The effect of anaerobic digestion in biogas plants on survival of pathogenic bacteria

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Abstract

The paper deals with the hygienic advantages of sanitation to treat dairy manure in full-scale biogas plants. Slurry samples were collected from two thermophilic biogas plants (55ºC) and two mesophilic biogas plants (38ºC) in Hokkaido, Japan. The detectable number of *Coli-aerogenes* group and *Enterococcus* in the slurries after anaerobic digestion could not be found in both thermophilic biogas plants. In both mesophilic biogas plants, however, the viable numbers of *Coli-aerogenes* group and *Enterococcus* were detected in the slurries even after anaerobic digestion. The mean decimation reduction time (T_{90}) values of *Coli-aerogenes* group and *Enterococcus* in the slurries during mesophilic digestion were 13.3 d and 16.7 d, respectively.

Keywords: biogas plant, pathogenic bacteria, *Coli-aerogenes* group, *Enterococcus*

Introduction

Anaerobic digestion in biogas plants (BGPs) is an alternative way to handle animal manure and reduce greenhouse gas emission. The digested manure may be used as a fertilizer on agricultural land. Animal manure is known to contain pathogenic bacteria that may be a health risk for both humans and animals. The digested manure must be proven hygienically safe in order to be recycled (Sahlstrom, 2003). However, there is no regulation concerning the hygienic standard of the BGPs residue in Japan. The growing interest in BGPs in Japan makes it important to consider biosecurity aspects of recycling digested residues. Umetsu et al. (2003) studied the survival of coliform bacteria during mesophilic anaerobic digestion of dairy manure slurry. Minato et al. (2003) also reported the monitoring of *Coli-aerogenes* group and *Enterococcus* in practical mesophilic BGPs, but there is no data on hygienic requirements in full-scale thermophilic BGPs in Hokkaido, Japan. In the thermophilic

process, the temperature is known to be beneficial for the conversion rate, but it was originally feared to be more difficult to control. Umetsu et al. (2005) and Aoki et al. (2006) demonstrated the practicality of thermophilic temperature of BGPs in the cold regions. Thus, the thermophilic process has become pervasive by this domination. The objective of the present paper is to clarify the survival of *Coli-aerogenes* group and *Enterococcus* in full-scale mesophilic and thermophilic BGPs in Hokkaido, Japan.

Materials and Methods

Indicator organisms

The bacterium used as the most common indicator for public health monitoring is fecal *E. coli*. *Enterococci* can validate the hygienic treatment of biowaste in BGPs (Larsen et al., 1994). In this study, indicator organisms were detected as the *Coli-aerogenes* group and *Enterococcus*. *Coli-aerogenes* group, and *Enterococcus* was isolated from the slurry samples on desoxycholate selective agar plates incubated at 35ºC for 20 h; M-*Enterococcus* selective agar plates were incubated at 35ºC for 48 h.

Sampling

The slurry samples were collected from the reception pit, digester, and storage tank in two thermophilic (55ºC) and two mesophilic (38ºC) BGPs on August 5, 2005. Specifications for these BGPs and the operating conditions of this study are presented in Table 1. Ambient temperature of the day was around 30ºC.

Table 1. Specifications and operating conditions of the biogas plants of this study.

Parameter		Plant A	Plant B	Plant C	Plant D
Digester temperature	˚C	55	55	38	38
Digester volume	m^3	60	540	424	671
Amount of feeding per day	m^3d^{-1}	4	45	16	18
Hydraulic Retention Time (HRT)	day	15	12	26.5	37.3

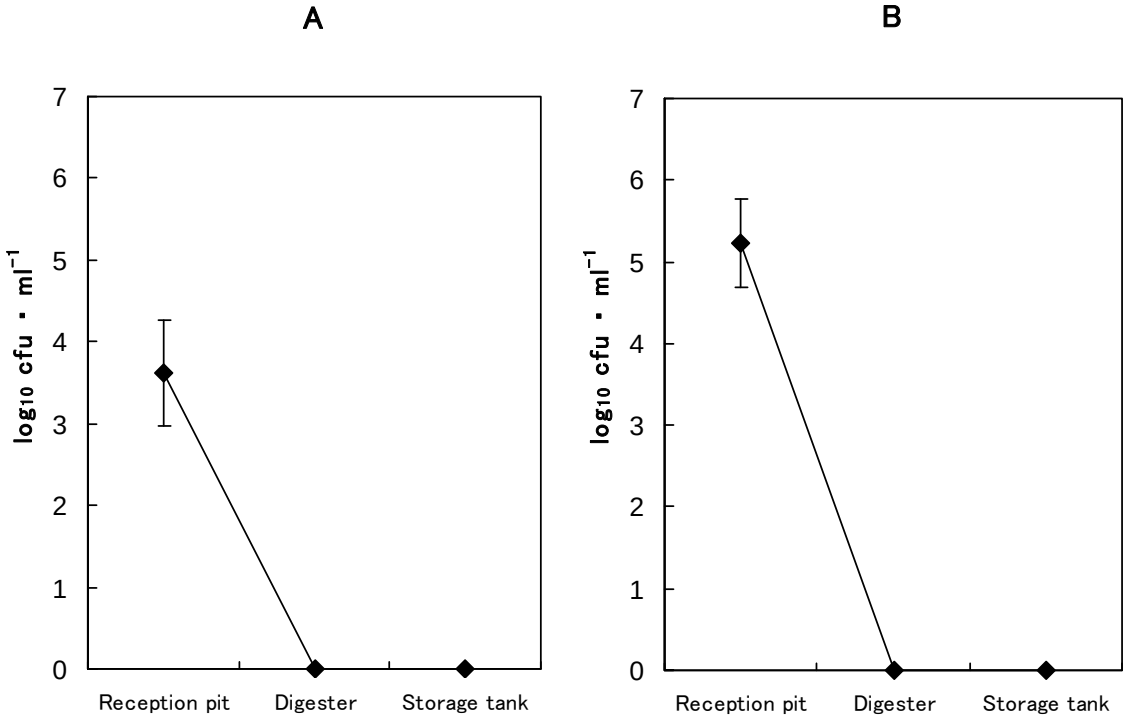
Analytical method

Total solids (TS) were determined by drying in a fan-assisted oven at 105ºC for 24 hours. Volatile solids (VS) were determined by combusting the oven-dried material at 550ºC for 4 hours. The pH values of the slurry samples were measured with pH meter (TOA Inc., Tokyo, Japan)**.** Total volatile acids (TVA) were determined with a Shimadzu HPLC (LC-10A), using a Shim-pack SGR-102H. The concentration of lactic, formamide, acetic, propionic and butyric acids were measured. The details of the analytical procedure for TVA were described

in a previous paper (Kimura et al., 1994).

Results and discussions

Anaerobic digestion can be performed under either mesophilic (30ºC-38ºC) or thermophilic (50ºC-55ºC) conditions. The advantages of thermophilic temperatures include increased reaction rates, and therefore less capital costs as a result of smaller digester size; another advantage is increased efficiency of organic matter destroyed. Disadvantages of thermophilic systems include higher energy requirements to heat influent substrate and maintain digester temperature. The survival patterns of *Coli-aerogenes* group and *Enterococcus* in the thermophilic (A, B) and mesophilic BGPs (C, D) are shown in Fig. 1 and 2. The number of *Coli-aerogenes* group and *Enterococcus* in the slurries after anaerobic digestion could not be found in either thermophilic BGP. In both mesophilic BGPs, the viable numbers of *Coli-aerogenes* group and *Enterococcus* in the slurries were detected, though the number declined to low levels after anaerobic digestion. The decay rate of viable bacteria has been reported to depend on temperature, retention time, pH, volatile acids, bacterial species, and available nutrients (Farrah and Bitton, 1983; Kearney et al., 1993). The temperature is the most important factor concerning survival of pathogenic bacteria during anaerobic digestion (Dumontet et al., 1999). Bacterial inactivation due to temperature is related to time (Olsen and Larsen, 1987).



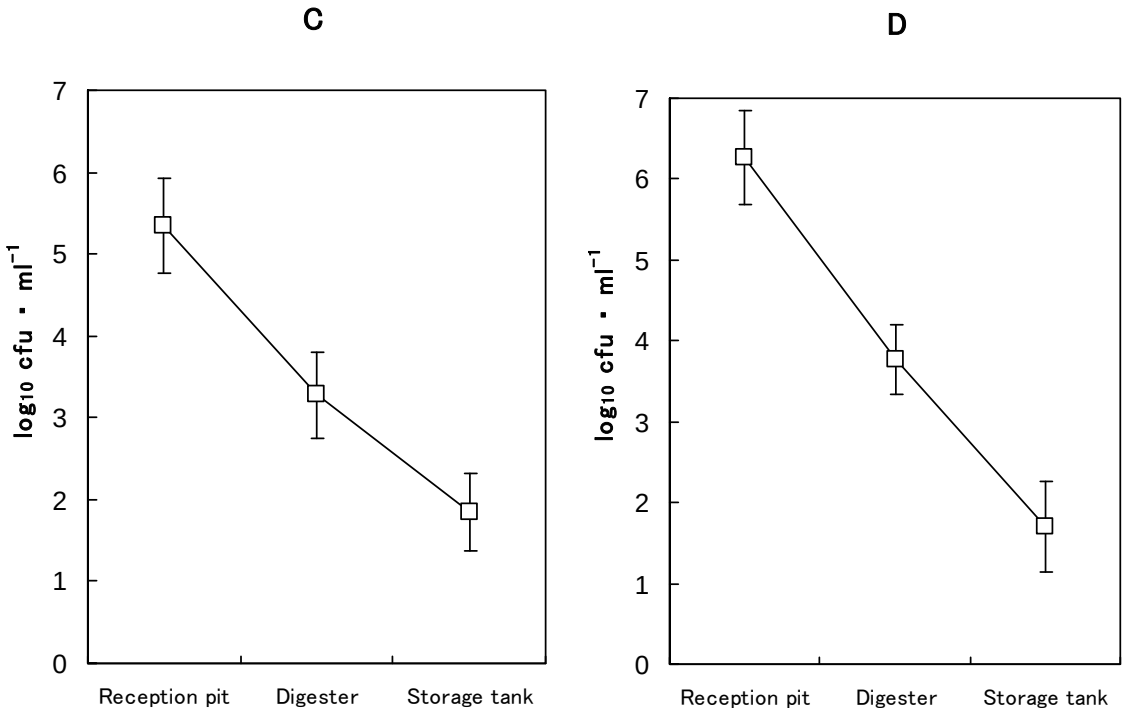
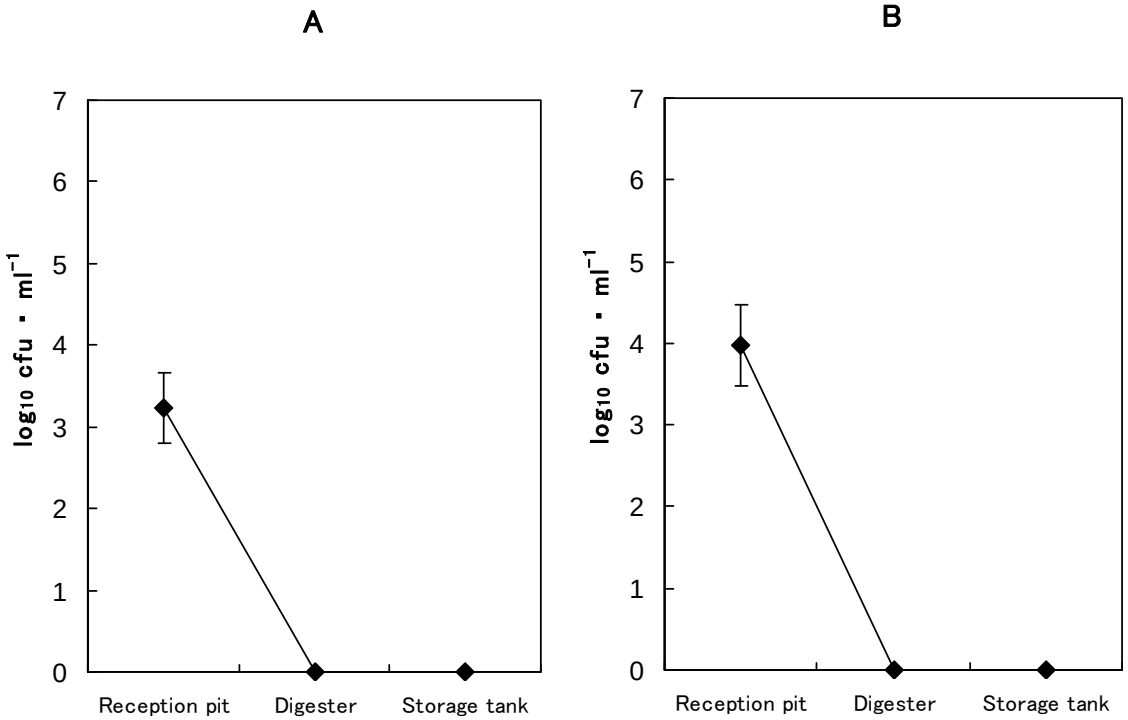


Figure 1. Arithmetic mean viable counts of Coli-aerogenes *group in the thermophilic (A ,B) and mesophilic (C, D) biogas plants.*



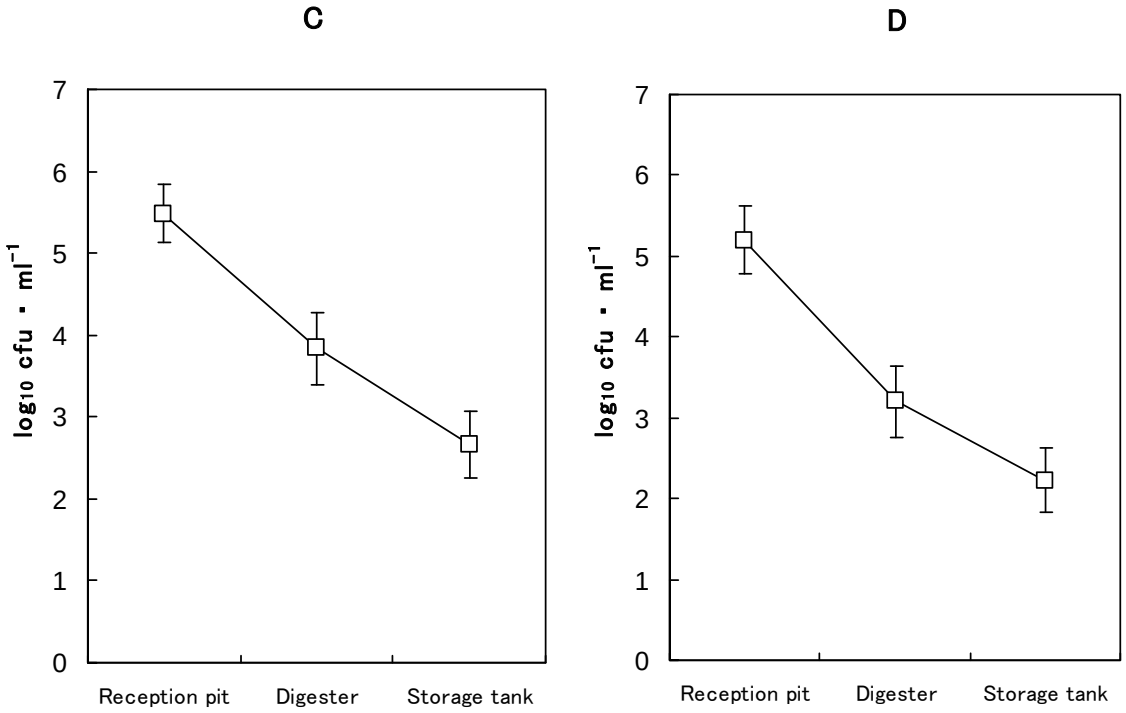


Figure 2. Arithmetic mean viable counts of Enterococcus *in the thermophilic (A ,B) and mesophilic (C, D) biogas plants.*

The time required for a 90% reduction of viable counts of a population of microorganisms—or a decrease by one logarithmic unit (log 10)—is called the decimation reduction time (T_{90}) (Schlundt, 1984). The T_{90} value indicates the differences of inactivation of bacterial pathogens in anaerobic digestion. T_{90} for many bacteria can be counted in hours in thermophilic digestion and in days in mesophilic digestion (Gibbs et al., 1995). In the present investigation, *Coli-aerogenes* group and *Enterococcus* in the digested slurries of thermophilic BGPs (A, B) were not detected. The mean T_{90} values of both *Coli-aerogenes* and *Enterococcus* in mesophilic BGPs (C, D) were 13.3 d and 16.7 d, respectively (Table 2). These findings agreed with the experimental results obtained by Olsen and Larsen (1987). *Salmonella* and *Mycobacterium paratuberculosis* are inactivated within 24 h in thermophilic AD compared to weeks and even months in mesophilic anaerobic digestion (Plym-Forshell, 1995; Olsen et al., 1985).

Figure 3 shows the changes in TS and VS in the slurries of the thermophilic (A ,B) and mesophilic (C, D) biogas plants. The TS contents of the slurries taken from the reception pits ranged from 3.97% to 11.95%, and the VS contents remained relatively constant averaging 83.0% of the TS. The slurries taken from the A plant had higher water content because of the dilution by milking parlor wastewater. On the other hand, the TS content of the slurries taken from the C plant, over 12%, was much higher because of no dilution. Figure 4 shows the

Table 2. The mean T_{90} values of Coli-aerogenes *and* Enterococcus *for each biogas plant.*

Plant		n	T_{90} for *Coli-aerogenes*	T_{90} for *Enterococcus*
	Reception pit	6		
A	Digester	5		
	Storage tank	5	-	-
	Reception pit	6		
B	Digester	6		
	Storage tank	6	-	-
	Reception pit	5		
C	Digester	6		
	Storage tank	5	14.5	18.3
	Reception pit	6		
D	Digester	6		
	Storage tank	6	12.1	15.0

n: Number of samples

changes in TVA in the slurries of the thermophilic (A , B) and mesophilic (C, D) biogas plants. The main volatile acids present in the slurries in the reception pits were acetic acid, propionic

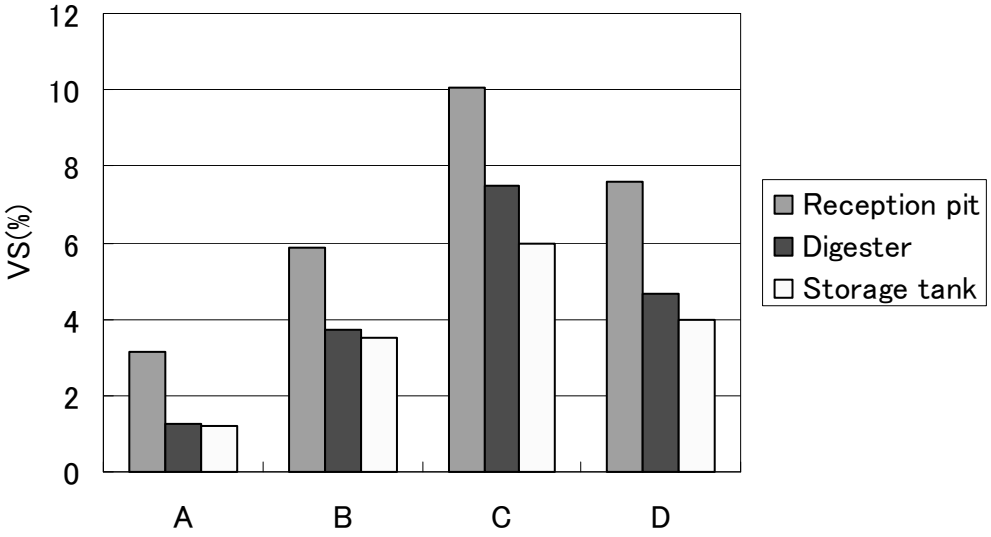


Figure 3. Changes in Total Solids (TS) and Volaitle Solids (VS) in the slurries of the thermophilic (A , B) and mesophilic (C, D) biogas plants.

acid, and butyric acid. The TVA content of the slurries taken from the reception pits was similar to those of TS or VS contents as shown in Fig. 3. During AD, the concentrations of TVA decreased sharply with the decomposition performed in each biogas plant.

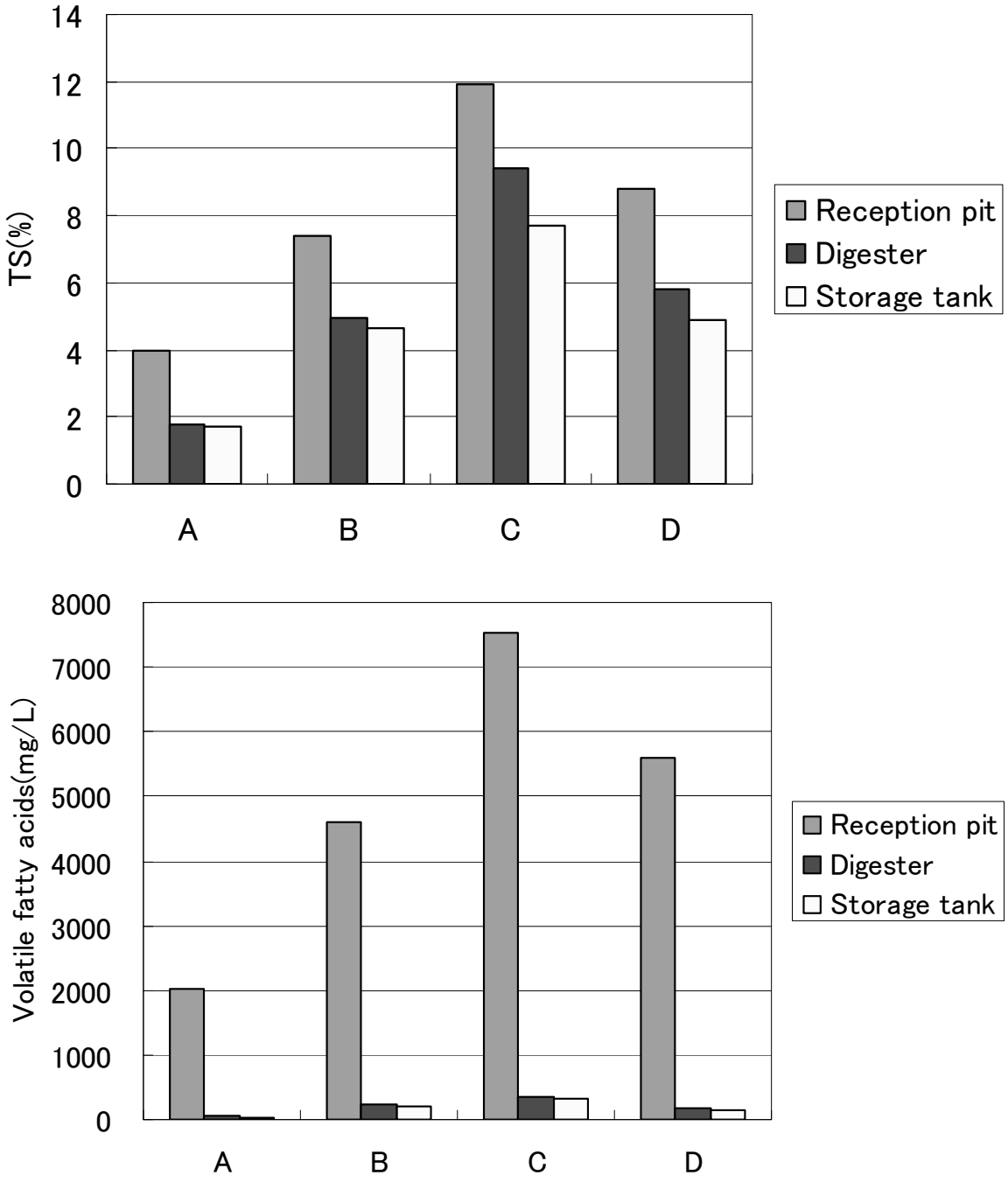


Figure 4. Changes in Total Volatile Acids (TVA) in the slurries of the thermophilic (A , B) and mesophilic (C, D) biogas plants.

Figure 5 shows the changes in pH value of the slurries for each BGP. The pH value is one of the important clues for understanding methane bacteria activity. In general, animal manure has sufficient alkalinity to maintain the pH in an anaerobic digester, so methane production from animal manure is a relatively stable process. However, as a result of overloading, excessive concentration of volatile acids and ammonia nitrogen can develop, and this has

been considered to hinder methane production because of the drastic changes in pH values. But in this observation, no special differences in pH were found between each of the biogas plants. From the data of composition of the digested slurries, the authors have concluded that each biogas plant was running favorably.

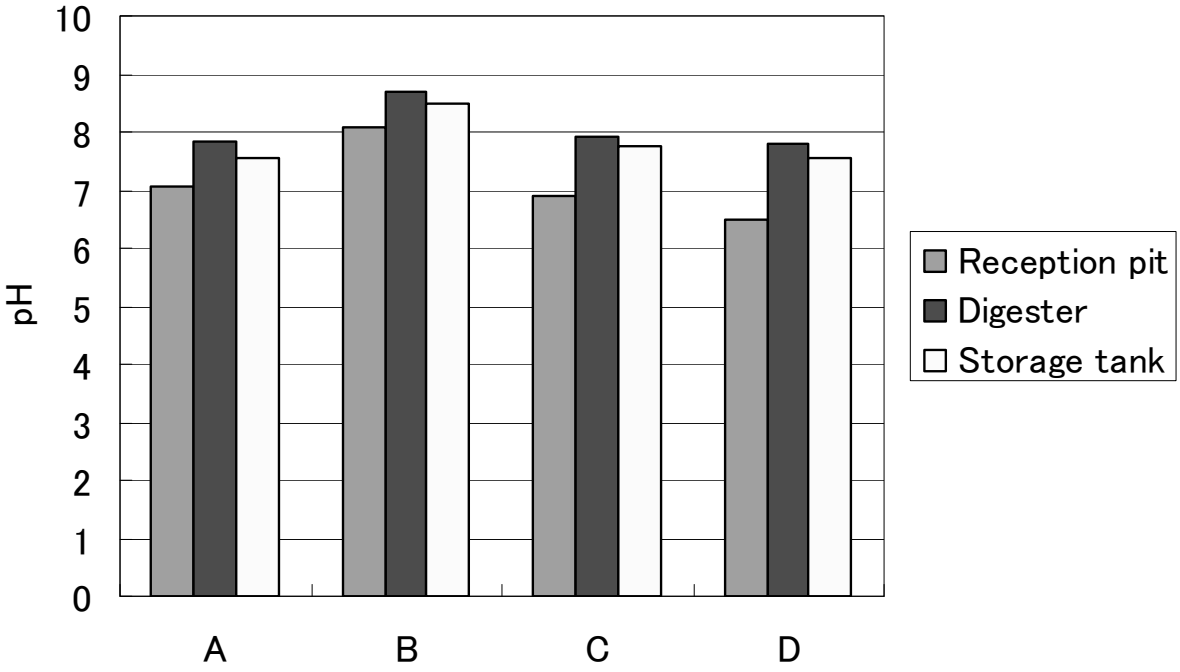


Figure 5. Changes in pH value of the slurries in the thermophilic (A , B) and mesophilic (C, D) biogas plants.

Conclusion

In Japan, many farmers spread raw or untreated manure straight to land. However, it is important that appropriate management practices are implemented to minimize the risks of pathogen transfer to the food chain from the management of animal manures. The present investigation was undertaken to study the hygienic and sanitation in full-scale biogas plants treating dairy manure. This paper reported the survival of *Coli-aerogenes* group and *Enterococcus* in full-scale mesophilic and thermophilic BGPs in Hokkaido, Japan. *Coli-aerogenes* group and *Enterococcus* in thermophilic BGPs were not detected. In both mesophilic BGPs, the viable numbers of *Coli-aerogenes* group and *Enterococcus* in the slurries declined to low levels after anaerobic digestion. In this study, high digester temperature would have a suppressing effect on the population of *Coli-aerogenes* group and *Enterococcus* in the slurries during anaerobic digestion. Findings of this study suggest that BGPs offers benefits towards net reduction of pathogenic bacterial numbers in dairy manure slurry.

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