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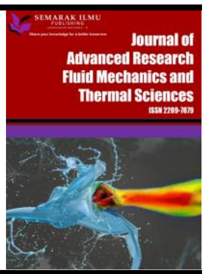

Potential of Biogas Production from Anaerobic Co-digestion of Hydrothermal Pre-treated OPEFB and Digested Sludge

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ABSTRACT

The palm oil industry plays a vital role in Malaysia, but it generates substantial amounts of biomass waste, which, if not properly managed, can lead to environmental pollution. Among these waste products, oil palm empty fruit bunch (OPEFB) is the most abundant and underutilized, being generated daily without any commercial value. The aim is to efficiently convert OPEFB into fermentable sugars for improved biogas production. Nevertheless, the challenge lies in efficiently degrading OPEFB within a short timeframe before fermentation into biogas can occur. This study aims to explore the potential of hydrothermal pre-treatment as a means to enhance biogas production from OPEFB. The pre-treatment process was optimized by varying reaction temperatures (100 to 250°C), and reaction times (10 to 40 minutes). The main objective was to maximize the total soluble sugar yield, a crucial precursor for efficient biogas production. The optimal conditions for hydrothermal pre-treatment are identified at a temperature of 175°C and a reaction time of 20 minutes, resulting in a yield of 49.2 mg of glucose per gram of OPEFB. Subsequent anaerobic co-digestion of the treated OPEFB with digested sludge increased methane yield to 100.53 ml/g-VS, representing a remarkable 392% increase compared to the low 20.44 ml/g-VS produced by untreated OPEFB. Nonetheless, elevated pre-treatment temperatures (200°C and above) led to reduced biogas production due to inhibitory compounds. This study demonstrates the effectiveness of hydrothermal pre-treatment as a low-temperature, green technology for enhancing OPEFB-based biogas production, contributing to Malaysia's renewable energy goals. Future research should focus on scale-up and validation of this integrated process.

1. Introduction

The Malaysian palm oil industry is a significant agricultural sector that has yielded substantial economic benefits. However, the generation and disposal of waste within this industry pose considerable environmental challenges. The waste produced from the palm oil extraction process

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can reach around 70-80 million tons annually, with approximately 25% of this waste originating from oil palm empty fruit bunches (OPEFB) [1]. OPEFB, a biomass generated daily in palm oil mills, has limited commercial value and a slow decomposition rate. Harnessing the potential of OPEFB within the agricultural sector could offer a solution to pollution issues and foster sustainable agricultural systems. Efforts are underway to transform this waste into valuable fuels and chemicals [2].

Due to its high moisture content of about 60%, OPEFB is unsuitable for use as boiler fuel due to issues such as fouling, corrosion, and incomplete combustion. These challenges reduce boiler efficiency and contribute to pollution [3], primarily by emitting greenhouse and hazardous gases, such as carbon dioxide, carbon monoxide, and nitrogen oxide, during the incomplete combustion process. Additionally, the presence of particulate matter in the emissions worsens air pollution, raising environmental concerns. Consequently, OPEFB is often either left to decompose naturally or utilized for mulching, serving as a soil conditioner. However, these practices can lead to pollution and pest attraction since OPEFB breaks down slowly owing to its high carbon-to-nitrogen ratio [4]. To address this, there is a growing interest in converting OPEFB into biogas, aligning with Malaysia's National Renewable Energy Policy and Action Plan to increase biogas production capacity by 2030 [5].

Numerous studies have explored OPEFB's potential as a renewable energy source, including its conversion into bioethanol, biobutanol, hydrogen, and organic fertilizer. Various methods such as hydrolysis, fermentation, gasification, pyrolysis, and anaerobic digestion have been employed in these investigations [6]. The anaerobic digestion of oil palm biomass stands out due to its economic and environmental advantages compared to conventional biodiesel and bioethanol conversion methods. This process employs anaerobic microorganisms that secrete enzymes to break down biomass and convert organic compounds into volatile fatty acids, subsequently transforming them into methane [7]. In light of the potential of anaerobic digestion for biogas production, a co-digestion approach is employed to enhance the process. Successful studies have been conducted on anaerobic digestion of oil palm biomass using various co-substrates like sewage sludge, animal manure, and food waste [8].

However, the high lignin content of OPEFB impedes the conversion of biomass into fermentable sugars [9]. Hydrothermal pre-treatment is proposed as a solution to break down the lignin-hemicellulose bond [10-11]. This process creates accessible surface area and carbon sources, enhancing enzymatic digestion and hydrolysis rates [12]. Hydrothermal pre-treatment involves the use of hot water to increase biomass biodegradability or facilitate the conversion into lignocellulosic sugar extract [13-14]. However, this process might generate inhibitory compounds that could affect microbial growth during anaerobic digestion.

This study aims to address these gaps by investigating the potential of anaerobic co-digestion using hydrothermal pre-treated OPEFB for biogas production. The study focuses on understanding how reaction temperature and time impact OPEFB hydrolysis into fermentable sugars and subsequent biogas yield.

2. Methodology

2.1 Sample Preparation

For this study, mill-processed OPEFB served as the raw material, obtained from Palm Oil Mill Seri Ulu Langat, Dengkil, Selangor, Malaysia (GPS Coordinates: 02.8507°N, 101.5501°E). The initially shredded OPEFB, collected with an approximate moisture content of 60%, was sun-dried for two days. Subsequently, the OPEFB was ground into loose fibers, sieved to obtain particle sizes between

0.15 and 0.50 mm, and further dried at 60°C overnight to minimize moisture content. It was then stored in an air-tight container until use.

For biogas production, digested sludge was collected from the sewage treatment plant in Shimodate, Ibaraki, Japan (GPS Coordinates: 36.2839°N, 139.9890°E) and stored at 4°C. The sludge was used as an inoculum for the co-digestion experiment.

2.2 Hydrothermal Pre-treatment

The hydrothermal pre-treatment process of OPEFB was investigated using a hot-compressed water system in a sealed stainless-steel reactor (OM LabTech Co., Ltd, Japan) with a volume of 200 ml. Each reaction comprised a mixture of 2.5 grams of dried sample, standardized to a particle size of 0.30 mm, and distilled water at a ratio of 1:20 w/v of solid to solvent. Reaction temperatures ranged from 100 to 250°C, with reaction times spanning between 10 and 40 minutes to identify the optimal soluble sugar content.

The heating rate to reach the desired temperatures varied based on the set temperature, typically within a standard range of 10 to 18°C per minute. The steam pressure inside the reactor adjusted correspondingly with the set temperatures. After the pre-treatment, the controlled cooling process involved circulating cooling water around the reactor jacket, ensuring a gradual decrease in pressure and temperature to cool the substrate to room temperature for subsequent use and analysis.

2.3 Anaerobic Co-digestion

Anaerobic co-digestion was carried out after hydrothermal pre-treatment of OPEFB under conditions yielding the highest total soluble sugars. The co-digestion involved mixing pre-treated OPEFB with anaerobic digested sludge as an inoculum. For the experiments, 100 ml glass bottles (Duran, Germany) were used, with approximately 45 ml of mixed pre-treated OPEFB (both solid and liquid) and 45 ml of digested sludge in each bottle. Untreated OPEFB was included as a control. Biogas volume was collected using 10 ml and 50 ml plastic disposable syringes and measured every 2 days, along with its composition using Gas Chromatography.

2.4 Analytical Methods

Total sugar content was analyzed using the phenol-sulfuric acid method. A small amount (0.1 ml) of the hydrolysate was mixed with 1.9 ml of distilled water, 1 ml of 5% w/v phenol, and 5 ml of concentrated sulfuric acid. The mixture was vortexed for 30 seconds. After a 10-minute incubation at room temperature, the absorbance of the mixture was measured at 490 nm using a UV-1800 Spectrophotometer (Shimadzu, Japan). Glucose was used as a standard as it is a major component of biomass.

For the analysis of biogas composition (methane, and carbon dioxide, hydrogen), gas chromatography with a thermal conductivity detector was employed (GC-8A, Shimadzu, Japan). A Porapak Q column with nitrogen as a carrier gas at a flow rate of 1 ml/min was used. Biogas samples (1 ml) were taken from the bottles and analyzed. The specific content of each biogas component was calculated using Eq. (1), where ‘Gas Composition’ represents percentage of each gas in the biogas mixture, 'Volume of Gas Collected' pertains to the measured volume of the resulting biogas, 'Volatile Solid' represents the percentage of volatile solids in the substrate, and 'Volume of Substrate' refers to the volume of the substrate used in the anaerobic digestion process, while 0.01 is a conversion factor of liter to millimeter. The biogas component is expressed as ml/g-VS. Standard gases (methane, carbon dioxide, and hydrogen) were separately injected into the equipment under similar conditions as mentioned above.

$$Biogas\ Component = \frac{(Gas\ Composition\ (\%) \times Volume\ of\ Gas\ Collected)}{(Volatile\ Solid\ (\%) \times Volume\ of\ Substrate \times 0.01)} \quad (1)$$

3. Result and Discussion

3.1 Physico-Chemical Properties of OPEFB and Sludge

Proximate and elemental analyses were conducted on OPEFB and digested sludge to determine their characteristics, which are crucial for their use in biogas fermentation. Table 1 presents the general physico-chemical characteristics of prepared OPEFB and digested sludge.

Table 1
Physico-chemical characteristics of prepared OPEFB and digested sludge

Analysis	OPEFB	Digested Sludge
Total solid (wt/wt%)	92.14	1.13
Volatile solid (wt/wt%)	3.91	70.14
Moisture content (wt/wt%)	7.85	98.23
Carbon (%, dwb)	42.62	34.27
Hydrogen (%, dwb)	6.09	6.27
Nitrogen (%, dwb)	1.72	5.87
*Oxygen (%, dwb)	49.57	53.59
pH	n.d	6.84

The proximate analysis of OPEFB revealed moisture, total solid, and volatile solid percentages of 7.85%, 92.14%, and 3.91%, respectively. Elemental analysis indicated that OPEFB had 42.62% carbon, 6.09% hydrogen, 1.72% nitrogen, and 49.57% oxygen on a dry weight basis. The resulting C/N ratio of 24.78 suggests OPEFB's suitability for effective biogas fermentation, aligning well with other commonly used biogas materials such as rice straw, palm pressed fiber, and corn stalks [15]. An excessively high C/N ratio impedes biogas production due to accelerated nitrogen consumption by acid-producing bacteria, while an excessively low ratio promotes rapid nitrogen utilization for growth, potentially elevating pH levels and adversely impacting biogas production [16].

For the inoculum, digested sludge was selected due to its reduced odor, lower pathogen risk, and improved dewatering properties compared to other sludge types [8]. The digested sludge, with 98.23% moisture and 1.13% total solids on a wet weight basis, contained volatile solids constituting 70.14% of the dry weight. Elemental analysis revealed carbon (34.27%), hydrogen (6.27%), nitrogen (5.87%), and oxygen (53.59%) on a dry weight basis, showcasing its potential contribution as an effective co-substrate for biogas production.

The high content of total solids in OPEFB and the substantial volatile solids in the sludge significantly contribute to the anaerobic co-digestion process. OPEFB is known to have a high total solid content, which consists of organic matter such as cellulose, hemicellulose, and lignin provide abundant organic matter, enhancing substrate availability for biogas production during co-digestion. Conversely, the elevated volatile solids in the sludge ensure a substantial concentration of biodegradable organic matter, fostering a favorable environment for methane generation [17].

3.2 Hydrothermal Pre-treatment of OPEFB on Total Soluble Sugar
3.2.1 Effect of reaction temperature and time

Figure 1 illustrates the total soluble sugars obtained from the hydrothermal pre-treatment of OPEFB at varying reaction temperatures and times. The results indicate a substantial increase in soluble sugars between 100°C and 175°C, suggesting cellulose and hemicellulose breakdown. Optimal conditions for maximum total soluble sugars were identified at 175°C and 20 minutes, yielding 49.2 mg of glucose per gram of dry matter. Similar trends were observed in the hydrothermal treatment of switchgrass by Basar *et al.*, [18]. This rapid delignification process exposes a significant portion of cellulose and hemicellulose for microbial conversion into biogas, rendering it a favorable precursor for biogas production.

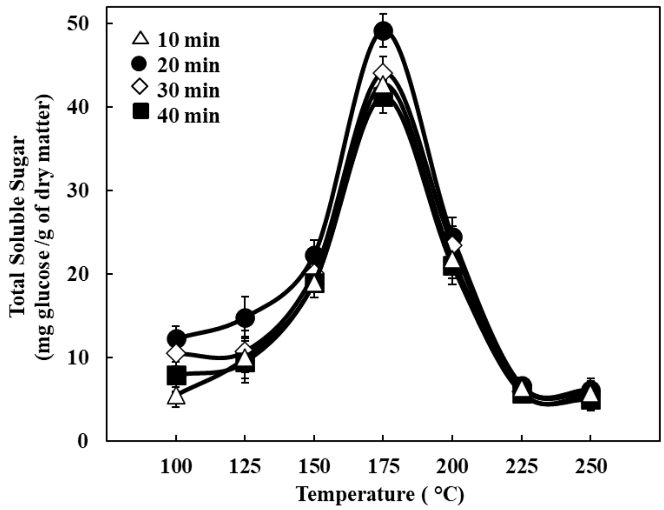

Fig. 1. Total soluble sugars recovered after the hydrothermal pre-treatment of OPEFB at various reaction temperatures and time

At temperatures exceeding 200°C, total soluble sugars began to decline to below 10 mg of glucose per gram of dry matter. This decrease could be attributed to further deterioration of sugars into other compounds like phenols and organic acids at higher reaction temperatures [19]. Moreover, the acidic nature of water at elevated temperatures might contribute to the breakdown of OPEFB into other compounds. This aligns with previous studies on hot-compressed water pre-treatment of OPEFB [20].

Prolonged reaction times of 30 and 40 minutes also led to a moderate reduction in total soluble sugars across all temperatures studied. The results may be attributed to the degradation of cellulose due to longer exposure to heat which was in line with the reports of Mariano *et al.*, [21] showing the adverse effect of excessive application of heat. Previous research by Ali *et al.*, [9] supports the need for optimizing reaction times to minimize the production of inhibitors and degradation-resistant compounds during hydrothermal pre-treatment. This suggests that higher reaction temperatures should be accompanied by shorter reaction times.

3.2.2 Biogas Production from Anaerobic Co-digestion

Anaerobic co-digestion of pre-treated OPEFB at various reaction temperatures (100 to 250°C) for 20 minutes was evaluated for biogas production, with untreated OPEFB used as a reference for comparison. Figure 2 illustrates the temporal profiles of biogas components (methane, carbon dioxide, and hydrogen) over a 10-day digestion period.

Figure 2 underscores the significant impact of hydrothermal pre-treatment temperatures on methane, carbon dioxide, and hydrogen production during the 10-days digestion period, highlighting the optimal conditions for enhanced biogas generation while emphasizing the limitations and challenges at elevated pre-treatment temperatures.

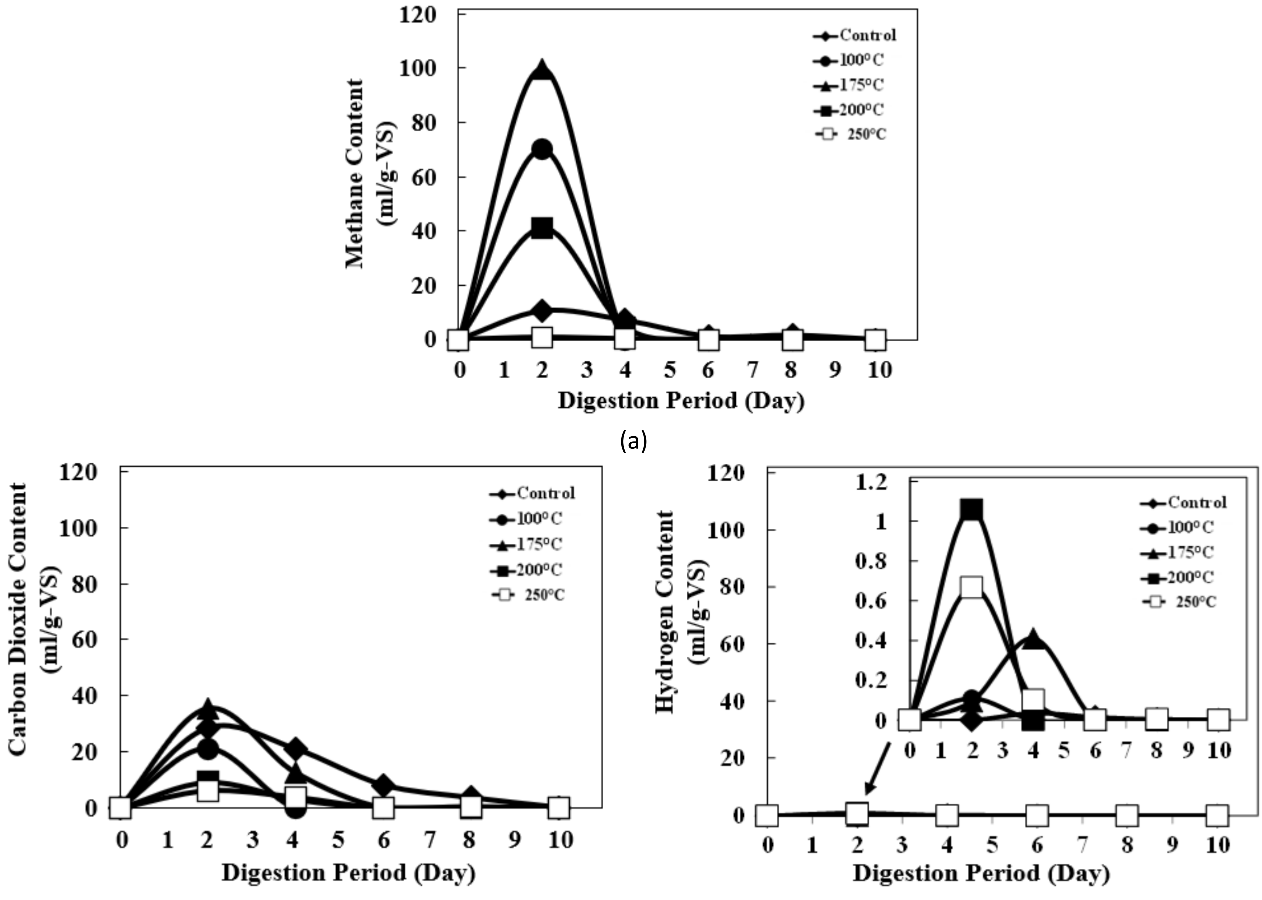

Fig. 2. Production of biogas components: (a) methane, (b) carbon dioxide, and (c) hydrogen for a digestion period of 10-days

The results indicate that methane synthesis during anaerobic digestion was dependent on the hydrothermal pre-treatment reaction temperature. In Figure 2(a), the highest methane production of 99.83 ml/g-VS occurred on day 2 of digestion using pre-treated OPEFB at 175°C and 20 minutes, highlighting increased methanogenic bacterial activity compared to untreated OPEFB. In total, methane production during the digestion period reached 100.53 ml/g-VS, representing a remarkable 392% increase compared to the low 20.44 ml/g-VS produced by untreated OPEFB.

However, methane production was significantly decreased for pretreatment above 175 ℃. This decline may be attributed to higher pre-treatment severity at elevated temperatures, causing substantial degradation of cellulose, hemicellulose, and lignin components, resulting in inhibitor concentrations surpassing tolerance thresholds [22]. Consequently, the growth of microorganisms essential for biogas generation was adversely affected. This finding aligns with the observations of Mustafa *et al.*, [23] who noted increased biogas production from sugarcane bagasse at hydrothermal treatment temperatures raised from 160°C to 180°C, but a considerable decrease above 180°C.

Furthermore, methane production stopped after 5 days for all pre-treatment conditions, likely due to volatile fatty acid accumulation, which lowered substrate pH and inhibited microorganisms responsible for carbon source conversion into biogas [24].

On the other hand, as shown in Figure 2(b), carbon dioxide, the second major biogas component, exhibited production ranging from 12.59 to 35.26 ml/g-VS for pre-treated OPEFB at 175°C, constituting 32% of total biogas produced. This aligns with Herout *et al.*, [25] suggesting that the carbon dioxide composition should fall between 20% and 35% of total biogas composition for efficient stripping and methane purification. Furthermore, excessive carbon dioxide composition of up to 50% in biogas generation may hinder the combustion process [26]. Meanwhile, Hydrogen production was relatively low for all substrates studied, as depicted in Figure 2(c). The maximum hydrogen production of 1.05 ml/g-VS was observed at 200°C of pre-treated OPEFB substrate after 2 days of digestion, accounting for up to 6% of the total biogas composition.

Overall, the results highlighted the significant impact of hydrothermal pre-treatment reaction temperature on biogas production. Severe pre-treatment conditions, such as high temperatures, should be avoided due to the potential for low biogas production resulting from toxic compound accumulation that inhibits microorganism growth [27]. Appropriate reaction temperature and time are crucial to enhancing methane yields compared to untreated biomass.

3. Conclusion

In conclusion, this study established optimal hydrothermal pre-treatment conditions at 175°C for 20 minutes, resulting in a significant yield of 49.2 mg of glucose per gram of OPEFB. The integration of this treated OPEFB with anaerobic co-digestion utilizing digested sludge showcased a remarkable 392% increase in methane production compared to untreated OPEFB. These findings underscore the potential of mill-processed OPEFB as a substantial and viable carbon source for enhancing biogas production.

Future investigations could focus on several promising areas, including mitigating inhibitory effects observed at higher pre-treatment temperatures, assessing the industrial scalability and feasibility of the process, and exploring biomass combinations or additives for enhanced biogas yields.

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