



## Optimizing Pretreatment Processes to Boost Bioethanol Yields from Lignocellulosic Biomass: A Review

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### Abstract

Fossil fuels remain the dominant global energy source, yet their depletion is projected within the next 40–50 years. Coupled with environmental challenges such as climate change, acid deposition, and air pollution, this has intensified the shift toward renewable energy alternatives, including solar, wind, and biofuels. Among these, bioethanol from lignocellulosic biomass (LCB) offers a sustainable solution, though its production faces challenges such as biomass logistics and the need for efficient pretreatment to overcome the recalcitrant structure of lignocellulose. Pretreatment is a critical step to enhance enzymatic hydrolysis and maximize sugar recovery, with numerous strategies available, each varying in mechanism, feasibility, and efficiency. This review provides a comprehensive overview of current pretreatment technologies for LCB, highlighting their advantages, limitations, and key considerations for developing cost-effective, high-performance processes for second-generation bioethanol production.

**Keywords:** Bioethanol, Biomass, Biorefinery, Inhibitor, Lignocellulose, Pretreatment

### 1 Introduction

The escalating challenge of carbon dioxide (CO<sub>2</sub>) emissions, coupled with concerns over energy security, has intensified interest in alternative energy sources that are not derived from petroleum. Biomass is uniquely positioned as a sustainable primary energy source for producing transportation fuels such as bioethanol and biodiesel [1]. Lignocellulose-based fuels present a promising solution, as they not only ensure fuel supply for transportation but also mitigate environmental pollution [2]. Agricultural residues generated during or after crop processing constitute a

renewable and abundant lignocellulosic biomass resource. Unlike conventional energy crops, converting agricultural waste into bioenergy does not compete with food production or require additional land, water, or energy inputs [3]. Lignocellulosic biomass represents the most prevalent form of renewable biomass, with an estimated global annual output of  $1 \times 10^{10}$  metric tons [4]. Major crops contributing to this resource include wheat, maize, rice, and sugarcane, which together yield more than 5,300 million tons of dry biomass annually [5].

Despite its potential, large-scale production of bioethanol from lignocellulose remains constrained by



economic and technical barriers [4]. Strategies to reduce production costs include maximizing raw material utilization, achieving higher ethanol yields and concentrations, and incorporating energy-efficient processing [6]. The production of lignocellulosic ethanol generally involves three steps: pretreatment to remove lignin and enhance cellulose and hemicellulose accessibility, hydrolysis to release fermentable sugars, and fermentation to convert these sugars into ethanol [7]. Lignocellulose is primarily composed of cellulose and hemicellulose, intricately bound by lignin, which provides structural integrity and contributes to resistance against enzymatic breakdown. Pretreatment aims to dismantle these complex structures, improving enzyme accessibility and enabling the conversion of carbohydrate polymers into fermentable sugars. The degree to which lignin and hemicellulose are removed depends on the specific pretreatment technique, process parameters, and treatment intensity [8]. Improving the separation of the major polymeric components within lignocellulose not only enhances bioconversion but also opens the potential for lignin to serve as a valuable source of bio-based chemicals and fuels. Pretreatment is a crucial step in the conversion of lignocellulosic biomass into ethanol, often contributing 30–70% of the total production cost and acting as a significant hurdle for commercial-scale deployment. The majority of current pretreatment strategies utilize physicochemical techniques, including steam explosion, dilute acid, alkaline, and oxidative processes, either independently or in various combinations [9].

Current pretreatment methods, however, face significant challenges such as high energy input, generation of inhibitory byproducts, environmental concerns, and limited scalability. These limitations underscore the motivation to develop innovative, cost-effective, and environmentally benign pretreatment strategies that can achieve high conversion efficiency while minimizing operational costs and downstream processing barriers. Achieving such advancements is essential not only for making lignocellulosic ethanol production commercially viable but also for supporting global efforts to reduce greenhouse gas emissions and dependence on fossil fuels. This review provides a succinct overview of pretreatment processes, with a focus on recent innovations in each method. It further evaluates the advantages and limitations of these technologies and discusses their application to a wide range of feedstocks, including herbaceous crops, agricultural

residues, hardwood, and softwood biomasses. Particular attention is given to the structural modifications of cellulose, hemicellulose, and lignin that occur during the major pretreatment processes.

## 2 Ethanol Production From Various Types of Biomass

Ethanol can be derived from a variety of biomass feedstocks rich in fermentable sugars or precursors that can be enzymatically or chemically converted into sugars, such as starches and cellulose. Currently, the primary sources for bioethanol include sucrose-rich crops—such as sugarcane, sugar beet, and sweet sorghum—as well as starch-based materials like corn, cassava, wheat, and rye (Figure 1). Among these, corn, sugar beet, and sugarcane are recognized as the most efficient in terms of ethanol yield and productivity per hectare [10]. Corn, one of the most widely cultivated crops globally, is especially prominent in the United States, where the corn belt offers optimal climatic conditions for high productivity. Sugarcane and sugar beet, while both highly productive, thrive in distinct environments: sugarcane flourishes in consistently warm regions, whereas sugar beet performs best in temperate climates [11]. Brazil is recognized as a model for sugarcane-based ethanol production due to its mature and economically viable program, which has succeeded with relatively limited government support. Meanwhile, in the European Union, sugar beet is the principal feedstock for ethanol, with France leading production, followed by Germany [12]. Recent restrictions imposed by the European Union on bioethanol imports have created a more supportive environment for domestic producers, enabling them to scale up operations and improve overall production efficiency. This shift has increased capacity utilization for EU-based bioethanol facilities, rising from 55% to 62% [13].



**Figure 1:** Biomass-derived energy production from diverse sources.

The selection of feedstocks plays a pivotal role in ethanol production, as the process and environmental impacts vary depending on the raw material used. Ethanol is generally classified into four generations, based on the type of feedstock and the technology employed (Table 1). First-generation (1G) ethanol is derived from edible crops, while the advanced generations, second (2G), third (3G), and fourth (4G), primarily utilize non-food-based resources. Each generation of biofuels offers distinct advantages. For example, 1G biofuels help manage surplus agricultural production, while all generations contribute to renewable energy supply and achieve notable reductions in greenhouse gas emissions compared with gasoline [14]. Nonetheless, significant challenges remain. 1G production is often criticized for intensifying the food-versus-fuel debate; 2G technologies can be resource-intensive, particularly in terms of energy and water requirements; 3G biofuels derived from algae face issues of high energy demand and nutrient imbalances; and 4G approaches, though promising, are still in early stages, with substantial costs and energy inputs hindering large-scale commercialization [15].

**Table 1:** Categorization of ethanol production methods.

Generations	Primary Raw Materials	Production Technique	CO <sub>2</sub> Balance
1 <sup>st</sup>	Biomass is derived from food sources rich in starch and sugar, such as wheat, maize, sugar beet, and sugarcane.	Fermentation	Positive
2 <sup>nd</sup>	Lignocellulosic biomass, such as agricultural and non-agricultural waste, along with a variety of grasses and tree species.	Hydrolysis and subsequent fermentation	Neutral
3 <sup>rd</sup>	Microscopic organisms, for example, microalgae, which have marine or aquatic habitats	Hydrolysis and subsequent fermentation	Negative
4 <sup>th</sup>	Microorganisms, for instance, microalgae, are genetically engineered.	Hydrolysis and subsequent fermentation	Negative

Ethanol is primarily used as a fuel additive, with blending ratios ranging from E1 (1% ethanol) to E100 (pure ethanol). The widespread use of pure ethanol is limited globally due to vehicle compatibility issues and performance challenges in colder climates. The global ethanol market is notably more consolidated

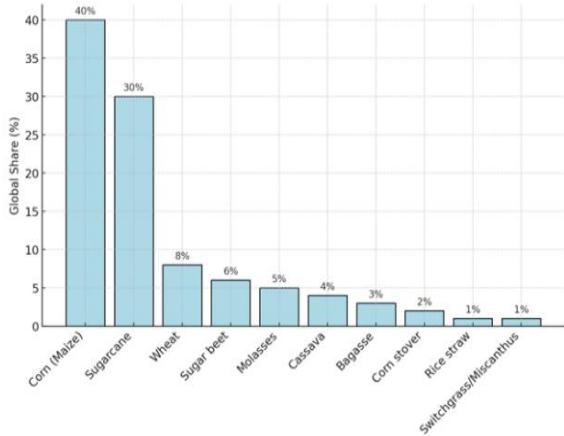
than the biodiesel industry, with two major producers—the United States and Brazil—accounting for 74% of global ethanol output (Table 2). In 2019, the U.S. led production with 59,809 million liters, comprising 46% of the global total, followed by Brazil with 36,238 million liters, or 28%. Other significant contributors included China (10,500 million liters), the European Union (6,370 million liters), and India (3,073 million liters), corresponding to 8%, 5%, and 3% of global output, respectively. Despite strong domestic demand, both the U.S. and Brazil produced ethanol surpluses in 2019, exceeding their internal consumption by 7.45% and 2.50%, respectively. Meanwhile, ethanol production in China, the EU, and India remained below their respective national demand levels.

**Table 2:** Global rankings of fuel ethanol production and their corresponding main biomass sources for the year up to 2023 (Sources: United States Department of Agriculture–Foreign Agricultural Service (USDA-FAS). (2023). Indonesia: Biofuels Annual 2023 (Report No. ID2023-0018).

Nation	Production Million Gallons	Primary Feedstock
United States	15,580	Corn
Brazil	8,470	Sugarcane
European Union	1,390	Likely Sugar beet
China	1,070	Corn/wheat
Canada	1,070	Corn/wheat
Thailand	340	Sugarcane/cassava
Argentina	310	Sugarcane & corn combinations
India	1,510	Molasses, cane juice, corn (diversifying)
The rest of the world	33%	Various

Sugarcane is one of the most important feedstocks for bioethanol production, with large-scale cultivation in tropical regions [16]. In temperate climates such as Europe, sugar beet is a key source of fermentable sugars, while molasses—a by-product of sugar refining—is widely used in India and other major sugar-producing countries. Growing attention is now being directed toward cellulosic and agricultural residue-based feedstocks, including wheat straw, corn stover, rice straw, sorghum stalks, and sugarcane bagasse, owing to their non-food nature and potential environmental benefits [17]. Among these, sugarcane bagasse—the fibrous residue left after juice extraction—is extensively utilized for advanced bioethanol production as well as power generation. In addition, dedicated energy crops such

as switchgrass and Miscanthus are cultivated in the United States and Europe to provide biomass for cellulosic ethanol production (Figure 2).

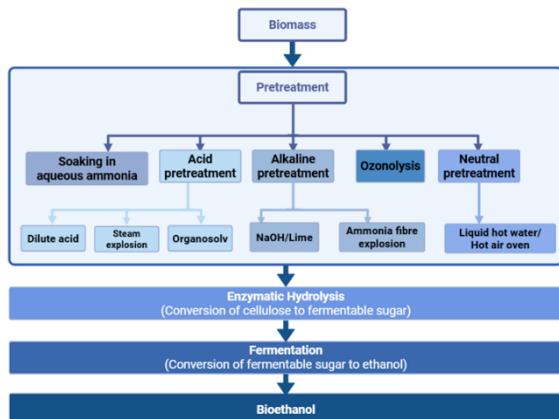


**Figure 2:** Most utilized substrates for bioethanol yield over the past decade (International Energy Agency (IEA) (2023)).

### 3 Pretreatment Methods

Lignocellulosic biomass is composed primarily of three complex biopolymers: cellulose, hemicellulose, and lignin. While these components are well characterized, their intricate interactions remain only partially understood and vary depending on species and environments. The bioethanol production process generally includes pretreatment, enzymatic hydrolysis of polysaccharides into simple sugars, fermentation to convert sugars into ethanol, and distillation for product purification [18] (Figure 3). Therefore, sugars are key intermediates, and it is essential to disrupt the biomass matrix to release fermentable sugars from cellulose and hemicellulose.

Among these, pretreatment is particularly critical for improving enzyme accessibility by increasing surface area and porosity, reducing cellulose crystallinity, and altering the structural integrity of the lignocellulosic network [19]. A well-designed pretreatment should render cellulose and hemicellulose more amenable to cellulolytic enzymes while minimizing the formation of inhibitory by-products, energy consumption, and chemical use. Additional considerations include wastewater treatment, catalyst recovery, and solvent recyclability, all of which influence process sustainability [20].



**Figure 3:** Presents a flow sheet that details the bioconversion pathway from biomass to bioethanol.

Over the past decade, a variety of pretreatment strategies have been investigated, each with distinct advantages and limitations (Table 3). Steam explosion, a widely adopted physico-chemical method, disrupts the rigid biomass structure at relatively low cost and has proven scalability and environmental compatibility. However, incomplete lignin removal and the generation of degradation products remain challenges [21].

Collectively, these three polymers form a robust composite network through multiple physicochemical interactions. Cellulose microfibrils are embedded within a matrix of hemicellulose and lignin, where hemicellulose functions as a structural bridge between the two [37]. Hydrogen bonding between cellulose and hemicellulose facilitates the coating and stabilization of cellulose microfibrils, while covalent bonds—including benzyl ether, benzyl ester, and phenyl glycosidic linkages—create lignin-carbohydrate complexes (LCCs) that interconnect lignin and hemicellulose. These LCCs form a rigid, cross-linked matrix that encases cellulose, restricting enzyme accessibility. Structurally, cellulose microfibrils are arranged in parallel, surrounded by hemicellulose that fills the interstitial spaces, with lignin polymerizing *in situ* to occupy the remaining voids and covalently bind to hemicellulose [38]. This intricate, interpenetrating architecture contributes to the intrinsic recalcitrance of lignocellulosic biomass, posing a major challenge for enzymatic hydrolysis and bioethanol production [39].

**Table 3:** Pretreatment applications for various types of biomass for cellulose conversion.

Pretreatment Method	Biomass Type	Pretreatment Conditions	Cellulose Conversion (%)	Enzyme Dosage	References
Dilute acid pretreatment (DAP)	Corn stover	140°C, 1.0 wt% H <sub>2</sub> SO <sub>4</sub> , 40 min	82.3 in 72 h	15 FPU/g cellulase, 26.25 CBU/g β-glucosidase	[22]
	Olive tree	210°C, 1.4 wt% H <sub>2</sub> SO <sub>4</sub> , 10 min	76.5 in 72 h	15 FPU/g cellulase, 15 CBU/g β-glucosidase	[23]
	Loblolly pine	180°C, 1.0 wt% H <sub>2</sub> SO <sub>4</sub> , 30 min	35 in 72 h	20 FPU/g cellulase, 40 IU/g β-glucosidase	[24]
	Sugarcane bagasse	121°C, 2.18 v% H <sub>2</sub> SO <sub>4</sub> , 29.49 min	72.06 in 72 h	20 FPU/g cellulase	[25]
Steam explosion pretreatment (SEP)	Sugarcane bagasse	121°C, 2.18 v% H <sub>2</sub> SO <sub>4</sub> , 29.49 min	72.06 in 72 h	20 FPU/g cellulase	[25]
	Wheat straw	190°C, 10 min	85 in 72 h	15 FPU/g cellulase, 12.6 IU/g β-glucosidase	[26]
	Poplar	220°C, 4 min	60 in 72 h	15 FPU/g cellulase, 12.6 IU/g β-glucosidase	[27]
	Douglas fir	4.5 wt% SO <sub>2</sub> , 195°C, 4.5 min	54.2 in 72 h	20 FPU/g cellulase, 35 CBU/g β-glucosidase	[22]
Organosolv	Bagasse	210°C, 2 wt% SO <sub>2</sub> , 5 min	70 in 72 h	18 FPU/g cellulase, 30 CBU/g β-glucosidase	[28]
	Miscanthus	170°C, 80 min, 1.2 wt% H <sub>2</sub> SO <sub>4</sub> , 50% ethanol	78 in 48 h	20 FPU/g cellulase, 40 IU/g β-glucosidase	[29]
	Poplar	180°C, 60 min, 1.25 wt% H <sub>2</sub> SO <sub>4</sub> , 50% ethanol	97 in 48 h	20 FPU/g cellulase, 40 IU/g β-glucosidase	[30]
	Lodgepole pine	170°C, 80 min, 1.1 wt% H <sub>2</sub> SO <sub>4</sub> , 65% ethanol	97 in 48 h	20 FPU/g cellulase (Spezyme CP), 40 IU/g β-glucosidase	[31]
Liquid hot water (LHW)	Bamboo	185°C, 75 min, 1.3 wt% H <sub>2</sub> SO <sub>4</sub> , 60% ethanol	83 in 48 h	18 FPU/g cellulase, 35 IU/g β-glucosidase	[32]
	Corn stover	190°C, 15 min	69.6 in 72 h	15 FPU/g cellulase (Spezyme CP), 65 IU/g β-glucosidase	[33]
	Poplar	200°C, 10 min	52 in 72 h	15 FPU/g cellulase, 40 CBU/g β-glucosidase	[34]
	Radiata pine	200°C, 30 min	27 in 72 h	20 FPU (C-30 cellulase), Novozyme-derived	[35]
	Clover-grass press cake	190°C, 10 min	90% in 72 h	24mg/g Cellic CTec3 HS	[36]

**Table 4:** The impact of various chemical pretreatment technologies on the structure of lignocellulose.

Pretreatment Approach	Enhanced Surface Area Accessibility	Cellulose Structural Changes	Hemicellulose Dissolution	Delignification	Formation of Inhibitory Compounds	Lignin Structure alteration
DAP	SI	LI	SI	LI	SI	SI
SEP	SI	LI	MI	LI	SI	MI
Organosolv	SI	LI	SI	SI	SI	SI
LHW	SI	LI	MI	LI	LI	MI
NaOH/ Ca(OH) <sub>2</sub>	SI	LI	MI	MI	LI	SI
Ammonia Fiber Explosion	SI	SI	LI	LI	LI	SI
Soaking in aqueous ammonia	SI	LI	LI	SI	LI	LI
Ozonolysis	SI	LI	MI	SI	LI	SI

Dilute acid pretreatment, particularly with sulfuric acid, is well established for solubilizing hemicellulose and enhancing cellulose digestibility, though issues such as equipment corrosion and the

formation of furfurals and hydroxymethylfurfural hinder large-scale application. Alkaline pretreatment (e.g., NaOH, Ca(OH)<sub>2</sub>) effectively solubilizes lignin under milder conditions compared to acid hydrolysis,



but often requires longer processing times and high reagent inputs, raising concerns about economic feasibility [40] (Table 4). Despite substantial progress, the initial deconstruction of biomass into fermentable sugars remains a major bottleneck in cost-effective bioethanol production. Continued innovation in pretreatment technologies is therefore essential to improve yields, reduce costs, and enable the broader commercialization of lignocellulosic biofuels.

## 4 Improvement Strategies in Pretreatment to Enhance Ethanol Production

### 4.1 Acid pretreatment

Acid pretreatment is one of the most widely applied methods for converting lignocellulosic biomass into fermentable sugars, which can subsequently undergo enzymatic or acid hydrolysis. It is generally classified into dilute and concentrated acid treatments, both of which operate under elevated temperatures to enhance cellulose conversion efficiency. Among these, dilute acid pretreatment (DAP) has gained prominence as a scalable and effective strategy, capable of reducing biomass recalcitrance by cleaving structural linkages, including covalent bonds within the lignocellulosic matrix [41]. Sulfuric acid remains the most commonly employed reagent due to its low cost and proven effectiveness, though alternatives such as nitric, hydrochloric, and phosphoric acids have also been investigated. Furthermore, sulfur dioxide has demonstrated potential as a catalytic additive in DAP, particularly for improving the pretreatment of softwood feedstocks.

Typical conditions for DAP involve operating temperatures of 120–210 °C, acid concentrations below 4 wt%, and residence times ranging from a few minutes to one hour. To optimize these parameters, the Combined Severity (CS) factor is commonly employed, integrating the effects of temperature, acid concentration, and residence time into a single index. Lower CS values generally favor the release of fermentable sugars from hemicellulose as monomers and oligomers, whereas higher CS values increase the risk of sugar degradation into inhibitory compounds such as furfural and 5-hydroxymethylfurfural (HMF), which negatively affect downstream enzymatic hydrolysis [42].

The primary role of DAP is to hydrolyze hemicellulose, releasing fermentable sugars while simultaneously enhancing cellulose accessibility. During the process, hemicellulosic oligomers are

initially solubilized, followed by further acid-catalyzed conversion into monosaccharides and, under harsher conditions, into furans and other volatile by-products. Hu *et. al.*, 2012 demonstrated that under severe pretreatment conditions, pseudo-lignin is generated primarily from carbohydrate degradation products rather than native lignin. Subsequent studies revealed that pseudo-lignin deposits as spherical particles rich in carbonyl groups on the biomass surface, creating additional barriers to enzymatic action and reducing hydrolysis efficiency [42]. Although effective in increasing the enzymatic digestibility of cellulose and hemicellulose, DAP also produces inhibitory by-products, requires pH adjustment before fermentation, and operates under corrosive conditions that necessitate specialized equipment [43]. Another limitation is the high energy input required for extensive particle size reduction, as most studies rely on finely milled biomass. Future investigations into the feasibility of applying DAP to larger biomass fractions, such as wood chips, could improve process sustainability and reduce energy demands.

DAP caused a significant reduction in the degree of polymerization (DP) of cellulose, indicating effective depolymerization of cellulose chains and improved accessibility for enzymatic hydrolysis. For instance, DP decreased from 7,300 to 2,600 in corn stover, from 3,500 to 600 in poplar [44], [45], and from 3,642 to 1,326 in loblolly pine [46], [47]. In contrast, the crystallinity index (CrI) showed only slight to moderate increases after pretreatment, with corn stover and poplar increasing marginally, while loblolly pine exhibited a more notable rise from 55.1% to 59.8% [46], [47]. This trend suggests that acid pretreatment preferentially removes amorphous components such as hemicellulose and lignin, thereby increasing the relative proportion of crystalline cellulose. Although different analytical techniques were used to evaluate CrI and DP, the results clearly demonstrate that the key structural modification induced by diluted acid pretreatment is cellulose chain depolymerization rather than a reduction in crystallinity.

In a recent study, dilute sulfuric acid pretreatment was applied to Rye straw and Bermuda grass at 10% (w/v) solid loading, using sulfuric acid concentrations of 0.6–1.5% (w/w) and residence times of 30–90 min at 121 °C [48]. Analysis of the liquid fractions revealed increasing concentrations of arabinose, galactose, and xylose with higher acid dosages and longer treatment times. In Bermuda grass,

glucose release also increased under more severe pretreatment, whereas rye straw exhibited relatively stable glucose levels. The cellulose-rich residues obtained were highly digestible by *Trichoderma reesei* cellulases, confirming that DAP significantly enhances enzymatic conversion efficiency. These results underscore the effectiveness of DAP in deconstructing hemicellulose and improving cellulose accessibility across diverse lignocellulosic feedstocks, though optimization is required to balance sugar recovery with by-product formation [48].

To optimize pretreatment conditions, a central composite design (CCD) approach was applied to microalgal biomass [49]. The study determined that treating 15 g/L of biomass with 1% (v/v) sulfuric acid at 140 °C for 30 minutes yielded a maximum bioethanol concentration of 7.20 g/L. The highest ethanol yield, approximately 52 wt%, was achieved using 10 g/L of biomass treated with 3% (v/v) sulfuric acid at 160 °C for 15 minutes. Statistical analysis indicated that temperature was the most influential factor affecting ethanol yield during acid pretreatment. Dilute sulfuric acid ( $H_2SO_4$ ) pretreatment of rice hulls was conducted at 120–190 °C, followed by enzymatic hydrolysis at 45 °C and pH 5.0 [32]. Optimal conditions—15% (w/v) slurry with 1% (v/v) acid for 72 h hydrolysis using a cocktail of cellulase,  $\beta$ -glucosidase, xylanase, esterase, and Tween 20—yielded 287 mg/g of sugars (~60% of initial carbohydrates) without detectable furfural or HMF. The hydrolysate contained 43.6 g/L fermentable sugars, which were fermented by recombinant *E. coli* FBR 5 to 18.7 g/L ethanol (0.43 g–ethanol/g–sugar, 0.13 g–ethanol/g–rice hulls). Overliming (pH 10.5, 90 °C, 30 min) improved fermentation, reducing SHF time to peak ethanol from 64 to 39 h (17 g/L from 42 g/L sugars) and increasing SSF ethanol from 7.1 g in 140 h to 9.1 g in 112 h [50]. These results demonstrate that careful optimization of acid concentration, temperature, and residence time in DAP, combined with enzymatic saccharification and appropriate detoxification strategies, can significantly enhance ethanol yields from both microalgal and lignocellulosic feedstocks.

#### 4.2 Steam explosion pretreatment

Steam explosion pretreatment (SEP) is a widely used method for disrupting the rigid structure of lignocellulosic biomass, enhancing its accessibility to chemical and enzymatic conversion. The process exposes biomass to high-pressure saturated steam,

followed by rapid depressurization, causing physical disruption of the material. Typical operating conditions range from 160–260 °C and 0.69–4.83 MPa, with residence times of a few seconds to several minutes, depending on the biomass type and desired outcomes [51]. SEP facilitates partial hemicellulose hydrolysis and structural modifications of lignin, thereby increasing cellulose exposure and improving enzymatic hydrolysis efficiency [52]. The treatment produces two primary fractions: a solid residue enriched in cellulose and lignin, and a liquid prehydrolysate containing hemicellulosic sugars [53]. However, partial degradation of carbohydrates and lignin can generate inhibitory byproducts that may reduce enzyme activity and hinder microbial fermentation in downstream processes [54].

The effectiveness of steam explosion pretreatment (SEP) is strongly influenced by operational parameters such as temperature and residence time, which are collectively expressed as the severity factor ( $Ro$ ) [55]. Higher temperatures facilitate hemicellulose removal and enhance cellulose digestibility, but they can also promote sugar degradation and the formation of inhibitory compounds. Studies indicate that optimal sugar release occurs within a severity range of  $\text{Log } Ro$  3.0–4.5 [55]. For example, wheat straw pretreated at 200 °C for 10 min achieved enzymatic hydrolysis efficiency of 91.7%, yielding 35.4 g glucose per 100 g raw biomass, albeit with substantial by-product formation [52]. A slightly lower severity ( $\text{Log } Ro = 3.65$ , 190 °C for 10 min) minimized sugar degradation and inhibitor formation, demonstrating that moderate severity combined with optimized enzymatic cocktails, including accessory enzymes, can maximize total sugar recovery [52].

In a related study, simultaneous saccharification and fermentation (SSF) of wheat straw was performed after impregnation with either dilute sulfuric acid (0.9% w/w  $H_2SO_4$ ) or water prior to SEP [25]. Thermal treatment at 160–200 °C for 5–20 min showed that the highest ethanol yields, around 80% of the theoretical value, were obtained at 190 °C for 10 minutes or 200 °C for 5 minutes with acid-impregnated biomass. Notable results included a peak ethanol production of 140 L per ton of wheat straw and maximum sugar recovery (300 g/kg) at 180 °C for 10 min under acidic conditions [56]. Similarly, vineyard pruning residues treated at pilot scale with severity factors of 3.83–4.69 produced cellulose-rich water-insoluble fractions for SHF. The highest ethanol yield, 8.9 g per 100 g raw material, was achieved at  $\text{Log } Ro$



4.24 [56]. These findings underscore the importance of optimizing severity conditions in SEP to balance sugar release, inhibitor formation, and ethanol yield, highlighting the potential of both agricultural residues and energy crops as feedstocks for sustainable bioethanol production.

### 4.3 Organosolv pretreatment

Organosolv pretreatment employs organic or aqueous-organic solvents, such as methanol, ethanol, acetone, ethylene glycol, or tetrahydrofurfuryl alcohol, to selectively solubilize lignin and hemicellulose, producing a cellulose-enriched solid residue suitable for enzymatic hydrolysis [57]. Introduced in the 1980s as a more environmentally sustainable alternative to conventional pulping methods like Kraft and sulfite processes, organosolv has since gained attention as a promising pretreatment for lignocellulosic feedstocks [58]. By removing lignin effectively, this method generates a cellulose-rich pulp that is highly digestible by cellulase enzymes, including endoglucanases, exoglucanases, and  $\beta$ -glucosidases, facilitating efficient conversion to glucose and subsequent fermentation into ethanol [59]. Beyond enhancing cellulose accessibility, organosolv fractionation enables the simultaneous recovery of multiple valuable components, including enzyme-digestible cellulose, high-purity lignin, and hemicellulose-derived compounds such as xylose, furfural, and acetic acid [60]. This integrated product recovery improves the economic viability of second-generation biofuel production, particularly for bioethanol, by creating opportunities for co-product valorization within biorefinery frameworks.

Pilot-scale organosolv applications include the Lignol process (formerly Alcell®) in Burnaby, Canada, which uses ethanol as the primary solvent [61], and the CIMV process in Pomacle, France, employing acetic and formic acids [62]. In one study, lignin was isolated from *Populus tremuloides* (aspen) treated at 165 °C for 1–2.5 hours with a 70:30 (v/v) methanol–water solvent mixture [63]. Catalysts such as sulfuric acid, phosphoric acid, or sodium bisulfate facilitated delignification. Lignin was recovered via water precipitation, yielding a water-insoluble fraction, and acetone solubilization, producing a smaller acetone-soluble fraction. Chemical characterization revealed low weight-average molecular weights, low polydispersity, and, under high-severity conditions, structural modifications including increased side-chain degradation and a

reduction in carbon atoms per repeating unit from nine to eight or fewer [63].

Organosolv pretreatment offers several advantages: (i) the use of sustainable, recoverable solvents, (ii) efficient fractionation of biomass into cellulose-rich solids, lignin, and hemicellulose-derived sugars for downstream applications [64]. The typical outputs include a cellulose-rich solid fraction, a solid lignin component, and a liquid stream containing hemicellulosic sugars, acid-soluble lignin, organic acids, and other degradation products. Most solvents used are bulk commodity chemicals and relatively cost-effective compared to specialty solvents such as ionic liquids. Nevertheless, the overall cost of the organosolv process and potential equipment corrosion present challenges, highlighting the need for robust solvent recovery and recycling strategies to enhance economic and environmental viability [65].

### 4.4 Liquid hot water pretreatment

Liquid hot water (LHW) pretreatment, also known as hydrothermal or autohydrolysis, employs only water without added chemicals, making it environmentally friendly and non-corrosive [66]. Typically conducted at 160–240 °C for several minutes up to one hour, LHW selectively solubilizes hemicellulose and enhances cellulose accessibility, thereby improving enzymatic hydrolysis efficiency. The process can be performed in batch reactors, where a biomass–water slurry is heated and maintained at the target temperature, or in flow-through systems, where hot water continuously passes over a fixed biomass bed. Following pretreatment, cellulose remains the primary carbohydrate and is converted to glucose via cellulolytic enzymes, including both cellulosomes and non-complex cellulases [67].

Optimization studies identified the ideal conditions at 188 °C for 40 minutes, achieving hemicellulose-derived sugar (HDS) recovery of 43.6% and enzymatic hydrolysis (EH) yield of 79.8% of the theoretical maximum [68]. When optimizing individual responses, HDS yield peaked at 71.2% at 184 °C for 24 min, while EH reached 90.6% at 214 °C for 2.7 min, suggesting potential for a two-step strategy to maximize fermentable sugar recovery, though economic and energy considerations must be addressed. Recycling spent LHW liquor up to three times improved glucose yield from 80.8% to 85.4%, with furfural and 5-hydroxymethylfurfural (HMF) concentrations remaining below inhibitory levels [69].

Additionally, the use of mild acetic acid (10 g/L) or surfactants during hydrolysis further enhanced sugar recovery in recycled systems.

A key advantage of LHW is its ability to solubilize hemicellulose while minimizing monosaccharide degradation by maintaining pH between 4 and 7, which can be stabilized using mild bases such as NaOH or KOH [70]. Organic acids released during pretreatment also contribute to partial delignification, reducing  $\beta$ -O-4 ether linkages and improving cellulose accessibility [71]. LHW avoids external catalysts, harsh chemicals, and neutralization steps, reducing the formation of fermentation inhibitors. However, the method is relatively energy-intensive due to the need for high temperatures, pressures, and large volumes of water, which may limit scalability and economic feasibility.

#### 4.5 Alkaline pretreatment

Alkaline pretreatment is increasingly favored for its low cost, moderate energy requirements, and broad applicability to various feedstocks, including agricultural residues and forages [72]. This approach promotes delignification, disrupts structural bonds, reduces cellulose crystallinity, and depolymerizes carbohydrates. Alkaline methods are generally classified into two categories: (1) those using NaOH or  $\text{Ca}(\text{OH})_2$  and (2) those employing ammonia. Both NaOH and  $\text{Ca}(\text{OH})_2$  have been shown to enhance cellulose digestibility [73]. Lime ( $\text{Ca}(\text{OH})_2$ ) is particularly attractive due to its low cost, approximately 6% that of NaOH, ease of handling, and recovery via carbonated wash water [74]. Compared to other pretreatment technologies, NaOH and lime often operate at lower temperatures and pressures, sometimes even under ambient conditions, though treatment durations can extend from several hours to days. Alkali recovery is possible but requires additional infrastructure, and biomass particle size reduction ( $\leq 10$  mm) is generally recommended to improve efficiency [75], [76].

Optimization studies on *Saccharum spontaneum* at 30 °C demonstrated maximum delignification efficiencies of 47.8% with 7% NaOH (48 h, 10% biomass loading), 51% using 7% NaOH combined with 10% urea (48 h, 10% biomass loading), and 48% with 30% aqueous ammonia over 40 days [72]. In microalgal biomass (*Chlorococcum infusionum*), NaOH pretreatment disrupted algal cell walls, releasing polysaccharides for subsequent fermentation [77]. Optimal conditions using 0.75% (w/v) NaOH at

120 °C for 30 min yielded a glucose concentration of 350 mg/g and ethanol production of 0.26 g ethanol/g algae [77].

Alkaline pretreatment has been successfully applied to diverse lignocellulosic feedstocks. For instance, coffee pulp waste treated with 4% (w/v) NaOH for 25 minutes retained 69.2% cellulose, 44.2% hemicellulose, and 25.2% lignin, yielding 38.1 g/L reducing sugars, 27.0 g/L glucose, and 13.7 g/L ethanol, corresponding to 0.4 g ethanol/g glucose [78]. Delignification efficiencies vary among biomass types: eucalyptus (11–51%), bagasse (22–90%), and straw (60–99%) using 5–15% NaOH [72]. Greater lignin removal in bagasse and straw is attributed to the higher abundance of free phenolic groups and ester linkages in grass-type lignin. Hemicellulose and cellulose losses ranged from 37–45% and 0.8–11% in bagasse, and 55–66% and 19–36% in straw, respectively [79].

Soaking in aqueous ammonia (SAA) is another effective alkaline pretreatment. Optimal conditions using 15 wt.% ammonia at 75 °C for 48 h with a 1:12 solid-to-liquid ratio achieved saccharification efficiencies of 83% for glucan and 63% for xylan using 15 FPU enzyme/g glucan [80]. Under similar conditions, 50–66% of lignin was removed while preserving 65–76% xylan and nearly all glucan [80]. SAA is particularly suitable for agricultural residues and herbaceous biomass, and supports simultaneous saccharification and co-fermentation (SSCF). Corn stover pretreated via SAA retained almost 100% glucan and over 80% xylan; subsequent two-phase SSCF achieved 88% xylan digestibility, limited glucan hydrolysis (10.5%), and an ethanol concentration of 22.3 g/L, corresponding to 84% of the theoretical yield [81], [82].

Structurally, alkaline pretreatment increases cellulose crystallinity by targeting amorphous regions, facilitates delignification akin to soda pulping, and enhances fiber porosity [73], [76]. Compared to dilute acid pretreatment, alkaline methods operate under milder temperatures and pressures. Nonetheless, challenges include the formation of unrecoverable salts, potential incorporation of these salts into biomass, and operational issues such as calcium oxalate precipitation in lime-based systems, which can impede industrial processes [75], [83].

#### 4.6 Ozonolysis pretreatment

Ozonolysis is a promising pretreatment method for lignocellulosic biomass, targeting lignin while



preserving cellulose integrity. By selectively degrading the lignin network, ozonolysis enhances delignification and exposes cellulose microfibrils, improving enzymatic hydrolysis and sugar recovery [84]. Although first explored in the 1980s, its use has resurged due to mild operating conditions and high efficiency. Unlike conventional pretreatments, ozonolysis does not generate inhibitors such as furfural or 5-hydroxymethylfurfural (HMF); instead, it primarily produces short-chain carboxylic acids, which can be removed by water washing [85]. Ozonolysis is relatively sustainable because it is typically conducted at ambient temperatures (20–30 °C) with ozone flow rates of 0.5–0.8 L/min [86]. Ozone, a strong oxidant with a standard redox potential of 2.07 V at 25 °C, can be conveniently generated from oxygen [87]. Its solubility in water is influenced by temperature (higher temperatures reduce solubility and accelerate decomposition), pressure (elevated pressure increases solubility), pH (acidic conditions favor solubility), and the presence of impurities [88], [89]. The resulting sugar-rich hydrolysate can serve as a feedstock for second-generation biofuels, including ethanol, methane, and hydrogen.

Ozonolysis has been successfully applied to a variety of lignocellulosic feedstocks, including wheat straw, bagasse, green hay, peanut shells, pine wood, cotton straw, and poplar sawdust [90], [91]. Ozone's high reactivity with conjugated double bonds and electron-rich functional groups makes it particularly effective for lignin removal [92]. Key advantages of this method include minimal generation of inhibitory byproducts, operation under ambient temperature and pressure, and the facile decomposition of residual ozone via thermal or catalytic means, supporting environmentally friendly process design. The primary limitation is the high ozone demand, which increases operational costs and may constrain industrial scalability.

After 2.5 h of ozonation, ozone consumption across various cereal straws averaged 0.10–0.12 g/g of dry substrate (DS) [93]. Wheat and rye straw exhibited the highest sugar release, yielding approximately 2–2.5 g glucose and 1.1–1.4 g xylose per gram of ozone applied, while barley and oat straw produced ~1.5 g glucose and 1.2 g xylose per gram of ozone. Studies on Japanese cedar and other residues showed effective lignin degradation, enabling enzymatic conversion of over 90% of polysaccharides into monomeric sugars. Ethanol production via simultaneous saccharification and fermentation (SSF) was also successfully

demonstrated with pretreated Japanese cedar [94]. Sugarcane straw has been investigated using a combined alkaline impregnation and ozonolysis approach [95]. The optimal procedure involved alkaline treatment at 80 °C for 8 h, followed by ozonation in a rotary reactor (0.24% w/w ozone, 35% moisture) for 60 min. Increasing ozone concentration to 1.3% w/w did not significantly enhance glucose yield (60.8% vs. 60.2%), highlighting the importance of optimizing ozone input for cost-effective saccharification [95].

Improvement strategies in pretreatment for ethanol production from lignocellulosic biomass focus on enhancing cellulose accessibility, maximizing fermentable sugar recovery, and minimizing inhibitory by-products. Likewise, the design of the process should also consider lowering the cost of operation and energy consumption, maintaining safety standards, minimizing waste and water usage, reducing operational time, and moving toward a green process. DAP remains one of the most widely applied methods, efficiently hydrolyzing hemicellulose and depolymerizing cellulose chains, though it is limited by inhibitor formation and equipment corrosion. Steam explosion and liquid hot water pretreatments provide chemical-free or low-chemical alternatives, where severity factor optimization is essential to balance sugar release and degradation. Organosolv and alkaline pretreatments are particularly effective in lignin removal, improving cellulose digestibility and enabling co-product valorization, while ozonolysis offers a mild and selective delignification route without producing furfural or HMF, though high ozone demand constrains scalability.

Progress in the development of the process depends on carefully tailoring conditions such as temperature, residence time, catalyst type, and severity index to specific feedstocks, as well as integrating pretreatment with enzymatic saccharification, fermentation, and detoxification. Combining methods (e.g., alkaline impregnation with ozonolysis or acid-catalyzed steam explosion) and developing solvent recovery strategies further enhance process efficiency and sustainability. Looking forward, green chemical pretreatments such as ionic liquids (ILs) and deep eutectic solvents (DES) are attracting attention due to their high delignification efficiency, tunable properties, and recyclability. DES, in particular, stands out as low-cost, biodegradable, and less toxic than many ILs [96], [97]. While challenges remain in scaling, solvent recovery, and enzyme compatibility, these approaches represent a

promising pathway toward environmentally friendly and economically viable bioethanol production within integrated biorefineries.

#### 4.7 Ionic Liquids (ILs) pretreatment

Ionic liquids (ILs) are molten salts composed of bulky organic cations and inorganic or organic anions that remain liquid below 100 °C. They have emerged as “green” and recyclable alternatives to conventional volatile organic solvents for lignocellulosic biomass pretreatment [98]. The efficiency of ILs largely depends on the nature of their anion; imidazolium-based ILs containing acetate, formate, or chloride anions are among the most widely studied. These ILs effectively disrupt the hydrogen-bonding networks among cellulose, hemicellulose, and lignin, leading to structural disintegration and improved enzyme accessibility [99]. Protic ILs, such as 1-ethylimidazolium chloride, have demonstrated reversible dissolution of whole biomass and substantial enhancement in enzymatic hydrolysability. High lignin extraction efficiencies have been reported, with [Hpy]Cl and [Hmim]Cl pretreatments achieving 60–61% lignin removal from poplar and 50–52% from bamboo, respectively [98].

The performance of ILs can be further enhanced through cost-effective molecular design. Protic ionic liquids (PILs) containing hydrogen sulfate ( $[\text{HSO}_4^-]$ ) anions, synthesized from inexpensive precursors such as sulfuric acid and simple amines, have demonstrated approximately 75% of the efficiency of benchmark IL systems while substantially lowering processing costs. Moreover, ILs can be efficiently recovered via vacuum distillation and reused across multiple pretreatment cycles without loss of activity, improving their economic feasibility. Recent developments also highlight the synthesis of bio-based ILs derived from lignin and hemicellulose, aligning with the principles of a circular biorefinery [99]. A major advantage of IL pretreatment lies in its ability to enhance enzymatic hydrolysis rates while minimizing the generation of inhibitory compounds—an improvement over many conventional chemical pretreatment methods.

#### 4.8 Deep Eutectic Solvents (DES) pretreatment

Deep eutectic solvents (DESs) are mixtures of hydrogen bond acceptors and donors that form low-melting eutectic liquids. DESs prepared from natural metabolites—such as choline chloride with organic acids or sugars—selectively remove lignin and

hemicellulose while reducing cellulose crystallinity [100]. DES-mediated hydrothermal treatment (DES-HTT) combines hydrothermal principles with DES chemistry, achieving efficient biomass fractionation and sugar release. Compared with ILs, DESs are cheaper, biodegradable, less toxic, and easily synthesized without purification. Their enzyme and microbe compatibility allows integrated pretreatment–biocconversion processes, and their tunable composition helps suppress lignin condensation reactions during processing [101].

#### 4.9 Biological pretreatment

Biological pretreatment employs microorganisms—mainly fungi, bacteria, microbial consortia, and their enzymes—to selectively degrade lignin and hemicellulose under mild, eco-friendly conditions without generating inhibitory compounds [102]. The process targets the complex lignin–carbohydrate matrix, particularly the cleavage of  $\beta$ -aryl ether bonds, which represent the most abundant linkages in lignin. White-rot fungi, such as *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus ostreatus*, are the most effective degraders, producing lignin-modifying enzymes including laccase, lignin peroxidase, and manganese peroxidase.

Bacterial pretreatment offers advantages such as adaptability and environmental resilience; however, individual strains often exhibit limited degradation capacity. To overcome this, microbial consortia combining multiple synergistic species are used to enhance overall efficiency. Direct enzyme application enables more targeted biomass deconstruction, employing cellulases, hemicellulases, and ligninases based on substrate composition [103].

Biological pretreatment offers several advantages—low energy demand, environmental compatibility, and elimination of toxic reagents—making it a sustainable alternative to chemical approaches. Nevertheless, the process is relatively slow, often requiring several days to weeks for substantial delignification. Recent studies have shown that integrating biological methods with chemical or catalytic pretreatments can significantly shorten processing time and improve biomass fractionation [104]. Advances in genetic engineering and strain development are further enhancing microbial activity, enzyme productivity, and substrate specificity, bringing biological pretreatment closer to viable industrial-scale applications [105].



Altogether, each pretreatment method has advantages and disadvantages (Table 5), so the selection of a suitable method requires the availability of the

current data and prerequisite experiments to find optimal conditions and appropriateness. Further evaluations of cost and feasibility are vital for real applications.

**Table 5:** Comprehensive research on biomass pretreatment methods, here is a detailed comparison table summarizing the advantages and disadvantages of each pretreatment approach [106], [107] and [108].

Pretreatment Method	Advantages	Disadvantages
Acid Pretreatment (Dilute Acid)	Fast hemicellulose hydrolysis; high xylose recovery; short residence time; increases porosity; effective at low temperatures; well-established technology.	Generates inhibitory compounds (furfural, HMF, phenolics); equipment corrosion; requires neutralization before fermentation; high acid consumption; environmental concerns with waste disposal.
Acid Pretreatment (Steam Explosion)	Cost-effective; low chemical usage; short residence time (seconds to minutes); high lignin transformation; no recycling of chemicals needed	Generates inhibitors; incomplete lignin removal; partial hemicellulose degradation; requires high temperature and pressure; energy intensive.
Acid Pretreatment (Organosolv)	Removes lignin and hemicellulose effectively; produces high-quality lignin; solvent can be recovered and reused; suitable for various biomass types.	High cost of organic solvents; solvent recovery required; potential fire hazard; requires expensive materials for construction; washing needed to remove solvents.
Neutral Pretreatment (Liquid Hot Water/Hot Air Oven)	No chemical addition required; environmentally friendly; minimal equipment corrosion; low inhibitor formation; hemicellulose recovery as oligomers; cost-effective	High energy consumption; requires high temperature (170–230°C); partial sugar degradation at extreme conditions; less effective for high-lignin biomass.
Alkaline Pretreatment (NaOH/Lime)	Effective lignin removal (25–38%); minimal cellulose loss; lower formation of inhibitory compounds; operates at mild temperatures; causes biomass swelling for better accessibility; affordable chemicals.	Long residence time (hours to days); requires washing to remove alkali; some sugar loss; chemical recovery needed; formation of phenolic compounds at high pH.
Alkaline Pretreatment (Ammonia Fiber Explosion–AFEX)	No inhibitor formation; increases accessible surface area dramatically; effective for agricultural residues; improves enzymatic digestibility; ammonia is recoverable.	High cost of ammonia; requires high pressure; not effective for high lignin content biomass; ammonia recovery necessary for economic viability.
Ozonolysis	Effective lignin reduction; operates at ambient temperature and pressure; no toxic residue formation; minimal inhibitor production; no chemical recovery needed.	High cost of ozone generation; large amounts of ozone required; not cost-effective for large-scale applications; difficult to scale up.
Ionic Liquids (ILs)	High dissolution efficiency; recyclable and reusable; operates at mild conditions; minimal inhibitor formation; breaks hydrogen bonds effectively; can dissolve whole biomass.	High cost of ILs; potential toxicity concerns; requires IL recovery for economic feasibility; viscosity issues; limited large-scale application data.
Deep Eutectic Solvents (DES)	Lower cost than ILs; biodegradable and low toxicity; easy synthesis without purification; excellent recyclability; effective lignin removal; enzyme-compatible.	Limited understanding of mechanisms; scaling challenges; potential solvent residues; requires optimization for each biomass type; relatively new technology.
Biological Pretreatment	Low energy consumption; environmentally friendly; no toxic chemicals required; no inhibitor production; low capital cost; mild operating conditions; selective lignin degradation.	Very slow process (days to weeks); requires careful control of growth conditions; low hydrolysis rate; loss of carbohydrates as microbial food; requires large space; difficult to scale up.

## 5 Conclusions

Lignocellulosic biomass has emerged as a sustainable and abundant alternative for bioethanol production,

addressing the limitations of conventional sugar- and starch-based feedstocks. Although theoretical ethanol yields from lignocellulose are generally lower, its widespread availability and renewability make it a

promising long-term solution to meet global energy demands. Pretreatment plays a critical role by disrupting the complex structure of cellulose, hemicellulose, and lignin, thereby enhancing the efficiency of subsequent enzymatic hydrolysis and fermentation. Despite significant technological progress, commercial-scale production of second-generation bioethanol remains economically challenging and often relies on policy incentives and financial support. Utilizing lignocellulosic wastes, such as agricultural residues and industrial byproducts, offers opportunities to reduce production costs while improving the environmental and social sustainability of the bioethanol supply chain. Future advancements will require the integration of process optimization, waste valorization, and efficient feedstock management to achieve economically viable and environmentally sustainable biofuel technologies.

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## Author Contributions

B.S.: Conceptualization, Investigation, Writing-Original draft; N.A.: Writing- Original draft, Data analysis; P.T., M.P.G.: Data curation; M.S.: Conceptualization, Data curation, Reviewing, Editing, and Funding acquisition.

## Conflicts of Interest

The authors declare no conflict of interest.

## Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized the ChatGPT tool to enhance the language and readability of the manuscript.

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