Biomethane potential laboratory-scale test (BMP) for the determination of biogas production from empty fruit bunches (EFB) derived during palm oil processing via co-digestion with palm oil mill effluent (POME)

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***Abstract*— The current thesis investigated biogas production from empty fruit bunches (EFB) as waste-to-energy valorization route using laboratory scale biomethane potential test (BMP), considering EFB pre-treatment and co-digestion with palm oil mill effluent (POME) as main strategies for enhancing methane yield. EFB pre-treatment using NaOH 0.1M at 80°C for 2 h with a recovery of 66.6% was carried out. $Ca(OH)_2$/Ash 60:40 (% wt.) was proposed for pH adjustment, leading to 4.82 ± 0.15 g used per litre of POME to reach a pH of 6.6 and 16.7% reduction on $Ca(OH)_2$ employed. Seven experiments were carried out during 22 days at mesophilic conditions and ambient temperature using the liquid displacement method to quantify CH_4 production. Lowest cumulative methane yield was 7.3 ± 0.3 mL CH_4/g VS for POME under facultative conditions while highest cumulative methane yield was 415 ± 34 mL CH_4/g VS for POME under anaerobic conditions. Alkaline pre-treatment showed no positive effect on biogas production from EFB and pre-treatment conditions should be reconsidered. Cumulative methane yield for co-digestion for POME and unpretreated EFB with a POME:EFB ratio of 6:2 in volatile solid (VS) basis was 369 ± 31 mL CH_4/g VS, equivalent to 46.5 m^3 CH_4 /ton of mixed EFB and POME, and simultaneous waste processing of 3.23% of total EFB and 4.98% to total ash produced on site via anaerobic co-digestion with a total solids and volatile solids removal of 81 ± 22% and 89 ± 18% respectively. Future research on pre-treatment parameters optimization, ash addition benefits for microbial community in anaerobic systems, and EFB:POME ratio evaluation will maximize methane yield.**

Keywords: Biogas, Methane Yield, Pre-treatment, Co-digestion, Palm Oil Mill Effluent (POME), Empty Fruit Bunches (EFB).

I. INTRODUCTION

World palm oil production has been increasing during the last 30 years starting at a value of 81.7 million tonnes for 1994 and reaching a value of 410.7 million tonnes for 2019, showing an increment in 403% during considered timespan [1]. Colombia production during the same period of time exhibited a similar behaviour for palm oil production, raising from 1.68 million tonnes in 1994 to 8.39 million tonnes in 2019, resulting in an 400% increment which is comparable to world palm oil production [1]. Colombia average palm oil production between 1994 and 2019 was 3.9 million tonnes per year, reaching 5th place in the world with Indonesia (104.8 million tonnes/year), Malaysia (74.4 million tonnes/year), Nigeria (8.3 million tonnes/year), and Thailand (7.6 million tonnes/year) as top four oil palm producers globally [2]. Regarding oil palm production area in the world for 2019, top five in thousands of hectares are: Indonesia 12780 (56.1%), Malaysia 5200 (22.8%), Thailand 920 (4.0%), Nigeria 525 (2.3%), and Colombia 486 (2.1%) [3]. As top five producer, Colombia has an important role in palm oil market.

During palm oil production, several liquid and solid waste streams are produced. During first step, fresh fruit bunches are sterilized and stripped obtaining fresh fruits and Palm Oil Mill Effluent (POME) and Empty Fruit Bunches (EFB) (22%) as waste streams. Fresh fruits are transformed into palm oil and press cake by means of digestion, clarification and pressing processes with POME as waste stream (Total: 67% combined with previous step). Then, press cake goes to depericarping step producing nut and fibre (13.5%) as solid waste stream. Last but not least, nuts are cracked to obtain kernel (6%) and shell (5.5%) as waste stream. To sum up, liquid waste stream produced are POME while EFB, fibre, and shell are solid waste streams [4].Other waste streams that could be considered are fronds, leaves, and trunks, which are produced on the field, and ash which is produced during fibre and shell combustion in the boiler [5].

Waste management for residues generated in palm oil mill (POM) are mostly focused on a waste-to-energy concept [6]. For POME, a wastewater treatment system with biogas production is normally considered while for fibre and shell are usually employed as solid fuel for the boilers [6]. However, EFB has not been considered in this approach mainly for its high moisture content which limits the application as solid fuel and high potassium content from EFB could lead to fouling and slagging problems in the boiler, limiting heat transfer in the boiler and increasing maintenance schedule [7] [8]. For these reasons, current uses in Colombia for EFB are mulching/soil amendment and composting [9] which are valorisation routes with no value-added products or energy production for a waste that reached 1.56 million ton EFB/year for 2019. Considering that Colombia's palm oil production was around 2% of world production for 2019 [1], it is estimated that 78 million ton EFB per year were generated in 2019 globally.

Figure 1 summarizes proposed approach for this research, where three waste streams are processed simultaneously leading to value added products (digestate) and energy generation via biogas production. No reports were found connected with biogas production utilizing these three waste types at the same time.

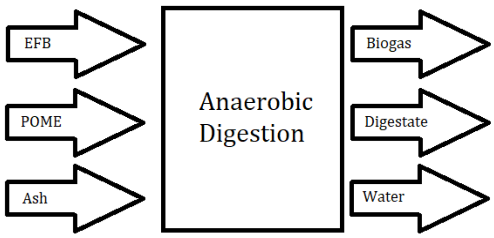

Figure 1. Waste-to energy valorisation routes via bigas production for research approach.

The current thesis is precisely motivated by waste-to-energy valorization route for EFB, which is non-existent in industrial scale, with an astonishing potential due to current uses in Colombia. In particular, the objective is to assess the biogas production as alternative use for EFB, considering the effect of alkaline pre-treatment and co-digestion with POME. The study aims at determining the conditions where the biogas production is maximized by means of laboratory-scale biomethane potential test (BMP).

To perform this research, section II comprised a state-of the-art literature review with current and promising uses for EFB, biogas technology and biomethane potential test (BMP) description, and biogas production from POME and EFB. Subsequently in section III, materials and methods were described. Sampling for EFB, POME, ash, and inoculum as well as characterization employed for each starting material was included. Alkaline pretreatment for EFB and pH trials for ash procedures were described. Fixed and study variables (pre-treatment and co-digestion) were defined for biogas production experimental design (7 experiments) as well as experimental set-up details and methane yield calculations. Results and discussion were collected in section IV. Data collected from material characterization, alkaline pre-treatment of EFB, and pH test for ash was compared to literature. Then, biomethane potential test (BMP) results were evaluated considering experimental set-up, biogas production from POME and EFB and the influence of alkaline pre-treatment and co-digestion on enhancing methane yield from EFB. Conclusions from this work and in connection with section IV were outlined in section V.

II. State of Art Literature Review

A. *Promising uses for EFB*

EFB (empty fruit bunches) is a non-wood lignocellulosic residue from palm oil processing and their average chemical composition is described in Table I [6]. EFB is mainly composed of cellulose and hemicellulose with a low lignin content (ca.15%). In addition, EFB has a high water content (up to 70%) which should be taken into account for technologies where water is removed prior to processing. Its high content of potassium makes EFB a good nutrient source when fertilizing options (i.e., mulching, composting) are considered. Since it is estimated that the amount of crude palm Oil (CPO) (18 - 24% wt.% of fresh fruit bunches (FFB)) is almost as the amount of EFB produced (18 - 24% wt.% of FFB) [7], and the total production of CPO in Colombia is 1.56 million tonnes of CPO per year [3], it is estimated that total production of EFB is 1.56 million ton EFB/year for 2019.

TABLE I.
EFB characterization [6]

Component	Mean	SD
Lignin (%)	15	8.9
Cellulose (%)	43	15.1
Hemicellulose (%)	21	6.3
Moisture (%)	36	28.8
Ash (%)	5.7	3.5
Volatiles (%)	80	5.8
C (%)	47	4.2
N (%)	0.6	0.4
S (%)	0.4	0.4
Mg (mg/kg)	913.5	-
P (mg/kg)	572.7	-
K (mg/kg)	5574.0	-

*SD: Standard deviation

Several technologies have been evaluated to convert EFB into fuels, energy carriers, or as a direct source of energy such as direct incineration, pelletizing [10] [11] [12], bioethanol [13] [13] [14] [15], bio-oil [16] [17] [18], biochar [19] [20], and hydrogen/syngas production [21] [22].

The direct use of EFB in combustion processes is not carried out and even banned in countries such as Indonesia and Malaysia [23] mainly due to heavy pollution effects. It is important to note that high content of water significantly reduces its high heating value (8.2 kJ/kg) compared to kernel shell (21.4 kJ/kg) and fiber (19.2 kJ/kg), which are residues that feed the boilers in Palm Oil Mills (POM) [7]. Additionally, high potassium content from EFB could lead to fouling and slagging problems in the boiler, limiting heat transfer in the boiler and increasing maintenance schedule [8]. Main target of pelletization is to reduce moisture content, which is energy intensive for high water content starting material and produced EFB pellets showed high ash content (2-5%). Current drawbacks for bioethanol production from EFB are low product yield, high recovery cost, and high CAPEX and energy consumption mostly connected with pre-treatment step. Barriers for bio-oil technology implementation are the wide variety of compounds present in the bio-oil, pilot scale research is limited, and high investment is required for pyrolysis process.

There are still few reports for biochar production from EFB, drying step is required and overall process is capital intensive. Hydrogen and syngas from biomass are produced by gasification [24] and for gasification, low moisture content (<10 wt.%) is required for the starting material as well as particle size reduction (between 0.3 mm and 1.0 mm). High initial investment is also mandatory for gasifier which could be considered as the main barrier for the process and further study is required to determine optimal conditions for maximizing hydrogen and syngas from EFB.

EFB in Colombia is mostly returned to the agricultural field as mulch/soil amendment (63.8%), transformed into compost (24.8%) or other uses such landfill disposal (11.4%) [9]. Agronomic use requires no pre-treatment of EFB and it is an inexpensive alternative for EFB disposal and use, providing nutrient recycling via slow decomposing of material on the field. However, phytosanitary issues connected with fly Stomoxys calcitrans multiplication in EFB disposed on field were reported in specific regions in Colombia, this implied additional solutions to this problem such as use of Sphalangia sp as parasite for fly control, fly traps, and plastic coverage to avoid fly multiplication, leading to cost increment due to materials and workforce employed for this purpose [25], as well as transportation cost for a material with high moisture content. Regarding composting of EFB [26] [27] [28] [29], the use of heavy machinery on a regular basis for aeration during composting and lack of market opportunities for final low-cost product are considered the main barriers for this alternative. Research reports showed different products derived from EFB: bio-composites, cellulose pulp and paper, bio-plastics, food for ruminants, enzyme production, chemicals via catalytic routes, among others [6]. Most of these products are still in early stages of development to be considered for industrial scale.

B. Biogas technology

Biogas production from EFB as alternative method of its utilisation exhibits several advantages when compared to the use and technologies described above:

Mild temperature (35-55°C) and pressure (ambient pressure) conditions, moisture removal not required to process EFB via anaerobic digestion., low capital investment compared to other technologies that require expensive reactors to handle extreme temperature and pressure, mature technology with presence in 7 out of 69 palm oil mill plants in Colombia in the form of methanogenic covered lagoons to process POME [3], self-sufficiency for energy consumption and energy excess production sold to the grid or near-by towns by 2 palm oil mill plants in Colombia [3], helping rural economy since most of these plants are located in regions with no connection to the national grid and production of digestate as by-product, promoting circular economy and nutrient recycling since anaerobic sludge could be used as fertilizer in the palm oil plantation or other farms. The main drawbacks for the process are low biogas production from EFB (0.20 m3 CH_4/ kg VS) [30] due to its lignocellulosic nature and large space/reactor requirements for biogas production.

Biogas is the main product from anaerobic degradation of organic matter and it is composed mainly of methane (45-70%), carbon dioxide (24-40%), and small amounts of other compounds (N_2, H_2, O_2, H_2S), where hydrogen sulfide concentration is relevant considering an upgrading step or further use [31]. The composition of biogas depends on the technological process and substrates used. Digestate is the solid and liquid product obtained from anaerobic digestion of organic matter [32]. Most of the elements different from carbon, nitrogen, oxygen, and sulphur from the original feedstock are concentrated and mineralized from the organically-bound nutrients in this by-product, making it a suitable fertilizer. The composition of the digestate is variable and it depends on the type of feedstock used since the chemical properties of the feedstock will be preserved in the resulting digestate, and the design of the digester. The recommendation is the evaluation of this organic fertilizer for each feedstock and biodigester case.

To determine biogas production on laboratory scale, biomethane potential test (BMP) is normally carried out [33]. Critical experimental parameters such as inoculum to substrate ratio (ISR), substrate concentration, temperature, blank control, reactor size, headspace, mode of mixing, and pH should be clearly defined and reported. pH adjustment is required since measured pH for POME ranges from 3.3 to 4.6 and low pH condition resulted in increased specific decay rate of methanogenic archaea 10 times more in a low pH environment (e.g. pH 5.1) compared to neutral pH (pH 7.0) [34]. For this purpose, oil palm ash addition derived from shell and kernel fed boiler. Using ash provided additional benefits such as savings in typically employed alkali chemicals for pH adjustment (i.e. slaked lime, lime, and sodium bicarbonate) and addition of alkali and trace metals (i.e. Fe and Co) that were relevant for increased number of methanogenic microorganisms and enzyme activity for biogas production [35].

C. Biogas production from EFB and POME

To overcome low biogas production from EFB, promising solutions such as pre-treatment, co-digestion, and a combination of both have been reported and are discussed.

Kim et al [36] evaluated biogas production from EFB and POME using co-digestion. Experimental conditions were mesophilic temperature (35°C) and EFB:POME ratio ranging from 0:1 to 1:1 in COD basis. For 40 days experiment, methane yield for EFB and POME solely were 221 mL CH_4/g COD and 301 mL CH_4/g COD respectively while the highest methane yield was 450 mL CH_4/g COD for EFB:POME ratio of 1:1 in COD basis leading to 1.5 times more methane produced compared to POME production and 2 times more compared to EFB production.

Co-digestion of EFB and POME under thermophilic conditions (55°C) was tested by O-thong et al [37]. Three different pre-treatment for EFB were analyzed: chemical (NaOH 1 wt %), hydrothermal (steam 230°C), and a combination of chemical and hydrothermal treatment. The best methane yields after 45 days for POME and EFB separately were 503 mL CH_4/g VS and 202 mL CH_4/g VS respectively. When EFB:POME ratio (in g VS basis) was evaluated in the range (0.4:1 to 11:1), the best methane production was 340 mL CH_4/g VS for 0.4:1 ratio. The authors evaluated EFB:POME ratio of 6.8:1 for pre-treatment experiments due to biomass availability in real conditions, obtaining a maximum of 392 mL CH_4/g VS methane yield using chemical and hydrothermal combination as pretreatment, high organic load (46 g VS/L) compared to similar studies, and 91% biodegradability. From this result, it was estimated a methane production of 82.7 m^3/ton mixed EFB and POME.

Co-digestion of EFB and POME was analyzed under facultative conditions (no inoculum added and air medium in the reactor) [38]. After 14 days and ambient temperature close to mesophilic conditions (27-30 °C), methane production from POME with a starting pH of 4.8 was 0.3668 mL CH_4/g VS while maximum methane yield was 0.5932 mL CH_4/g VS for a combination of 24 %w/w EFB and 76 %w/w POME (C/N ratio of 45) and starting pH of 5.7. Co-digestion resulted in 1.6 times more methane production compared to POME, however, when compared to methane production under anaerobic conditions, 392 mL CH_4/g VS [37] , methane yield was 660 times lower for facultative production.

Biogas production from EFB and POME via co-digestion was evaluated by Liew et al [39]. Alkaline pretreatment of EFB, mesophilic (35°C) and thermophilic (55°C) temperature conditions, and co-digestion in 35-day experiments were analyze in this research. Considering pretreatment effect on co-digestion with POME, it was observed that methane production was higher for untreated EFB compared to treated POME at EFB:POME ratio of 0.3:1 and 0.45:1 on COD (chemical oxygen demand) basis, regardless of the temperature. The authors concluded that pre-treatment employed should be reconsidered since no significant effect on biogas production was obtained. For mesophilic temperature and thermophilic temperature, maximum methane yields were 60.00 and 74.02 mL CH_4/g VS respectively (2.36 times more than methane production from POME), leading to a COD removal of 77% for both cases after anaerobic digestion. Maximum methane production was obtained for a EFB:POME ratio of 0.6:1 in COD basis or a C/N ratio of 10.72.

Co-digestion of EFB and POME was evaluated by Saelor et al [40] taking into consideration EFB particle size and EFB:POME ratio as main variables for the study at mesophilic conditions (37 °C). Particle size and EFB/POME VS concentration were evaluated between 0.5-6 cm and 2-10 g VS/L respectively. Highest methane production achieved was 52 m^3 /ton biomass, corresponding to a cumulative methane production of 282 mL CH_4/g VS and 40% biodegradability for 6:2 EFB mixing ratio and 3.3 cm EFB particle size.

Solid-state co-digestion of EFB and palm oil decanter cake (DC) was investigated by Tepsour et al [8]. Oil palm ash generated from combusting fiber and shell residues in a boiler was added to adjust pH, making the process suitable to include three different solid waste streams (EFB, DC, and ash). In mesophilic conditions (35°C), dried EFB produced 353.0 mL CH_4/g VS while co-digestion with DC at EFB:DC ratio of 1:1 (VS basis) and 5% ash addition produced 414.4 mL CH_4/g VS, showing a 17.4% biogas production rise for co-digestion.

Ali et al [41] reported an increment of 63% in biogas production when POME is co-digested with EFB changing from 152 L cumulative biogas for POME to 248 L cumulative biogas for EFB after 15 days in 50 L batch reactor at mesophilic conditions. However, relevant data such as volatile solids concentration, mixing frequency, starting pH, and EFB:POME ratio were missing in this research.

Singkhala et al [34] worked with biogas production from POME considering pH adjustment by means of effluent recycling or oil palm ash addition derived from shell and kernel fed boiler. Using ash provided additional benefits such as savings in typically employed alkali chemicals for pH adjustment (i.e. slaked lime, lime, and sodium bicarbonate) and addition of alkali and trace metals (i.e. Fe and Co) that were relevant for increased number of methanogenic microorganisms and enzyme activity for biogas production [35]. Methane yield after 45 days at thermophilic conditions (55°C) for raw POME (starting pH 4.3) was 132 mL CH_4/g VS. The best results achieved for methane production considering biogas effluent (20% v/v, starting pH of 6.5) and ash (%5 w/w, starting pH 6.6) addition separately were 351 mL CH_4/g VS and 440 mL CH_4/g VS respectively, reaching 3.3 times more methane production with the ash addition compared to raw POME. Furthermore, highest removal chemical oxygen demand (COD) was 94% for 5% w/w ash addition.

Nieves et al [30] studied alkaline pretreatment of EFB with high concentration of NaOH and acid pretreatment with H_3PO_4 in order to improve biogas production. Due to alkaline pretreatment, a reduction of cellulose crystallinity and partial solubilization of hemicellulose and lignin were found. After 30 days at thermophilic conditions (55°C), biogas production increased from 0.20 m^3 CH_4/ kg VS for untreated EFB to 0.28 m^3 CH_4/ kg VS for acid treated EFB. The best results were obtained for NaOH pretreatment during 60 min, 0.404 m^3 CH_4/ kg VS, which means 100% improvement in terms of methane yield and 97% of theoretical yield based on carbohydrate content. The authors estimated that energy production from pretreated EFB could reach 11700 MJ/ton EFB.

III. EXPERIMENTAL

A. *Material sampling and characterization*

5 kg of pressed EFB was taken from Alianza del Humea SA, Cabuyaro, The EFB was cut using scissors and dried at 105°C in the oven for one day to avoid fungi growth and microbial degradation. The dried EFB was sieved using a 19 mm mesh size test sieve. The sieved EFB was stored in plastic bags at ambient temperature until use. 6L of POME, 8L of inoculum (sludge from methanogenic lagoon), and 1 kg of ash were taken from Unipalma SA, Veracruz. The methanogenic system comprises two parallel covered lagoons with a 20,000 m^3 capacity each and 4.5 m depth. POME and inoculum were stored in sterilized containers at 4°C. Wetted ash was collected from the main boiler which is fed with mesocarp fiber and palm kernel shell before

use. Wetted ash was dried at 105°C in the oven for one day. Dried ash was stored in plastic bags at ambient temperature until use. The inoculum was stored at ambient temperature for seven days before the experiment to reduce background methane production from remaining organic matter in the sludge.

Table II summarizes analytical methods employed for characterization of starting materials. In addition, total solids, volatile solids, and pH were measured before and after anaerobic digestion in order to evaluate organic load removal. All parameters were measured in triplicate.

TABLE II.
CHARACTERIZATION METHODS AND PARAMETERS FOR COLLECTED MATERIALS [6]

Parameter	Method used*	EFB	POME	Inoculum	Ash
Total solids (TS)	APHA 2540B	x	x	x	
Volatile solids (VS)	APHA 2540E	x	x	x	
pH	APHA 4500H		x	x	x

* [42]

B. Alkaline pre-treatment for EFB

Considering pre-treatment suggested in literature [43], 17.2 g EFB (16 g EFB dry basis) were mixed with 182 g NaOH 0.1 M solution. The mixture was heated at 80°C for two hours at ambient pressure. After this time, the mixture was filtered and solid fraction was blended with distilled water for 30 seconds to ease washing. The previous step was repeated until pH 7 was reached. Pretreated EFB was then sun dried and stored in plastic bags before use.

C. pH tests for ash

In addition to pH determination for ash, it was determined the amount of ash required to increase the pH of POME. To do so, 40 mL or 50 mL POME were combined with ash with constant pH reading until the pH was 6.6, as it was shown in literature that biogas production using 6.6 as starting pH maximizes biogas production [34]. The ash mass added for this purpose was obtained by subtracting initial and final mass of ash container. The result is reported in g ash/L and carried out in duplicate.

D. Biogas production experimental design

Table III collects all fixed variables for BMP considered in this research.

TABLE III.
FIXED VARIABLES FOR BIOGAS PRODUCTION FROM EFB AND/OR POME

Fixed variable	Value	Notes
Temperature (°C)	Ambient	Max. and min. temperature is recorded
Inoculum:substrate ratio (ISR) (g VS)	1	
Substrate concentration (g VS/L)	8	
Working volume (mL)	300	
Headspace (%)	40	
Mixing (rpm)	500 for 2 minutes every two days	Vortex is employed
Starting pH	6.6	For POME and co-digestion experiments
EFB:POME ratio (g VS)	2:6	For co-digestion experiments

* g VS: Volatile solids mass expressed in grams

Two parameters were studied in the experiment: The effect of EFB pre-treatment and codigestión with POME. A total of seven experiments were proposed and described in Table IV. For experiment 1, blank experiment is considered combining inoculum and water. Experiment 2 is with POME only without the addition of inoculum. In the case of experiment 3, POME and inoculum are evaluated. For experiment 4 and 6, untreated EFB biogas production is assessed using EFB alone and codigestión with POME respectively. Similarly, in experiments 5 and 7, pre-treated EFB is employed without and with addition of POME respectively. All experiments were carried out in triplicate.

TABLE IV.
EXPERIMENT DESIGN FOR PRE-TREATMENT AND CO-DIGESTION EVALUATION

Experiment	Substrate			
	Water	*EFB*		*POME*
		Unpretreated	*Pre-treated*	
M1(blank)	x			
M2				x(no inoculum)
M3				x
M4		x		
M5			x	
M6		x		x
M7			x	x

E. Experimental set-up

A total of 21 batch experiments were carried out under ambient conditions near mesophilic temperature (24°C to 35°C) using the following set of elements per set-up: two 500 mL glass bottles, one for 2% NaOH alkaline solution with phenolphthalein as basic indicator and the other for biogas production, one intravenous catheter 14G x 45 mm, one intravenous catheter 24G x 19 mm, one 3-way stopcock, two butyl rubber stopper, one solution administration set, and one 250 mL graduated cylinder. All elements are assembled as shown in Figure 2.

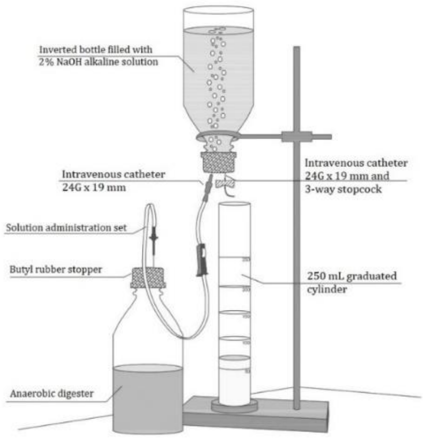

Figure 2. Experimental equipment for biogas production using liquid displacement method (Adapted from [44]).

To each digester were added substrate (EFB, POME, or both), water and inoculum, to reach a concentration of 8 g VS/L and an ISR of 1 expressed in g VS. After mixing, pH was adjusted to 6.6. To ensure anaerobic conditions, reactor was purged with N_2 for 5 minutes. The reactor was then sealed with a butyl rubber stopper and connected to the gas collection system using the solution administration set and intravenous catheter 24G x 19 mm.

F. Biogas production measurement

The methodology employed for methane quantification is liquid displacement method, where the biogas produced is collected in an inverted bottle with 2% NaOH alkaline solution where CO2 and H2S reacts completely to remove these gases from the biogas [45] and the amount of solution displaced to the graduated cylinder is the volume of CH_4 produced. For 30 days, the volume collected was checked daily and anaerobic digester was stirred at 500 rpm for 2 minutes using vortex every two days to ensure proper mixing and ease gas release from the medium. Gas and liquid leakage were evaluated twice per day and maximum and minimum temperature were recorded on a daily basis.

Cumulative volume is expressed at standard and pressure (STP) conditions (P = 1 atm, T = 273.15K) using (1):

$$V\ (STP, mL) = \frac{V_1 P_1 T_2}{P_2 T_1} \quad (1)$$

Where P2 and T2 were STP conditions and P1, T1, and V1 were local atmospheric pressure (0.995 atm), average experimental temperature for biogas production (273.15 + average T K), and methane volume at local conditions (mL) respectively [34]. The methane yield is the calculated using e (2). to be comparable to different literature reports:

$$Methane\ yield\ \left(mL\frac{CH_4}{gVS}\right) = \frac{Cumulative\ volume\ CH_4(mL)}{Starting\ volatile\ solids\ mass\ (g\ VS)} \quad (2)$$

IV. RESULT DISCUSSION

A. Material characterization

Total solids (TS), volatile solids (VS), and pH were measured for different starting materials and collected in Table V.

TABLE V.
CHARACTERIZATION PARAMETERS FOR THE COLLECTED MATERIALS

Parameter	**POME**	**Inoculum**	**EFB**	**Pre-treated EFB**	**Ash**
Total solids (TS) (%)	3.20 ± 0.07	6.36 ± 0.12	93.11 ± 0.20	95.02 ± 0.36	-
Volatile solids (VS) (%)	2.67 ± 0.05	3.33 ± 0.05	90.20 ± 0.24	93.94 ± 0.40	-
pH	4.04 ± 0.04	7.54 ± 0.01	-	-	10.67 ± 0.03

VS concentration for POME, EFB (untreated and pre-treated), and inoculum were employed to calculate the amount of each component to be added to a respective biogas experiment based on substrate concentration and inoculum to substrate ratio (ISR).

B. Alkaline pre-treatment

During alkaline pretreatment, an additional analysis for losses and water use was performed taking into consideration mainly the washing step. 2. Water consumption during washing step was about 2 liters of water for the starting mass of 16 g EFB. The concentration of total solids in this water was 2.43 g/L, representing 4.72 g of total solids in the resulting water, which is the main source of losses during the whole process, leading to a 66.6% recovery for the total process and a 5.34 g loss during the complete pre-treatment. These losses could be associated with lignin and hemicellulose partial solubilization [30] as well as solids that are below the filter pore size and should be considered for future solids recovery from water stream and decision-making regarding suitable wastewater treatment for this effluent.

C. pH tests for ash

Preliminary tests were carried out to determine the amount of ash required to increase pH in POME to reach a value of 6.6, which showed the best performance for biogas production based on literature [34]. It was required 94.80 ± 0.19 g ash per liter of POME. Considering that for a real palm oil mill (e.g. Unipalma), average flow rate of POME is 4 L/s and since it is required 95 g ash/L POME, calculations showed that the ash requirement on site for pH adjustment is 985 ton ash/month which clearly exceeds the produced amount of 400 ton/month, i.e. only 40% of this amount is covered with current ash production.

To overcome this issue, it was proposed to combine slaked lime ($Ca(OH)_2$) with ash in a proportion of 60 %wt. for $Ca(OH)_2$ and 40 %wt. for ash. An additional benefit is to promote a simpler transition from traditional chemicals, such as lime or slaked lime, for pH adjustment to an environmental-friendly and inexpensive option such as ash. pH measurement in water is higher for the proposed mixture (12.68 ± 0.06) compared to ash (10.67 ± 0.03) and it is almost the same pH value for slaked lime solely (12.63 ± 0.13). For pH adjustment of POME using mixture, it was required 4.82 ± 0.15 g mixture /L POME to increase pH over 6.6, whereas for $Ca(OH)_2$ the amount required is 3.47 ± 0.12 g/L POME. The mixture amount was 19.7 times lower compared to ash (94.80 ± 0.19 g/L POME) and 16.7% slaked lime consumption was diminished considering slaked lime alone (3.47 g/L POME). For these reasons, slaked lime/ash 60:40 mixture was employed for pH adjustment for biogas production in this thesis. Additional research should be carried out to evaluate the positive effect of ash addition on microbial community for biogas production from EFB and POME.

D. *Experimental set-up*

During preliminary tests, it was determined that the experimental set up proposed in literature showed a limited space for mixing, instability to support glass bottles containing alkaline solution, and longer times to refill these bottles. In order to improve these issues a different set up was proposed where alkaline solution was hanged using a plastic net. Figure 3 showed modified set up. After testing the set-up modification, mixing and refilling operation were simplified, and better stability for the bottles was observed.

Figure 3. Modified experimental set up.

Table VI summarized initial conditions for each proposed biogas production experiment. For those experiments where POME was not included (M1, M3, and M4), pH adjustment was not carried out. Inoculum concentration was always 8 g VS/L except for M2 experiment.

TABLE VI.
CHARACTERIZATION PARAMETERS FOR THE COLLECTED MATERIALS

Experiment	EFB:POME concentration (g VS/L)	Initial pH	pH after adjustment	Ash/$Ca(OH)_2$ concentration (g/L)
M1	0:0	7.54 ± 0.01	7.54 ± 0.01	NA
M2	0:27	4.06 ± 0.15	6.60 ± 0.04	13.15 ± 3.52
M3	0:8	5.88 ± 0.30	6.62 ± 0.02	3.75 ± 2.55
M4	8:0	7.47 ± 0.01	7.47 ± 0.01	NA
M5	8:0	7.61 ± 0.03	7.61 ± 0.03	NA
M6	2:6	6.26 ± 0.02	6.60 ± 0.07	0.92 ± 0.26
M7	2:6	6.25 ± 0.35	6.61 ± 0.04	1.13 ± 0.67

* NA: Not applicable, pH adjustment is not required

E. *Biogas production from EFB and POME*

For 22 days, cumulative methane production and cumulative methane yield were collected in Table VII.

TABLE VII.
SUMMARY OF BIOGAS PRODUCTION

Experiment	Brief description	Cumulative methane production (mL CH_4)	Cumulative methane yield (mL CH_4/g VS)
M2	POME, no inoculum	58 ± 2	7.3 ± 0.3
M3	POME	997 ± 81	415 ± 34
M4	Untreated EFB	310 ± 23	129 ± 9
M5	Pre-treated EFB	179 ± 23	74 ± 10
M6	Untreated EFB:POME	885 ± 75	369 ± 31
M7	Pre-treated EFB:POME	807 ± 69	336 ± 29

Cumulative methane yield for POME in absence of inoculum is the lowest among all experiments. Nurliyana et al [38] reported a maximum value for methane yield equal to 0.5932 mL CH_4/g VS which is 12.3 times lower than the methane yield in this research. The main difference between both procedures is starting pH since in literature the starting pH value was equal to 5.6 while starting pH for this experiment was 6.6 and working in the pH between 6.5-7.5 is recommended for appropriate methanogenic process [46].

For biogas production in experiment M3 (POME), the obtained result (415 mL ± 34 CH_4/g VS) was comparable to cumulative methane yield for adjusted POME in similar conditions (440 mL CH_4/g VS) [34]. Regarding methane yield for untreated EFB (experiment M4), the experimental value (129 ± 9 mL CH_4/g VS) was close to the untreated EFB methane yield range reported in literature (130 – 200 mL CH_4/g VS) [30].

Figure 4. shows cumulative methane yield evolution as a function of time, respectively. Cumulative methane yield from day 8 to day 22 in descending order is: M3 (POME) > M6 (Untreated EFB:POME) > M7 (Pre-treated EFB:POME) > M4 (Untreated EFB) > M5 (Pre-treated EFB) > M2 (POME, no inoculum).

The removal of total solids ranged from 63 ± 28 to 83 ± 15% and for volatile solids the removal ranged from 74 ± 30 to 90 ± 13%, showing high removal efficiencies during anaerobic digestion and additional benefits when considering organic load reduction as wastewater treatment. Final pH ranged from 6.67± 0.09 to 7.30 ± 0.06 was between the optimal pH range for biogas production which is between 6.5 and 7.5 [46].

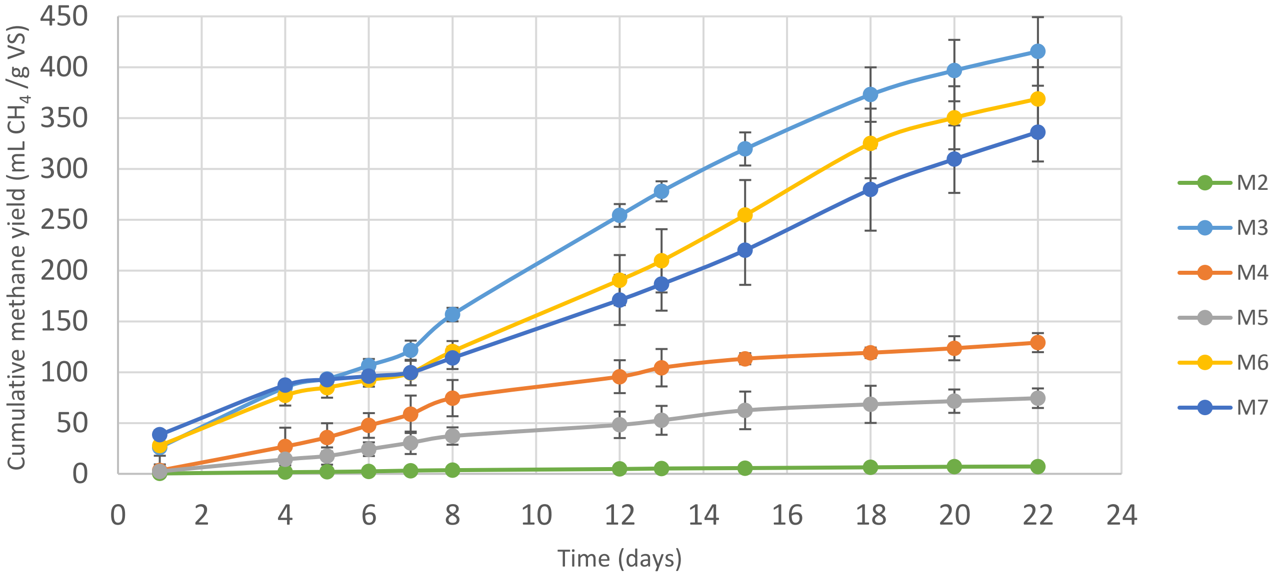

Figure 4. Cumulative methane yield from POME and/or EFB

F. Pre-treatment effect on EFB anerobic digestion

The cumulative methane yield for untreated EFB and pre-treated EFB were 129 ± 9 mL CH_4/g VS and 74 ± 10 mL CH_4/g VS, respectively, showing that methane yield for pre-treated EFB was 42.6 % lower. This behavior was also detected for co-digestion experiments where cumulative methane yield for untreated EFB was 369 ± 31 mL CH_4/g VS while for pre-treated EFB was lower (336 ± 29 mL CH_4/g VS). This result was similar to the biogas production from EFB under mesophilic conditions [39], where methane yield for untreated EFB was always higher compared to pre-treated EFB. Both results could relate to pre-treatment conditions that were not suitable for biogas production due to mild conditions (e.g., 80°C). Nieves et al [30] used a more concentrated NaOH solution (8%), higher temperature (100°C), and lower EFB particle size (<0.420 mm) reaching a methane production two times higher when compared with untreated EFB. Therefore, proposed alkaline pre-treatment conditions should be redefined to enhance biogas production and, at the same time, balancing costs regarding the use of more concentrated NaOH solution, higher temperature or reduced particle size.

G. Co-digestion effect on EFB anaerobic digestion

For co-digestion experiments, cumulative methane yield for untreated EFB and pre-treated EFB were 369 ± 31 mL CH_4/g VS and 336 ± 29 mL CH_4/g VS, respectively, and were comparable to the highest methane yield 415 ± 34 mL CH_4/g VS obtained for POME anaerobic digestion, showing a synergic effect for simultaneous digestion of EFB and POME. Methane yield in this experiment was higher than that reported in literature for mesophilic conditions at the same EFB:POME ratio which is 271 mL CH_4/g VS [40] and closer to EFB and POME co-digestion reported at thermophilic conditions (392 mL CH_4/g VS) [37]. When comparing pre-treatment and co-digestion as strategies for enhancing biogas production, co-digestion exhibited better performance and pre-treatment conditions in this experiment should be reviewed and additional improvement opportunities on methane yield could be considered for EFB:POME ratio optimization.

Since the maximum methane yield for co-digestion was 369 mL CH_4/g VS and taking into consideration a EFB:POME ratio of 2:6 in g VS basis, it was calculated that methane yield in terms of mass of POME and EFB was equal to 46.5 mL CH_4/g of mixed EFB and POME, which would be equal to 46.5 m^3 CH_4 /ton of mixed EFB and POME. Considering an energy content of 36 MJ/m^3 CH_4 [37], it was estimated that an energy content in produced methane would be 1674 MJ/ ton of mixed EFB and POME.

Considering a flow rate of 4 L POME/s and the EFB:POME ratio of 2:6 expressed in g VS, it was calculated that EFB could be processed at a rate of 0.21 ton/h via anaerobic co-digestion, leading to 3.23% consumption of EFB generated. Similarly, assuming that 4.82 g ash/slaked lime mixture per one liter of POME were used to pH adjustment (6.6), it was estimated that ash consumption for biogas production is 19.9 ton/month, which was equal to 4.98% of total ash produced per month. Besides biogas production, nutrients stored in ash from biomass boiler and EFB could be returned to the field in the water for irrigation and digestate for partial fertilization replacement, following nutrient recycling and circular economy approach, and reaching a zero-waste concept for anaerobic digestion process. Ash is commonly used as nutrient source in palm oil cultivation due to its high content of Ca, K, Mg, and P and, similarly, digestate (also known as sludge from anaerobic lagoon) is added in palm oil plantation as fertilization complement due to its high content of macro and micronutrients, contributing to an increase fruit production [47]. Additionally, the fertilizer values of liquid digestates lie between those of livestock manures and inorganic fertilizers [32].

V. CONCLUSIONS

Biogas production from empty fruit bunches (EFB) as waste-to-energy valorization route using laboratory scale biomethane potential test (BMP) was studied considering two main approaches: EFB pre-treatment and co-digestion with palm oil mill effluent (POME). Alkaline pre-treatment was considered as EFB pre-treatment using NaOH 0.1M at 80°C for 2 h with a recovery of 66.6%. Adjustment of pH for anaerobic digestion was evaluated using $Ca(OH)_2$/Ash 60:40 (% wt.) leading to 4.82 ± 0.15 g per liter of POME to reach a pH of 6.6 which was 19.7 times lower compared to ash solely, $Ca(OH)_2$ consumption was reduced by 16.7% considering $Ca(OH)_2$ alone (3.47 g/L POME),

Seven experiments were carried out during 22 days at mesophilic conditions using laboratory scale biomethane potential test (BMP) in order to determine biogas production. Fixed variables were inoculum to substrate ratio (ISR) equal to 1 g VS basis, ambient temperature, substrate concentration equal to 8 g VS/L, working volume equal to 300 mL, headspace (%) equal to 40, mixing equal to 500 rpm for 2 minutes every two days, starting pH of 6.6, and EFB:POME ratio of 2:6 in g VS basis. A simpler experimental set-up based on liquid displacement method was proposed, showing simplification of the regular tasks such as mixing and alkaline solution refilling.

Lowest cumulative methane yield was 7.3 ± 0.3 mL CH_4/g VS for POME under facultative conditions while highest cumulative methane yield was 415 ± 34 mL CH_4/g VS for POME under anaerobic conditions. Untreated EFB and pre-treated EFB methane yields were 129 ± 9 mL CH_4/g VS for POME and 74 ± 10 mL CH_4/g VS for POME respectively. Pre-treatment was not suitable as strategy for enhancing biogas production from EFB and pre-treatment parameters used in this research should be evaluated for improvement. Co-digestion of untreated EFB and pre-treated EFB with POME using a EFB:POME ratio of 2:6 in g VS basis resulted in methane yields of 369 ± 31 mL CH_4/g VS and 336 ± 29 mL CH_4/g VS respectively, showing co-digestion as alternative for biogas production using EFB as feedstock. Methane production of 369 mL CH_4/g VS was equivalent to 46.5 m^3 CH_4 /ton of mixed EFB and POME and represented an energy content of 1674 MJ/ ton of mixed EFB and POME, allowing waste processing of 3.23% EFB and 4.98% ash produced on site and nutrient recycling via water for irrigation and digestate for partial fertilizer replacement, leading to a future zero-waste concept with a total solids and volatile solids removal of 81 ± 22% and 89 ± 18% respectively.

Future research should be carried out to optimize pre-treatment conditions to enhance biogas production, including ash addition benefits for microbial community in anaerobic systems, and EFB:POME ratio evaluation to determine its optimal value maximizing biogas production from EFB and POME co-digestion.

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