International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 7, Issue. 3, May-June 2026, page no. 31-37

To cite this article: Hargono Hargono, Kristinah Haryani, Angga Satria Wibawa, Addila Arrofa'hiya Tri Atqiyani and Kevin Setiadi Seng (2026). ENHANCING BIOETHANOL PRODUCTION FROM SWEET SORGHUM: IMPACT OF METAL IONS SUPPLEMENTATION ON HYDROLYSIS AND FERMENTATION, International Journal of Applied Science and Engineering Review (IJASER) 7 (3): 31-37 Article No. 264 Sub Id 394

ENHANCING BIOETHANOL PRODUCTION FROM SWEET SORGHUM: IMPACT OF METAL IONS SUPPLEMENTATION ON HYDROLYSIS AND FERMENTATION

Hargono Hargono*, Kristinah Haryani, Angga Satria Wibawa, Addila Arrofa'hiya Tri Atqiyani and Kevin Setiadi Seng

Diponegoro University, Department of Chemical Engineering, Faculty of Engineering,
Semarang Prof. Soedarto Street 13, Central of Java, 50239, Indonesia,
*Corresponding author: hargono@che.undip.ac.id

DOI: https://doi.org/10.52267/IJASER.2026.7303

ABSTRACT

The growing demand for sustainable energy and the environmental concerns associated with fossil fuel consumption have encouraged the development of bioethanol from renewable biomass. This study investigates the production of bioethanol from sweet sorghum stalks through integrated pretreatment, enzymatic hydrolysis, and fermentation processes. The pretreatment stage involved drying, size reduction, and filtration to enhance hydrolysis efficiency. Enzymatic hydrolysis was conducted using cellulase (1%) at 60°C for 6 hours, with the addition of metal ions (Mg^{2+} and Zn^{2+} at concentrations of 0–0.6 g/L) to improve the conversion of cellulose into reducing sugars. The hydrolysate, with substrate concentrations of 80 and 100 g/L, was subsequently fermented for five days using Saccharomyces cerevisiae, with Fe^{2+} and K^{+} ions added to enhance ethanol production. The results showed that higher substrate concentration significantly increased reducing sugar levels, with the optimum obtained at 100 g/L. The addition of Mg^{2+} exhibited a more significant effect on sugar formation compared to Zn^{2+} during hydrolysis. In the fermentation stage, Fe^{2+} demonstrated a stronger influence on ethanol production than K^{+}. Overall, the combination of higher substrate concentration and metal ion supplementation effectively improved both hydrolysis and fermentation performance. These findings highlight the potential of sweet sorghum stalks as a promising lignocellulosic feedstock for bioethanol production and demonstrate that the use of metal ions as cofactors can enhance process efficiency in a more sustainable and cost-effective manner.

KEYWORDS: bioethanol, sweet sorghum, enzymatic hydrolysis, fermentation, metal ions, renewable energy

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 7, Issue.3, May-June 2026, page no. 31-37

1. INTRODUCTION

The increasing dependence on fossil fuels such as petroleum, coal, and natural gas has raised serious concerns regarding energy security and environmental sustainability. In many countries, including Indonesia, this dependence not only leads to instability in energy supply but also contributes significantly to greenhouse gas emissions and climate change. Consequently, the development of renewable and environmentally friendly energy sources has become a global priority. Among various alternatives, bioethanol has emerged as a promising renewable fuel due to its biodegradability, lower carbon emissions, and potential to be produced from abundant biomass resources.

Sweet sorghum (Sorghum bicolor) is considered one of the most attractive feedstocks for bioethanol production due to its high sugar and lignocellulosic content, rapid growth rate, and adaptability to diverse environmental conditions. The utilization of sweet sorghum stalks, in particular, offers significant potential for second-generation bioethanol production, especially in regions with abundant agricultural resources. However, the conversion of lignocellulosic biomass into bioethanol remains a complex process, requiring efficient pretreatment, hydrolysis, and fermentation steps to achieve optimal yields [1].

The production of bioethanol from sweet sorghum involves several key stages, including pretreatment, enzymatic hydrolysis, and fermentation. Pretreatment processes such as drying, size reduction, and filtration are essential to improve the accessibility of cellulose for enzymatic action. Enzymatic hydrolysis is then carried out to convert cellulose into fermentable sugars, typically using cellulase enzymes. However, the efficiency of hydrolysis is often limited by the recalcitrant structure of lignocellulosic biomass. To address this issue, the addition of metal ions such as Mg^{2+} and Zn^{2+} has been reported to enhance enzymatic activity and improve sugar yield [2].

Following hydrolysis, the resulting sugars are converted into ethanol through fermentation using microorganisms such as Saccharomyces cerevisiae. The performance of the fermentation process can be influenced by various factors, including substrate concentration, nutrient availability, and the presence of cofactors. Metal ions such as Fe^{2+} and K^{+} play important roles in microbial metabolism and can significantly affect ethanol production. Therefore, optimizing the concentration of these ions is crucial to improving fermentation efficiency.

Despite extensive research on bioethanol production, there is still a need to explore the combined effects of substrate concentration and metal ion addition on both hydrolysis and fermentation processes. Most studies have focused on individual process parameters, while integrated optimization remains limited. Therefore, this study aims to investigate the production of bioethanol from sweet sorghum stalks through

ISSN: 2582-6271

Vol. 7, Issue.3, May-June 2026, page no. 31-37

enzymatic hydrolysis and fermentation, with particular emphasis on the effects of substrate concentration and metal ion supplementation (Mg^{2+}, Zn^{2+}, Fe^{2+}, and K^{+}). The findings of this study are expected to contribute to the development of more efficient and sustainable bioethanol production processes from lignocellulosic biomass.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study were sweet sorghum stalk, distilled water, cellulase enzyme, KH_2PO_4, $MgSO_4$, $ZnSO_4$, $FeCl_2$, KCH_3COO, urea, baker's yeast, DNS reagent, NaOH, and glucose standard. The equipment used included a UV-Vis spectrophotometer, pH meter, Erlenmeyer flask, beaker glass, measuring pipette, dropper pipette, measuring cylinder, analytical balance, filter paper, and hydrolysis apparatus.

2.2 Methods

This study aimed to determine the effect of metal ions (Mg^{2+}, Zn^{2+}, Fe^{2+}, and K^{+}) on reducing sugar production and bioethanol yield from sweet sorghum stalk through enzymatic hydrolysis and fermentation processes. The experimental variables included metal ion types and concentrations (0; 0.2; 0.4; and 0.6 g/L), with reducing sugar content and bioethanol concentration as responses.

2.2.1 Pretreatment of Materials

Sweet sorghum stalks were dried under sunlight, ground into powder, and sieved to obtain uniform particle size.

2.2.2 Enzymatic Hydrolysis

A total of 20 and 25 g of sorghum powder was mixed with 250 mL distilled water in an Erlenmeyer flask. Cellulase enzyme (1%) and metal ions (Mg^{2+} or Zn^{2+}) were added with concentrations of 0; 0.2; 0.4; and 0.6 g/L. The mixture was heated at 60°C for 6 hours with continuous stirring to produce reducing sugars.

2.2.3 Starter Preparation

The starter was prepared by heating 70 mL of sweet sorghum juice until boiling and cooling it to room temperature (27°C). Nutrients (KH_2PO_4, $MgSO_4$, and urea) each 3.5 g were added, followed by pH adjustment to 5. Baker's yeast (5 g) was then added, and the mixture was incubated for 2 days.

2.2.4 Fermentation Process

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 7, Issue.3, May-June 2026, page no. 31-37

The hydrolysate was filtered and cooled to room temperature. A total of 100 mL of hydrolysate was mixed with 70 mL starter. Metal ions (Fe^{2+} or K^{+}) were added with concentrations of 0; 0.2; 0.4; and 0.6 g/L. The mixture was fermented for 5 days in a closed container.

2.2.5 Analysis Methods

Reducing sugar content was analyzed using the DNS method with a UV-Vis spectrophotometer at a wavelength of 570 nm. Bioethanol concentration was determined by measuring density using a pycnometer and converting it into ethanol concentration using standard density tables.

3. RESULTS AND DISCUSSION

3.1 Impact of Mg^{2+} and Zn^{2+} Supplementation on Reducing Sugar Yield

The quantitative impacts of Mg^{2+} and Zn^{2+} supplementation on the resulting reducing sugar yield at varying substrate loadings are illustrated in Figure 1 and Figure 2.

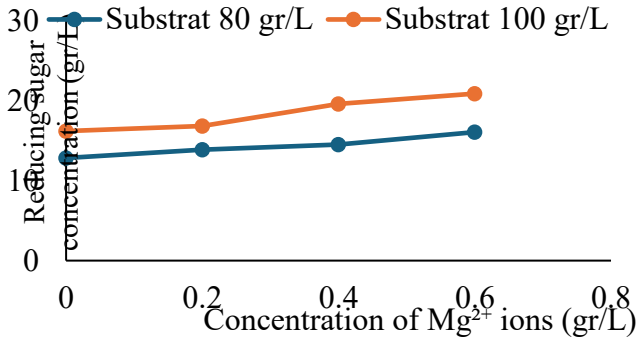
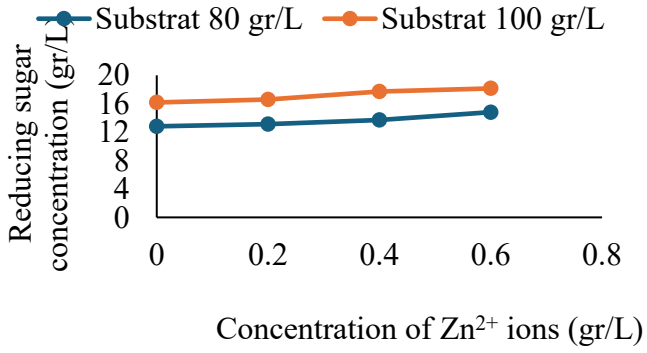


Figure 1. The Influence of Mg^{2+} Concentration on Reducing Sugar Production at Varying Substrate Loadings.



International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 7, Issue.3, May-June 2026, page no. 31-37

Figure 2. Impact of Zn^{2+} Supplementation on the Yield of Reducing Sugars during Enzymatic Hydrolysis.

The enzymatic hydrolysis of sweet sorghum stalks was significantly influenced by substrate concentration and the presence of divalent metallic cofactors. Experimental results (Fig. 1 and Fig. 2) demonstrate that increasing the substrate loading from 80 g/L to 100 g/L consistently enhanced the reducing sugar yield. At a 100 g/L substrate concentration, the addition of 0.6 g/L Mg^{2+} and Zn^{2+} yielded maximum reducing sugar concentrations of 20.86 g/L and 18.15 g/L, representing increases of 28.92% and 12.18% over the control, respectively.

The superior performance of Mg^{2+} over Zn^{2+} as a catalytic enhancer aligns with the findings of Milessi-Esteves et al. [3], who identified that magnesium ions effectively activate hydrolytic enzymes during polysaccharide conversion. Furthermore, the role of Mg^{2+} in maintaining the structural integrity of cellulase during hydrolysis is crucial for augmenting sugar formation [4]. While Zn^{2+} also provides a positive effect by enhancing beta-glucosidase activity and promoting enzyme adsorption onto the cellulose surface [5, 6], the higher affinity of Mg^{2+} within this specific reaction system resulted in a more substantial increase in saccharification yield. This synergistic effect between temperature (60°C) and metallic supplementation facilitates efficient glycosidic bond cleavage without inducing enzyme denaturation.

3.2 Impact of Fe^{2+} and K^{+} Supplementation on Bioethanol Fermentation

The influence of Fe^{2+} and K^{+} mineral enrichment on the fermentation performance and the final ethanol titer is quantitatively illustrated in Figure 4.

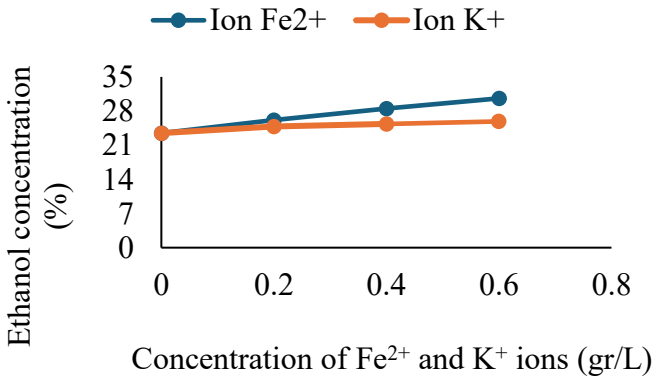


Figure 3. Evolution of bioethanol concentrations as a function of Fe^{2+} and K^{+} mineral supplementation.

ISSN: 2582-6271

Vol. 7, Issue.3, May-June 2026, page no. 31-37

The fermentation stage utilizing Saccharomyces cerevisiae exhibited a distinct response to the enrichment of Fe^{2+} and K^{+} ions (Fig. 3). Both ions successfully stimulated ethanol production, with Fe^{2+} demonstrating a more pronounced impact. Specifically, the introduction of 0.6 g/L Fe^{2+} resulted in a peak bioethanol concentration of 30.5989%, a significant 30.34% increase compared to the unsupplemented medium. In comparison, K^{+} supplementation at the same concentration achieved a maximum yield of 25.8726%.

The efficacy of K^{+} in accelerating the fermentation process and increasing ethanol titers has been previously documented by Hargono et al. [7] and Anggarini et al. [8]. However, the results suggest that Fe^{2+} plays a more critical role in this system by safeguarding yeast cells against environmental stressors, such as high osmotic pressure and thermal fluctuations. By bolstering the metabolic vigor and growth of the yeast population, these mineral ions facilitate a more complete conversion of reducing sugars into bioethanol, thereby optimizing the overall yield from sweet sorghum biomass.

4. CONCLUSION

This study demonstrates that the optimization of substrate concentration and metallic cofactor supplementation significantly enhances the production of second-generation bioethanol from sweet sorghum stalks. The results indicate that substrate concentration serves as a pivotal driver in saccharification efficiency, where a loading of 100 g/L yielded the most substantial increase in reducing sugar levels (26.21%) compared to lower concentrations. Furthermore, the introduction of divalent cations during enzymatic hydrolysis effectively promotes sugar release, with Mg^{2+} exhibiting a more pronounced catalytic effect, achieving a peak increment of 28.92% which significantly outperformed the 15.52% enhancement provided by Zn^{2+}. During the fermentation phase, the supplementation of Fe^{2+} and K^{+} underscored a positive correlation with ethanol titers. Fe^{2+} proved to be a highly effective metabolic enhancer for Saccharomyces cerevisiae, achieving a maximum bioethanol increment of 30.34%, whereas K^{+} supplementation resulted in a more moderate increase of 10.21%. In conclusion, the strategic integration of higher substrate loadings with Mg^{2+} assisted hydrolysis and Fe^{2+} enriched fermentation provides a robust framework for maximizing bioethanol yields, offering a viable and efficient pathway for the valorization of agricultural residues.

REFERENCES

[1] Szambelan, K., Nowak, J., Szwengiel, A., Jeleń, H., & Łukaszewski, G. (2018). Separate hydrolysis and fermentation and simultaneous saccharification and fermentation methods in bioethanol production and formation of volatile by-products from selected corn cultivars. *Industrial Crops and Products*, 118, 355–361. https://doi.org/10.1016/j.indcrop.2018.03.059

ISSN: 2582-6271

Vol. 7, Issue.3, May-June 2026, page no. 31-37

[2] Pejin, J. D., Mojović, L. V., Pejin, D. J., Kocić-Tanackov, S. D., Savić, D. S., Nikolić, S. B., & Djukić-Vuković, A. P. (2015). Bioethanol production from triticale by simultaneous saccharification and fermentation with magnesium or calcium ions addition. *Fuel*, 142, 58–64. https://doi.org/10.1016/j.fuel.2014.10.077

[3] Milessi-Esteves, J. L., Chandel, A. K., & de Carvalho, W. (2019). Synergistic effect of magnesium ions on cellulase activity and sugar release from pretreated lignocellulosic biomass. *Bioresource Technology Reports*, 6, 195. https://doi.org/10.1016/j.biteb.2019.195

[4] Gad, M. S., El-Shishtawy, R. M., & El-Sheikh, M. A. (2022). Enhancement of enzymatic hydrolysis of lignocellulosic biomass through metal ion cofactors and optimized pretreatment conditions. *Industrial Crops and Products*, 181, 114808. https://doi.org/10.1016/j.indcrop.2022.114808.

[5] Saroj, P., P, M., & Narasimhulu, K. (2022). Biochemical characterization of thermostable carboxymethyl cellulase and β-glucosidase from Aspergillus fumigatus JCM 10253. *Applied Biochemistry and Biotechnology*, *194*(6), 2503-2527.

[6] Zhang, P., Cui, M., Huang, R., Qi, W., Thielemans, W., He, Z., & Su, R. (2021). Enhanced enzymatic hydrolysis of cellulose by endoglucanase via expansin pretreatment and the addition of zinc ions. *Bioresource Technology*, *333*, 125139.

[7] Hargono, Santoso, A. K., Hidayat, A. L., Fransenda, A. R., Marpaung, A. H., & Sihombing, S. G. (2023). Optimizing bioethanol production through separated hydrolysis fermentation of rubber–cassava (*Manihot glaziovii*) starch with K^+ and Mg^{2+} mineral ion supplements. *International Journal of Innovative Science and Research Technology*, 8(10), 1972–1978.

[8] Anggarini, S., Hindun Pulungan, M., Wignyanto, W., Hidayat, N., Nurika, L., & Ihwah, A. (2016). Effect of temperature stress and metal ion supplement on ethanol fermentation by *Zymomonas mobilis*. *Industria : Jurnal Teknologi dan Manajemen Agroindustri*, 5(3), 125–131. https://doi.org/10.21776/ub.industria.2016.005.03.2