Biological pre-treatment for agricultural waste biomass: Novel Technologies and processes for resource recovery and circular bioeconomy

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Abstract

Global advancements in energy production are increasingly towards sustainable resource utilization and the adoption of circular economy principles. Innovative technologies have been developed to mitigate environmental impacts while simultaneously enhancing the economic viability of energy generation, particularly through the valorization of waste biomass. Agricultural residues and byproducts generated at various stages of crop cultivation and processing are recognized as promising alternative feedstocks to fossil fuels. Their utilization contributes significantly to greenhouse gas mitigation and facilitates resource recovery. The conversion of these residues into bioenergy involves several interdependent processes, among which biomass pre-treatment plays a pivotal role. This step is essential for the effective removal of lignin and the enhancement of biomass digestibility, thereby improving the overall efficiency

of bioenergy production systems. Given the rapid technological advancements in the field, there is a pressing need for a comprehensive evaluation of agricultural biomass as a feedstock, the biological conversion pathways employed, and the emerging pre-treatment methodologies. This review focuses on the current landscape of bioenergy production from agricultural biomass, emphasizing biological pre-treatment strategies within the framework of the circular economy. Furthermore, it discusses existing challenges and outlines potential avenues for future research and technological development

Keywords: Circular economy; Biomass; resource recovery; Renewable energy; Lignocellulosic biomass; Enzymes; Bioenergy; Biorefinery

1. Introduction

The use of agri-waste biomass for sustainable energy production has gained significant attention in recent years due to its potential to address energy and environmental challenges. Despite the efforts of competent agencies, increased population expansion, industrialization, faster urbanization and environmental deterioration have continued to raise serious problems to mankind on a scale never envisaged (Awogbemi and Von Kallon, 2022). These circumstances have given birth to difficulties that have remained the focus of people, groups, governments, and international organizations. Environmental degradation, poverty, energy instability, a lack of safe drinking water and sanitation, gender inequality, and a lack of peaceful cohabitation have all continued to affect the world's population. Waste disposal and management are inextricably linked to healthy living, air quality, sanitation, food and resource security, climate change and environmental sustainability (Awogbemi et al., 2022). In appropriate agricultural waste disposal and management have a detrimental influence on the environment and are a disturbance to environmental health. It is necessary to enforce policies and legislation that promote decrease in waste, reutilization, recycling, and regeneration, with the goal of eventually moving the manufacturing sector to zero waste (Guo et al., 2021). New waste management legislation and methods that encourage trash recovery and conversion to usable resources are required. Agriculture wastes, when managed and utilized properly, have a chance to become an environmentally friendly resource for added value as well as an important contributor to energy independence and environmental sustainability. Conversion and sensible use of agricultural wastes may promote resource conservation, economic growth, circular economy and human protection (Chilakamarry et al., 2022).

Utilizing agricultural waste as a viable, ecologically friendly substitute for an essential part for creating Ecofriendly products and energy keeping sustainability is in global focus (Yana et al., 2022). Numerous investigations have been carried out to uncover the diverse uses of trash. Recently, Awogbemi et al., surveyed a variety of waste management techniques to guarantee waste reduction, conversion, and usage (Awogbemi et al., 2022). Similarly, an extensive feasibility study on the transformation of citrus fruit waste was carried out by (Taghizadeh-Alisaraei et al., 2017). The researchers outlined a number of methods for turning agricultural wastes into products that are beneficial and looked into several methods for turning rice husk, rice straw, and sugarcane bagasse, respectively, into valuable goods and concluded that agricultural wastes had a wide range of applications in both industrial development and commercial purposes in daily life. These researchers concurred on the viability and advantages of converting different wastes for different uses. Nonetheless, they are aware that agricultural waste must first undergo pretreatment before conversion. To learn more about the method of mechanism, design, and conversion biomass must be characterized before and after pre-treatment (Zhou and Tian, 2022). Finding and identifying the mechanism responsible for the adaptation process remains a challenging endeavor. Reviewing the effects of pre-treating biomass and variables influencing the selected method is still hampered by the knowledges incapacity to assess and recognize the biomass residents that are pertinent to the construction of renewable energy and products with added value (Kumar et al., 2023b).

Globally, agricultural activities generate an estimated 4–5 billion tonnes of biomass residues annually, including crop residues, agro-industrial by-products, and livestock-derived wastes. When these residues are inadequately managed through practices such as open-field burning or uncontrolled disposal, they contribute substantially to greenhouse gas emissions, particulate matter ($PM_{2.5}$), soil and water contamination, biodiversity degradation, and the loss of valuable bioresources. Such practices also pose significant risks to human health by increasing the incidence of respiratory and cardiovascular diseases, particularly in regions where crop residue burning is widespread. Consequently, sustainable agricultural waste management has become a key priority within the framework of the United Nations Sustainable Development Goals (SDGs), particularly SDG 7 (Affordable and Clean Energy), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action), while also contributing to SDG

2 (Zero Hunger) through improved resource efficiency and SDG 3 (Good Health and Well-being) by reducing pollution-related health impacts. In this context, biological pretreatment technologies offer an environmentally friendly approach for converting agricultural residues into bioenergy and other value-added products, supporting the transition towards a circular bioeconomy.

Pre-treatment procedures are categorized by researchers into four separate categories: physical, physicochemical, chemical, and biological. While Beig et al., outline the key obstacles and creative advancements in the pretreatment of lignocellulosic residues (Beig et al., 2021). Devi et al., offer a thorough examination of pre-treatment strategies to produce ethanol (Devi et al., 2021) and Halder et al., provide an overview of the advancements made in pre-treatment methods utilizing ionic liquids (Halder et al., 2019). According to the previous reports that biological pre-treatments are showing better efficient through the microbes that generate the enzymes needed to break down lignocellulosic material, drastically altering the physical and chemical makeup of biomass (Halder et al., 2019). In addition, these techniques are more energy and environmentally-efficient compared to other categories (Beig et al., 2021). The studies of pertinent contemporary literature on biological pre-treatment methods used on agricultural wastes are still in the stage of infancy, Hence, this paper critically describes about recent technologies and methods about pretreatment of agricultural residues. In addition, content of different agricultural wastes production, decomposing, production of bioenergy, a detailed biological pre-treatment methods and future prospects are all covered in this study.

2. Literature Review Strategy

This review was conducted as a comprehensive narrative review to summarize recent advances in biological pretreatment technologies for agricultural waste biomass and their role in bioenergy production and circular bioeconomy applications. Relevant scientific literature was identified through comprehensive searches in major bibliographic databases, including Scopus, Web of Science, ScienceDirect, and Google Scholar. The search strategy employed combinations of keywords such as agricultural waste, lignocellulosic biomass, biological pretreatment, enzymes, microbial degradation, bioenergy, biorefinery, circular economy, and resource recovery. Priority was given to peer-reviewed research articles and review papers published primarily between 2015 and 2025, while seminal earlier publications were included where necessary to provide historical context. The collected literature was critically evaluated to identify recent technological developments, current challenges, and future research directions related to sustainable biomass valorization.

3. Agricultural waste production

Agricultural waste in India is generated from various farming activities and includes residues from crops, livestock, and other agricultural processes. The production of agricultural waste is substantial, and it has both positive and negative effects on the agricultural sector, the environment, and rural communities (Rani et al., 2023). Agriculture waste biomass is made up of vegetable wastes and their constituents, such as lignin, cellulose and hemicellulose. It may be further classified as waste paper, wood residues, grasses, etc (Sarangi et al., 2023).

Agricultural waste also includes polymeric materials like polyester and polypropylene. The development of bioenergy and other biofuels from biomass derived from agricultural waste has been the subject of research. However, because of the rising demand for food crops, there are now limitations on the use of food crops as a substitute source of energy (Zheng et al., 2022). As waste biomass is abundant, it is important to link waste biomass to the biomass economy because the majority of it can be wasted in the energy-production and pre-treatment processes. According to recent study, there is growing interest in using lignocellulosic biomass to produce different biofuels and other products with added value (Ashokkumar et al., 2022).
The production of agro-products is increased by the variety of agricultural operations, which has raised waste creation and environmental damage overall. The resources used and the trash produced are determined by a nation's physical and cultural characteristics. Large-scale pesticide deployment, advancements in irrigation systems, and contemporary agro-technologies have all contributed to the conversion of vast areas of wasteland into arable land (Nazir et al., 2022). India produces 500 million tons (Mt) of agricultural residue annually on average, according to the Ministry of New and Renewable Energy (MNRE) (Dabas et al., 2023). According to the same research, the bulk of this agricultural waste is actually used for industrial and residential fuel and feed. Nonetheless, there is still a 140 Mt excess, of which 92 Mt is burnt annually. It's also noteworthy that, in terms of volume, the amount of agricultural waste burned in India is far more than the total amount produced in other nations in the area.

Agriculture-related waste can be usefully applied to a variety of agricultural in nature uses and other industrial operations. However, the money made from the useful use of such garbage may be significantly outweighed by the costs of collecting, processing, and transportation. This paper focuses on the traditional instance of how economic factors have led to environmental instability in India and hampered the attainment of environmentally friendly disposal of agricultural waste. Two factors make this subject significant to a global audience outside of India: First, because of their organic makeup, crop residues constitute a significant component of waste from agriculture that may be utilized for the good of society. Another significant factor is that the amount of agricultural residue combined with unsustainable management techniques has severe negative environmental effects that extend well beyond India (Bhat et al., 2023). In particular, India is the world's second-largest producer of wheat and rice, two commodities that often yield significant amounts of residue.

4. Crop residue burning in India: Current status, environmental impacts and public health concerns

India, which makes up 17% of the global population and has an agricultural heritage, produces a lot of food grains, including wheat and rice, for both internal use and export (Bhuvaneshwari et al., 2019). Crop residue is burned annually in India at a rate of about 87.0 Mt, which is much more than the total amount of agricultural waste produced in Myanmar (19.0 Mt), Bangladesh (72.0 Mt) and Indonesia (55.0 Mt) combined (Teri, 2019). The quality of life in agricultural states and the areas around them is being negatively impacted by these emissions, which also raise the risk of serious respiratory infections and other connected diseases (Chakrabarti et al., 2019). Burning crop waste has recently been discovered to worsen the COVID-19 pandemic's detrimental effects on health (Chauhan et al., 2023). The specifics of the state-by-state data of crop residue created and surplus residue burnt are provided by the National Policy for Management of Crop Residues (NPMCR). According to NPMCR (Deshavath et al., 2022), it is clear that Uttar Pradesh produces the maximum agricultural residues (60 Mt) followed by Maharashtra (46 Mt) and Punjab (51 Mt). A total of 500 Mt of crop residues are generated annually, of which 92 Mt are burnt. About 70 percent of the agricultural leftovers come from rice and wheat (Fig. 1). The material that remains after using the residue for different reasons is referred to as the excess residue out of all the trash created. A portion of the excess garbage is burnt, leaving the residue on the field. The lack of laborers is the reason given by the Ministry of Agriculture for the rise in agricultural residue burning on farms (Deshavath et al., 2022).

Eighty percent of agricultural waste burning occurred in the post-harvest months of April-May and November-December, according to Jitendra et al (Majumdar, 2023).

This is explained by the crop patterns that are employed to guarantee greater economic returns, which leave a certain amount of time between two successive crop cultivations. A few farmers even turn to planting and harvesting three crops in one year with a little interval in between.

[Fig. 1. The percentage of dry biomass derived from various agricultural residues in India]

The relevant government authorities must be involved in order to device strict steps to decrease the burning of crops and further control agricultural waste management. Through government-initiated programs, the Indian management has completed numerous attempts to promote and instruct the farming community about the best methods of agricultural waste management. Environmentalists and government representatives also developed several forums and initiatives to discourage the burning of crop rest and to boost the use of another environmentally friendly cultivation techniques. Some of the law strictly followed by the Indian government, mainly in the states of Punjab, Haryana, Uttar Pradesh and Rajasthan to prohibit the burning of agricultural waste (Lohan et al., 2018).

In addition, air pollution causes significant premature mortality and illness burden worldwide, having a larger impact in low- and middle-income nations compared to high-income countries (Papadopoulos et al., 2023). India has one of the world's highest levels of exposure to air pollution. The primary sources of pollutants in the air are environmental particulate matter pollution, home air pollution, and, to a lesser extent, ozone in the troposphere, the lowest part of the atmosphere. In India, the biggest sources of surrounding particulate pollution are coal combustion for thermal energy manufacturing, industry emissions, building activity as well as brick kilns, automobiles, road dirt, commercial and residential combustion of biomass, combustion of waste, agriculture stubble combustion, and diesel generators (Anwar et al., 2021). Depending on the weather, the air pollution from burning crop wastes spread in every direction via long-range conveyance mechanisms, especially in the northwest Indian states of Delhi, Punjab, Haryana, and Uttar Pradesh. Much research on the effects of CRB on Delhi's and the neighboring areas' air quality, particularly in the Indo-Gangetic Basin, have been conducted recently and it was highlighted in map (Fig. 2). Domestic air pollution is mostly generated by the household use of solid combustibility for cooking and to a lesser degree, heating, with the most common forms being wood, dung, agricultural leftovers, coal, and charcoal. Ground-level ambient ozone is formed when the volatile organic compounds and nitrogen oxides released by cars, power plants, industries, and various other factors react in the presence of sunlight. Rapidly growing countries, such as India, have the combined dilemma of exposure to ambient and residential air pollution. The government along with other stakeholders in India have recently increased their attention on tackling air pollution (Das and Ghosh, 2023).

[Fig. 2. Impact of crop residues burning and their air pollution more spread in the Northern India (Figure adopted from (Sarkar et al., 2018)]

5. Method of Agriculture waste decomposing

General categories of agricultural leftovers generated by sugar cane and the major cereal crops. By being plowed directly into the soil or composted, these crop residues, especially as field residues, are a natural reserve that have historically subsidized to the stability and fertility of the soil. Effective field residue management can also improve erosion prevention and irrigation effectiveness. However, such conventional sustainable approaches are now economically and practically constrained by the large-scale and quick speed of agricultural production. Burning excess agricultural residue is a popular practice in many poor nations, particularly in Asia (Misra and Ghosh, 2024). Burning causes problems for the ecology, but it takes innovative and costly technical help to quickly till millions of hectares of farm residue into the ground. Lignin is nearly resistant and proposals basic support in contrast to cellulose and hemicellulose. Because lignin is so impervious to biological and chemical breakdown, it resists fermentation (Dharmaraja et al., 2023). Among the non-food components of crops are the stalks, straw, and husk; they are included under the term lignocellulosic biomass (Garg et al., 2023). The majority of lignocellulosic biomass is produced by the world's principal crops, which are sugarcane, wheat, rice, and maize, in that order. Because lignocellulosic biomass, which is made up of cellulose, hemicellulose, and lignin, is so readily available as a raw material for biofuel production, it is becoming more and more valued as a commodity.

Several nations make varied uses of the crop waste produced by agricultural operations.

Depending on the final usage, they are either treated or left unprocessed. Utilizing it for animal feed, composting, creating bioenergy, and implementing it in other prolonged agricultural endeavors like mushroom growing are among the potential uses (Ashokkumar et al., 2022). Compare India other countries like Malaysia, Thailand, Indonesia, China, Philippines, Nigeria and Japan uses its agricultural wastes to make compost and bioenergy. Pretreatment methods for lignocellulosic biomass for the production of biofuels have been the subject of much research (Ashokkumar et al., 2022).

The lignin layer is characteristically pretreated by the lignin-degrading microorganisms to interruption the lignin layer and reduce hemicellulose and cellulose substance to the consistent monomers and sugars for active biomass to fuel adaptation (Deng et al., 2023). This is because lignin is resistant to chemical and biological degradation by fungi, bacteria, and enzymes. Mechanical, chemical, physico-chemical, and biological pretreatments are all possible. These techniques lead to an increase in the cellulose and hemicellulose's accessible surface area, porosity, and degree of polymerization while decreasing their crystallinity.

Enhancing soil detoxification and lowering environmental pollution may also be achieved by using microorganisms to manage agricultural waste (Shahid et al., 2023). Microbial communities decompose the complex components of biomass into simpler forms that may be recycled or utilized by natural processes. The techniques employed may be aerobic or anaerobic, depending on the kind of bacteria, fungus, or algae causing the deterioration. (Kaur et al., 2023). By providing growth-accelerating metabolites, the microbial degradation strategies lessen the toxicity of the soil, encourage plant development, and supply nutrients to the plant by sequestering them from the soil. Thus, anaerobic and aerobic technologies, together with certain associated methods such composting purposes, worm composting, biogas generation, bio-methanation, and biopile farming, might be used to successfully carry out the bioremediation of agricultural waste (Kaur et al., 2023). By providing growth-accelerating metabolites, the microbial degradation strategies lessen the toxicity of the soil, encourage plant development, and supply nutrients to the plant by sequestering them from the soil (Kumar et al., 2023a). Thus, aerobic and anaerobic processes, together with certain associated methods like the process of composting vermicomposting, biogas generation, bio-methanation, and biopile farming, might be used to successfully carry out the bioremediation of agricultural waste.

Biogas, a sustainable energy source with about 50% methane, and the final solid residue that may be used as nutrient-rich fertilizer are both produced by anaerobic digesters from biomass. Because it can produce highly energetic biogas from nearly all kinds of biomass, including various forms of organic waste, slurry, and manure, anaerobic digestion is a viable method for valorizing biomass (Chozhavendhan et al., 2023). Given the large proportion of biodegradable components in agricultural by-products, it is a viable, ecologically appealing, and efficient method of recycling them. Microbial conversion occurs during anaerobic digestion in an aqueous environment, and it can be carried out without any prior treatment (Manyi-Loh and Lues, 2023). The utilization of agricultural residue as an energy source, both as a complement to thermal power plants and as biogas, has been the primary focus of previous government initiatives (Kumar et al., 2023c). Methane concentration of 40–70% is found in biogas produced by the anaerobic biodegradation of agricultural and municipal solid waste; this methane percentage is often increased to 70–99% in natural gas grade. Moreover, it may be fed into the natural gas infrastructure or utilized as vehicle fuel.

6. Substrate for the production of bioenergy

Agricultural biomass may be used as a feedstock to make goods with additional value. This includes biomass derived from fisheries and animals as well as biomass from vegetables, flowers, fruits and field crops (Cai et al., 2021). Both aquatic and terrestrial plants may be used as feedstock to produce energy. Waste biomass from the forest, municipal, and agricultural sectors that is disposed of serves as a useful fuel for the generation of bioenergy (Table.1) (Zhang et al., 2022). Depending on whether it is fed to animals or not, agricultural feedstock is categorized as either food or other than food raw materials. According to their yield or the goods made from them, feedstock can also be split (Van Raamsdonk et al., 2023). Feedstocks such as wheat, maize, sorghum, barley, sugars, sugar beet, etc. may be used to make biofuels like ethanol. Additionally, feedstock materials can be derived from oil-producing crops such sunflower, hemp, coconut, flax seeds, mustard, and palm oil. A small number of plant species are more suited for use as feedstock because they can adapt to different ecological situations (Sun et al., 2023). *Halopyrum mucronatum, Panicum turgidum, Phragmites karta* and other plants are a few examples. Agricultural leftovers and waste biomasses from starch, sugar, and oil-producing crops can also be used as feedstock. Furthermore, waste biomasses derived from sugar cane, corn, rice, wheat, and sugar beet are viable and appropriate sources for the generation of bioenergy.

[Table 1]

Hybrid sugarcane and Corn oil are becoming more popular for creating biofuels like methanol and ethanol because of their high production and potency. Massive amounts of lignocellulose are constantly released by microorganisms and plants. Enzymes break down cellulose to create glucose, which is used in the food and feed industries. Miscanthus sp. and other lignocellulosic energy crops have a high biomass production but highly specific cultivation requirements. Red giant grass is added harvest family with a large yield over an extended period. Another agricultural family with a high and sustained production is red giant grass (Sun et al., 2023). The transformation and manufacturing of biofuels and bioenergy is a common use for residues from the agricultural and forest sectors, such as stalk, husk, shells, stem and dry leaves, timbers, branches, etc., although sewage, animal manures and poultry composts can also be converted for energy construction (Alam et al., 2022). In addition to plant species, weed types and aquatic plants are another class of plants that have the potential to be used as feedstock for the production of bioenergy. These materials are advantageous to use as feedstock in agricultural enterprises because of their high adaptability, increased biomass content, and oil output. Assisting in the process of converting biomass into energy resources are the bacteria that are existing in the field and adjacent sources of water (Wu et al., 2023). The feedstock that comes from the agriculture industry has several characteristics. Agricultural biomass has some drawbacks, including handling, transportation, and collecting. The benefits, needs, and demands associated with the use of microbes and agricultural residue as feedstock in the production of bioenergy outweigh the few drawbacks and difficulties associated with their exploitation (Singh et al., 2023). To produce more bioenergy from biomass, more sophisticated methods must be developed in order to increase biomass productivity and yield. The high nutritious content of agricultural waste products also forces researchers to concentrate more on maximizing usefulness. The food sector is thought to be one of the main causes of ecological contamination and releases a significant quantity of food waste (Mohanty et al., 2022).

Large volumes of waste residues with unique characteristics are produced by a variety of industries, and to better understand and utilize these residues, they must be classified relative to their nature and attributes. Processing and field residues are two categories for agricultural residues. Field residues are materials that, following crop harvesting, are left in agricultural fields but are not used. These components consist of stalks, dried leaves, seeds, and stems. The amount of field leftovers is so great that improper management or lack of awareness can result in major environmental problems, contamination, and render the agricultural area unsuitable for further cultivation. The leftover crops after important materials are extracted are considered process residue. Rice farming is one instance, where the process leftovers are the paddy and husk. The leftovers from the procedure may be used to make biofertilizer, enhance the properties of the soil, feed animals, and other purposes (Kumar et al., 2022). The wastes from other manufacturing, including the food company, are rich in cellulose, lignin, moisture, ash, hemicellulose, and other components. When these high-value ingredients are used to make biofuels and bioenergy, more advantages result. The demand for fruit and vegetable processing has increased, leading to a growth in the industry and the release of massive wastes including seeds, pulps, peels, leaves, etc. Citrus fruit peels from the citrus processing industry are rich in bioactive components, making them an effective substrate for any fermentation process. Research has effectively demonstrated the viability of producing biofuels from a variety of fruit peels (Lin et al., 2021).

Another significant industry supporting the economy is the cultivation of oil seeds. Some main sources of vegetable oils are soybeans, groundnuts, mustard, etc. Research revealed that detergent stock from refineries that process oil may be used to make biofuel (Dhanke and Wagh, 2020). After pressing to remove oil, a cake is produced that is rich in minerals, proteins, fiber, vitamins, and antioxidants. They may be fed to animals as well as utilized as dietary supplements. To maximize the use of biomaterials, agricultural sectors need to concentrate on cutting-edge innovations (Sharma et al., 2023). Conflict between food production and bioresources has been pushed by increased demand. More sophisticated methods are needed to satisfy demand, however using lignocellulosic biomass from plants, agricultural wastes, lawns alongside heritably changed crops shows promise. The use of genetically changed crops and proper removal of agriculture wastes might lead to issues including improved soil erosion, deteriorated, pollution and soil quality from the widespread use of chemical fertilizers and pesticides. As genetic engineering methods progress, strategies for reducing their drawbacks need to be created.

6.1. The application of the nanotechnology in the generation of bioenergy

The field of nanotechnology is developing quickly. Worldwide, their important uses are widely dispersed. Numerous industries, including biofuels, food, healthcare, and agriculture, have great promise for nanotechnology (Arora et al., 2020). Fossil fuel consumption is rising steadily. There is an urgent requirement for a renewable energy source because the environment is severely harmed by these non-renewable sources. In addition to reducing environmental pollution, energy sources that are sustainable and renewable also help to satisfy the world's energy needs. This technique is more cost-effective, environmentally benign, and sustainable when LB feedstocks are used (Govil et al., 2020). For the manufacturing of biofuels, a variety of standard pretreatment techniques were employed; however, they had certain drawbacks, including time consumption, environmental contamination, and high catalyst/chemical cost. By using nanoparticles in the LB pretreatment, all these disadvantages may be solved while maintaining the process' affordability, sustainability, and environmental friendliness (Gou et al., 2020). Utilizing lignocellulosic raw materials, various nanomaterials, including nanoparticles, nanobiocatalysts, and magnetic-based nanomaterials, have been used to produce various forms of sustainable bioenergy, including bio-oil, bioethanol, biogas, biohydrogen and biodiesel. Several forms of renewable energy through the use of nanotechnology. The disintegration of LB is significantly influenced by the nanoscale size of the nanoparticles. They release a large amount of sugars as they interact with the components of LB and readily pass through its cell wall. For the enzymatic hydrolysis process, these liberated elements are further treated with the nanomaterial immobilized enzyme, which enhances the generation of renewable energy (Rai et al., 2017). via recovering and reusing very expensive enzymes, the hydrolysis process is made more inexpensive via the use of immobilized enzymes.

In the context of this review, nanotechnology is primarily considered as a complementary tool that enhances biological pretreatment rather than as an independent pretreatment strategy. Particular emphasis is placed on nanobiocatalysts and enzyme immobilization systems, where nanoparticles improve enzyme stability, catalytic activity, and reusability, thereby facilitating the biological disruption of the lignocellulosic matrix. Although selected examples involving nanoparticle-assisted anaerobic digestion and biohydrogen production are discussed, these are included to illustrate the broader integration of nanotechnology with biological conversion processes following pretreatment.

Magnetic nanoparticles functionalized with acid are utilized to immobilize the enzyme responsible for the hydrolysis of biomass lignocellulose. Important properties of magnetic nanoparticles are their large high specificity, thermal stability and surface area. Their magnetic properties indicate great promise for using a magnetic field to retrieve the enzyme (Attia et al., 2024). By hydrolyzing the mixed weed lignocellulose biomass during the gasification process, Ali and his colleagues were able to produce bio-oil, biogas and biodiesel through the use of the nickel and cobalt nanoparticles as a nanocatalyst. The temperature at which this entire process was conducted was 400 °C. The GC-MS method was used to test these bioproducts, and the results showed that the biodiesel had a greater ester content than regular biodiesel—roughly 65.47%. Several kinds of nanoparticles were employed in the synthesis of sustainable and clean bioenergy. One of them, zero valent iron nanomaterial, has important characteristics for the dark fermentation of grass to produce biohydrogen. It reduces the oxidation-reduction potential that promotes the development of fermentative microorganisms and increases the activity of oxygen-sensitive hydrogenase by eliminating the undesired quantity of oxygen from the system. Additionally, the microbial communities are strengthened by the zero valent iron nanoparticles, and this immediately improves the metabolic process that leads to the hyperproduction of biohydrogen. The highest amount of biohydrogen produced per gram of dry grass was 64.7 ml (Azahari et al.). Ashok and associates employed magnetic oxide nanoparticles in the biodiesel synthesis process. The co-precipitation process was utilized to create the magnetic oxide nanoparticles, which were then employed to transesterify cooking oil to produce biodiesel. The transesterification process was conducted for one hour with constant stirring at a temperature range of 25 to 75 °C. With 2 weight percent of magnetic oxide nanoparticles, the maximum biodiesel output was around 93.3% (Bai et al., 2021).

Attia et al. used nickel nanoparticles and graphitic carbon nitride nanosheets to improve biomass-to-biohydrogen conversion. The growth and activity of the purple non-sulfur bacteria are biostimulated by the laser-photoactivated nanomaterials, directly increasing the output of biohydrogen. More biohydrogen is produced by carbon nitride graphitic nanosheets than by nickel nanoparticles (Tian et al., 2022). Researchers employed bio-iron nanoparticles to produce biodiesel using micro-algal biomass in order to produce biofuels in a sustainable manner. Bio-nanocatalysts, or bio-iron nanoparticles, have a high catalytic activity, a large surface area, and tiny particle sizes that improve the transesterification process. A highly appropriate option for increasing the generation of biodiesel is the use of a bio-nanocatalyst, which transforms lipid extraction into fatty-acid methyl esters (Singhvi and Kim, 2020). Zinc oxide nanoparticles were employed by Hassaan and his colleagues to produce biogas. Barley, rapeseed, durum wheat, triticale, and abyssinian cabbage are among the five crops that have been utilized as biomass for the generation of biogas. The anaerobic digestion process's conversion efficiency is increased by these nanoparticles. Zinc oxide nanoparticle-treated durum wheat out of five biomasses exhibits the highest biogas generation, with 457 mL/g volatile solids, more than the control (Dey et al., 2022).

7. Biological Pre-treatment technologies

Pre-treatment, which involves structural alterations aimed at addressing the refractory character of biomass, is a crucial step in the biochemical and thermochemical conversion of agricultural biomass (Zhao et al., 2022). Pretreatment is required to improve the nature and qualities of biomass in order to increase the biomass's efficiency as an energy source. There are several approaches to pre-treatment procedures. The several pre-treatment techniques for converting biomass from agro-waste are available. Heat is used in several pre-treatment procedures to break down lignocellulosic biomass and liberate its sugar contents. The primary drawbacks of pre-treatment procedures are their expense and the need to optimize the process to achieve the least amount of deterioration of other essential components (Yoruklu et al., 2020). These issues must be resolved by evaluating the study team's work and figuring out new strategies that will advance the traditional biological pre-treatment procedure, which is necessary for the green society (Table.2). The kind, origin, and character of the biomass, as well as its broad use in the biorefinery and bioconversion processes, must be taken into consideration when selecting a pre-treatment method.

[Table 2]

Using enzymes generated by microorganisms, especially fungi, to break down, depolymerize, and cleave biomass apparatuses including cellulose, lignin, hemicellulose and is known as the biological approach of pre-treatment. When compared to the other two previously stated approaches, this type of pre-treatment offers a number of benefits, including the production of less harmful byproducts, enhanced productivity of necessary harvests, low energy ingesting, a simpler reaction process and substrate specificity (Xu et al., 2024). However, biological pre-treatment procedures are limited by a few factors, including sluggish processes, large space requirements, and maintaining ideal conditions for microbial development. The residence period for this procedure is typically close to 14 days, and since microorganisms break down the organic content of biomass, additional factors, such as techno-economic concerns, must be considered throughout the biological pre-treatment process (Fig.3) (Ruiz et al., 2023). As a result, this methodology is less appealing than other strategies and is not frequently used by researchers. Microorganisms that are frequently used in the pre-treatment process include microorganisms soft-rot fungi and brown, white-rot fungi, and rarely bacterial communities. These fungi are widely renowned for their ability to effectively remove lignin and hemicellulose while having little impact on cellulose. Even though a lot of pre-treatment techniques have been looked at, a lot of them are still in the development stage and need to be compared with other pre-treatment methodology and their availability verified. The majority of pre-treatment systems include upstream and downstream processing costs associated with original investment, recycling, and waste treatment processes. This approach, which employs enzymes generated from microorganisms to remove lignin instead of chemical compounds, has an efficiency range of 78 to 99%, depending on certain factors.

[Figure 3. Production of feedstock, biomass pretreatment, hydrolysis with enzymes, fermentation of sugar, and biofuel processing are the steps involved in the bioconversion of lignin-based biomass to biofuel]

White rot fungi have been used extensively for biomass pre-treatment because they are thought to be the most efficient microbes for lignin degradation because of their capacity to generate sufficient amounts of peroxidases and laccases, which aid in lignin oxidation. For instance, Yang et al examined the impact of applying the white rot fungus *Echinodontium taxodii* to the pre-treatment of biomass from maize stover and showed how it affected the distribution of pyrolytic products (Yang et al., 2011). After pre-treating around 10 g of dry biomass with 10 ml of the fungal seed culture at 28 °C, the biomass was left to grow for 30 days. After that, the resulting material was pyrolyzed for one minute at 340 °C. The findings exhibited that the fungus treatment of biomass enhanced cellulose, hemicellulose, and lignin pyrolysis, indicating that it had a degrading impact on each component. Notably, there was a significant increase in the pyrolytic product generation from cellulose and hemicellulose. When compared to untreated biomass, the treated biomass generated more long chain and polycyclic aromatic hydrocarbons, suggesting that the white rot fungus was responsible for the lignin's breakdown. Furthermore, the study's kinetic analysis demonstrated that, during low temperature pyrolysis, the biological treatment lowered the activation energy and raised the reaction rate (Dave et al., 2023). Beyond selective lignin removal, the effectiveness of biological pretreatment depends on the synergistic action of extracellular oxidative and hydrolytic enzymes, including laccases, lignin peroxidases, manganese peroxidases, cellulases, and hemicellulases. These enzymes progressively disrupt the lignocellulosic matrix, increase substrate porosity, reduce cellulose crystallinity, and improve enzyme accessibility during subsequent hydrolysis. The overall pretreatment efficiency is influenced by operational parameters such as fungal species, incubation time, temperature, moisture content, pH, and biomass particle size. More recently, corncob lignin was treated with a mixture of brown rot (*Fomitopsis pinicola*) and white rot (*Trametes orientalis*) fungi at 28 °C for 25 days, with pyrolysis taking place for one minute at 600 °C (You et al., 2019). The results of the investigation demonstrated that the brown rot fungus additionally promoted the decomposition of guaiacyl units whereas the white rot fungus efficiently broke down lignin into its component components. Moreover, a notable rise in the quantities of phenols and alkyl phenols was observed in the case of treated biomass. These chemicals are mostly obtained by the process of pyrolysis of lignin, suggesting that the fungi work in concert to break down lignin (You et al., 2019). There is a need to investigate the use of microbes for biomass pre-treatment and its impact on pyrolytic behavior as well as bio-oil upgrading further, as only a limited number of them have been employed for biomass pre-treatment and the thermal degradation process that follows.

In a different work, the production of pyrolytic compounds at 600 °C was examined using lignin that had been previously treated with *E. taxodii* (at 28 °C for 30 days) (Makepa et al., 2023). The FTIR outcomes established that the fungus had a substantial influence on lignin degradation, specifically the deformation of the aromatic skeleton side chain of lignin and carbon following the treatment, even though pyrolysis outcomes demonstrated that a greater number of lignin-derived pyrolytic compounds were produced with the treated biomass than the untreated biomass (Makepa et al., 2023). As was said in the discussion above, all of the biomass components' pyrolysis kinetics and process are enhanced by the biological processing of biomass. The increased pyrolytic product synthesis from lignin, hemicellulose, and cellulose suggests that biological treatment degrades each of these components. Thus, it is possible to propose that, given favorable pyrolysis conditions, processed biomass can generate a larger liquid yield and a greater quantity of organic molecules. However, because microbes take longer to break down biomass, the process might take a while. The need for a sizable quantity of room to do the microbe before to treatment at the pilot size application is another difficulty for this strategy. Microorganisms may use some portion of the carbohydrates for growth, which might reduce the bulk amount produced from the treated biomass (Papadopoulos et al., 2023).

Implementing biological pre-treatment technologies (Table 3) for agricultural waste biomass in India requires a holistic approach involving research, development, and adoption of sustainable practices. Factors such as regional variations in agricultural waste composition, climate, and the scale of operations need to be considered for effective implementation. Additionally, collaborations between research institutions, industry, and government bodies can contribute to the successful deployment of these technologies.

[Table 3]

8. Future perspectives and research gaps

The future development of biological pre-treatment technologies for agricultural waste presents promising prospects for sustainable waste management and bioenergy production. With improved enzyme engineering techniques, it may be possible to create customized enzymes that can effectively break down specific components of agricultural biomass, such as cellulose, hemicellulose, and lignin. Optimizing enzyme activity for various biomass feedstocks and comprehending the intricate enzymatic interactions are still unmet research needs. Higher yields of bioenergy and value-added products could result from substantial improvements to the efficacy and efficiency of biological pre-treatment procedures using these improved enzymes. Additionally, there is considerable promise for improving the breakdown of complex biomass materials through ongoing research on optimizing microbial consortia. However, further research is required to fully understand the dynamics of microbial consortia and their interactions with various biomass substrates in different environmental conditions.

By comprehending and leveraging mutualistic relationships among diverse microbes, scientists can develop consortia with enhanced capacities for biomass conversion. Another important direction for future research is the integration of biological pre-treatment with biorefinery principles. Nevertheless, research on integrating pre-treatment technologies with systems for product recovery and downstream processing within biorefinery frameworks still needs to be completed. By simultaneously producing multiple useful products from agricultural waste, including biofuels, biochemicals, and biopolymers, this approach improves the sustainability and economic viability of bioenergy production systems. Solving issues with industrial applications and scaling up is still important before biological pre-treatment technologies are widely adopted. Research initiatives focused on process parameter optimization, cost reduction, and scalability enhancement are crucial to overcome these obstacles and promote commercialization. Furthermore, investigating new microbial resources possessing distinct enzymatic properties presents stimulating prospects for innovation in biomass conversion procedures. Researchers can discover new pathways for biomass decomposition and identify enzymes with better performance characteristics by finding and analyzing new microorganisms. Furthermore, developing interdisciplinary cooperation among scientists in many domains, including microbiology, biochemistry, engineering, and agronomy, will be crucial for advancing biological pre-treatment technologies. Through the integration of disciplinary knowledge and resources, researchers may better tackle intricate problems and expedite the creation of sustainable solutions for the utilization of agricultural waste and the generation of bioenergy within the context of circular economies.

Conclusions

The sustainable handling of waste from agriculture has emerged as a significant concern, particularly for emerging nations like India that are experiencing rapid population increase, rising production rates, and economic expansion. Thus, a practical and sustainable approach to the processing of lignocellulose biomass is desperately needed. At the moment, this procedure is practical, economical, and environmentally benign due to the many uses of biotechnology and nanotechnology. Conversely, it is accountable for the least amount of harmful inhibitor generation in contrast to traditional pretreatment techniques. Therefore, the most advantageous procedure is thought to be the biological ones that employ nanoparticles for biomass pretreatment or to immobilize the enzyme involved in the saccharolytic process. The excellent recovery and reusability of immobilized enzymes can be attributed to their magnetic properties.

Because of their tiny size, nanomaterials readily pierce the cell walls of lignocellulosic biomass matrix. However, little is known about the precise mechanism behind the biological pretreatment process utilizing nanotechnological pathways. To improve the possibilities of biotechnology techniques for large-scale manufacturing of biofuels or biochemicals, a thorough investigation of their mechanism should be carried out in the future.

Future research should focus on bridging the gap between laboratory-scale innovations and industrial implementation through comprehensive techno-economic analyses, life cycle assessment, and pilot-scale validation of biological pretreatment technologies. Greater emphasis should also be placed on the development of engineered enzymes, optimized microbial consortia, and integrated biorefinery platforms capable of simultaneously producing bioenergy and high-value bioproducts from agricultural residues. Furthermore, interdisciplinary collaboration among microbiologists, chemical engineers, environmental scientists, and policy makers will be essential to accelerate the commercialization of sustainable biomass valorization technologies and to support the transition towards a resilient circular bioeconomy.

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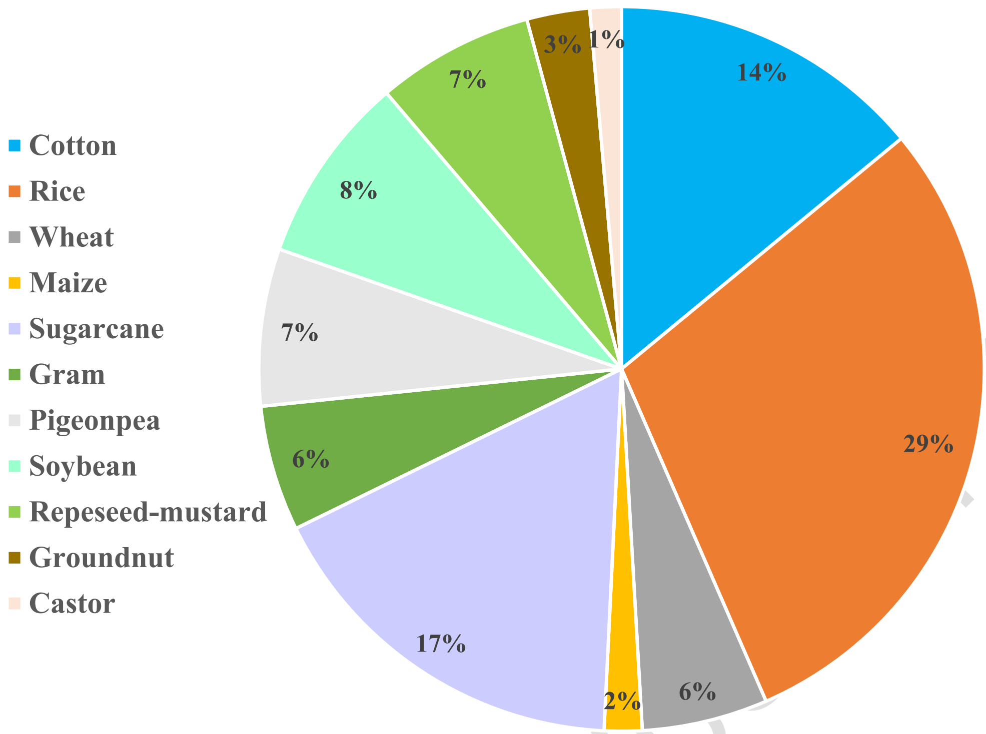

Figure 1. The percentage of dry biomass derived from various agricultural residues in India

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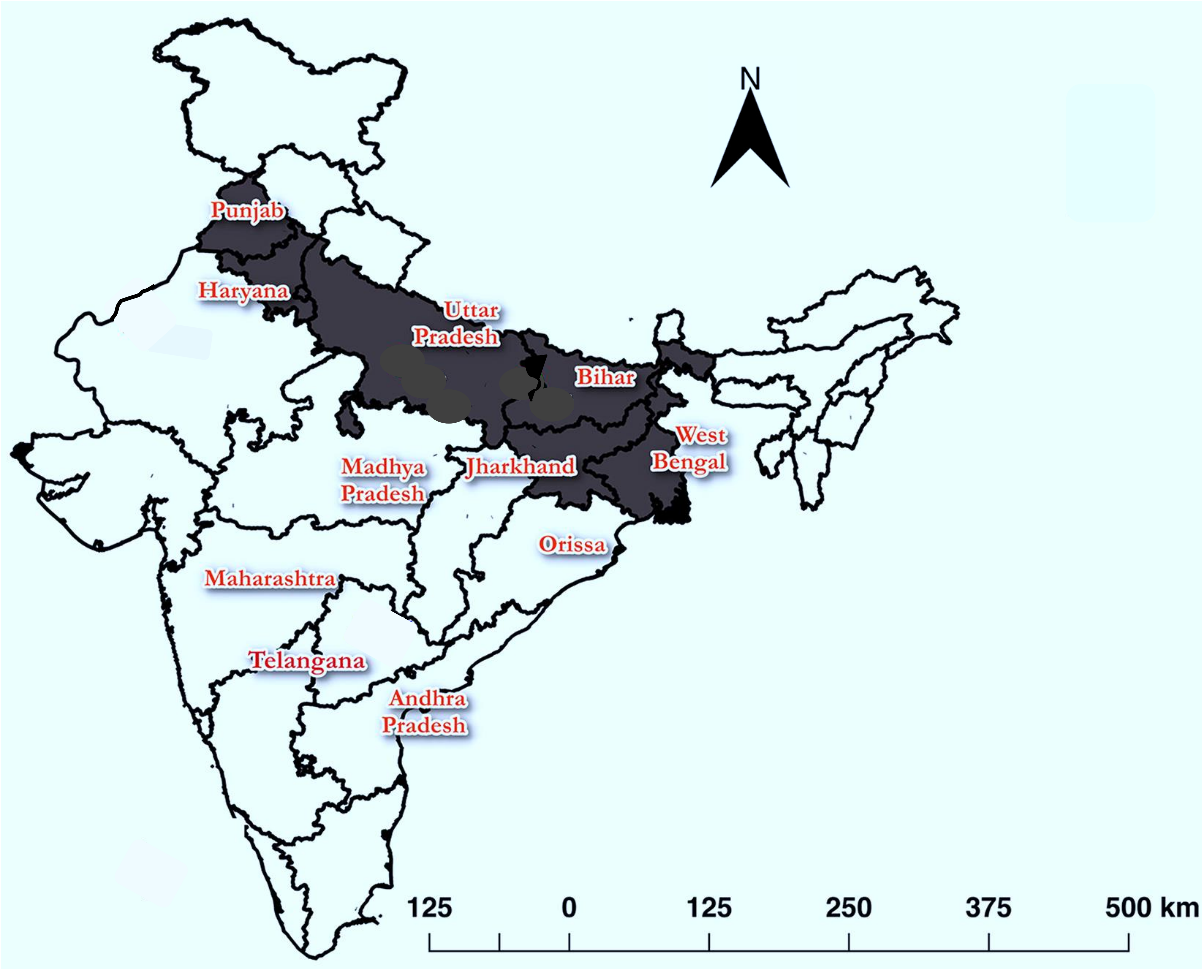

928 **Figure 2. Impact of crop residues burning and their air pollution more spread in the**
929 **Northern India (Figure adopted from (Sarkar et al., 2018))**

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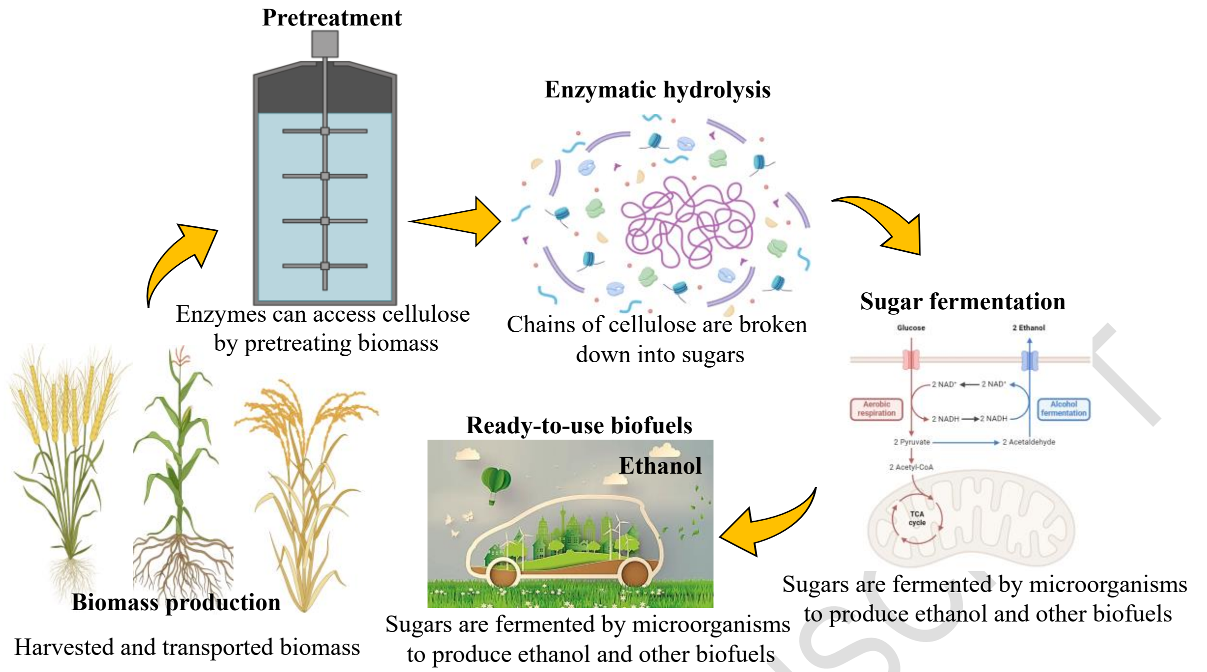

Figure 3. Production of feedstock, biomass pretreatment, hydrolysis with enzymes, fermentation of sugar, and biofuel processing are the steps involved in the bioconversion of lignin-based biomass to biofuel.

Table 1. Global bioenergy substrates and major producing countries

Substrate	Description	Type of bioenergy	Top producing countries	References
1. Crop residues	Stems, leaves, husks, stalks, and other plant parts left in fields after harvest. Examples include wheat straw, corn stover, rice husks, sugarcane bagasse, and soybean stubble.	Biomass for biofuels, biogas production	USA, China, Brazil	(Awogbemi and Kallon, 2023)
2. Woody biomass	Logging residues, forest thinning, sawdust, wood chips, and tree trimmings.	Biomass for biofuels	USA, Canada, Sweden	(Dudziec et al., 2023)
3. Energy crops	Fast-growing grasses (e.g., switchgrass, miscanthus), short-rotation woody crops (e.g., willow, poplar), and other dedicated energy crops.	Biomass for biofuels	Brazil, USA, China	(Mehmood et al., 2017)
4. Agricultural wastes	Byproducts and residues from food processing, such as fruit and vegetable trimmings, peels, cores, pulp, seeds, and meat and poultry processing residues.	Biomass for biofuels, biogas production	USA, China, India	(Lee et al., 2020)
5. Animal manure	Organic waste from livestock farming operations, including solid and liquid waste from barns, pens, and feedlots.	Biogas production	China, India, USA	(Pexas and Kyriazakis, 2023)
6. Algal biomass	Microalgae and macroalgae cultivated in ponds, tanks, or	Biomass for biofuels	USA, China, Japan	(Mahmood et al., 2023)

	open water systems for biofuel production.			
7. Municipal Solid Waste (MSW)	Organic fraction of household and commercial waste streams, including food waste, yard waste, and paper products.	Biogas production	USA, China, India	(Raj et al., 2023)
8. Sewage sludge	Organic matter removed from wastewater during treatment processes, often used for biogas production through anaerobic digestion.	Biogas production	USA, China, Japan	(Chen et al., 2023)
9. Industrial byproducts	Residues from industrial processes, such as paper mill sludge, brewery spent grains, and biodiesel glycerol.	Biomass for biofuels, biogas production	USA, China, Germany	(Rodrigues et al., 2023)
10. Landfill gas	Methane-rich gas produced by the anaerobic decomposition of organic waste in landfills.	Biogas production	USA, China, Germany	(Saravanakumar et al., 2023)

Table 2. Comprehensive overview of biological pre-treatment technologies for biomass conversion

Biological Pre-treatment Technologies	Description	Advantages	Challenges	References
Fungal enzymatic treatment	Utilizes enzymes produced by fungi to break down lignocellulosic biomass, including cellulose, hemicellulose, and lignin.	Production of less harmful byproducts - Enhanced productivity of necessary products - Low energy consumption	Slow processes - large space requirements-Need to maintain ideal conditions for microbial development	(Ahmad et al., 2023)
Bacterial pre-treatment	Utilizes bacteria to degrade biomass components, particularly lignin, to facilitate subsequent bioconversion processes.	Substrate specificity - Relatively simpler reaction processes	Optimization needed to prevent deterioration of other essential biomass components - longer residence periods	(Saini et al., 2023)
Microbial consortia	Utilizes a combination of microorganisms, including fungi, bacteria, and sometimes archaea, to synergistically degrade complex biomass.	Diverse enzymatic activities leading to efficient degradation of various biomass components - Potential for higher biomass conversion rates	Complexity in managing and optimizing consortia composition and interactions - Limited understanding of microbial dynamics and ecological interactions	(Zhou et al., 2024)
Enzyme engineering	Involves genetic modification or	Tailored enzymes for specific biomass	- Complex and costly enzyme	(Ranjan et al., 2023)

	optimization of enzymes to enhance their efficiency and specificity in degrading biomass components.	components and conditions - Higher enzymatic activity and stability under various conditions	engineering processes - Limited scalability and applicability of engineered enzymes - Regulatory and ethical considerations for genetic modification	
Solid-State fermentation (SSF)	Utilizes microbial fermentation processes in SSF conditions to degrade biomass, primarily lignocellulosic materials.	Simplified process with reduced energy and water requirements - Higher tolerance to inhibitors and variations in feedstock composition	Limited control over process parameters compared to liquid fermentation - Longer fermentation times - Challenges in scaling up to industrial levels	(Valle-Pérez et al., 2023)
Anaerobic digestion	Involves the breakdown of biomass by microorganisms in the absence of oxygen, producing biogas (methane and carbon dioxide) as a byproduct.	Production of biogas for energy generation - Reduction of biomass volume and pathogens	- Requires controlled anaerobic conditions and proper mixing - Variation in feedstock composition and loading rates may affect performance	(Kumar Sarangi et al., 2023)

Fermentation with yeasts	Utilizes yeast species to ferment sugars present in biomass into ethanol or other valuable products.	High conversion efficiency of sugars to ethanol - Compatibility with various types of biomasses	Need for pretreatment to release fermentable sugars - Sensitivity to inhibitors and variations in fermentation conditions	(Kumar Sarangi et al., 2023)

Table 3. Biological Pretreatment technologies and related application for agricultural biomass valorization

Pretreatment approach	**Biological agent / technology**	**Main mechanism**	**Advantages**	**Limitations**	**Typical biomass**	**references**
White-rot fungi	*Trametes*, *Phanerochaete*, *Echinodontium*	Selective lignin degradation (laccase, MnP, LiP)	High lignin removal, environmentally friendly	Slow process, long residence time	Corn stover, wheat straw, rice straw	Yang et al. (2011); Xu et al. (2024)
Brown-rot fungi	*Fomitopsis* spp.	Cell wall disruption via oxidative mechanisms	Improves cellulose accessibility	Lower lignin selectivity	Corncob, woody biomass	You et al. (2019)
Bacterial pretreatment	*Bacillus*, *Pseudomonas*, *Clostridium*	Enzymatic degradation of lignocellulose	Rapid growth, adaptable conditions	Lower lignin degradation efficiency	Agricultural residues	Kumar et al. (2023a); Shahid et al. (2023)
Enzymatic pretreatment	Cellulases, xylanases, laccases, peroxidases	Hydrolysis of cellulose, hemicellulose and lignin	High specificity, mild operating conditions	Enzyme cost	Lignocellulosic biomass	Ahmad et al. (2023); Ranjan et al. (2023)
Nanobiotechnology-assisted pretreatment	Magnetic nanoparticles, ZnO, Ni, Fe nanoparticles	Enzyme immobilization and catalytic enhancement	Improved efficiency, enzyme recovery	Scale-up and cost challenges	Mixed agricultural biomass	Attia et al. (2024); Dey et al. (2022); Singhvi & Kim (2020)

Anaerobic microbial conversion	Mixed microbial consortia	Biomass degradation and methane production	Bioenergy generation with waste stabilization	Sensitive to operational conditions	Organic agricultural residues	Chozhavendhan et al. (2023); Manyi-Loh & Lues (2023)

LiP: lignin peroxidase; MnP: manganese peroxidase