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Micro-Level Analysis through NIRS Sensor of Lignocellulosic Biomass for Bioreactor Application

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***Abstract*— Agricultural waste has great potential as renewable resource for production of bioproducts. Their proper utilization is necessary for studying their chemical composition. The reaction rate and efficiency of biodegradation are affected by presence of cellulose, lignin, hemicellulose. The traditional methods of knowing this content are time consuming, expensive, inappropriate and may give errors for quick results. Therefore, Near-infrared spectroscopy has gaining attention for quick, errors free, eco-friendly results. This method does not require chemical reagents or large samples. The study is based on characterization of lignocellulosic biomass from agricultural waste. This study highlights importance of Near-infrared spectroscopy based on quick analysis and process optimization.**

***Index Terms*— Lignin, Hemicellulose, Cellulose, Near-Infrared Spectroscopy, bioreactor, and lignocellulosic biomass.**

I. INTRODUCTION

Agricultural waste is becoming more efficient as the demand for biofuels as well as bioproduct is continuously increasing. However, there are significant obstacles because they come from a wide variety of sources and takes a long time to decompose, making it more difficult to use as a resource. Crops including rice, corn, wheat, and rapeseed, were used for study. Lignocellulosic material in plants are cellulose, hemicellulose, and lignin, makes up the majority of plant material. These components give plants their strength and structure, and they also influence how quickly living organisms may break down the material. Due of its speed, near infrared spectroscopy (NIR) is employed in numerous fields. No additional ingredients, chemicals, or specific preparation are required. It doesn't produce any garbage either. Biomass of First-generation is obtained from food crops; second-generation biomass is obtained from lignocellulosic materials; and micro algae is obtained from third and fourth generation. One ample and renewable resource derived from plants is lignocellulosic biomass. Most demanding agricultural products such as fruits, vegetables, meat, fowl, milk products, and crops results in agricultural wastes. Figure 1, depicts India's annual production of agricultural waste. Depending on the kind of agricultural product, these wastes can be solid, liquid, or a thick mixture. Fossil fuels are becoming more and more necessary as development advances. However, excessive usage of these finite fuels has led to an energy crisis. Climate change, contaminated air, and other environmental concerns are also brought about by it. There are two types of procedures for analysing lignocellulose: chemical methods and instrumental methods. Chemical procedures are less accurate than instrumental techniques like gas chromatography (GC) and excellent-performance liquid chromatography (HPLC).

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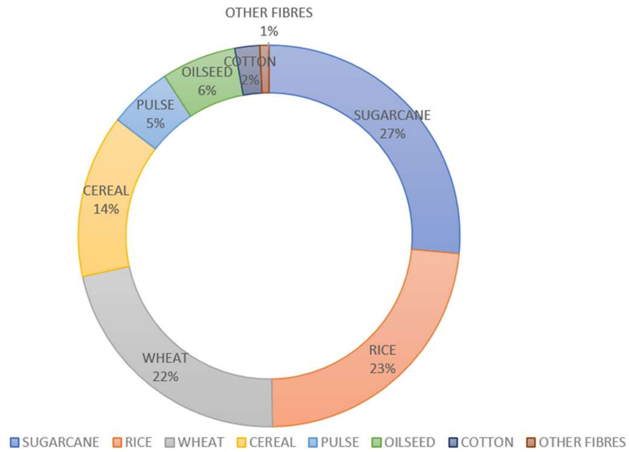

Figure 1. Approximate amount of agriculture waste production in India

II. METHODOLOGY

Fernández et al. observed the chemical composition of lignocellulosic biomass using Near Infrared Spectroscopy (NIRS), a rapid and non-destructive technique. Before conducting the spectrum measurements, they gathered the biomass samples, let them to dry, then crushed them until the particles were all the same size. The NIR spectra were collected within a specific range of wavelengths to identify the absorption patterns associated with the biomass's O–H, C–H, and N–H groups. To increase the efficiency of NIRS models they carried chemical tests on lignin, cellulose, hemicellulose, moisture. Partial Least Squares Regression (PLSR) method was used to find correlations between spectral information and chemical values. To enhance the reliability of predictions, scatter correction and normalization methods were employed as preprocessing tools to eliminate error in spectral information. The accuracy level of the models was determined by using statistical measures which includes the coefficient of determination (R^2) and prediction error values. The results showed that Near Infrared Spectroscopy (NIRS) is an efficient method for predicting various components and moisture content in lignocellulosic materials. Thus, making this method efficient for bioreactor and bioreactor applications [1]. Zhao et al. created model based on NIRS to measure lignocellulosic components in crop residues. The samples that used by Zhao et al. were obtained from different agricultural sources. The obtained sources were dried and ground it to uniform size particles before analysis. The work of Near-infrared spectra was to detect absorption bands related to O–H and C–H functional groups in biomass. At that exact time, laboratory chemical analysis was conducted to determine cellulose, hemicellulose, lignin, moisture content, total solids, and volatile solids. These values were taken as references for model calibration and validation. PLSR was employed to build models that correlated spectral information with laboratory data. To further improve the quality of the spectra, methods such as multiplicative scatter correction and derivative transformation were employed to reduce the effects of light scattering. The performance of the models was evaluated using the coefficient of determination and the root mean square error of prediction. The work demonstrated that the combination of NIRS with chemometric analysis provides a fast and accurate method for the analysis of lignocellulosic biomass for bioenergy and bioreactor applications [2]. Feng et al. also explored the application of NIRS for fast biomass analysis in bioreactor-related applications. The biomass samples were dried, ground, and sieved to ensure that the particle size was consistent to reduce the effects of spectral scattering. Diffuse reflectance NIR spectra were measured over a wide wavelength range to capture the vibration-sensitive absorption bands corresponding to O-H, C-H, and N-H bonds. To improve the quality of data, the various spectral preprocessing techniques like derivative transformation, smoothing and standard normal variate correction were used. To prove relation between chemical composition and spectral data, multivariate calibration models were developed using PLSR. For validation and accuracy of the model, statistical measures such as R^2 and prediction error were used [3]. Christy E. Manyi-Loh and Ryk Lues examine the anaerobic digestion of lignocellulosic biomass. The role of lignin, cellulose and hemicellulose in the biodegradation process during the anaerobic digestion is explain by the authors. For improving the efficiency of hydrolysis and defeating the biomass recalcitrance, various pretreatment methods are used. They also explain effect of pH, temperature and nutrient supplementation on methane and biogas production. Biochemical methane potential analysis was accepted as a standard technique for evaluating digestion efficiency [4]. To evaluate the conversion efficiency of lignocellulosic biomass through controlled processing Md. Wadud Ahmed, Vijay Singh and Mohammed Kamruzzaman perform experiments. Their work assisted in a better comprehension of biomass conversion, performance and its refinement for energy recuperation applications. To examine if the biomass feedstock was convenient for transformation in the first place it was checked for cellulose, hemicellulose, lignin, total solids and volatile solids. Prior treatment techniques were utilised to vary the structural intricacy of the biomass and surge its enzymatic and microbiological approachability. Anaerobic digestion studies were conducted in laboratory-scale reactors with controlled pH and temperature. The digestion process was regularly monitored to assess stability and performance using measurements of biogas volume and methane content [5]. Ning Ai et al. quickly assessed the cellulose, hemicellulose, and lignin content of Sargassum horneri using near-infrared spectroscopy. Ning Ai et al. gathered 74 samples of Sargassum horneri, cleaned, dried, powdered, and sieved them to create a uniform biomass powder. Ning Ai et al. used the Van Soest method to test the cellulose and hemicellulose contents and standard analytical method to measure the lignin concentration. Ning Ai et al. used the diffuse reflectance mode under controlled conditions to get near-infrared spectra in the 12,000–4000 cm^{-1} range. Spectral pretreatment methods such as Savitzky–Golay smoothing, derivatives, standard normal variate, and multiplicative scatter correction were used by Ning Ai et al. Partial least squares regression was used by Ning Ai et al. to create calibration models [6]. Xiaoli Li, Chanjun Sun, Binxiong Zhou, and Yong. He studied the contents of hemicellulose, cellulose, and lignin in moso bamboo using near-infrared spectroscopy (NIRS). The bamboo samples were taken from various sites and were grind into a fine powder. The chemical composition of hemicellulose, cellulose, and lignin was studied using the Van Soest method. The near-infrared spectra were scanned within the wavelength range of 1100-2498 nm. Chemometric models were developed using Partial Least Squares Regression (PLSR) with variable selection methods to increase the accuracy of the models [7].

III. PARAMETERS

A. pH

Microbial activity and biomass conversion in energy recovery mainly depends on pH. Biomass decomposition such as cellulose and hemicellulose are break down by maintaining a constant pH level. Large changes in pH may affect on how microbes use biomass [3].

B. Total Solids

The total quantity of solid matter, whether organic or inorganic, present in biomass is referred to as total solids (TS). The effect of solid concentration on biomass handling and processing capacity is relevant. The total solids concentration needs to be maintained at optimal levels [3].

C. Volatile Reduction

The volatile solids (VS) destruction in the biomass processing reflects the extent of biodegradable organic materials that have been decomposed. The greater the destruction of volatile solids, the better the use of biomass materials such as cellulose and hemicellulose. A method used to determine the effectiveness of biomass decomposition and energy recovery is the volatile solids destruction [3].

D. Lignin

According to Khaire et al. Lignin is a highly structured polymer that occurs in plants as well as algae. It is the major component that gives strength to plants. Figure 2 shows the chemical structure of lignin. Lignin is necessary for the development of plant cell walls. This protein support to plants stiffness and stability. Chemically speaking, lignin is a mostly interconnected phenolic polymer. It possess an amorphous, widely branched, and changeable aromatic structure but no clear underlying structure. The three fundamental phenylpropane units known as monolignols that comprise lignin are P-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Depending on the kind of biomass, different combinations of these monolignols are present. Coniferyl alcohol predominates in softwood lignin, sinapyl and coniferyl alcohols are present in hardwood lignin, and all three are present in grass lignin. The secondary cell wall contains the majority of lignin. It facilitates the passage of nutrients and water via xylem tissues by supporting vascular cells. Lignin is difficult to break down during biomass processing because of its great resistance to degradation, which helps shield plants from diseases and microorganisms. The composition of lignin differs among plant species. It usually carry a lot of carbon, hydrogen, oxygen, and a small amount of ash. Because of its very asymmetrical and heterogeneous molecular structure, lignin lacks a clearly defined structure in contrast to cellulose and hemicellulose. In industries, lignin is mostly used as fuel. Nevertheless, it can be chemically change and used for many things, like the extraction of heavy metals and the creation of adhesives, surfactants, and activated carbon. Plant biomass carry varying amounts of lignin [10]. The lignin content in woody plants is excessive than that of herbaceous plants, which influences the biomass's stiffness and ease of processing. Lignin is tightly associated with structural plant cells, involving wood that contain fibers and sclerenchyma cells, and it contributes to the strength and rigidity of plant cell walls. Lignin is generally utilized in procedures that don't require high purity and is produced in vast quantities all around the world. Plant defensive tissues contain lignin as well as other polymers such cutin, suberin, and waxes. These enhance the plant's resilience and preserve its surface [9]. Lignin is more tough for living organisms to decompose and has a more stable pattern in near-infrared (NIR) light than other plant material components like carbohydrates, according to Fernández et al. [1]. According to Zhao et al., it is difficult to predict lignin levels from NIR data since lignin signals can mix with cellulose and hemicellulose signals [2]. Lignin is a significant structural component of lignocellulosic biomass and is crucial in determining biomass recalcitrance, according to Kristoffer Mega Herdlevaer, Camilla Løhre, Egil Nodland, and Tanja Barth. Instead of focusing exclusively on tree species, they found that the lignin percentage changes greatly based on biomass fractions such wood, bark, needles, and twigs. According to the study, wood samples had somewhat lower lignin content than bark samples, which had the greatest lignin content [8].

E. Cellulose

Khaire et al. found that cellulose is main parts of the cell that present in plant wall and plays a big role in making plants strong and rigid. Figure 3, shows chemical structure of Cellulose. Cellulose makes up about 15 to 30 percent of the dry weight of the primary plant cell wall and is the main part of lignocellulosic biomass. Cellulose is a large molecule made of many glucose units linked together in long chains. Compared to hemicellulose, cellulose has a strong, crystal-like structure that doesn't break easily when exposed to water or acid, while hemicellulose is not as strong or stable. Cellulose microfibrils are arranged side by side in the cell wall, with sizes usually between 10 and 25 nanometers, and the smaller fibrils are about 2 to 4 nanometers wide. When cellulose fibers line up in this way, they form a structure called a micellar structure. Chemically, cellulose is an organic substance with the formula $(C_6H_{10}O_5)_n$ and does not dissolve in water or weak acid or alkali solutions at normal room temperatures. Cellulose makes up about 30 to 50 percent of total lignocellulosic biomass, and the world produces about 120 to 140 billion tons of it every year. The amount and type of cellulose can vary depending on the plant, its species, and how old it is, which affects the properties of the biomass and how easily it can be broken down [11]. The primary cell walls of flowering plants and cone-bearing plants are mostly made of cellulose-based sugars. Cotton fibers contain nearly 91 percent cellulose, making it one of the purest natural forms of cellulose compared to cellulose from wood. Cellulose is made up of long chains of many β-1,4-linked D-glucose units, forming a straight and regular structure. Each β-D-glucopyranose unit is rotated about 180 degrees compared to the one it was before it, which makes the molecule more stable and repeatable. These cellulose chains held together using hydrogen bonds and other weak forces between the molecules to form rigid microfibrils. Cellulose contains about 6,000 primary cell walls and can increase to 14,000 in secondary cell walls. use biomass [3]. Cellulose microfibrils are crystalline in structure and not water-attracting, that makes the lignocellulosic biomass hard to break down. Cellulose is not only an important part of higher plants but also it is found in algae, bacteria, oomycetes, and other microbes that forms biofilms [9]. Fernández et al. said that cellulose is the main type of polysaccharide in lignocellulosic biomass and has a big effect on how NIR spectra respond because of the O–H groups [1]. Zhao et al. found that cellulose levels can be predicted accurately with NIRS if strong calibration models are created using chemical data as a reference [2].

F. Hemicellulose

Khaire et al. said that hemicellulose, which is also called polyose, is a key structural polysaccharide found along with cellulose in the walls of all plant cells. Figure 4, shows chemical structure of Hemicellulose. Hemicellulose is made up of different types of heteropolysaccharides, like arabinoxylans, xylans, glucomannans, glucuronoxylans, and xyloglucans. These polymers include various sugar units besides glucose, such as arabinose, mannose, xylose, galactose, and rhamnose [12]. Hemicellulose has a high amount of D-sugars, mostly pentoses, and in some cases small amounts of L-sugars. Xylose is usually the most common sugar unit in hemicellulose, while mannose is the main one in softwood hemicellulose. Hemicellulose also contains modified sugar parts like glucuronic acid and galacturonic acid. Because hemicellulose is amorphous, it can be broken down easily by dilute acids, dilute bases, and specific enzymes that target hemicellulose. About 15 to 35 percent of all lignocellulosic biomass is made up of hemicellulose. In terms of structure, hemicellulose is a branched, single-chain, and amorphous heteropolysaccharide. Hemicellulose polymers consist of pentoses, hexoses, uronic acids, and acetylated sugars, with a length of between 500 and 3000 sugar units. The sugar parts in hemicellulose are mostly connected by β-1,4-glycosidic bonds at the O4 spot. Short side chains and lots of branching stop crystallization, so hemicellulose is not crystalline. The kinds and number of groups on the branches of hemicellulose depend on the type of plant material. Based on the main sugar in the main chain, hemicellulose is divided into arabinans, xylans, mannans, and xyloglucans. Xylan is the most common type of hemicellulose, and its makeup can differ a lot between plant types. Hemicellulose helps make plant cell walls stronger by linking with both cellulose and lignin [9].

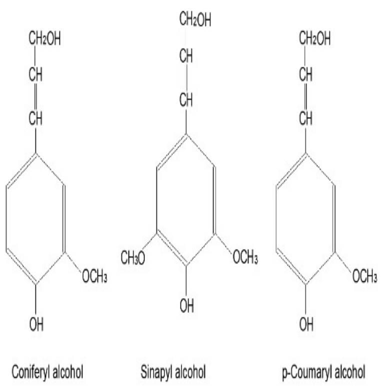

Figure 2. Chemical arrangement of Lignin

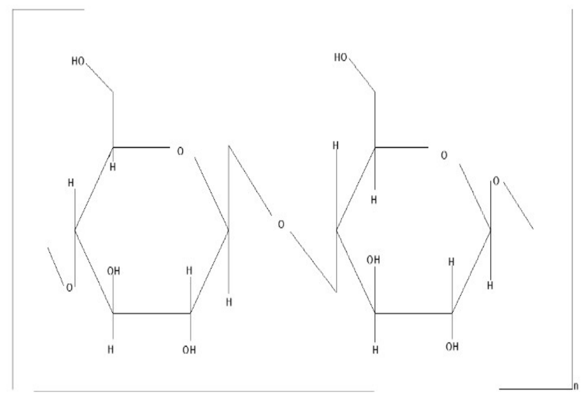

Figure 3. Chemical arrangement of Cellulose

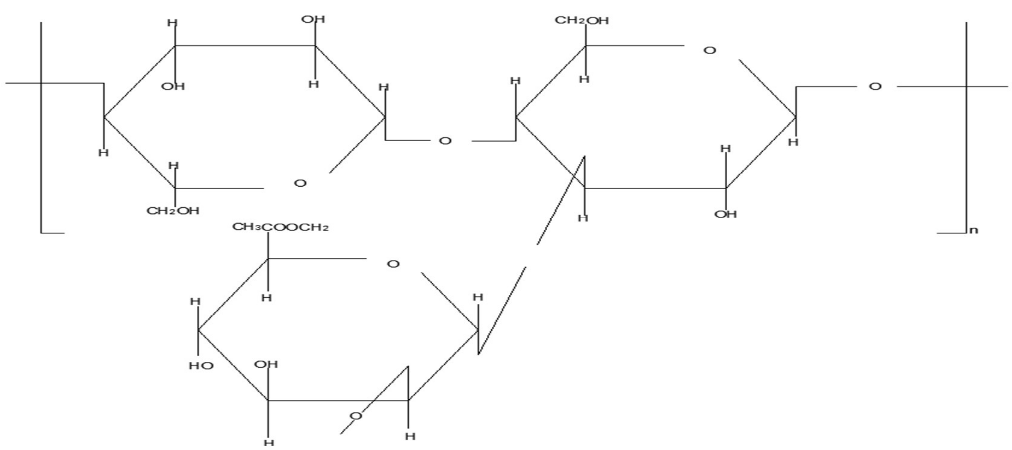

Figure 4. Chemical arrangement of Hemicellulose

G. Moisture Content

Fernández et al. found that moisture content has a big impact on NIR absorbance because of the strong interactions from the O–H bonds. In order to accurately forecast the biomass components, it is crucial to employ strong calibration models, as they found that the spectra overlap more when there is more moisture [1]. Moisture content is the amount of water in the biomass that is a very important factor in biomass processing and storage. High moisture content can have adverse effects on material handling, storage stability, and conversion efficiency in biofuel and bioproduct conversion processes [13]. Water has strong absorption properties in the near-infrared zone of the spectrum; hence, moisture content affects near-infrared spectra. It is important to estimate moisture content accurately for effective biomass analysis and process optimization. Near-infrared spectroscopy is a best possible technique for measuring moisture content in different types of biomass [5].

H. Fixed Solid

Fixed solids, also known as ash content, are the inorganic and non-combustible part of biomass that is left after complete combustion. High ash content can be detrimental to the overall performance of biomass because it reduces the amount of biodegradable material and the efficiency of energy conversion. Thus, fixed solids are important factor in biomass analysis. They can be analyzed by high-temperature furnace methods to determine the inorganic residue accurately [2].

III. RESULTS

Fernández et al. found that Near Infrared Spectroscopy (NIRS) is very effective for the prediction of moisture content as well as components of biomass including cellulose, hemicellulose, and lignin. The results indicated that the changes in moisture content had a significant effect on the regions of the spectrum related to O–H stretching vibrations. With increase in moisture content, overlapping of spectra also increased, which in turn affected the accuracy of prediction. However, after applying chemometric calibration techniques, the accuracy of prediction for cellulose, hemicellulose, and lignin was found to be significantly improved. The authors concluded that accurate control and correction for moisture content are required for obtaining accurate results when applying NIRS analysis for biomass characterization as per Table 1 [1]. Similarly, Zhao et al. showed that the combination of NIRS with Partial Least Squares Regression (PLSR) can be used for the accurate prediction of cellulose, hemicellulose, and lignin in agricultural residues. Their results revealed a high degree of agreement between predicted values and laboratory reference values for the key structural parts of plant biomass. Spectrum region involving related to C-H and O-H bonds were found great importance in enhancing efficiency of models. This paper includes NIRS capacity to handle compositional variations that are present in different crop residues. The author ensures that NIRS is promising method to assess agricultural biomass in bioenergy conversion plant [2]. Md. Wadud Ahmed, Vijay Singh, and Mohammed Kamruzzaman used strict method for experimental and analytical procedure to evaluate lignocellulosic biomass conversion. Their research focus on feedstock variability including plant characteristic such as lignin, cellulose, hemicellulose. This detailed study allows to measure effectiveness of biomass. It also helps to define most important factor that affect performance of process [5]. According to Ning Ai et al., the raw near-infrared spectra of Sargassum horneri presented a large overlap of signals, which made it difficult to interpret. The initial models for the prediction of cellulose, hemicellulose, and lignin had only moderate accuracy. But the selection of appropriate spectral variables helped to improve the accuracy of the models. The interval Partial Least Squares (iPLS) model performed the best, with high accuracy for cellulose and hemicellulose and moderate accuracy for lignin. The predicted values were in close agreement with the laboratory values, which proved the accuracy of the method. The study thus confirmed the effectiveness of NIRS in the rapid and accurate analysis of lignocellulosic components of marine biomass [14].

TABLE I: DIFFERENT FORMS OF LIGNOCELLULOSIC BIOMASS AND THEIR COMPOSITIONAL AND PHYSICOCHEMICAL FEATURES

Ref.	Biomass Type	pH	Total Solids (TS, %)	Volatile Solids (VS, % TS)	Lignin (%)	Cellulose (%)	Hemicellulose (%)
Fernández et al., 2019	Olive tree pruning	6.8–7.5	88–92	75–85	18–25	32–45	20–28
Zhao et al., 2018	Crop residues	6.5–7.2	85–93	70–88	12–22	35–48	18–32
Feng et al., 2018	Biofuel pellets	7.0–7.8	90–95	80–90	15–28	30–46	16–30
Manyi-Loh & Lues, 2023	Lignocellulosic biomass	6.5–8.0	80–92	65–85	10–30	30–50	15–35
Ahmed et al., 2025	Agricultural biomass	6.8–7.6	85–94	75–90	14–26	33–49	18–31
Ning Ai et al., 2022	Sargassum horneri	7.2–8.1	82–90	68–82	6–12	18–30	20–38
Li et al., 2015	Moso bamboo	6.4–7.0	90–95	78–88	22–30	40–50	20–28
Herdlevær et al., 2022	Wood, bark, needles	6.5–7.3	88–94	70–85	20–35	28–45	15–25
Khaire et al., 2021	Sugarcane tops	6.6–7.4	85–92	75–88	14–21	38–45	25–32

IV. CONCLUSION

The above studies show that Near Infrared Spectroscopy (NIRS) is a systematic, fast, and non-destructive method for micro-level analysis of lignocellulosic biomass components, making it very appropriate for bioreactor analysis. The results show that NIRS can accurately predict the values of moisture content, total solids, volatile solids, cellulose, hemicellulose, and lignin. The moisture content showed a major impact on NIR radiation absorption because O–H bond interactions caused water content problems which resulted in forecasting mistakes. The studies established that proper sample handling which includes homogenization and particle size reduction must be implemented to achieve better spectral stability and calibration results. The literature demonstrates that NIRS functions as an efficient method for quick biomass assessment which enables continuous measurement of biomass and serves as a faster option than traditional chemical testing methods that require extensive time. The development of strong calibration models needs to become the main area of research because they must handle biomass type variations and moisture content changes and structural heterogeneity. The combination of NIRS technology with existing online and offline bioreactor monitoring systems will enable real-time management and process improvement for anaerobic digestion and biomass transformation. Advanced chemometric and machine learning models can enhance the precision of predicting lignocellulosic fractions and key process parameters which include volatile solids and total solids. The scientific community requires more research to understand how NIR spectral patterns connect to micro-structural changes in lignin cellulose and hemicellulose throughout the pretreatment and biodegradation process.

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