مجلة جامعة بني وليد للعلوم الإنسانية والتطبيقية
Bani Waleed University Journal of Humanities and Applied Sciences
تصدر عن– جامعة بني وليد – ليبيا
Website:https://jhas-bwu.com/index.php/bwjhas/index

ISSN3005-3900 | الصفحات (234- 248) | المجلد العاشر ـ العدد الرابع ـ 2025

Recycling waste paper through the fermentation process for bioethanol Production as a renewable gasoline additive

Noorulhuda G. Al-Abdallah [1]*, Ahmed G. S. Al-Azzawi [2]
[1.2] Chemistry department, Education College for Pure Science, University of Mosul, Mosul, Iraq.
nooralhuda.23esp68@student.uomosul.edu.iq

إعادة تدوير نفايات الورق من خلال عملية التخمير لإنتاج الإيثانول الحيوي كمضاف متجدد للبنزين

نورالهدى العبد الله [1]*، أحمد العزاوي [2]
[2.1] قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق

Received: 07-07-2025	**Accepted: 05-08-2025**	**Published: 22-10-2025**

الملخص:

في هذا العمل، استُخدمت منتجات مخلفات الورق لإنتاج الإيثانول الحيوي باستخدام المعالجة الفيزيائية والكيميائية. اذ خضعت هذه النفايات الصلبة للمعالجة بحامض الكبريتيك المُخفف بتراكيز ونسب مُختلفة لاجل تحرير كمية كبيرة من السكريات الأحادية، والتي خُمِّرت لاحقًا إلى إيثانول حيوي بواسطة خميرة S. cerevisiae. اذ تم الحصول على أفضل إنتاج عند معاملة الورق المسبقة بتركيز حامضي 1.5% بنسبة 1 غم/10 مل (ورق صلب الى محلول حامضي)، حيث بلغت نسبة الإنتاج 32%. و لزيادة نقاء الإيثانول الحيوي، استُخدمت تقنيات التقطير والمناخل الجزيئية كعوامل تجفيف، مما أدى إلى زيادة تركيز الإيثانول الحيوي بنسبة 98%. تم تحليل وتشخيص الإيثانول الحيوي المُنتَج باستخدام مطيافية FTIR وNMR. بالإضافة إلى ذلك، اجري خُلط الإيثانول الحيوي مع الكازولين الاعتيادي بنسب مُختلفة لإنتاج مخاليط E0 وE6 وE8 وE10 وE12. اذ استُخدمت طريقة ASTM لتقييم هذه المخاليط، بما في ذلك RVP وRON والكثافة ومحتوى الماء. حيث لوحظ انخفاض قيم RVP، بينما ازدادت الكثافة مع زيادة محتوى الإيثانول في الخليط. ومع ذلك، ازداد محتوى الماء وRON في مخاليط الإيثانول الحيوي والكازولين تدريجيًا مع إضافة الإيثانول الحيوي. ويتمثل الجانب المهم في هذه الدراسة في إنتاج الإيثانول الحيوي باستخدام مواد خام متوفرة ورخيصة، وفي ظل ظروف مثالية.

الكلمات الدالة: الايثانول الحيوي، التخمير، نفايات الورق، المعالجة المسبقة والوقود الحيوي.

Abstract

In the work, discharged paper products were utilized to produce bioethanol using physicochemical treatment. This solid waste was subjected to the treatment with dilute sulfuric acid at varying concentrations and ratios to release a substantial amount of monosaccharides, which were subsequently fermented into bioethanol by S. cerevisiae. The best yield was obtained at a concentration of 1.5% acid at a ratio 1 gm/10 mL (solid to acid solution) that yielded 32%. To increase the purity of the bioethanol, distillation and molecular sieve as a drying agent were utilized, resulting in a bioethanol concentration of 98%. The produced bioethanol was characterized using FTIR and NMR spectroscopy. In addition, the bioethanol was blended with regular gasoline at different ratios to produce E0, E6, E8, E10 and E12 mixtures. The ASTM method was used to evaluate these mixtures, including RVP, RON, density and water content. It was observed that RVP values decreased while the density increased with increasing ethanol content in the mixture. However, the water content and RON of bioethanol-gasoline blends increased gradually with the addition of ethanol. The important aspect of this study is the production of bioethanol using an available and cheap feedstock using optimal conditions.

Keywords: Bioethanol, Fermentation, waste paper, Pretreatment, and Biofuel.

Introduction:

The increasing global demand for permanent energy sources in association with the environmental burden of fossil fuel dependence has created enough interest in producing biofuels from renewable resources (Subramaniam & Masron, 2021). Among various biofuels, bioethanol stands out due to its compatibility with the existing internal combustion engine, its relatively clean combustion profile and the ability to reduce greenhouse gas emissions (Mićić & Jotanović, 2015). Traditionally derived from food-based crops such as corn and sugarcane, bioethanol production has faced criticism due to the food-ban dilemma and its limited stability (Hattori & Morita, 2010). As a result, as the second-generation feedstocks for bioethanol production, especially the lignocellulosic biomass of waste materials, attention has been paid to the waste materials. Bioethanol production from waste paper has emerged as a promising strategy to address both environmental concerns associated with energy stability and paper waste accumulation (Wang, Sharifzadeh, Templer, & Murphy, 2012). The waste paper rich in cellulose acts as an excellent feedstock for bioethanol due to its abundance and low cost (Nishimura et al., 2016). Recent studies have focused on customizing pretreatment and hydrolysis processes to increase sugar yield for fermentation. For example, a study by Annamalai et al (Annamalai et al., 2020). It was demonstrated that in the hydrogen peroxide pretreatment at 121 ° C, the yield of the waste office has increased significantly from the paper and newspaper, leading to the ethanol concentration of 11.15 g/L and 6.65 g/L, respectively. Innovative approaches have also been explored to enhance process efficiency and stability. Wang et al (Wang, Templer, & Murphy, 2012). investigated the environmental stability of bioethanol production from various waste papers, highlighting that the enzyme production and process are significant contributors to the environmental heat impact. In addition, integrating bioethanol production in existing pulp and paper mills has been proposed to utilize the existing infrastructure, which has reduced capital investment and promotes a circular economy (Branco, Serafim, & Xavier, 2018). Integration of mechanical and acid pretreatment techniques is important in increasing the efficiency of bioethanol production from waste paper. (Beluhan, Mihajlovski, Šantek, & Ivančić Šantek, 2023). Dilute sulfuric acid is usually employed for hydrolysis and disrupting lignin structures, which releases fermentable sugars. A previous study by Thakare (Thakare & Trivedi, 2021), showed that treating waste paper with 5% sulfuric acid at 121 °C for 30 minutes effectively liberates monosaccharides suitable for fermentation. Similarly, a study by Alsaayigh and Al-Azzawi (Alsaayigh & Al-Azzawi, 2025) employed dilute sulfuric acid pretreatment on office waste paper to release fermentable sugars, followed by fermentation and distillation processes, achieving significant ethanol yields. Another research (Tadmourt, Khiari, Boulal, & Tarabet, 2024) optimized the acid hydrolysis conditions to maximize sugar yield from waste paper, highlighting the important parameters in bioethanol production. The co-operative application of mechanical and acid pretreatment increases the access of cellulose fibers, which improves enzymatic hydrolysis and high ethanol yields. This combined approach plays a vital role in changing waste paper, an abundant and renewable resource in bioethanol, which contributes to permanent energy solutions and waste management strategies. Saccharomyces cerevisiae is one of the most widely used microorganisms for converting feedstock into bioethanol from various wastes under different conditions (Park & Baratti, 1991).

The efficiency of bioethanol production by S. Cerevisiae is remarkably affected by several environmental factors, including temperature, substrate concentration and pH (Ezea, 2023).

These parameters play a vital role in regulating yeast metabolism and the fermentation process. Temperature is an important factor affecting yeast growth and enzymatic activity. The optimal ethanol production is usually between 30 ° C and 35 ° C. The temperature below or above this range can prevent cell viability and slow fermentation due to the requirement for specific enzymes or stress-induced metabolic changes. The substrate concentration affects the concentration of fermented sugars and bioethanol yield. While high sugar concentrations can an increase in theoretical ethanol production, it can also result in substrate inhibition, low fermentation efficiency, and delayed yeast growth. Therefore, balancing the level of sugar is necessary to maintain optimal osmotic conditions and avoid inhibited fermentation. PH levels similarly affect yeast activity. S. The optimal pH for S. cerevisiae fermentation ranges from 4.0 to 5.0. The deviations from this range can affect enzyme stability, eventually reducing ethanol productivity. Finally, maintaining optimal environmental conditions is important to maximise ethanol yield and ensure efficient fermentation performance by yeast (Gashaw, 2014). It is worth mentioning that bioethanol is one of the most common types of biofuels obtained from lignocellulosic biomass such as paper, cardboard and corn. Furthermore, it is used as an alternative to gasoline fuel or blended with gasoline in a concentration of 10% bioethanol to 90% gasoline (E10) to improve the quality of combustion and produce lower emissions of carbon monoxide. (Edeh, 2021) There are several advantages to using bioethanol as an alternative fuel, including being less toxic, derived from renewable feedstock, and reducing greenhouse gas emissions (Vohra, Manwar, Manmode, Padgilwar, & Patil, 2014).

In summary, the acid pretreatment process is a critical stage in the bioconversion pathway of waste paper to bioethanol, with ongoing research focusing on optimizing conditions to maximize sugar yields while minimizing the production of fermentation inhibitors. Furthermore, this study aims to produce biofuel as an additive to gasoline fuel from an available and cheap raw material as well as improving the quality of gasoline fuel combustion.

2. Methodology

2.1 Chemicals and reagents.

Reagents and chemicals were supplied by Fisher Ltd and Sigma- Aldrich, and then utilized as received without any further purification such as Sulfuric acid (H_2SO_4 98%), Sodium potassium tartrate, D (+) glucose, Urea reagent, 3, 5-dinitor salicylic acid. While the yeast (S. Cerevisiae -E 419) and 3 A° molecular sieve were available in the domestic markets.

2.2 Instrument used.

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

2.3 Experimental section:

2.3.1. Sample preparation.

Waste paper was locally collected in Mosul city and used as a raw material in this current study to produce bioethanol. The sample was cut into small pieces using scissor. Afterward, waste paper was taken and dried in the dryer at 90°C for 24 hours to remove the humidity from the waste After moisture content determination, the dried raw material was stored in plastic bags and placed in a dry place until the next stage (Awol & Abate, 2021) the dried feedstock (waste paper) was crushed using an electric mixer with water to obtain sludge and thick feedstock, after that the sludge feedstock was dried to afford dried material. The physical pre-treatment aims to reduce the crystalline structure of cellulose and maximize the exposed area, which improves the contact between the cellulose and hemicellulose with dilute acid (Gashaw, 2014).

2.3.2. Chemical pre-treatment of the feedstock.

The lignocellulosic biomass can be converted to simple sugar before fermentation and this stage called hydrolysis (Madson & Lococo, 2000), the acid pretreatment step was reported according to modified established literature by Awol (Awol & Abate, 2021) Waste paper was treated with sulfuric acid at various concentrations of acid (1%, 1.5%, 2%, 2.5%) respectively. The mixture was refluxed for 4 hours, after that the resulting mixture was allowed to cool down to normal temperature, filtered off, and then wash the sample with distilled water until a pH value of 4-5 was attained. This is followed by collecting and drying the sample to be ready for the fermentation process.

2.3.3. Fermentation.

Bioethanol production was executed according to the modified procedure by Saleh (SALEH & Ahmed, 2023). Yeast (Saccharomyces cerevisiae 10%) was activated in 200 mL of sugar solution with stirring. 2% of urea was added to the resulting yeast inoculum and stirred for 20 minutes at 40 °C for activation. The treated sample is placed in a 4 liter conical flask, and 3 liters of distilled water are poured into the sample. Followed by the addition of the yeast activating solution. The process was carried out in the absence of oxygen (anaerobic fermentation) at 32°C and kept for 3 days until maximum released sugars were converted into bioethanol. The pH of the fermented solution was adjusted to 4-5, and it was monitored using a pH meter. Sugar level was monitored using a spectrophotometer (Uv-Vis) at 540nm. It is worth mentioning that the fermentation process was carried out three times at each specific concentration, and the average bioethanol yield was determined to ensure accuracy and minimize the experimental errors.

2.3.4. Purification of bioethanol.

After fermentation completion, the bioethanol produced was purified from the mixture by the distillation techniques according to the procedure by Chen (Chen et al., 2014). The alcohol is separated using simple and fractional distillation at 78-80°C to obtain high-purity bioethanol 90-94%. The resulting alcohol concentration is measured by a hydrometer. In addition, the yield of bioethanol is dried using 3°A molecular sieve to obtain absolute bioethanol 98% also determined by the hydrometer tool. Bioethanol produced was analyzed and verified using FTIR analysis and ^{1}H, ^{13}C NMR spectroscopy. The percentage of bioethanol produced was calculated using the equation below (Alsaayigh & Al-Azzawi, 2025):

$$\left\{ \begin{aligned} &\% \, Bioethanol \\ &= \frac{volume\ of\ bioethanol\ obtained\ (ml) \times density\ of\ ethanol\ (0.789\ gm/ml)}{weight\ of\ feedstock\ (gm)} \times 100 \end{aligned} \right\}$$

2.3.5. Estimation of reducing sugar using the DNS method.

Reducing sugar values of fermented feedstock was estimated according to modified procedures as described by Garriga et al (Garriga, Almaraz, & Marchiaro, 2017). 0.5 of 3,5-Dinitrosalicylic Acid (DNS) reagent was dissolved in 10 mL of 2N sodium hydroxide and then stirred slowly and solution containing 15 gm of sodium potassiumtartaric acid ($KNaC_4H_4O_6.4H_2O$) and 25 mL of distilled water was added to the resultant mixture under stirring conditions; the resulting solution was heated up to 45 °C with stirring to obtain a pure solution and then cooled down to room temperature. Lastly, the distilled water was added to 50 mL to obtain a clear stock solution. The reagent solution was stored at a temperature below 10°C in the refrigerator. To establish the standard curve, glucose standard solutions with concentrations between 0.1 and 1 mg/mL were prepared using test tubes. 2 mL of distilled water and 1 mL of DNSA solution were added to each test tube, separately.

The solution in each tube was well mixed. All test tubes were heated at 90 °C for 10 minutes to allow colour development. Upon cooling, A volume of 5 mL distilled water was pipetted into each test tube. Afterwards,, absorbance was determined by a UV-Vis spectrometer at 540 nm.

2.3.6. Preparation and characterization of bioethanol-gasoline blends.

Blending of high-purity bioethanol with gasoline in different blended rates (0%, 6%, 8%, 10% and 12% (vol/vol) respectively, using a shaking machine at a fixed volume of 500 mL. Fuel characteristics of the tested base gasoline and bioethanol-gasoline blends were evaluated by American Society for Testing and Materials (ASTM) procedures. Comprehensive analyses were performed to document the fuel properties of the tested blends. Each binary mixture sample was measured to estimate the physico-chemical properties, including, density, RVP, water content and Research Octane Number RON.

Reid Vapour Pressure (RVP) test is an important tool for describing the starting conditions of a gasoline engine and vapor lock. It was estimated by the test method adopted by ASTM-D6378 (Mozaffari, Baird, Listak, & Oja, 2020) using a digital RVP analyzer (Eralytics Eravap). The density of each fuel sample was measured using a Rudolph Research Analytical density meter, according to ASTM D4052 (Andrade, Prada, & Muniategui, 1995). The density of the fuel sample at 15.5 °C was estimated by injecting it into a density analyzer. The ASTM method D6304 was used to describe the procedure used to measure the water content of fuel (M.-J. Lin, 2023). The moisture content was evaluated using a digital coulometric titrator (koehler AKF5000). RON is considered one of the main parameters to estimate the knocking characteristics of gasoline fuel. It is an important measurement for internal combustion engines at low speeds that reach 600 rpm (normal conditions). In the current study, RON of both gasoline and binary mixture fuel samples were measured following ASTM-D2699, using a CFR engine (Kolodziej & Wallner, 2017).

3. Results and discussion

Bioethanol was produced from paper waste after physical and chemical treatments, which were conducted on the feedstock to reduce the crystallinity domain of cellulose and increase the surface area to facilitate the hydrolysis process (Beluhan et al., 2023). The acid pre-treatment process was employed at various concentrations of sulfuric acid (1%, 1.5%, 2%, 2.5%) at a constant ratio (1:10 waste paper: acid solution wt./vol.) as shown in Table 1.

Table 1. Data obtained from acid pretreatment of office paper waste and fermentation parameters.

Sample	Acid concentration (H_2SO_4)	Solid waste to acid ratio(wt/vol.)	pH value	Fermentation period (days)	Bioethanol yield (%)
Sample 1	1.0%	1:10	4.2^b-4.3^a	3	30.5
Sample 2	1.5%	1:10	4.3^b-4.5^a	3	32.0
Sample 3	2.0%	1:10	4.5^b-4.6^a	3	23.6
Sample 4	2.5%	1:10	4.8^b-4.9^a	3	21.0

a: it refers to the pH level after a fermentation, **b**: the pH level before the fermentation.

It can be observed that the highest bioethanol yield from acid pretreated waste at 1.5% (Sample 2) was found to be 32.0% compared to other pretreated samples. The results suggested that the bioethanol productivity depended mainly on the concentration and ratio of acid used with the pretreated waste. In addition, the purity and yield of bioethanol were also influenced by fermentation conditions and purification methods. It can be noted that sample 2 showed the highest bioethanol yield. It was hypothesized that high concentrations of acid during the pretreatment process may generate more inhibitors such as furfural, Hydroxymethylfurfural (HMF), that affect severely the outcome of the process (Volynets, Ein-Mozaffari, & Dahman, 2017).

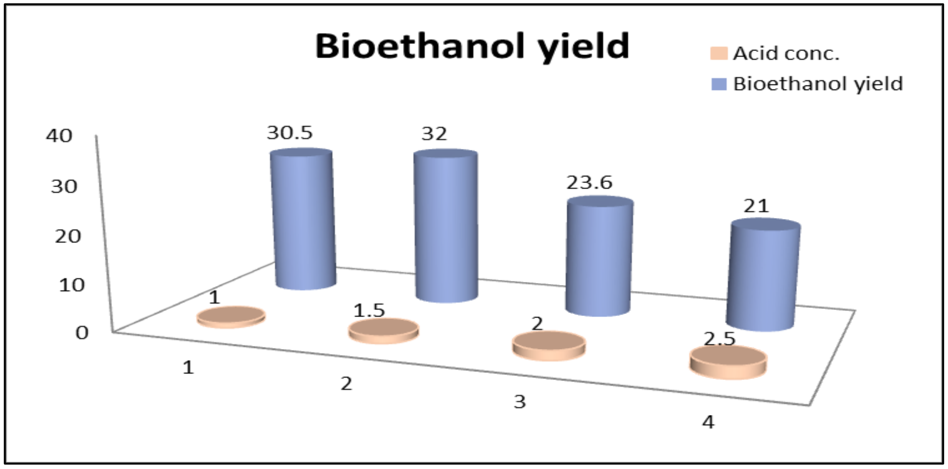


Figure 1. Bioethanol yield from waste paper samples using different acid pre-treatment conditions.

In fermentation, many researchers revealed that temperature plays a crucial role in anaerobic metabolism. Bioethanol yield improves as the temperature rises, attaining an optimal level at 35 °C; below this temperature, the fermentation rate is slow, while above 45 °C, the yeast used would denature (Y. Lin et al., 2012; Salihu, Usman, Abubakar, & Mansir, 2022; Torija, Rozes, Poblet, Guillamón, & Mas, 2003). The productivity of bioethanol can be theoretically performed using *S. cerevisiae* with a percentage yield that reaches 51% (Ruchala, Kurylenko, Dmytruk, & Sibirny, 2020). The current work showed a considerable enhancement in the bioethanol yield compared to previous studies (Annamalai et al., 2020; Dubey, Gupta, Garg, & Naithani, 2012; Obara, Okai, Ishida, & Urano, 2015). This can be attributed to improving the fermentation performance of yeast by a reduction of fermentation inhibitors, leading to improvements in yeast fermentation efficiency. In terms of the S. cerevisiae activity aspect, it can survive in acidic media that range from 4 to 6 (Janani, Ketzi, Megavathi, Vinothkumar, & Ramesh Babu, 2013). Previous studies indicated that the optimum pH range for maximum bioethanol productivity is between 4.5 and 5.0 (Y. Lin et al., 2012; C.-L. Wong, Yen, Lin, & Chang, 2014; Y. Wong & Sanggari, 2014). The glucose stock solution was prepared and used to generate a standard curve for estimating the reducing sugar content in each sample before and after fermentation. The sugar concentrations in both the glucose stock solution and the paper waste samples were determined by measuring their absorbance at 540 nm and comparing the values to the standard curve. Results from the standard graph showed a significant increase in reducing sugar levels in the treated samples after fermentation processes. The results of glucose concentration following acid pre-treatment of office paper waste are tabulated in Table 2. Among the samples, sample 1 exhibited the highest sugar concentration at 31.05 mg/mL, compared to 30.08 mg/mL in sample 2, 26.2 mg/mL in sample 3, and 21.1 mg/mL in sample 4. An increase in the acid concentration during the pre-treatment step led to a lower release of free sugars, which were subsequently converted to bioethanol through the fermentation process. The fermentation process of pre-treated samples was carried on under optimal conditions at 32 °C for three days using S. cerevisiae.

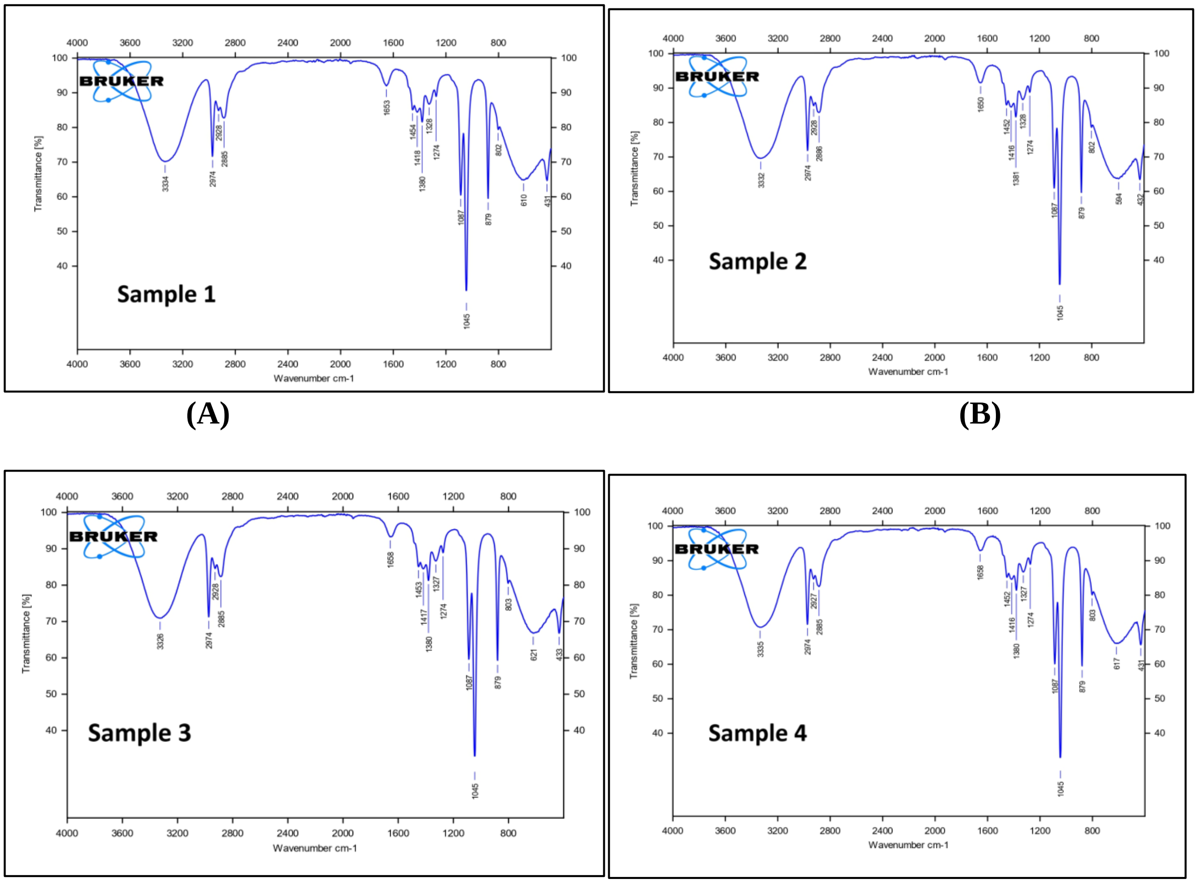
After 72 hours, the remaining sugar concentrations in the fermentation broth were 3.64 mg/mL for sample 1, 4.2 mg/mL for sample 2, 2.35 mg/mL for Sample 3, and 3.8 mg/mL for Sample 4. Overall, it was observed that there was a significant decrease in sugar levels after yeast fermentation, indicating substantial sugar consumption by sugars by the yeast (Ajit et al., 2018). Overall, the bioethanol yields correspond to the initial sugar concentrations.

Table 2. Data of released sugar level in treated paper waste before and after the fermentation process.

Fermented sample (mL)	Distilled water (mL)	DNS (mL)	Distilled water (mL)	Absorbance at 540 nm	Dilution (D) (mL)	Sugar content (mg/mL)
Sample 1^{b}(1.0)	1.0	1.0	5.0	0.910	27	31.05
Sample 1^{a}(1.0)				0.334	7	3.64
Sample 2^{b}(1.0)				0.409	28	16.5
Sample 2^{a}(1.0)				0.614	5	4.2
Sample 3^{b}(1.0)				0.606	32	26.2
Sample 3^{a}(1.0)				0.290	5	2.35
Sample 4^{b}(1.0)				0.740	22	21.1
Sample 4^{a}(1.0)				0.60	5	3.8

(b): it refers to glucose level before fermentation, (a): it refers to glucose level after fermentation, and (D): dilution factor.

The purity of the bioethanol from different samples was verified through FTIR analysis after undergoing distillation and dehydration. It can be observed that FTIR spectra exhibited similar trends as illustrated in Figure 4 A, B, C and D. The results obtained for all analysed samples (Samples 1, 2, 3, and 4) displayed the O-H, C-H, C-C, and C-O stretch absorption bands of bioethanol in the FTIR spectrum. As expected, the identified broad peak appeared around (3332-3336 cm^{-1}), which corresponds to the hydroxyl group (O-H) stretching vibration of bioethanol. Therefore, the presence of the functional group in alcohol was confirmed. The presence of distinctive absorption peaks between 2974 cm^{-1} and 2885 cm^{-1} was indicative of the presence of C-H stretching vibration. Moreover, the sharp band at a wavenumber of approximately 1380 cm^{-1} was also noted, which was associated with the C-OH band in bioethanol. These absorbance peaks 1087 and 1045 cm^{-1} were observed in the FTIR spectrum, which correspond to the C-O stretch. The FTIR results of the current study were similar to those of previous studies (Hamden, El-Ghoul, Alminderej, Saleh, & Majdoub, 2022; SALEH & Ahmed, 2023), which confirmed that the peaks that appeared in the region of 2,900 and 3,300 cm^{-1} were attributed to CH and OH molecule groups, respectively. Absorbance signals between 1045 cm^{-1} and 1380 cm^{-1} were observed, which are due to stretching bands of the -CH$_2$ functional group.

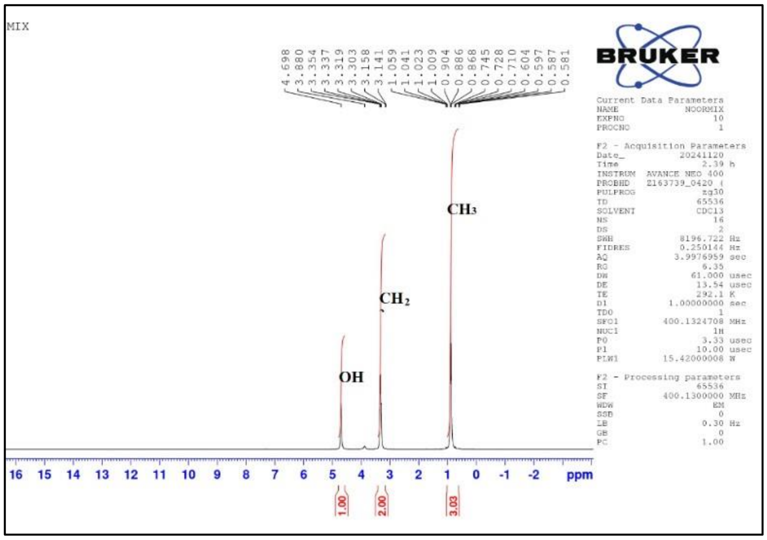


(A) (B)

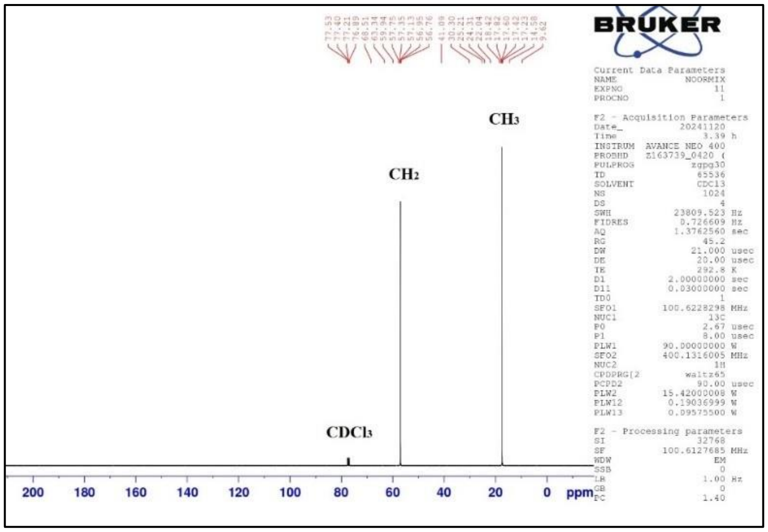
(C) (D)

Figure 2. FTIR images of bioethanol produced from sample 1 (A), sample 2 (B), and sample 3 (C), and sample 4 (D) with pre-treatment by H_2SO_4 at different concentrations.

These findings have been well aligned with the results obtained from ¹ NMR spectroscopy. The ^{1}H NMR spectrum (Figure 3a) of bioethanol displayed a triple peak at approximately 0.9 ppm, corresponding to the methyl proton (-CH_3), and a quartet peak around the 3.3 ppm, reflects the methylene proton (-CH_2-) adjacent to the hydroxyl group, Additionally, the broad singlet peak appearing around 4.6 ppm confirms the presence of exchangeable hydroxyl proton (-OH), supporting the identification of bioethanol. The results obtained agreed with previous research (Hamden et al., 2022; SALEH & Ahmed, 2023) that confirmed the chemical structure of bioethanol using the ^{1}H NMR technique. Furthermore, the ^{13}C NMR spectrum (Figure 3b) revealed two prominent peaks at 17 ppm and 57 ppm, corresponding to the methyl and methylene carbon atoms, respectively. Moreover, it can be noted that a signal was observed at 77 ppm, this corresponded to carbon in $CDCl_3$ solvent. The data obtained coincides with other existing results (Tabah, Pulidindi, Chitturi, Arava, & Gedanken, 2016).Combined spectroscopic data from FTIR and NMR decisively validate the formation of bioethanol, which demonstrates the effectiveness of the fermentation process in transforming the substrate into a structurally pure alcohol.



(a)



(b)

Figure 3. (a)^{1}H NMR spectra, (b) ^{13}C NMR spectra of the bioethanol produced from paper waste in deuterated chloroform ($CDCl_3$) at 400 MHz.

This work also investigated the performance of bioethanol-gasoline blends with different proportions of bioethanol, with the goal of examining their potential as alternative fuel options for internal combustion engines (ICE). The data obtained from the ASTM analysis methods, including density, RVP, RON, and water content, were tabulated in Table 3. It can be noted that density values of pure gasoline (E0) and bioethanol-gasoline blended fuel samples (E6, E8, E10, and E12) varied from 0.7260 g/cm^3 to 0.7381 g/cm^3, as represented in the density curve (Figure 6a). The densities of E6, E8, E10, and E12 were slightly higher than the untreated gasoline sample (E0) by 0.90%, 1.1%, 1.3%, and 1.6%, respectively. The observed increase in density with higher bioethanol volumes can be explained by the inherently greater density of bioethanol in comparison to pure gasoline (Kareem, Ahmed, & Mustafa, 2023). The results supported the findings of a previous research by Bassiouny et al. (El-Bassiouny, Aboul-Fotouh, & Abdellatief, 2015), the research exhibited that the bioethanol addition in gasoline of E5, E10, E15, and E20 would increase the density compared to base gasoline E0. However, Fotouh and his group (Aboul Fotouh, Mazen, &

Ashour, 2017) revealed that the volume percentage of bioethanol was 10% or less, and the density of the bioethanol-gasoline fuel sample became lighter.

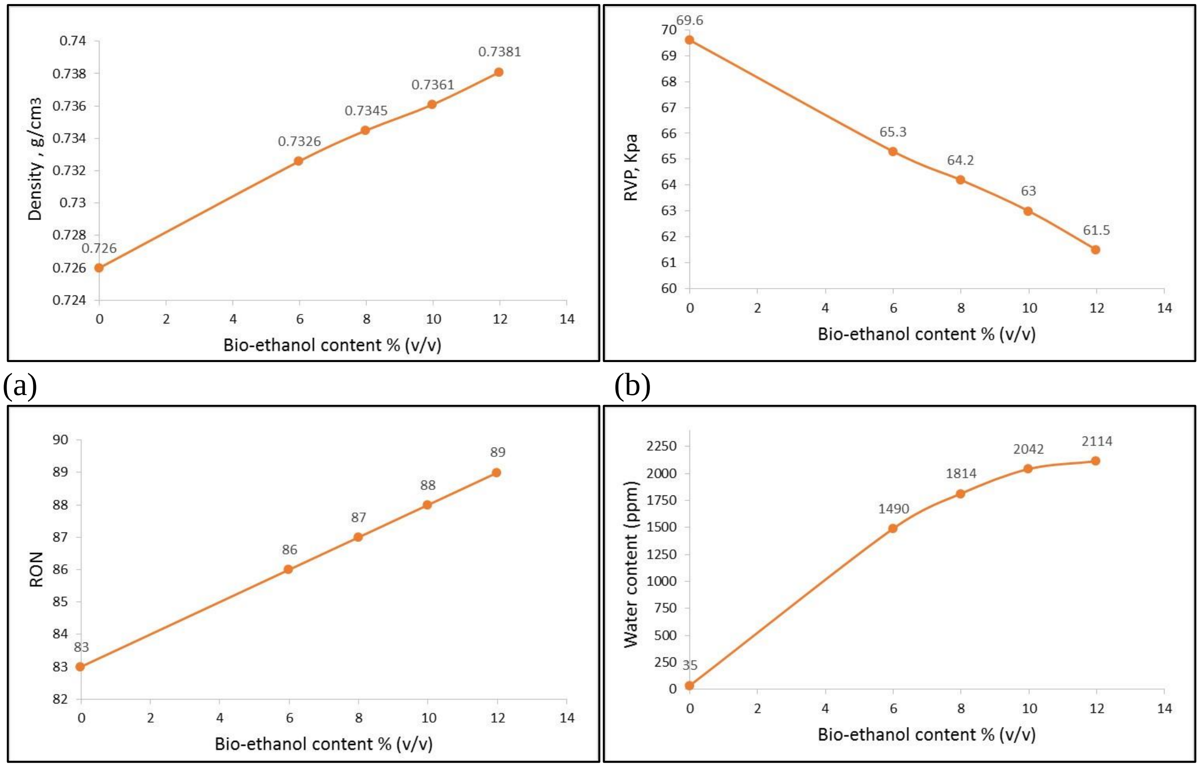
Table 3. Specifications of base gasoline and its blends with bioethanol at different ratios.

Characteristics	**Test method (ASTM)**	**Bioethanol ratio in the fuel**				
		E0(0%)	E6 (6%)	E8 (8%)	E10 (10%)	E12 (12%)
Density (g/cm^3 at 15.5 °C)	*ASTM-D4052*	0.7260	0.7326	0.7345	0.7361	0.7381
RVP (Kpa at 37 °C)	*ASTM-D5191*	69.6	65.3	64.2	63.0	61.5
RON	*ASTM-D2699*	83.0	86.0	87.0	88.0	89.0
Water content (ppm)		35	1490	1814	2042	2114
Color	**yellow**	**yellow**	**yellow**	**yellow**	**yellow**	**yellow**

The behavior of binary mixture fuel was significantly different from pure gasoline, although the RVP of bioethanol was much lower than that of normal gasoline. The RVP gradually decreased with the addition of bioethanol, as presented in Figure 6b. A decrease in the RVP of the fuel blends was linked to trace water content in the binary mixture, which rises with blend volume and may impact fuel volatility (water is more difficult to evaporate compared to bioethanol and gasoline) (Murachman, Pranantyo, & Putra, 2014a). On the other hand, the decrease in the RVP of fuel resulted in poor performance of ICE engines, especially in the winter season, as well as more emissions that are susceptible to evaporation. In contrast, Sharma and Bharj (Sharma & Bharj) revealed that RVP values increased when the percentage of ethanol was 5% and 10% v/v. This can be linked to the formation of azeotropes between ethanol and the standard hydrocarbon components of gasoline. Due to Azeotrope formation, ethanol-gasoline blends exhibit a positive deviation from ideal behavior, leading to higher vapor pressure in the binary mixture compared to pure gasoline for ethanol concentrations up to around 10% vol./vol.

The octane rating of bioethanol-gasoline blends varies from 83 to 89, with marked variations linked to the amount of ethanol incorporated into the base gasoline. Figure 6c represents the variation of RON with the percentage of bioethanol. The RON increased from 83 at E0 (100% gasoline) to 86 at E6. It was observed that the RON values for E6, E8, E10 and E12 were higher than the traditional gasoline by 3, 4, 5 and 6 points of RON value, respectively. As the bioethanol content increased, the RON value showed a steady increase, aligning with the high RON of absolute bioethanol at 108 (Sindhu et al., 2019). In comparison, the results obtained are in good agreement with a previous study that investigated the influence of feedstock blends on the value of gasoline's octane number (Wibowo, Sugiarto, Zikra, Budi, & Mulya, 2018). Eventually, bioethanol addition plays a vital role in improving spark ignition fuel in car engines if it is added within a limited range (Aboul Fotouh et al., 2017).

Figure 6d displayed that the water absorptive capacity of binary mixtures increased significantly with the increase of bioethanol content. It can be noticed that pure gasoline already holds up to 35 ppm, while the water solubility of E6 is approximately 41 times higher compared with base gasoline. Moreover, the water content of E8 increased by 21% compared to E6, but E12 showed the highest water solubility of 2114 ppm relative to other samples. Additives such as bioethanol can increase the absorption of water in a gasoline blend. It is probably due to the polar character of bioethanol and its ability to form hydrogen bonds with water. Ethanol is a strongly hydrophilic compound and therefore absorbs water easily in the form of air humidity during the storage process. It is important to highlight that bioethanol-gasoline blends' resistance toward moisture is considerably influenced by bioethanol content and blending temperature (Murachman, Pranantyo, & Putra, 2014b). The results from the current study coincided with the previous study by Muzikova et al.(Mužíková, Pospíšil, Černý, Šebor, & Zadražil, 2008).



(a) (b)

(c) (d)

Figure 4. Graphs showing density (a), RVP (b), RON (c), and water solubility of blended fuels (d) *vs* different bioethanol percentages of fuel blends.

High water content in bioethanol fuel can dramatically affect the performance and durability of the internal combustion engine ICE. When the water content exceeds the standard range, it causes a reduction in the heating value of the fuel, resulting in a decrease in the engine thermal efficiency and the fuel consumption to maintain power generation. Furthermore, water interferes with the combustion process by changing the air-fuel ratio, which may result in incomplete combustion, engine misfiring, and high emissions of carbon monoxide and unburned hydrocarbons. In ethanol-gasoline mixtures, excessive water phase can induce separation, producing a non-uniform fuel mixture that can cause knocking, stalling or engine damage. In addition, the presence of water accelerates the rust in the components of the fuel system, including fuel lines, injectors and combustion chambers, and rubber seals and gaskets may be degraded, eventually reducing engine lifespan. Therefore, controlling water content in ethanol fuel is necessary to ensure optimal engine performance, fuel stability and compliance with emission standards.

CONCLUSION

According to results from the current work, the acid hydrolysis of the paper waste released mono and di sugars that can be converted to bioethanol via the fermentation process. The acid hydrolysis with biomass waste at a ratio of 10:1 vol./wt. (sample 1) yielded more glucose molecules compared to other samples. ,Afterwards, the free glucose from the waste was subjected to a fermentation process using yeast at pH=4.5 and 32 °C for 72 hours. Upon completion, bioethanol was produced into a fermented solution. The use of simple distillation, followed by fractional distillation and dehydration resulted in high bioethanol yield and purity. Based on the results obtained, it can be concluded that waste paper is a suitable feedstock for bioethanol production. From the economic perspective, paper wastes are very abundant and low-cost lignocellulosic feedstocks with high cellulose and low lignin contents, which makes these materials a very attractive substrate for bioethanol production. Bioethanol was used as an additive to gasoline as bioethanol-gasoline blends at different ratios of bioethanol (0, 6, 8, 10, and 12%). Binary fuel blends containing up to 12 vol.% bioethanol can be used as alternative fuels in variable-speed spark ignition engines without requiring any modifications. The analytical and experimental performance of fuel blends was determined to evaluate the influence of using alternative fuel instead of pure gasoline. It was also observed that blending gasoline with bioethanol increased density, RON, and water content. Gasoline with a bioethanol content of 12% (vol./vol.) can boost the RON value by 6 points compared to base gasoline. The binary mixture fuel blends can be utilized by the car engine smoothly without any engine modification.

Conflicts of interest

No conflicts of interest are declared by the authors.

ACKNOWLEDGEMENT

We acknowledge Mosul University for funding support of this work. We are also most grateful to the north oil refinery in Baiji –Iraq for their cooperation.

References

Aboul Fotouh, T., Mazen, O., & Ashour, I. (2017). An experimental study on the influence of ethanol and automotive gasoline blends. *J Pet Environ Biotechnol, 7*(318), 2.

Ajit, E., Dominic, D., Farook, F., Promod, A., Kumar, B., Blesy, V., Chandran, P. (2018). Preparation of wine from fruits of Musa accuminata and Ananas comosus; its physico-chemical analyses and sensory evaluation. *Integrative Food, Nutrition and Metabolism, 5*(6), 1-5.

Alsaayigh, M., & Al-Azzawi, A. (2025). USING WASTE PAPER AS A PROMISING FEEDSTOCK FOR BIO-ETHANOL FUEL PRODUCTION WITH CONSEQUENCE PURIFICATION USING DISTILLATION TECHNIQUE AND MOLECULAR SIEVE. *Chemical Problems/Kimya Problemləri, 23*(1).

Andrade, J., Prada, D., & Muniategui, S. (1995). Manual or automatic methods for gravity determinations in heavy petroleum distillates? Structured evaluation of automation. *Laboratory Automation & Information Management, 31*(2), 89-98.

Annamalai, N., Al Battashi, H., Anu, S. N., Al Azkawi, A., Al Bahry, S., & Sivakumar, N. (2020). Enhanced bioethanol production from waste paper through separate hydrolysis and fermentation. *Waste and Biomass Valorization, 11*(1), 121-131.

Awol, Z. S., & Abate, R. T. (2021). Production of bio-ethanol from solid waste paper using biological activation.

Beluhan, S., Mihajlovski, K., Šantek, B., & Ivančić Šantek, M. (2023). The production of bioethanol from lignocellulosic biomass: Pretreatment methods, fermentation, and downstream processing. *Energies, 16*(19), 7003.

Branco, R. H., Serafim, L. S., & Xavier, A. M. (2018). Second generation bioethanol production: on the use of pulp and paper industry wastes as feedstock. *Fermentation, 5*(1), 4.

Chen, W.-C., Sheng, C.-T., Liu, Y.-C., Chen, W.-J., Huang, W.-L., Chang, S.-H., & Chang, W.-C. (2014). Optimizing the efficiency of anhydrous ethanol purification via regenerable molecular sieve. *Applied energy, 135*, 483-489.

Dubey, A. K., Gupta, P., Garg, N., & Naithani, S. (2012). Bioethanol production from waste paper acid pretreated hydrolyzate with xylose fermenting Pichia stipitis. *Carbohydrate Polymers, 88*(3), 825-829.

Edeh, I. (2021). Bioethanol production: An overview. *Bioethanol technologies, 1*.

El-Bassiouny, A., Aboul-Fotouh, T. M., & Abdellatief, T. M. (2015). Maximize the production of environmental, clean and high octane number gasoline-ethanol blends by using refinery products. *Int. J. Sci. Eng. Res, 6*(7), 1792-1803.

Ezea, I. B. (2023). Technologies and factors affecting bioethanol fermentation and its commercialization.

Garriga, M., Almaraz, M., & Marchiaro, A. (2017). Actas de Ingeniería. *Actas de ingenieria, 3*, 173-179.

Gashaw, A. (2014). Bioethanol Production from Fruit Wastes and FactorsAffecting Its Fabrication. *Int J Chem Natur Sci, 2*(5), 132-140.

Hamden, Z., El-Ghoul, Y., Alminderej, F. M., Saleh, S. M., & Majdoub, H. (2022). High-quality bioethanol and vinegar production from Saudi Arabia dates: Characterization and evaluation of their value and antioxidant efficiency. *Antioxidants, 11*(6), 1155.

Hattori, T., & Morita, S. (2010). Energy crops for sustainable bioethanol production; which, where and how? *Plant Production Science, 13*(3), 221-234.

Janani, K., Ketzi, M., Megavathi, S., Vinothkumar, D., & Ramesh Babu, N. (2013). Comparative studies of ethanol production from different fruit wastes using saccharomyces cerevisiae. *International Journal of Innovative Research in Science, Journal Engineering and Technology, 2*(12), 7161-7167.

Kareem, A. M. S., Ahmed, Z. K., & Mustafa, S. J. (2023). Effect of Blending Aromatic and Oxygenates Additives with Fuels to Enhance Fuel Properties. *Passer Journal of Basic and Applied Sciences, 5*(1), 30-37.

Kolodziej, C. P., & Wallner, T. (2017). Combustion characteristics of various fuels during research octane number testing on an instrumented CFR F1/F2 engine. *Combustion Engines, 56*(4), 164-169.

Lin, M.-J. (2023). Research the Instrument of Karl Fischer Titration How to Detect the Moisture Content from Transformer Insulating Oil. *Journal of Engineering Research and Reports, 24*(8), 46-51.

Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S., & Kong, H. (2012). Factors affecting ethanol fermentation using Saccharomyces cerevisiae BY4742. *Biomass and bioenergy, 47*, 395-401.

Madson, P. W., & Lococo, D. B. (2000). Recovery of volatile products from dilute high-fouling process streams. *Applied biochemistry and biotechnology, 84*, 1049-1061.

Mićić, V., & Jotanović, M. (2015). Bioethanol as fuel for internal combustion engines. *Zastita materijala, 56*(4), 403-408.

Mozaffari, P., Baird, Z. S., Listak, M., & Oja, V. (2020). Vapor pressures of narrow gasoline fractions of oil from industrial retorting of Kukersite oil shale. *Oil Shale, 37*(4), 288-303.

Murachman, B., Pranantyo, D., & Putra, E. S. (2014a). Study of gasohol as alternative fuel for gasoline substitution: characteristics and performances. *International Journal of Renewable Energy Development, 3*(3), 175.

Murachman, B., Pranantyo, D., & Putra, E. S. (2014b). Study of gasohol as alternative fuel for gasoline substitution: characteristics and performances. *International Journal of Renewable Energy Development, 3*(3), 175-183.

Mužíková, Z., Pospíšil, M., Černý, J., Šebor, G., & Zadražil, I. (2008). Water tolerance of petrol-ethanol blends. *Goriva i maziva, 47*, 34-53.

Nishimura, H., Tan, L., Sun, Z.-Y., Tang, Y.-Q., Kida, K., & Morimura, S. (2016). Efficient production of ethanol from waste paper and the biochemical methane potential of stillage eluted from ethanol fermentation. *Waste Management, 48*, 644-651.

Obara, N., Okai, M., Ishida, M., & Urano, N. (2015). Bioethanol production from mixed biomass (waste of Undaria pinnatifida processing and paper shredding) by fermentation with marine-derived Saccharomyces cerevisiae. *Fisheries science, 81*, 771-776.

Park, S., & Baratti, J. (1991). Batch fermentation kinetics of sugar beet molasses by Zymomonas mobilis. *Biotechnology and bioengineering, 38*(3), 304-313.

Ruchala, J., Kurylenko, O. O., Dmytruk, K. V., & Sibirny, A. A. (2020). Construction of advanced producers of first-and second-generation ethanol in Saccharomyces cerevisiae and selected species of non-conventional yeasts (Scheffersomyces stipitis, Ogataea polymorpha). *Journal of industrial microbiology and biotechnology, 47*(1), 109-132.

SALEH, S., & Ahmed, A.-A. (2023). Optimizing Bioethanol Production for High Octane Bioethanol-Gasoline Blended Fuel through Fermentation. *Journal of the Turkish Chemical Society Section A: Chemistry, 10*(2), 475-486.

Salihu, U., Usman, U., Abubakar, A., & Mansir, G. (2022). Effect of pH and temperature on bioethanol production: Evidences from the fermentation of sugarcane molasses using Saccharomyces cerevisiae. *Dutse Journal of Pure and Applied Sciences, 8*(4b), 9-16.

Sharma, S., & Bharj, R. Parametric Evaluation of Spark Ignition Engine with Ethanol Blended Gasoline Fuel. *Carbon (wt.%), 86*, 52.52.

Shukla, A., Kumar, D., Girdhar, M., Kumar, A., Goyal, A., Malik, T., & Mohan, A. (2023). Strategies of pretreatment of feedstocks for optimized bioethanol production: distinct and integrated approaches. *Biotechnology for biofuels and bioproducts, 16*(1), 44.

Sindhu, R., Binod, P., Pandey, A., Ankaram, S., Duan, Y., & Awasthi, M. K. (2019). Biofuel production from biomass: toward sustainable development *Current developments in biotechnology and bioengineering* (pp. 79-92): Elsevier.

Subramaniam, Y., & Masron, T. A. (2021). The impact of economic globalization on biofuel in developing countries. *Energy Conversion and Management: X, 10*, 100064.

Tabah, B., Pulidindi, I. N., Chitturi, V. R., Arava, L. M. R., & Gedanken, A. (2016). Utilization of solar energy for continuous bioethanol production for energy applications. *RSC advances, 6*(29), 24203-24209.

Tadmourt, W., Khiari, K., Boulal, A., & Tarabet, L. (2024). Waste paper valorization for bioethanol production: Pretreatment and acid hydrolysis optimization. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 46*(1), 16145-16164.

Thakare, K., & Trivedi, M. (2021). Bioethanol Production from Waste Paper. *Int. J. Sci. Res. in Biological Sciences Vol, 8*(2).

Torija, M. J., Rozes, N., Poblet, M., Guillamón, J. M., & Mas, A. (2003). Effects of fermentation temperature on the strain population of Saccharomyces cerevisiae. *International journal of food microbiology, 80*(1), 47-53.

Vohra, M., Manwar, J., Manmode, R., Padgilwar, S., & Patil, S. (2014). Bioethanol production: Feedstock and current technologies. *Journal of Environmental Chemical Engineering*, *2*(1), 573-584.

Volynets, B., Ein-Mozaffari, F., & Dahman, Y. (2017). Biomass processing into ethanol: pretreatment, enzymatic hydrolysis, fermentation, rheology, and mixing. *Green Processing and Synthesis*, *6*(1), 1-22.

Abujnah, R. E., & Hadidan, M. E. (2024). Reducing Greenhouse Gas Emissions and Ensuring Safe and Economic Waste Disposal in Libya by Converting Household Organic Waste into Fertilizer and Biogas through Anaerobic Digestion. Bani Waleed University Journal of Humanities and Applied Sciences, 9(5), 573-578.

Wang, L., Sharifzadeh, M., Templer, R., & Murphy, R. J. (2012). Technology performance and economic feasibility of bioethanol production from various waste papers. *Energy & Environmental Science*, *5*(2), 5717-5730.

Wang, L., Templer, R., & Murphy, R. J. (2012). Environmental sustainability of bioethanol production from waste papers: sensitivity to the system boundary. *Energy & Environmental Science*, *5*(8), 8281-8293.

Wibowo, C. S., Sugiarto, B., Zikra, A., Budi, A., & Mulya, T. (2018). *The Effect of Gasoline-Bioethanol Blends to The Value of Fuel's Octane Number.* Paper presented at the E3S Web of Conferences.

Wong, C.-L., Yen, H.-W., Lin, C.-L., & Chang, J.-S. (2014). Effects of pH and fermentation strategies on 2, 3-butanediol production with an isolated Klebsiella sp. Zmd30 strain. *Bioresource technology*, *152*, 169-176.

Wong, Y., & Sanggari, V. (2014). Bioethanol production from sugarcane bagasse using fermentation process. *Oriental journal of chemistry*, *30*(2), 507-513.