Research article

Changes in Surviving Microorganism in Cow Manure with Adding Lime Nitrogen

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Abstract Pathogenic bacteria known as *E. coli* may survive in cow manure and may efflux from upland fields where cow manure was applied. So, treating of *E.coli* through fermentation process has been focused. It has been considered that air drying method was effective method for decreasing *E.coli*. However, this treatment may possibly eliminate not only pathogenic bacteria but also useful bacteria for decomposition. This study aimed to investigate the changes in number of *E.coli*, coliform bacteria and general bacteria during the fermentation of cow manure with adding lime nitrogen (nitrolime). Samples used were fresh cow dung, 2 weeks and 12 weeks fermented manure. Lime nitrogen was added to all three types of dung samples then were kept in incubator within 14 days. Sampling, stirring and supplying of physiological saline were conducted. Based on the experimental results, there was a significant decrease in *E.coli* and coliform bacteria in cow manure after adding of lime nitrogen; however there was no decrease in general bacteria. It was observed that controlling to pH 9.0 in cow manure samples was appropriate for decreasing *E.coli* and coli form bacteria during cow manure fermentation with minimum damage to general bacteria. Therefore, it was concluded that treating of cow manure by adding lime nitrogen, especially controlling to around pH 9.0, is an effective strategy for sterilizing pathogenic bacteria such as *E.coli* and other coliform bacteria with minimum damage to general bacteria.

Keywords *Escherichia coli*, general bacteria, lime nitrogen, cow dung, pH

INTRODUCTION

Big amounts of cattle dung has been produced in farms. The point of view of organic agriculture, making manure was considered as proper treatment. However, bulk production of manure may possibly contain immature fermented manure. In addition, the pathogenic bacteria known as *Escherichia coli* (*E.coli*) or coliform bacteria may survive and remain in immature fermented manure (Chun-Ming et al., 2005; Indira et al., 1998). Especially, cow dung has an amount of *E.coli* than other cattle dung (Nakazawa and Sameshima, 2002). These bacteria may survive in soil (Islam, M et al., 2005) and may be released from grazed land and upland field applied with immature fermented manure (Mishra et al., 2007; Yagura et al., 2006; Ishikawa and Mihara., 2010). The efflux of *E.coli* causes water pollution or contamination of potable water sources affecting human health (Tamura et al., 2006; Mishina et al., 2007).

So, treatments to sterilize cow dung of *E.coli* during fermentation process were focused. Some treatments as air drying treatment (Saito and Mihara, 2010) were carried out. However, air drying treatment has possible affect to not only *E.coli* or coliform bacteria but also general bacteria (Ishikawa and Mihara, 2011). On the other hands, it was reported by Minato et al., (2001) that pH control by lime nitrogen adding treatment, it decreases not only *E.coli* but also beneficial bacteria. However, beneficial bacteria which affect fermentation of manure change in fermentation stage. So, it is necessary to observe the surviving of microorganisms in lime nitrogen adding treatment.

This study aimed to observe the survival of several microorganisms such as *E.coli,* coli form bacteria and general bacteria under lime nitrogen adding treatment.

METHODOLOGY

Cow dung and fermented manure used in this experiment were collected from Fuji Farm, a cattle farm of Tokyo University of Agriculture located in Shizuoka Prefecture (Fig.1). There were three types of cow dung collected for this experiment, fresh cow dung, 2 weeks, and 12 weeks fermented manure with water contents at 76%, 75% and 68% respectively. In addition, cow dung and manures contained 90%, 89% and 86% of organic matter. The colony forming units (cfu) of *E.coli* were 20×10^6 in cow dung, 1×10^3 in 2 weeks fermented manure. In 12 weeks fermented manure, *E.coli* was not observed. Also, the colonies of coli form bacteria was 1×10^5 cfu/g in cow dung and 0 cfu/g in 2 and 12 weeks fermented manures. The number of general bacteria was 128×10^6 in cow dung, 236×10^6 in 2 weeks fermented manure and 93×10^6 in 12 weeks fermented manure**.**

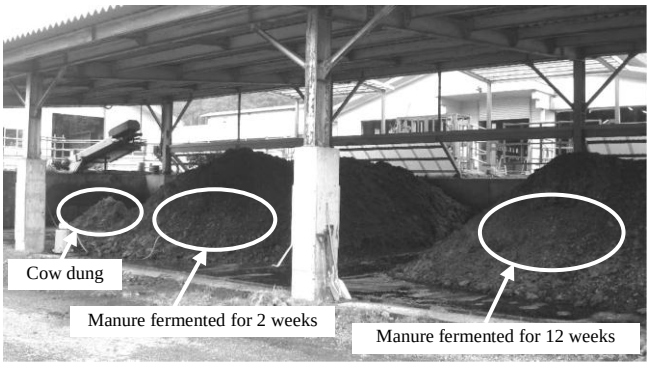

Fig.1 Situation of cow dung and manure

Table 1 Properties of cow dung and fermented manure

	Period of fermentation	*E.coli* (cfu/g)	Coliform bacteria (cfu/g)	General bacteria (cfu/g)	Water contents (%)	Organic matter (%)
Cow dung	0 days	20×10^6	1×10^5	128×10^6	76	90
Manure	2 weeks	1×10^3	0	236×10^6	75	89
Manure	12 weeks	0	0	93×10^6	68	86

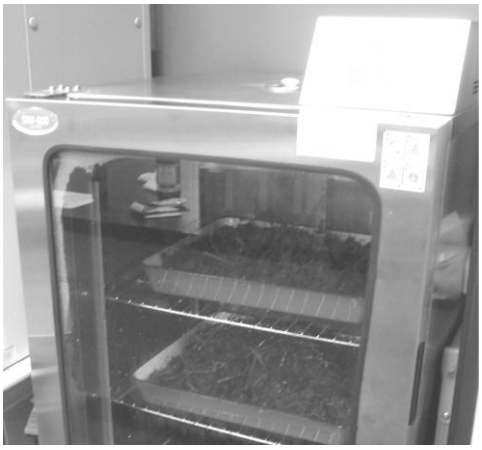

Fig. 2 Situation of incubator

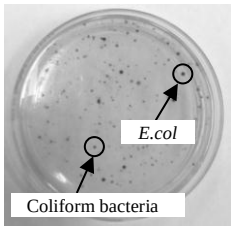

Fig. 3 Colonies of *E.coli* and coliform bacteria

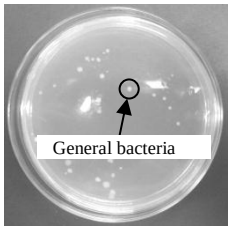

Fig. 4 Colonies of general bacteria

Both of cow dung and manure at 3 kg were mixed after adding 60 g of lime nitorogen. Then, they were put in incubator at 37 degree temperature for 14 days (Fig. 2). At 4th, 7th, 11th, 14th day, sampling, stirring and supplying of physiological saline were conducted.

The number of *E.coli*, coliform bacteria and general bacteria in sample were measured through laboratory experiments. The analysis of *E.coli* and coliform bacteria was carried out through counts of colonies on XM-G agar medium as shown in Fig.3. The analysis of general bacteria was carried out with general agar medium (Fig. 4). In addition, the pH in cow dung and manure were measured through laboratory experiments.

RESULTS AND DISCUSSION

The changes in the number of *E.coli* in each sample were summarized in Figs. 5-7. In cow dung, *E.coli* decreased after lime nitrogen was added then, *E.coli* was not observed. Based on the results of variance analysis, significant difference was observed. In 2 weeks fermented manure, *E.coli* was decreased after lime nitrogen was added as well as cow dung. However, significant difference was not observed. In 12 weeks fermented manure, *E.coli* was not observed during experiment.

Changes in coliform bacteria were shown in Figs. 8-10. In cow dung, coliform bacteria were sterilized by lime nitrogen adding treatment as shown in Fig. 8. Moreover, it showed a significant difference of 99% between before lime nitrogen adding and after. In addition, coliform bacteria were not detected from 2 weeks and 12 weeks fermented manure. Therefore, it was considered that the adding of lime nitrogen is an effective treatment to decrease pathogenic bacteria as *E.coli* or coliform bacteria. Figs. 11-13 show changes in general bacteria. In both of samples, it showed a tendency that the number of general bacteria was not decreased by adding of lime nitrogen. Based on results of variance analysis, significant difference was not observed during experiment. Therefore, it was considered that general bacteria which contain beneficial bacteria are not affected by lime nitrogen adding treatment.

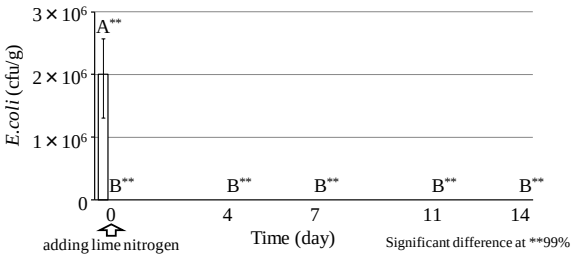

Fig. 5 Changes in *E.coli* (cow dung)

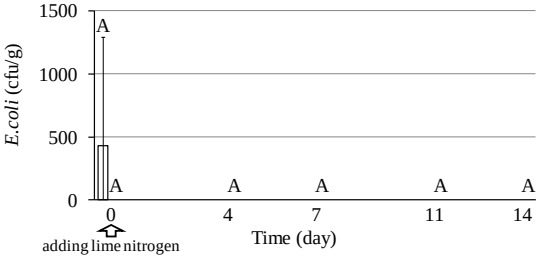

Fig. 6 Changes in *E.coli* (2 weeks fermented manure)

The pH in cow dung and manure are indicated in Fig. 14. In all of samples, pH was increased to around 9.0. It was reported that *E.coli* or coli form bacteria can survive from pH 4.4 to 9.0 (Nakanishi and Maruyama, 2009). Furthermore, based on a report of Fujita (1993), proper pH for fermentation of manure is 8 to 10. So, it was considered that pH controlling to around 9.0 is able to decrease *E.coli* and coliform bacteria without sterilize general bacteria which contain beneficial bacteria**.**

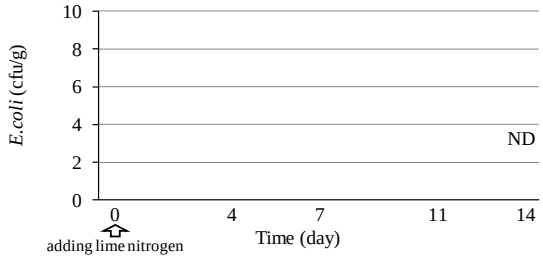

Fig. 7 Changes in *E.coli* (12 weeks fermented manure)

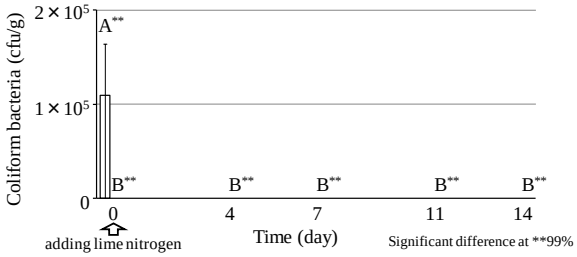

Fig. 8 Changes in coliform bacteria (cow dung)

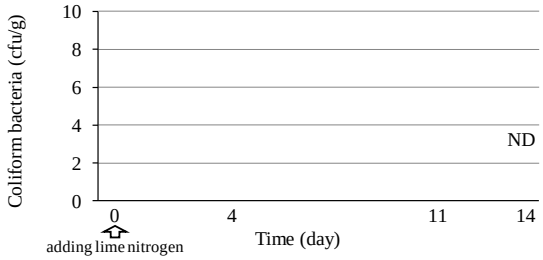

Fig. 9 Changes in coliform bacteria (2 weeks fermented manure)

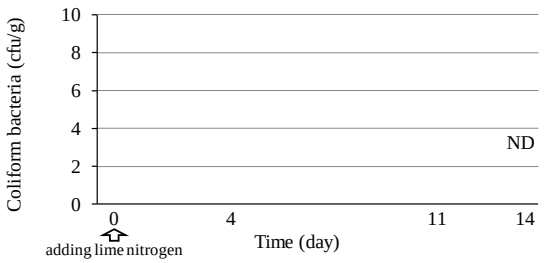

Fig. 10 Changes in coliform bacteria (12 weeks fermented manure)

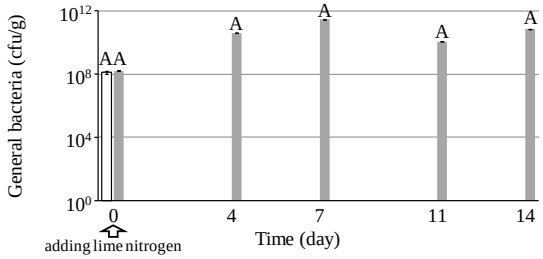

Fig. 11 Changes in general bacteria (cow dung)

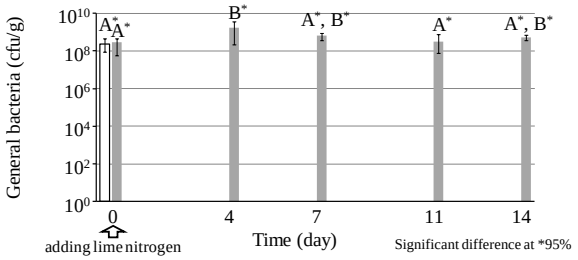

Fig. 12 Changes in general bacteria (2 weeks fermented manure)

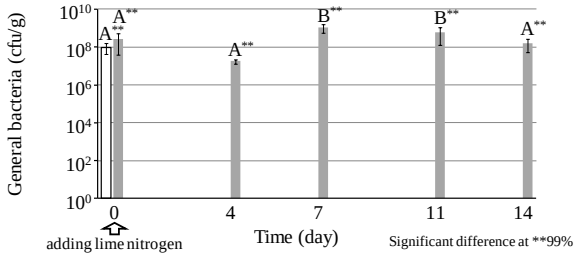

Fig. 13 Changes in general bacteria (12 weeks fermented manure)

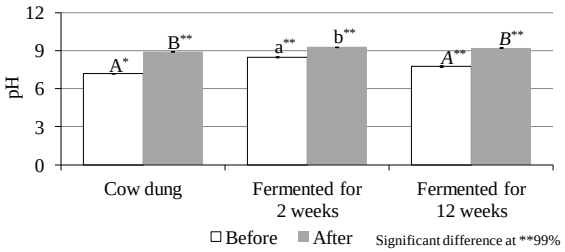

Fig. 14 Changes in pH of manure

CONCLUSION

This study aimed to observe the survival of *E. coli*, coli form bacteria and general bacteria under lime nitrogen adding treatment in cow dung, 2 weeks and 12 weeks fermented manure.

Based on experimental results, *E. coli* was decreased after lime nitrogen addition treatment. Moreover, there was a significant difference observed. Also, coli form bacteria were sterilized by lime nitrogen as well as *E. coli*. On the other hand, lime nitrogen has not affected the number of general bacteria which contain beneficial bacteria.

In addition, as the results of pH, there were tendencies that pH was increased to around 9.0 which were proper for decreasing *E. coli* or coli form bacteria with minimum damage to general bacteria.

Therefore, it was concluded that the pH control to around 9.0 using alkaline as lime nitrogen is an effective treatment for decreasing pathogenic bacteria in fermentation process.

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