Control of Enteric Bacteria in Source-Separated Human Urine

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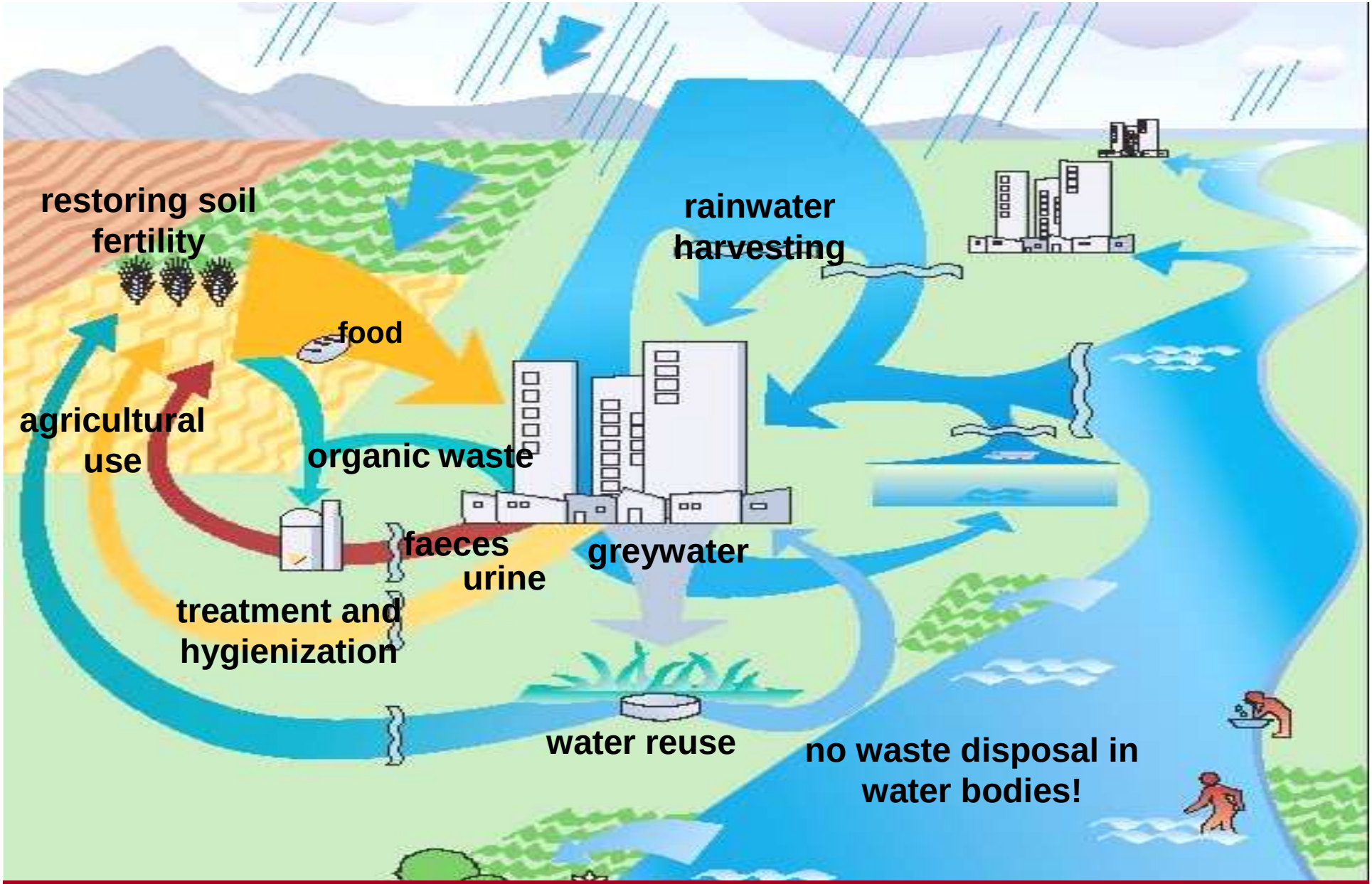
Ecological Sanitation



Source: Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH

Ecosan in the Philippines

La Union

Pangasinan

Negros Oriental

Sorsogon

Cebu

Bohol

Cagayan de Oro

Agusan del Sur

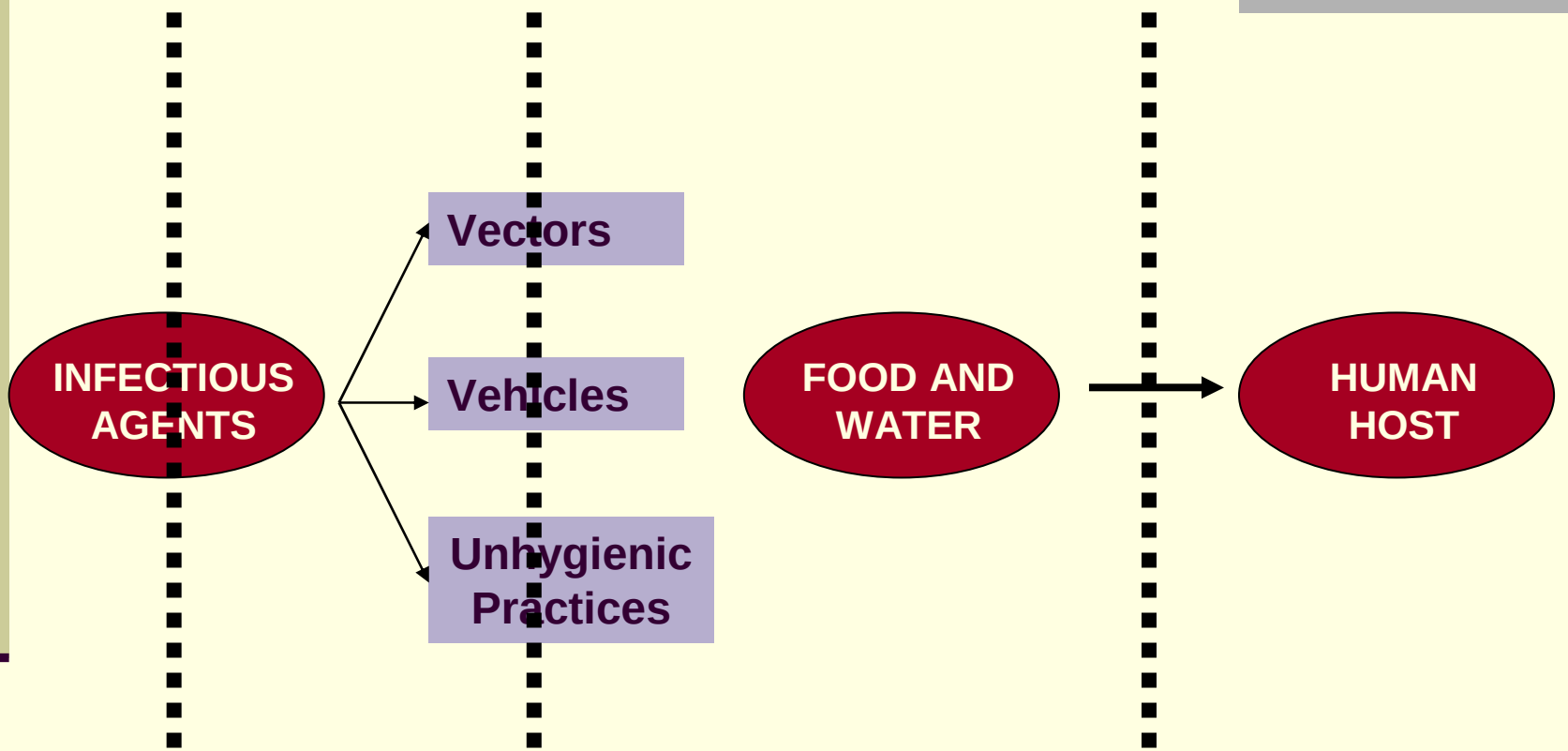
Source: Center for Advanced Philippine Studies

Problems encountered

- Low acceptance of the community on ecosan
  - Lack of political will
  - Lack of supportive environment

Pathogens can easily be transmitted to humans through vectors and vehicles

Public Health Risk



Infectious Agents in Urine

- *Escherichia coli*
- *Proteus mirabilis*
- *Enterococcus spp*.
- *Pseudomonas aeruginosa*
- *Klebsiella spp.*

Tabo, et al. 2006. *Occurrence and Survival of Enteric Bacteria in Source-separated Human Urine*

Ten Leading Causes of Morbidity in the Philippines

Disease	Number of Cases	Rate
		per 100,000 population
1. Pneumonia	670,231	828.8
2. Acute Watery Diarrhea	572,259	707.7
3. Bronchitis/Bronchiolitis	538,990	689.9
4. Hypertension	408,460	522.8
5. Influenza	339,881	435
6. TB Respiratory	132,725	169.9
7. Diseases of the Heart	384,82	49.3
8. Acute Febrile Illness	25,400	32.5
9. Malaria	22,284	27.6
10. Dengue fever	15,279	19.6

Department of Health, FHSIS Report. 2006

Treatment Methods

- Storage
  - Simplest method in controlling pathogens
- Increased temperature and pH
- Use of chemicals
- Biocontrols (on trials)
  - Use of *Bacillus spp*.
  - Use of *Bdellovibrio spp*.

General Objective

- Evaluate the antagonistic effect of *Bacillus subtilis* against the isolated enteric bacteria in source-separated human urine.

Specific Objectives

1. Isolate *B. subtilis* in urine-treated soil for biocontrol studies.
2. Determine the inhibitory effect of chemical (guava extract, cell-free extract, EM extract and urea) and biological (*B. subtilis*) control agents on enteric bacteria.
3. Identify the most effective treatment in the control of enteric bacteria.

METHODOLOGY

1. Research Procedures
   - Isolation of *Bacillus subtilis*
   - Antagonism Assay
   - Mixed-culture Experiment
     - Nutrient Broth
     - Urine
2. Research Statistics
   - Simple Linear Correlation
   - T-dependent Test
   - Analysis of Variance (ANOVA)
   - Post-Analysis of Variance (post ANOVA)

COLLECTION OF SAMPLES

URINE

Screened (Multistix™ Urinalysis Strips)

Placed in a 2.7-L amber bottle

Boil for 15 mins

Transferred to 300-mL bottle with 100 mL each.

Set aside

Screened Urine

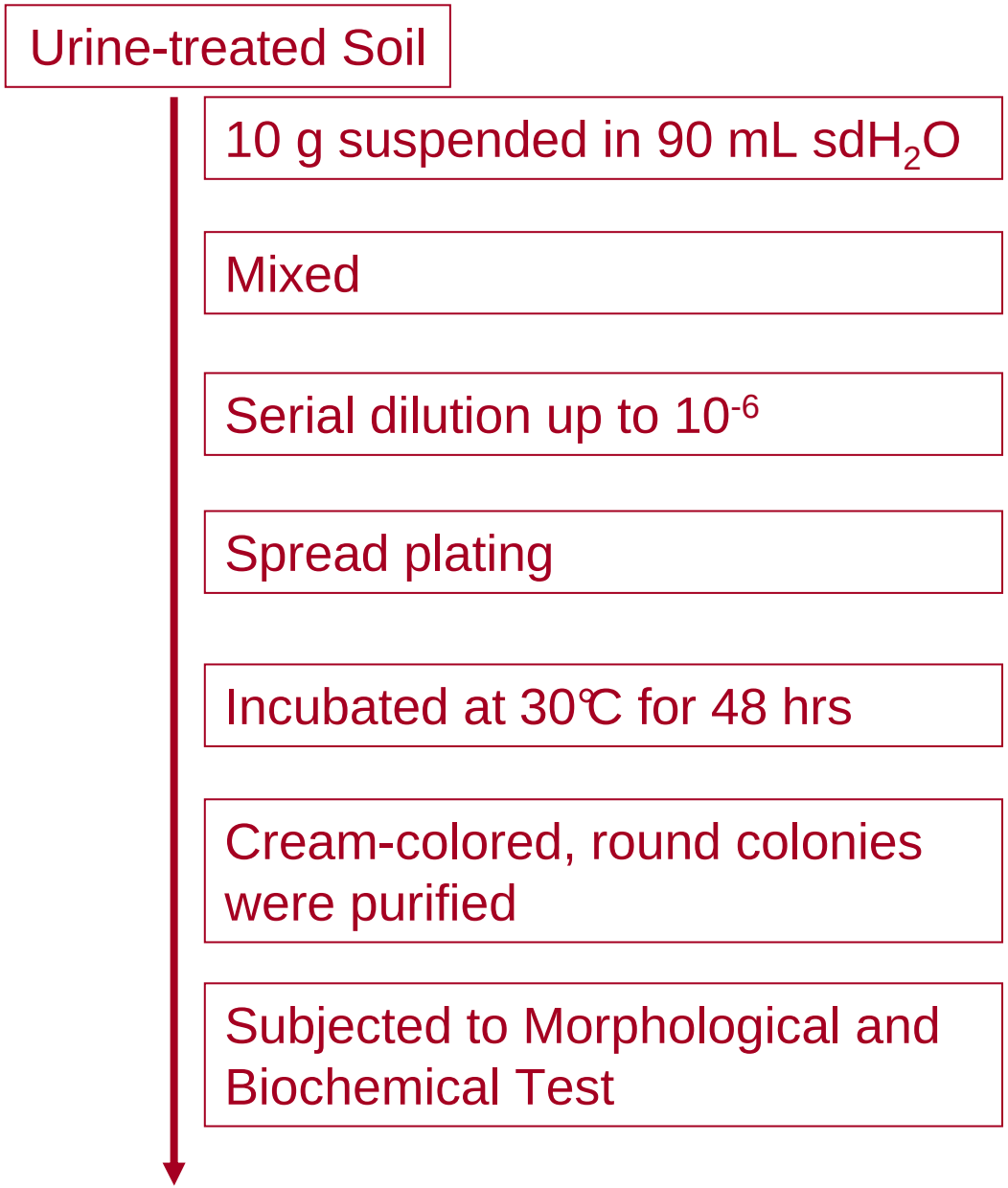
SOIL

Mixed with urine in a 1:10 vol/wt ratio

Incubated in an open area for 5 days

Urine-treated Soil

ISOLATION OF *Bacillus subtilis*

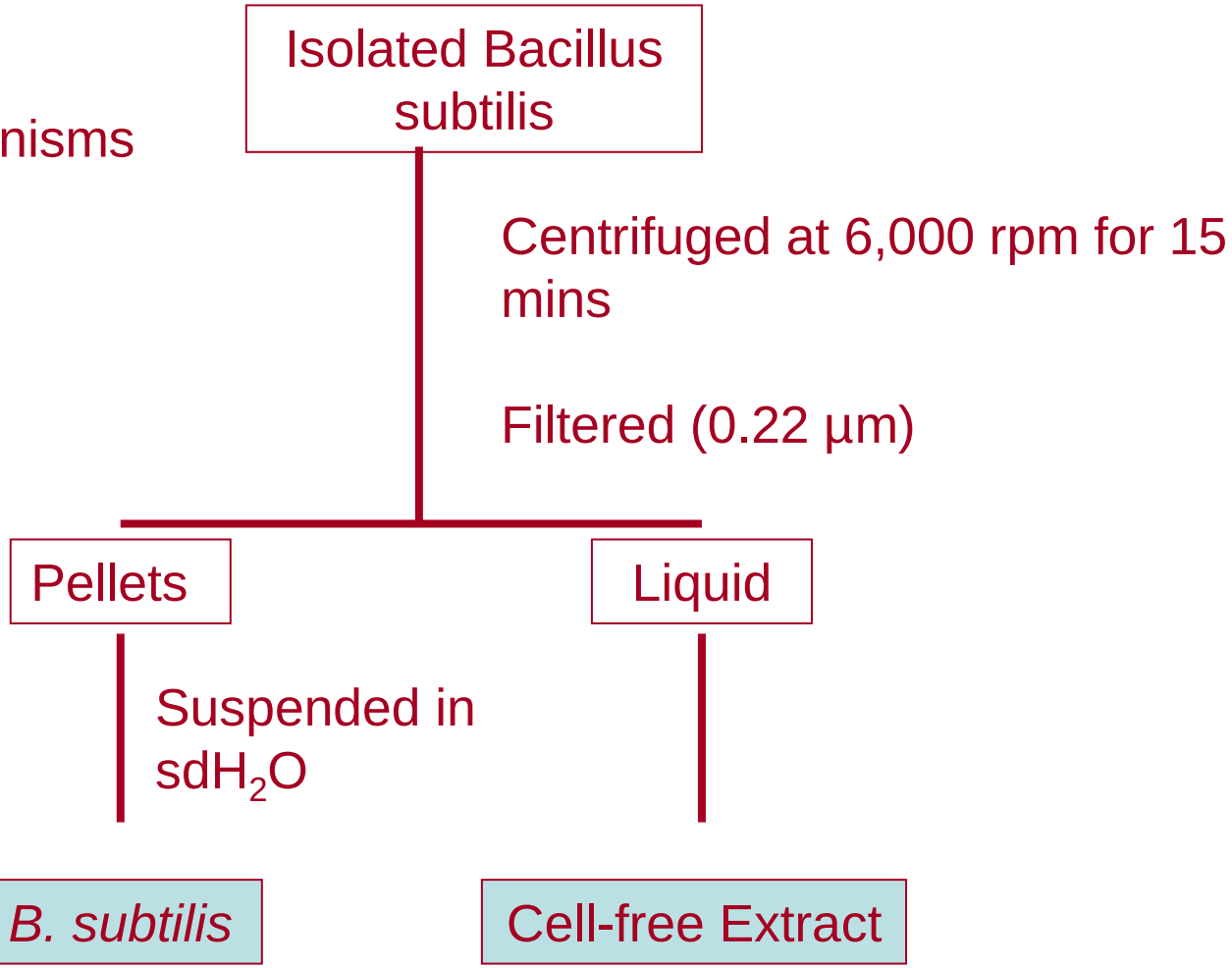


PREPARATION OF CONTROL AGENTS

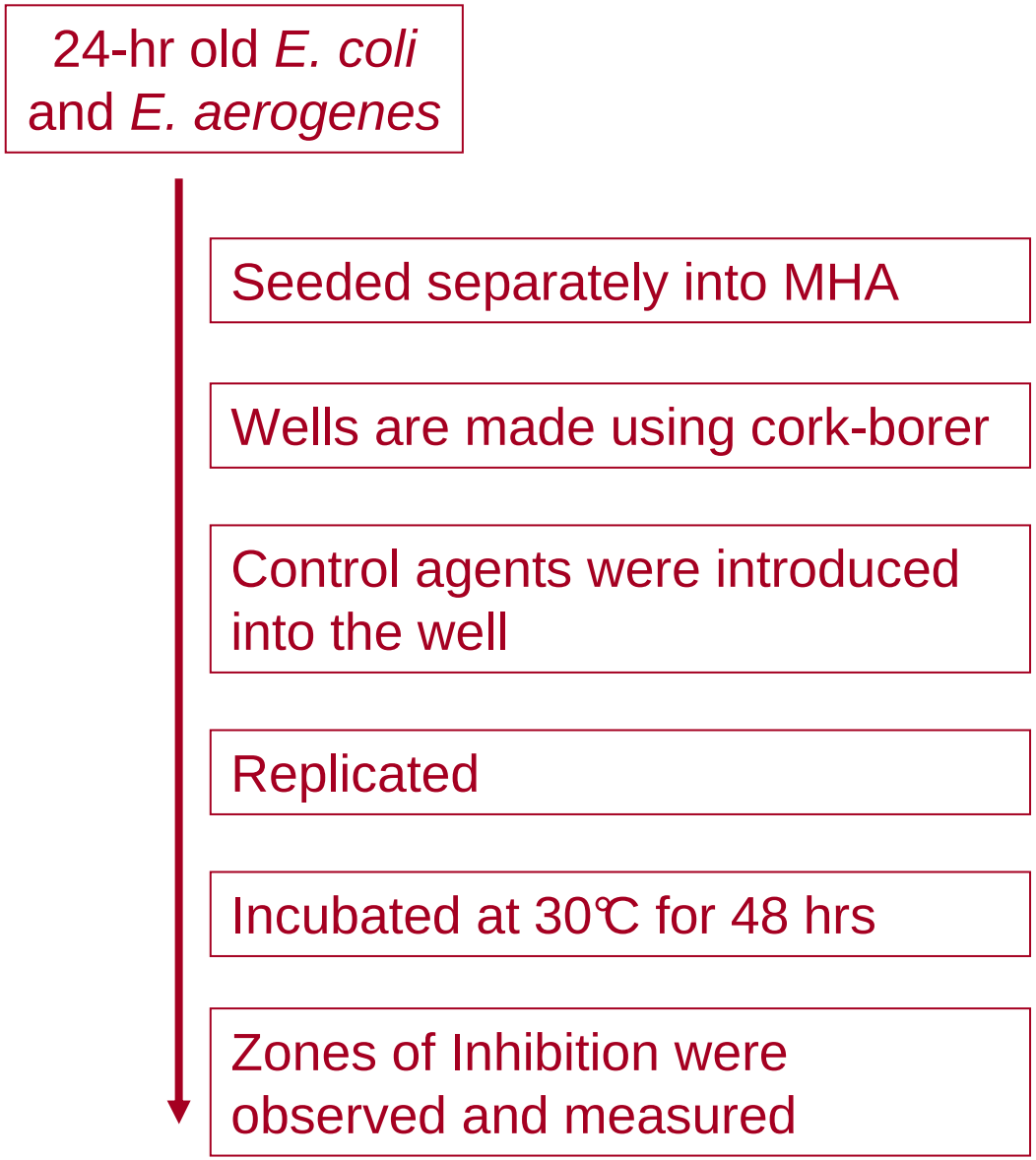
1. Guava Extract

   5% wt/vol
2. Cell-free Extract
3. Effective Microorganisms
4. Urea

   5% wt/vol
5. Bacillus subtilis



ANTAGONISM ASSAY

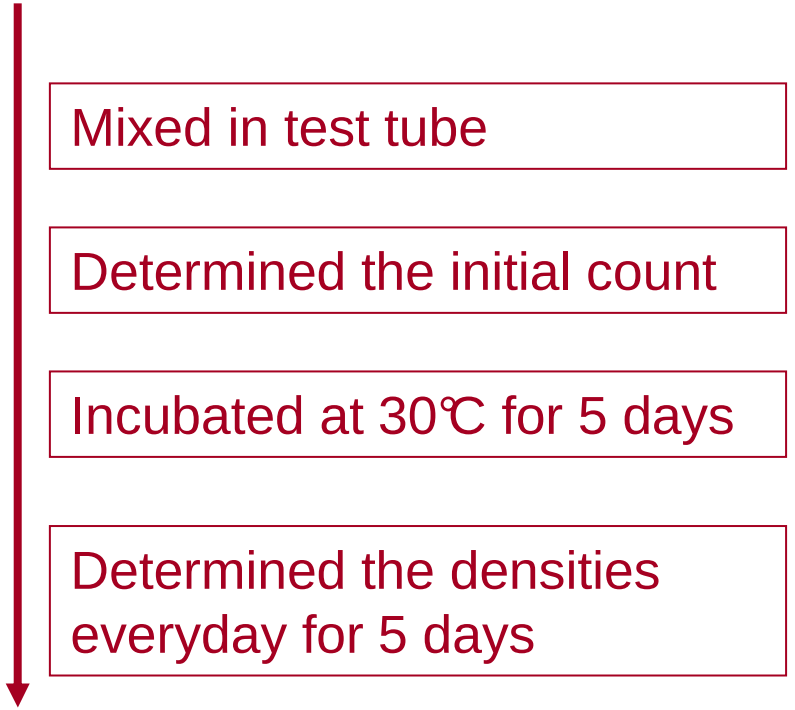


MIXED-CULTURE EXPERIMENT

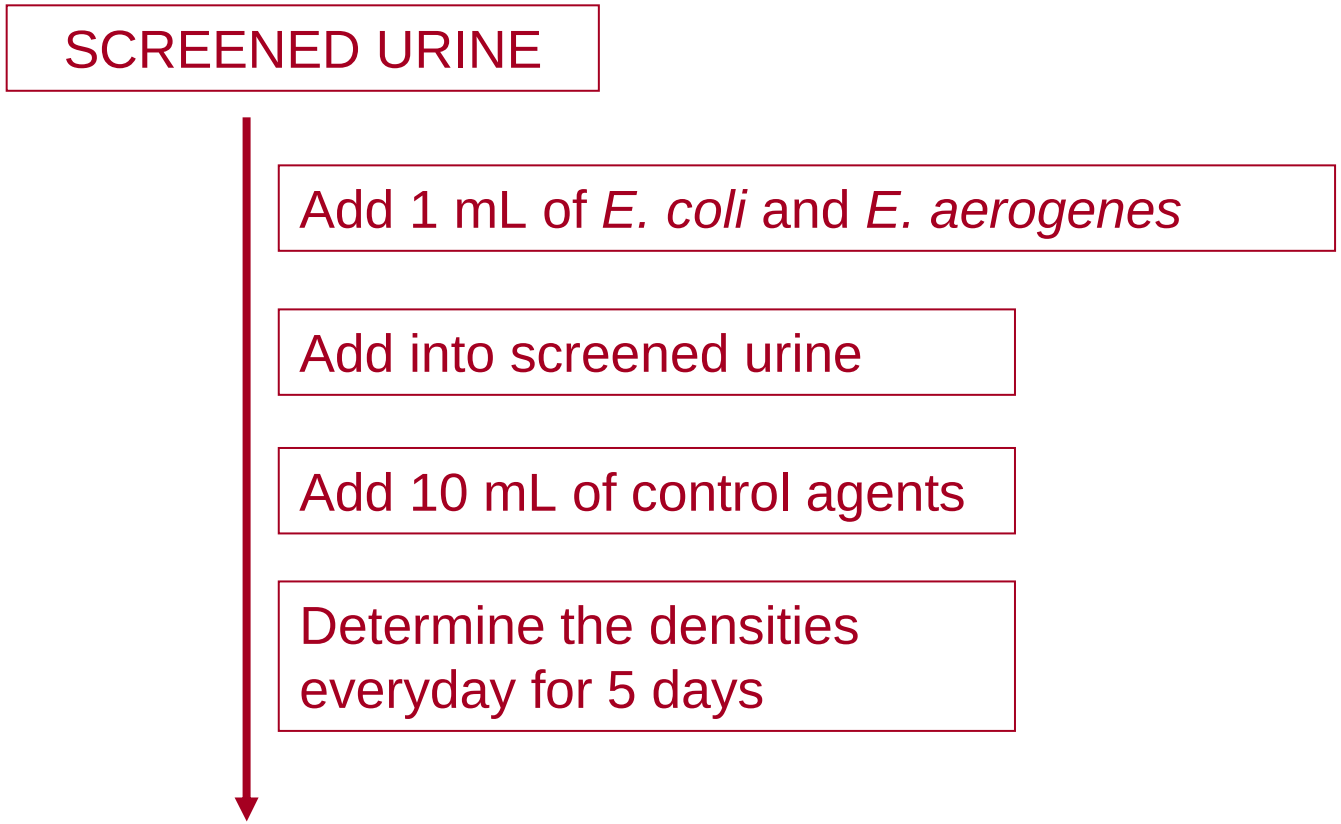
24-hr old, 0.5 Mc Farland
4 mL B. subtilis

24-hr old, 0.5 Mc Farland
2 mL E. coli

24-hr old, 0.5 Mc Farland
2 mL E. aerogenes



MIXED-CULTURE EXPERIMENT (URINE)



RESULTS

Table 1. Colonial and Morphological Characteristics of the Isolated Bacterium from Urine-treated Soil Cultured in Nutrient Agar.

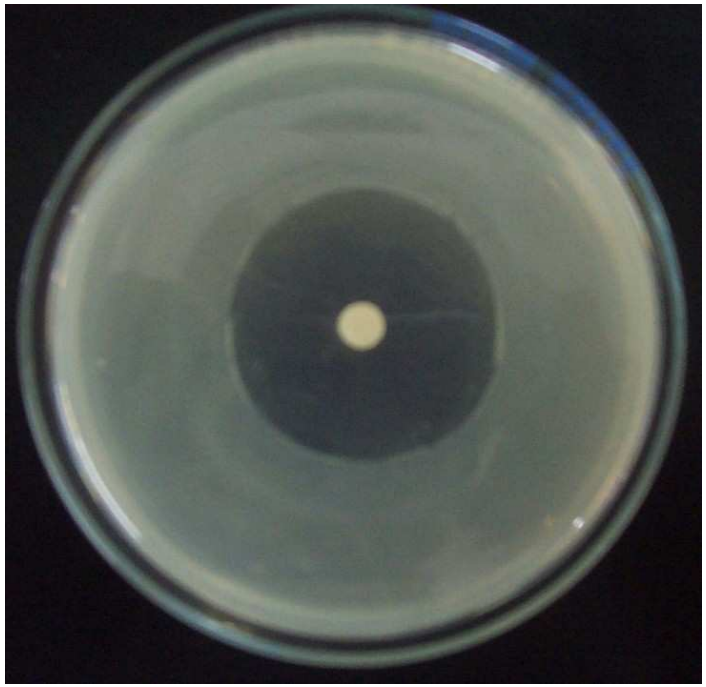
Characteristics	Results
Colonial Characteristics	
Size (colony)	5 mm
Margin	round to irregular
Elevation	Flat
Surface	dull and dry
Pigmentation	creamy-white
Morphological Characteristics	
Size (cell)	0.5 to 3.0 µm
Shape	rods
Arrangement	solitary
Oxygen Requirement	Aerobic
Endospore	Present
Gram Reaction	Positive

Table 2. Selected Biochemical Characteristics of the Isolated *Bacillus*.

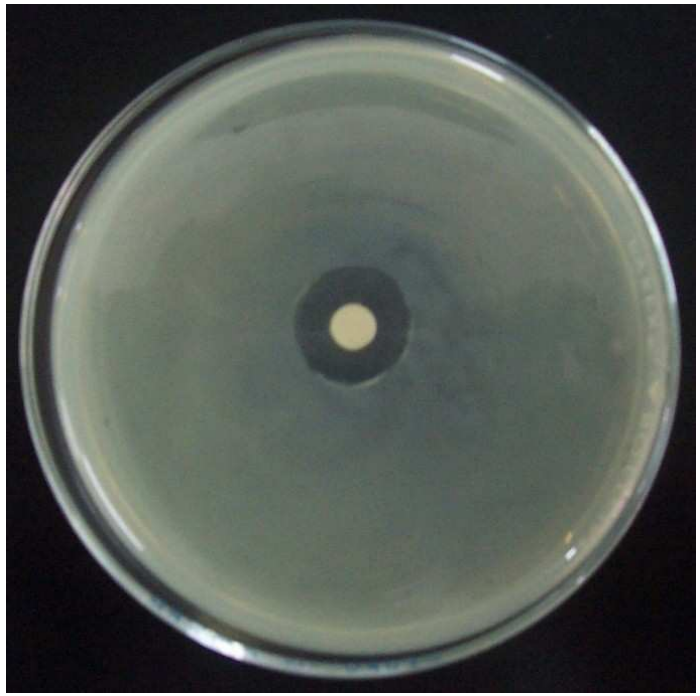
Biochemical Characteristics	Results
Catalase reaction	+
Acid from Glucose	+
Gas from Glucose	-
Acetoin Production	+
Growth in 7.0 % NaCl	+
Growth in Egg Yolk	-

Table 3. Antagonism Assay of the Control Agents on *E. coli* and *E. aerogenes*

Treatment	Zone of Inhibition (in mm)	Growth Inhibition
Treatment vs *E. coli* Water	7	-
T1 (Guava extract)	7	-
T2 (Cell-free extract)	15	+
T3 (EM)	12	+
T4 (Urea)	14	+
T5 (*Bacillus subtilis*)	40	+
Treatment vs *E. aerogenes* Water	7	-
T1 (Guava extract)	7	-
T2 (Cell-free extract)	9	+
T3 (EM)	8	+
T4 (Urea)	8	+
T5 (*Bacillus subtilis*)	14	+



BS suspension on EC



Cell-free extract on EC

Figure 1. Antagonism Assay of Bacillus subtilis and its extract on *E. coli* and *E. aerogenes*

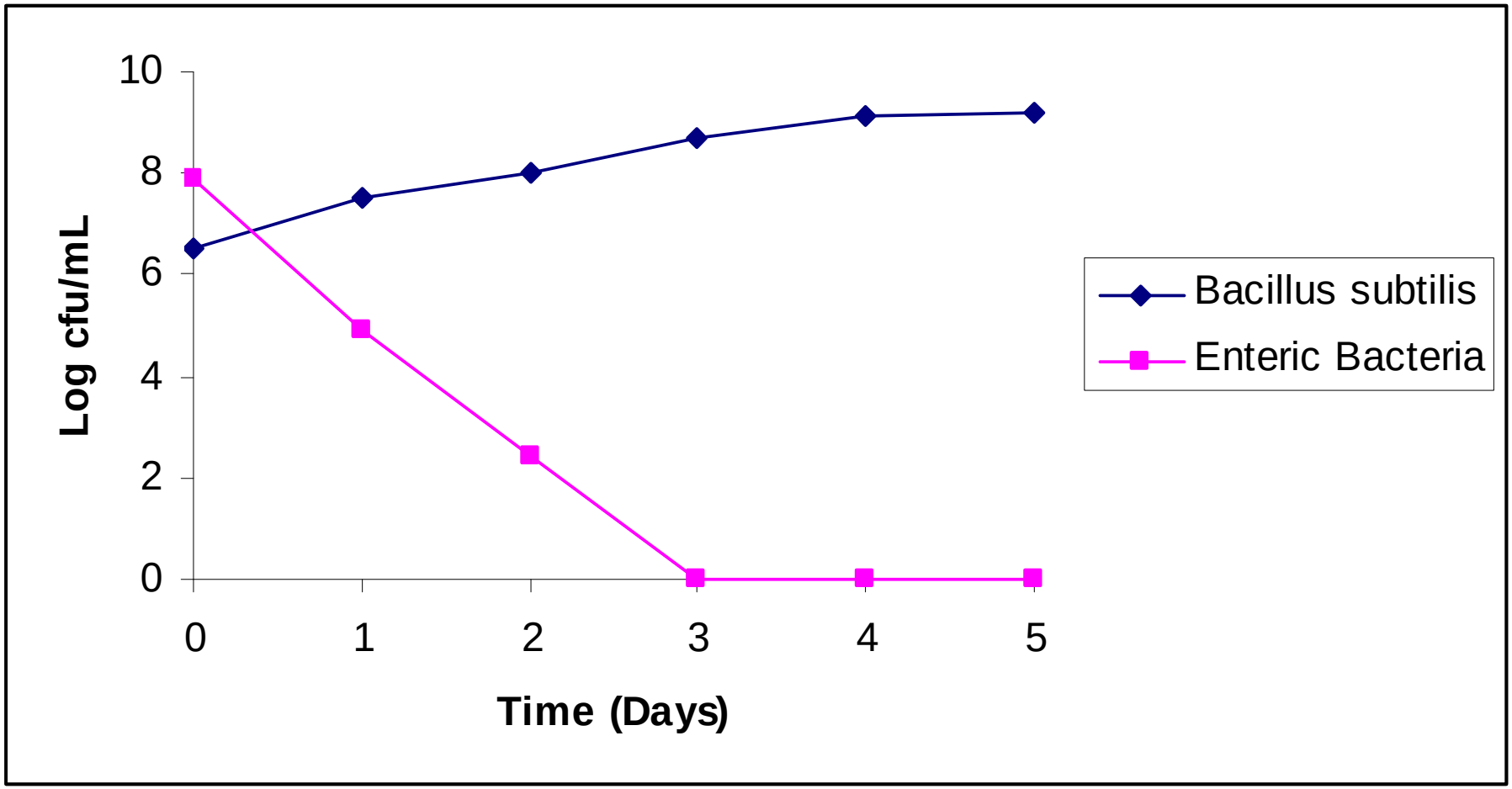


Figure 2. Mixed culture experiment in Nutrient Broth

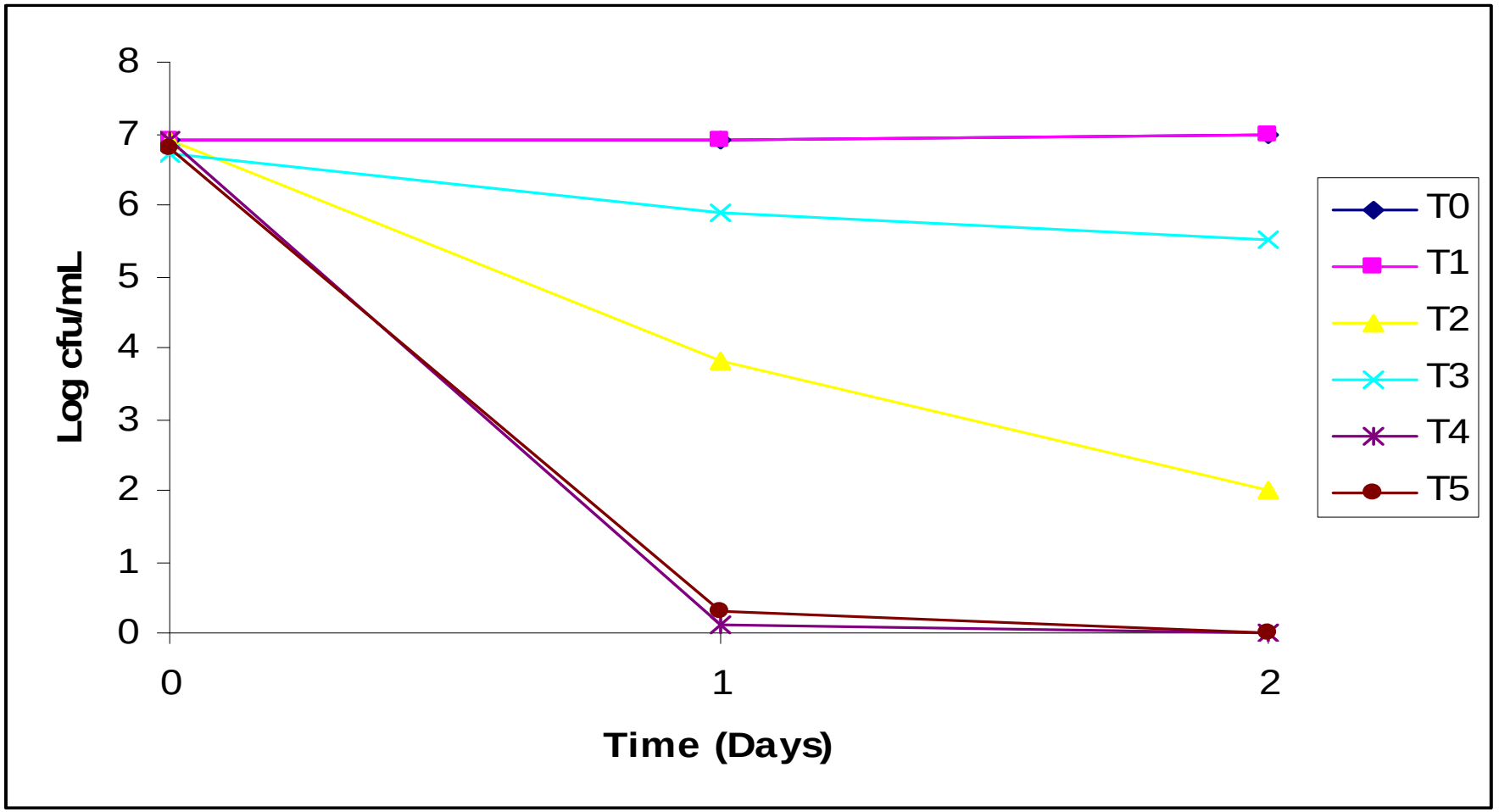


Figure 3. Survival of enteric bacteria in source-separated urine with control agents

Table 4. Identification of the most effective treatment in the control of enteric bacteria

Treatment	Initial Count	Final Count	Mean Difference (Initial vs Final)	Mean Difference (Treatments vs Control)
T0	8.7×10^6	8.5×10^6	2.0×10^5 ns	---
T1	6.7×10^6	6.7×10^6	0 ns	-2.0×10^5 ns
T2	7.7×10^6	5.7×10^3	7.7×10^6 **	7.5×10^{6}**
T3	5.5×10^6	7.8×10^5	4.7×10^6 **	4.6×10^{6}**
T4	7.7×10^6	1.2×10^0	7.7×10^6 **	7.5×10^{6}**
T5	6.5×10^6	2.0×10^0	6.5×10^6 **	6.3×10^{6}**

ns not significant
**** significant at 0.1%**

D value of the most effective treatment in the control of enteric bacteria

- T2 inhibited the growth of the test organisms very slowly with a D value of 15.53 hrs.
- T4 and T5 totally inhibited the growth of enterics with a D value of 7.05 hrs

CONCLUSION

1. *Bacillus subtilis* was not inhibited by enteric bacteria.
2. *B. subtilis* had the greatest inhibitory effect on enterics, as compared to chemical control agents such as guava extract, cell-free extract and EM extract. It is comparable to urea in terms of inhibitory effect and killing ability.
3. *B. subtilis* is a good alternative agent in the control of the growth of enteric bacteria in source-separated human urine.

RECOMMENDATION

1. Examine the growth of plants watered with *B. subtilis*-treated urine.
2. Test the synergistic effect of *Bacillus* with other non-pathogenic soil bacteria.
3. Determine the growth and survival of ammonia-reducing bacteria in urine with enteric bacteria.

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ecosan

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THANK YOU VERY MUCH !!!