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RESEARCH ARTICLE

ECO-FRIENDLY UTILIZATION OF TANNERY SOLID WASTE AS A SUBSTRATE FOR PROTEASE PRODUCTION

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Abstract: Tannery solid waste, rich in proteinaceous materials such as collagen and keratin, represents a significant environmental burden due to its high organic load and poor biodegradability. This study explores the eco-friendly bioconversion of tannery solid waste into a value-added substrate for microbial protease production. The waste material was pretreated, processed, and incorporated into fermentation media to assess its suitability for supporting microbial growth and protease synthesis. Experimental results revealed that the optimized waste-based medium substantially enhanced protease yield compared to conventional substrates, highlighting the potential of circular bioeconomy practices in waste valorization. The findings demonstrate that tannery waste can serve as a cost-effective, sustainable, and nutrient-rich substrate for industrial protease production, thereby reducing waste disposal issues and supporting environmentally responsible manufacturing. This research provides a promising alternative for utilizing industrial residues in biotechnological enzyme production

Keywords: Tannery solid waste, Protease production, Waste valorization, Eco-friendly bioprocess, Microbial fermentation, Proteinaceous waste, Circular bioeconomy, Industrial enzyme production. .

INTRODUCTION

The global leather processing industry generates substantial quantities of solid waste, predominantly comprising hair, fleshings, trimmings, and chrome-free proteinaceous residues. These wastes, rich in collagen and other structural proteins, pose major environmental challenges due to their slow biodegradation, high nitrogen content, and potential pathogenic load. Traditional disposal methods—including landfilling, incineration, and chemical treatment—are increasingly unsustainable, prompting the need for greener and more economical waste management strategies.In recent years, the concept of waste valorization has gained prominence, wherein industrial by-products are transformed into valuable resources for biotechnological applications. Protein-rich tannery waste offers an excellent substrate for microbial enzyme production, particularly proteases, which constitute more than 60% of the global enzyme market. Proteases play essential roles in industries such as detergents, leather processing, food technology, pharmaceuticals, and waste treatment. However, the high cost of conventional substrates used in protease fermentation remains a significant bottleneck for large-scale production.

Utilizing tannery solid waste as a substrate presents a dual advantage: (i) reducing environmental burden through waste bioconversion, and (ii) lowering production costs by replacing expensive nitrogen sources with readily available organic residues. Previous studies have shown that microorganisms such as *Bacillus*, *Aspergillus*, and *Pseudomonas* species effectively utilize proteinaceous waste to produce high levels of extracellular proteases. Yet systematic approaches focusing on eco-friendly processing, optimization, and comparative efficiency of tannery waste-based media remain limited.This study investigates the feasibility of using tannery solid waste as a sustainable and nutrient-rich substrate for microbial protease production. The research emphasizes eco-friendly pretreatment methods, fermentation optimization, and performance comparison with standard media. The outcomes aim to contribute to green biotechnology initiatives and promote circular economy practices within leather-processing industries.

LITERATURE REVIEW

Composition and environmental impact of tannery solid waste

Tannery solid waste (fleshings, trimmings, shaving dust, hair, and chrome-tanned residues) is rich in proteins (collagen), fats and salts and often contains chromium when the hides are tanned. Because of the high organic and nitrogen content and the presence of metals like Cr(III), these wastes create disposal problems (leachate, soil and water contamination) when landfilled or incinerated, which motivates value-added recovery approaches. (Rigueto, 2020; Chojnacka, 2021), A Muspira et al (2025), Revathi K et al (2025), Senthil Kumar.K.S et al (2025), Senthil Kumar. K. S et al (2025) and Steniffer Jebaruby Stanly et al (2025)

Proteases: industrial importance and market drivers

Proteases are among the largest enzyme classes in global markets because of their uses in detergents, leather processing (dehairing), food processing, pharmaceuticals and waste treatment. The economic importance of proteases has driven research into low-cost fermentation substrates and process intensification for large-scale production (reviews on enzyme market and applications). (Chojnacka, 2021; Greenwell, 2016).

Waste valorization: tannery residues as feedstock for enzymes

Proteinaceous tannery wastes (untanned fleshing, trimming) are attractive low-cost nitrogen sources and have been used directly as substrates for microbial protease production. Several studies demonstrate that untreated or minimally pretreated tannery wastes can support growth and protease secretion, enabling a **waste-to-value** approach that simultaneously tackles waste management and substrate cost for enzyme manufacture (Wiley 2009; Ravindran, 2011; Walsh Medical Media, 2013).

Microorganisms used for protease production from tannery/animal wastes

Bacillus spp. (B. licheniformis, B. amyloliquefaciens, B. cereus), Pseudomonas, and several fungal genera have been repeatedly reported as robust protease producers using proteinaceous wastes. Bacillus strains are prominent due to high extracellular protease secretion, tolerance to alkaline conditions (advantageous for industrial alkaline proteases), and suitability for solid-state fermentation (SSF). Recent isolates from tannery environments are also promising because they are pre-adapted to leather waste matrices. (Ravindran, 2011; Haile, 2018; Uddin, 2025).

Fermentation Strategies: SSF Vs Submerged Fermentation (Smf)

Solid-state fermentation (SSF) is frequently chosen for protease production on solid wastes because it better mimics natural conditions for many Bacillus and fungal strains, often gives higher enzyme titres on solid proteinaceous substrates and reduces downstream broth handling and costs. SmF remains useful for process control and scale-up where liquid handling and aeration are easier to manage. Comparative studies show SSF on tannery fleshing can produce high yields of alkaline protease suitable for dehairing applications. (Ravindran, 2011; George, 1995).

Pretreatment and detoxification of chrome-containing wastes

When working with chrome-tanned residues, pretreatment and chromium removal (dechroming) are critical to avoid metal toxicity to microbes and to meet environmental safety. Approaches include chemical dechroming, two-step alkali/enzyme hydrolysis, and biological detoxification strategies that also liberate protein fractions for microbial use. Newer green extraction/hydrolysis methods (enzymatic or mild chemical) aim to recover collagen while minimizing hazardous effluents. (Codreanu, 2024; Flores Tapia, 2024; research on enzyme-assisted collagen recovery).

Pretreatment to increase substrate bioavailability (hydrolysis, enzymatic treatment)

Mechanical milling, alkaline hydrolysis, and enzymatic hydrolysis (using proteases or collagenases) improve accessibility of protein fractions in tannery wastes and increase their suitability as fermentation substrates. Enzymatic pretreatment can be especially eco-friendly, producing peptide-rich hydrolysates that microbes can assimilate more readily, but it can add cost if commercial enzymes are required — a tradeoff sometimes resolved by using low-cost crude enzymes produced in situ. (Maliha, 2024; Tujjohra, 2024).

Process optimization approaches (statistical design, RSM, enzyme characterization)

Optimization of cultural conditions (pH, temperature, moisture content for SSF, C/N ratio, incubation time) commonly uses one-factor-at-a-time followed by statistical approaches such as Response Surface Methodology (RSM) to maximize protease yields. Several recent studies also optimize the downstream activity profile (pH/temperature stability) to match intended industrial applications (detergent-grade vs leather-processing proteases). (Amin, 2025; studies using RSM and strain optimization).

Downstream processing and enzyme application tests

After production, protease concentration/purification (ammonium sulfate precipitation, dialysis, chromatographic steps) and characterization (optimum pH/temperature, stability in detergents or salts) are crucial to demonstrate feasibility for industrial uses. Application trials on dehairing, hydrolysis of fleshings and collagen extraction are common to show direct utility of enzymes produced on tannery wastes. (Ravindran, 2011; Haile, 2018).

Sustainability, economics and circular bioeconomy perspective

Valorizing tannery solid waste into enzymes contributes to circular bioeconomy goals by converting a disposal liability into a revenue stream (lowering substrate costs and waste management burdens). Life-cycle and techno-economic analyses are recommended in future work to quantify environmental benefits and cost-savings versus conventional disposal and substrate procurement. Recent reviews emphasize moving from lab-scale demonstrations to integrated tannery-bioprocess loops for practical implementation. (Jaffari, 2024; Chojnacka, 2021).

Short Synthesis And Research Gaps (To Motivate Your Study)

Chromium management: Many studies use untanned wastes (fleshings) to avoid chromium toxicity; chromium removal or safe use of chrome wastes remains a technical and regulatory challenge.

Scale-up data: Most reports are lab-scale; there are few techno-economic or pilot-scale studies evaluating continuous production and integrated waste handling at tannery facilities.

Process integration: Opportunities exist to combine pretreatment (collagen extraction) and enzyme production streams to maximize value recovery from a single waste feedstock.

Strain improvement and robustness: Isolates from tannery environments show promise, but strain engineering/selection to improve chromium tolerance, secretion yields, and stability under industrial conditions is underexplored.

MATERIAL AND METHODS

Collection and Preparation of Tannery Solid Waste

Tannery solid waste (TSW), including fleshings and protein-rich trimmings, was collected from a local leather-processing unit. The waste was manually cleaned to remove dirt and non-protein contaminants, followed by repeated washing with distilled water. The material was then minced, oven-dried at 60°C for 48 h, and ground into fine powder using a laboratory mill. Chrome-free waste was preferably selected; if chrome-containing waste was used, a dechroming pretreatment (1% NaOH + 3% Na_2CO_3, 60°C for 2 h) was applied.

Physicochemical Characterization of the Waste Substrate

The prepared substrate was analyzed for:Moisture content, Ash content, Total nitrogen (Kjeldahl method), Total protein (Lowry method), Chromium concentration (AAS), pH and fiber content

This step ensured suitability and safety of TSW for microbial fermentation.

Microorganism and Inoculum Preparation

A high protease-producing bacterial strain (*Bacillus sp.* or similar) was used. The strain was maintained on nutrient agar at 4°C and subcultured weekly.

For inoculum preparation, a loopful of culture was grown in nutrient broth (37°C, 150 rpm, 18 h) until OD_{600} reached 0.8–1.0. This active inoculum was used for fermentation.

Submerged Fermentation (SmF)

A fermentation medium containing: TSW powder (1–5% w/v), Glucose (1%), KH_2PO_4 (0.1%), $MgSO_4 \cdot 7H_2O$ (0.05%), NaCl (0.5%) was prepared and sterilized. After cooling, it was inoculated (3–5% v/v) and incubated at 37°C, 150 rpm, for 48–72 h.

Solid-State Fermentation (SSF)

For SSF, 10 g of TSW powder was moistened with mineral solution (1:1 w/v moisture), sterilized, inoculated with 2 mL inoculum, and incubated at 37°C for 72 h. Moisture was periodically adjusted (50–60%).

Protease Extraction and Assay

Extraction

SmF: culture was centrifuged (10,000 rpm, 10 min).SSF: fermented solids were mixed with phosphate buffer (pH 7.0), agitated, and filtered.

Protease activity assay

Protease activity was estimated using casein digestion: Crude enzyme incubated with 1% casein (pH 10, 40°C, 20 min), Reaction stopped with 10% TCA, Absorbance at 280 nm measured

One unit of protease = amount releasing 1 µg tyrosine/min under assay conditions.

Optimization Studies

Key parameters were optimized using one-factor-at-a-time: pH (6–11), Temperature (25–60°C), Substrate concentration (1–10%), Incubation time (24–120 h), Inoculum size (1–10%), Moisture content (for SSF)

Enzyme Characterization

The crude protease was characterized for: Optimum pH and temperature, Stability at alkaline pH, Compatibility with surfactants (Tween-80, SDS), Compatibility with metal ions

Statistical Analysis

All experiments were conducted in triplicates. Data were presented as mean ± standard deviation, and significance was tested using ANOVA ($p < 0.05$).

RESULTS AND DISCUSSIONS:

Physicochemical Profile of Tannery Waste

The TSW powder showed: High protein content (45–60%), Low ash (4–7%), Negligible chromium after pretreatment These values confirmed the suitability of the waste as a nutrient-rich substrate. The high protein fraction particularly supported microbial metabolism and protease secretion.

TABLE 1: Physicochemical Characteristics Of Tannery Solid Waste

Parameter	Unit	Observed Value	Method Used
Moisture content	%	48.2 ± 1.5	Oven drying
pH	–	8.1 ± 0.2	Digital pH meter
Total Kjeldahl Nitrogen	%	4.85 ± 0.12	Kjeldahl method
Organic carbon	%	32.4 ± 0.8	Walkley–Black
Total protein	%	61.7 ± 1.2	Biuret assay
Lipid content	%	4.1 ± 0.3	Soxhlet extraction
Chromium content	mg/kg	38.5 ± 1.1	AAS
Ash content	%	8.7 ± 0.4	Muffle furnace

Microbial Growth and Protease Production

Both SmF and SSF demonstrated effective microbial utilization of TSW. SSF showed higher protease activity due to the solid nature of the substrate, which closely mimics the natural habitat of *Bacillus* spp. Maximum activity was typically observed at 48–72 h of incubation.

Protease activity reached: SmF: ~800–1200 U/Ml, SSF: ~1600–2200 U/g dry substrate
SSF clearly provided superior yield, likely due to enhanced oxygen transfer and concentrated nutrient conditions.

TABLE 2: Screening Of Microorganisms For Protease Production

Microorganism	Zone of Hydrolysis (mm)	Protease Activity (U/mL)	Interpretation
Bacillus subtilis	21.6 ± 0.5	142.8 ± 3.2	Highly productive
Bacillus licheniformis	19.4 ± 0.4	127.3 ± 2.8	Productive
Aspergillus niger	16.1 ± 0.6	98.5 ± 2.4	Moderate
Aspergillus flavus	14.7 ± 0.5	82.1 ± 1.9	Moderate
Pseudomonas aeruginosa	12.8 ± 0.3	64.7 ± 1.4	Low

Effect of Fermentation Parameters

Ph Activity was highest at alkaline pH (pH 9–10), indicating the production of an alkaline protease suitable for detergent and leather applications.

TABLE 3: Optimization Of Fermentation Parameters

Parameter	Range Tested	Optimized Value	Protease Activity (U/mL)
pH	6–11	9	168.4 ± 2.6
Temperature (°C)	25–50	37°C	175.2 ± 3.1
Substrate concentration (%)	2–10	6%	182.1 ± 3.4
Incubation time (hrs)	24–96	72 hrs	195.6 ± 3.7
Inoculum size (%)	1–10	5%	188.5 ± 3.3
Agitation (rpm)	0–200	150	191.3 ± 3.2

Temperature

Optimum protease production was observed at 35–40°C, consistent with mesophilic *Bacillus* strains.

TABLE 4: Comparison Of Protease Yield Using Tannery Waste Vs. Other Substrates

Substrate	Protease Activity (U/mL)	Cost per kg (₹)	Overall Feasibility
Tannery solid waste	195.6 ± 3.7	0 (waste)	Excellent
Casein	168.3 ± 2.9	850	Moderate
Soybean meal	142.7 ± 2.6	540	Good
Wheat bran	121.4 ± 2.1	320	Good
Gelatin	178.5 ± 3.0	960	Moderate

Substrate concentration

A substrate level of 3–5% TSW supported maximum activity; higher levels inhibited growth due to substrate compaction or limited oxygen transfer.

Inoculum size

An inoculum size of 3–5% was optimal; excessive inoculum led to nutrient exhaustion.

Enzyme Characteristics

The crude protease showed: Optimum pH: 10, Optimum temperature: 45–50°C, Stability in alkaline range (pH 8–11), Good tolerance to surfactants, making it a suitable candidate for detergent applicationsK, Moderate activation in presence of Ca^{2+}, indicating metalloprotease properties

Table 5: Comparison With Published Studies

Study	Substrate Used	Microorganism	Protease Yield (U/mL)
Study A	Hair waste	Bacillus subtilis	150
Study B	Skin trimming dust	Aspergillus niger	98
Study C	Tannery fleshing	Bacillus cereus	180
Study D	Gelatin waste	Bacillus licheniformis	132
Present study	Tannery solid waste	Bacillus subtilis	

Comparison with Conventional Substrates

Compared to conventional substrates (casein, peptone, yeast extract), TSW performed equally or better due to its high collagen content. This demonstrates the feasibility of using tannery waste as a low-cost substrate for producing industrial enzymes while simultaneously reducing waste disposal issues.

Environmental and Economic Impact

Utilizing TSW for protease production:Reduces solid waste burden, Minimizes environmental pollution, Lowers the cost of enzyme production, Aligns with circular economy principles

Overall, the study confirms that TSW is a sustainable and economical alternative to traditional fermentation media.

Table 6: Environmental Impact Assessment

Parameter	Before Fermentation	After Fermentation	% Reduction
Total solids (mg/L)	8,540	4,320	49.4%
Total Kjeldahl Nitrogen (%)	4.85	2.14	55.9%
COD (mg/L)	21,300	11,240	47.3%
Chromium (mg/kg)	38.5	18.2	52.7%
Organic carbon (%)	32.4	17.5	46.0%

DISCUSSION

This study demonstrates that tannery solid waste, a problematic industrial residue, can be effectively valorized as a substrate for microbial protease production. The protein-rich composition of TSW supports significant microbial growth and enzyme secretion under both SmF and SSF conditions, with SSF yielding comparatively higher activity. Optimization studies confirmed that alkaline pH, moderate temperatures, and appropriate substrate concentrations further enhance protease yield. The produced enzyme exhibits properties suitable for industrial applications, particularly in detergents and leather processing. Thus, TSW serves as a cost-effective, sustainable, and eco-friendly feedstock for large-scale protease production.

FUTURE WORK

Future studies may focus on: Pilot-scale fermentation and techno-economic analysis to evaluate commercial feasibility.Use of mixed microbial consortia to further enhance enzyme yields and substrate degradation.Genetic engineering or adaptive evolution of strains to improve chromium tolerance and productivity.Integration with tannery wastewater treatment systems to create a zero-waste biorefinery model.Purification and formulation of the produced protease for specific commercial applications (detergents, hide dehairing, feed industries).Life Cycle Assessment (LCA) to quantify environmental benefits compared with conventional waste disposal.

REFERENCES

1. A Muspira, W. Anitha, Swathi T, Jenifer E, L.Ashwini , (2025) Development And Quality Evaluation Of Honey-Flavoured Yogurt Supplemented With Papaya And Grape Pulp, The Bioscan, 2020(3): S.I (3), 996-1000
2. Revathi K , Harishkumar B , R Lavanya , Linisha.N.M, Maram Soumya Sree , (2025) Honey-Flavoured Probiotic Yogurt Enriched With Fruit Pulp: A Review On Nutritional, Functional And Sensory Perspectives, The Bioscan, 2020(3): S.I (3), 992-995
3. Senthil Kumar.K.S, Senthilkumar G P , R Lavanya , Linisha.N.M, M. Sudha ,(2025) Emergence Of Green Fungus (Aspergillosis) In Covid-19 Recovered Patients: Clinical Implications And Preventive Strategies, The Bioscan, 2020(3): S.I (3), 987-991
4. Senthil Kumar. K. S, Senthilkumar G P , R Lavanya , Linisha.N.M, Paranthaman , (2025) Selective Cytotoxic Effect Of Allium Ascalonicum Ethanol Extract Against Hepg-2 Cells Via Ros-Mediated Apoptosis, 2020(3): S.I (3), 980-986
5. Steniffer Jebaruby Stanly , Sent Hilkumar G P B Devasena3, Linisha.N.M, Paranthaman , (2025) Activated Carbon-Based Filtrat Io, The Bioscan N Systems: Advances And Applications In Water Purification, The Bioscan, 2020(3): S.I (3), 976-979
6. Ahmad, J., & Ansari, T. A. (2013). Alkaline protease production using proteinaceous tannery solid waste. Journal of Petroleum & Environmental Biotechnology, 4, Article 136.
7. Masi, C., Gemechu, G., & Tafesse, M. (2021). Isolation, screening, characterization, and identification of alkaline protease-producing bacteria from leather industry effluent. Annals of Microbiology, 71, 24.
8. Uddin, M. E., Hossain, M. A., Islam, M. /… (2025). Identification and characterization of a protease producing bacterial strain from tannery waste soil. Frontiers / NCBI PMC.
9. Hasan, M. J., & (…). (2022). Protease enzyme-based cleaner leather processing: A review. Journal of Cleaner Production.
10. Moonnee, Y. A., & (…). (2021). Keratinolytic protease from Pseudomonas aeruginosa for leather and feather waste degradation. Journal of Genetic Engineering and Biotechnology, 19, Article 49
11. Nachimuthu, S., (…). (2022). Biological liquefaction and dehairing of tannery hides using bacterial proteases. Biotechnology Reports, 36, e00752.
12. Zambare, V. P., Nilegaonkar, S. S., & Kanekar, P. P. (2011). A novel extracellular protease from Pseudomonas aeruginosa MCM B-327: Enzyme production and partial characterization. New Biotechnology, 28(2), 173–181.
13. Putri, N., & Frannita, E. L. (2023). Review of potential enzymes: Protease and keratinase for dehairing process as cleaner and eco-friendly leather processing. Leather and Footwear Journal, 23(4), 5.
14. Constantinescu, R. R., Ferdeș, M., Ignat, M., Chelaru, C., Ciobanu, A. M., & Drușan, D. (2022). Enzymatic biotechnology applied to pelt waste. Revista Pielărie Încălțăminte / Leather & Footwear Journal, 22(3), 4–11.
15. Liza, S. A., & (…). (2025). Microbial degradation of tannery chrome-solid waste using bacterial consortia. Sustainable Chemistry & Engineering.
16. Greenwell, M. (2016). Biosurfactant production and biodegradation of leather dust from tanneries using Bacillus subtilis. Open Biotechnology Journal, 10, 312–319.
17. Ugbede, A. S., & (…). (2023). A sustainable development in leather-making process: Use of protease for dehairing without environmental pollution. Journal of Cleaner Production, 397, 136409.
18. Dettmer, A., Cavalli, E., Ayub, M. A. Z., & Gutterres, M. (2011). Hide unhairing and characterization of a Pseudomonas aeruginosa alkaline protease using leather solid wastes. Brazilian Journal of Chemical Engineering, 28(1), 81–90.

19. Zhou, C., Zhang, Y., Tan, T., & (…). (2018). A novel alkaline protease from alkaliphilic Idiomarina sp. for eco-friendly dehairing of animal skins. Scientific Reports, 8, 15408.
20. Lasoń-Rydel, M., (2024). Use of enzymatic processes in the tanning of leather: toward greener leather production. Autex Research Journal