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Lignin from Sugar Process as Natural Antimicrobial Agent

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Abstract

An increase in health awareness, emerging communicable disease, and an increasing of older population support the important of product with antimicrobial characteristic. Market research reported an increase in global revenue of antimicrobial product to reach $16 million by the end of 2020. Currently, trend of antimicrobial agent is moving toward organic material but more research and investigation is required before it can completely replace traditional material, metallic compound. Lignin is one of the major natural resource and by-product from industrial process that exhibit antimicrobial activity. Variation of plant source play significant role in antimicrobial activity due to its variation of basic compound of lignin. In Thailand, it is estimated that approximately 7.5 million ton is available for utilization, annually, from sugar industry alone. Diversity of lignin composition, its antimicrobial activity and large supply of the material that is waiting to be utilized provide great opportunity for utilization of lignin as antimicrobial agent.

Keywords: Lignin; Antimicrobial agent; Sugar process; Thailand

Introduction

Lignin: natural extracts antimicrobial agent

Lignin, one of the major natural resource and industrial by-product among others, cellulose and CO_2, is an abundant natural resource found in all plant that capture scientific and industrial community as potential carbon source to replace current petroleum-based material for many applications e.g. carbon fiber and adhesive product. Lignin, a sub-category of polyphenol, [1] exhibits antimicrobial characteristic and has been continuously investigated as substitute antimicrobial agent for current metallic antimicrobial component because of its abundancy, renewable, and especially degradability. Organic antimicrobial agent gains significant attention as it does not stay in an environment unlike metallic antimicrobial agent [2] and shows lower toxicity compared to inorganic antimicrobial agents [3].

Supply of lignin

From an industrial point of view, lignin is a major by-product and has been commercially available in variety of grade (purity) with pricing in the range of $1,400 – $1,800 per ton [4]. Thailand sugar industry heavily uses large amount of sugarcane which subsequently produces sugarcane bagasse as by-product. Approximately 99 million tons per years of sugarcane was used by sugar industry [5]. As the bagasse is a major by-product from sugar process that accounted for approximately 28% of sugarcane that entering the factory [6] and normally contains 27% of lignin per mass of bagasse [7], it is estimated that 7.5 million tons of lignin is available each year in Thailand for utilization. In Thailand, there is no commercial product that adopt lignin as antimicrobial agent. To the best of our knowledge – in Thailand, lignin extraction is not widely implemented for further application and lignin generally stay within by-product, then enter processing line as fuel (Figure 1).

Important of lignin for Thailand's industry

In Thailand, natural extracts gain significant attention for medical and cosmetic application and receive generous opportunity for research and product development. Application of lignin and other by-product from sugar processing for non-food product are being evaluated by many investors and research agencies. With its highly functionalize characteristic and abundancy, it is likely that utilization of lignin in practical application in particularly coating of packaging items will be seen in the near future of Thailand.

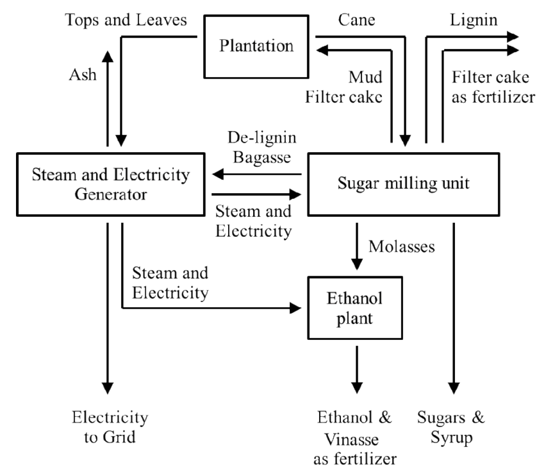

Figure 1: Products and by products of sugar cane processing with proposed lignin extraction unit inside sugar milling process.

Discussion

Antimicrobial activity of lignin

Lignin extracts usually has dark brown color. Lignin has been recognized to exhibit antimicrobial activity due to its two of the three basic structures, coniferyl alchol and sinapyl alcohol [8]. It was shown that softwood has high portion of coniferyl (>95%) and hardwood has relatively similar content between coniferyl alchol (25-50%) and sinapyl alcohol (45-75%) [9]. Based on this information, antimicrobial performance of lignin is relative to plant source (Figure 2). IR spectra of lignin extracts from sugarcane bagasse by alkali treatment method is shown in Figure 3.

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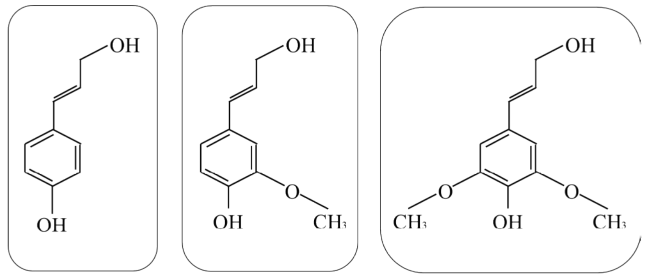

p-Coumaryl alcohol (4-hydroxyl phenyl) coniferyl alcohol (guaiacyl) sinapyl alcohol (syringyl)

Figure 2: Monomer of lignin.

Structural diversified of plant-based antimicrobial compound is large that leads to variation to antimicrobial action against microorganism. Most of phenolic compound that is considered as antimicrobial agent contains hydroxyl group. This functional group is currently considered as a cause to exhibit antimicrobial action by interacting with the cell membrane of bacteria to disrupt the cell membrane structure that is eventually cause the leakage of cell component [10]. Recent study also reported similar mechanism for antimicrobial activity of lignin to have similar effect by interacting with microbial cell wall that include a rupture of cell membrane and subsequently release cell content [11]. In addition, Ugartondo et al. conduct investigation on molecular weight distribution on cytotoxicity activity, reported showed heaviest lignin exhibited the least cytotoxicity [12]. This information suggests the fractionation of lignin is required to adjust and optimize lignin for antimicrobial product.

Performance of lignin compare with silver nanoparticles

Broth dilution method was implemented to identify minimum inhibitory concentration (MIC) value. Lignin extracts was dissolved in dimethyl sulfoxide and injected into nutrient broth containing bacteria. Series of lignin extracts solution was mixed and inoculated with targeted bacteria. The MIC value was identified by observing the lignin extracts concentration that changes the color of resazurin solution or observation of mixed solution turbidity, which is directly related to growth of microorganism. A positive and negative control well were prepared as a baseline to demonstrate microbial growth during the incubation period and sterilized medium, respectively.

Dong et al. [13] studied antimicrobial activity of lignin from corn stover from ethanol production. It was founded that lignin from corn stover yield MIC and MBC against *Staphylococcus aureus* at 1.250 and 3.125 µg/ml, *Listeria monocytogenes* above 10 µg/ml for both values, and *Candida lipolytica* at 3.75 and above 10 µg/ml. Boonruangrod et al. [14] investigated antimicrobial performance of lignin from sugarcane bagasse at different shelf life (fresh and 10-12 months old). According to the study, there were no different in antimicrobial performance between different shelf life of sugarcane bagasse prior to lignin extraction. Concentration to inhibit (MIC) and prevent (MBC) bacteria formation were evaluated as follow; *Staphylococcus aureus* (DMST 8840) 2,500 and above 2,500 µg/ml, *Escherichia coli* (DMST 4212) 1,250 and above 2,500 µg/ml, *Salmonella typhimurium* (DMST 562) 1,250 and above 2,500 µg/ml, *Vibrio cholera* (DMST 15778) 1,250 and 2,500 µg/ml.

Our previous study showed crude lignin extracts from sugarcane bagasse against *Staphylococcus epidermidis* (DMST 15505) yielded MIC at 4,096 µg/ml [15]. In our non-published results, a 50-liter pressurized tank can extract to obtain crude lignin from sugarcane bagasse by alkaline treatment process for $0.03/g of crude lignin. Alkaline treatment was selected due to its low-cost chemical recovery technique and effective waste water treatment [1]. MIC of silver nanoparticle with average particle diameter 26 nm was 1.69 µg/ml [16]. Retail price of commercial silver nanoparticle, 20 nm, for 0.5 mg was $164 [17]. Quick comparison between material price and amount based on MIC without accounting for actual content of antimicrobial agent in product

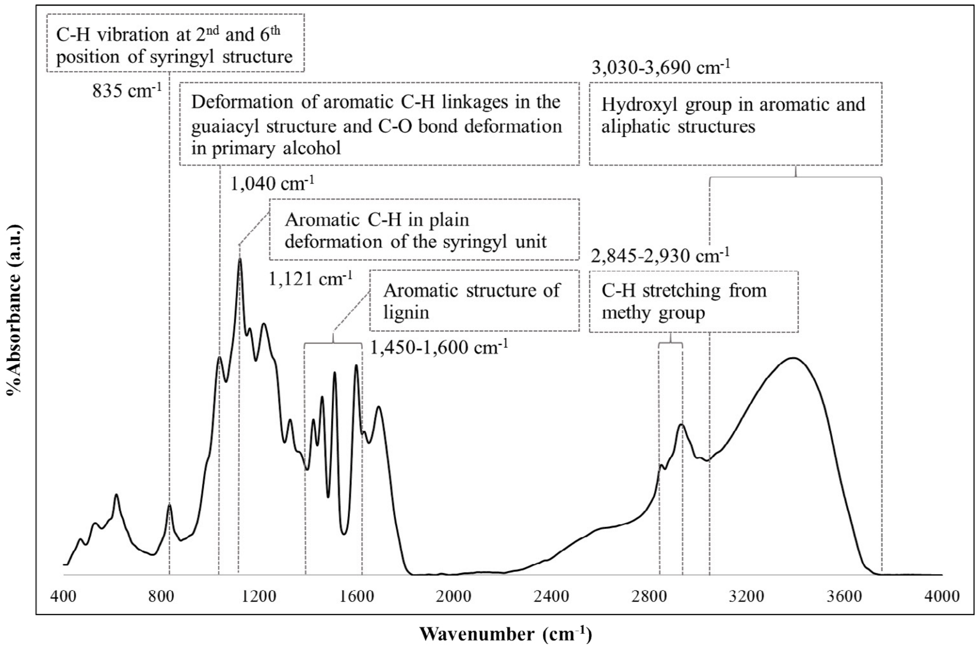

Figure 3: IR spectra of lignin from sugarcane bagasse.

(material cost*MIC), cost of lignin ($0.03/g*4,096 µg/ml) was cheaper than silver nanoparticle ($164/0.5 mg*1.69 µg/ml) by approximately 1,500 times. This shows the potential of utilize lignin as antimicrobial agent as replacement of traditional metallic antimicrobial compound. Effective amount of antimicrobial agent in product and shelf life may yield different number of this estimation but it confirms potential of lignin based on (i) material cost and its (ii) antimicrobial performance.

Product development

Lignin from different sources were investigated as potential antimicrobial agent against varieties of bacteria. Hui and Lincai [18] investigated antimicrobial performance of coated cellulose fiber by commercial lignosulfonate and chitosan by alternated immersion technique against *Escherichia coli*. Study showed product with outer coated layer as chitosan had better antimicrobial performance than product with outer coated layer as lignosulfonate. In addition to development of antimicrobial fabric from lignin from sugarcane bagasse against *Staphylococcus epidermidis*, we proposed a modified technique to evaluate antimicrobial activity as a function of lignin coating amount and period of contact as a tool for product development [15]. According to our investigation, it was possible to achieve bactericidal level within 6 hours of contact at specific concentration on fibrous.

Further development is required to compile the test according to standard protocol for antimicrobial textile product; ATCC Test Method 100 (Quantitative evaluation) and AATCC Test Method 147 (Qualitative evaluation) against gram positive *Staphylococcus aureus* and gram negative *Klebsiella pneumonia*, before commercialization phase.

Global trend of antimicrobial coating agent and market

An overall trend of antimicrobial product is expected to increase during the next decade. Due to an increasing in health awareness, emerging communicable disease, and an increasing of older population, it is expected that by the end of 2020, global revenue of this product will reach 16 billion [19].

Three types of major antimicrobial agents were investigated then combined into a comprehensive market report (source: NSTDA's subscriber to Frost & Sullivan) [20]. Metallic antimicrobial coating agent is currently the main component; which include silver, copper, zinc, selenium, palladium and its combination of these metals. Current key metallic material for antimicrobial coating is combination of metallic component with main composition is silver metal, according to number of publication and commercially available product by market survey. Polymeric coating material, polyurethane, polyphosphoethers, and poly(vinylpyrollidone) are major component in this product category. These polymers are not classified as antimicrobial agent but provide lower adhesion thus prevent adhesion of microorganisms on targeted surface. Organic coating agent, quaternary ammonium sulphates, biguanides, phenolics, and chlorhexidines are sold at lower price compare to metallic and polymeric coating agent that is likely to stimulate the consumption and sale volume.

Due to high price of coating process that is transferred to end user, process with lower manufacturing cost is required. Emerging coating agents and process are likely to be discarded if high investment cost is involved. High demand of product due to an increasing in awareness of personal hygiene and innovated technology are key factors to ensure an expansion of the market. Antimicrobial characteristic is being introduced to the commodity products such as plastic bottle and food packaging [21].

Conclusion

Lignin has long been recognized by Thailand industrial sector as valued by-product but development during the last decade on biorefinery of sugarcane revive interest in this material. Many industrial bodies invest heavily in collaborative research with research agencies and universities to implement innovated ideas for lignin-based product into practice. Direction of lignin-based product such as antimicrobial coating agent and substitute material for non-food application looks positive. Until now, there is an extensive research on resistance and shelf life assessment towards metallic antimicrobial agents and quaternary ammonium salts, similar apprehension regarding lignin is necessary before major replacement take places.

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