Biofuel Extraction methods from rice straw: Review

A . M. M. Abd-Elgawad

Mechanical Engineering Department, National Research Centre, Giza, Egypt

A R T I C L E   I N F O

A B S T R A C T

Burning rice straw to dispose of it harms the environment, but it holds promise as a valuable resource for biofuel production due to its lignocellulosic content. Biofuels derived from diverse lignocellulosic sources, including agricultural residues, forest byproducts, and wood, offer sustainable alternatives or supplements to gasoline. Rice straw, abundant and renewable, stands out as a prime candidate for biofuel according to higher content of cellulose and hemicellulose those are convertible into fermentable sugars via hydrolysis. However, unlocking these sugars requires pretreatment to disrupt the lignin barrier and expose the cellulose and hemicellulose for enzymatic conversion. Pretreatment methods are designed to achieve several objectives, including reducing cellulose crystallinity, enhancing biomass surface area, eliminating hemicellulose, and disrupting lignin barriers. This review delves into various pretreatment approaches for rice straw, encompassing physical, physicochemical, chemical, and biological methods. Highlighting the critical role of pretreatment, the subsequent enzymatic hydrolysis stage involves breaking down cellulose and hemicellulose polymers into fermentable sugars enzymatically.

1. Introduction

The increasing demand for renewable biomass energy stems from the ever-growing energy needs, the lessening of conventional fossil fuels, and the imperative to diminish gas emissions. However, handling logistics and combustion technology face significant challenges due to the natural characteristics of biomass feedstock having low density, great dust, bulk density was low, and diverse physical shapes, [1-3]. Biomass, originating from organisms that are currently or were recently alive, poses challenges when used in bio processes, especially plant-based materials. Lignocellulosic materials, which include cellulose, hemicellulose, pectin, and lignin polymers, need to be broken down into monomers. This breakdown can be achieved through biological means and/or physicochemical means. These materials are increasingly important for biofuels manufacturing, with estimates suggesting that up to 40% of fuel consumption in the USA could be replaced by fuels derived from lignocellulosic sources, contributing to reduced greenhouse gas emissions.

Rice serves as a fundamental staple food for more than 40% of the global population, generates large quantities of rice straw, often disposed of through burning, resulting in detrimental environmental effects such as the release of soot, smoke, greenhouse gases, and loss of plant nutrients. Agricultural wastes, categorized as crop residues or agro-industrial residues, present opportunities for energy production. Rice straw, categorized as a crop residue, contains cellulose, hemicellulose, and lignin and holds potential for ethanol production. Technologies for converting rice straw to ethanol exist, employing either the sugar or the syngas platforms. The first platform involves converting hemicellulose and cellulose to fermentable sugars, while the syngas platform converts biomass through gasification into a gas mixture containing carbon monoxide and hydrogen, which can be further processed to ethanol. Rice straw, with its annual global production estimated at about 731 million tons, has the potential to yield approximately 205 billion liters of bioethanol annually., [4-6].

The production of biofuel from lignocellulosic masses typically comprises from four leading steps: feedstock pretreatment, enzymatic saccharification, fermentation, and ethanol recovery as shown in Fig. (1). Integration of these

* Ahmed Mahfouz, Mechanical Engineering Department, National Research Centre, Egypt, +201555560244, ahmedian_1@hotmail.com

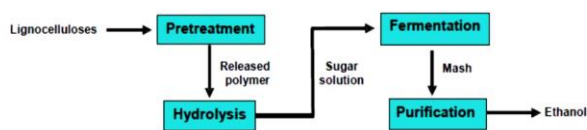process steps is crucial for reducing ethanol production costs, [7 and 8].

Figure (1) Ethanol production from lignocellulosic materials

2. Pretreatment:

Rice straw presents a complex blend of carbohydrate polymers, with cellulose and hemicellulose firmly attached by lignin layers, impeding enzymatic hydrolysis. A pretreatment step is imperative to disorder the lignin seal and expose cellulose and hemicellulose for enzymatic action. The objectives of pretreatment encompass reducing cellulose crystallinity, augmenting biomass surface area, eliminating hemicellulose, and breaking the lignin seal. Various pretreatment procedures like physicochemical, thermal, and their combinations, are developed. Recognized as one of the costliest processing stages in the conversion of cellulosic biomass to fermentable sugars, pretreatment plays a pivotal role in second-generation bioethanol production, significantly impacting operational costs, [9-10].

An optimal pretreatment process should meet several criteria:
(a) Simplicity and cost-effectiveness in operation;
(b) Cost-efficient size reduction, if necessary;
(c) lowest consumption of reactants and energy;
(d) Minimal corrosion;
(e) Favorable alterations to the lignocellulosic matrix;
(f) Limited losses of polysaccharides;
(g) Retrieval of hemicellulose-derived products;
(h) Controlled generation of undesirable by-products;
(i) Creation of pretreated solids having cellulose content;
(j) Retrieval of high-quality lignin or lignin-derived compounds;
(k) Minimal generation of wastes.

2.1. Physical pretreatment [11-13]

The Physical pretreatment approaches aim to improve the accessibility of the biomass surface area, reduce cellulose crystallinity, and decrease the polymerization degree of cellulose. Several techniques fall under this category:

a) **Torre faction**: This technology improves the properties of agricultural biomass to address issues such as high bulk volume, moisture content, and grind ability, particularly for thermochemical processing methods like combustion, co-combustion, or gasification, [14-16].

b) **Washing/Leaching**: This process removes problematic elements from the straw that can lead to slagging, fouling, and corrosion in furnaces and other thermal conversion systems, [12 and 13].

c) **Baling**: Field baling is a cost-effective method for harvesting and packing rice straw, commonly used to improve its characteristics for transportation and storage, [14 and 15].

d) **Pelletizing**: This compaction process creates homogeneous fuel with high energy density in various shapes such as squares, rectangles, or cubes, [16]. It addresses the low bulk density of biomass, reducing transportation costs and storage space requirements. However, it may limit the co-firing ratio due to boiler system capacity constraints, [17].

e) **Comminution**: This involves chipping, grinding, and/or milling to minimize the size of lignocellulosic materials. Typically, material sizes range from 10-30 mm after chipping to 0.2-2 mm after milling or grinding. Vibratory ball milling is more effective than ordinary ball milling in reducing cellulose crystallinity and improving digestibility. Disk milling is found to be more efficient than hammer milling. Milling studies have shown increased yields of biogas, bioethanol, and biohydrogen, [18-20].

2.2 *Physicochemical Pretreatment*

a) **Steam Explosion**:

Steam explosion stands as the greatest broadly utilized method for pretreating lignocellulosic supplies. Biomass undergoes with high-pressure steam treatment, followed by a sudden pressure reduction, leading to explosive decompression. Operating typically at temperatures of 160-260 °C and parallel to pressures of 0.69-4.83 MPa for few seconds to minutes, the method enhances hemicellulose hydrolysis and transforms lignin attributable to the high temperatures involved. Studies by Jin and Chen (2006) explored steam explosion treatment of rice straw to optimize grinding time, save energy costs, and enhance enzymatic hydrolysis. Similarly, other researchers like Kobayashi et al. mentioned that the use of steam explosion to improve fermentation processes for bamboo conversion into methane, while Ballestros et al. evaluated its effectiveness on herbaceous lignocellulosic biomass. Recent studies, such as Viola et al. who investigated the explosion via steam treatment on wheat, barley, and oat straws, have optimized the process at a batch scale for carbohydrate recovery, [21 and 22].

b) **Ammonia Fiber Explosion (AFEX)**:

AFEX, a physicochemical pretreatment process, involves subjecting lignocellulosic biomass to liquid ammonia under high temperature and pressure, followed by sudden pressure reduction, [23 and 24]. Similar to steam explosion, AFEX increases the accessible surface area and de-crystallizes cellulose, causing a phase change in the cellulose crystal structure, [25]. Teymouro et al. evaluated various parameters for optimizing the AFEX process, highlighting the significance of pretreatment temperature in biomass fiber structure disruption. AFEX-treated biomass exhibits high yields of glucose and xylose during hydrolysis of the enzym with minimal formation of inhibitory compounds, making it an active pretreatment process for the rice straw, [8, 25 and 26]

c) **Carbon Dioxide Explosion**:

The concept of supercritical CO2 explosion presents a potential alternative to steam and ammonia explosion methods, offering lower temperatures and possibly reduced costs. Supercritical CO2, when dissolved in water, forms carbonic acid, aiding in hemicellulose and cellulose hydrolysis. Dale et al. utilized CO2 explosion to pretreat alfalfa, achieving a significant release of glucose during enzymatic hydrolysis. Zheng et al. compared CO2 explosion with other methods, concluding its cost-effectiveness and lack of sugar degradation, unlike steam explosion. CO2 explosion involves delivering supercritical CO2 to biomass in a high-pressure vessel, enhancing digestibility through its penetration and dissolution in water, [23 ,24, 26-30].

Explosion stands as the most widely utilized method for pretreating lignocellulosic materials

2.3 *Chemical Pretreatment*:

Chemical pretreatment plays a crucial role in facilitating enzymatic transformation of lignocelluloses to fermentable sugars. Alkali and ammonia are the best chemicals for pretreating rice straw, [11].

a) **Ozonolysis**:

Ozone treatment offers a method for reducing lignin content in lignocellulosic wastes, enhancing their in vitro digestibility without producing toxic residues. This process degrades lignin and hemicellulose in various constituents like pine, wheat straw, and bagasse. Studies have shown that ozonation in hydrated fixed beds leads to further active oxidations compared to suspensions in water or in acetic acid solution. The major products identified from ozonized materials include oxalic and formic acids, among others, [11, 31-33].

b) ** Alkali **:

This pretreatment includes treating biomass with alkaline solutions such as NaOH or KOH. This process aims to remove lignin and a portion of hemicelluloses, which enhances enzyme accessibility to cellulose. By breaking ester bonds between lignin, hemicellulose, and cellulose, alkali pretreatment effectively eliminates acetyl and uronic acid substitutions that can impede enzyme access. Alkali pretreatment significantly increases yields of saccharification and can be achieved at relatively little temperatures accompanied with a high concentration of base, [8, 26,34-36].

c) **Acid **:

At ambient temperatures, lignocellulose is pretreated with acids to enhance anaerobic digestibility, particularly affecting hemicellulose with minimal lignin degradation. Dilute acid pretreatment is commonly employed and may require a two-stage process to reduce enzyme requirements. Different acids like, hydrochloric, sulfuric, and phosphoric acids are used for hydrolyzing biomass, with dilute concentrations preferred due to corrosiveness of concentrated acids, [8, 26, 37,38].

d) **Organosolv**:

Organosolv pretreatment involves delignification and elimination of hemicellulose, leaving rich residue of cellulose that could be enzymatically hydrolyzed to nearly theoretical glucose yield. Organic solvents like ethanol, methanol, and others are used, and pretreatment may require catalysts at lower temperatures. The process typically involves solvent removal to prevent inhibition of enzymatic hydrolysis or fermentation. Organosolv processes offer high rates of cellulose hydrolysis and recovery of lignin and hemicellulose for co-product production, [8, 39 and 40].

2.4 **Biological pretreatment**:

Biological pretreatments, particularly those involving fungi, are often employed to break down lignin, hemicellulose, and polyphenols in lignocellulosic biomass. Among the fungi, white-rot fungi have demonstrated remarkable effectiveness in this regard. They produce ligninolytic enzymes such as lignin and manganese peroxidase, and laccase, which efficiently break down lignin into smaller fragments. This process enhances the availability of cellulolytic enzymes into cellulose and hemicellulose, thereby facilitating subsequent enzymatic hydrolysis.

Selective degradation of lignin in wood and wheat straw by certain white-rot fungi has also been reported by Hatakka et al. (1993, 1994). These fungi, including Phanerochaete chrysosporium and Dichomitus squalens, demonstrate the ability to depolymerize lignin over several weeks, achieving significant delignification while maintaining selectivity and efficiency.

Despite the conceptual advantages of biological pretreatment, such as reduced chemical and energy usage, challenges remain in developing a controllable and rapid system. However, ongoing research continues to explore the potential of biological pretreatment as a sustainable approach for enhancing the conversion of lignocellulosic biomass into valuable yields like biofuels, [8, 39, 41-43]

3. Hydrolysis

3.1. *Enzymatic*

Enzymatic hydrolysis comprises breaking down cellulose and hemicellulose polymers with the aid of enzymes. Cellulose typically yields glucose, while hemicellulose produces various pentoses and hexoses. High content of lignin inhibits enzyme leading to reduced hydrolysis rates and yields.

Enzymatic hydrolysis is generally conducted under mild conditions, with varying acid concentrations and temperatures. Acid hydrolysis, such as with sulfuric acid, effectively breaks down hemicellulose, yielding monosaccharides suitable for fermentation. Recent advancements in enzyme technology have improved lignocellulosic ethanol research, with enzymes being used in combination to enhance hydrolysis efficiency.

Factors influencing hydrolysis yields include acid concentration, temperature, retention time, substrate size, and enzyme type. Research has shown that combining enzymes like cellulase, xylanases, and pectinases improves hydrolysis

---

efficiency, although this increases process costs. Despite challenges, enzymatic hydrolysis remains a key step in lignocellulosic ethanol production, with ongoing efforts to improve its efficiency and cost-effectiveness, [39, 44-48].

3.2. Cellulolytic Enzymes

The enzymatic breakdown of cellulose and hemicellulose involves the action of specific enzymes, including cellulases and hemicellulases. Cellulose degradation to glucose involves the synergistic action of endo-glucanases, exo-glucanases, and ß-glucosidases. Endoglucanases target low-crystallinity regions of cellulose, Endoglucanases create free chain-ends in cellulose, while exoglucanases degrade the sugar chain by removing cellobiose units. Subsequently, the produced cellobiose is cleaved to glucose by ß-glucosidase, completing cellulose depolymerization, [39].

Hemicellulose degradation is more complex due to its diverse sugar units, involving enzymes such as endo-1,4-β-D-xylanases, exo-1,4-β-D-xylosidases, and others. Various bacteria and fungi, including species like Clostridium, Trichoderma, and Penicillium, are capable of producing these enzymes.

Among cellulases, those from Trichoderma reesei or T. viride are well-studied and characterized. These enzymes provide advantages such as complete cellulase production and stability under hydrolysis conditions, although they may have suboptimal levels of ß-glucosidases. Aspergilli, on the other hand, are efficient ß-glucosidase producers. Combining Trichoderma cellulase with additional ß-glucosidases has shown improvement in enzymatic hydrolysis efficiency in several studies, [49].

4. Strategies for Hydrolysis and Fermentation

4.1. Separate Hydrolysis and Co-Fermentation

This treatment comprises of two primary stages. Initially, cellulose undergoes complete hydrolysis to glucose facilitated by cellulases under optimal conditions, usually around 50°C. This temperature facilitates enzymatic hydrolysis and reduces the enzyme dosage required. However, this temperature is not suitable for microorganisms performing ethanol fermentation as shown in Fig. (2), which typically prefer temperatures around 35°C.

Once cellulose is completely hydrolyzed, lignin remains, that can be improved by filtration. The resulting hydrolysate has a low viscosity, making it appropriate for high gravity (HG) fermentation. HG fermentation can reduce energy consumption for ethanol distillation and distillage treatment, as it significantly reduces the amount of distillage discharged from the distillation system.

The Iogen process, notable as the pioneering demonstration plant for bioethanol production through the biochemical conversion pathway, marked a significant milestone in the development of sustainable fuel technologies, tested this concept. It demonstrated the feasibility of SHCF for bioethanol production, highlighting its potential for efficient and sustainable ethanol production.
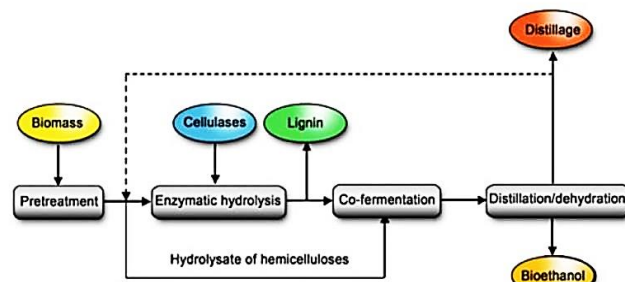

Figure (2) Separate Hydrolysis and Co-Fermentation

4.2. Simultaneous Saccharification and Co-Fermentation

During ethanol fermentation from starch-based feedstocks, the mash is subjected to liquefaction at elevated temperatures ranging from 90 to 110°C, facilitated by the action of thermo-tolerant amylase enzymes. This endoenzyme randomly hydrolyzes starch into dextrins. The resulting mixture is further hydrolyzed by glucoamylase, an exoenzyme that breaks down the dextrins into glucose at slightly lower temperatures (60–62°C) for 20–30 minutes, achieving a dextrose equivalent of 15–20. The solution is then cooled to around 30–32°C, in addition to transferring to fermenters to begin fermentation of ethanol; namely simultaneous saccharification and fermentation (SSF), that is common mentioned industrialy, [50].

Likewise, in the production of ethanol from lignocellulosic biomass, a comparable approach is utilized, known as simultaneous saccharification and co-fermentation (SSCF). However, SSCF accounts for the exceptional features of the hydrolysate, which contains C5 and C6 sugars. Although termed "simultaneous," The breakdown of dextrins or pretreated cellulose into sugars (saccharification) and the fermentation or co-fermentation of glucose, C5, and C6 sugars occur sequentially.

This process is simple in design and operation, but faces challenges. Due to the significantly different temperature requirements for enzymatic hydrolysis and ethanol fermentation, simultaneous optimization becomes unfeasible. Consequently, SSCF processes are constrained to lower temperatures ranging from 30 to 35°C to provide accommodations microbial progression and ethanol fermentation as shown in Fig. (3). As a consequence, the compromised enzymatic hydrolysis rate necessitates longer processing durations. Moreover, since lignin cannot be separated from cellulose prior to fermentation, the resulting fermentation broth becomes highly viscous, adversely affecting mixing, heat, and mass transfer efficiency. Consequently, SSCF operations cannot be conducted under high gravity (HG) conditions, leading to increased energy consumption for distillation of the fermentation broth with low ethanol concentrations, as well as for the treatment of distillate due to the larger volume. For instance, a fed-batch SSCF system reported a processing duration of 96 hours to convert pretreated wheat straw containing 11% water insoluble solids, yielding only 3.3% (w/v) ethanol.
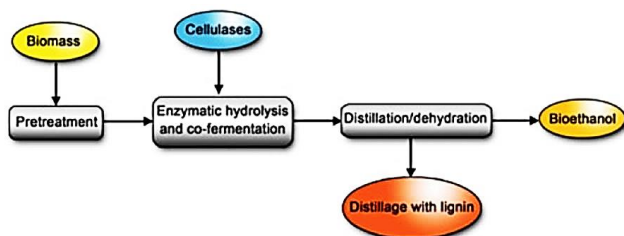
Figure (3) Saccharification and Co-Fermentation

4.3. Consolidated Bioprocessing

Cellulases serve as pivotal components in both the Separate Hydrolysis and Co-Fermentation (SHCF) and Simultaneous Saccharification and Co-Fermentation (SSCF) processes., where they are separately produced and then added in order to hydrolyze the pretreated biomass containinng cellulose. However, this approach poses a major barrier to cost saving in bioethanol production due to high initial cost of enzymes and their dosage required by these processes, [51 and 52].

Inspired by natural microorganisms that can utilize native cellulose for growth and metabolism, scientists have developed mimic systems known as consolidated bioprocessing (CBP) as shown in Fig.(4). CBP aims to directly convert lignocellulosic biomass into ethanol and other chemicals without pretreatment. This strategy addresses many challenges encountered in biochemical conversion processes.

CBP relies on the development of microbial strains capable of efficiently producing ethanol from lignocellulosic biomass. Two main strategies have been explored for this purpose:

1.**Engineering Cellulase Producers to be Ethanol Producers**: This strategy involves modifying anaerobic cellulolytic bacteria, such as those from the genus Clostridium, to produce ethanol. In metabolic engineering, the objectives include enhancing ethanol yield by enhancing ethanol tolerance and inhibiting the synthesis of major by-products., [53].

2. **Engineering Ethanol Producers to be Cellulolytic**: In this approach, ethanologenic species like Saccharomyces cerevisiae are engineered to express and secrete functional cellulases. Methods like cell surface display are utilized to incorporate genes that encode glycoside hydrolases, encompassing cellulases and hemicellulases, into the yeast. However, challenges remain in achieving efficient expression of certain cellulases, such as cellobiohydrolases (CBHI and CBHII) from Trichoderma reesei, in S. cerevisiae. The increase of CBP strains is crucial for advancing the CBP process, with ongoing research focusing on improving ethanol yield, tolerance, and cellulase expression in microbial hosts, [54-57].

5. Fermentation
5.1 Simultaneous saccharification and fermentation using Saccharomyces cerevisiae

In simultaneous saccharification and fermentation (SSF) experiments using Saccharomyces cerevisiae, Cellulase enzymes facilitated the conversion of cellulose into glucose, while S. cerevisiae concurrently metabolized the glucose into ethanol. Increasing the enzyme loading led to a 20% enhancement in the maximum ethanol concentration, observed in both aerobic and anaerobic conditions. The most rapid ethanol production rate was observed within the initial 25 hours of the process, with the ethanol concentration subsequently stabilizing. in anaerobic conditions but decreased slowly in aerobic conditions after the initial peak. The main byproduct from SSF by S. cerevisiae was Glycerol having higher concentrations observed under anaerobic conditions. The production of glycerol followed a similar pattern to that of ethanol, with no additional accumulation of glycerol noted after 80 hours in anaerobic simultaneous saccharification and fermentation (SSF). Glucose and cellobiose concentrations remained low throughout the experiments, with occasional increases observed in aerobic conditions, [58].

5.2 SSF using Mucor indicus

In SSF experiments using Mucor indicus, similar to S. cerevisiae, ethanol was the major metabolite, with glycerol as the main byproduct. The concentration of ethanol remained constant in anaerobic conditions but decreased slightly in aerobic conditions after the initial production phase. Higher enzyme loading had a notable impact on ethanol yield during anaerobic simultaneous saccharification and fermentation (SSF), whereas its effect was minimal in aerobic SSF. Additionally, M. indicus demonstrated higher glycerol production under anaerobic conditions compared to aerobic conditions. Glucose and cellobiose concentrations were low but slightly higher than those observed with S. cerevisiae, with accumulation observed in later stages of anaerobic SSF.

5.3 SSF using Rhizopus oryzae

Rhizopus oryzae, unlike S. cerevisiae and M. indicus, produced lactic acid as the main metabolite alongside ethanol. Ethanol production peaked within the first 2-3 days, with faster production in aerobic conditions, [58]. Enzyme loading affected ethanol yield, with doubling the enzyme concentration resulting in increased ethanol yield in both aerobic and anaerobic conditions. Lactic acid was produced simultaneously with ethanol, with higher yields observed in anaerobic conditions. R. oryzae produced lower levels of glycerol compared to S. cerevisiae and M. indicus. Glucose and cellobiose concentrations increased dramatically in the later stages of SSF, particularly with higher cellulase enzyme loading.

6. Conclusion

In summary, exploiting rice straw for bioethanol production shows great potential owing to its abundance and advantageous composition. Biological conversion facilitated by hydrolytic enzymes emerges as the most promising method, primarily due to its environmental benefits. However, the complex nature of rice straw, including high lignin and ash content, necessitates efficient pretreatment methods to remove unwanted components

and make sugars readily available. Significant progress has been made in developing efficient pretreatment methods.

Rice straw possesses the capacity to satisfy the bioethanol demand in the transportation sector, considering its substantial annual output. Increasing enzyme concentration can enhance ethanol yield from cellulose, but it's essential to evade lignocellulosic dryness as it may irreversibly collapse biomass pores. Careful selection and optimization of pretreatment, hydrolysis, and fermentation processes are crucial for maximizing efficiency.

With advancements in genetically modified yeast, synthetic hydrolyzing enzymes, and other sophisticated technologies, coupled with their efficient integration, bioethanol production from rice straw is poised to become a feasible and economically viable technology in the near future.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

References

1. F. R. Kargbo, J. Xing, Y. Zhang, "Pretreatment for energy use of rice straw", African Journal of Agricultural Research Vol. 4 (12), 1560-1565, 2009. DOI:10.5897/AJAR.9000558

2. P. C.A. Bergman, J. H.A. Kiel, "Torre faction for biomass upgrading", 14th European Biomass Conference and Exhibition, Paris, France, 17-21, ECNRX-05 -180, 2005-12-08.

3. Z. Pan, X. Li, L. Fu, Q. Li, X. Li, "Environmental sustainability by a comprehensive environmental and energy comparison analysis in a wood chip and rice straw biomass-fueled multi-generation energy system", Process Safety and Environmental Protection, Vol. 177, Pp 868-879, 2023, https://doi.org/10.1016/j.psep.2023.07.027.

4. P. Binod, R. Sindhu, R. R. Singhania, S. Vikram, L. Devi, S. Nagalakshmi, N. Kurien, R. K. Sukumaran, A. Pandey, "Bioethanol production from rice straw: An overview", resource Technology, 101, 4767–4774, 2010, DOI: 10.1016/j.biortech.2009.10.079

5. Balat, M., Balat, H., Oz, C., "Progress in bioethanol processing", Prog. Energy Combust. Sci. 34, 551–573, 2008. https://doi.org/10.1016/j.pecs.2007.11.001

6. K. Karimi, G. Emtiazi, M. J. Taherzadeh, "Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with Mucor indicus, Rhizopus oryzae, and Saccharomyces cerevisiae", Enzyme and Microbial Technology, 40, 138-144, 2006, https://doi.org/10.1016/j.enzmictec.2005.10.046.

7. Badal C. Saha_, Michael A. Cotta; "Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol", BIOMAS S AND BIOENERGY, 32, 971 – 977 2008, https://doi.org/10.1016/j.biombioe.2008.01.014.

8. J.P. Delgenes, R. Moletta, J.M. Navarro, "Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by Saccharomyces cerevisiae, Zymomonas mobilis, Pichia stipitis, and Candida shehatae", Enzyme and Microbial Technology, Vol. 19, Iss. 3, 220-225, 1996, https://doi.org/10.1016/0141-0229(95)00237-5.

9. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., "Features of promising technologies for pretreatment of lignocellulosic biomas" Bioresour. Technol. 96, 673–686, 2005, https://doi.org/10.1016/j.biortech.2004.06.025.

10. Lynd LR, Weimer PJ, Willem H. van Zyl, Isak S. Pretorius "Microbial cellulose utilization: fundamentals and biotechnology", Microbiol Mol Biol Rev 66, 506–577, 2002, **DOI:** https://doi.org/10.1128/mmbr.66.3.506-577.2002

11. Millet, M. A.; Baker, A. J.; Scatter, L. D. "Physical and chemical pretreatment for enhancing cellulose saccharification". Biotech. Bioeng. Symp, 6, 125–153, 1976, PMID: 1000062

12. D. KO, Koresgren JG, P. JBC, Jag IU, "The effects of fuel Washing techniques on alkali release from Biomass" Fuel 81, 137-142, 2002, https://doi.org/10.1016/S0016-2361(01)00132-6

13. T.R. Milles, T.R. Miles Jr., L.L. Baxter, B.M. Jenkins, L.L. Odens, "Alkali slagging problems with biomass fuels". First Biomass Conference of the Americans. Vol. I, 16, 406-421, 1993, ACNR/CP-16596

14. J. Dobie, P. S. Parsons, R. Curley, "Systems for handling and utilizing rice straw", Agricultural and Food Sciences, Transactions of the ASABE, 16, 533-536, 1973, DOI:10.13031/2013.37562

15. Dobie, A Haq, "Outside storage of baled rice straw", Agricultural and Food Sciences, Transactions of the ASABE, 22, 990-993, 1980, DOI:10.13031/2013.34702

16. Loo Van S, Koppejan J. "Handbook of Biomass combustion of co-firing prepared by task 32 of the Implementing Agreement on Biomass" under the auspices of the Int. Energy Agency Tent University press, 2004.

17. Bergman PCA, Boersma AR, Zwart RWR, Kiel JHA (2005). Palletizing for biomass co-firing in existing coal-fired power stations.

18. Y. Sun, J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: a review", Bioresour. Technol. 83, 1–11, 2002.

19. JY Zhua, GS Wang, XJ Pan, R. Gleisner, "Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification". Chem Eng Sci, 64, 474–485, 2009.

20. Delgenes JP, Penaud V, Moletta R. Pretreatment for the enhancement of anaerobic digestion of solid waster chapter 8. In Biomethanization of the organic fraction of municipal solid waste IWA publishing. 2002:201–28.

21. C. Cara, E. Ruiz, M. Ballesteros, P. Manzanares, JM Negro, E. Castro, "Production of fuel ethanol from steam-explosion pretreated olive tree pruning", Fue,87, 692–700, 2008.

22. S. Jin, H. Chen, "Superfine grinding of steam-exploded rice straw and its enzymatic hydrolysis", Biochem. Eng. J. 30, 225–230, 2006.

23. T.H. Kim and Y.Y. Lee, "Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures". Appl. Biochem. Biotechnol., 81–92, 2007.

24. T.H. Kim, F. Taylor, K.B. Hicks, "Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment", Bioresour. Technol. 99, 5694–5702, 2008.

25. C. Zhong, M.W. Lau, V. Balan, B.E. Dale, Y.J. Yuan, "Optimization of enzymatic hydrolysis and ethanol fermentation from AFEX-treated rice straw", Appl. Microbiol. Biotechnol. 84, 667–676, 2009.

26. V. B. Agbor, N. Cicek, R. Sparling, A. Berlin, D. B. Levin, "Biomass pretreatment: Fundamentals toward application", Biotechnology Advances, 29, 675–685, 2011.

27. P. Kumar, D. M. Barrett, M. J. Delwiche, P. Stroeve; "Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production", Ind. Eng. Chem. Res., 7, ASAP, 2009.

28. Y. Z. Zheng, H. M. Lin, G. T. Tsao, "Pretreatment for cellulose hydrolysis by carbon dioxide explosion". Biotechnol. Prog., 14, 890– 896, 1998.

29. Y. Z. Zheng, H. M. Lin, G. T. Tsao, "Supercritical carbon-dioxide explosion as a pretreatment for cellulose hydrolysis". Biotechnol Lett, 17, 845–50, 1995.

30. AT. Hendricks, G. Zeeman, "Pretreatments to enhance the digestibility of lignocellulosic biomass", Bioresour. Technol., 100, 10–8, 2009

31. W. C. Neely, "Factors affecting the pretreatment of biomass with gaseous ozone", Biotechnol. Bioeng., 26, 59–65, 1984.

32. P. F. Vidal, J. Molinier, "Ozonolysis of lignins Improvement of in vitro digestibility of poplar sawdust", Biomass, 16, 1–17, 1988,.

33. W. H. Morrison, D. E.Akin, "Water soluble reaction products from ozonolysis of grasses", J. Agric. Food Chem., 38, 678–681, 1990.

34. M. Gaspar, G. Kalman, K. Reczey, "Corn fibre as a raw material for hemicellulose and ethanol production", Process Biochem, 42, 1135–1139, 2007.

35. H.Tarkov, W.C. Feist, "A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia". Adv. Chem. Ser. 95, 197–218, 1969.

36. Q.Z. Zhang, W.M. Cai, "Enzymatic hydrolysis of alkali-pretreated rice straw by Trichoderma reesei ZM4-F3", Biomass Bioenergy 32, 1130–1135, 2008.

37. J. Sumphanwanich, N. Leepipatpiboon, T. Srinorakutara, A. Akaracharanya,"Evaluation of dilute-acid pretreated bagasse, corn cob and rice straw for ethanol fermentation by Saccharomyces cerevisiae", Ann. Microbiol. 58, 219–225, 2008.

38. J. D. McMillan, "Pretreatment of lignocellulosic biomass. In Enzymatic Conversion of Biomass for Fuels Production", American Chemical Society: Washington, DC, pp 292-324, 1994.

39. S. Ali, Mudasir A. Dar, F. Liaqat, S. Sethupathy, A. Rani, M. Ilyas Khan, M.d Rehan, D. Zhu, "Optimization of biomethane production from lignocellulosic biomass by a developed microbial consortium", Process Safety and Environmental Protection, Vol. 184, pp 1106-1118, 2024, https://doi.org/10.1016/j.psep.2024.02.037.

40. G.I. Mantanis, R.A. Young, M.R. Roger, "Swelling of wood part II Swelling organic liquids", Holzforschung 48, 480–490, 1994.

41. S.J. Patel, R. Onkarappa, K.S. Shobha, "Study of ethanol production from fungal pretreated wheat and rice straw". The Internet J. Microbiol. 4, 2007.

42. M. Taniguchi, H. Suzuki, D. Watanabe, K. Sakai, K. Hoshino, T. Tanaka, "Evaluation of pretreatment with Pleurotus ostreatus for enzymatic hydrolysis of rice Straw", J. Biosci. Bioeng., 100, 637–643, 2005.

43. M. Taniguchi, M. Tanaka, R. Matsuno, T. Kamikubo, "Evaluation of chemical pretreatment for enzymatic solubilization of rice straw", Eur. J. Appl. Microbio Biotechnol., 14, 35–39, 1982.

44. Z. Xin-Qing, Z. Li-Han, B. Feng-Wu, L. Hai-Long, H. Xiao-Ming, Y. Guo-Jun, H. Nancy W. Y., "Bioethanol from Lignocellulosic Biomass", Adv. Biochem. Engin. Biotechnol., 128, 25–51, 2012.

45. Wen F, Nair NU, Zhao H, "Protein engineering in designing tailored enzymes and microorganisms for biofuels production", Curr Opin Biotechnol 20, 412–419, 2009.

46. F. W. Bai, W. A. Anderson, M. Moo-Young, "Ethanol fermentation technologies form sugar and starch feedstocks", Biotechnol Adv., 26, 89–105, 2008.

47. A.S. Mamman, J.M. Lee, Y.C. Kim et al, "Furfural: Hemicellulose/xylose derived biochemical", Biofuel Bioprod Biorefin, 2, 438–454, 2008.

48. B.C. Saha, "Hemicellulose bioconversion", J Ind Microbiol Biotechnol 30, 279-291, 2003.

49. H. Talib Hamzah, V. Sridevi, D. Venkata Surya, P. Ramesh, C. Sankar Rao, S.r Palla, T. Adnan Abdullah, "Synergistic effects and product yields in microwave-assisted in-situ co-pyrolysis of rice straw and paraffin wax, Process Safety and

Environmental Protection, Vol. 182, pp 45-55, 2024, https://doi.org/10.1016/j.psep.2023.11.048.

50. K. Öhgren, R. Bura, G. Lesnicki   et al, "A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam pretreated corn Stover", Process Biochem, 42, 834–839, 2007.

51. L.R. Lynd, R.T. Elander, C.E. Wyman, "Likely features and costs of mature biomass ethanol technology", Appl Biochem Biotechnol., 57–58, 741–761, 1996.

52. L.R. Lynd, P.J. Weimer, W.H. van Zyl et al, "Microbial cellulose utilization: fundamentals and biotechnology", Microbiol Mol Biol Rev, 66, 506–577, 2002.

53. Q. Xu, A. Singh, M.E. Himmel, "Perspectives and new directions for the production of bioethanol using consolidated bioprocessing of lignocellulose", Curr Opin Biotechnol., 20, 364–371, 2009.

54. M. Jin, V. Balan, C. Gunawan et al, "Consolidated bioprocessing (CBP) performance of Clostridium phytofermentans on AFEX-treated corn stover for ethanol production", Biotechnol Bioeng., 108, 1290–1297, 2011.

55. A.J. Shaw, K.K. Podkaminer, S.G. Desai et al., "Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield", Proc Natl Acad Sci USA 105, 13769–13774, 2008

56. Y. Fujita, J. Ito, M. Ueda et al., "Synergistic saccharification and direct fermentation to ethanol of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme", Appl Environ Microbiol., 70, 1207–1212, 2004.

57. Katahira S, Fujita Y, Mizuike A et al., "Construction of a xylan- fermenting yeast strain through codisplay of xylanolytic enzymes on the surface of xylose-utilizing Saccharomyces cerevisiae cells", Appl Environ Microbiol., 70, 5407–5414, 2004.

58. H. Punnapayak, G.H. Emert, "Use of Pachysolen tannophilus in simultaneous saccharification and fermentation (SSF) of lignocellulosics" Biotechnol Lett; 8, 63–6, 1986.

Abbreviation and symbols

AFEX	**Ammonia Fiber Explosion**
PI CBP	**Consolidated bioprocessing**
HG	**High gravity**
SHCF	**Separate Hydrolysis and Co-Fermentation**
SHF	**Separate Enzymatic Hydrolysis and Fermentation**
SSCF	**Simultaneous Saccharification and Co-Fermentation**
SSF	**Simultaneous Saccharification and Fermentation**