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Extraction and Characterization of Lignin from Herbivorous Animal Manure

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Abstract: **Lignin is derived from an abundant and renewable resource like plants and agricultural crops which are having moderate lignin content, but in the present study lignin produced from herbivorous animal manure which is having highest lignin content (53-56%) is a cheap and easily available bioresource. Lignin was extracted by breaking the chemical bonds such as phenyl glycosidic linkages, acetal linkages, R-ether bonds and ester bonds between lignin and carbohydrates from nonwood cellulosic biomass i.e herbivorous animal manure by hydrothermal treatment followed by alkali treatment. The isolated lignin was purified to remove impurities and for the characterization of structural and compositional features of lignin were summarized by various spectroscopic methods, such as ultraviolet spectroscopy and Fourier-transformed infrared spectroscopy. The thermal properties and chemical composition were analysed by Thermo gravimetric analysis (TGA) and Differential scanning calorimetry (DSC) analysis and elemental analysis. The average molecular weight of the lignin (g/mol) and poly dispersity (PI) were determined using gel permeation chromatography (GPC).**

Keywords: Lignin, Herbivorous animal manure, Extraction, Isolation, Characterization.

I. INTRODUCTION

Lignin is a natural amorphous polymer, the second most abundant polymer after cellulose and the only biomass constituent based on aromatic units. Chemically, lignin is a hydrophobic branched hetero polymer reinforced with other major carbohydrate polymers (cellulose and hemicellulose fibers) of plant cell walls (Ganewatta M. S ,2019; Manimaran Ayyachamy ,2013; Satheesh Kumar M. N ,2009). Rcecent developments of incorporating lignin in promising advance applications such as nanoparticles, bioplastics, controlled release, in electro-chemical applications, composites, adsorbents, dispersants, carbon fibers. So, the extension of role of lignin is an environmental friendly functionalization for future biomass and biofuel applications (Norgren. M., 2014; Collins M. N, 2019). The efforts to use lignin derivatives in more prominent applications are currently booming. Lignin is derived from an abundant and renewable resource like plants, trees, and agricultural crops (Ndibewu P. P, & Tchieta P. G,2018). Growing demand for beef and milk has significantly encouraged the growth of the cattle industry over the past decades which directed the production of animal manure in large amount (Thornton P. K, 2010). Animal manure is the waste product excreted from herbivorous animal species that use plant material as feed. Most of the ingested carbohydrates present in green material is digested by rumen microorganisms in the animal for energy but some undigested food material enriched with lignin appears in animal manure (Ozbayram. E, 2018; Matthews. C, 2018). At present, mostly herbivorous animal manure is using as land applied fertilizer. However, excess use of animal manure for land application causes serious impact on environment, such as groundwater contamination due to heavy loads of phosphorus and nitrogen. (Szogi A.A, 2015). Therefore, animal manure management is ought to have an environmental challenge. Although animal manure is currently utilized for combustion, compost, pyrolysis and anaerobic co-digestion but more efforts are required for the development of new management strategies. (Font-Palma. C, 2019). The present invention describes the processes of extracting lignin from fresh manure of herbivorous animal. This lignin after extraction and purification can be exploited for various applications.

II. MATERIALS & METHODS

A. *Materials*

Natural biomass resource (fresh manure of herbivorous animals) was collected from local cattle fields near kukatpally, Hyderabad,India. The collected biomass was immediately dried in hot air oven for further experiments. Several chemicals used for extraction and isolation of lignin like

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sodium hydroxide, ortho phosphoric acid, 1,4-dioxane, Sulphuric acid and distilled water were obtained from Hi-Media, Hyderabad, India.

B. *Pretreatment Methods*

(1) *Hydrothermal Treatment*:

Biomass was initially subjected to a hydrothermal treatment at 180^{o}c temperature for 15 min in a batch reactor and thus obtained liquor was enriched with hemicellulose in the form of monomeric sugars (mostly xylose and pentosan polymeric sugars). The resulted hydrolysate also includes little quantities of degraded lignin which was precipitated by decreasing the pH of the hydrolysate to 4. The solid resulted from the treatment was enriched with cellulose and lignin.

(2) *Alkali Treatment*

The extraction and isolation of lignin from herbivorous animal manure was shown in Fig.1

(a). Extraction of lignin:

The solid obtain red from the hydrothermal treatment was subjected to alkali refluxing (4-6 wt% NaOH) at temperatures 80-100^{o}c for 3-5 h to liquefy the lignin. Finally, filtered the cooking liquor to separate the delignified fibers from cellulose and washed with hot water.

(b). *Isolation of lignin*

The spent liquor which was heated at 105◦C after pulping and delignification process. The black colored filtrate containing lignin was acidified with orthophosphoric acid to pH 2-4. Thereafter, the precipitate was separated by centrifuge. Finally, the separated lignin was washed with distilled water to pH 7 and was vacuum dried.

C. *Characterization*

(1). *FT-IR Analyse (FTIR)*

The dried lignin sample was mixed with IR grade KBr (SpectroInc) at a ratio of 2: 200 (lignin: KBr) to form a disc for analysis in FT-IR spectrometer (PerkinElmer). The background spectrum of control KBr was subtracted from that of the lignin spectrum. The sample was analyzed over a wave number range of 400 to 4000 cm^{-1} region to characterize the extracted lignin.

(2). *Elemental Analyze*

Carbon, Hydrogen and Oxygen analysis of lignin was carried out using a Rapid Elemental analyzer. 20mg of lignin was subjected to oxidative combustion at 950°C using Cerium oxide catalyst. The gases so produced were analysed and the concentration of each element was calculated from the integration of the corresponding peak.

(3). *Thermogravimetric Analysis (TGA)*

The thermal properties of the obtained lignin was analysed by S-II EXSTAR-6000, TG/ DTA-6300 thermal analyser. The analysis was carried out at a temperature range of 0 to 800^{o}C with heating rate of 10^{o}c/min.

(4). *Differential scanning calorimetry (DSC)*

The analysis was conducted in DSC2A-00163 equipment. The samples were placed in an aluminum crucible, under nitrogen atmosphere. The temperature range was 30 to 350°C at a heating rate of 10^{o}C.min-1.

(5). *Gel Permeation Chromatography (GPC)*

GPC analysis was performed using High performance liquid chromatography (HPLC) Waters, Austria equipped with HSP gel HR 4.0 column and an RID-10A refractive index detector (Waters, Ireland) using THF as mobile phase at the flow rate of 1mL/min to estimate the molecular (Mw) and number average (Mn).

III. RESULTS & DISCUSSION

A. *Chemical Composition and yield of lignin*

Herbivorous animal manure consists mainly lignin, cellulose and hemicelluloses and also includes 24 different minerals. The composition analysis of herbivorous animal manure were analysed according to the National Renewable Energy Laboratory Protocols (NREL-LAPs) (Sluiter. A et.al, 2018) which was shown in Table.I. When the biomass was hydrothermally treated, 91% of hemicellulose was extracted into liquid and the solid left was with cellulose and lignin. Further, all the solids obtained after the hemicellulose extraction was subjected to alkali treatment. After pretreatment methods 85% of lignin was extracted from the solids.

Table I: Chemical composition of herbivorous animal manure after pretreatment

Components on dry basis	%wt weight basis
Cellulose,	21.2
Hemicellulose,	11.2
Lignin (acid insoluble)	54.1
Ash,	7.6

B. *Fourier transform infrared spectroscopy (FT-IR)*

Fourier transform infrared spectra of lignin displays its chemical fingerprint as well as the purity (Fig.2). The wide band in range of 3200-3530 cm^{-1} indicated the stretching vibration of phenolic and aliphatic –OH groups. As compared to the reported standard lignin FT-IR spectrums (Kline.L.M, 2010), the lignin derived from the present process lacks a spectral band around 1700cm^{-1}, which is usually attributed to the carbonyl (aldehyde/ketone) moieties, signifying the chemical modification of most of the carbonyl groups under alkaline (high pH) conditions (Orton, CR, 2004). Bands centred at 2919 cm^{-1} and 2849cm^{-1} are related to C-Hstretching. Bands occurring at 1500 and 1600 cm−1 are aromatic compound characteristics (phenolic hydroxyl group) and are related to aromatic skeleton vibrations (Moubarik. A, 2013). Further, the bands in range of 1110-1130 cm^{-1} signifies the presence of syringyl (S) and guacyl (G) lignin (Zhao. H, 2009). The presence of a signal band around 1463cm^{-1}

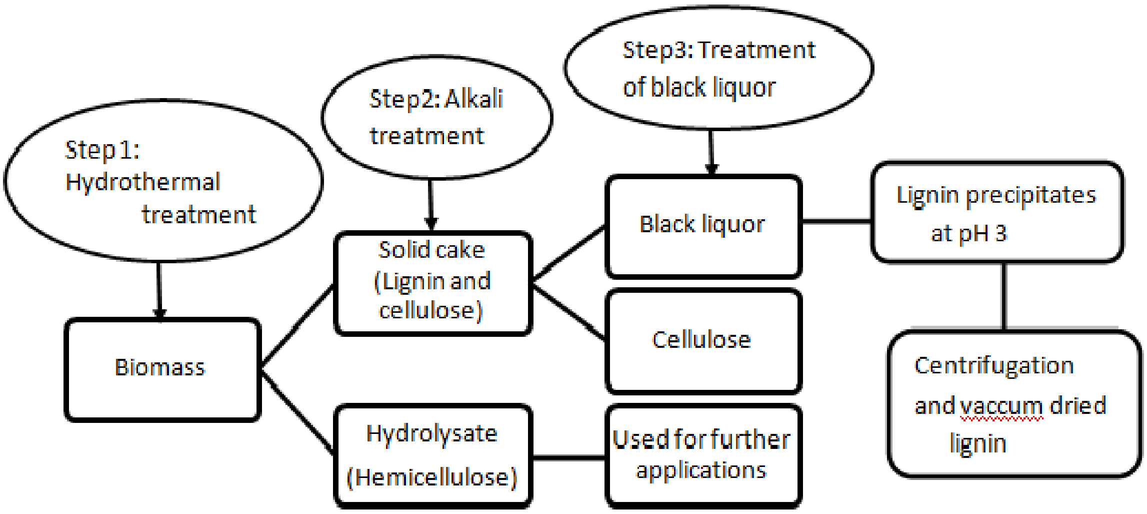

Fig 1: The schematic description Extraction and isolation of lignin from biomass (herbivorous animal manure)

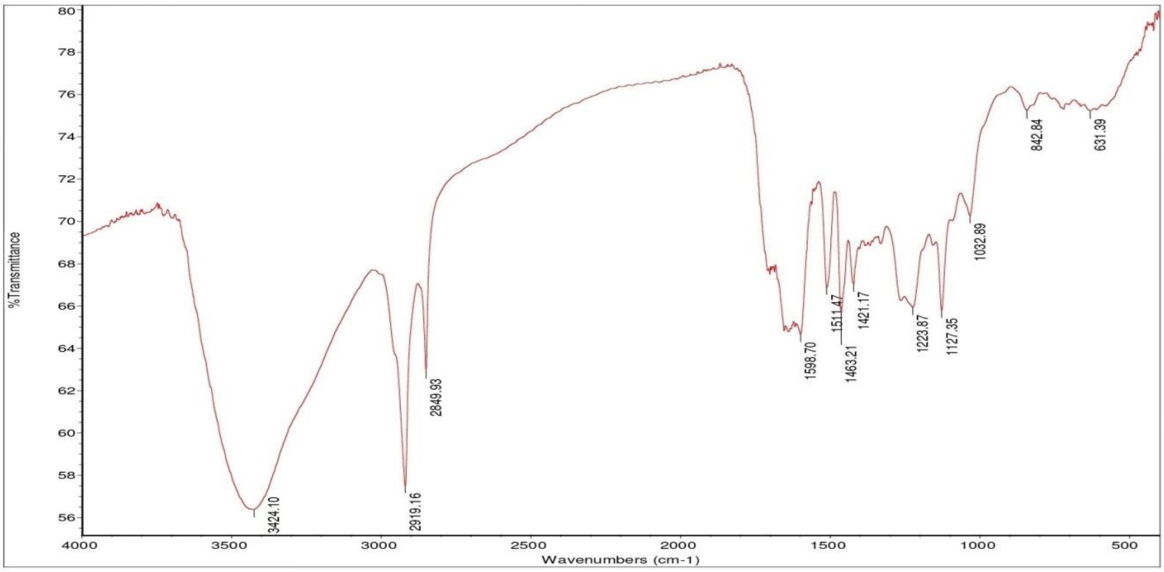

Fig.2: FTIR spectra of lignin

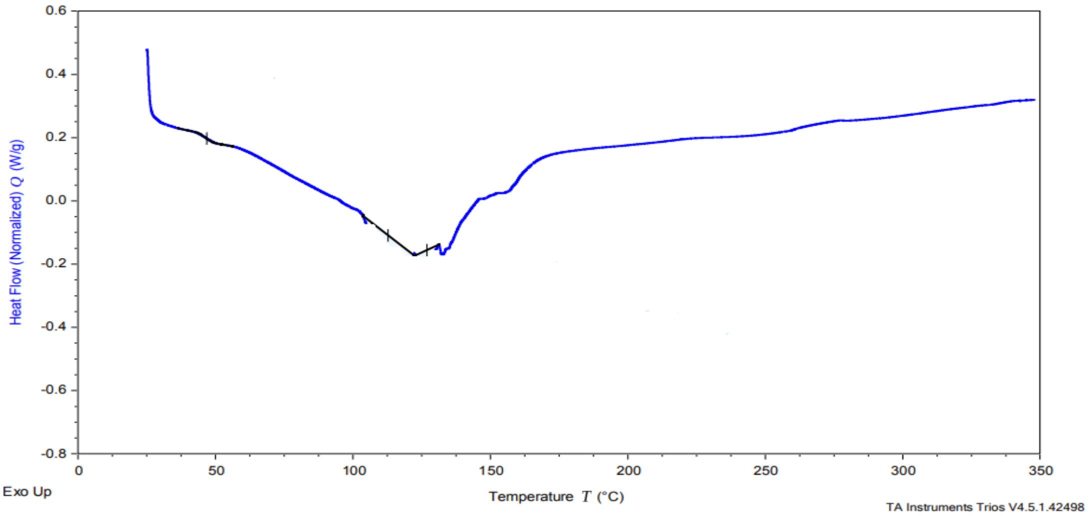

Fig 3: DSC thermograms of lignin

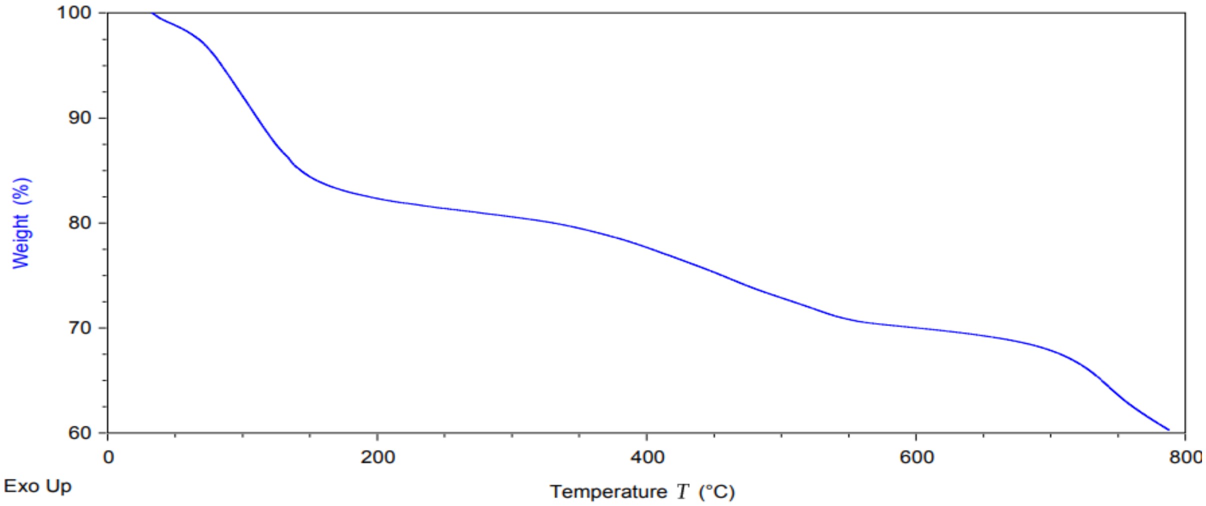

Fig. 4: TGA thermograms of lignin

(assigned to CH deformation/asymmetric in methyl, methylene and methoxyl groups), confirms that the aromatic structures of lignin did not change significantly during the alkaline extraction process.

C. *Differential scanning calorimetry (DSC)*

In this study thermal stability studies as shown in Fig.3 were conducted through DSC on lignin extracted from biomass (HAM) by measuring the heat of reaction. The measured enthalpy of lignin obtained from DSC was observed as 126.76 J/g which means that the more energy was required to break bonds present in the lignin composition. This yields lignin is a flame retardant and stable material.

D. *Thermogravimetric analysis (TGA)*

Under nitrogen conditions, thermal stability and decomposition of organic polymers were commonly calculated using TGA. TGA curve shows the percentage of material weight loss with respect to the temperature of thermal degradation. The molecular structure of lignin consists of mainly aromatic rings with different branching, these chemical bonds result in a wide range of temperature degradation from 100 to 1000 ° C as described by Yang, 2007. Lignin degradation can be divided into three stages (Tejado. A, 2007). In stage one, the initial weight loss step was due to water evaporation absorbed at 30–120 ° C. Second stage occurs around 180–350 ° C and is due to the degradation of carbohydrate components in the sample, which are transformed to volatile gasses such as CO_2, CO and CH_4. The final deterioration stage occurred above 350°C over a large range of temperatures. At this stage, degraded lignin derived volatile products including phenolics, aldehyde acids, alcohols and the formation of gaseous products have been removed. From Fig.4, it can also be shown that thermal degradation did not begin until the materials had been absorbed some amount of heat energy. Thus, heat initiated the processes of degradation and breakdown of the structure. The process of degradation involves fragmenting inter-unit linkages, releasing phenol derivatives and monomers into the phase of vapour. Finally, the process is associated with the decomposition of the aromatic rings at temperatures higher than 500°C (Tejado. A, 2007, El-Saied. H, 1993).

E. *Elemental analysis:*

From the elemental analysis Table. II shows the five elements of lignin i.e C, H, N, S and O, including various O/C and H/C elementary ratios. To use the biomass energy this elementary composition is significant as this energy produced by the thermal degradation is correlated with enthalpy of C, H and S. The low O/C and H/C ratios are therefore beneficial for the usage of lignocellulosic materials for energy and afflict on the higher heating value (HHV) (Xiao-hong Li , 2014).

Table II: Elemental analysis of lignin extracted from herbivorous animal manure

Elements	Lignin
C	38.6
H	4.7
N	1.8
S	0.1
O	36.1
O/C	0.9
H/C	0.12

F. *Molecular Weight Distribution Analysis*

The GPC is simple, fast and reliable method that involves the chromatographic fractionation of macromolecules based on their molecular size (Chen. Y, 2014). This detection method is useful for the measurement of the molecular weight distribution. For the obtained lignin, the weight-average molecular weight (Mw) was 16105g/mol, the numerical-average molecular weight (Mn) was 8920 g/mol, and the polydispersity (Mw/Mn) was 1.80 were the three fractions measured using GPC analysis.

CONCLUSION

An initial hydrothermal pretreatment for herbivorous animal manure was shown to recover 91% hemicellulose component, while subsequent alkali pretreatment for 85% lignin was shown in the present study. The results showed that the obtained lignin had similar chemical structure with commercial lignin. Therefore, lignin from herbivorous animal manure has considerable potential and possibility as feedstock for value added applications.

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