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*Review*

# Key Takeaways on the Cost-Effective Production of Cellulosic Sugars at Large Scale

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**Abstract:** The production of cellulosic sugars in lignocellulose biorefinery presents significant economic and environmental challenges due to the recalcitrant nature of biomass. The economic and facile production of renewable sugars with high yield and productivity is pivotal for the success of biorefinery. The cellulosic sugars are valorized either by biochemical routes or chemical routes or by hybrid (biological and chemical) routes into renewable chemicals, fuels, and materials. This manuscript focuses on the critical parameters affecting the economic viability of cellulosic sugar production at large scale, including biomass-specific pretreatment strategies and enzyme cost efficiency. High pretreatment costs, carbohydrate loss, and inhibitors production during pretreatment are identified as major contributors to overall production costs. To address these issues, we highlight the importance of developing cost-effective and efficient pretreatment methods tailored to specific biomass types and strategies for enzyme reuse and recycling. Future research should focus on innovations in pretreatment technologies, improved logistics for high-density feedstocks, biomass feeding systems, and advancements in enzyme technology to enhance the economic and environmental sustainability of lignocellulosic biorefineries. The findings highlight the need for continued innovation and optimization to make the commercial-scale production of cellulosic sugars more viable and sustainable.

**Keywords:** biorefinery; cellulosic sugar; lignocellulosic biomass; pretreatment; enzymatic hydrolysis

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## 1. Introduction

The cost-effective and environmentally friendly production of biofuels and biochemicals depends on sustainable biorefineries, which play a crucial role in achieving Sustainable Development Goals 7 and 13 (SDG 7 and 13). One major challenge in achieving sustainability in biorefineries is extracting fermentable sugars from lignocellulosic biomass (LCB) with high yield and productivity. The increased production of cellulosic sugars (second-generation sugars—2G sugars) relies on the specific chemical composition of LCB, the pretreatment and saccharification methods used, and the process integration techniques employed for various feedstocks [1].

Lignocellulosic biomass generally comprises cellulose (35–40%), hemicellulose (25–30%), and lignin (15–20%). The chemical composition of LCB varies with factors such as maturity, environmental conditions, and the genotype of plants and cultivars [2]. Feedstocks with higher lignin content are less preferred due to increased recalcitrance, eventually making it difficult for enzymatic hydrolysis to produce effective 2G sugar titers from the substrates. Besides the carbohydrate components, minor components present in the cell wall like silica, pectins, proteins, esters, extractables, and ash also affect sugar recovery. The presence of these minor components necessitates a combination of pretreatment strategies for efficient enzymatic hydrolysis [3].

A significant issue to address is the development of suitable pretreatment strategies to enhance cellulase accessibility to its substrates. Critical process parameters in pretreatment and enzymatic hydrolysis such as moisture content, temperature, pressure, pH, high biomass loading, and enzyme concentration play important roles in 2G sugar recovery [4]. Additionally, the efficiency of extracellular enzymes released by aerobic microorganisms or cellulosome formed by anaerobic microorganisms during saccharification is crucial. Factors such as the scalability of the pretreatment and hydrolysis process by adding the substrates in fed-batch/continuous mode, precise agitator design, inhibitor effects, and suitable enzymatic cocktails need standardization for commercial-scale biorefineries [5,6].

The bioprocess integration level is a critical parameter affecting the titer, rate, and yield (TRY) of fermentable sugars at a commercial scale [7]. Figure 1 illustrates the interrelationship of TRY with process parameters for desirable cellulosic sugar production from three potential feedstocks, viz. sugarcane bagasse (SCB), corn stover (CS), and rice straw (RS). Integrated bioprocesses help reduce capital and operating expenditures (CAPEX and OPEX). Various integrated technologies have been employed for extracting cellulosic sugars and their subsequent (co)-fermentation for biofuel and biochemical production. These include solid-state simultaneous saccharification and fermentation (SSSSF) [8], pre-saccharification simultaneous saccharification and fermentation (PSSSF) [9], non-isothermal fed-batch simultaneous saccharification and fermentation (SSSSF) [10], consolidated bio-saccharification [11], and consolidated bioprocessing [12]. Despite these advancements, achieving high TRY of fermentable sugars remains challenging due to low biomass loading, which limits feasibility at a commercial scale.

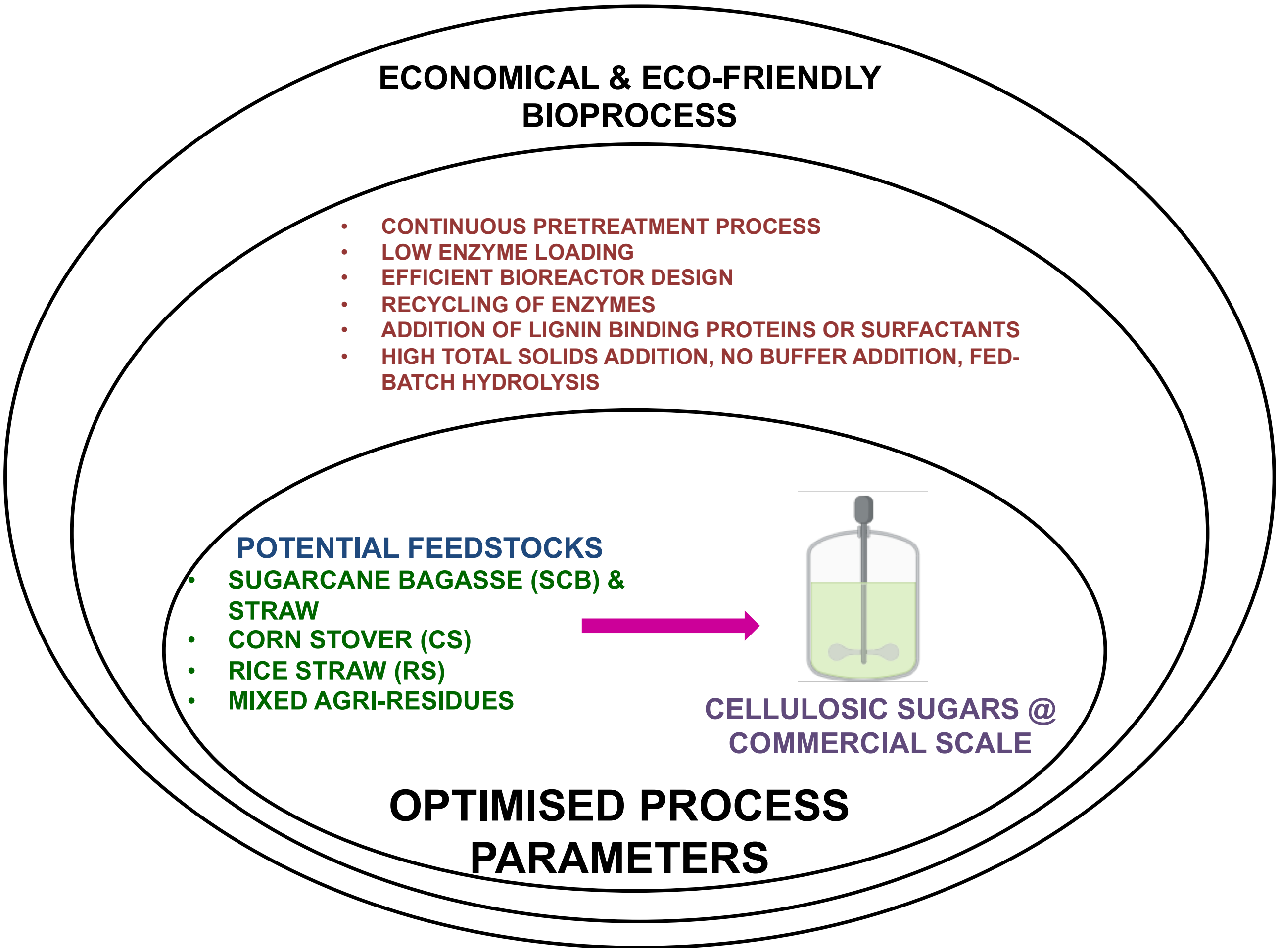


**Figure 1.** Titer, rate, and yield (TRY) interrelationship of process parameters for desirable level of cellulosic sugar production from potential lignocellulosic feedstocks.

To address these challenges, it is, thus, essential to focus on feedstock-centric approaches to enhance the titer, rate, and yield (TRY) from various LCBs. The pretreatment and saccharification processes for specific feedstocks are key factors in the optimization step of implementing a renewable carbon-centric biorefinery, in which the efficiency and scalability of cellulosic sugar production can be significantly improved. Process optimization at pilot scale is key before selecting the process parameters to be used at commercial scale operation for 2G sugar production. This approach can lead to advancements in the field, making the commercial-scale production of fermentable sugars more viable and economically feasible. Consequently, this manuscript will explore various feedstock-centric strategies, evaluating their potential to overcome the current limitations and enhance the production of fermentable sugars from LCBs.

## 2. Biomass Feeding and Feasible Pretreatment Technology at Commercial Scale

Biomass obtained from the mills or fields after preliminary milling or screening should be used as such for pretreatment process without any more milling or washing. This will save the processing cost, water footprints and overall time of the 2G sugar production process at large scale. Biomass feeding is critical for the economized production of 2G sugar at industrial scale. In batch feeding, biomass is added into the reactor in a single dose for the pretreatment and hydrolysis process. After the pretreatment process, the pretreated slurry or enzymatically hydrolyzed biomass is taken out from the reactor. Fed-batch feeding in the pretreatment and enzymatic hydrolysis stages is carried out by the step-wise addition of biomass; after completion of the process, the biomass is removed to separate the sugars from the solution. Fed-batch pretreatment is not conducted as regularly as enzyme hydrolysis. During the continuous operations involved in the pretreatment and enzymatic hydrolysis process, native biomass and pretreated biomass are fed continuously. In continuous pretreatment processes, biomass is regularly added to the pretreatment vessel, which is collected in the holding vessel and then sent either for filtration assembly or to the enzymatic hydrolysis tank.

For economic lignocellulosic biorefinery, it is important to develop the continuous biomass feeding process rather than the batch process [13]. Continuous biomass feeding is advantageous as it is time-saving, cost-saving, and increases productivity for a high amount of 2G sugar production. However, continuous biomass feeding poses several challenges, particularly the choking of feeding lines, erosion of feeding lines due to the silica present in the biomass and the failure of agitating motors in the continuous mixing of biomass and catalytic solution [14]. Biomass handling and feeding are important factors in achieving 2G sugar production targets (high TRY). Biomass is variable in size (particle size and shape), cohesive strength, bulk density, moisture, and impurities directly affecting smooth feeding into the pretreatment reactors [14,15]. Biomass with 50% moisture is usually fed into the reactors for pretreatment. The feeding of biomass through screw and rotary valves is usually used for pretreatment. However, some mechanical issues and the loss of biomass and steam are still issues encountered in the continuous/fed-batch mode of biomass feeding. The feeding of native biomass through screw feeders causes extreme heat generation and, thus, the biomass may change the cellulose and hemicellulose properties [16]. On the other hand, biomass feeding via rotary valves causes steam loss and the throwing out of solid biomass through a line before pushing the biomass into a steam-explosion (STEX) reactor or even simple stirred tank reactor. This problem has predominantly been encountered when dealing with sugarcane biomass, which is more recalcitrant than corn stover. Thus, feeding multiple feedstocks (mixed feedstock) into the pretreatment reactor is a significant challenge. A number of large-scale operations for producing 2G ethanol and biochemicals have failed recently because of the issues associated with biomass handling and feeding during biomass pretreatment, the filtration of pretreated biomass slurry, and the transference of the biomass slurry to the enzyme hydrolysis tanks.

For biomass pretreatment on an industrial scale, methods like dilute acid hydrolysis, alkaline pretreatment, and physicochemical pretreatment methods are quite feasible. However, pretreatment methods are usually biomass-centric, as the option to select the biomass pretreatment is not available for all biomasses. Each biomass pretreatment process has its own pros and cons, but for sugarcane biomass (bagasse and straw) and rice straw, dilute acid hydrolysis and steam explosion are commonly employed. Dilute acid hydrolysis and STEX are used to principally extract hemicellulose from the biomass. The hemicellulosic solution largely contains xylose and other hemicellulosic components. STEX process is also very effective in recovery of functional oligosaccharides such as xylo-oligosaccharides, gluco-oligosaccharides, among others. Production of these oligosaccharides are considered promising biomolecules for the development of holistic probiotics with lower cost. Xylose, which is a C5 sugar, is the major constituent in hemicellulosic hydrolysate, which is primarily used for the production of xylitol, ethanol, and some other renewable chemicals. For corn stover and grasses, AFEX is quite commonly used to pretreat the biomass. It removes lignin effectively and contains cellulose and hemicellulose together [16].

Pretreated slurry obtained using STEX and dilute acid hydrolysis can be directly used for enzymatic hydrolysis to consolidate the process in order to obtain high amounts of 2G sugars, minimizing the filtration process. This is contrary to alkaline pretreatment processes such as AFEX and Sodium hydroxide or any alkaline pretreatment, in which lignin is solubilized and, thus, pretreated slurry cannot be enzymatically hydrolyzed without removing the solubilized lignin stream. In enzymatic hydrolysis, it is essential to remove solubilized lignin by washing in order for the pretreated biomass (cellulose and hemicellulose) to be hydrolyzed into 2G sugar.

The transfer of pretreated slurry to an enzymatic hydrolysis reactor or filtration press is carried out via high-capacity feeding pumps. Thus, the pretreated slurry should have homogeneity and be effectively solubilized for the smooth functioning of the motor pumps. Figure 2 summarizes the key issues in biomass feeding for pretreatment, enzyme hydrolysis, and the filtration of pretreated slurry.

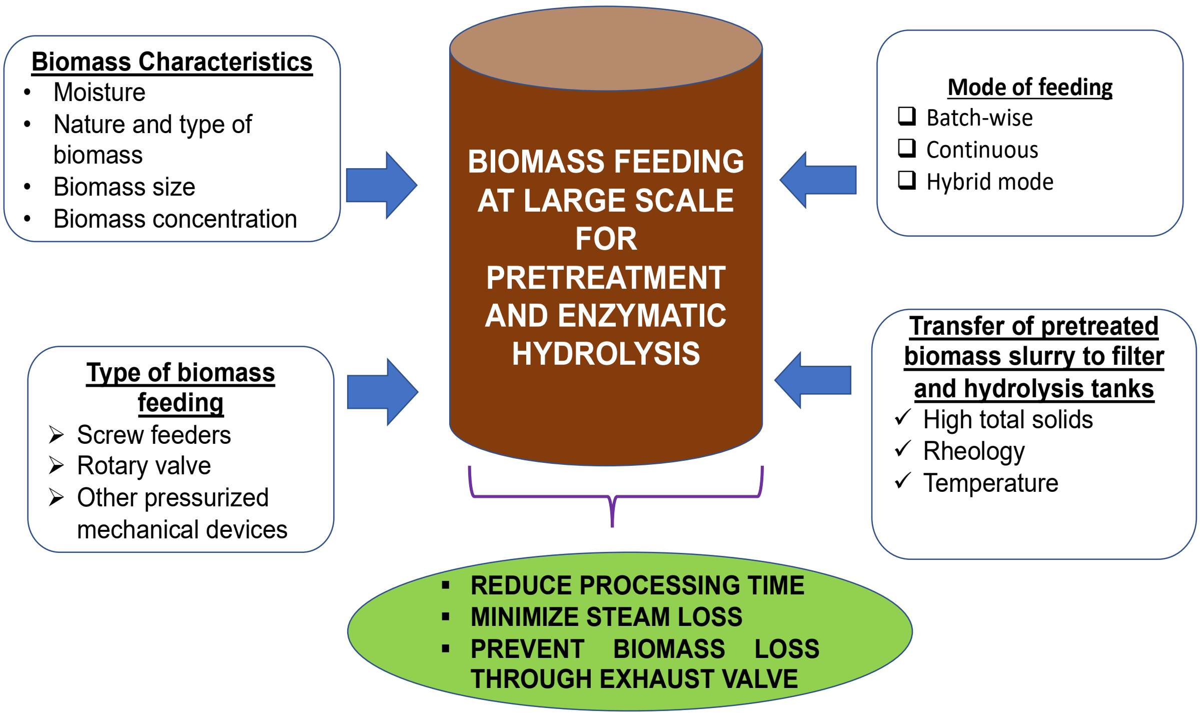


**Figure 2.** Important process considerations for biomass pretreatment, enzymatic hydrolysis, and filtration of 2G sugar from lignocellulosic feedstock at large scale.

## 3. Titer, Rate, and Yield (TRY) of Cellulosic Sugar Production

LCB can be a clean, renewable, and abundantly available resource with significant potential to replace fossil fuels for biofuel and biochemical production in second-generation (2G) biorefineries [17–19]. As previously stated, the technological deconstruction of complex LCB involves pretreatment and saccharification strategies to maximize the titer, rate, and yield (TRY) of cellulosic sugars. A summary of three major feedstocks for extracting the fermentable sugars sugarcane bagasse (SCB), corn stover (CS), and rice straw (RS) is presented in Table 1.

**Table 1.** Bioprocessing of the three major agro-residues sugarcane bagasse (SCB), rice straw (RS), and corn stover (CS) for cellulosic sugar production with high titer, rate, and yield (TRY). n.d.: not defined.

| Feedstock + Composition (% Dry Weight) | Pretreatment Details | Bioprocessing Details | Product Recovery (g/L) | Ref. |
|---|---|---|---|---|
| SCB, cellulose 47.2%, hemicellulose 19.59%, acid-soluble lignin 2.46%, acid-insoluble lignin 25.2% | Alkaline, sodium hydroxide, 2% NaOH, 15% solid loading, 121 °C, 0.5 h | Fed-batch, 20% TS (total solids), Cellic CTec2, 52.5 °C, 48 h, 15 mg protein $g^{-1}$ glucan | Glucose: 126.80 g/L, Xylose: 51.95 g/L | [20] |
| SCB, cellulose 36.7%, hemicellulose 20.5%, lignin 24.4%, ash 6.4%, extractive 4.8% | Physico-chemical, steam explosion sodium hydroxide and alkaline bleach, steam explosion: Feed rate 10 kg/h saturated steam at a rate of 25−30 kg/h at 15 bar (approx. 190 °C) with a residence time of 15 min; sodium hydroxide: 1% NaOH stirring at 80 rpm 100 °C 1 h; alkaline bleach: 80 °C 2 h 150 rpm 8% $H_2O_2$ 3% NaOH 5% solid loading | Fed-batch, 20% TS, Cellic CTec2, 50 mM sodium acetate buffer (pH 4.8), 50 °C, 20 rpm, 15.8 mg protein/g glucan | Glucose: 125.00 g/L | [21] |
| SCB, cellulose 41.4%, hemicellulose 23.3%, lignin 22.1% | Physico-chemical, al-AGO, 100 g SCB 1000 g glycerol (99.5% purity) and 2.2 g NaOH [i.e., 0.2% (*w*/*w*)] catalyst mixed in a 5-liter round-bottom flask cooked at 240 °C for 30 min | Fed-batch, initial 8% to final 20% TS, LT4, 72 h, 2mg protein $g^{-1}$ DM | Total sugars: 158 g/L | [22] |
| CS, cellulose 37.5%, hemicellulose 22.7%, lignin 18.2% | Physical, ball milling, 30% solid loading | batch, 30% TS, Cellic CTec2, 48 h, 10 FPU /g DM | Fermentable sugars: 130.5 g/L | [23] |
| CS, 37.5 wt% glucan, 21.6 wt% xylan, 20.4 wt% klason lignin | Physico-chemical, autoclave, 40% solid loading $Ca(OH)_2$ or $H_2SO_4$ followed by autoclave | Fed-batch, 20% TS, Cellic CTec3-HS, 72 h, n.d. | Fermentable sugars: 255 g/L | [24] |
| RS, n.d. | Alkaline, sodium hydroxide, 20% solid loading, 42 min, 84 °C | SSF, 10% TS, Cellic CTec2, 50 °C sodium citrate buffer pH 5.5, 24 h, 60 mg enzyme protein/g dry biomass | Reducing sugars: 36.93 g/L | [25] |
| RS, carbohydrates 58% | Thermo-alkaline, Sodium hydroxide and steam, 10% solid loading, 0.25 N NaOH steam, 15 psi, 1 h | batch, 10% TS, enzyme prepared using *Aspergillus niger* P-19, 50 °C, 200 rpm, 5 days, 5 FPU/g | Free sugars: 70 g/L | [4] |

Sugarcane bagasse (SCB) is a byproduct of sugarcane processing. It has a heterogeneous composition, primarily consisting of cellulose (40%), hemicellulose (28%), total lignin (20.5%), and ash (4%) [26]. Several studies have reported the fed-batch hydrolysis of SCB with a high titer, rate, and yield (TRY) of fermentable sugars. Mukasekuru et al. [27] reported producing glucose (125 g/L) and xylose (56 g/L) from alkaline-catalyzed atmospheric glycerol organosolv (al-AGO)-pretreated SCB (30%) using Cellic CTec2. In another study, a glucose yield of 105 g/L was obtained from al-AGO-pretreated SCB with 20% biomass loading, saccharified with Cellic CTec2 [28]. Similarly, 20% al-AGO-pretreated SCB was saccharified with the LT4 enzyme, along with accessory enzymes and additives, to maximize total sugar recovery (158 g/L) with minimal enzyme loading (2 mg protein/g) [26]. The timing of reactor feeding during fed-batch fermentation significantly affects saccharification efficiency. Baral et al. [29] found that alkali-pretreated SCB had maximum saccharification efficiency between 8 and 10 h, decreasing significantly beyond this due to feedback inhibition. The same research group estimated the cost of fermentable sugar production from SCB as 1.32 USD/kg sugar using alkaline pretreatment and fed-batch hydrolysis with 20% solid loading [30].

Corn stover (CS) is another promising feedstock for fermentable sugars with high TRY. Gong et al. [31] reported high concentrations of fermentable sugars using fed-batch alkaline organosolv pretreatment, which resulted in less effluent discharge, the preservation of holocellulose, and efficient delignification. Lu et al. [24] found that the ball milling pretreatment of CS reduced the degree of polymerization and disrupted lignin–carbohydrate complex (LCC) bonds, releasing fermentable sugars (130 g/L) with a solid loading of 30%. Another study optimized the dilute acid pretreatment of high-sugar-containing stover (HSS), reporting a high sugar concentration of 126.9 g/L along with the formation of inhibitors [32].

Rice straw (RS) is another abundant feedstock, primarily composed of cellulose (41.37%), hemicellulose (15.11%), and lignin (11.20%) [18]. However, its industrial use is limited due to its low bulk density and high mineral content. Additionally, its high silica and ash contents limit its use as animal feed and fuel. The open-field burning of RS is a common practice, resulting in environmental pollution and reduced soil fertility [33]. Due to its highly recalcitrant structure, RS requires specific pretreatment methods. Gabhane et al. [34] reported that glycerol thermal pretreatment (GTP) resulted in a 71.25% reduction in sugar yield with 94.36% holodigestibility. Cabrera-Villamizar [35] compared various RS pretreatments, finding maximum pure cellulose extraction by combining mild alkali (A), ozone (O), and enzymatic (engineered xylanase) treatments. Furthermore, Maibam and Goyal [36] formulated an efficient enzymatic cocktail of cellulases and xylanases for RS, reporting a saccharification efficiency of 72%. Despite these advancements, the cost-effective industrial-scale production of cellulosic sugars remains challenging.

## 4. SWOT Analysis of 2G Sugar Production at Large Scale

SWOT stands for strengths, weaknesses, opportunities, and threats, and is a situational analysis used to devise successful future strategies. It provides a clear picture based on internal (strengths and weaknesses) and external (opportunities and threats) factors, and can also be used for the economical production of cellulosic sugars and their derived products [37]. As a bottom line, it is important to highlight that cellulosic sugars also seek to integrate agricultural and industrial units on one platform, offering a potential solution for waste management. However, higher capital expenditure (CAPEX) and operating expenditure (OPEX), along with the lack of technical maturity of the bioprocesses, present significant weaknesses [1]. Another weakness is the lack of coordination between feedstock suppliers and stakeholders; therefore, standardizing biomass supply chains is essential.

High biomass loading for cellulosic sugar production offers enormous opportunities through a holistic approach [1]. It helps create jobs and knowledge, leading to major research achievements. Additionally, it provides opportunities to reduce greenhouse gas (GHG) emissions. Conversely, threats to cellulosic sugar production include technical uncertainties particularly successful biomass feeding operations at commercial scale, investors withdrawing support, and low product yield and productivity.

In the current scenario, researchers/engineers/techno-economic analysts need to reconsider the actual challenges that industries face. Developing suitable on-site processes for farmers, where they can provide primary treatment to their feedstock and gain monetary benefits by transporting it to industries, is crucial [38]. Furthermore, there is a need to fix the price of the feedstock. Figure 3 shows the empirical SWOT analysis of cellulosic sugar production from three major feedstocks: sugarcane bagasse, corn stover, and rice straw. In this sense, it is imperative to highlight that cellulosic sugar production requires more investment from both the public and private sectors, along with flexible government policies for ethanol producers. The investors should be willing to invest higher amounts, keeping in view the long-term perspectives for ethanol production. Further, the governmental should ensure that the policies impart maximum profits to the investors in the long run.

| | | |
|---|---|---|
| **Internal variables** | **S** | 1. Provides renewable and clean energy<br>2. Aligned with sustainable development goals (SDGs)<br>3. Brings agricultural and industrial units on one platform<br>4. Provides solution for waste management |
| | **W** | • Higher capital expenditure (CAPEX) and operating expenditure (OPEX)<br>• Immaturity at technical level<br>• Lack of coordination between stakeholders and feedstock suppliers<br>• Lack of system for collection and distribution of biomass |
| **External variables** | **O** | • Holistic approach for farmers and industrialists<br>• Job creation<br>• Knowledge creation<br>• Reduce greenhouse gas (GHG) emissions |
| | **T** | • Technical uncertainities<br>• Environmental uncertainities<br>• Investors backing out<br>• Low product yield |

**Figure 3.** Empirical SWOT analysis of cellulosic sugar production from three major feedstocks: sugarcane bagasse, corn stover, and rice straw.

## 5. Process Viability and Key Areas of Improvement

The recalcitrance of LCB poses a significant challenge for enzymatic saccharification. Despite advancements in the valorization of LCB to biofuels and biochemicals, commercial-scale technologies still face several techno-economic barriers. Tarasov et al. [39] identified lignin–carbohydrate complexes (LCC) as a key parameter contributing to recalcitrance. Effective pretreatment is crucial for hydrolyzing cellulose and hemicellulose, making them accessible to saccharifying enzymes. Petridis and Smith [40] emphasized the need for a rational pretreatment design for efficient high-solid saccharification. However, conventional alkaline pretreatment often eliminates the xylose fraction, reducing TRY [41]. Additionally, pseudo-lignin formation during dilute acid pretreatment increases crystallinity, reducing enzyme accessibility [42].

Effective saccharification strategies, including the use of accessory enzymes, enzyme additives, and optimized reactor configurations, are essential for cost-effective cellulosic sugar production with high solid loading. Enzyme additives enhance stability, prevent deactivation, and reduce unproductive lignin binding, thereby lowering enzyme loading and reaction time [11]. Brondi et al. [23] used 12% ($w/w$) soybean protein as an enzyme additive during the saccharification of steam-exploded sugarcane bagasse, achieving a 42% increase in glucose yield. Utilizing accessory enzymes can significantly enhance enzymatic saccharification, as demonstrated by a 19% higher glucose production with xylanase-fortified alkali-pretreated sugarcane bagasse [11].

The degradation of different LCBs requires substrate-specific Carbohydrate Active enZymes (CAZys) to cleave glycosidic bonds. However, the high cost of commercial enzymes remains a major barrier for the industry. Valdivia et al. [43] reported that cellulases account for 25–30% of the total operational costs in biorefineries.

High-solid-loading saccharification faces water constraints, as limited water activity in a viscous broth hampers efficient mass and heat transfer. This challenge can be mitigated by employing high solid pretreatment and saccharification in fed-batch mode, enhancing cellulase diffusivity. A pre-hydrolysis step is crucial to address mass and heat transfer issues during high solid saccharification. However, several parameters, including LCB type, enzyme cocktail composition, enzyme loading, and reaction time, influence saccharification efficiency at high solid loadings [29,30].

Improved strategies for pretreatment and saccharification are needed to overcome LCB recalcitrance. Two-step pretreatment strategies involving polysaccharide enrichment and delignification could address the shortcomings of conventional techniques [12,24,25]. Emerging pretreatment methods, such as using deep eutectic solvents (DESs) for enhanced lignin extraction, show promise for better saccharification [44].

Efficient saccharification is indispensable for the process economy, with research efforts focusing on high solid loading and reduced enzyme usage. Response surface methodology (RSM) is often employed to optimize bioprocess parameters [45–48]. Enzyme recycling during high biomass loading saccharification could enhance overall process performance, making it more cost-effective. Researchers have explored enzyme recycling for SCB [29] and CS [49]. Furthermore, enhancing fermentable sugar yield and reducing CAZy production costs are crucial for commercial viability. Lytic polysaccharide monooxygenases (LPMOs) can significantly boost enzymatic activity and support a circular carbon economy [50,51].

Integrated enzyme production and hydrolysis, or microbial saccharification (MS), using natural, adapted, and recombinant strains can significantly reduce saccharification costs at a commercial scale [52]. Advances in microbial metagenomics and metatranscriptomics are emerging areas for identifying efficient microbial cultures for LCB disruption. Additionally, fermentation with eukaryotic microorganisms characterized by temperature and product tolerance and a broad substrate range can benefit lignocellulosic biorefineries [7,53].

Developing sophisticated analytical methods to differentiate between oligomers and monomers is essential, too. Conventional biochemical assays, such as those based on 4-hydroxybenzoic acid hydrazide (PABAH) and 3,5-dinitrosalicylic acid (DNS), interfere with lignocellulosic substrates in raw or pretreated forms. Therefore, research into selective, reproducible, automatable, and fast methods for different LCBs is needed [50]. Semi-qualitative screening procedures for enzymatic hydrolysis also require improvement, as they currently suffer from longer reaction times, false zones, and variability in results.

Further studies should focus on understanding enzyme action mechanisms on biomass at the nanoscale. The application of the AA9 enzyme family during saccharification and the use of novel bioreactor designs could enhance saccharification efficiency. Standardizing enzyme loading based on glucan content rather than dry biomass is necessary for reproducible results.

In addition to the aforementioned factors involved in the production of cellulosic sugars, it is important to highlight the recycling and waste minimization that can provide potential economic and sustainability benefits to it. Recycling chemicals can reduce CAPEX and OPEX in biorefineries. The potential of LCB for producing biofuels and value-added chemicals, such as xylitol, xylene, glutamic acid, succinic acid, glucaric acid, fumaric acid, 2,3-butanediol, phenols, and eugenol, makes it an eco-friendly candidate. Akhtar et al. [54] optimized bioprocess parameters for succinic acid production using oil palm empty fruit bunches with high biomass loading.

LCBs can also serve as a carbon source for microbial enzyme production [50]. Nalawade et al. [55] proposed washing biomass after saccharification to ensure precise sugar recoveries, as significant amounts of sugars become entrapped in biomass post-pretreatment.

## 6. A Brief Case Study of Profitability and the Look Ahead: 1.5G Biorefineries

Ethanol biorefineries have gone through major transformations over the past two decades. In particular, those that operate under a dry-grind corn-to-ethanol process have implemented multiple mechanisms to increase the ethanol productivity per bushel of corn. One of the major implementations is the saccharification of cellulosic structures in the corn grain during the upstream and fermentation units, without major impacts on the conversion of starchy structures to dextrose units, which are further fermented to ethanol [56]. This simultaneous process, combining the efforts of typical first-generation (1G) biorefineries with the saccharification of cellulosic structures typically present in 2G, characterize a 1.5G process. Pure bioprocessing technologies for 2G feedstocks still face several economic gaps and have not been fully integrated due to techno-economic constraints at the commercial level [57]. Using of inhibitor tolerant ethanol producer further reduce the addition of antibiotics during ethanol fermentation. Further using thermotolerant ethanol producers can drastically reduce the cooling cost during fermentation process.

Integrating 1G and 2G biorefineries to produce 1.5G fermentable sugars could enhance overall sustainability and profitability [58]. In recent years, several researchers have explored the use of integrated biorefineries operating in a 1.5G mode for better process economics. Rodriguez Carpio et al. [59] proposed a model for optimizing process parameters to achieve maximum environmental and economic benefits during biomass hydrolysis. Elias et al. [60] applied retro-techno-economic-environmental analysis, reporting that improved operations in sugarcane biorefineries could facilitate the economical co-production of 1G–2G bioethanol and bioelectricity. Similar studies have been conducted by Oliveira et al. [58]. Recombinant ethanol producers (producing ethanol from glucose, xylose or even arabinose) can extremely useful in 1.5G or 2G ethanol production at large scale but their recycling and stability during fermentation processes are still major concerns. Further, the high cost incurred during operation and installation of facility (CAPEX and OPEX) are primary concerns as safety procedures have to be followed by the production unit considering the safety guidelines set by governments handling with genetically engineered microorganisms at large scale.

For the cost competitive sugars production, the addition of additives can further aid in the release of fermentable sugars in integrated (1.5G) biorefineries. Brondi et al. [61] reported that using soybean protein along with Tween 80 increased fermentable sugar release and decreased carbon dioxide emissions in an integrated 1G–2G biorefinery, thereby enhancing process economics. Based on these findings and our own experience, the implementation of 1.5-G biorefineries has a promising future in terms of environmental sustainability and a circular bioeconomy.

## 7. Governing Parameters for Techno-Economic Assessment of Biorefinery

The financial and environmental impacts of lignocellulosic biorefineries can be thoroughly assessed through techno-economic analysis (TEA) and life cycle assessment (LCA) [62]. These assessments help identify and address existing gaps in the technology and process chains. Brandt et al. [63] conducted a TEA for converting forest residues into cellulosic sugars and emphasized the significant role of electricity costs in achieving economic viability. Moreover, the overall costs could be further reduced if cellulosic sugars are utilized for biofuel production, highlighting the potential for cost savings through integrated bioprocessing.

Life cycle analysis plays a critical role in validating the eligibility of lignocellulosic biomass (LCB) feedstocks for Renewable Identification Number (RIN) credits, which can lower production costs or enhance returns. Additionally, the production of advanced biofuels can benefit from cellulosic waiver credits (CWC), further incentivizing the adoption of these technologies [64].

Operational expenditure (OPEX) and capital expenditure (CAPEX) are significantly influenced by the density of the LCB to be processed. Higher-density feedstocks reduce transportation costs, which is a substantial factor in the overall economic feasibility of biorefineries. Fully mechanized pretreatment strategies also offer advantages by minimizing the production of chemical inhibitors and reducing the need for multiple washings of pretreated biomass. This not only decreases environmental impact, but also cuts the costs and energy requirements for waste slurry treatment. However, the energy demand of pretreatment processes must be carefully evaluated to ensure overall efficiency [65,66].

On-site enzyme production is another promising strategy to reduce enzyme costs. This approach eliminates the need for downstream enzyme processing and promotes a circular economy by integrating enzyme production within the biorefinery [67–69]. Besides these strategies, several other factors significantly impact the economics of second-generation (2G) sugars. These include the total purchased equipment cost (TPEC), the assumed real discount rate, feedstock and labor costs, and the valorization of byproducts [70,71].

To enhance the economic viability and environmental sustainability of lignocellulosic biorefineries, future research and development should focus on optimizing these key cost drivers. In this sense, innovations in pretreatment technologies, improved logistics for high-density feedstocks, and advancements in on-site enzyme production are key [1]. Additionally, policy support in the form of incentives such as RIN and CWC credits will continue to play a role in making these biotechnologies more competitive. Integrating these strategies into a cohesive framework lead to sustainable and cost-effective production of cellulosic sugars and biofuels.

## 8. Conclusions

On the basis of several insights presented above, the critical parameters to access the process viability for the production of cellulosic sugars from LCB is the utilization of a biomass specific pretreatment strategy and the cost and efficiency of the enzymes used. Costly pretreatment contributes significantly to the overall cost in cellulosic sugar recovery. Moreover, the loss of carbohydrate fraction during pretreatment needs to be considered to assess the overall process viability. Thus, the selection of a biomass-specific pretreatment method will contribute to the overall feasibility of the bioprocess. To enhance the viability and sustainability of cellulosic sugar production, future research should prioritize the development and optimization of cost-effective and efficient pretreatment methods tailored to specific types of biomass [70]. Reducing carbohydrate loss during pretreatment is essential for maximizing yield and improving the overall economic feasibility. Moreover, advancements in enzyme technology, such as the development of more robust and efficient enzymes and strategies for enzyme reuse and recycling, are key to cost reduction. Integrating these approaches with a thorough techno-economic analysis (TEA) and life cycle assessment (LCA) will provide a comprehensive understanding of the process and identify opportunities for further optimization. Considering the environmental impact is equally important. Future studies should incorporate environmental assessments to ensure that the processes developed are not only economically viable, but also environmentally sustainable. This holistic approach will help in the transition towards a bio-based economy, contributing to a more sustainable and circular bio-economy. In conclusion, the path forward for cellulosic sugar production lies in the continued innovation and optimization of pretreatment and enzyme technologies, supported by comprehensive economic and environmental assessments. Following ten key parameters should be taken into consideration for the cost effective and facile production of 2G sugar at commercial scale:

- The feeding of high total solids (more than 50% total solids) in the pretreatment process (steam explosion or ammonia fiber expansion) under continuous mode. Use of biomass received as such from the sugar mills or corn milling processing farms, no further size reduction, screening and washing of biomass before pretreatment
- The robust mechanical feeding of biomass without steam or biomass loss from exhaust valves during pretreatment.
- Fewer chemicals for biomass pretreatment and using less severe process conditions; minimum generation of inhibitors; possible use of mixed feedstock.
- A reduction in the number of filtration and washing steps required for pretreated slurry and biomass.
- The loading of more than 20% total solids during fed-batch enzymatic hydrolysis.
- Avoidance of the use of buffer solutions and use of tap water in pretreatment and hydrolysis.
- Fed-batch enzymatic hydrolysis of pretreated slurry (liquified stream and cellulose solids) without filtration.
- The use of designer and highly potent cellulolytic enzyme cocktails (feedstock-centric) like Cellic Ctec3 or other commercial brands.

- The addition of cheap and easily available proteins or green surfactants into the biomass slurry before enzymatic hydrolysis.
- The use of thermostable cellulolytic enzyme cocktails with LPMOS (lytic polysaccharide monooxygenase), liquefaction enzymes, and ancillary proteins.

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