVERSITY
The University Of Queensland AUSTRALIA

Urban Water Security Research Alliance Technical Report ISSN 1836-5566 (Online)
Urban Water Security Research Alliance Technical Report ISSN 1836-5558 (Print)

The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to South East Queensland tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

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Sidhu, J.P.S. and Toze, S. (2011). *Concentration of Enteric Virus from Raw Wastewater and Treated Effluent with Silica Beads*. Urban Water Security Research Alliance Technical Report No. 56.

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ACKNOWLEDGEMENTS

This research was undertaken as part of the South East Queensland Urban Water Security Research Alliance, a scientific collaboration between the Queensland Government, CSIRO, The University of Queensland and Griffith University.

Thanks also go to the Brisbane City Council Wastewater Treatment Plant operators (now a part of Queensland Urban Utilities) for access to samples form the Oxley Creek Wastewater Treatment Plant.

FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

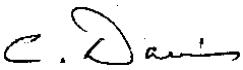
As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.



Chris Davis
Chair, Urban Water Security Research Alliance

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EXECUTIVE SUMMARY

The detection of enteric viruses from recycled water is hampered by the need to concentrate low numbers of viruses from large volumes of water. A number of virus concentration methods such as ultrafiltration, virus capture and release on glass wool, positively charged membranes and negatively charged membranes have been developed. However, most of the methods are developed to detect viruses from potable quality water but provide low recovery rates of viruses from lower quality water, such as primary and treated effluent, due to the presence of particulate matter, organics and other contaminants. The aim of this study was to develop a simple, efficient and inexpensive virus concentration method that can efficiently capture viruses in low quality water.

Initially, experiments were conducted with negatively charged filters, polystyrene beads and silica beads to compares their ability to capture enteric virus from tap water. Negatively charged HA type filters (1cm square) were found to result in a 16-18% recovery efficiency, whereas polystyrene beads resulted in 2% recovery rate of seeded adenovirus and coxsackievirus. Adenovirus and coxsackievirus recovery rates of 26 and 29% respectively were observed for with non-modified silica beads (SiO_2) in the presence of magnesium chloride ($MgCl_2$). Subsequently, silica beads were used as packing material in a glass column to capture viruses from primary and secondary treated wastewater.

At the column scale, coxsackievirus recovery rates of 18 and 26% were observed respectively for primary and secondary treated effluent in the presence of $MgCl_2$. The use of amino-functionalised, (*N*-3-(trimethoxysilylpropyl) diethylenetriamine) silica beads was tried to determine if the capture of viruses could be enhanced. The resulting recovery rate however, was lower than the $MgCl_2$ based method. The observed adenovirus recovery rate using the diethylenetriamine from the primary and secondary wastewater was up to 15%. In comparison, a slightly better recovery rate for coxsackievirus (16-28%) was observed from the secondary treated effluent. Pre-conditioning of the water samples with hydrochloric acid (HCl) (to pH 3) followed by capture on silica beads appears to be the most promising method for capture and release of virus from primary and secondary treated wastewater with recovery rates of around 40-50%. The virus capture and release mechanism appears to work better with coarse silica beads (60-100 mesh size) as compared to fine silica (3-6 mesh size), possibly due to less interference of particulate matter from the primary effluent. Acidification of samples is a relatively easier and less time consuming approach compared to the use of amino-functionalised silica with the added benefit of better virus recovery rates. Further research works is required to validate virus capture and release approached with different quantities of packing silica, flow rates, types of water and different virus types.

1. INTRODUCTION

Enteric viruses such as noroviruses, rotaviruses, adenovirus and astroviruses are major causes of non-bacterial gastroenteritis worldwide (Wilhelmi *et al*., 2003; Lopman *et al*., 2003). Norovirus in particular, and others in general, have been implicated in waterborne outbreaks (Bosch *et al*., 2008; La Rosa *et al*., 2008).

A numbers of studies have reported the presence of enteric viruses in high numbers in wastewater and treated effluent (Haramoto *et al*., 2006; da Silva *et al*., 2007; Katayama *et al*., 2008; Nordgren *et al*., 2009; Aw and Gin, 2010). Enteric viruses can find their way into aquatic environments through leaking sewers, septic systems, agricultural runoff and through discharge of treated wastewater into the environment. They have been detected in surface water, groundwater, and drinking water (Haramoto *et al*., 2005; Abbaszadegan *et al*., 2003; Lambertini *et al*., 2008; Albinana-Gimenez *et al*., 2009).

Bacteria, such as faecal coliforms and enterococci, are commonly used as indicators to evaluate pathogen removal efficiency of the wastewater treatment processes. However, the adequacy of these indicator bacteria for suggesting the presence and comparative survival of enteric viruses has been questioned in recent years (Tree *et al*., 2003; Robertson and Nicholson, 2005). Recent advances in molecular techniques have made it possible to detect viral pathogens in the environment; hence direct surveillance for viruses may be a better approach for protecting public health. Several studies have suggested that adenoviruses and polyomaviruses are present in higher numbers in wastewater compared to other enteric viruses and are thus a potentially suitable index microorganism for viral pollution in aquatic environments (Bofil-Mas *et al*., 2006; Symonds *et al*., 2009; Fong *et al*., 2010).

Due to the diffuse presence of enteric viruses in aquatic environments, typical virus detection methods necessitate the concentration of viruses from large volumes of water (Karim *et al*., 2009). Several virus concentration methods have been developed and applied, such as: ultrafiltration (Rodriguez-Diaz *et al*., 2009; Nordgern *et al*., 2009; Olszewski *et al*., 2005); adsorption-elution-based protocols with glass wool (Gantzer *et al*., 1997; Lambertini *et al*., 2008); positively charged membranes (Brassard *et al*., 2005; Bennett *et al*., 2010); negatively charged membranes (Katayama *et al*., 2008); and immunomagnetic capture (Casas and Sunen, 2002). However, most of the methods are developed to concentrate and detect viruses from relatively clean water (potable quality). Concentration of enteric viruses from low quality water with these techniques make them impractical due to the high cost of filters, co-concentration of PCR inhibitors, clogging of membranes and long sample processing time. Thus, there is a need for a simple, rapid and economical method for the concentration of enteric viruses in low quality water.

Enteric viruses are known to adsorb to a negatively charged membrane at low pH (3.0) conditions (Sobsey *et al*., 1973) or in the presence of multivalent cations (Lukasik *et al*., 2000; Katayama *et al*., 2002; Haramoto *et al*., 2004). Virus capture by the membrane occurs due to electrostatic interactions between microporous filters and virus capsid. Silicon dioxide (SiO_2) beads which exhibit a negative surface charge when immersed in water (Chen *et al*., 2006) offer a promising alternative as an adsorptive material for the concentration of enteric virus from low quality water. Reversal of the surface charge on the virus capsid with acidification of the water sample or by addition of multivalent cations, however, is required to promote virus capture. It has been reported that addition of aluminium chloride ($AlCl_3$) in water promotes capture of virus by lowering the pH and flocculating of suspended viral particles (Lukasik *et al*., 2000). Lukasik *et al*. (2000) evaluated the adsorption of different viruses onto various filters using three types of salt and observed that Cl^-ions associated with Na^+, Mg^{2+} or Al^{3+} promoted the hydrophobic interactions between viruses. They determined that an HA type membrane filter (Millipore) combined with $AlCl_3$ was the best for viral recovery from environmental waters, probably because the viruses were trapped more efficiently by the trivalent ion. However, Haramoto *et al*. (2007) observed that $MgCl_2$ was more sensitive for the capture of virions (intact virus) rather than naked RNA when compared with $AlCl_3$.

Amino-functionalised, electropositive silica particles have been used at small scale to capture bacteriophage MS2 and PRD1 from seeded water samples (Chen *et al.*, 2006). The applicability of this approach at column scale with low quality water, however, remains unknown. We have explored the potential capture of viruses with flow through a glass column packed with silica beads to improve the chances of detection of virus particles present in low numbers in wastewater. This approach allows the filtration of larger volumes of low quality water without clogging the filter, along with minimal chances of co-purification of PCR inhibitors. The objective of this study was to develop a simple, efficient and inexpensive virus concentration method based on silica beads that will efficiently capture viruses in low quality water.

2. MATERIALS AND METHODS

2.1. Wastewater Sample Collection

Primary effluent and secondary treated wastewater samples were collected from Luggage Point and Oxley Creek wastewater treatment plants (WWTP) in Brisbane, Australia. Two litres of both primary and secondary treated wastewater samples were collected in sterile borosilicate bottles, transported on ice to the laboratory and stored at 4ºC.

2.2. Standard Virus Cultures used in the Study

Coxsackievirus B3 and Adenovirus strain 41 used in this study were cultured in cell lines (African Green Monkey Kidney cells) by PathWest, Western Australia. The viruses were then harvested from the lawns and frozen at -80ºC until needed. The numbers of infective viral particles in the viral suspensions were determined using the MPN method in fresh cell culture lawns. The titre for each virus was determined by PathWest to be 10^9 plaque forming units (pfu) mL^{-1} for coxsackievirus and 10^7 pfu mL^{-1} for adenovirus. Virus cultures were washed twice in sterile phosphate buffer (P-buffer) to remove culture media and then re-suspended in P-buffer prior to seeding in water samples.

2.3. PCR Primers and Cloning of Target Gene Sequences

Adenovirus and coxsackievirus were quantified using published primer sets (Heim *et al.,* 2003; Abbaszadegan and Delong, 1997). The PCR amplified products (cDNA/DNA) were purified using the QIAquick PCR purification kit (Qiagen), and cloned into the pGEM®-T Easy Vector System (Promega Madison, WI, USA), transferred into *E. coli* JM109 competent cells and plated on LB agar ampicillin, IPTG (isopropyl-β-D-thio-galactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-β- D-galactopyranoside) as recommended by the manufacturer. The cloned plasmid was purified using a plasmid mini kit (Qiagen).

2.4. Preparation of Standard Curves

Purified plasmid DNA containing the enterovirus and adenovirus inserts were quantified using a spectrophotometer (NanoDrop ND-1000). Plasmid copies were calculated and a ten-fold serial dilution was prepared in DNase and RNase free water to a final concentration ranging from 10^0 to 10^6 copies/µL and aliquots were stored at -80ºC until use. A 3 µL template from each dilution was used to prepare a standard curve for qPCR.

2.5. Nucleic Acid Extraction and PCR Amplification

Viral RNA/DNA was extracted from the samples using a BD biosciences Clontech NucleoSpin® Virus nucleic acid extraction and purification kit. Viral RNA or DNA was extracted from 150 µL of sample according to the manufacturer's instructions. A final elution (50 µL) was collected in sterile RNase free centrifuge tubes and stored at -80°C prior to analysis.

Quantitative PCR assays were carried out to determine the gene copy numbers of seeded coxsackievirus and adenovirus numbers in the wastewater samples. Quantitative PCR reactions were performed on a Bio-Rad iQ5 thermocycler (Bio-Rad Laboratories, California, USA), using iScript RT-PCR mix and iQ Supermix (Bio-Rad). Each 25 µL PCR reaction mixture contained 12.5 µL of SuperMix, 120 nM of each primer, and 3 µL of template DNA. Bovine serum albumin (BSA) was added to each reaction mixture to a final concentration of 0.2 µg $µL^{-1}$ to relieve PCR inhibition (Kreader, 1996). RNAse inhibitor (Roche) was added to one step RT-PCR reaction mixture to a final concentration of 0.2 µg $µL^{-1}$ to inhibit the activity of RNAse.

Thermal cycling conditions for the detection of coxsackievirus and adenovirus were as outlined in Sidhu *et al*., (2010). A melt curve analysis was performed after the PCR run to differentiate between actual products and primer dimmers, and to eliminate the possibility of false-positive results. The melt curve was generated using 80 cycles of 10 seconds each starting at 55°C and increasing in 0.5°C intervals to a final temperature of 95°C. The Tm for each amplicon was determined using the iQ5 software (Bio-Rad).

2.6. Small Scale Virus Capture and Elution Experiments

Small scale virus capture and elution experiments were performed in duplicate by seeding adenovirus and coxsackievirus into tap water in 15 mL polypropylene centrifuge tubes. Enteric viruses adsorb to negatively charged membrane in the presence of cations (Na^{+}, Mg^{2+} or Al^{3+}). In this study, we have used negatively charged HA type membranes (Millipore), Polystyrene beads (3-5mm diameter) and silica beads (3-9 mesh) to capture viruses. In the first set of experiments, 10 squares of HA filters (1cm) were added to 10 mL tap water seeded with viruses. In the second experiment, 20 polystyrene beads were added to each 10 mL tap water sample in 15 mL centrifuge tubes. In the third experiment, five grams of silica beads were added to 10 mL of water sample to capture the seeded enteric virus. The virus capture and elution procedure used in this study was modified from the previously reported method by Katayama *et al*. (2002). The schematic of the virus capture and elution method adopted in this study is presented in Figure 1.

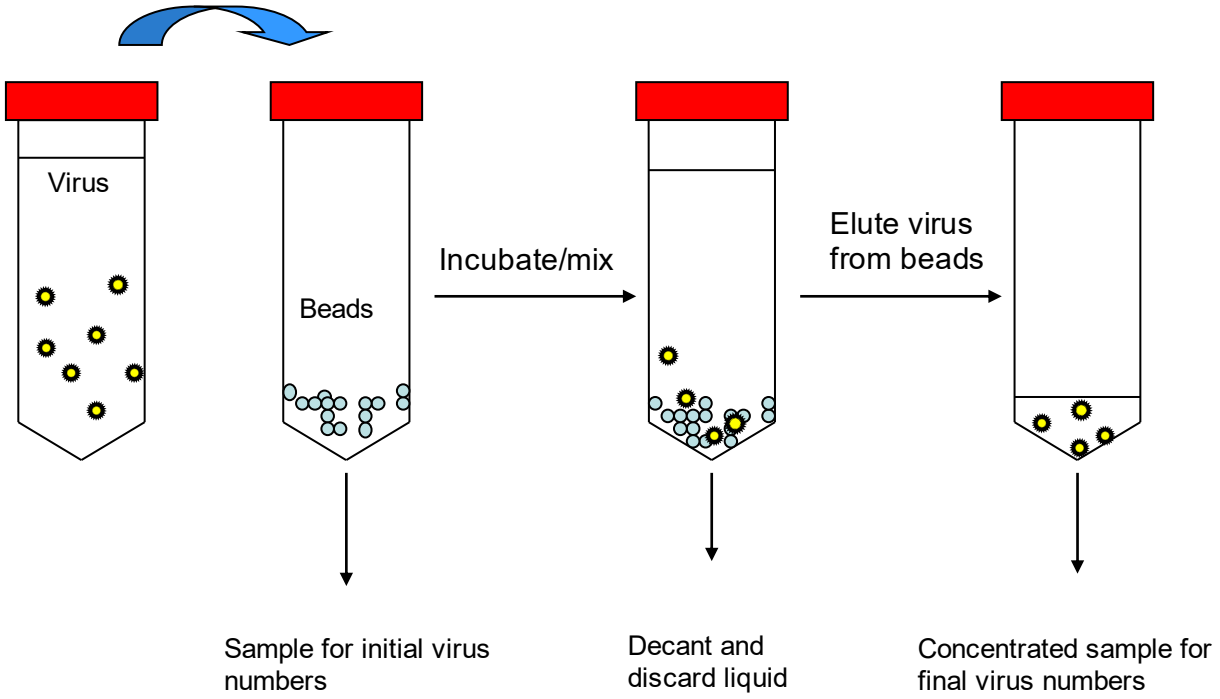

Figure 1. Schematic diagram of virus capture and release with silica beads.

Briefly, a tap water sample (10 mL) seeded with known numbers of adenovirus and coxsackievirus was conditioned with $MgCl_2$ to obtain a final concentration of 25 mM of $MgCl_2$ prior to addition of the virus capture material for each of the three sets of experiments. The tubes were then gently rotated on a Dynal sample mixer at slow speed (approximately 20 - 30 rpm) for 30 minutes (min.) at room temperature (Figure 2). Captured viruses were eluded with 5 mL of elution buffer (1 mM NaOH at pH 10.8). The pH of the eluent was then adjusted to neutral with 100 mM H_2SO_4 (pH 1.0). Finally, a Tris–EDTA buffer (pH 8.0) was added to stabilise the captured viruses. Further concentration of the captured viruses was carried out by centrifugation, using a Centriprep (Millipore) at 2500 rpm for 10 min to obtain a final volume of one mL.

Figure 2. Mixing of tubes on a Dynal sample mixer to capture seeded virus in tap water samples.

2.7. Silica Column Setup

A chromatography column (450 x 5 mm) was modified for circulation of wastewater samples seeded with enteric virus (Figure 3). The column was packed with 3 gm of glass beads (4 mm diameter) and 10 gm of silica (3-9 or 60-100 mesh size, Sigma–Aldrich) on top of the glass beads. A secondary treated wastewater sample (100 mL) was seeded with 50 µL each of coxsackievirus and adenovirus cultures to obtain a final number of approximately 10^7 PCR Detectable Units (pdu) mL^{-1}. Primary wastewater samples are known to contain 10^5 pdu mL^{-1} of adenovirus, therefore, primary wastewater samples (100 mL) were seeded with 50 µL of Coxsackievirus only (final number approximately 10^4 pdu mL^{-1}). The seeded 100 mL water sample was then circulated through the column for 20 min. with a peristaltic pump set at a speed of 60 mL per min. The water was added to the column from the top and flow was reversed for two minutes after every five minutes to ensure there was no preferential flow of water occurring through the column through the clogging of pore space with suspended solids.

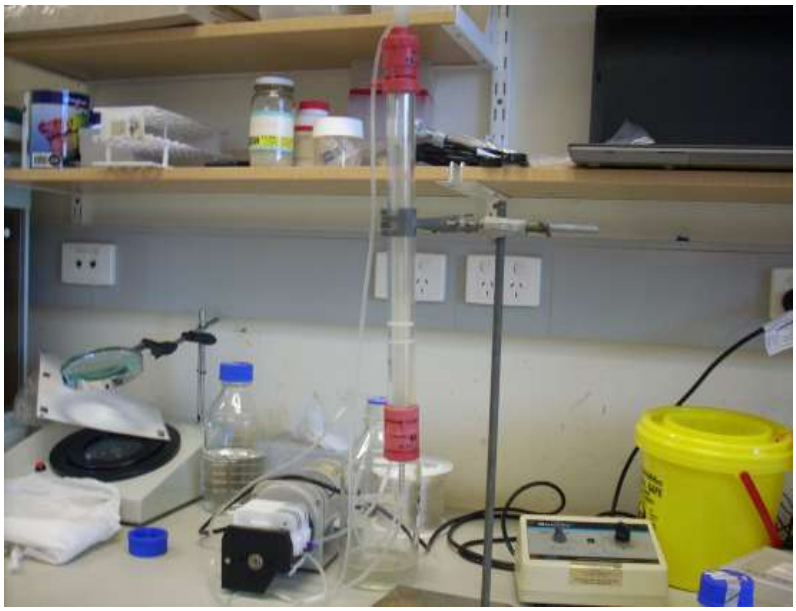

Figure 3. Modified chromatography column apparatus for virus concentration.

2.8. Virus Capture and Release Efficiency in Column Packed with Silica

The first method used for the capture of viruses from wastewater samples was based on the method described previously (Katayama *et al*., 2002). Briefly, 2 mL $MgCl_2$ (2.5 M) was added to primary or secondary wastewater samples to obtain a final concentration of 25 mM. The water sample (100 mL) was then circulated for 20 minutes through the column packed with non-modified silica beads. The column was drained and the silica beads were rinsed with 100 mL of 0.5 mM H_2SO_4 (pH 3.0) to remove excess $MgCl_2$. Captured viruses on the beads were eluted with 50 mL of elution buffer (1 mM NaOH at pH 10.8). The pH of the eluent was then adjusted to neutral with 100 mM H_2SO_4 (pH 1.0). Then Tris–EDTA buffer (pH 8.0) was added to stabilise captured viruses. Further concentration of the virus was carried out by centrifugation using a Centriprep (Millipore) at 2500 rpm for 10 min. to obtain a final volume of one mL.

The second virus capture and release method tested in this study was based on the surface charge reversal on virus particles by lowering the pH of the water sample (Sobsey *et al.,* 1976). Concentrated HCl was added drop-wise to lower the pH of the water sample (100 mL) to 3.0. The acidified sample was then circulated through the column packed with non-modified silica beads. After 20 min., the water was drained and captured viruses were eluted from the beads with 50 mL of 0.05 M glycine at pH 11.5. The eluate was adjusted to a neutral pH with 0.05 M glycine at pH 1.0. Further concentration of the virus in the sample was carried out using a Centriprep column as mentioned earlier to a final volume of one mL.

The third approach used in this study was based on the capture of virus on amino-functionalised silica as described previously (Chen *et al*., 2006). Briefly, 6 gm of silica (3-9 mesh) was dried overnight at 150°C and added to 150 mL of 3% *N*-3-(trimethoxysilylpropyl) diethylenetriamine solution (DPS) in 1 mM acetic acid. After three hours (h) of continuous stirring, the beads were filtered, washed and dried at 150°C for 4 h. The column was packed with the dried DPS silica and the seeded water sample was circulated through the columns as mentioned above. The captured viruses on the beads were eluted with 50 mL elution buffer containing 2.9% tryptose phosphate and 6% glycine at pH of 9.0. Further concentration of virus in the sample was carried out using a Centriprep column as mentioned earlier to a final volume of one mL.

3. RESULTS AND DISCUSSION

Enteric viruses are present in the wastewater matrix in relatively low numbers, therefore, for detection of virus in wastewater and environmental samples, collection and concentration of large sample volumes is generally required. Typically, the collected water sample is passed through the filter membranes to capture the virus on the basis of either charge or size. In both cases, clogging of the membranes is a major issue due to the presence of large amount of suspended solids.

In this study, we developed and evaluated a simple and inexpensive flow-through device packed with charged particles (silica beads) for the capture of enteric viruses in water samples without concentration of suspended solids. A number of charged materials were evaluated, however, silica beads were found to be most promising and hence were used as packing material for the glass column.

3.1 Small Scale Virus Capture and Release Experiments

The results of virus concentration in small scale setup (polypropylene centrifuge tubes) are presented in Figure 4. In order to make a direct comparison between virus capture efficiency of the new approaches used in this study and published literature, we reproduced the virus concentration approach used by Katayama *et al*. (2002), in 200 mL of tap water seeded with adenovirus and coxsackievirus.

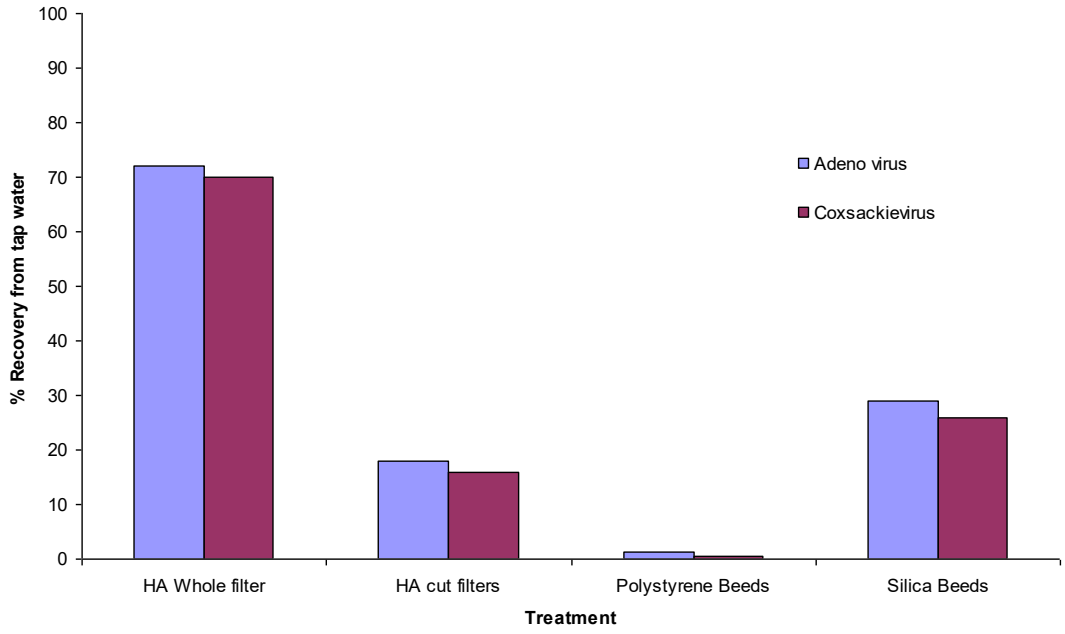

Figure 4. Comparative enteric virus concentration from tap water with different virus capture material.

The best recovery efficiency (70%) was observed when the water sample was passed through the negatively charged HA type filters, which is in agreement with the reported results in the literature (Katayama *et al*., 2002; Haramoto *et al*., 2005). However, virus capture efficiency dropped to 16-18% when 1 cm square HA type filters were added to the tap water seeded with viruses, which suggests that flow of water over the negatively charged HA type membranes is less efficient in capturing virus than when the water is passed through the filters. This is not unexpected as HA type filters are relatively hydrophobic (Lukasik *et al*., 2000) and the sticking of cut membranes to each other was also observed, which might have also reduced the surface area available for the virus capture and hence lower virus capture efficiency.

In the second set of experiments, polystyrene beads were evaluated for the capture of adenovirus and coxsackievirus as they have a small net negative surface charge. An added advantage is that beads repel each other due to the similar negative charge. Although the beads were not sticking to each other in the tap water, virus capture and release efficiency was found to be very low (< 2%) for both adenovirus and coxsackievirus. The observed low virus capture efficiency is possibly due to the hydrophobic nature of the polystyrene beads.

In the third set of experiments, with silica beads, a recovery efficiency of 26 and 29% was observed for coxsackievirus and adenovirus respectively in the presence of divalent cations ($MgCl_2$). Consequently, for the column based work, silica beads were identified as the most suitable virus capture material for trial.

3.2 Virus Capture and Release Experiments at Column Scale

3.2.1 Virus Concentration in the Presence of Divalent Cations

Enteric viruses are known to adsorb to negatively charged membranes in the presence of multivalent cations by promoting a hydrophobic interaction between the viruses and membranes (Katayama *et al*., 2002; Haramoto *et al*., 2004; Lukasik *et al*., 2000). In this study, we have used $MgCl_2$ to enhance the capture of virus on the negatively charged silica beads from the primary and secondary treated wastewater samples. The observed recovery rates for coxsackievirus and adenovirus are presented in Table 1.

Table 1. Virus recovery from primary (1°) and secondary (2°) treated wastewater with coarse silica beads (60-100 mesh size) after addition of $MgCl_2$ to sample.

	Initial Numbers (PDU mL^{-1})	Numbers in Eluate (PDU mL^{-1})	% Recovery
Adenovirus 1°			
Sample A	2.18×10^5	4.63×10^5	212
Sample B	2.18×10^5	4.43×10^5	203
Average SD			207 ±3.05
Coxsackievirus 1°			
Sample A	2.90×10^6	5.15×10^5	18
Sample B	2.34×10^6	4.23×10^5	18
Average SD			18 ±0.114
Adenovirus 2°			
Sample A	8.65×10^4	2.81×10^5	162
Sample B	9.40×10^4	2.45×10^5	130
Sample C	9.40×10^4	2.45×10^5	130
Average SD			152 ±18.35
Coxsackievirus 2°			
Sample A	7.20×10^6	9.56×10^5	27
Sample B	7.94×10^6	8.88×10^5	11
Average SD			21 ±5.20

The recovery rates for coxsackievirus were around 18%, whereas more than 100% recovery was observed for adenovirus for the primary effluent. Higher than 100% recovery can possibly be due to capture of adenovirus attached to suspended solids in the primary effluent as high numbers of adenovirus (10^5 to 10^8 L^{-1}) have been reported in wastewater (Fong *et al.*, 2010; Katayama *et al.*, 2008; Bofil-Mas *et al.*, 2006). This observation suggests that it is possible to capture coxsackievirus on the silica beads from primary wastewater with a relatively low recovery rate of around 18%, which is comparable to 23% recovery rate for poliovirus from primary wastewater reported by Katayama *et al.* (2008) where 100 mL of primary wastewater was filtered through 90 mm HA type filters. Harmoto *et al.* (2007) reported a recovery rate of 79% for poliovirus from tap water and 50% from secondary treated wastewater with an electronegative HA filter in the presence of $MgCl_2$. Therefore, lower recovery of RNA virus (coxsackievirus) is expected in low quality water as we observed higher recovery of coxsackievirus in secondary treated effluent than in the primary effluent.

The recovery efficiency from approximately 26% to more than 100% was observed for coxsackievirus and adenovirus, respectively, from secondary effluent. In comparison, Katayama *et al.* (2008) reported a recovery rate of 80% for poliovirus from secondary treated effluent. This suggests that the filtering of treated wastewater containing a low suspended solids content through a negatively charged membrane is more efficient for virus capture, as similar recovery rates (70-80%) for coxsackievirus were also observed in our laboratory experiments with tap water (Figure 4). The fact that more than 100% recovery rate was observed for adenovirus in primary and secondary effluent is most likely either due to the flocculation of adenovirus followed by entrapping of flocks in silica beads, rather then adsorption and release of virus from the silica beads, or the under-estimation of adenovirus numbers due to PCR inhibition in primary wastewater. However, as no PCR inhibition was observed for coxsackievirus in the same experiment, it appears that capture of flocks by silica beads is the most likely reason for higher recovery rates.

3.2.2 Concentration of Virus by Acidification of Samples

Dried silica beads, coarse (60-100 mesh size) and fine (3-6 mesh size), were used to concentrate virus from pre-acidified (pH 3.0) primary and secondary wastewater samples. The observed recovery rates for adenovirus and coxsackievirus with coarse and fine silica beads are presented in Tables 2 and 3. The average recovery rates for adenovirus and coxsackievirus from primary effluent were 54% and 5% respectively with coarse silica. Adenovirus recover rate of more than 100% was observed from the primary wastewater and 30% from secondary effluent with fine silica (Table 3). A possible cause for this greater than 100% recovery for adenovirus from primary wastewater sample could be due to the capture of flocs between the fine silica beads due to low pore space compared to the coarser silica. Additional experiments would be needed, however, to test this hypothesis.

Table 2. Virus recovery from primary (1°) and secondary (2°) treated wastewater with coarse silica beads (60-100 mesh size) after acidification of samples.

	Initial Numbers (PDU mL^{-1})	Numbers in Eluate (PDU mL^{-1})	% Recovery
Adenovirus 1°			
Sample A	2.84×10^5	1.43×10^5	50.24
Sample B	2.84×10^5	1.92×10^5	67.46
Sample C	2.84×10^5	1.26×10^5	44.39
Average SD			54.03 ±11.98
Coxsackievirus 1°			
Sample A	3.17×10^8	1.81×10^7	5.72
Sample B	1.87×10^8	1.14×10^7	6.06
Sample C	1.93×10^8	7.67×10^6	3.94
Average SD			5.24 ±1.13
Adenovirus 2°			
Sample A	6.76×10^6	1.08×10^6	16.00
Sample B	6.76×10^6	3.06×10^6	45.20
Sample C	6.50×10^6	2.57×10^6	39.51
Average SD			33.57 ±15.47
Coxsackievirus 2°			
Sample A	7.07×10^7	1.08×10^7	16.00
Sample B	2.40×10^8	9.03×10^7	37.56
Sample C	3.06×10^8	8.80×10^7	28.69
Average SD			27.56 ±10.6

With secondary treated effluent, similar average recovery rates of 27 and 33% were observed for both adenovirus and coxsackievirus with coarse silica (60-100 mesh). However, a higher recovery rate for adenovirus as compared to coxsackievirus (13%) was observed when fine silica was used as a packing material. The results from this experiment suggest that coarse silica work better than fine silica to capture and release virus as recovery rates for both virus, especially in the secondary treated wastewater, was more consistent.

Table 3. Virus recovery from primary (1°) and secondary (2°) treated wastewater with fine silica beads (3-6 mesh size) after acidification of samples.

	Initial Numbers 100 (PDU mL^{-1})	Numbers in Eluate 0.65(PDU mL^{-1})	% Recovery
Adenovirus 1°			
Sample A	3.30×10^5	4.03×10^5	159.21
Sample B	3.12×10^5	7.93×10^5	330.34
Average			244.78
Coxsackievirus 1°			
Sample A	4.72×10^8	8.73×10^7	18.53
Sample B	4.58×10^8	9.47×10^7	20.70
Average			19.61
Adenovirus 2°			
Sample A	1.50×10^7	5.20×10^6	34.67
Sample B	1.50×10^7	3.87×10^6	25.78
Average			30.22
Coxsackievirus 2°			
Sample A	5.12×10^8	4.53×10^7	8.85
Sample B	$3.82x\ 10^8$	7.32×10^7	18.94
Average			13.89

Although no direct comparison can be made with the reported literature as there is no report on the use of silica beads to capture virus form water samples, comparisons can be made with previously reported results with flow-through columns packed with glass wool (Lambertini *et al*., 2008; Albinana-Gimenez *et al*., 2009). In these studies, acidified drinking water was circulated thorough columns packed with glass wool, reported recovery rates ranged from 70% for poliovirus, 14% for coxsackievirus B5, 19% for echovirus 18, 21% for adenovirus 41, to 29% for norovirus (Lambertini *et al*., 2008). Consequently, our recovery rates from wastewater are comparable with virus capture efficiency reported with glass wool packed flow-through columns. In the present study, coxsackievirus recovery rates were found to be generally lower than adenovirus, especially from the primary wastewater. This is in agreement with reported literature where Lambertini *et al*. (2008) also observed virus type specific capture and recovery rates. In the present study, the average adenovirus recovery rate of 54% (Table 2) with coarse silica from primary wastewater was higher than 33% observed from the secondary treated wastewater.

3.2.3 Virus Capture and Release with Amino-Functionalised Silica

Amino-functionalised, (*N*-3-(trimethoxysilylpropyl) diethylenetriamine) electropositive silica beads were tested for the potential capture of negatively charged adenovirus and coxsackievirus from primary and secondary treated wastewater samples. The amino-functionalised silica was prepared using methodology described previously by Chen *et al*. (2006). The observed recovery rates for adenovirus and coxsackievirus on DPS silica beads from primary and secondary treated wastewater are presented in Table 4.

Table 4. Virus recovery from primary (1°) and secondary treated wastewater (2°) with amino-functionalised (DPS) silica beads.

	Initial Numbers (PDU mL^{-1})	Numbers in Eluate (PDU mL^{-1})	% Recovery
Adenovirus 1°			
Sample A	3.67×10^4	6.63×10^2	1.8
Sample B	2.30×10^5	2.88×10^2	0.1
Sample C	7.07×10^4	1.03×10^2	15
Average SD			5.5 ±7.93
Coxsackievirus 1°			
Sample A	2.44×10^8	8.05×10^5	0.3
Sample B	2.07×10^8	1.3×10^5	0.1
Sample C	3.18×10^8	2.23×10^5	0.01
Average SD			0.13 ±0.17
Adenovirus 2°			
Sample A	4.13×10^6	4.40×10^5	11
Sample B	4.70×10^6	1.54×10^5	3
Sample C	3.25×10^6	3.47×10^5	11
Average SD			8 ±4.3
Coxsackievirus 2°			
Sample A	2.53×10^8	7.27×10^7	29
Sample B	2.61×10^8	4.27×10^7	16
Sample C	2.57×10^8	4.27×10^7	17
Average SD			21 ±7.1

The observed adenovirus recovery rate from the primary treated wastewater was up to 15%, whereas, the recovery rate for coxsackievirus was extremely low (< 1%). The recovery rate for adenovirus from secondary treated wastewater was slightly less than from primary effluent (around 10%). However, the recovery rate for coxsackievirus improved significantly from < 1% to around 20% in secondary treated wastewater, which suggests that capture of coxsackievirus on DPS silica is more influenced by the interference from factors in primary wastewater such as suspended particulate matter than is adenovirus. The observed recovery rates for both adenovirus and coxsackievirus with DPS silica were lower than what was observed with acidification of samples in the previous experiments. The overall recovery rates observed in these experiments were also much lower compared to previously reported rates of 98% for bacteriophage MS2 and PRD1 in small-scale (1.5 mL tubes) laboratory based experiments with DPS silica (Cheng *et al.*, 2006). The potential reasons for lower recovery rates in our column study include high water turbidity, loss of virus during sample processing at a larger scale and PCR inhibition due to co-purification of PCR inhibitors from wastewater.

4. CONCLUSIONS

The comparative recovery rates of adenovirus and coxsackievirus from the wastewater matrix with three different capture and release mechanisms evaluated in this study are presented in Figures 5 and 6 respectively. In general, virus capture and release with all three approaches evaluated in this study works better with secondary treated wastewater. This could be potentially due to high suspended solid content and water chemistry which is different from secondary treated effluent. It is possible that suspended particulate matter either directly competes with binding sites on silica or interferes in binding of virus to silica particles. Acidification of samples followed by capture on silica particles appears to be the most promising approach for capture and release of virus from primary and secondary treated wastewater, as recovery rates with course silica of around 40-50% were observed for adenovirus from primary and secondary treated effluent.

Virus capture and release mechanism appears to work better with coarse silica beads (60-100 mesh size) as compared to fine silica beads (3-6 mesh size), possibly due to less clogging of pore spaces by particulate matter from the primary effluent, which would reduce the flow of water and viral particles past the fine silica beads. Acidification of samples is relatively easier and a less time-consuming approach compared to use of amino-functionalised beads, with added benefit of better virus recovery rates.

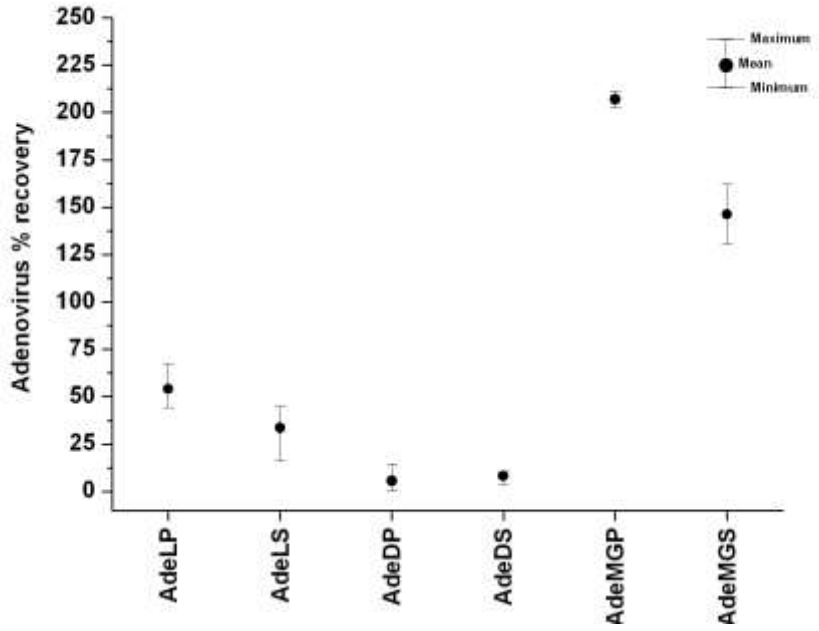

Figure 5. Percent recovery of adenovirus from primary and secondary treated wastewater samples with silica beads. Where, AdeLP = with Low pH from primary wastewater, AdeLS = with Low pH from secondary wastewater, AdeDP = DPS silica from primary wastewater, AdeDS = DPS silica from secondary wastewater, AdeMGP = with $MgCl_2$ addition from primary wastewater and AdeMGP = with $MgCl_2$ addition from secondary wastewater.

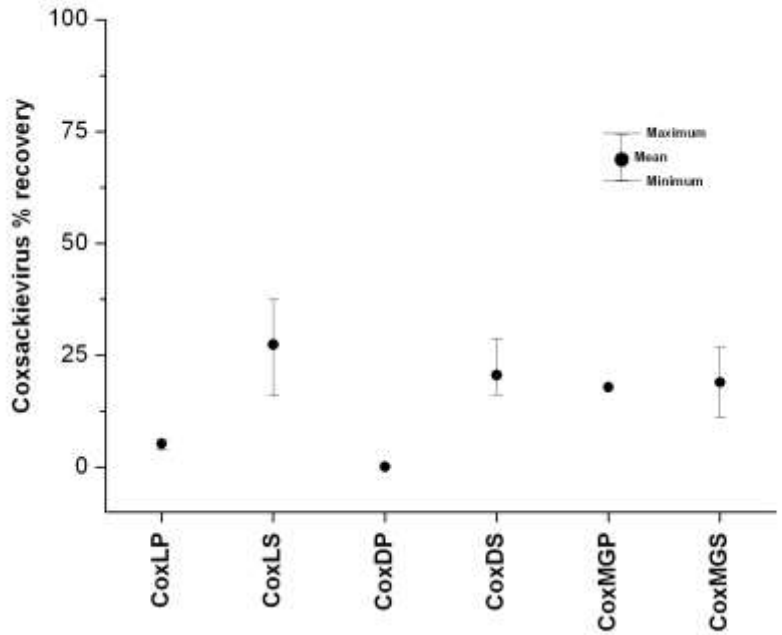

Figure 6. Percent recovery of coxsackievirus from primary and secondary treated wastewater samples with silica beads. Where, CoxLP = with Low pH from primary wastewater, CoxLS = with Low pH from secondary wastewater, CoxDP = DPS silica from primary wastewater, CoxDS = DPS silica from secondary wastewater, CoxMGP = with $MgCl_2$ addition from primary wastewater and CoxMGP = with $MgCl_2$ addition from secondary wastewater.

4.1 Future Research Directions

Further research works is required to validate virus capture and release from acidified primary and secondary treated effluent. Dependence of virus capture and release efficiency on the quantity of silica beads packed in the column needs to be explored further. Virus capture on the silica beads appears to depend upon the virus type. Consequently, further validation of methodology is required with other important enteric virus such as norovirus and rotavirus. The flow rate of water through the column is important for creating fluidised condition in the column - higher flow rates may result in higher virus capture efficiency. Although virus capture and release with silica beads works well with primary and secondary treated effluent, it still unknown if this technique could be applicable to other low quality water such as surface water and stormwater.

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