Pertanika J. Sci. & Technol. 27 (S1): 109 - 122 (2019)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Optimization of Hydrothermal Conditioning Conditions for *Pennisetum purpureum x Pennisetum americanum* (Napier PakChong1 grass) to Produce the Press Fluid for Biogas Production

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ABSTRACT

This study focused on the optimization of hydrothermal conditioning conditions for Napier PakChong1 grass to produce press fluid for biogas production. The integrated generation of solid fuel and biogas from biomass (IFBB) process was adopted to separate press fluid from the biomass. Napier PakChong1 grass was hydrothermally pretreated and then mechanically pressed. The press fluid was used for biochemical methane potential (BMP) test while the press cake could be utilized as the solid fuel. The full factorial design of experiment with center points and the Central Composite Design (CCD) were developed to obtain the best possible combination of harvesting time, grass to water ratio, temperature and soaking time for the maximum organic substance (as COD) in press fluid. It was found that the obtained model could satisfactorily predict the mass of COD in press fluid used as the model response. The optimum hydrothermal conditioning conditions were as follows; harvesting time 75 d, ratio of grass to water of 1:6 (by weight), ambient temperature (about 25°C) of the water and the soaking time of 355 min. The mass of COD obtained

ARTICLE INFO

Article history:
Received: 24 October 2018
Accepted:15 February 2019
Published: 21 June 2019

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ISSN: 0128-7680
e-ISSN: 2231-8526

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in these conditions was 226.42 g equating to 71.5% of the value predicted by the model (316.68 g). The microbial kinetic coefficients and biogas yield potential of press fluid at these optimum conditions were properly fitted with the modified Gompertz equation (adjusted R_2 = 0.995). The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 412.18 $mlCH_4/gVS_{added}$, 51.47 $mlCH_4/gVS_{added}$/d and 4.36 days, respectively.

Keywords: BMP, Grass liquor, hydrothermal conditioning, modified gompertz equation, *pennisetum purpureum* x *pennisetum americanum* (napier PakChong1 grass).

INTRODUCTION

Energy consumption in Thailand has been increased continuously since the year 2011 due to the expansion of domestic demands. Thailand's Alternative Energy Development Plan: AEDP 2015 has been launched with the target of using renewable and alternative energy to replace up to 30 percent of final energy consumption (in form of electricity, heat and bio-fuel) by 2036. This plan partly focuses on the utilization of energy crop, in which 680 MW of electricity is expected to be produced from biogas production using energy crop as the raw material (Energy Policy and Planning Office, 2017). Grass is one of the most important energy crops for Thailand because it is a perennial plant and can grow in every region of the country. Compared to other grass species, Napier PakChong1 grass has a higher production yield (up to 75 ton/ha-yr and carbohydrate content 36-38% as dry basis (Negawo et al., 2017; Rengsirikul et al., 2013). These characteristics make Napier PakChong1 grass to be suitable as a feedstock for biogas production and combustion. However, as grasses are lignocellulosic biomass, they are rather recalcitrant to anaerobic fermentation (Bruni et al., 2010). Low methane yields at long retention time have been observed from anaerobic biodegradation of grasses (Richter et al., 2011; Richter et al., 2009). In addition, long term of mono-digestion of grass may result in the decrease of biogas production due to the effect of trace element deficiency (Thamsiriroj et al., 2012). Moreover, system failure due to the floating of grasses could cause the blockage in the gas pipe (Thamsiriroj & Murphy, 2010). Most of biogas plants using grasses as the feedstock in Germany are co-digestion of grass silage with manure to stabilize the process and maintain biogas production. As the source for renewable energy production, grass can also be used as a substrate for combustion. However, there are many problems due to its high element concentrations which would cause ash slagging (K, Mg), corrosion (Cl, S) and emissions (Cl, S, N) (Jenkins et al., 1998; Obernberger et al., 2006).

The better method of utilizing Napier PakChong1 grass could be the integrated generation of solid fuel and biogas from biomass (IFBB) (Wachendorf et al., 2009),

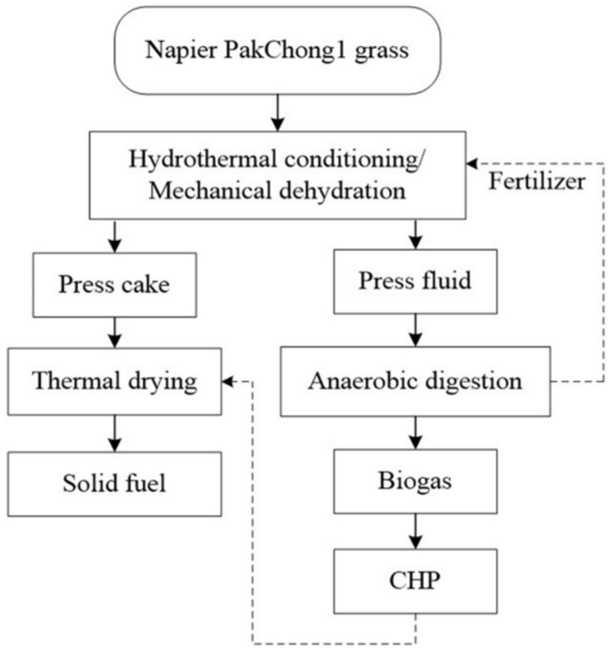

Figure 1. Process diagram of the IFBB (Wachendorf et al., 2008)

which has been developed for increasing efficiency of conversion and methane production yield. In IFBB method, grass is separated into two parts, i.e. press fluid and press cake (Figure 1). Hydrothermal conditioning, which is the process of grass soaking and heated under continuous stirring for cell wall maceration, and mechanical dehydration process are done in order to transfer the elements and organic compounds into the press fluid for efficient anaerobic digestion. Several works have been conducted using this hydrothermal conditioning and mechanical dehydration process with semi-natural grassland (Wachendorf et al., 2009); green cut material from landscape (Hensgen et al., 2011) and sward maturity (Richter et al., 2011). The process was reported to be able to efficiently transfer minerals and organic compounds required for biogas production to the press fluid. The press fluid of semi-natural grassland was found to contain high crude protein and had the methane yield (397-426 NL/kgVS after 13 day) two times higher than the whole crop grassland silage (218 NL/kgVS after 27 day) (Richter et al., 2009). Similarly, Reulein et al. (2007) observed the high value of methane yields 500 LCH4/kgVS from the press fluids of whole crop silages of maize and wheat. The press cake obtained after the mechanical dehydration process was a high solid fibrous fraction (cellulose, hemicellulose and lignin). This solid is a high quality fuel as it contains low element concentrations, e.g. potassium, magnesium and chloride (Bühle et al., 2012) which would produce less amounts of air pollution after combustion.

Efficiency of the dehydration process of a biomass depends on several factors, e.g. solid: liquid ratio, temperature, incubation time, mechanical pressing, detergent and harvesting time (Jia et al., 2013; King et al., 2012; Kuila et al., 2011; Wachendorf et al., 2009). This study aimed to utilise Napier PakChong1 grass to produce renewable energies using IFBB process. Therefore, the objective of this work was to (1) optimize the hydrothermal conditioning, i.e. harvesting time (day), ratio of solid to water (by weight), soaking time (min) and temperature (°C) and (2) determine the methane yield of the obtained press fluid.

MATERIALS AND METHODS

Napier PakChong1 grass

Napier Pak Chong1 grass was collected from Chiang Mai Fresh Milk farm, Lamphun, Thailand. After harvested and delivered to the laboratory, the grass was chopped by a hammer mill to the average size of 2 cm. Grass sample were stored at 4°C before each use.

Optimization of Hydrothermal Conditioning Conditions for Napier PakChong1 Grass

The 2-level full factorial design of experiment with center points and the Central Composite Design (CCD) were employed to obtain the optimum Napier PakChong1 grass pressing condition. For the full factorial experiments, the chopped Napier PakChong1 grass samples (harvesting time 30 and 60 d) were hydrothermally conditioned by mixing with water (solid: water = 1:3 and 1:5 by weight) at different temperatures (37 and 80°C) and different soaking times (10 and 240 minutes). Then the conditioned Napier PakChong1 samples were gravitationally separated from water.Subsequent mechanical dehydration of the Napier PakChong1 samples were conducted using screw press. Axial points used in the CCD for constructing the response surface to estimate the coefficients of quadratic terms are as follows; harvesting time (15 and 75 d), ratio of grass to water (1:2 and 1:6 by weight), soaking time (0 and 355 minutes) and soaking temperature (15.5 and 90°C). All experiments were developed and the results were analyzed using MINITAB version 16. Organic substance in form of mass of total COD (COD_t) was used as the response for optimization as it was the most pertinent parameter relating to biogas production potential. Mass of COD_t was calculated from the sum of mass of COD_t in press fluid and in drained water generated after the hydrothermal process.

Biochemical Methane Potential (BMP) Test

The press fluid obtained from the optimum condition for pressing conditioning was investigated for biogas production potential using the BMP test. The BMP test was conducted according to the German Standard Procedure VDI 4630 (Ingenieure, 2006) using

Table 1
Medium solution

Chemical	Concentration	Unit
KH_2PO_4	0.27	g/L
$Na_2HPO_4.12H_2O$	1.12	g/L
NH_4Cl	0.53	g/L
$CaCl_2.2H_2O$	0.075	g/L
$MgCl_2.6H_2O$	0.10	g/L
$FeCl_2.4H_2O$	0.02	g/L
$Na_2S.9H_2O$	0.10	g/L
$MnCl_2.4H_2O$	0.50	mg/L
H_3BO_3	0.05	mg/L
$ZnCl_2$	0.05	mg/L
$CuCl_2$	0.03	mg/L
$Na_2MoO_4.2H_2O$	0.01	mg/L
$CoCl_2.6H_2O$	1.00	mg/L
$NiCl_2.6H_2O$	0.10	mg/L
Ns_2SeO_3	0.05	mg/L

1000 ml glass bottle GL 45 (Schott Duran, Germany) with a working volume of 400 ml. Inoculum was collected from the final part of an anaerobic channel digester treating cow dung of Chiang Mai Fresh Milk farm in Lamphun province, Thailand. The inoculum was diluted to 20 gVS/L with medium solution (Table 1).

Press fluid and inoculum added in each bottle were 164 and 236 ml, respectively, equivalent to a ratio of press fluid to inoculum of 0.5 (by VS). Also, a bottle with only inoculum and distilled water was prepared and used as the control. All the experiments were done in triplicate including the control experiment. Microcrystalline cellulose was also used as a reference sample for checking the activity of inoculum.The gas production of this reference sample should be at least 80% of 740-750 mL_N/gVS_{added}. Nitrogen gas was used in flushing the headspace for 3 minutes to ensure the anaerobic condition. Each bottle was sealed with PTFE/silicone septa with PP screw cap and then kept in the temperature controlled room at 35°C. The gas volume was measured indirectly by pressure equipment (Kimo, model MP112) and then converted to that at STP condition (0°C and 1 atm). Complete anaerobic digestion was obtained when daily biogas production rate was less than 1% of total volume biogas production.

Analytical Method

Total solid, volatile solid and COD were analysed according to standard methods (Rice et al., 2012). The methane composition was measured using a gas chromatograph (Agilent 7890A) with a thermal conductivity detector (TCD). The temperature of the injector and detector were 120°C and 150°C, respectively. The carrier gas was He with the flow rate of 10 ml/min. Methane potential was calculated as $L_NCH_4/kgVS_{added}$. The modified Gompertz model (Eq.1) was used to predict the maximum methane yield (Ho & Shihwu, 2010).

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

Where, M is the cumulative methane yield (mL/gVS_{added}), P the maximum methane yield (mL/gVS_{added}), R_m the maximum methane production rate ($mL/gVS_{added}/d$), λ the lag phase (days), t the digestion time (days), e the exp(1)=2.718. All parameters (P, R_m, and λ) were estimated by the least square method using Solver function in Microsoft®Office Excel 2013. The sum of the squared errors (SSE) was set to minimize. The error value was the difference between the experimental value and predicted value.

RESULTS ANS DISCUSSION

Characteristic of Napier PakChong1 grass

Characteristics of Napier PakChong1 grass are shown in Table 2. The TS of Napier PakChong1 grass was in the range of 14.30-16.44%. This result was similar with those of Lounglawan et al. (2014) who found that the dry matter of King Napier grass was 13.37-18.39%. However, Ansah et al. (2010) reported TS values of four varieties of Napier grass in Ghana in the higher range (48-51%) at harvesting time 60-120 day. Lower TS values of Napier PakChong1 grass found in this current study could be attributed to the variety of Napier grass species, planting location and climate and, in particular, the shorter harvesting time. As founded in this current study, the 60 d-grass had higher lignin compared to those at shorter harvesting times, which could affect volumes and characteristics of the obtained grass juice.

Table 2
Characteristics of Napier PakChong1 Grass

Composition	Harvesting time (day)		
	30 (n=1)	45 (n=6)	60 (n=6)
Total solid (%)	14.42	16.44	14.30
VS (%)	12.56	14.27	12.60
Ether extract (% as dry matter)	3.63	3.72	3.40
Crude fiber (% as dry matter)	33.18	31.95	33.35
Crude protein (% as dry matter)	9.07	13.13	12.65
Ash (% as dry matter)	12.04	13.12	12.57

Table 2 *(Continued)*

Composition	Harvesting time (day)		
	30 (n=1)	45 (n=6)	60 (n=6)
Nitrogen-free extract, NFE (% as dry matter)	42.08	38.08	38.03
Cellulose (% as dry matter)	40.44	36.35	37.25
Hemicellulose (% as dry matter)	20.09	22.41	23.99
Lignin (% as dry matter)	4.04	4.74	4.92
Potassium (% as dry matter)	1.32	0.38	0.47

Optimization of Press Fluid

Normally, grasses have high water content up to 80-85%. The preservation methods of grasses, such as silage or drying, for use as a raw material is essential (Xiu & Shahbazi, 2015). For the green biorefinery of biomass, mechanical dehydration with screw press is the primary method used to press grasses to press fluid. To increase maceration of the cell walls a pretreating method of biomass by adding water needs to be applied before press juice separation is conducted by screw press (Arlabosse et al., 2011). Effects of harvesting time (A), grass to water ratio (B), soaking time (C) and temperature (D) on the organic substance (as mass of COD_t) obtained in the press juice were investigated. The experimental data and the regression model of the mass of COD_t (at confidence level of 90%) are shown in table 3 and table 4, respectively. The mathematical equation for the relationship between mass of COD_t and values of studied factors (uncoded values) gained from regression analysis can be shown in equation 2. The experimental data showed quadratic correlation between studied factors and the responses. Moreover, interaction effects between experimental variables had also been found.

$$Y_{COD} = 172.364 - 3.037\,(Harvested\ time) + 17.536\,(Grass{:}\,water\ ratio) + 0.176\,(Time) - 2.213\,(Temp) + 0.04\,(Harvested\ time)^2 + 0.0004(Time)^2 + 0.021(Temp)^2 - 0.003\,(Time\ x\ Temp) \quad [2]$$

As the press fluid from Napier PakChong1 grass was intended to be used for biogas production, COD_t was chosen as the response for optimization as it was the most pertinent characteristics for the bioreactor feedstock. The result showed that harvesting time, the ratio of grass to water and soaking time had significant effects on the total mass of COD_t ($P<0.1$). Optimum conditions obtained from the optimization were harvesting time 75 d, grass: water ratio 1: 6 (kg: L), soaking time 355 min and temperature 15.5°C. Under these conditions, the predicted maximum mass of COD_t was 85.06 g/kg wet weight Napier PakChong1

Table 3

Experimental data of design of experiment for the mass of COD_t of the press fluid, in term of coded factor

Run order	Harvesting time (d)	Grass to water ratio (w/w)	Soaking time (min)	Temperature (°C)	Mass of COD_t (g)	
					Experimental	Predicted
1	-1 (30)	-1 (1:3)	-1 (10)	-1 (37)	122.88	110.24
2	+1 (60)	-1 (1:3)	-1 (10)	-1 (37)	142.33	128.25
3	-1 (30)	+1 (1:5)	-1 (10)	-1 (37)	139.81	145.32
4	+1 (60)	+1 (1:5)	-1 (10)	-1 (37)	160.54	163.32
5	-1 (30)	-1 (1:3)	+1 (240)	-1 (37)	144.42	148.05
6	+1 (60)	-1 (1:3)	+1 (240)	-1 (37)	185.55	166.06
7	-1 (30)	+1 (1:5)	+1 (240)	-1 (37)	173.03	183.13
8	+1 (60)	+1 (1:5)	+1 (240)	-1 (37)	200.41	201.14
9	-1 (30)	-1 (1:3)	-1 (10)	+1 (80)	121.21	121.71
10	+1 (60)	-1 (1:3)	-1 (10)	+1 (80)	141.28	139.72
11	-1 (30)	+1 (1:5)	-1 (10)	+1 (80)	153.06	156.79
12	+1 (60)	+1 (1:5)	-1 (10)	+1 (80)	178.11	174.80
13	-1 (30)	-1 (1:3)	+1 (240)	+1 (80)	129.61	127.90
14	+1 (60)	-1 (1:3)	+1 (240)	+1 (80)	139.45	145.91
15	-1 (30)	+1 (1:5)	+1 (240)	+1 (80)	164.29	162.97
16	+1 (60)	+1 (1:5)	+1 (240)	+1 (80)	171.66	180.98
17	0 (45)	0 (1:4)	0 (125)	0 (58.5)	130.71	134.59
18	0 (45)	0 (1:4)	0 (125)	0 (58.5)	135.63	134.59
19	0 (45)	0 (1:4)	0 (125)	0 (58.5)	126.67	134.59
20	0 (45)	0 (1:4)	0 (125)	0 (58.5)	133.99	134.59
21	- α (15)	0 (1:4)	0 (125)	0 (58.5)	167.29	161.97
22	α (75)	0 (1:4)	0 (125)	0 (58.5)	189.84	197.99
23	0 (45)	- α (1:2)	0 (125)	0 (58.5)	92.26	109.04
24	0 (45)	α (1:6)	0 (125)	0 (58.5)	195.61	179.18
25	0 (45)	0 (1:4)	- α (0)	0 (58.5)	136.60	132.15
26	0 (45)	0 (1:4)	α (355)	0 (58.5)	181.92	166.11
27	0 (45)	0 (1:4)	0 (125)	- α (15.5)	179.14	188.30
28	0 (45)	0 (1:4)	0 (125)	α (90.0)	174.09	162.31
29	0 (45)	0 (1:4)	0 (125)	0 (58.5)	142.60	144.11
30	0 (45)	0 (1:4)	0 (125)	0 (58.5)	125.93	144.11

Table 4

Results of regression analysis of the mass of COD_t from the press fluid

Model term	Regression coefficient	Standard error coefficient	t-statistic	P-value
Constant	136.07	3.585	37.954	0.000
Block	-5.523	2.089	-2.644	0.016
A	9.004	2.143	4.201	0.000
B	17.536	2.143	8.182	0.000
C	9.530	2.415	3.946	0.001
D	-2.242	2.286	-0.981	0.338
A^2	9.093	1.964	4.631	0.000
C^2	4.977	2.622	1.898	0.072
D^2	9.911	2.409	4.114	0.001
CD	-7.907	2.625	-3.012	0.007
R^2 = 88.98%	R^2 (adj) = 70.65%			

grass. Hensgen et al. (2011) and Wachendorf et al. (2009) found that increase of water temperature in the range of 40-60°C did not increase of mass flow of minerals into the press fluid. Likewise, King et al. (2012) studied the effect of water temperature of hydrothermal conditioning process at 20, 40 and 60°C and reported that concentrations of elements in the press fluid from grass silage obtained at these temperatures were not significantly different. However, Richter et al. (2011) found that higher temperature of hydrothermal conditioning increased mass flows of elements into press fluids and decreased concentrations of elements in press cake when the soaking time and silage grass: water ratio were maintained at 10 min and 1:4 (w/w), respectively. Reasons for different effects of temperature on the quality of press fluid found in these studies are not clear. However, grass species (and structure), characteristics of soaking water and level of grass pretreatment might play some parts on the difference found. In this current study, it was found that the optimum temperature was lower than the normal ambient temperature in Thailand, which was not suitable for actual use. Therefore, the temperature was adjusted to the ambient temperature which was about 25°C. The mass of COD obtained in these adjusted conditions was 56.60 g/kg wet weight Napier PakChong1 grass equating to 71.5% of the value predicted by the model (79.17 g/kg wet weight Napier PakChong1 grass). Therefore, this is suitable conditions for producing press fluid from Napier PakChong1 grass to press fluid. Kuila et al. (2011) reported that increasing soaking time also increased the reducing sugar production from cashew apple bagasse. The maximum yield of 56.89 g reducing sugar/100 g dry substrate was obtained at liquid: solid of 3.26 (mL/g), pH 6.42, incubation time 6.30 h and temperature 52.27°C. Similarly, in this current study, increasing of solid: liquid ratio also resulted in the mass of COD_t and reducing sugar being increased (data not shown).

Biochemical Methane Potential (BMP) Test

The press fluid from the optimum hydrothermally conditioned grass (harvesting time 75 d, grass: water ratio 1: 6 (kg: L), soaking time 355 min and temperature about 25°C) was investigated for biogas production potential using the BMP test. The average methane yield of press fluid was 396.32±5.39 LCH_4/kgVS. The average methane content was 68.6%. Relatively, high methane yield could be attributed to the fact that press fluid contained mainly the biodegradable and soluble organic substances. The obtained methane yield is in the same range as those found in the studies of Hensgen et al. (2014), Hensgen et al. (2011), Nayono et al. (2010) and Richter et al. (2009) though different grass species and conditioning conditions before fluid pressing were used. Hensgen et al. (2014) found that methane yields of press fluid from IFBB for twelve European semi-natural grassland varied between 312-405 LCH_4/kgVS. In this work, the ensile samples were sprinkled with 25°C warm tap water and the ratio of biomass to mash water was 1:8. The previous study of Hensgen et al. (2011) reported that different water temperature in hydrothermal conditioning (40 and 60°C) did not affect the methane yields of the press fluid, in which 396-415 LCH_4/kgVS were obtained. Richter et al. (2009) also found that methane yields of press fluid from different types of semi-natural grassland conditioned under hydrothermal conditions were ranged 304-522 LCH_4/kgVS. This means that the optimum conditioning conditions achieved in this current study is as effective as those reported in previous works. Compared with the whole crop, methane yields of press fluid gained in this current study was significantly higher than that reported from the whole crop silage (218 LCH_4/kgVS) (Richter et al., 2009). Even though, Thamsiriroj and Murphy (2010) reported relatively high methane yield (455 LCH_4/kgVS) from the Irish silage, the organic loading rate used was only 1 kgVS/m^3.d and hydraulic retention time was more than 70 days. As the required digestion time of press fluid was only 15 d (time duration required to reach the maximum biogas production during the BMP test and the pipe clogging problem, normally found when the fibrous whole grass was used as the substrate) (Hensgen et al., 2014), is not the issue for press fluid, renewable energy production according to IFBB process is clearly more advantageous.

The cumulative methane data was used to fit with the modified Gompertz model (equation 1) to estimate the microbial kinetic parameters, with an assumption that biogas production is a function of the methanogens growth in batch digester. The best fit to modified Gompertz equation is compared with the experiment data as illustrated in Figure 2. The regression coefficient (R^2) was 0.995 demonstrating the suitability of the model for accurate estimation of the anaerobic digestion of press fluid. The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 412.18 $mLCH_4$/gVS_{added}, 51.47 $mLCH_4$/gVS_{added}/d and 4.36 days, respectively. Kacprzak et al. (2012) studied the kinetics of anaerobic digestion of canary grass by using modified Gompertz model. They found that the yield of biogas production and the lag phase were 648.44 L/kg VS and 14.67 d. In addition, Xie et al. (2011) reported specific methane yield and lag phase of co-digestion of pig manure and grass silage ratio at 1:0, 3:1, 1:1 and 1:3 which were equal to 279.8, 304.2, 302.8 and 267.3 $mLCH_4/gVS$ and 29.5, 28.1, 24.6 and 21.3 d, respectively. Furthermore, Prapinagson et al. (2017) found that the ratio of grass with cow dung and silage with cow dung at 3:1 by VS gave the maximum methane yield of 179.59 and 208.11 $mLCH_4/gVS_{added}$, respectively. The lag phase of these conditions were 11.9 and 5.9 day, respectively. Compared to microbial kinetic values obtained when grasses were used as either the sole substrate or codigested with animal manures, it is obvious that the grass press juice used in this current study rendered much higher maximum methane yield and shorter lag phase time. This means that, to produce the same amount of biogas, a reactor needed for biogas production from the grass press juice could be less complicated (as the mixing system is not necessary); smaller in size (as it is very likely to accepted higher organic loading rate); easier to start-up (as it needs shorter time for microbial acclimatization) and cheaper to operate (as elements required for microbial degradation are transferred into the press juice in soluble form).

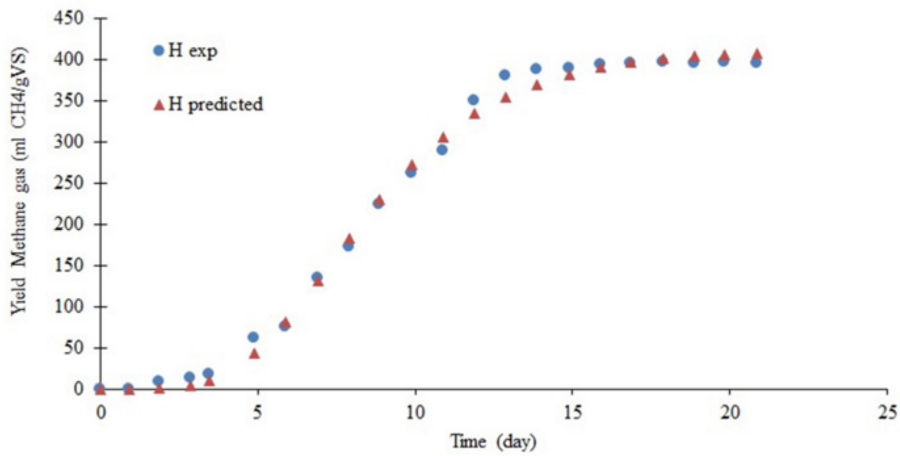

Figure 2. Comparison between the experimental data and modified Gompertz equation data

CONCLUSION

The study revealed that the optimum hydrothermal conditioning conditions for Napier PakChong1 grass were as follows; harvesting time 75 d, ratio of grass to water of 1:6 (by weight), ambient temperature (about 25°C) of the water and the soaking time of 355 min. The mass of COD in the press juice obtained in these conditions was 226.42 g equating to 71.5% of the value predicted by the model (316.68 g). Results from the BMP test showed that methane yield of press fluid was 396.32 L_NCH_4/kgVS with methane content of 68.6%. The microbial kinetic coefficients and biogas yield potential of press fluid were properly fitted with the modified Gompertz equation (adjusted $R^2 = 0.995$). The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 412.18 $mLCH_4/gVS_{added}$, 51.47 $mLCH_4/gVS_{added}$/d and 4.36 days, respectively. Producing biogas from the press fluid was clearly superior to that from the whole grass in a commercial scale.

ACKNOWLEDGEMENT

This study was financially supported by the Chiang Mai University and the Energy Research and Development Institute-Nakornping Chiang Mai University, Chiang Mai, Thailand. Authors wish to thank the Chiang Mai Fresh Milk farm for samples of grasses and inoculum provided.

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