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Municipal Solid Waste (Newspaper) — A Lignocellulosic Feedstock Conversion to Biofuel: A Prospective Solution to Energy and Waste Management Problem

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Abstract

Bioethanol, an alcohol-based fuel is produced from biowaste. The current research focuses on utilizing newspaper waste as a substrate for bioethanol production. The waste newspaper was pretreated and subjected to two important bioethanol production steps: (1) saccharification and (2) fermentation. *Aspergillus niger* utilized for the saccharification hydrolyzed the newspaper substrate by converting the complex compounds into simpler compounds, where lactose and chemically defined media enhanced the cellulolytic activity. The simpler sugars produced were fermented using the *Saccharomyces cerevisiae* and converted to bioethanol. The bioethanol samples were subject to different analyses, including dichromate oxidation tests, UV-Vis Spectroscopy, and High-Performance Liquid Chromatography (HPLC). The dichromate oxidation test confirmed the presence of bioethanol as the color of the samples changed from yellowish orange to green. The UV-Vis spectroscopy indicated the maximum bioethanol yield of 3.814% v/v (variant sample 1-lactose) and 5.049% v/v (variant sample 2-chemically defined media) on the 6th day of fermentation. HPLC further confirmed the bioethanol production as the peaks of bioethanol at retention times of 2.130 min (variant sample 1-lactose) and 2.112 min (variant sample 2-chemically defined media) matched the peak of standard ethanol at the retention time of 1.935 min. The present research concludes that newspaper waste is the potential substrate for bioethanol production.

Keywords: Bioethanol, Newspaper, Waste management, Lignocellulose

Introduction

The energy demand is continuously increasing with the ever-growing global population [1]. Energy plays an important role in improving the country's economy. It is also considered a major indicator of a country's social and economic development [2]. Fossil fuels – a non-renewable energy source dominates other energy sources to fulfill energy needs [3]. But with the depleting energy resources (non-renewable energy), countries are facing extra pressure to curb their energy needs.

The trend is therefore shifting towards utilizing renewable energy sources (alternative energy) for: (1) overcoming energy crisis, (2) promoting energy efficiency; and (3) reducing carbon emissions [4]. Among these energy sources, the prospect of using biofuels –the

energy-enriched compounds derived from the biomass or generated through the biological processes [5] as a substitute for conventional fossil fuels is high both in developed and developing countries [6].

Developed countries are: (1) extending their biofuel research and (2) expanding biofuel utilization in different energy sectors, especially the transport sector. Developed countries are biofuel leading producers: (1) USA in bioethanol (maize and sugarcane feedstocks) and (2) Germany in biodiesel (rapeseed feedstock) [7]. The potential of biofuel production is high in developing countries. Developing countries also seem interested in exploring biofuel potential in fulfilling energy needs [8, 9]. Brazil (a developing country) is also leading bioethanol production using the similar feedstocks as the USA [7].

Pakistan, like many other developing countries, is facing a severe energy crisis, a foundation of different socio-economic problems. Pakistan relied heavily on its depleting energy sources to fulfill its energy demands. But there resources were insufficient, and the country had to import the fuel (petrol and gas, etc.) [10]. Further, the ever-fluctuating petroleum prices have a marked impact on the country's energy budget. This whole situation demands the country's shift towards renewable energy sources [11], particularly biofuel. Despite the high potential for renewable energy sources [12], the country still has not managed to find the suitable feedstock and relevant methodology for biofuel production [10, 11].
Biofuels are grouped based on the feedstock used: (1) first generation biofuels are derived from edible feedstock (sugar cane and corn); (2) second-generation biofuels are produced from agricultural by-products or lignocellulosic material (grass, wood, and leaves); (3) third generation biofuels are obtained from aquatic feedstock (algae), and (4) fourth generation biofuels are made from bioengineered microorganisms (algae, fungi, and yeast). Second, third, and fourth-generation biofuels are believed to have a multitude of benefits over the first- generation biofuels [6].

Researchers aim at utilizing waste as a feedstock material for high-performance and eco-efficient biofuel production [13]. Therefore, second-generation biofuels are important as they can now be prepared from municipal solid waste [14, 15]. Solid waste derived biofuels do not negatively affect the food chain [16] and also provide a means for municipal waste management [17].

The lignocellulosic solid waste complex consists of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose (polysaccharides) are made primarily of monomeric sugars. While lignin is a complex of polyphenols (aromatic polymers) [18]. The monomeric sugars obtained from the fractionation of cellulose or hemicellulose used for the production of bioethanol (biofuel) through the microbial fermentation process [19].

Out of many options available in Pakistan; utilization of municipal waste feedstock newspaper [20], seems like a promising option for biofuel production. It will provide dual solution to energy and waste management problems. As solid waste management is also a key issue that needs immediate attention [21]. The country's annual solid waste production is approximately 0.283 to 0.612 kg/capita/day, which is continuously increasing at an annual rate of 2.4% [22].

Therefore, in the present study, an attempt has been made for the production of biofuel (bioethanol) from the solid newspaper waste.

Materials and Methods

Collection and Pretreatment of Newspaper

20 kg of newspaper waste was collected from households, offices, and universities. The newspapers were sun-dried to remove moisture and shredded using the electric grinder. The shreds were soaked in water for 24 h, filtered, and oven dried. For the de-inking, dried newspapers were soaked in 500 mL of 1% sodium hydroxide (NaOH) solution for 24 h, filtered, and oven dried [23].All chemicals and reagents used were of analytical grade

Preparation of Inoculant

Pure strains of *Aspergillus niger* obtained from the Mycology and Ecotoxicology Lab, Fatima Jinnah Women University, were cultured on agar plates using the loop and streak method. Plates were incubated in an incubator at 28 ºC for three days. A disc of *Aspergillus niger* was inoculated into the flask containing 125 mL of potato dextrose broth in duplet. The flasks were then incubated at 28.5 ºC for three days (72 h), and mycelial mats of fungus were obtained [24].

Saccharification

Cellulose in the newspaper acts as a growth medium for fungi. Fungi grow on cellulose and hydrolyze the raw materials. The variant solutions prepared in replicates for the optimized bioethanol production [25] are given in Table 1.

Table 1. **Variations in samples of newspaper.**

Variant solution no. 1	Variant solution no. 2
15 g of newspaper + 200 mL of distilled water + 0.25 g of lactose	15 g of newspaper + 200 mL of distilled water + 50 g of chemically defined media (CDM)

For the preparation of chemically defined media, materials and their quantities used are given in the Table 2.

Table 2. **Chemically defined media preparation.**

Chemicals	Quantity
Glycine	0.3 g
Ammonium nitrate	1.4 g
Potassium phosphate	2.0 g
Calcium chloride	0.3 g
Proteose peptone	7.5 g
Manganese sulfate	1.6 g
Zinc sulfate	1.4 g
Iron sulfate	5 g
Tween 20	3.3 mL

The solutions were autoclaved at 121 ºC for 15 min and cooled. Upon cooling, mycelial mats prepared earlier were added to the variant solutions. The solutions were agitated on an incubator shaker at 180 rpm for 6 days. The fungal mycelial mats were removed from the agitated solution under aseptic conditions [24]. After the completion of the saccharification process, the slurry of variant solutions was filtered using Whatman filter paper no. 42, and the solid residue was discarded [17].

Yeast Fermentation

The powder form of *Saccharomyces cerevisiae* was activated using potato dextrose broth. The 10% yeast media was prepared by adding 12.5 g of dry yeast in 125 mL of potato dextrose broth. 15 mL of yeast media was added to the filtered solutions and agitated on an incubator shaker at 28 ºC, at the shaking speed of 180 rpm for 6 days. The extraction of aliquots was done on the 4^{th}, 5^{th}, and 6^{th} days. The aliquots were filtered and centrifuged in the centrifugation machine at 4000 rpm for 10 min [24].

Purification and Distillation

It is the last step in bioethanol production. Distillation was performed using the Kjeldahl assembly; all impurities were removed, and purified samples were obtained. The temperature for this process was kept at 80-85 ºC [26].

Analysis of Bioethanol

The standard ethanol solutions at 2%, 4%, 6%, and 8% v/v concentrations were prepared by dissolving 2 mL, 4 mL, 6 mL, and 8 mL of pure ethanol, respectively, in 100 mL of distilled water. These solutions were used to plot a standard calibration curve for known ethanol concentrations. The standard solutions were then compared with purified newspaper samples (variant samples 1 and 2) to estimate the bioethanol presence and concentration. Various analytical techniques, including the dichromate oxidation method [27], UV-Vis spectroscopy, and HPLC [28], were employed to accurately assess the bioethanol content in the samples.

A flowchart explaining the procedure of the bioethanol production has been given in Fig. 1.

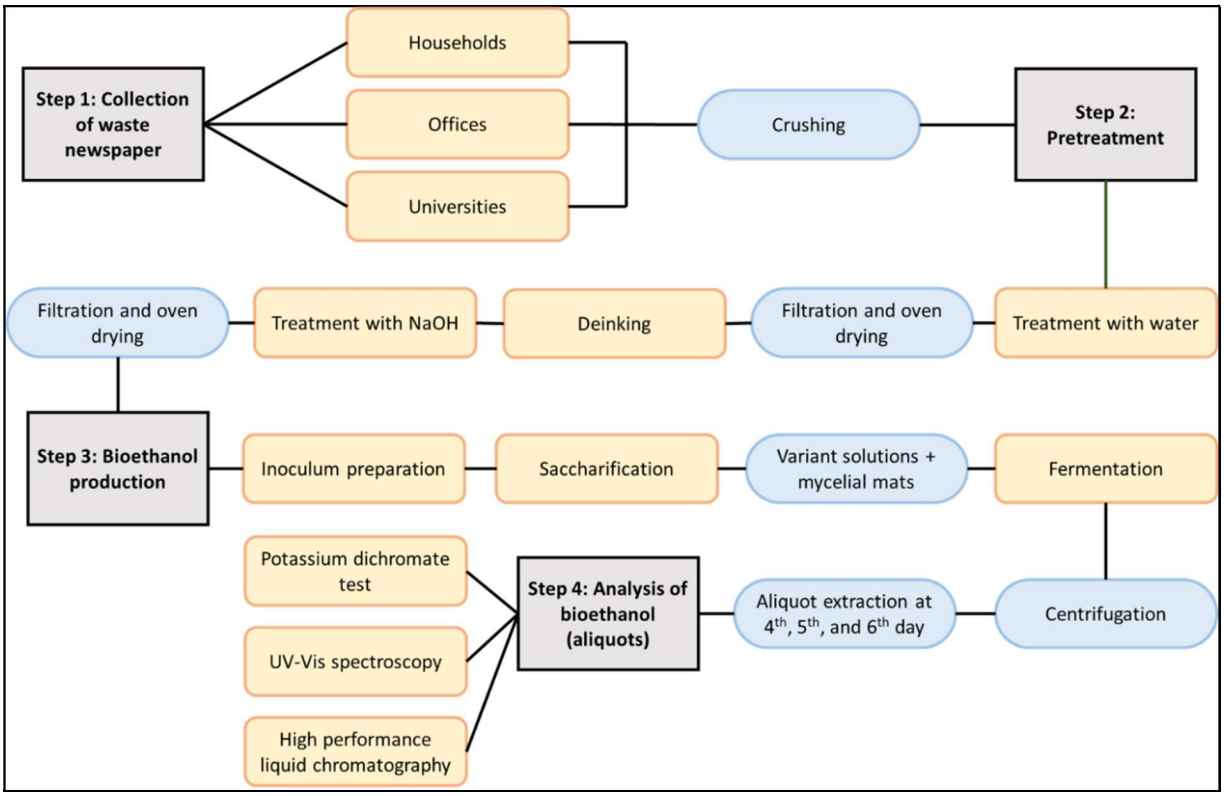

Figure 1. **Flowchart of the bioethanol production process**

Results and Discussions

The present study makes use of the cellulosic substrate a newspaper waste for bioethanol production through saccharification and fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae,* respectively. *Aspergillus niger* and was used in this study because it is more economical and a cheap alternative in comparison to other fungi used for the same purpose of saccharification. It also produces a high percentage of bioethanol.

Alabdalall et al. [29] indicated that *Aspergillus niger* shows high cellulosic activity and hydrolyzes the cellulose and other components present in the waste products into simpler compounds for bioethanol production. Further, *Saccharomyces cerevisiae* is a cheap and easily available fermenting agent used in the production of bioethanol from different waste substances. Jha et al. [25] and Prema et al. [30] effectively utilized *Saccharomyces cerevisiae* to convert the glucose formed from saccharification into bioethanol.

Dichromate Oxidation Analysis

The solvent dichromate acts as a reagent for the color change. The color changes from yellowish orange to green as dichromate oxidizes the ethanol in the solution [31]. The key indicator of ethanol presence in the samples is the green color. The change in the color of the standard samples is visible in Fig. 2. The color became darker with the increasing ethanol concentration in the standard samples. The ethanol concentration in the samples increased in the order 2% < 4% < 6% < 8%. The variant samples 1 and 2 extracted on the 4th day of fermentation upon oxidation showed a slight change in their color. On repeating the procedure, on the samples extracted on the 5th day of fermentation, it was observed that the intensity of the green color was higher in comparison to the previous samples. While examining the results of the 6th day fermented samples, it was noticed that the samples became much denser in color (green).

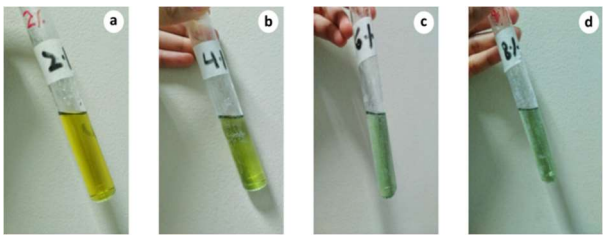

Figure 2. **Dichromate oxidation test indicating the color from yellow to green with the increasing the ethanol concentrations (a) 2%, (b) 4%, (c) 6%, and (d) 8% in the standard samples**

A study by Tulu et al. [32] conducted to produce bioethanol from Anchote *(Cocciniaabyssinica)* also indicated a change in color (yellowish orange to green) when treated in potassium dichromate solution. Cross-examination of the samples indicated that sample 1 containing chemically defined media had a darker green color in comparison to sample 2 containing lactose. These results are an indicator of the presence of bioethanol in variant samples. The increasing color intensity signifies that the concentration of bioethanol increased in the samples with the passage of time. The results of variant samples were further verified with the standard samples. It became evident that the color intensity of the samples increased with the increasing bioethanol concentration in the samples.

UV Spectroscopy Analysis

This analysis was performed to identify the bioethanol concentration present in the variant samples. The calibration curve obtained (standard and variant samples) at 320 nm absorbance wavelength indicated the precise ethanol concentration. The absorbance value at 320 nm increased (0.19, 0.24, 0.28, and 0.31) with increasing ethanol concentration in the standard samples (Fig. 3).

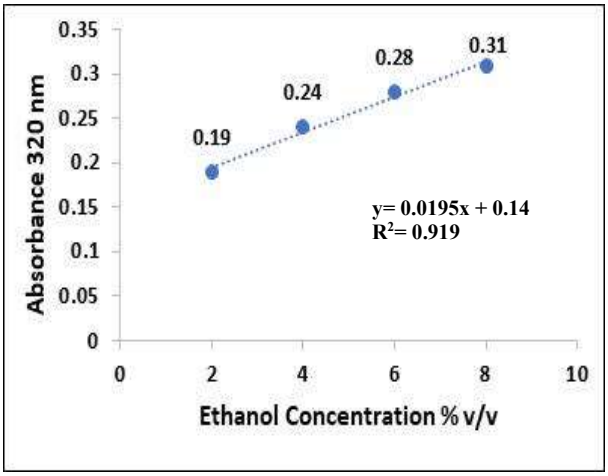

Figure 3. **Standard calibration graph of ethanol concentration**

Similarly, calibration curves were obtained for the variant samples on 4th, 5th, and 6th day. On the 4th day, the absorbance values of variant samples 1 and 2 were 0.193 and 0.230, respectively. The absorbance value for both variant samples on 5th day increased to 0.219 and 0.234. The absorbance value further increased (0.231 and 0.265) on 6th day (Table 3).

Table 3. **Absorbance value of variant samples on different fermentation days at 320 nm absorbance wavelength.**

Variant sample 1					
Fermentation	*Replicates*			*Mean*	*Standard Deviation*
	1	*2*	*3*		
4th day	0.192	0.195	0.193	0.193	±0.001
5th day	0.218	0.223	0.216	0.219	±0.003
6th day	0.230	0.232	0.232	0.231	±0.001
Variant sample 2					
Fermentation	*Replicates*			*Mean*	*Standard Deviation*
	1	*2*	*3*		
4th day	0.200	0.206	0.203	0.203	±0.003
5th day	0.232	0.235	0.236	0.234	±0.002
6th day	0.255	0.253	0.260	0.256	±0.003

The concentration of bioethanol in the variant samples at the corresponding absorbance values is given in Table 4. These results show an increasing trend in the bioethanol concentration from 4th day to 6th day. The increase in the concentration from 1.938% v/v to 3.814% v/v (variant sample 1) and 2.432% v/v to 5.049% v/v. The increase in the trend of the bioethanol yield from 1st day to 6th day has also been reported by Puttaswamy et al. [33]. The values also suggest that variant sample 1 containing lactose has a lower bioethanol concentration than the variant sample 2 containing chemically defined media. In a study by Ali and Khan [34], a high bioethanol yield of 4.0 g/100 g was obtained by the combination of groundnut hulls and chemically defined media compared to the combination of groundnut hulls and lactose that yielded 3.2 g/100 g of bioethanol. Lactose and chemically defined media provide nutrients to fugal species to work more efficiently (hydrolyze the raw material). It has been inferred that chemically defined media perform better than lactose.

Table 4. **Bioethanol concentration % (v/v) of samples on different days of fermentation.**

Day of fermentation	Variant sample	Absorbance value at 320 nm	Bioethanol concentration % (v/v)
4th day	1	0.193	1.938
	2	0.203	2.432
5th day	1	0.219	3.222
	2	0.234	3.962
6th day	1	0.231	3.814
	2	0.256	5.049

Bioethanol Estimation by HPLC

Ethanol peaks were detected with the mobile phase of methanol and water at 235 nm wavelength. The peak of the standard sample was obtained at the retention time of 1.935 min. Based on this information, the peaks of variant sample 1 and variant sample 2 were identified. The main peak of the variant sample 1 was obtained at the retention time of 2.130 min, and that of the variant sample 2 was obtained at the retention time of 2.112 min (same values Fig. 4).

The minor differences in the peaks formed in the chromatogram of samples appeared since different factors control sample retention. Bartzatt and Weidner [35] obtained the HPLC chromatogram of chloral hydrate and ethanol at the retention time of 2.1 min at 235 nm. This also proves that the compound detected and identified in the present study is bioethanol.

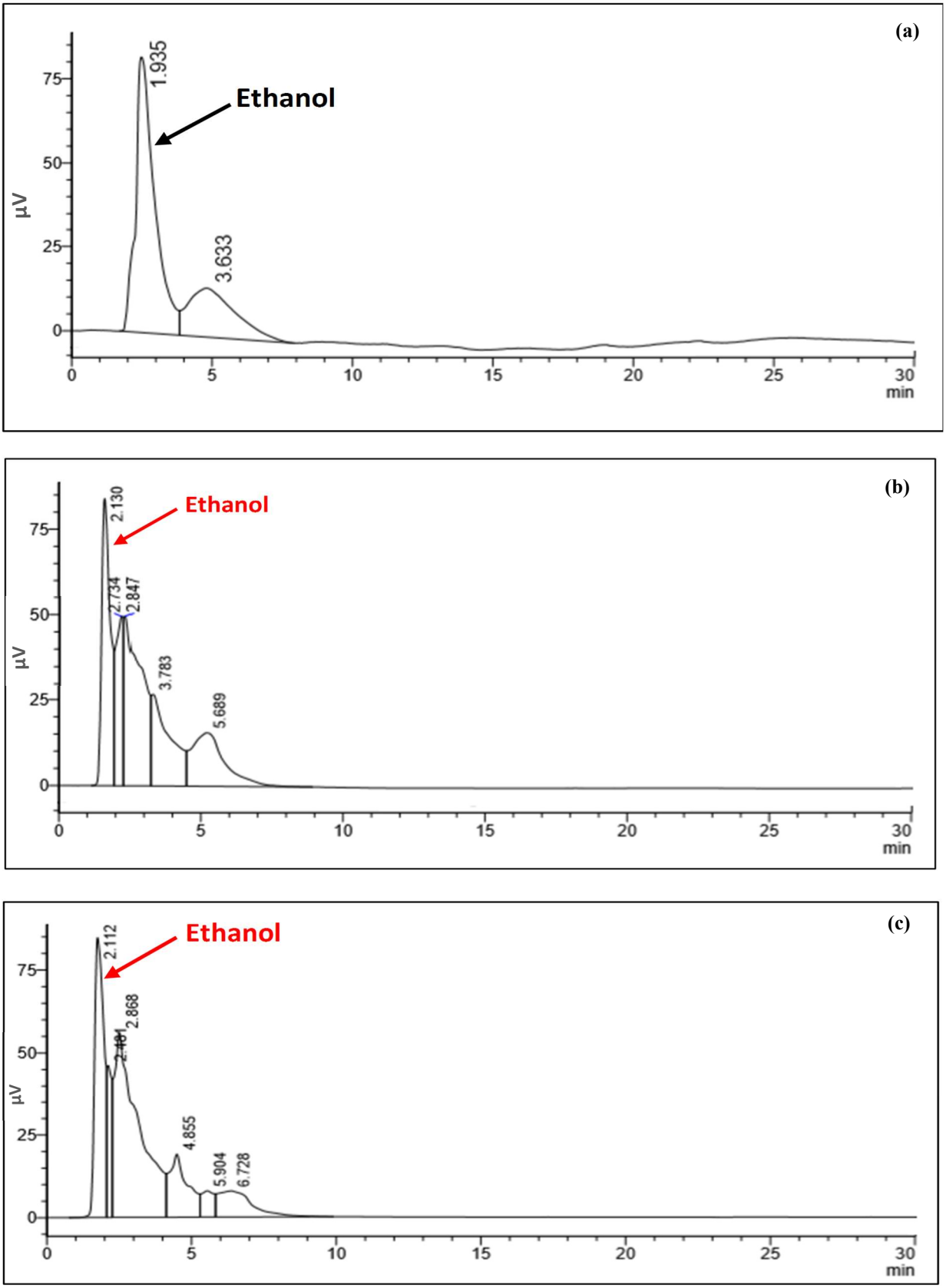

Figure 4. **HPLC chromatogram of (a) standard ethanol, (b) variant sample 1, and (c) variant sample 2**

Table 5. **Findings of the present and previous bioethanol production studies.**

Aspect of study	Current study	Previous study [24]	Previous study [25]
Substrate	Newspaper waste	Waste paper	Waste paper: office waste papers, newspapers and magazine waste papers
Pre-treatment method	Shredded, sun dried, and NaOH treated	Shredded, oven dried, and NaOH treated	H_2SO_4 pretreated
Microorganisms used	*Aspergillus niger, Saccharomyces cerevisiae*	*Pseudomonas aeruginosa, Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
Saccharification duration	6 days	1 day	-
Fermentation duration	6 days	2 days	4 days
Analytical methods	Dichromate oxidation, UV-Vis spectroscopy, HPLC	UV-Vis spectroscopy	UV-Vis spectroscopy, HPLC
Bioethanol yield	3.814% v/v (variant sample 1), 5.049% v/v (variant sample 2)	0.86 liters of 95% ethanol	Purity of bioethanol increased from 69.52% to 99.73% after the addition of calcium oxide.
Key findings	CDM showed higher bioethanol yield	Best to use waste papers which give a higher percentage of yields	Opportunity for preventing the noxious emissions during the chemical method of ethanol synthesis.

Comparison of the Bioethanol Production from Paper Waste

The aim of this comparison is to highlight different processing methods used, their efficiency, and the difference in the bioethanol yield. Table 5 summarizes the findings of the present and previous studies.

Conclusion

This study focused on the utilization of newspaper waste for bioethanol production. Newspaper waste is an economically viable renewable energy resource. *Aspergillus niger* converted the newspaper waste into simple sugars through the microbial process. Lactose and chemically defined media used as an inducer for *Aspergillus niger* improved the cellulolytic activity. Hence the yeast fermentation using the *Saccharomyces cerevisiae* yielded a good quantity of bioethanol. The bioethanol yield increased with the increasing duration, i.e., 4^{th} day < 5^{th} day < 6^{th} day. The results of the study are encouraging, indicating that newspaper waste should be used for fuel production on an industrial scale. This will not only help in overcoming the energy crisis but also help in effective solid waste management. Further, the successful application of the concept of bioethanol production from lignocellulosic waste (newspaper) should be ensured that will entice the private sector companies and farm partnerships to boost the growth and development of advanced bioenergy solutions and improve the developing economies.

Conflict of Interest

Authors declare that they don't have any conflict of interest.

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