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Evaluating Acid and Alkali Pretreatment Methods for Optimizing Bioethanol Production from Rice Husks

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Abstract

The growing demand for sustainable energy sources has highlighted bioethanol as a promising alternative to fossil fuels due to its renewable nature and lower environmental impact. This study investigates the effectiveness of acid and alkali pretreatment methods in enhancing bioethanol production from rice husks which is a readily available agricultural waste. Saccharomyces cerevisiae, a yeast isolated from palm wine, was employed for the fermentation process. Rice husks were pretreated with dilute sulfuric acid and sodium hydroxide, respectively, to facilitate hydrolysis and the subsequent conversion of cellulose to fermentable sugars. The fermentation parameters, including ethanol yield, glycerol production and inhibitor concentrations, were analysed using gas chromatography-mass spectrometry. Results indicated that alkali pretreatment consistently yielded higher ethanol production compared to acid pretreatment, with maximum ethanol concentrations of 20.13 mg/L and 17.59 mg/L, respectively. Additionally, glucose levels were higher in acid hydrolysates, while xylose and arabinose levels were elevated in alkali hydrolysates. The study also revealed significant variations in inhibitor concentrations, with acetic and formic acids present in both hydrolysates and furfural detected only in acid hydrolysates on the final day of fermentation. However, the findings support the potential of rice husks as a viable substrate for bioethanol production, thus emphasizing the importance of pretreatment methods in optimizing yield. Moreover, the co-inoculation of S. cerevisiae with pentose-metabolizing organisms is suggested to further enhance bioethanol yield and reduce by-product formation. Following this, the research contributes to the sustainable utilization of agricultural waste while advancing the understanding of lignocellulosic biomass conversion for bioethanol production.

Keywords: Acid and alkali pretreatment; Bioethanol production; Rice husks; *Saccharomyces cerevisiae.*

Introduction

The quest for sustainable energy sources has intensified in response to the depletion of fossil fuels and the environmental challenges associated with their continued use. Among the alternatives, bioethanol stands out as a promising solution due to its renewable nature and lower environmental impact. Derived from plant sugars, bioethanol offers a clean and sustainable alternative to petroleum-based fuels, with advantages including lower toxicity, easy biodegradability, and reduced greenhouse gas emissions (Vasic et al., 2021). According to Aliyu et al. (2021), the exploration of bioethanol as a viable alternative to traditional fossil fuels has garnered significant attention in recent years. As noted by Zuccotti & Fabiano (2011), bioethanol being a volatile and flammable liquid, is derived from the fermentation of sugars by microorganisms and their enzymes. With properties such as a density of 0.792 g/cm3 at 15.5˚C and miscibility in water and non-polar solvents, bioethanol offers promise as a versatile resource (Worfa et al., 2017). Notably, its lower evaporation heat, reduced hygroscopicity, and reasonable heat of combustion make it an attractive option (Sebayang et al., 2016). Beyond its industrial applications as engine fuel and fuel additive, bioethanol finds utility in various sectors. In medicine, it serves as an antiseptic in wipes and hand sanitizers, and even as an antidote for certain poisonings (Trevedi et al., 2021). Additionally, it features in liquid preparations for pharmaceuticals and as an antimicrobial preservative in numerous medicines (Zuccotti & Fabiano, 2011).

For Bajpai (2020), the history of bioethanol usage traces back to the 19th century, with periodic fluctuations in its prominence driven by factors like taxation, fuel shortages

and technological advancements. While its adoption has been sporadic, regions like Sub-Saharan Africa have demonstrated early successes, particularly with plants utilizing sugar cane molasses (Deenath et al., 2012). At the moment, bioethanol production has surged globally, with the United States and Brazil leading the charge (Sajid et al., 2021). On this, Martins et al. (2018) note that Brazil, in particular, boasts a mature ethanol industry, primarily derived from sugarcane. However, challenges such as economic downturns and adverse weather patterns have impacted production in both countries. In Europe, nations like Germany, Sweden, and France have embraced bioethanol, albeit with varying degrees of self-sufficiency (Bertrand et al., 2016). Meanwhile, emerging economies like Nigeria are investing heavily in bioethanol infrastructure, primarily utilizing first-generation feedstocks such as cassava and sugarcane (Adewuyi, 2020).

Although, the classification of bioethanol into first and second generations reflects evolving technologies and concerns. First-generation bioethanol, derived from edible crops, has faced criticism for its potential impact on food security and deforestation (Renzaho et al., 2017; Saini et al., 2022). Second-generation bioethanol, produced from non-food biomass, offers promise in addressing these concerns, albeit with its own set of challenges including high capital costs and slower yields (Padella et al., 2019).

Despite its potential, widespread adoption of bioethanol faces hurdles such as cost-effectiveness and scalability (Sandesh & Ujwal, 2021). Nonetheless, ongoing research and technological advancements hold promise for bioethanol to emerge as a sustainable alternative to conventional fuels.

Critical to bioethanol production is the conversion of lignocellulosic biomass, a process involving hydrolysis and fermentation (Lamichhane et al., 2021). Pretreatment of biomass, through physical, chemical, or biological means, enhances hydrolysis efficiency by altering biomass structure and composition (Madadi et al., 2017). Following this, the utilization of lignocellulosic biomass, such as agricultural and industrial waste materials, for bioethanol production has garnered significant attention. These biomass sources, including rice husks, wheat straw and sugarcane bagasse, present abundant and inexpensive feedstocks for bioethanol production (Deshavath et al., 2019). Essentially, rice husk, in particular, holds promise as a viable substrate for bioethanol production, given its widespread availability and high cellulose content.

In Nigeria, a country experiencing rapid growth in rice production, rice husks represent a substantial biomass resource. However, efficient bioethanol production from rice husks necessitates effective pretreatment methods to enhance the accessibility of cellulose for enzymatic hydrolysis. Acid and alkali pretreatment methods have emerged as viable approaches to break down the lignin barrier and facilitate the conversion of cellulose to fermentable sugars (Loow et al., 2016).

In the light of this, the study is aimed at investigating the efficacy of acid and alkali pretreatment methods for enhancing bioethanol production from rice husks. Saccharomyces cerevisiae, a commonly used ethanol-producing yeast isolated from palm wine was employed for fermentation. Therefore, by evaluating the fermentative ability of S. cerevisiae and analyzing the composition of rice husk hydrolysate, the research seeks to optimize the bioethanol production process and contribute to the sustainable utilization of agricultural waste resources.

Materials and Methods

Sample Collection and Processing

Rice husks were collected from Ekpoma in Edo State and taken to the laboratory for analyses. The rice husks were washed and oven dried at 60oC for 48 hours to reduce the moisture content. The oven-dried husk was then made into powder by grinding using an electric grinder and then sieved though a mesh sieve. The powdered rice husks were stored in a sealed plastic jar in preparation for treatment.

Isolation of Saccharomyces cerevisiae

The yeast, Saccharomyces cerevisiae was isolated from palmwine obtained in Obiaruku, Delta state. It was collected within 60 minutes of tapping in clean containers and transferred to the laboratory. Colonies were purified by subculturing onto freshly prepared media. The palmwine was serially diluted to a dilution factor of 105 which was then plated onto PDA + C medium containing - Potato extract (4.0g); Dextrose (20.0g); Agar (15.0g); Distilled water (1000ml); pH (5.6) and Chloramphenicol (0.05mg/ml). The plates were incubated at room temperature (28± 20C) for 48 hrs. Colonies suggestive of yeasts were identified by microscopic examination after which were purified by subculturing on PDA plates. The species was identified as Saccharomyces cerevisiae by studying morphological, biochemical and physiological characteristics (Frazier & Westhoff, 1998; Barnett & Hunter, 1998).

Microscopy Examination

A thin smear was prepared by emulsifying a loopful of an isolate on a clean slide with a drop of water. The film was spread to make a thin film and then air dried after which it was stained with a methylene blue dye and observed with a light microscope under X10 and X40 objective lenses (Frasier & Westhoff, 1998).

Carbohydrate fermentation by yeast isolate

Selected isolate was tested for its ability to ferment carbohydrate. Carbohydrates tested were glucose, sucrose, maltose and lactose. Forty-eight (48) hour old Isolate was inoculated into four different labeled test tubes, each containing an inverted Durham tube and the different fermentation broth. The medium was prepared with peptone (10g), NaCl (5g), sugar (10g) and Phenol red (0.25%) in 1000ml of distilled water. Broth was autoclaved at 121 °C for 15minutes and cooled before inoculation. Inoculated test tubes were incubated at 370C for 48hours. Trapping of gas in the Durham tubes was observed.

Acid hydrolysis of rice husk

Acid hydrolysis was carried out by soaking 10.00 g of the powdered rice husk in 100.00 ml of 0.5% H2SO4 for 24 hours. After which it was filtered and pH checked, then autoclaved at 1210C for 15 minutes. Stainless steel containers were used for the pretreatment to avoid corrosion (Lopez et al., 2011).

Alkali hydrolysis of rice husk

The powdered rice husk was pretreated with dilute sodium hydroxide (NaOH) by adding 10.00 g of the powdered rice husk to a beaker containing 1.00 ml of NaOH and 100.00 ml of water was added. The beaker was then closed and maintained at 100oC for 10 hours. The alkali solution was then filtered pH checked and filtrate autoclaved at 1210C for 15 minutes (Sun et al., 1995).

Detoxification

Detoxification was carried out by the rapid addition of Ca(OH)2 to the hydrolysates. The hydrolysates were held at 25oC in a temperature controlled water bath and mixed vigorously during the rapid addition of Ca (OH)2. After 30 min of incubation, the hydrolysate was cooled in a second water bath and used immediately for fermentation (Chandel et al., 2011).

Fermentation

The fermentation was carried out using a submerged fermentation process. The fermentation was small scale, carried out in Erlenmeyer flasks, each containing 100.00 ml of fermentation medium. Flasks were inoculated with 1% v/v of S. cerevisiae and incubated at 30oC using a rotary shaker at 180 rpm for 5 days (Fernanda et al., 2011). The flasks kept shaking to produce a homogenous solution and even distribution of the organisms in the substrates mixture.

Determination of fermentation parameters

Fermentation parameters including ethanol production, glycerol production, glucose, xylose, arabinose, furfural, HMF, acetic acid and formic acid was determined. 5.00 ml of the Samples was taken at 24-hour interval from day 0 to the final day of incubation and analyzed for the parameters mentioned above. Analysis was carried out using the gas chromatography mass spectrometry.

Gas chromatography – Mass spectrometry

All samples were analyzed on the HS-GC–MS instrumentation described with a headspace oven temperature of 50°C. The HS loop and transfer line temperatures were set at 70°C and 90°C, respectively. Vial equilibration was set at 20 min. The vial pressurization was set at 15 psi for 0.15 min. Injection, loop fill, and loop equilibration times were set at 0.50, 0.15, and 0.05 min, respectively. Multi HS Extraction and vial shaking were set to off. The GC cycle time was set at 13.5 minutes. For the GC, a constant helium flow rate of 3 mL/min was used. The injection port temperature was maintained at 90°C with a 5:1 split injection of the headspace and a septum purge flow of 3 mL/min. The initial GC oven temperature of 35°C was held for 2 min and then ramped at 25°C/min to a final temperature of 250°C, which was held for 8.4 min. The total GC run time was 19min/sample. Both restrictors were set at a constant helium flow of 2 mL/min. The MS transfer line was maintained at 280°C. The MS source and quadrupole were maintained at 230°C and 150°C, respectively. The MS electron multiplier voltage was set to a gain factor of 1 (tuned using Agilent Chemstation Gain Tune followed by Low Mass Auto Tune). The scan range was set at 20 to 700 with a threshold of 150 and a sample number of 4, which resulted in a scan rate of 2.02 scans/s (Tiscione et al., 2011).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, Version 25.0) was used for the analysis of data obtained.

Results

Cultural and Microscopic Characteristics of Fungal Isolate

Table 1 displays the cultural and microscopic characteristics of the fungal isolate observed after three days of incubation at room temperature (28 ± 20°C). The colonies exhibited a unique earthy smell, creamy texture, ovoid shape, raised elevation, and occurred singly. Microscopic observation revealed ovoid-shaped cells ranging from 2 to 6 micrometres with a multipolar budding pattern, and no spores were observed. The isolate fermented glucose, sucrose and maltose with gas production and lactose without gas production. Urea hydrolysis tested negative, with no colour change in the broth medium after incubation. The fungal isolate was identified as Saccharomyces cerevisiae.

Table 1: Cultural and Microscopic Characteristics of Fungal Isolate

Characteristics	Isolate
Cultural	White, creamy texture with raised elevation
Microscopic examination	Ovoid shape with multipolar budding pattern.
Carbohydrate Fermentation	
Glucose	+ with gas production
Sucrose	+ with gas production
Maltose	+ with gas production
Lactose	+ with no gas production
Urea Hydrolysis	-
Fungal isolate	Saccharomyces cerevisiae

Ethanol Yield During Fermentation

The ethanol yield during the fermentation process for both acid and alkali hydrolysates as depicted in figure 1 showed that no ethanol was produced on day 0. A gradual increase in ethanol yield was observed from day 0 to day 5, with alkali hydrolysates consistently yielding higher ethanol than acid hydrolysates.

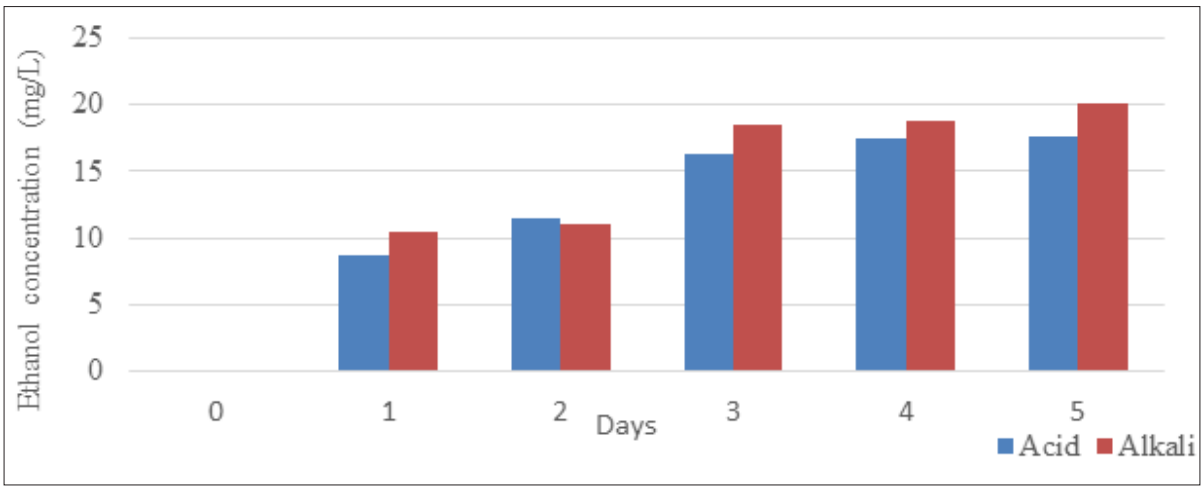

Figure 1: Ethanol yield during fermentation

Key:
Acid : acid hydrolysate
Alkali : alkali hydrolysate

Change in Parameters During Fermentation in Alkali Hydrolysates

Table 2 presents the changes in pH, ethanol and glycerol concentrations over the course of five days of fermentation in alkali hydrolysates. The pH decreased from 5.30 on day 0 to 5.00 on day 5, with ethanol and glycerol concentrations increasing significantly.

Table 2: Change in Parameters During Fermentation in Alkali Hydrolysates

Days	pH	Ethanol (mg/L)	Glycerol (mg/L)
0	5.30	-	0.06 ± 0.02
1	5.20	10.47 ± 0.02	0.80 ± 0.02
2	5.15	11.02 ± 0.02	1.69 ± 0.01
3	5.10	18.47 ± 0.04	5.87 ± 0.04
4	5.06	18.81 ± 0.03	5.96 ± 0.02
5	5.00	20.13 ± 0.03	6.12 ± 0.02

Change in Parameters During Fermentation in Acid Hydrolysates

Table 3 shows the variations in pH, ethanol, and glycerol concentrations during five days of fermentation in acid hydrolysates. Similar to the alkali hydrolysates, the pH decreased over time, with a notable increase in ethanol and glycerol concentrations.

Table 3: Change in Parameters During Fermentation in Acid Hydrolysates

Days	pH	Ethanol (mg/L)	Glycerol (mg/L)
0	5.10	-	0.69 ± 0.03
1	5.00	8.76 ± 0.02	0.88 ± 0.02
2	4.96	11.44 ± 0.03	0.59 ± 0.08
3	4.90	16.27 ± 0.02	0.71 ± 0.04
4	4.87	17.40 ± 0.01	0.86 ± 0.02
5	4.85	17.59 ± 0.05	2.71 ± 0.03

Glucose Concentration During Fermentation

Figure 2 depicts the daily glucose concentration throughout the fermentation process. Acid pretreatment yielded higher glucose amounts compared to alkali pretreatments initially, but the glucose levels decreased significantly by day 5.

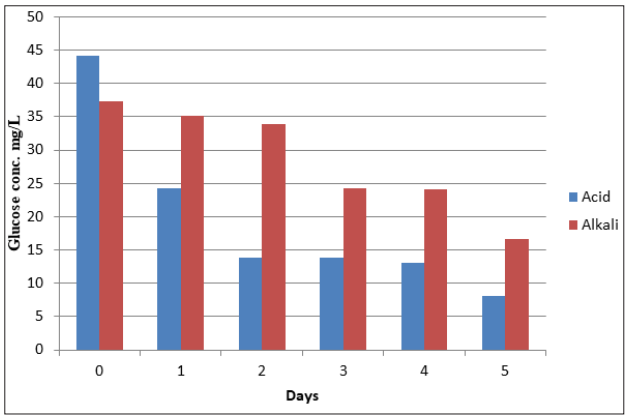

Figure 2: Glucose concentration during fermentation

Xylose Concentration During Fermentation

Figure 3 shows the xylose concentration during the fermentation process. Xylose yields were higher in alkali pretreatments compared to acid pretreatments, with a significant reduction in concentration by day 5.

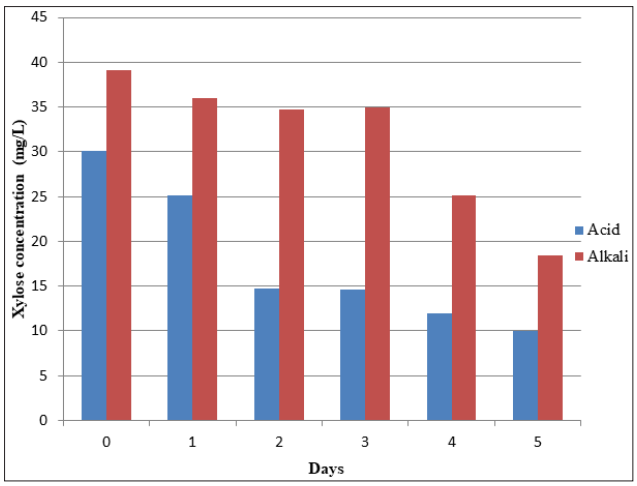

Figure 3: Xylose concentration during fermentation

Arabinose Concentration During Fermentation

Figure 4 illustrates the arabinose concentration throughout the fermentation process. Similar to xylose, arabinose yields were higher in alkali pretreatments and decreased over time.

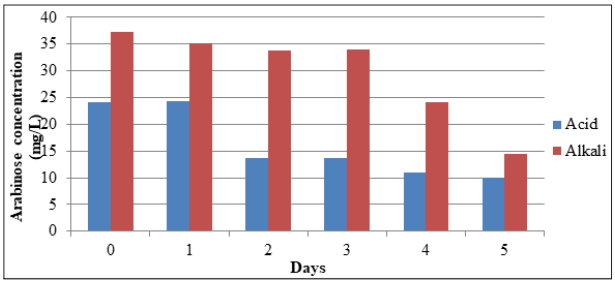

Figure 4: Arabinose concentration during fermentation

Mean Values for Inhibitor Concentration in Acid Hydrolysate (mg/L)

Table 4 summarizes the concentration of inhibitors such as formic acid, acetic acid, and furfural in acid hydrolysates. The concentrations of formic and acetic acids increased from day 0 to day 5, with furfural production observed only on day 5.

Table 4: Mean Values for Inhibitor Concentration in Acid Hydrolysate (mg/L)

Days	Formic Acid (mg/L)	Acetic Acid (mg/L)	Furfural (mg/L)
0	0.48 ± 0.03	0.89 ± 0.05	-
1	0.68 ± 0.04	1.08 ± 0.08	-
2	1.40 ± 0.05	1.77 ± 0.02	-
3	1.42 ± 0.01	1.79 ± 0.05	-
4	1.51 ± 0.05	1.88 ± 0.05	-
5	4.54 ± 0.02	4.92 ± 0.03	0.02 ± 0.01

Mean Values of Inhibitors Concentration in Alkali Hydrolysate (mg/L)

Table 5 presents the inhibitor concentrations in alkali hydrolysates. Formic acid levels increased from day 0 to day 5, with acetic acid production observed from day 1 onwards.

Table 5: Mean Values of Inhibitors Concentration in Alkali Hydrolysate (mg/L)

Days	Formic Acid (mg/L)	Acetic Acid (mg/L)
0	0.84 ± 0.03	
1	1.40 ± 0.01	1.66 ± 0.01
2	1.49 ± 0.04	1.86 ± 0.02
3	1.57 ± 0.03	2.26 ± 0.04
4	1.68 ± 0.06	3.04 ± 0.04
5	1.89 ± 0.03	3.26 ± 0.06

Discussion

In this study, rice husk was subjected to acid and alkali hydrolysis using dilute sulfuric acid and sodium hydroxide, respectively, before fermentation. Two distinct fermentation setups for acid and alkali hydrolysates were used, with temperature maintained at 28±20°C. The parameters measured included pH, the type and amount of reducing sugars, ethanol production, and the types and amounts of inhibitors produced. From the study, it was revealed that glucose, xylose and arabinose were the primary sugars obtained from rice husk pretreatment. Acid hydrolysates yielded higher glucose levels (44.13±0.05 mg/L) compared to alkali hydrolysates (37.34±0.04 mg/L). These findings align with the results of (Moreira et al., 2021), who observed higher glucose yields from acid hydrolysis of rice husk than from alkali hydrolysis. However, xylose and arabinose yields were higher in alkali hydrolysis which was in conformity with the findings of (de Figueiredo et al., 2017) in their study on lignocellulosic material breakdown by hydrolysis. The consumption rate for all sugars exceeded 50%, indicating significant utilization during fermentation. Notably, the S. cerevisiae strain used was a wild type, not genetically engineered, which complicates attributing pentose sugar utilization to this yeast. This is because the wild type S. cerevisiae lacks the necessary enzymes, such as transketolase and transaldolase for pentose metabolism (Kwak & Jin, 2017). Besides, it is more plausible that the pentose sugars decomposed into inhibitors, as previously noted by Moreira et al. (2021), although further investigation is required to confirm this. The pH during fermentation ranged from 4.85 to 5.30, with the highest ethanol yield observed at pH 5.0, supporting the notion that yeast thrives in slightly acidic conditions. According to Malik et al. (2022), acidic environments minimize contamination from competing microbes and enhance ethanol productivity. Although, Qin et al. (2020) maintain that both overly acidic and basic conditions hinder yeast metabolic pathways by reducing cell growth and ethanol production. Following this, Tang et al. (2017) assert that increased pH encourages microbial growth while adversely affecting fermentation. For Kim (2018), chemical pretreatment's primary drawback is sugar degradation and the formation of undesirable by-products, such as inhibitors and deactivators. The type and number of inhibitors depend on the lignocellulosic feedstock and pretreatment method used (Meenakshisundaram et al., 2021; Rajendran et al., 2018). In this study, inhibitors detected included acetic acid, formic acid and furfural, with no hydroxymethylfurfural (HMF) detected,

corroborating the findings of (Moreira et al., 2021). Furfural was observed in the acid hydrolysate only on day 5 (0.02±0.01 mg/L), likely due to the liming process with Ca(OH)2 before fermentation. However, this contrasts with the results of (Giraldeli et al., 2019), who found that HMF was the most potent inhibitor of H2 production and promoted the lactate and ethanol pathways. Furthermore, glycerol, a major by-product during alcoholic fermentation, increased steadily, with alkali hydrolysate producing the highest glycerol levels (6.12±0.02 mg/L). This is consistent with its role in osmoregulation and cellular redox balance (Mutton et al., 2019; Zhao et al., 2015). On the other hand, ethanol yield was higher in alkali hydrolysates (20.13±0.03 mg/L) than in acid hydrolysates (17.59±0.05 mg/L). this observation was in consonance with the findings of (Santos et al., 2021). In addition, the study agrees with Nikzad et al. (2013) who worked on different pretreatment methods of rice husk and observed that alkali (dilute NaOH) pretreatment produced higher ethanol yields than acid (dilute H2SO4) pretreatment. Although, Rahmani et al. (2022) note that dilute NaOH effectively enhances lignocellulosic digestibility by altering cellulose structure and separating lignin-carbohydrate linkages. Zhu et al. (2020) corroborated this view noting that NaOH pretreatment of lignocellulose converts lignin to aromatic monomers and alters cellulose structure, potentially enhancing digestibility.

Conclusion

In conclusion, the study supports the potential for bioethanol production from rice husk hydrolysates, with alkali pretreatment yielding higher pentose sugars and acid pretreatment yielding higher glucose. The co-inoculation of S. cerevisiae with pentose-metabolizing organisms could enhance bioethanol yield and reduce by-products like glycerol. Essentially, the study presents novel insights into the optimization of bioethanol production from rice husks using microbiological approaches. This posits that by evaluating the effectiveness of acid and alkali pretreatment methods and characterizing the fermentative behaviour of Saccharomyces cerevisiae, the research is targeted at advancing the overall understanding of lignocellulosic biomass utilization for bioethanol production.

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