

1 **A comprehensive review on the framework to valorise lignocellulosic**
2 **biomass through biorefinery techniques**

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6 Hang P. Vu^a, Luong N. Nguyen^{a*}, Minh T. Vu^a, Md Abu Hasan Johir^a, Robert
7 McLaughlan^a and Long D. Nghiem^{a,b}

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9
10 ^a Centre for Technology in Water and Wastewater, School of Civil and Environmental
11 Engineering, University of Technology Sydney, NSW 2220, Australia

12 ^b NTT Institute of Hi-Technology, Nguyen Tat Thanh University, Ho Chi Minh City,
13 Vietnam

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25 *Corresponding author

26 Luong N. Nguyen, Centre for Technology in Water and Wastewater, School of Civil and
27 Environmental Engineering, University of Technology Sydney, NSW 2007, Australia (E-mail:
28 luongngoc.nguyen@uts.edu.au)

30 **Abstract**

31 An effective pretreatment is the first step to enhance the digestibility of lignocellulosic
32 biomass – a source of renewable, eco-friendly and energy-dense materials – for biofuel and
33 biochemical productions. This review aims to provide a comprehensive assessment on the
34 advantages and disadvantages of lignocellulosic pretreatment techniques, which have been
35 studied at the lab-, pilot- and full-scale levels. Biological pretreatment is environmentally
36 friendly but time consuming (i.e. 15-40 days). Chemical pretreatment is effective in breaking
37 down lignocellulose and increasing sugar yield (e.g. 4 to 10-fold improvement) but entails
38 chemical cost and expensive reactors. Whereas the combination of physical and chemical (i.e.
39 physicochemical) pretreatment is energy intensive (e.g. energy production can only
40 compensate 80% of the input energy) despite offering good process efficiency (i.e. > 100%
41 increase in product yield). Demonstrations of pretreatment techniques (e.g. acid, alkaline, and
42 hydrothermal) in pilot-scale have reported 50-80% hemicellulose solubilisation and enhanced
43 sugar yields. The feasibility of these pilot and full-scale plants has been supported by
44 government subsidies to encourage biofuel consumption (e.g. tax credits and mandates). Due
45 to the variability in their mechanisms and characteristics, no superior pretreatment has been
46 identified. The main challenge lies in the capability to achieve a positive energy balance and
47 great economic viability with minimal environmental impacts i.e. the energy or product output
48 significantly surpasses the energy and monetary input. Enhancement of the current
49 pretreatment techno-economic efficiency (e.g. higher product yield, chemical recycling, and
50 by-products conversion to increase environmental sustainability) and the integration of
51 pretreatment methods to effectively treat a range of biomass will be the steppingstone for
52 commercial lignocellulosic biorefineries.

53 **Keywords:** Lignocellulosic biomass; Pretreatment; Biogas; Biofuel; Anaerobic digestion;
54 Ligninolytic enzyme; Valorisation.

55 **1. Introduction**

56 Lignocellulosic biomass is the most abundant resource of inexpensive, renewable, and high
57 energy-density raw materials that are often underutilized. It is a promising alternative to fossil-
58 based fuels [1-3]. Lignocellulosic biomass contains significant amounts of polysaccharides and
59 lignin, which can be converted to monomer sugars (e.g. glucose) and further valorised for the
60 synthesis of high value-added biochemicals (e.g. phenylpropanoids and levulinic acid) [4-6].
61 Most research on valorizing lignocellulosic biomass has focused on feedstocks that are wastes
62 or residues from human activities (e.g. agricultural, forestry, and industrial processes). The
63 utilization of these feedstocks eliminates the food versus fuel competition faced by energy
64 production from food crops and the land requirements for growing new plants [7]. Besides,
65 with most of the lignocellulosic wastes currently being burnt, landfilled, or discarded into
66 waterways, effective utilization of these feedstocks will bring about great environmental
67 impacts e.g. decrease greenhouse gas emissions and prevent water pollution [8].

68 Conversion of lignocellulosic biomass into valuable commodities has been explored at
69 bench-scale, demonstrated at pilot-scale, and implemented at full-scale in a few cases [1, 7, 9,
70 10]. Liquid biofuels (e.g. ethanol and diesel) and biogas (i.e. methane) are the major products
71 obtained from lignocellulosic materials through conversion processes (e.g. fermentation and
72 anaerobic digestion). The socioeconomic impacts of replacing fossil fuels with these
73 sustainable bioenergy sources are so great that scientists, economists, and politicians globally
74 have continuously invested in this research topic and passed policy and regulations to support
75 biofuels [7, 11]. The sufficient production of biofuels will ensure energy security, which is
76 under pressure due to the depletion of fossil fuel. The industrialization of this sector will also
77 provide new and ongoing employment, especially in the regional areas where the facilities are
78 likely to be located [7].

79 There are considerable challenges in full-scale bioenergy and biochemical production from
80 lignocellulosic biomass in terms of product yield and energy input into the process.
81 Lignocellulosic biomass is composed of cellulose and hemicellulose, tightly packed and
82 protected by phenol aldehyde lignin polymer [12]. The recalcitrant structure of lignocellulosic
83 biomass makes it difficult for enzymes to hydrolyse cellulose to simple sugars, thus lowering
84 product yield [13]. The inclusion of additional processes (e.g. pretreatment) has been
85 investigated to enhance the digestibility of lignocellulosic biomass. However, these processes
86 can be energy intensive as they require great mechanical forces or high temperature and
87 pressure to break down complex lignocellulose [14]. The capabilities to minimise energy input

88 and maximise product yield are essential to achieve a positive energy balance of lignocellulosic
89 biorefineries. It is the first step in ensuring the techno-economic viability of bioenergy and
90 biochemical production from lignocellulosic biomass.

91 Many pretreatment techniques have been explored to modify the lignocellulosic structure
92 and improve its bioconversion [13, 15, 16]. Pretreatment helps break down lignin and
93 glycosidic bonds, thus reducing the structural crystallisation and increasing the digestibility of
94 the biomass. They can be grouped into several categories including physical, chemical,
95 physicochemical, and biological treatments. These processes have proven to be effective at
96 enhancing the sugar and bioproduct yield of different lignocellulosic biomass, with each having
97 its advantages and disadvantages [17, 18]. Several reviews have comprehensively summarised
98 the mechanisms and properties of pretreatment techniques, as well as their performance in
99 modifying lignocellulosic biomass [13, 16, 19, 20]. However, a complete overview that
100 provides a framework on enhanced bioconversion of lignocellulosic biomass using
101 pretreatment techniques has not been presented.

102 This paper aims to provide a systematic perspective on lignocellulosic pretreatment
103 methods, their effective performance at bench-scale investigations, and the ongoing challenges
104 they are still facing. It also highlights the commercial outlook of pretreatment techniques
105 through evaluating studies conducted at the pilot and full-scale levels. Finally, strategies to
106 overcome the economic constraints of biofuel production from lignocellulosic biomass through
107 improving pretreatment process are also delineated.

108 **2. Lignocellulosic biomass characteristics**

109 **2.1. Lignocellulosic biomass resource**

110 Lignocellulosic biomass refers to plant biomass in the form of crop residues, agricultural
111 wastes, forestry waste, and urban wastes [21]. Due to the agricultural industrialization and the
112 increase in food demand, billions of tons of lignocellulosic biomass are produced every year,
113 making it the most abundant biomass on earth [22]. In the US, the annual yield of
114 lignocellulosic biomass from crops was estimated to be 1.4 billion dry tons alone [23]. Canada
115 produces 69 million dry tons of agricultural crop residue annually [24]. In Australia, the fruit
116 and energy crop industry such as banana, pineapple, and sugarcane also generate millions of
117 tons of lignocellulosic waste every year. In the past, the non-edible parts of the plants such as
118 leaves, pulps, stems, and peels are unavoidable food waste [25]. The majority of this non-
119 avoidable food waste biomass is being discarded or burned, contributing to the release of
120 greenhouse gases [26]. It, however, is a valuable source of cellulose and lignin (Table 1). The

121 abundant quantity of lignocellulosic biomass from annual agricultural production ensures a
 122 constant supply of feedstocks for large-scale applications.

123 **Table 1:** Chemical composition of various lignocellulosic biomass

Lignocellulosic biomass	Composition (% dry weight) ^a			Refs
	Cellulose	Hemicellulose	Lignin	
Agricultural wastes/residues				
Corn straw	42.6	21.3	10 – 20	[27]
Oat straw	39.4	27.1	20.7	[28]
Rice straw	31.1	22.3	13.3	[29]
Sugarcane bagasse	43.1	31.1	11.4	[30]
Wheat straw	35.9	23.9	19.3	[31]
Banana bunch stem	60 – 65	6 – 8	7.9	[25, 32]
Forestry residues				
Aspen	52.7	21.7	19.5	[33]
Eucalyptus	46.6 – 50.3	12.7 – 14.4	26.9 – 28.2	[34]
Japanese beech	43.9	28.4	24	[12]
Pine	42 – 50	24 – 27	20	[34]
Industrial/municipal wastes				
Brewer's spent grains	16.8 – 21.9	19.2 – 29.6	19.4 – 27.8	[35]
Newspaper	40 – 55	25 – 40	18 – 30	[36]
Solid cattle manure	1.6 – 4.7	2.7 – 5.7	1.4 – 3.3	[34]

124 ^a In addition to polysaccharides and lignin, lignocellulosic biomass also contains some inert materials (< 10
 125 wt%) [37, 38].

126 Effective pretreatment of available lignocellulosic biomass contributes to the generation of
 127 sustainable biorefineries and the decrease in environmental impacts caused by organic waste
 128 disposal. The polysaccharide fractions of lignocellulosic biomass including cellulose and
 129 hemicellulose can be broken down into sugar monomers [26]. They are then converted into
 130 biofuels, biogas, and biochemicals through biotechnologies such as anaerobic digestion and
 131 fermentation. The efficiency and cost-effectiveness of the bioconversion process depend on the
 132 transformation of polysaccharides to monomer sugars.

133 Commercial applications of lignocellulosic biomass are hindered by the resistance of
 134 polysaccharides to hydrolysis and the presence of recalcitrant lignin. A range of pretreatment
 135 methods has been developed and employed to increase conversion efficiency [1, 39]. The
 136 pretreatment of lignocellulosic biomass aims to decrystallise cellulose structure through lignin
 137 removal, increase cellulose and hemicellulose solubility, increase accessible surface area to
 138 enzymes, and chemicals, and minimise the loss of sugars [39, 40]. The anticipated end products

139 also determine the choice of pretreatment method as each method induces different effects on
140 different types of lignocellulosic biomass. Various by-products generated through these
141 processes can be recovered and utilized for other biochemical productions. The success in
142 identifying and applying effective pretreatment to lignocellulosic biomass can increase the
143 socioeconomic impacts and resolve global problems involving sustainable energy and
144 development.

145 2.2. Chemical structure and the associated challenges in lignocellulosic processing

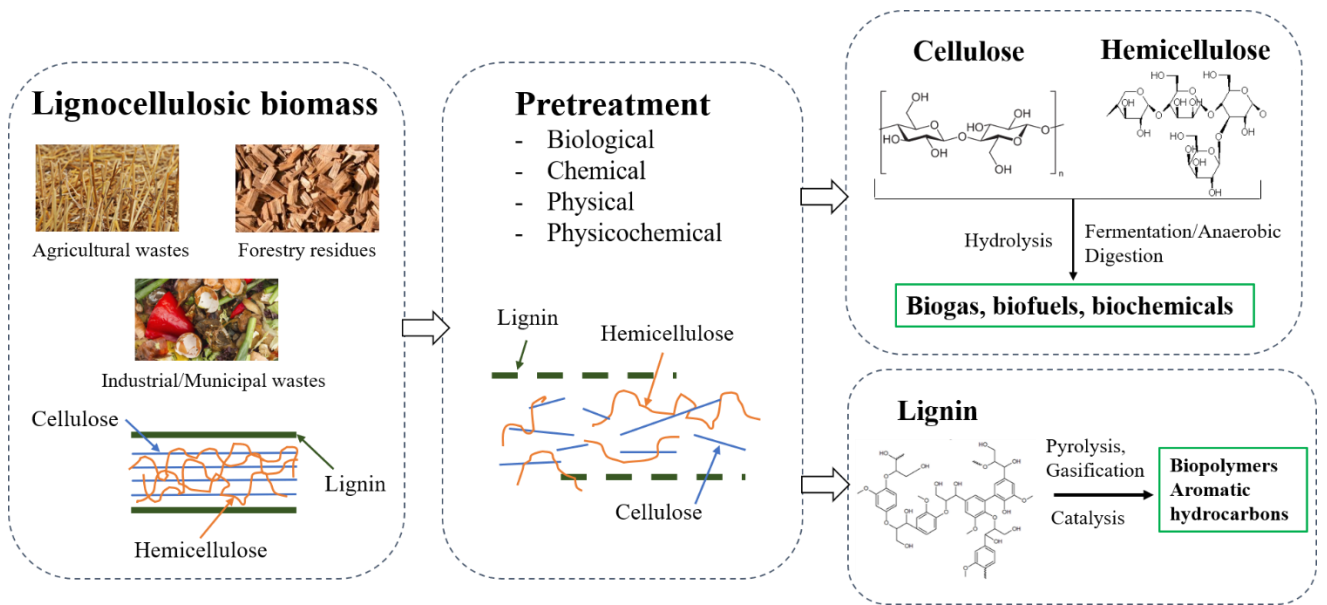
146 The main chemical building blocks of lignocellulosic biomass include cellulose (35-50
147 wt%), hemicellulose (20-35 wt%), and lignin (15-20 wt%) [17]. This composition varies and
148 depends on the cultivation conditions, geographical location, and the age of plants [41] (Table
149 1). Cellulose is the main constituent of lignocellulosic biomass, containing linear chains of D-
150 glucose linked to each other by β -(1,4)-glycosidic bonds. Intra- and intermolecular hydrogen
151 bonds connect cellulose strains to make cellulose microfibrils [17]. These are covered by
152 hemicelluloses. Hemicelluloses are heterogeneous biopolymer consisting of different
153 monosaccharides such as pentoses (β -D-xylose, α -L-arabinose), hexoses (β -D-mannose, β -D-
154 glucose, α -D galactose), and sugar acids [42]. Due to their amorphous and branched structure,
155 and low molecular weight, hemicellulose can be readily hydrolysed and is a major carbon
156 source for bioethanol production besides cellulose. The removal of hemicellulose increase
157 enzyme accessibility to cellulose fibrils, thus enhancing cellulose digestibility in biorefinery
158 processes [14]. Hemicelluloses are also the crosslinks between cellulose fibrils and the lignin
159 matrix. Lignin is an amorphous heteropolymer network of phenyl propane located in the plant
160 cell walls [13]. It provides structural support and acts as a natural, impermeable barrier to
161 microbial attack and oxidative stress on plant tissues [14]. Biopolymers cellulose,
162 hemicellulose, and lignin exist in complex lignin-carbohydrate linkages formed by the
163 hydrophobic and covalent interactions between lignin and carbohydrates [43, 44]. This tough
164 and tightly packed solid matrix of the biopolymers hinders the access to and the utilization of
165 cellulose.

166 The chemical composition of lignocellulosic biomass determines their potential as
167 biorefinery feedstocks. Agricultural residues such as wheat straw, rice straw, and sugarcane
168 bagasse have been commonly used for investigation of biofuel production due to their high
169 cellulose and hemicellulose content and a lower percentage of lignin (< 20 wt%) (Table 1) [31,
170 45, 29]. Biomass with lower lignin content is easier to break down, thus require less energy
171 intensive processes for pretreatment and conversion to biofuel. Forestry residues have also been

172 investigated for biorefineries, but their high lignin content is a major drawback regarding
 173 technical and economic outlooks (Table 1).

174 **3. Conversion of lignocellulosic biomass into valuable products**

175 The production of biofuels and biochemicals from non-edible lignocellulosic biomass (e.g.
 176 straw and bagasse) has emerged as an important pathway to develop a new economy
 177 independent of fossil fuels and without greenhouse gas emission [2]. Despite several full-scale
 178 plants and promising results from bench-scale experiments, improving the process efficiency
 179 is necessary to increase its cost-competitiveness, thus facilitating commercial applications
 180 without relying on government subsidies (e.g. lower tax rates than fossil fuels and mandate to
 181 encourage biofuel consumption). The focus lies on developing advanced pretreatment
 182 techniques that are eco-friendly and cost-effective to significantly enhance biofuel and
 183 biochemical production (Fig. 1).



184

185 **Figure 1:** Bioconversion of lignocellulosic biomass into value-added products with the
 186 inclusion of the pretreatment step.

187 **3.1. Biogas production**

188 There are several technical challenges to the commercial viability of biogas production
 189 from lignocellulosic biomass. The recalcitrant structure with complex lignin-carbohydrates
 190 linkages makes lignocellulose resistant to enzyme access, thus preventing it from achieving
 191 maximum theoretical methane yield [46]. About 70-80% of biomass (cellulose and lignin
 192 component) remains undigested in the solid residues (digestate). Although it may contain
 193 impurities and is often low solid content, the capability to convert this digestate to high-value

194 products is necessary for better economic returns [47]. In addition, the long digestion time (15
195 to 40 days) required for microbial growth under anaerobic conditions is also a major drawback
196 [48]. The choice of inoculum with the right microbial community is also important to reduce
197 the processing time and enhance biogas production. A balance between the abundance of
198 lignocellulolytic and methanogenic bacteria in the inoculum should be maintained [49].

199 Pretreatment techniques have been investigated as a strategy to improve biogas production
200 [19, 50, 51]. Pretreatment promotes lignin degradation, reduces hemicellulose crystallinity and
201 enhances the digestibility of biomass [19, 52]. Significant increases in biogas (methane) yield
202 from anaerobic digestion of lignocellulosic biomass after pretreatment have been reported in
203 the literature (Table 2). The diversity in biogas production is caused by the complexity and
204 variability of lignocellulosic compositions and the type of pretreatment techniques used [53].
205 For example, pretreated cardboard achieved a significant increase in biogas yield (140%)
206 compared to the untreated sample, while it is only a 40% increase for pretreated rice straw
207 (Table 2). Besides the difference in pretreatment technique applied, the variation in their
208 compositions also contributes to these results. Cardboard contains 57, 11, and 18 wt% of
209 cellulose, hemicellulose, and lignin, respectively [54]. Rice straw contains 31, 22, and 13 wt%
210 of cellulose, hemicellulose, and lignin, respectively [29]. Higher lignin content makes untreated
211 cardboard more resistant to digestion, thus having the lower initial biogas production than
212 untreated rice straw (Table 2). After pretreatment (i.e. lignin degradation), more carbohydrates
213 from cardboard (68 wt%) are made susceptible to digestion than from rice straw (53 wt%), thus
214 a larger increase in biogas yield. The inclusion of pretreatment step also resulted in shorter
215 digestion time [55, 56]. For example, 41.7% shorter digestion time than the untreated sample
216 was achieved for biologically pretreated corn stover [57]. Zhong et al. [56] were also able to
217 reduce the digestion time by 34.6% for corn straw pretreated with microbial agents (yeast and
218 cellulolytic bacteria). However, integrating a pretreatment step into the process will likely
219 increase the capital and operational costs for additional equipment and energy usage. Bench-
220 scale experiments (Table 2) are necessary to obtain an understanding of the underlying
221 mechanisms and achieve process optimisations for scale-up. The development of an effective
222 pretreatment and digestion process that produces greater energy density (biogas yield) without
223 greater energy use is essential. This will lead to the commercial expansion of biogas production
224 from lignocellulosic biomass (Section 7).

225 **Table 2:** Selected examples of increased biogas production from anaerobic digestion of
 226 pretreated lignocellulosic biomass

Pretreatment	2% H ₂ SO ₄ then Steam explosion at 190 °C	6% w/w NaOH	Ionic liquid [C ₄ mim]Cl	Hydrothermal pretreatment at 175 °C	Biological pretreatment using microbial agents ^a
Working volume of reactor (mL)	375	400	100	4000	750
Primary Substrate	Rape straw	Asparagus stem	Rice straw	Napier grass	Cardboard
Digestion duration (days)	36	18	30	42	55
Biogas production – untreated substrate (mL/g VS)	274.6	175.1	153	183.8	96
Biogas production – pretreated substrate (mL/g VS)	407.6	242.3	215.4	248.2	231
Increase in biogas (%)	48.4	38.4	40.8	35	140.6
Reference	[54]	[58]	[59]	[60]	[61]

227 ^a Contains *Clostridium straminisolvens* CSK1, *Clostridium* sp. train FG4b, *Pseudoxanthomonas* sp. strain
 228 M1-3, *Brevibacillus* sp. train M1-5, and *Bordetella* sp. strain M1-6 [62].
 229

230 Biogas purification or upgrading is the final important step to enhance the commercial
 231 value of the anaerobic digestion of lignocellulosic biomass [63]. It separates biomethane from
 232 other undesired chemical components of biogas (e.g. carbon dioxide, hydrogen sulfide,
 233 siloxanes and volatile organic compounds) which was produced during the digestion process
 234 [64]. Biomethane is a carbon dioxide neutral, renewable, and clean fuel that is a great
 235 alternative to fossil fuel [63, 65]. Available technologies to purify biogas include chemical
 236 scrubbing, organic physical scrubbing, pressure swing adsorption, cryogenic upgrading and
 237 membrane separation [63]. Incorporating a suitable biogas upgrading system into
 238 lignocellulosic biorefinery plants will contribute to the environmental sustainability and the
 239 development of a green economy.

240 3.2. Liquid biofuels

241 Liquid biofuels can be generated from lignocellulosic biomass include ethanol, butanol,
 242 and diesel. Extensive research has been conducted to achieve biofuel process optimisation
 243 through the advancement in pretreatment techniques. This will contribute to facilitating the full
 244 commercialisation of this sector. Tang et al. reported more than 80% lignin removal and a 7.5%
 245 increase in biobutanol concentration using 0.4% w/w NaOH-catalysed ethanol (60% v/v)

246 pretreatment of cornstalks at 110 °C for 90 min [66]. An eco-friendly pretreatment of forest
247 residues (spruce and oak sawdusts) at low temperature (45 °C) using [Emim][OAc] ionic liquid
248 also demonstrated an increase in the ethanol yield of 2.6–3.9 times compared to the
249 untreated samples [67]. In another study, Suko and Bura investigated steam-pretreatment
250 conditions for various feedstocks (mixed wood, switchgrass, and sugarcane bagasse) and
251 achieved enhanced ethanol yields up to 138% of the theoretical value [68]. These promising
252 laboratory results are the motivation for further research and applications of viable large-scale
253 biofuel production from lignocellulosic biomass.

254 Biofuel purification are necessary but costly processes in the final stage to obtain highly
255 concentrated biofuels from lignocellulosic biorefinery that meet fuel-grade standards and
256 specifications [69, 70]. Purified bioethanol (> 99%) can be achieved through distillation and
257 dehydration processes. Some emerging energy-saving technologies include heat integrated
258 distillation, membrane, feed splitting and ohmic-assisted distillation [69]. These technologies
259 are still under development and further research is required to validate their sustainability as
260 well as cost competitiveness. Successful implementation of advanced pretreatment and
261 purification processes will be the steppingstone to fully commercialised and greener bioethanol
262 production from lignocellulosic biomass.

263 There exist several commercial-scale lignocellulosic biofuel plants worldwide [7]. Notable
264 examples including the POET-DSM Advanced Biofuels plant in South Dakota, USA, and the
265 Raizen plant in Brazil. The POET-DSM plant produces 80 ML of bioethanol annually from
266 corn stover by fermentation. The corn stover was subjected to a pretreatment process but no
267 detailed information was available. The Raizen plant that produces 8 ML/year of ethanol from
268 bagasse also includes their state of the art pretreatment system. Several other biofuel plants are
269 under construction with expected production ranging from 40 to 160 ML/year. These plants
270 utilise municipal solid waste and agricultural wood waste for advanced biofuel production
271 through gasification, catalysis, and fermentation [7].

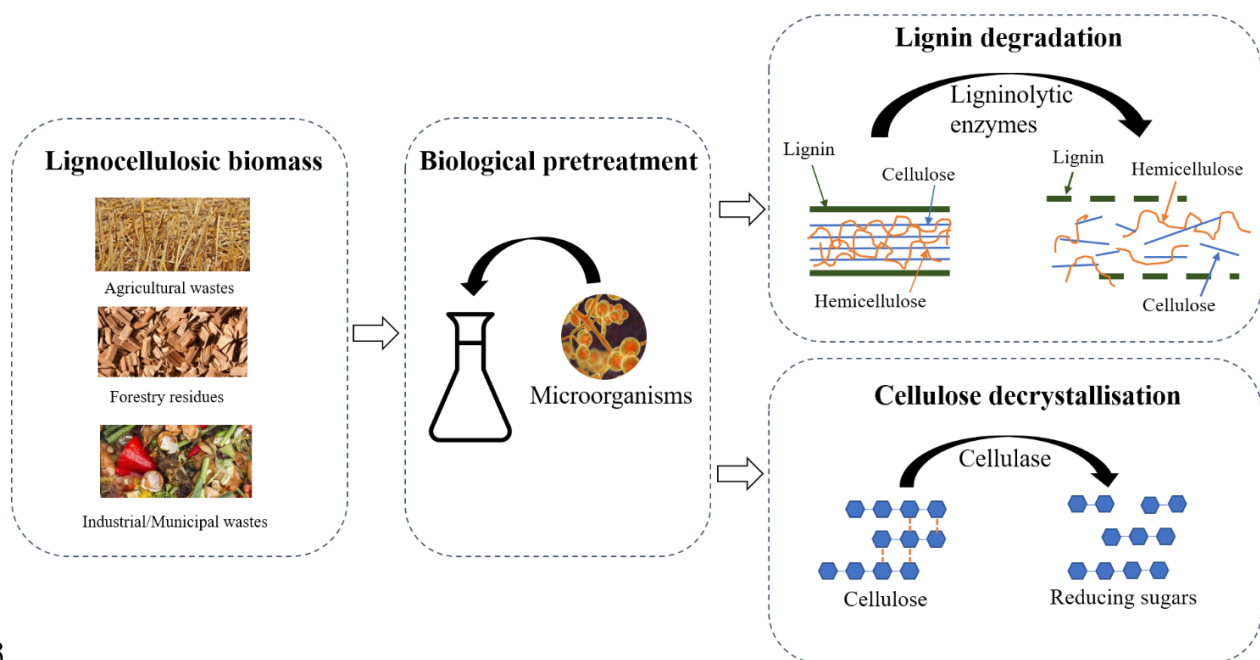
272 3.3. Bioproducts and biochemicals

273 The breakdown of lignocellulosic biomass can generate intermediate products such as
274 organic acids (e.g. acetic and lactic acid), microbial enzymes (e.g. cellulase, amylase, and
275 pectinase) and essential chemicals for bio-based polymers and bioactive compounds (e.g.
276 phenylpropanoids and phenolic compounds) [4, 71, 72]. These biochemicals can be obtained
277 through several bioprocesses such as separate hydrolysis and fermentation (SHF) and
278 simultaneous saccharification and fermentation (SSF). However, similarly to biofuel

279 production, these bioprocesses are confronted by the recalcitrant structure of lignocellulosic
 280 biomass that hinders their process efficiency and limits their economic viability [3]. Thus, the
 281 development of suitable, efficient, and cost-effective pretreatment techniques is the first crucial
 282 step in facilitating the valuable bio-based product market, which is expected to be 50 billion
 283 EURO by 2030 [3].

284 4. Biological approach to hydrolyse lignocellulose structure

285 Biological pretreatment using microorganisms is a promising approach to degrade
 286 lignocellulosic structure extracellularly, thus increasing the sugar conversion rate of the
 287 biomass [20] (Fig. 2). They have several attractive traits such as eco-friendly and simple
 288 operation, low capital cost, low energy requirement, and no chemical requirement [39, 52].
 289 Major drawbacks are long pretreatment time and strict microbial growth conditions. The
 290 extraction of lignin-degrading enzymes from microorganisms to be used directly on the
 291 biomass emerges as an alternative approach to eliminate the above problems. However, efforts
 292 in reducing the cost of enzyme extraction are necessary to make it a viable process.



293

294 **Figure 2:** Enzymatic attacks on lignocellulosic biomass incubated with microorganisms.

295 4.1. Cellulolytic and ligninolytic microorganisms

296 The commonly used microorganisms are bacteria and filamentous fungi (e.g. ascomycetes
 297 and basidiomycetes), which are found ubiquitous in soil, living plants, and lignocellulosic
 298 waste material [73, 74]. The fungi can be classified into brown rot, white rot, and soft rot fungi.
 299 These microorganisms secrete enzymes that are capable of selectively degrade lignin

300 (ligninolytic fungi) or hydrolyse cellulose (cellulolytic bacteria) [20]. The application of these
 301 species in biological pretreatment of various lignocellulosic biomass has been extensively
 302 studied (Table 3). Biological pretreatment using microorganisms and enzymes extracted from
 303 them also offers a great opportunity to produce various high value-added chemicals from the
 304 waste-by-product lignin. These products are useful for the generation of phenolic acid, vanillin,
 305 vanillic acid, cinnamic acid, benzoic acid, and syringaldehyde [75].

306 **Table 3:** Selected examples show the effect of lignin-degrading microorganisms on various
 307 types of lignocellulosic biomass (MC: moisture content).

Group	Microorg anism	Feedstock	Operation conditions	Effects	Refs.
White rot fungus	<i>Trametes versicolor</i>	Cow manure and selected cereal crops	25 °C 135 rpm 6 days 75% MC pH 4.2	80% increase in cellulose degradation 10-18% increase in methane yield	[76]
	<i>Ceriporio psis subvermis pora</i>	Miscanthus (Silvergrass)	21 days 28 °C 60-70 % MC	Degraded 30% of lignin 3 to 4-fold increase in glucose yield	[77]
	<i>Pleurotus ostreatus</i>	Rice straw	20 days 28 °C 75% MC	Degraded 33.4% of lignin content Methane yield increased by 120%	[78]
Soft rot fungus	<i>Trichoder ma reesei</i>	Rice straw	20 days 28 °C 75% MC	Degraded 23.6% of lignin content Methane yield increased by 78.3%	[78]
Brown rot fungus	<i>Coniopho ra puteana</i>	<i>Pine radiate</i> (Sapwood)	20 days 22 °C	3-fold increase in glucose yield	[79]
	<i>Postia placenta</i>	<i>Pine radiate</i> (Sapwood)	25 days 22 °C	3-fold increase in glucose yield	[79]
Endophytic fungus (ascomycetes)	<i>Pringshei mia smilacis</i>	<i>Eucalyptus globulus</i> wood	28 days 23 °C	Enhanced sugar production by 55.4%	[80]

Bacterium	<i>Cupriavidus basilensis</i> B-8	Acid-pretreated rice straw	3 days 30 °C	Biomass enzymatic digestibility increased 35–70% and 173–244% compared to acid-pretreated only and raw biomass, respectively	[81]
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308

309 Among the microorganisms, white-rot fungi have been extensively studied and proven to
310 be the most effective lignin-degrading species [20, 82]. During their growth, most white-rot
311 fungi (e.g. *Pleurotus ostreatus*, *Trametes versicolor*, *Phanerochaete chrysosporium*, and *P.*
312 *chrysosporium*) produce extracellular lignin-modifying enzymes including laccase, lignin
313 peroxidases (LiPs) and manganese peroxidases (MnPs) [83, 84]. These enzymes exhibit
314 specificity for lignin and catalyse enzymatic cleavage of lignin aromatic rings through
315 oxidation processes [85]. As a result, the linkages between polysaccharides (cellulose and
316 hemicellulose) and lignin are broken down, thus liberating the cellulose component, and
317 enhancing the hydrolysis of lignocellulose. In addition, some white rot fungi (*P.*
318 *chrysosporium*, *C. subvermispora*, and *Echinodontium taxodii* 2538) secrete cellulolytic
319 enzymes known to hydrolyse cellulose thus increasing its enzymatic digestibility [86]. This
320 activity, however, poses the risk of sugar loss due to the consumption of polysaccharides by
321 cellulolytic enzymes for fungal growth [82]. To minimise this problem, fungal strains that
322 exhibit low (< 1.0) selectivity value for lignin breakdown (the ratio of lignin loss to cellulose
323 loss) are not recommended for biological pretreatment of lignocellulosic biomass [87, 88]. A
324 techno-economic study on white-rot fungi pretreatment of corn stover to produce 75700 m³
325 fermentable sugars per year obtained a sugar production cost of \$1.60/kg [89]. Fungal
326 pretreatment does not require chemical addition and high energy demand. However, it
327 necessitates long pretreatment times and sterilisation requirements. The process's efficiency is
328 also lower than that of acid or alkaline pretreatment. These factors make the sugar production
329 cost of fungal pretreatment higher than that of conventional methods (< \$1/kg) [89].

330 Multiple approaches to enhance the efficacy of biological treatment and its suitability for
331 large-scale lignocellulosic biorefineries are identified. Microorganisms can be incorporated
332 into the seasonal biomass storage on the field (i.e. ensiling) to reduce the total treatment time.
333 Pretreatment using microbial co-culture instead of a single species to assimilate the synergistic
334 metabolic activities of microorganisms in nature also improves process efficiency [82]. Fungi

335 degrade lignin and hydrolyse holocellulose into monomer sugars, which are then converted to
 336 valuable products by the bacteria [20, 90, 91]. These mechanisms of biological pretreatment
 337 make it a suitable process prior to anaerobic digestion to enhance biogas production. Biological
 338 pretreatment increases biomass hydrolysis, which is the rate-limiting step in biogas production.
 339 By using natural microorganisms at room conditions, no additional step is required to remove
 340 toxic substances (e.g. acids) from the hydrolysates. Altogether biological pretreatment provides
 341 a cost-effective and high-solid lignocellulosic feedstock compatible with anaerobic digestion.

342 4.2. Ligninolytic enzymes

343 In an alternative approach to microorganism incubation, ligninolytic enzymes extracted from
 344 the fungal or bacterial cultures can be purified and used directly on the biomass as a
 345 pretreatment [74] (Table 4). These ligninolytic enzymes are capable of catalyzing various
 346 biochemical reactions to degrade selectively lignin with minimal cellulose consumption. Direct
 347 application of enzymes on the biomass eliminates the long growing period of microorganisms,
 348 thus significantly reducing the pretreatment time (e.g. from 15-40 days to 6-24 hours) [85, 92].
 349 Thus, enzymatic pretreatment can accelerate bioenergy production at minimal environmental
 350 impacts, no chemical addition, and lower energy demand. Modified lignin after enzymatic
 351 pretreatment can also be recovered for effective uses in fast pyrolysis to produce bio-oil [93].
 352 Common enzymes used for pretreating lignocellulosic biomass are mostly commercialized
 353 products from leading companies such as DuPont (Wilmington, USA), Novozymes
 354 (Bagsvaerd, Denmark) and DSM (Delft, the Netherlands) [18]. The capability to identify
 355 microorganisms and growth conditions to cost-effectively produce and purify a high amount
 356 of stable ligninolytic enzymes is critical for this pretreatment to be commercially viable [74,
 357 85].

358 **Table 4:** The properties of important ligninolytic enzymes

Ligninolytic Enzyme	Producer	Characteristics	Substrate specificity	Refs
Laccase	Higher plants Insects Fungi (e.g. <i>Phlebia radiata</i> , <i>Trametes versicolor</i>) Bacteria (e.g. <i>Azospirillum lipoferum</i>)	- Copper-containing (four atoms) blue oxidases - Extracellular - Catalyses oxidation of lignin by reducing O ₂ into H ₂ O	- Phenolic lignin (direct oxidation) - Nonphenolic lignin (oxidation through supplemented mediator system)	[83, 94, 95]
Lignin Peroxidase (LiPs)	Fungi (e.g. <i>Phanerochaete chrysosporium</i> ,	- Heme-containing glycoproteins	Phenolic and nonphenolic	[83, 94,

	<i>Phlebia radiata</i> , <i>Coriolus versicolor</i>) Bacteria (e.g. <i>Bacillus subtilis</i>)	- Requires hydrogen peroxide as an oxidant - High redox potential i.e. generating cation radical for C-bond cleavage	compounds (direct oxidation)	96, 97]
Manganese Peroxidase (MnPs)	Fungi (e.g. <i>Phanerochaete chrysosporium</i> , <i>Phlebia radiata</i>) Bacteria (e.g. <i>Cupriavidus basilensis</i>)	- Heme-containing glycoproteins - Most common ligninolytic enzymes in white rot fungi - Low redox potential	- Phenolic lignin (direct oxidation) - Nonphenolic lignin (in the presence of unsaturated lipid or thiols as mediators)	[83, 94, 95]

359

360 Major ligninolytic enzymes such as laccase, lignin peroxidase (LiPs), and manganese
361 peroxidase (MnPs) have been evaluated for their efficiency in delignifying lignocellulosic
362 biomass. Up to 50% lignin removal was attained by pretreatment with *P. ostreatus* laccases (10
363 U/mL, 28 °C, 24 h) for high lignin content biomass (29% and 33% for coffee silverskin and
364 potato peel, respectively) [98]. The pretreatment of wheat straw using a *Pycnoporus*
365 *cinnabarinus* laccase (65 U/g) and 1-hydroxybenzotriazole mediator (20%) system achieved
366 37% lignin removal, leading to an increase of 60% in glucose yield after enzymatic hydrolysis
367 [99]. Sugarcane bagasse pretreated with a ligninolytic enzyme extract from *Pleurotus ostreatus*
368 IBL-02 containing laccase, LiPs, and MnPs (0.83 mL/g dry biomass, 35 °C, 48 h) also reported
369 34% delignification and ethanol production of 16 g/L after fermentation process [100]. These
370 results suggest that pretreatment with ligninolytic enzymes is a promising technique to enhance
371 bioconversion of lignocellulosic biomass, especially the ones with high lignin content.

372 5. Chemical agents to promote structural modification

373 5.1. Enhance lignin solubility by alkaline solutions

374 Alkaline pretreatment is a very efficient and most traditional lignocellulosic biomass
375 pretreatment method widely used in commercial applications (e.g. pulp and paper processing)
376 [101]. It uses alkali such as sodium hydroxide, potassium hydroxide, and calcium hydroxide to
377 effectively solubilise lignin and part of the hemicellulose, thus reducing cellulose crystallinity
378 [102]. Alkaline pretreatment can be operated at mild conditions with a simple flow sheet, thus
379 lowering the cost for expensive equipment and special design to cope with severe reaction
380 conditions [103]. By fractionating the biomass into pure streams of saccharide (cellulose and
381 hemicellulose) recovery for biofuels and lignin by-products suitable for conversion into high-

382 value chemicals, alkaline pretreatment provides a promising opportunity to increase its
 383 economic viability and benefits [102]. These advantages make alkaline pretreatment a common
 384 process used in pilot-scale production of biogas and bioethanol. It is especially suitable for
 385 anaerobic digestion process as alkali limits acidification in the reactors (i.e. high stability)
 386 [104]. Washing steps are also not required to remove alkali in anaerobic digestion, thus
 387 reducing process complexity and cost.

388 Investigations have been conducted to study the effect of alkaline pretreatment and its
 389 operating conditions. It has shown to be effective in pretreating hardwood, herbaceous crops,
 390 and agricultural residues (Table 5). Sodium hydroxide is the most common alkali used for
 391 pretreatment due to its great delignification capability (60-80%) at mild concentration and non-
 392 production of any inhibitors [103, 105, 106]. Calcium hydroxide is a less expensive alternative
 393 to sodium hydroxide. It could also be easily recovered from the hydrolysates by reaction with
 394 carbon dioxide [107, 108]. Novel ammonia-based pretreatment (i.e. low liquid ammonia and
 395 low-moisture anhydrous ammonia) has also been extensively studied due to it being easily
 396 recoverable (high volatility), non-corrosive, non-toxic and inexpensive [103] (Table 5). In
 397 general, alkaline pretreatment of lignocellulosic biomass has shown to be effective at milder
 398 operating conditions than other pretreatment processes (e.g. acid and thermo-physical). The
 399 successful development of the solutions to overcome the drawbacks such as long pretreatment
 400 time and the conversion of alkali into irrecoverable salts during the reactions will increase the
 401 full-scale viability of alkaline pretreatment and its environmental friendliness [105].

402 **Table 5:** Performance of alkaline pretreatment in the bioconversion of lignocellulosic biomass
 403 (selected examples).

Pretreatment	Sodium hydroxide (NaOH)		Calcium hydroxide (Ca(OH)₂)		Low-liquid aqueous ammonia (NH₄OH)	Low-moisture anhydrous ammonia (NH₃)
Conc. (% w/w dry biomass)	0.2		0.1		50	10
Substrate	Sorghum straw	Bagasse	Wheat straw		Corn stover	Corn stover
Solid loading (% w/v)	10	10	6.67		20	100
Temperature (°C)	60	121	120	85	30	80

Duration (h)	1.5	1	1	3	672	84
Effects	4.3-fold increase in sugar yield	5.6-fold increase in sugar yield	4.3-fold increase in sugar yield	10-fold increase in sugar yield	Removed 55% of lignin Converted 86.5% of glucan to glucose	Yielded 25 g/L ethanol concentration (89% of theoretical yield)
Remarks	Temperature is the most critical factor followed by alkalinity		86% of added calcium was removed from the pretreated bagasse by ten washings		Increased glucan and xylan digestibility at high ammonia loading	Minimise ammonia and water input. No washing required to remove access ammonia
Refs	[106]		[109]		[110]	[111]

404

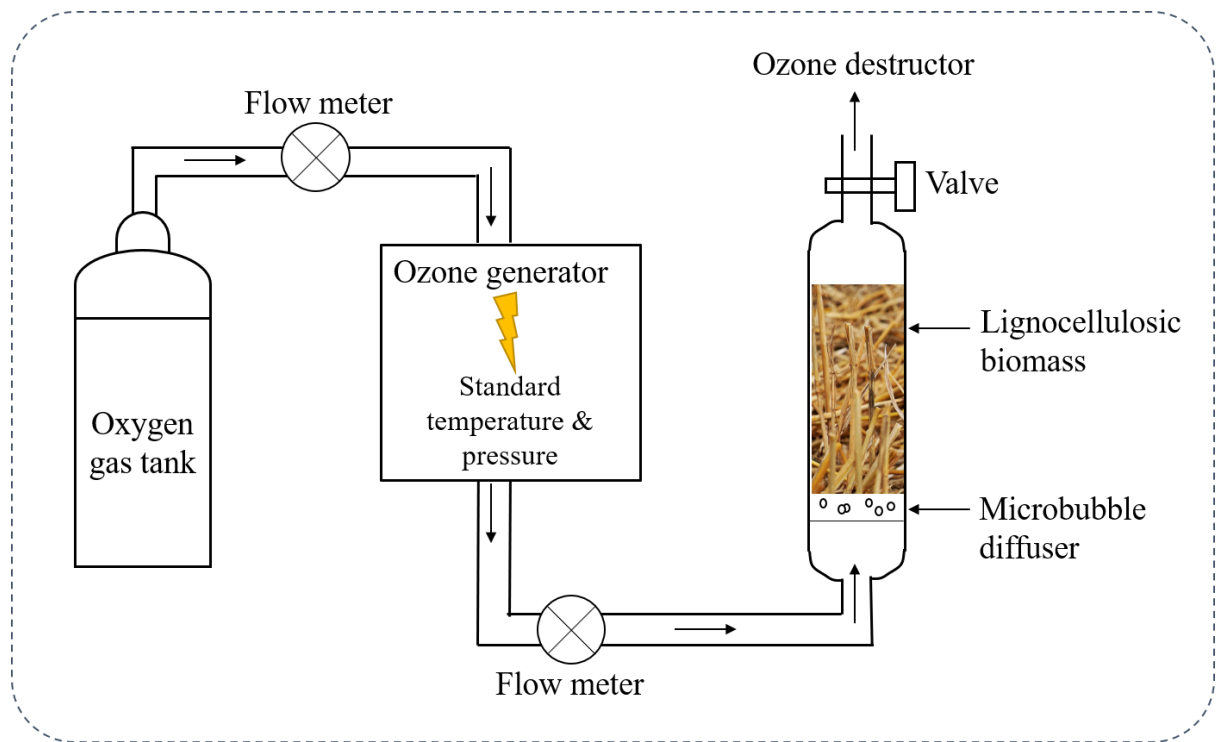
405 5.2. Hydrolysis using acidic solutions

406 Acid solutions solubilize hemicellulose component, and part of lignin through the cleavage
407 of glucosidic bonds thus making cellulose more accessible to enzymes [39, 101]. Highly
408 commercial inorganic (phosphoric acid, nitric acid, hydrochloric acid, and sulphuric acid) and
409 organic acids (citric acid, acetic acid, and oxalic acid) have been evaluated for lignocellulosic
410 biomass pretreatment. A study using phosphoric acid pretreatment on pinewood sawdust
411 achieved a maximum xylose production of 91% under optimal conditions of 107 °C for 5 h,
412 with 4.5% w/w of acid and a solution-to-feed ratio of 12.5 mL/g [112]. Baadhe et al. also
413 reported good reducing sugar production of 399 mg/mL from corncobs pretreated with 0.25 M
414 sulphuric acid at 121 °C for 20 min and solid to liquid ratio of 0.05 [113]. Acid hydrolysis of
415 lignocellulosic biomass can also produce a high yield of levulinic acid, which is an important
416 platform biochemical (i.e. building blocks for other chemicals) [5, 114]. A maximum levulinic
417 acid yield of 32 C mol was achieved using 0.135 M of sulphuric acid at 200 °C in a γ -
418 valerolactone and water solvent [5]. The pretreatment of lignocellulosic biomass can be done
419 with concentrated or dilute acids, depending on the desirable products. Dilute acid pretreatment
420 is a promising option for bioethanol production since the formation of inhibiting volatile
421 compounds (e.g. furfural and 5-hydroxymethylfurfural) in dilute acidic environment is
422 significantly less pronounced than in concentrated acidic conditions [13]. A study on optimised
423 dilute acid pretreatment (1.5% acid, 161 °C, 10 min) of fruit waste from the palm oil industry
424 reported 85% glucose conversion from biomass cellulose [115]. A bacteria-dilute acid
425 pretreatment strategy has also been studied on rice straw and showed enhanced lignocellulosic

426 digestibility [81]. The authors reported an increase of 70% and 244% in digestibility using this
427 strategy compared to dilute acid only pretreatment and untreated rice straw, respectively [81].
428 Acid pretreatment is also preferable for biogas production as the volatile inhibitors can be
429 converted to methane. Methanogens in anaerobic digestion process can tolerate a certain
430 concentration of furfural and 5-hydroxymethylfurfural [13]. However, sulphuric or nitric acids
431 are not recommended for anaerobic treatment as the reduction of sulphate and nitrate to H₂S
432 and N₂ respectively can hinder methane yield [13]. In general, acid pretreatment is a suitable
433 option for lignocellulosic biorefineries when high product yield within a short time frame (<
434 90 min) is the priority. However, there are on-going challenges in terms of environmental
435 impacts that hamper its application such as its toxicity (i.e. the necessity for chemical washing)
436 and corrosiveness (i.e. expensive resistant reactors) [105].

437 5.3. Ozone oxidation to promote delignification

438 Ozone is a promising reagent for the oxidation of lignocellulosic biomass due to its
439 selective reactivity with lignin [116]. Its powerful oxidizing property targets compounds with
440 functional groups with high electron densities such as lignin and overlook cellulose and
441 hemicellulose. Thus, no significant losses of carbohydrates occur and the sugar's accessibility
442 to enzymes and microbes is increased due to the destruction of lignocellulosic biomass
443 structure [116, 117]. This is the main advantage of ozone oxidation over some pretreatment
444 techniques (e.g. microbial pretreatment) which consume carbohydrates during the process.
445 Other advantages include no production of toxin residues, mild operating conditions (room
446 temperature and pressure), and easy on-site production (i.e. reduce transport cost, chemical
447 supply, and storage problems) [116, 45] (Fig. 3). These factors allow a promising valorisation
448 of completely lignocellulosic biomass through ozone oxidation to produce energy and
449 hemicellulose and lignin-derived products. On the contrary, ozone production requires high-
450 energy input (36 MJ/kg of ozone), and high dosages for pretreatment (e.g. 9 kg O₃/ton dry
451 biomass to produce 63 kg ethanol) [118, 119]. Some studies reported significantly higher ozone
452 dosages of 100-300 kg O₃/ton dry biomass to obtain 40-50% sugar yield [120, 121]. Thus, the
453 capability to minimize the energy input and ozone consumption is critical in making ozone
454 oxidation an economically viable and sustainable pretreatment process.



455

456 **Figure 3:** Schematic diagram of an ozone oxidation process for lignocellulosic biomass.

457 Studies have been conducted to explore the application of ozone oxidation in
 458 lignocellulosic biorefinery. It has been used to pretreat a wide range of lignocellulosic biomass
 459 to generate biogas, bioethanol, and biohydrogen (Table 6). The optimal moisture content of the
 460 substrate for effective ozone oxidation is about 40% [116]. High moisture content can block
 461 the pores on the biomass surface with thick water films and prevent ozone penetration. Whereas
 462 the diffusion of ozone in water to the substrate surface can be limited if the complex
 463 lignocellulosic biomass has low moisture content. Pretreatment time also has considerable
 464 influence on the performance of ozone oxidation. The inhibitory effect on dark fermentation of
 465 wheat straw was reported for prolonged ozone pretreatment (90 min) and caused lower
 466 biohydrogen yield than that of shorter ozone oxidation (45 min) [45]. Rosen et al. also reported
 467 reduced sugar conversion for long ozonation (6 h and beyond) compared to ozonation from 15
 468 to 90 min [119]. It is important to accommodate the optimal ozone operating parameters for
 469 different types of lignocellulosic biomass to achieve the best process efficiency.

470 **Table 6:** Selected examples of biofuel production from lignocellulosic biomass pretreated with
 471 ozone oxidation (MC: moisture content).

Product target	Substrate	Ozone pretreatment condition	Effects	Refs
----------------	-----------	------------------------------	---------	------

Biogas	Agave bagasse 0.60-0.70 mm 45% w/w MC	90 mg O ₃ /gTS 60 min 27 ± 2 °C	1.5-fold increase in sugar recovery Hydrolysate reached BMP of 219 mL CH ₄ /gCOD.	[122]
	Rice straw < 2 mm 40% w/w MC	35 ± 5 mg O ₃ /L 10 g O ₃ /h 90 min	134 mL/g TS cumulative biogas production	[123]
Bioethanol	Mixed municipal trimmings	15 min Room conditions	4-fold increase in saccharification efficiency vs non-ozonated sample	[119]
	Wheat straws	150 min Room conditions	3 to 3.5-fold increase in saccharification efficiency vs non-ozonated samples.	[124]
	Rye straws	conditions	Near negligible losses of cellulose.	
Biohydrogen	Wheat straw < 2 mm 40% w/w MC	4.94 mg O ₃ /g straw 45 min	158% increase in hydrogen production vs. non-ozonated sample	[45]

472

473 5.4. Novel green solvent-based pretreatment

474 Ionic liquids (ILs) have successfully emerged as the greener and recyclable organic
475 solvents for lignocellulosic biomass pretreatment due to its many advantages [125]. Ionic
476 liquids are natural organic salts constituting of anionic and cationic species and have a melting
477 point lower than 100 °C [67]. These salts are non-flammable, non-corrosive, non-toxic, have
478 very low vapour pressure, high viscosity, and high thermo-chemical stability [126]. Ionic
479 liquids are capable of dissolve cellulose, lignin and hemicellulose by breaking the linkages
480 among them and the highly ordered hydrogen bonds in cellulose fibers. This decreases
481 lignocellulosic crystallinity, facilitates enzyme access to cellulose and hemicellulose, and
482 enhances the bioconversion of lignocellulosic biomass [126, 127]. Smuga-Kogut et al. [128]
483 studied the impacts of Imidazolium-based ionic liquid [Emim][Cl] on rye straw under operating
484 conditions of 2 h, 120 °C and 1 mL/g of dry matter and recorded a three-time increase in sugar
485 yield compared to untreated rye [129]. Cornstalk pretreated with pyrrolidonium-based ionic
486 liquid at 90 °C for 30 min also achieved 86% lignin removal yielded 92% reducing sugar. In
487 addition, Brandt-Talbot et al. successfully recovered 99% of the utilised ionic liquid and reused
488 it four times in one of their studies. The current high cost of the process could be reduced by
489 the capability to maximise ionic liquid recovery and reuse as well as applying ionic liquids
490 with catalysts or co-solvents (e.g. dimethyl sulfoxide) to lower energy requirements [125, 130].
491 Baral and Shah [131] reported sugar production costs (\$/kg) from corn stover, switchgrass and
492 poplar pretreated with ionic liquids to be 2.7, 3.2, and 3.0, respectively. These figures can be

493 reduced to compete with acid pretreatment through the recovery of ionic liquids and waste heat
494 [131]. Ionic liquids have yet to be implemented at a pilot-scale plant, as the process still requires
495 further optimisation.

496 Deep eutectic solvents are another group of novel green solvents that was introduced as
497 promising alternatives to ionic liquids for lignocellulosic biomass pretreatment [132]. Deep
498 eutectic solvents are composed of two or three ionic compounds able of self-association to
499 form a eutectic mixture. They are mostly in fluid form and possess similar physicochemical
500 properties with ionic liquids, including the ability to solubilize lignin [133]. However, deep
501 eutectic solvents can be easily produced with nontoxicity and low cost, making it more
502 environmentally friendly and cost-effective than ionic liquid pretreatment [134, 135]. They
503 have shown great potential for bioconversion of lignocellulosic biomass in recent years based
504 on bench-scale experiments. Guo et al. [136] achieved a three-fold increase in sugar yield of
505 corncob pretreated with deep eutectic solvent system at 140 °C for 2 h. The liquids were also
506 reused for five recycled times without a significant effect on the enzymatic hydrolysis
507 efficiency of the corncob [136]. The recycling of deep eutectic solvents can increase its
508 economic efficiency for lignocellulosic biomass pretreatment. This process is still in its infancy
509 and requires more research to improve its technical and economic efficiency.

510 **6. Physical and physicochemical pretreatment**

511 Physical pretreatment of lignocellulosic biomass includes mechanical and irradiation-
512 based approaches. They both work towards reducing the particle size and increasing the surface
513 area of the biomass. This improves the digestibility of lignocellulose and its susceptibility to
514 enzymatic and microbial attacks [19]. Mechanical pretreatment such as milling and grinding
515 are highly effective in size reduction but showed lower efficiency in enhancing the product
516 yield compared to other pretreatments. This is because these techniques are not capable of
517 degrading lignin, the main polymer that forms recalcitrant lignocellulosic structure [1].
518 Additional delignification processes thus might be required and lead to an increase in the
519 overall cost. Microwave and ultrasound are commonly used for irradiation-based physical
520 pretreatment. Their properties (localized heating and pressurization) disrupt lignin and
521 hydrogen bonds within a short time, thus destructing cellulose crystallinity and increasing the
522 bioconversion of lignocellulosic biomass [1, 19]. However, these pretreatment techniques are
523 energy intensive (i.e. not sustainable) and require extreme conditions (high temperature and
524 pressure) which pose safety concerns and the potential formation of inhibitors (phenolic and
525 furfural). In general, mechanical pretreatment can be applied in combination or sequential order

526 with other pretreatment (e.g. hydrothermal, acid, or alkaline) to enhance process efficiency and
527 limit its disadvantages.

528 Hydrothermal pretreatment (i.e. hot water extraction) and steam explosion are
529 representatives of physicochemical pretreatment, which is a combined approach to breakdown
530 lignin-carbohydrate complex. Hydrothermal pretreatment uses water flow at elevated
531 temperature (150-300 °C) and pressure (0-60 bar) to disrupt the hydrogen bonds of
532 lignocellulosic biomass without the need for chemical addition [137]. Hemicellulose and lignin
533 start to solubilize at a temperature above 150 °C and 180 °C ,respectively, whereas cellulose
534 starts to decompose at a temperature above 230 °C [137, 138]. Similarly, steam explosion
535 pretreatment alters the structure of lignocellulosic biomass by subjecting the biomass to high
536 pressure (5-50 bar) steam at 160-270 °C for a specific period (few seconds to few minutes) [1,
537 19]. The sudden and rapid release of pressure once the process is completed causes the
538 autohydrolysis of the lignin-carbohydrate complex. The main hurdle of these pretreatments is
539 the cost required for water and energy supply to maintain high-temperature flow or steam.
540 Techno-economic analysis of biogas production with steam explosion pretreatment by Shafiei
541 et al. [139] reported that 46-58% of the total manufacturing cost (35 million USD) was
542 contributed to utility cost (i.e. consumption of high-pressure steam). An energy requirement of
543 0.7 kWh/kg biomass at 80% energy efficiency was also reported for the production in the same
544 study [139].

545 Hydrothermal and steam explosion pretreatment are eminent techniques to facilitate
546 bioconversion of lignocellulosic biomass. They have been investigated as the sole pretreatment
547 or in combination with other techniques for enhanced performance (Table 7). Pretreatments
548 that occurred at higher severity (> 150 °C) showed over 100% improvement in product yields
549 [140, 141]. This is due to the effective degradation of cellulose, hemicellulose, and lignin
550 through autohydrolysis. High temperature, however, promotes the formation of inhibitory
551 products such as furfural and 5-hydroxymethylfurfural [142]. It also increases the capital and
552 operational costs due to the necessity to uphold high temperatures in durable reactors and
553 additional processes to remove inhibitors. These are the key challenges in the scale-up of
554 hydrothermal processes. Using mild hydrothermal and steam explosion pretreatment (< 150
555 °C), hemicellulose is still readily degraded into xylose, and inhibitory products are limited
556 [143]. However, the minimal release of organic acids from lignocellulosic biomass restricts the
557 autohydrolysis of cellulose and lignin [144]. As a result, the improvement in product yield
558 after mild pretreatment is lower than that of pretreatment under high temperature (Table 7).

559 This suggests that a compromise among optimal conditions for effective lignocellulosic
 560 hydrolysis and low inhibitor formation is necessary to optimize the pretreatment process and
 561 maximise product yield. Both hydrothermal and steam explosion pretreatment have been
 562 implemented at pilot-scale level (Table 8). A pilot-scale bisabolene plant using
 563 hydrothermal/steam explosion pretreatment under 180 °C, 145 psi and 20 mins had gained 82%
 564 glucose conversion from wheat straw [9].

565 **Table 7:** Selected studies show the process efficiency of physicochemical pretreatment
 566 techniques for various lignocellulosic biomass.

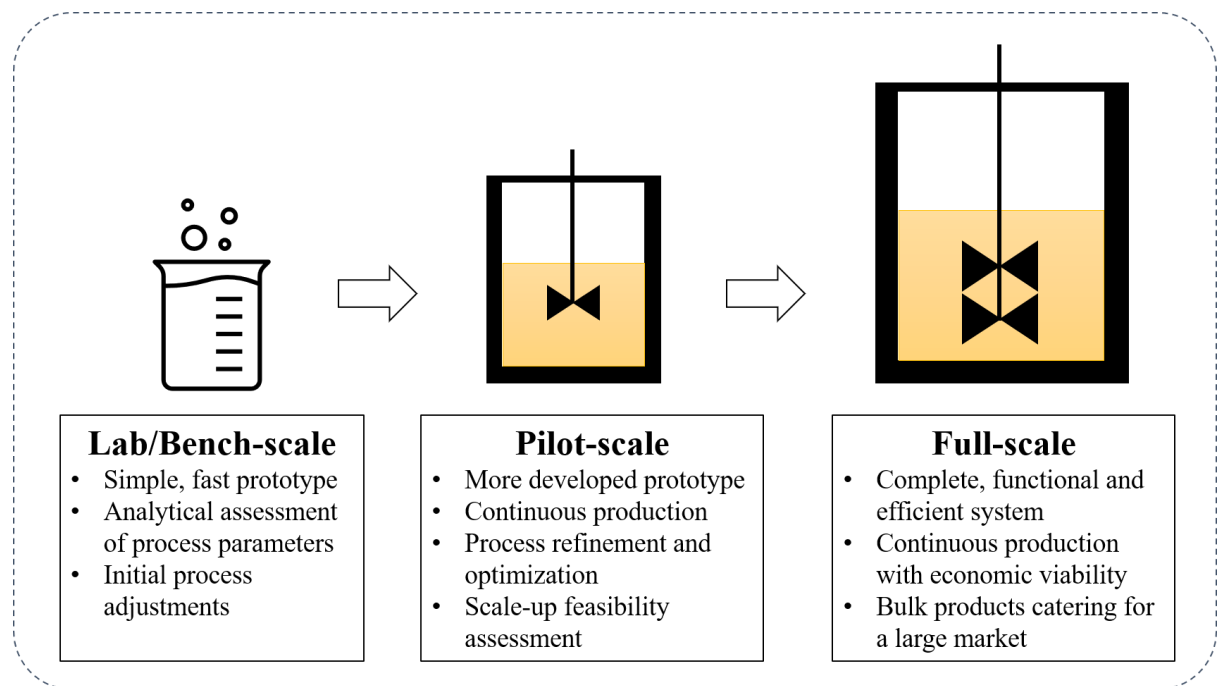
Pretreatment	Substrate	Product target	Operating conditions	Effects (versus untreated sample)	Reference (s)
Steam explosion	Reed biomass	Biogas	200 °C 34 bar 15 min	89% improvement in methane yield	[145]
	Rice straw	Biogas	280 °C 15 bar 10 min	147% improvement in methane yield	[140]
Hybrid organic solvent - steam explosion	Spruce biomass	Bioethanol	52% v/v ethanol 1% H ₂ SO ₄ w/w biomass 200 °C; 30 min	High ethanol concentration of 61.7 g/L	[146]
Hydrothermal	Safflower straw	Biogas	120 °C 60 min	70% improvement in methane yield	[143]
	Organic fraction of municipal solid waste	Bioethanol	160 °C 30 min	131% increase in glucose yield 141% increase in ethanol yield	[141]
Microwave-assisted hydrothermal	Brewer's spent grain (BSG)	Biobutanol	192.7 °C 5.4 min	Overall yield of 46 kg butanol/t BSG	[147]

567

568 7. Pretreatment practice at the pilot and full-scale level

569 The capability to perform pretreatment at a pilot-scale is essential to overcome techno-
 570 economic challenges and move from concept to full-scale implementation of the process (Fig.
 571 4). Most pretreatment studies are performed in small, batch-type reactors. While bench-scale
 572 experiments are effective in characterizing the effect of various operating conditions and
 573 parameters on pretreatment performance, the optimal conditions obtained from these
 574 experiments may not translate well to pilot and full-scale operation due to differences in heat
 575 and mass transfer characteristics [148]. Pilot-scale pretreatment process aims to provide
 576 reliable data for process scale-up in terms of equipment configuration and operating conditions

577 [9]. Since the products from pilot-scale reactors are highly similar to those of commercial
578 scales, evaluation of these materials for downstream processes will be useful for advancing
579 industrial biorefineries from lignocellulosic biomass.



580

581 **Figure 4:** The functionalities of each stage during a process scale-up.

582 Successful pilot and full-scale pretreatment systems for biofuel production from
583 lignocellulosic biomass have been reported (Table 8). These systems showed enhanced
584 lignocellulose degradation and improvement in sugar and biofuel yield. Ethanol concentration
585 of more than 40 g/L was achieved in some studies, which is minimum ethanol titers for the
586 process to become viable [149]. Do et al. has provided a conceptual process flow diagram of
587 the industrial bioethanol plant whichh consists of five main components: feed handling,
588 pretreatment and conditioning, saccharification and fermentation, product purification and
589 wastewater treatment [150]. Compared to liquid biofuel, biogas production has not attracted
590 many pilot or full-scale implementations despite its benefits. Due to necessary conditions for
591 microbial growth (long retention time and optimal temperature around 37 °C), biogas facilities
592 require large digester size (i.e. high capital cost) and often yield low gas production at cold
593 climate (i.e. low-cost efficiency). The development of advanced technologies is required to
594 simplify the process and make it abundant and cost effective. The detailed process designs of
595 a full-scale biogas plant implementing thermal-expansionary pretreatment have been provided
596 in a study by Kutsay et al. [151]. Their study suggested that the implementation of thermal
597 pretreatment had induced a 50% increase in biomethane production and allowed for effective

598 energy recycling and waste treatment within the plant [151]. Nonetheless, the success of existing
 599 systems provides promising outlooks for commercial biofuel productions from lignocellulosic,
 600 but further techno-economic analysis is still needed to validate their large-scale feasibility.

601 **Table 8:** Examples of pilot-scale systems for the pretreatment of lignocellulosic biomass.

Operation conditions	Feedstock & Desired product	Reactor	Effects of pretreatment	Ref.
Step 1: Acid pretreatment 1% w/w H ₂ SO ₄ 162 °C, 5 bar, 10 mins 10 kg/h rate Step 2: Enzymatic hydrolysis	Rice straw to Sugars for biorefinery	250 kg/day continuous horizontal reactor	- 87% decrease in hemicellulose content of dry biomass (converted to monomer sugars). - Enhanced sugar yield from 48 to 132 g/L	[152]
Acid pretreatment 0.26% w/w H ₂ SO ₄ 160 °C, 10 min	Deacetylated corn stover to Biofuel	1 ton/day horizontal reactor	- Total xylose yields after pretreatment is 100 g/L at 30% TS (i.e. 73.5% conversion from initial xylan concentration).	[148]
Step 1: Pretreatment 1st stage: 1% w/v H ₂ SO ₄ 121 °C, 30 mins 2nd stage: 3% w/v NaOH, 100 °C, 40 mins Step 2: Semi-simultaneous saccharification and fermentation (SFF)	Sugarcane bagasse to Bioethanol	80 L reactor	- 36% lignin degradation - 150 g/L glucose recovered after fed-batch enzymatic hydrolysis - 62 g/L of ethanol after 48 hours of SSF (i.e. ethanol productivity of 6.6 g/L/h)	[153]
Step 1: Hydrothermal pretreatment <i>Stage 1:</i> compression <i>Stage 2:</i> autohydrolysis <i>Stage 3:</i> steam explosion 180 °C, 145 psi, 20 min Step 2: Enzyme hydrolysis Step 3: Bisabolene fermentation	Agave bagasse (AB); Corn stover (CS); Sugarcane bagasse (SC); Wheat straw (WS) to Bisabolene	200 kg/d continuous tubular reactor	- Hemicellulose removal varied among biomass (e.g. 51% in SC, 20% in CS) - WS achieved the highest glucose conversion (82%); SC has the lowest (51%)	[9]

Step 1: Auto-catalysed steam explosion	Sugarcane bagasse to Sugars for biorefinery	65 L (80 kg/day) steam gun reactor	- 85% of hemicellulose was selectively solubilised	[154]
Step 2: Enzymatic hydrolysis			- An increase of 50.76% of ethanol/ton sugarcane bagasse is possible based on the results from this study	

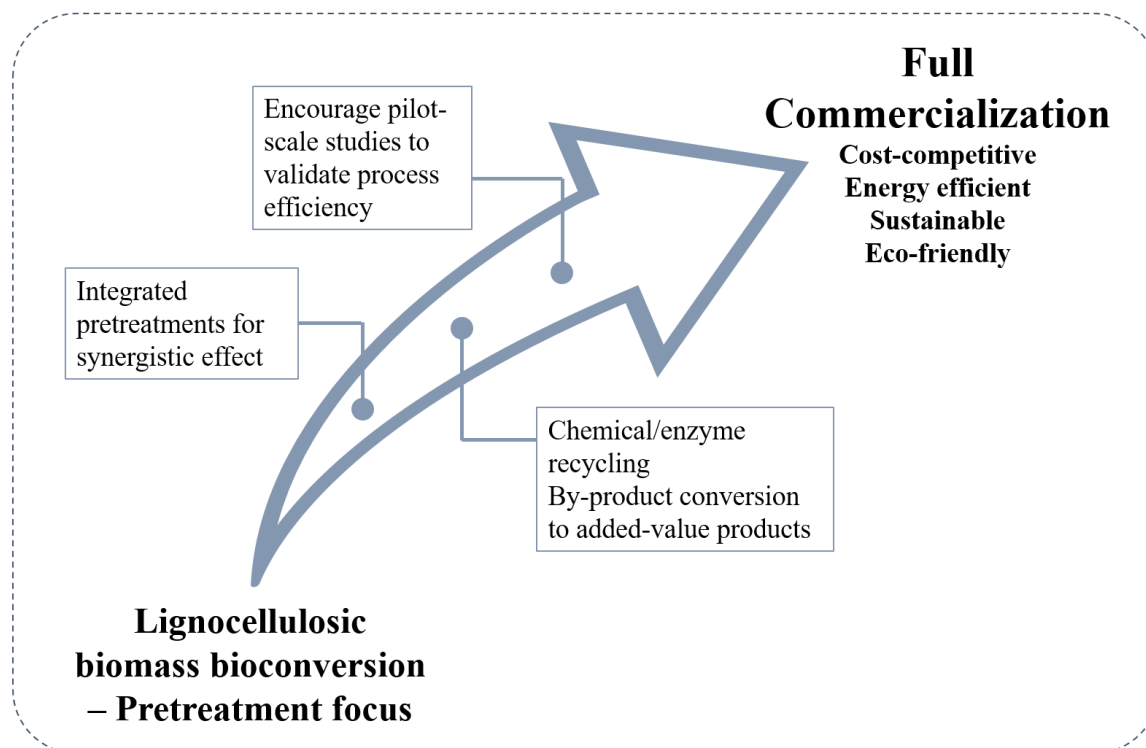
602 8. Research roadmap

603 The variability in the mechanisms and characteristics of current pretreatment techniques
604 makes it difficult to identify the best process. The choice of pretreatment depends on the desired
605 products, the type of feedstocks, and the advantages/disadvantages the process has to offer
606 (Table 9). The most vital considerations are the cost-effectiveness and environmental
607 sustainability of the pretreatment. These factors are often intertwined when considering the
608 process's energy consumption, chemical addition and water usage etc. It is also an important
609 hurdle to lignocellulosic biomass valorisation without government subsidy [19, 7]. Whether
610 the processes have been applied on industrial scales (e.g. steam explosion, acid/alkaline
611 pretreatment) or are novel and highly effective treatments (e.g. ionic liquids), there is still a
612 huge scope of improvement regarding the economic outlook [1, 126]. Efforts in developing
613 simpler and natural processes to reduce investment costs have been made (e.g. microbial and
614 enzymatic pretreatment). Besides, advancements in the capability to recover and reuse
615 chemicals and by-products from the pretreatment of lignocellulosic biomass are a great
616 approach to enhance cost benefits (Fig. 4). For example, recycling fungal enzymes used for
617 degrading lignin in lignocellulosic biomass can reduce the overall process expenditure as it is
618 costly to extract enzymes from fungi (Table 9). The effects of inhibitors such as phenolics,
619 weak acids and furfurals in acid, irradiation and ionic liquid pretreatment can also be mitigated
620 through removal strategies. Available technologies include evaporation, membrane, ionic
621 resins and biochar, etc. [126]. The removed inhibitors such as acetic acids are important
622 chemical reagents and suitable for recycling to improve the process sustainability. The
623 development of effective pretreatment and advanced detoxification process will contribute to
624 facilitating the full commercialisation of lignocellulosic biorefinery.

625 **Table 9:** The characteristics of currently lignocellulosic pretreatment techniques.

Pretreatment	Advantages	Disadvantages	Process optimisation
Microorganisms (Fungi and bacteria)	Low carbon footprint No chemical addition	Time consuming	In-storage pretreatment of wet biomass

	Low cost, low energy Selectively degrade lignin	Loss of carbohydrates Strict microbial growth conditions	providing year-long delignification (ensiling)
Ligninolytic enzymes	Selective lignin degradation Minimal inhibitors and toxins	High extraction and purification cost	Recycling of enzymes after pretreatment to reduce cost
Alkali	High rate of delignification Mild operating conditions	High cost Time consuming Conversion of alkali into irreversible salts	Recycling of alkali through washing steps Combined with hydrothermal processes
Acids	Effectively solubilize hemicellulose and lignin Short processing time	High chemical cost Expensive reactors Corrosive Inhibitor formation	Recycling of acids Inhibitors (e.g. acetic acids) can be converted to valuable products. Combined with steam explosion.
Ozone oxidation	Room conditions Low inhibitory formation Short processing time	Corrosive, flammable, toxic High energy demand for ozone production High dose	Combined with other pretreatments to reduce ozone consumption e.g. aqueous ammonia
Ionic liquids & Deep eutectic solvents	Eco-friendly, fast No hazardous by-products High solubilisation level of lignocellulose	High cost Large volume of liquids Inhibitory effect on hydrolytic enzymes	Recycling of liquids/solvents Combined with co-solvent to lower energy demand
Mechanical (e.g. milling, grinding)	Biomass size reduction Reduce crystallinity	Cannot degrade lignin Less effective in increase sugar yield Energy intensive	Combined with other pretreatments to enhance efficiency
Irradiation (e.g. microwave and ultrasound)	Disrupt lignocellulosic structure Short processing time	Energy intensive High temperature and pressure Inhibitor formation	Combined with other pretreatments to enhance efficiency
Hydrothermal (e.g. hot liquid, steam explosion)	Highly effective No chemical addition Short processing time	High water and energy demand Expensive reactors Inhibitor formation	Combined with microwave to reduce utility usage



626

627 **Figure 4:** Research roadmap to advance from research-based bioconversion of
 628 lignocellulosic biomass to full commercialization.

629 The performance of combining different techniques for lignocellulosic pretreatment has
 630 been studied to increase sugar yields and the overall process feasibility [29, 55, 81]. The
 631 advantages of each technique are complemented when operating in combination with each
 632 other, thus inducing a synergistic effect on the conversion of lignocellulosic biomass. However,
 633 due to the variability and complexity of lignocellulosic biomass structure, it is difficult to
 634 determine a universal combination of techniques that works effectively on most biomass.
 635 Moreover, the inclusion of multiple pretreatment techniques could increase the cost due to the
 636 additional equipment or chemicals needed. The success in identifying suitable integration of
 637 pretreatment techniques with minimal energy and resource input will contribute to the
 638 commercialization of biorefinery processes using lignocellulosic biomass (Fig. 4).

639 **9. Conclusion**

640 Full-scale conversion of lignocellulosic biomass into valuable products is currently limited
 641 due to its relatively high investment and operational cost. This problem is attributed to the
 642 inherent recalcitrant nature of lignocellulose that prevents enzyme penetration and microbial
 643 attack. Data and information corroborated in this review show that pretreatment is a necessary
 644 stage to overcome this challenge by enhancing the bioconversion of lignocellulosic biomass

645 into monomer sugars. Pretreatment techniques, however, possess both advantages and
646 disadvantages. Chemical and physicochemical pretreatment are effective (i.e. over 100%
647 improvement in sugar yields) but constrained by the high investments and environmental
648 impacts (e.g. expensive reactors, harsh chemicals such as acid, high water and energy
649 consumption) and the formation of inhibitors. The more eco-friendly and simpler process such
650 as microbial pretreatment is time consuming (15-40 days) thus reducing cost-effectiveness.
651 The successful development of universal cost-effective and sustainable pretreatment
652 techniques (single or combination) will facilitate the efficient biorefineries of lignocellulosic
653 biomass. The recovery of chemicals, enzymes, and by-products for reuse or conversion to
654 valuable products will also increase the revenues and reduce wastes. Besides, the capability to
655 scale-up the conversion system to a pilot or full-scale level, including a pretreatment step, will
656 validate the feasibility of the commercial applications.

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