

Official Use RADIATION AND OTHER PRETREATMENT EFFECTS ON ENZYMATIC
HYDROLYSIS OF CELLULOSIC MATERIALS

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5th Japan-Brazil Symposium on Sci. and Technology
Caxambu, MG, 27-29 Oct., 1986.



The search for alternative renewable sources of energy is receiving increasing attention in developed and less developed countries (1), (2), (3). One of the major obstacles encountered when converting lignocellulosic materials by enzymatic hydrolysis is the presence of lignin that binds cellulose and hemicellulose. On the other hand, molecular cristalinity limits the rate of attack on the cellulose in any kind of saccharification process. Consequently, prior to enzymatic hydrolysis it is necessary to break off the lignin and cellulose cristalinity barriers and make such process economically feasibly. Both effects can be achieved at least partially, through a combination of physical and chemical pretreatment(4), (5).
Effects of physical crushing of wood and sugarcane bagasse on enzymatic hydrolysis.

Wood chips were crushed to fine powder using an impact mill or a laboratory disc mill. Particle size distribution was determined by using a vibratory classifier with screens. The influence of the average particle size on the reducing sugar yield, after 2 h enzymatic hydrolysis at 50°C, is shown in figure 1, for irradiated and non irradiated samples. In all cases, reducing sugar yield, measured by DNS reagent (6), increases as the average particle size decreased down to about 300 µm. In the case of sugarcane bagasse, no noticeable influence of particle size on glucose yield could be found for particles smaller than 400 µm.

Electron beam irradiation.

Radiation dose has a direct effect on reducing sugar yield obtained by enzymatic hydrolysis of electron beam processed cellulosic substrates, as shown in figures 1, 2, 3, 4 and 5. An increase by a factor of 5 in the net yield of reducing sugar (difference between glucose equivalent yielded after incubation with and without enzyme) was obtained when the original wood fragments were pre-irradiated at 5×10^5 Gy (Fig. 2). Total reducing sugar yield varied from about 15 mg/g for 0 Gy to 57 mg/g for 5×10^5 Gy. The greater increment, within this range, occurred from 0 Gy to 2×10^5 Gy. A dose of 10^5 Gy seems to be a reasonable one for practical purposes, but 2×10^5 Gy might be more convenient for future experiments, since, at that value of dose, total reducing sugar yield obtained after 2 h enzymatic hydrolysis of wood increases by a factor greater than 5 as compared with a

factor of only 3 when a dose of 10^5 Gy is used. In the case of sugarcane bagasse (Fig. 3) total reducing sugar yield increased almost linearly with the dose, from about 75 mg/g at 10^5 Gy up to near 160 mg/g at 5×10^5 Gy after one hour of enzymatic hydrolysis.

Combination of electron beam irradiation and alkali pretreatments.

Figure 4 shows the effect on reducing sugar yield of combining diluted alkali impregnation of wood particles with electron beam irradiation and milling. Figure 5 exhibits the effect of combined treatments with 0 % to 3% NaOH solutions, irradiation up to 5×10^5 Gy and crushing below 300 μ m particle size, on the reducing sugar production from 10% w/v sugarcane bagasse powder, after 1 h incubation time with enzyme (36 mg/ml cellulase) at 50°C. Taking in mind the large amount of sugarcane bagasse available as waste from the sugar and alcohol industries (Brazil will be producing by 1990 about 114 million tons/year of sugarcane bagasse), the enzymatic hydrolysis of this product, after appropriated pretreatment, looks very promising. Other important point to be considered when dealing with industrial applications of the methods discussed above, is that the solid residue remaining after the enzymatic hydrolysis has also commercial value either as fuel or for other uses like in feeding ruminants. In most of the cases, low conversion efficiencies could be probably worthy since residues from forestry and allied industries and agricultural crop by-products are abundant and rather low cost renewable products.

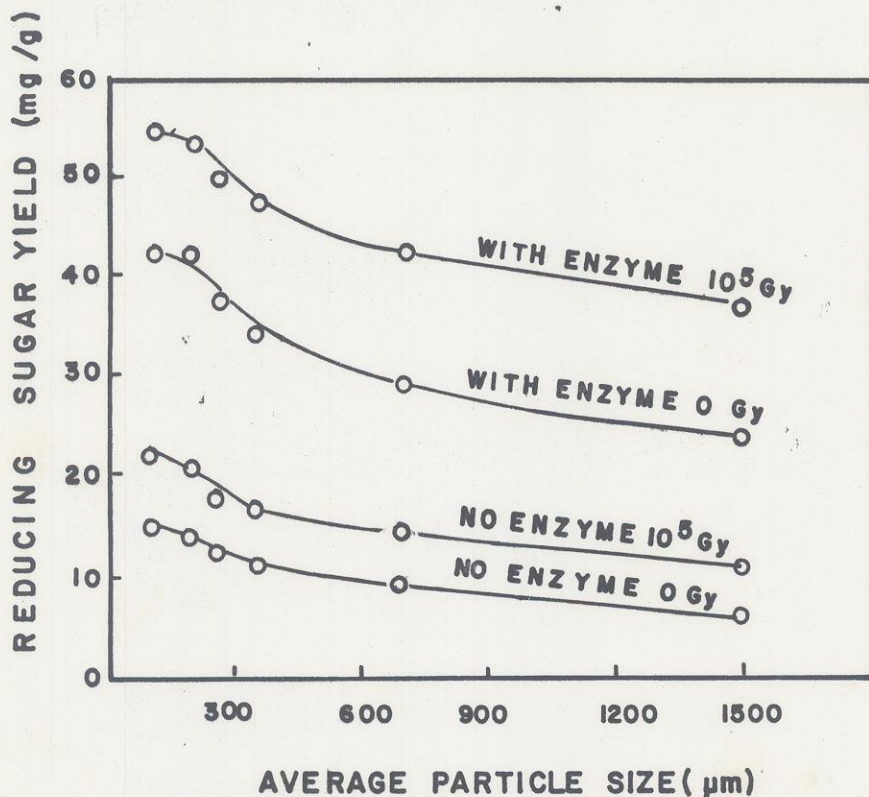


Fig. 1. Effect of average particle size and EB radiation, on sugar yield after 2h enzymatic hydrolysis of 10% w/v wood powder, at 50°C. Enzyme concentration: 36 mg/ml.

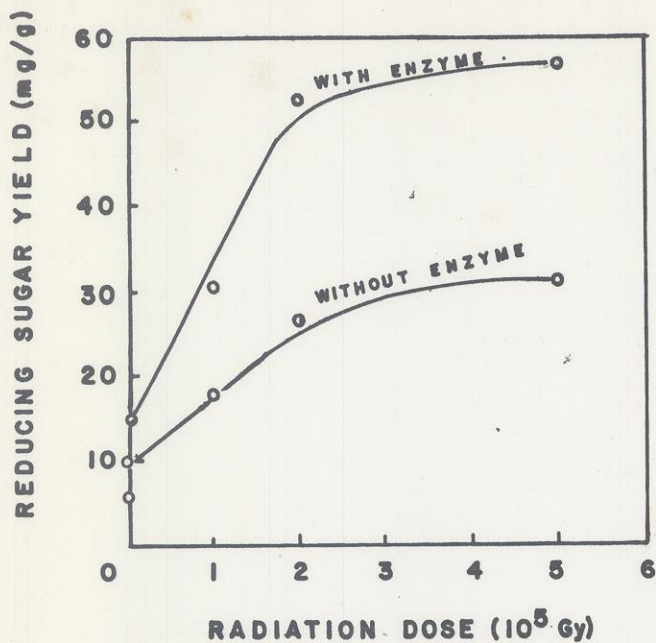


Fig. 2. Effect of EB irradiation on sugar yield of 10% w/v wood powder. Same conditions as Fig. 1.

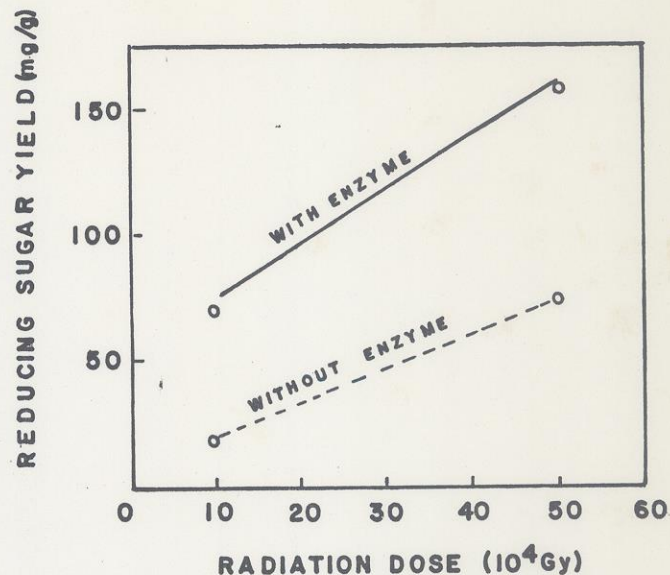


Fig. 3. Effect of EBI on sugar yield after 1h enzymatic hydrolysis of 10% w/v sugarcane bagasse powder (<300µm) at 50°C. Enzyme concentration 36mg/ml.

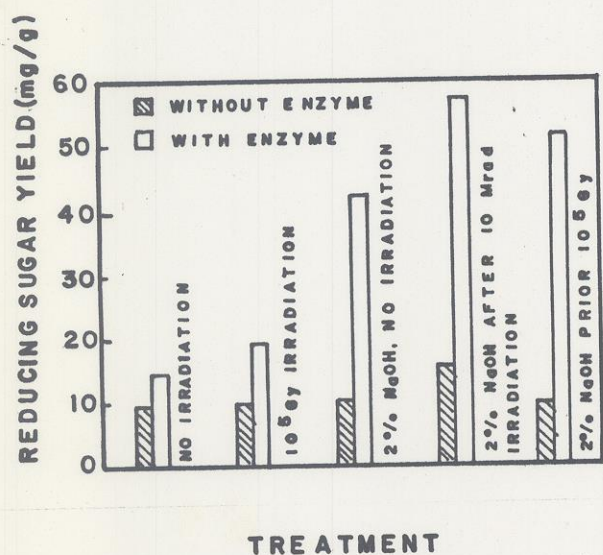


Fig. 4. Comparison of sugar yield obtained with eucalyptus wood powder (<300µm), for different pre-treatments. Conditions as in Fig. 1.

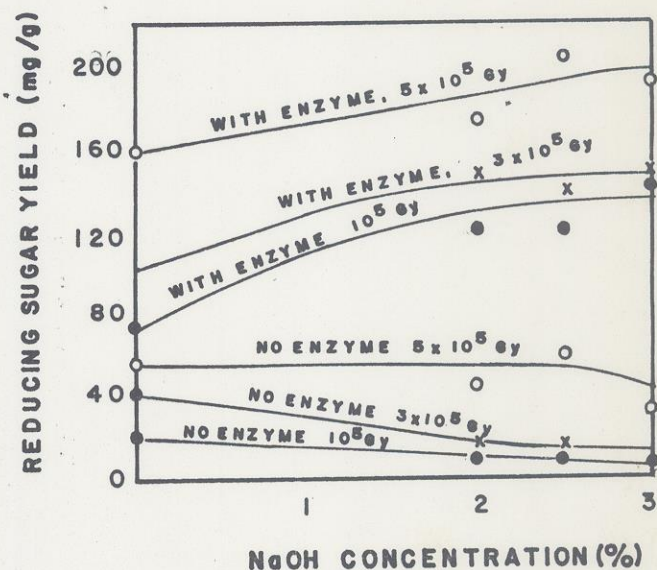


Fig. 5. Effect of treatment with NaOH before EBI, on the sugar yield from sugarcane bagasse. Conditions as in Fig. 3.

Acknowledgment: This work was partially supported by the International Atomic Energy Agency (Research Contract nº 3347/RB).

REFERENCES

1. BENTE Jr., In: Energy Seminar of the Aspen Institute for Humanistic Studies, Aspen, Colorado, 1982. Colorado, Bio-Energy Council, 1983. p. 21.
2. FAUST, U.; PRAVE, P.; SCHLINGMANN, M. Process Biochemistry, 31-7, 1983.
3. VARGAS, J. I. The Brazilian alcohol program and alternative energy sources. Secretaria de Tecnologia Industrial, Ministério da Indústria e Comércio, São Paulo, 1983, p. 32.
4. MILLET, M.A.; BAKER, A.J.; SATTER, L.D. Biotechnol. & Bioeng. Symp., 6:125 - 53, 1976.
5. MACHADO, O.L.T. Descompressão rápida como pré-tratamento de madeira para a hidrólise enzimática. Dissertação de Mestrado. Rio de Janeiro, Instituto de Química, Universidade Federal do Rio de Janeiro, 1982.
6. MANDELS, M.; ANDREOTTI, R.; ROCHE, C. Biotechnol. & Bioeng. Symp., 6: 21 - 33, 1976.