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A COMPARATIVE STUDY OF THE HYDROLYSIS OF GAMMA IRRADIATED LIGNOCELLULOSES

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Abstract - The effect of high-dose irradiation as a pretreatment method on two common lignocellulosic materials; hardwood (*Khaya senegalensis*) and softwood (*Triplochiton scleroxylon*) were investigated by assessing the potential of cellulase enzyme derived from *Aspergillus flavus* Linn isolate NSPR 101 to hydrolyse the materials. The irradiation strongly affected the materials, causing the enzymatic hydrolysis to increase by more than 3 fold. Maximum digestibility occurred in softwood at 40kGy dosage of irradiation, while in hardwood it was at 90kGy dosage. The results also showed that, at the same dosage levels ($p \leq 0.05$), hardwood was hydrolysed significantly better compared to the softwood.
Keywords: Gamma irradiation; Cellulase; *Aspergillus flavus*; Enzymatic hydrolysis.

INTRODUCTION

Now that the world is facing huge shortages and increasing cost of petroleum fuels, a potential solution to the ever-increasing energy demand is the use of materials from renewable sources. Lignocellulosics are one of these materials and are in vast supply. Their hydrolysis yields fermentable sugars which can serve as chemical feedstocks and energy sources (Solomon et al., 1990; Kim et al., 2000; Ojumu et al., 2003a and b). Nigerian forests contain a lot of softwood and hardwood timbers that are of great commercial importance (Gbile, 1984), namely: *Khaya senegalensis* (Mahogany), *Nauclea diderrichi* (Opepe), *Tectona grandis* (Teak), *Terminalia superba* (Afara), *Afzelia pachyloba* (Apa), *Khaya ivorensis* (Oganwo), *Chlorophora excelsa* (Iroko), *Mansonia altissima* (Masonia), *Distemonanthus benthamianus* (Ayan), *Entandrophrama candollei* (Omu), *Gossweilerodendron balsamiferum* (Agba) [hardwoods], and *Ceiba pentandra* (Araba), *Triplochiton scleroxylon* (Arere), *Funtumia africana* (Ire), *Albizia zygia* (Ayunre) [softwoods].

Sawdust generated by mill processing of these woods is in large supply and currently constitutes a large portion of municipal waste. Unfortunately, because of the recalcitrant nature of lignocellulosic materials, their hydrolysis is not readily achieved. Therefore, it is important to pretreat the biomass in order to obtain a material suitable for bioconversion. Attempts have been made by several workers to solve this non-degradation problem by using various pretreatment methods for enhancing bioconversion of lignocellulosics (Solomon et al., 1990; Ojumu et al., 2003a, 2003b; Yang and Wyman, 2006). The objective of the pretreatment is to destructure the

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lignocellulosic matrix to facilitate the separation of the constitutive polymers. Pretreatments are based on a controlled hydrolytic depolymerization in aqueous media, that is catalyzed by the acidic species in wood (autohydrolysis) or by the addition of catalytic amounts of mineral acids (prehydrolysis) (Ojumu et al., 2003a). Solomon et al. (1990) achieved hydrolysis of sawdust using cellulase with an activity of 0.056 IU/ml derived from *Triplochiton scleroxylon*. Ojumu et al. (2003b) produced cellulase enzyme of 0.0743IU/ml activity from *Aspergillus flavus* Linn isolate NSPR 101 using sawdust as substrate. In both cases, cellulase activity was determined by Filter Paper Activity (FPA) and the authors used a caustic swelling pretreatment method prior to the use of biological agents.

Recently it has been shown that about 70% glucose can be obtained from sawdust using steam explosion under extremely low concentration of acid, a method believed to be milder than acid hydrolysis (Ojumu et al., 2003a; Ojumu and Ogunkunle, 2005). The major components of lignocellulosic materials are cellulose, hemicellulose, lignin and extractives. Several reviews have been carried out on the structure of cellulose and it is still the subject of intense study (Kadla and Gilbert, 2000). Pretreatment of native lignocellulose causes reductions in crystallinity, decomposition of lignocellulosic biomass and removes secondary interactions between glucose chains (Fan et al., 1980). It has been reported that biodegradation of untreated natural lignocellulosic biomass is very slow, giving rise to the low extent of degradation, often under 20% (Fan et al., 1980). This low rate and extent of conversion inhibit the development of an economically feasible hydrolysis process.

Although various methods of pretreatment have been reported (Jeoh and Agblevor, 2001; Bigelow and Wyman, 2002; Martín and Thomsen, 2007), few reports exist on the use of gamma irradiation (Martfnez et al., 1995; Lam et al., 2000). Gamma radiation, if used in high dosage on lignocellulosics, causes a decrease in cell wall constituents or depolymerizes and delignifies the fiber (Al-Masri and Zarkawi, 1994). An increase in organic matter digestibility has been reported due to its cell wall degradation (Al-Masri and Guenther, 1995). However, its pasteurizing and sterilization capabilities for agricultural products have also been reported when used at low dosage (Kume et al., 1990; Kim et al., 2000). Kim et al. (2000) found that a gamma dose of 5–10 kGy was effective in reducing microbial contamination of medicinal herbs. However, research has shown that a higher cellulose degradation of agricultural by-products occurs for a combination of gamma radiation and chemical treatments as compared with chemical treatment or irradiation treatment alone (Banchorndhevakul, 2002). In this work, the extent of enzymatic hydrolysis of both softwood and hardwood was used to measure the effect of gamma irradiation as a pretreatment method. In addition, dosages for optimum recovery were also reported.

MATERIALS AND METHODS

Sawdust

Two types of sawdust were used for this study; softwood (*Triplochiton scleroxylon*) and hardwood (*Khaya senegalensis*). The sawdust samples (carefully collected from a Sawmill in Ibadan, Oyo State, Nigeria) were milled to yield fine particles. The fraction which passed 32-mesh but was retained by 42-mesh, was used in all the experiments. Samples were dried in a vacuum oven at 60^{o}C for 24 h before pretreatment. The cellulose components of a typical softwood and hardwood are reported to be 42% and 45%. The method proposed by Rivers et al. (1983) was used for the determination of cellulose content.

Sample Preparation for Photomicrography

The Tangential Longitudinal Section (TLS) of the wood samples was cut using a microtone. Maceration was carried out by using Schultze's method as reported in Faluyi (1992). The sections and fibres were stained with 1% (w/w) Toludiene Blue solution to characterise the lignified fibre and degree of lignifications in the samples. These stained sections were fixed on the glass slide and later exposed to photographic films in bright field on a Leitz Dialux research microscope equipped with a MO2 camera.

Pretreatment

The samples were exposed to γ-ray doses ranging from 10kGy to 100kGy emitted from ^{60}Co (cobalt-60 AECL), at a dose rate of 0.6 Gray per second. The irradiated samples were soaked in 1% (w/v) sodium hydroxide solution at a ratio of 1:10 (substrate:solution) for 2 h at room temperature, after which they were washed free of the chemical and autoclaved at 120ºC (15 psig steam) for 1 h as prescribed by Ojumu et al. (2003b). The samples used for the control study were also subjected to the above pretreatment methods except for exposure to

gamma radiation. This allows for the contribution of gamma radiation to be determined.

Enzyme Production

A pure culture of *Aspergillus flavus* Linn isolate NSPR101 was used for cellulase enzyme production. The details of the enzyme production have been described elsewhere (Ojumu et al., 2003b). The cellulase was harvested (and used immediately for the hydrolysis experiment) at the 12th hour of cultivation (when the activity is optimum) as observed in the previous study (Ojumu et al., 2003b).

Enzymatic Hydrolysis of Sawdust

Hydrolysis of the sawdust samples with cellulase was conducted by suspending a specified part of the dried samples in a 250 ml flask with 0.05 M citrate buffer (pH 5) for 1 h in an incubator at 45ºC before adding the cellulase enzyme produced above in an amount corresponding to 10 ml enzyme solution per 1 g of dry sample. The enzyme solution was considered to be impure, as no attempt was made to purity it. The hydrolysis was carried out at 45ºC in an incubator shaker at 200 rpm for 12 h. Samples of the hydrolyzate were withdrawn every 1 h and the supernatant was analysed for reducing sugar.

Cellulase Activity and Reducing Sugar Concentration Analysis

The cellulase activity was determined using Whatman No. 1 filter paper and was expressed as filter paper activity; this has been previously described in detail by Ghose (1987). The total amount of reducing sugars, which is expressed as equivalent glucose in 1.0 ml supernatant, was determined by the modified dinitrosalicylic acid (DNS) method of Miller (1959). The extent of hydrolysis was expressed as below (Mandels et al., 1976).

$$\text{extent of hydrolysis} = \frac{\text{weight of glucose formed} \times \left(162/180\right)}{\text{dry weight of cellulose used}} \times 100 \quad (1)$$

Statistical Analysis

The experiment was completely randomised and with three replicates. Sample means were used for statistical analysis using the software package SPSS for Windows® release 7.5.1 (1996). Analyses of variance (ANOVA) and multiple comparisons were performed for all measured parameters using Duncan's multiple range test.

RESULTS AND DISCUSSION

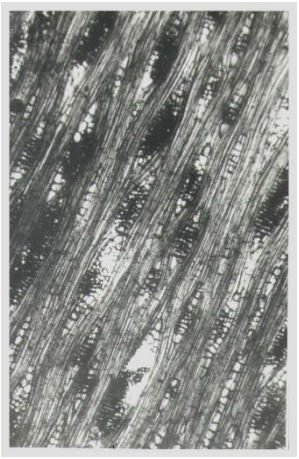
Comparison of the digestibility of the irradiated softwood and hardwood sawdust during hydrolysis was made on the basis of the reducing sugar productions. The hydrolysis time profile showed that the extent of hydrolysis was improved when the samples were irradiated, irrespective of the species of wood used (data not shown). Although previously reported experiments also indicated that irradiation improved the digestibility of lignocellulosic materials at high dosage (Lam et al., 2000), the results indicated that significant hydrolysis was obtained at low dosage (10 kGy) for both woods (Table 1).

Table 1: Mean for extent of conversion of irradiated softwood and hardwood sawdust after 8 hours at 45ºC[α]

Dosage Levels (kGy)	Mean Extent of Hydrolysis after 8hrs (softwood)[β]	Mean Extent of Hydrolysis After 8hrs (hardwood)[β]
0	0.272 ± 0.02^{c}	0.280 ± 0.02^{b}
10	0.643 ± 0.06^{a}	0.728 ± 0.10^{a}
20	0.661 ± 0.10^{a}	0.743 ± 0.08^{a}
30	0.646 ± 0.06^{a}	0.701 ± 0.09^{a}
40	0.673 ± 0.10^{b}	0.672 ± 0.07^{a}
50	0.653 ± 0.09^{a}	0.678 ± 0.06^{a}
60	0.667 ± 0.09^{b}	0.711 ± 0.03^{c}
70	0.663 ± 0.04^{b}	0.675 ± 0.06^{a}
80	0.658 ± 0.09^{a}	0.707 ± 0.09^{a}
90	0.632 ± 0.05^{b}	0.803 ± 0.10^{d}
100	0.642 ± 0.06^{b}	0.309 ± 0.03^{b}

[α] Mean ± standard deviation of triplicate analysis

[β] Values in the same column with the same superscripts are not significantly different at $p \leq 0.05$.



(a) Tangential Section of *Khaya senegalensis* (Hardwood) showing both multi-seriate. (x144)

(b) Tangential Longitudinal Section of *Triplochiton scleroxylon* (Softwood) with multi-seriate rays. (x144)

Figure 1: Photomicrographs of the hard- and soft- woods

The statistical analysis of the results obtained for irradiated woods at different dosage levels (20 to 90 kGy) revealed that by exposing the softwood to a 40 kGy dose, the highest hydrolysis rate and the maximum conversion of cellulose were obtained. Further increase in the irradiation dosage contributed insignificantly to the conversion, while the hardwood required 90 kGy radiation to obtain maximum digestibility (Table 1). Dunlap and Chiang (1980) observed this occurrence and stated that irradiation appears to be strongly species selective; for example, the digestion of aspen carbohydrate is essentially complete after a dosage of 108 rad, while spruce is only 14% digestible at this dosage.

A Student t-test analysis of the data revealed that the irradiated hardwood sawdust hydrolysed better compared with the irradiated softwood sawdust at 90kGray irradiation dosage level, as shown by the relatively higher yield of reducing sugar obtained from the irradiated hardwood. This observation could be attributed to percent composition of the cellulose in the wood samples, available specific surface area for the reaction, lignin content of the substrate, availability of the active site for enzymes, all of which favoured the hardwood sawdust (Cowling, 1975; Fan et al., 1980). In addition, from the photomicrographs (Figure 1a), it can be seen that most of the fibres of *Khaya senegalenesis* were moderately short (~0.81mm) and prominent and have pointed ends. They also exhibit light-gray colourations (Figure 1a), an indication of reduction in lignin barriers, while fibres in *Triplochiton scleroxylon* (Figure 1b) were moderately long (~1.76mm), but also with pointed ends and deep gray colouration characteristic of lignification (Cowling, 1975). This could be responsible for the observed higher digestibilities of the irradiated hardwood sawdust as compared to those of the softwood.

After six hours of hydrolysis, the data were found to deviate from the kinetic model of Ghose and Das (1971). This means that the kinetic pattern suggested by the empirical model for the initial phase of reaction may not be applicable to all the stages of hydrolysis beyond a certain period of hydrolysis. The possible cause of the deviation could be the existence of factors like the build-up of resistant cellulose during the course of hydrolysis, the onset of the effect of product inhibition, the heterogeneity of the lignocellulosic materials, interrelation between C_1 and C_x of the cellulase enzyme. Such a deviation was observed by Ghose (1969).

In this study, irradiated woods have been shown to be good candidates for cellulose biomass conversion into useful products; however, *Khaya senegalenesis* is more suitable for this process compared with *Triplochiton scleroxylon* as it gave the higher yield of the reducing sugar.

REFERENCES

Al-Masri, M. R. and Guenther, K. D., The effect of gamma irradiation on in vitro digestible energy of some agricultural residues, Das Wirtschaftseigene Futter, 41, pp. 61-68 (1995).

Al-Masri, M. R. and Zarkawi, M., Effect of gamma irradiation on cell-wall constituents of some

agricultural residues, Radiation Physics and Chemistry, 44, pp. 661-663 (1994).

Banchorndhevakul, S., Effect of urea and urea–gamma treatments on cellulose degradation of Thai rice strawand corn stalk, Radiation Physics and Chemistry, 64, pp. 417-422 (2002).

Bigelow, M. and Wyman, C. E., Cellulase production on bagasse pretreated with hot water, Applied Biochemistry and Biotechnology, 100, pp. 921-934 (2002).

Cowling, E. B., Physical and Chemical constraints in the hydrolysis of cellulose and lignocellulosic materials, Biotechnology and Bioengineering Symposium, 5, pp. 163-181 (1975).

Dunlap, C. E. and Chiang, L. C., Cellulose degradation - a common link. In: M. L. Schuler ed. Utilizing and Recycle of Agricultural Wastes and Residues, CRC Press, New York, pp. 19-65 (1980).

Faluyi, J. O., Principles and Practice of Photomicrography, Faluyi J. O, editor. Ile-Ife, Osun State, Nigeria.: Anchor Print Limited Ife-Ibadan Road, Ajebamidele (1992).

Fan L. T., Lee, Y-H. and Beardmore, D. H., Major chemical and physical features of cellulosic materials as substrates for enzymatic hydrolysis, Advances in Biochemical Engineering, 14, pp. 101-117 (1980).

Gbile, K. O., Botanical name of common trees in Nigeria, Class Handout, Iwalaye Press, Ibadan, Oyo State, Nigeria (1984).

Ghose, T. K., Continuous enzymatic saccharification of a cellulose with culture filtrates of *Trichoderma viride* QM 6a, Biotechnology and Bioengineering, 11, pp. 239-261 (1969).

Ghose, T. K., Measurement of cellulase activities, Pure and Applied Chemistry, 59, pp. 257-268 (1987).

Ghose, T. K. and Das, K. A., Simplified kinetic approach to cellulose-cellulase system, Advances in Biochemical Engineering, 1, pp. 55-76 (1971).

Jeoh, T., and Agblevor, F. A., Characterization and fermentation of steam exploded cotton gin waste, Biomass and Bioenergy, 21, pp. 109 -120 (2001).

Kadla, J. F. and Gilbert, R. D., Cellulose structure: A review, Cellulose Chemistry and Technology, 34, pp. 197-216 (2000).

Kim, M. J., Yook, H. S. and Byun, M. W., Effects of gamma irradiation on microbial contamination and extraction yields of Korean medicinal herbs, Radiation Physics and Chemistry, 57, pp. 55-58 (2000).

Kume, T., Ito, H., Ishigaki, I., LebaiJuri, M., Othman, Z., Ali, F., Mutaat, H. H., Awang, M. R. and Hashim A. S., Effect of gamma irradiation on microorganisms and components in empty fruit bunch and palm press fiber of oil palm wastes, Journal of Science Food and Agriculture, 52, pp. 147 (1990).

Lam, N. D., Nagasawa, N. and Kume, T., Effect of radiation and fungal treatment on lignocelluloses and their biological activity, Radiation Physics and Chemistry, 59, pp. 393-398 (2000).

Mandels, M., Andreotti, R. and Roche, C., Measurement of saccharifying cellulase, Biotechnology and Bioengineering Symposium, 6, pp. 21-33 (1976).

Martfnez, J. M., Granado, J., Montanr, D., Salvad, J. and Farriol, X., Fractionation of residual lignocellulosics by dilute-acid prehydrolysis and alkaline extraction: Application to almond shells, Bioresource Technology, 52, pp. 59-67 (1995).

Martín, C. and Thomsen, A. B., Wet oxidation pretreatment of lignocellulosic residues of sugarcane, rice, cassava and peanuts for ethanol production, Journal of Chemical Technology and Biotechnology, 82, pp. 174-181 (2007).

Miller, G. L., Use of dinitrosalicyclic acid reagent for determination of reducing sugars, Biotechnology and Bioengineering Symposium, 5, pp. 193-219 (1959).

Ojumu, T. V., Attah-Daniel, B. E., Betiku, E. and Solomon, B. O., Auto-hydrolysis of lignocellulosics using extremely low acid under high temperature in a batch process, Biotechnology and Bioprocess Engineering, 8, pp. 291-293 (2003a).

Ojumu, T. V., Solomon, B. O., Betiku, E., Layokun, S. K., Amigun, B., Cellulase production by *Aspergillus flavus* Linn isolate NSPR 101 fermented in sawdust, bagasse and corncorb, African Journal of Biotechnology, 2, pp. 150-152 (2003b).

Ojumu, T. V. and Ogunkunle, O. A., Production of Glucose from Lignocellulosic under Extremely Low Acid and High Temperature in Batch Process, Auto-hydrolysis Approach, Journal of Applied Sciences, 5, pp. 15-17 (2005).

Rivers, D. B., Zoldak, B. R., Evans II, R. S. and Emert, G. H., Determination of cellulose in municipal solid wastes contaminated with synthetic materials, Biotechnology Letters, 5, pp. 777-780 (1983).

Solomon, B. O., Layokun, S. K., Mwesigye, P. K. and Olutiola, P. O., Hydrolysis of sawdust by cellulase derived from *Aspergillus flavus* Linn Isolate NSPR 101: Beyond the initial fast rate period, Journal of Nigerian Society of Chemical Engineers, 9, pp. 46-50 (1990).

Yang, B. and Wyman, C. E., BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates, Biotechnology and Bioengineering, 94, pp. 611-617 (2006).