

Optimization of dilute acid hydrolysis for bioethanol production from selected agricultural wastes

Amina Ahmed El-Imam^{1,2}, Aishat Ajibola Rufai², Florence Oladeji², Latifa Saidu², and Eromosele Ighalo^{2*}

¹Department of Plant and Microbial Biology, North Carolina State University, 27607 USA

²Microbiology Department, Faculty of Life Sciences, University of Ilorin.

eromoseleighalo@gmail.com^{2*}

*Corresponding Author

Abstract:

The potential of bioethanol production from selected agricultural wastes (cassava peels, corn bran and millet bran) was investigated in this study. Firstly, yeasts were isolated from rotting orange and subjected to ethanol tolerance screening. Next, the substrates were subjected to dilute acid hydrolysis using different concentrations of HNO₃ (3 %, 4 % and 5 %) at 20 % solid loading and then autoclaved at 121 °C for 30 minutes. Dilute acid optimisation yielded varying amounts of reducing sugars. The 3 %, 4 % and 5 % HNO₃ solutions produced hydrolysates with the highest amounts of reducing sugars of 19.28 g/L, 16.36 g/L and 7.59 g/L from cassava peels, corn bran, and millet bran, respectively. Hydrolysate fermentation resulted in bioethanol concentrations of 7.73 g/L from cassava peels hydrolysate, 8.98 g/L from corn bran hydrolysate and 1.69 g/L from millet bran hydrolysate. This study demonstrates that various hydrolysates require different concentrations of catalyst for optimum sugar release. It also shows that bioethanol can be produced from millet bran and other abundant biomass, thus contributing valorising them.

Keywords: Bioethanol, optimisation, hydrolysis, food waste, valorisation.

1. Introduction

In order to achieve the sustainable development goal of renewable energy (SDG 7), renewable fuels are now used in substitution for fossil fuels (Ahmed El-Imam *et al.*, 2019a; Siacor *et al.*, 2020). Fossil fuels are not environmentally friendly and cannot be replenished after they run out, therefore using them as a source of energy has continued to pose major environmental issues (Awoyale and Lokhat, 2021). Additionally, the high cost of producing petroleum products, the shrinking of the world's oil supplies, and the negative effects of oil spills have all prompted researchers to look for alternate sources of fuel for the production of energy (Yildirim *et al.*, 2021). Additionally, the price of non-renewable fossil fuels has increased demand for affordable, renewable energy sources like solar, wind, and biofuels (Ahmed El-Imam *et al.*, 2019a). Due to the abundance of sunlight and wind, many nations around the world have adopted solar and wind energy (Hossain *et al.*, 2019). The widespread use of

these energy sources is, however, constrained by a number of factors, such as their high cost and poor battery efficiency (Hossain *et al.*, 2019; Obeta *et al.*, 2020). Due to these issues, biofuels have been introduced as a replacement (Milano *et al.*, 2016), as it addresses climate change, energy security, and also helps to diversify the economy (Obeta *et al.*, 2020).

The most significant and popular liquid biofuel is bioethanol (Ahmed El-Imam *et al.*, 2019a). In order to boost octane and lower carbon monoxide and other smog-causing emissions from gasoline, it is commonly used in Brazil and the United States (Shalaby, 2013). The predicted global bioethanol production for 2007 was 13.12 billion gallons, and it increased to 17.64 billion gallons for 2008 (Renewable Fuels Association, 2018). It is anticipated that the global production of bioethanol would expand to roughly 137 billion liters in 2026 due to increases in production in USA and Brazil (Dev *et al.*, 2019). In Nigeria, 134 million liters of ethanol are generated annually under the supposition that the factories operate year-round in ideal conditions (Ohimain, 2010). The use of bioethanol as fuel has decreased the overconsumption of crude oil and its impact on the environment (Bayu *et al.*, 2022).

First-generation bioethanol is the term used for bioethanol produced from glucose and starch (Ibeto *et al.*, 2011). Due to the detrimental effects on human and animal food chains, there are ethical questions around the utilization of food to produce bioethanol (Awoyale and Lokhat, 2021). Due to this, second-generation bioethanol was produced using lignocellulosic feedstocks (Ahmed El-Imam *et al.*, 2019a; Siacor *et al.*, 2020). In Nigeria, agricultural operations result in the production of large amounts of lignocellulosic biomass, the majority of which pollute the environment by littering or feeding animals with low-quality feed (Ahmed El-Imam *et al.*, 2019b; Hassan *et al.*, 2019). In order to disrupt the complex structure of lignocellulosic biomass and separate the complex sugars into their monomeric molecules, a pretreatment stage is required (Ahmed El-Imam *et al.*, 2013; Yildirim *et al.*, 2021).

The complex sugars in lignocellulosic biomass can be efficiently broken down into their monomeric unit by a process called Dilute Acid Hydrolysis (DAH), resulting in higher yields of simple sugars compared to enzymatic hydrolysis and these sugars can then be fermented to produce bioethanol. The two biggest benefits of DAH over enzymatic hydrolysis are its ability to penetrate lignin structure without the need for substantial pretreatment and its speed (Bayu *et al.*, 2022).

Efforts have been undertaken to transform agricultural wastes into value-added products. The potential of cassava peels, brans from cereals including sorghum bran, jatropha cake, corncobs, yam peels, plantain and mango peels, and husks from the fruit and vegetable sectors to produce useful products have been investigated (Ahmed El-Imam *et al.*, 2013; Ahmed El-Imam *et al.*, 2019a; Ahmed El-Imam *et al.*, 2019b). The high sugar content of these wastes creates a favorable environment for the fermenting microbes (Sadh *et al.*, 2018). These wastes are inexpensive and easily accessible in addition to having a high nutritional content, which lowers the cost of producing value-added products. This study examines the potential for producing bioethanol from commonly accessible agricultural wastes (cassava peels, corn bran, and millet bran) by optimising dilute acid hydrolysis for maximum reducing sugar yield.

2. Materials and Methods

2.1 Feedstock Collection and Processing

The feedstocks used in this study (cassava peels, corn bran and millet bran) were obtained from different locations in Ilorin, North-Central Nigeria. The brans were obtained by washing the grains thoroughly and soaking for four days. The soaked grains were then grinded in a petrol-operated Burr-plate mill into a fine slurry. Lastly, the slurry was sieved and the chaff obtained was rinsed, re-sieved and sundried (Ahmed El-Imam *et al.*, 2019a). The cassava peels were sundried and milled to -40 mesh size.

2.2 Isolation and Maintenance of Microorganisms

After being serially diluted in sterile distilled water, a sample of a rotting orange was plated on Yeast Extract Peptone Dextrose (YPD) agar plates and incubated at 30 °C for 48 h. By repeatedly streaking on new growth media, colonies with distinctive morphological traits were carefully chosen and purified. This was done in order to obtain pure culture, which was kept at 4 °C on PDA slants.

2.3 Ethanol Tolerance Test

By inoculating single colonies (of comparable sizes) of the isolates into 25 ml of YPD broth that contained 10 % (v/v) ethanol, ethanol tolerance screening was carried out. The YPD-ethanol used as a blank for the treatment was run in duplicate. Following a 72-hour incubation period at 30 °C, the cultures' growth was assessed by measuring the suspensions' absorbance at 600 nm.

2.4 Dilute Acid Hydrolysis Optimisation and Sugar Estimation

Different concentrations of HNO₃ (3 %, 4 %, and 5 %) were used to optimize the dilute acid hydrolysis in order to find the conditions that would result in the maximum sugar content in the hydrolysates. A slurry of 20 % (w/v) was made of each bran of the acid solutions and then autoclaved at 121 °C for 30 minutes. Whatman No. 1 filter paper was then used to filter the cooled slurry.

Using a glucose standard curve, the DNS approach described by Sana *et al.* (2017) was used to determine the sugar content of the hydrolysates. The hydrolysates were autoclaved and frozen if not used immediately.

2.5 Fermentation and Bioethanol Estimation

The optimum combination of hydrolysis factors for each substrate was then employed to produce a large hydrolysate for fermentation by the ethanol-tolerant strain. The pH of the sterile hydrolysates was adjusted to 5.5 using an 8 M NaOH solution. The yeast cells were then pitched to a final concentration of 1×10^7 cells/mL (Ahmed El-Imam *et al.*, 2019a) in 25 mL of hydrolysate in a 250 mL Erlenmeyer flask. The flasks were plugged with cotton wool and incubated at 30 °C for five days. All experiments were performed in triplicates, and ethanol yields were monitored by withdrawing samples at 24-hour intervals.

Ethanol concentration was estimated as described (Koshy *et al.*, 2014). Exactly 30 µL of the fermentation broth was filtered and made up to 500 µL with distilled water. Next, 2 mL and 1 mL of 2 N NaOH and potassium dichromate solutions were added to the samples, respectively. The solutions were incubated at 50 °C for 30 minutes. The A₆₀₀ was then read with a spectrophotometer, and the ethanol content was estimated with a standard curve.

3. Results and Discussion

3.1 Ethanol Tolerance Profile of Isolates

A *Pichia kudriavzevii* isolate was determined to be the most resistant to ethanol after 72 hours of incubation because it had the most abundant growth. This result compares with that of Lairón-Peris *et al.* (2021) who reported the most tolerant yeast strain, AJ4 in 10 % ethanol concentration. Additionally, Hassan *et al.* (2019) observed that yeast isolates (Strains S1–S5) grew at ethanol concentrations between 4 % and 20 %.

3.2 Dilute Acid Optimisation

Dilute acid optimisation of the substrates with different concentrations of 3 %, 4 % and 5 % solutions of nitric acid yielded varying amounts of reducing sugars, as shown in Figure 1.

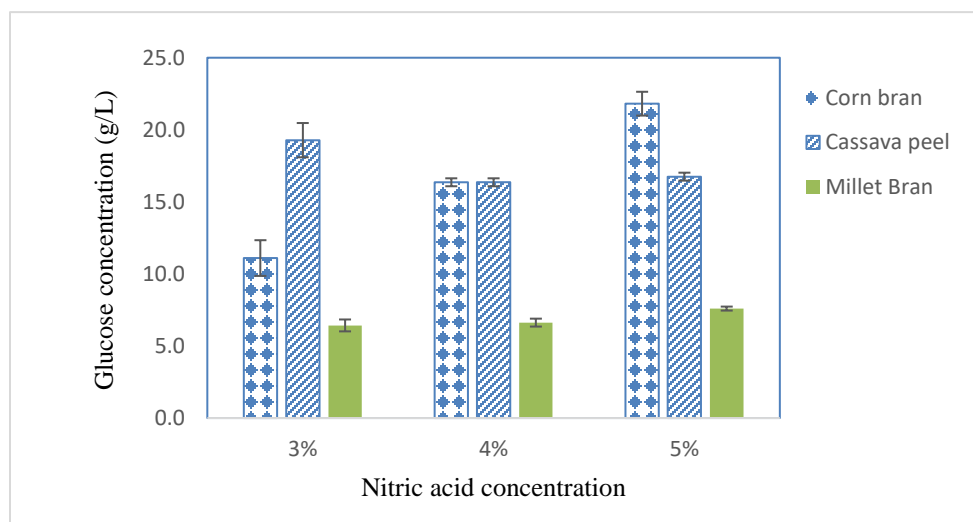


Figure 1: Reducing sugar yields from different biomass (values = Mean \pm SD).

According to the results of the hydrolysis, cassava peels hydrolyzed in 3 % nitric acid produced the highest amount of reducing sugars (19.28 g/L), corn bran hydrolyzed in 4 % nitric acid solution produced 16.36 g/L, and millet hydrolyzed in 5 % nitric acid produced the highest amount of reducing sugars (7.59 g/L) (Fig. 1). Despite the fact that Onyelucheya *et al.* (2017) also reported maximum yields from the hydrolysis of cassava peels using 3 % HNO₃, the various optimal conditions seen in this work show the necessity of first optimizing all novel biomass-catalyst combinations. Nitric acid hydrolysis of millet bran has not been documented in the literature. The differing substrate, acid concentrations, and reaction times may have contributed to the discrepancy in results.

3.3 Fermentation

Pichia kudriavzevii was employed in five-day fermentation using the three substrates. The results showed that on day four, cassava peel hydrolysate had a maximum bioethanol content of 7.73 g/L (Fig. 2a). This was more than the 3.67 g/L previously reported from the fermentation of cassava peel hydrolysate using *Saccharomyces cerevisiae* (Offor-Emenike *et al.*, 2020). Additionally, the results of this study support earlier publications on the appropriateness of cassava peels for the synthesis of bioethanol (Akinruli *et al.*, 2022; Oladipo *et al.*, 2022).

At day four of fermentation, corn bran hydrolysate produced a maximum bioethanol concentration of 8.98 g/L (Fig. 2b), whereas millet bran hydrolysate produced a maximum bioethanol concentration of 1.69 g/L (Fig. 2c). The findings of this study regarding the suitability of using cereal crop residues for the production of bioethanol are in agreement with earlier reports by Rana *et al.* (2014) and Ahmed El-Imam *et al.* (2019a). Using *Saccharomyces cerevisiae*, wet exploding corn stover fermentation produced a maximum bioethanol concentration of 15.6 g/L (Rana *et al.*, 2014). A maximum bioethanol concentration of 24.35 g/L was produced by the fermentation of sorghum bran enzymatic hydrolysate employing *Kluyveromyces marxianus* (Ahmed El-Imam *et al.*, 2019a). The ethanol yields recovered from lignocellulosic feedstocks are influenced by variables including temperature, fermentation duration, type of fermenting organism, carbon source concentration, and inoculum size (Hosny *et al.*, 2016), which may account for the discrepancies in results.

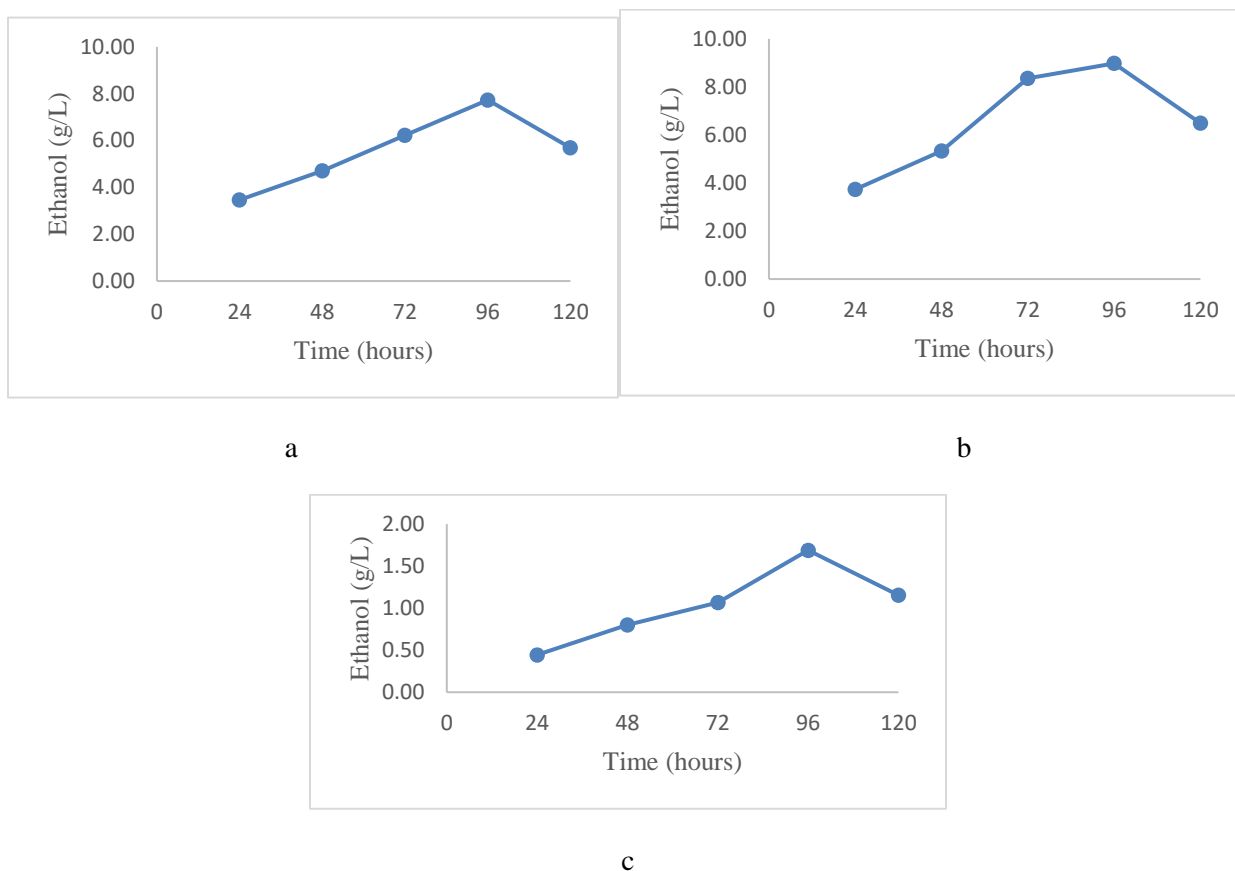


Figure 2: Trends in ethanol yields from different biomass (a): cassava peels (b): corn bran (c): millet bran.

After the fourth day of fermentation, it was noticed that the bioethanol concentrations for all the substrates decreased (Fig. 2), and this may be due to the accumulation of toxic substances in the fermentation medium (Zakpaa *et al.*, 2009). Due to the low sugar content of the biomass, millet bran hydrolysate fermentation produced the lowest ethanol concentration. This possibility is buttressed by the high sugar content of corn bran and cassava peel hydrolysate resulting in higher ethanol yields. This study successfully demonstrates that cassava peels, corn bran and millet bran can successfully be employed in clean bioethanol production with the additional benefit of the valorisation of these food processing wastes.

4. Conclusion

Lignocellulosic wastes, such as millet bran, corn cobs, and cassava peels, are frequently dumped into the environment, posing a pollution and esthetic risk. This study optimized the dilute acid hydrolysis process to examine the potential for these feedstocks to produce bioethanol. Cassava peels, corn bran, and millet bran all hydrolyzed to produce reducing sugar concentrations of 9.64 g/L, 11.10 g/L, and 3.41 g/L, respectively, in the hydrolysates. The sugar-rich hydrolysates were fermented, and from cassava peels, maize bran, and millet bran hydrolysates, respectively, a maximum bioethanol concentration of 7.73 g/L, 8.98 g/L, and 1.69 g/L was achieved. This study shows that it is feasible to convert these wastes into bioethanol, which might considerably improve the supply of alternative energy sources and the value chain for grains.

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