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Inactivation mechanisms of pathogenic bacteria in several matrixes during composting process in composting toilet

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Introduction

- In the world, 2.6 billion people are estimated to defecate in an open or in an unsanitary places (UNICEF, 2006)

- According to the MDGs (UN, 2008),
'*Reduce by half, in 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation.*'

- Composting toilet : inexpensive, sustainable method of treating human waste (feces)

- It is a naturally occurring aerobic process, whereby native microorganisms convert biodegradable organic matter into a humus like product

Introduction

- Composting toilet key technology of the OWDTS :
  - Preserve of water resource (dry toilet) ≠ flush toilet?
  - Organic fertilizer rich in (N, P, K ...)
  - Soil conditioner

- Using matrix for composting has the potential to trap pathogens (Zavala et al., 2004)

- And sawdust has been normally used, which plays a role of giving gas phase for aerial fecal decomposition with little odor (Otaki et al., 2006)

- To adapt the toilet in all over the world, alternative matrix is necessary because of limitation of sawdust availability

Introduction

- chopped corn stalk, rice husk and charcoal, which were regarded as a model of the alternative matrix, showed well fecal decomposition rate in the composting toilet (Hijikata et al, 2011)
- However, hygienic aspect of these alternative matrixes has not been observed, so far.
- TWe hypothesized that inactivation and its mechanism of pathogenic bacteria would differ with the type of matrix and the composting process
- Is the inactivation rate different:

  - several matrixes?

  - during the composting process?

  - which damage happened?

Introduction

- Main goal: To compare the inactivation rate and the mechanisms of pathogenic bacteria in different matrixes during composting process

- Specifically : To appreciate,

  - The inactivation rate of pathogens in different matrixes: sawdust, rice husk (chaff), charcoal

  - The parts and/or functions which were damage during composting process

Material and methods

- **Composting sampling**

(S)

Feces: pig feces

Pure Matrixes:

- (S) Sawdust
- (R) Rice husk (chaff of rice)
- (C) Charcoal (from rice husk)

(R)

(C)

Compost product in a bioreactor:

- 1 month compost (3)
- 2 month compost (3)

Material and methods

- ***Compost sample preparation and bacteria measurement***

0.3 mL of *E. coli* (about 10^8 CFU/mL)

↓

3g of feces + 50g of matrix or compost

(sterilize before)

↓

Incubation (37° C after adjusted MC at 50%)

↓

Measurement (0, 2, 4, 6, 8 hours) double agar layer method

Material and methods

- ***Bacteria culture preparation***

  A model microorganism of pathogenic bacteria: *Escherichia coli* 3301 was purchased from NBRC (National Institute of technology and Evaluation Biological resource center, Japan).

  E.coli culture was done in Tryptic Soy Broth (Difco) and incubated in a shaking water bath at 37° C overnight.

- ***Bacteria extraction from composting matrixes***

  - A 3% (w/v) beef for extract solution (otaki et al., 2002).

  - A weight (3g) sample in 20mL of extract solution

  - Phosphate buffer was used for serial dilution

Material and methods

- ***Estimation of the damage to E. coli***

  - According to (Kubo et al., 2005, 2006; Wang and otaki, 2007; Mizozoe et al., 2010).

Media	Mechanism can be detect	Damages assumed
TSA	Metabolism of proteins	Nucleic acid and/or Metabolism
DESO	Metabolism of lactose	Nucleic acid and/or Metabolism and/or Membrane
C-EC	metabolism of peptone, pyruvic acid and lactose	Nucleic acid and/or Metabolism and/or Enzyme activity

Material and methods

Data treatment

- Inactivation rate : $\ln (N/N_0) = -kt$
- inactivation rate constant : $k = -\ln (N/N_0) / t$

according to (Nakagawa et al., 2006; Otaki et al., 2007)

Statical analysis

- Analysis of variance (ANOVA) test
- Correlation test were applied

StatView software version 5.0

1. Inactivation rate of E. coli in three matrixes

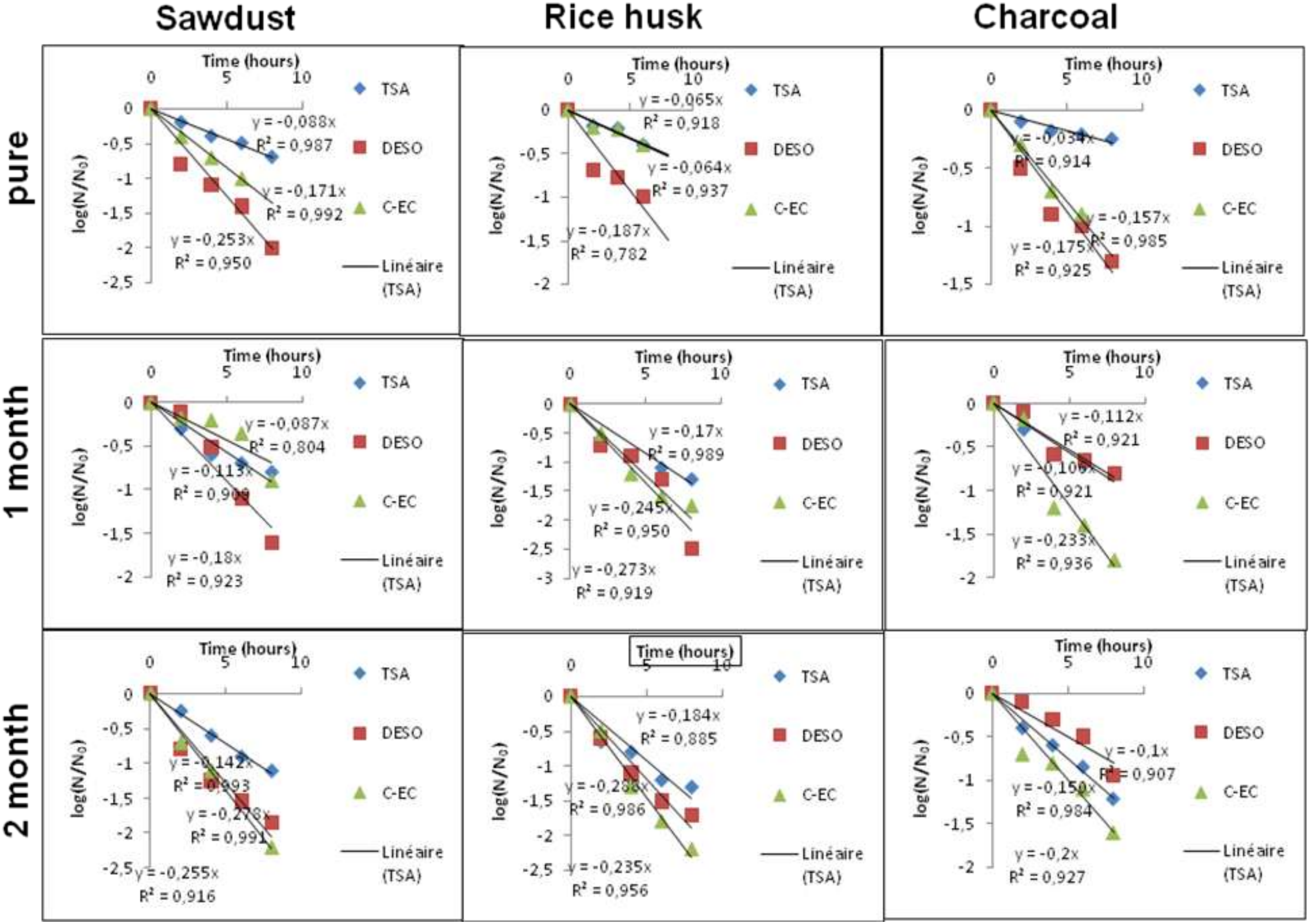

Figure 1- shows the change in the concentration of *E. coli* in three matrixes

Results and discussion

2. Normalized inactivation rate constant

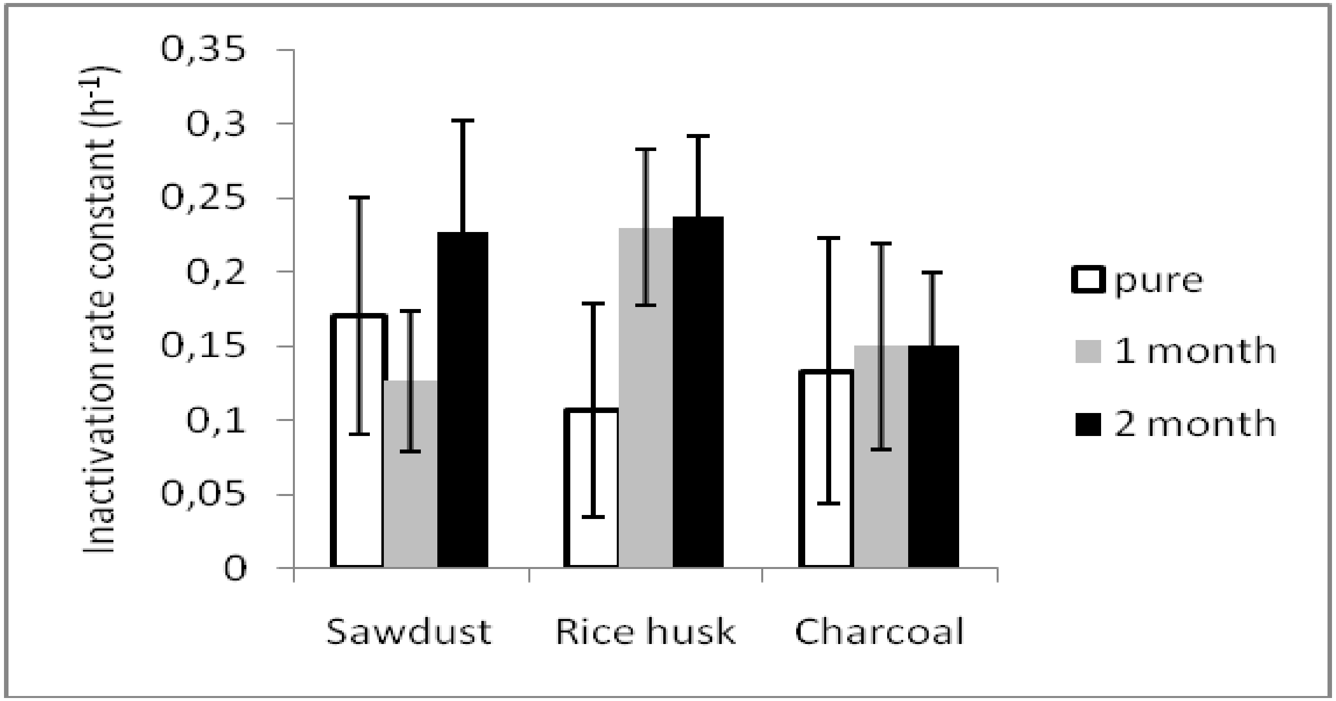

Figure 2- Normalized inactivation rate constant of three matrixes

- No significant difference in three matrixes and during each process (pure, 1 month and 2 month)

- However the value in rice husk was relatively increased during 2 month but no significant difference (ANOVA, p=0.07).

Results and discussion

3. Mechanism of damaging part *E. coli* during matrix change

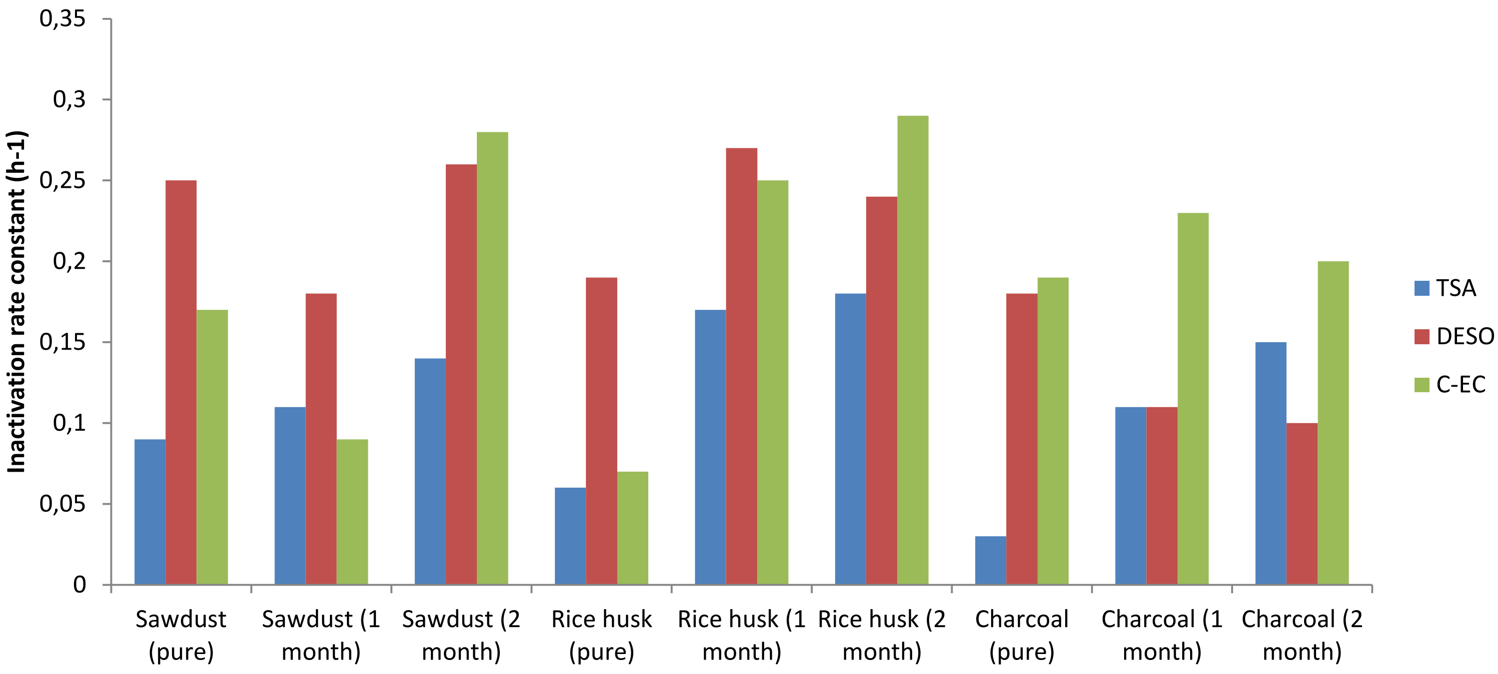

Figure 3- Inactivation rate constants by each media in differences matrixes

➢ *sawdust and rice husk :*TSA and C-EC value in pure matrix is lower than that of DESO But these value increased in 2 month compost; This indicated that damaging part was changed from outer membrane to enzymes and metabolisms

➢ *Charcoal:* TSA value in pure matrix is lower than that of DESO and C-EC, But the value increased in 2 month compost; This indicated that damaging part was changed from outer membrane and enzymes to metabolisms

Results and discussion

4. Mechanism of damaging part *E. coli* during pH change

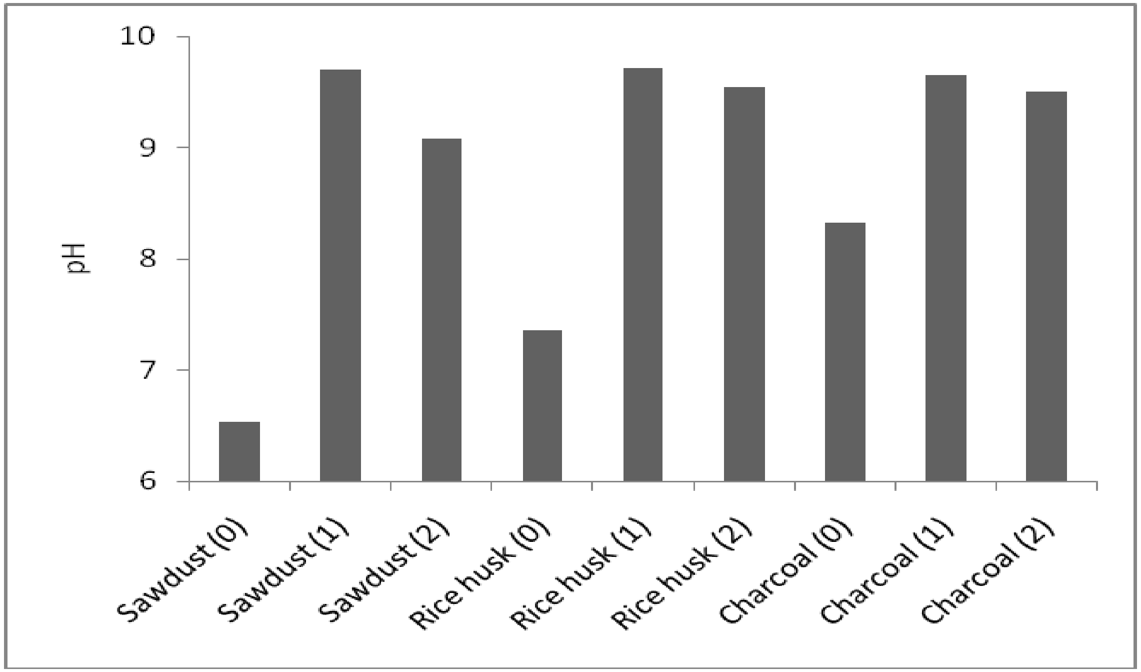

Figure 4- Inactivation rate constants by each media in differences pH

- pH in all composts was increased from pure (0 month) to 2 month. But no significant correlation (r2= 0.07) was not observed. This mean other factor also affected damaging part.

- However the damaging part of pure charcoal was different to other two pure matrix. pH in the charcoal was originally higher

Results and discussion

- Indigenous microflora of compost destroyed pathogenic bacteria
- Pure charcoal led the bacteria to more lethal damage than pure sawdust and pure rice husk: an originaly high pH because mineral is remained on its surface after carbonization
- The way of inactivation treatment for composting toilet should be further investigated from the viewpoint of risk assessment.

Conclusion

- The results suggested that composting matrixes (sawdust, rice husk and charcoal) and composting process did not significantly affect inactivation rate of pathogenic bacteria,

- However, these differences affected damaging part of the bacteria. Especially, composting process, accompanied with pH increase, changed the damaging part more lethally.

- This result could help to choose matrix and time from the viewpoint of hygienic aspect in composting toilet.

Acknowledgments

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Laboratory on Engineering for Sustainable Sanitation

' Thank you for your attention'