The Potential of Biomethane Recovery from Hemp Biomass Residue (*Cannabis sativa* L.)

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ABSTRACT

Hemp (*Cannabis sativa* L.) is a plant with significant potential for use in the textile industry, medical applications, food and health benefits, environmental conservation, and as biomass fuel for renewable energy production. The initial raw material for hemp processing must undergo an oil extraction process to obtain hemp oil, resulting in residual material known as hemp biomass residue (HBR). Researchers are interested in utilizing this residue to produce biofuel energy through biogas technology using the biochemical methane potential test in 120 mL serum bottles. The temperature-controlled was at 35 ± 2 °C, and the experiment was conducted for 45 days. The study found that TCOD, TS, and VS removals were 57.32 ± 2.61%, 42.59 ± 4.18%, and 47.21 ± 3.52%, respectively. Cumulative biogas and methane yield were 109.61 ± 5.02 and 48.89 ± 2.69 N mL/g VS_{added}. In addition, the maximum methane concentration was 71.64 ± 0.33%. Because of the above statement, future studies could focus on scaling up and studying technical and economic feasibilities. In addition, other anaerobic digestion strategies such as co-digestion, different inoculum sources, and different SIR to increase biomethane production potential should also be evaluated.

Keywords: Hemp biomass residue/ Biochemical methane potential/ Biofuel/ Biogas/ Anaerobic digestion

1. INTRODUCTION

The study of biochemical methane potential (BMP) from hemp biomass residue explores a promising avenue for sustainable energy production. Hemp, a versatile plant traditionally cultivated for fibers, seeds, and oil, has recently gained attention for its potential in bioenergy applications [1]. The efficient conversion of agricultural residues, such as those from hemp, into biogas through anaerobic digestion is an environmentally friendly approach that contributes to the circular economy [2]. This process helps manage waste and generates renewable energy through biomethane [2, 3]. Hemp biomass, characterized by its high cellulose content and rapid growth rate, presents a valuable feedstock for biogas production [3]. As the global energy demand continues to rise, there is an increasing need to explore alternative energy sources that are both renewable and sustainable. This study aims to evaluate the BMP of hemp biomass residues, providing insights into their potential as a feedstock for biomethane production [4].

The research investigates key parameters influencing the anaerobic digestion process, such as substrate composition, inoculum type, and process conditions. By understanding these factors, the study seeks to optimize the methane yield from hemp residues by enhancing biogas production's efficiency and viability. The findings of this research could contribute to the development of more sustainable energy systems and support the broader adoption of hemp as a bioenergy resource [4].

This study is significant for its potential contributions to renewable energy and its implications in sustainable agriculture and waste management. This research underscores the importance of integrating energy production with agricultural practices to achieve a more sustainable future by valorizing hemp residues.

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2. METHODOLOGY

2.1 Sample collection and preparation

The substrate used in this study was hemp biomass residue (HBR) or *Cannabis sativa* L. from the Faculty of Agricultural Technology, Chiang Mai Rajabhat University (Mae Rim campus) Chiang Mai, Thailand. The biomass was collected after extracting the hemp oil, as shown in Figure 1. After collection, the biomass was dried at 45 ± 2 ºC in a hot air oven (120BOF, Ponpe Instrument, Thailand) to decrease its moisture content to less than 10% [5]. The dried biomass was then ground using a commercial grinder. Finally, the prepared HBR was stored in a vacuum plastic bag and kept at room temperature to prevent decay. TS and VS were analyzed using APHA (2005) [6], the C/N ratio was set based on Walkley and Black [7], and the Kjeldahl method [8], and fiber composition was analyzed using the Detergent method [9]. The results are presented in Table 1.

The inoculum used in this study was collected from anaerobically digested pig manure at the Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand, shown in Figure 1. The inoculum was stored at 4 ± 2 ºC and reactivated at 35 ± 2 ºC for several days before being transferred to the serum bottles [10]. The characteristics of the inoculum used in this study are presented in Table 1.

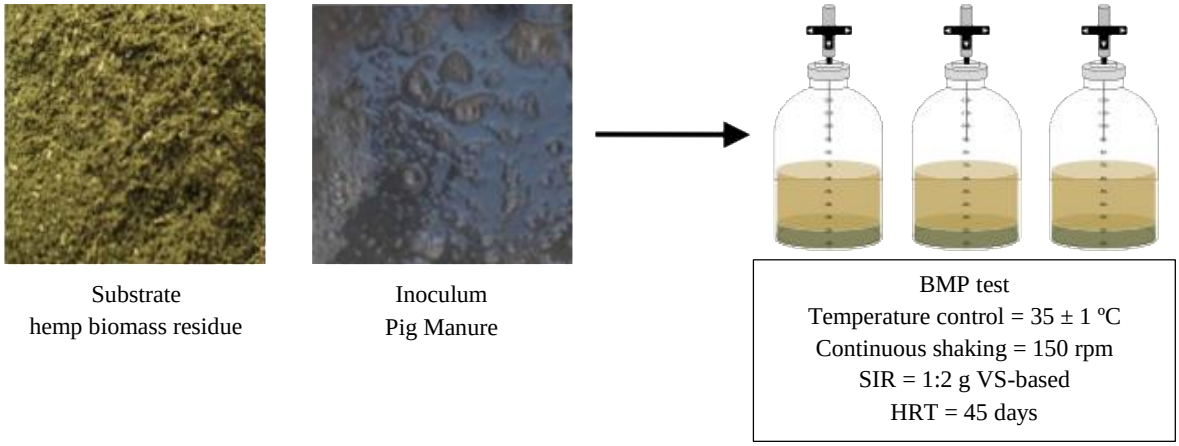

Figure 1. Diagram for biochemical methane potential test of hemp biomass residue

2.2 Experimental setup

The methane yield was evaluated in a series of serum bottle tests by digesting the substrate in a controlled environment. The biochemical methane potential (BMP) test was conducted in 120 mL serum bottles with a 60 mL working volume. The substrate-to-inoculum ratio (SIR) was set according to Moset et al. (2006) [11]. The BMP assays were carried out in triplicate. Stock nutrient solution 1% (v/v) and 50 g/L $NaHCO_3$ buffer solution 10% (v/v) were added to each serum bottle to ensure sufficient nutrition and acid buffer capacity during digestion. Stock nutrient solution at 5 times concentration contained NH_4Cl 1.4 g/L, K_2HPO_4 1.25 g/L, $MgSO_4 \cdot H_2O$ 0.5 g/L, $CaCl_2 \cdot 2H_2O$ 0.05 g/L, yeast extract 0.5 g/L, and trace element solution 5 mL/L. The trace element solution contained $FeCl_2 \cdot 4H_2O$ 2,000 mg/L, H_3BO_3 50 mg/L, $ZnCl_2$ 50 m/L, $CuCl_2.2H_2O$ 38 mg/L, $MnCl_2.4H_2O$ 500 m/L, $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ 50 mg/L, $AlCl_3 \cdot 6H_2O$ 90 mg/L, and $CoCl_2 \cdot 6H_2O$ 2,000 mg/L [12]. A small amount of 0.1 M NaOH or HCl was used to adjust the pH to 7.00 ± 0.01, and then deionized water was added to make up a final volume of 60 mL. The headspace of the serum bottles was purged with nitrogen gas for 1 min and sealed immediately to ensure anaerobic condition. All bottles were placed in an incubator shaker (WiseCube WIS10RL, DAIHAN Scientific Co., Ltd., Gangwon-do, Korea) at 35 ± 1 ºC with a continuous shaking of 150 rpm. The volume of the biogas produced and methane concentration were analyzed regularly. The BMP test was terminated when the daily methane production was below 1% of the cumulative methane production, which took 45 days. All parameters were analyzed in triplicate except biogas production.

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2.3 Analytical methods

The operating parameters (i.e., pH, TCOD, TS, VS, VFA, and Alkalinity) were determined following the Standard Methods APHA (2005) [6]. pH of the leachate was analyzed using a benchtop pH meter (Mettler Toledo [S220], Columbus, OH, USA). Daily biogas production was measured by a micromanometer (MP 112; KIMO Instrument, France). The biogas compositions were analyzed using the Gas Chromatography model 7820A with Agilent TU-AMPKS6FHQ Packed column and thermal conductivity detector (TCD). Methane production was calculated by multiplying methane content and biogas production, where helium was used as carrier gas. The standard calibration curve was made with gas mixtures containing CH_4 at 3 levels covering the range of 20-99.999%, and verified with a standard gas mixture of 5% N_2, 60% CH_4, and 35% CO_2. Methane potential was calculated as N mL/g VS $_{added}$ (at 0°C and 1 atm). All parameters were analyzed in triplicate except biogas production.

2.4 Kinetic study

The data of cumulative methane yield from the experiments was fit by the modified Gompertz equation, as presented in Eq. 1 [13].

$$Y = M\exp\left\{-\exp\left[\frac{Rme}{M}(\lambda - t) + 1\right]\right\} \qquad \text{Equation 1}$$

Where; Y is the accumulated methane volume (mL/g VS $_{added}$)
T is the experimental time (d)
M is the methane production potential (mL/g VS $_{added}$)
Rm is the maximum methane production rate (mL/g VS $_{added}$-d)
λ is lag phase period (d)
E is an Euler's number (2.718).

The variables were calculated using Microsoft Office Excel with the solver function.

2.5 Statistical data analysis

The experimental data were analyzed using analysis of variance (ANOVA) with a significance level (α) of 0.05; and a post hoc Tukey's test was conducted by using the Statistical Package for the Social Sciences (SPSS) version 22, IBM, USA.

3. RESULTS AND DISCUSSION

3.1 Characteristics of substrate and inoculum

The characteristics of HBR and inoculum are shown in Table 1. The HBR contained high VS, representing organic matter of 84.63 ± 4.88% of the total weight. Further, the VS/TS ratios of HBR and inoculum, indicators for evaluating biodigestibility, were high at 0.91±0.01 and 0.69±0.01, respectively. A substrate with a VS/TS ratio of over 0.80 isconsidered as a potential anaerobic digestion feedstock [14]. The C/N ratio, which indicates a proper amount of macronutrients to facilitate microbial growth, is one of the critical operating parameters for anaerobic digestion. Table 1 shows that the C/N ratios of HBR and inoculum were 18 ± 1 and 8 ± 1, respectively, which falls in the recommended range of 9 – 35 for the anaerobic digestion process [15]. In the fiber composition of HBR, cellulose was the main constituent, and the cellulose content of HBR was identical to Matassa et al., (2020) [4] the promising lignocellulosic substrate for anaerobic digestion process.

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Table 1. The Characteristics of substrate and inoculum before anaerobic digestion.

Characteristics	Substrate	Inoculum
TS	929653 ± 2516 mg/kg	128750 ± 1568 mg/L
VS	846274 ± 4884 mg/kg	88295 ± 1464 mg/L
VS/TS ratio	0.91 ± 0.01	0.69 ± 0.01
pH	NA	7.35 ± 0.02
VFA	NA	390 ± 20 mg/L as CH_3COOH
Alkalinity	NA	2033 ± 15 mg/L as $CaCO_3$
VFA/Alkalinity ratio	NA	0.19 ± 0.01
TCOD	NA	30667 ± 8269
C/N ratio	18 ± 1	8 ± 1
Cellulose	17.29 ± 0.86 % dry wt.	NA
Hemicellulose	21.45 ± 0.49 % dry wt.	NA
Lignin	33.78 ± 0.95 % dry wt.	NA
Ash	27.48 ± 0.78 % dry wt.	NA

Note: NA = Not Applicable

3.2 Removal efficiency of anaerobic digestion

Removal efficiencies for anaerobic digestion of HBR are shown in Table 2. The pH of the bottle content was maintained around neutral, which is in the optimization range for anaerobic digestion (6.8-7.2) [5, 10]. VFA concentrations before and after were 555 ± 64 and 445 ± 49 mg/L as CH_3COOH, and Alkalinity concentrations before and after were 3250 ± 141 and 3515 ± 213 mg/L as $CaCO_3$, respectively. Generally, VFA concentration in anaerobic digestion should not exceed 500 mg/L as CH_3COOH, but the maximum concentration could be as high as 2,000 mg/L as CH_3COOH. The system was kept in an anaerobic condition to decrease the excess VFA for a week until the produced biogas was undetected. The optimum alkalinity should be 1,000-5,000 mg/L as $CaCO_3$ for anaerobic digestion [5].

The VFA/Alkalinity ratios could be an indicator to determine the performance of the anaerobic digestion process. This study of before and after were 0.17 ± 0.08 and 0.13 ± 0.11, which is lower than the recommended value for anaerobic digestion, less than 0.4 [14] that could ensure system stability. Based on the characteristics of the bottle contents presented in Table 2, this study might not suffer from organic acid accumulation, which is one of the essential phenomena causing system failure during anaerobic digestion [10].

TCOD, TS, and VS removal strongly correlate with methane yield during anaerobic digestion. In this study, TCOD, TS, and VS removals of HBR were 57.32 ± 2.61%, 42.59 ± 4.18%, and 47.21 ± 3.52%, respectively is similar to that reported by Matassa et al. (2020) i.e., approximately 50% using hemp residue as the substrate.

Table 2. The characteristics of parameters analysis before and after anaerobic digestion.

Characteristic	Unit	Before	After	Efficiency
pH	–	7.00 ± 0.01	7.24 ± 0.01	NA
VFA	mg/L	555 ± 64	445 ± 49	NA
Alkalinity	mg/L	3250 ± 141	3515 ± 213	NA
VFA/Alkalinity ratio	mg/L	0.17 ± 0.08	0.13 ± 0.11	NA
TCOD	mg/L	90323 ± 1825	38550 ± 2828	57.32 ± 2.61
TS	mg/L	18455 ± 2496	10595 ± 1633	42.59 ± 4.18
VS	mg/L	11165 ± 1690	5895 ± 1294	47.21 ± 3.52
VS/TS ratio	–	0.61 ± 0.60	0.54 ± 0.48	NA

Note: NA is Not Applicable

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3.3 Biogas and methane yield

Maximum biogas and methane yield shown in Figure 2 were 39.59 ± 2.05 (day 9) and 18.04 ± 1.27 (day 9) N mL/g VS $_{added}$. Cumulative biogas and methane yield from the experimental shown in Figure 3 were 365.38 ± 16.72 and 162.97 ± 8.96 N mL/g VS $_{added}$, respectively. Average and maximum methane concentrations shown in Figure 4 were 51.69 ± 1.50% and 71.64% ± 0.33% (day 28). According to research by Matassa et al., (2020) [4], the different biomass residues (HBRs) were stalks, the unretted hurds, the retted hurds, the fibers, the inflorescences and the mix of leaves and inflorescences were evaluated as a potential feedstock for biomethane production. The specific cumulative biomethane production were 422 ± 20, 26 ± 5, 275 ± 7, 239 ± 10, 242 ± 13, and 118 ± 8 N mL/g VS $_{added}$, respectively. Moreover, according to Heiermann et al. (2009), the ensiling process might negatively influence biomethane formation due to the fermentation of plant sugars to lactic acid and other volatile fatty acids, thus obtaining a lower biomethane yield (259 N mL/g VS $_{added}$) under the same operating conditions. However, a general estimate of biogas production from lignocellulosic materials such as hemp typically consists of a methane concentration was 50-70% [16].

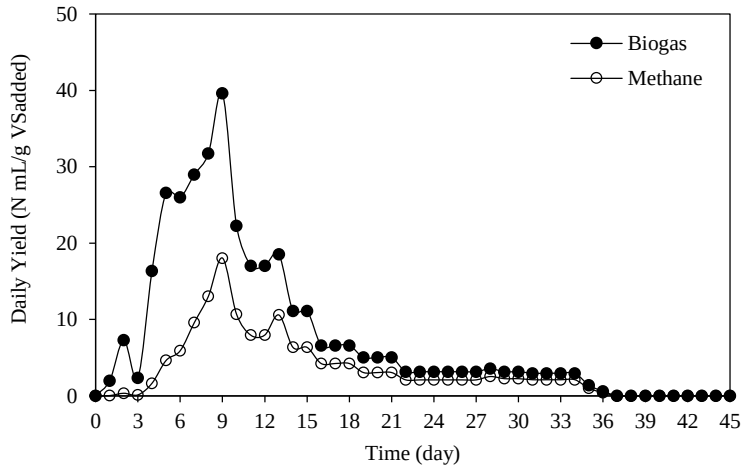

Figure 2. Daily biogas and methane yield

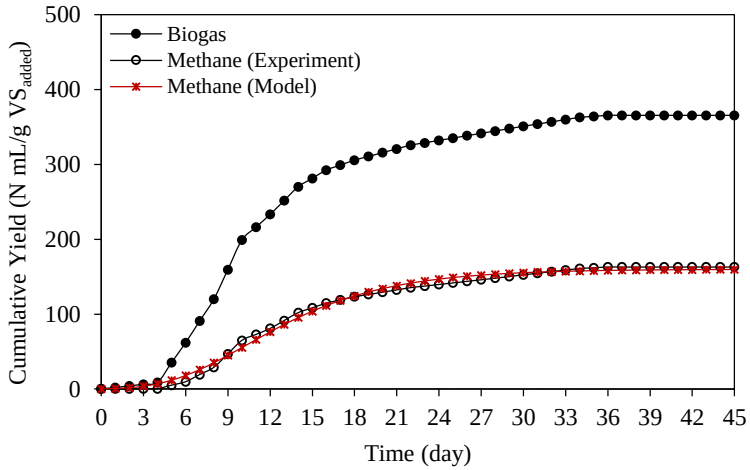

Figure 3. Cumulative biogas and methane yield

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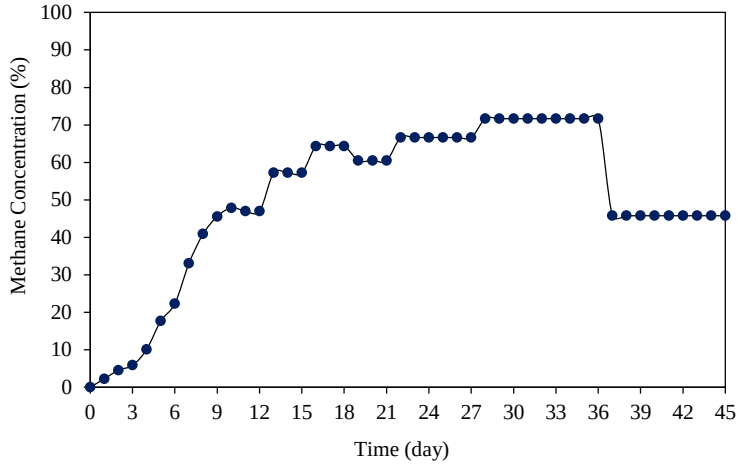

Figure 4. Methane concentration

3.4 Kinetic study

The information obtained from the kinetic study could be used to analyze and explain the cumulative methane yield (Model) from the anaerobic process shown in Figure 3 using the modified Gompertz model. Several models could be applied to fit the experimental data, including but not limited to first-order, logistic, and Gaussian equations [17, 18]. However, the modified Gompertz model was adopted to examine the kinetic parameters in this study. The modified Gompertz model has been widely used to fit the experimental data from the batch study of anaerobic digestion [18]. The lag phase during the acclimatization of microorganisms in the inoculum was included in this model; thus, it could effectively describe the anaerobic digestion process [4]. The kinetic parameters for anaerobic digestion of HBR are presented in Table 3.

Table 3. The kinetic parameters for biochemical methane potential

Substrate	M (N mL/g VS $_{added}$)	Rm (N mL/g VS $_{added}$-d)	λ (d)	R^2	Reference
Hemp biomass residue (mix of leaves and inflorescences)	160.0	10.59	4.75	0.9930	This study
Hemp Hurds (Narlısaray)	114.6	8.4	1.57	0.961	Arıç et al., (2014) [19]
Hemp Hurds (Futura)	108.0	8.1	1.49	0.963	Arıç et al., (2014) [19]
Oil palm empty fruit bunches	183.2	54.81	5.71	0.985	Wadchasit et al. (2020) [20]
Garden waste (Guinea grass (*Panicum maximum*), lawn grass (*Zoysia japonica*) and broadleaf carpet grass (*Axonopus compressus*))	267.0	33.6	0.1	0.996	Edwiges et al. (2019) [21]

The modified Gompertz model fitted well with the experimental data of all inoculum with a high R^2 of 0.9930. From Table 3, it is clear that HBRs presented the highest methane production potential of 160.00 N mL/g VS $_{added}$. The maximum methane production rates also showed the same trend with methane production potential. Typically, the lag phase of AD of the carbohydrate-rich substrate could be from VFA inhibition during the early stage of the anaerobic digestion process [22]. The short lag phase during the start-up period of the anaerobic digester could enhance the benefit of AD systems and the efficiency of biogas production [22]. HBR showed quite a short lag phase of 4.75 days.

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4. CONCLUSIONS

The study of biochemical methane potential test from hemp biomass residue (*Cannabis sativa* L.) obtained from the initial raw material for hemp processing must undergo an oil extraction process to obtain hemp oil, resulting in residual material known as hemp biomass residue was carried out in 120-mL serum bottles. The controlled at 35 ± 1 °C, and the experiment was carried out for 45 days. The study found that TCOD, TS, and VS removals were 57.32 ± 2.61%, 42.59 ± 4.18%, and 47.21 ± 3.52%, respectively. Cumulative biogas and methane yield were 365.38 ± 16.72 and 162.97 ± 8.96 N mL/g VS_{added}. In addition, the maximum methane concentration was 71.64 ± 0.33%. Because of the above statement, future studies on HBRs could scaled up and study technically and economical both technical and economical feasibilities. In addition, other anaerobic digestion strategies such as co-digestion, different inoculum sources, and different SIR to increase biomethane production potential should also be evaluated.

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