CHAPTER 4 – RESULTS AND DISCUSSIONS

4.1 Results

The results of this paper will be presented as in the design of experiment flowchart. The results will be presented in the order of empty fruit bunch (EFB), oil palm trunk (OPT) and oil palm frond (OPF). Each of the solid oil palm waste was categorized into its juice/sap feed and dried/lignocellulosic material as feed.

4.1.1 Empty Fruit Bunch (EFB)

4.1.1.1 Empty Fruit Bunch sap (EFBS)

As of the writing of this thesis, only the lignocellulosic material Empty Fruit Bunch (EFB) can be made into bioethanol. This is due the lack of sap present in the EFB. Further research should be performed to extract and or convert the sap of EFB so that it can be converted to bioethanol.

4.1.1.2 Lignocellulosic Empty Fruit Bunch (EFBC)

4.1.1.2.1 Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch

In 2015, Deliana Dahnum and team , conducted a study of empty fruit bunch to produce bioethanol. The research was conducted to find out the best fermentation method to produce bioethanol from lignocellulosic material of EFB. The fermentation method researched in this study was SHF and SSF. The EFB was obtained from Palembang, Indonesia.

EFB gathered was first milled to a size of 2mm. Milling of the EFB was to maximize the contact area of the feed. The milled EFB was subjected to alkaline pre-treatment. Alkaline pre-treatment using NaOH 10% (w/v) and solid to liquid ratio of 1:5 at 150°C and 4 bars for 30 minutes. The pre-treated EFB was then washed until a neutral pH was achieved and dried in the oven at 50–60°C.

Table 4.1 Contents of EFB after before and pre-treatment process(Dahnum *et al.*, 2015)

Substrate	Lignin (%)	Cellulose (%)	Hemicellulose (%)
Untreated EFB	25.52	35.84	15.48
Treated EFB	15.70	73.24	7.81

Table 4.1 shows the contents of EFB before and after the pre-treatment process. It shows that there was a 37.4% increase in cellulose content and a 9.82% decrease in lignin content. This is beneficial to the bioethanol process as lignin hinders bioethanol production while an increase in cellulose means an increase in glucose which is used to produce ethanol.

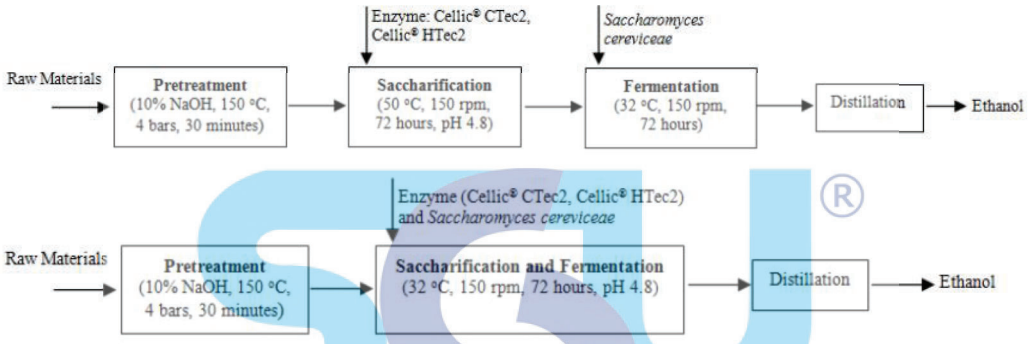

Figure 4.1 Process of bioethanol production by SHF and SSF(Dahnum *et al.*, 2015)

Figure 4.1 shows the process for each fermentation method. Both processes uses *Saccharomyces cerevisiae* as inoculum and Cellic® CTec2 and Cellic® HTec2 as agents for enzymatic hydrolysis. The saccharification process for the SHF method was conducted at 50°C for 72 hours with a pH level of 4.8 and agitation rate of 150 rpm. After the saccharification process, fermentation was done at 32°C for 72 hours with an agitation rate of 150 rpm. SSF was conducted at 32°C for 72 hours with a pH level of 4.8 and agitation rate of 150 rpm.

Results from both processes show that SSF was the better way to produce bioethanol. It was found that in 24 fours during the SSF period, 6.05% of bioethanol was produced while 72 hours of fermentation time was need to produce 4.74% bioethanol for SHF. This means that not only is SSF a faster process to produce bioethanol, but it is also produces a greater amount of bioethanol than SHF.

Table 4.2 Objective and summary of (Dahnum *et al.*, 2015)

Objective of the study: To discover which fermentation method is better to produce bioethanol from lignocellulosic EFB using *Saccharomyces cerevisiae* as inoculum	
Pre-treatment	Alkaline pre-treatment (NaOH 10%)
Cellulose content	73.24%
Lignin content	35.84%
Fermentation Method	SSF
Hydrolysis method	Enzymatic hydrolysis by Cellic® CTec2 and Cellic® HTec2
pH	4.8
Temperature	32°C
Inoculum	*Saccharomyces cerevisiae*
Fermentation Time	24 h
Agitation Rate	150 rpm
Bioethanol produced	6.05%

4.1.1.2.2 The Improvement of Sugar and Bioethanol Production of Oil Palm Empty Fruit Bunches through Microwave-Assisted Maleic Acid Pretreatment Lignocellulosic Empty Fruit Bunch

On 2018, Widya Fatriasari and team, conducted research about improvement of sugar and bioethanol from EFB through microwave assisted acid pre-treatment method. The purpose of this research was to find out about the optimum condition of pre-treating EFB to produce ethanol. The EFB obtained from Sukabumi in Indonesia.

The EFB was first milled until a size of 40-60 mesh was achieved. The milled EFB was then pre-treated with maleic acid assisted with the microwave. The pre-treatment process begins by soaking 3 g milled EFB into maleic acid and sealing it in a test tube before heating them in a microwave.

Pre-treatment under different conditions were performed. The conditions are as followed:

- Temperature
- Irradiation time
- Concentration of Maleic acid

Samples of milled EFB were heated to 160- 200°C for 2.5 minutes of irradiation time and maleic acid 1% (v/v) to get the optimum pre-treatment temperature. To obtain the optimum irradiation time, samples were exposed for 2.5-12.5 minutes at 190°C and maleic acid 1% (v/v). Lastly, samples were pre-treated with different concentrations of maleic acid ranging from 0.5-1.5%(v/v) at f190°C and exposed for 2.5 minutes of irradiation time.

Table 4.3 Effects of temperature on the content (w/w) EFB with 2.5 minutes of irradiation time and maleic acid 1%(Fatriasari *et al.*, 2018)

Temperature (°C)	Pulp Recovery (%)	Extractives (%)	ASL (%)	AIL (%)	α-Cellulose (%)	Hemicellulose (%)
160	80.32±1.01	3.30±0.56	2.79±0.03	21.63±1.41	52.51±1.95	16.61±2.12
170	70.32±2.97	5.38±0.10	2.55±0.13	18.69±2.37	51.75±5.14	14.66±4.42
180	66.97±1.11	7.25±1.16	3.13±0.15	19.47±2.00	49.02±3.80	16.09±0.18
190	68.80±3.62	11.40±2.08	2.66±0.58	20.07±1.02	57.50±2.81	12.77±2.72
200	50.40±0.75	10.96±0.69	2.49±0.45	18.58±2.07	44.04±2.67	12.04±5.52

Table 4.3 shows the contents of the pre-treated EFB under different temperatures with constant irradiation time and maleic acid concentration. It can be seen that the pulp yield is inversely proportional to the temperature. Pre-treatment at 160°C yields the highest amount of pulp while pre-treatment at 200°C yields the least amount of pulp. It can also be seen that pre-treatment at 190°C has the highest amount of alpha-cellulose content while pre-treatment at 200°C has the least amount of acid insoluble lignin (AIL) or lignin content.

Table 4.4 Effects of irradiation time on the content (w/w) EFB at 190°C and maleic acid 1%(Fatriasari *et al.*, 2018)

Irradiation Time (min)	Pulp Recovery (%)	Extractives (%)	ASL (%)	AIL (%)	α-Cellulose (%)	Hemicellulose (%)
2.5	68.80±3.62	11.40±2.08	2.66±0.58	20.07±1.02	57.50±2.81	12.77±2.72
5	65.51±2.31	5.29±0.36	1.40±0.06	20.97±0.96	53.92±1.71	12.13±1.18
7.5	63.58±1.23	9.07±0.01	1.24±0.01	20.74±0.84	55.05±0.99	11.16±0.71
10	63.50±0.65	9.85±0.29	1.04±0.11	20.03±0.74	49.36±5.87	20.56±5.30
12.5	57.15±0.35	10.73±0.05	1.33±0.06	16.62±4.05	56.89±2.97	13.50±2.55

Table 4.4 shows the contents of the pre-treated EFB under different irradiation times with constant temperature and maleic acid concentration. It can be seen that the pulp yield is inversely proportional to the irradiation time. EFB exposed for 2.5 minutes yields the highest amount of pulp while exposure for 12.5 minutes yields the least amount of pulp. It can also be seen that exposure for 2.5 minutes leads to the highest amount of alpha-cellulose content while exposure for 10 minutes leads to the least amount of AIL content.

Table 4.5 Effects maleic acid concentration (v/v) on the content (w/w) EFB with 2.5 minutes of irradiation time at 190°C (Fatriasari *et al.*, 2018)

Acid Concentration (%)	Pulp Recovery (%)	Extractives (%)	ASL (%)	AIL (%)	α-Cellulose (%)	Hemicellulose (%)
0.5	67.99±1.29	6.07±0.27	0.33±0.01	19.29±2.16	48.52±6.76	20.89±6.95
1	68.80±3.62	11.40±2.08	2.66±0.58	20.07±1.02	57.50±2.81	12.77±2.72
1.5	62.71±0.43	8.41±0.10	0.32±0.00	20.90±0.18	54.91±4.76	15.96±4.89

Table 4.5 shows the contents of the pre-treated EFB under different maleic acid concentrations with irradiation time and temperature. Pre-treatment with maleic acid concentration of 1% not only yields the highest amount of pulp yield, but also the highest amount of alpha-cellulose.

It can be concluded that the optimum pre-treatment process for this study is the pre-treatment of EFB through the acid pre-treatment process using 1% maleic acid at 190°C with an irradiation time of 2.5 minutes. After the pre-treatment process, the 15.3 g of pre-treated EFB with YP stock medium which consist of 200 g/L of peptone and 100

g/L of yeast extract. SSF using *Saccharomyces cerevisiae* was performed with a pH of 4.8 using meicelllase to hydrolyse the pre-treated EFB at 38°C for 72 hours with an agitation rate of 120 rpm. This process yields 0.43 g of ethanol per g cellulose (76.6% of maximum theoretical yield) and maximum concentration of 18.9 g/L of bioethanol after 48 hours.

Table 4.6 Objective and summary of (Fatriasari *et al.*, 2018)

Objective of the study: To discover the optimum parameter microwave-assisted maleic acid pretreatment Lignocellulosic Empty Fruit Bunch	
Pre-treatment	Microwave assisted acid pre-treatment
Temperature	190 °C
Irradiation time	2.5 minutes
Acid concentration	Maleic acid 1%(v/v)
Fermentation method	SSF
pH	4.8
Temperature	38°C
Inoculum	*Saccharomyces cerevisiae*
Hydrolysis method	Enzymatic hydrolysis by Meicelllase
Fermentation Time	48 hours
Agitation Rate	120 rpm
Bioethanol concentration	18.9 g/L
Bioethanol Yield	0.43 g ethanol/g cellulose

4.1.1.2.3 Bioethanol production from oil palm empty fruit bunch with SSF and SHF processes using *Kluyveromyces marxianus* yeast Lignocellulosic Empty Fruit Bunch

In 2020, Suwanan Sukhang and team, conducted a study of empty fruit bunch to produce bioethanol. The research was conducted to find out the best procedure to

produce bioethanol using lignocellulosic Oil Palm Trunk as feed. The research This research aimed to study bioethanol production from EFB by SSF and SHF. The EFB was obtained from Trang Palm Oil Industry Co., Ltd

Kluyveromyces marxianus was chosen to be the inoculum for this research. *Kluyveromyces marxianus* in agar slants with 20 g/L of glucose an, 3 g/L of yeast extract5 g/L of peptone, 3 g/L of malt extract and 1.5 g/L of agar at 4°C. Pre-treatment of EFB by acid pre-treatment using 0.2 M sulfuric acid at 121°C for 53 minutes followed by alkaline pre-treatment with 5% NaOH for 121°C for 20 min.

To produce bioethanol from EFB by SHF, hydrolysis of pre-treated EFB was done at 37.5°C for 72 hours with a pH level of 5. The hydrolysate was then filtered and pH level was adjusted to be 5 with the help of a citrate buffer. Fermentation was then done with an inoculum concentration of 1%(v/v) at 37.5°C for 96 hours with an agitation rate of 150 rpm. Bioethanol production by SSF was done at 36.94°C, inoculum concentration of 2.04%(v/v) for 96 hours with a pH level of 4.5, agitation rate of 150 rpm and 12.24% (w/v) of substrate loading.

Table 4.7 Contents (% w/w)of EFB during the pre-treatment process(Sukhang *et al.*, 2020)

Component of OPEFB	Untreated	1st pretreatment	2nd pretreatment
%Cellulose	41.11	65.70	72.10
%Hemicellulose	30.03	3.77	3.24
%Lignin	26.36	27.82	17.60

Table 4.7 shows the cellulose, hemicellulose and lignin content of the untreated, 1st-pre-treatment (acid method) and 2nd pre-treatment (acid and alkali method). The results show that after the 2nd pre-treatment process has the highest amount of cellulose while also having the lowest lignin percentage. There was also a 30.99% increase in cellulose and 8.76% decrease in lignin between the 2nd pre-treatment and untreated sample. This important as high cellulose and low lignin content is favourable for bioethanol production using lignocellulosic feed.

Table 4.8 Ethanol yield of pre-treated EFB(Sukhang *et al.*, 2020)

Substrate type	Microorganism	Operation mode	Performance
OPEFB	*K.marxianus* (TISTR5116)	SHF	0.258 g/g OPEFB
		SSF	0.281 g/g OPEFB

Table 4.8 shows that SSF is better than SHF for bioethanol production. SHF to produce bioethanol yields 0.258g/g pre-treated EFB while SSF yields 0.281g/g pre-treated EFB. The study also finds that SSF performed better than SHF with both quicker processing and higher bioethanol concentration.

A summary of this study can be shown in the table 4.9 below. It shows the best course of action to increase the cellulose level and decrease the lignin level of EFB so that it can be more suitable for bioethanol production as well as which method of fermentation alongside its parameters to optimally produce bioethanol.

Table 4.9 Objective and summary of (Sukhang *et al.*, 2020)

Objective of the study: To discover which fermentation method is better to produce bioethanol from lignocellulosic EFB as feed using *Kluyveromyces marxianus* as inoculum	
Pre-treatment	2 step pre-treatment of alkali and dilute acid method
Cellulose increase	30.99%
Lignin decrease	8.76%
Fermentation Method	SSF
Hydrolysis method	Enzymatic hydrolysis by cellulase enzyme
Substrate loading	12.24% (w/v)
pH	4.5
Temperature	36.94°C
Inoculum	*Kluyveromyces marxianus*

Fermentation Time	48 hours
Inoculum Concentration	2.04%v/v
Agitation Rate	150 rpm
Bioethanol concentration	34.39 g/L
Bioethanol Yield	0.281 g/g EFB

4.1.1.2.4 Integrated and partial process of xylitol and bioethanol production from oil palm empty fruit bunches

On 2022, Adela Bukhari and team, did a research about producing bioethanol from chemically pre-treated EFB with xylitol as a by-product. EFB is a very lignocellulosic biomass. The raw material used was OPEFB obtained from Incasi Raya Palm Oil Mills, West Sumatra, Indonesia. Table 4.10 shows the contents of EFB before the pre-treatment process.

Table 4.10 Contents (% w/w)of EFB (Mardawati *et al.*, 2022)

Lignocellulosic components	This Study
Cellulose	33.83 ± 1.02
Hemicellulose	17.07 ± 0.98
Lignin	26.71 ± 0.83

Debaryomyces hansenii and *Saccharomyces cerevisiae* had been rejuvenated in slant agar. Three loops of the yeast had been cultivated on a sterile Yeast Extract Peptone Dextrose liquid medium with the composition of 10 g/L peptones, 5% yeast extract, and 20% glucose. The inoculum was incubated at 30°C with the agitation of 150 rpm for 16-20 h for *Saccharomyces cerevisiae* for bioethanol production and 48 h for *Debaryomyces hansenii* for xylitol production.

EFB was then dried with an oven and was milled to a size of 60-80 mesh. Alkaline pre-treatment was then done by using 5% NH3 with a ratio of 1:10 of NH3 to EFB. After

the alkaline pre-treatment process, the fibres were then heated using an autoclave at 1.5 Bar for 15 minutes.

Saccharification was done by enzymatic hydrolysis with Cellic Htec (5% v/v of buffer volume) at 50°C for 48 hours with an agitation rate of 130rpm. The fermentation of EFB for bioethanol production was using SSF methods based on a previous study by Hanidah (2010), with some modifications. Fermentation at pH 4.8 at 60°C for 48 hours with an agitation rate of 130 rpm and EFB substrate used was 5% of the buffer amount. This process yields 0.32 g ethanol/g glucose and 0.10 g xylitol/g xylose.

Table 4.11 Objective and summary of (Mardawati *et al.*, 2020)

Objective of the study: To discover the optimum parameters to produce bioethanol with xylitol as a by-product	
Pre-treatment	2 step pre-treatment of alkali and autoclave method
Chemical agent	5 % NH3 (1:10 ratio of NH3:EFB)
Autoclave parameter	1.5 Bar for 15 min
Fermentation Method	SHF
Hydrolysis method	Enzymatic hydrolysis by Cellic Htec
Saccharification parameter	50°C for 48 hours with an agitation rate of 130rpm
Fermentation Parameter	pH 4.8 at 60°C for 48 hours with an agitation rate of 130 rpm and as *Saccharomyces cerevisiae* inoculum
Bioethanol yield	0.32 g ethanol/g glucose
Xylitol yield	0.10 g xylitol/g xylose

4.1.1.2.5 The Scaling up Model for Developing Second-Generation (2G) Bioethanol by using Palm Empty Fruit Bunches Feedstock

On 2018, Sawarni Hasibuan and Hermawan Thaheer, did a research about the development of a scaling model for bioethanol production using lignocellulosic EFB.

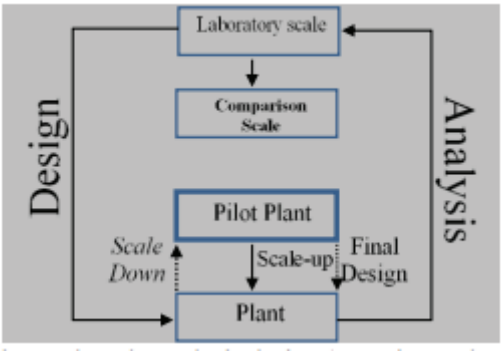

Figure 4.2 Scale multiplication process according to (Hasibuan and Thaheer, 2018)

Figure 4.2 shows the scale multiplication process, using math and knowledge calculations, a commercial scale configuration method is designed based on data gathering from data collecting equipment not for building, but for in-depth critical analysis.

Figure 4.2 illustrates the scaling up process, based on the data collected from data acquisition and welding of data by designing a of a commercial scale process for detailed critical analysis but not for construction and by using mathematical and knowledge-based calculations.

After multiple rounds of processing the data based on the scale and design calculations, scaling up the design is possible to a larger scale. Figure 4.2 shows the process of scaling up which are as follows:

1. Study of chemical process
2. Analysis of economic feasibility by following the business plan
3. Calculating the process through different simulations to get an idea of the commercial scale parameters
4. Validifying the commercial scale parameter by repeating as precise as possible through laboratory scale
5. Optimizing the process based on the range closest to the commercial scale
6. Validate the model using a medium scale

After simulation this study concludes that in an industrial scale model, production of 18.6 KL of bioethanol (99.6% ethanol content) per day would require the support of at least 3 crude palm oil plants with a capacity of 30-45 tonnes EFB per hour. is assumed

to be required. The model calculated starting material for approximately 600 tons of empty fruit bundles per day.

Table 4.12 Objective and summary of (Hasibuan and Thaheer, 2018)

Objective of the study: To find out the process of developing a scale up model for bioethanol production using EFBC as feed	
Feed required	600 ton/day.
Ethanol production	18.6 KL/day

4.1.2 Oil Palm Trunk (OPT)

4.1.2.1 Oil Palm Trunk sap (OPTS)

4.1.2.1.1 Old oil palm trunk: A promising source of sugars for bioethanol production

On 2010, Akihiko Kosugi and team, conducted a study of old oil palm trunk: A promising source of sugars for bioethanol production. The study aims to study the potential of old OPT to produce bioethanol by difference in trunk positioning, sectioning and storage time.

This study states that palm trees are replanted after 20-25 years. This is due to the decrease in palm oil production 20-25 years after the tree has been planted. During the replanting process, the palms are cut so that new trees can be planted. These old palms tend to be burned or just thrown away. Therefore, the waste can be used to produce bioethanol.

OPT was gathered at Ara Kuda, Kedah, Malaysia. The average height of the palm was 12m as shown in the figure 4.3 below. The log was then stored without direct sunlight and kept at 28-32°C with humidity ranging between 70-80%. After a certain amount of time (0-120 days), a 10cm disc was cut from the log with the end of each cut(5cm) being trimmed to avoid microbial contaminations. The cut was then section into inner (A), intermediate (B) and outer (C) sections as shown in below. The samples were then kept in an airtight plastic bag and kept in a freezer at 20°C until further analysis.

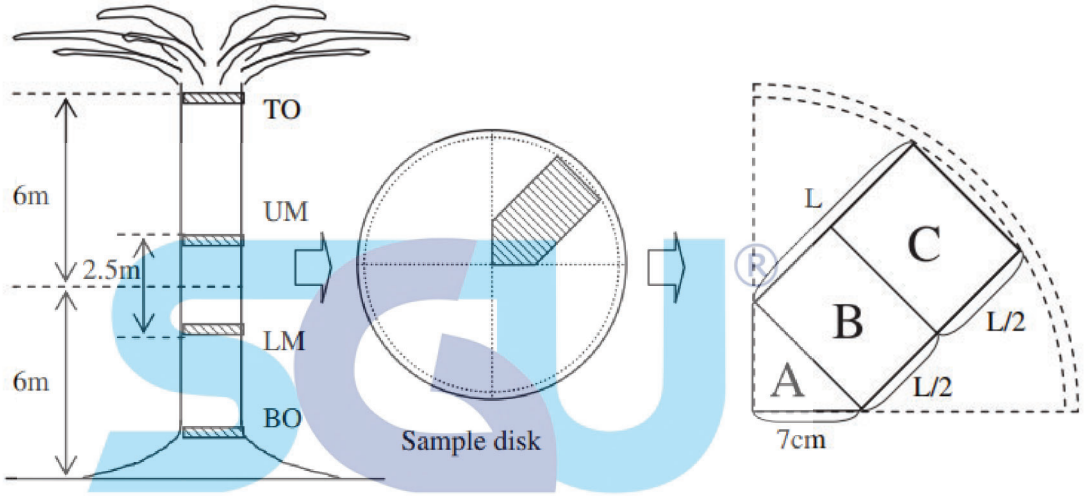

Figure 4.3 preparation of OPT(Yamada *et al.*, 2010)

Each sample was then dried at 105°C for 48 h and then cut into 2cm by 2cm by 5cm pieces. The samples were then squeezed with a laboratory scale press at 80 MPa to collect the sap. The sap is then centrifuged at 7,000G for 15 minutes before being analysed by phenol-sulfuric acid method for total sugar analysis. The table below shows that the inner part of the trunk produces the most sap while the upper middle part produces the most sugars.

Table 4.13 Total sugar content of different OPT parts (TO: top, UM: upper middle, LM: lower middle and BO:bottom)(Yamada *et al.*, 2010)

Total sugar(mg ml^{-1})				
	TO	UM	LM	BO
Inner (A)	111.8	129.9	129.2	93.0
Intermediate (B)	72.7	118.0	94.2	102.8
Outer (C)	71.1	103.6	81.6	107.7
Average	85.2	117.2	101.7	101.2
Sugars were analyzed by phenol-sulfuric acid method.				

Figure 4.4 below shows the total amount of sugars in OPTS in accordance to the storage time. There was a steady rise of the total amount of sugars from day 0 to day 30. The increase in sugars begins to slow down after 30 days up until 60 days. After day 60, the amount of sugar begins to decrease. This was due to microbial infections which was caused by fungal penetration after prolonged storage. The figure also shows that the approximate maximum amount of fermentable sugar was 128 mg/ml between day 30 and 60.

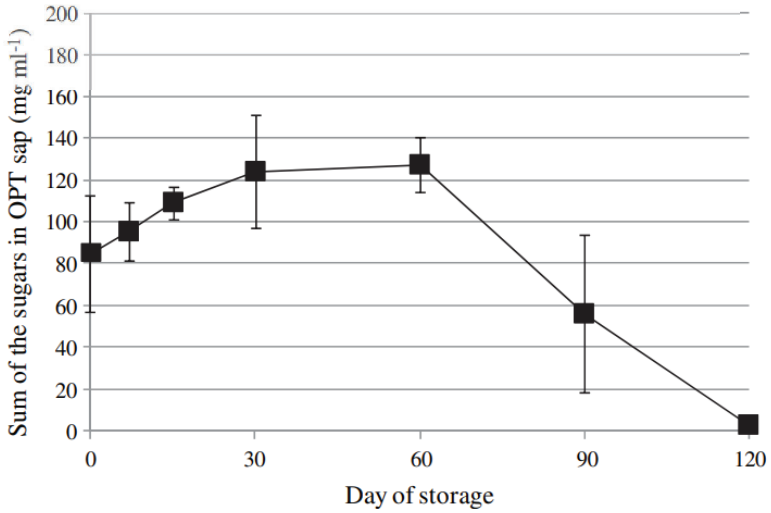

Figure 4.4 Sum of sugars in OPT sap(Yamada *et al.*, 2010)

A summary of this study can be shown in the table 4.14 below. It shows that the inner part of the upper middle trunk of the palm contains the most amount of sugar for

fermentation. It also shows that the trunk should be squeezed between 30-60 days after it has been cut to so that it contains approximately 128 mg/ml of fermentable sugars for bioethanol production.

Table 4.14 Objective and summary of (Yamada *et al.*, 2010)

Objective of the study: To find out the optimum storage duration of OPTS	
Trunk height	Upper Middle
Trunk section	Inner
Storage time	30-60 days
Fermentable sugars	128 mg/ml

4.1.2.1.2 Development of sap compressing systems from oil palm trunk

On 2013, **Yoshinori Murata**, conducted a study to develop a sap compressing system for OPT. The study aims to study to develop a sap compressing system for old OPT. The OPT obtained for this research was gathered from Johor, Malaysia. This study compares the different types of cutters and millers at different conditions to find out the best way to obtain sap from OPT.

3 cutters, 12mm in width, 100mm in with and polylayer blade was tested. It was shown that the 12mm width cutter produces a sap yield of 77.7% of the 70-80% moisture content of the trunk.

Table 4.15 Objective and summary of (Murata *et al.*, 2013)

Cutters[a]	Sap yield[b], %	Consumed power[c], MJ
12 mm width	77.7 ± 5.7	2.9 ± 0.5
100 mm width	69.6 ± 8.8	1.3 ± 0.2
Polylayer blade	71.6 ± 9.7	5.0 ± 1.7

Table 4.15 shows 3 shredder blades, 12mm in width, 100mm in with and polylayer blade was tested. It was shown that the 12mm width shredder produces a sap yield of 77.7%. The shredder which consumes the least amount of power however was the 100mm width cutter which consumed 1.3 MJ during the cutting process.

Table 4.16 Objective and summary of (Murata *et al.*, 2013)

Diameter of log	Diameter of log, mm	Shredded chips, kg	Moisture in chips[c], %	Sap yield[d], %		
				1st mill	2nd mill	Total[e]
150 mm av.[a]	151.4	16.9	79.5	44.8	27.1	71.9
150 mm SD[a]	8.4	3.3	3.2	4.5	11.6	8.6
200 mm av.[b]	199.9	29.5	77.1	48.6	20.3	68.9
200 mm SD[b]	13.3	3.4	5.5	6.7	9.8	7.3

Table 4.16 different diameters to compress the sap from OPT. This configuration uses 2 mills. It can be seen that the logs with a diameter of 150mm was able to obtain a sap yield of 71.9% and 68.9 for the logs with 200mm diameter. The study then states that there was no significant impact between the two lengths as the difference in sap yield was miniscule.

Table 4.17 Objective and summary of (Murata *et al.*, 2013)

	Mill 1		Mill 2		Shredded chips, kg	Moisture in chips[a], %	Sap yield, %		
	Rotation, rad s^{-1}	Pressure, MPa	Rotation rad, s^{-1}	Pressure, MPa			1st mill	2nd mill	Total
Condition 1	0.22	14.7	0.25	17.7	31.2	81.4	40.1	31.2	71.3
Condition 2	0.22	17.7	0.25	23.6	26.0	80.1	51.1	19.3	70.4
Condition 3	0.22	29.5	0.25	32.5	31.7	84.3	50.8	28.8	79.6
Condition 4	0.33	14.7	0.36	17.7	27.4	82.2	51.3	21.2	72.5
Condition 5	0.33	17.7	0.36	23.6	29.0	78.1	46.7	20.9	67.6
Condition 6	0.33	23.6	0.36	29.5	26.6	81.5	51.1	25.1	76.2
Condition 7	0.76	14.7	0.83	17.7	33.2	82.1	33.7	29.7	63.5
Condition 8	0.76	29.5	0.83	32.5	31.5	84.6	40.0	20.9	60.9

Logs with 200 mm in diameter were used for squeezing trials.
The rotation and the pressure of roll on mill mean the processing condition for compression on Mill 1 and Mill 2.
a Moisture in chips was calculated on basis of total mass.

To determine the optimum rotation speed and pressure, the system had undergone 8 conditions with different rotation speed and pressure. It was found that a rotation speed of 0.22 rad/s and pressure of 29.5 MPa for the first mill and 0.25 rad/s and pressure of 32.5 MPa for the second mill achieves a sap yield of 79.6% which was the highest among all the conditions

Therefore, this study finds that the 12mm blade should be used to cut the OPT and OPT logs with an average diameter of 200mm should be used. A combination of 2 mills the first rotating at a speed of 0.22 rad/s with a pressure of 29.5 MPa and 0.25 rad/s with a pressure of 32.5 MPa for the second mill achieves a sap yield of 79.6%

Table 4.18 Objective and summary of (Murata *et al.*, 2013)

Objective of the study: To discover the optimum parameters to compress sap from OPT	
Shredder	12mm blade
Log diameter	200mm
Number of mills	2
Rotation speed	0.22 rad/s for 1st mill and 0.25 rad/s for 2nd mill
Pressure	29.5 MPa for 1st mill and 32.5 MPa for 2nd mill

4.1.2.1.3 Influence of nutrient addition on the bioethanol yield from oil palm trunk sap fermented by Saccharomyces cerevisiae

On 2017, Mohd Nasir Nor and team, conducted a study on the influence of nutrient addition on the bioethanol yield from oil palm trunk sap fermented by *Saccharomyces cerevisiae*. This research aims to find out which nutrient is the most effective for bioethanol production. The nutrients studied in this research were $MgSO_4$, Na_2HPO_4, $(NH_4)_2SO4$ and C_3H7NO_2.

The OPT gathered for the research was a 30-year old tree from FELDA Jengka, Pahang, Malaysia. The tree was first peeled so that the core which is the softer and richer sap filled part of the trunk can be accessed. The core is then cut into 20cm x 20 cm x 1 cm pieces. It is then squeezed by using a sugar cane juice extractor to extract the sap and filtered before frozen at -20°C until further use.

Samples were created by adding the various nutrient into the sap before the fermentation process. The dosage for each sample was 2 g/L and the fermentation was carried out by using *Saccharomyces cerevisiae* as the inoculum with a pH level of 6 with an agitation rate of 170 rpm at 32°C in an incubator shaker.

A maximum of 20.23 g/L (45.05% of theoretical ethanol yield) was achieved with a fermentation time of 24 h. There was not much of a difference after 24 h as 93% of the sugars were used during this period. The OPTS with MgSO4 had the highest yield of

bioethanol being 76.13% and a bioethanol concentration of 34.64 g/L. The study finds that the $MgSO_4$, $C_3H_7NO_2$, $(NH_4)_2SO_4$ and Na_2HPO_4 were ranked in order of productivity with $MgSO_4$ being the most productive and Na_2HPO_4 being the least.

Table 4.19 Objective and summary of (Shahirah *et al.*, 2015)

Objective of the study: To find the best nutrient for bioethanol production	
Inoculum	*Saccharomyces cerevisiae*
Nutrient	MgSO4
Bioethanol concentration	34.64 g/L

4.1.2.1.4 Physical Factors Optimization of Saccharomyces cerevisiae Fermentation to Enhance the Production of Bioethanol: A Review

On 2015, Nazia Hossain and team, conducted a study Sugar and bioethanol production from oil palm trunk. The aim of the study was to research the utilization of old OPT to produce both sugar and bioethanol. This study utilizes different parts of the trunk to produce sugar and bioethanol which will give clear indication on which part of the trunk is best to produce bioethanol.

OPT was gathered from a 22 years old palm oil tree in Selangor, Malaysia. The tree was then divided into 3 parts: top, middle and bottom. After that, it was then cut into small pieces and squeezed to extract the sap. The sap was then filtered using a sieve and heated with a hot plate at 100°C for 3-4 hours. 2 samples were tested in this study, one with the nutrient and one without the nutrient. The nutrient filled with 1 g of Alanine amino acid and 1 g of $MgSO_4$ before the fermentation process. Both went through the same fermentation procedure. 50 ml of the samples were put in a flask alongside 1 g of *Saccharomyces Cerevisiae* and 30 ml of distilled water. Since an anaerobic fermentation needs to take place, cotton was used to make the flasks air tight. Both samples were fermented at 37°C for 96 hours with an agitation rate of 170 rpm (no pH correction was done). Every 24 hours, samples of each batch would be analysed for both ethanol and sugar percentage (w/w). The table below shows the batch without nutrients added.

Table 4.20 Percentage of Ethanol and Sugar content according to fermentation time without nutrients (Hossain and Jalil, 2017)

OPT parts	Percentage (%)	24 hours (1st Day)	48 hours (2nd Day)	72 hours (3rd Day)	96 hours (4th Day)
Upper	Ethanol	38.5	38.0	39.0	41.0
	Sugar	14.7	14.9	14.9	15.2
Middle	Ethanol	28.5	29.0	29.5	30.5
	Sugar	11.8	11.9	11.9	12.2
Bottom	Ethanol	31.5	31.5	32.0	32.5
	Sugar	12.4	12.5	12.7	12.7

The table below shows the batch with nutrients added. It can be seen that there was an increase in bioethanol percentage for all parts of the trunk with nutrients added compared to that without nutrients.

Table 4.21 Percentage of Ethanol and Sugar content according to fermentation time with nutrients (Hossain and Jalil, 2017)

OPT parts	Percentage (%)	24 hours (1st Day)	48 hours (2nd Day)	72 hours (3rd Day)	96 hours (4th Day)
Upper	Ethanol	51.0	58.34	53.0	49.34
	Sugar	20.34	20.6	21.2	21.14
Middle	Ethanol	42.67	44.84	45.84	45.17
	Sugar	16.24	16.77	16.87	16.97
Bottom	Ethanol	45.34	47.0	46.0	43.0
	Sugar	16.74	17.34	17.17	17.4

The table below is the summary of this study. It shows that the upper part of the trunk has the highest percentage(w/w) and is therefore the most efficient. The highest percentage was 58.34% (w/w) when fermented for 48 hours with the upper OPTS as feed. It also shows that nutrients increased the amount of bioethanol produced.

Table 4.22 Objective and summary of (Hossain and Jalil, 2017)

Objective of the study: To find out if nutrients increase the amount of bioethanol produced as well as the most efficient part to produce bioethanol	
Inoculum	*Saccharomyces cerevisiae*
Nutrient	MgSO4 and Alanine amino acid

Trunk position	Upper of the trunk
Fermentation time	48 hours
Bioethanol percentage(w/w)	58.34%

4.1.2.1.5 UTILIZATION OF OIL PALM TRUNK FOR BIOETHANOL PRODUCTION

In 2021, Muhammad Iqbal Herawan, conducted a review of oil palm trunk to produce bioethanol. The research was conducted to find out the best procedure to produce bioethanol using Oil Palm Trunk sap as feed. The research was approached with the literature study method by collecting and reviewing previous researches on the matter to safely get a reliable outcome.

As mentioned before, the study gathers various literature to get the wanted outcome in this case, the best parameters to produce bioethanol from Oil Palm Trunk sap as feed. The study finds that although different inoculum can be used to ferment OPTS, *Saccharomyces cerevisiae* to be the best to produce bioethanol due to its versatility and thermal resistant. The temperature of the fermentation found to be most effective for fermenting with *Saccharomyces cerevisiae* as the inoculum was shown to be between 30-37°C and the optimum pH level is a bit on the acidic side being 4-6.

This study also suggests that OPTS is more recommended to be used to produce bioethanol rather than dried OPT. It discusses that although dried OPT can produce a greater quantity compared to OPTS, the process of bioethanol production using OPTRS as feed is found to be much simpler than dried OPT. The complexity of bioethanol production using dried OPT as feed are the added chemicals which are not good for the environment, energy expenditure during the pre-treatment process as well as the specific equipment needed to break down cellulose.

A summary of this study can be shown in the table 4.23 below. It shows the best course of action to achieve optimum production of bioethanol by using OPTS as a feed. The table shows that to achieve a bioethanol production of 47.5 g/L, the pH should be 4.0 with a fermentation time of 24 hours and an agitation rate of 150 rpm at 30°C should be used.

Table 4.23 Objective and summary of (Herawan, 2021)

Objective of the study: To find out the best fermentation parameters for bioethanol production from OPTS	
pH	4.0
Temperature	30°C
Inoculum	*Saccharomyces cerevisiae*
Fermentation Time	24 hours
Agitation Rate	150 rpm
Bioethanol production	47.5 g/L

4.1.2.2 Lignocellulosic Oil Palm Trunk (OPTC)

4.1.2.2.1 The conversion of lignocellulosic biomass to bioethanol: pretreatment technology comparison

In 2020, Agustin Wardani and team, conducted a comparison of different pretreatment technology This research aims to find the pre-treatment process which increases the cellulose level and decreases the lignin level in OPT so that it can be more suitable as feed to produce bioethanol. The research compares 2 pre-treatment methods, subcritical water pre-treatment method and alkali peroxide method. The OPT was gathered from PT.Sampoerna Agro Tbk., Palembang. The trunks were sterilized and cut into 1cm pieces before the experiment.

The alkali peroxide pre-treatment was done using 2 concentration, H_2O_2 1% and 5%. NaOH was added to both solutions until a pH of 11.5 was reached. The pre-treatment process starts by adding 100ml of the prepared solution to 5 g of the sample (OPT) in an Erlenmeyer flask. It was then incubated for 2 different durations (3 hours and 72

hours) and temperatures (28°C and 80°C). The samples were then vacuum filtered and washed with distilled water. Finally, it was placed in an oven at 106 °C until the weight of the samples were constant.

The subcritical water pre-treatment method starts by adding 5g of the sample, 55ml of distilled water and magnetic stirrer into a reactor. After this, the reactor was heated at 170°C and nitrogen was injected until a desired pressure of 320 psi was achieved. The process was done for 20 minutes at 500 rpm. It was then let down to cool and passed through a vacuum filter to collect the sample. Finally, it was placed in an oven at 106 °C until the weight of the samples were constant.

Table 4.24 Increase/decrease in cellulose, hemicellulose and lignin content according to the pre-treatment method (Wardani, Tanaka and Sutrisno, 2020)

Pretreatment	Increase of cellulose level (%)	Decrease of hemicellulose level (%)	Decrease of lignin level (%)
H2O2 1% 80°C 3 hours	24,71	14,67	-
H2O2 5% 80°C 3 hours	35,23	5,76	-
H2O2 1% 28°C 72 hours	44,86	20,16	2,79
H2O2 5% 28°C 72 hours	47,89	47,96	16,89
Subcritical water	34,01	53,23	-

Table 4.24 below shows the increase in cellulose level, decrease in hemicellulose level and decrease in lignin level. The highest increase in cellulose level was the H2O2 5% at 28°C for 72 hours with an increase of 47.59%. The highest decrease in lignin level was also the H2O2 5% at 28°C for 72 hours with a decrease of 16.89%.

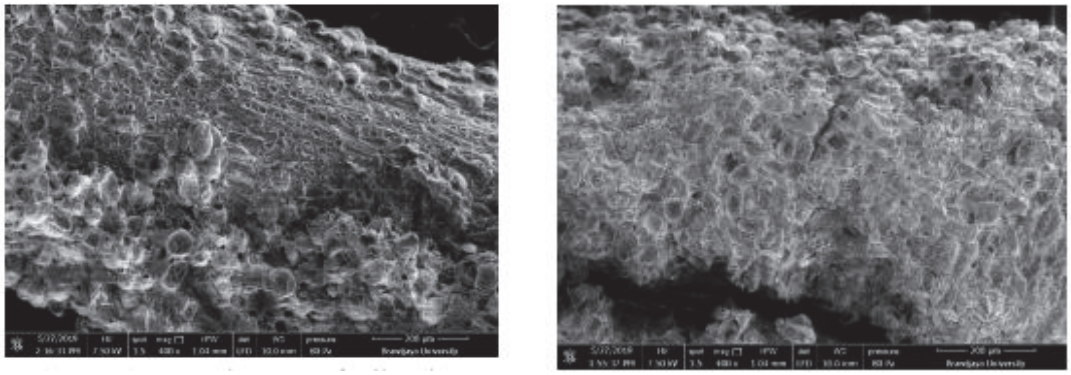

Figure 4.5 SEM images of OPT treated with alkali peroxide method (left) and subcritical water method (right) (Wardani et al., 2020)

SEM analysis was also done by comparing untreated OPT with 2 methods. As seen from the figure below, it can be seen that there was a clear different between the treated and untreated OPT. The untreated image shows clump like layers in the surface of the OPT where the image treated with the subcritical water pre-treatment method and alkali peroxide method shows holes. Although both images show holes, it can be seen that the one treated with the alkali peroxide method show more holes.

A summary of this study can be shown in the table 4.25 below. It shows the best course of action to increase the cellulose level and decrease the lignin level of OPT so that it can be more suitable for bioethanol production. It shows that the alkali peroxide method was chosen at a temperature of 28°C for 72 hours with a concentration of H2O2 of 5% will increase the cellulose level by 47.89% and decrease the lignin level by 16.89%. The OPT after pre-treatment will be more suitable due to the increase in cellulose level and decrease in lignin level.

Table 4.25 Objective and summary of (Wardani, Tanaka and Sutrisno, 2020)

Objective of the study: To discover which pre-treatment studied was more suitable for bioethanol production using OPTC as feed	
Pre-treatment Method	Alkali peroxide
Chemical agent	Hydrogen peroxide
Temperature	28°C

Incubation time	72 hours
Hydrogen peroxide concentration	5%
Cellulose increase	47.96%
Lignin decrease	16.89%

4.1.2.2.2 UTILIZATION OF OIL PALM TRUNK FOR BIOETHANOL PRODUCTION

In 2021, Muhammad Iqbal Herawan, conducted a review of oil palm trunk to produce bioethanol. The research was conducted to find out the best procedure to produce bioethanol using lignocellulosic Oil Palm Trunk as feed. The research was approached with the literature study method by collecting and reviewing previous researches on the matter to safely get a reliable outcome.

As mentioned before, the study gathers various literature to get the wanted outcome in this case, the best parameters to produce bioethanol from lignocellulosic Oil Palm Trunk as feed. The study finds that although different inoculum can be used. *Saccharomyces cerevisiae* and *Zymomonas mobilis* are popular choices because of how efficient they are in converting glucose to ethanol. *Saccharomyces cerevisiae* however is more ideal as the study finds that it can still efficiently convert glucose to ethanol while fermenting at room temperature and with a low pH level.

Hydrolysis is critical in breaking down cellulose and hemicellulose into sugars for fermentation. The study finds that acid hydrolysis and enzymatic hydrolysis are popular choices. Acid hydrolysis is done to usually break the link between cellulose and hemicellulose and proceeded by enzymatic hydrolysis which breaks down the cellulose and hemicellulose to sugars by help of enzymes. Acid hydrolysis is the effective but the most effective acids tend to be corrosive and hazardous. For hydrolysis to be more effective, the study finds that the OPT must be subjugated to pre-treatment first Physical pre-treatment, chemical pre-treatment, thermal pre-treatment care and a mix of some or all pre-treatment methods can be done for bioethanol production.

To optimally produce bioethanol from lignocellulosic OPT firstly, physical pre-treatment is done by shredding is done to reduce the size of the OPT proceeded by steam explosion method to let access to the enzymes so that it can convert it into sugars more easily. The alkaline extraction method was then done to extract unconvertible sugars. SSF was then performed using Enzymatic Hydrolysis by Celluclast and Novozyme 188 for 12 hours at 50°C and *Saccharomyces cerevisiae* SC90 as the inoculum. Fermentation was done for 96 hours at 40°C to achieve a yield of 44.25% (g ethanol / g substrate)

Table 4.26 Objective and summary of (Herawan, 2021)

Objective of the study: To discover the best parameters for bioethanol production from OPTC	
Pre-treatment Method	Steam Explosion and Alkaline Pretreatment
Hydrolosis Method	Enzymatic Hydrolysis by Celluclast and Novozyme 188
Fermentation method	SSF
pH	4.0
Inoculum	*Saccharomyces cerevisiae SC90*
Temperature	40°C
Fermentation Time	96 hours
Agitation Rate	150 rpm
Ethanol yield	0.46 g/g sugar
Bioethanol production	44.25 g/L

4.1.2.2.3 Utilization of urea as a nitrogen source for ethanol production from oil palm trunk using simultaneous saccharification and fermentation

In 2021, Afrasiab Khan Tareen and team, conducted a review of oil palm trunk to produce bioethanol. The research was conducted to study the utilization of urea at different concentrations as nitrogen source for ethanol production from oil palm trunk using simultaneous saccharification and fermentation. This research compares urea at different concentrations to yeast and peptone extract as a nitrogen source. OPT ages 25-26 which are deemed of no use were gathered and obtained from Krabi, Thailand. *Saccharomyces cerevisiae* SC90 was chosen as the inoculum for this experiment. The inoculum was grown at 30°C for 48–60 hours on agar plates containing yeast extract, peptone and dextrose (YPD). 20 g/L of agar, peptone and glucose was present while 10 g/L of yeast extract was present on the agar plate. It was then incubated at 30°C for 18 hours with an agitation rate of 150 rpm with a rotary shaker.

OPT was chopped into pieces of 20mmx20mmx5mm. Steam explosion pre-treatment was done to the chopped OPT using hot water at 80°C for 30 minutes with a solid to liquid ratio of 1:8. It was then subjected to alkaline extraction pre-treatment using NaOH 15%(w/v) at 90°C with a solid to liquid ratio of 1:8.

A 500 ml Erlenmeyer flask was filled with 10 g/L of yeast extract, 220 g/L of glucose and 20 g/L of peptone as control medium. The researched medium of 220 g/L of glucose, 2 g/L of KH_2PO_4, 5 g/L of $MgSO_4$ and urea at different concentrations(1,2,4 and 7 g/L) were used as a source for nitrogen. Sodium citrate buffer was used to bring the pH level to 4.8 and sterilization at 121°C for 15 minutes with the prepared starter culture 10% was added to the flask. Fermentation by SSF was conducted in a rotary shaker with a pH level of 4.8 at 40°C for 96 hours with an agitation rate of 150 rpm using enzymatic hydrolysis by 15 FPU/g cellulose of Celluclast 1.5 L and 15 IU/g β-glucosidase of Novozyme 188 were used.

Table 4.27 Fermentation kinetics of yeast extract and peptone and urea as nutrient(Tareen *et al.*, 2021)

Fermentation kinetics	Nitrogen source (g/L)	
	Yeast extract and peptone	Urea concentration at 1 g/L
μ (hr^{-1})	0.22 ± 0.04	0.18 ± 0.02
C_p (g/L)	43.79 ± 0.17	37.09 ± 0.18
Q_p (g/L/hr)	0.59 ± 0.09	0.51 ± 0.06
$Y_{p/s}$ (g/g)	0.49 ± 0.05	0.41 ± 0.02
Theoretical yield (%)	97.37 ± 1.07	81.24 ± 0.39

μ = specific growth rate (2–10 hr); C_p = maximum ethanol concentration; Q_p = ethanol productivity; $Y_{p/s}$ = ethanol yield

The table above shows that the fermentation kinetics of the sample from the yeast and peptone extract are higher than that of the one that of the urea concentration at 1g/L. Although urea as a nitrogen source is lacking compared to that of yeast and peptone as a nitrogen source, the paper concluded that urea can be used as an alternative for a low-cost nitrogen source instead of yeast extract and peptone may be used for batch ethanol fermentation in the SSF process.

Table 4.28 Objective and summary of(Tareen *et al.*, 2021)

Objective of the study: To discover if urea is viable as an alternative low-cost nitrogen source for bioethanol production	
Pre-treatment Method	Steam Explosion and Alkaline Pretreatment
Hydrolysis Method	Enzymatic Hydrolysis by Celluclast and Novozyme 188
Fermentation method	SSF
Nitrogen source	Urea
Urea concentration	1 g/L
Inoculum	*Saccharomyces cerevisiae* SC90

pH	4.8
Temperature	40°C
Fermentation Time	96 hours
Agitation Rate	150 rpm
Ethanol yield	0.41 g ethanol /g sugar
Bioethanol production	37.09 g/L

4.1.2.2.4 Comprehensive approach to utilize hydrogen peroxide sterilization and urea as nitrogen source for ethanol production from oil palm trunk

In 2022, Afrasiab Khan Tareen and team, conducted a review of oil palm trunk to produce bioethanol. The research was conducted to study the use of hydrogen peroxide for sterilization. This study builds upon the previous research of Utilization of urea as a nitrogen source for ethanol production from oil palm trunk using simultaneous saccharification and fermentation and compares sterilization using hydrogen peroxide to the autoclaving method. This research build upon (Tareen *et al.*, 2021) as shown in chapter 4.1.2.2.3 therefore, it has the same pre-treatment methods as well as fermentation parameters

Table 4.29 Fermentation kinetics of yeast extract and peptone and urea as nutrient (Danbamrongtrakool *et al.*, 2022)

Fermentation kinetics	Nitrogen medium (g/L)	
	Yeast extract and peptone	Urea concentration at 1 g/L
μ (h^{-1})	0.22 ± 0.05	0.18 ± 0.03
C_p (g/L)	43.83 ± 0.14	37.41 ± 0.19
Q_p (g/L/h)	0.60 ± 0.09	0.51 ± 0.01
$Y_{P/S}$ (g/g)	0.50 ± 0.01	0.52 ± 0.00
Theoretical yield (%)	97.24 ± 1.12	82.30 ± 0.40

μ = specific growth rate (2–10 hr); C_p = maximum ethanol concentration; Q_p = ethanol productivity; $Y_{P/S}$ = ethanol yield

The fermentation kinetics above shows approximately the same results as (Tareen *et al.*, 2021) as it uses the same materials methods with the exceptions of using both autoclave and H2O2 for sterilization.

Table 4.30 Fermentation kinetics of sterilization by autoclave and H2O2(Danbamrongtrakool *et al.*, 2022)

Fermentation kinetics	Sterilization					
	Autoclaving (control)	Sterilization time of media (hr)				
		0	4	8	12	24
μ (h^{-1})	0.18 ± 0.01^{b}	0.24 ± 0.04^{a}	0.24 ± 0.04^{a}	0.18 ± 0.06^{b}	0.17 ± 0.024^{c}	0.017 ± 0.01^{c}
C_p (g/L)	37.01 ± 0.19^{a}	26.14 ± 1.94^{d}	28.00 ± 2.71^{cd}	30.05 ± 3.39^{c}	33.48 ± 0.41^{b}	33.64 ± 0.07^{b}
Q_p (g/L/h)	0.51 ± 0.01^{a}	0.50 ± 0.18^{a}	0.39 ± 0.14^{a}	0.36 ± 0.03^{a}	0.37 ± 0.03^{a}	0.42 ± 0.05^{a}
$Y_{P/S}$ (g/g)	0.52 ± 0.01^{a}	0.38 ± 0.04^{d}	0.40 ± 0.01^{cd}	0.43 ± 0.04^{c}	0.47 ± 0.02^{b}	0.47 ± 0.01^{b}
Theoretical yield (%)	82.30 ± 0.40^{a}	61.02 ± 0.63^{c}	62.39 ± 3.67^{c}	68.87 ± 5.52^{b}	74.23 ± 2.66^{b}	74.35 ± 0.13^{b}

μ = specific growth rate (2–10 hr); C_p = maximum ethanol concentration; Q_p = ethanol productivity; $Y_{P/S}$ = ethanol yield;
Values (mean ± SD) within a row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Sterilization with hydrogen peroxide at different concentration (0 g/L, 0.1 g/L, 0.5 g/L and 1 g/L) was compared to that of the autoclaving method. The table above shows that sterilization with H2O2 at 0.5 g/L, the optimized period of sterilization before fermentation was 12 hr to produce the maximum values for ethanol concentration. Consequently, the cost of ethanol production based on SSF could be reduced through the utilization of urea as an alternative to yeast extract and peptone in conjunction with

H_2O_2 as a sterilizing agent as the cost of sterilization per cycle of hydrogen peroxide was only $18.98 to that of the cost of autoclaving per cycle costing $34.39.

A summary of this study can be shown in the table 4.31 below. It shows the best course of action to achieve optimum production of bioethanol by using OPTS as a feed. The table shows that to achieve a bioethanol production of 47.5 g/L, the pH should be 4.0 with a fermentation time of 24 hours and an agitation rate of 150 rpm at 30°C should be used.

Table 4.31 Objective and summary of(Danbamrongtrakool *et al.*, 2022)

Objective of the study: To discover if hydrogen peroxide as an alternative low-cost sterilization method for bioethanol production	
Pre-treatment Method	Steam Explosion and Alkaline Pretreatment
Sterilization method	Hydrogen peroxide (0.5 g/L)
Hydrolysis Method	Enzymatic Hydrolysis by Celluclast and Novozyme 188
Fermentation method	SSF
Nitrogen source	Urea
Urea concentration	1 g/L
Inoculum	*Saccharomyces cerevisiae* SC90
pH	4.8
Temperature	40°C
Fermentation Time	96 hours
Agitation Rate	150 rpm
Ethanol yield	0.47 g ethanol /g substrate

Bioethanol production	33.48 g/L

4.1.2.2.5 Kinetic study of ethanol production from different sizes of two-step pre-treated oil palm trunk by fed-batch simultaneous saccharification and fermentation

In 2022, Imrana Niaz Sultan and team, conducted a review of oil palm trunk to research the effect of particle size by way of fed-batch simultaneous saccharification and fermentation. The aim of this research was to find out the effects of particle size when using OPT as feed for bioethanol production. The Oil Palm trunk for this research was obtained from the Krabi province in southern Thailand.

The OPT gathered were first cut into 20mm x 20mm x 5mm pieces. The pre-treatment process is comprised of 3 pre-treatment processes being: steam explosion process, hot-water extraction process and the delignification process.

Pre-treatment begins by separating the OPT into batches of 150g and were steam-exploded using a 10L pressurized vessel at 210°C for 4 min. After the steam explosion process, the hot-water extraction process was done at 80°C for 30 minutes by using a solid liquid ratio of 1:8 respectively. After this process, the slurry mixture was filtered so that the OPT fibre can be separated and extracted from the slurry. They are then washed with tap water to neutralize the pH level Delignification was then done by using 15% of the weight per volume of the OPT fibre to NaOH 60%. The delignification process was done at 90°C for 60 minutes after which, the OPT fibres were oven dried at 80°C until a constant weight was achieved.

Before pre-treatment, the composition of OPT was 39.65% cellulose, 77.01% holocellulose, 23.47% lignin, 11.42% extractives and 1.46% ash on dry weight basis. After the pre-treatment process, the increase in cellulose was 77.59% and increase in holocellulose was 89.41% and the composition of OPT was 9.80% lignin, and 0.52% ash on dry weight basis. This important as the cellulosic material is important to produce bioethanol and increase of it would be beneficial and the decrease in lignin content is also beneficial as it hinders bioethanol production.

3 batches were then chosen less than 40mm in diameter, 40-60mm in diameter and more than 60 mm in diameter. The OPT fibres were processed into the desired sizes and bioethanol production can begin. To produce bioethanol, SSF method was chosen. It was done in a 500ml Erlenmeyer flask containing 300ml of YP media. The-pre-treated OPT was added simultaneously to separate flasks along with 10% starter culture by 15 FPU/g cellulose of Celluclast 1.5 L and 15 IU/g β-glucosidase of Novozyme 188 were used. SSF fermentation with pH of 4.8 was carried out in an incubator at 40 °C and agitation rate of 150 rpm for 98 h.

The table below shows the glucose concentration and conversion during SSF. It can be seen that the conversion rate and glucose concentration of the OPT fibre with the particle size of 40mm is the highest.

Table 4.32 Glucose conversion and concentration for each particle size (Sultana *et al.*, 2022)

Particle size (mesh)	Glucose concentration (g/L)	Conversion (%)
Control	83.37±2.12^{b}	94.37±3.01^{b}
40 mm	87.36±1.87^{a}	98.88±2.16^{a}
40–60 mm	77.24±2.75^{c}	87.81±1.98^{c}
60 mm	72.85±1.91^{d}	82.46±2.42^{d}

$^{a-d}$ Values (mean ± SD) within same column with different lowercase superscripts are significantly ($p < 0.05$) different

The table below shows that the sample with the particle size of 40mm has the highest ethanol concentration, ethanol productivity and ethanol yield compared to the other samples. It shows that the optimum size for this study would be 40mm for bioethanol production.

Table 4.33 Fermentation kinetics for each particle size (Sultana *et al.*, 2022)

Particle size (mesh)	Ethanol concentration (g/L)	Ethanol productivity (g/L/hr)	Ethanol yield (g/g)
Control	39.85±1.09^{b}	0.40±0.41^{b}	0.46±0.28^{a}
40	43.07±0.62^{a}	0.47±0.12^{a}	0.47±0.16^{a}
40–60	35.95±0.92^{c}	0.39±0.23^{ab}	0.39±0.22^{b}
60	32.35±1.28^{d}	0.36±0.58^{c}	0.34±0.13^{c}

Values (mean ± SD) within the same column with different lowercase superscripts are significantly ($p < 0.05$) different

A summary of this study can be shown in the table4.34 below. It shows the best course of action to achieve optimum production of bioethanol by using OPTC as a feed. The table shows that to achieve a bioethanol production of 43.7 g/L, ethanol productivity of 0.47 g/L/hr and ethanol yield of 0.47 g/g, particle size of 40mm mesh must be used. This study does not show if particle size lower than 40mm will be beneficial.

Table 4.34 Optimum production conditions in this study (Sultana *et al.*, 2022)

Objective of the study: To find out the best particle size for bioethanol production from OPTC	
Pre-treatment Method	Steam Explosion and Alkaline Pretreatment
Feed size	40mm
Hydrolosis Method	Enzymatic Hydrolysis by Celluclast and Novozyme 188
Fermentation method	SSF
Inoculum	*Saccharomyces cerevisiae* SC90
pH	4.8
Temperature	40°C
Fermentation Time	96 hours

Agitation Rate	150 rpm
Ethanol yield	0.47 g ethanol /g substrate
Bioethanol production	43.07 g/L

4.1.3 Oil Palm Frond (OPF)

4.1.3.1 Oil Palm Frond sap (OPFS)

4.1.3.1.1 Efficient utilization of oil palm frond for bio-based products and biorefinery

In2014, Mior Ahmad Khushairi Mohd Zahari and team, conducted a research on the Efficient utilization of oil palm frond for bio-based products and biorefinery. The research was conducted to know the effects of supplying nitrogen during the fermentation process. The experiment was done with *Saccharomyces cerevisiae* 10% (v/v). It was then separated into 3 mediums, medium A: heat sterilized, nitrogen source supplemented OPF juice, medium B: heat sterilized, no nitrogen source supplemented OPF juice and medium C: the control medium where commercial sugars were mixed to that of the same concentration in medium A. All mediums were cultivated with a rotary shaker at 30°C for 48 h with an agitation rate of 150 rpm.

To test the effect of supplementing nitrogen in bioethanol production, peptone and yeast extract was also supplemented to the fermentation medium. Medium A was shown to have a higher ethanol concentration than medium B due to the supplementation of nitrogen and the maximum ethanol production was shown to be faster by 12 hours as shown in the graph where medium A's maximum was in hour 24 while B was in hour 36. Nitrogen deficiency was shown to be the reason that medium B has a slower rate of sugar utilization. The yield for medium A was 0.49 g ethanol/g sugars. All of this can be seen in figure 4.6 shown below.

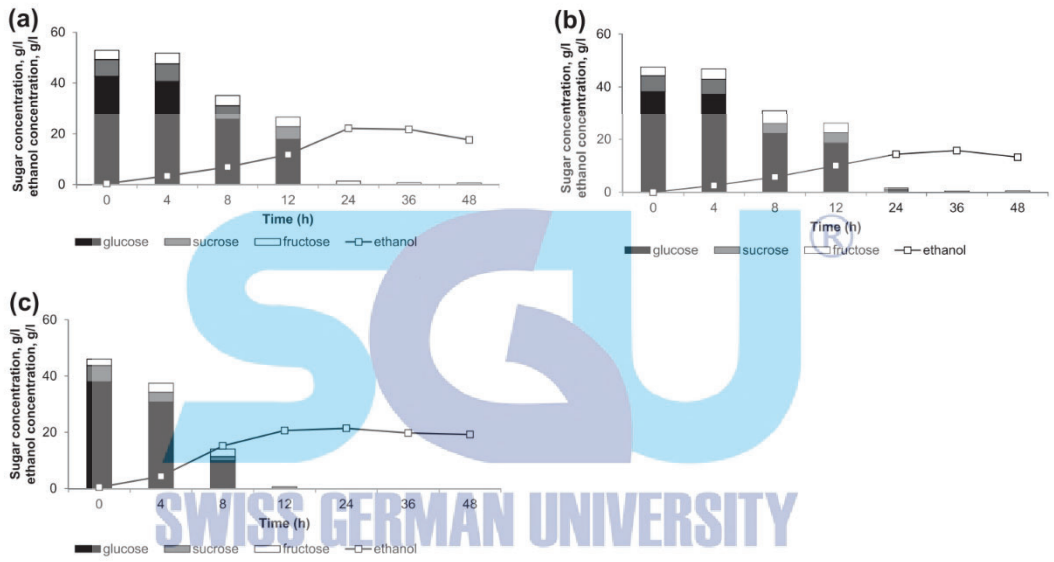

Figure 4.6 Ethanol production, sugars consumption and pH profile in (a) medium A (heat sterilized, nitrogen source supplemented OPF juice), (b) medium B (heat sterilized, no nitrogen source supplemented OPF juice), (c) control medium.(Zahari *et al.*, 2014)

A summary of this research can be shown in the table 4.35 below. It shows the best course of action to achieve optimum production of bioethanol by OPFS. The study shows that a yield of 0.49 g ethanol/g sugars can be achieved by supplying nitrogen during the fermentation to increase the sugar utilization rate.

Table 4.35 Optimum production conditions in this study (Zahari *et al.*, 2014)

Objective of the Study: The effects of supplying nitrogen during the fermentation process	
Supplementation	Nitrogen
Inoculum	*Saccharomyces cerevisiae*
Temperature	30°C
Fermentation time	24 hours
Agitation rate	150 rpm
Yield	0.49 g ethanol/g sugars

4.1.3.1.2 Fresh oil palm frond juice as a renewable, non-food, non-cellulosic and complete medium for direct bioethanol production

In 2015, Sharifah Soplah Syed Abdullah and team, conducted a study on fresh oil palm frond juice as a renewable, non-food, non-cellulosic and complete medium for direct bioethanol production. The study was conducted to combat the degradation of OPFS so that it can yield the best results for bioethanol production. The OPF for this research was obtained from Oil Palm trees at Selangor, Malaysia. To achieve the most accurate results, the bottom fronds were harvested just like how the planters harvest it during pruning. The petiole of the frond was the only part collected while the rest was used for the nutrition of the Oil Palm trees. The petiole was then squeezed by using a three-roller hydraulic press machine. 1 kg of petiole wield approximately 500g of OPFS. The juice was then centrifuged at 15,000 g, 4 ◦C for 15 min. This was done to remove solid particles from the juice. Lastly, the solid-free juice was stored at -20◦C for further use. The sugarcane research was bought from Seremban, Negeri Sembilan, Malaysia. Lastly, a control medium was concocted with commercial sugars that was comprised of glucose, sucrose, fructose and nitrogen supplements such as peptone and yeast extract. The fermentation for this experiment was conducted at 30 ◦C for 48 h with an agitation rate of 150 rpm. Figure 4.7 shows the potential of OPFS as feed resulted in a yield of 0.38 g ethanol/g sugars without any pH corrections and supplementations.

This value was not far off from the control medium which yielded of 0.40 g ethanol/g sugars.

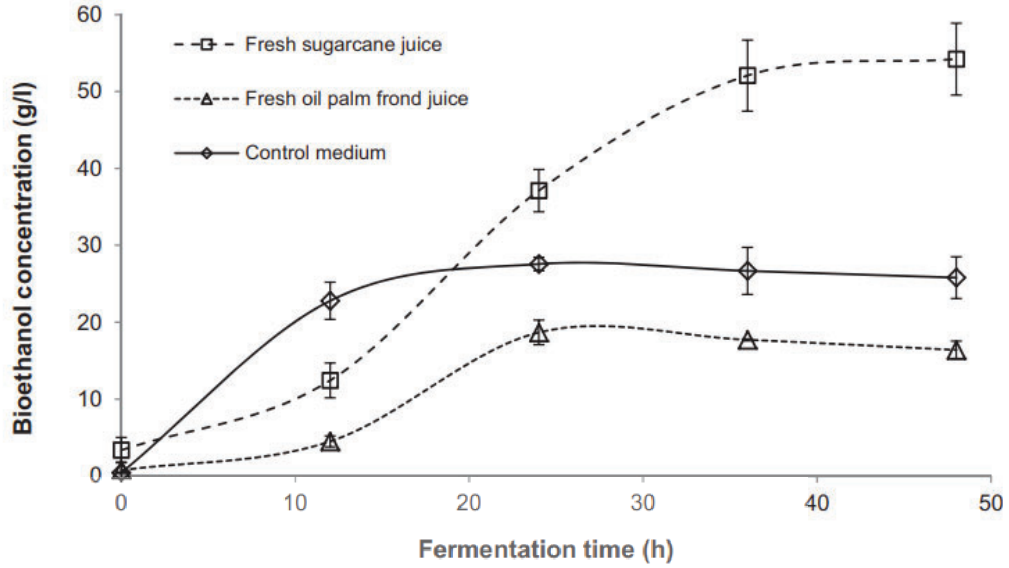

Figure 4.7 Bioethanol production during fermentation by *Saccharomyces cerevisiae* in OPFS, Sugarcane juice and control medium. (Abdullah *et al.*, 2015)

A summary of this research can be shown in the table 4.36 below. It shows the best course of action to achieve optimum production of bioethanol by OPFS. The study shows that a yield of 0.38 g ethanol/g sugars can be achieved with these parameters.

Table 4.36 Optimum production conditions in this study (Abdullah *et al.*, 2015)

Objective of study: To discover if OPF was a viable alternative to other juice feeds	
pH	4.84
Temperature	30°C
Fermentation Time	48 hours
Agitation Rate	150rpm
Yield	0.38 g ethanol/g sugars

4.1.3.1.3 OIL PALM FROND JUICE AS A FERMENTATION SUBSTRATE FOR BIOETHANOL PRODUCTION USING SACCHAROMYCES CEREVISIAE

In 2020, Nathalie Gloria, conducted a review of oil palm frond to produce bioethanol. The research was conducted to find out the best parameters to produce bioethanol using Oil Palm Frond Juice as feed. The research was approached with the literature study method by collecting and reviewing previous researches on the matter to safely get a reliable outcome.

As seen on table 4.37, the parameters set to achieve the optimum production in this research was pH, temperature, agitation rate and fermentation time while keeping the material, pre-treatment process and inoculum constant from all researches.

The study shows that of the 3 Oil Palm waste reviewed (EFB,OPT and OPF), OPF was the most suitable Oil Palm waste to be used as feed to produce bioethanol mainly due to it being the most inexpensive way for bioethanol production. The study states that equipment costs can be cut down because OPFS only needs to be obtained by simply pressing the frond. One other factor is that OPFS is shown to be made of approximately 77.69% overall free sugars while containing an array of simple sugars such as glucose and fructose.

The study also explains that there are 2 factors in determining the quality of OPFS. These factors are the age of the tree and its stem. The age of the tree directly corelates to the amount of OPFS produced as well as its sugar concentration. Trees age from 3-4 years old yield the highest sugar concentration whereas trees ranging from 5-25 years old produces has a lower sugar concentration. This shows that the OPFS yield as well as its sugar concentration is indirectly proportional to its age. The highest sugar concentration is located connected to the inner part of OPT as the inner part has been shown to contain a higher amount compared to the other part of the trunk.

A summary of this study can be shown in the table 4.37 below. It shows the best course of action to achieve optimum production of bioethanol by extracting the OPFS by pressing and then fermenting the juice with the inoculum *Saccharomyces cerevisiae*

with the optimal conditions of pH, temperature, agitation rate and fermentation duration.

Table 4.37 Objective and summary of (Gloria, Legowo and Kartawiria, 2020)

Objective of the study: To find out the best fermentation parameters for bioethanol production from OPFS	
pH	4,84
Temperature	30°C
Agitation Rate	150 rpm
Fermentation Time (hour)	24 hours
Bioethanol concentration	18.67 g/L

4.1.3.1.4 The influence of storage conditions on oil palm frond juice as a renewable feedstock for bioethanol production

In 2021, Sharifah Soplah Syed Abdullah and team, conducted a study on the influence of storage conditions on oil palm frond juice as a renewable feedstock for bioethanol production. The study was conducted to combat the degradation of OPFS so that it can yield the best results for bioethanol production. The OPF for this research was obtained from Oil Palm trees at Taman Pertanian Universiti, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. To achieve the most accurate results, the bottom fronds were harvested just like how the planters harvest it during pruning. The petiole of the frond was the only part collected while the rest was used for the nutrition of the Oil Palm trees. The petiole was then squeezed by using a three-roller hydraulic press machine. 1 kg of petiole wield approximately 500g of sap. The juice was then centrifuged at 15,000 g, 4 ◦C for 15 min. This was done to remove solid particles from the juice. Lastly, the solid-free juice was stored at -20◦C for further use.

As seen on table 4.38, the parameters set to achieve the optimum results in this research was the concentration of OPF juice/sap and storage temperature. To concentrate the prepared juice, a concentrated using a rotary evaporation system. The juice was then concentrated to remove 30%, 50%, 70% (v/v) of water from it. It was then named to

amount of water removed and 0% was used as a control in this experiment. The samples were then stored airtight and put at different temperatures for 20 days where the glucose content, initial and final pH as well as the bacterial count was monitored.

Figure 4.8 describes the glucose content of the various OPF juice concentration when stored at different temperatures. The degradation rate is found to be directly proportional to the water content and indirectly proportional to the temperature. It was also shown that microorganism grow cultivates best at the 30◦C–40 ◦C. This means that the higher the concentration and temperature is favoured for bioethanol production as it restricts the growth of bacteria and microorganism. To concentrate the prepared juice, a concentrated using a rotary evaporation system. The juice was then concentrated to remove 30%, 50%, 70% (v/v) of water from it. It was then named to amount of water removed and 0% was used as a control in this experiment. The samples were then stored airtight and put at different temperatures for 20 days where the glucose content, initial and final pH as well as the bacterial count was monitored. As seen on table 4.38, the parameters set to achieve the optimum results in this research was the concentration of OPF juice and storage temperature.

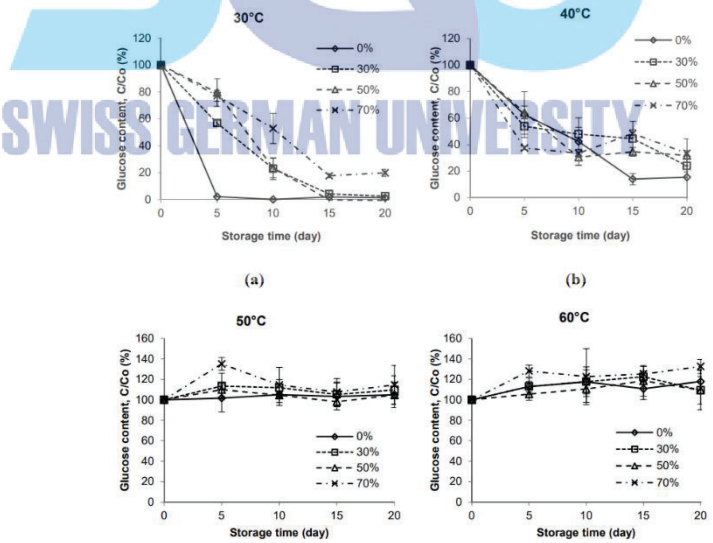

Figure 4.8 Glucose degradation profile in OPF juice stored at different temperatures (a) 30 ◦C, (b) 40 ◦C, (c) 50 ◦C and (d) 60 ◦C.(Abdullah *et al.*, 2021)

Figure 4.9 describes the initial and final pH level of the various OPF juice concentration when stored at different temperatures. All samples except for the one stored at 50 ◦C. This is important as the reduction in pH level is poor for bioethanol production.

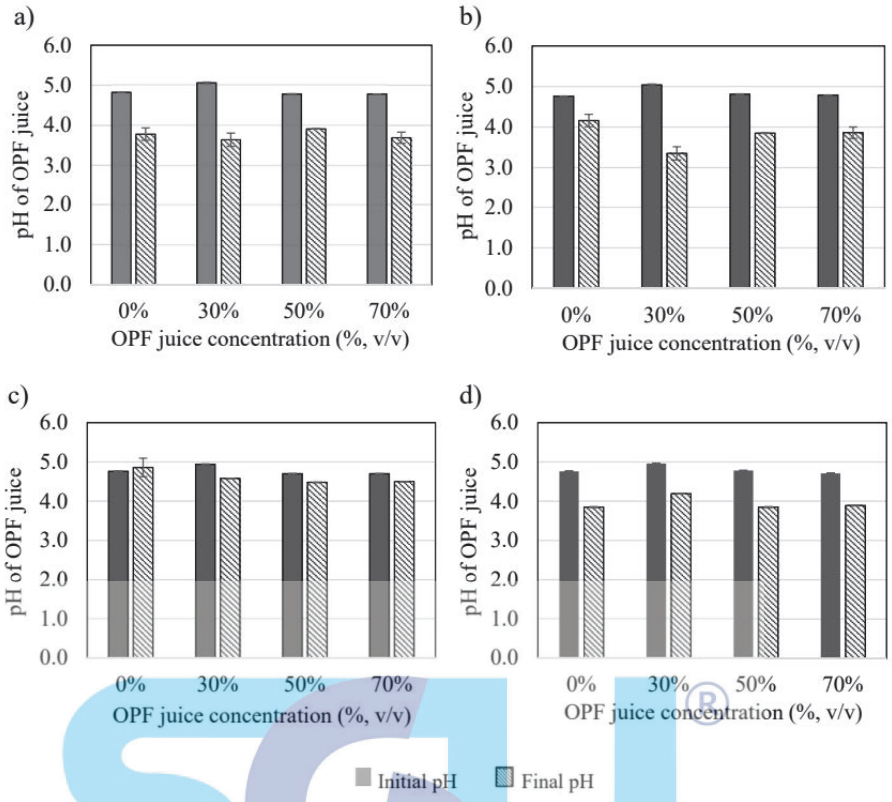

Figure. 4.9 The pH profiles of various concentrations of OPF juices stored at different temperatures a) 30 ◦C, b) 40 ◦C, c) 50 ◦C, d) 60 ◦C for 20 days.(Abdullah *et al.*, 2021)

A summary of this study can be shown in figure 4.10 and table 4.38 below. It shows the best course of action to achieve optimum results for bioethanol production. OPFS should be stored at 50 ◦C with at least 50% of the water removed from the juice to achieve the optimum results.

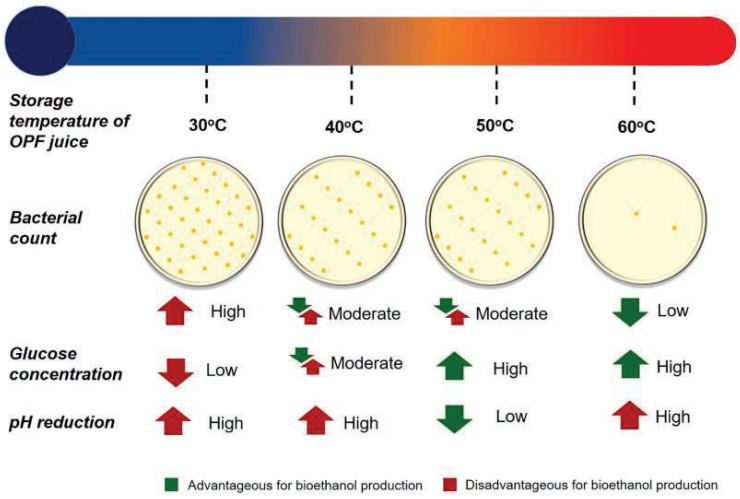

Fig. 4.10 Glucose and pH levels under different conditions (Abdullah *et al.*, 2021)

Table 4.38 Objective and summary of(Abdullah *et al.*, 2021)

Objective of the study: To discover the best parameters to store OPFS	
Water reduction(v/v%)	50-70%
Temperature	50°C

4.1.3.1.5 The influence of storage conditions on oil palm frond juice as a renewable feedstock for bioethanol production

In 2021, Poonsuk Prasertsan and team, conducted a study on Direct biotransformation of oil palm frond juice to ethanol and acetic acid by simultaneous fermentation of co-cultures and the efficacy of its culture filtrate as an antifungal agent against black seed rot disease. Unused OPF was used to produce bioethanol using *Saccharomyces cerevisiae* as an inoculum with a initial pH level of 4.83 conducted at room temperature (30±2 °C) for 72 h. 3 different methods of fermentation were tested, single- staged fermentation and two-stage fermentation focusing solely on ethanol production where the simultaneous fermentation model is used to produce acetic acid.

Figure 4.11 shows that the single-stage model has the highest yield of ethanol production being, 0.4 g ethanol/g sugars without any pH correction nor any supplement. It is highest of all 3 models presented.

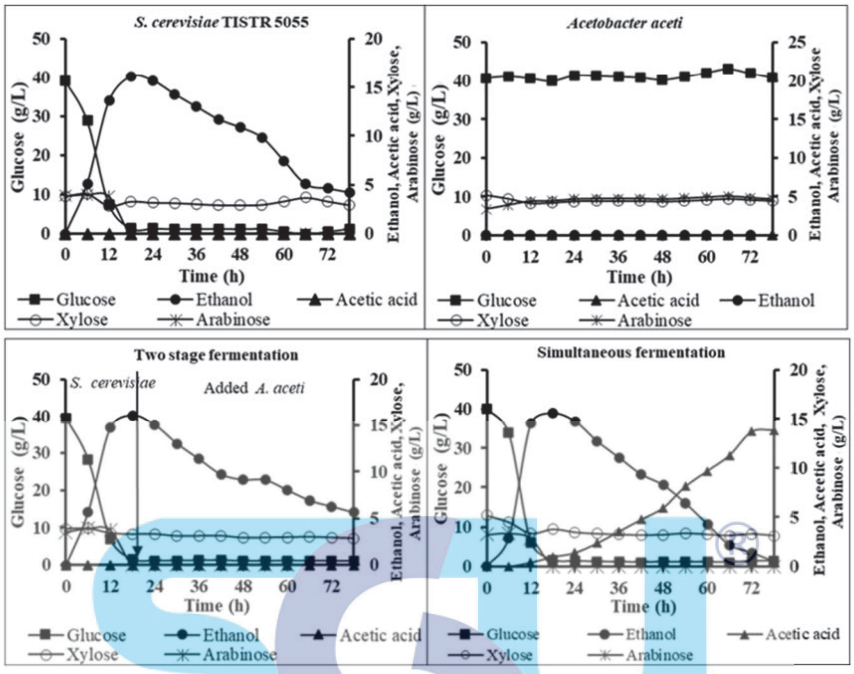

Fig. 4.11 Time course on sugar utilization, ethanol, and acetic acid production by S. cerevisiae TISTR5055 and A. aceti under single-stage, two-stage, and simultaneous fermentation from oil palm frond juice in shake-flask (150 rpm) culture at room temperature (30±2 °C) (Prasertsan *et al.*, 2022)

Figure 4.12 shows the simultaneous fermentation model with different shaking. The simultaneous fermentation model of OPFS which involves shaking did not have any impact to ethanol production but did have impact for acetic acid production. Therefore, it is not recommended to either shake or introduce aeration into the fermentation process for the optimum production of bioethanol.

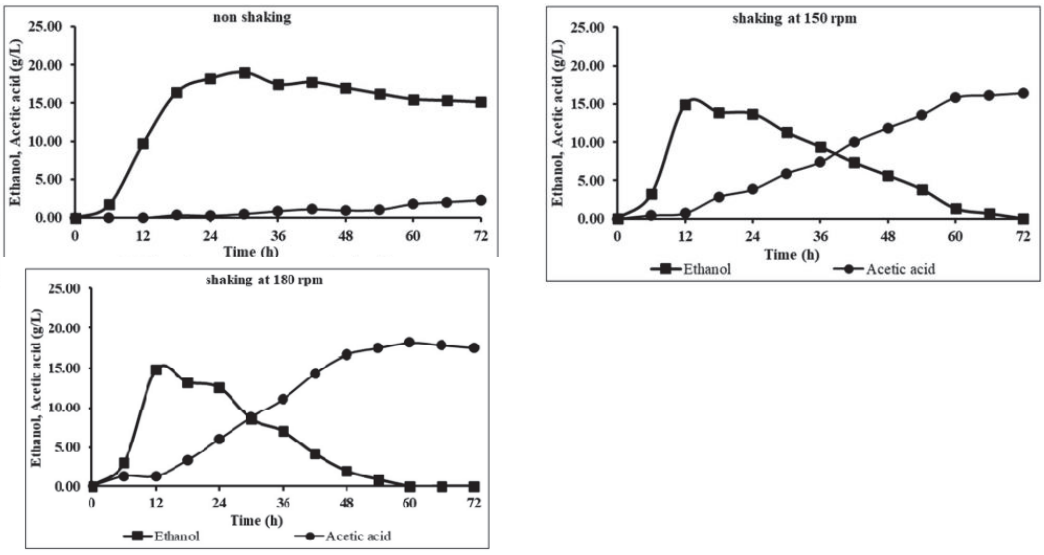

Fig. 4.12. Effect of shaking speed on ethanol and acetic acid production from oil palm frond juice by S. cerevisiae TISTR5055 and A. aceti under simultaneous fermentation at room temperature (30±2 °C) for 72 h (Prasertsan *et al.*, 2022)

Figure 4.13 shows the simultaneous fermentation model with different aeration rates. The simultaneous fermentation model of OPFS which involves aeration rates did not have any impact to ethanol production but did have impact for acetic acid production. Therefore, it is not recommended to either shake or introduce aeration into the fermentation process for the optimum production of bioethanol.

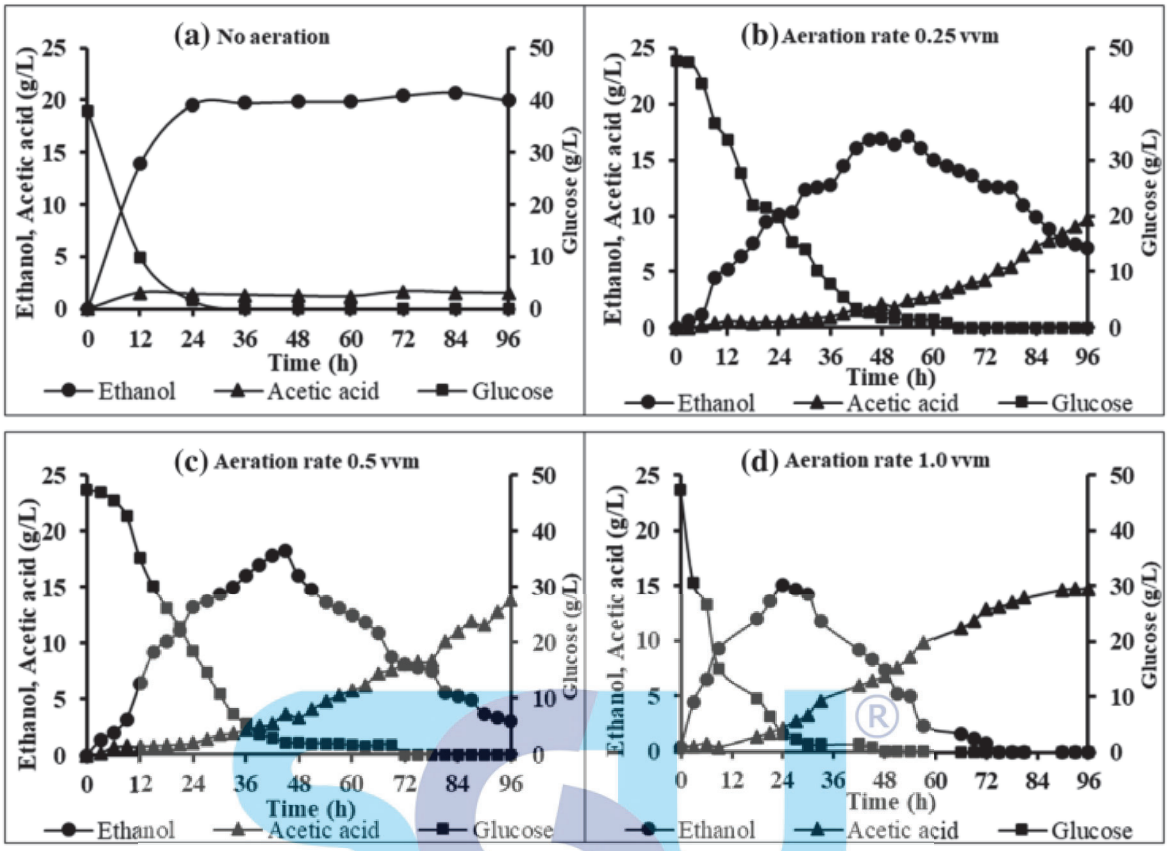

Fig. 4.13 Effect of aeration rate on ethanol and acetic acid production from OPF juice under simultaneous fermentation of S.cerevisiae TISTR5055 and A.aceti in 5L fermenter with an agitation speed of 180 rpm (Prasertsan *et al.*, 2022)

A summary of this research can be shown in the table 4.39 below. It shows the best course of action to achieve optimum production of bioethanol by OPFS. The study shows that a bioethanol concentration of 19.59 g/L can be achieved with the parameters below.

Table 4.39 Objective and summary of (Prasertsan *et al.*, 2022)

Objective of the Study: To find out if aeration and shaking is required for bioethanol production	
pH	4.83
Temperature	30°C

Fermentation Time	24 hours
Agitation Rate	180 rpm
Aeration	none
Bioethanol Concentration	19.59 g/L

4.1.3.2 Lignocellulosic Oil Palm Frond (OPFC)

4.1.3.2.1 Hot compressed water pretreatment of oil palm fronds to enhance glucose recovery for production of second-generation bio-ethanol

In 2010, Chun Sheng Goh and team, conducted a study on the effects of hot compressed water pre-treatment of oil palm fronds to enhance glucose recovery for production of second-generation bio-ethanol produce bioethanol. The research was conducted to find out what hot compressed as a pre-treatment will result in for bio-ethanol production.

The OPF was from the Engineering Campus of University Science Malaysia, Penang, Malaysia. The OPF was shredded to a size of less than 1mm and then dried by leaving it under the sun. It was then separated into 0.500–1.000 mm, 0.250– 0.500 mm, 0.125–0.250 mm and less than 0.125mm with 58.68%, 15.72%, 2.98% and 22.63% of its weight as percentage respectively. A control of hydrolized OPF was used with Novozyme 188 and Celluclast 1.5 L as the enzymes. The process was done under STC of pH 6 at 40°C with 30 minutes incubation time.

The Hot Compressed water (HCW) pre-treatment was done using a batch reactor. The reactor had a heating rate of 5°C /min. The dried OPF was fed into the reactor where deionized water mixed in to the desired solid to liquid ratio (weight to volume). The process woks at 10 bar to keep the water in a liquid phase. After the process, the reactor is rapidly cooled to 100 °C and depressurized. The mixture was then ran through a vacuo-filtrate to separate the solids and liquids. To compare the effectiveness of HCW pre-treatment, sets of experiments were prepared by treating the pre-hydrolized biomass with 3% (v/v) sulfuric acid at 120°C for 30 min and with HCW assisted by 0.07% (v/v) sulfuric acid at the optimum conditions identified in this study.

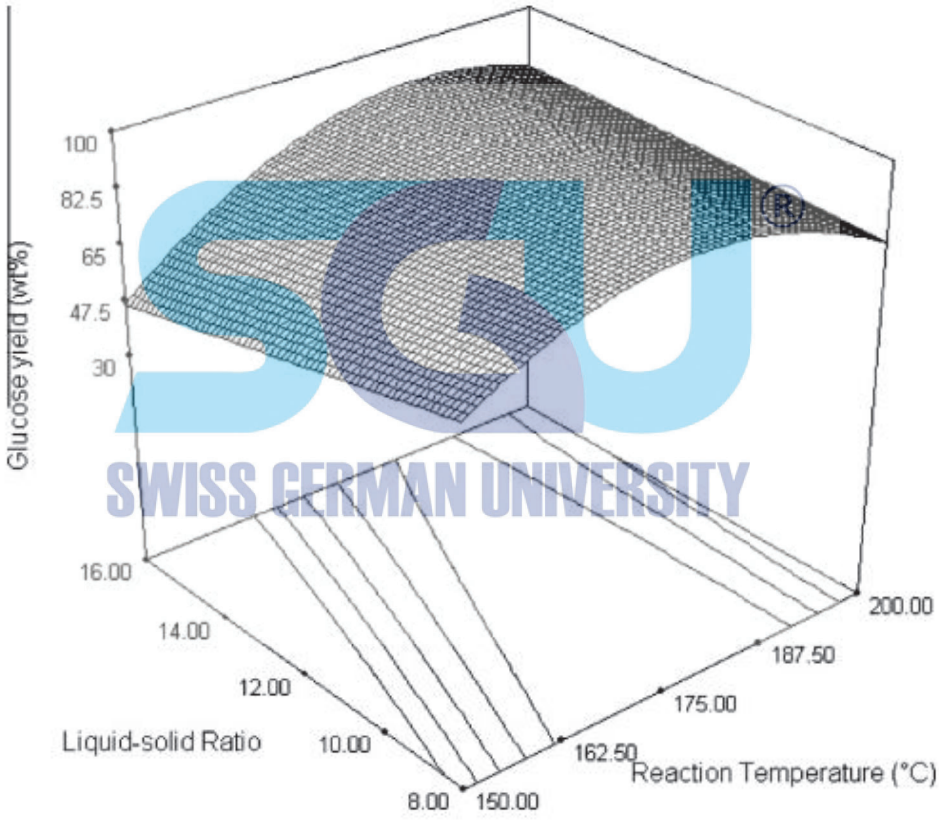

Figure 4.14 Three-dimensional response surface plot of glucose yield: effect of liquid–solid ratio and reaction temperature at a reaction time of 12.5 min.(Goh, Lee and Bhatia, 2010)

As seen in the image above, the optimization of glucose yield was done using three variables that were within the parameters of experimental runs. The program projected that the optimum reaction temperature, residence time, and liquid to solid ratio were at 178°C, 11.1 min, and 9.6, respectively, and that these circumstances would provide the optimal glucose yield, with a predicted glucose yield of 92.69 wt.%. By doing trial runs under the recommended ideal process parameters, the projected optimal yield was

confirmed. The experimental run produced an actual maximum output of 92.78%wt, which was in close accord with the figure determined by the model.

Table 4.40 Optimum production conditions in this study (Goh, Lee and Bhatia, 2010)

Objective of the Study: To study the effects of hot compressed water pre-treatment for bioethanol production	
Hydrolysis agent	Sulphuric acid (3% (v/v))
Residence time	11.1 min
Liquid to Solid ratio	9.6
Temperature	178°C
Optimum yield	92.78 w.%

4.1.3.2.2 Ultrasonic-assisted simultaneous saccharification and fermentation of pre-treated oil palm fronds for sustainable bioethanol production

In 2014, Cynthia Ofori-Boateng and team, conducted a study ultrasonic-assisted simultaneous saccharification and fermentation of pre-treated oil palm fronds for sustainable bioethanol production. The research was conducted to find out the effects of ultrasonic when used during the for simultaneous saccharification and fermentation process for bioethanol production.

The OPF for this study were obtained from the oil palm plantation at the Engineering campus of University Science Malaysia. The petioles of the frond were shredded into 10-20 mm long pieces and washed after the removal of the leaflets. It was the dried at 105°C for 16 h to achieve a moisture content of 10% with an oven and grounded. The grounded fronds were then passes through a sieve shaker of 1mm.

At a frequency of 37 kHz and a power of 200 W, ultrasonic-assisted organosolv/H2O2 pre-treatment (UOP) was performed in an ultrasonic bath. In a 500 ml Erlenmeyer flask, the extractive-free OPF (2 g) was combined with 40 ml of 1.4% aqueous NaOH and 80% aqueous ethanol (1:4 v/v). After that, the flask was immersed in the ultrasonic cleaning solution for 30 minutes at 75°C. The residue was repeatedly rinsed with distilled water before being delignified with 3% aqueous H2O2 at room temperature for

16 hours. After that, deionized water was used to wash the delignified OPF many times until the pH reached 7. This stage's dried residue served as the SSF substrate.

Hydrolysis of pre-treated OPF by enzymes Celluclast 1.5 L and Novozyme 188 were used to perform an enzymatic saccharification of the cellulose in order to measure the rate of sugar production and degradation in OPFs. The enzyme solutions were combined with 5% (w/v) pretreatment OPF in a sterilized flask with a total working capacity of 100 ml. The medium was then maintained at pH 4.5 using 0.05 M sodium citrate buffer after being autoclaved for 15 minutes at 121°C. Tetracycline 0.05 g/l was added to the fermentation medium to stop microbial development. The flask and its contents were incubated at 50°C for 72 hours with agitation at 150 rpm in an incubator shaker. Samples were collected throughout a range of time periods.

In a 500 ml Erlenmeyer flask with a total liquid volume of 100 ml, the ultrasonic-assisted SSF was performed twice. A solid loading of 2.5–15.0% (w/v) was employed. After being autoclaved for 15 minutes at 121°C, the pre-treated fronds were combined with the enzyme solutions and 10 ml of yeast inoculum (5-20 g/l) and kept at pH 5 using 0.05 M sodium citrate buffer. The flask's contents were ultrasonicated (at a frequency of 37 kHz and an ultrasonic power of 200 W) over varying periods of time and temperatures (30–50 °C) (30–360 min). Throughout the experiment, cold water was continuously circulated in the ultrasonic bath to maintain temperature. The fermentation broth was filtered to remove the solid by-products from the liquid after the SSF procedure. The supernatant was then diluted, and the content of ethanol was determined after being further filtered using 0.45 ml regenerated cellulose membranes.

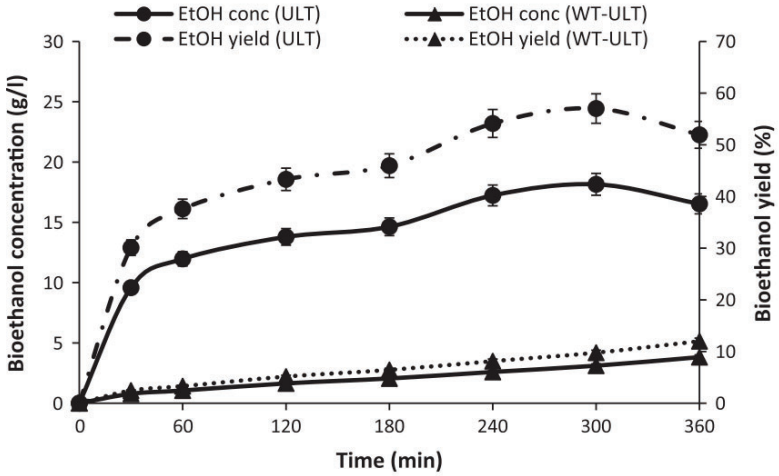

Figure 4.15 Comparison of optimized SSF process conditions for bioethanol production from organosolv/H2O2 pretreated OPF with and without ultrasound (ULT: SSF with ultrasonication, WT-ULT: SSF without ultrasound) (Ofori-Boateng and Lee, 2014)

The figure above shows that the ultrasonic assisted SSF has both a higher ethanol yield and concentration. Table 4.41 shows the summary of this study. It shows that a bioethanol yield of 57 % and bioethanol concentration of 18.2 g/L can be achieved with the set parameters.

Table 4.41 Optimum production conditions in this study (Ofori-Boateng and Lee, 2014)

Objective of the Study: To study the effects of ultrasonic-assistance during SSF for bioethanol production	
Pre-treatment	Ultrasonic-assisted organosolv/H2O2
pH	5
Enzymatic hydrolysis	Celluclast 1.5 L and Novozyme 188
Incubation time	5 h
Temperature	40°C
Yeast concentration	15 g/L(Saccharomyces cerevisiae)
Solid loading	10% (w/v)

Bioethanol yield	57%
Bioethanol Concentration	18.2 g/L

4.1.3.2.3 Increased Cellulose Levels in Organosolv Pretreatment Process in Bioethanol Production

In 2019, Saisa, conducted a study in increased Cellulose Levels in Organosolv Pretreatment Process in Bioethanol Production. The research was conducted to find out the effects of organosolv pre-treatment to produce bioethanol.

The OPF for this research was obtained from Oil Palm trees from PT. Fajar Baizuri & Brother in Nagan Raya District, Aceh. The material used for the study are the fibrous peeled part of the midrib. This material was then cut into to 2cm pieces to ease the drying process. An oven was used for the drying process. The cut-up pieces were then dried in the oven for 24 hours at 105°C and then crushed until they are made into a powder with a size of 250 mesh.

The pre-treatment process begin by determining the ratio of raw material to solvent ratio of 1:10 (mass of material/volume of the solvent). The raw material (powder dried OPF) is then delignified with ethanol with adjustments to the ethanol concentration, process time and temperature to determine the optimum parameters to increase the cellulose levels. After the pre-treatment process, holocellulose solids still in the cooking liquid were separated by filter and then washed with hot water. These holocellulose solids were then dried in the oven at 105°C.

Figure 4.16 shows the pre-treatment process at 100°C for 60 min with different ethanol concentrations (35%, 55%, 75%). When the concentration of ethanol is at 35% the lignin content and content was 17.4% and 41.15% respectively. At 55% the lignin content and content were 17.1% and 42.78% respectively and at 75%, the lignin content and content was 17.0% and 43.83% respectively. Although the lignin content decreases and cellulose content increases with the increase in ethanol concentration, there was a 2.68% difference in cellulose content between 35% and 75% ethanol concentration.

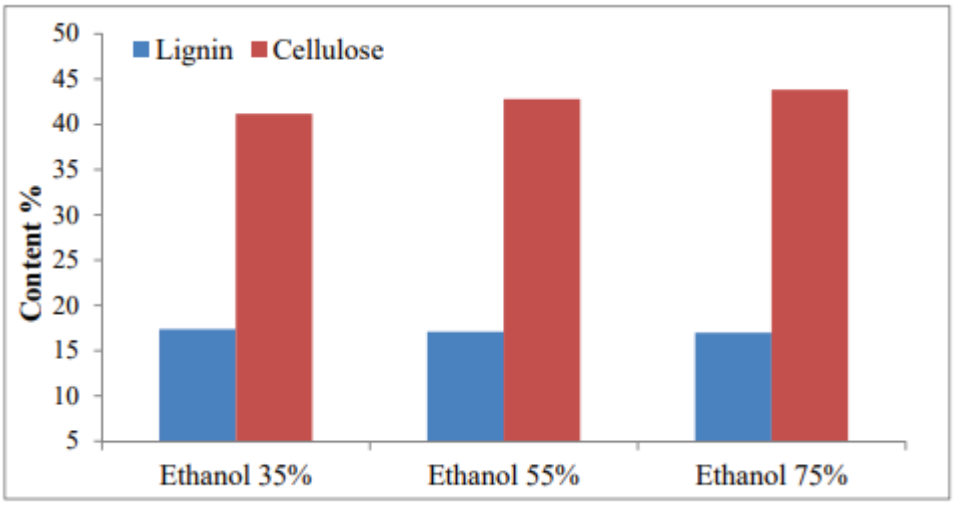

Figure 4.16 The results from analysis of lignin and cellulose content at100°C for 60 minutes (Husin, 2019)

Figure 4.17 shows the pre-treatment process at 150°C for 180 min with different ethanol concentrations (35%, 55%, 75%). At 75% the lignin content and content were 16.4% and 49.41%. There was a rise in cellulose content and a decrease in lignin content between the samples of 35% and 75% ethanol concentration. The increase in cellulose content was 1.63% and a decrease of 48.41% in lignin content. The results show that among all the experiments done in this study, the parameters with the highest amount of cellulose content and lowest amount of lignin content was the delignified dried OPF by use of 75% ethanol at 150°C with a process time of 180 min.

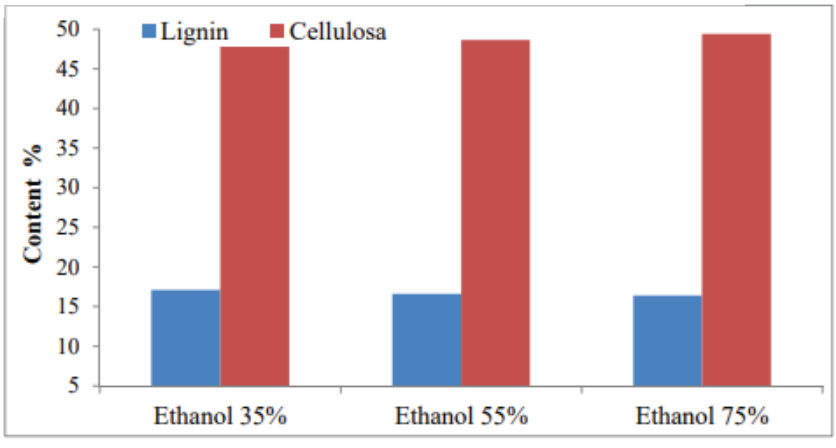

Figure 4.17. The results from analysis of lignin and cellulose content at 150°C C for 180 minutes(Husin, 2019)

Figure 4.18 shows the difference of the lignin and cellulose content between the untreated and treated dried OPF. The untreated sample has a lignin content of 19.8% and cellulose content of 34.3%. While the sample treated with 75% ethanol concentration at 150°C for 180 minutes has a lignin content of 16.4% and cellulose content of 49.41%. This shows that this pre-treatment process will be useful for bioethanol production as the higher the cellulose content, the higher the yield of bioethanol that will be produced by the feed.

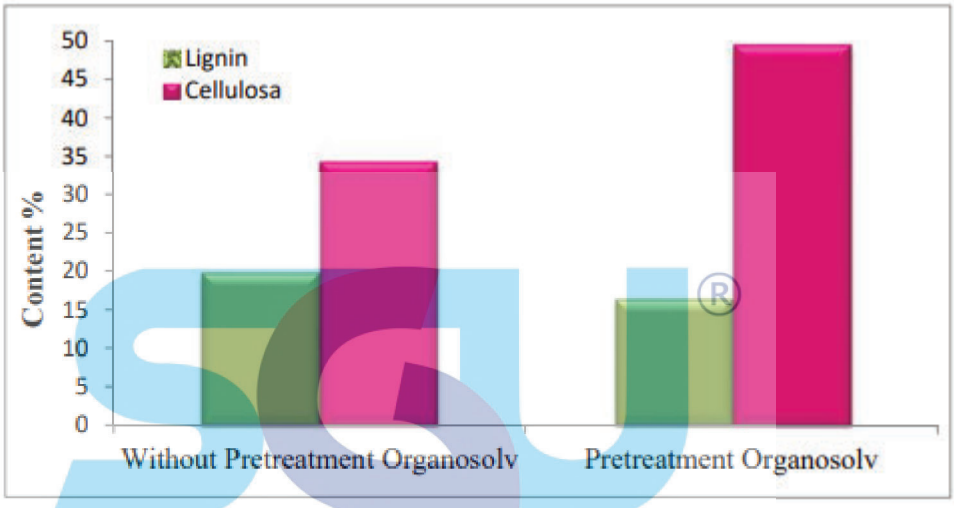

Figure 4.18 Effect of solvent concentration and optimum operating time on bioethanol(Husin, 2019)

Table 4.42 Optimum production conditions in this study (Husin, 2019)

Objective of the Study: The effects of organosolv pre-treatment Process in Bioethanol Production	
Ethanol Concentration	75%
Pre-treatment duration	180 min
Temperature	150°C
Cellulose content	49.41%
Lignin content	16.4%

4.1.3.2.4 OIL PALM FROND JUICE AS A FERMENTATION SUBSTRATE FOR BIOETHANOL PRODUCTION USING SACCHAROMYCES CEREVISIAE

In 2020, Nathalie Gloria, conducted a literature review of oil palm frond to produce bioethanol. The research was conducted to find out the best parameters to produce bioethanol using dried Oil Palm Frond as feed. The research was approached with the literature study method by collecting and reviewing previous researches on the matter to safely get a reliable outcome.

As seen on table 4.43, the parameters set to achieve the optimum production in this research was pH, temperature, agitation rate and fermentation time with the goal to find the best fermentation parameters for bioethanol production from fried OPF.

The study shows that of the 3 Oil Palm waste reviewed (EFB, OPT and OPF), OPFS was the most suitable Oil Palm waste to be used as feed to produce bioethanol mainly due to it being the most inexpensive way for bioethanol production and the lack of chemical use. The study states that OPFS was more favourable as a feed compared to dried OPF due to the lack of chemicals used during the pre-treatment process. The pre-treatment process is essential so that hemi-cellulose and lignin in the dried OPF can be broken down to cellulose which is important to produce bioethanol.

One other reason on why this study states that OPFS is the superior feed rather than dried OPF is the energy demand during the pre-treatment phase. The study states that during the pre-treatment phase, a great deal of energy was expended which makes it quite expensive compared to OPFS which does not need a pre-treatment phase.

A summary of this study can be shown in the table 4.43 below. It shows the best course of action to achieve optimum production of bioethanol by using dried Oil Palm Frond as a feed. The table shows that to achieve a bioethanol concentration of 59.20 g/L, the pH should be 4.8 with a fermentation time of 96 hours and an agitation rate of 150 rpm at 32°C should be used.

Table 4.43 Optimum production conditions in this study (Gloria, Legowo and Kartawiria, 2020)

Objective of the study: To find out the best fermentation parameters for bioethanol production from OPFC	
Fermentation method	SSF
Inoculum	*Saccharomyces cerevisiae*
pH	4.8
Temperature	32°C
Fermentation Time	96 hours
Agitation Rate	150 rpm
Hydrolysis method	Enzymatic hydrolysis (Cellic Ctec2)
Bioethanol Concentration	59.20 g/L

4.1.3.2.5 Ethanol Production through Optimized Alkaline Pretreated Elaeis guineensis Frond Waste from Krabi Province, Thailand

In 2022, Afrasiab Khan Tareen and team, conducted a literature review of oil palm frond to produce bioethanol. The research was conducted to optimize the parameters of alkaline pre-treatment to produce bioethanol using lignocellulosic. The OPF gathered was from Krabi, Thailand. The OPF through alkaline pre-treatment method was subjected to different concentration of NaOH, temperature and time. Table 4.44 shows the optimum pre-treatment process.

The first step is physical pre-treatment by shredding to reduce the size. The material, then the steams, exploded to expose cellulose for better access for the enzymes. OPT was steam exploded at 210°C for 4 min following alkaline pre-treatment. Alkaline pre-treatment process using 15% NaOH solution with a ratio of 1:8 OPF to NaOH was done

at 90°C for 60 minutes. The pre-treated OPF with this parameters yields the highest cellulose content. Pre-treated OPF after the pre-treatment process had a cellulose content of 80.74 and lignin content of 15.99%. The pulp yield of OPF after pre-treatment was 21.57%.

Saccharomyces cerevisiae SC 90 was chosen as the inoculum. It was grown on YPD medium which consist of 20 g/L glucose, 20 g/L peptone, 15g/L agar and 10 g/L yeast extract. It was then incubated at 30°C for 18 hours with an agitation rate of 150 rpm in an orbital shaker. OPF was then fermented at 40°C with pH of 5 using sodium citrate as buffer and enzymatic hydrolysis using Celluclast 15 FPU/ g substrate and Novozyme 188 15 IU/g substrate, agitation rate of 150 rpm for 96 hours through SSF with *Saccharomyces cerevisiae* SC 90 as inoculum. This process loads at 10% (w/v) of pre-treated OPF.

Table 4.44 Optimum production conditions in this study (Afrasiab, 2022)

Objective of the study: To optimize alkaline pre-treatment method using OPFC as feed for bioethanol production	
Pre-treatment	Steam Explosion and Alkaline Pretreatment
Steam explosion	210°C for 4 min
Alkaline method	15% NaOH at 90°C for 60 min
Fermentation method	SSF
Inoculum	Celluclast and Novozyme 188
pH	5
Temperature	40°C
Fermentation Time	96 hours
Agitation Rate	150 rpm
Hydrolysis method	Enzymatic hydrolysis (Cellic Ctec2)
Bioethanol Concentration	33.15 g/L

4.1.4 Economics and availability

4.1.4.1 Case study: Preliminary assessment of integrated palm biomass biorefinery for bioethanol production utilizing non-food sugars from oil palm frond petiole

In 2020, Sharifah Soplah Syed Abdullah and team, conducted a study preliminary assessment of integrated palm biomass biorefinery for bioethanol production utilizing non-food sugars from oil palm frond petiole. The conditions of the study is that there are 4 palm oil mills that are within an 80km radius of the bioethanol plant. The capacity of the assumed mills are that each mill can process 240,000 tons per year of FFB where 57,600 tons of OPF petiole can be per year for each mill.

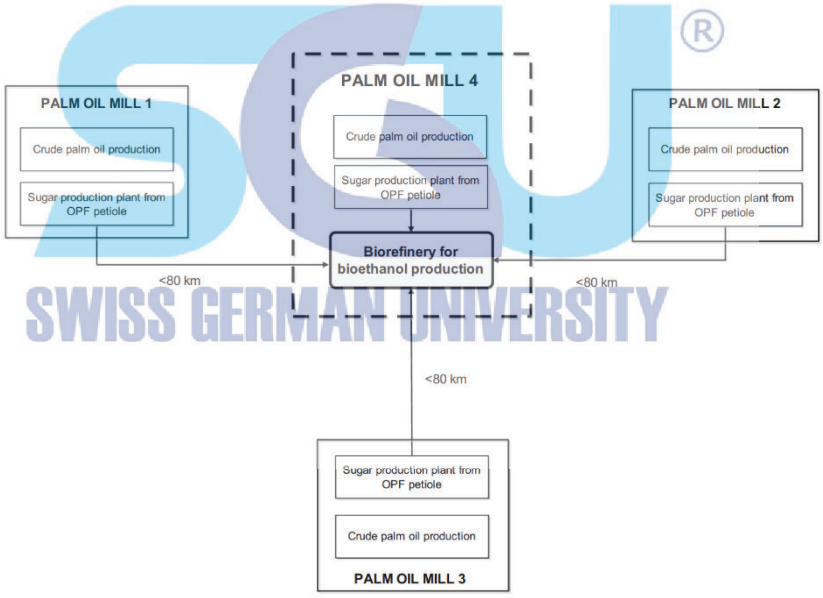

Figure 4.19 Conditions for biorefinery for bioethanol production (Abdullah *et al.*, 2016)

The bioethanol plant design proposed is that of one showed in figure 4.20. The plant would be getting the OPF from the 4 mills previously mentioned before. This study assumes that the petiole from trees ages from 8-20 years old yield 3kg of OPF petiole. The pressed juice would have 50% of its water content removed and approximately 14,400 tons of the concentrated juice can be gathered from one mill. The concentrated juice was then assumed to have a specific gravity value of 1100 kg/m^3 which then means that 13.1 million litres of concentrated juice containing 160 g/L of sugar or 2,100 tons

of sugar can be obtained from one mill. The study also states that the fibre of the OPF contains 80.58% holocellulose content. In this process, it was assumed that a 95% of the maximum concentration of glucose (0.469 g/g OPF) and xylose (0.298 g/g OPF) can be achieved through enzymatic hydrolysis. Therefore, it was then assumed that 7,200 tons of glucose and 4,600 tons of xylose can be gathered. Overall, the total amount of sugar collected was 55,600 tons per year. By assuming that 94% of the theoretical ethanol yield was achieved by this process, the plant can produce 33.9 million tons of bioethanol per year.

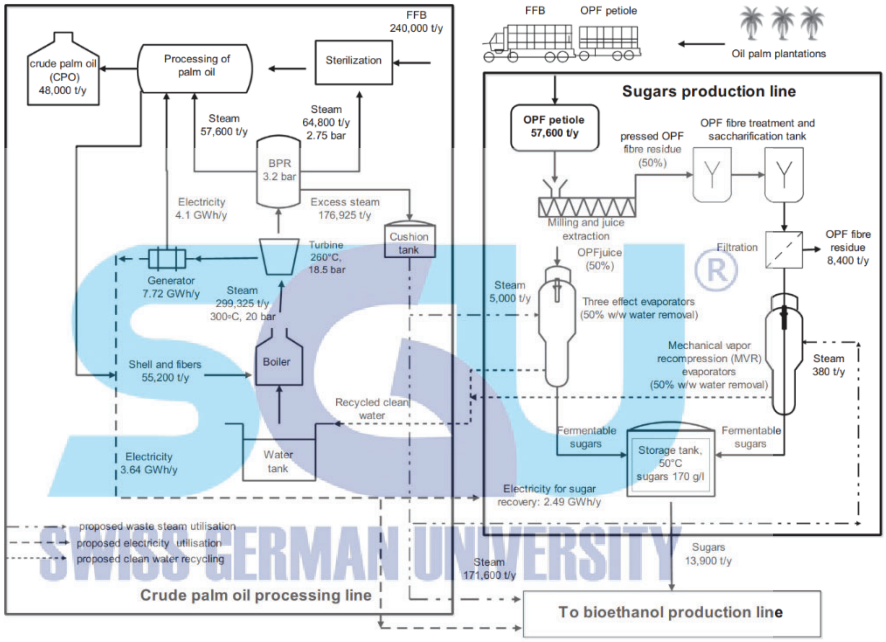

Figure 4.20 Process of bioethanol production (Abdullah *et al.*, 2016)

With the process showed in figure 4.20, economic analysis was performed which resulted in the price of $ 0.52/L bioethanol and a net energy ratio of 7.48.

Table 4.45 summary of this study (Abdullah *et al.*, 2016)

Objective of the Study: The effects of organosolv pre-treatment Process in Bioethanol Production	
Number of mills within a 80km radius	4
Petiole gathered per mill	57,600 t/y/mill.
Net energy ratio	7.48
Bioethanol production	33.9 millions tons/year

Bioethanol price	$ 0.52/l bioethanol

4.1.4.2 Techno-economic analysis of commercial-scale bioethanol production from oil palm trunk and empty fruit bunch

In 2022, Sharifah Soplah Syed Abdullah, conducted a analysis about the of a commercial-scale bioethanol production plant. The analysis of the plant was based on a 10,000 L per day ethanol plant which was 99% pure in terms of weight. The plant used lignocellulosic EFB and OPT as feed to produce bioethanol. The contents of both EFB and OPT can be seen as shown in table 4.46 below.

Table 4.46 Content of OPT and EFB (Suwajittanont, Thongrak and Srinophakun, 2022)

Composition (wt%)	OPT	EFB
Cellulose	38.67	38.85
Hemicellulose	30.22	26.14
Lignin	11.60	11.62
Ash	1.62	1.40
Other	17.89	21.99

Only simulations and basic design concepts were considered in this study. In this study, the process was split into three parts: pre-treatment, fermentation and purification. Pre-treatment of EFB was conducted by hot compressed water method while OPT was treated by steam explosion and hot water method. After the first pre-treatment process, both feeds were mixed and were pre-treated by alkaline method using hydrogen peroxide as the chemical agent. SSF was chosen as the fermentation method due to it producing a high yield of ethanol as well as how quick it is compared to conventional SHF. Two purification methods analysed, extractive distillation with using glycol and pervaporation. The two methods were chosen based on the economic parameters. OPT and EFB feed ratios were also examined, as the two feedstocks are dependent on seasonal plantation yields.

The feed ratio between OPT and EFB was also researched because both feedstocks depended on the seasonal plantation harvest. The ratio studied were: 75:25, 50:50, 25:75, and 100:0. Since the processing plant intended to run normally using EFB and to reserve utilizing OPT for particular seasons, the greatest designed percentage of OPT was 75%.

Table 4.47 Ratio feedstock, feedstock (Suwajittanont, Thongrak and Srinophakun, 2022)

OPT:EFB	Feedstock (kg/d)		Ethanol product (L/d)	
	OPT	EFB	Extractive distillation	Pervaporation
75:25	36,000	12,000	11,361	11,585
50:50	24,000	24,000	11,365	11,589
25:75	12,000	36,000	11,367	11,591
0:100	-	48,000	11,369	11,593

According to table 4.47, ethanol produced using a feed ratio of 0:100 of OPT to EFB was highest when using 100% EFB using pervaporation as purification method. The production of ethanol can also be seen to be directly proportional to the amount of EFB used where 100% EFB as feed produces the highest amount of bioethanol. The table also illustrates that pervaporation as the purification method produces more bioethanol compared to extractive distillation.

Table 4.48 Optimum production conditions in this study (Suwajittanont, Thongrak and Srinophakun, 2022)

OPT:EFB	Production cost (USD/L ethanol)	
	Extractive distillation	Pervaporation
75:25	1.14	1.10
50:50	1.16	1.11
25:75	1.17	1.12
0:100	1.18	1.12
Average	1.16	1.11

The table above shows that purification by pervaporation has a lower production cost. Like production rate, the cost of production per litre of ethanol was directly proportional to the ratio of EFB. The lowest production cost per litre of ethanol was with a feed ratio of 75:25 of OPT to EFB.

Table 4.49 Summary of (Suwajittanont, Thongrak and Srinophakun, 2022)

Objective of the Study: The effects of organosolv pre-treatment Process in Bioethanol Production

Feed ratio	0:100 ratio of OPT:EFB
Purification method	Pervaporation
Ethanol production	11,593 L/day
Production cost	$1.12/L ethanol

4.1.4.3 Availability assumptions

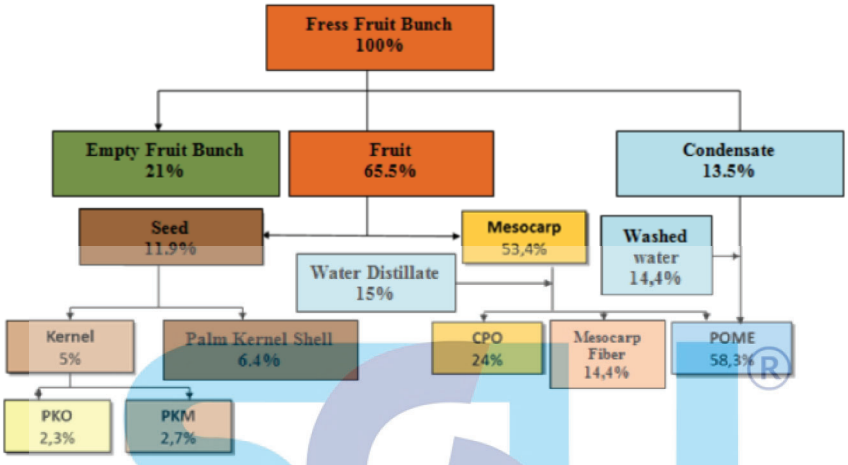

Figure 4.21 2 Waste generated from CPO production (Hambali and Rivai, 2017)

The figure above shows that CPO is 24% from the total 100% of FFB. It can also be seen that EFB is 21% from the total 100% of FFB. In 2019, Indonesia had 14.45 million hectares dedicated to palm oil plantation and it was estimated to increase to 15.08 million hectares in 2021. The production of CPO in 2019 was 47.12 million tons of CPO. It can then be assumed that approximately 43 million tons of EFB was produced in 2019.

Table 4.50 Optimum production conditions in this study (Hambali and Rivai, 2017)

Year	Area Coverage (Ha)	Frond Production (Tons)
2002	5,067,058	55,616,029
2003	5,283,557	57,992,322
2004	5,284,723	58,005,120
2005	5,453,817	59,861,095
2006	6,594,914	72,385,776
2007	6,766,836	74,272,792
2008	7,363,847	80,825,585
2009	7,873,294	86,417,275
2010	8,385,394	92,038,085
2011	8,992,824	98,705,236
2012	9,572,715	105,070,120
2013	10,465,020	114,864,060
2014	10,754,801	118,044,696
2015	11,300,370	124,032,861

The table above shows that in 2015, 124 million tons of OPF was produced. Calculations show that on average, 11.0 Tons of OPF was produced per hectare of land when data is substituted to eq.1. Therefore, it can then be assumed that 158.68 million tons of OPF was produced in 2019.

Table 4.51 Optimum production conditions in this study (Hambali and Rivai, 2017)

Year	Rejuvenation Area (Ha)	TrunkBiomass Production (Tons)
2002	202,682	15.302.515
2003	211,342	15.956.342
2004	211,389	15.959.863
2005	218,153	16.470.527
2006	263,797	19.916.640
2007	270,673	20.435.845
2008	294,554	22.238.818
2009	314,932	23.777.348
2010	335,416	25.323.890
2011	359,713	27.158.328
2012	382,909	28.909.599
2013	418,601	31.604.360
2014	430,192	32.479.499
2015*	452,015	34.127.117

The table above shows that in 2015, 34.1 million tons of OPF was produced. Calculations show that on average, 75.5 Tons of OPT was produced per hectare of the land used for rejuvenation when data is substituted to eq.1, (Hambali and Rivai, 2017) assumes that 4% of the total land used for CPO production will be used for rejuvenation per year. Therefore, it can then be assumed that 43.66 million tons of OPT was produced in 2019.

Table 4.52 Summary of (Hambali and Rivai, 2017)

Objective of the Study: The effects of organosolv pre-treatment Process in Bioethanol Production	
EFB biomass produced (2019)	43 million tons
OPF biomass produced (2019)	158.68 million tons
OPT biomass produced (2019)	43.66 million tons

4.2 Discussion

The discussion of the results of this study was separated into 2 categories. Fermentation of juice/sap and dried/lignocellulosic material. The most reliable feed for each category will be determined by comparing each feed in each category before comparing the two methodologies.

4.2.1 Discussion of the results of process methodology through juice fermentation

4.2.1.1 Feed OPFS

From all the research studied in this paper for the use of OPFS as feed for bioethanol production through juice fermentation, the ranges of the fermentation parameters can be seen as shown in Table 4.53. This paper studies the inoculum, pH level, temperature, fermentation time and agitation rate when using OPFS for feed for bioethanol production through juice fermentation.

Table 4.53 Range of study for OPFS

Inoculum	*Saccharomyces cerevisiae*
pH	4.5 - 6,62
Temperature	30°C-37°C
Fermentation Time	24-96 hours
Agitation rate	96.51 rpm - 180 rpm

From this research review, the most optimum conditions for bioethanol production from OPFS are as follows: pH 4.0, temperature 30 °C, agitation rate of 150 rpm and inoculum size of 10 % (v/v), and a fermentation period of 48 h. The process yields 0.5 g ethanol/ g sugar and produces 53 g/L of bioethanol. Study also shows that the age of the tree directly corelates to the amount of OPFS produced as well as its sugar concentration. Trees age from 3-4 years old yield the highest sugar concentration whereas trees ranging from 5-25 years old produces has a lower sugar concentration. To calculate the yield of ethanol per weight of feed, the amount of sap from OPF should be multiplied by the sugar content and the ethanol yield per gram sugar.

Yield of ethanol OPF = $0.5 \frac{L\ OPFS}{1\ kg\ OPF}$ x $76.09 \frac{g\ sugar}{L\ OPFS}$ x $0.49 \frac{g\ ethanol}{g\ sugar} = 19.02 \frac{g\ ethanol}{Kg\ OPF}$

Approximately 500 ml of OPFS can be obtained from 1 kg of OPF and a sugar content of 76.09g/L. We can then assume that the yield of ethanol is 19.02 g ethanol/ Kg OPF as seen in the calculations above.

Additional results from this review are:

1. The degradation rate of stored OPFS is found to be directly proportional to the water content and indirectly proportional to the temperature. OPFS should have its water content reduced by 30-70% while being kept at 50°C to reduce degradation rates.

2. Nutrient and sterilization effects bioethanol production. Comparison of three different samples with and without nutrient and sterilization. The study found that the sample which was supplemented with nitrogen and sterilized had a faster rate of sugar utilization.

3. Aeration and agitation were tested and different rates to test the effectiveness of it during bioethanol production. For bioethanol production, agitation has a positive impact while aeration was not needed during bioethanol production.

4.2.1.2 Feed OPTS

From all the research studied in this paper for the use of OPTS as feed for bioethanol production through juice fermentation, the ranges of the fermentation parameters can be seen as shown in Table 4.54. This paper studies the inoculum, pH level, temperature, fermentation time and agitation rate when using OPTS for feed for bioethanol production through juice fermentation.

Table 4.54 Range of study for OPTS

Inoculum	*Saccharomyces cerevisiae*
pH	4.5 - 6,62
Temperature	30°C-37°C
Fermentation Time	24-96 hours
Agitation rate	96.51 rpm - 180 rpm

From this research, the most optimum conditions for bioethanol production are as follows: pH 4.0, temperature 30 °C, and inoculum size of 10 % (v/v), and a fermentation period of 24 h with no nutrients added to the process. This process yields 0.49g ethanol/ g sugar and produces 47.5 g/l of bioethanol. The result may be caused by inoculum, pH, and temperature choice. *Saccharomyces cerevisiae* was used here because they are resilient and proven to have a good yield result. The pH control improved the reaction because *Saccharomyces cerevisiae* is more active when it is acidic, increasing the production rate. This is ideal as they produce a considerable amount of ethanol while minimizing chemicals and supplements. To calculate the yield of ethanol per weight of feed, the amount of sugar from OPT should be multiplied with the ethanol yield per gram sugar.

Yield of ethanol OPTS= 63 $\frac{g\ sugar}{Kg\ OPT}$ x 0.49$\frac{g\ ethanol}{g\ sugar}$ = 30.87 $\frac{g\ ethanol}{Kg\ OPT}$

63 g of sugar can be obtained from 1 kg of the inner part of the OPT. We can then assume that the yield of ethanol is 30.87 g ethanol/ g OPF by as seen in the calculations above.

Additional results from this review are:

1. Study shows that the OPTS should be stored between 30-60 days. Between this stored period, the maximum amount of fermentable sugars that can be obtained. There was an increase in presence of the 3 major sugars (glucose, fructose and sucrose) until 30 days and decrease after 60 days due to microbial infections which was caused by fungal penetration after prolonged storage. The study also shows that from the different section of the trunks, the upper middle section of the trunk contains the highest amount of sugars.

2. Nutrient supplementation during the fermentation process was shown to be effective in increasing bioethanol production. Popular nutrients include MgSO4, C3H7NO2, (NH4)2SO4 and Na2HPO4.The nutrients were ranked in order of productivity with MgSO4 being the most productive and Na2HPO4 being the least.

4.2.1.3 Comparison of OPF and OPT sap

When comparing OPTS and OPFS as juice feed for bioethanol production from Oil Palm waste, the most important criteria would be the yield of ethanol. Both OPTS and

OPFS have similar ethanol yield of 0.49 g sugar / g ethanol which was nearing the theoretical yield of 0.51 g sugar / g ethanol based on the stochiometric equation of sugar to ethanol. Yield of ethanol per gram sugar is a good indicator to know which process is more efficient but to determine the most suitable to produce bioethanol, yield of ethanol based on feed weight should be used. The yield of ethanol per weight of feed compared to that of OPFS where OPTS yields 30.87 g of ethanol / kg OPT while OPFS yields 19.02 g of ethanol / kg OPF.

Table 4.55 Range of study for EFB

Juice Feed	Yield of ethanol
Oil Palm Frond Sap	19.02 $\frac{g\ ethanol}{Kg\ OPF}$
Oil Palm Trunk Sap	30.87 $\frac{g\ ethanol}{Kg\ OPT}$

Although OPT produces a higher yield of ethanol per kilogram of feed as seen in the table above, the availability of OPFS is much greater than that of OPTS. This is due to the fact that when producing CPO, the frond of the Palm Tree is available all year round whereas the trunk of Palm tree can only be gathered when the tree is as its end stages of production (25-26 years). This means that OPT will not be available all year round. Therefore, OPF is the most suitable feed when producing bioethanol through the juice methodology with the exception that OPT can be used as feed is available or if it is in excess.

4.2.2 Discussion of the results of process methodology through lignocellulosic fermentation

4.2.2.1 Feed EFBC

From all the research studied in this paper for the use of EFBC as feed for bioethanol production through lignocellulosic process, the ranges of the parameters can be seen as shown in Table 4.55. This paper studies the inoculum, pre-treatment methods, hydrolysis methods, fermentation methods, pH level, temperature, fermentation time and agitation rate when using EFB for feed for bioethanol production through lignocellulosic fermentation.

Table 4.56 Range of study for EFB

Inoculum	*Saccharomyces cerevisiae, Kluyveromyces marxianus*, *Debaryomyces hansenii*
Pre-treatment methods	Alkaline method, acid method, microwave assisted acid method
Hydrolysis method	Enzymatic hydrolysis, acid hydrolysis
Fermentation methods	SHF, SSF
pH	4.5 – 4.84
Temperature	30°C-37°C
Fermentation Time	48-72 hours
Agitation rate	120 rpm - 180 rpm

The most efficient dry EFB conversion to bioethanol can be achieved via a series of processes. The pre-treatment step was acid pre-treatment with the assistance of a microwave. The empty fruit bunch was pre-treated with maleic acid at 1% concentration and irradiation time of 2.5 min at 190°C. The pre-treated EFB contains cellulose 57.5%, hemicellulose 12.77% and lignin 20.07% achieving a pulp yield of 68.8%.

From this research, the most optimum conditions for bioethanol production are as follows: pH 4.8, temperature 38 °C, and enzymatic hydrolysis with the cellulase enzyme, agitation rate of 120 rpm and a fermentation period of 48 h through SSF with, *Saccharomyces cerevisiae* as the inoculum. Through this process, the ethanol concentration of 18.9 g/L and ethanol yield of 0.43 g ethanol / g cellulose (76.6% of the theoretical yield) can be achieved. To get the concentration of biomass per litre of slurry, the data was substituted into eq.2 (% of theoretical yield = $\frac{\Delta EtOH}{0.51(f[biomass]1.111} \; x \; 100\%$), the calculations are as follows.

$$76.6\% = \frac{18.9\frac{g}{L}}{0.51(0.575[biomass]1.111} \text{ x } 100\%$$

Biomass= 75.73 $\frac{g}{L}$

After knowing the biomass in 1 litre of slurry, it is then substituted into eq.3 (Yield of ethanol=$\frac{EtOH\ x\ 1000}{biomass}$) to get the yield of ethanol per weight of EFB.

Yield of ethanol pre-treated EFB = $\frac{18.9\frac{g}{L}x1000}{75.73\ g/L}$ 249.56 $\frac{g\ ethanol}{Kg\ pre-EFB}$

Yield of ethanol EFB= 249.56 $\frac{g\ ethanol}{Kg\ pre-EFB}$ x 68.8%= 171.70 $\frac{g\ ethanol}{Kg\ EFB}$

It can be then assumed that an ethanol yield of 249.56 g ethanol / Kg pre-treated EFB can be achieved through this process. To get the true yield of ethanol, the yield of ethanol from pre-treated EFB multiplied with the pulp yield to achieve an ethanol yield of 171.70 g ethanol / Kg EFB.

Additional results from this review are:

1. SHF and SSF were compared to see which process was better for bioethanol production. Under the same conditions, not only does SSF take less time to complete, but SSF also produces higher yield and end concentration of bioethanol.
2. EFB has the potential to produce bioethanol with xylitol as a byproduct. This allows added economic viability as xylitol can be sold to add value to the existing process which can earn extra revenue.
3. An upscale model was researched by the principles of expansion, replication, and collaboration of the process. It was deemed that to produce 18.6 KL/day of 99.5% bioethanol requires three palm oil plants to provide 30-45 tons of EFB per hour.

4.2.2.2 Feed OPFC

From all the research studied in this paper for the use of OPFC as feed for bioethanol production through lignocellulosic process, the ranges of the parameters can be seen as shown in Table 4.56. This paper studies the inoculum, pre-treatment methods, hydrolysis methods, fermentation methods, pH level, temperature, fermentation time and agitation rate when using OPFC for feed for bioethanol production through lignocellulosic fermentation.

Table 4.57 Range of study for OPFC

Inoculum	*Saccharomyces cerevisiae, Saccharomyces cerevisiae SC 90, Zymomonas mobilis*
Pre-treatment methods	Alkaline method, steam explosion method, hot water method
Hydrolysis method	Enzymatic hydrolysis
Fermentation methods	SHF, SSF
pH	4.5 – 4.84
Temperature	30°C-37°C
Fermentation Time	48-72 hours
Agitation rate	120 rpm - 180 rpm

The most efficient OPFC conversion to bioethanol can be achieved via a series of processes. The first step is physical pre-treatment by shredding to reduce the size. The material, then the steams, exploded to expose cellulose for better access for the enzymes. OPT was steam exploded at 210°C for 4 min following alkaline pre-treatment. Alkaline pre-treatment process using 15% NaOH solution with a ratio of 1:8 OPF to NaOH was done at 90°C for 60 minutes. The pre-treated OPF had a cellulose content of 80.74 and lignin content of 15.99%. The pulp yield of OPF after pre-treatment was 21.57%.

From this research, the most optimum conditions for bioethanol production are as follows: temperature 40°C, pH of 5 and enzymatic hydrolysis using Celluclast 15 FPU/ g substrate and Novozyme 188 15 IU/g substrate, agitation rate of 150 rpm and a fermentation period of 96 h through SSF with *Saccharomyces cerevisiae* SC 90 as inoculum. This process loads at 10% (w/v) of pre-treated OPF. The fermentation method used was SSF. This process yields bioethanol concentration 33.15 g/L. To

calculate the yield of ethanol, data was substituted into eq.3 (Yield of ethanol=$\frac{EtOH\ x\ 1000}{biomass}$).

Yield of ethanol pre-treated OPFC = $\frac{33.15\ \frac{g}{L} x1000}{100\ g/L}$ = 331.5 $\frac{g\ ethanol}{Kg\ pre-OPF}$

Yield of ethanol OPFC= 331.5 $\frac{g\ ethanol}{Kg\ pre-OPF}$ x 21.57%= 71.50 $\frac{g\ ethanol}{Kg\ OPF}$

The calculation above shows the calculation for the yield of bioethanol per feed weight. It can be then assumed that an ethanol yield of 331.5 g ethanol / Kg pre-treated OPF can be achieved through this process. To get the true yield of ethanol, the yield of ethanol from pre-treated OPF multiplied with the pulp yield to achieve an ethanol yield of 71.5 g ethanol / Kg OPF.

Additional results from this review are:

1. Organosolv pre-treatment was studied to see the increase in cellulose and decrease in lignin content. This pre-treatment delignifies OPF with adjustments to the ethanol concentration, process time and temperature. At optimum temperature and duration of 150°C and duration of 180 min with an ethanol concentration of 75%, the cellulose content of and lignin content of the OPF was 49.41% and 16.4%. As of the writing of this paper, this method should be further developed before being used to delignify OPF.
2. Ultrasonic-assisted pre-treatment method was studied for bioethanol production. Ultrasonic-assisted organosolv/H2O2 pre-treatment (UOP) was carried out in an ultrasonic bath to delignify the OPF. The ultrasonic pre-treatment method and non-sonication were carried out using the pretreated fronds and the SSF process resulted in lower bioethanol concentration (3.1 g/l) and yield (14.0%) compared to the ultrasound SSF process. This pre-treatment method could be used in conjunction with other pre-treatment methods to increase bioethanol production.
3. SHF and SSF were compared to see which process was better for bioethanol production. Under the same conditions, not only does SSF take less time to complete, but SSF also produces higher yield and end concentration of bioethanol.

4.2.2.3 Feed OPTC

From all the research studied in this paper for the use of OPTC as feed for bioethanol production through lignocellulosic process, the ranges of the parameters can be seen as shown in Table 4.57. This paper studies the inoculum, pre-treatment methods, hydrolysis methods, fermentation methods, pH level, temperature, fermentation time and agitation rate when using OPTC for feed for bioethanol production through lignocellulosic fermentation.

Table 4.58 Range of study for OPTC

Inoculum	*Saccharomyces cerevisiae, Kluyveromyces marxianus ,Debaryomyces hansenii*
Pre-treatment methods	Alkaline method, acid method, microwave assisted acid method
Fermentation methods	SHF,SSF
pH	4.5 – 4.84
Temperature	30°C-37°C
Fermentation Time	48-72 hours
Agitation rate	120 rpm - 450 rpm

The most efficient OPTC conversion to bioethanol can be achieved via a series of processes. The first step is physical pre-treatment by shredding to reduce the size. The material, then the steams, exploded to expose cellulose for better access for the enzymes. OPT was steam exploded at 210°C for 4 min following alkaline pre-treatment with 15% NaOH at90°C for 60 minutes. The pre-treated OPT has a cellulose content of 87.14% and content lignin content of 6.13%. The pre-treatment process yields a pulp yield of 23.01%.

The next step is alkaline extraction to eliminate the unconvertible portion of sugar conversion so that the process is progressing effectively. Saccharification process was performed by enzymatic hydrolysis using Novozyme 188 50°C for 12 hours.

In this process, Saccharomyces cerevisiae was chosen as inoculum, fermentation conducted at room temperature (40°C) for 96 hours without pH control. This process loads at 10% (w/v) of pre-treated OPT. This process yields 0.46g ethanol/ g sugar and produces 44.25 g/l of bioethanol. To calculate the yield of ethanol, data was substituted into eq.3 (Yield of ethanol=$\frac{EtOH\ x\ 1000}{biomass}$).

Yield of ethanol pre-treated OPT = $\frac{44.25\ \frac{g}{L} x 1000}{100\ g/L}$ = 442.5 $\frac{g\ ethanol}{Kg\ pre-OPT}$

Yield of ethanol OPT= 442.5 $\frac{g\ ethanol}{Kg\ pre-OPT}$ x 23.01%= 101.82 $\frac{g\ ethanol}{Kg\ OPT}$

The calculation above shows the calculation for the yield of bioethanol per feed weight. It can be then assumed that an ethanol yield of 442.5 g ethanol / Kg pre-treated OPT can be achieved through this process. To get the true yield of ethanol, the yield of ethanol from pre-treated OPT multiplied with the pulp yield to achieve an ethanol yield of 101.82 g ethanol / Kg OPT.

Additional results from this review are:

1. Pre-treatment to reduce lignin content in OPTC is crucial for bioethanol production. One of the methods to reduce the lignin content would be the alkali peroxide method. 5% of hydrogen peroxide was the optimal concentration for increasing cellulose and decreasing lignin content. The pre-treatment method was conducted at 28°C for 72 hours which will increase cellulose level by 47.89% and decrease the lignin level by 16.89%.

2. To decrease the cost of bioethanol production, H2O2 as a sterilization media and urea as nitrogen source can be used. Different concentrations of urea were tested to and compared to the popular nitrogen source which is yeast extract and peptone. H2O2 at different concentrations were tested and compared to autoclaving method. Urea at 1 g/L and H2O2 at 0.5g/L was found to be the optimal concentration for bioethanol concentration.

3. A decrease in OPTC size can be used to ethanol concentration, productivity and yield. OPTC was shredded to a size of 40-60 mm and the results were compared

to the control. Results show that the 40mm OPTC had the highest ethanol concentration, productivity and yield. However, there is no data that going smaller than 40mm will result in higher results.

4.2.2.4 Lignocellulosic comparison

The comparison of yield for the lignocellulosic feed can be seen in the table 4.59 below.

Table 4.59 Comparison of lignocellulosic feed

Lignocellulosic feed	Yield of ethanol
Lignocellulosic Empty Fruit Bunch	171.70 $\frac{g\ ethanol}{Kg\ EFB}$
Lignocellulosic Oil Palm Trunk	101.82 $\frac{g\ ethanol}{Kg\ OPT}$
Lignocellulosic Oil Palm Frond	71.50 $\frac{g\ ethanol}{Kg\ OPF}$

When comparing lignocellulosic feed, as shown in the table above, the most important criteria would be the yield of ethanol. The yield of ethanol refers to the amount of ethanol which was produced from both the cellulose and hemicellulose content present in the feed. Although OPT has the most efficient process yielding 442.5 g ethanol / Kg pre-treated OPT followed by OPF and last lastly EFB. This however is not the true yield of ethanol as during the pre-treatment process, there is a loss of biomass. The difference in pulp yield is due to the difference in pre-treatment steps in where both OPTC and OPFC uses a two pre-treatment step arrangement whereas EFBC only uses a one pre-treatment step arrangement. When comparing the true yield of ethanol per weight of feed, it can be seen that EFB has the highest yield of ethanol achieving 171.70 g ethanol / Kg EFB followed by OPT (101.82 g ethanol / Kg OPT) and lastly OPF (71.5 g ethanol / Kg OPF). Although OPT and OPF has higher yield of ethanol per pre-treated biomass, the yields are considerably low making the true yield of ethanol lower than that of EFB.

4.2.3 Comparison of juice and lignocellulose & economy

The comparison of yield for juice and lignocellulosic feed can be seen in the table 4.60 below.

Table 4.60 Comparison of juice and lignocellulosic feed

Feed	Yield of ethanol
Lignocellulosic Empty Fruit Bunch	171.70 $\frac{g\ ethanol}{Kg\ EFB}$
Oil Palm Frond sap	19.02 $\frac{g\ ethanol}{Kg\ OPF}$

When comparing juice and lignocellulose feed, it is typically found that juice feed is more preferable to produce bioethanol than lignocellulosic feed. Both (Gloria, Legowo and Kartawiria, 2020) and (Herawan, 2021)which reviewed both juice and lignocellulosic feed for OPF and OPT respectively, chose juice feed to produce bioethanol. This decision was based on the fact that while lignocellulosic feeds have high ethanol yields per feed weight, the equipment and complexity of the process. The complexity and additional processes causes a loss of pulp yield which greatly affect the yield of ethanol pew weight of feed and the additional use of both chemicals and energy during the pre-treatment processes. Therefore, the studies conclude that the use of lignocellulosic oil palm residues was not preferred due to the complexity of the pre-treatments performed to extract lignin from dried biomass and the need for different equipment and materials.

However, as the aim of this study is to find the most suitable feed to produce bioethanol to keep up with the demand for the growing energy demand as well as the need for a sustainable alternative fuel source. Hence, it cannot be ignored that bioethanol production through its lignocellulosic material has vastly more potential than form its sap. The yield of bioethanol from the lignocellulosic material of EFB is 9 times greater than the yield of bioethanol from OPF sap. Therefore, it can be said that for a small-scale production where ease of process is prioritized, bioethanol from OPF sap should be used as feed but for industrial-scale production, which is the aim of this study, lignocellulosic EFB should be used as feed.