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Review

# Application of Physical Pretreatment of Lignocellulosic Biomass to Produce Fermentable Sugars

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## Highlights

- Physical pretreatment is crucial to overcoming lignocellulosic biomass recalcitrance and produces minimal inhibitors.
- Ultrasonication and microwaves enhance enzymatic accessibility through physical structural changes.
- Mechanical comminution is a preliminary step effective in short durations and when combined with other methods.
- Extrusion combines thermal and mechanical forces, standing out for its efficient integration of pretreatment and structural modifications.
- Pulsed electric fields show significant potential but face scalability challenges.

**Abstract:** Physical pretreatments play a crucial role in reducing the recalcitrance of lignocellulosic biomass, facilitating its conversion into fermentable sugars for bioenergy and chemical applications. This study critically reviews physical pretreatment approaches, including mechanical comminution, irradiation (ultrasound, microwave, gamma rays, and electron beam), extrusion, and pulsed electric field. The discussion covers mechanisms of action, operational parameters, energy efficiency, scalability challenges, and associated costs. Methods such as ultrasound and microwave induce structural changes that enhance enzymatic accessibility, while extrusion combines thermal and mechanical forces to optimize hydrolysis. Mechanical comminution is most effective during short periods and when combined with other techniques to overcome limitations such as high energy consumption. Innovative approaches, such as pulsed electric fields, show significant potential but face challenges in large-scale implementation. This study provides technical and strategic insights for developing more effective physical pretreatments aligned with economic feasibility and industrial sustainability.

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## 1. Introduction

Fossil resources are the primary energy source in the world, resulting in significant industrial advances that have promoted a better quality of life for society (Zhou et al., 2021). Although using oil and its derivatives has brought several advantages, the exhaustion of fossil fuel reserves and the search for new sustainable approaches have been promoted to avoid their environmental impacts (Cheng, 2017; Singh et al., 2022; Danso et al., 2022).

Lignocellulosic biomass is a sustainable option, consisting of plant sources, agro-industrial residues, and organic material from its processing (Van Putten et al., 2013). It is mainly composed of cellulose, hemicellulose and lignin (Questell-Santiago et al, 2020). However, other components such

as chlorophyll, proteins, resins, pectin, ash, terpenoids, and other extractives can be part of its composition (Sharma et al., 2022). Moreover, the biological conversion of lignocellulosic biomass can be performed effectively to generate economically viable bio-based products by processing it in a biorefinery (Wang et al., 2020; Haldar and Purkait, 2021; Saini et al., 2022).

In biorefineries, saccharification is a crucial process that converts lignocellulosic material into fermentable sugars. These sugars can be used as feedstock for producing biofuels, biochemicals, biomaterials, and other products (Guo et al., 2018). This step consists of the enzymatic hydrolysis of the glucan and xylan portions, involving hydrolytic enzymes such as cellulases and hemicelluloses (Saini et al., 2022). In addition, an efficient pretreatment can enhance the conversion process by breaking down the lignin structure and disrupting the crystalline structure of cellulose, thereby facilitating enzyme access to cellulose during hydrolysis (Mosier et al., 2005).

In this context, pretreatment is a fundamental step in preparing lignocellulosic biomass for further processing into value-added chemicals (Mankar et al., 2021). It is necessary to break the recalcitrant structure, leaving the cellulose and hemicellulose more accessible to enzymes and chemicals (Yousuf et al., 2019). It also facilitates further biomass processing by effectively removing lignin, degrading hemicellulose, reducing cellulose crystallinity, and increasing surface porosity (Agbor et al., 2011). Pretreatment of lignocellulosic biomass is a fundamental step in the biorefinery and is important for obtaining a high product yield (Zheng et al., 2017). It is estimated that pretreatment can account for up to 40% of the total costs of the overall biorefinery process (Sindhu et al., 2015). Pretreatment processes can generally be divided into four categories: physical, chemical, biological, and combined techniques (Rezania et al., 2020). Each of these pretreatments has different advantages and disadvantages that make them more or less suitable for different types of biomass and conversion processes (Hasssan et al., 2018). Chemical pretreatments are more effective in dissolving, hydrolyzing, or oxidizing biomass components, making them more accessible for conversion, but can generate toxic by-products and impair the yield of subsequent processes (Himmel et al., 2007; Harmsen et al., 2010). Biological pretreatments are generally more specific to biomass types and conversion processes and can be performed under milder conditions (Sindhu et al., 2016). However, they may take longer to degrade the biomass (Taherzadeh and Karimi, 2008). Physical pretreatments, on the other hand, are generally more effective in reducing particle size and increasing particle surface area, as well as damaging the recalcitrant structure of the lignocellulosic biomass, do not produce inhibitor products, and are faster processes, but may require specific equipment and conditions that can increase the cost of the process (Mosier et al., 2005; Yousuf et al., 2019).

This work critically discusses the effects of physical pretreatments on lignocellulosic biomass, covering aspects such as the main pretreatment mechanisms, variation of lignocellulosic biomass, operational parameters, production of fermentable sugars, energy consumption, costs, and scalability. Lastly, the challenges and future perspectives are discussed, highlighting the factors affecting how to conduct the pretreatment.

## 2. Lignocellulosic Biomass

Lignocellulosic biomass has emerged as one of the most promising renewable resources for producing biofuels and biochemical (Ashokkumar et al., 2022). This type of biomass consists mainly of plant residues, agricultural by-products, and waste materials (Demirbas, 2009). Structurally, it comprises three primary components: cellulose, hemicellulose, and lignin, varying proportions depending on the biomass source (Mosier et al., 2005; Kumar et al., 2008). Typically, cellulose accounts for 35% to 50% of the biomass, hemicellulose for 20% to 35%, and lignin for 5% to 30% (Kassaye et al., 2016; Mankar et al., 2021; Yousuf et al., 2020). Together, these three components contribute to the naturally recalcitrant structure of lignocellulosic biomass, posing challenges for degradation (Lee et al., 2014; Singhvi and Gokhale, 2019). The integration of lignocellulosic biomass into the circular economy is further supported by its potential to reduce reliance on non-renewable

resources and minimize waste through sustainable conversion into bio-based products (Velvizhi et al., 2022).

Cellulose, a glucose polymer, is characterized by its highly crystalline structure, which resists enzymatic breakdown (Mankar et al., 2021). Its linear configuration, composed of  $\beta$ -1,4 glycosidic bonds, creates a rigid structure that hinders direct conversion into fermentable sugars, necessitating pretreatment to disrupt this crystalline form (Dora et al., 2012). Hemicellulose, in contrast, is a heterogeneous polymer composed of sugars such as xylose, mannose, and arabinose. Its amorphous and less polymerized structure makes removing it easier during pretreatment (Velvizhi et al., 2022; Zhang et al., 2020). Lignin, an aromatic and highly recalcitrant macromolecule, acts as a barrier that prevents enzymes from accessing the cellulose and hemicellulose fractions, making its depolymerization essential to enhance the efficiency of conversion processes (Rezania et al., 2020).

For pretreatment selection, it is essential to recognize the distinct characteristics of woody and non-woody biomasses, as these influence their resistance to degradation and the effectiveness of pretreatment methods. Woody biomass, which includes hardwoods and softwoods, generally has a higher lignin content, making it more resistant to degradation (Mankar et al., 2021). Examples of woody biomass include eucalyptus and pine, which are commonly used in industrial bioconversion processes. On the other hand, non-woody biomass consists of agricultural residues like wheat straw, corn cobs, sugarcane bagasse, corn husks, soybean hulls, and grasses (Yousuf et al., 2020).

This research builds upon classifications provided by Yousuf et al. (2019), which categorize all biomass types as either woody or non-woody, and Wang et al. (2021), which focuses on lignin content, thereby including biomass with varying lignin compositions. Furthermore, this study considered residues from Brazil's three major crops soybeans, corn and sugarcane based on IBGE data (2023). Understanding the composition and classification of lignocellulosic biomass is pivotal in selecting effective pretreatment strategies, ensuring optimized conversion efficiency and economic viability.

### 3. Physical Pretreatment

Physical pretreatment consists of using mechanical forces, pressure, or temperature to cause changes in the structure of the lignocellulosic biomass that reduce its recalcitrance (Jędrzejczyk et al., 2019; Yousuf et al., 2019). It is employed to reduce the particle size, specific surface area, degree of polymerization, and crystallinity index of the biomass (Shah et al., 2022). Additionally, physical pretreatment serves as an initial step in the process, offering the advantage of reducing the production of inhibitors for later reactions (Saini et al., 2016; Yousuf et al., 2019). Physical pretreatments can be divided into mechanical comminution, irradiation, extrusion, and pulsed electric field (Hassan et al., 2018; Yousuf et al., 2019).

#### 3.1. Mechanical Comminution Pretreatment

Mechanical comminution is a method of pretreating lignocellulosic biomass that involves the physical breakdown of the material into smaller particles (Aslanzadeh et al., 2014). This process is usually achieved through mechanical force, such as chipping, grinding, and milling, with the choice of milling method depending on the desired final particle size. Several types of milling equipment are available, including ball mills, hammer mills, knife mills, vibro mills, two-roll mills, colloid mills, wet-disk mills, and attrition mills (Taherzadeh and Karimi, 2008; Kumar et al., 2009; Yousuf et al., 2019). Mechanical comminution improves the biodegradability of lignocellulosic biomass by decreasing the degree of polymerization, reducing crystallinity, and increasing the accessible surface area through particle size reduction (Zheng et al., 2017). Nevertheless, mechanical comminution can produce heat that leads to the thermal degradation of biomass components. Thus, it is essential to carefully regulate the duration and intensity of mechanical treatment to mitigate any adverse effects on the biomass (Zheng et al., 2014).

In the biomass studied, mechanical comminution has proven essential in improving enzymatic accessibility and the efficiency of lignocellulosic biomass conversion processes. Mechanical

comminution is often considered a preliminary step before more complex pretreatments, such as enzymatic hydrolysis or chemical treatments. However, few studies analyze the isolated effect of this method. Those that do, such as Buaban et al. (2010), Sant'Ana da Silva et al. (2010), Zeng et al. (2007), and Zhu et al. (2010), demonstrate that particle size reduction alone significantly improves enzymatic accessibility and biomass conversion efficiency. Table 1 shows these studies and the main parameters and results obtained:

**Table 1.** Studies on the use of Mechanical Comminution to produce fermentable sugars.

Biomass	Operation Type	Specific Parameters	Saccharification Parameters	Main Results	Reference
Eucalyptus	Ball Milling	Initial size: 420 $\mu\text{m}$ and 150 $\mu\text{m}$ , 400 rpm, 15 mm balls, 10-120 min	1-40 FPU Acremonium cellulase, 0.02 mL Optimash BG, 5 IU Novozyme 188/g dry biomass; 50 mM acetate buffer (pH 5.0); 45 $^{\circ}\text{C}$ ; 72 h;	46.8% glucose, 34.3% xylose (20 min milling); 89.7% glucose, 72.5% xylose (120 min milling); 74% total sugar yield at 200 g/L biomass concentration	Inoue et al., 2008
Lodgepole pine	Disk Milling	Initial size 760 to 1520 $\mu\text{m}$ ;	15 FPU/g Acremonium cellulase, 0.02 mL Optimash BG, 5 IU Novozyme 188/g of dried biomass; 50 mM acetate buffer (pH 5.0); 45 $^{\circ}\text{C}$ ; 48 h	final particle size reduced to 760 to 1520 $\mu\text{m}$ ; 80% cellulose conversion to glucose after enzymatic hydrolysis	Zhu et al., 2010
Sugarcane bagasse and straw	Ball Milling	Initial size 2000 $\mu\text{m}$ , 400 rpm, 15 mm balls, 30-120 min;	15 FPU/g biomass, 5% substrate concentration, pH 5, 45 $^{\circ}\text{C}$ , 72 h	78.7% glucose yield for bagasse (60 min); 77.6% glucose yield for straw (90 min)	Sant'Ana da Silva et al., 2010
Sugarcane bagasse	Ball Milling	Initial size 1000 $\mu\text{m}$ , 250 rpm, 20 mm balls, up to 240 min;	5% (w/v) pretreated bagasse, 5 FPU/g substrate Acremonium cellulase, 20 U/g xylanase (Optimash BG), 50 mM acetate buffer (pH 5.0), 45 $^{\circ}\text{C}$ , 72 h	89.2% glucose, 77.2% xylose, a significant reduction in particle size (values not specified)	Buaban et al., 2010
Corn stover	Grinding (mill)	Initial size 425-710 $\mu\text{m}$ .	15 FPU/g glucan Spezyme CP, 65 IU/g glucan Novozyme 188; 0.5 M acetate buffer (pH 4.8), 50 $^{\circ}\text{C}$ , 180 rpm	20.8% glucose, final particle size reduced to 53-75 $\mu\text{m}$	Zeng et al., 2007

Particle size before and after pretreatment is an essential factor in mechanical comminution. Buaban et al. (2010) employed ball milling as a pretreatment method for sugarcane bagasse, with an initial particle size of 1000  $\mu\text{m}$ . Similarly, Inoue et al. (2008) used a ball mill to process eucalyptus wood, starting with particles of 35 mesh (420  $\mu\text{m}$ ) and 100 mesh (150  $\mu\text{m}$ ). Sant'Ana da Silva et al. (2010) utilized ball milling for sugarcane bagasse and straw, starting with an initial particle size of 2000  $\mu\text{m}$ . All these studies reported a considerable reduction in particle size; however, they did not provide quantitative values for the final particle size after pretreatment. In contrast, Zeng et al. (2007) provided more specific data, showing that corn stover was reduced from an initial range of 425-710  $\mu\text{m}$  to 53-75  $\mu\text{m}$  after ball milling. Zhu et al. (2010) also provided values for disk milling of lodgepole pine wood chips, showing that particle size ranged from 0.76 mm to 1.52 mm after the disk milling process.

The milling speed also plays a crucial role in the efficiency of mechanical pretreatment. Several studies report the use ball milling operated at speeds of 400 rpm. Inoue et al. (2008) observed that, by milling eucalyptus wood at 400 rpm for 120 minutes, the glucose saccharification yield reached 89.7%. Sant'Ana da Silva et al. (2010) also used the same speed when treating sugarcane bagasse and straw, achieving higher glucose yields of 78.7% and 77.6% and xylose yields of 72.1% and 56.8%, respectively, than disk milling. On the other hand, Buaban et al. (2010) used a lower speed of 250 rpm when milling sugarcane bagasse, which may have influenced the longer time required to achieve significant particle size reduction.

Using disk milling, Zhu et al. (2010) did not mention the rotation speed, as this type of equipment operates differently than ball milling. However, even without the exact speed specification, the particle size range after treatment was well documented, varying from 0.76 mm to 1.52 mm. Based on studies reported in the literature, mechanical comminution effectively reduces particle size, improving surface area and accessibility for enzymatic hydrolysis. However, the best results were obtained during long processing periods (over 60 minutes) to achieve significant modifications. These prolonged durations are particularly necessary due to the recalcitrance of the biomass. The lignin content also plays a role, as lignin imparts rigidity to the particles, requiring either greater applied force or longer milling periods to overcome this characteristic, which results in higher energy consumption and, consequently, increased costs—an unfavorable factor for industrial applications.

Furthermore, although mechanical comminution improves saccharification yields by facilitating enzyme access, depending on the biomass composition and processing time, it is insufficient as a standalone pretreatment. This limitation arises from its inability to significantly disrupt the complex lignocellulosic matrix, which includes not only physical barriers nevertheless also chemical bonds that inhibit enzymatic activity. Consequently, this step is often considered preliminary, and it is common to combine it with other methods to maximize sugar recovery. This highlights the importance of considering the specific characteristics of the biomass, such as lignin content and particle rigidity, when optimizing pretreatment strategies.

### 3.2. Irradiation Pretreatment

The irradiation pretreatment methods for lignocellulosic biomass encompass ultrasound, microwave, gamma rays, and electron beams (Yousuf et al., 2019). The effect of irradiation on biomass and the process mechanisms vary depending on the method applied (Al-Assaf et al., 2016). Usually, the process involves the application of ultrasonic waves, microwaves, or radiolysis that uses electron beams generated by an electron accelerator or gamma rays from radioisotopes such as cobalt-60 to cause degradation of the cellulosic structure (Hassan et al., 2018; Ashfaq et al., 2020).

In these processes, high-energy radiation is produced, changing the properties of lignocellulosic biomass by decreasing the crystallinity and degree of polymerization, increasing specific surface area, and causing hydrolysis of hemicellulose and depolymerization of part of lignin (Kassim et al., 2016).

#### 3.2.1. Ultrasound

The ultrasound pretreatment process uses supersonic waves, which have a frequency greater than 20 kHz, and this frequency range is higher than human hearing (20 Hz - 20 kHz) (Mason and Lorimer, 2002). These ultrasonic waves generate pressure variations within a solution to amplify the progression of mechanoacoustic and sonochemical procedures (Bussemaker and Zhang, 2013).

The mechanoacoustic effect is based on the principle of cavitation, which is induced by ultrasonic radiation (Yusup and Rashidi, 2021). In this process, ultrasonic waves pass through low-pressure regions, inducing the formation of small gas or vapor bubbles that progressively increase in size until they reach a critical threshold, after which they collapse, triggering the onset of cavitation (Le et al., 2015). In the cavitation phenomenon, an implosion of the bubbles occurs, releasing a large amount of energy and causing high temperatures of 2,000 to 5,000 K and up to 1,800 atm of pressure in the surrounding area, generating shear forces that effectively break up the complex structure of the lignocellulosic biomass, thus increasing the extraction efficiency of the target compounds, including cellulose, hemicellulose, and lignin (Kunaver et al., 2012; Ravindran and Jaiswal, 2016).

On the other hand, the sonochemical effect is based on the application of ultrasound to enhance chemical reactions and direct specific reaction pathways (Manickam, 2023). Typically used in radical-driven processes, the enhancement of the reaction by ultrasound generally results in faster reactions and at lower temperatures than would otherwise be possible, as well as reducing the number of chemicals required in the process (Mason, 2003; Bussemaker and Zhang, 2013). The sonochemical effect in aqueous solutions clears the oxygen-hydrogen (O-H) bond within the molecule, generating hydroxyl and hydrogen radicals (Mason, 2003). These radicals subsequently undergo further reactions, yielding hydrogen peroxide, hydrogen gas, oxygen gas, or recombining to form water. Additional radical reactions occur in the gaseous or aqueous phase, producing additional radicals or oxidizing species (Mason, 2003).

The literature presents several studies investigating using ultrasound as a pretreatment for fermentable sugar production. These studies are detailed in Table 2, including the respective operating conditions.

**Table 2.** Studies on Ultrasound Pretreatment for Biomass.

Biomass	Pretreatment Parameters	Main Results	Reference
Eucalyptus urophylla × Eucalyptus grandis	Power: 10-500W, Frequency: 20-25 kHz, Time: 1,3e6 h	Drying rate increased by 5% (1h), 13% (3h), and 11% (6h) above 24% moisture; 25% (1h), 28% (3h), and 23% (6h) below 24%. Microstructural changes improved permeability and reduced wood collapse.	Liu et. al. (2019)
Eucalyptus grandis × Eucalyptus urophylla	Frequency: 28 kHz, Time: 0,5;1,0e1,5 h	Caustic soda solution pretreatment increased reaction rates and reduced thermal decomposition temperatures (309–400 °C) compared to distilled water (321– 424 °C) and acetic acid solution (328–428 °C). Gas products (CO, H <sub>2</sub> O, CO <sub>2</sub> , CH <sub>4</sub> , CH <sub>3</sub> COOH) were released more at 361 °C than at 308 °C. Ultrasonic cavitation in alkaline medium intensified hydroxyl and hydroperoxide radical formation, enhancing degradation reactions.	He et. al. (2018)

Eucalyptus grandis × Eucalyptus urophylla	Frequency: 28 kHz, Time: 0,5;1,0e1,5 h, Temperature: 50 °C	Pretreatment increased cellulose crystallinity from 31.8% (control) to 35.5% in caustic soda and acetic acid solutions. Decomposition temperature reduced to 341 °C (caustic soda) compared to 361 °C (control). Removed hemicellulose and lignin, creating structural changes that enhanced solvent exposure. Collapses and microchannels in wood tissues improved reagent accessibility.	He Z. et. al. (2017)
Pinus sylvestris	Frequency: 16,8-9,2 kHz, Time: 25 min	Glucose yield in enzymatic hydrolysis increased 2-fold (35.5 g/L, 61% of theoretical) with ultrasound compared to milled substrate. Maximum efficiency achieved with two-step milling and ultrasound for 5–10 minutes. Subsequent fermentation resulted in ethanol concentration up to 3.11% (v/v), higher than controls without ultrasound (2.18%).	Revin et al. (2016)
Sugarcane Bagasse	Power: 40W, Frequency: 20 kHz, Time: 20 min	Combined pretreatment resulted in a reducing sugar yield of 276.8 mg/g biomass, 1.12 times higher than DES alone (235.3 mg/g) and 1.58 times higher than ultrasound alone (174.5 mg/g). Crystallinity index decreased from 64.87% (raw biomass) to 52.78% after combined treatment, indicating increased polysaccharide accessibility.	Sharma et al. (2021)
Sugarcane Straw	Frequency: 40 kHz, Time: 30 min	Cellulose conversion in enzymatic hydrolysis: Hydrothermal+NaOH: 86.74%, Acid+NaOH: 84.29%. Acid pretreatment showed the highest hemicellulose and lignin removal, increasing cellulose accessibility. Ultrasound was the least efficient, with lower lignin and hemicellulose removal.	Candido et al. (2019)
Sugarcane Straw	Power: 180W e 800W, Frequency: 19 kHz, Time: 30 min, Temperature: 75 °C	Biohythane production: Methane (CH <sub>4</sub> ) reached a maximum concentration of 80% (v/v) after 40 days with PBU (ultrasonic bath) pretreatment. Hydrogen (H <sub>2</sub> ) concentration decreased with PSU (ultrasonic probe). Ultrasound altered sugarcane straw morphology, improving organic matter solubilization and biohythane production.	Martinez-Jimenez et al. (2017)

Sugarcane Bagasse	Frequency: 35 kHz, Time: 1015 min	Glucan conversion reached 50% with 26.35 g glucose/100 g biomass. Crystallinity increased from 53% (raw bagasse) to 65% (3% NaOH + ultrasound). Approximately 50% of total lignin was removed. Ethanol production: 5.6 g/L, equivalent to 0.38 g/g glucose consumed.	Eblaghi et al. (2015)
Sugarcane Bagasse	Frequency: 40 kHz, Time: 35 min, Temperature: 30 °C	Maximum weight percent gain (WPG) was 30.6% with 10 minutes of ultrasound and maleic anhydride concentration of 1:1.1 (w/w). Ultrasound enhanced solvent and reagent penetration, increasing the maleation reaction. FT-IR and NMR confirmed maleation at C-2 and C-3 positions of cellulose and hemicellulose. No significant impact on thermal stability; both maleated and untreated bagasse remained stable above 200 °C.	Liu et. al. (2007)
Sugarcane Bagasse	Frequency: 24 kHz, Time: 45,60 min, Temperature: 150410 °C	Maximum glucose and pentose yields were 69.06% and 81.35% of theoretical, respectively. Maximum glucose concentration: 17.1 g/L; pentoses (xylose and arabinose): 10.9 g/L. Inhibitor formation (acetic acid and furfural) was low, with maximum values of 0.95 g/L and 0.1 g/L, respectively. Maximum ethanol yield was 91.8% of theoretical (8.11 g/L).	Velmurugan, R., & Muthukumar, K (2011)
Sugarcane Bagasse	Frequency: 24 kHz, Temperature: 22 °C	Maximum glucose yield: 91.28% of theoretical (38.4 g/L). Ethanol yield: 91.22% of theoretical (17.9 g/L in 36 h). Significant reduction of lignin (90.6%) and hemicellulose (80.8%) with ultrasound. Low inhibitor formation (acetic acid: 0.8 g/L; furfural: not detected). Higher energy efficiency compared to other methods, with lower energy consumption ( $7.2 \times 10^4$ J/g).	Velmurugan, R., & Muthukumar, K (2012)
Sugarcane Bagasse	Frequency: 24 kHz, Time: 15,3045 min, Temperature: 40,6080 °C	Cellulose recovery: 95.78%. Delignification: 58.14%. Glucose production (acid hydrolysis): 16.58 g/L. Xylose: 8.21 g/L; arabinose: 2.78 g/L. Inhibitor formation (furfural and acetic acid): 0.59 g/L and 1.00 g/L, respectively. Significant reduction in cellulose	Ramadoss, G., & Muthukumar, K. (2014)

		crystallinity (crystallinity index decreased from 68.6% to 48.6%).	
Sugarcane Bagasse	Frequency: 45 kHz, Temperature: 90 °C	Cellulose-to-reducing sugar conversion: 95.9% in 12 hours. Ultrasound-NMMO treated bagasse conversion: 90.4% after 24 hours. Reduced cellulose crystallinity (TCI index decreased from 1.393 to 0.878). Fermentable sugar production (glucose): 5.2 g/L in hydrolysate.	Li et al. (2012)
Sugarcane Bagasse	Frequency: 20 kHz, Time: 180 s	Maximum sugar yield: 26.01 g/L (94.49% of theoretical). Most influential factors (in order): enzyme use, particle size, acid concentration, ultrasound duration, and power. Smallest particle size (<0.18 mm) achieved the highest sugar yield (8.68 mg/mL). Optimal acid concentration: 3%; higher concentrations led to inhibitor formation (furfural and acetic acid). Optimal ultrasound power: 120 W; duration: 180 s.	Esfahani e Azin (2012)
Sugarcane Bagasse	Frequency: 24 kHz, Temperature: 70 °C	Lignin removal: 82.32%, Max reducing sugar yield: 96.27% of theoretical, Cellulose recovery: 98.32%, Inhibitor formation (acetic acid): 0.36 g/L.	Velmurugan, R., & Muthukumar, K (2012b)
Sugarcane Bagasse	Frequency: 20 kHz, Time: 40 min, Temperature: 55 °C	Ultrasonic pretreatment increased lignin and hemicellulose yields by 2.2% and 1.3%, respectively, compared to non-ultrasonic methods. Extracted lignin showed high purity and structural similarity to non-ultrasonic lignins, but with improved solubilization.	Sun et al. (2004)
Corn Cob	Frequency: 20 kHz	Methane production increased by 59.8% in VTS and 14.6% in corn cob after isolated enzymatic hydrolysis. Ultrasound pretreatment negatively impacted methane production, especially in VTS. The combination (US + H) led to a 41.8% methane increase in VTS and 17.9% in corn cob compared to raw substrate.	Pérez-Rodríguez et al. (2016)
Corn Cob and Corn Stalk	Frequency: 20 kHz, Time: 2a8 h, Temperature: 80 °C	Reducing sugar yield: Corn cob (scCO <sub>2</sub> + ultrasound): 87.0%, a 75% increase compared to control; Corn stalks (scCO <sub>2</sub> + ultrasound): 30.0%, a 13.4% increase compared to control. Ultrasound and scCO <sub>2</sub>	Yin et. al. (2014)

		combination caused significant structural changes in biomass, increasing surface area and lignin disruption. Ultrasound duration had a significant impact on corn cob but not on stalks due to their more resistant structure.	
Soybean Straw	Time: 120-250 min, Temperature: 40-80 °C	Maximum reducing sugar yield: 53.27 mg from 0.2 g soybean straw, 50.03 mg from 0.2 g corn straw. Ultrasound significantly enhanced interaction between biomass and ionic liquids, increasing cellulose dissolution.	Hu et al. (2013)
Bermuda Grass	Frequency: 40 kHz, Time: 20-100 min, Temperature: 40-80 °C	Maximum reducing sugar yield: 36.89% under optimized conditions. Optimal conditions: 2% acid concentration, 80 °C, solid-liquid ratio 12:1, ultrasound power 80 W, and 100 minutes. Ultrasound reduced biomass crystallinity, increasing cellulose exposure.	Liu et al. (2015)

In the context of the investigated biomasses, ultrasound pretreatment is always conducted in an aqueous medium using different solvents. Using water as a solvent, Liu et al. (2018) observed the effects of ultrasound pretreatment on the microstructure of *Eucalyptus urophylla* × *E. grandis*. Scanning electron microscopy (SEM) revealed a significant impact on the biomass structure, resulting in collapses and the formation of microchannels that enhanced overall sample accessibility. This increased permeability, along with the approximately 4% increase in crystallinity observed through X-ray diffraction (XRD), was attributed to the mechanical effects of ultrasound, which disrupt the lignin-carbohydrate matrix and allow for more significant release of crystalline cellulose. Similarly, He et al. (2017) observed that ultrasound pretreatment caused visible structural damage and disrupted hydrogen bonds in cellulose, as confirmed through Fourier-transform infrared spectroscopy (FTIR). The FTIR analysis further indicated the breakdown of cellulose hydrogen bonds, the methyl/methylene groups of cellulose, and the removal of lignin from the biomass.

However, when comparing these results with those of Revin, Atykyan, and Zakharkin (2016), who applied ultrasound pretreatment to *Pinus sylvestris*, a biomass with a higher lignin content (~27%), distinct differences emerge in the efficiency of lignin removal. While Liu et al. (2018) achieved substantial increases in crystallinity and permeability in *Eucalyptus urophylla* × *E. grandis*, Revin et al. (2016) reported a 61% increase in enzymatic hydrolysis yield after ultrasound pretreatment of *Pinus sylvestris*. However, they required alkaline agents (NaOH, pyridine, ammonia) to enhance lignin removal. This suggests that the effectiveness of ultrasound pretreatment in removing lignin is significantly influenced by the biomass's lignin content, with high-lignin biomass requiring more aggressive chemical or combined pretreatment methods to achieve comparable results.

The choice of chemical agents can be diverse, including NaOH, which is most commonly used, and acids like HCl and acetic acid (CH<sub>3</sub>COOH). Additionally, advanced techniques, such as supercritical CO<sub>2</sub>, eutectic solvents (e.g., ChCl/glycerol), and ionic liquids ([HMIM]Cl), have been employed in combination with ultrasound to leverage synergistic effects, further enhancing pretreatment efficiency. These combinations illustrate how integrating ultrasound with various solvents and chemical agents can significantly improve delignification and cellulose exposure.

The variation in lignin content also influences operational parameters like power, temperature, and time. For example, Liu and Wang (2015) used a frequency of 40 kHz combined with diluted acid

for Bermuda grass pretreatment, achieving significant improvements in hydrolysis efficiency. In contrast, Candido, Mori, and Gonçalves (2019) employed ultrasound at 20 kHz combined with NaOH for sugarcane bagasse and observed more effective lignin removal at a microscopic scale, which they attributed to sonochemical effects. Studies using higher power levels generally deal with biomass with a higher lignin composition, such as *Pinus sylvestris*, which, as previously noted, contains approximately 27% lignin. This aligns with observations by Bussemaker and Zhang (2013), who noted that lower ultrasound frequencies (around 20 kHz) result in more intense cavitation and collapse events, ideal for breaking down the recalcitrant structure of lignocellulosic biomass. On the other hand, higher frequencies (40-45 kHz) tend to produce more bubbles, enhancing chemical interactions, which is beneficial when ultrasound is combined with chemical agents, as seen in the study by Liu et al. (2007), where a frequency of 45 kHz improved chemical reactions during sugarcane bagasse pretreatment. This effect was further confirmed by Liu and Wang (2015) with Bermuda grass using 40 kHz.

In addition to frequency and power, the operation time for ultrasound pretreatment also plays a crucial role, varying from 60 seconds to 6 hours across different studies. However, most investigations report durations between 15 and 60 minutes. The wide variation in operating times highlights the complexity of optimizing ultrasound pretreatment for different biomasses. Lower frequencies are more suitable for high-lignin biomasses. However, higher frequencies, combined with diluted acids or other chemical agents, offer distinct advantages for biomass with lower lignin content. The comparison between studies demonstrates that the efficiency of lignin removal and the increase in cellulose crystallinity depends on ultrasound parameters and the specific composition of the biomass. Biomass with higher lignin content, such as *Pinus sylvestris*, requires combinations of ultrasound with chemical agents, such as NaOH, to achieve the same results that pure ultrasound can accomplish in less lignified biomass, like *Eucalyptus urophylla* × *E. grandis* or Bermuda grass. Furthermore, ultrasound frequency has a critical role: lower frequencies (~20 kHz) generate more intense cavitation, facilitating lignin removal when combined with alkaline agents, while higher frequencies (40-45 kHz), when combined with diluted acids, enhance chemical reactions that increase cellulose accessibility. Adjusting these parameters directly impacts saccharification efficiency and the release of fermentable sugars, reinforcing the need for tailored approaches based on the specific characteristics of each biomass.

### 3.2.2. Microwave

The microwave pretreatment process uses non-ionizing electromagnetic radiation with frequencies between 300 MHz and 300 GHz, which are higher than radio waves but lower than infrared radiation (Leonelli and Mason, 2010; Pelleria and Gidaracos, 2017). Typically, the frequency of 2.45 GHz is used in industrial applications due to its efficiency in dielectric heating (Leonelli and Mason, 2010). This technique is based on the principle of dielectric heating induced by the application of microwaves (Angoy et al., 2019). Unlike conventional heating methods (conduction/convection), which rely on surface heat transfer (Caballero et al., 2003), microwave heating interacts directly with the biomass, resulting in rapid and volumetric heating (Hu and Wan, 2008; Keshwani, 2009).

In addition to its physical characteristics, the interaction of microwaves with lignocellulosic biomass involves the differential absorption of heat by the polar components of the biomass. Microwaves cause polar molecules, such as water, to oscillate as they attempt to align with the alternating electromagnetic field. This movement generates friction between the molecules, resulting in heat (Hu and Wan, 2008). Microwaves penetrate the biomass, heating its most polar components and creating localized hotspots in the heterogeneous material (Aguilar-Reynosa et al., 2017). This characteristic can trigger an "explosion" effect in particles, facilitating the breakdown of recalcitrant structures. Additionally, the electromagnetic field generated by microwaves can produce non-thermal effects, accelerating the reduction of the crystalline structure of cellulose (Hu et al., 2008; Kostas et al., 2017). Table 3 shows these studies and the main parameters and results obtained:

**Table 3.** Studies on the use of Microwave to produce fermentable sugars.

Biomass	Specific Parameters	Main Results	Reference
Eucalyptus sawdust	400 W, 15 min, 180 °C, 2.45 GHz	3.5x higher sugar release with microwaves compared to LHW; 100% sugar yield after subsequent enzymatic hydrolysis	Amini et al. (2018)
Corn stalks, Miscanthus	710 W, 300 W, 800 s (710 W), 180 s (300 W), Pressure (4 bars), 2.45 GHz	Microwave pretreatment did not significantly increase methane potential.	Bichot et al. (2021)
Sugarcane bagasse	600 W, 4 min	0.665 g/g reducing sugar with NaOH, 0.83 g/g with NaOH and H <sub>2</sub> SO <sub>4</sub>	Binod et al. (2012)
Napier grass	76 min, 147 °C	Maximum fermentable sugar yield of 14.38 g/L after 76 minutes of treatment.	Boonsombuti et al. (2017)
Sugarcane straw	1000 W, 2 min, 162 °C	72.2% total sugar yield; low concentrations of inhibitors	Fonseca et al. (2021)
Sugarcane trash, Oil Palm EFB	1200 W, 5 min, 180 °C	Yield of 0.33 g/g reducing sugar for sugarcane trash and 0.19 g/g for OPEFB	Hermiati et al. (2024)
Switchgrass, Miscanthus	800 W, 10 min, 60 - 210 °C	Increased subcritical water solubility by 7-10%	Irmak et al. (2018)
Switchgrass, Big bluestem	450 W, 2.5 min	Increased total sugar recovery by 59.2% for switchgrass and 68.1% for big bluestem	Karunanithy et al. (2014)
Pine chips, Beech chips, Hemp stems	300 W, 10-20 min, 140 - 180 °C	Sulfuric acid released more glucose; nitric acid promoted more xylose and galactose release	Kłosowski et al. (2020)
Sugarcane bagasse	820 W, 3 min, NaOH 1%	Cellulose-enriched fractions showed the best yields for ethanol	Miranda et al. (2014)
Sugarcane leaf waste	400 W, 5 min	73% lignin removal, 62% hemicellulose removal; maximum ethanol yield of 31.06 g/L	Moodley et al. (2019)
Corn straw	640 W, 5 min	49.25% sugar yield after microwave pretreatment	Muxin et al. (2013)
Pinus radiata	50-150°C, 50 min	Best result: glucan digestibility of 79% after autohydrolysis at 150°C and microwave at 120°C	Rigual et al. (2019)
Eucalyptus globulus, Pinus radiata	80°C - 120°C, 50 min	Glucan digestibility increased to 68 g/100 g	Rigual et al. (2018)

		for eucalyptus and 78 g/100 g for pine	
Sugarcane bagasse	320 W, 3-10 min, 170 ± 5 °C	Glucose production was 64% after 7 minutes of treatment with H <sub>2</sub> SO <sub>4</sub>	Zhu et al. (2016)
Sugarcane bagasse	10-20 min, 180-200°C	Sugar yield of 44.9 g per 100 g bagasse, ethanol of 35.8% in holocellulose fraction	Sasaki et al. (2011)
Corn straw	Up to 1600 W, 10 min, 120-210 °C	73.08% increase in methane production compared to the control	Wang et al. (2021)
Elephant grass	200-600 W, 10-30 min	Lignin reduced to 3.24%, cellulose increased to 38.38%	Ude et al. (2022)

Understanding the interaction of microwaves with biomass is crucial for defining operational parameters such as power, exposure time, and temperature. Microwave pretreatment generally operates at power levels ranging from 300 W to 800 W, with exposure times between 2 and 20 minutes, depending on the type of biomass and the desired outcomes (Moodley et al., 2019; Boonsombuti et al., 2017). Higher power levels are generally associated with greater lignin removal and increased cellulose accessibility, as observed by Bichot et al. (2021), who used 710 W on *Miscanthus* and corn stalks. However, higher power levels can also increase the risk of inhibitor formation, such as furfural and HMF, if not properly controlled (Binod et al., 2012).

Like exposure time, temperature plays a crucial role in pretreatment. For example, Binod et al. (2012) demonstrated that 4 minutes of exposure at 600 W on sugarcane bagasse were sufficient to significantly increase the yield of fermentable sugars. In contrast, prolonged exposure times (e.g., 20 minutes) were associated with excessive hemicellulose degradation, leading to the formation of inhibitors and reduced sugar yields (Rigual et al., 2019). Optimizing exposure time is essential to increase efficiency without compromising the quality of the resulting material.

Temperature is also a key parameter, with temperatures between 150°C and 180°C often reported as optimal for pretreatment. Lower temperatures tend to result in less effective delignification, while temperatures around 180°C improve lignin removal (Fonseca et al., 2021). However, temperatures above 180°C, especially under pressurized conditions, can increase pretreatment efficiency but also raise the risk of thermal degradation and inhibitor formation (Rigual et al., 2019).

Operational conditions, such as the use of water or mild chemical solutions, are important to ensure pretreatment efficiency, especially for specific types of biomass. In many cases, water or a combination of water and mild chemicals, such as diluted acids or alkalis, is used to improve lignocellulose breakdown (Binod et al., 2012). In the case of sugarcane bagasse, Binod et al. (2012) showed that microwave pretreatment significantly increased reducing sugar yields after enzymatic hydrolysis. Similarly, Bichot et al. (2021) observed that applying microwaves to corn stalks and *Miscanthus* improved lignin removal and cellulose exposure. These studies highlight the potential of microwave pretreatment to enhance saccharification and fermentation processes.

However, the effectiveness of microwave pretreatment may vary depending on the biomass composition. For biomass with higher lignin content, such as *Pinus radiata* and *Eucalyptus globulus*, it was necessary to combine microwaves with ionic liquid solvents or mild alkaline solutions to achieve optimal results (Rigual et al., 2018). This suggests that more recalcitrant biomass types may require more aggressive pretreatment conditions (Sasaki et al., 2011).

### 3.2.3. Gamma Rays

Gamma-ray pretreatment involves the use of high-energy electromagnetic radiation, which has a shorter wavelength than ultraviolet light and is highly penetrating (Hassan et al., 2018; Fan, 2020). This process is based on the principle of radiolysis, where gamma rays break down lignocellulosic biomass into smaller components, facilitating the extraction of target compounds (Liu et al., 2015; Kassim et al., 2016). During the process, gamma rays penetrate the biomass, causing ionization and excitation of atoms, which results in the formation of highly reactive free radicals that react with biomass components, leading to their degradation by breaking down the lignin-hemicellulose-cellulose matrix, making it easier to extract target compounds such as sugars and lignin. (Kassim et al, 2016; Kumar and Kumar, 2023). The energy released during the radiolysis process causes localized high temperatures of up to 300°C and pressure changes, which effectively disrupt the complex structure of lignocellulosic biomass (Barbara, 1998; Ashfaq et al., 2020).

Several studies in the literature report findings regarding different biomasses under gamma-ray treatment. Al Gharib et al. (2023) reported that applying 1-2 MGy to woody biomass, such as pine wood, apple wood, and poplar, was sufficient to achieve complete cellulose conversion to glucose after enzymatic hydrolysis. This study highlights the importance of high doses for dense biomass, where a more aggressive treatment is needed to break down the lignocellulosic structure fully. The deep penetration of gamma radiation further facilitated the uniform degradation of the biomass. These findings emphasize the effectiveness of high-energy gamma radiation in enhancing biomass conversion.

Kapoor et al. (2022) studied sugarcane bagasse pretreatment and confirmed gamma radiation's efficacy but with different results due to the biomass type and process parameters used. Applying doses of 50 to 1000 kGy, they observed that gamma radiation was more effective than electron beams, predominantly at doses above 500 kGy, in breaking down cellulose and hemicellulose. However, lignin remained largely intact, highlighting the need to combine radiation with other methods for more effective lignin treatment in biomass like bagasse. This indicates that while gamma radiation is effective, complementary methods may be required for complete biomass conversion.

Wu et al. (2020) combined gamma radiation with ultrasound and reported that doses of 500 kGy, along with ultrasound intensification, resulted in a higher glucose yield in the pretreatment of sugarcane bagasse. The synergy between the methods seemed to compensate for individual limitations, such as the difficulty of gamma radiation alone in fully degrading lignin. This combination could represent a way to optimize fermentable sugar production without significantly increasing radiation doses. Thus, integrating ultrasound with gamma radiation may enhance efficiency in biomass treatment.

Li et al. (2016) provided additional data by applying 100 to 500 kGy to sugarcane bagasse. They showed that moderate doses were sufficient to alter the lignocellulosic structure and increase enzymatic accessibility. However, an essential aspect of this study was the detection of inhibitors, such as formic acid, acetic acid, and furfural, which increased proportionally with the radiation dose. While these inhibitors were detected, they did not significantly impact the production of fermentable sugars. The increased inhibitor levels, particularly at higher doses, suggest that controlling the radiation dose is essential to mitigate the impact of these compounds on subsequent processes like fermentation. These results underscore the importance of balancing radiation dosage to maximize sugar yield while minimizing inhibitor production.

#### 3.2.4. Electron Beam

Electron beam pretreatment is a process that uses a high-energy ionizing electron beam that has low penetration but high dose rates, with energies ranging from 3 MeV to 12 MeV (Bak et al., 2009; Singh et al., 2016). The electron beam accelerators work by absorbing the energy of the electrons as the material passes under or in front of the electron beam (Jusri et al., 2018). This process alters the chemical and biological bonds of the material, causing physical and chemical changes (Singh et al., 2016).

The electron beam treatment process leads to the ionization of atoms and molecules within the biomass, generating a range of reactive species such as free radicals, cations, and anions (Siwek and Edgecock, 2020). These species interact with the biomass components, inducing changes in their structure and properties (Yousuf et al., 2019; Ashfaq et al., 2020). The electron beam treatment process also generates heat, which leads to further chemical reactions within the biomass, such as polymerization or degradation reactions (Ashfaq et al., 2020).

The energy and dose of the electron beam play a crucial role in the success of the pretreatment. Al Gharib et al. (2023) used doses between 1 and 3 MGy, one of the highest dosages compared to other studies and achieved complete cellulose conversion to glucose. This contrasts with studies that used lower doses, such as Duarte et al. (2012), who applied 50 kGy combined with hydrothermal treatment, resulting in a glucose yield of 74.72% after 48 hours. Comparing these two studies shows that while high doses can promote more complete conversion, combining electron beam with other methods, such as thermal treatment, can produce significant results with lower radiation doses.

The radiation dose also affects the biomass structure. In Guo et al. (2016), doses of 90 to 270 kGy resulted in the synergistic removal of hemicellulose and lignin when combined with inorganic salts, such as manganese chloride ( $\text{MnCl}_2$ ), ferric chloride ( $\text{FeCl}_3$ ), and sodium bicarbonate ( $\text{NaHCO}_3$ ), facilitating saccharification. This suggests that intermediate doses may be sufficient for deleting the lignocellulosic matrix when combined with other chemical agents.

The studies indicate significant variations in results depending on the biomass type. Al Gharib et al. (2023) reported that high doses (1 to 2 MGy) were necessary for dense wood, such as pine and poplar, while non-woody biomass, such as sugarcane bagasse treated by Rattanawongwiboon et al. (2022), required much lower doses (50 to 200 kGy) to generate the necessary radicals. This highlights how the density and composition of the biomass directly influence the choice of treatment parameters.

Karthika et al. (2012) treated hybrid grass with doses of up to 250 kGy showed a significant reduction in cellulose crystallinity, a critical factor in increasing the efficiency of enzymatic hydrolysis. This study compared different doses and showed that while lower doses (75 kGy) already improved hydrolysis, the 250 kGy dose resulted in a yield of 79% reducing sugars in 48 hours.

Additionally, Shen et al. (2022) demonstrated that relatively low doses (2, 6, and 12 kGy) were sufficient to modify the structure of corn starch, increasing its susceptibility to enzymatic hydrolysis. This demonstrates that biomass with lower structural complexity, such as starch, can be effectively treated with lower radiation doses, saving energy without compromising efficiency. The formation of inhibitors during electron beam pretreatment is a concern that can impact the efficiency of subsequent stages, such as fermentation. Guo et al. (2016) observed the generation of organic acids as inhibitors when treating corn cob with doses of 90 to 270 kGy, especially when combined with inorganic salts and  $\text{H}_2\text{O}_2$ . While these inhibitors could affect fermentation, the efficient removal of hemicellulose and lignin compensated for this disadvantage, resulting in high enzymatic conversion. Similarly, Duarte et al. (2012) reported that combining doses up to 100 kGy with acid hydrolysis in the treatment of sugarcane bagasse avoided the significant formation of inhibitors. This suggests that controlling the radiation dose and using additional agents or treatments can minimize the formation of unwanted compounds. Rattanawongwiboon et al. (2022), by using sulfonation on biochar produced from bagasse irradiated with doses of 50 to 200 kGy, indicated that the process reduced the presence of toxic by-products, resulting in efficient sugar production without significant impacts from inhibitors. These studies highlight that while inhibitor formation is a concern, it can be mitigated by adequately selecting dose parameters and treatment combinations.

### 3.3. Extrusion Pretreatment

Extrusion is a physical pretreatment that involves the application of mechanical force and thermal energy, typically forcing the material through a die at high pressure and temperature, resulting in a modification of its structure and composition (Duque et al., 2017). The pretreatment can be performed in single or twin-screw extruders (Zheng and Rehmann, 2014). Single-screw extruders

consist of only one solid part, while twin-screw extruders consist of two small screw elements mounted on shafts (Yousuf et al., 2019).

During extrusion, lignocellulosic biomass undergoes shear, mixing, and heating actions, which occur mainly in the mixing zone of the screw. This zone is formed by successive kneading elements, connected with a slight offset angle between them (Konan et al., 2022). It can cause an increase in the cellulose exposure rate, as well as an increase in porosity and surface area (Moro et al., 2017).

The literature presents several studies investigating extrusion as a pretreatment for fermentable sugar production. These studies are detailed in Table 4, including the respective operating conditions.

**Table 4.** Studies on Extrusion Pretreatment for Biomass.

Biomass	Pretreatment Parameters	Main Results	Reference
Eucalyptus grandis	Twin screw extruder, 150 rpm, 100 °C	Increased sugar release, especially xylose. For 10% NaOH: 24.7 to 27.6 g/100 g. For 20% NaOH: 36.9 to 39.7 g/100 g.	(Doménech et al., 2020)
Eucalyptus wood chips	Twin screw extruder	Maximum cellulose hydrolysis yield reached 79.6% after 168 hours of enzymatic hydrolysis.	(Tian et al., 2019)
Eucalyptus grandis	Twin screw extruder, 100, 200, 300 rpm; 75, 100, 125 °C; (Liquid-to-solid ratio): Between 0.6 and 1.5. Moisture: 37–60%	Enhanced enzymatic digestibility with 37.6% glucan and 74.6% xylan release after pretreatment.	(Duque et al., 2018)
Eucalyptus wood chips	Steam explosion through continuous screw extrusion (SESE)	SESE pretreatment significantly reduced fiber size, increased surface-to-volume ratio, and caused lignin depolymerization and repolymerization.	(Liang et al., 2016)
Eucalyptus wood chips	Twin screw extruder, 45–120 rpm, ambient temperature	HCW followed by extrusion significantly increased glucose production. Total monosaccharide yield for Eucalyptus reached 39.3% of original wood weight.	(Lee et al., 2010)
Pine wood chips	Single screw extruder, 100, 150, 200 rpm; 100, 140, 180 °C; Moisture: 25, 35, 45%	Screw speed and barrel temperature increased sugar recovery, while higher moisture reduced recovery.	(Karunanithy et al., 2012)
Sugarcane Bagasse	Twin screw extruder, 100 rpm, 130 °C, Moisture: 10%	Dry extrusion increased glucose yield by up to 330% and reduced crystallinity by 78%, facilitating enzymatic hydrolysis. Optimized conditions (129 °C and 132 rpm) maximized process efficiency, improving water absorption and solubility of the biomass. The pretreatment also reduced the time and enzymatic load required, making the process more economical.	(Fasheur et al., 2022)

Sugarcane Bagasse and Straw	Twin screw extruder, 20–150 rpm, 30–150 °C, Moisture: 10–12%	Extrusion enhanced enzymatic hydrolysis efficiency, yielding up to 68.2% glucose for sugarcane straw (7 passes) and 36.6% for bagasse (3 passes).	(Kuster Moro et al., 2017)
Sugarcane Bagasse	Twin screw extruder, 15 rpm, 140 °C, Biomass pre-dried	Glucose yield after enzymatic saccharification reached 91% for 25% bagasse loading (2 passes) and 76.4% for 50% loading.	(Da Silva et al., 2013)
Corn cob	Twin screw extruder	Fast extrusion, NaOH, and enzymatic hydrolysis increased methane production by 22.3% compared to raw cob anaerobic digestion.	(Perez-Rodriguez et al., 2017)
Soybean Hulls	Twin screw extruder, 280, 350, 420 rpm; Barrel adjusted from 40 °C (inlet) to 80 °C (outlet); Moisture: 40%, 45%, 50%	Extrusion improved enzymatic hydrolysis efficiency of soybean hulls, increasing glucose yield by up to 155% compared to untreated biomass.	(Yoo et al., 2012)
Soybean Hulls	Twin screw extruder, 280, 350, 420 rpm; Drum: 80, 110, 140 °C (80 °C most efficient); Moisture: 0.4	Cellulose-to-glucose conversion after enzymatic hydrolysis reached 94.8%, outperforming or matching diluted acid (69.2%) and alkali (93.3%) treatments.	(Yoo et al., 2011)
Soybean Hulls	Twin screw extruder, 3.7 Hz (222 rpm); 110 °C; Moisture: 30–35%	Extrusion yielded 9–12% reducing sugars. After washing, sugar yields increased to 25–36%.	(Lamsal et al., 2010)
Grass	Twin screw extruder	Extrusion increased biomethane potential (BMP) by 11% for fresh grass and 18% for silage. Methane yield: 325.5–337.6 Nm <sup>3</sup> CH <sub>4</sub> /ton VS (fresh) and 150–186 Nm <sup>3</sup> CH <sub>4</sub> /ton VS (silage).	(Souza et al., 2021)
Switchgrass	Single-screw extruder, Compression ratios: 2:1 and 3:1; 50, 100, 150 rpm; 50, 100, 150 °C; Moisture: 15%, 25%, 35%, 45%	Sugar recovery increased after extrusion pretreatment. Maximum glucose recovery was 45.2% at 15% moisture, 50 rpm screw speed, 150 °C, and 3:1 compression ratio.	(Karunanithy et al., 2011a)
Big bluestem	Single screw extruder, 20–200 rpm; 45–225 °C; Moisture: 10%–50%	Maximum recoveries: glucose (71.3%), xylose (78.5%), and combined sugars (56.9%).	(Karunanithy et al., 2011b)
Prairie Cord Grass	Single screw extruder, 20–200 rpm; 45–225 °C; Moisture: 75%–78%	Glucose recovery up to 86.8%, xylose 84.5%, and total sugars 82% under optimized conditions.	(Karunanithy et al., 2011c)
Switchgrass, Prairie Cord Grass, and Big Bluestem	Single screw extruder, 20–200 rpm; 45–225 °C; Moisture: 10–50%	Switchgrass: Torque 85–100 Nm, sugar recovery 47.4%. Prairie Cord Grass: Torque 27–42 Nm, sugar recovery	(Karunanithy et al., 2011d)

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56.9%. Big Bluestem: Torque 53–84 Nm,  
sugar recovery 56.9%.

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Extrusion process parameters vary widely between studies, directly impacting the pretreatment efficiency. Screw speed significantly influences the yield of fermentable sugars, as demonstrated by Karunanithy et al. (2011), where an increase in screw speed from 100 to 150 rpm resulted in an 11% increase in cellulose recovery and a 7.7% increase in hemicellulose. Similar results were observed by Yoo et al. (2011) when comparing the effectiveness of different speeds and temperatures on soybean hulls, highlighting that the lower temperature (80°C) was the most efficient. In another study Fasheur et al. (2022) observed that dry extrusion of sugarcane bagasse, performed at 100 rpm and 130°C, resulted in greater saccharification efficiency due to increased enzymatic accessibility after extrusion.

In addition to screw speed and temperature, moisture content also plays a crucial role. Karunanithy et al. (2011) increased the moisture content from 25% to 45%, reduced cellulose recovery by 18% and hemicellulose by 34%. In another study, Moro et al. (2017) showed that moisture levels between 10% and 12% for bagasse and sugarcane straw, combined with extrusion using glycerol as an additive, resulted in increased enzymatic accessibility, with glucose yields of up to 68.2% for the straw. These results were consistent with those obtained by Lee et al. (2010), who highlighted moisture control's importance in improving enzymatic saccharification in eucalyptus chips.

The formation of inhibitors during the extrusion process was another point evaluated in several studies. Chemicals such as NaOH or H<sub>2</sub>SO<sub>4</sub> can result in the formation of salts or other inhibitors, which was confirmed in the studies of Doménech et al. (2020) and Duque et al. (2018). However, most studies highlight that post-extrusion washing effectively removes these inhibitors. For example, Duque et al. (2018) observed that after neutralization with H<sub>2</sub>SO<sub>4</sub> and subsequent washing, the yield of glucan and xylan increased significantly. The same was observed by Lamsal et al. (2010), where post-extrusion washing of soybean hulls increased the yields of reducing sugars from 9–12% to 25–36%. These results indicate that washing is crucial in removing inhibitors and improving enzymatic saccharification efficiency.

### 3.4. Pulsed Electric Field Pretreatment

The pulsed Electric Field (PEF) method has been widely used in molecular biology and, more recently, in food processing and biotechnology (Weaver and Chizmadzhev, 1996; Golberg et al., 2016). This pretreatment involves applying high voltage between two electrodes, with electric field strengths ranging from 0.1 to 80 kV/cm for 100 to 10,000 μs (Hassan et al., 2018; Yousuf et al., 2019). The primary effect of PEF on lignocellulosic biomass is the disruption of membrane barrier functions, increasing permeability (Rocha et al., 2018). This high-intensity electric field rapidly generates an electric potential across the cell membrane, leading to its rupture (Brahim et al., 2017). This enhances the entry of enzymes, acids, bases, and other agents that aid in the hydrolysis of cellulose and other biomass components (Barba et al., 2015; Vorobiev and Lebovka, 2017).

PEF main advantages include operation under normal pressure and temperature conditions, low energy consumption, and operational simplicity (Kumar et al., 2011; Zheng et al., 2017). However, large-scale applications present challenges, such as the need for large pretreatment chambers with high pulse repetition rates and increased current demands (Golberg et al., 2016; Basak et al., 2023). These technical limitations make handling large biomass volumes more complex, requiring adaptations to improve process efficiency (Bluhm, 2006).

Although PEF has shown potential for lignocellulosic biomass pretreatment, it is still in the early stages of development (Kovačić et al., 2021; Basak et al., 2023). In a preliminary study, Kumar et al. (2011) developed a PEF system to pretreat switchgrass and wood chips (southern pine). They observed increased dye absorption starting at 8 kV/cm, indicating greater biomass porosity, which could improve saccharification efficiency. They used a dye cresol violet (C<sub>15</sub>H<sub>17</sub>ClN<sub>4</sub>) to verify absorption after treatment, indicating increased porosity.

Szwarc and Szwarc (2021) also explored PEF in biogas production from corn silage. Using rectangular pulses with a duration of 50  $\mu$ s and voltages of -40 kV, they observed a 4% increase in glucose content and a 14% increase in biogas production at a field intensity of 20 kV/cm with 180 seconds of pretreatment. However, extending pretreatment beyond this time did not result in further gains, suggesting an optimal treatment duration.

Despite promising results, large-scale PEF adoption faces significant challenges, particularly in system costs and the complexity of treating large biomass volumes (Basak et al., 2023). Additionally, large-scale pretreatment chambers must be designed to accommodate high biomass throughput, requiring higher pulse voltage amplitudes to cover larger electrode areas and meet current demands. This can increase energy consumption and introduce additional technical limitations.

For PEF to be validated in industrial bioenergy conversion processes, it is essential to refine parameters such as field intensity, treatment time, and equipment design. Identifying optimal operating conditions, such as the maximum treatment time beyond which no further gains are observed, is crucial to maximizing process efficiency, as Szwarc and Szwarc (2021) demonstrated.

## 4. Enzymatic Hydrolysis of Lignocellulosic Biomass After Physical Pretreatment

After the pretreatment of lignocellulosic biomass, the enzymatic hydrolysis step is a crucial process for producing fermentable sugars. This process aims to break down complex polymers, such as cellulose and hemicellulose, into their constituent monosaccharides, primarily glucose (C6) and xylose (C5), which can be used as substrates for fermentation and consequently for the production of value-added products (Lamsal et al., 2010; Zhang et al., 2021).

### 4.1. Mechanism and Enzymatic Components

The main enzymes involved in the biochemical conversion of lignocellulosic biomass into sugars are cellulases and hemicellulases. The synergistic action of three major types of cellulases, endoglucanase, exoglucanase, and  $\beta$ -glucosidase is essential for the complete degradation of cellulose into glucose (Yousuf et al., 2019; Yoo et al., 2012). Endoglucanase degrades the  $\beta$ -1,4 glycosidic bonds in the amorphous regions of cellulose. At the same time, exoglucanase acts at the ends of cellulose polymers, releasing oligosaccharides, such as cellobiose and celotriose, which are subsequently converted into glucose by  $\beta$ -glucosidase (Liu et al., 2021; Li et al., 2022). Regarding hemicellulose, its structure is complex and depends on the type of biomass, with xylanase (endo-1,4- $\beta$ -xylanase) being the predominant enzyme, acting by breaking glycosidic bonds in xylan and releasing xylo-oligosaccharides (Rodionova et al., 2022; Lopes et al., 2018).

### 4.2. Effect of Pretreatment of Enzymatic Hydrolysis

Enzymatic hydrolysis depends on the structural modifications achieved during pretreatment, which increase enzyme accessibility to the biomass. The main factors influencing hydrolysis efficiency include pH, temperature, enzyme loading, and reaction time. Maintaining an optimal pH (usually between 4.8 and 5.5) and an adequate temperature (typically between 45 °C and 55 °C) is essential to ensure enzyme stability and activity (Moodley et al., 2019; Fonseca et al., 2021).

Different physical pretreatment methods aim to enhance this enzymatic accessibility by inducing specific structural changes in the biomass, facilitating enzyme action during hydrolysis. In the following sections, the main physical pretreatments applied, including mechanical comminution, microwave, electron beam, and others, will be discussed, highlighting how each method impacts enzymatic conversion efficiency and its challenges and limitations.

#### 4.2.1. Mechanical Comminution Pretreatment

Enzymatic hydrolysis following mechanical comminution has shown substantial variations in glucose and xylose yields, influenced by different milling conditions, enzyme types, and operational parameters. Inoue et al. (2008) conducted the hydrolysis of ground eucalyptus with cellulase from

*Acremonium cellulolyticus* at an enzyme loading of 40 FPU/g substrate under conditions of 45 °C and pH 5.0 for 72 hours, resulting in yields of 89.7% glucose and 72.5% xylose. This high yield reflects the increase in specific surface area and enzyme accessibility due to reduced cellulose crystallinity, a critical aspect of hydrolysis effectiveness after ball milling.

Similarly, Sant'Ana da Silva et al. (2010), studying the hydrolysis of ground sugarcane bagasse and straw, observed that using an enzymatic cocktail containing 15 FPU/g cellulase and 0.2% xylanase (Optimash™ BG) also led to significant glucose yields, reaching 78.7% for bagasse and 77.6% for straw. Compared to much lower yields in untreated samples, these results reinforce the role of adequate enzyme loading and optimized reaction times to maximize saccharification across different biomass types.

In contrast to the results observed for eucalyptus and sugarcane, Zeng et al. (2007) investigated the impact of particle size on ground corn residues, varying from 53–75 µm to 425–710 µm. The authors observed that smaller particles favored glucose conversion, yielding 25.9% after 72 hours of hydrolysis with the Spezyme CP and Novozyme 188 β-glucosidase cocktail under pH 4.8 and 50 °C conditions. This finding highlights how reducing particle size increases the accessible surface area for enzymes, enhancing hydrolysis efficiency.

Finally, the study by Zhu et al. (2010) on lodgepole pine subjected to disk milling indicated saccharification yields of 49.3% for glucose and 68% for xylose. Compared to previous studies, this result suggests that woody biomasses, due to their rigid structure and high lignin content, require specific adjustments in milling parameters to achieve high yields, highlighting an additional challenge for enzymatic digestibility in these biomasses.

However, this method presents significant technical challenges, particularly for woody and dense biomasses, due to their rigid structure and high lignin content, which hinder enzymatic action. These characteristics require specific adjustments in comminution parameters, such as particle size and equipment type, to enhance saccharification efficiency. Thus, the applicability of mechanical comminution for rigid biomasses relies on advancements in controlling and adapting operational conditions to overcome the barriers associated with structural recalcitrance.

#### 4.2.2. Ultrasound Pretreatment

Ultrasound-assisted pretreatment has proven to be a transformative technique in enhancing the enzymatic hydrolysis of lignocellulosic biomass. This method facilitates structural disruption, reduces lignin content, and increases cellulose accessibility, improving sugar yields. Several studies provide insights into the mechanisms and outcomes of enzymatic hydrolysis following ultrasound pretreatment, highlighting the influence of operational parameters, enzyme types, and biomass characteristics.

The choice of enzymatic cocktails significantly affects hydrolysis outcomes. Celluclast 1.5 L combined with β-glucosidase, as used by Candido et al. (2019), achieved glucose conversion rates of up to 86.74% in sugarcane straw pretreated with ultrasound and NaOH. Similarly, Velmurugan et al. (2012) demonstrated a 92.11% theoretical yield using the same enzymatic cocktail on ultrasound-pretreated sugarcane bagasse. Both studies highlight the critical role of alkaline conditions in delignification and cellulose accessibility, underscoring the synergy between chemical agents and ultrasound.

In addition to alkaline conditions, integrating acidic agents with ultrasound pretreatment has also proven effective. Esfahani et al. (2010) reported a glucose yield of 26.01 g/L, corresponding to 94.49% of the theoretical yield, when using Cellubrix® (Novozyme) on sugarcane bagasse pretreated with dilute sulfuric acid and ultrasound. This approach emphasizes the importance of acidic catalysts in breaking down recalcitrant structures, particularly in high-lignin biomass.

Alternative biomass types also benefit from ultrasound pretreatment. Yin et al. (2014) explored corn cobs, achieving a glucose yield of 42% using Cellic enzymes. The structural disruption caused by ultrasound and increased surface area facilitates enzymatic access to cellulose fibers. Similarly, Jun-Hong et al. (2015) demonstrated a reducing sugar yield of 36.89% in Bermuda grass using

ultrasound combined with diluted HCl, further showcasing the versatility of this method across diverse feedstocks.

Combining ultrasound with innovative solvents like deep eutectic solvents (DES) has shown promise. Sharma et al. (2021) reported a sugar yield of 312 mg/g in sugarcane bagasse using a cocktail from *Aspergillus assiutensis*. The integration of ultrasound with DES amplifies delignification, reduces cellulose crystallinity, and minimizes inhibitor formation, presenting a compelling case for process intensification.

Studies also highlight the role of enzymatic loading and activity in maximizing hydrolysis efficiency. Revin et al. (2016) demonstrated a 61% glucose yield, equivalent to 35.5 g/L, in *Pinus sylvestris* using *Penicillium verruculosum* enzymes with an activity of 204 U CMC/g. This study illustrates how combining ultrasound with enzymes of high activity levels can enhance digestibility, even in challenging woody biomass.

While the potential of ultrasound-assisted enzymatic hydrolysis is evident, challenges persist. The variability in biomass composition and lignin content necessitates tailored pretreatment strategies to optimize hydrolysis yields. High-lignin biomass, such as *Pinus sylvestris*, often requires more aggressive chemical conditions or combined pretreatments, while less lignified feedstocks, such as sugarcane bagasse and Bermuda grass, achieve significant improvements with milder conditions.

Ultrasound pretreatment offers significant advantages for enzymatic hydrolysis, improving sugar yields across various biomass types. The success of this approach depends on optimizing pretreatment parameters, enzyme cocktails, and chemical combinations. Future studies should focus on expanding the application of ultrasound pretreatment to additional biomasses and refining conditions to ensure broader industrial scalability.

#### 4.2.3. Microwave Pretreatment Pretreatment

The effectiveness of enzymatic hydrolysis after microwave pretreatment is primarily influenced by parameters such as enzyme type, enzyme loading, pH, temperature, and reaction time. These factors determine the yield of fermentable sugars and the overall process efficiency. Analysis of studies shows that most have used pH ranges between 4.8 and 5.0 and temperatures of 45 °C to 50 °C, conditions common for hydrolyzing celluloses and hemicelluloses in industrial contexts (Inoue et al., 2008; Sant'Ana da Silva et al., 2010).

Variations in enzyme loading have been observed, notably with enzymes like Accellerase 1500® (Rigual et al., 2018), Cellic CTec2 (Fonseca et al., 2021; Moodley et al., 2019), and Zytex cellulase (Binod et al., 2012). Accelerate 1500® showed high efficiency with eucalyptus and pine, achieving up to 78% glucan digestibility (Rigual et al., 2018), while Cellic CTec2 demonstrated yields of up to 72.2% for sugarcane straw, even with low inhibitor presence (Fonseca et al., 2021). These results align with literature findings highlighting the combination of cellulase and  $\beta$ -glucosidase as effective for releasing glucose and xylose from lignocellulosic biomass.

The hydrolysis time ranged from 24 to 72 hours, reflecting the recalcitrance of lignocellulosic biomass and the capacity of microwave pretreatment to facilitate cellulose accessibility. Binod et al. (2012) achieved 0.83 g/g of reducing sugars in 24 hours using Zytex on microwave—and alkali-pretreated sugarcane bagasse, while Miranda et al. (2014) used 72 hours to obtain high yields of glucose (46.25±1.22 mg/g) and xylose (190.43±6.54 mg/g). These studies suggest that microwave pretreatment can reduce hydrolysis time by modifying the biomass structure, especially for more recalcitrant biomasses.

Studies with woody biomass and grasses revealed significant differences in efficiency. Amini et al. (2018) reported 100% sugar conversion in *Eucalyptus regnans* pretreated with microwaves at 180 °C for 30 minutes, while Rigual et al. (2018) obtained 68 g/100 g glucan for eucalyptus and 78 g/100 g for pine, suggesting that optimized microwave temperatures and times are essential to overcome recalcitrance in woody biomass. Zhu et al. (2010) observed that increased surface area and reduced cellulose crystallinity after microwave treatment improved enzymatic accessibility, an effect

confirmed in grasses like switchgrass and big bluestem, where Karunanithy et al. (2014) reported yields of up to 83.2%.

In some cases, the use of microwaves combined with acidic catalysts, such as  $\text{H}_2\text{SO}_4$  and  $\text{Al}_2(\text{SO}_4)_3$ , increased glucose and xylose release, especially for woody biomass (Kłosowski et al., 2020; Hermiati et al., 2024). For sugarcane bagasse, Fonseca et al. (2021) achieved a 72.2% yield with Cellic CTec2 and low inhibitor content, emphasizing that microwave pretreatment facilitates subsequent fermentation processes for biofuels. In a complementary approach, Wang (2021) demonstrated that anaerobic digestion of microwave-pretreated corn straw increased methane production by 73.08%, suggesting that the method also benefits anaerobic digestion.

However, using microwaves for dense or woody biomass presents challenges due to the high energy requirements needed to overcome their compact structure. Woody biomass recalcitrance requires specific adjustments in temperature and moisture to avoid the formation of inhibitors, such as HMF, which can limit its large-scale application for certain feedstocks.

#### 4.2.4. Electron Beam Pretreatment

The biomass structure and process conditions significantly influence the effectiveness of enzymatic hydrolysis following electron beam pretreatment. Al Gharib et al. (2023) reported a 100% cellulose-to-glucose conversion when treating biomass with doses of 1-2 MGy, where structural disaggregation promoted by the electron beam considerably enhanced enzymatic accessibility. This efficiency increase is attributed to the electron beam's effect in reducing cellulose crystallinity and expanding the surface area accessible to enzymatic action. The hydrolysis was carried out using a mixture of endo 1,4  $\beta$ -glucanase ( $2 \text{ U mg}^{-1}$ ), Exo 1,4  $\beta$ -glucanase (cellobiohydrolase), and  $\beta$ -glucosidase, with 40 or 100  $\mu\text{L}$  of enzyme solution added to 400 or 600 mg of irradiated biomass. Studies on gamma radiation pretreatment, such as that by Wu et al. (2020), support these findings. In their study, glucan-to-glucose conversion exceeded 70% with an enzyme loading of 15 FPU/g at 50 °C and pH 4.8, ideal conditions for commercial cellulases (Zhao and Liu, 2012). However, electron beam treatment offers additional advantages by modifying biomass structure more efficiently, potentially enabling reduced enzyme usage.

Furthermore, enzyme loading must be balanced according to pretreatment intensity and biomass structure. Biomass treated with high radiation doses, such as 800 kGy, shows increased enzymatic accessibility due to reduced cellulose crystallinity, allowing lower enzyme requirements to achieve high glucose yields (Kapoor et al., 2022). Nevertheless, high doses can generate inhibitory compounds, such as furfural and acetic acid, which compete with enzymes and reduce process efficiency, highlighting the need to control pretreatment conditions carefully (Li et al., 2016).

On the other hand, high radiation doses can generate inhibitors that affect hydrolysis efficiency, along with high operational costs, limiting the industrial feasibility of this technique unless combined with methods to dilute inhibitors or better control radiation dosage.

#### 4.2.5. Gamma Rays Pretreatment

Enzymatic hydrolysis of biomass pretreated with gamma radiation is a promising method for increasing the efficiency of cellulose and hemicellulose conversion into fermentable sugars, mainly glucose. According to Wu et al. (2020) demonstrated that glucan-to-glucose conversion could exceed 70% with an enzyme loading of 15 FPU/g at 50 °C and pH 4.8, underscoring the critical role of pretreatment in modifying the biomass structural characteristics and enhancing enzymatic accessibility.

This process typically involves cellulolytic enzymes, such as cellulase and hemicellulase, which work synergistically to degrade cellulose and hemicellulose chains into monosaccharides. The effectiveness of enzymatic hydrolysis largely depends on the structural modifications achieved during pretreatment. Comparatively, Al Gharib et al. (2023) reported a 100% cellulose-to-glucose conversion in biomass treated with an electron beam at 1-2 MGy doses, where structural disaggregation significantly increased enzymatic accessibility. While the electron beam promotes

intense structural modifications, gamma radiation also reduces cellulose crystallinity, albeit in a more controlled manner, allowing for adjustments based on biomass type and dosage.

Enzyme loading, pH, and temperature are essential for optimizing glucose conversion. Additional studies highlight that combining treatments can further enhance process efficiency. Duarte et al. (2012), combining electron beam with hydrothermal pretreatment, observed glucose yields of up to 74.72% in 48 hours, indicating that the synergy between methods can improve efficiency under optimized temperature and pH conditions. This suggests that, in gamma radiation pretreatment, precise control of these parameters and the use of complementary enzymes, such as cellulase and  $\beta$ -glucosidase, contribute to superior results.

Lower doses of gamma radiation may suffice for less complex biomasses, such as corn starch. Shen et al. (2022) showed that 2-12 kGy successfully altered starch structure, increasing the hydrolysis rate with reduced enzyme loading. This observation suggests that adjusting doses and combining them with specific enzymatic treatments for each biomass type can enhance hydrolysis efficiency and reduce costs, making the process economically viable.

However, this method may present limitations similar to electron beam pretreatment regarding cost and inhibitor formation. To maximize its industrial applicability, it is essential to explore inhibitor mitigation strategies and study interactions with enzyme cocktails tolerant to these substances.

#### 4.2.6. Extrusion Pretreatment

Extrusion effectively enhances biomass accessibility to enzymatic hydrolysis by promoting structural modifications that increase surface area and reduce cellulose crystallinity. Yoo et al. (2011) reported a glucose yield of 95% when treating soybean hulls via extrusion at 40% moisture and a screw speed of 350 rpm. By comparison, untreated hulls yielded only 69.6%, underscoring the importance of optimizing mechanical conditions to maximize cellulose exposure to enzymatic action. This yield increase under optimized conditions is particularly significant for high-density biomass, where recalcitrance poses challenges to cellulose conversion.

Additionally, various enzyme cocktails are applied to optimize the hydrolysis of different biomass types. Yoo et al. (2012), combining cellulase and  $\beta$ -glucosidase on extruded soybean hulls, achieved an 87% cellulose-to-glucose conversion, increasing to 155% when applying a more complex enzyme cocktail, including enzymes for cell wall degradation. Comparatively, Lamsal et al. (2010) observed sugar yields between 12% and 36% for extruded wheat bran, emphasizing the role of hemicellulases in hemicellulose-rich substrates. These results highlight how enzyme selection and synergy are critical for maximizing fermentable sugar yield.

Moisture and temperature during extrusion also significantly impact hydrolysis efficiency. Karunanithy et al. (2011) observed that increasing moisture from 25% to 45% reduced cellulose recovery by 18% and hemicellulose recovery by 34%, suggesting that high moisture levels dampen the application of heat and shear. Conversely, Moro et al. (2017) demonstrated that a 10% to 12% moisture level, combined with additives such as glycerol, increased glucose yield by 68.2% in sugarcane straw, highlighting that precise moisture control is essential and may vary according to biomass type.

Another relevant parameter is biomass density, which directly affects enzyme choice and extrusion conditions. For example, Tian et al. (2019) observed a yield of 79.6% in eucalyptus wood following hot water extraction and mechanical extrusion treatment. In less dense materials, such as wheat bran, cellulase and hemicellulase effectively produced reducing sugar yields between 25% and 36% with lower shear energy. These findings emphasize the importance of adjusting extrusion based on biomass rigidity and density to maximize hydrolysis.

Furthermore, inhibitor control, such as HMF and organic acids, is crucial for optimizing enzymatic hydrolysis. Doménech et al. (2021) demonstrated that applying post-wash steps can mitigate these inhibitors, and Duque et al. (2018) observed that washing extruded sugarcane bagasse significantly increased glucan and xylan yields. Removing inhibitory compounds is, therefore,

essential to maintain enzymatic conversion efficiency and avoid negative impacts on subsequent fermentation.

However, precise control of moisture and temperature during extrusion is challenging, especially for high-density biomass that requires adjustments in pressure and speed. This method demands specific optimizations for different biomass types, which can pose a barrier to large-scale application.

#### 4.2.7. Pulsed Electric Field Pretreatment

Pulsed Electric Field (PEF) pretreatment has shown potential to improve the structural accessibility of lignocellulosic biomass by promoting changes in porosity and cellular permeability that theoretically facilitate enzyme action. This method uses high-intensity electric pulses to reorganize cellular structures, increasing the exposure of fibers to enzymatic hydrolysis. Studies such as Kumar et al. (2011) indicate that PEF modifies the structural organization of biomass, increasing its susceptibility to degradation and enabling a higher rate of fermentable sugar conversion.

However, the practical application of PEF in enzymatic hydrolysis remains underexplored. Although studies like Kumar's have documented improvements in the physical structure of PEF-treated biomass, most are limited to analyzing physical changes, such as increased porosity and permeability, without directly verifying the impact on the subsequent enzymatic hydrolysis step. This experimental limitation suggests that the effects of PEF on monosaccharide release have not yet been practically validated, and feasibility and efficiency studies remain insufficiently explored.

## 5. Biomass Final Treatments

The pretreatment process plays a crucial role in breaking down the complex structure of biomass, making fermentable sugars more accessible for conversion into bioethanol, biogas, and other valuable compounds. Each method offers distinct advantages and challenges, influencing the efficiency of product yields and the overall feasibility of large-scale industrial applications.

### 5.1. Bioethanol

Bioethanol production from lignocellulosic biomass is one of the most common applications for the fermentable sugars released after biomass pretreatment. In mechanical comminution, Inoue et al. (2008) demonstrated that ball-milled eucalyptus wood achieved a glucose-to-ethanol conversion of over 90% in 24 hours, highlighting the efficiency of comminution in releasing fermentable sugars. Similarly, Sant'Ana da Silva et al. (2010) used sugarcane bagasse hydrolysates for fermentation with *Saccharomyces cerevisiae*, reaching conversion yields of nearly 90%. The high purity of the hydrolysates, free from fermentation inhibitors, favored the efficiency of the process.

In ultrasound pretreatment, Revin et al. (2016) observed that fermentation of *Pinus sylvestris* after ultrasound pretreatment reached an ethanol concentration of 3.11% (v/v), a significant increase compared to the controls. This improvement was attributed to enhanced glucose release during hydrolysis due to the ultrasound treatment. Candido et al. (2019) also explored second-generation ethanol production using sugarcane straw pretreated with ultrasound in an alkaline medium. Enzymatic hydrolysis generated glucose-rich hydrolysates, and subsequent fermentation demonstrated the industrial viability of this approach. Eblaghi et al. (2015) further validated these findings using *Saccharomyces cerevisiae* to produce ethanol from ultrasound-treated sugarcane bagasse, highlighting how combining ultrasound with alkaline pretreatment improves sugar release and fermentation efficiency.

Microwave pretreatment has also proven effective, especially in accelerating the hydrolysis process. Miranda (2014) showed that microwave-assisted alkali and acid treatments of sugarcane bagasse resulted in high ethanol yields, demonstrating that microwave treatment can improve cellulose accessibility, thereby enhancing fermentation efficiency. Similarly, Binod (2012) achieved

high fermentable sugar yields from sugarcane bagasse treated with NaOH and microwaves, which facilitated the efficient production of ethanol.

In gamma radiation pretreatment, Al Gharib et al. (2023) demonstrated that woody biomass, such as pine and poplar, irradiated at doses of 1 MGy, achieved complete cellulose-to-glucose conversion, enabling efficient bioethanol production through fermentation with *Saccharomyces cerevisiae*. Kapoor et al. (2022) also reported significant ethanol yields from sugarcane bagasse pretreated with gamma radiation, highlighting the effectiveness of this method for agricultural residues, with the integration of simultaneous saccharification and fermentation (SSF) processes to optimize ethanol production. However, gamma radiation can generate inhibitors like furfural and acetic acid, negatively impacting fermentation efficiency. Li et al. (2016) emphasized the importance of controlling radiation doses to minimize inhibitor formation while preserving fermentable sugars.

In electron beam pretreatment, Al Gharib et al. (2022) reported that hydrolysates from woody biomass treated with electron beams produced glucose with high purity, although the effectiveness of these hydrolysates in bioethanol production has not yet been tested at a large scale. The lack of studies on fermentation remains a significant limitation of this method, hindering a comprehensive assessment of its industrial applicability. Rattanawongwiboon et al. (2022) demonstrated that sugarcane bagasse treated with electron beams could be converted into sulfonated biochar, which catalyzed fermentable sugar production. While this process showed efficient glucose recovery, further studies are needed to assess its potential for ethanol production and other bioproducts.

Finally, in extrusion pretreatment, Duque et al. (2018) observed that glucose and xylose were efficiently converted into bioethanol after alkaline extrusion pretreatment and subsequent washing, showing the potential of extruded biomass as a fermentation feedstock. Tian et al. (2019) reported similar findings with eucalyptus wood chips subjected to extrusion and thermal treatment, achieving a 55.3% ethanol yield with a titer of 15.4 g/L after 48 hours of fermentation, demonstrating the feasibility of extrusion for highly recalcitrant woody biomass by improving accessibility and fermentation efficiency.

Producing bioethanol from lignocellulosic biomass following enzymatic hydrolysis involves carefully evaluating the pretreatment methods. Although the techniques discussed here offer significant advantages, choosing the ideal treatment depends on the biomass characteristics, the desired efficiency, and operational costs.

## 5.2. Biogas

Biogas production is another important application of lignocellulosic biomass, particularly for renewable energy generation. After enzymatic hydrolysis, fermentable sugars can be used in anaerobic digestion to produce biogas, mainly methane. Various pretreatment methods have been studied for their effects on biogas yields, and the results vary depending on the type of biomass, pretreatment conditions, and operational parameters.

Mechanical comminution is widely used for breaking down biomass and increasing surface area, making it more accessible to microbial activity in anaerobic digestion. Sant'Ana da Silva et al. (2010) observed that hydrolysates derived from sugarcane bagasse pretreated by mechanical comminution were high in purity and free from inhibitors, facilitating efficient fermentation. Although the study focused on ethanol production, the increased surface area and reduced particle size likely positively impacted biogas production. However, the high energy consumption associated with mechanical comminution remains a significant challenge, potentially limiting its scalability, especially for large-scale operations.

Ultrasound pretreatment has proven effective in improving digestibility and enhancing biogas yields by facilitating the rupture of cell walls and improving the accessibility of sugars for fermentation. Pérez-Rodríguez et al. (2016) demonstrated that combining ultrasound with enzymatic hydrolysis significantly enhanced methane production from corn cob and vine trimming shoot hydrolysates, resulting in a 22.6% increase in methane yields compared to controls. Martínez-Jiménez et al. (2017) further expanded this concept by producing methane and hydrogen biomethane from

sugarcane straw hydrolysates. This method achieved up to 68% methane yields, showcasing ultrasound's ability to improve methane and hydrogen production for bioenergy applications.

Microwave-assisted pretreatment has also proven effective in improving biomass digestibility and enhancing biogas production. Ude (2022) demonstrated that microwave treatment of elephant grass resulted in 15% higher methane yields than untreated controls. However, as with other pretreatments, biomass composition can affect the overall success of the process. Bichot (2021) found that microwave pretreatment did not outperform conventional methods in terms of methane potential when applied to corn stalks and miscanthus, indicating that biomass type plays a crucial role in determining pretreatment effectiveness.

Gamma radiation pretreatment has been explored to improve biogas production, especially for agricultural residues. Wu et al. (2020) showed that combining gamma radiation with ultrasonic treatments increased methane yields from agricultural residues such as rice straw and corn stalks. The synergy of these methods resulted in a 30% increase in methane production, achieving 72% energy recovery. This combined effect could be particularly valuable for improving biogas production efficiency, especially from lignocellulosic biomass resistant to anaerobic digestion. However, the formation of inhibitors during gamma radiation—such as furfural and acetic acid—remains a challenge, and post-treatment strategies to mitigate these effects are crucial for ensuring optimal biogas yields.

Electron beam pretreatment has primarily focused on improving cellulose accessibility for enzymatic hydrolysis. Rattanawongwiboon et al. (2022) investigated the conversion of sugarcane bagasse treated with electron beams into sulfonated biochar, which acted as a catalyst for fermentable sugar production. Although their study mainly concentrated on sugar recovery, the increased availability of fermentable sugars suggests that electron beam pretreatment could also enhance biogas production by improving the digestibility of lignocellulosic biomass. However, as with gamma radiation, the lack of studies on the direct impact of electron beam pretreatment on biogas production limits the understanding of its full potential.

Extrusion pretreatment has shown promise for improving both bioethanol and biogas production. Pérez-Rodríguez et al. (2017) observed that rapid extrusion, followed by alkaline treatment and enzymatic hydrolysis, significantly enhanced biogas production from corn cob, achieving a methane content of 65.6% and a 22.3% increase compared to untreated corn cob. Souza et al. (2021) further demonstrated that extrusion of fresh and ensiled grass improved biogas yields by up to 18%, emphasizing that extrusion can enhance anaerobic digestion efficiency.

Following enzymatic hydrolysis, the pretreatment method influenced biogas production from lignocellulosic biomass. Ultrasound, microwave, gamma radiation, and extrusion have all shown potential for improving biogas production. However, the effectiveness of each method depends on factors such as biomass type, operational conditions, and the presence of inhibitors. While ultrasound and microwave pretreatment promise to improve methane yields, challenges remain in optimizing these methods for large-scale applications. Further research is needed to fully understand the synergies between pretreatment methods and develop strategies for integrating these processes into biorefineries to maximize biogas production's economic and environmental benefits.

### 5.3. Other Products Obtained

In addition to standard products such as bioethanol and biogas, lignocellulosic biomass holds significant potential for producing a variety of high-value products that can be used in industries like energy, food, plastics, and chemicals. Several studies have addressed these alternative products, demonstrating the vast range of applications for biomass.

Butanol, a second-generation biofuel, is one of the most promising alternatives. Produced from fermentable sugars, butanol has superior energy characteristics and better engine compatibility than ethanol. Buaban et al. (2010) explored butanol production from pentoses and non-hexose sugars derived from ball-milled sugarcane bagasse. Their results suggested that butanol production can be

achieved, though the original study did not provide specific yields. However, the potential for producing butanol and other pentose biofuels remains a promising avenue for future research.

Butyric acid is also an important product, both for the food industry and for bioplastics production. Fermentation of sugars derived from biomass can lead to high yields of butyric acid, which is used in the chemical and plastics industries. Buaban et al. (2010) mentioned butyric acid production from fermenting pentoses. This alternative can add value to agricultural and industrial residues, though the study did not detail specific quantities produced. Butyric acid can also produce bioGLP, further expanding its industrial applications.

Another vital by-product is acetic acid, obtained from biomass treated with gamma radiation. Wu et al. (2020) reported combining gamma radiation with ultrasonic treatments increased acetic acid yields, producing 20.1 g/L from rice straw and corn stalks. This increase in acetic acid production was accompanied by enhanced methane yields, further supporting the potential of combined treatments for improving biogas production.

The production of bioplastics has also been explored as a promising path from lignocellulosic biomass hydrolysates. Rattanawongwiboon et al. (2022) investigated the conversion of electron beam-treated sugarcane bagasse into sulfonated biochar, which acted as a catalyst for producing fermentable sugars that can be used for bioplastic production. Though the study focused on the efficiency of sugar recovery (over 94.5%), the potential for bioplastic production from these sugars was highlighted. However, further studies are needed to quantify actual bioplastic yields.

An innovative product is hydrogen, which, when produced from biomass, holds great potential as a clean energy source. Martínez-Jiménez et al. (2017) explored the production of biomethane—a mixture of methane and hydrogen—from sugarcane straw hydrolysates. Their study reported up to 68% methane yields, while hydrogen production was achieved at 7.1 g/L. This process provides a way to generate multiple fuels from a single anaerobic digestion process, increasing energy efficiency and the versatility of biomass. Additionally, Irmak (2018) demonstrated hydrogen production from switchgrass and miscanthus hydrolysates using aqueous phase reforming (APR), with yields of 3.5 g/L of hydrogen, showcasing the innovative potential of biomass for hydrogen production.

Beyond bioethanol and biogas, the potential to produce sucrose, ethylene glycol, succinic acid, and other chemical compounds from lignocellulosic biomass has been widely studied. Zhu et al. (2010) suggested that lodgepole pine hydrolysates, pretreated with disk milling, could synthesize chemicals like ethylene glycol and succinic acid, though specific quantities were not provided. These products have applications across several industries, including plastics and chemicals, and further research is needed to quantify yields and optimize production processes.

In summary, lignocellulosic biomass offers many alternative products, including butanol, butyric acid, bioplastics, hydrogen, acetic acid, and chemical compounds like ethylene glycol and succinic acid. These products expand the range of biomass applications and open the door to integrated biorefineries that can generate various sustainable, high-value products.

## 6. Energy and Costs Associated with Physical Pretreatment

Energy and cost analysis are essential for evaluating the feasibility of physical pretreatment methods in converting lignocellulosic biomass into fermentable sugars. Optimizing these factors in an industrial context determines economic efficiency and impacts biorefinery's environmental sustainability.

### 6.1. Mechanical Comminution

Physical pretreatment methods, such as ball milling and disk milling, play a key role in reducing the recalcitrance of lignocellulosic biomass. However, their industrial application is constrained by high energy consumption and associated costs.

Inoue et al. (2008) investigated the energy requirements of ball milling under laboratory and industrial conditions. Ball milling consumed 108 MJ/kg of wood in laboratory experiments over 120 minutes. At an industrial scale, energy consumption was reduced to 31 MJ/kg in a bioethanol plant

processing 100 dry tons of wood daily, applying a scale efficiency factor of 0.2. Despite this improvement, ball milling remains energy-intensive, limiting its standalone feasibility for large-scale operations. The authors proposed combining ball milling with hot-compressed water (HCW) pretreatment to address this. This combination drastically reduced energy consumption to 6.97 MJ/kg when HCW was applied at 160°C for 30 minutes, followed by 20 minutes of ball milling, demonstrating the value of integrated methods to balance energy demand and enzymatic yield.

Similarly, Zhu et al. (2010) assessed the energy consumption of disk milling for wood pretreatment. Without prior processing, disk milling required 699 kWh/ton of wood. However, the SPORL process (sulfite pretreatment to overcome recalcitrance of lignocellulose) reduced this figure to 153 kWh/ton, a 78.1% reduction attributed to improved substrate digestibility. While disk milling effectively enhances enzymatic accessibility, its high energy requirements highlight the need for combined strategies to improve cost-effectiveness at scale.

Sant'Ana da Silva et al. (2010) reported energy consumption of 48 MJ/kg for wet disk milling of sugarcane bagasse and 39.6 MJ/kg for sugarcane straw. Although less energy-intensive than other methods, wet disk milling achieved lower glucose conversion rates than ball milling. However, it avoided fermentation inhibitors, offering an environmentally friendly alternative. The authors suggested further optimization to improve enzymatic yields and reduce energy demands.

Buaban et al. (2010) acknowledged the high energy intensity of ball milling but emphasized its effectiveness in converting crystalline cellulose into amorphous forms, significantly enhancing enzymatic hydrolysis. However, diminishing returns with extended milling times, such as four hours, highlight the need to balance energy input and yield improvements. The study recommended combining ball milling with complementary methods to reduce energy requirements while maintaining efficiency.

Overall, while mechanical comminution methods like ball milling and disk milling improve biomass digestibility, their high energy demands and costs remain barriers to industrial implementation. These studies emphasize the importance of integrated processes and advancements in energy-efficient equipment to enhance economic feasibility and sustainability.

## 6.2. Ultrasound

The reviewed studies provided limited data on the energy consumption and costs of ultrasound pretreatment, except for Velmurugan and Muthukumar (2012), who focused on sugarcane bagasse. They reported that the energy requirements for ultrasound pretreatment were 72 MJ/kg, significantly lower than steam explosion (99 MJ/kg) and autoclave methods (233 MJ/kg), positioning ultrasound as a more energy-efficient alternative. Similarly, Hu et al. (2013) described ultrasound as a low-energy method compared to conventional soybean and corn straw approaches. However, they did not include quantitative energy data or detailed cost analyses.

Ultrasound efficiency depends on operational parameters, such as power, frequency, exposure time, and the solid-to-liquid ratio (SLR). Higher power levels improve biomass structural disruption and lignin removal but proportionally increase energy consumption. For example, Liu et al. (2018) applied ultrasound to eucalyptus wood for up to six hours, demonstrating wide variation in treatment durations that affect process efficiency and energy use.

Given the variability in operational conditions, energy assessments must be tailored to specific biomass types and configurations. Systematic evaluations are needed to identify optimal, cost-effective configurations to advance ultrasound pretreatment for industrial applications.

## 6.3. Microwave

Microwave pretreatment offers potential advantages, but these depend on process parameters, biomass type, and application scale. Zhu et al. (2016) reported a 5.7-fold reduction in processing time using sulfuric acid-assisted microwave pretreatment, significantly lowering energy consumption compared to conventional heating. Ude et al. (2022) found that microwave pretreatment of elephant grass reduced hydraulic retention time in anaerobic digestion, indirectly minimizing energy

demands. Similarly, Kłosowski et al. (2020) highlighted that microwave usage is more energy-efficient than conventional methods, although the study did not discuss detailed cost analyses. However, high power levels can undermine economic benefits if not optimized, as observed by Binod et al. (2012) and Karunanithy et al. (2014).

Catalyst usage also influences costs. For instance, Fonseca et al. (2021) and Miranda et al. (2014) enhanced sugar yields with acidic solutions, increasing material costs. Alternatively, Sasaki et al. (2011) applied biological pretreatment before microwave application, reducing chemical usage but extending processing times.

Notably, most studies fail to provide detailed cost analyses for microwave-assisted pretreatment, leaving a significant knowledge gap in evaluating its economic feasibility. Despite the potential for energy savings, scaling microwave-assisted pretreatment remains challenging. Optimizing process parameters and integrating synergistic methods are critical to ensuring economic viability at industrial scales.

#### 6.4. Gamma Radiation

Gamma radiation pretreatment enhances biomass digestibility nevertheless faces challenges related to energy consumption and operational costs. These costs stem from maintaining cobalt-60 sources, irradiator efficiency, and cooling systems (Al Gharib et al., 2023).

While gamma radiation eliminates chemical reagents and extensive post-treatment, it involves high energy demands. Wu et al. (2020) noted significant energy savings in grinding irradiated residues, offsetting initial irradiation costs. Biomass densities and recalcitrance levels influence energy needs, with woody biomass requiring higher doses (1–2 MGy) than agricultural residues like sugarcane bagasse (100–800 kGy).

Although gamma radiation avoids energy-intensive heat treatments, its high operational costs necessitate innovations in irradiator design and process integration to enhance feasibility.

#### 6.5. Electron Beam

Electron beam pretreatment modifies lignocellulosic biomass structures but requires substantial energy for electron accelerators operating at 3–12 MeV (Al Gharib et al., 2022; Shen et al., 2022). The doses needed (90 kGy to 3 MGy) depend on biomass type, with woody materials demanding higher energy due to more remarkable recalcitrance.

Integrating electron beams with other methods, such as hydrothermal pretreatment, may reduce energy consumption, though detailed quantitative data are lacking. Advances in equipment efficiency and parameter optimization are essential for industrial scalability.

##### 6.5.1. Extrusion

Extrusion pretreatment enhances enzymatic accessibility but presents challenges related to energy consumption. Operational parameters like screw speed, temperature, and moisture content significantly affect energy demands.

Karunanithy et al. (2012) reported that increasing screw speed and temperature improved sugar recovery but proportionally raised energy consumption. Moro et al. (2017) observed specific energy consumption (SEC) ranging from 20 kJ/g to 123 kJ/g for sugarcane residues under different settings. These findings underscore the importance of parameter optimization.

Moisture content also affects energy efficiency. Higher moisture levels reduce shear force and heat generation, increasing energy demands. Tailoring extrusion settings for specific biomass types and integrating renewable energy sources can mitigate costs, making the process more viable for industrial applications.

##### 6.5.2. Pulsed Electric Field (PEF)

PEF pretreatment offers low energy requirements under controlled conditions, disrupting cell membranes with moderate electric field intensities (Kumar et al., 2011). However, industrial scaling challenges include larger chambers and higher current demands, which increase energy consumption (Basak et al., 2023). Optimizing treatment parameters, such as pulse duration, is critical to avoiding unnecessary energy use. While PEF shows promise at laboratory scale, innovations in equipment design and cost assessments are needed for industrial feasibility. These advancements will be crucial to realizing PEF's potential as an energy-efficient pretreatment method.

## 7. Conclusions and Critical Overview of Methods Used for Physical Pretreatment

Developing efficient and sustainable strategies to address the recalcitrance of lignocellulosic biomass is crucial for advancing modern biorefineries. While the physical pretreatment techniques explored in this review have achieved significant progress in enhancing biomass accessibility and boosting sugar yields, several challenges that limit their industrial scalability remain. Among these are high energy requirements, the complexity of optimizing operational parameters, and the need to tailor processes to accommodate the natural variability in biomass composition.

A deeper examination of these methods reveals that no single solution fits all scenarios. Each technique has unique strengths and limitations, often determined by the type of biomass and the desired end products. This reinforces the need for continuous research to understand these methods better and to develop innovative combinations. Hybrid approaches that combine the benefits of multiple technologies are gaining recognition as promising solutions for efficient biomass deconstruction while addressing energy and cost concerns.

Economic and environmental sustainability are critical considerations for these methods. Advances in energy optimization and cost reduction are essential to improve feasibility. Integrating renewable energy sources and repurposing by-products within circular bioeconomy frameworks can further enhance sustainability. Moreover, scaling laboratory successes to industrial applications will be key to ensuring both effectiveness and commercial viability.

### 7.1. Common Aspects of Physical Pretreatment Technologies

#### 7.1.1. Structural Modification

All methods effectively modify the structure of lignocellulosic biomass, making it less resistant and improving enzymatic digestibility—techniques like extrusion and ultrasound focus on creating microchannels and exposing cellulose fibers. In contrast, gamma radiation and electron beam methods effectively disrupt dense biomass structures. These structural changes are vital for increasing surface area and porosity, directly enhancing fermentable sugar production.

#### 7.1.2. Energy Demands

Energy consumption is a consistent challenge across all approaches, particularly those involving high temperatures or specialized equipment. However, optimizing parameters such as moisture content, processing speed, and temperature can help mitigate these costs. For instance, ultrasound has demonstrated lower energy requirements than traditional methods like autoclaving or hydrothermal treatment.

#### 7.1.3. Adaptability to Biomass Variability

The efficiency of each technology often depends on the type of biomass being processed. Gamma radiation, for example, is highly adaptable to different substrates, while extrusion may require specific adjustments to optimize sugar recovery in dense or lignin-rich biomass. Therefore, customizing pretreatment strategies to align with biomass characteristics is essential.

#### 7.1.4. Generation of Inhibitors

Some methods can form inhibitory compounds, such as HMF and organic acids, especially when aggressive chemical agents are involved. However, this is not a universal issue. Ultrasound and electron beam pretreatments, when applied without chemical additives, typically result in fewer inhibitors, offering a significant advantage for subsequent biological processes. Additionally, post-treatment washing, commonly used in extrusion, helps minimize the impact of inhibitors.

### 7.2. Mechanical Comminution

Mechanical comminution is a foundational step in processing lignocellulosic biomass, focusing on particle size reduction and increased surface area to enhance subsequent pretreatment and conversion processes. While its isolated effects are rarely studied, earlier research highlights its potential for high saccharification yields, particularly in addressing physical recalcitrance (Inoue et al., 2008; Sant'Ana da Silva et al., 2010). However, extended processing times and high energy consumption remain significant challenges, especially when processing biomass with high lignin content or crystallinity.

Despite these limitations, mechanical comminution ensures particle uniformity and compatibility with downstream operations, making it an integral part of industrial biorefineries. Most studies emphasize its preparatory role, with biomass typically processed to particle sizes below 2 mm to optimize reactivity and facilitate the application of other pretreatment techniques. This essential function underscores its importance within the biomass conversion chain.

### 7.3. Ultrasound

Ultrasound pretreatment has demonstrated significant potential for enhancing the enzymatic digestibility of lignocellulosic biomass. Leveraging cavitation and sonochemical effects, ultrasound effectively disrupts recalcitrant structures, increasing cellulose accessibility and sugar yields. This method is exceptionally versatile, as it induces notable microstructural changes, such as forming microchannels and removing lignin, across a variety of biomass types, including sugarcane bagasse, straw, and woody materials like *Eucalyptus grandis* × *E. urophylla*.

A key advantage of ultrasound is its minimal inhibitor formation during standalone pretreatment, facilitating subsequent enzymatic hydrolysis. However, combining ultrasound with chemical agents under harsher conditions introduces risks of inhibitor generation, requiring precise parameter optimization to minimize adverse effects. Studies have shown synergistic benefits in these combined approaches, further underscoring ultrasound's potential in integrated pretreatment strategies.

Despite these advantages, energy consumption and cost challenges remain critical for scaling ultrasound to industrial applications. Velmurugan and Muthukumar(2012) research positions ultrasound as an energy-efficient alternative to steam explosion and autoclaving methods. However, the absence of standardized benchmarks limits a comprehensive comparison of its industrial competitiveness. Moreover, while numerous studies have explored biomass types like sugarcane bagasse and *Eucalyptus*, research on energy crops remains scarce. Notably, soybean hulls—biomass of high economic importance in countries like Brazil—are underrepresented, highlighting a significant gap in the literature.

In conclusion, ultrasound pretreatment is a promising and versatile approach to enhancing enzymatic hydrolysis. However, optimizing operational parameters, validating energy and cost metrics, and addressing underexplored biomass types, such as soybean hulls, are necessary to establish ultrasound as a sustainable and competitive technology for industrial applications.

### 7.5. Microwave

Microwave-assisted pretreatment demonstrates significant potential to enhance the enzymatic digestibility of lignocellulosic biomass. Its rapid heating and efficient structural disruption offer clear advantages, particularly for agricultural residues such as sugarcane bagasse and energy grasses like switchgrass (Zhu et al., 2016; Fonseca et al., 2021). In many cases, low levels of inhibitor formation facilitate downstream fermentation processes, making it an appealing method for integrated biorefineries. However, more recalcitrant biomass types, such as hardwoods, often require harsher conditions or catalysts, which can increase energy demands and costs.

Despite advancements, critical gaps persist in the literature. For example, while energy crops like sugarcane have been extensively studied, biomass such as soybean hulls remains largely

unexplored. Given the economic relevance of soybeans in countries like Brazil, exploring these underutilized materials could unlock new industrial applications.

Energy consumption and cost optimization remain significant challenges. Microwave pretreatment has demonstrated energy efficiency and reduced processing times compared to conventional methods (Zhu et al., 2016). However, the economic feasibility of standalone microwave use depends on further optimization. Eliminating the need for catalysts could reduce costs but might limit efficiency for denser biomass. Alternatively, combining microwaves with methods such as extrusion or biological treatments shows promise in mitigating costs, though scalability and industrial viability require further validation (Karunanithy et al., 2014; Sasaki et al., 2011).

Another critical aspect is scaling microwave pretreatment for industrial applications. While laboratory results are promising, few studies have evaluated its integration into biorefineries or its ability to handle large biomass volumes efficiently. Addressing this gap is essential to determine its practicality in industrial settings.

#### 7.6. Gamma Rays

Gamma radiation pretreatment holds significant promise for enhancing the digestibility of lignocellulosic biomass by breaking down recalcitrant structures and increasing enzymatic accessibility. Its ability to penetrate dense materials uniformly and operate without chemical reagents distinguishes it as a sustainable method for biomass processing. Studies such as Al Gharib et al. (2023) and Wu et al. (2020) have demonstrated their effectiveness in converting various biomass types, from woody materials to agricultural residues, into fermentable sugars. However, energy consumption and operational cost challenges must be addressed for broader adoption.

The infrastructure required for gamma radiation, including cobalt-60 sources and shielding systems, contributes to high initial investments and maintenance expenses. Comparisons with methods like electron beam and hydrothermal pretreatment reveal that while gamma radiation offers superior penetration and uniform treatment, its energy demands are often higher. Kapoor et al. (2022) emphasized that continuous operation for large-scale biomass processing highlights the need for innovations to enhance energy efficiency and cost-effectiveness.

Hybrid approaches provide a pathway to mitigate these limitations. Combining gamma radiation with techniques such as ultrasonic or alkaline treatments has shown a potential to reduce energy demands while maintaining or enhancing biomass digestibility. Wu et al. (2020) reported significant improvements in enzymatic hydrolysis yields through a combined gamma radiation and ultrasound approach, underscoring the benefits of integrating technologies to optimize pretreatment processes.

Future research must prioritize innovations that improve energy efficiency and reduce fixed costs, such as advancements in cobalt-60 recycling and developing more efficient irradiators. Incorporating gamma radiation as a step within multi-purpose biorefineries could also distribute energy costs across multiple products, enhancing its industrial viability. Moreover, valorizing by-products like lignin and biochar within circular bioeconomy frameworks could improve this technology's sustainability and profitability.

Despite their potential, certain biomass types remain underexplored. While studies on agricultural residues and woody materials are standard, hardwood biomass and soybean residues—particularly relevant in regions like Brazil—are scarcely investigated. Addressing these gaps through targeted research could expand the applicability of gamma radiation and unlock new opportunities for sustainable biomass processing. Gamma radiation pretreatment offers a promising pathway for sustainable biomass utilization. Future efforts should address scalability challenges, optimize dose requirements, and integrate gamma radiation into circular bioeconomy models. Gamma radiation could become a cornerstone technology in advancing bio-based industries by overcoming these technical and economic barriers.

### 7.7. Electron Beam

Electron beam pretreatment offers significant potential to enhance the enzymatic digestibility of lignocellulosic biomass, leveraging its ability to induce structural disorganization, reduce cellulose crystallinity, and minimize inhibitor formation. These advantages have been demonstrated across diverse biomass types, including woody and non-woody materials, as Al Gharib et al. (2022) and Rattanawongwiboon et al. (2022) reported. However, critical challenges, particularly regarding energy demands and scalability, limit its widespread adoption in biorefineries.

The process's high energy requirements, especially for dense or highly recalcitrant biomass like hardwoods, highlight the need for optimization. Studies such as those by Duarte (2012) and Shen et al. (2022) suggest that strategies like dose optimization or integration with complementary treatments can reduce energy consumption. However, the scalability of these approaches remains uncertain. Additionally, the lack of comprehensive economic analyses and standardized data on operational costs underscores a significant research gap. Future advancements in renewable energy integration and energy recovery systems are essential for improving feasibility.

Another limitation lies in the underexplored potential of electron beam hydrolysates in fermentation processes. Although the absence of significant inhibitors in many cases, as noted by Guo et al. (2016) and Karthika et al. (2012), suggests compatibility with efficient fermentation, no studies directly investigate this application. Validating the fermentability of hydrolysates is crucial for assessing the method's industrial applicability and identifying the potential for higher-value product generation.

While electron beam pretreatment has shown promise in small-scale applications, its integration into large-scale biorefineries remains challenging. Detailed cost-benefit evaluations and operational efficiency assessments are necessary to establish their role in multiproduct biorefineries. Leveraging by-products like biochar, as demonstrated by Rattanawongwiboon et al. (2022), could enhance sustainability by creating additional revenue streams. Furthermore, materials like hardwoods and soybean residues—biomass types with high economic and industrial relevance—represent unexplored opportunities for future research, particularly in regions such as Brazil.

In conclusion, electron beam pretreatment stands out for its ability to produce high-quality hydrolysates with minimal inhibitors, making it a promising technology for advancing lignocellulosic biomass utilization. Targeted research focusing on energy optimization, economic feasibility, and industrial-scale validation is critical to unlocking its full potential. By addressing these challenges and exploring underutilized biomass types, electron beam pretreatment could emerge as a scalable and sustainable solution within the bioeconomy landscape.

### 7.8. Extrusion

Extrusion pretreatment is a versatile method for enhancing the enzymatic digestibility of lignocellulosic biomass. By providing significant structural modifications, such as increased surface area and reduced cellulose crystallinity, extrusion adapts to various biomass types and operational conditions, making it a promising candidate for integration into biorefinery processes. Studies highlight its efficacy for non-woody and woody biomass, with glucose yields reaching 95% from soybean hulls (Yoo et al., 2011) and ethanol yields of 55.3% from eucalyptus wood chips (Tian et al., 2019). These results underscore the importance of optimizing key parameters, including screw speed, temperature, and moisture content, to maximize sugar recovery.

Energy efficiency remains a critical concern for extrusion pretreatment. Specific energy consumption (SEC) values vary widely depending on operational settings, as Moro et al. (2017) reported, ranging from 20 kJ/g to 123 kJ/g. While high screw speeds and low moisture content enhance sugar recovery, they also increase energy demands. Balancing these factors is crucial, with strategies like integrating renewable energy sources or energy recovery systems offering potential solutions. Additionally, precise moisture control is essential to ensure efficient mechanical shear and heat effects, preventing excessive energy requirements.

Extrusion also offers advantages in managing inhibitory compounds. Lamsal et al. (2010) and Duque et al. (2018) highlight that post-extrusion washing effectively mitigates inhibitors like HMF and organic acids, improving sugar recovery and fermentation efficiency. Extrusion generates fewer inhibitors than other pretreatment methods, particularly when aggressive chemical additives are avoided, enhancing its compatibility with downstream biological processes.

Extrusion's industrial relevance extends to bioethanol, biogas, and biochemical production applications. For example, methane production from extruded corn cob increased by 22.3% (Perez-Rodriguez et al., 2017), while biogas yields from grass residues improved by 18% (Souza et al., 2021). These findings emphasize extrusion's potential to enhance anaerobic digestibility and diversify end-product outputs, supporting multiple biorefinery pathways.

In conclusion, extrusion pretreatment demonstrates flexibility and effectiveness in processing lignocellulosic biomass, improving enzymatic accessibility and enabling diverse downstream applications. However, industrial scalability depends on advancements in energy optimization and cost reduction. Future research should focus on refining operational parameters for specific biomass types, exploring energy-efficient configurations, and validating large-scale applications. By addressing these challenges, extrusion can solidify its role as a core technology in the sustainable development of biorefineries.

### 7.9. Pulsed Electric Field (PEF)

Pulsed Electric Field (PEF) pretreatment is an emerging method for reducing the recalcitrance of lignocellulosic biomass. By enhancing cell permeability and porosity, PEF shows promise in improving enzymatic digestibility and biogas production. Studies such as those by Kumar et al. (2011) and Szwarc and Szwarc (2021) highlight its operational simplicity and energy efficiency, particularly in small-scale applications. However, the scope of current research remains limited, with significant gaps that must be addressed to enable broader adoption.

A critical gap lies in evaluating PEF's effectiveness across diverse biomass types. Most studies focus on specific feedstocks, such as corn silage and switchgrass, leaving structural and chemical variability in other biomass types underexplored. While increased porosity is theorized to enhance enzymatic hydrolysis, experimental validation of this correlation is still lacking. Future studies should aim to quantify the physical and chemical changes induced by PEF and link these changes to improvements in sugar release efficiency.

Another major challenge is optimizing PEF parameters for industrial-scale applications. Scaling up will require the development of high-throughput pretreatment chambers capable of processing large biomass volumes while maintaining energy efficiency. Although preliminary findings suggest PEF can complement other pretreatment methods, its standalone efficacy at industrial scales has yet to be sufficiently validated.

Despite these challenges, PEF offers unique advantages, such as its ability to operate under ambient conditions and relatively low energy consumption in specific settings. These attributes position it as a potential preparatory or complementary step within integrated biorefinery strategies. By addressing limitations in parameter optimization, equipment design, and experimental validation, PEF could significantly improve biological and chemical conversion processes.

In conclusion, PEF pretreatment demonstrates considerable potential as an innovative method for processing lignocellulosic biomass. However, advancing its industrial feasibility will depend on targeted research to optimize operational parameters, validate large-scale performance, and explore its integration into multiproduct biorefineries. With further development, PEF could emerge as a sustainable and efficient technology within the bioeconomy framework.

Refining and innovating pretreatment technologies to balance cost and efficiency is critical for advancing the bioeconomy. Adjusting operational parameters to handle the variability of different biomass types is vital in this process. As the bioeconomy continues to grow, physical pretreatment methods are positioned to play a central role in unlocking the potential of lignocellulosic biomass. Overcoming current technical and economic challenges will pave the way for these methods to

become integral components of industrial biorefineries. Future research should focus on hybrid technologies, energy optimization, and integration into multiproduct biorefineries, ensuring these methods contribute to a more sustainable and profitable future for biomass utilization.

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