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Pretreatment of Biomass with Gamma Rays and Electron Beam for Ethanol Production via Enzymatic Hydrolysis: A Brief Review

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Abstract: Lignocellulosic biomass, sourced from non-edible plant materials like bagasse, straw, and other agricultural residues, represents a sustainable alternative to fossil fuels, contributing to a reduction in greenhouse gas emissions. Effective pretreatment is essential for modifying the structural integrity of biomass, thereby increasing the accessibility of cellulose and hemicellulose for enzymatic hydrolysis. This paper analyzes two pretreatment methodologies, highlighting the role of gamma-ray and electron beam irradiation. These methods leverage photons and high-energy particles to induce structural and chemical modifications in lignocellulosic biomass, which facilitate a more efficient breakdown into fermentable sugars during hydrolysis. This work showed that both irradiation methods not only increase the yield of fermentable sugars but also do it without the need for hazardous chemicals, thus presenting an environmentally benign alternative to conventional pretreatment methods and presents the potential of these irradiation techniques in streamlining bioethanol production processes, advocating for further research and technological development to fully harness their benefits in industrial applications.

Keywords: lignocellulosic biomass, irradiation pretreatment, second-generation ethanol, water radiolysis.

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Pré-tratamento de biomassa por raios gama e feixe de elétrons visando a produção de etanol por hidrólise enzimática: uma breve revisão

Resumo: A biomassa lignocelulósica, proveniente de materiais vegetais não comestíveis como o bagaço, a palha e outros resíduos agrícolas, representa uma alternativa sustentável aos combustíveis fósseis, contribuindo para a redução das emissões de gases com efeito de estufa. Um pré-tratamento eficaz é essencial para modificar a integridade estrutural da biomassa, aumentando assim a acessibilidade da celulose e da hemicelulose à hidrólise enzimática. O presente trabalho analisa duas metodologias de pré-tratamento, destacando o papel da irradiação com raios gama e feixe de elétrons. Estes métodos utilizam fótons e partículas de alta energia para promover modificações estruturais e químicas na biomassa lignocelulósica, que facilitam uma decomposição mais eficiente em açúcares fermentáveis durante a hidrólise. Este trabalho mostrou que ambos os métodos de irradiação não só aumentam o rendimento de açúcares fermentáveis, como também o fazem sem a necessidade de produtos químicos nocivos, apresentando assim uma alternativa ambientalmente benigna aos métodos convencionais de pré-tratamento, e apresenta o potencial destas técnicas de irradiação na racionalização dos processos de produção de bioetanol, defendendo mais investigação e desenvolvimento tecnológico para aproveitar plenamente os seus benefícios em aplicações industriais.

Palavras-chave: biomassa lignocelulósica, pré-tratamento via irradiação, etanol de segunda geração, radiólise da água.

1. INTRODUCTION

Considered clean, biofuels are renewable fuels composed of organic feedstock. Unlike fossil fuels, their use does not result (or there is a considerable drop) in releasing compounds hazardous to human health such as SO_X, NO_X, and fine particle matters. When it comes to global warming, the use of bioethanol reduces CO_2 emissions by more than three quarters [1].

The first-generation (1G) ethanol is derived from edible raw materials, such as grains, sugar crops, and vegetable oils. Since they are obtained directly from edible sources, there is some concern about the social and environmental impacts of competition for agricultural land, opening up the space for second-generation (2G) ethanol, also known as lignocellulosic ethanol. This 2G ethanol does not "fight" with food production, since it has no food purpose for humans, as it comes from the remains, such as bagasse, straw, and other cellulose-rich materials discarded or burned in the fields [2,3].

Biomass is all organic plant or animal matter used as a source of clean and sustainable energy [4]. Therefore, lignocellulosic biomass comes from materials rich in cellulose, hemicellulose, and lignin, which is the key to producing 2G ethanol. Cellulose is a linear homopolysaccharide composed of D-anhydroglucopyranose units linked together by β-1,4-glucosidic bonds [5]. Hemicelluloses are heterogeneous polymers of pentoses (e.g., xylose and arabinose), hexoses (e.g., glucose, mannose, and galactose), hexuronic acid, and deoxyhexoses [6]. Lignin is an amorphous three-dimensional macromolecule formed by the random condensation of p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, and its function is to give plant strength, rigidity, impermeability, and resistance to microbiological and mechanical attacks [6,7].

To produce ethanol from lignocellulosic biomass generally involves three interdependent stages [8]. The first one, and the subject of this paper, is pretreatment, where the material is submitted to processes to break down the complex structure of the cell wall and make the polysaccharides more accessible to the next step. The second stage is hydrolysis, in which specific enzymes are added to convert the polysaccharides into fermentable sugars, and, finally, the third stage, fermentation, takes place when microorganisms such as yeasts convert the sugars into ethanol.

The present paper reviews some existing literature based on biomass pretreatment using gamma radiation and electron beams. By examining the studies and advances in this area, this paper aims to provide a brief understanding of the effects of these pretreatment techniques on the structure and composition of lignocellulosic biomass, as well as their influence on the efficiency of subsequent conversion processes into biofuels, such as enzymatic hydrolysis for ethanol production.

2. BIOMASS PRETREATMENT

Pretreatment is the first and most critical step in biomass conversion, using techniques to make the feedstock more accessible to the enzymes or microorganisms used in the subsequent stages of the process. Different pretreatment increases the enzymatic access through multiple ways, which include (1) removing or altering lignin, (2) reducing the degree of polymerization of cellulose, (3) decrystallizing cellulose, and (4) increasing biomass surface area and pore size [1,9].

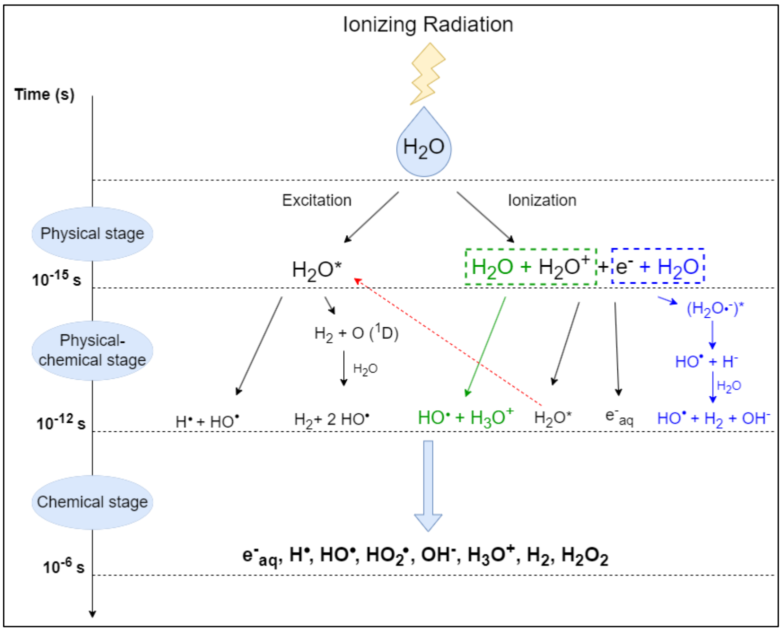
There are several types of pretreatments such as steam explosion, acid, or alkali, among others. These pretreatments can be classified into different groups, with authors categorizing them as physical, chemical, biological, and even combined, with the physicochemical combination being widely used [1,10,11]. Each of these methods offers unique advantages and challenges, depending on the specific characteristics of the biomass and the desired outcome. Physical pretreatments often involve mechanical processes, while chemical methods use reagents to break down biomass structures. Biological pretreatments utilize microorganisms or enzymes, making them environmentally friendly but sometimes slower. Authors consider pretreatment using radiation to be a physical type, which harnesses high-energy particles to alter the structure of biomass, thereby enhancing its accessibility for subsequent processing steps. This method has gained attention for its efficiency and potential to improve the overall yield of fermentable sugars.

3. GAMMA-RAY AND ELECTRON BEAM (EB) IRRADIATION PRETREATMENT

The two most studied and used pretreatments using ionizing radiation are gamma rays and electron beam (EB). Both techniques are based on the radiolysis of organic compounds and can occur through two primary mechanisms: direct and indirect interaction. In the direct interaction, the ionizing radiation acts directly with the main molecules of the organic compounds, leading to the cleavage of chemical bonds, resulting in the formation of smaller molecular fragments. In the indirect interaction, shown in Fig. 1, the radiation interacts with the water molecules present in the material, producing highly reactive radicals that subsequently will interact with the organic molecules [12,13,14].

As described by Al-Assaf [13] and Coqueret *et al.* [14], HO•, H•, and hydrated electrons (which are the most reactive products generated), have radiation-chemical yields (mol/J) identical for ^{60}Co γ rays and high energy electrons. This implies that both gamma radiation and electron beams are equally efficient in producing radicals through the radiolysis of water, allowing both techniques to be effectively used in various industrial and scientific applications.

Figure 1: Water radiolysis stages



Source: Varella *et al.* (2024).

3.1. Gamma irradiators

With a half-life of 5.27 years, ^{60}Co is extensively employed in industrial irradiation processes and small units for research purposes.137Cs is also used, but on a smaller scale due to reasons such as longer half-life (30.1 years), and lower photon energy. ^{60}Co is a synthetic radioactive isotope of cobalt, produced in nuclear reactors. The isotope undergoes β- decay into stable Ni-60, emitting an electron and two gamma rays with energies of 1.173 and 1.332 MeV [12].

The production of ^{60}Co begins with the sintering of natural cobalt powder (producing small pellets), then allocating inside zircaloy rods and introducing in a research nuclear power reactor, where they will be irradiated with neutrons for 18 to 24 months, depending on the neutron flux [15]. After this process, the irradiated pellets are encapsulated in corrosion-resistant stainless steel to produce the source pencils which only gamma radiation can pass through. The production and decay of ^{60}Co are shown below:

$$^{59}_{27}Co + ^{1}_{0}n \rightarrow ^{60}_{27}Co \rightarrow ^{60}_{28}Ni + e^- + 2\gamma\ (1.173\ MeV + 1.332\ MeV) \tag{1}$$

There are two main types of gamma irradiators: (1) self-contained and (2) panoramic irradiators [17].

3.1.1. Self-contained irradiators

Self-contained irradiators enclose the radiation source within a shield made of lead or another appropriate material. They are equipped with a mechanism to relocate the sample from the loading area to the irradiation area. Although it does not produce high doses, its great advantage is that it does not require high shielding and can be placed in a small room. The compact design of self-contained irradiators is especially beneficial in research settings, educational institutions, and medical facilities where space and safety are critical considerations. Their versatility allows for various experimental applications, ranging from sterilizing medical instruments to studying the effects of low-dose radiation on biological samples. This adaptability makes them an essential tool in environments that require precise and controlled radiation exposure without the need for extensive shielding or large spaces. An example of a self-contained irradiator is the Gammacell 220 (Fig.2).

Figure 2: Gammacell 200 (^{60}Co) Irradiator

Source: UM Radiation Laboratory (2024).

3.1.2. Panoramic irradiators

Panoramic irradiators are more appropriate and used for industries and research institutions that require higher doses and greater coverage of irradiated materials. These irradiators are larger and require more space, being located in a shielded room, constructed with a concrete wall thick enough to attenuate the radiation emitted from the source. When personnel need to enter the irradiation room, for any reason (work, maintenance, and so on) the source is retracted to a lower floor, where it is dry shielded by solid walls (dry storage) or water (wet storage).

While the source irradiates the products, it is located at the same level as the materials, radiating 360 degrees (panoramically). Uniform irradiation is achieved through several methods designed to ensure even exposure of all materials. The simplest yet efficient method is to use a turntable, powered by electric motors, positioned around the source. This setup ensures that every part of the material receives consistent radiation, enhancing the overall effectiveness of the process.

An example of a panoramic irradiator is employed at the Gamma Irradiation Laboratory (LIG) of the Nuclear Technology Development Center (CDTN), located on the UFMG campus in Belo Horizonte, as seen in Figure 3. Produced by the Canadian company MDS Nordion, the Category II Multipurpose Panoramic Irradiator is equipped with a dry-stored cobalt-60 source, with a maximum activity of 2,200 TBq or 60,000 Ci and a recharge time of 5.27 years. For the material to be uniformly exposed to radiation, rotating tables were used to place the materials to be irradiated [18, 19].

Figure 3: CDTN panoramic irradiator with turntables

a) b)

Source: Varella *et al.* (2022).

3.2. Electron beam accelerators

The electron beam accelerator functions as a charged particle accelerator. They have a cathode that generates electrons, an electric field that accelerates them through a vacuum region, and a magnetic field to guide their path. There are three types of electron accelerators: the DC type, which extracts a continuous beam; the microwave pulsed type, where the emitted beam is repeated at a low frequency (repetition rate); and the pulse or continuous wave type, where electrons are accelerated by a lower radiofrequency (100-200 MHz) at each amplitude [16].

Commercial EB accelerators have energies ranging from 80 keV to 10 MeV [14], with some managing to achieve slightly higher values (around 12 MeV) for industrial irradiation. Energies under 1 MeV are mainly employed for surface treatments, needing higher energies (around 2 MeV or higher) to penetrate more than 1 cm. As the product passes beneath or in front of the electron beam, energy from the electrons is absorbed. This energy absorption alters various chemical and biological bonds within the material.

Regardless of which type of EB is used, they have specific advantages and disadvantages compared to gamma irradiators. The most significant advantages of using electron beam accelerators in radiation facilities are their operational safety, the apparatus can be switched on and off, and the absence of radioactive waste [14], with no need to change the radioactive source. The main disadvantage is the limited penetration of electrons, where large objects cannot be irradiated. An example of an EB accelerator is the IBA Rhodotron (Fig. 4).

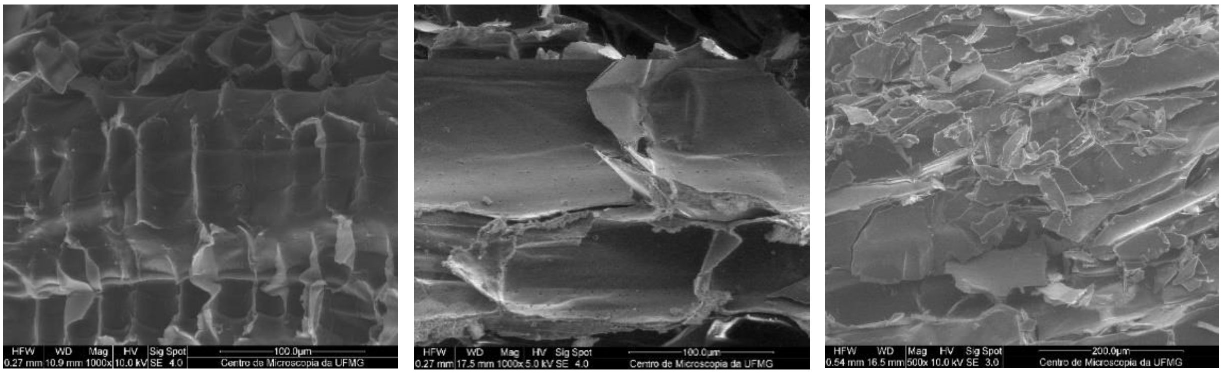
Figure 4: IBA Rhodotron

Source: Business Wire (2023).

4. GAMMA-RAY AND ELECTRON BEAM IRRADIATION FOR BIOMASS PRETREATMENT

Previous studies have shown that gamma radiation modifies the lignocellulosic complex at different dose levels. Han *et al.* [20] studied the physical and chemical properties of sugarcane bagasse exposed to gamma radiation between 10 kGy and 3000 kGy. They observed that the most significant effects of radiation were evident from 500 kGy onwards. Interestingly, radiation slightly increased the crystallinity of the material at 100 kGy, a phenomenon later corroborated by Kapoor *et al.* [21] and Varella *et al.* [22]. Despite minor alterations in holocellulose content at lower doses, lignin maintained its content even at higher doses (in the 1000 kGy and 2000 kGy range), which only shows that it is disassociated from the structure making it easier for hydrolytic enzymes to act, as can be seen in Fig. 5.

Figure 5: SEM images of (a) unirradiated bagasse, 1000x (b) 50 kGy, 1000x (c) 2000 kGy 500x



a) b) c)

Source: Varella *et al.* (2024).

Wang *et al.* [23] compared the use of gamma radiation with steam explosion technique on rice straw and other agricultural residues. Their study revealed significant differences between the two methods. In particular, irradiation pretreatment resulted in a more substantial reduction in cellulose content, in addition to obtaining a higher content of glucose and total reducing sugars. One particularly noteworthy finding was the absence of glucuronic acid formation when gamma rays were employed, in contrast to the significant formation of this compound observed with the steam explosion method.

Furthermore, Wang *et al.* [23] utilized both enzymatic and dilute acid hydrolysis to convert the polysaccharides into fermentable sugars, with gamma irradiation showing superior efficiency. For instance, the maximum concentration of glucose reached 43.3 mg/g and total reducing sugars 90.4 mg/g after enzymatic hydrolysis of gamma-irradiated rice straw, compared to 30.1 mg/g and 85.4 mg/g for steam explosion, respectively. Morphological analyses using SEM showed that gamma irradiation significantly disrupted the biomass structure, increasing the reactive surface area and facilitating hydrolysis.

Ribeiro *et al.* [24] examined the effect of electron beam irradiation on the pretreatment of sugarcane bagasse. Their study showed an increase in the liberation of free sugars and the conversion yield of cellulose to glucose, even in small doses. Bak *et al.* [25] also reported that EB irradiation improved the enzymatic hydrolysis of rice straw. Their research showed that the structural changes induced by the irradiation process facilitated greater enzyme accessibility, thereby increasing the efficiency of sugar release from the biomass.

Kapoor *et al.* [26] carried out a study comparing the use of electron beam and gamma radiation on sugarcane bagasse at the same doses. Although both were effective in pretreatment, gamma radiation showed better results in reducing hemicellulose at all doses. Specifically, the cellulose content experienced the most significant reduction up to 500 kGy with gamma rays, suggesting its efficacy at lower doses. However, at higher doses, such as 1000 kGy, electron beam irradiation outperformed gamma rays in reducing its content. Despite these differences in performance based on dosage levels, both pretreatment methods were successful in disrupting the lignocellulosic matrix of sugarcane bagasse. This disruption resulted in the creation of pores within the biomass structure, significantly enhancing the accessibility of hydrolytic enzymes. The increased porosity is crucial for subsequent enzymatic hydrolysis processes, as it allows enzymes to penetrate the biomass and break down complex carbohydrates into fermentable sugars.

5. CONCLUSIONS

This study highlights that both gamma radiation and electron beam are promising technologies for pretreating lignocellulosic biomass to produce bioethanol, each with its peculiarities and advantages. In general, irradiation stands out for its lack of toxicity, since reagents and additives such as acids are not required, in addition to non-formation of toxic compounds. Combining these technologies could further optimize the bioethanol production process, enhancing efficiency and reducing environmental impact. Continued research and development, along with policy support and investment in renewable energy, are crucial to fully leverage the benefits of irradiation for sustainable biofuel production. By advancing these technologies, we can significantly contribute to global efforts in reducing reliance on fossil fuels, decreasing greenhouse gas emissions, and promoting a more sustainable energy future.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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