Disinfection of Freshly Separated Fecal Matters by Applying Heat and Chemicals

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- Objectives
- Methodology
  - Experimental set-up
  - Operating condition
  - Lab analysis
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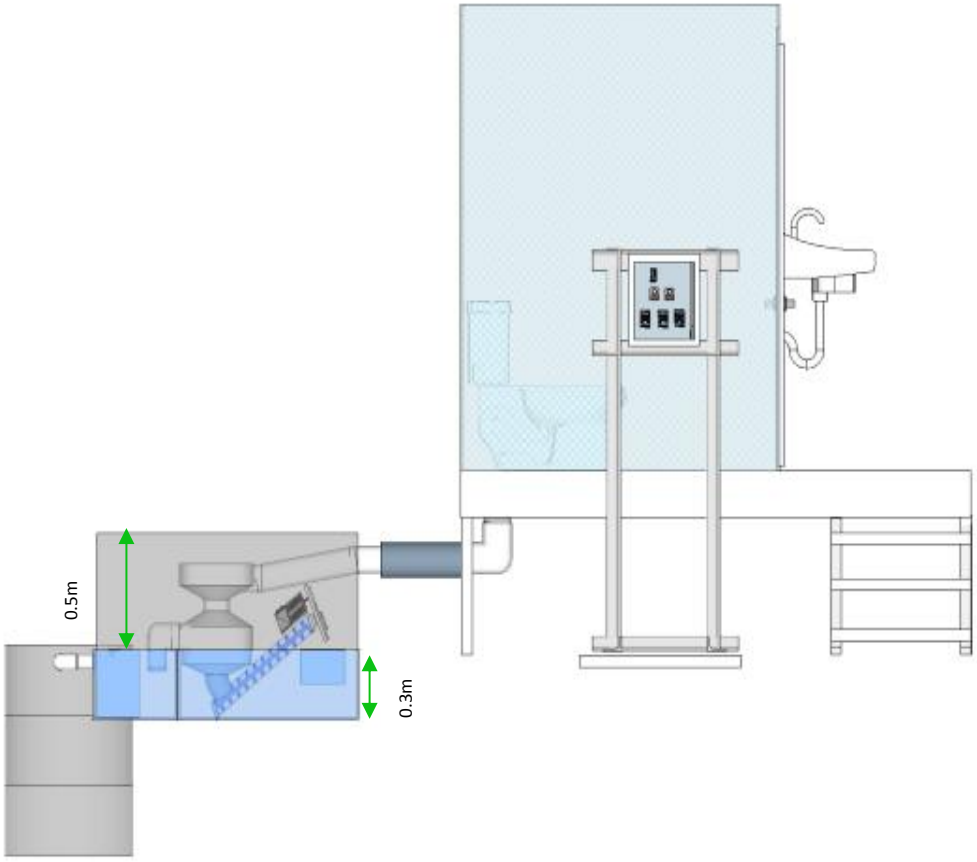
- Summary

Background: Zyclone cube toilet

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

- Concept of *Dry toilet* is being mimicked in *wet toilet*



Major challenges

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

Operating conditions

- Pathogenic Indictors: *E. coli,* Ascaris

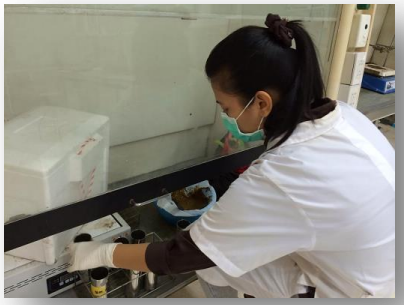
E. coli

- Temperature : 50^{o}C, 60^{o}C, 70^{o}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^{o}C, 70^{o}C, 80^{o}C
- *Ascaris* load: 400 eggs/g TS
- Time: 5, 10, 15, 20, 25, 30, 60, 120 min

Methods



Sampling

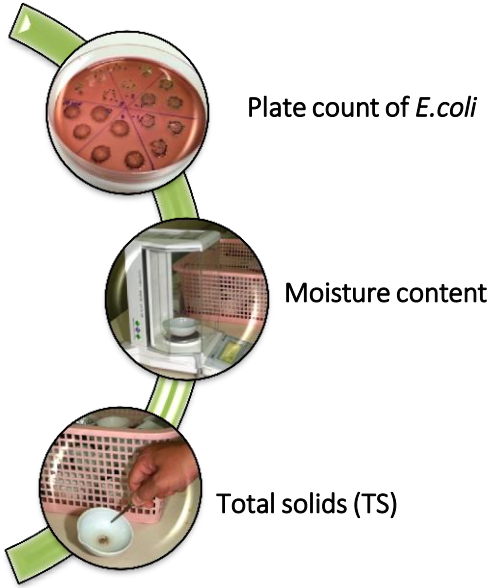
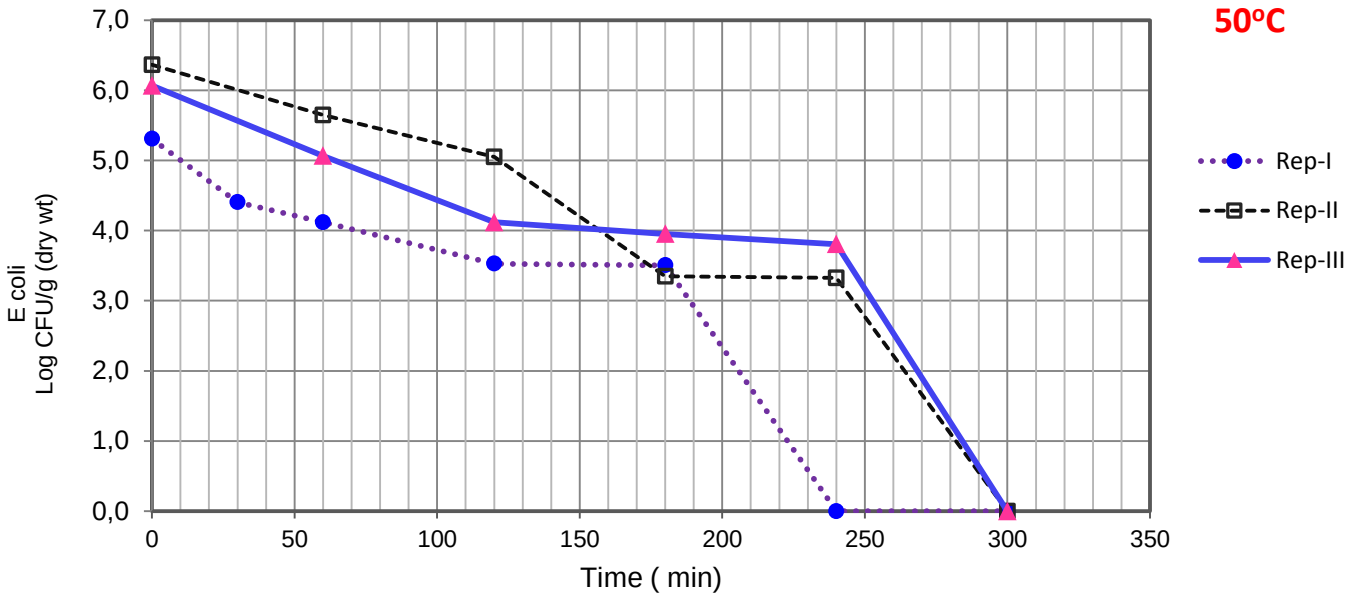


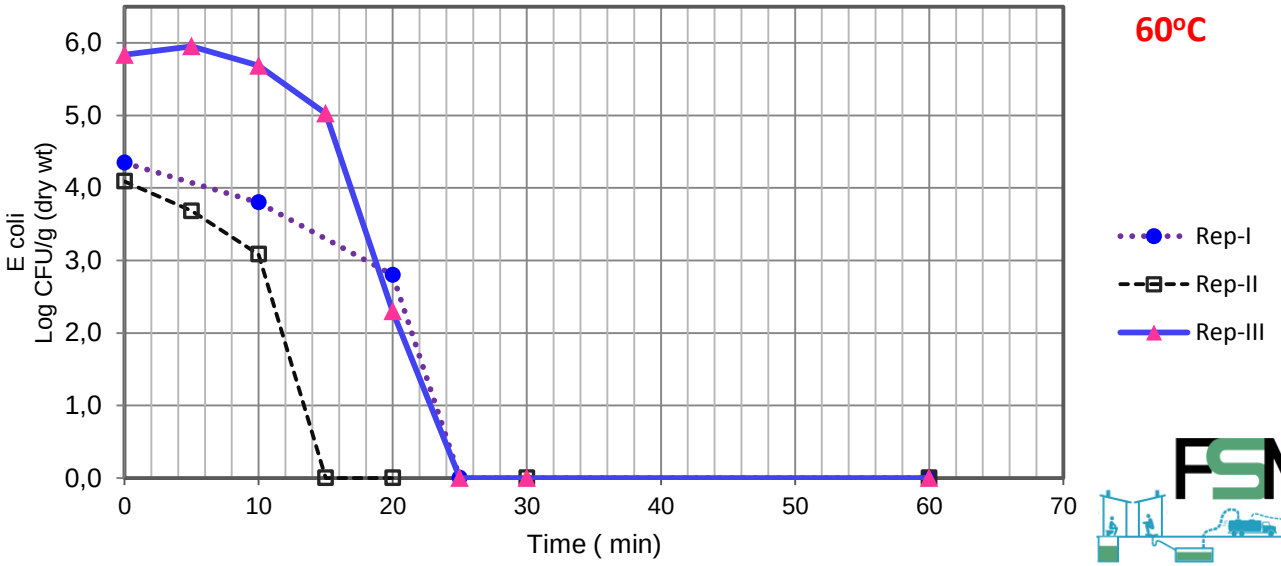
Plate count of *E.coli*

Moisture content

Total solids (TS)

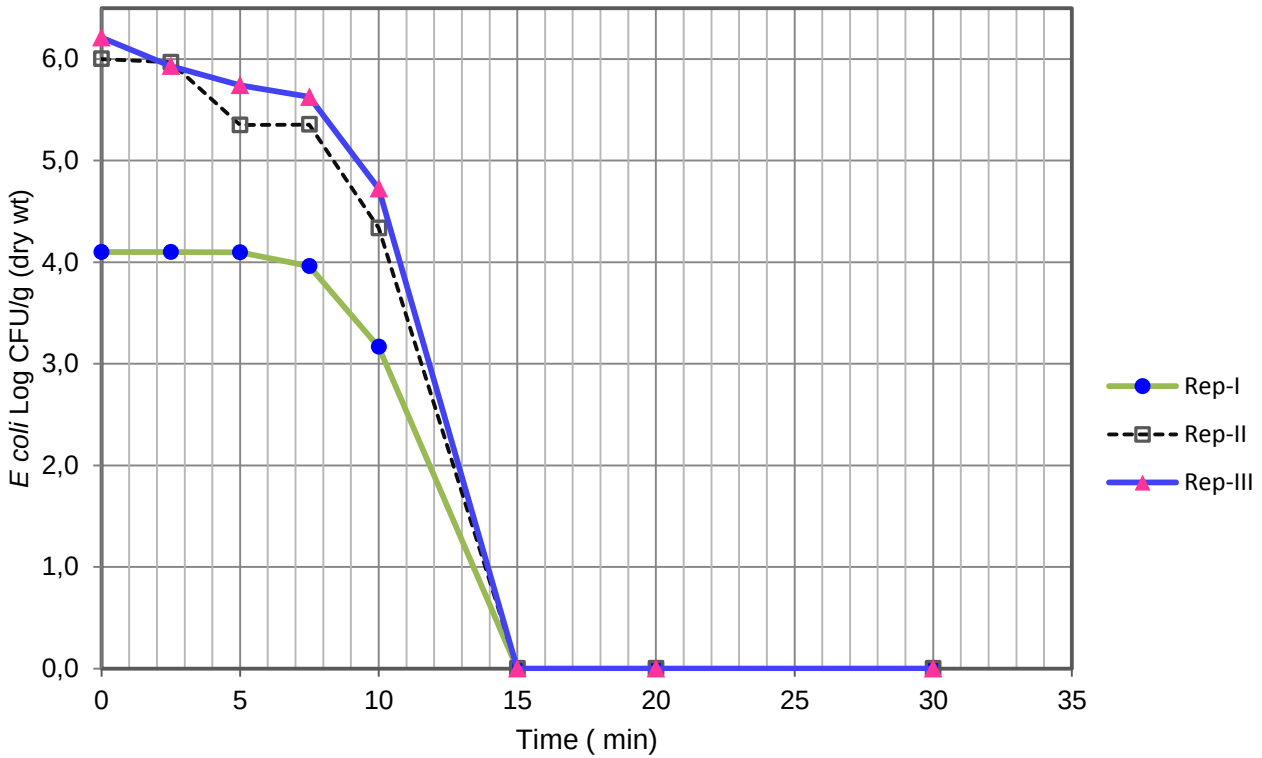
Results


50°C
E coli
Log CFU/g (dry wt)
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
0
50
100
150
200
250
300
350
Time (min)
Rep-I
Rep-II
Rep-III

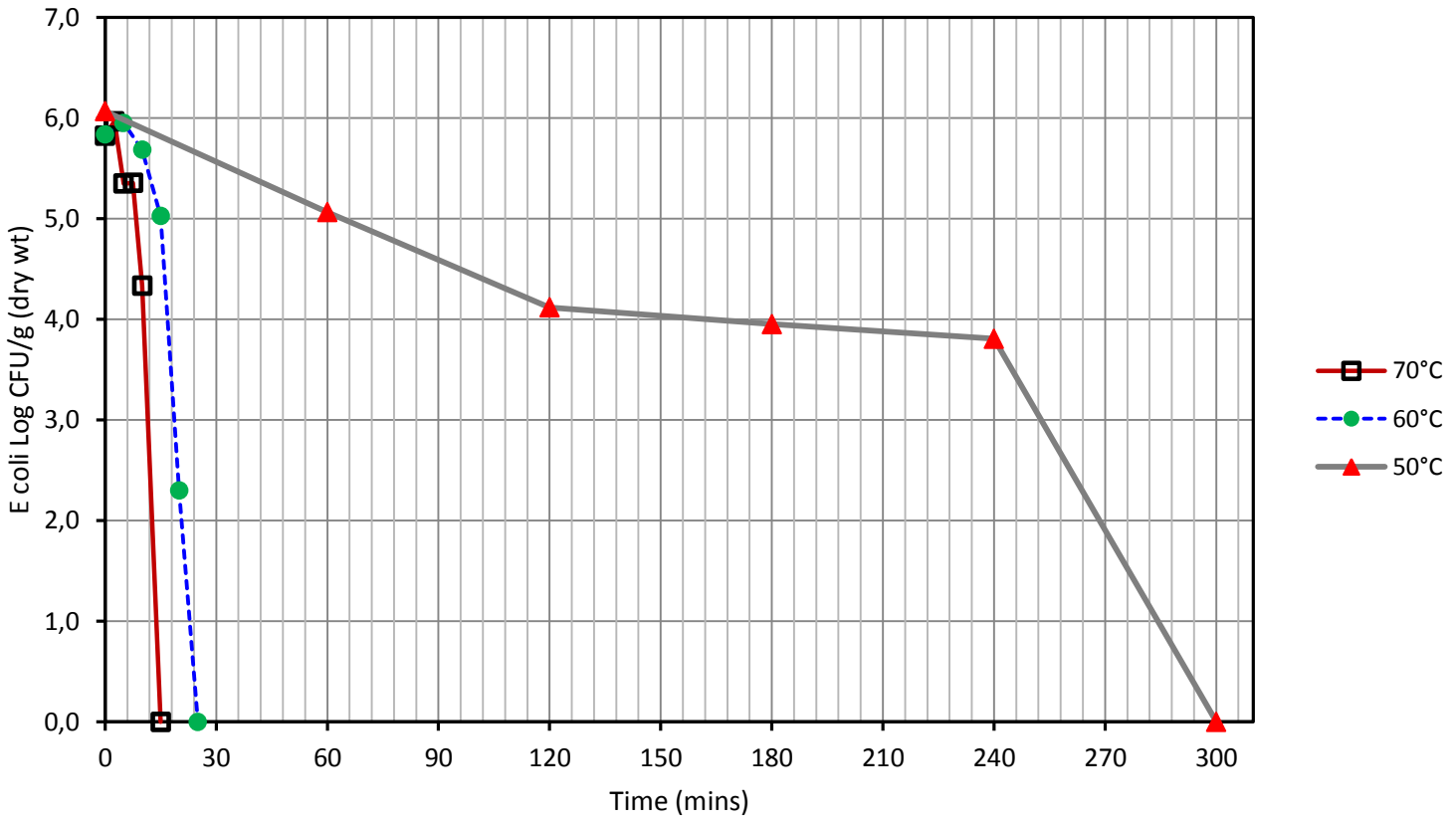

60°C
E coli
Log CFU/g (dry wt)
6,0
5,0
4,0
3,0
2,0
1,0
0,0
0
10
20
30
40
50
60
70
Time (min)
Rep-I
Rep-II
Rep-III

Results

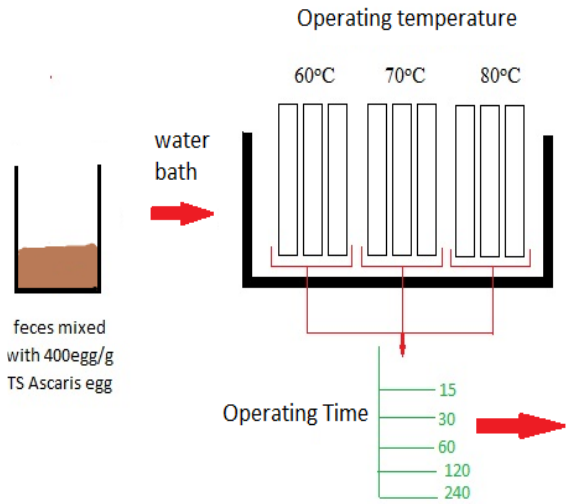
70°C


6,0
5,0
4,0
3,0
2,0
1,0
0,0
E coli Log CFU/g (dry wt)
0
5
10
15
20
25
30
35
Time (min)
Rep-I
Rep-II
Rep-III

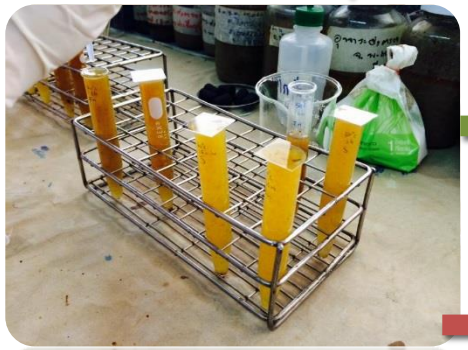
Results


7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
E coli Log CFU/g (dry wt)
0
30
60
90
120
150
180
210
240
270
300
Time (mins)
70°C
60°C
50°C

Method: Ascaris Egg Inactivation



Incubation 22-26°C for 28 days



Place 10 g of fecal sample and pour distilled water 30 mL in beaker, mix well and filtrate bydouble layer cheesecloth. Pour the suspension into centrifuge tube.

Centrifuge at 2000 rpm for 3 minutes.

Drain supernatant and fill ZnSO4 (sp.gr = 3.00*) to just over the top of the tube.

Cover the top of the tube with a coverslip and wait for 5-10 minutes.

Add 1-2 drops of iodine solution on a slide, move the coverslip from the tube and place on slide for microscopic examination.

Flotation Method ($ZnSO_4$)

- Eggs counted as viable- motile larva (active) and non-viable (inactive)
- Examined after 28 days

Microscopic examination

Method: Ascaris Egg Inactivation

Incubation 22-26°C for 28 days

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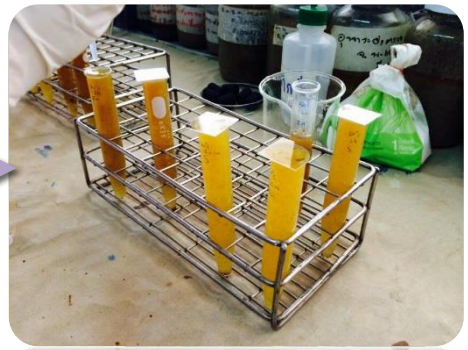

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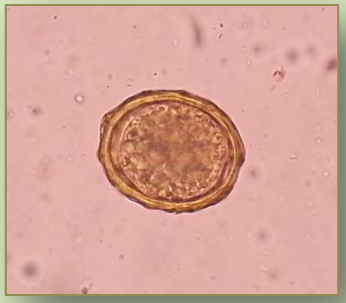
Microscopic examination

Results: Ascaris Egg Inactivation

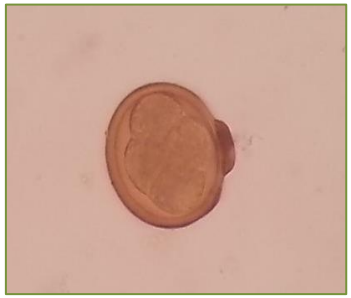


Control

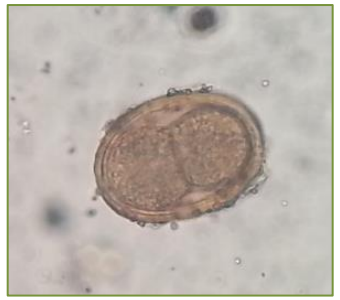
60°C
For 15 min

Infective larva

Infective larva



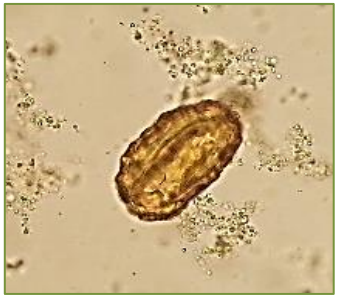
Cell division (four phase)

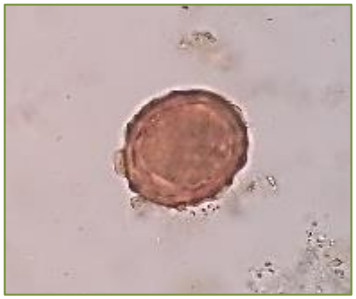


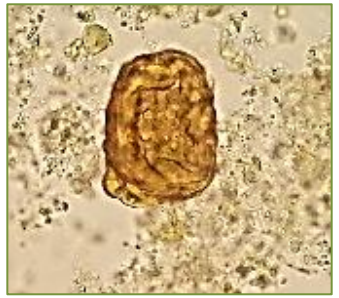
Cell division (two phase)

70°C
For 15 min

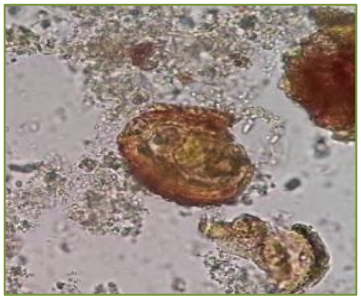
Inactive larva

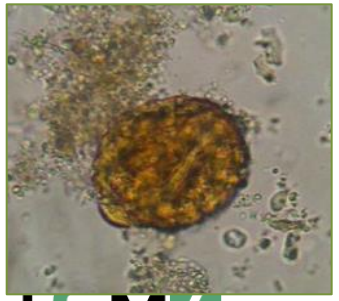




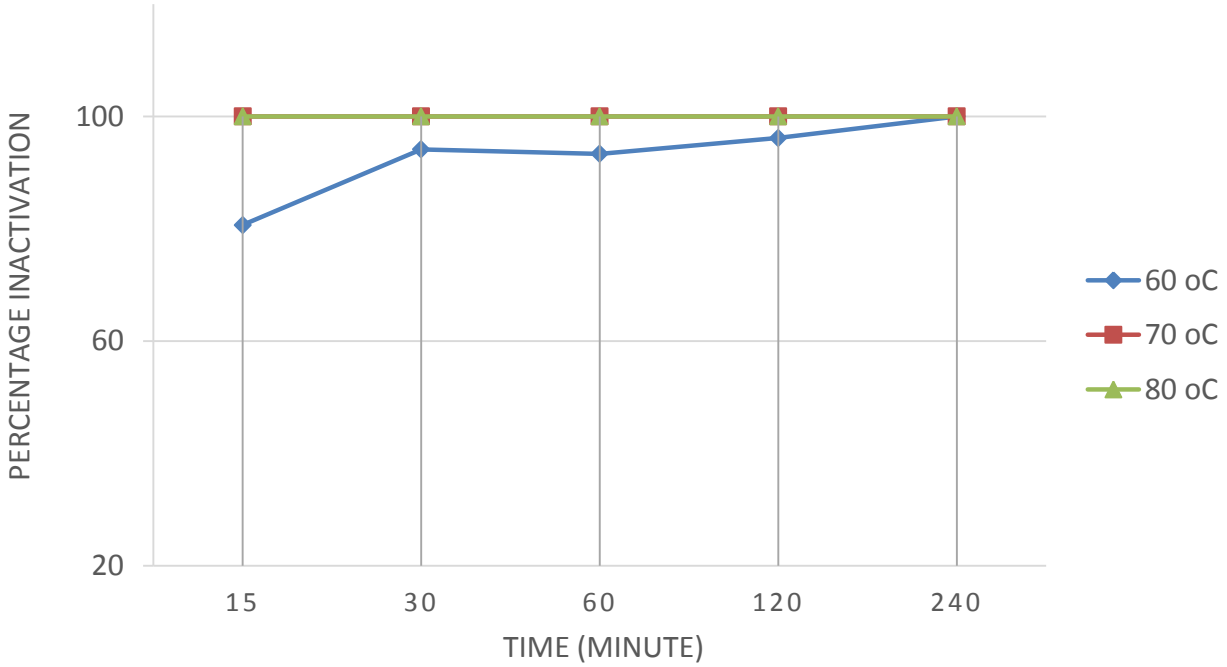


80°C
For 15 min





Results: Ascaris Egg Inactivation

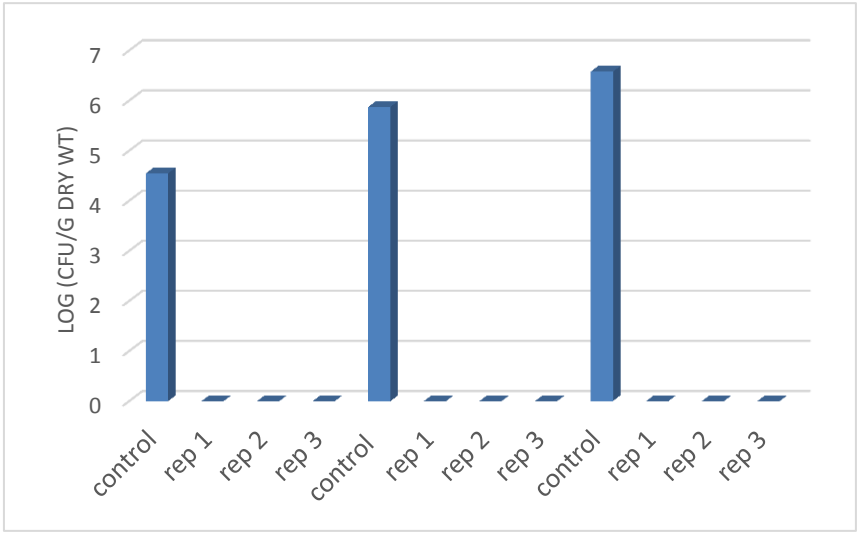


- 100% inactivation at 70°C and 80°C in 15 min
- 80% inactivation achieved under 60°C for 15 min, and requires 240 min for 100 % inactivation

Application in Actual condition

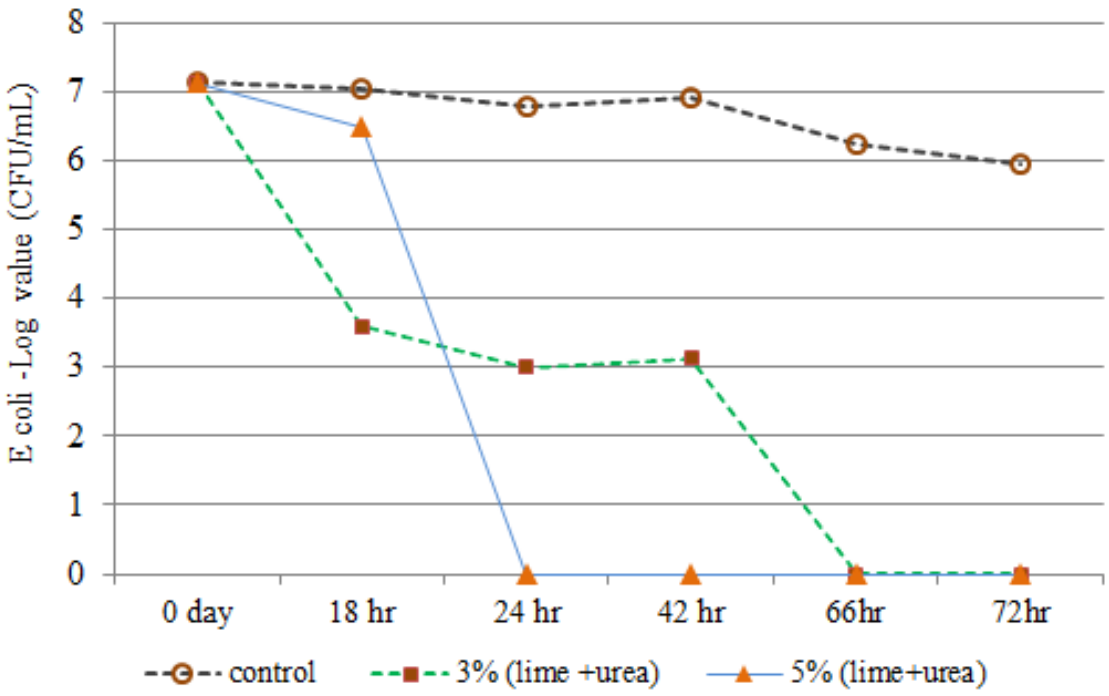
- *E coli*: 60°C for25 mins, 70°C for 15 mins

  Ascaris: 70°C for 15 mins
- Applied at 70°C for 15 mins



Solid Disinfection: Lime & Urea

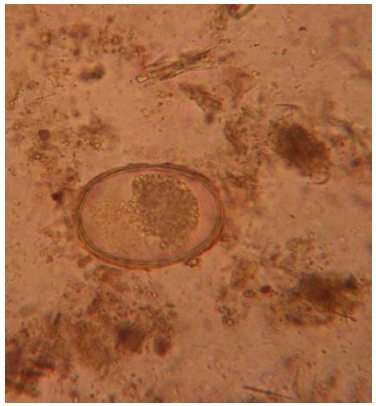
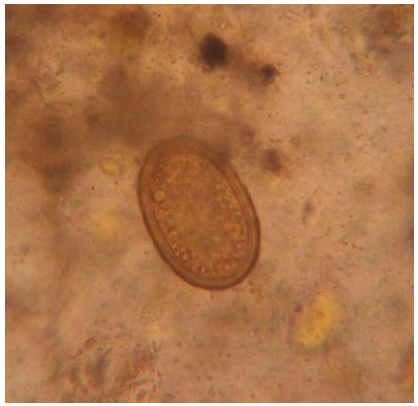
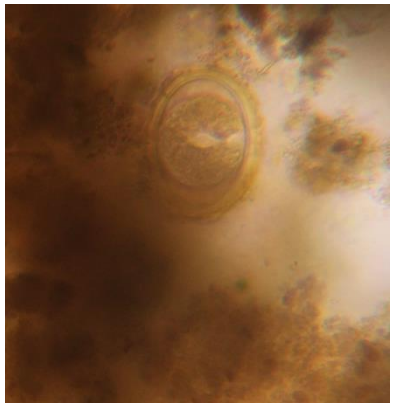
Chemicals: Lime & Urea application



Chemicals: Lime & Urea application

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

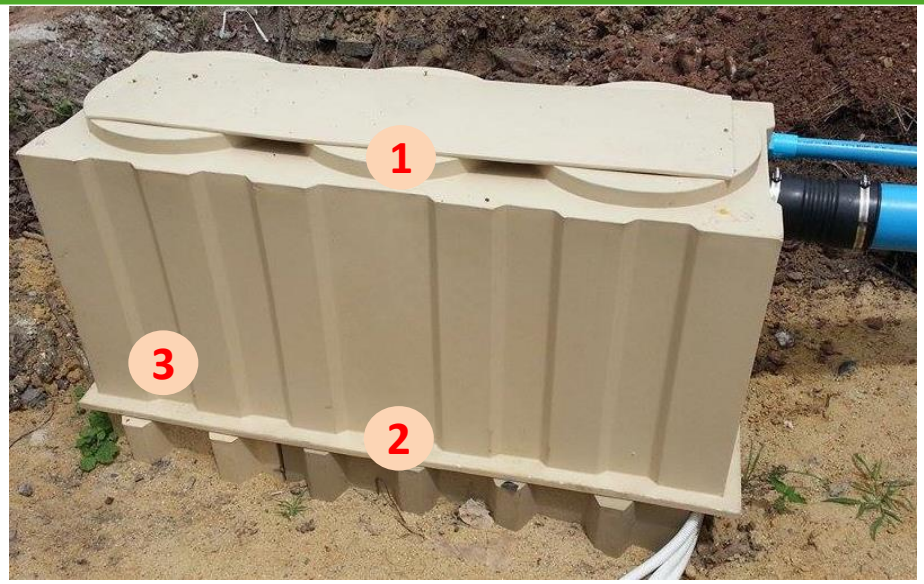
Take Away

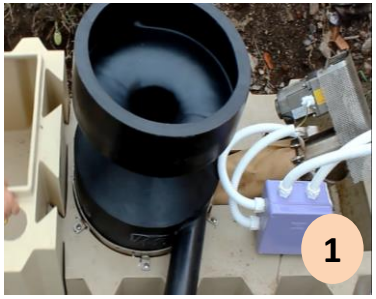
- Heating is effective for treating the freshly separated fecal matter
- Initial load of *E. coli* cause variation in time required for complete inactivation
- 60°C for 25 min was cost-effective, but 70°C for 15 min is ideal for complete disinfection (including *Ascaris*)
- Lime and urea @ 3% and 5%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

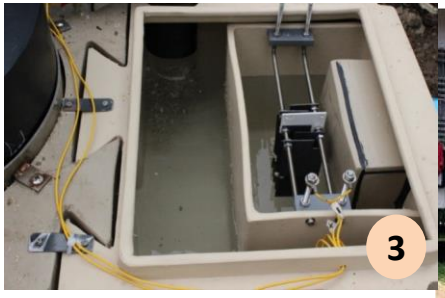
Thank you very much for your attention

Pilot scale Testing

Toilet Components







- Solid-liquid separator, and disinfection systems
- Cyclone based on centrifugal and gravity
- Heating and Electrochemical

Result

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:

$$P = Q.\Delta t / 860 \text{ Kcal.time.efficiency}$$

P= 2kg x (70-20)°C/860 x 0.25 hr x 0.9 = 100/193.5=0.5KW
1 Kw price in Thailand 3 bhat (9cent), India 4.9 IRS (8 cent)

Energy calculation

Amt to be treated	Initial temp	desired temp	time	Power		Price
Kg	° C	° C	hr	(KW)	3 TBH/kW	9 cent/kW
2	20	70	0.25	0.5168	1.55	4.65
2	20	60	0.33	0.3132	0.94	2.82
2	20	50	4	0.0194	0.06	0.17

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: $\text{CFU/g total solid} = \frac{\text{CFU/ml}}{\%\ \text{Total solid}}$

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.

Time (min)	wet moisture (%) at 50°C		Time (min)	wet moisture (%) at 60°C		Time (min)	wet moisture (%) at 70°C	
	2.6 cm dia.	5.2 cm dia.		2.6 cm dia.	5.2 cm dia.		2.6 cm dia.	5.2 cm dia.
0	86	86	0	85	85	0	84	84
30	86	84	10	85	85	5	85	84
60	85	85	20	85	85	7.5	84	83
120	85	85	30	85	85	10	85	84
180	85	84	60	85	86	15	84	81
240	86	85	90	86	85	20	84	81
300	85	85	180	85	85	30	85	83

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^{o}C with 15 minute.

Figures d-i illustrates inactive Ascaris ova at high temperature 70^{o}C and 80^{o}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 $ZnSO_4$ Solution (sp.gr = 1.30*), the solution (1.30 sp.gr.) was made by adding 500 grams of $ZnSO_4$ to 880 liter of deionized water. The mixture was checked with a hydrometer and adjusted to 1.30. The $ZnSO_4$ 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^{o}C.

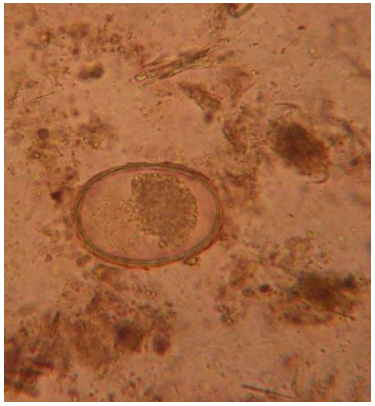
Ascaris inactivation (**@ lime 5% and 7 %)**

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)

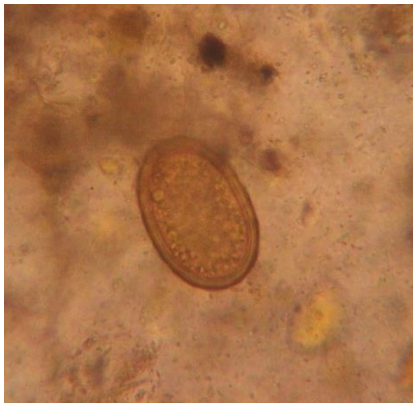
Ascaris eggs found in the bio-bag a) Control Ascaris egg with Larva formation b) Fertilized egg in bag with 5% dose c) Decorticated egg found in bag with 7% dose

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

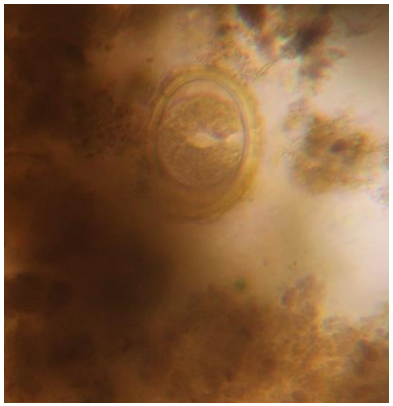
All the Ascaris where the larva is observed are counted as viable or the fertilized eggs as in Figure 7 a while the one with no larva development are counted as unfertilized eggs.



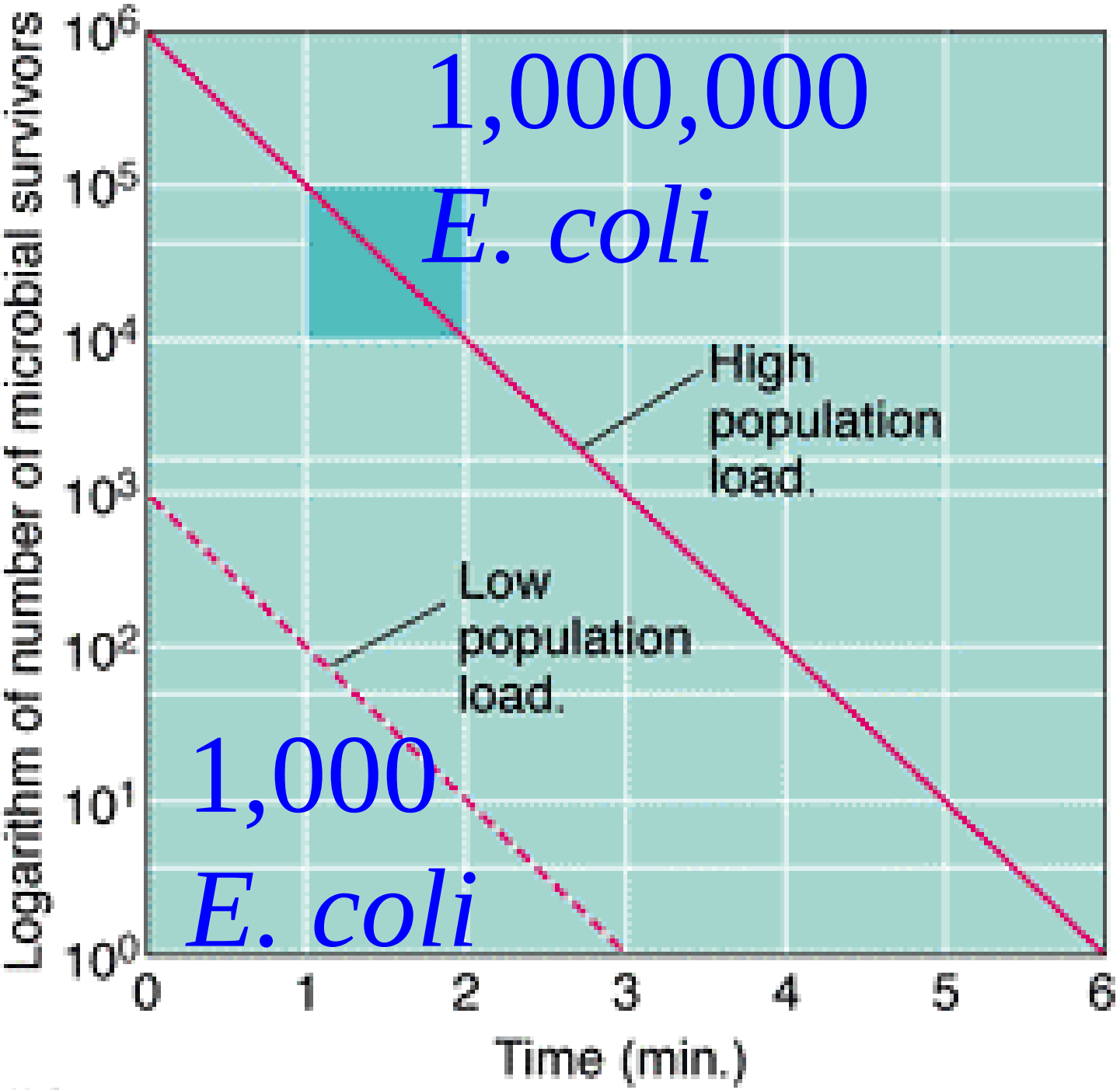
a)



b)



c)


1,000,000
E. coli
High population load.
Low population load.
1,000
E. coli
Logarithm of number of microbial survivors
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
1
2
3
4
5
6
Time (min.)

Background: Zyclone cube toilet

- 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

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