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Valorization of *Afzelia bella* oilseeds cake in bioethanol production using 1-butyl-3-methyl-imidazolium chloride ionic liquid and dilute sulfuric acid pretreatment

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Abstract

The use of renewable feedstocks and the variability of lignocellulosic feedstocks generating fermentable sugars also offer the advantages of the most abundant, sustainable and competitive non-food biomass. The aim of this study is to valorize the oilcake obtained from non-conventional Congolese *Afzelia bella* oilseeds, after extraction of the oil used in biodiesel production. The conversion of *A. bella* oilseeds cake into ethanol was carried out using the two methods of pretreatment (with the synthesized ionic liquid 1-butyl-3-methyl imidazolium chloride and dilute sulfuric acid), followed by hydrolysis with dilute sulfuric acid and fermentation by *Saccharomyces cerevisiae*. The lignocellulosic composition of the sample was 47.98% cellulose, 28.15% hemicellulose and 22.3% lignin. Pretreatment with 1-butyl-3-methyl-imidazolium chloride (ionic liquid) showed an increase in cellulose content of around 4.25% and hemicellulose content of around 7.7%, with simultaneous delignification of 12.25%. In contrast, with dilute sulfuric acid, the lignin and cellulose content of the sample fell to 3.88% and 1.88% respectively. Hemicellulose content, on the other hand, increased by only 3.58%. After dilute acid hydrolysis (5% H_2SO_4), the values for total reducing sugar, glucose and xylose were 66.06±1.34 $mg.g^{-1}$; 36.25 ± 1.2 $mg.g^{-1}$ and 29.71 ± 0.6 $mg.g^{-1}$ respectively. The percentage conversion of sugars was 55.16 ± 0.5% for cellulose to glucose and 53.76 ± 0.75% for hemicellulose to xylose. The hydrolyzed product of the complex polysaccharide was then converted to ethanol using commercial yeast. Results showed an ethanol yield of around 10.3%, and Fourier Transform Infrared (FTIR) spectroscopy results confirmed bioethanol production. *A. bella* oilseeds cakes are therefore a potential source for ethanol production.

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Keywords: *Afzelia bella* oilseeds cake; ethanol fermentation; Fourier Transform Infrared (FTIR) spectroscopy; ionic liquids; lignocellulosic biomass; pretreatment

Introduction

Since then, the demand for energy has continued to grow, leading researchers to develop alternative energy sources. Among all these energies, fossil fuels occupy an important place, as they are indispensable in transportation, heating and many other manufacturing operations (Dincer, 2018; Sakthivel *et al*., 2018). Global fossil fuel consumption was estimated at 84.3% in 2019, and could reach 19.08 billion liters per day by 2040 (Karmakar *et al*., 2019; Group, 2020; Yuwei *et al*., 2021). It is therefore important to use green, renewable biofuels. They offer the greatest potential, as they can use waste as a raw material and are renewable. There are several types of biofuels, including bioethanol, biodiesel, biogas and biohydrogen. Bioethanol is a clean, renewable energy resource produced by the microbial fermentation of plants or algae rich in starch and polysaccharides (Tse, 2021; Soleymani Angili *et al*., 2021; Kaviani, 2022).

The biomass utilized for production typically relies on the resources available in each region (Tse, 2021; Kaviani, 2022). The main bioethanol producers are the United States, Brazil and the European Union, which respectively utilize corn, sugarcane and potatoes (or corn) as biomass feedstocks (Tse, 2021; Kaviani, 2022; Vohra *et al*., 2014). Bioethanol produced from these edible biomasses is referred to as "first-generation bioethanol" and competes with food demand (Vohra *et al*., 2014). Therefore, it is important to use lignocellulosic biomasses, which are inexpensive, abundant, renewable and non-edible agricultural waste products (Sharma *et al*., 2021). However, these lignocellulosic substrates need to be pretreated to facilitate hydrolysis and subsequent bioethanol production (Sharma *et al*., 2021). There are several types of pretreatments, including chemical (acid or alkaline), physical and enzymatic (Saini *et al*., 2020; Rezania *et al*., 2020; Xu *et al*., 2020; Haq *et al*., 2021; Sharma *et al*., 2021). The latter pre-treatment is effective but very costly. For this reason, the first two are more widely used, as they are less costly (Saini *et al*., 2020; Rezania *et al*., 2020; Haq *et al*., 2021). Nowadays, a variety of ionic liquids with imidazolium, pyridinium, ammomium and phosphonium cations coupled with various anions such as chloride, acetate and phosphonate have been used in the pretreatment of lignocellulosic biomasses because they improve saccharification efficiency by removing lignin from lignocellulosic biomasses and they also have high thermal stability, negligible vapor pressures; they do not release toxic or explosive gases when used (Saini *et al*., 2020; Rezania *et al*., 2020; Xu *et al*., 2020; Haq *et al*., 2021; Sharma *et al*., 2021). After this pre-treatment, the lignocellulosic substrate is hydrolyzed into fermentable simple sugars (glucose, fructose, galactose, etc.), which are then fermented by yeast to give ethanol, carbon dioxide and other by-products (Tse, 2021; Maleki *et al*., 2021).

The non-timber forest products (NTFPs) of the Congo Basin Forest are rich in non-conventional oilseeds and many other plants that are underutilized, or are used in small quantities for therapeutic purposes (Mulula *et al*., 2017; Mulula *et al*., 2020; Mulula *et al*., 2021 a, b; Nsomue *et al*., 2022; Mulula *et al*., 2023 a, b; Mulula *et al*., 2024). One of these unconventional plants from Congo (DRC) is *A. bella*.

A. bella belongs to the Caesalpiniaceae family. It is known as 'Bolengu' in the central Kongo region of the Democratic Republic of Congo (DRC). It is widespread from eastern Liberia and eastern Guinea to the Central African Republic, the Democratic Republic of Congo and Angola (Cabinda) (Mulula *et al*., 2022). *A. bella* is inedible, non-toxic and biodegradable. In our previous study, we produced biodiesel from *A. bella* oilseeds. Thus, the aim of this study is to valorize the oilcake obtained from non-conventional Congolese *A. bella* oilseeds, after extraction of the oil used in biodiesel production.

Materials and Methods

Plant materials and extraction

A. bella fruits were collected in the Mayombe region of Kongo Central (Latitude: -4.767° and Longitude: 14.300°; Democratic Republic of Congo). The seeds were dried at 106 °C for 24 hours and subsequently ground. The oil from these dried seeds was extracted by the Soxlet method, using cyclohexane as solvent, according to the method described by Mulula *et al*. (2022).

The oil obtained was used for biodiesel production, as published in our previous article (Mulula *et al*., 2022). The cake obtained is used for bioethanol production in this study. The *Sachromyces cerevisiae* strain was obtained from the National Research Center and maintained on agar plates at 4 °C for the experiments.

Figure 1. *Afzelia bella* seeds (original picture by author Arnold MULULA)

Determination of cellulose, hemicellulose and lignin in Afzelia bella oilseeds cakes

Determination of cellulose

Cellulose in dried *A. bella* oilseeds cake was determined using the method described by Marzieh *et al*. (2010). One gram of dried *A. bella* oilseed cake was weighed and transferred to a 250 mL Erlenmeyer flask. Then, 50 mL of 96% ethyl alcohol and 25 mL of 65% nitric acid were added. The flask was connected to a condensing apparatus and heated on a heating mantle for 1 hour. After hydrolysis, the contents of the flask were filtered. Once again, the cellulose remaining on the filter paper was transferred to the flask, and the process repeated twice, with the celluloses and filter papers dried at 120 °C. The cellulose content was calculated from the following equation:

$$\%\ \text{Cellulose} = \frac{\text{Cellulose dry weight}}{\text{Sample dry weight}} \times 100$$

Determination of total lignin

The total lignin in the dried *A. bella* oilseeds cakes was obtained by the determination of the soluble and insoluble lignin according to the literature (Goering *et al*., 1975). The summation of the soluble and insoluble lignin gave the total lignin. In the insoluble lignin determination, 2 g of dried *A. bella* oilseeds cakes were impregnated with 3 mL of 72% sulphuric acid and placed in a water bath at a controlled temperature of 30 °C for 1h, after which 68 mL of deionized water was added to the mixture. The conical flask and its contents (mixture) were heated in an autoclave at 125 °C for 1 hr. The conical flask with its content was cooled and the lignin filtered. The insoluble lignin was washed with deionized water until neutral pH and then dried in an oven at a temperature of 80 °C until a constant weight. The lignin content was calculated by the following formula:

$$\% \text{ Insoluble lignin } = \frac{\text{W lignin}}{\text{W fibre}} \times 100$$

Where, W lignin = oven dry weight of insoluble lignin (g) and W fibre = oven dry weight of sample fibres (g).

The filtrate obtained from the insoluble lignin was used to determine the soluble lignin content in sulphuric acid by spectrophotometric method. In this method, 5 mL of 3% suphuric acid were added to 5 mL of the insoluble lignin filtrate. A UV spectrophotometer was used to measure the absorbance of the solution at a wavelength of 205 nm. The soluble lignin content was calculated by the following expression:

$$\% \text{ Soluble lignin } = \frac{\text{CV}}{1000 \times \text{W fibre}} \times 100$$

Where, C = concentration of soluble lignin in the filtrate (g/L). V = total volume of the filtrate (mL); W fibre = oven dry weight of sample fibres (g). The concentration of soluble lignin in the filtrate (C) is given by:

$$\text{C } = \frac{\text{A}}{110} \times \frac{\text{Vf}}{Vi}$$

Where, A = absorbance at a wavelength of 205 nm. Vf is the final volume of the solution (mL); Vi is the initial volume of the solution (mL).

The total lignin content was obtained by the addition of insoluble and soluble lignin obtained by both methods.

Determination of hemicellulose

The hemicellulose in the dried *Afzelia bella* oilseeds cakes was obtained according to the literature (Goering *et al*., 1975). Neutral detergent solution was prepared by weighing 18.61 g of disodium ethylenediamine tetraacetate and 6.81 g of sodium borate decahydrate into a 1000 mL beaker and dissolved in 200 mL distilled water by heating in an electromagnetic stirrer. To this a 150 mL solution containing 30 g of sodium lauryl sulphate, 10 mL of 2-ethoxy ethanol and 100 mL solution containing 4.5 g of disodium hydrogen phosphate were added. The volume was made up to 1000 mL and the pH of the solution kept at 7.

To 1.0 g of dried *A. bella* oilseeds cakes in a refluxing flask, 10 mL of cold neutral detergent solution was added followed by 0.5 g sodium sulphate. The mixture was heated to boiling and refluxed for 60 min. The solution was filtered through a Whatman filter paper No 42 (125 mm) and the residue in the paper washed twice with acetone. The filter paper with the residue was dried in an oven at a temperature of 100 °C for 8 hours. The filter paper and its content were cooled in a desiccator and weighed. Hemicellulose is calculated thus: Hemicellulose = Neutral Detergent Fibre (NDF) – Acid detergent Fibre (ADF); Where ADF value = Value of Lignin content.

Synthesis of 1-butyl-3-methyl imidazolium chloride [BMIM] chloride

The synthesis of 1-butyl-3-methyl imidazolium chloride was been performed according to the method described in the literature (Pinkert *et al*., 2009; Wei *et al*., 2012; Zhang *et al*., 2015) and the mechanism of this reaction is shown in Figure 2. A solution of 1- methylimidazole (8.21 g, 0.20 mol) and 1-chlorobutane (18.51 g, 0.10 mol) was refluxed under nitrogen for 24 hours. The unreacted starting materials were removed by extraction with diethyl ether to give 1-butyl-3-methyl imidazolium chloride (15.72 g, 90%). The purity of this compound was evaluated by using HPLC and thin layer chromatography and this compound have been characterized by Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) (Mulula *et al*., 2023 a, b). White low-melting solid, 88% yield, **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.59 (s, 1 H, C_2-H), 7.79 (t, J = 1.6 Hz,1 H, C_4-H), 7.62 (t, J = 1.6 Hz, 1 H, C_5-H), 4.35 (t, J = 7.2 Hz, 2 H, butyl C_1-H), 4.14 (s, 3 H, N-methyl), 1.91 (m, 2 H, butyl C_2-H), 1.38 (m, 2 H, butyl C_3-H), 0.96 (t, J = 7.6 Hz, butyl-CH_3), **^{13}C NMR** (101 MHz, $CDCl_3$) 137.83 (s, NCHN), 123.67 (s, NCHCHN), 121.98 (s, NCHCHN), 49.69 (s, NCH_2CH_2), 36.47 (s, NCH_3), 32.11 (s, $CH_2CH_2CH_2$), 19.38 (s, $CH_2CH_2CH_3$), 13.37 (s, CH_2CH_3).

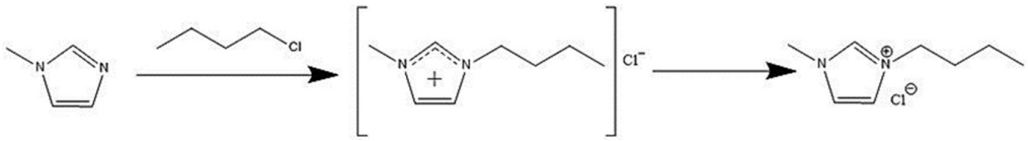

Figure 2. Synthesis of 1-butyl-3-methyl imidazolium chloride [BMIM][Cl]

Pretreatment of dried Afzelia bella oilseeds cakes

Pre-treatment of dried *A. bella* oilseeds cakes was carried out using two different pre-treatment methods (ionic liquid pre-treatment using 1-butyl-3-methyl imidazolium chloride and dilute acid pre-treatment using sulfuric acid) in order to compare the effectiveness of each method (lignin removed).

Ionic liquid pretreatment by using 1-butyl-3-methyl imidazolium chloride

Ten grams of dried *A. bella* oilseeds cakes were added to 50 mL of 1-butyl-3-methyl imidazolium chloride, homogenized, and dissolved at 120 °C for 2 h. After incubation, the samples were left to cool, and then deionized water was added to rinse the cellulose fibers and separate the biomass from the ionic liquid (1-butyl-3-methyl imidazolium chloride). During the addition of the deionized water, the ionic liquid dissolves in water, and the residue fraction precipitates. The water-IL solution with biomass was filtered using a Shot funnel with a filter (Whatman 1.0 paper). This procedure was repeated four times, where a significant increase in the plant-IL mixture was present. Such purified *A. bella* oilseeds cakes were subjected to analysis of Cellulose and Hemicellulose according the above-mentioned methods. Another part was subjected to hydrolysis (Verma *et al*., 2019; Cheah *et al*., 2020; Vereycken *et al*., 2022).

Dilute acid pretreatment by using sulfuric acid

Five grams of dried *A. bella* oilseeds cake were pre-treated with an equal volume of 0.5% sulfuric acid. These samples were heated at 121 °C and 15 psi for 90 minutes. The mixture was then dried in hot air at 30-35 °C. Samples were taken from the pre-treated material for analysis of cellulose and hemicellulose using the above methods. Another portion was subjected to hydrolysis.

Hydrolysis of Afzelia bella oilseeds cakes pretreated

Pre-treated *A. bella* oilseeds cake was hydrolyzed using the dilute acid hydrolysis method, by adding twice the volume of 5% H_2SO_4 to the pre-treated material and mixing well. The mixture was poured into glass bottles and sealed to prevent heat-induced vaporization of the acid. The mixture was kept at an elevated temperature of 55 °C for 3 days. The mixture was stirred regularly to prevent precipitation. Samples were taken from the hydrolyzed material for analysis. Total reducing sugars were assessed in accordance with the literature and expressed as glucose equivalent using standard glucose calibration curves (10-100 µg mL^{-1}, $r^2 = 1$) (Somogyi *et al*., 1952). The scheme of cellulose hydrolysis to glucose is shown in Figure 3.

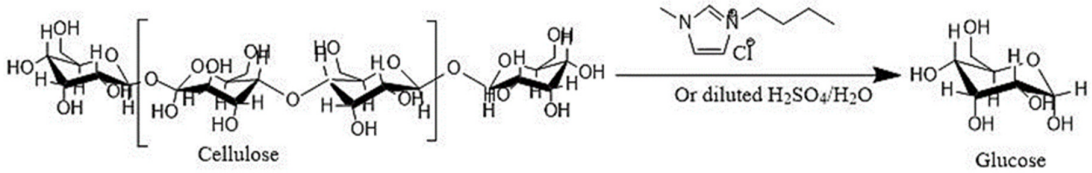

Figure 3. Cellulose hydrolysis to glucose

Glucose content was estimated by the glucose oxidase method and expressed as glucose equivalent using standard glucose calibration curves (10-100 µg mL^{-1}, $r^2 = 0.998$). Xylose was estimated by taking the difference between total reducing sugar and glucose (Somogyi *et al*., 1952). The hydrolysis rates of complex sugars,

cellulose and hemicellulose and the degree of conversion of these polymeric forms into simpler forms were calculated using the following formulas (Arthe *et al*., 2008).

$$V = \frac{dS}{dt} = \frac{\text{reducing sugar t} - \text{reducing sugar t}_i}{t - t_i}$$

Where reducing sugar t is the sugar concentration after time t, reducing sugar t_i is the reducing sugar concentration before hydrolysis, and t and t_i are the final and initial times in hours, respectively.

$$C.C = \frac{(\text{glucose t} - \text{glucose t}_i)}{C} \times 100$$

Where glucose t is the concentration of glucose after hydrolysis at time t and glucose t_i is the concentration of glucose before hydrolysis. C is the concentration of cellulose before hydrolysis.

$$H.C = \frac{(\text{xylose t} - \text{xylose t}_i)}{C} \times 100$$

Where xylose t is the concentration of xylose after hydrolysis time t and xylose t_i is the concentration of xylose before hydrolysis. H is the concentration of hemicellulose before hydrolysis.

Ethanolic fermentation

In order to convert the released sugars into ethanol, anaerobic fermentation was carried out using *Saccharomyces cerevisiae* for nine days, in accordance with the literature (Smuga-Kogut *et al*., 2021). The pH of the solution was raised to ~ 4.2 by adding the necessary amount of 4 M NaOH to allow yeast growth. Fermentation was carried out in a closed conical flask at a temperature of 32 °C with a stirring rate of 110 rpm. The amount of ethanol produced was determined by the potassium dichromate method using standard ethanol calibration curves (20-100 $mg.mL^{-1}$, $r^2 = 0.995$) and was further processed for distillation.

Infrared spectroscopy of bioethanol

The bioethanol distillate produced was subjected to Fourier Transform Infrared (FTIR) spectroscopy to establish the presence or otherwise of functional groups important in confirming or not that ethanol had indeed been produced. The PerkinElmer Spectrum 400 FTIR/FT-FIR spectrometer with a region of 4000-400 cm^{-1} was used to assess the chemical structure of bioethanol from *A. bella* oilseeds.

Results and Discussion

Extraction and lignocellulosic composition of Afzelia bella oilseeds cakes

Oil extraction from these *A. bella* oleaginous seeds was carried out by the Soxhlet method using cyclohexane as solvent. The percentage of oil extracted was 26.38±0.22%. This value is higher than that found in literature (Mulula *et al*., 2022). The lignocellulosic composition of *A. bella* oilseeds is shown in Figure 4.

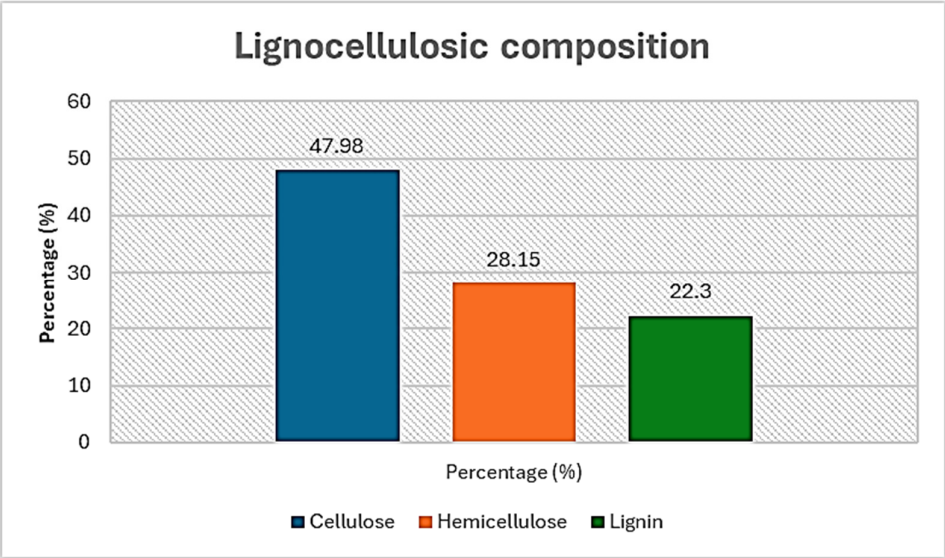

Figure 4. Lignocellulosic composition of *Afzelia bella* oilseeds cakes

A. bella oilseeds cake contains 47.98% cellulose, 28.15% hemicellulose and 22.3% lignin. This makes them an excellent source of cellulose and hemicellulose, which can be hydrolyzed to glucose and xylose respectively. The latter can be converted into bioethanol by fermentation.

Synthesis of 1-butyl-3-methyl imidazolium chloride [BMIM] chloride

Ionic liquids are environmentally friendly, reusable and reputedly very effective for the pretreatment of lignocellulosic biomasses (Rezania *et al*., 2020; Haq *et al*., 2021). 1-Butyl-3-methyl-imidazolium chloride was successfully synthesized according to the method described in the literature (Pinkert *et al*., 2009; Wei *et al*., 2012; Zhang *et al*., 2015). The ^{1}H-NMR spectrum of the synthesized compound is shown in Figure 5, revealing that the single characteristic proton on the carbon between the two nitrogens of the imidazole function has a chemical shift (δ) of 10.59 ppm. In contrast, the three n-methyl protons have a chemical shift (δ) of 4.14 ppm. These values are almost identical to those reported in the literature (Zhang *et al*., 2015).

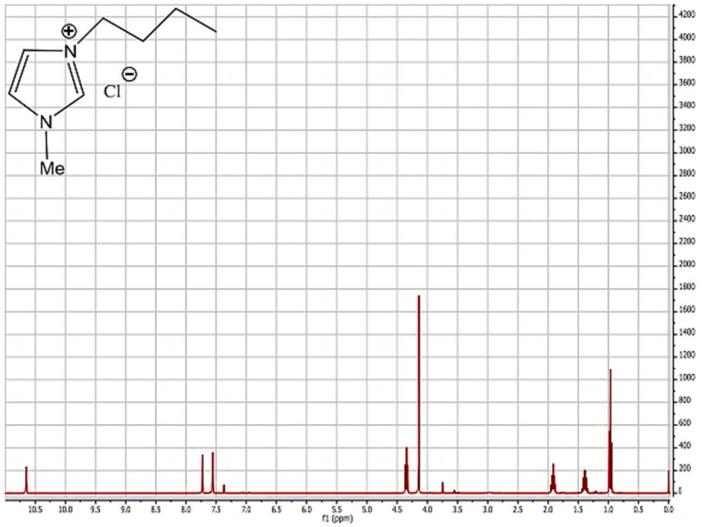

Figure 5. ^{1}H-NMR spectral of 1-butyl-3-methyl imidazolium chloride

Pretreatment of dried Afzelia bella oilseeds cakes

The use of agricultural biomass or lignocellulosic biomass for ethanol production as a biocomponent of liquid fuels is one of the most promising economic and environmental solutions, due to the low cost of raw materials and the reduction in greenhouse gas emissions during their combustion. However, the most problematic structural element of lignocellulose is lignin, which irreversibly binds the active sites of cellulolytic enzymes, significantly reducing the efficiency of the cellulose hydrolysis reaction, despite costly pre-treatment (Soleymani Angili *et al*., 2021). In order to remove lignin and separate it from cellulose and hemicellulose, two different pretreatment methods (ionic liquid pretreatment using 1-butyl-3-methyl-imidazolium chloride and dilute acid pretreatment using sulfuric acid) were applied comparatively. The percentage (%) of cellulose and hemicellulose before and after pre-treatment is shown in Table 1.

Table 1. The percentage (%) of cellulose, hemicellulose and lignin before and after pretreatment

Sample	Pretreatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Dried *A. bella* oilseeds cakes	Before pretreatment	47.98	28.15	22.3
	[BMIM] Chloride	52.23	35.85	10.05
	Diluted H_2SO_4	46.10	31.73	18.42

The values are means ± standard deviations (n = 3)

Non-pretreated dried *A. bella* oilseeds cakes contained an average of 47.98% cellulose, 28.15% hemicellulose and 22.3% lignin, while dried *A. bella* oilseeds cakes pretreated with 1-butyl-3-methyl imidazolium chloride contained 52.23% cellulose, 35.85% hemicellulose and 10.05% lignin. Dried *A. bella* oilseeds cakes subjected to the influence of 1-butyl-3-methyl imidazolium chloride ionic liquid resulted in an increase in cellulose content of around 4.25% and hemicellulose content of around 7.7%, with simultaneous delignification of 12.25%. However, when dilute sulfuric acid (0.5%) was used, the lignin and cellulose content of the sample decreased to 3.88% and 1.88% respectively. In contrast, the hemicellulose content increased by only 3.58%.

Hydrolysis of Afzelia bella oilseeds cakes pretreated

After pretreatment, *Afzelia bella* oilseed cake was hydrolyzed in accordance with the literature (Somogyi *et al*., 1952). Results concerning total reducing sugars, percentage sugar conversion (from cellulose to glucose and from hemicellulose to xylose) and hydrolysis rate are presented in Table 2.

Table 2. Total reducing sugars, percentage sugar conversion and hydrolysis rate of dried *Afzelia bella* oilseed cake

Sample	Reducing sugar release ($mg.g^{-1}$)			Sugar conversion percentage ($mg.g^{-1}$ %)		Rate of hydrolysis ($mg.g^{-1}.h^{-1}$)		
	Total reducing sugar	Glucose	Xylose	Cellulose to glucose	Hemicellulose to xylose	Total reducing sugar	Glucose	Xylose
Dried *Afzelia bella* oilseeds cakes	66.06± 1.34	36.25 ± 1.2	29.71 ± 0.6	55.16 ± 0.5	53.76 ± 0.7	0.82 ± 0.0	0.47 ± 0.0	0.36 ± 0.0

The values are means ± standard deviations (n = 3).

Dilute acid hydrolysis is more cost-effective and has been recognized as a feasible method for producing bioethanol from lignocellulosic biomass (Tse, 2021). After dilute acid hydrolysis (5% H_2SO_4), the values for total reducing sugar, glucose and xylose were 66.06±1.34 $mg.g^{-1}$; 36.25 ± 1.2 $mg.g^{-1}$ and 29.71 ± 0.6 $mg.g^{-1}$ respectively. The percentage conversion of sugars was 55.16 ± 0.5% for cellulose to glucose and 53.76 ± 0.75% for hemicellulose to xylose.

Ethanolic fermentation

Anaerobic fermentation was carried out using *Saccharomyces cerevisiae* for nine days, in accordance with the literature (Smuga-Kogut *et al*., 2021) Daily estimates of total reducing sugar, glucose and ethanol during fermentation are shown in Figures 6, 7 and 8, respectively.

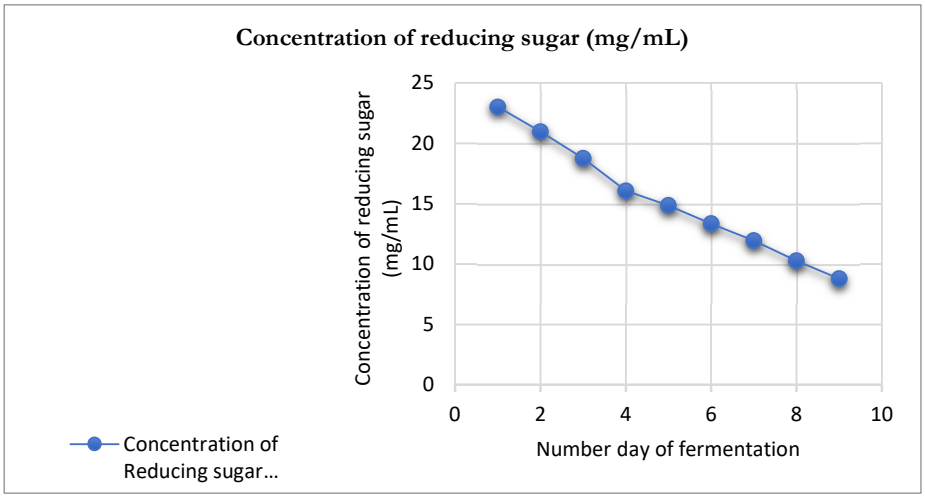

Figure 6. Day-wise estimation of total reducing sugar during fermentation

Based on the figures (Figures 6 and 7), it was observed that on the first day of fermentation, the broth contained 23.02 mg/mL and 18.31 mg/mL of total reducing sugar and glucose, respectively. Conversely, the amounts of total reducing sugar and glucose estimated on the last day (day 9) of fermentation were 8.79 mg/mL and 10.98 mg/mL, respectively.

These results demonstrate the degradation of these hydrolysates into ethanol by the microorganism.

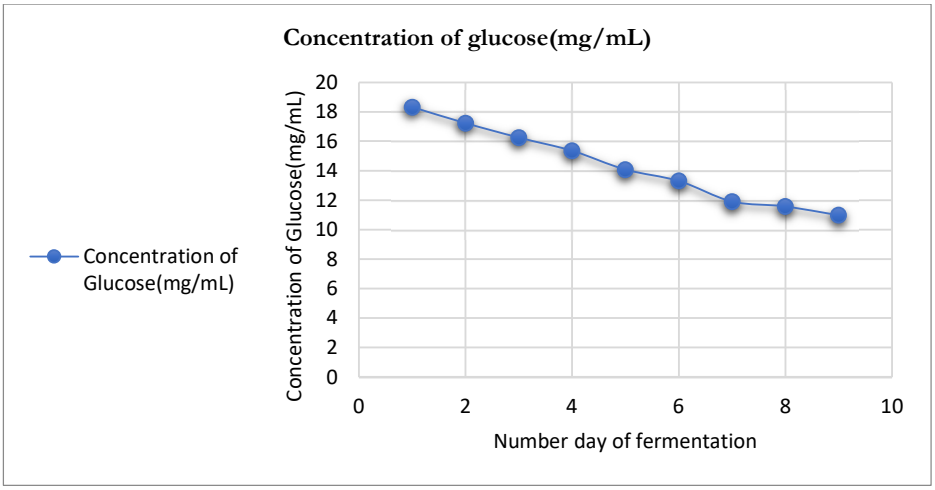

Figure 7. Day-wise estimation of glucose during fermentation

In Figure 6, it was observed that the estimated ethanol produced was 1.6% on the first day of fermentation and increased to 10.3% on the last day (day 9) of fermentation. This increase in the amount of ethanol in the broth was directly proportional to the decrease in hydrolysates.

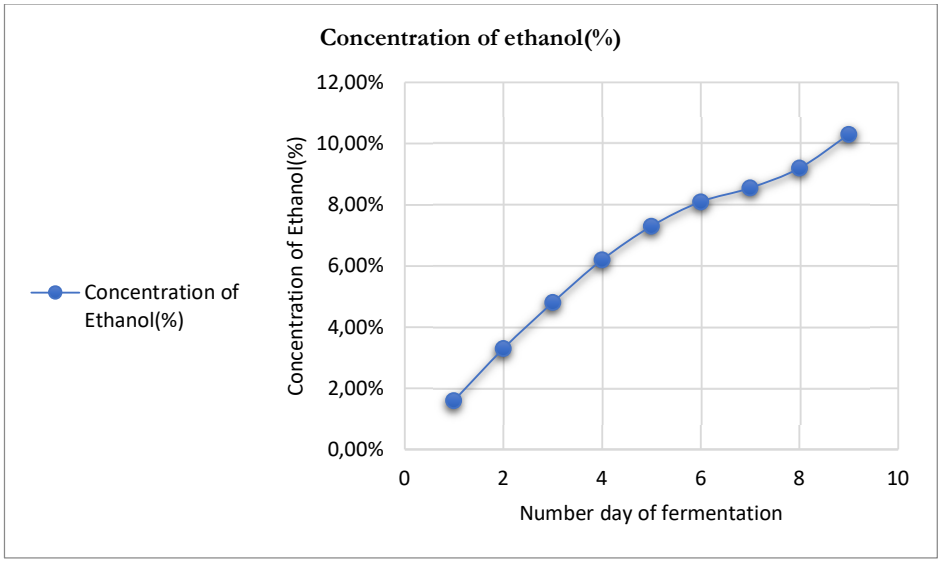

Figure 8. Day-wise estimation of ethanol produced during fermentation

Infrared spectroscopy of bioethanol

To examine bioethanol production and the functional group of alcoholic bonds existing in the samples after the distillation process, the bioethanol distillate produced was subjected to Fourier transform infrared spectroscopy (FTIR). FTIR results for bioethanol from dried *A. bella* oilseeds cake pre-treated with 1-butyl-3-methyl imidazolium chloride [BMIM]Cl and dilute sulfuric acid are shown in Figure 9.

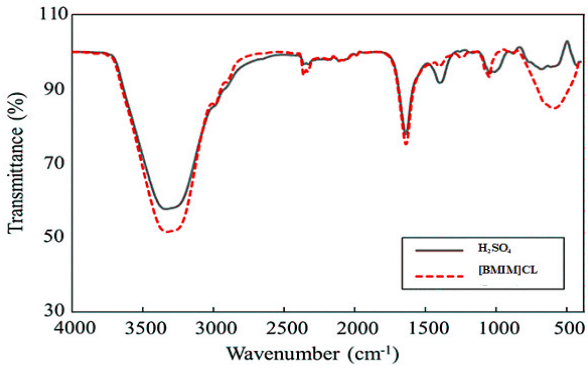

Figure 9. FTIR results of bioethanol from the pretreated dried *Afzelia bella* oilseeds cakes with 1-butyl-3-methyl imidazolium chloride and diluted sulfuric acid

The FTIR results obtained reveal the presence of the characteristic hydroxyl group with a frequency between 3400 and 3200 cm^{-1}, which is consistent with literature data (Sebayang *et al.*, 2017; Smuga-Kogut *et al.*, 2021). According to the literature, the peaks between 2356-2322 cm^{-1} and 1658-1638 cm^{-1} could be attributed to alkenes (Sebayang *et al.*, 2017). Conversely, peaks between 1300-1000 cm^{-1} could be attributed to C-O stretching and those between 1100-900 cm^{-1} to carbohydrates (Sebayang *et al.*, 2017). These spectroscopic

results confirm the production of bioethanol from pretreated dried *A. bella* oilseed cake. They also show that the sample pretreated with ionic liquid BMIM chloride produced a large amount of bioethanol.

Conclusions

Given the competitiveness of bioethanol production with the food sector, the utilization of non-food biomass, such as agricultural waste, is crucial due to its abundance and lower cost. This study revealed that *A. bella* oilseed cake is a good source of cellulose and hemicellulose. It can therefore be used for bioethanol production. In the future, *A. bella* oilseed cake, which is a by-product of biodiesel production, could be used as a good feedstock for ethanol production and solve the problem of safe disposal of the by-product. In addition, this work has provided essential information on the lignocellulosic composition of *Afzelia bella* oilseed cake, and on the pre-treatment method to maximize fermentable sugars for bioethanol production. Fourier transform infrared (FTIR) spectroscopy confirmed bioethanol production from dried and pretreated *A. bella* oilseed cake.

Authors' Contributions

Conceptualization and chemical analysis: AM; Biological analysis: AB; Data curation and Software: TN, BD, and AM; Writing-original draft: AM, BD and EM; Writing- review editing: JK, AZ, KT and KM; Supervision: AZ, Investigation: AM. All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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