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Effect of γ-irradiation dose on cellulose degradation of corn stover for bioethanol production

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Abstract

The effect of γ-irradiation on cellulose degradation of corn stover was evaluated on radiation doses of 0 kGy, 250 kGy, 500 kGy, 750 kGy, and 1000 kGy, respectively. Alkali pretreatment with NaOH solution 2% (w/v) was also employed before applying γ-irradiation. Research has indicated that a minimum of 250 kGy of γ-irradiation can produce glucose in corn stalk, corn husk, and corn cob. However, the glucose content of corn stalk was not statistically significantly affected by the radiation dose treatment (p>0.05). The impact of γ-irradiation dosage on cellulose degradation statistically influences the glucose content in corn husk and corn cob, with a minimum radiation dosage of 500 kGy and 250 kGy, respectively. Furthermore, a scanning electron micrograph image revealed structural differences in cellulose surface morphology between irradiation-pretreated and non-irradiated corn cob. We believe this study can be a further reference for selecting the best applications of γ-irradiation dose on corn stover for further research.

Keywords: γ-irradiation, corn stover, cellulose, glucose

Graphical Abstract:

1. Introduction

The escalating global demand for sustainable and environmentally friendly energy sources has intensified the focus on valorizing agricultural waste. Among the myriad options available for renewable energy, converting agricultural waste into cellulose and its subsequent transformation into fuels presents a promising avenue (Nunui et al., 2022). Corn stover is one of the agricultural wastes that can be used for bioethanol production. According to a statistical data report from Badan Pusat Statistik Indonesia on March 1st, 2024, corn production in Indonesia in 2023 reached 14.77 million tons (Indonesia, 2024). The high production of corn in Indonesia is a positive thing to utilize corn stover on a broader scale.

As a lignocellulosic material, corn stover also consists of cellulose, hemicellulose, and lignin components. Cellulose degradation breaks cellulose into simpler sugars that can be fermented into bioethanol. Degradation of cellulose is crucial in biofuel production as cellulose is one of the most abundant organic compounds and represents a renewable source of energy (Binder & Raines, 2009).

Various pretreatment methods have recently been designed for cellulose degradation and are divided into conventional and non-conventional methods. Steam explosion (Sun & Cheng, 2002), ammonia fiber expansion (Chundawat et al., 2020), alkali treatment (Zhao et al., 2018), acid treatment (Rezania et al., 2020), torrefaction (Klaas et al., 2020), biological (Baruah et al., 2018), hydrothermal (Sarker et al., 2021), supercritical CO² (Lü et al., 2013), Organosolv (Yang et al., 2023), Ozonolysis (Appels et al., 2012), and ionic liquids (Uppugundla et al., 2014) are included in conventional pretreatment. Meanwhile, non-conventional pretreatment is divided into four methods, namely microwave (Yan et al., 2021), gamma irradiation (Wang et al., 2012), electron beam (Guo et al., 2016), and ultrasound (Nakashima et al., 2016). Although numerous pretreatment methods have been reported, there are few reports on implementing γ -irradiation on corn stover. When γ -irradiation is used at a high dose (150) kGy) on lignocellulose biomass, the cell wall constituents decrease, and fiber depolymerization and delignification occur (Al-Masri & Zarkawi, 1994). Nevertheless, studies have shown that combining γ-irradiation and chemical treatments results in more significant cellulose degradation of lignocellulose biomass than either chemical or irradiation treatment alone (Banchorndhevakul, 2002).

Therefore, in this work, the effects of gamma irradiation on the degradation of cellulose were evaluated. The surface morphology of corn stalk, corn husk, and corn cob were also investigated to demonstrate irradiation pretreatment's effectiveness further. These investigations would be helpful in further understanding the applications and fundamentals of gamma irradiation pretreatment for bioethanol production from corn stover. This study aims to investigate gamma irradiation's effectiveness in enhancing cellulose degradation efficiency and its potential as a sustainable and environmentally friendly method for biofuel production.

2. Materials and Methods

2.1 Materials

Corn stover was gathered from Pamekasan Region, Madura Island, East Java Province, Indonesia (6°51' - 7°31' South latitude and 113°19' - 113°58' East longitude). The local corn type used in this study is a hybrid corn (Griliches, 1957). After being transported to the laboratory, various parts (stalk, husk, cob) of corn stover were separated, sun-dried, milled, and passed through a 30-mesh sieve.

2.2 Pretreatment methods

2.2.1 Alkali pretreatment

A total of 20 grams of each sample (corn stalk, corn husk, corn cob) was immersed in 160 ml of NaOH solution 2% (w/v) and heated at 120 $^{\circ}$ C for 30 minutes (Chen et al., 2009). The samples were then washed with distilled water until the pH was neutral. The neutral samples were then oven-dried at 121°C for 15 minutes until the weight was constant to remove the water content of the material. According to previous research (Chen et al., 2009), pretreatment with NaOH solution 2% (w/v) removed more lignin fractions and showed the highest lignin removal of 73.9%.

2.2.2 γ-irradiation

γ-irradiation pretreatment was performed using a Co^{60} γ-ray irradiator with a maximum source activity of 5444 Ci at the Polytechnic of Nuclear Technology, Yogyakarta, Indonesia. A total of 50 grams of each sample is contained in food-grade plastic. The samples were put into a sample holder, and then the holder was put into the gamma irradiator, which had a maximum dose rate in air of 2.57 kGy/h. The working principle of this gamma irradiator is that the sample approaches the Co-60 radioactive source. The specific irradiation doses were used at 0 kGy (control), 250 kGy, 500 kGy, 750 kGy, and 1000 kGy.

2.2.3 Dilute acid hydrolysis

The dilute acid hydrolysis of pretreated materials was modified according to the method by Wang et al., (2012). The gamma-pretreated lignocellulosic materials (7 grams) were mixed with H_2SO_4 1% (63 ml) in an autoclave at 124 °C for 60 min. After hydrolysis, the samples were air-cooled and filtered using Whatman paper. The filtrate was used to determine the glucose concentration.

2.3 Analysis methods

2.3.1 Glucose determination

Qualitative glucose analysis using Fehling A (CuSO4.5H2O) and Fehling B (a mixture of $KNaC_4H_4O_6.4H_2O$ and NaOH). Fehling's solution is used in a test to differentiate between water-soluble carbohydrate and ketone functional groups, and the two solutions are mixed in equal volumes to perform the test. One gram of the sample was dissolved in 30 ml of distilled water, then 10 ml of the solution was put into a reaction tube. Add Fehling A and Fehling B reagents to each reaction tube and heated in boiling water. Finally, observe the color change and precipitate in the reaction tube after heating.

Spectrophotometer UV-Vis (Shimadzu UV-Vis, Spectrophotometer Double Beam, UV-1780) was utilized to perform quantitative glucose analysis. Glucose and sample solutions were prepared, and each was added with distilled water and Benedict's solution as the reactant solution. Both solutions were heated using a water bath at 85-90 °C for 5 minutes until a precipitate formed.

2.3.3 Surface morphology analysis by scanning electron microscopy (SEM)

A scanning electron microscope (SEM) (Hitachi type SU3500) was used to observe the surface morphology of the samples before (0 kGy) and after gamma irradiation (1000 kGy). The samples were mounted on a specimen holder using a special double-sided tape and coated with gold at room temperature. The sample was vacuumed, inserted into the stage on the SEM, and observed using an applied tension of 3.00 kV.

2.3.2 Statistical analysis

The data were statistically analyzed using SPSS software. ANOVA (Analysis of Variance) was employed to determine if there are statistically significant $(p<0.05)$ differences between the means of three or more independent groups.

3. Results and Discussion

3.1 Effect of gamma irradiation on cellulose degradation

The reaction of glucose with Fehling's solution is a well-known chemical test used to detect the presence of reducing sugars, such as glucose, in a solution. Fehling's solution consists of two separate solutions: Fehling's A, which contains copper (II) sulfate, and Fehling's B, which contains potassium sodium tartrate and sodium hydroxide. When these two solutions are mixed, they form a deep blue complex. When glucose is present in the solution, it acts as a reducing agent. The aldehyde group of glucose reduces the copper (II) ions (Cu^{2+}) in Fehling's solution to copper (I) ions $(Cu⁺)$. The reduced copper (I) ions then precipitate out of the solution as red or orange cuprous oxide (Cu₂O), which is insoluble in water (Jacobs, 1912; Kooti & Matouri, 2010). This precipitate indicates a positive result for the presence of reducing sugars, as shown in [Fig.](#page-7-0) 1. It can be concluded that gamma irradiation with a minimum dose of 250 kGy can degrade cellulose to glucose with the presence of red precipitate in the samples.

3.2 Analysis of glucose concentration from corn stover before and after gamma irradiation

The application of gamma irradiation treatment showed increased glucose content of corn stalk, corn husk, and corn cob. Gamma radiation can degrade cellulose into glucose through chain cleavage and oxidation reactions. The high energy of gamma rays induces ionizations in cellulose that are not hindered by its crystallineamorphous structure (Blouin & Arthur, 1958, 1960; Orabi et al., 2017). The chain cleavage results in the formation of new reducing ends that can be further degraded into glucose. However, the radiation dose treatment statistically had no significant effect ($p>0.05$) on the glucose content of corn stalk, as shown in [Fig. 2a](#page-7-1). The cellulose fibers derived from corn stalks consist of single cells bound together by substances that might not have been eliminated by NaOH during the alkali treatment (Reddy $\&$ Yang, 2005). Consequently, the cellulose polymer bonds of the corn stalks may have been resistant to degradation by free radicals generated from gamma irradiation. A similar phenomenon was observed in corn husk, where the treatment with radiation at a radiation dose of 250 kGy and without radiation statistically also did not show significant differences. Nevertheless, the effect of gamma radiation on corn husk became evident when the radiation dose was applied at 500 kGy to 1000 kGy [\(Fig. 2b](#page-7-1)).

Meanwhile, increasing the radiation dose enhances the degradation of cellulose. Gamma irradiation doses ranging from 250 kGy to 1000 kGy caused a statistically significant increase in glucose concentration on corn cob when compared to without radiation, as depicted in [Fig. 2c](#page-7-1). The formation of free radicals possesses a significant effect of ionizing radiation on the degradation of gamma-irradiated cellulose. The ESR (Electron Spin Resonance) spectra of gamma-irradiated cellulose samples showed a doublet and a triplet as the main components, indicating the presence of different types of free radicals (Kameya et al., 2013; Rukhlya et al., 1983). These free radicals can undergo further reactions that lead to the degradation of cellulose. For example, the free radicals can initiate chain cleavage reactions, resulting in the formation of new reducing ends that can be further degraded into glucose (Kodama et al., 2016).

In addition, a similar investigation was performed by Darojati et al., (2022) on the lignocellulosic coconut coir biomass using gamma irradiation to separate lignin and to generate higher glucose content. The study found that the optimal dose to obtain glucose was achieved at an irradiation dose of 100 kGy with a glucose content of 5.09 mg. The optimal gamma irradiation dose for lignin separation was 50 kGy with a lignin separation percentage of 34.95%. Moreover, utilizing gamma irradiation on coconut coir biomass can produce higher bioethanol content than without irradiation. The study shows that the highest bioethanol content (34.93%) was obtained at a 200 kGy dose compared to without irradiation (2.25%) (Darojati et al., 2019).

3.3 Surface morphological study

The effect of γ-irradiation treatment on cellulose morphologies of corn cob was evaluated, and representative SEM images were shown in [Fig. 3](#page-8-0) using magnification of 500x and 1500x, respectively. Before irradiation treatment, the surface of the cellulose showed a rigid and smooth surface structure, indicating that the cellulose microfibrils were tightly coated with the cross-linked xylan and lignin matrix. Conversely, significant changes in cellulose morphology were observed after irradiation treatment. It can be seen that irradiation at 1000 kGy caused some fragmentation of cellulose fibers, the surface structure of cellulose fibers became loose, and their structure was damaged. These results confirm that γ-irradiation would break the structure of cellulose fibers to various extents and degrade into glucose, depending on the radiation dose employed.

K. Qin. Wang et al., (2011) demonstrated identical results when investigating the effect of Co^{60} - γ irradiation dose (0-2000 kGy) on the cellulose structure of *Phragmites Communis Trim* (PCT). Depending on the radiation dose, the surface morphology of PCT cellulose structures that have been gamma-irradiated appears structurally degraded and fragmented, as determined by SEM images. Furthermore, K. Qin Wang et al., (2012) also conducted a study on the morphological structures of rice straw, corn stalk, and bagasse. The structures were examined before and after pretreatment with a Co^{60} - γ irradiation dose of 1000 kGy. SEM observation reveals that Co⁶⁰- γ irradiation pretreatment has resulted in rough surfaces and extensive reactive areas in the three lignocellulosic biomasses. Some ultrastructure alterations caused by gamma irradiation pretreatment, such as cell wall component separation and increased porosity, have also been documented in the literature (Aouat et al., 2019; Betiku et al., 2009; Glegg & Kertesz, 1957; Takács et al., 1999, 2000).

4. Conclusion

The utilization of γ-irradiation on corn stover after alkali pretreatment has effectively enhanced cellulose degradation. Studies have demonstrated that γ-irradiation dose at a minimum of 250 kGy can generate glucose in corn stalk, corn husk, and corn cob, as evidenced by the presence of red precipitate at the bottom of the test tube. Nonetheless, the radiation dose treatment statistically had no significant effect $(p>0.05)$ on the glucose content of corn stalk. The effect of γ-irradiation dose on cellulose degradation significantly affects the glucose concentration in corn husk and corn cob with a minimum radiation dose of 500 kGy and 250 kGy, respectively. Moreover, the SEM image analysis illustrated that there were structural changes in cellulose surface morphology for irradiationpretreated corn cob compared with non-irradiation. The results of this study would be helpful in further understanding the applications of γ-irradiation pretreatment on corn stover for bioethanol production.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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Fig. 1. Glucose precipitation appearances of (a) Untreated corn stalk (0 kGy); (b) Irradiated corn stalk (250 kGy); (c) Untreated corn husk (0 kGy); (d) Irradiated corn husk (250 kGy); (e) Untreated corn cob (0 kGy); (f) Irradiated corn cob (250 kGy)

Fig. 2. Statistical analysis of glucose concentration before and after gamma irradiation of (a) corn stalk; (b) corn husk; (c) corn cob

3.00 kV 6.7 mm 100 µm (a) Untreated corn cob (0 kGy) (x 500)

3.00 kV 6.4 mm 100 µm (b) Irradiated corn cob (1000 kGy) (x 500)

3.00 kV 6.7 mm 30 µm (c) Untreated corn cob (0 kGy) (x 1500)

3.00 kV 6.4 mm 30 µm (d) Irradiated corn cob (1000 kGy) (x 1500)

Fig. 3. SEM images of before (0 kGy) and after (1000 kGy) gamma irradiation for corn cob