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Sustainable Biofuel Production from Giant Reed-Derived Nanocellulose

Eman Khammas[1*], Yusra M. Alobaidi[2], Mustafa R. Alshaheen[3]

[1,2] Department of Chemistry, College of Science, University of Anbar, Ramadi, Iraq;
[2] Nanomaterials Research Center, University of Anbar, Ramadi, Iraq.
[3] Department of Biotechnology, College of Science, University of Anbar, Ramadi, Iraq;
*E-mail: Ima21s3008@uoanbar.edu.iq

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ABSTRACT

Shifting from fossil fuels toward renewable energy sources depends heavily on sustainable biofuel production. Using nanocellulose from giant reed as feedstock is one potential strategy. One potential contender for sustainable biomass production is a giant reed. This annual plant is known for its prosperity, rapid growth rate, and adaptability to many temperatures and kinds of soil. In this research, a low-cost chemical-mechanical technique to synthesize nanocellulose (NC) was developed, included purification, alkali treatment, bleaching with sodium hypochlorite, hydrolysis using 30% H_2SO_4, and sonicating the sample for 80 min. The produced NC was characterized using a variety of techniques, such as field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD). FTIR spectroscopy showed that the amorphous portions of the cellulose fibers are eliminated. XRD technique confirmed that the crystalline content is increased, and it showed a crystallinity index for cellulose of 82.036% and that for nanocellulose 97.453%. The FESEM images showed that the prepared NC formed as nanoparticles with average diameters (55–84 nm). Nanocellulose was fermented to produce ethanol and compared to cellulose fermentation and concluded that the yield of bioethanol from nanocellulose fermentation is (76.3%) and the yield of bioethanol from cellulose fermentation is (36.5%). Then calcium oxide nanoparticles (CaO NPs) from egg shells were prepared and added to increase the concentration of ethanol to (91%).

Introduction

Recently, environmental concerns are increasing due to the usage of petroleum-based products [1]. To mitigate the impending severe shortage of fossil fuels, the entire scientific community is currently focusing on producing bioethanol from lignocellulosic biomass [2].

The environment and renewable energy sources are both wasted by this enormous waste output. However, by utilizing them as inputs to make biopolymers. Cellulose, which, due to its superior physical qualities, is employed in a variety of industries after being transformed into nanocellulose [3].

*Corresponding author at Department of Chemistry, College of Science, University Anbar, Ramadi, Iraq
ORCID:https:// https://orcid.org/0000-0000-0000-0000 ,
Tel: +964 7810860006
Email: Ima21s3008@uoanbar.edu.iq

Producing second-generation ethanol (2GE) from cellulosic biomass rather than first-generation ethanol (1GE) based on sugar and starch is preferable because no competition with food production occurs [4].

Cellulose is a significant, widely distributed, reasonably priced biopolymer with global economic significance, numerous studies have recently addressed various applications [5]. It has active hydroxyl groups (OH) above its surface chains, enabling hydrogen bonds between cellulose molecules. Cellulose also possesses active hydroxyl groups (OH) at the top of its surface chains, facilitating hydrogen bonding between cellulose molecules [6].

The most popular method of producing CNC is sulfuric acid due to its many benefits, which include producing a more uniform and shorter CNC with a narrow polydispersity, a higher crystallinity, and better solution stabilization against flocculation [7].

Giant reed, or *Arundo donax L.*, is a tall perennial cane that belongs to the Poaceae family [8]. It is known as a non-edible plant that grows quickly and is highly tolerant of different weather conditions because it is lignocellulosic. Their ability to be cultivated on marginal or degraded land prevents them from competing with agricultural land [9]. This plant reproduces either by root production on the nodes or through the rhizome [10]. The fibers are used in the production of cellulose because of the comparatively high cellulose content (leaf 38.9%, culm 39.4%) and low lignin content (leaf 12.1%, culm 13.0%) [11].

Bioethanol, with the chemical formula (CH_3-CH_2-OH), is produced by fermenting the glucose obtained from the hydrolysis process with yeast, when glucose is consumed, carbon dioxide is also produced as the simplified reaction equation (1) [12].

$C_6H_{12}O_6 \Longrightarrow 2C_2H_5OH + 2CO_2$---------(1)

Saccharomyces cerevisiae is a eukaryotic organism used in the bioethanol industry, because of its high osmotic pressure, resistance to inhibitory compounds, wide pH range, and excellent fermentative capacity [13]. It has strong resistance to various environmental stress factors, including high ethanol concentration, low pH, and low oxygen levels, as well as quick growth, effective glucose anaerobic metabolism, and high ethanol productivity [14]. Calcium oxide (CaO) is an alkali earth metal oxide that has a broad range of applications and is seen as promising. One of the primary advantages of CaO is its easy and affordable production [15]. Calcium carbonate makes up 94% of eggshells; the remaining material is made up of minerals and protein. One gram of eggshell contains 381–401 mg of calcium [16].

A few advantages of the thermal decomposition method are its low cost, simple process, and ease of producing high-purity products. It therefore has enormous potential and is simple to apply in business. Nevertheless, calcining $CaCO_3$ is a common way to directly obtain CaO for the thermal decomposition method. Calcinations require a high temperature [17].

Materials and Methods

Materials

Methods

Preparation of Nanocellulose.

Initially reed grass was gathered, air-dried, and ground, heated to 80 °C 4 h while stirring continuously and treating it with 2% NaOH. The sample was then repeatedly cleaned with distilled water until it attained a pH of 7, after which it was filtered, dried, and bleached with sodium hypochlorite. Subsequently, 30 °C was used to hydrolyze 10 g of fine powder with 30% sulfuric acid while vigorously stirring. The resultant sample was then repeatedly washed until the pH of their solution reached 7. To obtain NC, the final gel was sonicated for 80 minutes using an ultrasonic device (UP400S). The produced NC was characterized by various techniques [18].

Production of ethanol

The prepared NC was used to produce bioethanol using *Saccharomyces cerevisiae.* First, 0.1 grams of dry yeast were added to 2 ml of 5% sterilized glucose solution through filtration to activate Saccharomyces cerevisiae yeast, It was cooled to 38 °C before using it for the experiment after activating it at 38 °C for 1 h. The second step involved starting the fermentation process in two conical flasks, one containing 5 g of cellulose and the other containing 5 g of nanocellulose. Every conical flask was pretreated for 60 minutes at 121 °C using 0.5% H_2SO_4 and 100 ml of distilled water. NaOH was then added to raise the pH to 5, the remaining materials were cleaned with distilled water and activated yeast was added with 100 mL of distilled water [19]. After five days of fermentation, ethanol is produced. The concentration was then ascertained by gas chromatography (GC).

Distillation of produced pure ethanol

To increase the concentration of bioethanol, CaO nanoparticles (NPs) were prepared and added through distillation ethanol. CaO NPs were prepared from Iraqi egg shells using various processes including cleaning, removing their inner membranes, drying, grinding to fine powder and calcination by burning at 900 °C for 1 h [20].

The concentration of ethanol is raised during the distillation process. A digital heating mantle was used to heat 100 mL of fermented materials for 2 h at a predefined evaporation temperature of 80 °C, yielding exceptionally pure alcohol. The amount of ethanol present was determined. A total of 100 mL of fermented material yielded 25 mL of condensate.

A total of 0.5 g of CaO were added to the ethanol and mixed well. The mixture was distilled in 80 ºC to high purity, and the concentration of ethanol was determined using GC. This process was made to raise the ethanol concentration to high purity [19]. The concentration of ethanol was subsequently determined using GC. The objective of this process was to enhance the ethanol concentration to achieve high purity. Figure 1 depicts the sustainable and cost-effective method proposed in this work for converting reed grass waste into renewable energy sources.

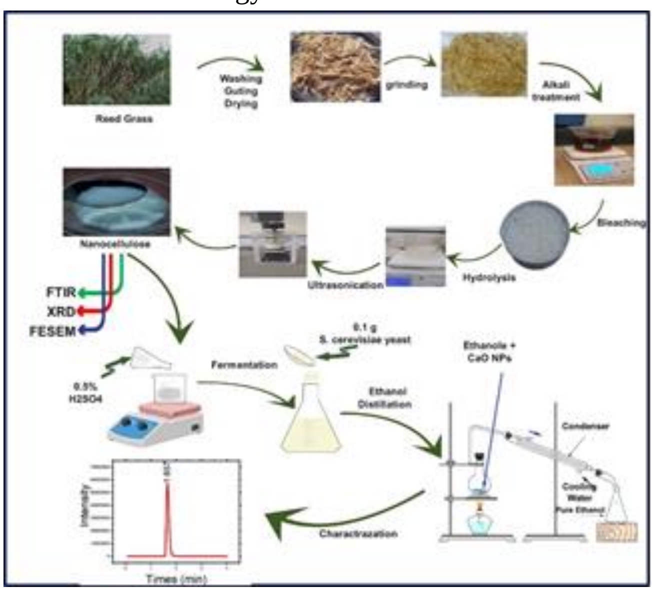

Figure (1). Schematic showing the conversion of reed grass into pure into bioethanol.

Characterization of prepared materials

Infrared Fourier transform was used to identify the functional groups in NC that were investigated [21], the crystallinity size and crystallinity index of NC was calculated from XRD diffraction (XRD) analysis [22]. Using the following formula, the crystallinity index was determined by [23]:

$$CI=\frac{Area_{crystalline}}{Area_{Crystalline}+Area_{Amorphous}}\times 100\% \quad ..(2)$$

where (Area $_{Crystallinity}$) is the integrated area under the crystalline peaks in the X-ray diffraction (XRD) pattern and (Area $_{Amorphous}$) is the integrated area under the amorphous halo in the XRD pattern.

Field emission scanning electron microscopy (ZEISS SIGMA VP model EM10C), was used to calculate the diameter of the prepared nanocellulose, and CaO NPs were measured. In GC, the relative concentration of an analyte can be determined using the area under the curve (AUC) of its chromatographic peak. The formula for calculating the concentration (C) of an analyte using the AUC is [24]:

$$C=\frac{Area_{\boldsymbol{sample}}}{Area_{standard}}\times C_{standard} \cdots \quad (3)$$

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.

Results and Discussion

1. FTIR Identification

Despite some variations in peak intensity, the FTIR spectra of cellulose and the synthesized nanocellulose revealed profiles that were remarkably similar [25]. OH stretching vibrations were also identified as the primary absorbance band at 3,330 cm^{-1} because of intramolecular and intermolecular hydrogen bonds. Cellulose and nanocellulose exhibit strong absorption bands here, but nanocellulose shows a slightly broader band due to increased hydrophilicity because the hydrogen bond works on the weakness of the (O-H) bond [26], additionally, the presence of a sulphate group in place of the OH group. The absorption peak at 2,917 cm^{-1} indicates the stretching of C–H bonds. The absorbance peak in the range of 1,608 $cm^{-1}$to represents the absorbed water's O-H bending [24]. The peak at 898 $cm^{-1}$was found to originate from the C-O-C

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stretching vibration of the β-glycosidic linkages in cellulose which represents the crystalline integrity of the cellulose structure [27].

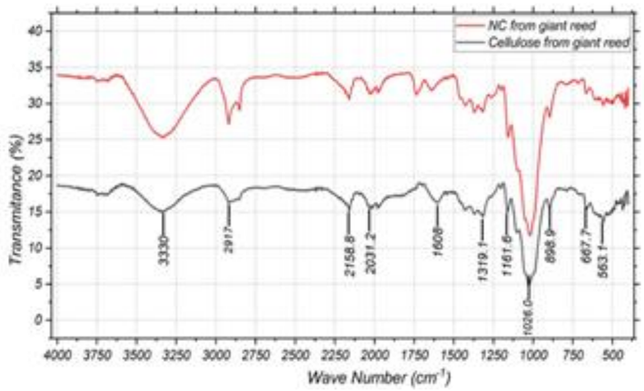

Figure (2). FTIR spectrum for cellulose and nanocellulose

2. X-ray diffraction (XRD) identification

XRD of NC

Cellulose, as opposed to lignin and hemicellulose, is naturally crystallized due to hydrogen bonds and van der Waals forces between neighboring molecules. Both crystalline and amorphous components make up cellulose, with the amorphous component being more susceptible to hydrolysis. When 30% acid is used for hydrolysis, the amorphous portion of cellulose is broken up and produces nanocellulose with a higher crystallinity index [25]. Crystallinity index for cellulose 82.036% while for nanocellulose 97.453%.

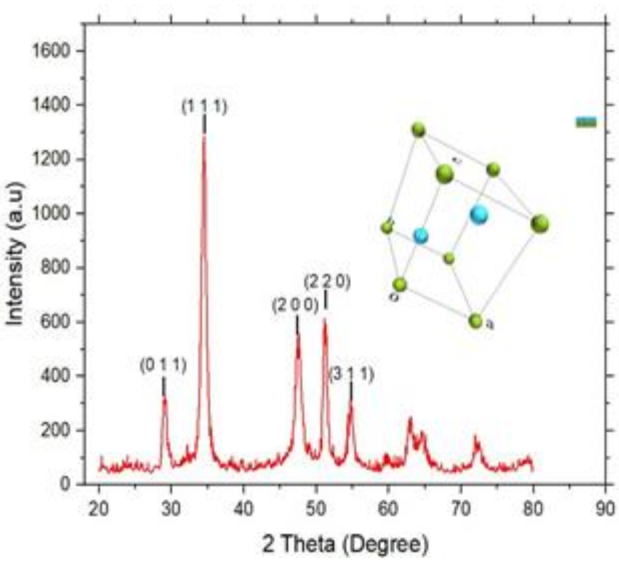

Figure (3) XRD image of cellulose.

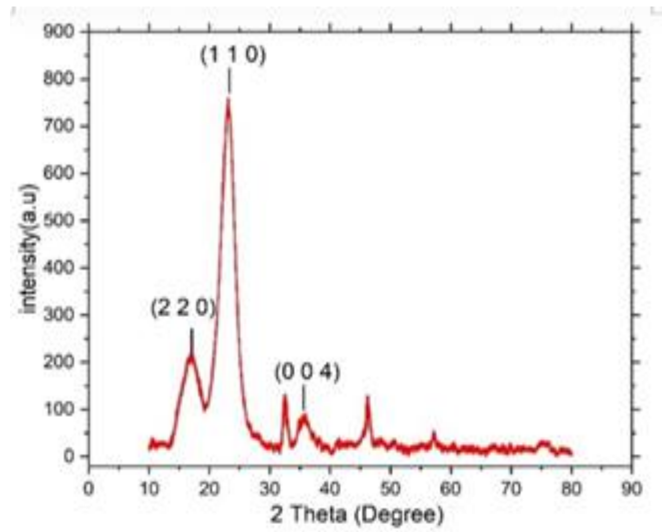

Figure (4) XRD image of nanocellulose.

XRD of CaO nano particles

Figure (5) illustrates that these correspond to the CaO phase, confirming the conversion of $CaCO_3$ into CaO, while the typical peak for $Ca(OH)_2$ emerged at 47 ºC [28]. The presence of $Ca(OH)_2$ in the calcined sample may have resulted from the CaO reaction with air during packaging and analysis [29].

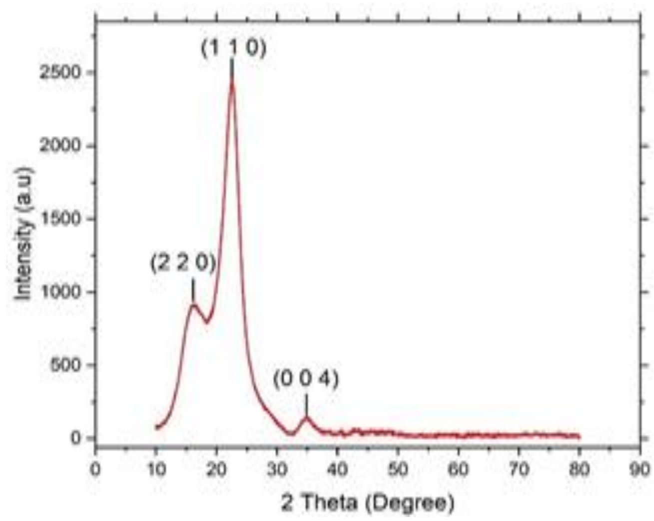

Figure (5). XRD of CaO NPs.

3. FESEM Identification

FESEM of NC

Figure (6) shows an SEM image of the macro fiber of cellulose before converting it to nanocellulose appearing as long narrow fibers, but during the acid hydrolysis, cellulose OH groups react and nanocellulose is sulfonated forming anionic sulfate ester groups(-oso$_3$-) due to the smaller particle size and different shapes [30].

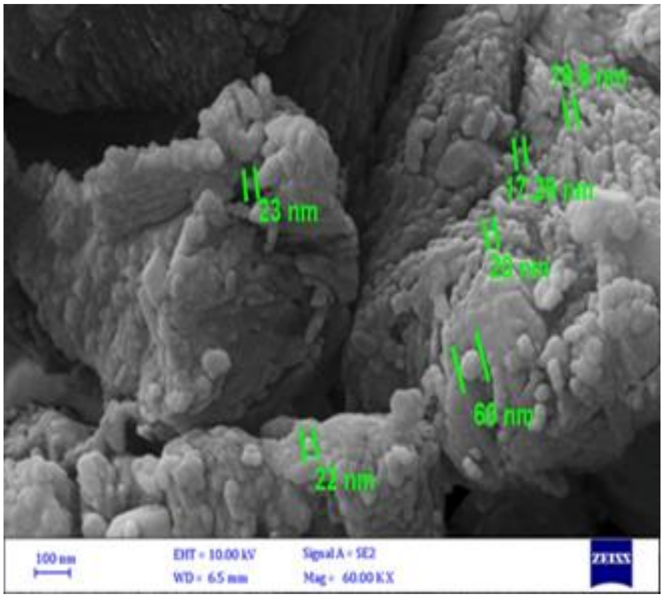

Figure (6). FESM image of cellulose

In Figure (7), the structure and cluster-like crystalline NPs of various sizes and shapes on the surface of NC are shown. Agglomerated particles with sharp edges that range in size from 55 to 84 nm were examined because the amorphous portions of cellulose were removed.

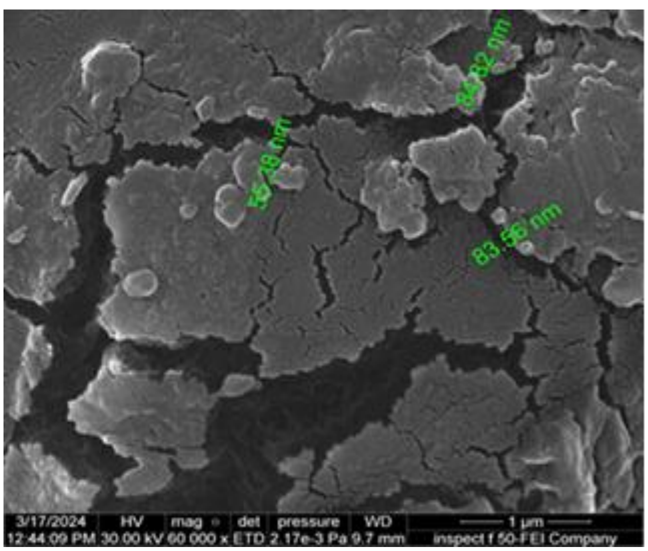

Figure (7). FESM image of nanocellulose

FESEM of CaO NPs

Figure (8) illustrates the morphology of CaO NPs formed from eggshells. The FESEM image indicated that the diameters for the CaO NPs was in the range 17.26–60 nm.

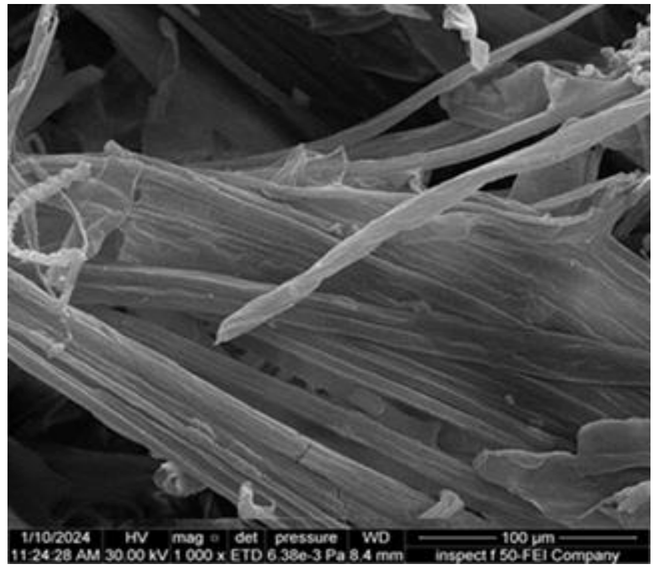

Figure (8) FESM image for CaO NPs prepared from eggshells.

Gas Chromatography Identification

The standard ethanol sample's GC and the bio-ethanol's GC retention times were compared. The graphs are displayed in Figure (8) for standard ethanol. Table (1) shows the distilled ethanol prepared from giant reed cellulose concentration was 36.5%. The distilled ethanol prepared from giant reed nanocellulose concentration was 76.3%.

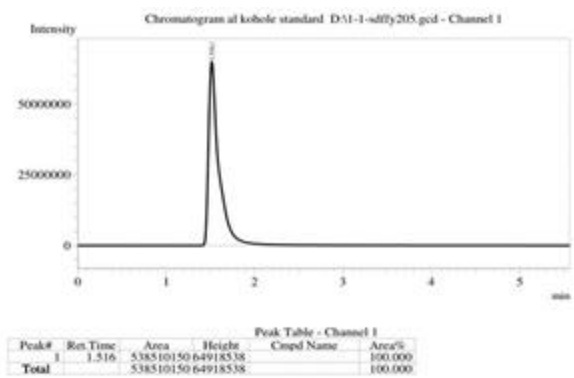

Fig (9) GC for standard ethanol

Table (1) Results of GC for bioethanol prepared from cellulose and nanocellulose

Subject	Ret.Time	Area	Height	Conc.
Standard ethanol	1.516	538510150	64918538	99%
Bioethanol prepared from cellulose	1.580	19864737	28975971	36.5%

Bioethanol prepared from nanocellulose	**1.428**	**415562485**	**64338121**	**76.3%**

The next stage of ethanol purification (dehydration) with CaO determined the ethanol concentration, and the result was 91%.

Table (2) Results of GC for bioethanol prepared from nanocellulose after adding calcium oxide nanoparticles

Subject	Ret..Time	Area	Height	Conc.
Standard ethanol(2)	**1.621**	**686543585**	**64918538**	**99%**
Bioethanol prepared from nanocellulose after purification	**1.647**	**631280243**	**28975971**	**91%**

Conclusions

This study used 30% sulfuric acid hydrolysis and 80 minutes of ultra-sonication to prepare NC from giant reed successfully. FTIR, XRD, and FESEM techniques were used to characterize the formed NC. According to the FESEM image, NC's diameters ranged from 55 to 84 nm. The crystallinity index values of cellulose and NC were confirmed by the XRD data to be 82.036% and 97.453%, respectively. The produced NC was utilized in a quick and easy process that involved fermenting nanocellulose and comparing the results to cellulose fermentation produced by saccharomyces cerevisiae GC was used to determine the concentration of produced ethanol. Bioethanol from nanocellulose and cellulose extracted from fermentation were (76.3%) and (36.5%), respectively. CaO NPs to raise the ethanol-produced purity were prepared from egg shells. Then added during distillation ethanol produced, Concentration of ethanol which produced from nanocellulose increased to (91%) after added CaO NPs.

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The authors provided their own financing.

Conflict of Interest

There are no conflicts of interest.

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إنتاج الوقود الحيوي المستدام من السليلوز النانوي المشتق من القصب العملاق

إيمان خماس العبيدي[1*] يسرى محمود العبيدي[1] مصطفى رياض الشاهين[2]

[1]قسم الكيمياء، كلية العلوم جامعة الأنبار – الأنبار – العراق

[2]قسم التقانة الأحيائية، كلية العلوم جامعة الأنبار – الأنبار –العراق

*الايميل : Ima21s3008@uoanbar.edu.iq

الخلاصة

يعتمد التحول من الوقود الأحفوري إلى مصادر الطاقة المتجددة بشكل كبير على إنتاج الوقود الحيوي المستدام. يعد استخدام النانوسليلوز من القصب العملاق (قصب الزل) كمادة اولية بوصفه منتجا مستداما للكتلة الحيوية. قصب الزل نبات سنوي معروف بازدهاره ومعدل نموه السريع وقابليته للتكييف مع درجات حرارة مختلفة وأنواع عديدة من التربة. في هذا البحث تم استخدام تقنية كيميائية ميكانيكية منخفضة التكلفة لتحضير النانوسليلوز (NC) من قصب الزل والتي تضمنت: التنقية، والمعاملة الألكيلية ، القصر باستخدام هايبوكلورات الصوديوم، والتحلل المائي باستخدام 30٪ H_2SO_4، والمعاملة بالموجات الفوق صوتية لمدة 80 دقيقة. تم تشخيص النانوسليلوز الناتج باستخدام مجموعة مختلفة من التقنيات، مثل المجهر الإلكتروني الماسح عالي الدقة ((FESEM)، حيود الأشعة السينية (XRD)،أظهر التحليل الطيفي بـــ FTIR أن الأجزاء غير المتبلورة من ألياف السليلوز تم التخلص منها. أكدت تقنية XRD زيادة المحتوى البلوري وأظهرت درجة التبلور للسليلوز 82.036٪ بينما النانوسليلوز 97.453٪. أظهرت صور FESEM أن NC المحضر يتكون على شكل جسيمات نانوية بأقطار (84–55 نانومتر). تم استخدام النانوسليلوز لإنتاج الإيثانول الحيوي عن طريق تخميره ومقارنته بتخمير السليلوز. كان تركيز الإيثانول الحيوي الناتج عن تخمير النانوسليلوز (76.3٪) و (36.5٪) من تخمير السليلوز. لتنقية الإيثانول الحيوي المنتج، تم تحضير جسيمات أوكسيد الكالسيوم النانوية (CaONPs) من قشور البيض وإضافتها أثناء عملية التقطير مما أدى إلى زيادة تركيز الإيثانول إلى (91٪).

الكلمات المفتاحية: الوقود الحيوي، القصب العملاق، النانوسليلوز ،التخمير ،كروموتوغرافيا الغاز