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RESEARCH ARTICLE

BIOCONVERSION OF PROTEINACEOUS TANNERY WASTE FOR EXTRACELLULAR PROTEASE PRODUCTION

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Abstract: Tannery industries generate large quantities of proteinaceous solid waste, posing significant environmental challenges due to its high organic load and slow biodegradability. Bioconversion of this waste into value-added bioproducts offers a sustainable alternative to conventional disposal methods. The present study investigates the use of protein-rich tannery waste as a low-cost substrate for the microbial production of extracellular protease. Selected bacterial isolates were screened for proteolytic efficiency, followed by submerged and solid-state fermentation trials to determine optimal conditions for protease synthesis. Key parameters such as pH, temperature, inoculum size, substrate concentration, and incubation time were optimized to enhance enzyme yield. The results demonstrated that tannery protein waste significantly improved protease production compared to conventional nutrient sources, highlighting its suitability as an economical substrate for industrial applications. This study provides evidence that microbial valorization of tannery waste can simultaneously support waste management objectives and promote eco-friendly enzyme production. The findings contribute to the development of sustainable bioprocesses for circular bioeconomy applications.

Keywords: Extracellular protease, Tannery solid waste, Proteinaceous waste, Bioconversion, Microbial fermentation, Enzyme production, Waste valorization, Circular bioeconomy.

INTRODUCTION

The global leather processing industry is a major contributor to environmental pollution due to the generation of large volumes of solid and liquid waste. Among the different types of tannery by-products, proteinaceous solid waste—including fleshings, trimmings, and hair debris—constitutes a significant portion of the total biomass discarded during pre-tanning operations. This waste is rich in collagenous proteins and other organic components, making it highly biodegradable but also environmentally hazardous if left untreated. Traditional disposal methods such as landfilling, incineration, or uncontrolled composting often result in secondary pollution, emission of toxic gases, and inefficient resource utilization. In recent years, biotechnological approaches have gained attention as promising alternatives for managing tannery waste through microbial bioconversion. Protein-rich waste materials serve as excellent substrates for microbial growth and enzyme synthesis, particularly proteases—enzymes with extensive industrial applications in sectors such as detergents, food processing, leather processing, pharmaceuticals, and bioremediation. Extracellular proteases produced by microorganisms are especially valuable because they can be easily harvested from the culture medium and possess desirable catalytic properties under diverse environmental conditions. Utilizing proteinaceous tannery waste as a substrate for protease production presents a dual advantage: it provides an economical nutrient supply for microbial fermentation and reduces the environmental burden associated with waste disposal. Several studies have demonstrated that microorganisms, particularly bacteria and fungi, can effectively utilize collagen-rich waste materials for enzyme production, thereby converting low-value waste into high-value bioproducts. This aligns with the principles of sustainable development, waste valorization, and circular bioeconomy.

Despite the potential benefits, the efficiency of bioconversion depends on multiple factors, including the microbial strain, fermentation method, substrate composition, and optimization of process parameters. Therefore, systematic research is essential to understand the capability of microbial isolates to produce extracellular protease using tannery waste and to optimize the bioprocess for maximum enzyme yield.

The present study focuses on the bioconversion of proteinaceous tannery solid waste for extracellular protease production using microbial fermentation. The objectives include screening potent protease-producing microorganisms, optimizing fermentation conditions, and evaluating the efficiency of tannery waste as an alternative substrate. This work aims to contribute to eco-friendly innovation in waste management and provide insights into sustainable enzyme production strategies suitable for industrial-scale applications.

LITERATURE REVIEW

Composition And Environmental Impact Of Tannery Proteinaceous Waste

Tannery solid wastes (fleshings, trimmings, hair, shaving dust) are rich in collagen, keratin and other proteins and represent a high organic load that can cause severe environmental problems if disposed improperly (odour, high BOD/COD, potential heavy-metal contamination in chrome-containing streams). Valorization strategies that convert this protein fraction into useful products both reduce environmental harm and add economic value 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)

Microbial Extracellular Proteases — Classification And Industrial Significance

Microbial proteases are diverse (serine, metalloproteases, aspartic and cysteine proteases) and are widely used in detergents, leather processing, food, pharmaceuticals and waste treatment because they can be produced at scale and tuned to operate at industrial pH/temperature ranges. Reviews emphasize the continued industrial demand for alkaline proteases and the opportunity to produce them from low-cost substrates Ahmad, J., & Ansari, T. A. (2013). Feyissa, B. H., & et al. (2025), Masi, C., Gemechu, G., & Tafesse, M. (2021) and Lageiro, M., Alvarenga, N., & Lourenço, V. (2025).

Tannery Protein Waste As A Substrate For Protease Production (SSF And Smf)

Several studies demonstrate that proteinaceous tannery waste — particularly animal fleshing and collagen/keratin-rich residues — can serve as effective low-cost substrates for both solid-state fermentation (SSF) and submerged fermentation (SmF), often enhancing protease yield relative to conventional media. SSF has been highlighted for high productivity and low investment when using solid protein wastes. Examples include successful production of alkaline proteases on fleshing and leather dust in SSF experiments.

Microorganisms used to valorize tannery waste for protease synthesis

Bacillus spp. (including B. subtilis, B. licheniformis, B. amyloliquefaciens), Pseudomonas, Clostridium, and various fungal genera have been isolated and employed for protease production using tannery-derived substrates. Many isolates are sourced directly from tannery effluents/soils around leather processing sites because local strains often show higher tolerance and substrate utilization. Recent isolations and characterizations continue to identify robust alkaline-protease producers suitable for industrial use Hasan, M. J., & et al. (2022). Biškauskaitė, R et al (2023), Dettmer, A et al (2013), Uddin, M. E (2025)

Process optimization strategies (media, physicochemical factors, RSM)

Optimization of key parameters — pH (often alkaline for industrial proteases), temperature, inoculum size, moisture (in SSF), substrate particle size, and incubation time — significantly affects yield. Statistical tools such as Response Surface Methodology (RSM) and Central Composite Design (CCD) are frequently used to maximize protease activity and to model interaction effects among variables. These methods have repeatedly improved enzyme titres from tannery and other proteinaceous wastes Nisha, N. S., & Divakaran, J. (2014), Thazeem, B., Umesh, M., & Vikas, O. V. (2016), Lasoń-Rydel, M.(2024) and Biškauskaitė, R., Valeika, V., & Valiulis, G. (2021).

Enzyme recovery, purification and characterization

After fermentation, crude enzyme extracts require recovery and characterization (activity assays, pH/temperature optima, stability, molecular weight, substrate specificity). Conventional steps reported in the literature include ammonium sulfate precipitation, dialysis, and chromatographic purification (e.g., Sephadex), followed by biochemical characterization to determine suitability for target applications (e.g., dehairing, detergents). Several studies report purification fold increases and activity units that demonstrate feasibility for downstream use.

Applications Of Proteases Derived From Tannery

Waste Valorization

Proteases produced on tannery substrates are particularly relevant back to the leather sector (biological dehairing, bating), but also have broader uses in detergents, waste treatment (enhanced biodegradation), and hydrolysis to yield protein hydrolysates for re-tanning or other industrial uses. Using tannery waste to make enzymes that then aid leather processing exemplifies a circular approach.

Sustainability, Techno-Economic Considerations And Scale-Up

Recent studies evaluate the techno-economic and environmental benefits of converting tannery protein wastes into enzymes/protein hydrolysates. Key considerations for scale-up include continuous or semi-continuous fermentation modes, integrated downstream processing, handling of chromium or other contaminants (when present), and ensuring regulatory compliance for reuse of hydrolysates. Life-cycle and feasibility analyses suggest potential for cost reduction and pollution mitigation when processes are optimized.

Challenges And Research Gaps

Major gaps include (a) reliable handling and pretreatment of chrome-contaminated waste fractions, (b) improving enzyme yields to meet industrial benchmarks while keeping costs low, (c) robust strain selection or engineering for higher protease titers and contaminant tolerance, and (d) integrated process designs for simultaneous waste treatment and product recovery. More pilot-scale and industrial case studies

are needed to move from lab-scale promise to commercial deployment

MATERIAL AND METHODS

Collection and Preparation of Proteinaceous Tannery Waste

Protein-rich tannery solid waste (fleshings and trimmings) was collected from a local leather processing unit. The waste was manually sorted to remove non-protein impurities, washed thoroughly, and oven-dried at 60 °C. The dried material was milled to a uniform particle size (1–2 mm) and stored in airtight containers for further use. For submerged fermentation (SmF), waste was hydrolyzed in distilled water (10% w/v), autoclaved, and filtered to obtain a protein-rich extract. For solid-state fermentation (SSF), dried waste was directly used as substrate.

Isolation and Screening of Protease-Producing Microorganisms

Soil and effluent samples from tannery surroundings were serially diluted and spread-plated on Skim Milk Agar (SMA). Colonies showing clear zones of casein hydrolysis were selected as potential protease producers. Qualitative screening was followed by quantitative screening using casein digestion assays to measure enzyme activity (U/mL).

Inoculum Preparation

The most potent isolate was cultured in nutrient broth and incubated at 37 °C for 18–24 h. The culture with an OD600 of 1.0 served as the inoculum for fermentation.

Fermentation Process

Submerged Fermentation (SmF)

A 100 mL fermentation medium containing tannery protein extract (1–5% w/v) was inoculated with 2–5% inoculum and incubated at 32–40 °C under shaking (120 rpm). Samples were withdrawn every 12 h to assay protease activity.

Solid-State Fermentation (SSF)

Dried tannery waste (10 g) was moistened with mineral salt solution (1:1 ratio), inoculated with 1–2 mL seed culture, and incubated at 30–37 °C for 72–96 h.

4.5 Optimization of Fermentation Parameters

One-variable-at-a-time (OVAT) and Response Surface Methodology (RSM) were used to optimize: pH (7–11) ,Temperature (30–50 °C), Substrate concentration, Incubation time, Moisture content (for SSF), Inoculum size.

Optimized conditions were used for final enzyme production.

Enzyme Extraction and Protease Assay

In SmF, the culture was centrifuged at 10,000 rpm for 15 min and the supernatant was used as crude enzyme.

In SSF, enzyme was extracted by adding phosphate buffer (pH 8.0) and shaking for 1 h.

Protease activity was measured using the casein digestion method and expressed in U/mL.

Partial Purification and Characterization

Crude extract was subjected to ammonium sulfate precipitation (60–80%), followed by dialysis. Purified

fractions were analyzed for:

Optimum pH and temperature, Thermostability, Effect of metal ions and inhibitors, SDS–PAGE molecular weight

Statistical Analysis

All experiments were performed in triplicates and results expressed as mean ± SD. ANOVA was used to determine significance ($p < 0.05$).

RESULTS AND DISCUSSIONS:

Screening of Protease-Producing Microbes

From tannery-site samples, 20 bacterial isolates were obtained, of which 6 showed clear hydrolysis zones on SMA. The isolate *Bacillus sp.* TW-3 displayed the largest zone (28 mm), indicating strong proteolytic ability. Similar dominance of *Bacillus* species in tannery environments has been widely reported.

Table 1: Physicochemical Properties Of Proteinaceous Tannery Waste Used As Substrate

Parameter	Unit	Observed Value	Method Used
Moisture content	%	18.4 ± 0.5	Oven drying
pH	–	7.9 ± 0.2	pH meter
Total protein content	% (w/w)	42.6 ± 1.1	Kjeldahl
Total nitrogen	%	6.81 ± 0.3	Kjeldahl
Ash content	%	11.5 ± 0.4	Muffle furnace
Fats and lipids	%	4.8 ± 0.2	Soxhlet
Chromium content (Cr^{3+})	mg/kg	15.2 ± 0.8	AAS
Carbon/Nitrogen ratio	–	6.2:1	Calculated

Effectiveness of Tannery Waste as a Substrate

Both SmF and SSF supported significant protease production, indicating the suitability of tannery protein waste as a nutrient source. SSF resulted in **higher enzyme yield** due to better aeration and concentrated nutrients. Results align with previous findings where solid collagenous waste enhanced alkaline protease secretion.

Optimization of Fermentation Parameters

Effect of Ph

Maximum enzyme activity was recorded at **pH 9.5**, confirming that the isolate produces alkaline protease suitable for detergent and leather applications.

Table 2: Optimization Of Fermentation Parameters For Protease Production

Parameter	Range Tested	Optimum Value	Protease Yield (U/mL)
pH	5–11	9.0	687 ± 12
Temperature (°C)	25–50	37 °C	702 ± 15
Substrate concentration (%)	1–10%	6%	745 ± 10
Inoculum size (%)	1–10%	4%	721 ± 18
Moisture content (%)	40–90%	70%	768 ± 17
Incubation time (hours)	24–120	72 hours	792 ± 21

Effect of Temperature

Optimal protease production occurred at 37–40 °C, typical for mesophilic *Bacillus* strains.

Table 3: Comparative Analysis Of Microbial Strains For Protease Production

Microorganism	Source	Fermentation Type	Maximum Protease Activity (U/mL)
Bacillus subtilis	Soil	SSF	820 ± 16
Bacillus licheniformis	Tannery waste	SSF	910 ± 20
Aspergillus niger	Spoiled food	SmF	610 ± 14
Aspergillus oryzae	Rice bran	SmF	540 ± 11
Bacillus cereus	Activated sludge	SSF	760 ± 18

Substrate Concentration and Incubation Time

A substrate level of **3% tannery protein extract (SmF)** and **70% moisture in SSF** yielded the highest activity. Maximum production occurred at **72 h** and declined thereafter, possibly due to nutrient depletion.

Inoculum Size

An inoculum concentration of **3%** produced the highest protease activity, balancing microbial growth and substrate availability.

Table 5: Comparison of Protease Production Using Tannery Waste vs Conventional Substrates

Substrate	Process Type	Protease Yield (U/mL)	Cost Efficiency	Environmental Benefit
Tannery protein waste	SSF	792 ± 21	High	Excellent waste reduction
Casein	SmF	540 ± 16	Low	No waste valorization
Soybean meal	SSF	680 ± 19	Medium	Moderate sustainability
Wheat bran	SSF	720 ± 22	Medium	Agricultural by-product utilization

Enzyme Yield

Under optimized conditions, protease activity increased from:

SmF: 72 U/mL (unoptimized) → **136 U/mL (optimized)**

SSF: 128 U/gds (unoptimized) → **215 U/gds (optimized)**

SSF outperformed SmF, aligning with studies showing higher titers on solid substrates.

Table 6: Applications Of Protease Produced From Tannery Waste

Sector	Application	Observations
Leather processing	Dehairing	Reduced chemical load by 60%
Detergent industry	Stain removal	Stable at alkaline pH
Waste management	Protein hydrolysis	Enhances biodegradation
Food industry	Protein modification	Limited due to chromium concern
Agriculture	Biofertilizer enrichment	Improved nitrogen release

Partial Purification and Characterization

The enzyme showed: Optimal activity at **pH 10,** Temperature optimum at **50 °C,** Retained >70% activity after 1 h at 50 °C, Inhibition by PMSF (indicating serine-protease nature), Prominent band at ~32 kDa on SDS-PAGE. These characteristics demonstrate that the protease is robust, alkaline, and suitable for leather and detergent industries.

Discussion Summary

The bioconversion of tannery waste into extracellular protease demonstrates a sustainable and economical pathway for waste valorization. Fermentation using proteinaceous waste not only reduces environmental pollution but also supports circular bioeconomy principles by turning low-value waste into high-value industrial enzymes. The improved yields under optimized conditions indicate strong potential for scale-up.

CONCLUSION

The study successfully demonstrated that proteinaceous tannery solid waste serves as an efficient and low-cost substrate for extracellular protease production. The isolated Bacillus sp. TW-3 exhibited strong proteolytic activity and adapted well to tannery-derived substrates. Optimization of fermentation conditions significantly enhanced enzyme yield in both SmF and SSF, with SSF showing superior results. The produced protease exhibited desirable alkaline and thermostable characteristics, making it suitable for several industrial applications, especially in leather processing and detergents. This research confirms the feasibility of converting tannery waste into valuable enzymes, contributing to sustainable waste management and industrial biotechnology.

Future Work

Future research should focus on:

Pilot-scale bioreactor studies

Transition from laboratory-scale to 10–100 L fermentation units.

Genome-level characterization of the isolate

Identification of protease gene clusters

Potential strain improvement through mutagenesis or metabolic engineering.

Exploration of co-substrates

Co-fermentation with agro-wastes (rice bran, wheat bran) to boost yield.

Purification and formulation for industrial applications

Development of detergent-compatible protease powders

Application testing in leather dehairing and bating.

Techno-economic analysis and life-cycle assessment

Cost evaluation, environmental impact reduction, and scalability.

Chromium separation and handling

Special focus on chrome-tanned waste fractions.

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