EFFECTS OF PHYSICAL AND CHEMICAL PRE-TREATMENTS ON XYLOSE AND GLUCOSE PRODUCTION FROM OIL PALM PRESS FIBRE

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ABSTRACT

Several physical and chemical pre-treatments were attempted to maximize the production of xylose and glucose from the hydrolysis and saccharification of hemicellulose and cellulose from oil palm press fibre. Bleached (holocellulose, PI), alkaline treated (PII) and untreated oil palm press fibre (PIII) of different sizes (<0.3 mm, 0.3 - 0.4 mm and >0.4 mm) were first pre-hydrolysed with H_2SO_4 to produce xylose. All the treatments showed that the maximum yield of xylose was obtained from fibre of >0.4 mm, and the lowest from fibre of <0.3 mm. The latter result is believed to be due to residual kernel, shell and other impurities present from the grinding. At the optimum size of >0.4 mm, sample PII gave the highest yield of xylose (67.8 g litre^{-1}) and the lowest yield was from PIII (29.2 g litre^{-1}) while PI gave 44.5 g litre^{-1}. The percentages of conversion of dry weight of oil palm press fibre were 23.9%, 9.3% and 22.3%, respectively. Saccharification of the residues from pre-hydrolysis of PI, PII and PIII (celluclast 1.5 L; 1500 IU g^{-1}, novozyme 188; 250 IU g^{-1}, pH 4.8, temperature 48ºC, agitation at 150 rpm for 48 hr) gave yields of glucose of 9.8, 35.8 and 18.8 g litre^{-1}, respectively. The percentages of conversion of dry weight of oil palm press fibre were 18.0%, 45.2% and 27.8%, respectively. The acid hydrolysed residue of PI treated with 2% (w/v) NaOH at 121ºC for 120 min (after soaking overnight at ambient temperature) gave the highest yield of glucose from saccharification (73.2 g litre^{-1}) (51.3% conversion of the dry weight of oil palm press fibre). The studies indicated that chemical pre-treatments of the fibre with mild acid followed by alkaline delignification before saccharification with enzyme, gave the maximum yields of xylose and glucose.

Keywords: oil palm biomass, hemicellulose, cellulose, pre-hydrolysis, saccharification.

INTRODUCTION

Oil palm press fibre (OPPF), or mesocarp fibre, is the fibre obtained after expressing oil from the fruit mesocarp. On average, for every tonne of fresh fruit bunches (FFB) processed, 200 kg empty fruit bunches (EFB), 670 kg palm oil mill effluent (POME), 120 kg mesocarp fibre, 70 kg shell and 30 kg palm kernel cake (PKC) are produced. In 1993, an estimated 4.74 million tonnes of FFB and 1.68 million tonnes of EFB were produced (Gurmit, 1994).

OPPF contains, on a dry weight basis, approximately 40% cellulose, 21% lignin, 24% pentosan and 5% ash (Kirkaldy and Sutanto, 1976). Cellulose, a polymer of α-D-1,4-linked anhydrous glucose units, $(C_6H_{12}O_5)n$, constitutes 40%-60% of the cell walls of woody plants. Basically, the individual cellulose molecules are linked together to form elementary microfibrils, which, in turn are aggregated by intermolecular hydrogen bonding

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into larger subunits called fibrils. The microfibrils contain alternating phases of highly ordered (crystalline) and randomly oriented (amorphous) cellulose embedded in a matrix of hemicellulose (Cole, 1983). This latter carbohydrate (also known as pentosan), constituting 20%-50% of the plant dry weight, is a branched polymer of pentose sugars, $(C_5H_{10}O_4)n$. The cellulosic and hemicellulose fractions are encrusted in an amorphous layer of lignin. Lignin, which constitutes 15%-25% of the plant material, is a complex three-dimensional polymer formed by carbon-carbon or ether bonding between phenyl-propane units (Sarkanen and Ludwig, 1971).

Two simple sugars - xylose and glucose - can be extracted from the hemicellulose and cellulose. Almost complete extraction is obtained by (i) pre-hydrolysis with mild acid to hydrolyse the hemicellulose into xylose, and (ii) pre-treatment of the substrate to increase the availability or susceptibility of the cellulose fraction, and enzymatic or concentrate acid hydrolysis of the cellulose to glucose.

Physical treatment to reduce the size of the OPPF (by grinding or milling) increases the hydrolysis rate by increasing the surface area for reaction (Phillip *et al.*, 1979). Mild acid is used to extract xylose by hydrolysing the hemicellulose (Gilbert *et al.*, 1952; Harris *et al.*, 1945). This process is also a pre-treatment for the fibre as removal of the hemicellulose (as xylose) and other low molecular extractives increases the cellulose susceptibility to chemical or enzymatic reactions.

Sodium hydroxide (NaOH) is used as an intracrystalline swelling agent for both the crystalline and amorphous cellulose (Millett *et al.*, 1976). Swelling the cellulose decreases its crystalinity and further increases its susceptibility to chemical or enzymatic reactions. Conversion of the cellulose into glucose can either be by acid hydrolysis or enzymatic saccharification. In this study, enzymatic saccharification was used as the specificity of the reaction avoided sugar degradation. The enzymes used were cellulase, which hydrolysed the cellulose at the β-1,4-glucan linkages into glucose and cellobiose, and cellobiase, which later converted the cellobiose into more glucose (Kanda *et al.*, 1979; Wood, 1968; Wood and McCrae, 1979).

The objective of this study was to investigate physical and chemical pre-treatments to promote the hydrolysis of hemicellulose and cellulose in OPPF to xylose and glucose, respectively.

MATERIALS AND METHODS

Sample Preparation

OPPF obtained from a palm oil mill in Nibong Tebal, Penang was washed with water and dried in an oven at 50°C - 60°C until constant weight, before grinding in a shredder and sifting into fractions of <0.3 mm, 0.3 - 0.4 mm and >0.4 mm. Three types of particles were prepared:

(a) Untreated OPPF (PI). The particles were used as ground and sieved.
(b) Holocellulose (PII). This was the residual OPPF fibre comprising only hemicellulose and cellulose after the lignin was removed by bleaching using the method of Wise and D'addieco (1946). It was prepared from all the sizes of OPPF.
(c) Alkali-treated OPPF (PIII). This was also prepared from all the different-sized OPPF. One portion (g) of OPPF was mixed with 20 portions (ml) of 1% (w/v) NaOH and heated in a boiling water bath for 2 hr. The treated fibre was then sieved out and pressed to remove its black liquor before washing with hot water until no traces of alkali were detected by litmus paper. The fibre was oven dried at 50°C-60°C until constant weight.

A ground but unsieved sample was used as the standard to assess the effect of sieving on the xylose yield during the mild acid hydrolysis.

Acid Hydrolysis

PI, PII and PIII of all sizes were hydrolysed by mild acid to produce a hydrolysate containing xylose (hydrolysis of hemicellulose). One portion (g) of each OPPF sample was soaked in five portions (ml) of sulphuric acid, H_2SO_4 (0.7% w/v), at 125°C for 120 min. The xylose was obtained in the filtrates from the hydrolysed samples. This preliminary study was to identify the chemical and physical pre-treatments for maximizing the yield of xylose from OPPF.

Saccharification

The optimum sized fibre (>0.4 mm) from the acid hydrolysis study above for each of the samples was used for conversion into glucose by enzymic saccharification. This was also to study the effect of the chemical pre-treatments towards the glucose yield from the saccharification process. Five portions (g) of each solid residue were added into 100 portions (ml) of acetate buffer solution (pH 4.8) at 48°C and mixed with 3 ml cellulase (celluclast 1.5 L, 1500 IU g^{-1}) enzyme and 1.5 ml cellobiase (Novozyme 188, 250 IU g^{-1}) enzyme and stirred at 150 rpm for 48 hr. The mixture was then heated to 100°C to denature the enzymes, followed by centrifugation at 4000 rpm for 20 min. The supernatant was used for estimation of the glucose produced. The non-treated fibre at the optimum size (>0.4 mm) was used as a blank.

Study on the Multistage Process

This was to study the effect of alkali treatment on the acid hydrolysed sample (PI) before saccharification. The process involved treating one portion (g) of the >0.4 mm PI with 20 portions (ml) of NaOH (0.5%-2.0%) at 121°C for 30 to 120 min. For comparison, another batch of PI was pre-soaked in the solution overnight prior to the heating process. The solid residue (designated PAI) was then filtered out and dried before the saccharification to glucose.

Chemical Analysis

Chemical analysis of the OPPF was carried out by the methods below:

- Moisture content - TAPPI Method T208 om-84 (Anon. ,1984a);
- Water solubility - TAPPI Method T207 om-81 (Anon., 1981);
- Sodium hydroxide (1%) solubility - TAPPI Method T212 om-83 (Anon., 1983a);
- Pentosan content - TAPPI Method T223 hm-84 (Anon., 1984b);
- Klason lignin - TAPPI Method T222 om-83 (Anon., 1983b);
- Holocellulose content - Wise *et al.* (1946); and
- α-Cellulose content - TAPPI Method T203 om-83 (Anon., 1983c).

The determination of glucose in the hydrolysate after saccharification was by the DNS method (Miller, 1959), and of furfural by calculation as for pentosan (TAPPI Method T223 hm-84).

RESULTS AND DISCUSSION

Table 1 shows the approximate chemical analysis of the OPPF. OPPF contains a high content of holocellulose (cellulose and hemicellulose) and lignin, like all lignocellulosic/woody materials. With the high content of holocellulose, it is to be expected that considerable sugars (glucose and xylose) can be extracted from it.

Furfural, or furafuraldehyde, $C_5H_4O_2$, is a side-product from the hydrolysis of pentosan (of which xylose is one), formed from the xylose under the extreme conditions of temperature and acidity as in *Figure 1* (Geissman, 1963). *Table 2* shows the yields of xylose and furfural from mild acid hydrolysis of the differently pre-treated OPPF.

Notwithstanding the effect of particle size, PII gave the highest concentration of xylose as well as furfural. The fibre of >0.4 mm gave the maximum concentration of xylose at 67.8 g litre^{-1}, while PIII and PI of that size gave only 29.2 g litre^{-1} and 44.5 g litre^{-1}, respectively. Although the xylose concentration of PII was significantly higher than that of PI, the conversions of OPPF for both the samples (from the initial dry weight) were not significantly different at 23.9% and 22.3%, respectively. This was because PII lost 25% of its weight in the bleaching process. The lowest yield of xylose from PIII (9.3% conversion from the initial OPPF weight) was because much of its hemicellulose was dissolved in the alkali solution.

The particle size of >0.4 mm gave the highest xylose yield regardless of chemical pre-treatment. This was unexpected, as the smaller particles with larger surface areas should have produced more (Millett *et al.*, 1976; Phillips and Humphrey, 1983). The only possible explanation for this was the presence of impurities, such as debris and shell, from the grinding. As no xylose can be produced from the shell, the higher the shell content, the lower would be the xylose concentration. This would be a difficult problem to avoid, as it would require the fibre to be totally shell-free. The solid impurities would have been grounded to a finer size, and, being more heavy and dense, would have mostly collected in the <0.3 mm fraction. This fact is supported by the xylose yields of the unsieved samples of the three pre-treatments, with each pre-treatment producing

TABLE 1. CHEMICAL ANALYSIS OF OIL PALM PRESS FIBRE (dry weight basis)

Parameter	(%)
Moisture	5.2
Hexane-alcohol solubility	3.6
Holocellulose	70.6
Pentosan	15.6
α-Cellulose	32.4
Lignin	20.5
1% NaOH solubility	20.0

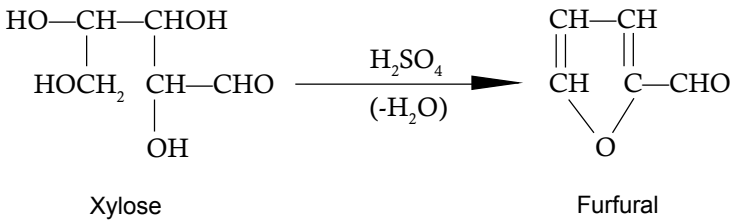

Figure 1. Conversion of xylose to furfural.

TABLE 2. YIELDS OF XYLOSE AND FURFURAL FROM ACID HYDROLYSIS OF DIFFERENT PREPARATIONS OF OIL PALM PRESS FIBRE (OPPF) (acid hydrolysis: 1 g PPF in 5 ml 0.7% H_2SO_4; autoclaved at 125°C at 120 min)

Treatment	Xylose (g litre^{-1})	Furfural (g litre^{-1})
Non-treated OPPF (PI)		
Ground without sieving	31.4	0.8
Ground and sieved		
>0.4 mm	44.5	1.0
0.3 - 0.4 mm	40.2	0.9
< 0.3 mm	28.2	0.5
Bleached OPPF (PII)		
Ground without sieving	39.3	1.1
Ground and sieved		
>0.4 mm	67.8	1.6
0.3 - 0.4 mm	44.4	1.8
<0.3 mm	30.6	1.3
Alkali treated OPPF (PIII)		
Ground without sieving	23.6	0.2
Ground and sieved		
>0.4 mm	29.2	0.5
0.3 - 0.4 mm	26.4	0.6
<0.3 mm	18.7	0.4

the second lowest yield from all the ground and sieved different-sized samples.

The residues from mild acid hydrolysis of the three chemically pre-treated OPPF (>0.4 mm fraction) were compared for their susceptibility to enzymic saccharification with the unhydrolysed OPPF as control. *Table 3* shows the yields of glucose and the percentages of conversion based on the initial dry weight of OPPF. The residue from the pre-hydrolysed PII gave the highest glucose yield of 35.8 g litre^{-1} (45.2% conversion of the initial OPPF weight). This result was expected, as PII was free of lignin. The oxidation agent, $NaClO_2$, used for bleaching had dissolved away the lignin leaving a white powder of holocellulose believed to contain only cellulose and hemicellulose (Ahlgren and Goring, 1971).

As expected, the residue from PIII gave the second highest glucose yield from saccharification. As mentioned earlier, NaOH swelled and converted the crystalline cellulose into the more reactive amorphous form (Millett *et al.*, 1976; Li and MacLeod, 1993).

The residue from PI produced more glucose (9.8 g litre^{-1}) than the control (2.3 g litre^{-1}). This was probably because leaching out the hemicellulose (as hydrolysed pentose sugars) left a porous structure in the lignocellulosic material, exposing more of the cellulose to enzymic saccharification. Pre-treatment of the lignocellulosic material with sulphuric acid had been attempted previously to achieve this (Han and Callihan, 1974; Knappert *et al.*, 1980).

On the study of the multistage process, the residue from the optimized mild acid hydrolysis (PAI) was treated with different concentrations of NaOH over various heating times (with/without pre-soaking overnight) before the saccharification process.

Figure 2 shows the weight loss of the OPPF residue from dissolving away the lignin with different concentrations of NaOH and autoclaving at 121°C for different times. There was a decrease in weight indicating that the lignin removal increased with the NaOH concentration and length of treatment. Conversely, there was a significant increase in the yield of glucose from saccharification (*Figure 3*). The NaOH first dissolved away the lignin encrusting the cellulose and protecting it from chemical attack. The exposed cellulose was then swollen by the alkali and transformed from its relatively inert crystalline form to the more reactive amorphous form (Millett *et al.,* 1976).

To optimize the alkali treatment, the effect of soaking the residue overnight in the alkali solution before cooking was investigated. The same range of alkali concentrations were studied but with the time of heating reduced to 90 and 120 min. *Figure 4* shows no significant difference in weight loss between the soaked and unsoaked samples for both the 90 and 120 min heating times; however, there was a significant increase in the yield of glucose by soaking (*Figure 5*). The highest glucose yield after soaking and immediately heating for 120 min was 49.2 g litre^{-1} compared to 73.2 g litre^{-1} for the pre-soaked sample, that is, an increase of 48.8%. This was a 51.3% conversion of the PPF into glucose, which made PAI

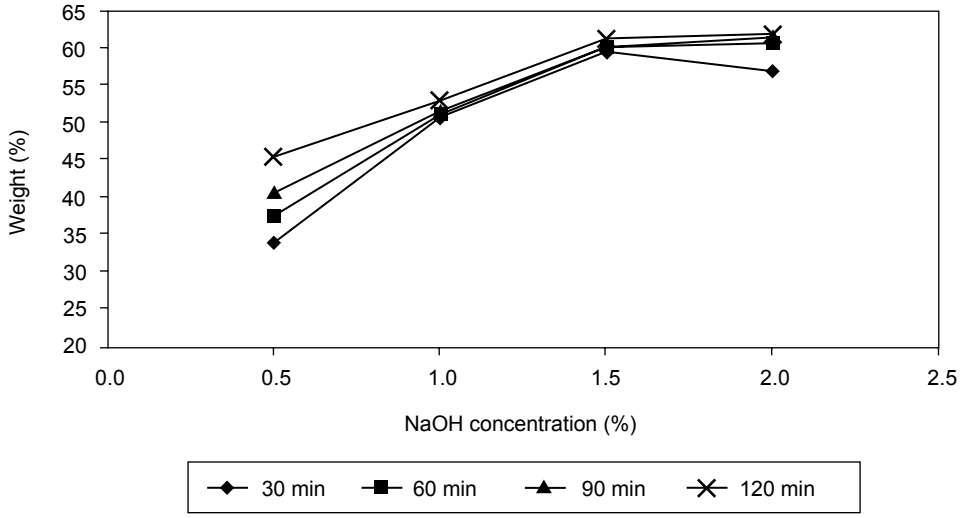

Figure 2. Weight loss of oil palm press fibre acid hydrolysed residue (PAI) after treatment with NaOH at different concentrations and times.

TABLE 3. YIELDS OF GLUCOSE AND PERCENTAGES OF CONVERSION OF RESIDUES FROM PRE-HYDROLYSED OIL PALM PRESS FIBRE (OPPF) UNDER OPTIMUM CONDITIONS (48°C, pH 4.8, stirring at 150 rpm for 48 hr, solid:liquid ratio of 1:20)

Sample	Concentration of glucose (g litre^{-1})	Percentage conversion of dry OPPF
Control (non-hydrolysed OPPF)	2.3	2.6
Residue from PI	9.8	18.0
Residue from PII	35.8	45.2
Residue from PIII	18.8	27.8

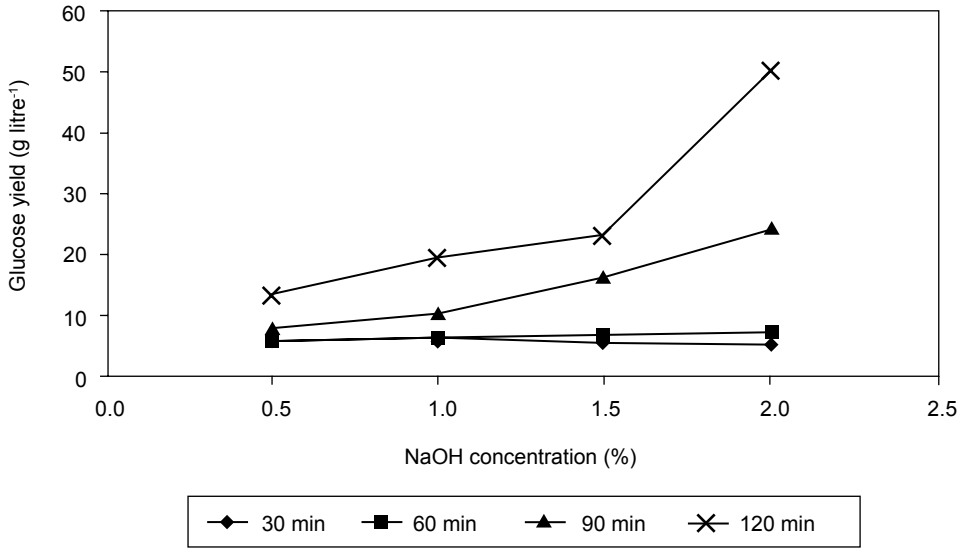

Figure 3. Yields of glucose from the saccharification of oil palm press fibre acid hydrolysed residue (PAI) after treatment with NaOH at different concentrations and times.

TABLE 4. YIELDS AND PERCENTAGES OF CONVERSION OF XYLOSE AND GLUCOSE FROM OIL PALM PRESS FIBRE (OPPF) BY VARIOUS METHODS OF CHEMICAL EXTRACTION

OPPF sample	Xylose (g litre^{-1})	Percent conversion of xylose (%)	Glucose (g litre^{-1})	Percent conversion of glucose (%)
PI	44.5	22.3	9.8	2.6
PAI	44.5	22.3	73.2	51.3
PII	67.8	25.4	35.8	45.2
PIII	29.2	9.3	18.8	27.8

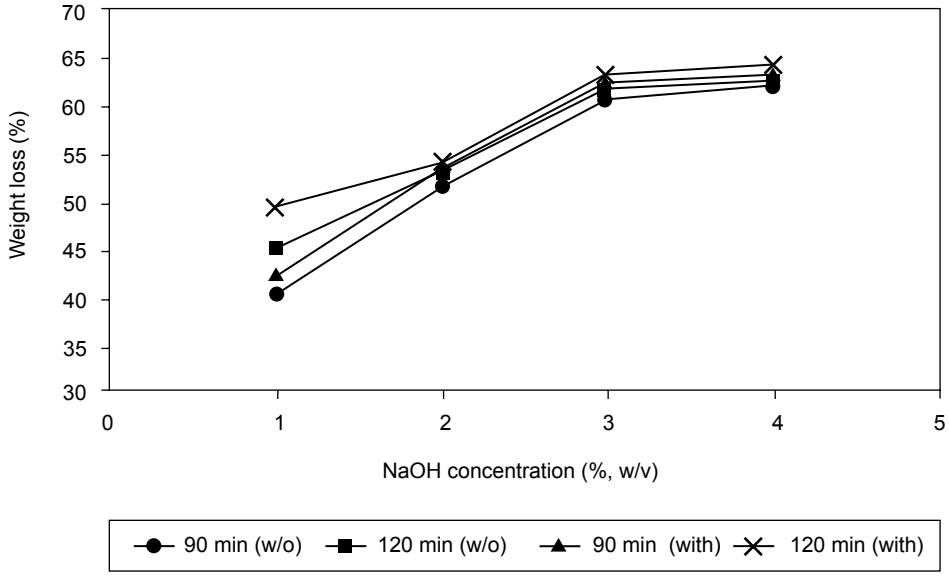

Figure 4. Weight loss of oil palm press fibre acid hydrolysed residue (PAI) after treatment with NaOH at various concentrations and times, with or without soaking overnight before cooking.

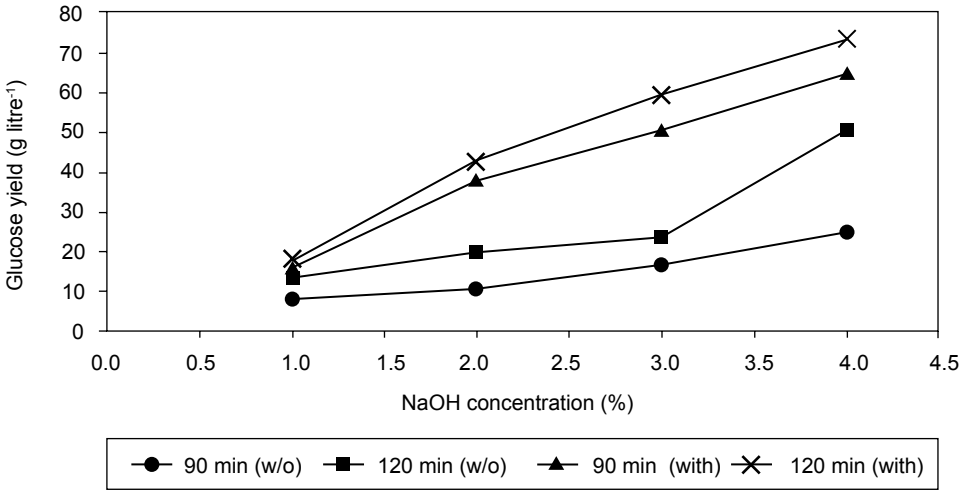

Figure 5. Yields of reducing sugars after saccharification of NaOH treated oil palm press fibre acid hydrolysed residue (PAI) at various NaOH concentrations and times, with or without soaking overnight before cooking.

the highest yielding sample for extracting glucose by the multi-stage process (*Table 4*).

CONCLUSION

Based on the xylose and glucose yields, a sequence of processes was needed to extract the cellulose, hemicellulose and lignin components from a lignocellulosic material. The lower molecular weight component, hemicellulose, was first extracted as a sugar, xylose and furfural by mild acid hydrolysis. Then, delignification, either by bleaching with sodium chlorite and acetic acid at 70°C or by autoclaving with NaOH, increased the accessibility of the cellulose part to enzymatic action to produce glucose (*Table 4*). However, apart from being a solvent for lignin, NaOH also acted as a swelling agent for the cellulose and enhanced its susceptibility to saccharification. This was shown by the samples PA1 and PII, both of which gave high glucose yields.

Alkali lignin was produced as a by-product, whereas the chlorous acid produced from using sodium chlorite and acetic acid oxidizes the lignin as well as the cellulose into lower molecular weight components (Ahlgren and Goring, 1971). Pre-hydrolysis of PPF with 0.7% (w/v) H_2SO_4 at 125°C for 120 min at a solid:liquid ratio of 1:5 (0.3 - 0.4 mm particle size), followed by treatment with 2% (w/v) NaOH at 121°C for 120 min (pre-soaked overnight before heating) was the optimum process yielding 40.2 g litre^{-1} and 73.2 g litre^{-1} of xylose and glucose, respectively.

Fractionation of cellulose, hemicellulose and lignin from OPPF by this multistage process was able to produce several useful chemical products, such as xylose, furfural, glucose and lignin, enhancing its economic feasibility (Tong and Cannel, 1983).

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