

Fungal and enzymatic pretreatments in hot-pressed lignocellulosic bio-composites: A critical review

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ABSTRACT

Fungal and enzymatic pre-treatments have long been used to activate the surface of lignocellulosic biomass particles or fibers to promote adhesion or develop novel composite materials and other products. Research into mycelium-based bio-composites, i.e. products in which constituents are bonded by fungal mycelium, is quite new and still growing. There has also been a significant amount of attention given to these materials by the industry and private sector. These bio-composites can be produced by enzymatically or fungally treating lignocellulosic substrates and then either molding them into a shape and letting the fungus grow or, alternatively, by hot-pressing the substrate into a panel product. This review article focuses on hot-pressed, higher density lignocellulosic bio-composites produced following enzymatic or fungal pretreatment. The definitions, raw materials, processing procedures, material properties, factors governing product properties, and potential adhesion mechanisms are summarized and discussed. Finally, current commercial products are introduced and roadblocks in the way of further development are presented along with the identification of knowledge gaps.

1. Introduction

The use of nature-based materials and, more specifically, biomass instead of petroleum-derived products is a prospective solution for sustainability and environmental issues faced by industries and humankind. From various sources of biomass available worldwide, including agricultural and forestry residues, animal residues, sewage, industrial residues, municipal solid waste, algae and aquatic crops (Tursi, 2019), the use of forestry and agricultural residues has become more convenient due to existing infrastructure and recent developments in the bio-composite industry. In the composites industry, lignocellulosic and fungal mycelium bio-composites are both promising candidates for replacing petroleum-derived products because they are made from natural, renewable, and biodegradable sources. Although the former (represented primarily by wood-based composites) have a longer history and are generally more available on commercial markets (Hemmilä et al., 2017; Rowell, 1992), the latter are an emerging class of

novel materials that has sparked significant research and commercialization interest since their introduction about ten years ago (Elsacker et al., 2020; Meyer et al., 2020).

Lignocellulosic bio-composites are manufactured from strands, flakes, particles, or fibers of wood or agricultural residues which are bonded together with a type of adhesive, conventionally in the form of panels (Fig. 1A). Woody biomass is the most traditionally used raw material in the lignocellulosic bio-composite industry, whereas composites based on non-wood fast-growing biomass feedstock were developed in response to the shortage of wood supply (Chaturvedi and Verma, 2013; Tye et al., 2016) and regional availability. Differences in properties, functions, and particle form yield categories such as insulation board, particleboard, fiberboard, oriented strand board (OSB), and plywood (Suchsland and Woodson, 1987) (Fig. 1). In 2019, over 355 million m³ of these products were produced from forest resources globally (FAO, 2020) for use in many applications, particularly in the building and construction sector either as interior or exterior components.

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Abbreviations

CNF	cellulose nanofibrils
DSC	differential scanning calorimetry
FSP	fiber saturation point
GPC	gel permeation chromatography
IB	internal bond strength
Lac	laccase
LiP	lignin peroxidase
MDF	medium-density fiberboard
MnP	manganese peroxidase
MOE	modulus of elasticity
MOR	modulus of rupture
OSB	oriented strand board
SEM	scanning electron microscopy
TS	thickness swelling
TSR	<i>triarrhena sacchariflora</i> residue
VP	versatile peroxidase
WA	water absorption
XPS	X-ray photoelectron spectroscopy

To bond reconstituted lignocellulosic biomass, adhesives are usually applied to the furnish and are cured under heat and pressure. The dominant adhesives in lignocellulosic bio-composite manufacturing are formaldehyde and isocyanate-based synthetic resins, which raise environmental and health concerns and are typically not bio-based (EPA, 2017; Mantanis et al., 2018; Solt et al., 2019). There is a perceived consumer desire for completely bio-based alternatives, reflected in the commitment of IKEA, one of the world's largest furniture retailers, to achieve 40% natural raw materials for all their adhesives by 2025 and 80% by 2030 (Bruck, 2017).

Much effort has been devoted to replacing formaldehyde-based adhesives with bio-based adhesives (Ferdosian et al., 2017; He, 2017) or developing binder-less lignocellulosic bio-composites (Hubbe et al., 2017; Pintiaux et al., 2015). Additional benefits may be achieved if lignocellulosic composites can be produced in a binder-less manner as opposed to those made with bio-based adhesives. In the quest for adhesion promotion, pretreatments are often applied to the raw materials to “activate” their surfaces either physically, i.e. making already available surfaces more accessible (Pelaez-Samaniego et al., 2013), or

chemically, i.e. adding additional functional groups to the surface (Saha et al., 2015; Widsten and Kandelbauer, 2008). Conventional activation methods such as steam explosion, acid, and alkali pretreatment usually require large inputs of energy and cause pollution. For this reason, biological pretreatments are expected to be generally a milder, safer, and more environmentally friendly alternative (Maurya et al., 2015; Shirkavand et al., 2016). These processes typically require the “activation” of lignocellulosic raw materials by fungi or enzymes derived from fungi before hot pressing.

The concept of “mycelium-based bio-composites” (Fig. 1B), or products where constituents are bonded by fungal mycelium, is quite new but has received significant research and industrial attention. The most well-studied and commercialized mycelium-based products are as-grown mycelium-based composites (Elsacker et al., 2020; Jones et al., 2020). Typically, their manufacturing starts with a low-cost lignocellulosic biomass substrate. Then the mycelia of filamentous fungi are added to a mixture of the substrate and nutrients in a mold with a predetermined shape where the mycelium partially digests and adheres to the surface of the substrate particles. As they grow, the mycelia form entangled networks and bind the substrate particles into a whole aggregation. In both industry and research labs, oven drying is often used to inactivate fungal growth and to produce the final product (Elsacker et al., 2020). Fully processed composites typically have a density range of 0.06–0.30 g m⁻³ (Jones et al., 2020), depending on the substrate material. More recently, a growing number of research projects have focused on developing mycelium-based bio-composites at higher density. One attempt involved adding pressure during the growth step (Bajwa et al., 2017; Pelletier et al., 2017). Others mainly densified the composites by applying heat and pressure afterward (Liu et al., 2019; Sun et al., 2019).

It is not difficult to notice that the predominant component in this “novel mycelium bio-composite” is still lignocellulosic biomass. In fact, the weight percentage of fungal biomass in the system is usually very low (e.g. around 5% for 7-day growth of *Trametes (Coriolus) versicolor* on wheat grains, according to Jones et al. (2019)). It is therefore reasonable to categorize them in the same class as traditional lignocellulosic bio-composites, particularly in the case of hot-pressed products where the mycelium development and enzyme secretion during fungal growth are the same as the fungal or enzymatic pretreatment in lignocellulosic bio-composite manufacturing. The only difference between these cases is that in mycelium bioproducts the mycelia themselves act as the binder.

Multiple reviews focusing on binder-less lignocellulosic bio-

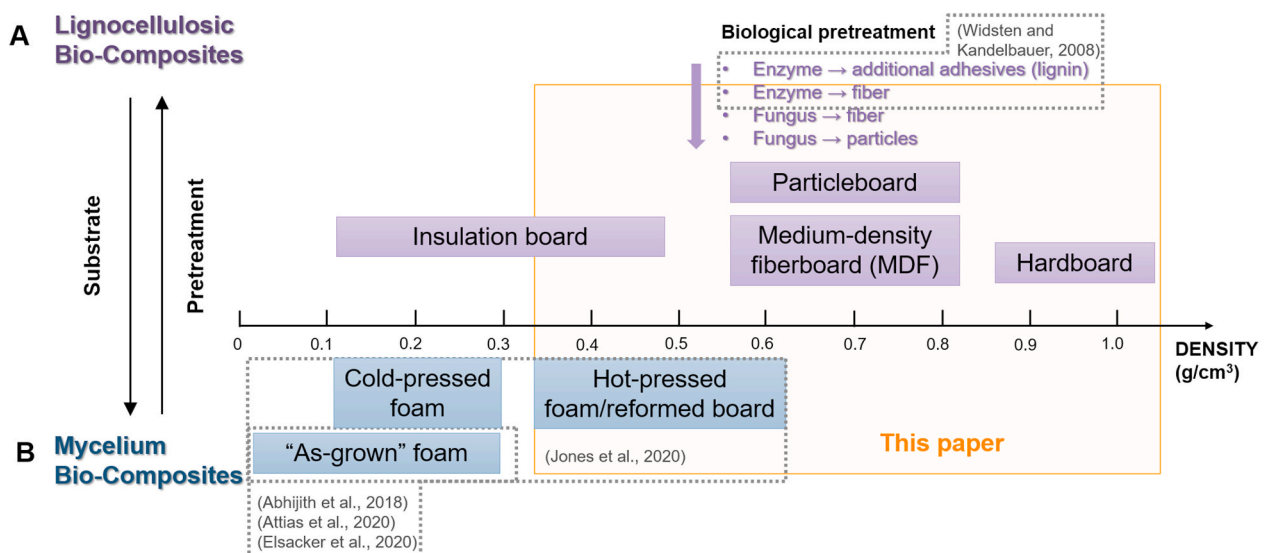


Fig. 1. Classifications of common lignocellulosic (A) and mycelium (B) bio-composites, their relationships and the scope of this paper.

composites have been published over the past 20 years (Hubbe et al., 2017; Nasir et al., 2019; Pintiaux et al., 2015; Zhang et al., 2015). Most of these publications also include small sections on enzymatic activation of lignin, a major component of any lignocellulosic material. Widsten and Kandelbauer (2008) specifically summarized the adhesion improvement of lignocellulosic bio-composites by enzymatic pretreatment. Their two main topics were the oxidation of wood surface lignin before hot-pressing of fiberboard and the use of enzymatically pretreated lignin as adhesive in particleboard production. Since then, more research articles on enzymatic pretreatment have been published with explorations extended into binder-less particleboard and direct use of fungi for pretreatment. Therefore, the present review covers the latest literature in the field since the last reviews were published. For the “mycelium bio-composites”, recent progress has been reviewed by several research groups in the past two years covering manufacturing processes, material properties, applications, and life cycle assessment (Abhijith et al., 2018; Attias et al., 2020; Elsacker et al., 2020; Girometta et al., 2019; Jones et al., 2020). However, there has been a lack of attention paid to the higher-density category (density above 0.30 g m⁻³), particularly how higher-density hot-pressed panels compare to biologically pretreated traditional lignocellulosic bio-composites. The present review covers this gap.

In view of the intrinsic similarities of lignocellulosic and mycelium bio-composites, the objective of this paper is to be the first to consider them both part of the same category, hot-pressed higher-density bio-composites, and summarize literature with that classification in mind (Fig. 1). The discussion will focus on the changes in lignocellulosic biomass after fungal and enzymatic pretreatments and their possible links to specific adhesion mechanisms. Finally, current industrial developments, limitation and roadblocks will be presented. In Section 2, we will introduce raw materials and production systems, while Section 3 will focus on the properties of the composites as affected by processing and other relevant factors. Section 4 will delve into the current understanding of relevant adhesion mechanisms involved and, finally, Section 5 will provide information on unresolved issues and commercial limitations.

2. Raw materials and production procedure

2.1. Lignocellulosic biomass

For hot-pressed bio-composites, it is better for the initial density of the biomass to be closer to the density of the final product. Lower-density biomass would require more compression which the manufacturers typically do not prefer; higher-density biomass would need less but would leave air in the structure (Hubbe et al., 2017). Woody biomass has an advantage over non-wood biomass as it has higher bulk density and is more abundant and available year-round (Suchsland and Woodson, 1987). In addition, as woody biomass can also be sourced from primary wood processing facilities such as sawmills, there is an established collection, storage, and end-use application for these materials.

To take advantage of enzymes added to promote bonding, the surface chemistry of the biomass is important. In particular, lignin radicals that are created by enzymes are believed to form covalent bonds between lignocellulosic biomass particles during hot pressing (Widsten and Kandelbauer, 2008). Fibers used for the production of fiberboard usually carry more water-extractable aromatic lignin on their surface because of the fiberizing process, which is more beneficial for the reactivity of the enzyme laccase (Suchsland and Woodson, 1987; Widsten, 2002). These details are particularly critical in the use of living fungi to promote bonding, as the composition and complexity of the carbon sources greatly influence the growth behavior and properties of fungi feeding on them (Ahmed et al., 2020; Antinori et al., 2020).

For the reasons outlined above and many more, different substrates may find optimal pairing with different fungi or enzymes for the most efficient surface activation as well as the most suitable application for the resulting product. For example, it was found that *Pleurotus ostreatus* grew faster in wheat bran and sugarcane than in sawdust (Joshi et al., 2020). As shown in Table 1, higher density woody substrates are generally more favored in producing medium-density fiberboard, while lower density agricultural plants are more often used in low-density particleboard production. A range of densities from 0.35 g/cm³ to 1.2 g/cm³ has been reported.

2.2. Selection of fungi and enzymes

2.2.1. Fungi

Among different lignocellulosic-degrading microorganisms, white-rot fungi are often favored in pretreatments in lignocellulosic bio-composite manufacturing. The most commonly used white-rot fungi (*Trametes* spp, *Pleurotus* spp, and *Ganoderma* spp) are listed in Table 1. The frequent use of white-rot fungi is attributed to their three outstanding features: first, for more traditional fiberboard and particleboard manufacturing, lignin oxidative enzymes that are secreted at the initial stage of white-rot colonization are believed to activate the surface by creating lignin radicals that improve bonding during hot-pressing (Fig. 2) (de Paula et al., 2019; Narayanaswamy et al., 2013; Wesenberg et al., 2003). Second, white-rot fungi also have a tendency to grow elongated mycelial networks which bind the substrates mechanically and are particularly preferred for as-grown low-density mycelium bio-composites (Abhijith et al., 2018; Sauerwein et al., 2017). Finally, in contrast to brown rot, white rot does little damage to the mechanical integrity of the wood substrate at early stages of decay. For example, at a mass loss of 4%, brown rot can cause up to 60–70% loss of impact bending strength in hardwoods while white rot only causes 44% reduction (Wilcox, 1978; Zabel and Morrell, 2020).

To activate lignin and hemicellulose while maintaining the strength of the biomass particle, a selective white-rot fungus is preferable as it will degrade lignin and hemicellulose and will leave cellulose, the component that provides most of the strength, mostly unmodified. In contrast, non-selective white-rot fungi, which degrade all major wood constituents at similar rates (Fackler et al., 2007; Watanabe et al., 2007), are not favorable. However, the definition of “selective fungi” is ambiguous and sometimes contradictory in the literature. For instance, *Trametes (Coriolus) versicolor* has been defined as both a selective and non-selective fungus in different reports (Bhatt et al., 2016; Fackler et al., 2011; Nadir et al., 2019). It appears that the selectivity of different fungi varies with the type of lignocellulosic biomass and the pretreatment time (Wan and Li, 2012; Zhang et al., 2007). In other words, “non-selective fungi” are usually defined based on a long degradation period (weeks to months) in the traditional wood deterioration field. However, they also have a “selective” stage of only degrading hemicelluloses and lignin at the very beginning of colonization. For this reason, “non-selective” fungi should not be entirely excluded.

The ability to be selective appears to differ among fungal strains and also when these strains are applied to different substrates. Wu et al. (2016) compared three lignocellulosic biomasses pretreated by five white-rot fungi and revealed that only *Trametes (Coriolus) versicolor*-treated *Triarrhena sacchariflora* residue could be further produced into fiberboard with no apparent issues. Jones et al. (2019) compared the growth of *Trametes (Coriolus) versicolor* and *Polyporus brumalis* on different lignocellulosic substrates (wheat grains, wheat straw, rice hulls, and sugarcane bagasse) and reported that only wheat grains could result in sufficient growth. The other characteristic difference leading to more attention to white-rot fungi is the hyphal extension and growth density, which is mainly important for as-grown products. Jones et al.

Table 1
Summary of fungal/enzymatic pretreated hot-pressed lignocellulosic bio-composites studies.

Biological pretreatment		Substrate and pretreatment time		Hot-pressing condition			Final product type and properties								Reference
Fungal/ Enzymatic	Type	Species	Time	Pre-processing, Moisture content	t, min	T, °C	Type	ρ , g/cm ³	Thickness, mm	MOR ^b , MPa	MOE ^c , MPa	IB ^d , MPa	24 h TS ^e , %	24 h WA ^f , %	
Enzymatic	Laccase	Beech	1 h	Cold pressed (33 %MC)	5	180	Fiberboard	1.04	3	44.6	3360	1.55	57 ^a	72	Felby et al. (1997)
Enzymatic	Laccase	Beech	1 h	Forced air dried, 40 °C, 18 h, 12% MC	5	200	Fiberboard	0.90	3	41.7	4020	1.57	19 ^a	72	Felby et al. (1997)
Enzymatic	Laccase	Mixed ^g	12 h	De-watered	5	190	Fiberboard	0.78	5.4	–	–	0.95	21 ^a	–	Kharazipour et al. (1997)
Enzymatic	Laccase	Mixed ^g	12 h	Dried at 50 °C (20–25% MC	5	190	Fiberboard	0.77	6.0	–	–	0.59	22 ^a	–	Kharazipour et al. (1997)
Enzymatic	Peroxidase	Mixed ^g	4 h	De-watered	5	190	Fiberboard	0.8	5	41.7	4020	0.63	28 ^a	–	Kharazipour et al. (1998)
Enzymatic	Laccase	Rape straw	–	Dry	–	–	Fiberboard	0.8	–	13 ^a	–	–	50 ^a	–	Unbehaun et al. (2000)
Enzymatic	<i>Trametes versicolor</i> medium	Rape straw	–	Dry	–	–	Fiberboard	0.8	–	20	–	–	50 ^a	–	Unbehaun et al. (2000)
Enzymatic	Laccase	Beech	0.5 h	11–13%	3.4	200	Fiberboard	0.87	8	46.0	3950	0.93	46 ^a	92	Felby et al. (2002)
Enzymatic	Laccase	Aspen	–	7%	5	170	Fiberboard	0.75	12	16.1	2170	1.02	33 ^a	–	Widsten et al. (2003)
Enzymatic	Laccase	Birch	–	6%	4.7	190	Fiberboard	0.71	12	19.8	2730	0.86	39 ^a	–	Widsten et al. (2003)
Enzymatic	Laccase	Spruce	–	7%	4.1	170	Fiberboard	0.91	12	23.5	3700	1.26	39 ^a	–	Widsten et al. (2004)
Enzymatic	Laccase	Plantain plants	1 h	Dried at 40 °C, 24 h	8	200	Fiberboard	1.1	3	18.7	3600	–	30 ^a	81	Álvarez et al. (2010)
Enzymatic	Laccase	Pine wood	20–30 min	Dried at 120 °C, 10%	–	200	Fiberboard	0.8	10	20	–	0.39	65 ^a	–	Euring, M. et al. (2011)
Fungal	<i>Trametes hirsute</i>	Corn stalk	21 d	Fresh, wet	5	170	Fiberboard	0.39	3	2.6 ^a	413 ^a	–	–	–	Wu et al. (2011)
Fungal	N/A ^h	Coconut fibers	7 d	Dry	5	165	Fiberboard	–	–	10.9 ^a	2784	–	–	–	Lokko (2016)
Fungal	<i>Trametes (Coriolus) versicolor</i>	TSR ⁱ	14 d	Fresh after incubation	8	185	Fiberboard	0.91	3	18.1	4300	–	–	–	Wu et al. (2016)
Enzymatic	Laccase	Mixed ^j	120 min	13%	15	170	Fiberboard	1	5	20.8	2944	0.25	–	–	Yang et al. (2017)
Enzymatic	Laccase + xylanase	Bamboo	2 h	14%	10	200	Particleboard	0.7	10	33.7	6100	0.77	–	–	Song et al. (2018)
Fungal	<i>Trametes multicolor</i>	Rapeseed straw	24 d	Fresh, wet	20	150	Particleboard	0.35	8.8	0.86 ^a	80 ^a	–	–	–	Appels et al. (2019)
Fungal	<i>Pleurotus ostreatus</i>	Cotton fibers	14 d	Fresh, wet	20	150	Particleboard	0.35	8.0	0.87 ^a	34 ^a	–	–	–	Appels et al. (2019)
Fungal	<i>Pleurotus ostreatus</i>	Rapeseed straw	14 d	Fresh, wet	20	150	Particleboard	0.39	9.5	0.62 ^a	72 ^a	–	–	–	Appels et al. (2019)
Fungal	<i>Ganoderma lucidum</i>	Cotton stalk	14 d	Dried at 65 °C, 10 h	7	200	Particleboard	0.6	12	4.6	680	0.18	–	–	Liu et al. (2019)
Fungal	N/A ^h	SPF ^k	N/A	Dried at 43 °C, re-immersed in water, 62%	5	180	Particleboard	0.6	9.4	1.46 ^a	224 ^a	–	70	158	Sun et al. (2019)
Fungal	<i>Ganoderma lucidum</i>	Rubberwood sawdust	N/A	Sun dried, blended into powder	20	160	Particleboard	1.2	–	–	–	2.5	3.1	–	Khoo et al. (2020)
Fungal	<i>Ganoderma lucidum</i>	cotton stalk	14 d	Dried at 65 °C, 10 h first; re-immersed in water; 30%	7	200	Particleboard	0.6	12	6.0	860	0.34	–	–	Liu et al. (2020)

(continued on next page)

Table 1 (continued)

Biological pretreatment		Substrate and pretreatment time		Hot-pressing condition		Final product type and properties							Reference		
Fungal/ Enzymatic	Type	Species	Time	Pre-processing, Moisture content	t _p , min	T, °C	Type	ρ, g/cm ³	Thickness, mm	MOR ^b , MPa	MOE ^c , MPa	IB ^d , MPa	24 h TS ^e , %	24 h WA ^f , %	
Fungal	<i>Pleurotus ostreatus</i>	Rubberwood sawdust	45 d	Fresh after incubation	40	130	Particleboard	0.80	–	3.9	–	–	–	–	Shakir et al. (2020)
Fungal	<i>Trametes (Coriolus) versicolor</i>	Poplar	21 d	Fresh after incubation, 65%	10	170	Fiberboard	0.92	3	22.7	–	–	12	–	Wu et al. (2020)
Enzymatic	Laccase	Poplar	4 h	Fresh after incubation, 65%	10	170	Fiberboard	0.92	3	19.6	–	–	–	–	Wu et al. (2020)

The best property results and the corresponding conditions from different papers are presented in this table.

^a Values that lower than the required values according to American National Standard values (minimum) of particleboard (ANSI A208.1–2016) and medium-density fiberboard (MDF) (ANSI A208.1–2016) for interior applications.

^b MOR: modulus of rupture (Typical standards: ASTM D1037, ISO 16893, and GB/T 4897).

^c MOE: modulus of elasticity (Typical standards: ASTM D1037, ISO 16893, and GB/T 4897).

^d IB: internal bond strength (Typical standards: ASTM D1037, ISO 16893, and GB/T 4897).

^e TS: thickness swelling (Typical standards: ASTM D1037, ISO 16893, and GB/T 4897).

^f WA: water absorption (Typical standards: ASTM D1037, ISO 16893, and GB/T 4897).

^g Commercially produced wood fibers: 80% softwood (spruce and pine) and 20% hardwood (beech).

^h Provided from Ecovative Design LLC (Green Island, NY, USA).

ⁱ TSR: Triarthra sacchariflora residue.

^j 80% wheat straw fibers and 20% bamboo fibers.

^k SPF: commercially available mixture of spruce, pine, and fir.

(2018) investigated the mycelium growth performance of various traditional and non-traditional fungal species and concluded that trimitic (presenting three types of hyphae) and dimitic (presenting two types of hyphae) species that have skeletal hyphae presented higher hyphal extension rates than those that only presented one type of hyphae. In this work, *Trametes (Coriolus) versicolor* and *Polyporus brumalis* were selected as the highest-performing species for further applications.

2.2.2. Enzymes

The selection of enzymes is much more straightforward than the selection of fungal species for several reasons: first, because there are fewer types of enzymes than species of fungi; second, because enzymes are very specific in terms of their target functionality; and finally, because unlike fungal hyphae, enzymes themselves are too small to affect other properties of the bio-composite. Enzymes are believed to activate the surface of the biomass by breaking down molecules and generating more reaction sites such as radicals. There are four main lignin oxidative enzymes produced by white-rot fungi: laccase, manganese peroxidase (MnP), lignin peroxidase (LiP), and versatile peroxidase (VP) (Fig. 2) (Asina et al., 2017; Janusz et al., 2017). The enzymes are able to cleave both phenolic and non-phenolic moieties of the lignin structure. Among these, laccase has the longest utilization and application history because it directly uses O₂ in the reaction process (Komal et al., 2018; Slagman et al., 2018). There are also hydrolytic enzymes in the white-rot degradation system, including cellulases and hemicellulases, which digest carbon sources for fungal growth and metabolism (Julia et al., 2016; Schimpf and Schulz, 2016). Because the cellulases would cause the loss of cellulose, which negatively influences the mechanical properties of the final product and is therefore undesirable (Sisti et al., 2018), only hemicellulases, such as xylanases, have received much attention in the literature (Song et al., 2018).

As shown in Table 1, only four types of enzymes and fungi have been investigated in the fabrication of hot-pressed, higher-density lignocellulosic boards. However, in the field of bio-pretreatment for the production of biofuels (Narayanaswamy et al., 2013; Tian et al., 2012; Zhang et al., 2007) and ruminant feed ingredients (van Kuijk et al., 2015), many more combinations have been explored. This body of work may provide some useful information for bio-composite production as well, as it is evident that there might be many more fungal species that remain unexplored.

2.3. Production procedure

The production procedure for fungal or enzymatic pretreatment of hot-pressed lignocellulosic bio-composites is shown in Fig. 3, using particleboard as an example. The basic procedure includes three steps: 1) fungal or enzymatic incubation; 2) preparation for hot-pressing, and 3) hot-pressing.

2.3.1. Fungal and enzymatic incubation

As shown in Table 1, fungal incubation usually takes a much longer time than enzymatic incubation (days vs. hours) and requires an additional decontamination step for lignocellulosic biomass to prevent contamination and growth of unwanted microorganisms (Elsacker et al., 2020). Ground mycelial tissue is mixed with the substrate and the entire mixture is packed in containers or molds before being incubated in a controlled environment (20–30 °C, dark, humid, with forced ventilation for larger volumes). To accelerate the colonization of fungi, a solution of nutrients may also be added to the mixture. Although enzymes come from fungi, the ones used in research are usually purchased from commercially available sources while in industry they usually are generated by the fungi on site. The enzymes are usually in solutions and are either mixed with or sprayed onto the biomass. The incubation is carried out at a set pH and temperature and generally lasts from 30 min to several hours (Table 1).

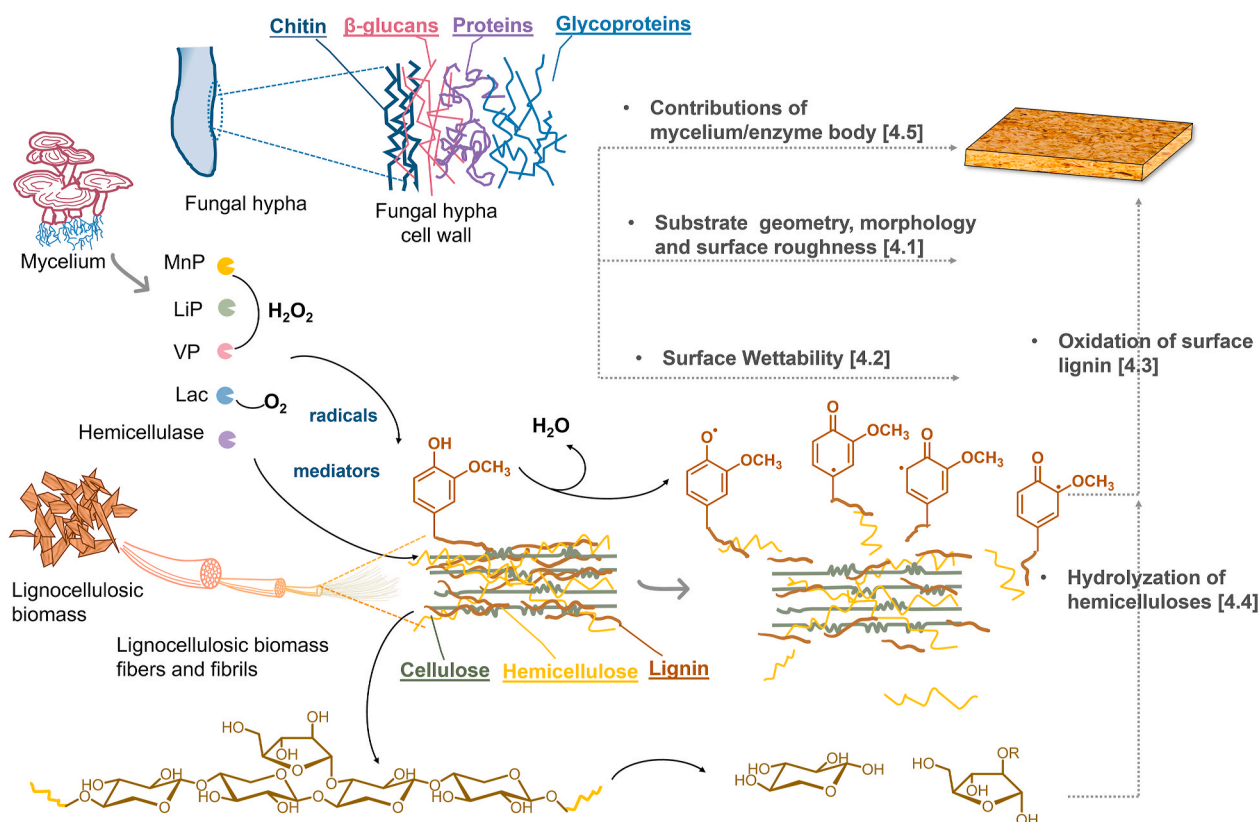


Fig. 2. Schematic structure of lignocellulosic biomass and fungal hyphae cell walls, changes happening during fungal and enzymatic pretreatments and possible corresponding mechanisms. MnP: manganese peroxidase; LiP: lignin peroxidase; VP: versatile peroxidase; Lac: laccase. The numbers in brackets correspond to the subsection headings in the paper.

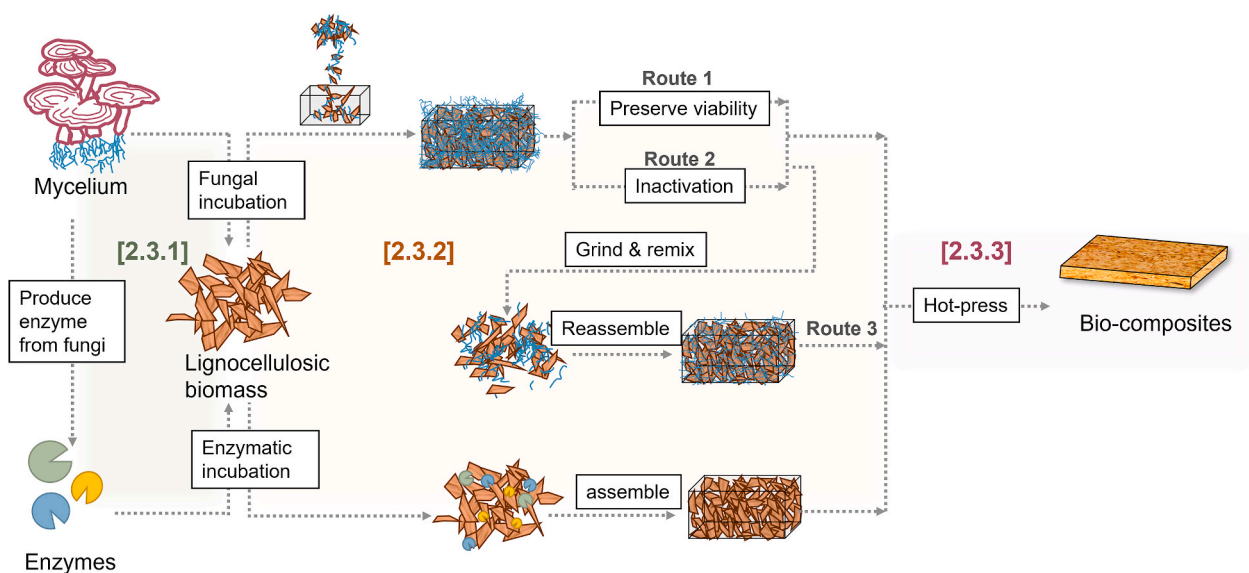


Fig. 3. Production routes for fungal and enzymatically pretreated lignocellulosic bio-composites (using particleboard as an example). The numbers in brackets correspond to the subsection headings in the paper.

2.3.2. Preparation for hot-pressing

The preparation step after incubation and before hot-pressing varies in different studies. For enzymatically pretreated substrates as well as some of those pretreated with fungi, the activated lignocellulosic biomass substrates are directly formed into mats, with the water content adjusted by filtration, squeezing, or low temperature drying (Fig. 3). In other methods involving fungal incubation, the substrate is shaped

before the growth phase so that after incubation the mycelia form connections between the particles and adhere them together. Three possible routes may be followed after that: 1) directly hot-press the fresh, auto-packed mixture; 2) heat-treat the mixture to inactivate the fungi but keep the formed mixture shape and then hot-press; or 3) inactivate the mixture, grind it down, and repack in molds before hot-pressing. There are currently no studies that directly compare these

different procedures in terms of the properties of the final bio-composites.

2.3.3. Hot-pressing

The purpose of the hot-pressing step is to densify the composite, create more adhesive bonds, and inactivate the enzymes or fungi in those cases where there is no pre-inactivation step. The hot-pressing temperature varies from 130 °C to 200 °C, as listed in Table 1. Two major different processes, “wet process” and “dry process”, differ in the moisture content of the mat as it enters the hot press. Typically, when the water content is above ~25% and plays a significant role in bond formation and heat transfer, the term “wet process” is used. Wet processes are more favored in combination with the fungal pretreatment of substrates as fungal incubation itself requires high humidity. In contrast, dry processes are more developed in combination with enzymatic pretreatments, particularly on the pilot-scale (Euring et al., 2013; Felby et al., 2002). Dry processes are of increasing interest as they do not require process water (Suchsland and Woodson, 1987), the energy requirement is far less as there is no need to boil water, and the enzyme solution can be easily sprayed onto the substrate.

3. Factors affecting composite properties

Table 1 lists the characteristic properties of fiberboards and particleboards made from various lignocellulosic biomass types through fungal or enzymatic pretreatment with no additional adhesive. It should be noted that the data are limited and based on different substrates, conditions, and processing parameters, which makes them difficult to

compare. Below we summarize some of the findings in the literature as they relate to the composite properties and factors governing them.

3.1. Mechanical properties

For the mechanical properties, the strength of bio-composites is usually given priority due to the typical applications in which the products are expected to be used (Pintiaux et al., 2015). The most commonly evaluated property is the modulus of rupture (MOR), determined via three-point bending tests. Fig. 4 presents a comparison of the change in the MOR with density and other typical parameters, including temperature, type of composites, and substrate. Fiberboards are usually produced for higher-density applications (0.71–1 g/cm³) and usually exhibit higher strength (MOR 13–44.6 MPa). In contrast, lower-quality agricultural wastes are preferred for the fabrication of lower-density particleboard or insulation, which generally result in a lower strength of the final product (MOR 0.62–33.7 MPa).

Enzymatically pretreated bio-composites generally show a higher strength (MOR 13–44.6 MPa) than low-density particleboard, but this is mostly caused by the density difference among samples: enzymes are more favored in pretreating higher-density fiberboards whereas fungi are more often used in lower-density particleboards (Table 1). Wu et al. (2020) treated poplar wood with both laccase and *Trametes (Coriolus) versicolor*; the fungally treated products showed a higher MOR value than the enzymatically treated products (22.7 MPa vs. 19.6 MPa, respectively, as shown in Table 1). However, the efficiency of both fungal and enzymatic pre-treatments is highly dependent upon a number of parameters (time, pH, temperature, relative humidity, etc.)

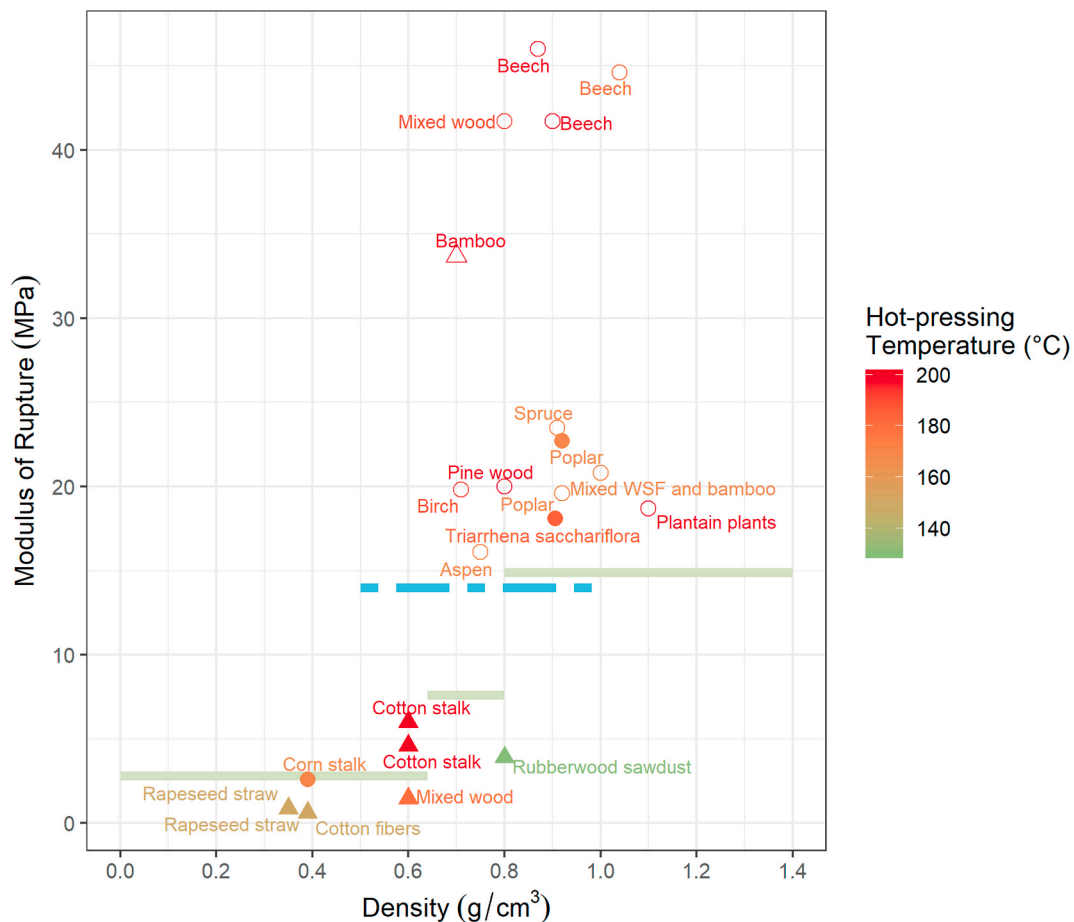


Fig. 4. Density (g/cm³) and modulus of rupture (MPa) of hot-pressed lignocellulosic bio-composites. Board type: ● ○ = fiberboard; ▲ Δ = particleboard. Pretreatment type: ○ Δ = Enzymatic; ●▲ = Fungal. All data come from Table 1, only the data that contain both density and MOR values are included. Solid line: ANSI standard MOR value for particleboard (Table 2); dashed line: ANSI standard MOR value for MDF (Table 2).

making it difficult to compare the results directly.

During the hot-pressing process, the use of higher temperatures usually brings about a higher MOR, which is attributed to the formation of more hydrogen and covalent bonds. Liu et al. (2019) compared *Ganoderma lucidum* pretreated cotton stalk bio-composites made at different hot-pressing temperatures (160, 180, and 200 °C) with controlled density and thickness. They showed that the interfacial bonding strength increased significantly with the increased temperature (230% increase in MOR from 160 °C to 200 °C). They also observed more repolymerization, esterification, and formation of hydrogen bonds from Fourier transform infrared (FTIR) spectra.

Enzymatic and fungal pretreatments are both wet processes, but the substrate may be pre-dried before pressing. The presence of moisture may lower the glass transition temperature (T_g) of the polymeric materials as water can act as a plasticizer (Jakes et al., 2019). A wet substrate can soften and flow, increasing the contact area to enhance bonding. Liu et al. (2020) reported that increasing the water content of the substrate by immersing in water before hot-pressing increased the mechanical properties (MOR, MOE and IB) of the final product by 1.5 times. They also reported that the increase in mechanical properties due to moisture plateaued at around 30% water content. They explained that beyond 30% moisture content, the excessive water only remained in the cell lumen and therefore had little effect to the properties of the fibers. This agrees with the generally reported fiber saturation point (FSP) of lignocellulosic materials. It should be mentioned that the FSP value was redefined by Engelund et al., in 2013 to be at about 40%; nevertheless, the 10% difference between these values can be explained by water entering the cell wall without breaking cell wall polymer hydrogen bonds (Engelund et al., 2012). Felby et al. (1997) compared wet and dry processing methods with 33% and 12% water content, respectively. The average MOR of the dry-produced bio-composites was not significantly different from that of the wet-produced ones.

Table 2 lists the minimum required values from the American National Standard for particleboard (ANSI A208.1–2016) and medium-density fiberboard (MDF) (ANSI A208.1–2016) for interior applications (ANSI, 2016a, b). In addition to the MOR, the modulus of elasticity (MOE), and internal bonding (IB) values are summarized in Table 2. Comparing these values with the ones presented in Table 1, it can be seen that most of the produced fiberboards or particleboards were able to meet the required standard values. For the ones with values lower than required (marked ^a in Table 1), they either used hot-pressing temperatures lower than 180 °C (Appels et al., 2019) or ground and reassembled the fungus-pretreated substrate (Sun et al., 2019) which are both believed to have negative effects on bonding. In the latter case, it was hypothesized that it was the disruption of the 3D structure of the filamentous mycelia holding the lignocellulosic particles together that resulted in the deterioration of the bio-composites' mechanical performance.

Table 2

American National Standard values (minimum required) of particleboard (ANSI A208.1–2016) and medium-density fiberboard (MDF) (ANSI A208.1–2016) for interior applications.

Product	Density, g/cm ³	MOR, MPa	MOE, MPa	IB, MPa	TS, %
Particleboard (H)	>0.8	14.9	2160	0.81	N/A
Particleboard (M)	0.64–0.8	7.6	1380	0.31	N/A
Particleboard (L)	<0.64	2.8	500	0.10	N/A
MDF	0.5–1.0	14	1400	0.30	10

^a letters H, M and L indicate high density, medium density and low density panels, respectively.

3.2. Physical properties

While the mechanical properties of fungus- and enzyme-pretreated bio-composites are nearly as good as composites bonded by traditional adhesives, the physical properties are far from equivalent. Water absorption (WA) and thickness swelling (TS) are common measures of water resistance in panel products. As shown in Table 1, these parameters have been only rarely reported for bio-composites, particularly when the product being manufactured is particleboard. This may be due to the fact that WA and TS measurements are not required for interior grade particleboard (Table 2). For fiberboards, although fungal and enzymatic pretreatments were reported to decrease these values, all reported 24 h TS values were still much higher than the values required by ANSI standard. One exception is from a recently published paper (Khoo et al., 2020) where the 24 h TS of 160 °C hot-pressed fungal pretreated rubberwood sawdust was only 3.1%. This value is dramatically lower than the data obtained from other research groups. This low value might be a technical error as there are a few other technical errors in this particular paper including contact angle values.

3.3. Thermal stability

The thermal stability of mycelium-based bio-composites highly depends on the substrate. The pretreatments are mild; the proportion of enzymes and fungal body is negligible compared to the other components of the mixture and therefore have little influence on the thermal properties of the final composite. Appels et al. (2019) reported that a colonized substrate thermally degraded faster than an uncolonized group. One possible reason is that fungal degradation makes the substrate more accessible for thermal degradation, but it also might be due to the lower thermal stability of the mycelial body. Appels et al. (2019) also reported that there was no noticeable impact on the thermal degradation profiles of the bio-composite after hot-pressing at 150 °C. However, Liu et al. (2019) reported that hot-pressing at 160 °C and 180 °C caused a higher weight loss and decreased residue as determined via thermogravimetric analysis (TGA), but this would be reversed when increasing the hot-pressing temperature to 200 °C. The authors hypothesized that the highest hot-pressing temperature might have already released the most unstable compounds, leading to higher thermal stability.

3.4. Additives to enhance properties

To further improve the physical and mechanical properties of fungus- and enzyme-pretreated bio-composites to compete with commercial formaldehyde-based adhesive-bonded products, different additives have been investigated. There have also been attempts to add traditional formaldehyde-based adhesives (Nasir et al., 2014, 2015; Zhu et al., 2017), which are outside the scope of this review. In the following sections, we review non-formaldehyde-based additives and how they may improve composite properties.

3.4.1. Mediators

In laccase systems, mediators are often added to promote enzymatic reactions. Mediators are small molecules which are oxidized by enzymes and act as electron carriers to oxidize other molecules. Mediators can increase the rate and number of chemical reactions within biomass. The group of Euring et al. conducted several studies adding mediators including 4-hydroxybenzoic acid (HBA), 1-hydroxybenzotriazole (HBT), acetosyringone (AS), caffeic acid (CA) and vanillic alcohol (VAI) to medium-density fiberboard systems (Euring, M. et al., 2011; Euring et al., 2011; Euring et al., 2013; Kirsch et al., 2016). All the mediators were found to improve both physical (TS, up to 17%) and mechanical (MOR, up to 15% and IB, up to 18%) properties of the bio-composites. Of these mediators, HBA, CA, and VAI were considered the best choices because of their higher efficiency.

3.4.2. Lignin derivatives

Based on the previously described laccase-mediator pretreatment results, Euring et al. (2016) upgraded the system by adding calcium-ligninsulfonate to MDF. More phenoxy radicals were observed, which contributed to the bonding and further improved the mechanical properties (MOR and IB). The resulting bio-composites performed as well as fiberboards made with 10% urea-formaldehyde as the adhesive.

3.4.3. Hydrophobic agents

Wax is typically added to commercial particleboard, OSB, and fiberboard to improve water resistance (Mantanis et al., 2017). Felby et al. (2002) added 1% liquid paraffin wax to a laccase-treated fiber mixture, finding a negative effect on bonding strength (19% in MOR, 7% in MOE, and 66% in IB) and no improvement in dimensional stability. Because the total amount of radicals generated by the laccase was not changed, the authors attributed this observation to a reduced number of contact points and other interactions among fibers caused by the wax coating. Kirsch et al. (2016) made a similar attempt but changed the paraffin wax to hydrowax. They reported a noticeable improvement compared to previous work with no wax addition (Euring, 2008). Although these studies were performed by the same research group, it is always difficult to compare numbers from different studies as maintaining the exact same conditions is a challenge.

3.4.4. Nanocellulose

Sun et al. (2019) found that adding cellulose nanofibrils (CNFs) significantly improved the mechanical properties of mycelium-pretreated particleboard. At the optimum level of 2.5 wt% CNFs, the MOR increased from 1.5 MPa to 3.5 MPa and the MOE from 220 MPa to 575 MPa. Despite their hydrophilicity, both the TS and WA exhibited slight decreases compared with the control group in which no CNFs were added. This can be attributed to the hornification of cellulose nanofibrils upon drying in the hot press.

4. Contribution of fungal/enzymatic pretreatment to adhesion

When lignocellulosic substrates are treated with fungi or enzymes, certain changes in their physical, chemical, and morphological properties may occur which can contribute to adhesion. These changes are summarized in the following sections.

4.1. Substrate geometry, morphology and surface roughness

Mechanical interlocking and polymer interdiffusion are two of the main adhesion theories in lignocellulosic adhesive bonding and have been extensively used for low viscosity adhesives such as urea-formaldehyde and phenol formaldehyde resins (Gardner et al., 2014). Lignocellulosic biomass possesses a porous and irregular structure, which has air spaces between and within individual particles or fibers. Rough surfaces provide more surface area for mechanical interlocking and can expose more porous structure on the surface for the diffusion and penetration of adhesives. Because the cell wall of lignocellulosic biomass is an aggregate of microfibrils surrounded by a matrix of lignin and hemicelluloses (Fig. 1), the matrix breakdown or removal that occurs during fungal pretreatment would cause the exposure of more microfibrils and result in a rougher surface. At the same time, the growth of aerial hyphae on the surface also contributes to changes in the roughness by adding a microscale network of hyphae on the original biomass surface (Sun et al., 2019; Wu et al., 2016). Sun et al. (2019) measured particle size distribution and analyzed the dimensions of both untreated and mycelium-colonized softwood particles. They did not find significant differences in wood particle size parameters and concluded that changes seen in physical and mechanical properties were not caused by wood particle size.

Enzymatic treatments have been reported to have widely different effects on lignocellulose surfaces. When the incubation time is longer,

the surface will become rougher as the surface layer is lost. Zhu et al. (2017) treated poplar fibers with laccases for 11 days and found that the surface of the fibers became loose, as confirmed by scanning electron microscopy (SEM) images. Rajak and Banerjee (2016) investigated the change of surface properties of Kans grass after laccase incubation. They found that the surface area (measured using Brunauer-Emmett-Teller (BET)), pore volume, and pore diameter (measured using differential scanning calorimetry (DSC)) all increased after the treatment, which could be explained by the opening of the outer layer of the matrix in a manner similar what occurs in fungal pretreatment. In contrast, Álvarez et al. (2010) treated leaf sheath fiber bundles with laccase for 24 h but were not able to find a noticeable change in fibrillation and integrity using SEM. However, Nasir et al. (2015) treated rubberwood fibers with laccase for 30 min, and 60 min and found that laccase treated fibers had a smoother surface compared with the untreated fibers (in SEM). Similar observations are also reported by Nasir et al. (2014). It was believed that the loosely bonded lignin was removed by the enzyme, the aliphatic and aromatic ring molecules were broken down, and the hydrolyzed lignin was re-precipitated onto the surface.

4.2. Surface wettability

Wettability describes the spreading and uniformity of the surface bonding agent. Lignocellulosic biomass generally has high surface energy due to its chemical composition. For this reason, it requires adhesives with polar molecules for better molecular contact. Lower-viscosity adhesives are generally preferred due to their better spreading and penetration properties (Gardner et al., 2014; Hunt et al., 2018). In binder-less lignocellulosic bio-composite systems, the adhesion agents are most likely heat-plasticized lignin and hemicelluloses (Hubbe et al., 2017). As these materials are already well distributed on the surface of each particle or fiber, there are no issues of contact and distribution. The hot-press process also promotes adhesion by plasticizing and compressing the surface components so that new bonds can be formed.

Aerial mycelia contain amphiphilic hydrophobin proteins which mediate the escape of hyphae from aqueous environment into the air. These proteins make the aerial hyphae hydrophobic on their surfaces (Appels et al., 2018; Sammer et al., 2016) with water contact angles over 120° (Antinori et al., 2020; Haneef et al., 2017). Therefore, the presence of aerial hyphae on the surface of lignocellulosic materials can significantly change the materials' wettability and interactions with bonding agents and adhesives (Sun et al., 2020).

Laccase incubation decreases the surface energy of lignocellulosic biomass substrates. Zhu et al. (2017) reported that the initial water contact angle of poplar fibers increased from 114° to 136° following 5-day to 11-day laccase treatments. Felby et al. (2004) investigated the change of surface free energy components of beech fibers after laccase treatment. They reported a 55% reduction in hydrogen dipole (hydrogen bonds) and a 77% reduction in Lewis acid-base interactions (covalent bonds), indicating that the amount of Lewis acid-base interactions and hydrogen- or dipole-induced bonding in boards were lower compared to boards made from control untreated fibers.

4.3. Oxidation of surface lignin

One-electron (radical) oxidation of lignin by enzyme systems secreted by white-rot fungi and covalent bonds formed by lignin radicals during hot-pressing are the most accepted and well-studied mechanisms of adhesion in hot-pressed bio-composites. As introduced in Section 2.2, enzymes are often secreted in relatively large quantities during fungal colonization because they are needed for the initial breakdown of the lignin and hemicelluloses of the cell wall. This selective stage is the critical period of direct fungal pretreatment, where the main goal is to take full advantage of the lignin oxidation without damaging the cellulose.

As expected, secretion of enzymes differs by fungal species, substrate, nutrient availability, culture conditions, age, etc. In Lekounougou

et al. (2008)'s work treating beech with *Trametes (Coriolus) versicolor*, there was no peroxidase activity at all in the first ten days, suggesting that laccase was the only contributor to lignin degradation in the initial stages of decay. However, when using the same fungus on poplar wood, Wu et al. (2020) reported that the activities of laccase and LiP are too low to be determined in the first 28 days, whereas the activity of MnP increased in the first 14 days then decreased in the following 14 days. This difference is also revealed through measurements of the physical properties of the bio-composites produced using *Trametes (Coriolus) versicolor* pretreatment. Some studies reported direct positive correlations between the activity of a specific enzyme (laccase) and improved mechanical properties (Wu et al., 2011; Zhu et al., 2017), while Wu et al. (2020) revealed positive correlations between MnP and bending strength but with a 7-day lag.

Directly treating the substrate with specific lignin-degrading enzymes is more favored than growing fungi as it can help avoid potential damage to carbohydrates. It is also easier to test for adhesion mechanisms. The generation of more free radicals after enzymatic pretreatment of fibers was detected by electron paramagnetic resonance (EPR) spectroscopy (Felby et al., 1997, 2002). Fibers treated by higher dosages of enzymes showed a higher radical generation and the bio-composite made from them had improved mechanical properties compared to those treated with a lower dosage (Felby et al., 2002). The oxidation of surface lignin was confirmed by the increase of the surface O/C ratio, measured by X-ray photoelectron spectroscopy (XPS analysis) (Felby et al., 2004; Liu et al., 2020). Finally, gel permeation chromatography (GPC) revealed an increasing molecular weight of laccase-pretreated lignin in the resulting bio-composites compare with untreated control samples, which confirmed additional polymerization during the hot-pressing process (Felby et al., 2002).

4.4. Hydrolyzation of hemicelluloses

Another possible source of covalent bond formation is the degraded hemicelluloses produced by the action of white-rot fungi. Because hemicelluloses naturally have low thermostability, they are believed to partially decompose in the hot-pressing of binder-less composite manufacturing process and thus contribute to covalent bond formation. Many studies have shown evidence of hydrolysis and thermal decomposition of hemicelluloses in binder-less bio-composites production (Brazdauskas et al., 2015; Cristescu and Karlsson, 2013; Dolan et al., 2015). Hubbe et al. (2017) summarized the likely linking reactions involved in the presence of hemicellulose degradation products (such as furfural and 5-hydroxymethylfurfural) and lignin fragments. One possible explanation for the effectiveness of fungal pretreatment maybe that lignin does not provide carbon and energy to the fungus in earliest stages of colonization (Lekounougou et al., 2008), but the fungus secretes hemicellulases that can hydrolyze surface hemicelluloses into smaller molecules, which decompose more easily during hot-pressing to provide further reaction sites (Mishra et al., 2017; Xie et al., 2017).

In two of their published studies, Wu et al. investigated the change of water-soluble hemicelluloses, treating corn stalk with *Trametes hirsuta* and *Triarrhena sacchariflor* with *Trametes (Coriolus) versicolor* (Wu et al., 2011, 2016). They found that the content of water-soluble polysaccharides first increased then decreased, corresponding well with the mechanical properties of the resulting bio-composites. However, in their recently published research using *Trametes (Coriolus) versicolor*-pretreated poplar wood shavings, the ethanol-extracted polysaccharide content decreased throughout the 28-day incubation period in correlation with the strength of the composite, while the reducing sugars in the filtrate first increased then decreased (Wu et al., 2020). Many factors (surface chemistry, morphology, components from biomass and fungi, etc.) change throughout the decay process. Thus, there is unlikely to be a direct relationships between the changes of polysaccharides and the composite properties, making this an interesting topic for further research.

4.5. Fungus/enzyme physical structure contributions

When incubating fungi or enzymes with biomass, the physical components of the fungus or enzyme may also contribute to the bonding and should therefore be considered. As shown in Fig. 4, the typical white-rot fungal mycelium consists of two elemental layers. The inner layer is composed of chitin and branched β -(1,3) and β -(1,6) glucans, which work as the scaffold for the cell. The outer layer contains a protein layer, a glycoprotein layer, and a slime layer, which vary among the species (Gow et al., 2017; Steudler and Bley, 2015). In fungal pretreatment of lignocellulosic substrates, fungal mycelium covers the surface and the protein and carbohydrate components in the outer layer of hyphae may contribute to the formation of adhesive bonds. Sun et al. (2020) studied the functionality of surface mycelium interfaces in wood bonding by growing mycelium on model wood veneer surface. They found that the presence of surface mycelium improved the wood bonding even in the absence of wood degradation. In a hot-pressed composite system, Liu et al. (2019) investigated changes due to mycelial colonization of cotton stalks. Examining the FTIR spectra, they found that there were new peaks appearing at 2978 cm^{-1} and 1086 cm^{-1} assigned to C-NH and C-O stretching, respectively, after hot-pressing at $200\text{ }^{\circ}\text{C}$, which they suggested were the results of reactions such as esterification between the mycelia and cotton stalk particles. However, these reactions could also occur within the fungal body itself and could have therefore had no connection to the cotton stalk biomass. More evidence needs to be gathered to confirm the bonding mechanism. Sun et al. (2019) also attempted to use pure mycelium as adhesive by mixing 10% pure mycelium particles with mixed softwood particles, then using the mixture to create particleboards via hot-pressing. The resulting panels had inferior mechanical properties that did not reach the required industry-standard values. One possible reason for this result may have been that the pure mycelium particles were not evenly distributed throughout the mixture, a conclusion supported by electron microscopy imaging of the panels. Another reason could have been that the aerial mycelia did not provide the same level of binding as wet mycelia would potentially have (Sun et al., 2020).

When using enzymes alone to pretreat biomass, the proportion of non-substrate material in the system is even lower than if a full fungal pretreatment had been used. It is unlikely that the bonding chemistry would be significantly altered. Felby et al. (1997) and Kirsch et al. (2016) confirmed this by comparing fiberboard made from untreated fibers and fibers pretreated with inactivated laccase (using same concentration as active laccase) and observed no significant difference in the physical and mechanical properties.

5. Knowledge gaps and commercial limitations

In the previous sections of this review, we discussed the current state of the art for production routes, raw materials, and factors affecting high-density hot-pressed composite properties, as well as potential reasons behind the substrate changes which have been shown to contribute to adhesion. In this section, we present our understanding of the current knowledge gaps as well as the hurdles that might be faced by commercial industries seeking to promote wider adoption of this technology.

5.1. Knowledge gaps

5.1.1. Fungal vs. enzymatic pretreatment

Generally, fungal pretreatment requires longer incubation times and results in a less targeted treatment, while enzymatic pretreatment, although more targeted, generally costs more due to more complicated and costly enzyme production processes. The question remains, then, whether such precision in lignocellulosic bio-composite manufacturing is, in fact, needed. If so, how much improvement in properties can be achieved? A detailed comparison of the pretreatment efficiency versus

cost of time and energy would help to answer these questions.

5.1.2. Fresh vs. inactivated substrate

Radicals are generally not stable; however, recent research has shown that enzymatically generated lignin radicals can be stabilized within lignocellulosic biomass for up to several days (Perna et al., 2019; Shi et al., 2020). Furthermore, as enzymatic pretreatment usually occurs right before hot-pressing, there is little concern about the loss of generated radicals over time. Additional pre-drying can result in a decrease in radical concentration; however, some groups have shown that as the initial amount of radicals is considerable, bonding is still promoted (Felby et al., 2002). In the fungal pretreatment process, the situation is quite different. A number of studies inactivated the fungal growth by heat first, then proceeded with the hot-pressing. It is understandable that this approach can make the production process more flexible and also reduce the hot-pressing time, but it is not clear how this step would affect the bonding as the adhesion mechanism in fungal pretreatment system is more complicated and not well-understood. A direct comparison of bio-composites made from freshly compressed versus heat-inactivated substrates would provide valuable information to answer these questions.

5.1.3. Adhesion mechanism-related questions

As described in Section 4.5, the effects caused by the physical structure of the mycelium itself have rarely been considered in biologically pretreated lignocellulosic hot-pressed bio-composite systems. Although the presence of living surface mycelia has been confirmed to work as a binder for wood veneers, no confirmation has been demonstrated in more complex bio-composite systems where a 3D structure of particles are to be bonded. There are also several remaining questions: are there certain characteristics of mycelia such as specific proteins and carbohydrates that make them bond to the substrate more effectively? If so, can we guide the fungi to express these characteristics when growing on the biomass? And how much do various bonding agents contribute to MOR, MOE, IB, TS, WA, and creep, either in dry or in wet/high humidity conditions? How can various bonding mechanisms be maximized and promoted? Could there be a synergistic effect of mycelial physical structure, lignin radicals, and hemicelluloses at a particular degradation stage? Thus, a thorough investigation of the adhesion mechanism in the composite system is critical to the further targeted improvement of hot-pressed bio-composite systems enhanced through fungal pretreatment.

5.2. Roadblocks to commercialization

Although some studies have demonstrated comparable mechanical properties of bio-composites pretreated with enzymes and fungi to those of traditional adhesive-bonded composites, several problems remain. Water resistance, performance at high relative humidity, and high press temperatures are issues that all must be addressed before biologically pretreated lignocellulosic hot-pressed bio-composite systems can become widely available on commercial markets.

5.2.1. Moisture resistance

Nearly all bio-composites reported on in the literature have extremely low moisture resistance and are thus not suitable for use in humid or wet conditions. This is unsurprising as there is significantly less covalent bond formation in bio-based composites compared to the more traditional adhesive-bonded systems. The moisture resistance problem is less of an issue for particleboard-related applications as the majority of commercialized particleboards are designed for interior use. However, for MDF-related applications, great improvements in the thickness swelling values are needed. Indeed, improvements in moisture/water resistance will benefit the commercialization of bio-based composite products even if such performance is not required by relevant standards.

5.2.2. Processing temperature

High temperature (140–200 °C) is favorable in both the enzymatic and fungal pretreatment systems. In “dry process” systems and most enzymatic pretreatment systems, the goal of high temperature processing is primarily to activate the surface components. When bio-composites incubated with fungi are directly pressed, the higher temperature also facilitates the evaporation of water to reduce the time needed to manufacture the compressed panel. The high processing temperature requires much more energy and limits the potential of commercialization. As most synthetic adhesives being used in the industry for current commercial panels cure at much lower temperatures (120–140 °C) (Mantanis et al., 2017), processing temperatures for bio-based composites need to be reduced to make them competitive. Alternatively, other energy-saving techniques or approaches could be considered in the larger life cycle analysis of these products.

5.2.3. Cost and process time

There is currently no straightforward method of calculating the manufacturing cost of enzymatic or fungal pretreated bio-composites. One possible reason is that, at the time of the writing of this review, “mycelium-based bio-composites” are relatively new in the market and have not yet been considered together with enzymatic pretreatment. Because the raw materials are the same as the commercial products, the cost of enzymes and fungi are the main contributors to the raw material cost. Fungi have a great advantage over enzymes as they do not require an additional extraction process and the most commonly used white-rot fungal species for fungal pretreatment are the same as commercially available mushrooms. In addition, mushroom spawns are easy to obtain and reusable. The long incubation time (days) is one of the major disadvantages of fungal pretreatment as compared with enzymatic treatments. However, most of the species can be grown in ambient conditions, which only require energy for ventilation, but not for heating.

5.2.4. Commercial products and potential improvement approaches

In the recent years, some compressed fungal mycelium-lignocellulosic bioproducts have been developed by a number of companies. Ecovative Design LLC successively developed several fungal mycelium-bonded particleboard-like bio-composites named MycoBoard™ (Ecovative, 2017) and mCore™ (Ecovative, 2018), in the past five years. In one technical report published in 2014, they reported the laminated MycoBoard™ achieved competitive mechanical properties to the MDF panels (Tudryn, 2014). However, no moisture-resistance related properties were reported and no related product is currently advertised on their website. MOGU s.r.l developed floor tiles using fungal mycelium-bonded fiber waste as a core material. The floor tiles include a top coating layer (90% bio-based resin), a bio-polyurethane (PU) layer, the mycelium composite core, and a moisture barrier layer. With the added layers, the density is reported as 0.7 g/cm³ and the dimensional stability was 0.10% (MOGU, 2019). They also claimed that the bio-PU layer can be separated to enable the biodegradation of the core. Adding barrier layers is definitely a potential approach for commercialization of biologically pretreated bio-composites. These layers can be specially designed to impart different functionalities to the composite.

6. Conclusions

This review evaluated the current state of the art around mycelium-based bio-composites produced through enzymatic or fungal pretreatment of lignocellulosic materials. We have critically reviewed major findings related to raw materials, processing, issues, and roadblocks, particularly as they pertain to hot-pressed, higher-density mycelium-based bio-composites. We expect that this review will provide a reasonable basis for future research and development of these novel and interesting composite materials. Bio-composite manufacturing and

associated technologies have great potential to produce all-natural bio-composite materials for an array of applications. However, the complexities of the system require more investigation into both the raw material and processing procedure, as well as further evaluation of the adhesion mechanisms and bonding theories. As a good number of the chemical reactions and material transformations occur in both biodegradation and pressing processes, many different possible mechanisms must be considered when seeking to better understand the optimum processing parameters for mycelial bio-composites. This is further complicated by the fact that different adhesion theories in the literature can contradict one another. Addressing the questions and limitations listed in Section 5 would be an important step toward achieving a better knowledge of this complicated system. Nevertheless, a deeper investigation of the important factors for specific applications and an informed selection of raw materials, pretreatment conditions, and additives will be critical to realizing the mass-production of commercially-viable mycelium-based bio-composites.

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CRediT authorship contribution statement

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Declaration of competing interest

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