



Biochemical Methane Potential of Fresh and Silage 4190 Grass Under Thermophilic Conditions

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ABSTRACT

Rapid population growth and expansion of industry have led to higher energy consumption while natural resources have been continuously decreased. Thus, making the pursuit of new energy sources is an urgent need. Thailand is noted as a country of agriculture, having high potential for producing energy crops that can be used to create renewable energy. The relatively high yield and fast growing 4190 grass is an interesting alternative for this purpose. This research was conducted to study the biochemical methane potential of 4190 grass. The modified Biochemical Methane Potential (BMP) test was conducted under thermophilic condition (55.4 ± 0.73 °C) using 4190 grass aged 45, 70 and 90 d both in fresh and silage forms. The obtained methane yields could perfectly be fitted with modified Gompertz model ($R^2 \geq 0.93$). Total methane productions calculated from the model for the fresh and silage grasses of the same age were not significantly different, presumably thanks to the effect of digestion reaction at the thermophilic temperature. The highest methane yield of 199 ± 7.77 NL/kg VS_{added} was attained when the fresh 4190 grass (45-d) was used as a feedstock. The bioreactor design and operation can be implemented from results gained for further study of the reactor performance under the continuous operation.

Keywords : 4190 grass; Fresh grass; Silage; Thermophilic temperature; Biochemical Methane Potential

INTRODUCTION

Energy security is an important factor in the development of the country, due to the demand for more energy and the growth of the industry and population. As a result, seeking energy sources is an urgent need. Thailand is a suitable country for agriculture, as it has the potential to grow energy crops that can be used as raw materials for biogas production. Furthermore, energy crops, especially grass, are an interesting alternative because they are suitable for use as raw materials for biogas production. Grass can be easily planted, have high yield and is easy to harvest. There are many grass species that have been studied for biogas production, such as miscanthus grass, miscanthus giganteus, elephant grass, timothy grass, tall fescue grass, cocksfoot grass, reed canary grass, foxtail meadow grass, rye grass [1-5] and napier grass [6, 7]. It can be seen that many grass species can be used as raw materials for the production of biogas. However, in Thailand, it was found that most of the studies were carried out to specifically investigate the biogas production from Napier grass while there are other potential grass species, especially the 4190 grass that has been supported by the Electricity Generating Authority of Thailand (EGAT) to be used as a raw material for power generation. This grass species has relatively higher yields (≥ 75 tons/rai) and can be easily grown in the environment of Thailand, even in low soil quality without continuous irrigation [8].

The main objective of this research was to determine the biogas production potential (BMP) of the 4190 grass. As large amounts of high temperature condensate are continually generated during electricity generation process by EGAT, utilisation of this condensate for increasing the bioreactor temperature for higher conversion efficiency is very appealing. Therefore, the BMP test was conducted under

thermophilic condition. Results of this study can be used for the design of lab-scale reactor and experimental setup for further investigation.

METHODOLOGY

Inoculum and Substrate

Inoculum used in this study was collected from an anaerobic digester co-digesting Napier Pak Chong 1 grass and dairy waste under ambient temperatures at Chiang Mai Fresh Milk farm, Lamphun, Thailand. The collected samples were cultured under thermophilic condition for 2 months in a completely mixed reactor fed with Napier Pak Chong1 grass before being used in the BMP test. Characteristics of the inoculum are presented in Table 1.

The samples of the first-cut 4190 grass were obtained from a planting area at Sansai District, Chiang Mai, Thailand. Different grass ages, i.e. 45, 70 and 90 d, were harvested and grinded to a size of 10-30 mm with a CLEO blender model CCB403/404. One part of these grinded grass was kept frozen at -20°C for being used as fresh grass and the other part was made into silage by putting in a plastic bag before the air was totally sucked out by a vacuum pump. The bag was kept at room temperature ($28.1 \pm 1.40^{\circ}\text{C}$) for 21 d. The samples were then kept frozen at -20°C until use.

Biochemical Methane Potential

The experiments were conducted to evaluate the potential of grass for biogas production using the modified BMP test in a 1,000 ml glass bottle with a working volume of 700 ml under thermophilic conditions ($55.4 \pm 0.73^{\circ}\text{C}$). Each bottle was added with 240 ml of inoculum and a sample of grass harvested at each age. The volatile solids ratio of inoculum to grass was controlled at 2:1. Distilled water was then added to the working

volume (700 ml). Alkalinity was adjusted with a solution of sodium bicarbonate to pH around 7.0. The bottle was purged with nitrogen gas for 3 minutes and then sealed with a septum. The experiment using only inoculum was used as the controlled experiment (blank). All experiments including blank were done in 3 replicates.

Amounts of biogas produced was measured via the measurement of gas pressure in the bottle using Digital Manometer version DM9200. Each bottle was manually shaken once a day before measuring the pressure. When the pressure in the bottle was up to 300 mbar, the biogas composition was measured by Multichannel Portable Gas Analyzer version GFM406. Conversion of bottle pressures into volumes of biogas produced at standard temperature and pressure was done using Equation (1) [9].

$$V_0^{tr} = \frac{V(P - P_w)T_0}{P_0T} \quad (1)$$

Where V_0^{tr} = Volume of dry gas at normal state (mL)
 V = Volume of gas as read off (mL)
 T_0 = Normal temperature (K), 273 K
 T = Temperature of the fermentation gas or ambient space (K),
 at 55 °C = 328 K
 P_w = Vapor pressure of water as function of temperature of ambient space (hPa),
 at 55 °C = 157.52 hPa
 P_0 = Normal pressure (hPa), 1013 hPa
 P = Pressure of gas phase at the time of reading (hPa)

Analyses of the results of BMP test were conducted by mathematical modelling to predict the amount of methane produced per weight of organic matter. Kinetic analysis was performed on the cumulative production curves produced from

each BMP assay. Kinetic modelling allowed a measure of the relative biodegradability of each substrate. It was also used as a method of physical comparison for the cellulose BMP assays from each run. The modified Gompertz model (Equation 2) was used to determine the remaining kinetic biodegradability values associated with BMP assays [10].

$$M = M_0 \times \exp \left\{ - \exp \left[\frac{R_m \times e}{M_0} (\lambda - t) + 1 \right] \right\} \quad (2)$$

Where M = Accumulative methane production (NL/kg VS_{added})
 M_0 = Maximum methane production potential established in the BMP (NL/kg VS_{added})
 R_m = Maximum methane production rate (NL/kg VS_{added}-d)
 λ = Lag Phase (d)
 t = Digestion time since the startup of BMP tests (d)

Microbial kinetic coefficients obtained were used for comparing performances of the microorganisms in transforming each grass sample into biogas and assessing the demand of the bioreactor system for the production of biogas from 4190 grass.

Statistical analysis

All results were analyzed and differences among results from different groups of samples were tested using either Student's t test or ANOVA at 95% confidence level.

RESULTS AND DISCUSSIONS

Characteristics of 4190 grasses

Grasses at different ages had different amounts of some elements (Table 1). While amounts of total solids and volatile solids of grasses were not significantly different ($P=0.103$ and 0.122 respectively), lignin content of the 90 d

grass was significantly higher than those of other grasses, i.e. 45 and 70 d. In addition, 4190 grasses were mostly composed of cellulose ($\geq 29.6\%$) and hemicellulose ($\geq 19.8\%$). These components are essential for biogas production, especially cellulose as biomethane potential of cellulose is higher than that of hemicellulose [11]. Nonetheless, hemicellulose can be hydrolysed more quickly than cellulose, while lignin is very difficult to be digested [11]. Compared to the 45 d- Napier Pak Chong 1 grass (comprising 3.9% of lignin), 4190 grasses at all ages found in this study contained notably higher lignin contents and thus expected to render lower methane yields.

Methane production from organic substrates also depends on the content of nutrients (crude protein, crude fat, crude fiber, N-free extracts) which can be degraded to CH_4 and CO_2 . The content of these nutrients determine the degradability and thus the methane yield that can be produced through anaerobic digestion [12]. Results showed that the highest crude protein and fat content ($P = 0.00$) were detected in the 45 d grass while amounts of carbohydrates and crude fiber were not significantly different among the studied grasses.

Table 1 Characteristics of the inoculum and substrate used in the experiment

Parameter	Inoculum	Fresh grass		
		45 day	70 day	90 day
MLSS (mg/L)	14,264±1,729	-	-	-
MLVSS (mg/L)	11,183±1,516	-	-	-
Density (kg/m^3)	990±5.40	925±102 ^a	906±5.10 ^b	890±16.3 ^b
TS (mg/kg)	19,800±669	182,477±136 ^a	185,030±606 ^a	189,838±978 ^a
VS (mg/kg)	12,120±606	158,465±697 ^a	160,280±544 ^a	162,172±552 ^a
Moisture Content (%)	-	82.3±0.43 ^a	75.2±0.59 ^b	82.8±0.11 ^a
C/N ratio	-	50.0±0.98 ^b	66.0±0.98 ^a	69.9±3.29 ^a
Carbohydrate, NEF (%)	-	43.3±0.69 ^b	46.0±1.04 ^a	40.1±0.60 ^c
Crude Protein (%)	-	5.50±0.10 ^a	4.47±0.12 ^b	4.27±0.15 ^b
Ether Extract, EE (%)	-	2.30±0.04 ^a	1.89±0.07 ^b	1.82±0.05 ^b
Crude Fiber (%)	-	33.7±0.89 ^a	35.9±1.11 ^a	36.1±0.88 ^a
Lignin (%)	-	13.8±0.60 ^b	9.25±0.11 ^c	18.2±1.27 ^a
Cellulose (%)	-	29.6±1.75 ^b	47.6±2.06 ^a	32.8±0.67 ^b
Hemicellulose (%)	-	26.3±1.55 ^a	19.8±3.53 ^b	22.6±1.05 ^{a,b}
Iron (mg/kg)	-	929±19.7 ^a	544±13.5 ^b	285±47.4 ^c
Nickel (mg/kg)	-	1.17±0.13 ^b	0.22±0.02 ^c	2.31±0.58 ^a
Cobalt (mg/kg)	-	<0.075	<0.075	<0.075

Results shown as Mean \pm SD, Different superscripts in the same row indicate differences ($P < 0.05$)

Considering that grass yield per area for the 90 d 4190 grass was much higher than those of the younger age grasses while differences of amounts of other important components affecting biodegradation were less significant, the 90 d grass has relatively high potential for being used as the feedstock for biogas production.

Biochemical Methane Potential

Accumulative methane yields obtained from the modified BMP test is shown in Figure 1. The highest specific methane yields (199 ± 7.77 NL/kg VS_{added}) was found when the 45 d fresh grass was used as the substrate, while 90 d silage grass gave the lowest value at 138 ± 9.82 NL/kg VS_{added} . Grass ages were found to affect the degradation of organic matter, in which the older the grasses were, the less methane yields were attained. Lower methane yields could partly be attributed to amounts of lignin. Lignin is a very recalcitrant part of any biomass and it is the main component responsible in protecting the whole plant from biodegradation [13]. In order to biologically convert the biomass

organic components efficiently into biogas, destruction of lignin is required [14].

The specific methane yields obtained from the 4190 grass under thermophilic conditions were relatively high. It was even higher than values obtained from the Napier Pak Chong1 grass aged 35-55 d, which was reported to be in the range of 32.9-84.5 NL/kg VS_{added} under mesophilic temperature [6]. Considering that the 4190 grass contained significantly higher amounts of lignin (9.25-18.2%) compared to those of the Napier Pak Chong1 grass (9.83-14.3%), advantages of thermophilic biodegradation were very obvious [15]. There are many grass species that have been studied for biogas production. Perennial ryegrass silage and Meadow grass were investigated under the thermophilic state and the specific methane yields up to 364-392 NL/kg VS_{added} and 254.57 ± 12.72 NL/kg VS_{added} were reported [3, 15]. Different methane yields found were attributed to grass ages and components contained in each grass.

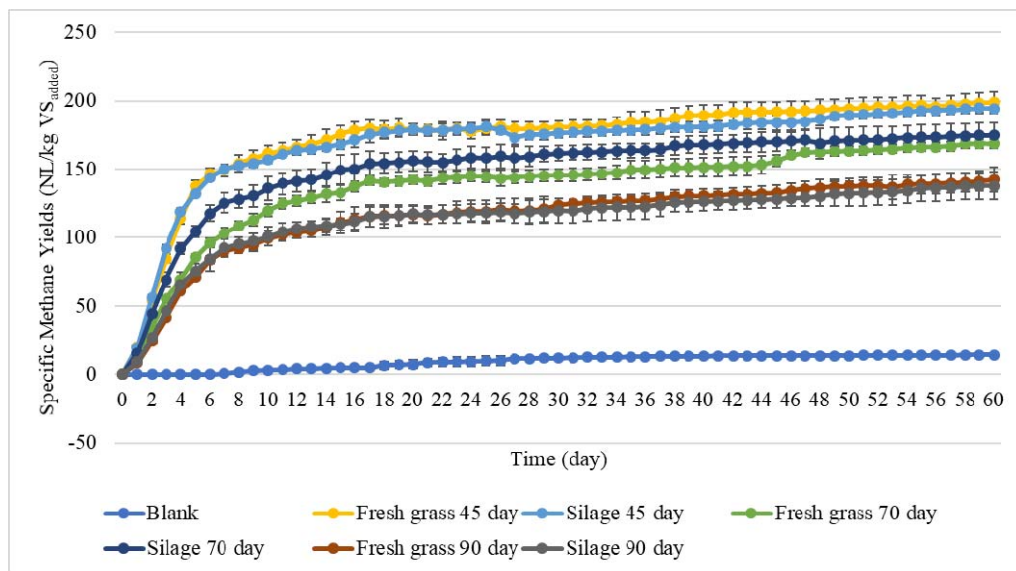


Figure 1 Accumulative methane yields of all studied grasses

In order to analyze the results of the BMP test to assess the potential of biogas production from the 4190 grass, a model to predict the results of the experiment was constructed using the modified Gompertz model.

When substituting the obtained values to create the equation for predicting specific methane yields from the experiment using the modified Gompertz model, it was found that the obtained equations could satisfactorily predict the experimental results (coefficients of the determination (R^2) were in the range of 0.93-0.95, as shown in Table 2 and Figure 2).

Results from the modified Gompertz model showed that the highest ultimate methane production (M ; 186 ± 6.08 NL/kg VS_{added}) was gained when fresh grass was used as the substrate while the grass silage had lower M value. Superiority of fresh grass over grass

silage could be attributed to the higher microbial activities and increase of hydrolysis reaction under thermophilic conditions that outweighed the advantages of transformation of grass components into the more easily digested grass silage.

In addition, the Lag phase times (λ) or the time that the microorganisms need to acclimatise to the new substrate for both fresh and silage grass was found to be zero. This could partly be explained by usage of inoculum from anaerobic reactor digesting Napier Pak Chong1 grass as a seed in the BMP test. Results gained were very promising because microorganisms could still digest the 4190 grass without acclimatisation even though the 4190 grass was found to contain higher lignin contents than Napier Pak Chong1 grass.

Table 2 The microbial kinetic coefficients obtained using the modified Gompertz model

Substrate	M (NL/kg VS_{added})	R_m (NL/kg VS_{added} -d)	λ (day)	R^2
Fresh grass 45 day	186 ± 6.08^a	25.8 ± 1.01^a	0.00 ± 0.00^a	0.95
Silage 45 day	181 ± 3.80^a	26.7 ± 0.53^a	0.00 ± 0.00^a	0.95
Fresh grass 70 day	153 ± 3.77^b	14.4 ± 0.66^b	0.00 ± 0.00^a	0.94
Silage 70 day	164 ± 7.48^b	19.1 ± 1.39^c	0.00 ± 0.00^a	0.95
Fresh grass 90 day	129 ± 5.88^c	$11.8 \pm 0.60^{c,d}$	0.00 ± 0.00^a	0.93
Silage 90 day	125 ± 7.67^c	13.2 ± 0.90^d	0.00 ± 0.00^a	0.94

Results shown as Mean \pm SD, Different superscripts in the same column indicate differences ($P < 0.05$)

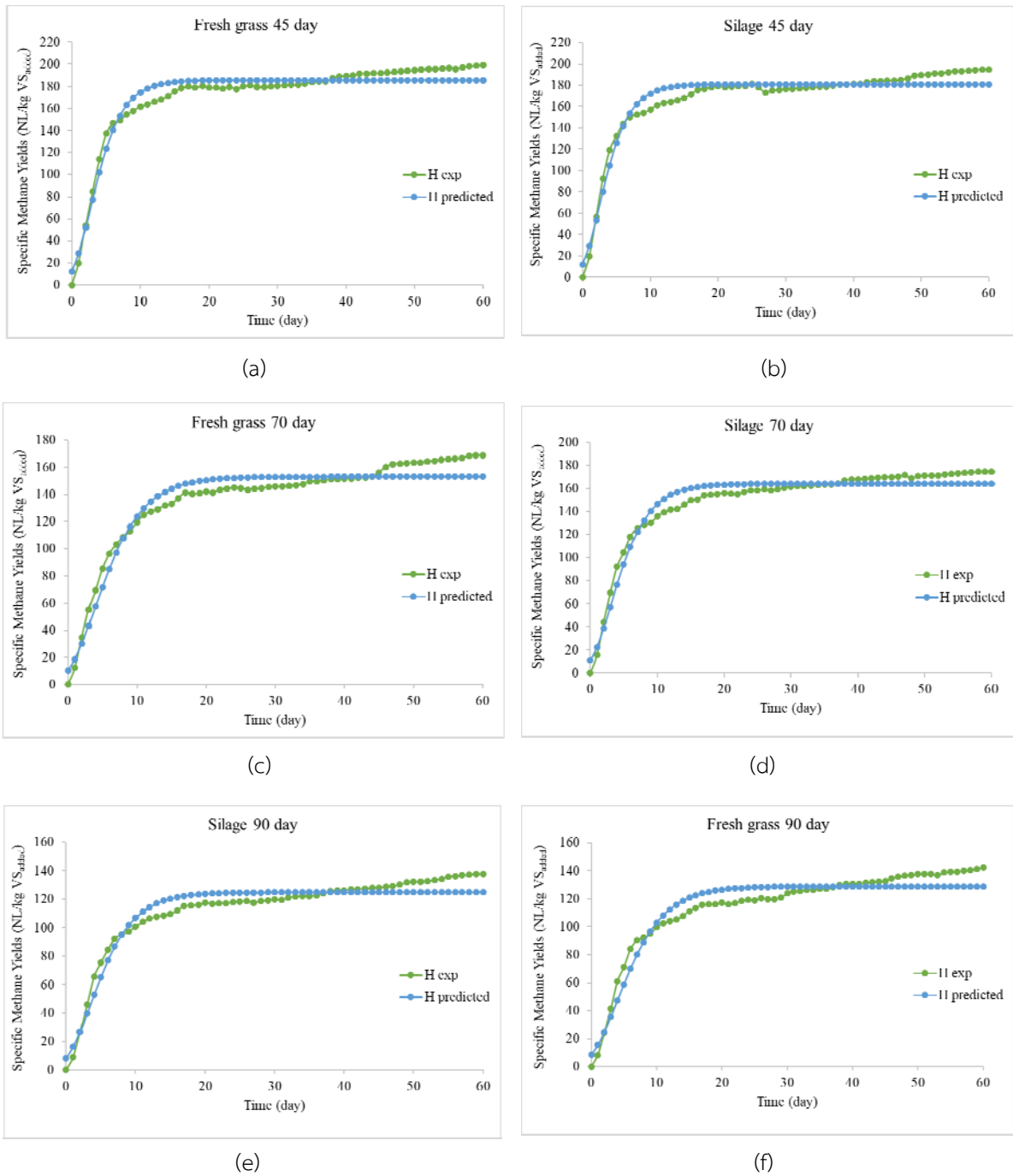


Figure 2 The GM model comparison of the predicted and experimental values
 (a) Fresh grass 45-d (b) Silage 45-d (c) Fresh grass 70-d (d) Silage 70-d
 (e) Fresh grass 90-d (f) Silage 90-d

CONCLUSION

The 4190 grass could be efficiently utilised as a substrate for biogas production under thermophilic condition. The highest methane yield of 199 ± 7.77 NL/kg VS_{added} was obtained when the fresh 4190 grass (harvesting time 45-d) was used as a feedstock. The bioreactor design and operation can be implemented from results gained for further study.

ACKNOWLEDGEMENT

Authors wish to thank the Electricity Generating Authority of Thailand for providing the 4190 grass scion and information required for this research. This work is a part of the research project "Determination of Potential and Suitable Condition for Biogas Production from Grass as the Energy Crop", which is financially supported by the Electricity Generating Authority of Thailand and Graduate School of Chiang Mai University.

REFERENCES

- [1] Bułkowska, K., et al. 2012. Optimization of anaerobic digestion of a mixture of *Zea mays* and *Miscanthus sacchariflorus* silages with various pig manure dosages. *Bioresource Technol.* 125: 208-216.
- [2] Seppälä, M., et al. 2009. Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. *Bioresource Technol.* 100(12): 2952-2958.
- [3] Feng, L., et al. 2017. Anaerobic co-digestion of cattle manure and meadow grass: Effect of serial configurations of continuous stirred tank reactors (CSTRs). *Biosyst. Eng.* 160: 1-11.
- [4] Himanshu, H., et al. 2018. Synergies from co-digesting grass or clover silages with cattle slurry in in vitro batch anaerobic digestion. *Renew. Energ.* 127: 474-480.
- [5] Dussadee, N., K. Reansuwan, and R. Ramaraj. 2014. Potential development of compressed bio-methane gas production from pig farms and elephant grass silage for transportation in Thailand. *Bioresource Technol.* 155: 438-441.
- [6] Chanpla, M., et al. 2017. Effect of harvesting age and performance evaluation on biogasification from Napier grass in separated stages process. *KSCE J. Civ. Eng.* 22(1): 40-45.
- [7] Sawasdee, V. and N. Pisutpaisal. 2014. Feasibility of Biogas Production from Napier Grass. *Energy Proced.* 61: 1229-1233.
- [8] KwaZulu-Natal Department of Agriculture and Rural Development, Giant Juncao Grass Cultivation in KwaZulu-Natal, in *AGRI UPDATE, Research and Technology Bulletins.* 2010.
- [9] VDI 4630, Fermentation of organic materials-characterisation of the substrate, sampling, collection of material data, fermentation tests. *VDI Guideline 4630.* 2006, Verein Deutscher Ingenieure, Düsseldorf.
- [10] Allen, E., et al., A detailed assessment of resource of biomethane from first, second and third generation substrates. *Renewable Energy*, 2016. 87: p. 656-665.
- [11] Li, W., et al. 2018. Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose,

- hemicellulose and lignin. *Appl. Energ.* 226: 1219-1228.
- [12] Amon, T., et al. 2007. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technol.* 98(17): 3204-3212.
- [13] Herrmann, C., C. Idler. and M. Heiermann. 2016. Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics. *Bioresource Technol.* 206: 23-35.
- [14] Mancini, G., et al. 2018. Increased biogas production from wheat straw by chemical pretreatments. *Renew. Energy.* 119: 608-614.
- [15] Voelklein, M.A., D. Rusmanis, and J.D. Murphy. 2016. Increased loading rates and specific methane yields facilitated by digesting grass silage at thermophilic rather than mesophilic temperatures. *Bioresource Technol.* 216: 486-493.