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# Nanocellulose-Based Biofuel Production from Sugarcane Bagasse: A Sustainable Approach

Sustainable biofuel production has many advantages, including that it accelerates the worldwide transition away from fossil fuels and toward renewable forms of energy. The current study included the synthesis and characterization of nanocellulose (NC) from sugarcane bagasse. The prepared NC was used to produce biofuel using a simple and rapid method including fermentation cellulose and NC using the *Saccharomyces cerevisiae* yeast. Gas chromatography was used to determine the concentration of produced ethanol. About 49% and 79.5% ethanol were obtained when fermentation cellulose and NC, respectively. To increase concentration of produced ethanol, calcium oxide nanoparticles (CaONPs) were prepared from egg shells and added during the distillation of ethanol. The concentration of ethanol was increased to %97 after added CaONPs due to the fact that CaONPs works as adsorbent water molecules.

**Keywords:** Nanocellulose; Biofuel; Calcium oxide; Gas chromatography

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## 1. Introduction

Production of agricultural goods rises in tandem with the ever-increasing global population. Agricultural wastes amount to millions of tons as a consequence of this growth in crop production [1]. The near future of intelligent solid waste management for a green and circular economy includes the use of nanomaterials that aid in the generation and storage of energy from agricultural wastes [2], such as fruit waste and skin, rice straw, wheat straw, corn cob, corn stalk, and bagasse [3]. Cellulose is the most common biopolymer and can be found in trees, crop wastes, and other biomass [4]. Cellulose is a structural component of green plant cell walls and can be produced by algae, acetobacter, and rhizobium. All plant matter has a cellulose concentration of approximately 33% [5]. The emergence of NC obtained from various natural resources has gained increased interest for a wide range of applications [6].

Out of 640 million tons of sugarcane, over 160 million tons of sugarcane bagasse (SCB) is generated; this accounts for over 80% of the global sugar output. This has led to a great deal of interest in the idea of processing SCB to extract useful elements. Using natural biomass resources, this method lessens environmental contamination. [7]. As a result of sugarcane refining, a byproduct known as sugarcane bagasse (SCB) is produced. Its composition consists of lignin, cellulose, hemicellulose, and ash, in the range of 19-25% [8]. Bioethanol, which has the molecular formula  $\text{CH}_3\text{-CH}_2\text{-OH}$ , is a bio-based product that greatly aids in worldwide transition from the use of fossil fuels. Bioethanol is at the forefront of its field, with yearly sales reaching 58 billion [9]. The use of bioethanol, instead of gasoline, has many advantages, such as rapid vaporization rate and high-octane number (108), which prevent the cylinder

from banging early during combustion [10]. Moreover, the high oxygen content (35%) of bioethanol allows its mixing with gasoline and reduces the emissions of carbon monoxide and hydrocarbons. Bioethanol may be seen as a sustainable energy source since it is made from cheap and easily accessible lignocellulosic waste biomass [11]. Calcium oxide (CaO) shows promise in a variety of fields. The manufacturing of CaO from trash is both straightforward and affordable [12]. One of the main sources of CaO is eggshells, which contain 94% calcium carbonate and 6% proteins and minerals. Each gram of eggshell contains 381–401 mg of calcium [13].

## 2. Experimental Part

SCB was collected from local markets in Amman, Jordan. Sodium hydroxide was purchased from HI media. Sulfuric acid was obtained from BDH Chemicals. Sodium hypochlorite was supplied by Sigma–Aldrich, and instant dry yeast was provided by Star Maya.

SCB was collected, sun-dried, ground, treated with 2% NaOH, and heated at 80 °C for 4 hrs under continuous stirring to increasing its internal surface area, and causing lignin breakdown [14]. The sample was washed several times with distilled water until it reached pH=7 when measured with pH meter. The sample was then filtered, dried, and bleached with sodium hypochlorite. It was washed repeated until pH be 7 and dried. About 10 g of fine powder was hydrolysed with 30% sulfuric acid at 30 °C under vigorous stirring. The amorphous regions of cellulose are fast hydrolysis while the crystalline regions are retained [15]. The resulting sample was washed multiple times until the pH reached 7. The resulting solution was sonicated by a UP400S ultrasonic device

for 80 mins to obtain gel, namely, NC, which was stored for later use [16].

*Saccharomyces cerevisiae* was activated by adding 0.1 g of it to 2 mL of 5% sterilised glucose solution through filtration. The yeast was activated at 38 °C for 1 h and cooled to 30 °C prior to use in the experiment. Fermentation was then conducted using two conical flasks. About 5 g of cellulose was added to the first flask, and 5 g of NC was added to another conical flask. Each conical flask was pre-treated with diluted H<sub>2</sub>SO<sub>4</sub> (0.5%) at 121 °C for 60 min and 100 mL of distilled water. The remaining materials were then cleaned with distilled water. After adding NaOH to increase the pH to 5, the activated yeast was mixed with 100 mL of distilled water [17]. After 5 days of fermentation, ethanol was produced and its concentration was measured by gas chromatography. Figure (1) shows the steps of production of ethanol from prepared nanocellulose.

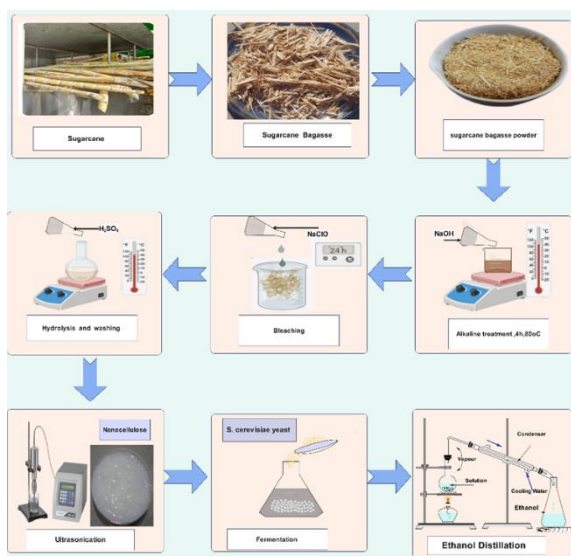


Fig. (1) Steps of production of ethanol from prepared nanocellulose

CaO nanoparticles (NPs) were synthesised through calcination of eggshell. Iraqi eggshells were collected after home use, washed, and removed the inner membranes attached to them. The shells were dried at room temperature and ground for 5 min with an electrical milling machine. The fine powder was burned for 1 hour at 900°C [18] in the process referred to as calcination, which decomposes calcium carbonate by heating it to high temperatures to form CaO NPs and CO<sub>2</sub> gas, [19].

The distillation process of ethanol from fermentation solution aims to increase the amount of ethanol produced. Columns are designed to separate and vaporize ethanol by using different boiling points of ethanol and water as shown in Fig. (2). Distillation involves concentrating alcohol to achieve high purity. In this experiment, 100 mL of fermented material was heated for 2 hours at a controlled evaporation temperature of 80°C by using a digital heating mantle

to produce ethanol. For every 100 mL of fermented material, 25 mL of condensate was produced. About 0.5 g of CaO NPs was added to the ethanol and mixed thoroughly to enhance purification. The mixture was then distilled at 80°C to achieve high purity. Ethanol concentration was determined using gas chromatography (GC).

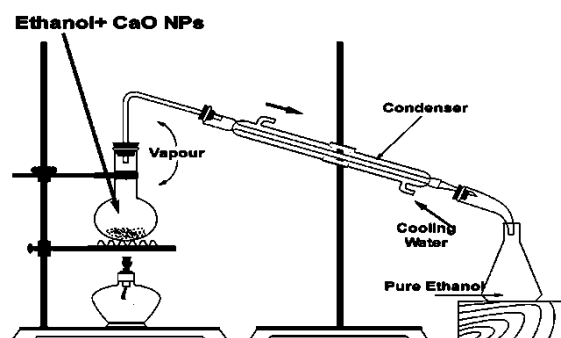


Fig. (2) Distillation lab instrument for Ethanol Preparation from nanocellulose

Fourier-transform infrared (FTIR) spectroscopy determines the molecular composition by measuring the absorbed IR radiation [20]. A ZEISS SIGMA VP EM10C field-emission scanning electron microscope (FE-SEM) was used to determine the diameter of the prepared NC and CaO NPs. Crystallinity size and crystallinity index of NC were calculated based on the XRD diffraction (XRD) data [21]. Crystallinity index was calculated using the following formula [22]:

$$CI = \frac{Area_{crystalline}}{Area_{Crystalline} + Area_{Amorphous}} \times 100\% \quad (1)$$

where  $Area_{Crystalline}$  is the integrated area under the crystalline peaks in the XRD pattern, and  $Area_{Amorphous}$  is the integrated area under the amorphous halo

In GC technique, the relative concentration of an analyte material was determined using the area under the curve (AUC) of the chromatographic peak. The formula for calculating the concentration (C) of an analyte using the AUC is [23]:

$$C = \frac{Area_{sample}}{Area_{standard}} \times C_{standard} \quad (2)$$

where  $Area_{sample}$  is the area under the curve of the analyte peak in the sample chromatogram,  $Area_{standard}$  is the area under the curve of the peak in the standard chromatogram, and  $C_{standard}$  is the known concentration of the standard

#### 4. Results and Discussion

Figure (3) shows the SEM image of the macrofibre of cellulose before conversion into NC, with diameter ranging from 3 to 10.4 µm. Figure (4) shows the morphology and size of NC prepared from SCB. The FE-SEM images demonstrated the formation of tiny and spherical granular NC nanoparticles, with diameters ranging from 29 to 43nm. The morphology and size of CaO NPs prepared from eggshells are depicted in Fig. (5). The

FE-SEM image indicated that CaO NPs have diameters ranging from 29 to 70 nm.

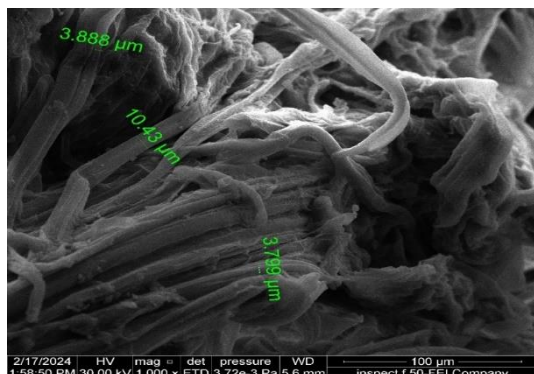


Fig. (3) FE-SEM image of cellulose

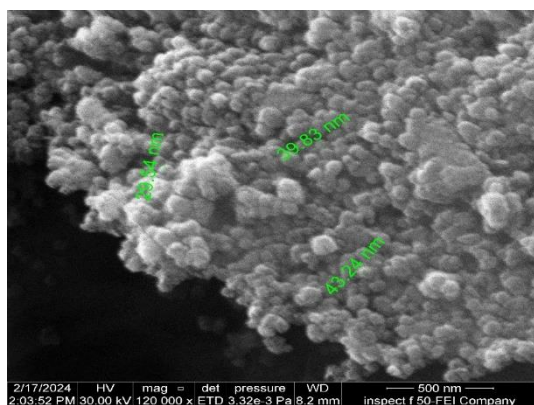
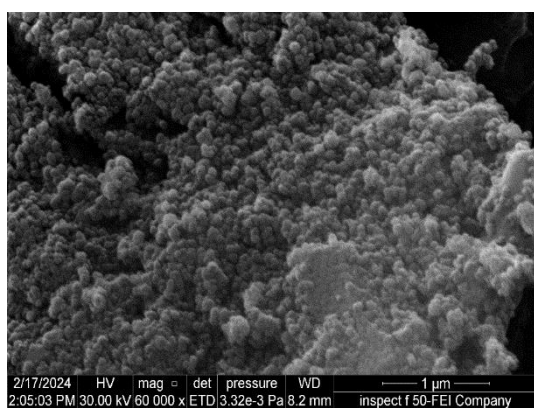


Fig. (4) FE-SEM images of nanocellulose prepared from sugarcane bagasse

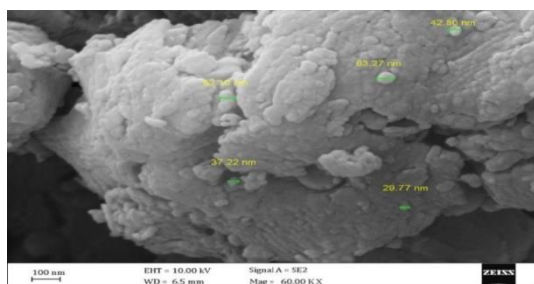


Fig (5) FE-SEM image of the prepared CaO NPs

Cellulose, in contrast with hemicellulose and

lignin, has a crystalline structure that is generated by bonds of hydrogen and van der Waals reactions among molecules within it. The amorphous part is more hydrolysable than the crystalline one. Hydrolysis process using 30%  $H_2SO_4$  and treated with ultrasonication breaks down the amorphous cellulose, leading to NC with a high crystallinity index [24]. Crystallinity index of NC calculated using Eq. (3) was found to be 80.98%, while that of cellulose was 59.62%. The diffraction peaks indexed to the lattice planes (220), (110), (004) associated with the characteristic peaks of cellulose at  $2\theta$  of  $16.68^\circ$ ,  $23.31^\circ$  and  $35.88^\circ$  compared to NC slightly displaced peaks at  $2\theta$  values of  $15.82^\circ$ ,  $22.28^\circ$ , and  $34.67^\circ$  [25], as shown Fig. (6). It was evident from the sharp and intense peak shifts that NC had a greater crystallinity than cellulose.

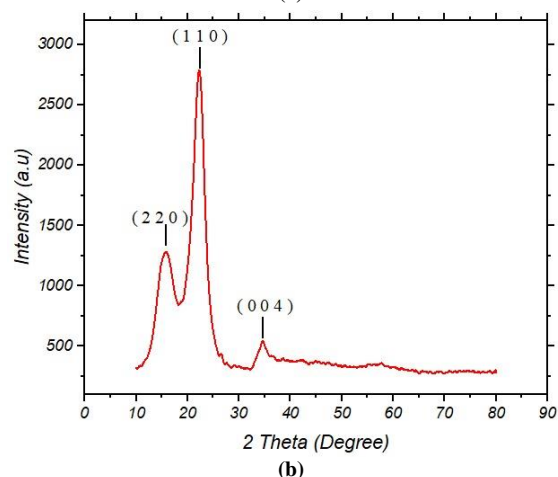
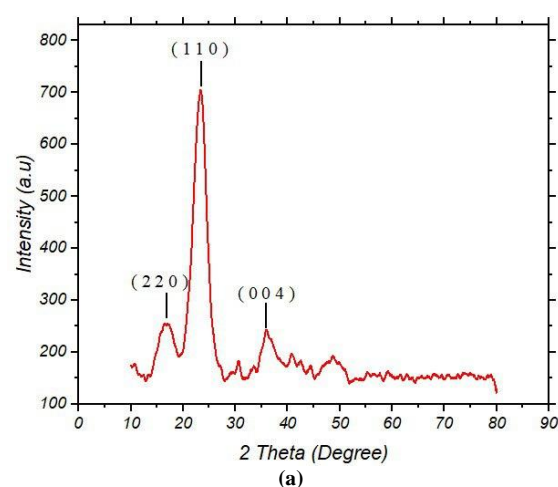


Fig. (6) XRD patterns of (a) cellulose and (b) NC prepared from sugarcane bagasse

As shown in Fig. (7), the FTIR spectra of cellulose and the synthesised NC showed strikingly similar profiles, although the peak intensity differed [26]. The stretching vibration of OH groups is indicated by the broad absorption band at  $3339\text{ cm}^{-1}$  [27]. In NC, the peak will be at a low intensity. Hydrolysis causes a reduction in band intensity because intermolecular hydrogen bonds are broken [28]. An absorption peak at  $2900\text{ cm}^{-1}$  is indicative of C-H bond stretching



vibration [27]. The bending mode of the absorbed water is responsible for the absorption band at  $1617\text{ cm}^{-1}$  while the peak in NC became less intense, indicating a decreased tendency to absorb water. The peak at  $1318\text{ cm}^{-1}$  refers to C=C stretching vibration of the aromatic ring, and those seen at  $1160$  and  $1026\text{ cm}^{-1}$  are attributed to C-O and C-C stretching vibration [29]. The C-O-C stretching vibration of the  $\beta$ -glycosidic linkages in cellulose symbolizes the crystalline integrity of the cellulose structure and was identified as the source of the peak at  $896\text{ cm}^{-1}$  [30].

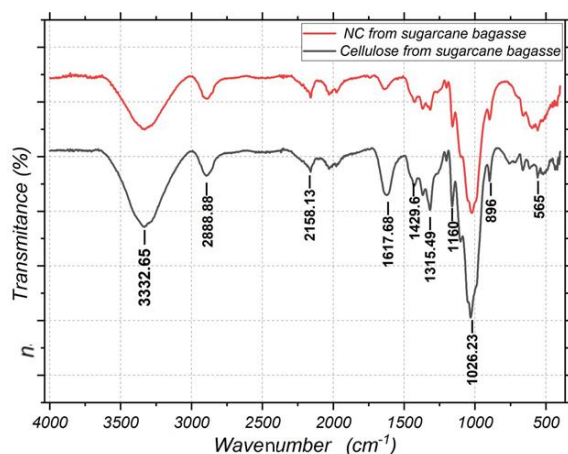


Fig. (7) FTIR spectra for cellulose and NC prepared from SCB

The GC retention times of standard ethanol and bioethanol were compared. Figures (8a,b) shows the graphs for standard ethanol and ethanol prepared from SCB cellulose respectively. Figure (9a) represents the standard ethanol and figure (9b) represents the ethanol prepared from NC.

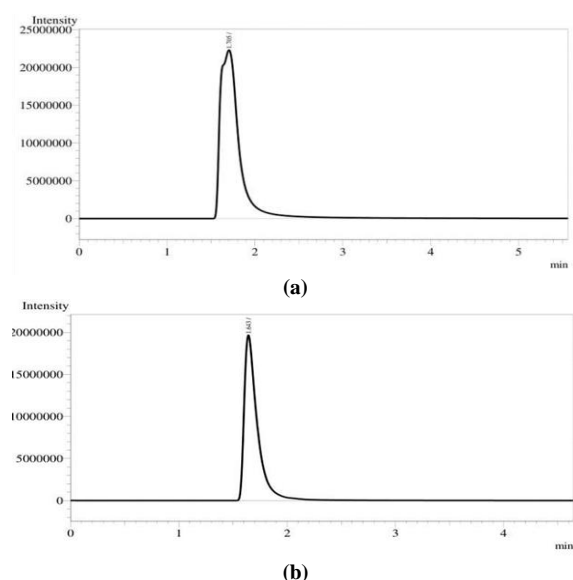


Fig. (8) GC spectra for (a) standard bioethanol, (b) bioethanol prepared from cellulose

The concentrations of distilled ethanol prepared from cellulose and ethanol prepared from NC are 49% and 79.5%, respectively, while the graph

displayed in Figures (10a,b) for standard ethanol and distilled ethanol prepared from sugarcane bagasse nanocellulose after adding CaO NPs, respectively, the bioethanol concentration increased to 97%.

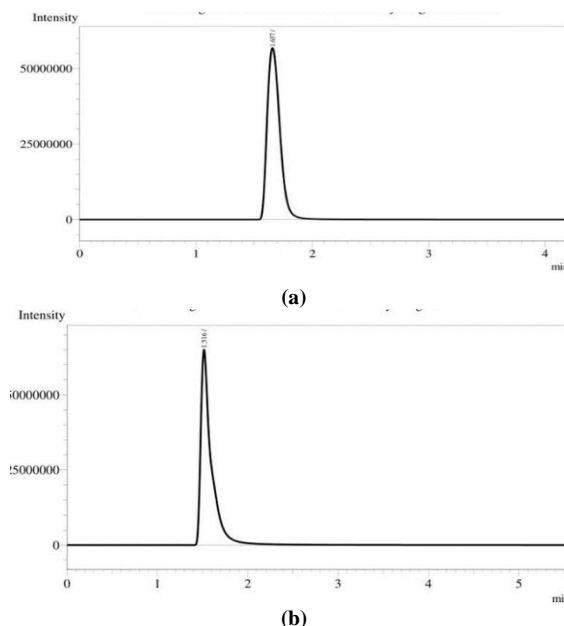


Fig. (9) GC spectra for (a) standard bioethanol, (b) bioethanol prepared from nanocellulose

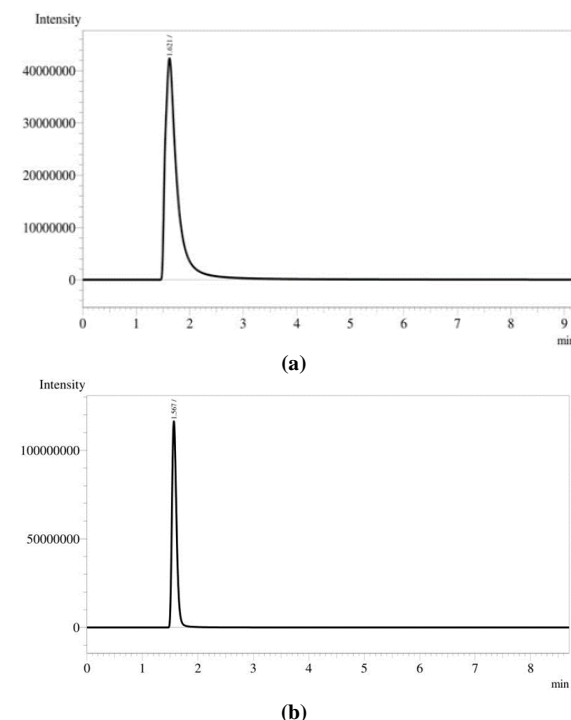


Fig. (10) GC spectra for (a) standard bioethanol, (b) bioethanol prepared from nanocellulose

#### 4. Conclusion

In this study, NC was prepared successfully from SCB through hydrolysis with 30% sulfuric acid followed by ultrasonication for 80 min. The NC had diameters ranging from 29 to 43 nm. The crystallinity index values of cellulose and NC were equal to 59.62% and 80.9815%, respectively. The produced

ethanol concentrations were 49% and 79.5% when using cellulose and NC, respectively. The GC technique proved that the concentration of ethanol was increased to 97% when CaO NPs added during the distillation process. By integrating CaO nanoparticles into the distillation process, manufacturers may get increased ethanol yields with improved purity, thus enhancing the total energy advantages of bioethanol. This development enables the more widespread embrace of bioethanol as a renewable energy source, hence enhancing energy stability and ecological sustainability.

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### References

- [1] N. Karić et al., "Bio-waste valorisation: Agricultural wastes as biosorbents for removal of (in)organic pollutants in wastewater treatment", *Chem. Eng. J. Adv.*, 9 (2022) 100239.
- [2] Sonu et al., "Agro-waste to sustainable energy: A green strategy of converting agricultural waste to nano-enabled energy applications", *Sci. Total Environ.*, 875 (2023) 162667.
- [3] S. Abdelhamid et al., "Hydrolysis of Cellulose Rich Agricultural Waste Using Two Potent Local Bacterial Isolates", *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.*, 93 (2023) 225.
- [4] L. Solhi et al., "Understanding Nanocellulose–Water Interactions: Turning a Detriment into an Asset", *Chem. Rev.*, 123 (2023) 1925–2015.
- [5] T. Aziz et al., "A Review on the Modification of Cellulose and Its Applications", *Polymers (Basel)*, 14 (2022) 3206.
- [6] M. Alalousi, Y. Al-Obaidi and M. Jehad, "Modification of Cellulose Nanofibers by ZnO Nanoparticles for Gas Sensing", *J. Phys.: Conf. Ser.*, (2020) 12089.
- [7] T. Nguyen et al., "Highly efficient and straightforward conversion of sugarcane bagasse into high value-added chemicals using carbonaceous catalyst in deep eutectic solvent", *Biomass Bioenergy*, 180 (2024) 107004.
- [8] J. Paschoa et al., "Production and purification of xylooligosaccharides from sugarcane bagasse and antioxidant potential assessment for functional ingredient application in the food industry", *Ind. Crops Prod.*, 208 (2024) 117844.
- [9] M. Rastogi and S. Shrivastava, "Recent advances in second generation bioethanol production: An insight to pretreatment, saccharification and fermentation processes", *Renew. Sustain. Energy Rev.*, 80 (2017) 330-340.
- [10] M. Moghaddam, K. Shayesteh and H. Seddighi, "A Review of the Bioethanol Production Process from Sugar and Lignocellulosic Base Materials and their Comparison", *J. Environ. Sci. Stud.*, 9 (2024) 8388-8406.
- [11] D. Bitew et al., "Ethanologenic yeasts from Ethiopian fermented beverages and optimization of fermentation conditions", *Renew. Sustain. Energy Rev.*, 190 (2024) 114090.
- [12] S. Bano and S. Pillai, "Green synthesis of calcium oxide nanoparticles at different calcination temperatures", *World J. Sci. Technol. Sustain. Dev.*, 17(3) (2020) 283-295.
- [13] K. Mustapha et al., "Mechanical properties of calcium carbonate/eggshell particle filled polypropylene Composites", *MRS Adv.*, 5 (2020) 2783–2792.
- [14] P. Meléndez-Hernández et al., "Comparative of alkaline hydrogen peroxide pretreatment using NaOH and Ca(OH)<sub>2</sub> and their effects on enzymatic hydrolysis and fermentation steps", *Biomass Convers. Biorefin.*, 11 (2021) 1897–1907.
- [15] H. Wang et al., "Highly Efficient Preparation of Functional and Thermostable Cellulose Nanocrystals via H<sub>2</sub>SO<sub>4</sub> Intensified Acetic Acid Hydrolysis", *Carbohydr. Polym.*, 239 (2020) 116233.
- [16] A. AlTarbuli, Y. Alobaidi and H. Abdullah, "Electrochemical study of redox stream antioxidant effect of nanocellulose membranes prepared from wheat straw in blood medium", *AIP Conf. Proc.*, 2660(1) (2022) 020125.
- [17] Y.M. Yousif and H.S. Abdulhay, "Bioethanol Production from Banana Peels using Different Pretreatments", *Asian J. Biol. Life Sci.*, 6 (2017).
- [18] Y.M. Alobaidi, M.M. Ali and A.M. Mohammed, "Synthesis of Calcium Oxide Nanoparticles from Waste Eggshell by Thermal Decomposition and their Applications", *Jordan J. Biol. Sci.*, 15(2) (2022) 269-274.
- [19] N. Razali et al., "Production of Calcium Oxide from Eggshell: Study on Temperature, Weight and Contact Time", in *RSU Int. Res. Conf.*, (2021) pp. 624-637.
- [20] M. Guerrero-Pérez and G. Patience, "Experimental methods in chemical engineering: Fourier transform infrared spectroscopy—FTIR", *Can. J. Chem. Eng.*, 98(1) (2020) 25-33.
- [21] H. Khan et al., "Experimental methods in chemical engineering: X-ray diffraction spectroscopy—XRD", *Can. J. Chem. Eng.*, 98(6) (2020) 1255–1266.
- [22] F. Schweingruber, "Wood Structure and Environment", Springer Science & Business Media, T. Timell (2019), pp. 389-400.

- [23] J. Swinley and P. Coning, **“A Practical Guide to Gas Analysis by Gas Chromatography”**, Elsevier (2019), pp. 310-320.
- [24] W. Wulandari, A. Rochliadi and I. Arcana, “Nanocellulose prepared by acid hydrolysis of isolated cellulose from sugarcane bagasse”, *IOP Conf. Ser.: Mater. Sci. Eng.*, 107 (2016) 012045.
- [25] H. Kargarzadeh et al., **“Handbook of Nanocellulose and Cellulose Nanocomposites”**, Wiley VCH (2017) pp. 325-326.
- [26] N. Ishak et al., “A correlation on ultrasonication with nanocrystalline cellulose characteristics”, *Carbohydr. Polym.*, 246 (2020) 116553.
- [27] R. Zhabankov, **“Infrared Spectra of Cellulose and Its Derivatives”**, Springer (2013), pp. 7-8.
- [28] S. Lu., “Facile extraction and characterization of cellulose nanocrystals from agricultural waste sugarcane straw”, *J. Sci. Food Agric.*, 102(1) (2022) 312–321.
- [29] M. Fan, D. Dai and B. Huang, in *Fourier Transform. Anal.*, 3 (2012) 45–68.
- M. Fan, D. Dai and B. Huang, “Fourier Transform Infrared Spectroscopy for Natural Fibers”, *Fourier Trans.-Mater. Anal.*, 3 (2012) 45-68.
- [30] N. Abidi, **“FTIR Microspectroscopy”**, Springer (USA, 2021), pp. 21-22.
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