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# Utilization of Agro-Waste for Lignin Production and Assessment of Its Antibacterial Activity

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**KEYWORDS**

Lignin, biomass, antibacterial, stubble

**ABSTRACT:**

**Introduction**: Agricultural sector plays a vital role in GDP of most of the Asian and African country. Agricultural practices produce a huge number of lignocellulosic wastes which is mostly burnt that adds to the air pollution. In order to add value to it, effective extraction process is needed to be carried out. Lignin, an important component of this biomass is always regarded as a non-useful component. However, studies have indicated its multipurpose application. Lignin is known to have antimicrobial activity, owing to the presence of phenolic group in it. Such a study of extracting lignin from agricultural waste, will not only add value to lignin but also contribute towards a sustainable development by having a scientific approach towards air quality index.

**Objectives**: to extract lignin by organosolv method and assess its antibacterial activity.

**Methods**: Rice stubble was taken for extraction of lignin. The stubble was powdered and treated with organic acid, made from a combination of acetic acid and formic acid. The residue collected after filtration is again treated with peroxy-organic acid and finally bleached with hydrogen peroxide to increase the brightness of the residue. The liquor collected was mixed with excess amount of water to precipitate the lignin. The precipitated lignin was filtered and dried. For characterization, FTIR, XRD and FE-SEM was done. Antimicrobial activity was conducted by estimating MIC.

**Results**: The organic acid-based extraction of lignin depends on the ratio FA/AA. The lignin extracted is called organosolv lignin and is the purest from of lignin. Through FTIR and XRD characterization it can be concluded that lignin was not modified and extracted in purest form. The structure was also amorphous. The anti-bacterial studies indicates that as lignin concentration is increased, antibacterial property enhances.

**Conclusions**: This method of extracting lignin from biowaste is an efficient method where pure lignin with no modification can be extracted. This is one of the few works where lignin was found to be effective against both gram positive and gram-negative bacteria.

## 1. Introduction

Agricultural sector produces huge amount of waste which remains unutilized mostly. The agricultural biomass which is non-consumable is termed as agro-waste. More than 600 MT of agricultural waste is produced around the world. The agro waste is a good source of chemical oxygen demand, biological oxygen demand and other solid particles which are a great threat to food security, water bodies and source of other pollutions [1,2]. One of the world sustainable development goals is to explore the potentials in agro waste for addressing agro waste management. Due to

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lack of economic viability, agro-waste are always neglected and therefore are discarded largely contributing to environmental pollution. Agro wastes always end up in unplanned landfills and are burnt that contributes to air pollution and increase in green house gases. Therefore, effective outlook is required for extraction of value added products from agro waste biomass that would attract economic sectors to invest in waste management. Different agro wastes have different compositions that can be utilized in the production of different types of value added products of commercial importance [3,4].

At present residues rice, corn, wheat, sugar beet, and sugar cane are the major agro waste produced world wide. All agrowaste generally contains cellulose (45%), hemicellulose (32%) and lignin (23%). Apart from these main constituents waste biomass also contains some amount of ashes[5]. The concentrations of these biopolymers may vary according to source material. Since they are derived from living organisms, they offer advantages such as being antimicrobial, biocompatible, biodegradable, environmentally friendly, non-toxic, bioadhesive, etc [6].

As discussed above lignin is one of the constituting material of agrowaste biomass. Its is a biopolymer that binds to cellulose and hemicellulose and provide mechanical strength to the plant. It is estimated that about $3 \times 10^{11}$ MT of lignin is produced world wide [7]. It is the second most abundant biopolymer after cellulose. The term lignin is derived from a latin word *lignum* that means wood. It is present in all plants and helps in transport of nutrients and water, protect plants from pathogens apart from providing mechanical support. Lignin is phenolic compound that is amorphous in nature and has a complex structure [8,9]. Lignin in its native form is known as protolignin, a polyphenolic material which is made up of monolignols – coumaryl, coniferyl, and sinapyl alcohol which have structures from hydroxyphenyl, guaiacyl, and syringyl[10]. These structures are linked together by ester, ether and carbon-carbon bonds [11].

These structural complexity makes it challenging for the extraction of lignin and therefore value added products from it are low from commercial point of view. However, at industrial level, kraft lignin and sulfite lignin is produced which are the most abundant from of lignin available for commercial application. Such lignin contain sulphur in their structure and are foul smelling. This causes hinderance in its usage at mass scale [12,13]. Owing to its structure, lignin poses antibacterial activity and thus have a huge application in biomedical industry for development of antibacterial products. Moreover, several co-workers have suggested that antibacterial effectivity of lignin depends on its extraction process [14]. Therefore here, we tried extracting lignin from rice stubble by organic acid based method for extraction of pure lignin and then looking at its application in antibacterial activity against both gram positive and gram negative bacteria.

## 2. Objective

Following are the objectives of studies: (1) To extract lignin from rice stubble using organic acid, (2) To characterize the extracted lignin using FTIR, XRD, and FE-SEM, (3) To study its antibacterial activity.

## 3. Material and Method

### *Material*

Glacial acetic acid ($CH_3COOH$), formic acid (HCOOH), hydrogen peroxide$H_2O_2$, sodium hydroxide (NaOH), tryptone soya broth (TSB) were obtained from himedia. All are of analytical grade. Rice stubble (husk and straw) was obtained from Jawaharlal Agricultural university, Jabalpur. The microbial cultures *S. Aureus* (ATCC 6538-0485P) and *E. Coli* (ATCC 25922) sticks were acquired from HiMedia Laboratories Pvt. Ltd., India.

### *Method*

#### *Lignin Extraction:*

Rice stubble was first washed and dried and then grounded in a mixer grinder and converted to fine powder. This powder was mixed with 85% organic acid, which is made from formic acid:acetic acid (70:30), and kept at 80°C for 120 minutes on a hot air oven and then filtered. The formic acid:acetic acid ratio were altered to see the effect of changing concentration on lignin yield. The liquor (filtrate) was collected and residue was treated with peroxy-organic acid in a hot water bath for 60 minutes. It was filtered again and residue was bleached using 35% hydrogen peroxide in water bath till the residue turns to bright color. Peroxy-organic acid is prepared by mixing 35% hydrogen peroxide in 85%

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organic acid. The liquor from all the above steps were collected and excess amount of distilled water was added to precipitate organosolv lignin. The precipitated lignin was filtered out and dried in hot air oven for 24 hours and stored.

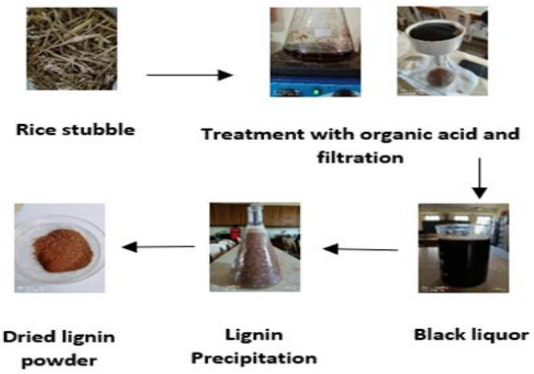


**Fig 1: Extraction process of lignin from rice stubble.**

***Lignin Characterization:*** For structural analysis he extracted lignin was characterized using Shimadzu IRAffinity 1 compact FTIR scanned in a range between 400 – 4000 $cm^{-1}$ with 50 scans in each scan with a resolution of 4 cm. The crystallinity of extracted lignin was analysed by X-ray Diffractometer (Phillips Diffractometer 3200). The samples were scanned at a step size of 3°, between the grade range (2Θ) of 5° to 70°. FE-SEM images were developed using Carl Zeiss UHR FESEM model Gemini SEM 500 KMAT to understand the morphology of the extracted lignin.

***Minimum Inhibitory concentration:*** The extracted lignin was analysed for its antibacterial activity against both gram positive and gram negative bacteria. *S.aureus* (gram positive) and *E.coli* (gram negative) were revived in nutrient broth media. Later three different concentration of lignin (0.05 mg/ml , 0.1 mg/ml, and 0.15 mg/ml) based TSB media was prepared. The bacterial cultures were inoculated in each tube separately and incubated for 24 hours in rotatory incubator at 37°C. after incubation the cultures were streaked on TS agar plates and incubated for 24 hours. The number of colonies in each plate were calculated using colony counter.

## 4. Result

### *Lignin extraction*

Organic acid composed formic acid /acetic acid (FA:AA) was used for extraction of lignin from rice stubble (straw and husk). Organic acid is actively involved in breakage of ether bonds [15]. Through pulping of rice stubble by organic acid, lignin dissolves in organic acid and is retrieved by precipitation by water as lignin is hydrophobic in nature. However, the extracted lignin is slightly soluble in lignin but largely insoluble in water. This makes it possible to precipitate most of the lignin .Ma et al. through their studies have indicated that solubility of lignin decreases as AA<FA<$H_2O$ [16]. Studies by other researcher have indicated that lignin is not completely removed by formic acid treatment. Therefore mixing of hydrogen peroxide with organic acid increases the delignification process from lignocellulose biomass. $OH^+$ ion formed from $H_2O_2$ acts as a strong electrophilic agent and attacks bond between lignin and hemicellulose thus delignifying the lignocellulosic biomass completely[17–21]. Further to increase the brightness of the delignified biomass, it was bleached using $H_2O_2$ in alkaline condition. Under alkaline condition hydrogen peroxide completely removes the color imparting agents from biomass, thus increasing the brightness [22,23].

The study on the effect of FA:AA concentration on lignin concentration was studied under steady conditions of treatment time and temperature. Table 1 shows the result of the analysis.

| FA:AA | Yield % |
|---|---|
| 70:30 | 13.22 |
| 75:25 | 8.5 |
| 80:20 | 1.8 |

**Table 1: percentage yield against the FA:AA concentration**

From the results it is evident that as the acetic acid concentration decreases and formic acid concentration increases the lignin yield decreased. The maximum yield was obtained when the FA/AA ratio was 70:30. Studies have shown that lignin dissolves better in AA than in FA. Therefore decreasing the concentration of AA affects the lignin yield [24].

### *FTIR*

The FTIR analysis of rice stubble showed characteristic peaks at 1269cm-1, 854 cm-1,817 cm-1 indicating the presence of guaiacyl units. The peaks at 1369 -1371 cm-

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1 shows the presence of phenolic OH and aliphatic C-H methyl groups. Presence of condensed syringyl and guaiacyl ring is confirmed by the peaks at 1325 – 1327 cm-1. Aromatic ring vibration was evident by absorption at 1460 cm-1. However, aromatic skeleton vibration was not observed. The conjugated and unconjugated carbonyl-carboxyl stretching can be observed by the peaks at 1679 cm-1 and 1705 cm-1 respectively. Peak at 3001 cm-1 indicates the CH stretching in aromatic methoxyl group. Our work corresponds to Boeriu et al., Rana et al., and Pandey and Pitman [25–27].

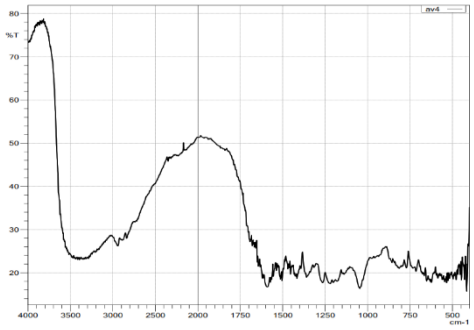



**Fig 2: FTIR of extracted lignin**

***XRD***

To analyse the morphology of extracted lignin XRD was conducted. Since lignin does not have any regularity in its structure therefore no high intensity peaks were observed in lignin XRD. Only one broad peak at 2Θ of 20°. Therefore, through XRD analysis amorphous structure was evident. During FTIR analysis, cellulose contamination was indicated. However, in XRD no intense peak indicative of cellulose was seen. Moreover, less intense peak are observed at 2Θ of 21.8°, that may correspond to the presence of silica in sample [28].

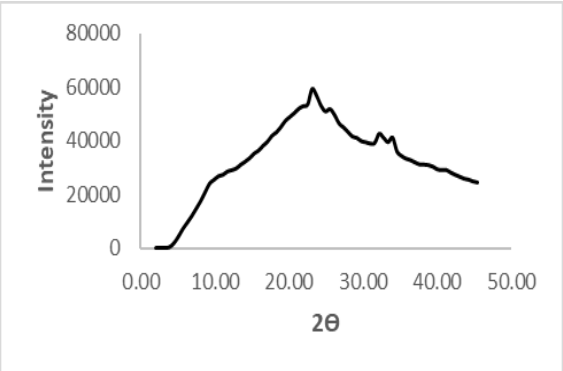
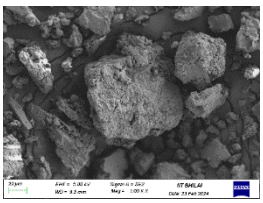



**Fig 3: XRD of extracted lignin**

***FE-SEM***

The FE-SEM images shows that no definitive structure for extracted lignin. It has a rough shape with smooth surface morphology. The shape and size of the lignin depends on the extraction process.



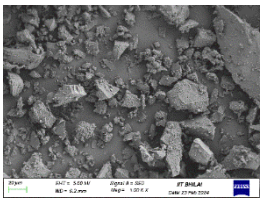


**Fig 4: FE-SEM images of extracted lignin**

***Antibacterial Activity Assessment***

Organosolv lignin showed inhibitory activity against both gram positive and gram negative bacteria. Three different concentrations of organosolv lignin were taken to assess minimum inhibitory concentration. Though complete inhibition was absent but the number of CFU/ml decreased with increase in incubation time. At lower concentration, antibacterial activity was more evident than at higher concentrations for both gram positive and gram negative bacteria. At higher concentrations, lignin may start to precipitate, rather being in soluble state. Therefore, cannot show any effective antibacterial effect. The antibacterial activity of lignin may be attributed to its structure that binds effectively to bacterial membrane and the phenolic group present in lignin structure may assert inhibitory effect [29,30]. Thus lignin finds great application in biomedical and material research as biopolymer that can be useful in developing products related to antibacterial effects.

| **Lignin Conc (TSB with lignin)** | **Bacteria** | |
|---|---|---|
| | **E.coli** | **S. Aureus** |
| 0.05g/ml | + | + |
| 0.1g/ml | ++ | ++ |
| 0.15g/ml | +++ | +++ |

**Table 2 : inhibition zones of lignin against *S. aureus* and *E. coli.* - - shows lawn formation, - not effective inhibition, + moderate inhibition, ++ good inhibition, and +++ most effective inhibition.**

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## 5. Conclusion

This method of extracting lignin from biowaste is an efficient method where pure lignin with no modification can be extracted. Lignocellulosic waste biomass is generally burnt contributing to environmental pollution and thus needs a remedy to encourage a scientific way of disposing it. Extracting lignin by organosolv method provides a way of adding value to biomass waste with effective use in biomedical and pharmaceutical industries that are in search of novel biological compounds having antibacterial property. This is one of the few works where lignin was found to be effective against both gram positive and gram negative bacteria.

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## References

(1) Madurwar, M. V.; Ralegaonkar, R. V.; Mandavgane, S. A., 2013. Application of Agro-Waste for Sustainable Construction Materials: A Review. *Constr. Build. Mater. 38*, 872–878. https://doi.org/10.1016/j.conbuildmat.2012.09.011.

(2) Dey, T.; Bhattacharjee, T.; Nag, P.; Ritika; Ghati, A.; Kuila, A., 2021. Valorization of Agro-Waste into Value Added Products for Sustainable Development. *Bioresour. Technol. Rep. 16*, 100834. https://doi.org/10.1016/j.biteb.2021.100834.

(3) Phiri, R.; Mavinkere Rangappa, S.; Siengchin, S., 2024. Agro-Waste for Renewable and Sustainable Green Production: A Review. *J. Clean. Prod. 434*, 139989. https://doi.org/10.1016/j.jclepro.2023.139989.

(4) Afolalu, S. A.; Salawu, E. Y.; Ogedengbe, T. S.; Joseph, O. O.; Okwilagwe, O.; Emetere, M. E.; Yusuf, O. O.; Noiki, A. A.; Akinlabi, S. A., 2021. Bio-Agro Waste Valorization and Its Sustainability in the Industry: A Review. *IOP Conf. Ser. Mater. Sci. Eng. 1107* (1), 012140. https://doi.org/10.1088/1757-899X/1107/1/012140.

(5) Zielińska, M.; Bułkowska, K., 2024. Agricultural Wastes and Their By-Products for the Energy Market. *Energies. 17* (9), 2099. https://doi.org/10.3390/en17092099.

(6) Nqoro, X.; Taziwa, R.; Popoola, P., 2024. Recent Progress in the Conversion of Agricultural Waste into Functional Materials. *Biomass Convers. Biorefinery. 14* (24), 30881–30895. https://doi.org/10.1007/s13399-023-05044-5.

(7) Shah, S. W. A.; Xu, Q.; Ullah, M. W.; Zahoor; Sethupathy, S.; Morales, G. M.; Sun, J.; Zhu, D., 2023. Lignin-Based Additive Materials: A Review of Current Status, Challenges, and Future Perspectives. *Addit. Manuf. 74*, 103711. https://doi.org/10.1016/j.addma.2023.103711.

(8) Ez-Zahraoui, S.; Semlali Aouragh Hassani, F.-Z.; El Achaby, M.; Qaiss, A. el kacem; Bouhfid, R. 1 - Natural Fiber Reinforcements: Classification, Extraction, Treatment, and Properties. In *Multiscale Textile Preforms and Structures for Natural Fiber Composites*; Midani, M., Hamouda, T., Hassanin, A. H., Seyam, A.-F. M., Eds.; Woodhead Publishing Series in Composites Science and Engineering; Woodhead Publishing, 2023; pp 3–29. https://doi.org/10.1016/B978-0-323-95329-0.00004-1.

(9) Ribeiro, A. M.; Santos, A. I.; Veiga, F.; Figueiras, A. 3 - Lignin Nanoparticle–Based Nanocomposite Hydrogels for Biomedical Applications. In *Functional Nanocomposite Hydrogels*; Kumar, A., Thakur, V. K., Eds.; Nanotechnology in Biomedicine; Elsevier, 2023; pp 69–90. https://doi.org/10.1016/B978-0-323-99638-9.00003-4.

(10) Bernardi, M.; Tangorra, R. R.; Palmisano, P.; Bogliano, A. Chemicals From Renewable Sources. In *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*; Elsevier, 2016. https://doi.org/10.1016/B978-0-12-409547-2.11722-6.

(11) Shaily; Shahzaib, A.; Zafar, F.; Nishat, N. Chapter 28 - Biobased Advanced Coating Materials. In *Advanced Applications of Biobased Materials*; Ahmed, S., Annu, Eds.; Elsevier, 2023; pp 673–

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JCHR (2024) 14(6), 2465-2471 | ISSN:2251-6727

697. https://doi.org/10.1016/B978-0-323-91677-6.00032-5.

(12) Gaspar, R.; Fardim, P., 2023. Lignin-Based Materials for Emerging Advanced Applications. *Curr. Opin. Green Sustain. Chem. 41*, 100834. https://doi.org/10.1016/j.cogsc.2023.100834.

(13) Norgren, M.; Edlund, H., 2014. Lignin: Recent Advances and Emerging Applications. *Curr. Opin. Colloid Interface Sci. 19* (5), 409–416. https://doi.org/10.1016/j.cocis.2014.08.004.

(14) Chen, M.; Li, Y.; Liu, H.; Zhang, D.; Shi, Q.-S.; Zhong, X.-Q.; Guo, Y.; Xie, X.-B., 2023. High Value Valorization of Lignin as Environmental Benign Antimicrobial. *Mater. Today Bio. 18*, 100520. https://doi.org/10.1016/j.mtbio.2022.100520.

(15) Nuruddin, M.; Chowdhury, A.; Haque, S. A.; Rahman, M.; Farhad, S. F.; Jahan, M. S.; Quaiyyum, A., 2011. EXTRACTION AND CHARACTERIZATION OF CELLULOSE MICROFIBRILS FROM AGRICULTURAL WASTES IN AN INTEGRATED BIOREFINERY INITIATIVE. *Cellul. Chem. Technol. 45* ((5-6)), 347–354.

(16) Ma, Q.; Wang, L.; Zhai, H.; Ren, H., 2021. Lignin Dissolution Model in Formic Acid–Acetic Acid–Water Systems Based on Lignin Chemical Structure. *Int. J. Biol. Macromol. 182*, 51–58. https://doi.org/10.1016/j.ijbiomac.2021.03.179.

(17) Li, M.-F.; Sun, S.-N.; Xu, F.; Sun, R.-C., 2012. Formic Acid Based Organosolv Pulping of Bamboo (*Phyllostachys Acuta*): Comparative Characterization of the Dissolved Lignins with Milled Wood Lignin. *Chem. Eng. J. 179*, 80–89. https://doi.org/10.1016/j.cej.2011.10.060.

(18) Xu, F.; Sun, J.-X.; Sun, R.; Fowler, P.; Baird, M. S., 2006. Comparative Study of Organosolv Lignins from Wheat Straw. *Ind. Crops Prod. 23* (2), 180–193. https://doi.org/10.1016/j.indcrop.2005.05.008.

(19) Kham, L.; Le Bigot, Y.; Delmas, M.; Avignon, G., 2005. Delignification of Wheat Straw Using a Mixture of Carboxylic Acids and Peroxoacids. *Ind. Crops Prod. 21* (1), 9–15. https://doi.org/10.1016/j.indcrop.2003.12.002.

(20) Jahan, M. S.; Chowdhury, D. A. N.; Islam, M. K., 2007. Atmospheric Formic Acid Pulping and TCF Bleaching of Dhaincha (*Sesbania Aculeata*), Kash (*Saccharum Spontaneum*) and Banana Stem (*Musa Cavendish*). *Ind. Crops Prod. 26* (3), 324–331. https://doi.org/10.1016/j.indcrop.2007.03.012.

(21) *Peroxyformic Acid Formation: A Kinetic Study | Industrial & Engineering Chemistry Research*. https://pubs.acs.org/doi/10.1021/ie801163j (accessed 2024-12-25).

(22) Shatalov, A. A.; Pereira, H. *Arundo Donax* L., 2005. Reed: New Perspectives for Pulping and Bleaching. Part 4. Peroxide Bleaching of Organosolv Pulps. *Bioresour. Technol. 96* (8), 865–872. https://doi.org/10.1016/j.biortech.2004.09.005.

(23) Roncero, M. B.; Torres, A. L.; Colom, J. F.; Vidal, T., 2003. TCF Bleaching of Wheat Straw Pulp Using Ozone and Xylanase. Part A: Paper Quality Assessment. *Bioresour. Technol. 87* (3), 305–314. https://doi.org/10.1016/S0960-8524(02)00224-9.

(24) Mao, L.; Zhang, L.; Gao, N.; Li, A., 2012. FeCl3 and Acetic Acid Co-Catalyzed Hydrolysis of Corncob for Improving Furfural Production and Lignin Removal from Residue. *Bioresour. Technol. 123*, 324–331. https://doi.org/10.1016/j.biortech.2012.07.058.

(25) Boeriu, C. G.; Bravo, D.; Gosselink, R. J. A.; van Dam, J. E. G., 2004 Characterisation of Structure-Dependent Functional Properties of Lignin with Infrared Spectroscopy. *Ind. Crops Prod. 20* (2), 205–218. https://doi.org/10.1016/j.indcrop.2004.04.022.

(26) Rana, R.; Langenfeld-Heyser, R.; Finkeldey, R.; Polle, A., 2010. FTIR Spectroscopy, Chemical and Histochemical Characterisation of Wood and Lignin of Five Tropical Timber Wood Species of the Family of Dipterocarpaceae. *Wood Sci. Technol. 44* (2), 225–242. https://doi.org/10.1007/s00226-009-0281-2.

(27) Pandey, K. K.; Pitman, A. J., 2003. FTIR Studies of the Changes in Wood Chemistry Following Decay by Brown-Rot and White-Rot Fungi. *Int.*

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JCHR (2024) 14(6), 2465-2471 | ISSN:2251-6727

*Biodeterior. Biodegrad. 52* (3), 151–160. https://doi.org/10.1016/S0964-8305(03)00052-0.

(28) Singh, S. K.; Dhepe, P. L., 2016 Isolation of Lignin by Organosolv Process from Different Varieties of Rice Husk: Understanding Their Physical and Chemical Properties. *Bioresour. Technol. 221*, 310–317. https://doi.org/10.1016/j.biortech.2016.09.042.

(29) Morena, A. G.; Bassegoda, A.; Natan, M.; Jacobi, G.; Banin, E.; Tzanov, T., 2022. Antibacterial Properties and Mechanisms of Action of Sonoenzymatically Synthesized Lignin-Based Nanoparticles. *ACS Appl. Mater. Interfaces 14* (33), 37270–37279. https://doi.org/10.1021/acsami.2c05443.

(30) *Antimicrobial Activity of Lignin and Lignin-Derived Cellulose and Chitosan Composites against Selected Pathogenic and Spoilage Microorganisms*. https://www.mdpi.com/2073-4360/11/4/670 (accessed 2024-12-26).