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Research article

Influence of Inhibitors from Microwave Pretreatment of Oil Palm Frond Pulping (OPFP) on Bioethanol Production

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Abstract: This research aims to analyze the influence of inhibitors from microwave pretreatment of oil palm frond pulping (OPFP) on the efficiency of bioethanol fermentation by *S.cerevisiae* in the simultaneous saccharification and fermentation (SSF) processes. OPFPs were achieved at different ages: 3-4, 4-7, 7-10, 10-20, and 20-25 years old. OPFP was pretreated with a microwave and sulfuric acid (MW/SF), microwave and hydrogen sulfide (MW/HP), and microwave and water (MW/W). The results showed that the main inhibitors formed during the pretreatment process of OPFP were acetic acid, furfural, 5-hydroxymethylfurfural (HMF), furfural, formic acid, and phenol. The pretreatment of OPFP with MW/W had the lowest concentrations of inhibitors compared to the other pretreatment methods. The highest bioethanol yields at all ages of OPFP were in the range of 0.41-0.42 g-bioethanol/g-glucose, corresponding to more than 80% fermentation efficiency. At these conditions, the concentrations of the acetic acid were 0.09-0.19 g/l, HMF =0, furfural =0, formic acid 0.05-0.28 g/l, and phenol 0.22-0.47 g/l. The MW/W was the suitable pretreatment of OPFP for bioethanol production due to the lowest to generate the inhibitor and high ethanol production yield.

Keywords: Bioethanol production; Oil palm frond pulping; Inhibitors; Microwave pretreatment

1. Introduction

It is generally known that fossil resources decrease continuously, and fossil-based fuels cause environmental problems. An alternative way to solve these issues is using alternative energy, such as ethanol. Ethanol is particularly important as a liquid biofuel, which can be mixed with gasoline with different ratios (e.g., E10, E20, E85). Ethanol has been produced from sugar or starch-based raw materials on the industrial scale. However, using starch and sugar for ethanol production has led to a "food vs. fuels" conflict due to the increasing global population [1]. Therefore, using lignocellulosic raw materials is attractive due to the large quantity, low prices, and no rapacious land for the plantation of humans.

Biomass resources are organic materials containing carbon, hydrogen, and oxygen atoms in their structure [2]. The process of biofuel production and consumption results in zero net CO_2 emissions because the amount of CO_2 released during combustion equals the amount of CO_2 absorbed by trees and plants through photosynthesis [2]. In Thailand, an oil palm frond (OPF) is a sustainable agricultural waste obtained from the harvest of fresh fruit bunch. OPF contains many carbohydrates in the form of simple sugars, which could certainly be used as a raw material for producing bioethanol. However, using lignocellulosic materials to produce bioethanol requires a pretreatment process. The pretreatment step aims to open up the structure and decrease the crystal structure of lignocelluloses via the solubilization of hemicellulose and lignin [3]. On the other hand, access to the cellulose surface area increased for the following saccharification and fermentation processes. The pretreatment process is a key step in the biochemical conversion of lignocellulose to produce bioethanol [4]. During pretreatment, inhibitors are somewhat generated depending on the pretreatment method. Inhibitors in lignocellulosic hydrolysates consist of aliphatic acids (i.e., acetic, formic, and levulinic acid), furaldehydes (i.e., 5-hydroxymethylfurfural (HMF), and furfural), aromatic compounds (i.e.phenolics) and extractives; all of which affect bioethanol fermentation [5]. Jonsson and Matin [6] reported that the disadvantage of using sulfuric acid pretreatment was the formation of inhibitory by-products. Additionally, the advantage of hydrothermal pretreatment by controlling the pH around neutral values was the decreased amount of fermentation inhibitors [7]. Microwave pretreatment is a potential alternative to conventional heating due to its efficiency and ease of use. Energy consumption is reduced as treatment time is 10 times shorter than other heating systems [2]. Additionally, the combined method (microwave-assisted) can improve enzymatic hydrolysis and effectively remove lignin and hemicellulose, which is the maximum utilization of lignocellulosic components [8]. Biomass pretreatment provides a practical and clean process to increase surface area and improve access to enzyme binding sites. [9].

This research analyzes the influence of inhibitors formed during the different methods in the pretreatment of oil palm frond pulping (OPFP). These included microwave treatment with sulfuric acid (MW/SF), microwave treatment with hydrogen peroxide (MW/HP), and microwave treatment with water (MW/W). The research focuses on the effect of inhibitors (acetic acid, furfural, 5-hydroxymethylfurfural (HMF), formic acid, and phenol) on the efficiency of the bioethanol fermentation by *S.cerevisiae* in the following simultaneous saccharification and fermentation (SSF) processes.

2. Materials and Methods

2.1 Preparation of oil palm frond

The fresh oil palm frond (OPF) without leaves was cut to 1.0 m in length. The OPF was selected from oil palm trees having different ages: 3-4, 4-7, 7-10, 10-20, and 20-25 years old as shown in Figure 1A. The oil palm frond juice was removed from OPF by pressing OPF with a conventional sugarcane press machine. The remaining OPF solid, oil palm frond pulping (OPFP), was then cut into small pieces and dried at 103^{0}C for 24 hours to remove the moisture. After that, it was ground to 0.2 - 2 mm in size and put in a plastic bag at room temperature to protect the sample from moisture before use (Figure 1B). The initial chemical components in OPFP are shown in Table 1.

Figure 1. Raw material, (A) the fresh oil palm frond (OPF) without leaves used in the experiment, (B) Preparation of oil palm frond pulping (OPFP)

Table 1. The initial chemical components in OPFP

Oil palm age (years)	**Components %(w/w)**				
	Cellulose	**Hemicellulose**	**Holocellulose**	**Lignin**	**Ash**
3-4	38.84	34.68	73.52	24.74	1.74
4-7	39.82	33.58	73.40	25.56	1.04
7-10	40.44	31.06	71.50	26.94	1.56
10-20	42.38	28.68	71.06	27.52	1.42
20-25	43.50	26.34	69.84	29.44	1.24

2.2 Pretreatment of oil palm frond pulping

In this study, there are 3 types of pretreatments of OPFP:(i) using microwave and sulfuric acid (97%, Merck)(MW/SF),(ii)using microwave and hydrogen peroxide(37%, Merck) (MW/HP), and (iii) using microwave and water (MW/W). All pretreatments were carried out in 100 ml solutions (sulfuric acid, hydrogen peroxide, or aqueous solutions) in which 10% w/v of OPFP was loaded (fixed amount of OPFP at 10 g). Different sulfuric acid and hydrogen peroxide concentrations were studied at 0, 1, 2, 3, and 4 vol.%. The microwave was generated by the Samsung Home Model (frequency 2.45 GHz multimode cavity and largest 800 W) by putting the samples in the microwave and setting it up at 500 W for 15 minutes to study the effect of different pretreatment processes.

2.3 Enzyme hydrolysis and bioethanol production

The pretreated OPFP was forwarded to the hydrolysis and bioethanol production by simultaneous saccharification and fermentation (SSF). Bioethanol production from OPFP cellulose by SSF was tested in a batch mode. The enzymatic hydrolysis was done in 250 ml volumetric flasks using 10% (w/v) of OPFP. 5 ml of 5 M citrate buffer solution (pH 4.8) and 85 ml of distilled water were added to the flasks. Afterward, the flasks were sterilized in an autoclave (Model SA 300 VL Brand sturdy) at 121 ^{0}C for 15 min. The cellulosic enzyme used in this study was Cellic^R CTec2 (Cellulase + β-glucosidase). The enzymatic loading was 3% w/w (g-enzyme/g-cellulose). The mixtures were incubated at 55 ^{0}C with shaking (Shaking Incubator Model BJPX Series)at 150 rpm for 96 hours. Then, 10% (v/v) of *S. cerevisiae* and 1% (v/v) yeast extract were added. Finally, the mixtures were incubated at 37 ^{0}C with shaking at 150 rpm for 24 hours. Samples were collected at 0, 24, 48, 72, and 96 hours to determine the amounts of reducing sugar and bioethanol. Bioethanol yield and fermentation efficiency (%) were calculated using Eq. (1) and (2), respectively [10].

$$\text{Ethanol yield }\left(\text{g}-\text{ethanol}/\text{g}-\text{glucose}\right)=\frac{\text{Maximum ethanol concentration}\left(\text{g}/\text{l}\right)}{\text{Utilized glucose }\left(\text{g}/\text{l}\right)} \tag{1}$$

$$\text{Fermentation efficiency }\left(\%\right)=\frac{\text{Actual ethanol yield }\left(\text{g}/\text{l}\right)}{\text{Theoretical ethanol yield}\left(\text{g}/\text{l}\right)}x100 \tag{2}$$

2.4 Analytical methods

The total sugars were analyzed by high-performance liquid chromatography (HPLC) with a refractive index detector at 50 ^{0}C. 5.0 μl of samples were injected into a column (SH1011, 8.0x300 nm, Shodex) with 0.04 N H_2SO_4 as the mobile phase (flow rate 0.8 ml/min). The running time of the samples was 20 min. The dichromate reagent method determined the bioethanol concentration [11]. Formic acid, acetic acid, HMF, and furfural were determined by HPLC using a Bio-Rad HOX-87P ion-exclusion column, a Waters 2414 refractive index detector, and 0.01 N H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min and 60 ^{0}C. The phenolic compounds in samples were determined by the Folin-Cio-Cateu method [12].

3. Results and Discussion

3.1 Effect of microwave-hydrogen peroxide pretreatment (MW/HP) on the formation of inhibitors

The effect of microwave-hydrogen peroxide (MW/HP) pretreatment of OPFP obtained from different oil palm ages (3-4, 4-7, 7-10, 10-20, and 20-25 years) on the formation of inhibitors in OPFP hydrolysates after 96 hr of enzyme hydrolysis is shown in Figure 2. Compared with microwave-water (MW/W) pretreatment, the pretreatment of OPFP with MW/HP promoted the formation of inhibitors. Increasing the concentration of HP combined with microwave resulted in the increased concentrations of inhibitors found in hydrolysate at all oil palm ages. The inhibitors found with MW/HP were formic acid, acetic acid, and phenol. All of these compounds inevitably occurred even with water pretreatment. Matin and Jonsson [15] reported that the main inhibitors in lignocellulosic hydrolysates were formic acid, acetic acid, 5-hydroxymethylfurfural (HMF), furfural and phenol, which could be produced in different concentrations depending on the pretreatment methods. Indeed, the concentration of the inhibitors significantly affected enzyme digestion and the fermentation process, which will be discussed later. Additionally, Table 2 shows the concentration of inhibitors found in this study with other experiments [13].

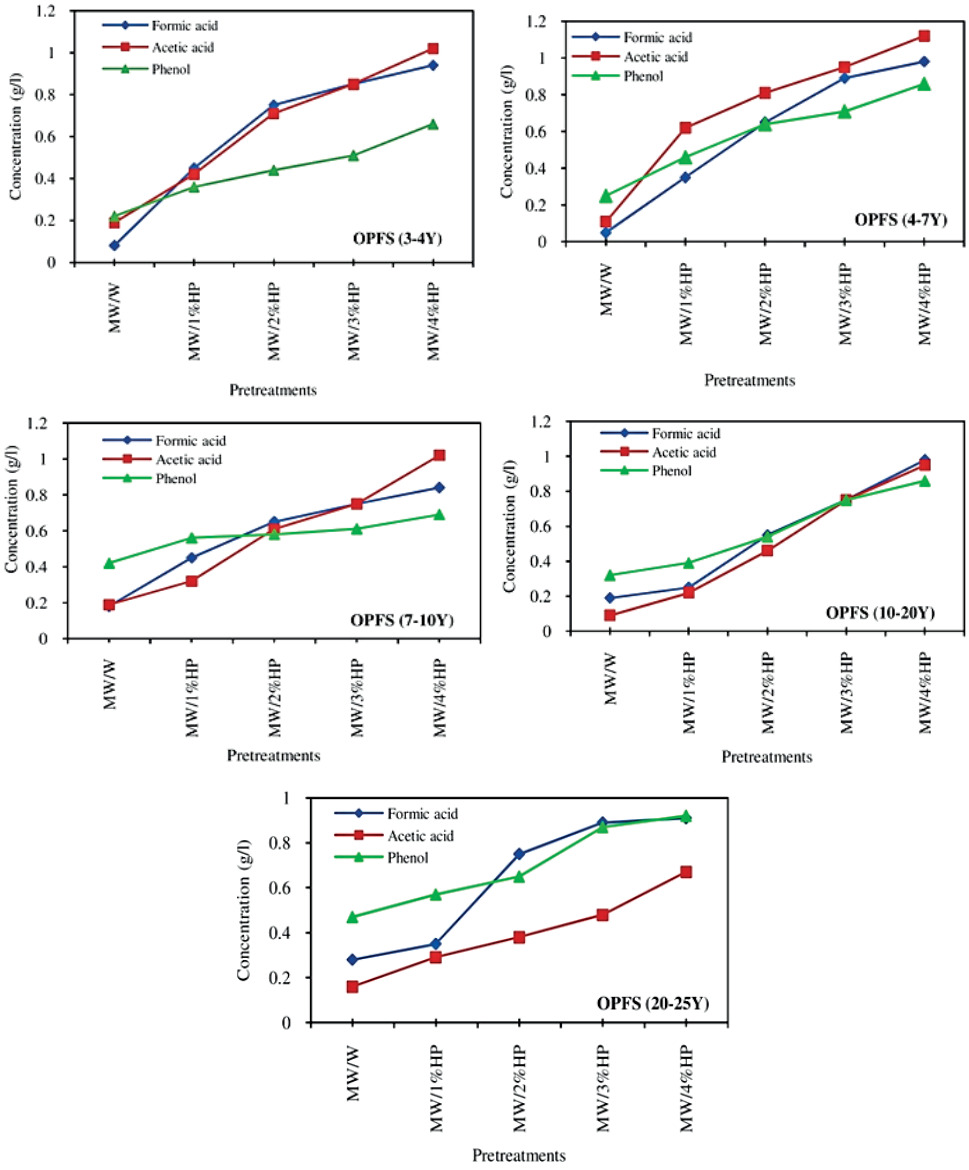

Figure 2. Inhibitors concentrations in hydrolysates of OPFP aged 3-4, 4-7, 7-10, 10-20, and 20-25 years using the different OPFP pretreatment methods with MW/HP.

Table 2. Comparison inhibitors concentration between all palm age of OPFP and other experiments

Sample	Pretreatment	Inhibitors concentration (g/l)					Reference
		Formic acid	Acetic acid	HMF	Furfural	Phenol	
OPFP (3-4 Y)	MW+W (15 min)	0.08	0.19	0.00	0.00	0.22	This study
	MW+4%HP (15 min)	0.94	1.02	0.00	0.00	0.66	This study
	MW+4%SF (15 min)	1.56	2.75	0.45	3.50	1.85	This study
OPFP (4-7 Y)	MW+W (15 min)	0.05	0.11	0.00	0.00	0.25	This study
	MW+4%HP (15 min)	0.98	1.12	0.00	0.00	0.86	This study
	MW+4%SF (15 min)	1.66	2.65	0.75	3.65	1.75	This study
OPFP (7-10Y)	MW+W (15 min)	0.18	0.19	0.00	0.00	0.42	This study
	MW+4%HP (15 min)	0.84	1.02	0.00	0.00	0.69	This study
	MW+4%SF (15 min)	1.66	2.45	0.65	3.35	1.75	This study
OPFP (10-20 Y)	MW+W (15 min)	0.19	0.09	0.00	0.00	0.32	This study
	MW+4%HP (15 min)	0.98	0.95	0.00	0.00	0.86	This study
	MW+4%SF (15 min)	1.66	2.65	0.85	3.25	1.67	This study
OPFP (20-25 Y)	MW+W (15 min)	0.28	0.19	0.00	0.00	0.47	This study
	MW+4%HP (15 min)	0.91	0.67	0.00	0.00	0.92	This study
	MW+4%SF (15 min)	1.75	2.85	0.75	3.65	1.77	This study
wheat straw	Thermal autoclaving (60 min)	0.07	1.02	0.00	0.00	0.50	[14]
	1.5%HCL autoclaving (60 min)	0.18	0.82	0.04	0.84	0.96	[14]
	1%NaOH autoclaving (60 min)	2.06	3.59	0.00	0.00	3.24	[14]
	5% H_2O_2 (60 min)	0.62	0.69	0.00	0.00	0.29	[14]
Eucalyptus residue	065%H_2SO_4 (15 min)	0.00	3.10	0.20	1.23	0.00	[15]

3.2 Effect of microwave-sulfuric acid pretreatment (MW/SF) on the formation of inhibitors

The effect of microwave-sulfuric acid (MW/SF) pretreatment of OPFP obtained from different oil palm ages (3-4, 4-7, 7-10, 10-20, and 20-25 years) on the formation of inhibitors in OPFP hydrolysates after 96 hr of enzyme hydrolysis is shown in Figure 3. Compared with microwave-water (MW/W) pretreatment, the pretreatment of OPFP with MW/SF promoted the formation of inhibitors. Increasing the concentration of SF combined with microwave resulted in the increased concentrations of inhibitors found in hydrolysate at all oil palm ages. The inhibitors found with MW/SF were formic acid, acetic acid, phenol, HMF, and furfural. Compared with MW/HP, sulfuric acid promoted more types of inhibitors, such as HMF and furfural. However, HMF and furfural have been reported to be less toxic to *S. cerevisiae* than phenol, acetic acid, and formic [6]. Behera et al. [16] similarly reported that the formation of inhibitors depended on the conditions used in the acid pretreatment process, such as the acid type and concentration.

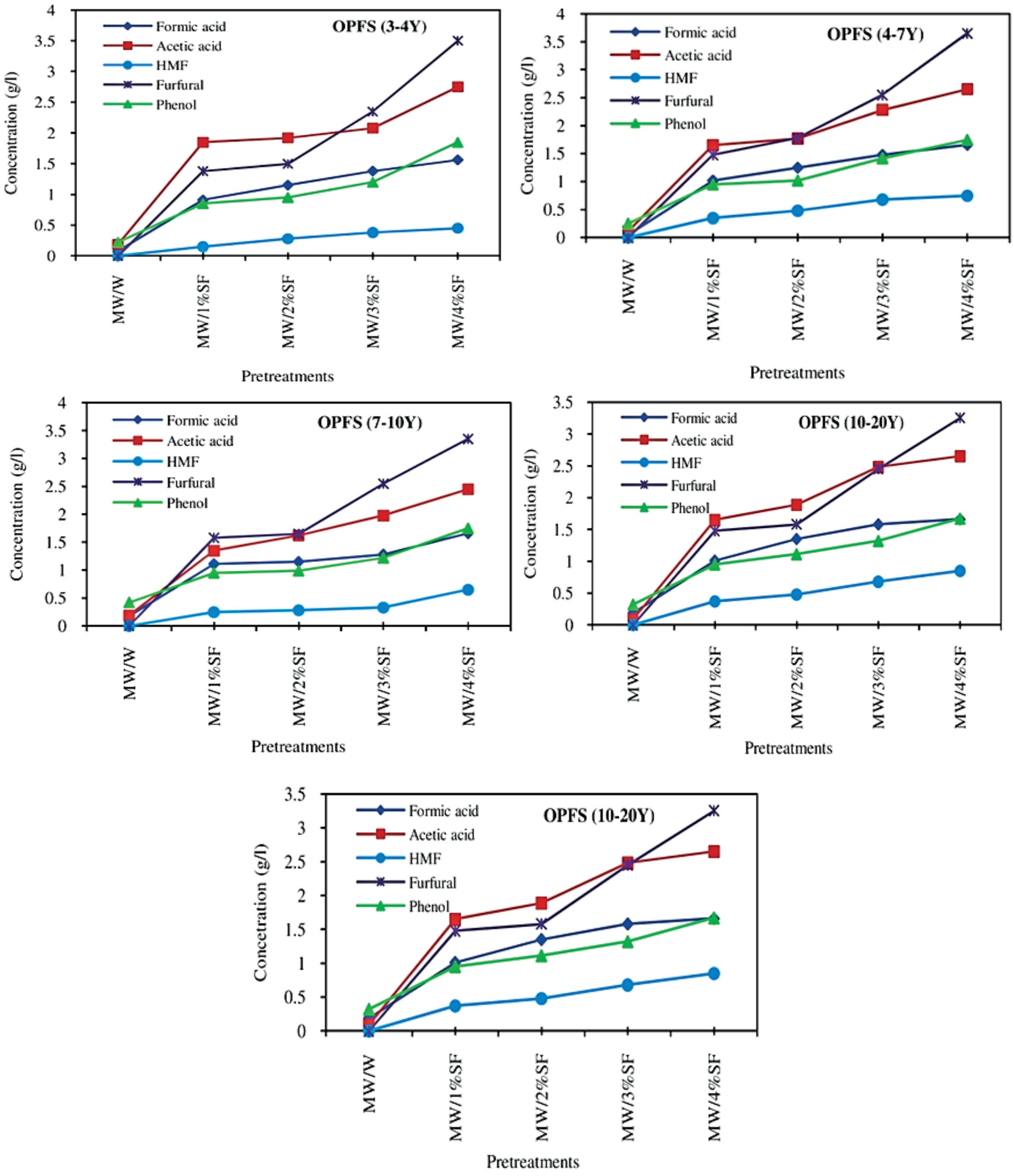

Figure 3. Inhibitors concentration in hydrolysates of OPFP aged 3-4, 4-7, 7-10, 10-20, and 20-25 years using the different OPFP pretreatment methods with MW/SF.

3.2.1 Acetic acid

All the pretreatment methods gave concentrations of acetic acid in the range of 0.09-2.85 g/l at various oil palm ages (Figures. 2 and 3). Therefore, it can be concluded that acetic acid is inevitably generated regardless of the type of pretreatment methods and the age of the oil palm. Compared to the other pretreatment methods, the pretreated OPFP with MW/W generated the lowest concentrations of acetic acid in the range of 0.09-0.19 g/l. The concentrations of acetic acid after the pretreatment of OPFP with MW/HP and MW/SF were in the range of 0.32-1.12 g/l and 1.65-2.84 g/l, respectively. Sulfuric acid generated a greater amount of acetic acid than hydrogen peroxide. The pretreatment of OPFP with MW/SF at the same concentration produced a more severe reaction than MW/HP, which was affected by a higher reaction temperature, resulting in the generation of acetic acid inhibitors than the pretreatment by MW/HP. The nature and concentration of inhibitors depend highly on the amount of solids in the reactor, pretreatment conditions such as time, pH, temperature, and the concentrations of chemicals and raw materials used [17]. It has been reported that the cellular growth of yeast and ethanol production was inhibited completely when the concentration of acetic acid was about 3.5 g/l [5]. Therefore, the pretreatment of OPFP with MW/W, MW/HP, and MW/SF (concentrations of HP and SF not more than 4%) in this study can be applied to practice.

3.2.2 Furfural

Furfural was formed only by the pretreatment of OPFP with MW/SF. During the thermochemical pretreatment, inhibitors such as furfural and HMF are produced by dehydration of the pentose and hexose(Palmqvist, E., Hahn-Hagerdal, 2000)[17]. The concentrations of furfural were found in the range of 1.38-3.65 g/l for oil palm ages 3-4, 4-7, 7-10, 10-20, and 20-25 years (Figure. 3). The concentration of furfural increased with the acid concentration. This is consistent with the previous study [18]. Furfural harmed the ethanol production rate and such impact increased with concentration [16],[19]. Decreased ethanol production occurred at 2 g/l of furfural and the complete inhibition was reported at 4 g/l [20]. Therefore, furfural generated from MW/SF in this study has a significant effect on the production of bioethanol. Furfural inhibited the growth of *S. cerevisiae*, leading to a slower sugar consumption rate of yeast [21]. This, in turn, reduces the production of bioethanol. The pretreatment with MW/W and MW/HP did not generate furfural (Figure 2). Toquero and Bolado [13] similarly reported that no furfural was formed after the pretreatment of wheat straw with dilute alkali. The pretreatment of rice hulls with alkaline peroxide neither generated furfural [22].

3.2.3 5-hydroxymethylfurfural (HMF)

HMF was formed only with the pretreatment of OPFP with MW/SF; the concentrations of HMF were found in the range of 0.15-0.85 g/l at various oil palm ages (Figure 3). The pretreatment with MW/W and MW/HP generated no HMF (Fig. 2). The generation of HMF is believed to be associated with the formation of furfural when SF was used in the pretreatment. The pretreatment with dilute acids similarly generated furfural and HMF [23]. However, the concentrations of HMF were lower than those of furfural at all oil palm ages. This agrees with a previous study in which furfural was formed more easily than HMF in acid pretreatment at high temperatures [24]. HMF was formed from the degradation of glucose catalyzed by sulfuric acid [25]. The HMF concentrations that have an inhibiting effect on bioethanol production were reported to be higher than 1 g/l [26]. Since the HMF concentrations in this study were lower than 1 g/l, it is not severely toxic compared to furfural. Furfural has more potent toxicity than HMF [19]. However, HMF was reported to have a synergistic effect when combined with other inhibitors [16].

3.2.4 Formic acid

All the pretreatment methods gave concentrations of formic acid in the range of 0.05-1.75 g/l at various oil palm ages (Figures 2 and 3). The lowest concentrations of formic acid were in the 0.05-0.28 g/l range when OPFP was pretreated with MW/W. The concentrations of formic acid after pretreated OPFP with MW/HP and MW/SF were 0.25-0.98 g/l and 0.91-1.75 g/l, higher than the pretreatment with MW/W. Similar to the case of acetic acid, SF generated a greater amount of formic acid than HP. This is partly due to the formation of furfural and HMF. It has been reported that formic acid could occur from the degradation of furfural and HMF and had a more inhibitory effect than acetic acid [16], [27]. The complete inhibitory effect of formic acid on ethanol production was reported to occur at a concentration of 2 g/l [10]. However, if the formic acid concentration was less than 1 g/l, a higher bioethanol yield than fermentation without formic acid was observed [14]. This is consistent with our result. The pretreatment of OPFP with MW/SF, which gave concentrations of formic acid higher than 1 g/l, caused a decrease in the bioethanol yields compared to the pretreatment with MW/HP and MW/W.

3.2.5 Phenol

The phenol concentrations of all pretreatment methods were in the range of 0.22-1.77 g/l for oil palm ages 3-4, 4-7, 7-10, 10-20, and 20-25 years(Figures 2 and 3). The minimum phenol concentrations were in the range of 0.22-0.47 g/l after the pretreatment of OPFP with MW/W. After the pretreatment with MW/HP and MW/SF, the phenol concentrations were in the range of 0.36-0.92 g/l and 0.85-1.77 g/l, respectively, higher than the pretreatment with MW/W. Phenolic compounds were formed during the pretreatment due to the partial breakdown of lignin [16].

In conclusion, the concentrations of inhibitors after all pretreatment conditions in this study were in the range of 0.09-2.85 g/l of acetic acid, 1.38-3.65 g/l of furfural, 0.15-0.85 g/l of HMF, 0.05-1.75 g/l of formic acid and 0.22-1.77 g/l of phenol. These ranges are consistent with previous research. In diluted acid pretreatment, using 0.65%H_2SO_4 (20 min) to treat the Eucalyptus residue generated 3.10 g/l of acetic acid, 1.23 g/l of furfural and 0.20 g/l of HMF [28]. Interestingly, the increased ages of OPF produced more inhibitors. Increasing the age of OPF increased the initial components in OPFP, which affected the synergism to have the inhibitors after the pretreatment with MW/HP and MW/SF. The pretreatment with MW/W created the lowest inhibitors when compared to the others. Similar to the liquid hot water (LHW) pretreatment, the advantage of the combined pretreatment with water and microwave was to avoid the formation of inhibitors [3], [29]. These inhibitors harm the hydrolytic process by reducing the adsorption of an enzyme [30].

3.3 Glucose concentration

The OPFP from different pretreatment methods was subjected to enzymatic hydrolysis and bioethanol production by simultaneous saccharification and fermentation (SSF), and the glucose concentrations were measured after 96 hours of enzymatic hydrolysis. The results are shown in Figure 4. It was found that OPFP from the pretreatment with MW/HP (4%)gave the highest glucose concentrations, which were 13.35, 19.61, 21.73, 25.07, and 28.67 g/l for oil palm aged 3-4, 4-7, 7-10, 10-20 and 20-25 years, respectively. Regardless of the age of the oil palm, the glucose concentration was enhanced with the increased concentration of hydrogen peroxide used in the pretreatment. The pretreatment with HP gave higher concentrations of glucose than the pretreatments with MW/W, which were 11.10, 15.10, 16.75, 19.56, and 22.30 g/l for oil palm aged 3-4, 4-7, 7-10, 10-20 and 20-25 years, respectively.

For OPFP with MW/SF pretreatment, the highest glucose concentrations were found with 4% SF that was 11.91, 18.27, 19.07, 23.46, and 24.76 g/l for oil palm aged 3-4, 4-7, 7-10, 10-20 and 20-25 years, respectively. Increasing the sulfuric acid concentration from 1-4% for the combined microwave and chemical pretreatment process positively affected the glucose concentration. The pretreatment with SF gave lower glucose concentrations than with HP at the same concentrations as the pretreating agent. This is because the lignin that inhibited the enzymatic hydrolysis was removed with acid pretreatment but to a lesser extent than the alkali treatment [31]. When the lignocellulose structure is destroyed with the pretreatment process, the enzyme will work more effectively. Using hydrogen peroxide in the pretreatment was found to have more effect on the enzymatic digestibility than using sulfuric acid in the pretreatment [29]. Moreover, the pretreatment with MW/SF at some concentrations was better than that with water in terms of glucose concentrations released from enzymatic hydrolysis.

All types of pretreatment methods gave higher glucose concentrations than without pretreatment. This is because the high energy radiation of microwave pretreatment leads to more change in cellulosic materials by increasing specific surface area and decreasing the degree of polymerization of cellulose [32]. Taherzadeh and Karimi [3] reported that the pretreated bagasse with microwave radiation resulted in a double glucose concentration compared to the untreated bagasse, corresponding to this study's result (Figures 4 and 5).

3.4 Bioethanol production from the SSF process

Production of bioethanol from cellulose hydrolysate by *S. cerevisiae* in the combination of pretreated OPFP with MW/W gave the most significant bioethanol concentrations: 4.55, 6.28, 6.95, 8.02, and 9.17 g/l at different oil palm ages of 3-4, 4-7, 7-10, 10-20 and 20-25 years, respectively (Figures 4 and 5). The bioethanol yields based on the amount of glucose are also shown. The pretreatment of OPFP with MW/W gave the highest bioethanol yields in the range of 0.41-0.42 g-bioethanol/g-glucose regardless of the ages of OPFP. Similarly, the studies of Kaparaju et al. [33] and Kadar et al. [34] reported that the ethanol yields from rice straw and industrial wastes using *S. Cerevisiae* in simultaneous saccharification and fermentation (SSF) were 0.41 and 0.31-0.36 g-bioethanol/g-glucose. The bioethanol yield obtained from sago pith waste using microwave hydrothermal hydrolysis was 0.47 g-bioethanol/g-glucose [10].

The pretreated OPFP with MW/W gave the highest bioethanol yield compared to MW/HP and MW/SF pretreatments. Although the MW/HP and MW/SF pretreatments showed higher glucose concentrations than the MW/W pretreatment in enzymatic hydrolysis, they generated greater amounts of inhibitors. These inhibitors significantly affected the efficiency of enzymatic hydrolysis, as seen from the higher glucose concentrations in the pretreatments with MW/HP and MW/SF. However, the inhibitors harmed the growth of yeast in fermentation. As a result, increasing the concentrations of hydrogen peroxide and sulfuric acid decreased bioethanol yield. The combined pretreatment with MW/HP and MW/SF could increase the amount of cellulose. Still, it inevitably generated more toxicity (furfural, HMF, formic acid, acetic acid, phenol, etc.), negatively affecting the SSF's yeast activity. Inhibitory components such as acetic acid, furfural, HMF, formic acid, and phenolic compounds caused a lag phase in the cell growth of *S. cerevisiae*, resulting in a slow sugar consumption rate [35]. Therefore, the combined pretreatment of OPFP with MW/W is attractive. Furthermore, this type of pretreatment could save the cost of chemicals.

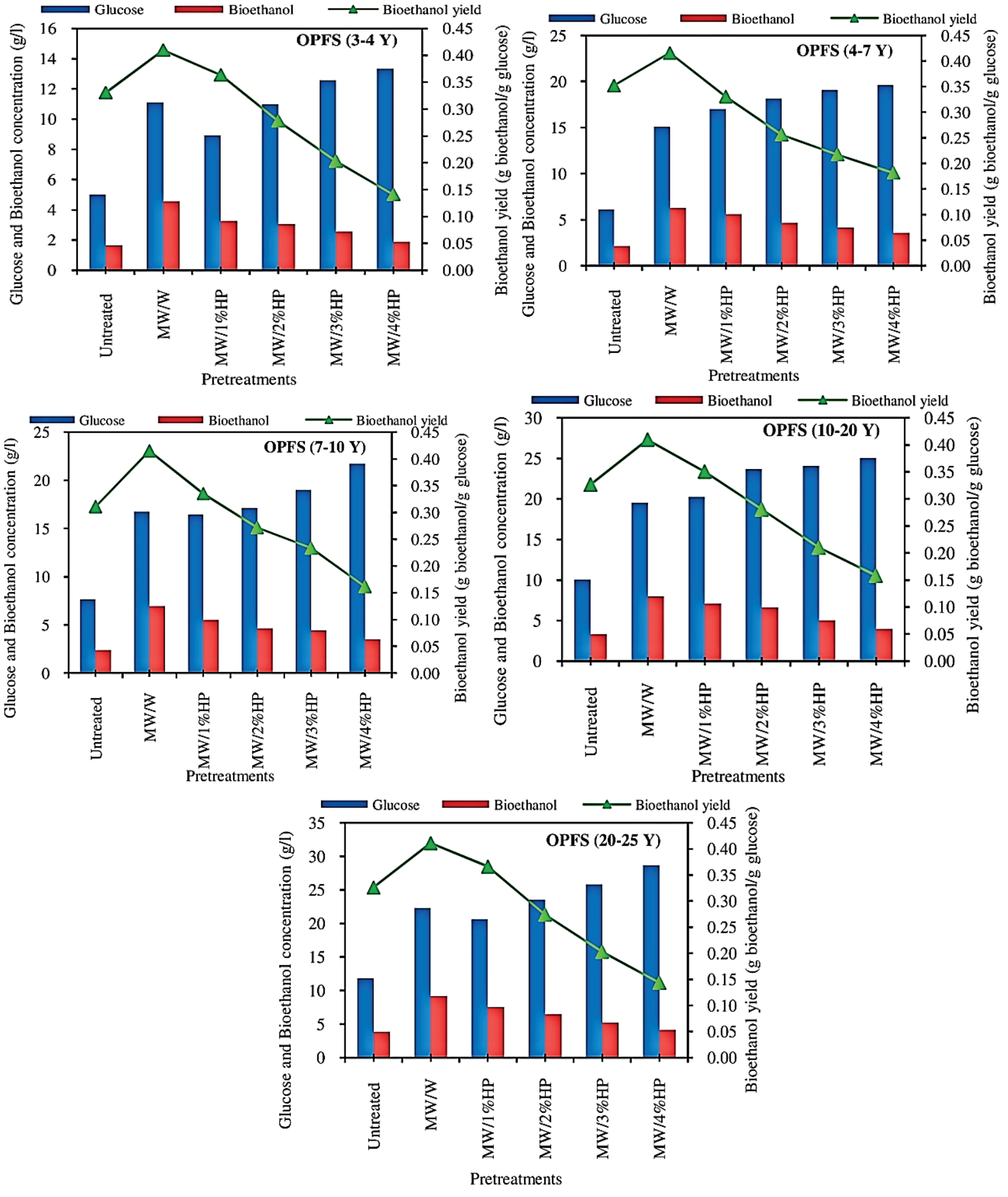

Figure 4. Glucose, bioethanol concentration, and bioethanol yield in SSF process of OPFP at oil palm aged 3-4, 4-7, 7-10, 10-20, and 20-25 years using the different OPFP pretreatment methods with MW/HP.

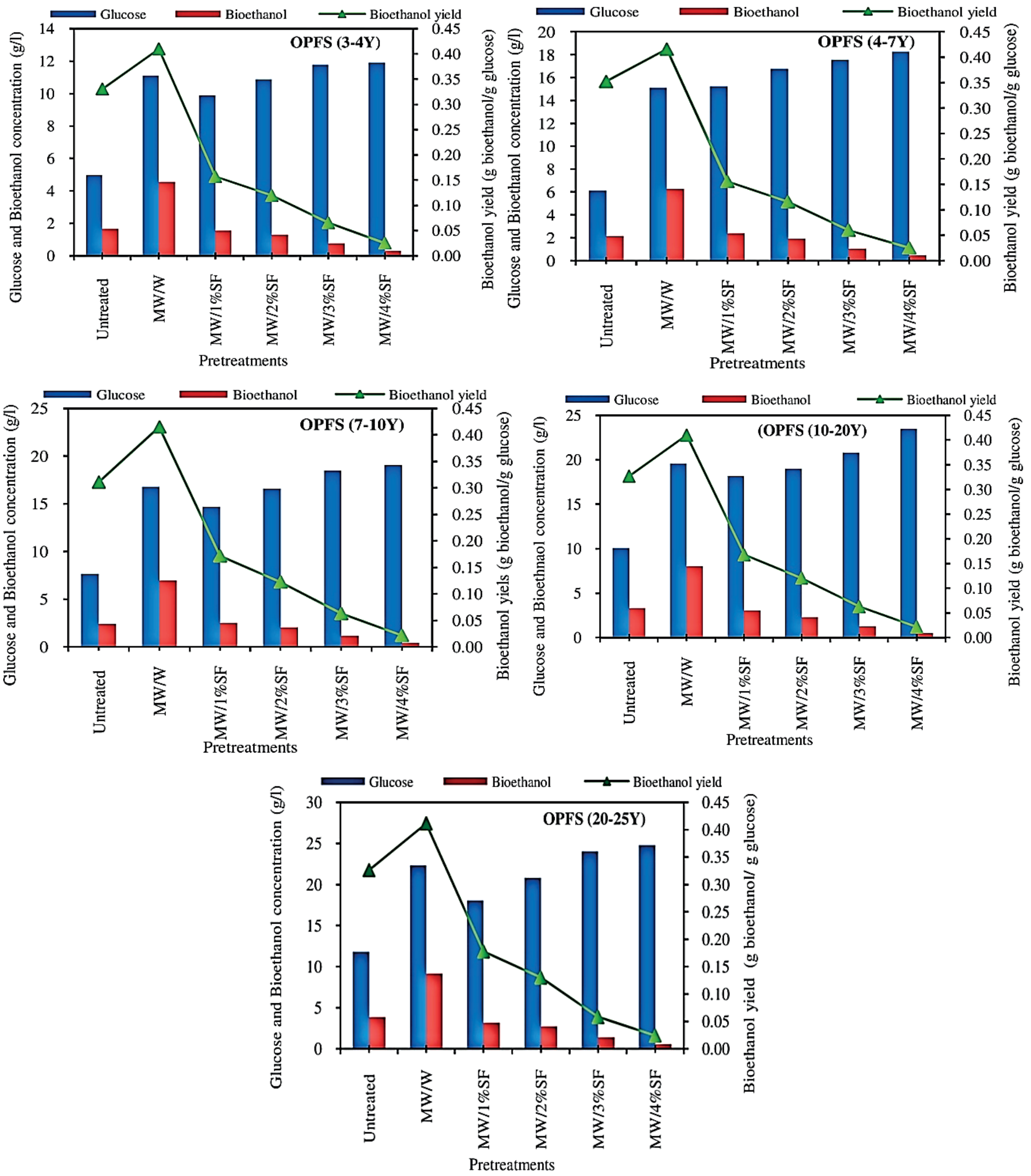

Figure 5. Glucose, bioethanol concentration, and bioethanol yield in the SSF process of OPFP aged 3-4, 4-7, 7-10, 10-20, and 20-25 years using the different OPFP pretreatment methods with MW/SF.

Comparisons between the concentrations of bioethanol from the experiment with the theoretical values were made and shown in Figures 6 and 7. The fermentation efficiency (%) is also included. The results showed that combining the pretreated OPFP with MW/W gave the highest efficiencies, about 82% of the theoretical bioethanol yield (0.51g-bioethanol/g-glucose), regardless of the ages of OPFP. Jung et al. [21] reported that the combined pretreatment with 1% (w/v) sulfuric acid and microwave at 1,200W and a heating time of 3 min resulted in 52.5% of theoretical ethanol yield after 72 hr of SSF.

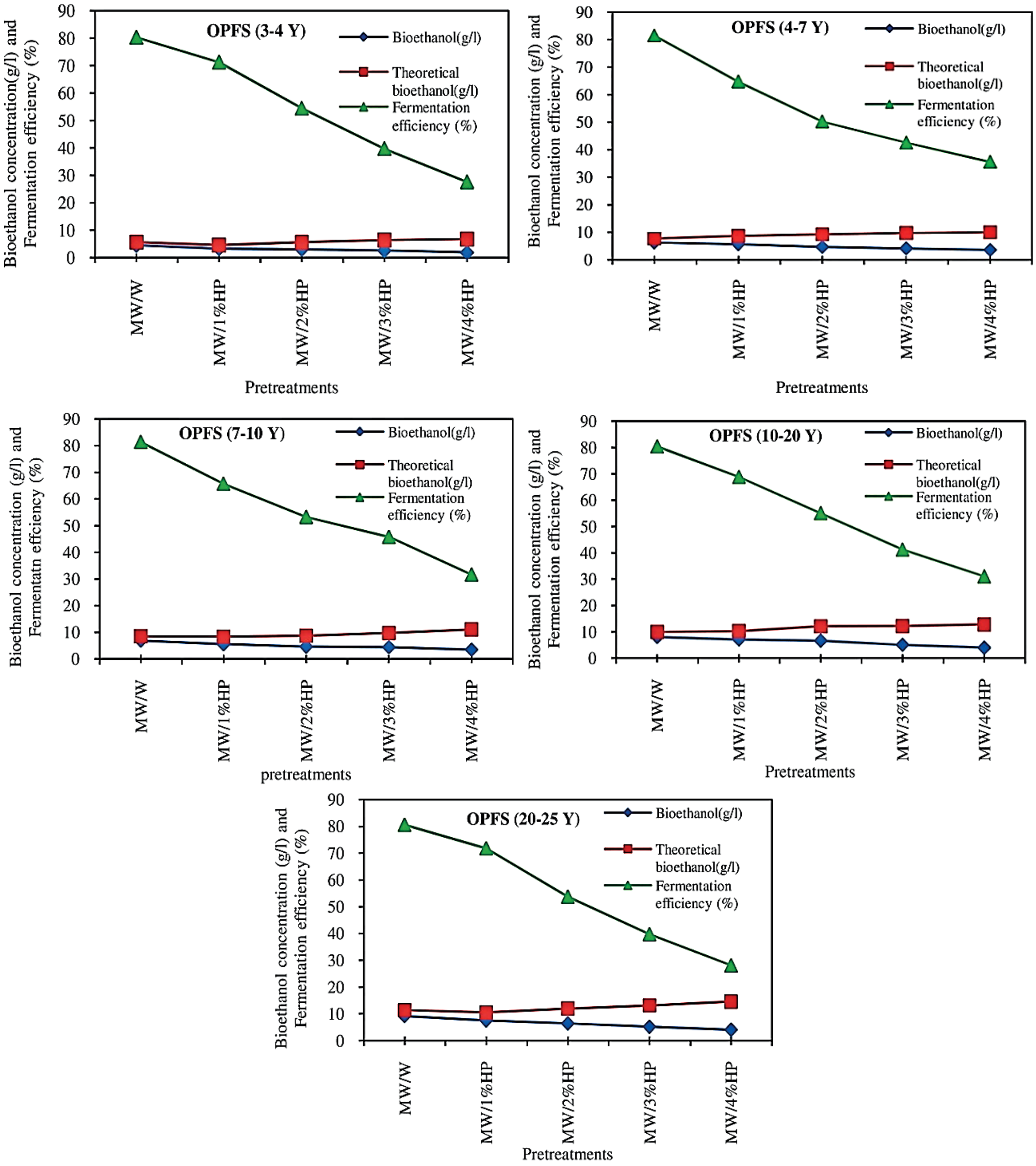

Figure 6. Comparison of experimental and theoretical concentrations of ethanol and fermentation efficiency (%) at different oil palm ages: 3-4, 4-7, 7-10, 10-20, and 20-25 years using the different OPFP pretreatment methods with MW/HP.

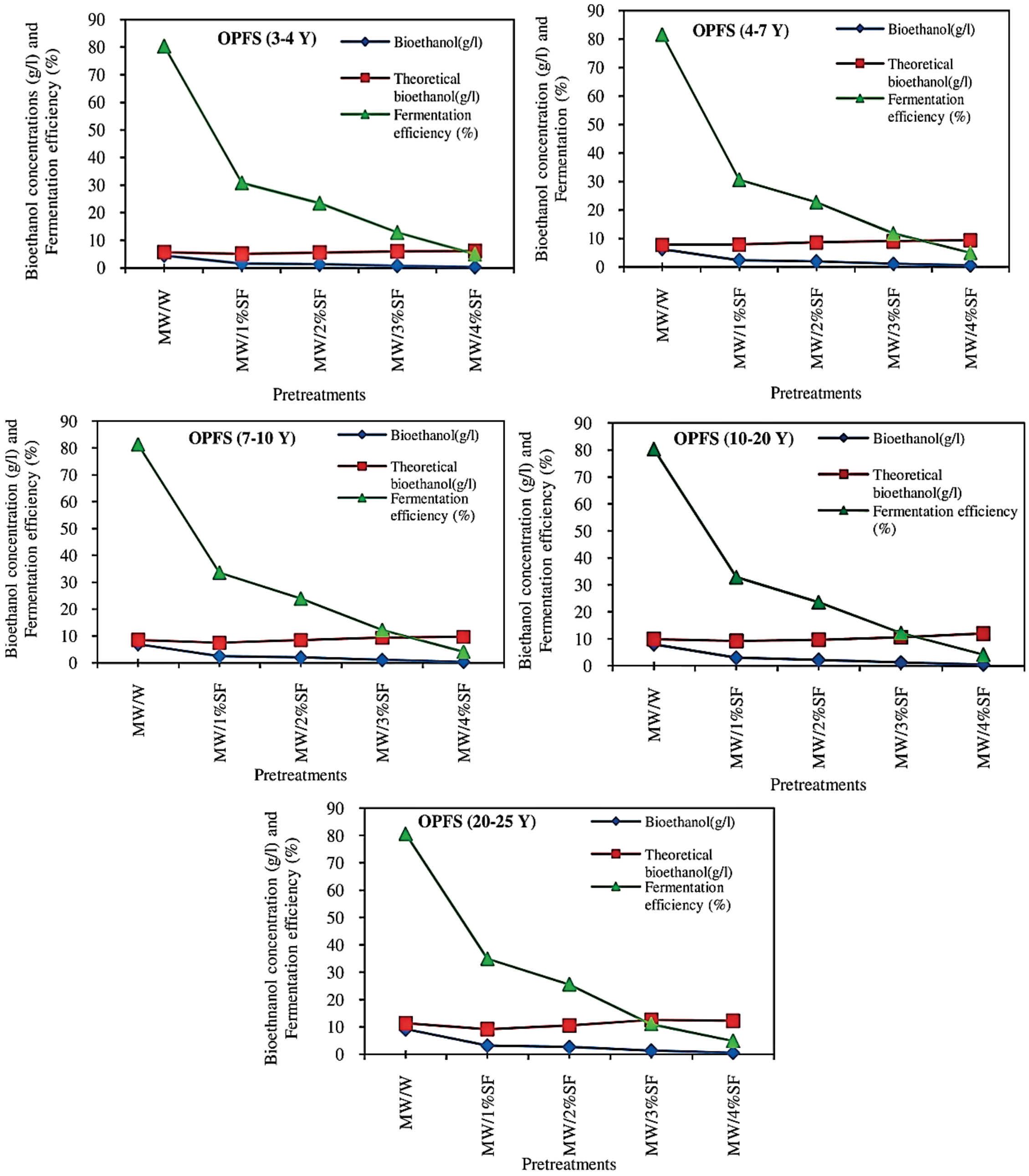

Figure 7. Comparison of experimental and theoretical concentrations of ethanol and fermentation efficiency (%) at different oil palm ages: 3-4, 4-7, 7-10, 10-20, and 20-25 years using the different OPFP pretreatment methods with MW/SF

4. Conclusions

OPFP pretreated with MW/W showed the highest bioethanol yield compared to MW/HP and MW/SF pretreatments. MW/HP and MW/SF pretreatments yielded higher glucose concentrations during enzymatic hydrolysis than MW/W pretreatments but produced large amounts of inhibitor. These inhibitors significantly affected the efficiency of enzymatic hydrolysis, as evidenced by the high glucose concentrations in the MW/HP and MW/SF pretreatments. However, the inhibitor adversely affected yeast growth during fermentation. As a result, bioethanol yield decreased with increasing hydrogen peroxide and sulfuric acid concentrations. Combined MW/HP and MW/SF pretreatments can increase the amount of cellulose but inevitably produce more toxic substances (furfural, HMF, formic acid, acetic acid, phenol, etc.) and reduce yeast activity in the SSF.

5. Acknowledgements

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