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High-solids anaerobic mono-digestion of riverbank grass under thermophilic conditions

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ABSTRACT

The purpose of this study was to investigate the potential of high-solids anaerobic mono-digestion of riverbank grass under thermophilic conditions, focusing on the effects of the strength and the amount of inoculum. Ensiled grass was inoculated with three different inocula; inoculum from liquid anaerobic digester (LI), inoculum from dry anaerobic digester (DI), and mixture of LI and DI (MI), at feedstock-to-inoculum ratio (FIR) of 1, 2 and 4. The ensiling process of riverbank grass reduced moisture content ($p > 0.05$), while the hemicellulose content was significantly increased from 30.88% to 35.15% ($p < 0.05$), on dry matter basis. The highest methane production was at an FIR of 2 with MI (167 L/kg VS_{added}), which was significantly higher ($p < 0.05$) than with DI, but not significant compared to LI ($p > 0.05$). At an FIR of 4, digesters inoculated with LI and DI failed to produce methane, whereas 135 L_{CH4}/kg VS_{added} was obtained with MI. The kinetic studies showed that at an FIR of 1 with LI and MI, the inoculum had less of effects on the hydrolysis rate constant (0.269 day^{-1} and 0.245 day^{-1}) and methane production (135 versus 149 L/kg VS_{added}); rather, it affected the lag phase. In a thermophilic HS-AD of riverbank grass, the mixture of inoculum with low and high total solids content (TS) helps increase the TS of inoculum and digestion process. An FIR of 2 was deducted to be the limit for a better startup time and higher volumetric productivity of methane.

Introduction

Cellulosic biomass is an essentially inexhaustible source of raw material; with an annual production of 1.5 trillion tons (Kumar et al., 2008), which can be used for sustainable production of environmentally friendly bioenergy such as biogas. Among feedstock, it is of interest for bioenergy production because it does not compete with human food or animal feed (Yang et al., 2015). Composed mainly of cellulose and hemicellulose, and small quantity of lignin (5%–7%) (Frigon and Guiot, 2010), cellulosic biomass is a good feedstock for anaerobic digestion. Based on total solids content (TS), anaerobic digestion (AD) can be categorized as liquid AD (L-AD) when the TS is lower than 15%; otherwise, it is categorized as high-solids AD (HS-AD) (Li et al., 2011a). Both systems have their own advantages and disadvantages; however, the choice of either L-AD or HS-AD is principally based on the characteristics of the feedstock. For example, for substrates that are low in TS, such as animal manure, sewage sludge and food waste, L-AD is preferable, whereas HS-AD is

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more attractive for OFMSW and grass (Li et al., 2011b; Kothari et al., 2014) because they tend to scum, float and stratify during L-AD (Chanakya et al., 1999).

Riverbank grass is among the cellulosic biomass that is widely available during summer and fall in Japan. For example, on the bank of the Satsunai River, Obihiro, the yearly production of grass is estimated to be 600 tons. Generally, it is cut, dried, rolled and incinerated, which obviously causes adverse effects on the environment. Therefore, the use of this grass for HS-AD may help recover the energy contained in the grass as a form of methane gas and a valuable compost-like effluent as a biofertilizer. However, it is a challenging process because at a TS of more than 12%, the biogas production of mono-fermentation of ensiled grass, under mesophilic conditions, drops remarkably, as reported by Koch et al. (2009).

Among the disadvantages of HS-AD are its requirements for higher quantities of inoculum and longer hydraulic retention times (Li et al., 2011b). The strength and amount of inoculum have been reported to have a remarkable impact on the overall performance of the AD process (Forster-Carneiro et al., 2007; Pozdniakova et al., 2012; Dechrugsa et al., 2013). In the case of mono-digestion of grass, which has a low buffer capacity, high C/N ratio and low macro- and micro-nutrient contents (Wilkie et al., 1986; Demirel and Scherer, 2011), some challenges are addressed, such as the longer acclimation period of the inoculum to the substrate, slower startup, and longer retention time. However, with a good quality and appropriate quantity of inoculum, mono-digestion of grass can be made more efficient and stable (Yang et al., 2015). For HS-AD, the solids content in the inoculum is an important parameter for obtaining high TS content in the digester when it is mixed with the substrate. Up to now, many laboratory works on HS-AD have used centrifuged inoculum from L-AD to inoculate HS-AD of different types of feedstock (Li et al., 2013; Shi et al., 2013; Motte et al., 2013). The main objective of centrifugation is to increase the TS content of the inoculum to obtain a high TS content in the digester (Chen et al., 2014), which may affect the microbial community and the macro- and micro-nutrient contents in the inoculum. Another alternative for increasing the TS of the inoculum is through the mixture of two different inocula, such as low TS inoculum originating from the L-AD process and high TS inoculum originating from the HS-AD process. The effect of this mixture inoculum on methane production for HS-AD is investigated and reported on in this study.

The feedstock-to-inoculum ratio (FIR) has been reported to have a remarkable influence on methane production (Dechrugsa et al., 2013; Raposo et al., 2009; Kawai et al., 2014). It is strictly dependent on substrate characteristics and operating temperature (Li et al., 2011a; Zhu et al., 2014). The amount of inoculum, on a volatile solids (VS) basis, is very important in assuring a good startup of AD that helps prevent acidic conditions in the digester (Angelidaki et al., 2009). In some cases, inoculum is the only source of nitrogen, heavy metals and the microbial population that guarantees a balanced microbial community in the digester (Zhu et al., 2014; Xu et al., 2013), especially for high C/N ratio substrates. For HS-AD of lignocellulosic biomass, the start-up phase is strictly dependent on the FIR (Motte et al., 2013). In many cases, a high FIR is related to an overloading of the feedstock that leads to the accumulation of volatile fatty acids (VFAs) and to acidic conditions in the digester (Dechrugsa et al., 2013; Shi et al., 2014). In contrast, a low FIR is favorable for a faster startup and increased process performance (Motte et al., 2013; Cui et al., 2011). However, lowering the FIR reduces the reactor's efficiency, which can be defined as the methane production per reactor volume (Li et al., 2013). Therefore, investigating the optimum FIR is of interest for increasing the process performance of HS-AD of riverbank grass.

In HS-AD, digestion temperature is an important factor as it determines the design of digester. For example, Dranco and Kompogas are operated under thermophilic condition, whereas Valorga is operated under mesophilic condition (Karthikeyan and Visvanathan, 2013). It is believed that thermophilic condition is more reliable for HS-AD over mesophilic condition because it enhances the AD process, increases digester's efficiency. Although, higher input energy is required under thermophilic condition, the energy balance can be offset by the higher biogas yield under this condition (Li et al., 2011b; Karthikeyan and Visvanathan, 2013). Therefore, the main objective of the study was to investigate the potential of riverbank grass for methane production under thermophilic HS-AD conditions. In particular, the study focused on the effects of the inoculum mixture and its quantity on methane production. Therefore, the specific objectives were (1) to determine the best inoculum for HS-AD of riverbank grass and (2) to determine the appropriate riverbank grass and inoculum ratio for methane production. Since riverbank grass is seasonally available substrate, ensiling is an important conservation process to preserve the energy content of the grass and ensure a constant supply for AD plants (Vervaeren et al., 2010; McEniry et al., 2014). Therefore, the effect of ensiling process on the characteristics of grass was also investigated.

1. Materials and methods

1.1. Feedstock preparation

Grass was collected in October 2013 on the bank of the Satsunai River, Obihiro, Japan (42°55′N, 143°12′E). Fresh grass was chopped into 20-mm lengths and then 500 g of Si-Master-LP (Snow Brand Seed Co. Ltd., Japan), fermentative lactic acid bacteria composed of *Lactococcus lactis* and *Lactobacillus paracasei*, per ton of grass was added to ensure the lactic fermentation of the grass. The grass was fermented under anaerobic conditions for 3 months under room temperature. Since inception of experiment was several months after ensiling process, the ensiled grass was kept in a freezer at −20°C until use in order to prevent any change in terms of characteristics and components. Before use, the silage was thawed at 4°C for 24 hr and was air-dried until the moisture content was lower than 10% in order to reduce the specific energy requirements for milling (Barakat et al., 2013). The dried silage was coarse-milled using a centrifugal mill to pass through a 1-mm sieve, which is the optimum particle size to make AD economically viable (Barakat et al., 2013) and enabled more accessible surface for microbial attack (Li et al., 2011a). The dried silage was kept in airtight bags at room temperature prior to use.

Table 1 – Characteristics of inoculum.

Parameters	LI before centrifugation	LI after centrifugation [a]	DI	MI
TS	6.42%	9.90%	23.24%	10.80%
VS	4.52%	7.41%	19.80%	8.39%
pH	8.01	7.93	7.89	7.97

LI: Inoculum from active liquid anaerobic digestion plant; DI: Inoculum from active dry anaerobic digestion plant; MI: mixture of LI and DI at a ratio of 45% and 55% (TS basis), respectively.
[a] Centrifuged inoculum from liquid anaerobic digestion plant at 3000 rpm for 15 min.

1.2. *Inocula*

n the study, inocula from two different active anaerobic digesters were chosen: one from a wet biogas digester and another one from the high-solid digester of a Kompogas® system. The characteristics of inocula are presented in Table 1. Both digesters are farm-scales and use dairy manure as the sole substrate. Part of the inoculum from the wet biogas digester (TS 6%) was centrifuged at 3000 rpm for 15 min, and the solid fraction (TS 10%) was used. From the high-solid digester, inoculum was squeezed to separate the liquid and the solid part, and the solid fraction (TS content of 23%) was used. To investigate the effect of these inocula and their mixture, ground ensiled grass was inoculated with three different inocula; (1) centrifuged inoculum from the wet digester (LI), (2) solid inoculum from the Kompogas® digester (DI) after adjusting the TS to 10% by dilution with deionized water, and (3) a mixture of non-centrifuged inoculum from the wet digester and the solid part of the inoculum from the Kompogas® digester (MI) at a ratio of 45% and 55% (VS basis), respectively, with TS of 10%. In order to reactivate the inocula, digesters were fed with 500 g of inocula and were kept in water bath at 55°C for one week prior to use.

1.3. *Experimental set up*

Three groups of experiments were conducted related to the three types of inocula described above. Each group consisted of three different ratios of ensiled grass and inoculum, namely 50:50, 67:33 and 80:20 (on a TS basis), related to a feedstock-to-inoculum ratio (FIR) of 1, 2, and 4, respectively. The pre-mixed inoculum and grass were added into 1-L laboratory-scale batch digesters, made from polypropylene, with an active volume of 500 mL. The TS content in the digesters was adjusted through the addition of deionized water and was maintained at 16%. Control digesters were fed only with 500 mL inoculum and were run simultaneously with the test digesters. The detail of the amount of mixtures is illustrated in Table 2. Each digester was connected to 5-L Tedlar® gas-sampling bags and kept in a water bath at 55°C for 20 days. The digesters were manually agitated twice a day to ensure the homogeneity of substrate in digester, and increase the contact between inoculum and substrate. Aliquot samples were taken before and after digestion and were analyzed for pH, TS and volatile solids content (VS). Particularly after digestion, aliquot samples were used to analyze individual volatile fatty acids (VFAs), as well as the volatile organic acids (FOS) to the total inorganic carbonate (TAC) ratio.

Biogas was determined every day for the first 10 days and thereafter every 2 to 3 days. The biogas in the gasbag was vacuumed using Multi Air Station MAS-1 (As One Corp., Japan) that was attached to a high precision flow gas meter model Wet Gas Meter W-NK-1B (Sinagawa Corp., Japan) to measure the volume of biogas. The gas compositions were analyzed using gas chromatography (GC).

1.4. *Analytical procedure*

Raw riverbank grass and silage were analyzed to determine their protein, lipid, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), non-fiber carbohydrate, calcium, magnesium and potassium contents using the procedures applied in the laboratory of Tokachi Agricultural Cooperation (Appendix A S1). Cellulose and hemicellulose contents were calculated by subtracting ADF from NDF and ADL from ADF, respectively.

TS and VS were measured according to the standard methods (part 2540G) (APHA, 2005). The pH was measured using a Horiba D-55 pH meter. FOS and TAC were measured according to the

Table 2 – Experimental design.

	Type of inoculum	Reactor	Quantity of inoculum (g)	Quantity of grass (g)	Quantity of water (g)
Controls	LI	R1	500	0	0
	DI	R2	500	0	0
	MI	R3	500	0	0
FIR 1	LI	R4	400	42	58
	DI	R5	400	42	58
	MI	R6	400	42	58
FIR 2	LI	R7	266	56	177
	DI	R8	266	56	177
	MI	R9	266	56	177
FIR 4	LI	R10	160	68	272
	DI	R11	160	68	272
	MI	R12	160	68	272

LI: Inoculum from active liquid anaerobic digestion plant; DI: Inoculum from active dry anaerobic digestion plant; MI: mixture of LI and DI at a ratio of 45% and 55% (TS basis), respectively.

Nordmann-titration method (Kafle et al., 2012), and the FOS to TAC ratio was used to characterize the digestates, along with pH and VFA (Lossie and Pütz, 2008; Xu and Li, 2012).

Gas compositions (methane, carbon dioxide, hydrogen, nitrogen and oxygen) were determined using a GC-14A (Shimadzu, Japan) equipped with a thermal conductivity detector (stainless column and Porapak Q packing). The operational temperatures of the injector port, the column and the detector were 220, 150 and 220°C, respectively. Argon was the carrier gas at a flow rate of 50 mL/min. Individual VFA (formic acid, acetic acid, propionic acid, and butyric acid) of samples before and after anaerobic digestion were analyzed with a high-performance liquid chromatograph (HPLC, Shimadzu LC-10AD, Japan) with a Shim-Pack SCR-102H column. Kimura et al. (1994) described the analytical procedures in detail.

1.5. *Data analysis*

Gas volume was adjusted at standard temperature and pressure (STP) condition (273 K, 1 atmospheric pressure, dry) using Eq. (1) (El-Mashad and Zhang, 2010):

$$B_{STP} = B_{exp} \cdot \frac{P.C}{R.T} \tag{1}$$

where, B_{STP} (L) is the volume of gas adjusted at STP, P (1000 mbar) is the atmospheric pressure, B_{exp} (L) is the volume of gas at temperature T, C (22.41 L/mol) is the molar volume at STP, R (83.14 L mbar/K/mol) is the universal gas constant, and T (K) is the observed biogas temperature.

The first-order kinetic model (Eq. (2)) was used to characterize the methane production from riverbank grass inoculated with different inocula. When Eq. (1) was logarithmized (Eq. (3)), a straight line would be obtained against time and showed the value of the slope, which is equal to the hydrolysis rate constant (Angelidaki et al., 2009).

$$C_t = C_{max} . (1 - \exp(-kt)) \tag{2}$$

$$\ln\left(\frac{C_{max}}{C_{max} - C_t}\right) = kt \tag{3}$$

where, t (day) is the time, C_{max} (L/kg VS_{added}) is the cumulative methane yield obtained in 20 days, C_t (L/kg VS_{added}/day) is the methane yield obtained at time t, and k is the hydrolysis rate constant.

Gompertz' modified equation (Eq. (4)) was used to calculate the lag phase and methane production potential (Zhu et al., 2014):

$$M_{(t)} = M. \quad \exp\left\{-\exp\left[\frac{R_{max}.e}{M_0}(\lambda - t) + 1.\right]\right\} \tag{4}$$

where, $M(t)$ (L/kg VS_{added}) is the cumulative methane yield at time t, e is exp(1) = 2.71828, R_{max} (L/kg VS_{added}/day) is the maximum specific methane production rate, M (L/kg VS_{added}) is the methane production potential, and λ (day) is the lag phase time. The parameters in this equation (M, R_{max} and λ) were estimated by the least squares method using the Solver Function of Microsoft® Office Excel 2010 (Ohuchi et al., 2014).

VS degradations of inoculum alone and mixture (grass + inoculum) were calculated using Eq. (5) (Koch, 2015):

$$VS_r = 1 - \frac{VS_d.(1 - VS_f)}{VS_f.(1 - VS_d)} \tag{5}$$

where, VS_r (%) is the VS reduction, VS_d (% of TS) is the VS of digestate and VS_f (% of TS) is the VS of feed. The VS reduction of grass alone was calculated using Eq. (6):

$$r_{sub} = VS_r + \frac{VS_{inoc.ad.}}{VS_{sub.ad.}}.(VS_r - r_{inoc.}) \tag{6}$$

where r_{sub} (%) is the VS reduction of grass alone, VS_r (%) is the total VS reduction of the mixture (grass + inoculum), $VS_{inoc.ad.}$ (g/kg) is the VS of inoculum added, $VS_{sub.ad.}$ (g/kg) is the VS of ensiled grass added, and $r_{ionc.}$ (%) is the VS reduction of inoculum alone. In the case that there is no residual digestible VS in inoculum, which means that $r_{inoc.}$ is zero, the $VS_{inoc.ad.}$ is assumed to be zero. Therefore, the VS reduction of

Table 3 – Grass and ensiled grass characteristics.

Parameters	Grass		Ensiled grass	
pH			4.50	
Total solids (g/kg)	230.75		276.00	
Volatile solids (g/kg)	209.25	(90.68%) [a]	252.00	(91.30%) [a]
Crude protein (g/kg)	32.00	(13.87%)	32.00	(11.59%)
Crude fat (g/kg)	6.25	(2.71%)	8.00	(2.90%)
Cellulose (g/kg)	62.42	(27.05%)	67.00	(24.28%)
Hemicellulose * (g/kg)	71.25	(30.88%)	97.00	(35.15%)
Lignin * (g/kg)	11.33	(4.91%)	22.00	(7.97%)
Non Fibers Carbohydrate (g/kg)	36.67	(15.89%)	36.00	(13.04%)
Ca (g/kg)	0.09		0.10	
Mg (g/kg)	0.05		0.05	
K (g/kg)	0.29		0.32	
K/(Ca + Mg)	2.07		2.13	
Butyric acid (g/kg)	ND		0.00	
Lactic acid (g/kg)	ND		13.60	
Acetic acid (g/kg)	ND		0.50	
Propionic acid (g/kg)	ND		0.00	

ND: Not determined.
* Indicates that there is significant difference between fresh and ensiled grass at $p < 0.05$.
[a] Percent value on dry matter basis.

the substrate is the same as the VS reduction of the mixture as shown in Eq. (7).

$$r_{sub.} = VS_r \quad (7)$$

All experiments were conducted in triplicate and results are reported in means. Statistical analysis was performed using STATA version 12.0 (StataCorp LP, USA). Significant difference was determined at p value of less than 0.05.

2. Results and discussion

2.1. Feedstock characterization

The characteristics of raw grass and ensiled grass are presented in Table 3. The ensiling process increased the TS content from 23.1% to 27.6%. However, there was no significant difference ($p > 0.05$). The hemicellulose and lignin contents increased significantly ($p < 0.05$) from 30.88% to 35.15% and from 4.91% to 7.97% (on dry matter basis), respectively. In contrast, the cellulose contents were similar before and after the process ($p > 0.05$). After ensiling process, the pH was 4.50, and lactic acid and acetic acid concentrations were 13.6 and 0.5 g/kg (on fresh matter basis), respectively. The pH of ensiled grass is in the range of grass silages (4.3–4.7) reported by Kung and Shaver (2001). However, the lactic acid concentration is lower than reported in literatures for a good quality silage (Ohshima et al., 1997; Kung and Shaver, 2001). This is assumed as the result of ensiling temperature, which was under room temperature, that causes an unstable fermentation process (Ohshima et al., 1997). When the ensiled grass was air-dried, it is assumed that VFA decreased more remarkably than lactic acid (Kreuger et al., 2011) and the remaining VFA and lactic acid were considered to have negligible effect on the overall methane production (Koch et al., 2009; Kreuger et al., 2011).

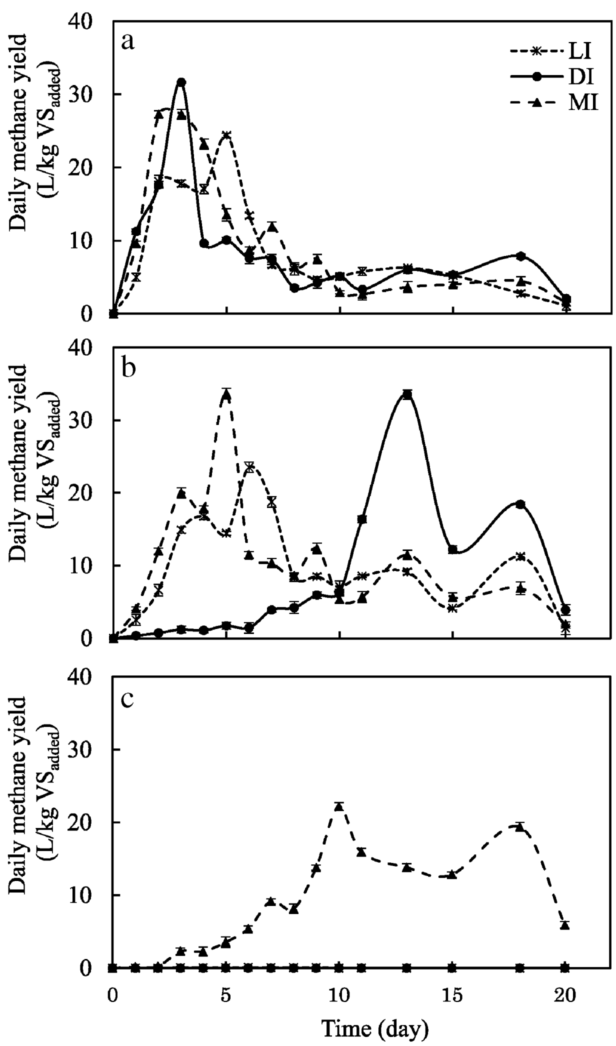

Fig. 1 – Daily methane yield at different feedstock to inoculum ratios: (a) FIR of 1, (b) FIR of 2, and (c) FIR of 4. FIR: feedstock-to-inoculum ratio.

2.2. Methane production and digestate properties

2.2.1. Daily methane production

Fig. 1 shows the means of daily methane production over the period of 20 days of residence time. Generally, peak values, peaking time and peak appearance are dependent on the FIR and the inoculum sources (Gu et al., 2014). At an FIR of 1 (Fig. 1a), apparent methane peaks of 27.27, 31.63 and 24.38 L/kg VS_{added}/day were observed on days 2, 3 and 5 for digesters inoculated with MI, DI, and LI, respectively, which may be the result of the fast growth of hydrolytic and acidogenic bacteria in the early stages (Brown et al., 2012). Thereafter, daily methane production decreased gradually due to the depletion of an easily available fraction in the substrate. Compared with the results of mesophilic HS-AD of different lignocellulosic feedstock reported by Brown et al. (2012), the peaking times in this study were reduced by almost half, which can be attributed to the fast growth of cellulolytic bacteria, as well as the high microbial activity in the reactor under thermophilic conditions (Shi et al., 2013; Heeg et al., 2014). At an FIR of 2 (Fig. 1b), two major peaks were observed due to the presence of a readily available component such as a soluble fraction (for the first peak) and the subsequent degradation of easily biodegradable components such as cellulose (for the second peak) in the substrate. The days of highest peaks were delayed approximately 1.2, 2.5, and 4.3 times for digesters inoculated with LI, MI, and DI, respectively (Fig. 1b) compared to those at an FIR of 1. This indicates that (1) an FIR of 1 had a faster hydrolysis process than an FIR of 2 (Zhang et al., 2014) and (2) there was a higher amount of easily digestible material that required a longer acclimation period of methanogenic archaea at an FIR of 2 because of the higher percentage of ensiled grass. At an FIR of 4 (Fig. 1c), the digesters inoculated with DI and LI failed to produce methane, while the methane peak was on day 10 with MI.

2.2.2. Methane content and cumulative methane production

Fig. 2 illustrates the means of methane content during this study. After an adaptation period, the methane contents in the digesters were between 50% and 70%, indicating a balanced growth of acidogenic bacteria and methanogenic archaea. However, at an FIR of 4 (Fig. 2c), digesters inoculated with LI and DI failed to produce methane due to the "sour" condition in the digesters. Shi et al., 2014 investigated the anaerobic digestion of corn stover with total effluent and reported that at an FIR of 4.4, the methane content was 40%, which is lower than the methane content with MI at an FIR of 4 in this study. This was attributed to the higher buffer capacity of the inoculum (MI) used in the study that favored the growth of methanogens. Generally, digesters with DI showed higher fluctuation of methane content, whereas approximately 55% and 58% were the average of the methane contents for digesters with LI and MI at an FIR of 1 and 2, respectively. This indicates that the type of inoculum affects the methane content and therefore affects the overall performance of HS-AD of ensiled grass.

The means of cumulative methane yield (CMY) is illustrated in Fig. 3. There was no significant difference ($p > 0.05$) between the values of the CMY at an FIR of 1 for digesters inoculated with LI (139.28 L/kg VS_{added}), DI (132.67 L/kg VS_{added}) and MI (154.61 L/kg VS_{added}) (Fig. 3a). At an FIR of 2, the CMP with MI (167.40 L/kg VS_{added}) was significantly higher ($p < 0.05$) higher than with DI, whereas it is only 108% higher than that of LI ($p > 0.05$) (Fig. 3b), indicating the presence of inhibition in the digesters with DI and LI. At FIR of 4, digesters inoculated with LI and DI failed to produce methane, whereas 135 L_{CH4}/kg VS_{added} was obtained with MI (Fig. 3c). The CMP reported in this study is slightly lower than that reported by Xu et al. (2013) (238.5 L/kg VS_{added}) but higher than that reported by Shi et al., 2014 (100–103 L/kg VS_{added}). However, it is comparable to that obtained by Borowski et al. (2014) (185 L/kg VS_{added}).

2.2.3. *Digestate properties*

The characteristics of digestate are presented in Table 4. The initial pH in the digesters decreased when the FIR was increased, confirming the acidic property of the ensiled grass (Table 4). Because the initial pH was not adjusted to neutral and buffer capacity was not increased, the increase in final pH was entirely dependent on the buffer capacity of the inocula. In general, the final pH varied between 7.40 and 7.92 at FIRs of 1 and 2, which were in the range of "healthy" AD. However, at an FIR of 4, the pH decreased by approximately 1 for digesters inoculated with LI and showed almost no change with DI, whereas it increased by 1 with MI. The decrease in pH at a high FIR has been reported in the literature (Shi et al., 2014; Brown and Li, 2013) and agrees with the results of this study. However, the types of inocula are important for recovering a "sour" digester. Therefore, in HS-AD of grass, the quantity and quality of the inocula are determinant factors for a "healthy" AD process.

In general, the VFA concentrations were in the range of 46.05 to 144.22 mg/L in most digesters, which were in the range of a stable AD process and harmonized microbial activity. It was observed that all VFA in digesters with MI were low, indicating a better balance between fermentative bacteria and methanogenic archaea. In contrast, at an FIR of 4, digesters inoculated with LI had the highest VFA, 133 times than that of an FIR of 1 with LI, while they had 37 times than that with DI at an FIR of 4. The concentration of acetic acid was between 25.90 and 104.65 mg/L, which is far lower than the inhibition concentration (800 mg/L) (Hill et al., 1987). In the failed digesters, the propionate to acetate ratios were 0.13 and 0.14 at an FIR of 4 with LI and DI, respectively, which are 10 times lower than the inhibition concentration (1.4) reported by Hill et al. (1987). This might be caused by the difference in the feedstock that was used. In their study, the feedstock used was swine manure, which is hardly degradable compared to ensiled grass (*e.g.*, VS/TS for swine manure was 0.73 (Dechrugsa et al., 2013; Xie et al., 2011) versus 0.91 for the ensiled grass used in this study).

The FOS to TAC ratios of the effluents are also presented in Table 4. Generally, the ratios were in the range of 0.22 to 0.39, indicating a stable process in the digesters (Lossie and Pütz, 2008), except for the digester with LI at an FIR of 4 with a FOS to TAC ratio of 6.47. This result confirms the decrease in pH at an FIR of 4 with LI, showing an overloading of ensiled grass.

2.3. *Volumetric productivity of methane and volatile solids reduction*

The volumetric methane production (VMP) is given in Fig. 4. The highest VMP was obtained at an FIR of 2 when ensiled grass was inoculated with MI (17.8 $L_{CH4}/L_{reactor}$/day), which is in agreement with the results reported by Brown and Li (2013).

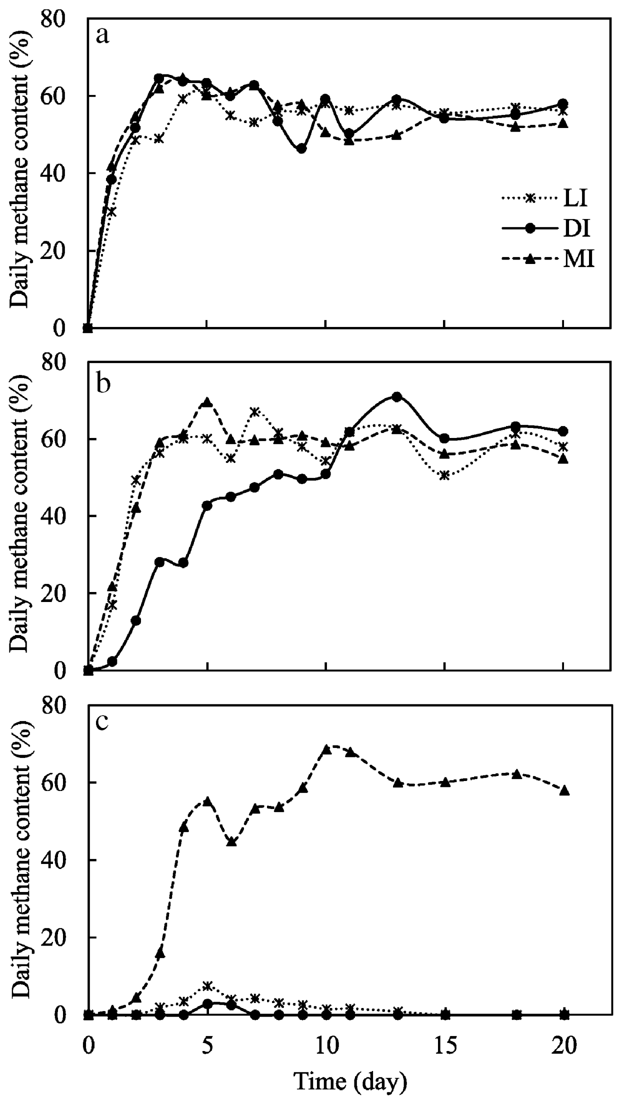

Fig. 2 – Methane concentration at different feedstock to inoculum ratios: (a) FIR of 1, (b) FIR of 2, and (c) FIR of 4.

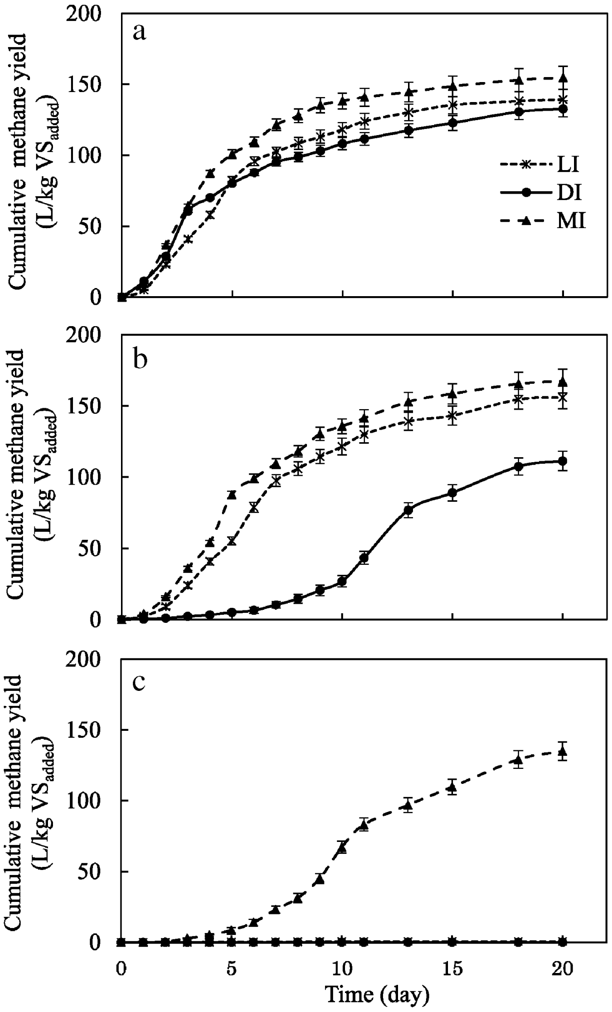

Fig. 3 – Cumulative methane production at different feedstock to inoculum ratios: (a) FIR of 1, (b) FIR of 2, and (c) FIR of 4. ratio.

In their study, the highest VMP of HS-Aco-D of 90% yard waste and 10% food waste was at an FIR of 2. The VMP at an FIR of 2 were significantly higher ($p < 0.05$) than at an FIR of 1 for a digester with LI and MI, whereas no difference ($p > 0.05$) was observed for a digester with DI. At the same FIR (FIRs of 1 and 2) no significant differences ($p > 0.05$) were observed between digesters with LI and MI, whereas lower VMP ($p < 0.05$) was obtained from digesters with DI at an FIR of 2. At an FIR of 4, digesters with LI and DI failed to produce methane, while no significant difference ($p > 0.05$) was observed between an FIR of 2 and 4 for digesters with MI (Fig. 4).

The VS reductions after the HS-AD of riverbank grass are given in Table 4. It is shown that an increase in FIR from 1 to 2 is associated with an increase in VS reduction of 1.3- to 1.4-fold for digesters inoculated with LI and MI, respectively. However, a slight decrease in VS reduction was observed for a digester inoculated with DI (0.9-fold). The percentage of VS reduction is correlated with the VMP, which is in agreement with the results reported by Chen et al. (2014) and Brown and Li (2013). In contrast, at an FIR of 4, although no methane production was observed in the digester inoculated with LI (Fig. 4), there was a 10.5% VS reduction. This means that the feedstock was biologically degraded and was produced intermediate products such as VFA. For digesters inoculated with DI, no VS reduction was observed. The possible reason was the slow biological activity due to low kinetic reactions as a consequence of low water content and low substrate flowability in the digesters. The average degradation of VS in the control digesters was around 7%, which is lower than the degradation of feedstock. The reason might be the reactivation process where bacteria involved in anaerobic digestion used almost the remaining VS in the inocula prior to the mixture with the feedstock.

2.4. Kinetic study of methane production

The coefficients of determination (r^2) and the hydrolysis rate constants (k) are presented in Table 5, while the logarithmic plot of methane production against time is illustrated in Fig. 5. The r^2 values obtained in this study were between 0.74 and 0.98. Digesters inoculated with LI at an FIR of 1 and with MI at FIRs of 1 and 2 showed a linear relationship between methane production and reaction time ($r^2 > 0.96$), and the logarithmic methane production in these digesters followed the first-order kinetic model. However, at an FIR of 2 with LI and DI, at an FIR of 1 with DI, and at an FIR of 4 with MI, the logarithmic methane production did not follow the model, with lower linear correlations (r^2 between 0.74 and 0.93) indicating a biphase profile of methane production in these digesters (Liew et al., 2012). The k were between 0.173 and 0.269 day^{-1}, and the highest k values were observed from digesters inoculated with LI at an FIR of 1 (0.27 day^{-1}) and 2 (0.25 day^{-1}) followed by MI at an FIR of 1 and 2 (0.25 day^{-1}). The k values in this study were lower than those reported by

Table 4 – Initial pH, characteristics of effluents and volatile solids reduction.

	Inocula	pH		FOS/TAC	VFA (mg/L)	HAc (mg/L)	HPr/HAc	VS reduction (%)
		Initial	Final					
FIR 1	LI	7.46	7.70	0.34	104.65	104.65	0	28.04 ± 0.75
	DI	6.7	7.40	0.39	61.14	25.90	0	24.94 ± 2.20
	MI	7.24	7.92	0.22	46.88	50.95	0	25.53 ± 0.64
FIR 2	LI	7.32	7.69	0.27	144.22	78.72	0	35.30 ± 2.65
	DI	5.88	7.41	0.39	101.65	60.03	0	24.27 ± 1.83
	MI	6.78	7.61	0.28	50.95	46.88	0	34.73 ± 1.18
FIR 4	LI	6.49	5.53	6.47	14,032.20	6809.78	0.13	10.49 ± 2.09
	DI	4.98	4.91	ND	3223.65	1001.50	0.14	0.02 ± 0.01
	MI	6.22	7.51	0.33	46.04	46.04	0	42.34 ± 1.98

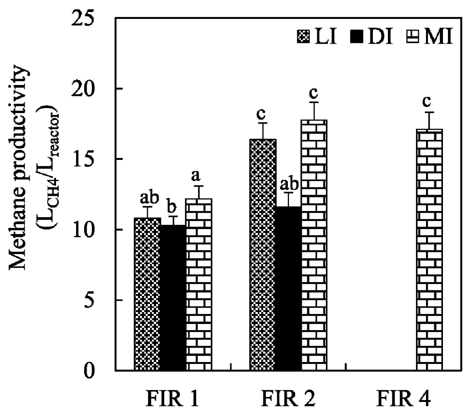

Fig. 4 – Volumetric productivity of methane. Means with different letters are significantly different at $p < 0.05$.

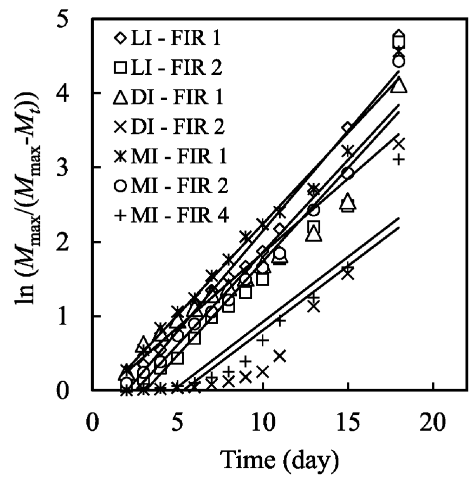

Fig. 5 – Logarithmic plot of methane production against time for the three types of inoculum at different feedstock to inoculum ratios.

Xie et al. (2011) (k between 0.34 and 0.56 day^{-1}) for Aco-D of pig manure and grass silage, but higher than those reported by Liew et al. (2012) (k between 0.12 and 0.13 day^{-1}) for HS-AD of lignocellulosic biomass, indicating the consistency of these results with other studies.

The parameters of the kinetic study using the modified Gompertz model are presented in Table 5. The results showed that the correlation coefficients were all approximately 0.99, indicating that the measured values were well-fitted with the regression model. At the same FIR value, the lag phase of methane production varied according to the types of inocula. At an FIR of 1, there was no lag phase for a digester inoculated with DI, whereas 0.3-day and 0.7-day lag phases were present for digesters inoculated with MI and LI, respectively. When FIR was increased to 2, the lag phases were delayed 2.4, 3.7, and 7.7 times for LI, MI, and DI, respectively. In this study, digesters inoculated with MI were proven to have shorter lag phases and higher methane production at all FIR values. As opposed to AD of meat and bone meal, where the lag phase was increased with solid contents (Wu et al., 2009), in the HS-AD of riverbank ensiled grass, the lag phase was strictly dependent on the types and the amounts of inoculum. For HS-AD of riverbank grass, an FIR of 1 with MI would be suggested to shorten the effective methane production time and improve the methane production rate.

In short, based on the results of the two models, k obtained from MI and LI at an FIR of 1 were slightly different, indicating that the inoculum had less of an effect on the hydrolysis rate of riverbank grass under thermophilic conditions. In contrast, it had a significant effect on the lag phase, which is an important parameter to be considered for the application of the results of batch experiment to a large-scale biogas plant.

3. Conclusions

Riverbank grass was successfully ensiled under room temperature. The methane production was dependent on both the strength and the amount of inoculum. The highest methane yield (167 L/kg VS_{added}) was with MI at an FIR of 2, while the shortest lag phase was at an FIR of 1. The highest hydrolysis rate constant (0.27 day^{-1}) was with LI at an FIR of 1. For HS-AD of riverbank grass, the best inoculum and appropriate FIR was MI and 2, respectively. The results of this study enable us to deduce that the mixture of low and high TS

Table 5 – Results of kinetic studies.

Inocula	FIR	First-order kinetic model		Modified Gompertz model			
		r^2	k (day^{-1})	λ (day)	r^2	R_{max} (L/kg VS)	M (L/kg VS)
LI	1	0.973	0.269	0.74	0.99	17.99	135.41
	2	0.920	0.252	1.72	0.99	17.68	150.84
	4	ND	ND	ND	ND	ND	0.00
DI	1	0.932	0.200	0.00	0.98	15.74	123.70
	2	0.744	0.173	7.18	0.99	11.23	125.70
	4	ND	ND	ND	ND	ND	0.00
MI	1	0.983	0.245	0.30	0.99	21.93	148.83
	2	0.964	0.248	1.10	0.99	19.41	162.27
	4	0.855	0.174	5.20	0.99	12.75	138.32

ND: Not determined.

inocula is an effective method to increase TS of inoculum destined for HS-AD and to ensure the improvement of the process. Therefore, for a laboratory scale experiment of HS-AD, the increase of the TS of inoculum from active liquid anaerobic digester by centrifugation process can be avoided. Similarly, for a large-scale high-solids anaerobic digester mixture inoculum can be used to start the process, which reduces the time required to obtain the target TS in the digester.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.05.005.

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