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Antimicrobial Activity of Lignin-Derived Polyurethane Coatings Prepared from Unmodified and Demethylated Lignins

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Abstract: Due to global ecological and economic challenges that have been correlated to the transition from fossil-based to renewable resources, fundamental studies are being performed worldwide to replace fossil fuel raw materials in plastic production. One aspect of current research is the development of lignin-derived polyols to substitute expensive fossil-based polyol components for polyurethane and polyester production. This article describes the synthesis of bioactive lignin-based polyurethane coatings using unmodified and demethylated Kraft lignins. Demethylation was performed to enhance the reaction selectivity toward polyurethane formation. The antimicrobial activity was tested according to a slightly modified standard test (JIS Z 2801:2010). Besides effects caused by the lignins themselves, triphenylmethane derivatives (brilliant green and crystal violet) were used as additional antimicrobial substances. Results showed increased antimicrobial capacity against *Staphylococcus aureus*. Furthermore, the coating color could be varied from dark brown to green and blue, respectively.

Keywords: antimicrobial activity; brilliant green; crystal violet; demethylation; lignin; polyurethane coatings; triphenylmethane dyes

1. Introduction

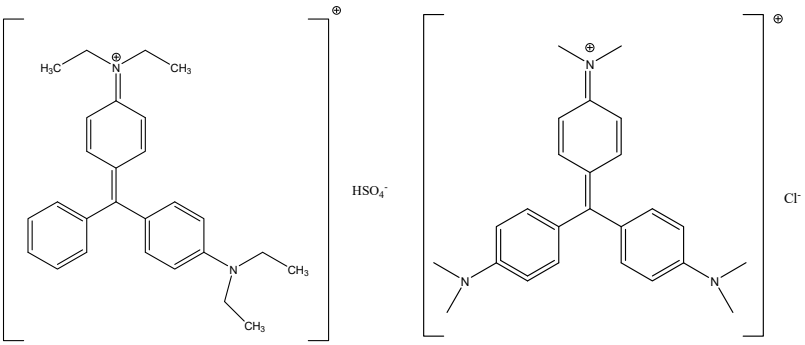
Lignin, the most abundant natural resource next to cellulose and hemicellulose [1–4] contains various functional groups that provide active sites for chemical modification such as polarity adjustment to enhance the compatibility of lignin with other polymeric matrices in lignin/polymer composites [4,5] or to improve antioxidant properties [6–11]. Furthermore, studies reported lignin-derived encapsulation of various drugs for biomedical and agricultural applications. Richter et al. reported the encapsulation of silver nanoparticles in lignin-coated polymers [12]. Gregorova et al. studied the encapsulation of lignin nanoparticles in polyethylene films (Björkman lignin from beech wood flour) [13]. In other studies, the delivery of Resveratrol® [14], the controlled release of Avermectin® [15], lignin–polyurea microcapsules with anti-photolysis and sustained-release performances [16], montmorillonite–lignin hybrid hydrogel as super-sorbent for dye removal from wastewater [17], cellulose–lignin hydrogels and their controlled release of polyphenols [18], lignin-stimulated protection of polypropylene films

and DNA in cells of mice against oxidation damage [19] have been tested. Gao [20] and Bshena [21] studied the antimicrobial activity of various textiles, using lignin incorporated into polyethylene films and applied in the finishing processes. For textiles, there are special requirements such as non-toxicity to the consumer, namely cytotoxicity, allergy or irritation and sensitization. In other recent studies, lignosulfonic acid is reported to exhibit broad-spectrum anti-HIV (human immunodeficiency virus) and anti-HSV (herpes simplex virus) properties [22,23]. Thus, Qiu investigated the anti-HIV-1 activity-potential of lignosulfonates as a microbicide to prevent HIV-1 sexual transmission [23]. Another recently reported study revealed that the antimicrobial capacity of lignin correlates with the phenolic components, specifically the side chain structure and the nature of further functional groups [24]. Typically, the presence of a double bond in α, β positions of the side chain and a methyl group in the γ position grants the phenolic fragments with the most potency against microorganisms. However, none of the hitherto published studies included the investigation of the antibacterial activity of lignin when included in polymeric matrices.

Unmodified lignin is widely studied as a component for polymer production with a focus on phenol–formaldehyde resins and polyurethanes (PUs) [25], where lignin is used as polyol substitute due to the high amount of hydroxyl groups resulting in high crosslinking densities and variable mechanical properties [26–28]. In previous studies, lignin-derived polyurethane coatings have been prepared using Kraft lignin isolated at room temperature from aqueous media (black liquor) at different pH values [29]. In addition, their antioxidative activity has been investigated using the Folin–Ciocalteu (FC) assay [30]. Although lignin contains many functionalities, they are often difficult to access due to rather strong steric hindrance. So far, various procedures have been explored to incorporate more OH groups into the lignin structure including hydroxymethylation, phenolation, demethylation, oxidation and reduction [31]. These modifications have been studied primarily in conjunction with phenol–formaldehyde (PF) resins or PU research using lignin as a replacement for fossil-based phenols and polyols [32].

In 2016, Li et al. reported using demethylation to enhance the chemical reactivity at atmospheric pressure to produce fast curing phenolic resins [33]. Another possibility for lignin demethylation is an enzymatically catalyzed reaction using fungi (i.e., white and brown rot fungi) or bacteria (i.e., *Pseudomonas*, *Sphingomonas*). Mainly laccase was investigated, which oxidizes the guaiacyl into catechol units [34]. Industrially, demethylated lignin is recovered as a byproduct in dimethylsulfoxide (DMSO) production. For this purpose, black liquor is mixed with molten sulfur at about 230 °C. Two methyl groups are transferred from the lignin to the sulfur, forming dimethyl sulfide, which is oxidized to DMSO with nitrogen dioxide. Based on this process, Kraft lignin was demethylated with sulfur at 225 to 235 °C under high pressure and successfully increased its reactivity for the synthesis of phenol–formaldehyde (PF) resins [34]. Sulfur and halogen compounds are also used as nucleophiles for the chemical demethylation of lignin. For example, Chung and Washburn have demethylated softwood Kraft lignin with hydrobromic acid under the catalytic action of hexadecyltributylphosphonium bromide at 115 °C for 20 h, resulting in an increase in the OH content of 28% [35]. PU foams synthesized from the modified Kraft lignins showed a higher compressive strength than conventional ones [36,37]. Song et al. used the same method for white straw alkali lignin, with results that showed a significant increase in the total hydroxy content of demethylated lignin [38] when samples were explored for the synthesis of bio-based PF resins by demethylations with sulfur-containing compounds (sulfur, *n*-dodecyl mercaptan, sodium hydrogen sulfide and sodium sulfite). Here, soda lignin was heated with the reagent for 1 h at 90 °C. This research aimed to provide a cost-effective and efficient method for the chemical demethylation of lignin. The best results in terms of an increase in OH content and use for PF resins was the sample demethylated with Na_2SO_3. Other authors used Na_2SO_3 for demethylation performed under high-pressure reactors [39] or under reflux [15,40]. Podschun et al. chose a different approach in which organosolv lignin was demethylated under microwave radiation [41].

The antimicrobial properties of various dyes, in particular triphenylmethane (TPM) derivatives such as malachite green and crystal violet, have been studied since their first successful application as bioactive additives more than a hundred years ago (Figure 1, Table 1).



(**a**) (**b**)

Figure 1. Molecular structure of (**a**) brilliant green and (**b**) crystal violet (see also Table 1).

In 1891, methylene blue, another TPM derivative was discovered by Paul Ehrlich to be efficient in malaria treatment, a few years later followed by the discovery of the antiseptic capacity of brilliant green (BG) [42–44]. For many years, malachite green was one of the most frequently used disinfectants in aquaculture due to its fungicidal effects. Due to the discovery of antibiotics and biocide polymers, antimicrobial dyes have not only been displaced in biomedicine but also other applications. Bolous et al. intensively studied the mechanisms of antimicrobial effects [45,46]. In detail, it was reported that the evidence to link the antimicrobial properties of TPM dyes, especially brilliant green, to the activity of mechanosensitive ion channel (MIC) of large conductance, which is known to be highly specific and ubiquitous in various bacterial species [47]. In 2012, Vilela et al. reported a study using methylene blue (MB) and malachite green photosensitizer microbial reduction of *Staphylococcus aureus* by synthesizing biofilms with it. The best results showed microbial reduction with 3000 μM of malachite green with a microbial reduction of 1.6–4.0 $\log_{10}$ [48]. In oral cavities, biofilm formation is considered to cause resistance to antimicrobial agents. Photodynamic therapies using phenothiazinic photosensitizers first confirmed the antimicrobial effect in biofilms. Malachite green was then compared with the phenothiazinic photosensitizers (methylene blue and toluidine blue) on *Staphylococcus aureus* and *Escherichia coli* biofilms. Noimark et al. reported the synthesis of modified photobactericidal silicones for medical applications. In detail, crystal violet and/or methylene blue were incorporated into the silicone bulk and gold nanoparticles were coated using a dipping method. The polymers showed good photostability, the photobactericidal activity was determined against *Staphylococcus epidermidis* and *Escherichia coli*. The results showed that these multi-dye–nanogold-polymers exhibit strong photobactericidal activity both under light and dark conditions [49]. Bartoszewicz et al. filed a patent claiming lubricious antimicrobial coatings containing silver, pyrrolidone carboxylic acid (PCA) and a TPM dye (malachite green). The coating composition of the invention provides photostability to the silver ions contained therein and is hydrophilic and antimicrobial [50]. In 2016, Santos et al. comprehensively reviewed various classes of antimicrobial polymers and discussed their bioactivity mechanisms including biocidal activity, antifungal and antibacterial capacity against numerous microorganisms (i.e., gram positive and gram negative bacteria and fungi) [51]. Table 1 summarizes literature reporting the antimicrobial activity of lignins and triphenylmethane derivatives such as malachite green, brilliant green, methylene blue and crystal violet.

Table 1. Literature studies regarding antimicrobial activity of lignins and triphenylmethane derivatives (i.e., malachite green, brilliant green, methylene blue and crystal violet).

Sample Composition	Studied Activity (Antibacterial, Antifungal)	Microorganisms (Bacteria, Fungi)	Results	References
Triphenylmethane (TPM) dyes	Mechanistic studies of the antimicrobial effects of triphenylmethanes (crystal violet, methylene blue, malachite green, brilliant green).	Various gram positive and gram negative bacteria	Evidence to link the antimicrobial properties of TPM dyes, especially brilliant green, to the activity of mechanosensitive ion channel (MIC) of large conductance, known to be highly specific/ubiquitous in various bacterial species.	Bolous et al. [45–47]
TPM dyes (i.e., methylene blue, malachite green) used as photosensitizer for acrylic resins	Microbial reduction of biofilms.	*S. aureus*	Best microbial reduction with 3000 μM malachite green with microbial reduction of 1.6–4.0 $\log_{10}$.	Vilela et al. [48]
TPM-based antimicrobial surfaces	Antimicrobial effects of crystal violet and methylene blue.	*S. epidermidis* (RP62a) and *E. coli* (NCTC 25522)	Light-activated antimicrobial surfaces with enhanced efficacy induced by a dark-activated mechanism.	Noimark et al. [49]
TPM-based coating additives (i.e., brilliant green, crystal violet) for polymeric substrates including PU	Antimicrobial activity of photo-stable composition used for coating a variety of medical materials.	Not specified	The coating composition comprising silver and TPM dyes (malachite green) provided photostability to the silver ions and antimicrobial activity.	Bartoszewicz et al. WO 2009/015476 Al [50]
Antimicrobial polymers	Bioactive polymers including biocidal activity, antifungal and antibacterial capacity.	Various gram positive and gram negative bacteria	Comprehensive review discussing different mechanisms regarding antimicrobial effects in polymer materials.	Santos et al. 2016 [51]
Lignin/HPMC and HPMC/lignin/chitosan composites	Antibacterial effects.	*E. coli* and *S. aureus*, *B. thermosphacta* and *P. fluorescens*	Testing the films against spoilage bacteria that grow at low temperatures revealed the activity of the 30% addition on HPMC/lignin against *B. thermosphacta* and *P. fluorescens*. HPMC/lignin/chitosan films (5% lignin) showed activity against both *B. thermosphacta* and *P. fluorescens*.	Alzagameem et al. 2019 [52]
Cellulose and lignin effects on disintegration, antimicrobial and antioxidant properties of PLA active films	Antimicrobial, antioxidant and disintegrability activities	Gram negative bacteria: *Xanthomonas axonopodis* pv. *vesicatoria* and *Xanthomonas arboricola* pv. *pruni*	Inhibition capacity for Gram negative bacteria (*Xanthomonas axonopodis* pv. *vesicatoria* and *Xanthomonas arboricola* pv. *pruni*) for lignin-modified PLA films.	Yang et al. 2016 [53]
Lignin derivatives (epoxides, esters, ether)	Antimicrobial activity of chemically modified lignins (by acetylation, epoxidation and hydroxymethylation reactions).	*Bacillus aryabhattai* and *Klebsiella*	Epoxy/lignin was found to be the most effective antibacterial among modified lignin with minimum inhibitory concentration of 90 and 200 μg/disc.	Kaur et al. 2017 [54]
Lignin for benign encapsulation	Antimicrobial activity of nanoparticles coated with LignoBoostTM softwood Kraft lignin.	*E. coli* and *Pseudomonas aeruginosa*	Nanoparticle flash precipitation with subsequent silver ion infusion and polyelectrolyte coating including lignin.	Richter et al. 2015 [12]
Antibacterial lignin–polyethylene (PE)	Lignin nanoparticles embedded in polyethylene films (Björkman lignin from beech wood flour).	*E. coli* and *S. aureus*	Lignin particles exhibit antibacterial effect against *E. coli* and *S. aureus* in the same order of magnitude as other antibacterial agents such as Bronopol® and Chlorohexidine®.	Gregorova et al. 2011 [13]

In a recently published study, chitosan/hydroxypropylmethylcellulose (HPMC) composites with varying ratio up to 30% of Kraft lignins (isolated from black liquor and purified via solvent extraction) were prepared and tested against spoilage bacteria that grow at low temperatures. The results revealed the activity against both *B. thermosphacta* and *P. fluorescens* for samples with 30% lignin. In HPMC/lignin/chitosan films, the 5% addition exhibited activity against both *B. thermosphacta* and *P. fluorescens* [52]. Currently, these lignin-derived composites are studied regarding their applications as scaffold component for mesenchymal stem cell differentiation and bone regeneration [55]. To do so, lignin as feedstock component has to be specified including protocols for quality control using novel chemometric data analysis methods [56,57].

In the present study, lignins isolated from black liquor at different pH values were used to explore the potential of these compounds as an antimicrobial component in polyurethane coatings. First, the extraction conditions that favored high lignin yields were optimized. Unmodified and demethylated lignins were used to prepare the lignin–polyurethane (LPU) coatings. The last part of the study aimed to correlate the antimicrobial properties with extraction conditions (i.e., pH value) and molecular structures (unmodified versus demethylated lignins). Furthermore, the influence of additional antimicrobial dyes (brilliant green and crystal violet) on the LPU coating bioactivity, color and morphology was studied.

2. Materials and Methods

2.1. Extraction of Kraft Lignin (KL) and Organosolv Lignin (OL)

The Kraft lignin (KL) was extracted through the acidic precipitation from black liquor according to a procedure reported by Garcia et al. [58]. First, about 450 mL of black liquor was filtered with a vacuum filter. The filter cake was rejected. Of the filtrate, 400 mL was heated to 50–60 °C. Sulfuric acid (160 mL, 25 vol.%) was added while stirring. The mixture was stirred for another hour at room temperature and then vacuum filtered. The filter cake was washed with distilled water and sulfuric acid (25 vol.%) until the requested pH value was reached (pH 2 to pH 5). Finally, the precipitated lignin was dried in a freeze dryer for 48 h. The organosolv lignin (OL) was isolated according to a procedure recently reported [10].

2.2. Synthesis of Demethylated Kraft Lignin

For the demethylation, a procedure reported by Li et al. was used and slightly modified [33]. The sample (1 g), 0.1 g of Na_2SO_3 as the demethylating reagent and 6 g of 2.5 mol NaOH solution were introduced into a 15 mL rolled rim glass on an analytical balance and homogenized. The solution was heated with stirring to 90 or 72 °C and stirred for 1 h at this temperature. After cooling to room temperature (RT), the pH was adjusted to pH 2 by means of 1% HCl. The demethylated lignin precipitated as a brown solid. The suspension was transferred to a 45 mL tube and centrifuged for 10 min at 3000 rpm to separate the demethylated lignin from the aqueous solution. The lignin was washed with distilled water and the pH adjusted to pH 7 with 2.5 molar NaOH solution. It was again centrifuged (for 30 min at 4000 rpm) to separate the aqueous phase from the demethylated lignin. The product was first stored at 40 °C in a drying oven and then freeze-dried at 80 °C and 0.10 mbar. Subsequently, the samples were homogenized and transferred for storage in rolled edge glasses, which were closed with snap lids. Furthermore, the samples were protected against UV radiation.

2.3. Size Exclusion Chromatography

Size exclusion chromatography was used to determine the number-average (M_n) and weight-average (M_w) molecular weights of lignins and their polydispersities, analogue to recently reported methods [29,30]. A PSS SECurity^2 GPC System was used with tetrahydrofuran as the mobile phase, a run time of 30 min and an injection volume of 100 µL. The system was calibrated using polystyrene standards at different molecular weights.

2.4. Determination of Hydroxyl Groups

The content of hydroxyl groups was determined via two different methods. ISO 14900:2001(E) developed for polyether polyols with steric hindrance was recently reported [29]. Shortly, each lignin sample was boiled under reflux in 25 mL of acetylation reagent solution with a blank sample simultaneously under the same conditions. After three hours at reflux, the flasks were left to cool down to room temperature. Twenty-five milliliters of sample and blank, respectively, were filled up with water to 100 mL and were titrated with sodium hydroxide (0.5 M). The split up of the acetylated samples and blanks allowed a triple determination via titration. Different amounts of sample and blank were needed. The differences were used to determine the total hydroxyl content.

2.5. Antibacterial Activity of Lignin

The antimicrobial activity of the lignin powders samples was analyzed in a quantitative way by modifying the test for antimicrobial activity and efficacy (JIS Z 2801:2010) of liquid samples [59]. The JIS is based on a comparison of bacteria counts in saline solution on reference and sample materials after a defined incubation temperature and time. *Staphylococcus aureus* (DSM No. 799) was applied as the test organism. The inoculum was prepared in the same way as described above. According to the McFarland-standard the inoculum was adjusted in physiological saline solution with tryptone (Blank, Vörstetten, Germany; VWR International, Darmstadt, Germany) to a concentration of 108 cfu mL^{-1}. This inoculum suspension was diluted in physiological saline solution with tryptone (Blank, Vörstetten, Germany; VWR International, Darmstadt, Germany) to a final concentration of 105 cfu mL^{-1}. Lignin powder was added into tubes with 5 mL physiological saline solution with tryptone to a final concentration of 0.1, 0.01 and 0.001 g mL^{-1}. Each tube was inoculated with 50 µL of the inoculum. The same measurements were done in nutrient broth instead of physiological saline solution. The measurements were carried out in triplicates.

The inoculum (1 mL) was incubated at 37 °C for 24 h in a mixture of 9 mL nutrient broth (Merck KGaA, Darmstadt, Germany) and 1 mL of sample or reference. Afterwards viable counts were determined by counting the colonies on plate-count agar after incubation at 37 °C for 24 h.

The value of antimicrobial activity was calculated by subtracting the logarithmic value of viable counts of the sample from the logarithmic value of reference material after inoculation and incubation:

$$log_{10} - reduction = log_{10}\left(\frac{c_{gew}(reference)}{c_{gew}(sample)}\right) \quad (1)$$

where as $c_{gew}(reference)$ = arithmetic mean of bacterial counts of reference 24 h after inoculation, and $c_{gew}(sample)$ = arithmetic mean of bacterial counts of sample material 24 h after inoculation. According to the JIS Z 2801:2010 a material can be characterized as antimicrobial, if the calculated log_{10}-reduction is ≥2.0 after 24 h at 37 °C [59].

2.6. Hemmhoff Test

The antimicrobial activity of the lignin was tested according to the disk diffusion test of the National Committee for Clinical Laboratory Standards (NCCLS) standard method. The disk diffusion test is based on the diffusion of the sampling material in agar. If the bacterium is sensitive to the tested substance, the growth of the bacterium is inhibited and a visible inhibition zone arises. The inhibition zone is the defined area between the punched out area and the beginning of the grown bacterium. If there is no inhibition zone, the bacterium is not sensitive to the tested substance.

Staphylococcus aureus (DSM No. 799) was used as a test organism. The inoculum was prepared by transferring a frozen culture to 10 mL of nutrient broth (Merck KGaA, Darmstadt, Germany). The nutrient broth with the inoculum was incubated at 37 °C for 24 h. According to the McFarland-standard the inoculum was adjusted in physiological saline solution with tryptone (Blank, Vörstetten, Germany; VWR International, Darmstadt, Germany) to a final concentration

of 108 cfu mL^{-1}. In each Petri dish (Sarstedt AG, Nümbrecht, Germany) 100 µL of the inoculum was spatulated on Mueller–Hinton agar (VWR International, Darmstadt, Germany) which were impregnated with the different lignins and blank filter papers as references and were put on the inoculated agar plates.

The agar plates were incubated at 37 °C for 24 h. Afterwards, the diameter of the inhibition zone was measured with a digital caliper (Traceable Digital Caliper 6, VWR International, Darmstadt, Germany).

2.7. Synthesis of Lignin-Based Polyurethane Coatings

PEG400 was obtained from Sigma-Aldrich (Steinheim, Germany). 4,4-Diphenylmethane diisocyanate (MDI, for synthesis) was purchased from Merck in Darmstadt and triethylamine (TEA, for synthesis) was received from Carl Roth GmbH in Karlsruhe. All chemicals were used without further purification. PEG400 was mixed with lignin to obtain 1 g of polyol blend. Coatings prepared from lignins isolated at different pH values were produced analogously to the previously described procedure, with the MDI amount adapted to the hydroxyl number of the lignin and the resulting polyol blend. Lignin-based PU coatings were prepared using unmodified and demethylated lignins, respectively, and 4,4-diphenylmethandiisocyanate (MDI). The NCO:OH ratio was 1.7. The calculation was performed according to literature reference [60,61]:

$$\frac{\mathrm{NCO}}{\mathrm{OH}} = \frac{w_{\mathrm{MDI}} \times [\mathrm{NCO}]_{\mathrm{MDI}}}{w_{\mathrm{L}} \times [\mathrm{OH}]_{\mathrm{L}} + w_{\mathrm{P}} \times [\mathrm{OH}]_{\mathrm{P}}} \quad (2)$$

where w_{MDI}, w_{L} and w_{P} are the weights (g) of MDI, lignin and polyol, respectively. $[\mathrm{NCO}]_{\mathrm{MDI}}$ is the molar content of isocyanate groups in MDI, 8.0 mmol/g for 4,4′-MDI. $[\mathrm{OH}]_{\mathrm{L}}$ and $[\mathrm{OH}]_{\mathrm{P}}$ are the molar contents of total hydroxyl groups in the lignin and the polyol, respectively. Masses of lignin and polyol were kept constant. Thus, 1 g of lignin was dissolved in 6 mL THF under constant stirring. MDI was added and the mixture was transferred on a polyethylene (PE) transparency and dried for 1 h at room temperature. Finally, the pre-films were cured at 37 °C for 3 h to obtain the final lignin PU films. The synthesis of lignin-modified PU coatings with brilliant green (BG) and crystal violet (CV) followed the same procedure, using 0.8% (*w*/*v*) of the corresponding triphenylmethane derivative.

Analogously, 1 g of demethylated lignin was dissolved in 6 mL of THF under constant stirring to prepare the LPU coatings. MDI was added and the mixture was transferred onto a PE-transparency and dried for 1 h at room temperature. Finally, the pre-films were cured at 35 °C for 3 h to obtain the final lignin-derived PU films.

2.8. Antimicrobial Activity of the LPU Coatings

The antimicrobial activity of the coatings was analyzed based on the Japanese Industrial Standard (JIS) Z 2801:2010 [59]. The JIS is based on the comparison of bacteria counts on sample coating/surface and reference material after a defined storage temperature and time (35 °C, 24 h). The reduction of bacteria counts were calculated and represented as $\log_{10}$-reduction. The $\log_{10}$-reduction is a measure for the antimicrobial activity and effectiveness of the coatings. According to the JIS a material is called antimicrobial when the $\log_{10}$-reduction is ≥2 $\log_{10}$.

Staphylococcus aureus (DSM No. 799) and *Listeria monocytogenes* were used as test organisms. The inoculum was prepared by transferring a frozen culture to 10 mL of nutrient broth (Merck KGaA, Darmstadt, Germany). The nutrient broth with the inoculum was incubated at 37 °C for 24 h. According to the McFarland-standard the inoculum was adjusted in physiological saline solution with tryptone (Blank, Vörstetten, Germany; VWR International, Darmstadt, Germany) to a final concentration of 108 cfu mL^{-1}. This inoculum suspension was diluted in physiological saline solution with tryptone to a final concentration of 105 cfu mL^{-1}.

The coatings and references were inoculated with 400 µL of the inoculum suspension. To enlarge the contact area of the coatings with the inoculum, the inoculum was covered with a sterile foil (Interscience,

Saint-Nom-la-Bretèche, France). The plates were incubated at 37 °C for 24 h. After incubation the inoculated suspension was washed out with 10 mL soybean casein lecithin polysorbate 80 broth (SCDLP) solution (Merck KGaA, Darmstadt, Germany). This served as the first solution stage and was used for further decimal solution series. The bacteria counts were determined by using the drop-plate-technique and counting the colonies on plate-count agar (Merck KGaA, Darmstadt, Germany) after incubation at 37 °C for 24 h.

The value of antimicrobial activity was calculated by subtracting the logarithmic value of viable counts of the sample from the logarithmic value of reference material after inoculation and incubation:

$$log_{10} - reduction = log_{10}(\frac{c_{gew}(reference)}{c_{gew}(sample)}) \quad (3)$$

where c_{gew} *(reference)* = arithmetic mean of bacterial counts of reference 24 h after inoculation, and c_{gew}*(sample)* = arithmetic mean of bacterial counts of sample material 24 h after inoculation. According to the JIS Z 2801:2010 a material can be characterized as antimicrobial, if the calculated $\log_{10}$-reduction is ≥2.0 after 24 h at 37 °C.

2.9. Thermogravimetric Analysis

TGA measurements were performed with about 10 mg of lignin using a Netzsch (Selb, Germany) TGA 209 F1 with a heating rate of 10 °C min^{-1} under a nitrogen atmosphere. The temperature ranged from ambient to 800 °C.

2.10. Optical Contact Angle

Static optical contact angle (OCA) measurements were performed on the PU films at room temperature using an OCA device equipped with a charge-coupled device (CCD) photocamera (DataPhysics Instruments, Filderstadt, Germany). A 40 µL volume of distilled water was used to dispense liquid droplets.

2.11. Scanning Electron Microscopy

Scanning electron microscopy (SEM) from ThermoFischer was combined with X-ray analysis (SEM-EDX). Characterization of the texture, phases and the thin LPU layer were determined by SEM-EDX microscopy using an ESEM Quanta FEG 250 FEI with Apollo XL30 EDX (Thermo Fisher Scientific Inc., Huntsville, AL, USA).

3. Results and Discussion

3.1. Antibacterial Activity of Kraft Lignin

Kraft lignins were demethylated (DL) and characterized regarding their molecular weight and hydroxyl content (Table 2). In addition, Table 2 shows Kraft lignins isolated at different pH values [29].

Studies of the antimicrobial activity were performed following procedures reported to investigate intrinsically antimicrobial polymers based on poly((tertbutyl-amino)-methyl-styrene) [62–66] and coatings based on HPMC/lignin/chitosan [52]. Two different nutritions were used: sodium chloride (NaCl) and physiological saline solution (NB) of different concentrations (Figure 2).

Results for both solutions (NaCl, NB) clearly showed an increase in antimicrobial activity against *Staphylococcus aureus* for the lignins isolated at different pH values (pH 2 to pH 5) with the highest activity ($\log_{10}$ reduction of 7.0) for the pH 5 samples. This tendency could also be confirmed for the corresponding LPU coatings prepared from the different lignin samples (see next paragraph). Due to the measurement procedure, the study started using the highest concentrations (0.1 mol/L), then the concentration decreased down to 0.001 mol/L. Obviously, the lowest concentrations were sufficient for the observed antimicrobial effects. Similar results could be observed for the HPMC/lignin coatings

recently reported [52] and also for organosolv lignins (not yet published). Further studies are required to clarify the correlation of concentration and antimicrobial activity.

Table 2. Weight-average (M_w) and number-average (M_n) molecular weight and polydispersity (PDI) obtained by gel permeation chromatography (GPC) measurements, and OH content according to ISO 14900 for demethylated lignins (DL) and Kraft lignins isolated at different pH values [29].

Lignin	M_w (g/mol)	M_n (g/mol)	PDI	OH content (ISO 14900) (mmol·g^{-1})	OH content (ISO 14900) (mg KOH) g^{-1}	Reference
pH2	1879	574	3.3	2.67	150	[29]
pH3	1732	538	3.2	4.48	251	[29]
pH4	1570	441	3.6	5.02	282	[29]
pH5	1502	490	3.0	5.34	300	[29]
DL-pH2	5417	1299	4.2	4.75	266	–
DL-pH3	5461	1318	4.1	4.00	224	–
DL-pH4	5522	1335	4.1	5.51	309	–
DL-pH5	5610	1347	4.2	4.80	269	–

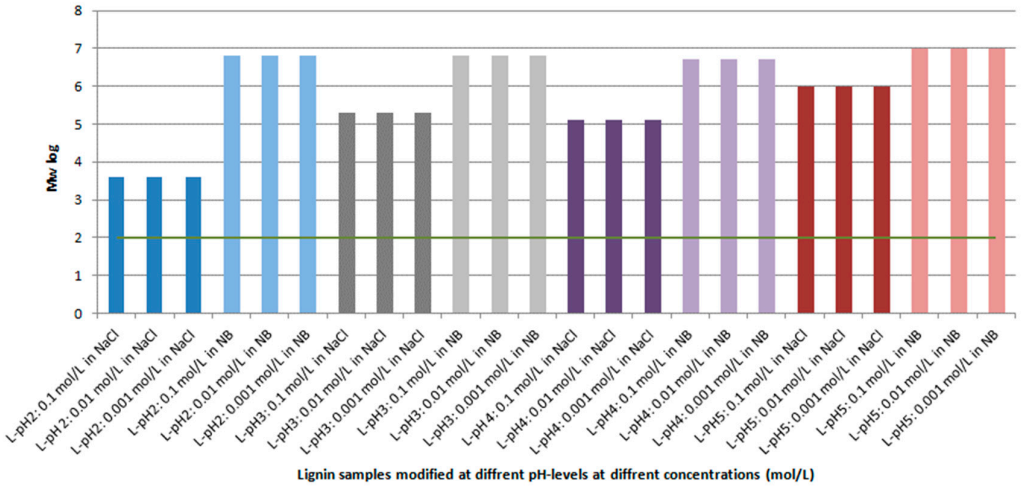


Figure 2. Antimicrobial activity of unmodified Kraft lignins isolated at different pH levels (varying from 2 to 5). Activity tested against *Staphylococcus aureus* in NaCl and physiological saline solution (NB)), respectively, in concentrations ranging between 0.1–0.001 mol/L.

3.2. Antibacterial Activity of LPU Coatings

For the comparability of subsequent investigations, first the antimicrobial effect on different reference surfaces was tested according to Japanese Industrial Standard Z 2801:2000 [59]. The results are shown in Table 3 and Figure 3.

Table 3. Results of antimicrobial activity of different reference systems surfaces against *S. aureus*.

Reference Systems (blank)	Kbe mL^{-1}	Ø log Kbe mL^{-1}
Petri dish	1.18×10^5	5.05
Glass	1.07×10^7	6.71
Plastic dish (PP *)	4.07×10^6	6.48
Transparencies (PS **)	9.62×10^6	6.94
Stainless steel	2.60×10^1	1.41

* Polypropylene, ** Polystyrene

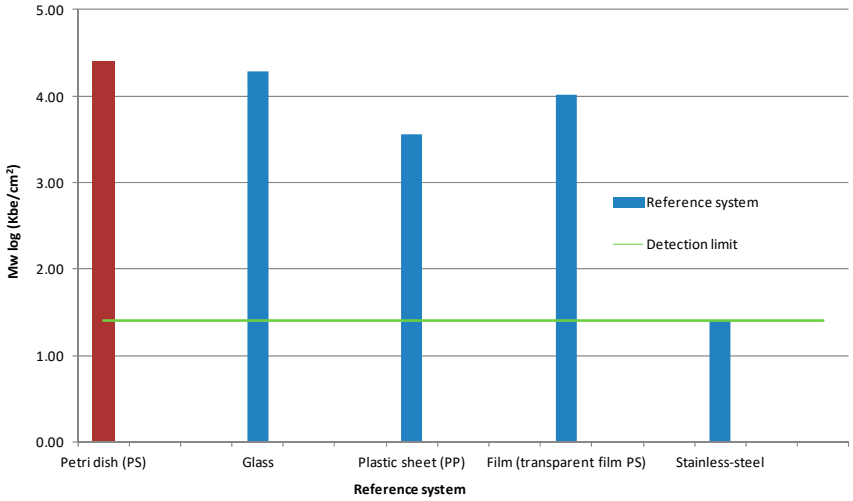


Figure 3. Antimicrobial activity (log Kbe mL^{-1}) of different blank surfaces (used as reference systems) against *S. aureus* and *Listeria monocytogenes*. The green line represents the detection limit for the determination of the antimicrobial activity and is 1.4 log (Kbe cm^{-1}).

As suggested, the results showed normal bacterial growth on blank surfaces: untreated glass, plastic sheets (polypropylene, PP), transparent polystyrene films (PS) and stainless steel (Figure 3). Notable was an emerging germ resistance on the untreated stainless-steel surfaces which underlines the natural antimicrobial effect of stainless-steel surfaces for different bacteria, known as oligodynamic effect. The oligodynamic effects describes the damaging effect of various metal ions on different bacteria, viruses and fungi, most probably due stainless-steel alloy formation initiated by different metal cations [64].

Furthermore, lignin-modified PU coatings prepared from demethylated lignins were applied to various surfaces and analyzed for their antimicrobial action. The results are listed below in Table 4 and Figure 4.

Table 4. Results of antimicrobial activity of demethylated lignin-based polyurethane (PU) coatings against *S. aureus*.

Lignin–PU Coatings	Kbe/cm²	Ø log Kbe/cm²
DL-pH2-060718	2.07×10^3	3.03
DL-pH3-060718	1.17×10^3	2.51
DL-pH4-060718	3.09×10^1	2.36
DL-pH5-060718	6.25×10^{-1}	0

The results showed significant microbial reduction against *S. aureus* for the PU coatings synthesized from demethylated lignins. It is also noticeable that the germ reduction can be correlated to the pH value for lignin isolation: lignins isolated at pH 3, 4 and 5 showed a higher germ reduction and antimicrobial activity, respectively, than the reference (blind value: polypropylene glycol (PPG) as polyol without lignin). One reason for this could be the improved homogeneity of the coatings, caused by higher crosslinking density of the LPU due to high OH numbers, analogous to the correlation of OH number and antioxidant activity of LPU coatings [29,30]. Besides LPU coatings, it was recently reported that the antimicrobial activity of various lignin-derived cellulose and cellulose/chitosan composites against *S. aureus* and *E. coli* (Table 1) [52]. A comparison of hydroxypropylmethyl cellulose/lignin films were blended with Kraft lignin in different amounts up to 30 wt.%. Comparing both systems (HPMC versus PU), the capacity against *S. aureus* was highest for the lignin isolated at pH 5 (Table 5). As supposed, the addition of triphenylmethane derivatives (BG, crystal violet (CV)) resulted in increased antimicrobial activity against *S. aureus*.

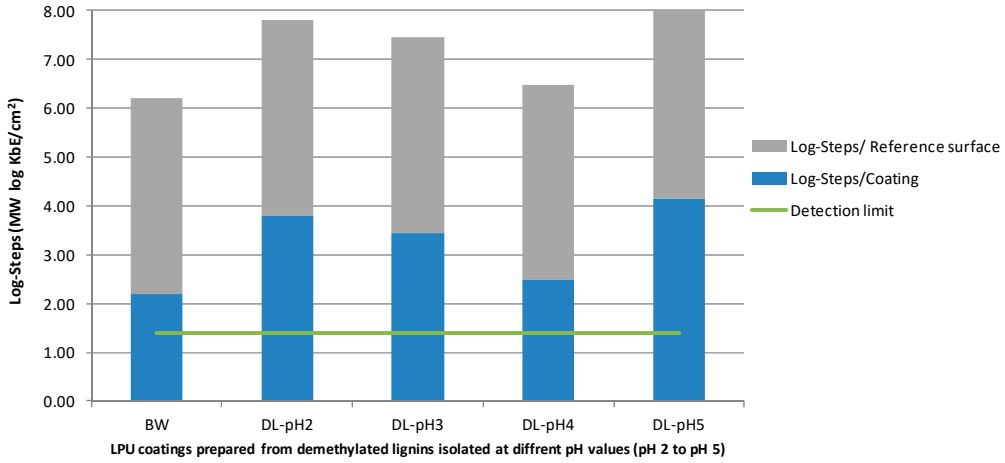


Figure 4. Results of antimicrobial activity against *S. aureus* of lignin-based PU coatings prepared demethylated lignins (DL) isolated at different pH values ranging from pH 2 to pH 5. BV:blind value of PU without lignin. The green line represents the detection limit for the determination of the antimicrobial activity (1.4 log Kbe cm^{-1}).

Table 5. Antimicrobial activity of lignin and lignin-derived PU coatings against *S. aureus*. The lignins used for lignin–polyurethane (LPU) coating preparation were isolated from aqueous solution at different pH values. For comparison, the antimicrobial activity of hydroxypropylmethylcellulose (HPMC)/lignin coatings was added in this table, previously reported in [52].

Antimicrobial Activity	Lignin (Isolated at pH 5)	LPU Coating (DL-pH5)	LPU Coating (KL-pH5)	LPU with 0.8% (*w*/*v*) BG	LPU with 0.8% (*w*/*v*) CV	HPMC/lignin (15 wt.% L1) [52]
Log_{10} reduction	7.00	4.12	2.62	8.31	8.60	2.50

3.3. Thermal Properties (TGA)

TGA measurements were performed to describe and evaluate the thermal stability of the corresponding LPU coatings with antimicrobial additives and the coatings prepared from demethylated lignins (Figure 5).

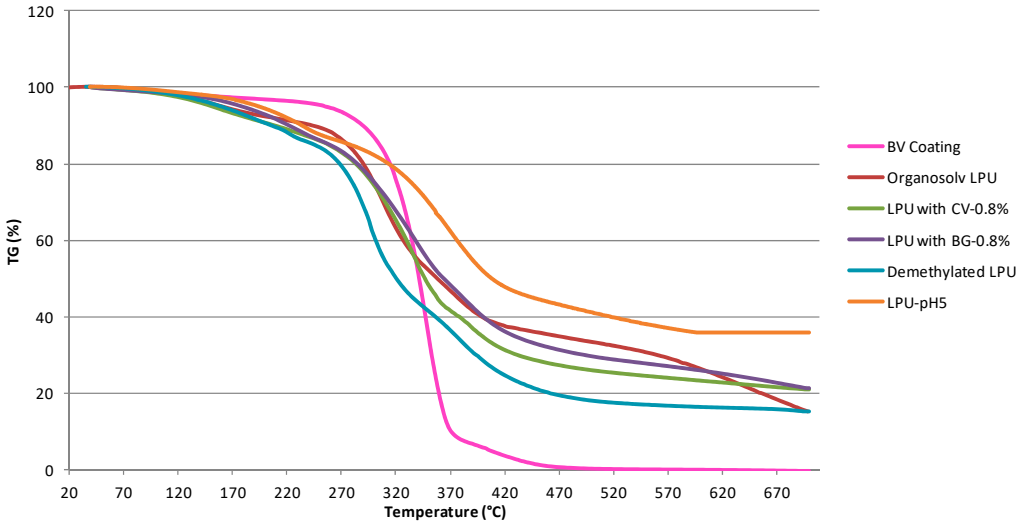


Figure 5. TGA results of different modified lignin-based LPU coatings containing brilliant green (BG) and crystal violet (CV); BV (blind value: PU without lignin).

TGA results showed thermal stability between 143–165 °C which are reasonable temperature stabilities for applications in construction and packaging (Table 6). Coatings with CV as antimicrobial additive showed the highest temperature stability with 165 °C in contrast to LPU coatings prepared with BG with a stability of 146 °C. The residual mass of both LPU coatings was between 20%–21.5%.

Table 6. Thermal stability for lignin and various LPU coatings with/without triphenylmethane (TPM) derivatives.

Lignin Coatings	Temperature (°C)	Δ*m* (%)	Residual Mass (%)
Blank (PU coating without lignin)	250	−4.75%	0.39
LPU-pH 5	166	−3.31%	35.99
Lignin coating CV	165	−4.05%	20.80
Lignin coating BG	146	−4.49%	21.43
Lignin coating organosolv	143	−4.03%	8.00
Lignin-DLPU coating	153	−4.55%	21.43

Obviously, decomposition temperature and residuals are influenced not only by the pulping process used for lignin isolation (Kraft versus organosolv), but also by demethylation and added antimicrobial triphenylmethane derivatives (brilliant green and crystal violet). Further quantification of these effects by DSC measurements is under investigation.

3.4. Contact Angle of LPU Coatings

The wettability properties of the surfaces of all lignin-based PUs were investigated by means of static contact angle measurements against water (Table 6). The LPU coatings possessed a rather hydrophobic character with water contact angles θH_2O up to 92 degrees, higher than literature data for LPUs reported by Jia et al. prepared from organosolv lignin (61°). The PU coatings with demethylated lignins showed a contact angle of 84.22 ± 0.51°. Table 7 summarizes the contact angle data of all LPU with antimicrobial additives. The results revealed that the LPU with brilliant green had a better wettability (87.36 ± 0.15°) compared to the LPU with crystal violet (67.40 ± 0.18°).

Table 7. Results of contact angle measurements of the different LPU. Abbreviations: polyurethane (PU), Kraft lignin (KL), organosolv lignin (OL), beech wood (BW), brillant green (BG), crystal violet (CV).

Sample	Contact Angle (°)
PU-KL-pH 2	92.28 ± 0.49
PU-KL-pH 3	80.49 ± 1.03
PU-KL-pH 4	83.28 ± 0.24
PU-KL-pH 5	86.01 ± 0.22
PU-OL	61,59 ± 0.69
PU-KL-Demethylated	84.22 ± 0.51
LPU Coatings with TPM dyes	
PU-BV-BG	62.93 ± 0.34
PU-BV-CV	80.19 ± 0.28
PU-KL-pH2-BG	87.36± 0.15
PU-KL-pH2-CV	81.11 ± 0.18

3.5. Morphology of the LPU Coatings

To get a first idea of the homogeneity of the lignin-derived coatings, the coatings were observed via reflected light microscopy showing that homogeneous coatings could be obtained using lignins of number-average molecular weight (M_n) < 500 g/mol (equivalent weight-average (M_w) < 1570 g/mol) and OH content above 5 mmol/g (samples isolated at pH 4 and pH 5). Using scanning electron microscopy (SEM), the thickness of the casted films was determined to range between 150–160 µm [29].

Figure 6 shows the prepared LPU coatings: (a) with 0.8% brilliant green added resulted in homogeneous films of greenish color and smooth surface; (b) with 0.8% crystal violet added also

resulted in homogeneous films of smooth surface colored in dark blue. Reference coatings are shown containing PU/CV (no lignin) and PU (no lignin, no crystal violet). The antimicrobial activities were determined according to Japanese Industrial Standard (JIS) Z 2801:2010 [59]. Figure 6c shows coatings on different surfaces: stainless steel, wood, plastic (polypropylene). On all surfaces, the coatings showed smooth homogeneous surfaces. In ongoing studies, the adhesion strength will be quantified.

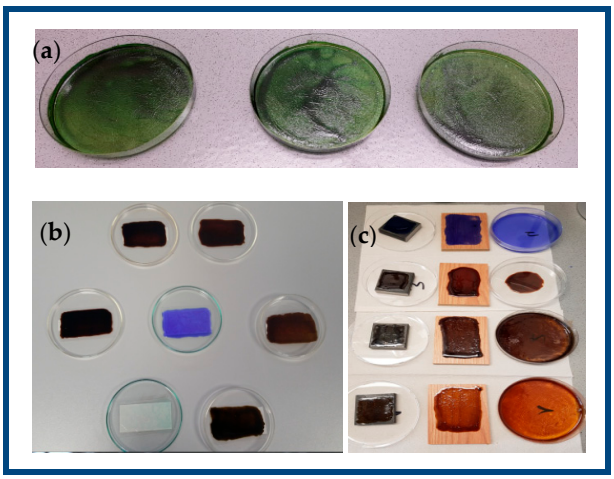


Figure 6. (**a**) Three lignin–PU coatings with brilliant green as antimicrobial additive; (**b**) lignin–PU coatings with crystal violet as an antimicrobial additive (blue sample in the middle: PU-CV without lignin; clockwise starting with the white sample (PU without lignin), LPU coatings prepared from organosolv-lignin and lignins isolated at pH 2 to pH 5; (**c**) lignin–PU–CV coatings on different surfaces: steel (left), wood (middle) and polystyrene (PS) petri dishes (right); top-down: PU-CV, LPU-CV-pH5, LPU-CV-pH4, LPU-CV-pH2.

4. Conclusions

The results of the antimicrobial activity study of lignin-based polyurethane coatings confirmed the capacity of Kraft lignin against special microorganisms such as *S. aureus*. Triphenylmethane derivatives (brilliant green, crystal violet) significantly increased this antimicrobial effect. The coating color changed from dark brown to green (in case of BG) and blue (in case of CV). Wettability tests using contact angle measurements confirmed the hydrophobic character of the lignin-derived PU coatings.

Author Contributions: S.E.K. mainly contributed to the manuscript, performed the experiments and analyzed the data; A.A. contributed analytical data regarding the lignins extracted from organic solvents; J.R. contributed in PU coating preparation. I.K. and J.K. contributed antimicrobial analyses; M.S. conceived and designed the experimental studies and contributed in writing the manuscript.

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Conflicts of Interest: There are no conflicts to declare.

References

1. Ralph, J.; Lapierre, C.; Boerjan, W. Lignin structure and its engineering. *Curr. Opin. Biotechnol.* **2019**, *56*, 240–249. [CrossRef] [PubMed]
2. Rinaldi, R.; Jastrzebski, R.; Clough, M.T.; Ralph, J.; Kennema, M.; Bruijnincx, P.C.A.; Weckhuysen, B.M. Paving the way for lignin valorisation: Recent advances in bioengineering, biorefining and catalysis. *Angew. Chem. Int. Ed.* **2016**, *55*, 2–54. [CrossRef] [PubMed]

3. Alzagameem, A.; El Khaldi-Hansen, B.; Kamm, B.; Schulze, M. Lignocellulosic biomass for energy, biofuels, biomaterials, and chemicals. In *Biomass and Green Chemistry*, 1st ed.; Vaz, S., Jr., Ed.; Springer International Publishing: Basel, Switzerland, 2018; pp. 95–132.
4. Hansen, B.; Kamm, B.; Schulze, M. Qualitative and quantitative analysis of lignins from different sources and isolation methods for an application as a biobased chemical resource and polymeric material. In *Analytical Techniques and Methods for Biomass Products*; Vaz, S., Jr., Seidl, P., Eds.; Springer: Berlin, Germany, 2017; pp. 15–44.
5. Naseem, A.; Tabasum, S.; Zia, K.M.; Zuber, M.; Ali, M.; Noreen, A. Lignin-derivatives based polymers, blends and composites: A review. *Int. J. Biol. Macromol.* **2016**, *93*, 296–313. [CrossRef] [PubMed]
6. Ko, F.K.; Goudarzi, A.; Lin, L.-T.; Li, Y.; Kadla, J.F. Lignin-based composite carbon nanofibers. In *Lignin in Polymer Composites*, 1st ed.; Faruk, O., Sain, M., Eds.; Elsevier B.V: Amsterdam, The Netherlands, 2016; pp. 167–194.
7. Ponomarenko, J.; Dizhbite, T.; Lauberts, M.; Viksna, A.; Dobele, G.; Bikovens, O.; Telysheva, G. Characterization of softwood and hardwood lignoboost kraft lignins with emphasis on their antioxidant activity. *Bioresources* **2014**, *9*, 2051–2068. [CrossRef]
8. Benzie, I.F.; Devaki, M. The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: Concepts, procedures, limitations and applications. In *Measurement of Antioxidant Activity & Capacity*, 1st ed.; Apak, R., Capanoglu, E., Shahidi, F., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2017; pp. 77–106.
9. Alzagameem, A.; El Khaldi-Hansen, B.; Büchner, D.; Larkins, M.; Kamm, B.; Witzleben, S.; Schulze, M. Lignocellulosic biomass as source for lignin-based environmentally benign antioxidants. *Molecules* **2018**, *23*, 2664. [CrossRef] [PubMed]
10. Bergs, M.; Völkering, G.; Kraska, T.; Do, X.; Monakhova, Y.; Konow, C.; Pude, R.; Schulze, M. *Miscanthus x giganteus* stem versus leave-derived lignins differing in monolignol ratio and linkage. *Int. J. Mol. Sci.* **2019**, *20*, 1200. [CrossRef]
11. Hansen, B.; Kamm, B.; Schulze, M. Qualitative and quantitative analysis of lignin produced from beech wood by different conditions of the Organosolv process. *J. Polym. Environ.* **2016**, *24*, 85. [CrossRef]
12. Richter, A.P.; Brown, J.S.; Bharti, B.; Wang, A.; Gangwal, S.; Houck, K.; Cohen Hubal, E.A.; Paunov, V.N.; Stoyanov, S.D.; Velev, O.D. An environmentally benign antimicrobial nanoparticle based on a silver-infused lignin core. *Nat. Nanotechnol.* **2015**, *10*, 817–824. [CrossRef]
13. Gregorova, A.; Redik, S.; Sedlarik, V.; Stelzer, F. Lignin-containing polyethylene films with antibacterial activity. In Proceedings of the 3rd International Conference on Thomson Reuters of NANOCON, Brno, Czech Republic, 21–23 September 2011. Available online: http://konference.tanger.cz/data/nanocon2011/sbornik/lists/papers/1366.pdf (accessed on 5 May 2019).
14. Dai, L.; Liu, R.; Hu, L.-Q.; Zou, Z.-F.; Si, C.-L. Lignin nanoparticle as a novel green carrier for the efficient delivery of resveratrol. *ACS Sustain. Chem. Eng.* **2017**, *5*, 8241–8249. [CrossRef]
15. Li, Y.; Yang, D.; Lu, S.; Lao, S.; Qiu, X. Modified lignin with anionic surfactant and its application in controlled release of avermectin. *J. Agric. Food Chem.* **2018**, *66*, 3457–3464. [CrossRef]
16. Pang, Y.; Li, X.; Wang, S.; Qiu, X.; Yang, D.; Lou, H. Lignin-polyurea microcapsules with anti-photolysis and sustained-release performances synthesized via pickering emulsion template. *React. Funct. Polym.* **2018**, *123*, 115–121. [CrossRef]
17. Wang, Y.; Xiong, Y.; Wang, J.; Zhang, X. Ultrasonic-assisted fabrication of montmorillonite-lignin hybrid hydrogel: Highly efficient swelling behaviors and super-sorbent for dye removal from wastewater. *Colloids Surf. A Physicochem. Eng. Asp.* **2017**, *520*, 903–913. [CrossRef]
18. Ciolacu, D.; Oprea, A.M.; Anghel, N.; Cazacu, G.; Cazacu, M. New cellulose–lignin hydrogels and their application in controlled release of polyphenols. *Mater. Sci. Eng. C* **2012**, *32*, 452–463. [CrossRef]
19. Kosikova, B.; Labaj, J. Lignin-stimulated protection of polypropylene films and DNA in cells of mice against oxidation damage. *Bioresources* **2009**, *4*, 805–815.
20. Gao, Y.; Cranston, R. Recent advances in antimicrobial treatments of textiles. *Text. Res. J.* **2008**, *78*, 60–72. [CrossRef]
21. Bshena, O.; Heunis, T.D.; Dicks, L.M.; Klumperman, B. Antimicrobial fibers: Therapeutic possibilities and recent advances. *Future Med. Chem.* **2011**, *3*, 1821–1847. [CrossRef]

22. Gordts, S.C.; Férir, G.; D'huys, T.; Petrova, M.I.; Lebeer, S.; Snoeck, R.; Andrei, G.; Schols, D. The low-cost compound lignosulfonic acid (LA) exhibits broad-spectrum anti-HIV and anti-HSV activity and has potential for microbicidal applications. *PLoS ONE* **2015**, *10*, e0131219. [CrossRef]
23. Qiu, M.; Wang, Q.; Chu, Y.; Yuan, Z.; Song, H.; Chen, Z.; Wu, Z. Lignosulfonic acid exhibits broadly anti-HIV-1activity-potential as a microbicide candidate for the prevention of HIV-1 sexual transmission. *PLoS ONE* **2012**, *7*, e35906. [CrossRef]
24. Kai, D.; Tan, M.J.; Chee, P.L.; Chua, Y.K.; Yap, Y.L.; Loh, X.J. Towards lignin-based functional materials in a sustainable world. *Green Chem.* **2016**, *18*, 1175–1200. [CrossRef]
25. Lau, P.C.K. Lignin: A platform for renewable aromatic polymeric materials. In *Quality Living Through Chemurgy and Green Chemistry. Green Chemistry and Sustainable Technology*; Lau, P.C.K. Springer: Berlin/Heidelberg, Germany, 2016; pp. 221–263.
26. Ten, E.; Vermerris, W. Recent developments in polymers derived from industrial lignin. *J. Appl. Polym. Sci.* **2015**, *132*, 1–13. [CrossRef]
27. Jia, Z.; Lu, C.; Zhou, P.; Wang, L. Preparation and characterization of high boiling solvent lignin-based polyurethane film with lignin as the only hydroxyl group provider. *RSC Adv.* **2015**, *5*, 53949–53955. [CrossRef]
28. Griffini, G.; Passoni, V.; Suriano, R.; Levi, M.; Turri, S. Polyurethane coatings based on chemically unmodified fractionated lignin. *ACS Sustain. Chem. Eng.* **2015**, *3*, 1145–1154. [CrossRef]
29. Klein, S.E.; Rumpf, J.; Kusch, P.; Albach, R.; Rehahn, M.; Witzleben, S.; Schulze, M. Utilization of unmodified kraft lignin for the preparation of highly flexible and transparent polyurethane coatings. *RSC Adv.* **2018**, *8*, 40765. [CrossRef]
30. Klein, S.E.; Rumpf, J.; Rehahn, M.; Witzleben, S.; Schulze, M. Biobased flexible polyurethane coatings prepared from kraft lignin: One-pot synthesis and antioxidant activity. *J. Coat. Technol. Res.* **2019**. [CrossRef]
31. Hu, J.; Zhang, Q.; Lee, D.-J. Kraft lignin biorefinery: A proposal. *Bioresour. Technol.* **2017**, *247*, 1181–1183. [CrossRef]
32. Sain, M.; Faruk, O. *Lignin in Polymer Composites*, 1st ed.; Elsevier: Kidlington, UK, 2016.
33. Li, J.; Wang, W.; Shifeng, Z.; Qiang, G.; Zhang, W.; Li, J. Preparation and characterization of lignin demethylated at atmospheric pressure and its application in fast curing biobased phenolic resins. *RSC Adv.* **2016**, *6*, 67435–67443. [CrossRef]
34. Laurichesse, S.; Avérous, L. Chemical modification of lignins: Towards biobased polymers. *Prog. Polym. Sci.* **2014**, *39*, 1266–1290. [CrossRef]
35. Chung, H.; Washburn, N.R. Improved lignin polyurethane properties with Lewis acid treatment. *ACS Appl. Mater. Interfaces* **2012**, *4*, 2840–2846. [CrossRef]
36. Zou, L.; Ross, B.M.; Hutchison, L.J.; Christopher, L.P.; Dekker, R.F.; Malek, L. Fungal demethylation of Kraft lignin. *Enzyme Microb. Technol.* **2015**, *73–74*, 44–50. [CrossRef]
37. Ibrahim, V.; Mendoza, L.; Mamo, G.; Hatti-Kaul, R. Blue laccase from *Galerina* sp.: Properties and potential for Kraft lignin demethylation. *Process Biochem.* **2011**, *46*, 379–384. [CrossRef]
38. Song, Y.; Wang, Z.; Yan, N.; Zhang, R.; Li, J. Demethylation of wheat straw alkali lignin for application in phenol formaldehyde adhesives. *Polymers* **2016**, *8*, 209. [CrossRef]
39. An, X.; Schroeder, H.A.; Thompson, G.E. Demethylated kraft lignin as a substitute for phenol in wood adhesive. *Chem. Ind. For. Prod.* **1995**, *15*, 36–42.
40. Ferhan, M.; Sain, M.; Yan, N. A new method for demethylation of lignin from woody biomass using biophysical methods. *J. Chem. Eng. Process. Technol.* **2013**, *4*, 160. [CrossRef]
41. Podschun, J.; Saake, B.; Lehnen, R. Catalytic demethylation of organosolv lignin in aqueous medium using indium triflate under microwave irradiation. *React. Funct. Polym.* **2017**, *119*, 82–86. [CrossRef]
42. Webb, C.H.S. A note on the value of brilliant green as an antiseptic. *Br. Med. J.* **1917**, *1*, 870. [CrossRef]
43. Sneader, W. *Drug Discovery: A history*; John Wiley and Sons Ltd.: Chichester, UK, 2005; p. 468.
44. Schirmer, R.H.; Coulibaly, B.; Stich, A.; Scheiwein, M.; Merkle, H.; Eubel, J.; Becker, K.; Becher, H.; Müller, O.; Zich, T.; et al. Methylene blue as an antimalarial agent. *Redox Rep.* **2003**, *8*, 272–275. [CrossRef]
45. Boulos, R. Bacterial Mechanosensitive Channels as Novel Targets for Antibacterial Agents. Ph.D. Thesis, The University of Western Australia, Perth, Australia, December 2011.
46. Boulos, R.A. Antimicrobial Compounds. U.S. Patent 20120329871 A1, 27 December 2012.
47. Boulos, R.A.; Eroglu, E.; Chen, X.; Scaffidi, A.; Edwards, B.R.; Toster, J.; Raston, C.L. Unravelling the structure and function of human hair. *Green Chem.* **2013**, *15*, 1268–1273. [CrossRef]

48. Vilela, S.F.G.; Junqueira, J.C.; Barbosa, J.O.; Majewski, M.; Munin, E.; Jorge, A.O.C. Photodynamic inactivation of *Staphylococcus aureus* and *Escherichia coli* biofilms by malachite green and phenothiazine dyes: An in vitro study. *Arch. Oral Biol.* **2012**, *57*, 704–710. [CrossRef]
49. Noimark, S.; Allan, E.; Parkin, I.P. Light-activated antimicrobial surfaces with enhanced efficacy induced by a dark-activated mechanism. *Chem. Sci.* **2014**, *5*, 2216. [CrossRef]
50. Bartoszewicz, L. Antimicrobial Photo-Stable Coating Composition. WO2009015476A1, 5 February 2009.
51. Santos, M.R.E.; Fonseca, A.C.; Mendonça, P.V.; Branco, R.; Serra, A.C.; Morais, P.V.; Coelho, J.F.J. Recent developments in antimicrobial polymers: A review. *Materials* **2016**, *9*, 599. [CrossRef]
52. Alzagameem, A.; Klein, S.E.; Bergs, M.; Do, X.T.; Korte, I.; Dohlen, S.; Kreyenschmidt, J.; Kamm, B.; Larkins, M.; Schulze, M. Antimicrobial activity of lignin and lignin-derived cellulose and chitosan composites against selected pathoge nic and spoilage microorganisms. *Polymers* **2019**, *11*, 670. [CrossRef]
53. Yang, W.; Fortunati, E.; Dominici, F.; Giovanale, G.; Mazzaglia, A.; Balestra, G.M.; Kenny, J.M.; Puglia, D. Effect of cellulose and lignin on disintegration, antimicrobial and antioxidant properties of PLA active films. *Int. J. Biol. Macromol.* **2016**. [CrossRef]
54. Kaur, R.; Uppal, S.K.; Sharma, P. Antioxidant and antibacterial activities of sugarcane bagasse lignin and chemically modified lignins. *Sugar Tech.* **2017**, *19*, 675–680. [CrossRef]
55. Witzler, M.; Alzagameem, A.; Bergs, M.; El Khaldi-Hansen, B.; Klein, S.E.; Hielscher, D.; Kamm, B.; Kreyenschmidt, J.; Tobiasch, E.; Schulze, M. Lignin-derived biomaterials for drug release and tissue engineering. *Molecules* **2018**, *23*, 1885. [CrossRef]
56. Monakhova, Y.; Diehl, B.W.K.; Do, X.T.; Witzleben, S.; Schulze, M. Novel method for the determination of average molecular weight of natural polymers based on 2D DOSY NMR and chemometrics: Example of heparin. *J. Pharm. Biomed. Anal.* **2018**, *149*, 128–132. [CrossRef]
57. Alzagameem, A.; Bergs, M.; Do, X.T.; Klein, S.E.; Rumpf, J.; Larkins, M.; Monakhova, Y.; Pude, R.; Schulze, M. Low-input crops as lignocellulosic feedstock for second generation biorefineries and the potential of chemometrics in biomass quality control. *Appl. Sci.* **2019**, *9*, 2252. [CrossRef]
58. Garcia, A.; Toledano, A.; Serrano, A.; Egüés, I.; González, M.; Marín, F.; Labidi, J. Characterization of lignins obtained by selective precipitation. *Sep. Purif. Technol.* **2009**, *68*, 193–198. [CrossRef]
59. Japanese Industrial Standard. Z 2801:2000. ICS 07.100.10; 11.100 Descriptors: Bacteriocide-Activity Determination, Microbiological-Resistance Tests, Biological Hazards, Culture Techniques. Available online: http://lotusyapi.com.tr/Antibacterial/JIS%20Z%202801%202000.pdf (accessed on 5 May 2019).
60. Pan, X.; Saddler, J.N. Effect of replacing polyol by organosolv and kraft lignin on the property and structure of rigid polyurethane foam. *Biotechnol. Biofuels* **2013**, *6*, 12–21. [CrossRef]
61. Tavares, L.B.; Boas, C.V.; Schleder, G.R.; Nacas, A.M.; Rosa, D.S.; Santos, D.J. Bio-based polyurethane prepared from Kraft lignin and modified castor oil. *eXPRESS Pol. Lett.* **2016**, *10*, 927–940. [CrossRef]
62. Dohlen, S.; Braun, C.; Brodkorb, F.; Fischer, B.; Ilg, Y.; Kalbfleisch, K.; Kreyenschmidt, M.; Lorenz, R.; Kreyenschmidt, J. Effect of different packaging materials containing poly-[2-(tert-butylamino) methylstyrene] on the growth of spoilage and pathogenic bacteria on fresh meat. *Int. J. Food Microbiol.* **2017**, *257*, 91–100. [CrossRef]
63. Dohlen, S.; Braun, C.; Brodkorb, F.; Fischer, B.; Ilg, Y.; Kalbfleisch, K.; Kreyenschmidt, M.; Lorenz, R.; Robers, O.; Kreyenschmidt, J. Potential of the polymer poly-[2-(tert-butylamino) methylstyrene] as antimicrobial packaging material for meat products. *J. Appl. Microbiol.* **2016**, *4*, 1059–1070. [CrossRef]
64. Hüwe, C.; Schmeichel, J.; Brodkorb, F.; Dohlen, S.; Kalbfleisch, K.; Kreyenschmidt, M.; Lorenz, R.; Kreyenschmidt, J. Potential of antimicrobial treatment of linear low-density polyethylene with poly((tert-butyl-amino)-methyl-styrene) to reduce biofilm Formation in the Food industry. *Biofouling* **2018**, *34*, 378–387. [CrossRef]
65. Braun, C.; Dohlen, S.; Ilg, Y.; Brodkorb, F.; Fischer, B.; Heindirk, P.; Kalbfleisch, K.; Richter, T.; Robers, O.; Kreyenschmidt, M. Antimicrobial activity of intrinsic antimicrobial polymers based on poly((tertbutyl-amino)-methyl-styrene) against selected pathogenic and spoilage microorganisms relevant in meat processing facilities. *J. Antimicrob Agents* **2017**, *3*, 1000136. [CrossRef]
66. Song, W.; Ge, S. Application of antimicrobial nanoparticles in dentistry. *Molecules* **2019**, *24*, 1033. [CrossRef]