# Comparing Low-Temperature Hydrothermal Pretreatments through Convective Heating versus Microwave Heating for Napier Grass Digestion

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*Keywords:* energy balance, Napier grass, anaerobic digestion, enzymatic hydrolyzability, lignocellulose, microwave pretreatment, hydrothermal pretreatment

*Abstract:*

This study investigates the effects of convective hydrothermal pretreatment (CHTP) compared to microwave pretreatment (MWP) on the anaerobic digestion of hybrid Napier grass for biomethane production. For rapid estimation of methane yield (YCH4), enzymatic hydrolyzability (EH), whose test lasts only 2 days was used as a surrogate parameter instead of the biochemical methane potential (BMP) assay that normally takes 45?60 days. The relationship between EH and BMP was successfully modeled with satisfactory accuracy (R2 = 0.9810). From CHTP results, quadratic regression characterised by $p < 0.0001$ and R2 = 0.8364 shows that YCH4 increase was clearly sensitive to detention time at all CHTP temperatures. The maximal YCH4 achieved of 301.5 ± 3.0 mL CH4/gVSadd was 53.2% higher than the control. Then, MWP was employed at various power levels and microwave exposure times. Changes in lignocellulosic structure by Fourier-transform infrared spectroscopy (FTIR) and energy balance demonstrate that MWP caused more damage to plant cells, which proved more effective than CHTP. In the best conditions, approximately 50% of energy was needed for MWP to achieve the equivalent improvement in YCH4. However, CHTP is a more suitable option since waste heat, i.e., from a biogas CHP (combined heat and power) unit, could be used, as opposed to the electricity required for MWP.

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# Comparing Low-Temperature Hydrothermal Pretreatments through Convective Heating versus Microwave Heating for Napier Grass Digestion

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**Abstract:** This study investigates the effects of convective hydrothermal pretreatment (CHTP) compared to microwave pretreatment (MWP) on the anaerobic digestion of hybrid Napier grass for biomethane production. For rapid estimation of methane yield ($\mathrm{Y_{CH4}}$), enzymatic hydrolyzability (EH), whose test lasts only 2 days was used as a surrogate parameter instead of the biochemical methane potential (BMP) assay that normally takes 45–60 days. The relationship between EH and BMP was successfully modeled with satisfactory accuracy ($R^2 = 0.9810$). From CHTP results, quadratic regression characterised by $p < 0.0001$ and $R^2 = 0.8364$ shows that $Y_{CH4}$ increase was clearly sensitive to detention time at all CHTP temperatures. The maximal $Y_{CH4}$ achieved of 301.5 ± 3.0 mL $\mathrm{CH_4/gVS_{add}}$ was 53.2% higher than the control. Then, MWP was employed at various power levels and microwave exposure times. Changes in lignocellulosic structure by Fourier-transform infrared spectroscopy (FTIR) and energy balance demonstrate that MWP caused more damage to plant cells, which proved more effective than CHTP. In the best conditions, approximately 50% of energy was needed for MWP to achieve the equivalent improvement in $\mathrm{Y_{CH4}}$. However, CHTP is a more suitable option since waste heat, i.e., from a biogas CHP (combined heat and power) unit, could be used, as opposed to the electricity required for MWP.

**Keywords:** hydrothermal pretreatment; microwave pretreatment; lignocellulose; Napier grass; anaerobic digestion; enzymatic hydrolyzability; energy balance

## 1. Introduction

Energy consumption has increased over the last century as the world population has continuously grown, and many countries are adopting digital transformation driven by an even higher energy supply. Crude oil, natural gas, and coal have been the primary resources used to respond to the increased demand [1]. Renewable energy is, however, a promising supply for future generations as we strive toward a low-carbon society. Biogas, a methane-rich biofuel produced from biomaterials by means of anaerobic decomposition, has received great attention [2,3]. Without an additional step for end-product separation, as in ethanol fermentation, anaerobic digestion is an efficient yet simple way of both recovering energy and recycling various biodegradable materials back into nature. This is accomplished via the cooperation of various prokaryotic microorganisms in hydrolysis, acidogenesis, acetogenesis, and methanogenesis, yielding mainly gaseous $CH_4$ and $CO_2$ [4]. While industrial wastewaters are the prime material for anaerobic digestion, fast-growing crop biomass is expected to play a significant role as a future source for anaerobic digestion (AD) feedstock [5]. Hybrid Napier grass (*Pennisetum purpureum Schumach* × *Pennisetum americanum* cv. Pakchong 1) is a fast-growing perennial crop that can regrow quickly after harvest for four to five years without replanting [6]. With its high productivity of 87 t/ha/year [7], this grass has become an appealing feedstock for anaerobic digestion. Industries possessing large arable land can cultivate it as a means to treat or dispose of their final effluent, in combination with the production of feedstocks for animal or energy conversion, which has now become an integral approach in the circular economy. Many countries in the European Union have promoted this bioeconomy scheme for their state members for quite some time. Recently, emerging economies such as Thailand have started embracing this powerful strategic mindset in response to the Sustainable Development Goals (SDGs) of the United Nations by launching their own bio–circular–green (BCG) economic policy [8].

Currently, wastewaters are extensively converted to biogas with ease, while the conversion of solid biomaterials is far more complicated. Major challenges facing the production of biogas from lignocellulosic feedstocks are the inherent recalcitrant structure and the complex chemical composition, pertaining to resistance to hydrolysis and further conversion by anaerobic microorganisms [9,10]. The biochemical conversion of lignocellulosic materials into methane involves the hydrolysis of cellulose into fermentable sugars and the subsequent fermentation of these sugars into methane. This hydrolysis reaction is carried out by carbohydrolases that break down structural carbohydrates into monomeric sugars such as glucose and xylose. Since lignocellulosic materials contain cellulose, hemicellulose, and lignin that form such a complex crystalline structure, the efficiency of hydrolysis is reduced due to limited accessibility of the enzymes to the inner core substrate [11].

Pretreatment can be applied to overcome this limiting step and increase digestibility of the biomass by inducing changes in the physical properties that aid anaerobic digestion. The structure of lignocellulosic material is compromised causing an increase in surface area [12], which allows faster enzyme hydrolysis of cellulose and hemicellulose [13]. Hydrothermal pretreatment via convective heating was found to be a simple and relatively effective method to increase the methane yield of biomasses such as rice straw (22.8%), corn stover (22.7%), and seaweed (14.3%) [14–16]. The term "hydrothermal pretreatment" loosely means using water as a medium to carry heat to the biomass, without a specific temperature and pressure range. Conditions of the water medium can be divided at its critical point at 374 °C and 22.1 MPa, which then separate it into subcritical and supercritical hydrothermal treatments [17]. However, even below 100 °C, disintegration of biomass starts to occur. While intense hydrolysis starts above 150 °C, a variety of rigorous thermochemical transformations take place at higher temperature and pressure, which are currently beyond the economical point for biofuel production. This work focuses on the low temperature range to conserve the net energy output from anaerobic digestion. Studies indicate that there is a trend of improvement in biogas production with an increase in temperature up to 100 °C by 30% and 12% for dewatered pig manure and sunflower cake, respectively, while it is rather insignificant beyond that level to 150 °C [18,19]. The use of hydrothermal biomass (Napier grass) in a high temperature range (125–200 °C) showed a 35% increase in biodegradability at 175 °C compared to non-pretreated biomass, but resulted in furfural derivative formation at 200 °C, which is inhibitory for anaerobic digestion [20]. Without a special pressurised vessel required for temperatures below 100 °C, low-range hydrothermal pretreatment could be a more feasible approach if a sufficient exposure time is given to the biomass [21].

Alternatively, microwave heating was reported to enhance enzymatic saccharification through fiber swelling and fragmentation as a result of the internal uniform and rapid heating of biomass particles [22]. Microwave radiation has recently emerged as a pretreatment method for improving the degradability of biomasses. It employs electromagnetic irradiation with a frequency between 300 MHz and 300 GHz and a wavelength ranging from 0.001 to 1 m [23,24]. This energised wave induces a transfer of heat through the vibration of dipolar molecules and the migration of ions [23,25]. Microwave pretreatment (MWP) was proven to be highly effective in breaking organic molecules and disrupting complex structures, which increases the accessibility and bioavailability of substrates [26,27].

Prior studies on the effects of microwave pretreatment of agricultural residuals at various temperatures of 90–200 °C were reported [28,29]. There were different degrees of improvement in biodegradation depending on the total energy applied. Albeit effective at such high power, no comparison with other hydrothermal methods was carried out.

In this study, convective hydrothermal pretreatment and microwave pretreatment were implemented to improve the digestibility of a lignocellulosic biomass, Napier grass. In the first part of the study, the effects of temperature and pretreatment time in a convective hydrothermal approach with an industrial practical range of <100 °C were evaluated. In this process, the relationship between enzymatic hydrolysis and biochemical methane potential (BMP) was established in order to enable the prediction of methane production potential of the biomass. This greatly reduced the time required to obtain the BMP of the lignocellulosic biomass. In the second part of the study, microwave pretreatment to the biomass was independently conducted, and a comparison with the convective hydrothermal pretreatment was performed on the basis of specific energy input and methane yield. Analysis of the effectiveness of the two methods was discussed in terms of their technical and industrial aspects.

## 2. Materials and Methods

### 2.1. Substrate

Ensilaged Napier grass was kindly provided by Satun Animal Nutrition Development Station, Satun Province, Thailand. The grass was a hybrid cultivar *Pennisetum purpureum Schumach* × *Pennisetum americanum* and aged sixty-day when harvested. The ensilaged grass was used because silaging is the standard preservation method for biomass feedstock to animal farm and anaerobic digester. The silage was milled with grinder to size 100 mesh (<0.2 mm), oven dried at 60 °C until constant weight (approximately 48 h) and stored in sealed plastic bags at room temperature until use for the entire experiment. Composition of Napier grass silage is presented in Table 1. The analytical procedures employed are outlined in Section 2.7.

**Table 1.** Composition of Napier grass silage used in this study.

| Component | Unit | Napier Grass Silage |
|---|---|---|
| **Ultimate analysis** | | |
| Carbon (C) | % dry wt. | 45.31 ± 0.11 |
| Hydrogen (H) | % dry wt. | 5.82 ± 0.11 |
| Oxygen (O) | % dry wt. | 40.27 ± 0.73 |
| Nitrogen (N) | % dry wt. | 0.58 ± 0.02 |
| Sulphur (S) | % dry wt. | n.d. |
| **Proximate analysis** | | |
| Moisture | % fresh wt. | 76.48 ± 2.47 |
| Total Solids | % fresh wt. | 23.52 ± 2.47 |
| Volatile Solids | % fresh wt. | 21.31 ± 2.27 |
| **Fiber composition** | | |
| Cellulose | % dry wt. | 43.78 ± 0.37 |
| Hemicellulose | % dry wt. | 35.29 ± 1.11 |
| Lignin | % dry wt. | 4.36 ± 0.82 |
| Ash | % dry wt. | 1.03 ± 0.03 |

Note: n.d. = not detected, *n* = 3.

### 2.2. Convective Hydrothermal Pretreatment (CHTP) of the Substrate

The prepared Napier grass was brought to mix with distilled water at a ratio of 1:10 (*w*/*w*) before being hydrothermally treated as a slurry in the reactor at temperatures (Temp) of 60, 70, 80, 90 and 100 °C with detention time (DT) of 15, 30, 45 and 60 min. It is noted that this range of DT (0–60 min) was studied as an attempt for design of heating apparatus for biomass pretreatment. The hydrothermal reactor was made of glass with an inner diameter of 6.0 cm and a total volume of 2.0 L. An adjustable 25-watt gear motor was mounted to the top of the reactor with a shaft impeller submerged in the slurry (Figure S1 in Supplementary Materials). It was operated at 20 revolutions per minute to provide constant agitation and ensure homogeneous distribution of temperature during the entire period of designed DT. A thermocouple rod was immersed into the reactor to control the heating plate to the preset temperatures. The reactor was removed from the heating plate immediately after the designed DT was reached. In all cases, the time to reach all target temperatures was under 30 min. After treatment, distilled water was added to the original level to compensate for evaporation loss prior to analyses or further use in all experiments.

The experiment was carried out in full factorial design. Changes in properties of the liquid portion as well as the solid portion were monitored. The control was set at room temperature of 30 °C where the sample was submerged into and immediately removed from the liquid for analyses.

### 2.3. Enzymatic Hydrolysis Assay

The Napier grass samples prepared in Section 2.2 were used as substrate in enzymatic hydrolysis assay. The slurry samples of 3 g (dry wt. basis) were placed in a glass reactor where the commercial enzyme Celli CTec2 (Novozyme) was applied. The enzyme has a standard filter paper activity of 255 FPU/mL (filter paper unit per mL), measured using the method described by Ghose [30], Selig et al. [31] and Yu et al. [32]. The cellulase enzyme was added at concentration 30.0 FPU/$g_{substrate}$ and filled with 0.05 M citrate buffer to a total volume 50 mL in each reactor. The reaction mixture incubated at 50 ± 1 °C for 48 h with orbital shaking at 150 revolutions per minute continuously [33]. At the end of hydrolysis, the mixed liquor was filtered to separate the solid residue and liquid hydrolysate. The experiment was performed in triplicate and the results are presented as the average values which are statistically compared at $\alpha = 0.05$. From the results, enzymatic hydrolysibility (EH) of pretreated samples was calculated according to Equation (1) [34].

$$\text{Enzymatic hydrolysibility (EH, \%)} = \frac{C_{glucose} \cdot V_{hydrolysis} \cdot 0.9}{M_{cellulose}} \times 100\% \quad (1)$$

where $C_{glucose}$ is glucose concentration (g/L), $V_{hydrolysis}$ is remaining hydrolysis volume on the calculation hour (L), $M_{cellulose}$ is initial cellulose mass (g) and 0.9 is the correction factor used for the process of conversion of monosaccharide to polysaccharide due to water uptake during hydrolysis.

### 2.4. Biochemical Methane Potential (BMP) Assay

Inoculum used in the BMP assay was the anaerobic sludge collected from a full-scale digester in a local pig farm in Songkhla Province, Thailand. The sludge was screened to remove large solids and impurities using a standard ASTM 2.36 mm sieve (Thermo Fisher Scientific Inc., Waltham, MA, USA). Only the sludge passed through the screen was collected, then left to degas in a container until the biogas production ceased at under 1 mL/gVS.d. This was done to minimise the interference from organic impurity in the inoculum sludge during the assay. The sludge sample was then taken to analyze for total solids (TS) and volatile solids (VS) prior to use as inoculum in all BMP assays.

The BMP assay was performed according to the procedure described by Angelidaki et al. [35] using 120-mL glass bottles with an effective volume of 60 mL. Each BMP reactor was provided with 1% (*v*/*v*) of nutrients and trace element solution, and a buffer solution of 50 g/L $NaHCO_3$ at 10% (*v*/*v*). Seed inoculum of 15 gVS/L was added to each bottle, and 15 gVS/L of the substrates were used. The substrate used in BMP was the whole slurry from the experimental treatments. The pH was adjusted to 7.0 using small drops of 0.1 M NaOH or 0.1 M HCl. Effective volume of 60 mL was attained by the addition of distilled water. Each reactor was flushed with nitrogen gas for 60 s and sealed immediately to attain anaerobic condition. All reactors were placed in an incubator shaker with continuous shaking at 150 revolutions per minute and at a temperature of 35 ± 1 °C. The assays were run against a control of blank containing only 15 gVS/L of inoculum, nutrient, and trace element solution with no substrate and filled to the effective volume with distilled water. All BMP assays were run in triplicate. The biogas produced was collected and measured for volume and methane content during the entire digestion period. Methane yield was derived from dividing the cumulative volume of methane by the initial weight of the sample (VS) added to the reactor. In order to evaluate the kinetics of methane evolution, the experimental data were fitted using the modified Gompertz kinetics model (Equation (2)) [36] as follows:

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

where $P(t)$ is cumulative methane production (mL) at day $t$, $P$ is the methane production potential (mLCH$_4$/gVS), $R_m$ is the maximum methane production rate (mLCH$_4$/d), $\lambda$ is the lag phase time (days), $e$ is exp (1) = 2.71828 and $t$ is the time lapse from start of the assay (days).

### 2.5. Determination of Biomethanation Potential from Enzymatic Hydrolysibility, and Quadratic Prediction Model

In order to save time for anaerobic digestion of all solid samples, which normally takes 45–60 days for each sample, the relationship between EH and BMP in terms of methane yield ($Y_{CH4}$, mL CH$_4$/gVS$_{add}$) of the sample was investigated. Biomass samples from CHTP at 30 (control), and 80 and 100 °C with DT of 15, 30, 45 and 60 min were randomly matched and tested by the BMP assays. These conditions were, however, chosen to guarantee the coverage of enzymatic hydrolysibility values from the entire set of CHTP biomass tested in the experiment. The data of EH and BMP were plotted to observe their relationship. A linear regression was then performed where a coefficient of determination ($R^2$) was calculated using Design Expert version 7.0 (Sate-Ease, Inc, Minneapolis, MN, USA). At a satisfactory precision, the correlation developed was then used to predict the methane yield of the samples at other CHTP conditions as well as those from microwave-pretreated biomass which will be discussed in the following section.

Subsequently, the effects of CHTP temperature and DT on the methane yield were evaluated. The EH-predicted methane yield data were then regressed with the CHTP temperature and DT using a quadratic model using Design Expert version 7.0, as shown in Equation (3).

$$Y_{CH4} = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (3)$$

where $X_i$ and $X_j$ are the input variables (CHTP temperature and detention time), which influence the response variable $Y_{CH4}$ (methane yield), $\beta_0$ is the offset term, $\beta_i$ is the linear coefficient, $\beta_{ii}$ is the quadratic coefficient, $\beta_{ij}$ is coefficient for the $ij$ interaction effect, $n$ is the number of studied independent variables (in this work $n$ = 2).

### 2.6. Comparison between Convective and Microwave Hydrothermal Pretreatments of the Napier Grass Biomass

#### 2.6.1. Microwave Pretreatment (MWP)

The Napier grass slurry prepared according to Sections 2.1 and 2.2 (1:10 *w*/*w* mixed with distilled water) was placed in a 500 mL glass vessel before exposed to the microwave irradiation. The power-adjustable microwave oven (Panasonic Corporation, Model NN-S954) was employed to deliver electromagnetic energy at variable levels and durations, i.e., 330 W at 60–720 s, 600 W at 32–821 s, and 770 W at 50–389 s. A glass lid was loosely placed on the vessel top to prevent pressure build-up and minimise water evaporation. Microwave energy delivered to the sample was calculated according to Equation (4). Distilled water was added to the glass vessel after the treatment to the original level before analyses or use as substrate in the experiment. Only the MWP results at the same range of energy intensity, defined in Section 2.6.2, with CHTP were selected for detailed examination in this study so as to enable sensible comparison between MWP and CHTP.

### 2.6.2. Energy Consumption Calculations

In order to compare the energy input of pretreatment by convective or microwave methods, the energy exerted to a unit mass of the substrate or so-called energy intensity (EI) was used. EI was calculated based on the change in temperature of the treated sample according to Pellera and Gidarakos [29] (Equation (4)). This gave an actual energy the sample received by discounting for losses thru inefficiency of the heating equipment used.

$$EI = \frac{[m_s \cdot C_{p,s} \cdot \Delta T + m_w \cdot C_{p,w} \cdot \Delta T] + m_{w(added)} \cdot L}{m_s} \tag{4}$$

where $EI$ is energy intensity (kJ/kg$_{\text{substrate}}$), $m_s$ is the mass (kg) of solid substrate (dry wt.) subjected to pretreatment, $m_w$ is the mass (kg) of water in the vessel used in pretreatment (including the water contained in the substrate), $C_{p,s}$ and $C_{p,w}$ are the specific heat capacities of solid (1.89 kJ/kg·°C) and water (4.18 kJ/kg·°C), respectively, and $\Delta T$ is the temperature difference (°C) between the temperature of the slurry before (room temperature) and at the end of pretreatment. The calculation of latent heat (kJ) constitutes of $m_{w(added)}$ (the mass of water evaporated, kg) and $L$ (latent heat of evaporation of water, 2257.2 kJ/kg).

### 2.6.3. Biodegradability Index of Substrate

The term biodegradability index (BI) was used to determine the changes in substrate biodegradability after pretreatment. Biodegradability index represents the degree of degradation in the tested system relative to the maximum degradation, hence it is a percentage ratio of methane potential from BMP assay (mL $CH_4$/g VS) and theoretical methane potential (mL $CH_4$/g VS) of the test substrate, as shown in Equation (5).

$$BI\ (\%) = \frac{BMP}{TMP} \times 100 \tag{5}$$

TMP is theoretical methane potential which represents a complete conversion of the substrate from its original elements under anaerobic digestion. In this study, TMP was calculated from elemental compositions (C, H, O, N, and S) using the modified Buswell and Mueller equation, as shown in Equations (6) and (7) [37].

$$\mathrm{C_cH_hO_oN_nS_s + yH_2O \rightarrow xCH_4 + zCO_2 + nNH_3 + sH_2S} \tag{6}$$

$$\begin{gathered} \mathrm{x = 0.125(4c + h - 2o - 3n - 2s),} \\ \mathrm{y = 0.250(4c - h - 2o + 3n + 2s),\ and} \\ \mathrm{z = 0.125(4c - h + 2o + 3n + 2s)} \end{gathered} \tag{7}$$

where c, h, o, n, and s are the molar ratio of carbon, hydrogen, nitrogen, sulphur and oxygen, respectively, assigning s = 1. TMP was reported in mL of methane per gram vs. of substrate.

## *2.7. Analytical Procedures*

TS and VS were determined using standard methods for the examination of water and wastewater [38]. Napier grass silage was dried at 60 °C to a constant weight and ground for the analysis of elemental compositions (C, H, O, N and S) using a CHNS-O Analyzer (CE Instruments Flash EA 1112 Series, Thermo Scientific (Thermo Quest), Milan, Italy) by dynamic flash combustion method. Quantification of soluble chemical oxygen demand (SCOD) concentration was performed according to the open reflux colorimetric method outlined in Standard Methods [38]. The volume of biogas generated was measured using a 10 mL loss-of-resistance (LOR) syringe with 0.2 mL scale (B. Braun Medical Inc., Bethlehem, PA, USA). Biogas sample was injected to a gas chromatography (GC Agilent$^{\text{TM}}$ 7820 A Agilent Technologies, Santa Clara, CA, USA), equipped with a thermal conductivity detector (TCD) and a stainless steel packed column SS Hayesep Q80/100 (6 m × 1/8 in.) using helium gas (He) as carrier gas to determine its composition. The standard calibration curve was made with pure $CH_4$, $CO_2$, and $N_2$ gases, and verified with a standard gas mixture of 5% $N_2$, 60% $CH_4$, and 35% $CO_2$. Total reducing sugar (TRS) concentration was determined following the 3,5-dinitrosalicylic acid (DNS) method described in [39]. Soluble sugars contained in enzymatic hydrolysates were quantified according to the Laboratory Analytical Procedure (LAP) by the National Renewable Energy Laboratory (NREL) [31].

### 2.8. Statistical Analysis

All experiments in this study were conducted in triplicate as all error bars presented are based on one standard deviation from mean. The means were compared using SPSS software version 22 with the one-way analysis of variance (ANOVA) and Duncan's multiple range test at $p < 0.05$. The multivariate regression model was generated by Design Expert version 7.0 (Stat-Ease, Inc., Minneapolis, MN, USA).

## 3. Results and Discussion

### 3.1. Effects of Temperature and Detention Time of Convective Hydrothermal Pretreatment (CHTP) on Methane Yield

At this stage, CHTP was tested at 30, 80 and 100 °C to find the workable correlation of pretreatment temperature to the biomass degradation at this low range hydrothermal application. Results show that the CHTP induced a dramatic increase in SCOD concentration, which represents the organic constituent in the liquid hydrolysate. The highest increase of SCOD was around 70% at CHTP temperature 80 °C and DT 60 min (117 ± 0.2 $mg/g_{substrate}$) compared to the control (non-treated) (69 ± 0.35 $mg/g_{substrate}$). In general, most of the organic matters solubilised due to the thermal pretreatment are the easily soluble biodegradable compounds [40] consisting of lactic acid in the silage sample and partial disintegration of plant cells [41]. Even though the concentration of SCOD and total reducing sugar were in the same trend, they were not proportional to the methane yield derived from the BMP assay. The effects of temperature and DT to methane yield based on the 42-day BMP assay are shown in Figure 1.

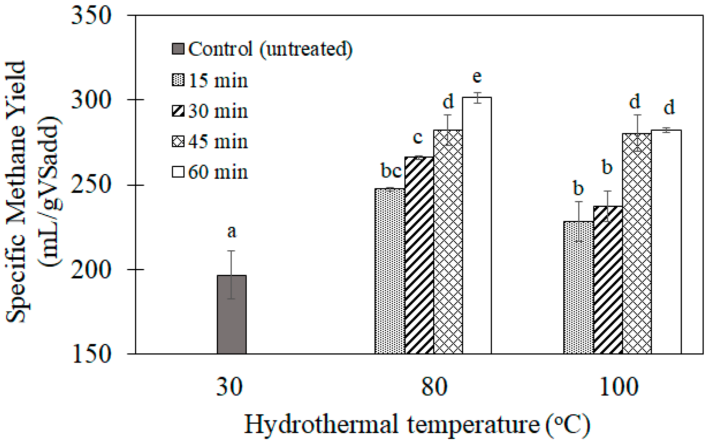

**Figure 1.** Specific methane yield from a silage Napier grass hydrothermally treated with various temperature and detention time. (Different letters on each bar represent significant difference by Duncan's multiple range test at α = 0.05).

Under CHTP, the biomass structure was disrupted, although not so intensively, so that anaerobic microorganisms and their enzymes could gain access and able to decompose organic matters at a higher rate. Faster biodegradation and biogas production profiles of the CHTP biomass were observed at an early stage of digestion during the BMP assay conducted. At equal CHTP temperature, the eventual increase of methane yield corresponded to the longer DT. As expected, higher temperature (80 and 100 °C) could improve $CH_4$ yield and anaerobic digestibility compared to the control. Pairwise comparison between CHTP 80 and 100 °C at the same DT reveals that CHTP at 80 °C was mostly more effective in improving methane yield. The highest $CH_4$ yield of 301.5 ± 3.0 $mLCH_4/gVS_{add}$ was obtained at 80 °C, DT 60 min, which was 53.2 ± 1.5% higher than control and statistically higher than any other conditions ($\alpha = 0.05$).

The lower methane yield at CHTP 100 °C, DT 60 min was probably caused by a destruction of some organics at a more severe condition [42], especially at a hot surface of the heating element for a long period. It is noted that the temperature at the hotplate surface was roughly 400–550 °C while the heater was on although the bulk liquid in the reactor was maintained at 100 °C. Treatments at high temperatures could lead to decreased sludge biodegradability despite achieving high solubilisation efficiencies. One of the most well known phenomena is the Maillard reaction, the non-enzymatic browning reaction which occurs between carbohydrates and amino acids, resulting in the formation of complex substrates that are difficult to be biodegraded. This reaction can also occur at extreme longer treatment time at lower temperatures (<100 °C) [43]. The soluble carbohydrates reacted among themselves or with soluble proteins and formed organic compounds which are like melanoidins, resulting in coloring the supernatant brownish.

### 3.2. Enzymatic Hydrolysis and Correlation to Methane Yield

The fact that sugars and methane, which are the end products of enzymatic hydrolysis and AD, respectively, are mostly originated from the same cellulose in the biomass suggests the possible correlation between the EH and methane yield ($Y_{CH4}$). Enzymatic hydrolysis assay were conducted for samples tested with BMP in Section 3.1, and EH values (Equation (1)) were then correlated with the $Y_{CH4}$ from these conditions. Results reveal a strong linear relationship ($R^2 = 0.9810$) between EH and $Y_{CH4}$ of the Napier grass silage, as shown in Figure 2. This reiterates that the rate-limiting step for anaerobic digestion of this substrate is hydrolysis, which dictates the overall digestion. It should be noted that the commercial enzyme Celli CTec2 used in EH assay possesses not only cellulase activity but also endo-xylanase activity which is able to degrade hemicullulose in biomass [44,45]. Lignin degradation under anaerobic condition is very limited by specific group of anaerobes [46] and Celli CTec2 is not capable of attacking the lignin structure. These facts imply the mechanistic relationship between EH and BMP. Thus, the linear model in Equation (8) was used to predict the $Y_{CH4}$ of the samples from EH values. The advantage of the enzymatic hydrolysis in contrast with the BMP assay is its shorter time and relatively simpler protocol. Although there can be a general trend between enzymatic hydrolysis and methane yield, this kind of relationship is specific to individual biomass (type, specific properties and preconditioning) and the workable relationship must contain the range of EH and $Y_{CH4}$ data to be predicted. Comparison with the previous study by Liew et al. [47] indicates that the generic trend reported for different biomasses was not in good agreement with the data from this present study and their hydrolysibility range was only 10.5–17.0%, which is lower than the data here (up to 30%). No mathematical relationship was reported in Liew et al. [47], thus, use of the new linear model with high $R^2$ developed specifically for this present study could be justified.

$$\text{Methane Yield} = 11.098 \times (\text{Enzymatic Hydrolysibility}) - 32.128 \tag{8}$$

### 3.3. Modelling Methane Yield in Convective Hydrothermal Pretreatment

In order to maximise the pretreatment efficiency, a quadratic model of variables, hydrothermal temperature (Temp) and detention time (DT), the exposed samples were established. Methane yield ($Y_{CH4}$) was regressed as a response function by the F-test, and the resulting ANOVA is shown in Table 2. Given the low $p$-value (<0.0001), the ANOVA of the model was highly significant, indicating that this quadratic model possesses good predictability. A good fit to the experimental data by Temp and DT is expressed by the high determination coefficient ($R^2$) of 0.8364 for $Y_{CH4}$. In addition, the coefficient of variance (C.V.) of 4.28 for $Y_{CH4}$ confirmed a satisfactory precision and reliability for the experiments performed. The polynomial equation, describing the predicted methane yield as a simultaneous function of the Temp and DT, is shown in Equation (9) and was used to generate a three-dimensional surface plot in Figure 3.

$$Y_{CH4}\ (\%) = 82.1401 + 4.0080\ \mathrm{Temp} - 0.0548\ \mathrm{DT} + 0.0092\ \mathrm{Temp{*}DT} - 0.0258\ \mathrm{Temp}^2 - 0.0009\ \mathrm{DT}^2 \quad (9)$$

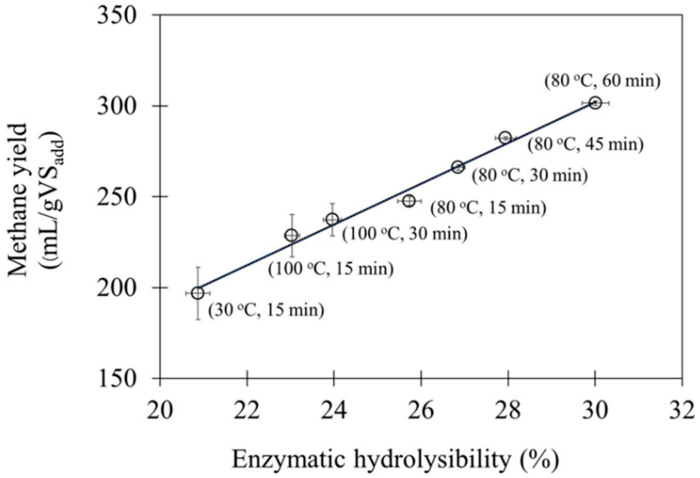

**Figure 2.** Relationship between specific methane yield ($Y_{CH4}$) and enzymatic hydrolysibility (EH) of Napier grass silage treated at 80 and 100 °C for 15–60 min. (Note: Error bars represent one standard deviation from means).

**Table 2.** Estimated regression coefficients and analysis of variance (ANOVA) of the fitting model for predicted methane yield ($Y_{CH4}$).

| Source | Predicted Methane Yield (%) | |
|---|---|---|
| | **Coefficient Estimate** | **Probability** |
| $b_0$ | 82.1401 | <0.0001 |
| Temp | 4.0080 | <0.0001 |
| DT | −0.0548 | 0.0039 |
| Temp*DT | 0.0092 | 0.2385 |
| $\mathrm{Temp}^2$ | −0.0258 | <0.0001 |
| $\mathrm{DT}^2$ | −0.0009 | 0.9116 |
| F-significant | <0.0001 | |
| $R^2$ | 0.8364 | |
| $R^2$ adjusted | 0.8131 | |
| Coefficient of variance (CV) | 4.28 | |

The 3D response surface and the contour plots by Equation (9) depict the nature and extent of the interaction between the independent variables Temp and DT on the response. It is clear that CHTP temperature had a higher impact on methane yield over the detention time. Increasing DT started to show some influence at higher temperature, i.e., approximately above 65 °C. At lower temperature, merely the duration of wetting does very little to cause structural changes of the biomass without the help of other factors. Water activity will assist the function of enzymes or chemicals to attack the microfibril of the lignocellulose provided that enzymes are present in the environment. It must be recognised that the Napier grass used in this experiment was ensilaged and contained hydrolytic bacteria. Thus, at higher temperature, the activity of hydrolytic enzymes become higher [48] and thus DT played a role in pretreatment as shown in the upper range of temperature (above 65 °C). These facts reflect in the coefficient probability of $\mathrm{DT}^2$ and interaction term Temp*DT which are not significantly influential to the accuracy of the model (see Table 2). It must be noted that too high a temperature will denature the enzymes, which are essentially proteins.

It was also observed in Figure 3 that methane yield decreased when CHTP temperature was over 88 °C. The highest methane yield (277.3 $mLCH_4/gVS_{add}$) was obtained at 88.4 °C, 60 min from the model, which is in the proximity of the observed values in Figure 1 (Section 3.1). However, this contour suggests that the pace to reach the optimal of 88.4 °C is rather slow as seen by the lowering slope from around Temp 65 °C onward. Using the heat source, i.e., from the CHP (combined heat and power) unit or biogas, to around 60 °C could have an insignificant difference in methane yield increase in anaerobic digester compared to the optimal condition (88.4 °C). Although longer DT gave some advantage, too long a DT, i.e., over 24 h, could cause loss of the carbon from microbial degradation of the substrate to $CO_2$ or $CH_4$, especially in an open or unsealed tank setup.

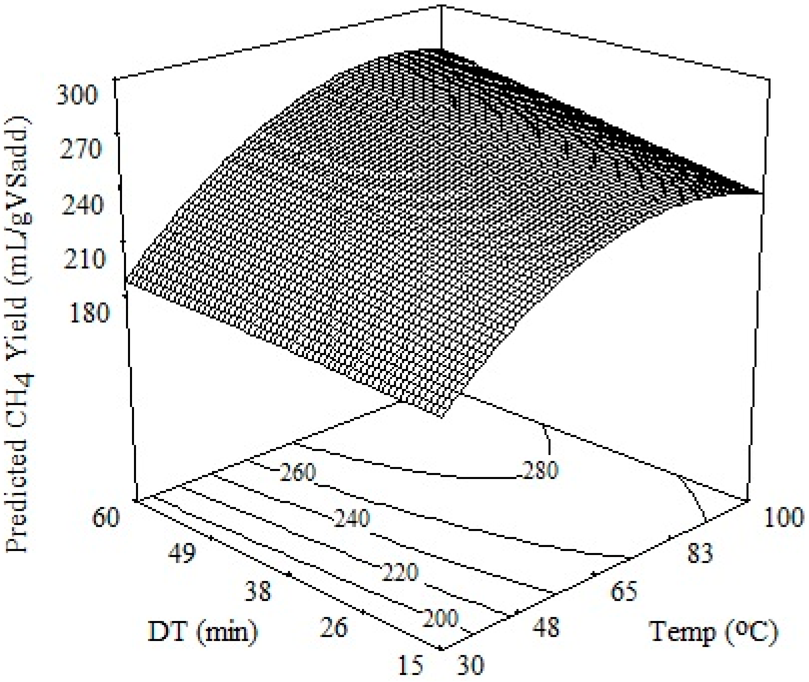

**Figure 3.** Interrelation of temperature (Temp) and detention time (DT) on methane yield by convective hydrothermal pretreatment of Napier grass biomass.

### 3.4. Comparison between Convective Hydrothermal and Microwave Pretreatments

In order to evaluate the comparative effectiveness of CHTP and MWP, the sample must be treated at a comparable level of the energy input. Thus, EI defined in Equation (4) was used to estimate the energy that the sample received in both CHTP and MWP. This calculation was based on the change in temperature and loss of water thru the latent heat of evaporation, thus ignoring the efficiency of the equipment either heater or microwave machine. The conditions for this comparison were CHTP at EI 3006, 3443, 4727 and 5136 $kJ/kg_{substrate}$ and MWP at EI 2344, 2743 and 3462 $kJ/kg_{substrate}$. Additionally, to confirm the workable relationship developed from CHTP (outlined in Section 2.5) with the Napier grass from MWP, random treated samples from MWP were tested for EH and BMP. The data were found to fit well with those established from CHTP, and hence used to predict the BI of MWP biomass in the same fashion.

#### 3.4.1. Release of Sugars and Lignocellulose Derivatives into Liquid Hydrolysate

The hydrolysate after pretreatment was sampled to measure the total reducing sugar (TRS) concentration so as to indicate the degree of hydrolysis. They are presented in Figure 4 as dash lines. The overall trend clearly shows the positive relationship between TRS release to EI applied to the biomass sample. Hu and Wen [49], by using scanning electron microscope images, showed that when switchgrass was treated by microwave, many granule-like spots appeared on the surface, indicating the local destruction of the lignocellolosic structure, probably from intracellular moisture vaporization. These changes would facilitate the enzyme's access to the potentially hydrolysable components. In our case, highest applied power either MWP or CHTP always caused some loss in the saccharification yield (Figure 4). Microwave pretreatment at 2743 kJ/kg$_{\text{substrate}}$ produced an increase in TRS of 39% compared to the control. Similar effects were obtained at low to medium power with long exposure time, or medium to high power with short exposure time [50] but it is necessary to be aware of an upper limit so as to avoid adverse effects of inhibitor formation and loss of carbon through over oxidation. The threshold EI value of around 2700 kJ/kg from this experiment could be used as a guideline for MWP of grass biomass. Coincidentally, TRS concentration declined after EI of MWP went from 2743 to 3462 kJ/kg$_{\text{substrate}}$ (−7.5% TRS), and of CHTP went from 4727 to 5136 kJ/kg$_{\text{substrate}}$ (−10.8% TRS). These TRS concentrations were, however, still substantially higher than control (10.72 ± 0.22 mg/g$_{\text{substrate}}$).

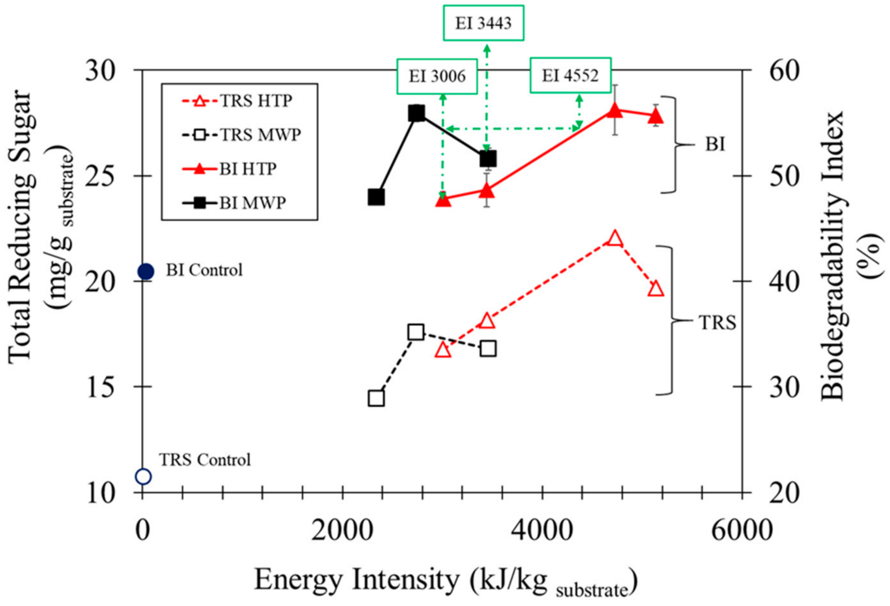

**Figure 4.** Effects of energy intensity on total reducing sugar release and biodegradability (BI) of Napier grass biomass by convective hydrothermal pretreatment (CHTP) and microwave pretreatment (MW).

Higher energy intensity applied to the biomass caused intensive hydrolysis of cellulose and hemicellulose into fermentable sugars while phenolic compounds and dissolved lignin were undesirably released from lignin degradation. In this present study, inhibitory by-products of hydroxymethylfurfural (HMF) and furfural, which are the dehydration products from fermentable sugar (glucose and xylose, respectively), were not detected in the hydrolysate at this EI level. Nevertheless, other inhibitors such as phenolic compounds and dissolved lignin were released from the biomass during the pretreatments, which were revealed by the Fourier-transform infrared (FTIR) spectroscopy results (Figure S2 in Supplementary Materials). The peak located at 1518–1593 cm$^{-1}$ was attributed to the aromatic skeletal vibrations of lignin [51,52]. The transmittance signal of the pretreated biomass indicates that the dissolution of lignin took place during CHTP and MWP. It was reported that the weakening in hydrolysis efficiency and acidogenesis began at a dissolved lignin level of 1 g/L [53]. Furthermore, the digestion of biomass with a combination of initial concentrations of furans and total phenolic compounds higher than 0.33 g/L and 0.25 g/L, respectively, had a negative effect on anaerobic digestion. Both compounds greatly disturbed the acetoclastic pathway. While a hydrogenotrophic activity could still thrive, it resulted in the overall drop of methane production [44]. Additionally, these newly formed compounds would be harder to degrade biologically as shown by the concurrent decline of BI in Figure 4. The findings of this present study, suggest that the CHTP and MWP with amounts of energy intensity exceeding 10,963 and 3462 MJ/kg$_{substrate}$ posed the potential inhibition to the digestion system and should, therefore, be avoided. Compared to other studies with high temperatures, only small changes of the lignin structure were detected by the lower heat but were still effective at disturbing the cellulose-hemicellulose bond. This range of temperature is, nevertheless, feasible for self-sustained heat source within the biogas plant or a small fraction of external energy input for methane production improvement. The difference in TRS releases suggests that EI is also specific to the method of heat transfer. It is noted that the CHTP conditions selected for this comparison were from the effective temperature of 60–80 °C, as lower temperature was insufficient to effectively cause these biomass structural changes, which was evidenced in the control set.

### 3.4.2. Difference in Biodegradability of the Pretreated Grass Biomass

Generally, there will be a portion of plant cells that would not be converted biologically under any test conditions. The degree of conversion in any pathway depends on various parameters and can be represented by the term biodegradability index (BI) under a defined condition. From the stoichiometric equation generated from CHNS-O elemental composition (Equation (10)), the theoretical methane yield of Napier grass silage is 477.8 mLCH$_4$/gVS.

$$C_{91.1}H_{139.2}O_{60.8}N + 26.7H_2O \rightarrow 47.4CH_4 + 43.7CO_2 + NH_3 \tag{10}$$

The highest BIs of microwave and hydrothermal treatment were 57.21 ± 0.70% and 56.98 ± 2.33% which were obtained at EI 4727 and 2743 kJ/kg$_{substrate}$, respectively (Figure 4). Statistical analysis indicates no significant difference between the two BI values but they were significantly higher than that of the control (BI 39.72 ± 0.24%) at $\alpha = 0.05$. These two methods of thermal pretreatment could improve the biodegradability of the biomass by about 17%. The fact that there was still a big gap between the theoretical and methane yield around 43% (100 − BI) suggests that either there was still a great potential to improve the digestion of this material, and there remained the valuable degradable materials in the digested solids. It was obvious that the energy input by means of microwave irradiation was more effective than convective hydrothermal. It is apparent in Figure 4 that less energy is required for MWP to achieve the same BI as the CHTP. This issue with regard to energy balance will be discussed further in Section 3.4.3.

In contrast, a decrease in methane yield was reported by Li et al. [54] where a 13.8% drop in methane yield resulted from microwave treatment of *Pennisetum* hybrid grass at 1180 W, frequency 2450 MHz, and temperature up to 260 °C. MWP can generate inhibitory by-products such as HMF and furfural and cause losses of volatile and biodegradable matter due to exposure to the high microwave irradiation without temperature control especially in intense heating rate represented by high wattage and lack of sufficient liquid to distribute the heat [55,56]. These factors, solid to liquid ratio, exposure time, and microwave power, all constitute energy intensity. Thus, careful method/equipment selection and operation must be taken seriously in substrate pretreatment. It is of the utmost importance to recognise that excessive pretreatment not only leads to the formation of inhibitors and loss of carbon, but also a great waste of energy and money. Low EI such as demonstrated in this study would not cause inhibition, only mild loss of carbon and sugar transformation to melanoidins could occur [43]. Selection of pretreatment condition to minimize or avoid such instance should be practised.

### 3.4.3. Relative Energy Gain from Thermal Pretreatments

Table 3 demonstrates the energy gain and the energy input by the two methods of thermal pretreatment to the Napier grass biomass. The conditions displayed in the table are selected by their equal EI values over the range that overlaps (see Figure 4) or equal value of BI (EI 4552 kJ/kg) to enable a direct comparison. The values outside the experimental conditions were derived from linear interpolation of the data to enable comparison. At the equivalent EI (3006 and 3443 kJ/kg), the predicted $Y_{CH4}$ and BI of MWP were higher than CHTP that led to the increase in energy gain as high as 2.36 $\mathrm{MJ/kg_{substrate}}$ of MWP versus only 1.29 $\mathrm{MJ/kg_{substrate}}$ of CHTP at EI 3006 $\mathrm{kJ/kg_{substrate}}$. The ratio between energy gain and energy input calculated in the last column reveals that neither methods are energy efficient, with an energy ratio (ER) $< 1$. The most efficient process in terms of energy recovery was MWP at 3006 $\mathrm{kJ/kg_{substrate}}$ which is 0.79 or 79%.

**Table 3.** Estimated performance and energy balance of convective hydrothermal pretreatment (CHTP) versus microwave pretreatment (MWP) of Napier grass biomass at equivalent energy intensity or biodegradability index (BI).

| Method | Energy Intensity | Predicted Methane Yield | BI [a] | Energy Yield from $CH_4$ in Biogas Produced | | Increased Energy Gain ① | Energy Input [b] ② | ER [c] = Output/Input ①/② |
|---|---|---|---|---|---|---|---|---|
| | ($\mathrm{kJ/kg_{substrate}}$) | ($\mathrm{mLCH_4/gVS}$) | (%) | (MJ/kgVS) | ($\mathrm{MJ/kg_{substrate}}$) | ($\mathrm{MJ/kg_{substrate}}$) | ($\mathrm{MJ/kg_{substrate}}$) | |
| Untreated | Untreated | 189.8 | 39.7 | 6.79 | 6.16 | - | - | - |
| CHTP | 3006 | 227.8 | 47.5 | 8.22 | 7.45 | 1.29 | 3.0 | 0.43 |
| | 3443 | 235.9 | 49.4 | 8.65 | 7.84 | 1.68 | 3.4 | 0.49 |
| | 4552 | 265.2 | 55.5 | 9.49 | 8.52 | 2.36 | 4.6 | 0.52 |
| MWP | 3006 | 265.2 | 55.5 | 9.49 | 8.52 | 2.36 | 3.0 | 0.79 |
| | 3443 | 250.8 | 52.5 | 8.98 | 8.14 | 1.98 | 3.4 | 0.57 |

Note: [a] Biodegradability index, [b] Energy input is calculated from EI of CHTP or MWP, [c] ER = Energy ratio.

Another scenario for comparison is at the equivalent BI (55.5%) of MWP where EI of CHTP was 4552 $\mathrm{kJ/kg_{substrate}}$. The energy input for MWP was only 3006 $\mathrm{kg/kg_{substrate}}$. This clearly shows the specific effect of microwaves on the disruption of biomass structure. The major benefit of microwave heating is that microwave irradiation could penetrate lignocellulosic biomass and directly vibrates the water molecules inside causing an increase in temperature, shattering the lignocellulosic structure from within. This vibration breaks inter- and intra-molecular hydrogen bonds, leading to enhanced accessibility of enzyme to cellulose core in AD [57].

However, if the recovered waste heat from the CHP (combined heat and power generation) unit, which has only around 30% efficiency to produce electricity, and a portion of the biogas (heating value of around 21.5 $\mathrm{MJ/m^3}$ at 60% $CH_4$ content) from the biogas plant itself are to be exploited as a heat source, the external energy input term of CHTP can be greatly reduced. In contrast, microwaves need to be generated from electricity that will be the major expenditure of the microwave machine system. The potential thermal pretreatment of lignocellulosic substrate from waste heat could be technically feasible at low temperature range with an appropriate detention time.

## 4. Conclusions

Low-range convective hydrothermal pretreatment (CHTP) of grass biomass was found to be effective in promoting methane yield for anaerobic digestion. In this study, enzymatic hydrolysis was proven and used as a surrogate parameter for methane potential to circumvent the long and tedious BMP assay. A temperature range of CHTP under 100 °C with appropriate detention time was sufficient and practical for industrial application. In contrast, microwave pretreatment (MWP) although more effective in terms of energy expenditure, requires an energy source from electricity. Waste heat from a biogas power plant and biogas itself should be utilized for CHTP which would greatly improve the energy balance for pretreatment.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2227-9717/8/10/1221/s1: Figure S1: Reactor setup for convective hydrothermal pretreatment, and Figure S2: FTIR spectrograms of Napier grass under convective hydrothermal pretreatment (CHTP) and microwave pretreatment (MWP) at different energy intensities.

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