Adsorption of viruses to soil: impact of anaerobic treatment

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Abstract The adsorption of viruses in untreated flushed dairy manure wastewater (FDMW), anaerobically digested flushed dairy manure wastewater (ADFDMW) and groundwater to sandy soil was investigated. Batch adsorption studies showed differential adsorption of viruses in groundwater to soil. Less than 75% of PRD1 and MS2 added to groundwater adsorbed after 1 h, but greater than 95% of ΦX174 and poliovirus 1 adsorbed to the soil. Adsorption differences in groundwater were related to the isoelectric points of the viruses. Suspending phages in untreated and treated wastewater reduced adsorption compared with groundwater. For MS2, more phages were adsorbed using ADFDMW than with FDMW. Adsorption of poliovirus 1 was not affected by FDMW and ADFDMW. Small column studies (6×2.5 cm) produced a similar trend in that adsorption was observed with groundwater and both FDMW and ADFDMW reduced virus adsorption. Groundwater, FDMW or ADFDMW did not affect the adsorption of poliovirus 1 in column studies. The major difference between FDMW and ADFDMW was in mobilisation of adsorbed viruses. The application of FDMW to soil columns with adsorbed viruses caused significantly more viruses to be mobilised than did the application of rainwater or ADFDMW. These results showed that treating FDMW by anaerobic digestion increased the adsorption of viruses to soil and decreased detachment of adsorbed viruses. As the potential for new zoonotic pathogens becomes known, the treatment of animal wastes may become mandatory. The assessment and management of viruses in manure for addressing possible risk to animal and human health is of interest.

Keywords Fixed-film anaerobic digestion; flushed dairy manure wastewater; sandy soil; virus adsorption

Introduction

Intensification and concentration of large dairy operations have led to issues with manure management. Two major concerns are nuisance odours and possible contamination of groundwater resources following land application of manure. In an attempt to reduce these problems, a fixed-film anaerobic digester has been constructed at the University of Florida's Dairy Research Unit (DRU). Freestall barns at the DRU are hydraulically flushed with water to remove animal manure. The fixed-film digester then treats the flushed dairy manure wastewater (FDMW) before it is land applied for forage crop production. Anaerobic digestion significantly reduces the COD of flushed dairy manure (Wilkie, 2005). Previous studies have shown that this unit can also reduce the levels of indicator and pathogenic bacteria and bacteriophages by approximately 90% (Davis *et al.*, 2001). However, the use of fixed-film anaerobic digesters on flushed manure is a relatively new technology and the effect on transport of residual viruses following land application of anaerobically digested flushed dairy manure (ADFDMW) is unknown.

Viruses are known to be associated with and survive in animal manure (Elliott and Ellis, 1977; Mawdsley *et al.*, 1995; Pesaro *et al.*, 1995). These viruses may be markers for animal manure, risks to animal health, and possibly, as more zoonotic pathogens are identified, risk to human health. Furthermore, viruses have been shown to survive anaerobic treatment conditions that are known to cause decimation of pathogenic bacteria.

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Spillmann *et al*. (1987) found that anaerobic digestion of sewage sludge at mesophilic temperatures was ineffective for inactivation of rotavirus and coxsackievirus. However, the effect of FDMW and ADFDMW on the adsorption of viruses to soil following land application has not been studied. The purpose of this study, therefore, was to investigate the adsorption of viruses in FDMW before and after anaerobic treatment.

Materials and methods

Collection and analysis of soil samples

Soil was collected from the DRU in Hague, FL, in an area mapped as typic Quartzipsamments. Samples were taken at 0.8 m depth from a sprayfield that received ADFDMW following short-term storage. Soil samples were thoroughly mixed to yield a composite sample. The soil was air dried at room temperature and sieved. Batch adsorption studies were performed on soil with particle size less than 0.2 mm. To increase the flow rate through columns, soil with particle size of 0.2–0.8 mm was used for column studies. Soil pH and conductivity were measured as described by Mylavarapu and Kennelley (2002).

Collection and analysis of wastewater, groundwater and rainwater samples

One-litre samples of FDMW and ADFDMW were collected from the DRU manure management facility. Grab samples of FDMW were collected from a wet well prior to anaerobic treatment. ADFDMW was collected from an effluent port located on the fixed-film anaerobic digester. Supernatant fractions were used for experiments. Groundwater was collected from wells located at the DRU and rainwater was collected during storm events. Sample pH and conductivity were measured according to standard methods (Standard Methods, 1998).

Viruses and viral assays

Bacteriophages and their hosts used in this study were MS2 (ATCC 15597-B1), host *E. coli* C3000 (ATCC 15597); ΦX174 (ATCC 13706-B5), host *E. coli* (ATCC 13706); and PRD1, host *Salmonella typhimurium* (ATCC 19585). Phages were quantified as plaque-forming units (PFU) using the agar overlay technique, as previously described by Hurst (1997). Poliovirus 1 LSc (ATCC VR-59) was assayed as PFU on Buffalo green monkey kidney cells, as previously described by Hurst (1997).

Batch studies

Batch adsorption studies were performed with groundwater, FDMW and ADFDMW. The study was designed using nine batches so that each experimental condition was performed in triplicate. The entire experiment was performed twice to yield a total of six observations for each experimental condition. Two-millilitre aliquots of groundwater, FDMW or ADFDMW inoculated with viruses were added to 1 g soil samples (<0.2 mm). Samples were mixed on a reciprocating shaker at 55 rpm for 1 h, followed by centrifugation at $3{,}000 \times g$ for 10 min. The supernatant fraction was collected and assayed. The soil pellets were mixed with 10 mL 3% beef extract (BE), pH 7, for 30 min to recover adsorbed viruses. The samples were centrifuged again and the supernatant fraction was collected and assayed.

To determine the influence of groundwater, FDMW and ADFDMW on desorption of enteroviruses and bacteriophages from soil, 1 g soil samples were set up as before. Each soil sample was mixed with 2 mL of inoculated groundwater for 1 h and centrifuged as previously described. The supernatant fraction was assayed and the soil pellets were treated with 10 mL of either groundwater, 3% BE, FDMW or ADFDMW, and the eluants were assayed after 30 min.

Column studies

Adsorption studies with soil columns were performed using 45 g of soil (0.2–0.8 mm) packed in 60 mL syringes with a borosilicate fiberglass filter in the bottom of the syringe. Each experimental condition was performed in triplicate. One pore volume (20 mL) of either groundwater, FDMW or ADFDMW was inoculated with viruses and passed through the columns at 1 mL min^{-1}. The percolate was collected and assayed to determine the percentage of viral adsorption. Each column was then removed from the syringes and placed in a 500 mL centrifuge bottle. From each column, 5 g was removed, dried and then mixed with 10 mL of deionized water for 2 h to measure soil pH. The remaining 40 g of soil was mixed for 30 min with 40 mL 10% buffered BE (100 g L^{-1} BE, 13.4 g L^{-1} sodium phosphate dibasic, 1.2 g L^{-1} citric acid) (Hurst, 1997). The samples were centrifuged at 5,000 × *g* for 10 min and then the supernatant fraction was collected and assayed.

To study potential mobilization of viruses by FDMW and ADFDMW, one pore volume (20 mL) of groundwater inoculated with viruses was added to each column. The percolate was collected and assayed to determine the percentage of viral adsorption. Six pore volumes of either rainwater, FDMW or ADFDMW were passed through triplicate columns. The columns were then mixed with BE and assayed for recovered viruses.

Statistical analysis

A comparison of the percentage of viruses adsorbed in groundwater and various virus characteristics (i.e. isoelectric point, type of interaction and structure) was subjected to regression analysis to determine correlations. The percentage of viruses eluted in the batch and column studies was calculated by dividing the amount of viruses in the eluant by the amount of viruses adsorbed. The mean percentages were analysed by Student's *t*-test with a significance level of 0.05. All analysis was performed using ProStat© v3.5.

Results

Batch studies

The adsorption of viruses in groundwater, FDMW and ADFDMW to soil are shown in Figure. 1. When the viruses were suspended in groundwater, there was greater adsorption of ΦX174 (99%) and poliovirus 1 (96%) than of MS2 (72%) and PRD1 (48%).

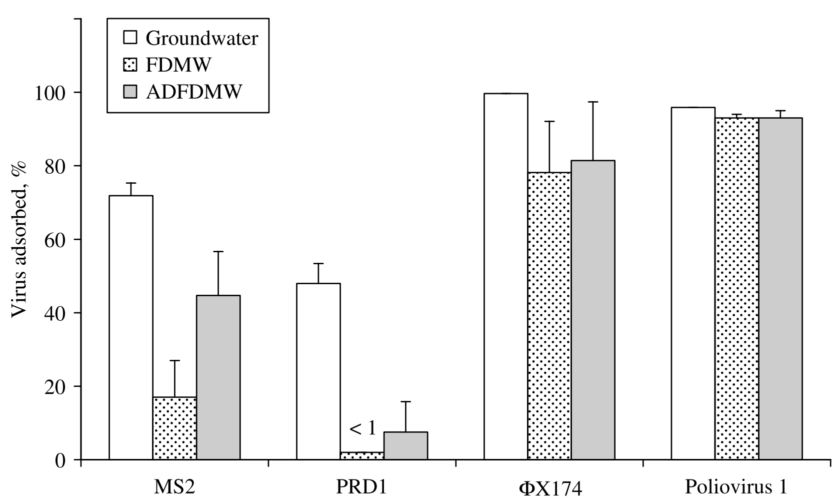

Figure 1 Adsorption of viruses in groundwater, flushed dairy manure wastewater (FDMW) and anaerobically digested flushed dairy manure wastewater (ADFDMW) to soil

In FDMW, adsorption decreased to 17% for MS2 and $<1\%$ for PRD1. The presence of FDMW also decreased the adsorption of ΦX174 (78%) but had little effect on the adsorption of poliovirus 1 (93%). The adsorption of MS2 (45%) and PRD1 (8%) was less in ADFDMW than in groundwater, but greater than in FDMW. The adsorption of ΦX174 and poliovirus 1 was similar in FDMW and ADFDMW. These results indicate that the adsorption of some viruses was greater in ADFDMW than in FDMW.

The phages adsorbed to soil in the presence of groundwater were desorbed by FDMW (Table 1). The percentage of viruses mobilised by FDMW was significantly higher ($p > 0.05$) than the percentage mobilised by ADFDMW and groundwater. The percentage eluted by ADFDMW and groundwater was not significantly different ($p < 0.05$) for MS2 and PRD1. Poliovirus 1 adsorbed to the soil was not eluted by groundwater, FDMW or ADFDMW. These results show that, except for poliovirus 1, untreated wastewater can detach more adsorbed phages from soil as compared with anaerobically treated wastewater.

Column studies

The adsorption of viruses in groundwater, FDMW and ADFDMW to soil columns is shown in Figure. 2. The adsorption pattern for the viruses studied was similar to that observed in the batch studies in that the adsorption of ΦX174 (97%) and poliovirus 1 (99%) in groundwater was greater than the adsorption of MS2 (88%) and PRD1 (54%). In contrast with the batch studies, adsorption of MS2 and ΦX174 in FDMW and ADFDMW to soil was low.

As observed in the batch studies, FDMW was found to mobilise adsorbed viruses (Table 2). Mobilisation of MS2, PRD1 and ΦX174 by FDMW was significantly higher ($p < 0.05$) than ADFDMW and rainwater. FDMW mobilised 2% of poliovirus 1, whereas no mobilisation was observed with ADFDMW and rainwater.

Conductivity and pH

The initial pH of the soil samples was considerably lower than the pH of groundwater, rainwater, FDMW and ADFDMW samples (Table 3). In both the batch and column experiments, there was an increase in pH in all soil samples. However, we found that normalising the pH of the soil during the experiment did not affect adsorption and elution (data not shown). Furthermore, during percolation, conductivity of the soil samples decreased with rainwater and increased with FDMW and ADFDMW (Table 3).

Discussion

The adsorption of viruses to soil and virus transport through soil columns has been reported in several studies (Lance *et al.*, 1976; Gerba and Lance, 1978; Landry *et al.*, 1980). Factors that influence virus adsorption include the characteristics of the viruses,

Table 1 Elution of viruses adsorbed to soil

Virus	Initial amount, PFU	Adsorbed, %	Eluted, % of adsorbed			
			GW	FDMW	ADFDMW	3% BE
MS2	$3.2 \pm 0.4 \times 10^4$	44 ± 25	41 ± 26^B	100 ± 0^A	50 ± 12^B	89 ± 23^A
PRD1	$1.1 \pm 0.1 \times 10^6$	33 ± 16	33 ± 12^B	97 ± 4^A	37 ± 10^B	100 ± 0^A
ΦX174	$1.1 \pm 0.1 \times 10^4$	99 ± 0.2	8 ± 2^D	92 ± 2^A	21 ± 5^C	47 ± 9^B
Poliovirus 1 LSc	$5.1 \pm 1.9 \times 10^4$	99 ± 0.5	0	0	0	37 ± 1

GW, groundwater; FDMW, flushed dairy manure wastewater; ADFDMW, anaerobically digested flushed dairy manure wastewater; BE, beef extract. Values are average ± standard deviation ($n = 6$). Values with the same letter within the same row are not significantly different ($p < 0.05$)

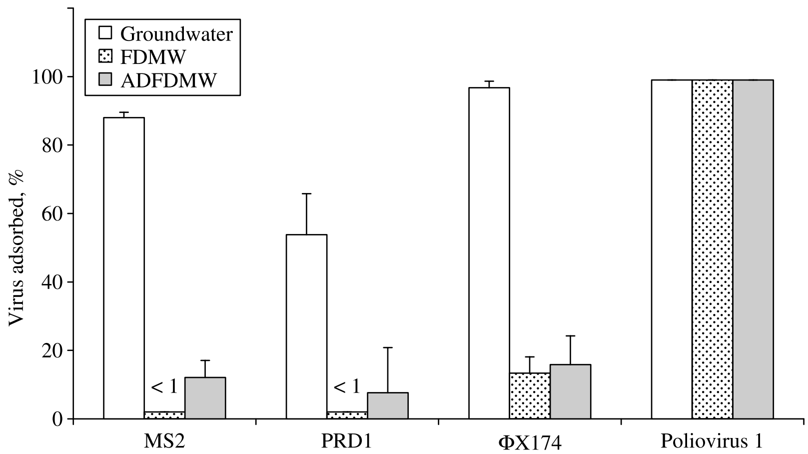

Figure 2 Adsorption of viruses in groundwater, flushed dairy manure wastewater (FDMW) and anaerobically digested flushed dairy manure wastewater (ADFDMW) to soil columns

the properties of the soil and the chemical composition of the solution that contains the viruses.

In the batch studies, we have confirmed that the isoelectric point of viruses influences their adsorption to soil ($r^2 = 0.97$). Also, the presence of FDMW reduces virus adsorption and promotes mobilisation of viruses adsorbed to the soil columns.

The current study provided evidence demonstrating the adsorption and desorption behaviour of selected viruses. In both the batch and column studies, FDMW was shown to decrease viral adsorption to sandy soil. These results suggest that viruses present in untreated dairy manure wastewater may have potential for groundwater contamination during land application. Furthermore, FDMW was shown to increase the release of viruses attached to soil. Thus, land application of untreated dairy manure wastewater may increase the movement of retained viruses through soil, potentially contaminating groundwater.

The viruses studied demonstrated varying adsorption and elution behaviour. Changes in pH of suspending solution and soil columns have been shown to influence the adsorption and elution behaviour of viruses (Kinoshita *et al.*, 1993). In the current study, increases in pH were observed during the experiment. However, the observed pH changes did not influence virus adsorption and elution behaviour. The presence of dissolved ions may also influence adsorption and elution of viruses. The conductivity of the soil decreased when mixed with rainwater and increased when mixed with FDMW and ADFDMW. Increased mobilisation of phages was observed with FDMW as compared with ADFDMW. However, the conductivity of the soil mixed with both wastewaters was

Table 2 Elution of viruses adsorbed in soil columns

Virus	Initial amount, PFU	Adsorbed, %	Eluted, % of adsorbed		
			Rainwater	FDMW	ADFDMW
MS2	$7.6 \pm 1.6 \times 10^3$	91 ± 1	1 ± 0.6^C	63 ± 13^A	16 ± 3^B
PRD1	$2.4 \pm 0.3 \times 10^5$	50 ± 6	21 ± 7^C	79 ± 14^A	36 ± 7^B
ΦX174	$1.4 \pm 0.2 \times 10^5$	98 ± 0.3	10 ± 5^B	48 ± 20^A	5 ± 4^B
Poliovirus 1 LSc	$1.8 \pm 0.1 \times 10^4$	99 ± 0.1	0	2	0

FDMW, flushed dairy manure wastewater; ADFDMW, anaerobically digested flushed dairy manure wastewater. Values are average ± standard deviation ($n = 3$). Values with the same letter within the same row are not significantly different ($p < 0.05$)

Table 3 Conductivity and pH changes in soil during batch and column experiments

Parameter	Experiment	Initial soil	Soil mixed with:			
			Groundwater	Rainwater	FDMW	ADFDMW
pH	Batch	5.02	6.86 (7.71)	NA	7.27 (7.77)	7.41 (7.20)
	Column	5.02	6.50 (7.71)	5.81 (7.12)	8.04 (7.33)	7.82 (7.49)
Conductivity, μS/cm	Column	473	452 (353)	122 (39)	628 (3370)	700 (3600)

FDMW, flushed dairy manure wastewater; ADFDMW, anaerobically digested flushed dairy manure wastewater. Values in parentheses are initial values prior to mixing with soil; NA, not applicable similar. Therefore, conductivity changes did not appear to contribute to the mobilisation patterns of phages observed in the current study.

Conclusions

The present study demonstrates the benefit of anaerobic treatment in regard to reducing potential groundwater contamination by viruses in sandy soils. Anaerobic treatment alters the physicochemical characteristics of FDMW (Wilkie *et al.*, 2004). However, it is unknown which characteristic(s) is responsible for the observed differences in adsorption/desorption behaviour. We conclude from the current study that anaerobic treatment of FDMW increases the retention of some viruses to soil and decreases the transport of some adsorbed viruses through the soil matrix, thereby decreasing the potential for groundwater contamination.

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