ANTIMICROBIAL PROPERTIES OF LIGNIN COMPOUNDS

RESEARCH PROPOSAL

A research proposal submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Biosystems Engineering
at the University of Kentucky

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Abstract

The overuse of antibiotics in agriculture has become an emerging concern to our society, due to the detrimental impact caused by antibiotics to the environment and ecosystems. Lignin, accounting for about 1/3 of the plant biomass, is the most abundant natural aromatic polymer on earth. The fate of lignin could take a turn from being disposed as a waste to a valuable commodity. Development of antimicrobial properties from lignin one of the focus areas surrounding this organic material. This study aims to look at the potential use of lignin-derived compounds to combat microbial contaminations in corn ethanol bio refinery. We will use a 48-well plate-screening platform to scan a range of lignin model compounds on the growth of yeast, E. coli, and lactobacillus. Next, we will extract and test the actual lignin derived compounds from three different lignin breakdown methods (pyrolysis, oxidation and hydrogenolysis). Results from this study will provide a better understanding of how lignin can replace the antibiotics currently used in corn ethanol fermentation.

Background

Lignin is a compound that is found in all plants. It is that part of the plant that is responsible for the plants rigid structure. This compound is made up of three main monolignol monomers: p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S). Different plants have different ratios of these three monolignol monomers. In recent years, the idea that these could have antimicrobial properties has come to the forefront of research interest.

Literature Review

<u>Lignin</u>

As the most abundant source of renewable aromatic compounds on the planet, lignin has the potential to replacing petroleum-based chemicals and products. It is however an under-utilized resource due to its structural heterogeneities and difficulty to work with. Understanding the structures of lignin will aid in better fabrication of lignin applications. A deeper understanding of the lignin properties will lead to better utilization of this resource. (Zhao, 2016).

<u>Oxidation Break Down</u>

The processes called pulping and bleaching are required for preparing industrial scale pure cellulose from biomass. The oxidation reaction of these bleachable components consume stoichiometric volumes of oxidant and take hours to react. The addition of catalysis can increase the efficiency of oxidants. Catalytic oxidation has different effects at different pH, temperatures, and oxidant dosage. (Chenna *et al.,* 2013).

<u>Hydrogenolysis Break Down</u>

The hydrogenolysis of lignin should be performed in an autoclave with 65 vol.% ethanol/water as solvent, with 5% Ru/C, Pd/C and Pt/C as catalysts. The influences of catalysts, pretreated lignin, and reaction conditions can effect target compound yields. (Ye *et al.,* 2012).

<u>Pyrolysis Break Down</u>

Pyrolysis takes place between 400-700°C. Three main products come out of this process: pyrolysis oil, char, and gas. The pyrolysis oil can be broken down into heavy oil and light oil. As the temperature gets closed to 700°C more pyrolysis oil and less char is produced. The aliphatic OH, carbonyl and methoxyl groups, and the ether bonds in the lignin are the targets to breakdown during this process. (Haoxi and Ragauskas, 2012).

Different biomass has different lignin types, this type effects the thermal behavior. Degradation of aspen lignin starts above 200 °C, forming acetic acid, methanol and methylacetate, with a maximum rate around 340°C. Throughout pyrolysis, the formation of particular compounds is difficult to distinguish. (Brebu *et al.,* 2011).

Microbial Break Down

The search for alternative sources of fuels that are inexpensive, ecofriendly, and that can replace fossil fuels is increasing as the demand for energy continues to rise. A major bottleneck is lignin, which is a protective covering and makes cellulose and hemicellulose recalcitrant to enzymatic hydrolysis. A number of biomass breakdown processes have been utilized to break the framework of plants and depolymerize lignin. (Chaturvedi and Verma, 2013).

Lignin is an amorphic three-dimensional substance. The chemical structure of lignin has also been difficult to determine, and even very recently, new bonding patterns have been described in softwood lignin. The results obtained from using microbes on lignin not easy to quantify. Little is known about what happens when micro-organisms, like white-rot fungi, degrade lignin in wood. (*Biodegradation,* 2005*).*

The feasibility of the combination of fungal and mild acid pretreatments are an increasing area of study. Combined pretreatment with Phanerochaete Chrysosporium and 2.5% sulfuric acid has been shown to be more effective than the acid-only pretreatment. (Xiaohua, 2013).

Objective

There are two main objectives for this study. The first objective is to determine if the degradation compounds of lignin (monomers and dimers) have antimicrobial properties. Six different lignin monomers will be tested: guaiacol, vanillin,vanillic acid, syringaldehyde, 2,6-dimethoxyphenol, syringic acid. These compounds will be screened against yeast, E.coil, and lactobacillus. The second objective will be to identify different lignin breakdown compounds. An acid and alkaline pretreated lignin from corn stover will undergo pyrolysis, oxidation, and hydrogenolysis. The product of these methods will then be analyzed.

Methods

In order to test lignin's components against microbes, the lignin will first have to be broken down. Three pretreatment methods have been chosen for this: phrolysis, hydrogenation, and catalytic oxidation. Each of these pretreatments will cause lignin to breakdown in a slightly different way and each method will produce a slightly different mixture of lignin compounds. These different compound mixtures were measured by gas chromatography – mass spectrometry (GC-MS) to determine what percentage of different monomers and dimers lignins are present in each of the different mixtures.

Six monomers will be screened, guaiacol, vanillin,vanillic acid, syringaldehyde, 2,6-dimethoxyphenol, and syringic acid, against three different microbe, yeast, E.coil, and lactobacillus, to test from antimicrobial properties. This screening will also be done with the different lignin breakdown mixtures themselves. As it is nearly impossible to separate the different compounds in the mixtures, the mixtures themselves will be used as a whole.

The microbe in question will be grown up in a 250mL flask. Using a spectrophotometer, the wavelength will be set to 600nm and then the OD will be read. Using a 48 well plate, each column in the plate will have a different lignin compounds. Starting with zero concentration at the top of the column and increasing concentration going down the column. The concentration of microbe in each well will be kept constant in each row. The first well in each column having no lignin compounds present, it will be considered as the control. The 48 well plate will be read every half hour until 24 hours have been reached. The pure lignin mono compounds will be compared to the mixture screening to see if there is a relation to a certain compound having a selective antimicrobial property.

Appendix A

Budget

Direct Costs	Year 1	Year 2
A. Salaries and Wages		
(1) Grad student	$16,000	$16,000
(2) Advisor	$24,500	$24,500
Total Salaries and Wages	**$40,500**	**$,500**
B. Fringe Benefits		
(1) Grad Student		
Health insurance	$2,500	$2,500
Tuition	$14,190	$14,190
Social Security	$1,224	$1,224
(2) Advisor		
Health insurance	$11,064	$11,064
Social Security	$6,464.25	$6,464.25
Retirement	$8,450	$8,450
Total Fringe Benefits	**$46,892.25**	**$46,892.25**
C. Travel		
ASABE meeting	$1,000	$1,000
Total Travel	**$1,000**	**$1,000**
D. Materials and Supplies		
Chemicals	$1,000	$1,000
Enzymes	$1,000	$1,000
Test plates	$500	$500
Total Materials and Supplies	**$2,500**	**$2,500**
E. Equipment		
Spectrophotometer	$5,000	
Total Equipment	**$5,000**	
F. Other Direct Costs		
Publication costs		$1,500
Subcontracted	$1,500	$1,500
Total Other Direct Costs	**$1,500**	**$3,000**
Total Direct Costs	**$56,892**	**$53,392**
Indirect Costs	**$28,446**	**$26,696**
Total Costs	**$85,338**	**$80,088**

Appendix B

<u>Research Plan</u>

There are three main milestones for this research project. The first one is to figure out the lignin composition based on three different breakdown processes: hydrogenolysis, catalytic oxidation, and pyrolysis. The second milestone is to perform the anti-microbial test. Once both of these milestones are finished, the third one, finalizing data and doing an analysis, can begin.

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