Contents list available at IJRED website

Int. Journal of Renewable Energy Development (IJRED)

Journal homepage: http://ejournal.undip.ac.id/index.php/ijred

Research Article

Physicochemical Characterization of Native and Steam Explosion Pretreated Wild Sugarcane (*Saccharum spontaneum*)

Aruna Selvaraj, Sriramulu Gobikrishnan*

Department of Food Processing Technology, School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore, India

ABSTRACT. The technology of biomass conversion to bioethanol primarily based on pretreatment, enzymatic hydrolysis, and fermentation. This study was to investigate the effectiveness of the steam explosion pretreatment of *Saccharum spontaneum* L., which accomplishes the greater efficacy of physicochemical and structural properties. The collected plant material was processed and analyzed for ash, moisture, carbon content, and other elements. The cellulose content of pretreated biomass was increased to 54.31% when compared to native wild sugarcane 41.23% due to the removal of lignin. SEM and FTIR results identified the changes in structural and functional groups also the BET analysis confirmed the increased surface area of Pretreated biomass is 55.541m²/g whereas the surface area of native biomass is 17.939 m²/g, this is due to the increase in pore volume and pore diameter of pretreated wild sugarcane which is 0.260 cc/g and 9.712 nm when compared to pore volume and Pore Diameter Dv(d) of raw material is 0.040 cc/g and 3.650 nm. XRD crystallinity pattern of pretreated wild sugarcane showed an increase in the crystallinity index due to the breakage of lignin during pretreatment. This comparative study has been carried out to know the effect of steam explosion pretreatment over the physicochemical composition and structural changes of wild sugarcane for sustainable bioethanol production.

Keywords: Wild sugarcane; Steam explosion; *Saccharum spontaneum*; Native biomass; Cellulose; Bioethanol

Article History: Received: 22nd April 2020; Revised: 19th June 2020; Accepted; 23rd June 2020; Available online: 25th June 2020
How to Cite This Article: Selvaraj, A. and Gobikrishnan, S. (2020). Physicochemical Characterization of Native and Steam Explosion Pretreated Wild Sugarcane *(Saccharum spontaneum). International Journal of Renewable Energy Development*, 9(3), 353-359.
https://doi.org/10.14710/ijred.2020.30240

1. Introduction

In recent years, lignocellulosic based bioethanol production has gained interest due to a variety of reasons like the high cost of petroleum-based fuel, sustainable energy, less pollution (Sukumaran and Pandey, 2009). Bioethanol produced from lignocellulosic biomass serves as an alternative promising carbon-neutral biofuel (Sindhu *et al*, 2019). In India, agricultural crop residues were considered as the most abundant available feedstocks for bioethanol production (Pandey *et al*, 2009). The agricultural biomass as lignocelluloses characterizes an effective reservoir of carbon-rich material (Rebello *et al.*, 2020). The lignocellulosic biomass conversion to biofuel provides economic and environmental sustainability compared to fossil fuels. The bioethanol production from lignocellulose biomass includes three significant steps, *viz.* biomass pretreatment, enzymatic hydrolysis, fermentation, and a distillation process. The pretreatment is considered a bottleneck in the process of obtaining a higher yield of bioethanol production. It involves hydrolyzing the carbohydrate to monomeric sugars through alteration of the structural and chemical composition of biomass.

Wild sugarcane *(Saccharum spontaneum)* is considered as a wasteland weed, which is a long perennial grass with deep roots. It is understood as a precursor of *S. officinarum L.* (cultivated sugarcane) and considered as a common regional weed (Sastri and Kavathekar, 1990; Hammond, 1998). *S. spontaneum* is suitable for biofuel production since its cell walls are rich in carbohydrates (67.85% on dry basis), (Chandel et al., 2009a, b; Scordia et al., 2010). The genus *Saccharum* comprised 13 species and found to be the same as the genus of *Miscanthus*; both this genus can be differed by the disposition of its spikelets (Scally *et al.*, 1997). Also, they show a higher yield after the second generation of crop establishment whereas in the *Miscanthus* genus it happens only after third-generation (Cosentino *et al.*, 2006). The studies of effective pretreatment have been carried out on different sugarcane hybrids, but for wild species, no reports were found so far. However, the efficiency of hydrolysis for the conversion of carbohydrates into monomeric sugars depends on the utilization of biomass (substrate) which is a major step

* Corresponding authors: suguraj200@gmail.com

that includes the changes in its structure, size, and nature of the biomass (Moiser *et al.*, 2005).

Generally, the steam explosion was considered as a successful pretreatment method due to the sudden explosive decompression (Chen and Liu, 2007a,b). NaOH is commonly used in the chemical pretreatment of lignocelluloses because of its ability to delignify biomass (Sindhu *et al.*, 2015). In this process, the lignin was redistributed and removed from the biomass at high temperatures by the acetic acids whereas the hemicellulose was hydrolyzed (Mosier *et al.*, 2002). The enzyme accessibility of cellulose microfibrils increases due to the removal of hemicelluloses by exposing the cellulose surface (Alvira *et al.*, 2010). The objective of the present study was to analyze the efficiency of pretreatment using a steam explosion for efficient bioethanol production. To investigate the changes in biomass after steam explosion pretreatment, characterization studies of native and pretreated samples were carried out by SEM, FTIR, BET, and XRD spectrum (Ragavi *et al.*, 2016).

2. Materials and Methods

2.1. Sample preparation

The plant *Saccharum spontaneum* L (wild sugarcane) was collected from Ash Lake, Neyveli, Tamilnadu, India, and authenticated by the Botanical Survey of India (BSI), TNAU – BSI/SRC/2804. The stem part of the feedstock was collected by removing leaves and flowers, chopped into small pieces, and sun-dried. The sun-dried raw material was subjected to mechanical size reduction and stored at room temperature as shown in (Fig.1).

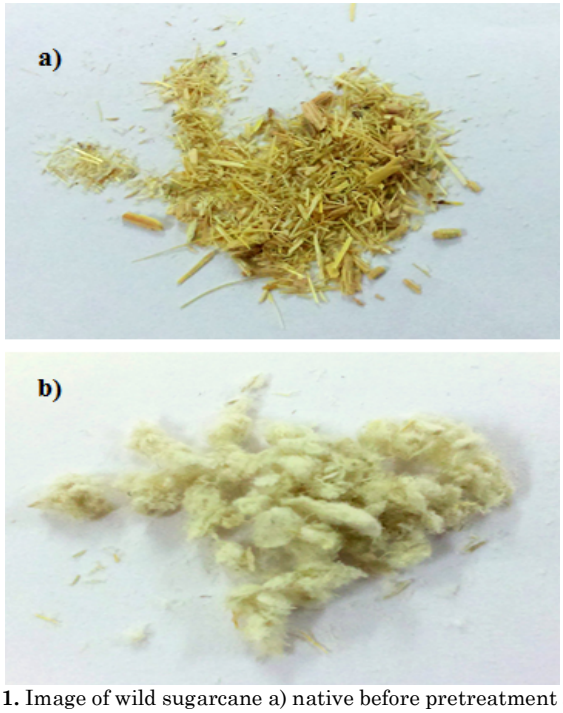

Fig 1. Image of wild sugarcane a) native before pretreatment and b) after steam explosion pretreatment

2.2. Apparatus and experimental procedure

Briefly, 5 g of the fresh sample was collected, the final biomass weight was noted after drying at 70°C in a hot air oven. The percentage of moisture content was determined by the method described by AOAC (1990).

2.3. Ash Content

In a crucible, 3 g of dried wild sugarcane sample was taken and heated to oxidize flames until smoke dissipated. The crucible was placed for 6 hrs in the muffle furnace at 550°C, then cooled and weighed in a desiccator. The composition of ash content was determined by the standard method (AOAC 1990).

2.4 Estimation of Cellulose

To the known quantity (0.5 g or 1 g) of wild sugarcane biomass, 3 mL of acetic/nitric reagent (150 mL 80% acetic acid and 15 mL concentrated nitric acid) were added in a test tube, mixed and placed at 100°C for 30 minutes in the boiling water bath. The content was cooled and then centrifuged for 15 – 20 minutes at 5000 rpm. After centrifugation, the biomass residue was washed using distilled water and the supernatant was collected separately. One mL supernatant was diluted to 100 mL of the above solution.

Ten mL of anthrone reagent was applied to 1 mL of this diluted solution and blended well. The contents are kept for heating in the boiling water bath for 10 minutes and measured the color at 630 nm and the amount of cellulose (Norman and Jenkins 1933).

2.5 Estimation of Lignin

2.5.1 Acid Detergent Fibre (ADF)

One gram of wild sugarcane was added to a round bottom container then 100 mL of acid detergent solution was prepared and added to mixture (20 g CTAB in 1 L of 1 N sulfuric acid). The content was warmed to simmer for 5 to 10 min and refluxed until boiling for 1 hour. The contents were drained by suction through a pre-weighed sintered glass crucible and washed twice with acetone accompanied by hot water until the filtrate was colorless and kept for overnight drying at 100 °C. The fiber was weighted after cooling (Norman and Jenkins 1933).

2.5.2 Acid Detergent Lignin (ADL)

To the obtained acid detergent fiber (ADF), 25-50 mL of 72% H_2SO_4 and 1 g of asbestos was added and kept it to stand for 3 hours. The content was diluted with distilled water, filtered with pre-weighed filter paper Whatman No.1 and dried at 100°C. The filter paper was weighed after cooling. The filter paper was added to a pre-weighed silica crucible and kept in a muffle furnace for ashing for 3 hours at 550°C and the ash content was measured. For a blank, 1 g asbestos with 72% H_2S0_4, was taken (Norman and Jenkins, 1933)

2.6 Estimation of hemicellulose

To 1 g of the powdered wild sugarcane sample, 10 mL of cold neutral detergent solution was added and kept in a refluxing flask. About 0.5 g of sodium sulfite and 2 mL of decahydronaphthalene were added to the mixture. The content was heated to the warm condition and refluxed for 60 minutes. The sample was washed with acetone and hot water by suction filtration using sintered glass crucible. The residue was kept in a silica crucible and dried at 100°C for eight hours. The crucible was cooled and weighed (O'dwyer, 1923).

2.7 EDAX analysis

The qualitative analysis of the chemical elements was performed using an energy dispersive spectrometer (EDAX System), which is coupled with SEM (JOEL – JSM 6390, Japan). For the EDX analysis, elements present in the sample absorb the X-ray beam, resulting in the movement of electrons from a ground state, causing dislocation of the electrons and thereby creating a hole that was filled by electrons from other higher energy state. This creates a difference in energy, which results in peak formation.

2.8 Estimation of CHN

CHNS analyzer (Thermo Finnigan FLASH EA 1112 series, Italy) was used to estimate the total carbon, hydrogen, and nitrogen content of the wild sugarcane at the SAIF, IIT Bombay. The CHNS(O) analyzer determined the percentages of carbon, hydrogen, nitrogen, sulphur and oxygen of organic compounds by instantaneous oxidation of the sample at 1800°C by "flash combustion". The combustion products were separated by a chromatographic column and detected by the thermal conductivity detector.

2.9 Pretreatment by steam explosion

The pretreatment was carried out for the powdered raw material as described by (Kaushik and Singh, 2011).

2.9.1 Preparation of Steam Exploded fibers

Alkaline treatment was done out in two steps. In the first step, 10g samples were soaked overnight in 2% NaOH solution and then autoclaved for four hours at 15 lb pressure after treating with 10% NaOH. The pressure was then immediately released and the residues were cleaned. In the first treatment, fibers are swollen due to the removal of excess impurities from the surface which makes further treatment easy. Lignin was removed in the second treatment from the fiber. The fibers were then washed in distilled water for the removal of alkali.

2.9.2 Preparation of Steam exploded bleached fiber

The pulp obtained after alkali treatment was kept for overnight soaking in 8% H_2O_2 (v/v) for bleaching. This bleached pulp was washed with distilled water to get rid of acid.

2.9.3 Treatment of Steam exploded bleached fiber in Acidic medium

After bleaching, the pulp was kept in ultrasonicator for mixing at a temperature of around 60 ± 1°C for 5 hr after treating with 10% HCl (1N) solution. Fibers were dried (Fig. 1) after neutralizing the final pH by washing with distilled water.

2.10 BET Analysis

The BET surface area, pore-volume, and porosity of wild sugarcane were measured using the (Brunauer–Emmett–Teller) BET surface area analyzer. BET-N_2 adsorption experiments were carried out manometrically using an Autosorb (Quanta Chrome Crop). Before the measurements of gas adsorption, the samples were kept in a vacuum condition at 200°C for a period of 24 hrs at least for degassing. Nitrogen adsorption isotherms were measured at a series of different pressures at -196°C. And the BET surface area was determined using the standard BET equation as described by (Kibami *et al.,* 2017).

2.11 Scanning electron microscopy

The SEM images were taken using a scanning electron microscope (JOEL – JSM 6390, Japan) to study the differences in the morphology of both pretreated and native wild sugarcane. The images were acquired at 20kV of an accelerating voltage at 500X magnification.

2.12 X-ray diffraction

X-ray diffraction analysis was performed using (XRD-6000 Shimadzu diffractometer) for the native and pretreated wild sugarcane. Diffraction patterns were reported utilizing 40 kV and 30 mA Cu-Kα radiation and 10-30 grade scale with a 0.03 phase scale. The crystallinity index was determined based on the empirical method (Segal *et al.,* 1959).

2.13 Fourier Transmission Infrared Analysis

FT-IR spectroscopy (Shimadzu Spectrometer, Japan) was done to study the difference in structural and functional groups through the FT-IR spectrum which acts as a molecular fingerprint of the sample. This will detect the changes in functional groups that may have been caused during pretreatment. The FTIR analysis was performed with the detector at 4 cm^{-1} resolution, 50 scans per sample, and the spectrum between 4000 and 400 cm^{-1}. Discs were prepared by using 300 mg of spectroscopic grade KBr to 5 mg of dried specimens and mixed in an agate mortar. The obtained mixture was pressed for 3 min at 10 Mpa (Sindhu *et al*., 2014).

3. Results and Discussions

3.1 Composition of wild sugarcane

Initially, *S. spontaneum L* was characterized for its moisture, ash content, Carbon, surface area, and elemental composition. The results indicated that the

native wild sugarcane contains 13.12% of moisture and 3.6% of ash content. CHN analysis revealed the presence of carbon 43.75%, Hydrogen 6.242%, Nitrogen 50.003%. From SEM - EDAX results it is found to be the total amount of elements are (99.99%) that is O (64.666%), Mg (1.15%), Si (6.81%), Si (4.5%), Cl (4.61%), K (16.83%), Ca (1.43%).

3.2 Effect of steam explosion pretreatment on wild sugarcane

Steam explosion pretreatment favors the reduction of lignin and hemicellulose by increasing the amount of cellulose in the biomass. Table 1 summarizes the dry basis composition of native and pretreated *S. spontaneum* L. The result shows the changes in biochemical composition of native and pretreatedS. spontaneum. The cellulose, hemicelluloses, and lignin composition in native was found to be 41.23%, 23.26%, 11.21%, whereas after pretreatment the composition was found as 54.31% of cellulose, 17.22% of hemicellulose and 7.14% of lignin, respectively. After pretreatment, the results showed increased cellulose content, reduced hemicellulose and lignin content compared to native raw material. Results from repeated measurements of wild sugarcane composition prior and after pretreatment showed excellent reproducibility. The pretreated carbohydrate content in the *S.spontaneum* biomass is highly comparable to other lignocellulosic substrates such as agricultural residue and other woody biomass which make this feedstock as a suitable substrate for the production of bioethanol.

Table 1
Composition of native and steam explosion pretreated wild sugarcane

Components	Native Wild sugarcane (% dry basis)	Steam Explosion Pretreated Wild sugarcane (% dry basis)
Cellulose	41.23	54.31
Hemicellulose	23.26	17.22
Lignin	11.21	7.14

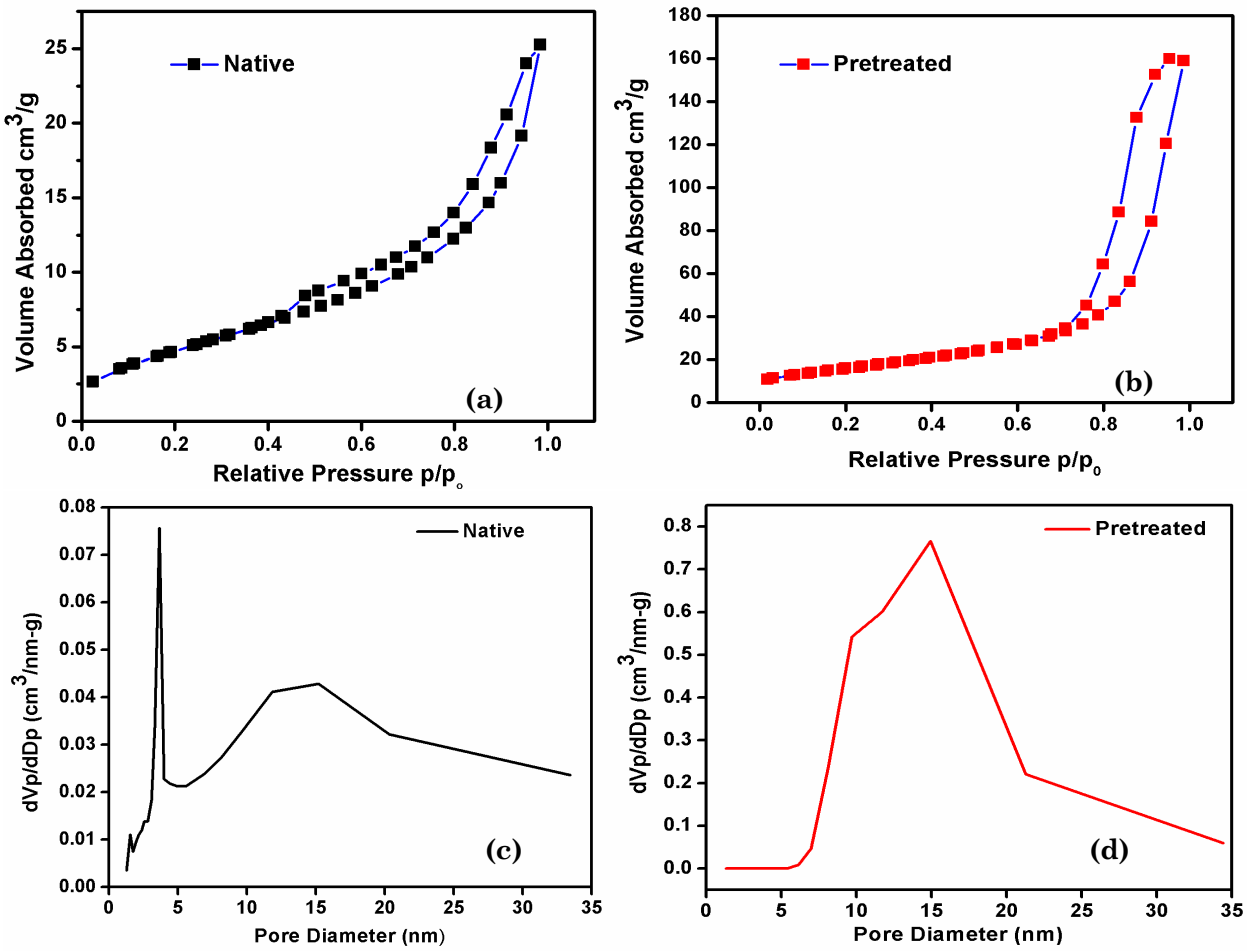

Fig 4. Adsorption–desorption isotherms of native and steam explosion pretreated wild sugarcane (a) native, (b) pretreated, and the corresponding pore size distribution curves of (c) native, (d) pretreated.

3.3 BET Surface characterization and nitrogen sorption of wild sugarcane

BET analysis revealed the measurement of the surface area of both native and pretreated wild sugarcane. The surface area of pretreated biomass increased to be 55.54 m^2/g whereas the surface area of native biomass is 17.94 m^2/g. Fig. 4a and 4b represent the adsorption–desorption isotherms of both native and pretreated biomass. Fig.4c and 4d represent the pore size distribution curve of native and pretreated wild sugarcane respectively. From the results, it was found that the pore volume and pore diameter-Dv(d) of raw material is 0.040 cc/g and 3.650 nm,respectively, whereas the pore volume and pore diameter-Dv(d) of pretreated biomass is 0.260 cc/g and 9.712 nm,respectively and found that pore volume was increased when compared with the untreated. The hydrolysis process will be more optimal due to the increase in specific surface area, this is because of the effective interaction between native and pretreated biomass. The pretreatment acts as a swelling agent and simultaneously removes the lignin, this results is an increase in a specific area that is suitable for enzyme action (Saha *et al.,* 2005). The internal specific surface area is observed based on the capillary structure of cellulosic fiber; while the external specific surface area depends on the particle size and shape.

3.4 Morphological analysis of wild sugarcane

SEM images show that most surface (lignin) substances have been removed, and linear cellulose crystal segments have been exposed after pretreatment. Pretreatment of the cellulosic biomass will make it much more available to enzymes for the degradation of cellulose and hemicellulose.

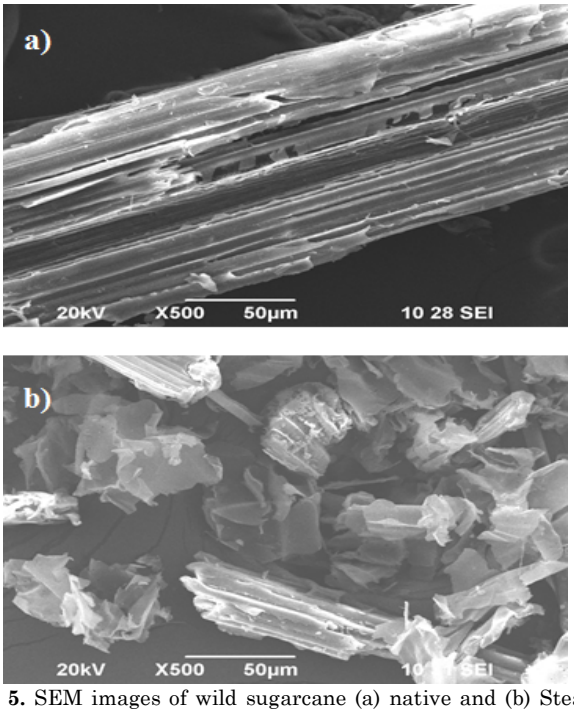

Fig 5. SEM images of wild sugarcane (a) native and (b) Steam exploded wild sugarcane

Pretreatment with a steam explosion caused more damage to the microstructure of the cellular matter. The original hard structure was broken after steam explosion pretreatment with a high temperature and the individual fibers vanished (Fig. 5). Such morphological modifications led to increased external surface area and porosity of the cellulosic material similar to previous NaOH pretreatment experiments in rice straw and sugar cane bagasse (Lin *et al.,* 2013; Zhang *et al.,* 2008). The effects of the steam explosion pretreatment were recorded by observing the difference between the ultra-structure of the native plant cell wall and the possible disruption of the pretreated *S. spontaneum* cell wall. The SEM image reveals the short anatomy of chopped and untreated *S. spontaneum,* with the sheath leaves surrounded by straw itself as thick-walled fiber cells. Exposing cellulose by structural modification or alteration of hemicelluloses and lignin tends to be a critical factor in saccharification of the remaining fraction of carbohydrate contained in *S. spontaneum* cell walls. Similar observations were reported earlier regarding the exposure of fibrous cell walls by pretreatment of barley hull (Kim *et al.,* 2008) and corn stover (Kim *et al.,* 2003).

3.5 Crystallinity analysis of wild sugarcane

XRD spectrum of the native and pretreated samples was presented on (Fig. 6). The crystallinity index of biomass has a major impact during hydrolysis (Kim *et al.,* 2003). X-ray diffractograms results show the changes in the crystallinity index before and after the steam explosion pretreatment of wild sugarcane. The crystallinity index of native and pretreated samples was 36.13% and 41.1% respectively. The increase in the crystallinity index after pretreatment is based on the increase of amorphous polymer it is due to the hydrolysis of glycosidic linkages of cellulose fibers. The amorphous region of cellulose is easy for enzymes to digest. The increase in the crystallinity index after pretreatment of lignocellulose has been observed in other studies, such as sugarcane tops pretreated with acid (Sindhu *et al.,* 2011), pretreatment of switchgrass with acid (Sindhu *et al.,* 2012).

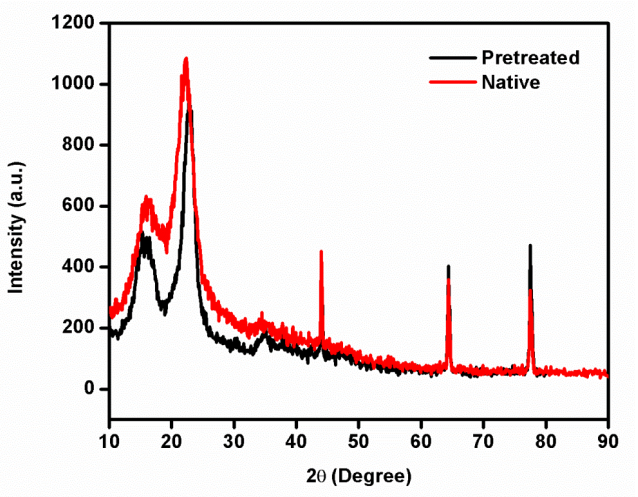

Fig 6. X-ray diffraction pattern of native and steam explosion pretreated wild sugarcane

3.6 FT-IR analysis of wild sugarcane

The FT-IR spectroscopy is commonly used for the characterization of lignocellulose, as it provides a relatively easy tool for direct information on chemical change during different chemical pretreating processes (Ristolainen *et al.,* 2002). The FTIR spectra of native and pretreated biomass revealed a significant difference was found in the fingerprint region (1659.13 – 895 cm^{-1}) for lignin's IR spectrum (Fig. 7). The peak at 1462.69 cm^{-1}, shows that the acetyl group linkage was reduced in this band which is present in between lignin and hemicellulose due to the breakage of the ester group. A significant reduction was also observed at the peak 1287.01 cm^{-1} which indicates the C–O stretching in lignin and hemicellulose. A sharp peak at 895 cm^{-1} is characteristic of ß-glycosidic linkages between the sugar units (Gupta *et al.,* 1987). The pretreated substrate also showed a reduction in the linkage of hemicellulose–lignin and C=O stretching due to carbohydrate linked with lignin via shift and decrease of the band at 3115.57 cm^{-1} and 2578.17cm^{-1}, respectively.

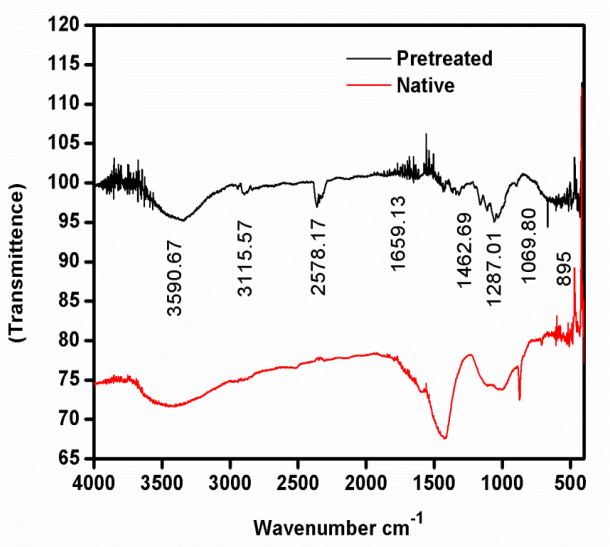

Fig 7. FTIR spectra of (a) native and (b) steam explosion pretreated wild sugarcane.

4. Conclusion

Saccharum spontaneum is considered as low-cost biomass since it is a wasteland weed which can be used as a renewable and potential source of feedstock for ethanol production. In this study, the steam explosion pretreatment of wild sugarcane increases the cellulose content by the removal of lignin and hemicellulose compared to the native wild sugarcane. The structural changes in the SEM image will enhance the biomass reaction during hydrolysis. Also, the release of lignin was detected in FTIR in pretreated biomass. The XRD results confirmed the increase in the crystallinity index after pretreatment than the native, this is due to the increase of amorphous nature of cellulose which improves the accessibility of cellulose surface which improves more cellulose hydrolysis to enable more theoretical yield by providing free β-glucosidic sites. The changes in the physicochemical, biochemical, and structural composition of wild sugarcane indicated that it is a suitable feedstock for bioethanol production.

Acknowledgments

This work was supported by the Department of Food Processing Technology, School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences - 641114, Coimbatore, India.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure Statement

The authors declare that they have no conflict of interest.

References

Alvira, P., Tomas-Pejo, E., Ballesteros ,M., Negro ,M.J., (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresour. Technol.(101),*4851–61. https://doi.org/10.1016/j.biortech.2009.11.093

AOAC., (1990). Official methods of analysis of the Association of Official Analytical Chemists. https://doi.org/10.1002/0471740039.vec0284

Chandel, A.K., Narasu, M.L., Chandrasekhar, G., Manikyam, A., Rao, L.V., (2009a). Use of Saccharum spontaneum (wild sugarcane) as biomaterial for cell immobilization and modulated ethanol production by thermotolerant Saccharomyces cerevisiae VS3. *Bioresour. Technol. (100),* 2404–2410. https://doi.org/10.1016/j.biortech.2008.11.014

Chandel, A.K., Narasu, M.L., Rudravaram, R., Ravindra, P., Narasu, M.L., Rao, L.V., (2009b). Bioconversion of de-oiled rice bran (DORB) hemicellulosic hydrolysate into ethanol by Pichia stipitis NCIM3499 under optimized conditions. *Int. J. Food Eng. (2),* 1–12. https://doi.org/10.1515/energyo.0035.00001

Chen, H.Z., Liu, L.Y., (2007a). Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. *Bioresour Technol. (98),* 666–76. https://doi.org/10.1016/j.biortech.2006.02.029

Chen, H.Z., Liu, L.Y., (2007b.) Technology of steam explosion: principle and application. Beijing: *Chemical Industry Press.*

Cosentino, S.L., Copani, V., D'Agosta, G.M., Mantineo, M., Litrico, A., (2006). Valutazione di germoplasma di specie del genere Miscanthus e Saccharum per la produzione di biomassa. *Italus. Hortus. (13),* 433–436 https://doi.org/10.3280/riv2018-071003

Gupta, S., Madan, R.N., Bansal, M.C., (1987). Chemical composition of Pinus caribae hemicelluloses. *J. Tappi. (70),* 113–114. https://doi.org/10.2524/jtappij.41.867

Hammond, B.W., (1998). Saccharum spontaneum (Gramineae) in Panama. *J. Sust. Forest. (8),* 23–38. https://doi.org/10.1300/j091v08n03_03

Kaushik, A., Singh, M., (2011). Isolation and characterization of cellulose nanofibrils from wheat straw using steam explosion coupled with high shear homogenization. *Carbohydr. Res. 346*(1), 76–85. https://doi.org/10.1016/j.carres.2010.10.020

Kibami, D., Pongener, C., Rao, K.S., Sinha, D., (2017). Surface characterization and adsorption studies of Bambusa vulgaris-a low-cost adsorbent. *J. Mater. Environ. Sci.* 8(7), 2494–2505.

Kim, H.T., Kim, J.S., Sunwoo, C., Lee, Y.Y., (2003). Pretreatment of corn stover by aqueous ammonia. *Bioresour. Technol. (90),* 39–47. https://doi.org/10.1016/s0960-8524(03)00097-x

Kim, H.T., Taylor, F., Hicks, K.B., (2008). Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. *Bioresour. Technol. (99),* 5694– 5702. https://doi.org/10.1016/j.biortech.2007.10.055

Lin, Y.S., Lee,W.C., Duan, K.J., Lin, Y.H., (2013). Ethanol production by simultaneous saccharification and fermentation in rotary drum reactor using thermotolerant Kluveromyces marxianus, *Appl. Energy (105).* 389-394. https://doi.org/10.1016/j.apenergy.2012.12.020

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y,Y., Holtzapple, M., et al., (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol. (96),* 673–86. https://doi.org/10.1016/j.biortech.2004.06.025

Mosier, S.N., Ladisch, C.M., Ladisch, M.R., (2002). Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng. (79),* 610–618. https://doi.org/10.1002/bit.10316

Norman, A.G., Jenkins, S.H., (1933). A new method for the determination of cellulose, based upon observations on the removal of lignin and other encrusting materials. *Biochem. J. 27(3),* 818–831. https://doi.org/10.1042/bj0270818

O'dwyer, M.H., (1923). The hemicelluloses. III. The hemicellulose of American white oak. *Biochem. J. 17(4–5),* 501–509. https://doi.org/10.1042/bj0170501

Pandey, A., Biswas, S., Sukumaran, R.K., Kaushik, N., (2009). Study on the Availability of Indian Biomass Resources for Exploitation: A Report Based on Nationwide Survey. TIFAC, New Delhi.

Raghavi, S., Sindhu, R., Binod, P., Gnansounou, E., Pandey, A., (2016). Development of a novel sequential pretreatment strategy for the production of bioethanol from sugarcane trash. *Bioresour. Technol. (199),*202–210. https://doi.org/10.1016/j.biortech.2015.08.062

Raveendran Sindhu, Ashok Pandey, Parameswaran Binod., (2015). Alkaline Treatment. *Pretreament of Biomass: Processes and Technologies.* Chapter 4, pp. 51 – 60. https://doi.org/10.1016/B978-0-12-800080-9.00004-9

Raveendran Sindhu, Parameswaran Binod, Ashok Pandey, Snehalata Ankaram, Yumin Duan, Mukesh Kumar Awasthi, (2019). Biofuel Production From Biomass:Toward Sustainable Development. *Current Developments in Biotechnology and Bioengineering: Waste Treatment Processes for Energy Generation.* Chapter 5, pp. 79 -92. https://doi.org/10.1016/B978-0-444-64083-3.00005-1

Ristolainen, M., Alen, R., Malkavaara, P., Pere, J.,(2002). Reflectance FTIR microspectroscopy for studying effect of xylan removal on unbleached and bleached birch kraft pulps. *Holzforschung (56),* 513–521. https://doi.org/10.1515/hf.2002.079

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., (2005). Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Proc. Biochem. (40),* 3693–3700. https://doi.org/10.1016/j.procbio.2005.04.006

Sastri, C.S.T., Kavathekar, K.Y., (1990). Plants for reclamation of wastelands. *CSIR,* New Delhi, India. pp. 360–362.

Scally, L., Hodkinson, T., Jones, M.B., (1997). Origin and taxonomy of Miscanthus. In: Jones, M.B., Walsh, N. (Ed.), Miscanthus for Energy and Fibre. Earthscan Publications Ltd., London, UK, pp. 1–45.

Scordia, D., Cosentino, S.L., Jeffries, T.W., (2010). Second-generation bioethanol production from Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. *Bioresour. Technol. (101),* 5358–5365. https://doi.org/10.1016/j.biortech.2010.02.036

Segal, L., Creely, J.J., Martin, A.E., Conrad, C.M., (1959). An empirical method for estimating the degree of crystallinity of native cellulose using X-ray Diffractometer. *J. Text. Res. (29),* 786–794. https://doi.org/10.1177/004051755902901003

SharrelRebello, AnoopkumarA.N, Embalil MathachanAneesh, RaveendranSindhu, Parameswaran Binod and Ashok Pandey, (2020). Sustainability and life cycle assessments of lignocellulosic and algal pretreatments. *Bioresource Technology. (301),* 122678. https://doi.org/10.1016/j.biortech.2019.122678

Sindhu, R., Binod, P., Janu, K.U., Sukumaran, R.K., Pandey, A., (2012). Organosolvent pretreatment and enzymatic hydrolysis of rice straw for the production of bioethanol. World *J. Microb. Biotechnol. (28),*473-83. https://doi.org/10.1007/s11274-011-0838-8

Sindhu, R., Kuttiraja, M., Binod, P., Janu, K.U., Sukumaran, R.K., Pandey, A., (2011). Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. *Bioresour. Technol. (102),* 10915-21. https://doi.org/10.1016/j.biortech.2011.09.066

Sindhu, R., Kuttiraja, M., Binod, P., Sukumaran, R.K., Pandey, A., (2014). Physicochemical characterization of alkali pretreated sugarcane tops and optimization of enzymatic saccharification using response surface methodology. *Renew. Ener. (62),* 362-368. https://doi.org/10.1016/j.renene.2013.07.041

Sukumaran, R.K., Pandey, A., (2009). Ethanol from biomass. In: Biswas, S., Basak, P.R., Kaushik, N. (Eds.), Biomass and Bioproducts–Emerging trends. TIFAC, New Delhi, pp. 11–36.

Zhang, Q.Z., Cai, W.M., (2008). Enzymatic hydrolysis of alkali-pretreated rice straw by Trichoderma reesei ZM4-F3, *Biomass Bioenergy 32 (12),* 1130-1135. https://doi.org/10.1016/j.biombioe.2008.02.006