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Original Article

Sustainable and Enhanced Production of Glucose Oxidase by *Aspergillus niger* Using Pineapple Rind as an Economical Substrate for Biomedical and Biosensor Applications

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ABSTRACT

Objectives: Glucose Oxidase (GOx) is a essential enzyme used in several industries, including food, pharmaceuticals, and biotechnology. However, commercial-scale production continues to face challenges due to high processing costs and the environmental impact linked with conventional manufacturing approaches. **Materials and Methods:** In this work, a simple and sustainable method was explored to enhance GOx production using *Aspergillus niger* cultivated on pineapple rind. **Results:** The findings indicated that pineapple rind served as an excellent fermentation substrate, producing a noticeably higher enzyme yield compared to routinely used media. These results highlight the potential of agricultural waste materials such as pineapple rind as an efficient, low-cost option for large-scale GOx production. Utilizing this agro-industrial residue not only lowers production expenses but also offers an eco-friendly solution by converting waste biomass into a useful resource. **Conclusion:** The study establishes the feasibility of achieving enhanced GOx production, reaching up to 136 IU/mL, thereby highlighting a greener and more economical approach for industrial enzyme manufacture.

Keywords: *Aspergillus niger*, Glucose Oxidase enzyme, Pineapple rind, Sustainable production.

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INTRODUCTION

Enzymes have played a central role in advancing industrial biotechnology, owing to their remarkable precision in catalyzing biochemical reactions. Among various industrially relevant enzymes, Glucose Oxidase (GOx) has gained importance as a member of the oxidoreductase group. It facilitates the conversion of β-D-glucose into glucono-δ-lactone, simultaneously producing hydrogen peroxide as a byproduct. GOx has found extensive use in the food industry for improving product preservation and flavor, in the pharmaceutical field for glucose estimation and diabetes management, and in bioanalytical systems such as biosensors and diagnostic assays. Its high stability, non-toxic nature, and strong substrate specificity have made it a versatile and dependable enzyme for both traditional applications and innovative biotechnological developments (Mayel *et al*., 2025; Markwell *et al*., 1989).

Although glucose oxidase holds great industrial potential, its large-scale synthesis remains difficult, primarily due to the high expenses involved in production. The reliance on purified substrates and expensive fermentation ingredients significantly limits its commercial viability. These challenges emphasize the need to develop sustainable and cost-effective strategies for GOx production that minimize both economic and ecological impacts (Kriaa *et al*., 2020; Zia *et al*., 2012, Maye *et al*., 2025; Ramzan *et al*., 2009).

In recent years, agro-industrial by-products have gained considerable attention as promising raw materials for microbial fermentation. These residues, which are often treated as waste, are naturally rich in carbohydrates, fibers, and essential nutrients that support microbial proliferation and enzyme synthesis. Employing such materials not only lowers production costs but also promotes sustainable waste utilization and resource recovery, aligning well with the goals of a circular bioeconomy. Among the diverse agricultural residues available, pineapple rind stands out as a valuable, yet underexploited substrate derived from fruit processing industries. It contains appreciable amounts of fermentable sugars, dietary fibers, and bioactive compounds, making it a promising and sustainable substrate for microbial fermentation (Khatami *et al*., 2022; Li *et al*., 1994).

Aspergillus niger is a well-known filamentous fungus of great industrial relevance, recognized for its ability to produce a wide range of enzymes, including glucose oxidase (Timothy *et al*., 2022; Bankar *et al*., 2008). This species has been widely studied

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and extensively employed in fermentation-based industries due to its robust enzyme-producing ability and rapid growth under environmentally favorable conditions. The glucose oxidation pathway catalyzed by *A. niger* shares similar characteristics with glucose oxidase derived from other microbial sources, facilitating the conversion of glucose into gluconic acid with the concurrent formation of hydrogen peroxide (Garay-Flores *et al*., 2014; Dubey *et al*., 2017). Nevertheless, specific attributes such as the enzyme's optimal pH, production efficiency, and temperature stability can vary depending on the fermentation environment and the strain of *A. niger* used (Mayel *et al*., 2020).

The production of microbial enzymes is strongly influenced by multiple fermentation parameters, including pH, temperature, nutrient availability, substrate concentration, and incubation period. In this study, an attempt was made to establish a sustainable and economical process for the production of glucose oxidase by *Aspergillus niger* using pineapple rind as the main fermentation substrate (Ahmad *et al*., 2014; Hossain *et al*., 2020). The work aimed to evaluate the suitability of pineapple rind as a carbon source, optimize key fermentation parameters, and compare the enzyme yields obtained from this unconventional material with those produced using traditional media (Khurshid *et al*., 2011; Banerjee *et al*., 2018; Ajayi *et al*., 2021).

MATERIALS AND METHODS

Culture growth conditions

Aspergillus niger was initially obtained through isolation from soil samples and subsequently cultivated in flasks containing 100 mL of sterile water supplemented with yeast extract. The cultures were incubated at 30°C for 96 hr to promote vigorous fungal growth. After achieving sufficient biomass development, the strain was transferred to a liquid nutrient medium formulated according to the composition described by Reese and Mandels, to facilitate further growth and enzyme production. The formulation of the Reese and Mandels medium per liter was as follows: Ammonium Sulfate $(NH_4)_2SO_4$) at 1.5 g, Potassium Dihydrogen Phosphate (KH_2PO_4) at 3 g, Magnesium Sulfate ($MgSO_4$) at 0.3 g, Calcium Chloride ($CaCl_2$) at 0.3 g, peptone at 2.5 g, urea at 0.3 g, yeast extract at 2 g, Ferrous Sulfate Heptahydrate ($FeSO_4.7H_2O$) at 0.005 g, Zinc Sulfate Heptahydrate ($ZnSO_4.7H_2O$) at 0.0014 g, manganese sulfate Monohydrate ($MnSO_4.H_2O$) at 0.0016 g, and Cobalt Chloride ($CoCl_2$) at 0.0012 g. The pH of the media altered to 5.0 earlier to sterilization.

Later autoclaving, a sterile glucose solution was added into the medium to attain a concluding glucose concentration of 1% (w/v). The medium was then inoculated with *A. niger* culture and then incubated at 30°C for another 96-hr period to prolong biomass accumulation. Backing the incubation, the fungal biomass was parted from the culture broth by centrifugation method. Culture broth was centrifuged at 6000 rpm for 20 min. The extracted mycelial pellet was suspended in sterile 10 mM potassium phosphate buffer, adjusted to a pH of 5. This biomass slurry was set aside at 4°C and subsequently made use of the inoculum for experiments. Experimental combinations for media preparations shown in Table 1.

Enzyme production by submerged fermentation

Submerged Fermentation (SmF) was selected as the technique for producing glucose oxidase using *Aspergillus niger*. In this process, pineapple rind used as the principal carbon source, chosen for its high carbohydrate content and richness as an agro-industrial byproduct. Prior to fermentation, the pineapple rind was pretreated by drying and milling to a uniform particle size for fermentation. The fermentation parameters (pH, temperature and substrate concentration) were optimized for glucose oxidase production. The fermentation was done in orbital shaker under controlled laboratory conditions at 30°C. Samples were collected at regular 4-hr intervals during the fermentation process to evaluate glucose oxidase activity, yield, and overall productivity.

Glucose oxidase purification

The fungal culture was then processed for glucose oxidase by filtering the broth, removing the mycelial biomass; the supernatant, which was clear and contained extracellular enzymes, was collected and used as crude extract of glucose oxidase. The process for partial purification began with ammonium sulfate slowly added to the extract to saturation at 75%, precipitating proteins. The solution was centrifuged for 10 min at 6000 revolutions per minute after the ammonium sulfate was added to separate the precipitated proteins from the solution. The pellet of protein was very gently resuspended in 10 mL of the appropriate buffer. The resuspended sample was dialyzed against the same buffer to remove free salts and small molecular weight impurities. After dialysis, the enzyme solution was further concentrated with the addition of sucrose powder. The concentrated enzyme solution was purified further by cation exchange chromatography.

Glucose Oxidase activity assay

Glucose Oxidase (GOD) is a flavin-containing enzyme that facilitates the oxidation of β-D-glucose into D-glucono-1,5-lactone, which spontaneously converts into gluconic acid through hydrolysis. The biochemical reaction often involved the reduction of molecular oxygen to hydrogen peroxide as the by-product. In the present analytical method, benzoquinone is used as a substitute electron acceptor for oxygen. During the enzymatic oxidation of glucose, benzoquinone is reduced to hydroquinone. The production of hydroquinone is measured by measuring the increase in absorbance at 290 nm using a UV-visible spectrophotometer. The rate of increase in absorbance at 290 nm is linearly correlated to the glucose oxidase activity in the sample. This approach has allowed a sensitive and reproducible means to evaluate GOX activity as an indicator, either of crude extracts or partially purified enzyme preparations.

Ethical statement

This research did not involve human participants or animal subjects; therefore, ethical approval was not required.

Statistical analysis

The data was presented as Mean±SD. Every outcome was determined to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Glucose Oxidase production

The production of Glucose Oxidase (GOx) was carried out using *Aspergillus niger* in Submerged Fermentation (SmF) to optimize enzyme production. Several fermentation parameters were systematically optimized to achieve maximum glucose oxidase production. Different inoculum concentrations of *A. niger* (1%, 3%, and 5% v/v) were evaluated to determine the biomass level that yielded the highest enzyme activity. The influence of the initial pH of the fermentation medium (4.0, 6.0, and 8.0) was also investigated, as pH plays a crutial role in fungal metabolism and enzyme biosynthesis. Pineapple rind, was used as an inexpensive agro-industrial residue, served as the main carbon source, replacing conventional substrates. Its concentration was varied to assess its effect on substrate utilization and enzyme productivity. Although pineapple rind has been explored in previous research, this study specifically examined the impact of varying its concentrations on the growth of *A. niger* and its potential for glucose oxidase synthesis.

The submerged fermentation process was carried out for seven days under controlled incubation. After fermentation, the culture broth was filtered to separate the fungal mycelia from the liquid phase. The filtrate, containing extracellular enzymes i.e., glucose oxidase, was subjected to ammonium sulphate precipitation to obtain a concentrated crude enzyme extract for further purification and activity assays. Overall, the optimization of parameters including inoculum size, pH, and pineapple rind concentration demonstrated that this agricultural residue is a viable, sustainable, and cost-effective substrate for local-scale enzyme production.

Purification of Glucose oxidase

Following the initial concentration of extracellular proteins by ammonium sulphate precipitation, Glucose Oxidase (GOx) was subjected to further purification using ion exchange chromatography to enhance both its purity and enzymatic activity. The protein pellet obtained from precipitation was carefully resuspended in an appropriate buffer and dialyzed extensively to remove residual salts that could interfere with subsequent chromatographic separation.

The dialyzed enzyme solution was then applied to a CM-Sephadex C-50 cation exchange column. The column was first equilibrated with 10mM potassium phosphate buffer at the to facilitate binding of positively charged proteins. Upon loading the enzyme mixture, glucose oxidase adsorbed to the resin based on its net charge, while unbound proteins and other contaminants were removed through washing with the 10mM potassium phosphate buffer equilibration buffer. The bound GOx was subsequently eluted by gradually increasing the ionic strength of the buffer, using a 0 M to 0.5 M NaCl gradient, resulting in a purified enzyme suitable for activity assays and further characterization.

During elution, fractions were collected and analysed spectrophotometrically, and those showing glucose oxidase activity were pooled for further assessment. This purification step substantially enhanced the specific activity of the enzyme by effectively removing contaminating proteins (Figure 1).

Impact of inoculum size on enzyme production

Inoculum size plays a crucial role in determining the efficiency of microbial fermentation, as it influences biomass development, substrate utilization, and enzyme synthesis. In this study, the effect of varying inoculum sizes of *Aspergillus niger* (1%, 3%, and 5% v/v) on Glucose Oxidase (GOx) production was evaluated under submerged fermentation, with pineapple rind serving as the principal carbon source. The results demonstrated a clear influence of inoculum size on enzyme production. At the lowest inoculum level (1%), glucose oxidase yield was limited, likely due to insufficient fungal biomass, which reduced substrate degradation and enzyme secretion. In contrast, the highest inoculum level (5%) resulted in the highest GOx activity, indicating that this concentration provided adequate fungal growth while maintaining effective nutrient and oxygen availability. This balance facilitated optimal fermentation conditions and led to maximal enzyme production. Consequently, it was found that a 5% inoculum size was ideal for optimising *A. niger* synthesis of glucose oxidase, highlighting the critical significance that inoculum concentration plays in the fermentation process's overall success (Figure 2).

Impact of substrate concentration

The amount of glucose oxidase produced by submerged fermentation significantly decreases when the initial concentration of pineapple rind powder is increased from 1% to 5%. Optimal productivity of glucose oxidase was observed with 1% pineapple rind powder with an activity of 136 IU/mL (Figure 3).

Impact of additional carbon source on enzyme productivity

Adding extra carbon source i.e., dextrose showed minimal effect on overall productivity of Glucose oxidase. We observed a slightly higher concentration of Glucose oxidase produced with 5% dextrose in broth but comparatively lesser the without additional carbon sources. These results indicate higher percentage of dextrose addition might be triggering the organism to carry out

alternative metabolic pathways and to reduce overall productivity of glucose oxidase (Figure 4). GOX activity of experimental combinations were shown in Table 2.

DISCUSSION

The present study demonstrates the feasibility of utilizing pineapple rind, an underexploited agro-industrial residue, as a cost-effective substrate for Glucose Oxidase (GOx) production by *Aspergillus niger*. The findings provide evidence that agro-wastes rich in carbohydrates and fibers can serve as excellent carbon sources for fungal fermentation, thereby reducing dependence on conventional, high-cost synthetic media. The optimized fermentation process in this study achieved a maximum enzyme activity of 136 IU/mL with 1% pineapple rind powder and a 5% inoculum size, confirming that appropriate substrate concentration and inoculum density are critical for enhancing enzyme productivity.

The influence of inoculum size was particularly significant. A higher inoculum (5%) supported vigorous fungal growth and efficient substrate utilization, whereas lower inoculum levels were insufficient to generate the biomass required for substantial enzyme secretion. However, further increases in substrate concentration beyond 1% led to a decline in GOx productivity. This reduction may be attributed to substrate inhibition or accumulation of inhibitory metabolites, conditions that can adversely affect metabolic pathways and restrict enzyme synthesis. Comparable patterns have been reported in previous

Table 1: Experimental combinations for media preparation.

Flask. No	Pineapple rind Powder (%)	Yeast Extract (%)	Inoculum Size (%)	Dextrose (Additional Carbon) (%)
1	1%	1%	1%	-
2	3%	1%	1%	-
3	5%	1%	1%	-
4	1%	1%	3%	-
5	3%	1%	3%	-
6	5%	1%	3%	-
7	1%	1%	5%	-
8	3%	1%	5%	-
9	5%	1%	5%	-
10	1%	1%	5%	1%
11	1%	1%	5%	3%
12	1%	1%	5%	5%

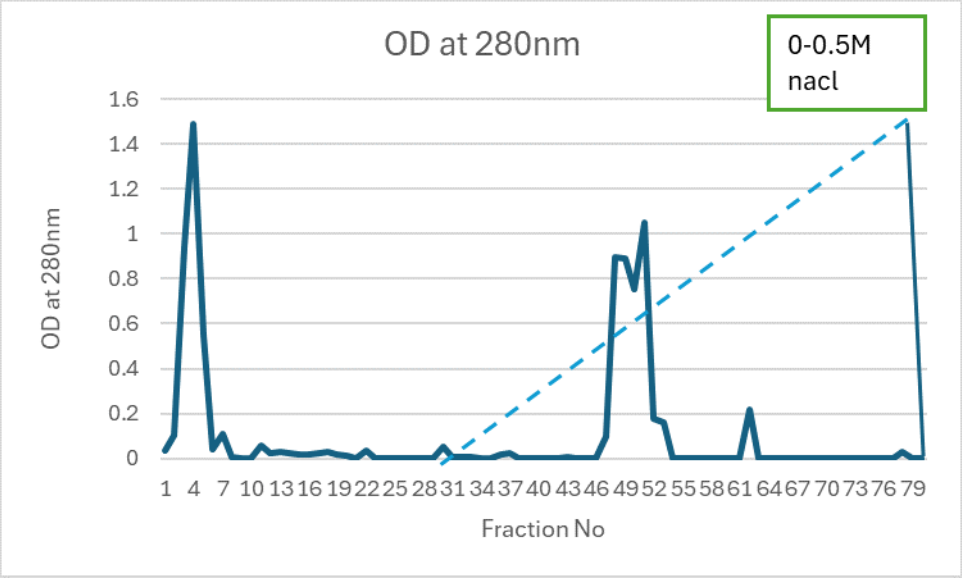

Figure 1: Chromatogram of Gox enzyme purification using cation exchange chromatography on CM Sephadex C-50 resin.

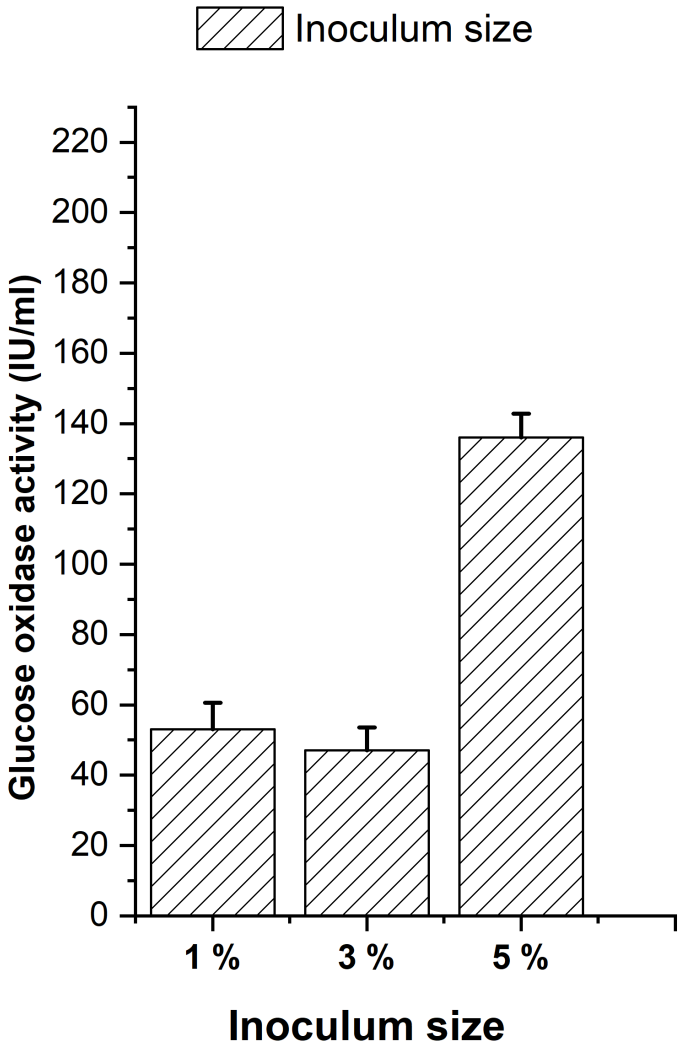

Figure 2: Culture initial concentration on glucose oxidase productivity.

studies where excessive substrate concentrations interfered with fungal metabolism, reducing yields of GOx and other hydrolytic enzymes (Ramachandran *et al.*, 2004; Friedrich *et al.*, 1990; Pandey *et al.*, 1999).

Interestingly, the supplementation of dextrose as an additional carbon source did not significantly enhance GOx production. On the contrary, higher dextrose levels resulted in decreased enzyme yields, suggesting the onset of catabolite repression. This phenomenon is consistent with earlier reports where readily metabolizable sugars diverted fungal metabolism toward primary growth rather than secondary metabolite formation. Such findings highlight the suitability of pineapple rind alone as a carbon source and reinforce the importance of selecting substrates that not only support fungal growth but also direct metabolic flux toward enzyme biosynthesis (Singh *et al.*, 2003; Kubicek *et al.*, 1988; Strauss *et al.*, 1995).

The purification of GOx through ammonium sulfate precipitation followed by ion-exchange chromatography yielded enzyme

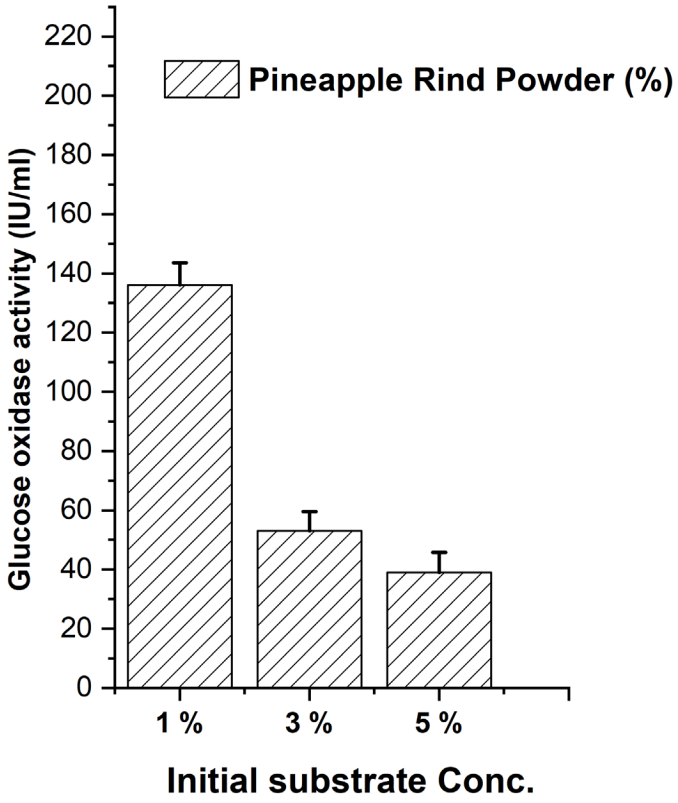

Figure 3: Impact of substrate concentration on enzyme production.

preparations with improved activity and purity, aligning with earlier purification strategies reported in fungal enzyme research. The approach adopted in this study confirms that low-cost substrates can still support the production of enzymes of sufficient quality for industrial and biomedical applications.

Beyond industrial processing, the biomedical significance of GOx is noteworthy. Its role in glucose biosensors for diabetes management underscores the relevance of developing sustainable production platforms that ensure affordability without compromising quality. The results of this study thus not only advance sustainable enzyme technology but also have potential to strengthen the bioeconomy by integrating waste valorization with healthcare applications.

Overall, this work confirms that pineapple rind is an effective substrate for fungal GOx production and demonstrates that optimizing simple fermentation parameters can achieve substantial improvements in enzyme yield. The dual benefits of reducing environmental waste and lowering production costs highlight the significance of this approach for large-scale, eco-friendly enzyme manufacturing. Future studies could investigate the use of combined agro-residues, large-scale cultivation in bioreactors, and strain improvement approaches to further increase enzyme yields and expand the scope of this sustainable bioprocess.

Table 2: GOX Activity of different experimental combinations.

Sl. No.	Pineapple rind Powder (%)	Yeast Extract (%)	Inoculum Size (%)	Dextrose (%)	Absorbance at 290 nm	IU/mL
1	1%	1%	1%	-	0.122	83
2	3%	1%	1%	-	0.102	44
3	5%	1%	1%	-	0.235	101
4	1%	1%	3%	-	0.110	47
5	3%	1%	3%	-	0.126	54
6	5%	1%	3%	-	0.175	75
7	1%	1%	5%	-	0.315	136
8	3%	1%	5%	-	0.124	53
9	5%	1%	5%	-	0.091	39
10	1%	1%	5%	1%	0.036	15
11	1%	1%	5%	3%	0.063	27
12	1%	1%	5%	5%	0.076	33

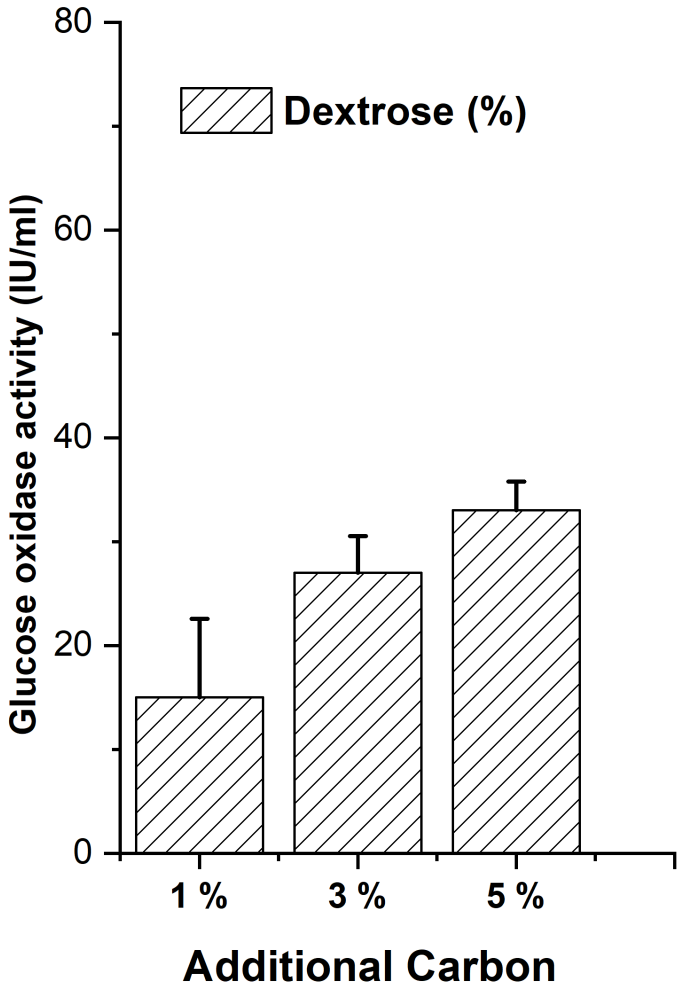

Figure 4: Effect of additional carbon sources on glucose oxidase productivity.

CONCLUSION

This study demonstrates that pineapple rind, an inexpensive agro-industrial by-product, can serve as an effective substrate for glucose oxidase production by *Aspergillus niger*. Utilizing this waste material reduces dependence on conventional nutrient sources while offering an eco-friendly strategy for converting organic residues into valuable products. The approach supports sustainable bioprocessing by linking waste management with enzyme production. Maximum glucose oxidase activity was recorded on the fifth day of fermentation, with peak yields achieved at 40°C and pH 5. Optimal productivity of glucose oxidase was observed with 1% pineapple rind powder with an activity of 136 IU/mL. Additional carbon source decreased overall glucose oxidase productivity. Beyond its industrial utility, glucose oxidase has considerable importance in the biomedical field, most notably in glucose biosensors that are widely applied for diabetes monitoring and management. By combining affordability, sustainability, and biomedical relevance, the present work highlights a practical strategy for enzyme production with direct implications for healthcare and bioeconomy.

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ABBREVIATIONS

SD: Standard Deviation; **IU/mL:** International Units per milliliter; **GOx:** Glucose Oxidase; **SmF:** Submerged Fermentation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Chandrasekhar Chanda conceived the idea and supervised the finding of this work. Harika Gadiparthi, Hema Gayatri Simhadri carried out the research work. All three authors involved in manuscript writing and statistical analysis. Chandrasekhar Chanda involved in manuscript corrections. All authors discussed the methodology and results and contributed to the final manuscript.

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