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Preliminary Studies on the Steam Explosion Pretreatment of the Oil Palm Stem

HALIMAHTON MANSOR[1], K.SUDO[2] AND M.ISHIHARA[2]
[1]*Forest Research Institute Malaysia*
Kepong, 52109 Kuala Lumpur, Malaysia

[2]*Wood Chemistry Department.*
Forestry and Forest Products Research Institute, P.O. Box 16,
Tsukuba, Norin Kenkyu Danchi-Nai, Ibaraki-Ken,
305 Japan

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ABSTRAK

Peletupan stim telah didapati sebagai suatu kaedah yang berkesan bagi meningkatkan hidrolisis enzim ke atas selulosa di dalam batang kelapa sawit. Pengolahan ini juga menyebabkan degradasi hemiselulosa dan menjadikannya larut di dalam air. Melalui pengestrakan akueus ke atas bahan yang diletupstimkan, 23-31% hemiselulosa yang mengandungi xilosa sehingga 83%, boleh diperolehi selepas hidrolisis asif. Perubahan-perubahan morfologi yang berlaku di dalam pengolahan ini juga dibincangkan.

ASTRACT

Steam explosion was found to be an effective method of improving the enzymatic hydrolysis of cellulose in the oil palm stem. The pretreatment also caused the degradation of hemicelluloses and rendered them water-soluble. By aqueous extraction of the exploded material, 23-31% hemicelluloses with xylose contents up to 83% after acid hydrolysis could be recovered. This paper also discusses some morphological changes which occurred in the pretreatment.

INTRODUCTION

The economic life of the oil palm (*Elaeis guineensis* Jacq.) is approximately 25 years after which its felling and replanting results in a large availability of the oil palm stem (OPS). In order to convert OPS into value-added materials, studies have been conducted on its characteristics and properties (Killmann and Lim 1985; Lim and Khoo 1986), pulp and papermaking (Khoo and Lee 1985) and production of reconstituted board (Chew and Ong 1985; Rahim *et al.* 1987). Studies on the carbohydarate content of OPS (Halimahton and Abdul Rashih 1990) revealed that this lignocellulosic material provides 50-60% monosaccharides after acid hydrolysis; thus it could also turn out to be a source of free sugars, especially glucose and xylose.

Steam explosion has been known to be one of the most promising pretreatments of various lignocellulosic materials in order to increase the rate and extent of cellulose hydrolysis for production of sugars for fermentation or digestible fiber for use as animal feed (Schults *et al.* 1983; Playne 1984; Tanahashi *et al.* 1985; Rolz *et al.* 1986; Kling *et al.* 1987)

This paper describes the investigation on the possibility of applying steam explosion as a pretreatment technique for OPS and reports on the effectiveness of the process in terms of enhancement of enzymatic hydrolysis and hemicelluloses solubilisation.

MATERIALS AND METHODS

Equipment

A batch type of steam explosion was used in this study. The apparatus consists of a reactor, a collecting chamber and a steam generator, all of which are made of stainless steel. The steam generator can produce pressures of up to 40 kgf/cm^2 (Nitto Koatsu Corp., Japan).

Materials

The oil palm stem (OPS) used in this study was collected from a plantation near Kepong, Selangor. The stem was divided into three portions, namely, the bottom (about 10% from the base), middle (50% from the base) and top (approximately 80% from the base). The wood samples were air-dried for two days before they were cut into matchstick-size chips. The chips were then oven-dried (40°C) for another two days for the pretreatment.

Substrate Pretreatment

Pretreatment by steam explosion was conducted by heating the OPS (300g) with superheated steam. The lignocellulosics were heated for a predetermined period, after which the treated material was exploded and released into the collecting chamber by instantaneous decompression of the reactor. The pretreatment time, which corresponded to the residence time of the material in the reactor, was varied with the pretreatment steam pressure. After the process, the effects of the pretreatment on hemicelluloses solubilisation and enhancement of enzymatic hydrolysis were evaluated.

Enzymatic Saccharification

The exploded wood (200mg moisture-free basis) was hydrolysed in a 0.1M acetate buffer solution (pH 5.0, 10ml) containing 50mg of a commercial enzyme preparation, "Meiselase-P" (Meiji Seika Co. Ltd.) derived from **Trichoderma viride** at 40°C for 48 h. The saccharification yield was based on the total polysaccharides in the treated sample. The enzymatic hydrolysate was analysed by HPLC for the determination of glucose and other reducing sugars.

Determination of Water Solubles and Dioxane Lignin

The steam-exploded wood (100g) was extracted with distilled water (300ml) at 70°C for 2 h. The mixture was filtered and the solution reduced to a small volume, under reduced pressure, freeze-dried, weighed and analysed by GPC and HPLC. About 300ml of 90% dioxane solution was added to the water-extracted sample and the mixture was heated at 70°C for 4 h. The dioxane extracts were evaporated under reduced pressure and weighed.

Acid Hydrolysis of Water-soluble Extracts

This was conducted according to the method of Paice *et al.* (1982). The water extracts (5mg) were mixed with trifluoroacetic acid (2M, 1.6ml) and the resulting solution was kept at room temperature for 48 h. It was then heated in air at 90°C for 2 h. The acid was removed under reduced pressure and the hydrolysis products dissolved in distilled water and analysed by HPLC.

Determination of Klason Lignin and Holocellulose

The determination of Klason Lignin was conducted according to TAPPI T222 in TAPPI Testing Procedures (Anon. 1978). The content of holocellulose was determined by the acid chlorite method (Browning 1967).

Analysis by HPLC

The relative amount of monosaccharides in the enzymatic hydrolysates and the acid hydrolysates of the water extracts was determined using a liquid chromatograph (Dionex 2000i, USA) with an ion-exchange column (Dionex AG-7 and AS-7, USA) and a pulsed amperometric detector. Distilled water was used as an eluant with a flow rate of 1.0 ml/min. To obtain adequate sensitivity of the electrochemical detection, 0.3N sodium hydroxide solution was added to the column eluant with a flow rate of 1.0 ml/min.

Analysis by GPC

Solutions of the water extracts (100 μl) were analysed for the presence of oligosaccharides using gel permeation chromatograph (Shimadzu, LC-3A) with two connected columns

(Shodex Ionpak KS-802, Showa Denkok K.K., Japan; length 30mm x diameter 8mm). Distilled water was used for the elution, at a flow rate of 0.8 ml/min. The eluant was monitored by refractive index.

Examination by Scanning Electron Microscopy
Fiber samples selected for study were mounted on brass specimen stubs. The fiber samples were then coated with platinum using an Ion Sputter JEOL JFC-110. The samples were examined on a JEOL Model JXA-840A scanning electron microscope with a 10-kv acceleration voltage.

RESULTS AND DISCUSSION

The analyses of the steam-exploded OPS are given in Table 1. Only the middle and the bottom section of the stem were investigated and not the top portion since the quantity of the latter was found to be insufficient for the pretreatment.

A part of the steam-exploded fiber was hydrolysed with a commercial cellulase, yielding a hydrolysate comprising mainly glucose (75-100%) and xylose (8-22%) (Table 3). The residual polysaccharides of the steam-exploded wood were highly susceptible to enzymatic attack compared to the corresponding untreated wood (Table 2). Based on the percentage of enzymatic susceptibility, the most efficient operating conditions were 20 kgf/cm^2 (4min) for the middle part of the OPS and 25 kgf/cm^2 (3 min) for the bottom portion. Under these reaction conditions, the level of enzymatic digestibility of the steam-exploded fiber was increased by altering the conditions of

TABLE 1
Enzymatic susceptibility and chemical composition of steam exploded oil palm stem

Samples	Condition		Suscep-tibility	Water extract	K.L.	S.L.
	Pressure (kgf/cm)	Time (min)	(%)[a]	(%)	(%)	(%)
Middle	20	4	85.9	22.5	26.4	43.1
	25	3	67.5	30.1	31.1	25.3
Bottom	20	4	81.0	30.5	31.9	29.9
	25	3	98.3	27.9	27.7	20.0

a Based on the residual polysaccharides in the fibre material
K.L. Klason lignin; values based on the residue.
S.L. Soluble lignin-content of extractable lignin with 90% dioxane to the total lignin in steamed wood; values based on the residue.

TABLE 2
Enzymatic saccharification and chemical composition of the untreated oil palm stem.

Samples	Moisture content	Suscep-tibility	Hot-water solubles		K.L.	Holocellulose
	(%)	(%)[a]	(%)[b]		(%)	(%)[c]
Middle	4.6	36.8	30.0[d]	31.1[e]	16.33	51.4
Bottom	5.1	25.4	19.3	20.0	20.58	68.6

a Based on the residual polysaccharides in the fibre material
b Based on the oven-dried material
c Based on extractives-free woodmeal
d Based on difference in weight of the sample before and after the extraction
e The weight of the water extract after evaporation under reduced pressure.

TABLE 3
Composition of reducing sugars in the enzymatic hydrolysates of steam-expoded oil palm stem

Samples	Condition		Reducing Sugars (ppm)				
	Pressure (kgf/cm^2)	Time (min)	Ara	Gal	Glc	Xyl	Man
Middle	10	10	-	-	433.3	76.1	-
	10	20	-	-	707.3	104.9	-
	20	4	-	-	525.0	72.2	-
	25	3	-	-	935.6	-	-
Bottom	10	10	43.5	11.4	1426.2	220.4	26.8
	10	20	-	-	632.8	53.7	-
	20	4	-	-	253.0	72.8	-
	25	3	-	8.6	696.3	198.8	28.6

Ara - arabinose
Gal - galactose
Glc - glucose
Xyl - xylose
Man - mannose

steam explosion. The cellulose in the pretreated fibre became more susceptible to enzymatic attack probably as a result of hemicellulose extraction and lignin redistribution. A subsequent loss in susceptibility, brought by structural collapse or further lignin redistribution and condensation, resulted in a corresponding loss in digestibility (Wong *et al.* 1988).

The Klason lignin content of the residual cellulosic materials ranged from 26-32%, indicating that the lignin was retained mostly in the residues after the pretreatment. Quite a high portion of the lignin could be extracted with 90% dioxane. However, earlier work has established that the removal of lignin did not increase the enzymatic digestibility of steam-exploded materials (Shimizu *et al.* 1983; Puls *et al.* 1983).

Table 4 shows the main sugar components in the water extracts after acid hydrolysis with trifluoroacetic acid. Depending on the part of the OPS, 23-32% hemicelluloses with xylose contents up to 83% could be recovered by aqueous extraction of the steam-exploded materials. Glucose seems to be the other major sugar present in the extracts while galactose, arabinose and fructose were found in very small or trace amounts. In the case of the middle part of OPS, the amount of xylose increased and that of glucose decreased with increasing steam pressure. However, for the bottom part of OPS both the sugars decreased with increasing steam pressure. These results differ from those of *A.mangium* and rubberwood in which the amounts of xylose and glucose in the water extracts decreased and increased respectively under more drastic reaction conditions (Halimahton *et al.* 1990).

Morphological Characteristics of the Steam-exploded OPS

The morphology of the steam-exploded OPS was found to be significantly different from that of the steam-exploded *Acacia mangium* and rubberwood (*Hevea brasiliensis*) (Halimahton *et al.* 1990). For the latter two species, at low steam pressure the steam-exploded material consisted mainly of fiber bundles; with increasing steam pressure fully separated fibers could be observed in addition to fiber fragments. Under more drastic conditions, the products appeared to be a dark brown to black amorphous mass, much like mud.

However, for OPS, the appearance of the steam-exploded material was the same at 20 kgf/cm^2 for 4 min and at 25 kgf/cm^2 for 3 min. The material was observed to consist of undegraded fiber bundles and a pale brown fibrous mass. Electron microscopy showed that the fibrous mass consisted of separated fibers and fiber fragments, with some of the fibers entangled into clusters together with scattering of parenchyma cells (*Fig.1*). There were also

TABLE 4
Sugar composition in the water extracts after steam explosion and acid hydrolysis

Samples	Condition		Water Extracts (%)	Reducing Sugars (%)[a]				
	Pressure (kgf/cm^2)	Time (min)		Glc	Xyl	Gal	Ara	Fruc
Middle	20	4	22.5	68.5	31.5	-	-	-
	25	3	30.1	61.8	38.2	-	-	-
Bottom	20	4	30.5	17.4	82.6	-	-	-
	25	3	27.9	4.4	68.0	-	12.6	13.1

a Based on the total solubilised sugars
Glc - glucose
Xyl - xylose
Gal - galactose
Ara - arabinose
Fruc - fructose

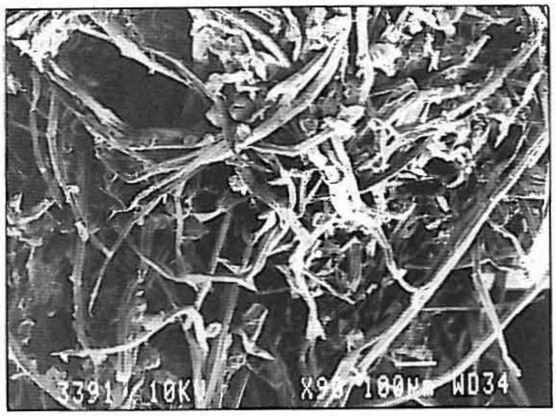

Fig 1: Wood fiber of oil palm stem, after steam explosion at 25 kgf/cm² for 3 min. Parenchyma cells are seen scattered among the fiber fragments.

regions containing intact fibres as shown in *Fig. 2.* An electron micrograph of a vascular bundle is shown in *Fig 3.* The vascular bundles seemed to be unaffected by the pretreatment.

The reason for the resistance displayed by the vascular bundles in the oil palm stem towards steam explosion was that the steam was unable to penetrate the wood chips sufficiently during the process. This arose because the chips used had been thoroughly dried and as a result they could not swell easily when in contact with steam. Hence, it it suggested that the use of fresh, green or soaked wood samples should be used for the pretreatment in the case of the oil palm stem.

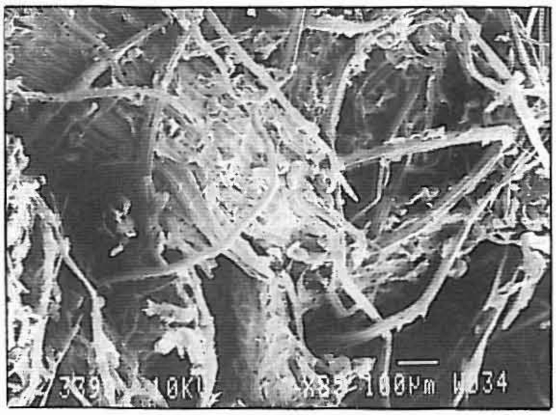

Fig 2: Wood fiber of oil palm stem after steam explosion at 25 kgf/cm² for 10 min. The area "A" shows a bundle of intact fibers.

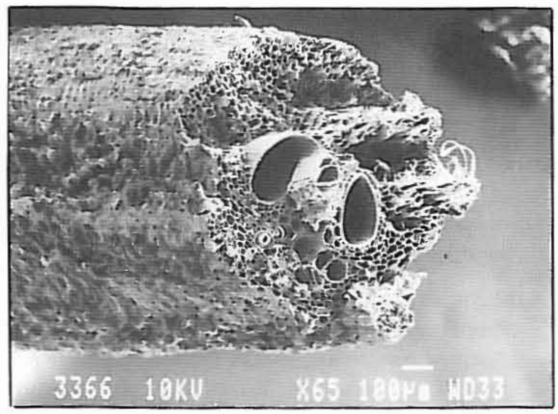

Fig 3: Vascular bundle of steam-exploded oil palm stem, at 25 kgf/cm² for 3 min

CONCLUSION

The results indicate that steam explosion as a pretreatment for the oil palm stem appears to have some promise for practical application.

The process seems to be very effective in preparing the substrate for further enzymatic and biological degradation, not only because of a high degree of delignification but also of the enhanced susceptibility of cellulose to enzymatic hydrolysis. This study also indicates that a marked improvement of sugar yield can be made when OPS is pretreated by steam explosion.

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