Supplemental Material for:

**Opportunities for Optimization: Fate of Manure-Borne Pathogens during Anaerobic Digestion and Solids Separation**

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Elution Procedure for Separated Solids and Solids Having Undergone Secondary Treatment

First, 10 g of sample (wet mass) was added to 200 mL of 3% beef extract solution (wt/vol) containing 0.05 M glycine (pH 9.5) and inverted to mix. This mixture was incubated at room temperature for 15 minutes, then centrifuged at 1,000 × g for 5 minutes. Following centrifugation, we filtered the supernatant with a coffee filter and adjusted the filtrate pH to between 7.0 and 7.2 using 1 N HCl. This mixture was flocculated with 8% polyethylene glycol (wt/vol) and 0.2 M NaCl, mixed at 4°C for 1 hour, and allowed to settle overnight at 4°C. It was then centrifuged at 4,700 × g for 45 minutes, the supernatant was discarded, and the remaining final concentrated sample volume (FCSV) was stored at -80°C until nucleic acid extraction.
Quantitative PCR (qPCR) and Two-step Reverse Transcription-qPCR

Reverse transcription was carried out in 50-µL reactions using previously described chemistry (Lambertini et al., 2008), except Superscript III reverse transcriptase (Invitrogen Life Technologies, Rockville, MD) was used in the current work rather than Superscript II. Reverse transcription incubation temperatures were 42°C for 60 min followed by 95°C for 5 min. qPCR was carried out using a Lightcycler 480 (Roche Diagnostics, Mannheim, Germany) in 20-µL reaction volumes. Each reaction was prepared using LightCycler 480 Probes Master reaction mix (Roche Diagnostics), 6 µL of DNA or cDNA template, and target-specific primers and TaqMan probes (Table 2 of main text). Thermocycling began at 95°C for 5 min followed by 45 cycles of 10 s at 95°C and 1 min at 60°C.
Inhibition Controls

Inhibition controls were conducted by spiking samples with a known quantity of bovine viral diarrhea virus 1 (BVDV1) after nucleic acid extraction, which was then reverse transcribed and quantified using qPCR. These reverse transcription reactions consisted of 0.35 µL random primers, 2.35 µL nuclease-free water, and 2 µL BVDV1 vaccine (Pfizer Animal Health, Exton, PA) added to 4.3 µL of sample. This mixture was heated at 95°C for 5 min to denature the viral capsid, then combined with 16 µL of reverse transcription master mix consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 70 µM concentration of each deoxynucleoside triphosphate (ProMega), 30 U RNAsin (ProMega), and 100 U SuperScript III reverse transcriptase. The total reaction volume was 25 µL; it was incubated at 42°C for 30 min and 95°C for 5 min. qPCR was carried out as described above using primers and probes for BVDV1 (Table 2 of main text).
Inactivation Papers found during Literature Review


**Mass Balance on Solids Separators**

Consider a solids separator operating at steady state with a negligible residence time (i.e., no internal accumulation or reactions). A diagram for the mass balance on manure entering this separator looks like:

![Mass Balance Diagram](image)

Where $m$ refers to the mass flow rate of manure (g manure/day) and $C$ refers to the concentration of total solids in manure (g total solids/g manure). The subscripts $L$ and $S$ refer to the liquid and solid fractions of separated manure, respectively. This mass balance is defined by two equations:

1. $m = m_L + m_S$  \[1\]
2. $mC = m_L C_L + m_S C_S$  \[2\]

From our related study described in Section 2.1, we have measurements for all three concentrations (i.e., the $C$ values), but lack measurements for the three manure flow rates (the $m$ values). Thus, as currently formulated, this mass balance consists of two equations and three unknowns; it cannot be solved in these terms.

To fix this problem, we can rewrite Equations 1 and 2 using two new terms: $f_L$ and $f_S$. These represent the fraction of total manure in the separated liquids and separated solids, respectively. That is, $f_L = m_L/m$ and $f_S = m_S/m$. Starting with Equation 1, first divide by $m$:

\[ l = m_L/m + m_S/m \]  \[3\]

Then substitute in the two new terms:

\[ l = f_L + f_S \]  \[4\]
Following the same procedure for Equation 2 yields:

\[ C = f_L C_L + f_S C_S \]  \hspace{1cm} [5]

This new formulation of the mass balance contains two equations and just two unknowns \((f_L\) and \(f_S\)), so it can be solved. To do so, start by rearranging Equation 4 as \(f_S = 1 - f_L\), then substitute this expression into Equation 5 to yield:

\[ C = f_L C_L + (1 - f_L)C_S \]  \hspace{1cm} [6]

Then, solve Equation 6 for \(f_L\):

\[ f_L = \frac{C - C_S}{C_L - C_S} \]  \hspace{1cm} [7]

Average measurements of \(C\), \(C_L\), and \(C_S\) for the separators in our study were 0.06, 0.04, and 0.3 g total solids/g manure. These correspond to total solids concentrations of 6%, 4%, and 30%, respectively, and they represent biweekly measurements on all nine separators between September 2011 and May 2012. Substituting these values into Equation 7 yields:

\[ f_L = \frac{0.06 - 0.3}{0.04 - 0.3} = 0.92 \]

Thus, on average, 92% of the unseparated manure entering these separators exits in the liquid fraction, and – using Equation 4 to find \(f_S\) – the other 8% exits in the solid fraction.