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Sustainability Spotlight Statement

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Sustainable treatment of biowastes or organic wastes like food wastes, municipal solid wastes, sewage sludge, plant's materials, animal biomasses, aquatic and terrestrial wastes, agricultural and forestry wastes, and many others are the need of time and different countries are working on the various related project in order to treat these biowastes and sustainably produce energy, biogases, methane, ethanol, carbohydrates, protein and several other useful chemicals. This type of sustainable literature review may helpful to better understand the goals of sustainable development. This critical review may be well aligned with various sustainable development goals of United Nations like '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 **Fungal Pretreatment Methods for Organic Wastes: Advances and**
2 **Challenges in Biomass Valorization**

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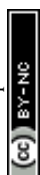
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37 **ABSTRACT**

38 Food wastes, municipal solid wastes, sewage sludge, plant's materials, animal
39 biomasses, aquatic and terrestrial wastes, agricultural and forestry wastes, industrial and
40 domestic wastes and many other lignocellulosic biomasses are considered under the category
41 of organic wastes or bio-wastes. Various techniques, mainly mechanical pretreatment (high
42 pressure homogenization and ultra-sonication), thermal (temperature based) pretreatment,
43 microwave technology of pretreatment, chemical pretreatment, and biological pretreatment
44 are found to be effective in the organic wastes valorization. Fungal pretreatment of organic
45 wastes is a promising biological technology because of its excellent efficiency of the
46 decomposition of various types of organic wastes like food wastes, ligno-cellulosic
47 biomasses, hemicellulose, agricultural wastes, hardwoods, softwoods, switchgrass, spent
48 coffee grounds, park wastes, cattle dung, and solid digestate which have been reviewed
49 pointedly. Fungal pretreatment of the organic waste materials may give advantageous
50 products like biogases, energy source, sugars' monomeric or oligomeric products, different
51 type of acids, and many more. Major challenge associated with the fungal pretreatment
52 technology is the requirement of higher time for greater degree of biomass valorization which
53 raises the cost as well as vulnerability of contamination but the use of fungal pretreatment
54 with other pretreatment techniques may reduce the time and enhance the functionality of the
55 method with higher rate of biomass valorization. Heat generation in fungal pretreatment
56 process and need of feedstock sterilization before fungal pretreatment are some other
57 challenges to be properly tackled for its efficient application at industrial scale. In this review,
58 use of different fungal pretreatment methods for the valorization of different types of
59 biomasses and production of valuable products have been evaluated and discussed. Authors
60 have provided an inclusive assessment of the fungal pre-treatment of various types of organic
61 wastes along with the concise but effective discussions on the organic solid wastes and



62 different pretreatment techniques involved in the bio-waste digestion processes. Techno-
63 economic analysis, challenges and future perspectives have also been discussed.

64

65 **Keywords:** Fungal pretreatment, organic solid waste, bio-waste, ligno-cellulosic biomass,
66 cellulose, biogas, methane gas.

67

68 1 INTRODUCTION

69

70 Solid wastes materials or organic wastes or bio-wastes materials are composed of
71 large size organic molecules (macromolecules) like lignocellulose, lignin, hemicellulose,
72 proteins, fats, and vitamins etc. and they are being produced due to the various reasons and
73 their decomposition produces harmful gases and constituents which are needed to be resolved
74 properly. Proper management of these bio-wastes may also produce several beneficial
75 products like biogases, sugars, electricity (*via* biogases or other fuel sources derived from
76 biowastes' valorization), short chain carboxylic acids, fertilizers and other several products.
77 The growth of the generation of municipal solid wastes is expected to rise from 2.1 billion
78 tonnes in year 2023 to 3.8 billion tonnes by the year 2050.¹ On other side, food loss and
79 associated waste are global challenges and it is a prediction that 13% of the globally
80 generated food is wasted per year from harvest up to, but not counting retail, with about four
81 hundred billion US Dollars' economic value.² Additionally, households, restaurants and
82 many other food services are responsible for about 19% of the food loss or food waste
83 generation.² Disposed food wastes in 2017 were 38.1 million tons.³ So, it is crucial to
84 carefully manage the waste of food in order to avoid the health issues and contamination of
85 environment.^{4,5} The biggest waste produced in the course of the process of sewage treatment
86 is sewage sludge which is very rich in the organic content and due to which it is thought as



87 organic municipal solid waste.⁶ The sewage sludge's generation has grown throughout the
88 year. As for instance, sewage sludge's dry mass has heightened annually from ten million
89 tons (in 2010) to 13.5 million tons (in 2020) in the twenty seven European Union countries.⁷
90 The process of composting and anaerobic digestion is preferred because waste can be
91 processed by these ways into the safe products like organic fertilizers and soil improvers.⁸

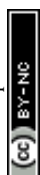
92 A series of management strategies are being promoted by the various countries and
93 technologies for food waste treatment are being developed. In Germany, there were greater
94 than nine thousand pertinent food waste anaerobic digestion projects in action by 2015,
95 accounting for greater than eighty percent of the biogas based projects in Europe.⁹
96 Additionally, food waste produces around fertilizer's 5 million tons every year.^{10,11} By 2025,
97 rate of recycling of food waste is intended to be increased by UK from current 10% to
98 70%.^{12,13} Organic macromolecules like proteins, sugars and fats are found in the food waste
99 materials. Food wastes also have trace elements like Fe (iron) and Co (cobalt), which
100 facilitate the microorganisms' growth.¹⁴

101 Huge generations of bio-wastes are serious problems for the world, specifically for
102 developing countries where managements are not as good as developed countries. But, they
103 may also be converted into the useful form if proper managements of the bio-wastes'
104 pretreatment are up to the mark because bio-pretreatment of such wastes may give huge
105 amount of energy and useful products. There are some important pretreatment techniques
106 known for the pretreatment of organic bio-wastes (**Fig. 1**) i.e. mechanical techniques of
107 pretreatments including high pressure homogenization and ultrasonication, thermal technique
108 of pretreatment, microwave assisted pretreatment, combined technology of pretreatment,
109 chemical technology of pretreatment, biological method of pretreatment including bacterial
110 pretreatment, enzymatic pretreatment, and fungal pretreatment techniques. Complexity of the
111 lignocellulosic structures and problems linked with pretreatment's chemical as well as



112 physical methods make the biological techniques very useful because they are environment
113 friendly, economical, effective and does not require or release any toxic chemicals.¹⁵ Aerobic
114 method of pretreatment, temperature-phased anaerobic digestion, and enzyme-mediated
115 pretreatment processes are promising biological pretreatment processes¹⁶ which may be used
116 as favorable alternatives of chemical, physical, or thermal processes. Aerobic process is
117 depended on sludge's inherent enzymatic activity, which requires oxygen,¹⁷ on the other hand
118 temperature-phased anaerobic digestion is done through the configuration composed of two
119 digesters in a series where thermophilic condition is applied for first digester while
120 mesophilic ones for the second digester.¹⁶ Enzyme-mediated process of pretreatment causes
121 the improvement of the sludge bioconversion with the assistance of exogenous enzymes,
122 contributing in the refractory compounds' degradation.¹⁶ For the production of biogas from
123 sludge, a critical review on various pretreatment methods has been presented by Mitraka et
124 al.¹⁸

125 Fungi and their enzymatic systems are known for their huge biotechnological
126 application due to their versatile capability and several enzymes producing ability.¹⁹⁻²² Fungi
127 have also the great roles in the environment restoration by eliminating or degrading the
128 several toxic environmental pollutants like organic molecules or heavy metals.^{23,24} Along
129 with these applications, fungi also have strong potential in the biological pretreatment of
130 organic wastes or bio-wastes. Out of several physical, chemical and biological pretreatment
131 techniques,^{18,25} fungal based biological methods may play an emerging role in this area.
132 Fungal pretreatment method is an important conventional pretreatments' alternative that is
133 mostly worked at 25–30 °C temperature range, in minimum use of water, at atmospheric
134 pressure, and without the use of any chemicals.²⁶ Wood rot fungi like soft, white, or
135 brown fungi have the most precious role in the fungal pretreatment technique because of
136 their potential to change the lignocellulosic biomass' constituents.²⁷ After the fungal



137 pretreatment, detoxification and/or washing are not generally necessary because the fungal
138 treatment's mild conditions are unlikely to generate microbial inhibitory compounds. At
139 higher feedstock's particle sizes, fungal technique of pretreatment is more effective than the
140 most conventional methods of pretreatment.²⁶ Fungal pretreatment have also some
141 disadvantages along with aforementioned advantages. Requirement of long time for reaction,
142 smaller yields of sugars and the need of sterilization of feedstock are the potential
143 shortcomings of the fungal pretreatment technique in comparison with traditional one.²⁸

144 Utilization of solid state procedure with small energy and chemical involvements,
145 fungal technique of pretreatment is considered as a low cost technique²⁷ but above described
146 shortcomings, mainly, the longer time requirement in fungal pretreatment process may make
147 it more costly which may be eliminated using the combined pretreatment technology.
148 Sterility requirement, long time of residence, significant heat generation by fungal metabolic
149 rate and need of the high rate of aeration for efficient delignification may have significant
150 role in the techno-economic study of the fungi based pretreatment at the level of commercial
151 scale, and it is still to be studied.²⁷ Even after a few shortcomings or challenges of the fungal
152 pretreatment methods, several researches show that fungal pretreatment techniques may be a
153 noteworthy biological pretreatment way for the treatment of varieties of biowastes or
154 biomasses^{29,30} because it provides a gentle, ecofriendly, and low cost future solution for the
155 biomass conversion and it is widely utilized in the chemicals and biofuels production due to
156 its role in the degradation of lignin, saccharification, lipid accumulation and fermentation.²⁹

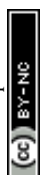
157 Observing the crucial involvement of fungi in the pretreatment of ligno-cellulosic
158 biomasses, agricultural wastes, food wastes, saccharification process, biogas production,
159 bioethanol production, and sugar production etc., authors have decided to prepare a
160 comprehensive review on the fungal pretreatment of such biowastes materials. The principle
161 objective of this review is to analyze the significance of the fungal pretreatment in the



162 management of the problem of huge organic wastes or bio-waste materials and presents the
163 techno-economic analysis, potential future challenges and its applicability. During the fungal
164 pretreatment process, production of biogases, bioethanol, sugar molecules, and other
165 biologically valuable components have great future to be used in the welfare of humanity.
166 Several recent literatures have been discussed here in details in order to open and understand
167 the promising possibilities of fungal pretreatment methods. Furthermore, brief but effective
168 discussions have also been made here on topics of organic wastes and various pretreatment
169 techniques.

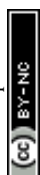
171 2 ORGANIC WASTES OR BIO-WASTES OR SOURCES OF BIOMASS

172
173 Wide ranges of materials come under the topic of organic solid wastes or bio-wastes.
174 Wastes of foods, sewage sludge, plants and animal biomasses, fungal and algal biomasses,
175 several aquatic and terrestrial wastes, agricultural wastes, industrial wastes, and many other
176 waste materials which are rich in organic components like polysaccharides, lignin, proteins,
177 fats, and vitamins are considered as organic waste or organic solid wastes. **Fig. 2** presents the
178 picture of huge plant biomasses from nature (dead plant materials as well as green plants). In
179 a comprehensive critical review, Lizundia et al. have well-described the organic waste
180 valorization.³¹ Algae and crustaceans among the great diversity of aquatic organisms are
181 considered as the good source of organic waste. The vast probabilities to get varied
182 polysaccharides are offered by the marine algae's high quantity reaching the coast and
183 discarded marine. About 9.1 million tonnes are discarded by the fisheries annually³² whereas
184 seafood accounts for thirty one percent of the consumer- level food's losses in United State of
185 America (USA).³³ Wastes derived from forestry (softwood, grass, sawdust, and hardwood)
186 offers biomasses with high ligno-cellulosic nature at small cost. As opposite to the waste



187 from agriculture, intensive physico-chemical treatments are required by the forestry
188 lignocellulosic waste given the more complex structure of cell wall.³⁴ In order to extract the
189 important and valuable materials, hydrolysis or thermo-chemical processes are applied. With
190 a projected global production of egg of ninety million tons by 2030,³⁵ eggshells denote
191 terrestrial animal wastes' classical example with portions still usable after discarding. It is a
192 hydroxyapatite's resource after the calcination and following treatment with salts, for
193 instance $\text{Ca}_3(\text{PO}_4)_2$.^{36,37} Good access to hydroxyapatite is also offered by the porcine or
194 bovine bones. After the removal of residual protein through alkaline process and calcination
195 at large temperature, yields of ~65 wt% are attained.³⁸ Opposite to synthetic hydroxyapatite,
196 bio-derived hydroxyapatite displays traces of Na^+ , Mg^{2+} , K^+ , Zn^{2+} , Al^{3+} , Sr^{2+} , F^- , Cl^- ,
197 SO_4^{2-} , and CO_3^{2-} which are valuable for stimulating the proliferation functions of cell.³⁹
198 Bovine bones may be utilized for the extraction of collagen fibres which are mineralized.⁴⁰

199 There are a numbers of sources of bio-wastes with promising compositions from
200 aquatic origin like fishes (hyaluronic acid, skin's collagen, gelatin after denaturation), red
201 algae (carrageenans and agarose in the cell walls), brown algae (cell walls' based alginate),
202 chitin (chitosan found upon chitin deacetylation), cephalopods endoskeleton (β -chitin,
203 proteins, lipids), and cuticles from marine-arthropod (varied proteins enfolded alpha-chitin
204 nanofibrils). Agricultural bio-wastes are cereals (rice, corn, wheat, rye, barley, oats etc), fruits
205 (grape, orange, apple, coffee, mango, banana, apricot, pineapple etc), vegetables (carrots,
206 tomato, olive husk, onions, potato, red beet), and legumes (lentils, cow pea, lupins, beans
207 chickpea etc). Bio-wastes from the forestry origin are grass, softwood, hardwood, sawdust;
208 cellulose, lignin, and hemicelluloses). Fungal bio-wastes have composition of glucans, chitin,
209 glycoproteins, and melanin. Bio-wastes from the terrestrial animal origin are eggshell
210 (CaCO_3 , organic matter, MgCO_3 , $\text{Ca}_3(\text{PO}_4)_2$), bones with natural hydroxyapatite and collagen
211 fibres which are mineralized, feathers with beta-keratin, manure (carbon, oxygen, hydrogen,



212 nitrogen, sulphur), wool with alpha-keratin, fat, impurities etc, structural proteins derived
213 from animal (silk, collagen, and gelatin), and exoskeletons of terrestrial-arthropod (proteins
214 enfolded chitin fibres).³¹

215 Parts of the plants like fruits, tubers, and seeds, from different crops like sunflower,
216 rapeseed, palm, cotton, corn and soybean have been utilized in the generation of first-
217 generation ethanol and biodiesel (**Fig. 3**)⁴¹ while on the other hand, complete biomass of
218 above-ground plant called as ligno-cellulosic feedstock (inedible leaves and stems) has been
219 utilized for creation of the biofuels of second-generation.⁴¹ Switchgrass, sorghum,
220 miscanthus, and eucalyptus are some biofuel crops and a few of them have adaptability to
221 deprived soils and marginal agronomic lands.⁴² Olives, coconut and eastern black walnut's
222 endocarps/shells are the feedstocks with great density and contain the maximum content of
223 lignin of all recognized organs of plants and endocarp derived energy is equivalent to coal.⁴³
224 Worldwide, there is the availability of several million tons of the biomass of drupe endocarp
225 (24-31 million tons),⁴⁴ that is greatly underutilized and countries could be benefited with
226 energy scarcity by its proper management.⁴³ Agricultural and industrial wastes are also the
227 important sources of lignocellulosic materials and may be used for the generation of biofuels.
228 Lignin, cellulose, hemicellulose are the major components of plant cell wall while proteins,
229 organic acids, tannins along with secondary metabolites are its minor components.
230 Lignocellulosic material's composition varies with species, plant parts and ecological
231 situations.^{43,45}

233 3 PRETREATMENT METHODS FOR ORGANIC WASTES

234
235 Mainly two types of waste are generated by our daily garbage in which first type
236 include non-biodegradable wastes (i.e. metals, plastics, glass etc.) while second one includes



237 biodegradable wastes like leftover foods, dried leaves, fruits etc.⁴⁶ Several animal based
238 products, plant linked products, garden wastes, food wastes, and degradable carbon are the
239 examples of biodegradable organic wastes. Energy may be provided as biogas by using the
240 organic waste's recycling methods of anaerobic digestion.⁴⁶ Efficiency of non-organic
241 recycling is also improved by organic and non-organic wastes' separation.⁴⁷ Minimization of
242 pollution in the water, air, and land by the recycling of organic wastes is its one of the
243 promising advantages. For less effective techniques (disposal and incineration), amount of
244 the garbage left over is also minimized by the recycling of the organic wastes. Organic
245 waste's stabilization offers value by enhancing the content of nutrient and obtainability for
246 utilization as fertilizer in the area of agriculture. Popular concepts like zero-waste strategies,
247 cleaner production, sustainability, and bio-based circular economy are uplifted by the
248 recycling of organic waste.^{46,48,49} Different barriers in the management of the organic waste
249 have also been assessed by the Kharola et al. (2022).⁴⁶

250 The biogas generation from organic wastes may be a promising factor for a
251 sustainable future. Organic wastes utilization for biogas generation applying the methods of
252 mono-and co-digestion has been broadly reported.²⁵ In spite of the reactions of many-stages
253 related with the production and hydrolysis is crucial as 1st step and helps in enhancing the
254 complete yield. Hydrolysis step's optimization causes the complex organic matter's
255 decomposition into the large quantities of monomeric/oligomeric components which can
256 simply be used in anaerobic situation for biogas production. The aim of pretreatment
257 approaches is making the existing nutrients reachable to the maximum species of microbes
258 that accelerate the use of biomass for the duration of anaerobic digestion.⁵⁰ In a review,
259 Mitra et al. (2022) have comprehensively discussed the several pretreatment methods for
260 the increased production of biogas from sewage sludge¹⁸. There are many methodologies of



261 pretreatments of organic wastes for the biogas production^{18,25} which have been concisely
262 discussed here (**Table 1**).

263

264 **3.1 Mechanical Pretreatment**

265

266 This type of pretreatment method causes the reduction of the size of particles of
267 organic wastes and does not generate any products.²⁵ It is a process which consumes energy
268 and this is the main drawback of this method. Developments in milling approaches display
269 that in compare with dry milling procedure, wet milling is better because of its greater
270 pulverization properties with minimal consumption of energy.⁵¹ de Oliveira *et al.* (2022)
271 investigated 02 wet mechanical pre-treatments on bio-wastes from urban household that were
272 air-compressed press and worm screw press.⁵² In each experiment, they studied 02
273 liquid/solid ratios. Enhancement in the biodegradable organic matter's proportion extracted
274 from bio-waste was allowed by enhancing the ratio of liquid to solid in pre-treatments up to
275 949 gCOD.kgTS⁻¹ from the household bio-waste. In constantly stirred-tank based reactor, a
276 very good COD load conversion (81%) and methane's high production up to 345
277 LCH₄.kgVS⁻¹ was shown by the anaerobic digestion.⁵² Cesaro *et al.* (2021) assessed the
278 press-extrusion pretreatment's potential to upturn the concert of anaerobic degradation of
279 organic part of solid municipal waste.⁵³ Among the methods of mechanical pretreatment,
280 great interest was recently raised by the press-extrusion for its probable use to either increase
281 the organic weight to digester or enhance the overall stability of process and methane's
282 yields.⁵³ For the improvement in the production of bio-methane, Chevalier *et al.* (2023)
283 successfully evaluated the influence of mechanical method of treatment *via* twin-screw
284 extrusion using lignocellulosic biomasses of different types and they tested the 02 dissimilar
285 shear stress screw profiles.⁵⁴ Specific rate were found to be enhanced by both extrusion



286 mechanical treatments which was proved by the kinetic assessment of the production of methane.
287 methane.
288

289 **3.1.1 High Pressure Homogenization Technique (HPH)**

291 High potential of disintegration, minimal costs of operation, handling and operation's
292 simplicity without chemical variations are a few of the benefits linked to the method.^{25,55} Sun
293 et al. (2022)⁵⁶ used HPH to treat the soybean protein isolate as potential method. Spatial
294 structure of insoluble soybean protein isolate was destroyed by the pretreatment using HPH;
295 particle size of the soybean protein isolate dispersion was significantly decreased.⁵⁶ Nabi et
296 al. (2022)⁵⁷ explored the improvement of sludge's anaerobic breakdown by combining the
297 HPH with FNA (free nitrous acid) pretreatment. In comparison with individual HPH
298 treatment and FNA treatment, triggered sludge was solubilized by HPH-FNA pretreatment
299 efficiently and thus, there was subsequent improvement in the anaerobic breakdown
300 process.⁵⁷ Biogas's cumulative generation from combined HPH-FNA pretreated sewage
301 sludge was 154, 108, and 284% higher than free nitrous acid, HPH, and raw sludge single
302 pretreatment, respectively. Content of methane in biogas was 45%, 51%, 55% and 65% for
303 the raw sludge, free nitrous acid, HPH, and HPH-free nitrous acid pretreated sludge,
304 respectively.⁵⁷ HPH has also been utilized for the recovery of the agri-food remains.⁵⁸ Malik
305 et al. (2023)⁵⁹ explores the potential of HPH technology in the functional foods development.
306 In another study, HPH was optimized and used for the intensification of the bioactive
307 components recovery from tomato byproducts.⁶⁰ Significant improvement in the biogas yield
308 of sludge's anaerobic digestion was found using HPH methods.⁶¹ Also, there are number of
309 other studies on HPH utilization in biowaste management which have been omitted to be
310 focused on the main subject.



311 3.1.2 Ultrasonication

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313 Ultrasonication was described as the most effective method of pretreatment by Pili
314 *et al.* (2011)⁶² and effectiveness of process is exclusively reliant on the biowaste/sludge's
315 features. On the basis of literature availability, ultrasonication is extensively described for
316 wastewater pretreatment, sludge pretreatment, and manure pretreatment in anaerobic
317 digestion processes for generation of biogas.^{25,63-65} There is a recent review on the ultrasonic
318 processing of food waste by Wu *et al.* (2022)⁶⁶ who presents detailed insights on the use of
319 ultrasound in the processing of food wastes. Karouach *et al.* (2020)⁶⁷ evaluated the influence
320 of CMUP (combined mechanical and ultrasonic pretreatment) on household organic waste
321 fraction's anaerobic digestion. They compared the results gained by the experiment with
322 mechanical pretreatment and CMUP. Mechanical pretreatment was taken as control in this
323 study. The 382 mL CH₄ g⁻¹VS (at 0 °C temperature, 1 atm pressure), 72%, and 493 mL CH₄
324 g⁻¹VS (at 0 °C temperature, 1 atm pressure), 86% were the yields of methane and control and
325 combined mechanical & ultrasonic pretreatment (CMUP) biodegradability, respectively
326 which displays the biodegradability enhancement and enhancement in the production of
327 methane by CMUP. Result also suggests that hydrolysis stage and methanogenesis stage of
328 procedure are upgraded by the combined way of pretreatment.⁶⁷ Ultrasonication was
329 successfully utilized in order to improve the methane production from sewage sludge.⁶⁸
330 Ultrasound assisted technology has been reviewed for the valorization of bio-wastes
331 purposes.⁶⁹ There are also several studies on the application of ultrasound.^{70,71}

332

333 3.1.3 Utilities of Mechanical Pretreatment in Biological (Fungal) Pretreatment

334 There are several commonly used mechanical mechanisms for the size reduction of
335 lignocellulosic organic wastes like cutting, shearing between flat surfaces, tearing,



336 compression and breaking the materials.⁷² Milling, grinding, ultrasonications, refining and
337 many others are also the widely utilized mechanical methods for particle reduction.
338 Mechanical treatment of lignocellulosic materials or organic biowastes is a necessary step in
339 the process of pretreatment in order to maximize the material's valorization potential.⁷² Thus,
340 it has great application in the biorefinery. Milling is a mechanical process which is generally
341 applied to the materials before the start of any other treatment process. Thus, it may have
342 great applicability in the fungal based biological pretreatment process to reduce the size of
343 biowastes materials for the efficient use of these reduced materials in the bioreactor with
344 fungal system (fungal mycelia and/or fungal enzymes). The use of mechanical treatment
345 before the fungal or other treatment does not cause any chemical alteration in the materials to
346 be treated and improves the effectiveness of other pretreatment processes which are applied
347 after mechanical method.⁷² Need of the lignocellulosic materials or biowastes in bulk
348 amounts cause the difficulties in handling, transportation and processing for its fungal or
349 other biological pretreatment process in bioreactors but the use of mechanical treatment
350 before the fungal pretreatment process make the easy handling, transfer, and processing of
351 these biowastes in large density. Optimization of the mechanical pretreatment process is
352 needed because it is a process which consumes high energy. Various types of mills can be
353 utilized but selection of equipment is based on the material's type and properties, final
354 required size of the materials, and operational systems like continuous or batch system or
355 bioreactor.⁷² In literature, different types of equipment have been used for the continuous
356 milling before the biological or other methods (as the final treatment process) like disc
357 refiner,⁷³ screw extruder,⁷⁴ knife mill,⁷⁵ hammer mill,⁷⁶ and many others.⁷²

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361 3.2 Thermal Pretreatment

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363 Thermal method of pretreatment helps in hydrolyzing the organic wastes' complex
364 organic components and has been detected to increase the anaerobic digestion.²⁵ El Gnaoui et
365 al. (2022)⁷⁷ evaluated the thermal pretreatment's effect, including thermal pretreatment at
366 60 and 80 °C temperatures for sixty minutes, and thermal pretreatment at 100, 120, and
367 140 °C temperatures for thirty minutes, as well as pre-hydrolysis (biological) at temperatures
368 37, 55, 37 °C followed by temperatures 55 and 55 °C temperature followed by temperature
369 37 °C for forty hours on the performance of anaerobic breakdown of food waste in batch test.
370 An enhancement in the soluble COD and efficiency of hydrolysis was caused by the pre-
371 hydrolysis (biological) and thermal way of pretreatment. There was increase in the yield of
372 methane from 371.17 mL CH₄/g VS for untreated food waste to 471.95 mL CH₄/ g VS. There
373 was greatest methane yield for biological pre-hydrolysis at 37 °C temperature for twenty
374 hours followed by 55 °C temperature for twenty hours. Rate of formation of biogas was
375 increased and lag phase was decreased by the pretreatments.⁷⁷ There are also many other
376 recent literatures on thermal pretreatment.⁷⁸⁻⁸⁰

377

378 3.3 Microwave Pretreatment

379 This is also a type of heat pretreatment method. Besides the generally utilized thermal
380 method of pretreatment, microwave pretreatment are effective in the stabilization of the
381 organic wastes and biogas formation.²⁵ There are a few benefits with this method which
382 includes quick heating and penetration, simplicity of handling as well as control, pathogens
383 elimination, effective dewaterability of sludge and sludge reduction⁸¹ and due to which this
384 method is more efficient in comparison with conventional thermal methods of pretreatment.
385 Microwave pretreatment's effect on the model food waste's anaerobic fermentation to



386 organic acids with small chains and ethanol was investigated.⁸² Microwave pretreatment was
387 studied at 120 °C, 150 °C, and 180 °C (three temperatures) and two, five and eight minutes
388 residence times. The highest decrease in the volatile suspended solids (VSS) was 20%,
389 representing organic matter solubilisation. There was greater (17.5% COD COD⁻¹) total
390 product yield in the fermentation batch tests in comparison with for untreated substrate
391 (11.1% COD COD⁻¹).⁸² Influence of the microwave based pretreatment on sludge as well as
392 food waste's anaerobic co-digestion was described by Liu et al. (2020).⁸³ Results displayed
393 that microwave pretreatment was useful to the organic materials' dissolution, protein
394 conversion to NH₄⁺-N, cumulative production of CH₄, unit yield of bio-methane, and
395 methane's formation reaction rate in sludge and food based waste's anaerobic system of co-
396 digestion. In the co-digestion system, CH₄ maximum cumulative production reached
397 3446.3 ± 172.3 mL (thirty five days) that was 19.93% greater in compare with control.
398 Moreover, microwave method of pretreatment considerably enhanced the volatile fatty acids
399 accumulation and content of butyric acid in the anaerobic-digested effluent.⁸³ Hydrolysis of
400 the cassava pulp was studied by Prasertsilp et al. (2023)⁸⁴ using microwave method for the
401 effectual utilization of natural materials and four different factors like liquid-solid ratio, acids
402 types, watt power and time were investigated. High glucose amount was provided by this
403 study having 88.1% conversion. There are several other relevant studies on the microwave
404 assisted pretreatment technology.^{85,86}

405

406 3.4 Chemical Pretreatment Methods

407

408 In this method, oxidants, alkali, and/or acids are used to breakdown the organic
409 components and it is found to be very effective for the purpose. Ozonation and peroxidation
410 (oxidation) are found to be beneficial in the pretreatment causing in solubilisation of sludge.²⁵



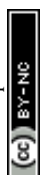
411 Up to a certain limit, there are a dose-reliant association between concentration of oxidant
412 and solubilization of sludge. Therefore, peroxidation and ozonation process tends to display
413 greater rate of sludge degradation having compromised biogas's yield.^{87,88} In a study, Alino
414 et al. (2022)⁸⁹ assessed the effectiveness of a low-cost and greater sustainable method to
415 enhance the sugarcane bagasse biodegradability and enhance methane's generation by its pre-
416 collection with organic bio-wastes of acidic types (like cheese whey, wastes of fruit, and
417 waste of vegetable). They got the best result with sugarcane bagasse plus waste of fruit and
418 vegetable (5:95 ratio), being 520 ± 7 NL CH₄ kg VS⁻¹ (27.6% greater in compare with
419 control) with time of degradation (T90) decreased from thirteen days to seven days. Yield of
420 methane was increased by 21.2% and 34.1% by the alkaline pretreatment with sodium
421 hydroxide at 5% and 10% concentrations, respectively.⁸⁹ Jankovičová et al. (2022)⁹⁰
422 performed the work on material's hydrolysis in NaOH (0.5% and 5%) and H₂SO₄ (0.5% and
423 5%) at 90–100 °C for 2 hours. Influence of these techniques on the lignocellulosic
424 constitution of rapeseed straw, maize based waste, and wheat straw and the yields of biogas
425 were compared by them. The 0.5% NaOH pretreatment enhanced the production of biogas
426 the most (for rapeseed straw by 159%, wheat straw by 240% and maize waste by 59%),
427 furthermore, the solubilization degrees were greater.⁹⁰ Studies of Sreevathsan et al., (2023)⁹¹
428 on ozonation effects on the biodegradability improvement and bio-methanation ability of the
429 wastewater pretreatment and study of Qiao et al. (2023)⁹² pretreatment of landfill leachate
430 state the advantages of chemical pretreatment.

431

432 **3.5 Combinations of Chemical Pretreatment Methods with Other Pretreatment**

433

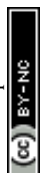
434 Chemical methods of pretreatment including the utilization of alkali and acid are
435 typically used in amalgamation with additional method of treatments.⁹³⁻⁹⁵ The combined



436 techniques of pretreatment are beneficial in improving the solubilization of sludge, its
437 sanitation, dewaterability and anaerobic way of digestion.²⁵ Influence of thermal and
438 combined thermal-chemical pretreatments was investigated by Ahmed et al. (2022)⁹⁶ on
439 solubilization of organics, yield of biogas, formation of recalcitrant, and efficiency of energy
440 at changeable temperatures and alkali dosage. Municipal solid waste's organic fractions were
441 applied to the thermal pretreatment (i.e. 100-200°C temperature, 1.6–15.8 bar pressure, and
442 30–120 minutes reaction time) alone and in conjugation with dosage of alkali (1–7 g/L
443 NaOH). Over control (331 mL/gVS_{added}), the maximum biogas production increase of 43%
444 (474 mL/gVS_{added}) and 87% (618 mL/gVS_{added}) was noticed at temperature 125°C and
445 125°C+3g/L sodium hydroxide (NaOH) dose, respectively.⁹⁶ Pham *et al.* (2021)⁹⁷ performed
446 acid-catalyzed hydrolysis at temperatures 120, 150, and 180 °C utilizing H₂SO₄ (sulfuric
447 acid) with 0–0.5 M concentrations within 90–180 minutes reaction time to yield bio-based
448 chemicals from sludge of sewage. The maximum yield of xylose was 7.69 mol% while the
449 maximum yield of glucose was 5.22 mol% at temperature 120 °C, 0.5 M H₂SO₄ in the course
450 of 180 minutes of reaction time. Moreover, under acid-catalyzed hydrolysis at 180 °C
451 temperature and 180 minutes, at 0.5 M H₂SO₄, levulinic acid production touched a highest
452 level of 0.48 mol%, and at 0.1 M H₂SO₄, the maximum production of 5-
453 hydroxymethylfurfural was 1.66 mol%.⁹⁷ Qiao et al., (2023)⁹² describes the importance of
454 combined physicochemical methods for the landfill leachate pretreatment process.
455 Combination methods of pretreatment are more useful techniques in the pretreatment
456 processes in compare with individual methods of pretreatment. In another study, plant wastes
457 were studied by the combined pretreatment using physical, enzymatic and chemical
458 techniques and this study also proved that the combined methods are more favorable.⁹⁸

459

460



461 3.6 Biological Pretreatment Methods

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462

463 Biological pretreatments of organic wastes or bio-wastes are the promising
464 ecofriendly methods which are based on the use of mainly fungi, bacteria, and enzymes. Use
465 of them in the pretreatment of organic waste is very effective in decomposition of the large
466 cellulosic or other organic materials into the monomeric or smaller units and generating
467 valuable biogases. **Table 2⁷⁸** shows the variation in the production of biogas from some
468 lignocellulosic biomass after low temperature pretreatment process under biological
469 condition.⁹⁹⁻¹⁰⁶ In biological pretreatment and among various microorganisms, fungi are of
470 utmost significance and filamentous fungi specifically *Basidiomycetes* have great potential in
471 delignification and lignocellulosic biomass conversions with the role of effective involvement
472 of their enzymatic machinery.¹⁵ In bio-waste processing, microbial roles are promising in the
473 improvement of production of biogas and the process can be enhanced by using various
474 metagenomics approaches.¹⁰⁷ Literatures of Mitraka et al. (2022)¹⁸ and Salihu and Alam
475 (2016)²⁵ have also detailed discussions on biological pretreatment. Bacterial pretreatments
476 have been very concisely discussed here with a few recent literatures because main purpose is
477 to focus comprehensively on the fungal pretreatment techniques.

478

479 3.6.1 Bacterial Pretreatment of Organic Wastes

480 Bacteria have been found as effective candidates for the pretreatment of organic solid
481 wastes. *Bacillus licheniformis* and *Bacillus oryzaecorticis* may be utilized in bio-waste
482 managements as they have shown the promising role in the food waste degradation. Starch
483 was degraded by the *Bacillus oryzaecorticis* and reducing sugars' big amount was found to be
484 released, providing hydroxyl and COOH to fulvic acid molecules while positive result on the
485 structure of humic acid was shown by *Bacillus licheniformis*, which had greater hydroxyl



(OH), methyl (CH₃), and aliphatics.¹⁰⁸ Liu *et al.* (2022)¹⁰⁹ isolated nitrogen (N)-fixing and lignin-decomposing bacterial strain *R. ornithinolytica* RS-1, from an abandoned termite colony. In order 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 the further hemicellulose depletion. After only 7 days, this bacterial strain RS-1 degraded lignin with a 19% reduction whereas relative cellulose content was enhanced with 21%. Moreover, conversion of the cellulose of the corn stover was found up to 48.58% through a process of 2-stage using sodium hydroxide (2.5%) pretreatment. In the meantime, considerable removal of lignin and hemicellulose was observed. Furthermore, the highest activity of manganese peroxidase was found on day 3 (181.0256 U/L) while highest activity of lignin peroxidase was on day 5 (37.473 U/L), respectively.¹⁰⁹ Song *et al.* (2021)¹¹⁰ presented an evaluation of paper waste's anaerobic and micro-aerobic pretreatment with various oxygen loadings through 5 microbial agents like composting inoculum, cow manure, straw-decomposing inoculum, digestate effluent, and sheep manure. Result showed that paper waste pretreated by digestate effluent with a 15 ml/gVS oxygen loading demonstrated the maximum cumulative yield of CH₄ of 343.2 ml/gVS, with a biodegradability of 79.3%. Besides digestate effluent, straw-decomposing inoculum and sheep manure were likewise observed as promising microbial agents due to the quickening of the production of methane at anaerobic digestion's early stage. It was demonstrated by the analysis of microbial community that after anaerobic way of pretreatment *via* 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 were all contributable to the degradation of cellulose. Besides, degradation of lignin was probably promoted by aerobic *Bacillus* in straw-decomposing inoculum and *Acinetobacter* in sheep manure and digestate effluent only in

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511 micro-aerobic conditions. In the course of anaerobic digestion *C. sensu stricto* 1, *VadinBC27*,
512 *Caldicoprobacter*, and *Fastidiosipila* were the key bacteria that enabled the paper waste's
513 bio-decomposition.¹¹⁰ These works demonstrate the potential of bacteria in biological
514 pretreatment of organic wastes and methane gas production.

515

516 3.6.2 Fungal Pretreatment

517 This is one of the important biological methods of pretreatment. Bio-wastes or organic
518 wastes or lignocellulosic structures may be effectively treated with fungal system (fungi
519 and/or associated enzymes). There are a number of significant studies on the fungal role in the
520 pretreatment of such waste materials. As the key reason behind this review was to presents an
521 insight view on the role of fungi in the pretreatment process of biomasses, this has been
522 comprehensively and independently discussed in next section as main heading.

523

524 4 FUNGAL PRETREATMENT OF ORGANIC WASTES

525

526 Due to minimum energy condition and negligible toxicity, fungal pretreatment
527 processes drew promising attention for their role in the biomass conversion. It prevents
528 chemicals use, hinders the compounds production and has good selectivity towards lignin
529 degradation.^{111,112} Several choices are provided by the useful and copious species of fungi for
530 the biomass conversion. Rice straw, wheat straw, corn straw, peanut shell, coconut shell,
531 bagasse, spent coffee ground, food digestate, food wastes, hardwoods, softwoods and
532 switchgrass etc are the recognized biowastes upon which fungal treatments techniques have
533 been successfully applied. **Table 3** shows the major chemical compositions of various types
534 of biowastes/organic waste.¹¹³⁻¹²¹ After the degradation of ligno-cellulose structure, cellulose
535 and hemicellulose efficiency in the biomass rises.²⁹ **Fig. 4** shows the complex structure of



536 lignin¹²² while **Fig. 5** shows the schematic presentation of cellulose degradation. Competence
537 of subsequent fermentation and accumulation of lipid is promoted by the saccharification,
538 and thus various downstream products can be formed from the biomass conversion.^{123,124}
539 However, its popularization has been hindered due to low efficacy of fungal pretreatment
540 process, but applying the useful strategies may result in to the improvement of the efficiency
541 of fungal technology.²⁹ This section has a comprehensive approach for the fungal
542 pretreatment technology in the biomass conversion based on recent literature studies.
543 Different methods based on fungi have been adopted and successfully performed the process
544 of biomass/bio-waste valorization by several researches which have been reviewed and
545 presented in this section with their results. Fungal pretreatments of organic wastes or
546 biomasses has been discussed here as a main heading because the principle target of this
547 review was to comprehensively evaluate the various studies already done on the fungal
548 pretreatment technology in solving the problem of different organic waste or bio-wastes and
549 production of many useful products like biogas, bioethanol, sugars, acids, and others.

551 **4.1 Fungal Pretreatment of Lignocellulosic Biomass of Agricultural Wastes**

552
553 Fungi play significant roles in the decomposition of the various cellulosic materials
554 (like wheat straw, willow chips, rice straw, sugar bagasse, corn stover, and plants materials
555 etc.) as well as agricultural wastes. Lignocellulosic biomass contains high amount of complex
556 carbohydrates i.e. 55–75% in total solids (TS) and is renewable as well as widely available,
557 that is why, it could be excellent feedstock for the purpose of the production of energy.¹²⁵ It is
558 well established that WRF may be involved in the improvement of enzyme based hydrolysis
559 and its subsequent sugar's yield.¹²⁶ Due to this reason, their involvement in pretreating the
560 substrates, mainly for the generation of bioethanol^{125,127} has been studied, though hardly for



561 anaerobic digestion.¹²⁸ In case of submerged fermentation for pretreatment, Solid State
562 Fermentation (SSF) process is found better which permits for greater loads of feedstock,
563 prefers the fungal enzymes' attachments to substrate, along with diffusion of oxygen. SSF
564 needs less aeration, mixing, water, and heating due to which there are lower cost than liquid
565 culture.¹²⁹

566 Pretreatment of lignocellulosic materials like wheat straw, woody willow chips, and
567 corn stover was studied by Kovács et al. (2022)¹³⁰ with the help of four filamentous fungi,
568 namely, *Penicillium aurantiogriseum*, *Gilbertella persicaria* (SZMC11086), *Trichoderma*
569 *reesei* (DSM768), and *Rhizomucor miehei* (SZMC11005). Excellent production of hydrolytic
570 enzyme and maximum yield of biogas from the partly decomposed substrates were shown by
571 *P. aurantiogriseum*. Corn stover was the finest material for the breakdown of biomass and
572 generation of the biogas. Highest effective strain for the pretreatment and biogas was *P.*
573 *aurantiogriseum*. All the tested fungi preferred the corn stover substrate for the productivity
574 of methane.¹³⁰ In 60 mL batch fermentation, within the first twenty days, a noteworthy
575 portion of the generated methane (95%) was found to be evolved. Maximum yield of
576 methane was for corn stover fed reactors. The maximum average production of methane (281
577 mL_N/g oTS) was yielded by the reactors pretreated with *P. aurantiogriseum*. During 300 mL
578 batch fermentations, influence of the five-fold enhancement in volumetric scaling-up was
579 also studied in the succeeding step. But, there was a point of attention that only 13.3%
580 maximum difference (for the case of *P. aurantiogriseum* reactors) was observed between the
581 outcomes of two reactor sizes which show the insensitiveness of the overall process to scaling
582 up. *G. persicaria*, *T. reesei*, and *R. miehei* based pretreatment of all substrates also showed same
583 behavior.¹³⁰ Biogas production from corn silage was found to be enhanced by *Pleurotus*
584 *ostreatus* and *Dichomitus squalens* while negative impact was shown by *Trametes versicolor*
585 and *Irpex lacteus*.¹³¹ Increase in the cumulative production of CH₄ was 1.55 fold with *P.*



586 *ostreatus* after ten days and at 28 °C while longer duration of pretreatment (30, 60 days) View Article Online
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587 showed lower effect. With distinctive corn silage, CH₄ production was increased from 0.301
588 to 0.465 m³kg_{VS}⁻¹ due to depolymerization of lignin.¹³¹

589 Pallín *et al.* (2024)¹³² utilized *Irpex lacteus* for the lignocellulosic biomass's (wheat
590 straw) pretreatment and assessed the feasibility at a demonstration scale. Like submerged
591 cultures, scaling up SSFs is not forthright. Before choosing the best design for bioreactor,
592 several issues like microbes' growth kinetics, need of agitation, solid substrate's physical
593 properties, conditions of sterilization, and agitation generated mechanical stress should be
594 tackled. During the process, agitation based design of SSF reactor was ruled out because of
595 the agglomeration of *Irpex lacteus* into wheat straw. After considering the several points
596 about SSFs reactor, an autoclavable vertical bioreactor of 22 L capacity was designed.¹³²
597 Digestibility of the wheat straw after 21 days of the pretreatment in the solid state
598 fermentations bioreactor (60.6%) was alike to that found on a small scale i.e. 57.9%. In the
599 03 bioreactor experiments (B1, B2 and PB), sugars evolution was completely different after
600 21 days of treatment.¹³² The 26.5% lignin's reduction was observed. There was greater
601 lowering of lignin in experiment B2 i.e. 34.90 ± 0.87% in comparison with that of experiment
602 B1 i.e. 26.7 ± 3.08%. A rise was noticed for all the compounds in the PB fermentation except
603 lignin, which was lowered by 52.3 ± 0.69%. It was the biggest lessening of any biomass
604 constituent obtained in 03 solid state fermentations reactor experiments. There was alike
605 reduction of lignin i.e. 53.2 ± 0.60% in the flask-scale pretreatment.¹³²

606 Two isolated species of fungi, namely, *Trichoderma harzanium* and *Aspergillus terreus*
607 were used to investigate the degradation of the cellulose followed by bioethanol generation
608 from acid-thermal pretreated rice straw and the experiment was conducted in two phases.¹³³
609 In first phase, *Aspergillus terreus* and *Trichoderma harzanium*, isolated cellulose degrading
610 fungi were used for the pretreated rice straw's enzymatic hydrolysis which was parted into



611 HL (hydrolysate liquid) and RP (residual pulp) while in the second phase of the experiments
612 substrate (enzymatically hydrolyzed) was applied to the yeast fermentation for the generation
613 of bioethanol. Results showed the 80% degradation of cellulose by these fungi. *Aspergillus*
614 *terreus* performance upon cellulose lowering with hydrolysate liquid and residual pulp was
615 92% and 80%, respectively while for *Trichoderma harzanium* it was 93% and 82%,
616 respectively. With the use of *A. terreus*, glucose formation from cellulose during enzyme
617 hydrolysis was 12.15 g/L and 16 g/L while it was 10.8 g/L and 21.6 g/L using *T. harzanium*.
618 Mechanism in this process involves the action on β -1, 4- glycosidic bonds by the cellulose
619 degrading fungi through enzymatic activities only after the pretreatment process. Rice straw's
620 RP treated with *T. harzanium* has led to in greater bioethanol of 5.4 g/L while for *Aspergillus*
621 *terreus*, it was 4.7 g/L.¹³³ The pHs of the enzymatically pretreated reactors using *A. terreus*
622 were 3.8 for control, 4.8 for hydrolysate liquid, and 4.5 for residual pulp while in the case of
623 *T. harzanium* base reactors, pH values were 5.0, 4.9, and 4.8. Substrate and inoculum types
624 are the major factors for the production of bioethanol between pH range of 3.5-6. In reactors
625 based on hydrolysate liquid as substrate, *A. terreus* and *T. harzanium* performances in
626 production of bioethanol was same as 4.5 g/L.¹³³

627 A genuine challenge in the bioethanol production from rice straw is to remove lignin
628 properly from biomass through pretreatment process because lignin removal is necessary to
629 generate bioethanol from rice straw effectively by the saccharification and fermentation
630 process.¹³⁴ This challenge may be tackled through the use of alkali assisted pretreatment and
631 acid thermal pretreatment of rice straw. Devi and Munjam¹³³ have successfully used *A.*
632 *terreus* and *T. harzanium* in the production of bioethanol from rice straw which was
633 pretreated in acid-thermal way while study of Takano and Hoshino¹³⁴ shows the use of
634 enzyme cocktail (optimized) and *Mucor circinelloides* (xylose fermenting fungus) in the
635 ethanol production from alkali pretreated rice straw by concurrent saccharification and solid



636 state fermentation. Abo-State et al.¹³⁵ subjected the rice straw to steam treatment
637 (autoclaving) and various gamma irradiation doses like 50 and 70 Mrad. Now, different
638 fungal isolates were used to enzymatically treat the steam treated rice straw throughout SSF
639 process.¹³⁵ Therefore, any pretreatment processes which are effective in the removal of lignin
640 from rice straw may be used and combined with the fungal treatment process for the
641 production of bioethanol by saccharification and SSF process.

642 Furthermore, the effect of alkali sodium hydroxide (NaOH)/hydrogen peroxide
643 (H₂O₂) based various pretreatment approaches on willow sawdust biomass was described by
644 Atitallah et al. (2022)¹³⁶ utilizing the conventional yeast i.e. *Saccharomyces cerevisiae* and
645 03 non-conventional strains of yeasts like *Pachysolen tannophilus*, *Wickerhamomyces*
646 *anomalous* X19, and *Pichia stipitis*. Result shows that greater delignification i.e. $38.3 \pm 0.1\%$
647 and efficiency of saccharification i.e. $31.7 \pm 0.3\%$ and greater concentration of ethanol and
648 yield was led by the 2-stage pretreatment method i.e. 0.5% w/v NaOH for twenty four hours
649 and 0.5% v/v H₂O₂ for twenty four hours. Yields of ethanol ranging from 11.67 ± 0.21 to
650 13.81 ± 0.20 g/100 g TS was observed by the *Saccharomyces cerevisiae* or
651 *Wickerhamomyces anomalous* X19 monocultures and co-cultures with *Pichia stipitis*. *W.*
652 *anomalous* was selected as non-conventional strain due to its high efficiency of bioethanol
653 production whereas *S. cerevisiae* was utilized as the highest exploited strain of yeast for the
654 production of bioethanol from sugar fermentation. There was reduction of hemicellulose to
655 1.3%, 18.9%, 25.1%, and 21.4% when willow sawdust was applied to different pretreatment
656 approaches i.e. A (sodium hydroxide), C (sodium hydroxide and hydrogen peroxide mixture),
657 D (initially sodium hydroxide followed by hydrogen peroxide) and E (initially hydrogen
658 peroxide followed by sodium hydroxide), respectively (approach B i.e. H₂O₂ is omitted here).
659 For the approach D, there was maximum removal of lignin (38.3%).¹³⁶ Co-cultures use for
660 the bioethanol fermentation is generally considered beneficial over the monocultures because



661 of the synergistic action of the involved microorganism's metabolic pathways.¹³⁷ In another
662 study, filamentous fungi like *Rhizomucor miehei*, *Aspergillus nidulans*, *Gilbertella*
663 *persicaria*, and *Trichoderma reesei* were tested for the pretreatment of dry CS (corn stover),
664 WS (wheat straw) and WWC (willow wood chip).¹³⁸ *A. nidulans* based pretreatment doubled
665 the yield of methane in compare with the untreated corn stover. Pretreatment with *G.*
666 *persicaria* and *T. reesei* also gave noteworthy differences in the production of bio-methane in
667 comparison with the samples having only untreated plant substrates, respectively.
668 Outstanding great activity of endo-(1,4)- β -D-glucanase on willow wood chip and corn stover,
669 and great activity of β -glucosidase on willow wood chip are shown by the *A. nidulans*. As the
670 pretreatment's consequence, *A. nidulans* based pretreatment of samples generated highest
671 biogas for all involved raw substances. This study recommended the use of short time
672 pretreatment for cellulose-abundant substances, which in definite cases may double the yield
673 of biogas.¹³⁸

674 *Phanerochaete chrysosporium*, among the WRF, is recognized for its choosy lignin's
675 breakdown and has ability to show numerous applications in biotechnology.¹³⁹ Pretreatment
676 of richly found wheat straw (WS) can be done by applying WRF, which transforms the
677 cellulose (complex plant biomass) in glucose.¹⁴⁰ This sugar can be used by the *Pichia*
678 *fermentans* and IAA (indole-3-acetic acid) may be produced in tryptophan's presence.
679 Besides effective WS pretreatment in the course of primary fermentation, *Phanerochaete*
680 *chrysosporium* may also generate IAA in tryptophan's presence,¹⁴¹ which may further
681 participate in the enhancement of the creation of IAA in the course of secondary
682 fermentation.¹⁴² In a study, *P. chrysosporium* (150 $\mu\text{g/ml}$) based pretreatment of WS showed
683 nine folds enhancement in IAA in comparison with untreated WS (16.44 $\mu\text{g/ml}$).¹⁴² IAA was
684 produced in 1.99–129.33 $\mu\text{g/ml}$ range.¹⁴² The WS was pretreated with *Phanerochaete*
685 *chrysosporium* for releasing the sugar in medium that could be used by *Pichia fermentans* for



686 the production of IAA. Considerable sugar amount was released by *P. chrysosporium* from
 687 2nd day onwards and was highest on 9th day (0.89 mg/ml).¹⁴² Production of IAA using yeasts
 688 has also been described earlier.¹⁴³ Lesser than 25 µg/ml was produced by the *Pichia*
 689 *guilliermondii* and *Hanseniaspora uvarum* when inoculated in the medium based on yeast
 690 extract-dextrose after incubation's seven days.¹⁴⁴ Furthermore, an endophytic yeast found in
 691 roots of maize, *Williopsis saturnus* was observed to be able of generating indole-3-acetic acid
 692 (22.51 µg/ml) *in vitro* in GPB medium (glucose-peptone broth).¹⁴⁵

693 In the natural substrates' degradation, the consortium of effective microbes are found
 694 to be effective than a single organism.^{146,147} Ramarajan and Manohar (2017)¹⁴⁸ found good
 695 lignocellulolytic activity for the fungal isolates, namely, GK1 (*Chaetomium*
 696 *globosum*), GK2 (*Chaetomium brasiliense*), G4 (*Engyodontium*
 697 *album*), G10 (*Metarhizium anisopliae*), G13 (*Engyodontium album*), M155 (*Acremonium*
 698 *persicinum*), M158 (*Acremonium minutisporum*), and M2E
 699 (*Inonotus tropicalis*). They evaluated the activity of these isolates and isolate 2a, *Cerrena*
 700 *unicolor*¹⁴⁹ in the liquid culture media and nice growth and ligninolytic activity were shown
 701 by the M2E and 2a while exceptional cellulolytic activity was shown by the isolates GK1 and
 702 GK2 on the lignocellulosic substrates i.e. RS (rice straw) and SCB (sugarcane bagasse). Upon
 703 treatment with individual isolates, highest sugar yield observed from SCB with GK2 was
 704 1.35 g L⁻¹ while it was less than 1 gL⁻¹ sugar yield from RS and SCB after the treatment with
 705 individual isolates except GK2. Amongst the different consortia, the highest yield of sugar
 706 (4.39 gL⁻¹) was given by M2E+GK2 on sugarcane bagasse followed by the yield of 2.64 gL⁻¹
 707 on rice straw by 2a+GK2. This enhanced yield of sugar in case of consortia M2E+GK2 and
 708 2a+GK2 could be due to the high manganese peroxidase activity on SCB by the consortium
 709 M2E+GK2 and enhanced activity of laccase by consortium 2a+GK2 on RS followed by
 710 noteworthy cellulolytic nature. Thus, developed lignolytic and cellulolytic marine-derived



711 fungal consortium shows the potential for the application in the agricultural wastes.¹⁴⁸
712 Comparative study between biological and physical pretreatment were also performed by
713 researchers. Yield of sugar was moderately increased from the substrates by physical and
714 combination of physical and biological pretreatment but it was lesser than developed
715 consortia-based biological pretreatment which demonstrates the potential of fungal isolates in
716 the biological pretreatment.¹⁴⁸ Rouches, Zhou *et al.* (2016)¹²⁶ performed study on the
717 pretreatment of wheat straw using different strains of fungi for studying the probability of
718 increase in the production of methane. Anaerobic digestion was found to be improved up to
719 20% by *Polyporus brumalis* BRFM 985 even after the mass loss. Using this strain, they
720 obtained up to 43% extra methane (CH₄)/gram of pretreated VS in comparison with control
721 straw. Considering the dry weight loss studied in the pretreatment course in non-optimized
722 conditions, there was up to 21% extra methane per gram of initial TS (total solids). For the
723 fixed culture condition, there was lowering in the delignification upon increase in glucose
724 addition between fifty and four hundred milligram/gram straw in a strain dependent way.¹²⁶

726 4.2 Pretreatment of Hardwoods, Softwoods and Switchgrass

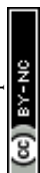
727 Lignin degradation ability was found to be increased by co-culturing of
728 *Paracremonium* sp. LCB1 and *Clonostachys compactiuscula* LCN1 and pronounced drop of
729 76.37% in the weight of lignin was observed for the bamboo culms pretreatment using this
730 co-culture at 30°C of temperature, 40 days of culture time and pH 5. There was high loss
731 ratio of lignin/cellulose (>10).¹⁵⁰ It was also observed that co-culturing of two or three fungi
732 gave higher degree of weight loss of lignin in comparison with single fungal strain culture.
733 During the process of pretreatment, interacting fungi's co-cultivation over-expresses
734 lignolytic enzymes and this may generate synergistic and combinatorial influence for



735 effective delignification.¹⁵¹ Resultantly, combination of LCB1+LCN1 gave the maximum
736 loss of lignin weight.¹⁵⁰

737 *Trametes versicolor* (a white rot fungus), and *Gloeophyllum trabeum* and *Rhodonia*
738 *placenta* (two brown rot fungi) were used for the pre-treatment process of two softwood,
739 namely, *Pinus yunnanensis* and *Cunninghamia lanceolata* and two hardwoods, namely,
740 *Populus yunnanensis* and *Hevea brasiliensis* with different period of conversion.¹⁵² Selective
741 degradation in softwood was shown by *T. versicolor* where lignin and hemicellulose was
742 converted preferentially while cellulose was selectively retained. On the other hand, in
743 hardwood simultaneous conversion was achieved for cellulose, hemicellulose and lignin by
744 *T. versicolor*. Carbohydrates were converted preferentially by the brown rot fungal species
745 but cellulose conversion was selectively shown by the *R. placenta*. Wood cells accessibility
746 was improved and porosity was enhanced by the fungal pre-treatment. It was concluded that
747 cellulose content may be maximized by the use of *T. versicolor* pretreatment while
748 pretreatment via using brown rot fungi (especially *R. placenta*) may be gainful for the
749 biofuels, chemicals based on gasoline and other bio-chemicals.¹⁵² Both brown rot fungi cause
750 higher mass loss of softwoods in compare to *T. versicolor* as 28.59%, 36.19%, and 13.09%,
751 decaying by *G. trabeum*, *R. placenta*, and *T. versicolor*, respectively in *P. yunnanensis* while
752 in the wood of *Cunninghamia lanceolata*, there were 66.52%, 45.87%, and 35.57% decaying
753 by *G. trabeum*, *R. placenta*, and *T. versicolor*, respectively. But, the case was reverse for
754 hardwoods where hardwoods mass conversion by white rot fungi was higher than brown rot
755 fungi. Nature of lignin and different pathways of bio-degradation between hard woods and
756 softwoods may be the reasons behind this discrepancy in the degradation percentage by
757 different group of fungi.¹⁵²

758 On the other hand, a technoeconomic analysis was done by Olughu et al. (2023)¹⁵³
759 for the fungal pretreatment-dependent production of cellulosic ethanol where processing



760 capacity of the plant was 2000 tonnes switchgrass/day. Ethanol yield of plant was projected
761 to be 211.9 L/t of switchgrass and fungal pretreatment was the main contributor to the total
762 capital investment. Switchgrass-based ethanol production's profitability was observed to be
763 sensitive to the changes in the cost of feedstock, yield of glucose and yield of xylose.
764 Growing yield of glucose from 60 to 80% resulted in a five-fold enhancement in the net
765 present value. Additional, study on fermentation time's optimization in the course of fungi-
766 based pretreatment and subsequent glucose yield optimization upon enzyme catalysed
767 hydrolysis would be essential to improve the economic feasibility of this type of ethanol
768 plant.¹⁵³

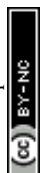
770 4.3 Fungal Pretreatment of Spent Coffee Grounds (SCGs)

771
772 SCGs are biowastes materials produced after the coffee brewing which is generated in
773 noteworthy volume each year globally. Exact volume of SCGs generated each year is not
774 well known but probably its 6 million tons are globally produced each year on the wet
775 basis.^{154,155} Approximately, its 50% production comes from small-scale shops of coffee,
776 cafeterias, individuals, or restaurants.¹⁵⁶ The discarded SCGs pose considerable challenges
777 for the environment. Now, new technologies and policies are working on to develop SCGs as
778 worthwhile feedstock for the bioproducts synthesis, platform for chemicals production, and
779 value-added energy materials generation.^{157,158} Furthermore, due to the rich source of
780 polysaccharides, proteins, and lipids, SCGs are promising feedstock for bio-based and
781 chemical processes to get great value products for cosmetics industries, pharmaceutical
782 industries, and food industries.¹⁵⁹ Hemicellulose and lignin in SCG are found as 39.75%, and
783 23.1%, respectively while protein and caffeine are found as 10.82 and 1.83%, respectively.¹⁶⁰
784 Actually, coffee Valorization of SCGs can be done in many ways, including *via* the SCOAs



785 (short-chain organic acids) production.¹⁶¹ These organic acids with small chain are
786 monocarboxylic acids (aliphatic) with 2 to 6 C-atoms (i.e. acetic, propionic, butyric,
787 isobutyric, valeric, caproic, and lactic acids) having industrial applications either by the direct
788 involvements or by uses as building block for further transformations.¹⁶² Usually
789 petrochemical processes are used for the production of these molecules but production *via*
790 biological processes are being promoted due to the crude oil's growing cost and
791 environmental impact, specially utilizing the organic wastes as substrate.¹⁶³

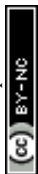
792 There is a promising work on biological pretreatment of coffee waste's acidogenic
793 fermentation¹⁶¹ using two fungi i.e. *Paecilomyces variotii* NRRL-115 and *Trametes*
794 *versicolor* CBS 109428. The production of SCOA (short chain organic acid) was positively
795 influenced by the utilization of SCG_TvSmF (Spent coffee ground_submerged fermentation
796 by *T. versicolor*) as pretreatment, getting a maximum of 2.44 gCOD/L that was high
797 enhancement (87%) related to the control. There were the generation of acetic acids,
798 propionic acids, and butyric acids in an average proportion (59.9/33.8/6.3%). Production of
799 acetic acid throughout the assay was happened while appearance of butyric acids and
800 propionic acids occurred after the 9th day and 18th day, respectively.¹⁶¹ As observed before in
801 another studies,¹²⁵ celluloses and hemicelluloses of spent coffee grounds were possibly
802 broken down and consumed. Study shows that a pretreatment step's inclusion may assist to
803 make spent coffee grounds an appropriate material for valorization and this work is nice
804 contribution towards lessening the cost of enzymatic hydrolysis utilization as complex
805 feedstock's pretreatment.¹⁶¹ Afriliana *et al.* (2021)¹⁶⁰ studied the composting spent coffee
806 ground using aerobic static batch composting with temperature control with help of
807 *Aspergillus sp.*, and *Penicillium sp.* The basis for selecting these activator fungi in
808 composting was the hemicellulose and lignin's high contents. Study was performed *via* three
809 samples analysis (control, C1, and C2) and greater degradation was observed for lignin in C2.



810 In comparison with alike rates 35.56% in C1 and 31.1% in control, this led to improved
811 global breakdown of lignin 40.28% in C2. Protein percentages decompositions i.e. 85.44%
812 (sample C2), and 83.02% (sample C1) were greater than that of control (81.82%).
813 Macromolecules decomposition rate were more than 40% in case of lignin while 70% in case
814 of cellulose. With the help of this method, composting time can be speed up and results of the
815 produced compost can be optimized.¹⁶⁰

816 In a study, *Pleurotus ostreatus* capacity in the degradation of lignocellulosic nature of
817 combined spent coffee grounds (SCG) and olive pruning residues (OLPR) was assessed by
818 Fayssal *et al.* (2021).¹⁶⁴ They adopted the complete randomized design with 5 treatments i.e.
819 S1:100% wheat straw (control), S2:33% wheat straw + 33% spent coffee grounds + 33%
820 olive pruning residues, S3:66% wheat straw + 17% spent coffee grounds + 17% olive pruning
821 residues, S4:17% wheat straw + 66% spent coffee grounds + 17% olive pruning residues, and
822 S5: 17% wheat straw + 17% spent coffee grounds + 66% olive pruning residues, and ten
823 replicates per treatment. Only S1, S2, and S3 were observed as productive. With the rise in
824 the OLPR and SCG proportions, loss of organic matter reduced. Lignin loss percentage was
825 greater in S1 in compare with S2 and S3 i.e. 53.51, 26.25, and 46.15%, respectively.
826 Mushrooms' combined production yield harvested from 2 flushes of *Pleurotus ostreatus*
827 cultured in grass and coffee pulp created a biological efficiency changing between 59.9 and
828 93%.¹⁶⁵ For accessing the holocellulose, the fungus requires firstly to break lignin¹²⁵ and a
829 greater loss of lignin means for greater mycelial activity.¹⁶⁶ In all the studied substrates,
830 degradation of hemicellulose was favorably occurred with respect to cellulose which was
831 steady to the early results of Thompson *et al.* (2003)¹⁶⁷ found on the WS.¹⁶⁴

832 Above discussions demonstrates the efficiency of fungal system in SCGs pretreatment
833 as biological method. In recent years, a considerable attention has been received by the SCGs
834 utilization as bio-resource for the many value added bio-products but there are certain



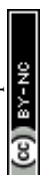
835 noteworthy challenges which are required to be solved for effective industrial applications.
836 SCGs' heterogeneity from their different sources and collection from coffee shops,
837 consumers and other small-scale sources are the primary challenges. Inconsistency in SCG
838 composition, factors like type of the coffee, method used for brewing and conditions required
839 for the processing are some of the factors which generate difficulties to standardize the
840 process of extraction and optimize the creation of value added bio-products. Furthermore,
841 development of effective techniques is needed to sort and preprocess SCGs to make sure their
842 reliable excellence and composition.¹⁵⁸ A big quantity of SCGs is achieved from the shops of
843 coffee and domestic consumers and thus, logistical challenges are faced by the bioprocessing
844 plants in concentrating the SCGs' huge volumes to the level of processing. Innovative
845 approaches and more research are required to overcome these challenges.¹⁵⁸

846

847 **4.4 Fungal Digestion of Food Wastes**

848

849 Various sources are responsible for the generation of the food wastes like canteens,
850 households, hotels, function halls, gated communities, different industries of food processing,
851 and many other.¹⁶⁸ Its decentralized treatment at source utilizing the best probable anaerobic
852 digestion (AD) technique makes it remunerative¹⁶⁹ and food waste's diversion to landfills
853 may be arrested to a high extent.¹⁷⁰ There are three configurations of AD process based on TS
854 concentration in organic waste i.e. wet anaerobic digestion (total solids \leq 10%), semi dry
855 anaerobic digestion (total solids between 10%–15%), and dry anaerobic digestion (total solids
856 $>$ 15%).¹⁷¹⁻¹⁷³ Dry anaerobic digestion (solid-state digestion) is a positive technique, owed to
857 various benefits in compare with wet anaerobic digestion (total solids $<$ 10%) making it
858 especially striking for food waste's treatment, municipal solid waste's organic fraction
859 treatment, and treatment of agricultural wastes.¹⁷⁴



860 Different pretreatment methods (namely, autoclaving, acid based, alkali based, aeration, and fungi based methods of pretreatment) were applied by the Bhurat et al. (2023)¹⁷⁵
861 for the pretreatment of food wastes. In comparison with control, the 3.8-fold improvement in
862 the yield of hydrogen and 1.7-fold enhancement in the yield of methane were shown by the
863 fungal treatment. In order to study the fungal succession and their ecological as well as
864 engineering value, food wastes' AD bioreactors were performed by Yang *et al.* (2022).¹⁷⁶ It
865 was observed that deterministic procedures slowly dominated fungal assembly succession
866 (i.e. at the final stage up to 84.85%), signifying varying environmental status accountable for
867 dynamics of the fungal community and specially, structure, diversity and biomass of the
868 fungal community were controlled by the various environmental variables or the same
869 variables with opposite influences.¹⁷⁶ A work on the fungal mash enzymatic pretreatment
870 combined with pH adjusting approach was performed by Zhang *et al.* (2022)¹⁷⁷ using
871 *Aspergillus awamori* (CICC 41363) to generate fungal mash enzymes *via* SSF. Complex
872 amylase (CA) was the crude enzyme produced from this fungus that was added to the food
873 waste fermentation's short-term anaerobic system. There was 116.9% enhancement in
874 concentration of SCOD with CA addition relative to the control. After 24 hours, TOC and
875 SCOD concentrations considerably increased with complex amylase (CA) addition under an
876 extensive range of pH conditions. Here, total organic carbon and SCOD mean concentration
877 were 12.5 g/L and 34.5 g/L, respectively that were 1.65 and 1.81 times greater than control
878 (7.6 g/L and 19.1 g/L), respectively. The pH 8 was the optimal pH condition for the yield of
879 VFAs that was reliable with the finding of Chen and co-workers.¹⁷⁸ This study may be an
880 economical way to increase the yield of VFAs for the FW valorization in the course of
881 anaerobic fermentation.¹⁷⁷

883 Furthermore, fungal mash (in-situ produced) was also utilized by Yin *et al.*
884 (2016)¹⁷⁹ showing the nice presence of hydrolytic enzymes to pretreat activated sludge, FW,



885 and their combination before AD. Enzyme catalyzed pretreatment of activated sludge
886 combined with FW caused in the generation of 3.72 g/L glucose and 51 mg/L free amino
887 nitrogen, equivalent to SCOD (7.65 g/L) within twenty four hours, accompanied with 19.9%
888 of the reduction of VS (volatile solids). The lowering of VS was found as 19.1% and 21.4%
889 after the activated sludge and FW pretreatment, respectively through fungal mash. Moreover,
890 yield of bio-methane of fungal mash pretreated mixed waste was 2.5 times greater than the
891 activated sludge receiving no pretreatment, with as further decrease of volatile solids of
892 34.5%. These put forward a total reduction of volatile solids of 54.3% in suggested anaerobic
893 system with fungal mash pretreatment. This study demonstrate that in the enhancement of the
894 production of bio-methane as well as in the maximization of the mixed waste's volume
895 reduction *via* anaerobic co-digestion, in-situ produced fungal mash based combined activated
896 sludge and FW pretreatment would be a promising option.¹⁷⁹

897 Effective role of fungi in the pretreatment of food biowastes and its conversion into
898 the several useful bio-products has been well demonstrated. But, in order to reduce the
899 harmful impacts of food wastes on environment and human health and conversion of food
900 wastes into value added bio-products, certain challenges are needed to be resolved like bulk
901 collection of food wastes from various sources, their proper separation from other types of
902 inessential materials, their bulk storage and processing at biorefinery plants and elaborate the
903 researches and studies at the level of industrial scale from the laboratory-scale experiments.

904

905 **4.5 Saccharification of Grain Stillage**

906

907 In terms of fiber's composition, grain stillage is mainly made of hemicellulose (15-
908 25%) and cellulose (35-45%) which depends on the sources like rice, corn, sorghum, and
909 wheat.¹⁸⁰ It is also considered as a feedstock for the bio-refinery because of its big content of



910 carbohydrate [163].¹⁸¹ Pretreatment of grain stillage using the microwave-assisted
911 hydrothermal (MH) pretreatment, fungus based pretreatments, and their amalgamation was
912 done by Ren et al. (2020)¹⁸¹. Superior reducing sugar yield (25.51 g/100 g) and efficiency of
913 saccharification (66.28%) were achieved by microwave-assisted hydrothermal +
914 *Phanerochaete chrysosporium* (microwave-assisted hydrothermal prior to the *Phanerochaete*
915 *chrysosporium*) pretreatment. Considerable loss of mass i.e. 23.54 and 39.43% was caused by
916 the joint pretreatments as *Phanerochaete chrysosporium* + microwave-assisted hydrothermal
917 and microwave-assisted hydrothermal + *Phanerochaete chrysosporium*, respectively. The
918 degrees of delignification were considerably enhanced to 32.80 (for *Phanerochaete*
919 *chrysosporium* + microwave-assisted hydrothermal) and 43.34% (for microwave-assisted
920 hydrothermal + *Phanerochaete chrysosporium*) after the combined pretreatment.
921 Furthermore, the degree of delignification of the microwave-assisted hydrothermal +
922 *Phanerochaete chrysosporium* was considerably greater than that of *Phanerochaete*
923 *chrysosporium* + microwave-assisted hydrothermal pretreatment.¹⁸¹ This may be because
924 microwave-assisted hydrothermal pretreatment results the hydrogen bonds breakage and
925 lignocellulose structure's destruction through explosion and disruption, which stimulates the
926 subsequent attack of *P. chrysosporium* for the delignification.^{182,183} In order to enable the
927 utilization of the cost effective grain stillage, use of joint microwave-assisted hydrothermal
928 and *Phanerochaete chrysosporium* pretreatment may be an excellent method.¹⁸¹

929

930 4.6 Symbiotic Digestion of Lignocellulose

931

932 In world's tropical and subtropical areas, fungus-growing termites have ability to
933 consume 20-90% of dead plant's materials.¹⁸⁴⁻¹⁸⁶ Lignocellulosic materials can be completely
934 degraded and digested by *Termitomyces* fungi with resulting ecological influences on the



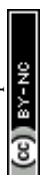
935 processes of the ecosystem, chiefly Carbon-cycling.¹⁸⁷ For investigating the digestion of
936 lignocellulose in fungus-growing termite *O. formosanus* (Shiraki) symbiotic system and to
937 equate the bacterial communities across various phases during degradation's process, Ahmad
938 *et al.* (2022)¹⁸⁸ did many analytical works on the plant's biomass components' fate and
939 performed 16S rRNA gene's amplicon sequencing. Young workers' digestive tract initiates
940 the lignocellulose degradation but leaves maximum of the cellulose, lignin, and
941 hemicellulose, which comes in the fresh fungus comb, where principally the decomposition
942 happens. The consumed samples of lignocellulose (fresh, mature, and old comb) from three
943 colonies were compared¹⁸⁸ with the original wood of mulberry through compositional
944 analysis of lignocellulose with fiber detergent technique.¹⁸⁹ It was shown by the examination
945 of the comb material that in all three colonies, there was considerable degradation of
946 lignocellulosic constituents.¹⁸⁸ There was on average reduction of lignin, cellulose, and
947 hemicellulose in fresh comb by 18.9%, 11.1%, and 15.0%, in the mature comb by 56.9%,
948 41.0%, and 32.5%, and in the old comb by 63.0%, 65.5%, and 53.4%, respectively.¹⁸⁸

950 4.7 Fungal Pretreatment of Solid Digestate

951
952 Digestate fractions' fate generally involves agricultural aims like soil amendment
953 and organic fertilizer.¹⁹⁰ Zanellati *et al.* (2020)¹⁹¹ studied the fungal pretreatment on non-
954 sterile solid digestate and inoculated the fungi *Coprinopsis cinerea* MUT 6385,
955 *Cephalotrichum stemonitis* MUT 6326, and *Cyclocybe aegerita* MUT 5639 in the digestate's
956 non-sterile solid fraction with aim to reuse it as feedstock for anaerobic digestion. In the
957 *Cyclocybe aegerita*, *Cephalotrichum stemonitis*, and *Coprinopsis cinerea* pretreated samples,
958 there were noteworthy reductions in the concentration of total solids (TS) i.e. 23.8%, 25.4%,
959 and 28.5%, respectively. In the *C. cinerea* pretreated samples for ten days and



960 *Cephalotrichum stemonitis* pretreated samples for twenty days, NDF (neutral detergent fiber) losses percentage was ranged from 1.6% to 10.4%, respectively. Alike behavior was shown
961 by the different strains towards the PCWP (Plant cell wall polymer), causing greater
962 lessening in hemicellulose (18.5–59.3%) in compare with cellulose (0.2–8.2%) and lignin
963 (1.0–9.6%). *C. stemonitis* based pretreatment for 20 days gave the maximum lowering in all
964 the PCWP components and resulted in the reductions of hemicellulose, lignin, and cellulose
965 as 59.3%, 9.6%, and 8.2%, respectively. The anaerobic digestion functioned superior with
966 solid fraction of digestate treated by the fungal strain *Cephalotrichum stemonitis* for twenty
967 days, that led to about 3-fold greater yields of biogas and CH₄ i.e. +182% and +214%,
968 respectively in compare with untreated solid fraction of digestate. Cumulative methane
969 formed with fungal strain *C. stemonitis* was considerably greater than that attained with
970 fungal strains *Cyclocybe aegerita* and *Coprinopsis cinerea* for both ten and twenty days.¹⁹¹
971 *M. isabellina* ATCC 42613 was applied by Zhong *et al.* (2016)¹⁹² for accumulating the lipids
972 on detoxified hydrolysate medium. Characteristics of digestates (solid and liquid) and AD
973 effluent showed that soild digestate has 30.60% TS content and carbohydrate contents i.e.
974 cellulose (26%), xylan (13%), and lignin (30%) to be utilized for fungal lipid accumulation as
975 the lignocellulosic feedstock. After the pretreatment and hydrolysis processes, the mixture
976 feed at the total solids of 10% produced a hydrolysate having glucose (13.85 g/L), xylose
977 (8.95 g/L), and acetate (2.67 g/L). Study shows the substrate's consumption of *Mortierella*
978 *isabellina* on hydrolysates.¹⁹² Without detoxification, there was no consumption of sugars
979 and acetate in the hydrolysate during culture period of 89 hours. In comparison with the
980 culture of synthetic medium (consumption of all sugars and acetate in 66 hours), a delay
981 (23 hours) of the consumption of substrate was noticed from the cultures on detoxified
982 hydrolysates. There was complete consumption of glucose and acetate in 49–54 hours,
983 respectively. At the batch culture's end (77 hours), xylose 1.79 g/L stayed in the broth, and



985 biomass 8.98 g/L and lipid 1.50 g/L were accumulated. The corresponding yields of lipid and
986 biomass were 0.07 g/g and 0.42 g/g, respectively.¹⁹² Conclusively, fungi shows the promising
987 presence in the pretreatment process of solid digestate with anaerobic digestion process.

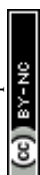
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989 **4.8 Fungal Pretreatment of Park Wastes and Cattle Dung**

990

991 For treating the cellulosic biomass to enhance its digestibility, Ali and Sun (2015)¹⁹³
992 studied the physic-chemical pretreatment's influence on the degradation of cellulose followed
993 by treatment utilizing fungi *Trichoderma viride* and *Aspergillus terreus*. In their experimental
994 set up, mixture of new leaves (125 g), dry leaves (125 g), and cattle dung (250 g) was present
995 in each of the two digesters. Park wastes' fungal treatment was applied for seven days at 25
996 °C followed by both digesters' incubation for seventy days on incubator shaker (35° C
997 temperature and 120 rpm) in order to help the mixing. Pre-treated and untreated substrate's
998 biogas and CH₄ yields were measured. Three pre-treatment stages improved the production
999 yields of daily biogas and CH₄ from the substrate. In comparison with untreated substrate, the
1000 pretreated substrate gave maximum yields of biogas and CH₄ of 2.6 and 1.9 L/KgVS,
1001 respectively in the 28th day. There was 102.6 L/KgVS biogas cumulative production for
1002 untreated substrate that was found to be improved to 125.9 L/KgVS for the pretreated
1003 substrate and in this way, there was 22.7% enhancement in comparison with the yield of
1004 biogas from untreated substrate. The pretreated and untreated substrate's cumulative
1005 production of CH₄ was 79.8 and 61.4 L/KgVS, respectively and in comparison with the yield
1006 of methane from untreated substrate, there was 30 % enhancement.¹⁹³ This study may be
1007 useful for the treatment of cattle dung and park waste and in the production of biogas with
1008 further improvement, optimization and/or with combined technology.

1009



1010 4.9 Card Waste's Fungal Pretreatment

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1011

1012 Card wastes are also a good source of hemicellulose, lignin, and cellulose. Suthar and
1013 Singh (2022) studied the waste cardboard's fungal pretreatment in the monoculture and
1014 mixed culture and then composted for 35 days after mingling with cow dung in various
1015 ratios.¹⁹⁴ They utilized fungi *Oligoporus placenta* and *Tremetes hirsuta* for fungal
1016 pretreatment. There was considerable lowering in contents of cellulose (28.3–35.8%),
1017 hemicellulose (61.4–68.4%), and lignin (67.5–69.3%) in waste card board. The pretreated
1018 waste cardboard displays better decrement' rates in TOC (26.02–47.92%), C-N ratio (19.4–
1019 23.5), and contents of lignocellulose, in addition to incensement in total N (40.48–63.31%),
1020 total K (51.92–73.91%), germination index (88.5–102.0%), and levels of elements i.e.
1021 copper, iron, zinc, chromium, and manganese. Thus, after the pretreatment with a white rot
1022 fungi consortium, waste cardboard could be utilized as an important substrate for the
1023 preparation of valuable-added compost.¹⁹⁴

1024 Therefore, now, it is very clear that fungi have a great future in the biological
1025 pretreatment technology for the management of various aforementioned organic wastes and
1026 the production of valuable energy, biogases and compounds. But there are also several
1027 associated challenges before such pretreatment technologies. There are some other literatures
1028 that may be significant for the readers in the field of bio-wastes degradation as well as
1029 opportunities and challenges.¹⁹⁵⁻²⁰⁸ **Table 4** summarizes the effective brief descriptions on the
1030 myco-pretreatment of different type's organic wastes. Along with an advantageous biological
1031 solution of the problems of bio-wastes management, fungal based bio-pretreatment processes
1032 may also generate various types of valuable products after the pretreatment processes from
1033 their macromolecules lignins, cellulose, hemicellulose, starch, pectins etc.

1034



1035 **5 TECHNO-ECONOMIC ANALYSIS AND SCALE-UP ISSUES FOR INDUSTRIAL**
1036 **IMPLEMENTATION OF FUNGAL PRETREATMENT METHODS**

1037 Fungal pretreatment process at industrial level requires big capital investments. From
1038 grasses to hardwoods, total capital investment ranges from 700 million (in dollars) to 1.2
1039 billion (in dollars), respectively and this is approximately five to ten time greater than the
1040 previously estimated for the conventional treatment at parallel scale.²⁰⁹ High cost are due to
1041 the expenses on large equipment's purchasing, installation, construction, engineering, many
1042 units' requirements for each of the main processes like autoclaving, fungal pretreatment and
1043 enzymatic hydrolysis expenses and other requirements. Packed-bed bioreactors utilized in the
1044 fungal pretreatment process are responsible for the majority of the cost value, mainly because
1045 of longer residence time. In the fungal pretreatment process, fermentable sugar's estimated
1046 price was 1.6-2.8 dollars/kg which were 4-5 times greater than the previously stated
1047 production cost of sugar utilizing the conventional pretreatment methods.^{209,210} Due to the
1048 minimum requirement of energy and chemicals, fungal pretreatment process is believed to be
1049 a pretreatment method which requires low cost but analysis showed that it needs noticeably
1050 higher cost than conventional pretreatments and about one order of magnitude over that
1051 anticipated for a pretreatment to be feasible at commercial level.²¹⁰

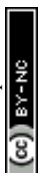
1052 High cost of facility arrangement for the fungal pretreatment is primarily responsible
1053 for the need of high capital investment while feedstock cost is the second highest contributor
1054 to the cost of sugar production which contributed 18-22% of the complete cost of sugar
1055 production.²⁷ However, continuous advancements in the technologies may reduce the total
1056 cost of sugar production using fungal pretreatment techniques and it will make fungal
1057 pretreatment methods a commercially profitable environmentally safe and green technique.
1058 Longer time required for the fungal pretreatment, enhancement of the yield of sugar, and
1059 sterilization requirement of the feedstock before fungal pretreatment are in several bottleneck



1060 which are required to be overcome in order to expand and improve the fungal based
1061 pretreatment technique. Studies on the combination with different other pretreatments with
1062 low severity has achieved greater yield of sugar with significant reduced need of energy and
1063 chemicals.²¹¹ Optimization of these processes are not only required at laboratory scale from
1064 technical perspective but also at commercial scale utilization from techno-economic
1065 perspective.²⁷ High temperature may affect the fungal based pretreatment processes because
1066 fungal enzymes are strongly involved in the enzymatic degradation process of lignocellulosic
1067 materials or other biowastes which are inactivated at higher temperatures so during the fungal
1068 based treatment plant set up, special attention should also be given on the control of
1069 temperature rise during the process.

1071 6 CONCLUSION, CHALLENGES AND FUTURE PERSPECTIVES

1073 Fungi are effective in the decomposition of lignocellulosic biomasses, food wastes,
1074 sewage sludge, polysaccharides, lignin, and hemicelluloses etc and found to be efficiently
1075 involved in saccharification, biogas productions, glucose production, ethanol production, and
1076 bio-fertilizer developments utilizing the organic wastes. They have significant contribution to
1077 the biomass valorization²⁹ via generation of alcohol,²⁰² biodiesel,²⁰³ organic acids,²⁰⁴ and gas
1078 fuels.²⁰⁵ But, there are several challenges before the efficient use of pretreatment technologies
1079 in the digestion of organic wastes or bio-wastes and solid waste managements. Anaerobic
1080 decomposition of organic wastes occurs when dumped in landfills.⁴⁶ Harmful greenhouse
1081 gases like methane are produced by the decomposition of green waste in anaerobic condition,
1082 which are the main participant to the global warming.²⁰⁶ Speedily depleting landfill space is
1083 another problem with the direct landfill. In proper management of organic wastes, there are
1084 various considerable barriers. Poor infrastructure, poor planning of strategy, capacity of staff,



1085 registration, programme engagement, information system, and unsystematic management of
1086 waste make it a difficult job.⁴⁶ Also, there is absence of participation in the initiatives of the
1087 separation of garbage and inadequate communication between the homeowners and
1088 municipality²⁰⁷ which makes the management of organic waste very difficult. For allowing
1089 more effective value extraction and recycling process, separation of waste should be at
1090 source.²⁰⁷ Separation of the types of wastes like dry wastes or wet wastes (biodegradable)
1091 make the pretreatment process more effective and significant. So, it is the main responsibility
1092 of producers of wastes along with the government's effective involvements. There are the
1093 needs of combined efforts of urban local bodies, governments, private sectors, and non-
1094 governmental bodies for the long term waste managements and visionary project
1095 developments are strongly needed in this regards. There is also strong need of well-defined
1096 roles and responsibility to work on waste management with continuous monitoring and
1097 assessment.²⁰⁷ Kumar *et al.* (2017)²⁰⁷ have nicely reviewed the challenges and opportunities
1098 related to the management of the waste materials in Indian scenario. Thus, in order to
1099 implement perfectly the fungal pretreatment technology either individually or in combination
1100 with other technology for the green and sustainable environment, the above barriers need to
1101 be tackled because in poor countries organic waste disposal is a very difficult task. Consistent
1102 and properly managed involvements of the system (government, industries and public) in
1103 organic waste management with appropriate methodologies are very essential to pretreat the
1104 organic or bio-wastes biologically. Higher pretreatment time is generally required by the
1105 fungal technology to gain the high removal rates of lignin and saccharification of cellulose
1106 which generate problems regarding cost rise and contamination by bacteria.¹²⁵ Except longer
1107 pretreatment time; requirement of feedstock's sterilization before the pretreatment process,
1108 heat generation during the fungal pretreatment process, and lower yield of sugar are some
1109 other challenges and shortcomings of fungal pretreatment which are needed to be properly



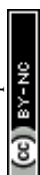
1110 tackled in future to make the fungal pretreatment technology more efficient and cost-
1111 effective. Biomass' big solids content is the principal reason restraining its solid
1112 fermentation, which generates ethanol. Similar to glucose fermentation, yield of ethanol and
1113 titer can be achieved through the amalgamation of cosolvent-improved lignocellulose
1114 fractionation and the following saccharification and fermentation.²⁰⁸ Fungal pretreatment in
1115 combination with other methods of pretreatment may increase the enzymatic digestibility and
1116 lessen the time of fungal pretreatment.²⁹ Fungal technology has great advantages in the field
1117 of biomass conversion because several derived products from biomass's hydrolyzed sugar are
1118 important from the point of energy generation. 5-hydroxymethylfurfural, levulinic acid, and
1119 furfural have the ability as great-worth chemicals *via* the fungal technology.²⁹ Future
1120 development will require the more upgraded fungal technology with lesser pretreatment time,
1121 lesser or no sterilization requirements, enhanced enzymatic digestibility, reduced chances of
1122 contamination, and more yields of the products and such upgraded fungal pretreatment
1123 technologies should break more efficiently the biomass's complex structures enhancing the
1124 production of various valuable products.²⁹ Biogases (like methane) and other useful organic
1125 components (bioethanol, monomeric sugars etc.) produced during the process of fungal
1126 pretreatment technology may be utilized in human welfare with opting the consistent
1127 management techniques.

1128

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1138 **Authors Contributions**

1139 Dr. Pankaj Kumar Chaurasia and Dr. Shashi Lata Bharati prepared the original
1140 manuscript's draft, wrote major sections, supervised, and edited the manuscript; Dr. Sunita
1141 Singh wrote specific sections and performed formal analysis and editing; Dr. Azhagu
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1147 **Competing Interests**

1148 No any competing interests

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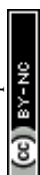


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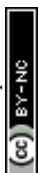
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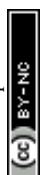
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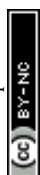
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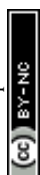
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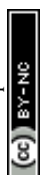


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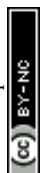


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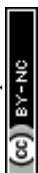
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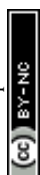
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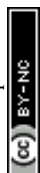
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Figures Legends

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

Fig. 2: Plants (dead and/or green) as the huge source of ligno-cellulose, lignin, cellulose, hemicellulose, small carbohydrates along with 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).

Fig. 3: Different type of biomasses and their application in biofuels, bioenergy and bio-products generation.⁴¹

Fig. 4: A model structure of lignin.¹²²

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



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Tables Legends

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

Table 2: Variation in the production of biogas/methane from lignocellulosic biomass after low temperature pretreatment process.⁷⁸

Table 3: Major components in some agricultural wastes and food wastes.

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

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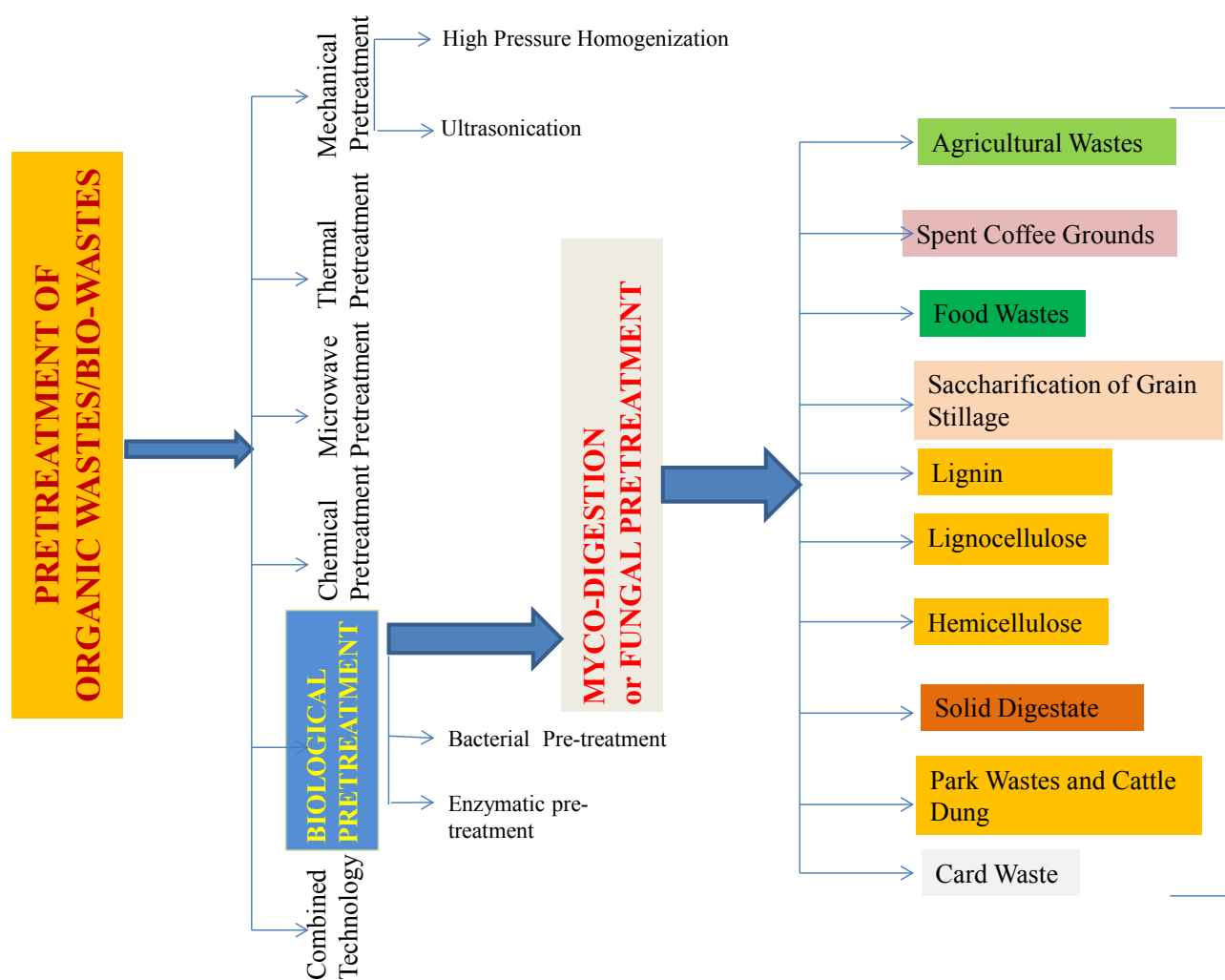


Fig. 1



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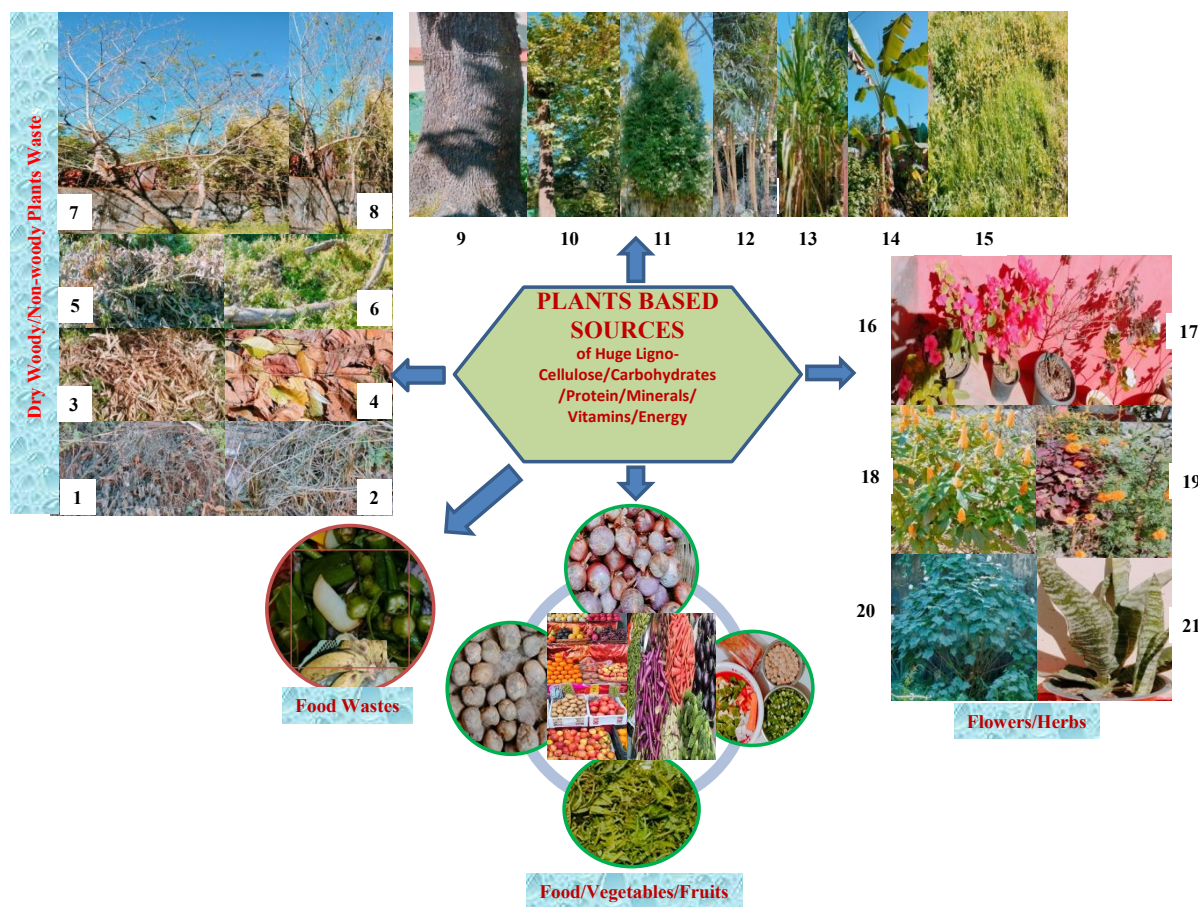


Fig. 2

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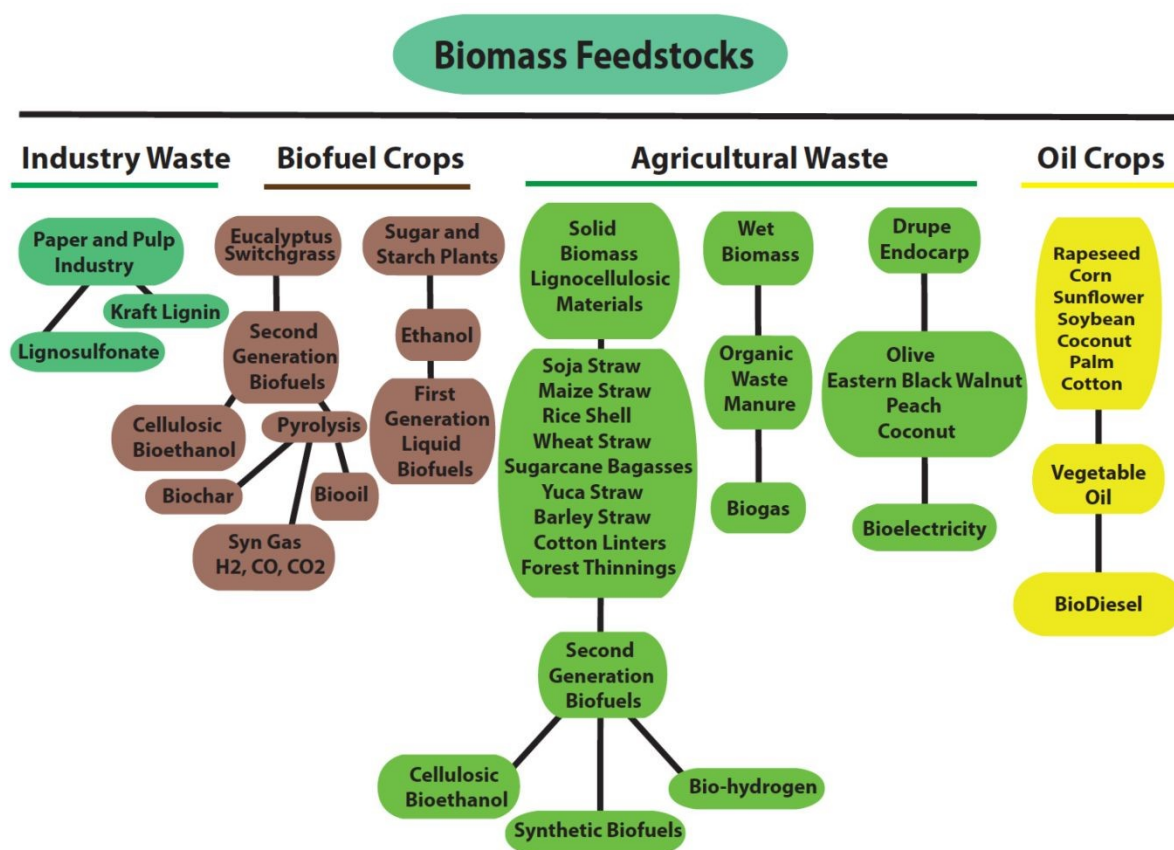


Fig. 3

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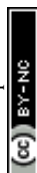
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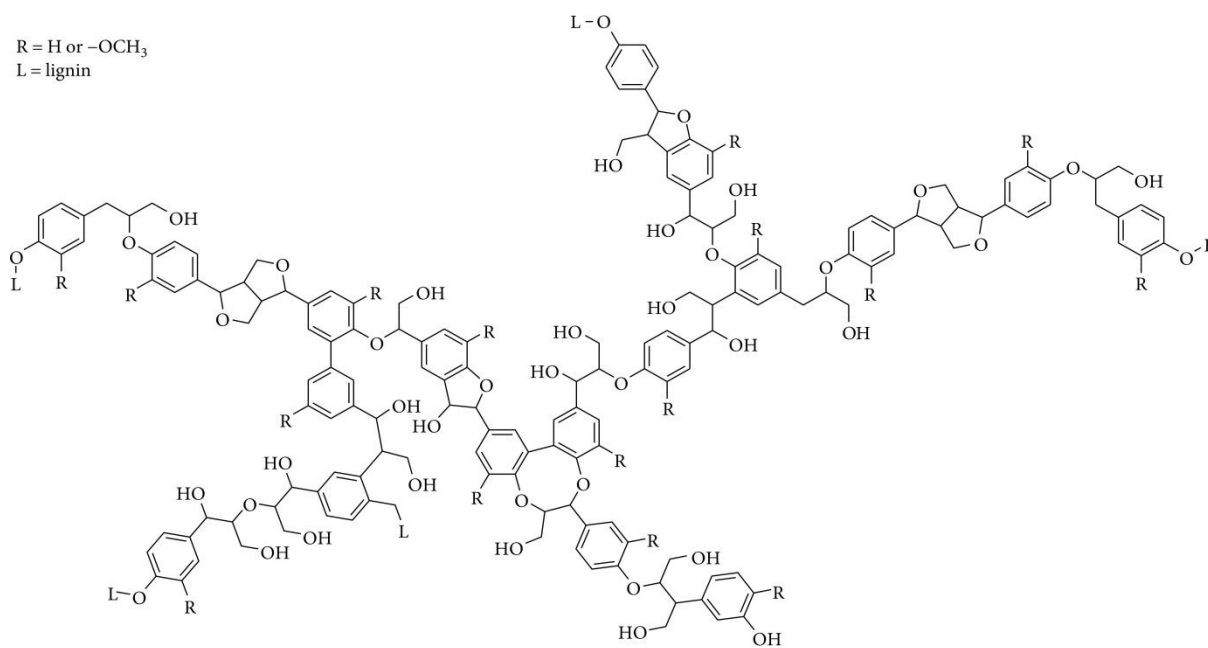
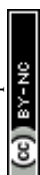
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**Fig. 4**

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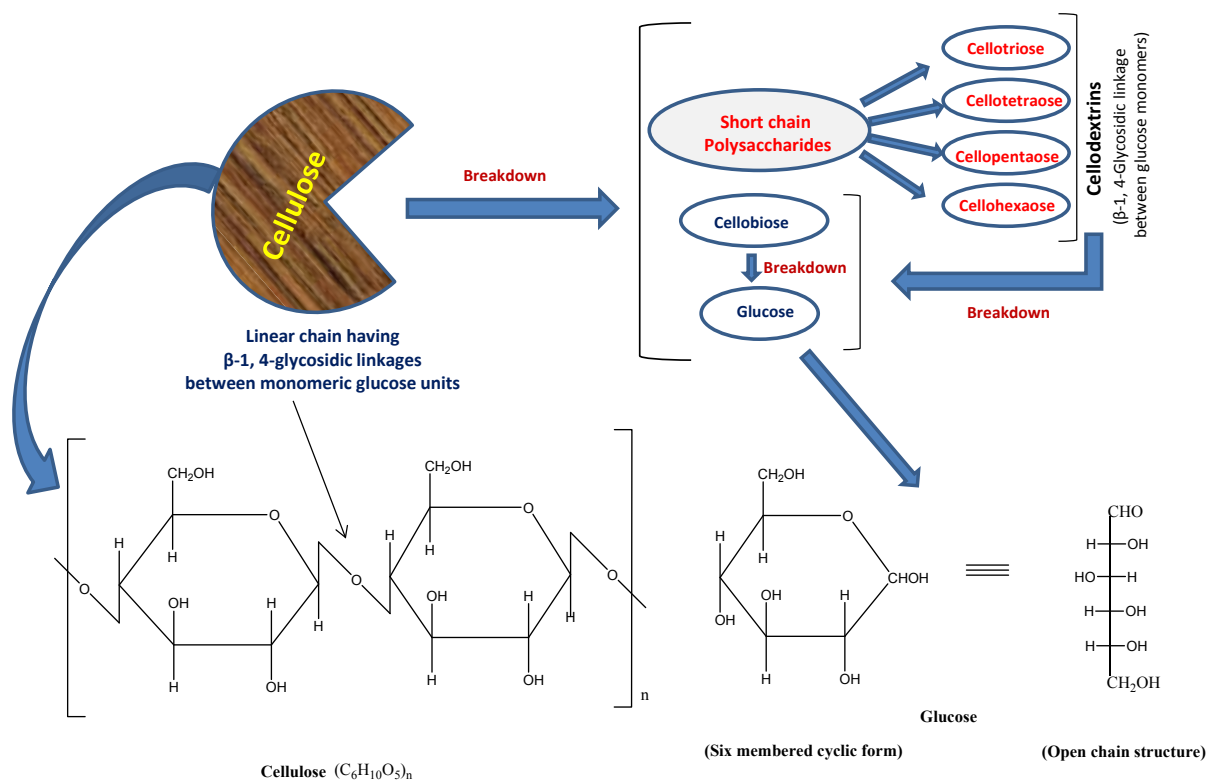
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Fig. 5

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2036 **Table 1**

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) It's use before biological or other pretreatment methods is needed and makes 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 wastes. (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 wastes. (ii) More effective and efficient.	Depends on methods combined together for pretreatment.	Thermal-chemical methods, Chemical-biological techniques, Physical-chemical-biological methods, Other possible methods
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S.N.	Lignocellulosic sources	AD condition/Mode	Temperature /Time	Yield of Biogas/methane	Rise	References
1	67% Wheat straw and 33% sunflower meal	Mesophilic/Batch mode (45 days)	120 °C/ 1 hour	-/ 370 mL·g ⁻¹ VS	8.8%	99
			140 °C/ 1 hour	-/ 390 mL·g ⁻¹ VS	14.7%	
2	Bean straw	Mesophilic/Continuous mode (Hydraulic retention time 4.5 d)	121 °C/ 1 hour	145 mL·g ⁻¹ COD /-	-	100
3	Rice straw	Mesophilic /Batch mode (30 days)	80 °C/ 6 hours	372.5 mL·g ⁻¹ VS /-	12.4%	101
4	Rice straw	Mesophilic/Batch mode (35 days)	100 °C/ 150 minutes	128 L·kg ⁻¹ TS /-	22.8%	102
			130 °C/ 150 minutes	125 L·kg ⁻¹ TS /-	19.8%	
5	Rice straw	Mesophilic/Batch mode (50 days)	90 °C/ 15 minutes	307 mL·g ⁻¹ TS /-	3.0%	103
6	Wheat straw	Mesophilic/Batch mode (45 days)	120 °C/ 60 minutes	496 mL·g ⁻¹ 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

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S.N.	Common bio-wastes	Composition	References	
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

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2051 **Table 4**

S.N.	Fungi	Enzyme activity	Substrates	Some Outputs	References
1	<i>Trametes versicolor</i> (WRF), <i>Gloeophyllum trabeum</i> and <i>Rhodonia placenta</i> (Brown rot fungi)	-	Softwoods and hardwoods	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Yield of ethanol was projected to be 211.9 L/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	<i>Paracremonium</i> sp. LCB1 and <i>Clonostachys compactiuscula</i> LCN1	Hemicellulase and ligninolytic enzyme	Bamboo culms	<ul style="list-style-type: none"> Significant lowering in the lignin weight (76.37%). Lignin/cellulose ratio showed high loss (>10). 	150
4	<i>Penicillium aurantiogriseum</i> , <i>Gilbertella persicaria</i> (SZMC11086), <i>Rhizomucor miehei</i> (SZMC11005), and <i>Trichoderma reesei</i> (DSM768)	Endoglucanase, β -glucosidase, and cellobiohydrolase	Wheat straw, woody willow chips, and corn stover	<ul style="list-style-type: none"> Excellent production of hydrolytic enzyme and maximum yield of biogas from the partly decomposed substrates were shown by <i>P. aurantiogriseum</i> 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	<i>Pleurotus ostreatus</i> and <i>Dichomitus squalens</i>	-	Corn silage	<ul style="list-style-type: none"> Biogas production was enhanced. Methane gas was found to be increased by 1.55 fold. Lignin de-polymerization increased the production of methane 0.301 to 0.465 m³kg_{VS}⁻¹. 	131
6	<i>Irpex lacteus</i>	-	wheat straw	<ul style="list-style-type: none"> Wheat straw digestibility found after 21 days of the pretreatment in the solid 	132



				<p>state fermentations bioreactor (60.6%) was alike to that found on a small scale i.e. 57.9%.</p> <ul style="list-style-type: none"> • In the 03 bioreactor experiments, sugars evolution was completely different after 21 days of treatment. • Greater lowering of lignin in experiment B2 i.e. $34.90 \pm 0.87\%$ in comparison with that of experiment B1 i.e. $26.7 \pm 3.08\%$. • Alike reduction of lignin i.e. $53.2 \pm 0.60\%$ in the flask-scale pretreatment 	
7	<i>Aspergillus terreus</i> and <i>Trichoderma harzanium</i>	-	Rice straw	<ul style="list-style-type: none"> • Degradation of cellulose • Production of bioethanol • The 80% degradation of cellulose by the isolated fungi <i>A. terreus</i> and <i>T. harzanium</i>. • <i>A. terreus</i> performance on the decrease of cellulose with HL and RP as substrate was 92% and 80%, respectively. • <i>T. harzanium</i> performance on the decrease of cellulose with HL and RP as substrate was 93% and 82%, respectively. • Rice straw's RP treated with <i>T. harzanium</i> has resulted in greater bioethanol of 5.4 g/L 	133
8	<i>Saccharomyces cerevisiae</i> , <i>Pichia stipitis</i> , <i>Pachysolen tannophilus</i> , and <i>Wickerhamomyces anomalus</i> X19	-	Willow sawdust	<ul style="list-style-type: none"> • Bioethanol production • Yields of ethanol ranging from 11.67 ± 0.21 to 13.81 ± 0.20 g/100 g TS was shown by the <i>S. cerevisiae</i> or <i>W. anomalus</i> X19 monocultures and co-cultures with <i>P. stipitis</i>. • For the approach D, there was maximum removal of lignin (38.3%). • Co-cultures was useful 	136
9	<i>Rhizomucor miehei</i> , <i>Aspergillus nidulans</i> , <i>Gilbertella persicaria</i> ,	β -Glucosidase and endoglucanase	Dry CS (corn stover), WS	<ul style="list-style-type: none"> • β-Glucosidase and endo-(1,4)-β-D-glucanase activity • Yield of methane 	138

	and <i>Trichoderma reesei</i>		and WWC		
10	<i>Pichia fermentans</i> , <i>Phanerochaete chrysosporium</i>	-	Wheat straw	<ul style="list-style-type: none"> • Auxin production by <i>P. fermentans</i> • Indole-3-acetic acid (IAA) production • The wheat straw was pretreated by <i>P. chrysosporium</i> for releasing the sugar in the medium which may be used by <i>P. fermentans</i> for the production of IAA • Sugar amount was released by <i>P. chrysosporium</i> from 2nd day onwards and was highest on 9th day 	142
11	GK1 (<i>Chaetomium globosum</i>), GK2 (<i>Chaetomium brasiliense</i>), G4 (<i>Engyodontium album</i>), G10 (<i>Metarhizium anisopliae</i>), G13 (<i>Engyodontium album</i>), M155 (<i>Acremonium persicinum</i>), M158 (<i>Acremonium minutisporum</i>), and M2E (<i>Inonotus tropicalis</i>).	LiP (lignin peroxidase), laccase, CMCCase, MnP (manganese peroxidase), and xylanase	Rice straw and sugarcane bagasse	<ul style="list-style-type: none"> • LiP (lignin peroxidase), laccase, CMCCase, MnP (manganese peroxidase), and xylanase activity • Amongst the different consortia, the highest yield of sugar (4.39 gL⁻¹) was given by M2E+GK2 • ligninolytic and cellulolytic marine-derived fungal consortium were effective for agricultural wastes 	148
12	<i>Polyporus brumalis</i> BRFM 985	-	Wheat straw	<ul style="list-style-type: none"> • Enhancement in the production of methane • They obtained up to 43% extra methane (CH₄) per gram of pretreated VS in comparison with control straw 	126
13	<i>Paecilomyces variotii</i> NRRL-115 and <i>Trametes versicolor</i> CBS 109428	Enzymatic extracts	Spent coffee grounds	<ul style="list-style-type: none"> • 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	<i>Aspergillus sp.</i> , and <i>Penicillium sp</i>	-	Spent coffee	<ul style="list-style-type: none"> • Chemical composition of SCG (%) as hemicellulose (39.75 ± 0.007), lignin 	160





			grounds	<p>(23.1 ± 0.007), caffeine (1.83 ± 0.007), and protein (10.82 ± 0.007).</p> <ul style="list-style-type: none"> • Study <i>via</i> 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 	
15	<i>Pleurotus ostreatus</i>	-	Spent coffee grounds and olive pruning residues	<ul style="list-style-type: none"> • <i>Adopted the complete randomized design with 5 treatments</i> • 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 was preferentially occurred with respect to cellulose 	164
16	<i>Aspergillus awamori</i> (CICC 41363)	Complex amylase (CA)	Food wastes	<ul style="list-style-type: none"> • 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 and 34.5 g/L, 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
17	Fungal mash	Hydrolytic enzymes	Food wastes	<ul style="list-style-type: none"> • Enzymatic pretreatment of activated sludge mixed with FW caused in the production of glucose (3.72 g/L) • The reduction of VS was found as 19.1% and 21.4% after the activated sludge and FW pretreatment, respectively by the fungal mash 	179

				<ul style="list-style-type: none"> • 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%. 	
18	<i>Phanerochaete chrysosporium</i>	Ligninolytic enzyme activity	Grain stillage	<ul style="list-style-type: none"> • Pretreatment of grain stillage • The maximum activities of ligninolytic enzyme was achieved by the fungal pretreatment with <i>Phanerochaete chrysosporium</i> 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	<i>Coprinopsis cinerea</i> MUT 6385, <i>Cephalotrichum stemonitis</i> MUT 6326, and <i>Cyclocybe aegerita</i> MUT 5639	-	Solid digestate	<ul style="list-style-type: none"> • In the <i>C. aegerita</i>, <i>C. stemonitis</i>, and <i>C. cinerea</i> 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 <i>C. stemonitis</i> for twenty days • Cumulative yields of biogas and methane also studied 	191
20	<i>Mortierella isabellina</i>	-	Anaerobic digestate	<ul style="list-style-type: none"> • 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), xylose (8.95 g/L), and acetate (2.67 g/L). • Complete consumption of glucose and acetate in 49–54 hours, respectively 	192





21	<i>Aspergillus terreus</i> and <i>Trichoderma viride</i>	-	Park wastes	<ul style="list-style-type: none"> • Physico-chemical pretreatment's influence on the degradation of cellulose followed by fungal treatment • TS, VS, TOC etc were studied • Study on biogas and CH₄ yields 	193
22	<i>Oligoporus placenta</i> and <i>Tremetes hirsuta</i>	-	Card wastes	<ul style="list-style-type: none"> • 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

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Data availability statements

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

