Chapter 19

RADIATION PRETREATMENT OF BIOMASS

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1. INTRODUCTION

Biomass is organic matter derived from living or recently living organisms. With growing global concerns over renewable energy sources, biomass can be used as part of the energy supply chain. The main constituents of biomass are cellulose, hemicellulose and lignin. The relative amounts of these constituents change with type of lignocellulosic material as well as with where the biomass was grown. Cellulose is a polysaccharide made up of D-glucose units, linked by β-1,4 glycosidic bonds, and organized into crystalline and amorphous regions. Lignin and hemicellulose increase the mechanical properties of lignocellulosic materials.

Both physical and chemical methods are used to treat lignocellulosic material. Physical treatment is used to separate cellulose by eliminating lignin and hemicellulose as well as breaking down the crystallinity of the cellulose, so as to increase the accessible surface area and the size of pores in cellulosic materials. An effective pretreatment should break down the lignocellulose structure to give a digestible raw material, avoiding degradation of the target material and the formation of toxic by-products. Any pretreatment must be economically worthwhile [1]. The yield in the separation of the main components of lignocellulosic material depends on some parameters, such as the ratio of cellulose, hemicellulose and lignin to each other, the crystallinity of the cellulose and its water content. The general structure of lignocellulose is shown in Fig.1. The most important limiting parameter is the available surface area which influences the next processes, as when producing fine chemicals.

Several types of physical processes have been developed, mainly by using milling [2, 3], steam or fiber explosion [4-6], exposure to supercritical fluids [7, 8], irradiation [9-11] as well as chemical processes [12-14]. Some of these methods are not often satisfactory unless used with chemical ones in order to improve the process efficiency.

High energy irradiation (electron beams, X-rays or gamma rays) can produce ions and/or radicals in the biomass which initiate some chemical reactions

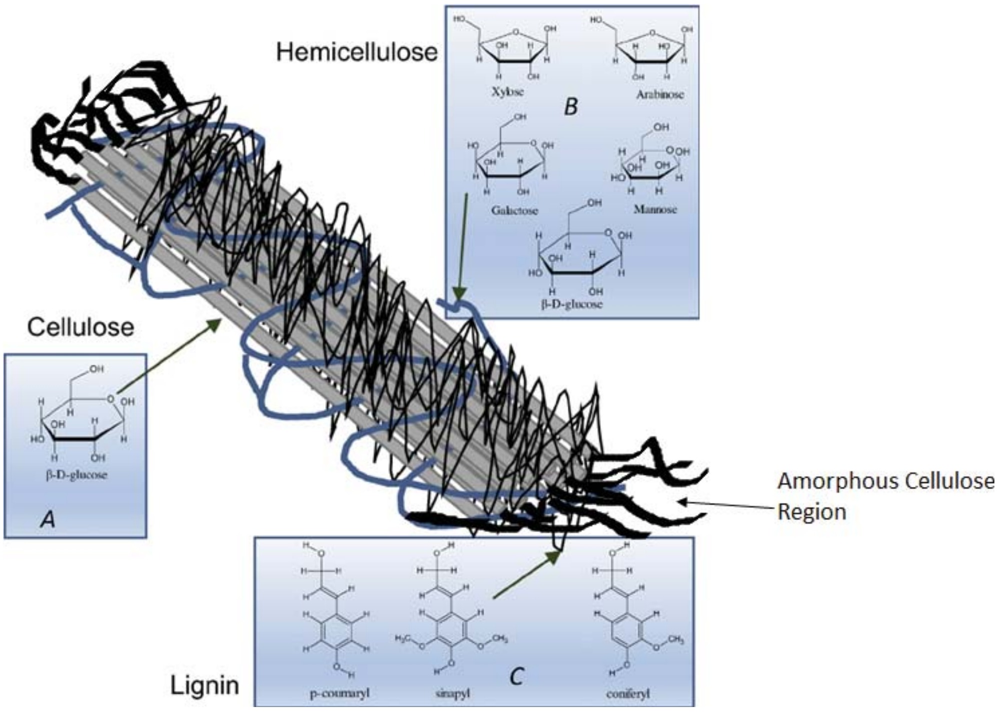

Fig.1. General structure of lignocellulose.

that mainly result in chemical bond cleavages [15-19] and decreasing molecular weight with increasing irradiation dose. Ionizing radiation is an effective method for the pretreatment of lignocellulosic biomass which can modify and disrupt the structure of biomass. The radiation-induced reactions in cellulosic materials are based on short- and long-lived radicals which produce secondary degradation through chain scissioning and crosslinking [20] which depend on the polymeric structure and radiation dose [21]. The irradiation dose needed can be reduced by adding low concentrations of sodium hydroxide. The degradation of the lignocellulosic material becomes accelerated and the process is more economically feasible [22]. The high degradation yield of lignocellulosic compounds using small irradiation doses is attributed to the weak chemical bonds between the lignin, the hemicellulose and the cellulose units and to alkali swelling [23]. Figure 2 illustrates this process.

During or soon after irradiation, radicals, which are produced mainly in amorphous regions of cellulose structure, extinguish quickly as well as radicals in the crystalline and semicrystalline regions which decay after a certain period which mainly causes the degradation aftereffect of lignocellulosic materials [24]. Investigations of the radiation-induced degradation of lignocellulosic materials include analyses of the formed radicals. A significant decrease of cellulosic radicals was observed with time after irradiation and the rate was dependent on temperature, humidity, the presence of oxygen and other envi-

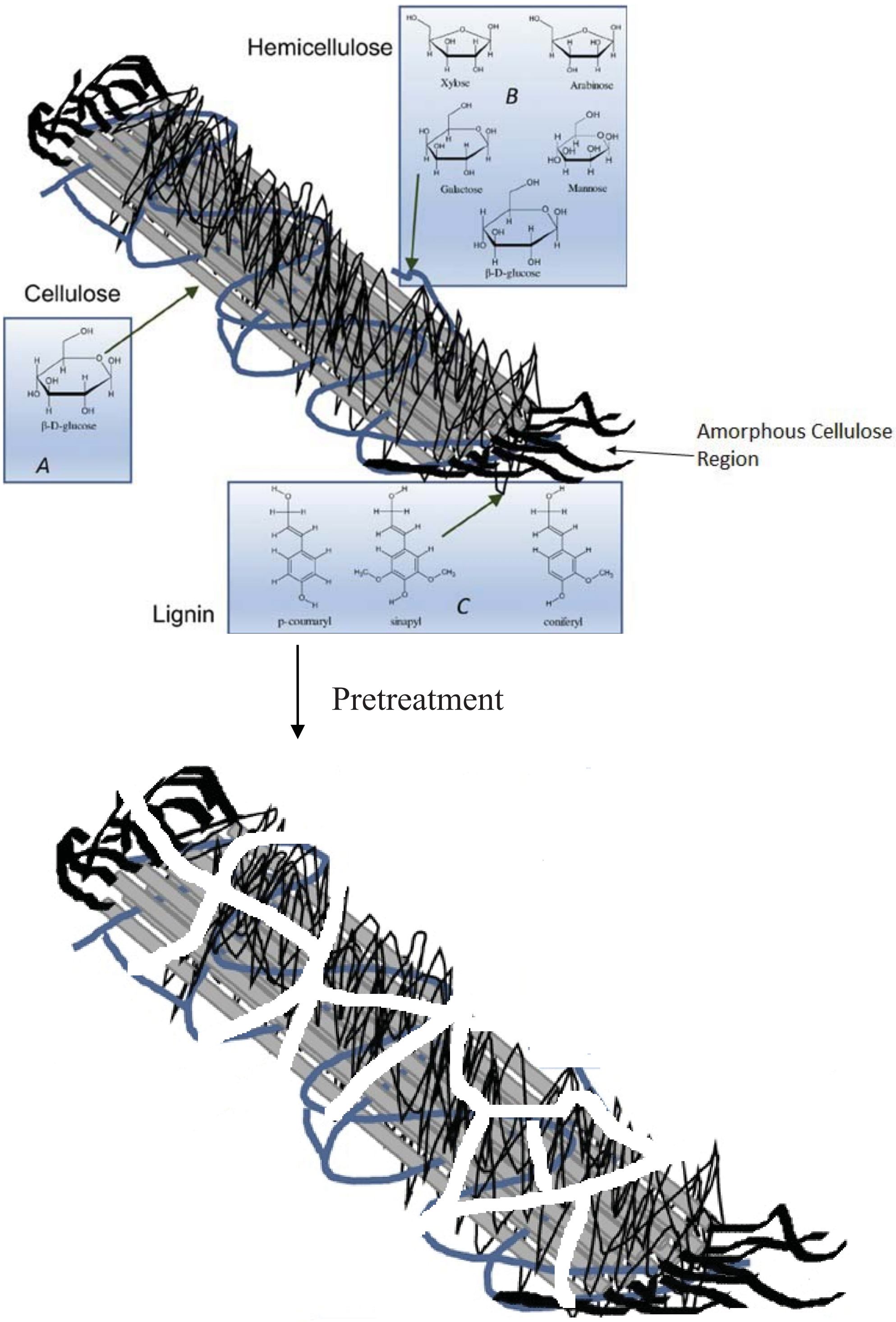

Fig.2. Pretreatment of lignocellulose biomass. Lignin and hemicellulose decompose before hydrolysis.

ronmental parameters [25-28]. A polyphenolic material, lignin, has some stabilizing effect on the radiation of lignocellulosic biomass [29] in which phenoxy radicals importantly become transformed into the o-quinonoid structures in lignin [30, 31].

1.1. GAMMA IRRADIATION

The ^{60}Co and ^{137}Cs isotopes undergo radioactive decay to produce gamma rays (γ-rays), ^{60}Co, which has a half-life of 5.26 years, is widely used in industrial irradiation processes as compared to other radioactive isotopes. Irradiation systems can be a batch (discontinuous), closed system process using a gamma chamber. This limits the amount of biomass that can be treated. In a batch-continuous process, the amount of irradiated biomass would be useful for further large-scale processing. Such systems have safety features and a long history of use in other areas. The energy of the gamma rays is transferred to the atoms of the biomass and results in the formation of radicals. The scission of glycosidic bonds results in the degradation of polysaccharides [21] as well as destructing cell walls [32, 33]. Gamma irradiation alone is an effective technique for biomass pretreatment and when the irradiation process is combined with other ones (chemical, physical) there is an increase in the efficiency of the entire process which can produce the same results using a lower dose.

1.2. ELECTRON BEAM IRRADIATION

Electron beam processing relies on electrically accelerated electrons, not on radioactive isotopes. When biomass is exposed to accelerated electrons, energy is transferred to the atoms of the biomass. The interaction of biomass with accelerated electrons results mainly in chain scission [34]. In addition, any heat generated in the target material may have a synergic effect in biomass treatment. As a result, both irradiation and physical transformations may occur together [35].

2. USES OF BIOMASS PRETREATMENT BY IRRADIATION

The are several uses of irradiation for biomass conversion:

- to facilitate enzymatic hydrolysis,
- to produce bioethanol,

- to facilitate protein synthesis,
- others.

The radiation pretreatment of biomass is mainly for bioethanol production, as well as for environmental purposes, and the combined use of radiation pretreatment with chemical pretreatment are discussed in the literature.

2.1. ENZYMATIC HYDROLYSIS

Kim *et al.* investigated radiation effects on mutated lignocellulose *Brachypodium* stems using a ^{60}Co gamma source. Changes in the cell walls and decrease in cell size were observed along with an increase in broken regions on cell surfaces with increase in irradiation dose [36]. This was due to the effect of reactive oxygen species formed after gamma irradiation and its synergistic effect on enzymatic hydrolysis. The reduction in lignin content was one of the most important steps for the development of bioethanol feedstock as well as for other aims. Kim *et al.* observed between 2.4 and 18.4% decrease of lignin content in irradiated cell walls [36]. Enzymatic hydrolysis of *Brachypodium* biomasses was performed to determine the irradiation yield of lignocellulosic biomass conversion to fermentable sugars by the amount of glucose change in structure. Samples were exposed to 0, 50, 100, 150, 200, 250 and 300 kGy doses of gamma rays. There was an increase in glucose yield with irradiation doses of 200, 250 and 300 kGy. At 50 and 100 kGy irradiation doses there was a lower glucose yield than the control plant samples (0 kGy, no dose). This lower glucose yield was attributed to insufficient pretreatment which failed to break the surface of lignocellulose or to the conversion of glucose to other by-products, as earlier reported by Yang *et al.* [23]. Crystallinity may affect enzymatic hydrolysis of lignocellulose [37] and irradiation pretreatment of *Brachypodium* may change the crystallinity of the lignocellulose surface. Very low dose-rate irradiation, which requires a long period of low energy irradiation, leads to depolymerization and further degradation as well as increases in the lignocellulose surface area which then yields enzymatic hydrolysis.

Yang *et al.* [23] investigated the effect of gamma irradiation pretreatment on the enzymatic hydrolysis of wheat straw. Increasing the irradiation dose increased the weight loss of the biomass as well as producing a more fine particle size distribution after pulverizing. The weight loss of the irradiated biomass wheat straw at 500 kGy was 3.70% due to the emission of by-product gases, including CO, CO_2 and H_2 as well as the loss of bounded water during chain scissioning process. The emission of molecular hydrogen among some volatile gases has been observed by various researchers [38, 39] after irradiation of cellulose. The yield of powder yield below 200 mesh screen size increased with irradiation dose and reached a value of 61.3% fine powder at 500 kGy from 15.2% of the control. This indicates the radiolytic degradation of biomass wheat straw resulted in a brittle structure and the combination of irradiation degradation and pulverization was effective in reducing the size of wheat straw particles. Yang *et al.* [23] also observed an increase in glucose yield with irradiation dose, which was moderate at 50 to 300 kGy but increased between 300 and 500 kGy irradiation doses with a maximum yield of 13.40% and then decreased with greater irradiation doses. This decrease of conversion to glucose was due to increased by-products which can act as inhibitors for the enzymatic hydrolysis of cellulose. The effect of the granularity of biomass wheat straw on radiation pretreatment was also examined by Yang *et al.* [23]. Powder fractions of 1 cm, 20, 60, 100, 140 and 180 mesh were studied. Biomass samples were irradiated to 500 kGy irradiation dose and the enzymatic hydrolysis was immediately studied. The correlation of granularity with glucose yield for unirradiated biomass powdered wheat straw between the 20 to 180 mesh sizes was relatively higher than that of 1 cm biomass. This can be explained by the increase in the surface area of cellulosic material after pulverizing or grinding which decreased the crystallinity that enhances the enzymatic hydrolysis. A synergistic effect between irradiation and grinding was observed for a granularity of 140 mesh biomass wheat straw irradiated to 500 kGy which gave a glucose yield of 10.24%. The aftereffect of radiation pretreatment of biomass wheat straw was also examined by Yang *et al.* [23]. The aftereffect was defined as the change of glucose (or reducing sugar) yield of a sample that was stored for different times after irradiation and compared with primary effects at the same irradiation dose. 140 mesh size biomass powder was irradiated to 400 kGy and the aftereffect at different storage times (0, 1, 4, 9, 15, 22, 30 days) was examined. The glucose yield increased up to twenty-second day after irradiation with a maximum value of 10.21% and then decreased at the thirtieth day, indicating the post-degradation of biomass proceeds with time. The decrease in glucose yield at the thirtieth day may be due to the accumulation of by-products that inhibit enzymatic hydrolysis.

Chung *et al.* examined the enzymatic hydrolysis of poplar bark by the combined use of gamma rays and dilute sulfuric acid for bioethanol production [40]. The combined pretreatment of biomass poplar bark at 1000 kGy gamma irradiation and dilute sulfuric acid process (3% w/w H_2SO_4) remarkably increased the yield of fermentable sugars. The dry basis content of poplar bark was 20.2% lignin. The xylose content of the biomass poplar bark with irradiation only decreased from 23.8 to 14.3%. When the use of a 3% dilute sulfuric acid solution was combined with 1000 kGy gamma irradiation, the xylose content was significantly reduced to 3.4%. In addition to this, the combined pretreatment process produced cellulose enriched solids. The xylose loss indicates the removal of hemicelluloses and provides a higher surface area of the cellulosic material for hydrolysis.

2.2. CONVERSION TO BIOETHANOL

There are many studies in the literature about the conversion of lignocellulosic biomass to bioethanol by various pretreatment methods including high energy radiation pretreatment.

Yoon *et al.* examined the production of saccharides from biomass *Undaria sp.* using gamma irradiation for bioethanol production [41]. The effect of low acid concentration for biomass conversion to fermentable sugars can be found elsewhere in literature as well as the use of a low pH to inhibit the microbial growth. The fermentation results in lower ethanol yields and this process requires acid recovery for economic reasons. Biomass samples were irradiated at various irradiation doses (0, 10, 50, 100 and 500 kGy) and the concentration of reducing sugar increased with irradiation dose from 0.017 to 0.048 g/L when irradiated to 500 kGy. Irradiation leads to the degradation of the starch in the *Undaria sp.* biomass through the cleavage of glycosidic bonds in the presence of water [42, 43]. Irradiation processing is free of toxic additives and is easier and more environmentally friendly since there is no need for additives, to tightly control temperature and for the use of special chemicals [22]. Irradiation of biomass in the presence of a dilute acid (1% sulfuric acid) enhances the degradation of the biomass to glucose. The sugar concentration was 0.017 g/L for 500 kGy irradiated biomass but this value increased to 0.235 g/L when combined with acid hydrolysis. This study showed that irradiation increased the saccharide yield from raw materials, biomass with high lignin content, that can be used for ethanol or biogas production.

Wang *et al.* examined the effect of gamma irradiation and steam explosion pretreatment on biomasses from agricultural residues for ethanol production [44]. Using a rice straw biomass, the degradation ratio for cellulose, hemicellulose and lignin increased with irradiation as could be done in the steam explosion process by increasing processing time or pressure. When compared, gamma irradiation was more efficient.

When compared with steam explosion pretreatment, irradiation is more useful for the enzymatic hydrolysis for bioethanol production since toxic compounds are not generated during the process. When treated biomass samples were compared with controls of untreated materials, the maximum concentrations of glucose and total reducing sugar were 43.3 mg/g of glucose and 90.4 mg/g of reducing sugar by enzymatic hydrolysis of biomass rice straw irradiated to 800 kGy. Using biomass samples which initially had 19.0 mg/g glucose and 37.2 mg/g reducing sugar, the steam explosion process at 2 MPa pressure for 2 min yielded 30.1 mg/g glucose and 85.4 mg/g reducing sugar. The concentrations of glucose and of total reducing sugar decrease with irradiation doses above 800 kGy and up to 2000 kGy. Toxic materials, such as glucuronic acid and galacturonic acid, were not found when using the irradiation pretreatment process. The concentrations of glucose and of total reducing sugar were at acceptable levels, indicating that irradiation processing is feasible for ethanol production. Morphology studies by scanning electron microscopy (SEM) compared lignocellulosic biomasses from rice straw, corn stalk and bagasse after gamma irradiation pretreatment at a dose of 1000 kGy with control samples. The irradiated biomasses show a wider distribution of lignin with an uneven surface and covered with small droplets, that may be ascribed to changes in lignin structure at an irradiation dose of 1000 kGy. The separation of cell wall components and an increase in the porosity were also found. Elimination of the interference of lignin droplets showed that three lignocellulosic biomasses have rough surfaces and large reactive areas after gamma irradiation treatment, which is very important for enzymatic hydrolysis. One of the important problems in industrial-scale bioethanol production from lignocellulose is the low efficiency from normally structured cellulose [45]. The gamma irradiation pretreatment process of biomass overcomes from this problem by degrading fibers and increasing the surface area needed to access cellulose. Acid hydrolyzed, gamma irradiation pretreatment of biomasses showed that the concentration of hydrolyzed sugars (glucose, xylose and arabinose) varied with agricultural types. The maximum concentration of glucose from bagasse was 0.18 g/L at a dose 2500 kGy followed by 1.0 (w/v) dilute sulfuric acid hydrolysis for 60 min. Under same conditions, the maximum concentration of xylose was 0.18 g/L for bagasse. Glucose, xylose and arabinose yields increase in the acid hydrolysis step with increasing irradiation doses from 500 to 2000 kGy. The effect of enzymatic hydrolysis on the radiation pretreatment for three lignocellulosic biomasses rice straw, corn stalk and bagasse was also studied. The maximum conversion ratios to hydrolyzed sugars were higher by 69% for the three investigated lignocellulosic biomasses. Glucose and xylose conversion was much higher and increased with irradiation dose from 500 to 2500 kGy following enzymatic hydrolysis. Arabinose conversion rate was relatively lower that decreased with irradiation dose from 500 to 2500 kGy following enzymatic hydrolysis. The pretreatment of lignocellulosic biomass with gamma irradiation was found to be more efficient than steam explosion. In addition, no toxic by-products are formed in the irradiation pretreatment process. Scanning electron microscopy showed the changes in lignocellulosic biomasses after the gamma irradiation pretreatment process which seems more suitable for industrial-scale bioethanol production.

2.3. CONVERSION TO PROTEINS

Some researcher has focused on the study of radiation pretreatment of lignocellulosic biomass for the bioconversion to protein rich materials for environmental purposes.

Awafo *et al.* examined the effect of radiation pretreatment of biomass corn stover to obtain protein rich organic substances [46]. Gamma irradiation at 100 kGy did not show an effect on the bioconversion of biomass corn stover into protein rich organic material. The irradiated material was equivalent to the unirradiated biomass. With additional irradiation, the protein profile becomes richer as discussed above because of a decrease in crystallinity, depolymerization and an increase in specific surface area available for bioconversion.

2.4. OTHER APPLICATIONS

Duarte *et al.* examined the effect of electron beam irradiation combined with a hydrothermal treatment process on the pretreatment of sugarcane bagasse [47]. The composition of biomass sugarcane bagasse was found to be 41.9% cellulose, 31.3% hemicellulose, 19.5% lignin, 6.3% soluble compounds and 1.0% ash. An increase in solubility was found with irradiation dose due to the hemicellulose cleavage that forms water soluble cello-oligosaccharides from xylanes. The main by-products of hemicellulose degradation were acetic acid, furfural and formic acid. When irradiated, moisture in the biomass forms an $^{\bullet}OH$ radical that contributes to the radiation effects.

El-Batal *et al.* investigated the effect of radiation pretreatment of biomass *Aspergillus tamarii* on its adsorption capacity of dyes from textile manufacturing wastewater [48]. The cell walls of the biomass play an important role in dye adsorption and some pretreatment methods are used to increase the adsorption capacity by decomposition of such components as lignocellulosic biomass. The degraded cells have a larger surface area with more surface binding sites. The effects of ionizing radiation can be explained by the change in charges of the biomass which are based on the formation of more electrostatic charges that change the overall surface charge and modify binding sites. These enhance the formation of electrostatic bonds between the biomass surface and the dye molecules [49].

Ouallouche *et al.* investigated the effect of gamma irradiation pretreatment of *Rhizopus Stolonifer* biomass on the removal of mercury and lead from aqueous solutions and made a comparison with other methods [50]. All of the investigated pretreatment methods showed efficient removal of lead and mercury form water. The irradiation pretreatment of biomass increased adsorption of lead and mercury ions by 10% and by 33%, respectively. Although, the adsorption yield is relatively low compared to other pretreatment methods, the irradiation pretreatment is feasible since it easy to operate, economic over long periods of time, no additional chemicals are introduced into the system and is environment friendly without toxicity.

2.5. DEGRADATION

Khan *et al.* examined the changes in the physical and chemical properties of lignocellulose for jute after gamma irradiation [20]. The radiolytic degradation of biomass jute resulted in a biodegradable natural lignocellulose fiber formed mainly from 58-63% cellulose, 20-24% hemicelluloses and 12-15% lignin. The effect of gamma irradiation on jute was noted by several analytical methods which are given below. Tensile strength and elongation of jute was reduced with irradiation. The change in the mechanical strength of jute with irradiation was significant up to 30 kGy, but additional irradiation did not change the mechanical properties significantly due to concurrent chain scissioning and crosslinking reactions. Fluorescence and Fourier transform infrared (FTIR) spectrometry showed a decrease in the number and integral area of analytical peaks of pure jute with irradiation dose corresponding to radiolytic degradation. The thermal stability of jute was also decreased with irradiation since pyrolytic degradation occurs by glycosidic chain scission and the breakdown of other groups [51, 52]. Thermal reactions proceed more rapidly above 315°C in which glycosidic bond scissioning occurs. X-ray studies showed a decrease in crystallinity with irradiation dose which corresponds to a structural change in the lignocellulosic material.

Orozco *et al.* studied gamma irradiation induced degradation of orange peels [53]. A color change from yellow to brown was observed because of the formation of reducing sugars with irradiation. Sucrose, glucose, and fructose were determined by HPLC (high-performance liquid chromatography) analysis after irradiation of the orange peel biomass. Further irradiation of orange peels (1800 kGy) resulted in a decline in the amount of both glucose and fructose in the sugar concentration which was due to glycolytic degradation. Irradiated and unirradiated biomass orange peels both have the similar FTIR spectra with small changes in functional group. These changes were attributed to a decrease in chemical linkages by gamma irradiation. Thermal gravimetric results showed that when the irradiation dose reached 600 kGy or higher, hemicellulose and cellulose undergo chain cleavage but lignin is less affected by gamma irradiation. Lignin is more radiation resistant and protects the biomass structure from radiation. The formation of carbonyl groups on cellulose structure in the presence of oxygen enhances the conversion of cellulose to lower molecular weight compounds.

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