

# Advances in safe processing of sugarcane bagasse for the generation of bio-products and biofuels: An overview

## Abstract

One of the main agricultural products grown in tropical nations is sugarcane. An estimated 1.6 billion tons of sugarcane are produced worldwide each year, and from that, 279 million metric tons (MMT) of biomass residues—bagasse and leaves—are produced. Sugarcane residues have been investigated for both non-biotechnological and biotechnological uses, in particular sugarcane bagasse (SB) and leaves (SL). The use of SB and SL in lignocellulosic bioconversion has been investigated for the past thirty years. This process presents prospects for the profitable use of leftover substrates in the synthesis of bioethanol and value-added commercial products like xylitol, specialty enzymes, organic acids, single-cell protein, etc. However, the development of bio-based commercial processes using SB and SL as basic substrates still faces significant technological and financial obstacles. The need to find a cost-effective and environmentally safe gasoline substitute stems from the depletion of fossil fuel reserves, frequent increases in gasoline prices, and harm to the environment. Due to the growing needs for fuel, food, feed, and other resources, the use of ethanol as an energy source does not appear appropriate in the long run. Ethanol is created from substrates that are derived from food and feed, such as grains, sugars, and molasses. However, the development of bio-based commercial processes using SB and SL as basic substrates still faces significant technological and financial obstacles. In order to better understand their industrial consequences, their application in commercial products, including commercial evaluation, and their potential to improve sustainable bio-based fuel systems, this article will examine SB and SL as less expensive sources of carbohydrates in developing countries.

**Keywords:** Bagasse; bio-products sugarcane residues; ethanol; xylitol; value added product

**About the abstract:**

## Introduction

Since the world's population has increased over the past century and food consumption has been rising, it is critical to develop economical and environmentally friendly processes, like thermal conversion processes, to produce high-value bio-based materials from agricultural waste or byproducts. Utilizing sustainable and less expensive resources for their bioconversion into value-added products of commercial interest through fundamental methods of microbial bioconversion has become necessary due to the ongoing need for non-food and feed-based substrates (Hatti-Kaul et al., 2007). Numerous items have been produced with this goal using renewable resources like biomass. The agricultural sectors have advanced to the point where millions of tons of trash and byproducts are produced annually, which might be used as

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Comment [u5]: authors have to reorganize the abstract: Introduce 1st and 2nd generation biofuels by showing the growing interest in the 2nd generation. Follow up with agricultural residues such as bagasse and sugar cane leaves. Specify the recovery channels for the last thirty years (ethanol, xylitol, others). Announce the technological and environmental challenges of each recovery sector and show how the ethanol sector has more challenges to overcome

inexpensive sources of material and energy (Pandey et al., 2000). Among these byproducts is sugarcane bagasse, which has applications in the manufacturing of xylitol, ethanol, organic acids, industrial enzymes, and more (Parameswaran, 2009). After sugarcane is crushed to extract juice for making sugar and ethanol, the residue left over is called bagasse. The leaves that are typically left in agricultural areas after sugarcane is harvested are another significant sugarcane residue (Krishna et al., 1998). One hectare of sugarcane crop yields 6–8 tons of dried leaves, also known as sugarcane trash (ST). Typically, leaves are burned in the fields, creating fly ash, seriously reducing the diversity of soil microbes, and creating environmental issues.

A significant amount of cellulose and hemicellulose can be broken down into simple sugar monomers (glucose, xylose, arabinose, mannose, galactose, etc.) by chemical or enzyme cocktails. This process can be applied to sugarcane bagasse (SB) and sugarcane leaves/trash (SL or ST). (2008) Singh et al. Based on the previously indicated data, a significant amount of SB and SL are kept in stockyards and on the ground during the sugarcane harvesting season. For **SCB** reprocessing facilities that eliminate the pith, the same is true. These raw materials' smell and dust particles have the potential to contaminate the environment and infect inhabitants with infectious diseases. These days, agricultural food crops serve as the primary feedstock for the manufacturing of liquid biofuels. For instance, sugar cane, corn, wheat, and sugar beets are frequently used to produce bioethanol. Oil seeds like soybean, rapeseed, sunflower, and palm oil are also utilized in the manufacturing of biodiesel (Demirbas, 2008). Climate-related variables cause regional variations in the availability of various feed supplies. As per Ajanovic's (2013) findings, the predominant feedstock utilized for bioethanol production is wheat, constituting 70% of the total. Barley is used for only 15% of bioethanol production, while corn and rye account for 10% and 5% of the total bioethanol production in the European Union in 2008. **Seventy-nine percent of the biodiesel produced in the European Union is made from rapeseed, with soybeans accounting for eighteen percent and sunflowers for just three percent.** Natural (organic) and non-natural undesired materials, mostly from agricultural operations, were included in agroindustrial wastes (Sarkar et al., 2012). Solid agroindustrial wastes include sugarcane bagasse, potato starch residues, grape pomace, sugar beet pomace, and raw corn starch, among other waste materials (Visioli et al., 2014). Moreover, agricultural wastes may include residues that include starch and sugar, such as sugarcane (Visioli et al., 2014). Furthermore, according to Rabi et al. (2009), agroindustrial wastes might be either non-degradable or degradable.

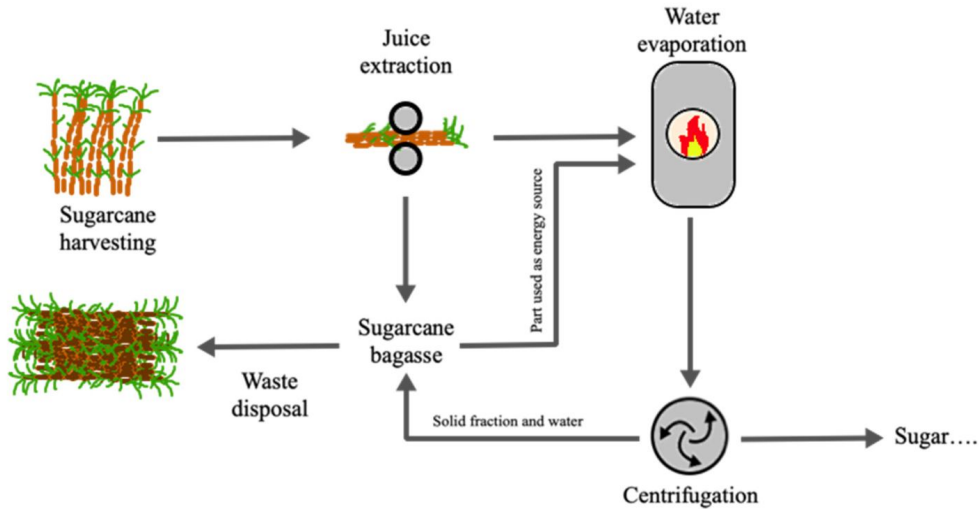
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The biological process that turns lignocellulose biomass into fuel ethanol generally entails the following steps: (1) pretreatment to liberate cellulose by removing lignin or hemicellulose; (2) cellulase-mediated depolymerization of carbohydrate polymers to produce free sugars; (3) fermentation of hexose and/or pentose sugars to produce ethanol; and (4) distillation of ethanol. Since it delivers energy that is renewable and less carbon intensive than gasoline, ethanol made from sugarcane leftovers is one of the best substitutes for fossil fuels when used in part. Because it emits fewer greenhouse gases into the atmosphere, bioethanol helps to combat climate change and reduce air pollution. The important facts about sugarcane bagasse, including the predicted biomass of bioproducts and biofuel, are reviewed in this work.

### **Sugarcane Leaves and Sugarcane Bagasse Chemistry**

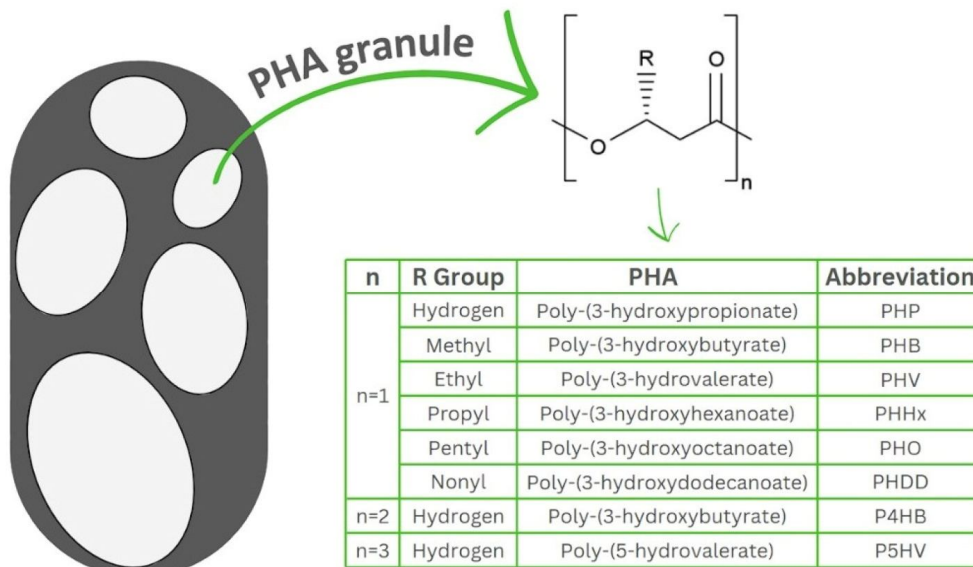
Compared to other straws like wheat, rice, sorghum, etc., the intricate chemical makeup of SB's cell walls restricts its suitability as cow and ruminant fodder, making it a more desirable substrate for commercialization. Generally speaking, hemicellulose (26.2–35.8), cellulose (35–45), lignin (11.4–25.2), and other materials (2.9–14.4) make up SB (% w/w dry basis) (Zhao et al., 2009). Numerous variables, such as crop variety, climatic circumstances, growth site and method, fertilizer use, and soil composition—both physical and chemical—all affect the uneven chemical composition of bagasse (Canilha et al., 2011). Determining the chemical composition of bagasse may also be significantly influenced by the method of chemical composition analysis. The cellulosic component must be harnessed to provide ready-to-fermentable sugars in order to transform the LB into value-added products (Zhao et al., 2009). It is clear that a significant obstacle to accessing the carbohydrate fraction of the plant cell wall is the high lignin content (figure 1). This essentially necessitates pretreatment, which involves higher chemical loadings along with longer reaction times and temperatures, and higher cellulase loadings make the process unprofitable (Chandel et al., 2010). Processing costs are raised by the high cost of cellulolytic enzymes and the high quantity of cellulases needed. According to Zhao et al. (2009), the elimination of lignin improves cellulose accessibility and boosts cellulase's amenability to the plant cell wall's carbohydrate skeleton. Compared to other agricultural residues like rice straw (17.5% ash) or wheat straw (11.0% ash), SB's low ash concentration (1.4%) was shown to be significantly favorable (Pandey et al., 2010).



**Figure 1.** Steps in the application process for using SB to create a variety of industrially significant products

### Sugarcane Structure

Straw (or garbage) and stem make up sugarcane. The substance that is removed from sugarcane stems before to the cane being ground into a juice that is then utilized to produce either sugar (sucrose) or alcohol (ethanol). SB is what's left behind after the juice is extracted from the stems. Fresh leaves, dry leaves, and tips that are accessible prior to harvesting make up SS, sometimes known as garbage. According to Neto (2005), the tops of cane plants are the portion between the top end and the last stalk node. Fresh leaves are green and yellow in color. Dry leaves are often brownish in color (figure 2). The leaves can be used as (1) a fuel for direct burning or (2) a raw material for pyrolysis, which produces char, oil, and/or gas; (3) as a starting point for the synthesis and gasification of methanol. The tops can be used for the following purposes: (1) as fresh or dried ruminant feed; (2) as a substrate for anaerobic fermentation to produce methane; and (3) after water content is reduced, for the energy uses specified for cane garbage (Triana et al., 1990). After the crop is harvested, SB and SS are often burned in an open agricultural field. In certain situations, they are also exploited as an untapped source of simple sugars that can be used to produce alcohol.



**Figure 2.** General structure of polyhydroxyalkanoates accumulated in bacteria in the form of granules. Polyhydroxyalkanoates are classified as short chain length (scl-PHAs), medium chain length (mcl-PHAs) and long chain length (lcl-PHAs). The table insert shows different PHA derivatives.

### Physical and Chemical Compositions of Sugarcane

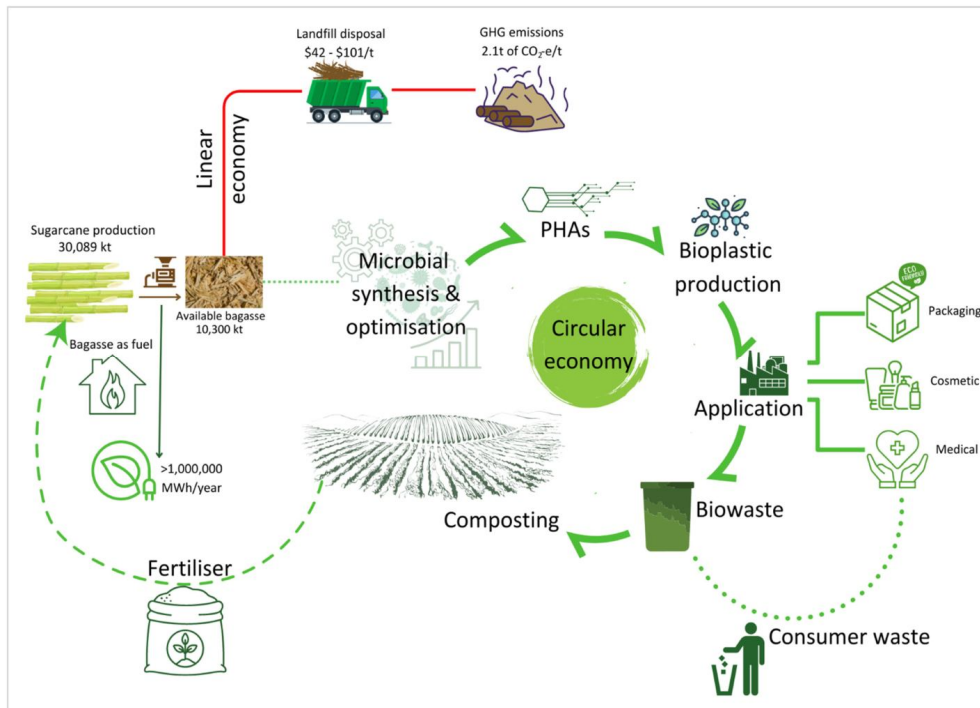
Physically, sugarcane is made up of four main fractions: fiber, nonsoluble solids, soluble solids, and water. The proportion of each fraction varies depending on the sugar agroindustrial process (figure 3). The fiber is made up of the entire organic solid component, which was once present in the stem of the cane and is distinguished by its distinct heterogeneity. The majority of the fraction that is insoluble in water, or nonsoluble solids, are made up of inorganic components such as rocks, soil, and extraneous materials. The conditions in which agricultural cane is processed, as well as the kinds of cutting and harvesting, have a significant impact on this fraction. The majority of the soluble solids fraction that dissolves in water is sucrose, with minor amounts of other chemical components such waxes (Triana et al., 1990). The main component of 2G ethanol synthesis, SB or SS, are lignocellulosic materials made up of cellulose, hemicelluloses, and lignin.



Figure 3: A comparative evaluation on the physicochemical properties of sugarcane residues (Najafi et al., 2023).

### Bagasse Pretreatment for Industrial Applications

To increase the total hydrolysis efficiency, exposing the celluloses by removing lignin or hemicellulose is an efficient pretreatment (Kumar et al 2009). For maximum hydrolysis of lignocellulosic material, essential parameters include pretreatment, an efficient cellulolytic enzyme cocktail, the amount of enzyme loading, hydrolyzing conditions, and the kind of lignocellulosic material (Zhao et al., 2009). With pretreated substrate, significant increases in lignin removal and hemicellulose depolymerization into simpler sugars have frequently been recorded (figure 4). Additionally, pretreated SB has been used as an immobilization carrier (Santos et al., 2008) and as an inert support material for fungal biomass in the solid-state fermentation process (Panday 1999). After a cultivation cycle is over, high product titers with relatively high purity are produced by the mechanical application of prepared SB impregnated with appropriate liquid medium, which creates uniform aerobic conditions throughout the bioreactor.



**Figure 4.** Comparison of linear economy and circular economy models arising from bagasse generation

### Value-Added Goods and Commercially Significant Sugarcane Bagasse

#### Ethanol

SB has long been researched for use in industry, particularly the manufacturing of ethanol. Up to 300 L of ethanol might theoretically be produced from one ton of SB (Cerqueira-Leite et al., 2009). However, a number of factors, like the bagasse's quality and the method used to produce the ethanol, have a direct impact on the amount of ethanol produced (Cerqueira-Leite et al., 2009). For the generation of ethanol, SB has been used in two different methods: simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) (Chande et al., 2007). In pre-saccharification assisted SSF from SB, Santos et al. [34](#) reported highest cellulose-to-ethanol conversions (~60%) with volumetric productivity 0.29–0.30 g L<sup>-1</sup>h<sup>-1</sup>. The significance of process integration in sugarcane processing mills was proposed by Dias et al. (2009). SB was used for the traditional technique of producing ethanol

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from sugarcane juice after being hydrolyzed in an organosolv method under thermal integration, which was aided by diluted acid. These investigations demonstrated how lignin and SB are used as supplementary fuels to meet the biorefinery's energy needs.

### **Xylitol**

According to Carvalho et al. (2008) and Prakasham et al. (2009), another value-added product that may be made from SB/SL by microbial fermentation to meet the high demand in the food and pharmaceutical industries is d-xylitol. Various fruits and vegetables naturally contain this five-carbon sugar alcohol. D-xylitol has been used extensively in the food, pharmaceutical, and odontological industries because of its anti-carcinogenicity, tooth rehardening, and remineralization qualities (Prakasham et al., 2009). Xylitol, a sugar alternative, has been incorporated into food, particularly for individuals with insulin insufficiency (Carvalho et al., 2008 and Prakasham et al., 2009). As noted by Prakasham et al. (2009), yeast species, primarily *Candida* sp., have been reported to generate xylitol from pentose-rich SB hydrolysates. Additionally, it has been discovered that SB is a highly effective carrier for ensnaring *Candida guilliermondii* cells to produce xylitol continuously (Santos et al., 2008). Though SB has been mentioned several times in research and development as a possible substrate for xylitol synthesis, xylitol from SB has not yet reached the industrial level.

### **Industrial enzymes**

Utilizing solid-state fermentation (SSF) or submerged fermentation (SmF) systems, SB has been used to produce industrial enzymes by specific bacteria and fungi, including xylanase, cellulase, amylase, and laccase. Among others, xylanase and cellulase have been thoroughly researched for SB generation (Singh et al., 2010). Cellulase production from SB was compared to that from other lignocellulosic materials, including rice straw, wheat bran, and cassava bagasse, under SSF by *Trichoderma reesei* NRRL 11 460, by Singhania et al. (2006). SB was shown to have the highest cellulase production (154.58 U/gds-1), with rice straw, wheat bran, and cassava bagasse coming in second and third, respectively. The success of biorefineries is significantly influenced by the cost of cellulase. There is still work to be done to find a viable technology that can produce cellulase with high titers at a reasonable cost.

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### **Commercially Interesting Microbial Metabolic Products Derived from Sugarcane Bagasse**

In addition to ethanol, xylitol, enzymes, and organic acids, SB has been used to produce antibiotics, animal feed, biohydrogen, alkaloids, and pigments, among other value-added

products of commercial significance (Pandey et al., 2000). In an SSF system, Nampoothiri and Pandey<sup>50</sup> produced 80 mg L<sup>-1</sup> glutamic acid g<sup>-1</sup> dry bagasse by using SB to create L-glutamic acid. Additionally, in SmF cultivation, the synthesis of a single-cell protein with *Candida langeronii* RLJY-019 using SB has been established (2009). SB has also been investigated for the synthesis of several organic acids (citric acid, lactic acid, gluconic acid, etc.) utilizing a range of microbes and growth methods. Borges and Pereira (2010) used *Actinobacillus succinogenes* to produce succinic acid using SB hemicellulosic hydrolysate. In order to produce gluconic acid under semi-solid state fermentation (SmSF) and SSF, Singh et al. (2003) employed SB as an inert support for the growth of *Aspergillus niger* mycelium in a stabilized mutant strain called ORS-4.410. Under SSF circumstances, *Gibberella fujikuroi*, NRRL 2278, synthesized gibberellic acid (GA), a plant hormone, using SB. Excellent biomass growth was seen, but during the fermentation reaction, only a little amount of GA was detected (Nampoothiri and Pandey, 1996).

#### **Bio-Industrial Significance of Sugarcane Leaves/Trash**

SL has not yet been investigated for biological functions. Because 57.5% of SL's cell walls are made of carbohydrates, this indicates that it may be possible to bioconvert important goods, like ethanol to biofuel. Nonetheless, the high concentration of silica (6.96%) and lignin (36.1%) may restrict SL's applicability in the veterinary and industrial sectors.<sup>61</sup> Sulfuric acid hydrolyzed SL at different temperatures, acid loading, hydrolysis periods, and solid:liquid ratios in a fractional factorial and central composite design, regardless of the complicated chemical makeup of its cell wall. Ethanol (2% w/v) was produced from SL using SSF and cellulases from *S. cerevisiae* NRRL-Y-132 and *Trichoderma reesei* QM 9414, according to Krishna et al. (1998). After pretreating SL with steam at 220 °C for 5 min, Ferreira-Leitao et al. (2010) assessed the saccharification of SL into glucose (97.2% theoretical yield). Mane et al. (1988) used ST to produce oxalic acid (42.9–51.6% w/w) among compounds of economic significance by the use of a nitric oxide oxidation method. A low-density biomass gasification system for thermal purposes was investigated using SL (Jorapur and Rajvanshi, 1995). The system's effectiveness was evaluated for over 700 hours ex situ, producing output levels between 288 and 1080 MJ h<sup>-1</sup>.

#### **Future Perspectives**

The SB and SL byproducts produced during the agro-industrial processing of sugarcane are potential sources of carbohydrates that can be utilized to create value goods with a

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marketable appeal. Advances in microbiotechnology seem to provide enormous potential for sustainable, economic, environmental, and strategic advantages in bio-industrial applications. Numerous value-added products, including ethanol, xylitol, organic acids, industrial enzymes, and other significant specialized chemicals, have previously been effectively produced from SB. But scaling up to the pilot stage is still required. In the underdeveloped world, this would be a very fulfilling step forward. For example, it has already been predicted that if the alcohol business exploited the complete sugarcane plant, including SB in the process, 16 times more energy could be produced. If SL were included in the processing cycle, the amount of energy generated may be raised even further. Biofuel produced from SB could soon be able to offset the rising cost of gasoline. Integration of process steps such as hydrolysis, detoxification, fermentation, and distillation would be a useful tactic in the sugar and alcohol industries to optimize the effective use of raw substrates. SB is currently largely used in boilers as a less expensive energy source. The technique currently in use to produce bioethanol from SB polysaccharides is unable to provide "second-generation ethanol" at a price that is competitive. Consequently, several methods for transforming SB and SL/ST into valuable products with high market value—like xylitol, enzymes, organic acids, antibiotics, and single-cell protein—would help to streamline the entire process.

The last thirty years have seen rapid advancements in pretreatment techniques, microbial biotechnology, and downstream processing that have made it possible to utilize sugarcane residues for the large-scale, commercially significant production of numerous products without compromising the supply of food and feed. One major obstacle to the effective use of these wastes is biomass recalcitrance. Pretreatment is a necessary step to improve the accessibility of carbohydrates for the enzymatic hydrolysis reaction that follows, which produces fermentable sugars, and to overcome the biomass recalcitrance. There are a number of reliable pretreatment techniques available; however, the best pretreatment process will depend on factors such as efficient hemicellulose or delignification removal, minimal inhibitor generation, minimal sugar loss, cost-effectiveness, and reduced environmental pollution. The appropriate ethnologic strain turns the sugars that are liberated following hemicellulose depolymerization and enzymatic hydrolysis into ethanol. The ethnologic strains should be able to use pentose and hexose sugars, have high osmotolerance, and be resistant to inhibitors in order to produce the required ethanol yields. To create a long-term, sustainable second-generation ethanol manufacturing process from

sugarcane scraps, the 10 characteristics listed below are essential. (1) Maximum use of the nation's SB and SS production for improved management. (2) Choosing the appropriate detoxification and pretreatment plan. (3) Internal synthesis of cellulase and the creation of ethanol-producing and cellulolytic strains from pentose and hexose sugars that exhibit tolerance to ethanol, resistance to inhibitors, and quicker rates of sugar conversion. (4) Process intensification: simultaneous fermentation and hydrolysis in one location. (5) Ethanol distillation is inexpensive, quick, and efficient. (6) Combining sugar/distilleries with bioethanol production facilities to share machinery, reactors, and other equipment. (7) Maximum use of products (cell mass of yeast, furans, and lignin). (8) Preservation of the environment.

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### Conclusion

Several countries, including India, offer sugarcane bagasse (SB) and sugarcane leaves (SL) as an appealing renewable feedstock that is of the second generation. When utilized responsibly, this feedstock might offer a steady supply of industrial enzymes, drop-in ethanol, organic acids, single-cell proteins, and other products. Nonetheless, a sizeable portion of this biomass is used by industry to produce steam and power. The remainder constitutes the optimal feedstock for producing high-value goods.

### References

1. [37] Prakasham RS, Rao RS and Hobbs PJ, Current trends in biotechnological production of xylitol and future prospects. *Curr Trends Biotechnol Pharm* 3:8–36 (2009).
2. Ajanovic A (2013) Renewable fuels - A comparative assessment from economic, energetic and ecological point-of-view up to 2050 in EU-countries. *Renewable Energy* 60:733–8.
3. Borges ER and Pereira N Jr, Succinic acid production from sugarcane bagasse hemicellulose hydrolysate by *Actinobacillus succinogenes*. *J Ind Microbiol Biotechnol* 38:1001–1011 (2010).
4. Canilha L, Santos VTO, Rocha GJM, Silva JBM and Giulietti M, A study on the pretreatment of a sugarcane bagasse sample with dilute sulfuric acid. *J Ind Microbiol Biotechnol* 38:1467–1475 (2011).
5. Carvalho W, Canilha L and Silva SS, Semi-continuous xylose-to-xylitol bioconversion by Ca-alginate entrapped yeast cells in a stirred tank reactor. *Bioprocess Biosyst Eng* 31:493–498 (2008).
6. Cerqueira-Leite RC, Leal MRLVL, Cortez L, Griffin WM and Scandiffio MIG, Can Brazil replace 5% of the 2025 gasoline world demand with ethanol? *Energy* 34:655–661 (2009).

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7. Chandel AK, Singh OV, Chandrasekhar G, Rao LV and Narasu ML, Key-drivers influencing the commercialization of ethanol based biorefineries. *J Comm Biotechnol* 16:239–257 (2010).
8. Demirbas A (2008a) Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Conversion and Management* 49 : 2106–2116.
9. Dias MOS, et al, Production of bioethanol and other bio-based materials from sugarcane bagasse: integration to conventional bioethanol production process. *ChemEng ResDesign* 87:1206–1216 (2009)
10. Ferreira-Leitao V, Perrone CC, Rodrigues J, Franke APM, Macrelli S and Zacchi G, An approach to the utilization of CO<sub>2</sub> as impregnating agent in steam pretreatment of sugarcane bagasse and leaves for ethanol production. *Biotechnol Biofuels* 3:1–8 (2010).
11. Hatti-Kaul R, Tornvall U, Gustafsson L and Björjesson P, Industrial biotechnology for the production of bio-based chemicals a cradle-to-grave perspective. *Trends Biotechnol* 25:119–124 (2007).
12. Jorapur RM and Rajvanshi AK, Development of sugarcane leaves gasifier for electricity generation. *Biomass Bioenergy* 8:91–8 (1995).
13. Krishna SH, Prasanthi K, Chowdary GV and Ayyanna C, Simultaneous saccharification and fermentation of pretreated sugarcane leaves to ethanol. *Process Biochem* 33:825–830 (1998).
14. Krishna SH, Prasanthi K, Chowdary GV and Ayyanna C, Simultaneous saccharification and fermentation of pretreated sugarcane leaves to ethanol. *Process Biochem* 33:825–830 (1998).
15. Kumar P, Barrett DM, Delwiche MJ and Stroeve P, Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res* 48:3713–3729 (2009).
16. Mane JD, Modak HM, Ramaiah NA and Jadhav SJ, Utilisation of sugarcane trash and other cellulosic wastes for production of oxalic acid. *Biol Wastes* 25:171–76 (1988).
17. Najafi, H., Golrokh Sani, A. & Sobati, M. A. (2023). A comparative evaluation on the physicochemical properties of sugarcane residues for thermal conversion processes. *Industrial Crops and Products* 202, 117112
18. Nampoothiri KM and Pandey A, Solid state fermentation for L-glutamic acid production using *Brevibacterium* sp. *Biotechnol Lett* 18:199–204 (1996).
19. Neto, M. A. T. "Characterization of sugarcane trash and bagasse," in *Biomass Power Generation. Sugarcane Bagasse and Trash*, S. J. Hassuani, M. L. R. V. Leal, and I. C. Macedo, Eds., p. 24, PNUD and CTC, Piracicaba, Brazil, 1st edition, 2005.
20. Nigam JN, Cultivation of *Candida lutzeri* in sugarcane bagasse hemicellulosic hydrolyzate for the production of single cell protein. *World J Microbiol Biotechnol* 16:367–372 (2000).
21. Pandey A, Selvakumar P, Soccol CR and Nigam P, Solid state fermentation for the production of industrial enzymes. *Curr Sci* 77:149–162 (1999).

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22. Pandey A, Soccol CR, Nigam P and Soccol VT, Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. *Bioresource Technol* 74:69–80 (2000).
23. Parameswaran B, Sugarcane bagasse. in *Biotechnology for AgroIndustrial Residues Utilization*, ed. by Nigam P and Pandey A. Springer, Netherlands (2009).
24. Rabi JA, Santos SF, Tonoli GHD, Savastano Jr H (2009) *Agricultural wastes as building materials: properties, performance and applications. Agriculture Issues and Policies Series* Pp. 219.
25. Santos DT, Sarrouh BF, Rivaldi JD, Converti A and Silva SS, Use of sugarcane bagasse as biomaterial for cell immobilization for xylitol production. *J Food Eng* 86:542–548 (2008).
26. Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012) *Bioethanol production from agricultural wastes: An overview. Renewable Energy* 37:19–27.
27. Singh A, Bajar S, Bishnoi NR and Singh N, Laccase production by *Aspergillus heteromorphus* using distillery spent wash and lignocellulosic biomass. *J Hazard Mater* 176:1079–1082 (2010).
28. Singh OV, Jain RK and Singh RP, Gluconic acid production under varying fermentation conditions by *Aspergillus niger*. *J Chem Technol Biotechnol* 78:208–212 (2003).
29. Singh P, Suman A, Tiwari P, Arya N, Gaur A and Shrivastava AK, Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. *World J Microbiol Biotechnol* 24:667–673(2008)
30. Triana, O. Leonard, M. Saavedra, F., I. C. Acan, O. L. Garcia, and A. Abril, *Atlas of Sugarcane Bagasse*, Geplacea and ICIDCA, Mexico, 1990.
31. Visioli LJ, Stringhini FM, Salbego PRS, Chielle DP, Ribeiro GV, Gasparotto JM (2014) *Use of Agroindustrial Residues for Bioethanol Production. Bioenergy Research: Advances and Applications* 49–56.
32. Zhao X, Peng F, Cheng K and Liu D, Enhancement of the enzymatic digestibility of sugarcane bagasse by alkali-peracetic acid pretreatment. *Enzyme Microb Technol* 44:17–23 (2009).
33. Zhao X, Peng F, Cheng K and Liu D, Enhancement of the enzymatic digestibility of sugarcane bagasse by alkali-peracetic acid pretreatment. *Enzyme Microb Technol* 44:17–23 (2009).

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