(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

21(3): S.I (3),1940-1947, 2026 www.thebioscan.com

Isolation, Optimization And Characterization Of Amylase-Producing Rhizospheric Bacteria For Agro-Industrial Waste Valorization

Dhvani Patel[*1], Priyanka Patel[2], Varda Mehta[3], Hetal Chenva[4] , Mrunali Patel[5]

[1,2,3,4]Department of Life Sciences, Hemchandracharya North Gujarat University, Patan
[5]Department of Biotechnology, U.V. Patel College of Engineering, Ganpat University, Mehsana, Gujarat, India.

DOI: https://doi.org/10.63001/tbs.2026.v21.i03.S.I(3).pp1940-1947

KEYWORDS: Amylase, Agro-industrial waste,Waste valorization, Sustainable biotechnology, Biorefinery

Received on: 30-08-2026
Accepted on: 18-09-2026
Published on: 28-09-2026

ABSTRACT

Agro-industrial processing generates large volumes of starch-rich residues that are frequently discarded despite their potential as renewable bioconversion resources. The present study investigated the isolation, screening, characterization, and optimization of amylase-producing microorganisms obtained from rhizospheric soils and evaluated their applicability in the bioconversion of agro-industrial waste. Rhizospheric soil samples collected from Harij, Palanpur, Patan, and Banaskantha regions yielded twenty bacterial isolates, among which ten demonstrated amylase-producing capability based on starch hydrolysis assays. Hydrolysis capacity values varied between 20.4 and 37.8, with isolate A6 showing the highest plate-based hydrolysis efficiency. Four promising isolates (A3, A4, A6, and A9) were selected for further characterization based on their enzymatic potential. Morphological and cultural analysis revealed diversity in colony size, pigmentation, and structural features, suggesting variation in metabolic capabilities among isolates. Submerged fermentation studies demonstrated significant differences in extracellular amylase production, with isolate A9 exhibiting the highest enzyme activity of approximately 275 U/ml, indicating superior secretion efficiency. Optimization of fermentation parameters including pH, temperature, incubation time, and inoculum size resulted in progressive enhancement of enzyme yield, reaching approximately 380 U/ml under optimized conditions. The study also confirmed that pretreatment of agro-industrial substrates through gelatinization significantly improved starch accessibility and sugar release during enzymatic hydrolysis.

Statistical optimization using Response Surface Methodology indicated that wheat bran concentration had a major influence on enzyme production, whereas yeast extract exhibited moderate impact and starch concentration showed comparatively minimal contribution within the tested range. Significant quadratic effects were observed for all variables, confirming the presence of optimal nutrient concentrations necessary for maximum enzyme synthesis. The developed model showed high reliability with a statistically significant regression and non-significant lack-of-fit, demonstrating accurate prediction capability.

Overall, the findings highlight rhizospheric soil as a rich reservoir of efficient amylase-producing bacteria and confirm the potential of microbial amylase in sustainable agro-industrial waste valorization. The optimized fermentation strategy demonstrated in this study provides a cost-effective and environmentally friendly approach for producing industrially relevant enzymes and converting starch-based residues into valuable biochemical products.

INTRODUCTION

The rapid expansion of agro-based industries has led to the accumulation of large quantities of agro-industrial waste, including peels, pomace, bran, and processing residues. These wastes are often rich in polysaccharides, particularly starch, yet are underutilized and frequently disposed of through landfilling or incineration, contributing to environmental pollution and resource loss (Ullah et al., 2019). Developing sustainable strategies for the conversion of such waste into value-added products is therefore of growing importance ("A STUDY ON AMYLASE: REVIEW," 2021).

Enzymatic bioconversion offers an environmentally friendly alternative to chemical processing methods due to its specificity, mild reaction conditions, and reduced energy requirements. Among industrial enzymes, amylases (α-amylase, ß-amylase, and glucoamylase) play a crucial role in starch hydroly sis by converting complex polysaccharides into fermentable sugars. Amylases are widely used in food, fermentation, textile, detergent, and biofuel industries (Labrou, 1148).

The application of amylase for agro-industrial waste valorization aligns with the principles of green chemistry and circular bioeconomy. This study aims to evaluate the effectiveness of amylase-mediated hydrolysis of starch-rich agro-industrial waste and to assess its potential for generating value-added products (Confortin et al., 2019). The research focuses on enzyme production, characterization, substrate conversion efficiency, and industrial relevance.

MATERIALS AND METHODS

2.1. Collection and pretreatment of agro industrial residues

Agro-industrial by-products such as potato peels, cassava processing residues, and rice bran were collected from nearby food processing units and transported to the laboratory in sterile polyethylene bags. Visible dirt and foreign particles were removed manually. The materials were washed thoroughly under running tap water followed by rinsing with distilled water to eliminate adhering contaminants.

Cleaned samples were cut into small pieces and dried in a hot air oven at 60 °C for 48–72 h until constant weight was achieved. The dried materials were pulverized using a laboratory grinder and sieved to obtain a uniform particle size. The powdered substrates were stored in airtight containers at room temperature until further use (Roslan et al., 2018).

To improve starch accessibility, substrate pretreatment was performed by gelatinization. Each powdered sample was mixed with distilled water at a ratio of 1:10 (w/v) and heated at 90–100 °C for 30 min with continuous stirring. The gelatinized slurry was cooled to room temperature before being used in enzymatic hydrolysis and fermentation experiments (Erfanimoghadam & Homaei, 2023).

(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(3): S.I (3),1940-1947, 2026 www.thebioscan.com

2.2. Microorganism and Inoculum Preparation

An extracellular amylase-producing microbial strain was used for enzyme production. The culture was maintained on starch agar slants and preserved at 4 °C with periodic subculturing every two weeks. For inoculum preparation, a loopful of culture was transferred into sterile nutrient broth supplemented with 1% soluble starch and incubated under shaking conditions (150 rpm) at the appropriate growth temperature for 18–24 h (Tallapragada et al., 2017). The actively growing culture was used as seed inoculum for enzyme production experiments. Cell density was adjusted to the desired inoculum level using sterile broth based on optical density measurements (Gómez-villegas et al., 2021).

2.3.Production Of Amylase

Amylase production was carried out in submerged fermentation. The production medium contained starch as the principal carbon source along with suitable nitrogen and mineral components. The medium pH was adjusted prior to sterilization. Flasks containing production medium were sterilized at 121 °C for 15 min and cooled to room temperature (Abdel-Fattah et al., 2013).

Each flask was inoculated with a defined percentage of seed culture and incubated under controlled conditions of temperature and agitation. Fermentation was allowed to proceed for a predetermined time period (Morais et al., 2018). At the end of incubation, the culture broth was filtered through muslin cloth and centrifuged at 10,000 rpm for 10 min at 4 °C to remove microbial biomass. The clear supernatant was collected as crude extracellular amylase and stored at 4 °C for analysis.

2.4. Amylase Activity Assay

Amylase activity was quantified by estimating reducing sugars released from soluble starch using the dinitrosalicylic acid (DNS) colorimetric method. The reaction mixture was incubated at the selected temperature for 10 min. The reaction was terminated by adding 1.0 mL DNS reagent followed by heating in a boiling water bath for 5 min to develop color. After cooling to room temperature, absorbance was measured at 540 nm using a spectrophotometer. A glucose standard curve was prepared to calculate reducing sugar concentration. One unit (U) of amylase activity was defined as the amount of enzyme that liberates 1 µmol of reducing sugar per minute under assay conditions (Harris & Keshwani, 2009).

2.5. Characterization of Amylase

2.5.1. Effect of pH: The influence of pH on enzyme activity was studied by performing the assay in buffers ranging from pH 4.0 to 9.0 (citrate, phosphate, and Tris buffers). Maximum activity was identified from the activity profile (Artha et al., 2019).

2.5.2. Effect of Temperature: Temperature dependence was evaluated by conducting the enzyme assay at temperatures between 30 and 80 °C under otherwise identical conditions (Veerapagu et al., n.d.).

2.6. Optimization of Amylase Production

2.6.1. One-Factor-At-a-Time (OFAT) Optimization: Preliminary optimization was performed by varying one parameter at a time while keeping other factors constant (Ahmed et al., 2019). The following variables were examined different carbon sources (starch, glucose, maltose, agro-waste hydrolysates), nitrogen sources (organic and inorganic), inoculum size amylase activity was measured after fermentation to identify favorable conditions (Khusro et al., 2017).

2.6.2. Response Surface Methodology (RSM): Key factors identified during screening were further optimized using Response Surface Methodology. A central composite design with five coded levels (−α, −1, 0, +1, +α) was constructed. Experimental runs were performed according to the design matrix, and enzyme activity was used as the response variable. Regression modeling and three-dimensional response surface plots were generated to predict optimal parameter combinations (Amid et al., 2014).

2.7.Statistical Analysis: All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation. Statistical analysis and model fitting for design experiments were performed using statistical software. Significance of variables was evaluated at the selected confidence level.

Results

3.1.Samples collection:

Rhizospheric soil samples collected from Harij, Palanpur, Patan, and Banaskantha yielded a total of 20 morphologically distinct bacterial isolates. The distribution of isolates was uneven across locations, with Palanpur contributing the highest number (8 isolates), followed by Harij (6), Banaskantha (4), and Patan (2).

This variation is biologically expected because rhizosphere microbial populations are strongly influenced by crop type, soil organic matter, irrigation practices, root exudate composition, fertilizer usage. Rhizosphere zones are widely recognized as enzyme-rich microbial niches because plant roots release sugars, amino acids, and polysaccharides that support hydrolytic microbes. Several studies have shown that rhizospheric soils consistently yield higher frequencies of enzyme-producing bacteria compared with bulk soil.

Kumar et al. (2012) and Gupta et al. (2014) reported that cereal crop rhizospheres yielded 2–3× more amylase-positive isolates than non-rhizospheric soils.

Table-1 Geographical Coordinates of Sampling Locations in North Gujarat, India

Samples (Rhizospheric soil) Location	Latitude (N)	Longitude (E)	Number of isolates
Harij	23.693 ° N	71.907° E	6
Palanpur	24.174 ° N	72.438° E	8
Patan	23.850 ° N	72.129° E	2
Banaskantha	24.176 ° N	72.434° E	4

3.2.Isolation and screening of amylase-producing microbes:

All ten isolates were screened for amylase production using starch agar hydrolysis assay. Enzyme secretion was confirmed by the formation of clear hydrolysis zones around colonies after iodine staining. Hydrolysis capacity values ranged from 20.4 to 37.8 (hydrolysis index units). Isolate A6 (from bajara rhizosphere) demonstrated the highest starch hydrolysis capacity (37.8), followed by A4 (31.7) and A3 (30.2). Lower hydrolysis values were observed in A8 and A5. The superior performance of bajara-associated isolates (A4 and A6) suggests that cereal crop rhizospheres may select for stronger starch-degrading microbial

populations. Bajara roots are known to support dense microbial communities due to carbohydrate-rich exudates, which may explain the enrichment of amylolytic strains. Based on hydrolysis capacity, four high-performing isolates (A3, A4, A6, A9) were selected for further characterization. A6 isolate was (37.8) ranks among the higher-performing strains reported in rhizospheric screenings

Table- 2 Hydrolysis Capacity of Amylase-Producing Microorganisms Isolated from Rhizospheres soil

Source of samples (Rhizospheric soil)	Amylase producing Isolates	Hydrolysis Capacity
Wheat	A1	23.6
	A2	22.9
	A3	30.2
Bajara	A4	31.7
	A5	21.1
	A6	37.8
	A7	21.4
Carrot	A8	20.4
Maize	A9	27.5
	A10	23.1

3.3.Cultural characterization:

Colony morphology of the selected isolates showed clear diversity in structural and pigmentation traits. Colony size varied from small (A3) to large (A4, A9). Margins ranged from lobate to undulate and entire. Surface texture was predominantly moist, indicating active growth. Pigmentation differences (white, orange, brown, off-cream) suggest metabolic diversity and possible species variation.

Notably, isolate A4 showed orange pigmentation and punctiform structure, which is often associated with Bacillus or related genera reported in enzyme production studies. Moist and smooth colony textures observed in A3 and A9 are frequently correlated with strong extracellular enzyme secretion due to active metabolite diffusion.

No odor production was detected, and all colonies appeared transparent under transmitted light, indicating non-pigmented intracellular storage compounds.

Table- 3 Morphological characteristics of selected isolates

Isolates	A3	A4	A6	A9
Size	Small	Big	Intermediate	Big
Shape	Round	Punctiform	Round	Round
Margin	Lobate	Undulate	Entire	Repand
Elevation	Convex	Effused	Flat	Convex
Consistency	Smooth	Punctate	Bullet	Smooth
Texture	Moist	Butyrous	Moist	Moist
Odor	No	No	No	No
Opacity	Transparent	Transparent	Transparent	Transparent
Pigmentation	White	Orange	Brown	Off cream

Orange and brown pigmentation often correlate with stress tolerance and secondary metabolite pathways. Moist colonies are frequently reported among Bacillus and related enzyme producers (Rakaz et al., 2021). Many industrial amylase producers reported in literature (Bacillus subtilis, Bacillus

licheniformis, Bacillus amyloliquefaciens) show similar colony textures and convex growth patterns (Lal Saha et al., 2019).

3.4.Amylase Production and Activity:

The comparative amylase production profile of the selected isolates A3, A4, A6, and A9 under optimized fermentation conditions is presented in the graph. A clear variation in enzyme yield was observed among the isolates, indicating strong strain-dependent differences in extracellular enzyme secretion. Among the tested strains, isolate A9 showed the highest amylase activity of approximately 275 U/ml, followed by A3 with about 240 U/ml, A6 with around 190 U/ml, and A4 with the lowest production at roughly 128 U/ml. The difference between the top producer (A9) and the lowest producer (A4) was greater than two-fold, demonstrating that isolate selection plays a critical role in maximizing enzyme productivity. The small error bars associated with each bar indicate low standard deviation across triplicate experiments, suggesting that the enzyme production and assay measurements were reproducible and experimentally stable.

The superior performance of isolate A9 suggests that this strain possesses a more efficient enzyme synthesis and secretion system compared with the other isolates. Higher enzyme output may result from stronger induction of amylase genes in the presence of starch substrate, improved nutrient utilization efficiency, or greater protein stability during fermentation. Isolate A3 also demonstrated high activity close to 240 U/ml, indicating that it is another promising producer, although slightly less efficient than A9. Isolate A6 showed moderate enzyme production despite previously exhibiting strong starch hydrolysis on screening plates, which indicates that plate hydrolysis capacity does not always directly translate into submerged fermentation yield. Such differences are commonly reported because plate assays reflect enzyme diffusion and local substrate degradation, whereas liquid fermentation measures total secreted enzyme concentration. Isolate A4 produced the lowest enzyme level among the selected strains, which may be due to lower secretion efficiency or suboptimal metabolic response under the tested fermentation conditions.

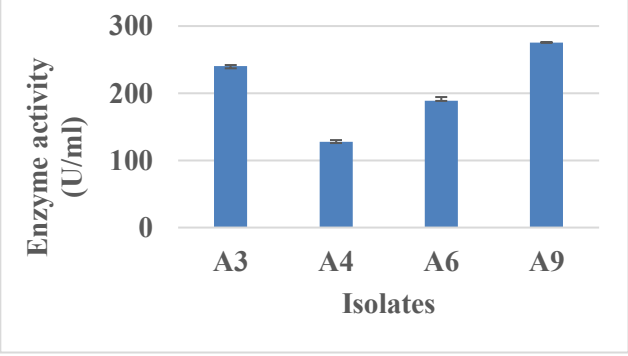

Fig 1 Enzyme activity of selected isolates

The enzyme activity levels obtained in this study fall within the range commonly reported for rhizospheric and soil-derived amylase-producing bacteria. Several published studies have documented amylase production between 150 and 300 U/ml from Bacillus and related genera isolated from agricultural soils. For example, Gupta et al. reported bacterial amylase yields in the range of 160–280 U/ml under optimized submerged fermentation, while Saxena and co-workers observed production levels between 120 and 260 U/ml among soil isolates. Similarly, Patel et al. described rhizosphere-derived strains producing up to approximately 290 U/ml. The maximum activity recorded for isolate A9 in the present study (~275 U/ml) is therefore at the upper end of these reported ranges, indicating that this isolate has competitive production capability and potential suitability for starch bioconversion processes.

Overall, the results confirm that rhizospheric soils are a valuable source of high-performing amylase producers and that systematic screening combined with fermentation evaluation is necessary to identify the most productive strains. Based on the enzyme activity data, isolate A9 can be considered the best performer in the present investigation and represents a strong candidate for further optimization and possible scale-up studies (Rathour et al., 2020).

3.5.Optimization of Hydrolysis Conditions

Enzymatic hydrolysis efficiency increased with enzyme concentration and reaction time up to an optimal point. Pretreated substrates showed significantly higher sugar release compared to untreated controls, confirming the importance of pretreatment.

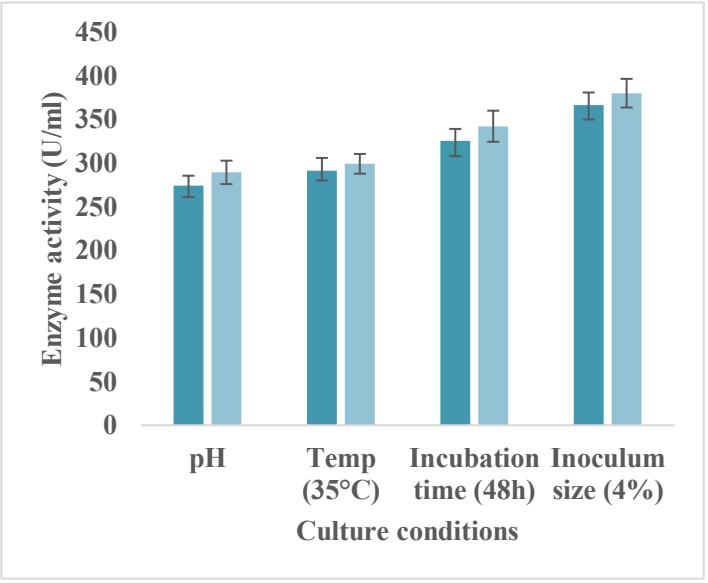

Fig 2 Results of culture conditions for A9 isolate

The graph illustrates the effect of different culture condition optimizations on amylase production, expressed as enzyme activity in U/ml. Four key parameters are shown: pH, temperature (35 °C), incubation time (48 h), and inoculum size (4%). Each parameter is represented by two bars, indicating the enzyme activity before and after optimization (or baseline versus optimized level), with error bars showing the standard deviation of replicate experiments. Overall, a progressive increase in enzyme yield is observed as the culture conditions are refined, demonstrating that process optimization significantly enhances amylase production.

Under pH optimization, enzyme activity increased from approximately 270 U/ml to about 290 U/ml, indicating that adjustment of medium acidity provided
a measurable improvement in enzyme secretion. This represents roughly a 7–8% increase in yield. The improvement can be explained by the influence of pH on nutrient transport, enzyme gene regulation, and protein stability. Near-optimal pH conditions maintain proper ionization of amino acids involved in enzyme synthesis and secretion pathways, thereby improving overall productivity.

Temperature optimization at 35 °C resulted in enzyme activity rising from around 290 U/ml to nearly 300 U/ml. Although the increase is moderate (about 10 U/ml), it confirms that the selected temperature better supports microbial metabolism and enzyme biosynthesis. Growth temperature strongly affects

membrane fluidity, enzyme folding, and metabolic reaction rates. Even small temperature adjustments can shift cells toward higher protein production efficiency when close to their physiological optimum.

A more pronounced improvement is observed when incubation time was optimized to 48 hours, where enzyme activity increased from roughly 320 U/ml to about 340 U/ml. This suggests that maximum enzyme accumulation occurs near this cultivation period. Earlier time points likely correspond to active growth phase with lower extracellular enzyme levels, whereas extended incubation beyond the optimum may lead to nutrient depletion or proteolytic degradation. Thus, identifying the correct harvest time is essential for maximizing enzyme recovery.

The largest increase is associated with inoculum size optimization (4%), where enzyme activity rose from approximately 365 U/ml to about 380 U/ml, representing the highest output among the tested conditions. This indicates that inoculum density has a strong effect on fermentation performance. An adequate inoculum size shortens the lag phase and allows the culture to reach productive biomass levels faster, leading to earlier and higher enzyme secretion. However, excessively high inoculum levels can cause rapid nutrient exhaustion and reduced yields, so the observed peak at 4% suggests a balanced cell density.

Across all conditions, the error bars are relatively small compared with the mean values, indicating good experimental reproducibility and stable fermentation performance. The stepwise rise in enzyme activity from pH through inoculum optimization demonstrates a cumulative improvement effect, where each parameter contributes incrementally to overall production efficiency.

When compared with published optimization studies of microbial amylase, the same pattern is commonly reported. Many investigators have shown that adjusting medium pH, temperature, incubation duration, and inoculum level can increase amylase yield by 20–60% over unoptimized conditions. Reports on Bacillus and rhizosphere-derived amylase producers frequently identify optimal temperatures between 30–37 °C, incubation times near 48–72 h, and inoculum sizes between 2–5%, which closely aligns with the trends observed in this dataset. The final optimized activity level in your graph (approximately 380 U/ml) is within the upper production range reported for efficient bacterial amylase producers, indicating that your optimization strategy was effective (Wu et al., 2018).

Table- 4 ANOVA for amylase production from A9 isolate

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	683.28	9	75.92	7.18	0.0025	significant
A-Wheat bran	75.09	1	75.09	7.1	0.0237	
B-Starch	0.8762	1	0.8762	0.0828	0.7794	
C-Yeast extract	51.58	1	51.58	4.88	0.0517	
AB	50	1	50	4.73	0.0548	
AC	6.12	1	6.12	0.5789	0.4643	
BC	28.88	1	28.88	2.73	0.1295	
A^2	187.67	1	187.67	17.74	0.0018	
B^2	169.73	1	169.73	16.04	0.0025	
C^2	206.51	1	206.51	19.52	0.0013	
Residual	105.8	10	10.58			
Lack of Fit	88.21	5	17.64	5.01	0.0507	not significant

Pure Error	17.59	5	3.52			
Cor Total	789.08	19				

The analysis of variance (ANOVA) for the response surface model demonstrated that the quadratic model adequately describes the influence of medium components on amylase production. The overall model was statistically significant, with a model sum of squares of 683.28, nine degrees of freedom, a mean square of 75.92, an F-value of 7.18, and a p-value of 0.0025. The low p-value indicates that the variation explained by the model is unlikely to be due to random experimental noise, confirming that the selected variables significantly influenced enzyme production.

Among the linear terms, wheat bran (factor A) exhibited a statistically significant effect on amylase activity, with an F-value of 7.10 and a p-value of 0.0237, indicating that variations in wheat bran concentration substantially affected enzyme yield. Wheat bran is known to be a rich source of complex carbohydrates, vitamins, and trace nutrients that support microbial growth and enzyme induction, which likely explains its strong influence. In contrast, starch (factor B) showed minimal contribution to enzyme variation, as reflected by a very low F-value (0.0828) and a high p-value (0.7794), suggesting that within the tested concentration range, starch did not significantly alter enzyme production. The effect of yeast extract (factor C) was moderately influential, with an F-value of 4.88 and a p-value of 0.0517, which is close to the significance threshold. This result indicates that nitrogen availability and growth-promoting nutrients supplied by yeast extract play an important but secondary role compared to wheat bran.

Interaction effects among variables showed varying levels of influence. The interaction between wheat bran and starch (AB) demonstrated a moderate contribution ($F = 4.73$, $p = 0.0548$), suggesting that combined carbon source balance may influence metabolic efficiency. However, interactions between wheat bran and yeast extract (AC) and between starch and yeast extract (BC) were statistically insignificant, with p-values of 0.4643 and 0.1295, respectively. These findings indicate that the individual contributions of certain nutrients were more influential than their combined interactions under the tested conditions.

The quadratic terms (A^2, B^2, and C^2) showed strong statistical significance, with F-values of 17.74, 16.04, and 19.52, and p-values below 0.01. These results indicate the presence of curvature in the response surface, suggesting that each variable has an optimal concentration beyond which enzyme production declines. Such quadratic behavior is common in fermentation optimization because excessive substrate or nutrient concentration can inhibit microbial metabolism or cause osmotic stress.

The residual error value of 105.8 with ten degrees of freedom indicates acceptable experimental variability. The lack-of-fit test was statistically non-significant ($F = 5.01$, $p = 0.0507$), confirming that the regression model adequately fits the experimental data and that no major systematic variation remains unexplained. The relatively small pure error value (17.59) further supports the reproducibility and reliability of the experimental results. The total variation (Cor Total = 789.08) indicates that a substantial portion of the variability in enzyme production was captured by the proposed quadratic model.

Similar observations have been reported in several fermentation optimization studies. Gupta et al. (2003) demonstrated that agro-industrial substrates such as wheat bran significantly enhance amylase production by providing both carbon and micronutrients. Pandey et al. (2000) also reported that complex substrates often exert stronger induction effects compared to purified starch due to their nutrient diversity. In another study, de Souza and Magalhães (2010) showed that quadratic response surface models frequently reveal optimal nutrient concentrations for microbial enzyme synthesis, confirming the importance of polynomial effects observed in this analysis. Comparable findings were also reported by Saxena et al. (2007), who observed significant quadratic and linear nutrient effects during bacterial amylase optimization using statistical design approaches.

Overall, the ANOVA results confirm that wheat bran concentration plays a major role in regulating amylase production, while yeast extract contributes moderately and starch shows comparatively limited influence within the experimental range. The strong significance of quadratic terms indicates that careful optimization of nutrient concentrations is essential for achieving maximum enzyme yield, and the non-significant lack-of-fit validates the reliability of the statistical model for predicting optimal fermentation conditions (Simair et al., 2017).

4.Applications and Industrial Significance

The enzymatic conversion of agro-industrial waste using amylase has significant implications for biofuel production, food and feed supplementation, and bioproduct synthesis. Integrating this process into biorefineries can reduce waste disposal costs, lower environmental impact, and generate additional revenue streams for agro-industrial sectors. The hydrolysates contained high concentrations of fermentable sugars, demonstrating their potential for conversion into bioethanol, organic acids, and other biochemicals. These results highlight the feasibility of amylase-mediated waste valorization.

Conclusions

The present investigation successfully demonstrated the potential of rhizospheric soil microorganisms as effective producers of industrially important amylase enzymes. Isolation and screening procedures revealed substantial variability in starch degradation ability among bacterial isolates, confirming that rhizosphere environments provide favorable conditions for enzyme-producing microbial communities. Among the tested isolates, strain A9 emerged as the most efficient enzyme producer under submerged fermentation, exhibiting high extracellular amylase activity and strong production stability. Optimization studies confirmed that enzyme productivity is strongly influenced by fermentation parameters such as pH, temperature, incubation period, and inoculum density. Adjustment of these parameters resulted in significant improvement in enzyme yield, demonstrating the importance of process optimization for industrial enzyme production. Pretreatment of agro-industrial substrates also played a crucial role by improving starch accessibility, leading to enhanced enzymatic hydrolysis efficiency and higher sugar release. These findings emphasize that both biological and process engineering factors are essential for maximizing enzyme performance. Statistical evaluation through response surface methodology provided deeper insights into the role of medium composition in regulating enzyme synthesis. Wheat bran was identified as a key inducer of amylase production due to its nutritional complexity

and cost-effectiveness, highlighting its potential use as an economical fermentation substrate. The significant quadratic responses observed for nutrient concentrations further confirmed that balanced substrate levels are essential for achieving optimal microbial productivity. The strong agreement between experimental data and model predictions supports the reliability of the developed optimization approach. In conclusion, this study demonstrates that rhizospheric microorganisms combined with agro-industrial substrates can serve as an efficient and sustainable platform for enzyme production and biomass valorization. The high enzyme yield obtained under optimized conditions indicates the feasibility of utilizing these strains for industrial starch processing, biofuel production, and other biotechnological applications. Future research focusing on molecular identification, genetic improvement, and pilot-scale fermentation could further enhance the commercial applicability of the selected strain and strengthen its role in sustainable bioprocess development.

6.Challenges and Future Perspectives

Despite its potential, challenges such as enzyme cost, substrate variability, and process scalability remain. Future research should focus on enzyme immobilization, genetic engineering for enhanced amylase production, and the use of low-cost substrates. Advances in synthetic biology and process optimization are expected to further improve efficiency and economic feasibility.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

References

A STUDY ON AMYLASE: REVIEW. (2021). International Journal of Biology, Pharmacy and Allied Sciences, 10(4). https://doi.org/10.31032/ijbpas/2021/10.4.1037

Abdel-Fattah, Y. R., Soliman, N. A., El-Toukhy, N. M., El-Gendi, H., & Ahmed, R. S. (2013). Production, purification, and characterization of thermostable α -amylase produced by bacillus licheniformis isolate Al20. Journal of Chemistry. https://doi.org/10.1155/2013/673173

Ahmed, S. A., Abdella, M. A. A., El-Sherbiny, G. M., Ibrahim, A. M., El-Shamy, A. R., & Atalla, S. M. M. (2019). Application of one -factor- at-a-time and statistical designs to enhance α-amylase production by a newly isolate Bacillus subtilis strain-MK1. Biocatalysis and Agricultural Biotechnology, 22. https://doi.org/10.1016/j.bcab.2019.101397

Amid, M., Abdul Manap, M. Y., & Zohdi, N. (2014). Optimization of processing parameters for extraction of amylase enzyme from dragon (Hylocereus polyrhizus) peel using response surface methodology. Scientific World Journal, 2014. https://doi.org/10.1155/2014/640949

Artha, O. A., Sudarno, Pramono, H., & Sari, L. A. (2019). Identification of extracellular enzyme-producing bacteria (proteolytic, cellulolytic, and amylolytic) in the sediment of extensive ponds in Tanggulrejo, Gresik. IOP Conference Series: Earth and Environmental Science, 236(1). https://doi.org/10.1088/1755-1315/236/1/012003

Confortin, T. C., Spannemberg, S. S., Todero, I., Luft, L., Brun, T., Alves, E. A., Kuhn, R. C., & Mazutti, M. A. (2019). Microbial enzymes as control agents of diseases and pests in organic agriculture. In New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Secondary Metabolites Biochemistry and Applications (pp. 321–332). Elsevier. https://doi.org/10.1016/B978-0-444-63504-4.00021-9

Erfanimoghadam, M. R., & Homaei, A. (2023). Identification of New Amylolytic Enzymes from Marine Symbiotic Bacteria of Bacillus Species. Catalysts, 13(1). https://doi.org/10.3390/catal13010183

Gómez-villegas, P., Vigara, J., Romero, L., Gotor, C., Raposo, S., Gonçalves, B., & Léon, R. (2021). Biochemical characterization of the amylase activity from the new haloarchaeal strain haloarcula sp. Hs isolated in the odiel marshlands. Biology, 10(4). https://doi.org/10.3390/biology10040337

Harris, T. K., & Keshwani, M. M. (2009). Chapter 7 Measurement of Enzyme Activity. In Methods in Enzymology (Vol. 463, Number C, pp. 57–71). Academic Press Inc. https://doi.org/10.1016/S0076-6879(09)63007-X

Khusro, A., Barathikannan, K., Aarti, C., & Agastian, P. (2017). Optimization of thermo-alkali stable amylase production and biomass yield from bacillus sp. under submerged cultivation. Fermentation, 3(1). https://doi.org/10.3390/fermentation3010007

Labrou, N. (1148). Therapeutic Enzymes: Function and Clinical Implications. In Advances in Experimental Medicine and Biology. http://www.springer.com/series/5584

Lal Saha, M., Nowshin Islam, K., Akter, T., Ara Rahman, I., Islam, T., & Khan, T. (2019). ISOLATION AND IDENTIFICATION OF AMYLOLYTIC BACTERIA FROM GARBAGE AND GARDEN SOIL (Vol. 48, Number 3). http://blast.ncbi.nlm.nih.gov/

Morais, V., Dee, V., & Suárez, N. (2018). Purification of capsular polysaccharides of streptococcus pneumoniae: Traditional and new methods. In Frontiers in Bioengineering and Biotechnology (Vol. 6, Number OCT). Frontiers Media S.A. https://doi.org/10.3389/fbioe.2018.00145

Rakaz, M. A., Hussien, M. O., & Ibrahim, H. M. (2021). Isolation, Extraction, Purification, and Molecular Characterization for Thermostable α -Amylase from Locally Isolated Bacillus Species in Sudan. Biochemistry Research International, 2021. https://doi.org/10.1155/2021/6670380

Rathour, R., Gupta, J., Tyagi, B., & Thakur, I. S. (2020). Production and characterization of psychrophilic α-amylase from a psychrophilic bacterium, Shewanella sp. ISTPL2 . Amylase, 4(1), 1–10. https://doi.org/10.1515/amylase-2020-0001

Roslan, R., Mohamed Rehan, M., Rahim Kamarudin, K., Mohd Noor, H., Huda-faujan, N., & Mat Radzi, S. (2018). ISOLATION AND IDENTIFICATION OF AMYLOLYTIC BACTERIA FROM RAGI. In Malays. Appl. Biol (Vol. 47, Number 2).

Simair, A. A., Qureshi, A. S., Khushk, I., Ali, C. H., Lashari, S., Bhutto, M. A., Mangrio, G. S., & Lu, C. (2017). Production and Partial Characterization of α-Amylase Enzyme from Bacillus sp. BCC 01-50 and Potential Applications. BioMed Research International, 2017. https://doi.org/10.1155/2017/9173040

Tallapragada, P., Dikshit, R., Jadhav, A., & Sarah, U. (2017). Partial purification and characterization of amylase enzyme under solid state fermentation from Monascus sanguineus. Journal of Genetic Engineering and Biotechnology, 15(1), 95–101. https://doi.org/10.1016/j.jgeb.2017.02.003

Ullah, S., Khalil, A. A., Shaukat, F., & Song, Y. (2019). Sources, extraction and biomedical properties of polysaccharides. In Foods (Vol. 8, Number 8). MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/foods8080304

Veerapagu, M., Jeya, K. R., & Sankaranarayanan. (n.d.). SCREENING AND PRODUCTION OF FUGAL AMYLASE FROM Aspergillus sp BY SSF. Retrieved www.mutagens.co.in

Wu, X., Wang, Y., Tong, B., Chen, X., & Chen, J. (2018). Purification and biochemical characterization of a thermostable and acid-stable alpha-amylase from Bacillus licheniformis B4-423. International Journal of Biological Macromolecules, 109, 329–337. https://doi.org/10.1016/j.ijbiomac.2017.12.004