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Sustainable Biomass Conversion: Impact of NaCl Pretreatment on Cabbage Waste

Sunder, Sangita Yadav and Jitender Pal†
Department of Environmental Sciences and Engineering, Guru Jambheshwar University of Science and Technology, Hisar-125001, Haryana, India
†Corresponding author: Jitender Pal; j_pal2k1@yahoo.com

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ABSTRACT

Vegetable waste, particularly cabbage waste (CW), is a valuable raw material for various applications, including bioenergy production, owing to its high lignocellulosic content. However, the potential of lignin in biomass conversion remains largely untapped. This study is significant as it aims to optimize the pretreatment of CW biomass using different chemical reagents and concentrations (sulphuric acid, acetic acid, sodium hydroxide, potassium hydroxide, and sodium chloride) at 12 and 24 h for 50, 75, and 100°C. In this study, a novel pretreatment approach was introduced with 2% NaCl at 50°C for 12 h for CW biomass. At this optimized condition, 2% NaCl led to 28% delignification for CW biomass. The study examined the impact of pretreatment efficacy on biomass characterization using SEM, XRD, and FTIR analytical techniques. Results showed that 2% NaCl pretreatment significantly improved digestibility, increased surface area and porosity, altered the crystallinity index, and confirmed delignification through shifts in peaks and intensity changes. Furthermore, reduced hemicellulose and reduced lignin were noted in comparison to untreated biomass. This reassures us of the effectiveness of the pretreatment method. This promising result underscores the feasibility, economics, sustainability, and environmental friendliness of this pretreatment method. The method not only offers a cost-effective solution but also aligns with the principles of sustainability and environmental protection, thereby reassuring the researchers about its potential for various industrial applications.

INTRODUCTION

India is a country that grows more than 70 different kinds of vegetables. One of the most popular vegetables grown worldwide is cabbage including India, a cruciferous vegetable used as an herbal medicine and nutritional supplement (Šamec et al. 2017). People eat cabbage worldwide since it is a healthful vegetable renowned for its high nutritional value, delicious taste, and medicinal properties (Pradhan 2020). It provides essential nutrients and fiber. It is usually cooked like a green vegetable, consumed raw, and frequently turned into a pickle (Sarkar et al. 2021). Cabbage makes up around 6.5% of global vegetable production (Pradhan 2020). It is grown on 0.40 million hectares in India, accounting for 4% of the total vegetable area, and produces 9.04 million metric tonnes annually (Patra et al. 2024). Although cabbage meets quality criteria and is typically consumed by humans as food, over thirty percent of the cultivated cabbage is rejected as solid waste. CW comprised 50 million metric tonnes, or 8%, of all vegetable and fruit garbage in 2017 (Eom et al. 2024). CW is often collected from various sources and disposed of in landfills or dumpsites, leading to severe environmental impacts such as greenhouse gas emissions, groundwater pollution, malodor, and microbial contamination (Bamisaye et al. 2022). The dumping of food waste also affects the environment's health in ways such as acidification and eutrophication of local water bodies. These impacts underscore the urgent need for sustainable waste management solutions.

Crop waste biomass, including (CW), contains high concentrations of cellulose, hemicelluloses, and lignin, making them potential value-added products for lignocellulose biorefinery, as per research by Bamisaye et al. (2022). The stalks of cabbage have about 37% cellulose on a dry weight basis, making it relatively cellulose-rich (Pradhan 2020). The hydrolysis of cellulose in plant cells is hindered by lignin and hemicellulose, which bind to the cellulose microfibrils, making pretreatment crucial for enhancing efficiency by solubilizing these components (Dharmalingam et al. 2023). Pretreatment procedures are also crucial to converting lignocellulosic feedstocks into reducing sugars. Pretreatments with alkali, acid, organic solvent, and mechanical methods have all been shown to improve the release of structural carbohydrates from different types of lignocellulosic biomass (He et al. 2021). Plant materials undergo various pretreatment methods, including thermal and ultrasonic, to improve structural modifications, but thermal pretreatment can potentially cause bioactive compound degradation, while ultrasonic pretreatment disrupts cell walls (Pongmalai et al. 2015). As a result, the development of alkali treatment utilizing NaOH, CaO, NaCl, ammonia, urea, etc., was required to modify the biomass matrix (Bamisaye et al. 2022). Organic salts have certain qualities, such as low volatility, high stability, recyclability, non-toxic, and non-explosive (Xie et al. 2020).

This research analyses chemical pretreatment in converting lignocellulosic biomass, considering factors like research breakthroughs, technology development, application strengths and weaknesses, and potential economic and environmental impacts. Cellulose, lignin, and hemicellulose yields from acid, base, and salt pre-treatment processes were compared 1stly. Then, this study introduces a novel pretreatment method for CW biomass degradation with sodium chloride within 24 h. The method optimizes the pretreatment of cabbage residue based on temperature, time, and chemical reagent. Before and after pretreatment, the selected biomass samples were characterized by FTIR, XRD, and SEM for their physicochemical composition. This study offers the development of a cost-effective, environmentally friendly pretreatment method that contributes to the circular economy by using agro-waste as raw material while solving the waste management problem. The results offer valuable insights for designing pretreatment processes for agricultural waste.

MATERIALS AND METHODS

Sample Collection and Processing

Lignocellulose biomass (CW) was collected from the nearby village of Dhana in the district of Hisar. The biomass samples were prepared by washing them with distilled water to remove dirt and surface impurities and then drying them in the sun. After that, biomass was chopped and ground into powdered form, and it was sieved using a 40–60 mesh sieve. All the samples were prepared in atmospheric conditions. For subsequent use, powdered CW was kept in hermetically sealed bags.

Chemicals

The chemicals employed in this study are acetic acid, Sulfuric acid, Sodium hydroxide, Potassium hydroxide, sodium chloride, and ethanol in the pretreatment process. EDTA, sodium borate, sodium dodecyl sulfate, disodium phosphate, and 2-ethoxyethanol were used to analyze lignin, cellulose, and hemicellulose. All the chemicals used were analytical grade and procured by Merck (Sigma-Aldrich, Chemicals Pvt. Ltd., Bangalore, India). The experiments were conducted with deionized water.

Pretreatment Optimisation Experiment Design

The purpose of statistically designing an experiment is to collect common relationships between various factors affecting the process of finding the most suitable conditions. An experimental design methodology must be economical for extracting the maximum amount of complex information, significantly reducing experimental time and saving material and cost. Four factors, namely, chemicals, chemical concentration, temperature, and time, were studied to optimize the process parameters for pretreatment, as shown in Table 1. The experimental scheme employed in this study is depicted in Fig. 1.

Estimation of Lignocellulosic Biomass

The lignocellulosic composition of lignin, cellulose, and hemicellulose in biomass in powder form was investigated, and data were analyzed both before and after the biomass was pretreated. The lignocellulosic content, lignin, cellulose, and hemicelluloses in CW were investigated using a modified version of the Van Soest method.

Calculation for Lignocellulosic Biomass (dry weight percentage):

Hemicelluloses = neutral detergent fiber (NDF) - acid detergent fiber (ADF)

Cellulose = ADF-ash

Lignin = acid detergent lignin (ADL)

Characterization of Biomass

The X-ray diffractometer (Smart Lab 3kW/Rigaku) was used to analyze the crystalline structure of lignocellulosic biomass,

Table 1: Experimental design for optimization of the process parameters for pretreatment.

Type of Biomass	Pretreatment Conditions			
	Chemical	Concentration [%]	Temperature [^oC]	Time [h]
CW	Control	0	37	12
				24
	CH_3COOH	2	37	12
				24
	H_2SO_4	2	37	12
				24
	KOH	2	37	12
				24
	NaOH	2	37	12
				24
	NaCl	2	37	12
				24
CW	Control	0	50	12
	NaCl	2	50	12
	NaCl	4	50	12
	NaCl	6	50	12
CW	Control	0	75	12
	NaCl	2	75	12
	NaCl	4%	75	12
	NaCl	6%	75	12
CW	Control	0	100	12
	NaCl	2%	100	12
	NaCl	4%	100	12
	NaCl	6%	100	12

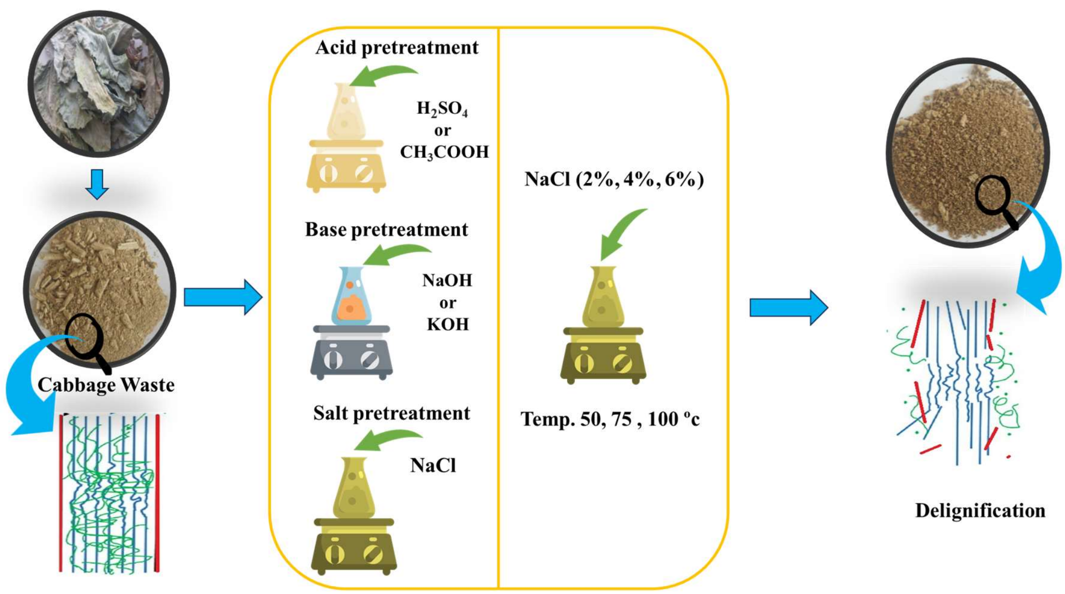

Fig. 1: Schematic illustration of experimental design for pretreatment of biomass.

examining its inherent crystalline nature across a 2θ scan range of 3° to 60° (Vydrina et al. 2023). The crystallinity index (CrI) was determined using the following equation;

$$\text{CrI (\%)} = \frac{A_C}{A_{C+A_\alpha}} \times 100 \quad \ldots(1)$$

Where CrI represents the relative degree of crystallinity (%), A_C is the under-crystalline peak, and Aα is the area under the amorphous peak at 2θ. FTIR (PerkinElmer, USA) analysis was performed in a range of 400 to 4000 cm^{-1} to analyze the functional groups of biomasses before and after pretreatment. The morphology of lignocellulosic biomass was analyzed using scanning electron microscopy (Leica EM SCD050) before and after pretreatment.

RESULTS AND DISCUSSION

This study investigates the impact of different pretreatment technologies on CW biomass. NaCl pretreatment is best suited for the delignification of the CW, which was further proved using characterization like FTIR, XRD, and SEM.

Optimization Study

Optimization of chemical reagents and time: The effect of five different chemicals, acetic acid, Sulfuric acid, Sodium hydroxide, Potassium hydroxide, and sodium chloride, and time on the lignin, cellulose, and hemicellulose was studied for CW at 37°C while varying time (12 and 24 h), as presented in Table 1. The lignin in the supernatant was measured as an indicator of pretreatment efficiency, as depicted in Fig. 2. The pretreatment process leads to the disruption of the fiber, which is demonstrated by a drop in the biomass's lignin concentration as well as a reduction in the biomass matrix's degree of polymerization and crystallinity (Bamisaye et al. 2022). These were the preliminary experiments conducted to determine a suitable time and chemical for the pretreatment process.

The study reveals that the pretreatment of CW biomass leads to delignification, a reduction in lignin, and an increase in cellulose composition. This is attributed to the observed delignification of the biomass samples. Fig. 2 shows that when NaCl pretreatment is compared with the control and other chemical reagents, it is observed to have the most delignification. Yiga et al. (2021) indicated that the biomass samples' observed delignification, a decrease in their lignin and extractive contents, and the con.2comitant rise in their cellulose composition are caused by the alkali pretreatment of cabbage waste. Fig. 2 shows that 12 h and 24 h of pretreatment do not differ much; that is why 12 h is selected as an optimized condition to make the process economical, sustainable, and environmentally friendly. The findings show that pretreatment with NaCl for 12 h was selected as the optimized condition for CW biomass.

Optimization of salt concentration and temperature: After optimizing chemical reagents and time, this study also optimizes chemical concentration and temperature. As discussed in the above section 3.1.1, NaCl is the optimized chemical at 12 h. So, the effect of the different concentrations of NaCl (2, 4, and 6% w/w) at different temperatures on the lignin, cellulose, and hemicellulose was studied for CW at 12 h, as presented in Table 1. The lignin in the supernatant was measured as an indicator of pretreatment efficiency. From Fig. 3, it can be concluded that lignin degradation also increased with an increase in temperature from 50 to 100°C. This is because a higher reaction temperature enhances hemicellulose solubilization and modifies lignin in biomass. It was found that 50°C is best suited for the pretreatment experiments to make the process more economical and environmentally friendly. Especially

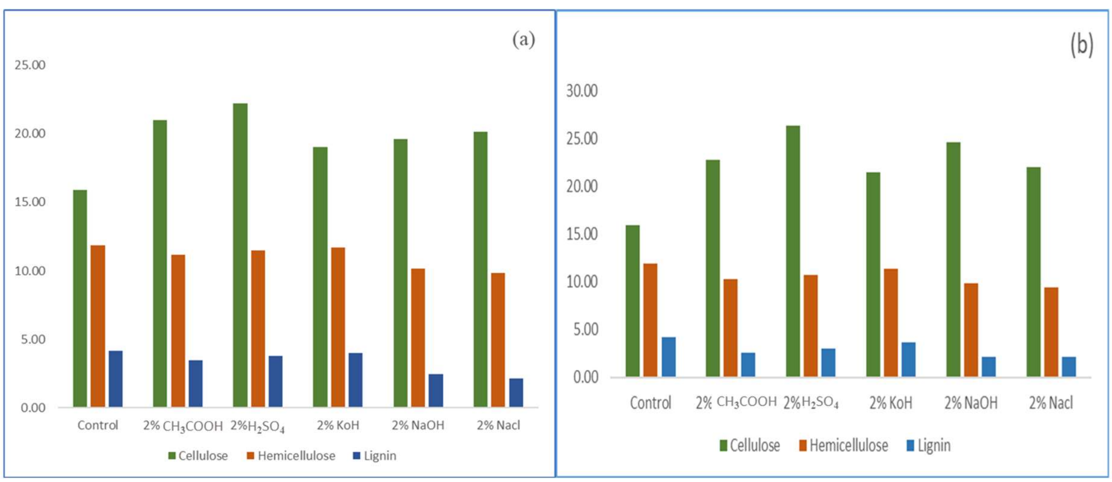

Fig. 2: Optimization study of different chemicals for CW biomass at 12 h (a) and 24 h (b).

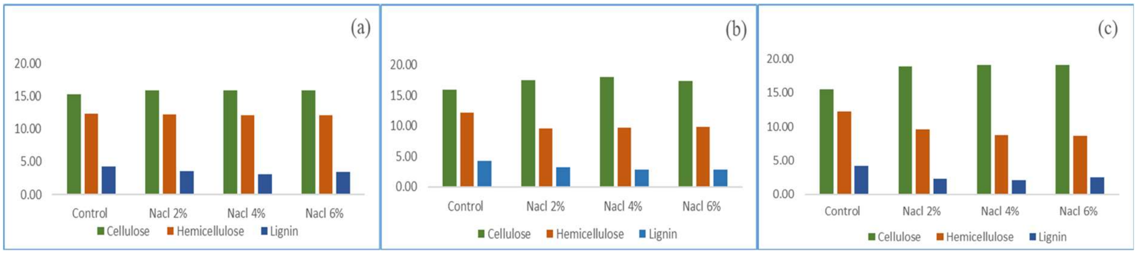

Fig. 3: Optimization study of NaCl concentration for CW biomass at 50°C (a), 75°C (b) and 100°C (c).

at higher temperatures, specifically in hydrothermal pretreatment, the lignin recondensation occurred in the solid matrix, and biomass browning at higher temperatures was observed in this study. The browning effect can be attributed to carbohydrate degradation products, caramelization of polysaccharides, or the recondensation of pseudo-lignin aromatic polycondensates (Batista et al. 2019). From Fig. 3, it can also be observed that with an increase in NaCl conc, there is a decrease in lignin degradation, and 2% NaCl shows maximum lignin degradation when compared to control and 4 and 6% NaCl conc. Dharmalingam et al. (2023) also showed that the reducing sugar yield decreased for samples pretreated with acetic acid when the temperature increased from 100 to 140°C and the acid concentration increased from 2 to 10% w/v. Thus, 2% NaCl at 50°C is an optimized condition for further pretreatment experiments.

Effect of Pretreatment on the Lignocellulosic Biomass Characteristics

The simplest method of overcoming biomass recalcitrance is to modify the structural properties through an appropriate pretreatment process. Milling biomass samples could increase specific surface area and decrease cellulose crystallinity, but these changes were not significant enough to have a significant pretreatment effect. Therefore, a more effective method should be applied to alter both structural and chemical properties significantly (Lee & Park 2020). Table 2 shows the CW lignocellulosic biomass characteristics, determining weight percentage composition for raw and pretreated biomass with 2% NaCl at 50ºC for 12 h using a modified Van Soest approach. The composition of lignocellulosic biomass varies globally due to factors like soil type, nutrient availability, and crop harvesting timing. NaCl pretreatment decreases lignin concentration with the increase in the availability of cellulose and hemicellulose. Meng et al. (2020) also reported the delignification in biomass of Populus trichocarpa deltoides through organosolv pretreatment. Saratale et al. (2020) suggested the alkaline pretreatment as a potential treatment for wheat waste biomass. Table 3 presents the comparison of previous studies with the present study to show the crucial benefits of this study,

Table 2: Characteristics of NaCl treated biomass at optimized conditions.

Biomass structural characteristics	Agro waste (Cabbage)		
	Raw [%]	Pretreated [%]	Change [%]
Lignin	4.33	3.10	28
Cellulose	15.34	15.95	2.9
Hemicellulose	12.39	12.05	2.7

Characterization of Wheat and Rice Straw before and after Pretreatment

The study assessed CW's surface morphology, functional groups, and crystallinity before and after NaCl pretreatment at optimum conditions using FTIR, XRD, and SEM analytical techniques. The results were used to understand the lower and higher saccharification yields after pretreatment. The efficiency of saccharification performance was linked

Table 3: Pretreatment efficiency comparison with previous studies.

Pretreatment methods	Reagent Used	Pretreatment conditions	Change [%]			Reference
			Lignin	Cellulose	Hemicellulose	
Acidic	H_2SO_4	2%,4%	10.6	22.8	21.8	Song et al. 2014
Acidic	H_2SO_4	1.1%	41.36	31.2	47.5	Christopher et al. 2023
Alkali	NaOH	4%	10.66	28.3	17.36	Song et al. 2014
Alkali	NaOH	8%	62	--	81	Mansour et al. 2024
Salt Solution	NaCl CW biomass	2%, 12 hr, 50°C	28	2.9	2.7	Present Study

to the substrate's structural characteristics, with biomass pretreatment causing structural changes like the removal of lignin and modification of cellulose crystallinity (Saratale et al. 2020, Selvakumar et al. 2022).

Surface Morphological Analysis

Scanning Microscope Electronic Microscopy (SEM) examined the morphology of the untreated and pretreated biomass samples, as depicted in Fig. 4. A smooth, fibrillary, stiff, and dense structure was seen in the SEM analysis of the raw biomass (Fig. 4a), which may have been caused by the lignin and hemicellulose in the tightly wrapped cellulose. However, after NaCl pretreatment, the pretreated biomass surfaces were disordered, rougher, conglomerated, and agglomerated. SEM micrographs showed increased porosity and surface area. However, pretreatment with NaCl increased biomass fragmentation and cellulose fiber surface roughness, suggesting that NaCl improved the depolymerization effect on CW biomass, as presented (Fig. 4 b, c, d, e, f). Pongmalai et al. (2015) showed that ultrasonic-assisted pretreatment of CW biomass also showed space, cell rapture, and cell structure collapse. Blanched cabbage showed a wilt appearance and flattened surface, while pretreated samples with acetic acid showed deformation, with higher acid concentrations causing more deformation (Chiewchan et al. 2010). Eom et al. (2024) showed that the untreated mixed cabbage residue (MCR) had a rigid, smooth structure, limiting enzyme access, while the pretreated MCR had a rough, conglomerated, and agglomerated surface morphology. This may be due to matrix disruption brought on by the dissolution of lignin and hemicellulose (Ziaei-Rad et al. 2021). Additionally, the structural modification enhanced the porosity of cellulose, increasing its hydrolyzability (Eom et al. 2019).

Fig. 4 shows that 2% and 4% NaCl pretreatment do not differ much; that is why 2% NaCl is taken as Optimised conditions to make the process economical, sustainable, and environmentally friendly. The figure also depicts the images taken at different magnifications for optimized 2% NaCl for CW biomass, showing the profound changes in biomass surface compared to raw biomasses. Overall, incorporating salt solutions facilitated the breakdown of surface structure and enhanced biomass depolymerization.

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was utilized to analyze the functional groups of raw and pretreated biomass samples, specifically CW, with NaCl pretreatment. It revealed conformational variations of functional groups. FTIR is particularly useful for qualitative analysis of cellulose, hemicellulose, and lignin in lignocellulosic biomass, focusing on structural analysis. The FTIR spectra ranged from 4000-500 cm^{-1}. FTIR analysis revealed significant chemical changes in CW biomass samples, with an increase or decrease in cellulose, hemicellulose, and lignin peaks in the pretreated samples compared to raw CW biomass, as depicted in Fig. 5 (a) and analysis of FTIR spectra are presented in Table 4. The FT-IR spectra indicate the presence of alkali lignin, which range from 1850 to 500 cm^{-1} (Wang et al. 2016). Changes in C=O bonds following pretreatment (Xie et al. 2020) might also be connected to hemicellulose's partial elimination. The intermolecular decomposition of hydrogen bonding due to

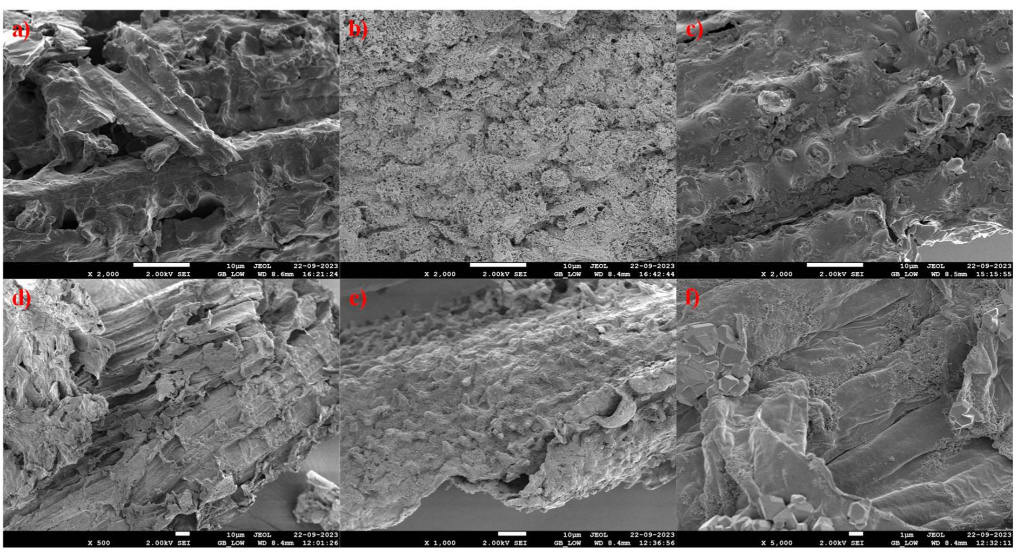

Fig. 4: SEM images of cabbage waste biomass; a) Raw, b) 2% NaCl, c) 4%NaCl, (d-f) Optimized 2% NaCl at different magnifications.

Table 4: FTIR analysis of CW biomass.

Raw CW biomass	2% pretreated CW biomass	4% pretreated CW biomass	Range [cm^{-1}]	Functional group	Characteristic and Reference
3412.3	3411	3417	3570-3200	O-H	Alcohol/water molecules
2917.9	2922.3	2917.9	3000-2840	C-H	Alkane
2855	2841.7	2855	3000-2800	N-H	Amine
1735.8	1744	1747.5	1750-1735	C=O	Esters
1631.3	1611.1	1627.6	1650-1566	C=C	Cyclo alkene
1382.08	1377.6	1382.08	1390-1380	C-H	Aldehyde
1060.44	1104.4	1096.2	1250-1020	C-N	Amine

the change in peak in the 3200-3570 cm^{-1} range is associated with hemicellulose and cellulose (Eom et al. 2024). The change in all the peaks presented in Table 4 before and after the pretreatment could be attributed to the delignification in the biomass surface, increasing the particle size and porosity. From the FTIR figure, it can also be concluded that 2% NaCl pretreatment is more effective as the change in peak intensity and shifting of the peaks is more profound for CW.

X-Ray Diffraction (XRD) Analysis

X-ray diffractograms (XRD) are crucial for analyzing cellulosic fraction morphology and conformational changes. XRD is also an essential analytical technique for determining the materials' structure, i.e., crystalline or amorphous. Alkali delignification treatments can affect the crystalline structure of cellulose. The XRD spectra of wheat and rice straw before and after pretreatment are presented in Fig. 5 (b). The XRD pattern showed distinct peaks at 2θ are 20.74, 29.54, 31.74, and 45.34 for raw CW biomass, 20.76, 29.54, 31.72, and 45.44 for 2% NaCl pretreatment, and 20.28, 27.46, 31.84 and 45.56 for 4% NaCl pretreatment of CW biomass. Compared with the control, the positions of these peaks of different pretreated samples had no noticeable change, while their intensities varied greatly. This phenomenon indicated that the crystal form of cellulose was not affected by pretreatment, while cellulose structures were changed to different degrees after pretreatments (Zhang et al. 2020).

The untreated and pretreated CW biomass was characterized using XRD to calculate the cellulose crystallinity index (CrI). The CrI value changed from 19.37% for raw to 15.14% for 2% NaCl pretreatment and 24% for 4% NaCl pretreatment for the CW biomass. According to these results, the amorphous areas of the CW biomass, such as lignin, hemicellulose, and a small portion of cellulose, were removed during pretreatment (Eom et al. 2024). These results are consistent with those of earlier research. Eom et al. (2024) showed that the pretreated MCR had a higher CrI (44.4% vs. 38.03%) than untreated MCR. A 3-hour ionic liquid (IL0 pretreatment of wheat straw increased crystallinity by about 3% (Ziaei-Rad et al. 2021). Quinoa straw treated with IL has more crystallinity than untreated biomass (40.0% vs. 33.3%), according to Xie et al. (2020).

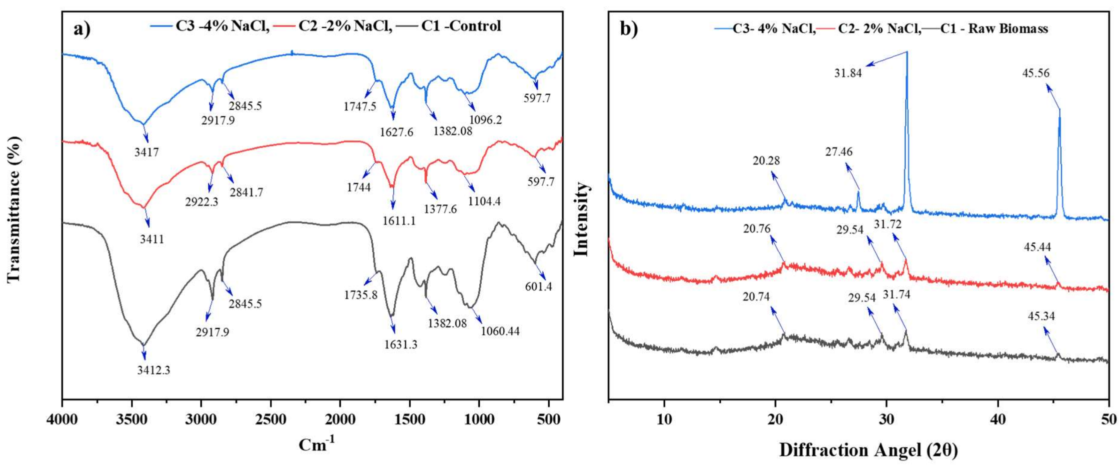

Fig. 5: Characterization of cabbage waste biomass before and after pretreatment a) FTIR spectra b) XRD spectra.

CONCLUSION

Mixed vegetable and food wastes left from consumption and processing were important uncontrolled sources of greenhouse gas emissions. The application of vegetable waste is ineffective due to the recalcitrant structure of lignocellulose. However, this biomass must be pretreated to break down the lignin structure and interrupt the cellulose crystalline makeup. The CW biomass was obtained locally, dried, and pretreated using acid, base, and salt-based chemical processes, with salt being the most effective for delignification at 12 h and 50°C temperature. The results of the FTIR and composition analysis of the biomass indicated that hemicellulose and lignin were extracted from the biomass by pretreatment with various chemical reagents. NaCl pretreatment was observed to be more effective in removing lignin than other chemical reagents. Compared to untreated CW biomass, the pretreated biomass showed a greater cellulose conversion. The SEM picture, XRD pattern, and FT-IR spectra of the CW biomass after pretreatment revealed a noteworthy impact of the NaCl pretreatment, as pretreated biomass showed a significant structural and morphological difference from the raw biomass. This study provides valuable insights into the effectiveness of NaCl pretreatment and offers a promising avenue for sustainable biomass applications, inspiring further research and development in the field.

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