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Bioethanol Production from Graminoid Weeds in Bayero University Kano: A Potential Feedstock for Future Sustainable Aviation Fuel Applications

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Abstract: This study evaluates the potential of selected graminoid weeds from Bayero University, Kano (BUK), Nigeria, as feedstock for bioethanol production. Ten (10) abundant weed species, including Cenchrus species, Cynodon dactylon, and Setaria barbata, were subjected to physicochemical pretreatment (ultrasonication, hydrothermal treatment, and enzymatic hydrolysis) followed by fermentation using Bacillus subtilis, Saccharomyces cerevisiae, and their co-culture. Pretreatment significantly enhanced reducing sugar yield, increasing to a range of 19.2–24.43 mg/g, with S. barbata recording the highest value. Bioethanol production varied across substrates and microbial systems, with Cenchrus species yielding the highest ethanol concentration of 29.87% under co-culture conditions, compared to 26.84% and 26.83% obtained using S. cerevisiae and B. subtilis, respectively. In contrast, Dactyloctenium aegyptium recorded the lowest yields (20.68–22.91%). Statistical analysis showed significant differences ($p<0.05$) among the treatments. The enhanced performance observed in co-culture fermentation suggests improved substrate utilization and process efficiency. These findings demonstrate that graminoid weeds can serve as a viable, low-cost biomass resource for bioethanol production, which can serve as a precursor for sustainable aviation fuel generation via the Alcohol-to-Jet pathway in subsequent stages of the research.

Keywords: Bioethanol, Graminoid Weeds, Fermentation, Saccharomyces Cerevisiae, Bacillus Subtilis

Introduction

A sustainable and reliable energy supply is a fundamental requirement for human development. However, the continuous increase in global population has intensified energy demand, placing considerable pressure on the limited natural resources, particularly fossil fuels (Olujobi, 2023). The environmental consequences associated with fossil fuel utilization, including greenhouse gas emissions and climate change, further highlight the need for cleaner and more sustainable energy alternatives (Tope et al., 2023; Olujobi et al., 2023). Renewable energy sources such as solar, wind, hydropower, and biomass are widely recognized as viable substitutes due to their reduced environmental impact (Awoyale and Lokhat, 2021). Biofuels, which are fuels derived from biomass, have gained significant attention as sustainable energy carriers capable of reducing dependence on fossil resources (Dong et al., 2022; Obi et al., 2023). They include bioethanol, biodiesel, and biogas, each offering distinct advantages depending on their application (Olujobi et al., 2023). Among these, bioethanol is one of the most widely produced and utilized liquid biofuels globally, particularly in the transportation sector, where it enhances combustion efficiency and reduces exhaust emissions (Ali et al., 2014). In addition to its direct use as a fuel, bioethanol also serves as an important intermediate in advanced fuel technologies such as the Alcohol-to-Jet (ATJ) pathway for sustainable aviation fuel production (Obi et al., 2023; Olujobi et al., 2023).

Bioethanol is typically produced through microbial fermentation of fermentable sugars obtained from biomass (Zhang et al., 2023). During this process, microorganisms metabolize simple sugars such as glucose under anaerobic conditions, converting them into ethanol and carbon dioxide. Different microorganisms possess varying metabolic capabilities; for example, Saccharomyces cerevisiae is highly efficient in ethanol production from hexose sugars, while Zymomonas mobilis offers high conversion efficiency with reduced biomass formation (Zhang et al., 2023; Naik et al., 2010). Although Bacillus subtilis is not a conventional ethanol producer, it contributes to biomass degradation and can enhance overall substrate utilization when used in co-culture systems. Therefore, the selection and combination of microorganisms play a critical role in improving bioethanol yield and process efficiency (Obi et al., 2023).

The production of bioethanol from lignocellulosic biomass involves a series of processes, including pretreatment, hydrolysis, and fermentation. Pretreatment is particularly important, as it disrupts the rigid structure of lignocellulose by breaking down lignin and hemicellulose components, thereby increasing the accessibility of cellulose to enzymatic action (Naik et al., 2010; Zhang et al., 2023). This facilitates the release of fermentable sugars, which are subsequently converted into ethanol during fermentation (Zhang et al., 2022; Kumar et al., 2021; Gupta et al., 2022). The integration of efficient pretreatment methods with optimized fermentation strategies is essential for improving overall bioethanol production (Naik et al., 2010; Obi et al., 2023).

Considerable research has focused on conventional lignocellulosic feedstocks such as agricultural residues and energy crops (Zhang et al., 2023). However, herbaceous weeds remain largely underexplored despite their widespread availability and minimal economic value. These plants are typically regarded as unwanted vegetation and are commonly found along roadsides, industrial areas, residential environments, and institutional premises, where they often create management challenges (Gao and Su, 2024; Lewandrowski et al., 2020). In Northwestern Nigeria, particularly within Bayero University Kano (BUK), herbaceous weeds are abundant and are frequently removed through clearing or open burning, contributing to environmental pollution without any beneficial utilization (Zhang et al., 2023; Ogbonda and Kiin-Kabari, 2013). Harnessing these weeds as feedstock for bioethanol production presents a dual advantage: It provides a low-cost renewable energy resource while addressing waste management challenges (Damayanti et al., 2012; Zhang et al., 2023). In addition, the conversion of such biomass into bioethanol supports emerging applications such as sustainable aviation fuel production, thereby contributing to global efforts toward decarbonization of the energy sector.

Despite existing studies on lignocellulosic bioethanol production, limited attention has been given to graminoid weeds within institutional environments in Nigeria. Furthermore, the combined use of Bacillus subtilis and Saccharomyces cerevisiae for improving bioethanol yield from such feedstocks remains insufficiently explored (Dong et al., 2022; Zhang et al., 2023). This study addresses these gaps by evaluating the bioethanol production potential of selected graminoid weeds collected from Bayero University Kano using single and co-culture fermentation systems. Therefore, the main objective of this study is to assess the suitability of locally available graminoid weeds as feedstock for bioethanol production through integrated pretreatment and microbial fermentation processes. Specifically, the study aims to determine the reducing sugar yield following pretreatment, evaluate bioethanol production using different microbial systems, and compare the efficiency of single and combined cultures in enhancing ethanol yield. The findings are expected to provide new insights into the utilization of underused biomass resources for sustainable energy production and contribute to the advancement of biofuel technologies.

Materials and Methods

Samples Collection and Identification

The study area (BUK, located at 12.0453°N, 8.5235°E) was surveyed, and ten (10) most abundant graminoid weed species were selected. The selected weeds were clipped using scissors and placed in labeled paper bags and then transported to the Herbarium of the Department of Plant Biology, BUK, where they were identified.

Pretreatment of the Sampled Plant Biomass

The samples were thoroughly washed to remove any contaminants and soil particles and air-dried for 5 days. The drying was done under shade in a clean, well-ventilated area to prevent mould growth and ensure uniform drying. After drying, grinding was performed using a blender (Preethi Taurus, MGA-217, India) and then sieved using a 2 mm mesh size sieve to achieve a uniform particle size, which is important for consistent and efficient processing in later stages of bioethanol production.

Proximate Analyses

These analyses were carried out to determine the concentrations of the various proximate parameters, including moisture, ash, crude fibre, crude protein, crude lipid, and carbohydrate, according to the Association of Official Analytical Collaboration, using analytical grade reagents.

Sourcing of the Ferming Microorganisms

Pure cultures of B. subtilis, A. niger, and S. cerevisiae were prepared at the Department of Microbiology, BUK, Nigeria. B. subtilis was aseptically inoculated into 100 mL of nutrient broth (containing 0.5% peptone, 0.3% yeast extract, and 0.5% NaCl), while 100 mL of Potato Dextrose Broth (containing 2% potato infusion and 2% dextrose) was used for A. niger, and 100 mL of Yeast Extract-Peptone-Dextrose (YPD) broth (containing 1% yeast extract, 2% peptone, and 2% dextrose) was used for S. cerevisiae, with 1% (v/v) inoculum size and incubated for 48 hours to develop pure suspensions of the individual organisms. A. niger was used in the pretreatment of the graminoid weed samples, while B. subtilis and S. cerevisiae were used in the fermentation process (Suranska et al., 2012; Duque et al., 2021; Chauhan et al., 2023; Ndudi et al., 2024).

Pretreatment of the Prepared Samples

This was carried out according to the method described by Debiagi et al. (2020). Twenty grams (20 g) of each powdered sample was suspended in 250 mL of distilled water. The suspensions were subjected to ultrasonic treatment using an ultrasonicator (SW-30 H, Switzerland). The sonication process was carried out at a frequency of 20 kHz and power of 200 W for 60 minutes. After ultrasonication, the suspensions were then autoclaved at 121ºC for 15 minutes for hydrothermal hydrolysis. The autoclaved suspensions were allowed to cool, and then inoculated with a pure culture of Aspergillus niger (obtained from the Department of Microbiology, BUK) for enzymatic hydrolysis. These were incubated at 37ºC for 5 days with periodic agitation (3 times a day), allowing the fungus to proliferate and produce the necessary hydrolytic enzymes (Ballesteros et al., 2014). After the hydrolysis period, the resulting mixture was filtered using a clean sieve (Hamouda et al., 2015).

Reducing Sugar Concentration of the Hydrolysate

This was carried out using UV-Spectrophotometry as described by Leonald et al. (2020). From a stock solution of 20 mmol/L, standard glucose concentrations of 0, 2.5, 5, 7.5 and 10 mmol/L were prepared, and 5 mL of each was transferred to a test tube. Then 2 mL of Benedict's reagent was added to each tube and placed in a boiling water bath for 5 minutes. Absorption in each tube was read with a spectrophotometer at 477 nm. The readings were used to plot a graph of absorbance against concentrations. The blank (i.e. 20 mmol/L), which was 10 mL distilled water and 2mL Benedict's reagent, was used to zero the spectrophotometer (UV670, Germany). In line with this, glucose concentrations in samples were determined by adding 2 mL Benedict's reagent in 5 mL of each filtrate, and the mixture was placed in a boiling water bath for 5 minutes. Absorption of a portion of each mixture was then read using a spectrophotometer at 477 nm, and the glucose concentration was extrapolated from the standard plot (Aggarwal et al., 2022).

Bioethanol Production

The hydrolysed samples were transferred to 500 mL capacity bottles, covered with cotton wool and aluminium foil, and autoclaved at 121ºC for 15 minutes. After autoclaving, the bottles were cooled to room temperature, and 20 mL suspensions of the pure culture of B. subtilis and S. cerevisiae were inoculated accordingly into the flasks containing the hydrolysate, and the setups were duplicated. The fermentation was conducted at a temperature ranging between 30°C and 37°C (Tenkolu et al., 2022); pH of 6.5 to 7.5 (Ridwan et al., 2023), for a 14-day retention time (Kumar et al., 2013), which were described to be suitable for bioethanol production with improved yield (Ogundari et al., 2022).

Determination of Concentration and Estimation of Bioethanol Produced

This was carried out using UV-VIS quantitative analysis of alcohol using dichromate reagent according to the method described by Sriariyanun et al. 2019). Various concentrations of the standard ethanol (0.2%, 0.4%, 0.6%, 0.8% and 1%) were prepared in test tubes, and 500µl of potassium dichromate (10%) solution was added to each concentration. These were heated at 60ºC to ensure the complete oxidation of ethanol to acetic acid and the reduction of dichromate to chromium (III). Sulfuric acid (5M) was then added to each concentration, and their absorbances were measured at 595nm wavelength using a spectrophotometer (UV VIS spectrophotometer - 6705 Germany), which was used to develop a standard curve. The samples were treated in a similar way, and their absorbances were also measured and used to determine the concentration of the bioethanol produced using the slope of the standard curve.

Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics (version 29.0, IBM Corp., Armonk, NY, USA). One-way Analysis of Variance (ANOVA) was used to evaluate differences among treatments. Where significant differences were observed, Tukey's post-hoc test was applied for multiple comparisons. Statistical significance was considered at $p<0.05$.

Results and Discussion

This section presents and interprets the findings from the evaluation of selected graminoid weeds as feedstock

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for bioethanol production, with emphasis on biomass composition, pretreatment efficiency, and microbial fermentation performance.

The distribution of graminoid weeds within Bayero University Kano (Fig. 1) highlights the predominance of Cenchrus species and Cynodon dactylon, indicating their strong adaptability to the local environment. Their abundance suggests a reliable and cost-effective biomass supply, particularly in resource-limited settings where feedstock availability is a major constraint in biofuel production. Similar observations have been reported in rangeland ecosystems in Maradi, Niger Republic (Alassan et al., 2019), and in floristic surveys conducted at the University of Abuja, Nigeria (Jimin et al., 2022).

The proximate composition of the selected species (Table 1) revealed appreciable carbohydrate content across most samples, with Dactyloctenium aegyptium and Bothriochloa ischaemum exhibiting the highest values. While carbohydrate content is fundamental for fermentable sugar generation, biomass digestibility is also influenced by structural components such as lignin and crude fibre. For instance, the relatively high fibre content observed in Cenchrus species may have provided substantial polysaccharide reserves while remaining amenable to breakdown during pretreatment. In addition, lower moisture content in some species may enhance processing efficiency by minimizing dilution effects and reducing the risk of microbial contamination during fermentation. Comparable bioethanol yields have been reported for other graminoids, including Pennisetum purpureum (18.6 g/L) and Saccharum spontaneum (22.5 g/L) (Olugbenga et al., 2018; Kumar et al., 2019).

The effectiveness of the pretreatment process is demonstrated by the significant increase in reducing sugar concentrations across all samples following ultrasonication and hydrothermal treatment (Fig. 2).

Table 1: Proximate Compositions of the Selected Graminoid Species (%)

Plant Samples	Moisture	Ash	Crude Protein	Crude Fibre	Fat Content	Carbohydrate
Cyperus rotundus	13.72	2.13	27.91	17.37	7.62	31.25
Setaria barbata	21.49	3.13	16.89	16.10	3.97	38.42
Dactyloctenium aegyptium	10.67	7.02	8.63	13.46	4.31	55.91
Bothriochloa ischaemum	12.63	5.08	9.33	10.67	6.96	55.33
Heteropogon contortus	12.70	2.74	7.27	15.97	10.55	50.77
Hyperrhenia hirta	9.87	5.21	6.67	17.02	9.33	51.90
Cenchrus species	11.69	3.93	17.34	19.22	7.05	40.77
Digitaria species	17.33	2.11	13.67	11.33	13.97	41.59
Eleusine indica	26.67	4.73	11.96	15.24	19.96	21.44
Cynodon dactylon	8.32	17.46	9.67	13.09	11.24	40.22
Andropogon species	23.35	11.41	6.22	21.67	7.33	30.02

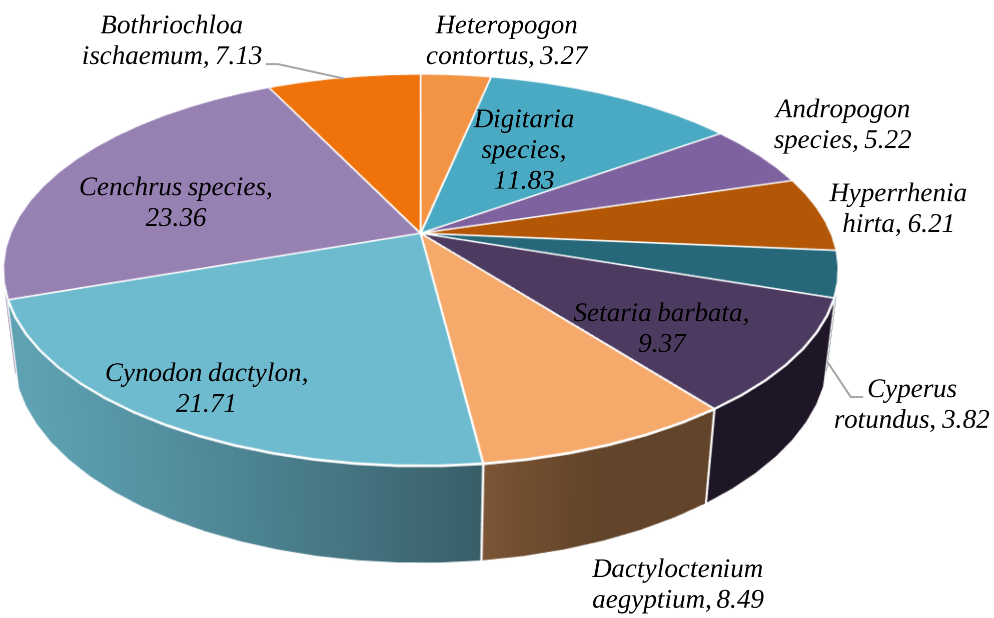

Fig. 1: Distribution of Graminoid Weeds in Bayero University Kano, Nigeria

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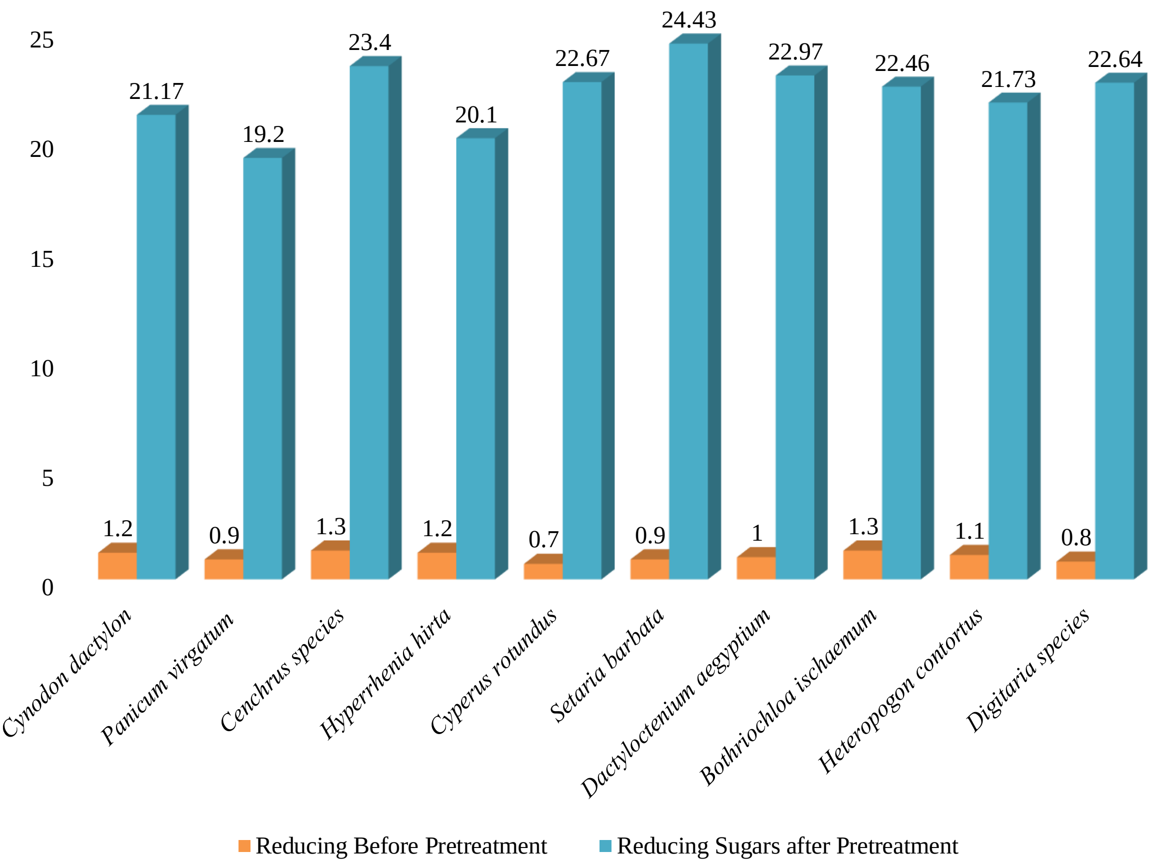

Fig. 2: Results of Reducing Sugars Yields from the Plant Samples Before and After Pretreatment

This indicates efficient disruption of the lignocellulosic matrix, resulting in enhanced enzyme accessibility and improved hydrolysis. Notably, Setaria barbata exhibited the greatest increase in reducing sugar yield, suggesting a biomass structure that is particularly responsive to physicochemical pretreatment. These findings are consistent with previous studies reporting the effectiveness of hydrothermal and ultrasonic pretreatment methods in improving sugar release from lignocellulosic materials (Alhassan, 2023; Yahaya et al., 2024; Zaafouri et al., 2016; Scherzinger et al., 2020). In addition, the role of microbial enzymes, particularly from filamentous fungi such as Aspergillus spp., has been widely recognized in enhancing biomass degradation and facilitating fermentable sugar production (Behera et al., 2017; Moretti et al., 2012).

Bioethanol production results (Fig. 3) revealed marked variation among the tested species and fermentation systems. Cenchrus species consistently produced the highest ethanol yield, particularly under co-culture conditions, whereas Dactyloctenium aegyptium recorded the lowest yield. These differences can be attributed to variations in substrate composition, sugar availability, and the presence of inhibitory compounds. The superior performance of Cenchrus species suggests an optimal balance between carbohydrate content and structural characteristics, enabling efficient conversion during both hydrolysis and fermentation stages. Previous studies have similarly demonstrated the advantages of co-culture systems in enhancing bioethanol production. For example, co-culturing Saccharomyces cerevisiae with Wickerhamomyces anomalus increased ethanol yield from 29% to 53% (Li et al., 2020), while other combinations, including Kluyveromyces marxianus with Bacillus coagulans, have shown substantial improvements in substrate utilization and product yield (Kumar et al., 2022; Narisetty et al., 2022).

The co-culture of Bacillus subtilis and Saccharomyces cerevisiae in this study resulted in the highest ethanol yield, indicating a synergistic interaction between the organisms. While S. cerevisiae is primarily responsible for ethanol fermentation, B. subtilis likely enhances substrate hydrolysis and increases the availability of fermentable sugars. This complementary interaction promotes more efficient substrate utilization and reduces the limitations commonly associated with single-strain fermentation systems.

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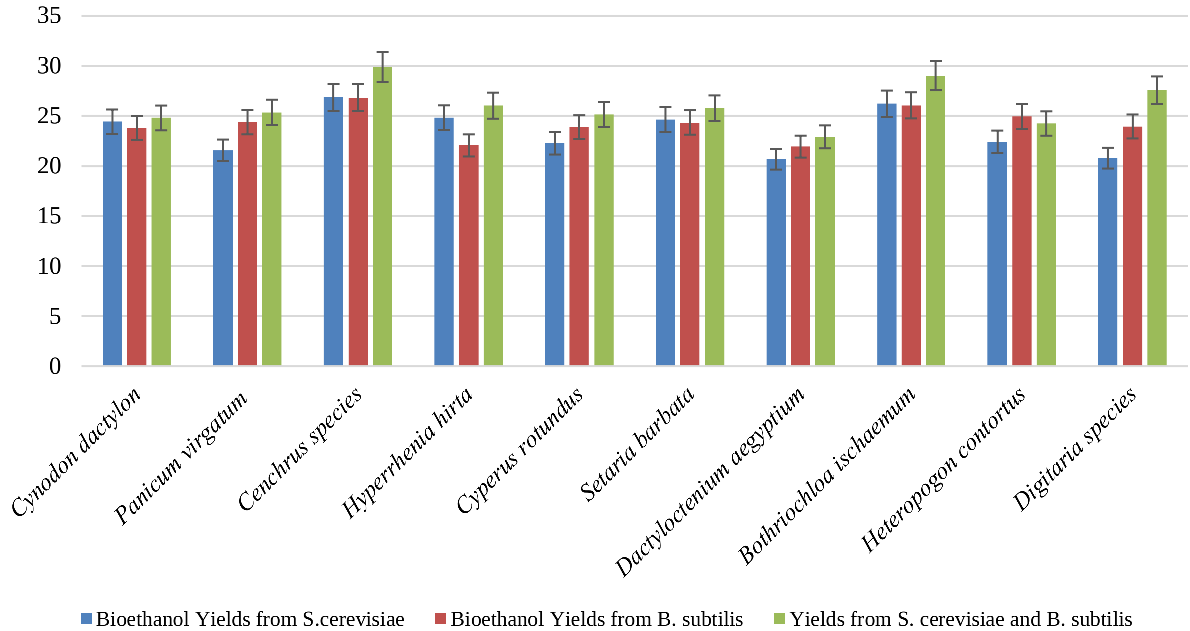

Fig. 3: Results of Bioethanol Production from the Graminoid Weed Samples using B. Subtilis and S. cerevisiae

Statistical analysis ($p<0.05$) confirmed that the differences observed in both reducing sugar yield and ethanol production were significant across the different species and microbial treatments. This indicates that both feedstock type and fermentation strategy are critical determinants of process performance. The results emphasize the need for careful selection of biomass and microbial systems in order to achieve optimal outcomes.

From an application perspective, the results demonstrate that locally available graminoid weeds can serve as viable feedstock for bioethanol production. This has important implications for waste management and renewable energy development, particularly in regions where such biomass is abundant but underutilized. The conversion of these weeds into bioethanol not only provides an alternative energy source but also reduces environmental pollution associated with their disposal. Furthermore, the produced ethanol has potential for downstream applications, including its use as a precursor in Alcohol-to-Jet fuel production, thereby contributing to sustainable aviation fuel development. Overall, this study highlights the potential of integrating effective pretreatment methods with co-culture fermentation systems to enhance bioethanol production from unconventional biomass sources. The findings contribute to ongoing efforts to diversify feedstock options and improve the sustainability of biofuel production systems.

Strengths and Limitations of the Study

This study demonstrates several strengths, including the utilization of locally abundant and underexplored graminoid weeds as feedstock, the integration of physicochemical and biological pretreatment methods, and the application of co-culture fermentation to enhance ethanol yield. These approaches contribute to improving biomass valorization and sustainable biofuel production. However, some limitations should be acknowledged. The study was conducted at laboratory scale without process optimization or scale-up validation. In addition, no techno-economic analysis was performed to assess commercial feasibility. Furthermore, while bioethanol was identified as a potential precursor for sustainable aviation fuel via the Alcohol-to-Jet pathway, no downstream conversion or fuel characterization was conducted. Future studies should address these limitations by incorporating process optimization, scale-up experiments, and fuel conversion analysis.

Conclusion

This study demonstrates that graminoid weeds from Bayero University Kano represent a viable and sustainable feedstock for bioethanol production. Among the species evaluated, Cenchrus species exhibited the highest ethanol yield, particularly under co-culture fermentation using Bacillus subtilis and Saccharomyces cerevisiae, confirming the effectiveness of synergistic microbial interactions in enhancing substrate conversion efficiency. In contrast, Dactyloctenium aegyptium showed comparatively lower performance, highlighting the influence of feedstock composition on bioethanol yield. The integration of physicochemical and enzymatic pretreatment significantly improved reducing sugar availability, thereby enhancing fermentation outcomes. Statistical analysis further confirmed that both biomass type and microbial strategy are critical determinants of process efficiency.

Overall, the findings highlight the potential of underutilized graminoid weeds as low-cost biomass resources for renewable energy production. Their conversion into bioethanol offers dual benefits of waste management and sustainable fuel generation, with potential for future downstream applications such as Alcohol-to-Jet fuel production, which was not investigated in the present study. Future research should focus on process optimization, scale-up, techno-economic assessment, and downstream conversion of bioethanol to aviation fuel via the Alcohol-to-Jet pathway, including fuel characterization.

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Declaration of AI Use

During the preparation of this work, the author(s) used Meta AI and ChatGPT in order to assist with language editing and structuring of this manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Author's Contributions

All the authors have contributed to aspects related to their respective expertise.

Ethics

All the ethical issues have been observed in the course of carrying out the present research.

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