Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 22(3) September-December 2024]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

ADVISORYBOARD

Patron
Dr. Manmohan Singh Chauhan, Vice-Chancellor, G.B. Pant University of Agriculture and Technology, Pantnagar, India
Members
Dr. A.S. Nain, Ph.D., Director Research, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. Jitendra Kwatra, Ph.D., Director, Extension Education, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. S.K. Kashyap, Ph.D., Dean, College of Agriculture, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. A.H. Ahmad, Ph.D., Dean, College of Veterinary & Animal Sciences, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. K.P. Raverkar, Ph.D., Dean, College of Post Graduate Studies, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. Sandeep Arora, Ph.D., Dean, College of Basic Sciences & Humanities, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. Alaknanda Ashok, Ph.D., Dean, College of Technology, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. Alka Goel, Ph.D., Dean, College of Home Science, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. Avdhesh Kumar, Ph.D., Dean, College of Fisheries, G.B. Pant University of Agri. & Tech., Pantnagar, India
Dr. R.S. Jadoun,Ph.D., Dean, College of Agribusiness Management, G.B. Pant University of Agri. & Tech., Pantnagar, India

EDITORIALBOARD

Members
Prof. A.K. Misra, Ph.D., Chairman, Agricultural Scientists Recruitment Board, Krishi Anusandhan Bhavan I, New Delhi, India
Dr. Anand Shukla, Director, Reefberry Foodex Pvt. Ltd., Veraval, Gujarat, India
Dr. Anil Kumar, Ph.D., Director, Education, Rani Lakshmi Bai Central Agricultural University, Jhansi, India
Dr. Ashok K. Mishra, Ph.D., Kemper and Ethel Marley Foundation Chair, W P Carey Business School, Arizona State University, U.S.A
Dr. B.B. Singh, Ph.D., Visiting Professor and Senior Fellow, Dept. of Soil and Crop Sciences and Borlaug Institute for International Agriculture, Texas A&M University, U.S.A.
Prof. Binod Kumar Kanaujia, Ph.D., Professor, School of Computational and Integrative Sciences, Jawahar Lal Nehru University, New Delhi, India
Dr. D. Ratna Kumari, Ph.D., Associate Dean, College of Community / Home Science, PJTSAU, Hyderabad, India
Dr. Deepak Pant, Ph.D., Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Belgium
Dr. Desirazu N. Rao, Ph.D., Professor, Department of Biochemistry, Indian Institute of Science, Bangalore, India
Dr. G.K. Garg, Ph.D., Dean (Retired), College of Basic Sciences & Humanities, G.B. Pant University of Agric. & Tech., Pantnagar, India
Dr. Humnath Bhandari, Ph.D., IRRI Representative for Bangladesh, Agricultural Economist, Agrifood Policy Platform, Philippines
Dr. Indu S Sawant, Ph.D., Director, ICAR - National Research Centre for Grapes, Pune, India
Dr. Kuldeep Singh, Ph.D., Director, ICAR - National Bureau of Plant Genetic Resources, New Delhi, India
Dr. M.P. Pandey, Ph.D., Ex. Vice Chancellor, BAU, Ranchi & IGKV, Raipur and Director General, IAT, Allahabad, India
Dr. Martin Mortimer, Ph.D., Professor, The Centre of Excellence for Sustainable Food Systems, University of Liverpool, United Kingdom
Dr. Muneshwar Singh, Ph.D., Project Coordinator AICRP- LTFE, ICAR - Indian Institute of Soil Science, Bhopal, India
Prof. Omkar, Ph.D., Professor, Department of Zoology, University of Lucknow, India
Dr. P.C. Srivastav, Ph.D., Professor, Department of Soil Science, G.B. Pant University of Agriculture and Technology, Pantnagar, India
Dr. Prashant Srivastava, Ph.D., Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, University of South Australia, Australia
Dr. Puneet Srivastava, Ph.D., Director, Water Resources Center, Butler-Cunningham Eminent Scholar, Professor, Biosystems Engineering, Auburn University, U.S.A.
Dr. R.C. Chaudhary, Ph.D., Chairman, Participatory Rural Development Foundation, Gorakhpur, India
Dr. R.K. Singh, Ph.D., Director & Vice Chancellor, ICAR-Indian Veterinary Research Institute, Izatnagar, U.P., India
Prof. Ramesh Kanwar, Ph.D., Charles F. Curtiss Distinguished Professor of Water Resources Engineering, Iowa State University, U.S.A.
Dr. S.N. Maurya, Ph.D., Professor (Retired), Department of Gynecology & Obstetrics, G.B. Pant University of Agric. & Tech., Pantnagar, India
Dr. Sham S. Goyal, Ph.D., Professor (Retired), Faculty of Agriculture and Environmental Sciences, University of California, Davis, U.S.A.
Prof. Umesh Varshney, Ph.D., Professor, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India
Prof. V.D. Sharma, Ph.D., Dean Academics, SAI Group of Institutions, Dehradun, India
Dr. V.K. Singh, Ph.D., Head, Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi, India
Dr. Vijay P. Singh, Ph.D., Distinguished Professor, Caroline and William N. Lehrer Distinguished Chair in Water Engineering, Department of Biological Agricultural Engineering, Texas A& M University, U.S.A.
Dr. Vinay Mehrotra, Ph.D., President, Vinlax Canada Inc., Canada

Editor-in-Chief
Dr. Manoranjan Dutta, Head Crop Improvement Division (Retd.), National Bureau of Plant Genetic Resources, New Delhi, India

Managing Editor
Dr. S.N. Tiwari, Ph.D., Professor, Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Assistant Managing Editor
Dr. Jyotsna Yadav, Ph.D., Research Editor, Directorate of Research, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Technical Manager
Dr. S.D. Samantray, Ph.D., Professor, Department of Computer Science and Engineering, G.B. Pant University of Agriculture and Technology, Pantnagar, India

PANTNAGAR JOURNAL OF RESEARCH

Vol. 22(3) September-December 2024

CONTENTS

Thermal stability and catalytic efficiency of β-Glucosidase extracted from biogas slurry: Implications for biomass conversion

GAURAV SINGH RANA, A. K. VERMA* and ASHUTOSH DUBEY

Department of Biochemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145 (U.S. Nagar, Uttarakhand)
**Corresponding author's email id: akv72@rediffmail.com*

ABSTRACT: Biogas slurry (BGS), a nutrient-rich byproduct of anaerobic digestion, contains valuable enzymes like β-glucosidase, essential for lignocellulosic biomass degradation. This study investigated the kinetic and thermodynamic properties of β-glucosidase extracted from BGS. The enzyme was purified through lyophilization and ammonium sulfate precipitation, resulting in an 8.3-fold increase in activity. Optimal enzyme activity was observed at 60°C and pH 7.0, with a V_{max} of 4.207±0.07 mM/min, K_m of 2.51±0.01 mM and a catalytic efficiency (K_{cat}/K_m) of 0.36±0.003 s/M. β-glucosidase exhibited high thermal and pH stability, retaining significant activity after prolonged exposure to high temperatures and varying pH levels. The activation energy (E_a) of 14.97±0.67 kJ/mol and a temperature coefficient (Q_{10}) of 1.16±0.06, was indicating enzyme stability across a range of temperatures. These findings suggested that β-glucosidase from BGS could be a cost-effective and sustainable enzyme source for biofuel production from lignocellulosic biomass and could be suitable for large-scale waste valorization.

Key words: Bio-Gas Slurry (BGS), catalytic efficiency, enzyme kinetics, optimum temperature and pH, residual activity, thermodynamic analysis

Biogas slurry (BGS) is a nutrient-rich byproduct generated from the anaerobic digestion of organic materials, particularly livestock manure and agricultural residues. It is characterized by a high concentration of essential nutrients such as nitrogen (N), phosphorus (P) and potassium (K) that are crucial for plant growth and soil health (Kumar *et al.*, 2023). Traditional uses of BGS primarily focus on its application as a fertilizer, enhancing soil nutrient profiles and improving crop yields (Yadav *et al.*, 2023). Recent studies indicate that BGS also holds significant potential as a source of valuable enzymes, specifically β-glucosidases, which are vital for the degradation of lignocellulosic biomass (Srivastava *et al.*, 2019). Lignocellulosic biomass, which is primarily composed of cellulose, hemicellulose and lignin, represents an abundant yet challenging feedstock for conversion processes due to its complex structural composition (Periyasamy *et al.*, 2018). The key challenge lies in breaking down these polymers effectively, which is essential for transforming biomass into fermentable sugars for biofuels and biochemical products (Zang *et al.*, 2018; Lopes *et al.*, 2017). β-Glucosidase plays a central role in the enzymatic hydrolysis of lignocellulosic materials by catalyzing the hydrolysis of cellobiose into glucose (Konar *et al.*, 2019; Teugjas and Valjamae, 2013). This step is crucial, as the accumulation of cellobiose can inhibit the activity of other cellulases, impeding the overall hydrolysis process (Wang and Lu, 2016). By converting cellobiose into glucose, β-glucosidase not only alleviates this inhibitory effect but also improves the overall efficiency of cellulose degradation, thereby facilitating the production of biofuels such as bioethanol and biogas (Singhania *et al.*, 2013). The resulting glucose can be readily fermented by microorganisms, including yeast and bacteria, leading to bioenergy production (Shin *et al.*, 2024; Lopes *et al.*, 2017). In addition to its significant role in biofuel production, β-glucosidase is applied in various biochemical contexts, including the synthesis of oligosaccharides and the formation of pharmacologically active glycosides (Tran *et al.*, 2023; Kim *et al.*, 2022). β-glucosidase has also role the food industry for processing and enhancing flavors, as well as in the pharmaceutical sector for the production of drug precursors through glycosylation reactions (Kannan *et al.*, 2023; Amer *et al.*, 2017). The extraction of β-glucosidase from BGS presents a sustainable and economically viable alternative to traditional enzyme production

methods, which often involve high manufacturing and purification costs (Kumar *et al.*, 2023). The utilization of biogas slurry not only offers a solution to waste management but also promotes the recycling of nutrients and the production of enzymes that can help in the valorization of lignocellulosic biomass (Stumpe *et al.*, 2012). This study aims to explore the kinetic and thermodynamic properties of â-glucosidase extracted from BGS, focusing on optimal temperature and pH conditions, kinetic parameters such as K_m, V_{max} and K_{cat} and thermodynamic properties like activation energy (E_a) and the temperature coefficient (Q_{10}), to determine the enzyme's stability and functionality under different conditions. By characterizing â-glucosidase from BGS, the study seeks to establish its potential as a cost-effective and sustainable enzyme source for lignocellulosic biomass degradation, promoting the use of BGS-derived enzymes in bioenergy production and other industrial sectors.

MATERIALS AND METHODS

Source of Biogas Slurry

Ten liters of biogas slurry (BGS) was collected from four functional cow-dung-based biogas plants after 60-75 days of digestion at the experimental Dairy Farm Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar. The BGS was sonicated at 4°C for 5 minutes, followed by centrifugation at 10, 000 g for 30 minutes (Jeilu *et al.*, 2024). The supernatant was used as crude enzyme extract. A portion of the extract was lyophilized for 48 hours, while another portion was precipitated at 0-80% ammonium sulfate saturation, dissolved in phosphate buffer (pH 6.0) and dialyzed for 48 hours at 4°C (Zhang *et al.*, 2024).

β-glucosidase Activity Assay

β-glucosidase activity was measured by incubating 1 mL of enzyme extract with 1 mL of 30 mM pNPG (4-Nitrophenyl-β-D-glucopyranoside) in 100 mM phosphate buffer (pH 6.6) at 50°C for 10 minutes. The reaction was terminated by adding 2 mL of 1 M Na_2CO_3 and absorbance was measured at 410 nm **(**Kim *et al.*, 2022). One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol per minute under assay conditions (Teugjas and Valjamae, 2013).

Determination of Optimum Temperature and pH

The optimum temperature for β-glucosidase activity was determined by incubating the enzyme extract at temperatures ranging from 30°C to 90°C in phosphate buffer (pH 6.6) (Singhania *et al.*, 2013; Goswami *et al.*, 2016). Similarly, the optimum pH was determined by incubating the enzyme at pH values ranging from 3.0 to 9.0 at the optimum temperature (60°C) (Singh *et al.*, 2023).

Temperature and pH Stability

The temperature stability of β-glucosidase was assessed by incubating the enzyme at various temperatures (40°C to 80°C) in phosphate buffer (pH 7.0) without substrate for 1 hour (Dong *et al.*, 2023). The residual enzyme activity was measured under standard assay conditions. For pH stability, the enzyme was pre-incubated in buffers of different pH (4.0 to 8.0) for 1 hour, followed by the measurement of residual activity (Lopes *et al.*, 2017).

Kinetic Parameters

The kinetic parameters of β-glucosidase, including the Michaelis constant (K_m) and maximum reaction velocity (V_{max}), were determined by measuring the initial reaction rates at various substrate (pNPG) concentrations (0.5-10 mM) using the Lineweaver-Burk plot (de Andrades *et al.*, 2019; Yapi *et al.*, 2009). The turnover number (K_{cat}) and catalytic efficiency (K_{cat}/K_m) were calculated from the measured V_{max} and K_m values (Jeilu *et al.*, 2024; Liu *et al.*, 2012). The reaction was conducted at the optimal temperature (60°C) and pH (7.0) (Klimes *et al.*, 2017; Zhang *et al.*, 2011).

Thermodynamic Constants

The activation energy (E_a) and temperature coefficient (Q_{10}) for β-glucosidase were determined using the Arrhenius equation (Paz-Ferreiro *et al.*, 2015). The reaction rates were measured at various temperatures and a plot of the natural logarithm of the reaction rate versus the reciprocal of temperature (in Kelvin) was used to calculate E_a (Mfombep *et*

al., 2013). The Q_{10} was calculated by comparing the reaction rates over a 10°C temperature range (Dong *et al.*, 2023).

Statistical Analysis

Standard error was calculated manually for all the experiments. All treatments were completed in triplicate. One-way analysis of variance (ANOVA) and post-hoc tests (Bonferroni Tukey, Duncan, Least Significant Difference) with a significance level (alpha) of 0.05 were conducted.

RESULTS AND DISCUSSION

Enzyme Activity and Specific Activity

The crude extract of β-glucosidase exhibited an activity of 0.42±0.01 U/mL and a specific activity of 0.77±0.03 U/mg. After lyophilization, the enzyme activity increased to 0.92±0.02 U/mL and the specific activity rose to 1.21±0.03 U/mg (Table 1). Lyophilization, a process known to concentrate the enzyme by removing water, stabilizes the enzyme and reduces degradation, leading to enhanced activity (Fischer *et al.*, 2014; Aguiar-Oliveira *et al.*, 2013). Following precipitation, the enzyme activity further increased to 3.49±0.06 U/mL, with a corresponding specific activity of 1.42±0.02 U/mg, signifying an 8.3-fold increase in enzymatic activity compared to the crude extract. This substantial enhancement underscores the effectiveness of the precipitation process in purifying and concentrating the enzyme by removing inhibitory compounds or impurities. Karami *et al.* (2020) demonstrated a similar purification process, achieving an 86-fold increase in β-glucosidase activity from a crude extract, highlighting the effectiveness of such purification strategies. β-glucosidase activity is also known to be present in biogas slurry, a byproduct of anaerobic digestion of agricultural residues and biowaste (Zhang *et al.*, 2024). The significant increases in both enzyme activity and specific activity after lyophilization and precipitation can thus be attributed to the concentration of the enzyme and the removal of interfering substances that hinder its activity in the crude extract.

Table 1: Enzyme Activity and Specific Activity of β-Glucosidase Enzymes in Biogas slurry

Sample	Enzyme Activity (Unit/ml)	Specific Activity (Unit/mg)
Crude	0.42±0.01^{c}	0.77±0.03^{c}
Lyophilized	0.92±0.02^{b}	1.21±0.03^{b}
Precipitated	3.49±0.06 ^{a}	1.42±0.02^{a}

Note: Means in a column followed by a different letter in the superscript are significantly different at $p<0.05$.

Optimum Temperature and pH

The optimal temperature for β-glucosidase activity was determined to be 60°C, with an enzyme activity of 4.45±0.12 U/mL and a specific activity of 1.81±0.05 U/mg. Similarly, the optimal pH was found to be 7.0, yielding an enzyme activity of 4.77±0.13 U/mL and a specific activity of 1.94±0.05 U/mg (Fig.1). These optimal temperature and pH conditions are crucial for the enzyme's application in biowaste processing and biomass hydrolysis, particularly in biogas slurry. β-glucosidases from various microbial sources often exhibit optimal activity within specific temperature and pH ranges, influencing their efficiency in converting cellulose into glucose, a critical step in biofuel production. Del Pozo *et al.* (2012) and Mendez-Liter *et al.* (2018) similarly reported the optimal temperature for β-glucosidase activity as 60°C, while the optimal pH for activity has been noted to vary between 4.0 and 7.0, depending on the enzyme source (Yan *et al.*, 2016). These findings reinforce that the enzyme performs best within a narrow range of physiological conditions, maximizing its role in biomass degradation.

Enzyme Stability

β-glucosidase exhibited the highest residual activity at pH 7.0, with significant losses in activity at extreme pH levels. The enzyme retained its peak activity at 60°C, but a notable decline occurred at 70°C, indicating that it is sensitive to temperatures above its optimal range (Fig. 2). Enzyme stability under different pH and temperature conditions is essential for its practical application in industrial processes involving biogas slurry and biowaste. Yu *et al.* (2007) and Bai *et al.* (2013) reported similar findings, indicating that β-glucosidase displays optimal activity at pH 5.0 and remains stable across temperatures ranging from 50°C to 80°C. Tiwari *et*

al. (2017) showed that β-glucosidase from *Bacillus subtilis* retained 78% of its activity at 80°C and 68.32% at 50°C after 48 hours, which is consistent with the thermostability observed in this study. Such stability is beneficial for prolonged industrial applications, ensuring the enzyme's performance under varying conditions.

Kinetic Parameters

The maximum reaction velocity (V_{max}) for β-glucosidase was measured at 4.207±0.07 mM/min, while the Michaelis constant (K_m) was 2.51±0.01 mM, indicating high substrate affinity. The enzyme exhibited a turnover number (K_{cat}) of 0.9±0.01/min, with a catalytic efficiency (K_{cat}/K_m) of 0.36±0.003 s/M (Table 2). These kinetic parameters reflect the enzyme's efficiency, particularly at low substrate concentrations. β-glucosidases from different sources exhibit varied kinetic properties, shaped by their adaptation to specific functional roles or ecological niches. Ten Kate *et al.* (2024) reported V_{max} values of 1.6 and 22.1 µmol/min/mg for β-glucosidase from *Thermotoga maritima*, with corresponding K_m values of 0.4 and 12.9 mM, highlighting the variability across different enzyme sources. A lower K_m, such as the 2.51±0.01 mM observed in this study, signifies a stronger affinity for the substrate. Similarly, Bai *et al.* (2013) reported a K_m of 0.37 mM for β-glucosidase from *Penicillium occitanis*, reflecting a very high affinity, though with a lower V_{max}. In comparison, β-glucosidase from rye has a K_m of 83 µM, further illustrating the diversity of kinetic profiles among β-glucosidases from different origins (Purushothaman and Siddalinga, 2011). This suggests that β-glucosidase in this study demonstrates a balanced affinity and catalytic efficiency suitable for industrial applications.

Thermodynamic Constants

The activation energy (E_a) for β-glucosidase was calculated to be 14.97±0.67 kJ/mol, while the temperature coefficient (Q_{10}) was 1.16±0.06 (Table 2), indicating that the enzyme is relatively stable across temperature fluctuations compared to other lignocellulosic enzymes. Thermodynamic parameters such as E_a and Q_{10} offer valuable insights into the enzyme's stability and activity under various temperature conditions, which is crucial for optimizing its performance in biogas slurry and biowaste applications. Yan *et al.* (2010) and Bai *et al.* (2013) studied these thermodynamic properties for β-glucosidase from different sources, highlighting the importance of such metrics in evaluating enzyme stability. Yapi *et al.* (2009) reported an activation energy of 68.78 kJ/mol for β-

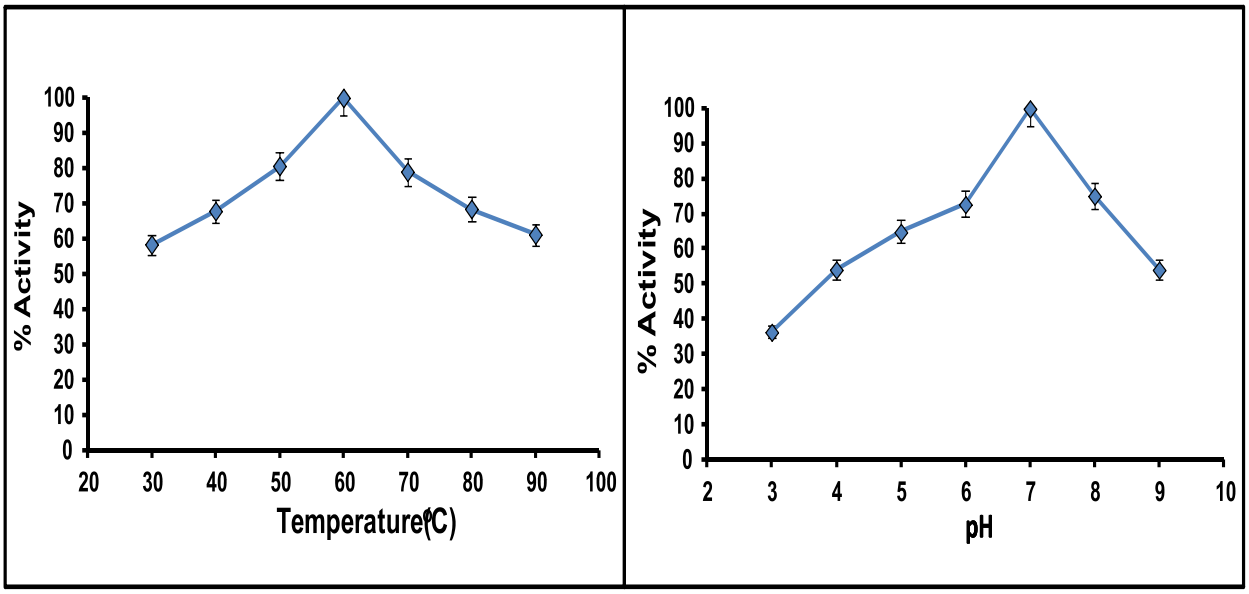

Fig. 1: Effect of Temperature and pH on β-Glucosidase Activity (in % activity)

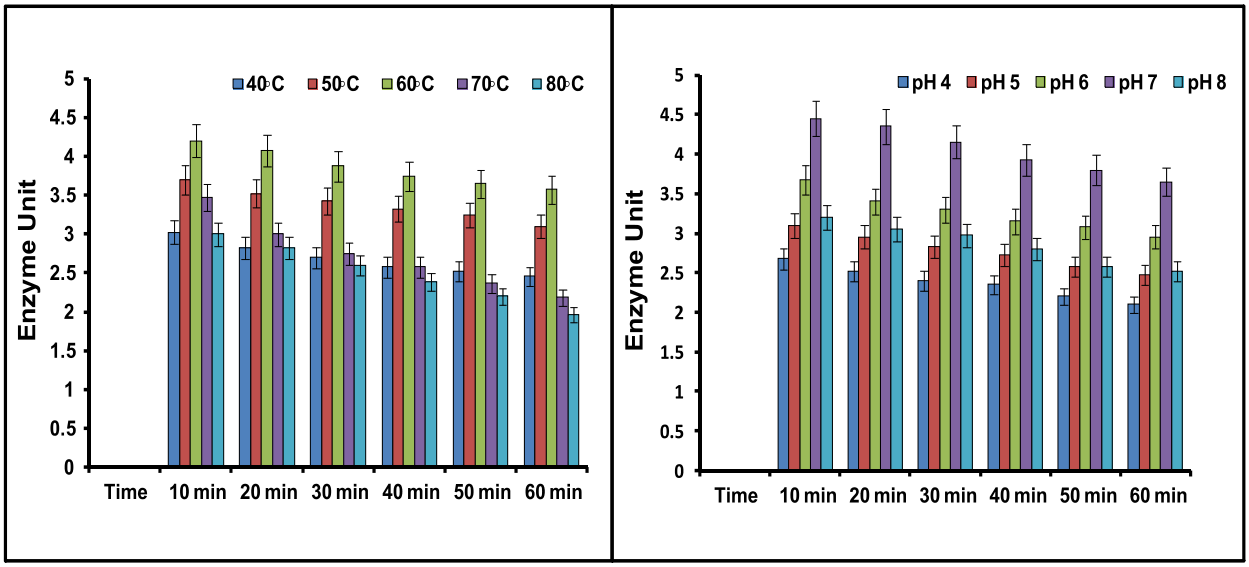

Fig. 2: Effect of Temperature and pH on the Long-Term Stability of β-Glucosidase

Table 2: Different kinetic and thermodynamic parameters of β-Glucosidase Enzyme in Biogas Slurry

	V_{max}	K_m	k (/sec)	K_{cat} (/min)	K_{cat}/K_m (/s/M)	E_a (KJ/mole)	Q_{10}
β-Glucosidase	4.207±0.07 mM/min	2.51±0.01 mM	0.028±0.002	0.9±0.01	0.36±0.003	14.97±0.67	1.16±0.06

glucosidase, while Chauve *et al.* (2010) calculated an E_a of 53.2 kJ/mol for β-glucosidase from Aspergillus niger over a temperature range of 30°C to 60°C. Additionally, Eivazi and Tabatabai (1988) recorded an E_a of 30.8 kJ/mol for soil-derived β-glucosidase. These values underscore the variability in thermodynamic constants depending on the enzyme's source and environmental conditions. The relatively low activation energy and moderate Q_{10} value observed in this study suggest that β-glucosidase is well-suited for catalysis under a range of temperatures, making it a robust candidate for industrial processes such as biomass hydrolysis.

The present study on β-Glucosidase extracted from biogas slurry (BGS) demonstrated promising kinetic and thermodynamic properties, including high substrate affinity (K_m: 2.51 mM) and notable stability at 60°C and pH 7.0, making it highly suitable for lignocellulosic biomass degradation. The enzyme's relatively low activation energy (E_a: 14.97 kJ/mol) and temperature coefficient (Q_{10}: 1.16) suggested robust performance under varied industrial conditions. These findings position BGS-derived β-glucosidase as a cost-effective and sustainable alternative for bioenergy production and other industrial applications, particularly in biomass conversion processes. Future studies could focus on enhancing the enzyme's thermostability through genetic or chemical modifications to expand its usability in harsher conditions. Additionally, exploring its potential for large-scale industrial applications in biofuel production and the synthesis of bioactive compounds can further contribute to waste valorization and circular economy models, offering environmentally friendly solutions to energy and biochemical production challenges.

REFERENCES

Aguiar-Oliveira, E. and Maugeri, F. (2013). Effects of lyophilization on catalytic properties of immobilized fructosyltransferase from *Rhodotorula* sp. LEB-V10. *Food and Bioproducts Processing*, 91(4): 609-616. https: //doi.org/10.1016/j.fbp.2013.03.003

Amer, A., Kashfa, B. and Bibi, A. (2017). Microbial β-glucosidase: sources, production and

applications. *Journal of Applied and Environmental Microbiology*, 5(1): 31-46.

Bai, H., Wang, H., Sun, J., Irfan, M., Han, M., Huang, Y., ... and Yang, Q. (2013). Production, purification and characterization of novel beta glucosidase from newly isolated *Penicillium simplicissimum* H-11 in submerged fermentation. *EXCLI journal*, 12: 528.

Chauve, M., Mathis, H., Huc, D., Casanave, D., Monot, F. and Lopes Ferreira, N. (2010). Comparative kinetic analysis of two fungal β-glucosidases. *Biotechnology for Biofuels*, 3: 1-8. https: //doi.org/10.1186/1754-6834-3-3

de Andrades, D., Graebin, N. G., Ayub, M. A., Fernandez-Lafuente, R. and Rodrigues, R. C. (2019). Physico-chemical properties, kinetic parameters and glucose inhibition of several beta-glucosidases for industrial applications. *Process Biochemistry*, 78: 82-90. https: //doi.org/10.1016/j.procbio.2019.01.008

Del Pozo, M. V., Fernandez-Arrojo, L., Gil-Martinez, J., Montesinos, A., Chernikova, T. N., Nechitaylo, T. Y., ... and Golyshin, P. N. (2012). Microbial β-glucosidases from cow rumen metagenome enhance the saccharification of lignocellulose in combination with commercial cellulase cocktail. *Biotechnology for Biofuels*, 5: 1-13. https: //doi.org/10.1186/1754-6834-5-73

Dong, R., Abdelkerim-Ouba, D., Liu, D., Ma, X. and Wang, S. (2023). Impacts of Partial Substitution of Chemical Fertilizer with Organic Manure on the Kinetic and Thermodynamic Characteristics of Soil β–Glucosidase. *Agronomy*, 13(4): 1065. https: //doi.org/10.3390/agronomy13041065

Eivazi, F. and Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, 20(5): 601-606. https: //doi.org/10.1016/0038-0717(88)90141-1

Fischer, C., Krause, A. and Kleinschmidt, T. (2014). Optimization of production, purification and lyophilisation of cellobiose dehydrogenase by *Sclerotium rolfsii*. *BMC Biotechnology*, 14: 1-12. https: //doi.org/10.1186/s12896-014-0097-5

Goswami, S., Gupta, N. and Datta, S. (2016). Using the β-glucosidase catalyzed reaction product glucose to improve the ionic liquid tolerance of β-glucosidases. *Biotechnology for Biofuels*, 9: 1-12. https: //doi.org/10.1186/s13068-016-0484-3

Jeilu, O., Alexandersson, E., Johansson, E., Simachew, A. and Gessesse, A. (2024). A novel GH3-β-glucosidase from soda lake metagenomic libraries with desirable properties for biomass degradation. *Scientific Reports*, 14(1): 10012. https: //doi.org/10.1038/s41598-024-60645-y

Kannan, P., Achudhan, A. B., Gupta, A. and Saleena, L. M. (2023). A review on applications of β-glucosidase in food, brewery, pharmaceutical and cosmetic industries. *Carbohydrate Research*, 530: 108855. https: //doi.org/10.1016/j.carres.2023.108855

Karami, F., Ghorbani, M., Mahoonak, A. S. and Khodarahmi, R. (2020). Fast, inexpensive purification of β-glucosidase from *Aspergillus niger* and improved catalytic/physico-chemical properties upon the enzyme immobilization: Possible broad prospects for industrial applications. *Lwt*, 118: 108770. https: //doi.org/10.1016/j.lwt.2019.108770

Kim, I. J., Bornscheuer, U. T. and Nam, K. H. (2022). Biochemical and structural analysis of a glucose-tolerant β-glucosidase from the hemicellulose-degrading *Thermoanaerobacterium sacchar-olyticum*. *Molecules*, 27(1): 290. https: //doi.org/10.3390/molecules27010290

Klimes, P., Mazura, P., Turek, D. and Brzobohaty, B. (2017). An automated method to evaluate the enzyme kinetics of β glucosidases. *Protein Science*, 26(2): 382-388. https: //doi.org/10.1002/pro.3078

Konar, S., Sinha, S. K., Datta, S. and Ghorai, P. K. (2019). Probing the effect of glucose on the activity and stability of β-glucosidase: An

all-atom molecular dynamics simulation investigation. *ACS omega*, 4(6): 11189-11196.

Kumar, A., Verma, L. M., Sharma, S. and Singh, N. (2023). Overview on agricultural potentials of biogas slurry (BGS): applications, challenges and solutions. *Biomass Conversion and Biorefinery*, 13: 13729-13769. https: //doi.org/10.1007/s13399-021-02215-0

Liu, D., Zhang, R., Yang, X., Zhang, Z., Song, S., Miao, Y. and Shen, Q. (2012). Characterization of a thermostable β-glucosidase from *Aspergillus fumigatus* Z5 and its functional expression in *Pichia pastoris* X33. *Microbial cell factories*, 11: 1-15. https: //doi.org/10.1186/1475-2859-11-25

Lopes, D. B., de Queiros, L. D., de Avila, A. R., Monteiro, N. E. and Macedo, G. A. (2017). The importance of microbial and enzymatic bioconversions of isoflavones in bioactive compounds. *In Food Bioconversion*, Pp. 55-93. Academic Press. https: //doi.org/10.1016/b978-0-12-811413-1.00002-4

Mendez-Liter, J. A., de Eugenio, L. I., Prieto, A. and Martinez, M. J. (2018). The β-glucosidase secreted by *Talaromyces amestolkiae* under carbon starvation: a versatile catalyst for biofuel production from plant and algal biomass. *Biotechnology for Biofuels*, 11: 1-14. https: //doi.org/10.1186/s13068-018-1125-9

Mfombep, P. M., Senwo, Z. N. and Isikhuemhen, O. S. (2013). Enzymatic activities and kinetic properties of β-glucosidase from selected white rot fungi. DOI: 10.4236/abc.2013.32025

Paz-Ferreiro, J., Fu, S., Mendez, A. and Gasco, G. (2015). Biochar modifies the thermodynamic parameters of soil enzyme activity in a tropical soil. *Journal of Soils and Sediments*, 15: 578-583.

Periyasamy, K., Santhalembi, L., Mortha, G., Aurousseau, M., Boyer, A. and Subramanian, S. (2018). Bioconversion of lignocellulosic biomass to fermentable sugars by immobilized magnetic cellulolytic enzyme cocktails. *Langmuir*, 34(22): 6546-6555.

Purushothaman, S. and Siddalinga Murthy, K. R. (2011). Extraction of β-galactosidase and βglucosidase from the seeds of *Tamarindus indica*. *International Journal of Biomolecules and Biomedicine (IJBB)* ISSN, 2221-1063.

Shin, J., Liao, S., Kuanyshev, N., Xin, Y., Kim, C., Lu, T. and Jin, Y. S. (2024). Compositional and temporal division of labor modulates mixed sugar fermentation by an engineered yeast consortium. *Nature communications*, 15(1): 781. https: //doi.org/10.1038/s41467-024-45011-w

Singh, N., Sithole, B., Kumar, A. and Govinden, R. (2023). A glucose tolerant β-glucosidase from a newly isolated *Neofusicoccum parvum* strain F7: Production, purification and characterization. *Scientific Reports*, 13(1): 5134. https: //doi.org/10.1038/s41598-023-32353-6

Singhania, R. R., Patel, A. K., Sukumaran, R. K., Larroche, C. and Pandey, A. (2013). Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. *Bioresource technology*, 127: 500-507. https: //doi.org/10.1016/j.biortech.2012.09.012

Srivastava, N., Rathour, R., Jha, S., Pandey, K., Srivastava, M., Thakur, V. K., ... and Mishra, P. K. (2019). Microbial beta glucosidase enzymes: recent advances in biomass conversation for biofuels application. *Biomolecules*, 9(6): 220. https: //doi.org/10.3390/biom9060220

Stumpe, B., Werner, S., Jung, R., Heinze, S., Jüschke, E., Strippel, C. and Marschner, B. (2012). Organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge. https: //doi.org/10.4236/as.2012.31014

Ten Kate, G. A., Sanders, P., Dijkhuizen, L. and van Leeuwen, S. S. (2024). Kinetics and products of Thermotoga maritima β-

glucosidase with lactose and cellobiose. *Applied Microbiology and Biotechnology*, 108(1): 1-12.

Teugjas, H. and Väljamäe, P. (2013). Selecting β-glucosidases to support cellulases in cellulose saccharification. *Biotechnology for Biofuels*, 6: 1-13.

Tiwari, R., Singh, P. K., Singh, S., Nain, P. K., Nain, L. and Shukla, P. (2017). Bioprospecting of novel thermostable β-glucosidase from *Bacillus subtilis* RA10 and its application in biomass hydrolysis. *Biotechnology for biofuels*, 10: 1-18. https: //doi.org/10.1186/s13068-017-0932-8

Tran, T. N. A., Son, J. S., Awais, M., Ko, J. H., Yang, D. C. and Jung, S. K. (2023). β-Glucosidase and Its Application in Bioconversion of Ginsenosides in Panax ginseng. Bioengineering, 10(4): 484. https: //doi.org/10.3390/bioengineering10040484

Wang, M. and Lu, X. (2016). Exploring the synergy between cellobiose dehydrogenase from *Phanerochaete chrysosporium* and cellulase from *Trichoderma reesei*. *Frontiers in microbiology*, 7: 620.

Yadav, R., Sudhishri, S., Khanna, M., Lal, K., Dass, A., Kushwaha, H. L., ... and Nag, R. H. (2023). Temporal characterization of biogas slurry: a pre-requisite for sustainable nutrigation in crop production. *Frontiers in Sustainable Food Systems*, 7: 1234472. https: //doi.org/10.3389/fsufs.2023.1234472

Yan, F. Y., Xia, W., Zhang, X. X., Chen, S., Nie, X. Z. and Qian, L. C. (2016). Characterization of β-glucosidase from *Aspergillus terreus* and its application in the hydrolysis of soybean isoflavones. *Journal of Zhejiang University. Science. B*, 17(6): 455. https: //doi.org/10.1631/jzus.b1500317

Yan, J., Pan, G., Ding, C. and Quan, G. (2010). Kinetic and thermodynamic parameters of β-glucosidase immobilized on various colloidal particles from a paddy soil. Colloids and Surfaces B: Biointerfaces, 79(1): 298-303. https: //doi.org/10.1016/j.colsurfb.2010.04.015

Yapi, D. Y. A., Gnakri, D., Niamke, S. L. and Kouame, L. P. (2009). Purification and biochemical characterization of a specific β-glucosidase from the digestive fluid of larvae of the palm weevil, *Rhynchophorus palmarum*. *Journal of Insect Science*, 9(1): 4. https: //doi.org/10.1673/031.009.0401

Yu, H. L., Xu, J. H., Lu, W. Y. and Lin, G. Q. (2007). Identification, purification and characterization of β-glucosidase from apple seed as a novel catalyst for synthesis of O-glucosides. *Enzyme and Microbial Technology*, 40(2): 354-361.

Zang, X., Liu, M., Fan, Y., Xu, J., Xu, X. and Li, H. (2018). The structural and functional contributions of β-glucosidase-producing microbial communities to cellulose degradation in composting. Biotechnology for Biofuels, 11: 1-13. https: //doi.org/10.1186/s13068-018-1045-8

Zhang, M., Cui, J., Mi, M., Jin, Z., Wong, M. H., Shan, S. and Ping, L. (2024). Persistent effects of swine manure biochar and biogas slurry application on soil nitrogen content and quality of lotus root. *Frontiers in Plant Science*, 15: 1359911. https: //doi.org/10.3389/fpls.2024.1359911

Zhang, Y., Chen, L., Wu, Z. and Sun, C. (2011). Kinetic parameters of soil β-glucosidase response to environmental temperature and moisture regimes. *Revista Brasileira de Ciência do Solo*, 35: 1285-1291.

Received: October 16, 2024
Accepted: November 28, 2024