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Fungal pretreatment methods for organic wastes: advances and challenges in biomass valorization

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Food wastes, municipal solid wastes, sewage sludge, plant materials, animal biomasses, aquatic and terrestrial wastes, agricultural and forestry wastes, industrial and domestic wastes and many other lignocellulosic biomasses are grouped under the category of organic wastes or bio-wastes. Various techniques, mainly mechanical (high-pressure homogenization and ultra-sonication), thermal (temperature-based), microwave-assisted, chemical, and biological pretreatments, have been found to be effective in organic waste valorization. Fungal pretreatment of organic wastes is a promising biological technology because of its excellent efficiency in the decomposition of various types of organic wastes, such as food wastes, ligno-cellulosic biomasses, hemicellulose, agricultural wastes, hardwoods, softwoods, switchgrass, spent coffee grounds, park wastes, cattle dung, and solid digestate, which are specifically reviewed. Fungal pretreatment of organic waste materials can generate advantageous products such as biogas, alternative energy sources, monomeric or oligomeric sugar products, and different types of acids. However, the major challenge associated with fungal pretreatment

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technology is the requirement of a longer time to achieve a greater degree of biomass valorization, which increases the cost and vulnerability to contamination. However, the use of fungal pretreatment with other pretreatment techniques may shorten the time and enhance the functionality of the method with a higher rate of biomass valorization. Heat generation in the fungal pretreatment process and need for feedstock sterilization before fungal pretreatment are some other challenges that need to be properly addressed for its efficient application on an industrial scale. In this review, the use of different fungal pretreatment methods for the valorization of different types of biomasses and production of valuable products is evaluated and discussed. We performed a comprehensive assessment of the fungal pre-treatment of various types of organic wastes together with a concise but effective discussion on organic solid wastes and different pretreatment techniques involved in bio-waste digestion processes. Furthermore, techno-economic analysis, challenges and future perspectives are discussed.

Sustainability spotlight

Sustainable treatment of biowaste or organic waste such as food waste, municipal solid waste, sewage sludge, plant materials, animal biomass, aquatic and terrestrial waste, agricultural and forestry waste, and many others is the need of the hour. Thus, different countries are working on various related projects to treat this biowaste and sustainably produce energy, biogases, methane, ethanol, carbohydrates, protein and several other useful chemicals. This type of sustainable literature review may be helpful to better understand the goals of sustainable development. This critical review is well-aligned with various sustainable development goals of the United Nations, including 'affordable and clean energy' (UN's SDG 7), 'sustainable cities and communities' (UN's SDG 11) and 'responsible consumption and production' (UN's SDG 12).

1 Introduction

Solid waste, organic waste or bio-waste materials are composed of large-size organic molecules (macromolecules) such as lignocellulose, lignin, hemicellulose, proteins, fats, and vitamins. These materials are produced owing to various reasons; however, their decomposition produces harmful gases and constituents, which need to be properly treated. The appropriate management of this bio-waste may also produce several beneficial products such as biogas, sugars, electricity (*via* biogas or other fuel sources derived from biowaste valorization), short chain carboxylic acids, fertilizers and several other products. The generation of municipal solid wastes is expected to increase from 2.1 billion tonnes in 2023 to 3.8 billion tonnes by 2050.[1] Besides, food loss and associated waste are global challenges. It is predicted that 13% of the globally generated food is wasted annually from harvest up to, but not counting retail, accounting for an economic value of about four hundred billion USD.[2] Additionally, households, restaurants and many other food services are responsible for about 19% of food loss or food waste generation.[2] Disposed food waste reached 38.1 million tons in 2017.[3] Therefore, it is crucial to carefully manage food waste to avoid health issues and environmental contamination.[4,5] The biggest waste produced in the process of sewage treatment is sewage sludge, which is rich in organic content, and considered organic municipal solid waste.[6] The generation of sewage

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sludge has increased annually. For instance, the dry mass of sewage sludge has increased annually from ten million tons (in 2010) to 13.5 million tons (in 2020) in the twenty seven countries of the European Union.[7] In this case, the process of composting and anaerobic digestion is preferred because waste can be processed by these ways into safe products such as organic fertilizers and soil improvers.[8]

Currently, a series of management strategies is being promoted by various countries and technologies for food waste treatment are being developed. In Germany, there were greater than nine thousand pertinent food waste anaerobic digestion projects in action by 2015, accounting for greater than 80% of the biogas-based projects in Europe.[9] Additionally, food waste produces around 5 million tons of fertilizer annually.[10,11] By 2025, the rate of recycling of food waste is intended to be increased in the UK from the current 10% to 70%.[12,13] Organic macromolecules such as proteins, sugars and fats are found in food waste materials. Food wastes also have trace elements such as Fe (iron) and Co (cobalt), which facilitate the growth of microorganisms.[14]

The huge generation of bio-waste is a serious problem worldwide, particularly in developing countries where its management is not as good as developed countries. However, it may also be converted into a useful form by the proper management of bio-waste *via* pretreatment because the bio-pretreatment of this type of waste may generate a large amount of energy and useful products. There are some important pretreatment techniques available for the pretreatment of organic bio-waste (Fig. 1), *i.e.* mechanical pretreatment techniques including high-pressure homogenization and ultrasonication, thermal pretreatment, microwave-assisted pretreatment, combined pretreatment technology, chemical pretreatment technology, and biological pretreatment methods including bacterial pretreatment, enzymatic pretreatment, and fungal pretreatment techniques. However, considering the complexity of the lignocellulosic structures and problems linked with pretreatment *via* chemical and physical methods, biological techniques are very useful because they are environmentally friendly, economical, effective and do not require or release any toxic chemicals.[15] Among them, aerobic pretreatment, temperature-phased anaerobic digestion, and enzyme-mediated pretreatment processes are promising biological pretreatment processes,[16] which can be used as favorable alternatives to chemical, physical, and thermal processes. The aerobic process is dependent on the inherent enzymatic activity of sludge, which requires oxygen.[17] Alternatively, temperature-phased anaerobic digestion is done through a configuration composed of two digesters in a series, where thermophilic conditions are applied for the first digester, while mesophilic conditions for the second digester.[16] The enzyme-mediated process of pretreatment causes am improvement in the sludge bioconversion with the assistance of exogenous enzymes, contributing to the degradation of refractory compounds.[16] Regarding the production of biogas from sludge, a critical review on various pretreatment methods has been presented by Mitraka *et al.*[18]

Fungi and their enzymatic systems are known for their huge biotechnological application due to their versatile capability and ability to produce several enzymes.[19–22] Fungi also play great roles in environment restoration by eliminating or degrading several toxic environmental pollutants such as organic molecules and heavy metals.[23,24] Together with these applications, fungi also show strong potential in the biological pretreatment of organic waste or bio-waste. Among the numerous physical, chemical and biological pretreatment techniques,[18,25] fungal-based biological methods may play an emerging role in this field. The fungal pretreatment method is an important alternative to conventional pretreatments, which is mostly performed in the temperature range of 25–30 °C, with the minimum use of water, at atmospheric pressure, and without the use of any chemicals.[26] Wood rot fungi such as soft, white, and brown fungi play the most important role in the fungal pretreatment technique because of their potential to change the constituents of lignocellulosic biomass.[27] After fungal pretreatment, detoxification and/or washing are not generally

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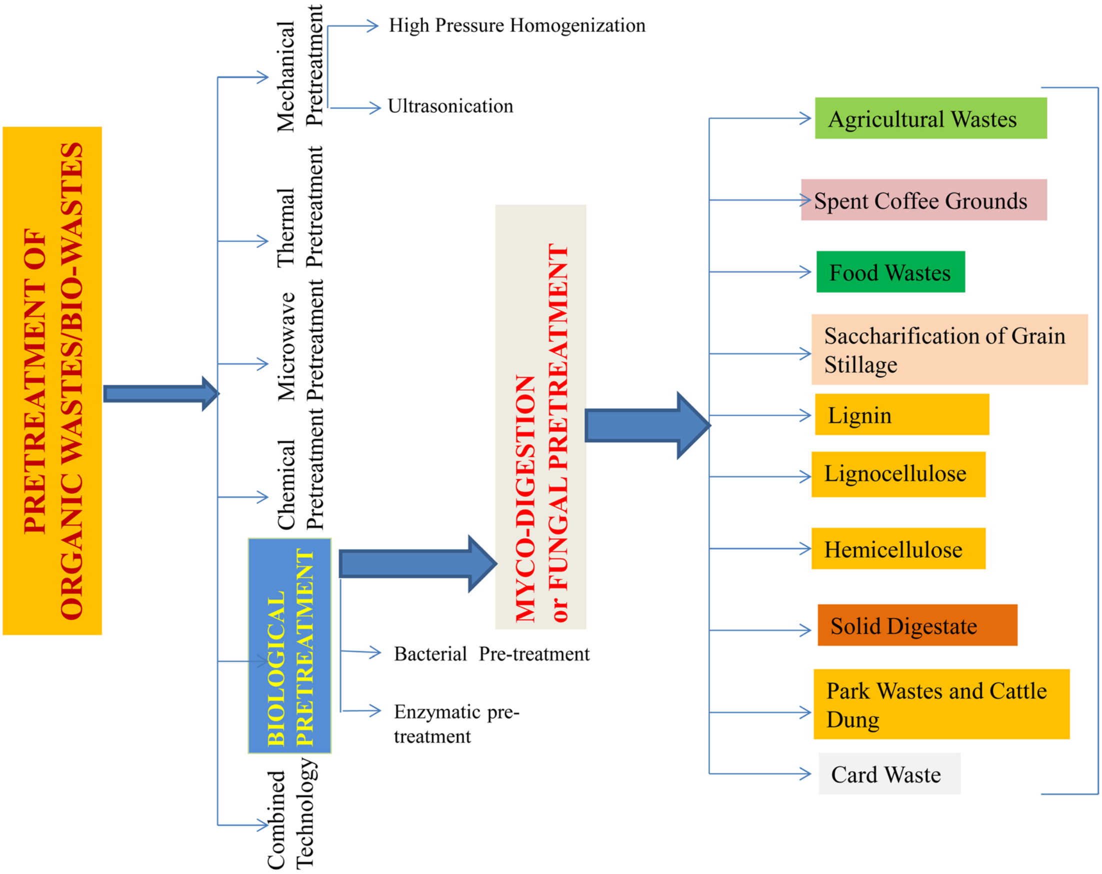

Fig. 1 Different pretreatment techniques involved in the pretreatment process of organic or bio-waste.

necessary because the mild conditions of fungal treatment are unlikely to generate microbial inhibitory compounds. At higher feedstock particle sizes, the fungal pretreatment technique is more effective than most conventional pretreatment methods.[26] However, fungal pretreatment also has some disadvantages together with the aforementioned advantages. These shortcomings include the requirement of a long reaction time, lower sugar yields and the need for sterilization of the feedstock in comparison with the traditional pretreatment methods.[28]

As a solid-state procedure with low energy and chemical consumption, the fungal pretreatment technique is considered a low-cost technique[27] but its above-described shortcomings, mainly the longer time requirement, may make it more costly, which can be eliminated using combined pretreatment technology. The sterility requirement, long residence time, significant heat generation by the fungal metabolic rate and need for a high rate of aeration for efficient delignification may play a significant role in the techno-economic study of fungus-based pretreatment at the commercial scale, which still need to be studied.[27] However, despite the shortcomings or challenges of fungal pretreatment methods, several studies have shown that fungal pretreatment techniques may be a noteworthy biological pretreatment method for the treatment of diverse biowaste or biomass[29,30] because they provide a gentle, ecofriendly, and low cost future solution for biomass conversion. Also, they are widely utilized in the production of chemicals and biofuels due to their role in the degradation of lignin, saccharification, lipid accumulation and fermentation.[29]

Considering the crucial involvement of fungi in the pretreatment of lignocellulosic biomass, agricultural waste, food waste, saccharification process, biogas production, bioethanol production, and sugar production, *etc.*, we decided to prepare a comprehensive review on the fungal pretreatment of these biowaste materials. The principle objective of this review is to analyze the significance of the fungal pretreatment in the management of the problem of huge organic waste or bio-waste materials and present a techno-economic analysis, potential future challenges and its applicability. During the fungal pretreatment process, the production of biogases, bioethanol, sugar molecules, and other biologically valuable components has a great future owing to their use in the welfare of humanity. Several recent reports are discussed herein in detail to highlight and understand the promising possibilities of fungal pretreatment methods. Furthermore, a brief but effective discussion is also presented on the topics of organic waste and various pretreatment techniques.

2 Organic waste or bio-waste or sources of biomass

A wide range of materials come under the topic of organic solid waste or bio-waste. Waste from food, sewage sludge, plants and animal biomass, fungal and algal biomass, several aquatic and terrestrial waste, agricultural waste, industrial waste, and many other waste materials that are rich in organic components such as polysaccharides, lignin, proteins, fats, and vitamins are considered organic waste or organic solid waste. Fig. 2 presents a picture of huge plant biomass from nature (dead plant materials and green plants). In a comprehensive critical review, Lizundia *et al.* well-described the valorization of organic waste.[31] Among the great diversity of aquatic organisms, algae and crustaceans are considered a good source of organic waste. Vast probabilities to get varying polysaccharides are offered by the high quantity of marine algae reaching the coast and discarded. About 9.1 million tonnes are discarded by fisheries annually.[32] whereas seafood accounts for 31% of the consumer-level food losses in the United States of America (USA).[33] Waste derived from forestry (softwood, grass, sawdust, and hardwood) offers biomass with a high lignocellulosic nature at a low cost. Compared to waste from agriculture, forestry-lignocellulosic waste requires intensive physico-chemical treatments given the more complex structure of the cell wall.[34] Thus, to extract important and valuable materials, hydrolysis or thermo-chemical processes are applied to this type of waste. Considering the projected global production of eggs of ninety million tons by 2030,[35] eggshells are a classic example of terrestrial animal waste with portions still useable after they are discarded. Eggshells are a source of hydroxyapatite after calcination and following treatment with salts, for instance $Ca_3(PO_4)_2$.[36,37] Porcine or bovine bones also offer good access to hydroxyapatite. After the removal of residual protein through alkaline process and calcination at a high temperature, yields of $\sim$65 wt% are attained.[38] Compared to synthetic hydroxyapatite, bio-derived hydroxyapatite displays traces of Na^+, Mg^{2+}, K^+, Zn^{2+}, Al^{3+}, Sr^{2+}, F^-, Cl^-, SO_4^{2-}, and CO_3^{2-}, which are valuable for stimulating the proliferation functions of cell.[39] Bovine bones may be utilized for the extraction of collagen fibres that are mineralized.[40]

There are numerous sources of bio-waste with promising compositions from aquatic origin such as fishes (hyaluronic acid, skin collagen, and gelatin after denaturation), red algae (carrageenans and agarose in the cell wall), brown algae (cell wall-based alginate), chitin (chitosan found upon chitin deacetylation), cephalopod endoskeleton (β-chitin, proteins, and lipids), and cuticles from marine arthropods (varying protein-enfolded alpha-chitin nanofibrils). Agricultural bio-waste include cereals (rice, corn, wheat, rye, barley, oats, *etc.*), fruits (grapes, oranges, apples, coffee, mangoes, bananas, apricots, pineapples, *etc.*), vegetables (carrots, tomatoes, olive husks, onions, potatoes, and red beet), and legumes (lentils, cow peas, lupins, beans, chickpeas, *etc.*). Bio-waste from forestry origin includes (grass, softwood, hardwood, sawdust, cellulose,

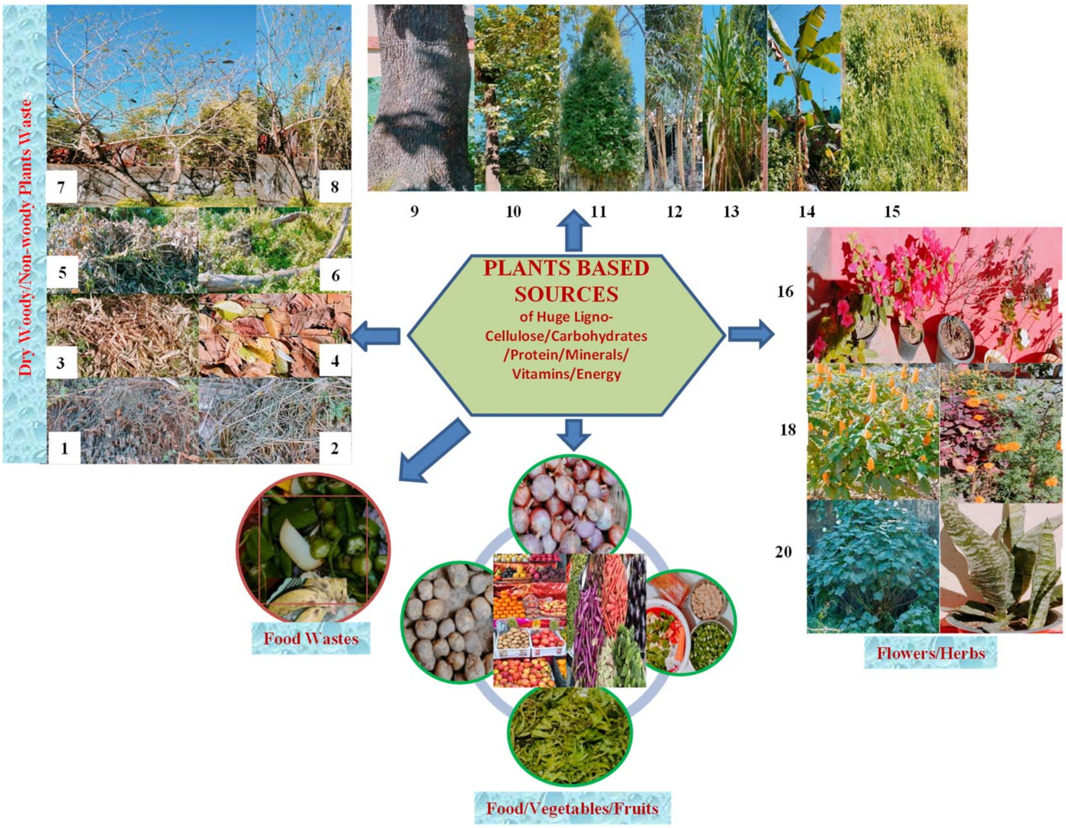

Fig. 2 Plants (dead and/or green) as a huge source of lignocellulose, lignin, cellulose, hemicellulose, small carbohydrates proteins, vitamins and several biologically organic compounds (1–8: dead or dry woody or non-woody plants/leaves as bio-wastes; 9–15: green woody and non-woody plants; 16–21: varieties of flowers/herbs (green) become bio-wastes).

lignin, and hemicelluloses). Fungal bio-waste is composed of glucans, chitin, glycoproteins, and melanin. Bio-waste from terrestrial animal origin include eggshells ($CaCO_3$, organic matter, $MgCO_3$, and $Ca_3(PO_4)_2$), bones with natural hydroxyapatite and collagen fibres, which are mineralized, feathers with beta-keratin, manure (carbon, oxygen, hydrogen, nitrogen, and sulphur), wool with alpha-keratin, fat, impurities, *etc.*, structural proteins derived from animals (silk, collagen, and gelatin), and exoskeletons of terrestrial-arthropods (protein-enfolded chitin fibres).[31]

Parts of plants such as fruits, tubers, and seeds, from different crops such as sunflower, rapeseed, palm, cotton, corn and soybean have been utilized in the generation of first-generation ethanol and biodiesel (Fig. 3),[41] while complete biomass of above-ground plants called lignocellulosic feedstock (inedible leaves and stems) has been utilized for the creation of second-generation biofuels.[41] Switchgrass, sorghum, miscanthus, and eucalyptus are some biofuel crops and a few of them have adaptability to deprived soils and marginal agronomic lands.[42] Olives, coconut and endocarps/shells of eastern black walnut are feedstocks with a high density and contain the maximum content of lignin among the recognized organs of plants, where endocarp-derived energy is equivalent to coal.[43] Worldwide, several million tons of biomass from drupe endocarp are available (24–31 million tons),[44] which is greatly underutilized, and thus countries with energy scarcity can benefit from its proper management.[43] Agricultural and industrial waste are also important sources of lignocellulosic materials and can be used for the generation of biofuels. Lignin, cellulose, and hemicellulose are the major components of the plant cell wall, while proteins, organic acids, and tannins together with secondary metabolites are its minor components. The composition of lignocellulosic materials varies with species, plant parts and ecological conditions.[43,45]

3 Methods for the pretreatment of organic waste

Mainly two types of waste are generated by daily garbage, in which the first type includes non-biodegradable wastes (*i.e.* metals, plastics, and glass), while the second includes biodegradable wastes such as leftover foods, dried leaves, and fruits.[46] Several animal-based products, plant-linked products, garden waste, food waste, and degradable carbon are examples of biodegradable organic waste. Energy may be provided as biogas by using the organic waste recycling method of anaerobic digestion.[46] The efficiency of non-organic recycling is also improved by the separation of organic and non-organic wastes.[47] Minimizing the pollution in the water, air, and land by recycling organic waste is its one of the promising advantages. In the case of less effective techniques (disposal and incineration), the amount of garbage left over is also minimized by the recycling of organic waste. Organic waste stabilization offers value by enhancing the content of nutrients and availability for utilization as fertilizer in the agriculture sector.

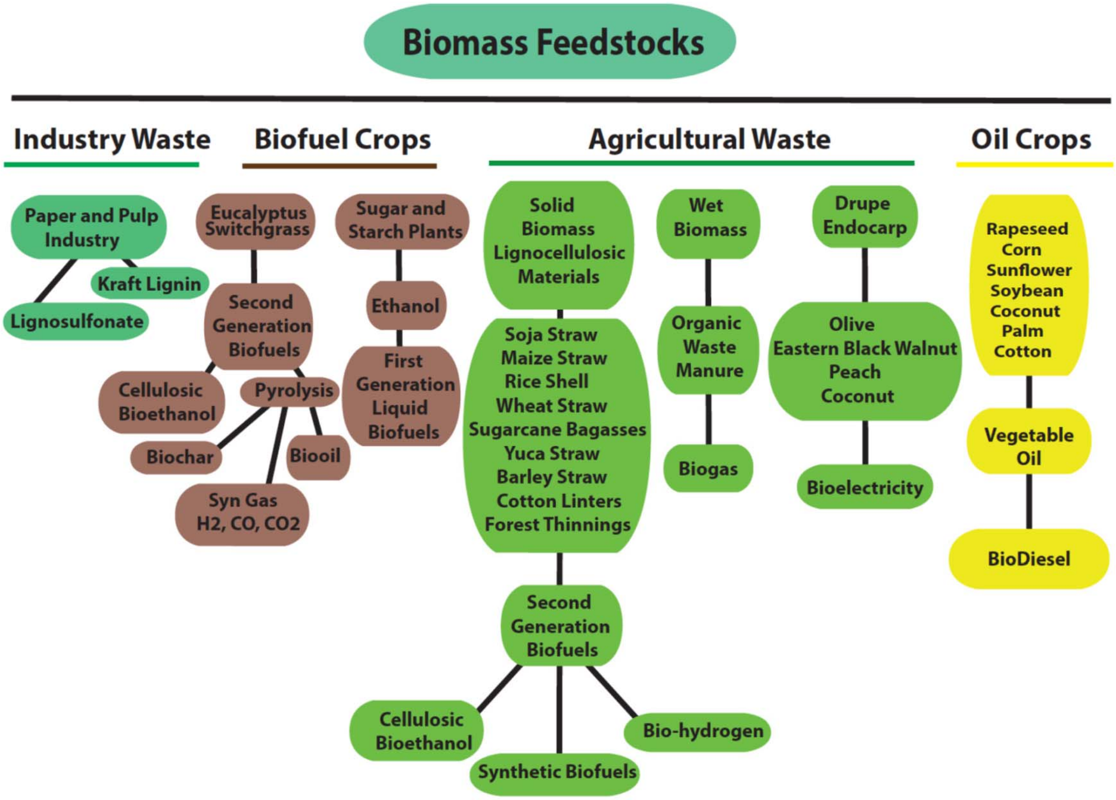

Fig. 3 Different types of biomass and their application in biofuel, bioenergy and bio-product generation.[41]

Table 1 Simplified and comparative presentation of some pretreatment techniques, their potential features, drawbacks and types

S. N.	Pretreatment techniques	Potential features	Potential drawbacks	Potential types
1	Mechanical treatment	(i) Size reduction without producing any products and chemical alteration (ii) No chemical needed (iii) Its use before biological or other pretreatment methods is needed and enables the easy handling, transport and processing of even a big density of lignocellulosic materials	High energy consumption	High pressure homogenization technique, ultrasonication, *etc.*
2	Thermal pretreatment	(i) Helps in complex structures' hydrolysis (ii) Helps in enhancing the anaerobic digestion	Mostly high temperature requirement	Low temperature-based treatment, high temperature-based pretreatment
3	Microwave-assisted pretreatment	A type of heat pretreatment and helpful in the stabilization of wastes	Electromagnetic radiation required	—
4	Chemical based pretreatments	(i) Chemical constituents of bio-wastes are broken down using oxidants, alkali, and acids (ii) Helping in the solubilisation of sludge	Harmful/toxic chemicals or reagents required Used chemicals may be harmful for environment and human health	Pretreatment using alkali, pretreatment using acids, ozonation, peroxidation, *etc.*
5	Biological methods of pretreatment	(i) Microbial utilization for the degradation of organic waste (ii) No adverse conditions are required (iii) Efficient in biodegradation, valuable products formation, bioconversion and biogas production (iv) Bacterial, fungal and enzymatic methods are very effective techniques	Comparatively time consuming process, contamination possibility, need of sterilization, works in optimized conditions like pH, temperature, concentrations, *etc.*	Bacterial method of pretreatment, fungal method of pretreatment, enzymatic ways, combined methods
6	Combination of methods	(i) Utilization of two or more different methods for the pretreatment of organic waste (ii) More effective and efficient	Depends on methods combined for pretreatment	Thermal-chemical methods, chemical–biological techniques, physical–chemical–biological methods, other possible methods

Popular concepts such as zero-waste strategies, cleaner production, sustainability, and bio-based circular economy are supported by the recycling of organic waste.[46,48,49] Different barriers in the management of organic waste have also been assessed by Kharola *et al.* (2022).[46]

The generation of biogas from organic waste may be a promising factor for a sustainable future. Organic waste utilization for biogas generation applying the methods of mono- and co-digestion has been broadly reported.[25] The reactions in the production of biogas occur in many stages, among which hydrolysis is crucial as the 1st step and helps in enhancing the complete yield. The optimization of the hydrolysis step causes the decomposition of complex organic matter into large quantities of monomeric/oligomeric components, which can simply be used under anaerobic conditions for biogas production. The aim of pretreatment approaches is making the existing nutrients available to the maximum species of microbes that accelerate the use of biomass for the duration of anaerobic digestion.[50] In the review by Mitraka *et al.* (2022), they comprehensively discussed several pretreatment methods for the increased production of biogas from sewage sludge.[18] There are many methodologies for the pretreatment of organic wastes for biogas production,[18,25] which are concisely discussed herein (Table 1).

3.1 Mechanical pretreatment

Mechanical pretreatment causes a reduction in the size of organic waste particles and does not generate any products.[25] It is a process that consumes energy, which is its main drawback. Developments in milling approaches display that compared with the dry milling procedure, wet milling is better because of its greater pulverization properties with minimal consumption of energy.[51] de Oliveira *et al.* (2022) investigated two wet mechanical pre-treatments on bio-waste from urban household, which were air-compressed press and worm screw press.[52] In each experiment, they studied two liquid/solid ratios. An enhancement in the biodegradable organic matter proportion extracted from bio-waste was observed with an increase in the ratio of liquid to solid in the pre-treatment up to 949 g COD per kg TS from the household bio-waste. In a constantly stirred tank-based reactor, a very good COD load conversion (81%) and high methane production of up to 345 L CH_4 per kg VS were achieved by anaerobic digestion.[52] Cesaro *et al.* (2021) assessed the potential of press-extrusion pretreatment to enhance the anaerobic degradation of the organic part of solid municipal waste.[53] Among the mechanical pretreatment methods, recently press-extrusion attracted great interest for its probable use to either increase the organic weight in the digester or enhance the overall stability of the process and methane yield.[53] To improve the production of bio-methane, Chevalier *et al.* (2023) successfully evaluated the influence of the mechanical treatment method *via* twin-screw extrusion using different types of lignocellulosic biomass and they tested two dissimilar shear stress screw profiles.[54] The specific rate was found to be enhanced by both extrusion mechanical treatments, which was proven by the kinetic assessment of the production of methane.

3.1.1 High pressure homogenization technique (HPH). The high potential of disintegration, minimal costs of operation, handling and operation simplicity without chemical variations are a few of the benefits associated with the HPH method.[25,55] Sun *et al.* (2022)[56] used HPH as a potential method to treat soybean protein isolate. The spatial structure of insoluble soybean protein isolate was destroyed by pretreatment using HPH; consequently, the particle size of the soybean protein isolate dispersion significantly decreased.[56] Nabi *et al.* (2022)[57] explored the improvement of the anaerobic breakdown of sludge by combining HPH with FNA (free nitrous acid) pretreatment. In comparison with individual HPH treatment and FNA treatment, triggered sludge was efficiently solubilized by HPH–FNA pretreatment, and thus there was subsequent improvement in the anaerobic breakdown process.[57] The cumulative generation of biogas from combined HPH–FNA pretreated sewage sludge was 154%, 108%, and 284% higher than that by free nitrous acid, HPH, and raw sludge single pretreatment, respectively. The content of methane in the biogas was 45%, 51%, 55% and 65% for the raw sludge, free nitrous acid, HPH, and HPH-free nitrous acid pretreated sludge, respectively.[57] HPH has also been utilized for the recovery of agri-food remains.[58] Malik *et al.* (2023)[59] explored the potential of HPH technology in the development of functional foods. In another study, HPH was optimized and used for the intensification of the recovery of bioactive components from tomato byproducts.[60] A significant improvement in the biogas yield from the anaerobic digestion of sludge was achieved using the HPH method.[61] Also, there are numerous other studies on HPH utilization in biowaste management, which have been omitted herein to maintain the focus on the main subject.

3.1.2 Ultrasonication. Ultrasonication was described as the most effective pretreatment method by Pilli *et al.* (2011),[62] where the effectiveness of this process is exclusively reliant on the features of the biowaste/sludge. Based on the literature availability, ultrasonication has been extensively described for wastewater pretreatment, sludge pretreatment, and manure pretreatment in anaerobic digestion processes for the generation of biogas.[25,63–65] A recent review on the ultrasonic processing of food waste was reported by Wu *et al.* (2022),[66] presenting detailed insights into the use of ultrasound in the processing of food waste. Karouach *et al.* (2020)[67] evaluated the influence of CMUP (combined mechanical and ultrasonic pretreatment) on the anaerobic digestion of the household organic waste fraction. They compared the results gained by the experiment with mechanical pretreatment and CMUP. Mechanical pretreatment was taken as control in this study. The yield of methane from the control and combined mechanical and ultrasonic pretreatment (CMUP) biodegradation was 382 mL CH_4 per g VS (at 0 °C temperature, 1 atm pressure), 72%, and 493 mL CH_4 per g VS (at 0 °C temperature, 1 atm pressure), 86%, respectively, displaying the enhancement in biodegradability and the production of methane by CMUP. The results also suggest that the hydrolysis stage and methanogenesis stage of the procedure were upgraded by the combined pretreatment method.[67] Ultrasonication was successfully utilized to improve the methane production from sewage sludge.[68] Ultrasound-assisted technology has been reviewed for the valorization of bio-waste.[69] There are also several studies on the application of ultrasound.[70,71]

3.1.3 Utility of mechanical pretreatment in biological (fungal) pretreatment. There are several commonly used mechanical mechanisms for the size reduction of lignocellulosic organic waste such as cutting, shearing between flat surfaces, tearing, compression and breaking the materials.[72] Milling, grinding, ultrasonication, refining, and many others are also the widely utilized mechanical methods for particle reduction. Mechanical treatment of lignocellulosic materials or organic biowaste is a necessary step in the process of pretreatment to maximize the valorization potential of materials.[72] Thus, it has great application in biorefineries. Milling is a mechanical process generally applied to materials before the start of any treatment process. Thus, it may have great applicability in the fungal-based biological pretreatment process to reduce the size of biowaste materials for the efficient use of these reduced materials in bioreactors with fungal systems (fungal mycelia and/or fungal enzymes). The use of mechanical treatment before the fungal or other treatment does not cause any chemical alteration in the materials to be treated and improves the effectiveness of other pretreatment processes that are applied after the mechanical method.[72] The need for lignocellulosic materials or biowaste in bulk amounts causes difficulties in handling, transportation and processing for the fungal or other biological pretreatment process in bioreactors but the use of mechanical treatment before the fungal pretreatment process makes the handling, transfer, and processing of these biowaste easier on a large scale. Optimization of the mechanical pretreatment process is necessary because it is a process that consumes a large amount of energy. Various types of mills can be utilized but the selection of equipment is based on the type and properties of materials, final required size of materials, and operational systems such as continuous or batch systems and bioreactors.[72] In the literature, different types of equipment have been used for continuous milling before the biological or other methods (as the final treatment process) such as disc refiner,[73] screw extruder,[74] knife mill,[75] hammer mill,[76] and many others.[72]

3.2 Thermal pretreatment

The thermal pretreatment method helps in hydrolyzing the complex organic components in organic waste and has been employed to increase their anaerobic digestion.[25] El Gnaoui *et al.* (2022)[77] evaluated the effect of thermal pretreatment, including thermal pretreatment at temperatures of 60 °C and 80 °C for 60 min, and thermal pretreatment at 100 °C, 120 °C, and 140 °C for 30 min, as well as pre-hydrolysis (biological) at temperatures of 37 °C, 55 °C, 37 °C, followed by temperature of 55 °C and 55 °C followed by 37 °C for 40 h on the anaerobic breakdown of food waste in a batch test. The pre-hydrolysis (biological) and thermal pretreatment method resulted in an enhancement in the soluble COD and efficiency of hydrolysis. There was an increase in the yield of methane from 371.17 mL CH_4 per g VS for untreated food waste to 471.95 mL CH_4 per g VS. The greatest methane yield was observed for biological pre-hydrolysis at a temperature of 37 °C for 20 h, followed by 55 °C for 20 h. The rate of formation of biogas increased and the lag phase decreased applying the pretreatments.[77] There are also many other recent reports in the literature on thermal pretreatment.[78–80]

3.3 Microwave pretreatment

Microwave pretreatment is also a type of heat pretreatment method. Besides the generally utilized thermal pretreatment method, microwave pretreatment is effective in the stabilization of organic waste and biogas formation.[25] This method has a few benefits, which include quick heating and penetration, simple handling and control, pathogen elimination, and effective dewaterability of sludge and sludge reduction,[81] due to which it is more efficient in comparison with the conventional thermal pretreatment methods. The effect of microwave pretreatment on the anaerobic fermentation of model food waste to organic acids with small chains and ethanol was investigated.[82] Microwave pretreatment was studied at 120 °C, 150 °C, and 180 °C (three temperatures) and residence times of 2, 5 and 8 min. The highest decrease in the volatile suspended solids (VSS) was 20%, representing the solubilisation of organic matter. There was a greater (17.5% COD per COD) total product yield in the fermentation batch tests in comparison with the untreated substrate (11.1% COD per COD).[82] The influence of microwave-based pretreatment on the anaerobic co-digestion of sludge and food waste was described by Liu *et al.* (2020).[83] The results displayed that microwave pretreatment was beneficial for the dissolution of organic materials, protein conversion to NH_4^+–N, cumulative production of CH_4, unit yield of bio-methane, and methane formation reaction rate in the sludge and food-based waste anaerobic system of co-digestion. In the co-digestion system, the maximum cumulative production of CH_4 reached 3446.3 ± 172.3 mL (35 days), which was 19.93% greater than that of the control. Moreover, the microwave pretreatment method considerably enhanced the accumulation of volatile fatty acids and content of butyric acid in the anaerobic-digested effluent.[83] Hydrolysis of the cassava pulp was studied by Prasertsilp *et al.* (2023)[84] using the microwave method for the effective utilization of natural materials and four different factors such as liquid–solid ratio, acid type, watt power and time were investigated. A high glucose content was provided by this study, exhibiting 88.1% conversion. There are several other relevant studies on microwave-assisted pretreatment technology.[85,86]

3.4 Chemical pretreatment methods

In the chemical pretreatment method, oxidants, alkali, and/or acids are used to breakdown the organic components of organic bio-waste, which has been found to be very effective. Ozonation and peroxidation (oxidation) are found to be beneficial in the pretreatment, causing the solubilisation of sludge.[25] Up to a certain limit, there is a dose-dependent association between the concentration of oxidant and solubilization of sludge. Therefore, the peroxidation and ozonation process tends to display a greater rate of sludge degradation, this compromising the biogas yield.[87,88] In the study by Alino *et al.* (2022),[89] they assessed the effectiveness of a low-cost and more sustainable method to enhance the biodegradability of sugarcane bagasse and enhance the generation of methane by its pre-collection with acidic types of organic bio-waste (such as cheese whey, fruit waste, and vegetable waste). They obtained the best result with sugarcane bagasse plus fruit and vegetable waste (5 : 95 ratio) of 520 ± 7 NL CH_4 per kg VS (27.6% greater than the control) with a decrease in the degradation time (T90) from 13 days to 7 days. The yield of methane increased by 21.2% and 34.1% upon alkaline pretreatment with sodium hydroxide at concentrations of 5% and 10%, respectively.[89] Jankovičová *et al.* (2022)[90] performed a study on the hydrolysis of materials in NaOH (0.5% and 5%) and H_2SO_4 (0.5% and 5%) at 90–100 °C for 2 h. The influence of these techniques on the lignocellulosic constitution of rapeseed straw, maize based waste, and wheat straw and the biogas yield was compared. The 0.5% NaOH pretreatment enhanced the production of biogas the most (for rapeseed straw by 159%, wheat straw by 240% and maize waste by 59%); furthermore, the solubilization degrees were greater.[90] The study by Sreevathsan *et al.*, (2023)[91] on the effects of ozonation on the biodegradability improvement and bio-methanation ability of wastewater pretreatment and study by Qiao *et al.* (2023)[92] on the pretreatment of landfill leachate state the advantages of chemical pretreatment.

3.5 Combination of chemical pretreatment with other pretreatment methods

Chemical pretreatment methods including the utilization of alkali and acid are typically used in combination with additional treatment methods.[93–95] The combination of pretreatment techniques is beneficial for improving the solubilization, sanitation, dewaterability and anaerobic digestion of sludge.[25] The influence of thermal and combined thermal-chemical pretreatments was investigated by Ahmed *et al.* (2022)[96] on the solubilization of organics, yield of biogas, formation of recalcitrant, and energy efficiency at varying temperatures and alkali dosages. The organic fractions of municipal solid waste were subjected to thermal pretreatment (*i.e.* 100–200 °C temperature, 1.6–15.8 bar pressure, and 30–120 min reaction time) alone and in conjugation with different dosages of alkali (1–7 g per L NaOH). Compared to the control (331 mL per g VS_{added}), the maximum biogas production increase of 43% (474 mL per g

VS_{added}) and 87% (618 mL per g VS_{added}) was observed at a temperature of 125 °C and 125 °C + 3 g per L sodium hydroxide (NaOH) dose, respectively.[96] Pham *et al.* (2021)[97] performed acid-catalyzed hydrolysis at temperatures of 120 °C, 150 °C, and 180 °C utilizing H_2SO_4 (sulfuric acid) with concentrations in the range of 0–0.5 M within 90–180 min reaction time to yield bio-based chemicals from sewage sludge. The maximum yield of xylose was 7.69 mol%, while the maximum yield of glucose was 5.22 mol% at a temperature of 120 °C, H_2SO_4 concentration of 0.5 M within 180 min reaction time. Moreover, under acid-catalyzed hydrolysis at a temperature of 180 °C and 180 min, at 0.5 M H_2SO_4, levulinic acid production reached the highest level of 0.48 mol%, while at 0.1 M H_2SO_4, the maximum production of 5-hydroxymethylfurfural was 1.66 mol%.[97] Qiao *et al.* (2023)[92] described the importance of combined physicochemical methods for the landfill leachate pretreatment process. Combined pretreatment methods are more useful techniques in pretreatment processes compared with individual pretreatment methods. In another study, the effect of combined pretreatment on plant waste was studied using physical, enzymatic and chemical techniques, which also proved that combined methods are more favorable.[98]

3.6 Biological pretreatment methods

The biological pretreatment of organic waste or bio-waste is a promising ecofriendly method, which is based on the use of mainly fungi, bacteria, and enzymes. Their use in the pretreatment of organic waste is very effective in the decomposition of large cellulosic or other organic materials into monomeric or smaller units and generating valuable biogas. Table 2 (ref. 78) shows the variation in the production of biogas from some lignocellulosic biomass after a low-temperature pretreatment process under biological conditions.[99–106] In biological pretreatment, among the various microorganisms, fungi are of the utmost significance and filamentous fungi, specifically *Basidiomycetes*, have great potential in delignification and lignocellulosic biomass conversion owing to the effective involvement of their enzymatic machinery.[15] In bio-waste processing, microbial play promising roles in the improvement of the production of biogas and the process can be enhanced by using various metagenomics approaches.[107] The reports by Mitraka *et al.* (2022)[18] and Salihu and Alam (2016)[25] also presented a detailed discussion on biological pretreatment. Bacterial pretreatments are concisely discussed herein with a few recent reports because the focus is to comprehensively discuss fungal pretreatment techniques.

3.6.1 Bacterial pretreatment of organic waste. Bacteria have been found to be effective candidates for the pretreatment of organic solid waste. *Bacillus licheniformis* and *Bacillus oryzaecorticis* can be utilized in bio-waste management given that they have shown to play a promising role in the degradation of food waste. Starch was degraded by *Bacillus oryzaecorticis* and a large amount of reducing sugars was found to be released, providing hydroxyl and COOH to fulvic acid molecules, while a positive result on the structure of humic acid was shown by *Bacillus licheniformis*, which had greater hydroxyl (OH), methyl (CH_3), and aliphatic groups.[108] Liu *et al.* (2022)[109] isolated the nitrogen (N)-fixing and lignin-decomposing bacterial strain *R. ornithinolytica* RS-1 from an abandoned termite colony. To increase the enzymatic saccharification and degradation in corn stover, they utilized this strain for lignin depletion, combined with mild NaOH (2.5%) pretreatment for further hemicellulose depletion. After only 7 days, bacterial strain RS-1 degraded lignin with 19% reduction, whereas the relative cellulose content was enhanced by 21%. Moreover, the conversion of cellulose in corn stover was found to reach 48.58% through a 2-stage process using sodium hydroxide (2.5%) pretreatment. Meanwhile, the considerable removal of lignin and hemicellulose was observed. Furthermore, the highest activity of manganese peroxidase was found on day 3 (181.0256 U L^{-1}), while the highest activity of lignin peroxidase was on day 5 (37.473 U L^{-1}).[109] Song *et al.* (2021)[110] presented an evaluation of the anaerobic and micro-aerobic pretreatment of paper waste with various oxygen loadings using 5 microbial agents including composting inoculum, cow manure, straw-decomposing inoculum, digestate effluent, and sheep manure. The results showed that the paper waste pretreated by digestate effluent with a 15 mL per g VS oxygen loading demonstrated the maximum cumulative CH_4 yield of 343.2 mL per g VS, with biodegradability of 79.3%. Besides digestate

Table 2 Variation in the production of biogas/methane from lignocellulosic biomass after a low temperature pretreatment process[78]

S. N.	Lignocellulosic sources	AD condition/mode	Temperature/time	Yield of biogas/methane	Increase	Reference
1	67% wheat straw and 33% sunflower meal	Mesophilic/batch mode (45 days)	120 °C/1 hour 140 °C/1 hour	-/370 mL per g VS -/390 mL per g VS	8.8% 14.7%	99
2	Bean straw	Mesophilic/continuous mode (hydraulic retention time 4.5 days)	121 °C/1 hour	145 mL per g COD/-	—	100
3	Rice straw	Mesophilic/batch mode (30 days)	80 °C/6 hours	372.5 mL per g VS/-	12.4%	101
4	Rice straw	Mesophilic/batch mode (35 days)	100 °C/150 minutes 130 °C/150 minutes	128 L per kg TS/- 125 L per kg TS/-	22.8% 19.8%	102
5	Rice straw	Mesophilic/batch mode (50 days)	90 °C/15 minutes	307 mL per g TS/-	3.0%	103
6	Wheat straw	Mesophilic/batch mode (45 days)	120 °C/60 minutes	496 mL per g VS/-	22.8%	104
7	Wheat straw	Mesophilic/batch mode (30 days)	121 °C/60 minutes	—	29%	105
8	Sugarcane bagasse	Mesophilic/batch mode (30 days)	121 °C/60 minutes	—	11%	105
9	Switchgrass	Mesophilic/batch mode (1100 hours)	100 °C/6 hours	—	25.9%	106

effluent, straw-decomposing inoculum and sheep manure were likewise observed as promising microbial agents due to the quickening of the production of methane in the early stage of anaerobic digestion. It was demonstrated by the analysis of the microbial community that after anaerobic pretreatment using the straw-decaying inoculum, *Clostridium sensu stricto 10* and *Clostridium sensu stricto 1* possessed great relative abundance, whereas after micro-aerobic pretreatment by sheep manure, *Macellibacteroides* and *Bacteroides* were enriched, which all contributed to the degradation of cellulose. Besides, the degradation of lignin was probably promoted by aerobic *Bacillus* in the straw-decomposing inoculum and *Acinetobacter* in the sheep manure and digestate effluent only under micro-aerobic conditions. In the course of anaerobic digestion, *C. sensu stricto* 1, *VadinBC27*, *Caldicoprobacter*, and *Fastidiosipila* were the key bacteria that enabled the bio-decomposition of paper waste.[110] These works demonstrate the potential of bacteria in the biological pretreatment of organic waste and methane gas production.

3.6.2 Fungal pretreatment. Fungal pretreatment is one of the important biological pretreatment methods. Bio-waste, organic waste or lignocellulosic structures may be effectively treated with fungal systems (fungi and/or associated enzymes). There are numerous significant studies on the role of fungi in the pretreatment of these waste materials. Given that aim of this review is to presents insight into the role of fungi in the pretreatment process of biomass, this is comprehensively and independently discussed in next section as main heading.

4 Fungal pretreatment of organic waste

Due to their minimum energy condition and negligible toxicity, fungal pretreatment processes have attracted significant attention for their role in biomass conversion. They avoid the use of chemicals, hinder the production of compounds and show good selectivity towards lignin degradation.[111,112] Several choices are provided by the useful and copious species of fungi for biomass conversion. Rice straw, wheat straw, corn straw, peanut shells, coconut shells, bagasse, spent coffee grounds, food digestate, food waste, hardwoods, softwoods, switchgrass, *etc.* are the recognized biowaste successfully subjected to fungal treatment techniques. Table 3 shows the major chemical compositions of various types of biowaste/organic waste.[113–121] After the degradation of the lignocellulose structure, the cellulose and hemicellulose degradation efficiency in the biomass increases.[29] Fig. 4 shows the complex structure of lignin,[122] while Fig. 5 shows a schematic presentation of the degradation of cellulose. The competence of subsequent fermentation and accumulation of lipid is promoted by saccharification, and thus various downstream products can be formed from the conversion of biomass.[123,124] However, its popularization has been hindered due to the low efficacy of the fungal pretreatment process, but applying useful strategies may result in an improvement in the efficiency of fungal technology.[29] This section presents a comprehensive approach for fungal pretreatment technology in biomass conversion based on recent literature studies.

Table 3 Major components in some agricultural waste and food waste

S. N.	Common bio-waste	Composition		Reference
1	Wheat straw	Cellulose: 44.2%, hemicellulose: 26.5%, lignin: 22.4%, ash: 2.8%		113
2	Rice straw	Cellulose: 36.1%, hemicellulose: 27.2%, lignin: 19.7%, ash: 12.1%		113
3	Rice husk	Cellulose: 50%, lignin: 25–30%, moisture: 10–15% and silica: 15–20%		114
4	Poplar	Cellulose: 39.2%, hemicellulose: 18.8%, lignin: 29.6%, ash: 1.5%		113
5	Corn straw	Cellulose: 33%, lignin: 19.47%, cellulose sugar: 23.58%, ash: 5.12%		115
6	Peanut shell	Cellulose: 44.8%, lignin: 36.1% and hemicellulose: 5.6%		116
7	Bagasse	Cellulose: 35.2%, hemicellulose: 24.5%, lignin: 22.2%, ash: 20.9%		117
8	Coconut shell	Cellulose: 36.13%, lignin: 32.33%, hemicellulose: 20.36%, content soluble in water: 11.17%		118
9	Walnut shells	Polysaccharides: 49.7%, lignin: 30.1%, extractives: 10.6%		119
10	Almond shells	Polysaccharides: 56.1%, lignin: 28.9%, extractives: 5.7%		119
11	Pine nut shells	Polysaccharides: 48.7%, lignin: 40.5%, extractives: 4.5%		119
12	Corn stover fractions	Cobs	Cellulose: 37.8%, lignin: 13.5%	120
		Husk	Cellulose: 38.1%, lignin: 12.6%	
		Leaves	Cellulose: 39.3%, lignin: 17.6%	
		Stalks	Cellulose: 44.9%, lignin: 19.9%	
13	Food wastes	Fruits and vegetables wastes	Protein: 5.20%, fat: 1.36%, carbohydrates: 39.01%	121
		Waste of mixed vegetables	Protein: 15.3%, fat: 0.87%, carbohydrates: 83.83%	
		Dairy related products	Protein: 14.05%, fat: 28.43%, carbohydrates: 57.51%	
		Waste of cereal products	Protein: 11.71%, fat: 3.83%, carbohydrates: 84.98%	
		Bakery wares related wastes	Protein: 12.92%, fat: 6.03%, carbohydrates: 81.05%	
		Wastes of meat related products	Protein: 25.17%, fat: 57.74%, carbohydrates: 17.10%	
		Wastes of fish related products	Protein: 27.48%, fat: 65.53%, carbohydrates: 6.98%	
		Wastes from egg related products	Protein: 19%, fat: 73.06%, carbohydrates: 7.94%	
		Wastes from restaurants	Protein: 15.59%, fat: 19.05%, carbohydrates: 65.36%	
14	Rice wastes	Carbohydrates: 91%, protein: 8%		121
15	Spent coffee grounds	Protein: 39.88%, fat: 60.12%		121
16	Tea wastes	Carbohydrates: 76.59%, protein: 23.04%		121

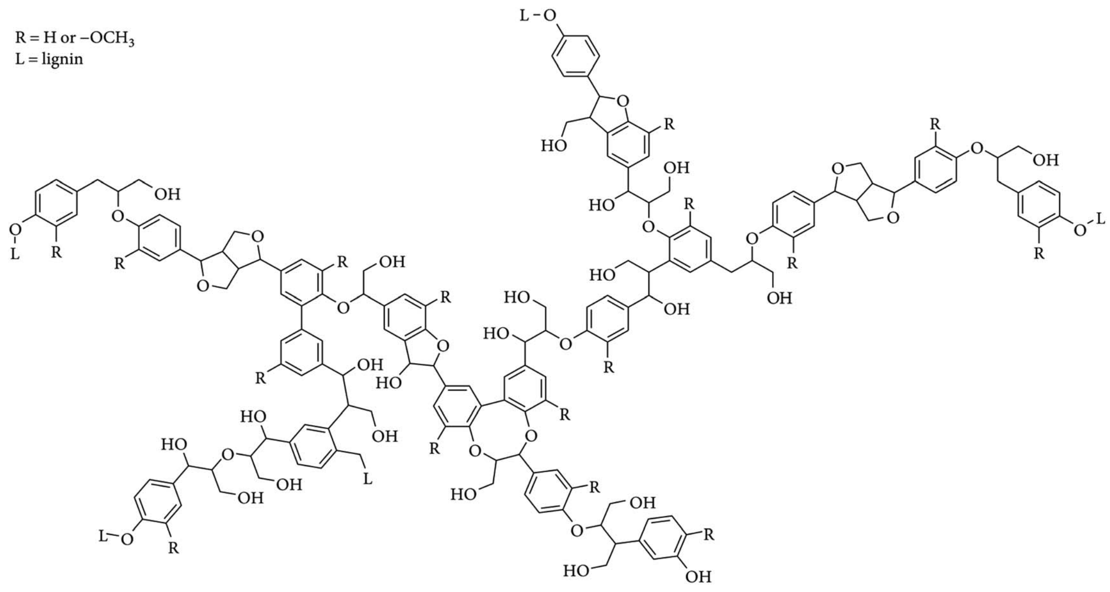

Fig. 4 Model structure of lignin.[122]

Different methods based on fungi have been adopted and the process of biomass/bio-waste valorization successfully performed in several studies, which are reviewed and their results presented in this section. Fungal pretreatment of organic waste or biomass is discussed here as the main heading because the principle target of this review is to comprehensively evaluate the various studies reported on fungal pretreatment technology in solving the problem of different organic waste or bio-waste and production of many useful products such as biogas, bioethanol, sugars, acids, and others.

4.1 Fungal pretreatment of lignocellulosic biomass of agricultural wastes

Fungi play a significant role in the decomposition of the various cellulosic materials (such as wheat straw, willow chips, rice straw, sugar bagasse, corn stover, and plant materials) and

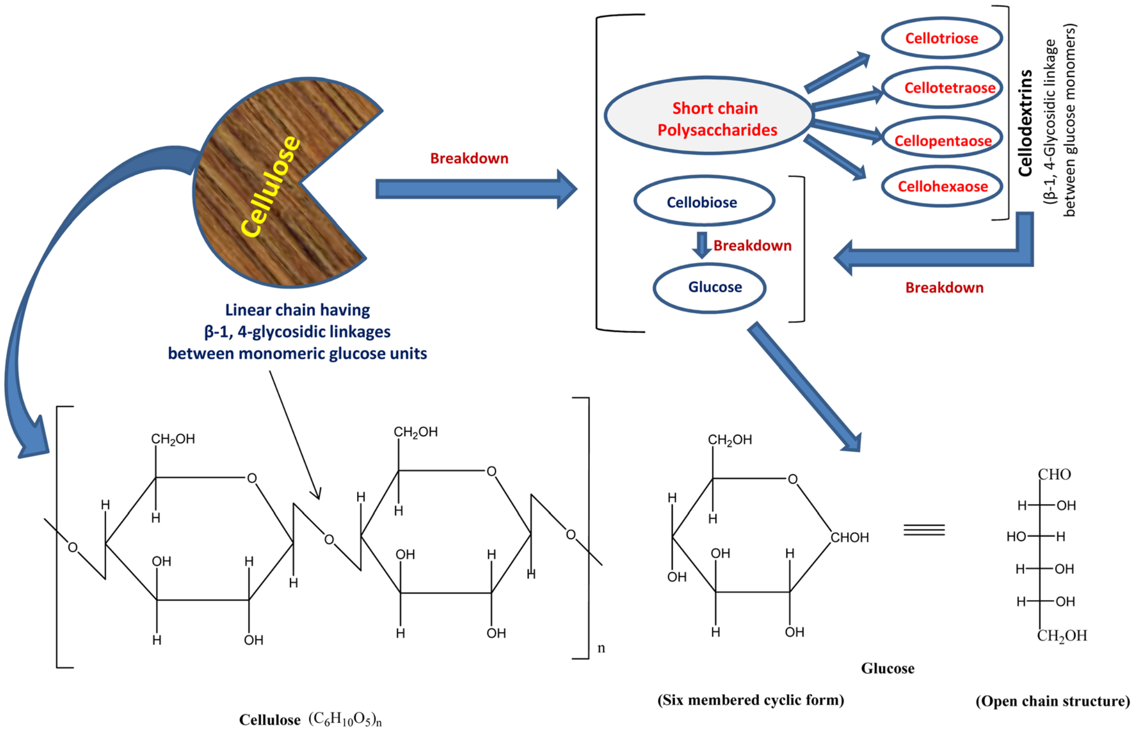

Fig. 5 Schematic presentation of cellulose degradation and its possible products.

agricultural waste. Lignocellulosic biomass contains a large amount of complex carbohydrates, *i.e.* 55–75% in total solids (TS), and is renewable as well as widely available, and thus it can be excellent feedstock for the production of energy.[125] It is well established that WRF may be involved in the improvement of enzyme-based hydrolysis and its subsequent sugar yield.[126] Therefore, its involvement in pretreating substrates, mainly for the generation of bioethanol,[125,127] has been studied but hardly for anaerobic digestion.[128] In the case of submerged fermentation for pretreatment, the solid-state fermentation (SSF) process has been found to be better, which permits greater loads of feedstock, prefers fungal enzyme attachment to the substrate, and the diffusion of oxygen. SSF needs less aeration, mixing, water, and heating, and consequently less expensive than liquid culture.[129]

Pretreatment of lignocellulosic materials such as wheat straw, woody willow chips, and corn stover was studied by Kovács *et al.* (2022)[130] with the help of four filamentous fungi, namely, *Penicillium aurantiogriseum*, *Gilbertella persicaria* (SZMC11086), *Trichoderma reesei* (DSM768), and *Rhizomucor miehei* (SZMC11005). The excellent production of a hydrolytic enzyme and maximum yield of biogas from the partly decomposed substrates were shown by *P. aurantiogriseum*. Corn stover was the best material for the breakdown of biomass and generation of the biogas. The most effective strain for the pretreatment and biogas production was *P. aurantiogriseum*. All the tested fungi preferred the corn stover substrate for the productivity of methane.[130] In 60 mL batch fermentation, within the first 20 days, a noteworthy portion of the generated methane (95%) was found to be evolved. The maximum yield of methane was observed in the corn stover-fed reactors. The maximum average production of methane (281 mL N per g oTS) was observed in the reactors pretreated with *P. aurantiogriseum*. During 300 mL batch fermentation, the influence of a 5-fold enhancement in volumetric scaling was also studied in the succeeding step. However, it was notable that only a 13.3% maximum difference (for the case of *P. aurantiogriseum* reactors) was observed between the outcomes of the two reactor sizes, showing the insensitivity of the overall process to scaling up. *G. persicaria*, *T. reesei*, and *R. miehei*-based pretreatment of all the substrates also showed the same behavior.[130] Biogas production from corn silage was found to be enhanced by *Pleurotus ostreatus* and *Dichomitus squalens*, while a negative impact was shown by *Trametes versicolor* and *Irpex lacteus*.[131] The cumulative production of CH_4 increased by 1.55-fold with *P. ostreatus* after 10 days at 28 °C, while a longer pretreatment duration (30 and 60 days) showed a lower effect. With distinctive corn silage, the CH_4 production increased from 0.301 to 0.465 m^3 per kg VS due to the depolymerization of lignin.[131]

Pallín *et al.* (2024)[132] utilized *Irpex lacteus* for the pretreatment of lignocellulosic biomass (wheat straw) and assessed its feasibility at a demonstration scale. Similar to submerged cultures, scaling up SSFs is not straightforward. Before choosing the best bioreactor design, several issues such as the microbe growth kinetics, agitation requirement, physical properties of the solid substrate, sterilization conditions, and agitation-generated mechanical stress should be addressed. During the process, the agitation-based design of the SSF reactor was ruled out because of the agglomeration of *Irpex lacteus* into wheat straw. After considering the several factors associated with the SSF reactor, an autoclavable vertical bioreactor of 22 L capacity was designed.[132] The digestibility of the wheat straw after 21 days of pretreatment in a solid-state fermentations bioreactor (60.6%) was similar to that found on a small scale, *i.e.* 57.9%. In the three bioreactor experiments (B1, B2 and PB), the sugar evolution was completely different after 21 days of treatment.[132] A 26.5% reduction in lignin was observed. There was greater reduction in lignin in experiment B2, *i.e.* 34.90% ± 0.87%, in comparison with that in experiment B1, *i.e.* 26.7% ± 3.08%. In the case of the PB fermentation, an increase was noticed for all the compounds in except lignin, which decreased by 52.3% ± 0.69%. This was the largest reduction in all the biomass constituents obtained in the three solid-state fermentation reactor experiments. There was similar reduction in lignin, *i.e.* 53.2% ± 0.60%, in the flask-scale pretreatment.[132]

Two isolated species of fungi, namely, *Trichoderma harzianum* and *Aspergillus terreus*, were used to investigate the degradation of cellulose, followed by bioethanol generation from acid-thermal pretreated rice straw, and the experiment was conducted in two phases.[133] In the first phase, *Aspergillus terreus* and *Trichoderma harzianum*, isolated cellulose degrading fungi, were used for the enzymatic hydrolysis of pretreated rice straw, which was divided into HL (hydrolysate liquid) and RP (residual pulp), while in the second phase of the experiments, the substrate (enzymatically hydrolyzed) was subjected to yeast fermentation for the generation of bioethanol. The results showed the degradation of 80% cellulose by these fungi. The performance of *Aspergillus terreus* in the degradation of cellulose with hydrolysate liquid and residual pulp was 92% and 80%, while that for *Trichoderma harzianum* was 93% and 82%, respectively. With the use of *A. terreus*, the glucose formation from cellulose during enzyme hydrolysis was 12.15 g L^{-1} and 16 g L^{-1}, while it was 10.8 g L^{-1} and 21.6 g L^{-1} using *T. harzianum*, respectively. The underlying mechanism of this process involves the action on the β-1,4-glycosidic bonds by the cellulose-degrading fungi through enzymatic activities only after the pretreatment process. The treatment of rice straw RP with *T. harzianum* led to a greater bioethanol yield of 5.4 g L^{-1}, while that for *Aspergillus terreus* was 4.7 g L^{-1}.[133] The pH of the enzymatically pretreated reactors using *A. terreus* was 3.8 for the control, 4.8 for the hydrolysate liquid, and 4.5 for the residual pulp, while in the case of the *T. harzianum*-based reactors, the pH values were 5.0, 4.9, and 4.8, respectively. The substrate and inoculum types are the major factors in the production of bioethanol in the pH range of 3.5–6. In the reactors based on hydrolysate liquid as the substrate, the performance of *A. terreus* and *T. harzianum* in the production of bioethanol was the same at 4.5 g L^{-1}.[133]

A genuine challenge in the production of bioethanol from rice straw is to remove lignin properly from biomass through a pretreatment process because lignin removal is necessary to generate bioethanol from rice straw effectively by the saccharification and fermentation process.[134] This challenge can be addressed through the use of alkali-assisted pretreatment and acid thermal pretreatment of rice straw. Devi and Munjam[133] successfully used *A. terreus* and *T. harzianum* in the production of bioethanol from rice straw, which was pretreated using an acid-thermal method. Alternatively, the study by Takano and Hoshino[134] showed the use of an enzyme cocktail (optimized) and *Mucor circinelloides* (xylose fermenting fungus) in the production of ethanol from alkali-pretreated rice straw by concurrent saccharification and solid state fermentation. Abo-State *et al.*[135] subjected rice straw to steam treatment (autoclaving) and various gamma irradiation doses of 50 and 70 mrad. Subsequently, different fungal isolates were used to enzymatically treat the steam-treated rice straw throughout the SSF process.[135] Therefore, any pretreatment processes that are effective in the removal of lignin from rice straw can be used and combined with the fungal treatment process for the production of bioethanol by saccharification and SSF process.

Furthermore, the effect of alkali sodium hydroxide (NaOH)/hydrogen peroxide (H_2O_2)-based various pretreatment approaches on willow sawdust biomass was described by Atitallah *et al.* (2022)[136] utilizing the conventional yeast, *i.e.* *Saccharomyces cerevisiae*, and three non-conventional strains of yeasts including *Pachysolen tannophilus*, *Wickerhamomyces anomalus* X19, and *Pichia stipitis*. The results showed that greater delignification, *i.e.* 38.3% ± 0.1%, saccharification efficiency, *i.e.* 31.7% ± 0.3%, and ethanol yield were achieved by the 2-stage pretreatment method, *i.e.* 0.5% w/v NaOH for 24 h and 0.5% v/v H_2O_2 for 24 h. Ethanol yields in the range of 11.67 ± 0.21 to 13.81 ± 0.20 g/100 g TS were observed by the *Saccharomyces cerevisiae* or *Wickerhamomyces anomalus* X19 monocultures and co-cultures with *Pichia stipitis*. *W. anomalus* was selected as the non-conventional strain due to its high efficiency in bioethanol production, whereas *S. cerevisiae* was utilized as the highest exploited strain of yeast for the production of bioethanol from sugar fermentation. There was reduction in hemicellulose to 1.3%, 18.9%, 25.1%, and 21.4% when willow sawdust was subjected to different pretreatment approaches, *i.e.* A (sodium hydroxide), C (sodium hydroxide and hydrogen peroxide mixture), D (initially sodium hydroxide, followed by hydrogen peroxide) and E (initially hydrogen peroxide, followed by sodium hydroxide), respectively (approach B, *i.e.* H_2O_2, is omitted here). Among them, the maximum removal of lignin (38.3%) was observed for approach D.[136] The use of co-cultures for bioethanol fermentation is generally considered beneficial over monocultures because of the synergistic action of the metabolic pathways of the involved microorganisms.[137] In another study, filamentous fungi such as *Rhizomucor miehei*, *Aspergillus nidulans*, *Gilbertella persicaria*, and *Trichoderma reesei* were tested for the pretreatment of dry CS (corn stover), WS (wheat straw) and WWC (willow wood chip).[138] *A. nidulans*-based pretreatment doubled the yield of methane compared with the untreated corn stover. Pretreatment with *G. persicaria* and *T. reesei* also showed noteworthy differences in the production of bio-methane in comparison with the samples having only untreated plant substrates, respectively. Outstanding activity by endo-(1,4)-β-D-glucanase on willow wood chip and corn stover, and great activity by β-glucosidase on willow wood chip were shown in the case of *A. nidulans*. Consequently, the *A. nidulans*-based pretreatment of the samples generated the highest biogas yield for all the involved raw substances. This study recommended the use of a short pretreatment time for cellulose-abundant substances, which in definite cases may double the yield of biogas.[138]

Phanerochaete chrysosporium, among the WRF, is recognized for its selective lignin breakdown and numerous applications in biotechnology.[139] Pretreatment of the abundant wheat straw (WS) can be done by applying WRF, which transforms cellulose (complex plant biomass) into glucose.[140] This sugar can be used by *Pichia fermentans* and IAA (indole-3-acetic acid) may be produced in the presence of tryptophan. Besides effective WS pretreatment in the course of primary fermentation, *Phanerochaete chrysosporium* may also generate IAA in the presence of tryptophan,[141] which may further participate in enhancing the production of IAA in the course of secondary fermentation.[142] In one study, the *P. chrysosporium* (150 μg mL^{-1})-based pretreatment of WS resulted in a 9-fold enhancement in IAA in comparison with untreated WS (16.44 μg mL^{-1}).[142] IAA was produced in the range of 1.99–129.33 μg mL^{-1}.[142] The WS was pretreated with *Phanerochaete chrysosporium* for releasing the sugar in medium, which could be used by *Pichia fermentans* for the production of IAA. A considerable amount of sugar was released by *P. chrysosporium* from the 2nd day onwards and was the highest on the 9th day (0.89 mg mL^{-1}).[142] The production of IAA using yeasts was also described earlier.[143] Less than 25 μg mL^{-1} was produced by *Pichia guilliermondii* and *Hanseniaspora uvarum* when they were inoculated in the medium based on yeast extract-dextrose after incubation for seven days.[144] Furthermore, an endophytic yeast found in roots of maize, *Williopsis saturnus*, was observed to generate indole-3-acetic acid (22.51 μg mL^{-1}) *in vitro* in GPB medium (glucose-peptone broth).[145]

In the degradation of natural substrates, a consortium of effective microbes has been found to be more effective than a single organism.[146,147] Ramarajan and Manohar (2017)[148] observed good lignocellulolytic by the fungal isolates, namely, GK1 (*Chaetomium globosum*), GK2 (*Chaetomium brasiliense*), G4 (*Engyodontium album*), G10 (*Metarhizium anisopliae*), G13 (*Engyodontium album*), M155 (*Acremonium persicinum*), M158 (*Acremonium minutisporum*), and M2E (*Inonotus tropicalis*). They evaluated the activity of these isolates and isolate 2a, *Cerrena* unicolor,[149] in the liquid culture media, where good growth and ligninolytic activity were shown by M2E and 2a, while exceptional cellulolytic activity was shown by the isolates GK1 and GK2 on the lignocellulosic substrates, *i.e.* RS (rice straw) and SCB (sugarcane bagasse). Upon treatment with individual isolates, the highest sugar yield observed from SCB with GK2 was 1.35 g L^{-1}, while the sugar yield was less than 1 g L^{-1} from RS and SCB after treatment with individual isolates except GK2. Amongst the different consortia, the highest yield of sugar (4.39 g L^{-1}) was produced by M2E + GK2 on sugarcane bagasse, followed by the yield of 2.64 g L^{-1} on rice straw by 2a + GK2. This enhanced yield of sugar in the case of the M2E + GK2 and 2a + GK2 consortia could be due to the high manganese peroxidase activity on SCB by the M2E + GK2 consortium and enhanced activity of laccase by the 2a + GK2 consortium on RS, followed by noteworthy cellulolytic nature. Thus, the developed lignolytic and cellulolytic marine-derived fungal consortium shows potential for application in agricultural waste.[148] A comparative study between biological and physical pretreatment was also performed by researchers. The yield of sugar moderately increased from the substrates *via* physical pretreatment and a combination of physical and biological pretreatment but it was less than the developed consortium-based biological pretreatment, which demonstrates the potential of fungal isolates in biological pretreatment.[148] Rouches, Zhou *et al.* (2016)[126] performed a study on the pretreatment of wheat straw using different strains of fungi to determine the probability of increasing the production of methane. Anaerobic digestion was found to be improved up to 20% by *Polyporus brumalis* BRFM 985 even after the mass loss. Using this strain, they obtained up to 43% extra methane (CH_4)/g of pretreated VS in comparison with the control straw. Considering the dry weight loss studied in the pretreatment course under non-optimized conditions, there was up to 21% extra methane per g of initial TS (total solids). In the case of the fixed culture condition, there was a decrease in delignification with an increase in glucose between 50 and 400 mg per g straw in a strain-dependent way.[126]

4.2 Pretreatment of hardwoods, softwoods and switchgrass

The lignin degradation ability was found to be increased by co-culturing of *Paracremonium* sp. LCB1 and *Clonostachys compactiuscula* LCN1 and pronounced drop of 76.37% in the weight of lignin was observed for the pretreatment of bamboo culms using this co-culture at a temperature of 30 °C, 40 days of culture time and pH 5. There was a high loss ratio of lignin/cellulose (>10).[150] It was also observed that co-culturing of two or three fungi gave higher degree of weight loss of lignin in comparison with a single fungal strain culture. During the process of pretreatment, the co-cultivation of interacting fungi results in the over-expression of lignolytic enzymes, which may generate a synergistic and combinatorial influence for effective delignification.[151] Consequently, the combination of LCB1 + LCN1 gave the maximum loss of lignin weight.[150]

Trametes versicolor (a white rot fungus), and *Gloeophyllum trabeum* and *Rhodonia placenta* (two brown rot funguses) were used for the pre-treatment of two softwoods, namely, *Pinus yunnanensis* and *Cunninghamia lanceolata*, and two hardwoods, namely, *Populus yunnanensis* and *Hevea brasiliensis*, with different conversion periods.[152] Selective degradation in softwood was shown by *T. versicolor*, where lignin and hemicellulose were converted preferentially, while cellulose was selectively retained. Alternatively, in hardwood, simultaneous conversion was achieved for cellulose, hemicellulose and lignin by *T. versicolor*. Carbohydrates were converted preferentially by the brown rot fungal species but cellulose conversion was selectively shown by *R. placenta*. The accessibility to wood cells was improved and the porosity was enhanced by the fungal pretreatment. It was concluded that the cellulose content may be maximized by the use of *T. versicolor* pretreatment, while pretreatment using brown rot fungi (especially *R. placenta*) may be beneficial for the production of biofuels, chemicals based on gasoline and other bio-chemicals.[152] Both brown rot fungi caused higher mass loss of softwoods compared to *T. versicolor* of 28.59%, 36.19%, and 13.09%, decaying by *G. trabeum*, *R. placenta*, and *T. versicolor* in *P. yunnanensis*, while in the wood of *Cunninghamia lanceolata*, 66.52%, 45.87%, and 35.57% decay was observed by *G. trabeum*, *R. placenta*, and *T. versicolor*, respectively. However, the case was reverse for hardwoods, where the hardwood mass conversion by white rot fungi was higher than brown rot fungi. The nature of lignin and different pathways of bio-degradation between hard woods and softwoods may be the reasons for this discrepancy in the degradation percentage by the different groups of fungi.[152]

Alternatively, a technoeconomic analysis was done by Olughu *et al.* (2023)[153] for the fungal pretreatment-dependent production of cellulosic ethanol, where the processing capacity of the plant was 2000 tonnes switchgrass per day. The ethanol yield of the plant was projected to be 211.9 L per t of switchgrass and fungal pretreatment was the main contributor to the total capital investment. The profitability of switchgrass-based ethanol production was observed to be sensitive to the changes in the cost of the feedstock, yield of glucose and yield of xylose. An increase in the yield of glucose from 60% to 80% resulted in a 5-fold enhancement in the net present value. Additionally, a study on the optimization of the fermentation time in the course of fungi-based pretreatment and subsequent glucose yield optimization upon enzyme-catalysed hydrolysis will be essential to improve the economic feasibility of this type of ethanol plant.[153]

4.3 Fungal pretreatment of spent coffee grounds (SCGs)

SCGs are biowaste materials produced after coffee brewing, which are generated in a noteworthy volume each year globally. The exact volume of SCGs generated each year is not well known but it is estimated that 6 million tons are produced globally each year on a wet basis.[154,155] Approximately, 50% production comes from small-scale coffee shops, cafeterias, individuals, and restaurants.[156] However, discarded SCGs pose a considerable threat to the environment. Presently, new technologies and policies are devoted to developing SCGs as worthwhile feedstock for the synthesis of bioproducts, platform for the production of chemicals, and generation of value-added energy materials.[157,158] Furthermore, given that they are a rich source of polysaccharides, proteins, and lipids, SCGs are promising feedstock for bio-based and chemical processes to get great-value products for the cosmetics, pharmaceutical, and food industries.[159] The contents of hemicellulose and lignin in SCGs were found to be 39.75%, and 23.1%, while protein and caffeine were found to be 10.82% and 1.83%, respectively.[160] Actually, the valorization of SCGs can be done in many ways, including *via* the production of SCOAs (short-chain organic acids).[161] These organic acids with small chains are monocarboxylic acids (aliphatic) with 2 to 6 C-atoms (*i.e.* acetic, propionic, butyric, isobutyric, valeric, caproic, and lactic acids), having industrial application either by direct involvement or use as building blocks for further transformations.[162] Usually, petrochemical processes are used for the production of these molecules but their production *via* biological processes is being promoted due to the growing cost and environmental impact of crude oil, specially utilizing organic waste as the substrate.[163]

A promising work demonstrated the biological pretreatment of coffee waste by acidogenic fermentation[161] using two fungi, *i.e.* *Paecilomyces variotii* NRRL-115 and *Trametes versicolor* CBS 109428. The production of SCOA (short chain organic acid) was positively influenced by the utilization of SCG_TvSmF (spent coffee ground_submerged fermentation by *T. versicolor*) as the pretreatment, getting the maximum of 2.44 g COD per L, which was a significant enhancement (87%) compared to the control. Also, the generation of acetic acids, propionic acids, and butyric acids in an average proportion of 59.9%/33.8%/6.3% was observed. The production of acetic acid happened throughout the assay, while the appearance of butyric acids and propionic acids occurred after the 9th day and 18th day, respectively.[161] As observed before in other studies,[125] celluloses and hemicelluloses of spent coffee grounds were possibly broken down and consumed. A study showed that the inclusion of a pretreatment step may assist to make spent coffee grounds an appropriate material for valorization and this work is a nice contribution towards lessening the cost of enzymatic hydrolysis as a complex feedstock pretreatment.[161] Afriliana *et al.* (2021)[160] studied composting spent coffee grounds using aerobic static batch composting with temperature control using *Aspergillus* sp. and *Penicillium* sp. The basis for selecting these activator fungi in compositing was the high contents of hemicellulose and lignin in the substrate. The study was performed *via* the analysis of three samples (control, C1, and C2), where the greatest degradation was observed for lignin in C2. In comparison with the similar rates of 35.56% in C1 and 31.1% in the control, this led to the improved global breakdown of lignin of 40.28% in C2. The percentage protein decomposition, *i.e.* 85.44% (sample C2) and 83.02% (sample C1), was greater than that of the control (81.82%). The macromolecule decomposition rate was more than 40% in the case of lignin, while 70% in the case of cellulose. With the help of this method, the composting time can be sped up and the results of the produced compost can be optimized.[160]

The capacity of *Pleurotus ostreatus* in the degradation of the lignocellulosic nature of combined spent coffee grounds (SCG) and olive pruning residues (OLPR) was assessed by Fayssal *et al.* (2021).[164] They adopted the complete randomized design with 5 treatments, *i.e.* S1: 100% wheat straw (control), S2: 33% wheat straw + 33% spent coffee grounds + 33% olive pruning residues, S3: 66% wheat straw + 17% spent coffee grounds + 17% olive pruning residues, S4: 17% wheat straw + 66% spent coffee grounds + 17% olive pruning residues, and S5: 17% wheat straw + 17% spent coffee grounds + 66% olive pruning residues, with 10 replicates per treatment. Among them, only S1, S2, and S3 were observed to be productive. With an increase in the OLPR and SCG proportions, the loss of organic matter decreased. The lignin loss percentage was greater in S1 compared with S2 and S3, *i.e.* 53.51%, 26.25%, and 46.15%, respectively. The combined production yield of mushrooms harvested from 2 flushes of *Pleurotus ostreatus* cultured in grass and coffee pulp created a biological efficiency change of 59.9% and 93%, respectively.[165] To access holocellulose, the fungus needs to firstly break lignin,[125] where a greater loss of lignin means greater mycelial activity.[166] In all the studied substrates, the degradation of hemicellulose favorably occurred with respect to cellulose, which was consistent with the early results reported by Thompson *et al.* (2003)[167] for WS.[164]

The previous discussion demonstrated the efficiency of fungal systems in the pretreatment of SCGs as a biological method. In recent years, the utilization of SCGs as a bioresource for many value-added bio-products has attracted considerable attention but there are certain noteworthy challenges, which need to be solved for its effective industrial application. The heterogeneity of SCGs from different sources and collection from coffee shops, consumers and other small-scale sources are the primary challenges. Inconsistency in the SCG composition, factors such as type of coffee, the method used for brewing and conditions required for processing are some of the factors generating difficulties to standardize the process of extraction and optimize the creation of value-added bio-products. Furthermore, the development of effective techniques is necessary to sort and preprocess SCGs to ensure their reliable excellence and composition.[158] A large quantity of SCGs is produced by coffee shops and domestic consumers, and thus logistical challenges are encountered by bioprocessing plants in concentrating the huge volumes of SCGs to the level of processing. Thus, innovative approaches and more research are required to overcome these challenges.[158]

4.4 Fungal digestion of food wastes

Various sources are responsible for the generation of food waste such as canteens, households, hotels, function halls, gated communities, different food processing industries, and many others.[168] Thus, its decentralized treatment at the source utilizing the best probable anaerobic digestion (AD) technique makes it remunerative,[169] and consequently the diversion of food waste to landfills may be avoided to a high extent.[170] There are three configurations of the AD process based on the TS concentration in organic waste, *i.e.* wet anaerobic digestion (total solids ≤10%), semi dry anaerobic digestion (total solids in the range of 10–15%), and dry anaerobic digestion (total solids >15%).[171–173] Dry anaerobic digestion (solid-state digestion) is a positive technique, owing to its various benefits compared with wet anaerobic digestion (total solids <10%), making it especially striking for food waste treatment, municipal solid waste organic fraction treatment, and treatment of agricultural waste.[174]

Different pretreatment methods (namely, autoclaving, acid based, alkali-based, aeration, and fungi-based methods of pretreatment) were applied by Bhurat *et al.* (2023)[175] for the pretreatment of food waste. In comparison with the control, a 3.8-fold improvement in the yield of hydrogen and 1.7-fold enhancement in the yield of methane were shown by fungal treatment. To study the fungal succession and their ecological and engineering value, food waste AD bioreactors were investigated by Yang *et al.* (2022).[176] It was observed that deterministic procedures slowly dominated the fungal assembly succession (*i.e.* at the final stage up to 84.85%), signifying the varying environmental status accountable for the dynamics of the fungal community, and specially, the structure, diversity and biomass of the fungal community were controlled by the various environmental variables or the same variables with opposite influences.[176] A work on fungal mash enzymatic pretreatment combined with pH adjustment was performed by Zhang *et al.* (2022)[177] using *Aspergillus awamori* (CICC 41363) to generate fungal mash enzymes *via* SSF. Complex amylase (CA) was the crude enzyme produced from this fungus, which was added to the food waste fermentation short-term anaerobic system. There was 116.9% enhancement in the concentration of SCOD with the addition of CA relative to the control. After 24 h, the TOC and SCOD concentrations considerably increased with the addition of complex amylase (CA) under an extensive range of pH conditions. Here, the total organic carbon and SCOD mean concentration were 12.5 g L^{-1} and 34.5 g L^{-1}, which were 1.65 and 1.81-times greater than the control (7.6 g L^{-1} and 19.1 g L^{-1}), respectively. pH 8 was the optimal pH condition for the yield of VFAs, which was consistent with the finding by Chen and co-workers.[178] This study may be an economical way to increase the yield of VFAs for the valorization of FW in the course of anaerobic fermentation.[177]

Furthermore, fungal mash (*in situ* produced) was also utilized by Yin *et al.* (2016),[179] showing the nice presence of hydrolytic enzymes to pretreat activated sludge, FW, and their combination before AD. The enzyme-catalyzed pretreatment of activated sludge combined with FW resulted in the generation of 3.72 g per L glucose and 51 mg per L free amino nitrogen, equivalent to SCOD (7.65 g L^{-1}) within 24 h, accompanied with 19.9% reduction in VS (volatile solids). The decrease in VS was found to be 19.1% and 21.4% after the activated sludge and FW pretreatment, respectively, through fungal mash. Moreover, the yield of bio-methane from the fungal mash pretreated mixed waste was 2.5-times greater than the activated sludge receiving no pretreatment, with a further decrease in the volatile solids of 34.5%. This resulted in a total reduction in volatile solids of 54.3% in the suggested anaerobic system with fungal mash pretreatment. This study demonstrates that in the enhancement of the production of bio-methane and the maximization of the mixed waste volume reduction *via* anaerobic co-digestion, in situ-produced fungal mash-based combined activated sludge and FW pretreatment is a promising option.[179]

The effective role of fungi in the pretreatment of food biowaste and its conversion into several useful bio-products has been well demonstrated. However, to reduce the harmful impacts of food waste on the environment and human health and conversion of food waste into value-added bio-products, certain challenges need to be resolved such as the bulk collection of food waste from various sources, their proper separation from other types of inessential materials, their bulk storage and processing at biorefinery plants and further research on an industrial scale from the laboratory-scale experiments.

4.5 Saccharification of grain stillage

In terms of the composition of fiber, grain stillage is mainly made of hemicellulose (15–25%) and cellulose (35–45%), depending on its source such as rice, corn, sorghum, and wheat.[180] It is also considered a feedstock for bio-refineries because of its large content of carbohydrates.[181] Pretreatment of grain stillage using the microwave-assisted hydrothermal (MH) pretreatment, fungus-based pretreatment, and their combination was done by Ren *et al.* (2020).[181] A superior reducing sugar yield (25.51 g/100 g) and saccharification efficiency (66.28%) were achieved by the microwave-assisted hydrothermal + *Phanerochaete chrysosporium* (microwave-assisted hydrothermal prior to *Phanerochaete chrysosporium*) pretreatment. A considerable loss of mass, *i.e.* 23.54% and 39.43%, was caused by the joint pretreatment of *Phanerochaete chrysosporium* + microwave-assisted hydrothermal and microwave-assisted hydrothermal + *Phanerochaete chrysosporium*, respectively. The degrees of delignification were considerably enhanced to 32.80% (for *Phanerochaete chrysosporium* + microwave-assisted hydrothermal) and 43.34% (for microwave-assisted hydrothermal + *Phanerochaete chrysosporium*) after the combined pretreatment. Furthermore, the degree of delignification by microwave-assisted hydrothermal + *Phanerochaete chrysosporium* was considerably greater than by *Phanerochaete chrysosporium* + microwave-assisted hydrothermal pretreatment.[181] This may be because microwave-assisted hydrothermal pretreatment results in hydrogen bond breakage and lignocellulose structure destruction through explosion and disruption, which stimulates the subsequent attack of *P. chrysosporium* for delignification.[182,183] To enable the utilization of the cost-effective grain stillage, the use of joint microwave-assisted hydrothermal and *Phanerochaete chrysosporium* pretreatment may be an excellent method.[181]

4.6 Symbiotic digestion of lignocellulose

In tropical and subtropical areas of the world, fungus-growing termites have the ability to consume 20–90% of dead plant materials.[184–186] Lignocellulosic materials can be completely degraded and digested by *Termitomyces* fungi with a resulting ecological influence on the processes of the ecosystem, chiefly the carbon cycle.[187] To investigate the digestion of lignocellulose in a fungus-growing termite *O. formosanus* (Shiraki) symbiotic system and to equate the bacterial communities across various phases during the degradation process, Ahmad *et al.* (2022)[188] performed many analytical works on the fate of the components of plant biomass and performed 16S rRNA gene amplicon sequencing. The digestive tract of the young workers initiates the degradation of lignocellulose but leaves the maximum cellulose, lignin, and hemicellulose, which are principally decomposed in the fresh fungus comb. The consumed samples of lignocellulose (fresh, mature, and old comb) from three colonies were compared[188] with the original wood of mulberry through compositional analysis of lignocellulose using the fiber detergent technique.[189] It was shown by the examination of the comb material that in all three colonies, the considerable degradation of the lignocellulosic constituents occurred.[188] There was on average a reduction in lignin, cellulose, and hemicellulose in the fresh comb by 18.9%, 11.1%, and 15.0%, in the mature comb by 56.9%, 41.0%, and 32.5%, and in the old comb by 63.0%, 65.5%, and 53.4%, respectively.[188]

4.7 Fungal pretreatment of solid digestate

The fate of the digestate fractions generally involves agricultural aims such as soil amendment and organic fertilizer.[190] Zanellati *et al.* (2020)[191] studied fungal pretreatment on non-sterile solid digestate and inoculated the fungi *Coprinopsis cinerea* MUT 6385, *Cephalotrichum stemonitis* MUT 6326, and *Cyclocybe aegerita* MUT 5639 in the digestate non-sterile solid fraction with aim to reuse it as feedstock for anaerobic digestion. In the *Cyclocybe aegerita*, *Cephalotrichum stemonitis*, and *Coprinopsis cinerea* pretreated samples, there were noteworthy reductions in the concentration of total solids (TS), *i.e.* 23.8%, 25.4%, and 28.5%, respectively. In the *C. cinerea* samples pretreated for 10 days and *Cephalotrichum stemonitis* samples pretreated for 20 days, the NDF (neutral detergent fiber) loss percentage ranged from 1.6% to 10.4%, respectively. Similar behavior was shown by the different strains towards the PCWP (plant cell wall polymer), causing a greater reduction in hemicellulose (18.5–59.3%) compared with cellulose (0.2–8.2%) and lignin (1.0–9.6%). *C. stemonitis*-based pretreatment for 20 days gave the maximum reduction in all the PCWP components and resulted in the reduction of hemicellulose, lignin, and cellulose of 59.3%, 9.6%, and 8.2%, respectively. The anaerobic digestion showed a superior function with solid fraction of digestate treated by the fungal strain *Cephalotrichum stemonitis* for 20 days, which led to about 3-fold greater yield of biogas and CH_4, *i.e.* +182% and +214%, respectively, compared with the untreated solid fraction of digestate. The cumulative methane formed with the fungal strain *C. stemonitis* was considerably greater than that attained with the fungal strains *Cyclocybe aegerita* and *Coprinopsis cinerea* for both 10 and 20 days.[191] *M. isabellina* ATCC 42613 was applied by Zhong *et al.* (2016)[192] for accumulating lipids on detoxified hydrolysate medium. The characteristics of the digestates (solid and liquid) and AD effluent showed that the solid digestate had a 30.60% TS content and carbohydrate content, *i.e.* cellulose (26%), xylan (13%), and lignin (30%), to be utilized for fungal lipid accumulation as the lignocellulosic feedstock. After the pretreatment and hydrolysis processes, the mixture feed with the total solids of 10% produced a hydrolysate having glucose (13.85 g L^{-1}), xylose (8.95 g L^{-1}), and acetate (2.67 g L^{-1}). This study showed the substrate consumption by *Mortierella isabellina* on hydrolysates.[192] Without detoxification, there was no consumption of sugars and acetate in the hydrolysate during the culture period of 89 hours. In comparison with the culture of synthetic medium (consumption of all sugars and acetate in 66 h), a delay (23 h) of the consumption of the substrate was noticed from the cultures on detoxified hydrolysates. There was complete consumption of glucose and acetate in 49–54 h, respectively. At the end of the batch culture (77 h), 1.79 g per L xylose remained in the broth, and 8.98 g per L biomass and 1.50 g per L lipid were accumulated. The corresponding yields of lipid and biomass were 0.07 g g^{-1} and 0.42 g g^{-1}, respectively.[192] Conclusively, fungi show promise in the pretreatment of solid digestate with anaerobic digestion process.

4.8 Fungal pretreatment of park waste and cattle dung

To treat cellulosic biomass and enhance its digestibility, Ali and Sun (2015)[193] studied the influence of physico-chemical pretreatment on the degradation of cellulose, followed by treatment utilizing fungi *Trichoderma viride* and *Aspergillus terreus*. In their experimental set up, a mixture of new leaves (125 g), dry leaves (125 g), and cattle dung (250 g) was present in each of the two digesters. Park waste fungal treatment was applied for 7 days at 25 °C, followed by the incubation of both digesters for 70 days on an incubator shaker (temperature of 35 °C and 120 rpm) to help the mixing. The pre-treated and untreated substrate biogas and CH_4 yields were measured. The three pre-treatment stages improved the production yields of daily biogas and CH_4 from the substrate. In comparison with the untreated substrate, the pretreated substrate gave maximum yields of biogas and CH_4 of 2.6 and 1.9 L per kg VS, respectively, on the 28th day. There was 102.6 L per kg VS biogas cumulative production for the untreated substrate, which was found to be improved to 125.9 L per kg VS for the pretreated substrate. Consequently, there was 22.7% enhancement in comparison with the yield of biogas from the untreated substrate. The pretreated and untreated substrate cumulative production of CH_4 was 79.8 and 61.4 L per kg VS, respectively, and in comparison with the yield of methane from the untreated substrate, there was a 30% enhancement.[193] This study may be useful for the treatment of cattle dung and park waste and in the production of biogas with further improvement, optimization and/or with combined technology.

4.9 Cardboard waste fungal pretreatment

Cardboard waste is also a good source of hemicellulose, lignin, and cellulose. Suthar and Singh (2022) studied the fungal pretreatment of waste cardboard in a monoculture and mixed culture, and then composted for 35 days after mingling with cow dung in various ratios.[194] They utilized fungi *Oligoporus placenta* and *Trametes hirsuta* for fungal pretreatment. There was considerable decrease in the contents of cellulose (28.3–35.8%), hemicellulose (61.4–68.4%), and lignin (67.5–69.3%) in the waste cardboard. The pretreated waste cardboard displayed better reduction rates in TOC (26.02–47.92%), C–N ratio (19.4–23.5), and contents of lignocellulose, in addition to an increase in total N (40.48–63.31%), total K (51.92–73.91%), germination index (88.5–102.0%), and levels of elements *i.e.* copper, iron, zinc, chromium, and manganese. Thus, after the pretreatment with a white rot fungi consortium, waste cardboard could be utilized as an important substrate for the preparation of value-added compost.[194]

Therefore, now, it is very clear that fungi have a great future in biological pretreatment technology for the management of the aforementioned organic waste and the production of valuable energy, biogases and compounds. However, there are also several associated challenges before these pretreatment technologies. There are some other reports that may be significant for readers in the field of bio-waste degradation as well as opportunities and challenges.[195–208] Table 4 summarizes the effective brief descriptions of the myco-pretreatment of different types of organic waste. Together with being an advantageous biological solution to the problems of bio-waste management, fungal-based bio-pretreatment processes may

Table 4 Comparative table of different recent studies on the fungal pretreatment of various organic wastes and their outcomes

S. N.	Fungi	Enzyme activity	Substrates	Some outputs	References
1	*Trametes versicolor* (WRF), *Gloeophyllum trabeum* and *Rhodonia placenta* (brown rot fungi)	—	Softwoods and hardwoods	• Softwoods mass conversion by two brown fungal species was greater than white rot fungi • Hardwoods mass conversion by WRF exceeded that by BRF	152
2	Fungal source	—	Switchgrass	• Yield of ethanol was projected to be 211.9 L per t of switchgrass and fungal pretreatment was the main contributor to the total capital investment • Growing yield of glucose from 60 to 80% resulted in a five-fold enhancement in the net present value	153
3	*Paracremonium* sp. LCB1 and *Clonostachys compactiuscula* LCN1	Hemicellulase and ligninolytic enzyme	Bamboo culms	• Significant lowering in the lignin weight (76.37%) • Lignin/cellulose ratio showed high loss (>10)	150
4	*Penicillium aurantiogriseum*, *Gilbertella persicaria* (SZMC11086), *Rhizomucor miehei* (SZMC11005), and *Trichoderma reesei* (DSM768)	Endoglucanase, β-glucosidase, and cellobiohydrolase	Wheat straw, woody willow chips, and corn stover	• Excellent production of hydrolytic enzyme and maximum yield of biogas from the partly decomposed substrates were shown by *P. aurantiogriseum* • Maximum yield of methane was for corn stover fed reactors • All the tested fungi preferred the corn stover substrate for the productivity of methane	130
5	*Pleurotus ostreatus* and *Dichomitus squalens*	—	Corn silage	• Biogas production was enhanced • Methane gas was found to increase by 1.55 fold • Lignin de-polymerization increased the production of methane to 0.301 to 0.465 m^3 per kg VS	131
6	*Irpex lacteus*	—	Wheat straw	• Wheat straw digestibility found after 21 days of the pretreatment in the solid state fermentations bioreactor (60.6%) was alike to that found on a small scale *i.e.* 57.9% • In the 03 bioreactor experiments, sugars evolution was completely different after 21 days of treatment • Greater lowering of lignin in experiment B2 *i.e.* 34.90 ± 0.87% in comparison with that of experiment B1 *i.e.* 26.7 ± 3.08% • Alike reduction of lignin *i.e.* 53.2 ± 0.60% in the flask-scale pretreatment	132

Table 4 (*Contd.*)

S. N.	Fungi	Enzyme activity	Substrates	Some outputs	References
7	*Aspergillus terreus* and *Trichoderma harzianum*	—	Rice straw	• Degradation of cellulose • Production of bioethanol • The 80% degradation of cellulose by the isolated fungi *A. terreus* and *T. harzianum* • *A. terreus* performance on the decrease of cellulose with HL and RP as substrate was 92% and 80%, respectively • *T. harzianum* performance on the decrease of cellulose with HL and RP as substrate was 93% and 82%, respectively • Rice straw's RP treated with *T. harzianum* has resulted in greater bioethanol of 5.4 g L^{-1}	133
8	*Saccharomyces cerevisiae*, *Pichia stipitis*, *Pachysolen tannophilus*, and *Wickerhamomyces anomalus* X19	—	Willow sawdust	• Bioethanol production • Yields of ethanol ranging from 11.67 ± 0.21 to 13.81 ± 0.20 g/100 g TS was shown by the *S. cerevisiae* or *W. anomalus* X19 monocultures and co-cultures with *P. stipitis* • For the approach D, there was maximum removal of lignin (38.3%) • Co-cultures was useful	136
9	*Rhizomucor miehei*, *Aspergillus nidulans*, *Gilbertella persicaria*, and *Trichoderma reesei*	β-Glucosidase and endoglucanase	Dry CS (corn stover), WS and WWC	• β-Glucosidase and endo-(1,4)-β-ᴅ-glucanase activity • Yield of methane	138
10	*Pichia fermentans*, *Phanerochaete chrysosporium*	—	Wheat straw	• Auxin production by *P. fermentans* • Indole-3-acetic acid (IAA) production • The wheat straw was pretreated by *P. chrysosporium* for releasing the sugar in the medium which may be used by *P. fermentans* for the production of IAA • Sugar amount was released by *P. chrysosporium* from 2nd day onwards and was highest on 9th day	142
11	GK1 (*Chaetomium globosum*), GK2 (*Chaetomium brasiliense*), G4 (*Engyodontium album*), G10 (*Metarhizium anisopliae*), G13 (*Engyodontium album*), M155 (*Acremonium persicinum*), M158 (*Acremonium minutisporum*), and M2E (*Inonotus tropicalis*)	LiP (lignin peroxidase), laccase, CMCase, MnP (manganese peroxidase), and xylanase	Rice straw and sugarcane bagasse	• LiP (lignin peroxidase), laccase, CMCase, MnP (manganese peroxidase), and xylanase activity • Amongst the different consortia, the highest yield of sugar (4.39 g L^{-1}) was given by M2E + GK2 • Ligninolytic and cellulolytic marine-derived fungal consortium were effective for agricultural wastes	148
12	*Polyporus brumalis* BRFM 985	—	Wheat straw	• Enhancement in the production of methane • They obtained up to 43% extra methane (CH_4) per gram of pretreated VS in comparison with control straw	126

Table 4 (*Contd.*)

S. N.	Fungi	Enzyme activity	Substrates	Some outputs	References
13	*Paecilomyces variotii* NRRL-115 and *Trametes versicolor* CBS 109428	Enzymatic extracts	Spent coffee grounds	• Biological pretreatment for coffee waste's acidogenic fermentation • Generation of acetic acids, propionic acids, and butyric acids in an average proportion (59.9/33.8/6.3%) • Production of SCOA in the course of acidogenic fermentation	161
14	*Aspergillus* sp., and *Penicillium* sp.	—	Spent coffee grounds	• Chemical composition of SCG (%) as hemicellulose (39.75% ± 0.007%), lignin (23.1% ± 0.007%), caffeine (1.83% ± 0.007%), and protein (10.82% ± 0.007%) • Study *via* three samples analysis (control, C1, and C2) • Greater degradation of lignin in C2 • Composting time can be speed up and results of the produced compost can be optimized by this composting method	160
15	*Pleurotus ostreatus*	—	Spent coffee grounds and olive pruning residues	• Adopted the complete randomized design with 5 treatments • They observed only S1, S2, and S3 as productive • Lignin loss percentage was greater in S1 in compare with S2 and S3 *i.e.* 53.51%, 26.25%, and 46.15%, respectively • Degradation of hemicellulose preferentially occurred with respect to cellulose	164
16	*Aspergillus awamori* (CICC 41363)	Complex amylase (CA)	Food wastes	• Solubility and degradability of the organics in food waste were considerably enhanced by CA addition • The 116.9% increase in concentration of SCOD with the CA addition relative to the control • TOC and SCOD mean concentration were 12.5 g L^{-1} and 34.5 g L^{-1}, respectively • Under weakly basic and neutral conditions, a greater VFAs concentration was found • In enhancing FW hydrolysis, the pretreatment method of adding CA could be an effective method	177

Table 4 (*Contd.*)

S. N.	Fungi	Enzyme activity	Substrates	Some outputs	References
17	Fungal mash	Hydrolytic enzymes	Food wastes	• Enzymatic pretreatment of activated sludge mixed with FW caused in the production of glucose (3.72 g L^{-1}) • The reduction of VS was found as 19.1% and 21.4% after the activated sludge and FW pretreatment, respectively by the fungal mash • Yield of bio-methane of fungal mash pretreated mixed waste was 2.5 times greater than the activated sludge receiving no pretreatment, with as further reduction of VS of 34.5%	179
18	*Phanerochaete chrysosporium*	Ligninolytic enzyme activity	Grain stillage	• Pretreatment of grain stillage • The maximum activities of ligninolytic enzyme was achieved by the fungal pretreatment with *Phanerochaete chrysosporium* digestion (PC) in six days with 10% inoculum size at which yield of reducing sugar and efficiency of saccharification reached 19.74 g/100 g and 36.29%, respectively • The degrees of delignification were considerably enhanced to 32.80% (for PC + MH) and 43.34% (for MH + PC) after the combined pretreatment • Use of combined MH and PC pretreatment could be an excellent method	181
19	*Coprinopsis cinerea* MUT 6385, *Cephalotrichum stemonitis* MUT 6326, and *Cyclocybe aegerita* MUT 5639	—	Solid digestate	• In the *C. aegerita*, *C. stemonitis*, and *C. cinerea* pretreated samples, there was significant decrease in the concentration of total solids (TS) *i.e.* 23.8%, 25.4%, and 28.5%, respectively • The anaerobic digestion functioned superior with SFD treated by the fungal strain *C. stemonitis* for twenty days • Cumulative yields of biogas and methane also studied	191
20	*Mortierella isabellina*	—	Anaerobic digestate	• Production of lignocellulosic biodiesel • Accumulation of lipid • After the process of pretreatment and hydrolysis, the mixture feed at the total solids of 10% produced a hydrolysate having glucose (13.85 g L^{-1}), xylose (8.95 g L^{-1}), and acetate (2.67 g L^{-1}) • Complete consumption of glucose and acetate in 49–54 hours, respectively	192

Table 4 (*Contd.*)

S. N.	Fungi	Enzyme activity	Substrates	Some outputs	References
21	*Aspergillus terreus* and *Trichoderma viride*	—	Park wastes	• Physico-chemical pretreatment's influence on the degradation of cellulose followed by fungal treatment • TS, VS, TOC, *etc.* were studied • Study on biogas and CH_4 yields	193
22	*Oligoporus placenta* and trametes hirsuta	—	Card wastes	• Fungal pretreatment of waste cardboard • Considerable lowering in cellulose (28.3–35.8%), hemicellulose (61.4–68.4%), and lignin (67.5–69.3%) content in waste card board	194

also generate various types of valuable products after the pretreatment processes from macromolecules lignins, cellulose, hemicellulose, starch, pectins, *etc.*

5 Techno-economic analysis and scale-up issues for industrial implementation of fungal pretreatment methods

Fungal pretreatment processes at the industrial level require large capital investments. From grasses to hardwoods, the total capital investment ranges from 700 million (dollars) to 1.2 billion (dollars), respectively, which is approximately 5- to 10-times greater than the previously estimated cost for conventional treatment on a parallel scale.[209] This high cost is due to the expenses related to purchasing large equipment, installation, construction, engineering, requirement of many units for each of the main processes such as autoclaving, fungal pretreatment and enzymatic hydrolysis and other requirements. Packed-bed bioreactors utilized in the fungal pretreatment process are responsible for the majority of the cost mainly because of their longer residence time. In the fungal pretreatment process, the estimated price of fermentable sugar was 1.6–2.8 dollars per kg, which is 4–5 times greater than the previously stated production cost of sugar utilizing the conventional pretreatment methods.[209,210] Due to its minimum requirement of energy and chemicals, the fungal pretreatment process is believed to be an inexpensive pretreatment method but the analysis showed that it has a noticeably higher cost than conventional pretreatments and about one order of magnitude over that anticipated for a pretreatment to be feasible at the commercial level.[210]

The high cost of facility arrangement for fungal pretreatment is primarily responsible for the need of high capital investment, while the feedstock cost is the second highest contributor to the cost of sugar production, which contributed 18–22% of the total cost of sugar production.[27] However, continuous advancements in technologies may reduce the total cost of sugar production using fungal pretreatment techniques, making them a commercially profitable environmentally safe and green technique. The longer time required for fungal pretreatment, enhancement of the yield of sugar, and sterilization requirement of the feedstock before fungal pretreatment are several bottlenecks that need to be overcome to expand and improve the fungal-based pretreatment technique. Studies on its combination with other pretreatments with low severity achieved a greater yield of sugar with a significantly reduced need for energy and chemicals.[211] Optimization of these processes is not only required on the laboratory scale from a technical perspective but also at the commercial scale utilization from a techno-economic perspective.[27] High temperature may affect fungal-based pretreatment processes because fungal enzymes are strongly involved in the enzymatic degradation process of lignocellulosic materials or other biowaste, which are inactivated at higher temperatures, and thus during the fungal-based treatment plant set up, special attention should also be given to the control of temperature increase during the process.

6 Conclusions, challenges and future perspectives

Fungi are effective in the decomposition of lignocellulosic biomass, food waste, sewage sludge, polysaccharides, lignin, hemicelluloses, *etc.*, and found to be efficiently involved in saccharification, biogas productions, glucose production, ethanol production, and bio-fertilizer development utilizing organic waste. They have a significant contribution to the valorization of biomass[29] *via* the generation of alcohol,[202] biodiesel,[203] organic acids,[204] and gas fuels.[205] However, there are several challenges in the efficient use of pretreatment technologies for the digestion of organic waste or bio-waste and solid waste management. The anaerobic decomposition of organic waste occurs when dumped in landfills.[46] Harmful greenhouse gases such as methane are produced by the decomposition of green waste under anaerobic condition, which are the main contributors to global warming.[206] The rapidly depleting landfill space is another problem with direct landfill disposal. In the proper management of organic waste, there are various considerable barriers. Poor infrastructure, strategy planning, staff capacity, registration, programme engagement, information system, and unsystematic management of waste make it a difficult job.[46] Also, there is a lack of participation in the initiatives of the separation of garbage and inadequate communication between homeowners and the municipality,[207] which make the management of organic waste very difficult. To allow a more effective value extraction and recycling process, the separation of waste should occur at the source.[207] The separation of different types of wastes such as dry wastes and wet wastes (biodegradable) makes the pretreatment process more effective and significant. Thus, this is the main responsibility of producers of wastes together with effective government involvement. There is a need for combined efforts from urban local bodies, governments, private sectors, and non-governmental bodies for long-term waste management, and visionary project developments are strongly needed in this regard. There is also a strong need for well-defined roles and responsibility to work on waste management with continuous monitoring and assessment.[207] Kumar *et al.* (2017)[207] nicely reviewed the challenges and opportunities related to the management of waste materials in India. Thus, to perfectly implement fungal pretreatment technology either individually or in combination with other technologies for a green and sustainable environment, the above-mentioned barriers need to be tackled because in poor countries organic waste disposal is a very difficult task. The consistent and properly managed involvement of the system (government, industry and public) in organic waste management with appropriate methodologies is essential to pretreat organic or bio-waste biologically. A longer pretreatment time is generally required by fungal technology to achieve high removal rates of lignin and saccharification of cellulose, which generates problems regarding cost increase and contamination by bacteria.[125] Besides a longer pretreatment time, the requirement of feedstock sterilization before the pretreatment process, heat generation during the fungal pretreatment process, and lower yield of sugar are some other challenges and shortcomings of fungal pretreatment, which needed to be properly tackled in the future to make fungal pretreatment technology more efficient and cost-effective. The large solid content in biomass is the principal reason restraining its solid fermentation, which generates ethanol. Similar to glucose fermentation, the yield of ethanol and titer can be achieved through the amalgamation of cosolvent-improved lignocellulose fractionation, and subsequently saccharification and fermentation.[208] Fungal pretreatment in combination with other pretreatment methods may increase the enzymatic digestibility and shorten the time for fungal pretreatment.[29] Fungal technology has great advantages in the field of biomass conversion because several derived products from biomass hydrolyzed sugar are important from the point of energy generation. For example, 5-hydroxymethylfurfural, levulinic acid, and furfural are considered value-added chemicals produced *via* fungal technology.[29] Future development will require further upgraded fungal technology with a shorter pretreatment time, little or no sterilization requirements, enhanced enzymatic digestibility, reduced chance of contamination, and greater product yields. These upgraded fungal pretreatment technologies should more efficiently break the complex structures of biomass, enhancing the production of various valuable products.[29] Biogases (methane) and other useful organic components (bioethanol, monomeric sugars, *etc.*) produced during the process of fungal pretreatment technology may be utilized in human welfare using consistent management techniques.

Data availability

This is a review article and during the preparation of this article, no new data were generated or analysed as part of this review.

Author contributions

Dr Pankaj Kumar Chaurasia and Dr Shashi Lata Bharati prepared the original manuscript's draft, wrote major sections, supervised, and edited the manuscript; Dr Sunita Singh wrote specific sections and performed formal analysis and editing; Dr Azhagu Madhavan Sivalingam, and Dr Shiv Shankar performed formal analysis and editing. Dr Ashutosh Mani was involved in supervision, editing and formal analysis. All authors also reviewed the manuscript.

Conflicts of interest

No competing interests.

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