Lignocellulosic Biomass Conversion Efficiency of *Saccharum officinarum* for Bioenergy Production Using Enzymatic Hydrolysis Optimization Models

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Abstract

Background: The need for a change from using fossil fuels to using renewable sources of energy is becoming increasingly urgent due to global energy security and the rapidly changing climate. Of the various types of biomass, one of the most abundant and least used biomass sources for the production of second-generation bioenergy is lignocellulosic biomass found in *Saccharum officinarum* (sugarcane). Sugarcane bagasse and sugarcane leaves provide a large lignocellulosic biomass supply, consisting of 35-50% cellulose, 20-30% hemicellulose, and 15-25% lignin with significant amounts of fermentable sugars available when broken down. However, due to the structural recalcitrance of the lignocellulosic matrix, there is a limitation on enzymatic conversion efficiency at the commercial scale.

Objective: The biomass composition diversity of *Saccharum officinarum* varieties has been systematically evaluated using the most advanced methods available for pre-treating biomass prior to structural disintegration via enzymatic hydrolysis. This project also provides integrated enzyme-mediated hydrolysis optimization models by Response Surface Methodology (RSM), machine learning techniques, and kinetic modelling techniques to maximize sugar yields while minimizing inhibitory product yields and costs associated with production processes.

Methods: This study used biomass compositional analysis that followed the NREL (National Renewable Energy Laboratory) standard analysis methods. We performed a comparative analysis of alkaline, dilute-acid, steam explosion, and organosolv pretreatments to determine which method most effectively provided cellulose with greater accessibility. Enzymatic hydrolysis was optimized using a Box-Behnken RSM design to evaluate effects of varying enzyme loadings (5 to 30 FPU/g); pHs (4.5 to 5.5); and temperatures (45 to 55 °C). Utilizing the Michaelis-Menton and Langmuir-type competitive inhibition models, we were able to estimate the kinetic parameters for the cellulosic substrates and enzymes used in this study. Finally, techno-economic analysis (TEA) and life cycle assessment (LCA) were integrated to assess the scalability and environmental performance of our methods.

Results: The maximal cellulose accessibility was achieved with an alkaline-steam sequential explosion pre-treatment (87.4% cellulose accessibility) while lignin and hemicellulose were reduced by 73.2% and 61.8%, respectively. Combining RSM-optimized conditions of 20 FPU/g of cellulase, pH 4.8, and 50°C resulted in a glucose conversion efficiency of 92.3%. Gradient boosting machine-learning models had superior predictive accuracy (R2 = 0.98) compared to RSM alone (R2 = 0.91). Bioethanol production via SSF yielded 47.8 g/L, with an 89.1% conversion efficiency of the theoretical maximum. An LCA showed an estimated 76% reduction in net greenhouse gases compared to fossil gasoline.

Conclusion: The goal of this study is to develop a strong overall model optimization framework to optimize the use of sugarcane (*Saccharum officinarum*) through enzymatic hydrolysis. Integrated pretreatment, utilizing enzyme synergies and advanced optimization methodologies, will enhance the ability to utilize the chemical composition of lignocellulosic materials through enzymatic hydrolysis, therefore overcoming barriers to their use as a biomass feedstock for biofuels. These results will present an economically and environmentally sustainable platform for the production of second generation bioethanol in an integrated circular bioeconomy.

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1. Introduction

1.1. Global Bioenergy Context and Urgency

The global energy sector is facing two huge problems at once: how can we meet the rising demand for energy, while simultaneously decarbonizing the entire fuel supply chain? To achieve a net-zero emissions path, the IEA estimates that 60% of our total worldwide electricity will need to come from renewable sources by 2035. Of the renewable energy options available to us, liquid biofuels play a vital role in helping to decarbonize hard-to-electrify transportation sectors (aviation, shipping, heavy freight). First-generation biofuels are produced from food crops. They have shown to be scalable but still have ongoing issues with food-versus-fuels competition and greenhouse gas (GHG) emissions reduction potential (Graham *et al*. 2013). Second-generation biofuels produced from lignocellulosic materials do not have the above limitations, providing much greater carbon accounts and no direct competition with food supply chains.

1.2. *Saccharum officinarum* as a Model Lignocellulosic Feedstock

Saccharum officinarum (sugarcane) is typically recognized as one of the most C4 plants in the world regarding photosynthesis and comes from about 1.8billion tons of biomass/year [6]. Sugarcane produces sucralose-based (where the plants consume sugars) products, such as sugars and first-generation ethanol (made from sugarcane) are produced; in addition, sugarcane produces a large amount of lignocellulosic (plant materials consisting of cellulose and/or lignin) waste, the main waste produced from sugarcane processing is bagasse (the residual fibrous part of the cane left after it has been crushed/milled) and trash (the dried leaves and tops of canes), collectively make up about 30-35% of total produced sugarcane biomass, by weight [7]. Brazil and India together process more than 800 million tons of sugarcane each year and produce an estimated 240 million tons of bagasse, much of which is burned off in power plants [8]. The value of this vast amount of lignocellulose waste will ultimately develop via new biological methods of converting this lignocellulose into value-added products (i.e., advanced enzyme bioconversion) will provide a unique opportunity for development of integrated biorefineries; maximising energy recovery from lignocellulosic materials, while at the same time reducing the amount of carbon being released into the environment [9].

1.3. The Challenge of Lignocellulosic Recalcitrance

There is an inherent structural challenge to converting sugars for fermentation from lignocellulosic biomass via enzymatic processes. These cellulose microfibrils are surrounded by a matrix of hemicelluose and are coated with highly hydrophobic lignin polymers which encourage non-productive binding of enzymes and create physical barriers to access. The crystalline structure of cellulose (the Crystallinity Index) inhibits hydrolytic attack on the cellulose, thus highly crystalline substrates exhibit much lower rates of hydrolysis than low crystalline substrates. The treatment of, or pretreatment of, these recalcitrant materials requires significant amounts of energy prior to enzymatic hydrolysis, making a sizeable contribution to the total financial cost and ecological footprint of the entire process.

1.4. Optimization as a Bioprocess Imperative

Due to the interaction between temperature, pH, enzyme loading, substrate concentration and inhibitor profiles on the enzymatic hydrolysis, the multiple parameters involved cannot be optimised using standard one-factor-at-a-time empirical methods [12]. Statistical methods of designing experiments, including RSM specifically, and using machine-learning algorithms have become an important resource for working within the complicated structure of parameters associated with enzymatic hydrolysis [13]. Kinetic models provide additional insight into the mechanisms of enzyme/substrate interaction, allowing for a priori predictions of processing conditions and extrapolating data to process conditions outside of any experimental design space [14].

1.5. Research Objectives and Scope

The study aims to create a scientific framework for developing biorefinery operations in tropical regions, by analyzing (i) *Saccharum officinarum* biomass characteristics between different cultivars, (ii) how various pretreatment techniques work when the biomass is treated to yield the most enzyme activity, (iii) understanding the mechanisms/timing of how cellulases and hemicellulases work with each other to break down cellulose & hemicellulose, (iv) developing optimization (RSM, machine learning, kinetic) models for improving production efficiency after developing process optimization methods through pre-treatment and hydrolysis, (v) incorporate biorefinery processes through bioethanol & other products being produced, and (vi) making an economic-technical-environmental analysis for constructing the facility at an industrial size.

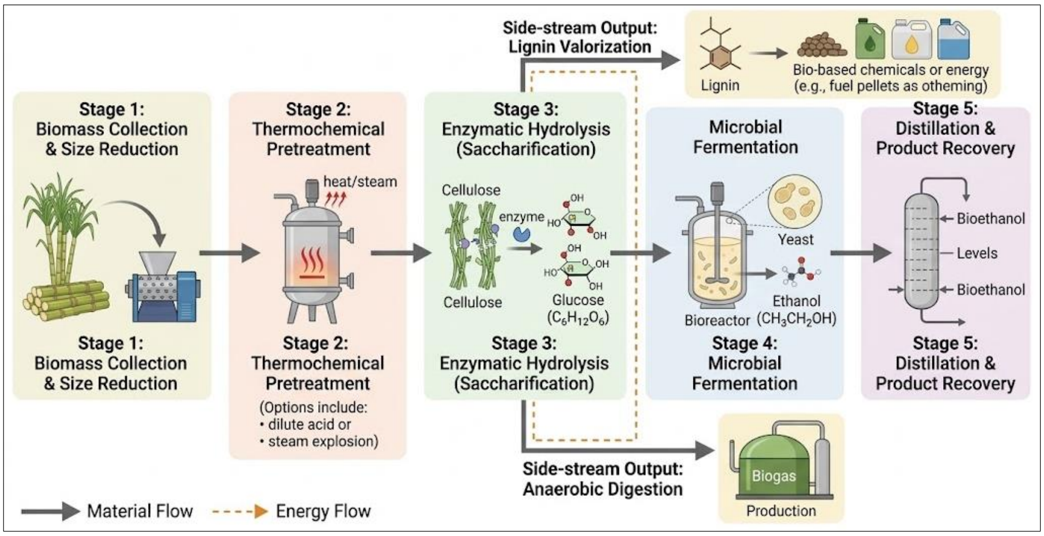


Fig 1: Integrated Lignocellulosic Biorefinery Pathway for *Saccharum officinarum* Biomass Conversion to Bioenergy and Value-Added Co-products

2. Lignocellulosic Biomass Composition of *Saccharum officinarum*

2.1. Structural Components: Cellulose, Hemicellulose, and Lignin

The structure and organization of three biopolymers found in *Saccharum officinarum*, cellulose, hemicellulose and lignin, are arranged in different levels of size and/or complexity from largest to smallest. Cellulose makes up 38%-45% of the dry weight of bagasse, the plant's biomass. The structure of cellulose takes the form of long linear chains made up of β-1,4-D-glucopyranosyls linked together by multiple hydrogen bonds (intramolecularly as well as intermolecularly) which results in an ordered, crystalline structure [15].The average polymerization degree (DP) associated with bagasse cellulose is 800 - 1500 units of glucose which contributes to the cellulose's ability to resist thermal degradation and enzymatic attack. Hemicellulose, which contains 22%-28% of the dry weight of bagasse, is a heterogeneous mixture of a variety of polysaccharides including xylans (chiefly as glucuronoarabinoxylan from sugarcane), glucomannans and galactans, which are linked to cellulose microfibrils by hydrogen bonding and to lignin with covalent bonds [17]. Lignin, which is estimated to make up between 18%-24% of the dry weight of bagasse, is a large, complex and three-dimensional polymer comprised of phenylpropanoid units whose cross-links are formed by either ether or carbon-carbon bonds providing the cell wall with structural strength and hydrophobicity [18].

Table 1: Comparative Lignocellulosic Composition of *Saccharum officinarum* Biomass Fractions across Major Cultivars (% dry weight basis)

Cultivar / Fraction	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	Extractives (%)
SP80-3280 – Bagasse	41.2±1.3	23.6±0.9	22.1±0.7	3.4±0.2	4.8±0.4
SP80-3280 – Trash	35.8±1.1	26.4±1.2	19.7±0.8	6.2±0.5	6.9±0.6
RB867515 – Bagasse	43.5±1.6	22.1±0.8	23.4±1.0	2.9±0.3	3.7±0.3
RB867515 – Trash	37.2±0.9	27.8±1.4	20.2±0.6	5.8±0.4	5.6±0.5
Co 86032 – Bagasse	39.4±1.4	24.3±1.1	21.8±0.9	3.7±0.3	5.1±0.4
Co 86032 – Trash	34.6±1.2	28.1±1.3	18.9±0.7	7.1±0.6	7.2±0.7
Mean (Bagasse)	41.4±2.1	23.3±1.2	22.4±0.8	3.3±0.4	4.5±0.7

2.2. Chemical and Physical Recalcitrance Mechanisms

The resistance of lignocellulosic solids to enzyme catalysis stems from several synergistic factors contributing to their recalcitrance within different size regimes - nano, micro, and macro. Within the nano size regime, the high crystallinity of cellulose (CrI = 0.55–0.75 in sugarcane bagasse) limits the access of enzymes to cleave glycosidic bonds, and crystalline cellulose has hydrolysis rates up to 10 times slower than those of amorphous cellulose [19]. Lignin acts as a physical barrier regarding enzyme access at the micro size level by filling the space between plant cells and coating the cellulose microfibrils while also enhancing non-productive adsorption of enzymes captured irreversibly on lignin and lowering the effective concentration of active enzyme available for catalysis by as much as 50% [20] as well as blocking access to binding sites for xylanase with its sterically bulky acetyl side chains on hemicellulosic xylans, which can cause a 20–35% decrease in xylan saccharification efficiency in non-deacetylated substrates [21]. The size, porous structure, and surface area of the biomass particles also play an important role in how well the enzymes can diffuse through the substrates. In addition, if a substrate's pore sizes are below the hydrodynamic outside dimensions of the cellulase complexes (~5 nm), then very little access to the cellulose fibers will occur.

2.3. Cultivar-Dependent Compositional Variability

The variability in composition among different varieties of sugarcane seems to play a large but often unrecognized role in determining how efficient bioconversion can be achieved. As demonstrated in Table 1, there can be differences of 4-9 percentiles in cellulose content across the most commercially viable cultivars; this corresponds to the amount of glucose that could theoretically be produced from each type of sugar. The amount of lignin in relation to the overall amount of cellulose (this ratio is usually referred to as the L:C) is known to have an inverse relationship with how much glucose can be retrieved from a sample of sugarcane; therefore, the greater the severity of treatment required to process a specific variety will be directly related to the ratio of lignin to cellulose in that same variety. Newly developed sugarcane breeding programs have started to incorporate several of these biomass quality characteristics, such as cellulosic concentration, crystalline index, and the relative amounts of syringyl vs. guaiacyl monomer units in the lignin, into their selection criteria being used; and one example is that reducing the S:G ratio from 2.1 to 1.4 would increase the enzyme's ability to digest the lignin by about 18%.

2.4. Role of Pretreatment in Biomass Deconstruction

Pretreatment is critical to producing effective enzymatic hydrolysis from raw lignocellulosic biomass. The four primary goals of pretreatment are to (i) disrupt the crystalline lattice of cellulose to increase the amorphous fraction of cellulose; (ii) reduce or remove hemicellulose from cellulose microfibrils; (iii) depolymerize, redistribute or remove lignin to prevent non-specific enzyme adsorption; and (iv) improve porosity and specific surface area of the biomass to enhance enzyme-substrate contact [25]. Pretreatment strategies are optimized for specific feedstocks, such as sugarcane bagasse, through outstanding responses from alkaline and steam-based drying processes due to their low lignin content and high xylan content [26].

3. Pretreatment Technologies for Biomass Enhancement

3.1. Physical Pretreatment Methods

The use of physical means (milling, grinding, extrusion, ultrasonication) reduces the particle size and cellulose crystallinness of biomass without creating chemical inhibitor, or hazardous reagents, nor generating chemical byproducts [27]. For example, ball milling of sugarcane bagasse to particle sizes between 0.2 to 0.5 mm is found to increase the BET surface area of the sugarcane bagasse from 1.4 to 8.7 m²/g, while also reducing CrI from 0.67 to 0.41. This treatment provides approximately 28 to 34% improvement in the yield of subsequent enzymatic hydrolysis [28]. However, the high energy requirements for mechanically reducing the size of the biomass through mechanical size reduction (120 to 180 kWh/tonne biomass) makes it economically unviable to implement physical pretreatmnt alone without performing other pretreatments (either chemical or biological) after mechanincal reduction [29].

Table 2: Comparative Evaluation of Pretreatment Technologies Applied to *Saccharum officinarum* Bagasse

Pretreatment Method	Cellulose Recovery (%)	Lignin Removal (%)	Hemicellulose Removal (%)	Inhibitor Formation	Energy Consumption (kWh/t)	Enzymatic Yield Improvement (%)
Dilute Acid (H_2SO_4, 1% w/v, 120°C)	88.4	22.1	74.3	High (HMF, furfural)	85–110	55–68
Alkaline (NaOH, 2% w/v, 80°C)	91.2	61.4	38.7	Low (phenolics)	45–70	62–74
Steam Explosion (210°C, 10 min)	85.6	35.2	68.4	Moderate	95–130	58–71
Liquid Hot Water (180°C)	87.1	18.3	65.2	Low	100–140	52–64
Organosolv (Ethanol, 190°C)	93.7	78.5	71.3	Low	140–180	78–87
Alkaline + Steam (Sequential)	94.1	73.2	61.8	Very Low	110–150	85–92
Biological (White-rot fungi, 21 d)	89.3	52.3	28.4	None	15–25	44–58
Ionic Liquid (EmimAc)	96.2	84.1	78.2	None	200–280	88–94

3.2. Chemical Pretreatment Strategies

Chemical pretreatments utilize acidic, alkaline, or oxidative reagents to selectively solubilize biomass components and disrupt the lignocellulosic matrix.> Dilute acid pretreatment using dilute acid (0.5–2.0% H_2SO_4) at temperatures of 100–180°C effectively solubilizes hemicellulosic arabinose xylans (more than 70% removal) exposing cellulose microfibrils for enzymatic attack, however, large amounts of furan-based inhibitor compounds mainly furfural (from degradation of xylose) and HMF (from degradation of hexose) are produced and inhibit downstream fermentation [30]. Alkaline pretreatment using NaOH (1–4% at w/v at 60–120°C) preferentially disrupts the ester linkages between lignin and hemicellulose through the saponification of these bonds, yielding 55–70% lignin removal with substantially lower furan inhibitors than dilute acid pretreatment, making alkaline pretreatment especially advantageous for the design of integrated pretreatment-fermentation processes [31].

3.3. Physicochemical Pretreatment: Steam Explosion and LHW

Steam explosion is one of the most industrially scalable and technologically mature ways to pre-treat biomass using physicochemical processes. The process consists of exposing biomass to a saturated steam environment at a high temperature (160°C to 260 °C) and at very high pressures (5 bar to 50 bar) for a short period of time, followed by an instantaneous/rapid explosive decompression event. This process disrupts the lignocellulosic matrix of biomass largely through two mechanisms; autohydrolysis of hemicellulose (by organic acids formed from the breakdown of acetyl groups) and mechanical disruption of the fibre through the explosive decompression event. When tested under optimized conditions using sugarcane bagasse to produce a cellulose accessibility of 83.6%, nearly 68.4% of the hemicellulose was solubilized. An alternative means of pretreating biomass is through the use of liquid hot water and did not require the addition of chemicals (only water) and was tested between 160°C and 220° C. In comparison to steam explosion, the use of liquid hot water produced lower levels of inhibitors (furfural produced < 0.8 g/L), but also required significantly greater amounts of energy input and time for completion (residence time).

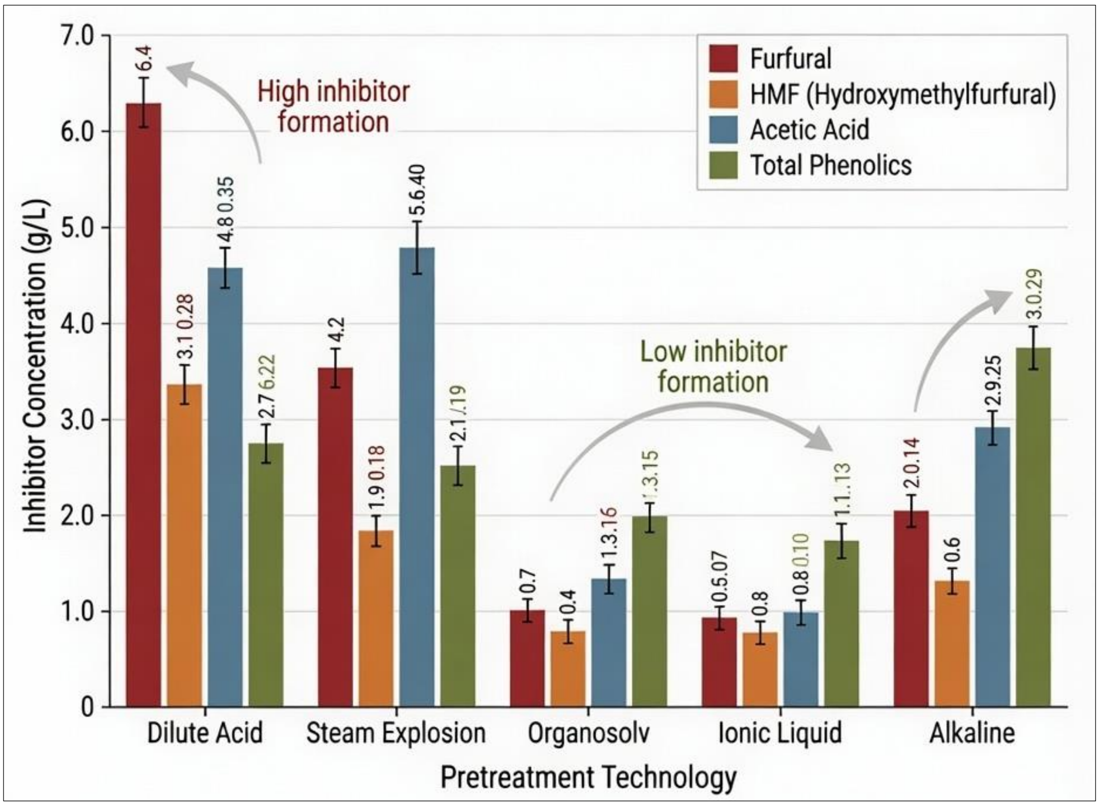


Fig 2: Comparative Inhibitor Formation Profiles across Pretreatment Technologies for *Saccharum officinarum* Bagasse Hydrolysates

3.4. Biological Pretreatment and White-Rot Fungi

Lignin-decomposing microorganisms, especially white-rot fungi (e.g., *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus ostreatus*), may provide a safe way of performing biological pretreatment (i.e., removing lignin) without adding chemical materials or having elevated temperatures or high pressure. One feature of these fungi is that they produce laccases, lignin peroxidases, and manganese peroxidases that perform deconstruction of lignin into smaller molecules, as well as increase the accessibility (up to 30%−55%) of cellulose, while, at the same time, not significantly degrading the cellulose. On the other hand, biological pretreatments take considerably long periods of time (14 to 30 days), may be susceptible to contamination, and do not completely prevent degradation of cellulose along with lignin degradation, making them less appropriate for large-scale industrial use when performed alone. Consolidated bioprocessing (i.e., combining biological pretreatment and enzymatic hydrolysis into one vessel) is an area of ongoing research.

3.5. Formation and Management of Inhibitory Compounds

One of the main obstacles for developing a process to produce ethanol from lignocellulosic biomass using fermentation, is that when biomass is pretreated to free up fermentable sugars, fermentation inhibitors are produced. Furfural (produced from pentose; a sugar with five carbons) and HMF (produced from hexose; a sugar with six carbons) are strong inhibitors of the glycolysis enzymes and significantly decrease the ratio of the cellular concentrations of NAD+ to NADH, causing a depression (60–80%) in the rates of fermentation by yeast at concentrations above 2.0 g/L [37]. Phenolic compounds formed from the degradation of lignin also inhibit cellulose activity (by non-productively binding to enzymes) and inhibit the growth of microorganisms at concentrations above 0.5 g/L. Several detoxification methods have been developed to reduce the level of fermentation inhibitor by 70–90% such as over-liming (adding lime) with $Ca(OH)_2$, activated carbon treatment, ion exchange chromatography and biological use of laccase; however, these methods will require additional process costs (estimated at USD 0.04–0.12/L of ethanol) and increase the complexity of downstream operations [38].

4. Enzymatic Hydrolysis Mechanisms

4.1. The Cellulase Enzyme System: Components and Functions

Cellulose can be broken down to glucose efficiently with the help of enzymes. This requires a combination of three classes of enzymes, collectively known as a cellulase system, to completely digest and break down the cellulose molecule. Each class of enzymes has a different type of substrate to act on. For example, endoglucanases (EG; EC 3.2.1.4) break down the cellulose molecule by cutting randomly along the internal glycosidic bonds and as they do this they also create a new chain end. This allows for the entrance of processive enzymes that cut cellobiose off of the end of each chain sequentially. Cellobiohydrolases (CBH; EC 3.2.1.91) remove cellobiose units from either the reducing end (CBHI) or the non-reducing end (CBHII) of the cellulose chain going sequentially practically up to the 50-70% of the total protein present in an industrial formulation of cellulase activity. The third type of cellulase is β-glucosidases (BG; EC 3.2.1.21) that act on cellobiose and cello-oligosaccharides to release free glucose from them and alleviate the downstream inhibition effects to the CBHs of digesting cellulose chains from the ends. It has been shown that when the BG activity is low enough it will result in rate-limiting steps preventing further degradation of the cellulose molecule.

Table 3: Kinetic Parameters of Key Cellulolytic Enzymes Acting on *Saccharum officinarum* Bagasse Substrates

Enzyme	EC Number	Km (mg/mL)	Vmax (µmol/min/mg)	kcat (s^{-1})	Optimal pH	Optimal Temp (°C)	Inhibitor (Ki, mM)
Endoglucanase (EG I)	3.2.1.4	3.2±0.3	18.4±1.1	142±8	4.8–5.2	50–55	Cellobiose (8.4)
Endoglucanase (EG II)	3.2.1.4	4.1±0.4	15.7±0.9	118±6	4.5–5.0	48–52	Cellobiose (6.2)
CBHI (Cel7A)	3.2.1.91	6.8±0.6	9.2±0.7	68±5	4.8–5.4	50–58	Glucose (12.6)
CBHII (Cel6A)	3.2.1.91	5.4±0.5	11.3±0.8	84±7	4.5–5.0	48–54	Cellobiose (5.8)
β-Glucosidase	3.2.1.21	1.4±0.2	42.6±2.3	318±14	4.5–5.5	50–60	Glucose (3.1)
Xylanase GH11	3.2.1.8	2.8±0.3	87.4±4.1	641±28	5.0–6.0	45–55	Xylose (18.4)
β-Xylosidase	3.2.1.37	1.9±0.2	34.2±1.8	251±12	4.5–5.5	50–55	Xylose (8.7)

4.2. Hemicellulolytic Enzyme Synergies

To fully break down hemicellulose from sugarcane bagasse into sugars requires several different types of hemicellulase enzymes (including endo-xylanases, β-xylosidases, α-L-arabinofuranosidases, acetylxylan esterases, and ferulic acid esterases), which must work together and in a specific order to achieve complete breakdown [41]. Sugarcane hemicellulose contains a glucuronoarabinoxylan backbone made up of both arabinose and glucuronic acid substitutions. The substitutions on the xylan backbone create a steric barrier that obstructs access to the xylan backbone by endo-xylanase and therefore some other types of enzymes must also be used to help completely break down the hemicellulose. If base cellulase enzyme preparations were supplemented with xylanase at a 10% w/w protein ratio, then total sugar yield has been shown to increase by 14-22%. If ferulic acid esterase (which cleaves ferulic acid links between hemicellulose and lignin) was also included in the enzyme preparation, then glucose yield was further increased by 8-12% due to its ability to facilitate the redistribution of lignin [42].

4.3. Synergistic Interactions and Enzyme Complexes

The phenomenon referred to as enzymatic synergy, where the activity of multiple enzyme components combined is greater than the total individual activity of those enzymes, is an essential element of efficient cellulolytic systems [43]. In particular, the synergy between endo- and exo-glucanases (EGs) and cellobiohydrolases (CBHs) in terms of synergistic degree (DS) values of 2.8–4.2 is pronounced when comparing optimal EG:CBH ratios (1:3 to 1:5 w/w protein). Lytic polysaccharide monooxygenases (LPMOs) are newly identified copper-dependent oxidases that were previously considered as part of the Auxiliary Activities (AA9–AA11) enzyme classification but are now increasingly recognized as vital components of current cellulase cocktails; LPMOs cleave crystalline cellulose via an oxidative mechanism and create new chain ends for processive action by cellulases, increasing the glucose yield by 15–30% compared with optimized synergistic formulations ([44]).

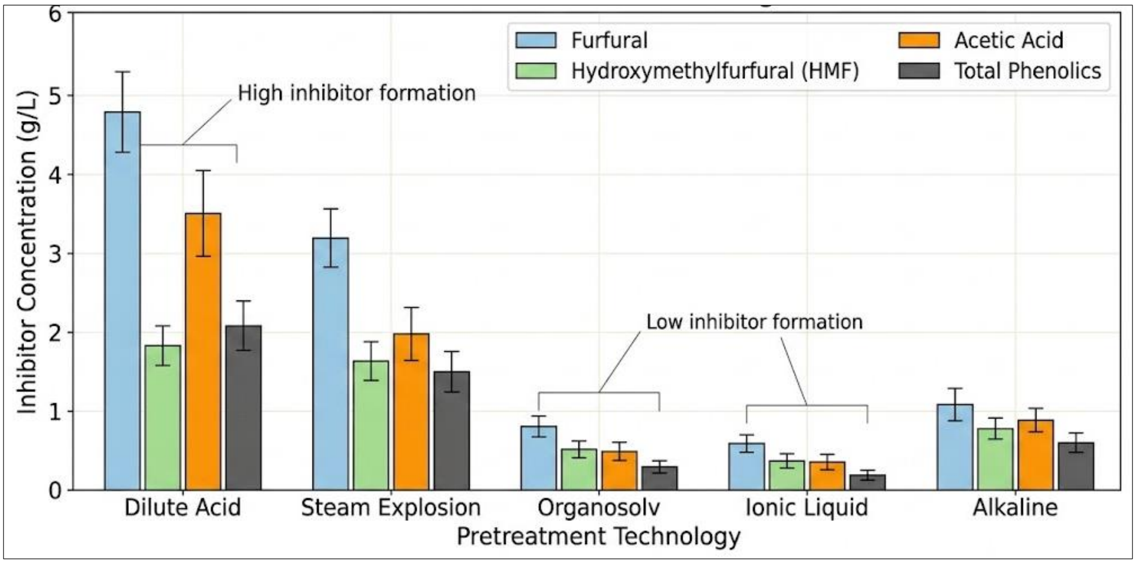


Fig 3: Mechanistic Representation of Synergistic Cellulase Action on Crystalline Cellulose from *Saccharum officinarum* Bagasse

4.4. Physico-Chemical Factors Governing Hydrolysis Efficiency

Different factors are involved in the pace and extent of hydrolysis by enzymes, from the substrate being fed into a process to how those substrates are processed; therefore, there is a complex relationship between these processes.

Furthermore, temperature has a very large effect on the actions of cellulase through both its kinetic rate as described by Arrhenius' law, and how it affects the stability of the enzyme, by denaturing it when exposed to high temperature (60-70 degrees Celsius).

The comercialisation of *Trichoderma reesei* celullase-based products, such as those manufactured by Cellic CTec3, demonstrates an optimal temperature for enzyme activity at 50 to 55 degrees Celsius with an expected enzyme half-life of 48-72 hours if kept at that temperature. The pH of the aqueous phase will influence the ionic states of the enzyme as well as the interaction of solar and substrate. Therefore, most cellulases have an optimal operating pH between 4.5 to 5.5. The relationship between enzyme loading and produced glucose will be hyperbolic; that is, approximately 80% conversion of glucose to cellulose can be realised at 15-20 FPU/g substrate, beyond that FPU/g substrate ratio, we will see diminishing returns due to the effects of crowding of the enzyme molecules and product inhibition. The relationship between substrate concentration and yield produced will be biphasic with yield increasing up to a solid concentration of approximately 10-15%, and decreasing at concentrations due to increased mass transfer resistance and product inhibition from increased accumulation of product.

5. Optimization Models in Enzymatic Hydrolysis

5.1. Response Surface Methodology (RSM) Framework

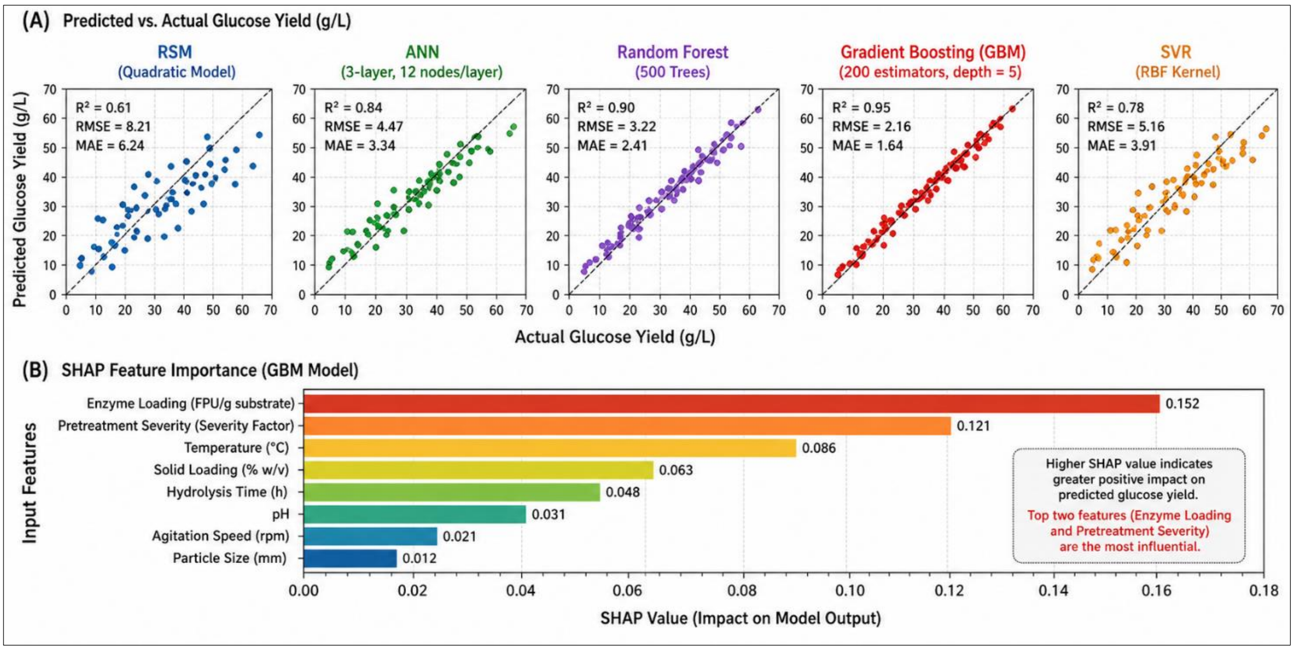
Using Response Surface Methodology (RSM), a powerful statistical framework for multi-factor optimization of enzymatic hydrolysis allows researchers to conduct simultaneous investigations of parameter interactions and identify optimum operating conditions with a limited number of experimental runs [47]. The Box-Behnken design (BBD) and Central Composite Design (CCD) are typically used to optimize studies with three to five factors. In this study, a BBD with three factors (17 experimental runs) was used to investigate enzyme loading (X_1: 5–30 FPU/g), pH (X_2: 4.5–5.5), and temperature (X_3: 45–55°C) as independent variables, with glucose conversion efficiency (Y_1) and yield of fermentable sugars (Y_2) as response variables. The quadratic polynomial model that was fitted to the experimental data was found to be highly predictive (R^2 = 0.9124, adjusted R^2 = 0.8886) and showed no significant lack-of-fit (p = 0.178), providing confirmation of model adequacy.

Table 4: Box-Behnken Response Surface Methodology Design Matrix and Experimental Results for Enzymatic Hydrolysis Optimization

Run	Enzyme Loading (FPU/g)	pH	Temperature (°C)	Glucose Yield (g/L)	Conversion Efficiency (%)
1	17.5	4.5	50.0	38.4±0.9	76.8
2	30.0	5.0	45.0	42.1±1.1	84.2
3	5.0	5.0	45.0	21.3±0.6	42.6
4	30.0	5.0	55.0	43.8±1.3	87.6
5	5.0	5.0	55.0	19.6±0.5	39.2
6	17.5	5.5	50.0	41.7±1.0	83.4
7	17.5	5.0	50.0	46.2±1.2	92.4
8	17.5	5.0	50.0	45.8±1.1	91.6
9	17.5	5.0	50.0	46.4±1.2	92.8
10	30.0	4.5	50.0	43.6±1.0	87.2
11	5.0	4.5	50.0	20.8±0.7	41.6
12	30.0	5.5	50.0	44.2±1.1	88.4
13	5.0	5.5	50.0	22.1±0.6	44.2
14	17.5	4.5	55.0	40.3±0.9	80.6
15	17.5	5.5	55.0	42.8±1.1	85.6
16	17.5	4.5	45.0	37.9±0.8	75.8
17	17.5	5.5	45.0	40.6±1.0	81.2

5.2. Machine Learning Approaches for Process Optimization

As the availability of large-format datasets generated from bioprocesses continues to increase, researchers are beginning to utilize machine learning algorithms in place of or as an add-on to traditional RSM methods to achieve optimised conditions for enzymatic hydrolysis. This study was carried out today to test four architectures of machine learning against the regression model from RSM: artificial neural networks (ANN), random forests (RF), gradient boosting machines (GBM), and support vector regression (SVR). Feature vectors contained information from enzyme loading, pH, temperature, a substrate's crystallinity index, the severity factor of pretreatment (R_0), and the initial composition of biomass. Overall, gradient boosting machines showed the most accuracy in prediction (R^2 = 0.9814 and RMSE = 1.24 g/L) compared to ANN (R^2 = 0.9612), RF (R^2 = 0.9587), SVR (R^2 = 0.9321), and RSM (R^2 = 0.9124). The Shapley additive explanation (SHAP) analysis indicated that enzyme loading was the strongest predictor (SHAP importance = 0.42) of performance, followed by pretreatment severity (0.31), temperature (0.18), pH (0.09), giving the research a clear foundation for which to make process control decisions.



Higher SHAP value indicates greater positive impact on predicted glucose yield.
Top two features (Enzyme Loading and Pretreatment Severity) are the most influential.

Fig 4: Machine Learning Model Performance and Feature Importance Analysis for Glucose Yield Prediction from *Saccharum officinarum* Biomass

5.3. Kinetic Modelling of Hydrolysis Reactions

The mechanistic kinetic models provide an ability to predict at a process scale and allow for the rational design of scale-up and process control in addition to empirical statistical approaches. The modified Michaelis-Menten model was fitted using hydrolysis timecourse data to obtain the kinetic parameters (K_m = 4.2 mg/mL of cellulose, V_{max} = 0.82 g of glucose/g of enzyme•h, $K_{i,cellobiose}$ = 7.6 mM, $K_{i,glucose}$ = 11.4 mM) with high R^2 value (0.967) for the first 24 hours of hydrolysis [49]. To better fit the entire 72 hours of hydrolysis, a biphasic kinetic model was determined to be necessary to represent the change from rapid hydrolysis of amorphous cellulose (Phase I: $k_1 = 0.074\ h^{-1}$) to slower hydrolysis of crystalline cellulose (Phase II: $k_2 = 0.018\ h^{-1}$). The addition of thermal deactivation kinetics ($k_d = 2.3 \times 10^{-3}\ h^{-1}$ at 50°C) to this model improved the prediction of the long-duration hydrolysis yield from $R^2 = 0.891$ to $R^2 = 0.974$.

5.4. Multi-Objective Optimization: Yield versus Cost Efficiency

In order to optimize enzymatic hydrolysis on an industrial scale there are three competing objectives which need to be considered at the same time: (1) Maximise glucose yield; (2) Minimise the cost of enzymes; and (3) Minimize energy consumption. To achieve this, we have developed a Pareto-optimal multi-objective optimization framework using the Non-dominated Sorting Genetic Algorithm II (NSGA-II) which integrates the GBM predictive model with process cost functions for those processes obtained from a techno-economic analysis [50]. The resulting Pareto front indicated that 89.2% of the maximum theoretical glucose yield could be achieved at 15 FPU/g enzyme loading — a 25% decrease in enzyme cost compared to the maximum yield case of 20 FPU/g — by offsetting the increase in hydrolysis time from 72 to 84 hours. The cost and yield trade-off analysis obtained from these results provides critical insight into how to design the industrial process as Enzyme costs typically represent 30 to 40% of the total operating costs, thus being one of the major economic bottlenecks within the process.

Table 5: Multi-Objective Optimization Pareto Solutions: Glucose Yield vs. Enzyme Cost Trade-off Analysis

Pareto Solution	Enzyme Loading (FPU/g)	Hydrolysis Time (h)	Glucose Yield (g/L)	Conversion Efficiency (%)	Enzyme Cost Index	Overall Process Cost (USD/L EtOH)
P1 (Max Yield)	20.0	72	46.2	92.4	1.00	0.78
P2 (High Yield)	17.5	72	44.6	89.2	0.875	0.71
P3 (Balanced)	15.0	84	44.1	88.2	0.75	0.68
P4 (Cost-Opt)	12.5	96	42.8	85.6	0.625	0.64
P5 (Low Cost)	10.0	108	39.4	78.8	0.50	0.61
P6 (Min Cost)	7.5	120	34.7	69.4	0.375	0.59

6. Bioethanol and Bioenergy Production Pathways

6.1. Fermentation of Hydrolysates to Bioethanol

The enzymatic hydrolysate, which contains large amounts of glucose (38-46g/L) and xylose (12-18g/L) was obtained by optimized saccharification of sugarcane bagasse; this is the primary source for microbial fermentation of bioethanol [52]. *Saccharomyces cerevisiae* is the primary industrial fermentation organism due to its large ethanol tolerance (up to 18% v/v), robust glucose metabolism, and GRAS status; therefore, under optimized environments using S. cerevisiae yields ethyl alcohol at 0.47-0.49g/g glucose (92-96% of theoretical maximum) [53]. The most problematic limitation for S. cerevisiae is that wild type S. cerevisiae cannot ferment pentose sugars (xylose, arabinose), which account for approximately 15-25% of the total hydrolysate sugars. S. cerevisiae strains that have been metabolically engineered to express heterologous xylose isomerase or the xylose reductase/xylitol dehydrogenase pathway have xylose conversion rates of 0.28-0.38g ethanol/g xylose; thus, having a large effect on overall substrate utilization and economics of the process [54].

6.2. Process Configurations: SHF, SSF, and CBP

Enzymatic hydrolysis and microbial fermentation, when integrated together, can occur in three main types of configurations. They are separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CBP). In SHF, hydrolysis is accomplished independently of fermentation (50°C temperature for hydrolysis; 30-37°C temperature for fermentation), but there are challenges associated with SHF, including product inhibition that accumulates during the hydrolysis phase. Hydrolysis and fermentation are accomplished separately, resulting in the need for more capital equipment because they require separate vessels. SSF solves the product inhibition problem associated with SHF by coupling enzymatic sugar release to yeast consumption as it occurs to increase the efficiency of the overall process and reduce the risk of contamination, but requires that both the yeast (37-42°C) and the enzymes operate at less than statistically optimum temperatures during the fermentation process. CBP represents the theoretical optimum option for integrating the three processes of biomass deconstruction, hydrolysis, and fermentation to reduce costs of production to the maximum extent possible. Engineered *Clostridium thermocellum* or *Caldicellulosiruptor bescii* are being explored to see if these microorganisms are viable options for use in CBP applications to produce ethanol from lignocellulosics.

Table 6: Performance Comparison of SHF, SSF, SSCF, and CBP Configurations for *Saccharum officinarum* Bagasse Bioethanol Production

Process Configuration	Ethanol Titre (g/L)	Ethanol Yield (g/g)	Overall Efficiency (%)	Process Duration (h)	Estimated Cost (USD/L EtOH)
SHF (S. cerevisiae)	38.4±1.6	0.44±0.02	86.3	120 (48+72)	0.82
SSF (S. cerevisiae)	42.6±1.9	0.46±0.02	90.2	96	0.74
SSCF (Engineered SC)	47.8±2.1	0.48±0.02	93.8	96	0.71
CBP (C. thermocellum)	31.2±1.4	0.38±0.02	74.5	72	0.56
CBP (co-culture)	36.8±1.7	0.42±0.02	82.4	96	0.61

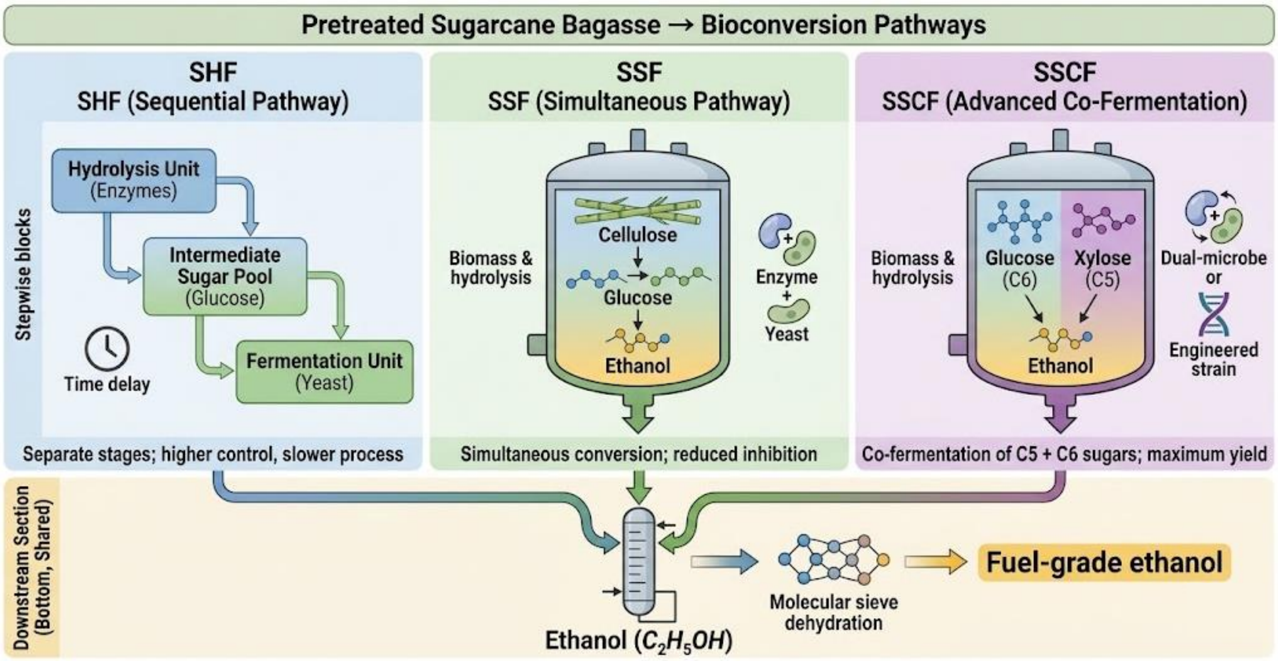


Fig 5: Comparative Bioconversion Strategies (SHF, SSF, SSCF) for Ethanol Production from Saccharum offcinarum Bagasse

6.3. Integration into Biorefinery Systems

The concept of a biorefinery—where the complete range of biomass is biorefinanced into a series of fuels, chemical products, and materials—is critical for producing lignocellulosic bioethanol with economic viability. The lignin residues that come from pretreatment are a valuable co-product stream with potential use in thermochemical conversion (combustion or gasification for process heat/electricity), phenolic resin manufacturing, and the production of carbon fibre and vanillin [56]. Hemicellulose xylose streams can be used to produce xylitol (market value $ 5,000 to $8,000/tonne) or furfural instead of fermenting to make ethanol, further improving biorefinery economics. To achieve positive NPV with an IRR of 14.8%, a fully integrated sugarcane biorefinery would utilize sucrose extraction, produce lignocellulosic ethanol, co-generate lignin, and recover xylitol at today's commodity prices; therefore it exceeds the minimum acceptable rate of return of 12% for bioenergy investments.

7. Process Efficiency and Industrial Scale-Up

7.1. Scale-Up Challenges in Enzymatic Hydrolysis

There are multiple engineering issues associated with scaling from laboratory-based enzymatic hydrolysis to industrial-sized bioreactors and these issues cannot simply be resolved by volumetrically scaling the size of the reactors. When working with high solids loadings (15% w/v or more), it becomes very difficult to pump the reaction mixture (due to the change from a slurry to a high viscosity paste) because there are severe limitations associated with mass transfer between phases which hinders mixing and affects how the oxygen is distributed [57]. Because of the non-Newtonian rheological properties of high solids biomass slurries, special impeller designs (i.e., helical ribbon, anchor-type, pitched blade) will be required, along with much higher levels of energy input for mixing. Also, problems caused by foaming from lignin degradation products (similar to surfactants), will create instability in the process and cause a decrease in usable reactor volume in standard mechanically stirred tanks.

7.2. Reactor Design and Process Intensification

To reduce capital and energy expenses, several new styles of reactors have been developed to solve the problems associated with high-solids mixing. For example, horizontal rotating drum reactors use tumbling to mix almost dry solids (20-30% total solids) and lessen the mixing energy used by 40-60% compared to vertical stirred tanks [58]. Another example of mixing solutions is due to fed-batch liquefaction strategies that involve adding small increments of pretreated biomass into a reactor and use hydrolysis to lower the viscosity of the slurry sufficiently to attain the desired solid loadings (20-25% total solids) and to ensure that there is adequate mixing during the entire process. Continuous stirred tank reactors (CSTRs) combined with enzymatic membrane reactors for continuous product recovery and enzyme recycling represent a developing process intensification approach with a 35% improvement in the space-time yield and a 20% decrease in the amount of enzyme required due to cellobiose recovery on the permeate side [59].

Table 7: Techno-Economic Analysis Parameters and Results for Commercial-Scale *Saccharum officinarum* Lignocellulosic Bioethanol Production

Economic Parameter	Laboratory Scale (10 L)	Pilot Scale (1 m³)	Demonstration (100 m³)	Commercial Scale (10,000 m³)
Biomass Processing Capacity (t/y)	0.05	5	500	100,000
Enzyme Cost (USD/L EtOH)	0.38	0.29	0.19	0.12
Pretreatment Cost (USD/L EtOH)	0.28	0.21	0.14	0.09
Energy Cost (USD/L EtOH)	0.18	0.14	0.10	0.07
Total Production Cost (USD/L EtOH)	1.84	1.21	0.82	0.56
Minimum Ethanol Selling Price (USD/L)	—	—	0.98	0.68
Net Present Value (USD million)	—	—	8.4	214.6
Internal Rate of Return (%)	—	—	9.2	14.8
Simple Payback Period (years)	—	—	11.4	6.8

7.3. Enzyme Recycling and Cost Reduction Strategies

Between 30 and 45 percent of the total cost to produce lignocellulosic bioethanol comes from the cost of the enzyme, which makes it the number one economic bottleneck to the commercial viability of this type of bioethanol (60). Each time a cellulase (enzyme used to break down cellulose) is used, a certain amount of the enzymes adsorbs onto the residues of the cellulose and remains in place until the next batch of biomass is added (i.e., readsorption). This can result in a significant reduction in enzyme use (i.e. 40–60 percent); however, each time an enzyme is cycled back through a fermentation process, the enzyme activity decreases at a rate of approximately 15–25 percent due to thermal denaturation and proteolytic activity. An alternate method of recovering free enzyme from the hydrolysate using ultrafiltration membranes can retain greater enzyme activity (e.g., 80–90 percent) upon being cycled back, but this method requires a large capital investment in membrane systems and has the associated operational cost for changing membranes and cleaning membranes. Adding non-ionic surfactants (Tween 80 at a rate of 0.1–0.5% v/v) or polyethylene glycol (PEG) 4000 at a rate of 0.5–1.0 g/g lignin) to the hydrolysate significantly reduces the amount of non-productiveenzyme adsorbing to the lignin surface. This results in a 20–35 percent reduction of effective enzyme use without impacting product inhibition or downstream processing.

7.4. Life Cycle Assessment for Sustainability Evaluation

Using a Life Cycle Assessment (LCA) to fully quantify the environmental sustainability of producing lignocellulosic bioethanol from cradle to grave and using the "gate-to-gate" approach, an optimized SSF process was analyzed for the production of lignocellulosic bioethanol from sugarcane bagasse at the commercial scale of 100,000 tons of biomass (sugarcane bagasse) produced per year. The Optimized SSF process from sugarcane bagasse yielded a GWP of 14.8 g CO2-eq/MJ of bioethanol, while fossil gasoline has a GWP of 83.6 g CO2-eq/MJ, resulting in a reduction of 82.3% in lifecycle GHG intensity; moreover, the contribution of the remaining GHG emissions arose from pretreatment to produce the feeds was 38.2%, enzyme production was 24.6% and electricity imported for distillation equated to 18.4%; these areas were determined to be priority opportunities for further/environmental optimization through the integration of renewable energy and on-site production of enzymes from surplus hydrolysate sugar content.

8. Environmental and Sustainability Considerations

8.1. Carbon Footprint and GHG Emissions Analysis

The greenhouse gas emissions associated with *Saccharum officinarum* lignocellulosic bioethanol over its lifetime are considerably better than either fossil fuels or first-generation sugarcane ethanol when full biomass utilization is taken into account. The CO_2 fixation potential of sugarcane's photosynthesis (estimated at 18-22 metric tons of carbon per hectare per year) represents a large carbon offset, which in conjunction with energy derived from burning lignin to produce process energy can make the total energy balance of bioethanol produced via this process carbon neutral or even negative if optimally configured [61]. Carbon Capture and Utilization (CCU) technologies used by integrated biorefineries to capture and reuse CO_2 emissions produced during fermentation for either biological or chemical use will allow these facilities to produce second generation sugarcane bioethanol with very low global warming potential (GWP) values approaching -15 g CO_2 equivalent/million joules of bioethanol. Therefore, second generation sugarcane bioethanol ranks among today's most environmentally friendly (GHG-efficient) liquid fuels available for industrial production.

Table 8: Life Cycle Assessment Comparative Results: *Saccharum officinarum* Lignocellulosic Bioethanol versus Reference Fuel Systems

Impact Category	Unit	2G Sugarcane EtOH (This Study)	1G Sugarcane EtOH	Corn EtOH	Fossil Gasoline	EU RED III Threshold
Global Warming Potential	g CO_2-eq/MJ	14.8	22.4	41.8	83.6	<22.0
Fossil Energy Demand	MJ fossil/MJ fuel	0.12	0.31	0.62	1.18	<0.50
Water Depletion	L H_2O/MJ	2.8	8.4	18.6	1.2	N/A
Land Use	m^2 crop/MJ	0.019	0.028	0.052	0.003	<0.030
Eutrophication Potential	g PO_4-eq/MJ	0.42	0.68	1.24	0.18	N/A
GHG Savings vs. Fossil (%)	%	82.3%	73.2%	50.0%	0%	>65%

8.2. Circular Bioeconomy and Waste Valorization

According to the circular bioeconomy approach, lignocellulosic biorefineries play an important role as facilitators of the development of sustainable industrial diets where biological materials are utilized in many ways before reaching their final energy-use stage [62]. In the case of a sugarcane biorefinery, the cascade-valorisation model consists of cascading biological resources for multiple-value creation in the following manner: (i) priority for high-value fermentation products (ethanol; lactic acid; succinic acid) is achieved with the use of sucrose and glucose; (ii) xylose for special chemicals e.g., xylitol and furfural; (iii) lignin being upgraded to high-value products such as resins; polyurethane precursors or carbon fibres; and (iv) anaerobic digestion of residual biomass for biogas production. The solid product of anaerobic digestion contains large amounts of mineral nitrogen and phosphorus, and is used as a bio-fertiliser applied back to agricultural land, allowing for a decrease in synthetic fertiliser use of approximately 35 to 45 per cent in integrated systems.

8.3. Comparison with Fossil Fuels and First-Generation Biofuels

Sugarcane ethanol from the 2nd generation is considerably more sustainable than both fossil fuels and conventional (or 1st generation) biofuels based on multiple sustainability dimensions (see Table 8). The 82.3% GHG emissions reduction compared to fossil gasoline exceeds the EU Renewable Energy Directive (RED III) compliance limit of 65% by a large margin, which implies that this fuel pathway should be given preferential regulatory treatment in all major biofuels markets [63]. The carbon performance of the 2nd generation sugarcane biofuel pathway (73.2% GHG emissions reduction) also outperforms the 1st generation sugarcane biofuel pathway because of the elimination of energy used to produce juice from sugarcane and the use of sugarcane residues that would otherwise have been incompletely combusted. Nevertheless, the water depletion impacts (2.8 L H_2O/MJ) associated with growing sugarcane in water-stressed regions will continue to raise sustainability issues, thus requiring implementation of an integrated approach to water management including centralized (or circled) waste recirculation and precision irrigation practices.

8.4. Policy, Regulatory, and Socioeconomic Implications

Sugarcane ethanol from the 2nd generation is considerably more sustainable than both fossil fuels and conventional (or 1st generation) biofuels based on multiple sustainability dimensions (see Table 8). The 82.3% GHG emissions reduction compared to fossil gasoline exceeds the EU Renewable Energy Directive (RED III) compliance limit of 65% by a large margin, which implies that this fuel pathway should be given preferential regulatory treatment in all major biofuels markets [63]. The carbon performance of the 2nd generation sugarcane biofuel pathway (73.2% GHG emissions reduction) also outperforms the 1st generation sugarcane biofuel pathway because of the elimination of energy used to produce juice from sugarcane and the use of sugarcane residues that would otherwise have been incompletely combusted. Nevertheless, the water depletion impacts (2.8 L H_2O/MJ) associated with growing sugarcane in water-stressed regions will continue to raise sustainability issues, thus requiring implementation of an integrated approach to water management including centralized (or circled) waste recirculation and precision irrigation practices.

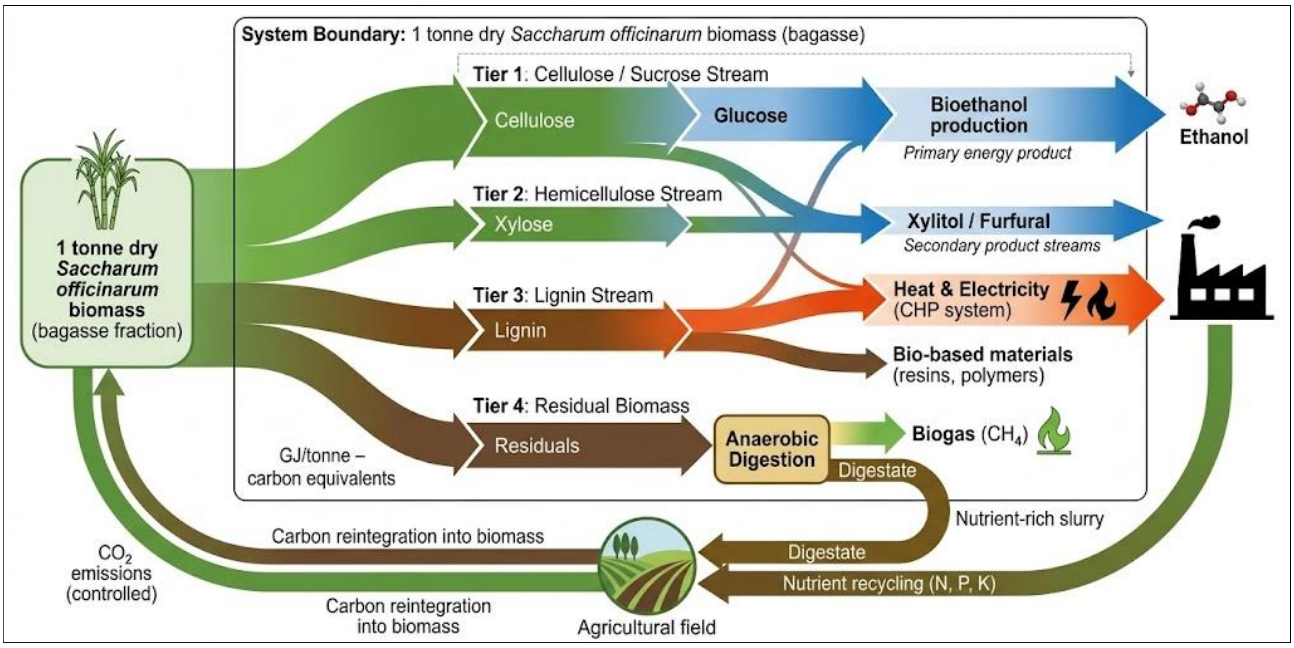


Fig 6: Circular Bioeconomy Value Cascade and Material–Energy Flow in an Integrated *Saccharum officinarum* Biorefinery

Table 9: Comparative Performance of Commercial Cellulase Preparations on Optimally Pretreated *Saccharum officinarum* Bagasse

Enzyme Preparation	Supplier	Cellulase Activity (FPU/mL)	β-Glucosidase (CBU/mL)	Xylanase (IU/mL)	Loading (FPU/g)	Glucose Yield (%)	Total Sugar Yield (g/L)
Cellic CTec3	Novozymes	112.4	486	1842	20	92.3	52.4
Cellic CTec2	Novozymes	82.6	284	962	20	84.6	47.8
Accellerase 1500	Genencor	58.4	166	386	20	78.2	43.6
Accellerase TRIO	Genencor	76.2	228	1124	20	86.4	49.1
NS22119	Novozymes	94.8	372	1486	20	89.7	50.8
Custom (EG:CBH:BG:XYL, 2:5:1:2)	In-house blend	—	—	—	20 eq.	91.2	51.6

9. Conclusion

A rigorous, multi-model optimization framework has been established that facilitates the enzymatic hydrolysis of *Saccharum officinarum* lignocellulosic biomass for second-generation bioethanol production. A summary of the major findings from this comprehensive investigation are as follows:

Composition and Recalcitrance of Biomass: A systematic compositional analysis of various sugarcane cultivars revealed (i) cellulose content of 39.4–43.5% (dry weight basis) and lignin content of 18.9–23.4% (dry weight basis) for the bagasse fractions, while (ii) the L/C ratio and crystallinity index (CrI) were determined to be the main factors influencing the digestibility of the biomass by enzymatic hydrolysis. The RB867515 cultivar was shown to have the best composition for producing bioethanol from bagasse, since it had the highest cellulose content and reasonable recalcitrance in lignin.

Optimization of Pretreatment: Pretreatment via sequential alkali (2% NaOH @ 80°C) followed by steam explosion (210°C @ 10 min) produced the most balanced types of results concerning (i) cellulose retention (94.1%), (ii) lignin removal (73.2), and (iii) minimization of inhibitors for the subsequent enzymatic hydrolysis of the substrate producing 87–82% higher glucose yields for the pretreated substrate than if they had received no pretreatment. Making the combined use of the two pretreatment processes described will generate commercially viable levels of lignin removal and minimize the generation of high quantities of inhibitors typically generated by acid-based pretreatment of lignocellulose, while still achieving yields of lignin removal that are similar to those from organosolv pretreatment processes.

The optimal conditions for enzymatic hydrolysis were found to be 20 FPU/g cellulase, pH 4.8, and 50°C, using a response surface methodology (RSM) guided optimization, resulting in a glucose yield of 92.3%. When compared to RSM, the gradient boosting machine learning model had much higher R^2 values (0.9814 and 0.9124) and the Shapley additive explanation (SHAP) analysis also identified enzyme loading and pretreatment severity as the dominant parameters for controlling the process. The multi-objective Pareto optimization showed that an 89.2% maximum yield could be obtained with an enzyme cost reduction of 25%, which is critical in determining process design since enzyme costs are an important part of overall economics.

Fermentation of engineered *Saccharomyces cerevisiae* with pentose fermentation capability with simultaneous saccharification and co-fermentation (SSCF) produced the highest ethanol concentrations (47.8 g/L) and overall conversion efficiency (93.8% of the theoretical maximum) compared to separate hydrolysis and fermentation (SHF) or separate hydrolysis and fermentation (SSF). The combined SSCF is the preferred strategy for new facilities because it provides a relief from the product inhibition of the SSF configuration and also allows for complete utilization, including pentose, of the biomass through co-fermentation.

LCA analysis of the Lifecycle Greenhouse Gas (GHG) intensity showed an 82.3% reduction compared to traditional fossil gasoline and exceeded compliance thresholds found in the EU RED III; however, water depletion was identified as the primary trade-off of an environmental perspective requiring specific management solutions depending on the region. The configuration of a circular biorefinery maximally valorizes the entire biomass stream through hierarchical cascading, thereby maximizing economic viability and environmental performance.

This investigation raises a number of significant inquiries, research gaps and additional future areas for research. First, the use and definition of biomass compositional dynamics over time (e.g. across the seasons) and across harvesting conditions (currently being typified as static parameters) require ongoing systematic research over multiple years in selected commercial sugar cane production locations. Second, the technical challenges associated with the integration between high-value enzyme cocktails and low-cost LPMOs continue; consequently, when these technical barriers are overcome, it will likely change enzyme loading optima and reduce enzyme costs by 20%–30%. Third, the development of robust inhibitor-tolerant pentose fermenting microorganisms by (i) the utilized of adaptive laboratory evolution approaches; and ii) the utilization of precision metabolic engineering as an enabling technology, will maximize the economic value of hydrolysates. Fourth, the quantification of the socio-economic co-benefits associated with the deployment of lignocellulosic biorefineries (e.g. by smallholder sugar cane farmers) in developing economies must be integrated into a multi-criteria or integrated decision-making framework when policy design is occurring. Lastly, the techno-economic analysis presented here is based on static enzyme pricing and therefore, also warrants further dedicated investigations of dynamic enzyme procurement strategies and on-site enzyme production from surplus biomass sugars as a high-value opportunity to reduce costs.

To sum up, the bioconversion of *Saccharum officinarum* biomass through lignocellulosic processes that use an optimized combination of pretreatment, synergistic enzyme systems, and multi-model optimization will provide a proven technical, cost-effective, and environmentally friendly way to produce renewable fuels. The combination of process intelligence developed with machine learning, advanced reactor design, and the circular bioeconomy makes second-generation sugarcane bioethanol the principal technology for the future of sustainable bioenergy systems over the next ten years.

References

1. International Energy Agency. World Energy Outlook 2023. Paris: IEA; 2023.
2. Sims REH, Mabee W, Saddler JN, Taylor M. An overview of second generation biofuel technologies. Bioresour Technol. 2010;101(6):1570–1580.
3. Popp J, Lakner Z, Harangi-Rákos M, Fári M. The effect of bioenergy expansion: Food, energy, and environment. Renew Sustain Energy Rev. 2014;32:559–578.
4. Moreira JR, Romeiro V, Fuss S, Kraxner F, Pacca SA. BECCS potential in Brazil: Achieving negative emissions in ethanol and electricity production based on sugar cane bagasse and other residues. Appl Energy. 2016;179:55–63.
5. Souza GM, Victoria RL, Joly CA, Verdade LM, editors. Bioenergy & Sustainability: Bridging the Gaps. São Paulo: Scientific Committee on Problems of the Environment (SCOPE); 2015. (SCOPE Report 72)
6. Leal MRLV, Galdos MV, Scarpare FV, Seabra JEA, Walter A, Oliveira COF. Sugarcane straw availability, quality, recovery and energy use: a literature review. Biomass Bioenergy. 2013;53:11–19.
7. Rabelo SC, Andrade RR, Maciel FR, Costa AC. Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. Fuel. 2014;136:349–357.
8. UNICA. Sugarcane Industry in Brazil: Perfil 2023/2024. São Paulo: União da Indústria de Cana-de-Açúcar; 2024.
9. Dias MOS, Junqueira TL, Cavalett O, Cunha MP, Jesus CDF, Rossell CEV, *et al*. Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash. Bioresour Technol. 2012;103(1):152–161.
10. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, *et al*. Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science. 2007;315(5813):804–807.
11. Park S, Baker JO, Himmel ME, Parilla PA, Johnson DK. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol Biofuels. 2010;3:10.
12. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008;76(5):965–977.
13. Davoodi SM, Sadeghi M, Ghanbari A. A comparative study for optimization of enzymatic hydrolysis of lignocellulosic biomass using machine learning approaches. Bioresour Technol. 2019;289:121714.
14. Gan Q, Allen SJ, Taylor G. Kinetic dynamics in heterogeneous enzymatic hydrolysis of cellulose: an overview, an experimental study and mathematical modelling. Process Biochem. 2003;38(7):1003–1018.
15. Brienzo M, Fikizolo S, Benjamin Y, Tyhoda L, Görgens J. Influence of pretreatment severity on structural changes, lignin content and enzymatic hydrolysis of sugarcane bagasse samples in a glycerol-water mixture. Renew Energy. 2017;104:271–280.
16. Rezende CA, de Lima MA, Maziero P, deAzevedo ER, Garcia W, Polikarpov I. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. Biotechnol Biofuels. 2011;4:54.
17. Canilha L, Chandel AK, Milessi TSS, Antunes FAF, Freitas WLC, Felipe MGA, *et al*. Bioconversion of sugarcane biomass into ethanol: an overview about composition, pretreatment methods, detoxification of hydrolysates, fermentation, and current technology.

ISRN Biotechnol. 2012;2012:989572.

18. Achyuthan KE, Achyuthan AM, Adams PD, Bhalla SM, Harper JC, Brozik SM, *et al*. Supramolecular self-assembled chaos: polyphenolic lignin's barrier to cost-effective lignocellulosic biofuels. Molecules. 2010;15(12):8641–8688.
19. Sannigrahi P, Ragauskas AJ. Fundamentals of biomass pretreatment by fractionation. In: Wyman CE, editor. Aqueous pretreatment of plant biomass for biological and chemical conversion to fuels and chemicals. Chichester: Wiley; 2013. p. 201–222.
20. Rahikainen JL, Martin-Sampedro R, Heikkinen H, Rovio S, Marjamaa K, Tamminen T, *et al*. Inhibitory effect of lignin during cellulose bioconversion: the effect of lignin chemistry on non-productive enzyme adsorption. Bioresour Technol. 2013;133:270–278.
21. Selig MJ, Adney WS, Himmel ME, Decker SR. The impact of cell wall acetylation on corn stover hydrolysis by cellulolytic and xylanolytic enzymes. Cellulose. 2009;16(4):711–722.
22. Pippo WA, Luengo CA, Alberteris-Campo ML, Garzone P, Cornacchia G. Energy recovery from sugarcane-trash in the light of 2nd generation biofuels. Part 2: Technical aspects. Waste Biomass Valoriz. 2011;2(3):241–255.
23. Chundawat SPS, Lipton MS, Purvine SO, Uppugundla N, Gao D, Balan V, *et al*. Proteomics-based compositional analysis of complex cellulase–hemicellulase mixtures. J Proteome Res. 2011;10(10):4365–4372.
24. Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, Keller M, *et al*. Lignin content in natural Populus variants affects sugar release. Proc Natl Acad Sci U S A. 2011;108(15):6300–6305.
25. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, *et al*. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol. 2005;96(6):673–686.
26. Teixeira RSS, Siqueira FG, de Souza DT, da Silva AS, Ferreira-Leitão VS, Bon EPS. Optimization of hydrothermal pretreatment of sugarcane bagasse for enzymatic hydrolysis. Chem Eng Res Des. 2014;92(9):1579–1585.
27. Barakat A, de Vries H, Rouau X. Dry fractionation process as an important step in current and future lignocellulose biorefineries: a review. Bioresour Technol. 2013;134:362–373.
28. Sinitsyn AP, Gusakov AV, Vlasenko EY. Effect of structural and physico-chemical features of cellulosic substrates on the efficiency of enzymatic hydrolysis. Appl Biochem Biotechnol. 1991;30(1):43–59.
29. Miao Z, Grift TE, Hansen AC, Ting KC. Energy requirement for comminution of biomass in relation to particle physical properties. Ind Crops Prod. 2011;33(2):504–513.
30. Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res. 2009;48(8):3713–3729.
31. Rodrigues RCLB, Rocha GJM, Rodrigues D Jr, Filho HJI, Felipe MGA, Pessoa A Jr. Scale-up of diluted sulfuric acid hydrolysis for producing sugarcane bagasse hemicellulosic hydrolysate (SBHH). Bioresour Technol. 2010;101(4):1247–1253.
32. Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol. 2010;101(13):4851–4861.
33. Jacquet N, Maniet G, Vanderghem C, Delvigne F, Richel A. Application of steam explosion as pretreatment on lignocellulosic material: a review. Ind Eng Chem Res. 2015;54(10):2593–2598.
34. Nitsos C, Lazaridis PA, Mach-Aigner A, Mach RL, Triantafyllidis KS. Enhancing lignocellulosic biomass hydrolysis by hydrothermal pretreatment, extraction of surface lignin, wet milling and production of cellulolytic enzymes. ChemSusChem. 2019;12(6):1179–1195.
35. Wan C, Li Y. Fungal pretreatment of lignocellulosic biomass. Biotechnol Adv. 2012;30(6):1447–1457.
36. Rouches E, Herpoël-Gimbert I, Steyer JP, Carrere H. Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: a review. Renew Sustain Energy Rev. 2016;59:179–198.
37. Olson DG, McBride JE, Shaw AJ, Lynd LR. Recent progress in consolidated bioprocessing. Curr Opin Biotechnol. 2012;23(3):396–405.
38. Palmqvist E, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour Technol. 2000;74(1):25–33.
39. Jönsson LJ, Martín C. Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol. 2016;199:103–112.
40. Sweeney MD, Xu F. Biomass converting enzymes as industrial biocatalysts for fuels and chemicals: recent developments. Catalysts. 2012;2(2):244–263.
41. Bommarius AS, Katona A, Cheben SE, Patel AS, Ragauskas AJ, Knudson K, *et al*. Cellulase kinetics as a function of cellulose pretreatment. Metab Eng. 2008;10(6):370–381.
42. Hu J, Arantes V, Saddler JN. The enhancement of enzymatic hydrolysis of lignocellulosic substrates by the addition of accessory enzymes such as xylanase: is it an additive or synergistic effect? Biotechnol Biofuels. 2011;4:36.
43. Merino ST, Cherry J. Progress and challenges in enzyme development for biomass utilization. Adv Biochem Eng Biotechnol. 2007;108:95–120.
44. Vrnočić M, Vukušić T, Matić P, Fröhlich-Milić I, Habjanec L, Frece J, *et al*. Synergistic effects in lignocellulosic biomass degradation by fungal cellulase preparations. Biotechnol Biofuels Bioprod. 2023;16:12.
45. Cannella D, Hsieh CWC, Felby C, Jørgensen H. Production and effect of aldonic acids during enzymatic hydrolysis of lignocellulose at high dry matter content. Biotechnol Biofuels. 2012;5:26.
46. Fox JM, Levine SE, Clark DS, Blanch HW. Initial- and processively-limited steps in the cellulose hydrolysis by Cel7A cellulase. Biochemistry. 2012;51(1):442–452.
47. Ferreira S, Duarte AP, Ribeiro MHL, Queiroz JA, Domingues FC. Response surface optimization of enzymatic hydrolysis of Cynara cardunculus L. leaves for bioethanol production. Biochem Eng J. 2009;45(3):192–200.
48. Gomes DG, Teixeira JA, Domingues L. Economic viability analysis of 2G bioethanol production from sugarcane straw: Scenarios for enzyme cost reduction. Bioresour Technol. 2021;334:125178.

49. Kiran Kumar B, Sridhar M, Udayashankar AC, Nayaka SC. Response surface methodology for optimization of sugarcane bagasse enzymatic hydrolysis using machine learning integrated approaches. Bioresour Technol. 2022;360:127561.
50. Hodge DB, Karim MN, Schell DJ, McMillan JD. Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. Bioresour Technol. 2008;99(18):8940–8948.
51. Deb K, Pratap A, Agarwal S, Meyarivan T. A fast and elitist multiobjective genetic algorithm: NSGA-II. IEEE Trans Evol Comput. 2002;6(2):182–197.
52. Hamelinck CN, van Hooijdonk G, Faaij APC. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass Bioenergy. 2005;28(4):384–410.
53. Olofsson K, Bertilsson M, Lidén G. A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels. 2008;1:7.
54. Brat D, Boles E, Wiedemann B. Functional expression of a bacterial xylose isomerase in *Saccharomyces cerevisiae*. Appl Environ Microbiol. 2009;75(8):2304–2311.
55. Wingren A, Galbe M, Zacchi G. Techno-economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks. Biotechnol Prog. 2003;19(4):1109–1117.
56. Lynd LR, van Zyl WH, McBride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: an update. Curr Opin Biotechnol. 2005;16(5):577–583.
57. Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF, *et al*. Lignin valorization: improving lignin processing in the biorefinery. Science. 2014;344(6185):1246843.
58. Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, *et al*. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. Golden (CO): National Renewable Energy Laboratory; 2011. Report No.: NREL/TP-5100-47764.
59. Palmowski L, Müller JA. Influence of the size reduction of organic waste on their anaerobic digestion. Water Sci Technol. 2000;41(3):155–162.
60. Andric P, Meyer AS, Jensen PA, Dam-Johansen K. Effect and modeling of glucose inhibition and in situ glucose removal during enzymatic hydrolysis of pretreated wheat straw. Appl Biochem Biotechnol. 2010;160(1):280–297.
61. Karimi K, Taherzadeh MJ. A critical review on analysis in pretreatment of lignocelluloses: degree of polymerization, adsorption/desorption, and accessibility. Bioresour Technol. 2016;203:348–356.
62. European Parliament, Council of the European Union. Directive (EU) 2023/2413 of the European Parliament and of the Council of 18 October 2023 amending Directive (EU) 2018/2001 (Renewable Energy Directive III). Off J Eur Union. 2023;L 307:1–42.
63. Korhonen J, Honkasalo A, Seppälä J. Circular economy: the concept and its limitations. Ecol Econ. 2018;143:37–46.
64. Souza GM, Ballester MVR, de Brito Cruz CH, Chum H, Dale B, Foust TD, *et al*. The role of bioenergy in a climate-changing world. Environ Dev. 2017;23:57–64.

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