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ENZYMATIC HYDROLYSIS OF SOME MALAYSIAN WOODS

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TOMIMURA, Y., KHOO,K.C. & PUTRI FARIDATUL AKMAR. 1988. Enzymatic hydrolysis of some Malaysian woods. Steamed wood fibres from some Malaysian woods were subjected to enzymatic hydrolysis. The yield of reducing sugars from fibres of rubberwood *(Hevea brasiliensis)* and fast-growing trees such as *Acacia mangium, Paraserianthes falcataria,* and *Gmelina arborea* was under 20% of total fibre weight. On the other hand, fibres from oil palm stem showed a high yield of about 50% of the total fibre weight. Extraction of hemicellulose fraction with dilute alkaline solution improved the accessibility of enzyme for rubberwood but not for oil palm. Extraction by hot water increased slightly the saccharification of rubberwood and oil palm. With simultaneous enzymatic hydrolysis and fermentation of oil palm fibres, the glucose produced was rapidly consumed by yeast and converted to ethanol. The concentration of the ethanol reached a maximum of about 1% of the solution after three days reaction time and then decreased gradually.

Key words: Malaysian woods - steamed fibres - enzymatic hydrolysis - fermentation.

Introduction

Little work has been done to investigate the possibility of using the rich wood and plant resources in Malaysia for animal feed production despite the huge quantities of residues generated in wood harvesting and processing operations. In line with the effort to upgrade and develop the animal husbandry industry in the country, the potential of wood resources for animal feed was examined.

Enzymatic hydrolysis can be used to convert ligno-cellulosic materials for animal feed (Dietrichs *et al.* 1978). It is also applied in the saccharification of ligno-cellulosic materials to produce sugars for subsequent fermentation to alcohol (Kuwahara *et al.* 1988). The raw material, however, has to be pretreated to enhance its susceptibility to enzymic action. Steaming of lignocellulosic materials at high

temperature and pressure is one of the effective pretreatments frequently employed. Since Bender and his coworkers used this method in 1970 to increase the digestibility of the lignocellulosic materials for ruminants, it has been widely applied to various wood species.

The oil palm (*Elaeis guineensis*) and rubberwood (*Hevea brasiliensis*), largely available in Malaysia, are not fully used. Others include acacia (*Acacia mangium*), batai (*Paraserianthes falcataria*) and yemane (*Gmelina arborea*), the three most common tree plantation species being established throughout the country. This study examines the response of these five plants to enzymatic hydrolysis after suitable pretreatment by steaming.

Materials and methods

Preparation of fibres

Wood chips of the five plants were used for fibre preparation. The chips, about 300 *g* per lot, were treated in an Asplund defibrator with saturated steam at 10 $kg\ cm^{-2}$ for 20 *min* and then defibrated for 2 *min*. The fibrous product was dried in an oven at *ca.* 40° *C*.

Extraction of fibres

In hot water extraction, the fibres were washed in a Buchner funnel with ten parts boiling water to one part oven dry material. In alkaline extraction, the fibres were soaked in 1% w/v sodium hydroxide solution for 30 *min*, filtered in a Buchner funnel and then neutralised with dilute acetic acid.

Determination of holocellulose and lignin

The holocellulose and Klason lignin contents of the fibres were determined according to the method of Wise *et al.* (1946) and Tappi T 222, respectively.

Enzymatic hydrolysis

Fibres (about 0.2 *g*) and cellulase (Meicelase CEPB - 5081, 50 *g*) were placed in an Erlenmeyer flask and 0.1 M acetate buffer (pH 5.0, 10 *ml*) was added. The flask was shaken in a reciprocating shaking water bath at 40° *C* for 48 *h*. The loss in weight of fibres was determined by filtration and the filtrate was analysed for sugars by the Somogyi - Nelson method (Somogyi 1952) and HPLC.

Simultaneous enzymatic hydrolysis fermentation

Hot water extracted oil palm and rubberwood fibres were used. Fibres (10 *g*) and cellulase (1 *g*) were suspended in 0.1 M phosphate buffer solution (pH 5.0,

100 *ml*). For the culture of yeast, phosphate buffer was used instead of acetate buffer, and a few drops of Tween - 20 (polyoxyethylene sorbitan monolaurate) were added as an emulsifier (Kuwahara *et al.* 1988). Commercially available dry yeast of strain *Saccharomyces cerevisiae* (0.3 *g*) was added into the reaction mixture in a 250 *ml* Erlenmeyer flask. The whole mixture was shaken at 40°*C* for 10 days in a reciprocating shaking water bath incubator. At intervals a portion (5 *ml*) of reaction mixture was withdrawn with a pipette and filtered and the filtrate was analysed by HPLC.

Analysis by HPLC

Liquid chromatograph (HEWLETT-PACKARD, 1084B) with ion exchange column (AMINEX HPX-87P, BIO-RAD)and a refractive index detector (1037A, HEWLETT-PACKARD) were used for analysing 10 *ml* of reaction solution. Distilled water was used for the elution.

Results and discussion

Table 1 shows the yields and analysis of fibres. All the yields of the Asplund fibres were over 90%. Rubberwood and oil palm gave the lowest yields due probably to their relatively higher contents of sugars and starch. The fast growing trees, acacia, batai and yemane, contained over 25% Klason lignin. On the other hand, rubberwood and oil palm had lignin contents less than 20%. Acacia and oil palm showed lower holocellulose contents when compared to batai, yemane and rubberwood, but contained a larger amount of hot water solubles indicating the removal of a considerable amount of carbohydrates by hot water in the determination of holocellulose.

Table 1. Asplund yield and analysis of fibres

Wood Species	Asplund Yield (% of O.D. material)	Chemical Analysis(% of O.D. fibres)		
		Klason Lignin	Holocellulose	Hot Water Solubles
Acacia	95.3	26.3	55.5	21.4
Batai	96.7	26.6	61.8	18.8
Yemane	97.0	26.7	62.5	18.1
Rubberwood	91.0	19.4	67.3	18.3
Oil Palm	90.5	17.0	49.4	37.0

Figure 1 shows the HPLC chromatogram of the enzymatic hydrolyzate of oil palm fibres. Glucose and xylose were the main products in the enzymatic hydrolysis. All wood species showed the same tendency. Table 2 shows the yield of reducing sugars. In all species except oil palm, the yield of reducing sugars was less

than 2%. In oil palm, almost half of the dry fibres were converted to reducing sugars. This means that oil palm is an excellent raw material for conversion to animal feed or for fermentation. Rubberwood and acacia also gave good yields although much less than in oil palm.

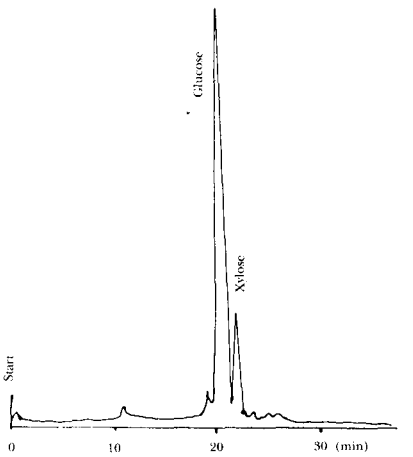

Figure 1. High-performance liquid chromatogram of enzymatic hydrolyzate of oil fibre

All these species are especially important to Malaysia. Malaysia is the leading producer of oil palm in the world and has about 1.5 million *ha* of oil palm plantations (Mohamad Husin *et al.* 1986). Malaysia, the largest producer of natural rubber in the world, is capable of producing an estimated at 8-10 million m^3 per annum including branch wood greater than 15 *cm* diameter. This is assured through the systematic replanting programme of the 20.0 million *ha* of existing plantation (Anonymous 1986). Further investigations were carried out on these two species on account of their large availability in Malaysia.

Table 2. Results of enzymatic hydrolysis (% of O.D. fibres)

Wood Species	Saccharification * Yield
Acacia	18.2
Batai	16.5
Yemane	12.8
Rubberwood	18.7
Oil Palm	49.6

$$\text{*Saccharification yield} = \frac{\text{amount of reducing sugars produced}}{\text{O.D. weight of fibres used}} \times 100\%$$

Table 3 shows the effect of alkaline extraction of fibres. Treatment of fibres with 1% sodium hydroxide solution increased the yield of reducing sugars of rubberwood but decreased that of oil palm. In alkaline extraction, both carbohydrates and lignin were partially removed. In oil palm, the large amount of weight loss of about 40% indicated an excessive removal of carbohydrates by this treatment which was therefore too drastic for this material.

Table 3. Effect of alkaline extraction on the lignin content and saccharification yield

Wood Species	1% NaOH Solubles (% of O.D. original fibres)	Klason Lignin	Saccharification Yield (% of O.D. extracted fibres)
Rubberwood	25.0	21.9	29.1 (21.8)
Oil Palm	40.5	17.3	33.8 (20.1)

( ) Saccharification yield, % of O.D. original fibres

Table 4 shows the results of hot water extraction. The substance which was extracted with hot water was mainly hemicellulose. By this extraction the yield of reducing sugars in the enzymatic hydrolysis of rubberwood fibres was increased from 18.7 to 23.5% (Table 2). Oil palm fibres showed only a marginal increase from 49.6 to 50.3% indicating that the accessibility to enzyme had attained its maximum during the initial steaming stage, the fibre preparation. Hence the subsequent treatment with hot water had little effect.

Table 4. Effect of hot water extraction on the lignin content and saccharification yield

Wood species	Klason Lignin (% of O.D. extracted fibres)	Saccharification Yield		
		Residue	Hot water extract (% of O.D. original fibres)	Total
Rubberwood	23.3	23.4	4.4	23.5
Oil Palm	23.5	52.6	17.2	50.3

Figure 2 shows the chromatogram of enzymatic hydrolysis of the hot water soluble fraction of oil palm fibres. The main products were xylobiose, xylose and glucose. Xylobiose and xylose are derived from xylan and glucose may be derived from cellulose as well as starch which is present in both species. The chromatogram for rubberwood fibres showed the same products. Therefore, to pretreat fibres prior to enzymatic hydrolysis (to increase their accessibility), hot water extraction was better than alkaline extraction in the case of rubberwood fibres.

The chromatogram of reaction solution of simultaneous enzymatic hydrolysis and fermentation of oil palm fibres is represented in Figure 3. A consecutive method was not used since the accumulation of glucose in the reaction system hindered the degradation process from the cellulose to glucose,

but on the other hand, in a simultaneous process, glucose formed by enzymatic hydrolysis was immediately consumed by yeast and converted to ethanol (Kuwahara *et al.* 1988). After four hours of reaction time, the main peaks were xylobiose, glucose, xylose and ethanol. After ten days, these peaks were altered; a large part of xylobiose was degraded to xylose, glucose was almost totally converted by the yeast to ethanol which gave a remarkable increase. Xylose was left in the solution because it cannot be used by yeast. In the case of rubberwood the chromatogram showed the same profile.

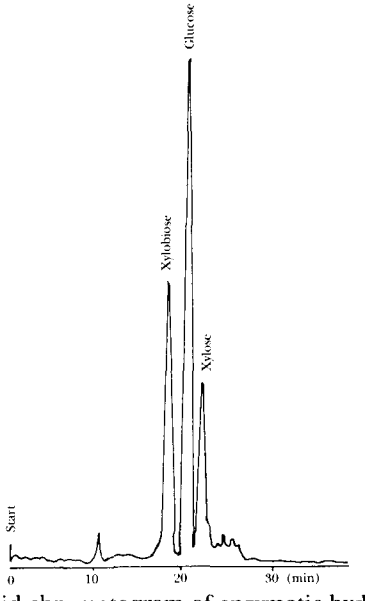

Figure 2. High-performance liquid chromatogram of enzymatic hydrolyzate of hot water soluble fraction of oil palm fibre

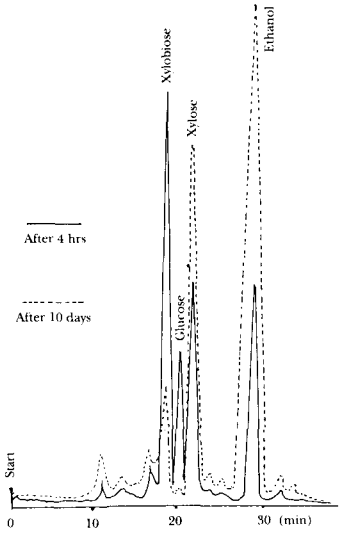

Figure 3. High-performance liquid chromatogram of simultaneous saccharification and fermentation hydrolyzate of oil palm fibre

Table 5 shows the changes in concentration of glucose and ethanol in the reaction solution of oil palm and rubberwood fibres during the process of simultaneous enzymatic hydrolysis and fermentation. In 24 hours, glucose was consumed rapidly with substantial production of ethanol. The concentration of ethanol reached a maximum after three days at 1% for oil palm and 0.62% for rubberwood and then decreased gradually. For rubberwood, glucose could not be detected after 24 hours, but in the case of oil palm a small amount of glucose was detected throughout the whole reaction time. Higher concentrations of alcohol could be obtained by distillation. After fermentation, xylose (which was left in the reaction mixture) could be recovered and converted by hydrogenation into xylitol, an expensive industrial chemical.

Table 5. Yields of glucose and ethanol on simultaneous enzymatic hydrolysis and fermentation (% reaction solution)

Reaction Time	Rubberwood		Oil Palm	
	Glucose	Ethanol	Glucose	Ethanol
4 *h*	0.16	0.20	0.07	0.26
1 day	0.02	0.57	0.02	0.74
2 days	-	0.57	0.02	0.91
3 days	-	0.62	0.02	1.00
4 days	-	0.42	0.02	0.90
10 days	-	0.31	0.02	0.67

Conclusion

Among the five wood materials examined, oil palm showed the best response and yemane the least to enzymatic hydrolysis. Through the process of simultaneous enzymatic hydrolysis and fermentation, oil palm fibres also yielded a high amount of ethanol (up to 1.0% of reaction solution). Rubberwood fibres as substrate for enzymatic hydrolysis were not so remarkable but nevertheless not too far behind in their response. The saccharification yield was only about half that of oil palm fibres (after hot water extraction) and the amount of ethanol produced on simultaneous enzymatic hydrolysis and fermentation was just above 0.6% of reaction solution. These preliminary findings indicate good potential for using these plant materials for alcohol production in Malaysia, especially when considering their large availability in the country.

References

ANONYMOUS. 1986. *Malaysian Rubberwood. A Useful and Versatile Timber.* Malaysian Timber Industry Board, Kuala Lumpur.

BENDER, F., HEANEY, D.P. & BOWDEN, A. 1970. The potential of steamed wood as feed for ruminants. *Forest Products Journal* 20 (4) : 36 - 41.

DIETRICHS, H.H., SINNER, M. & PULS, J. 1978. Potential of steaming hardwoods and straw for feed and food production. *Holzforschung* 32 (6) : 193 - 199.

KUWAHARA, M., SAWADA, T., ASADA, Y., NAKAMURA, Y., USHIKOSHI, M. & KOMATSU, M. 1988. Enzymatic saccharification and fermentation of NaOH-treated Ezomatsu bark. *Mokusai Gakkaishi* 34 (1) : 75 - 81.

MOHAMAD HUSIN , ABD. HALIM HJ. HASSAN & AHMAD TAMIZI MOHAMAD. 1986. Availability and potential utilization of oil palm trunks and fronds up to the year 2000. *PORIM Occasional Paper* No. 20.

SOMOGYI, M. 1952. Notes on sugar determination. *Journal of Biological Chemistry* 195 : 19 - 23.

WISE, L.E., MURPHY, M. & D' ADDIECO, A.A. 1946. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and studies on the hemicelluloses. *Paper Trade Journal* 122 (2) : 35 - 43.