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The utilization of cotton stalks as a renewable feedstock for the production of bioethanol and its use as an additive to improve the properties of gasoline

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استخدام سيقان القطن كمواد خام متجددة لإنتاج الإيثانول الحيوي واستخدامه كمضاف لتحسين خصائص الكازولين

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الملخص:
يهدف هذا العمل الى اجراء دراسة شاملة حول إمكانية انتاج الايثانول الحيوي من نفايات سيقان القطن باعتبارها مادة أولية ليكنوسليلوزية تمتاز بوفرتها وانخفاض كلفتها. تضمنت هذه الدراسة معالجة أولية كيميائية لمسحوق سيقان القطن باستخدام حامض الكبريتيك بنسبة 6:1 وبتراكيز **(1.5%,1.0%,0.5%)** لتفكيك البنية الليكنوسليلوزية المعقدة وإزالة اللجنين والهيميسليلوز تلت ذلك اجراء عملية التخمير للمادة الأولية لمدة **3** ايام باستخدام خميرة جافة من نوع **Saccharomyces cerevisiae** التجارية المستخدمة لتحويل السكريات الأحادية الى ايثانول حيوي. حيث أظهرت النتائج تغيراً ملحوظاً في انتاج الايثانول الحيوي باستخدام تراكيز مختلفة من الحامض ، والهدف من هذه الدراسة هو تحديد التركيز الأمثل للحامض المستخدم لانتاج اعلى مردود من الايثانول الحيوي، والذي بدوره يستخدم كمادة مضافة الى وقود الكازولين النقي بنسب حجمية مختلفة 0% ، 5% ، 7% و10%. وقد تم اجراء العديد من الاختبارات التجريبية لتقييم خصائص الوقود الناتج باستخدام معايير الجمعية الامريكية للاختبار والمواد **(ASTM)** وذلك لتحديد خصائصه الفيزيائية والكيميائية . حيث أظهرت النتائج زيادة قيمة رقم الاوكتان البحثي للوقود الناتج مع زيادة نسبة الايثانول المضاف.

الكلمات الدالة: الإيثانول الحيوي ، الكتلة الحيوية الليكنوسليلوزية ، الوقود الحيوي ، سيقان القطن ، عملية التخمير.

Abstract
The objective of this study is to investigate the feasibility of producing bioethanol from cotton stalk waste, a lignocellulosic feedstock characterized by its abundance and low cost. The study involved preliminary chemical pretreatment of cotton stalk powder using sulfuric acid at a solid-to-liquid ratio

of 1:6 and concentrations of 1.5%, 1.0%, and 0.5%, in order to break down the complex lignocellulosic structure and partially remove lignin and hemicellulose. This was followed by fermentation of the pretreated material for 3days using commercial dry yeast (*Saccharomyces cerevisiae*) to convert monosaccharides into bioethanol. The results showed significant variation in bioethanol production depending on the acid concentration used. The objective of this study is therefore to determine the optimal acid concentration that yields the highest bioethanol production.
The produced bioethanol was then used as an additive to pure gasoline at different volume fractions (0%, 5%, 7%, and 10%). Numerous experimental tests were conducted to evaluate the properties of the resulting fuel in accordance with American Society for Testing and Materials (ASTM) standards, in order to determine its physical and chemical properties. The results showed an increase in the research octane number of the fuel blend as the percentage of ethanol increased.

Keywords: Bioethanol , Biofuel , cotton stalk , fermentation process , Lignocellulosic biomass.

Introduction

In recent years, climate change has become a major environmental threat due to rising temperatures and increasing levels of greenhouse gases, which contribute to global warming. These challenges are intensifying as a result of human activities that overexploit natural resources and rely heavily on fossil fuels (Venezia et al., 2021). Therefore, it has become necessary to seek alternative energy sources to replace fossil fuels. One important alternative is biomass, a widely available and carbon-neutral resource. Energy production from biomass results in lower greenhouse gas emissions compared to fossil fuels (Stöcker, 2008). Some of the most important potential biomass sources for energy production include wood and its byproducts, agricultural and forestry residues, food and animal waste, municipal solid waste, and energy crops. These resources are considered sustainable alternatives to fossil fuels due to their abundance and their ability to be converted into various forms of bioenergy through physical, chemical, and biological processes (Ibitoye et al., 2023). Currently, cotton stalks and other agricultural residues are often burned in the field, leading to the loss of valuable resources, environmental pollution, and health problems for farmers. Therefore, it is essential to explore methods for increasing the value of biomass and reducing environmental pollution. One potential approach is to utilize cotton stalks as a renewable energy source. These stalks can be converted into bioethanol through processing methods that meet biofuel production requirements (Wang & Tuohedi, 2020). Recent statistics indicate that the global production of cotton is 24.2×10^6 tons, cultivated across 31.92×10^6 hectares in 80 countries. Annual sales are estimated at $5.68 billion (Vitale et al., 2025). Bioethanol is a biofuel derived from plant biomass and is produced through the fermentation of simple sugars into alcohol. It can be used in modified vehicles (E85 technology), originally developed in Brazil, either as a blend with fossil fuels or in pure form (Bertrand & Dussap, 2022). Bioethanol is the most widely produced biofuel, with an estimated global production of 30 billion gallons in 2023. The United States and Brazil are the leading producers worldwide (Ghazali & Mustafa, 2025). Due to its high octane rating, ethanol allows for higher compression ratios in engines, improving combustion efficiency. Its chemical structure contains a hydroxyl group, which enhances combustion, making it faster and more complete. However, ethanol has a lower calorific value compared to gasoline (Vanzela et al., 2017). The objective of this study is to convert cotton stalk waste into an inexpensive source of bioethanol through fermentation. The produced ethanol was then purified via distillation to obtain high-purity ethanol. Subsequently, it was blended with gasoline in different proportions to improve the combustion quality of fuel used in internal combustion engines. Finally, the ethanol–gasoline blends were analyzed using various techniques to evaluate the effect of ethanol on fuel performance.

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2. Methodology

2.1 Chemicals and Reagents: The chemicals and reagents were supplied by Sigma-Aldrich and Fisher Ltd. and used without further purification. These included phosphoric acid (85%), sulfuric acid (H_2SO_4, 98%), urea reagent, sodium potassium tartrate, 3,5-dinitrosalicylic acid, and D-glucose. Yeast (Saccharomyces cerevisiae E419) and 3A molecular sieves were obtained from local markets.

2.2 Instrument used. UV-Vis spectrometer (Model T92+), Digital pH meter (Eutech instruments- PC 700), FTIR spectrometer (Bruker Alpha II-ATR, Germany), and NMR spectroscopy (Bruker, 500 MHz) were employed to investigate the obtained results in this current research

2.3Experimental section:

2.3.1 Sample preparation.

Cotton stalks were collected as raw material from agricultural fields after the harvest season in the city of Kirkuk. The stalks were washed with water to remove any remaining dirt and then left under sunlight for several days to dry. They were subsequently ground into a powder using an electric blender to facilitate the subsequent processing steps.

The resulting powder was then dried in an electric oven at 100°C for 5 hours to completely remove moisture and determine its moisture content prior to fermentation, in order to calculate the actual weight of the raw material required for the anaerobic fermentation process. Finally, the dried cotton stalk powder was stored in airtight plastic bags for subsequent acid treatment (Baig & DHArMADHikAri, 2014).

2.3.2 Chemical pre-treatment of the feedstock.

A preliminary acid treatment was performed on cotton stalk powder using sulfuric acid at various concentrations (0.5%, 1%, and 1.5%). Each sample was then subjected to heat treatment at temperatures ranging from 120 to 130 degrees Celsius for 4 hours. After completing the acid pretreatment of each sample, the resulting solid residue was filtered and continuously washed with distilled water until the pH reached a value between 4 and 5. This step was intended to remove yeast inhibitors that formed during the acid treatment of the raw material. These inhibitory compounds include furfural derivatives and acetic acid, which must be eliminated to ensure that the fermentation medium is suitable for yeast activity.

2.3.3. Fermentation.

The fermentation process and bioethanol production were carried out according to the modified method described by Saleh & Al-Azzawi (2023), in which Saccharomyces cerevisiae yeast was activated by mixing 200 g of white sugar and 7 g of urea reagent in a 500 mL beaker containing 400 mL of distilled water. The mixture was placed on an electric hot plate at a temperature ranging from 35–40 °C under continuous stirring for 15 minutes. Afterwards, 10% dry yeast (20 g, relative to the weight of the dry starting material used) was added. Heating and stirring were continued for an additional 15 minutes to ensure the growth and multiplication of yeast cells until bubbles appeared, indicating yeast activation. This was followed by the addition of the activated yeast to the fermentation system, consisting of a 3-liter conical glass flask that had been evacuated of air and tightly sealed, containing acid-treated cotton stems and samples 1–3, each processed separately in individual fermentation runs. The mixture was then diluted with distilled water to a final volume of 2.5 liters. Anaerobic fermentation was carried out under controlled conditions at 32 °C and pH 4–5 in the absence of oxygen, with continuous stirring at 100–150 rpm. The fermentation process lasted for 3 days and was accompanied by the release of carbon dioxide bubbles from the fermentation mixture, indicating the conversion of released sugars into bioethanol.

2.3.4. Purification of bioethanol.

After the anaerobic fermentation process is completed, the resulting ethanol is purified and concentrated using the method developed by Chen et al. (2014). Simple and fractional distillation techniques were employed, respectively, to obtain bioethanol with a purity of 94%. This was followed by drying the resulting bioethanol and further increasing its concentration using 3Å molecular sieves. The obtained alcohol was placed in direct contact with the molecular sieves for two days, with occasional stirring. Finally, the bioethanol was filtered to obtain ethanol with a purity of 99%.

It is worth noting that the concentration of the resulting bioethanol was measured using a hydrometer, while the composition and purity of the obtained bioethanol were verified using FTIR and NMR techniques. The percentage of ethanol obtained was calculated using the equation below (Alsaayigh & Al-Azzawi, 2025).

$$\%\ Bioethanol\ (wt/wt) = \frac{volume\ of\ bioethanol\ obtained\ (ml) \times density\ of\ ethanol\ (0.789\ gm/ml) \times 100}{weight\ of\ feedstock\ (gm)}$$

2.3.5. Preparation and characterization of bioethanol-gasoline blends.

Gasoline–ethanol fuel samples were prepared by blending pure ethanol with conventional gasoline at different blending ratios of 0%, 5%, 7%, and 10% (v/v). The purpose of this blending process was to determine the optimal volume ratio of ethanol added to conventional gasoline to improve its chemical and physical properties. Subsequently, a comprehensive analysis was conducted on each sample of the prepared binary mixture to determine its physical and chemical properties, including the Research Octane Number (RON), which is one of the key parameters for internal combustion engine fuels used to assess their resistance to knocking. Additionally, the Reid Vapor Pressure (RVP), which reflects fuel volatility, was measured. Consequently, it is used to determine gasoline engine operating conditions in both winter and summer. In addition, the density and water content of the prepared blends were measured, as shown in Table 1.

Physicochemical Properties	Measurement Method	Reference
Research Octane Number (RON)	ASTM D2699	)Kolodziej & Wallner, 2017)
Raid vapor pressure (RVP)	ASTM D6378	)Mozaffari et al., 2020)
Density	ASTM D4052	)Andrade et al., 1995)
Water content	ASTM D6304	)Lin, 2023)

Tables 1: Standards for Measuring Physical and Chemical Properties.

3. Results and discussion

Bioethanol was obtained through the anaerobic fermentation of cotton stalks pretreated with sulfuric acid at concentrations of 0.5%, 1%, and 1.5% for samples 1–3, using a fixed ratio of 1:6 (acid solution: cotton stalks) (volume/weight). The results shown in Tables 2 were obtained.

Samples	Acid concentration (%)	Acid/Solid ratio (ml/g)	Ph value	Fermentation period (days)	Bioethanol yield (%0)
Sample1	0.5	6:1	4.3^{b}-4.5^{a}	3	27.4
Sample2	1.0	6:1	4.4^{b}-4.6^{a}	3	18.0
Sample3	1.5	6:1	4.3^{b}-4.6^{a}	3	28.9

Tables 2 : Data obtained from acid pretreatment of cotton salks and fermentation parameters

a) represnted the value of Ph after fermentation, b) represnted the value of Ph before fermentation.

After completing the acid pretreatment step for Samples 1 through 3, anaerobic fermentation of these samples was conducted using *S. cerevisiae* yeast under controlled conditions, including a temperature of 32 °C, a pH range of 4–5, and an incubation period of three days. The results showed that the bioethanol yield from cotton stalk residues treated with 1.5% sulfuric acid (Sample 3) was the highest compared to the other treated samples, reaching 28.9%. This was attributed to the release of a higher amount of glucose as a result of the acid pretreatment. Sample 1 ranked second, with a yield of 27.4%, while Sample 2 recorded the lowest yield at 18.0%, which was attributed to the formation of inhibitory compounds such as furfural, hydroxymethylfurfural (HMF), organic acids (e.g., acetic acid), and phenolic compounds resulting from lignin degradation. These inhibitors negatively affect yeast growth, thereby reducing fermentation efficiency and decreasing bioethanol yield. Figure 1 illustrates the percentage of ethanol obtained following acid pretreatment.

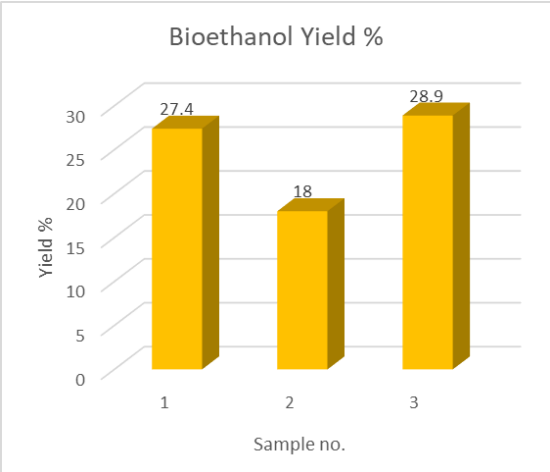

Figure 1: Bioethanol yield from cotton stalks samples using different acid pre-treatment conditions.

Bioethanol production is influenced by many factors, including the concentration of the raw material, fermentation duration, pH, and temperature (Baig & Dharmadhikari, 2014). Temperature plays a key role in the fermentation process; high temperatures inhibit or kill yeast cells, while low temperatures slow down biochemical reactions (Jain & Kumar, 2024). Other studies also indicate that the concentration of acid used in the pre-acidification process directly affects ethanol yield. While increasing the concentration improves the efficiency of acid hydrolysis of the raw material, it also leads to the formation of inhibitory compounds that negatively affect the fermentation process (Porninta et al., 2024). According to a study conducted by Sritrakul et al. (2017), pre-acidification using sulfuric acid at concentrations ranging from 1–5% resulted in bioethanol production levels of 21–25 g/L. The study also clarified that increasing acid concentration does not necessarily lead to increased ethanol yield. This is due to the formation of inhibitory compounds such as furfural and hydroxymethylfurfural, which is consistent with the results of our study.

After distilling and drying the bioethanol produced from fermentation, an FTIR spectroscopic analysis was conducted to verify the composition and purity of the extracted alcohol. The spectral results were consistent across the three samples under study, as shown in Figures 2(A, B, and C). Typical absorption bands characteristic of the extracted ethanol were observed. A distinct, broad absorption band (stretching) appeared at a wavelength ranging between 3310–3325 cm^{-1} , and the presence of this band is attributed to the hydroxyl group (-OH) in the bioethanol sample. The broadening of this band is attributed to the hydrogen bonding between bioethanol molecules. As for the sharp absorption bands at wavelengths between 2881 cm^{-1} and 2973 cm^{-1}, they are attributed to the methyl group of the carbon–hydrogen (C–H) bond, which is consistent with the findings of the study conducted by (Manzoor et al., 2020). The results also revealed two sharp absorption bands (C-O) at wavelengths between 1045 cm^{-1} and 1087 cm^{-1}, both of which are attributed to the methylene group ($–CH_2$) in ethanol, corresponding to the C-O bond. A sharp absorption band was also observed at a wavelength of 1379 cm^{-1}, and the appearance of this band is attributed to the characteristic C-O-H bond in alcohols. The infrared spectroscopy results for the ethanol produced in our study are in good agreement with previous studies (Hamden et al., 2022; Rabiu et al., 2021; Saleh & Al-azzawi, 2023). These studies have shown that the spectral absorption bands of ethanol in the 2900 cm^{-1} and 3300 cm^{-1} regions are attributable to CH and OH groups, respectively. Absorption bands were also observed in the spectral region between 1045 cm^{-1} and 1379 cm^{-1}, which are mainly attributed to the stretching bonds of the methylene ($–CH_2$) functional group linked to the hydroxyl (OH) group.

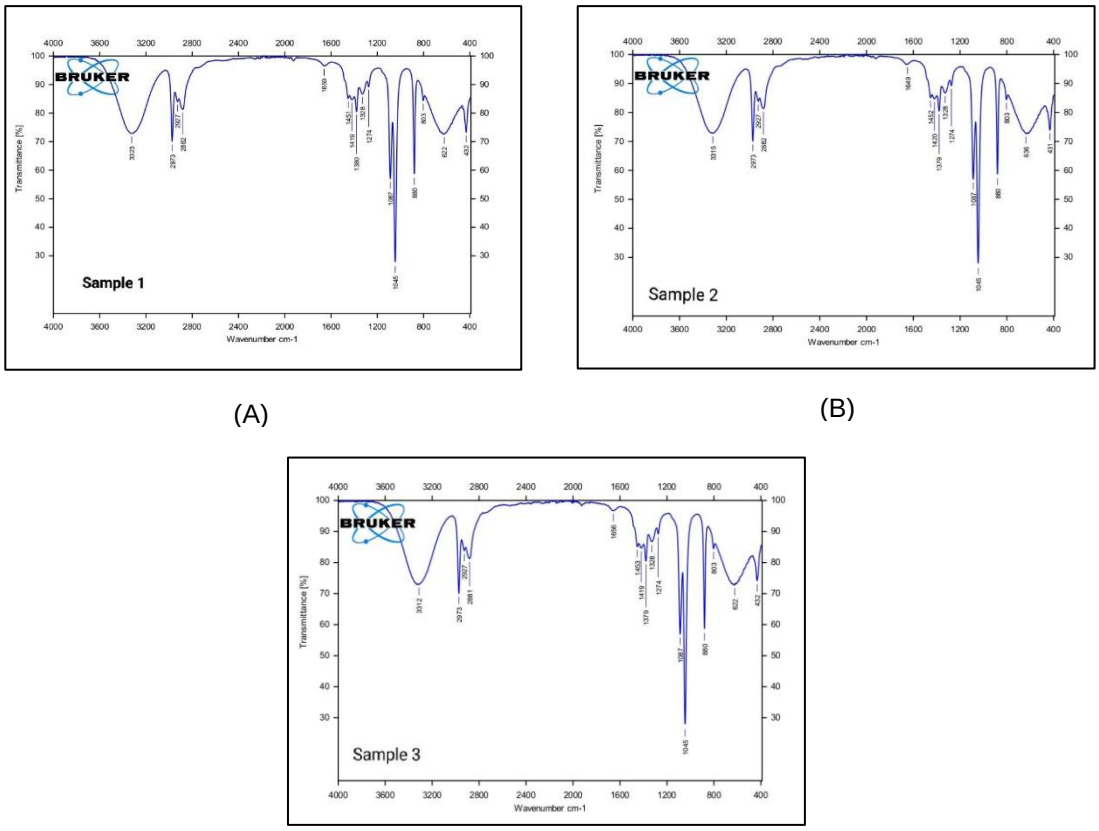

Figure 2: FTIR images of bioethanol produced from sample 1 (A), sample 2 (B), and sample 3 (C) with pre-treatment by H_2SO_4 at different concentrations.

The FTIR results obtained are consistent with those from proton and carbon NMR spectroscopy. HNMR spectroscopy revealed three absorption bands at different chemical shifts, as shown in Figure 3A . The first absorption band appeared as a triplet at 0.9 ppm, which is attributed to the methyl group protons (-CH_3) in the ethanol molecule. The second absorption band appears as a multiple signal at 3.3 ppm, which is attributed to the protons of the methylene group (–CH_2) in the ethanol molecule. The third absorption band, which appears as a broad singlet, is attributed to the proton of the hydroxyl group (OH), which is isolated and appears at 4.6 ppm. As for the C-NMR technique, it revealed the presence of two main absorption peaks, as shown in Figure 3B. The first absorption peak appeared at a chemical shift of 17 ppm and is attributed to the carbon atom in the methyl group (–CH_3). The second band appeared at a chemical shift of 57 ppm and is attributed to the methylene group (–CH_2) in the ethanol produced, respectively. A small band signal was also observed at 77 ppm, as shown in the figure below, which is attributed to the carbon atom in the solvent used in NMR spectroscopy, namely chloroform-deuterium ($CDCl_3$). The findings of this study are fully consistent with those of other studies (Alsaayigh & Al-Azzawi, 2025; Hamden et al., 2022; Saleh & Al-azzawi, 2023; Tabah et al., 2016)

(A) (B)

Figure 3: The ^{1}H and ^{13}C NMR Spectrum of the pure bio-ethanol obtained from cotton stalks.

Our study also involved analyzing the prepared gasohol blends and selecting the most suitable ratio for use as an alternative fuel in internal combustion engines. Gasoline–alcohol blends were prepared at different ratios (0%, 5%, 7%, and 10%). The properties of the prepared blends were examined using standard ASTM methods to determine their physical and chemical characteristics, including density, Reid vapor pressure (RVP), research octane number (RON), and water content. The results of these measurements are presented in Table 4.

Properties	**ASTM standard test**	**Bioethanol ratio in the fuel**			
		E0 (0%)	E5 (5%)	E7 (7%)	E10 (10%)
Density (g/cm^3) at 15.5C^o	ASTM-D4052	0.7220	0.7290	0.7344	0.7371
RVP (Kpa at 37C^o)	ASTM-D5191	70.0	66.2	64.9	63.1
RON	ASTM-D2699	82.0	85.0	87.0	89.0
Water content (ppm)	ASTM-D6304	35	1340	1710	2110
Color	Yellow	Yellow	Yellow	Yellow	Yellow

Figure 3. Physiochemical characteristics of pure gasoline and bioethanol-gasoline blends.

The table above shows that the density values of the prepared blends increase gradually as the proportion of ethanol increases, compared to pure gasoline. The density values of the blends (E0, E5, E7, and E10) ranged between 0.7220 g/cm³ and 0.7371 g/cm³, as shown in Figure A5. This trend is commonly observed because the density of ethanol is higher than that of pure gasoline (Alsaayigh & Al-Azzawi, 2025). The results of our study are consistent with those of Calvin et al. (2022), who confirmed that the density of gasohol blends increases as the proportion of bioethanol increases. Although bioethanol has a much lower vapor pressure than pure gasoline, its addition to fuel alters the Reid vapor pressure (RVP). A slight gradual decrease in vapor pressure was observed as the ethanol percentage increased, as shown in Figure B5. This behavior is attributed to the presence of water in the gasoline–ethanol blends, resulting from the hygroscopic nature of ethanol, which affects the volatility of the fuel (Manzoor et al., 2020). Regarding Research Octane Number (RON), the values of the gasohol samples ranged from 82 to 89, with a gradual increase observed as the ethanol content increased, as shown in Figure C5. An overall increase of 7 octane units was recorded, which is attributed to the high octane rating of bioethanol (RON ≈ 105). These results are consistent with previous studies investigating the effect of gasohol blends on the octane number of gasoline (Alsaayigh & Al-Azzawi, 2025; Wibowo et al., 2018). As for water content (Figure D4), it was observed that the water content in the fuel mixture increases with increasing ethanol concentration, which negatively affects combustion quality and may reduce the effective octane performance of the blend. Excess water can also cause phase separation, resulting in the formation of two distinct phases: an ethanol–water phase and a hydrocarbon-rich gasoline phase. This separation deteriorates combustion performance, may cause corrosion in fuel system components, and can ultimately lead to engine malfunction or damage.

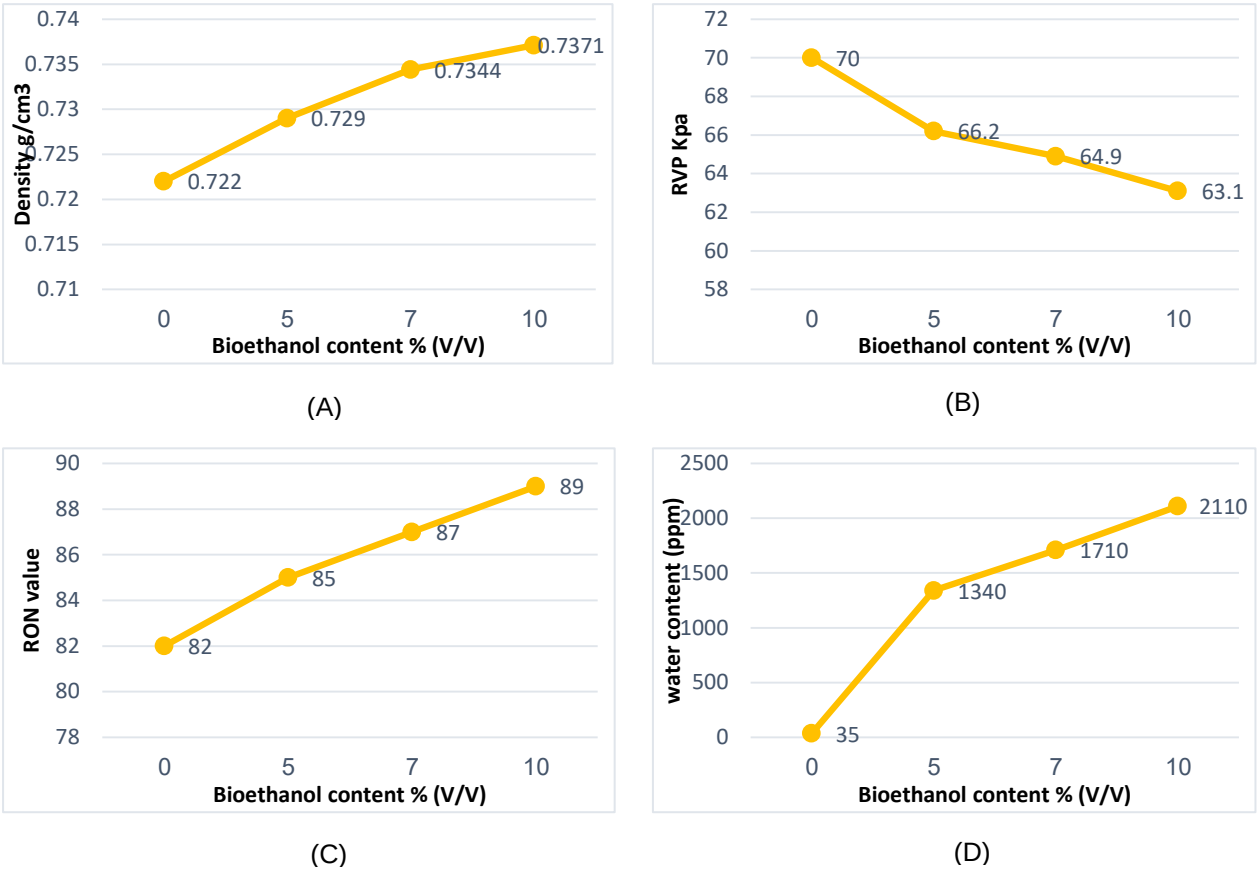

Figure 4. Diagrams showing the density (A), RVP (B), RON, and water solubility of the mixed fuel (D) as a function of different ratios of bioethanol in the fuel mixture.

4. Conclusions.

This study examines the use of cotton stalks as a widely available lignocellulosic waste material that can serve as a feedstock for bioethanol production via anaerobic fermentation using *S. cerevisiae* yeast. This was achieved after subjecting the stalk powder to acid pretreatment using sulfuric acid at different concentrations (1.5%, 1.0%, and 0.5%) with an acid-to-feedstock ratio of 6:1 (v/w) for three hours. The acid pretreatment resulted in an increased release of glucose, which is subsequently converted into ethanol and carbon dioxide during the fermentation process. This was followed by distillation and the use of molecular sieves to separate and dehydrate the produced ethanol, thereby obtaining high-purity ethanol. The obtained ethanol was then blended in various ratios (0%, 5%, 7%, and 10%) with pure gasoline and used as an alternative fuel in compression-ignition engines without any modifications. The use of alternative fuels reduces the growing demand for fossil fuels and, consequently, reduces harmful emissions from vehicle exhausts. According to the results of this study, the addition of ethanol to gasoline led to an increase in the Research Octane Number (RON), with slight changes in Reid vapor pressure (RVP) and density compared to conventional gasoline.

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