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<https://doi.org/10.5109/1801783>

出版情報：九州大学大学院農学研究院紀要. 62 (1), pp.197-203, 2017-02-24. Faculty of Agriculture, Kyushu University

バージョン：

権利関係：



Effect of Substrate to Inoculum Ratio on Methane Production and Organic Matter Removal during Solid State Anaerobic Digestion of Beef Manure and Sawdust Mixture

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(Received October 20, 2016 and accepted November 4, 2016)

This study was conducted to determine the optimal substrate/inoculum (S/I) ratio for solid state anaerobic digestion (SS-AD) of beef manure and sawdust bedding mixtures. The organic material removal and methane yield performance were evaluated at five different S/I levels (1, 2, 4, 50, and beef manure alone on a VS basis) for 49 days at 37°C. Results showed that biodegradable volatile solids (BVS) reduction of beef manure alone test unit was 76% which was not significantly different from the rest of all inoculated test units. The methane production potential of all five test units showed similar levels (135 to 154 mL/g VS), regardless of S/I ratio. Although it showed similar methane production potential, the beef manure alone and S/I ratio 50 test units showed the highest methane yields of 7.3 and 7.2 based on the volume of methane produced per volume of digester, respectively. In addition, when considering utilization efficiency of the available digester volume, the beef manure alone treatment was 15% higher than that of the S/I ratio 50 treatment. These results reveal that the beef manure and sawdust bedding mixture does not require inoculation to improve SS-AD performance.

Key words: Beef manure, inoculum, solid state anaerobic digestion, biogas, biodegradability

INTRODUCTION

Approximately 14 million tons of beef manure was produced in South Korea in 2014, which accounted for about 31% of total livestock manure production (Ahn, 2015). About 90% of beef farms in South Korea contain bedded pack barns (sawdust bedding), and composting is the most popular method for treating mixtures of manure and bedding material. However, several problems have been observed in the composting process. Because of the increase in bedding material price and stocking rate, the moisture content of collected manure and sawdust mixture is very high, which leads to difficulty in composting. Moreover, about 70% of farms discharge manure into the field without going through the proper composting process (Ahn, 2015). This practice has led to complaints from surrounding local residents regarding the environment. To increase the utilization of beef manure collected in solid form, advanced alternative technology for conventional composting must be developed.

Anaerobic digestion is advantageous for the treatment of high strength organic wastes such as manure because it produces energy (methane) and solid digestate that can be recycled such as compost or bedding material (Angelodaki and Ahring, 1994; Bonmati *et al.*, 2001). However, only 0.5% of the total livestock manure produced in South Korea is subjected to anaerobic digestion treatment, and all anaerobic digestion plants are equipped with only liquid anaerobic digesters for treat-

ing swine slurry (Ahn, 2015). Anaerobic digestion processes are classified according to the total solids content. Liquid anaerobic digestion generally occurs at solids content between 0.5 to 15%, whereas solid state anaerobic digestion process occurs feed stocks with solids content higher than 15% (Li *et al.*, 2011a).

Due to low moisture content of beef manure, it was not considered as a main feedstock material of liquid anaerobic digester. However, solid state anaerobic digestion (SS-AD) system is suitable for treating beef manure because it works well with feed stocks have low moisture content (less than 85% w.b.).

When compared with liquid phase anaerobic digestion (L-AD), the SS-AD process can treat more organic materials in the same capacity digester while using less energy for heating (Li *et al.*, 2011a). However, the SS-AD requires a long digestion time to decompose organic material because of the high amount of non-biodegradable materials such as cellulose, hemicellulose and lignin. Previous studies have attempted to overcome the disadvantages of solid state anaerobic digestion through physicochemical pretreatment, inoculation, mixing, and moisture content control (Delia and Osman, 2004; Nges and Liu, 2009; Li *et al.*, 2011b).

Overall, inoculation can improve the digestive efficiency, accelerating the methane production rate and reducing digestion time (Raposo *et al.*, 2006). However, depending on the type and characteristics of livestock manure to be treated, selection of an appropriate inoculation ratio is important. If the ratio of the substrate is very low, the utilization efficiency of the available digester volume will be reduced by the excessive amount of inoculum used. Conversely, an excessively high substrate ratio could cause inhibition by accumulation of volatile fatty acids (Alzate *et al.*, 2012). González-

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Fernandez and García-Encina (2009) suggested that anaerobic digestion of swine slurry should be conducted at an S/I ratio of around 1 (COD/VS basis) to prevent from process imbalance results from VFA accumulation. Stabnikova *et al.* (2008) reported the proper S/I ratio of food waste for anaerobic digestion was 7.7 (VS basis), whereas an S/I ratio of 50 (VS basis) was reported as the optimal S/I ratio for cattle manure (Demirer and Chen, 2008). In previous studies, it should be noted that a lower amount of inoculum was required for cattle manure than for other feedstock materials. This occurs because the digestive organs of ruminants discharge anaerobes with manure that influence the S/I ratio. McGarvey *et al.* (2007) and Girtija *et al.* (2013) reported that fresh cattle manure is composed of 30% to 70% phyla Firmicutes (strict anaerobic microorganisms). S/I ratio suggested by the Demirer and Chen (2008) cannot be directly applied to the manure collected from unique bedded pack barn prevailed in S. Korea because the characteristics of manure collected from S. Korean style bedded pack barn is different from the cattle manure reported by them.

As time goes on, excreted dairy manure and bedding mixtures are gradually decomposed, the air permeability becomes low, and beef manure that has accumulated on a daily basis to a bedded pack barn will form an anaerobic condition by maintaining moisture content of more than 70%. Moreover, bedding and manure is stepped on by cattle, resulting in an indirect mixing effect that enhances the activity of anaerobic microorganisms in discharged manure. Due to the high activity of anaerobic microorganisms, it may increase the S/I ratio more than the value reported by previous research (Forster-Carneiro *et al.*, 2008).

The purpose of the present study was to look at the optimal substrate to inoculum ratio of beef manure and sawdust bedding mixture as one of the factors likely to induce removal of organic materials, methane production characteristics, and digester stability.

MATERIALS AND METHODS

Materials

The beef manure (mixed with sawdust) used in this study was obtained from a beef farm at the Animal Resource Research Center of Chungnam National University and digestate used as the seed material was collected from a sewage treatment plant in Dae-jeon city. Digestate was subjected to starvation under anaerobic conditions at 37°C after centrifuging for 30 minutes

at 3500 rpm. The feedstock was stored at 4°C prior to use. Characteristics of the beef manure and inoculum used in this study are described in Table 1.

Experimental configuration

In this study beef manure and inoculum were mixed to achieve different S/I ratios of 1, 2, 4, 50, and 100 (beef manure alone) on a VS basis (Table 2). A lab-scale solid-state anaerobic digestion system with a 2 L capacity mass cylinder composed of PP (polypropylene) material and digester filled with beef manure and inoculum mixture was sealed using a silicone stopper to maintain anaerobic condition. An internal solid state anaerobic digester was able to separate the liquid and solid phase using PP cylindrical fine-mesh screen (diameter 70 mm, length 200 mm, volume 770 mL, screen opening 1 mm). The solid state anaerobic digesters were then maintained for 49 days at 37°C. The top of the cylinder had a 3-way valve connected to a Tedlar bag to collect the biogas.

Table 2. Experimental conditions

Items	Substrate [†] /Inoculum ratio(VS basis)
Substrate only	Beef manure 100%
Treatment 1	1 : 1, ratio = 1
Treatment 2	2 : 1, ratio = 2
Treatment 3	4 : 1, ratio = 4
Treatment 4	50 : 1, ratio = 50

note: [†]Beef manure and sawdust mixture.

Sampling and analysis

Approximately 1 mL liquid samples were taken from the anaerobic digestion reactor using a syringe. Samples were centrifuged at 3500 rpm for 30 min, and then filtered through 1.2 µm filter paper. The samples were subsequently analyzed for Soluble Chemical Oxygen Demand (SCOD), Volatile Fatty Acids (VFAs), NH₄-N, and Alkalinity. Moreover, moisture contents and volatile solids were determined from the loss of weight after drying the samples for 24 hours in an oven at 105°C and then for 8 h at 550°C, respectively.

SCOD analysis was performed using the closed reflux titrimetric method (APHA, 1998) and NH₄-N, alkalinity were analyzed using Gallery™ automated photometric analyzer (Thermo Fisher Scientific).

VFA was analyzed by injecting the supernatant into an iGC 7200 (DS Science, Korea) with a BP20 column (bore 0.32 mm, length 30 m, film thickness 1.0 µm) and FID (flame ionization detector). Methane contents were analyzed using an iGC 7200 (DS Science, Korea).

Ultimate Biodegradability

A long time is required to completely decompose the organic material. Total volatile solids (TVS) are composed of biodegradable volatile solids (BVS) and non-biodegradable volatile solids, but only the BVS are

Table 1. Characteristics of beef manure and sawdust mixture and inoculum (mean±S.D., n=3)

Characteristics	Beef manure	Inoculum
Moisture Contents (% w.b.)	71.3±0.3	90.3±0.7
Volatile Solids (% d.b.)	83.5±0.4	64.3±1.0
Bulk density (kg m ⁻³)	692±6.93	—

decomposed into biogas. Therefore, the decomposition rate of organic materials is more suitable based on the BVS rather than the TVS basis. In this study, the ultimate biodegradability (UB) of a beef manure and sawdust mixture was determined using a graphical statistical method (Tritt and Kang, 1991) to estimate the initial BVS mass. The amount of removed organic materials can be assumed to be the same as the mass of biogas produced due to biodegradable organic materials that were completely decomposed (CH_4 and CO_2 ; Eq. 1–Eq. 3). In this case, trace gases contained in the biogas were excluded. UB is calculated by a graphical method, linear regression of the remaining volatile solid portion (TVSe) of the initial volatile solid (TVS₀) at time *t*, as the operating time of the test approached infinity. TVS remaining at infinity was assumed to be the refractory fraction of feedstock (R_0), which is NBVS.

Next, a linear plot of TVSe/TVS₀ versus 1/time to the y-axis was extrapolated to show the refractory fraction as the value of the y-intercept. The remaining portion of TVSe/TVS₀ at any time was calculated by the biogas produced during each interval. The ultimate biodegradability of a substrate is estimated to be $\text{UB} = (1 - R_0)$.

$$\text{BMR (biomass removed)} = \text{CH}_4 \text{ mass} + \text{CO}_2 \text{ mass} \quad \text{..... (Eq.1)}$$

$$\text{BMR} = \frac{V_0 \times \left(\frac{16\text{g}}{1\text{mole}} \times \frac{\text{CH}_4}{100} + \frac{44\text{g}}{1\text{mole}} \times \frac{\text{CO}_2}{100} \right)}{\frac{22.413\text{l}}{\text{mole}}} \quad \text{..... (Eq.2)}$$

BMR = biomass removed (g)

V_0 = normal state (0°C, 1atm) biogas volume (L)

CH_4 , CO_2 = CH_4 and CO_2 contents (%)

Substituting the CO_2 (%) =

$100 - \text{CH}_4$ (%) in Eq. (2) yields Eq. (3)

$$\text{BMR} = V_0 \times \{1.963 - (0.0124 \times \text{CH}_4)\} \quad \text{..... (Eq.3)}$$

Kinetic modeling (Modified Gompertz)

Based on the measured methane production, kinetic modeling was conducted using the Gompertz equation to evaluate methanogenesis during SS-AD. Specifically, this was applied to estimate the cumulative methane production by using the characteristics of the methanogenic bacteria growth rate.

$$M = P \times \exp \left\{ -\exp \left[\frac{R_m \times e}{P} (\lambda - t) + 1 \right] \right\} \quad \text{..... (Eq.4)}$$

In this equation, *M* is the cumulative CH_4 production, $\text{ml g}^{-1}\text{vs}$; *P* is potential CH_4 production, $\text{ml g}^{-1}\text{vs}$; R_m is the rate of maximum CH_4 production ($\text{ml g}^{-1}\text{vs day}$); λ is the lag phase period (minimum time to produce biogas) in days; and *t* is the cumulative time for CH_4 production in days. The parameters *P*, λ , and R_m were estimated using the Solver function in MS-Excel.

Statistical analysis

The Origin Pro software (Origin-Lab, version 8.1) was used for Tukey test. Comparison of experimental

accumulative biogas and Gompertz modeling results were done by adjusted regression analysis.

RESULTS AND DISCUSSION

Variation of pH

pH is an important factor that can influence the activity of anaerobes. The ideal pH for anaerobic digestion is 6.6 to 7.8, and proper pH is always necessary to maintain the activity of the digester (Lay *et al.*, 1997). If the activity of methanogenic bacteria is inhibited by changes in the environment, VFA accumulates in the digester, resulting in reduced pH and failure of the digester. Fig. 1 depicts variations in pH owing to the difference in S/I ratio during SS-AD. In all treatments, the pH value decreased during the first 5 days because of VFA accumulation in response to hydrolysis and acidification of organic matter. It was steadily increased after 5 days then maintained around pH 7.8 due to the VFA consumption by methanogens.

Although pH decreased more with increasing S/I ratio (increasing beef manure content), pH level maintained proper range (6.8 – 7.8) throughout the entire experimental period.

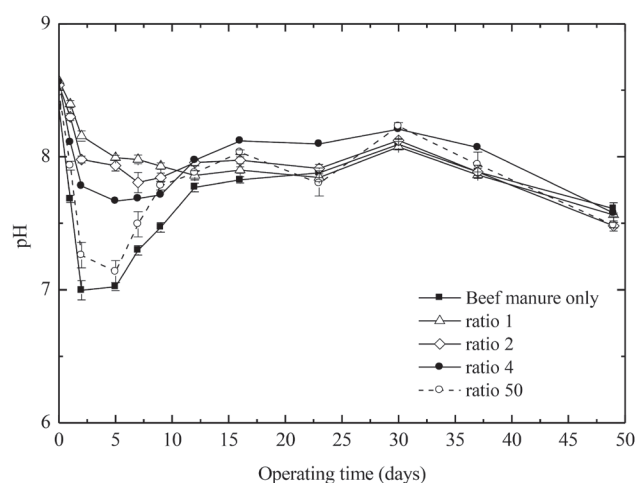


Fig. 1. Variation of pH in different beef manure to inoculum ratios assayed.

Variation of $\text{NH}_4\text{-N}$

Fig. 2 depicts variations in $\text{NH}_4\text{-N}$ concentrations at different S/I ratios during SS-AD. $\text{NH}_4\text{-N}$ concentrations of all treatments increased rapidly during the first five days as a result of protein hydrolysis by proteolytic bacteria. In the case of non-inoculated treatment (beef manure alone), the $\text{NH}_4\text{-N}$ concentration was about 2.4 times higher than the initial concentration after five days, whereas inoculated treatments (S/I ratio 1, 2, 4 and 50) showed increases of 3.2, 3.7, 4.0 and 4.3 times, respectively. These findings indicate that inoculation can be improved by hydrolysis of protein (Siebert and Toerien, 1969). At the end of the anaerobic digestion period, the $\text{NH}_4\text{-N}$ concentrations ranged from 764 to 883 mg/L. Based on these results, the $\text{NH}_4\text{-N}$ concentrations of all treatments were lower than 1500 mg/L, which

has been reported to have toxic effects on the anaerobic digestion process (McCarty, 1964). These findings demonstrated that neither non-inoculated nor inoculated treatments could cause significant $\text{NH}_4\text{-N}$ inhibition.

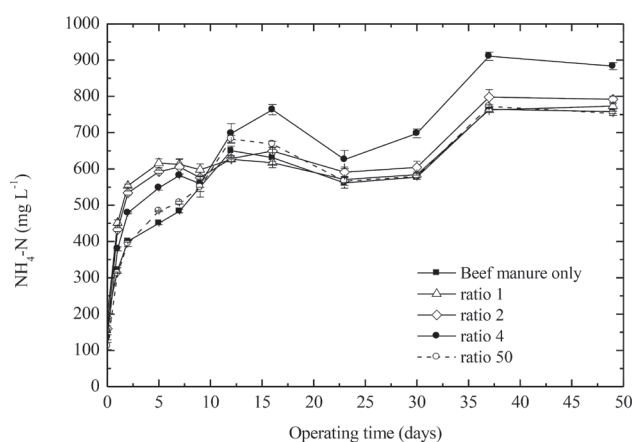


Fig. 2. Variation of $\text{NH}_4\text{-N}$ in different beef manure to inoculum ratios assayed.

Variation of VFA

Volatile fatty acids (VFAs) are one of the important factors for stable anaerobic digestion. In general, VFAs can be used to determine anaerobic digestion process stability as the concentration of VFAs changes in the digester. VFAs are intermediate products of anaerobic digestion that are decomposed by acetogenic and methanogenic bacteria. If the activity of methanogenic bacteria is inhibited, the pH is decreased by VFA accumulation, eventually leading to failure of anaerobic digestion. Fig. 3 depicts variations in total VFAs (TVFA) concentrations at different S/I ratios during SS-AD. Although the times to reach the maximum TVFA concentration showed the tendency to a steady reduction with decreasing S/I ratio, the maximum TVFA concentration showed trend of significant increment in proportion to S/I ratio. The maximum TVFA concentrations obtained were 2087, 267, 544, 1527, and 2760 mg/L for beef manure alone and treatments with S/I ratios of 1, 2, 4 and 50, respectively.

After the maximum VFA concentration was reached, the TVFA concentration decreased and was eventually removed from all treatments due to VFA was consumed by methanogens. The VFA/Alkalinity ratio is also an important indicator of stable anaerobic digestion. In general, when the VFA/Alkalinity ratio is lower than 0.4, the anaerobic digestion process is stable without accumulation of VFA, while when this ratio is higher than 0.8, it can lead to failure of anaerobic digestion (Karthikeyan and Visvanathan, 2013). Fig. 4 shows variations in the VFA/Alkalinity ratios at different S/I ratios during SS-AD. Continuous increases in VFA/Alkalinity were presented for all treatments during the first 5 days owing to VFA accumulation by accelerating hydrolysis and acidification of organic matter. Regardless of S/I ratio, VFA/Alkalinity values maintained appropriate range to lead to stable anaerobic digestion throughout the entire experi-

mental period.

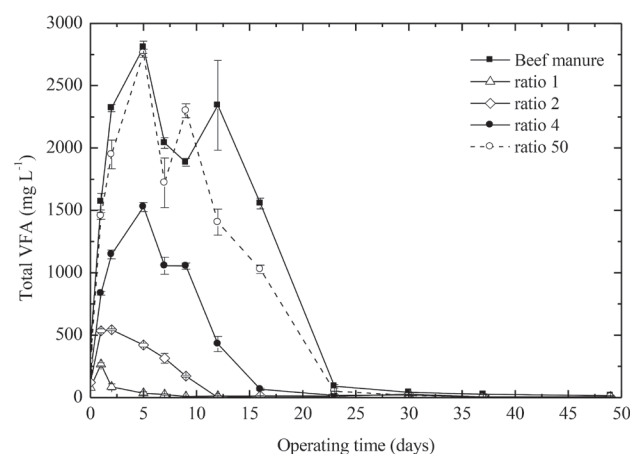


Fig. 3. Variation of TVFA in different beef manure to inoculum ratios assayed.

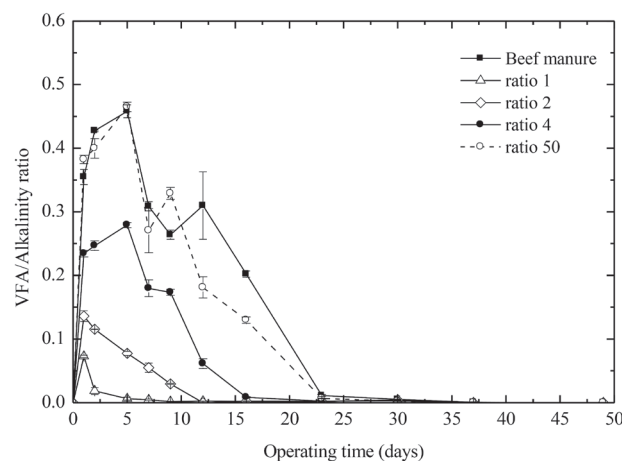


Fig. 4. Variation of VFA/Alkalinity ratio in different beef manure to inoculum ratios assayed.

Organic matter removal

Table 3 shows the ultimate biodegradability and volatile solids removal achieved under the different S/I ratios during SS-AD. The ultimate biodegradability of the beef manure and sawdust mixture ranges from 32 to 36%, indicating about 32–36% of the beef manure and sawdust bedding mixture is composed of biodegradable volatile solids, while about 64–68% of the remainder is composed of recalcitrant volatile solids. Total volatile solids (TVS) are composed of biodegradable volatile solids (BVS) and non-biodegradable volatile solids (NBVS). Only the BVS can be converted into biogas during anaerobic digestion, therefore BVS removal is more correlated with biogas production than TVS. BVS removal ranges from 68 to 79%. Although beef manure alone and an S/I ratio of 50 showed slightly higher BVS removal performance than others, they were not significantly different ($p > 0.05$).

Methane production rate and kinetic modeling

Fig. 5 shows the variations in CH_4 content at differ-

Table 3. Summary of ultimate biodegradability and volatile solids removal at different S/I ratios

(Mean±S.E., n=3)

	Initial VS(g)	BMR(g)	UB (%)	VS removal (%)	BVS* removal (%)
Beef manure only	127.9	35.2±0.2	33.4±0.2 ^a	27.5±0.2 ^{ab}	76.2±0.4 ^{ab}
ratio 1	32.8	8.0±0.3	31.6±0.8 ^a	24.5±1.1 ^a	67.7±2.4 ^a
ratio 2	52.1	12.8±0.3	32.0±0.91 ^a	24.5±0.6 ^a	67.8±1.4 ^a
ratio 4	74.1	20.1±0.7	35.8±1.2 ^a	27.1±1.1 ^{ab}	75.1±2.5 ^{ab}
ratio 50	110.5	31.5±0.5	36.1±0.6 ^a	28.5±0.6 ^b	78.9±1.3 ^b

note: 1) ^{a-b} Means with different superscripts in the same column of each group are significantly different (p<0.05).

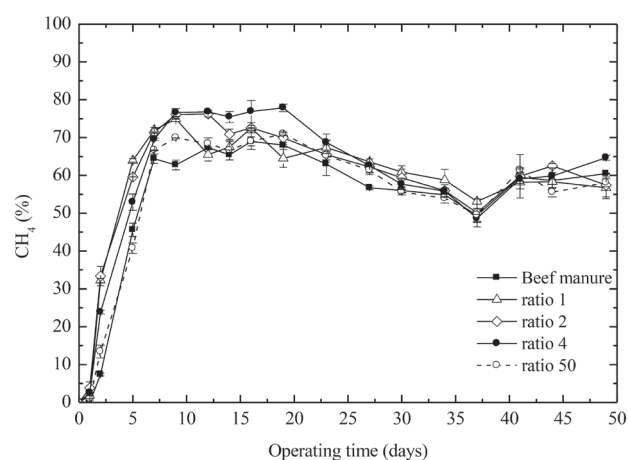
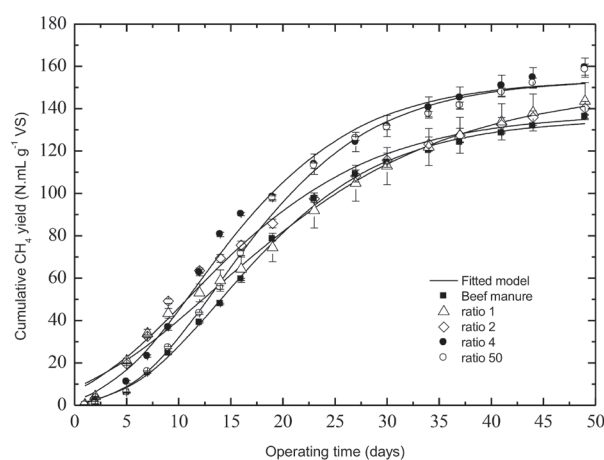
2) *S/I ratio 50 UB basis.

ent S/I ratios during SS-AD. A sharp increase in CH₄ concentration was observed in all treatments during the first 7 days, it reached above 60% regardless of S/I ratios.

Fig. 6 presents the measured and predicted cumulative methane yield with modified Gompertz model. The methane production potential (P) did not differ significantly among groups, with values of 135, 151, 138, 154 and 154 mL CH₄/g VS being observed for beef manure alone and with an S/I ratio of 1, 2, 4 and 50, respectively (p>0.05).

As shown in Table 4, the lag phase (λ) of the S/I ratio 1 test units was less than 1 day. Although it showed the shortest start-up period, it required significantly

longer T95 than other test units (p<0.05). In the case of the S/I ratio 1 test unit, T95 was reached in 52 days, whereas the rest of test units reached maximum methane production at 36 to 40 days. Based on the shortest T95, 36 days, cumulative methane production did not differ significantly among all test units (p>0.05), with values of 126, 128, 128, 147 and 145 mL/gVS being observed for beef manure alone, S/I ratio of 1, 2, 4 and 50, respectively. Although it showed similar cumulative methane production at HRT of 36 days, the beef manure alone and S/I ratio 50 test units showed the highest methane yields of 7.3 and 7.2 based on the volume of methane produced per volume of digester(V/V), respec-

**Fig. 5.** Variation of methane content in different beef manure to inoculum ratios assayed.**Fig. 6.** Measured and predicted cumulative methane yield with modified Gompertz model.**Table 4.** Experimental and Gompertz model parameters of cumulative methane production

(Mean±S.E., n=3)

Parameter	Beef manure only	ratio 1	ratio 2	ratio 4	ratio 50
Cumulative methane yield (mL/ gVS)	136.2±0.7 ^a	143.6±7.1 ^{ab}	140.3±2.6 ^{ab}	159.4±3.7 ^b	158.4±2.1 ^b
P (mL/gVS)	135.2±0.9 ^a	150.5±5.8 ^a	137.9±2.8 ^a	153.8±5.0 ^a	153.9±1.9 ^a
Rm (mL/gVS)	5.8±0.1 ^a	4.4±0.4 ^b	5.1±0.1 ^{ab}	6.8±0.1 ^c	7.0±0.0 ^c
Λ (day)	5.4±0.1 ^a	0.8±0.4 ^b	1.0±0.1 ^b	3.5±0.2 ^c	5.6±0.1 ^a
T95 (day)	39.6±0.7 ^a	51.8±2.5 ^b	40.5±0.5 ^a	36.2±1.0 ^a	37.9±0.3 ^a
V/V ¹ in HRT 36 days	7.3±0.0 ^a	1.9±0.1 ^b	3.1±0.1 ^c	4.9±0.1 ^d	7.2±0.1 ^a

Note: 1) ^{a-d} Means with different superscripts in the same row of each group are significantly different (p<0.05).2) ¹Volume of methane produced/Volume of digester.

Table 5. Utilization efficiency of the available digester volume

	Beef manure (g)	Inoculum (g)	Total amount (g)	Beef manure volume (mL)	Rate of digester utilization (%)
Beef manure only	533.5	0.0	533.5	770.0	100.0
ratio 1	136.6	572.8	709.4	197.2	25.6
ratio 2	217.6	456.0	673.6	314.0	40.8
ratio 4	309.1	323.9	633.0	446.1	57.9
ratio 50	461.0	104.7	565.7	665.3	86.9

tively. However, when considering utilization efficiency of the available digester volume, the beef manure alone treatment was 15% higher than that of the S/I ratio 50 treatment (Table 5). Therefore, the beef manure alone test unit was determined to be most efficient for treatment of a large amount of beef manure and sawdust mixture within a short period.

CONCLUSION

The effects of different S/I ratios (beef manure alone, 1, 2, 4 and 50) on methane production and organic matter removal of beef manure and sawdust bedding mixture were evaluated using SS-AD system operated in the batch mode under mesophilic conditions (37°C) for 49 days. All treatments demonstrated adequate stability of anaerobic digestion, regardless of the inoculum ratio, based on parameters indicating AD process stability including $\text{NH}_4\text{-N}$, TVFA and TVFA/Alkalinity ratio. Although beef manure only test unit showed similar methane production and BVS reduction to the rest of all inoculated test units, it showed the best available digester volume utilization performance. The results reveal that beef manure and sawdust bedding mixture can achieve successful SS-AD performance without inoculation because it contains enough anaerobic microorganisms originated from ruminant to activate anaerobic digestion.

ACKNOWLEDGEMENTS

This research was funded by the Rural Development Administration (Project No. PJ11694012015) Republic of Korea. However, we received an enormous help from Prof. Myung-Gyu Lee, Sangji University during our study. In particular, a special thanks to Prof. Yabe, Kyushu University in Japan for his collaboration in the experimental design and interpretation of results and help to write research paper in this study.

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