Disinfection of Freshly Separated Fecal Matters by Applying Heat and Chemicals

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- Summary
Solid-liquid separation is a common process in wastewater treatment

Concept of *Dry toilet* is being mimicked in *wet toilet*
Fresh-fecal matter

• High concentration of pathogens in the fresh fecal matter
• High water content

• Reuse of human excreta (nutrients) without appropriate treatment, can increase the risk of health impacts in food and feed production (WHO, 2006)
• Selection of appropriate treatment is a crucial
Disinfection: Heating

- A range of disinfection techniques namely: UV radiation, chemical treatment, drying, heating
- Heat inactivation is one of the reliable methods (Vinneras et al. 2002)
- Affects the enzyme-catalyzed reaction in microbes
- However, when it comes to fresh fecal matters, there is still only limited information on pathogen inactivation
Objectives

- Establish **time-temperature relationship**, pathogen inactivation in fresh fecal matters
  - *Heating temperature*
  - *Initial E coli load*
- Select optimum time-temperature particularly for Zyclone cube toilet
- To determine pathogens **inactivation by chemicals** (lime and urea) in fresh fecal matters
- To **evaluate the feasibility** of these methods in actual conditions
Pathogenic Indictors: *E. coli*, Ascaris

**E. coli**
- Temperature: 50°C, 60°C, 70°C
- *E. coli* load: 3-4, 4-5, 5-6.5 log value
- Time: 3, 5, 10, 15, ..... 180, 240, 300 min

**Ascaris**
- Temperature: 60°C, 70°C, 80°C
- *Ascaris* load: 400 eggs/g TS
- Time: 5, 10, 15, 20, 25, 30, 60, 120 min
Methods

Sampling

Plate count of *E. coli*

Moisture content

Total solids (TS)
Results

**50°C**

![Graph showing E. coli Log CFU/g (dry wt) over time at 50°C for Rep-I, Rep-II, and Rep-III.]

**60°C**

![Graph showing E. coli Log CFU/g (dry wt) over time at 60°C for Rep-I, Rep-II, and Rep-III.]

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The graphs illustrate the log colony-forming units per gram (CFU/g) of dry weight of E. coli (E. coli) over time at 50°C and 60°C. The graphs are labeled as Rep-I, Rep-II, and Rep-III, representing different replicates or conditions.
Results

70°C

![Graph showing the log CFU/g (dry wt) of E. coli over time at 70°C for different repetitions (Rep-I, Rep-II, Rep-III).](image)
Results

![Graph of E. coli Log CFU/g (dry wt) vs. Time (mins) at different temperatures (70°C, 60°C, 50°C)]
**Method: Ascaris Egg Inactivation**

### Flotation Method (ZnSO₄)

- Eggs counted as viable - motile larva (active) and non-viable (inactive)
- Examined after 28 days
Method: Ascaris Egg Inactivation

Incubation 22-26°C for 28 days

Flotation Method (ZnSO₄)

- Eggs counted as viable- motile larva (active) and non-viable (inactive)
- Examined after 28 days
Results: Ascaris Egg Inactivation

Control

- 60°C For 15 min
  Infective larva

- 70°C For 15 min
  Inactive larva

- 80°C For 15 min

14°C For 15 min

Results:

- Egg Inactivation
Results: Ascaris Egg Inactivation

- **100% inactivation** at 70°C and 80°C in 15 min
- **80% inactivation** achieved under 60°C for 15 min, and requires 240 min for 100% inactivation
Application in Actual condition

- *E. coli*: 60°C for 25 mins, 70°C for 15 mins
- Ascaris: 70°C for 15 mins
- Applied at 70°C for 15 mins
Solid Disinfection: Lime & Urea
Chemicals: Lime & Urea application

![Graph showing the effect of lime and urea application on E.coli Log value (CFU/mL) over time.]

- **Control**
- **3% (lime + urea)**
- **5% (lime + urea)**
Ascaris inactivation: @ lime 5% and 7%

- Not-effective
- Larva is observed, counted as viable or the fertilized eggs
Conclusions

- Time-temperature relationship for *E. coli* inactivation in fresh feces was established.

- 50°C, 60°C and 70°C require an inactivation time of 300, 30 and 15 minutes, respectively.

- Effect of Initial *E. coli* load was prevalent for low temperature

- *Ascaris* were inactivated at 70°C and 80°C in 15 minutes

- Lime (3%) and urea (5%) inactivated *E. coli* in 66, and 24 hours, respectively

- Lime and urea were not effective for *Ascaris* egg inactivation
Take Away

- Heating is effective for treating the freshly separated fecal matter.
- Initial load of *E. coli* cause variation in time required for complete inactivation.
- 60°C for 25 min was cost-effective, but 70°C for 15 min is ideal for complete disinfection (including *Ascaris*).
- Lime and urea @ 3% and 5% inactivates *E. coli* but do not ensure Ascaris inactivation.
Thank you very much for your attention
Pilot scale Testing
Toilet Components

- Solid-liquid separator, and disinfection systems
- Cyclone based on centrifugal and gravity
- Heating and Electrochemical
Energy calculation and relevant cost at different heating time under different heating temperature

Assume that 70°C for 15 min exposure energy requirement;
Formula used for calculation of power required:

\[ P = \frac{Q \cdot \Delta t}{860 \text{ Kcal.time.efficiency}} \]

\[ P = 2\text{kg} \times \frac{(70-20)^\circ\text{C}}{860} \times 0.25\text{hr} \times 0.9 = \frac{100}{193.5} = 0.5\text{KW} \]

1 Kw price in Thailand 3 bhat (9cent), India 4.9 IRS (8 cent)

<table>
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<th>Amt to be treated</th>
<th>Initial temp</th>
<th>desired temp</th>
<th>time</th>
<th>Power (KW)</th>
<th>3 TBH/kW</th>
<th>9 cent/kW</th>
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Back up-information

II) Collection of Fresh feces

Fresh feces were collected voluntarily at Asian Institute of Technology. The feces were stored at room at 5°C temperature immediately after the collection. Prior to apply heat treatment, the stored feces were kept outside of room so that temperature could be adjusted as per ambient condition. The feces were then well mixed, and E. coli. Inoculums were added externally to vary the initial E coli loads.

iii) Inoculation of E coli.

Initial E coli levels were differed by adding the mixed cultures of E. coli in fresh fecal samples. E.coli cultures were grown separately overnight at 37°C in 20 ml of Luria Broth (LB), which were inoculated into 1.5 kg of fresh feces with 70% ethanol and rinsing with sterile DI water. After the enrichment, the fresh feces were mixed continuously for 10 min on sterile polypropylene trays by hand, wearing sterile gloves. The inoculated levels of the pathogens in feces were enumerated by serial dilutions of fresh feces in DI water.

Total coliform and E. coli analysis

Following steps were followed in analysis of E coli and total coliform

Serial dilution technique

- Label the test tube 10\(^{-1}\)-10\(^{-8}\) indicating dilution factor.
- Aseptically add 1 ml of sample to the 9 ml of DI water and mix gently.
- Take 1 ml of this solution and add to the next tube (10\(^{-2}\)) and mix gently.
- Repeat this procedure for the remaining tube (10\(^{-3}\)-10\(^{-8}\)).

Drop plate method

- Label sample name, date and serial dilution.
- After serial dilution, mix the last dilution (10\(^{-8}\)) using a vortex mixer and take 10μl and drop on section 10\(^{-8}\) in eosin-methylene blue (EMB) agar plates (3 drop, 10μl/each). The surface of the EMB agar plates needs to be sufficiently dry to be absorbed in 15–20 minutes.
- Mix 10\(^{-7}\) dilution on vortex mixer. Using the same pipette tip, take 10μl of 10\(^{-7}\) dilution and drop on EMB agar plates.
- Repeat this procedure for the remaining dilutions (sequentially from 10\(^{-6}\)-10\(^{-1}\))
- The plates are left upright on the bench to dry before inversion and incubation at 37°C for 18 – 24 hour.
- Colonies are counted in the sector where the highest number of full-size discrete colonies can be seen (usually sectors containing between 2-20 colonies are counted).
- The following equation is used to calculate the number of colony forming units CFU/ml from the original sample:

\[
\text{CFU/ml} = \frac{\text{Average number of colonies for a dilution}}{\text{dilution factor}}
\]

Convert to CFU/g total solid: CFU/g total solid = \(\frac{\text{CFU/ml}}{\% \text{Total solid}}\)
Moisture in function of heating

The moisture of fresh fecal matters heating at different temperature and time during the inactivation test are calculated. The moisture percentage of samples at various stage of heat treatment is shown in Table 7.

Table 7: Moisture % of fresh fecal matter at different time and temperature of heating

The table 7 shows that changes in moisture levels are not prominent with heating temperatures and time. Only 1% of moisture was decreased even though long period of heating (300 minutes) in case of 50°C and 70°C, however, it remained almost the same over a time in case of 60°C temperature. Literally, heating is also considered effective towards the microbial inactivate, which is not suitable for reduction of moisture levels.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>wet moisture (%) at 50°C</th>
<th>Time (min)</th>
<th>wet moisture (%) at 60°C</th>
<th>Time (min)</th>
<th>wet moisture (%) at 70°C</th>
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<td></td>
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<tr>
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<td>180</td>
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The first 3 Figures (a-c) illustrates: The control which is the stage of fertilized (a); infective larva in ova (b); cell division in ova (c) at 60°C with 15 minute.

Figures d-i illustrates inactive Ascaris ova at high temperature 70°C and 80°C within 15 minute. The changing characteristic of inactive Ascaris ova on this study could be divided into four forms i.e. vacuolations inside the ova, irregular over-cell sized, ova cell cracked and bubbling of the cell.

**Method for enumeration and viability of *Ascaris lumbricoides***

The method used in this study is efficient and cost effective for the detection, enumeration and determination of viability of Ascaris ova in wastewater, sludge, and compost which is based on a protocol published by (Bowman et al., 2002). Method is based on three fundamental principles: (1) washing, (2) filtration and (3) floatation and sedimentation of the parasites.

For the lab analysis, 10g of fecal sample was weighed and placed in a beaker. 30ml of distilled water was gradually added to the beaker. The mixture was mixed well and filtrated by using double layer cheesecloth. The suspension was then poured into centrifuge tube. The tubes were then centrifuged for 3 minutes at 2000rpm (photo 3).

Then the supernatant was drained and filled with ZnSO₄ (sp.gr=1.30*) just over the top of the tube. For the preparation of the ZnSO₄ Solution (sp.gr = 1.30*), the solution (1.30 sp.gr.) was made by adding 500 grams of ZnSO₄ to 880 liter of deionized water. The mixture was checked with a hydrometer and adjusted to 1.30. The ZnSO₄ solution was stored tightly capped to prevent evaporation.

The top of the tube was covered with a coverslip and then waited for 5-10 minutes. Later 1-2 drops of iodine solution was added on a slide and coverslip from the tube was moved and placed on the slide for microscopic examination. All Ascaris eggs which has motile larva (active) were counted as viable and the ones with no larva development (inactive) were counted as non-viable Ascaris in the eggs under microscope after 28 days of incubation at 26-27°C.
Ascaris inactivation (@ lime 5% and 7 %)

The Ascaris egg disinfection was not found effective within 28 days contact time as Previous research has also found a similar lack of correlation between concentration of the lime and Ascaris die-off in human excreta for pH below 12 (Polprasert C, et al 1980)

Ascaris eggs found in the bio-bag a) Control Ascaris egg with Larva formation b) Fertilized egg in bag with 5% dose c) Decorticated egg found in bag with 7% dose

It can be concluded that the treatment of lime and urea is still ineffective at reducing helminth eggs at both 5% and 7 %.

All the Ascaris where the larva is observed are counted as viable or the fertilized eggs as in Figure 7 a while the one with no larva development are counted as unfertilized eggs.
Solid-liquid separation is a common process in wastewater treatment.

Concept of Dry toilet is being mimicked in wet toilet.

Zyclone cube toilet involves active separation of solid and liquid matter from toilet wastewater.

Separated solid and liquid are stored separately.

Treatment of these separated portions is done on-site before their safe discharge.
Background: Zyclone cube toilet