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Research Paper

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## **Sustainable transformation for horticultural / agricultural crop residue/ waste matters into biofuels/bioethanol**

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**Abstract**

Due to more demand for food products and flowers designed decoration, efforts have been made for huge cultivation of crops and different flowering plants. It has pushed for generation of different types of residues in the form of lignocellulosic matter. This becomes a big challenge due to huge generation and accumulation in the environment and its disposal strategies at the worldwide level, creating environmental burden and pollution. This has further pushed to climate change and global warming effect with great/ nutrient loss. This situation has forced researcher groups to create more awareness with efficient ways of mitigation and transformation into value-added products like fuels. Next benefit of these efforts can provide the circular bioeconomy and biorefinery concept via reducing these waste matter accumulation. This review focuses on the sustainable mode of horticultural and agricultural crop residue hydrolysis with transformation into value-added products recovery/ generation. This can be utilized for the best opportunity to provide good revenue to farmers and also jobs to the local people via reducing these waste accumulation. It cannot generate any environmental issues with clean environment maintenance. Microbial fermentation and saccharification approaches can be applied for these waste transformations into valuable products. This review talks about the different sources of horticultural and agricultural crop residues and generation, conversion / transformation techniques and also bioethanol production process with emphasis on different microbial systems (wild and engineered strains) contribution.

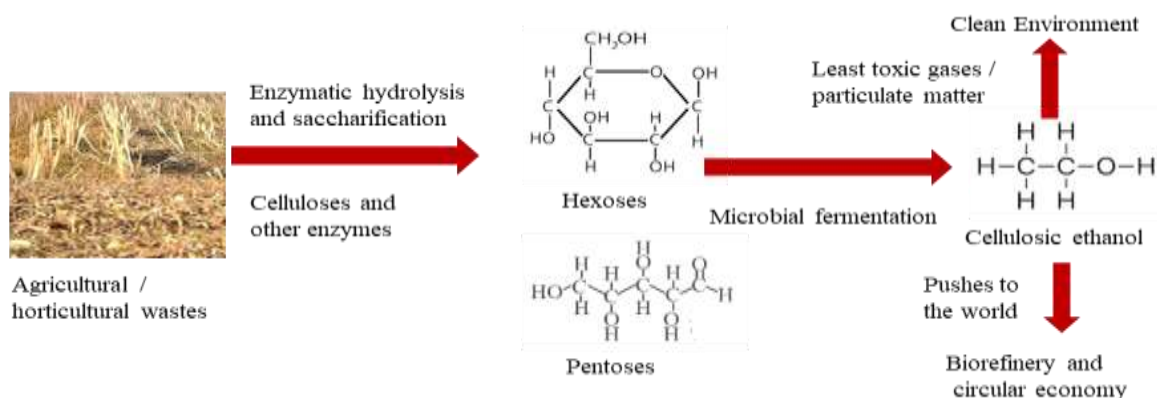
**Keywords:** Agricultural residue, crop wastes, Microbial transformation, Bioethanol, Environment, Value-added products

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## Highlights

- Different agricultural and horticultural wastes discussed with their valorization approach.
- Different pretreatment and hydrolysis approaches emphasized with their merits/ demerits.
- Chemical/ physical with enzymatic hydrolysis showed high efficiency in ethanol yield/ titer.
- Different fermentation technique in ethanol synthesis discuss their advantages/ limitations.

## Graphical abstract



## 1. Introduction

In cropland, wheat and rice straw/ husk, corn stover and sugarcane bagasse generation is reported as important agricultural residues. These are found as left portions in farming lands and are recognized as waste matters, with further utility in landfilling and fodder materials. Some of these wastes are burnt in many places. Various types of waste residues are made up of branch, bark, leaves and other parts of wood/ shrub/herb plants (Fang et al., 2018). These wastes are the main source of lignocellulosic biomass/ matter that contains hemicelluloses (25 to 40%), cellulose (35 to 55%) and lignin (15-25%) with little quantity % of ashes, protein and extractives. These components have shown a high value of energy (12 to 20 MJ/kg) and this value can also depend on ash %/ quantity in these waste matter. Waste plant biomass utility in the pyrolysis process requires a low % of moisture/ water and most of the wood waste residues need to be free from dirt like leaves/ needles. Then, wood waste matters were compared for ash contents (contain less than 2%) (Matías et al., 2015; Srivastava et al., 2019). But most agricultural residues like rice husks can contain more than 20% ash content. Many researchers have calculated the elemental composition details in various agricultural and horticultural wastes and woody wastes contained a little bit high % of carbon and hydrogen element compared to agricultural wastes/ residues. Agricultural wastes and horticultural residues are available in huge quantities as inexpensive and renewable lignocellulosic biomass/ fiber resources. These can be utilized as alternative options to woody lignocellulosic biomass (Matías et al., 2015; Fang et al., 2018). Bioethanol generation can be obtained from hydrolysis of agro-foods wastes and it can contribute to minimize the fossil fuel sources dependency with reduction in its combustion impact to environment/ atmosphere. Further it can mitigate the plant wastes accumulation via pushing/ promoting fuel generation concept from its transformation. Various studies have been done with aims to investigate residual and inexpensive nature of agro-food waste biomasses with their huge availability. This can be done by utilization as feed source of second-generation/ advanced bioethanol plants, located in specific areas of North Eastern Italy (Cripwell et al., 2020).

In these studies, various identification approaches have been applied for various natures of crops and its waste matter (more than 40 agro-waste residues) with systematic analysis for their compositions/ availability like water %, sugars % and polysaccharides %. These analyses were found to benefit with potentially conversion/ transformation into ethanol biosynthesis (Myburgh et al., 2019). In this context, huge quantity residual wet lignocellulosic biomasses (more than  $5.74 \times 10^5$  Metric tons~ MT) were converted into dry material of lignocellulosic matter ( $2.97 \times 10^5$  MT) and these were found to suitable substrates for bioethanol conversion. In this regard, wheat straw and vine shoots are applied as the most promising substrate and these are basically more suitable due to their chemical compositions determination, showing potential ethanol conversion/ attainable capability (Myburgh et al., 2019; Cripwell et al., 2020). From a theoretical point of view, potential estimation of ethanol production was found to be nearly  $7.2 \times 10^5$  Metric gallon (MG)/year. This production capacity of ethanol from lignocellulosic biomass was found to exceed from previously reported production capacity of sustainable bioethanol plant (i.e., 50 MG/ year) (Senila et al., 2020a). By comparing last few years results, it was shown the best analysis of areas that contained huge agro-food residues (in their quantities/ amount) with plenty of availability. These can help to minimize the limit of bioethanol production in specific and restricted districts/ locations. Now, evaluation of techno-economical techniques capabilities can be applied for evaluation of progress of actual feasibility of installing a second generation /2G- bioethanol production plant in a specific area of interest (Senila et al., 2020a; Cripwell et al., 2020).

In China, different nature of crop residue generation is reported with huge quantities and their abundance form. It is one of the lucrative waste biomass resources in China and it can expect to decrease its dependency or reliance on petroleum or coal resources. These can be done due to more uses of crop

residues in China or other countries (Fang et al., 2019). This data is supported by the National Bureau of Statistics (NBS) in China that has done systematic study on crop production capacity with their residue/ waste generation. In this context, they have done nearly 1417 valid face to face questionnaires on various crops residues with effective utilization for fuel production (Cunha et al., 2019). Huge quantities of crop residue generation is reported from the last few years as an increasing trend (725 MT in 2007 to 897.06 MT in 2016) with a good increase in annual rate (2.63%). In China, various crop residues was accounted to total 897.06 MT quantity that is consisted of huge portions of field residues (781.32 MT) and smaller portion of processed crop residues (115.74 MT) in 2016 (Cunha et al., 2019; Fang et al., 2019). Further, these waste field residues in farming lands have exhibited a high ratio (35.2%) with less than 1% utility in bioenergy development tasks. Researchers did the estimation of total quantities of available field crop residues (AFCR) that was used for bioethanol production (nearly 254.6 MT) in 2016 (Bojic et al., 2018; Ko et al., 2016). And in this context, maize crop residue has provided the highest/ largest quantities of AFCR (95.0 MT) and then followed by rice crop residue (79.0 MT) and wheat crop residue (19.0 MT) also and these have contributed/ accounted to 76% of total AFCR quantities in China (Liu et al., 2018).

Further these were assessed at location-wise level contribution like in Heilongjiang (40 MT), then followed by Henan (31.03 MT) and Jilin (22.51 MT). These locations or areas-wise showed the different density of CFCR like high in NEC (Northeast China), CSC (central south China) and southeast provinces of SWC (southwest China) (Liu et al., 2018; Bojic et al., 2018). This review mainly focuses on different lignocellulosic sources of waste matters like agricultural residues and also horticultural wastes. Further it discusses the different microbial cells system and transformation techniques for efficient hydrolysis and saccharification tasks with final conversion into bioethanol. Later, environmental impacts of different sources generated from bioethanol can also be discussed that can keep the environment clean/ greenery with promotion of biorefinery concept.

## **2. Source of crop residues from agriculture/ horticulture sector**

Horticultural crops can include different fruits, vegetables and also ornamental plants and these crops are shown to have high values to human culture and health. These crops are found to be affected with changing climatic conditions. These are due to the bringing of unexpected stress conditions/ events like heat waves, chilling or frost, excess radiation, flood or salinity and drought conditions (Bashir et al., 2023). These are good examples of major devastating abiotic stresses with impact to productivity and quality of horticultural crops. These crops are responsible for generating huge quantities of waste matter/ residues that are thrown to the many environmental components like water, creating a quantic ecosystem issue. (Haq et al., 2023). And other source of waste residues come from woody lignocellulosic biomass and these have shown the similar composition, properties and structures and it is suitable for composite development, textile; paper and pulp sector application (Haq et al., 2023; Bashir et al., 2023). Now, due to several challenges like deforestation issue from woody plant uses, demand for agro-residues as an alternative option to woody cellulosic fiber, is found to rise/ increase in recent decades. And, it is due to increased awareness of environmental concern against burning of these wastes and also deforestation issue at global level (Borovkova et al., 2022). Further, the benefits of agricultural waste is low cost of residue, compared to other plant fibers. Next, utilization/ uses of agro-waste/ residue can minimize the shortage of wood resources in various countries that showed low /few numbers of forestry resources (El Hage et al., 2023).

Several characteristics of lignocellulosic biomass from agricultural residues/ waste are reported and straw of rice, wheat/ barley crops, sorghum stalk with corn stover and coconut husks are good examples with sugarcane bagasse, banana and pineapple leaves. Different natures of agro-wastes fibers

are found with huge availability at worldwide level and these are cultivated to different regions, and are depending on climatic conditions (El Hage et al., 2022; Borovkova et al., 2022). Wheat crops are cultivated at huge quantities that generate the wheat straw and other wastes as important agro-wastes. This crop generated residues contain high % cellulose content as fiber sources and also its hydrolysed form is a good source of glucose (Bari et al., 2018; Sharma et al., 2021).

This can provide the promised alternative to virgin wood fiber and can be utilized in composite industries. Wheat straw has been explored to lignocellulosic resources for composites material development and it is highly available with valuable utilization in composites material and it has best structures and composition with more suitability (Čabalová et al., 2021; Sharma et al., 2021). In India, field stagnation is found in major cropping systems and it has shown the decline of soil fertility and soil health. This has been found to be a prime threat to sustainable food security and environment. Other issues in the world are consistent rising in population and it has increased the food demand from limited land resources suitability (Bari et al., 2018; Čabalová et al., 2021; Sharma et al., 2021). This situation has made a huge gap between the availability of optimal nutrients and also demand to sustain food security and healthy crops. Now worldwide, it becomes a big need of hour for recovering and recycling of nutrients that have been mined/ removed from soils/ lands. It needs the recycling of surplus crops. And horticultural residues and animal excreta can find the viable option for minimization of nutrient gaps via helping in high crop productivity (Čabalová et al., 2021; Husaini and Sohail, 2023). And **table 1** shows different sources waste biomasses with potential source of bioethanol.

**Table 1.** Different agro waste and horticultural waste for potential feedstock for sustainable biofuel recovery with systematic wastes mitigations

Waste biomass	Processing conditions	Potential for biofuel	References
<i>Jerusalem artichoke</i> ( <i>Helianthus tuberosus</i> L.) finds plantation size of 20.1–35.0 ha in size in both Gansu and Shandong, respectively	Enzymatic digestibility with pretreatments of ultrasonic-assisted dilute sodium hydroxide, alkaline peroxide, and ultrasonic assisted alkaline peroxide is shown.	These waste biomasses shows better energy productivity (0.77 kg /MJ in Gansu and .74 kg/MJ in Shandong)	(Fang et al., 2018; Li et al., 2016)
The highest cellulose and hemicellulose content (almost 65%) from vine shoots wastes (VSWs)	The autohydrolysis fractionation method applied for the cellulosic and hemicellulosic sugars separation from the VSW varieties	It is used for the production of important biofuels with recovered solid ranges (60.5-75.0%) after auto-hydrolysis	(Senila et al., 2020)
Hemicelluloses of aspen wood ( <i>Pópulus trémula</i> ) wastes	Oxidative delignification is reported with an acetic acid water-hydrogen peroxide medium at temperatures (70-100°C / process time (1-4 h)	The xylose, mannose, galactose, and glucose monomer units of 9 % of hemicelluloses reported	(Borovkova et al., 2022)
Waste biomass of beech wood with chemical, and photochemical study	This wood wastes were exposed to <i>Pleurotus ostreatus</i> and <i>Trametes versicolor</i> with lignin most degraded polymer followed	Lignin and carbohydrates in beech wood supported chemical alteration is reported by both fungi	(Bari et al., 2018)

	by cellulose and hemicelluloses	decay wood in a simultaneous pattern	
Chemical and morphological composition of Norway Spruce Wood wastes	Steam pretreatments by two different temperatures (180°C and 210°C), with and without the addition of various acids (CH <sub>3</sub> COOH, H <sub>3</sub> PO <sub>4</sub> , H <sub>2</sub> SO <sub>4</sub> , SO <sub>2</sub> ) is reported	The extractives, cellulose, hemicelluloses, and lignin contents is determined for biofuel synthesis	(Čabalová et al., 2021; Caputo et al., 2022)
Different agricultural wastes like wheat and rice straw with sugarcane bagasse.	Microwave heating assisted pyrolysis is applied for releasing of huge amount of fermentable sugars/ oils and it is considered as economically viable technique	Agricultural wastes (AW) as lignocellulosic biomass with weight (%) hemicellulose (10.5–40.4%), cellulose (25.0–44.2.%), and lignin (21.7- 44.%) is shown	(Ge et al., 2021)
Wastes leaves of cellulose fibers as glucose source from (Phoenix canariensis) palm plant	Hydrothermal process for period of 25–33 min with hot water at 70 °C. can decrease the crystallinity of α-cellulose and hemicellulose that released sugars	This plant palm leaves, most common plants in the local environment of the Alicante region (Spain), good feedstock for biofuels	(PérezLimiñana et al., 2022)
Chemical pretreatment of rice straw is reported	Ammonia pretreatment of rice straw significantly enhanced the enzymatic accessibility and liberation of reducing sugars at 60°C after 48 h.	Pretreatment methods followed by saccharification from autoclaved ammonia pretreatment liberated reducing sugar (233.8 mg/g)	(Anu et al., 2020)
Dilute acid hydrolysis of rice straw	The pretreatment of biomass with H <sub>2</sub> SO <sub>4</sub> (0.5% v/v) at 140°C for 90 minutes is reported for this biomass hydrolysis	Optimized process conditions for hydrolysis of rice straw resulted best recovery of pentose sugar (xylose~80-95%) in aqueous fraction	(Gupta et al., 2016)

Plant breeding techniques in horticultural crops are applied for its increased productivity with generation of huge residues and it can improve the related traits of these crops. In this context, vegetable and fruit quality are important parameters that are better changed by this technique. Further it can improve the perishability, consumer acceptance and also market values fulfilment for fruit and vegetable crops with more residue generation. Improved nutritional values in horticultural crops like various fruits/vegetables can be found to be beneficial to undernourished and underprivileged communities (Cameron et al., 2019). Reports are shown in a declined genetic base and due to this reason, conventional plant breeding techniques cannot make a bigger contribution in quality improvement. And existing crossing barriers and natural allelic variation can be found between cultivated and wild nature species to a limited level (Yoo et al., 2020). Last few decades modern and omics biotechnological based transformations/techniques have been applied to decode the complex genomes of crop plants, develop genome-wide

DNA markers, and assign functions for unknown genes. In this context, genetic engineering techniques can help to validate these genes with introduction of crucial agronomic traits/ characteristics that can influence the quality parameters in indirect and direct ways (Yoo et al., 2020; Bashiret al., 2023).

### **3. Transformation techniques for waste matters**

Efforts are done for conversion of agricultural waste residues into value-added products. And these are required for eco-friendly, sustainable and cost-effective modes for various nature of plant derived biomasses/ residues. Waste management for plant matters can start by application of pretreatment practices with achieving improved biodegradability and digestively of agricultural lignocellulosic matters/ biomasses (Awogbemi and Kallon, 2022). Researchers have applied physical, chemical or physicochemical and biological modes in combined/ synergistic modes. Further this step can be done with application of novel green solvent-based pre-treatment that can result in practical and application forms (Ge et al., 2021). Various pre-treatment approaches are discussed with their benefits and limitations that can be stimulated to renewed investigation with scope in research fields/ spaces. From these outcome benefits, more investment in agricultural waste conversion and utilization can be achieved. And it can help in the development of a feasible waste management strategy for a clean environment and also a sustainable ecosystem (Ge et al., 2021; Awogbemi and Kallon, 2022). Last few years, more research has been done on targeted investigations for innovative techniques. And these can help in conversion of various agricultural wastes into a number of valuable products (Ismail and Mohamad, 2021). It needs government involvement, policymaker, stakeholder, environmentalists and jurisdictions people for better management of agricultural wastes. This required sensitization to disclose the cultural and religious barrier against the waste conversion effort (Ismail and Mohamad, 2021; Ge et al., 2021).

#### **3.1. Pre-treatment and enzymatic hydrolysis techniques**

##### **3.1.1. Pre-treatment by chemical reagents hydrolysis**

Researchers have applied optimal experimental conditions for grass biomass hydrolysis by utilization of several enzymes. These biomasses was grown at different stages and then used it for hydrolysis with the help of Taguchi methodology. In this context, rye grass silage and three stages of Italian ryegrass were utilized for hydrolysis tasks at optimal conditions (Vasić et al., 2021). In this biomass hydrolysis process, five factors like enzymatic composition, pretreatment, incubation temperature, pH and pre-treatment time was considered and these are found to influence the hydrolysis of rye grass. This study was done at individual and interactive levels of grass biomass (Maitan-Alfenas et al., 2015). Researcher has set up some experiments with some hydrolysis factors of grass biomass. Efforts was done at individual level for grass pretreatment task by use of sodium hydroxide solution and enzyme compositions and it showed greater influence (75% and 14.7% of the variance respectively) on enzymatic hydrolysis of this grass biomass (Maitan-Alfenas et al., 2015; Vasić et al., 2021). Next, various hydrolysis factors have different influenced on this grass hydrolysis such as incubation temperature (showed 8.1%), pre-treatment time (2.2%) and pH (0.055%). Report for pH and incubation temperature has shown more significant interaction effect (more than 60%) on enzymatic mode of hydrolysis (Chiaramonti and Goumas, 2019). Least influencing individual factor can be shown significant interaction effect on enzymatic mediated hydrolysis process for grasses. Further studies was done on improved hydrolysis process for rye glass at optimal conditions for different stages of Italian grass. Pre-treatment steps can be completed by combination of several pre-treatment techniques and it can improve the susceptibility for enzymatic degradation of grass hydrolysis (Chiaramonti and Goumas, 2019; Vasić et al., 2021).

In this context, enzyme biosynthesis from *Trichoderma reesei* fungal strain is reported and this enzyme is utilized for degradation of waste biomass of lignocellulosic matter that can release huge fermentative sugars. These enzymes are not effective for hemicelluloses and lignin components hydrolysis in lignocellulose matter (de Paula et al., 2019). For enzymatic hydrolysis of lignocellulosic matter, the optimal pH range is 5.0-6.5 and it can result in the best hydrolysis. Normally in pretreatment process of lignocellulosic matter, hydrolysis can result in simple sugar formation and it utilizes a complex process of secreted enzyme from filamentous fungi especially for *Trichoderma* species (dos Santos Castro et al., 2014; de Paula et al., 2019). This fungal species can high levels of cellulases enzymes (like cellobiohydrolases and endoglucanases) with less amount of enzyme with capability to attack the non-cellulosic polysaccharides like hemicelluloses and pectin (Aro, 2016). Efficient enzymatic hydrolysis can occur by systematic pre-treatment tasks for lignocellulosic substrates. And it can be limited by different process parameters like pH, temperature, substrate loads, product recovery plan and also high lignin quantities (Phwan et al., 2018). Further physicochemical properties (like thermal stability and specific activities) of enzymes can influence the hydrolysis rate. Enhanced bioconversion of plant biomass can occur by use of a combination of enzymes (Aro, 2016; Phwan et al., 2018). For enzymatic hydrolysis processes, process optimization can be achieved by application of statistical techniques/ analysis (Sarkar et al., 2012). And researchers have gathered the experimental data from design experiments and then these were utilized for statistical analysis. These analyses of experimental data can help to enhance bioconversion of yield with suitable/ optimal process requirements (Sarkar et al., 2012; Vasić et al., 2021). Enzymatic hydrolysis of several types of lignocellulosic biomass can help to generate a huge amount of fermentable sugars and then its conversion into bioethanol as the final fermentation process. Enzymatic hydrolysis process can find more innovative ways of fermentative sugar formation and it requires more new enzymes of effective nature and some advanced research is going on to get the cost-effective process of enzymatic hydrolysis (Zoghalmi and Paës, 2019). In this context, many enzyme hydrolysis strategies are discussed with implementation of hydrolysis protocol as their monitoring capabilities for different lignocellulosic biomass like wood/ horticultural feedstock and different agricultural residues. These are utilized as potential substrates for efficient way of bioethanol production (Zoghalmi and Paës, 2019; Vasić et al., 2021)

### 3.1.2. Steam and alkali solution

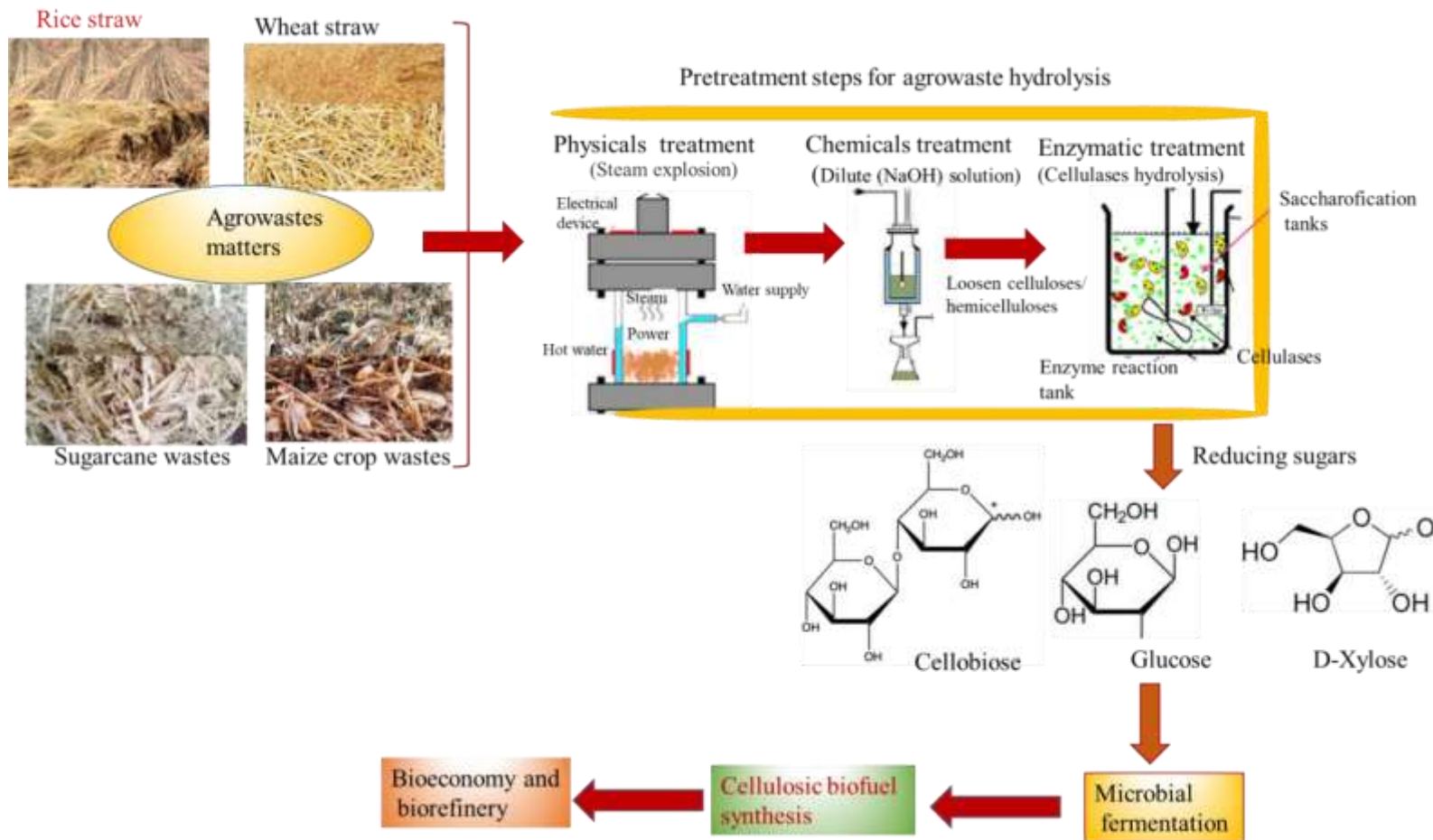
Some researchers have applied 5% (w/v) plant biomass samples in water. These solutions in two flasks were prepared to a working volume of 800 ml. First flask was autoclaved for 15 min at 121°C and the second flask was also autoclaved at the same temperature for thirty min. These flask samples (after heat treatment at two different temperatures) were gone to filtrate and then grass residues were gone to an oven dried task at 60°C of temperature for 16 h (Hassan et al., 2018). Two different conical flasks with two different concentrations of sodium oxide solution (0.5 % w/v and 1% w/v) with 5% w/v feedstock samples were sent to the pre-treatment task. These were kept at a working volume of 600 ml. Two flasks sets (each flask contains 0.5% w/v and 1% w/v NaOH solution) were autoclaved for high temperature at 121°C for 15 min and also 30 min of reaction time (Mihiretu et al., 2019). These combinations of steam and alkali solution pre-treatment have impacted hydrolysis. After heat treatment, suspension can be filtered with keeping for drying of grass residues at 60°C in the oven for 16 h. And similar experiments were also conducted for each of the Italian rye grass hydrolysis with high glucose releases (Mihiretu et al., 2019; Hassan et al., 2018). 1% of NaOH solution has shown highest % mass reduction in Italian ryegrass and ryegrass silage samples, but in steam-pretreated these grass samples were shown lower hydrolysis results at 15 min of autoclaved temperature (121°C) (Pérez-Limiñana et al., 2022).



During the steaming experiment of rye grass silage sample, extended time has caused huge sample weight reduction. Various samples of these grass biomass were compared during the pretreatment process with uses of different concentrations of sodium hydroxide solution. And there is no significant difference in hydrolysis results of rye biomasses while increasing the concentration sodium hydroxide solution (from 0.5 to 1% w/v) at 15 min of hydrolysis reaction time (Park and Kim, 2012). Also these samples showed lower weight in steam-treated samples during the pre-treatment process. Later comparison was done for weight loss for Italian rye growth (high/ greater %) samples and rye grass silage (lower %) during the pre-treatment process (Zabed et al., 2017). Variation in hydrolysis results between two grass biomass and it can occur better at acidic pH for ryegrass silage samples. Switchgrass sample for sodium hydroxide solution was checked for hydrolysis capacities and efficient reduction in lignin, hemicellulose and celluloses is shown by this pretreatment process (Park and Kim, 2012; Zabed et al., 2017). Another waste biomass resource like rice straw hydrolysis was done by optimal approaches of chemical and physical assisted agents/ reagents. After this pretreatment process, saccharification steps were done. These tasks were done with autoclaved ammonia reagent/ solution based pre-treatment that enhanced the liberation/ generation of reducing sugars (233 mg/g substrates) as compared to other approaches/ techniques (Anu et al., 2020). These processes are gone to optimize by response surface methodology (RSM) approaches as statistical optimal technique. It helped to optimize the value of ammonia concentration, substrates concentration and autoclaving time that resulted in enhanced saccharification (more than 1.9 times or more %~ 451 mg/ g substrates) (Gupta et al., 2016).

These values for optimal parameters like 12% for (NH<sub>3</sub>), 5% (rice straw) and 30 min (autoclave time). After the 6 h saccharification process, at temperature of 48°C and 48 h as optimal condition, liberation of reducing sugars was found to 635.4 mg/g substrate as maximum value in hydrolysis process (Gupta et al., 2016; Anu et al., 2020). After these treatments, researchers have applied a few analytical techniques like X-ray diffraction, scanning electron microscopy and also Fourier-transform infrared spectroscopy technique for confirmation of removal of lignin in pretreated biomass. Ammonia assisted pretreatment with enzymatic hydrolysis solution was fermented by *Saccharomyces cerevisiae* strain at temperature of 30°C, pH of 7.0, and speed rate of 150rpm, 20% rice straw hydrolysate has resulted to high concentration of ethanol (24.4 g/L) after 72 hour of fermentation process (Houfani et al., 2020; Anu et al., 2020). RSM technique was applied for optimization of input parameters in enzymatic hydrolysis process of lignocellulosic biomass. These input parameters are enzyme loading value (20-100 U/g), yeast titer (1- 5 times) and temperature (30 to 50°C). These set up parameters were checked for improved bioethanol production yield/ titer (Jugwanth et al., 2020).

A maximum bioethanol titer (4.88 g/L) was claimed at optimal condition under the optimal value of yeast titer (1 time), enzyme loading value (100U/g) and temperature (39°C). Further RSM model has helped to keep optimal enzyme loading value, influenced at high rate in bioethanol production. This has helped to determine the kinetic of specific growth rate (0.15 h<sup>-1</sup>) and also maximum cell biomass concentration X<sub>max</sub>-2.58 g/L (Periyasamy et al., 2024). Next, a modified Gompertz model helped to achieve the maximum bioethanol (P<sub>m</sub>~3.12 g/L) and maximum bioethanol production rate (0.29 g/L/h). These studies are best found with demonstration of sugarcane waste valorization capacity/ potential via insight of biofuel production and process design determination for large scale operation (Jugwanth et al., 2020; Periyasamy et al., 2024). Researchers have done the pretreatment task for pine (*Pinus sylvestris*) and ground lignocellulosic matters (*Miscanthus giganteus*) and they applied the ionic liquid mixture of two different compounds like 1-butyl-3-methylimidazolium methyl sulfate and also 1-butyl-3-methylimidazolium hydrogen sulfate (Hou et al., 2017).



**Figure 1.** Different agrowastes sources utilization for generation of reducing sugars that can ferment for biofuel synthesis

After this solution treatment, a solid portion of cellulose components was recovered subject to enzymatic hydrolysis task. Nearly 90% of glucose and 25 % of hemicellulose was released from original biomass after combined action of ionic liquid solution treatment and also enzymatic hydrolysis task (Brandt et al., 2011). Huge portion of lignin and also hemicellulose component was present in ionic liquid liquor after completion of this combined pretreatment task. Later lignin portion was gone to partially precipitate from liquor upon dilution with water. In ionic liquid liquor, the amount of monomers of hemicellulose was determined and then later, it was converted into a furfural compound with proper examination (Brandt et al., 2011; Hou et al., 2017). Later combination of ionic liquid-water mixture like acetate with 1, 3-dialkylimidazolium ionic liquid was checked with other chloride anions, trifluoromethanesulfonate and methanesulfonate (Gschwend et al., 2018). But lignocellulosic pretreatment was found to be more effective in 1-butylimidazolium hydrogen sulfate solution. This solution has contained methyl sulfate, hydrogen sulfate and methanesulfonate anions and these are more effective for lignocellulosic degradation/ fractionalization with induced cellulose digestibility (Hou et al., 2017; Gschwend et al., 2018). And **figure 1** shows the different waste matters from agriculture sector that is used for reducing sugar generation with cellulosic ethanol promotion.

### 3.1.3. Enzymatic hydrolysis of lignocellulosic biomass

Rye grass silage was used for enzymatic hydrolysis and some experimental conditions were set up to complete hydrolysis. Later it was applied for cut grass samples for hydrolysis. And it was done in a 60 ml labeled syringe (Oamen et al., 2019). Each experiment set up contained 5% (w/v) pretreated rye grass silage in 0.1 M Na-citrate buffer and also 180  $\mu$ l of tetracycline solution. Later it was inoculated with various enzyme mixtures (Zhao et al., 2012). And then each syringe was fitted with rubber tubing and clip for ease of collection of hydrolysates. The syringe was placed in an incubator and then it was operated at various temperatures (30, 50, 70°C) and time (16, 40, and 72 h) of experiment start time and analysis. (Zhao et al., 2012; Oamen et al., 2019). In recent years, a number of research works are done on better enzymatic hydrolysis with good analysis and it was set up at experimental and theoretical models /concepts. During experimental plans, pre-treatment tasks are done on consideration of physiochemical or acid type of reagents and these can be found to be better preferable tasks for different nature of herbaceous feedstocks (Zhang et al., 2021). In this study alkaline pretreatment was found more suitable for lignin-rich wood/ herbaceous feedstock. For such lignocellulosic biomass, hydrothermal and chemical pre-treatment steps can decrease the energy consumption of mechanical refining tasks. In these biomasses, the recalcitrance properties are found different (Wang et al., 2022).

It needs to consider different parameters like substrate and enzyme properties that can help to keep enzyme adsorption and hydrolysis analysis at optimal reaction conditions. Further theoretical models helped to elucidate the mass transfer mechanism at molecular level and provide the cue in reaction mechanism in enzymatic hydrolysis. This model can help to determine the key rate- limiting steps in hydrolysis. Further analysis was done of experimental set up and model studies with indication of accessible cellulose surface area and pore volume (Zhang et al., 2021; Wang et al., 2022). These are the main parameters for limiting hydrolysis reactions. Next limiting parameter is enzyme diffusion and it can occur at the beginning of the reaction. There are little effects of particles with radii smaller than  $5 \times 10^{-3}$ cm. This study can help in deep analysis of link between experimental and model designs studies via helping conversion process optimization task (Selvakumar et al., 2022; Zhang et al., 2021).

Hydrothermal pretreatment was performed at lower temperature (value~170°C) and later it was combined with ultrafine grinding tasks. This effort has achieved high glucose yield (i.e., 80.4%) at low

enzyme loads (i.e., 5 filter paper unit~ FPU/g substrate) as favorable concentration (Zhang et al., 2018). During the enzymatic hydrolysis process, relative significant structural parameters are reported like specific surface area (SSA) > pore volume > cellulose crystallinity (CrI) /cellulose > particle size. But, pore volume and specific surface area has shown the logarithmic correlation for final enzymatic hydrolysis yield (Zhang et al., 2018; Selvakumar et al., 2022).

#### 4. Microbial cells and their enzymes

Microbial cellulases have exhibited more potential application in different industries and these industries are textile, laundry, biofuels production, food and feed, paper/ pulp, brewing industries and agriculture sector. Cellulases are utilized as potential candidates for research for academic and industries sectors and it is due to the more complex nature of enzymes and also immense potential applications (Kuhad et al., 2011). Recent years, significant attention on cellulases is shown by researcher groups for current knowledge on its activity and its reactions. Next, production of cellulases is also shown many challenges with current knowledge updates in research. These can improve the reaction process with better economics for various industries applications (Bhardwaj et al., 2021). In this context, various genera of microbes like fungi and bacteria /actinomycetes are discussed. And in categories of soft rot fungi are discussed with few examples like species of *Aspergillus* (like *A. oryzae*, *A. niger* and *A. nidulans*) are reported with *Fusarium* species (like *F. oxysporum*, and *A. solani*). *Trichoderma* species (like *T. harzianum*, *A. longibrachiatum*, *T. atroviride*, and *T. reesei*) is also reported to produce cellulases (Crognale et al., 2019; Bischof et al., 2016).

Next, same fungal category, *Humicola* species (like *H. grisea* and *insolens*), *Penicillium* species (like *P. occitanis*, *P. decumbans*, *P. myces*, *P. ifumigosum* and *P. brasilianum*), *Melanocarpus albomyces*, *Chaetomium* species (like *C. cellulolyticum* and *C. thermophilum*), *Neurospora crassa* and *Thermoascus aurantiacus* (Kuhad et al., 2011; Bhardwaj et al., 2021). Few more species of the same fungal category are *Mucor circinelloides*, *Paecilomyces* (*P. inflatus* and *P. echinulatum*) are known to produce to cellulases enzymes with strong cellulolytic abilities. Next fungi for strong cellulases enzyme producer capacity is brown rot fungi and few species like *Coniophora puteana*, *Poria placenta*, *Lenzites trabeum*, *Fomitopsis species* and *Tyromyces palustris* (Maeda et al., 2013; Crognale et al., 2019). White rot fungi are also best producer of cellulases enzyme like *Phanerochaete chrysosporium*, *Trametes versicolor*, *Sporotrichum thermophile*, *Agaricus arvensis*, *Pleurotus ostreatus* and *Phlebia gigantea* species (Maeda et al., 2013; Bischof et al., 2016). In this context, various species aerobic bacteria are reported like *Acinetobacter* species (*A. aminitratus* and *A. junii*), *Bacillus* species (*B. subtilis*, *B. pumilus*, *B. circulans*, *B. flexus*, *B. licheniformis*, and *B. amyloliquefaciens*) (Sharma et al., 2016), *Acidothermus cellulolyticus*, *Bacteroides species*, *Microbispora bispora*, *Geobacillus species*, *Salinivibrio species*, *Rhodothermus marinus*, *Cellulomonas biazotea*, *Cellvibrio gilvus*, *Eubacterium cellulosolvens*, *Pseudomonas cellulose*, and *Paenibacillus cudlanolyticus* (Kuhad et al., 2011; Sharma et al., 2016). These bacterial species are very good producers of celluloses. And anaerobic bacteria species are also good producer of cellulases enzymes and these are such as *Clostridium* species (like *C. thermocellum*, *C. acetobutylium*, *C. papyrosolvens* and *C. cellulolyticum*), *Acetivibrio cellulolyticus*, *Fibrobacter succinogenes*, and *Ruminococcus albus*. Next, *Streptomyces drozdowiczii*, *S. lividans*, *Thermomonospora fusca*, *T. curvata*, *Cellulomonas fimi*, *C. bioazotea*, *C. uda*, and *Cellulomonas fimi* is good example of *Actinomycetes* species with good cellulases enzyme producer capabilities (Islam and Roy, 2018).

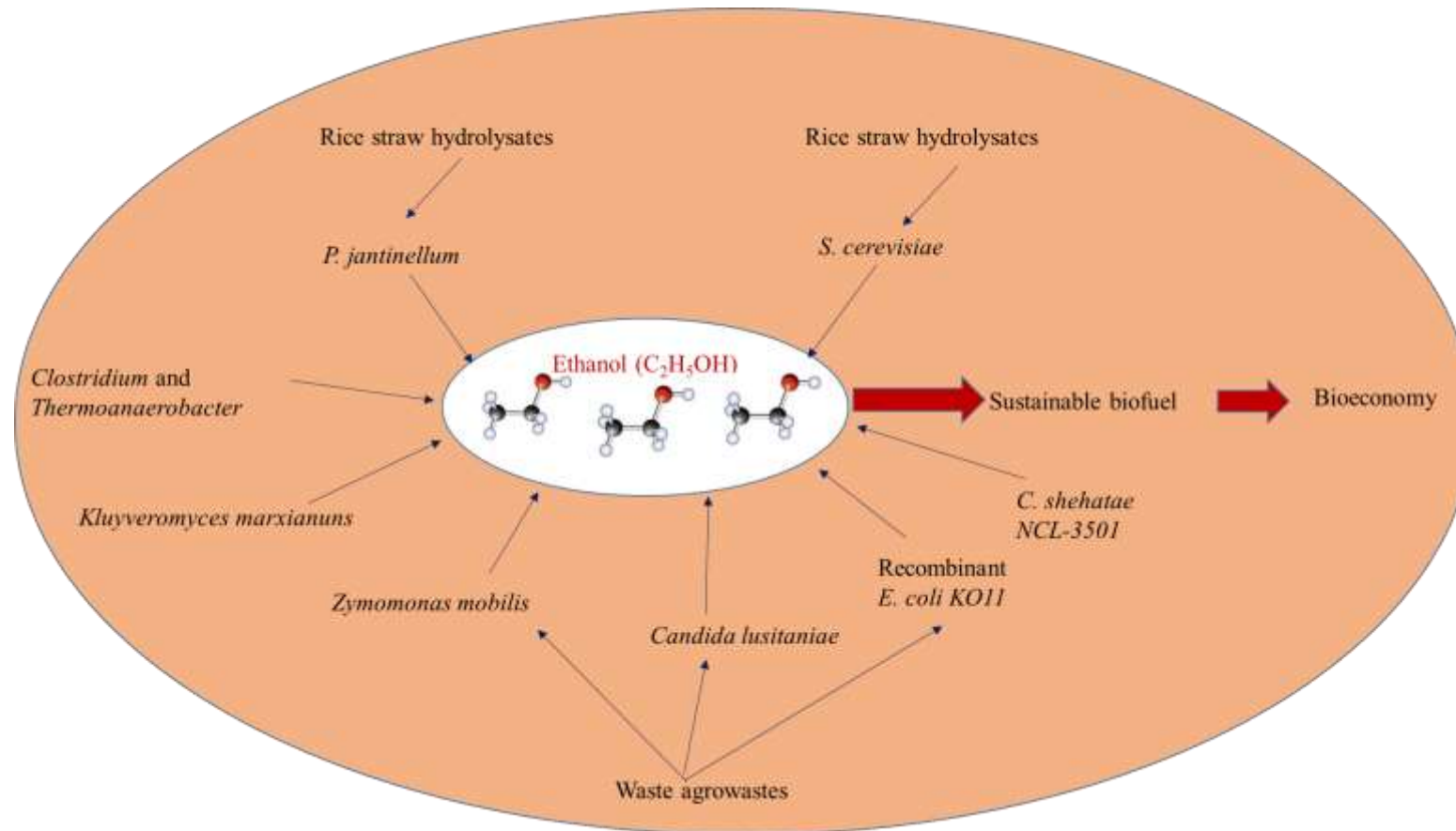
From these microbial systems, various efficiency of cellulases production is found and these enzyme production are inducible enzymes with huge diversity of microbial systems like several fungi and bacterial species and produced during microbial growth stages on utilization of cellulosic material.

These bacterial species are mesophilic, thermophilic and aerobic or anaerobic nature (Sheldon et al., 2011). Fungal originated cellulases are found simpler form as compared to bacterial cellulases system like cellulosomes. Further studies was done for fungal cells system derived cellulases and is found to contain two separate domain such as a catalytic domain (CD) and cellulose binding modules (CBM) (Raj and Krishnan, 2018). These domains are joined by a short polylinker regions to the catalytic domain at the N-terminal. Next analysis was done on CBM structures and this structure is consisted of 35 amino acids with containing of linker regions like rich in threonine and serine amino acid.

Researchers have discussed the main difference between cellulosomes and also free cellulose enzyme. These components can be part of cellulosomes-cohesion and it is consisted of scaffolding and dockerin parts in this enzyme (Sheldon et al., 2011; Raj and Krishnan, 2018). **Figure 2** shows the microbial system capability for reducing sugars generation with ethanol synthesis. The Free cellulases is consisted of cellulose bind domains (CBMs) and it can replace by a dockerin in cellulosomal complex. Now a single scaffolding-born CBM can direct the entire cellulosomes complex with best capacity to cellulosic biomass hydrolysis. Further studies was done on cellulases enzyme with its family of three groups of enzyme like endo-(1, 4)- $\beta$ -D-glucanase,  $\beta$ glucosidases and exo-(1, 4)- $\beta$ -D-glucanase (Cruys-Bagger et al., 2013). Action of exo-(1, 4)- $\beta$ -Dglucanase is also reported to find on the ends of cellulose chains and then it can release many units of  $\beta$ -cellobiose as end product. Enzyme endoglucanase (EG) can randomly attack the internal O-glycosidic bonds that can release glucan chains of different carbon chains lengths (Amore et al., 2017). And enzyme  $\beta$ -glucosidases can act in a specific manner on the  $\beta$ -cellobiose disaccharides that can produce glucose. Different mechanisms of cellulose degradation are also reported by aerobic bacteria with similar fashion of aerobic fungal species hydrolysis.

Cellulosomes can be located on the cell surface and it can mediate via adherence of anaerobic cellulolytic bacterial species to the substrate (CruysBagger et al., 2013; Amore et al., 2017). This can undergo a supramolecular reorganization with redistribution of cellulosomal subunits with interaction with the different target substrates. In the last few decades, researchers have tried to improve the cellular enzyme production capability at commercial scale and these enzyme production and activity considerations were taken at industrial and academic level research tasks (Park et al., 2010). Number studies were done on basic and applied concepts of cellulolytic enzymes with demonstration of their potential application for biological processes in various industries like food and animal feeds, brewing/ wine producing, biomass refining, pulp/ paper, textile, agriculture and laundry industry. Number of reviews have been done on cellulases applications at industrial operations (Hussain et al., 2023). Cellulases are applied alone or in combination with xylanases enzymes and these can provide benefits for delinking of different types of biomass waste (including paper processing wastes). People have applied cellulases and hemicellulases enzymes and it can help to release color components from waste plant biomass via achieving partial hydrolysis of carbohydrate molecules (Park et al., 2010; Hussain et al., 2023).

Further people have found the utility of cellulose enzyme for dewatering and delinking of various waste biomass (including pulp) and it has resulted in the peeling of the individual fibrils and bundles for biomass that have high affinity for the surrounding water and also ink particles (Fouda et al., 2024). This enzymatic delinking task application showed many advantages like reduced or complete elimination of alkali solution usages with improved fiber brightness, enhanced strength properties and pulp freeness. Further this task has been done to cleanliness and reduce fine particles in the pulp process (Hussain et al., 2023; Fouda et al., 2024). Normally the delinking process can be done by using enzymes at acid pH and it can prevent the alkaline yellowing with easy completion of this task (delinking of pulp). Further changes from this enzyme combination treatment are ink particle size distribution changes and also reduced environmental pollution.



**Figure 2.** Efficient microbial system for waste biomass utilization for ethanol production in microbial fermentation processes

Enzyme delinking tasks can lower the need of deinking chemicals and also reduce the adverse impact to the environment and also paper industries contaminations (Cruys-Bagger et al., 2013; Fouda et al., 2024).

## 5. Biofuel production

Researchers have applied the microwave pyrolysis (MP) and this technique in context to biofuels production is a promising technique with capacity to valorize the agricultural wastes into biofuels like biochar, bio-oils and syngas. This researcher put efforts to minimize/ fill the research gap, the state-of-the-art MP technique was utilized to convert the agricultural wastes (AWs) into different nature of value-added biofuels and these conversions were dependent on feedstock compositions, catalytic applications, reaction mechanism, new reactor designs and operating conditions (Ge et al., 2021). Discussion was done on techno-economic and environmental impacts with key implications for future development for biomass hydrolysis and then its conversion into biofuel production. MWassisted valorization of AWs can be found to be economically viable and also eco-friendly techniques. This can apply to high availability of AWs sources with good scalable process possibility (Naik et al., 2021). This technique can further utilize a big potential for continuous operation with a positive energy ratio for thermochemical processes. During the continuous MP process, microwave (MW) heating distribution and reactor design have not been completely explored and it can be due to limited understanding of MW radiation propagation pattern, varying feedstock composition and also material handling tasks (Naik et al., 2021; Ge et al., 2021).

In recent years, utilization of AW with uses of this technique can provide a new route for biofuel conversion with this feedstock. This technique and AWs can provide several environmental advantages like improved biomass utilization, lower sulfur emission and enhanced carbon sequestration. Next, toxicity of bio-oils can be minimized by addition of metal oxide catalysts (like MgO, CuO and CaO) (Chandel et al., 2018). This effort can lessen its content of polycyclic aromatic hydrocarbons (PAHs). Effort for optimal process of continuous MP was achieved by coupling shaftless auger and multiples magnetron. This has improved the biofuel quality with maintaining the uniformity of the MW heating system/ process. In continuous MP process, AWs conversion into sustainable biofuel production was done with keeping low carbon footprint and alternative energy generation routes. But it requires appropriate catalyst, effective condenser and self-purging conditions selection (Chandel et al., 2018; Ge et al., 2021). And **table 2** shows various sources of ethanol productions from different microbial system.

**Table 2.** Different concentration / yield of cellulosic ethanol from different waste biomass and microbial system

Waste biomass	Microbial system and processes	Cellulosic ethanol yield/ concentration	References
Ethanol production from Jerusalem artichoke	Commercial inulinase (17 U g <sup>-1</sup> ) and a juice heating at 52.5 °C for 60 min. Reports for fermentation at 30 °C by <i>S. cerevisiae</i> is shown	Ethanol yields of 0.458 and 0.454 g g <sup>-1</sup> from autumn juice and winter ones.	(Matías et al., 2015)

Waste rice crops residue and raw broken rice	Recombinant enzymes (10% dosage ) sufficiently hydrolysed raw broken rice	During the fermentation of 20% dw/v broken rice starch is yielded high ethanol titers (93% of the theoretical ethanol yield after 96 h.	(Myburgh et al., 2019)
Vine shoots waste for ethanol	The highest cellulose and hemicellulose content (almost 65%) after auto- hydrolysis pretreatment treatment	The maximum bioethanol production (6%) reported by autohydrolysis (at 165 °C), chlorite delignification and SSF process at 37 °C , solid loading~10% /72 h	(Senila et al., 2020a and b)
China's crop (maize, rice, / wheat) residue increased from in 2007 (725.5 Mt) to 2016 (897.1 Mt)	<i>S. cerevisiae</i> utilization with recent advances in pretreatment, hydrolysis and fermentation of wheat straw is reported with better sugar yield (74–99.6%)	Bioethanol production potential from field residue (124.3 Mt) in total. This ethanol yield is ranging from 65% to 99% of theoretical value.	(Fang et al., 2019)
Corn cob as waste biomass	Xylose isomerase (XI) found to increase the fermentative capacity of two robust industrial <i>S. cerevisiae</i> strains in synthetic media at 30 °C and 40°C	Best cofactor equilibrium between the XDH (xylose reductase) and furan detoxifying enzymes, has increased the ethanol yield (by more than 38%)	(Cunha et al., 2019)
Bioethanol production from rice straw and hardwood (oak) hydrolysis	Recently engineered isomerasebased xylose utilizing strain ( <i>S. cerevisiae</i> ) is reported in mixed sugars (70 g/L of glucose and 40 g/L of xylose)	SXA-R2P-E strain has produced better ethanol (50 g/L) with an yield (0.43 g ethanol/g sugars) at 72 h	(Ko et al., 2019)
Agricultural and forestry wastes to bioethanol	Recombinant diploid <i>S. cerevisiae</i> strain with E2 xylose isomerase is reported with better co- fermentation of glucose (162 g.L <sup>-1</sup> ) and xylose ( 95 g.L <sup>-1</sup> )	Better ethanol titer (120.6 g L <sup>-1</sup> ) in 23 h with higher sugar conversion (99%), ethanol yield (0.47 g g <sup>-1</sup> ), / productivity (5.26 g. L <sup>-1</sup> ·h <sup>-1</sup> ).	Liu et al., 2018
Bioethanol from wheat straw	Optimal co-culturing of <i>B. licheniformis</i> and <i>S. cerevisiae</i> with simultaneous saccharification and fermentation is best bioethanol production (~ 14.70 g/l).	Co-culturing parameters like, time, pH, substrate concentrations and nitrogen source concentrations has given bioethanol better (a net yield of ~ 4.11 g/l)	(Sharma et al., 2021)
Pretreatment of rice straw is reported with ethanol	Autoclaved ammonia pretreatment has enhanced the liberation of reducing sugars ( 233.76 ± 1.23 mg/g substrate)	Fermentation of enzymatic hydrolysate (20%) resulted better ethanol (24.37 g/L) using <i>S. cerevisiae</i>	Anu et al., 2018



In the last few decades, waste generation trends from agricultural and horticultural crops cultivation and processing tasks are generated and found as unwanted or worthless matter, without further use, as defective products. These wastes look as identical raw materials and inaccurate without any usefulness (Kalkanis et al., 2022). But it requires best management tasks that can be transformed into value-added products like fuel/ biofuel. Waste management becomes a more critical challenge for the municipal and agricultural sector to deal with the different nature of waste like radioactive or explosive form (Salmenperä et al., 2021). At municipal level effort is done for solid waste management with systematic and proper collection and later these are gone to sorting and separating tasks for very important waste like metals, glass or plastics or other valuable products (Salmenperä et al., 2021; Kalkanis et al., 2022). These can be utilized with recovery and then recycled into new products. These efforts can be achieved via lowering the production cost and also environmental impact (negative form). Number of case studies have been done successfully in waste management tasks in various countries at worldwide level and these have shown as showcases for optimal waste transformation to wealth products like fuels (Rybicka et al., 2016).

Applied waste management is explored in a specified way with suitable action and adaptation for handling of the plant originated wastes for the agriculture sector. During managerial level actions need to be applied for potential resources or commodities that can result in economic, social or environmental level value-added products recovery (D'Adamo et al., 2022). In the transformation of plant waste context, effort was made for identification and presentation of value-added products/ resources in waste streams of the agriculture sector. These can be focussed at their monetary values, and it applied the literature for better information or data for waste product/ matters. Further it can be taken from raw material stock markets (Rybicka et al., 2016; D'Adamo et al., 2022). Efforts were done for MSW, non-hazardous commercial and industrial waste with agricultural wastes. These were utilized with application of better methodology with suitable identification and analysis approaches for various cities, communities and countries (Chaliki, et al., 2016).

### **5.1. Waste to energy**

Adaptation of plant waste management policies can help to push toward circular bioeconomy and waste to wealth/ value-added products conversion effort. Few alternative options in plant waste management effort are discussed for energy/ fuel recovery/ generation from plant waste utilization. Energy recovery from plant wastes/ residues can be done by application of thermo-chemical and also biological processes (Francés et al., 2013). In biological process techniques, anaerobic digestion and fermentation can be applied as green approaches in an eco-friendly way. In thermochemical processes, pyrolysis, combustion and gasification is found to apply for energy recovery/ generation efforts. These conversion/ transformations are more efficient for recovery of energy from waste with the concept of waste to energy (WTE) (Francés et al., 2013; Chaliki, et al., 2016).

In thermal treatment tasks / technologies, combustion is very common and frequently applied for waste mitigation tasks. Another recovery for variable wastes is recycling technique and it helps in energy recovery tasks with proven or effective ones. During the waste to energy plant/ facilities, optimal operation can enhance the recycling rate with its mitigation (Psomopoulos, 2014). This approach is applied for waste materials that come from municipal solid waste (MSW) and these are plastic and paper. In this technique, these showed specific potential for transformation with reaching their maximum value into combustible materials (Chen, 2013). Waste to energy facilities can help in huge reduction (by 90%) and weight (75%) of MSWs with the additional benefit of avoiding land coverage/ occupation in sanitary landfills (Chen, 2013; Psomopoulos, 2014). This facility can provide electricity and heat energy recovery and this form of energy from wastes can be considered as a renewable energy

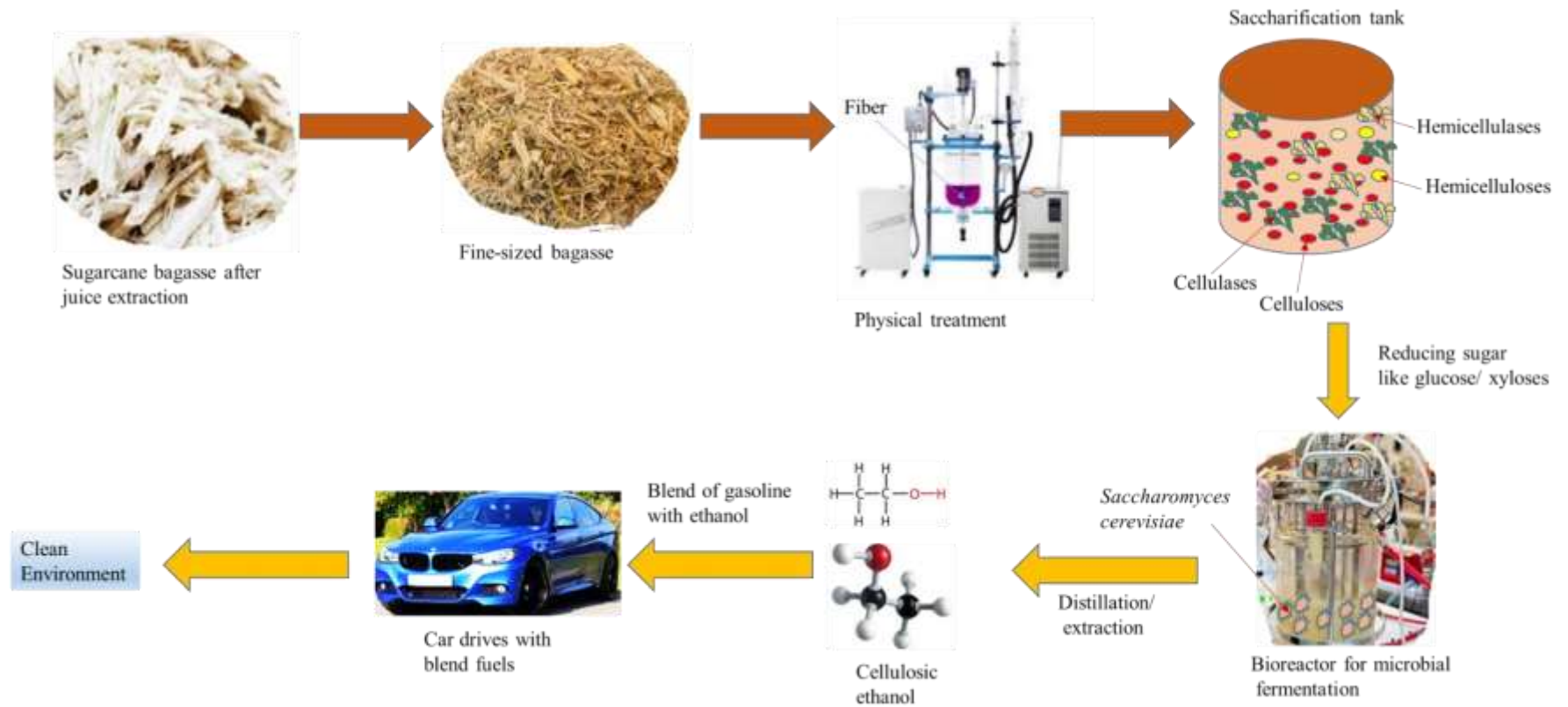
source as mentioned by the US-DOE (department of energy) agency. These energies come from plant waste biomass (i.e., huge % of organic matters) and lesser quantities of fossil derived materials (Agll et al., 2014). In recent period, renewable energy development can find public agenda or preference compared the other energies form and it help to protect human health and environment. This has discussed in Europe 2020 plan/ strategy with introduction of specific goals in different sectors. This transformation efforts can reduced the 20% or more% of GHG emission while compared to 1990 level (Chen, 2013; Agll et al., 2014). In this context, 20% share of energy production can achieved by renewable resources in EU with aims to further improve in energy efficiency among various member states. In this discussion, it considered to power sector especially for greenhouse gases (GHGs) emission reduction by 21% or more compared to 2005 level (Psomopoulos, 2014; Agll et al., 2014).

Many case studies are done for MSW treatment effort with leading to simultaneous way of renewable energy production. It can be done with better WTE facilities with keep objective to top ranks in waste management methods. These treatments need to satisfy EU environmental and energy directives. Efforts are done for energy supply with high security and renewable sources. It needs intermittent energy production from several renewable sources due to its stochastic nature and also lower capacity factor (70%) with high energy recovery (Chaliki, et al., 2016; D'Adamo et al., 2022). Energy from MSW source can be reached up to 9 to 10 MJ/ kg but refuse derived fuel (RDF)/ solid recovered fuel (SRF) can be reached to 16 MJ/ kg. This was compared to conventional fuel and lignite, these energy content can be reached to higher (Salmenperä et al., 2021; D'Adamo et al., 2022).

## 5.2. Bioethanol

In current period, lot of efforts are done for mitigation of agricultural residue like corn and sugarcane crops residues/ waste matters and these residues utility can help to generate demand of cellulosic nature bioethanol and it can provide option to fulfill the fuel/ energy demand at global level (Sarkar et al., 2012). Earlier people put efforts for their crops grains for bioethanol but this trends/ mode of ethanol is found to reduce/ minimize due to rise of hungry issue to many nations. Lignocellulosic matters like agricultural waste are found to attractive feedstock for bioethanol production (Panda and Maiti, 2024). Further these wastes/ matters are cost-effective, abundance and renewable in nature. Bioethanol from agricultural waste matter can be achieved by application of innovative and promising technology. This process has several challenges and limitation like handing, biomass transport and also application of efficient and effective pre-treatment process. These can help for delignification process to various lignocellulosic biomass (Sarkar et al., 2012; Panda and Maiti, 2024). And **Figure 3** shows ethanol production from sugarcane bagasse hydrolysate.

Reports for systematic and proper pretreatment are discussed that can help in increasing the concentration of the fermentable and reducing nature of sugars. This sugar release is only possible after enzymatic saccharification via improving overall efficiency of agricultural residues hydrolysis (Jugwanth et al., 2020). Next effort was done for conversion of glucose and xylose sugars into ethanol and people applied new and innovative fermentation technologies to enhance the yield and co-digestion of mixed substrates. This type of efforts can keep to whole fermentation process cost-effective with minimal byproducts generation. Till period efforts was put to utilize the available technologies that can help to produce the bioethanol from agricultural wastes/residues and people have discussed the commercial viable ethanol production techniques (Jugwanth et al., 2020;; Panda and Maiti, 2024).



**Figure 3.** Cellulosic ethanol synthesis from microbial fermentation that used sugarcane bagasse residue with sustainable fuels.

This can only utilize the ideal or modified microbial system with capability to consume or utilize a broad range of substrates together or sequential manners and this arrangement can enhance the ethanol yield and productivity. Further, microbial systems have to withstand the high temperature and concentration of ethanol (Lamichhane et al., 2021). Next these microbes need to keep the tolerance capability for several inhibitors in hydrolysates and these are generated during the hydrolysis process with high cellulolytic activity. In this context, the report showed engineered or modified microbial cells with capacity to achieve the complete utilization of sugars in hydrolysate with better bioethanol production benefits (Sarkar et al., 2012; Lamichhane et al., 2021).

### 5.2.1. Microbial fermentation and production process

In effort to bioethanol production, people have been employed in microbial fermentation for utilizing lignocellulosic hydrolysates. And simultaneous saccharification and fermentation (SSF) and also separate hydrolysis and fermentation (SHF) are also discussed for cellulosic ethanol. Normally researchers are discussing the traditional or conventional approach like SHF and it was applied for bioethanol but this approach was not better than the SSF process. This is due to the SSF process with capacity to superior performance for ethanol production in terms of improved yield (Méndez et al., 2021). The SSF process can exhibit the removal of end product inhibition with elimination of the need for a separate reactor. SSF is a cost-effective technique but it has shown the difference in optimal temperature conditions of enzymatic hydrolysis and also fermentation as some limitations (Nadeem et al., 2015).

SSF technique can provide higher ethanol yield coefficients and it is due to partial conversion of xylose to xylitol. Next, SSF or SHF process can applied for bioethanol production but alternative to these processes, consolidated bioprocessing (CBP), simultaneous saccharification and co-fermentation (SSCF) can be also applied for bioethanol production (Nadeem et al., 2015; Méndez et al., 2021). In CBP process, researchers put effort for cellulose enzyme production, biomass hydrolysis and also bioethanol production during microbial fermentation and these are carried out in a single bioreactor and it is also know direct microbial conversion (DMC) technique (van Zyl et al., 2007; Maleki et al., 2021). People applied the mono or co-culture of microbial cell systems that can be applied to ferment the cellulose component into ethanol products (Shrivastava et al., 2014; Nadeem et al., 2015). In this effort, CBP technique is found effective with no requirement of capital investment for enzyme purchasing or its production. And in this context, some bacterial system like *Clostridium thermocellum* and some fungi like *Neurospora crassa*, *Paecilomyces species* and *Fusarium oxysporum* are found to effective for bioethanol production with cellulose enzyme activity (Svetlana et al., 2012; Maleki et al., 2021).

CBP technique is not found to be an effective and efficient process due to poor ethanol yield and also long fermentation period of time duration (3 to 12 days) (Maleki et al., 2021; Sakwa et al., 2018). In the SSCF process, microbial cells systems with co-fermentation capability are applied and they need to be more compatible at operating pH and temperature. In this process, a combination of *Candida shehatae* and *Saccharomyces cerevisiae* strains are found to be suitable for bioethanol production (Sakwa et al., 2018). Next, sequential fermentation with two different microbial systems with two different time's period of fermentation are found as better options. These can achieve the better utilization of sugars with help of *S. cerevisiae* in the first phase of hexose fermentation process and *C. shehatae* utilization is found in the second phase of fermentation for pentose sugar fermentation but yield may not be gained high. Among many microbial strains with bioethanol producer capacity, yeast and bacteria are known that use hexoses. These capacities are found in *S. cerevisiae* and *Z. mobilis* strains that can produce the ethanol (Koppram et al., 2013).

*S. cerevisiae* is unable to utilize the five carbon sugars like xylose in biomass hydrolysates. Some wild/native microbial strain like *Candida* and *Pichia* species can be applied in place of *S. cerevisiae* strain that can effectively consume the xylose sugar/ other sugars for bioethanol synthesis (Koppram et al., 2013; Sakwa et al., 2018). But these strains showed at least fivefold than *S. cerevisiae* strain for bioethanol yield/ titer. Some studies were done on genetically modified microbial strain like *P. stipitis* BCC15191, *P. stipitis* NRRL Y-7124, *C. shehatae* NCL-3501 and recombinant *E. coli* KO11 with *S. cerevisiae* ATCC26603. These yeast strains are effective for bioethanol production (Gong et al., 2023). Next strict anaerobic hemophilic bacteria like *Clostridium* and *Thermoanaerobacter* species were also modified that showed the best capacity at high temperature stability during ethanol production of microbial fermentation process. Further, studies were done on some thermo-tolerant microbial strains like *K. marxianus*, *Z. mobilis* and *Candida lusitanae* that are applied for bioethanol production (Zhang et al., 2018).

Several efforts on reduction of cost are done for cellulose enzymes with optimization of enzymatic hydrolysis process. Fermentation configuration case studies were done with considering the challenges of xylose and glucose co-fermentation process and also uses of recombinant microbial strain for bioethanol production (Zhang et al., 2018; Gong et al., 2023). Researchers have solved the technology bottlenecks of the transformation / conversion process with utilization of novel scientific and efficient technologies (Koppram et al., 2013). These efforts can be found to improve the bioethanol production process from agricultural wastes hydrolysates with the development of optimal processes in future. In this context, a novel strategy was applied with the use of cyclic shifting of temperature in the SSF process during bioethanol production (Sharma et al., 2013). This has used rice straw hydrolysates and in-situ cellulose production was done with carrying of saccharification and fermentation process. This process has used the two microbial strains like *P. jantinelum* and *S. cerevisiae* and these mixed cultures process has gained 14.9 g/L of ethanol titer (Singhania et al., 2021).

This bioprocess has a base followed by acid pretreatment task for rice straw and then it employs the cycling shifting of temperature strategy (i.e., 30°C for 2h and 40°C for 2h setup). The holding time was further tuned to enhance the productivity at 30°C (for 1.7h) - 40°C (for 2h) (Yoon et al., 2019). This arrangement has changed the bioethanol titer to 15.9 g/L. And there is also a change in productivity to 2.8 to 5.1 fold increment of bioethanol compared to known approaches. From these published works, SSF process at mutually optimal temperature and prolonged prehydrolysis can be applied to follow the microbial fermentation for bioethanol production from waste biomass hydrolysates (Yoon et al., 2019; Singhania et al., 2021). Next study of application of cyclic shifting of temperature strategy/ plan can again explore the great potential in yield and efficiency enhancement task with keeping of sustainable bioethanol production from waste lignocellulosic biomass hydrolysates (Sharma et al., 2019; Singhania et al., 2021).

## 6. Impact of bioethanol blends combustion to environment

Reports are shown blending a mixture of bioethanol and gasoline and then it was analyzed under different combustion conditions like fully premixed combustion that eliminate the physical effect of fuels. During studies, fuel was injected in direct mode into the cylinder and then it was investigated for physical properties of blended fuel in terms of particulate matter formation from this blended fuel (Sakai and Rothamer, 2019). And the engine was operated at a fixed load with phasing and equivalence ratio. An end of injection time variation was done. From this blended fuel results, it was found that increased contents of ethanol in gasoline fuel can lead to decrease in engine-out particulate matters. Next it also showed significant changes in fuel properties (Jin et al., 2017). Particulate matters from engine operation are shown as output history and till now it was not taken into account for implication into research and

real world applications. Most studies were done on various gasoline-ethanol blend mixtures with particulate matter formation research/ investigation and research in engine operations. Low ethanol blend % (less than 20%) are applied with various in different engine operations with particulate matter (PM) formation analysis. Further studies on little or no sensitivity to ethanol contents (10% by volume) are done at engine operating conditions and it has played a critical role in engine PM emission impacts at low blend levels (Jin et al., 2017; Sakai and Rothamer, 2019). No change in PM formation for ethanol level (up to 30%) with large reduction for E85 (means ethanol ~5183%+gasoline~17 to 49%) with consistent decrease in PM with different ethanol % (Szybist et al., 2011). Some researchers for E50 and E85 claimed for more particulate matter formation than neat gasoline based engine operation conditions. Some studies discussed the role of engine operation parameters like fuel vaporization, wetting surfaces, and air-fuel mixture preparation that decide the PM formation rate during combustion (Szybist et al., 2011; Sakai and Rothamer, 2019).

Various ethanol-gasoline blend effects were checked with analysis of hazardous air pollutants (HAPs) emissions that come from wall-guided injection passenger vehicles. Later, the fuel economy at regulated and unregulated gaseous emission was checked with evaluation on a chassis dynamometer that used the federal test procedure (FTP-75). In this context, five fuels combine different ethanol % with gasoline and these are shown as E0, E10, E30 and E50/ E85 (Szybist et al., 2011; Plácido et al., 2024). These blends are prepared by adding ethanol into commercial gasoline on a volumetric basis. Later these were gone for analysis of each blend fuel impact with their specification. In this study, the engine control scheme of fuel injection quantity was checked for various ethanol blends and later these were adjusted to optimal condition of various parameters: line engine start capability, emission performance and vehicle drivability (Plácido et al., 2024). Studies were done on engine efficiency (FE) of the E85 fueled vehicle and this fuel has shown to better decrease (29% or more %) in HAPs emission compared to gasoline fuel, due to the low energy contents of ethanol. Blended ethanol with gasoline mixture showed a decrease in high rate of particulate matter (Sakai and Rothamer, 2022). And it is known for ethanol fuels without any aromatic compounds and also lesser carbon content compared to gasoline. During these studies, nano-scale particle emission is found less % in blended mixture than 30% ethanol during its combustion in vehicle tests (Sakai and Rothamer, 2022; Plácido et al., 2024). Carbonyl compounds emission is reported due to partial or incomplete oxidation of ethanol. Sometimes it can be sharply increased with reduced emission of volatile organic compounds (VOCs). It is due to formation of medium and high-ethanol formulations with lower proportion of aromatic compounds in such blended fuels (Su et al., 2022).

In another study, people have investigated the fuels nature including baseline emission of certified gasoline. And a blend of 20% (by vol.) ethanol with gasoline (E20%) and also a blend of 85% (vol.) ethanol with gasoline (E85). These fuels are gone for invested operating strategies and it has reflected the versatility of emerging cam-based variable valve actuation technology (Yadav et al., 2021). This technology is capable of unthroted operation with late/ early intake valve close (LIVC/ EIVC). Further particle emission characterization done with particle number and size distribution as measured criteria. For this study, scanning mobility particles was used with filter smoke number (FSN) (Easter et al., 2020). Particle emission for the part fuel injection (PFI) system was shown to be very low quantity and it compared for all the fuels and breathing conditions. Next study was done with a direction injection system with the use of gasoline and E20 fuels system and then particle number emission was increased by one to two orders /fold as compared to PFI fueling system (Easter et al., 2020; Yadav et al., 2021; Srivastava, and Srivastava, 2010). This change in emission can be dependent on the fuel injection time period. Later DI fueling system was checked with gasoline and E20 fuels and then particle number emission was found to be low while compared to PFI fueling system. Uses of E85 fuel are shown with better efficiency and power advantages of DI (direct injection) fueling systems. This study can provide

better inputs in emission study and then it showed without huge particle emission compared to gasoline and E20 fuels (Easter et al., 2020; Plácido et al., 2024).

## 7. Conclusion

This review is emphasized with different types of agricultural and horticultural wastes/ residues and these waste transformations are discussed with efficient approaches of hydrolysis and fermentation. During these wastes utilization with impacts to environmental impact, these studies can provide valuable information of waste mitigation with generation of value-added like bioethanol. This study has focused on enzymatic, chemical and physical approaches of pre-treatment processes with microbial mediation of bioethanol generation. This study has explored different microbial systems that showed the best potential of sustainable mode of fuel generation with systematic approaches of waste mitigation. This can help in clean environment maintenance with a sustainable fuel surplus option for world energy demand. Different microbial systems and also fermentation/ hydrolysis processes have impacted bioethanol yield and productivity with the impact of engineered strains contribution. This study has emphasized cellulose enzyme efficiency in waste biomass hydrolysis with reduced toxic compound formation in biomass hydrolysates. Further this studies has shown the combustion impact of blended fuels like ethanol and gasoline. This has shown in minimization of toxic particulate matters and also toxic gas emission.

## 8. Abbreviations

**AFCR:** Available field crop residues; **AWs:** Agricultural wastes; **CaO:** Calcium oxide; **CBM:** Cellulose binding modules; **CBP:** Consolidated bioprocessing; **CD:** Catalytic domain; **CrI:** Crystallinity; **CSC:** Central south China; **CuO:** Copper oxide; **DI:** Direct injection; **DMC:** Direct microbial conversion; **DNA:** Deoxynucleic acid; **EG:** Endoglucanase; **EIVC:** Early intake valve close; **FE:** Engine efficiency; **FPU:** Filter paper unit; **FSN:** Filter smoke number; **FTP:** Federal test procedure; **GHGs:** Greenhouse gases; **HAPs:** Hazardous air pollutants; **LIVC:** Late intake valve close; **MG:** Metric gallon; **MgO:** Magnesium oxide; **MP:** Microwave pyrolysis; **MSW:** municipal solid waste; **MT:** Metric tons; **MW:** Microwave; **NaOH:** Sodium hydroxide; **NBS:** National Bureau of Statistics; **NEC:** Northeast China; **NH<sub>3</sub>:** Ammonia; **PAHs:** Polycyclic aromatic hydrocarbons; **PFI:** Part fuel injection; **PM:** Particulate matter; **RDF:** refuse derived fuel; **RSM:** Response surface methodology; **SHF:** Separate hydrolysis and fermentation; **SRF:** Solid recovered fuel; **SSA:** Specific surface area; **SSCF:** Simultaneous saccharification and co-fermentation; **SSF:** Saccharification and fermentation; **SWC:** Southwest China; **US-DOE:** U.S. Department of energy; **VOCs:** Volatile organic compounds; **WTE:** Waste to energy;

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