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Original Research Article

Structural Characterisation of Raw and Acid-Pretreated Invasive Plant Biomass (*Ludwigia hexapetala* and *Ricinus communis*)

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ABSTRACT
This study focused on the structural characterization of Raw and Acid-Pretreated Invasive Plant Biomass (*Ludwigia hexapetala* and *Ricinus communis*) and its impact on biofuels production. Raw and acid-pretreated biomass samples were characterized using various techniques, including FTIR, SEM, TEM, XRD, TGA, and BET surface area analysis. Acid pretreatment significantly altered the biomass structure, increasing cellulose content, decreasing lignin content, and enhancing surface area and porosity. The results obtained in this analysis highlight the crucial role of structural features in determining biofuel potential. This study demonstrates the feasibility of utilizing invasive plants as sustainable feedstocks for biofuel production and underscores the importance of structural analysis in optimizing bioethanol production processes.

INTRODUCTION

Some leading plants available for bio fuel production may not be the most sustainable. Alternatively, the removal of existing invasive plant species biomass and processing into the forms for combustion or liquid fuel conversion maybe more sustainable as it would comply with the US Executive Order 13112 on invasive species [1] support climate change initiatives [2], and expand economic opportunities in rural areas by helping fulfill the mandate by the US Renewable Fuels Standards [3].

Looking at the vast natural vegetation, potentially promising new areas of bioenergy production exist in the invasive plant species. The addition of invasive plant species as a bioenergy source will help to diversify the nation's energy dependence and help in the reduction of the negative environmental and social impacts from energy crop production.

With the great energy crisis and alarmingly rapid climate change, there is a growing awareness of the search for more sustainable and distributed methods of energy production, waste minimization, air pollution reduction, the protection of native forests, and a reduction in greenhouse gas (GHG) emissions, all achievable by bio energy production from Alien Invasive Plants [4]

The lack of economic return is an important reason for less intensive management in non crop areas like invasive plants. With new markets emerging for cellulosic energy sources and advances in equipment technology, this increased incentive could help improve the level of management of these plant species for subsequent harvest or removal of excess biomass source for bio fuels. Using invasive plant species in bioenergy facilities would provide enticing opportunities for land managers and business developers. Though the characteristics of invasive plants such as rapid growth have been shown to be

identical to those of bioenergy crops [5], there are still inadequate scientific studies on the use of most invasive plant species in bio energy production hence the needs for structural characterization of invasive plant which is essential for understanding their potential for biofuel production. Advanced analytical techniques, such as Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), Thermogravimetric Analysis (TGA), and Brunauer-Emmett-Teller (BET) surface area analysis, provide valuable insights into the chemical composition and physical structure of the biomass [6, 7, 8].

FTIR spectroscopy analyzes the vibrational frequencies of chemical bonds, revealing functional groups present in the biomass, such as hydroxyl, carbonyl, and carboxyl groups, which are indicative of cellulose, hemicellulose, and lignin content. SEM and TEM provide high-resolution images of the surface and internal structure of the biomass, respectively, revealing information about pore size, surface area, and crystallinity. XRD analyzes the diffraction patterns of X-rays, revealing the crystalline structure of cellulose, a key factor influencing enzymatic hydrolysis. TGA measures the weight loss of the biomass as a function of temperature, providing information about thermal stability and decomposition characteristics. BET surface area analysis determines the specific surface area and pore volume of the biomass, which are crucial factors influencing enzymatic accessibility and reactivity.

The findings from the structural characterization of *Ludwigia hexapetala and Ricinus communis* biomass will provide valuable insights into their suitability for biofuel production. The acid pretreatment will increase the surface area, pore volume, and hydrophilicity of both plants, enhancing enzymatic accessibility and hydrolysis for higher yields of the bio fuels.

MATERIALS AND METHODS

Ludwigia hexapetala and *Ricinus communis* Biomass Collection and Preparation

Ludwigia hexapetala (LU) and *Ricinus communis* (RI) biomass were harvested from Abuja environs. Samples were air-dried to a constant weight, then ground and sieved to the smallest particle size. A portion of each raw biomass was subjected to acid pretreatment by mixing with 10% (w/v) sulfuric acid at a 1:10 solid-to-liquid ratio. This mixture was autoclaved at 121°C for 15 minutes, cooled, and the solid residue separated by filtration and washed thoroughly with distilled water until a neutral pH was achieved. Both the raw and pretreated biomass were stored for further analysis and bioethanol production processes.

Structural Characterization of *Ludwigia hexapetala* and *Ricinus communis*

Several techniques were used for structural characterization. Fourier Transform Infrared (FTIR) spectroscopy, using a Shimadzu IRAffinity-1S spectrometer with a QATR-10 accessory, analyzed functional groups in the 4000-500 cm^{-1} range, providing information about the biomass composition and pretreatment effects [4]. X-ray Diffraction (XRD), employing a Philips XPERT-PRO diffractometer with Cu Kα radiation, assessed cellulose crystallinity via the Segal method [9], scanning from 10-80° 2θ. Scanning Electron Microscopy (SEM) using a Tescan Vega 3 LMH at 20.0 kV and Transmission Electron Microscopy (TEM) using a JEOL TEM-2100F at 200 kV visualized the surface morphology and microstructure, respectively. Samples were carbon-coated for SEM and sonicated in ethanol for TEM. Thermogravimetric Analysis (TGA) with a Perkin-Elmer STA 6000, heating from 25°C to 1000°C at 10°C/min under nitrogen flow, evaluated thermal stability. Brunauer-Emmett-Teller (BET) surface area analysis, performed on a Micrometrics ASAP 2460 with nitrogen adsorption-desorption at 77 K after sample degassing, determined specific surface area and pore volume.

RESULTS AND DISCUSSION

Brunauer–Emmett–Teller (BET) Analysis

The structural characteristics of biomass, including surface area, pore volume, and pore size distribution, significantly influence the accessibility of cellulose for enzymatic hydrolysis and, consequently, biofuel production [10]. The Brunauer-Emmett-Teller (BET) analysis provides valuable insights into these structural properties, enabling a better understanding of the relationship between biomass structure and bioethanol yield. The nitrogen adsorption-desorption isotherms, shown in Figure 1, were used to determine the BET surface area, pore volume, and pore size distribution of raw and pretreated *Ludwigia hexapetala* (LU) and *Ricinus communis* (RI) biomass.

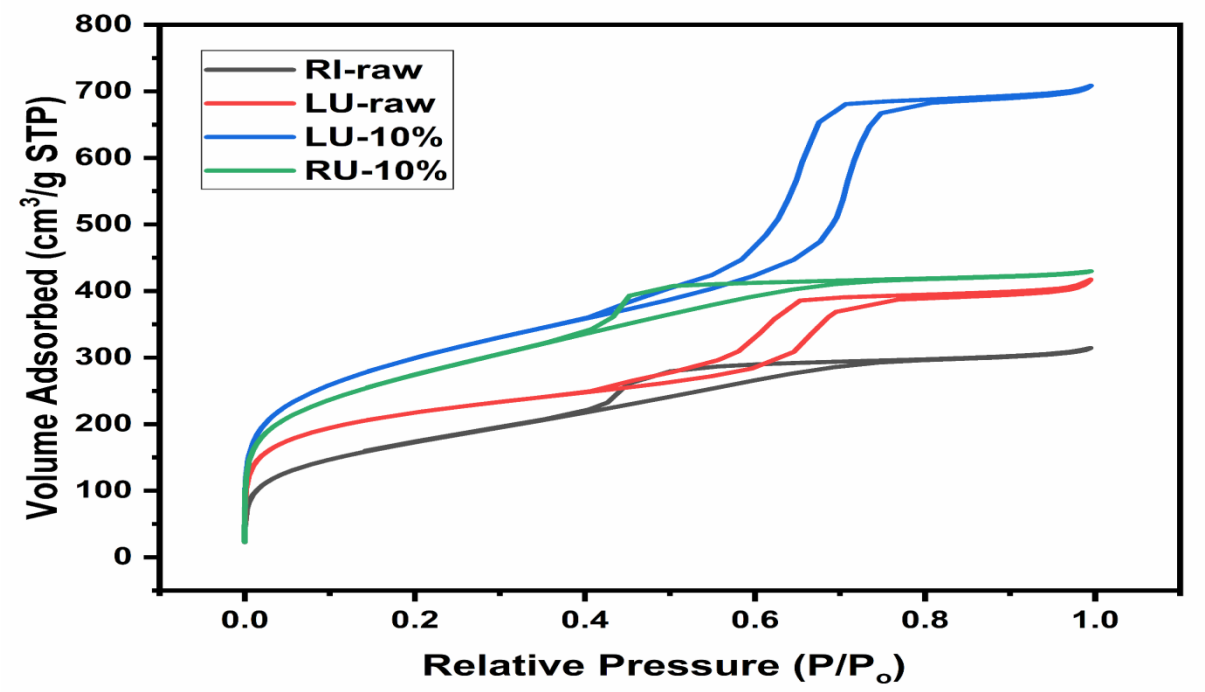

Figure 1: Nitrogen-sorption isotherms of the biomass

The nitrogen sorption isotherms (Figure 1) exhibit typical Type IV isotherm characteristics with a H3 hysteresis loop, which is indicative of mesoporous materials with slit-shaped pores [11]. Pretreatment of LU and RI with 10% H_2SO_4 resulted in a significant increase in BET surface area and pore volume. This increase can be attributed to the removal of hemicellulose and lignin, which exposes more cellulose fibers and creates additional pores within the biomass structure, making it more accessible to enzymes during hydrolysis. pore size distribution further reveals that the pretreatment process creates a broader range of pore sizes, including a higher proportion of mesopores (2-50 nm). These mesopores are particularly important for enzyme access and activity, as they provide sufficient space for enzymes to diffuse into the biomass structure and interact with the cellulose substrate [12]. The larger pore volume observed in pretreated biomass accommodates a greater volume of enzyme solution, promoting more effective hydrolysis.The increased accessibility of cellulose, facilitated by the larger surface area and pore volume, enhances enzymatic hydrolysis and subsequently boosts bioethanol production. The improved enzyme accessibility to the cellulose substrate in pretreated biomass leads to a more efficient breakdown of cellulose into fermentable sugars, ultimately resulting in a higher ethanol yield.

Scanning Electron Microscopy (SEM) Analysis and Bioethanol Production

Surface morphology and microstructure of biomass are shown by the SEM analysis, which reveal details about the effects of pretreatment on the structure and accessibility of cellulose fibers. SEM analysis can correlate structural changes with the efficiency of enzymatic hydrolysis and bioethanol production. Figure 2 displays representative SEM images of raw and pretreated *Ludwigia hexapetala* (LU) and *Ricinus communis* (RI) biomass.

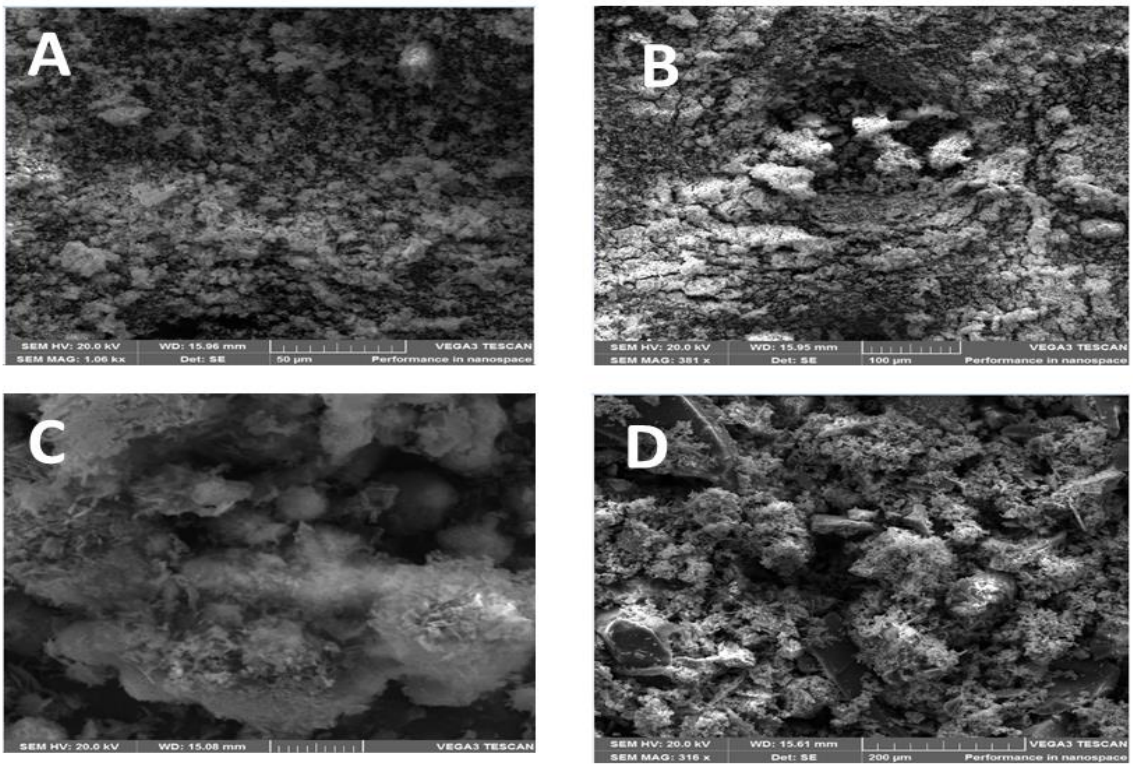

Figure 2: Representative SEM images of the biomass: (A) Raw LU, (B) Pretreated LU, (C) Raw RI and (D) Pretreated RI

The SEM images (Figure 2) reveal distinct differences in the surface morphology of raw and pretreated LU and RI biomass. Raw LU and RI exhibit a relatively smooth surface with intact cell walls and a compact structure. Pretreatment with 10% H_2SO_4 causes significant structural changes, including disruption of cell walls, increased surface roughness, and the formation of pores and cracks. These structural modifications are attributed to the removal of hemicellulose and lignin, exposing cellulose fibers and increasing the surface area accessible to enzymes during hydrolysis [13].The increased surface roughness and porosity observed in pretreated LU and RI are consistent with the BET analysis results (Figure 1), which showed a significant increase in surface area and pore volume. These structural changes facilitate enzyme access to the cellulose substrate, promoting more efficient hydrolysis and higher sugar yields. The SEM images provide visual evidence of the effectiveness of acid pretreatment in enhancing the accessibility of cellulose for enzymatic attack. The increased surface area and porosity, visualized by SEM, enable greater enzyme penetration and interaction with cellulose fibers, leading to more efficient hydrolysis and ultimately higher ethanol production. The SEM analysis provides valuable information about the relationship between biomass structure and bioethanol yield, complementing the BET analysis and further supporting the importance of structural analysis in optimizing bioethanol production processes.

Transmission Electron Microscopy (TEM) Analysis and Bioethanol Production

Transmission Electron Microscopy (TEM) provides high-resolution images of the internal structure of biomass, allowing for a more detailed examination of the effects of pretreatment on cellulose crystallinity and microfibril structure. TEM analysis can reveal changes in the arrangement and organization of cellulose chains, which can impact enzymatic hydrolysis efficiency. Figure 3 presents high-resolution TEM (HRTEM) images of raw and pretreated *Ludwigia hexapetala* (LU) and *Ricinus communis* (RI) biomass.

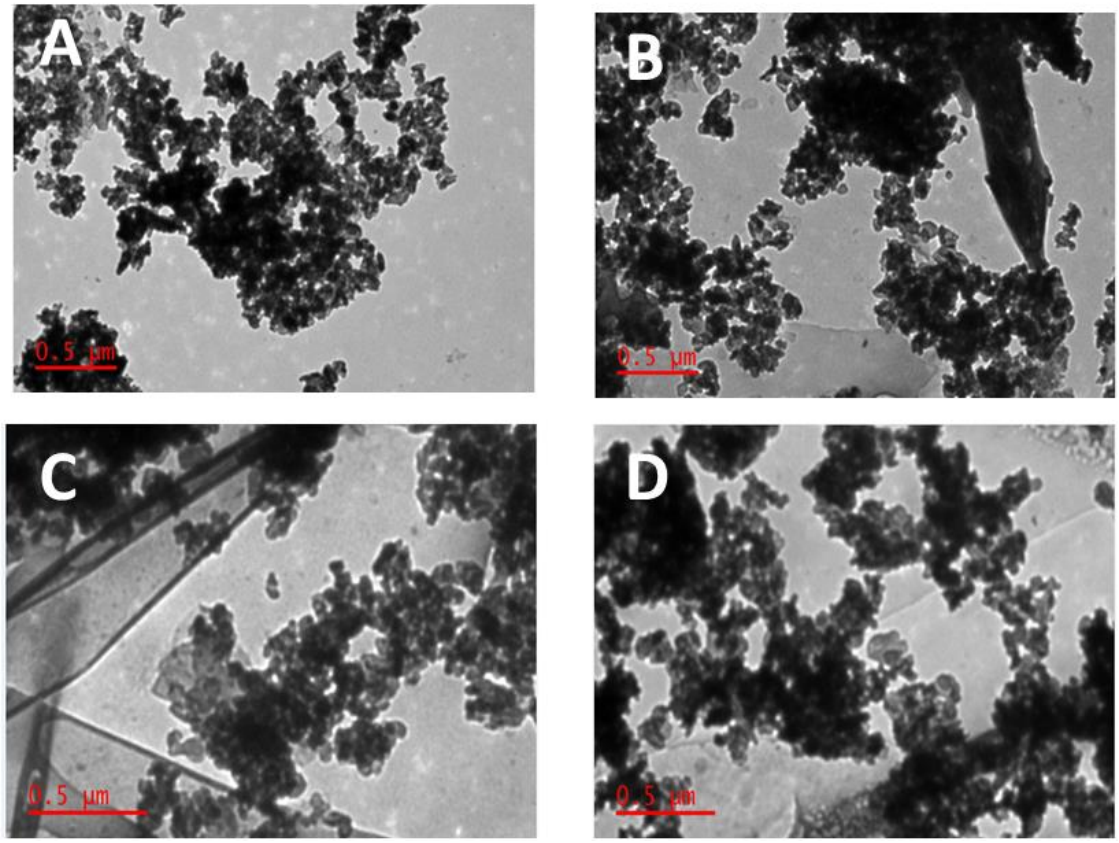

Figure 3: Representative TEM images of the biomass: (A) Raw LU, (B) Pretreated LU, (C) Raw RI and (D) Pretreated RI

The HRTEM images (Figure 3) provide further insights into the structural changes induced by acid pretreatment. Raw LU and RI show ordered cellulose microfibrils within the cell walls, indicative of crystalline cellulose. Pretreatment with 10% H_2SO_4 causes a disruption of the ordered structure, leading to a decrease in cellulose crystallinity and an increase in amorphous regions. This decrease in crystallinity is attributed to the partial hydrolysis of cellulose chains and the removal of hemicellulose and lignin, which disrupt the hydrogen bonding network that stabilizes the crystalline structure [14].

The decrease in cellulose crystallinity observed in pretreated LU and RI is consistent with the XRD analysis, which also revealed a reduction in the crystallinity index (CrI). The reduction in crystallinity enhances enzymatic hydrolysis, as amorphous cellulose is more accessible to enzyme attack compared to crystalline cellulose [15]. The HRTEM images offer visual confirmation of these structural changes, further supporting the effectiveness of acid pretreatment in improving enzymatic hydrolysis efficiency. The decreased crystallinity and increased amorphous regions, visualized in the TEM images, enhance enzyme accessibility and activity, contributing to more efficient hydrolysis and higher ethanol yields. The TEM analysis provides valuable information about the nanoscale structural changes in biomass during pretreatment, complementing the SEM and BET analyses and highlighting the importance of understanding the structural properties of biomass for optimizing bioethanol production.

Powder X-ray Diffraction (PXRD) Analysis and Bioethanol Production

Valuable information about the crystallinity of cellulose within the biomassare revealed by the Powder X-ray Diffraction (PXRD) analysis. Cellulose crystallinity significantly influences the accessibility of cellulose chains to enzymes during hydrolysis, affecting the overall bioethanol yield [16]. Higher crystallinity makes cellulose more resistant to enzymatic attack, while lower crystallinity enhances enzyme accessibility and promotes hydrolysis. Figure 4 presents the wide-angle XRD diffraction patterns of raw and pretreated*Ludwigia hexapetala* (LU) and *Ricinus communis* (RI) biomass.

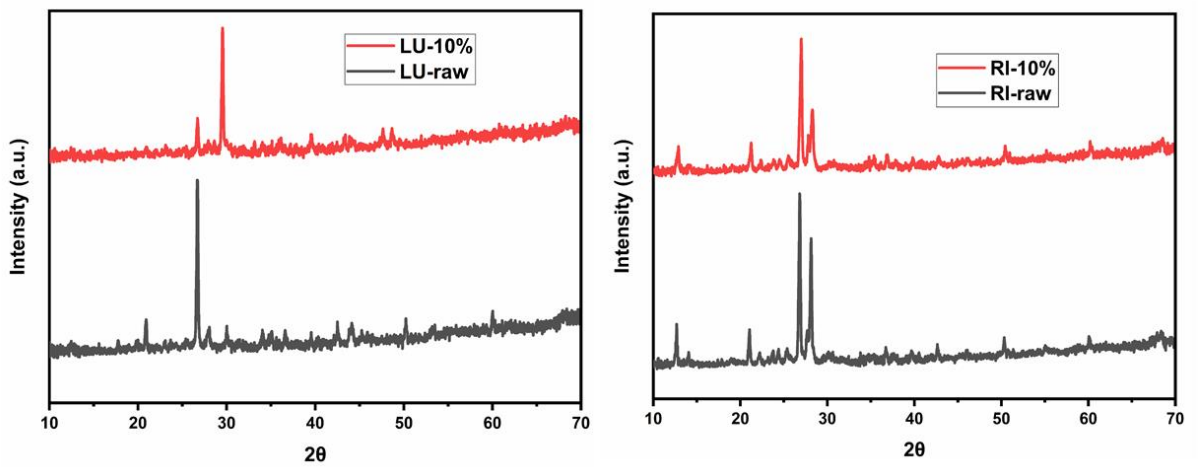

Figure 4: Wild angle, XRD diffraction patterns of the nanomaterials

The XRD diffraction patterns (Figure 4) reveal characteristic peaks corresponding to cellulose I, the predominant crystalline allomorph in plant biomass [16]. The intensity of these peaks reflects the degree of cellulose crystallinity. Raw LU and RI exhibit relatively sharp peaks, indicating a higher degree of crystallinity. Pretreatment with 10% H_2SO_4 leads to a decrease in peak intensity and broadening of the diffraction peaks, suggesting a reduction in cellulose crystallinity and an increase in the amorphous cellulose fraction [17]. This decrease in crystallinity, observed in the XRD patterns, is consistent with the TEM analysis (Figure 3), which also showed a disruption of the ordered cellulose microfibril structure in pretreated biomass. The reduction in crystallinity enhances the accessibility of cellulose chains to enzymes during hydrolysis, promoting more efficient breakdown and higher sugar yields. The PXRD analysis confirms the effectiveness of acid pretreatment in reducing cellulose crystallinity, further supporting its positive impact on enzymatic hydrolysis and bioethanol production. The crystallinity index (CrI), calculated from the XRD data, could be used to quantify the changes in cellulose crystallinity and correlate them with enzymatic hydrolysis efficiency and ethanol yield.

Thermogravimetric Analysis (TGA) and Bioethanol Production

Thermogravimetric analysis (TGA) provides information about the thermal stability and decomposition behaviour of biomass, which can be relevant for understanding the pretreatment process and optimizing reaction conditions [18]. TGA measures the weight loss of a sample as a function of temperature, providing insights into the different components of biomass and their thermal degradation characteristics. Figure 5 displays representative TGA spectra for raw and pretreated*Ludwigia hexapetala* (LU) and *Ricinus communis* (RI) biomass.

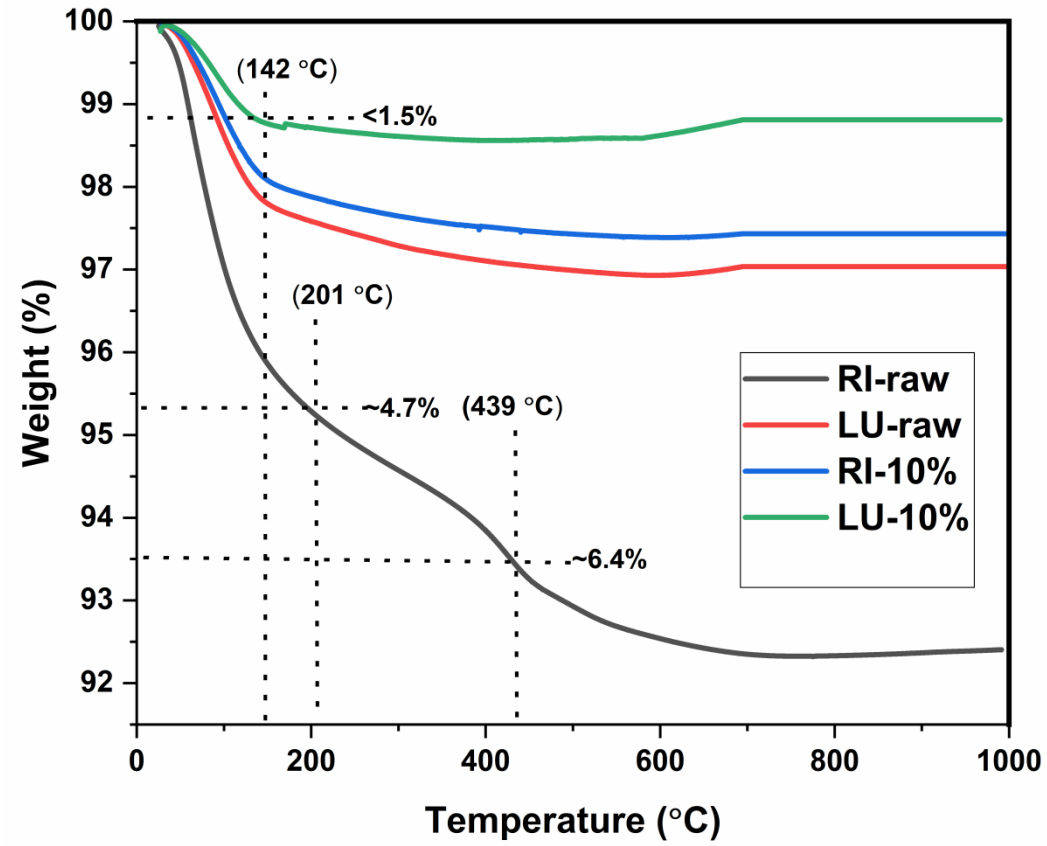

Figure 5: Representative TGA spectra for the *Ludwigia hexapetala and Ricinus communis* biomass

The TGA spectra (Figure 5) reveal distinct weight loss stages corresponding to the decomposition of different biomass components. The initial weight loss, occurring at lower temperatures (below 150°C), is attributed to the evaporation of moisture. The subsequent weight loss, occurring at higher temperatures (200-400°C), corresponds to the decomposition of hemicellulose and cellulose. The final weight loss stage, occurring at even higher temperatures (above 400°C), is associated with the decomposition of lignin [19].

Pretreatment with 10% H_2SO_4 affects the thermal decomposition behaviour of both LU and RI. The pretreated samples exhibit a lower initial weight loss due to the removal of moisture during the pretreatment process. The decomposition of hemicellulose and cellulose occurs at slightly lower temperatures in pretreated biomass compared to raw biomass, indicating increased susceptibility to thermal degradation. This is consistent with the reduction in cellulose crystallinity observed in the PXRD analysis (Figure 4) and the structural changes observed in the SEM and TEM images (Figures 2 and 3). The lower decomposition temperature of cellulose in pretreated samples suggests that the pretreatment process makes the cellulose more accessible for both thermal and enzymatic degradation.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Biomass

Fourier Transform Infrared (FTIR) spectroscopy provides information about the chemical functional groups present in biomass, offering insights into the composition and changes induced by pretreatment [20]. FTIR analysis can identify characteristic peaks associated with cellulose, hemicellulose, and lignin, allowing for a qualitative assessment of the biomass components and the effectiveness of pretreatment in removing or modifying these components. Figure 6 presents the FTIR spectra of raw and pretreated*Ludwigia hexapetala* (LU) and *Ricinus communis* (RI) biomass.

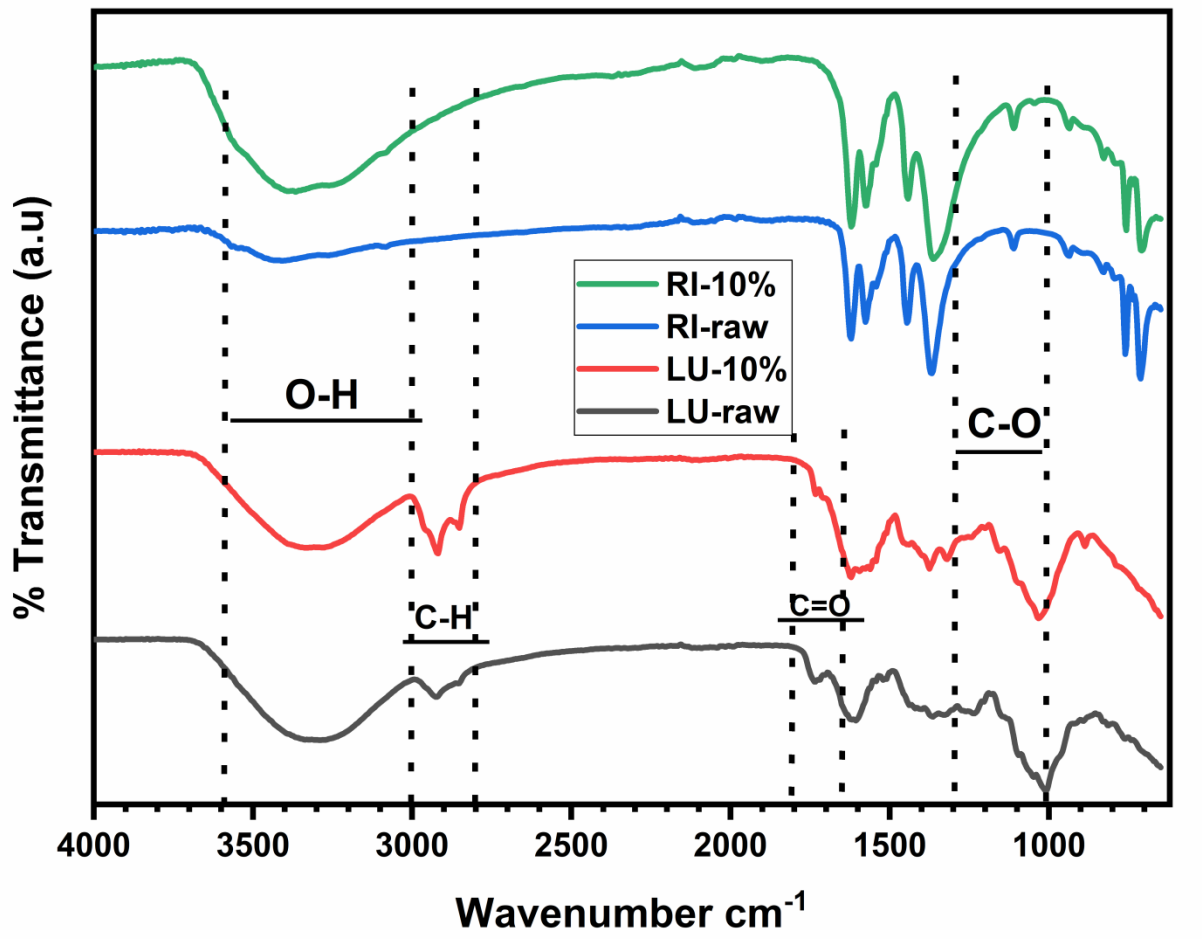

Figure 6: FTIR spectra for the *Ludwigia hexapetala and Ricinus communis* biomass

The FTIR spectra (Figure 6) reveal several characteristic absorption bands associated with different functional groups in the biomass. The broad band around 3400 cm^{-1} corresponds to O-H stretching vibrations, primarily from cellulose and hemicellulose [21]. The band around 2900 cm^{-1} is attributed to C-H stretching vibrations from aliphatic groups in all three components – cellulose, hemicellulose, and lignin. The band around 1730 cm^{-1} represents C=O stretching vibrations, typically associated with carbonyl groups in hemicellulose and lignin. The band around 1510 cm^{-1} corresponds to aromatic skeletal vibrations in lignin [22]. The bands in the region of 1000-1200 cm^{-1} are associated with C-O stretching and C-O-C vibrations in cellulose and hemicellulose.

Comparing the FTIR spectra of raw and pretreated LU and RI reveals changes induced by the acid pretreatment. The intensity of the band around 1730 cm^{-1} decreases in pretreated samples, indicating a reduction in carbonyl groups, likely due to the removal of hemicellulose and some lignin. The band around 1510 cm^{-1}, associated with lignin, also shows a decrease in intensity after pretreatment, further confirming lignin removal. These changes in the FTIR spectra correlate with the compositional analysis (Table 1), which showed a decrease in lignin and hemicellulose content after acid pretreatment. Furthermore, the changes observed in the FTIR spectra are consistent with the results from SEM, TEM, and XRD analyses, confirming the efficacy of acid pretreatment in altering the chemical and structural characteristics of the biomass.The FTIR analysis provides valuable qualitative information about the chemical composition of the biomass and the effects of pretreatment, complementing the other structural characterization techniques used in this study. The FTIR data, combined with the results from other analyses, contribute to a comprehensive understanding of the biomass properties and their influence on enzymatic hydrolysis and bioethanol production.

CONCLUSION

This study investigated the structural characterization of Raw and Acid-Pretreated Invasive Plant Biomass *(Ludwigia hexapetala and Ricinus communis)*. The detailed analyses, including FTIR, SEM, TEM, XRD, TGA, and BET, provided valuable insights into the structural modifications induced by acid pretreatment and their impact on enzymatic

hydrolysis and fermentation. Acid pretreatment effectively enhanced the cellulose content, reduced lignin content, increased surface area and porosity, and decreased cellulose crystallinity in both LU and RI.. Notably, LU and RI, have more favourable structural characteristics after pretreatment for the production of Biofuels.

The study's findings highlight the importance of pretreatment optimization and structural characterization for efficient bioethanol production from lignocellulosic biomass. The comprehensive structural analyses provide a deeper understanding of the relationship between biomass structure and bioethanol yield, offering valuable information for tailoring pretreatment and hydrolysis strategies for specific feedstocks. This research contributes to the growing body of knowledge on sustainable biofuel production and demonstrates the potential of utilizing invasive plant species as a valuable resource for bioethanol, while simultaneously addressing environmental concerns associated with their proliferation. Further research, including techno-economic analysis and life-cycle assessment, is warranted to fully evaluate the commercial viability and environmental sustainability of bioethanol production from these invasive plant species.

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