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Enzymatic Hydrolysis: A Sustainable Approach for Paddy Straw-Based Ethanol Production and its Advancement

V. C. Verma*

Dept. of Agricultural Engineering, Bhola Paswan Shastri Agricultural College (Purnea), Bihar Agricultural University, Sabour, Bihar (802 136), India

Corresponding Author

V. C. Verma

e-mail: vikashvermaiitkgp@gmail.com

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Abstract

The objective of this review paper was to highlight the importance of paddy straw as raw material and the treatment practices to convert into bio-ethanol. Enzymatic hydrolysis played a significant role in converting lignocellulosic biomass into sugars, which were subsequently fermented to produce bioethanol. This process provided a sustainable alternative to fossil fuels, using renewable biomass sources such as rice straw. Rice straw was mainly composed of cellulose (33–47%), hemi-celluloses (19–27%), lignin (5–24%) and silica (18.3%) and were bonded through a gel matrix to form bio-composites, and created complication for converting rice straw into bio-ethanol. Therefore, Enzymatic hydrolysis followed by pre-treatment process was essential to disrupt lingo-cellulosic complex, to remove lignin, to reduce cellulose crystallinity and to increase the porosity of the materials and digestibility of the substances. The sugar released during hydrolysis was converted into the ethanol through fermentation depending on the percentage of sugar recovery, type of simple sugar. Advance process technology had been employed to optimize the various aspects of fermentation from higher yield to better and wide substrate utilization to increased recovery rates. Bio-ethanol had higher octane number and higher heat of vaporization and so it could be easily blended with the gasoline or also used as alcohol in dedicated engines. In India, till now ethanol was primarily produced by sugar- cane molasses, so that it might be proved as an attractive alternative option for fossil fuels.

Keywords: Rice straw, enzymatic hydrolysis, fermentation, bio-ethanol

1. Introduction

“Sustainable agriculture” is holistic approach, representing economically viable and environmentally safe approach that produces socially accepted food, fibre or fuel. The growing world economy is facing highly increased greenhouses (GHG) gases so there is need of technology or methodology so that we can reduce the GHG emission for mitigating the climate change and move towards the sustainable growth of economy (Aditya et al, 2016). Therefore, we have to promote biomass-based energy resources (bio-ethanol, bio-diesel, bio-hydrogen). Bio-ethanol has higher octane number and higher heat of vaporization and so it can be easily blend with the gasoline or use as alcohol in dedicated engines (Hahn-Hägerdal et al., 2007). Further, it can be produced from sugars, starch and lignocellulosic materials, abundant in nature as agricultural residue but the high economic costs of these technologies and variable substrate composition limit their implementation at commercial scale (Carrillo-Nieves et al., 2019). Therefore, bio-ethanol as alternative sources of energy fulfil the criteria of sustainable development. We have to focus the bio-ethanol production from inedible potential feed stocks to avoid pressure on food crops, otherwise food security issues will arise for rapid growing world population (Sun and Cheng, 2002). Lignocellulosic biomass (sugarcane bagasse, rice hull, timber species, willow, salix, switchgrass, softwood, rice straw, wheat straw etc.) may be proved more sustainable technologies for bio-ethanol production] because their utilization does not compete with the food crops and animal feed and moreover these cellulosic materials contribute to environmental sustainability (Sassner et al., 2008; Singh et al., 2014; Sahu et al., 2022). Furthermore, Paddy straw will be best suitable alternative among all the lignocellulosic substances (consist cellulose and hemicellulose around 33% and 28% respectively) mentioned above due to abundant supply, cheap and locally available (Sun et al., 2010; Park et al., 2011). Bio ethanol results into zero GHG emission as CO_2 is released through burning of fossil fuel fixed again by the following harvest. Enzymatic hydrolysis is defined as a chemical interaction of an enzymes (cellulase or trypsin), that loosen the connections of the cell wall and enable easy extraction. Further, non cellulose components such as lignin and hemicelluloses, crystallinity of cellulose make the limited adsorption of cellulose so these should be removed by promising pre-treatment technology. Therefore, Enzymatic hydrolysis may be the most promising method for the saccharification of paddy straw where polysaccharides are hydrolysed into mono saccharides and further can be subsequently converted to bio ethanol by microorganisms. It needs less energy and milder operational conditions (approximately under the temperature of 40–50 °C and pH of 4–5), while compared to traditional acid hydrolysis (Nakazawa et al., 2009). Furthermore, it shows low toxicity, low deterioration and corrosion, low utility cost, no inhibitory by-product, and no environmental damages compared to acid saccharification (Yan et al., 2003). Advancements in enzymatic hydrolysis have been significant. For example, optimizing pretreatment conditions such as acid concentration and time, as well as enzymatic hydrolysis parameters, has been shown to improve the yield of fermentable sugars from rice straw. Studies using various substrates, including barley and wheat straws, have demonstrated the importance of pretreatment in increasing bioethanol yields by enhancing the efficiency (Hawrot-Paw et al., 2023; Rusanescu et al., 2024). Moreover, advancements are being made in co-product generation such as lignin and silica during the bioethanol production process, which enhances economic viability, sustainability and profitability of the process, making it a zero-waste model biorefinery (Le et al., 2022; Schutyser et al., 2018). The objective of this review paper was to highlight the importance of paddy straw as raw material and the treatment practices to convert into bio-ethanol.

2. Paddy Straw: Structural Characterisation

Paddy straw possesses cellulose, and hemicellulose (around 33% and 28% respectively) besides having 10% lignin and 12% silica (Domínguez-Escribá and Porcar, 2010). Paddy straw composition and morphological characteristics are influenced by cultivation practices such as fertiliser application, irrigation and time of harvesting (Vadiveloo and Phang, 1996). In addition, weather, climate, and plant age related to the cultivation season are also playing important role in affecting the composition and quality of straw (Vadiveloo and Phang, 1996).

Cellulose and hemicellulose are both carbohydrates. The main building blocks of the lignin structure are of phenolic monomers, and so called monolignols (Schutyser et al., 2018; Minu et al., 2012). Native lignin have three monolignols as *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, present in varying proportion. Presence of these monolignnols makes the lignin as potential resource of aromatic compounds production or bioethanol. Additionally, paddy straw has significant amount of silica, originated from soil and entering into the roots ofthe paddy plants as mono silicic acid, Si $(OH)_4$.

2.1. *Cellulose*

Cellulose consists of anhydrous 1->4 glucose. Cellulose exists as of D-glucose subunits, linking by β-1,4 glycosidic bonds (Fengel and Wegener, 1984). Plants Cellulose consisting of two structures namely, crystalline (organized) structure and amorphous (not well organized) structure. When cellulose strains are bundled together calledcellulose fibrils or cellulose bundles, are mostly independent as bounded through weak hydrogen bonding (Laureano-Perez et al., 2005) (Figure 1).

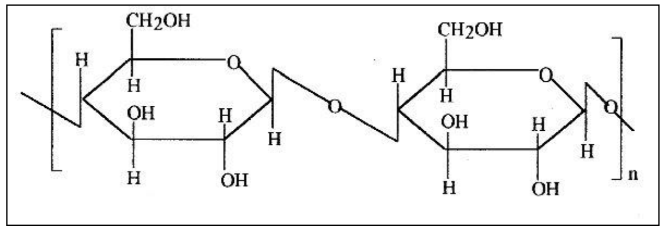

Figure 1: Chemical structure of cellulose

2.2. *Hemicellulose*

Hemicellulose is connecting the lignin and the cellulose fibres and providing more rigidity to the cellulose-hemicellulose-lignin network (Laureano-Perez et al., 2005), so acomplex carbohydrate structure consisting various polymers like pentoses (like xylose and arabinose), hexoses (like mannose, glucose and galactose), and sugar acids. Unlike cellulose, hemi celluloses are not chemically homogeneous and are a low molecular weight with short lateral chains branches of different sugars, which are easily hydrolysable polymers (Fengel and Wegener, 1984). The solubility of the different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose which increase with increasing temperature. The solubilities of higher molecular polymers could not be predicted, due to their unknown melting points (Gray et al., 2003). The solubilisation of hemicellulose compounds into the water starts around 180 °C under neutral conditions according to Bobleter (1994). The solubilization of lignocelluloses components not only depends on temperature, but also on other aspects like moisture content and pH (Fengel and Wegener, 1984). The dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw, is xylan (Fengel and Wegener, 1984; Saha, 2003). The xylan of hemicellulose can be extracted easily in an acid or alkaline environment (Balaban and Ucar, 1999; Fengel and Wegener, 1984; Lawther et al., 1996b). The hemicelluloses are the most thermal-chemically sensitive among cellulose, hemicellulose and lignin (Levan et al., 1990; Winandy, 1995). The side groups of hemicellulose reacted firstly followed by the hemicellulose backbone during thermochemical pretreatment (Figure 2).

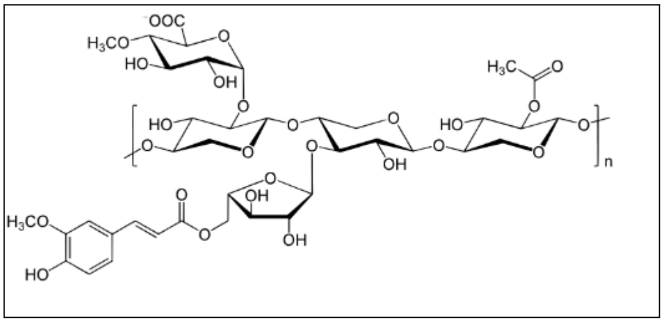

Figure 2: Chemical structure of hemicellulose-xylan

2.3. Lignin

Lignin is present in cellular wall, most abundant polymers in nature. It is an amorphous hetero polymer, of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) holding together by different kind of linkages. Lignin supports the plant structure, impermeable (due to its hydrophobic nature), and provides resistance against microbial attack and oxidative stress. The amorphous heteropolymer is non-water soluble and optically inactive, so the degradation of lignin is found to be very tough (Fengel and Wegener, 1984). Lignin started to dissolve normally around 180 °C under neutral conditions (Bobleter, 1994). The solubility of the lignin in acid, neutral or alkaline environments depends on its precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them) (Grabber, 2005) (Figure 3).

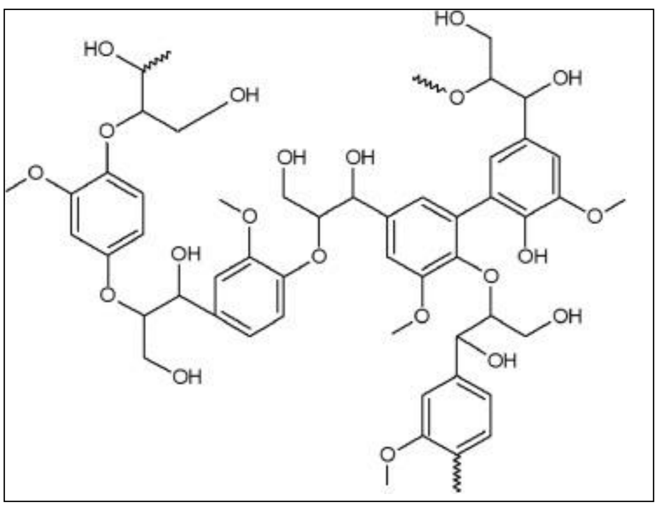

Figure 3: Chemical structure of Lignin

2.4. Silica

Silica content in straw is identified as indicators of straw quality. The biogenic Si content may reduce the biodegradability by lowering the cell wall digestibility (Van Soest and Jones, 1968; Jackson, 1977; Van Soest, 1981), especially in paddy straw. Therefore, Silica also found in rice straw may inhibit the degradability and reduce the palatability (Widyastuti and Abe, 1989; Doyle and Chanpongsang, 1990). So, they cannot be used for animal feeding purposes also. High yielding varieties have high degree of resistance against pathogens and against lodging of the plants resulted into relatively high silica content (Deren et al., 1994). Silica contentis also stimulating the absorption of elements such as N, P and Kin the soil.

3. Pre-treatment Technologies

Rice straws have compact packed structure consisting of cellulose, hemi-celluloses and lignin, causing difficulties in bioethanol production (Himmel et al., 2007). Therefore, pre treatment is essentially required to disrupt lignocellulosic complex, to remove lignin, to reduce cellulose crystallinity and to increase the porosity of the materials (Kumar et al., 2009). This treatment makes the lignocellulosic biomass more amenable for hydrolysis, results into high rate ofrate of production as well as the total yield of sugars (Yang et al., 2008). Furthermore, it also increases the digestibility of the substances for enzymatic hydrolysis (Jørgensen et al., 2007). The pretreatment technologies can be classified as physical, chemical, biological and combined pre-treatment techniques.

3.1. Physical pre treatment

Physical pre treatment includes grinding and milling, irradiation, steaming, temperature, and pressure. Milling process reduces the particle size resulted into increase in specific surface area and reduces the degree of polymerisation (DP) (Chang and Holtzapple, 2000). Mostly, the increased surface area and the shearing increase the total hydrolysis yield of lignocelluloses (depending on kind of biomass, kind, and duration of milling) as well as reduces the technical digestion time i.e. results in hydrolysis rate (Delgenés et al., 2003). Wet disk milling is preferred due to the fact that energy consumption of was found lower than that of other milling practices (ball milling) (Hideno et al., 2009), for the enzymatic hydrolysis of herbaceous biomass, speciallyfor rice straw (Hideno et al., 2009).

The Irradiation reduces DP and the crystallinity of lignocellulose (Bouchard et al., 2006) resulted into more accessibility of the cellulose crystalline regions (Stepanik et al., 1998). The application of electron beam irradiation pre treatment has also been reported to improve enzymatic hydrolysis of rice straw (Bak et al., 2009). Microwave irradiation develops a change into the ultrastructure of cellulose, degrade the lignin, and hemicelluloses as well as increase the enzymatic susceptibility of rice straw (Intanakul et al., 2003).

3.2. Chemical pre treatments

Chemical pre-treatment methods include lime, acid, steam explosion, sulphur dioxide explosion, ammonia fibre explosion, ionicLiquid and others have been developed for pre treatment of biomass (Behera et al., 2014). Sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca $(OH)_2$) are generally used for alkali pre- treatment. Hydrochloric acid, phosphoric acid and nitric acid are an acid pre treatment agent (Mosier et al., 2005). Sodium hydroxide increases the internal surface of cellulose, decreases the degree of polymerization and crystallinity and thereby disrupting the lignin structure. Calcium hydroxide removes the acetyl groups from hemicelluloses, reduces steric hindrance of enzymes and increases cellulose digestibility (Mosier et al., 2005). Chang and Holtzapple (2000) and Silverstein et al. (2007) reported that alkali pretreatment demonstrated minor cellulose and hemi- celluloses solubilisation than acid or hydro thermal process. The acid pre treatment increases the recovery of hemicelluloses as monomers in liquid fraction and digestible cellulose in solid fraction (Sassner et al., 2008; Linde et al., 2008 and Yang and Wyman, 2008).

3.3. Biological pre treatments

Biological pre-treatment is a safe and environmentally friendly process, as there is possibility to utilize the abundant source of cellulolytic and hemicellulolyticmicroorganism makes biological pre-treatment an intelligent method also. These microorganisms includes white rot fungi , *Phanerochaete chrysosporium , Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus, Pleurotus ostreaus P. chrysosporium, Trametes versicolor* which serve as the agents for pre-treatment procedure in mostlignocellulosic based material (Kumar et al., 2009; Shi et al., 2008) and show up as filamentous being growing in the very soil (Vats et al., 2013).This results into low chemical and energy requirement, higher yield of desired product and greater substrate and reaction specificity (Kirk and Chang, 1981). Keller et al (2003) reported that white rot fungi such as *Phanerochaete chrysosporium, Ceriporiopsis subvermispora, and Pleurotus ostreatus* can competently remove lignin. *Dichomitus squalens* increases the enzymatic digestibility of lignocelluloses and promote cellulose hydrolysis of rice straw (Bak et al., 2010). The main disadvantage is that the rate of hydrolysis declines due to its sluggishness and inefficiency rendering it impractical for industrial application (Sun and Cheng, 2002) and so not recommended so much. Much research must be done in isolation procedure for instance, utilizing *basidiomycetes* organisms for their capacity to break the plant material rapidly and productively. Thus, biological pre-treatment is not compelling enough due to its sluggishness and inefficiency (Dashtban et al., 2010).

4. Enzymatic Hydrolysis and its Limiting Factor

4.1. Enzymatic hydrolysis

Enzymatic hydrolysis is the second step in the production of ethanol from lignocellulosic materials. It involves cleaving the polymers of cellulose and hemicellulose using cellulytic enzyme such as cellulases and hemicellulases, to separate starch or cellulose into sugar (Zhang and Lynd 2004). Cellulose normally contains glucans, while hemicellulose contains polymers of a few sugars, such as mannan, xylan, glucan, galactan, and arabinan. Therefore, hydrolysis of cellulose results into glucose formation while for hemicelluloses it is pentose and hexose. However, high lignin content blocks enzyme accessibility, causes end-product inhibition, and reduces the rate and yield of hydrolysis. Cellobiose and glucose also act as strong inhibitors of cellulases (Knauf and Moniruzzaman, 2004) in addition to lignin. Pre-treatment of cellulosic material may change the crystallinity of cellulose including some different factors such as liquid to solid ratio, particle size, temperature, length of cellulose polymer, arrangement of the cellulose chain, association of cellulose with other protective polymeric structures inside the plant cell wall, for example, lignin, pectin, hemicellulose, proteins, and mineral components, which influence the yields of lignocellulose to monomeric sugars and the by-products. Zhu et al. (2006) reported that the microwave-assisted alkali pre-treatment removed more lignin and hemicellulose from wheat straw resulted into enhanced enzymatic hydrolysis. But in the other study it has been reported that mechanical crushed irradiate pre-treated wheat straw also improve enzymatic hydrolysis (Yang et al., 2008). Enzymatic hydrolysis rate of alkali assisted photocatalysis of rice straw is 2.56 times more than that of alkali process (Niu et al., 2009) whereas, ammonia treated rice straw results into 61% monomeric sugars while comparing with the untreated one (11%) (Sulbaran-de-Ferrer et al., 2003). Hydrolysis efficiency of lignocellulosic biomass increases, when applied in combination of enzymes, such as cellulase, xylanases, and pectinases as compared to application of only cellulase (Sulbaran-de-Ferrer et al., 2003).

4.2. Limiting factor

Bioethanol production by conventional process of SHF at commercial scale may be major challenge especially from recalcitrant biomass like paddy straw (Singh et al., 2016). Further, its large-scale production, and commercialization is additionally a crucial task because of requirements of efficient reactors, high temperature maintenance (for enzyme and thermophilic strains), cost of enzyme and fermentative microorganisms, distillation, additionally major challenges of large volume required for storage due to low density 20–40 kg m^{-3}), and associated problems of transportation, chopping, bailing, etc. (Kargbo et al., 2010). High silica content of paddy straw makes a real challenge of its bio processing. Silica layer presents shearing problems to the knife of mill/chaff cutter, inhibits enzymatic and microbial actions, and thus prevents its microbial degradation and even hydrolysis more difficult than lignin resulting into lesser saccharification and ethanol yields. Thus, silica removal and enzymatic degradation are hardertasks for SSF process especiallyfor paddy straw. Therefore, some significant pre-treatment methods should be investigated to soften and take away the silica in addition to reducing lignin and crystallinity of paddy straw. Inoculum and hydrolytic enzymes are key contributors of high cost of process, along with no recovery in SSF due to its permanent amalgamation with biomass. Further, Enzymes could be recycled using desorption technique with surfactants (Xu et al., 2008). However, cells could not be recycled but immobilized cells could be used for SSF process to enable the separation of cells out of biomass (Kumar et al., 2011). There are diffusion restrictions of immobilized cells while comparing to free cell systems with high solid loading, viscosity and mobility difficulties of paddy straw, reduction in productivity and consequently insignificant yields has been observed.

5. Bio-ethanol Production and Process Optimization

Sugarcane, sugar beet juice or other starch containing materials such as corn, wheat are traditionally used for bio ethanol production. The foremost important parameters for the economic outcome are the feedstock cost. Paddy straw as crop residues reduces the potential conflict between the land use for food or feed, rich in lignocellulosic components, less expansive raw materials, easily available in Asian countries may be proved more suitable. Therefore, recent research is targeted on conversion of bio-ethanol from lignocellulosic crop residue materials. So far, two processes were investigated regarding ethanol production from lignocellulosic materials. The major difference is the way through which the cellulose chain is broken apart; either with dilute sulphuric acid or cellulolytic enzymes are used to hydrolyse the cellulose molecules. As already discussed above that the enzymatic process is operated with milder conditions and the yield is much higher and formation of toxic products is also reduced. So, there is focus on economic aspects of ethanol production and to provide targets where the process can be improved.

The process steps e.g. distillation, evaporation, drying and incineration, are considered to be technically mature, i.e. their operational performance is wellknown. But, lignocellulose-to-ethanol process involves not only vapour and liquid phases but also a solid phase, including a typical compounds like cellulose, lignin and yeast. Therefore, the modelling of a lignocellulose-to-ethanol process poses a number of unique challenges and so while simulating such type of process it is necessary to use a flow sheeting program that is able to handle solid components. Flow sheeting programs is an invaluable tool in studying how changes in process design affect the overall performance of a plant. For this purposes, Aspen Plus from Aspen Technologies has been recommended. It is based on a modular approach where each module is a mathematical model of a unit operation. The fundamental equations is needed to accurately describe standard process equipment, such as columns (distillation, absorption, etc.), heat exchangers, pumps, reactors and splitters, are normally available as part of the program. The actual simulation is performed by arranging different unit operation modules into a complete flowsheet that represents the process to be simulated.

Lynd et al. (1996) reported a more advanced improvements in conversion technology by the use of a microorganism capable of not only fermenting sugars to ethanol but also of hydrolysing the cellulose (direct microbial conversion), and shorter residence times in the process steps. According to a techno-economic evaluation of ethanol production from biomass performed by "SRI Consulting's Process Economic Program" (PEP), the capital investment required for a plant producing ethanol is about 2.5 times higher than the investment cost of a corn-based ethanol plant with the same feed capacity. Improvements were mainly expected to be in enhanced by pre treatment and bioconversion steps, changing from SSF to SSCF of hexose and pentose sugars and finally Consolidated Bio Processing (CBP), resulting in higher ethanol yield and reduced capital costs, based on improvements of both enzyme efficiency and fermentation microorganisms.

The ethanol production cost for the lignocellulosic process would be reduced by using co-product instead disposing off unutilized. Pellets from the excess solids is used to generate heat and electricity, makes the process energy efficient. For this there is need to integrate ethanol production unit with another suitable plant, e.g. a combined heat and power plant, a starch-based ethanol plant or a pulp and paper mill.

Another option is to integrate cellulosic ethanol production with starch-based ethanol production to utilize the whole agricultural crop. This will increase the production capacity drastically and it may also help to boost the ethanol concentration resulting from the lignocellulosic process.

It is, however, difficult to compare production costs from different studies due to the many assumptions made in the simulations, such as ethanol yield, productivity and concentration, as no commercial-scale plants are in existence.

6. Bio-ethanol Application: Present Status and Future Prospects

Presently, paddy straw is utilized as animal feed with some additives, enhancing nutritional quality after that still huge part is burnt in the open field. Open burning is creating air population with increase of GHG worldwide. At present, transport bio-fuel provided 4% of the world demand for transporting purposes while very few countries such as United States, Brazil, and china are focussing on the production of this alternative fuel.It has been expected that transport fuel demand is going to be increased by 9% and estimated that bio-fuel could provide 26% from alternative sources by 2050. Therefore, bio ethanol mayprove a good alternative as it is a renewable and sustainable bio-fuel with a promising future to alleviate the global energy crisis and to improve environmental quality (Aditiya, et al., 2016). We have already discussed that different pre-treatment for conditioning of lignocellulosic materials to improve the overall efficiency of the process and make the cellulose and hemicellulose more accessible for the subsequent process of enzymatic hydrolysis. Several studies have reported the benefits of bio processing methodology due to its overall benefits. Different fungi are also used as biological pre-treatment to break cellulose and lignin in different lignocellulosic biomass. Therefore, it is now considered important to find a model strain able to produce various enzymes involved in enzymatic hydrolysis to become solution for consolidated bio-processing.

Bio-ethanol has great potential if implemented nationally.

In this regard, Centre for Innovation in Bio-energy, Mexico has developed an institutional framework that contemplates the incorporation of clean and renewable energies in the national energy matrix, as well as the replacement of fossil fuels. The Energy Transition Law establishes that by 2021, 30% of electricity generation must comefrom clean sources and by 2024 it must reach 35% (Carrillo-Nieves et al., 2019). But, mostly in developing countries there is urgent need of significant investments in technology, infrastructure and building capacity so that they could increase the productivity and makes the agricultural sector more sustainable. These steps might allow dedicated agricultural land to increase food production and, and consequently, more feedstock for secondary production of ethanol in the coming future. For this purposes, more detailed, paddy straw specific studies are needed by country to assess the economic feasibility of collecting and pre-processing for bio ethanol production. The cost of bio ethanol production depends on supply of straw on production site, related with the total area of theregion, infrastructure with regard to transport network and its utilization, and the availability of multimodal plant sites that have access toroads, rail. This infrastructure related to plant capacity, if this increases consequently decreases the total capital investment. There are not specific market established for agricultural residue so they may vary significantly between different emerging and developing countries (Anonymous)

Therefore, enzymatic hydrolysis could be attractive solution for bio-ethanol production from lignocellulosic waste with low cost of operation by integrating several steps into one. However, the challenges of this process are precisely related to the selection of pre-treatment methods, efficient microorganisms and thecontrol of the conditions involving during the process. Enzymatic hydrolysis for bio-ethanol production from lignocellulosic waste, still a focussed area of research as this methodology has not been fully commercialized, thus this maybe framed as a promisingtechnology for the future production of bio-ethanol worldwide.

7. Conclusion

The enzymatic hydrolysis of paddy straw presented viable and sustainable method for ethanol production and also embodied a shift towards sustainable and eco-friendly energy solutions. This approach leveraged rice straw as renewable resource, helped to address the growing demand for alternative energy sources while minimizing the ecological impact of fossil fuels as well as managing the agricultural waste. The development of pilot-scale biorefineries indicated the feasibility of this approach on larger scales, advocating for a zero-waste model.

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