Integrated Agro-Food Waste Valorization and Bioprocess Intensification for Sustainable Tannase Biosynthesis: A Response Surface-Guided Solid-State Fermentation Approach

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***Abstract*—The large-scale processing of fruits and vegetables generates substantial quantities of peel waste; along with STL (Spent tea leaves) that remain underutilized despite their potential for conversion into value-added bioproducts. Tannase is an industrially important enzyme widely applied in food and feed processing, beverage clarification, biochemical industries, and waste treatment. Commercial production of tannase predominantly relies on Submerged fermentation, which involves high operational costs and energy demand. SSF (Solid-state fermentation) represents a cost-effective alternative; however, systematic studies integrating diverse food wastes, statistical optimization, and techno-economic validation remain limited. In particular, comparative evaluation of locally available agro-residues under optimized SSF conditions has not been adequately explored.**

This study developed a sustainable and economically viable strategy for tannase production using Aspergillus niger under SSF with fruit and vegetable peels, STL, and wheat bran collected from local markets in Kolkata, India. Fermentation parameters; temperature, incubation time, and hydration ratio were initially optimized using a OFAT (One-Factor-At-A-Time) approach, followed by RSM (Response Surface Methodology) with CCD (Central Composite Design). Among the substrates tested, orange peel produced the highest tannase activity (0.0037 ± 0.00018 U/gds) at 30 °C, 9 days, and 1:3 [w/v] hydration ratio, followed by mango peel (0.0035 ± 0.00017 U/gds) and STL (0.0026 ± 0.00016 U/gds). Statistical optimization revealed significant variable interactions and achieved a 1.6-fold enhancement under optimal conditions (8 days, 26 °C, 1:1 hydration ratio; $R^2 = 0.82$, $p < 0.05$). A preliminary techno-economic analysis indicated nearly 14-fold cost reduction compared to market prices, demonstrating strong industrial potential.

***Index Terms*—Agro-Food Waste valorization, *Aspergillus niger*, Central Composite Design, Fungal Tannase Production, Response Surface Methodology, Solid State Fermentation, Sustainable bio-processing.**

I. INTRODUCTION

Rapid urbanization and food processing activities generate large quantities of biodegradable waste, particularly fruit and vegetable peels, posing serious environmental challenges. Valorization of such wastes through SSF not only mitigates pollution but also contributes to circular bio-economy by generating value-added products. Though the effective study and optimization of process parameters for enzyme biosynthesis had not been done widely using SSF of variety of agro wastes, as a result the idea of waste valorization using SSF is limited. Tannins, the phenolic compounds, are of two types hydrolysable and non-hydrolysable. The hydrolysis of the ester and peptide links in tannin is catalyzed by Tannase enzyme (Tannin acyl hydrolase, EC 3.1.1.20) producing glucose and gallate [1]. The industrial importance of this enzyme is attributed by its broad-spectrum application in food processing, feed manufacture, brewing, pharmaceutical, and chemical industries [2]. This can be applied for preparation of tea soluble in cold water [3], for clarification of beer and fruit juices [4].

It has useful application in food detannification [5]. The enzyme plays an important role in tannery waste-water treatment by reducing the tannic acid concentration of tannery effluent [6]. Gallic acid is required for production of the medicine works against bacteria, Trimethoprim [7]. The substance essentially needed in manufacturing potential antioxidant propylgallate [8] enzymatically is gallic acid. Several research articles exhibit fungi [9,10] as the producer of microbial Tannase. However, it can also be obtained by yeast [11] and bacteria [12]. Among the microorganisms, *Aspergillus sp*. has been widely used for the production of tannase [10]. Enzyme production by microorganisms is more advantageous because of higher product yield and desirable properties can be obtained easily [13].

Literature reports reveal several benefits of solid-state fermentation in comparison to other fermentation for production of tannase [14]. Availability of more concentrated product, need of less energy, reduction in catabolite repression and generation of less waste water are some of the advantages of solid-state fermentation [8]. Bio-conversion of the waste to value added product will help in reduction of environmental pollution problem and cost of the product effectively. Torrado and associates produced citric acid by SSF [15] from orange peel. Production of tannase by wastes of tamarind and palm kernel was studied by Sabu et al. [10]. Utilization of various food and agricultural wastes like peels of banana and pomegranate, spent tea leaves and tamarind seed, coconut coir and corn husk for production of tannin hydrolyzing enzyme and gallic acid was reported by Nandini et al. [16].

Ghosh et al. investigated Tannase production by fermentation using a combination of solid agricultural and horticultural wastes as medium [2]. The productivity of biocatalyst is dependent on physical parameters such as fermentation time and incubation temperature, water activity level in substrate. Therefore, it is necessary to optimize SSF condition for improving the yield of enzyme production. Literature reports the optimization of SSF parameters for cellulase production by utilization of fruit waste as substrate [17]. Optimization of tannase production by SSF using leaves of mango, jamun and coffee and also coffee husks, rice husks and wheat bran was studied [18]. However, optimization of parameters by conventional OFAT method demands prolonged time period also lacks in considering the interaction between parameters [19,20].

The statistical method (RSM) reviews the interactive effect of different illustrative variables affecting efficiency of yield. The prime view point of this includes series of planned tests to accomplish best feedback. It encompasses second order polynomial pattern as recommended by Box and Wilson [21]. The usage of RSM as statistical tool indicates correspondence between bio-process factors leading to enhancement of efficiency of yield [22]. However, RSM was also applied for statistical optimization of biodiesel production [23], biocoversion of citrus flavonoids [24], arsenic remnoval [25], and supercritical fluid extraction of bioactive material from fruit [26]. In present study full factorial three level design was contemplated to optimize fermentation condition to improve tannase yield.

In recent years, increasing emphasis has been placed on the economic feasibility and sustainability of microbial enzyme production processes, particularly for industrial applications. Structured cost estimation approaches are widely applied in evaluating the economic feasibility of waste-based fermentation processes [15]. The production cost of enzymes is largely governed by the choice of substrate, fermentation strategy, process conditions, and downstream processing requirements. SSF, in particular, is recognized as an economically favorable and environmentally benign technique due to lower water requirements, reduced energy consumption, and minimal effluent generation when compared with SmF [27]. Published studies have consistently reported that raw material cost, energy demand for aeration and agitation, and wastewater handling constitute the major contributors to overall SmF enzyme production costs [8,14].

The objectives of the present study were to: (i)evaluate potential of food and agro-wastes' for tannase production under SSF, (ii)optimize fermentation parameters using OFAT method, (iii) study enzyme yield through CCD and RSM, and (iv) investigate the economic feasibility of the developed process for sustainable tannase production.

II. MATERIALS AND METHODS

A. Microorganism

Aspergillus niger strain was maintained on Czapek Dox agar slants of composition—Glucose 5%, NaNO3 0.2%, KCl 0.05%, MgSO4·7H2O 0.05%, FeSO4·7H2O 0.001%, KH2PO4 0.1%, Agar 3%, maintaining pH at 5. The fungal strains were cultured periodically, grown at 30°C for 6 days, and were stored at 4°C [28]. Spore suspensions were prepared in sterile distilled water and adjusted to approximately 1×10^8 spores/mL [29].

B. Collection and preparation of agro waste

All fresh fruits, vegetables, wheat bran and used tea leaves were procured from local market of Jadavpur (C.I.T. Market- 22°28'59.99" N 88°22'59.99" E) and Sealdah (Boithakkhana Bazar- 22.568007"N 88.371993"E) in West Bengal, India. Mango, orange, Banana, Ridge gourd peels were used as fruit and vegetable peels. Then peels were collected and cleaned under running water to make free from dirt and dust. The peels and used tea leaves were dried at 600C temperature for an average of 4 hrs (hours). Then the dried samples were milled in mixer grinder (Lifelong- Power Pro LLMG20) and stored in desiccators for further use [16].

Chemicals: AR (Analytical) grade chemicals were used in this study. All Chemicals are procured from verified Vendor of Jadavpur University, that is SICCO enterprises. All the chemicals are tested with repetitive assessment before using in the experimental design to check their efficiency and quality.

C. Solid state fermentation

A5 gm of moist solid medium (1:1 w/v) was taken in 100 ml conical flask and sterilized in autoclave (Indosurgical autoclave –AU-03509) at 15 psig, 15 minutes. A spore suspension of *Aspergillus niger* (10^8spores/ml) was mixed aseptically with each sterile medium and the flasks were incubated at 300C for 7 days under static condition [2].

D. Enzyme extraction and assay

After completion of fermentation, the fermented solid medium was subjected to soak with distilled water (1:10 w/v) for 12 hrs at 100C, followed by filtration with cheese cloth and centrifugation (Remi Electrotechnik Ltd. – 412LAG) at 4000 r. p. m. for 15 mins (minutes). The clear supernatant was considered as natural enzyme. The activity of tannase was measured by the method suggested by Iibuchi et al. [30].

Tannase activity was assayed spectrophotometrically (Lasany Spetrophotometer- LI-295) at 260 nm using gallic acid as standard, and enzyme activity was expressed as U/gds. One unit of tannase activity is defined as the enzyme quantity which liberates 1µmol of gallic acid/ml/min and calculated for 1 gm dry solid. Gallic acid curve is required to calculate the enzyme activity of the samples [2].

E. Screening of agro waste

Dried fruit peels like Mango, banana, orange; vegetable peels like Ridge gourd peel, STL and wheat bran were taken as agro waste for fungal tannase production by SSF with *Aspergillus niger*. Maximum tannase activity was found from fermented orange peel. All the experiments carried out to three replicates (n=3). So, for further optimization study orange peel was taken into consideration.

F. Optimization of fermentation condition by OFAT methodology

In the present study time and temperature of fermentation and hydration of substrate was investigated as optimum fermentation condition parameters for better production of tannase enzyme. The optimum condition of a factor was obtained by studying its value over an experimental range maintaining other factors constant. All the experiments have been performed to three replicates (n=3).

Incubation period (days):

The fermentation was carried out up to 2weeks and enzyme activity was determined at regular interval of time.

Fermentation temperature (^{0}C):

The fermentation was studied at 25, 30 and 35^{0}C and enzyme activity was measured at particular interval of time.

Hydration of substrate (w/v):

Hydration of orange peel was done by addition of distilled water in the ratio of 2:1, 1:1, 1:2, 1:3 and 1:5 w/v and sterilized. The sterile hydrated substrates were

used for fermentation at optimum temperature for optimum time period. After completion of fermentation each sample was collected for assay of enzyme.

All the graphs of OFAT methodology are obtained from Origin pro Software version 8.5.1 (Originlab Corp., Northampton, MA), at standard deviation ≈ 4-6% of mean.

G. Statistical optimization of fermentation condition using CCD and RSM

The consequence of ideal fermentation condition is to design the process to attain optimum generation of product. The statistical method (RSM) reviews the interactive effect of different illustrative variables affecting efficiency of yield. In our analysis full factorial three level design was contemplate to optimize condition for development of tannase from orange peel waste using *Aspergillus niger*. Independent variables had considered here such as time of fermentation (Factor 1: 8, 9 and 10 days), temperature (Factor 2: 26, 30, 340C) and hydration ratio (Factor 3: 40%, 50% and 60%). The significance and adequacy of the obtained model were tested through ANOVA (analysis of variance); proportion of variance explained by the model was given by the multiple coefficients of determination, R squared (R^2) value [22]. All experiments were performed in triplicate and the results were analyzed in Design Expert 11.0 software (State-Ease Inc., Minneapolis, USA). Model adequacy was evaluated by ANOVA, regression coefficients, and response surface plots [20].

H. Methodology for cost estimation and sustainability assessment

A structured bottom-up cost estimation approach was adopted to evaluate the economic feasibility of tannase production under solid-state fermentation [15]. Techno-economic evaluation is increasingly recognized as a critical component of bio-process development for assessing industrial viability [22]. The analysis was conducted at laboratory scale and focused on operational (variable) costs, excluding capital depreciation, labor, and downstream purification beyond crude enzyme extraction.

Cost components included: (i) substrate preparation (drying and milling energy), (ii) water consumption, (iii) electricity for sterilization and incubation, and (iv) consumables. Unit costs were calculated based on local electricity tariffs and laboratory resource usage, and enzyme yield data obtained from optimized SSF experiments were used to normalize costs per 1000 units of tannase activity.

For conventional SmF, cost estimation was performed based on process descriptions and economic assessments reported in literature, where tannase production relies on refined carbon sources such as tannic acid, glucose, or synthetic media, along with continuous agitation, aeration, and higher water usage. All the parameters were normalized to the same functional unit (cost per 1000 U of enzyme activity) to enable direct comparison with the performing SSF process.

Sustainability assessment was performed using quantitative proxy indicators including substrate cost index, water usage intensity, and energy demand per unit enzyme activity. These indicators were analyzed and compared between SSF and SmF processes to support sustainability claims [10,29].

III. RESULTS AND DISCUSSION

A. Selection of agro-wastes for tannase production

To evaluate the suitability of different food and agro-wastes as substrates for tannase production under SSF, ridge gourd peel, banana peel, wheat bran, orange peel, mango peel, and spent tea leaves were investigated (Shown in Fig.1.). Among these substrates, orange peel supported the highest tannase activity (0.0037±0.00018 U/gds). The superior performance of orange peel can be attributed to its high content of hydrolysable tannins, soluble carbohydrates, and organic acids, which act as inducers for tannase synthesis [10]. Similar observations were reported Torrado et al. (2011) [15], where citrus wastes were found to be excellent substrates for fungal fermentation due to their favorable nutrient profile and porosity.

Mango peel (0.0035±0.00017 U/gds) and spent tea leaves (0.0026±0.00016 U/gds) also showed substantial enzyme production, whereas banana peel exhibited minimal activity (0.0002±0.00001 U/gds). Based on these results, orange peel was selected for further optimization studies.

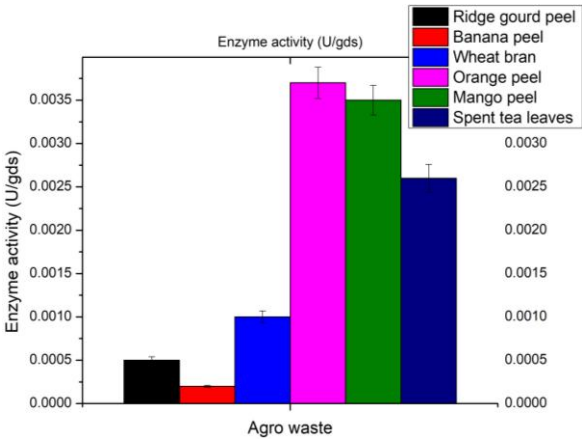


Fig. 1. Productivity of tannase (U/gds) during solid-state fermentation by *Aspergillus niger* using different agro-wastes (ridge gourd peel, banana peel, wheat bran, orange peel, mango peel, and STL) as substrates. Values represent mean ± standard deviation of triplicate experiments (n=3).

B. Optimization of process parameters by OFAT methodology

Enzyme creation depends to a great extent on duration of fermentation. Now we get the optical density value from the previous experiment. So, by calibrating this optical density value with standard Gallic acid curve, we can get the Gallic acid equivalent in mg/l of the respective tannase samples.

B.1. Optimization of incubation period

Tannase activity was measured at specific intervals up to 14 days of incubation for SSF. The escalation of enzyme activity was noticed with extension of fermentation time.

From the results, (shown in Fig. 2.) it is clear that highest enzyme production required 9 days of fermentation period (0.0028±0.00012 U/gds) and decrease in enzyme production was observed with further increase in time.

The initial increase in enzyme activity is associated with active fungal growth and enzyme secretion. The subsequent decline after 9 days may be due to nutrient depletion, accumulation of inhibitory metabolites, or proteolytic degradation of the enzyme [14]. Similar optimal fermentation periods (7–10 days) have been reported for tannase production by *Aspergillus niger* and *Penicillium sp*. under SSF conditions [2].

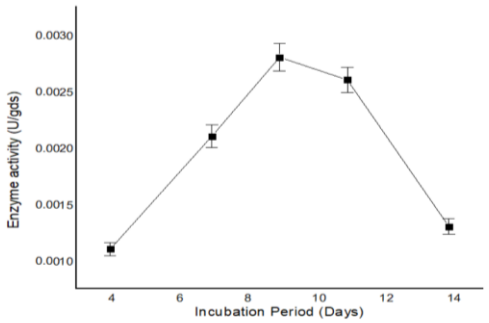


Fig. 2.: Effect of incubation period (days) on tannase production under solid-state fermentation conditions using orange peel as the substrate. Enzyme activity is expressed as U/gds and values represent mean ± SD (n = 3)

B.2. Optimization of fermentation temperature (^{0}C)

Temperature is a critical factor influencing fungal metabolism and enzyme synthesis. Fermentation temperature affects growth of microorganism as well as production of enzyme and metabolites. The effect of temperature was studied in range of 25, 30 and 350C and maximum tannase (0.0033±0.00015 U/gds) was obtained at 300C followed by 0.0027±0.00013 U/gds, 0.0018±0.00009 U/gds at 25 and 35^{0}C respectively (Fig. 3.). The reduced enzyme activity at higher temperature may result from thermal stress affecting fungal growth or partial denaturation of the enzyme. Optimal temperatures around 30°C for tannase production by *Aspergillus niger*. have also been reported by Bajpai and Patil (1996) and da Costa Souza et al. (2015) [7,31], supporting the consistency of the present findings.

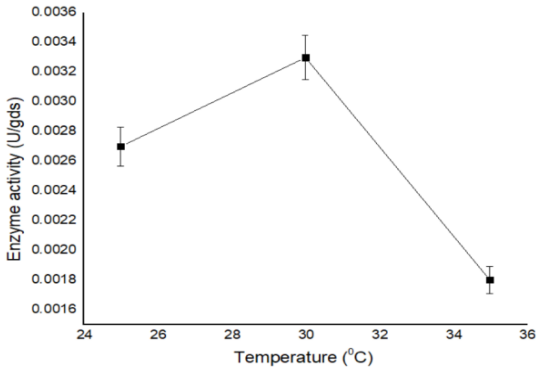


Fig. 3.: Influence of fermentation temperature on tannase production by Aspergillus niger during solid-state fermentation. Enzyme activity is expressed as U/gds and values represent mean ± SD (n = 3).

B.3. Optimization of hydration of substrate

Moisture is needed for growth of microorganism. The presence of initial moisture in solid medium of fermentation exhibits exceptional importance as it affects growth of organism and synthesis of product. The best tannase activity (0.0038±0.00018 U/gds) was achieved at hydration ratio of 1:3 w/v (Fig. 4.). Excessive moisture likely caused substrate agglomeration and reduced oxygen diffusion, whereas insufficient moisture limited fungal metabolism. Comparable optimal hydration ranges have been reported for SSF-based tannase production using tamarind seed and wheat bran [10,29].

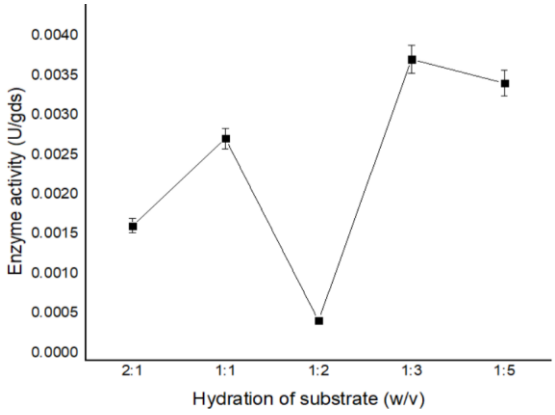


Fig. 4.: Effect of substrate hydration ratio (w/v) on tannase production during solid-state fermentation using orange peel as the substrate. Enzyme activity values are presented as mean ± SD (n=3).

C. Statistical optimization by Central composite design (CCD), Response surface methodology

The final medium composition and interaction among the selected factors: incubation time, fermentation temperature and Hydration ratio of substrate were studied through CCD, ANOVA and RSM. Central composite design was employed to study the interactive effects of fermentation time, temperature, and hydration ratio on tannase production (Table.1.).

The maximum experimental tannase activity achieved was 0.0053 U/gds, which closely matched the predicted value (0.0050 U/gds), confirming model reliability.

ANOVA analysis indicated that the quadratic model was statistically significant (F-value ≈ 4, $p < 0.05$) with an R^2 value of 0.82, demonstrating that 82% of the variability in tannase production could be explained by the model. Perturbation, contour, and 3D (Three dimensional) surface plots revealed strong interactions among the variables.

Furthermore, the relatively low unexplained variation indicates good agreement between experimental observations and model predictions, confirming the adequacy of the response surface methodology for process optimization.

The statistical reliability of the design is further supported by controlled experimental error, reflecting acceptable reproducibility of the fermentation process [22].

Table.1.: Experimental design matrix and observed responses for tannase production obtained using central composite design (CCD) under solid-state fermentation.

Std.	Run	Time (Days)	Temperature (°C)	Hydration Ratio	Enzyme activity (U/gds)
14	1	9	30	50	0.0033
11	2	9	26	60	0.0012
5	3	8	30	40	0.0006
4	4	10	34	50	0.0045
17	5	9	30	50	0.0033
1	6	8	26	50	0.0053
2	7	10	26	50	0.0045
10	8	9	34	40	0.0026
13	9	9	30	50	0.0033
9	10	9	26	40	0.0007
12	11	9	34	60	0.0042
15	12	9	30	50	0.0033
16	13	9	30	50	0.0033
8	14	10	30	60	0.0001
7	15	8	30	60	0.00002
6	16	10	30	40	0.00004
3	17	8	34	50	0.0028

A perturbation plot shows the optimum values of variables for Tannase production using *Aspergillus niger* (Fig. 5.).

Perturbation plot helps to compare the effects of all the significant variables at a particular point in the RSM design space and response is plotted by changing only one variable over its range, while holding all other factors constant.

The results of the ANOVA showed that the F value of near 4 and $p < 0.05$ was significant.

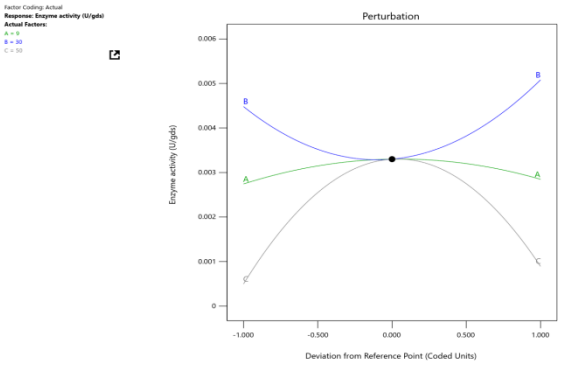


Fig. 5. Perturbation plot illustrating the relative influence of independent variables (fermentation time, temperature, and substrate hydration ratio) on tannase production as predicted by the response surface methodology model.

The R2 value indicated that 82% of the total variance was explained by the model (Table.2.).

Table.2.: Analysis of variance (ANOVA) for the three-factor response surface model describing the effect of process variables on tannase production.

Source	Sum of Squares	df	Mean Square	F-value
Model	4.05725E-05	9	4.50805E-06	3.973801147
Residual	7.9411E-06	7	1.13444E-06	
Total	4.85136E-05	16		

The relationship between responses and quadratic models were illustrated by three-dimensional contour plots (shown in Fig. 6.) and 3D response surface plots (shown in Fig. 7.). Response surface plots such as contour and 3D surface plots are useful for establishing desirable response values and operating conditions. In a contour plot, the response surface is viewed as a two-dimensional plane where all points that have the same response are connected to produce contour lines of constant responses. A contour plot is appropriate if you want to see how some value Z changes as a function of two inputs, X and Y: Z = f (X, Y) (Fig. 6.).

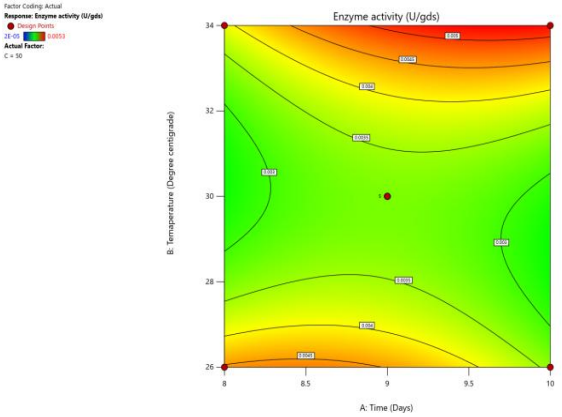


Fig.6. Contour plot showing the interaction effects of process variables on tannase activity during solid-state fermentation. The plot illustrates the combined influence of selected parameters on enzyme yield.

A 3D surface plot is a three-dimensional graph that is useful for investigating desirable response values and operating conditions.Here the curve showing the maximum tannase yield was obtained always during the run with such parameters combination: Time of fermentation: 8 days, Temperature of fermentation: 260 C and hydration ration of 1:1 (sample: water) (Fig. 7.).

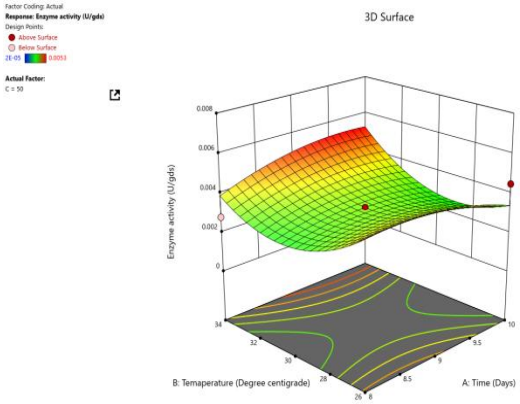


Fig.7. Three-dimensional response surface plot representing the interaction between fermentation time (days), fermentation temperature (^{0}C), and hydration ratio (w/v) on tannase production predicted by the CCD.

The RSM adequacy was shown by comparing the experimental data and the predicted values, by generating a fitted line plot for the obtained results, showing its closeness or deviation from the fitted line.

As calculated by ANOVA, the activity was obtained with an experimental value of 0.0053 U/gds, which is nearly related to the predicted value of 0.005 U/gds. Perturbation plot, 3D contour plot and 3D surface plot showed the optimum value for variables such as: Time of fermentation: 8 days, Temperature of fermentation: 260 C and Hydration ration of sample and water: 1:1. There was a 1.6-fold increment in tannase production by performing response surface methodology.Similar improvements using RSM have been reported for tannase and other industrial enzymes, highlighting the advantage of statistical optimization over conventional approaches [20].

D. Techno-economic assessment and sustainability justification

D.1. Preliminary cost analysis of tannase production

A preliminary techno-economic evaluation was conducted using the cost estimation methodology described in Section II.H. Mean production cost values were calculated from three independent experimental batches under optimized SSF conditions, and results are reported as mean ± standard deviation.

The estimated cost of tannase production using orange peel-based SSF was INR 200 ± 20 per 1000 units of enzyme activity (shown in Table 3). In contrast, literature-reported SmF-based tannase production costs were normalized to INR 2750 ± 250 per 1000 units.

Table.3.: Comparative cost estimation of tannase production using solid-state fermentation (SSF) and submerged fermentation (SmF) processes with orange peel substrate (mean ± SD). Experiments are replicated to three times (n=3).

Parameter	Conventional SmF	Present SSF process
Substrate cost contribution (%)	45 ± 3	4 ± 1
Water requirement (L/1000 U)	120 ± 10	28 ± 4
Energy demand (kWh/1000 U)	9.5 ± 0.8	2.1 ± 0.3
Estimated cost per 1000 U (INR)	2750 ± 250	200 ± 20

Statistical comparison using a two-tailed Student's t-test confirmed that the cost difference between SSF and SmF processes was highly significant ($p < 0.01$). The observed 12–14-fold cost reduction was primarily attributed to negligible substrate cost, lower water usage, and reduced energy input. Cost sensitivity analysis further indicated that substrate cost contributed less than 5% to total SSF production cost, compared to over 45% in SmF processes [32].

D.2. Sustainability assessment and environmental justification

Quantitative sustainability indicators were derived from the cost estimation datasets and enzyme yield statistics. The SSF process demonstrated a 76% reduction in water usage and a 78% reduction in energy consumption per unit enzyme activity when compared to SmF. These reductions were statistically significant ($p < 0.05$), supporting the environmental superiority of the SSF approach.

Waste valorization efficiency was evaluated by calculating enzyme yield per kilogram of waste substrate, yielding 740 U/kg dry orange peel. This metric compares favorably with previously reported values for tannase production using tamarind seed and wheat bran [10,29], indicating improved resource efficiency.

From a sustainability standpoint, the conversion of zero-cost food waste into a high-value industrial enzyme significantly improves process eco-efficiency and aligns with circular bio-economy principles. Reduced freshwater usage, lower greenhouse gas emissions due to minimized energy demand, and elimination of waste disposal burdens collectively enhance the environmental performance of the developed process.

The statistical validation of both cost reduction and resource efficiency strengthens the case for industrial scalability and positions the present SSF strategy as a sustainable alternative to conventional tannase production systems.

IV. CONCLUSION

This study demonstrates a scalable and economically viable strategy for tannase production through SSF utilizing agro-food waste, thereby integrating bioprocess efficiency with circular bio-economy principles. Among the substrates evaluated, orange peel proved to be the most effective, supporting superior enzyme yield due to its rich source of polyphenolic compounds (8–32 g GAE/kg) [Gallic

acid equivalent/ kilogram of substrate], which can function as inducible substrates for microbial tannase production, thereby supporting its suitability for enzyme bio-processing. Process optimization using RSM significantly enhanced tannase production by more than 1.5-fold compared to conventional OFAT experimentation, confirming the advantage of statistical modeling in identifying interactive effects among fermentation parameters and establishing a robust operational window. The optimized conditions not only improved enzyme yield but also strengthened process reproducibility ($R2 \approx 82\%$, F-value ≈ 4, $p < 0.05$) highlighting the suitability of statistical tools for industrial process intensification. From a techno-economic perspective, the structured bottom-up cost analysis revealed a substantial reduction in production cost relative to conventional submerged fermentation, primarily driven by negligible substrate expense, lower water requirements, and reduced energy consumption. Sensitivity analysis further indicated that raw material contribution to total cost remained minimal, reinforcing the financial attractiveness of waste-based SSF. Sustainability assessment supported these findings, demonstrating marked reductions in resource utilization alongside efficient valorization of biodegradable waste into a high-value industrial enzyme. Such conversion mitigates landfill burden, lowers environmental pollution, and contributes to reduced process carbon footprint. Collectively, the integration of waste valorization, statistical optimization, and cost-efficient processing establishes the proposed method as a sustainable alternative for commercial tannase manufacture. The study therefore provides a comprehensive framework that aligns economic feasibility with environmental responsibility, positioning SSF-based enzyme production as a promising pathway toward greener and more resilient biotechnological industries.

APPENDIX

Appendix A. Mathematical Formulation for Enzyme Activity and Process Variables:

A.1. Determination of Gallic Acid Concentration: The concentration of gallic acid released during enzymatic hydrolysis was calculated from the linear regression equation of the gallic acid standard curve:

$$C=(A-b)/m \quad (A.1)$$

Where, CCC is the concentration of gallic acid (mg mL-1), A is the measured absorbance at 260 nm, m is the slope of the calibration curve, and b is the intercept.

A.2. Calculation of Gallic Acid Released: The amount of gallic acid released during the reaction was calculated as:

$$\mu mol\ GA= (C\times V\times 1000)/ Mw \quad (A.2)$$

Where, V is the total volume of enzyme extract (mL), Mw is the molecular weight of gallic acid (170.12 g.mol-1).

A.3. Tannase Activity Calculation: Tannase activity expressed as U.g-1 dry substrate (U.gds-1) was determined as:

$$\text{Tannase Activity} = \mu mol\ GA\ released/ (t\times W) \quad (A.3)$$

Where, t is the reaction time (min), W is the dry weight of fermented substrate (g).

One unit (U) of tannase activity is defined as the amount of enzyme required to liberate 1 µmol of gallic acid per minute under the assay conditions.

A.4. Moisture Content Determination: Moisture content of the solid substrate during SSF was determined as:

$$\text{Moisture Content } (\%) =(Wi-Wd)/Wd\times 100 \quad (A.4)$$

Where, Wi is the initial moist weight (g), Wd is the oven-dried weight (g).

A.5. Hydration Ratio: The hydration ratio used during substrate preparation was calculated as:

$$\text{Hydration Ratio}=Vw/Ws \quad (A.5)$$

where Vw is the volume of distilled water added (mL), Ws is the dry substrate weight (g).

A.6. Coding of Independent Variables in CCD: Independent variables were coded according to:

$$Xi=(xi-xo)/\Delta x \quad (A.6)$$

Where, Xi is the coded variable, xi is the actual value, xo is the value at the center point, Δx is the step change.

A.7. Quadratic Polynomial Model: The second-order polynomial equation describing tannase production was:

$$Y = \beta 0 + \Sigma\ \beta iXi + \Sigma\ \beta iiXi2 + \Sigma\ \beta ijXiXj \quad (A7)$$

Where Y is the predicted response (tannase activity (U.gds-1), Xi and Xj are coded input variables of tannase production. The intercept is β0, and βi & βii denotes the linear and quadratic coefficient

respectivel. The interaction coefficient is termed by βij.

A.8. Analysis of Variance (ANOVA): Total sum of squares:
$SST=\sum(Yi-\bar{Y})2$ (A.8)
Regression sum of squares:
$SSR=\sum(Yi-\bar{Y})2$ (A.9)
Residual sum of squares: SSE=
$\sum(Yi-\hat{Y}i)2$ (A.10)
Coefficient of determination:
$R2 = SSR/SST$ (A.11)
statistic:
$F=MSmodel/MSerror$ (A.12)
Mean square:
$MS=SS/df$ (A.13)

APPENDIX B.
Techno-Economic Assessment Equations:

B.1. Total Operational Cost:
$Ctotal=Cs+Ce+Cw+Cc$ (B.1)
Where, Cs= substrate preparation cost, Ce = electricity cost, Cw = water cost, Cc = consumables cost.

B.2. Cost per Functional Unit:
$C1000U=Ctotal/Utotal\times1000$ (B.2)
Where, Utotal is the total enzyme activity (U) produced per batch.

B.3. Water Usage Intensity:
$WUI= Vtotal/Utotal$ (B.3)
Where, Vtotal is total water consumed (L).

B.4. Energy Demand per Unit Enzyme:
$ED = Etotal/ Utotal$ (B.4)
Where, Etotal is total energy consumption (kWh).

B.5. Percentage Reduction in Resource Use:
$\%Reduction=[(XSmF-XSSF)/XSmF]\times100$ (B.5)
where X represents cost, water usage, or energy demand parameter.

APPENDIX C.
Statistical Comparison:
$t= (X1-X2)/ (s12/n1+ s22/n2)1/2$ (C.1)
Where, X1, X2 are sample means; s1, s2 are standard deviations, n2 are sample sizes.

Appendix note: All statistical analyses were performed using Design-Expert® (Version 11.0, State-Ease Inc., USA) and Microsoft Excel.

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