Doctoral Thesis

Bioenergy Potential of Slaughterhouse Waste and Enhancement of Anaerobic Digestion Parameters through Substrate Co-digestion

기질 혼합 분해를 통한 도축장 폐기물의 바이오 에너지 잠재성 및 혐기성 소화 변수의 개선방안

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Bioenergy Potential of Slaughterhouse Waste and Enhancement of Anaerobic Digestion Parameters through Substrate Co-digestion

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ABSTRACT

Bioenergy Potential of Slaughterhouse Waste and Enhancement of Anaerobic Digestion Parameters through Substrate Co-digestion

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Drastic change in food consumption pattern in South Korea since 1980 has led to increasing amount of livestock slaughtered causing increased waste generation in slaughterhouse. The total amount of slaughterhouse waste generated from South Korea in 2017 was around 260,018 and 127,539 tons for pig and beef cattle, respectively. Proper treatment of slaughterhouse waste in South Korea became an issue because previous disposal method (ocean dumping) was prohibited in 2012. One alternative solution was to treat slaughterhouse waste via anaerobic digestion. However, this process often varied in CH_4 production due to variation in waste characteristics. Thus, determination of waste characteristics was essential.

Around 13 million tons of swine slurry were produced in South Korea every year. Swine slurry accumulation might lead to an environmental pollution

that needs a proper treatment. Anaerobic digestion is an alternative technology to treat swine slurry. However, it was characterized with low CH_4 production that makes anaerobic digestion plant to be uneconomical. Substrate co-digestion might become solution regarding this problem.

Since there was high production of beef cattle slaughterhouse waste (BCSW) and pig slaughterhouse waste (PSW), this study was not aimed to compare the characteristics of BCSW and PSW but rather to determine the characteristics and BCSW or PSW co-digestion effect with livestock waste. As such, this study was divided into two main parts. In the first part, characterization of PSW and swine slurry (SS) was performed by examination of chemical, proximate, ultimate, and energy content. The energy content includes heating value determination by bomb calorimeter and CH_4 potential production by batch anaerobic digestion. In addition, effect of PSW co-digestion with SS to anaerobic digestion parameters was also determined. The PSW content in the co-digested mixture was 67%, 50%, or 33% weight per weight volatile solid basis (w/w VS basis). SS was added as remaining substrate to obtain 100% volatile solid in substrate mixture.

PSW heating value was 37 to 93.5% higher than heating value of various energy crops. However, high moisture content (81.8% of fresh matter) of PSW indicated that energy valorization by thermal conversion is uneconomical. In addition, PSW had high volatile solid content (94.5% of dry matter) that might complement the characteristics of SS that had low volatile solid content (67.6% of dry matter) during anaerobic co-digestion. Low volatile solid content in SS indicated that it had high minerals content that might be useful for anaerobic digestion.

Co-digestion experiment showed that high volatile solid content within PSW resulted in 22 to 84% increase of CH_4 production potential compare to SS sole digestion. However, low CH_4 production potential of SS, 48 to 62% of PSW CH_4 production potential, resulted in 7 to 32% decrease of CH_4 production potential compare to PSW sole digestion. Still, co-digestion shortened the lag phase period (3.3 to 8.5 days shorter) and effective digestion time (6.5 to 9.1 days faster) compare to PSW sole digestion. Short lag phase and faster digestion time during anaerobic co-digestion might be due to higher cobalt (Co) and nickel (Ni) concentration in SS than PSW. Co and Ni are important cofactor in CH_4 production (methanogenesis) stage during anaerobic digestion. It was concluded that, anaerobic digestion was a suitable option to treat PSW. In addition, co-digestion results in improved anaerobic digestion efficiency as seen from increased CH_4 production potential with no significant effect on effective digestion time compare to sole SS digestion. From PSW perspective, co-digestion reduced CH_4 production potential but improved digestion efficiency as seen from reduced lag phase period and effective digestion time.

In the second part, characterization of BCSW was performed with similar parameters with the first part study. In addition, effect of BCSW co-digestion with SS or cattle dung (CD) to anaerobic digestion parameter was also determined. The BCSW content in the co-digested mixture was 66%, 50%, or

33% w/w OM basis. SS or CD was added as remaining substrate to obtain 100% volatile solid in substrate mixture.

BCSW heating value was 41 to 99% higher than heating value of various energy crops. However, high moisture content (83.3% of fresh matter) of BCSW indicated that energy valorization by thermal conversion is uneconomical. In addition, BCSW had high volatile solid content (93% of dry matter) that might complement the characteristics of SS during anaerobic co-digestion. CD also had high volatile solid content (91% of dry matter).

Co-digestion experiment showed that high volatile solid content within BCSW resulted in 30.7 to 75.8% increase of CH_4 production potential compare to SS sole digestion. However, low CH_4 production potential of SS, 50 to 64% of BCSW CH_4 production potential, resulted in 3.64 to 25.6% decrease of CH_4 production potential compare to BCSW sole digestion. Still, co-digestion with SS shortened the lag phase period (2.8 to 7.8 days shorter) and effective digestion time (6.5 to 8.3 days faster) compare to BCSW sole digestion. BCSW anaerobic co-digestion with CD resulted in 3 to 27% decrease of CH_4 production potential compare to BCSW sole digestion. In addition, there was no significant effect to lag phase period and effective digestion time indicating CD was not suitable to be co-digested with BCSW. High Co and Ni concentration in the SS was the cause of improved anaerobic digestion efficiency (short lag phase and faster effective digestion time) during anaerobic co-digestion of BCSW and SS. Low Co and Ni concentration in CD was the cause of no improvement in anaerobic digestion efficiency. It was concluded that, anaerobic digestion was a suitable option to treat BCSW. In addition, co-digestion with SS results in improved anaerobic digestion efficiency as seen from increased CH_4 production potential with no significant effect on effective digestion time compare to sole SS digestion. From the BCSW perspective, co-digestion with swine slurry reduced CH_4 production potential but improved digestion efficiency as seen from reduced lag phase period and effective digestion time.

Based on the results from the first and second experiment, it was concluded that anaerobic digestion was an appropriate technology to treat PSW and BCSW. In addition, co-digestion provides solution to low CH_4 production associated with large scale anaerobic digestion of SS. Slaughterhouse waste co-digestion with SS increased CH_4 production potential without any adverse effect to the duration of lag phase period and effective digestion time indicating improved digestion efficiency. High concentration of Co and Ni in SS combined with high volatile solid content of slaughterhouse waste were the causes of these observations. This indicated that co-digestion of slaughterhouse waste and SS resulting in complementary effect in term of volatile solid and micro-nutrient availability for anaerobic digestion.

Keywords: Anaerobic digestion, beef cattle slaughterhouse waste, co-digestion, CH_4 production, heating value, pig slaughterhouse waste, swine slurry

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LIST OF ABBREVIATIONS

ABS	adult bovine serum
ADF	acid detergent fiber
BGP	biogas production
BMP	bio-methane potential
C	carbon
C/N ratio	carbon to nitrogen ratio
Co	cobalt
CSW	beef cattle slaughterhouse waste
DM	dry matter
FBS	fetal bovine serum
Fe	iron
H	hydrogen
HHV	higher heating value
N	nitrogen
NDF	neutral detergent fiber
Ni	nickel
O	oxygen
PSW	pig slaughterhouse waste
S	sulfur
SMY	specific CH_4 yield
STP	standard temperature at pressure of 0 °C and 1 atm

TKN	total kjedahl nitrogen
TMY	theoretical CH_4 yield
TS	total solid
VS	volatile solid

NOMENCLATURES AND UNITS

%DM	% of dry matter (total solid)
%FM	% of fresh matter
°C	degree Celsius
CH_4	methane gas
CO_2	carbon dioxide
d	days
D_{deh}	degree of anaerobic degradation
g	gram
H_2S	hydrogen sulfide
K	kelvin
mg/kg	milligram per kilogram
MJ/kg DM	mega joule per kilogram dry matter
MJ/kg FM	mega joule per kilogram fresh matter
ml CH_4/g VS_{added}	milliliter CH_4 per gram volatile solid added
ml	milliliter
M_{max}	maximum CH_4 production potential
NH_3	ammonia
Nml CH_4/g VS_{added}	Normalized milliliter methane gas per gram volatile solid added (CH_4 production at standard temperature and pressure of 273 K and 1 atm)
ppm	parts per million

R_{max}	maximum CH_4 production rate
S/I Ratio	substrate to inoculum ratio
t_0	temperature at standard temperature and pressure (273 K)
T_{90}	time required to obtain 90% M_{max}
T_{eff}	effective digestion time
t_i	temperature during experiment (308 K)
w/w VS basis	weight per weight volatile solid basis
λ	lag phase period

CHAPTER 1. GENERAL INTRODUCTION

1.1. Background of Research

1.1.1. Slaughterhouse Waste Status in Korea

Drastic change in food consumption pattern in South Korea since 1980 has led to increasing amount of slaughtered livestock (**Figure 1.1**). It was reported that meat consumption per capita in South Korea was increased from 13.9 to 43.5 kg in 2010 (Lee et al., 2015).

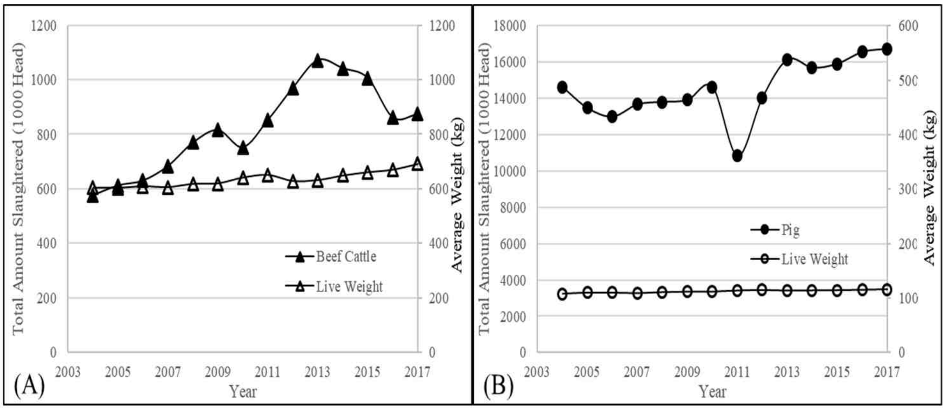

Figure 1.1. Total amount and average live weight of (A) Beef cattle and (B) Pig slaughtered in South Korea from 2004 until 2017 (APQA, 2018)

Figure 1.1 shows the amount of beef cattle and pigs slaughtered in South Korea from 2004 until 2017 (APQA, 2018). There is increasing trend in beef cattle and pigs slaughtered in Korea from 2003. The total number of beef cattle and pigs slaughtered in Korea in 2017 was 873,483 and 16.7 million heads, respectively (**Figure 1.1**). The average livestock weight slaughtered also has increased. It was reported that the live weight of beef cattle slaughtered increased from 603 kg/head in 2004 to 692 kg/head in 2017. Similarly, the live weight of pig slaughtered increased from 108 kg/head in 2004 to 116 kg/head in 2017 (APQA, 2018).

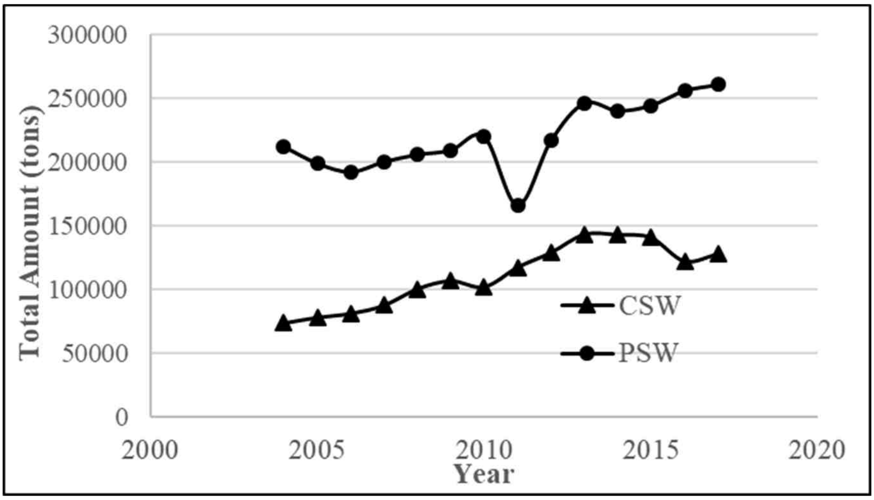

Figure 1.2. Estimation of slaughterhouse waste production in South Korea from 2004 until 2017 (APQA, 2018; Edstrom et al., 2003; Wang et al., 2018)

Other than meat and meat products for human consumption, slaughterhouses also generate other by-products such as blood, skins, fats, inedible animal tissues, and bones not intended for human consumption (Alvarez & Liden, 2008; Cuetos et al., 2008). The by-products, excluding bone, from slaughtered livestock was around 13.4% and 21.1% of live weight for pig and beef cattle, respectively (Edström et al., 2003; Wang et al., 2018). Thus, the total amount of by-products (slaughterhouse waste) generated from South Korea in 2017 was around 260,018 and 127,539 tons for pig and beef cattle, respectively (**Figure 1.2**).

1.1.2. Treatment and Disposal Method for Slaughterhouse Waste

The considerable amount of generated slaughterhouse waste requires proper treatment since improper and unsafe disposal can lead to serious environmental problems such as water, air, and soil pollution (Arvanitoyannis & Ladas, 2008). Several technologies had been utilized to properly treat slaughterhouse waste. These technologies include rendering, composting, alkaline hydrolysis, and anaerobic digestion (Franke-Whittle & Insam, 2013).

Rendering converts waste material into carcass meal, tallow, and water by separating the fat part from bone and protein. It includes several steps of chemical, mechanical, and thermal process such as cooking, decanting, drying, evaporating, grinding, mixing, pressing, separating, and solvent extraction (Franke-Whittle & Insam, 2013). The protein meal was previously utilized as component of animal feed. However, due to bovine spongiform encephalopathy (BSE) problem, utilization of protein meal to beef cattle was prohibited resulting in less role of rendering plant for the disposal of slaughterhouse waste (Franke-Whittle & Insam, 2013).

Composting is an aerobic process utilized microorganisms to degrade volatile solid. The end product of composting is called compost has beneficial effect as fertilizer and soil conditioner for agricultural practice (Haug, 1993). Since it is an aerobic process, composting requires continuous aeration in order to properly treat the organic waste. Still, it provides an economical and environmental friendly method to treat slaughterhouse waste (Franke-Whittle & Insam, 2013).

Alkaline hydrolysis (AH) utilizes sodium or potassium hydroxide (NaOH or KOH) and high pressure (103-486 kPa) and temperature (100-150°C) to hydrolyze protein, nucleic acids, carbohydrate, and lipids into amino acids, sugar, and soaps. The end products of AH can be released into sanitary sewer after post-treatment such as carbon dioxide injection to reduce pH to around 8 (Kaye et al., 1998). In addition, it contains peptide, amino acid, and fatty acids that are excellent nutrient for microbial growth (Franke-Whittle & Insam, 2013). Still, the high pH of end product requires further consideration for proper disposal and application of AH to treat slaughterhouse waste.

1.1.3. Anaerobic Digestion Technology

The slaughterhouse waste was characterized by high protein and fat content and have gained interest to be utilized as substrate for anaerobic digestion (Borowski & Kubacki, 2015; Rodríguez-Abalde et al., 2017; Wang et al., 2018). Anaerobic digestion is a biological process occurs in absence of oxygen to converts volatile solid into stabilized digestate and energy in form of CH_4 (Mata-Alvarez et al., 2014). The produced digestate can be utilized as fertilizer in agricultural practice. Moreover, the produced CH_4 can be utilized for heat and electricity production which can reduce fossil fuel consumption (Achinas et al., 2017). This technology has been applied to treat livestock, agriculture, industrial, and municipal waste (Franke-Whittle & Insam, 2013).

There are four major processes occur during the anaerobic digestion of organic waste which are hydrolysis, acidification, acetogenesis, and methanogenesis (**Figure 1.3**). This process was initially learned and almost similar to the fermentation that occurs in the rumen of ruminant. During hydrolysis process, hydrolytic bacteria convert macromolecules (organic waste) in the livestock waste such as carbohydrates, lipids, and protein into its associated monomers. For example, carbohydrate into glucose, lipid into fatty acids, and protein into amino acids (Renggaman et al., 2015).

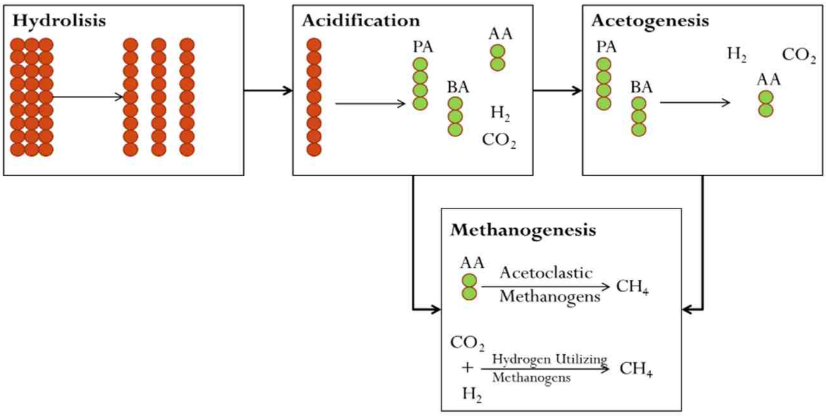

Figure 1.3. Illustration of the processes occurs during anaerobic digestion of organic waste

During acidification, acid-forming bacteria convert the monomers (glucose, fatty acids, and amino acids) into acetic acid, propionic acid, and butyric acid. This process also generates hydrogen (H_2) and carbon dioxide (CO_2) gas. The acetic acid, H_2, and CO_2 gas are directly utilized in the last step which is methanogenesis. Meanwhile, acetogenic bacteria convert the propionic and butyric acids into acetic acid in the acetogenesis process. This process also generates H_2 and CO_2 gas (Guo et al., 2015). The H_2 partial pressure needs to be maintained below 10^{-3} atm to ensure the acetogenesis process occurs well. This is achieved with the help of hydrogenotrophic methanogen which efficiently consume the produced H_2 gas and convert it into CH_4 gas.

The main pathways for CH_4 production (methanogenesis) in anaerobic digestion are acetoclastic and hydrogenotrophic pathway. Acetoclastic pathway converts acetate into CH_4 and contributes 70% to overall CH_4 production in anaerobic digestion. The remaining 30% is contributed by hydrogenotophic pathway that converts H_2 and CO_2 into CH_4 (Choong et al., 2016). Microbial group that responsible during these pathways are called methanogens.

Apart from volatile solid, anaerobic digestion requires adequate essential nutrients (trace element) for microbial growth and metabolism (Choong et al., 2016; Thanh et al., 2016) Among whole microbial trophic groups in anaerobic digestion, methanogens are considered to be the most sensitive to nutrient deficiency and less efficient in utilizing the nutrients compare to hydrolytic and acid forming bacteria due to their slow growth rate (Thanh et al., 2016). Inadequate nutrient during anaerobic digestion resulted in low CH_4 production and might lead to digestion failure (Romero-Güiza et al., 2016).

Table 1.1 summarized the role of trace elements and its recommended concentration in anaerobic digestion. The trace elements act as cofactor, that facilitates volatile solid conversion into CH_4. However, in regards to methanogenesis, adequate concentration of iron (Fe), molybdenum (Mo), tungsten (W), Nickel (Ni), cobalt (Co), and zinc (Zn) are very important.

Table 1.1. Trace elements role and recommended concentration in anaerobic digestion (Choong et al., 2016; Romero-Güiza et al., 2016)

Enzyme/ Cofactor	Process	Trace Element(s)	Recommended Concentration (mg/kg)	Reaction
Formate dehydrogenase	Acetogenesis	Fe Se W	<0.3 <0.04 <0.04	$HCOOH \leftrightarrow CO_2 + 2e^- + 2H^+$
Carbon monoxide dehydrogenase	Acetogenesis	Fe Zn Ni	<0.3 0.03<Zn<7.5 0.03<Ni<35	$CO + H_2O \leftrightarrow CO_2 + 2e^- + 2H^+$
Hydrogenase	Acetogenesis and methanogenesis	Fe Ni	<0.3 0.03<Ni<35	$H_2 \leftrightarrow 2e^- + 2H^+$
Carbonic anhydrase	Methanogenesis	Zn	0.03<Zn<7.5	$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$
Formylmethanofuran dehydrogenase	Methanogenesis	Mo/W	Mo<0.05/W<0.04	CO_2 + MF ↔ CHO-MF + H_2O
Methyltransferase/factor III	Methanogenesis	Co	0.03<Co<35	MeOH + CoM ↔ CH_3-CoM + H_2O
Methyl-CoM reductase/F_{430}	Methanogenesis	Ni	0.03<Ni<35	CH_3-CoM + [H] ↔ CH_4 + CoM

Anaerobic digestion of single substrates has some drawbacks such as inadequate volatile solid or nutrient concentration and high concentration of inhibitory compounds such as NH_3 or fatty acid (FA) (Mata-Alvarez et al., 2014). Inadequate nutrient concentration can be solved by nutrient supplementation into the anaerobic digestion. However, proper technique and knowledge regarding supplementation requirement are necessary in order to achieve optimal results (Choong et al., 2016). Excessive nutrients supplementation might result in digestion failure due to their toxic effect at high concentration to the microorganisms involved in anaerobic digestion.

Another method is by combining two or more organic wastes as substrate for anaerobic digestion. This practice is also known as substrate co-digestion (Mata-Alvarez et al., 2011). Anaerobic co-digestion is feasible strategy to overcome the drawback of mono-digestion through combining two or more substrates with complementary characteristics (Mata-Alvarez et al., 2014). The complementary characteristics might be based on carbon to nitrogen ratio (C/N ratio), macro- and micro-nutrient content, volatile solid content, pH value or alkalinity content, inhibitors content, and dry matter content (Mata-Alvarez et al., 2011).

Anaerobic co-digestion not only improves CH_4 production but also improves process stability and efficiency. In addition, it also might improve economic benefit due to equipment and infrastructures are utilized to treat several wastes (Mata-Alvarez et al., 2011). Another important consideration to determine co-digested substrate is the organic waste availability around the anaerobic digestion plant. This is related to waste transportation that affects overall operational cost (Mata-Alvarez et al., 2014).

1.1.4. Treatment and Disposal Method for Slaughterhouse Waste in South Korea

Previously, disposal of slaughterhouse waste in South Korea includes ocean dumping, composting, recycling as animal feed, and landfilling disposal (Lee et al., 2015; Yoon et al., 2014). However, in accordance with Marine Environment Management Act regulated by Ministry of Land, Transport, and Maritime Affairs in 2012, ocean dumping has been prohibited in South Korea. This makes all waste generated in lands, including slaughterhouse waste, needs to be treated and prevented from entering the ocean (Jeon et al., 2013; Yu et al., 2013).

Utilization of slaughterhouse waste as animal feed requires thermal pre-treatment (rendering) that has high operational cost. The transportation and storage of slaughterhouse waste for the treatment also proved to be costly that it is cheaper to utilize alternative material such as soybeans and corn (Urlings et al., 1992). Landfilling also has been criticized due to its high operational cost and insanitary issues (Lee et al., 2015).

Slaughterhouse waste was characterized by its high fat and protein content indicating it is an energy-rich organic material. As such, economic utilization of slaughterhouse waste is important. In recent years, several research had been conducted in Korea to convert slaughterhouse waste into economical products. For example, Jeon et al. (2013) conducted experiment to convert pig blood from slaughterhouse into amino acid liquefied fertilizer through crushing and protease decomposition. The product, rich in small peptide and amino acids, can be utilized to fertilize soil in agricultural practice. Amino acid fertilizer application to Bentgrass increased the nitrogen utilization rate (70.6-90.1% increase) and its chlorophyll content (22-39% increase) (Kim et al., 2003).

Yu et al. (2013) also showed potential alternative utilization of beef cattle blood from slaughterhouse as media for mammalian cell culture. The experiment was to compare the performance of adult bovine serum (ABS) and fetal bovine serum (FBS) for culturing mammalian cell. The performance of ABS was comparable with FBS in culturing astrocytes and fibroblasts. This results indicated that slaughterhouse blood also has potency to be utilized as biological agent.

In regards to solid waste from slaughterhouses such as viscera and offal, there are growing interest to utilize them as substrate for anaerobic digestion in South Korea. Lee et al. (2015) utilized cattle offal consists of internal organs such as liver, bronchus, intestines, liver, heart, and stomach as substrate for anaerobic digestion. The experiment was conducted in mesophilic batch system equipped with pH controller with substrate inoculum (S/I) ratio of 100:1 w/w VS basis. The experiment was conducted for 220 days in which CH_4 production was started at day 60 with exponential CH_4 production occurs at day 80. This indicated that at high S/I ratio, there was lag phase of around 70 days (**Table**

1.2). Still, the specific methane yield (SMY) was observed at 520 ml CH_4/kg VS_{added}.

Table 1.2. Summary of previous results regarding anaerobic digestion of slaughterhouse waste in South Korea

Substrate	S/I Ratio (w/w VS basis)[1]	Specific CH_4 Yield (ml CH_4/g VS)	Lag Phase (days)	References
Cattle offal	100:1	520	70	Lee et al. (2015)
Pig blood	0.1-1.5:1	450-799	0-0.3	Yoon et al. (2014)
Pig intestines residue	0.1-1.5:1	511-848	0-26.9	
Pig digestive tract content	0.1-1.5:1	357-1076	0-0.5	

[1] S/I ratio: Substrate to inoculum ratio; w/w VS basis: weight per weight volatile solid basis

Yoon et al. (2014) utilized pig blood, pig intestine residue, and pig digestive tract content separately as substrate for anaerobic digestion. The experiment was also conducted in mesophilic batch anaerobic digestion. Interestingly, the results showed that different waste has their own SMY and there was effect of S/I ratio to the SMY. The highest SMY was observed at 1,076 ml CH_4/g VS with S/I ratio of 0.1 (**Table 1.2**). The lowest SMY was observed at 357 ml CH4/g VS with S/I ratio of 1.5. Both results were observed from pig digestive tract content. The lag phase was only observed in pig intestines residues as substrate with the highest lag phase of 26.9 days observed at S/I ratio of 1.5.

Results from previous experiment indicated that different slaughterhouse waste and S/I ratio affect the SMY and lag phase during anaerobic digestion. In addition, the slaughterhouse waste from one livestock species usually mixed together when taken into the disposal or treatment site. As such, it is important to analyze the characteristics and bio-methane potential of slaughterhouse waste at different S/I ratio. Since there was high production of beef cattle slaughterhouse waste (BCSW) and pig slaughterhouse waste (PSW), this study was not aimed to compare the characteristics of BCSW and PSW but rather to determine characteristics and suitable treatment for each waste.

In addition, it is better to reduce the lag phase observed in the anaerobic digestion of slaughterhouse waste since prolong lag phase affect the overall time required to treat the waste. One possible solution regarding this matter is by utilizing co-digestion to treat slaughterhouse waste with other organic substrates. The co-digestion showed benefit in regards to anaerobic digestion process such as improving the balance of macro- and micro-nutrients, providing buffer capacity during the digestion process, reducing inhibitory and/or toxic material in the substrate, and increasing digestion and stabilization rate (reducing lag phase and improving SMY) (Alvarez & Liden, 2008; Borowski & Kubacki, 2015; Cuetos et al., 2008).

1.2. Research Objectives

The objectives of this study are:

1. Determine the characteristics of pig slaughterhouse waste and swine slurry.
2. Determine the co-digestion effect of pig slaughterhouse waste with swine slurry to anaerobic digestion parameters.
3. Determine the characteristics of beef cattle slaughterhouse waste and cattle dung.
4. Determine the co-digestion effect of beef cattle slaughterhouse waste with swine slurry or cattle dung to anaerobic digestion parameters.

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CHAPTER 2. CHARACTERISTICS OF PIG SLAUGHTERHOUSE WASTE AND ENHANCEMENT OF ANAEROBIC DIGESTION PARAMETERS THROUGH CO-DIGESTION WITH SWINE SLURRY

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2.1. Abstract

This study was done to determine the characteristics (chemical, proximate, ultimate, and energy content) of pig slaughterhouse waste (PSW) and swine slurry (SS) and determine PSW co-digestion effect with SS to anaerobic digestion parameters. PSW showed high volatile solid (VS) content of 94.6 % of dry matter (%DM). Meanwhile, SS had low VS content of 67.6 %DM. PSW also showed high energy content of 28 MJ/kg dry matter. It was 37 to 93.5% higher than energy content of energy crops and livestock waste. In term of anaerobic digestion parameters, both PSW and SS showed specific CH_4 yield (SMY) in the range of previously reported. Specifically, the SMY of PSW was 611 and 711 Nml CH_4/g VS_{added} for substrate to inoculum (S/I) ratio of 1 and 0.5 weight per weight volatile solid (w/w VS) basis, respectively. While SS showed SMY of 310 and 516 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5 w/w VS basis, respectively. Higher SMY in S/I ratio of 0.5 indicated that S/I ratio had significant effect during anaerobic digestion of PSW and SS. In addition, there was lag phase period (λ) of 9.7 and 9 days (d) for S/I ratio of 1 and 0.5, respectively, during anaerobic digestion of PSW. While, SS only showed λ of 0.5 and 1.5 d for S/I of 1 and 0.5, respectively. However, the difference of λ at different S/I ratio for PSW and SS was not statistically significant ($p>0.05$). PSW co-digestion with SS resulted in maximum CH_4 production potential (M_{max}) increased from 289.8 to 453.2 ml CH_4/g VS_{added} (SS sole digestion) to between 405 and 672 ml CH_4/g VS_{added} (PSW and SS co-digestion). However, low M_{max} of SS, 48 to 62% of PSW M_{max}, resulted in 7 to 32% decrease of CH_4 production potential compare to PSW sole digestion. Still, co-digestion shortened the λ from 9 and 9.7 d (PSW sole digestion) to between 1.2 and 5.7 d (PSW and SS co-digestion). It also shortened the effective digestion time (T_{eff}) from 20.9 and 24.0 d (PSW sole digestion) to between 13.5 and 15.7 d (PSW and SS co-digestion). Short λ and faster T_{eff} during anaerobic co-digestion might be due to higher cobalt (Co) and nickel (Ni) concentration in SS than PSW. It was concluded that anaerobic digestion was a suitable option to treat PSW. In addition, co-digestion results in improved anaerobic digestion efficiency as seen from 22 to 84% increase of M_{max} with no significant effect on T_{eff} compare to sole SS digestion. From PSW perspective, co-digestion reduced M_{max} but improved digestion efficiency as seen from reduced λ (3.3 to 8.5 d shorter) and T_{eff} (6.5 to 9.1 d faster).

Keywords: anaerobic digestion, pig slaughterhouse waste, energy content, swine slurry, co-digestion

2.2. Introduction

Drastic change in meat consumption per capita in South Korea from 13.9 kg in 1980 to 43.5 kg in 2010 has led to increasing amount of livestock slaughtered in the slaughterhouse (Lee et al., 2015). This also led to increased of by-products (slaughterhouse waste) generation from the slaughterhouse. It was reported that 16.7 million heads of pigs were slaughtered in South Korea equal to around 260,018 tons of pig slaughterhouse waste (PSW) generation in 2017 (APQA, 2018).

PSW consists mainly of blood, viscera (digestive tract tissues), and offal (internal organs) not intended for human consumption (Alvarez & Liden, 2008; Cuetos et al., 2008). It is characterized by high volatile solid content consist mainly of fat and protein indicated that PSW is rich energy waste.

The potential energy content of a substance is called heating value (Choi et al., 2014). Heating value is a standard energy measurement for a fuel. It is defined as quantity of energy when substance is burned or combusted (Choi et al., 2014; Sanli et al., 2014). The heating value can be determined theoretically through theoretical oxygen demand (ThOD) approach in which 13.64 MJ of energy was produced per 1 kg of ThOD (Haug, 1993). It is also can be determined experimentally using adiabatic bomb calorimeter (Choi et al., 2014). It is interesting to see the energy content of PSW in comparison to other organic substrate utilized in bioenergy production.

Previously, PSW in South Korea was disposed through ocean dumping, composting, recycling as animal feed, and landfilling. However, ocean dumping has been prohibited in South Korea in 2012. In addition, there was interest of utilizing slaughterhouse waste as substrate for anaerobic digestion in recent years (Lee et al., 2015; Yoon et al., 2014).

Around 13 million tons of swine slurry (SS) were produced in South Korea every year (Suresh & Choi, 2012). SS contains large amount of organic matter. Its accumulation leads to greenhouse gas and odor emission during storage and soil and water pollution through run-off and leachate (Renggaman et al., 2015). In addition, previous research showed that SS has 0.6 to 12.6% of total solid that makes it exists mainly in liquid form (Suresh et al., 2009). This means slurry transportation is uneconomical that on-site treatment or treatment near swine farming complex is more preferable (Renggaman et al., 2015).

Kim et al. (2012) reported that among 49 biogas plants available in South Korea in 2010, only 9 biogas plants were treating livestock manure. In addition, 13 biogas plants were treating mix of swine manure and food waste or any other mixtures. These biogas plants were characterized as uneconomical because the biogas production is lower than expected or the operational cost are too high. It is also reported that biogas production using sole substrate such as livestock manure is not economically sustainable (Møller et al., 2007). Substrate co-digestion might provide solution to this problem.

Co-digestion is defined as simultaneous digestion of two or more organic wastes to improve digestion stability and CH_4 production (Borowski & Kubacki, 2015; Mata-Alvarez et al., 2011; Pagés-Díaz et al., 2015). The positive effect of co-digestion is caused by dilution of inhibitory compounds, better macro- and micro-nutrient balance, and improvement of buffering capacity and volatile solid content of the co-digested mixture (Mata-Alvarez et al., 2014).

Co-digestion offers technological and economic advantage such as utilization of same infrastructure and equipment for treating several wastes. In regard to livestock waste, anaerobic digestion plants must produce as much CH_4 as possible to ensure its economic viability. Co-digestion results in higher CH_4 production than total amount of CH_4 produced from sole substrates (Mata-Alvarez et al., 2011).

The substrates utilized for co-digestion is selected based on several factors such as compatibility and geographical availability. Compatibility depends on whether the characteristics of organic wastes utilized for the experiment complement each other or not. Geographical availability depends on type of agro-food industry developed near the biogas plants or organic waste sources (Rodríguez-Abalde et al., 2017).

SS is characterized by high macro-, micro-nutrient and buffering capacity, but low volatile solid that results in low CH_4 yield during anaerobic digestion process (Rodríguez-Abalde et al., 2017). In large scale application, this often led to uneconomical operation. While PSW has low macro-, micro-nutrient, and buffering capacity but high volatile solid content.

In term of geographical availability, slaughterhouse is usually located around or near livestock farming facility to reduce the transportation cost and livestock stress during the transportation. This indicated that co-digestion between livestock waste and slaughterhouse waste are a feasible option to improve the anaerobic digestion parameters such as maximum CH_4 potential production (M_{max}), lag phase period (λ), and effective digestion time (T_{eff}).

The objectives of this study were to determine the characteristics (chemical, proximate, ultimate, and energy content) of PSW and SS and determine PSW co-digestion with SS effect to anaerobic digestion parameters.

2.3. Material and Method

2.3.1. Samples and Inoculum

The PSW samples were collected from a slaughterhouse in Yeongcheon City, Gyeongsang Province, South Korea. The facility slaughter around 189,466 swine in 2017 (APQA, 2018). The samples in these studies consist of livestock remains excepts for blood, brain, bones, and spinal cord. Blood was omitted since it has been processed separately by the slaughterhouse. Brain and spinal cord were also omitted due to safety reason. Once arrived it was ground and sieved into smaller pieces (<5 mm) and mixed thoroughly without any separation to emulate actual condition in the slaughterhouse. The samples then dried at 105°C for the chemical, ultimate, and higher heating value (HHV) analysis while untreated samples were utilized for the proximate and bio-methane potential (BMP) analysis. Remaining untreated samples were stored at -20°C until further used.

SS was collected from swine farm in Hoengseong, County, Gangwon Province, Republic of Korea. SS was filtered with 5 mm to ensure uniformity.

SS was dried at 105°C for the chemical, ultimate, and HHV analysis while untreated samples were utilized for the proximate and BMP analysis.

Table 2.1. Inoculum characteristics utilized in these studies

Parameter[1]	BMP Experiment[2]	Co-digestion Experiment[2]
TS (mg/L)	39,111±798	39,786±1515
VS (mg/L)	21,515±602	25,354±5035
VS/TS	0.55	0.64
pH	7.73±0.0	7.69±0.0

[1] TS: Total solid; VS: Volatile solid (organic matter)

[2] Values expressed as mean ± standard deviation

The inoculum used in the BMP experiment was coming from mesophilic anaerobic digestion treating swine slurry. The inoculum was transferred into serum bottle inside the laboratory and maintained by adding new substrate consist of swine slurry and slaughterhouse waste once a month. Prior to the experiment, the inoculum was degassed for two weeks to deplete any remaining organic materials and gas production from the inoculum. **Table 2.1** showed the characteristics of the inoculum utilized in both BMP and co-digestion experiments.

2.3.2. Experimental Design and Co-Digestion Experiment

Bio-methane Potential Analysis

Methane potential analysis was done in 250 ml serum bottles. Substrate (PSW or SS) and inoculum were added into the serum bottle. The S/I ratio utilized in this experiment were 1 and 0.5 (w/w VS basis). For S/I ratio of 0.5, 0.25 g VS of slaughterhouse waste was added into 0.50 g VS inoculum. Whereas, around 0.5 g VS slaughterhouse waste was added into 0.5 g VS inoculum for S/I ratio 1. Distilled water was then added to reach 200 ml total volume. Control bottle was prepared from the mixture of inoculum and distilled water to correct the biogas production. Sample of 50 ml was taken from each bottle for further analysis. During the sampling, the headspace was gassed with mixture of CO_2 and N_2 gas (20:80% volume per volume (v/v)) to create and maintain anaerobic condition inside the bottle. The serum bottles were then closed and sealed with rubber cap and aluminum crimps.

Co-Digestion Experiment

The batch anaerobic co-digestion of PSW and SS were carried out in five different PSW per SS (PSW/SS) ratio of 1:0, 0:1, 2:1, 1:1, and 1:2 weight per weight volatile solid basis (w/w VS basis). This equal to 100, 0, 67, 50, and 33% VS basis of PSW content in co-digested mixture. The experiments were also done at S/I ratio of 1 and 0.5 w/w VS basis. Similar with methane potential experiment, around 0.5 g VS of co-digested mixture was added to 0.5 g VS of inoculum for S/I ratio of 1. Whereas, around 0.25 g VS of co-digested mixture was added to 0.5 g VS inoculum for S/I ratio of 0.5. The summary of experimental design for co-digestion experiment can be seen in **Table 2.2**. The anaerobic digestion results for PSW and SS utilized as sole substrate was reported in the bio-methane potential experiment. Those results were compared with the results obtained from co-digestion experiment.

The substrate mixture and inoculum were added to 250 ml serum bottle. Distilled water was added so that the volume in each bottle reached 200 ml. Then, 50 ml of the mixture was sampled for further analysis. Control bottle was prepared from the mixture of inoculum and distilled water to correct the biogas production. During the sampling, the serum bottle headspace was gassed with mixture of CO_2 and N_2 (20:80% v/v) to create anaerobic condition. The serum bottles were then closed and sealed with rubber cap and aluminum crimps.

Table 2.2. Mixture composition and experimental design for anaerobic co-digestion experiment of pig slaughterhouse waste (PSW) with swine slurry (SS)

Code	Substrate (% Volatile Solid basis)[1]	
	PSW	SS
P1	100	0
P2	0	100
P3	67	33
P4	50	50
P5	33	67

[1] The co-digestion experiment was done in S/I ratio of 1 (A) and 0.5 (B) with similar mixture composition. All experiments were done in triplicate

2.3.3. Analytical Methods

Chemical, Proximate, and Ultimate Analysis

Chemical analysis constitutes the determination of samples fat, protein, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Fat content was determined with soxhlet extraction with ether as solvent. Protein content was determined with total kjedahl nitrogen (TKN). NDF and ADF content were determined following the procedure described in (Fernández-Cegrí et al., 2012). While hemicellulose content was calculated following **Equation 2-1**.

$$\%\mathrm{Hemicellulose} = \%\mathrm{NDF} - \%\mathrm{ADF} \qquad \textbf{(2-1)}$$

where:
%NDF = Neutral detergent fiber content, in % of dry matter (%DM)
%ADF = Acid detergent fiber content, in %DM

Proximate analysis constitutes the determination of moisture, total solid (TS), volatile solid (VS), and fixed solid (FS) content. The analysis was done following APHA standard method for total, fixed, and volatile solid determination in solid and semi solid samples (method number 2450G) (APHA, 2005). The analysis included drying the samples at 105°C overnight and combusting the dried samples at 550°C for 3 hours. The moisture, TS, VS, and FS content were determined following **Equation 2-2**, **Equation 2-3**, **Equation 2-4**, and **Equation 2-5**, respectively.

$$\mathrm{Moisture} = (\mathrm{X} - \mathrm{Y})/\mathrm{A} \times 100\% \qquad \textbf{(2-2)}$$

$$\mathrm{TS} = \mathrm{Y}/\mathrm{X} \times 100\% \qquad \textbf{(2-3)}$$

$$\mathrm{VS} = (\mathrm{Y} - \mathrm{Z})/\mathrm{Y} \times 100\% \qquad \textbf{(2-4)}$$

$$\mathrm{FS} = \mathrm{Z}/\mathrm{Y} \times 100\% \qquad \textbf{(2-5)}$$

where:

Moisture	= Moisture content, in % of fresh material (%FM)
TS	= Total solid content, in %FM
VS	= Volatile solid content, in % of dry matter (%DM)
FS	= Fixed solid content, in %DM
X	= Initial Sample weight, in gram (g)
Y	= Sample weight after drying at 105°C, in g
Z	= Sample weight after combustion at 550°C, in g

Prior to ultimate (elemental) analysis, samples were pretreated following the procedure described in Choi et al. (2014). The carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content of pretreated samples were analyzed with Elemental Analyzer (Flash EA 1112, Thermo Fisher Scientific, Germany) via combustion at 1014°C. The oxygen (O) content of the samples was analyzed with another instrument (Flash 2000 Elemental Analyzer, Thermo Fisher Scientific, Germany).

Higher Heating Value (HHV) Analysis

Dried samples were ground using mortar and pestle and then screened through sieves with 5 mm opening (DH.Si8021, DAIHAN Scientific). Samples were then pelletized using pellet press (2811, Parr Instrument) and analyzed with an oxygen bomb calorimeter (Model 1341 plain jacket calorimeter, Parr Instrument). Benzoic acid pellets (3415, Parr Instrument) were utilized to standardize the oxygen bomb calorimeter prior to the analysis.

Mineral Content Analysis

The mineral content such as cobalt (Co), iron (Fe), molybdenum (Mo), nickel (Ni), tungsten (W), and zinc (Zn) of PSW and SS were analyzed with inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ICP-7510, Shimadzu Corp., Kyoto, Japan). Prior to the analysis, the samples were digested with strong acid following the standard nitric acid-hydrochloric acid digestion (method number 3030F) (APHA, 2005).

Biogas Production, Composition, and Specific CH_4 Yield

The biogas production was analyzed using manometric method in which constant volume was maintained and headspace pressure increased was measured with pressure transducer (Himanshu et al., 2017). Still, high pressure in the headspace must be prevented because it can affect the microbial growth and activity. Thus, the excess gas needs to be released regularly (Valero et al., 2016). During these studies, the excess gas was released using a glass syringe until the pressure was similar to the start of incubation. Using this method, the amount of excess gas can be simultaneously determined. Since constant volume was maintained, the excess gas is equal to biogas production.

The gas composition from the headspace was analyzed using a gas chromatograph HP 6890N (Agilent Technologies) equipped with a HP-PLOT Q column (Agilent Technologies) and a thermal conductivity detector. The inlet, oven, and detector temperature were 40, 35, and 200°C, respectively. The CH_4 content was then utilized to determine CH_4 production using **Equation 2-**

6. In addition, CH_4 production was utilized to determine specific methane yield (SMY) using **Equation 2-7**.

$$\mathrm{MP} = \%\mathrm{CH_4} \times \mathrm{BGP} \quad \textbf{(2-6)}$$

$$\mathrm{SMY} = (\mathrm{MP}/\mathrm{VS})\,(\mathrm{t_0}/\mathrm{t_i}) \quad \textbf{(2-7)}$$

where:

MP	= CH_4 production, in ml
$\%CH_4$	= CH_4 content in the gas, in %
BGP	= Biogas production, in ml
SMY	= Specific CH_4 yield, in Nml CH_4/g VS_{added} or NL CH_4/kg VS_{added}
VS	= volatile solid content of initial samples, in g
t_0	= Temperature at standard condition, 273 K
t_i	= Temperature where the experiment was conducted, 308 K

Theoretical CH_4 Yield

Elemental analysis data was utilized to determine the chemical formula of organic waste (Yoon et al., 2014). Theoretical CH_4 yield can be determined empirically from chemical formula of organic waste suggested by Symons and Buswell (1933) and further developed by Boyle (1977). The equation is shown in **Equation 2-8**. This equation was still utilized to determine theoretical CH_4 from chemical formula of organic waste until today (Moukazis et al., 2018; Yoon et al., 2014). This equation considered the production of carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulfide (H_2S) gas that are the by-products of anaerobic digestion complex substrates.

$$C_aH_bO_cN_dS_e + xH_2O \rightarrow yCH_4 + zCO_2 + dNH_3 + eH_2S \qquad \textbf{(2-8)}$$

The reaction coefficient for H_2O (x), CH_4 (y), and CO_2 (z) can be determined using **Equation 2-9**.

$$x = \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)$$

$$y = \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) \qquad \textbf{(2-9)}$$

$$z = \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)$$

The theoretical CH_4 yield (TMY) of organic waste at standard temperature and pressure (STP) of 1 atm and 273 K can be determined using **Equation 2-10** as suggested by Pellera and Gidarakos (2016).

$$\mathrm{TMY} = 1000y/(12a + b + 16c + 14d + 32e) \qquad \textbf{(2-10)}$$

where:

TMY = Theoretical CH_4 yield, in Nml CH_4/g VS

The degree of anaerobic degradation (D_{deg}) was determined using **Equation 2-11**.

$$D_{deg} = \mathrm{TMY/SMY} \times 100 \qquad \textbf{(2-11)}$$

where:

D_{deg}= Degree of anaerobic degradation, in %

Kinetic model

Biogas production curve during anaerobic digestion of complex organic material corresponds to slower flat curve (Rao et al., 2000). Thus, lag phase (λ) is also an important factor determining anaerobic digestion efficiency apart from cumulative CH_4 yield and CH_4 production rate (Zhang et al., 2014). λ can be estimated by modified Gompertz formula as follows (Kafle et al, 2013):

$$M(t) = M_{max} \exp\left\{-\exp\left[\frac{R_{max} e}{M_{max}}(\lambda - 1) + 1\right]\right\} \quad \textbf{(2-12)}$$

where:

$M(t)$ = Cumulative CH_4 yield at digestion time t, in NmL CH_4/g VS_{added}

M_{max} = Maximum CH_4 production potential, in NmL CH_4/g VS_{added}

R_{max} = Maximum CH_4 production rate, in NmL CH_4/g VS_{added}/day

λ = Lag phase period, in days (d)

t = Obervation Time, in d

e = exp(1)= 2.7183

A non-linear least-square regression analysis was performed using Excel solver add-in (Microsoft Excel 2016) to determine M_{max}, R_{max}, λ, and correlation coefficient (R^2) of produced model. In addition, the Excel solver was also utilized to estimate T_{90} that is time required to obtain 90% M_{max}. Utilizing T_{90} and λ, effective digestion time (T_{eff}) can be calculated by **Equation 2-13:**

$$T_{ef} = T_{90} - \lambda \qquad \textbf{(2-13)}$$

where:

T_{eff} = Effective digestion time, in d

T_{90} = Time required to obtain 90% M_{max}, in d

The simulated maximum CH_4 production potential from the co-digested mixture (M_{sim}) was calculated by the proportion of PSW and SS in the mixture and the M_{max} estimated by the modified Gompertz formula for the sole PSW or SS anaerobic digestion, as showed in **Equation 2-14** (Zhang et al., 2014):

$$M_{sim} = M_{PSW} \times \%Y_{PSW} + M_{SS} \times \%Y_{SS} \qquad \textbf{(2-14)}$$

where:

M_{sim} = Simulated maximum CH_4 production potential of co-digested mixture obtained from the modified Gompertz formula, in NmL CH_4/g VS_{added}

M_{PSW} = M_{max} of PSW obtained from the modified Gompertz formula, in NmL CH_4/g VS_{added}

$\%Y_{PSW}$ = Percentage of PSW in the mixture, in %

M_{SS} = M_{max} of SS obtained from the modified Gompertz formula, in NmL CH_4/g VS_{added}

$\%Y_{SS}$ = Percentage of SS in the mixture, in %

Synergistic Effect

Synergistic effect is inner reactions produced by the co-digestion of different components. Each co-digested substrate can influence the CH_4 production rate (Nielfa et al., 2015). Synergistic effect was calculated using **Equation 2-15**:

$$\alpha = M_{co-digestion} / M_{sim} \qquad \textbf{(2-15)}$$

where:

$M_{co\text{-}digestion}$ = Experimental M_{max} obtained from modified Gompertz formula (**Equation 2-12**) of co-digested substrate, in NmL CH_4/g VS_{added}

The α value determines type of synergistic relation among co-digested substrate. Specifically, **α** > 1 indicated that co-digested substrates have synergistic effect, **α** = 1 indicated that co-digested substrates work independently during the digestion process, and **α** < 1 indicated that co-digested substrates have antagonistic effect (Nielfa et al., 2015).

Statistical Analysis

One-tail t-test was done to compare the anaerobic digestion parameter (SMY, M_{max}, R_{max}, λ, T_{90}, T_{eff}) of PSW and SS at different S/I ratio. One-way ANOVA followed by Tukey HSD was done to determine the effect of PSW co-digestion with SS to the anaerobic digestion parameters at the same S/I ratio (1 or 0.5). P-value of 0.05 was utilized for all test.

2.4. Results and Discussion

2.4.1. Characteristics of Pig Slaughterhouse Waste and Swine Slurry

Pig slaughterhouse waste (PSW) and swine slurry (SS) characteristics were shown in **Table 2.3**. PSW had total solid (TS) content of 18.2 % of fresh weight (%FM) with high volatile solid (VS) content (94.57 % of dry matter (%DM)).

The VS (organic matter) mainly constituted of protein (30.44 %DM) and fat (53.64 %DM). The TS content was actually lower than those reported in previous worked. It was reported that TS content from PSW was ranged between 27.9 and 55 %FW (Hejnfelt & Angelidaki, 2009; Rodríguez-Abalde et al., 2017; Ware & Power, 2016). This might be due to washing and cleaning that was done to the offal for sanitary purpose by the slaughterhouse prior to sample collection.

The fat and protein content observed in PSW were in ranged and even higher than previous report. Yoon et al. (2014) reported that pig intestinal residue and digestive tract content from slaughterhouse in South Korea had protein content between 15.1 and 40.1 %DM and fat content between 4 and 15.3 %DM. The difference might be caused by the PSW in this study not only consisted of intestine and digestive tract content but also offal that increased its fat content. Previous studies found that pig offal has fat content between 41.8 and 65.76 %DM and protein content between 20.1 and 31.6 %DM, respectively (Rodríguez-Abalde et al., 2017; Ware & Power, 2016). High fat and protein content in the PSW indicating it was an energy-rich substrate.

SS had low TS content of 4.1 %FM. In addition, VS content in SS was only 67.6 %DM indicating high mineral (fixed solid) content. The VS was mainly constituted from protein (28.1 %DM) and neutral detergent fiber (27.4 %DM). SS is a mixture of urine, manure, wasted feed, and flushing water (Renggaman et al., 2015; Suresh & Choi, 2012). The protein and neutral detergent fiber of SS were come from the manure and wasted feed. Manure itself is undigested feed material from the livestock digestive tract. The TS and VS content of SS were within range of previously reported. SS collected from South Korea had TS content between 0.6 and 12.6 %FM with VS content between 56 and 84 %DM (Suresh et al., 2009).

Table 2.3. Characteristics of pig slaughterhouse waste and swine slurry utilized in this study

Parameters[1]	**Pig Slaughter Waste**[2]	**Swine Slurry**[2]
Total Solid (%FM)	18.20±0.7^{B}	4.1±0.2^{A}
Volatile Solid (%DM)	94.57±0.2^{B}	67.6±0.8^{A}
Fixed Solid (%DM)	6.82±0.6^{A}	32.4±0.8^{B}
Total Kjedahl Nitrogen (%DM)	4.87±1.0^{A}	4.5±0.1^{A}
Protein (%DM)	30.44±6.1^{A}	28.1±0.9^{A}
Fat (%DM)	53.64±1.9^{B}	5.6±0.2^{A}
Neutral Detergent Fiber (%DM)	7.26±1.8^{A}	27.4±0.8^{B}
Acid Detergent Fiber (%DM)	3.45±1.0^{A}	10.7±0.0^{B}
Hemicellulose (%DM)	3.81±0.9^{A}	16.7±0.8^{B}

[1] %FM: % of fresh matter, %DM: % of dry matter

[2] Values expressed as mean ± standard deviation

[A,B] Means in the same row with different uppercase letter differs significantly ($p<0.05$)

Compare to SS, PSW had higher VS and fat content. While SS had higher fixed solid (FS), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose content than PSW. Fat is an energy-rich substance indicating PSW might have higher energy content than SS. In addition, high VS content indicating that more organic matter is available in PSW to be converted into CH_4 during anaerobic digestion. However, low FS content in PSW indicating PSW had low mineral content that might inhibit CH_4 generation rate. In contrary, SS had low VS with high FS content indicating that less organic matter is available in SS to be converted into CH_4. However, SS might have adequate mineral content for anaerobic digestion.

2.4.2. Energy Content of Pigs Slaughterhouse Waste and Swine Slurry

PSW showed high energy content (HHV) of 28.43 MJ/kg DM (**Figure 2.1**). This was higher than energy content of various renewable resources. **Figure 2.1** showed that energy crops had HHV between 14.69 and 20.71 MJ/kg DM (Nhuchhen & Salam, 2012; Yin, 2011). Whereas, livestock manure collected in South Korea had HHV between 11.92 and 19.44 MJ/kg DM (Choi et al., 2014). Palm kernel had the highest HHV among the energy crops, while swine manure had the highest HHV among the livestock waste group. High HHV of PSW indicated that it has potential to be utilized as substrate for bioenergy production.

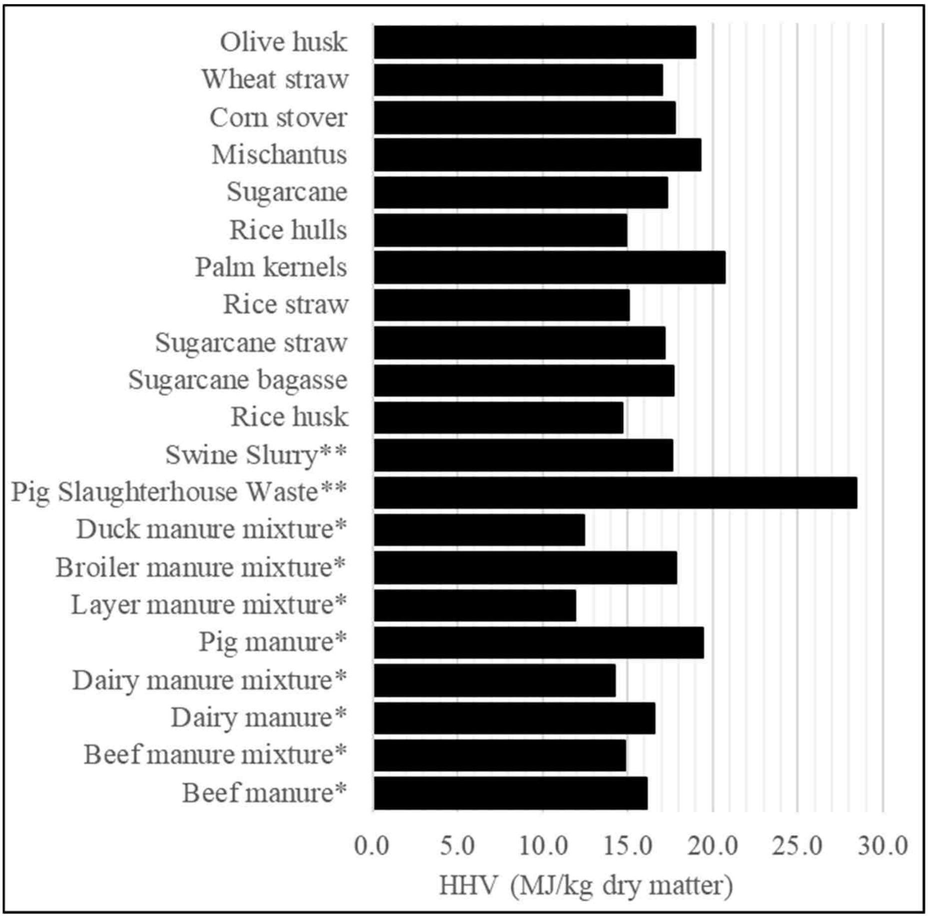

Figure 2.1. Energy content (HHV) of pig slaughterhouse waste and swine slurry in comparison to various renewable resources (Choi et al., 2014; Nhuchhen & Salam, 2012; Yin, 2011). * Average value of 12 samples; ** This study

SS had HHV of 17.6 MJ/kg DM (**Figure 2.1**). This was within ranged of HHV from livestock waste sampled in South Korea. Choi et al. (2014) reported that HHV of various livestock waste in South Korea was between 11.9 and 19.44 MJ/kg DM. The highest HHV was observed in pig manure. SS in this experiment had low VS content of 67.6% that might cause lower HHV than those observed previously. The HHV had positive correlation with VS content

(Choi et al., 2014; Nhuchhen & Salam, 2012; Yin, 2011).

The HHV was reported in MJ/kg DM because the samples were dried prior to the analysis. However, low TS content in PSW (18.2 %FM) and SS (4.1 %FM) (**Table 2.3**) indicated that the HHV of PSW and SS was only 5.17 and 0.72 MJ/kg FM, respectively. This showed that physical energy valorization from PSW and SS is not sustainable (Moukazis et al., 2018). This because, only small amount of energy can be recovered from thermal treatment of fresh PSW and SS. Thus, alternative technology to recover energy from PSW and SS is necessary.

Energy crops are common substrate for anaerobic digestion because of its high biogas potential. In addition, utilization of energy crop (maize) as substrate for anaerobic digestion had been successfully applied in large-scale plants across Europe (Lijó et al., 2017). Livestock waste was also characterized by low TS content (Suresh et al., 2009). It has been utilized as substrate for anaerobic digestion. Thus, anaerobic digestion can be an alternative technology to recover energy from PSW and SS.

2.4.3. Anaerobic Digestion of Pig Slaughterhouse Waste and Swine Slurry

Table 2.4 showed the ultimate analysis results, empirical chemical formula, theoretical CH_4 yield (TMY), specific CH_4 yield (SMY), and degree of anaerobic digestion (D_{deg}) of pig slaughterhouse waste (PSW) and swine slurry (SS). S/I ratio had significant effect on the SMY and D_{deg} for PSW and SS

($p<0.05$).

Table 2.4. Ultimate analysis, empirical formula, theoretical methane yield (TMY), specific methane yield (SMY), and degree of anaerobic digestion (D_{deg}) from pig slaughterhouse waste (PSW) and swine slurry (SS)

Parameter[1]	PSW[2]	SS[2]
Carbon (%DM)	58.45±1.92	37.3±0.3
Hydrogen (%DM)	8.83±0.54	5.2±0.0
Oxygen (%DM)	22.14±3.48	23.7±0.7
Nitrogen (%DM	3.66±0.00	4.8±0.1
Sulfur (%DM)	0.64±0.16	1.0±0.1
Empirical formula	$C_{37.1}H_{65}O_{9.5}N_{3.2}S_{0.1}$	$C_{9.8}H_{16.5}O_{4.7}N_{1.1}S_{0.1}$
TMY (Nml CH_4/g VS_{added})	725.5 ± 0.51^{B}	529.54 ± 9.0^{A}
SMY at S/I ratio of 1 (Nml CH_4/g VS_{added})	$611.5\pm13.2^{a,B}$	$310.1\pm9.0^{a,A}$
SMY at S/I ratio of 0.5 (Nml CH_4/g VS_{added})	$711.2\pm9.9^{b,B}$	$516.3\pm11.1^{b,A}$
D_{deg} at S/I ratio of 1 (%)	$84.3\pm1.8^{a,B}$	$58.6\pm1.7^{a,A}$
D_{deg} at S/I ratio of 0.5 (%)	$98.0\pm1.4^{b,A}$	$97.5\pm2.1^{b,A}$

[1] %DM: % of dry matter

[2] Values are expressed as mean ± standard deviation

[a,b] Means in the same column with different lowercase letter differ significantly ($p<0.05$)

[A,B] Means in the same row with different uppercase letter differ significantly ($p<0.05$)

PSW had TMY of 725.5 Nml CH_4/g VS. The SMY of PSW at S/I ratio of 1 and 0.5 was 611.5 and 711.2 Nml CH_4/g VS_{added}, respectively. The D_{deg} of PSW at S/I ratio of 1 and 0.5 was 84.3 and 98%, respectively (**Table 2.4**). The SMY of PSW was comparable with previous results. Previous results showed that SMY of PSW was between 357 and 866 Nml CH_4/g VS_{added}. Specifically, Yoon et al. (2014) reported that anaerobic digestion of pig intestine residue and digestive tract content showed SMY of 357 to 589 Nml CH_4/g VS_{added}. The anaerobic digestion of pig offal from Spain showed high SMY of 866 Nml CH_4/g VS_{added} (Rodríguez-Abalde et al., 2017). Anaerobic digestion of mixed PSW (blood, meat, fat, and flour) in Denmark showed the highest SMY of 620 Nml CH_4/g VS_{added} (Hejnfelt & Angelidaki, 2009). Anaerobic digestion of pig slaughterhouse mixture (meat tissue, fat, bristles, and intestinal wastes) from Poland showed SMY of 839.2 Nml CH_4/g VS_{added} (Borowski & Kubacki, 2015). The wide range of SMY from PSW occurred because of variations in the PSW characteristics.

The TMY of SS was 529.5 Nml CH_4/g VS. The SMY of SS was 310.1 and 516.3 Nml CH_4/g VS_{added} at S/I ratio of 1 and 0.5, respectively. These corresponded to D_{deg} of 58.6 and 97.5% at S/I ratio of 1 and 0.5, respectively. At S/I ratio of 1, SS had low degradability in comparison to PSW (84%). This might be due to low VS content observed in the SS or production of inhibitory compound during anaerobic digestion process. Anaerobic digestion of SS often resulted in ammonia production due to protein degradation.

The SMY value of SS observed in this study was within the range of previously reported. Zhang et al. (2014) reported that the SMY from anaerobic digestion of pig manure was 358.7 Nml CH_4/g VS_{added}. SMY of 204 Nml CH_4/g VS_{added} was also reported during anaerobic digestion of SS (Rodríguez-Abalde et al., 2017). Chae et al. (2008) observed at feed loads between 5 and 40% (v/v reactor) the SMY was in ranged of 228 to 437 Nml CH_4/g VS.

The D_{deg} of 84.3 and 98% from PSW after 50 days of anaerobic digestion indicated that PSW was highly degradable. In addition, SMY of PSW was significantly higher than SMY of SS at S/I ratio of 1 and 0.5. This indicated that PSW had potency as substrate for anaerobic digestion. Still, other anaerobic digestion parameters need to be analyzed to determine the applicability and necessary improvement strategies for PSW.

The improvement of D_{deg} and SMY at low S/I ratio were also observed in previous study. Yoon et al., (2014) reported that the D_{deg} of pig intestine residue and pig digestive tract content improved from 77.0 to 85.8% and from 69.9 to 86.3% when S/I ratio reduced from 1 to 0.5, respectively. The SMY of pig digestive content was also improved from 361 ml CH_4/g VS_{added} at S/I ratio of 1 to 446 ml CH_4/g VS_{added} at S/I ratio of 0.5.

High inoculum concentration during batch anaerobic digestion can prevent VFA accumulation from hydrolysis and acidification process at the initial stage of anaerobic digestion through rapid conversion of organic acid into CH_4. It was also reported that anaerobic digestion can run successfully even without pH adjustment at high inoculum concentration as also observed in this study. It was recommended that S/I ratio of 0.5 or 1 to be utilized for batch anaerobic digestion (Neves et al., 2004). Toxic compound dilution from the substrate or during the anaerobic digestion was also another advantage of utilizing high inoculum concentration during batch anaerobic digestion. Thus, it might explain the SMY and D_{deg} improvement at S/I ratio of 0.5 in comparison of S/I ratio of 1 from PSW and SS.

Table 2.5. Anaerobic digestion parameters of pig slaughterhouse waste and swine slurry estimated by modified Gompertz formula (**Equation 2-12**)

Parameter*	**Pig slaughterhouse waste****		**Swine slurry****	
	S/I ratio of 1	**S/I ratio of 0.5**	**S/I ratio of 1**	**S/I ratio of 0.5**
M_{max} (Nml CH_4/g VS_{added})	$598.7\pm13.3^{a,B}$	$723.7\pm17.0^{b,2}$	$289.8\pm8.6^{a,A}$	$453.2\pm11.0^{b,1}$
R_{max} (Nml CH_4/g VS_{added}/d)	$34.3\pm2.9^{a,B}$	$36.2\pm2.8^{a,1}$	$20.1\pm0.9^{a,A}$	$35.0\pm2.4^{b,1}$
λ (day)	$9.7\pm2.4^{a,B}$	$9.0\pm0.2^{a,2}$	$0.2\pm0.1^{a,A}$	$1.5\pm0.2^{b,1}$
Correlation Coefficient (R^2)	0.999	0.999	0.989	0.991
T_{90} (d)	$30.7\pm0.7^{a,B}$	$33.0\pm2.2^{a,2}$	$17.4\pm0.3^{a,A}$	$17.0\pm0.8^{a,1}$
T_{eff} (d)	$20.9\pm1.8^{a,B}$	$24.0\pm2.3^{a,2}$	$17.2\pm0.2^{b,A}$	$15.5\pm1.0^{a,1}$

* M_{max}: Maximum CH_4 potential production, R_{max}: Maximum CH_4 production rate, λ: Lag phase period, T_{90}: Time required to obtain 90% of M_{max}, T_{eff}: Effective digestion time (T_{90} - λ)

** Values are expressed as mean ± standard deviation

a,b Means of the same substrate (PSW or SS) at different S/I ratio with different lowercase letter differ significantly ($p<0.05$)

A,B Means at S/I ratio of 1 with different uppercase letter differ significantly ($p<0.05$)

[1,2] Means at S/I ratio of 0.5 with different number differ significantly ($p<0.05$)

Modified Gompertz formula (**Equation 2-12**) was utilized to estimate the maximum CH_4 production potential (M_{max}), maximum CH_4 production rate (R_{max}), lag phase period (λ), time required to obtain 90% M_{max} (T_{90}), and effective digestion time (T_{eff}). The anaerobic digestion parameters estimated by modified Gompertz formula were shown in **Table 2.5**.

The estimated cumulative CH_4 yield (CMY) from modified Gompertz formula were plotted against the experimental CMY of PSW and SS to test the model accuracy. The results were shown in **Figure 2.2**. The correlation coefficient (R^2) was in ranged of 0.989 to 0.999 (**Table 2.5**) indicating that modified Gompertz formula had the best fit to the substrate utilized in the experiment. Modified Gompertz formula was shown to have better fitness for predicting CH_4 production from complex organic substrates. For example, CMY curve from anaerobic co-digestion of SS and dewatered sewage sludge was best fitted with modified Gompertz formula (Zhang et al., 2014). Kafle and Kim (2013) also showed that CMY curve from anaerobic co-digestion of SS and apple waste was best fitted with modified Gompertz formula.

The maximum CH_4 production potential (M_{max}) showed similar pattern with SMY. Low S/I ratio resulted in higher M_{max} for PSW and SS ($p<0.05$). PSW had significantly higher M_{max} than SS at both S/I ratio (P<0.05]. PSW had M_{max} of 598.7 and 723.7 Nml CH_4/kg VS_{added} at S/I ratio of 1 and 0.5, respectively. High M_{max} at low S/I ratio was due to inhibitory compound dilution by high inoculum concentration.

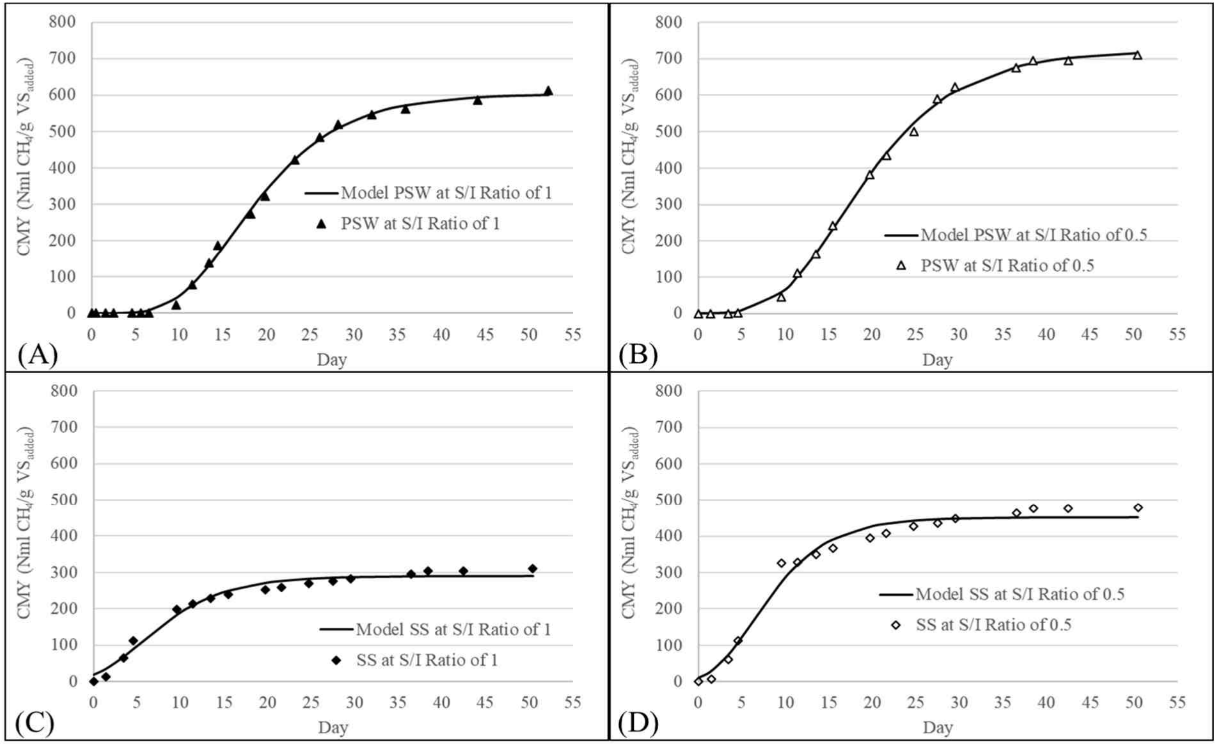

Figure 2.2. Cumulative CH_4 yield (CMY) estimated by modified Gompertz formula (line) plotted against experimental CMY (bullet points) of pig slaughterhouse waste (PSW) and swine slurry (SS). (A) PSW at S/I ratio of 1, (B) PSW at S/I ratio of 0.5, (C) SS at S/I ratio of 1, (D) SS at S/I ratio of 0.5

Aside from M_{max}, λ and digestion time (T_{90} and T_{eff}) are also important anaerobic digestion parameters (Xie et al., 2011). λ is an indicator of methanogens adaption to the environment. In addition, it also represents the substrate bioavailability during anaerobic digestion (Ware & Power, 2016). **Table 2.5** showed that λ of 9 to 9.7 days (d) was estimated during anaerobic digestion of PSW at S/I ratio of 1 and 0.5, respectively. Long λ observed in this study indicated that VS in PSW was not readily available for the methanogen. This could be related to high fat content in the PSW (53.6 %DM). Macromolecule such as fat needs to undergo hydrolysis, acidogenesis, and acetogenesis to produce acetic acid, CO_2, and H_2 which are substrates for methanogenesis process (Guo et al., 2015). Thus, there was lag phase observed during the CH_4 production in batch anaerobic digestion. Rodríguez-Abalde et al. (2017) observed 7 d of λ occurred during batch anaerobic digestion of PSW with fat content of 65.7 %DM. One of the possible caused is microbial adaptation to high fat content (Silvestre et al., 2011).

PSW showed potential as substrate for anaerobic digestion based on the SMY, D_{deg}, and M_{max} values. However, long λ might affect overall anaerobic reactor performance especially the digestion time. **Table 2.5** showed T_{90} and T_{eff} values for anaerobic digestion of PSW. The technical digestion time, T_{90}, is defined as required time to obtain 90% of M_{max} (Kafle & Kim, 2013). The T_{90} calculated in this study was 30.7 and 33 days for PSW at S/I ratio of 1 and 0.5, respectively. Subtracting T_{90} with λ gave T_{eff} (effective digestion time). The T_{eff} for PSW at S/I ratio of 1 and 0.5 was 20.9 and 24 days, respectively. There was no significant difference for T_{90} and T_{eff} at different S/I ratio ($p>0.05$). Still, the λ, T_{90}, and T_{eff} of PSW was significantly higher than those of SS at S/I ratio of 1 and 0.5.

T_{eff} result indicated that most CH_4 production from anaerobic digestion of PSW requires only 20.9 to 24 days assuming no λ occurred at the beginning of the digestion process. Thus, λ presence made the digestion process to be longer than necessary. Long λ, T_{90}, and T_{eff} might be caused by high fat and low mineral content in the PSW. As explained before, fat needs to be converted into volatile fatty acid prior to the methanogenesis process. In addition, low FS content might result in inadequate available nutrient for anaerobic digestion. In practice, long λ, T_{90}, and T_{eff} for waste treatment increase the operational cost that reduces economic benefit of the systems. Thus, long λ, T_{90}, and T_{eff} during anaerobic digestion of PSW must be reduced or, if possible, eliminated. Still, high M_{max} indicated that PSW can be utilized as substrate for anaerobic digestion. Substrate co-digestion might be the solution to reduce the λ, T_{90}, and T_{eff} during anaerobic digestion of PSW.

Higher R_{max} at S/I ratio of 0.5 observed in SS indicated that there was rapid degradation of VS occurred after λ of 1.5 d. λ of 0.2 d was estimated during anaerobic digestion of SS at S/I ratio of 1. These indicated that SS might have more available organic compound for methanogen at S/I ratio of 1 than at S/I ratio of 0.5. However, rapid CH_4 production rate and lower T_{eff} from anaerobic digestion of swine slurry at S/I ratio of 0.5 indicated that there was no inhibition that the R_{max} was also higher than those at S/I ratio of 1.

Compared to PSW, SS had less M_{max} at both S/I ratio. At S/I ratio of 1, M_{max} of swine slurry was only 48.4 % of PSW M_{max}. However, SS had lower λ, T_{90}, and T_{eff} indicated that VS in SS was available for the methanogen at the beginning of anaerobic digestion. In addition, low TS content also indicated that SS might have more soluble VS and nutrient that make it easier to be utilized by the microorganisms during anaerobic digestion.

However, SS had low M_{max} and SMY compared to PSW ($p<0.05$) due to its low VS content. High fixed solid (FS) content (32.4 %DM) indicated SS rich in mineral that might benefit anaerobic digestion process. In addition, low λ, T_{90}, and T_{eff} estimated from anaerobic digestion of SS might help to achieve better digestion efficiency of PSW by reducing the λ, T_{90}, and T_{eff}. From SS perspective, low SMY and M_{max} might indicate anaerobic digestion of SS to be uneconomical due to low CH_4 production. Thus, it requires additional VS that can enhance CH_4 production. As such, co-digestion of SS with PSW can be a viable option.

2.4.4. Pig Slaughterhouse Waste Co-Digestion with Swine Slurry

Table 2.6 showed the effect of pig slaughterhouse waste (PSW) co-digestion with swine slurry (SS) to anaerobic digestion parameters estimated by modified Gompertz formula. The results for anaerobic digester parameters of sole PSW and SS were taken from **Table 2.5**. One-way ANOVA followed by Tukey HSD test was utilized to determine the significant difference from anaerobic digestion parameter between treatments (individual and co-digested substrate) at the same S/I ratio (S/I ratio of 1 or 0.5).

From SS perspective, co-digestion increased M_{max} from 289.8 and 453.2 Nml CH_4/g VS_{added} (SS sole digestion at S/I ratio of 1 and 0.5, respectively) to between 405.1 and 672.4 Nml CH_4/g VS_{added} (PSW and SS co-digestion at both S/I ratio). This was equal to 22 to 84% M_{max} increase compare to SS sole digestion. In addition, co-digestion had no significant effect to T_{90} and T_{eff} compare to sole SS digestion at both S/I ratio ($p>0.05$). This indicated, that PSW co-digestion with SS improved digestion efficiency in term of higher CH_4 generation compare to sole SS digestion. Higher M_{max} of co-digested mixture compare to SS sole digestion was due to VS addition from the PSW. SS had low VS content compare to PSW. Combining PSW and SS increased the VS content that can be converted into CH_4 during anaerobic digestion. In addition, high fat content in the PSW also contributed to the M_{max} increased observed in the co-digested mixture compare to sole SS digestion. Fat has higher CH_4 production potential than protein and carbohydrates (Alves et al., 2009).

The M_{max} of co-digested substrate at both S/I ratios were significantly lower compared to M_{max} obtained from sole anaerobic digestion of PSW. The M_{max} obtained from co-digestion of PSW and SS was 535.1, 482.9, and 405.1 Nml CH_4/g VS_{added} at S/I ratio of 1 for co-digested mixture contains 67, 50, and 33% of PSW, respectively. At S/I ratio of 0.5, the M_{max} was 672.4, 634.8, and 555.5 Nml CH_4/g VS_{added} for co-digested mixture contains 67, 50, and 33% of PSW, respectively. The M_{max} of co-digested mixture showed 7 to 32% decrease of compare to M_{max} of PSW sole digestion.

Table 2.6. Anaerobic digestion parameter from co-digestion of pig slaughterhouse waste (PSW) with swine slurry (SS). The PSW content in co-digested mixture was 100% (P1), 0% (P2), 67% (P3), 50% (P4), and 33% (P5) in volatile solid (VS) basis

Parameters[1]	S/I Ratio of 1[2,3]					S/I Ratio of 0.5[2,4]				
	P1A	P2A	P3A	P4A	P5A	P1B	P2B	P3B	P4B	P5B
M_{max} (Nml CH_4/g VS_{added})	598.7 ±13.3[e]	289.8 ±8.6[a]	535.1 ±3.4[d]	482.9 ±4.0[c]	405.1 ±5.3[b]	723.7 ±17.0[D]	453.2 ±11.0[A]	672.4 ±11.3[C]	634.8 ±9.3[C]	555.5 ±17.0[B]
R_{max} (Nml CH_4/g VS_{added}/d)	34.3 ±2.9[b]	20.1 ±0.9[a]	44.7 ±4.0[b]	42.9 ±1.5[b]	37.1 ±9.5[b]	36.2 ±2.8[A]	35.0 ±2.4[A]	54.4 ±7.4[B]	48.3 ±1.3[B]	42.1 ±6.2[AB]
λ (d)	9.7 ±2.4[c]	0.2 ±0.1[a]	5.2 ±2.3[b]	2.4 ±0.8[a]	1.2 ±0.7[a]	9.0 ±0.2[E]	1.5 ±0.2[A]	5.7 ±0.4[D]	4.0 ±0.2[C]	3.0 ±0.2[B]
R^2	0.999	0.989	0.999	0.998	0.997	0.999	0.991	0.998	0.997	0.996
T_{90} (d)	30.7 ±0.7[b]	17.4 ±0.3[a]	19.6 ±1.0[a]	15.9 ±1.4[a]	14.9 ±4.6[a]	33.0 ±2.2[B]	17.0 ±0.8[A]	20.6 ±2.0[A]	19.7 ±0.4[A]	18.9 ±1.7[A]
T_{eff} (d)	20.9 ±1.8[b]	17.2 ±0.2[ab]	14.4 ±1.3[a]	13.5 ±0.6[a]	13.7 ±3.9[a]	24.0 ±2.3[B]	15.5 ±1.0[A]	14.9 ±2.4[A]	15.7 ±0.2[A]	15.9 ±1.8[A]

[1] M_{max}: Maximum CH_4 potential production, R_{max}: Maximum CH_4 production rate, λ: Lag phase period, R^2: Correlation coefficient, T_{90}: Time required to obtain 90% of M_{max}, T_{eff}: Effective digestion time (T_{90} - λ), d: days

[2] Values are expressed as mean ± standard deviation

[3] Means in the same row at S/I ratio of 1 (A) with different lowercase letter differs significantly ($p<0.05$)

[4] Means in the same row at S/I ratio of 0.5 (B) with different uppercase letter differs significantly ($p<0.05$)

Lower M_{max} obtained during anaerobic co-digestion of slaughterhouse waste with other substrates than sole slaughterhouse waste digestion had been reported. Borowski and Kubacki (2015) observed that as sole substrate, anaerobic digestion of PSW gave M_{max} of 839 Nml CH_4/g VS_{added}. However, when co-digested with sewage sludge at mixing ratio of 30 and 50% weight per weight (w/w), the M_{max} of co-digested mixture in ranged of 472.8 to 608.6 Nml CH_4/g VS_{added}. During anaerobic co-digestion of PSW and swine manure (SM) at mixing ratio of 67% SM per 36% PSW in VS basis, Rodríguez-Abalde et al. (2017) reported M_{max} of 430 Nml CH_4/g VS_{added}. This was lower than PSW sole digestion that had M_{max} of 809 Nml CH_4/g VS_{added}. Lower M_{max} observed in the co-digested substrate than the sole substrate digestion was caused by inherently low M_{max} from the sewage sludge, SM, or in this study case, SS.

Still, the co-digestion of PSW and SS had significant effect on lag phase period (λ), time required to obtain 90% of CH_4 potential (T_{90}), and effective digestion time (T_{eff}) (**Table 2.6**). Anaerobic digestion of sole PSW had the highest λ, T_{eff}, and T_{90} at both S/I ratio. All co-digested mixture had significantly lower ($p<0.05$) λ, T_{90}, and T_{eff} compare to sole PSW digestion (**Table 2.6**). Co-digestion shortened the λ from 9 and 9.7 d (PSW sole digestion at S/I ratio of 1 and 0.5, respectively) to between 1.2 and 5.7 d (PSW and SS co-digestion at both S/I ratio). It also shortened T_{eff} from 20.9 and 24.0 d (PSW sole digestion at S/I ratio of 1 and 0.5, respectively) to between 13.5 and 15.7 d (PSW and SS co-digestion at both S/I ratio). T_{90} was also shortened from 30.7 and 33 d (PSW sole digestion at S/I ratio of 1 and 0.5, respectively) to 14.9 to

20.6 d (PSW and SS co-digestion at both S/I ratio). Increased of SS composition in co-digested mixture reduced the λ at S/I ratio of 0.5. However, it had no significant effect on T_{90} and T_{eff}. Shorter λ, T_{90}, T_{eff} indicated that co-digestion of PSW and SS improved anaerobic digestion efficiency in term of shorter digestion time from PSW perspective.

Improved anaerobic digestion efficiency compared to sole PSW digestion might be due to more soluble substrate and nutrient were available for the microorganisms. SS had high moisture content (95.9 %FW) indicating the VS and nutrient were might be mostly present in soluble form. In addition, **Table 2.7** showed that SS had higher cobalt (Co), iron (Fe), molybdenum (Mo), nickel (Ni), tungsten (W), and zinc (Zn) content compare to PSW.

The last stage for methanogenesis (CH_4 generation) in both acetoclastic and hydrogenotrophic pathways requires methyl group (CH_3) transfer to Coenzyme M (CoM) forming CH_3-CoM complex. The CH_3-CoM complex is then reduced by methyl coenzyme M reductase (MCR) into CH_4 (Guo et al., 2015). The CH_3-CoM formation was facilitated by enzyme methyltransferase. Co was known as cofactor of methyltransferase, while Ni was known as cofactor for MCR (**Table 1.1**) (Choong et al., 2016). This indicates adequate concentration of Co and Ni during anaerobic digestion is necessary.

Table 2.7 showed that PSW only had Co and Ni content of 0.3 and 2.9 mg/kg, respectively. This was much lower than Co and Ni content of 10.4 and 25 mg/kg, respectively, obtained from SS. The recommended Co and Ni content for anaerobic digestion was actually between 0.03 and 35 mg/kg (**Table 1.1**)

(Romero-Güiza et al., 2016). This indicates low Co and Ni content in PSW was still adequate for anaerobic digestion. This was confirmed from the M_{max}, D_{deg}, and SMY of sole PSW that showed high CH_4 production and anaerobic degradability (**Table 2.4** and **2.5**). However, long λ, T_{90}, and T_{eff} observed during anaerobic digestion of PSW might indicate that both Co and Ni might not be readily available from the start of anaerobic digestion.

Table 2.7. Comparison of mineral content between pig slaughterhouse waste (PSW) and swine slurry (SS)

Mineral	PSW[1] (mg/kg)	SS[1] (mg/kg)
Cobalt (Co)	0.3±0.0[a]	10.4±0.1[b]
Iron (Fe)	229.3±46.4[a]	10872±136[b]
Molybdenum (Mo)	3.6±3.4[a]	13.0±0.1[b]
Nickel (Ni)	2.9±0.4[a]	25.0±0.1[b]
Tungsten (W)	0.0±0.0[a]	22.1±0.0[b]
Zinc (Zn)	200.5±34.2[a]	2239±20[b]

[1] Values are expressed as mean ± standard deviation

[a,b] Means in the same column with different lowercase letter differ significantly ($p<0.05$)

Apart from its total concentration, mineral availability was also an important factor in anaerobic digestion. During anaerobic digestion, mineral can exist as soluble (free ions) form, complex from (organic or inorganic), and precipitates (Choong et al., 2016; Ortner et al.,2015). High VS content of PSW might form mineral complex with Co and Ni that makes it not available from the start of anaerobic digestion. After the VS was digested through hydrolysis and acidification process, the Co and Ni then become available to be utilized by the microorganisms. This might be one cause of long λ, T_{90}, T_{eff} observed during anaerobic digestion of PSW.

SS, on the other hand, had low VS with high moisture, Co, and Ni content. This indicates the Co and Ni in SS might be more available than Co and Ni in PSW. These might be the cause of improved digestion efficiency (shorter λ, T_{90}, and T_{eff}) of co-digested mixture compared to sole PSW digestion. However, only total concentration of Co and Ni was analyzed in this study. As stated before, this study was focus on the PSW and SS co-digestion effect to anaerobic digestion parameters. Thus, the effect of PSW and SS co-digestion to mineral availability and anaerobic digestion parameters seems to be interesting topic for future study to clearly understand the reasons for shorter λ and digestion time during anaerobic co-digestion of PSW and SS.

Substrate co-digestion can produce either synergistic or antagonistic effect. Antagonistic effect occurred when the experimental M_{max} was lower than the simulated M_{max}. Whereas, synergistic occurred when the experimental M_{max} was higher than the simulated M_{max} (Nielfa et al., 2015). Experimental M_{max} of PSW co-digestion with SS at S/I ratio of 1 and 0.5 showed α value more than 1 indicating that synergistic effect occurred during the anaerobic co-digestion of PSW and SS **Table 2.8**. Combination of high VS and fat content of PSW and high Co and Ni content of SS was the reason for the synergistic effect.

Table 2.8. Results of the synergistic or antagonistic effect produced by the co-digestion of pig slaughterhouse waste (PSW) with swine slurry (SS)

Reactor[1]	Experimental M_{max}[2]	Simulated M_{max}[2]	α[2,3]
P1A	598.7±13.3	598.7±13.3	
P2A	289.8±8.6	289.8±8.6	
P3A	535.1±3.4	495.8±6.6	1.08±0.01
P4A	482.9±4.0	444.3±3.7	1.09±0.01
P5A	405.1±5.3	392.8±2.9	1.03±0.02
P1B	723.7±17.0	723.7±17.0	
P2B	453.2±11.0	453.2±11.0	
P3B	672.4±11.3	633.5±14.6	1.07±0.02
P4B	634.8±9.3	588.4±13.5	1.08±0.03
P5B	555.5±17.0	543.4±12.5	1.02±0.02

[1] The PSW content in co-digested mixture was 100% (P1), 0% (P2), 67% (P3), 50% (P4), and 33% (P5) in volatile solid basis. A indicated the experiment was conducted at S/I ratio of 1, while B indicated the experiment was conducted at S/I ratio of 0.5.

[2] Values are expressed as mean ± standard deviation; unit in Nml CH_4/g VS_{added}

[3] α= Experimental M_{max}/Simulated M_{max} (**Equation 2-15**)

2.5. Conclusion

It was reported that around 260,018 tons of pig slaughterhouse waste (PSW) was generated in 2017. In addition, around 13 million tons of swine slurry (SS) were produced in South Korea every year. Anaerobic digestion is considered as suitable technology to treat PSW and SS. However, wide diversity of substrate characteristics resulted in high variation in CH_4 production. As such, this study was done to determine the characteristics (chemical, proximate, ultimate, and energy content) of PSW and SS and determine PSW co-digestion with SS effect to anaerobic digestion parameters. The PSW content in the co-digested mixture was 67%, 50%, or 33% weight per weight volatile solid basis (w/w VS basis). SS was added as remaining substrate to obtain 100% VS in substrate mixture.

PSW had high energy content of 28 MJ/kg dry matter. This equal to 37 to 93.5% higher than heating value of various energy crops and livestock waste. However, high moisture content (81.8% of fresh matter) of PSW indicated that energy valorization by thermal conversion is uneconomical. In addition, PSW had high volatile solid (VS) content (94.5% of dry matter) that might complement the characteristics of SS that had low VS content (67.6% of dry matter) during anaerobic co-digestion. Low VS content in SS indicated that it had high minerals content that might be useful for anaerobic digestion.

In term of anaerobic digestion parameters, both PSW and SS showed specific CH_4 yield (SMY) in the range of previously reported. Specifically, the SMY of PSW was 611 and 711 Nml CH_4/g VS_{added} for substrate to inoculum (S/I) ratio of 1 and 0.5 weight per weight volatile solid (w/w VS) basis, respectively. While SS only showed SMY of 310 and 516 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5 w/w VS basis, respectively. S/I ratio of 1 and 0.5 was recommended to be utilized for batch anaerobic digestion to test the presence of inhibitory compounds in the substrate or its production during the anaerobic digestion. Higher SMY in S/I ratio of 0.5 indicated that S/I ratio had significant effect during anaerobic digestion of PSW and SS. High inoculum concentration might dilute toxic or inhibitory compounds during anaerobic digestion.

There was lag phase period (λ) of 9.7 and 9 days (d) for S/I ratio of 1 and 0.5, respectively, during anaerobic digestion of PSW. While, SS only showed λ of 0.5 and 1.5 d for S/I of 1 and 0.5, respectively. Moreover, PSW also had longer effective digestion time (T_{eff}) than SS. In practice, long λ and T_{eff} for waste treatment increases the operational cost that reduces economic benefit of the systems. Thus, long λ and T_{eff} during anaerobic digestion of PSW must be reduced or, if possible, eliminated. Low SMY from SS also results in uneconomical plant operation. Substrate co-digestion might be the solution to reduce the λ and T_{eff} during anaerobic digestion of PSW. In addition, it might also become solution to increase SMY of SS.

PSW co-digestion with SS resulted in maximum CH_4 production potential (M_{max}) increased from 289.8 to 453.2 Nml CH_4/g VS_{added} (SS sole digestion) to between 405 and 672 Nml CH_4/g VS_{added} (PSW and SS co-digestion) with no significant effect to T_{eff}. High VS and fat content in PSW might be the cause of M_{max} increased compare to SS sole digestion.

However, low M_{max} of SS, 48 to 62% of PSW M_{max}, resulted in 7 to 32% decrease of M_{max} compare to PSW sole digestion. Still, co-digestion shortened the λ (3.3 to 8.5 d shorter) and T_{eff} (6.5 to 9.1 d faster) compare to PSW sole digestion. Shorter lag phase and faster digestion time during anaerobic co-digestion might be due to higher cobalt (Co) and nickel (Ni) concentration and its availability in SS than PSW. Co and Ni are important cofactor in CH_4 production (methanogenesis) stage during anaerobic digestion.

It was concluded that anaerobic digestion was a suitable option to treat

PSW due to its high SMY and M_{max}. Co-digestion results in improved anaerobic digestion efficiency as seen from 22 to 84% increase of M_{max} with no significant effect on T_{eff} compare to sole SS digestion. From PSW perspective, co-digestion reduced M_{max} but improved digestion efficiency as seen from reduced λ and T_{eff}. As such, co-digestion can be alternative solution to reduce long λ and T_{eff} associated with anaerobic digestion of PSW and increase low CH_4 production associated with anaerobic digestion of SS. Still, the mechanisms of shorter λ and T_{eff} of PSW co-digestion with SS was not clear yet. Thus, the PSW and SS co-digestion effect to mineral availability and anaerobic digestion parameters seems to be interesting topic for future research. Moreover, application of co-digestion technology to increase CH_4 production and its economic benefit in field scale anaerobic digester also seems to be another interesting topic for future study.

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CHAPTER 3. CHARACTERISTICS OF BEEF CATTLE SLAUGHTERHOUSE WASTE AND ENHANCEMENT OF ANAEROBIC DIGESTION PARAMETERS THROUGH CO-DIGESTION WITH LIVESTOCK WASTE

This chapter will be published in SCI(E) journal as a partial fulfillment of Agricultural Biotechnology's Ph.D. program for Anriansyah Renggaman

3.1. Abstract

This study was done to determine the characteristics (chemical, proximate, ultimate, and energy content) of beef cattle (BCSW) and cattle dung (CD) and determine BCSW co-digestion effect with swine slurry (SS) or CD to anaerobic digestion parameters. BCSW and CD showed high volatile solid (VS) content of 93.1 and 91.2 % of dry matter (%DM), respectively. BCSW also showed high energy content of 29.2 MJ/kg dry matter. It was 41 to 99% higher than energy content of energy crops and livestock waste. In term of anaerobic digestion, both BCSW and CD showed specific CH_4 yield (SMY) in the range of previously reported. Specifically, the SMY of BCSW was 582 and 702 Nml CH_4/g VS_{added} for substrate to inoculum (S/I) ratio of 1 and 0.5 weight per weight volatile solid (w/w VS) basis, respectively. While CD showed SMY of 431 and 505 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5, respectively. Higher SMY in S/I ratio of 0.5 indicated that S/I ratio had significant effect during anaerobic digestion of BCSW and CD. In addition, there was long lag phase period (λ) of 10.2 and 11 days (d) for S/I ratio of 1 and 0.5, respectively, during

anaerobic digestion of BCSW. CD also showed long λ of 8.3 and 8.2 d for S/I ratio of 1 and 0.5, respectively. However, the difference of λ at different S/I ratio for BCSW and CD was not statistically significant ($p>0.05$). BCSW co-digestion with SS resulted in maximum CH_4 production potential (M_{max}) increased from 289.8 and 453.2 Nml CH_4/g VS_{added} (SS sole digestion) to between 431 and 685 Nml CH_4/g VS_{added} (BCSW and SS co-digestion). However, low M_{max} of SS, 50 to 64% of BCSW M_{max}, resulted in 3.7 to 25.6% decrease of M_{max} compare to BCSW sole digestion. Still, co-digestion shortened the λ from 10.2 and 11 d (BCSW sole digestion) to between 2.4 and 8.5 d (BCSW and SS co-digestion). It also shortened the effective digestion time (T_{eff}) from 21.8 and 22.6 d (BCSW sole digestion) to between 13.7 and 16.8 d (BCSW and SS co-digestion). BCSW co-digestion with CD, however, resulted in 3 to 27% decrease of M_{max} compare to BCSW sole digestion and there was no significant effect to λ and T_{eff} indicating CD was not suitable to be co-digested with BCSW. Shorter λ and faster T_{eff} during BCSW co-digestion with SS might be due to higher cobalt (Co) and nickel (Ni) concentration and its availability in SS than BCSW. While CD had similar Co and Ni concentration to BCSW resulting in no improvement in λ and T_{eff}. It was concluded that anaerobic digestion was a suitable option to treat BCSW. In addition, co-digestion results in improved anaerobic digestion efficiency as seen from 22 to 84% increase of M_{max} with no significant effect on T_{eff} compare to sole SS digestion. From BCSW perspective, co-digestion reduced M_{max} but improved digestion efficiency as seen from reduced λ (3.3 to 8.5 d shorter) and

T_{eff} (6.5 to 9.1 d faster).

Keywords: Beef cattle slaughterhouse waste, swine slurry, anaerobic digestion, co-digestion, energy content

3.2. Introduction

Drastic change in meat consumption per capita in South Korea from 13.9 kg in 1980 to 43.5 kg in 2010 has led to increasing amount of waste generation from the slaughterhouse (Lee et al., 2015). It was reported that 873,483 heads of beef cattle were slaughtered in South Korea equal to around 127,539 tons of beef cattle slaughterhouse waste (BCSW) generation in 2017 (APQA, 2018).

BCSW consists mainly of blood, digestive tract tissues, and internal organs not intended for human consumption (Alvarez & Liden, 2008; Cuetos et al., 2008). It is characterized by high organic matter, fat, and protein content indicating it is an energy-rich waste. Still, it is interesting to compare energy content of BCSW to other organic substrates. The energy content can be determined using adiabatic bomb calorimeter (Choi et al., 2014; Sanli et al., 2014).

BCSW was previously disposed through ocean dumping, composting, recycling as animal feed, and landfilling in South Korea. However, ocean dumping has been prohibited since 2012. In addition, there was interest in converting slaughterhouse waste into economical product. One example is to convert slaughterhouse waste into CH_4 that can be utilized to produce heat and electricity (Lee et al., 2015; Yoon et al., 2014).

Livestock waste is one of the main sources of greenhouse gas emission (GHG), mostly as methane (CH_4) and nitrous oxide (N_2O), in livestock production. GHG produced during livestock production consist of carbon dioxide from livestock respiration, CH_4 gas from enteric fermentation, and N_2O from partial waste treatment (Philippe & Nicks, 2015). CH_4 also produced from the manure decomposition in the storage or pit. This happens because anaerobic condition of storage and slurry pit favors the methanogenesis process that produces CH_4 (Monteny et al., 2006). Despite its disadvantages of CH_4 emission to the atmosphere, CH_4 also beneficial as renewable energy resources.

Every year, 13 million tons of swine slurry (SS) were generated in South Korea (Suresh & Choi, 2012). Previous research showed that SS mainly exists in liquid form (Suresh et al., 2009). SS accumulation leads to environmental pollution such as GHG and odor emission and soil and water pollution through run-off and leachate (Renggaman et al., 2015).

Kim et al. (2012) reported that 18.4% of biogas plants available in South Korea in 2010 were treating livestock manure. In addition, 26% of biogas plant were treating mixture of SS and food waste. However, the biogas production of these plants was lower than expected that the plants were considered to be uneconomical. Møller et al. (2007) also reported that anaerobic digestion using only livestock waste is not economically sustainable. Co-digestion might be solution to this problem.

Co-digestion is simultaneous digestion of two or more organic wastes with complementary characteristics to improve CH_4 production or digestion efficiency (Borowski & Kubacki, 2015; Mata-Alvarez et al., 2011; Pagés-Díaz et al., 2015). Co-digestion can lead to dilution of inhibitory compounds, addition of macro-, micro-nutrient, and organic matter, and improvement of buffering capacity of the co-digested mixture (Mata-Alvarez et al., 2014). In many cases, co-digestion results in higher CH_4 production than sole substrate digestion (Mata-Alvarez et al., 2011).

Substrates compatibility and geographical availability are basic criteria to select suitable substrate for co-digestion. Compatibility depends on whether the wastes characteristics (nutrient content) complement each other or not. Geographical availability depends on whether waste is available near the biogas plants or other organic wastes (Rodríguez-Abalde et al., 2017).

SS is characterized by high nutrient but low volatile solid (VS) content. This results in low CH_4 yield during anaerobic digestion (Rodríguez-Abalde et al., 2017). BCSW, on the other hand, has low nutrient but high VS content. This indicates SS and BCSW had compatible characteristics that might beneficial for anaerobic co-digestion.

In term of geographical availability, slaughterhouse is located near livestock farm to reduce stress and transportation cost. It was reported that 97% of slaughterhouse that slaughtered beef cattle also slaughtered swine indicating that those places are in vicinity of swine farm (APQA, 2018). Thus, BCSW can be co-digested with cattle dung (CD) or SS. Co-digestion between BCSW and livestock waste (CD or SS) might be a feasible option to improve the anaerobic digestion parameters such as maximum CH_4 potential production (M_{max}), lag phase period (λ), and effective digestion time (T_{eff}).

The objectives of this study were to determine the characteristics (chemical, proximate, ultimate, and energy content) of BCSW and CD and determine BCSW co-digestion with SS or CD effect to anaerobic digestion parameters.

3.3. Material and Method

3.3.1. Samples and Inoculum

BCSW was collected from a slaughterhouse in Yeongcheon City, Gyeongsang Province, South Korea. The facility slaughter around 10,710 beef cattle in 2017 (APQA, 2018). The BCSW was mixture of viscera (digestive tract tissue) and offal (internal organs). Other waste such as blood, brain, bones, and spinal cord were not included in this study. Blood was already treated separately by the slaughterhouse, while brain, bones, and spinal cord were not utilized due to safety reason. BCSW was ground and sieved into smaller pieces (<5 mm) and mixed thoroughly without any separation to emulate actual condition in the slaughterhouse. Samples then dried at 105°C for the ultimate and higher heating value (HHV) analysis while untreated samples were utilized for the proximate analysis and co-digestion analysis.

SS was collected from swine farm in Hoengseong, County, Gangwon Province, Republic of Korea. Cattle dung (CD) was collected for Hanwoo cattle farm nearby the swine farm. Both CD and SS sample were screened with 5 mm mesh to ensure uniformity. The samples then undergo treatment and analysis similar to BCSW. The characteristics of SS was already reported in **Chapter 2**. The data were compared with the characteristics of BCSW and CD obtained in this study.

The inoculum used in this experiment was was similar to the inoculum utilized in **Chapter 2**. **Table 2.1** showed the characteristics of the inoculum utilized in this experiment.

3.3.2. Experimental Design and Anaerobic Co-Digestion Experiment

Bio-methane Potential Analysis

Bio-Methane potential (BMP) analysis for SS was already reported in **Chapter 2**. Still, the data from **Table 2.2** was utilized to compare SS methane potential with CD and BCSW. BMP analysis for BCSW and CD was done in 250 ml serum bottles. BCSW or CD and inoculum were added into the serum bottle. The substrate per inoculum (S/I) ratio utilized in this experiment were 1 and 0.5 weight per weight volatile solid basis (w/w VS basis). Around 0.25 g volatile solid (VS) of BCSW or CD was added into 0.50 g VS inoculum for S/I ratio of 0.5. Whereas, for S/I ratio of 1, around 0.5 g VS of BCSW or CD was added into 0.5 g VS inoculum. Distilled water was then added to reach 200 ml total volume. Control bottle was prepared to correct the biogas production. Only mixture of inoculum and water was added into the control bottle. Sample of 50 ml was taken from each bottle for further analysis. Mixture of CO_2 and N_2 gas (20:80% volume per volume (v/v)) were inserted into the headspace during the sampling to create and maintain anaerobic condition inside the bottle. The serum bottles were then closed and sealed with rubber cap and aluminum crimps.

Table 3.1. Mixture composition and experimental design for anaerobic co-digestion experiment of beef cattle slaughterhouse waste (BCSW) with cattle dung (CD) or swine slurry (SS)

Code	Substrate (%VS)[1]		
	CSW	SS	CD
C1	100	0	0
C2	0	100	0
C3	0	0	100
C4	67	33	0
C5	50	50	0
C6	33	67	0
C7	33	0	67
C8	50	0	50
C9	67	0	33

[1] The co-digestion experiment was done in S/I ratio of 1 (A) and 0.5 (B) with similar mixture composition. Only co-digestion of cattle slaughterhouse waste and cattle dung conducted in S/I ratio of 1. All experiments were done in triplicate

Co-Digestion Experiment

The batch anaerobic co-digestion of BCSW and livestock waste (SS or CD) were carried out in five different BSCW/SS or CD weight per weight volatile solid basis (w/w VS basis) of: 1:0, 0:1, 2:1, 1:1, and 1:2. This equal to 100, 0, 67, 50, and 33% VS basis of BCSW content in co-digested mixture. The co-digestion experiment between BCSW and SS was done at S/I ratio of 1 and 0.5, while co-digestion experiment between BCSW and CD was only done at S/I ratio of 1. Similar with methane potential experiment, around 0.5 g VS of co-digested mixture was added to 0.5 g VS of inoculum for S/I ratio of 1. Whereas, around 0.25 g VS of co-digested mixture was added to 0.5 g VS inoculum for S/I ratio of 0.5.

The summary of experimental design for co-digestion experiment can be seen in **Table 3.1**. The anaerobic digestion results for BCSW, CD, and SS utilized as sole substrate was reported in the bio-methane potential experiment. Those results were compared with the results obtained from co-digestion experiment.

The substrate mixture and inoculum were added to 250 ml serum bottle. Distilled water was added so that the volume in each bottle reaches 200 ml. Then, 50 ml of the mixture was sampled for further analysis. Control bottle was prepared from the mixture of inoculum and distilled water to correct the biogas production. During the sampling, the serum bottle headspace was gassed with mixture of CO_2 and N_2 (20:80% v/v) to create anaerobic condition. Bottles were then closed and sealed with rubber cap and aluminum crimps.

3.3.3. Analytical Methods

Chemical, Proximate and Ultimate Analysis

Chemical analysis constitutes the determination of samples fat, protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose content.

Fat content was determined with soxhlet extraction with ether as solvent. Protein content were determined with total kjedahl nitrogen (TKN). NDF and ADF content were determined following the procedure described in (Fernández-Cegrí et al., 2012). Hemicellulose content was calculated following **Equation 2-1**.

Parameters for proximate analysis were moisture, total solid (TS), volatile solid (VS), and fixed solid (FS) content. Similar to **Chapter 2**, proximate analysis was done following method number 2450G in APHA Standard Method (APHA, 2005). **Equation 2-2** and **2-3** were utilized to determine the moisture and TS content, respectively. While, **Equation 2-4** and **2-5** were utilized to determine VS and FS content, respectively.

The carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content were analyzed with Elemental Analyzer (Flash EA 1112, Thermo Fisher Scientific, Germany). The oxygen (O) content was analyzed with another instrument (Flash 2000 Elemental Analyzer, Thermo Fisher Scientific, Germany).

Higher Heating Value (HHV) analysis

Dried samples were ground and screened through sieves with 5 mm opening (DH.Si8021, DAIHAN Scientific). Samples were then pelletized using pellet press (2811, Parr Instrument). Pelletized samples were analyzed with an oxygen bomb calorimeter (Model 1341 plain jacket calorimeter, Parr Instrument). Prior to the analysis, the oxygen bomb calorimeter was standardized with benzoic acid pellets (3415, Parr Instrument).

Mineral Content Analysis

The mineral content such as cobalt (Co), iron (Fe), molybdenum (Mo), nickel (Ni), tungsten (W), and zinc (Zn) of the samples were analyzed with inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ICP-7510, Shimadzu Corp., Kyoto, Japan). Prior to the analysis, standard nitric acid-hydrochloric acid digestion (method number 3030F) was utilized to digest the samples with strong acid (APHA, 2005).

Biogas Production, Composition, and Specific CH_4 Yield

The biogas production was analyzed using manometric method. This method maintained constant volume and measured increase in headspace pressure with pressure transducer (Himanshu et al., 2017). High pressure in the headspace can affect microbial activity. Thus, excess gas must be removed regularly (Valero et al., 2016). When glass syringe was utilized to remove excess gas, the amount of excess gas can be measured. This was equal to biogas production because constant volume was maintained.

The gas composition was analyzed using a gas chromatograph HP 6890N (Agilent Technologies) equipped with a HP-PLOT Q column (Agilent Technologies) and a thermal conductivity detector. The inlet, oven, and detector temperature were 40, 35, and 200°C, respectively. The CH_4 content was then utilized to determine CH_4 production using **Equation 2-6**. Specific methane yield (SMY) was determined using **Equation 2-7**.

Theoretical and Simulated CH_4 yield

Results from elemental analysis were utilized to determine the chemical formula of organic waste (Yoon et al., 2014). Theoretical CH_4 yield (TMY) can be determined empirically from chemical formula of organic waste suggested by Symons and Buswell (1933) and further developed by Boyle (1977). The equation is shown in **Equation 2-8**. The reaction coefficient for CH_4 can be determined using **Equation 2-9**. TMY of organic waste at standard temperature and pressure (STP) of 1 atm and 273 K can be determined using **Equation 2-10** as suggested by Pellera and Gidarakos (2016). TMY then utilized to determine degree of anaerobic degradability (D_{deg}) utilizing **Equation 2-11**.

Similar to **Chapter 2**, modified Gompertz formula (**Equation 2-12**) were utilized to determine maximum CH_4 production potential (M_{max}), maximum CH_4 production rate (R_{max}), lag phase period (λ), and correlation coefficient (R^2) of produced model. Excel solver add-in (Microsoft Excel 2016) was utilized to obtain the results. In addition, time required to obtain 90% of M_{max} (T_{90}) was estimated utilizing Excel solver add-in. The results then utilized to estimate effective digestion time (T_{eff}) utilizing **Equation 2-13**.

The simulated maximum CH_4 production potential (M_{sim}) from the co-digested mixture was calculated by the proportion of BCSW and livestock waste (SS or CD) in the mixture and the M_{max} of sole BCSW and SS or CD, as showed in **Equation 2-14**.

Synergistics Effect

Synergistic effect is inner reactions produced by the co-digestion of different components. Each co-digested substrate can influence the CH_4 production rate (Nielfa et al., 2015). Synergistic effect was calculated using **Equation 2-15**.

Statistical Analysis

One-tail t-test was done to compare the anaerobic digestion parameters (SMY, M_{max}, R_{max}, λ, T_{90}, T_{eff}) at different S/I ratio. One-way ANOVA followed by Tukey HSD was done to determine the effect of BCSW co-digestion with SS or CD to the anaerobic digestion parameters at the same S/I ratio (1 or 0.5). P-value of 0.05 was utilized for all test.

3.4. Results and Discussion

3.4.1. Characteristics of Beef Cattle Slaughterhouse Waste and Livestock Waste

Table 3.2 showed the characteristics of beef cattle slaughterhouse waste (BCSW) and livestock waste utilized in this experiment. BCSW had total solid (TS) content 16.67% of fresh weight (%FM) with high volatile solid (VS) content (93.18% of dry matter (%DM)). Similar to pig slaughterhouse waste (PSW), the VS was constituted mainly by fat (57.4 %DM) and protein (19.83 %DM). The TS content observed in this study was lower than previous works. BCSW had TS content between 26 and 65.2 %FW (Lee et al., 2015; Pagés-Díaz et al., 2015; Ware & Power, 2016). Similar to PSW, this might be due to washing and cleaning that was done to the offal for sanitary purpose by the slaughterhouse prior to the sample collection.

Table 3.2. Characteristics of beef cattle slaughterhouse waste. cattle dung, and swine slurry utilized in this study

Parameters[1]	Beef Cattle Slaughter Waste[2]	Cattle Dung[2]	Swine Slurry[2,3]
Total Solid (%FM)	16.7±0.3^{B}	32.1±0.2^{C}	4.1±0.2^{A}
Volatile Solid (%DM)	93.2±0.6^{B}	91.2±2.1^{B}	67.6±0.8^{A}
Fixed Solid (%DM)	5.4±0.2^{A}	8.8±2.1^{A}	32.4±0.8^{B}
Total Kjedahl Nitrogen (%DM)	3.2±0.3^{A}	2.5±0.3^{A}	4.5±0.1^{B}
Protein (%DM)	19.8±1.9^{A}	15.4±1.7^{A}	28.1±0.9^{B}
Fat (%DM)	57.4±0.3^{C}	3.1±0.1^{A}	5.6±0.2^{B}
Neutral Detergent Fiber (%DM)	18.9±0.6^{A}	61.3±0.1^{C}	27.4±0.8^{B}
Acid Detergent Fiber (%DM)	13.1±0.5^{B}	23.5±0.6^{C}	10.7±0.0^{A}
Hemicellulose (%DM)	5.8±0.2^{A}	37.9±0.5^{C}	16.7±0.8^{B}

[1] %FM: % of fresh matter, %DM: % of dry matter

[2] Values expressed as mean ± standard deviation

[3] The data was also reported in **Table 2.3**

[A,B,C] Means in the same row with different uppercase letter differs significantly ($p<0.05$)

Nevertheless, the protein and fat content of BCSW observed in this study were comparable with previous results. Previous results showed that the fat content of BCSW was between 17.5 and 58.1 %DM. The protein content was also reported to be in the range of 13 to 33 %DM (Lee et al., 2015; Pagés-Díaz et al., 2015; Ware & Power, 2016). High fat and protein content in the BCSW indicated it was also an energy-rich substrate.

Similar to BCSW and PSW, cattle dung (CD) showed high VS content (91 %DM). The VS was mainly constituted from protein (15.4 %DM) and neutral detergent fiber (61.3 %DM). The protein and neutral detergent fiber of CD were from undigested feed from the digestive tract. In addition, the TS content of CD was relatively higher than those observed in SS and BCSW. The TS content of CD was 32 %FM.

BCSW had the highest fat content compared to CD and SS. Fat is an energy-rich substance indicating BCSW might have higher energy content than CD and SS. Compare to SS and BCSW, CD had higher neutral and acid detergent fiber but had low fat content. Low fat content indicated CD was not an energy-rich substrate unlike BCSW even though CD also had high VS content (**Table 3.2**). The VS, protein, and fixed solid (FS) content of CD was comparable with BCSW. High VS content in BCSW and CD indicated that there might be more organic matter to be converted into CH_4 during anaerobic digestion. However, low FS content in BCSW and CD might result in inhibition of CH_4 production.

3.4.2. Energy Content of Beef Cattle Slaughterhouse Waste and Cattle Dung

BCSW showed high energy content (HHV) of 29.26 MJ/kg dry matter (MJ/kg DM) (**Figure 3.1**). This was higher than energy content of various renewable resources. It was reported that energy crops had HHV between 14.69 and 20.71 MJ/kg DM. Palm kernel had the highest energy content among energy crops (Nhuchhen & Salam, 2012; Yin, 2011). Choi et al. (2014) reported that livestock manure collected in South Korea had HHV between 11.92 and 19.44 MJ/kg DM. Swine manure had the highest energy content among livestock waste (**Figure 3.1**). High energy content of BCSW indicated that it has potential to be utilized for bioenergy production.

The HHV of CD was 17.5 MJ/kg DM (**Figure 3.1**). This value was within the range of reported HHV from various livestock waste. Choi et al. (2014) reported that average HHV of 12 cattle manure samples was 16.1 MJ/kg DM. This indicated that the CD utilized in this study had higher HHV than the HHV reported previously. This was due to high VS content of the CD utilized in this study. The average VS content of 12 cattle manure samples was 80.4 %DM (Choi et al., 2014), while the VS content of CD utilized in this study was 91 %DM (**Table 3.2**). It was reported that VS content had positive correlation with HHV of organic waste (Choi et al., 2014; Nhuchhen & Salam, 2012; Yin, 2011).

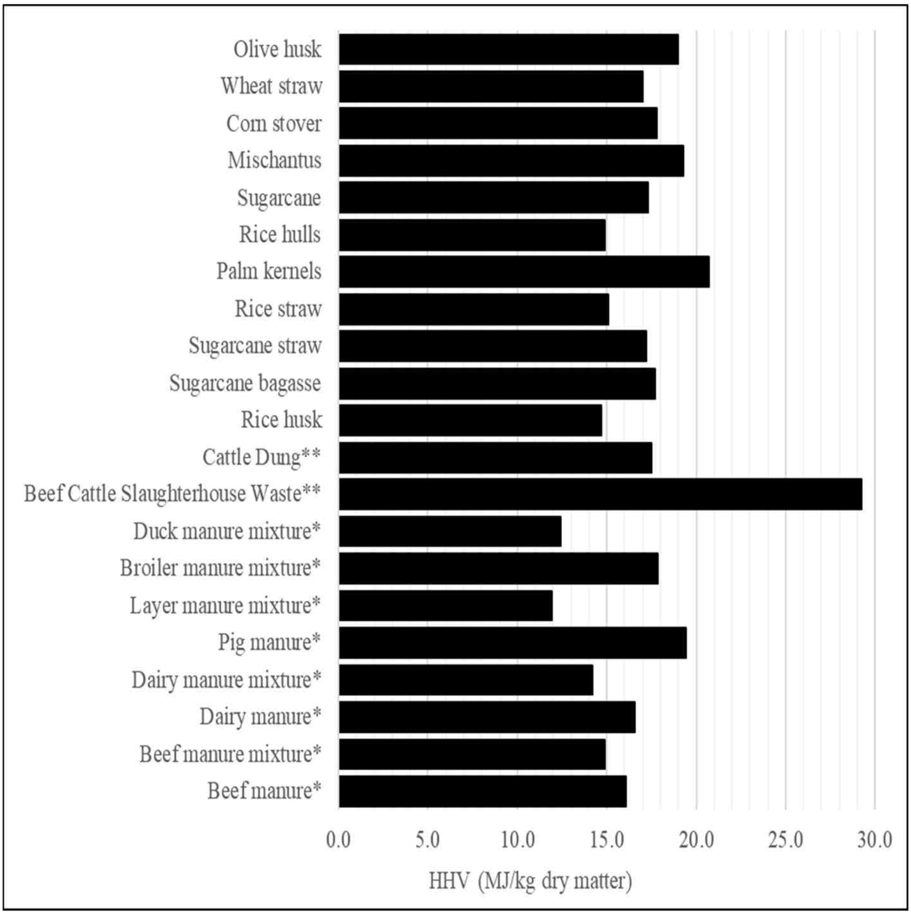

Figure 3.1. Energy content (HHV) of beef cattle slaughterhouse waste and cattle dung in comparison to various renewable resources (Choi et al., 2014; Nhuchhen & Salam, 2012; Yin, 2011). * Average value of 12 samples; ** This study

Similar to HHV of PSW and SS reported in **Chapter 2**, BCSW and CD HHV were reported as MJ/kg DM. This was because dried samples were utilized for HHV analysis utilizing bomb calorimeter. Low TS content in BCSW and CD indicated that the HHV of BCSW and CD was 4.88 and 5.61 MJ/kg of fresh matter (MJ/kg FM), respectively. As such, energy valorization from fresh BCSW and CD through thermal treatment will be uneconomical

(Moukazis et al., 2018). Anaerobic digestion has been applied to treat energy crops and livestock waste which has similar characteristics (high energy and moisture content) as BCSW and CD. Thus, anaerobic digestion might be an alternative technology to treat BSCW and CD.

Table 3.3. Ultimate analysis, empirical formula, theoretical methane yield (TMY), specific methane yield (SMY), and degree of anaerobic digestion (D_{deg}) of beef cattle slaughterhouse waste (BCSW), cattle dung (CD), and swine slurry (SS)

Parameter[1]	BCSW[2]	CD[2]	SS[2,3]
Carbon (%DM)	59.35±0.60	45.1±2.0	37.3±0.3
Hydrogen (%DM)	8.68±0.23	5.9±0.3	5.2±0.0
Oxygen (%DM)	20.28±0.48	31.9±0.1	23.7±0.7
Nitrogen (%DM	6.14±0.68	2.8±0.4	4.8±0.1
Sulfur (%DM)	0.43±0.02	0.2±0.0	1.0±0.1
Empirical formula	$C_{25}H_{45}O_7N_1S_{0.1}$	$C_{60}H_{94}O_{32}N_3S_{0.1}$	$C_{10}H_{16}O_5N_1S_{0.1}$
TMY (Nml CH_4/g VS_{added})	738.8±54.7	533.5±22.3	529.54±9.0
SMY at S/I ratio of 1 (Nml CH_4/g VS_{added})	582.2±3.3^{a,C}	431.5±15.4^{a,B}	310.1±9.0^{a,A}
SMY at S/I ratio of 0.5 (Nml CH_4/g VS_{added})	702.2±10.6^{b,B}	505.6±7.3^{b,A}	516.3±11.1^{b,A}
D_{deg} at S/I ratio of 1 (%)	78.8±0.4^{a,B}	80.9±2.9^{a,B}	58.6±1.7^{a,A}
D_{deg} at S/I ratio of 0.5 (%)	95.0±1.4^{b,A}	94.8±1.4^{b,A}	97.5±2.1^{b,A}

[1] %DM: % of dry matter

[2] Values are expressed as mean ± standard deviation

[3] The data was also reported in **Table 2.4**

[a,b] Means in the same column with different lowercase letter differ significantly ($p<0.05$)

[A,B] Means in the same row with different uppercase letter differ significantly ($p<0.05$)

3.4.3. Anaerobic Digestion of Beef Cattle Slaughterhouse Waste, Cattle Dung, and Swine Slurry

Table 3.3 showed the ultimate analysis results, empirical chemical formula, theoretical CH_4 yield (TMY), specific CH_4 yield (SMY), and degree of anaerobic digestion (D_{deg}) of beef cattle slaughterhouse waste (BCSW), cattle dung (CD), and swine slurry (SS). Substrate to inoculum (S/I) ratio had significant effect on the SMY and D_{deg} of BCSW, CD, and SS ($p<0.05$).

BCSW had TMY of 738.8 Nml CH_4/g VS_{added}. The SMY of BCSW at S/I ratio of 1 and 0.5 was 582.2 and 702.2 Nml CH_4/g VS_{added}, respectively. The D_{deg} of BCSW at S/I ratio of 1 and 0.5 was 78.8 and 95%, respectively (**Table 3.3**). The SMY of BCSW was comparable with previous results. Previous results showed that SMY of BCSW was between 410 and 609 Nml CH_4/g VS_{added}. Specifically, Lee et al. (2015) reported that anaerobic digestion of cattle offal had SMY of 520 Nml CH_4/g VS_{added}. Anaerobic digestion of untreated and pasteurized cattle offal had SMY of 515.5 and 650.9 Nml CH_4/g VS_{added}, respectively (Ware & Power, 2016). During the semi-continuous anaerobic digestion of cattle slaughterhouse waste, 410 Nml CH_4/g VS_{added} was obtained (Pagés-Díaz et al., 2015). This was lower than the SMY obtained from batch anaerobic digestion of 609 Nml CH_4/g VS_{added} by the same research group (Pagés-Díaz et al., 2014). The wide range of SMY from BCSW occurred because of variations in waste characteristics.

TMY of CD was 533 Nml CH_4/g VS_{added}. The SMY of CD was 431.5 and 506.5 Nml CH_4/g VS_{added} at S/I ratio of 1 and 0.5, respectively. These corresponded to high D_{deg} of 81 and 95% at S/I ratio of 1 and 0.5, respectively. The SMY value was within the range of previously reported. Cavinato et al. (2010) reported that anaerobic digestion of cattle manure resulted in SMY between 420 and 620 Nml CH_4/g VS_{added}.

The D_{deg} of 78.8 and 95% from BCSW after 50 days of digestion indicated that it was highly degradable. In addition, SMY of BCSW was significantly higher than SMY of CD and SS at S/I ratio of 1 and 0.5. This indicated that similar to PSW, BCSW also had potency as substrate for anaerobic digestion. Still, other anaerobic digestion parameters need to be analyzed to determine the applicability and necessary improvement strategies for BCSW.

Compare to SS, CD had higher SMY at S/I ratio of 1 ($p<0.05$). Interestingly, at S/I ratio of 0.5 SMY of CD was not significantly different from SMY of SS ($p>0.05$). This might be caused by at high S/I ratio, there was toxic compound production during anaerobic digestion of SS. SS digestion resulted in ammonia production that can inhibit anaerobic digestion process. At low S/I ratio (S/I ratio of 0.5), the produced ammonia might be diluted that no inhibition was observed. The increased of D_{deg} value during anaerobic digestion of SS from 58.6% at S/I ratio of 1 to 97.5% at S/I ratio of 0.5 indicated no inhibition was observed at low S/I ratio.

The improvement of D_{deg} and SMY at low S/I ratio was also observed during anaerobic digestion of PSW (**Chapter 2**). S/I ratio of 0.5 or 1 was recommended to be utilized for batch anaerobic digestion (Neves et al., 2004). High inoculum concentration might dilute toxic or inhibitory compound during digestion process that explained the SMY and D_{deg} improvement at S/I ratio of 0.5 in comparison of S/I ratio of 1 from BCSW, CD, and SS.

Modified Gompertz formula (**Equation 2-12**) was utilized to estimate the maximum CH_4 production potential (M_{max}), maximum CH_4 production rate (R_{max}), lag phase period (λ), time required to obtain 90% M_{max} (T_{90}), and effective digestion time (T_{eff}). The estimated anaerobic digestion parameters from anaerobic digestion of BCSW, CD, and SS were shown in **Table 3.4**.

M_{max} and R_{max} showed similar pattern with SMY. Low S/I ratio resulted in significantly higher M_{max} and R_{max} for BCSW, CD, and SS ($p<0.05$) (**Table 3.4**). BCSW had significantly higher M_{max} than CD and SS at both S/I ratio of 1 and 0.5 ($p<0.05$). M_{max} of BCSW was 575.5 and 711.4 Nml CH_4/kg VS_{added} at S/I ratio of 1 and 0.5, respectively. High M_{max} and R_{max} at low S/I ratio were due to inhibitory compound dilution by high inoculum concentration.

Aside from M_{max} and R_{max}, λ and digestion time (T_{90} and T_{eff}) were also important anaerobic digestion parameters (Xie et al., 2011). λ is an indicator of methanogens adaption to the environment. In addition, it also represents the substrate bioavailability during anaerobic digestion (Ware & Power, 2016). **Table 3.4** showed that λ of 10.2 and 11 were estimated for anaerobic digestion of BCSW at S/I ratio of 1 and 0.5.

Table 3.4. Anaerobic digestion parameters of beef cattle slaughterhouse waste (BCSW), cattle dung (CD), and swine slurry (SS) estimated by modified Gompertz formula (**Equation 2-12**)

Parameter*	**BCSW**[**]		**CD**[**]		**SS**[**,***]	
	S/I ratio of 1	**S/I ratio of 0.5**	**S/I ratio of 1**	**S/I ratio of 0.5**	**S/I ratio of 1**	**S/I ratio of 0.5**
M_{max} (Nml CH_4/g VS_{added})	578.5±14.4[a,C]	711..4±13.7[b,3]	397.2±15.3[a,B]	501.2±4.1[b,2]	289.8±8.6[a,A]	453.2±11.0[b,1]
R_{max} (Nml CH_4/g VS_{added}/d)	30.8±2.6 [a,B]	39±0.7[b,2]	22.0±0.8[a,A]	32.2±3.6[b,1]	20.1±0.9[a,A]	35.0±2.4[b,12]
λ (days)	10.2±1.7[a,B]	11.0±0.0[a,3]	8.3±0.4[a,B]	8.2±1.4[a,2]	0.2±0.1[a,A]	1.5±0.2[b,1]
R^2	0.999	0.999	0.998	0.999	0.989	0.991
T_{90} (days)	32.7±2.4[a,B]	32.8±0.5[a,3]	29.9±0.4[a,B]	27.0±0.7[a,2]	17.4±0.3[a,A]	17.0±0.8[a,1]
T_{eff} (days)	22.5±2.3[a,B]	21.8±0.5[a,3]	21.6±0.4[a,B]	18.7±1.8[a,2]	17.2±0.2[b,A]	15.5±1.0[a,1]

[*] M_{max}: Maximum CH_4 production potential, R_{max}: Maximum CH_4 production rate, λ: Lag phase period, R^2: Correlation coefficient, T_{90}: Time required to obtain 90% of M_{max}, T_{eff}: Effective digestion time (T_{90} - λ)

[**] Values are expressed as mean ± standard deviation

[***] The data was also reported in **Table 2.4**

[a,b] Means of the same substrate (BCSW, CD, or SS) at different S/I ratio with different lowercase letter differ significantly

(p<0.05)

[A,B] Means at S/I ratio of 1 with different uppercase letter differ significantly (p<0.05)

[1,2] Means at S/I ratio of 0.5 with different number differ significantly (p<0.05)

Long λ indicated that VS in BCSW was not readily available for the methanogen. This could be related to high fat content in the BCSW (57.4 %DM). Fat needs to undergo hydrolysis, acidogenesis, and acetogenesis to produce acetic acid, CO_2, and H_2 which are substrates for methanogenesis process. Long λ was also observed during anaerobic digestion of PSW (**Chapter 2**). One possible cause for long λ is microbial adaptation to high fat content (Silvestre et al., 2011).

BCSW showed potential as substrate for anaerobic digestion based on the SMY, D_{deg}, and M_{max} values. However, long λ might affect overall anaerobic reactor performance especially the digestion time. **Table 3.4** showed T_{90} and T_{eff} values for anaerobic digestion of BCSW. The technical digestion time, T_{90}, is defined as required time to obtain 90% of M_{max} (Kafle & Kim, 2013). The T_{90} estimated in this study was 32.7 and 32.8 days (d) for BCSW at S/I ratio of 1 and 0.5, respectively. The T_{eff} for BCSW estimated n this study was 22.5 and 21.8 d at S/I ratio of 1 and 0.5, respectively. Compare to SS and CD, BCSW had significantly higher ($p<0.05$) λ, T_{90}, and T_{eff} at S/I ratio of 0.5 indicating low degradation rate. However, at S/I ratio of 1, there was no significant different of λ, T_{90}, and T_{eff} between BCSW and CD. Still, λ, T_{90}, and T_{eff} of BCSW and CD was higher than those of SS at S/I ratio of 1.

High λ, T_{90}, and T_{eff} might be caused by high fat and low mineral content in the BCSW. As explained before, fat needs to be converted into volatile fatty acid prior to the methanogenesis process. In addition, low FS content might result in inadequate available nutrient for anaerobic digestion. Long λ, T_{90}, and

T_{eff} during anaerobic digestion of BCSW need to be avoided since it might increase plant operational cost that reduces economic benefit of the treatment. Still, high M_{max} indicated that BCSW can be utilized as substrate for anaerobic digestion. Substrate co-digestion might be the solution to reduce the λ, T_{90}, and T_{eff} during anaerobic digestion of BCSW.

Compared to BCSW and CD, SS had less M_{max} at both S/I ratio. At S/I ratio of 1, M_{max} of SS was only 50 % of BCSW M_{max}. However, SS had lower λ, T_{90}, and T_{eff} indicated that VS in SS was available for the methanogen at the beginning of anaerobic digestion. In addition, high moisture (low TS) content also indicated that the VS and nutrient in SS might mostly exist in soluble form that makes it easier to be utilized by the microorganisms during anaerobic digestion.

Low VS content in SS resulting in low M_{max} and SMY compared to BCSW and CD ($p<0.05$). Low VS content indicated SS rich in mineral that might benefit methanogen growth and metabolism. In addition, low λ, T_{90}, and T_{eff} estimated from anaerobic digestion of SS might also help to reduce the λ, T_{90}, and T_{eff} from anaerobic digestion of BCSW to achieve better digestion efficiency. From SS perspective, low SMY and M_{max}, caused by low VS content, indicated that SS anaerobic digestion was uneconomical. Thus, SS requires additional VS that can enhance CH_4 production. As such, co-digestion of SS with BCSW can be a viable option.

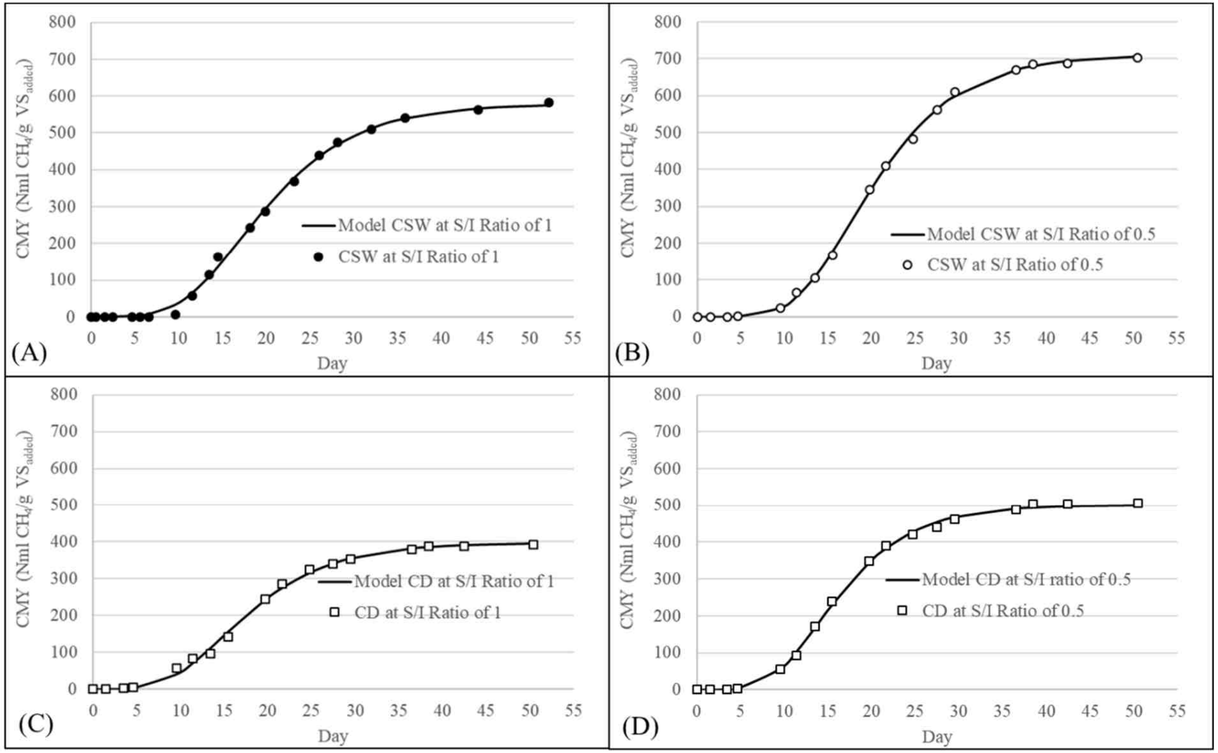

Figure 3.2. Cumulative CH_4 yield (CMY) estimated by modified Gompertz formula (line) plotted against experimental CMY (bullet points) of beef cattle slaughterhouse waste (BCSW) and cattle dung (CD). (A) BCSW at S/I ratio of 1, (B) BCSW at S/I ratio of 0.5, (C) CD at S/I ratio of 1, (D) CD at S/I ratio of 0.5

CD showed higher M_{max} than SS at both S/I ratio (**Table 3.4**). The M_{max} of CD was 397 and 501.2 Nml CH_4/g VS_{added} at S/I ratio of 1 and 0.5, respectively. This was around 68% of BCSW M_{max}. However, similar to BCSW, there was estimated λ of 8.3 and 8.2 d at S/I ratio of 1 and 0.5, respectively. This indicated that, VS in CD was also not readily available for the methanogen from the beginning of digestion process. CD had high TS and neutral detergent fiber (NDF) compare to SS (**Table 3.2**). High TS and NDF content also requires long hydrolysis and acidogenesis stage to produce volatile fatty acid, H_2, and CO_2 for the methanogens similar to fat. In addition, low FS content in CD compare to SS also indicated that it might contain inadequate available nutrient for the methanogens. As such, CD might not be a viable option as co-digestion substrate for anaerobic digestion of BCSW. Still, the anaerobic co-digestion of cattle dung and cattle slaughterhouse waste was conducted at S/I ratio of 1.

The predicted cumulative CH_4 yield (CMY) from modified Gompertz formula were plotted against the experimental CMY from BCSW and CD to test the model accuracy (Zhang et al., 2014). The correlation coefficients (R^2) were in ranged of 0.998 to 0.999 (**Table 3.4**), indicating modified Gompertz formula also had best fit for BCSW and CD. The results were shown in **Figure 3.2**.

3.4.4. Co-digestion of Beef Cattle Slaughterhouse Waste with Cattle Dung or Swine Slurry

Table 3.5 showed the co-digestion effect of beef cattle slaughterhouse waste

(BCSW) with swine slurry (SS) to anaerobic digestion parameters estimated by modified Gompertz formula. The results for anaerobic digester parameters of individual BCSW and SS were taken from **Table 3.4**. One-way ANOVA followed by Tukey HSD test was utilized to determine the significant difference from anaerobic digestion parameters between treatment (individual and co-digested mixture) at S/I ratio of 1 or 0.5.

From SS perspectives, anaerobic co-digestion increased the M_{max} from 289.8 and 453.2 Nml CH_4/g VS_{added} (SS sole digestion) to between 431 and 685 Nml CH_4/g VS_{added} (BCSW and SS co-digestion). The increased of M_{max} from co-digestion experiment was equal to 30 to 75.5% increase compared to M_{max} of individual SS digestion. In addition, co-digestion did not negatively affect the T_{eff} compared to individual SS digestion. However, BCSW co-digestion with SS affects the T_{90} compared to individual SS digestion.

Co-digestion resulted in significantly higher T_{90} at BCSW content of 67 (C4) and 50 (C5) %VS basis in the co-digested mixture at both S/I ratio compare to individual SS digestion (**Table 3.5**). This indicated that high BCSW content in the mixture resulted in longer digestion time. Still, the highest T_{90} was observed at individual BCSW digestion. High fat content in the co-digested mixture due to higher BCSW concentration might be the cause of this observation. Although fat had high CH_4 potential, it is associated with low degradation rate (Alves et al., 20009). Interestingly, the T_{90} of co-digested mixture with BCSW content of 33% VS basis (C6) showed no significant difference with T_{90} of individual SS digestion at both S/I ratio.

Table 3.5. Anaerobic digestion parameters from co-digestion of beef cattle slaughterhouse waste (BCSW) with swine slurry (SS). The BCSW content in co-digested mixture was 100% (C1), 0% (C2), 67% (C4), 50% (C5), and 33% (C6) in volatile solid (VS) basis

Parameters[1]	**S/I Ratio of 1**[2,3]					**S/I Ratio of 0.5**[2,4]				
	C1A	**C2A**	**C4A**	**C5A**	**C6A**	**C1B**	**C2B**	**C4B**	**C5B**	**C6B**
M_{max} (Nml CH_4/g VS_{added})	578.5 ±14.4^{d}	289.8 ±8.6^{a}	508.5 ±35.8^{c}	484.0 ±8.9^{c}	430.7 ±16.9^{b}	711.4 ±13.7^{D}	453.2 ±11.0^{A}	685.5 ±7.7^{CD}	667.4 ±12.9^{C}	592.7 ±17.1^{B}
R_{max} (Nml CH_4/g VS_{added}/d)	30.8 ±2.6^{b}	20.1 ±0.9^{a}	42.5 ±1.9^{c}	34.5 ±0.9^{b}	35.0 ±0.7^{b}	39.0 ±0.7^{A}	35.0 ±2.4^{A}	59.7 ±1.2^{D}	52.5 ±1.7^{C}	46.3 ±2.2^{B}
λ (d)	10.2 ±1.7^{e}	0.2 ±0.1^{a}	7.4 ±0.1^{d}	4.9 ±0.6^{c}	2.4 ±0.1^{b}	11.0± 0.0^{E}	1.5 ±0.2^{A}	8.5 ±1.1^{D}	5.3 ±0.4^{C}	3.4 ±0.2^{B}
R^2	0.999	0.989	0.998	0.998	0.997	0.999	0.991	0.998	0.997	0.997
T_{90} (d)	32.7 ±2.4^{c}	17.4 ±0.3^{a}	21.7 ±0.5^{b}	21.7 ±0.3^{b}	17.1 ±0.4^{a}	32.8 ±0.5^{D}	17.0 ±0.8^{A}	22.3 ±1.0^{C}	20.5 ±0.3^{B}	18.7 ±0.3^{A}
T_{eff} (d)	22.6 ±2.4^{b}	17.2 ±0.2^{a}	14.3 ±0.5^{a}	16.8 ±0.2^{a}	14.7 ±0.3^{a}	21.8 ±0.5^{C}	15.5 ±1.0^{B}	13.7 ±0.3^{A}	15.2 ±0.4^{AB}	15.3 ±0.4^{B}

1 M_{max}: Maximum CH_4 potential production, R_{max}: Maximum CH_4 production rate, λ: Lag phase period, R^2: Correlation coefficient, T_{90}: Time required to obtain 90% of M_{max}, T_{eff}: Effective digestion time (T_{90} - λ), d: days

2 Values are expressed as mean ± standard deviation

3 Means in the same row at S/I ratio of 1 (A) with different lowercase letter differs significantly (p<0.05)

4 Means in the same row at S/I ratio of 0.5 (B) with different uppercase letter differs significantly (p<0.05)

Higher M_{max} of co-digested mixture than individual SS digestion was due to VS and fat addition from the BCSW to the co-digested mixture. SS had low VS and fat content compare to BCSW. VS and fat addition in the co-digested mixture resulted in increased M_{max} because fat had the highest CH_4 potential among the organic compounds (Alves et al., 2009).

Table 3.6. Anaerobic digestion parameters from co-digestion of beef cattle slaughterhouse waste (BCSW) with cattle dung (CD). The BCSW content in co-digested mixture was 100% (C1), 0% (C3), 67% (C7), 50% (C8), and 33% (C9) in volatile solid (VS) basis

Parameters[1]	**S/I Ratio of 1[2,3]**				
	C1A	**C3A**	**C7A**	**C8A**	**C9A**
M_{max} (Nml CH_4/g VS_{added})	578.5 ±14.4[c]	397.2 ±15.3[a]	422.0 ±13.8[a]	496.8 ±12.2[b]	557.9 ±16.7[c]
R_{max} (Nml CH_4/g VS_{added}/d)	30.8±2.6[b]	22.0±0.8[a]	24.7±0.3[a]	29.6±1.8[b]	30.8±1.4[b]
λ (d)	10.2±1.7[a]	8.3±0.4[a]	7.1±1.6[a]	7.5±1.7[a]	8.2±1.0[a]
R^2	0.999	0.998	0.999	0.999	0.998
T_{90} (d)	32.7±2.4[b]	29.9±0.4[b]	27.5±1.7[a]	27.5±1.5[a]	29.9±1.7[b]
T_{eff} (d)	22.6±2.4[a]	21.6±0.4[a]	20.4±0.8[a]	20.1±0.9[a]	21.7±0.8[a]

1 M_{max}: Maximum CH_4 potential production, R_{max}: Maximum CH_4 production rate, λ: Lag phase period, R^2: Correlation coefficient, T_{90}: Time required to obtain 90% of M_{max}, T_{eff}: Effective digestion time (T_{90} - λ), d: days

2 Values are expressed as mean ± standard deviation

3 Means in the same row with different lowercase letter differs significantly ($p<0.05$)

Table 3.6 showed the co-digestion effect of BCSW with cattle dung (CD) to anaerobic digestion parameters estimated by modified Gompertz formula. The results for anaerobic digester parameters of individual BCSW and CD were taken from **Table 3.4**. Anaerobic co-digestion of BCSW and CD had M_{max} of 557.9, 496.8, and 422 Nml CH_4/g VS_{added} for co-digested mixture contains 67, 50, and 33% of BCSW, respectively. The M_{max} of co-digested mixture contains BCSW and CD showed 3 to 27% decrease of M_{max} compare to individual BCSW digestion. In addition, co-digestion between BCSW and CD had no significant effect to λ and T_{eff} compare to individual BCSW digestion ($p>0.05$). This indicated that CD was not suitable to be co-digested with BCSW.

Most of the co-digested mixture of BCSW and SS at both S/I ratio had M_{max} lower than M_{max} of individual BCSW digestion ($p<0.05$). The only exception was observed from mixture between BCSW and SS at BCSW content of 67% volatile solid (VS) basis in the mixture at S/I ratio of 0.5 (C4B). The M_{max} of C4B was not significantly lower than M_{max} of individual BCSW digestion ($p>0.05$).

The M_{max} obtained from co-digestion of BCSW and SS was 508.5, 484, and 430.7 Nml CH_4/g VS_{added} at S/I ratio of 1 for co-digested mixture contains 67, 50, and 33% of BCSW, respectively. At S/I ratio of 0.5, the M_{max} was 685.5, 667.4, and 592.7 Nml CH_4/g VS_{added} for co-digested mixture contains 67, 50, and 33% of BCSW, respectively. These values were equal to 3.7 to 25.6% decrease of M_{max} compare to BCSW individual digestion.

Lower M_{max} obtained during anaerobic co-digestion of BCSW with other substrates than individual BCSW digestion had been reported. Pagés-Díaz et al. (2018) reported that anaerobic co-digestion of cattle slaughterhouse waste with mixture of livestock manure (2:1 VS basis) gave M_{max} of 570 Nml CH_4/g VS_{added}. This was lower than M_{max} from individual cattle slaughterhouse waste that had M_{max} of 668 Nml CH_4/g VS_{added}.

Low M_{max} observed from the co-digestion between BCSW waste with CD or SS was caused by low M_{max} and fat content of CD and SS. Fat has CH_4 potential of 990 Nml CH_4/g VS_{added}. This was higher than carbohydrate and protein which has CH_4 potential of 415 and 633 Nml CH_4/g VS_{added}, respectively (Alves et al., 2009). Fat content in CD and SS was only 3.1 and 5.6 %DM, respectively. In addition, CD had the highest neutral detergent fiber content (61.3 %DM) that has lower CH_4 than protein. This resulted in low M_{max} of CD even though its VS content was comparable to BCSW.

Still, the co-digestion of BCSW and SS had significant effect on lag phase period (λ), effective digestion time (T_{eff}), and time to obtain 90% of CH_4 potential (T_{90}) (**Table 3.5**). Anaerobic digestion of BCSW had significantly higher λ, T_{eff}, and T_{90} than co-digested mixture of BCSW and SS at both S/I ratio ($p<0.05$). Co-digestion shortened the λ from 10.2 and 11 d (BCSW sole digestion at S/I ratio of 1 and 0.5, respectively) to between 2.4 and 8.5 d (BCSW and SS co-digestion at both S/I ratio). It also shortened T_{eff} from 22.6 and 21.8 d (BCSW sole digestion at S/I ratio of 1 and 0.5, respectively) to between 13.7 and 16.8 d (BCSW and SS co-digestion at both S/I ratio). T_{90} was also shortened from 32.7 and 32.8 d (BCSW sole digestion at S/I ratio of 1 and 0.5, respectively) to 17.1 to 22.3 d (BCSW and SS co-digestion at both S/I ratio). Increased of SS composition in co-digested mixture reduced the λ at both S/I ratio. However, it had no significant effect on T_{90} and T_{eff}. Shorter λ, T_{90}, T_{eff} indicated that co-digestion of BCSW and SS improved anaerobic digestion efficiency in term of faster digestion rate from BCSW perspective.

Improved digestion rate of co-digested mixture consists of BCSW and SS compared to sole BCSW digestion might be due to more VS and nutrient were available for the microorganisms. **Table 3.7** showed that BCSW had lower cobalt (Co), iron (Fe), molybdenum (Mo), nickel (Ni), tungsten (W), and zinc (Zn) content than SS. This indicated that, co-digested mixture had high nutrient content compare to BCSW. In addition, SS had high moisture content (95.9 %FW) indicating most of VS and nutrient in the co-digested mixture might be in soluble form.

Adequate Co and Ni content are important for anaerobic digestion. Co is co-factor of metyltransferase. Ni is cofactor for methyl coenzyme M reductase (MCR) (Choong et al., 2016). These enzymes have significant role at the last stage of methanogenesis process. Methyltransferase catalyzes the formation of methyl Coenzyme M (CH_3-CoM) complex and MCR catalyzed the reduction of CH_3-CoM complex into CH_4 (Guo et al., 2015).

Table 3.7 showed that BCSW only had Co and Ni content of 0.7 and 3.4 mg/kg, respectively. This was much lower than Co and Ni content of 10.4 and 25 mg/kg, respectively, obtained from SS. The recommended Co and Ni content for anaerobic digestion was 0.03 to 35 mg/kg (**Table 1.1**) (Romero-Güiza et al., 2016). This indicated that BCSW actually had adequate Co and Ni content for anaerobic digestion. In addition, BCSW had high CH_4 production and anaerobic degradability (**Table 3.3** and **3.4**). However, long λ, T_{90}, and T_{eff} observed during anaerobic digestion of BCSW might indicate that both Co and Ni might not be readily available from the start of anaerobic digestion.

Table 3.7. Comparison of mineral content between beef cattle slaughterhouse waste (BCSW), cattle dung (CD), and swine slurry (SS)

Mineral	BCSW[1] (mg/kg)	CD[1] (mg/kg)	SS[1,2] (mg/kg)
Cobalt (Co)	0.7±0.3[a]	2.2±0.1[b]	10.4±0.1[c]
Iron (Fe)	1,111.5±14.7[a]	975±346.1[a]	10,872±135.8[b]
Molybdenum (Mo)	8.9±5.8[a]	0.9±0.1[a]	13.0±0.1[a]
Nickel (Ni)	3.4±0.7[a]	4.9±0.4[a]	25.0±0.1[b]
Tungsten (W)	0.0±0.0[a]	5.5±0.5[b]	22.1±0.0[c]
Zinc (Zn)	626.8±195.3[a]	576.3±43.2[a]	2,239±19.9[b]

[1] Values are expressed as mean ± standard deviation

[2] The data was also reported in **Table 2.7**

[a,b] Means in the same column with different lowercase letter differ significantly ($p<0.05$)

BCSW had high VS content that might form mineral complex with Co and Ni. This made hydrolysis and acidification process was required to make the mineral become available to the methanogen. Similar to PSW, this might cause long λ, T_{90}, T_{eff} observed during anaerobic digestion of BCSW.

Combining SS and BCSW during co-digestion might increase the availability of Co and Ni in the co-digested mixture. This because, SS was in liquid form (moisture content of 95.1 %FM) that indicates most of VS, Co, and Ni in SS might be in soluble form. This might be the cause of improved digestion efficiency (shorter λ, T_{90}, and T_{eff}) of co-digested mixture compared to individual BCSW digestion.

However, only total concentration of Co and Ni was analyzed in this study. As stated before, this study was focus on the BCSW and SS co-digestion effect to anaerobic digestion parameters. Thus, the effect of BCSW and SS co-digestion to nutrient availability and its correlation with anaerobic digestion parameters seems to be interesting topic for future study to clearly understand the reasons for shorter λ and digestion time during anaerobic co-digestion of BCSW and SS.

In regards to BCSW and CD co-digestion, **Table 3.7** showed that both substrates had lower Co and Ni content than SS. Still, Ni and Co content in both BCSW and CD were within the recommended Co and Ni content for anaerobic digestion. This was confirmed by the M_{max}, D_{deg}, and SMY of BCSW and CD that showed high CH_4 production and anaerobic degradability. However, long λ, T_{90}, and T_{eff} observed during anaerobic co-digestion of BCSW and CD indicated that both Co and Ni might not be readily available from the start of anaerobic digestion. CD had high TS and VS content compare to SS indicated the Co and Ni might form mineral complex with VS and were not in soluble form unlike SS. Hydrolysis and acidification process were required to make Co and Ni available to methanogens resulting in long λ, T_{90}, and T_{eff} during BCSW and CD co-digestion that comparable to individual BCSW digestion. Combined with low M_{max} of CD, this resulted in lower M_{max} in the co-digested mixture compare to individual BCSW digestion with no significant effect on digestion time indicating CD was not suitable to be co-digested with BCSW.

Table 3.8. Results of the synergistic or antagonistic effect produced by the co-digestion of beef cattle slaughterhouse waste (BCSW) with swine slurry (SS)

Reactor[1]	Experimental M_{max}[2]	Simulated M_{max}[2]	α[2,3]
C1A	578.5±14.4	578.5±14.4	
C2A	289.8±8.6	289.8±8.6	
C3A	508.5±35.8	482.3±10.7	1.05±0.05
C4A	484.0±8.9	434.2±9.3	1.12±0.02
C5A	430.7±16.9	386.1±8.4	1.12±0.02
C1B	711.4±13.7	711.4±13.7	
C2B	453.2±11.0	453.2±11.0	
C3B	685.5±7.7	625.3±12.8	1.10±0.03
C4B	667.4±12.9	582.3±12.3	1.15±0.01
C5B	592.7±17.1	539.3±11.9	1.10±0.01

1 The BCSW content in co-digested mixture was 100% (C1), 0% (C2), 67% (C3), 50% (C4), and 33% (C5) in volatile solid basis. A indicated the experiment was conducted at S/I ratio of 1, while B indicated the experiment was conducted at S/I ratio of 0.5.

2 Values are expressed as mean ± standard deviation; unit in Nml CH_4/g VS_{added}

3 α= Experimental M_{max}/Simulated M_{max} (**Equation 2-15**)

Substrate co-digestion can produce either synergistic or antagonistic effect. Antagonistic effect occurred when the experimental M_{max} was lower than the simulated M_{max} ($\alpha<1$). Whereas, synergistic occurred when the experimental M_{max} was higher than the simulated M_{max} ($\alpha>1$) (Nielfa et al., 2015). Experimental M_{max} of co-digested BCSW and SS at both S/I ratio showed α value more than 1 indicated that synergistic effect occurred during the anaerobic co-digestion of BCSW and SS (**Table 3.8)**. Complementary characteristics of BCSW (high VS and fat content) and SS (high Co and Ni content) was the reason for the synergistic effect.

3.5. Conclusion

In recent years, there was interest to utilize beef cattle slaughterhouse waste (BCSW) to produce economical product. Anaerobic digestion can convert BCSW into CH_4 that can be utilized to produce heat and electricity. However, wide diversity of substrate characteristics resulted in high variation in CH_4 production. Thus, this study was done to determine the characteristics (chemical, proximate, ultimate, and energy content) of BCSW and cattle dung (CD) and determine BCSW co-digestion effect with swine slurry (SS) or CD to anaerobic digestion parameters. The BCSW content in the co-digested mixture was 67%, 50%, or 33% weight per weight volatile solid basis (w/w VS basis). SS or CD was added as remaining substrate to obtain 100% VS in substrate mixture.

BCSW had high energy content of 29.26 MJ/kg dry matter. This was equal to 41 to 99% higher than heating value of various energy crops. However, high moisture content (83.3% of fresh matter) of BCSW indicated that energy valorization by thermal conversion is uneconomical. In addition, BCSW had high VS content (93% of dry matter) that might complement the characteristics of SS during anaerobic co-digestion. CD also had high VS content (91% of dry matter).

In term of anaerobic digestion parameters, both BCSW and CD showed specific CH_4 yield (SMY) in the range of previously reported. Specifically, the SMY of BCSW was 582 and 702 Nml CH_4/g VS_{added} for substrate to inoculum (S/I) ratio of 1 and 0.5 weight per weight volatile solid (w/w VS) basis, respectively. While CD showed SMY of 431 and 505 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5 w/w VS basis, respectively. Higher SMY in S/I ratio of 0.5 indicated that S/I ratio had significant effect during anaerobic digestion of BCSW and CD. High inoculum concentration might dilute toxic or inhibitory compounds during anaerobic digestion.

In addition, there was lag phase period (λ) of 10.2 and 11 days (d) for S/I ratio of 1 and 0.5, respectively, during anaerobic digestion of BCSW. CD also showed λ of 8.3 and 8.2 d for S/I ratio of 1 and 0.5, respectively. However, the difference of λ at different S/I ratio for BCSW and CD was not statistically significant ($p>0.05$). Moreover, BCSW also had long effective digestion time (T_{eff}) of 22.5 and 21.8 d for S/I ratio of 1 and 0.5, respectively. In practice, long λ and T_{eff} for waste treatment increases the operational cost that reduces economic benefit of the systems. Thus, long λ and T_{eff} during anaerobic digestion of BCSW must be reduced. Substrate co-digestion might be the solution to reduce the λ and T_{eff} during anaerobic digestion of BCSW. In addition, high VS content of BCSW might increase CH_4 production from SS.

Co-digestion experiment showed that high VS and fat content within BCSW resulted in M_{max} increased from 289.8 and 453.2 Nml CH_4/g VS_{added} (SS sole digestion) to between 431 and 685 Nml CH_4/g VS_{added} (BCSW and SS co-digestion) with no negative effect to T_{eff}. However, low CH_4 production potential of SS, 50 to 64% of BCSW CH_4 production potential, resulted in 3.64 to 25.6% decrease of CH_4 production potential compare to BCSW sole digestion. Still, co-digestion with SS shortened λ (2.8 to 7.8 d shorter) and T_{eff} (6.5 to 8.3 d faster) compare to BCSW sole digestion. BCSW anaerobic co-digestion with CD resulted in 3 to 27% decrease of M_{max} compare to BCSW sole digestion with no positive impact on λ and T_{eff} indicating CD was not suitable to be co-digested with BCSW. Improved digestion efficiency (short λ and faster T_{eff}) during anaerobic co-digestion of BCSW and SS might be due to high Co and Ni concentration and its availability in the SS. While CD also had low Co and Ni concentration that resulted in no improvement in anaerobic digestion efficiency.

It was concluded that anaerobic digestion was a suitable option to treat BCSW due to its high SMY and M_{max}. In addition, co-digestion with SS results in improved anaerobic digestion efficiency as seen from 30.7 to 75.8% increase of M_{max} with no negative effect on T_{eff} compare to individual SS digestion. From the BCSW perspective, co-digestion with SS reduced M_{max} but improved digestion efficiency as seen from reduced λ and T_{eff}. As such, co-digestion can be alternative solution to reduce long λ and T_{eff} associated with anaerobic digestion of BCSW and increase low CH_4 production associated with anaerobic digestion of SS. Still, the mechanisms of shorter λ and T_{eff} of BCSW co-digestion with SS was not clear yet. Thus, the PSW and SS co-digestion effect to mineral availability and anaerobic digestion parameters seems to be interesting topic for future research. Moreover, application of co-digestion technology to increase CH_4 production and its economic benefit in field scale anaerobic digester also seems to be another interesting topic for future study.

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CHAPTER 4. GENERAL CONCLUSION

Drastic change in food consumption pattern in South Korea since 1980 has led to increasing amount of livestock slaughtered causing increased waste generation in slaughterhouse. The total amount of slaughterhouse waste (SW) generated from South Korea in 2017 was around 260,018 and 127,539 tons for pig and beef cattle, respectively. There was interest to convert SW into economical products. Anaerobic digestion converts waste into CH_4 that can be utilized to produce heat and electricity. It had been applied to treat swine slurry (SS). However, anaerobic digestion of SS was considered to be uneconomical due to its low CH_4 production. Still, anaerobic digestion might be an alternative treatment for SW. However, wide diversity of substrate characteristics resulted in high variation in CH_4 production. Thus, this study was done to determine the characteristics (chemical, proximate, ultimate, and energy content) of pig slaughterhouse waste (PSW), beef cattle slaughterhouse waste (BCSW), swine slurry (CD), and cattle dung (CD) and determine the effect of SW co-digestion with livestock waste to anaerobic digestion parameters.

PSW and BCSW showed high volatile solid (VS) content rich in fat indicating both are an energy-rich substrate. The VS and fat content of PSW was 94.6 and 53.6 % of dry matter (%DM). The VS and fat content of BCSW was 93.18 and 57.4 %DM, respectively. PSW and BCSW had high energy content of 28.6 and 29.2 MJ/kg dry matter. However, direct energy production from fresh slaughterhouse waste through thermal conversion is not economical due to its high moisture content. Anaerobic digestion might become alternative solution to obtain energy from BCSW and PSW.

CD also showed high VS content of 91.2 %DM. However, the fat content in CD was only 3.1 %DM. VS in CD mainly consisted of neutral detergent fiber (NDF) (61.3 %DM) and protein (15.4 %DM). SS had low VS content (67.6 %DM) rich in protein (28.1 %DM) and NDF (27.4 %DM). The energy content of CD and SS was within range of previously reported. Specifically, CD had energy content of 17.5 MJ/kg dry matter, while SS had energy content of 17.63 MJ/kg dry matter.

In term of anaerobic digestion parameters, both PSW and SS showed specific CH_4 yield (SMY) in the range of previously reported. Specifically, the SMY of PSW was 611 and 711 Nml CH_4/g VS_{added} for substrate to inoculum (S/I) ratio of 1 and 0.5 weight per weight volatile solid (w/w VS) basis, respectively. While SS only showed SMY of 310 and 516 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5, respectively. S/I ratio of 1 and 0.5 was recommended to be utilized for batch anaerobic digestion to test the presence of inhibitory compounds in the substrate or its production during the anaerobic digestion. Higher SMY in S/I ratio of 0.5 indicated that S/I ratio had significant effect during anaerobic digestion of PSW and SS. High inoculum concentration might dilute toxic or inhibitory compounds during anaerobic digestion.

There was lag phase period (λ) of 9.7 and 9 days (d) for S/I ratio of 1 and 0.5, respectively, during anaerobic digestion of PSW. While, SS only showed λ of 0.5 and 1.5 d for S/I of 1 and 0.5, respectively. Moreover, PSW also had longer effective digestion time (T_{eff}) than SS. In practice, long λ and T_{eff} for waste treatment increases the operational cost that reduces economic benefit of the systems. Thus, long λ and T_{eff} during anaerobic digestion of PSW must be reduced or, if possible, eliminated. Low SMY from SS also results in uneconomical plant operation. Substrate co-digestion might be the solution to reduce the λ and T_{eff} during anaerobic digestion of PSW. In addition, it might also become solution to increase SMY of SS.

PSW co-digestion with SS showed that high VS and fat content within PSW resulted in 22 to 84% increase of maximum CH_4 production potential (M_{max}) compare to SS sole digestion. However, low M_{max} of SS, 48 to 62% of PSW M_{max}, resulted in 7 to 32% decrease of M_{max} compare to PSW sole digestion. Still, co-digestion shortened λ (3.3 to 8.5 d shorter) and T_{eff} (6.5 to 9.1 d faster) compare to PSW sole digestion. Short λ and faster digestion time during anaerobic co-digestion of PSW and SS might be due to high cobalt (Co) and nickel (Ni) concentration in SS. Co and Ni are important cofactor in CH_4 production (methanogenesis) stage during anaerobic digestion.

In term of anaerobic digestion parameters, both BCSW and CD showed SMY in the range of previously reported. Specifically, the SMY of BCSW was 582 and 702 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5, respectively. While CD showed SMY of 431 and 505 Nml CH_4/g VS_{added} for S/I ratio of 1 and 0.5 w/w VS basis, respectively. Higher SMY in S/I ratio of 0.5 indicated that S/I ratio had significant effect during anaerobic digestion of BCSW and CD. High inoculum concentration might dilute toxic or inhibitory compounds during anaerobic digestion. In addition, there was λ of 10.2 and 11 d for S/I ratio of 1 and 0.5, respectively, during anaerobic digestion of BCSW. CD also showed λ of 8.3 and 8.2 d for S/I ratio of 1 and 0.5, respectively. Moreover, BCSW also had long T_{eff} of 22.5 and 21.8 d for S/I ratio of 1 and 0.5, respectively. CD also had long T_{eff} of 21.6 and 18.7 d for S/I ratio of 1 and 0.5, respectively. In practice, long λ and T_{eff} for waste treatment increases the operational cost that reduces economic benefit of the systems. Thus, long λ and T_{eff} during anaerobic digestion of BCSW must be reduced. Substrate co-digestion might be the solution to reduce the λ and T_{eff} during anaerobic digestion of BCSW. In addition, high VS content of BCSW might increase CH_4 production from SS.

BCSW co-digestion with SS also showed that high VS and fat content within BCSW resulted in 30.7 to 75.8% increase of M_{max} compare to SS sole digestion. However, low M_{max} of SS, 50 to 64% of BCSW M_{max}, resulted in 3.64 to 25.6% decrease of M_{max} compare to BCSW sole digestion. Still, co-digestion with SS shortened the λ (2.8 to 7.8 d shorter) and T_{eff} (6.5 to 8.3 d faster) compare to BCSW sole digestion. BCSW co-digestion with CD resulted in 3 to 27% decrease of M_{max} compare to BCSW sole digestion. In addition, there was no significant effect to λ and T_{eff} indicating CD was not suitable to be co-digested with BCSW. High Co and Ni concentration in the SS might be the cause of improved anaerobic digestion efficiency (short λ and faster effective digestion time) during anaerobic co-digestion of BCSW and SS. While CD had low Co and Ni concentration that resulted in no improvement in anaerobic digestion efficiency

It was concluded that anaerobic digestion was an appropriate technology to treat PSW and BCSW due to their high SMY and M_{max}. However, long λ and T_{eff} during anaerobic digestion of SW need to be avoided. From SS perspective, SW co-digestion with SS, at SW content of 67, 50, and 33% VS basis in the mixture, increased M_{max} without any adverse effect to λ and T_{eff} indicating improved digestion efficiency. From SW perspective, co-digestion with SS, at similar SW content in the mixture, reduced M_{max} but improved digestion efficiency as seen from reduced λ and T_{eff}.. As such, co-digestion can be a solution to increase low CH_4 production associated with large-scale anaerobic digestion of SS and reduce long λ and T_{eff} associated with anaerobic digestion of SW. High concentration of Co and Ni in SS combined with high VS and fat content of SW might be the causes of these observations. This indicated that co-digestion of SW and SS resulting in complementary effect in term of VS and micro-nutrient availability for anaerobic digestion.

The SW and SS co-digestion effect to mineral availability and its correlation with anaerobic digestion parameters seem to be interesting topic for future research to clearly understand the reason for the improvement in digestion efficiency. Moreover, application of co-digestion technology to increase CH_4 production from SS and its economic benefit analysis during field scale anaerobic digestion also seems to be another interesting topic for future research.

요약 (국문초록)

기질 혼합 분해를 통한 도축장 폐기물의 바이오 에너지 잠재성 및 혐기성 소화 변수의 개선방안

1980년 이후 한국의 음식 소비 패턴의 급격한 변화는 도축장 폐기물을 증가시키는 도축되는 가축의 양의 증가를 야기했다. 지난 해 국내에서 발생한 도축장 폐기물의 총량은 돼지와 육우의 경우 각각 26만 18톤과 12만 7539톤 이었다. 기존의 처리방법(해양 투기)이 2012년에 금지됨에 따라 적절한 도축장 폐기물 처리가 큰 이슈가 되었다. 한 가지 대안은 혐기성 소화를 통해 도축장 폐기물을 처리하는 것이었다. 그러나 이 과정은 폐기물 특성 차이로 인해 여러 CH_4 생성이 발생했다. 따라서, 폐기물 특성 판단이 필수적이었다.

매년 한국에서 약 1300만 톤의 돼지 슬러리(SS)가 생산되었다. SS 축적은 적절한 처리가 필요해지는 환경 오염으로 이어질 수 있다. 혐기성 소화는 SS를 치료하는 대체 기술이다. 그러나, 이것은 혐기성 소화 공장을 비경제적으로 만드는 낮은 CH_4 생성이라는 특징이 있었다. 기질 혼합 분해는 이 문제에 대한 해결 방법이 될 수 있다.

소 도축장 폐기물(BCSW) 및 돼지 도축장 폐기물(PSW)의 생산이 높기 때문에 본 연구의 목표는 BCSW 및 PSW의 특성을 비교하는 것이 아니라 가축 폐기물과 BCSW 또는 PSW의 통합 소화의 효과 및 특성을

밝히는 것이다. 본 연구는 두 개의 주요 부분으로 나뉜다. 첫 번째 부분에서는, 돼지 도축장 폐기물(PSW)과 돼지 슬러리(SS)의 특성을 화학, 근접, 최종과 에너지 함량에 따라 검사하여 수행했다. 에너지 함량은 봄베 열량계에 따른 발열량 측정과 일괄 (한 회의) 혐기성 소화에 의한 CH_4의 생산 가능성을 내포한다. 또한 혐기성 소화 변수에 대한 PSW 혼합 분해와 SS의 영향도 파악되었다. 혼합 분해된 혼합물의 PSW 함량은 중량 당 중량(질량 백분율 VS 기준)으로 67%, 50% 또는 33%이다. SS는 기질 혼합물에 100% 유기물을 얻기 위해 잔여 기질로 첨가되었다.

PSW 발열량은 다양한 에너지 작물의 발열량보다 37%에서 93.5% 더 높았다. 그러나 PSW의 높은 수분 함량(81.8%)은 열 변환에 의한 에너지 연산은 비경제적이라는 것을 나타냈다. 또한 PSW는 혐기성 생물 통합 분해 시 낮은 유기물 함량(건조 물질의 67.6%)을 가진 SS의 특성을 보완할 수 있는 높은 유기물 함량(건조 물질의 94.5%)을 가지고 있었다. SS의 낮은 유기 물질 함량은 혐기성 분해에 유용할 수 있는 높은 미네랄 함량을 가지고 있다는 것을 나타냈다.

혼합 분해 실험은 PSW 내의 높은 유기 물질 함량이 SS의 단독 분해에 비해 CH_4 생산 가능성을 22%에서 84%까지 증가시킨다는 것을 보여주었다. 그러나, PSW CH_4 생산 가능성의 48~62%인 SS의 낮은 CH_4 생산 가능성은 PSW 단독 분해 대비 CH_4 생산 가능성을 7~32% 감소하게 했다. 그러나 혼합 분해는 PSW의 단독 분해에 비해 지연 단계 기간(3.3일에서 8.5일 더 짧음)과 유효 소화 시간(6.5일에서 9.1일 더

빠름)을 단축시켰다. 혐기성 생물 통합 분해가 진행되는 동안 짧은 지연 단계와 빠른 소화 시간은 PSW보다 SS의 코발트(Co)와 니켈(Ni) 농도가 높기 때문일 수 있다. Co와 Ni는 혐기성 분해 시 CH_4 생산(메탄 생성) 단계에서 중요한 공동 인자이다. 혐기성 분해가 PSW를 처리하는 데 적합한 선택이라고 결론을 내렸다. 또한, 혼합 분해는 CH_4 생산 가능성 증가에서 보여지듯이 단독 SS 분해에 비해 효과적인 소화 시간에 유의미한 영향을 주지 않고 혐기성 분해 효율을 향상시킨다. PSW 관점에서 보면, 통합 분해는 CH_4 생산 잠재성을 감소시켰지만, 지연 단계 기간 감소와 효과적인 소화 시간에서 보듯이 분해의 효율을 향상시켰다.

두 번째 부분에서는, 첫 번째 부분 연구에서 유사한 매개변수로 소 도축장(BCSW)의 특성화가 수행되었다. SS 또는 소의 배설물(CD)에 대한 BCSW 생물 통합 분해가 혐기성 분해 변수에 미치는 영향 또한 파악되었다. 함께 분해된 혼합물의 BCSW 함량은 질량 백분율 VS 기준 66%, 50% 또는 33% 이었다. 기질 혼합물에 100% 유기물을 얻기 위해 잔여 기질로 SS 또는 CD를 첨가했다.

BCSW 발열량은 다양한 에너지 작물의 발열량보다 41~99% 더 높았다. 그러나 BCSW의 높은 수분 함량(83.3%)은 열 변환에 의한 에너지 연산이 비경제적이라는 것을 나타냈다. 또한 BCSW는 혐기성 혼합 분해 시 SS의 특성을 보완할 수 있는 높은 유기물 함량(건조 물질의 93%)을 가지고 있었다. CD는 또한 유기물 함량이 높았다 (건조 물질의 91%)

혼합 분해 실험은 BCSW 내의 높은 유기 물질 함량이 SS 단독 소화

대비 CH_4 생산 가능성 30.7~ 75.8 % 증가를 초래했다는 것을 보여주었다. 그러나 BCSW CH_4 생산 가능성의 50-64%인 낮은 CH_4 생산 가능성은 BCSW 단독 분해 대비 3.6-25%를 감소하였다. 하지만, SS와 혼합 분해는 BCSW의 소화에 비해 지연 단계 기간(2.8일에서 7.8일 단축)과 유효 소화 시간(6.5일에서 8.3일 빠름) 에서 더 빨라졌다. CD와의 BCSW 혐기성 생물 통합 분해는 BCSW의 단독 분해에 비해 CH_4 생산 가능성이 3%에서 27%로 감소하는 결과를 낳았다. 또한, 지연 단계 기간에 유의미한 영향은 없었으며, CD를 BCSW와 함께 분해하기에 적합하지 않음을 나타내는 효과적인 소화 시간은 없었다. BCSW와 SS의 혐기성 통합 분해가 진행되는 동안 SS의 높은 Co와 Ni 농도는 혐기성 소화 효율성 (짧은 지연 단계와 빠른 유효 소화 시간)의 개선의 원인이었다. 반면에 CD는 Co와 Ni의 농도가 낮아서 혐기성 분해 효율이 개선되지 않았다. 혐기성 분해가 BCSW 처치에 적합한 방법이라고 결론을 내릴 수 있다. 또한 SS와 혼합 분해는 CH_4 생산 가능성 증가에서 보여지듯이 단독 SS 분해에 비해 유효 시간에 유의미한 영향을 주지 않고 혐기성 소화의 효율성을 향상시킨다. BCSW의 관점에서 돼지 슬러리와의 혼합 분해는 CH_4 생산 가능성을 감소시켰지만, 지연 단계 기간 감소와 효과적인 분해 시간에서 보듯이 분해의 효율을 향상시켰다.

첫 번째와 두 번째 실험의 결과에 따르면, 혐기성 분해가 PSW와 BCSW를 처치하기에 적절한 기술이라는 결론을 내렸다. 또한, 혼합 분해는 SS의 대규모 혐기성 분해와 관련된 낮은 CH_4 생산에 대한

해결책을 제공한다. SS와 함께 도축장 폐기물도 CH_4 생산 가능성을 증가시켰으며, 이는 지연 단계 지속시간과 효과적인 소화시간에도 전혀 나쁜 영향을 미치지 않았다. 도축장 폐기물의 높은 유기물 함량과 SS의 높은 Co와 Ni의 농도는 이러한 관찰의 원인이었다. 이는 도축장 폐기물과 SS의 혼합 분해가 유기물 및 혐기성 분해를 위한 미시적 가용성 측면에서 상호 보완적인 효과를 초래한다는 것을 의미한다.

핵심 단어: 혐기성 소화, 쇠고기 도축장 폐기물, CH_4 생성, 혼합 분해, 발열량, 돼지 도축장 폐기물, 돼지 슬러리

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