

## Research Article

# Techno-Economic Fermentative Microbe-Based Industrial Production of Lactic Acid (LA): Potential Future Prospects and Constraints

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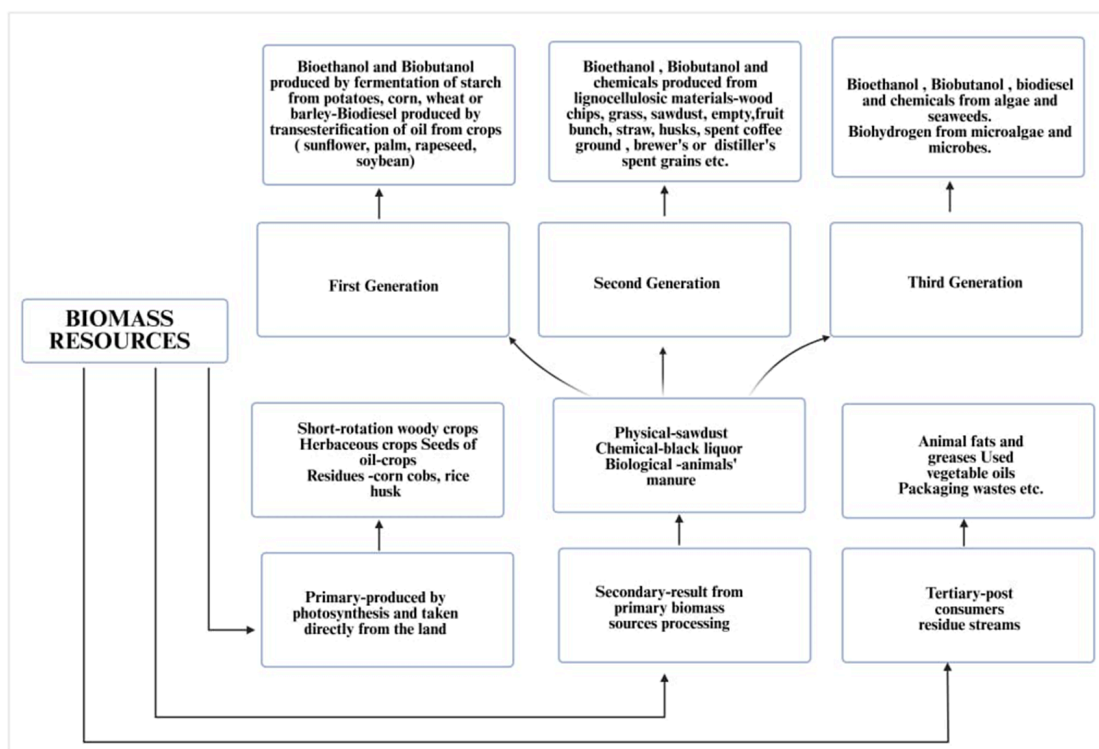
Sugarcane bagasse, as lignocellulosic biomass encompassing sixty percent carbohydrates, is a substantial waste and a renewable source of fermentable sugars. Sugarcane bagasse is mainly utilized for co-generation because of its special chemical composition. Anaerobic digestion of sugarcane bagasse can produce biogas and fertilizer, but pretreatment is necessary to overcome recalcitrance. The literature often uses milled biomass as a substrate, which doesn't accurately represent the impact of pretreatment type on biogas generation. Sugars are used as a feedstock for the fermentation-based manufacture of several renewable chemicals and fuels that are important for accelerating industrialization. Lactic acid is a major industry for biomass-derived chemicals. Fermentation dominates ninety percent of lactic acid production by utilizing abundant feedstock and sugar-rich food. The microbial production of lactic acid is gaining interest due to its exceptional optical purity, cost-effectiveness, and enhanced efficiency. However, challenges include feedstock costs, energy consumption, substrate and end-product inhibition, inhibitory compounds, and lower optical purity. Lactic acid-based low-cost manufacturing benefits developing nations. The current analysis highlights biochemical advances in commercializing lactic acid production using bagasse feedstock. This review identifies these limitations and discusses solutions for industrial lactic acid production. The study also explores pretreatment, saccharification, and fermentation techniques for industrial and lab-scale lactic acid production. This study encapsulates the sugarcane bagasse-derived lactic acid processing, highlighting the potential of 2G lactic acid in expanding sugar industries and bio-based fuel production.



enzyme hydrolysis. Conventional pretreatment methods like dilute acid and steam explosion fail to effectively remove lignin and hydrogen linkages in crystalline cellulose, causing low digestibility and high cellulase loading. Organosolv fractionation, an eco-friendly pretreatment for sugarcane bagasse, selectively removes hemicellulose and liquefies bagasse carbohydrates and lignin [1].

Brazil and India are the world's largest sugarcane producers, accounting for 59% of the world's total, with Brazil's production increasing by 12 mmt in 2020-21 [2]. Brazil, India, China, Thailand, and Pakistan dominate the market with respective cultivation of sugarcane in areas of 10.18, 4.39, 1.38, 1.37, and 1.22 million hectares. Pakistan leads in sugarcane production with 67 million tons annually. Currently, nearly ninety sugar mills are operating in Pakistan. During 2020-2021, sugarcane cultivation took place on 1.16 million acres of land [3]. Pakistan's sugar industry, an agro-based sector, employs 1 million farmers, 1,200 employees, and 1 million individuals, providing livelihoods for 15,000-25,000 families [3]. The International Energy Agency urges governments to transition from fossil fuels to bio-economies, with Pakistan ranking fifth globally in sugarcane production, with an annual yield of 67 million tons, producing significant by-products [4]. According to reports, crushing one ton of sugarcane results in the production of roughly 0.3 tonnes of SCB [5]. Biomass, abundant globally, offers a diverse range of energy and environmental sustainability applications [1].

Biomass, the organic matter found in all living species, is abundant and sustainable. It consists of carbon, hydrogen, oxygen, and nitrogen and can be used for compound synthesis. Biomass can be categorized into primary, secondary, and tertiary resources. Plants primarily use lignocellulose, cellulose derivatives, lignin, pectin, and ash. The distribution of biomass resources and their subsequent role in 1G, 2G, and 3G bioethanol production has been depicted through a flow chart in Figure 1. SCB is a lignocellulosic biomass with varying percentages of cellulose, hemicellulose, and lignin, ranging from 40-50%, 25-35%, and 20-30% [6]. Lignin is a major constituent, accounting for 60%. SCB is a renewable, non-edible source of fermentable and organic carbon, suitable for high-value goods. Co-generation of SCB for heat and power is common, while depolymerization of hemicellulose, cellulose, and polysaccharides can produce xylose and glucose [7]. Bio-based chemicals are a profitable and extraordinary class of organic compounds with reactive functional groups [8]. They are transformed into novel chemical objects with numerous commercial applications [9]. Lactic Acid (LA) is primarily found in the "L (+)" assimilative form [10]. Microbial fermentation is the dominant industrial application, producing pure LA and D (-) compounds [11]. LA is crucial for producing various compounds, including  $\text{CH}_3\text{CHCH}_2\text{O}$ ,  $\text{CH}_2\text{CHCOOH}$ , and ethyl lactate, as well as biodegradable polymers like polylactic acid (PLA). LA and PLA have become key products in agriculture, electronics, electrical, textile, packaging, cosmetic, pharmaceutical, food, and biomedical industries [10].



**Figure 1.** The distribution of biomass resources and their subsequent role in 1G, 2G, and 3G bioethanol production has been depicted through a flow chart

The PLA market is predicted to reach \$4.69 billion by 2031, while LA generates \$11.51 billion USD through sugar-rich fermentation <sup>[11]</sup>. A promising compound with a Technological Readiness Level of 8 is under development for commercial production using the LCB approach <sup>[12]</sup>. The development of biorefineries faces challenges in sustainable supply and logistics in LCB. By selecting SCB as the initial feedstock and focusing on bio-based products like lactic acid, the sugar sector can capitalize on this opportunity. Four main modules ensure SCB valorization to lactic acid, resulting in economic, technologically competitive, ecologically safe, and long-lasting business success. This review explores SCB-based modules and commercial LA manufacture, focusing on lignocellulosic feedstocks for lactic acid production through fermentation to reduce petroleum-derived chemicals. Corn stover is the primary feedstock, with pretreatment methods such as acid and enzyme hydrolysis. Challenges include lignin extraction, inhibitory substances, byproduct production, and fermentation broth composition. Advancements include ionic liquids, genetically engineered microbes, and process integration. Environmentally friendly solvents are being utilized, with the most favorable approach being pre-treatment with DES and then implementing SSCF (or SSF) in either continuous or fed-batch mode. Integrating fermentation and separation phases can

increase productivity and reduce energy consumption in extended fermentation. Techniques for eliminating lactic acid include ion exchange, membrane bioreactors, and liquid-liquid extraction. Results for second-generation biomass production are limited. Table 1 presents the advantages and adverse effects of various fermentation modes of process organizations.

## 2. Biomass Recalcitrance and Carbohydrate Recovery

A study reported exploring fermentative lactic acid production using corn stover feedstocks to reduce petroleum dependence, overcoming challenges such as lignin separation and complex broth composition. Pre-processing schemes reduce biomass recalcitrance and recover the most carbohydrates while requiring low investment and operational expenditure. High-solids pretreatment and chemical recycling can make pre-treatment strategies more environmentally and economically sustainable. The *Amorphotheca resinae* ZN1 strain is utilized for biological detoxification due to its ability to assimilate hazardous oxidized sugar compounds, using high-solids preprocessed techniques for ten years [13].

Advancements in pretreatment have increased solid loadings in biomass, allowing for efficient mass and heat transfer. Single or twin-screw extrusion systems enable fast pretreatment of large amounts of biomass with high solids, using minimal chemical loadings. Strong shearing pressures promote proper defibrillation and increased digestibility. Extruder systems are better for processing bulk biomass in a shorter time, with comparable glucan conversion yields in twin-screw extruder systems. The study emphasizes the importance of biomass surface area in achieving significant saccharification yields. Preconditioning, Biomass Recalcitrance and Carbohydrates Recovery (percentage) indicating optimized valorization of Sugarcane Bagasse have been shown in Table 2.

## 3. Biodetoxification and Pretreatment with Dry Acid

Pelletization-based biomass densification (BD) is crucial for improving 2G biorefinery industrial viability. Innovative methods like COBRA and autoclave-pretreated lignocellulosic biomass densification demonstrate SCB's effectiveness [14]. SCB, H<sub>2</sub>SO<sub>4</sub>, and water were combined in a DLCA technique, resulting in a pellet with 84% xylan hydrolysis and 95% glucan digestibility [15]. An ammonia-based pretreatment approach named the COBRA-LE method is a next-generation technique that densifies SCB to 560 kg/m<sup>3</sup> and heats biomass pellets to 100°C for 3.5 hours. This process yields 65.7±1.8 kg of total sugars, increasing ethanol fermentability [14].

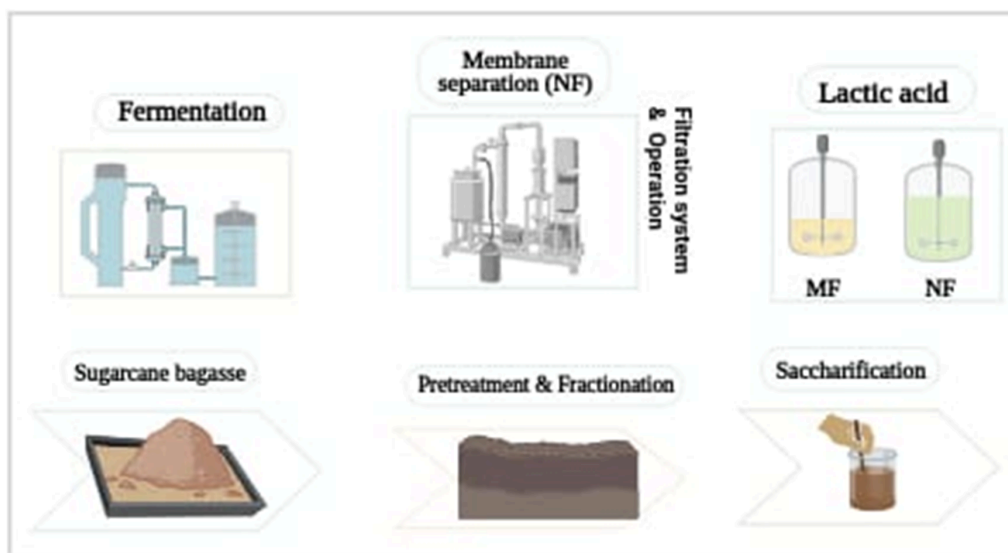
Solids handling techniques like H<sub>3</sub>PO<sub>4</sub> acid and hydrogen peroxide, C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>, acidified-Valero lactone, and basic organosolv have been successful with LCBs, but their effectiveness in lowering SCB resistance to use as

feedstock remains unknown. Chemical recycling is a commercially viable strategy for chemicals with negative environmental effects or expensive ionic liquids, reducing waste production. However, it is crucial to ensure recycled chemicals perform as well as virgin or pure chemicals during pretreatment [16]. The recycling and performance of protic IL monoethanolammonium acetate (MEA) during IL pretreatment are influenced by the acid-base ratio (ABR). Excessive use of  $\text{CH}_3\text{COOH}$  and acetate leads to unfavorable glucan conversion ratios. After three recycles, IL  $\text{C}_2\text{H}_5\text{NO}$  production is limited to 30%, while pretreatment SCB yields good glucan and xylan conversion [17]. FA produced 45.6% XOS in an ideal setup, easily recovered in a rotary evaporator, and furoic acid rain storming was used for subsequent runs, releasing 83 grams from 15% solids [17].

It has been found that most processed biomasses show significant carbohydrate conversion through saccharification, but inadequacies exist in pretreatment techniques and diverse biological products. Most methods are efficient at the pilot level, increasing the chances of commercialization. Few economically feasible techniques yield more than 400 grams of sugar per kilogram of biomass, according to reaction chain flux analysis. Table 2 indicates the reagents recycling processes, pretreatment strategies, and their respective effects.

## 4. Valorization of Bagasse

Enzyme-based saccharification at high solids (15%) is crucial for producing concentrated second-generation sugar syrups and economically accessible sugar bases. However, the expenditure on cellulase manufacturing, particularly in the biorefinery segment, and the rate-determining phases of the process are crucial [18][19]. High-solid saccharification resolves rheological shortcomings using enzymes, multiple-step techniques, and auxiliary enzymes for polysaccharides [18]. A unique case in saccharification involves 30 percent SCB solids pretreated with alkaline hydrogen peroxide, resulting in 70% carbohydrate conversion rates [20]. Figure 2 illustrates the process of obtaining lactic acid with a high level of purity from lignocellulosic biomass.



**Figure 2.** The process of obtaining lactic acid with a high level of purity from lignocellulosic biomass.

Sugar recovery during pretreatment is often prioritized over high sugar yields during saccharification, which determines lignocellulosic sugar prices. Simultaneous intensification of both processes is crucial for increasing sugar recovery from SCB. Integrated processes have been developed to recover 75% of sugar or 540 grams of main bioactive sugars per kilogram of raw SCB. The theoretical maximum sugar recovery is 725 g. HSES effectively retrieves sugars from SCB at enzyme loadings ranging from 12.5–20 mg protein per gram of glucan [14][18]. Sodium methoxide solution in glycerol preprocessing retains glucan and xylan fractions, increases delignification, and yields 566.6 grams per kilogram of sugarcane bagasse [21]. SCB pretreated with alkali-peracetic acid lost 41% of xylan [22]. The study suggests slowing down and fine-tuning the method to preserve the xylan portion. Pretreatment with ethylene glycol and water with iron chloride ( $\text{FeCl}_3$ ) as a catalyst resulted in a 3.2 times increase (from 28.3% to 91.5%) in glucose production by the saccharification process. Similarly, when the enzyme concentration was at 12.37 FPU g<sup>-1</sup> glucan, adding a mechanical filtering step after autohydrolysis boosted the glucan exchange ratios of SCB rich in cellulolignin by 17% [23].

A new saccharification and co-fermentation process using *B. coagulans* and *L. rhamnosus* can produce high-titer lactic acid, improving sugar yield and lactic acid production from cassava bagasse. This process has potential for industrial applications and can be feasible under optimal conditions like 40°C, pH 5.8, 800 rpm, and 10 g/L biomass [24]. The study proposes a batch SSF process for converting RPS into LA, achieving 330 g LA per kilogram. The process was enhanced with a pulsed-fed batch strategy, improving the paper industry's economics and reducing environmental impact. Further process intensification could be

achieved [25]. The valorization potential of sugarcane bagasse through microbial fermentation and their respective production capacity and yield have been shown in Table 3.

A study suggests a sequential solid-state fermentation method for converting renewable plant sources into lactic acid, resulting in 330 grams of LA per kilogram, which enhances the paper sector's economic viability and reduces its environmental impact [26]. Research shows that increasing pretreatment and saccharification modules in SCB bioprocessing can reduce market prices, costs, and environmental impact while identifying eco-friendly hotspots, which is crucial for creating a sustainable sugar foundation [27].

## 5. Microbes-based LA Production

Microbial processes produce 90% of industrial LA, but barriers prevent its technologically and economically feasible synthesis using 2G carbs due to metabolic problems, xylose transfer genes, carbon catabolite repression, byproduct generation, and product cleanliness [28]. LA fermenting bacteria face genetic engineering techniques to address inherent problems, including deteriorating SCB, dissolved and insoluble inhibitors, and potential threats to commercial-scale LA production [28][29]. To improve cellulase cocktails, experiments involving various fermentation techniques, switching to one-step feeding, detoxification of 2G sugars, selection of microbes from different metabolic environments, and subjecting LA fermenting microbes to adaptive evolution may tend to produce desirable outcomes. There are two pathways for cellulase cocktails, which include co-fermentation and synchronized saccharification. Enzymatic pre-hydrolysis initiates sugar release, followed by fermentation by a bacterium, improving efficiency and minimizing OPEX and CAPEX [28][30].

## 6. Methods for Producing High-Performing LA-Microbial Strains

### 6.1. CRISPR/CAS9 Mediated Engineered Microorganisms for Lactic Acid Production

D-lactic acid, a type of lactic acid, is primarily produced by bacteria by converting carbohydrates into L-lactic acid. The growing demand for D-lactic acid in polylactic blends has led to the development of genetically modified strains, such as a successfully cloned *Lactobacillus plantarum* strain [31]. Currently, the most often utilized methods for transforming LAB genes and manipulating their metabolism are single-stranded genome recombination, double-stranded red/RecET-mediated DNA recombination, and plasmid-based homologous recombination. The CRISPR/Cas9 gene-editing technology, which has been recently established, is notable for its precision, effectiveness, and user-friendliness [32].



The three major methods of producing high-performing LAB strains are metabolic engineering, mutagenesis screening, and adaptive evolution. Each one of these approaches has benefits and drawbacks of its own. The post-mutagenesis screening procedure can be made faster by using high-throughput screening methods, but it still mostly depends on random mutation. Conversely, strategies like adaptive evolution and metabolic engineering were designed to make up for the deficiencies in mutagenesis screening [32].

Hemicellulosic hydrolysates are inefficient substrates for lactobacilli, as they cannot ferment pentose sugars. Certain highly productive strains produce a mixture of both lactic acids (L- and D), but the presence of by-products from hetero-fermentative lactic acid synthesis reduces efficiency and increases production costs. Factors such as yield, substrate specificity, optical component purity, and acid tolerance can hinder lactic acid synthesis. Biochemical engineering is a promising approach, enabling pure lactic acid isomer synthesis using lignocellulosic feedstocks by genetically engineering strains with pentose integration genes, unwanted metabolic pathway branches, or lactate dehydrogenase gene deletion. Future genetic tools for generating recombinant LA-bacteria and genome editing approaches for lactobacilli design are being developed [33].

A study identifies four main challenges in lactic acid production: ensuring acid purity, developing acid-tolerant bacteria, finding suitable carbon sources, and optimizing production parameters. It also discusses potential solutions, for instance, by using metabolic engineering, genetically modifying bacteria for producing LA from lignocellulosic-derived biomass using natural cellulolytic methods and recombinant techniques. The fermentative potential of *Lactobacillus pentosus* was improved through adaptive laboratory evolution [34][35].

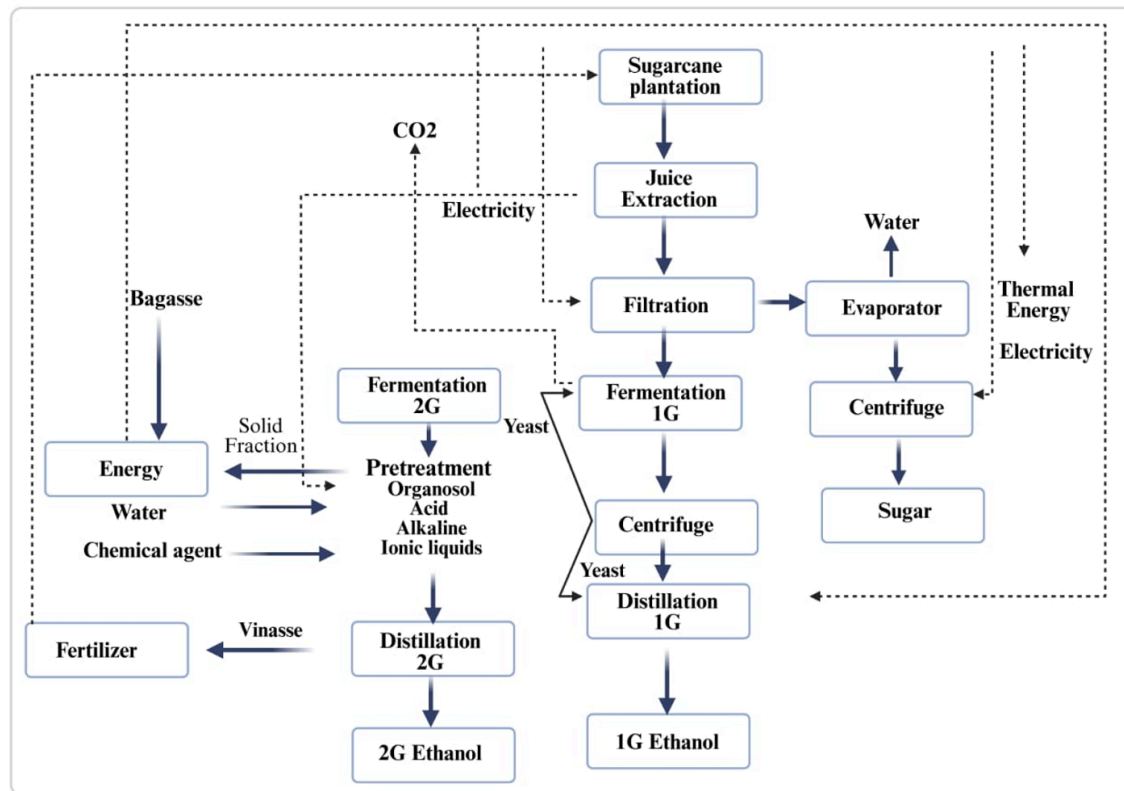
A study reports the genetic modification of *Kluyveromyces marxianus* to efficiently synthesize L-lactic acid from corncob. This was achieved by saccharifying and co-fermenting corncob residue, producing 103.00 grams per liter of L-LA, indicating good optical purity (99.5%). *Kluyveromyces marxianus*' D-lactate dehydrogenase was disrupted by the change [36]. Thirteen out of 26 rotten fruit and soil isolates were tested for wood hydrolysate fermentation. *Lactobacillus paracasei* 7B was selected with the aim of modifying it further by inhibiting ldhD due to its high lactic acid production and tolerance [37][38]. The batch culture increased the xylose-to-glucose ratio, leading to the selection of the most successful cells. The selected strain (MAX2) increased xylose production, transformed blended sugars into wheat straw hydrolysate, and demonstrated resistance to acidic conditions, demonstrating the potential of genetic modification [39].

A study modified the NL01 strain of *Bacillus coagulans* to resist inhibitors' effects on maize stover hydrolysates. The GKN316 mutant strain has high inhibitor tolerance and can convert hydrolysates to lactic acid. The syringyl compound is most poisonous when inhibited by furfural [40]. A study utilized a genetically

engineered strain JU15 of *Escherichia coli* to convert sugarcane bagasse and maize stover hydrolysates into D-lactic acid. The modified strain, AV03, exhibited simultaneous sugar intake without acetic acid production, with a D-lactic acid output of approximately 0.95 g/g sugars. The transformed strain JU15 was utilized to produce both lactic and acetic acids [41]. Scientists developed a mutated strain of *Lactobacillus plantarum* NCIMB 8826 ldhL1 with a xylose assimilation plasmid (DldhL1-pCU-PxylAB) for lactic acid production. This strain can ferment both xylose and glucose simultaneously, allowing for large quantities of D-lactic acid using xylose. Sorghum stalk hydrolysates and maize stover hydrolysates were used for D-lactic acid synthesis [31].

The study utilized a mutant *L. plantarum* strain and a genetically modified *Lactobacillus gasseri* JCM 1131T strain to produce D-lactic acid from delignified hardwood pulp. The strains produced LA from 2–84.6 g per liter and effectively fermented wheat straw hydrolysate, resulting in 0.37–0.42 g of lactate per gram [42]. Previous studies have explored the potential of producing D-lactic acid using modified *Lactobacillus plantarum* strains. A strain with a defective L-lactate dehydrogenase was modified to produce D-lactic acid from cellulosic materials. A plasmid-secreting endoglucanase produced 1.27 g/l of D-lactic acid when transferred to *L. plantarum* using cellohexaose by using  $\beta$ -glucan. Acetic acid was the main by-product in the end [43].

A study successfully synthesized D-lactic acid from xylose and glucose using *Lactobacillus pentosus* and L-lactate dehydrogenase-deficient *Lactobacillus plantarum*. The process yielded homo-D-lactic acid at 41 grams per liter, with an 88% yield and 98.7% optical purity. The study also utilized saccharification and fermentation methods to produce D-lactic acid from delignified hardwood pulp. 2G ethanol is a promising alternative to increase biofuel production and aligns with global goals to expand renewable energy. Bioethanol production from sugarcane bagasse is depicted in Figure 3.



Research on the use of *Lactobacillus bulgaricus* for producing D-lactic acid using rice straw is limited. The genetically modified strain demonstrated the ability to function at a temperature of 45°C, making it suitable for use in a simultaneous saccharification and fermentation (SSF) process. This strain effectively reduced the amount of enzyme required. The rice straw biomass was subjected to pretreatment using dilute acid and, subsequently, steam explosion. Rice straw was treated with sulfuric acid for 24 hours, then heated to 175°C. Different fermentation methods were used, yielding D-lactic acid in the range of 11.8–32.4 g/L. The strain JU15 of *E. coli* was genetically modified to produce D-lactic acid from algal biomass, achieving 86% of the expected glucose-to-lactate conversion [44][45].

CRISPR-Cas9 gene-editing technology was used to create a strain of L-LA encompassing a high percentage of optical purity. NCBI001-M2-ldhL1-HT, a strain with adaptive evolution, demonstrated efficient production at 45°C, yielding 221.0 g per liter and exceeding 99.1% optical purity [32].

## 6.2. Fermentation of Microbial Strains and High Product Yield

Conventional split-batch fermentation involves adjusting the medium level of substrate concentration to the strain's ideal concentration in order to maximize product fermentation in the lab. However, this process is frequently labor- and energy-intensive. Strains should produce LA efficiently in a high-substrate-content medium, but this excessive concentration may lead to a reduction in the efficiency of the strain due to prolonged lag periods and higher osmotic stress [40]. Therefore, various research has addressed the suppression of strains that thrive at elevated concentrations of substrate by screening and modifying the strains, and by adjusting the fermentation procedure. The batch fermentation effectively controlled the ratio of solute to unsaturated fatty acid pools, consuming all 248 grams per liter of glucose and yielding 223.7 grams per liter of LA [41]. Regarding the method of production, two well-established procedures now in use are fed-batch fermentation and solid-state filtration. When fed-batch fermentation is used instead of continuous fermentation, the substrate is used more efficiently. By regulating the nutrient content during fermentation, it ensures that the bacteria are growing in an environment that is conducive to their best development. *L. rhamnosus* ATCC 7469 has been shown to produce L-LA from brewer's waste grain hydrolysates as well as malt rootlet extract [46][47]. The batch fermentation resulted in a maximum LA concentration of 25.73 g/L, yielding 0.95 g/(L.h) and an 86.31% conversion rate. In contrast, fed-batch fermentation produced a higher LA concentration of 58.01 g/L, a higher yield of 1.19 g/(L.h), and an 88.54% conversion rate. The starch hydrolysis process proceeded well, and SSF made it possible to use the sugars efficiently. The fermentation technique used a low initial sugar concentration and increased polysaccharide hydrolysis, promoting microbial development, but high-temperature saccharification is a limitation. Thus, strains that can withstand high temperatures must be used in order to comply with this procedure. After being isolated from soil, strain *B. coagulans* WCP10-4 was fermented by SSF at 50°C, consuming 200 g/L of maize starch to yield 202.0 g/L of L-LA [42]. Overall, strains exhibiting a high capacity to tolerate glucose, along with the utilization of simultaneous saccharification and fermentation (SSF) and fed-batch fermentation techniques, offer significant benefits in terms of lactic acid (LA) production.

## 6.3. Fermentation of Immobilized Microbial Strains

Cell immobilization enhances fermentation processes by providing advantages like high cell concentration, biocatalyst stability, easier separation, and higher fermentation rates. The studies explored lactic acid production from biomass using adhesion, encapsulation, and gel entrapment methods, while evaluating LA production from renewable substrates like *L. plantarum* [48]. A study on lactic acid fermentation from *Chlorella vulgaris* biomass, using sugarcane bagasse and microalgal biomass, achieved a 72% yield using a fed-batch process combined with ion exchange [49]. The study utilized *Lactobacillus casei* cells immobilized

with calcium alginate to synthesize lactic acid from sugarcane molasses, achieving an optimal yield of 128.45 g/L [50].

Several studies have described fermentation setups for synthesizing Lactic Acid (LA) from pretreated SCB or SCB by using 2G sugars (Table 4). To develop a commercially feasible 2G LA technique, the strain of microbes used must have the potential for increased sugar intake and improved metabolic production. Sterilization and maintaining sterility are crucial steps in the process. Most processes showed positive TYP characteristics, with high returns. However, only a few methods have achieved a titer > 100 g/L and productivity > 2 g/L/h [51]. Figure 4 gives a pictorial representation of the pathways involved in two-stage SSF and batch SSF fermentation.

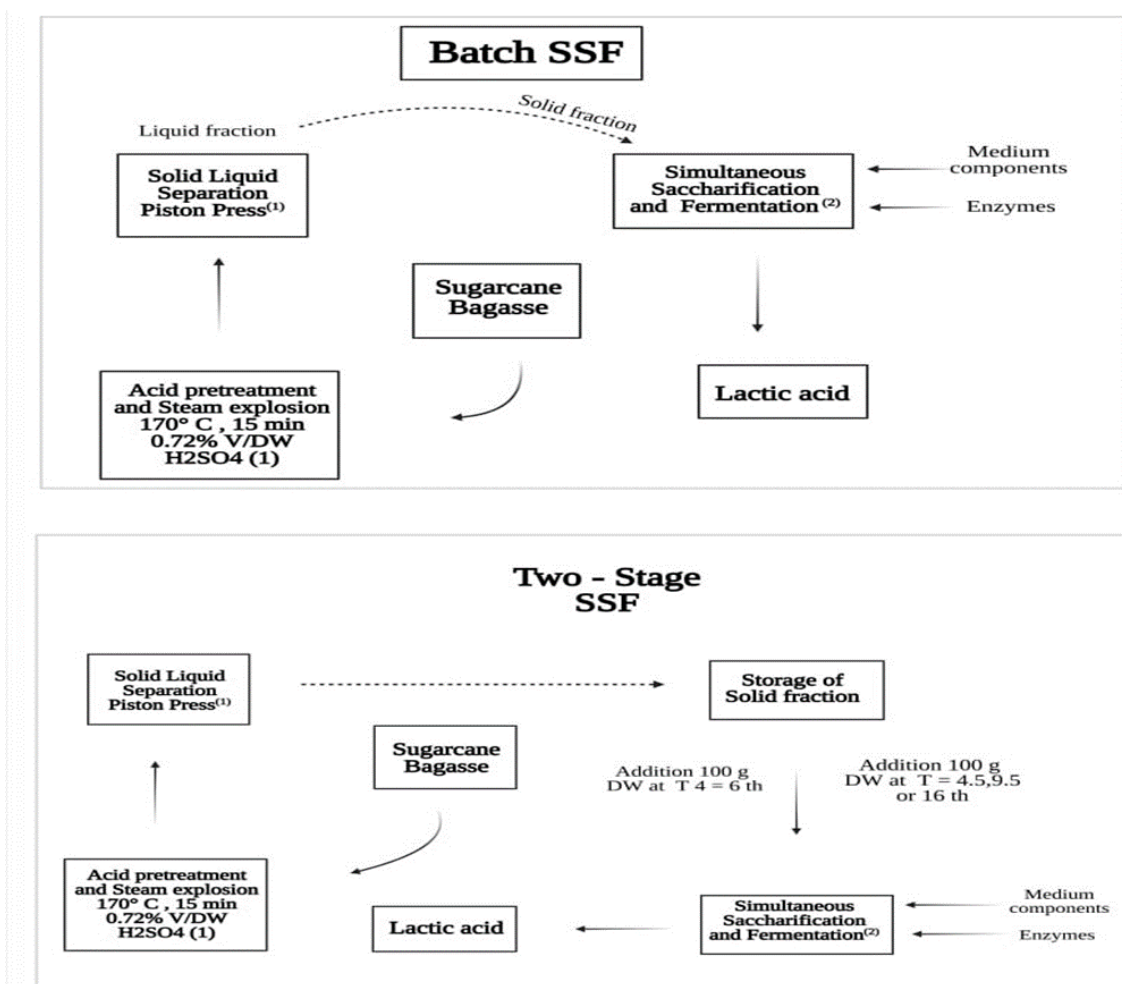


Figure 4. Pathways involved in two-stage SSF and batch SSF fermentation.

A study indicated the production of LA using SCB hydrolysate, using H<sub>2</sub>SO<sub>4</sub> acid and NaOH alkali. Commercial cellulases were used for enzyme-catalyzed hydrolysis of the solid fraction. The hydrolysate

contained glucose, xylose, CH<sub>3</sub>COOH, and lignin. Fermentation using SCB hydrolyzed cotton seed meal, *Bacillus* sp. strain P38 achieved high LA intensity, 100% L-isomer efficiency, and no D-isomer [52]. It was also found that *Bacillus coagulans* CC17 LA fermentation with bagasse sulfite pulp (BSP) was more cost-effective than traditional sulfite pulp (SHF) due to the absence of additional  $\beta$ -glucosidase. Simultaneous Saccharification and Fermentation (SSF) was employed, with cellulase added gradually during the fermentation process. The culture produced 110 grams per liter of Lactic Acid, with a total yield of 0.60 g g<sup>-1</sup> [53]. However, the Lactic Acid yield for cellulose was 0.72 g per gram. It was found that LA derived from SCB costs \$3.27 USD per kg with alkali as the pretreatment option. However, they identified hotspots that could reduce LA prices and environmental impact. The price of fermentable sugars decreased by 39% due to exaggerated preprocessing and saccharification procedures, impacting the price of Lactic Acid from SCB and contributing to global warming. The strain *Bacillus coagulans* NCIM 5648 was found to be unable to utilize xylose during fermentation, resulting in an unutilized value [54].

The fermentation process of *Lactobacillus aeruginosa* (LA) requires a pH-managed atmosphere for consistent production. Acid-tolerant bacteria, such as yeasts, are being studied for their environmental benefits and reduced alkali usage. Eukaryotic systems, such as yeasts, are being investigated due to their chemical tolerance and ability to host various gene expressions. A recent study introduced the D-Lactate dehydrogenase gene from *Leuconostoc mesenteroides* into *Saccharomyces cerevisiae* [51]. Valorization of sugar through microbial fermentation and their respective production capacity and yield have been given in Table 4.

The introduction of the D-LDH gene in yeast allows it to produce the D-LDH enzyme and incorporate it into its metabolic pathway. Two specific genes in *Saccharomyces cerevisiae*, *gdh1* and *gdh2*, were targeted for knockout, reducing the yeast's ability to produce glycerol. A genetically modified strain was cross-mated with *S. cerevisiae* BCC39850, resulting in a highly effective hybrid strain for producing D-lactic acid in environments not neutralized using sugarcane bagasse preprocessed with a base through the Simultaneous Saccharification and Fermentation technique. The LA2 strain was created by deleting the *pdc1* and *pdc5* pyruvate decarboxylase genes and introducing an exogenous LDH gene. The maximum LA production from the recombinant strain was 25.34 3.25 g L<sup>-1</sup> L (+), using crop and availability of 0.51 and 1.69 per gram per liter per hour. The demand for acid-tolerant microorganisms in industrial LA fermentation is expected to rise, but achieving consistent and reproducible results from sugarcane bagasse remains a challenge. Recent studies have used a specific thermophilic *Bacillus coagulans* JI12 strain to synthesize Lactic Acid (LA) from oil palm empty fruit bunches. This strain hydrolyses polysaccharides and co-ferments OPEFB natural furfural to furoic acid, absorbing glucose and xylose simultaneously. When switched from batch to continuous mode,

the lactic acid yield increased significantly, lowering its value from 17.8% to 107.5 g/L. This represents a significant step towards commercial LA synthesis using Lignocellulosic Biomass (LCB) technology<sup>[55]</sup>.

MICF fermentation of Lactic Acid in glucose-rich maize with *Bacillus coagulans* showed superior advantages over batch fermentation. Batch mode yielded only 3.28 g L<sup>-1</sup> h<sup>-1</sup>, while MICF yielded 98.25 g/L. Further investigation is needed for various Lactic Acid fermentation configurations using Sugarcane Bagasse or Sugarcane Bagasse-generated sugars<sup>[56]</sup>.

Downstream processing is crucial for a bioprocess's commercial viability, accounting for 30–70% of the overall cost. 2G lactic acid fermentation broth contains chemicals from biomass, hindering effective, continuous, and cost-efficient production of Lactic Acid. Hybrid short-path evaporation techniques are used to separate LA from sugarcane bagasse hemicellulosic but are negatively impacted by leftover sugar. However, researchers prefer dealing with simulated fermentation liquors and do not anticipate difficulties when managing real-time fermentation liquors containing 2G of LA throughout development. In the future, the focus should be shifted to the utilization of real LA fermentation liquors, which are acquired by using the LCB-route<sup>[57]</sup>.

## 7. Conclusions and Future Perspectives

The study explores fermentative microbes-induced industrial lactic acid production using lignocellulosic feedstocks, aiming to reduce petroleum dependence. Challenges include the separation of lignin, inhibitory compounds, and complex broth composition. Progress is being made using green solvents and ionic liquids. It is pertinent to diversify the sugar industry, which could be a crucial first step towards the successful exploitation of low-budget sources or raw products. This would inevitably shift the focus from supplying energy needs through products like steam, ethanol, electricity, biogas, and bio-CNG. Therefore, the current study highlights the significance of the sugarcane biorefinery as a favorable theory and potential speculation. Manufacturing 2G lactic acid may prove a profitable alternative, as it is commercially produced through microbial fermentation. The least endowment (MSP) of LAs is comparatively more than that of ethanol, but the potential of ethanol in the market as an energy-dense chemical product for energy with a wide range of uses is clear. In Pakistan, 30–40 million tonnes of sugarcane are crushed, producing 12 million tonnes of sugarcane biomass (SCB). A theoretical scenario suggests that using 50% of SCB, comprising 45% glucan and 20% xylan, can create a sugar platform with a combined pretreatment and saccharification efficiency of 70% fermentable sugars of 40–50 lakh tonnes. Developing a sugar platform using domestically produced, inexpensive, and effective cellulase cocktails is essential for increasing microbial biodiversity prospecting through classical or metagenomic and proteomic approaches. The best method for 2G LA downstream processing purifies the product with little waste and has a defined process that is reliable,

error-free, and simple enough for industrial scale-up. Academic-industrial cooperation is highly required to focus on developing a downstream processing method for 2G LA that is both commercially and environmentally sustainable. Sugarcane, which accounts for 50% of major crop yields worldwide, can contribute to at least three sustainability development goals (SDGs) if enhanced and integrated with sugarcane industries in the next five years.

Lactic acid (LA) is a vital chemical with a rapidly expanding global market. It is a far-off possibility to manufacture LA commercially by utilizing 2G feedstocks, even though LA is a bio-based product generated on an industrial scale through fermentation. Its low cost of manufacture is required to broaden its market base, and it can be decreased by employing less expensive substrates. An evident by-product from the sugarcane industry and related businesses is sugarcane bagasse (SCB), and it is easily valorized. The lignocellulosic nature of SCB makes it a potent bio-resource to synthesize bio-based materials, and it also exhibits characteristics like plentiful availability, sustainability, and synergy with food production. The current study clarifies the technological procedures that have given the biorefineries based on SCB commercialization—with 2G LA as the desired chemical—the necessary push. Although LA build-up from SCB has shown encouraging outcomes both upstream and downstream, research and development still need to be done. Several obstacles to industrial 2G lactic acid yield, such as costly polysaccharide hydrolysis, cost-effective downstream processing, and elevated metrics that match industrial requirements, have been partially overcome by ad hoc initiatives, but as a whole, integration of the method is yet difficult. Therefore, academia and researchers should collaborate with the sugar industry and related sectors to better understand the difficulties that arise when scaling up each process module, address engineering and technical roadblocks, and provide long-term solutions for the growth of biorefineries. LA demand is skyrocketing, and developments in the manufacturing of sugar from lignocellulosic biomass and engineering of microbial strains have heightened expectations for a profitable LA production via SCB-based industry. Sugar refineries can be economical, reduce debris, and expand their product line if they demonstrate a technologically and commercially viable procedure for producing 2G lactic acid. Moreover, it may also provide indirect benefits such as promoting bio-based economic activities in rural areas, improving the socioeconomic standing of collaborators, generating more employment positions, and promoting environmental sustainability. LA manufacturing using renewable resources faces challenges in concentrations, yields, productivity, and optical purity. Cost reduction, appropriate raw materials, fermentation techniques, strain tolerance screening, and effective processes are crucial.



## Tables

Mode of Fermentation	Feedstocks generation	Pretreatment	Advantages	Disadvantages
Fed-batch fermentation	1st	Not required	<ul style="list-style-type: none"> <li>Decreased inhibition of substrate</li> <li>Improved cell concentration</li> <li>Less process time required.</li> <li>High growth of cells</li> </ul>	<ul style="list-style-type: none"> <li>Product inhibition</li> <li>Problems faced while controlling procedure conditions.</li> <li>Low productivity when batch number is increased.</li> <li>Issues with viability and stability of cells</li> </ul>
	2nd	Solids, including food waste and corn stover, are milled, dissolved, mixed with other feedstocks, detoxified using substrate and lignocellulose, sterilized, and saccharified.		
	3rd	Liquid (including whey & molasses); mix (with other base materials) – sterilization – saccharification Mill – dissolve – mix (with other base materials) – detoxification – sterilization – saccharification (or SSF)		
Continuous fermentation	1st	///	<ul style="list-style-type: none"> <li>Under control growth</li> <li>Elevated product yield</li> </ul>	<ul style="list-style-type: none"> <li>Failure of complete substrate consumption</li> <li>Cells washing requirement or accumulation of product</li> </ul>
	2nd			
	3rd			
Cell immobilization fermentation	1st	///	<ul style="list-style-type: none"> <li>Fermentation Reliability and Product Yield</li> <li>Reliable for batch or continuous fermentation.</li> <li>High product yield.</li> </ul>	<ul style="list-style-type: none"> <li>Limitations associated with mass transfer.</li> <li>Mechanical instability</li> </ul>
	2nd	Feedstocks pre-treatment for example in case of lignocellulose, may generate inhibitory substances that's why it is not suggested.		

Mode of Fermentation	Feedstocks generation	Pretreatment	Advantages	Disadvantages
	3rd	///	<ul style="list-style-type: none"> <li>No cell washing required.</li> </ul>	
Cell-Recycled batch fermentation	1st	///	<ul style="list-style-type: none"> <li>High cell density.</li> </ul>	<ul style="list-style-type: none"> <li>Requires a separation device, for example centrifuge.</li> </ul>
	2nd	Feedstocks pre-treatment for example in case of lignocellulose, may generate inhibitory substances that's why it is not suggested.	<ul style="list-style-type: none"> <li>High cell density</li> <li>High product yield</li> </ul>	
	3rd	///		

**Table 1.** Advantages, disadvantages, and subsequent feedstock productions through different Fermentative mode of Process organizations.<sup>[40][58][59][60][61]</sup>

///= Same as above

Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] % -age	Carbohydrate Conversion ratio (% -age), Procedure Primacy & Shortcomings	References
NaOH (Alkali) Cellulase Hydrolysis	CS (Corn Stover)	75°C/3h	20% SL [5% NaOH] Cellic Ctec2	-	[62]
Sodium hydroxide (FBH)			20 (SL%), Cellic CTec3 (EL)	Xylan (93) Glucan (83), PEG* (E/A), 124.9+183.5 (G+X), 2.60	[18]
NaOH (Alkali) Cellulase Hydrolysis	DDGS	121°C for 15 min	10% loading of DDGS	1,500 Cellulase-Accellerase® cellulose: enzyme ratio 1:3@ 50°C	[63]
al-AGO (FBH)	-	-	20 (SL %), LT4 (EL), BSA, Endo xylanase, BG, Tween, PEG (E/A)	Glucan (83), 1.48 Sugar*/ glucose. (g/L.h), 107 Glucose titer (g/l), 1.48 Productivity Sugar*/ glucose (g/L.h)	[28]
AlCl <sub>3</sub> - Aq. Glycerol (acidified)	Organosolv lignin	150°C1h	10 SL (%) precipitated through ethanol-water (3:9) conversion. Ethanol and water vacuum removed at 50°C.	Recycled glycerol (acidified) shows 96-100% glucan recovery efficiency. The recycling process experienced a significant reduction in delignification capacity by 8-14% compared to fresh solvent, and a	[64]

Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] % -age	Carbohydrate Conversion ratio (% -age), Procedure Primacy & Shortcomings	References
			Three pretreatments recycle acidified glycerol.	significant decrease in xylan removal efficiency.	
SE, NaOH, & bleaching (Sequential) (BH)	-	-	17.5 (SL%), Cellic CTec <sup>2</sup> (EL)	Glucan (80%), 125 Glucose titer (g/l), 1.74 Productivity Sugar* / glucose (g/L.h)	[65]
Sodium hydroxide (FBH)	-	-	20 (SL%), Cellic CTec <sup>2</sup> (EL)	Xylan (82.6%) Glucan (84%), PEG (E/A), 126.8+166.7 (G+X) Glucose titer (g/l), 2.64 Productivity Sugar* / glucose (g/L.h)	[27]
Enzymatic hydrolysis/ PA Phosphoric Acid	Rye straw 75-80%	50-75°C/15-45 min	-	PA /enzymatic hydrolysis, Ratio 1:3	[66]

Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] % - age	Carbohydrate Conversion ratio (% - age), Procedure Primacy & Shortcomings	References
Lactic acid or TEBA		6.67 SL (%) / 120°C / 4h	The solids, based on EtOH, were washed with DES-rich hydrolysate, and then recovered as a solid, which was then recovered five times.	<ul style="list-style-type: none"> <li>• 5th DES recycling reduces glucan digestibility by 22%.</li> <li>• 20% and 46% reduction in alkali and water usage.</li> <li>Seven recycles achieved approx. 78.5% delignification, compared to approx. 80% with fresh sodium hydroxide</li> </ul>	[67]
Alkaline/ H <sub>2</sub> O <sub>2</sub> (Hydrogen Peroxide)	Sugar Beet Pulp	24h, pH 11.5 @ 30°C	Liquid/Solid (20:1)	hydrogen peroxide (1%)	[68]
Alk. NaOH		10 SL % / 121°C / 1h /	<p>100 mL BL produced using Ist cycle, pH adjusted to 13.7.</p> <p>Vacuum pretreatment with fresh NaOH for positive control with VALBR repeated seven times.</p>	<ul style="list-style-type: none"> <li>• VALBR enhances Xylan recovery by 14%.</li> <li>Glucan recovery was similar, 79.94 ± 0.96% via VALBR and 81.7 % via fresh sodium hydroxide.</li> <li>Post-saccharification occurs when proportionate glucose is used.</li> </ul>	[69]
Alkali (NaOH)	SCB	121°C / 30m	15 [2]%	Over 85% of sugars are produced through	[18]

Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] % -age	Carbohydrate Conversion ratio (% -age), Procedure Primacy & Shortcomings	References
				20% solids via fed-batch hydrolysis. (21 % Xln* <sup>2</sup> lost in BL)	
COBRA (BH)	-	-	21 (SL%), Cellic CTec <sup>3</sup> (EL),	Sugar (> 90), Xylan (> 95) Glucan (> 95), Multifect pectinase, HTec3 & Cellic (E/A) 0.62 Glucose titer (g/l), 2.64 Productivity Sugar* / glucose (g/L.h)	[18]
Sodium hydroxide (FBH)	-	-	21 (SL%), Cellic CTec3 (EL)	Xylan (93%) Glucan (86%), Multifect pectinase, HTec3 & Cellic (E/A), Glucose titer (g/l)=61+97 (G+X), 0.63= Productivity Sugar* / glucose (g/L.h)	[18]
Sulfuric Acid (2%)	SCB	121 °C/ 1.5hr	1:2 Solid: liquid ratio	The bead coating method at optimal conditions can be repeated 4 to 9 times, resulting in a lactose content of 128.45 g/L.	[70]
$\alpha$ -amylase, Cellulase, glucoamylase	<i>Arthrospira platensis</i> (Microalga)	90°C, 60°C, & 50°C, for 2h, 2h, & 40h	-	-	[71]

Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] % -age	Carbohydrate Conversion ratio (% -age), Procedure Primacy & Shortcomings	References
EMIM Ac	—	2h/5 SL %/120°C	Utilizing ultra-filtration and electrolysis in combination.  94% of the IL that was recycled four times was recovered.	•delignification (61.97 ± 0.68%) achieved from four IL recycles.  Recycled IL outperforms fresh IL in delignification efficiency.	[72]
$\alpha$ -amylase, cellulase, glucoamylase	Cassava bagasse	105°C for 1hr	n/a	Cellulose & Glucoamylase added	[73]
Alkali (NaOH)	n/a	121°C/30	2 %	Above 85% of sugars are produced through 20% solids via fed-batch hydrolysis.  (35 % Xln* <sup>2</sup> lost in BL)	[27]
COBRA-LE (BH)	-	-	, Cellic CTec <sup>3</sup> (EL)	Multifect pectinase, HTec3 & Cellic (E/A), Glucose titer (g/l) 61+97 (G+X), 0.63 Productivity Sugar*/glucose (g/L.h)	[14]
Enzymatic Hydrolysis	Corn Stover	pH 5.0, 50°C, 72 h	-	Crude lignocellulolytic	[58]
Basic-ASE (Sequential) (BH)			15 (SL%), Cellic CTec2	Glucan (84%), 75Glucose titer (g/l), 1.6 Productivity	[74]

Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] % -age	Carbohydrate Conversion ratio (% -age), Procedure Primacy & Shortcomings	References
				Sugar*/ glucose (g/L.h)	
Enzyme hydrolysis <sup>#1</sup>	Waste of Date pulp	pH 5.0-to-5.5, 50°C,	150 g/L TS	n/a	[60]
Glycerin and DA	SCB	170°C/15 (Gly.) and 130°C/15 (DA)	20 [80% (Gly), and 2.4% (DA)]	66.5 % xylose yields and 99.2% gln* <sup>1</sup> digestibility. (> 45% unrecovered CH fraction)	[75]
Enzymatic Hydrolysis/ H <sub>2</sub> SO <sub>4</sub>	Wheat Straw	160°C, 40 min	1:10 ratio Solid-liquid, Cellulose (5 FPU per g)	H <sub>2</sub> SO <sub>4</sub> (1.5%), 72h @ 150 rpm Cellulase Cellic	[76]
DA- Glycerol (Sequential)		130°C/15 (DA), 150°C/15 (Gly)	23.8 [3.33%]	AH 70% (gln* <sup>1</sup> digestibility (69%))	[75]
Enzyme hydrolysis, Acid, Alkali	SCB	27 min @ 121°C	Liquid: Solid (2.8:1), sulfuric acid (1%)	NaOH (4%) sodium hydroxide @ 121°C, liquid: solid 20:1 Enzymatic hydrolysis, 24h, pH 5.0 @ 50°C & Enzyme Cocktail	[59]
Glycerol		150°C/15	20 [80%]	92.5 gln* <sup>1</sup> digestibility achieved with 72% lignin. (Xylose loss in pre-hydrolysate glycerol)	[75]



Pre-Conditioning Method	Lignocellulosic Biomass/ Biomass Recalcitrance	Temp (°C) / Duration (min)	Loading Wt. (SL) & [Chemical loading] %age	Carbohydrate Conversion ratio (%age), Procedure Primacy & Shortcomings	References
Hydrothermalmagnetic solid acid (carbon-based)	SCB	10 min. @ 170°C	Cellulase 72h @ 50°C	Water:Catalyst: SCB (25:1:1) (ml/g/g)	[77]

**Table 2.** Preconditioning, biomass recalcitrance, and carbohydrates recovery (%age) indicating optimized valorization of sugarcane bagasse.

\*<sup>1</sup> Glucan; \*<sup>2</sup>Xylan; \*<sup>3</sup>Klason lignin; # demonstrate Residual solids after the process of pre-treatment; AH: acid hydrolysis; SL: solid loading; TSE: twin screw extruder; DA: dilute H<sub>2</sub>SO<sub>4</sub>; S:L: solid to liquid ratio; BL: black liquor; LHW: liquid hot water; IL: Ionic Liquid; WDM: Wet Disc Milling; Ox-B: hypochlorite-hydrogen peroxide pretreatment, TS: Total Solids, SCB: Sugar-cane Bagasse, \* EtOH stands for Ethanol, BL for black liquor DES for deep eutectic solvent; TEBA: Tri-ethyl benzyl ammonium chloride; EMIM-Ac: 1-ethyl-3-methylimidazolium acetate. #<sup>1</sup>Cellic® CTec2, EL; Enzyme Loading, E/A: Enzymes/ additives used, PEG\* PEG 6000; SL: solid loading; al-AGO; Atmospheric glycerin stimulated by alkali; SE: Steam bursting; ASE: alkaline sulfite pretreated ethanol; WP for Whey Protein; COBRA; Compacted biomass recovered (through) ammonia; Lignin Extraction (LE); Calcium Lignosulfonate (CL); Sophorolipid (SPL); BG--glucosidase; HC-hemicellulose; Blood Serum Albumin (BSA); Polyethylene glycol (PEG); FBH and BH indicate Fed-batch and batch hydrolysis (BH).

Fermentation Operation Procedure	Microbial Strain	Pre-treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
Fed-batch	<i>Lactobacillus sakei</i> <sup>#1</sup> <i>Lactobacillus rhamnosus</i> , <i>Weissella sp.</i> <sup>#2</sup> <i>Weissella paramesenteroides</i> <sup>#3</sup>			0.73	6.25	A novel pretreatment hydrodynamic cavitation (HC) vortex-based method for bagasse pretreatment has been successfully processed, resulting in a net energy gain of 373 kWh/ton and enhanced biomethane production.	[78]
				0.85	7.53		
				0.81	7.20		
SSCF	<i>Lactobacillus plantarum</i> <sup>#10</sup>	Sulphuric Acid 20 min. @ 121°C	Glucose & Hydrolysates	0.94	1.94	LA-fermentation using <i>Lactobacillus plantarum</i> <sup>#10</sup> cells, using glucose and <i>Chlorella vulgaris</i> <sup>#11</sup> hydrolysate as carbon sources. Batch fermentation	[49]

Fermentation Operation Procedure	Microbial Strain	Pre- treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
						improved LA titer and yield by 43% and 39%, respectively. Fed-batch culture within situ LA removal increased productivity by 72%. The highest LA productivity was achieved with glucose and hydrolysate.	
Fed-batch SSF	<i>Bacillus coagulans</i> #6	CS-Pulp (NaOH 2%)  @ 118 °C	lignocellulosic  L-LA	0.39	1.25	A non-sterilized fed-batch SSF - membrane separation integration process to reduce cellulase consumption in lignocellulosic L-LA production. The process recycled residual	[49]

Fermentation Operation Procedure	Microbial Strain	Pre- treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
						cellulase and solid residues from CS- resulted in a 1.20 times higher LA yield and reduced wastewater discharge and nutrient consumption.	
B-SHF	<i>B. coagulans</i> <sup>1</sup>	NaOH –DA	Glucose	0.90	2.99	The study aimed to maximize carbohydrate content recovery from SCB by evaluating various pretreatment procedures and later focusing on LA production.	[51]
		NaOH		0.92	2.86		
		DA- NaOH		0.88	2.79		
		Cavitation with alkali		0.92	2.6		
B-SHF	<i>B. coagulans</i> <sup>1</sup>	NaOH	Glucose	0.76	2.88	The seed medium was prepared using a wash fraction of enzyme-catalyzed	[79]

Fermentation Operation Procedure	Microbial Strain	Pre-treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
						hydrolysis and high-cell density fermentation was performed.	
B-SScF	<i>L. pentosus</i> <sup>2</sup>	Sequential DA- NaOH	Glucose and Xylose	0.93	1.01	The strain was modified to improve its fermentation capacity and inhibitor resistance by being adapted to acid-hydrolysate.	[80]
SSCF	Mixed Culture <i>Bacillus coagulans</i> <sup>#5</sup> <i>Lactobacillus rhamnosus</i> <sup>#4</sup>	N/A	Starch & Lignocellulosic sugars	0.88	2.74 (112.5g/L Productivity)	A by-product cassava bagasse, with starch and lignocellulosic fibers, for lactic acid production through simultaneous saccharification and co-fermentation. The mixed culture of <i>B. coagulans</i> & <i>L. rhamnosus</i> was significantly	[49]

Fermentation Operation Procedure	Microbial Strain	Pre- treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
						improved lactic acid concentration and productivity, achieving a highest yield of 0.88 g/g, making it a promising waste disposal method.	
SSF	<i>L. rhamnosus</i> <sup>#7</sup>	RPS <sup>#8</sup>	LA	0.76	0.81	LA produced lactic acid from RPS <sup>#8</sup> using a bench-scale bioreactor. The process improves both hydrolysis and fermentation, resulting in higher conversion rates and increased yield. The bioreactor also enhances the process by applying a pulsed fed-	[81]

Fermentation Operation Procedure	Microbial Strain	Pre- treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
						batch strategy, resulting in a 62% yield after 120 hours. This makes large-scale upgrading of RPS more realistic.	
SHF	<i>Lactobacillus delbrueckii</i> ssp.	OPW#9 hydrolysates	d-Lactic Acid (Galactose and Fructose uptake)	0.95	6.72	D-LA production involving resting or immobilized <i>delbrueckii</i> cells is feasible under optimal conditions like 40°C, 800 rpm, pH 5.8, and 10 g/L biomass. This method yields good results on fructose and galactose, eliminating nitrogen sources and purification processes.	[82]

Fermentation Operation Procedure	Microbial Strain	Pre-treatment Method/ Optimal conditions	Sugar Valorized	Sugar yield (g/g)	Production Capacity (g/L/h)	Significant Potential Features	References
B-SHF	<i>B. coagulans</i> <sup>3*</sup>	Dilute HCl	Xylose	0.87	1.7	<i>B. coagulans</i> transformed a detoxifying pre-hydrolysate rich in xylose, containing furfural and HMF, into Lactic acid through fermentation.	[83]
FB-SSF	<i>B. coagulans</i> <sup>9</sup>	Sulphite pulping	Glucose	0.72	0.57	The enzyme dose in FB-SSF decreased from 15-10 FPU/g DM compared to SHF.	[52]

**Table 3.** Valorization potential of sugarcane bagasse through microbial fermentation and their respective production capacity and yield.

<sup>1</sup>NCIM-5648; <sup>2</sup>ATCC-8041; <sup>3</sup>DSMID14-300; <sup>4</sup>DSM-2314; <sup>5</sup>Uc-3; <sup>6</sup>17C5; <sup>7</sup>P38; <sup>8</sup>RM2-24; <sup>9</sup>CC17; \*SSF: Simultaneous saccharification & fermentation; AH stands for acid hydrolysate; B stands for batch; DA is for dilute acid; SHF; Separate hydrolysis & fermentation; SScF: Simultaneous saccharification & co-fermentation. \$: production process for D (-) lactic acid; # production process for L(+) lactic acid. <sup>#1</sup> 25, <sup>#2</sup> 28, <sup>#3</sup> 24, <sup>#4</sup> LA-04-01, <sup>#5</sup> LA-15-2, <sup>#6</sup> LA-1507, <sup>#7</sup>ATCC 7469, RPS<sup>#8</sup>: Recycled Paper Sludge, OPW-hydrolysate<sup>#9</sup>: Orange Peel Waste Hydrolysate, CS: Corn Starch, <sup>#10</sup> =23, <sup>#11</sup> = ESP-31



## Abbreviations

Abbreviation	Meaning
ABR	Acid-base ratio
ASE	Alkaline Sulfite Ethanol
BD	Biomass densification
BH	Batch Hydrolysis
BL	Black liquor
BSA	Blood Serum Albumin
BSP	Bagasse Sulfite Pulp
CL	Calcium Lignosulfonate
COBRA	Compacted Biomass Recovered Ammonia
DES	Deep Eutectic Solvent
EMIM-Ac	1-ethyl-3-methylimidazolium acetate
EtOH	Ethanol
FBH	Fed-batch Hydrolysis
HC	Hemicellulose
LA	Lactic Acid
LCBs	Lignocellulosic Biomass
LE	Lignin Extraction
MEA	Mono-Ethanol-Ammonium Acetate
MMT	Million Metric Tonnes
PEG	Polyethylene glycol
PLA	Polylactic acid
SCB	Sugarcane Bagasse
SDGs	Sustainability Development Goals
SE	Steam Bursting
SHF	Sequential Hydrolysis Fermentation

Abbreviation	Meaning
SL	Solid Loading
SPL	Sophorolipid
SScF	Simultaneous Saccharification & Co-fermentation
SSF	Simultaneous Saccharification Fermentation
TEBA	Tri-ethyl benzyl ammonium chloride
WP	Whey Protein
XOS	Xylo-oligosaccharides

## Statements and Declarations

**Ethical Approval and Consent to Participate:** Not Applicable

**Consent for Publication:** Not Applicable

**Data and Material Availability:** The authors will provide the information and material sources used in their current work upon reasonable request.

**Competing Interests:** The authors declare no competing interests.

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**Author's Contribution:** The current study involved formal analysis (I.P.; M.Z.), conceptualization (M.N.; H.S.; T.A.F.); draft preparation (I.P.; T.A.F.; H.S.; & M.N.), and supervision (I.P., M.Z., N.A, S.H., Y.S., Q.S., & S.H.A.), and editing (M.Z.; M.N.; H.S.; T.A.F.). The current version has been approved by all authors.

**Consent for Publication:** Not Applicable.

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## Declarations

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