ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Efficient Conversion of Oil Palm Trunk and Frond to Bioethanol and Biogas Using Two-Stage Steam Explosion Pretreatment

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Citation:
Srimachai, T., Meengam, C., Konjan, P., Rattanadilok Na Phuket, K. Efficient conversion of oil palm trunk and frond to bioethanol and biogas using two-stage steam explosion pretreatment. *ASEAN J. Sci. Tech. Report.* **2023**, *26*(4), 11-20. https://doi.org/10.55164/ajstr.v26i4.249622

Article history:
Received: May 26, 2023
Revised: August 16, 2023
Accepted: August 25, 2023
Available online: September 30, 2023

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Abstract: Bioethanol and biogas production from oil palm trunk (OPT) and oil palm frond (OPF) investigated within a biorefinery concept. Firstly, bioethanol production from OPT and OPF pretreated two-stage steam explosion by comparing with enzyme hydrolysis and without enzyme hydrolysis in the SSF process, which used *S. cerevisiae* in fermentation. The amount of bioethanol increased rapidly, which was stable when the fermentation process entered 96 hrs, resulting in the highest amount of bioethanol produced from OPF and OPT equal to 0.31 and 0.40 g ethanol/g glucose of bioethanol yield, which was about 24.96% and 25.99% higher than the non-enzymatic fermentation of OPF and OPT. The total stillage from OPT and OPF distillation produced a methane yield of 164.38 ml/CH_4 g COD at 30 days of HRT and an organic loading rate (OLR) of 12.45 g COD/L-day. Biofuels in the biorefinery concept of OPF/OPT material at 1,000 kg produced bioethanol, biogas, and solid residue as 49 L at 95% bioethanol, 7,116 L at 63.05% of methane composition, and 13 kilograms of solid residue.

Keywords: Bioethanol; Biogas; Two-stage steam explosion; Biorefinery

1. Introduction

Problems arising from the use of renewable energy in the form of bioethanol are mainly problems such as raw material problems. Presently, bioethanol production mainly uses sugarcane and cassava, the main raw material that is a food source for humans [1]. Still, it would not be easy to use sugarcane and cassava to produce all bioethanol because it infringes on the food supply of people. It affects the food security of the country. Therefore, there should be new sources of raw materials to replace such raw materials to achieve food and energy security at the same time. Another exciting raw material is biomass from palm oil. The government has focused on promoting the planting area to 10 million rai by the year 2029 to increase the amount of palm bunch raw materials that will be supplied to the palm oil industry for crude oil for processing into biodiesel. The palm oil industry has a large amount of lignocellulose biomass waste. Significantly, the oil palm trunk is cut after the oil palm tree is over 25 years old and the oil palm leaf is cut down every 2

weeks or with the harvesting of the oil palm fruit. Each year, approximately 1,000 palm oil palms are cut down 20,000-45,000 rai with biomass from oil palm trunks of about 400,000-900,000 million trees and biomass from oil palm leaves of approximately 7,050,000 tons, which biomass not used maximizes the benefits in the industry. Considering the composition of biomass from different parts of oil palm trunk and oil palm fronds, it was found that the main constituents were cellulose 40-50%, hemicellulose 20-35%, and lignin 16-29% [2]. The hemicellulose content of palm oil palm trees is as high as 34%, with hemicellulose being an exciting part as its main constituents are pentose and hexose sugar. If processed or pretreated, it can be used as a substrate in the fermentation process to produce bioethanol. Biorefineries, which produce various products and co-products such as biofuels, heat, and electricity, have attracted attention in recent years [3]. Biorefineries can convert biomass into valuable biomaterials and energy carriers in an integrated manner. This maximizes the economic value of the biomass used while reducing the waste stream generated [4]. Developing several biofuel-based lignocellulosic biorefineries is a significant opportunity to increase material and energy efficiency and reduce the cost of biomass options to reduce greenhouse gas emissions [5]. However, the main problem of bioethanol production from lignocellulosic materials. is the problem of raw materials. There is also a problem with efficiency and the cost of the pretreatment process [6]. A good hydrolysis process will affect the bioethanol yield because good hydrolysis and pretreatment have the least amount of inhibitors to occur. Inhibitors in lignocellulosic hydrolysates consist of aliphatic acids (i.e., acetic, formic, and levulinic acid), for aldehydes (i.e., 5-hydroxymethylfurfural (HMF), and furfural), aromatic compounds (i.e.phenolics) and extractives; all of which affect ethanol fermentation [7]. These substances are toxic to fermenting microorganisms, resulting in decreased bioethanol yield. Jonsson and Matin [8] reported that the disadvantage of using sulfuric acid pretreatment was the formation of inhibitory by-products. Additionally, the advantage of hydrothermal pretreatment by controlling the pH around neutral values was the decreased amount of fermentation inhibitors [9].

In this experiment, the methods of conditioning and hydrolysis of oil palm biomass, including oil palm trunk and oil palm frond, were selected by the two-stage steam explosion, which is likely to be effective in destroying the structure of lignocellulose materials well and have less toxicity compared to other methods such as using acids or alkali pretreatment. In addition, this method is likely to cost less than using enzymes. Using two-step pretreatment can reduce the cost of the bioethanol production process.

2. Materials and Methods

2.1 Preparation of materials

Prepare raw materials from oil palm trunk (OPT) and oil palm frond (OPF) of fresh oil palm by OPTs are peeled and OPFs cut off leaves by using OPT and OPF aged 25-30 years from farmers in Khuan Kalong District, Satun Province. After that, it is cut into pieces about 50-100 cm be able to enter the chipper, which will obtain a sample size of about 2-4 cm. After that, the samples of OPT and OPF were squeezed by a screw press juicer. After that, the juice from the OPT and OPF is filtered and stored in a cold room at -20 °C for preservation. The residues or solids of OPT and OPF were dried at 103-105 °C for 24 hrs. The samples were stored in zipped plastic bags to prevent moisture.

2.2 Pretreatment method

Steam explosion pretreatment was performed in a 98 L capacity reactor with a maximum steam output of 12 bar. OPF and OPT samples amounting to 150 g were treated for the first and two steps by steam explosion pretreatment (Figure 1) by steam explosion conditions as in Table 1, then filtered to separate the solid from the solution to remove only the insoluble solids and washed with distilled water until the pH was neutral. The solids were then dried at 103-105°C for 24 hours to evaporate moisture. The samples were analyzed for cellulose, hemicellulose, and lignin content. Samples undergoing a 2-stage steam explosion are fed into the fermentation process for bioethanol. It will be divided into 2 parts to compare the use of enzymes and non-enzymes during fermentation.



Figure 1. Steam explosion equipment

Table 1. Conditions for pretreatment method using experiment

First-steam explosion pretreatment			Second- steam explosion pretreatment		
Temperature (°C)	Pressure (bar)	Time (min)	Temperature (°C)	Pressure (bar)	Time (min)
160	4	30	230	10	3

2.3 Inoculum

Yeast cultures were stored on YM agar medium at 4 °C (refrigerator). *Saccharomyces cerevisiae TISTR 5339* was transferred from a solid medium into a liquid medium (YM broth) and cultured at 37 °C for 48 hrs at a rate of 100 rpm. Then, it measured the turbidity at the absorbance (OD_{620}) using 10% (v/v) as the starter culture.

2.4 Simultaneous Saccharification and Fermentation (SSF) for bioethanol production

2.4.1 SSF without enzymatic hydrolysis

Take 10% by weight of the samples from section 2.2 (OPT and OPF) without enzymatic hydrolysis in a 250 ml flask, add 5 ml of 5M Citrate buffer (pH 4.8) and 85 ml of distilled water, and sterilize at 121 °C for 15 minutes. Set aside to cool to room temperature, 10% (v/v) *S. cerevisiae* and 1% (v/v) yeast extract (10.0 g/l) was added and shaken at 150 rpm for 72 hrs in a 250 ml flask. Samples were collected and analyzed at 0, 24, 48, 72, and 96 hrs for analysis of reducing sugar content and bioethanol content.

2.4.2 SSF using enzymatic hydrolysis

Take the sample from 10% by weight, put it in a 250 ml flask, and add 5 ml of 5M Citrate buffer (pH 4.8) and 85 ml of distilled water. Sterilized at 121 °C for 15 min, then 3% wt% of CellicR CTec2 (Cellulase + β-glucosidase) was added and incubated at 55 °C and shaken at 150 rpm for 72 hrs. Samples were analyzed at 0, 24, 48, 72, and 96 hrs. After that, 10% (v/v) *S. cerevisiae* and 1% (v/v) yeast extract (10.0 g/l) were added and stirred at 150 rpm for 48 hrs. Samples were collected and analyzed at 0, 24, 48, 72, and 96 hrs to explore reduced sugar and bioethanol content.

2.5 Biogas production

Take the total stillage from the bioethanol distillation process and operate the system with a 200-liter CSTR reactor for biogas production. The conditions will be studied in different HRTs as follows: 35, 30, 25, and 20 days to define the optimal conditions of the experiment in the highest biogas production.

2.6 Analytical methods

The Van Soest method determined the chemical composition (cellulose, hemicellulose, and lignin) [10]. The chemical composition of the distillery slop was analyzed using the Standard method [11]. The total sugars were analyzed by high-performance liquid chromatography (HPLC) with a refractive index detector at 50 °C. 5.0 µl of samples were injected into a column (SH1011, 8.0x300 nm, Shodex) with 0.04 N H_2SO_4 as the mobile phase (flow rate 0.8 ml/min). The running time of the samples was 20 min. The dichromate reagent

method determined the bioethanol concentration [12]. pH was measured by using a pH meter (Horiba, Japan). Chemical oxygen demand (COD), total solid (TS), volatile solid (VS), total nitrogen (TN), protein, carbohydrate, and fat were analyzed following the procedures explained in the Standard Method [11]. The volume of biogas was measured by water replacement and biogas composition was monitored by gas chromatograph GC-8APT with a thermal conductivity detector (TCD)[13]. Gas chromatograph GC-8APF analyzed the VFA with a flame ionization detector (FID) [13].

3. Results and Discussion

3.1 Effect of pretreated first-steam explosion

OPF (untreated) before conditioning contained cellulose hemicellulose and lignin were 35.83, 26.79, and 26.33 percent, respectively, and OPT (untreated), before pretreatment, had cellulose hemicellulose and lignin were 43.76, 29.53, and 21.64 %, respectively (Table 1) which cellulose after pretreatment of OPF and OPT increased as 44.17 and 53.82 %. Because the result of the first-steam explosion pretreatment caused internal swelling to increase the reaction surface area of cellulose was increased porosity, reduced crystallinity, and degraded macro polymerization (Figure 2), affecting the amount of cellulose increased. After pretreatment, hemicellulose of OPF and OPT was decreased by 18.35% and 20.62% due to the hemicellulose portion dissolving with steam when pretreated by steam explosion. Lignin after pretreatment of OPF and OPT increased to 35.00% and 23.77% due to the limitation of the steam explosion pretreatment. Degradation of lignin is often incomplete because the pressure used for first-steam explosion pretreatment was not high enough when reducing the pressure effect of lignin did not escape.

Table 1. Effect of two-stage steam explosion pretreatment on the chemical composition of OPT and OPF.

Pretreatment	Composition	Percent (w/w)		
		Raw material (Untreated)	First stream explosion	Second stream explosion
OPF	Cellulose	35.83 ± 1.53	44.17 ± 3.25	55.15 ± 1.27
	Hemicellulose	26.73 ± 1.32	18.35 ± 3.50	5.78 ± 1.50
	Lignin	26.33 ± 1.25	35.00 ± 2.17	38.44 ± 2.17
OPT	Cellulose	43.76 ± 1.05	53.82 ± 2.45	63.22 ± 2.14
	Hemicellulose	29.53 ± 1.28	20.62 ± 5.50	4.58 ± 1.25
	Lignin	21.64 ± 1.32	23.77 ± 3.82	31.08 ± 1.58

3.2 Effect of pretreated second-steam explosion

Take the sample in the first stream explosion for further conditioning with the second stream explosion (230 °C, pressure 10 bar, for 3 mins). The results showed that by steam explosion pretreatment in step 2, the cellulose content of OPF and OPT increased by 55.15% and 63.22% (Table 1). The second pretreatment resulted in a marked decrease in hemicellulose after the pretreatment of OPF and OPT to only 5.78% and 4.58%. The hemicellulose content decreased due to the dissolution of the hemicellulose by the second steam explosion pretreatment (Figure 3). The lignin content after the pretreatment of OPF and OPT increased to 38.44 and 31.08 percent because the condition of the steam explosion was more severe. Still, the second steam explosion pretreatment depolymerized lignocellulose polymer structures and breakage of crosslinking between macromolecules affecting the amount of cellulose enhanced.

SEM images of untreated OPF and OPT are shown in A1 and A2 in Figures 2 and 3. It observed that the structure of the tested sample remains intact and firmly connected. The untreated samples contain higher amounts of lignin, which protects cellulose and hemicellulose from degradation. Therefore, lignin removal in sugar recovery required pretreatment by steam explosion. After the second-steam explosion pretreatment, the structure mesocarp fiber of OPT and OPF was destroyed, and the wall of the fibers was ruptured entirely, as shown in B1 and B2 in Figures 2 and 3. Jacque et al. [14] reported that pretreated steam explosion is a thermo-mechanicochemical pretreatment that allows the destruction of lignocellulose structure by heating, the formation of organic acids in the process, and shearing forces resulting in the expansion of the moisture, resulting in

more surface area and higher effect to enhance the enzyme hydrolysis process, increased to produce reducing sugar in bioethanol production.

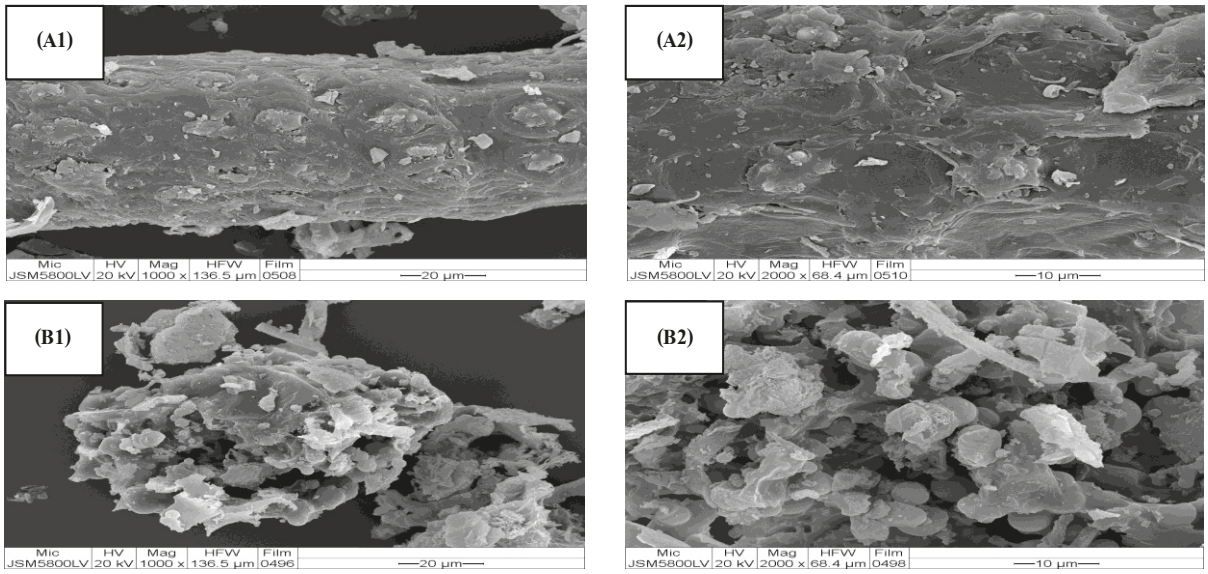


Figure 2. Morphology of OPT at 1,000 and 2,000 x magnification: (A1) and (A2) Untreated OPT, (B1) and (B2) Pretreated first-steam explosion

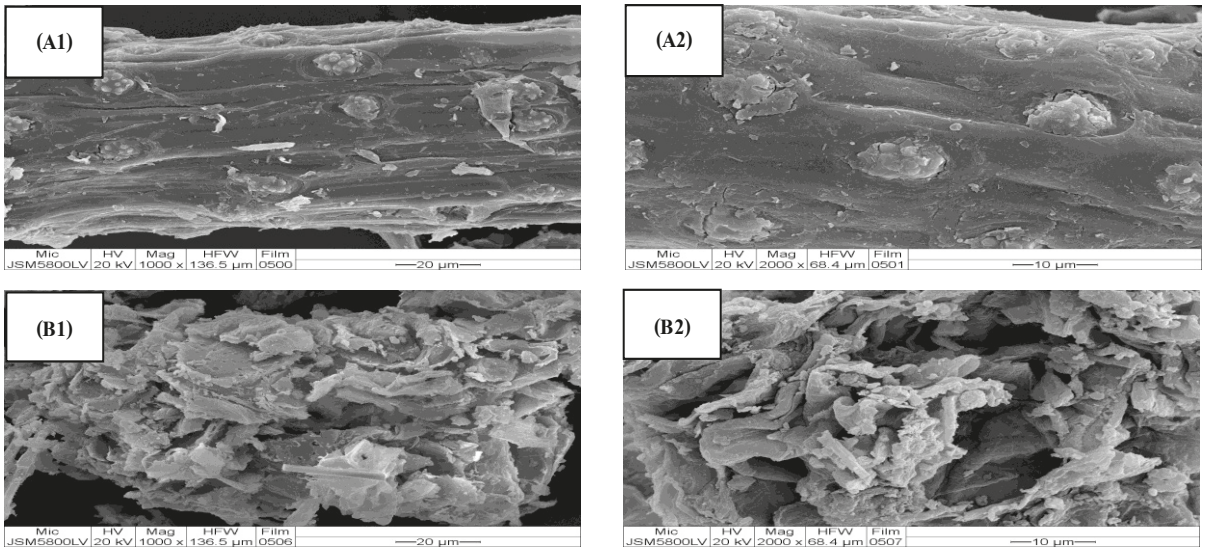


Figure 3. Morphology of OPF at 1,000 and 2,000 x magnification: (A1) and (A2) Untreated OPF, (B1) and (B2) Pretreatted second-steam explosion pretreatment

3.3 SSF without enzymatic hydrolysis to produce bioethanol

After a two-stage steam explosion, samples of OPF and OPT were studied in SSF by non-adding enzymatic to hydrolysis process for producing bioethanol. As a result of the two-stage steam explosion, the cellulose content explodes violently and breaks down to more glucose without the use of enzymes. The glucose contents of OPF and OPT after the two-stage steam explosion were 21.75 and 35.65 g/l, which the *S. cerevisiae* could use for bioethanol production. After 96 hours, 27.12 and 35.44 g/l of bioethanol (0.25 and 0.33 g ethanol/g glucose of bioethanol yield) from OPF and OPT could be produced without enzyme addition (Figure 4). The maximum bioethanol content of sugarcane bagasse was 21 g/l by H_2O_2 pretreatment using *S. cerevisiae* in SSF fermentation [15]. Zuo et al. [16] reported that corn stover could produce bioethanol 36.1 g/l by 1 % NaOH+8 % NH_4OH pretreatment using *Pichiastipitis* in SSF fermentation, similar to this experiment.

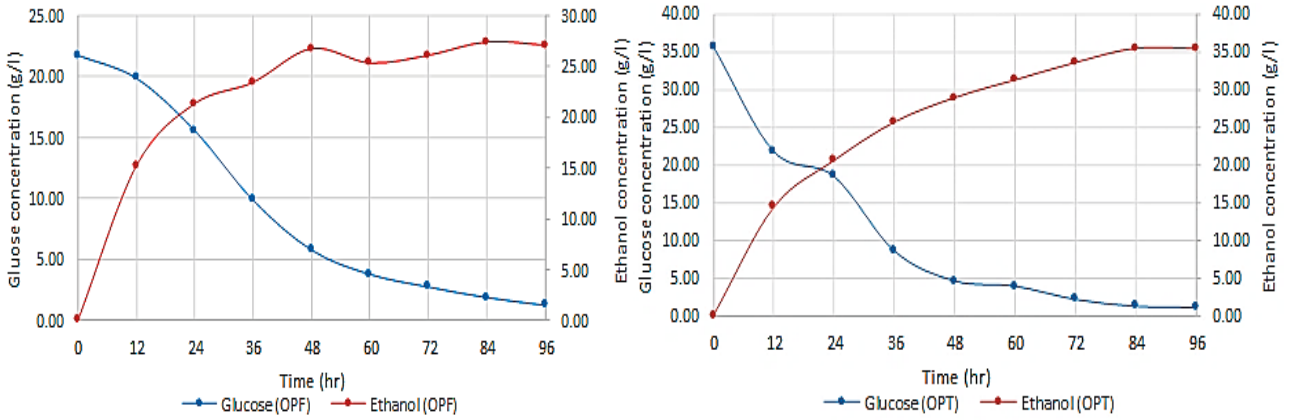


Figure 4. Glucose utilization and bioethanol production of OPF and OPT after the two-stage steam explosion (without enzyme)

3.4 SSF using enzymatic hydrolysis to produce bioethanol

Samples of OPF and OPT after a pretreated two-stage steam explosion were studied in SSF, in which enzymes are added to the hydrolysis process to produce bioethanol. Samples were collected and analyzed for glucose and bioethanol content at 0, 12, 24, 36, 48, 60, 72, 84, and 96 hrs. The experimental results showed cellulose was digested into glucose after adding an enzyme. The OPF glucose content increased from 21.75 to 35.76 g/l, and OPT increased from 35.65 to 46.88 g/l within 12 hours. After 12 hrs, the glucose decreased sharply because of the use of glucose by *S. cerevisiae* to produce bioethanol. As a result, the amount of bioethanol increased rapidly, which was stable when the fermentation process entered 96 hrs, resulting in the highest amount of bioethanol produced from OPF and OPT were 33.89 and 44.65 g/l (Figure 5) or 0.31 and 0.40 g ethanol/g glucose of bioethanol yield, which was about 24.96% and 25.99% higher than compared with the non-enzymatic fermentation (27.12 and 35.44 g/l of bioethanol) of OPF and OPT, respectively. Similarly, enzymatic hydrolysis and subsequent fermentation of cellulose yielded 0.41 g-ethanol/g-glucose from wheat straw[17].

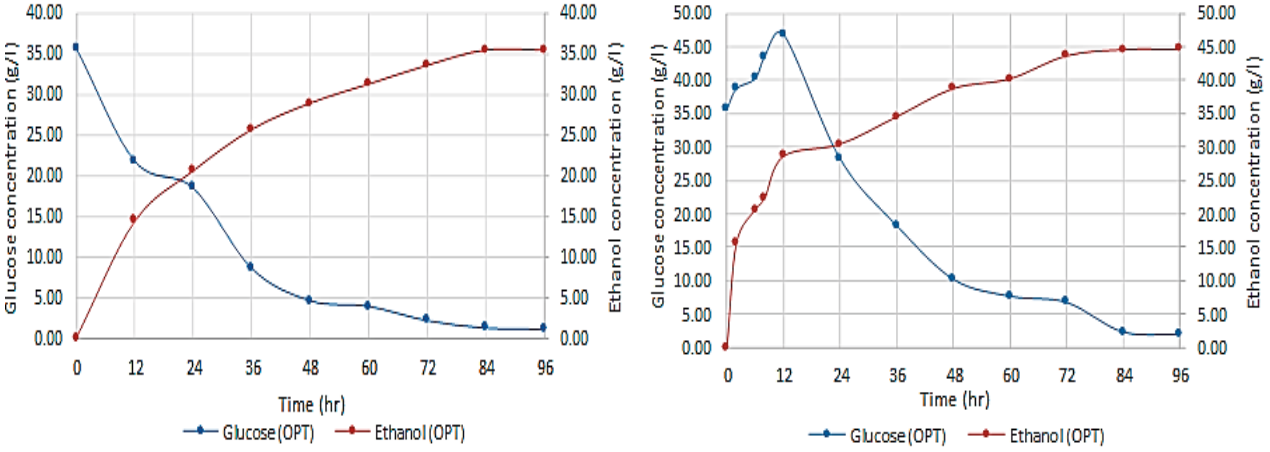


Figure 5. Glucose utilization and bioethanol production of OPF and OPT after the two-stage steam explosion (using enzyme)

3.5 Biogas production from total stillage

Total stillage is the total wastewater obtained after distillation of OPF and OPT of the bioethanol. The chemical characterization of the total stillage is shown in Table 2. The total stillage was operated in a CSTR reactor sizing 200 L to produce biogas in a continuous system. The operation was performed during the HRT as follows: 35, 30, 25, and 20 days, respectively. During 30 days of HRT, the daily methane production and maximum methane production were 116,000 ml CH_4/day (116 L CH_4/day) at 63.05 percent methane

concentration (Figures 6 and 7), followed by a hydraulic retention time of 35 days, with the highest daily methane yield and methane production rate of 88,000 ml CH_4/day (88 L CH_4/day) at 59.06% methane concentration. At 25 days of HRT, the maximum methane production was 64,000 ml CH_4/day (64 L CH_4/day) at 54.20% methane concentration. HRT at 20 days had the highest daily methane yield and methane production rate of 40,000 ml CH_4/day (40 L CH_4/day) at 52.87 % concentration of methane gas, respectively. Daily methane yield from continuous fermentation of distillery slop was studied using a 200-liter CSTR reactor. It found that day 63 yielded the highest daily methane yield. The 30-day of HRT yielded the highest daily methane yield of 164.38 ml/CH_4 g COD (Figure 7), which was calculated from the organic loading rate (OLR) of 12.45 g COD/L-day, followed by the hydraulic retention time of 35 days, 25, and 20 days, which result in the highest daily methane yield of 149.88 ml/CH_4 gCOD, 70.87 ml/ CH_4 gCOD and 32.07 ml/CH_4 g COD, calculated from organic loading rates (OLR) of 9.96, 16.44 and 24.90 g COD/L-day, respectively. At 30 days of HRT (optimal), the methane yield was 164.38 ml CH_4/g COD. Kaparaju et al. [17] reported that methane yield from the stillage of wheat straw fermentation was 381 ml CH_4/g VS-added.

Table 2. The chemical characterization of the total stillage

Chemical characterization	Total stillage	Stillage wheat straw [17]
pH	4.8	4.0
COD (g/L)	153	170.7
VFA (g/L)	5.25	0.37
TKN (g/L)	13.28	6.20
TS (g/L)	28	19.6
VS (g/L)	18	17.8
Protein (g/L)	10.54	38.80
Carbohydrate(g/L)	81.22	129.30
Lipids(g/L)	1.67	0.93
C/N ratio	13	-

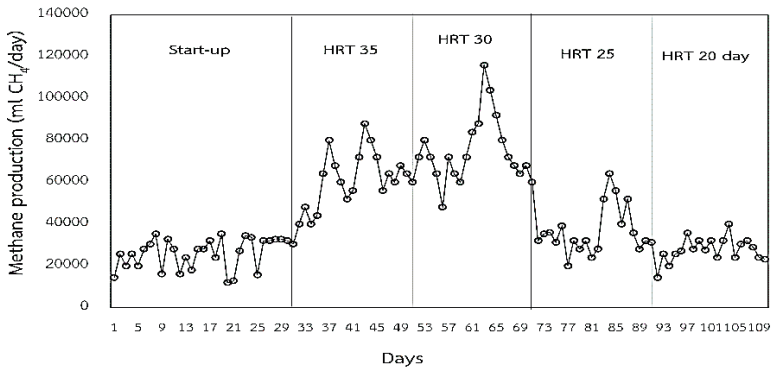


Figure 6. Methane production in a continuous system

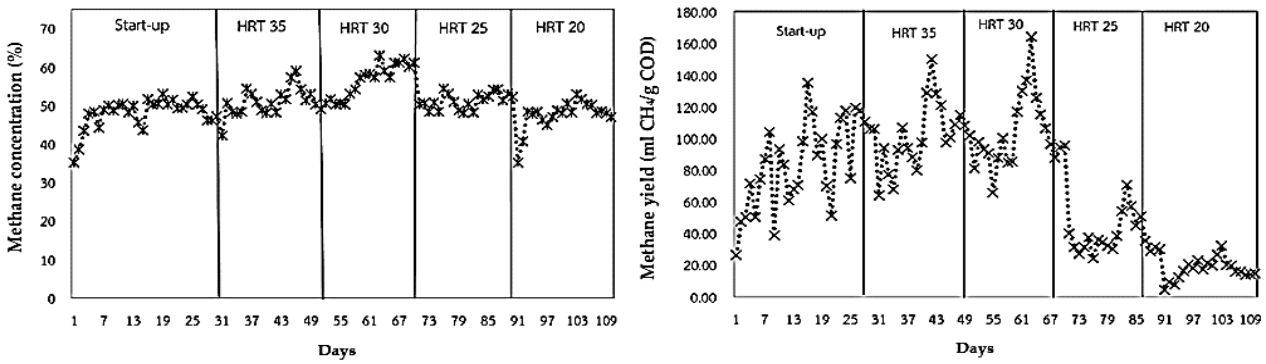


Figure 7. Methane concentration and methane yield in a continuous system

The volatile fatty acid (VFA) content study results showed that VFA composition increased with increasing organic matter input rate. The retention period was 30 days (maximum methane production). The VFAs in the system consisted of acetic and butyric acid as the major constituents, which ranged from 50-130 mg/l (Figure 8), indicating that methane-producing microorganisms were good efficiently for using volatile fatty acids to produce methane. There is no accumulation of volatile fatty acids in the system, which results in the pH value of the system being neutral. This corresponds to the pH in the 30-day retention period, which is 7.6-7.9 (Figure 8), indicating that the pH in the system is neutral. It is suitable for the growth of methane-producing microorganisms. The optimum pH range for methanogenesis in anaerobic systems is usually between 6 and 8 [18]. Franke Whittle et al. [19] noted that accumulation in most situations reflects an imbalance between acid producers and consumers. This leads to decreased pH within the system, inhibiting methanogen growth. Mass flow rates in the studied biorefinery concept were calculated based on the number of sugars and their conversion to various biofuels/products [17]. So, biofuels in the biorefinery concept of OPF/OPT material at 1,000 kg could produce bioethanol, biogas, and solid residue as 49 L at 95% bioethanol, 7,116 L at 63.05% of methane composition, and 13 kilograms of solid residue (Figure 9).

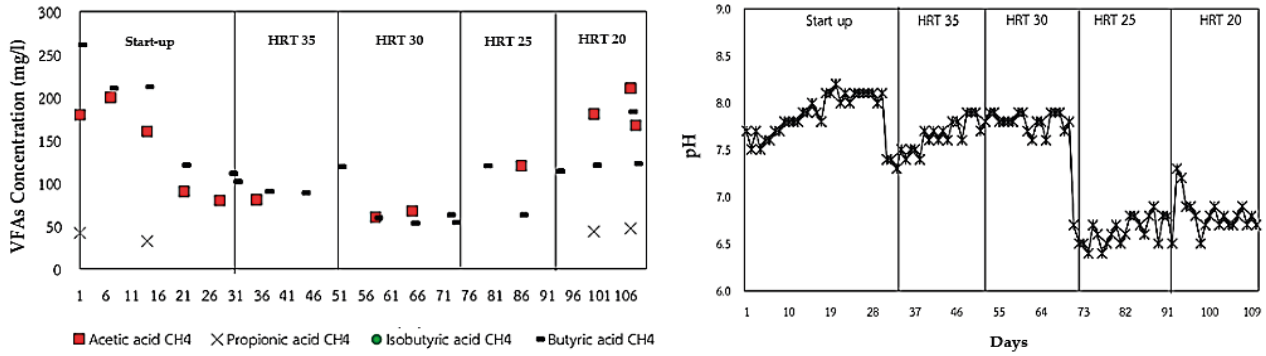


Figure 8. VFA concentration in a continuous system

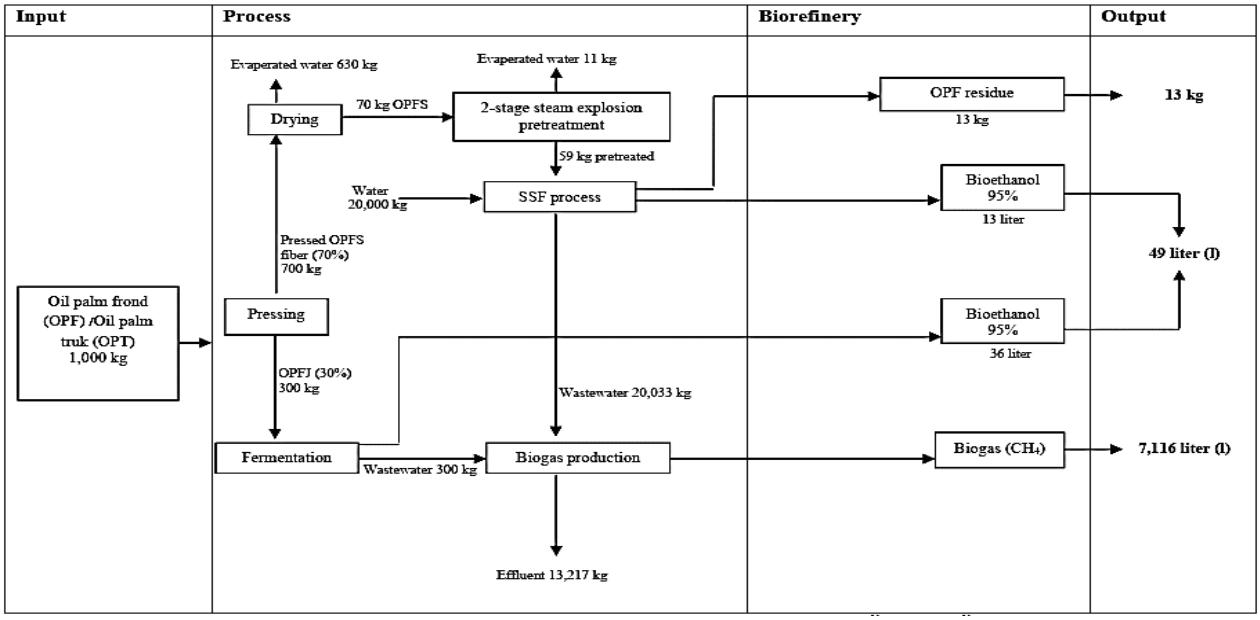


Figure 9. Mass flow in the biorefinery process

4. Conclusions

Two-stage steam explosion pretreatment of OPF and OPT could destroy the pores structure formed on the fiber when the lignin was solubilized and removed, resulting in more surface area and higher effect to enhance the enzyme hydrolysis process, increased to produce reducing sugar in bioethanol production. The optimization in bioethanol production compared between using enzyme and without enzyme SSF, without enzyme could produce maxed bioethanol from OPF and OPT was 27.12 (0.25 g ethanol/g glucose) and 35.44 g/l (0.33 g ethanol/g glucose) and with enzyme could produce maxed bioethanol 33.89 and 44.65 g/l (0.31 and 0.40 g ethanol/g glucose) of OPF and OPT which were 24.96 and 25.99 % higher than those of without enzymatic fermentation. Methane yield from total stillage in the CSTR reactor was 164.38 ml/CH_4 g COD. Increasing sugar content discharged from OPF and OPT by two-stage steam explosion pretreatment has produced biofuels (bioethanol and biogas) in production-based biorefineries.

5. Acknowledgements

The authors would like to thank Songkhla Rajabhat University and the Faculty of Science and Technology, Prince of Songkhla University (Pattani campus), for the opportunity to use the laboratory to do this research.

Author Contributions: Conceptualization, K.R.; methodology T.S. and K.R.; formal analysis, T.S.; investigation, P.K., and C.M.; writing—original draft preparation, K.R.; writing—review and editing, K.R.

Funding: The Energy Policy and Planning Office (EPPO) for funding this research.

Conflicts of Interest: The authors declare no conflict of interest.

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