

Using biology to add value to waste lignin streams



Geoff Dumsday

CSIRO Molecular & Health
Technologies
Clayton VIC 3168
Tel: (03) 9545 2344
Fax: (03) 9545 2109
Email: geoff.dumsday@csiro.au

Arising from the need to find renewable sources of fuels and chemicals, a number of biological and chemical processes are in development or have been commercialised that enable the use of biomass as a starting material. Examples that have reached full commercial scale include production of 1,3-propanediol (a monomer for production of a plastic similar to the polyethylene used in plastic bottles) ¹ and lactic acid which is also a monomer used in the production of polylactic acid – a biodegradable plastic ².

A somewhat more important and larger scale requirement is the conversion of biomass into liquid transport fuels. Whilst in recent times commercial efforts have been largely directed towards conversion of starch and sugar to ethanol or oilseed and tallow to biodiesel, these processes are widely recognised as being unsustainable ³. This is due mainly to the fact that there is insufficient feedstock to meet more than a small fraction of transport fuel needs and conflicting use of food crops for fuel production. On the other hand, lignocellulose derived from either waste biomass or dedicated energy crops grown on marginal land is seen as having the potential to provide a significant proportion of liquid fuel requirements. However, the resistance of lignocellulose to physical and chemical breakdown results in the cost of production of liquid fuels from this starting material being significantly higher, and the technological hurdles are somewhat more difficult to overcome.

To offset the production cost increase, the concept of biorefineries has been developed. This has come about from the realisation that economical and sustainable use of biomass as a starting material for fuels and chemicals will require extracting the maximum possible value from the highest possible proportion of the biomass feedstock and subsequent waste streams. Adding value to lignin, a major and largely underutilised component of lignocellulose, is seen as an opportunity to improve the economics

of converting lignocellulose to ethanol. Living organisms have already established processes that modify lignin structure, and better exploitation of these processes has the potential to both increase the utility of lignin streams and to generate renewable products that can be used in chemical and other industries.

The biorefinery

In many ways the biorefinery concept is similar to current crude oil refineries, where a range of different streams are used to derive a number of fuels and chemical feedstocks. In the future, many of the roles presently played by the oil refinery may well be replaced by the biorefinery ⁴. Essentially the biorefinery concept defines optimal use of a biomass feedstock via a range of processes to deliver a diverse array of products including fuels, chemicals and even agricultural products such as fertiliser and animal feed. An example of a biorefinery that uses lignocellulose as a feedstock is shown in Figure 1 ⁵.

As shown in Figure 1, rather than fuel ethanol being the major product derived from lignocellulose, the biorefinery produces a vast array of products (including fuels) that have a wide range of applications. The system also has in-built flexibility which enables product outputs to be easily changed, ensuring that maximum economic value can be captured from each product type. The lignin stream, which can represent up to 30% (by weight) of the biomass, is largely underutilised and is potentially a rich source of aromatic and other compounds. Most lignocellulosic-based processes use waste lignin streams as a fuel that can be used as an energy source to generate heat and electricity for the process. Partially dried lignin has around 80% of the combustion efficiency of hard coal, but its attractiveness as a fuel is somewhat reduced by the requirement for drying to around 60% solids before burning. The heat generated by combustion of the lignin streams generated by the pulp and paper industry is only marginally greater than that required to evaporate the water from spent liquors ⁶. Adding value to lignin waste streams is therefore seen as an opportunity to improve the economics of converting lignocellulose to ethanol by providing a range of products that have extensive utility in the chemical industry.

Lignin

Globally, around 10¹¹ to 10¹² tons of terrestrial biomass are produced every year, of which 15-36% is the lignin component of woody plants (gymnosperms and angiosperms) ⁷. Lignin is the most abundant natural aromatic polymer on earth and is poorly degraded by most microorganisms because of its complex random structure, which lacks regular hydrolysable bonds and has

a high degree of hydrophobicity⁸. Although lignin demonstrates a high degree of resistance to microbial attack, dead plant tissue is eventually degraded to humus, carbon dioxide and water.

Chemically, lignin is a heterogenous, optically inactive polymer that consists of phenylpropanoid interunits which are linked by several covalent bonds including aryl-ether, aryl-aryl and carbon-carbon bonds with the composition of being largely dependent upon the type of biomass⁹. The lignin from softwoods is generally harder to degrade due to a larger number of reactive sites resulting in greater cross-linking of the lignin (Figure 2)⁷.

Current uses of lignin

A number of uses for waste lignin streams have been identified, including adhesives, agricultural chemicals, water treatment, cement additives, drilling, production of rubbers, dispersing agents, tanning and batteries. Despite at least 4000 references being published on the utilisation of lignin for polymeric materials¹⁰, its large-scale use is limited and, until recently, lignin streams have been seen as having little or no value. The main driving force behind this vast amount of research is the pulp and paper industry which recognises that up to 50% of their feedstock is not effectively utilised. Some of the uses of polymeric lignin are outlined in more detail in Table 1 (see also study by Hu¹¹).

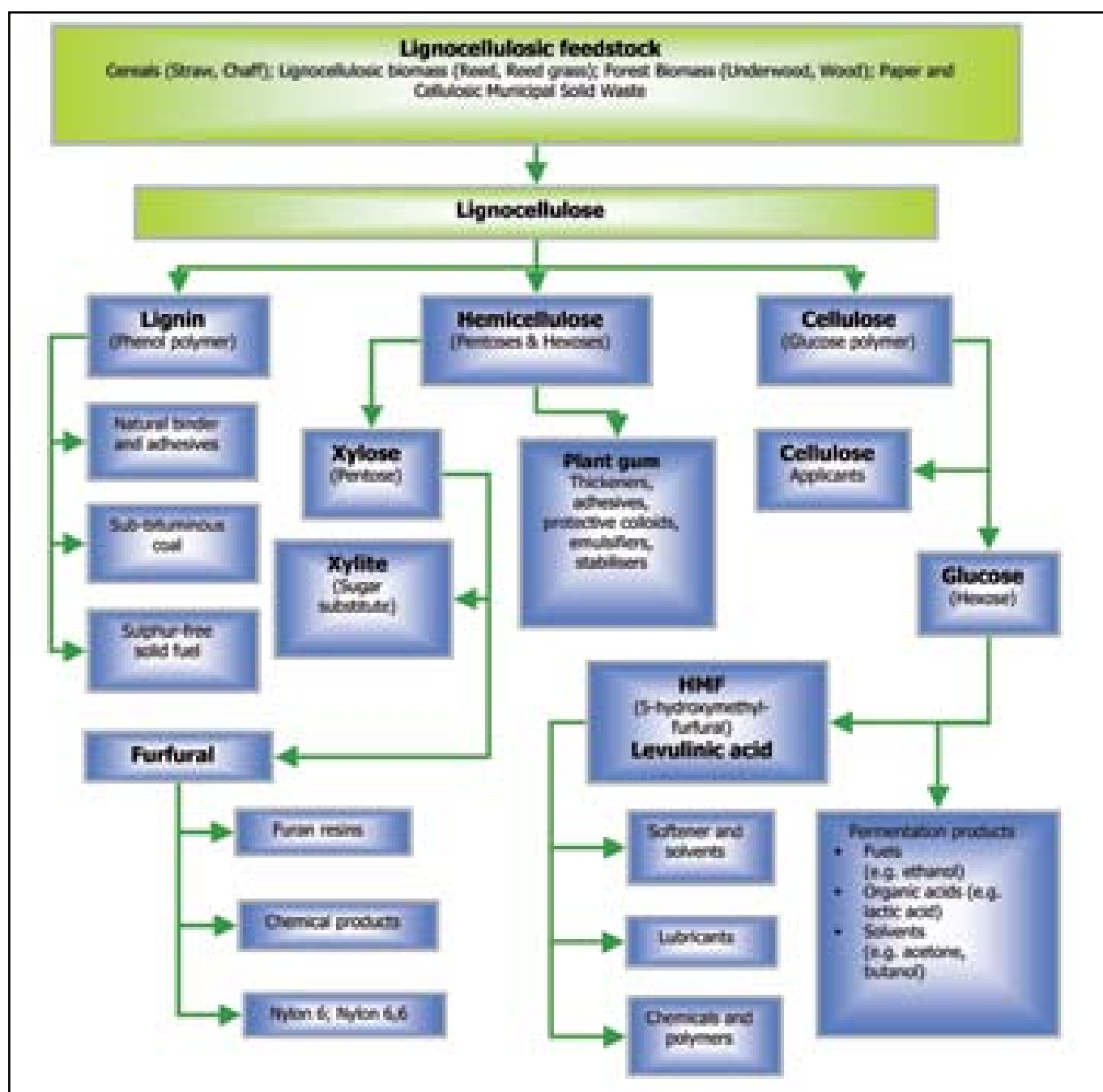


Figure 1. Schematic representation of a biorefinery using lignocellulosics as the starting material (adapted⁵).

Biotransformation of small molecules derived from lignin

As oil becomes more expensive, the cost of chemicals derived from crude oil also increases and some supply chains are becoming saturated or unreliable. Large multinational chemical companies are also looking to source more of their starting materials from renewable resources. These global trends represent a significant opportunity for new sources and production methods for many of the commodity chemicals currently sourced from oil. As a starting material, lignin has the potential to provide many of the chemicals required by the chemical industry and, where possible, the use of biological processes will enable production of these chemicals to be cleaner, greener and more energy efficient.

In terms of the biological processes associated with lignin, perhaps the best known and most widely studied group of microorganisms capable of degrading lignin are the white-rot fungi. This group are currently the only known organisms that are capable of efficient lignin mineralisation with CO_2 and H_2O as the end products¹⁸.

A number of enzymes are involved in lignin degradation by white-rot fungi, including manganese peroxidase, lignin peroxidase and

laccase. The enzymes do not act directly on the lignin structure with the degradation process being non-specific and mediated by mobile highly reactive small molecules produced by the enzymes. For example, manganese peroxidase exhibits specificity for complexed Mn^{2+} , oxidising it to trivalent Mn^{3+} ¹⁸. The Mn^{3+} is stabilised by organic acids such as oxalate and malonate and the Mn^{3+} -organic acid complex diffuses from the enzyme and oxidises phenolic substrates such as lignin substructures and aromatic pollutants¹⁹, a process that is essentially enzyme-mediated combustion.

Reports of lignin degradation by bacteria are rare. However, Don Crawford, after discovering *Streptomyces* species that can degrade lignocellulose, spent many years elucidating the mechanisms of partial lignin breakdown by this group of microorganisms^{20, 21}. Biological breakdown of lignocellulose and lignin are typically slow processes and therefore it is likely that a combination of thermochemical and biological processes will be required to realise the full potential of lignin streams. Thermochemical treatment of lignin can release a number of small aromatic molecules such as aromatic aldehydes and phenols.

At CSIRO we have used such compounds as substrates for selecting microbes capable of degrading them under a

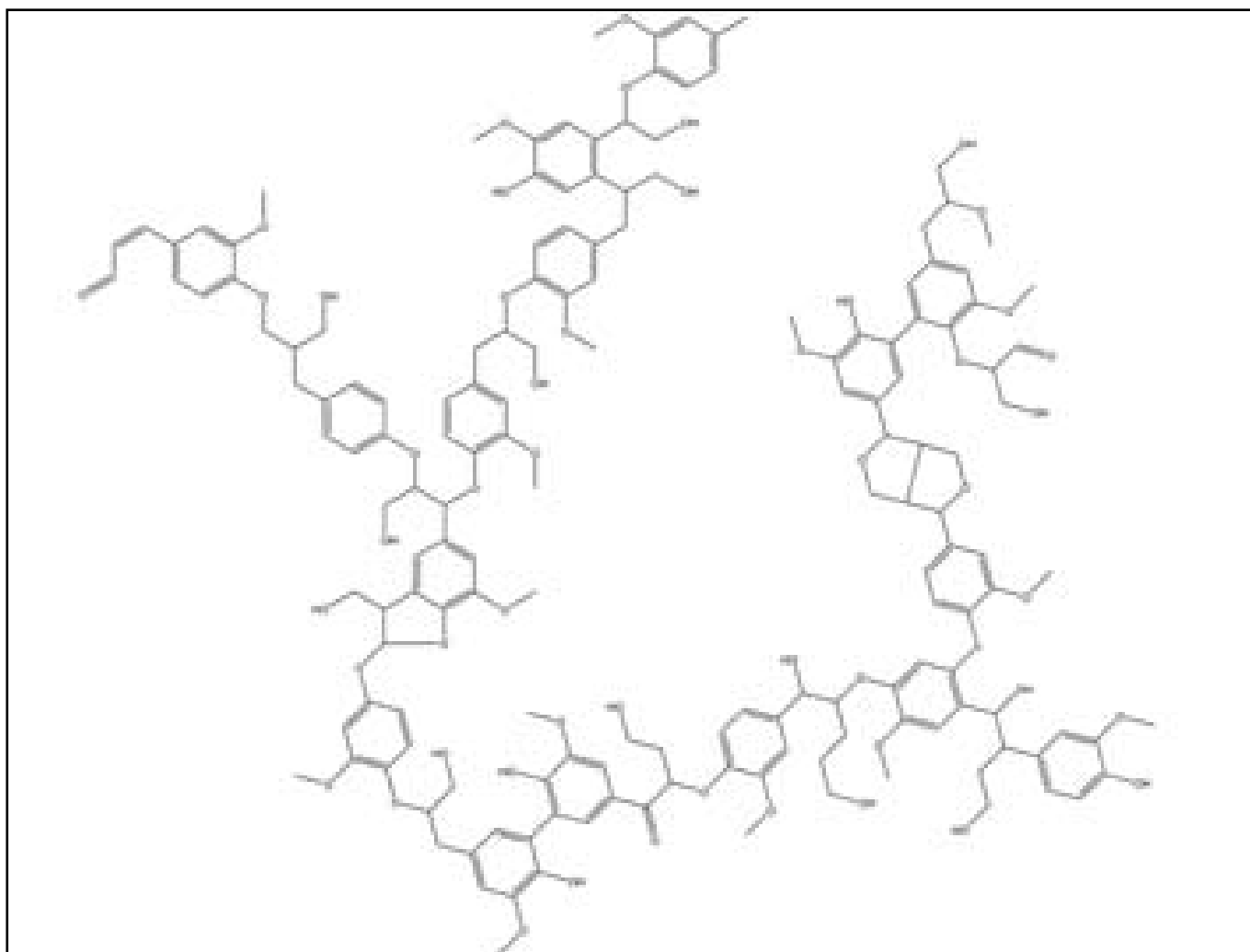


Figure 2. Schematic formula for a section of spruce lignin highlighting the complexity of the structure and the diverse chemical functionality (adapted⁷).

range of environmental conditions. To date, more than 160 microorganisms has been isolated which are capable of metabolising at least one of the lignin-derived molecules, and these include examples which grow in acidic, alkaline or salty environments and at low (16°C) and high (55°C) temperatures. CSIRO's wood rot fungal culture collection (comprised of over 2000 isolates) was also screened for the capacity to degrade the aromatic compounds. Several metabolic intermediates were detected in cultures of microbes capable of degrading the small molecules; these are now being identified using conventional analytical chemistry. Identification of the compounds will enable the determination of the value of these metabolites in their own right and provide insight into metabolic pathways used to degrade the aromatic compounds. The metabolic pathways may provide processes for large-scale conversion of lignin-derived small molecules into the commodity chemicals that are widely used today, and potentially other compounds with novel applications.

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Geoff Dumsday is a Senior Research Scientist at CSIRO. His research interests are focused on industrial microbiology specifically the use of microbial systems for production of useful products ranging from vaccines and enzymes to the production of small molecules from a range of starting materials, particularly biomass.

Table 1. Examples of use of polymeric lignin.

Lignin source and reference	Process	Use
Pulp and paper ¹²	Water insoluble lignins with the addition of small amounts of petrochemical resins	Particle board production
Organosolv lignin ¹²	Co-polymerisation of lignin and acrylamide in aqueous dioxane. Laccase was used to generate lignin radicals and dioxane peroxides	Grafted copolymers
Beech pre-hydrolysate – viscose pulp production ¹³	Blending	Composite with polypropylene
Steam exploded <i>Eucalyptus globulus</i> ¹⁴	Epoxidation	Lignin epoxy resin
Not stated ¹⁵	Enzyme treatment	Removal of substances from waste water
Pulp and paper ¹⁶	Firing (microwave/plasma processing)	Carbon fibre composite for cars
Acetosolv and Formacell lignin from <i>Eucalyptus grandis</i> ¹⁷	Phenol/formaldehyde/NaOH treatment	Resol-type resins