Recent Advancements in Lignin Valorization, Bioengineering, Catalysis and Biorefining

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Abstract – Most of the research studies in the field of lignin valorization has been achieved on lignin from paper and pulp industries. The key merit of utilizing lignin from the facilities is that resources are already centralized, and the costs of transportation to further process are fundamentally less compared to possible sources. Biomass composed of lignin, hemicelluloses, and cellulose (also known as lignocellulosic biomass) is both plentiful and renewable. The three polymer groups may be isolated and chemically transformed to provide a wide variety of bio-generated value-added materials, chemicals, and fuels. To maximize the value of the biomass feedstock as a whole, it is best to extract these products via a streamlined, integrated models of pathways concerned with catalytic reactions i.e., bio-refinery. In this article, upstream processing is analyzed in depth, including the phenylpropanoid route, bioengineered lignins, and lignin bioengineering. The last section addresses the difficulties inherent in lignin bioengineering from a technical and practical standpoint.

Keywords – Lignin, Lignin Bioengineering, Bioengineered Lignins, Lignin Valorization, Metabolic Pathways, Lignocellulosic Biomass.

I. INTRODUCTION

Methoxylated hydroxyxycinnamyl alcohols, the prototype monolignols, are the primary building blocks from which lignin; a water-insoluble and complex aromatic polymer is produced. In contrast to the well-illustrated monomeric unit sequence in cellulose, which is connected by a 4-glycosidic regular β-1 bond, lignin is featured by different and chemically varied bonding motifs, each of which necessitate distinct cleavage conditions whenever selective depolymerisation is beleaguered. Despite its greater structural complexity, lignin is a desirable feedstock for chemicals and biofuels synthesis because of its lower oxygen-content and higher carbon-content in comparison to holocellulose fraction or polysaccharides.

As lignin is already highly functionalized and aromatic, it may be possible to manufacture aromatic speciality and fine chemicals without first having to be fully defunctionalized to "BTX" (xylene, benzene, and toluene) and then refunctionalized to the required platforms chemically. However, effectual separations from the oxygenated aromatics using methods like distillation present challenges, hence critical defunctionalisation to alkanes and arenes will also be of much significance for the processing of fuel and chemical components from lignin and its derivatives. Because of its availability and potential for economic growth, lignin has been the subject of intensive study for its catalytic valorisation.

The bioenergy sector has concentrated heavily on plant cell walls as part of its endeavor to reduce the cost of producing cellulosic biofuels. Lignin, found mostly in cell walls, is the greatest source of natural aromatic polymers and the third most prevalent biopolymer. The guaiacyl (G), p-hydroxyphenyl (H), and syringyl (S), units of lignin comes from sinapyl, monolignols p-coumaryl, and sinapyl alcohols, correspondingly. A coating of lignin with cell wall polysaccharides is integrated, rendering then increasingly resistant to extractions and enzymatic hydrolysis, and this is the key contribution to the high lignocellulosic sugar manufacturing costs. Moreover, lignin is often considered a waste product since it has essentially little economic value beyond its use as a heat source.

Since lignin levels in biomass are inversely connected to their kappa value and fodder digestibility within the pulping industry, they have been the subject of genetic modification for decades. All enzymes needed for the manufacturing of the three foundational units of lignin, considered monolignols, are highly-conserved and well-characterized across different vascular plants. Regrettably, removing lignin from plants in progress would have a negative impact on their growth and development. Trials of genetic modification utilizing natural mutations or silencing techniques have failed because to the severe and nonselective reduction of lignin content. Nonetheless, in certain circumstances, light genetic interventions have been utilized to considerably lower lignin concentration or vary its biomass composition, somewhat enhancing fodder digestibility, pulping output, and saccharification efficiency.
To further decrease lignin concentration without affecting plant growth or having unintended consequences, new ways need to be devised. The expression or activity of lignin biosynthesis genes is often suppressed in conventional lignin-modification techniques. To solve these problems, researchers must either find naturally occurring faulty alleles, screen mutant populations for single-nucleotide polymorphisms (SNPs) (a time-consuming procedure), or use RNA interference (RNAi)-based gene-silencing strategies. All of these methods are limited by their inability to target a particular tissue, since the same faulty allele or silenced gene is present in every cell due to the ubiquitous nature of RNAi throughout the plant. They have an impact on the phenylpropanoid and monolignol metabolic pathways, which in turn affects the lignin production process.

The phenylpropanoid pathway, for instance, produces a plethora of secondary metabolites that play a role in every stage of plant growth and in the plant's response to both biotic and abiotic stressors. Collaboration between scientists from many fields (such as genetic engineering to boost polymers homogeneity, or the fractions of cleavable connections, the enhancement of effective analytical tools for reactor engineering, lignin model determination) is necessary to gain insight into and command of the complex series of processing stages involved in transforming raw biomass into the final lignin-derived product.

This article provides an in-depth examination of upstream processing, including such topics as the phenylpropanoid pathway, bioengineered lignins, and lignin bioengineering. In the last part, we discuss the pragmatic challenges of lignin bioengineering. The rest of the paper is organized as follows: Section II focuses on bioengineering of lignins. Section III discusses the phenylpropanoid pathway, while Section IV enumerates the concept of bioengineered lignin. In Section V, practical, and technical challenges of lignin bioengineering are discussed. Section VI draws final remarks to the article.

II. BIOENGINEERING OF LIGNINS

Biorefinery to be financially successful, all of its biomass, including the lignin stream, should be transformed to high-value products. Connectivity (not just in terms of physical arrangement, but also via covalent bonding) between lignin, hemicellulose, and cellulose complicates direct form of enzymic cellulose to glucose saccharification and other processes, such as increasing the forage crop digestibility for animal feeds. Li et al. [1] working with plants have tried fiddling with phenylpropanoid biosynthesis, a complex biosynthetic process that begins with the amino acid phenylalanine and culminates in the production of propenylated p-hydroxyphenyl derivatives, as a means of overcoming this obstacle (or tyrosine). Changes to the phenylpropanoid route may affect plant fitness since its forks provide fuel to several metabolic pathways necessary for development and expansion.

Hardwoods, softwoods, monocots (grasses), and dicots (including Arabidopsis and alfalfa/truncatula) are all being examined in connection to biomass conversion, and the bulk of phenylpropanoid genetic techniques have concentrated on reducing lignin levels in these plants. Nevertheless, in case lignin fraction that composes 15 wt% to 30 wt% of drier biomass, become increasingly intractable, then an increment in saccharification yield could not boost biorefinery operations and economics.

New and important genetic alterations to the lignin biosynthetic pathway are reviewed here, including the effects of down-regulation and up-regulation of different genes encoding enzymes fundamental to the generation of lignin’s building blocks. Yin et al.’s [2] latest research into structural changes of lignin reveals almost endless possibilities for better use of both the carbohydrate and lignocellulosic lignin fractions, via catalytic processing. Actual genetic modification combinations may result to plants with greater or similar development and growth as a wild type. Including a limited subset of links or precursor units, for example, might make lignin deconstruction possible under gentler circumstances than are now necessary, without increasing or decreasing the lignin fraction's overall amount. As a result, bioengineering lignin might increase the lignin's valorisation by later catalytic treatment and boost the saccharification yield from biomass.

Whether evolutionary stream the targeted species originate from—the lignin or carbohydrate streams—will have a significant impact on the optimal circumstances for any bioengineering method.

Lignin-engineering pathways

Whilst overexpression of the synthesis of type III polyketide for the production of flavonoids, stilbenes, benzalacetones, coumarins, and curcuminoinds has been studied in other contexts, its effect on tissues forming lignified secondary cell walls has scarcely been studied. The enzymes might be utilized to divert hydroxycinnamoyl-CoAs from the pathways of lignin, but only if there is an enough co-substrate malonyl-CoA supply. Lignin precursors such as cinnamate, coniferyl, and phenylalanine alcohol may be transformed into phenylpropanoid/benzenoid volatiles by enzymes involved in the production of neolignans, and lignans, diverting these compounds away from lignin formation.

For monolignol candidates’ generation that is cleavable, the tissue-based overexpression of different enzymes from the family of BAHD acyl-CoA transferase is of a significant interest. Among the transferases in this family are those responsible for the formation of hydroxycinnamate esters like coniferyl coumarate/ferulate, and rosmarinic acid. Even though two enzymes have been noticed to connect hydroxycinnamoyl-CoAs to hydroxylanthranilates, there is no BAHD that catalyze the production of hydroxycinnamate amide utilizing the aromatic acceptor have yet been explored. However, many plant species contain amino acid amides N-phenylpropenoyl-aromatic such as (deoxy)clovamide. Instead, the GCN5-based N-acyltransferase family was identified to include enzymes involved for the generation of the tyramine hydroxycinnamate amide. In a broader sense, monomers might be produced by overexpressing (yet-to-be-discovered)
monolignol acetyltransferase, which utilizes hydroxybenzoyl-CoA as a source to decrease lignin DP and increase the amount of useful moieties, which may be contained in the processing of biomass.

Plants have been shown to possess biosynthetic enzymes for the synthesis of C6C1 molecules. In particular, vanillin is synthesized from coumarate via the intermediates protocatechuicde and 4-hydroxybenzaldehyde, with the involvement of three vanilla orchid enzymes. Lignifying tissues that co-express these enzymes may be able to redirect coumarate towards the production of C6C1 aromatics. Hydroxycinnamoyl-CoAs may also be converted into C6C1 hydroxybenzaldehydes with the help of HCHL enzymes. Transcriptional analysis of the HCHL gene in Arabidopsis revealed that C6C1 hydroxybenzaldehydes were easily metabolized by endogenous enzymes to C6C1 acids and could go through the process of methoxylation and hydroxylation of their heterocyclic ring after being expressed. As a conclusion, microbes’ chorismate pyruvatelyase, e.g. Escherichia coli’s UbiC, may be utilized to accumulate 4-hydroxybenzoate from chorismate in plants, where 4-hydroxybenzoate 3-hydroxylases could be utilized to generate protocatechuic.

Overexpressing shikimate reductase from Juglans regia (walnut) from Ecoli causes a fivefold rise in gallate contents in tobacco plants, according to Chen, Chen, Yu, and Huo [3] on the biosynthesis of pyrogallol groups. In a recent study, we found that the Saccharothrix espanaensis bacteria coumarate 3-hydroxylase SAM5 could caffeate in order to produce 3, 4, or 5-trihydroxycinnamates whenever produced in Ecoli. This finding provides a means through which pyrogallol-containing compounds may be synthesized in plants, diverting coumarate from the lignin path.

III. THE PHENYLPROPANOID PATHWAY

The enzymes directly integrated in the archetypal lignin monomer processing are outlined as comprehensive phenylpropanoid pathways. Sinapyl, P-coumaryl, and coniferyl alcohols are all examples of monolignols; they are integrated in the process of polymerization, which amounts to lignin polymers, and provides the "H," "G," and "S" units. In dicots, phenylalanine (Phe) is the amino acid of choice for the phenylpropanoid pathway, but in monocots, tyrosine (Tyr) may be used instead. The PAL (phenylalanine ammonia-lyase) enzyme deaminates phenylalanine to cinamate, and subsequently cinamate is thus hydroxylated to the p-coumarate (through C4H, cinamate 4-hydroxylases). In case tyrosine is utilized as the substrate, the two-phase enzymatic procedure is skipped and p-coumarate is produced directly from Tyr by deaminations (through TAL –tyrosine ammonia-lyase, but also through PAL that is not significantly categorical for Phe).

Depending on whether enzyme is present, the pathway from p-coumarate may either yield p-coumaroyl-CoA (4-coumarate: 4CL, CoA ligase) or caffeates (2-hydroxyaromatic ring hydroxylation) (C3H or C4H). The enzyme hydroxycinnamoyltransferase (HCT) converts p-coumaroyl quinic/shikimic acid from p-coumaroyl-CoA whereas CCR (Cinnamoyl-CoA Reductase) transforms to p-coumaraldehyde alcohol from p-coumaraldehyde that is therefore integrated into lignin to generate H-units.

Although feruloyl-CoA (4CL) is typically considered to originate from CCoAOMT – caffeoyl-CoA methylation, caffete could also be changed to ferulates through 3-hydroxy group methylation of rings (COMT, caffeic acids O-methyltransferase). Reduced feruloyl-CoA yields coniferaldehyde (by CCR). G units, formed from coniferyl alcohol, and S units, derived from sinapyl alcohol, both originate from coniferaldehyde. The primary process toward sinapaldehyde involves the coniferaldehyde hydroxylation and successive methylation of results (through COMT). Conversion of the aldehyde moiety to the main alcohol is the last step in the synthesis of S and G units, and it is catalyzed by CAD. The aldehyde may be oxidized to produce ferulic acid, which can then be recycled to phenylpropanoid pathways (by hydroxycinnamaldehyde dehydrogenase, HCALDH).

While it is generally accepted in the lay literature that approximately three monolignols are the foundational unit of lignins, a distinction of substitute monomers could be introduced into lignin structures in typical wild-type plants or through their genetic modifications; the latter, whereby incomplete monolignol generation results are incorporated into lignin (and could mostly also be available in wild-type plants of low quantities), is explored in Section V.

Natural substitute monomers could also integrate different structures, the importance of which is often overlooked despite its role in the formation of lignin in some plants. To be specific, p-hydroxybenzoates, monolignol acetates, p-coumarates and acylated monolignols, stand out. These final two products have caused some confusion since they are often mismeasured as H-lignin units. These units are generated from p-coumaryl alcohols, which are a type of monolignol. Conversely, p-hydroxybenzoic and p-coumaric acid ester conjugates produce monolignol (often coniferyl and sinapyl alcohol) conjugates.

While p-coumarate esters are generated from p-coumaroyl-CoA in the entire process, p-coumarate itself is neither a monolignol nor a lignin monomer. The conjugate's monolignol moiety couples with lignin as expected, but the p-coumarate moieties and p-hydroxybenzoate do not; instead, they act as free-phenolic pendant decorates on γ-OH groupings on C3-sidechain on the varied units of lignin. The simpler radical transfers (to more stabilized S and G radicals) compared to radical coupling is thought to be the cause. Because of their potential abundance and the potential profit they may bring to a company, pendant esters should be considered a component of lignin itself. These are not, however, the same as the monomers, which stimulate radical coupling occurrences, which are features of lignification and amount to polymer foundational unit.

Except when - coupling or cross-coupling with a monolignol occurs, the structures of the radical coupling products produced from the monolignol conjugate are the same as those generated from monolignols themselves. In each instance,
lignin is produced through unconventional tetrahydrofuran (THF) structures rather than the usual resinols, and these structures prove that the acylated lignin is generated from precouylated monolignol. In maize, the -coupling seems to originate nearly entirely from the dimerization of sinapyl p-coumarate, and the THF structures predominate over the resinol moieties. Even in extremely naturally acetylated lignins (such as curaua), whereby monomers are typically monolignol acetates, THF structures predominate. Although the p-hydroxybenzoxylation genes and acetylation of similar molecules are still unknown, the gene necessary for p-coumaroylation of monolignols has been identified and characterized.

Dihydroconiferyl alcohol within softwoods, that, under the circumstances of oxidative lignification, could also create 3-diols, guaiacylpropan-1; tricin, flavone from a totally other mechanism, just recently found in grasses. Moreover, as reviewed, tyramine ferulates, which are noticed incorporate into various Solanaeae species, including higher oligomers (dehydromers), which originates from them, should similarly be considered lignin monomers in its complete sense (such as tomato, and tobacco).

While a great deal of study has added to our understanding of the phenol derivatives in plants and metabolic pathways of lignin, the interaction between numerous genes (both generic and plant-specific) and the impacts upon lignin synthesis remain only partly explored. It is rather remarkable, though, that novel processes steps and enzymes continue to present themselves, such as CSE-catalyzed quinic acid conversion or caffeic acid to caffeoyl shikimic.

IV. BIOENGINEERED LIGNINS
When lignin has been biosynthesized from its component parts, the monomers tend to be transported to a secondary cell walls for the plants, where they undergo radical polymerization processes catalyzed by laccases and peroxidases. At now, only a fragmentary picture of the involvement of peroxidases or laccases in the ultimate polymerization of lignin monomers exists. There is a wide variety of laccases, each of which is able to stimulate radical processes necessary for the lignin sub-unit polymerization. Since they serve several purposes throughout plant growth, isolating lignin-specific laccases is challenging. However, lignification has been drastically changed when certain laccases have been silenced, suggesting that they play a role in the process.

The specific peroxidases (or groups of peroxidases) that contribute to the manufacture of lignin also need to be isolated, which will need further research. In Arabidopsis thaliana, for instance, Peroxidase 4 was only recently shown to have a role in the synthesis of syringyl lignin; however, a mutant's reduced fraction of syringyl components was only visualized under optimal illumination circumstances and was age-based. This discovery illustrates the complexities of the interaction with abiotic and biotic stress interactions and the reality that even genetically improved plants don't always exhibit the predicted change for reasons unrelated to genetic enhancement. Several efforts have been documented to determine holistic impacts (i.e., including bio-engineering and an assessment of the effects on other pathways, if not on exterior, "ambient" elements) in this setting.

Reduced lignin yields are the consequence of reduced flux through the phenylpropanoid biosynthesis pathway, which is caused by gene encoding down-regulation the enzymes tasked for the first three stages in the production of phenylpropanoids. While this strategy of creating less of the troublesome component has been favored by academics for some time, plants need lignin, and if it is reduced to an excessive degree, it may have negative agronomic impacts. Overaccumulation of Phe is the consequence of downregulation of PAL. Nevertheless, downregulating PAL in Arabidopsis thaliana resulted in a considerable reduction in lignin concentration but had no discernible effect on the plant phenotype. This finding could be due to the addition of Tyr to the phenylpropanoid pathway.

It was previously assumed that C3H 3-hydroxylated p-coumarate at either CoA or acid levels; however, recent studies have shown the existence of a novel enzyme, HCT, across a wide range of plant species; this enzyme is responsible for the production of quinic acid or p-coumaroyl shikimic conjugates, the selected C3H substrates. It was hypothesized that HCT, after the hydroxylation, would restore the products to CoA levels as the caffeoyl-CoA. On the other hand, the route seems to be complex since CSE (an enzyme) has been well-derived in particular plants as recycling the findings of C3H reactions back to caffeic acids, of which 4CL should again operate to create CoA derivatives.

Discovery of all the genes involved in the enzyme expression in the generation of lignin building units has the benefit of expanding the range of possible perturbations to the system. However, in most cases, a rise in the relative H-unit level is the primary effect of down-regulating C3H, CSE, or HCT. The downregulation or lack of HCT causes a large loss in growth, a drop in the amount of lignin, an overaccumulation of flavonoids, and a forecasted increment in relative H-unit levels. A c3h mutant, Arabidopsis ref8, is a dwarf plant, which does not produce seeds and has lignins that solely contain the H isotope. It is intriguing to note that the agronomic problems do not originate from the shift to H-lignin; rather, co-downregulating mediator gene pair restores seed production and alleviates most of dwarfing while maintaining the unique or high-H lignins properties.

The feruloyl-CoA, p-coumaroyl-CoA, and sinapoyl-CoA are abridged to their respective aldehydes before progressing toward the monolignols (via CCR). As subsequent plants such as Arabidopsis considered to be readily and noticeable saccharifiable, even without a pretreatment, CCR-downregulation has gained substantial attention, notably in Europe. Even if plants suffer a minimal developmental effect and have minimized lignins levels, the most interesting feature is the incorporation of ferulic acid as a monomer into the lignins. By analyzing CCR-deficient plants, scientists have discovered
a new method for creating lignins that are more amenable to chemical degradation; the integration resulted in unique acetal branch-point in polymers, and acetal are cleaved with acids.

An essential fork in the lignin biosynthesis process, the enzymatic conversion of coniferaldehyde may result in either G-units, S-units (F5H, CAD, and COMT), or a regeneration to HCALDH (ferulic acids). When F5H is overexpressed using strong lignin promoters, the resulting lignin is nearly entirely made up of S-units, but when F5H is downregulated or absent, the G-units tend to predominate. Nevertheless, a loss of secondary structures of the plant wall in the xylem and inter-fascicular fibers was identified in both Arabidopsis situations, resulting to a minimization in plant complexity and stiffness. There was no change in total lignin content for G-unit-rich lignins, but the saccharification yield of the biomass minimized, recommending the establishment of a more resilient biomass.

The 5-methoxyl and 3-methoxyl groups on S and G monomers are synthesized by two types of O-methyltransferases, namely the CCoAOMT and COMT enzymes. Lack of 5-O-methylation in 5-hydroxyconiferaldehyde causes the reduction of the compound to 5-hydroxyconiferyl alcohols, which is subsequently integrated into lignin into COMT-scarce plants. However, the novel 5-OH internalizes the intermediate quinone methide product, leading to the production of benzodioxane polymer structures rather than the expected 4-O—coupling between the resulting 5-hydroxyguaiacyl units and any of the monomers of hydroxycinnamyl alcohols (5-hydroxyconiferyl alcohol or prototypical monolignols). Amplification of benzodioxane levels is possible by both overexpression of F5H and downregulation of COMT. The resultant Arabidopsis plants have been shown to assimilate as much as 70% of the monomers for 5-hydroxyconiferyl alcohols and to produce different levels of benzodioxane as higher as 90% of the polymer.

Nonetheless, at this complex phenotypical level, aberrant plant development resulted. Caffeyl alcohols are integrated into lignins by OMT CCoAOMT (appealed initially in the pathways), but this only happens in softwoods (that do not compose S-lignin); tries to down-regulate both the genes (and OMTs) in the hardwood, or the dicot as a whole have amounted to any authentic proof for the integrated of caffeyl alcohols. Even after considerable down regulation of COMT and CCoAOMT, the normal S and G monolignols are produced, although at a reduced level. This suggests that additional genes (or OMTs aside from those targeted) are involved.

In many cases, hydroxycinnamaldehydes are introduced into lignification due to the CAD down-regulation, which produces CAD enzyme integrated in the last phase of synthesis for all traditional lignin monomer. Lignin may include either coniferaldehyde or sinapaldehyde as monomers. Interestingly, it was shown that coniferaldehyde is poorly incorporated into gymnosperm (G-only) lignins because it does not -O-4-cross-couple with G units in vitro or in vivo. Nevertheless, dicot lignins make good use of coniferaldehyde and sinapaldehyde due to their propensity to cross-couple with S units and G units, respectively. Several recent instances of CAD misregulation demonstrate the extent to which non-canonical monomers may be present in plants and yet allow for normal growth. Around 95% of the lignin in a Medicago truncatula mutant lacking in the enzyme responsible for making CAD is said to come from hydroxycinnamaldehydes. Moreover, plants whose lignin is formed nearly exclusively from coniferaldehyde or sinapaldehyde have been produced by manipulating the S and G monomer syntheses in conjunction with CAD deficit in Arabidopsis.

Lignification may make use of monomers that are completely unique or non-traditional, creating lignin polymers with completely new structures. In reality, some of these are also available in physically occurring wild-form plants, albeit at much lower concentrations (through sensitive analytical approaches). For instance, independent of plant species, incorporating monomers other than the standardized three S, G, or H monolignols into the structures of lignin was the consequence of downregulating/suppressing C6H, CCR, C3H, CCoAOMT, or COMT. Depending on the species, lignin may include monomers such as ferulic acid (C6H8O2), coniferaldehyde (C3H2O2), sinapaldehyde (C3H10O2), 5-hydroxyconiferyl alcohol (5OH-CA), caffeyl alcohol (C140, 143), or monolignol ferulates (C7H18O4). In addition, the recoverable low molecule weight metabolites from continuously lignifying tissues undergo constant change, with the appearance of both new compounds and increased levels of existing ones.

V. LIGNIN BIOENGINEERING CHALLENGES

Practical Challenges

Most bioengineering efforts are now focused on reducing lignin content for enhanced fermentability and increased saccharification yields. However, lignin fraction recalcitrance should also be taken into account to guarantee the biorefinery's economic viability. As this is the case, it is intriguing to examine the possibility of creating lignin with a chemically labile model by, for example, adding some ester bonding to polymers' backbone other than of the more difficult ether connections.

Studies aimed at optimizing plant lignin processing are far from easy. The lengthy period needed for biomass development and output is a significant obstacle. This ranges from around 5-30 years for most hardwoods and softwoods. In order to get around this problem, scientists have focused their efforts on bioengineering lignin for shorter-lived angiosperms (e.g., Arabidopsis thaliana, the structure dicots, and, recently, Brachypodium distachyon, the structure monocot). Both Arabidopsis and Brachypodium may serve as models for longer-lived plants like Poplar and Aspen, as well as the commercially generated monocots (such as grasses), since they have many of the same genetic and biochemical characteristics. Structure plants are helpful because they point the way toward potential genetic engineering targets.
Nevertheless, it is fundamental to not presume feature portability over monocots, gymnosperms and dicots, and the methods must be employed to the actual grasses, or hardwood or softwood trees, at some point in the process. Even among the same type of plant, there may be noticeable differences in phenotypic behavior depending on whether the plants are cultivated in a greenhouse or the wild. Poplar, aspen, and eucalyptus (hardwoods) and monocots (switch grass), barley, and maize are common examples of plants that may be transformed easily and are also rapidly growing and economically significant. For softwoods, pinus taeda (lobolly pine), and pinus radiate (radiata pine) are among the finest uses, although development is still challenging and sluggish.

When the phenylpropanoid pathway is disrupted in a plant, phenotypic changes (i.e., modifications in morphology, growth, or behavior) often occur (even though particular details for personal phenotypic transformations are challenging to identify). Reduced water transfer is a common symptom of dwarfism, which is caused by the collapse of xylem vessels. Over-accumulation of flavonoids, however, is not always linked to stunted or dwarf plant development, contrary to popular assumption. Dwarfism's exact origin is still unknown.

High-throughput multi-trait genetic alteration has been proposed as a solution to the difficulties in forecasting the physiology of plants, and long-term developmental performance after minor genetic change. This method involves analyzing a massive sample of genetically modified plants during the course of their development to identify characteristics that contribute to optimal growth. Both biological and environmental factors may cause damage to plants. Plants that show tolerance are kept for future research (evaluated for growth, saccharifiability, and/or lignin composition), whereas those that do not are thrown away. In a short amount of time, we can find out whether plants have lignins, which may be chemically disassembled using our high-throughput screening technology. If this plant is found, research may begin to attempt to pinpoint the precise genetic modifications that brought about the plant's enhanced characteristics. **Fig 1** is an example of a high-throughput approach for multi-feature genetic engineering that has been used for lignin screening.

![Fig 1. Multi-gene Engineering With High Throughput](image)

The latter phases of field trials are crucial for determining environmental implications (e.g., insects’ toxicity, effects on the chemistry of soils). Neutral sugar concentrations, structures, polymerization degree, and molecular weights are all factors in how immunostimulatory a certain lignin is. Several studies have looked at how the amount of lignin was influenced by abiotic stress (such as drought, salt, wounding, cold, and UV-B radiations), but these research findings have not reviewed structural basis of lignins, thus there are still many unanswered concerns. The phenylpropanoid and lignin biosynthesis pathways are directly affected by abiotic stress, which has an effect on lignin production. Comprehending full biological relevance of lignin in plants require knowledge of the feedback of the pathways to the abiotic stressors. Analyzing the effects of abiotic stress may be done in different forms, such as the integration of artificial stressors (such as stimulating the development of plants in a high-salinity medium) and the comparison of the plants' responses to growth in a greenhouse and in the wild. The effects of both abiotic and biotic strain of the fitness of plants during transition from a greenhouse to outdoor trials have been compared in recent reviews of the literature. However, it is difficult to foretell the performance and behavior mature trees from the investigations of greenhouse, investigations, and this is true even for non-transgenic tree kinds.

**Technical Challenges**

While there are many high value uses for technical lignins, they are mostly burned as fuel. To begin with, the economic potential of these applications is out of sync with current manufacturing of lignins; for instance, the generation of lignins is high and insufficient prospective uses exist to completely fulfil the potential of lignin. However, lignin-based goods aren't necessarily better to current products' quality even in well-established industries like adhesives, sorbents, and dispersants. Obviously, there is a need for scalable applications that make use of technological lignins. The emergence of high-quality
lignin products that outperform petroleum-produced products could increase the likelihood of commercial success. Rather, we may focus on creating items with a higher value-to-weight ratio. The use of technological lignin is limited not only by financial but also by technical considerations. Most of these issues originate from technical lignin structures and, to a lesser degree, delignification techniques. All of the issues that have been found have been categorized into one of four broad types.

Recovery from the Products Stream

Being a byproduct of both pulp mills and biorefineries, technical lignins are often overlooked. Several components in the lignin-rich streams from these locations should be filtered out or reused. Twenty to forty percent of the dry solids in lignin-containing streams produced from the main sources (such LS, soda, and kraft lignins) are dissolved lignin, with the remaining 60 to 80 percent made up of undissolved pulp and extractives. Extractives, made up of rosin and fatty acids, are scanned off the top of the liquid after being separated from the pulp in multistage pressured filters. Lignin, inorganic cooking residue, and some extractives are what remain in the lignin-reach stream once these processes are complete. The pH of black liquor, the liquid stage, which is wasted from the pulping of kraft, is between 9 and 10. The pH of spent sulfite liquor varies from 2 to 12 and is affected by the kind of cooking technique used.

Separating lignin from kraft products is a breeze when using black liquor. Filtration and precipitation are the two most common methods used in the separating process. The current standard procedure for lignin isolation is precipitation of kraft lignin. As a rule, a two-stage acidification process is used to precipitate. Then, carbon dioxide is used to lower the pH of the black liquid to 9 or 10; this causes 75% of the lignin to precipitate out as a sodium salt. In a second procedure, sulfuric acid is added to a lignin-water suspension until the pH drops below 3. Lignin treated with sulfuric acid has a higher sulfur concentration and should not be used in sulfur-sensitive applications. Lastly, lignin is extracted using filtering. It's important to note that the black liquor content has a huge role in the success of the precipitation recovery procedure. Experts agree that, in terms of dry solids, a concentration of 27-30% is ideal for precipitation. Costs related with CO2 precipitation extraction of lignin have been estimated at between €25.50 and €38.50 per tonne of lignin by Lee, Lee, Ryu, and Choi [4].

Moreover, ultrafiltration is a potential route for isolating kraft lignin from the rest of the kraft pulp. Recovering kraft lignin using ultrafiltration has been extensively researched by [5]. The main benefits of this technology are that it is adaptable (it may be used at any stage of the mill) and that lignin can be separated without requiring any changes to the mill's pH or temperature. Ultrafiltration has been deemed financially viable at an industrial scale. The cost of ultrafiltration for the generation of hardwood lignins from the black liquor was projected to be 60 €/tons of lignins at an approximation of 90% volume minimization by Jönsson, Nordin, and Wallberg [6]. In this case, a 66% volume decrease results in an extraction cost of only 17 euro cents per ton of lignin. Nevertheless, the overall cost of separation would rise due to the necessity for further processes for purifying of lignin since lignins recovered by ultrafiltration include ash (about 0.47g/g solid and large hemicelluloses quantity (about 50 g/L).

In any case, the separated technical lignins need further care to remove odors and discoloration. If not, their utility in a number of sensitive contexts is severely diminished. Lignins' dark hue derives from their chemical composition. Chromophores, the light-absorbing bonds and groups in technical lignin, are responsible for coloration. Bleaching chemicals such as chlorine dioxide, hydrogen peroxide, and ozone may be used to remedy this issue; it should be noted that bleaching is also an option for mechanical pulps with high lignin concentrations. The smelly chemicals that result from the separation methods are not the technical lignins themselves, but rather the compounds that were generated throughout the process. The smell of sulphur-containing lignins makes this a particularly crucial consideration. Smells may be eliminated by more filtration or the use of oxidizing different chemicals such as chlorine dioxide and hydrogen peroxide.

Lignin from soda ash may be extracted in the same manner as kraft lignin is. In this instance, deodorizing the booze is unnecessary. Because of its high carboxylic group concentration, which makes lignin very hydrophilic, filtering and centrifugation retrieval of non-wood soda lignins could be challenging. Minimal non-wood-generated mills without any chemical retrieval structure may greatly benefit the environment by separating lignin from wasted liquors. Soda lignin isolation by precipitation is a patented process developed by Dominguez-Robles et al. [7].

Ultrafiltration is the standard method for removing lignosulphonates from wasted liquor. As lignosulphonates include lignin fragments with a larger molecular weight, they can be more easily isolated than other types of lignin. To get the most out of ultrafiltration, it's best to filter out the carbs in the liquor first. It is possible to efficiently separate lignosulphonates and sugars using membrane separation techniques. Sugar retention could be significantly lower as 3% in case just higher molecular LS weight fraction is isolated from sugars and recovered. Other technical lignins, such as ionic lignins, and organosolv, could be retrieved by integrating non-solvent, such as water. Dissolved air flotation has been successfully employed by Tindall et al. [8] to separate lignins at temperatures, which are lower than 35 °C for organosolyn lignin recovery from the Salix Shwerinii.

The residues left behind after hydrolysis of lignin are solid. Hydrolysis lignin with higher residual cellulose contents might be considered "cellululignin", and it is important to note that hydrolysis lignin may include considerable levels of unhydrolyzed cellulose, up to 15%. The proportion of residual cellulose is significantly more for hydrolysis lignin that is enzymatic. As hydrolysis lignin develops in its solid condition, there is minimal requirement for the application of
complex recovery processes; it could be filtered via the fine mesh. However, instinctively recovered lignin integrates diversified contaminants. Jia, Li, Wang, Yin, and Zhang [9] have utilized NaOH extraction to retrieve lignin for application within polyurethane manufacturing. Another challenge of the recovery of hydrolysis lignin is the fundamental water retention capacity of the lignins, restricting hydrolysis lignin application as a fuel.

Table 1 summarizes the various techniques for extracting technical lignins. As can be observed from the Table 1, only lignosulphonates, and kraft lignin have been recovered at industrial sizes, whereas the most of the technologies have only been shown in the lab or/and at pilot scale. However, none of these techniques can reliably isolate clean lignins, which could be conveniently used in chemical syntheses. Retrieved lignins often have high levels of organic and inorganic contaminants. Although technologies like precipitation and ultrafiltration have been around for a long, newer techniques for processing lignin are still in the experimental stages. The quality and cost-effectiveness of retrieved lignins are two primary objectives in technological lignin separation.

**Purification of Lignins**

Sugars, proteins, sulphur, silicates, ash, and other chemicals extracted from either the delignification process or the raw materials are examples of impurities that may be found in technical lignins. First, technical lignins must be purified so that they may be employed in chemical or biological conversion procedures. Cleansing entails the elimination of contaminants such sugars, extractives, and inorganics.

<table>
<thead>
<tr>
<th>Lignin Type</th>
<th>Status</th>
<th>Separation Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILL</td>
<td>Lab</td>
<td>Precipitation (integration of non-solvent)</td>
</tr>
<tr>
<td>OSL</td>
<td>Lab/Pilot</td>
<td>Precipitation (integration of non-solvent)</td>
</tr>
<tr>
<td>HL</td>
<td>Lab</td>
<td>Dissolved air flotations</td>
</tr>
<tr>
<td></td>
<td>Pilot/Industrial</td>
<td>NaOH extraction</td>
</tr>
<tr>
<td>HL</td>
<td>Industrial</td>
<td>Ultra-filtration</td>
</tr>
<tr>
<td>Soda</td>
<td>Lab</td>
<td>Ultra-filtration</td>
</tr>
<tr>
<td></td>
<td>Lab/Pilot</td>
<td>pH change (precipitation)</td>
</tr>
<tr>
<td>Kraft</td>
<td>Industrial</td>
<td>Ultra-filtration</td>
</tr>
<tr>
<td></td>
<td>Industrial</td>
<td>pH change (precipitation)</td>
</tr>
</tbody>
</table>

In case lignins are going to be applied in chemical syntheses of low-molecular polymers or weight compounds, then they need to be purified to the utmost possible degree. Impurities, if left untreated, may reduce yields, degrade product quality, and even lead to the development of unwanted byproducts. In addition, some contaminants, such as sulfur, may kill catalysts in chemical reactions.

The value of technical lignins as raw materials may be drastically diminished by the presence of sugar residues. Notwithstanding this challenge, effective techniques for sugar and lignin isolation are available. Acid/base extraction may be used to separate the precipitated sugars from the lignin due to their dissimilar solubilities. While extraction is often used for this purpose, it may be somewhat challenging to separate alkaline lignins from other sugars when the carbohydrates are chemically bound to the lignin. A two-step process (enzymatic hydrolysis followed by acid hydrolysis) has been developed by Yun et al. [10] for isolating kraft lignin; they claim it removes almost all of the sugars present in the compound. Hemicelluloses are abundant in wasted sulphite liquor, and the sugars in hemicelluloses may form chemical bonds with lignosulphonates. Due to their shared solubility in water, lignosulphonates and sugars provide significant challenges when trying to isolate one from the other. To extract sugars from sulphonated lignin, fermentation of sugars is utilized in sulphite pulp mills. Organic acids and aldehydes are only two of the many fermentation inhibitors found in sulfite wasted liquors, necessitating the use of specialist techniques to render them fermentable. While ultrafiltration and precipitation are often utilized, there are other techniques that may be used to isolate the lignosulphonates.

Water washing is all that's needed to get rid of ash and other inorganic contaminants that dissolve in water. Organic nitrogen (proteins and amino acids) is the source of the nitrogen found in lignins. Proteolytic enzymes, including proteases, might be used to break down lignin, therefore removing any nitrogen-containing compounds. Sulfur is chemically bound to lignin, making it extremely challenging to extract from kraft lignin and lignosulphonates. Around 70% of the sulphur in kraft lignin is biologically attached sulphur, whereas 30% may be washed away. Sulphur may be eliminated from lignin by a process called Raney nickel reduction. However, this technique is both expensive and difficult to use in industry. The bacterium Thiobacillus has shown effective in removing sulphur from coal, suggesting that it might be employed for microbial removal of sulphur from lignin. For employment where the deficiency of sulphur is fundamental, the sulphur-free lignin has to be employed because none of the aforementioned processes can assure the complete elimination of sulphur.
Silica may be extracted from non-wood pulping waste liquors using a variety of techniques. The most common technique is carbonation, which may eliminate up to 70% of the silica. Calcium oxide and sulphuric acid are two other desilication options. Loganathan, Hedley, Clark, and Bolan [11] provide a comprehensive overview of these techniques. For the most part, silica does not need to be removed since it is an inert substance. Treatment with hot water or a protease may remove nitrogen from lignin. There are a variety of techniques available for removing contaminants from lignin. Regrettably, there are cases when these techniques would not be economically viable to utilize. Separation techniques also have limitations when it comes to removing chemicals that are chemically bound to lignin.

Non-Uniform Structure
Variation in polymer size, content, cross-linkage degree, and availability of the functional groups, as well as other structural features, characterize the wide range of technical lignins. Variations in monomer makeup and bond type among lignin fragments account for the majority of the key variations. Additionally, lignin composition differs from plant to plant and even within a single plant's tissues. Whereas guaiacyl units make up about 95% of softwood lignin, the composition of other plants (hardwoods, straw) is more diverse, resulting in a blend of three different phenylpropanoid units. Hydroxyl, methoxyl, carbonyl, and carboxyl are the four most common functional groups in lignin. The quantity and composition of these groupings are context-specific and extraction-specific. The lignins used in technical applications are a composite of many lignins fragmentation with different molecular weight and polydispersities. Organosolv lignin has the lowest polydispersity, followed by Kraft lignin, lignosulphonates, and soda lignin, in that order.

One way to get the structural homogeneity of lignin fragments to a certain point is by controlled degradation. Either a chemical or an enzymatic approach may be used to accomplish degradation. Lignin fragments of desired molecular weight (Mw) intervals may be obtained by controlled degradation; the Mw intervals should be determined by the lignin's intended use. There are a few different ways to get gradual deterioration under control. By integrating a single solvent, or solvent combination with a predefined attraction concentrated on a certain fragments Mw, the lignin fragments within the given Mw interval may be identified. At high temperatures, Watanabe et al. [12] treated alkali lignin using an ethanol-NaOH combination.

The original lignin had a molecular weight (Mw) of 10,000 to 60,000 g/mol, but after degradation, its molecular weight distribution narrowed to a range of 450 to 1000 g/mol, making it more soluble. When naphtol-2 is added to the lignin separation process, condensation processes are inhibited, the reactivity of lignin is increased, and the molecular weights of the lignin fragment are significantly reduced. In this case, the carbonium ions are being removed by the naphtol-2. Nevertheless, since pyrolysis involves free-radical processes, it is not possible to create lignin pieces with a pre-defined structure during lignin degradation. Chemical oxidation combined with microwave irradiation is another another technique for the controlled breakdown of lignin. Narrow Mw distributions are achievable with this kind of therapy.

The lignin structure may be altered by enzymatic processes, as was discussed before. Nonetheless, substantial research efforts are needed at the current time to develop these techniques. Recently, Zhang et al. [13] examined the state-of-art application of enzymatically-corrected lignin. Certain processes call for fragments having a molecular weight (Mw) greater compared to Mw of the beginning technical lignins. Lignin polymers may have their molecular weight increased by oxidative polymerization by laccases. None of these techniques can reliably generate a uniform set of lignin chain fragments; hence it is unclear how to achieve structural homogeneity in lignin. Effective approaches for lignin homogenization are urgently required.

Unique Reactivity
Technical lignins' chemical reactivity is essential to several uses. As lignins are structurally disordered, they often act in unanticipated ways. For a technical lignin to be employed in the formulation of synthetic resin, structural homogeneity is of paramount importance. Due to the presence of many functional groups, technical lignins may respond in numerous ways, only some of which may be beneficial. Lignin structure is intrinsically linked to its reactive properties. Fewer ortho and para reactive sites in poorly accessible locations reduce lignin's reactivity. The molecular weight can be reduced by oxidative and other techniques, the structure may be modified to enhance the number of particular functional segment, and the lignin fragment with targeted structure and Mw can be isolated to improve reactivity.

There are currently two primary methods for enhancing lignin's reactivity: Depolymerizing lignin into oligomers and monomers is the first technique, whereas phenolation introduces reactive sites into lignin molecules. Reactivity in the ortho and para locations of lignin stays unchanged despite the depolymerization that increases accessibility to reactive sites. Lignin reactivity may be increased with the use of enzymes, including laccases, which encourage lignin oxidation processes. In the process of these reactions, new reactive sites are created.

According to Gao et al. [14], depolymerization and a phenolation process may be used together. In this instance, phenol was employed to seal the bottle. Depolymerized lignin with high reactivity was obtained by the treatment. The presence of phenolic hydroxyl (OH) groups in lignin is necessary for its high reactivity, whereas the presence of aliphatic hydroxyl (OH) groups, which facilitate carbohydrate bonding, may have a detrimental effect on reactivity. Chemical reduction using sodium dithionite may increase the number of phenolic hydroxyl groups. He, Li, Zhou, Gu, and Jiang [15] suggest using genetic manipulation of the CAI5dh (coniferyl-aldehyde 5-hydrolase) gene family in order to influence the
lignin’s reactivity and structure. Because of the potential for genetic manipulation, this method has great promise. However, this approach is unlikely to be used anytime soon. In other contexts, GM lignins have been considered.

VI. CONCLUSION

This paper has provided a discussion of upstream processing, including the phenylpropanoid route, bioengineered lignins, and lignin bioengineering. In addition, this paper addresses the challenges inherent in lignin bioengineering from a practical and technical standpoint. Integrating chemically-labile connections deliberately to lignin’s chemical structure is, thus, an intriguing prospect for further study. In addition, advances in "high-throughput multi-gene engineering" will help in determining which particular successful tactics may be used to lessen the native lignin polymers’ recalcitrance. The extremely interdisciplinary character of the study (from the first integration of genetic mutations to the final chemical production) and the richness of factors connected with every phase of valorization (and the challenges linked with attributing the experiential phenotypic changes to certain biotic or abiotic effects) make any bioengineering method problematic. It takes a wide range of knowledge and skill sets to successfully genetically design a plant species with lignin that is more easily chemically deconstructible without compromising the plant’s viability. There must be a focus on the long term, guaranteed financing, and the dedication of numerous complementing research teams for this to happen.

Data Availability
No data was used to support this study.

Conflicts of Interests
The author(s) declare(s) that they have no conflicts of interest.

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