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Pre-Treatment and Characterization of Water Hyacinth Biomass (WHB) for Enhanced Xylose Production Using Dilute Alkali Treatment Method

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Abstract: Lignocellulosic biomass from water hyacinth, a readily available waste material, holds potential for producing commercial products such as xylose, which can be further converted into value-added products like xylitol. However, the complex structure of lignocellulosic biomass necessitates energy-intensive processes to release fermentable sugars. Chemical pre-treatment methods, such as alkali pre-treatment, offer a viable approach to degrade lignocellulose biomass. In this study, water hyacinth biomass (WHB) was treated with 3% potassium hydroxide and subjected to autoclaving to hydrolyse the sample. The total xylose released during the process was quantified using a UV-Vis spectrophotometer and was found to 0.253 g/g of water hyacinth biomass when the sample was treated for 20 min at 2% biomass concentration. The morphological changes in the treated biomass compared to the untreated sample were analysed using Field Emission Scanning Electron Microscopy (FE-SEM). Crystallinity alterations were evaluated through X-Ray Diffraction (XRD), while Fourier-Transform Infrared Spectroscopy (FTIR) was employed to study the changes in chemical states of the biomass. The primary objective of this study was to identify a reliable pre-treatment method for processing water hyacinth biomass, facilitating the efficient release of fermentable sugars for downstream applications.

Keywords: water hyacinth; alkali pre-treatment; UV-Vis spectrophotometer; XRD; FE-SEM; FTIR

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1. Introduction

Water hyacinth, scientifically known as *Eichhornia crassipes*, is a highly invasive alien species (IAS) that poses significant challenges worldwide. Originating from the Amazon region in South America, it has spread extensively, causing widespread ecological, economic, and public health issues. The plant's rapid and unchecked growth results in the depletion of tourism and fishing activities, the obstruction of water transport routes, and blockages in irrigation systems [1,2]. Its decaying matter further degrades water quality, making it unsafe for human, animal, or wildlife use. Additionally, stagnant water in infested areas becomes a prime breeding ground for mosquitoes, contributing to the spread of diseases [2]. Traditional disposal methods for water hyacinth, such as incineration, landfilling, and composting, face significant challenges due to the plant's high moisture content and heavy metal accumulation. These practices often result in the release of contaminative gases and heavy metal runoff into soil and water systems, exacerbating environmental damage. Consequently, the management of water hyacinth has emerged as one of the most pressing issues in urban environmental management [3]. However, despite its drawbacks, water

hyacinth offers a unique opportunity as a non-terrestrial biomass. Since it does not compete with food crops for arable land, it holds promise as a renewable resource for industrial applications [4]. It is a fast-growing invasive plant that propagates and spreads rapidly in a short duration in any fresh water body, making it readily available. Pre-treatment is a crucial step in unlocking this potential, as it removes the plant's recalcitrant properties that limit enzymatic digestion. An effective pre-treatment method must be cost-effective, environmentally sustainable, and efficient [5,6]. Water hyacinth is a rich source of cellulose and hemicellulose, making it suitable to produce bioethanol, methane, hydrogen, and xylitol. In addition, it can be used to manufacture paper, fibreboard, animal feed, and fertilizers [7]. Among these applications, xylitol—a sugar alcohol widely used in the food, pharmaceutical, and healthcare industries—stands out for its high commercial value. Xylitol is commonly used in sugar-free chewing gums, candies, jellies, and other confectionery products due to its organoleptic properties, good solubility, and dental benefits. Currently, xylitol is primarily produced through the catalytic hydrogenation of commercial xylose. However, this process is costly, yielding only about 60% due to the separation of xylitol from other chemical compounds formed during production. The low natural content of xylitol in fruits and vegetables (less than 9 mg/g) further increases production costs [8,9]. With a global xylitol market valued at approximately USD 537 million annually, finding more efficient production methods is critical [10]. This study investigates the use of water hyacinth as a sustainable and cost-effective source of xylose, the precursor to xylitol, which itself is an industrially important product [8]. The pre-treatment process involves hydrolysing water hyacinth biomass using a dilute potassium hydroxide (KOH) solution. This chemical pre-treatment method breaks down structural links between lignin and polysaccharides, removes lignin and acetyl groups, and saponifies ester bonds between lignin and hemicellulose, making it more efficient than biological and physical pre-treatments [11]. Biomass pre-treatment is a critical step in converting plant-based materials into value-added products. Alkali hydrolysis, commonly performed with sodium hydroxide (NaOH), is one of the most effective techniques for breaking down lignocellulosic biomass [4,6,11–13]. When coupled with steam explosion, the combined effect enhances the accessibility of cellulose and hemicellulose fractions [4,14]. However, prolonged exposure to high temperatures during steam explosion risks degrading sugars such as xylose, which are essential intermediates for downstream applications. The use of an autoclave enables the precise control of reaction conditions, facilitating high temperatures over shorter durations to mitigate sugar degradation. While traditional steam explosion processes are typically conducted at temperatures of 180–240 °C for 5–10 min, the integration of autoclaves has been reported to extend the reaction time to 15–60 min, balancing efficiency and sugar preservation [4,6,9]. Water hyacinth, a fast-growing invasive aquatic weed, has attracted significant attention for its potential as a renewable biomass source. However, the combined application of KOH-based alkali hydrolysis and steam explosion within optimized reaction times has not been extensively explored. To analyse the outcomes, Fourier-Transform Infrared Spectroscopy (FTIR) was used to study its molecular components [7,12,13,15,16] and bonding structures, while Field Emission Scanning Electron Microscopy (FE-SEM) was used to examine the morphological changes during the pre-treatment process [13,14], and XRD analysis was carried out to investigate the effects of chemical treatment on the structures of the lignocellulosic biomass of water hyacinth [16–19]. By addressing the environmental challenges posed by water hyacinth and exploring its potential as an industrial resource, this research demonstrates the dual benefits of ecological management and economic value creation. Through sustainable and efficient methods, water hyacinth can be transformed from a problematic invasive species into a valuable raw material for industrial applications.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

The chemicals used in this study were of analytical grade, obtained from Sigma Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany), and HiMedia (Maharashtra, India). Various chemical agents were tested to identify the most effective treatment for water hyacinth biomass. For alkali hydrolysis, sodium hydroxide (NaOH), potassium hydroxide (KOH), and calcium hydroxide ($Ca(OH)_2$) were evaluated. Acid hydrolysis was performed using hydrochloric acid (HCl), nitric acid (HNO_3), and sulfuric acid (H_2SO_4). Among the alkali agents, potassium hydroxide (KOH) demonstrated superior efficacy in treating lignocellulosic biomass and was selected for further experiments. For xylose estimation, a combination of hydrochloric acid (HCl), orcinol, and ferric chloride ($FeCl_3$) was employed. The selection of these agents ensured precise hydrolysis and the accurate quantification of xylose, which is a key outcome of the pre-treatment process. This study highlights the suitability of KOH for the alkali hydrolysis of water hyacinth biomass and establishes a robust approach for xylose estimation.

2.1.2. Equipment

A UV-Vis spectrophotometer (Shimadzu UV spectrophotometer UV-1800, Kyoto, Japan), digital weighing machine (Sartorius BSA323S, Göttingen, Germany), pH meter (Eutech Cyberscan pH Tutor, Thermo, MA, USA), magnetic stirrer with a hot plate, hot air oven, and autoclave were used.

2.2. Methods

2.2.1. Sample Preparation

Water hyacinth was collected manually from a pond in Panagarh, West Bengal, India (coordinates: 23.452002, 87.446756 (23°27′07.2″ N 87°26′48.3″ E)). The leaves were separated from the stems and roots, then washed five times with tap water and twice with distilled water to remove dirt and contaminants. To ensure thorough cleaning, they were rinsed once with 50–70% ethanol. The cleaned leaves were air-dried initially, followed by drying in a hot air oven at 55 °C for 48 h. Once dried, the leaves were ground into a fine powder. The powder was sieved to achieve a uniform particle size of 250–355 microns. These prepared samples were then stored in airtight containers for further use. This method ensures purity and consistency for experimental applications.

2.2.2. Pre-Treatment of WHB

The chemical pre-treatment of water hyacinth biomass (WHB) was conducted using 2% (W/V) alkali (NaOH, KOH, $Ca(OH)_2$) and acid (H_2SO_4, HCl, HNO_3) hydrolysis agents. The process was carried out in an autoclave at 121 °C and 15 psi for 20 min [16]. After cooling, the hydrolysed material was centrifuged and filtered through cheesecloth to separate the solid residue. The residue was washed multiple times with distilled water to neutralize its pH. The neutralized biomass was dried at 55 °C on aluminium foil to prepare it for further analysis [4]. This method effectively breaks down lignocellulosic material, enhancing its suitability for biochemical studies. The pre-treatment combines chemical hydrolysis and autoclave conditions for efficient substrate conversion.

2.2.3. Effect of Process Parameters on Xylose Production

The process parameters or conditions plays a vital role in the outcome or yield. In this study, for the hydrolysis of WHB, three parameters were selected. Pre-treatment time (10, 20, 30, 40, 60) in minutes, KOH conc. (1–10%), and WHB conc. (1–5%). Each of the

experiments were performed in triplicate and the produced xylose conc. (mg/mL) was determined using a spectrophotometric method.

2.2.4. Spectrophotometric Determination of Xylose

A xylose stock solution was prepared by dissolving xylose in distilled water, and various dilutions were made by pipetting a known volume of the standard. Total xylose produced by the hydrolysis of WHB was analysed at 620 nm with Orcinol reagent [20].

2.2.5. Surface Morphology Observation

The dried WH was examined both before and after pre-treatment for differences in surface appearance and microscopic structural conditions and the dried WHB samples were mounted and coated with platinum. SEM images at 5 K magnification were recorded [13,14].

2.2.6. XRD and Crystallinity Index Measurement

The XRD analysis was performed at 40 kV and 130 mA within the scanning angle of 5–50°, at the scanning speed of 0.5°/min [21]. The degree of crystallinity (CI) was calculated, according to Segal's method, from the XRD profiles using the following equation [19,22]:

$$\text{CI } (\%) = \left(\frac{I_{200} - I_{AM}}{I_{AM}} \right) \times 100 \quad (1)$$

where I_{200} is the maximum intensity of the crystalline peak located at 2θ between 22 and 23°, and I_{AM} is the minimum intensity of the amorphous region at 2θ between 18 and 19° [17,18].

2.2.7. FTIR Analysis

Fourier-Transform Infrared Spectroscopy (FTIR) was used to investigate and quantify chemical changes in the pretreated and treated samples. IR spectra were studied. Samples were prepared by grinding the samples to obtain a fine powder. The analysis was conducted using the Attenuated Total Reflectance (ATR) technique. Each sample was pressed firmly to ensure proper contact, and spectra were recorded over the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. Background spectra were collected prior to each measurement and automatically subtracted from the sample spectra. This method was selected for its ease of use and ability to analyse samples in their native state, ensuring reliable and reproducible results [15,16].

3. Results and Discussion

3.1. Spectrophotometric Analysis of Hydrolysate

The samples were analysed at 620 nm with orcinol reagent. The samples were diluted for analysis. Table 1 and Figure 1 summarizes the results of the different pre-treatment methods; it was found out that KOH gave better results compared to the other chemical agents.

Lignocellulosic biomass pre-treatment is essential for improving the accessibility of hemicellulose. Acid pre-treatment, especially with diluted acids, alters the lignin–carbohydrate linkages. However, high acid concentrations can degrade polysaccharides, leading to a loss of valuable carbohydrates [11]. In contrast, alkali pre-treatment focuses on removing lignin without significantly affecting cellulose or hemicellulose content. It works by breaking the bonds between lignin and polysaccharides, making cellulose more accessible for further processing [11]. Additionally, alkali treatment saponifies ester bonds between lignin and hemicellulose, further aiding the separation of these components. This selective approach of alkali pre-treatment preserves the carbohydrate fraction while

effectively reducing lignin content. While both methods alter the lignin structure, alkali pre-treatment is more focused on lignin removal and maintains polysaccharide integrity. The choice between acid and alkali pre-treatment depends on the desired balance between lignin modification and carbohydrate preservation.

Table 1. Effect of different pre-treatment agents for xylose production.

Sr. No	Pre-Treatment Agent (2%)	Xylose Conc. (mg/mL)
1	NaOH	5.8029
2	KOH	8.0587
3	$Ca(OH)_2$	2.7739
4	H_2SO_4	4.6469
5	HCl	4.7827
6	HNO_3	7.4625
7	Autohydrolysis	2.1785

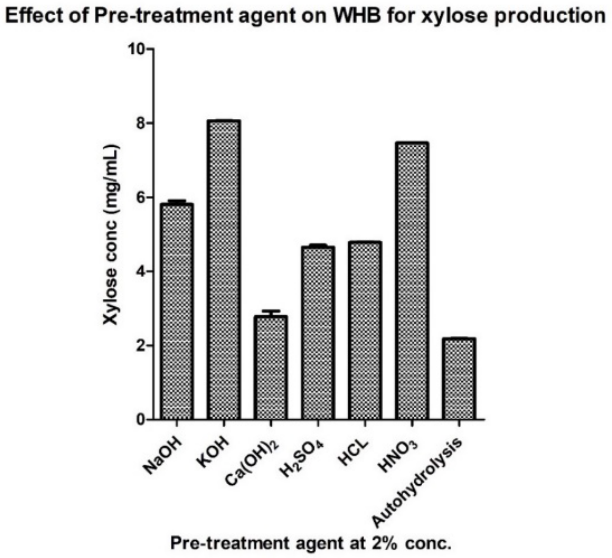

Figure 1. Effect of different hydrolytic agents on xylose production.

3.2. Effect of Process Parameters on Xylose Production

In this study, the Change of One Variable at a Time (COVT) method was employed to optimize the production of xylose. The primary focus was on the effect of varying potassium hydroxide (KOH) concentration on the hydrolysis process, while maintaining the concentration of WHB and the pre-treatment time constant. KOH concentrations ranging from 1% to 10% were tested to determine the most effective concentration for xylose production. The results, presented in Table 2 and Figure 2, revealed that a KOH concentration of 3% yielded the highest production of xylose. Therefore, 3% KOH was identified as the optimal concentration for the hydrolysis process. Based on these findings, subsequent experiments were conducted using a 3% concentration of KOH for the further optimization of xylose production.

The pre-treatment time plays a crucial role in the steam explosion process, which is conducted in an autoclave under specific conditions of 121 °C, 15 psi, and 20 min. This high-temperature environment can lead to the degradation of fermentable sugars, which is undesirable for the process. Therefore, identifying the optimal pre-treatment time is essential. In this study, the optimal time was found to be 20 min (Table 3, Figure 3) when wheat husk biomass (WHB) was treated with 3% KOH. The results indicate that steam explosion significantly contributes to the release of fermentable sugars, as evidenced by

the autohydrolysis data presented in Figure 1 and Table 1. This highlights the importance of both the pre-treatment time and the steam explosion process itself in maximizing the efficiency of sugar release.

Table 2. Effect of varying KOH conc. on xylose production.

Sr. No	KOH Conc. (%)	Xylose Conc. (mg/mL)
1	1	2.0329
2	2	4.0641
3	3	4.4130
4	4	3.3116
5	5	3.3715
6	6	3.7323
7	7	3.7221
8	8	3.6636
9	9	3.5469
10	10	3.4883

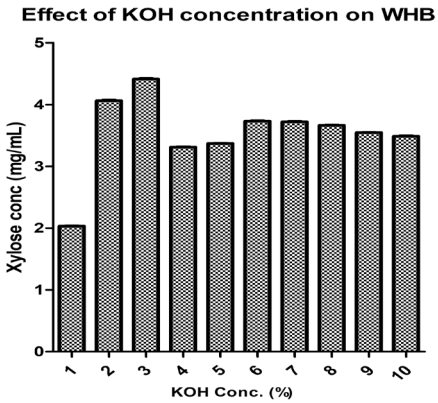

Figure 2. Effect of KOH conc. on xylose production.

Table 3. Effect of pre-treatment time on xylose production.

Sr. No	Pre-Treatment Time (min.)	Xylose Conc. (mg/mL)
1	10	3.0943
2	20	3.1833
3	30	2.8463
4	40	2.7646
5	60	2.2213

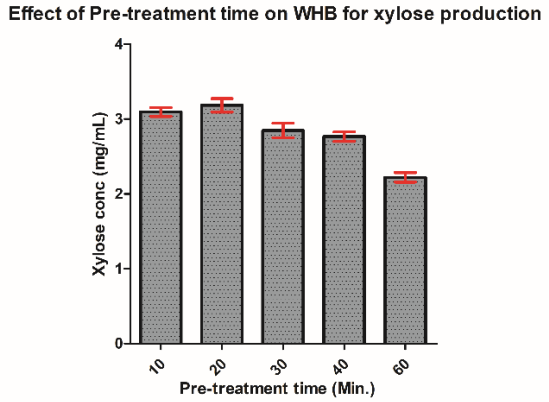

Figure 3. Effect of pre-treatment time on xylose production.

This study explored the optimization of water hyacinth biomass (WHB) concentration for an effective pre-treatment process under steam explosion conditions. It was observed that concentrations of WHB above 5% were unsuitable for pre-treatment, as the biomass formed a semi-solid mixture, complicating the process. To identify the optimal conditions, WHB concentrations in the range of 1–5% were treated with 3% potassium hydroxide (KOH) for 20 min. Spectrophotometric analysis revealed that a 2% WHB concentration, when combined with 3% KOH, provided the most efficient hydrolysis within the 20 min treatment period. This finding suggests that the ratio of the biomass sample to the pre-treatment agent plays a crucial role in determining the yield of the desired product. The results highlight the importance of balancing these parameters to optimize the pre-treatment process. Table 4 and Figure 4 summarizes the findings from the optimization studies, offering further insights into the effects of WHB concentration on the efficiency of the pre-treatment process.

Table 4. Effect of WHB conc. on xylose production.

Sr. No	WHB Conc. (%)	Xylose Conc. (mg/mL)
1	1	5.6307
2	2	6.4549
3	3	5.4647
4	4	4.4746
5	5	3.9528

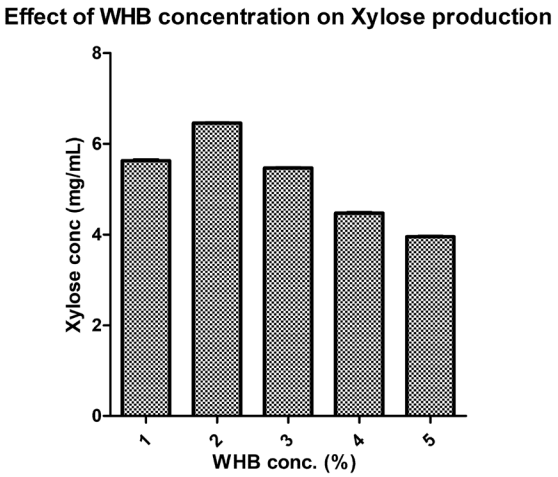

Figure 4. Effect of WHB conc. on xylose production.

3.3. FE-SEM Analysis

Scanning electron microscopy (SEM) provided valuable insights into how pre-treatment methods affect lignocellulosic biomass. When comparing untreated and treated samples under 5 K magnification, it was clear that alkali-treated samples underwent significant structural changes. The combination of steam explosion and chemical pre-treatment was especially effective in breaking down the complex structure of the biomass, leading to the release of fermentable sugars like xylose [14]. Figure 5 highlights the differences between untreated and treated water hyacinth biomass (WHB). The alkali-treated samples showed visible damage. These changes occurred because the alkali broke down the xylan matrix, which holds the cellulose microfibrils together. By weakening the cell wall through this process, known as delignification, the biomass became more accessible, yielding higher amounts of fermentable sugars. This is largely due to the easier solubilization of cellulose and hemicellulose [13]. The treated samples also showed granules forming on the surface,

as captured in the SEM images. This structural weakening and the appearance of granules are likely caused by the dissolution of lignin and the breaking of bonds between lignin and carbohydrates. An increase in internal surface area is experienced due to the interaction with potassium hydroxide (KOH) [12]. These findings emphasize the importance of alkaline pre-treatment in preparing lignocellulosic biomass for further processing. By breaking down its rigid structure and creating a more porous surface, the treatment makes it easier to access and extract sugars. This step is critical for improving the efficiency of xylitol production and other applications that rely on fermentable sugars.

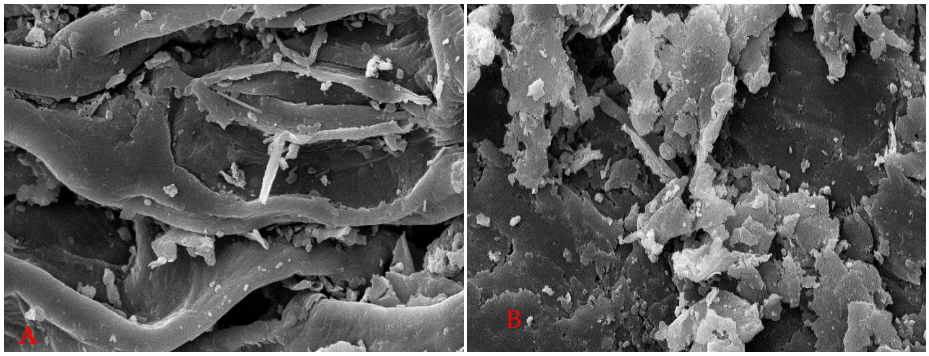

Figure 5. FE-SEM analysis of untreated (**A**) and treated (**B**) WHB samples.

3.4. XRD Analysis

XRD analysis was carried out to investigate the effects of chemical treatment on the structure of the lignocellulosic biomass of water hyacinth. In water hyacinth, cellulose and hemicellulose constitute about 65% to 70% of lignocellulosic biomass. The semi-crystalline profile of water hyacinth is due to the lignin in its structure, because lignin is amorphous while cellulose is predominantly crystalline. The peak height method, also called the Segal method, is the most widely used analysis approach to characterize the crystallinity of samples. It was found that the Crystallinity Index (CI) of the untreated sample was 25.90%, and for alkali-treated sample it was 52.02% (Figure 6). Through the intensive analysis of peaks between the region of 22–23° and 18–19°, the total increment of crystallinity was found to be 26.12%. The additional peaks observed in the XRD spectra may correspond to the inorganic substances present in the water hyacinth biomass. While water hyacinth is known to accumulate heavy metals from contaminated water bodies, confirmation of such elements requires additional characterization techniques like XRF or ICP-MS. It is also possible that residual salts from the alkali treatment contributed to these peaks. Through the XRD analysis, it could be seen that the alkaline pre-treatment that was used for the hydrolysis of WHB was successful, in line with the increase in the CI value.

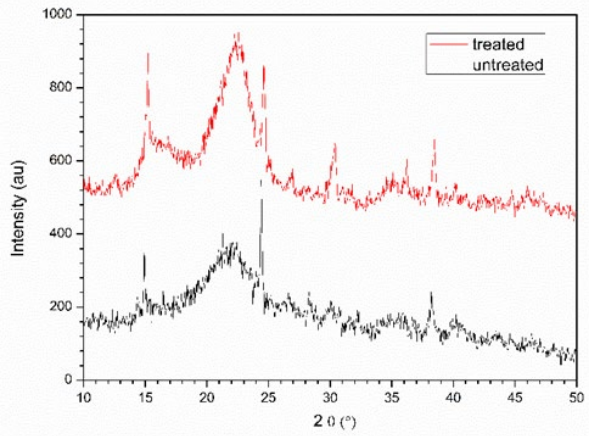

Figure 6. XRD analysis of treated and untreated WHB samples.

3.5. FTIR Analysis

Peaks were found in the regions of 3400–3200 cm^{-1}, 2900 cm^{-1}, 1700 cm^{-1}, and 1050 cm^{-1}, corresponding to the chemical bonds of the cellulose. Hemicellulose is indicated by the peaks in the wavenumber range of 1765–1715 cm^{-1}, and lignin is indicated by three bonds referring to the bands at 1270 cm^{-1} and 1430–1470 cm^{-1} [15].

The peaks and the indications are as follows: 3000–2800 cm^{-1} for C-H symmetric stretching; 1640–1650 cm^{-1} for O-H bonding, which also equated to water adsorption; 1500–1600 cm^{-1} for aromatic vibrations due to lignin content; 1365–1375 cm^{-1} for CH_2 symmetrical bonding in treated and untreated samples; 1150–1160 cm^{-1} for the C-C bond in treated and untreated samples; 1104 cm^{-1} for the C-O-C glycosidic ether bond (this behaviour is due to the polysaccharide components); 1030–1050 cm^{-1} for C-O-C stretching vibrations (this stretching is known to be due to pyranose ring cellulose); and 894–897 cm^{-1} for -CH vibrations of cellulose [12]. The 3400 cm^{-1} band represents hydroxyl (-OH); 2900 cm^{-1} is the -CH bond; and 1700 cm^{-1} represents either acetyl or uronic ether bonds of carboxylic groups on ferulic acids and p-coumaric acids. Both acids can be found in lignin. The 1050 cm^{-1} region usually indicates a biomass decrease due to hemicellulose solubilization. This observation indicates that the pre-treatment removed lignin and broke cellulose and hemicellulose from their tight matrices. Singh J. K. et al. (2020) [13] reported that the peaks in the wavenumber range of 2800–3000 cm^{-1} and the small band at 2373 cm^{-1} are due to the stretching vibrations of -OH and C-H in CH_2 and CH_3, which are slightly more absorbed due to the alkaline pre-treatment, while 1417 cm^{-1} represents CH_2 scissoring at C(6) in cellulose. The reductions in the two bands at 1325 cm^{-1} and 1240 cm^{-1} are due to C-H deformation in hemicellulose and C-O stretching in lignin and hemicellulose, respectively, with the increment in the band at around 1050 cm^{-1} representing the C-OH stretching vibration of cellulose stretching (Figure 7).

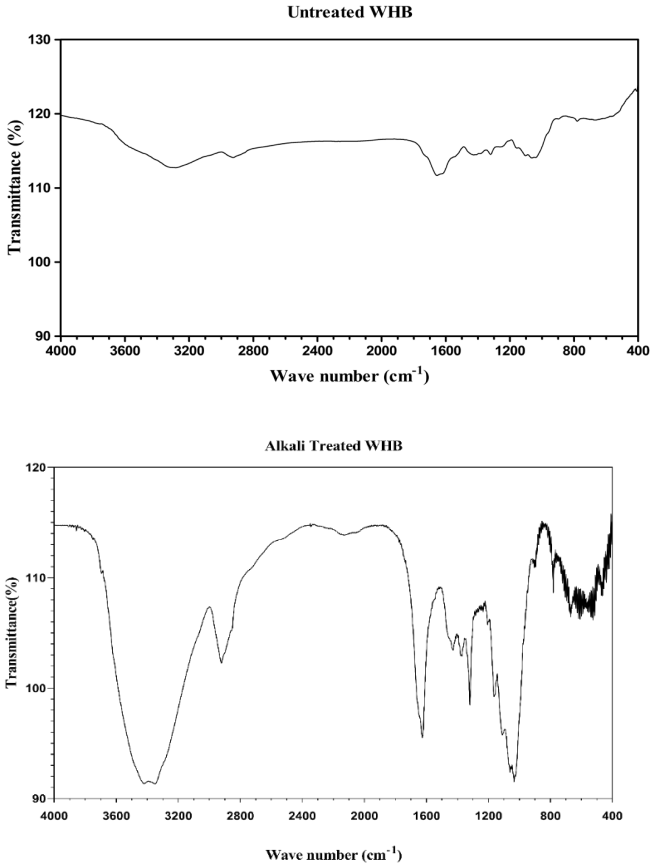

Figure 7. FTIR analysis of treated and untreated WHB samples.

4. Conclusions

Water hyacinth biomass (WHB), a problematic aquatic plant, was utilized as a resource for producing xylose, a value-added fermentable sugar. Among the tested chemical agents, potassium hydroxide (KOH) was the most effective for alkali hydrolysis, especially under steam explosion conditions (Table 1, Figure 1). Autohydrolysis (using water alone) was less efficient, highlighting the importance of chemical treatment. Optimal conditions were established for hydrolysis: 3% KOH concentration (Table 2, Figure 2) and a 20 min pre-treatment time (Table 3, Figure 3). Longer exposure to high autoclave temperatures led to sugar degradation. A 2% WHB concentration was found to be ideal, as higher concentrations formed semi-solid slurries unsuitable for experiments (Table 4, Figure 4). The total xylose yield was 0.253 g/g of WHB. Structural changes observed through FE-SEM (Figure 5) indicated lignin dissolution, increased surface granularity, and bond separation between lignin and carbohydrates. XRD analysis showed a rise in crystallinity from 25.90% to 52.09% (Figure 6), demonstrating the successful removal of amorphous regions and improved sugar accessibility. FTIR analysis (Figure 7) revealed chemical changes, with new peaks and increased transmittance indicating bond alterations. These findings confirm that dilute alkali pre-treatment is an effective method for delignifying WHB and enhancing fermentable sugar production.

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References

1. Carneiro, M.T.; Barros, A.Z.B.; Morais, A.I.S.; Melo, A.L.F.C.; Bezerra, R.D.S.; Osajima, J.A.; Silva-Filho, E.C. Application of Water Hyacinth Biomass (*Eichhornia crassipes*) as an Adsorbent for Methylene Blue Dye from Aqueous Medium: Kinetic and Isothermal Study. *Polymers* **2022**, *14*, 2732. [CrossRef] [PubMed]
2. Ilo, O.P.; Simatele, M.D.; Nkomo, S.L.; Mkhize, N.M.; Prabhu, N.G. The Benefits of Water Hyacinth (*Eichhornia crassipes*) for Southern Africa: A Review. *Sustainability* **2020**, *12*, 9222. [CrossRef]
3. Zhang, C.; Ma, X.; Chen, X.; Tian, Y.; Zhou, Y.; Lu, X.; Huang, T. Conversion of Water Hyacinth to Value-Added Fuel via Hydrothermal Carbonization. *Energy* **2020**, *197*, 117193. [CrossRef]
4. Singh, A.; Bishnoi, N.R. Comparative Study of Various Pretreatment Techniques for Ethanol Production from Water Hyacinth. *Ind. Crops Prod.* **2013**, *44*, 283–289. [CrossRef]
5. Jin, X.; Chen, X.; Shi, C.; Li, M.; Guan, Y.; Yu, C.Y.; Yamada, T.; Sacks, E.J.; Peng, J. Determination of Hemicellulose, Cellulose and Lignin Content Using Visible and near Infrared Spectroscopy in Miscanthus Sinensis. *Bioresour. Technol.* **2017**, *241*, 603–609. [CrossRef]
6. Zhang, Q.; Wei, Y.; Han, H.; Weng, C. Enhancing Bioethanol Production from Water Hyacinth by New Combined Pretreatment Methods. *Bioresour. Technol.* **2018**, *251*, 358–363. [CrossRef]
7. Wang, Z.; Zheng, F.; Xue, S. The Economic Feasibility of the Valorization of Water Hyacinth for Bioethanol Production. *Sustainability* **2019**, *11*, 905. [CrossRef]

8. Arcaño, Y.D.; García, O.D.V.; Mandelli, D.; Carvalho, W.A.; Pontes, L.A.M. Xylitol: A Review on the Progress and Challenges of Its Production by Chemical Route. *Catal. Today* **2020**, *344*, 2–14. [CrossRef]
9. Dey, A.; Das, S.; Das, K.; Ghosh, P.; Ganguly, A.; Chatterjee, P.K.; Chatterjee, K. Studies on the Optimization of Phenolics during Production of Xylitol from Water Hyacinth. *Eur. J. Biotechnol. Biosci.* **2015**, *25*, 25–33.
10. Dalli, S.S.; Patel, M.; Rakshit, S.K. Development and Evaluation of Poplar Hemicellulose Prehydrolysate Upstream Processes for the Enhanced Fermentative Production of Xylitol. *Biomass Bioenergy* **2017**, *105*, 402–410. [CrossRef]
11. Chinwatpaiboon, P.; Savarajara, A.; Luengnaruemitchai, A. Enzymatic Hydrolysate of Water Hyacinth with NaOH Pretreatment for Biobutanol Production via ABE Fermentation by Clostridium Beijerinckii JCM 8026. *Biomass Bioenergy* **2023**, *173*, 106782. [CrossRef]
12. Packiam, K.K.; Murugesan, B.; Sundaramoorthy, P.M.K.; Srinivasan, H.; Dhanasekaran, K. Extraction, Purification and Characterization of Nanocrystalline Cellulose from *Eichhornia crassipes* (Mart.) Solms: A Common Aquatic Weed Water Hyacinth. *J. Nat. Fibers* **2022**, *19*, 7424–7435. [CrossRef]
13. Singh, J.K.; Chaurasia, B.; Dubey, A.; Noguera, A.M.F.; Gupta, A.; Kothari, R.; Upadhyaya, C.P.; Kumar, A.; Hashem, A.; Alqarawi, A.A.; et al. Biological Characterization and Instrumental Analytical Comparison of Two Biorefining Pretreatments for Water Hyacinth (*Eichhornia crassipes*) Biomass Hydrolysis. *Sustainability* **2020**, *13*, 245. [CrossRef]
14. Rezania, S.; Alizadeh, H.; Cho, J.; Darajeh, N.; Park, J.; Hashemi, B.; Din, M.F.M.; Krishnan, S.; Yadav, K.K.; Gupta, N.; et al. Changes in Composition and Structure of Water Hyacinth Based on Various Pretreatment Methods. *Bioresources* **2019**, *14*, 6088–6099. [CrossRef]
15. Bronzato, G.R.F.; Ziegler, S.M.; Silva, R.C.; Cesarino, I.; Leão, A.L. Characterization of the Pre-Treated Biomass of *Eichhornia crassipes* (Water Hyacinth) for the Second Generation Ethanol Production. *Mol. Cryst. Liq. Cryst.* **2017**, *655*, 224–235. [CrossRef]
16. S, L.M.; Baas-López, J.M.; Barbosa, R.; Pacheco, D.; Escobar, B. Activated Carbon from Water Hyacinth as Electrocatalyst for Oxygen Reduction Reaction in an Alkaline Fuel Cell. *Int. J. Hydrog. Energy* **2021**, *46*, 25995–26004. [CrossRef]
17. Rongpipi, S.; Ye, D.; Gomez, E.D.; Gomez, E.W. Progress and Opportunities in the Characterization of Cellulose—An Important Regulator of Cell Wall Growth and Mechanics. *Front. Plant Sci.* **2019**, 9. [CrossRef]
18. Nam, S.; French, A.D.; Condon, B.D.; Concha, M. Segal Crystallinity Index Revisited by the Simulation of X-Ray Diffraction Patterns of Cotton Cellulose Iβ and Cellulose II. *Carbohydr. Polym.* **2016**, *135*, 1–9. [CrossRef]
19. Tanpichai, S.; Biswas, S.K.; Witayakran, S.; Yano, H. Water Hyacinth: A Sustainable Lignin-Poor Cellulose Source for the Production of Cellulose Nanofibers. *ACS Sustain. Chem. Eng.* **2019**, *7*, 18884–18893. [CrossRef]
20. Pham, P.J.; Hernandez, R.; French, W.T.; Estill, B.G.; Mondala, A.H. A Spectrophotometric Method for Quantitative Determination of Xylose in Fermentation Medium. *Biomass Bioenergy* **2011**, *35*, 2814–2821. [CrossRef]
21. Dey, A.; Ting, Y.-P. Characterization of Water Hyacinth Biomass and Microbial Degradation of the Biomass under Solid State Fermentation Using a Lignocellulolytic Fungus (Alterneria Spp NITDS1). *J. Chem. Biol. Phys. Sci.* **2014**, *4*, 2279–2293.
22. Asrofi, M.; Abral, H.; Kasim, A.; Pratoto, A. XRD and FTIR Studies of Nanocrystalline Cellulose from Water Hyacinth (*Eichornia crassipes*) Fiber. *J. Metastable Nanocrystalline Mater.* **2017**, *29*, 9–16. [CrossRef]

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