

Original Research Paper

doi https://doi.org/10.46488/NEPT.2024.v23i03.029 **Open Access Journal**

Production of Amylase by Solid State Fermentation Using Agricultural Waste

M. M. Morbia[*](https://orcid.org/0000-0001-7446-384X)† **.A.** A. Pandey[*](https://orcid.org/0009-0005-8584-9795) **.**[,](https://orcid.org/0009-0004-1108-9554) P. K. Mahla* **a**nd S. N. Gohil** **0**

*Microbiology Department, Swarrnim Startup and Innovation University, Bhoyan Rathod, Opp IFCO, near ONGC WSS, Adalaj-Kalol Highway, Gandhinagar, Gujarat, 382420, India

**Vitely Corp LLP, Microbiology laboratory, HO, Commerce House 2, Satya Marg, Opposite Pushparaj Tower,

Judges Bungalow Road, Bodakdev, Ahmedabad-380054, Gujarat, India

†Corresponding author: M.M. Morbia; morbiamayur@gmail.com

Nat. Env. & Poll. Tech. Website: www.neptjournal.com

Received: 28-12-2023 *Revised:* 08-02-2024 *Accepted:* 10-02-2024

Key Words: Amylase **Optimization** Agricultural waste Solid state fermentation

ABSTRACT

This study presents a comprehensive investigation into the production of amylase, a crucial enzyme with wide-ranging industrial applications, using locally sourced substrates from Kachchh, Gujarat. The research employed the *Bacillus licheniformis* strain and substrates such as coconut, rice husk, wheat bran, paddy straw, and maize straw. The study found paddy straw to be the most promising substrate for amylase production. The research also systematically optimized various process parameters for amylase production in Solid-State Fermentation (SSF) using the One Variable at a Time (OVAT) method. These parameters included incubation period, temperature, inoculum level, additional carbon sources, starch concentrations, additional nitrogen sources, initial pH, different mineral salt ions, initial moisture level, and surfactants. The results showed that the optimal conditions for maximum amylase yield were an incubation period of 48 hours, an incubation temperature of 35°C, an inoculum level of 10%, starch as the additional carbon source, a starch concentration of 2.5%, yeast extract as the additional nitrogen source, an initial pH of 7, NaCl as the mineral salt, an initial moisture level of 75%, and Tween 80 as the surfactant. This research provides a reliable and sustainable approach to enzyme production, offering valuable insights for the optimization of the solid-state fermentation process for maximum amylase production.

INTRODUCTION

Enzymes play a pivotal role in numerous industrial applications, catalyzing biochemical reactions with remarkable specificity and efficiency. Among these enzymes, amylase holds a significant position due to its wide-ranging applications in various industries, including food, textiles, and biofuel production. Solid-state fermentation (SSF) has emerged as an environmentally friendly and economically viable approach for amylase production, using agricultural waste as a substrate (Souza & Magalhaes 2010).

Amylase, an enzyme that hydrolyzes starch into simpler sugars, is crucial for many industrial processes. Traditionally, amylase has been produced using submerged fermentation; however, SSF offers distinct advantages, such as reduced water consumption, higher enzyme stability, and the use of solid substrates, particularly agricultural waste. This shift towards SSF aligns with the global efforts to develop sustainable and eco-friendly processes in the biotechnological industry (Saxena & Singh 2011).

Agricultural waste, a substantial byproduct of farming activities, poses environmental challenges when not effectively managed. The utilization of these agricultural residues as substrates for SSF not only addresses the issue of waste disposal but also transforms these residues into valuable resources for enzyme production. The incorporation of agricultural waste into the SSF process adds an extra layer of sustainability to the bioprocess, contributing to the concept of a circular economy (Sadh et al. 2018).

In this context, this research paper aims to investigate the production of amylase through solid-state fermentation, using agricultural waste as a cost-effective and sustainable substrate. The study focuses on optimizing the fermentation conditions to enhance amylase yield and activity, thereby contributing to the development of efficient and environmentally friendly enzymatic processes. The utilization of agricultural waste not only aligns with the principles of sustainable biotechnology but also addresses the dual challenges of enzyme production and waste management in the agricultural sector.

Through a comprehensive exploration of the potential of solid-state fermentation using agricultural waste, this research looks to provide insights into the optimization of amylase production, offering a sustainable alternative to traditional enzyme production methods. The findings

of this study could have far-reaching implications for the biotechnological industry, fostering a greener and more efficient approach to enzyme production.

MATERIALS AND METHODS

Selection of Microbial Culture

The research initiative involved the meticulous collection of soil specimens from six distinct Talukas, namely Gandhidham, Rapar, Bhuj, Nakhatrana, Mandvi, and Mundra, situated within the Kachchh District of Gujarat, India. For isolation and screening purposes designated 24-72 h incubation period on the starch agar plates was subjected to meticulous visual examination for growth.

Selection of Substrate

This study used locally sourced substrates like coconut, rice husk, wheat bran, paddy straw, and maize straw from Kachchh, Gujarat, for amylase production. Their abundant availability ensures a steady supply for enzyme production. Using these agricultural residues promotes sustainability by repurposing waste and economically utilizing local resources.

Pretreatment of Substrate

 The substrate was pretreated through several steps. It was washed with tap water and distilled water, treated with 1% NaOH to remove impurities, and dried at 80°C for two days. After being ground into fine particles, it was autoclaved at 121.6°C for 20 min (Mushtaq et al. 2023).

Preparation of Bacterial Inoculum

A 500 mL flask was filled with 250 mL of nutrient broth and autoclaved at 121.6°C for 20 min. After cooling, 1 mL of a 24-h-old broth was added and incubated at 37°C with 150 rpm agitation. Post -24hour incubation, 1 mL of the broth culture was added to the dry substrate for enzyme production (Saxena & Singh 2011).

Effect of Various Substrates on Enzyme Production

Four substrates-coconut, rice husk, wheat bran, paddy straw, and maize straw-were selected based on their starch content. After preparing the substrates, each underwent Solid-State Fermentation as per the below procedure.

Solid-state Fermentation (SSF)

SSF was conducted in 500 mL flasks, each containing 5 g of substrate. The moisture content was kept at 50%. After autoclaving at 121.6°C for 20 min and cooling, 1 mL of bacterial inoculum was added. The setup was then incubated for 48 h at 37°C (Cerda et al. 2016).

Purification of Crude Enzyme

Post-incubation, 50 mL of pH 7.4 phosphate buffer was added for crude enzyme extraction. The slurry was strained using a damp cheesecloth and centrifuged at 10000 rpm for 15 min to separate cells and particles. The cell-free supernatant, having the exoenzyme amylase, was used as the crude enzyme (Ramapriya et al. 2018).

Amylase Activity

Amylase activity was assessed using the 3,5-dinitrosalicylic acid (DNSA) method (Elyasi Far et al. 2020), which measures reducing sugars produced in the enzyme-substrate reaction. This systematic approach, from determining amylase activity to purifying the crude enzyme and conducting detailed assays, provided a reliable method for evaluating the amylase production of bacterial strains.

The amylase assay was conducted using a reaction mixture with 1% soluble starch in a 50 mM phosphate buffer at pH 7.2. The mixture was incubated for 10 minutes at 37°C, and the reaction was stopped by adding 2 mL of DNSA reagent. The mixture was then heat-treated at 100°C for 10 min and cooled. The optical density of each sample was measured at 540 nm using a spectrophotometer. Enzyme activity was quantified in units, with 1 unit.mL $^{-1}$ represents the enzyme amount that releases 1μ mole of glucose under the assay conditions. The substrate with the highest amylase production was identified for further study.

Optimization of Amylase Production Using the One Variable at A Time (OVAT) Method

This study systematically optimized various process parameters for amylase production in solid-state fermentation (SSF) (Table 1). Physicochemical parameters like fermentation period, initial pH, moisture level, and inoculum concentration were adjusted to study their effects on α-amylase production. Nutritional parameters like carbon and nitrogen sources, surfactants, and metal ions were also fine-tuned. The goal was to maximize enzyme production in SSF, enhancing the efficiency and yield of α-amylase, which is crucial for practical applications of SSF processes (Dike et al. 2022).

RESULTS AND DISCUSSION

Selection of Microbial Culture

Among various pure cultures, the *Bacillus licheniformis* was utilized for the production of amylase enzyme. *Bacillus licheniformis* was screened out as a potent one and identified (16 S rRNA) from a soil sample.

Effect of Various Substrates on Enzyme Production

Four substrates-coconut, rice husk, wheat bran, paddy straw, and maize straw-were evaluated for amylase production. Paddy straw showed the highest enzyme activity at 1.002 IU.g⁻¹, making it a promising substrate for amylase production. Wheat bran also showed good results with an activity of 0.812 IU.g-1. Coconut substrates and rice husk had lower activities at 0.513 IU.g⁻¹ and 0.612 IU.g⁻¹, respectively, while maize straw had a moderate activity of 0.849 IU.g⁻¹. [\(Fig. 1\)](#page-2-0).

Optimization of Amylase Production Using The One Variable at A Time (OVAT) Method

Effect of incubation period on enzyme production: The effect of the incubation period on enzyme production was investigated by varying the duration of fermentation at 24, 48, 72, and 96 h. The enzyme production, measured in $IU.g^{-1}$, varied at different incubation times, as outlined in [Fig. 2](#page-3-0).

The results show that amylase production varies across different incubation periods. The highest yield was observed at the 48-h mark, reaching 1.40 IU.g⁻¹, indicating that this is the optimal duration for enzyme production in this process. The lower yield at 24 hours $(0.07 \text{ IU} \cdot \text{g}^{-1})$ suggests that the fermentation process might not have reached peak efficiency in this short duration. The decline in enzyme production at 72 h (0.36 IU.g⁻¹) and 96 h (0.10 IU.g⁻¹) could be due to factors like substrate depletion, accumulation of by-products, or other inhibitory factors. Dike et al. (2022) also found a similar kind of pattern during the production of amylase enzyme.

Effect of incubation temperature on enzyme production: The study found that incubation temperature significantly affects amylase production. The highest yield was observed at 35 $^{\circ}$ C, with an enzyme production of 0.20 IU.g⁻¹, suggesting this is the optimal temperature for this process. At 30°C and 40 $^{\circ}$ C, the production was relatively high (0.18 IU.g⁻¹ and 0.15 IU.g⁻¹, respectively), indicating these temperatures are also favorable for amylase synthesis. However, a decrease in enzyme production was noted at 45° C (0.10 IU.g⁻¹) and

Table 1: Optimization parameters for amylase production.

Parameter	Levels
Incubation Period	24, 48, 72, 96 h
Incubation Temperature	30, 35, 40, 45, 50, 55° C
Inoculum Level (Bacillus licheniformis)	5%, 10%, 15%, 30%
Additional Carbon Sources [1%]	Starch, lactose, glucose, and fructose
Starch Concentrations	0.5, 1, 1.5, 2, and 2.5%
Additional Nitrogen Sources [1%]	$NH4Cl$, $(NH4)2SO4$, $NH4NO3$, Yeast extract, and peptone
Initial pH	4, 5, 6, 7, and 8
Different Mineral Salts Ions [100 µM]	NaCl, KH_2PO_4 , FeCl ₂ , CaCl ₂ , and MgSO ₄
Initial Moisture Level	25%, 50%, 75%, and 100%
Surfactants	Tween 20, Tween 80, SDS, and PEG

Fig. 1: Amylase production with different substrates.

Fig. 2: Results of optimized parameters for amylase production.

55 $\rm{^{\circ}C}$ (0.09 IU.g⁻¹), implying reduced enzymatic activity at higher temperatures. Notably, at 50°C, no measurable amylase production was observed, indicating a temperature beyond the optimal range for enzymatic activity under these conditions [\(Fig. 2\)](#page-3-0).

In a study on amylase production by *Bacillus subtilis*, it was found that the optimal activity temperature was 40°C. However, the enzyme yield dropped down more quickly with the increase in incubation period at 50°C (Salman et al. 2016). It is important to note that the optimal incubation temperature can vary depending on the specific strain of microorganisms used, the substrate, and the specific conditions of the fermentation process.

Effect of inoculum level on enzyme production: The study found that the inoculum level of isolated *Bacillus licheniformis* significantly impacts amylase production. The highest yield was observed with a 10% inoculum, reaching 1.80 IU.g⁻¹, suggesting this is the optimal level for this process. At lower inoculum levels of 5% and 15%, the production remained relatively high $(1.70 \text{ IU}.g^{-1})$ and 1.40 IU.g⁻¹, respectively), indicating these levels are also favorable for amylase synthesis. However, a slight decrease in enzyme production was noted at a higher inoculum level of 30% $(1.20 \text{ IU}.g^{-1})$, implying that an excessively high inoculum may not be conducive to maximum amylase yield [\(Fig. 2\)](#page-3-0).

Amylase is an enzyme that hydrolyses starch to glucose units. The production of amylase is influenced by several factors, including the inoculum level (Ozdemir et al. 2012).

In a study on amylase production by Aspergillus awamori under solid-state fermentation, an inoculum level of 10% (volume per mass) was found to be optimum for α -amylase production (Kalaiarasi & Parvatham 2015).

Effect of additional carbon sources on enzyme production: The investigation into the effect of additional carbon sources on amylase production revealed varying outcomes based on different carbon supplements (Starch, lactose, glucose, and fructose) with paddy straw. The enzyme production, measured in $IU.g^{-1}$, in the presence of different carbon sources, is summarized in [Fig. 2](#page-3-0).

The study found that the type of carbon source significantly impacts amylase production. Starch was the most effective, yielding the highest enzyme production at 4.01 IU.g⁻¹, suggesting that it, along with paddy straw, creates an optimal environment for amylase synthesis. Lactose also showed substantial effectiveness, yielding 3.56 IU.g⁻¹ of amylase, indicating its potential as a supplementary carbon source. However, glucose and fructose resulted in lower amylase production (2.96 IU.g⁻¹ and 2.88 IU.g⁻¹, respectively), suggesting they might not be as

conducive to amylase synthesis as starch and lactose under these experimental conditions.

In a study on amylase production by *Trichoderma viride*, it was found that the different commercial carbon substrates significantly affected the concomitant syntheses of amylase (Juwon & Emmanuel 2012).

Effect of different starch concentrations on enzyme production: The exploration of different starch concentrations for amylase production revealed varying outcomes, emphasizing the importance of starch concentration in optimizing enzyme yield. The enzyme production, measured in $IU.g^{-1}$ at different starch concentrations, is presented in [Fig. 2](#page-3-0).

The study found a clear concentration-dependent effect on amylase production. As the starch concentration increased, so did the enzyme yield. Specifically, at starch concentrations of 2% and 2.5%, amylase production significantly increased, reaching 11 IU.g⁻¹ and 11.5 IU.g⁻¹, respectively, suggesting that higher starch concentrations are favorable for maximizing amylase synthesis.

At lower concentrations (0.5%, 1%, and 1.5%), a progressive increase in enzyme production was also observed, indicating a positive correlation between starch concentration and amylase yield. However, the most substantial enhancement was observed at higher concentrations.

The control experiment with a starch concentration of 0% yielded an amylase production of 1.95 IU.g⁻¹, serving as a baseline for comparison. This reinforces the notion that starch is a critical substrate for amylase production and its absence results in significantly lower enzyme yields.

A study on amylase production by *Bacillus licheniformis* ZB-05 under solid-state fermentation found that as additional carbon sources, 2% soluble starch enhanced α-amylase production (Karatas et al. 2013).

Effect of additional nitrogen sources on enzyme production: The investigation into the effect of additional nitrogen sources on amylase production revealed distinct outcomes, highlighting the influence of different inorganic nitrogen sources. The enzyme production, measured in $IU.g^{-1}$, with various nitrogen sources at a 1% concentration, is summarized in [Fig. 2](#page-3-0).

The study found that nitrogen sources significantly impact amylase production. Yeast extract was the most effective, yielding the highest enzyme production at 17 IU.g-1, suggesting it provides optimal conditions for amylase synthesis. Peptone also showed substantial effectiveness, producing $12.5 \text{ IU}.g^{-1}$ of amylase.

Ammonium chloride and ammonium sulfate resulted in moderate amylase production (10.1 IU.g⁻¹ and 9.65 IU.g⁻¹, respectively), indicating their suitability as nitrogen sources, albeit with a lower impact compared to yeast extract and peptone.

However, ammonium nitrate led to lower amylase production $(1.2 \text{ IU}.g^{-1})$, suggesting it might be less conducive to amylase synthesis under these experimental conditions.

The control experiment with no added nitrogen yielded an amylase production of 8.8 IU.g⁻¹, serving as a baseline for comparison. This reinforces the notion that the presence of specific nitrogen sources positively influences amylase production.

In a study on amylase production by *Brevibacillus borstelensis* R1, it was found that the addition of different nitrogen sources greatly affected the production of amylase (Suribabu et al. 2014).

Effect of Initial pH on Enzyme Production

The investigation into the effect of initial pH on amylase production revealed distinct outcomes, emphasizing the significance of pH in optimizing enzyme yield. The enzyme production, measured in $IU.g^{-1}$ at different pH levels, is summarized in [Fig. 2.](#page-3-0)

The study found that the initial pH significantly impacts amylase production. The optimal pH was found to be 7, yielding the highest enzyme production at $20.1 \text{ IU}.g^{-1}$, suggesting a slightly acidic to neutral pH environment is conducive for maximum amylase synthesis.

At pH 6 and 8, substantial amylase production was observed (16.8 IU.g⁻¹ and 15.5 IU.g⁻¹, respectively), indicating that the enzyme is still active and efficient at these pH levels.

However, lowering the pH to 5 and 4 resulted in decreased amylase production $(14.5 \text{ IU}.g^{-1})$ and 12.1 $IU.g^{-1}$, respectively), suggesting that excessively acidic conditions may adversely affect amylase synthesis.

A published study emphasized the of pH influence the production of alpha-amylase (Kalaiarasi & Parvatham 2015, Suribabu et al. 2014)

Effect of different mineral salt ions on enzyme production: The investigation into the effect of different mineral salts on amylase production provided insights into the influence of specific metal ions on enzyme yield. The enzyme production, measured in $IU.g^{-1}$, with various mineral salts at a concentration of 100 µM is summarized in [Fig. 2.](#page-3-0)

The study found that the addition of specific mineral salts significantly impacts amylase production. Here are the results:

- NaCl (Sodium Chloride): The presence of NaCl resulted in the highest yield at $22.15 \text{ IU}.g^{-1}$, suggesting that sodium ions play a positive role in promoting amylase synthesis.
- K2HPO₄ (Potassium Hydrogen Phosphate): The addition of K_2HPO_4 yielded 19.15 IU.g⁻¹, indicating that potassium ions contribute to the enhancement of amylase synthesis.
- FeCl₂ (Iron (II) Chloride): Iron ions resulted in an α-amylase production of 16.5 IU.g⁻¹, demonstrating a positive impact on amylase synthesis.
- CaCl₂ (Calcium Chloride): Calcium ions positively influenced α-amylase production, yielding 20.1 IU.g⁻ ¹, suggesting that calcium plays a role in enhancing amylase synthesis.
- MgSO₄ (Magnesium Sulphate): Magnesium ions resulted in an α-amylase production of 19.15 IU.g⁻¹, indicating that, like potassium and calcium, magnesium ions contribute to the positive regulation of amylase synthesis.

The control experiment, without the addition of specific mineral salts, produced α -amylase at 19.5 IU.g⁻¹, serving as a baseline for comparison. A Published study shows a significant effect on the production of amylase enzyme (Saxena & Singh 2011).

Effect of initial moisture level on enzyme production: The investigation into the effect of different levels of initial moisture on amylase production elucidated the impact of varying substrate moisture content on enzyme yield. The enzyme production, measured in $IU.g^{-1}$ at different moisture levels, is summarized in [Fig. 2.](#page-3-0)

The study found that the initial moisture level of the substrate significantly impacts amylase production. Here are the results:

- 25% Moisture: A moisture level of 25% resulted in an amylase production of 21.5 $IU.g^{-1}$, suggesting that lower moisture levels still support significant amylase synthesis.
- 50% Moisture: Increasing the moisture level to 50% positively impacted amylase production, yielding 22.75 IU.g⁻¹, indicating that a moderate increase in substrate moisture enhances enzyme yield.
- 75% Moisture: The highest amylase production was observed at a moisture level of 75%, reaching 28.2 IU.g⁻¹, suggesting that the fermentation process

is particularly efficient when the substrate has a higher moisture content.

• 100% Moisture: A further increase to 100% moisture resulted in a slight reduction in amylase production to 25.5 IU.g⁻¹, indicating that excessively high moisture levels may not be optimal for enzyme synthesis.

This information is crucial for optimizing the solid-state fermentation process for maximum amylase production. It shows the importance of carefully controlling the moisture level of the substrate to achieve the best results (Gangadharan et al. 2007).

Effect of various surfactants on enzyme production: The investigation into the effect of various surfactants on amylase production provided insights into the impact of different surface-active agents on enzyme yield. The enzyme production, measured in $IU.g^{-1}$, with various surfactants is summarized in [Fig. 2.](#page-3-0)

The study found that different surfactants significantly impact amylase production. Here are the results:

- Tween 20: The addition of Tween 20 enhanced amylase production, yielding $35.2 \text{ IU}.g^{-1}$, suggesting it positively influences amylase synthesis.
- Tween 80: Tween 80 was the most effective, yielding the highest enzyme production at 38.35 IU.g⁻¹, indicating it's particularly effective in promoting amylase production.
- SDS (Sodium Dodecyl Sulphate): The addition of SDS also positively influenced amylase production, yielding $35.5 \mathrm{~IU}.g^{-1}$, suggesting it contributes to the enhancement of amylase synthesis.
- PEG (Polyethylene Glycol): PEG resulted in slightly lower amylase production at $30.1 \text{ IU}.g^{-1}$ compared to the other surfactants, indicating its impact on amylase synthesis is less pronounced under these experimental conditions.

This information is crucial for optimizing the solid-state fermentation process for maximum amylase production. It shows the importance of carefully selecting the surfactants to achieve the best results (Singh & Kumar Brahman 2013).

CONCLUSION

The comprehensive study we conducted aimed to optimize amylase production through a systematic exploration of a range of factors and conditions. The final set of media identified to maximize enzyme yield includes:

• Substrate Composition: Paddy straw (5 g) and Starch (3%) .

- Mineral Salt and Additives: NaCl $(100 \mu M)$, Tween-80 (1%) , and Yeast Extract (1%) .
- Moisture Content: Initial Moisture (75%).
- Incubation Conditions: Incubation Time (48 hours), Temperature $(35^{\circ}$ C), and pH (7) .

This optimal combination ensures significant enzymatic activity and provides insights for process scalability and industrial applications. The established parameters serve as a valuable foundation for further studies and applications in the field of enzyme production. This research offers a systematic and tailored approach to maximize amylase yield, contributing to the advancement of biotechnological processes and applications.

REFERENCES

- Cerda, A., El-Bakry, M., Gea, T. and Sanchez, A. 2016. Long-term enhanced solid-state fermentation: Inoculation strategies for amylase production from soy and bread wastes by Thermomyces sp. in a sequential batch operation. J. Environ. Chem. Eng., 4(2): 2394-2401. DOI:10.1016/j. jece.2016.04.022
- Dike, P., Ogugbue, C., Akaranta, O. and Oji, A. 2022. Optimization of B. cereus PW4 alpha-amylase production by OVAT technique. GSC Biol. Pharm. Sci., 20(1): 083-090. DOI:10.30574/gscbps.2022.20.1.0305
- Elyasi Far, B., Ahmadi, Y., Yari Khosroshahi, A. and Dilmaghani, A. 2020. Microbial alpha-amylase production: progress, challenges, and perspectives. Adv. Pharm. Bull., 10(3): 350-358. DOI:10.34172/ apb.2020.043
- Gangadharan, D., Nampoothiri, K. and Pandey, A. 2007. Alpha amylase production by Aspergillus oryzae employing solid-state fermentation. J. Sci. Ind. Res., 66: 621-626.
- Juwon, A. D. and Emmanuel, O. F. 2012. Experimental investigations on the effects of carbon and nitrogen sources on concomitant amylase and polygalacturonase production by Trichoderma viride BITRS-1001 in submerged fermentation. Biotechnol. Res. Int., 2: 1-8. DOI:10.1155/2012/904763
- Kalaiarasi, K. and Parvatham, R. 2015. Optimization of process parameters for α-amylase production under solid-state fermentation by Aspergillus awamori MTCC 9997. J. Sci. Ind. Res., 74: 286-289.
- Karatas, H., Uyar, F., Tolan, V. and Baysal, Z. 2013. Optimization and enhanced production of α -amylase and protease by a newly isolated Bacillus licheniformis ZB-05 under solid-state fermentation. Ann. Microbiol., 63(1): 45-52. DOI:10.1007/s13213-012-0443-6
- Mushtaq, Q., Joly, N., Martin, P. and Qazi, J. I. 2023. Optimization of alkali treatment for the production of fermentable sugars and phenolic compounds from potato peel waste using topographical characterization and FTIR spectroscopy. Molecules, 28(21): 7250. DOI:10.3390/ molecules28217250
- Ozdemir, S., Matpan, F., Okumus, V., Dündar, A., Ulutas, M. S. and Kumru, M. 2012. Isolation of a thermophilic Anoxybacillus flavithermus sp. nov. and production of thermostable α-amylase under solid-state fermentation (SSF). Ann. Microbiol., 62(4): 1367-1375. DOI:10.1007/ s13213-011-0385-4
- Ramapriya, R., Thirumurugan, A., Sathishkumar, T. and Manimaran, D. R. 2018. Partial purification and characterization of exoinulinase produced from Bacillus sp. J. Genet. Eng. Biotechnol., 16(2): 363-367. DOI:10.1016/j.jgeb.2018.03.001
- Sadh, P. K., Duhan, S. and Duhan, J. S. 2018. Agro-industrial wastes and their utilization using solid-state fermentation: a review. Bioresour. Bioprocess., 5(1): 1. DOI:10.1186/s40643-017-0187-z
- Salman, T., Kamal, M., Ahmed, M., Siddiqa, S. M., Khan, R. A. and Hassan, A. 2016. Medium optimization for the production of amylase by *Bacillus subtilis* RM16 in shake-flask fermentation. Pak. J. Pharm. Sci., 29(2): 439-444.
- Saxena, R. and Singh, R. 2011. Amylase production by solid-state fermentation of agro-industrial wastes using Bacillus sp. Braz. J. Microbiol., 42(4): 1334-1342. DOI:10.1590/S1517-838220110004000014
- Singh, P. and Kumar Brahman, L. 2013. Effect of carbon, nitrogen sources, and surfactant on production of α-amylase by *Bacillus subtilis*. J. Kalash Sci., 1(2): 83-86.
- Souza, P. M. and Magalhães, P. 2010. Application of microbial α-amylase in industry - A review. Braz. J. Microbiol., 41(4): 850-861. DOI:10.1590/ S1517-83822010000400004
- Suribabu, K. Govardhan, T. L. and Hemalatha, K. 2014. Optimization of various nitrogen sources for the production of amylase using *Brevibacillus borstelensis* R1 by submerged fermentation. Int. J. Curr. Microbiol. App. Sci., 3(4): 791-800.

ORCID DETAILS OF THE AUTHORS

- M. M. Morbia: https://orcid.org/0000-0001-5339-5868
- A. A. Pandey: https://orcid.org/0009-0004-1108-9554
- P. K. Mahla: https://orcid.org/0009-0005-8584-9795
- S. N. Gohil: <https://orcid.org/0000-0001-7446-384X>

