RADIATION-INDUCED CELLULOSE DEGRADATION

A. M. dela Rosa, L.V. Abad, R. B. Banzon
A. S. dela Mines-Bulos and Z. F. Simbul-Nuguid
Philippine Atomic Energy Commission
Diliman, Quezon City, Philippines

ABSTRACT

The effects of radiation pretreatment on the acid and enzymatic saccharification of cellulose and some agricultural cellulosic wastes were investigated. The saccharification of these materials increased with increase in radiation dose. The extent of the radiation effect varied with the cellulosic material. Rice straw exhibited the greatest sensitivity, and sawdust, the greatest resistance to gamma radiation. The radiation-treated rice straw was found to be a better substrate for the growth of cellulolytic microorganisms than the unirradiated sample. The data show that gamma radiation induces some changes in the molecular and supermolecular structures of cellulose and agricultural cellulosic wastes resulting in their increased susceptibility to hydrolytic attack by either acid or enzyme.

Introduction

Biofuels derived from plant biomass offer promising alternatives to fossil fuels. The utilization of agricultural cellulosic wastes and forestry products as renewable energy sources presents an interesting challenge in the near future.

It is known that pretreatment of these raw materials is necessary in order to reduce the crystalline structure of cellulose as well as the cementing effect of other plant components, such as lignin and hemicellulose, which render the cellulose relatively inert to hydrolytic attack. One method of physical pretreatment is gamma irradiation.

Several studies have been reported on the radiation-induced decomposition of cellulose (Kaputskii *et al.*, 1975; Klimentov *et al.*, 1981; Kunz *et al.*, 1972), but very few studies dealt with the application of the radiation technique to enhance the hydrolysis of cellulose for subsequent production of ethanol (Kumakura and Kaetsu, 1979). In the present work, we report the results of our investigation on the effects of gamma radiation on the acid and enzyme-catalyzed saccharification of cellulose and agricultural cellulosic wastes.

Materials and Methods

Cellulose materials

Rice straw, rice hull, corn husk, sawdust, and microcrystalline cellulose (Avicel Type PH 105) were used as the cellulosic samples. The agricultural cellulosic wastes were air-dried, and ground in a Wiley mill to particle size of 0.5 mm diameter. The microcrystalline cellulose powder was used as received from the manufacturer. The moisture content of the samples was determined from the loss in weight after drying at 105°C to constant weight. The cellulose content was analyzed according to Doree (1947), while the lignin content was determined using the method of Adams (1965).

Gamma irradiation of samples

The ground samples were packed and sealed individually in polyethylene bags in 5-gram aliquots. The samples were then irradiated in the dose range of 0-500 KGy (0-50 Mrad) at 1.92 KGy/hr using the Gamma Cell 220 (Atomic Energy of Canada, Ltd.) of the Philippine Atomic Energy Commission.

Acid saccharification

The cellulosic samples were hydrolyzed with H_2SO_4 using the optimum conditions established previously (Dela Rosa *et al.*, 1983). To 5-gram aliquot of the sample was added 1% H_2SO_4 (v/v) at a substrate to acid ratio (w/v) of 1:5, and autoclaved at 20 psig for 30 minutes. The acid-treated sample was neutralized with NH_3, filtered under suction through Whatman filter No. 1 qualitative paper, and washed sparingly with distilled water. The filtrate was analyzed for reducing sugars according to the method of Somogyi as modified by Nelson using glucose as the standard (Nelson, 1944). The composition of the filtrate was determined by high performance liquid chromatography (Waters Associates PTY., Ltd.) as well as by thin layer chromatography (TLC).

Enzyme preparation

Crude extract of cellulase was prepared from culture filtrates of *Myrothecium verrucaria* and *Trichoderma reesei* (obtained from the culture collection of the Natural Science Research Institute, University of the Philippines, Quezon City) according to the procedure described previously (Dela Rosa, *et al.*, 1985). The protein content of the enzyme extract was determined by the method of Lowry and co-workers (1951). The specific activity of the enzyme preparation was determined using cellulose as the substrate.

Enzymatic saccharification

The cellulosic samples were hydrolyzed with the crude extract of cellulase using the optimum conditions for enzymatic saccharification established previously

(Dela Rosa *et al.*, 1985). The substrate (0.4 g) was suspended in 3 ml of 0.2 M sodium acetate buffer, pH 4.5, and incubated with 10 ml of crude cellulase extract (2.4 – 4.7 mg protein/ml) at 50°C for 48 hours in a water bath shaker. The reaction was stopped by placing the incubation mixture in boiling H_2O for 10 minutes. The incubation mixture was filtered, and the filtrate was analyzed for reducing sugars by the Somogyi-Nelson method. The composition of the filtrate was also determined by TLC. In some experiments, commercial enzyme preparations (crude cellulase from *T. reesei*, Tokyo-Kasei) were used for saccharification of samples. Results obtained were comparable with those using the crude enzyme prepared in the laboratory.

Effect of irradiated rice straw on the production of cellulase by T. reesei

Spore suspensions (1.0 ml) from 8-day old slants of *T. reesei* were distributed in flasks containing 2.5 g of irradiated rice straw and 50 ml of liquid mineral media. The fungus was allowed to grow at 30°C in a water bath shaker for 3 days. The enzyme was harvested as described previously (Dela Rosa, *et al.*, 1985). The protein content of the enzyme extracts was determined by the Lowry method and the relative specific activities of the enzyme extracts were obtained using cellulose as the substrate.

Results and Discussion

Cellulose is the most abundant organic material in nature. It makes up approximately 50% of the cell wall material of wood and plants, and between 25-50% of agricultural cellulosic materials. Cellulose is a linear array of glucose residues linked in β 1, 4 configuration to form side-by-side but anti-parallel chains. These microfibers comprise the highly crystalline structure of cellulose which is occasionally interspersed with packets of amorphous structure. Cellulose is associated with other carbohydrates notably hemicelluloses. The cellulose fibers also interact with lignin, a polyphenol cementlike material which forms a protective matrix around the microfibers.

The accessibility of native cellulose to hydrolytic agents is affected, among other factors, by a) its crystallinity and extent of lignification, and b) the nature of structural relationships among the cell wall components. Gamma radiation may induce changes in the molecular as well as supermolecular structures of cellulose, thereby resulting in its increased rate of conversion to glucose and/or other fermentable saccharides.

Gamma radiolysis of agricultural cellulosic wastes

Table 1 shows the concentration of reducing sugars extracted from cellulose with water after irradiation. The formation of reducing sugars in the cellulose

Table 1. Percent reducing sugar produced from radiation-induced bond cleavage in agricultural cellulosic wastes [1]

Dose (KGy)	Reducing sugar, %[2] Rice straw	Corn husk	Rice hull
0	0.00	0.00	0.00
100	0.03	1.38	0.22
200	2.85	2.69	0.13
300	9.06	3.15	0.54
400	9.73	3.52	0.59
500	3.13	7.13	1.20

[1]The sample was suspended in distilled H_2O, and autoclaved for 30 minutes at 20 psig. After filtration, the filtrate was analyzed for reducing sugar.

[2]Mean values of three replicates, each of duplicate trials for all samples except corn husk for which mean values are 2 replicates, each of 2 trials.

materials increased with increasing radiation doses. There was however, a decrease in the reducing sugar of rice straw irradiated at 500 KGy. The HPLC data shown in Table 2 indicate the presence of glucose, xylose, cellobiose and other unidentified compounds. Our data agree quite well with those reported in the literature. Schuchmann and von Sonntag (1978) found glucose, gluconic acid, arabinose, xylose and L-threo-tetradialdose in measurable amounts in irradiated cellobiose which is the disaccharide unit of cellulose. Furthermore, Bludovsky & Duchacek (1979) detected glucose, xylose, arabinose, glucuronic acid, formic acid and malondialdehyde in irradiated cotton cellulose.

Table 2. Identification of water-extracted saccharides from cellulosic samples by HPLC[1]

Dose (KGy)	Saccharides Rice straw	Rice hull
0 – 100	unidentified peaks	unidentified peaks
200	glucose, unidentified peaks	glucose, xylose
300	glucose, unidentified peaks	unidentified peaks
400	glucose, xylose, cellobiose, malondialdehyde	unidentified peaks
500	glucose, malondialdehyde	unidentified peaks

[1]The sample was suspended in distilled H_2O, and autoclaved for 30 minutes at 20 psig. HPLC was undertaken on the filtrate with the following parameters:

column: Dextropak; solvent: H_2O, 2 ml/min.

detector: RI

The presence of soluble reducing sugars in the irradiated agricultural cellulosic wastes indicates that gamma radiation cleaves the β 1,4 glycosidic bonds of cellulose. Other workers noted the decrease in the degree of polymerization of cellulose upon irradiation which is another indication of the occurrence of radiation-induced scission of these bonds (Klimentov *et al.*, 1981; Focher *et al.*, 1981; Kaputskii *et al.*, 1975).

The data presented in Table 1 further show that the three cellulosic materials are affected by radiation to varying degrees; the yield of reducing sugar is highest in rice straw, and lowest in rice hull. The results imply that the supermolecular structure of cellulose varies in the three materials, and that radiation modifies this structure differently. The observed differential effect of radiation on the structure of cellulose is reflected in the subsequent acid and enzymatic saccharification of the cellulosic materials.

Effect of radiation pretreatment on the acid saccharification of agricultural cellulosic wastes

Fig. 1 shows the observed radiation effects on the acid saccharification of cellulosic materials. The increase in percent saccharification of cellulosic samples after the radiation treatment is greatest in rice straw and about the same in rice hull and corn husk. Sawdust exhibits the least increase in percent saccharification. An earlier study has shown a significant increase ($p < 0.01$) in reducing sugar content of acid-hydrolyzed rice straw, rice hull and corn husk with increase in radiation dose (Dela Rosa *et al.*, 1985.) The samples when irradiated at 500 KGy, contained 41.9% , 14.9% and 19.8% reducing sugar, respectively, after acid hydrolysis. The corresponding unirradiated samples yielded 9.9%, 3.5%, and 8.6% reducing sugar, respectively.

The data suggest that the cellulose in rice straw is the most susceptible while that of sawdust is the most resistant to radiolytic attack. It is interesting to note that while rice straw and corn husk contain comparable amounts of cellulose and produce the same amount of reducing sugar without the radiation pretreatment, their response to radiation varies. Thus, the susceptibility of cellulose to radiation and to hydrolytic attack may depend, to some extent, on the nature of its supermolecular structure.

The response of microcrystalline cellulose powder to radiation was studied in order to determine the role of the crystalline state alone on cellulose radiolysis. Fig. 1 shows that cellulose is less hydrolyzed than rice straw with or without gamma irradiation. Thus, microcrystalline cellulose is relatively inert to acid hydrolysis, although gamma radiation increases the hydrolytic reaction. In an earlier study, it was shown that the unirradiated sample yielded 1.3% reducing sugar while the irradiated sample yielded 8.3% reducing sugar (Dela Rosa *et al.*, 1985).

The data indicate that microcrystalline cellulose may contain less packets of the amorphous state than rice straw. Differential susceptibility of the cellulose samples may be attributed to a) inherent crystalline state in the cellulose structure,

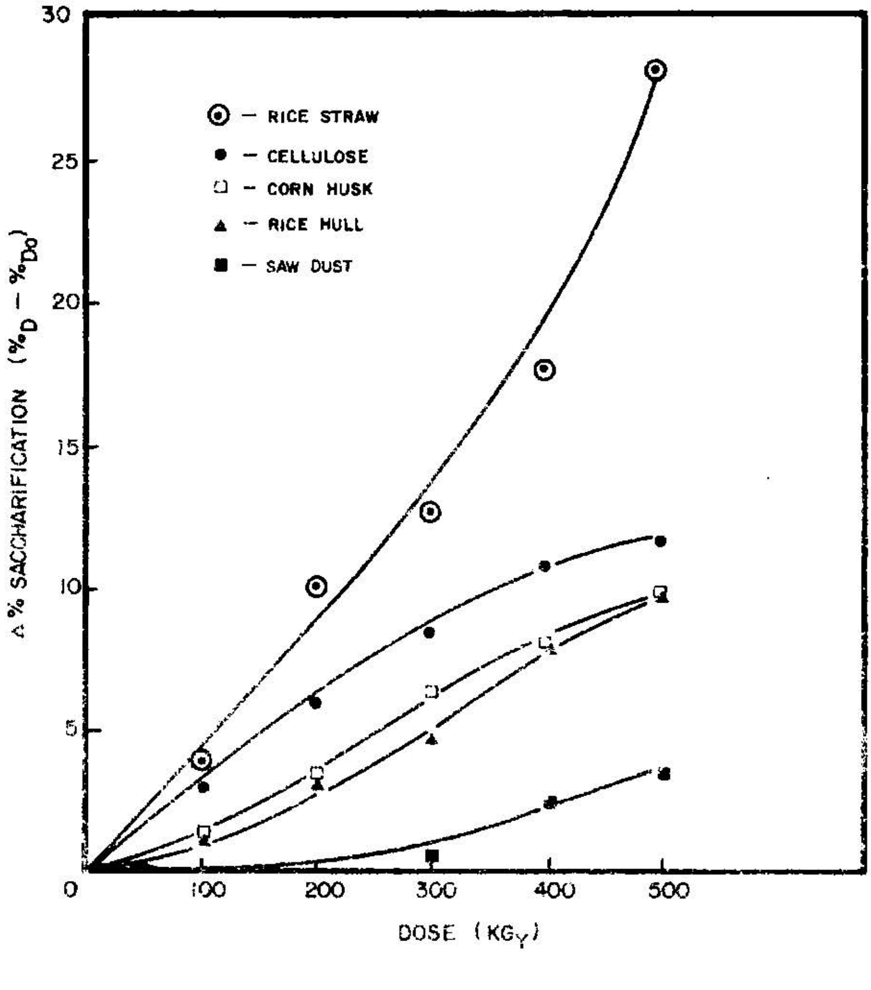

Fig. 1. Effect of radiation pretreatment on the acid hydrolysis of cellulose and agricultural cellulosic wastes.
Points represent averages of three replicates. D = radiation dose and Do = zero dose

and b) the relative contribution of lignin and other cell wall components on the supermolecular structure of cellulose.

The constituents of the acid hydrolysates of the unirradiated and irradiated samples indicate certain mechanisms in the gamma-radiolysis of cellulose and rice straw. As shown in Table 3, cellulose and rice straw yield cellobiose, glucose and

Table 3. Concentration of sugars produced after acid hydrolysis of unirradiated and irradiated cellulosic materials[1]

A. Rice straw

	Sugar %[2]			
Dose, KGy	*Cellobiose*	*Glucose*	*Arabinose*	*Xylose*
0	7.00 ± 2.04	15.80 ± 1.20	5.08 ± 1.46	28.38 ± 4.67 ab
100	5.37 ± 3.58	17.45 ± 1.47	5.50 ± 1.50	28.90 ± 3.83 ab
200	5.36 ± 3.94	16.33 ± 1.05	6.50 ± 1.17	26.30 ± 3.27 a
300	5.90 ± 3.89	16.83 ± 0.62	6.72 ± 0.71	33.42 ± 3.25 bc
400	5.33 ± 3.63	17.60 ± 0.57	6.82 ± 2.49	35.93 ± 3.29 c
500	5.56 ± 3.91	17.60 ± 1.97	4.80 ± 0.67	31.30 ± 4.29 abc

B. Cellulose

	Sugar %[2]		
Dose, KGy	*Cellobiose*	*Glucose*	*Xylose*
0	0.28 ± 0.57	4.95 ± 0.74 a	0.28 ± 0.43
100	0.09 ± 0.18	7.77 ± 0.56 ab	0.31 ± 0.48
200	0.42 ± 0.66	11.16 ± 1.19 bc	0.75 ± 0.60
300	1.08 ± 0.80	12.42 ± 1.11 c	1.20 ± 0.64
400	2.09 ± 1.48	18.30 ± 0.93 d	1.77 ± 1.21
500	1.80 ± 1.35	20.64 ± 6.02 d	1.92 ± 1.47

[1] The constituents were separated by TLC using Kieselguhr plates and the solvent system, ethyl acetate-isopropanol-water (65 + 23.5 + 11.5). Aniline Phthalate was used as the spray reagent.

[2] Mean values ± S.D. of four replicates, each with two trials.

[3] Means followed by the same letter are not significantly different from each other at 5% level using DMRT.

[a] Any two means having a common letter are not significantly different at 1% level (DMRT).

xylose after acid hydrolysis. Arabinose was also present in the hydrolysate of rice straw. One spot was not identified because of lack of corresponding standard. Glucose is the main component in the hydrolysate of cellulose, while in the rice straw hydrolysate, xylose is the predominant component. The presence of xylose in the hydrolysate of cellulose may be due either to the hydrolysis of the radiolytic products of cellulose or to the direct radiolytic attack on the glucose moieties of cellulose resulting in the liberation of free xylose. On the other hand, the xylose present in the hydrolysate of rice straw comes mainly from the acid hydrolysis of hemicellulose which is a component of the latter. For both cellulose and rice straw, the contribution of a direct radiolytic attack on the monomeric units to liberate free xylose may be minimal as may be noted later in enzyme-catalyzed samples wherein the xylose produced which is a measure of the above mechanism, is present in relatively non-quantifiable amounts (Table 4).

Effect of radiation pretreatment on the enzymatic saccharification of agricultural cellulosic wastes

As shown in Fig. 2, the change in percent saccharification of the samples increases linearly with dose up to 300 KGy, then levels off at higher doses. Rice straw exhibited the greatest increase in percent saccharification with radiation dose while rice hull, the smallest increase. In an earlier study (Dela Rosa *et al.*, 1985) it was found that the radiation treatment significantly enhanced ($p < 0.01$) the

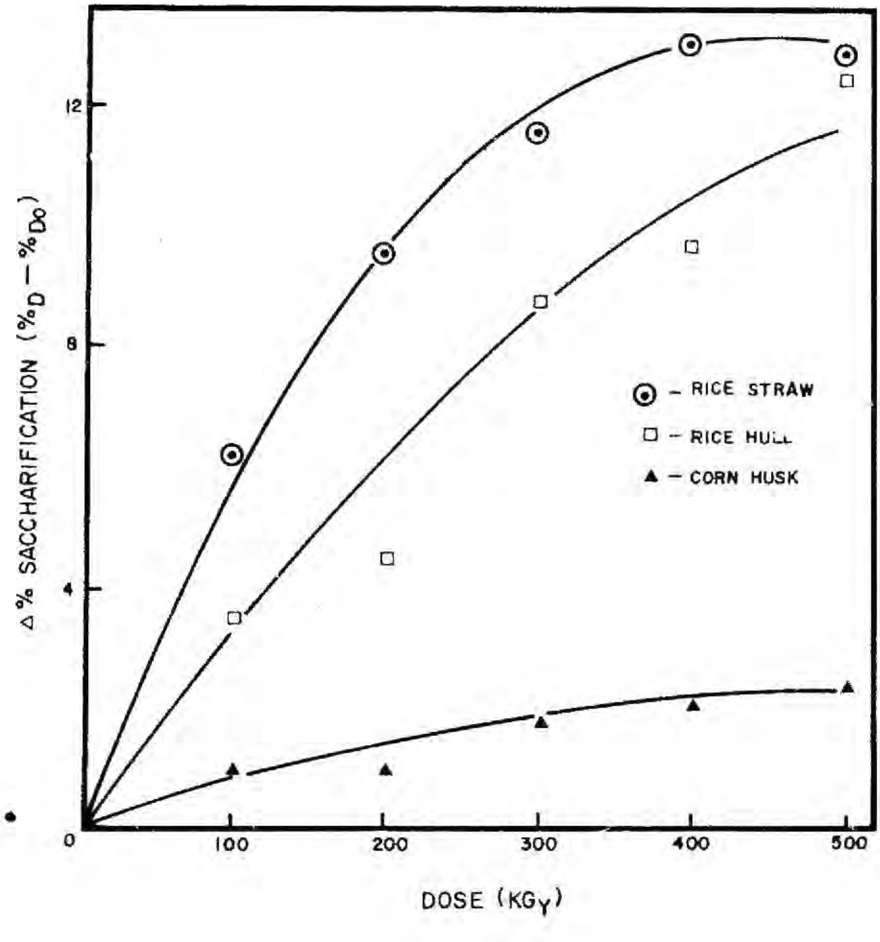

Fig. 2. Effect of radiation pretreatment on the enzymatic hydrolysis of rice straw, rice hull and corn husk.
Points represent averages of three replicates, D = radiation dose and Do = Zero dose.

enzyme-catalyzed saccharification of rice straw, rice hull and corn husk. Sawdust was not significantly affected by radiation. A radiation dose of 500 KGy resulted in the production of 25.8%, 24.0%, 4.0% and 1.6% reducing sugar from the hydrolysis of rice straw, corn husk, rice hull and sawdust, respectively. The corresponding unirradiated samples yielded 11.0%, 10.0%, 2.0% and 0.4% reducing sugars, respectively.

Table 4 shows the constituents of the hydrolysates of cellulose and rice straw. As expected, glucose and cellobiose make up the enzyme hydrolysates of unirradiated cellulose and rice straw. Gamma irradiation enhances the enzymatic hydrolysis of rice straw as indicated by a significant increase in glucose content with increasing radiation dose. The enzymatic hydrolysis of cellulose is not affected by radiation. Faint spots due to xylose were observed, but these were present in

Table 4. Concentrations of sugars produced after enzymatic saccharification of unirradiated and irradiated cellulosic materials.[1]

A. Rice Straw Straw

Dose, KGy	Cellobiose	Sugar %[2] Glucose	Xylose
0	1.69 ± 2.93	5.63 ± 1.03 a[3]	–[4]
100	1.89 ± 1.91	9.52 ± 3.52 abc	–
200	2.18 ± 1.41	11.01 ± 2.71 bcd	–
300	2.93 ± 1.95	12.26 ± 2.20 cde	–
400	4.89 ± 4.72	12.58 ± 0.93 de	–
500	4.48 ± 4.53	16.52 ± 3.36 c	–

b. Cellulose

Dose, KGy	Cellobiose	Sugar, 1%[2] Glucose	1 Xylose
0	9.17 ± 2.75	28.67 ± 4.75	–[4]
100	8.17 ± 6.79	32.50 ± 7.50	–
200	10.50 ± 4.77	30.33 ± 10.75	–
300	13.17 ± 6.23	39.17 ± 18.76	–
400	16.67 ± 1.44	43.50 ± 10.21	–
500	12.00 ± 11.65	32.17 ± 4.65	–

[1]The constituents were separated by TLC using Kieselguhr plates and the solvent system, ethyl acetate-isopropanol-water (65 + 23.5 + 11.5). Aniline phthalate was used as the spray reagent.

[2]Mean value ± S.D. of three replicates, each of two trials.

[3]Any two means having a common letter are not significantly different at 1% level (DMRT).

[4]Not quantifiable.

trace amounts below the detection limit. No xylose can be produced by cellulase-catalyzed hydrolysis of rice straw since this enzyme is specific for cellulose. Thus, the low xylose content in the hydrolysates suggests that the direct radiolytic attack on the glucose and xylose moieties of cellulose and hemicellulose respectively, resulting in the liberation of free xylose is a minor mechanism in the gamma-radiolysis of these macromolecules. Thus while radiation can exert an effect on the monomeric units of the macromolecules, its enhancing action on the acid and enzymatic hydrolyses of these macromolecules may be attributed to its effeet on their supermolecular structures.

The radiation-induced changes in the structures of the agricultural cellulosic wastes may result in the conversion of these wastes into better substrates for cellulolytic microorganisms. To test this postulate, *T. reesei* was allowed to grow in unirradiated and irradiated rice straw, and its production of the enzyme cellulose measured. Table 5 shows that *T. reesei* produces more enzyme when grown in irradiated rice straw. The data discount the possibility that high radiation doses may generate substances that may become toxic to the growth of the microorganism. In addition to making the cellulose molecule more accessible to the microorganism, the radiation treatment may induce other changes that result in more nutrients for the microorganism. The results of this study have wide applications in the various aspects of biomass conversion.

The data just presented clearly show that gamma irradiation enhances the acid and enzymatic saccharification of some cellulosic materials. The extent of the observed radiation effects depends on the type of substrate used. The differential response of the substrates cannot be due to radiation-induced changes in the molecular structure of cellulose alone. Such response may be attributed also to changes in the supermolecular structure of cellulose molecules relative to one another and to other components of the cellulosic materials.

Table 5. Effect of radiation on the production of cellulase by *Trichoderma reesei*[1]

Dose, KGy	*Concentration of Cellulase, mg/ml*
0	2.11 ± 0.52 a[2]
100	2.69 ± 0.70 b
200	3.03 ± 0.41 cd
300	3.22 ± 0.34 d
400	3.34 ± 0.27 de
500	3.68 ± 0.43 e

[1]Mean values ± S.D. of four replicates, each of duplicate trials.

[2]Any two means having a common letter are not significantly different at 1% level (DMRT).

In conclusion, the present study has demonstrated the enhancing effect of gamma irradiation on the acid and enzymatic saccharification of cellulose and agricultural cellulosic wastes. The data indicate that the observed radiation effect results from the action of gamma radiation on the molecular and supermolecular structures of the cellulosic materials.

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