Ecological sanitation technologies can be effective in providing health and environmental pollution control if they can efficiently reduce the pathogenicity of microorganisms carried in fecal material to safe levels. This study evaluated the sanitizing effects of different additives for dry treatment of feces from urine-diverting dry toilets, based on inactivation of Enterococcus faecalis, Salmonella enterica serovar Typhimurium, bacteriophages MS2 and ΦX, and Ascaris suum. The additives, ash (A) and oyster shell (O) in different amounts and urea (U) to optimize the process, were compared with no additive, solely urea, and sawdust as controls (C) and were covered ([x]%) or uncovered (x%). The main inactivation factors found were desiccation, ammonia content, and pH. S. Typhimurium and E. faecalis were more affected by the ammonia content. A combination of neutral to high pH and desiccation was most effective for inactivation of MS2, and desiccation was most effective for inactivation of ΦX and A. suum. The inactivation rate was modeled for all combinations studied. The most promising treatments were [150%O:A+U], 150%O:A+U, and 150%O:A. According to the models, these could inactivate, for example, 7 log units of all bacteria and bacteriophages within 83, 125, and 183 days, respectively. The inactivation of A. suum was modeled, albeit the measured decay in egg viability was low.

On-site sanitation is still the main solution for the majority of the world’s population, with ventilated improved pit latrines (VIPS) being the most common solution, serving over 3 billion people, mainly in low- and mid-income countries (WHO/UNICEF Joint Monitoring Program for Water Supply and Sanitation [http://www.wssinfo.org/]). The aim of most sanitation solutions is to keep excreta away from the household. The misconception that human excreta are wastes with no useful purpose has resulted in unsustainable sanitary systems. However, the recovery and agricultural use of the organics and nutrients contained in human excreta can improve soil structure and fertility, increasing agricultural productivity and thus contributing to food security, benefits that can be obtained once excreta are treated sufficiently to inactivate pathogens and make them safe (1,2). Wastewater segregation and treatment of feces in dry toilets, for example, reduce the use of water and facilitate the reuse of gray water, feces, and urine (2–4).

Sanitation technologies with an ecological and low-cost perspective can be effective in providing health and environmental pollution control if they can efficiently reduce the pathogenicity of microorganisms carried in fecal material to safe levels (5, 6). The success of a given process in reducing pathogens depends, in general, on its retention time and on the creation of an environment hostile to particular organisms that can inactivate and avoid re-growth (7). Common technologies applied in sanitation for treatment of feces or combined feces and urine include different types of latrines, dry or composting toilets, and septic tanks (8–10). However, there is still a lack of information about the effectiveness of these systems.

A study in a community in Vietnam found that the presence of latrines alone (98% coverage) was not sufficient to reduce the prevalence of diseases caused by fecal contamination, since acceptable treatment did not occur (11). A microbial evaluation of the sludge accumulated in 20 distinct septic tanks in Can Tho City, southern Vietnam, revealed high levels of pathogenic bacteria, bacteriophages, and helminth ova in the sludges, even after many years of storage (12). These examples show that just collecting and treating wastewater by processes where the dynamics of pathogen removal is not known does not guarantee improved sanitation for the respective populations. In composting, the thermophilic conditions that can effectively reduce pathogens are reached only on addition of amendments. Even then, it is difficult to ensure a homogeneously high temperature and to achieve a uniform reduction in organisms in feces (13,14), which is essential for full sanitation in that process (15).

It is common in dry sanitation to sprinkle dry substances on top of the feces in order to decrease odor and repel insects. The most common additive is ash, which has alkaline characteristics, but several other alternatives are available, such as sand and ground oyster shell. Oyster shell consists almost completely (99%) of calcium carbonate and thus has neutral to alkaline characteristics, which can increase and buffer the pH of feces during storage and also promote desiccation, improving the sanitization process.

The lack of information about different systems applied as on-site and ecological solutions indicates the necessity for further studies on the processes and question of promoting sanitation in a safe way. From a risk management perspective, it is preferable if treatment of the fecal fraction occurs within the collection chamber, ensuring minimal contact with potentially contaminated feces. The aim of this study was thus to evaluate options for dry treatment of feces within urine-diverting dry toilets (UDDTs).

The evaluation was based on inactivation of Enterococcus faecalis, as a model organism.
Salmonella enterica serovar Typhimurium, bacteriophages MS2 and ϕX, and Ascaris suum.

MATERIALS AND METHODS

The treatments studied involved the use of different additives (oyster shells alone or mixed 50-50 by weight with ash), added to feces in different amounts (in terms of wet mass in relation to fecal wet mass), and the use of urea to optimize the process. Ammonia-based sanitation has previously produced promising results in the sanitization of human feces (16–20). The urea used for optimizing the process was added at a low concentration (0.5% feces [wet mass]) compared with those in other studies (16, 17, 20–22). The urea was mixed with the additive before application and was used in treatments 6, 7, 8, 9, 10, and 11 (Table 1). For evaluation of the ammonia losses, some of the treatments with urea were kept in closed boxes.

Figure 2 presents a diagram with the configuration of the experiment. The experiment was run for 130 days (19 weeks) at a temperature of 22°C ± 2°C and an average relative humidity of 45% ± 5%.

The fecal material utilized in the experiment was collected daily from volunteers over a 3-week period and then frozen at −20°C. In total, 20 kg of feces was collected. Before the start of the experiment, the entire amount was allowed to defrost naturally at room temperature (22°C ± 2°C) for 24 h and then mixed homogeneously.

The sanitization effect was evaluated for all treatments and controls by determining the inactivation of the microorganisms Salmonella Typhimurium CGUG3169, Enterococcus faecalis ATCC 29212, bacteriophages MS2 ATCC 15597-B1 and ϕX 174 ATCC 13706-B1, and Ascaris suum.

Inactivation of Ascaris suum eggs was evaluated for four of the treatments (150%O, [75%O + U], [150%O + U], and [150%O:A + U]) and two of the controls ([C2-F] and [C4-F+U]). Replicates were evaluated in just one layer.

Inoculation and sampling. At the start of the experiment, bacterial and bacteriophage suspensions were added to each 200-g layer of feces in volumes corresponding to 1% of the total wet mass. This resulted in initial concentrations of S. Typhimurium, E. faecalis, ϕX, and MS2 of 10^6 CFU g⁻¹, 10^5 CFU g⁻¹, 10^6 PFU g⁻¹, and 10^6 PFU g⁻¹, respectively. The A. suum eggs were added as approximately 10^7 eggs inside permeable nylon bags (35-μm mesh) placed in the middle of the fecal layer. The eggs were

![FIG 1 Scheme of a urine-diverting dry toilet.](image)

### Table 1 Main characteristics of the treatments and controls in the experiment

<table>
<thead>
<tr>
<th>Treatment or control</th>
<th>% Additive</th>
<th>Additive</th>
<th>Addition of 0.5% urea</th>
<th>Open or closed system</th>
</tr>
</thead>
<tbody>
<tr>
<td>75%O</td>
<td>75</td>
<td>Oyster shells</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>75%O:A</td>
<td>75</td>
<td>Oyster shells + ash</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>150%O</td>
<td>150</td>
<td>Oyster shells</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>150%O:A</td>
<td>150</td>
<td>Oyster shells + ash</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>300%O</td>
<td>300</td>
<td>Oyster shells</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>300%O + U</td>
<td>75</td>
<td>Oyster shells</td>
<td>Yes</td>
<td>Closed</td>
</tr>
<tr>
<td>75%O + U</td>
<td>75</td>
<td>Oyster shells</td>
<td>Yes</td>
<td>Closed</td>
</tr>
<tr>
<td>75%O:A + U</td>
<td>75</td>
<td>Oyster shells + ash</td>
<td>Yes</td>
<td>Closed</td>
</tr>
<tr>
<td>150%O + U</td>
<td>150</td>
<td>Oyster shells</td>
<td>Yes</td>
<td>Closed</td>
</tr>
<tr>
<td>150%O:A + U</td>
<td>150</td>
<td>Oyster shells + ash</td>
<td>Yes</td>
<td>Closed</td>
</tr>
<tr>
<td>C1-F + WC</td>
<td>150</td>
<td>Wood chips</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>C2-F</td>
<td>150</td>
<td>Wood chips</td>
<td>No</td>
<td>Closed</td>
</tr>
<tr>
<td>C4-F</td>
<td>150</td>
<td>Wood chips</td>
<td>No</td>
<td>Open</td>
</tr>
<tr>
<td>C4-F + U</td>
<td>150</td>
<td>Wood chips</td>
<td>Yes</td>
<td>Closed</td>
</tr>
</tbody>
</table>

*The percentage of additive was calculated in terms of wet mass in relation to fecal wet mass.

* In C1, the percentage of wood chips was calculated in terms of the volume of wet feces because of low material density.

* The additives were prepared before use; the oyster shells were ground, and the ashes were sieved. Mixtures of oyster shells and ash had a 1:1 ratio in terms of mass.

* The percentage of urea was calculated in terms of wet mass of feces. The amount of urea was the same in all treatments.
obtained from adult Ascaris suum worms, which were collected from the intestines of slaughterhouse pigs. The eggs were harvested by dissection of the posterior 2 cm of the worm uterus. Washing was conducted in addition to the use of sodium hypochlorite.

Bacteria were cultured in nutrient broth for 14 h, and bacteriophages MS2 and ΦX174 were propagated on their respective host strains, i.e., Salmonella Typhimurium WG49 NCTC12484 and Escherichia coli ATCC 13706. After addition of microorganisms to the feces, they were allowed to adapt to the material overnight before the treatments were applied.

Treatment performance was measured in terms of analytical parameters (pH, total solid [TS] content, and ammonia) and survival of the microorganisms added. During sampling, it was important to evaluate the fecal layer in the treatments without mixing it with the additive layers, as this could have interfered with the results. Therefore, each paper box was sliced through transversely, allowing just the feces from each layer to be sampled. All sampling was carried out using a separate set of sterile instruments for each treatment/control. The boxes were always sliced in a different place from one sampling to the next, and once the procedure was completed, each box was reconstructed by tightly taping the sliced parts together. To avoid type II errors in the statistical analysis of data, all treatments and samplings were carried out by following identical procedures. All boxes were also placed randomly in a different place in the experiments and samplings were carried out by following identical procedures.

To avoid type II errors in the statistical analysis of data, all treatments and controls increased during the experiment. The calculated uncharged ammonia concentration was higher in feces with a higher pH (Table 2). In the treatments with added ammonia, losses were detected. Furthermore, in comparing the open and closed systems, treatments presented below were made on the assumption that both layers in each system had the same behavior.

### RESULTS

The ANOVA tests confirmed that there were no differences between each pair of layers for all treatments. The same finding was made for all organisms. Therefore, all statistical analyses of the treatments presented below were made on the assumption that both layers in each system had the same behavior.

**Physicochemical parameters.** The feces used in the experiment had an initial pH of 5.85, a total solid content of 20%, and a total ammonia concentration of 18 mM. After contact with the additives, the pH value of the feces started to increase, including that of the controls. Treatments with ash addition achieved the highest pH values (Table 2). The average pHs of the additives were 8.8, 10.2, and 12.4, for oyster shells, the mixture of ash and oyster shells, and solely ash, respectively. The presence of urea in the additives had no effect on the pH.

The average ammonia concentration in all treatments and controls increased during the experiment. The calculated uncharged ammonia concentration was higher in feces with a higher pH (Table 2). In the treatments with added ammonia, losses were detected. Furthermore, in comparing the open and closed systems, losses were noted in the open system, e.g., the 75%O system lost approximately 37% of the ammonia.

Desiccation was observed in all open treatments, resulting in an average TS content of 87% by the end of the 130-day experimental period. The feces in the closed systems also dried out, mainly due to the additives, reaching 42% TS by the end. The C2 system maintained the moisture characteristics of the initial feces, i.e., 20% TS (Table 2).

**Salmonella Typhimurium.** The salmonella count decreased according to the method described in Standard Methods for the Examination of Water and Wastewater (24), and ammonia was quantified spectrophotometrically on a Genesys 20-4001/4 spectrophotometer (Thermo Spectronic), using the indophenol blue method (Merck, Whitehouse Station, NJ).

Before microbiological analysis, 1 g of sample was diluted with 9 ml buffered NaCl-peptone solution with Tween, pH 7.0, followed by a 10-fold dilution series using the same buffer solution. Samples for S. Typhimurium determination were spread on xylose-lysine-desoxycholate (XLD) agar containing novobiocin and cultivated at 37°C for 24 h. Samples for Escherichia coli determination were spread on Slanetz-Bartley (SlaBa) agar and cultivated at 44.5°C for 48 h.

The bacteriophage assay used the double agar method (25), and the bacteria were cultured at 37°C for 3 to 5 h in nutrient broth as a host strain for enumeration. The plates were then incubated at 37°C for 18 h.

**Data evaluation.** A general evaluation was conducted using descriptive statistics for initial considerations of data from physicochemical analyses. Inactivation rate coefficients were determined for all organisms evaluated for all treatments and controls, based on accepted multiple-correlation coefficients (0.65) in a linear regression, log-linear scale, according to first-order exponential decay. Zero values were not used. The software utilized was Microsoft Excel.

Statistical one-way analysis of variance (ANOVA) was used to evaluate differences between the two layers of each treatment individually and between the 11 treatments and controls, with a 95% confidence level. Analyses were done for each time point or sample. ANOVA between the treatments was followed by the Tukey range test to evaluate the differences in greater depth. The treatments and controls were grouped (groups a, b, c, d, and e) according to similar efficiencies for each different microorganism, except for A. suum. Before these tests, the data were tested for distribution fitting, and all data had to be normalized by logarithmic transformation [where zero x' = log10(x + 1)]. The software utilized was Statistica 7.0.
with all combinations tested. Each set of six samples for all treatments (from both layers of the three replicates) was tested for each sampling (ANOVA), and the results showed that the different treatments started to behave differently between days 6 and 15. Before that, they could be considered the same (P = 1.7406). From day 15 until the end of the experiment, all tests showed significant differences between the different treatments. However, after 30 days, the ranking test showed that despite the significant differences between the 11 treatments (P = 0.000003), [75%O+U], [75%O:A+U], and [150%O+U] could be grouped (group a) and considered to have the same performance. The reduction of S. Typhimurium within 30 days in the [75%O+U], [75%O:A+U], and [150%O+U] systems was 7 log units, which was the highest inactivation performance of the 11 treatments tested.

The decimal reductions (Dr values) for [75%O+U], [75%O:A+U], and [150%O+U] were 5.1, 4.6, and 4.8 days, respectively, while the corresponding control, where urea was added to feces ([F+U]), had a Dr value of 5.9 days. Addition of oyster shells solely gave a long survival of Salmonella, with a Dr value of about 30 days. All decimal reductions calculated for treatments and controls and the results of the Tukey rank test are shown in Table 3.

**Table 3** Results of ranking Tukey test for bacteria and bacteriophages, with inactivation coefficients (k), Dr values, and multiple-correlation coefficients (R²) calculated for treatments and controls based on data collected from the experiment

<table>
<thead>
<tr>
<th>Treatment or control</th>
<th>pH (avg ± SD)</th>
<th>Total NH₄-N concn (mM) (avg ± SD)</th>
<th>NH₃-N concn (mM) (avg ± SD)</th>
<th>Total solids (%) (130 days of treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75%O</td>
<td>7.55 ± 0.72</td>
<td>137.3 ± 84.1</td>
<td>1.7 ± 1.1</td>
<td>93</td>
</tr>
<tr>
<td>75%O:A</td>
<td>8.37 ± 1.54</td>
<td>63.4 ± 18.7</td>
<td>5.1 ± 1.6</td>
<td>84</td>
</tr>
<tr>
<td>150%O</td>
<td>7.85 ± 0.74</td>
<td>114.9 ± 32.8</td>
<td>2.9 ± 0.9</td>
<td>81</td>
</tr>
<tr>
<td>150%O:A</td>
<td>8.59 ± 1.54</td>
<td>87.0 ± 35.4</td>
<td>11.0 ± 4.7</td>
<td>90</td>
</tr>
<tr>
<td>300%O</td>
<td>8.13 ± 0.47</td>
<td>128.5 ± 21.0</td>
<td>6.0 ± 1.1</td>
<td>83</td>
</tr>
<tr>
<td>75%O+U</td>
<td>7.64 ± 0.83</td>
<td>148.5 ± 74.0</td>
<td>2.2 ± 1.2</td>
<td>92</td>
</tr>
<tr>
<td>[75%O+U]</td>
<td>7.93 ± 0.90</td>
<td>238.8 ± 76.0</td>
<td>6.7 ± 2.3</td>
<td>33</td>
</tr>
<tr>
<td>[75%O:A+U]</td>
<td>8.13 ± 1.30</td>
<td>147.0 ± 74.5</td>
<td>6.7 ± 3.6</td>
<td>41</td>
</tr>
<tr>
<td>[150%O+U]</td>
<td>8.37 ± 0.50</td>
<td>254.7 ± 81.9</td>
<td>18.6 ± 6.5</td>
<td>36</td>
</tr>
<tr>
<td>[150%O:A+U]</td>
<td>8.61 ± 1.44</td>
<td>80.0 ± 28.0</td>
<td>10.4 ± 3.9</td>
<td>83</td>
</tr>
<tr>
<td>[150%O:A+U]</td>
<td>8.35 ± 1.55</td>
<td>97.3 ± 39.0</td>
<td>7.3 ± 3.1</td>
<td>56</td>
</tr>
<tr>
<td>C1-F+W</td>
<td>7.46 ± 0.67</td>
<td>81.1 ± 14.6</td>
<td>0.9 ± 0.2</td>
<td>87</td>
</tr>
<tr>
<td>C2-F</td>
<td>7.25 ± 1.01</td>
<td>108.8 ± 25.9</td>
<td>0.7 ± 0.2</td>
<td>20</td>
</tr>
<tr>
<td>C3-F</td>
<td>6.85 ± 1.20</td>
<td>86.5 ± 12.7</td>
<td>0.2 ± 0.0</td>
<td>91</td>
</tr>
<tr>
<td>[C4-F+U]</td>
<td>7.68 ± 0.81</td>
<td>212.1 ± 20.0</td>
<td>3.4 ± 0.4</td>
<td>26</td>
</tr>
</tbody>
</table>

* Calculated by taking into account pH, temperature, total ammonia, and total carbonate.

**Table 2** pH, total ammonia nitrogen concentration, uncharged ammonia concentration, and total solid content in treatments and controls of the experiment

**Enterococcus faecalis.** The E. faecalis count increased with all treatments until day 20, when it started to decrease at different rates, as reported by J. Fidjeland, M. E. Magri, H. Jonsson, A. Albinh, and B. Vinnerås (unpublished data). For all samples, the ANOVA test detected differences between the combinations (P < 0.05) during the whole experimental period. At 100 days, [75%O+U], 150%O:A+U, and [150%O:A+U] achieved approximately 6-log₁₀ reductions. Tukey rank testing confirmed that the performances of those treatments (group a) in inactivation of E. faecalis could be considered equal. Their Dr values were 15.6, 16.0, and 9.8 days, respectively (Table 3). Following the ranking of the Tukey test, the next group of treatments (group b) was 150%O:A, [75%O:A+U], [150%O:A+U], and [C4-F+U]. The following groups in the ranking are listed in Table 3. There was no significant reduction in the C1-150%WC system.

**Bacteriophage MS2.** The treatments could be considered statistically different for all samples (P < 0.05) for the entire period of the experiment. The inactivation varied substantially until approximately day 50, with no stable tendency for the ranking test. However, from day 50 until day 130, the treatments could be grouped, with clearly different tendencies in inactivation. The fastest inactivation was achieved with 150%O:A+U and [150%O:A+U] (group a), with Dr values of 17.9 and 11.9 days, respectively, corresponding to a 6.5- to 7.5-log₁₀ reduction in 130 days. The next group of treatments in the ranking contained [75%O:A+U] and 150%O:A (group b). The following groups in the ranking are listed in Table 3.

**Bacteriophage ΦX.** Until day 15 of the experiment, the treatments showed no difference in inactivation of bacteriophage ΦX (P > 0.05), which could be considered almost null (<0.5 log₁₀ unit). After this first period, the treatments differed significantly in inactivation (P < 0.05). From day 20 until the end of the experiment, the first two groups could be identified by the ranking test. With its higher inactivation, 150%O:A+U could not be paired with any other treatment to form group a. Its decimal reduction

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* Coefficients were calculated between days 20 and 130 of treatment, not considering the growing phase at the beginning of the evaluation (0 to 20 days).
The initial viability of the eggs used in the experiment was 98%. At the first sampling (10 days), the viability with all treatments had declined to 50%, on average. In the following 30 days, the viability increased in very similar and linear ways with 150%O, [75%O+U], and [150%O+U], reaching 70%, on average. The [150%O:A+U] system presented a different behavior from the others, with egg viability decreasing to 32% within the first 20 days (two samplings), followed by a fast increase to 85% in the next 20 days. At the fourth and fifth samplings (80 and 120 days), egg viability had decreased at different rates for all treatments, achieving 14, 15, 20, and 32% viability for 150%O, [75%O+U], [150%O+U], and [150%O:A+U], respectively, by day 120. The Dr values for 150%O, [75%O+U], [150%O+U], and [150%O:A+U] were 120, 139, 125, and 192 days. The following groups in the ranking are listed in Table 3.

**Ascaris suum.** The initial viability of the eggs used in the experiment was 98%. At the first sampling (10 days), the viability with all treatments had declined to 50%, on average. In the following 30 days, the viability increased in very similar and linear ways with 150%O, [75%O+U], and [150%O+U], reaching 70%, on average. The [150%O:A+U] system presented a different behavior from the others, with egg viability decreasing to 32% within the first 20 days (two samplings), followed by a fast increase to 85% in the next 20 days. At the fourth and fifth samplings (80 and 120 days), egg viability had decreased at different rates for all treatments, achieving 14, 15, 20, and 32% viability for 150%O, [75%O+U], [150%O+U], and [150%O:A+U], respectively, by day 120. The Dr values for 150%O, [75%O+U], [150%O+U], and [150%O:A+U] were 120, 139, 125, and 192 days. The [C2-F] and [C4-F+U] controls could not be modeled because of the inconsistency of data.

**DISCUSSION**

In terms of the factors affecting inactivation of the tested microorganisms, inactivation of *E. faecalis* and *S. Typhimurium* was more affected by the combination of neutral to high pH and urea, which consequently gave the highest concentrations of uncharged ammonia. *Enterococcus faecalis* had a slower response to the factors than *S. Typhimurium*. Control 4, which had feces and urea, presented similar levels of inactivation, while in control 3, where mainly desiccation was acting, no significant decrease of bacteria was observed (Fig. 3 and 4). Treatments grouped as group a in the ranking test for both bacteria had urea in the additive mixture.

The concentration of ammonia in feces increased for all treatments and controls, probably due to further degradation and mineralization of organic nitrogen during the storage period, since no other ammonia source was added. The ammonia concentration was higher when oyster shells were added to feces, mainly in the closed systems, than with the addition of oyster shells plus ash. The higher pH of ash was a possible reason for why urea was not completely hydrolyzed into ammonia by pH-related urease inhibition. Studies report that pHs above 12 inhibit urease (26). There were also no indications that more ammonia was produced when ash was used and then lost by volatilization, because in comparing treatments 150%O:A+U and [150%O:A+U], i.e., uncovered and covered treatments, similar ammonia concentrations were found.

The use of oyster shells with urea as an additive was more effective for the inactivation of *S. Typhimurium*, since it promoted higher ammonia concentrations in the feces. The use of oyster shells, ash, and urea improved the inactivation of *E. faecalis*, since this mixture also raised the pH in the fecal layer. Figure 5 presents the positive correlation between pH and Dr values for *E. faecalis*. Another study has shown that *E. faecalis* is affected mainly by pH rather than the ammonia concentration at temperatures below 20°C (21).

After 130 days, bacteria were still only slightly reduced in treatments with oyster shell addition only (300%O, 150%O, and 75%O) and in two controls (C1-150%WC and C3-F), which had in common a higher TS content and lower pH, and consequently a lower uncharged ammonia concentration, than treatments with oyster shells and ash. The single inactivation factor in these treatments was identified as desiccation. Bacteria, e.g., some serovars of *Salmonella*, possess a number of defenses against the effects of desiccation. Certain cell components can help to slow the drying process and prevent complete desiccation, while others appear to
maintain cell viability by protecting membranes and proteins in a desiccated state (27). The use of solely oyster shells as an additive was not effective because it promoted mainly desiccation, although when enriched with urea it was shown to be effective.

The higher degree of inactivation of *E. faecalis* achieved with [150%O:A + U], [150%O:A + U], and [75%O + U] gave very similar decimal reduction values (13.8 days, on average) to those found in studies treating feces with approximately 250% ash, which greatly increased the pH, at 30°C (28). Comparing the results of using just ash with the present study, one advantage of using oyster shells solely or mixed was that similar inactivation was achieved with about half of the additive under a more limiting temperature.

Addition of urea at percentages above the 0.5% level used in the present study has a confirmed effect on inactivation of *Enterobacteriaceae*. One study achieved decimal reduction values of 0.7 and 3 days for *Salmonella* spp. and *Enterococcus* spp., respectively, by adding 3% urea to feces in a closed system at 20°C (15). Lower addition rates result in longer treatment periods being required (16,17). On the other hand, in untreated fecal storage, the decimal reduction value for *Enterococcus* spp. was reported to be 50 days (19). Another study that treated feces with 0.5% urea at 14°C (the same percentage as that in the present study) achieved decimal reduction values of 13 and 71 days; comparing these data with our values of 6 and 20 days for *Salmonella* spp. and *Enterococcus* spp., respectively, also confirms the direct influence of temperature on inactivation (17). The same study also showed considerably faster inactivation at 34°C (17). The temperature increased the concentration of uncharged ammonia, but the increase was not linear relative to the effect of the uncharged ammonia, as a higher temperature increased the inactivation per mM uncharged ammonia.

The inactivation of bacteriophages MS2 and ΦX was influenced more by the combination of high desiccation and high pH (which slightly increased the NH₃-N content) than by the total ammonia content. Treatments grouped in group a for MS2 and ΦX had low average total ammonia concentrations (89 and 80 mM, respectively) and uncharged ammonia concentrations of 10.4 and 8.8 mM, respectively. In control 4, where feces was exposed just to urea, there was a much slower decimal reduction (65.8 days for MS2 and 67.6 days for ΦX) than the average decimal reductions for treatments in groups a and b (Dr value = 17.5 days for MS2 and 9.7 and 11.5 days for ΦX) (Fig. 6 and 7). The reduction in control 2, where just feces was kept in closed boxes, was also slow for ΦX (Dr value = 71.9 days), and it was insignificant for MS2, while in control 3, where the desiccation effect was strong, there was a significant inactivation. The differences between these controls and treatments confirm desiccation and pH as the main factors in inactivation (Fig. 8 and 9) and show that uncharged ammonia at these low concentrations does not affect bacteriophages. Studies of ammonia inactivation of animal viruses indicate a considerably higher sensitivity to ammonia than...
that of phages (29). This indicates that phages are conservative microorganisms, and further studies of the survival of animal and human viruses in human feces with different additives are recommended.

The inactivation response of \( \Phi X \) against the drying factor was stronger than the response of MS2 (Fig. 9). The fastest inactivation of \( \Phi X \) (Dr value = 9.7 days) occurred with the 150%O:A+U treatment, which had 83% TS and a maximum pH of 10.2 (mean, 8.6). Despite urea being included in the additive mixture, the total ammonia content in feces was low. The prevalence of the factors affecting inactivation can be seen more clearly in comparing 150%O:A+U against [150%O:A+U]. The two treatments had all the same characteristics, except that one was open and the other closed. When the system was open, the moisture content in the treatment decreased, making inactivation more efficient. Preliminary studies have produced similar findings about the moisture content in compartments of UDDTs with natural ventilation pipes, where the dry matter content increased to 70 to 80% in 200 days (30).

The use of oyster shells and ash rather than oyster shells alone improved the inactivation of MS2, since the mixture raised the pH in the fecal layer. Studies on bacteriophage inactivation in composting toilets with sawdust as an additive showed a higher dependency on water content and temperature, with a Dr value of approximately 3.4 days for MS2 in a matrix with 50% water content at 30°C (31). Inactivation studies with urine reported that reducing the moisture content by 90% gave 75% inactivation (32), confirming the effects of desiccation on the inactivation of bacteriophages, similar to what was detected with 150%O:A+U and in the controls that were kept open and achieved higher TS contents, such as C1-F+WC.

The behavior of \( A. \text{suum} \) in the present study highlights the importance of evaluating this helminth, one of the most conservative indicators of fecal pathogens, for longer periods. The treatments (most clearly 150%O, [150%O+U], and [75%O+U]) showed a significant decrease in egg viability in the first 10 days, followed by an increase. This behavior suggests either a certain capacity for self-recovery after a stress situation in the environment or that the nonviable eggs were degraded, leading to a falsely high viability, as the correlation between viable and nonviable eggs, not the concentration, was measured. A decrease in viability was detected again only by day 80. Similar behavior has been reported regarding the survival of \( A. \text{suum} \) in ensiled swine manure (45% TS and pH 3.9 to 4.4 at 15 to 25°C). In that case, the viability decreased from 100 to 40% in the first 28 days, followed by an increase to 70% in 56 days (33). The high resistance of ascarid ova has been attributed mainly to the thick wall that surrounds the developing larvae (34).

Temperature, drying, and UV light are the main factors influencing die-off of \( A. \text{suum} \) in natural systems (35). The highest level of inactivation in the treatments evaluated here occurred in the open system, where the TS content reached 82%. In all samplings with 150%O, the unviable eggs had damage to the membranes, whereas in the other treatments unviable eggs were identified simply by a failure of larvae to develop. The effect of ammonia was probably lower as a result of the lower concentrations tested. A previous study reported that an estimated uncharged ammonia concentration of 610 mM has a sufficient effect on ascarid inactivation (4 log_{10} within 4 weeks in human feces at a temperature of 24°C (20). The pH probably had no effect in our case, as it was never above 10, and the inactivation rate was strongly dependent on temperature. The addition of ammonia caused an increase in inactivation rates that was dose dependent. The minimum total ammonia nitrogen concentration where some effect can be detected at 20°C is reported to be 294 mM, with no effect on inactivation at pH values of 7 to 12 at 20°C (36).

Sprinkling large amounts of additive (except for very highly alkaline additives, e.g., lime) on feces in dry toilets can create a protective layer that preserves the characteristics of feces, allowing microorganisms to survive longer rather than actually killing them off. This was shown by the treatment with 300% oyster shells, which had lower inactivation rates for \( S. \text{Typhimurium}, E. \text{faecalis} \), and MS2 than all other treatments and controls. It is common practice for people to add large amounts of additive to cover the feces, but our results indicate that less additive should be used for better inactivation.

The most promising treatments tested were [150%O:A+U], 150%O:A+U, and 150%OA. With the calculated models, these three treatments could inactivate, e.g., \( 7 \log_{10} \) units of all bacteria and bacteriophages evaluated over a maximum period of 83, 125, and 183 days, respectively, with MS2 being the limiting organism for the first two cases and \( E. \text{faecalis} \) the limiting organism for the third. Among these treatments, \( A. \text{suum} \) was evaluated only with [150%O:A+U], and there was a small reduction during this period (Dr value = 192 days). When \( A. \text{suum} \) organisms are considered a risk, treatments based on desiccation and urea, e.g., 150%O:A+U and 150%O:A, should be prioritized. The addition of urea at higher percentages (0.75 to 2.00%) is also recommended if the time of treatment needs to be shortened. In addition, in using higher ammonia concentrations and to combine that factor with desiccation, it is recommended to have nonforced ventilation pipes in the toilet and to cover the feces container once it is full to minimize ammonia losses. Thus, during the filling period, both desiccation and urea could act in the inactivation, and when the container is full, urea could act more strongly.

Conclusions. A combination of factors (pH, desiccation, and ammonia content) showed promising results for sanitizing feces. Maximization of individual effects of those factors in a combined process can make it possible to produce a safe material in a low-cost and low-maintenance technology suitable for application in urine-diverting dry toilets.

Using organic additives such as wood chips or using no additive resulted in small reductions of the organisms evaluated. The most promising treatment alternative was 150%O:A+U, as it showed large reductions of all the bacteria and phages in the study, and indications are that it would also be efficient for inactivation of \( A. \text{suum} \); however, further studies are required.

REFERENCES


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